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Syntheses of 2,6-Anhydro-3-deoxy-5-*O*-phosphono-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonic Acid, Its Dimeric Analogue, and Related Compounds

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Abstract: Pyran carboxylic acid analogues of GLA-60 (**12a**, **12b**, and **17**) and their dimeric analogues (**21** and **24**) were synthesized in a stereocontrolled manner. Compounds **12a** and **17** showed endotoxin antagonistic activity toward human monoblastic U937 cells as an index of the inhibition of LPS-induced TNFα production. Compounds **12b** and **24** were less active than **12a** and **17**. Dimeric ester **21** was practically inactive. © 1997 Elsevier Science Ltd.

Introduction

Lipopolysaccharides (LPS)[1] cover the outer surface membrane of such Gram-negative bacteria as *Salmonella minnesota*, *Salmonella typhimurium*, and *Escherichia coli*, and are highly potent stimulators of the immune system. A variety of responses, both beneficial and harmful, can be elicited by LPS. One of these harmful responses is fatal endotoxic shock (bacterial sepsis) caused as a consequence of acute inflammatory response, which has precluded the clinical use of LPS. Most of the biological activities of LPS reside in a relatively small portion of the molecule, that is, the terminal disaccharide phospholipid subunit known as lipid A,[2] which is a hydrophobic anchor substance holding an essentially linear polysaccharide chain to the cell wall. Lipid A was chemically synthesized by both Shiba *et al.* and Achiwa *et al.*[3]

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

Recently, Qureshi's group[5] has isolated a lipid A-related compound from *Rhodobacter sphaeroides* as an inseparable mixture of three compounds, which showed potent LPS antagonist activity. Furthermore, an Eisai group has developed a related compound, E-5531,[6] as a highly potent anti-septicemia drug.

In early studies, endotoxin and its related compounds were investigated for their potential as anti-cancer medicines that function as LPS-agonists by activating macrophages. However, in recent years, endotoxin-related compounds have been studied as LPS-antagonists, which may be useful in treating inflammation, autoimmune diseases or septicemia, by deactivating LPS-induced aggressive macrophages.

During our investigation of the biological activity of compounds related to GLA-60, we found that most of them had LPS-agonistic activity, but a few of them behaved as LPS antagonists. Among them, carboxymethyl 2-deoxy-2-(2,2-difluorotetradecanamido)-4-*O*-phosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetra-decanoyl]-α-D-glucopyranoside exhibited fairly strong LPS antagonistic activity.[7] By analogy, we designed pyran carboxylic acids (**12a** and **12b**) as related LPS-antagonists. In this paper, we would like to describe the synthesis of 2,6-anhydro-3-deoxy-5-*O*-phosphono-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonic acid (**12a**),[8] some related compounds (**12b** and **17**) and its dimeric analogues (**21** and **24**).

Synthesis

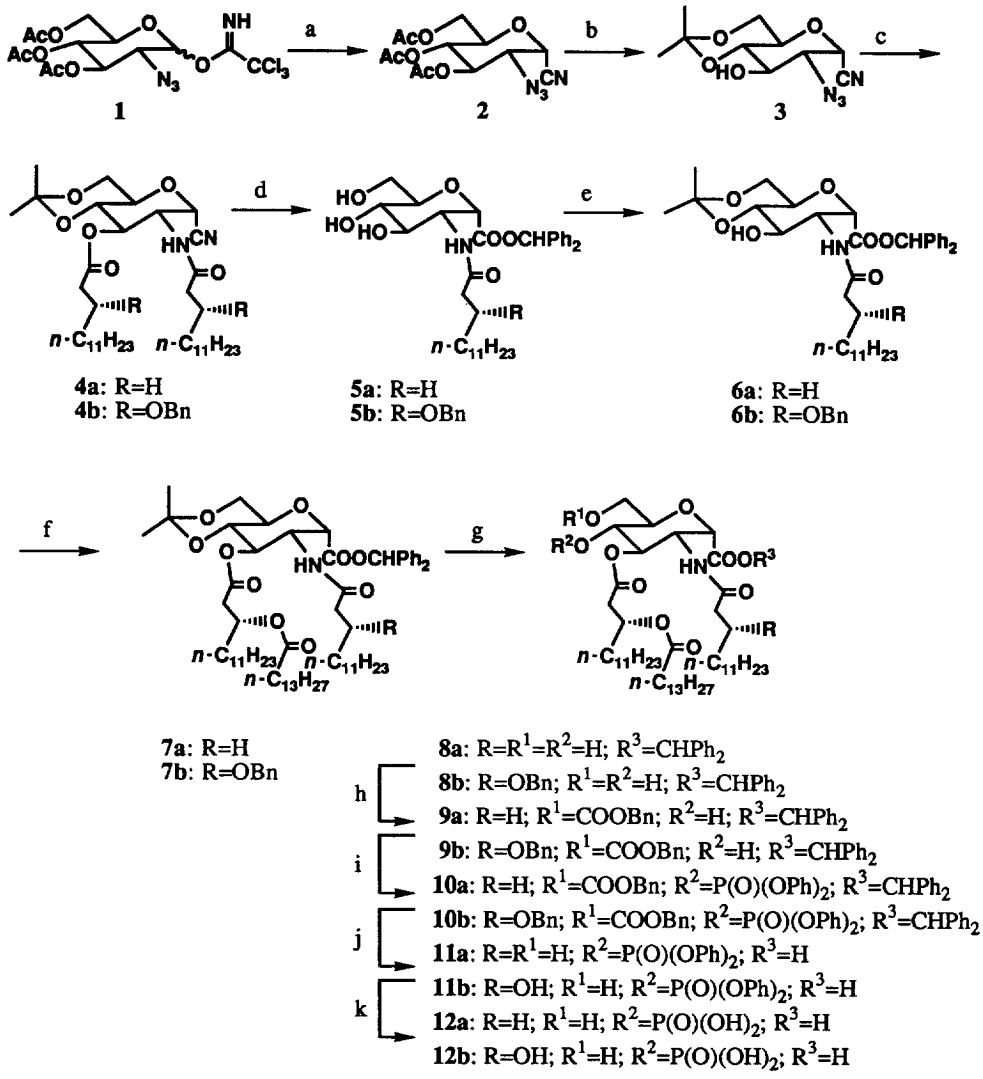
1,3,4,6-Tetra-*O*-acetyl-2-azido-2-deoxy-D-glucopyranoside, obtained from D-glucosamine hydrochloride using the method reported by Vasella,[9] was converted to 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-D-glucopyranoside according to Excoffier's procedure,[10] and then further converted to a mixture of α- and β-trichloroacetimidates (**1**).[11] The mixture was partially separated by silica gel chromatography into **1**-α (mp. 130-131 °C) and **1**-β (mp. 136-137 °C). The next stage is the most critical step in the synthesis, because an α-oriented carboxylic acid equivalent is needed. Schmidt's group[11] has already reported that treatment of 3,4,6-tri-*O*-benzyl-2-azido-2-deoxy-α-D-glucopyranosyl trichloroacetimidate with trimethylsilyl cyanide using trimethylsilyl trifluoromethane-sulfonate as a catalyst yielded a corresponding α-cyanide (anomeric J=5.4 Hz). Application of this reaction to compound **1**-α gave an α-cyanide **2** as expected. Moreover, application of this reaction to **1**-β exclusively formed **2**. Also, the mixture of **1**-α and **1**-β gave **2** stereospecifically in quantitative yield. Deacetylation of **2** with a catalytic amount of KOH in EtOH, and isopropylidene formation between C5-OH and C7-OH with 2,2-dimethoxypropane using *p*-TsOH as a catalyst formed **3** (mp. 172-173 °C). The NMR coupling constant between C2-H (anomeric position, δ 4.94) and C3-H (δ 3.95) of **2** was J=6.0 Hz, which was a little bit larger than that of the tri-benzyl analogue. However, the α-cyano configuration of **3** was confirmed from observation of the NOE between C2-H (anomeric position, δ 4.83) and C3-H (δ 3.73) of **3**. Treatment of **3** in THF with (i) PPh_3 and H_2O, (ii) tetradecanoic acid or (*R*)-3-(benzyloxy)tetradecanoic acid, DCC and DMAP, and (iii) tetradecanoyl chloride or (*R*)-3-(benzyloxy)tetradecanoyl chloride and Et_3N yielded **4a** (mp. 59-61 °C) or **4b**, respectively.

An alternative stepwise treatment of **3** with (i) PPh_3 and NH_4OH-H_2O, (ii) 1 equivalent each of tetradecanoic acid or (*R*)-3-(benzyloxy)tetradecanoic acid and DCC gave **13a** or **13b**, respectively.

Hydrolysis of both nitriles **4a** and **13a** with 4M HCl in dioxane-H_2O (v/v, 10:1), and successive esterification of the resulting carboxylic acid with Ph_2CN_2 gave a diphenylmethyl ester **5a** (mp. 176-179 °C). Also, the same treatment of nitriles **4b** and **13b** yielded a diphenylmethyl ester **5b**.

