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The First Syntheses of GLA-60 Positional Isomers and Their Biological Activities

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Six GLA-60 positional isomers (**8**, **14**, **14′**, **20**, **26**, and **26′**) were synthesized to investigate their biological activities. Compound **8** exhibited potent agonistic activity, while compounds **26** and **26′** exhibited slight agonistic activity on TNFα production toward human monoblastic U937 cells. TNFα production (% control; 10 ng ml^{-1} of LPS = 100) of compound **8** in the concentration of 10 μM was 611, and that of lipid A in the same concentration was 651. In contrast, the difluorinated compounds **14**, **14′**, and **20** showed little agonistic activity on TNFα production. And neither compound **8** nor compounds **26** and **26′** showed antagonistic activity. On the other hand, the difluorinated compounds **14**, **14′**, and **20** showed potent antagonistic activity, and inhibited the LPS-induced TNFα production dose-dependently. Compound **14′** (10 μM) inhibited in excess of 80% of the LPS-induced TNFα production.

Lipopolysaccharide (LPS),[1] an outer surface membrane component present in Gram-negative bacterial cells such as *Salmonella minnesota*, *Salmonella typhimurium*, and *Escherichia coli*, is the causative agent of fever and lethal shock in higher animals afflicted by septicemia. On the other hand, LPS is a highly potent stimulator of the immune system, but its propensity to induce endotoxic shock has precluded it from clinical use (Fig. 1).

Most of the biological activities of LPS reside in a relatively small portion of the molecule known as lipid A, a disaccharide unit bearing constituent lipid chains. Lipid A, which was first isolated by Westphal and Luderitz[2] and later chemically synthesized by both Shiba[3] and Achiwa[4] groups, exists as a hydrophobic anchor substance holding an essentially linear polysaccharide chain to the cell wall, and is responsible for eliciting many physiological effects in Gram-negative bacteria; endotoxicity, adjuvanticity, and certain immunostimulating properties are all derived from the lipid A stimulatory effect on macrophages. Nishijima and Raetz[5] isolated lipid X from a mutant of *Escherichia coli*. Lipid X, the reducing part of lipid A, is one of the intermediates in the biosynthesis of LPS.[6]

In a series of structure-activity relationship studies on nonreducing subunit analogues of lipid A, Hasegawa and Kiso[7] have demonstrated that several LPS agonistic activities are expressed by certain 4-*O*-phosphono-D-glucosamine derivatives pertaining to the structure of GLA-60 (Fig. 2).[7] Recently it has been shown that some lipid A and some GLA-60 analogues show potency as endotoxin antagonists.[8] In related studies, it has been recognized that the antimicrobial property of antibiotics induces the release of LPS from the outer membrane of Gram-negative bacteria, and in turn causes an acute inflammatory response following the release of TNFα. This remains a serious unsolved clinical problem, despite the availability of potent antibiotics.

We have been investigating nontoxic compounds that display LPS agonist activity for cancer therapy or LPS antagonist activity for septicemia and antiinflammation, wherein such activity is ascribable to their GLA-60 structural resemblance. In this paper, we report the first syntheses and biological activities of GLA-60 positional isomers, namely, *N*-[(*R*)-3-(hydroxy)tetradecanoyl]-3-*O*-phosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (**8**),[9] and its fluorinated derivatives (**14**, **14′**, **20**, **26**, and **26′**). The positions of the equatorial substituents of these compounds were each shifted one position compared with GLA-60 or lipid A. Moreover, the substitutions at the C1 (anomeric amido), C2 (ester), and C3 (phosphono) positions of these compounds are alined with C1-β, C2-α, and C3-β, respectively, and also those of GLA-60 and lipid A are alined with C2-α (amido), C3-β (ester), and C4-α (phosphono), respectively. The 2-monofluorinated or 2,2-difluorinated tetradecanoyl group was expected to enhance biological activity based on our previous work.[10]

Synthesis. Compound **1**[11] was treated with (*R*)-3-(benzyloxy)tetradecanoyl chloride,[12] (±)-3-benzyloxy-2,2-difluorotetradecanoyl chloride, 2,2-difluorotetradecanoyl chloride,[13] or (±)-*threo*-3-benzyloxy-2-fluorotetradecanoyl chloride, and triethylamine in dichloromethane, and yielded amides **2**, an inseparable diastereomeric mixture of **9** and **9′**,

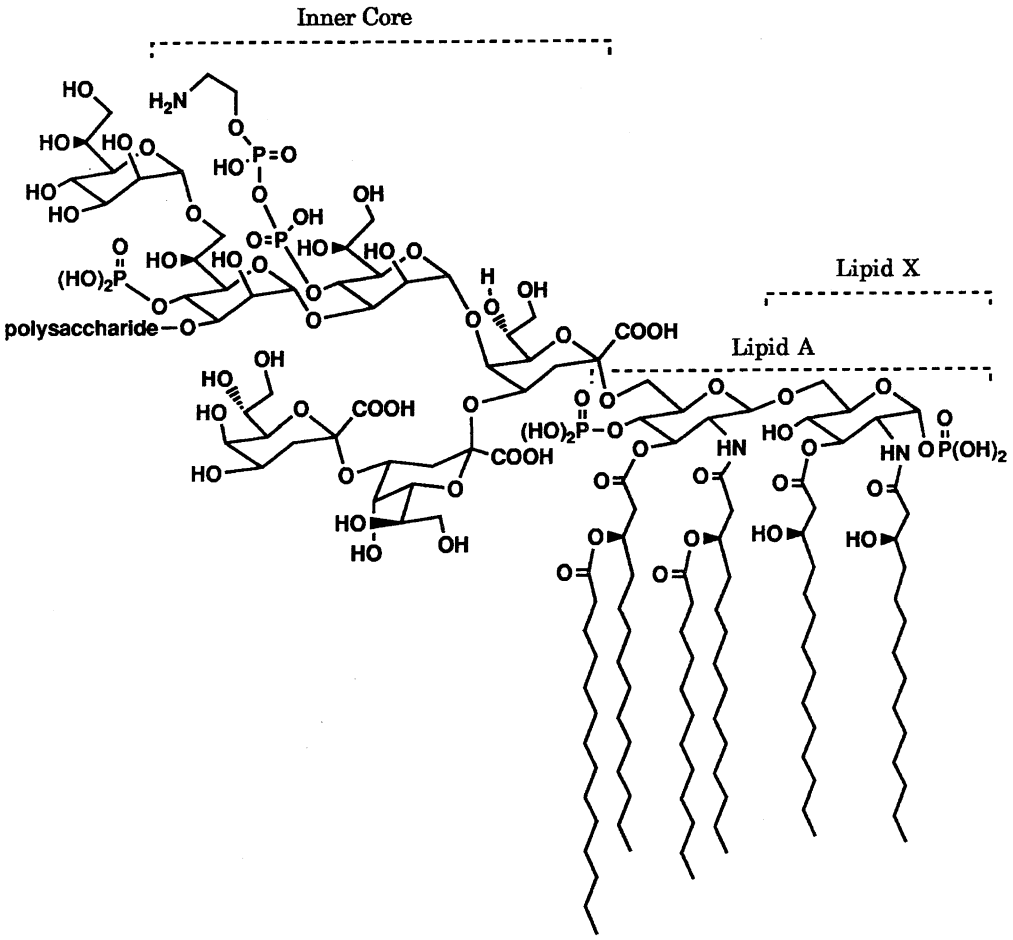


Fig. 1. Lipopolysaccharide (LPS).[1)]

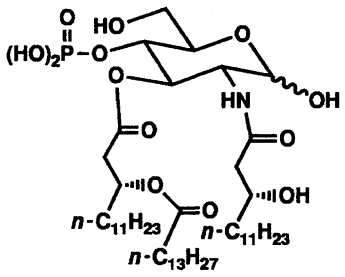
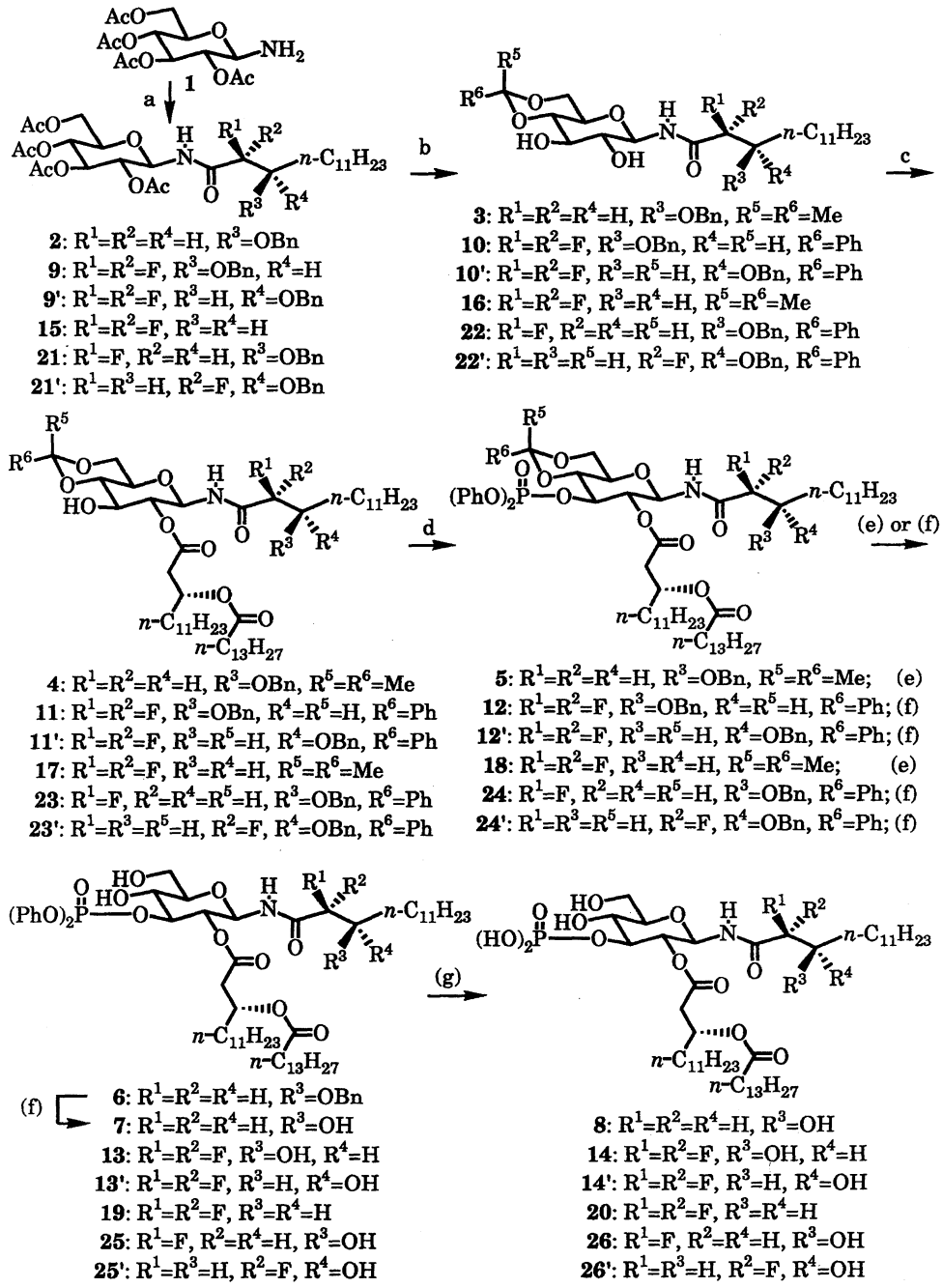


Fig. 2. GLA-60.

15, **21**, and **21′**, respectively (Scheme 1). The mixture (**9** and **9′**) was converted to a separable mixture **11** and **11′** via an inseparable mixture **10** and **10′**. At this stage, compounds **11** and **11′** were separated chromatographically. We used the two racemates as the mono- and di-fluorinated compounds. Because they were prepared easily, and moreover, each of them was able to yield a pair of diastereomers as the products in one-pot.

