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Syntheses of 1-*O*-carboxyalkyl GLA-60 analogues

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Abstract

As part of our ongoing study to survey potent LPS antagonists, the following six compounds were synthesized in an efficient manner: 3-carboxypropyl and carboxymethyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4-*O*-phosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α- and β-D-glucopyranosides (**11** and **23**; **32** and **36**), as well as the non-fluorinated equivalents, carboxymethyl 2-deoxy-4-*O*-phosphono-2-tetradecanamido-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**44**) and carboxymethyl 2-deoxy-2-[(*R*)-3-(hydroxy)tetradecanamido]-4-*O*-phosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**48**). Of these compounds, **32** was most pronounced in terms of LPS-antagonistic activity.

Keywords: GLA-60; GLA-60, 1-*O*-carboxyalkyl; LPS antagonist; Olefin carboxylation; Ruthenium tetroxide

1. Introduction

Lipopolysaccharides (LPS) [1], an outer surface membrane component present in Gram-negative bacterial cells such as *Salmonella minnesota*, *Salmonella typhirium*, and *Escherichia coli*, cause fever and lethal shock in the septicemia of higher animals. Despite LPS being a highly potent stimulator of the immune system, its ability to induce endotoxic shock has precluded it from clinical use. In related studies, it has been recognized that the antimicrobial property of antibiotics induces the release of LPS from

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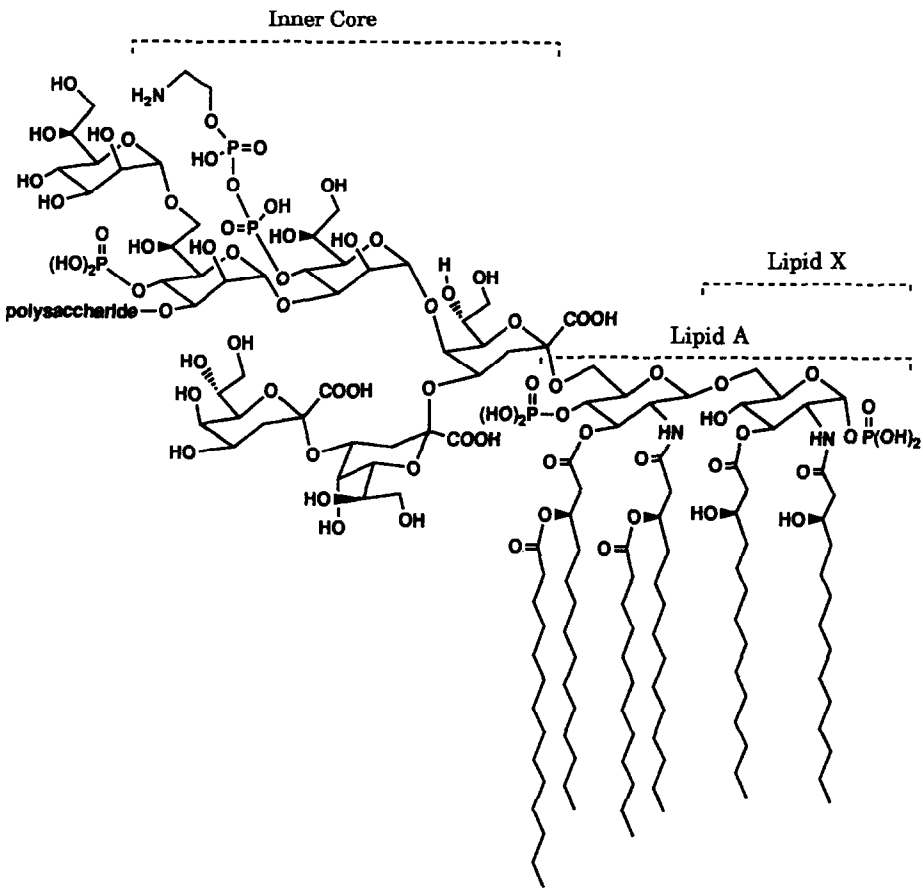
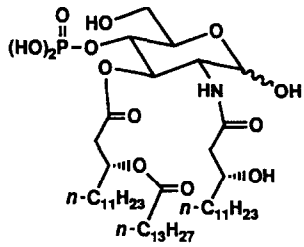
the outer membrane of Gram-negative bacteria, and in turn causes an acute inflammatory response following the release of TNFα [2]. This remains a serious unsolved clinical problem, despite the availability of potent antibiotics.

An example of a LPS structure is shown below [3]. Most of the biological activities of LPS reside in a relatively small portion of the molecule known as lipid A, a disaccharide unit bearing the constituent lipid moiety. This same unit, lipid A, which was first isolated by Westphal and Luderitz [1] and later chemically synthesized by Imoto et al. [4] and Achiwa and coworkers [5], exists as a hydrophobic anchor substance holding an essentially linear polysaccharide chain to the cell wall [3]. Nishijima and Raetz [6] isolated lipid X from a mutant of *Escherichia coli*. Lipid X, the reducing part of lipid A, is a biosynthetic precursor of the latter [7].



In a series of structure–activity relationship studies on nonreducing subunit analogues of lipid A, Hasegawa, Kiso and coworkers [8] have demonstrated that several of the biological activities of LPS, mainly having LPS-agonistic activity, are expressed by

certain 4-*O*-phosphono-D-glucosamine derivatives pertaining to the structure of GLA-60 [8]. Recently it has been shown that some lipid A analogues show potency as an endotoxin antagonist [9].



GLA-60

We have been investigating compounds related to GLA-60 that have LPS-antagonist activity. In this paper, we describe the syntheses and biological activities of the difluoro compounds, namely, 3-carboxypropyl and carboxymethyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4-*O*-phosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α- and β-D-glucopyranosides (**11**, **23**, **32**, and **36**), and their nonfluorinated equivalents, carboxymethyl 2-deoxy-4-*O*-phosphono-2-tetradecanamido-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**44**), and carboxymethyl 2-deoxy-2-[(*R*)-3-(hydroxy)tetradecanamido]-4-*O*-phosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**48**). These compounds are common to each other in that they bear either a substituted or nonsubstituted tetradecanoyl group at the 2-amido position, an (*R*)-3-(tetradecanoyloxy)tetradecanoyl group at *O*-3, a phosphoric acid at *O*-4, and a carboxymethyl or a 3-carboxypropyl group at the anomeric position. The 2,2-difluorinated tetradecanoyl group was expected to enhance the biological activity based on our previous work [10].

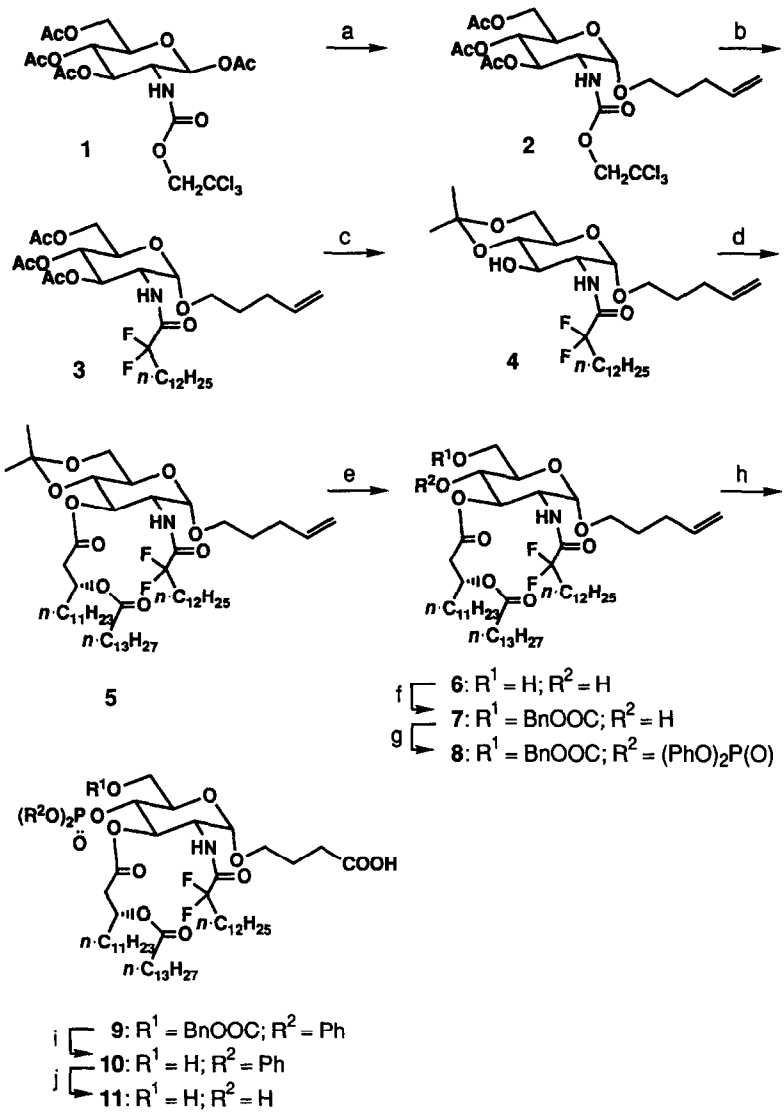
2. Synthesis

The starting 2-deoxy-1,3,4,6-tetra-*O*-acetyl-2-[(2,2,2-trichloroethoxycarbonyl)amino]-β-D-glucopyranose (**1**) was employed for the synthesis of the α-pentenyl derivative according to the reported method [11], and 2-deoxy-1,3,4,6-tetra-*O*-acetyl-2-trifluoroacetamido-β-D-glucopyranose (**12**) was used for the preparation of the β-pentenyl anomeric equivalent. Neighbouring group participating effects of the trichloroethoxycarbonyl and trifluoroacetyl groups, protecting the C-2 amine function, gave rise to stereospecific formation of the α-pentenyl and β-pentenyl glycosides, respectively. On the other hand, α- (**24**) and β-allyl compounds (**33** and **45**) were synthesized from allyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-α- and β-D-glucopyranosides, respectively, according to the reported methods [12].

Compound **1** was brominated using 30% HBr in acetic acid, and treatment of the resultant bromide with 4-penten-1-ol, using silver perchlorate and calcium sulphate as Lewis acids, in methylene chloride, stereospecifically gave an α-pentenyl compound **2** (see Scheme 1 for structures **1–11**). We cannot explain the stereospecificity of this reaction. However, this procedure has already been reported for the other glycosyl acceptor by Kusama et al. [11]. The amine protecting group, trichloroethoxycarbonyl, was subsequently cleaved by zinc dust in acetic acid, and the free amine was acylated by treatment with 2,2-difluorotetradecanoyl chloride and triethylamine to give compound **3**. Deacetylation of **3** (sodium methoxide in methanol), and reprotection of the C-4,6 positions with 2,2-dimethoxypropane in *N,N*-dimethylformamide (DMF), using pyridinium *p*-toluenesulphonate as a catalyst, gave an isopropylidene derivative **4**. The C-3 free hydroxyl group of **4** was acylated with (*R*)-3-(tetradecanoyloxy)tetradecanoic acid and 4-dimethylaminopyridine (DMAP), using *N,N'*-dicyclohexylcarbodiimide (DCC) as a condensing reagent, to give **5**. The isopropylidene group of **5** was deprotected by aq 90% acetic acid in tetrahydrofuran at 60 °C, and the primary alcohol of **6** was selectively protected in a good yield with benzyl chloroformate and pyridine to afford **7**. With the free secondary alcohol at the C-4 position remaining, phosphorylation with diphenyl chlorophosphate and DMAP was performed to generate **8**. In the course of optimizing conditions for the benzyloxycarbonylation reaction, by resorting to DMAP as a base, dibenzyloxycarbonylation was found to occur at both the C-6 and C-4 hydroxy groups, and was further hampered by the extremely low yield of **7**. The crucial step which followed was oxidizing the olefin in the 4-pentenyl group to a carboxylic acid function. Treatment of **8** with ruthenium tetroxide, formed in situ from ruthenium dioxide and sodium periodate, gave a carboxylic acid **9**. Hydrogenolysis of **9**, using 10% palladium-on-carbon as catalyst, yielded **10**, and successive hydrogenolysis of the diphenyl phosphate of **10** gave the objective compound **11** (see Scheme 2 for structures **12–23**).