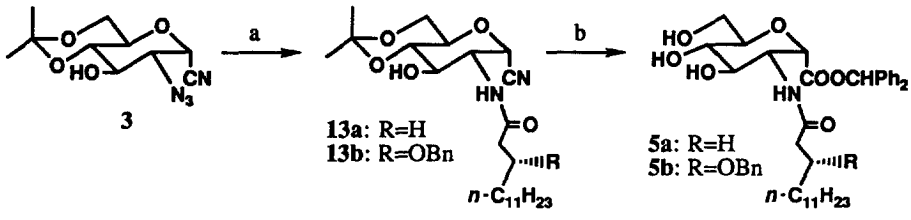
Isopropylidene formation between C5-OH and C7-OH of **5a** or **5b** with 2,2-dimethoxypropane using *p*-

Scheme 1



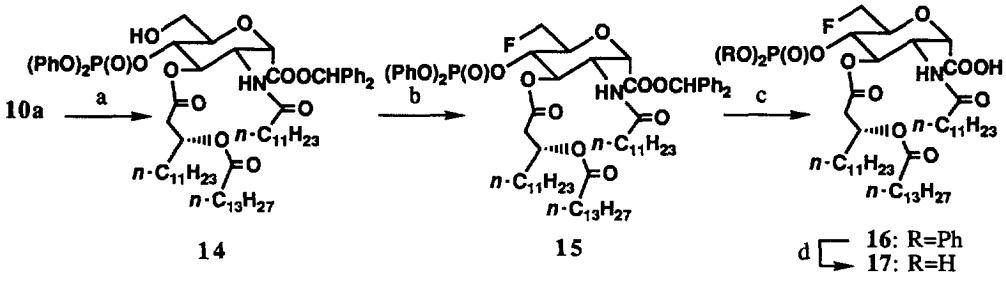
Reagents and Conditions: a) TMSCN, cat. TMSOTf, 24 °C, 15 h, CH_2Cl_2, quantitative; b) (i) cat. KOH, EtOH, 24 °C, 30 min; (ii) $Me_2C(OMe)_2$, cat. *p*-TsOH·H_2O, 24 °C, 16 h, DMF, 65%; c) (i) Ph_3P, THF-H_2O, 24 °C, 16 h; (ii) tetradecanoic acid or (*R*)-3-(benzyloxy)tetradecanoic acid, DCC, DMAP, 24 °C, 3 h, THF; (iii) tetradecanoyl chloride or (*R*)-3-(benzyloxy)tetradecanoyl chloride, Et_3N, 24 °C, 16 h, THF, 43% (**4a**), 85% (**4b**); d) (i) 4M HCl in dioxane-H2O (10:1), 55-60 °C, 4 h; (ii) Ph_2CN_2, 55-60 °C, 1.5 h, DMF, 53% (**5a**), 33% (**5b**); e) $Me_2C(OMe)_2$, cat. *p*-TsOH·H_2O, 25 °C, 16 h, DMF, 56% (**6a**), 83% (**6b**); f) (*R*)-3-(tetradecanoyloxy)tetradecanoic acid, DCC, DMAP, 24 °C, 16 h, CH_2Cl_2, 96% (**7a**), 86% (**7b**); g) aq. 85% AcOH, 70-75 °C, 1 h, 58% (**8a**), 37% (**8b**); h) ClCOOBn, pyridine, 0-5 °C, 30 min, CH_2Cl_2, 97% (**9a**), 97% (**9b**); i) $ClP(O)(OPh)_2$, DMAP, 24 °C, 4 h, CH_2Cl_2, 87% (**10a**), 91% (**10b**); j) H_2, Pd/C, 25 °C, 10 h, THF, 89% (**11a**), 75% (**11b**); k) H_2, PtO_2, 25 °C, 3-10 h, THF, 92% (**12a**), 96% (**12b**).

Scheme 2



Reagents and Conditions: a) (i) Ph_3P, THF, 24 °C, 1 h; then 28% NH_4OH-H_2O (1:3), 24 °C, 16 h; (ii) tetradecanoic acid or (*R*)-3-(benzyloxy)tetradecanoic acid, DCC, DMAP, 24 °C, 16 h, THF, 82% (**13a**), 49% (**13b**); (b) 4M HCl in dioxane-H_2O (10:1), 55-60 °C, 4 h; (ii) Ph_2CN_2, 55-60 °C, 4 h, DMF, 61% (**5a**), 41% (**5b**).

Scheme 3

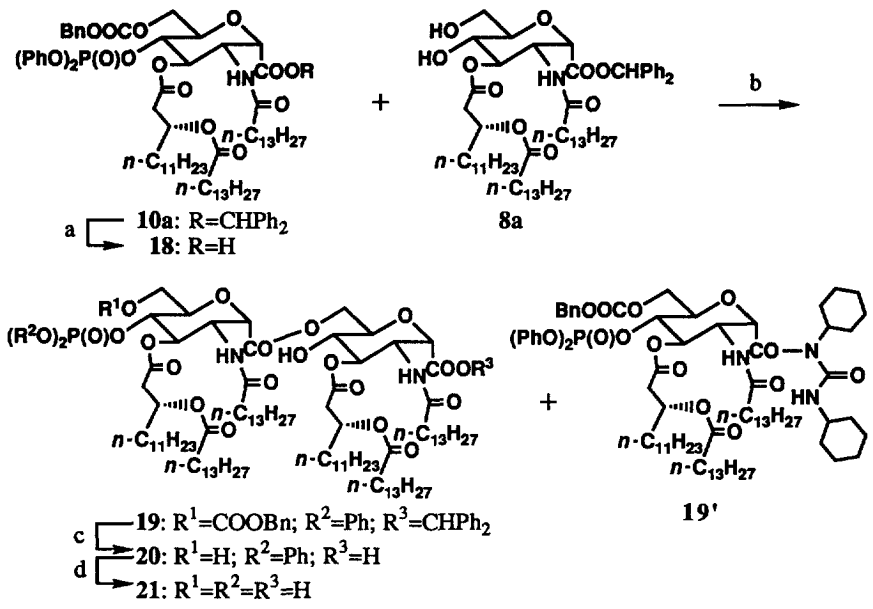


Reagents and Conditions: a) (i) H_2, Pd/C, 24 °C, 10 h, THF; (ii) Ph_2CN_2, 24 °C, 16 h, THF, two steps quantitative; b) DAST, 0-5 °C, 3 h, CH_2Cl_2, 87%; c) H_2, Pd/C, 24 °C, 16 h, THF, 65%; (d) H_2, PtO_2, 24 °C, 17 h, THF, quantitative.

TsOH as a catalyst gave **6a** (mp. 113-115 °C) or **6b**, respectively. Esterification of **6a** or **6b** with (*R*)-3-(tetradecanoyloxy)tetra-decanoic acid, DCC and DMAP formed **7a** or **7b**, respectively. Deprotection of acetonide **7a** or **7b** with aqueous 85% AcOH gave **8a** (mp. 105-106 °C) or **8b**, respectively. Treatment of **8a** or **8b** with benzyl chloroformate and pyridine yielded **9a** or **9b**, respectively. Treatment of **9a** or **9b** with diphenyl chlorophosphate and DMAP formed **10a** or **10b**, respectively. Hydrogenolysis of **10a** and **10b** using 10% Pd/C as a catalyst gave **11a** or **11b**, respectively. Finally, hydrogenolysis of **11a** or **11b** gave **12a** or **12b**, respectively.

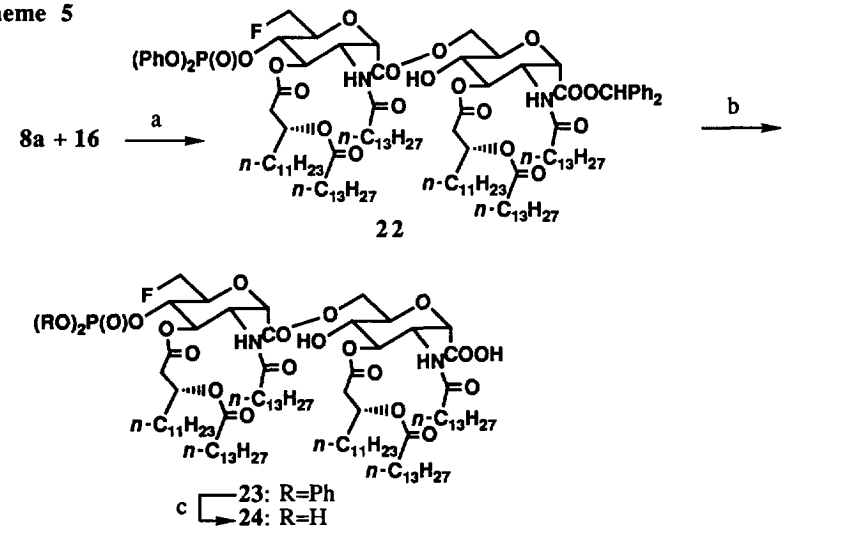
The synthesis of 6-fluorinated compound **17** was accomplished as follows. The diphenylmethyl ester of 1-carboxylic acid and the 7-benzyloxycarbonyl protective group of the hydroxy part in **10a** were deprotected by hydrogenolysis, and the resulting carboxylic acid was reprotected with Ph_2CN_2 to give **14**. The hydroxy group at the C7 position of **14** was fluorinated with diethylaminosulfur trifluoride (DAST) to give **15**. Deprotection of the diphenylmethyl ester of **15** by hydrogenolysis gave carboxylic acid **16**. The

Scheme 4



Reagents and Conditions: a) CF_3COOH, CH_2Cl_2, 24 °C, 1 h, 56%; b) DCC, DMAP, 24 °C, 16 h, CH_2Cl_2, THF, **19** (11%), **19'** (22%); c) H_2, Pd/C, THF, 24 °C, 6 h, quantitative; d) H_2, PtO_2, THF, 24 °C, 16 h, 80%.

Scheme 5



Reagents and Conditions: a) DCC, DMAP, 24 °C, 16 h, CH_2Cl_2, THF, 11%; b) H_2, Pd/C, THF, 24 °C, 6 h; c) H_2, PtO_2, THF, 24 °C, 16 h, two steps quantitative.