Compounds **2** and **15** were converted to acetonides (4, 6-*O*-isopropylidene derivatives) **3** and **16**, respectively, by treatment with sodium methoxide in methanol, and with 2, 2-dimethoxypropane, using pyridinium *p*-toluenesulfonate as a catalyst in *N*,*N*-dimethylformamide (DMF), in succession. On the other hand, compounds **9**, **9′**, **21**, and **21′**, were converted to 4,6-*O*-benzylidene derivatives **10**, **10′**, **22**, and **22′**, respectively, by treatment with sodium methoxide in methanol, and then with benzaldehyde dimethyl acetal using 10-camphorsulfonic acid in DMF. These 2,3-diol compounds, **3**, **10**, **10′**, **16**, **22**, and **22′**, were treated with (*R*)-3-(tetradecanoyloxy)tetradecanoic acid, 4-dimethylaminopyridine (DMAP) and dicylohexylcarbodiimide (DCC) in DMF regioselectively to give 2-*O*-monoacylated compounds **4**, **11**, **11′**, **17**, **23**, and **23′**, respectively. Verification that acylation had occurred at the 2-position of these 2,3-diol compounds came from proton NMR data, showing that the C2 protons of products shifted to lower magnetic fields (in the case of **4**, shifted from $\delta = 3.20$ to $\delta = 4.70$). The remaining 3-hydroxy groups of **4**, **11**, **11′**, **17**, **23**, and **23′** were phosphorylated with diphenyl phosphorochloridate and DMAP to give **5**, **12**, **12′**, **18**, **24**, and **24′**, respectively. The 4,6-*O*-isopropylidene groups of compounds **5** and **18** were cleaved by treatment with 90% acetic acid to give 4,6-diols **6** and **19**, respectively. Compound **6** in turn was hydrogenolytically debenzylated under hydrogen using 10% palladium on carbon in tetrahydrofuran (THF) to give **7**. The same two-step procedure was employed to remove the 4,6-*O*-benzylidene and *O*-benzyl groups of compounds **12**, **12′**, **24**, and **24′** to afford compounds **13**, **13′**, **25**, and **25′**, respectively. Finally, hydrogenolysis of these phosphate esters **7**, **13**, **13′**, **19**, **25**,



2: $R^1=R^2=R^4=H$, $R^3=OBn$
9: $R^1=R^2=F$, $R^3=OBn$, $R^4=H$
9': $R^1=R^2=F$, $R^3=H$, $R^4=OBn$
15: $R^1=R^2=F$, $R^3=R^4=H$
21: $R^1=F$, $R^2=R^4=H$, $R^3=OBn$
21': $R^1=R^3=H$, $R^2=F$, $R^4=OBn$

3: $R^1=R^2=R^4=H$, $R^3=OBn$, $R^5=R^6=Me$
10: $R^1=R^2=F$, $R^3=OBn$, $R^4=R^5=H$, $R^6=Ph$
10': $R^1=R^2=F$, $R^3=R^5=H$, $R^4=OBn$, $R^6=Ph$
16: $R^1=R^2=F$, $R^3=R^4=H$, $R^5=R^6=Me$
22: $R^1=F$, $R^2=R^4=R^5=H$, $R^3=OBn$, $R^6=Ph$
22': $R^1=R^3=R^5=H$, $R^2=F$, $R^4=OBn$, $R^6=Ph$

4: $R^1=R^2=R^4=H$, $R^3=OBn$, $R^5=R^6=Me$
11: $R^1=R^2=F$, $R^3=OBn$, $R^4=R^5=H$, $R^6=Ph$
11': $R^1=R^2=F$, $R^3=R^5=H$, $R^4=OBn$, $R^6=Ph$
17: $R^1=R^2=F$, $R^3=R^4=H$, $R^5=R^6=Me$
23: $R^1=F$, $R^2=R^4=R^5=H$, $R^3=OBn$, $R^6=Ph$
23': $R^1=R^3=R^5=H$, $R^2=F$, $R^4=OBn$, $R^6=Ph$

5: $R^1=R^2=R^4=H$, $R^3=OBn$, $R^5=R^6=Me$; (e)
12: $R^1=R^2=F$, $R^3=OBn$, $R^4=R^5=H$, $R^6=Ph$; (f)
12': $R^1=R^2=F$, $R^3=R^5=H$, $R^4=OBn$, $R^6=Ph$; (f)
18: $R^1=R^2=F$, $R^3=R^4=H$, $R^5=R^6=Me$; (e)
24: $R^1=F$, $R^2=R^4=R^5=H$, $R^3=OBn$, $R^6=Ph$; (f)
24': $R^1=R^3=R^5=H$, $R^2=F$, $R^4=OBn$, $R^6=Ph$; (f)

6: $R^1=R^2=R^4=H$, $R^3=OBn$
7: $R^1=R^2=R^4=H$, $R^3=OH$
13: $R^1=R^2=F$, $R^3=OH$, $R^4=H$
13': $R^1=R^2=F$, $R^3=H$, $R^4=OH$
19: $R^1=R^2=F$, $R^3=R^4=H$
25: $R^1=F$, $R^2=R^4=H$, $R^3=OH$
25': $R^1=R^3=H$, $R^2=F$, $R^4=OH$

8: $R^1=R^2=R^4=H$, $R^3=OH$
14: $R^1=R^2=F$, $R^3=OH$, $R^4=H$
14': $R^1=R^2=F$, $R^3=H$, $R^4=OH$
20: $R^1=R^2=F$, $R^3=R^4=H$
26: $R^1=F$, $R^2=R^4=H$, $R^3=OH$
26': $R^1=R^3=H$, $R^2=F$, $R^4=OH$

Reagents and conditions :
(a) (*R*)-3-benzyloxytetradecanoyl chloride, (±)-3-benzyloxy-2,2-difluorotetradecanoyl chloride, 2,2-difluorotetradecanoyl chloride or (±)-*threo*-3-benzyloxy-2-fluorotetradecanoyl chloride, Et_3N, CH_2Cl_2, 30 min, 25 °C, 84%, 94% (9+9′), 84%, 83% (**21** : **21′** = 6 : 5); (b) 1) MeONa, MeOH, 30 min, 25 °C; 2) 2,2-dimethoxypropane or benzaldehyde dimethyl acetal, PPTS or 10-camphorsulfonic acid, DMF, 25 °C, 3—16 h, 72%, 70% (**10**+**10′**), 85%, 42%, 71%; (c) (*R*)-3-(tetradecanoyloxy)tetradecanoic acid, DMAP, DCC, Et_2O, 1 h, 25 °C, 67%, 57% (**11** : **11′** = 11 : 8), 62%, 75%, 67%; (d) diphenyl phosphorochloridate, DMAP, CH_2Cl_2, 0.5—12 h, 25 °C, 94%, 85%, 96%, 93%, 73%, 73%, (e) AcOH–H_2O (9 : 1), 5 h, 60 °C, 76% (**6**), 99% (**19**); (f) H_2, 10% Pd/C, THF, 3—18 h, 25 °C, 77% (**7**), 81% (**13**), 87% (**13′**), 95% (**25**), 86% (**25′**); (g) H_2, PtO_2, THF, 3—12 h, 25 °C, 99%, 96%, 98%, 81%, 93%, 92%, respectively.

Scheme 1.

and **25′**, using platinum(IV) oxide as a catalyst, yielded the phosphono compounds **8**, **14**, **14′**, **20**, **26**, and **26′**, respectively.

The above synthetic procedure demonstrates a synthetic generality for preparing six GLA-60 positional isomers (**8**, **14**, **14′**, **20**, **26**, and **26′**) from β-D-glucosylamine tetraacetate

(**1**).

Finally, we would like to describe how to determine the configurations on the anomeric amide side chains of the two pairs of diastereomers : **21** and **21′**, and **11** and **11′**.

Both compound **28′**, obtained from compound **21′**, and that obtained from the authentic (2*R*,3*S*)-compound (**27′**) by hydrogenolysis were the same compound in all respects (Scheme 2). Reaction of **1** with (±)-*threo*-3-[(benzyloxycarbonyl)oxy]-2-fluorotetradecanoic acid[14] in CH_2Cl_2 using DCC and DMAP as condensing agents gave an upper R_f compound **27** and a lower R_f compound **27′**. The upper R_f compound (**27**) was identical in all respects with that obtained by the reaction of **1** and optically active (2*S*,3*R*)-3-[(benzyloxycarbonyl)oxy]-2-fluorotetradecanoic acid.[15] Accordingly, we could determine the configuration of **27′** to be (2*R*,3*S*), and we could correlate the configurations of compounds **21** and **21′** as (2*S*,3*R*) and (2*R*,3*S*), respectively.

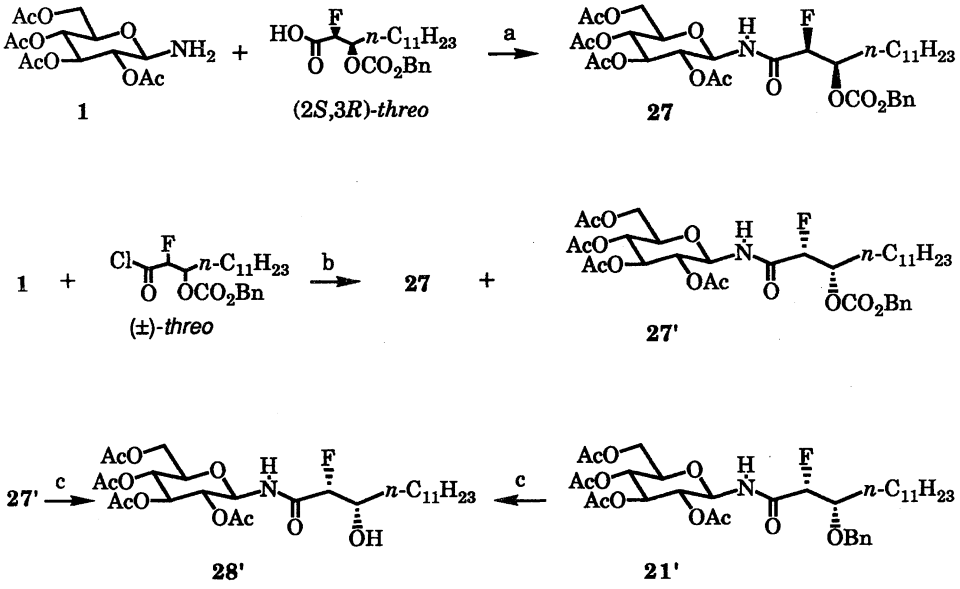
Also, configurations of the amide side chain of **11** and **11′**, which were prepared from **1** (Scheme 3) and (±)-3-benzyloxy-2,2-difluorotetradecanoic acid, were determined as (*R*) and (*S*), respectively, by coincidence in all respects with the lower R_f compound **11′** and the authentic **11′** prepared via compound **10′** from the compound **9′** obtained from **1** and optically active (*S*)-3-benzyloxy-2,2-difluorotetradecanoic acid (**32**) in the same procedure as mentioned in Scheme 1.

The optically active **32** was prepared as follows. Treatment of (*S*)-2,2-difluorotetradecane-1,3-diol (**29**)[16] with 1.1 molar amounts of *t*-butyldimethylsilyl chloride and DMAP gave monosilylated **30** (81% yield) which was treated with benzyl bromide in THF containing a catalytic amount of tetrabutylammonium iodide using NaH as a base, and the benzylated compound was successively treated with concd aqueous HCl in 1,4-dioxane to give **31** (62% yield). The alcohol **31** was oxidized with Jones reagent to yield a carboxylic acid **32** (32% yield).

Biological Activity of Six GLA-60 Positional Isomers. The TNFα-inducing activities of GLA-60 derivatives were investigated in vitro, using human monoblastic U937 cells. As shown in Fig. 3, a lower concentration of 10 ng ml^{-1} of LPS showed potent TNFα-inducing activity. Lipid A also induced TNFα production in a dose-dependent manner. Compound **8** exhibited potent agonistic activity, while compounds **26** and **26′** exhibited slight agonistic activity on TNFα production. TNFα production (% control; 10 ng ml^{-1} of LPS = 100) of compound **8** in the concentration of 10 μM was 611, and that of lipid A in the same concentration was 651. In contrast, the difluorinated compounds **14**, **14′**, and **20** showed little agonistic activity on TNFα production. GLA-60 showed minimal effects on LPS-induced TNFα production at the concentration range of 0.01 to 10 μM.

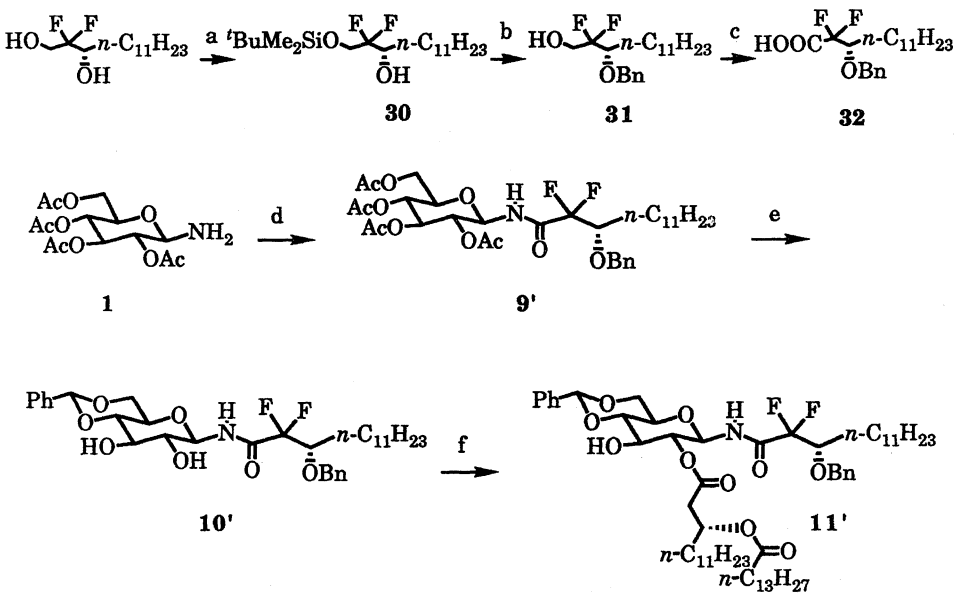
The inhibitory activities of GLA-60 positional isomers on LPS-induced TNFα production were also investigated, as shown in Fig. 4. Lipid A and GLA-60 showed no antagonistic activity on the LPS-induced TNFα production. Neither compound **8**, with a potent agonistic activity on TNFα production, nor compounds **26** and **26′**, with weak agonistic activity, showed antagonistic activities. In contrast, the difluorinated compounds **14**, **14′**, and **20**, with no agonistic activity, inhibited the LPS-induced TNFα production dose-dependently. Ten μM of compound **14′** inhibited in excess of 80% of the LPS-induced TNFα production.

These results indicate that the difluorinated compounds **14**, **14′**, and **20** are the LPS antagonists on LPS-induced TNFα production by U937 cells, whereas compounds **8**, **26**, and



Reagents and conditions:
(a) DCC, CH_2Cl_2, 30 min, 25 °C, 85%; (b) Et_3N, CH_2Cl_2, 1 h, 25 °C, 45% (**27**), 43% (**27′**); (c) H_2, 10% Pd/C, THF, 15 h, 25 °C, 98% (from **27′**), 85% (from **21′**).