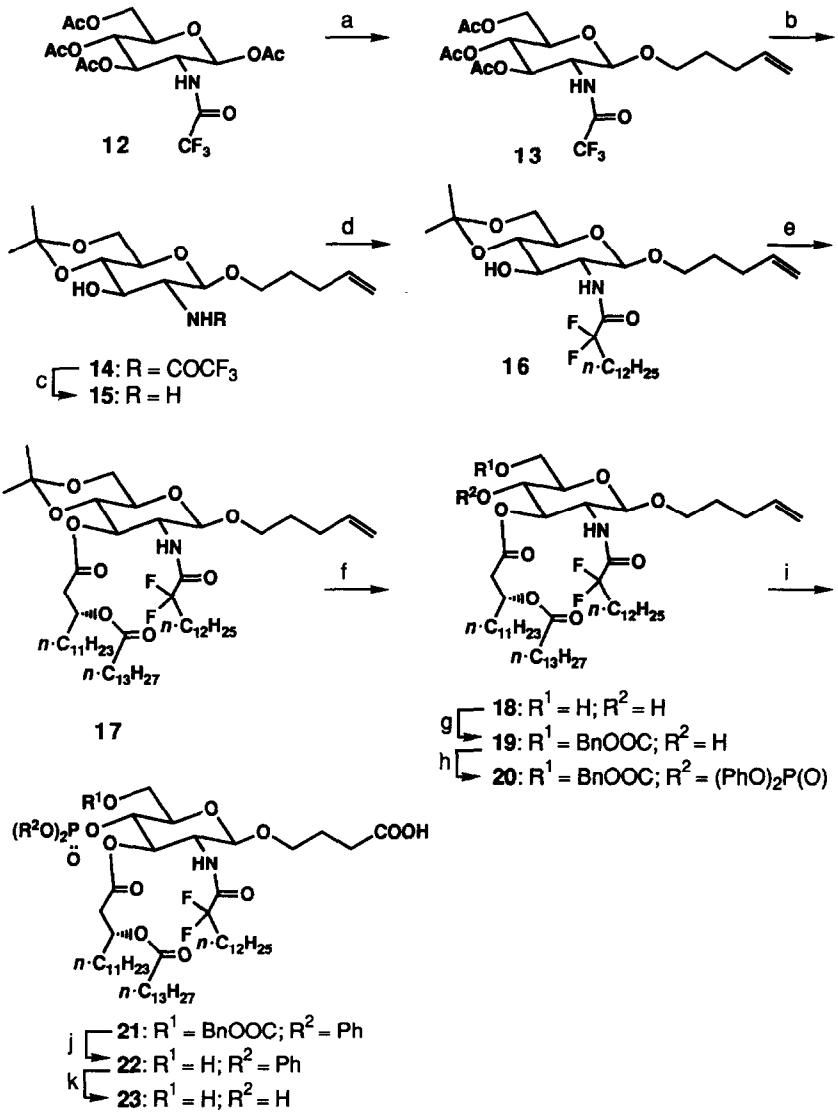
The β-anomer of **11** was obtained from the compound **16**, which was synthesized from 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranoside (**12**). Hence treatment of **12** with 4-penten-1-ol, using ferric chloride and calcium sulphate as Lewis acids, gave **13**. The trifluoroacetamido group at the C-2 position stereospecifically resulted in the β-pentenyl group being at the anomeric position. The stereospecificity of this reaction is unexplained. Removal of the 3,4,6-tri-*O*-acetyl groups of **13** with ammoniun hydroxide in methanol, and reprotection at the C-4,6 positions with 2,2-dimethoxypropane in DMF, using pyridium *p*-toluenesulphonate as catalyst, afforded the isopropylidene compound **14**. The trifluoroacetyl group of **14** was removed to yield an amine **15** with 1 M potassium hydroxide in ethanol, and the amine was reacylated to give the 2,2-difluorotetradecanamide group by treatment with 2,2-difluorotetradecanoyl chloride and triethylamine. The β-anomer **16** thus obtained was further converted to the carboxylic acid **23** over seven steps, according to the analogous procedure for the synthesis of the α anomer **11** from α anomer **4** (see Scheme 3 for structures **24–36**).

The α- and β-carboxymethyl pyranosides **32** and **36** were synthesized from the α-allyl derivative **24** [12,13], and the β-allyl derivative **33** [14], respectively. Both the C-2 amino and C-3 hydroxy positions of **24** were converted to lipid functions to yield **26**, according to the above-mentioned procedure (from **15** to **17**). However, unlike in the above case of pentenyl oxidation to generate the carboxylic acid function, the carboxylic



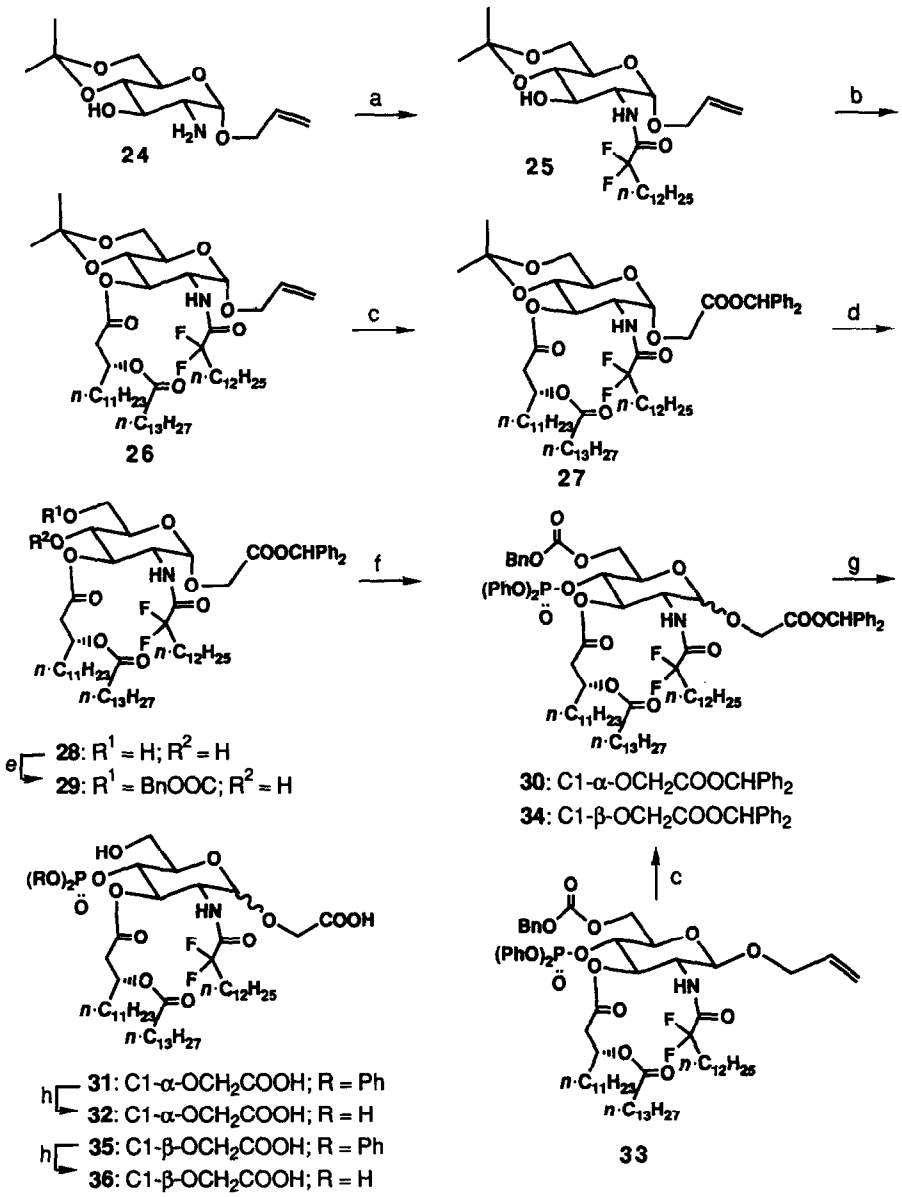
Scheme 1. Conditions: (a) (1) HBr–AcOH, 24 °C, 2 h; (2) $AgClO_4$–$CaSO_4$, 4-penten-1-ol, CH_2Cl_2, 24 °C, 1 h, 66%; (b) (1) Zn–AcOH, 60 °C, 1.5 h; then 4 M HCl–dioxane; (2) 2,2-difluorotetradecanoyl chloride, Et_3N, CH_2Cl_2, 24 °C, 30 min, 85%; (c) (1) MeONa–MeOH, 25 °C, 30 min; (2) 2,2-dimethoxypropane, PPTS, Me_2NCHO, 25 °C, 5 h, 88%; (d) (*R*)-3-(tetradecanoyloxy)tetradecanoic acid, DCC, DMAP, CH_2Cl_2, 24 °C, 1 h, 98%; (e) 9:1 AcOH–H_2O and THF, 60 °C, 8 h, 82%; (f) ClCOOBn–pyridine, THF, 24 °C, 1 h, 92%; (g) ClP(=O)$(OPh)_2$, DMAP, CH_2Cl_2, 24 °C, 1 h, 92%; (h) RuO_2–$NaIO_4$, 2:2:3 MeCN–CCl_4–H_2O, 24 °C, 5 h, 58%; (i) H_2, 10% Pd/C, THF, 25 °C, 3 h, 82%; (j) H_2, PtO_2, THF, 24 °C, 6 h, 99%.

acid derived from **26** in situ was esterified with diphenyldiazomethane to yield the diphenylmethyl ester **27**. Successive treatment of **27**, namely: (1) deprotection of the 4,6-*O*-isopropylidene group, (2) C-6-selective benzyloxycarbonylation (3) C-4-diphenyl-

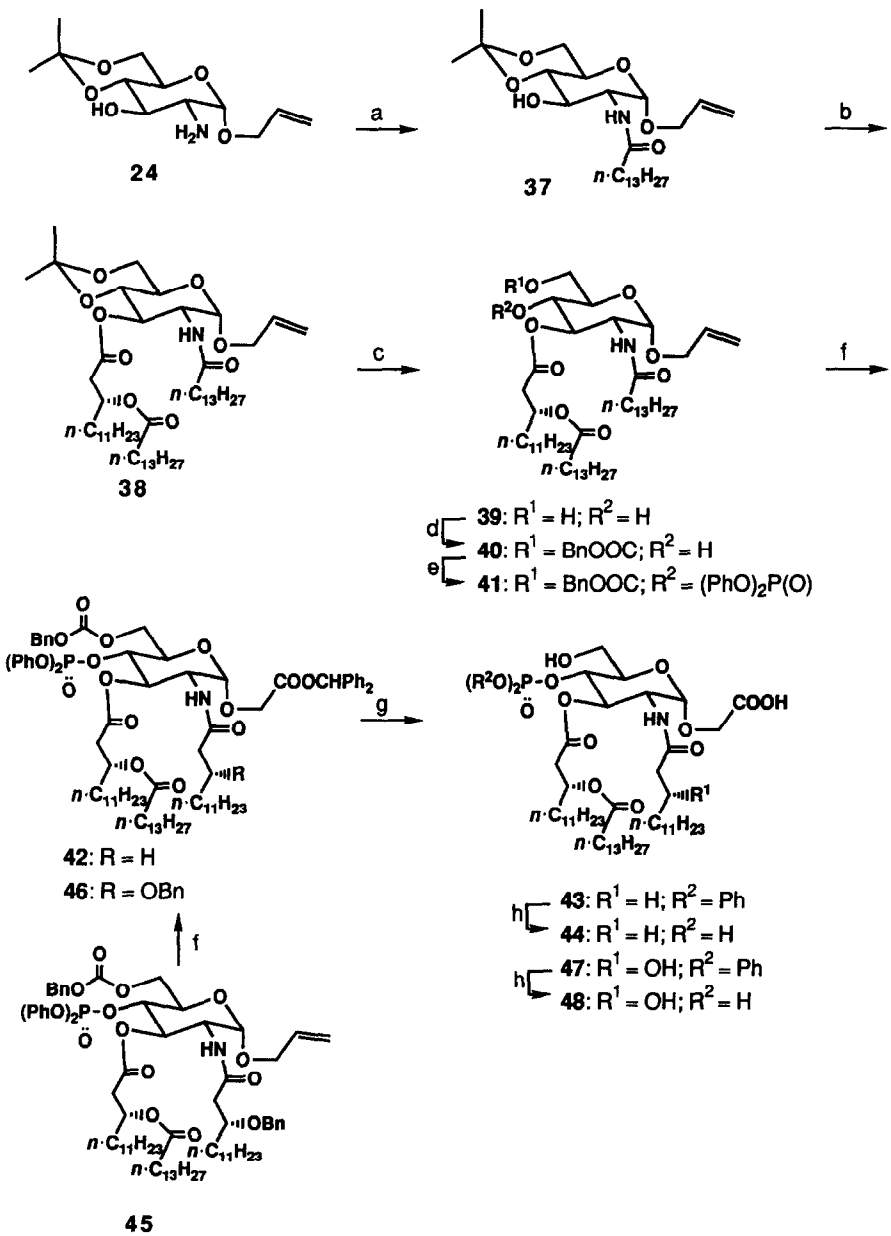


Scheme 2. Conditions: (a) $FeCl_3$–$CaSO_4$, 4-penten-1-ol, CH_2Cl_2, 24 °C, 5 h, 74%; (b) (1) MeOH–NH_4OH–H_2O, 25 °C, 6 h; (2) 2,2-dimethoxypropane, PPTS, DMF, 24 °C, 6 h, 77%; (c) M KOH, EtOH, 24 °C, 1 h, 88%; (d) 2,2-difluorotetradecanoyl chloride, Et_3N, CH_2Cl_2, 24 °C, 1 h, 87%; (e) (*R*)-3-(tetradecanoyloxy)-tetradecanoic acid, DCC, DMAP, CH_2Cl_2, 24 °C, 1 h, 84%; (f) 9:1 AcOH–H_2O and THF, 60 °C, 8 h, 98%; (g) ClCOOBn–pyridine, THF, 24 °C, 1 h, 68%; (h) ClP(=O)$(OPh)_2$, DMAP, CH_2Cl_2, 24 °C, 1 h, 96%; (i) RuO_2–$NaIO_4$, 2:2:3 MeCN–CCl_4–H_2O, 24 °C, 5 h, 98%; (j) H_2, 10% Pd/C, THF, 25 °C, 3 h, 79%; (k) H_2, PtO_2, THF, 24 °C, 6 h, 89%.

phosphorylation, (4) tandem hydrogenolysis of the C-6 benzyloxycarbonyl group and the diphenylmethyl ester, and finally (5) hydrogenolysis of the diphenylphosphate ester, gave the objective α anomer **32**.



Scheme 3. Conditions: (a) 2,2-difluorotetradecanoyl chloride, Et_3N, CH_2Cl_2, 24 °C, 1 h, 88%; (b) (*R*)-3-(tetradecanoyloxy)tetradecanoic acid, DCC, DMAP, CH_2Cl_2, 24 °C, 2 h, 64%; (c) (1) RuO_2–$NaIO_4$, 2:2:3 MeCN–CCl_4–H_2O, 24 °C, 3 h; (2) Ph_2CN_2, THF, 24 °C, 16 h, **27**: 68%; **34**: 55%; (d) 9:1 AcOH–H_2O, 60–65 °C, 3 h, 55%; (e) ClCOOBn–pyridine, THF, 24 °C, 1 h, 90%; (f) $ClP(=O)(OPh)_2$, DMAP, CH_2Cl_2, 24 °C, 1 h, 97%; (g) H_2, 10% Pd/C, THF, 25 °C, 3 h, **31**: 87%, **35**: 81%; (h) H_2, PtO_2, THF, 24 °C, 6 h, **32**: 97%, **36**: 93%.