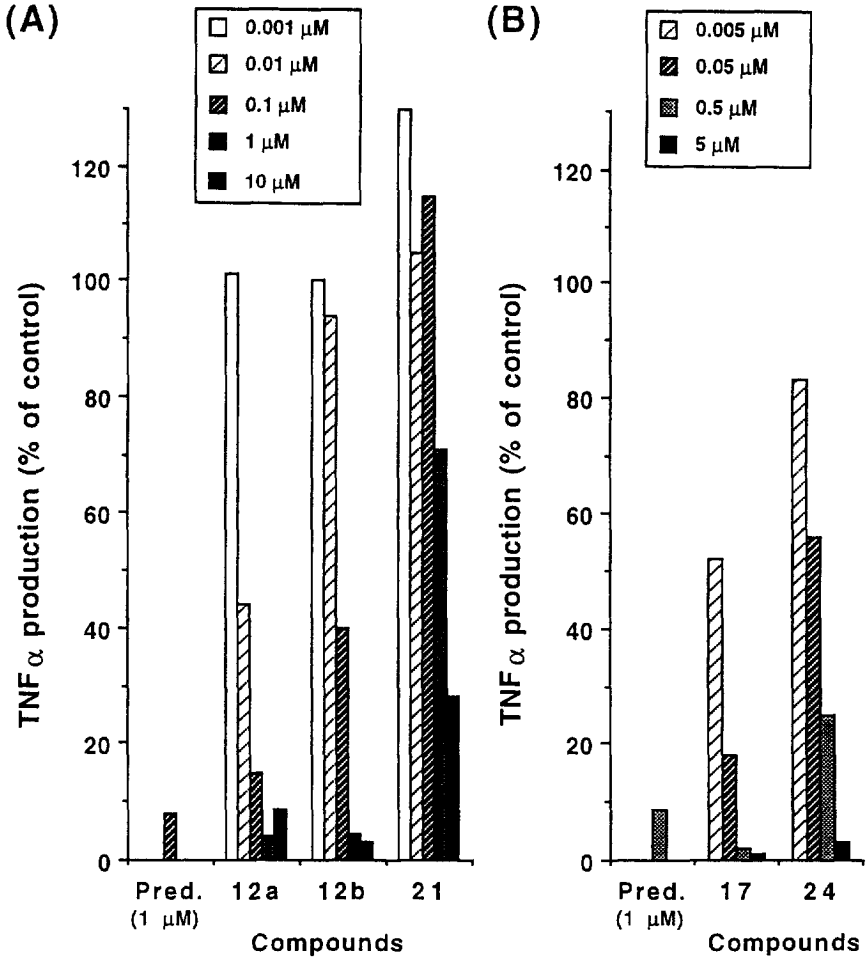


Fig. A and B. LPS antagonistic activity of compounds on TNFα production. TPA-treated U937 cells were stimulated with 10 ng/mL of LPS in the presence of the indicated concentrations of the isomers or prednisolone (Pred.) at 1 μM. After incubation for 4.5 h, the amounts of TNFα in the culture supernatant were measured by ELISA. As a control, the amount of TNFα produced by U937 cells, which were stimulated with LPS alone, was used. The relative amounts of TNFα produced by U937 cells were indicated as percentages of the control.

diphenylphosphono group of **16** was converted to phosphono compound **17** by hydrogenolysis using PtO_2 as a catalyst.[12]

Dimeric compounds **21** and **24** were synthesized as follows. Treatment of diphenylmethyl ester **10a** with trifluoroacetic acid gave carboxylic acid **18**. Esterification of **18** with 5,7-diol **8a** using dicyclohexylcarbodiimide (DCC) as a condensing agent and 4-dimethylaminopyridine (DMAP) as a catalyst gave dimeric ester **19** in 11% yield and urea **19'**, which was obtained by the side-reaction of carboxylic acid **18** with DCC. The detected dimer was an ester with a primary C7-OH. However, the yield was very low. The same tendency was observed in the reaction of **8a** with **16** to form fluorinated dimer **22** in 11% yield. Compounds **19** and **22** were converted to carboxylic acids **20** and **23** by hydrogenolysis using Pd on carbon as a catalyst. Finally, compounds **20** and **23** were further converted to phosphono compounds **21** and **24**, respectively, by hydrogenolysis using PtO_2 as a catalyst.

Biological Activity

The inhibitory activities of compounds on LPS-induced TNFα production were investigated *in vitro*, using human monoblastic U937 cells. As shown in Fig. A and B, all five compounds inhibited the LPS-induced TNFα production dose-dependently. The concentrations (μM) of compounds **12a**, **12b**, **17**, **21**, and **24** required to inhibit the LPS-induced TNFα production by U937 cells by 50% (IC_{50}) were 0.0079, 0.065, 0.0057, 3.1, and 0.078, respectively. Prednisolone (an antiinflammatory steroid known to potently inhibit TNFα production by stimulated monocytes)[13] used as a positive control reagent, inhibited the TNFα production by U937 cells by over 90%. None of these compounds showed agonistic activities on the TNFα production (data not shown). These compounds did not exhibit cytotoxic effects on U937 cells at the concentrations used in this study (data not shown).

Experimental Section

Melting points were uncorrected. 270 MHz ^{1}H-NMR spectra were recorded using tetramethylsilane as an internal reference. IR absorption spectra were recorded on a Jasco IR A-2 spectophotometer, and mass spectra were obtained with a JMS-O1SG mass spectrometer. Elemental analyses were performed by the Institute of Science and Technology, Inc. Separation of the compounds by column chromatography was carried out with Silica Gel 60 (Merck, 230-400 mesh ASTM) at slightly elevated pressure (1.2-1.5 atom) for easy elution, and the quantity of the used silica gel was 50-100 times the weight of the product purified. Analytical chromatography was performed on Merck Art 5715 silica gel 60-F_{254} plates.

2,6-Anhydro-3-azido-3-deoxy-4,5,7-tri-*O*-acetyl-D-*glycero*-D-*ido*-heptononitrile (2).

To a solution of α- or β-imidate, or a mixture of α- and β-imidates (**1**, 8.44g, 17.7 mmol), and trimethylsilyl cyanide (2.83 mL, 21.3 mmol) in CH_2Cl_2 (84 mL) was added trimethylsilyl trifluoromethanesulfonate (130 mg) at 24 °C with stirring. After stirring for 3-15 h at room temperature, the solution was concentrated in vacuo to one third the volume, and diluted with EtOAc. The solution was washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (2:1) gave **2** (6.22 g, quantitatively)

as a viscous oil. IR ν_{max}(film) 2200 (w), 2130, 1750, 1600 (w) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.06 (3H, s), 2.09 (3H, s), 2.12 (3H, s), 3.95 (1H, dd, *J*=6.0, 10.5 Hz), 4.07-4.19 (2H, m), 4.30-4.38 (1H, m), 4.94 (1H, d, *J*=6.0 Hz), 5.01-5.11 (1H, m), 5.34-5.44 (1H, m); MS (FAB, positive) *m/z* 341 $(M+H)^+$. *Anal.* Calcd. for $C_{13}H_{16}N_4O_7$ (340.3): C, 45.87; H, 4.74; N, 16.47. Found: C, 45.75; H, 4.31; N, 16.22.

2,6-Anhydro-3-azido-3-deoxy-5,7-*O*-isopropylidene-D-*glycero*-D-*ido*-heptononitrile (3). To a solution of **2** (5.36 g, 15.8 mmol in EtOH, 200 mL) was added KOH (290 mg, 5.2 mmol). After stirring for 30 min at 24 °C, the solution was concentrated in vacuo to give a residue which was dissolved in DMF (36 mL) and 2,2-dimethoxypropane (36 mL). To this solution, *p*-TsOH·H_2O (1.44 g, 7.6 mmol) was added, and the mixture was stirred for 16 h at 25 °C, diluted with EtOAc, which was washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (2:1) gave **3**, (2.60 g, 65% yield) as a solid, mp 172-173 °C (from EtOAc-hexane). IR ν_{max}(Nujol) 3510, 2230 (w), 2130 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.45 (3H, s), 1.51 (3H, s), 2.98 (1H, bs, OH), 3.50-3.60 (1H, m), 3.65-3.80 (3H, m), 3.84-4.07 (2H, m), 4.83 (1H, d, *J*=5.9 Hz, C2-H. NOE was observed between the protons, C3-H (δ 3.73) and C2-H. Therefore, the configuration at C2 position is *S*, and the nitrile group is *axial.*); MS (FAB, positive) *m/z* 255 $(M+H)^+$, 239. *Anal.* Calcd. for $C_{10}H_{14}N_4O_4$ (254.2): C, 47.24; H, 5.55; N, 22.04. Found: C, 47.29; H, 5.52; N, 22.20.

2,6-Anhydro-3-deoxy-5,7-*O*-isopropylidene-3-tetradecanamido-4-O-tetradecanoyl-D-*glycero*-D-*ido*-heptononitrile (4a). To a solution of **3** (2.21 g, 8.84 mmol) in THF (80 mL) was added Ph_3P (4.37 g, 16.68mmol). After stirring for 3 h at 24 °C, H_2O (8.0 mL) was added to this solution. The mixture was stirred for 16 h at 24 °C, and concentrated in vacuo to give a mixture which was dried with a pump. The mixture was dissolved in THF (80 mL). To this solution, tetradecanoic acid (2.00 g, 8.76 mmol), DCC (1.81 g, 8.76 mmol), and DMAP (1.07 g, 8.76 mmol) were added with stirring at 24 °C. Furthermore, after 3 h stirring at 24 °C, to this reaction mixture, tetradecanoyl chloride (2.16 g, 8.76 mmol) and Et_3N (1.68 g, 16.68 mmol) were added. The stirring was continued for 16 h at 24 °C. The reaction mixture was diluted with EtOAc, which was washed with H_2O, aqueous $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a mixture. The mixture was chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (9:1, then 4:1) gave **4a** (2.33 g, 43% yield) as an amorphous solid, mp 59-61 °C (from *iso*-Pr_2O). IR ν_{max}(Nujol) 3420, 3360, 1727, 1670, 1655 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (6H, t, *J*=6.2-7.0 Hz), 1.26 (40H, bs), 1.40 (3H, s), 1.49 (3H, s), 1.50-2.02 (5H, m), 2.18 (1H, m), 2.30-2.45 (2H, m), 3.70-3.80 (3H, m), 3.96 (1H, m), 4.24 (1H, m), 5.11 (1H, m), 5.14 (1H, d, *J*=6.3 Hz), 6.27 (1H, d, *J*=6.9 Hz, NH); MS (EI) *m/z* 648 (M^+), 633 (M^+-Me); MS (FAB, positive) *m/z* 671 $(M+Na)^+$, 649 $(M+H)^+$; High Resolution MS (FAB, positive), Calcd. for $C_{38}H_{68}N_2O_6Na$: 671.4975. Found: 671.4955, and Calcd. for $C_{38}H_{68}N_2O_6$: 649.5156. Found: 649.5165. *Anal.* Calcd. for $C_{38}H_{68}N_2O_6$ (648.9): C, 70.33; H, 10.56; N, 4.32. Found: C, 70.57; H, 10.66; N, 4.69.