Scheme 2.



Reagents and conditions:
(a) TBDMS-Cl, DMAP, CH_2Cl_2, 16 h, 25 °C, 81%; (b) BnBr, NaH, cat. Bu_4NI, THF, 25 °C, 16 h; then aq HCl, dioxane, 50 °C, 4 h, 62%; (c) Jones reagent, acetone, 6 h, 24 °C, 32%; (d) **32**, $(COCl)_2$, CH_2Cl_2, 1 h, 24 °C; then Et_3N, CH_2Cl_2, 30 min, 25 °C, 94%; (e) (1) MeONa, MeOH, 30 min, 25 °C; 2) $PhCH(OMe)_2$, 10-camphorsulfonic acid, DMF, 16 h, 24 °C, 70%; (f) (*R*)-3-(tetradecanolyloxy)tetradecanoic acid, DCC, DMAP, Et_2O, 1 h, 25 °C, 60%.

Scheme 3.

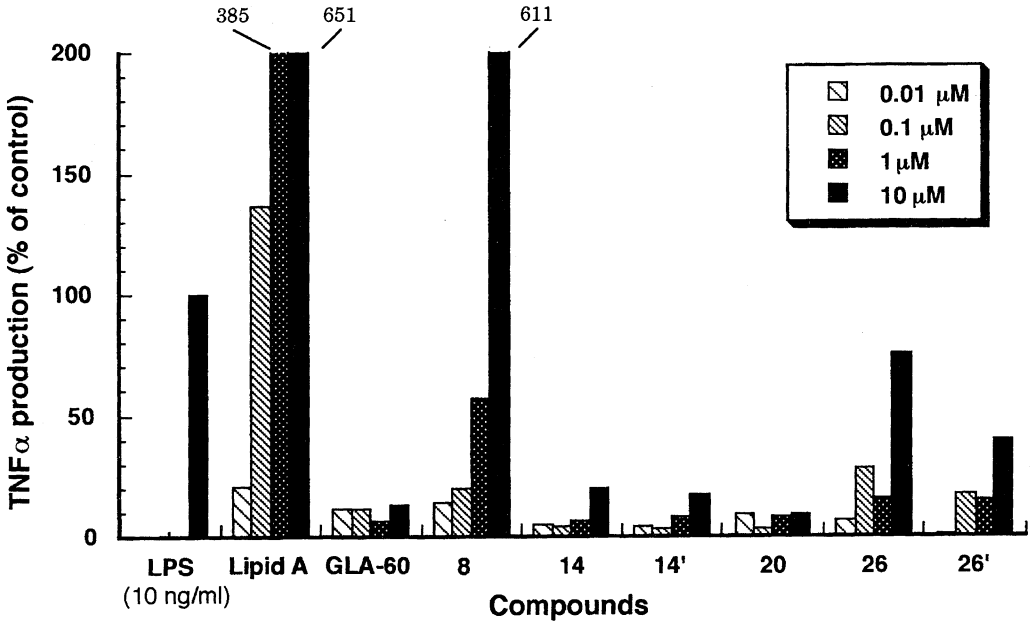


Fig. 3. LPS agonistic activities of GLA-60 positional isomers on TNFα production by U937 cells. TPA-treated U937 cells were stimulated with LPS (10 ng ml^{-1}), or the indicated concentrations of lipid A, GLA-60 or its positional isomers. After 4.5 h incubation, the amounts of TNFα in the culture supernatant were measured by ELISA. As a control, the amount of TNFα produced by U937 cells stimulated with 10 ng ml^{-1} of LPS in the absence of compounds was used (270 pg ml^{-1}). The relative amounts were calculated and indicated as percentages of the control amount.

26′ are the agonists.

Experimental

General. Melting points were determined on a Yanagimoto micro melting point apparatus and were uncorrected. ^{1}H NMR (270 MHz) spectra were recorded with a JEOL JNM-270 spectrometer using Me_4Si as the 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. Detection involved spraying the silica gel-coating chromatograph glass plate with a solution of 17% H_2SO_4 in water (w/w), containing ammonium molybdate (2.3%) and cerium(IV) sulfate (0.9%), and

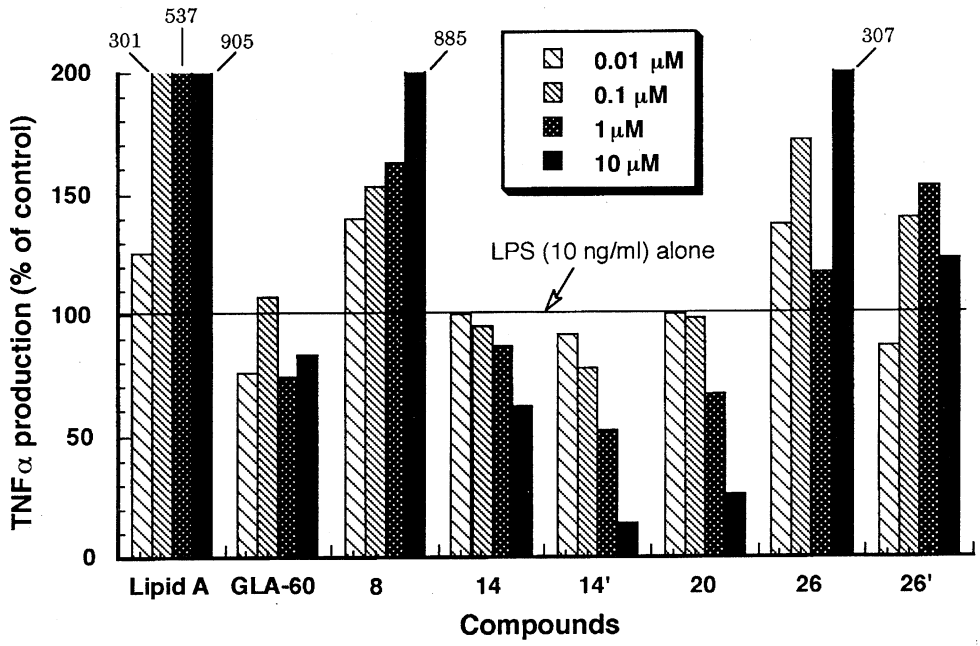


Fig. 4. LPS antagonistic activities of GLA-60 positional isomers on TNFα production by U937 cells. TPA-treated U937 cells were stimulated with the indicated concentrations of lipid A, GLA-60 or its positional isomers in the presence of LPS (10 ng ml^{-1}). After 4.5 h incubation, the amounts of TNFα in the culture supernatant were measured by ELISA. As a control, the amount of TNFα produced by U937 cells stimulated with 10 ng ml^{-1} of LPS alone was used (270 pg ml^{-1}). The relative amounts were calculated and indicated as percentages of the control amount.

heating the plate for several minutes at ca 180 °C. Separation of the compounds by column chromatography was performed with Silica Gel 60 (230—400 mesh ASTM, E. Merck) under slightly elevated pressure (1.2—1.5 atm) for easy elution. The weight of the silica gel was 50—100 times that of the substrate purified.

***N*-[(*R*)-3-(Benzyloxy)tetradecanoyl]-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (2).** A solution of (*R*)-3-(benzyloxy)-tetradecanoic acid (1.06 g, 3.17 mmol) and oxalyl chloride (1.0 ml) in CH_2Cl_2 (10 ml) was stirred for 30 min at 24 °C. This mixture was concentrated in vacuo to give an acid chloride, which was dissolved in CH_2Cl_2 (10 ml). This solution was added dropwise at 0—5 °C to a solution of **1** (1.00 g, 2.88 mmol) and Et_3N (0.380 g, 3.74 mmol) in CH_2Cl_2 (10 ml). The mixture was further stirred for 30 min at 25 °C, and diluted with EtOAc (100 ml). The EtOAc solution was washed with sat. aqueous $NaHCO_3$ (10 ml) and brine (10 ml), dried over anhydrous $MgSO_4$ (4 g), filtered, and concentrated in vacuo to give a residue, which was separated by silica-gel column chromatography. Elution with hexane–EtOAc (3 : 1) gave **2** (1.61 g, 84%) as a powder: IR ν_{max} (KBr) 3344, 2923, 2853, 1749, 1672 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ = 0.88 (3H, t, J = 6.6 Hz), 1.18—1.44 (18H, m), 1.45—1.73 (2H, m), 1.89 (3H, s), 2.01 (3H, s), 2.03 (3H, s), 2.07 (3H, s), 2.36 (1H, d, J = 7.3, 15.2 Hz), 2.46 (1H, dd, J = 4.0, 15.2 Hz), 3.74—3.88 (2H, m), 4.07 (1H, dd, J = 2.6, 12.5 Hz), 4.29 (1H, dd, J = 4.0, 12.5 Hz), 4.54 (2H, s), 4.87 (1H, t, J = 9.2—9.9 Hz), 5.04 (1H, t, J = 9.9 Hz), 5.24—5.33 (2H, m), 6.86 (1H, d, J = 9.9 Hz), 7.26—7.43 (5H, m). Found: C, 63.14; H, 8.40; N, 2.10%. Calcd for $C_{35}H_{53}NO_{11}$ (663.8): C, 63.33; H, 8.05; N, 2.11%.

***N*-[(*R*)-3-(Benzyloxy)tetradecanoyl]-4,6-*O*-isopropylidene-β-D-glucopyranosylamine (3).** To a solution of **2** (1.30 g, 1.96 mmol) in MeOH (10 ml) was added NaOMe (455 mg, 8.42 mmol). After stirring for 30 min at 25 °C, the reaction mixture was quenched with AcOH (0.60 ml), concentrated in vacuo, and diluted with EtOAc (100 ml). Then the solution was washed with sat. aqueous $NaHCO_3$ (10 ml), and brine (10 ml) dried over anhydrous $MgSO_4$ (4 g), filtered, and concentrated in vacuo to give a residue, which was dissolved in DMF (20 ml). To this solution were added 2, 2-dimethoxypropane (5.00 ml) and pyridinium *p*-toluenesulfonate (50 mg). After stirring for 3 h at 25 °C, the reaction mixture was diluted with EtOAc (100 ml), washed with sat. aqueous $NaHCO_3$ (10 ml) and brine, dried over anhydrous $MgSO_4$ (4 g) and filtered. The filtrate was concentrated in vacuo to give a residue, which was separated by silica-gel column chromatography. Elution with EtOAc gave **3** (570 mg, 72%) as a gum: IR ν_{max} ($CHCl_3$) 3440, 2930, 2850, 2851, 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ = 0.88 (3H, t, J = 6.6 Hz), 1.10—1.85 (21H, m), 1.43 (3H, s), 1.49 (3H, s), 2.39 (1H, dd, J = 7.9, 15.2 Hz), 2.51 (1H, dd, J = 3.3—3.9, 15.2 Hz), 3.00 (1H, broad s), 3.20 (1H, t, J = 8.6—9.2 Hz, C_2-*H*), 3.32—3.41 (1H, m), 3.46 (1H, t, J = 9.2 Hz), 3.56—3.73 (2H, m), 3.82—3.97 (2H, m), 4.51 (1H, d, J = 11.2 Hz), 4.60 (1H, d, J = 11.2 Hz), 5.08 (1H, t, J = 8.6—9.2 Hz, C_1-*H*), 6.93 (1H, d, J = 8.6 Hz, N*H*), 7.28—7.42 (5H, m). Found: C, 66.65; H, 9.33; N, 2.65%. Calcd for $C_{30}H_{49}NO_7 \cdot 0.2H_2O$ (539.3): C, 66.80; H, 9.23; N, 2.60%.

***N*-[(*R*)-3-(Benzyloxy)tetradecanoyl]-4,6-*O*-isopropylidene-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (4).** To a solution of **3** (540 mg, 1.01 mmol) in Et_2O (10 ml) were added (*R*)-3-(tetradecanoyloxy)tetradecanoic acid (504 mg, 1.11 mmol), DCC (416 mg, 2.02 mmol), and DMAP (12.3 mg, 0.10 mmol). The mixture was stirred for 1 h at 25 °C, concentrated in vacuo, and diluted with EtOAc (50 ml). The precipitate was filtered, and the filtrate was washed with sat. aqueous $NaHCO_3$ (5 ml), water (5 ml), and brine (5 ml), and then dried over anhydrous $MgSO_4$ (2 g). The solution was filtered, and concentrated in valuo to give a residue, which was separated by silica-gel column chromatography. Elution with hexane–EtOAc (3 : 1) gave **4** (660 mg, 67%) as a gum: IR ν_{max} ($CHCl_3$) 3500, 3301, 2925, 2850, 1740, 1725, 1685 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ = 0.88 (9H, t, J = 6.6

Hz), 1.16—1.68 (62H, m), 1.46 (3H, s), 1.52 (3H, s), 2.22—2.50 (6H, m), 3.33—3.46 (1H, m), 3.54—3.82 (4H, m, containing OH), 3.84—4.00 (2H, m), 4.51, 4.57 (2H, AB-q, J = 11.9 Hz, OCH_2Ph), 4.70 (1H, t, J = 8.6—9.2 Hz, C_2-H), 5.19 (1H, t, J = 9.2 Hz, C_1-H), 5.24 (1H, m, C_3-H-OCO), 6.88 (1H, d, J = 9.2 Hz, NH), 7.26—7.38 (5H, m). Found: C, 71.54; H, 10.31; N, 1.47%. Calcd for $C_{58}H_{101}NO_{10}$ (972.4): C, 71.64; H, 10.47; N, 1.44%.