Scheme 4. Conditions: (a) tetradecanoyl chloride, Et_3N, CH_2Cl_2, 24 °C, 1 h, 91%; (b) (*R*)-3-(tetradecanoyloxy)tetradecanoic acid, DCC, DMAP, CH_2Cl_2, 24 °C, 2 h, 76%; (c) 9:1 AcOH–H_2O, 60 °C, 6 h, 80%; (d) ClCOOBn–pyridine, THF, 24 °C, 1 h, 92%; (e) ClP(=O)(OPh)$_2$, DMAP, CH_2Cl_2, 24 °C, 2 h, 85%; (f) (1) RuO_2–$NaIO_4$, 2:2:3 MeCN–CCl_4–H_2O, 24 °C, 3 h; (2) Ph_2CN_2, THF, 24 °C, 1–3 h, **42**: 58%; **46**: 78%; (g) H_2, 10% Pd/C, THF, 25 °C, 3–10 h, **43**: 43%, **47**: 70%; (h) H_2, PtO_2, THF, 24 °C, 16 h, **44**: 96%, **48**: 90%.

The β-anomer **36** was synthesized from **33** according to essentially the same procedure for the preparation of **11** from **8** (see Scheme 4 for structures **37–48**).

At this stage, we found that compounds **11**, **23**, and **36** did not show significant biological activities. However, the α-carboxymethyl anomer **32** showed a fairly strong LPS-antagonistic activity. Therefore, we intended to synthesize the other α-carboxymethyl analogues **44** and **48**, which did not contain any fluorine atom in the C-2 amido group. Such compounds would also obviate whether fluoride substitution has any significant influence towards promoting LPS-antagonistic activity. The procedure for the synthesis of **44** and **48** from **24** and **45** was essentially the same method described in Schemes 1–3.

3. Results and discussion

We were able to synthesize six 1-*O*-(carboxy)alkyl GLA-60 analogues (**11**, **23**, **32**, **36**, **44**, and **48**). The critical step was the oxidation of the allyl or 4-pentenyl double bond by ruthenium tetroxide. However, it was found that these oxidation conditions were highly chemoselective towards the double bonds to give their corresponding

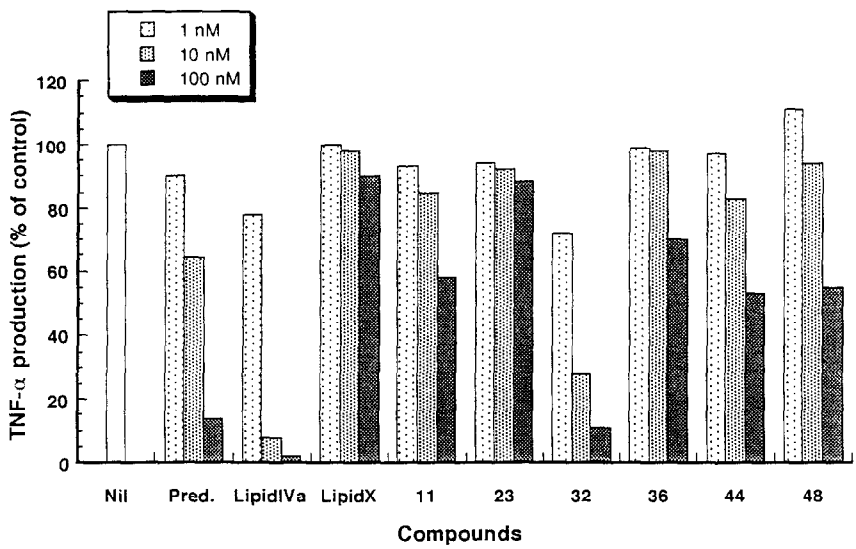
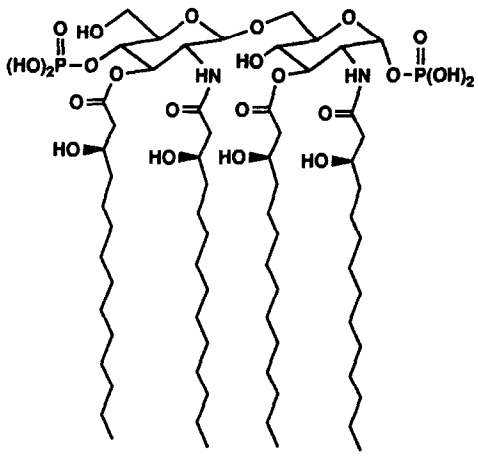


Fig. 1. Inhibition of LPS-induced TNFα production by GLA-60 analogues. U937 cells were preincubated in RPMI-1640 medium containing 10% FBS and 30 ng/mL of TPA for 72 h. The cells were then cultured in RPMI-1640 medium coating 10% NBBS, 10 ng/mL of LPS and graded concentrations of GLA-60 analogues (compounds **11**, **23**, **32**, **36**, **44**, and **48**), lipid X, lipid IVa or prednisolone. The amount of TNFα produced by the cells was determined by ELISA analysis. 1.0×10^5 U937 cells produced 600–800 pg of TNFα in the absence of compounds (control). Each value is a percentage of the control. The data are expressed as an average of their duplicate determinations.

carboxylic acid functions in considerably good yields at any stage without affecting isopropylidene, ester, amide, phosphate ester, phenyl, benzyl, or hemiacetal groups in the molecules. Furthermore, we found that we could perform benzyloxycarbonylation selectively at the C-6 hydroxy group without resorting to C-4 hydroxyl group protection, in high yields, by using pyridine as a base. When pyridine was used under these conditions, no C-4 benzyloxycarbonylation product was obtained. However, in the case of DMAP as base, benzyloxycarbonylated products at both the C-4 and C-6 positions were obtained as byproducts.

Biological activity.—The inhibitory activity of these compounds on LPS-induced TNFα production in U937 cells was measured in comparison with both predonisolone and lipid IVa as standards. The latter is a well-known LPS antagonist in human monocytes [15]. Compound **32** revealed fairly strong LPS-antagonist activity, while the activity of compounds **11**, **23**, **36**, **44**, and **48** was of no comparison as shown in Fig. 1. The conclusion, therefore, based on this study is obviously that the length of the methylene group, the configuration at the anomeric position, and the difluorinated amide at C-2 position affects the LPS-antagonist activity.



Lipid IVa

4. Experimental

General methods.—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 JNN-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 chromatogram with a solution of 17% H_2SO_4 in water (w/w), containing ammonium molybdate (2.3%) and ceric sulphate (0.9%) (Hanessian dip), and heating the plate for several minutes at ca. 180 °C. Separation of the compounds by column chromatography was done with Silica Gel 60 (230–400 mesh ASTM, E. Merck) under a slightly elevated pressure (1.5 atm) for easy elution, and the quantity of the used silica gel was 50–100 times the weight of the product purified. Concentrations were conducted in vacuo.

4-Pentenyl 3,4,6-tri-O-acetyl-2-deoxy-2-[(2,2,2-trichloroethoxycarbonyl)amino]-α-D-glucopyranoside (**2**).—To a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-[(2,2,2-trichloroethoxycarbonyl)amino]-β-D-glucopyranose (**1**, 9.0 g, 17.22 mmol) in CH_3COOH (5.0 mL), was added a solution (1.0 mL) of 30% HBr in AcOH, and the mixture was stirred for 2 h at room temperature; then it was diluted with EtOAc. The solution was neutralized with aq $NaHCO_3$. The organic layer was washed with water and brine, then it was dried over $MgSO_4$, filtered, concentrated, and chromatographed on a silica gel column. Elution with 2:1 hexane–EtOAc gave a bromide (7.35 g). To a suspension of $CaSO_4$ (Drierite) (7.36 g, 54.1 mmol) and $AgClO_4$ (3.64 g, 17.58 mmol) in CH_2Cl_2 (120 mL), was added a solution of the above-prepared bromide (7.35 g) in CH_2Cl_2 (100 mL). After 5 min, 4-penten-1-ol (2.8 mL, 27.04 mmol) was added to this suspension, and the mixture was stirred for 1 h at room temperature, neutralized with aq $NaHCO_3$ and filtered through Celite. The organic layer was washed with water, dried over $MgSO_4$, and concentrated to give a residue, that was chromatographed on a silica gel column. Elution with 2:1 hexane–EtOAc gave **2** (6.19 g, 66% in two steps). ^{1}H NMR ($CDCl_3$): δ 1.65–1.82 (m, 2 H), 1.98–2.22 (m, 11 H, containing, s, 3 H at δ 2.01, s, 3 H at δ 2.04, and s, 3 H at δ 2.10), 3.43–3.52 (m, 1 H), 3.68–3.79 (m, 1 H), 3.94–4.16 (m, 3 H), 4.26 (dd, 1 H, *J* 4.6, 11.9 Hz), 4.67 (d, 1 H, *J* 11.9 Hz), 4.80 (d, 1 H, *J* 11.9 Hz), 4.87 (d, 1 H, *J* 3.3 Hz) 4.98–5.17 (m, 3 H), 5.17–5.32 (m, 2 H), 5.72–5.90 (m, 1 H); IR ν_{max} ($CHCl_3$) 3430, 1745, 1643 (w) cm^{-1}. Anal. Calcd for $C_{20}H_{28}Cl_3NO_{10}$ (548.8): C, 43.77; H, 5.14; N, 2.55; Cl, 19.38. Found: C, 44.06; H, 5.29; N, 2.47; Cl, 19.21.

4-Pentenyl 3,4,6-tri-O-acetyl-2-deoxy-2-(2,2-difluorotetradecanamido)-α-D-glucopyranoside (**3**).—To a solution of **2** (5.61 g, 10.22 mmol) in AcOH (50 mL), was added Zn dust (6.0 g), and the mixture was stirred vigorously for 1.5 h at 60 °C. The mixture was filtered, concentrated, diluted with toluene, and again concentrated to give a residue to which was added excess 4.0 M HCl in dioxane, and the resulting solution was again concentrated to give an HCl salt of the amine. The amine–HCl salt so obtained was dissolved in CH_2Cl_2 (100 mL); and then Et_3N (3.12 mL, 22.49 mmol) and a solution of 2,2-difluorotetradecanoyl chloride (3.18 g, 11.24 mmol) in CH_2Cl_2 (20 mL) were added to this solution. After stirring for 30 min at room temperature, the mixture was concentrated to one-third volume and diluted with EtOAc. The solution was washed, successively, with aq M HCl and brine, dried over $MgSO_4$, filtered, concentrated, and chromatographed on a silica gel column. Elution with 2:1 hexane–EtOAc gave **3** (5.38 g, 85%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3 H, *J* 5.9–7.3 Hz), 1.18–1.45 (m, 20 H), 1.64–1.81 (m, 2 H), 1.94–2.20 (m, 13 H, containing, s, 3 H at δ 2.00, s, 3 H at δ 2.04, and s, 3 H at δ 2.10), 3.40–3.54 (m, 1 H), 3.68–3.90 (m, 1 H), 3.94–4.06 (m, 1 H), 4.06–4.18 (m, 1 H), 4.22–4.40 (m, 2 H), 4.86 (d, 1 H, *J* 3.3 Hz), 4.98–5.20 (m, 3 H),

5.28 (t, 1 H, *J* 9.9 Hz), 5.72–5.88 (m, 1 H), 6.55 (d, 1 H, *J* 9.2 Hz); IR ν_{max} (film): 3440, 3340, 2927, 2856, 1752, 1713, 1641 (w) cm^{-1}. Anal. Calcd for $C_{31}H_{51}F_2NO_{10}$ (619.7): C, 60.08; H, 8.30; N, 2.26; F, 6.13. Found: C, 59.82; H, 8.40; N, 2.28; F, 6.14.

*4-Pentenyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4,6-*O*-isopropylidene-α-*D*-glucopyranoside* (**4**).—To a solution of **3** (3.75 g, 6.05 mmol) in MeOH (30 mL) was added NaOMe (0.98 g, 18.15 mmol). After 30 min of stirring at room temperature, AcOH (1.0 mL) was added, and the solution was concentrated to give a residue, which was dissolved in EtOAc. The EtOAc solution was washed with aq $NaHCO_3$, water, and brine, dried over $MgSO_4$, filtered, concentrated to give a residue, which was dissolved in DMF (30 mL) and 2,2-dimethoxypropane (30 mL). To this solution was added pyridinium *p*-toluenesulphonate (0.20 g), and after 5 h of stirring, the mixture was concentrated, and diluted with EtOAc. The solution was washed successively with saturated $NaHCO_3$, water, and brine; then it was dried over $MgSO_4$, filtered, and concentrated to give a residue that was chromatographed on a silica gel column. Elution with 3:1 hexane–EtOAc gave **4** (2.83 g, 88%). ^1H NMR ($CDCl_3$): δ 0.88 (t, 3 H, *J* 6.6 Hz), 1.18–1.56 (m, 26 H, containing, s, 3 H at δ 1.44, and s, 3 H at δ 1.52), 1.65–1.76 (m, 2 H), 1.98–2.21 (m, 4 H), 2.43 (d, 1 H, *J* 3.3 Hz, OH), 3.34–3.47 (m, 1 H), 3.56–3.92 (m, 6 H), 4.17 (tdd, 1 H, *J* 4.0, 9.2, 9.9 Hz), 4.80 (d, 1 H, *J* 3.3 Hz, H-1), 4.90–5.12 (m, 2 H), 5.73–5.90 (m, 1 H), 6.59 (d, 1 H, *J* 8.6 Hz); IR ν_{max} (KBr): 3475, 3451, 2958, 2923, 2853, 1720, 1642 (w) cm^{-1}. Anal. Calcd for $C_{28}H_{49}F_2NO_6$ (533.7): C, 63.01; H, 9.25; N, 2.62; F, 7.12. Found: C, 63.14; H, 9.05; N, 2.66; F, 7.11.