2,6-Anhydro-3-deoxy-5,7-*O*-isopropylidene-3-tetradecanamido-D-*glycero*-D-*ido*-heptononitrile (13a). To a solution of **3** (127 mg, 0.50 mmol) in THF (5 mL) was added PPh_3 (328 mg, 1.25 mmol). After stirring for 1 h at 24 °C, H_2O (0.3 ml) and 28% NH_4OH (0.1 ml) were added.

The mixture was stirred for 16 h at 24 °C. The reaction mixture was concentrated in vacuo, and diluted with EtOAc, and then concentrated in vacuo to give a mixture. The residual mixture was diluted with THF (5 ml), and tetradecanoic acid (137 mg, 0.60 mmol) and DCC (124 mg, 0.60 mmol) were added. After stirring for 16 h, the mixture was filtered, concentrated, and chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (1:1) gave **13a** (179 mg, 82%) as a gum. IR ν_{max}(KBr) 3430, 3327, 2925, 2853, 1650, 1627 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (3H, t, *J*=6.4-6.8 Hz), 1.25 (20H, bs), 1.45 (3H, s), 1.52 (3H, s), 1.59-1.69 (2H, m), 2.26 (2H, t, *J*=7.3-7.9 Hz), 2.79 (1H, d, *J*=2.3 Hz, OH), 3.55-3.96 (5H, m), 4.14 (1H, m), 5.28 (1H, d, *J*=5.9 Hz, C2-H), 5.94 (1H, d, *J*=5.6 Hz, NH); MS (FAB, positive) *m/z* 439 $(M+H)^+$; High Resolution MS (FAB, positive), Calcd. for $C_{24}H_{43}N_2O_5$: 439.3172. Found: 439.3171

Diphenylmethyl 2,6-Anhydro-3-deoxy-3-tetradecanamido-D-*glycero*-D-*ido*-heptonate (5a). (a) To a solution of **4a** (2.31 g, 3.56 mmol) in dioxane (18 mL), 4M HCl in dioxane (18 mL) and H_2O (1.8 mL) were added. The solution was warmed at 55-60 °C for 4 h. The reaction mixture was concentrated, and dried in vacuo to give a mixture which was dissolved in DMF (20 mL). Ph_2CN_2 (2.5 g) was added to this solution, which was warmed at 55-60 °C for 1.5 h, concentrated in vacuo, and chromatographed on a silica gel column. Elution with EtOAc, then 5% MeOH in EtOAc gave **5a** (1.11 g, 53% yield) as a solid, mp 176-179 °C (from EtOAc). IR ν_{max}(Nujol) 3325 (broad), 2470, 2420, 1733, 1637 cm^{-1}; ^{1}H-NMR (DMF-d7) δ 0.88 (3H, t, *J*=6.4-6.8 Hz), 1.27 (20H, bs), 1.43-1.52 (2H, m), 1.94-2.11 (2H, m), 3.44 (1H, m), 3.60-3.80 (3H, m), 3.80-3.92 (1H, m), 3.92-4.07 (1H, m), 5.20 (1H, d, *J*=4.9 Hz, OH), 6.88 (1H, s), 7.27-7.49 (10H, m), 7.60 (1H, d, *J*=7.3 Hz, NH); MS *m/z* 583 (M^+). *Anal.* Calcd. for $C_{34}H_{49}NO_7$ (583.7): C, 69.96; H, 8.46; N, 2.40. Found: C, 69.71; H, 8.80; N, 2.81.

(b) A solution of **13a** (154 mg, 0.264 mmol) in dioxane (1.5 ml), 4M HCl-dioxane (1.5 mL) and H_2O (0.15 mL) was warmed at 55-60 °C for 2 h, and this reaction mixture was concentrated in vacuo, then diluted with EtOAc, and concentrated to dryness. This procedure was repeated two more time, and the resulting material was dried in vacuo, and then dissolved in DMF (2 mL). To this mixture, Ph_2CN_2 (230 mg,) was added, and warmed at 55-60 °C for 2 h. The reaction mixture was concentrated in vacuo, and chromatographed on a silica gel column. Elution with EtOAc, and then 5% MeOH in EtOAc gave **5a** (125 mg, 61%) as a solid.

Diphenylmethyl 2,6-Anhydro-3-deoxy-5,7-isopropylidene-3-tetradecanamido-D-*glycero*-D-*ido*-heptonate (6a). A solution of **5a** (1.10 g, 1.88 mmo) in DMF (4 mL) and 2,2-dimethoxypropane (8 mL) containing *p*-TsOH·H_2O (80 mg, 0.42 mmol) was allowed to stand for 16 h at 25 °C, and diluted with EtOAc, which was washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (1:1) gave **6a** (635 mg, 56% yield) as a solid, mp 111-113 °C (from EtOAc-hexane). IR ν_{max}(Nujol) 3380, 1750, 1640 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (3H, t, *J*=6.3-6.9 Hz), 1.05-1.33 (20H, m), 1.37 (3H, s), 1.49 (3H, s), 1.53 (2H, broad), 2.07-2.13 (2H, m), 2.56 (1H, bs, OH), 3.26 (1H, dt, *J*=5.5, 9.8 Hz), 3.58 (1H, t, *J*=8.9-9.6 Hz), 3.70 (1H, t, *J*=10.1-10.5 Hz), 3.78-3.87 (2H, m), 4.40 (1H, dt, *J*=5.9, 9.7 Hz, C3-H), 4.62 (1H, d, *J*=5.9 Hz, C2-H), 6.20 (1H, d, *J*=9.0 Hz, NH), 6.93 (1H, s), 7.32-7.39 (10H, m); MS (EI) *m/z* 623 (M^+), 608 $(M^+$-Me). *Anal.* Calcd. for $C_{37}H_{53}NO_7$ (623.8): C, 71.24; H, 8.56; N, 2.25. Found: C, 71.32; H, 8.43; N, 2.27.

Diphenylmethyl 2,6-Anhydro-3-deoxy-5,7-*O*-isopropylidene-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonate (7a). To a solution of **6a** (624 mg, 1.00 mmol) in CH_2Cl_2 (10 mL) was added (*R*)-3-(tetradecanoyloxy)tetradecanoic acid (546 mg, 1.20 mmol), DCC (248 mg, 1.20 mmol), and DMAP (146 mg, 1.20 mmol) with stirring at 24 °C. The mixture was stirred for 16 h at 25 °C, and diluted with EtOAc, which was washed with aqueous 0.1M HCl, water, aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (5:1) gave **7a** (1.02 g, 96% yield) as a gum. IR ν_{max}(film) 3310, 2925, 2860, 1730, 1655 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (9H, t, *J*=6.4-6.9 Hz), 1.15-1.37 (61H, m), 1.45 (3H, s), 1.50-2.00 (7H, m, containing 3H singlet at δ 1.61), 2.20-2.30 (2H, m), 3.32-3.41 (1H, m), 3.65-3.84 (3H, m), 4.38-4.47 (1H, m), 4.67 (1H, d, *J*=6.1 Hz, C2-H), 5.14 (1H, t, *J*=6.3 Hz), 5.33 (1H, t, *J*=9.5-10.4 Hz), 6.08(1H, d, *J*=8.8 Hz, NH), 6.95 (1H, s), 7.30-7.37 (10H, m); MS (FAB, positive, addition of 1M KI aq solutiion) *m/z* 1098 $(M+K)^+$. *Anal.* Calcd. for $C_{65}H_{105}NO_{10}$ (1060.6): C, 73.61; H, 9.98; N, 1.32. Found: C, 73.64; H, 10.16; N, 1.49.

Diphenylmethyl 2,6-Anhydro-3-deoxy-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonate (8a). A suspension of **7a** (1.00 g, 0.943 mmol) in aqueous 85% ACOH (100 mL) was stirred for 1 h at 70-75 °C, and the resulting solution was concentrated in vacuo with a pump, and chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (2:1, then 1:1) gave **8a** (555 mg, 58% yield) as an amorphous solid, mp 105-106 °C (from hexane-cyclohexane). IR ν_{max}(Nujol) 3340, 1732, 1722 (shoulder), 1644 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 0.88 (9H, t, *J*=6.4-6.8 Hz), 1.26 (61H, bs), 1.40-1.50 (1H, m), 1.50-1.65 (2H, m), 1.96 (2H, t, *J*=7.3-8.1 Hz), 2.04 (1H, t, *J*=5.9 Hz, OH), 2.29 (2H, t, *J*=7.5 Hz), 2.50-2.54 (2H, m), 3.36 (1H, d, *J*=4.0 Hz, OH), 3.42-3.48 (1H, m), 3.66 (1H, dt, *J*=4.0, 9.3-9.7 Hz, changed to t, *J*=9.3-9.7 Hz, on addition of D_2O), 3.73 (1H, m, changed to dd, *J*=4.0, 12.1 Hz, on addition of D_2O), 3.81 (1H, m, changed to dd, *J*=3.2, 12.1 Hz, on addition of D_2O), 4.37 (1H, m), 4.72 (1H, d, *J*=5.9 Hz, C2-H), 5.09 (1H, m), 5.23 (1H, dd, *J*=9.O, 11.0 Hz), 6.26 (1H, d, *J*=8.6 Hz, NH), 6.95 (1H, s), 7.29-7.39 (10H, m); MS (FAB, positive, addition of 1M KI aq solution) *m/z* 1058 $(M+K)^+$. *Anal.* Calcd. for $C_{62}H_{101}NO_{10}$ (1020.5): C, 72.97; H, 9.98; N, 1.37. Found: C, 72.62; H, 10.22; N, 1.42. H, 10.16; N, 1.49.