***N*-[(*R*)-3-(Benzyloxy)tetradecanoyl]-3-*O*-diphenylphosphono-4,6-*O*-isopropylidene-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (5).** To a solution of **4** (540 mg, 0.555 mmol) in CH_2Cl_2 (5 ml) and DMAP (137 mg, 1.11 mmol) was added a solution of diphenyl chlorophosphate (298 mg, 1.11 mmol) in CH_2Cl_2 (3 ml) at 25 °C. After 30 min of stirring at 25 °C, the reaction mixture was concentrated in vacuo and diluted with EtOAc (50 ml). The solution was washed with sat. aqueous $NaHCO_3$ (5 ml) and brine, dried over anhydrous $MgSO_4$ (2 g), and filtered. The filtrate was concentrated in vacuo to give a residue, which was separated by silica-gel column chromatography. Elution with hexane–EtOAc (3 : 1) gave **5** (630 mg, 94%) as a gum: IR ν_{max} ($CHCl_3$) 2920, 2850, 1745, 1730, 1685 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (9H, t, J = 6.6 Hz), 0.98—1.68 (68H, m), 2.12—2.59 (6H, m), 3.34—3.48 (1H, m), 3.60—3.80 (3H, m), 3.94 (1H, dd, J = 5.3, 10.6 Hz), 4.50 (1H, d, J = 11.9 Hz), 4.64 (1H, d, J = 11.9 Hz), 4.78 (1H, q, J = 9.2 Hz), 4.97 (1H, t, J = 9.2 Hz), 5.00—5.11 (1H, m), 5.22 (1H, t, J = 9.2 Hz), 7.09—7.42 (16H, m).

***N*-[(*R*)-3-(Benzyloxy)tetradecanoyl]-3-*O*-diphenylphosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (6).** A solution of **5** (88 mg, 0.073 mmol) in aqueous 90% AcOH (2.0 ml) was stirred for 5 h at 60 °C. Then it was concentrated in vacuo to give a residue, which was separated by silica-gel column chromatography. Elution with hexane–EtOAc (1 : 1) gave **6** (65 mg, 76%) as a gum: IR ν_{max} ($CHCl_3$) 3350 (broad), 2920, 2850, 1730, 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (9H, t, J = 5.9—7.3 Hz), 1.05—1.67 (62H, m), 1.99 (2H, broad), 2.13—2.55 (6H, m), 3.42—3.53 (1H, m), 3.65—3.91 (4H, m), 4.52 (1H, d, J = 11.9 Hz), 4.60 (1H, d, J = 11.9 Hz), 4.66 (1H, q, J = 7.3 Hz), 4.89 (1H, t, J = 9.2 Hz), 5.00—5.12 (1H, m), 5.22 (1H, t, J = 9.2 Hz), 7.10—7.42 (16H, m). Found: C, 68.71; H, 9.33; N, 1.18; P, 2.56%. Calcd for $C_{67}H_{106}NO_{13}P$ (1164.5): C, 69.10; H, 9.81; N, 1.20; P, 2.66%.

3-*O*-Diphenylphosphono-*N*-[(*R*)-3-(hydroxy)tetradecanoyl]-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (7). A solution of **6** (120 mg, 0.103 mmol) in THF (3 ml) containing 10% Pd on carbon (50 mg) was stirred under H_2 for 3 h at 25 °C, and then filtered. The filtrate was concentrated in vacuo to give a residue, which was separated by silica-gel column chromatography. Elution with hexane–EtOAc (1 : 2) gave **7** (85 mg, 77%) as a powder: IR ν_{max} (KBr) 3381 (broad), 2922, 2852, 1745, 1677 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (9H, t, J = 6.6 Hz), 1.12—1.65 (62H, m), 1.77 (3H, broad, OH×3), 2.15—2.39 (5H, m), 2.54 (1H, dd, J = 5.3, 15.8 Hz), 3.48—3.59 (1H, m), 3.78—4.02 (4H, m), 4.67 (1H, q, J = 7.3 Hz), 4.92—5.02 (2H, m), 5.22 (1H, t, J = 9.2 Hz), 6.78 (1H, d, J = 8.6 Hz), 7.13—7.43 (10H, m). Found: C, 66.67; H, 9.43; N, 1.29; P, 2.71%. Calcd for $C_{60}H_{100}NO_{13}P$ (1074.4): C, 67.07; H, 9.38; N, 1.30; P, 2.88%.

***N*-[(*R*)-3-(Hydroxy)tetradecanoyl]-3-*O*-phosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (8).** A solution of **7** (70 mg, 0.065 mmol) in THF (3 ml) containing PtO_2 (20 mg) was stirred under H_2 for 3 h at 25 °C, and then filtered. The filtrate was concentrated in vacuo to give **8** (59.5 mg, 99%) as a powder: IR ν_{max} (KBr) 3323, 2922, 2852, 1740, 1672 cm^{-1}; ^1H NMR (pyridine-d_5) δ = 0.89 (9H, t, J = 6.6 Hz), 1.05—1.60 (56H, m), 1.60—1.82 (6H, m), 2.51 (2H, t, J = 7.3 Hz), 2.77 (1H, dd, J = 4.4, 14.7 Hz), 2.84 (1H, dd, J = 6.6, 14.7 Hz), 2.95 (1H, dd, J = 7.3, 16.1 Hz), 3.05 (1H, dd, J = 7.3, 16.1 Hz), 3.93—4.00 (1H, m), 4.20—4.52 (4H, m), 5.12 (1H, q, J = 8.8 Hz), 5.57—5.68 (2H, m), 6.10 (1H, t, J = 9.5 Hz), 6.21 (5H, broad, OH×5), 9.81 (1H, d, J = 8.8 Hz). FAB MS (negative) m/z 920 $(M-H)^-$. Found: C, 6.65; H, 10.05; N, 1.52; P, 3.23%. Calcd for $C_{48}H_{92}NO_{13}P\cdot 0.7H_2O$ (934.8): C, 61.67; H, 10.07; N, 1.50; P, 3.31%.

***N*-[(*R*)-3-Benzyloxy-2,2-difluorotetradecanoyl]-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (9) and *N*-[(*S*)-3-Benzyloxy-2,2-difluorotetradecanoyl]-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (9′).** A solution of (±)-3-benzyloxy-2,2-difluorotetradecanoic acid (1.85 g, 5.00 mmol) and oxalyl chloride (2.0 ml) in CH_2Cl_2 (10 ml) was stirred for 1 h at 24 °C. Then it was concentrated in vacuo to give an acid chloride, which was dissolved in CH_2Cl_2 (20 ml). This solution was added dropwise at 0—5 °C to a solution of **1** (1.58 g, 5.55 mmol) and Et_3N (0.60 g, 5.91 mmol) in CH_2Cl_2 (20 ml). The mixture was concentrated in vacuo and diluted with EtOAc (150 ml). The EtOAc solution was washed with sat. aqueous $NaHCO_3$ (15 ml) and brine (15 ml), dried over anhydrous $MgSO_4$ (6 g), and filtered. The filtrate was concentrated in vacuo to give a residue, which was separated by silica-gel column chromatography. Elution with hexane–EtOAc (3 : 1) gave an inseparable mixture of diasteremers **9** and **9′**, (2.98 g, 94%) as a powder: IR ν_{max} (KBr) 3311, 2923, 2854, 1752, 1737, 1710, 1697 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (3H, t, J = 6.6 Hz), 1.19—1.70 (20H, m), 1.80—2.10 (12H, m), 3.79—4.00 (2H, m), 4.00—4.14 (1H, m), 4.23—4.39 (1H, m), 4.47—4.59 (1H, m), 4.70 (1H, d, J = 11.2 Hz), 4.94—5.15 (2H, m), 5.15—5.38 (2H, m), 7.21—7.40 (6H, m). Found: C, 60.17; H, 7.56; N, 2.07; F, 5.50%. Calcd for $C_{35}H_{51}F_2NO_{11}$ (699.8): C, 60.07; H, 7.35; N, 2.00; F, 5.43%.

4,6-*O*-[(*R*)-Benzylidene]-*N*-[(*R*)-3-benzyloxy-2,2-difluorotetradecanoyl]-β-D-glucopyranosylamine (10) and 4,6-*O*-[(*S*)-Benzylidene]-*N*-[(*R*)-3-benzyloxy-2,2-difluorotetradecanoyl]-β-D-glucopyranosylamine (10′). To a solution of a mixture of diastereomers (**9** and **9′**) (1.81 g, 2.58 mmol) in MeOH (20 ml) was added NaOMe (600 mg, 11.12 mmol). After stirring for 30 min at 24 °C, the reaction mixture was quenched with AcOH, concentrated in vacuo, and diluted with EtOAc (200 ml). The solution was washed with sat. aqueous $NaHCO_3$ (20 ml) and brine (20 ml), dried over $MgSO_4$ (4 g), and filtered. The filtrate was concentrated in vacuo to give a residue, which was dissolved in DMF (20 ml). To this solution were added benzaldehyde dimethyl acetal (0.77 ml, 5.17 mmol) and (±)-10-camphorsulfonic acid (100 mg). After stirring for 16 h at 24 °C, the reaction mixture was concentrated in vacuo, then diluted with EtOAc (180 ml). This solution was washed with sat. aqueous $NaHCO_3$ (18 ml) and brine (18 ml), dried over $MgSO_4$ (5 g), and filtered. The filtrate was concentrated in vacuo to give a residue, which was separated by silica-gel column chromatography. Elution with hexane–EtOAc (3 : 1) gave an inseparable mixture of diastereomers (**10** and **10′**), (1.12 g, 70%) as a powder: IR ν_{max} (KBr) 3583, 3405, 3311, 3069, 3035, 2923, 2853, 1694 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (3H, t, J = 6.6 Hz), 1.13—1.90 (20H, m), 3.02 (2H, broad, OH×2), 3.21—3.73 (4H, m), 3.79 (1H, td, J = 3.3, 8.6 Hz), 3.88—4.08 (1H, m), 4.23—4.39 (1H, m), 4.51—4.60 (1H, m), 4.64—4.77 (1H, m), 5.14 (1H, t, J = 9.2 Hz), 5.50 (1H, s), 6.94 (0.5H, d, J = 8.6 Hz), 7.07 (0.5H, d, J = 8.6 Hz), 7.25—7.57 (10H, m). Found: C, 65.79; H, 7.72; N, 2.35; F, 6.25%. Calcd for $C_{34}H_{47}F_2NO_7$ (619.4): C, 65.89; H, 7.64; N, 2.26; F, 6.13%.

4,6-*O*-[(*R*)-Benzylidene]-*N*-[(*R*)-3-benzyloxy-2,2-difluorotetradecanoyl]-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-

β-D-glucopyranosylamine (11) and 4,6-*O*-[(*S*)-Benzylidene]-*N*-[(*R*)-3-benzyloxy-2,2-difluorotetradecanoyl]-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (11′). A mixture of diastereomers **10** and **10′** (1.75 g, 2.82 mmol) was treated with (*R*)-3-(tetradecanoyloxy)tetradecanoic acid as described in the formation of **4** from **3** to give **11** (0.99 g, 33%, R_f=0.58, hexane/EtOAc=3/1) and **11′** (0.71 g, 24%, R_f=0.54, hexane/EtOAc=3/1). Physical data of **11**: $[\alpha]_D^{24}$ +5.8° (*c*=1.2, $CHCl_3$) as a powder; IR ν_{max} (KBr) 3469, 3328, 2923, 2853, 1730, 1713 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.88 (9H, t, *J*=5.9—7.3 Hz), 1.13—1.72 (62H, m), 2.18—2.38 (4H, m), 3.50—3.78 (4H, m, containing OH), 3.82—3.99 (1H, m), 4.08 (1H, d, *J*=8.6 Hz), 4.33—4.42 (1H, m), 4.55 (1H, d, *J*=11.2 Hz), 4.75 (1H, d, *J*=11.2 Hz), 4.83 (1H, t, *J*=9.2 Hz), 5.12—5.33 (2H, m), 5.55 (1H, s), 7.22—7.41 (9H, m), 7.47—7.55 (2H, m). Found: C, 70.51; H, 9.15; N, 1.20; F, 3.47%. Calcd for $C_{62}H_{99}F_2NO_{10}$ (1056.5): C, 70.49; H, 9.45; N, 1.33; F, 3.60%. Physical data of **11′**: $[\alpha]_D^{24}$ −6.9° (*c*=1.2, $CHCl_3$); IR ν_{max} (KBr) 3473, 3320, 2954, 2852, 1737, 1701 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.88 (9H, t, *J*=6.6 Hz), 1.14—1.67 (62H, m), 2.25 (2H, t, *J*=7.3 Hz), 2.39—2.58 (2H, m), 3.52—3.75 (4H, m, containing OH), 3.81—3.97 (1H, m), 4.09 (1H, d, *J*=8.6 Hz), 4.25—4.36 (1H, m), 4.49 (1H, d, *J*=11.2 Hz), 4.67 (1H, d, *J*=11.2 Hz), 4.86 (1H, t, *J*=9.2 Hz), 5.19—5.35 (2H, m), 5.54 (1H, s), 7.18 (1H, d, *J*=9.2 Hz), 7.28—7.41 (8H, m), 7.45—7.54 (2H, m). Found: C, 70.50; H, 9.15; N, 1.31; F, 3.44%. Calcd for $C_{62}H_{99}F_2NO_{10}$ (1056.5): C, 70.49; H, 9.45; N, 1.33; F, 3.60%.