*4-Pentenyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4,6-*O*-isopropylidene-3-*O*-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-*D*-glucopyranoside* (**5**).—To a solution of **4** (2.70 g, 5.06 mmol) in CH_2Cl_2 (30 mL), were added (*R*)-3-(tetradecanoyloxy)tetradecanoic acid (2.53 g, 5.56 mmol), DCC (1.36 g, 6.58 mmol), and 4-dimethylaminopyridine (0.62 g, 5.06 mmol). After 1 h of stirring at room temperature, the reaction mixture was concentrated and diluted with EtOAc, and the solution was washed with saturated $NaHCO_3$, water, and brine; then it was dried over $MgSO_4$, filtered, and concentrated to give a residue that was chromatographed on a silica gel column. Elution with 6:1 hexane–EtOAc gave **5** (4.82 g, 98%). ^1H NMR ($CDCl_3$): δ 0.88 (t, 3 H, *J* 5.9–7.3 Hz), 1.10–1.76 (m, 70 H, containing, s, 3 H at δ 1.39, and s, 3 H at δ 1.49), 1.91–2.19 (m, 2 H), 2.26 (t, 2 H, *J* 7.3–7.9 Hz), 2.48 (dd, 1 H, *J* 6.6, 15.8 Hz), 2.64 (dd, 1 H, *J* 6.6, 15.8 Hz), 3.36–3.42 (m, 1 H), 3.66–3.95 (m, 5 H), 4.21 (dt, 1 H, *J* 3.3, 9.9 Hz), 4.80 (d, 1 H, *J* 4.0 Hz), 4.97–5.10 (m, 2 H), 5.11–5.29 (m, 2 H), 5.79 (m, 1 H), 6.67 (d, 1 H, *J* 9.2 Hz); IR ν_{max} (KBr): 3445, 2956, 2920, 2851, 1736, 1716, 1643 (w) cm^{-1}. Anal. Calcd for $C_{56}H_{101}F_2NO_9$ (970.4): C, 69.31; H, 10.49; N, 1.44; F, 3.92. Found: C, 69.49; H, 10.60; N, 1.55; F, 3.92.

*4-Pentenyl 2-deoxy-2-(2,2-difluorotetradecanamido)-3-*O*-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-*D*-glucopyranoside* (**6**).—A solution of **5** (2.22 g, 2.29 mmol) in 9:1 AcOH–H_2O (30 mL) and THF (20 mL) was warmed to 60 °C for 8 h; then it was concentrated and diluted with EtOAc. The solution was washed successively with saturated $NaHCO_3$, water, and brine; then it was dried over $MgSO_4$, filtered, and concentrated to give a residue that was chromatographed on a silica gel column. Elution with 2:1 hexane–EtOAc gave **6** (1.65 g, 82%). ^1H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 6.6–7.3 Hz), 1.17–1.77 (m, 64 H), 1.93–2.19 (m, 5 H, containing OH), 2.31 (t, 2 H, *J*

7.3–7.9 Hz), 2.42–2.58 (m, 2 H), 3.35–3.47 (m, 1 H), 3.49 (d, 1 H, *J* 3.3 Hz, OH), 3.65–3.94 (m, 5 H), 4.11–4.23 (m, 1 H), 4.84 (d, 1 H, *J* 3.3 Hz), 4.97–5.20 (m, 4 H), 5.73–5.88 (m, 1 H), 6.77 (d, 1 H, *J* 9.2 Hz); IR ν_{max} (KBr): 3465, 3301, 3080 (w), 3280, 2956, 2920, 2851, 1737, 1721, 1697, 1686, 1644 (w) cm^{-1}. Anal. Calcd for $C_{53}H_{97}F_2NO_9$ (930.3): C, 68.42; H, 10.51; N, 1.51; F, 4.08. Found: C, 68.18; H, 10.21; N, 1.51; F, 4.10.

4-Pentenyl 6-O-benzyloxycarbonyl-2-deoxy-2-(2,2-difluorotetradecanamido)-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**7**).—To a solution of **6** (1.50 g, 1.60 mmol) in THF (20 mL) and pyridine (0.20 mL, 2.42 mmol) was added benzyl chloroformate (0.40 mL, 2.26 mmol) at 5 °C. After 1 h of stirring at 24 °C, the reaction mixture was concentrated and diluted with EtOAc. The solution was washed successively with M HCl, saturated $NaHCO_3$, and brine; then it was dried over $MgSO_4$, filtered, and concentrated to give a residue that was chromatographed on a silica gel column. Elution with 5:1 hexane–EtOAc gave **7** (1.57 g, 92%). ^1H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 6.6 Hz), 1.18–1.74 (m, 64 H), 1.91–2.17 (m, 4 H), 2.26–2.32 (m, 2 H), 2.42–2.57 (m, 2 H), 3.34–3.43 (m, 1 H), 3.51 (d, 1 H, *J* 4.0 Hz, OH), 3.58–3.75 (m, 2 H), 3.82–3.91 (m, 1 H), 4.12–4.24 (m, 1 H), 4.41–4.52 (m, 2 H), 4.82 (d, 1 H, *J* 3.3 Hz), 4.94–5.16 (m, 4 H), 5.18 (s, 2 H), 5.71–5.86 (m, 1 H), 6.73 (d, 1 H, 8.6 Hz), 7.32–7.45 (m, 5 H); IR ν_{max} (KBr): 3530, 3471, 2956, 2919, 2850, 1745, 1734, 1721, 1696, 1684, 1644 (w) cm^{-1}. Anal. Calcd for $C_{61}H_{103}F_2NO_{11}$ (1064.5): C, 68.83; H, 9.75; N, 1.32; F, 3.57. Found: C, 68.55; H, 9.81; N, 1.30; F, 3.55.

4-Pentenyl 6-O-benzyloxycarbonyl-2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-diphenylphosphono-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**8**).—To a solution of **7** (1.40 g, 1.32 mmol) in CH_2Cl_2 (15 mL), were added 4-dimethylaminopyridine (241 mg, 1.97 mmol) and a solution of diphenyl chlorophosphate (425 mg, 1.58 mmol) in CH_2Cl_2 (2.0 mL). After 1 h of stirring at room temperature, the reaction mixture was concentrated and diluted with EtOAc, and then the solution was washed successively with 0.2 M HCl, saturated $NaHCO_3$, and brine; then it was dried over $MgSO_4$, filtered, and concentrated to give a residue that was chromatographed on a silica gel column. Elution with 5:1 hexane–EtOAc gave **8** (1.57 g, 92%). ^1H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 6.6–7.2 Hz), 1.11–1.76 (m, 64 H), 1.92–2.18 (m, 6 H), 2.38–2.54 (m, 2 H), 3.35–3.46 (m, 1 H), 3.65–3.74 (m, 1 H), 3.97–4.09 (m, 1 H), 4.19–4.37 (m, 3 H), 4.74 (q, 1 H, *J* 9.2–9.9 Hz), 4.85 (d, 1 H, *J* 3.3 Hz), 4.98–5.18 (m, 5 H), 5.46 (dd, 1 H, *J* 9.2, 10.6 Hz), 5.72–5.87 (m, 1 H), 6.69 (d, 1 H, 8.6 Hz), 7.13–7.43 (m, 15 H); IR ν_{max} (KBr): 3447, 2920, 2852, 1753, 1738, 1717, 1643 (w) cm^{-1}. Anal. Calcd for $C_{73}H_{112}F_2NO_{14}P$ (1296.7): C, 67.62; H, 8.71; N, 1.08; F, 2.93; P, 2.39. Found: C, 67.58; H, 8.88; N, 1.05; F, 2.85; P, 2.17.

3-Carboxypropyl 6-O-benzyloxycarbonyl-2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-diphenylphosphono-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**9**).—To a solution of **8** (960 mg, 0.74 mmol) in 1:1:1.5 MeCN–CCl_4–H_2O (15 mL), were added $NaIO_4$ (10 g) and RuO_2 (20 mg). After 5 h of stirring at room temperature, the reaction mixture was diluted with EtOAc, and then the solution was washed successively with water and brine; then it was dried over $MgSO_4$, filtered, and concentrated to give a residue that was chromatographed on a silica gel column. Elution with 1:1 hexane–EtOAc, and then 1:2 gave **9** (568 mg, 58%). ^1H NMR ($CDCl_3$): δ

0.88 (t, 9 H, *J* 6.6 Hz), 1.08–1.57 (m, 62 H), 1.90–2.18 (m, 6 H), 2.31–2.55 (m, 4 H), 3.36–3.51 (m, 1 H), 3.71–3.85 (m, 1 H), 3.97–4.08 (m, 1 H), 4.15–4.38 (m, 3 H), 4.73 (q, 1 H, *J* 9.2–9.9 Hz), 4.80 (d, 1 H, *J* 3.3 Hz), 5.02–5.17 (m, 3 H), 5.42 (dd, 1 H, *J* 9.2, 10.6 Hz), 7.00 (d, 1 H, *J* 9.2 Hz), 7.11–7.38 (m, 16 H); IR ν_{max} ($CHCl_3$): 3430, 3400–2500 (containing 2925, 2850), 1743, 1710, 1590 cm^{-1}. Anal. Calcd for $C_{72}H_{110}F_2NO_{16}P$ (1314.6): C, 65.78; H, 8.43; N, 1.07; F, 2.89; P, 2.36. Found: C, 65.73; H, 8.70; N, 1.07; F, 2.81; P, 2.42.

*3-Carboxypropyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4-*O*-diphenylphosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-*D*-glucopyranoside* (**10**).—A solution of **9** (790 mg, 0.60 mmol) in THF (10 mL), containing 10% Pd-on-charcoal (50 mg) as a catalyst, was hydrogenolyzed for 3 h at 25 °C; then it was filtered and concentrated to give a residue that was chromatographed on a silica gel column. Elution with EtOAc gave **10** (580 mg, 82%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 5.9–7.3 Hz), 1.06–1.60 (m, 62 H), 1.91–2.21 (m, 6 H), 2.32–2.55 (m, 4 H), 3.41–3.51 (m, 1 H), 3.63 (s, 2 H), 3.71–3.87 (m, 2 H), 4.31 (dt, 1 H, *J* 3.3, 10.6 Hz), 4.77 (q, 1 H, *J* 9.2–9.9 Hz), 4.85 (d, 1 H, *J* 3.3 Hz, H-1), 5.12 (m, 1 H), 5.44 (dd, 1 H, *J* 9.2, 11.2 Hz), 6.98 (d, 1 H, 9.2 Hz), 7.13–7.40 (m, 10 H); IR ν_{max} (KBr): 3493, 3445, 3339, 3069, 2956, 2922, 2852, 1743, 1725, 1702, 1693 (shoulder) cm^{-1}. Anal. Calcd for $C_{64}H_{104}F_2NO_{14}P$ (1180.5): C, 65.12; H, 8.88; N, 1.19; F, 3.22; P, 2.62. Found: C, 64.89; H, 8.87; N, 1.19; F, 3.08; P, 2.54.

*3-Carboxypropyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4-*O*-phosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-*D*-glucopyranoside* (**11**).—A solution of **10** (212 mg, 0.18 mmol) in THF (5 mL) containing PtO_2 (20 mg) was hydrogenolyzed under hydrogen atmosphere at 24 °C for 3 h; then it was filtered and concentrated to give **11** (184 mg, quant). The powder **11** (15 mg) was dissolved in 0.1 M HCl (4 mL) and 1:2 $CHCl_3$–MeOH (15 mL). $CHCl_3$ (5 mL) and 0.1 M HCl (5 mL) were added to this solution to separate the mixture into two phases. The lower chloroform phase was collected and concentrated to give 14 mg of **11**. For test of biological activity, this compound was dissolved in aq 0.1% triethylamine (v/v) solution in order to prepare the 5 mM solution of **11**. ^{1}H NMR (pyridine-d_5): δ 4.90–5.05 (m, 1 H), 5.14–5.30 (m, 2 H, containing d, 1 H, *J* 3.4 Hz, at δ 5.26), 5.61–5.74 (m, 1 H), 6.12 (dd, 1 H, *J* 9.3, 10.7 Hz), 8.34 (m, 4 H, OH × 3, COOH), 9.29 (d, 1 H, 9.3 Hz); IR ν_{max} (KBr): 3271, 2957, 2920, 2851, 1743, 1704, 1561, 1468 cm^{-1}. FABMS (negative): *m/z* 1026 $(M-1)^-$, 798, 590, 572. Anal. Calcd for $C_{52}H_{96}F_2NO_{14}P$ (1028.3): C, 60.74; H, 9.41; N, 1.36; F, 3.70; P, 3.01. Found: C, 60.64; H, 9.08; N, 1.39; F, 3.57; P, 3.02.