Diphenylmethyl 2,6-Anhydro-7-*O*-benzyloxycarbonyl-3-deoxy-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonate (9a). To a solution of **8a** (200 mg, 0.196 mmol) in CH_2Cl_2 (30 mL) and pyridine (3.0 mL) was added dropwise ClCOOBn (1.6 mL) at 0-5 °C under nitrogen with stirring. After 30 min, the solution was concentrated in vacuo, and diluted with EtOAc, which was washed with aqueous 0.2M HCl, water, aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (4:1) gave **9a** (219 mg, 97% yield) as a wax. IR ν_{max}(film) 3550, 3290, 2920, 2840, 1730, 1652 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (9H, t, *J*=6.2-7.0 Hz), 1.25 (61H, bs), 1.40-1.51 (1H, m), 1.51-1.65 (2H, m), 1.95 (2H, t, *J*=7.2-8.1 Hz), 2.28 (2H, t, *J*=7.4-7.6 Hz), 2.45-2.57 (2H, m), 3.34 (1H, d, *J*=3.8 Hz, OH), 3.53-3.67 (2H, m), 4.30-4.45 (3H, m), 4.71 (1H, d, *J*=6.0 Hz, C2-H), 5.08 (1H, m), 5.18 (1H, s), 5.21 (1H, m), 6.22 (1H, d, *J*=8.6 Hz, NH), 6.93 (1H, s), 7.21-7.38 (15H, m); MS (FAB, positive, addition of 1M KI aq solution) *m/z* 1192 $(M+K)^+$. *Anal.* Calcd. for $C_{70}H_{107}NO_{12}$ (1154.6): C, 72.82; H,

9.34; N, 1.21. Found: C, 72.60; H, 9.71; N, 1.27.

Diphenylmethyl 2,6-Anhydro-7-*O*-benzyloxycarbonyl-3-deoxy-5-*O*-diphenylphosphono-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetra-decanoyl]-D-*glycero*-D-*ido*-heptonate (10a). To a solution of **9a** (174 mg, 0.15 mmol) and DMAP (42 mg, 0.344 mmol) in CH_2Cl_2 (5 ml) was added dropwise diphenyl chlorophosphate (90 mg, 0.335 mmol). After stirring at 24 °C for 4 h, the reaction mixture was diluted with EtOAc, washed with aqueous 1M HCl, water, aqueous $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (4:1) gave **10a** (182 mg, 87% yield) as an amorphous solid. IR ν_{max}(Nujol) 3340, 1737, 1660 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (9H, t, *J*=6.5-6.8 Hz), 1.25 (61H, bs), 1.37-1.57 (3H, m), 1.80-1.97 (2H, m), 2.16 (1H, t, *J*=7.2-7.8 Hz), 2.33-2.48 (2H, m), 3.90 (1H, dm, *J*=7.9, <1 Hz), 4.09 (1H, dd, *J*=4.3, 12.1 Hz), 4.23 (1H, dd, =2.0, 12.1 Hz), 4.37-4.47 (1H, m), 4.70 (1H, q, *J*=9.2 Hz), 4.81 (1H, d, =6.0 Hz), 5.01, 5.10 (2H, AB-q, *J*=12.0 Hz), 5.07 (1H, m), 5.55 (1H, dd, *J*=8.9, 11.0 Hz), 6.11 (1H, d, *J*=7.9 Hz, NH), 6.94 (1H, s), 7.11-7.39 (25H, m); MS (FAB, positive, addition of 1M KI aq solution) *m/z* 1424 $(M+K)^+$. *Anal.* Calcd. for $C_{82}H_{116}NO_{15}P$ (1386.8): C, 71.02; H, 8.43; N, 1.01; P, 2.23. Found: C, 70.77; H, 8.63; N, 0.99; P, 2.32.

2,6-Anhydro-3-deoxy-5-*O*-diphenylphosphono-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonic Acid (11a). To a solution of **10a** (159 mg, 0.115 mmol in THF, 4 mL) was added 10% Pd on carbon (115 mg), and the mixture was stirred for 10 h under hydrogen at 25 °C, and the whole was filtered. The filtrate was concentrated in vacuo, and chromatographed on a silica gel column. Elution with EtOAc, and then with 2% AcOH in EtOAc gave **11a** (111 mg, 89% yield) as an amorphous solid. IR ν_{max}(Nujol) 3700-3000, 1733, 1660 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (9H, t, *J*=6.4-6.7 Hz), 1.24 (61H, bs), 1.37-1.60 (3H, m), 2.08-2.17 (4H, m), 2.38 (1H, d, *J*=6.2 Hz), 2.71 (1H, bs, OH), 3.58 (1H, m), 3.66-3.83 (2H, m), 4.43-4.73 (3H, m), 5.10 (1H, m), 5.50-5.63 (1H, m), 6.50-6.65 (1H, broad, NH), 7.17-7.37 (10H, m); MS (FAB, positive) *m/z* 1108 $(M+Na)^+$. *Anal.* Calcd. for $C_{61}H_{100}NO_{13}P$ (1086.4): C, 67.44; H, 9.28; N, 1.29; P, 2.85. Found: C, 67.49; H, 9.28; N, 1.30; P, 2.79.

2,6-Anhydro-3-deoxy-5-*O*-phosphono-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonic Acid (12a). A solution of **11a** (91 mg, 0.084 mmol) in THF (4 ml) was stirred for 3 h at 25 °C under hydrogen using PtO_2 (22 mg) as a catalyst. The reaction mixture was filtered, and the filtrate was concentrated in vacuo to give **12a** (72 mg, 92% yield) as an amorphous solid. IR ν_{max}(Nujol) 3360 (broad), 1735, 1650 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (9H, t, *J*=6.0-6.8 Hz), 1.26 (58H, bs), 1.57 (6H, m), 2.10-2.80 (6H, m), 3.60-4.10 (3H, m), 4.20-4.70 (3H, m), 5.06-5.40 (2H, m), 7.00 (1H, bs, NH); MS (FAB, positive) *m/z* 956 $(M+Na)^+$, 935 $(M+H)^+$. *Anal.* Calcd. for $C_{49}H_{92}NO_{13}P$ (934.2): C, 63.00; H, 9.93; N, 1.50; P, 3.32. Found: C, 63.13; H, 9.87; N, 1.18; P, 3.11.

2,6-Anhydro-3-[(*R*)-3-(benzyloxy)tetradecanamido]-4-*O*-[(*R*)-3-(benzyloxy)tetradecanoyl]-3-deoxy-5,7-*O*-isopropylidene-D-*glycero*-D-*ido*-heptononitrile

(4b). To a solution of **3** (1.19 g, 4.68 mmol) in THF (47 mL) was added Ph_3P (2.46 g, 9.36 mmol). After stirring for 3 h at 24 °C, H_2O (4.7 mL) was added to this solution. The mixture was stirred for 16 h at 24 °C, concentrated in vacuo, diluted with EtOAc, and concentrated in vacuo again. This procedure was repeated 3 times to remove the water completely. The residual mixture was dissolved in THF (47 mL). To this solution, (*R*)-3-(benzyloxy)tetradecanoic acid (1.72 g, 5.15 mmol), DCC (1.06 g, 5.15 mmol), and DMAP (629 mg, 5.15 mmol) were added with stirring at 24 °C. After 3 h, to this reaction mixture, (*R*)-3-(benzyloxy)tetradecanoyl chloride, which was obtained from the corresponding acid (1.72 g, 5.15 mmol) and excess oxalyl chloride in CH_2Cl_2, and Et_3N (1.5 mL, 10.8 mmol) were added at 24 °C with stirring. The stirring was continued for 16 h at 24 °C. The reaction mixture was filtered, and the filtrate was diluted with EtOAc, which was washed with H_2O, aqueous $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a mixture. The mixture was chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (9:1, then 4:1) gave **4b** (3.44 g, 85% yield) as an amorphous solid. IR ν_{max}(film) 3280, 2920, 2860, 1738, 1652 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (6H, t, *J*=6.3-6.9 Hz), 1.25 (36H, bs), 1.33 (3H, s), 1.42 (3H, s), 1.52 (4H, bm), 2.27-2.44 (2H, m), 2.45 (1H, dd, *J*=5.7, 15.1 Hz), 2.65 (1H, dd, *J*=6.6, 15.1 Hz), 3.68-3.85 (5H, m), 3.92-3.97 (1H, m), 4.26 (1H, dt, *J*=10.8, 6.4 Hz), 4.45, 4.58 (2H, AB-q, *J*=11.9 Hz), 5.08 (1H, d, *J*=6.1 Hz), 5.20 (1H, dd, *J*=8.8, 10.5 Hz), 6.79 (1H, d, *J*=6.8 Hz, NH), 7.27-7.40 (10H, m); *Anal.* Calcd. for $C_{52}H_{80}N_2O_8$ (861.2): C, 72.51; H, 9.37; N, 3.25. Found: C, 72.38; H, 9.44; N, 3.28.

2,6-Anhydro-3-deoxy-5,7-*O*-isopropylidene-3-[(*R*)-3-(benzyloxy)tetra-decanamido]-D-*glycero*-D-*ido*-heptononitrile (13b). Compound **3** was treated as described in the formation of **13a** using (*R*)-3-(benzyloxy)tetradecanoic acid in place of tetradecanoic acid to give **13b** in 49% yield.