4,6-*O*-[(*R*)-Benzylidene]-*N*-[(*R*)-3-benzyloxy-2,2-difluorotetradecanoyl]-4-*O*-diphenylphosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (12). Compound **11** (410 mg, 0.388 mmol) was treated as described in the formation of **5** from **4** to give **12** (425 mg, 85%) as a gum: $[\alpha]_D^{24}$ +10.6° (*c*=1.4, $CHCl_3$); IR ν_{max} ($CHCl_3$) 2925, 2850, 1750, 1725, 1590, cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.88 (9H, t, *J*=5.9—7.2 Hz), 0.96—1.63 (62H, m), 2.12—2.39 (4H, m), 3.60—3.95 (4H, m), 4.40 (1H, dd, *J*=3.3, 9.2 Hz), 4.55 (1H, d, *J*=11.2 Hz), 4.77 (1H, d, *J*=11.2 Hz), 4.95—5.07 (2H, m), 5.13 (1H, dd, *J*=8.6, 9.2 Hz), 5.23 (1H, t, *J*=8.6 Hz), 5.49 (1H, s), 6.98 (5H, s), 7.12—7.45 (16H, m). Found: C, 68.93; H, 8.50; N, 1.24; F, 2.98; P, 2.24%. Calcd for $C_{74}H_{108}F_2NO_{13}P$ (1288.6): C, 68.97; H, 8.45; N, 1.09; F, 2.95, P, 2.40%.

4,6-*O*-[(*R*)-Benzylidene]-*N*-[(*S*)-3-benzyloxy-2,2-difluorotetradecanoyl]-4-*O*-diphenylphosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (12′). Compound **11′** (350 mg, 0.331 mmol) was treated as described in the formation of **5** from **4** to give **12′** (410 mg, 96%) as a gum; $[\alpha]_D^{24}$ +7.7° (*c*=1.3, $CHCl_3$); IR ν_{max} ($CHCl_3$) 2925, 2850, 1750, 1725, 1590, cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.88 (9H, t, *J*=6.6 Hz), 0.98—1.69 (62H, m), 2.19 (2H, t, *J*=7.3—7.9 Hz), 2.30 (1H, dd, *J*=4.0, 17.2 Hz), 2.48 (1H, dd, *J*=8.6, 17.2 Hz), 3.60—3.82 (3H, m), 3.86—4.02 (1H, m), 4.30 (1H, q, *J*=5.9 Hz), 4.48 (1H, d, *J*=12.1 Hz), 4.63 (1H, d, *J*=12.1 Hz), 4.96—5.09 (2H, m), 5.13 (1H, t, *J*=9.2 Hz), 5.28 (1H, t, *J*=9.2 Hz), 5.48 (1H, s), 7.00 (5H, s), 7.13—7.48 (16H, m). Found: C, 69.16; H, 8.48; N, 1.19; F, 2.90; P, 2.32%. Calcd for $C_{74}H_{108}F_2NO_{13}P$ (1288.6): C, 68.97; H, 8.45; N, 1.09; F, 2.95, P, 2.40%.

4,6-*O*-[(*R*)-Benzylidene]-*N*-[(*R*)-2,2-difluoro-3-(hydroxy)tetradecanoyl]-4-*O*-diphenylphosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (13). A solution of **12** (300 mg, 0.233 mmol) in THF (3 ml)–MeOH (3 ml) containing $Pd(OH)_2$ on carbon (50 mg, wet Degussa type, Pd content 20%) was stirred under hydrogen at 25 °C for 6 h, and then filtered. The filtrate was concentrated in vacuo to give a residue, which was separated by silica-gel column chromatography. Elution with hexane–EtOAc (2 : 3) gave **13** (207 mg, 81%) as a gum: $[\alpha]_D^{24}$ +25.2° (*c*=1.2, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3425, 2920, 2850, 1745, 1720, 1590 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.88 (9H, t, *J*=6.6 Hz), 1.08—1.80 (63H, m, containing OH×1), 2.18—2.34 (3H, m), 2.59 (1H, dd, *J*=4.6, 17.8 Hz), 3.50—4.12 (7H, m, containing OH×2), 4.64—4.75 (1H, m), 4.86—4.98 (1H, m), 5.06 (1H, t, *J*=9.2 Hz), 5.22 (1H, t, *J*=9.2 Hz), 7.10—7.43 (11H, m). Found: C, 64.80; H, 8.92; N, 1.26; F, 3.34; P, 2.56%. Calcd for $C_{60}H_{98}F_2NO_{13}P$ (1110.4): C, 64.90; H, 8.90; N, 1.26; F, 3.42, P, 2.79%.

4,6-*O*-[(*R*)-Benzylidene]-*N*-[(*S*)-2,2-difluoro-3-(hydroxy)tetradecanoyl]-4-*O*-diphenylphosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (13′). Compound **12′** (100 mg, 0.078 mmol) was treated as described above to give **13′** (75 mg, 87%); $[\alpha]_D^{24}$ +20.3° (*c*=1.2, $CHCl_3$) as a gum: IR ν_{max} ($CHCl_3$) 3420, 2920, 2845, 1745, 1720, 1585 cm^{-1}; ^1H NMR ($CDCl_3$) δ=0.88 (9H, t, *J*=6.6 Hz), 1.01—1.80 (63H, m, containing OH×1), 2.18—2.33 (3H, m), 2.52 (1H, dd, *J*=5.9, 17.2 Hz), 3.37 (1H, broad, OH), 3.49—3.60 (1H, m), 3.66—4.10 (5H, m, containing OH×1), 4.70 (1H, q, *J*=7.9—8.6 Hz), 4.95—5.25 (3H, m), 7.15—7.42 (11H, m). Found: C, 64.75; H, 8.60; N, 1.25; F, 3.37; P, 2.67%. Calcd for $C_{60}H_{98}F_2NO_{13}P$ (1110.4): C, 64.90; H, 8.90; N, 1.26; F, 3.42, P, 2.79%.

4,6-*O*-[(*R*)-Benzylidene]-*N*-[(*R*)-2,2-difluoro-3-(hydroxy)tetradecanoyl]-4-*O*-phosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (14). Compound **13** (155 mg, 0.140 mmol) was treated as described in the formation of **8** from **7** to give **14** (128 mg, 96%) as a powder: IR ν_{max} (KBr) 3528, 3308, 2957, 2922, 2853, 1745, 1705 cm^{-1}; ^1H NMR (pyridine-d_5) δ=0.89 (9H, t, *J*=6.8 Hz), 0.99—2.05 (62H, m), 2.50 (1H, dd, *J*=7.6, 15.8 Hz), 2.97 (1H, dd, *J*=5.8, 16.6 Hz), 3.04 (1H, dd, *J*=7.1, 16.6 Hz), 3.09—4.03 (1H, m), 4.21—4.42 (3H, m), 4.55—4.70 (1H, m), 5.13 (1H, q, *J*=8.6 Hz), 5.58—5.65 (1H, m), 5.79 (1H, t, *J*=9.3 Hz), 6.16 (1H, t, *J*=9.3 Hz), 7.41 (5H, broad, OH×5), 10.98 (1H, d, *J*=9.2 Hz). Found: C, 58.59; H, 9.47; N, 1.45; F, 3.70; P, 3.38%. Calcd for $C_{48}H_{90}F_2NO_{13}P{\cdot}5H_2O$ (985.2): C, 58.51; H, 9.51; N, 1.42; F, 3.86, P, 3.14%.

4,6-*O*-[(*R*)-Benzylidene]-*N*-[(*S*)-2,2-difluoro-3-(hydroxy)tetradecanoyl]-4-*O*-phosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (14′). Compound **13′** (112 mg, 0.101 mmol) was treated as described in the formation of **8** from **7** to give **14′** (95 mg, 98%) as a powder: IR ν_{max} (KBr) 3302, 2957, 2923, 2854, 1745, 1705 cm^{-1}; ^1H NMR (pyridine-d_5) δ=0.78—0.99 (9H, m), 1.02—1.50 (54H, m), 1.53—2.08 (8H, m), 2.46—2.63 (2H, m), 3.01 (1H, dd, *J*=5.6, 16.6 Hz), 3.09 (1H, dd, *J*=7.3, 16.6 Hz), 3.92—4.03 (1H, m), 4.20—4.42 (3H, m), 4.61—4.77 (1H, m), 5.14 (1H, q, *J*=8.6 Hz), 5.58—5.71 (1H, m), 5.80 (1H, t, *J*=9.3 Hz), 6.15 (1H, t, *J*=9.3 Hz), 6.75 (5H, broad, OH×5), 11.02 (1H, d, *J*=9.2 Hz); FAB MS (negative) *m/z* 956 $(M-H)^-$. Found: C, 59.85; H, 9.52; N, 1.47; F, 3.78; P, 2.97%. Calcd for $C_{48}H_{90}F_2NO_{13}P$ (958.2): C, 60.17; H, 9.47; N, 1.46; F, 3.97, P, 3.23%.

***N*-2,2-Difluorotetradecanoyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (15).** A solution of 2,2-difluorotetradecanoic acid (838 mg, 3.17 mmol) and oxalyl chloride (1.5 ml) in CH_2Cl_2 (5 ml) was stirred for 1 h at 24 °C. It was then concentrated in vacuo to give an acid chloride, which was dissolved in CH_2Cl_2 (5 ml). This solution was added dropwise at 0—5 °C to a solution of **1** (1.00 g, 2.88 mmol) and Et_3N (0.480 ml, 3.43 mmol) in CH_2Cl_2 (8 ml). The mixture was concentrated in vacuo and diluted with EtOAc (80 ml). The EtOAc solution was washed with sat. aqueous $NaHCO_3$ (8 ml) and brine (8 ml), dried

over anhydrous $MgSO_4$ (2 g), and filtered. The filtrate was concentrated in vacuo to give a residue, which was separated by silica-gel column chromatography. Elution with toluene–EtOAc (3 : 1) gave **15** (1.44 g, 84%) as a crystalline solid: Mp 99—102 °C; $[\alpha]_D^{24}$ +10.7° (c = 1.3, $CHCl_3$); IR ν_{max} ($CHCl_3$) 2925, 2850, 1750 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (3H, t, J = 6.8 Hz), 1.25 (20H, broad), 1.95—2.13 (2H, m), 2.03 (3H, s), 2.04 (6H, s), 2.09 (3H, s), 3.84 (1H, ddd, J = 2.6, 4.6, 9.9 Hz), 4.07 (1H, dd, J = 2.6, 12.5 Hz), 4.33 (1H, dd, J = 4.6, 12.5 Hz), 4.99 (1H, dd, J = 9.2, 9.9 Hz), 5.09 (1H, t, J = 9.9 Hz), 5.22 (1H, t, J = 9.2 Hz), 5.33 (1H, dd, J = 9.2, 8.6 Hz), 7.09 (1H, d, J = 8.6 Hz, N*H*). Found: C, 56.69; H, 7.47; N, 2.43; F, 6.30%. Calcd for $C_{28}H_{45}F_2NO_{10}$ (593.7): C, 56.65; H, 7.64; N, 2.36; F, 6.40%.

***N*-2, 2-Difluorotetradecanoyl-4, 6-*O*-isopropylidene-β-D-glucopyranosylamine (16).** Compound **15** (1.32 g, 2.22 mmol) was treated as described in the formation of **10** from **9** to give **16** (0.82 g, 85%) as a crystalline solid: mp 127—129 °C; IR ν_{max} (Nujol®) 3577, 3552, 3363, 3345, 3035, 1694 cm^{-1}; ^1H NMR ($CDCl_3/CD_3OD$ = 9/1) δ = 0.88 (3H, t, J = 5.9—6.6 Hz), 1.26 (20H, broad s), 1.40—1.50 (8H, m, containing two Me singlets at δ = 1.44 and 1.51), 1.98—2.16 (2H, m), 3.36—3.44 (2H, m), 3.52 (1H, dd, J = 8.6, 9.2 Hz, C2-H), 3.61 (1H, t, J = 8.6 Hz), 3.67—3.75 (4H, m), 3.94 (1H, dd, J = 5.3, 11.2 Hz), 5.01 (1H, d, J = 9.2 Hz, C1-H). Found: C, 58.60; H, 9.08; N, 2.90; F, 8.05%. Calcd for $C_{23}H_{41}F_2NO_6 \cdot 0.3H_2O$ (471.0): C, 58.66; H, 8.90; N, 2.97; F, 8.07%.

***N*-2,2-Difluorotetradecanoyl-4,6-*O*-isopropylidene-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (17).** To a solution of **16** (608 mg, 1.30 mmol) in Et_2O (14 ml) and THF (7 ml) were added (*R*)-3-(tetradecanoyloxy)tetradecanoic acid (624 mg, 1.37 mmol), DCC (404 mg, 1.96 mmol), and DMAP (16 mg). The reaction mixture was stirred at 24 °C for 2 h under nitrogen. The precipitated 1,3-dicyclohexylurea was filtered off by passing the mixture through Celite. The filtrate was diluted with EtOAc (60 ml), which was washed with sat. aqueous $NaHCO_3$ (6 ml), and brine (6 ml), dried over anhydrous $MgSO_4$ (2 g), and evaporated in vacuo to give a residue. This residue was separated by silica-gel column chromatography. Elution with hexane–EtOAc (3 : 1) gave **17** (731 mg, 62%) as a crystalline solid: Mp 78—79 °C; $[\alpha]_D^{24}$ +2.1° (c = 1.7, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3400, 2900, 2850, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (3H, t, J = 6.6 Hz), 1.25 (60H, broad), 1.46 (3H, s), 1.48—1.65 (2H, m), 1.53 (3H, s), 1.90—2.13 (2H, m), 2.26 (2H, t, J = 7.6 Hz), 2.43—2.60 (2H, m), 3.38—3.48 (2H, m), 3.61—3.81 (2H, m), 3.89—3.99 (2H, m, C4-H, OH), 4.81 (1H, t, J = 9.2 Hz, C2-H), 5.14 (1H, dd, J = 8.6, 9.2 Hz, C1-H), 5.28 (1H, m), 6.97 (1H, d, J = 8.6 Hz, NH); FAB MS (negative) m/z 900 $(M-H)^-$.