*4-Pentenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(trifluoroacetamido)-β-*D*-glucopyranoside* (**13**).—To a suspension of $CaSO_4$ (1.84 g, 13.53 mmol) and $FeCl_3$ (1.10 g, 6.77 mmol) in CH_2Cl_2 (50 mL) were added 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranose (**12**, 2.00 g, 4.50 mmol) and 4-pentene-1-ol (2.33 mL, 22.6 mmol). This suspension was stirred for 5 h at room temperature; then it was poured into a mixture of $CHCl_3$ (50 mL) and saturated $NaHCO_3$ (50 mL). The mixture was stirred vigorously for 1 h at room temperature. The organic layer was collected, washed with saturated $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated to give a residue that was chromatographed on a silica gel column. Elution with 3:1 hexane–EtOAc gave **13** (1.56 g, 74%). ^{1}H NMR ($CDCl_3$): δ 1.62–1.76 (m, 2 H), 2.02–2.22 (m, 11 H,

containing, s, 6 H at δ 2.04 and s, 3 H at δ 2.10), 3.43–3.54 (m, 1 H), 3.69–3.78 (m, 1 H), 3.84–4.06 (m, 2 H), 4.15 (dd, 1 H, *J* 2.0–2.6, 11.9 Hz), 4.29 (dd, 1 H, *J* 4.6, 11.9 Hz), 4.64 (d, 1 H, *J* 7.9 Hz, H-1), 4.95–5.03 (m, 2 H), 5.11 (dd, 1 H, *J* 9.2, 9.9 Hz) 5.29 (dd, 1 H, *J* 9.2, 10.6 Hz), 5.69–5.88 (m, 1 H), 6.50 (d, 1 H, *J* 8.6 Hz, NH); IR ν_{max} (KBr): 3316, 1752, 1709, 1644 (w) cm^{-1}. Anal. Calcd for $C_{19}H_{26}F_3NO_9$ (469.4): C, 48.62; H, 5.58; N, 2.98; F, 12.14. Found: C, 48.76; H, 5.68; N, 3.07; F, 11.94.

4-Pentenyl 2-deoxy-4,6-O-isopropylidene-2-trifluoroacetamido-β-D-glucopyranoside (**14**).—A solution of **13** (6.79 g, 14.46 mmol) in MeOH (70 mL) and aq 28% ammonium hydroxide (7 mL) was stirred for 6 h at 25 °C; then it was concentrated to give a residue that was chromatographed on a short column of silica gel. Elution with EtOAc gave a triol (ca. 4.0 g). The triol was dissolved in Me_2NCHO (25 mL) and 2,2-dimethoxypropane (30 mL) containing pyridinium *p*-toluenesulphonate (100 mg). The mixture was stirred for 6 h at 24 °C; then it was concentrated and diluted with EtOAc. The solution was washed successively with saturated $NaHCO_3$ and brine; then it was dried over $MgSO_4$, filtered, and concentrated to give a residue that was chromatographed on a silica gel column. Elution with 2:1 hexane–EtOAc gave **14** (4.26 g, 77%). ^1H NMR ($CDCl_3$): δ 1.43 (s, 3 H), 1.51 (s, 3 H), 1.60–1.75 (m, 2 H), 2.03–2.14 (m, 2 H), 2.90 (d, 1 H, *J* 3.3 Hz, OH), 3.32 (dt, 1 H, *J* 5.3–9.9 Hz), 3.44–3.60 (m, 3 H), 3.75–3.99 (m, 3 H), 4.05–4.15 (m, 1 H), 4.77 (d, 1 H, *J* 7.9 Hz, H-1), 4.94–5.05 (m, 2 H), 5.70–5.85 (m, 1 H), 6.54 (d, 1 H, *J* 6.6 Hz, NH); IR ν_{max} (KBr): 3483, 3323, 2944, 2886, 1705, 1644 (w) cm^{-1}. Anal. Calcd for $C_{16}H_{24}F_3NO_6$ (383.4): C, 50.13; H, 6.31; N, 3.65; F, 14.87. Found: C, 49.95; H, 6.21; N, 3.75; F, 14.90.

4-Pentenyl 2-amino-2-deoxy-4,6-O-isopropylidene-β-D-glucopyranoside (**15**).—A solution of **14** (3.04 g, 10.59 mmol) in EtOH (60 mL) and aq M KOH (30 mL) was stirred for 1 h at 24 °C; then it was concentrated and diluted with EtOAc–H_2O, and the organic layer was washed with water and then brine; then it was dried over $MgSO_4$, filtered, and concentrated to give a residue that was chromatographed on a silica gel column. Elution with EtOAc, and then 19:1 EtOAc–MeOH, gave **15** (2.68 g, 88%). ^1H NMR ($CDCl_3$): δ 1.43 (s, 3 H), 1.51 (s, 3 H), 1.67–1.80 (m, 2 H), 1.88 (s, 3 H, OH, NH_2), 2.09–2.20 (m, 2 H), 2.75 (dd, 1 H, *J* 8.6, 9.2 Hz), 3.26 (dt, 1 H, *J* 5.3, 9.9 Hz), 3.42–3.63 (m, 3 H), 3.76–3.96 (m, 3 H), 4.23 (d, 1 H, *J* 7.9 Hz, H-1), 4.95–5.08 (m, 2 H), 5.73–5.90 (m, 1 H); IR ν_{max} (KBr): 3380, 3320, 3120, 3000–2800, 1642 (w) cm^{-1}. Anal. Calcd for $C_{14}H_{25}NO_5$ (287.4): C, 58.52; H, 8.77; N, 4.87. Found: C, 58.24; H, 9.07; N, 4.81.

4-Pentenyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4,6-O-isopropylidene-β-D-glucopyranoside (**16**).—To a solution of **15** (2.47 g, 8.60 mmol) in CH_2Cl_2 (25 mL), were added Et_3N (1.55 mL, 11.2 mmol) and a solution of 2,2-difluorotetradecanoyl chloride (2.68 g, 9.46 mmol) in CH_2Cl_2 (25 mL) at 5 °C with stirring. After stirring for 1 h at 24 °C, the mixture was concentrated and diluted with EtOAc. The solution was washed successively with aq M HCl, saturated $NaHCO_3$ and brine; then it was dried over $MgSO_4$, filtered, concentrated, and chromatographed on a silica gel column. Elution with 2:1 hexane–EtOAc gave **16** (3.97 g, 87%). ^1H NMR ($CDCl_3$): δ 0.88 (t, 3 H, *J* 6.6 Hz), 1.14–1.55 (m, 26 H, containing, s, 3 H at δ 1.44 and s, 3 H at δ 1.52), 1.60–1.76 (m, 2 H), 1.98–2.19 (m, 4 H), 3.20 (d, 1 H, *J* 2.6 Hz, OH), 3.31 (dt, *J* 5.3, 9.9 Hz), 3.40–3.64 (m, 3 H), 3.75–4.10 (m, 4 H), 4.70 (d, 1 H, *J* 8.6 Hz, H-1), 4.93–5.07 (m, 2 H), 5.70–5.87 (m, 1 H), 6.52 (d, 1 H, *J* 6.6 Hz); IR ν_{max} (KBr): 3488,

3330, 2924, 2853, 1683, 1644 (w) cm^{-1}. Anal. Calcd for $C_{28}H_{49}F_2NO_6$ (533.7): C, 63.01; H, 9.25; N, 2.62; F, 7.12. Found: C, 62.73; H, 9.25; N, 2.63; F, 7.09.

*4-Pentenyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4,6-*O*-isopropylidene-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-*D*-glucopyranoside* (**17**).—Compound **16** (3.68 g, 6.90 mmol) was treated as described in the formation of **5** from **4** to give **17** (5.62 g, 84%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3 H, *J* 6.6 Hz), 1.17–1.52 (m, 68 H, containing, s, 3 H at δ 1.37, and s, 3 H at δ 1.48), 1.52–1.70 (m, 2 H), 1.92–2.14 (m, 4 H), 2.26 (t, 2 H, *J* 7.3–7.9 Hz), 2.49 (dd, 1 H, *J* 6.6, 15.2 Hz), 2.63 (dd, 1 H, *J* 6.6, 15.2 Hz), 3.28–3.52 (m, 2 H), 3.68–4.02 (m, 5 H), 4.61 (d, 1 H, *J* 8.6 Hz, H-1), 4.91–5.05 (m, 2 H), 5.11–5.27 (m, 2 H), 5.68–5.87 (m, 1 H), 6.52 (d, 1 H, *J* 9.2 Hz, NH); IR ν_{max} (KBr): 3448, 2955, 2921, 2873, 2851, 1735, 1684, 1643 (w) cm^{-1}. Anal. Calcd for $C_{56}H_{101}F_2NO_9$ (970.4): C, 69.31; H, 10.49; N, 1.44; F, 3.92. Found: C, 69.55; H, 10.21; N, 1.50; F, 3.97.

*4-Pentenyl 2-deoxy-2-(2,2-difluorotetradecanamido)-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-*D*-glucopyranoside* (**18**).—Compound **17** (5.20 g, 5.36 mmol) was treated as described in the formation of **6** from **5** to give **18** (4.88 g, 98%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 6.6 Hz), 1.12–1.43 (m, 62 H), 1.52–1.71 (m, 2 H), 1.92–2.19 (m, 5 H, containing OH), 2.30 (t, 2 H, *J* 7.3 Hz), 2.43–2.60 (m, 2 H), 3.40–3.55 (m, 2 H), 3.60 (m, 2 H, containing OH), 3.77–4.04 (m, 4 H), 4.55 (d, 1 H, *J* 7.9 Hz, H-1), 4.92–5.16 (m, 4 H), 5.68–5.86 (m, 1 H), 6.60 (d, 1 H, *J* 8.6 Hz, NH); IR ν_{max} (KBr): 3505, 3290, 2956, 2921, 2815, 1732, 1684, 1643 (w) cm^{-1}. Anal. Calcd for $C_{53}H_{97}F_2NO_9$ (930.3): C, 68.42; H, 10.51; N, 1.51; F, 4.08. Found: C, 68.19; H, 10.59; N, 1.54; F, 4.00.

*4-Pentenyl 6-*O*-benzyloxycarbonyl-2-deoxy-2-(2,2-difluorotetradecanamido)-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-*D*-glucopyranoside* (**19**).—Compound **18** (4.80 g, 5.16 mmol) was treated as described in the formation of **7** from **6** to give the recovered **18** (0.83 g, 17%) and **19** (3.71 g, 68%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 6.6–7.3 Hz), 1.12–1.70 (m, 64 H), 1.92–2.14 (m, 4 H), 2.29 (t, 2 H, *J* 7.3–7.9 Hz), 2.43–2.59 (m, 2 H), 3.38–3.48 (m, 1 H), 3.54–3.67 (m, 3 H, containing OH), 3.79–4.02 (m, 2 H), 4.36–4.45 (m, 1 H), 4.48–4.58 (m, 2 H), 4.90–5.12 (m, 4 H), 5.18 (s, 2 H), 5.67–5.83 (m, 1 H), 6.62 (d, 1 H, 9.2 Hz), 7.33–7.43 (m, 5 H); IR ν_{max} (KBr): 3479, 3328, 2923, 2852, 1728, 1691, 1643 (w) cm^{-1}. Anal. Calcd for $C_{61}H_{103}F_2NO_{11}$ (1064.5): C, 68.83; H, 9.75; N, 1.32; F, 3.57. Found: C, 68.60; H, 9.73; N, 1.39; F, 3.48.

*4-Pentenyl 6-*O*-benzyloxycarbonyl-2-deoxy-2-(2,2-difluorotetradecanamido)-4-*O*-diphenylphosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-*D*-glucopyranoside* (**20**).—Compound **19** (3.48 g, 3.27 mmol) was treated as described in the formation of **8** from **7** to give **20** (4.06 g, 96%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 6.6 Hz), 1.10–1.69 (m, 64 H), 1.90–2.13 (m, 4 H), 2.17 (t, 2 H, *J* 7.3–7.9 Hz), 2.26–2.47 (m, 2 H), 3.38–3.50 (m, 1 H), 3.56–3.70 (m, 1 H), 3.73–3.89 (m, 2 H), 4.17–4.28 (m, 1 H), 4.32–4.42 (m, 1 H), 4.70 (q, 1 H, *J* 9.9 Hz), 4.91–5.20 (m, 6 H), 5.60 (dd, 1 H, *J* 9.2, 10.6 Hz), 5.68–5.85 (m, 1 H), 6.77 (d, 1 H, 7.9 Hz, NH), 7.10–7.40 (m, 15 H); IR ν_{max} (KBr): 3462, 2955, 2920, 2851, 1746, 1728, 1687, 1644 (w), 1590 cm^{-1}. Anal. Calcd for $C_{73}H_{112}F_2NO_{14}P$ (1296.7): C, 67.62; H, 8.71; N, 1.08; F, 2.93; P, 2.39. Found: C, 67.91; H, 8.58; N, 1.08; F, 2.83; P, 2.25.

*3-Carboxypropyl 6-*O*-benzyloxycarbonyl-2-deoxy-2-(2,2-difluorotetradecanamido)-4-*

O-diphenylphosphono-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranoside (**21**).—Compound **20** (5.20 g, 5.36 mmol) was treated as described in the formation of **9** from **8** to give **21** (4.88 g, 98%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 6.6 Hz), 1.05–1.61 (m, 62 H), 1.78–2.16 (m, 4 H), 2.17 (t, 2 H, *J* 7.3–7.9 Hz), 2.28–2.46 (m, 4 H), 3.45–3.58 (m, 1 H), 3.62–3.89 (m, 3 H), 4.15–4.26 (m, 1 H), 4.38 (dd, 1 H, *J* 2.0, 9.9 Hz), 4.71 (q, 1 H, *J* 9.2–9.9 Hz), 4.95 (d, 1 H, *J* 7.9 Hz, H-1), 5.03, 5.11 (AB-q, 2 H, *J* 12.2 Hz), 5.13 (m, 1 H), 5.58 (dd, 1 H, *J* 9.2, 9.9 Hz), 6.94 (d, 1 H, 7.9 Hz, NH), 7.09–7.43 (m, 15 H); IR ν_{max} ($CHCl_3$): 3349, 3400–2500 (containing 2957, 2923, 2853), 1753, 1728, 1694, 1591 cm^{-1}. Anal. Calcd for $C_{72}H_{110}F_2NO_{16}P$ (1314.6): C, 65.78; H, 8.43; N, 1.07; F, 2.89; P, 2.36. Found: C, 65.56; H, 8.38; N, 1.22; F, 2.82; P, 2.28.