Diphenylmethyl 2,6-Anhydro-3-[(*R*)-3-(benzyloxy)tetradecanamido]-3-deoxy-D-*glycero*-D-*ido*-heptonate (5b). (a) A solution of **4b** (3.44 g, 3.99 mmol) in dioxane (22 mL), 4M HCl in dioxane (22 mL), and H_2O (2.2 mL) was warmed at 60-65 °C (bath temperature) for 5 h. The mixture was concentrated in vacuo, diluted with EtOAc, and concentrated. This procedure was repeated 3 times to remove the water completely, and the residue was dissolved in DMF (30 mL). Ph_2CN_2 (2.00 g, 10.3 mmol) was added to this solution, which was warmed at 55-60 °C for 1 h, concentrated in vacuo, and chromatographed on a silica gel column. Elution with EtOAc, then 5% MeOH in EtOAc gave **5b** (897 mg, 33% yield) as an amorphous solid. IR ν_{max}(Nujol) 3330, 1735, 1640 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (3H, t, *J*=6.4-6.7 Hz), 1.20-1.60 (22H, m), 1.92-2.08 (2H, m), 3.44 (1H, m), 3.60-3.80 (3H, m), 3.80-3.92 (1H, m), 3.92-4.07 (1H, m), 4.53 (1H, m, OH), 4.80 (1H, d, *J*=5.9 Hz), 5.00 (1H, bs, OH), 5.11 (1H, bs, OH), 6.85 (1H, s), 7.25-7.47 (10H, m), 7.57 (1H, d, *J*=7.3 Hz, NH); MS (FAB, positive) *m/z* 712 $(M+Na)^+$. *Anal.* Calcd. for $C_{41}H_{55}NO_8$ (689.9): C, 71.37; H, 8.04; N, 2.03. Found: C, 71.25; H, 8.17; N, 2.01.

(b) Compound **13b** was treated as described in the formation of **5a** from **13a** to give **5b** in 41% yield.

Diphenylmethyl 2,6-Anhydro-3-[(*R*)-3-(benzyloxy)tetradecanamido]-3-deoxy-5,7-*O*-isopropylidene-D-*glycero*-D-*ido*-heptonate (6b). A solution of **5b** (897 mg, 1.30 mmol) in DMF (4 mL) and 2,2-dimethoxypropane (8 mL) containing *p*-TsOH·H_2O (100 mg, 0.526 mmol) was allowed to stand for 16 h at 25 °C, and diluted with EtOAc, which was washed with aqueous $NaHCO_3$ and brine, dried

over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (3:2) gave **6b** (422 mg, 44% yield) as an amorphous solid. IR ν_{max}(Nujol) 3380, 1727, 1640 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (3H, t, *J*=6.4-6.8 Hz), 1.23-1.29 (18H, m), 1.36-1.65 (8H, m, containing two 3H, s, at δ 1.37 and 1.48), 2.29-2.44 (2H, m), 2.54 (1H, d, *J*=2.8 Hz, OH), 3.17-3.26 (1H, m), 3.56 (1H, t, *J*=9.3 Hz), 3.62-3.86 (4H, m), 4.35-4.51 (3H, m, containing 1H, d, *J*=5.9 Hz, at δ 4.41), 4.56 (1H, d, *J*=5.9 Hz), 6.83 (1H, d, *J*=6.9 Hz, NH), 6.84 (1H, s), 7.24-7.38 (15H, m); MS (EI) *m/z* 729 (M^+). *Anal.* Calcd. for $C_{44}H_{59}NO_8$ (730.0): C, 72.40; H, 8.15; N, 1.92. Found: C, 71.95; H, 7.85; N, 2.22.

Diphenylmethyl 2,6-Anhydro-3-[(*R*)-3-(benzyloxy)tetradecanamido]-3-deoxy-5,7-*O*-isopropylidene-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonate (7b). To this solution of **6b** (422 mg, 0.578 mmol) in CH_2Cl_2 (4 mL) was added (R)-3-(tetradecanoyloxy)tetradecanoic acid (289 mg, 0.636 mmol), DCC (140 mg, 0.679 mmol), and DMAP (83 mg, 0.679 mmol) was added with stirring at 24 °C. The mixture was stirred for 16 h at 25 °C, and filtered. The filtrate was diluted with EtOAc, which was washed with aqueous 0.1M HCl, water, aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (5:1) gave **7b** (583 mg, 86% yield) as a wax. IR ν_{max}(film) 3400, 2930, 2860, 1736, 1660 cm^{-1}; ^{1}H-NMR (CDCl3) δ 0.88 (9H, t, *J*=6.3-6.8 Hz), 1.25 (60H, s), 1.30 (3H, s), 1.35-1.84 (7H, m, containing 3H, s, at δ 1.44), 2.13-2.31 (4H, m), 2.46 (1H, dd, *J*=6.2, 15.3 Hz), 2.60 (1H, dd, *J*=6.7, 15.6 Hz), 3.27 (1H, td, *J*=4.2-5.5, 14.0 Hz), 3.63-3.81 (5H, m), 4.37 (2H, s), 4.48 (1H, m), 4.60 (1H, d, *J*=5.9 Hz, C2-H), 5.35 (1H, t, *J*=9.6-10.3 Hz), 6.55 (1H, d, *J*=8.8 Hz, NH), 6.85 (1H, s), 7.25-7.40 (15H, m); MS (FAB, positive) *m/z* 1166 $(M+H)^+$. *Anal.* Calcd. for $C_{72}H_{111}NO_{11}$ (1166.7): C, 74.12; H, 9.59; N, 1.20. Found: C, 74.10; H, 9.63; N, 1.18.

Diphenylmethyl 2,6-Anhydro-3-[(*R*)-3-(benzyloxy)tetradecanamido]-3-deoxy-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonate (8b). A suspension of **7** (152 mg, 0.13 mmol) in aqueous 85% AcOH (16 mL) was stirred for 1 h at 70-75 °C, and the resulting solution was concentrated in vacuo with a pump, and chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (5:3) gave the starting **7b** (60 mg recovery, 39%), a lower R_f isomer (19 mg, 13%) of **8b**, and **8b** (55 mg, 58% yield) as an amorphous solid, mp 93-94 °C (from hexane-EtOAc). IR ν_{max}(Nujol) 3420 (shoulder), 1730, 1648 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (9H, t, *J*=6.4-6.8 Hz), 1.25 (61H, bs), 1.41-1.62 (3H, m), 2.21-2.32 (4H, m), 2.45-2.49 (2H, m), 3.30 (1H, d, *J*=4.0 Hz, OH), 3.38 (1H, dt, *J*=9.6, 3.8 Hz), 3.58-3.84 (4H, m), 4.38 (2H, s), 4.43 (1H, m), 5.24 (1H, dd, *J*=9.0, 11.0 Hz), 6.66 (1H, d, *J*=8.7 Hz, NH), 6.85 (1H, s), 7.23-7.39 (15H, m); MS (FAB, positive) *m/z* 1126 $(M+H)^+$, 1148 $(M+Na)^+$. *Anal.* Calcd. for $C_{69}H_{107}NO_{11}$ (1126.6): C, 73.56; H, 9.57; N, 1.24. Found: C, 73.48; H, 9.51; N, 1.30.

Diphenylmethyl 2,6-Anhydro-7-*O*-benzyloxycarbonyl-3-[(*R*)-3-(benzyloxy)tetradecanamido]-3-deoxy-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonate (9b). To a solution of **8b** (200 mg, 0.178 mmol) in CH_2Cl_2 (30 mL) was added pyridine (1.5 ml, 18.5 mmol) and ClCOOBn (1.6 mL, 11.2 mmol) at 5-7 °C under nitrogen with stirring. After 30 min at this temperature, the reaction mixture was concentrated in vacuo, and diluted with EtOAc. The

solution was washed with aqueous 0.2M HCl, water, aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (9:1, then 4:1) gave **9b** (217 mg, 97% yield) as a wax. IR ν_{max}(Nujol) 3525, 3310, 1735, 1648 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (9H, t, *J*=6.4-6.8 Hz), 1.25 (61H, bs), 1.50-1.63 (3H, m), 2.15-2.31 (4H, m), 2.41-2.54 (2H, m), 3.35 (1H, bs, OH), 3.55 (2H, bs), 3.67 (1H, quintet, *J*=5.3-5.8 Hz), 4.29-4.50 (5H, m, containing 2H, s, at 5 4.38), 4.64 (1H, d, *J*=5.9 Hz, C2-H), 5.06 (1H, m), 5.14-5.26 (3H, m, containing 2H, s, at δ 5.18), 5.21 (1H, m), 6.65 (1H, d, *J*=8.8 Hz, NH), 6.83 (1H, s), 7.22-7.40 (20H, m); MS (FAB, positive) *m/z* 1260 $(M+H)^+$, 1282 $(M+Na)^+$. *Anal.* Calcd. for $C_{77}H_{113}NO_{13}$ (1260.7): C, 73.36; H, 9.03; N, 1.11. Found: C, 73.18; H, 9.31; N, 1.14.