***N*-2,2-Difluorotetradecanoyl-3-*O*-diphenylphosphono-4,6-*O*-isopropylidene-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (18).** Compound **17** (610 mg, 0.676 mmol) was treated as described in the formation of **5** from **4** to give **18** (714 mg, 93%) as a crystalline solid: Mp 82—84 °C; IR ν_{max} ($CHCl_3$) 2950, 2850, 1755 (shoulder), 1730, 1590 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (9H, t, J = 6.6 Hz), 1.05—1.58 (62H, m), 1.28 (3H, s), 1.34 (3H, s), 1.94—2.13 (2H, m), 2.19 (2H, t, J = 7.3 Hz), 2.31 (1H, dd, J = 4.0, 17.2 Hz), 2.46 (1H, dd, J = 8.3, 17.2 Hz), 3.45 (1H, dt, J = 5.3, 15.2 Hz), 3.71 (1H, t, J = 9.9 Hz), 3.78 (1H, t, J = 9.9 Hz), 3.98 (1H, dd, J = 5.3, 10.6 Hz), 4.81 (1H, dd, J = 8.9, 18.2 Hz), 5.01 (1H, m), 5.08 (1H, t, J = 8.9 Hz), 5.15 (1H, dd, J = 8.6, 8.9 Hz), 7.07 (1H, d, J = 8.6 Hz, NH), 7.14—7.38 (10H, m). Found: C, 66.47; H, 9.13; N, 1.26%. Calcd for $C_{63}H_{102}F_2NO_{12}P$ (1134.5): C, 66.70; H, 9.06; N, 1.23%.

***N*-2,2-Difluorotetradecanoyl-3-*O*-diphenylphosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (19).** A solution of **18** (193 mg, 0.170 mmol) in CH_2Cl_2-90% aq CF_3COOH (20 : 1, 30 ml) was stirred for 2 h at 25 °C, then neutralized with sat. aqueous $NaHCO_3$ (1 ml) and diluted with EtOAc (100 ml). The organic layer was washed with H_2O (10 ml) and brine (10 ml), dried over anhydrous $MgSO_4$ (2 g), and filtered. The filtrate was concentrated in vacuo to give a residue, which was separated by silica-gel column chromatography. Elution with toluene–EtOAc (1 : 1) gave **19** (184 mg, 99%) as a gum: R_f = 0.42 (toluene : EtOAc = 1 : 1); $[\alpha]_D^{25}$ +40.4° (c = 0.25, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3400, 2900, 2850, 1730 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (9H, t, J = 6.6—7.3 Hz), 1.05—1.50 (62H, m), 1.80—2.15 (3H, m, containing OH×1), 2.17—2.31 (3H, m), 2.45 (1H, dd, J = 7.6, 16.8 Hz), 3.53 (1H, m), 3.80—3.94 (4H, m), 4.67 (1H, dd, J = 8.9, 15.5 Hz), 5.00 (1H, t, J = 9.2 Hz), 5.06 (1H, m), 5.17 (1H, t, J = 9.2 Hz), 7.05 (1H, d, J = 8.6 Hz, NH), 7.16—7.41 (10H, m); FAB MS (negative) m/z 1017 (M−Ph), 1016 $(M-Ph-H)^-$.

***N*-2, 2-Difluorotetradecanoyl-3-*O*-phosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (20).** Compound **19** (61 mg, 0.056 mmol) was treated as descibed in the formation of **8** from **7** to give **20** (43 mg, 81%) as a powder. Further purification (22 mg) was performed by chromatography on a Sephadex (LH-20) column. Elution with MeOH–THF (1 : 1) gave 10 mg of product as a powder: R_f = 0.33 ($CHCl_3$: EtOH : AcOH : H_2O = 8 : 5 : 1 : 1); $[\alpha]_D^{25}$ −10.9° (c = 0.23, MeOH : pyridine = 1 : 1); IR ν_{max} (Nujol®) 3500—3400, 1735, 1699 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5 + D_2O) δ = 0.89 (9H, m), 1.28 (56H, broad), 1.61—1.84 (6H, m), 2.30—2.44 (2H, m), 2.53 (2H, t, J = 7.2 Hz), 3.01 (1H, dd, J = 7.7, 16.7 Hz), 3.12 (1H, dd, J = 4.7, 16.7 Hz), 3.97 (1H, m), 4.24 (2H, m), 4.38 (1H, d, J = 11.0 Hz), 5.11 (1H, m), 5.65 (1H, m), 5.76 (1H, t, J = 9.3 Hz), 6.01 (1H, t, J = 9.3 Hz, changed to a doublet on addition of D_2O), 11.01 (this peak appears in the absence of D_2O, bs, NH). FAB MS (negative) m/z 940 $(M-H)^-$.

***N*-[(2*S*,3*R*)-3-Benzyloxy-2-fluorotetradecanoyl]-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (21) and *N*-[(2*R*,3*S*)-3-Benzyloxy-2-fluorotetradecanoyl]-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (21′).** A solution of (±)-*syn*-3-benzyloxy-2-fluorotetradecanoic acid (1.57 g, 4.45 mmol) and oxalyl chloride (2.0 ml) in CH_2Cl_2 (10 ml) was stirred for 1 h at 24 °C. It was then concentrated in vacuo to give an acid chloride, which was dissolved in CH_2Cl_2 (6 ml). This solution was added dropwise at 0—5 °C to a solution of **1** (1.40 g, 4.04 mmol) and Et_3N (0.670 g, 4.81 mmol) in CH_2Cl_2 (6 ml). The mixture was stirred for 15 h at 24 °C, concentrated in vacuo, and diluted with EtOAc (150 ml). The EtOAc solution was washed with sat. aqueous $NaHCO_3$ (15 ml) and brine (15 ml), dried over anhydrous $MgSO_4$ (3 g), and filtered. The filtrate was concentrated in vacuo to give a residue, which was separated by silica-gel column chromatography. Elution with toluene–EtOAc (3 : 1) gave **21** (1.25 g, 45.3%, R_f = 0.48 (toluene : EtOAc = 3 : 1)) as a powder and **21′**, (1.04 g, 37.8%, R_f = 0.43 (toluene : EtOAc = 3 : 1)) as a powder.

Physical Data of 21: $[\alpha]_D^{25}$ −5.2° (c = 1.0, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3400, 2900, 2850, 1750, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (3H, t, J = 5.9—7.3 Hz), 1.25 (18H, broad), 1.62—1.69 (2H, m), 2.00 (3H, s), 2.04 (3H, s), 2.09 (3H, s), 3.84—3.91 (2H, m), 3.92—4.03 (1H, m), 4.11 (1H, dd, J = 1.0, 12.5 Hz), 4.32 (1H, dd, J = 4.6, 12.6 Hz), 4.47, 4.52 (2H, AB-q, J = 11.4 Hz), 4.89 (1H, dd, J = 1.0, 48.5 Hz), 4.98 (1H, t, J = 9.6 Hz), 5.09 (1H, t, J = 9.6 Hz), 5.23 (1H, t, J = 9.3 Hz), 5.32 (1H, t, J = 9.2 Hz), 7.10—7.35 (6H, m, containing NH). Found: C, 62.05; H, 7.53; N, 2.04; F,

2.80%. Calcd for $C_{35}H_{52}FNO_{11}$ (681.8): C, 61.66; H, 7.69; N, 2.05; F, 2.79%.

Physical Data of 21′: $[\alpha]_D^{25}$ 11.3° (c = 1.6, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3400, 2900, 2850, 1750, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (3H, t, J = 5.9—7.3 Hz), 1.25 (18H, broad), 1.53—1.86 (2H, m), 1.95 (3H, s), 2.02 (3H, s), 2.03 (3H, s), 2.05 (3H, s), 3.82—4.03 (3H, m), 4.25 (1H, dd, J = 4.6, 12.5 Hz), 4.45, 4.59 (2H, AB-q, J = 10.9 Hz), 4.82 (1H, dd, J = 1.0, 48.2 Hz, CHF), 5.00 (1H, t, J = 9.9 Hz), 5.08 (1H, t, J = 9.9 Hz), 5.21—5.37 (2H, m), 7.17 (1H, d, J = 7.9 Hz, NH), 7.18—7.22 (5H, m). Found: C, 61.26; H, 7.56; N, 2.01; F, 2.72%. Calcd for $C_{35}H_{52}FNO_{11}$ (681.8): C, 61.66; H, 7.69; N, 2.05; F, 2.79%.

4,6-*O*-[(*R*)-Benzylidene]-*N*-[(2*S*,3*R*)-3-benzyloxy-2-fluorotetradecanoyl]-β-D-glucopyranosylamine (22). Compound **21** (1.24 g, 1.82 mmol) was treated as described in the formation of **10** from **9** to give **22** (0.45 g, 42%) as a gum: R_f = 0.45 (toluene : EtOAc : MeOH = 20 : 20 : 1); $[\alpha]_D^{25}$ −38.8° (c = 0.5, $CHCl_3$); IR ν_{max} (Nujol®) 3566, 3522, 3327, 1675 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.89 (3H, t, J = 6.6 Hz), 1.26 (18H, broad), 1.65 (2H, m), 3.28 (2H, broad, OH×2), 3.43—3.77 (5H, m), 3.95 (1H, td, J = 7.0, 29.7 Hz), 4.29 (1H, dd, J = 4.3, 9.6 Hz), 4.45, 4.52 (2H, AB-q, J = 11.2 Hz), 4.91 (1H, d, J = 48.2 Hz), 5.11 (1H, t, J = 8.6 Hz, C1-H), 5.45 (1H, s), 7.20 (1H, dd, J = 3.3, 8.6 Hz, NH), 7.27 (5H, s), 7.34—7.38 (3H, m), 7.47—7.49 (2H, m); FAB MS (positive) m/z 602 $(M+H)^+$; (negative) m/z 600 $(M-H)^-$. Found: C, 67.05; H, 7.85; N, 2.41%. Calcd for $C_{34}H_{48}FNO_7$ (601.8): C, 67.86; H, 8.04; N, 2.33%.

4,6-*O*-[(*R*)-Benzylidene]-*N*-[(2*S*,3*R*)-3-benzyloxy-2-fluorotetradecanoyl]-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (23). Compound **22** (424 mg, 0.704 mmol) was treated as described in the formation of **11** from **10** to give **23** (547 mg, 75%): R_f = 0.46 (hexane : EtOAc = 3 : 1) as a gum: $[\alpha]_D^{25}$ −21.2° (c = 0.5, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3500, 3425, 2850, 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (9H, t, J = 5.3—7.3 Hz), 1.25 (58H, broad), 1.41—1.78 (4H, m), 1.88 (1H, dd, J = 2.6, 14.5 Hz), 2.07 (1H, d, J = 9.9, 14.5 Hz), 2.22 (2H, t, J = 7.3 Hz), 3.61—3.75 (4H, m), 3.94 (1H, dtd, J = 2.0, 7.3, 28.4 Hz), 4.09 (1H, ddd, J = 2.0, 8.6, 10.6 Hz), 4.40 (1H, dd, J = 2.6, 8.6 Hz), 4.51 (2H, s, PhCH_2), 4.85 (1H, t, J = 9.2 Hz, C2-H), 4.89 (1H, dd, J = 2.6, 48.2 Hz), 5.08 (1H, m), 5.21 (1H, t, J = 9.2 Hz, C1-H), 5.56 (1H, s, PhC*H*), 7.21—7.53 (11H, m). Found: C, 71.51; H, 10.00; N, 1.57%. Calcd for $C_{62}H_{100}FNO_{10}$ (1038.5): C, 71.71; H, 9.71; N, 1.35%.

4,6-*O*-[(*R*)-Benzylidene]-*N*-[(2*S*,3*R*)-3-benzyloxy-2-fluorotetradecanoyl]-3-*O*-diphenylphosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (24). Compound **23** (525 mg, 0.505 mmol) was treated as described in the formation of **12** from **11** to give **24** (466 mg, 73%): R_f = 0.47 (hexane : EtOAc = 3 : 1) as a gum: $[\alpha]_D^{25}$ −8.0° (c = 0.8, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3400, 2900, 2850, 1750, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (9H, t, J = 6.6 Hz), 1.25 (58H, broad), 1.50—1.82 (4H, m), 2.01—2.04 (2H, m), 2.09—2.25 (2H, m), 3.66—4.01 (4H, m), 4.42 (1H, m), 4.51 (2H, s), 4.89 (1H, dd, J = 2.0, 47.5 Hz), 4.91 (1H, m), 5.04 (1H, t, J = 8.6 Hz), 5.16—5.29 (2H, m), 5.50 (1H, s, PhC*H*), 6.97 (5H, s), 7.01—7.44 (16H, m, containing N*H*). Found: C, 70.06; H, 8.91; N, 1.23; F, 1.53; P, 2.21%. Calcd for $C_{74}H_{109}FNO_{13}P$ (1270.6): C, 69.95; H, 8.65; N, 1.10; F, 1.50; P, 2.44%.