3-Carboxypropyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-diphenylphosphono-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**22**).—Compound **21** (630 mg, 0.479 mmol) was treated as described in the formation of **10** from **9** to give **22** (444 mg, 79%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 5.9–7.3 Hz), 1.05–1.60 (m, 62 H), 1.81–2.15 (m, 4 H), 2.17 (t, 2 H, *J* 7.3 Hz), 2.30–2.51 (m, 4 H), 3.49–3.67 (m, 3 H), 3.68–3.82 (m, 2 H), 3.83–3.95 (m, 1 H), 4.75 (q, 1 H, *J* 9.2 Hz), 4.92 (d, 1 H, *J* 8.6 Hz, H-1), 5.14 (m, 1 H), 5.61 (dd, 1 H, *J* 9.2, 9.9 Hz), 6.99 (d, 1 H, 7.9 Hz, NH), 7.12–7.42 (m, 10 H); IR ν_{max} ($CHCl_3$): 3400, 2930, 2850, 1715, 1630 cm^{-1}. Anal. Calcd for $C_{64}H_{104}F_2NO_{14}P$ (1180.5): C, 65.12; H, 8.88; N, 1.19; F, 3.22; P, 2.62. Found: C, 64.89; H, 9.02; N, 1.20; F, 3.12; P, 2.61.

3-Carboxypropyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-phosphono-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**23**).—Compound **22** (150 mg, 0.127 mmol) was treated as described in the formation of **11** from **10** to give **23** (116 mg, 89%). ^{1}H NMR (pyridine-d_7): δ 0.82–0.98 (m, 9 H), 1.18–2.03 (m, 62 H), 2.08–2.23 (m, 2 H), 2.25–2.55 (m, 4 H), 2.63–2.77 (m, 2 H), 3.01 (dd, 1 H, *J* 6.4, 16.1 Hz), 3.32 (dd, 1 H, *J* 5.9–6.4, 16.1–16.6 Hz), 3.67 (d, 1 H, *J* 9.8 Hz), 3.78 (td, 1 H, *J* 6.3, 9.8 Hz), 4.07–4.21 (m, 2 H), 4.49 (dd, 1 H, *J* 2.4, 10.3 Hz), 4.66 (dd, 1 H, *J* 8.7, 9.0 Hz), 5.08–5.27 (m, 2 H, containing d, 1 H, *J* 8.3 Hz, at δ 5.13), 5.73 (m, 1 H), 6.16 (t, 1 H, *J* 9.8–10.3 Hz), 7.34 (broad, 4 H, D_2O exchanged), 10.21 (d, 1 H, 9.3 Hz); IR ν_{max} (KBr): 3271, 2957, 2920, 2851, 1743, 1704, 1561, 1468 cm^{-1}. FABMS (negative): *m/z* 1026 $(M-1)^-$, 798, 590, 572. Anal. Calcd for $C_{52}H_{96}F_2NO_{14}P \cdot H_2O$ (1046.3): C, 59.70; H, 9.44; N, 1.34; F, 3.63; P, 2.96. Found: C, 59.85; H, 9.31; N, 1.35; F, 3.61; P, 2.77.

Allyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4,6-O-isopropylidene-α-D-glucopyranoside (**25**).—Allyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (**24**) was treated as described in the formation of **16** from **15** to give **25** (88%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, *J* 6.6 Hz, 3 H), 1.13–1.57 (m, 62 H, containing s, 3 H, at δ 1.44 and s, 3 H, at δ 1.52), 1.94–2.20 (m, 2 H), 2.49 (d, 1 H, *J* 3.3 Hz, OH), 3.56–4.28 (m, 8 H), 4.87 (d, 1 H, *J* 4.0 Hz, H-1), 5.12–5.37 (m, 2 H), 5.78–5.98 (m, 1 H), 6.61 (d, 1 H, *J* 9.2 Hz, NH). Anal. Calcd for $C_{26}H_{45}F_2NO_6$ (505.6): C, 61.74; H, 8.97; N, 2.77; F, 7.52. Found: C, 61.87; H, 8.75; N, 2.73; F, 7.62.

Allyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4,6-O-isopropylidene-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**26**).—Compound **25** was treated as described in the formation of **17** from **16** to give **26** (64%). ^{1}H NMR ($CDCl_3$): δ

0.88 (t, *J* 6.6 Hz, 9 H), 1.25 (m, 62 H), 1.38 (s, 3 H), 1.49 (s, 3 H), 1.55–1.65 (m, 4 H), 1.90–2.12 (m, 2 H), 2.26 (t, 2 H, *J* 7.3–7.9 Hz), 2.48 (dd, 1 H, *J* 6.6, 15.8 Hz), 2.64 (dd, 1 H, *J* 6.6, 15.2 Hz), 3.67–4.29 (m, 7 H), 4.87 (d, 1 H, *J* 3.3 Hz, H-1), 5.09–5.33 (m, 4 H), 5.75–5.94 (m, 1 H), 6.69 (d, 1 H, *J* 9.2 Hz, NH). Anal. Calcd for $C_{54}H_{97}F_2NO_9$ (942.4): C, 68.83; H, 10.38; N, 1.49; F, 4.03. Found: C, 68.97; H, 10.22; N, 1.51; F, 3.96.

(Diphenylmethyloxycarbonyl)methyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4,6-O-isopropylidene-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**27**). —To a solution of **26** (500 mg, 0.530 mmol) in 1:1:1.5 MeCN–CCl_4–H_2O (35 mL), were added $NaIO_4$ (4.0 g) and RuO_2 (15 mg). After 3 h of stirring at 24 °C, the reaction mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to give a residue that was dissolved in THF (3 mL). To this solution was added Ph_2CN_2 (150 mg, 0.800 mmol). The mixture was stirred for 16 h at 23–26 °C, quenched with AcOH, concentrated, and chromatographed on a silica gel column. Elution with 9:1 then 5:1 hexane–EtOAc gave **27** (409 mg, 68%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 5.9–7.0 Hz), 1.18–1.65 (m, 68 H, containing, s, 3 H at δ 1.48, and s, 3 H at δ 1.57), 1.89–2.13 (m, 2 H), 2.25 (t, 2 H, *J* 7.3 Hz), 2.47 (dd, 1 H, *J* 7.3, 15.2 Hz), 2.63 (dd, 1 H, *J* 7.3, 15.2 Hz), 3.67–3.89 (m, 4 H), 4.20–4.36 (m, 3 H, containing, s, 2 H at δ 4.29), 4.90 (d, 1 H, *J* 3.3 Hz), 5.10–5.29 (m, 2 H), 6.89–6.99 (m, 2 H), 7.26–7.40 (m, 10 H); IR ν_{max} ($CHCl_3$): 2925, 2860, 1745, 1710 cm^{-1}. Anal. Calcd for $C_{66}H_{105}F_2NO_{11}$ (1126.6): C, 70.37; H, 9.39; N, 1.24; F, 3.37. Found: C, 70.31; H, 9.57; N, 1.41; F, 3.43.

(Diphenylmethyloxycarbonyl)methyl 2-deoxy-2-(2,2-difluorotetradecanamido)-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**28**).—A solution of **27** (690 mg, 0.612 mmol) in 9:1 AcOH–H_2O (10 mL) was warmed to 60–65 °C for 3 h and concentrated to give a residue that was chromatographed on a silica gel column. Elution with 2:1 hexane–EtOAc gave **28** (363 mg, 55%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 5.9–7.3 Hz), 1.12–1.67 (m, 62 H), 1.87–2.13 (m, 3 H, containing OH), 2.25–2.33 (m, 2 H), 2.41–2.58 (m, 2 H), 3.51 (d, 1 H, *J* 3.3 Hz, OH), 3.66–3.90 (m, 4 H), 4.16–4.28 (m, 1 H), 4.32 (s, 2 H), 4.94 (d, 1 H, *J* 4.0 Hz), 5.02–5.23 (m, 2 H), 6.96 (s, 1 H), 7.04 (d, 1 H, *J* 8.6 Hz), 7.26–7.42 (m, 10 H); IR ν_{max} ($CHCl_3$): 3430, 2920, 2850, 1742, 1710 cm^{-1}. Anal. Calcd for $C_{63}H_{101}F_2NO_{11}$ (1086.5): C, 69.65; H, 9.37; N, 1.29; F, 3.50. Found: C, 69.49; H, 9.45; N, 1.29; F, 3.49.

(Diphenylmethyloxycarbonyl)methyl 6-O-benzyloxycarbonyl-2-deoxy-2-(2,2-difluorotetradecanamido)-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**29**).—Compound **28** (418 mg, 0.385 mmol) was treated as described in the formation of **7** from **6** to give **29** (422 mg, 90%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 5.9–6.6 Hz), 1.16–1.67 (m, 62 H), 1.91–2.12 (m, 2 H), 2.24–2.33 (m, 2 H), 2.41–2.57 (m, 2 H), 3.50 (d, 1 H, *J* 4.0 Hz, OH), 3.58–3.68 (m, 1 H), 3.92–4.02 (m, 1 H), 4.16–4.47 (m, 5 H), 4.90 (d, 1 H, *J* 4.0 Hz), 5.01–5.25 (m, 4 H), 6.94 (s, 1 H), 7.01 (d, 1 H, *J* 9.2 Hz), 7.24–7.42 (m, 15 H); IR ν_{max} ($CHCl_3$): 3440, 2925, 2855, 1745, 1710 cm^{-1}. Anal. Calcd for $C_{71}H_{107}F_2NO_{13}$ (1220.6): C, 69.86; H, 8.84; N, 1.15; F, 3.11. Found: C, 69.69; H, 8.55; N, 1.40; F, 2.90.

(Diphenylmethyloxycarbonyl)methyl 6-O-benzyloxycarbonyl-2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-diphenylphosphono-3-O-[(R)-3-(tetradecanoyloxy)tetradecano-

yl]-α-D-glucopyranoside (**30**).—Compound **29** (177 mg, 0.145 mmol) was treated as described in the formation of **8** from **7**, except that it was eluted with 3:1 hexane–EtOAc on silica gel chromatography to give **30** (204 mg, 97%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 6.6 Hz), 1.16–1.60 (m, 62 H), 1.90–2.16 (m, 4 H), 2.39–2.52 (m, 2 H), 4.08–4.35 (m, 6 H), 4.76 (q, 1 H, *J* 9.2 Hz), 4.93 (d, 1 H, *J* 4.0 Hz), 4.99 (m, 3 H, containing d, 1 H, *J* 11.9 Hz, at δ 5.01, and d, 1 H, *J* 11.9 Hz, at δ 5.09), 5.47 (dd, 1 H, J 9.2, 10.6 Hz), 6.89–6.99 (m, 2 H), 7.10–7.43 (m, 25 H); IR ν_{max} ($CHCl_3$): 2920, 2850, 1750, 1710, 1590 cm^{-1}. Anal. Calcd for $C_{83}H_{116}F_2NO_{13}P$ (1452.8): C, 68.60; H, 8.05; N, 0.96; F, 2.62; P, 2.13. Found: C, 68.57; H, 8.07; N, 1.00; F, 2.59.

Carboxymethyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-diphenylphosphono-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**31**).—Compound **30** (145 mg, 0.100 mmol) was treated as described in the formation of **10** from **9**. After the reaction, the crude product was eluted with 9:1 EtOAc–MeOH on silica gel chromatography, the **31** so obtained was dissolved in EtOAc, and the solution was washed with H_2O to remove contaminating silica gel from the chromatography. Concentration of the solution in vacuo gave **31** (100 mg, 87%). ^{1}H NMR ($CDCl_3$ + D_2O): δ 0.88 (t, 9 H, *J* 5.9–7.2 Hz), 0.98–1.56 (m, 62 H), 1.88–2.17 (m, 4 H), 2.27–2.54 (m, 2 H), 3.50–3.74 (m, 2 H), 3.94–4.21 (m, 3 H), 4.24–4.38 (m, 1 H), 4.57–4.79 (m, 1 H), 4.95 (d, 1 H, *J* 3.3 Hz), 5.06–5.19 (m, 1 H), 5.57 (t, 1 H, *J* 9.9 Hz), 7.12–7.38 (m, 10 H); IR ν_{max} ($CHCl_3$): 3420, 2920, 2845, 1725, 1705, 1590 cm^{-1}. Anal. Calcd for $C_{62}H_{100}F_2NO_{14}P$ (1152.4): C, 64.62; H, 8.75; N, 1.22; F, 3.30; P, 2.69. Found: C, 64.58; H, 8.83; N, 1.23; F, 3.35; P, 2.66.

Carboxymethyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-phosphono-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**32**).—Compound **31** (58 mg, 0.050 mmol) was treated as described in the formation of **11** from **10** to give **32** (49 mg, 97%). ^{1}H NMR ($CDCl_3$ + D_2O): δ 0.78–1.05 (m, 9 H), 1.07–2.03 (m, 62 H), 2.31–2.52 (m, 4 H), 3.02–3.16 (m, 1 H), 3.28–3.39 (m, 1 H), 4.01–4.22 (m, 2 H), 4.35–4.63 (m, 3 H), 4.86–4.96 (m, 1 H), 5.01–5.21 (m, 1 H), 5.48 (d, 1 H, *J* 2.9 Hz), 5.65–5.79 (m, 1 H), 6.17 (d, 1 H, *J* 9.3 Hz); IR ν_{max} (KBr): 3333, 2957, 2924, 2854, 1738, 1709 cm^{-1}; FABMS (negative): *m/z* 998 $(M-H)^-$, 770, 562, 544, 227; (positive): 1000 $(M+H)^+$, 1022 $(M+Na)^+$. High-resolution FABMS (positive); Calcd. for $C_{50}H_{92}F_2NNaO_{14}PNa$: 1022.6121. Found: 1022.6166. Anal. Calcd for $C_{50}H_{92}F_2NO_{14}P$ (1000.3): C, 60.04; H, 9.27; N, 1.40; F, 3.80; P, 3.10. Found: C, 59.87; H, 9.39; N, 1.31; F, 3.64; P, 3.01.