Diphenylmethyl 2,6-Anhydro-7-*O*-benzyloxycarbonyl-3-[(*R*)-3-(benzyloxy)tetradecanamido]-3-deoxy-5-*O*-diphenylphosphono-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonate (10b). To a solution of **9b** (207 mg, 0.04 mmol) and diphenyl chlorophosphate (144 mg, 0.164 mmol) in CH_2Cl_2 (12 mL) was added DMAP (80 mg, 0.655 mmol) . After stirring at 24 °C for 2 h, the reaction mixture was concentrated in vacuo, and diluted with EtOAc, The solution was washed with aqueous 1M HCl, water, aqueous $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (5:1) gave **10b** (223 mg, 91% yield) as an oil. IR ν_{max}(film) 3440, 2930, 2850, 1750 (shoulder), 1730, 1670, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (9H, t, *J*=6.6-7.0 Hz), 1.25 (60H, bs), 1.25-1.60 (4H, m), 2.11-2.26 (4H, m), 2.41 (1H, d, *J*=6.3 Hz), 3.73 (1H, t, *J*=5.3-6.4 Hz), 3.75 (1H, td, *J*=2.1, 11.7 Hz), 4.08 (1H, dd, *J*=4.4, 12.0 Hz), 4.21 (1H, dd, *J*=1.8, 12.0 Hz), 4.37 (2H, s), 4.49 (1H, m), 4.67 (1H, t, *J*=9.2 Hz), 4.71 (1H, d, *J*=5.7 Hz, C2-H), 5.01, 5.10 (2H, AB-q, =12.1 Hz), 5.08 (1H, m), 5.56 (1H, dd, *J*=9.0, 11.0 Hz), 6.57 (1H, d, *J*=8.3 Hz, NH), 6.83 (1H, s), 7.10-7.41 (30H, m); MS (FAB, positive) *m/z* 1492 $(M+H)^+$, 1514 $(M+Na)^+$. *Anal.* Calcd. for $C_{89}H_{122}NO_{16}P$ (1492.9): C, 71.60; H, 8.24; N, 0.94; P, 2.07. Found: C, 71.48; H, 8.41; N, 0.90; P, 2.18.

2,6-Anhydro-3-deoxy-5-*O*-diphenylphosphono-3-[(*R*)-3-(hydroxy)tetradecan-amido]-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonic Acid (11b). To a solution of **10b** (49 mg, 0.03 mmol) in EtOAc (4 mL) was added 10% Pd on carbon (50 mg), and the mixture was stirred for 18 h under hydrogen at 25 °C, and then filtered. The filtrate was concentrated in vacuo, and chromatographed on a silica gel short column. Elution with EtOAc, and then with 3% AcOH in EtOAc gave **11b** (27 mg, 75% yield) as an amorphous solid. IR ν_{max}(Nujol) 3360, 2930, 2850, 1735, 1640, 1600 (shoulder) cm^{-1}; ^{1}H-NMR (CDCl3) δ 0.88 (9H, t, *J*=6.1-6.9 Hz), 1.25 (60H, bs), 1.30-1.60 (4H, m), 2.1-2.41 (6H, m), 3.58 (1H, dd, *J*=2.6, 13.0 Hz), 3.72 (1H, d, *J*=13.0 Hz), 3.84-4.00 (2H, m), 4.52 (1H, m), 4.69-4.79 (2H, m), 5.10 (1H, m), 5.60 (1H, t, *J*=9.9 Hz), 7.05 (1H, d, *J*=8.7 Hz, NH), 7.08-7.38 (10H, m); MS (FAB, positive) *m/z* 1102 $(M+H)^+$. *Anal.* Calcd. for $C_{61}H_{1003}NO_{13}P$ (1102.4): C, 66.44; H, 9.15; N, 1.27; P, 2.81. Found: C, 66.31; H, 9.28; N, 1.27; P, 2.81.

2,6-Anhydro-3-deoxy-5-*O*-phosphono-3-[(*R*)-3-(hydroxy)tetradecanamido]-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonic Acid (12b). A solution of **11b** (23 mg, 0.02 mmol) in THF (3 mL) was stirred for 6 h at 25 °C under hydrogen using PtO_2 (25 mg) as a

catalyst. The reaction mixture was filtered, and the filtrate was concentrated in vacuo to give **12b** (19 mg, 96% yield) as a powder. IR ν_{max}(Nujol) 3360 (broad), 1720, 1638 cm^{-1}; ^{1}H-NMR (pyridine-d5) δ 0.86-0.91 (9H, m), 1.26 (52H, bs), 1.27-1.60 (3H, m), 1.63-1.88 (10H, m), 2.46 (2H, t, *J*=7.5 Hz), 2.79 (1H, d, *J*=1.1 Hz), 2.81 (1H, s), 3.13 (1H, dd, *J*=6.3, 15.9 Hz), 3.26 (1H, dd, *J*=6.5, 15.9 Hz), 4.20 (1H, d, *J*=9.1 Hz), 4.45-4.52 (3H, m), 5.11 (1H, d, *J*=5.9 Hz, C2-H), 5.26-5.34 (2H, m), 5.71-5.74 (1H, m), 6.52 (1H, dd, *J*=9.2, 10.7 Hz), 8.68 (1H, d, *J*=9.3 Hz, NH); MS (FAB, positive) *m/z* 972 $(M+Na)^+$, 950 $(M+H)^+$; MS (FAB, negative) 948 $(M-H)^-$. *Anal.* Calcd. for $C_{49}H_{92}NO_{13}P$ (950.2): C, 61.94; H, 9.76; N, 1.47; P, 3.26. Found: C, 61.25; H, 9.88; N, 1.34; P, 3.17.

Diphenylmethyl 2,6-Anhydro-3-deoxy-5-*O*-diphenylphosphono-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonate (14). A solution of **10a** (260 mg, 0.187 mmol) in THF (30 mL) containing 10% Pd on carbon (300 mg) was stirred vigorously under hydrogen at 24 °C for 10 h. After filtration, Ph_2CN_2 (200 mg, 1.03 mmol) was added to this solution, and the mixture was stirred for 16 h at 24 °C, concentrated in vacuo, and chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (2:1) gave **14** (235 mg, 100%) as a gum. IR ν_{max}(film) 3600-3200, 2925, 2850, 1735, 1658, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (9H, t, *J*=6.2-6.9 Hz), 1.25 (56H, bs), 1.35-1.72 (8H, m), 1.90-1.99 (2H, m), 2.19 (2H, t, *J*=7.4-7.7 Hz), 2.34-2.37 (2H, m), 3.19 (1H, bs, OH), 3.38-3.59 (3H, m), 4.47 (1H, m), 4.73 (1H, q, *J*=9.5 Hz), 4.80 (1H, d, *J*=6.0 Hz), 5.10 (1H, quintet, *J*=6.1 Hz), 5.60 (1H, dd, *J*=9.4, 10.8 Hz), 6.12 (1H, d, *J*=8.3 Hz, NH), 6.94 (1H, s), 7.10-7.38 (20H, m); MS (FAB, positive) *m/z* 1251 $(M^+)°$, 1274 $(M+Na)^+$. *Anal.* Calcd. for $C_{74}H_{110}NO_{13}P$ (1252.7): C, 70.95; H, 8.85; N, 1.12; P, 2.47. Found: C, 70.59; H, 8.70; N, 1.28; P, 3.00.

Diphenylmethyl 2,6-Anhydro-3,7-dideoxy-5-*O*-diphenylphosphono-7-fluoro-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonate (15). To a solution of **14** (251 mg, 0.20 mmol) in CH_2Cl_2 (10 mL) was added dropwise a solution of DAST (1.29 g, 8.0 mmol) in CH_2Cl_2 (5 mL) at 0-5°C with stirring. After stirring for 3.5 h at 0 °C, the reaction mixture was diluted with EtOAc, washed with aqueous $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, concentrated in vacuo, and chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (4:1) gave **15** (218 mg, 87%) as a gum. IR ν_{max}(Nujol) 3340 (w), 1738, 1663, 1590 (w) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (9H, t, *J*=6.4-6.8 Hz), 1.25 (60H, bs), 1.35-1.60 (4H, m), 1.80-2.00 (2H, m), 2.16 (2H, t, *J*=7.4-7.7 Hz), 2.33-2.50 (2H, m), 3.85 (1H, dd, J=9.9, 24.6 Hz), 4.37-4.48 (2H, m), 4.68 (1H, dd, *J*=9.2, 18.7 Hz), 4.86 (1H, d, *J*=6.0 Hz), 5.08 (1H, m), 5.58 (1H, dd, *J*=8.9, 11.0 Hz), 6.11 (1H, d, *J*=7.9 Hz, NH), 6.96 (1H, s), 7.12-7.41 (20H, m); MS (FAB, positive) *m/z* 1254 $(M+H)^+$; High Resolution MS (FAB, positive) *m/z*: Calcd. for $C_{74}H_{110}NO_{12}FP$: 1254.7750; Found: 1254.7793; *Anal.* Calcd. for $C_{74}H_{109}NO_{12}FP$ (1254.7): C, 70.84; H, 8.76; N, 1.12; F, 1.51; P, 2.47. Found: C, 70.58; H, 8.46; N, 1.18; F, 1.33; P, 2.45.

2,6-Anhydro-3,7-dideoxy-5-*O*-diphenylphosphono-7-fluoro-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonic Acid (16). A solution of **15** (187 mg, 0.149 mmol) in THF (40 mL) containing 10% Pd on carbon (700 mg) was stirred for 18 h at 24 °C. The reaction mixture was filtered, and concentrated in vacuo to give 167 mg of a mixture of **16** and Ph_2CH_2. This mixture was pure enough for the next hydrogenolysis. However, when short column

chromatography was performed, a fair amount of the carboxylic acid was absorbed. Elution with EtOAc-MeOH (95:5) gave pure **16** (107 mg, 66%) as a gum. IR ν_{max}(film) 3380, 1736, 1680, 1640, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (9H, t, *J*=5.8-7.0 Hz), 1.10-1.70 (64H, m, containing bs at δ 1.25), 2.14-2.20 (4H, m), 2.39-2.50 (2H, m), 4.22-4.60 (4H, m), 4.66 (1H, d, *J*=6.0 Hz), 4.74 (1H, dd, *J*=8.9, 17.5 Hz), 5.09 (1H, quintet, *J*=6.0 Hz), 5.63 (1H, dd, *J*=9.0, 10.9 Hz), 6.65 (1H, d, *J*=8.9 Hz, NH), 7.14-7.38 (10H, m); MS (FAB, positive) *m/z* 1110 $(M+Na)^+$; High Resolution MS (FAB, positive) *m/z*: Calcd. for $C_{61}H_{99}NO_{12}FPNa$: 1110.6785; Found: 1110.6770. *Anal.* Calcd. for $C_{61}H_{99}NO_{12}FP$ (1088.4): C, 67.31; H, 9.17; N, 1.29; F, 1.75; P, 2.85. Found: C, 67.01; H, 9.01; N, 1.29; F, 1.80; P, 2.69.