***N*-[(2*S*,3*R*)-2-Fluoro-3-(hydroxy)tetradecanoyl]-3-*O*-diphenylphosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (25).** Compound **24** (446 mg, 0.351 mmol) was hydrogenolyzed for 48 h at 25 °C in THF (5 ml), MeOH (5 ml), and AcOH (1 ml) using $Pd(OH)_2$ on carbon (75 mg, wet Degussa type, Pd content 20%) as a catalyst to give **25** (365 mg, 95%) as a wax: R_f = 0.40 (hexane : EtOAc = 1 : 2); $[\alpha]_D^{25}$ +18.6° (c = 1.1, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3425, 2900, 2850, 1750, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (9H, t, J = 6.6 Hz), 1.25 (58H, broad), 1.39—1.73 (4H, m), 2.21—2.31 (4H, m), 2.64 (1H, dd, J = 4.6, 17.8 Hz), 3.19 (1H, d, J = 6.6 Hz, OH), 3.54 (1H, td, J = 3.7, 9.2 Hz), 3.77—4.10 (5H, m, containing OH×2), 4.71 (1H, dd, J = 8.6, 16.5 Hz), 4.76 (1H, d, J = 48.8 Hz), 4.96 (1H, m), 5.08 (1H, t, J = 9.2 Hz), 5.28 (1H, t, J = 9.2 Hz), 7.09 (1H, dd, J = 4.0, 9.2 Hz, N*H*), 7.14—7.39 (10H, m). Found: C, 65.76; H, 9.38; N, 1.36; F, 2.00%. Calcd for $C_{60}H_{99}FNO_{13}P$ (1092.4): C, 65.97; H, 9.13; N, 1.28; F, 1.74%.

***N*-[(2*S*,3*R*)-2-Fluoro-3-(hydroxy)tetradecanoyl]-3-*O*-phosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (26).** Compound **25** (79 mg, 0.072 mmol) was treated as described in the formation of **8** from **7** to give **26** (63 mg, 93%) as a powder: R_f = 0.34 ($CHCl_3$: EtOH : AcOH : H_2O = 8 : 5 : 1 : 1); $[\alpha]_D^{25}$ −9.5° (c = 0.2, pyridine); IR ν_{max} ($CHCl_3$) 3525, 3313, 1739, 1684 cm^{-1}; ^1H NMR (400 MHz, pyridine-d_5 + D_2O) δ = 0.90 (9H, t, J = 6.0—6.1 Hz), 1.16—1.46 (56H, m), 1.50—2.05 (8H, m), 2.50—2.62 (2H, m), 3.03—3.19 (2H, m), 3.93 (1H, m), 4.13—4.25 (2H, m), 4.37 (1H, d, J = 11.1 Hz), 4.51 (1H, m), 5.08 (1H, m), 5.22 (1H, dd, J = 2.0, 48.5 Hz), 5.68 (1H, m), 5.72 (1H, t, J = 9.4 Hz), 5.99 (1H, d, J = 9.4 Hz). FAB MS (positive) m/z 962 $(M+Na)^+$, 940 $(M+H)^+$; (negative) m/z 938 $(M-H)^-$.

4,6-*O*-[(*R*)-Benzylidene]-*N*-[(2*R*,3*S*)-3-benzyloxy-2-fluorotetradecanoyl]-β-D-glucopyranosylamine (22′). Compound **21′** (1.03 g, 1.50 mmol) was treated as described in the formation of **10** from **9** to give **22′** (0.64 g, 71%) as a powder: R_f = 0.61 (EtOAc); $[\alpha]_D^{25}$ +7.0° (c = 1.3, $CHCl_3$); IR ν_{max} (Nujol®) 3330, 3034, 1672 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (3H, t, J = 6.6 Hz), 1.26 (18H, broad), 1.57—1.80 (2H, m), 3.22 (1H, broad s, OH), 3.32 (1H, broad s, OH), 3.41—3.64 (4H, m), 3.79—4.03 (2H, m), 4.25 (1H, m), 4.47, 4.55 (2H, AB-q, J = 11.2 Hz), 4.92 (1H, d, J = 46.9 Hz), 5.19 (1H, t, J = 9.2 Hz), 5.48 (1H, s, PhC*H*), 7.12 (1H, dd, J = 3.3, 9.2 Hz, NH), 7.26—7.30 (5H, m), 7.34—7.37 (3H, m), 7.44—7.50 (2H, m). FAB MS (positive): m/z 624 $(M+Na)^+$, 602 $(M+H)^+$; (negative): m/z 600 $(M-H)^-$. Found: C, 67.14; H, 7.70; N, 2.53%. Calcd for $C_{34}H_{48}FNO_7$ (601.8): C, 67.86; H, 8.04; N, 2.33%.

4,6-*O*-[(*R*)-Benzylidene]-*N*-[(2*R*,3*S*)-3-benzyloxy-2-fluorotetradecanoyl]-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (23′). Compound **22′** (127 mg, 0.212 mmol) was treated as described in the formation of **11** from **10** to give **23′** (147 mg, 67%) as a gum: R_f = 0.38 (hexane : EtOAc = 3 : 1); $[\alpha]_D^{25}$ +3.3° (c = 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ = 0.88 (9H, t, J = 6.6 Hz), 1.26 (58H, broad), 1.51—2.01 (4H, m), 2.26 (2H, t, J = 7.3—7.9 Hz), 2.50—2.64 (2H, m), 3.50—3.72 (4H, m), 3.83—4.12 (2H, m), 4.28 (1H, m), 4.48, 4.55 (2H, AB-q, J = 11.2 Hz), 4.82 (1H, dd, J = 1.5, 48.2 Hz), 4.88 (1H, t, J = 9.2 Hz), 5.20—5.33 (2H, m), 5.54 (1H, s), 7.17 (1H, dd, J = 4.3, 9.6 Hz, N*H*), 7.25-7.56 (10H, m).

4,6-*O*-[(*R*)-Benzylidene]-*N*-[(2*R*,3*S*)-3-benzyloxy-2-fluorotetradecanoyl]-3-*O*-diphenylphosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (24′). Compound **23′** (139 mg, 0.133 mmol) was treated as described in the formation of **12** from **11** to give **24′** (127 mg, 73%) as a gum: R_f = 0.47 (hexane : EtOAc = 3 : 1); $[\alpha]_D^{25}$ +13.3° (c = 3.6, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3400, 2900, 2850, 1750, 1730, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (9H, t, J = 6.6 Hz), 1.25 (58H, broad), 1.46—1.82 (4H, m), 2.19 (2H, t, J = 7.3 Hz), 2.27 (1H, dd, J = 4.6, 17.2 Hz), 2.45 (1H, dd, J = 7.9, 17.2 Hz), 3.61—3.80 (3H, m), 3.92 (1H,

td, J = 2.0, 27.7 Hz, C*H*OBn), 4.30 (1H, m), 4.84, 4.54 (2H, ABq, J = 11.2 Hz), 4.85 (1H, dd, J = 1.33, 48.2 Hz, C*H*F), 4.97—5.07 (2H, m). 5.15 (1H, t, J = 9.2 Hz), 5.32 (1H, t, J = 9.2 Hz), 5.48 (1H, s, PhC*H*), 6.99 (5H, s), 7.16—7.44 (16H, m, containing N*H*); FAB MS (negative) m/z 1268 $(M-H)^-$. Found: C, 69.79; H, 9.10; N, 1.06; F, 1.66; P, 2.39%. Calcd for $C_{74}H_{109}FNO_{13}P$ (1270.6): C, 69.95; H, 8.65; N, 1.10; F, 1.50; P, 2.44%.

***N*-[(2*R*,3*S*)-2-Fluoro-3-(hydroxy)tetradecanoyl]-3-*O*-diphenylphosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (25′).** (a) Compound **24′** (185 mg, 0.146 mmol) was hydrogenolyzed for 18 h at 25 °C in THF (20 ml) using 10% Pd on carbon (30 mg) as a catalyst, and the reaction mixture was filtered. The filtrate was concentrated in vacuo to give a debenzylated compound. The ^{1} NMR of this compound showed the benzylidene group remaining; therefore, this benzylidene compound was dissolved in 90% CF_3COOH aq-CH_2Cl_2 (1 : 20, 10 ml), and allowed to stand for 2 h at 24 °C. The reaction mixture was diluted with EtOAc (30 ml), washed with sat. aqueous $NaHCO_3$ (3 ml) and brine (3 ml), dried over anhydrous $MgSO_4$ (1 g), and filtered. The filtrate was evaporated in vacuo to give a residue, which was separated on preparative silica gel TLC plates. Development with hexane–EtOAc (1 : 3) gave **25′** (93 mg, 58%, R_f = 0.22) as a gum: $[\alpha]_D^{25}$ +21.7° (c = 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ = 0.88 (9H, t, J = 6.6 Hz), 1.25 (58H, broad), 1.50—1.90 (4H, m), 2.15—2.27 (3H, m), 2.44 (1H, dd, J = 7.6, 16.8 Hz), 3.27 (1H, broad, OH), 3.59 (1H, m), 3.71—4.18 (5H, m), 4.72 (1H, t, J = 7.9—8.6 Hz), 4.76 (1H, d, J = 49.5 Hz), 5.00—5.12 (2H, m), 5.20 (1H, t, J = 9.2 Hz), 7.16-7.38 (11H, m, containing N*H*).

(b) Compound **24′** (60 mg, 0.047 mmol) was treated as described in the formation of **25** from **24** to give **25′** (44 mg, 86%) as a gum.

***N*-[(2*R*,3*S*)-2-Fluoro-3-(hydroxy)tetradecanoyl]-3-*O*-phosphono-2-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosylamine (26′).** Compound **25′** (21 mg, 0.019 mmol) was treated as described in the formation of **8** from **7** to give **26′** (17 mg, 92%) as a powder: R_f = 0.32 ($CHCl_3$: EtOH : AcOH : H_2O = 8 : 5 : 1 : 1); $[\alpha]_D^{25}$ +4.0° (c = 0.1, pyridine); IR ν_{max} (KBr) 3333, 1742, 1675 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-d_5 + D_2O) δ = 0.89 (9H, t, J = 6.5—6.9 Hz), 1.05—2.15 (62H, m), 2.50—2.60 (2H, m), 3.02—3.07 (2H, m), 3.90 (1H, m), 4.10—4.32 (3H, m), 4.65 (1H, td, J = 7.0, 28.3 Hz), 5.10 (1H, d, J = 46.9 Hz, CF*H*), 5.62—5.75 (2H, m), 6.00 (1H, d, J = 9.0 Hz, C1-*H*); FAB MS (positive) m/z 962 $(M+Na)^+$, 940 $(M+H)^+$; (negative) m/z 938 $(M-H)^-$.

***N*-[(2*S*, 3*R*)-3-(Benzyloxycarbonyloxy)-2-fluorotetradecanoyl]-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (27).** To a solution of **1** (8.2 mg, 0.024 mmol) and (2*S*,3*R*)-3-[(benzyloxycarbonyl)oxy]-2-fluorotetradecanoic acid[15] (10.3 mg, 0.026 mmol) in CH_2Cl_2 (1 ml) was added DCC (8.2 mg, 0.040 mmol). This mixture was stirred for 30 min at 25 °C, diluted with EtOAc (30 ml), washed with sat. aqueous $NaHCO_3$ (3 ml) and brine (3 ml), and dried over anhydrous $MgSO_4$ (1 g). After filtration, the filtrate was concentrated in vacuo to give a mixture, which was purified by preparative TLC on a silica gel plate. Development with toluene–EtOAc (3 : 1) gave **27** (14.6 mg, 85%) as a gum, which was identical with the upper R_f (= 0.46) material obtained from the reaction of **1** with (±)-*syn*-3-(benzyloxycarbonyloxy)-2-fluorotetradecanoic acid mentioned below.

***N*-[(2*S*, 3*R*)-3-(Benzyloxycarbonyloxy)-2-fluorotetradecanoyl]-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (27) and *N*-[(2*R*,3*S*)-3-(Benzyloxycarbonyloxy)-2-fluorotetradecanoyl]-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (27′).** A solution of (±)-*threo*-3-[(benzyloxycarbonyl)oxy]-2-fluorotetradecanoic acid[16] (126 mg, 0.317 mmol) and oxalyl chloride (0.150 ml) in CH_2Cl_2 (1 ml) containing DMF (3 mg) was stirred for 1 h at 24 °C. This reaction mixture was concentrated in vacuo to give an acid chloride, which was dissolved in CH_2Cl_2 (1 ml). This solution was added dropwise at 0—5 °C to a solution of **1** (100 mg, 0.288 mmol) and Et_3N (40 mg, 0.400 mmol) in CH_2Cl_2 (1 ml). The mixture was stirred for 1 h at 24 °C, concentrated in vacuo, and diluted with EtOAc (39 ml). This solution was washed with sat. aqueous $NaHCO_3$ (3 ml) and brine (3 ml), dried over anhydrous $MgSO_4$ (1 g), and filtered. The filtrate was concentrated in vacuo to give a residue, which was separated by silica-gel column chromatography. Elution with toluene–EtOAc (3 : 1) gave **27** [95 mg, 45%, R_f = 0.46 (toluene : EtOAc = 3 : 1)] as a gum and **27′** [91 mg, 43%, R_f = 0.38 (toluene : EtOAc = 3 : 1)] as a gum. The upper R_f compound (**27**) was identical in all respects with that obtained from the rection of **1** with optically active (2*S*,3*R*)-3-[(benzyloxycaronyl)oxy]-2-fluorotetradecanoic acid. Therefore, the configurations at carbon C2 and C3 of compound **27′** were necessarily determined as (2*R*,3*S*).