(Diphenylmethyloxycarbonyl)methyl 6-O-benzyloxycarbonyl-2-deoxy-2-(2,2-difluorotetradecanamido)-4-O-diphenylphosphono-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranoside (**34**).—To a solution of allyl 6-*O*-benzyloxycarbonyl-2-deoxy-2-(2,2-difluorotetradecanamido)-4-*O*-diphenylphosphono-3-*O*-[(3*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranoside [14] (**33**, 1.92 g, 1.51 mmol) in 1:1:1.5 MeCN–CCl_4–H_2O (35 mL), were added $NaIO_4$ (10 g) and RuO_2 (20 mg). After 2 h of stirring at 24 °C, the reaction mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to give a residue that was dissolved in THF (10 mL). To this solution was added Ph_2CN_2 (590 mg, 3.02 mmol). The mixture was stirred for 16 h at 23–26 °C; then it was quenched with AcOH, concentrated, and chromatographed on a silica gel column. Elution with 5:1 hexane–EtOAc gave **34** (1.20

g, 55%). ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 9 H, J 5.9–7.3 Hz), 1.11–1.65 (m, 62 H), 1.87–2.07 (m, 2 H), 2.14 (t, 2 H, J 7.3–7.9 Hz), 2.33–2.50 (m, 2 H), 3.65–3.78 (m, 1 H), 3.90 (q, 1 H, J 9.9 Hz), 4.18 (dd, 1 H, J 4.6–5.3, 11.9–12.5 Hz), 4.26–4.33 (m, 3 H, containing, s, 2 H at δ 4.34), 4.71 (q, 1 H, J 9.2–9.9 Hz), 4.90 (d, 1 H, J 8.6 Hz, H-1), 5.00–5.18 (m, 3 H, containing, d, 1 H, J 11.9 Hz, at δ 5.02, and d, 1 H, J 11.9 Hz, at δ 5.09), 5.43 (dd, 1 H, J 9.2, 10.6 Hz), 7.10–7.21 (m, 5 H), 7.22–7.46 (m, 20 H); IR ν_{max} (KBr): 2921, 2851, 1760, 1744, 1727, 1699 cm^{-1}. Anal. Calcd for $C_{83}H_{116}F_2NO_{16}P$ (1452.8): C, 68.62; H, 8.05; N, 0.96; F, 2.62; P, 2.13. Found: C, 68.66; H, 8.13; N, 1.00; F, 2.57; P, 2.10.

*Carboxymethyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4-*O*-diphenylphosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-*D*-glucopyranoside* (**35**).—Compound **34** (530 mg, 0.365 mmol) was treated as described in the formation of **31** from **30** to give **35** (340 mg, 81%). ^{1}H NMR (Me_2SO-d_6 + D_2O): δ 0.88 (t, 9 H, J 6.6 Hz), 0.95–1.55 (m, 62 H), 1.86–2.11 (m, 4 H), 2.30–2.41 (m, 2 H), 2.33–2.50 (m, 2 H), 3.35–3.50 (m, 1 H), 3.57–3.68 (m, 2 H), 3.70–3.77 (m, 1 H), 3.83–3.95 (m, 2 H), 4.64 (q, 1 H, J 8.8 Hz), 4.90 (d, 1 H, J 8.1 Hz), 4.92–5.05 (m, 1 H), 5.32 (t, 1 H, J 9.5 Hz), 6.75 (d, 1 H, J 8.8 Hz), 7.12–7.28 (m, 6 H), 7.35–7.43 (m, 4 H); IR ν_{max} ($CHCl_3$): 3330, 2920, 2845, 1745, 1725, 1700, 1590 cm^{-1}. Anal. Calcd for $C_{62}H_{100}F_2NO_{14}P$ (1152.4): C, 64.62; H, 8.75; N, 1.22; F, 3.30; P, 2.69. Found: C, 64.50; H, 8.51; N, 1.13; F, 2.98; P, 2.48.

*Carboxymethyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4-*O*-phosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-*D*-glucopyranoside* (**36**).—Compound **35** (50 mg) was treated as described in the formation of **11** from **10** to give **36** (40 mg, 93%). ^{1}H NMR (pyridine-d_5): δ 0.86–0.90 (m, 9 H), 1.14–1.54 (m, 56 H), 1.65–1.75 (m, 4 H), 1.82–1.98 (m, 2 H), 2.36–2.44 (m, 4 H), 3.06 (dd, 1 H, J 7.0, 16.4 Hz), 3.33 (dd, 1 H, J 6.0, 16.4 Hz), 3.70 (d, 1 H, J 9.7 Hz), 4.16 (d, 1 H, J 12.5 Hz), 4.46 (d, 1 H, J 12.5 Hz), 4.64, 4.78 (AB-q, 2 H, J 16.7 Hz), 4.85 (dd, 1 H, J 9.1, 19.1 Hz), 5.23 (dd, 1 H, J 9.7, 19.9 Hz), 5.49 (d, 1 H, J 8.3 Hz), 5.75 (m, 1 H), 6.24 (t, 1 H, J 9.9 Hz), 6.44–7.03 (broad, 3 H, D_2O exchanged), 10.24 (d, 1 H, J 9.0 Hz, NH); IR ν_{max} (KBr): 3293, 2957, 2920, 2851, 1733, 1690 cm^{-1}; FABMS (positive): m/z 1000 $(M + H)^+$, 924, 919. Anal. Calcd for $C_{50}H_{92}F_2NO_{14}P$ (1000.3): C, 60.04; H, 9.27; N, 1.40; F, 3.80; P, 3.10. Found: C, 59.91; H, 9.04; N, 1.65; F, 3.71.

*Allyl 2-deoxy-4,6-*O*-isopropylidene-2-tetradecanamido-α-*D*-glucopyranoside* (**37**).—Allyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (**24**, 4.68 g) was treated as described in the formation of **16** from **15** to give **25** (7.73 g, 91%): R_f = 0.16 (1:1 cyclohexane–EtOAc). $[\alpha]_D^{25}$ +52.6° (c 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3 H, J 6.6 Hz) 1.24–1.74 (m, 22 H), 1.43 (s, 3 H), 1.52 (s, 3 H), 2.23 (t, 2 H, J 7.3 Hz), 3.02 (d, 1 H, J 3.3 Hz, OH), 3.60–4.83 (m, 8 H), 4.82 (d, 1 H, J 4.0 Hz), 5.20–5.32 (m, 2 H), 5.82–5.95 (m, 1 H); IR ν_{max} (KBr): 3476, 3311, 2918, 1648, 1542 cm^{-1}. Anal. Calcd for $C_{26}H_{47}NO_6$ (469.7): C, 66.49; H, 10.09; N, 2.98. Found: C, 66.09; H, 10.37; N, 2.94.

*Allyl 2-deoxy-4,6-*O*-isopropylidene-2-tetradecanamido-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-*D*-glucopyranoside* (**38**).—Compound **37** was treated as described in the formation of **17** from **16** to give **38** (76%): R_f = 0.25 (5:1 cyclohexane–EtOAc); $[\alpha]_D^{25}$ +15.3° (c 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 9 H, J 6.4–6.9

Hz) 1.25 (bs, 60 H), 1.38 (s, 3 H), 1.48 (s, 3 H), 1.56–1.64 (m, 4 H), 2.12 (t, 2 H, *J* 7.4–7.8 Hz), 2.27 (t, 2 H, *J* 7.4–7.7 Hz), 2.48 (dd, 1 H, *J* 6.0, 15.4 Hz), 2.63 (dd, 1 H, *J* 6.7, 15.4 Hz) 3.65–3.98 (m, 5 H), 4.13–4.30 (m, 2 H), 4.84 (d, 1 H, *J* 3.7 Hz, C1–H), 5.11–5.31 (m, 4 H), 5.81 (d, 1 H, *J* 9.7 Hz, NH), 5.84 (m, 1 H); IR ν_{max} (KBr): 3298, 2919, 2851, 1735, 1650 cm^{-1}; FABMS (positive): *m/z* 928 $(M + Na)^+$, 906 $(M + H)^+$. Anal. Calcd for $C_{54}H_{99}NO_9$ (906.2): C, 71.56; H, 11.01; N, 1.55. Found: C, 71.56; H, 11.81; N, 1.64.

*Allyl 2-deoxy-2-tetradecanamido-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-*D*-glucopyranoside* (**39**).—Compound **38** was treated as described in the formation of **18** from **17** to give **39** (80%): $R_f = 0.13$ (2:1 cyclohexane–EtOAc); $[\alpha]_D^{25}$ +65.0° (*c* 0.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 6.4–6.9 Hz) 1.26 (bs, 60 H), 1.56–1.64 (m, 4 H), 2.05 (t, 1 H, *J* 5.9–6.6 Hz, OH), 2.14 (dt, 2 H, *J* 2.0, 7.8 Hz), 2.30 (t, 2 H, *J* 7.5 Hz), 2.45–2.60 (m, 2 H), 3.33 (d, 1 H, *J* 3.2 Hz, OH), 3.63–4.01 (m, 5 H), 4.15–4.25 (m, 2 H), 4.89 (d, 1 H, *J* 3.6 Hz, H-1). 5.06–5.33 (m, 4 H), 5.86 (m, 1 H), 5.90 (d, 1 H, *J* 9.1 Hz, NH); IR ν_{max} (KBr): 3470, 3294, 2920, 1736, 1721, 1701, 1652 cm^{-1}; FABMS (positive): *m/z* 888 $(M + Na)^+$, 866 $(M + H)^+$. Anal. Calcd for $C_{51}H_{95}NO_9 \cdot 0.5H_2O$ (866.3 + 9.0): C, 69.98; H, 11.05; N, 1.60. Found: C, 70.27; H, 11.15; N, 1.69.

*Allyl 6-*O*-benzyloxycarbonyl-2-deoxy-2-tetradecanamido-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-*D*-glucopyranoside* (**40**).—Compound **39** was treated as described in the formation of **19** from **18** to give **40** (65%): $R_f = 0.55$ (2:1 cyclohexane–EtOAc); $[\alpha]_D^{25}$ −16.4° (*c* 0.5, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 6.2–7.1 Hz), 1.25 (bs, 60 H), 1.53–1.60 (m, 4 H), 2.01–2.15 (m, 2 H), 2.29 (t, 2 H, *J* 7.5 Hz), 2.45–2.59 (m, 2 H), 3.33 (d, 1 H, *J* 4.0 Hz, OH), 3.61 (dt, 1 H, *J* 3.9, 9.5 Hz), 3.83–3.98 (m, 2 H), 4.12–4.27 (m, 2 H), 4.39–4.49 (m, 2 H), 4.86 (d, 1 H, *J* 3.5 Hz, H-1). 5.04–5.30 (m, 6 H, containing s, 2 H at δ 5.18), 5.83 (m, 1 H), 5.87 (d, 1 H, *J* 8.6 Hz, NH), 7.33–7.42 (m, 5 H); IR ν_{max} (KBr): 3485, 3314, 2920, 1733, 1724, 1654 cm^{-1}; FABMS (positive): *m/z* 1022 $(M + Na)^+$, 1000 $(M + H)^+$. Anal. Calcd for $C_{59}H_{101}NO_{11} \cdot 1.5H_2O$ (1000.3 + 27.0): C, 68.97; H, 10.20; N, 1.36. Found: C, 68.93; H, 9.67; N, 1.62.

*Allyl 6-*O*-benzyloxycarbonyl-2-deoxy-4-*O*-diphenylphosphono-2-tetradecanamido-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-*D*-glucopyranoside* (**41**).—Compound **40** was treated as described in the formation of **20** from **19** to give **41** (85%): $R_f = 0.35$ (4:1 cyclohexane–EtOAc); $[\alpha]_D^{25}$ +38.4° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 6.6 Hz) 1.25 (bs, 60 H), 1.40–1.60 (m, 4 H), 2.10–2.17 (m, 4 H), 2.41 (d, 2 H, *J* 6.6 Hz), 3.91–4.04 (m, 2 H), 4.11–4.35 (m, 4 H), 4.72 (dd, 1 H, *J* 6.6, 19.1 Hz), 4.92 (d, 1 H, *J* 3.3 Hz, H-1). 5.01–5.43 (m, 6 H), 5.84 (m, 1 H), 5.85 (d, 1 H, *J* 8.6 Hz, NH), 7.14–7.35 (m, 15 H); IR ν_{max} (KBr): 3352, 2920, 1734, 1655, 1528 cm^{-1}; FABMS (positive): *m/z* 1254 $(M + Na)^+$, 1232 $(M + H)^+$.

*(Diphenylmethyloxycarbonyl)methyl 6-*O*-benzyloxycarbonyl-2-deoxy-4-*O*-diphenylphosphono-2-tetradecanamido-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-α-*D*-glucopyranoside* (**42**).—Compound **41** was treated as described in the formation of **21** from **20** to give **42** (58%): $R_f = 0.48$ (4:1 cyclohexane–EtOAc); $[\alpha]_D^{25}$ +37.0° (*c* 0.6, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 6.4–6.8 Hz), 1.25 (bs, 60 H), 1.37–1.63 (m, 4 H), 2.04–2.16 (m, 4 H), 2.42 (d, 2 H, *J* 6.3 Hz), 4.12–4.36 (m, 6 H), 4.73 (dd, 1

H, *J* 9.3, 18.8 Hz), 4.88 (d, 1 H, *J* 3.6 Hz, H-1), 4.99–5.11 (m, 6 H), 5.41 (dd, 1 H, *J* 9.3, 10.7 Hz), 6.14 (d, 1 H, *J* 8.9 Hz, NH), 6.93 (s, 1 H), 7.10–7.53 (m, 25 H); IR ν_{max} (KBr): 3426, 3350, 3281, 3068, 3035, 2922, 2852, 1739, 1652 cm^{-1}; FABMS (positive): *m/z* 1438 $(M + Na)^+$, 1416 $(M + H)^+$. Anal. Calcd for $C_{83}H_{118}NO_{16}P$ (1416.8): C, 70.36; H, 8.39; N, 0.99; P, 2.19. Found: C, 70.21; H, 8.24; N, 0.97; P, 2.10.

Carboxymethyl 2-deoxy-4-O-diphenylphosphono-2-tetradecanamido-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**43**).—Compound **42** was treated as described in the formation of **22** from **21** to give **43** (43%): $R_f = 0.40$ (9:1 CH_2Cl_2–MeOH); $[\alpha]_D^{25}$ +25.8° (*c* 0.55, $CHCl_3$); IR ν_{max} ($CHCl_3$): 3431, 2928, 2856, 1744, 1673, 1592 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 6.4–6.9 Hz), 1.19–1.31 (m, 56 H), 1.40–1.42 (m, 2 H), 1.52–1.54 (m, 4 H), 1.95 (bs, 2 H), 2.13–2.23 (m, 4 H), 2.35–2.39 (m, 2 H), 3.56–3.68 (m, 2 H), 3.91 (m, 1 H), 4.17 (s, 2 H), 4.31 (m, 1 H), 4.74 (m, 1 H), 4.99 (s, 1 H), 5.12 (m, 1 H), 5.45 (t, 1 H, *J* 10.0 Hz), 6.57 (bs, 1 H, NH), 7.14–7.36 (m, 10 H). FABMS (positive): *m/z* 1116 $(M + H)^+$, 1138 $(M + Na)^+$. High-resolution FABMS $(M + Na)^+$; Calcd for $C_{62}H_{102}NO_{14}PNa$: 1138.6952. Found: 1138.6936.

Carboxymethyl 2-deoxy-4-O-phosphono-2-tetradecanamido-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**44**).—Compound **43** was treated as described in the formation of **23** from **22** to give **44** (96%): $R_f = 0.33$ (8:5:1:1 $CHCl_3$–EtOH–H_2O–AcOH); $[\alpha]_D^{25}$ +41.8° (*c* 0.33, 2:1 MeOH–$CHCl_3$); ^1H NMR (400 MHz, pyridine-d_5): δ 0.86–0.91 (m, 9 H), 1.26–1.50 (m, 58 H), 1.64–1.79 (m, 2 H), 1.80–1.98 (m, 4 H), 2.46 (t, 2 H, *J* 7.4 Hz), 2.60 (t, 2 H, *J* 7.7 Hz), 3.08 (dd, 1 H, *J* 6.6, 16.0 Hz), 3.27 (dd, 1 H, *J* 6.4, 16.0 Hz), 4.13 (d, 1 H, *J* 12.1 Hz), 4.30 (d, 1 H, *J* 9.9 Hz), 4.41, 4.51 (AB-q, 2 H, *J* 15.4 Hz), 4.52 (m, 1 H), 5.08 (m, 1 H, H-2), 5.24 (dd, 1 H, *J* 9.9, 20.1 Hz), 5.51 (d, 1 H, *J* 3.6 Hz, H-1), 5.72 (m, 1 H), 6.16 (t, 1 H, *J* 9.9–10.2 Hz), 6.31 (bs, 4 H, OH × 4), 8.70 (d, 1 H, *J* 9.3 Hz, NH); IR ν_{max} (KBr): 3295, 2957, 2920, 2851, 1740, 1656 cm^{-1}; FABMS (positive): *m/z* 964 $(M + H)^+$; (negative): *m/z* 962 $(M - H)^-$. High-resolution FABMS (positive); Calcd. for $C_{50}H_{95}NO_{14}P$: 964.6445. Found: 964.6490. Anal. Calcd for $C_{50}H_{94}NO_{14}P$ (964.3): C, 62.28; H, 9.83; N, 1.45; P, 3.21. Found: C, 62.35; H, 9.77; N, 1.40; P, 3.39.

(Diphenylmethyloxycarbonyl)methyl 6-O-benzyloxycarbonyl-2-[(R)-3-(benzyloxy)tetradecanamido]-2-deoxy-4-O-diphenylphosphono-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**46**).—Allyl 6-*O*-benzyloxycarbonyl-2-[(*R*)-3-(benzyloxy)tetradecanamido]-2-deoxy-4-*O*-diphenylphosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)-tetradecanoyl]-α-D-glucopyranoside (**45**) [12] was treated as described in the formation of **27** from **26** to give **46** (78%): $R_f = 0.62$ (7:1 benzene–EtOAc); $[\alpha]_D^{25}$ +29.3° (*c* 0.15, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.88 (t, 9 H, *J* 6.6–7.1 Hz), 1.20–1.35 (m, 56 H), 1.40–1.64 (m, 6 H), 2.14 (t, 2 H, *J* 7.1–7.9 Hz), 2.34–2.38 (m, 2 H), 2.42 (d, 2 H, *J* 6.4 Hz), 3.84 (m, 1 H), 3.89, 3.94 (AB-q, 2 H, *J* 16.5 Hz), 4.10–4.29 (m, 3 H), 4.32 (m, 1 H), 4.40, 4.49 (AB-q, 2 H, *J* 11.6 Hz), 4.71 (t, 1 H, *J* 9.1 Hz), 4.75 (d, 1 H, *J* 3.6 Hz, H-1), 4.99, 5.07 (AB-q, 2 H, *J* 12.0 Hz), 5.10 (m, 1 H), 5.40 (dd, 1 H, *J* 9.4, 10.8 Hz), 6.58 (d, 1 H, *J* 9.2 Hz, NH), 6.87 (s, 1 H), 7.10–7.38 (m, 30 H); IR ν_{max} (KBr): 2925, 2854, 1751, 1685, 1590 cm^{-1}; FABMS (positive): *m/z* 1522 $(M + H)^+$. High-resolution FABMS $(M + Na)^+$; Calcd. for $C_{90}H_{124}NO_{17}PNa$: 1544.8492; Found:

1544.8504. Anal. Calcd for $C_{90}H_{124}NO_{17}P$ (1522.9): C, 70.79; H, 8.21; N, 0.92; P, 2.04. Found: C, 70.83; H, 8.02; N, 0.99; P, 2.21.

Carboxymethyl 2-deoxy-4-O-diphenylphosphono-2-[(R)-3-(hydroxy)tetradecanamido]-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**47**).—Compound **46** (60 mg) was treated as described in the formation of **10** from **9**. Chromatography was carried out on a preparative TLC plate (silica gel). Development with 5:1 CH_2Cl_2–MeOH, and the product band was collected, eluted with 5:1 CH_2Cl_2–MeOH, and the eluent was concentrated to give a product that contained a considerable amount of silica gel as a contaminant. The product was dissolved in EtOAc, and the solution was washed with water to remove the containing silica gel, dried over $MgSO_4$, and concentrated to give **47** (70%): $R_f = 0.33$ (9:1 CH_2Cl_2–MeOH); $[\alpha]_D^{25}$ +27.7° (*c* 0.13, $CHCl_3$); ^1H NMR ($CDCl_3 + D_2O$) δ 0.85–0.89 (m, 9 H) 1.14–1.49 (m, 62 H), 2.10 (t, 2 H, *J* 7.5 Hz), 2.24–2.44 (m, 4 H), 3.55–3.58 (m, 1 H), 3.66–3.70 (m, 1 H), 3.87 (d, 1 H, *J* 9.3 Hz), 3.95–4.04 (m, 3 H), 4.29–4.31 (m, 1 H), 4.71 (q, 1 H, *J* 9.4 Hz), 4.97 (d, 1 H, *J* 2.7 Hz, H-1), 5.08–5.14 (m, 1 H), 5.46 (t, 1 H, *J* 9.9 Hz), 7.11–7.33 (m, 10 H); IR ν_{max} (KBr): 3305, 3071, 2925, 2854, 1740, 1721, 1591 cm^{-1}; FABMS (positive): *m/z* 1154 $(M + Na)^+$. High-resolution FABMS $(M + Na)^+$; Calcd for $C_{62}H_{102}NO_{15}PNa$: 1154.6884. Found: 1154.6885. Anal. Calcd for $C_{62}H_{102}NO_{15}P$ (1132.5): C, 65.76; H, 9.08; N, 1.26; P, 2.74. Found: C, 65.64; H, 9.13; N, 1.52; P, 2.70.

Carboxymethyl 2-deoxy-2-[(R)-3-(hydroxy)tetradecanamido]-4-O-phosphono-3-O-[(R)-3-(tetradecanoyloxy)tetradecanoyl]-α-D-glucopyranoside (**48**).—Compound **47** was treated as described in the formation of **11** from **10** to give **48** (90%): $R_f = 0.27$ (8:5:1:1 $CHCl_3$–EtOH–H_2O–AcOH); $[\alpha]_D^{25}$ +41.9° (*c* 0.16, 4:25 MeOH–$CHCl_3$); ^1H NMR (pyridine-d_5): δ 0.85–0.91 (m, 9 H) 1.26–1.93 (m, 62 H), 2.47 (t, 2 H, *J* 7.4 Hz), 2.84–2.92 (m, 2 H), 3.12 (dd, 1 H, *J* 6.5, 16.0 Hz), 3.26 (dd, 1 H, *J* 6.5, 16.0 Hz), 4.12 (d, 1 H, *J* 12.4 Hz), 4.32 (d, 1 H, *J* 9.8 Hz), 4.34, 4.46 (AB-q, 2 H, *J* 16.7 Hz), 4.49–4.59 (m, 2 H), 5.08 (ddd, 1 H, *J* 3.6, 9.6, 10.8 Hz, H-2), 5.24 (q, 1 H, *J* 10.1 Hz), 5.46 (d, 1 H, *J* 3.6 Hz, H-1), 5.73 (m, 1 H), 6.06 (broad, 5 H, OH), 6.18 (dd, 1 H, *J* 9.6, 10.6 Hz), 9.01 (d, 1 H, *J* 9.6 Hz); IR ν_{max} (KBr): 3294, 2957, 2920, 2852, 1736, 1648 cm^{-1}; FABMS (positive): *m/z* 1002 $(M + Na)^+$, 980 $(M + H)^+$; (negative): *m/z* 978 $(M - H)^-$. High-resolution FABMS $(M + Na)^+$; Calcd for $C_{50}H_{94}NO_{15}PNa$: 1002.6268. Found: 1002.6258. Anal. Calcd for $C_{50}H_{94}NO_{15}P$ (980.3): C, 61.26; H, 9.67; N, 1.43; P, 3.16. Found: C, 61.12; H, 9.91; N, 1.40; P, 3.07.

Bioassay.—Materials and methods. Reagents. The sources of the materials used in this study are as follows: lipopolysaccharide (LPS) from *Escherichia coli* serotype 026; B6, phorbol 12,13-dibutyrate (TPA) and prednisolone were from SIGMA, St. Louis, MO. Lipid IVa, a gift received from Daiichi Pure Chemical Co., Ltd., Tokyo Japan, is a biosynthetic precursor of *E. coli* lipid A [16]. The RPMI-1640 medium and fetal bovine serum (FBS) were from GIBCO, Grand Island, NY. Newborn bovine serum (NBBS) was from ICN Biomedicals, Inc., Costa Mesa, CA. Human TNFα ELISA kit was from ENDOGEN Inc., Boston, MA.

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

Production of TNFα by U937 cells: 5.0×10^3 U937 cells, in Corning 96-well plates containing 200 mL of growth medium, were incubated with 30 ng/mL of TPA in a humidified atmosphere of 5% CO_2 for 72 h at 37 °C. The supernatant of the culture medium was thereafter aspirated. The cell culture was incubated with 200 mL of RPMI-1640 medium containing 10% NBBS, 10 ng/mL of LPS and graded concentrations of the compound under study in a humidified atmosphere of 5% CO_2 for 6 h at 37 °C. After incubation, the amount of TNFα produced in the culture medium was determined by an ELISA kit. The amount of TNFα produced by 10^5 U937 cells in each condition (picogram order) was calculated by subtracting the amount of TNFα produced in the absence of LPS as background. We defined the amount of TNFα produced by 10^5 U937 cells stimulated with 10 ng/mL of LPS in the absence of the test compound, as the control amount. The relative amount was calculated and indicated as a percentage of the control amount.

Results. Inhibitory activity of the GLA-60 analogues on LPS-induced TNFα production in U937 cells: U937 cells produced TNFα dose-dependently upon LPS-stimulation in the presence of 30 ng/mL of TPA, and TNFα production almost peaked and saturated at a concentration of 10 ng/mL LPS. The above TNFα production in U937 cells stimulated with 10 ng/mL LPS was almost completely abrogated by anti-CD14 monoclonal antibody treatment, indicating the central role of CD14 during the LPS-induced production of TNFα in U937 cells (data not shown). The central role of CD14 was also implicated in a study involving monokine production by LPS-stimulated human monocytes [17,18].

As shown in Fig. 1, lipid IVa, a well-known potent LPS antagonist in human monocytes [15], markedly inhibited the LPS-induced production of TNFα in U937 cells, whereas lipid X, which is thought to be an LPS antagonist of relatively low potency and limited efficacy [19], did not inhibit the LPS-induced production of TNFα by U937 cells within the concentration range of 1 to 100 nM. Compounds **11**, **23**, **36**, **44**, and **48** also showed a weak or little inhibitory activity towards TNFα production. On the other hand, compound **32** dose-dependently inhibited the TNFα production within the concentration range of 1 to 100 nM, and its inhibitory activity on TNFα production was almost comparable to that of predonisolone under the present experimental conditions.

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