Physical Data of 27: $[\alpha]_D^{25}$ +89.0° (c = 9.5, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3425, 2925, 2850, 1750, 1710 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ = 0.88 (3H, t, J = 6.3 Hz), 1.25 (18H, broad), 1.70—1.85 (2H, m), 1.82 (3H, s), 2.01 (3H, s), 2.04 (3H, s), 2.08 (3H, s), 3.81 (1H, ddd, J = 2.0, 4.3, 9.9 Hz, C5-H), 4.10 (1H, dd, J = 2.0, 12.5 Hz, C6-H), 4.30 (1H, dd, J = 4.3, 12.5 Hz, C6-H), 4.95 (1H, dd, J = 2.0, 47.5 Hz, CF*H*), 4.96 (1H, t, J = 9.2 Hz), 5.01—5.33 (6H, m), 7.25 (1H, m, N*H*), 7.32—7.43 (5H, m). Found: C, 59.66; H, 7.14; N, 2.08%. Calcd for $C_{36}H_{52}NO_{13}F$ (725.8): C, 59.57; H, 7.22; N, 1.93%.

Physical Data of 27′: $[\alpha]_D^{25}$ −70.0° (c = 9.1, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3400, 2900, 2850, 1750, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ = 0.88 (3H, t, J = 6.6 Hz), 1.20—1.40 (18H, m), 1.72—1.82 (2H, m), 2.00 (3H, s), 2.03 (6H, s), 2.04 (3H, s), 3.78 (1H, ddd, J = 2.3, 4.3, 9.9 Hz, C5-H), 3.94 (1H, dd, J = 2.3, 12.5 Hz, C6-H), 4.28 (1H, dd, J = 4.3, 12.5 Hz, C6-H), 4.89 (1H, dd, J = 3.3, 47.5 Hz, CF*H*), 4.93-5.34 (7H, m), 7.13 (1H, dd, J = 4.0, 9.2 Hz), 7.35 (5H, s). Found: C, 59.19; H, 7.01; N, 1.91; F, 2.53%. Calcd for $C_{36}H_{52}FNO_{13}$ (725.8): C, 59.57; H, 7.22; N, 1.93; F, 2.62%.

***N*-[(2*R*,3*S*)-2-Fluoro-3-(hydroxy)tetradecanoyl]-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (28′).** (a) To a solution of **27′** (55 mg, 0.076 mmol) in THF (0.5 ml) was added 10% Pd on carbon (6 mg). The mixture was stirred under an atmosphere of hydrogen at 25 °C for 15 h. The reaction mixture was filtered through Celite, which was washed with a small amount of THF. The combined filtrate was evaporated in vacuo to give **28′** (44 mg, 98%) as a gum: $[\alpha]_D^{25}$ +59.0° (c = 2.2, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3550, 3400, 3000, 2850, 1750, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ = 0.88 (3H, t, J = 6.6 Hz), 1.26 (18H, broad), 1.45—1.70 (2H, m), 2.03 (3H, s), 2.04 (3H, s), 2.05 (3H, s), 2.08 (3H, s), 2.19 (1H, d, J = 7.9 Hz, O*H*), 3.83 (1H, m, C*H*OH), 4.00—4.14 (2H, m, C5-H, C6-H), 4.32 (1H, dd, J = 4.0, 12.5 Hz, C6-H), 4.78 (1H, dd, J = 1.0, 48.2 Hz, CF*H*), 5.01 (1H, t, J = 9.2—9.9 Hz), 5.09 (1H, t, J = 9.9 Hz), 5.25 (1H, t, J = 9.2 Hz, C1-H), 5.32 (1H, dd, J = 9.2, 9.9 Hz), 7.17 (1H, dd, J = 4.0, 9.2 Hz, N*H*). Found: C, 56.73; H, 7.96; N, 2.38%. Calcd for $C_{28}H_{46}FNO_{11}$ (591.7): C, 56.84; H, 7.84; N, 2.37%.

(b) To a solution of **21′** (50 mg, 0.073 mmol) in THF (0.6 ml) was added 10% Pd on carbon (27 mg). The mixture was stirred under an atmosphere of hydrogen at 25 °C for 15 h. The reaction mixture was filtered through Celite, which was washed with a small amount of THF. The combined filtrate was evaporated in vacuo to give **28′** (37 mg, 85%) as a gum, which was identical in all respects to that obtained in procedure (a) mentioned above. Therefore, we could correlate the configurations of compounds **21** and **21′** as (2*S*,3*R*) and (2*R*,3*S*), respectively, because the configurations on the amide side chain of both **21′** and **27′** were also (2*R*,3*S*).

(*S*)-1-(*t*-Butyldimethylsilyloxy)-2,2-difluoro-3-(hydroxy)-tetradecane (30). To a solution of 2,2-difluorotetradecane-1,3-diol[16] (**29**, 232 mg, 0.871 mmol) in CH_2Cl_2 (30 ml) was added TBDMS-Cl (145 mg, 0.958 mmol) and DMAP (128 mg, 1.05 mmol). The mixture was stirred for 16 h at 25 °C, concentrated in vacuo, and diluted with EtOAc (50 ml). The solution was washed with H_2O (5 ml) and brine (5 ml), dried over anhydrous $MgSO_4$ (1 g), and filtered. The filtrate was concentrated in vacuo to give a mixture which was separated by silica-gel column chromatography. Elution with cyclohexane–EtOAc (9 : 1) gave **30** (267 mg, 81%) as a syrup: IR ν_{max} (film) 3400, 2940, 2860 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.10 (6H, s), 0.88 (3H, t, J = 6.8 Hz), 0.91 (9H, s), 1.20—1.40 (18H, m), 1.40—1.75 (2H, m), 2.18 (1H, d, J = 6.8 Hz, OH), 3.77—4.04 (3H, m). Found: C, 62.83; H, 11.21; F, 9.85%. Calcd for $C_{20}H_{42}F_2O_2Si$ (380.6): C, 63.11; H, 11.12; F, 9.98%.

(*S*)-3-Benzyloxy-2,2-dilfuorotetradecane-1-ol (31). To a solution of **30** (76 mg, 0.20 mmol) in THF (2 ml) were added NaH (20 mg, 55% oil dispersion) and Bu_4NI (10 mg). The mixture was stirred for 16 h at 25 °C, diluted with EtOAc (50 ml), washed with H_2O (5 ml) and brine (5 ml), dried over anhydrous $MgSO_4$ (1 g), and filtered. The filtrate was concentrated to give a residue, which was dissolved in dioxane (7 ml). To this solution was added H_2O (0.18 ml) and concd aqueous HCl (0.35 ml), and this mixture was stirred for 4 h at 50 °C, and diluted with EtOAc (50 ml). This solution was washed with sat. aqueous $NaHCO_3$ (5 ml), and brine (5 ml), dried over anhydrous $MgSO_4$ (1 g), and filtered. The filtrate was concentrated in vacuo to give a residue, which was separated by silica-gel column chromatography. Elution with cyclohexane–EtOAc (4 : 1) gave **31** (44 mg, 62%) as a viscous oil: IR ν_{max} (film) 3400, 2920, 2860 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (3H, t, J = 6.6 Hz), 1.26 (18H, broad), 1.50—1.70 (2H, m), 1.99 (1H, t, J = 6.8 Hz, OH), 3.68—4.04 (3H, m), 4.61, 4.76 (2H, AB-q, J = 11.2 Hz), 7.34 (5H, broad). Found: C, 70.23; H, 9.79; F, 10.57%. Calcd for $C_{21}H_{34}F_2O_2 \cdot 0.17\ H_2O$ (356.5+3.0): C, 70.16; H, 9.63; F, 9.86%.

(*S*)-3-Benzyloxy-2,2-dilfuorotetradecanoic Acid (32). The solution of **31** (39 mg, 0.11 mmol) in acetone (2 ml) and Jones reagent (0.50 ml) was stirred for 6 h at 24 °C. This reaction mixture was diluted with EtOAc (50 ml), washed with brine (5 ml), dried over anhydrous $MgSO_4$ (1 g), and filtered. The filtrate was concentrated in vacuo to give a mixture, which was separated on a short silica gel column. The column was eluted with EtOAc or EtOAc–MeOH (19 : 1, v/v). The product-containing fractions, were concentrated in vacuo and diluted with EtOAc (50 ml). The solution was washed with aqueous 1 M HCl (5 ml), H_2O (5 ml), and brine (5 ml), dried over anhydrous $MgSO_4$ (1 g), and filtered. The filtrate was concentrated in vacuo to give **32** (13 mg, 32%) as a viscous oil: IR ν_{max} (film) 3600—3400, 1745 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 0.88 (3H, t, J = 6.6 Hz), 0.91 (9H, s), 1.26 (18H, broad), 1.60—1.70 (2H, m), 3.82—3.94 (1H, m), 4.64, 4.78 (2H, AB-q, J = 11.2 Hz), 7.31—7.37 (5H, m). FAB MS (negative) m/z 369 $(M-H)^-$.

This compound **32** was used for identification of the configurations on the anomeric side chains of compounds **11** and **11′** showed in Scheme 1. Compound **32** was converted to compound **11′** via compounds **9′** and **10′** in the same procedure as Scheme 1. Thus obtained authentic (3*S*)-**11′** was identical in all respects with the lower R_f compound **11′**, and compound **11** consequently has (3*R*)-configuration.

(±)-3-Benzyloxy-2,2-difluorotetradecanyl Chloride. To a solution of (±)-ethyl 2,2-difluoro-3-hydroxytetradecanoate[9] (3.0 g, 9.7 mmol) in DMF (30 ml) were added NaH (510 mg, 55% oil dispersion) and benzyl bromide (1.4 ml). The mixture was stirred for 3 h at 25 °C, quenched with AcOH under ice cooling temperature, and diluted with EtOAc (300 ml), washed with sat. aqueous $NaHCO_3$ (30 ml) and brine (30 ml), dried over anhydrous $MgSO_4$ (5 g), and filtered. The filtrate was concentrated in vacuo, and separated by silica-gel column chromatography. Elution with hexane–EtOAc (9 : 1) gave (±)-ethyl 3-benzyloxy-2,2-difluorotetradecanoate (2.77 g, 72%), which was saponified in EtOH (50 ml) and 1 M KOH (25 ml) for 1 h at 25 °C. The reaction mixture was concentrated to half volume, and diluted with EtOAc (250 ml), washed with brine (25 ml), dried over anhydrous $MgSO_4$ (4 g), and filtered. The filtrate was concentrated in vacuo to give an oily mixture, which was separated by silica-gel column chromatography. Elution with EtOAc gave (±)-3-benzyloxy-2,2-difluorotetradecanoic acid (2.33 g, 87%), which was treated with excess oxalyl chloride in CH_2Cl_2 to give (±)-3-benzyloxy-2,2-difluorotetradecanoyl chloride. ^1H NMR data of (±)-3-benzyloxy-2,2-difluorotetradecanoic acid was the same as that of optically active (3*S*)-**32**.

(±)-*threo*-3-Benzyloxy-2-fluorotetradecanoic Acid. (i) (±)-*threo*-2-Fluoro-3-hydroxytetradecanoic acid[14] (5.0 g, 19.1 mmol) in cold MeOH (250 ml) was esterified with CH_2N_2 in Et_2O to yield (±)-methyl *threo*-2-fluoro-3-hydroxytetradecanoate (3.38 g, 64%) after silica-gel chromatography (elution with cyclohexane : EtOAc = 4 : 1). (ii) To a solution of this methyl ester (3.30 g, 11.9 mmol) in CH_2Cl_2 (60 ml), benzyl 2,2,2-trichloroacetimidate (4.52 g, 17.9 mmol) and trifluoromethanesulfonic acid (0.2 ml) were added. The mixture was stirred for 16 h at 25 °C under N_2. Coevaporation with toluene (50 ml×3) in vacuo gave a residue, which was directly purified by silica-gel column chromatography. Elution with cyclohexane–EtOAc (9 : 1) gave a mixture, which was separated by silica-gel column chromatography. Elution with cyclohexane–EtOAc (9 : 1) gave (±)-methyl *threo*-3-benzyloxy-2-fluorotetradecanoate (2.81 g, 64%). (iii) To this methyl ester (2.75 g, 7.50 mmol) in EtOH (50 ml) was added aqueous 1 M NaOH (30 ml). This suspension was stirred for 1—3 h at 50 °C. The resulting clear solution was evaporated in vacuo to half the volume, made acidic by addition of conc. aqueous HCl, and extracted with EtOAc (150 ml×2). The organic layer was washed with brine (30 ml), dried over anhydrous $MgSO_4$ (4 g), and filtered. The filtrate was concentrated in vacuo, and separated by silica-gel column chromatography. Elution with cyclohexane–EtOAc (9 : 1), then EtOAc gave (±)-*threo*-3-benzyloxy-2-fluorotetradecanoic acid (1.59 g, 60%).

Materials and Methods for Measurement of Biological Activity. The sources of the materials used in the study (Figs. 3 and 4) are as follows: Lipopolysaccharide (LPS) from *E. coli* serotype 026 : B6, and 12-*O*-tetradecanoyl phorbor acetate (TPA) were from SIGMA, St. Louis, MO. Lipid A, compound 506, was purchased from Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan. RPMI-1640 medium, fetal bovine serum (FBS), and newborn calf serum (NBCS) were from GIBCO, Grand Island, N Y. 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 $\mu g\ ml^{-1}$ of streptomycin (growth medium).

Production of TNFα by U937 Cells: U937 cells (1×10^4/200 μl/well) were plated in 96-well plates (Corning, Cambridge, MA), and were cultured in the presence of TPA (30 $ng\ ml^{-1}$) for 72 h at 37 °C. After the removing of the supernatant, the cells were incubated with 200 μl of fresh RPMI-1640 medium containing 10% NBCS, 10 $ng\ ml^{-1}$ of LPS and graded concentrations of compounds in a humidified atmosphere of 5% 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 kits. As a control, the amount of TNFα produced by U937 cells stimulated with 10 ng ml^{-1} of LPS in the absence of compounds was used. The relative amounts were calculated and are indicated as percentages of the control amount.

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