2,6-Anhydro-3,7-dideoxy-7-fluoro-5-*O*-phosphono-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonic Acid (17). A solution of **16** (16 mg, 0.015 mmol) in THF (3 mL) containing PtO_2 (22 mg) was hydrogenolized with stirring for 17 h at 24 °C, filtered, and concentrated in vacuo to give **17** (14 mg, quantitatively) as an amorphous solid. IR ν_{max}(Nujol) 3570, 3350, 3000-2500 (broad), 1742, 1727, 1710 (shoulder), 1654 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (9H, t, *J*=6.1-7.0 Hz), 1.26 (58H, bs), 1.50-1.63 (6H, m), 2.16-2.25 (2H, m), 2.27-2.32 (2H, m), 2.55-2.75 (2H, m), 3.89 (1H, m), 4.31-4.50 (2H, m), 4.58-4.61 (2H, m), 4.76 (1H, bs), 5.12 (1H, m), 5.36 (1H, m), 6.91 (1H, d, *J*=9.5 Hz, D_2O exchanged, NH); MS (FAB, positive) *m/z* 958 $(M+Na)^+$; MS (FAB, negative) *m/z* 934 $(M-H)^-$; High Resolution MS (FAB, positive) *m/z*: Calcd. for $C_{49}H_{91}NO_{12}FPNa$: 958.6102; Found: 958.6132. *Anal.* Calcd. for $C_{49}H_{91}NO_{12}FP$ (936.2): C, 62.86; H, 9.80; N, 1.50; F, 2.03; P, 3.31. Found: C,62.55; H, 9.68; N, 1.32; F, 1.87; P, 3.01.

2,6-Anhydro-7-*O*-benzyloxycarbonyl-3-deoxy-5-*O*-diphenylphosphono-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonic Acid (18). A solution of **10a** (65 mg, 0.047 mmol) in CH_2Cl_2 (2 mL) and CF_3COOH (0.20 mL, 2.60 mmol) was stirred for 2 h at 0-5 °C. To this reaction mixture, was added powdered $NaHCO_3$ (218 mg, 2.60 mmol) and H_2O (2 mL), and diluted with EtOAc, which was washed with H_2O and brine, dried over $MgSO_4$, filtered, concentrated and chromatographed on a silica gel column. Elution with EtOAc, and then EtOAc-MeOH (95:5) gave fractions of product, which were concentrated in vacuo, and diluted with EtOAc, washed with H_2O, dried over $MgSO_4$, filtered, and concentrated in vacuo to give **18** (32 mg, 56%) as a gum. IR ν_{max}(film) 3400 (broad), 2935, 2860, 1750, 1660 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (9H, t, *J*=5.7-6.9 Hz), 1.24 (58H, bs), 1.37-1.59 (6H, m), 2.07-2.19 (4H, m), 2.38-2.44 (2H, m), 4.10 (1H, m), 4.26-4.55 (4H, m), 4.68 (1H, dd, *J*=9.5, 18.8 Hz), 5.02-5.17 (3H, m), 5.59 (1H, m), 6.84 (1H, broad, NH), 7.10-7.33 (15H, m); MS (FAB, positive) *m/z* 1242 $(M+Na)^+$. *Anal.* Calcd. for $C_{69}H_{106}NO_{15}P$ (1220.6): C, 67.90; H, 8.75; N, 1.15; P, 2.54. Found: C, 67.65; H, 8.70; N, 1.13; P, 2.68.

Diphenylmethyl 2,6-Anhydro-7-*O*-[2,6-anhydro-7-*O*-benzyloxycarbonyl-3-deoxy-5-*O*-diphenylphosphono-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyl-oxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonyl]-3-deoxy-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonate (19). To a solution of acid **18** (183 mg, 0.15 mmol) and diol **8a** (30 mg, 0.02 mmol) in CH_2Cl_2 (8 mL) were added DMAP (18.3 mg, 0.15 mmol) and DCC (39 mg, 0.19 mmol) under nitrogen with stirring at 24 °C. After stirring for 16 h, the mixture

was concentrated in vacuo, and chromatographed on a preparative silica gel plate. Development with cyclohexane-EtOAc (3:1) gave **19** (35 mg, 11%, R_f=0.364) as a gum and **19'** (46 mg, 22%, R_f=0.509) as a gum. Physical data of **19**: IR ν_{max}(film) 3320, 2930, 2850, 1740, 1657, 1626 (w), 1590 (w) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (18H, t, *J*=6.4-6.8 Hz), 1.25 (120H, bs), 1.37-1.61 (8H, m), 1.92 (2H, t, *J*=7.3-8.2 Hz), 2.05 (2H, t, *J*=7.3-8.2 Hz), 2.19 (2H, t, *J*=7.5-7.7 Hz), 2.27 (2H, t, *J*=7.4-7.7 Hz), 2.45-2.60 (4H, m), 3.69 (2H, bs), 3.91 (1H, bs, OH), 4.20-4.54 (7H, m), 4.69-4.75 (3H, m), 5.03-5.23 (5H, m, containing 2H, AB-q, *J*=12.3 Hz at δ 5.05 and 5.11), 5.43 (1H, t, *J*=7.5-8.2 Hz), 6.22 (1H, d, *J*=8.3 Hz, NH), 6.38 (1H, d, *J*=9.0 Hz, NH), 6.93 (1H, s), 7.15-7.36 (25H, m); MS (FAB, positive) *m/z* 2222 $(M+H)^+$, 2221; *Anal.* Calcd. for $C_{131}H_{205}N_2O_{24}P$ (2223.0): C, 70.78; H, 9.30; N, 1.26. Found: C, 71.50; H, 8.71; N, 1.34. ^{1}H-NMR of **19'**: ($CDCl_3$) δ 0.88 (9H, t, *J*=6.3-6.9 Hz), 1.25 (58H, bs), 1.39-1.97 (26H, m), 2.06-2.17 (4H, m), 2.38-2.42 (2H, m), 3.60 (1H, m), 3.82 (1H, m), 3.97-4.16 (2H, m), 4.40-4.63 (3H, m), 4.77 (1H, d, *J*=5.9 Hz, C2-H), 5.10 (2H, s), 5.10 (1H, m), 5.95 (1H, t, *J*=9.5-9.9 Hz), 6.13 (1H, d, *J*=8.9 Hz, NH), 7.15-7.40 (16H, m); MS (FAB, positive) *m/z* 1426 $(M+H)^+$, 1448 $(M+Na)^+$.

2,6-Anhydro-7-*O*-[2,6-anhydro-3-deoxy-5-*O*-diphenylphosphono-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonyl]-3-deoxy-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetra-decanoyl]-D-*glycero*-D-*ido*-heptonic Acid (20). Hydrogenolysis of **19** (51 mg, 0.023 mmol) in THF (8 mL) containing 10% Pd on carbon (32 mg) for 6 h at 24 °C under hydrogen gave **20** (46 mg, containing Ph_2CH_2) as a gum after filtration and concentration. This mixture was pure enough, and employed for the next reaction without further purification. For analytical sample, the mixture (11 mg) was partly chromatographed to remove Ph_2CH_2 on a silica gel short column. Elution with EtOAc-MeOH (95:5) gave 4 mg of **20**. Most of **20** was absorbed on a silica gel column. IR ν_{max}(film) 3380 (broad), 2925, 2850, 1735, 1650, 1590 (w) cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (18H, t, *J*=6.3-6.8 Hz), 1.25 (120H, bs), 1.43-1.63 (8H, m), 1.89-2.57 (12H, m), 3.42 (1H, t, *J*=9.0 Hz), 3.52-3.70 (3H, m), 3.80-3.94 (2H, m), 4.45 (2H, m), 4.55 (1H, m), 4.56-4.62 (2H, m), 4.72 (1H, m), 5.07-5.17 (2H, m), 5.26 (1H, t, *J*=9.0 Hz), 5.59 (1H, m), 6.44 (1H, d, *J*=9.2 Hz, NH), 6.52 (1H, d, *J*=7.4 Hz, NH), 7.14-7.39 (20H, m); MS (FAB, positive) *m/z* 1922, 1921 $(M+H)^+$, 1828; High resolution MS (FAB, positive) *m/z*: Calcd. for $C_{110}H_{189}N_2O_{22}PNa$; 1944.3367. Found: 1944.3370. *Anal.* Calcd. for $C_{110}H_{189}N_2O_{22}P$ (2223.0): C, 68.72; H, 9.91; N, 1.46; P, 1.61. Found: C, 68.20; H, 10.05; N, 1.82; P, 164.

2,6-Anhydro-7-*O*-[2,6-anhydro-3-deoxy-5-*O*-phosphono-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonyl]-3-deoxy-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonic Acid (21).

Hydrogenolysis of **20** (42 mg, above obtained and containing Ph_2CH_2) in THF (6 mL) containing PtO_2 (50 mg) as a catalyst at 24 °C for 16 h gave 40 mg of residue after concentration. The residue was dissolved in $CHCl_3$ (13 mL), MeOH (26 mL) and aqueous 0.1 M HCl (10.4 mL). To this solution, was added another volume of $CHCl_3$ (13 mL) and aqueous 0.1 M HCl (13 mL) to separate the solution into two phases. The lower $CHCl_3$ phase was collected and concentrated to give **21** (31 mg, 80%) as a foam. IR ν_{max}(film) 3420, 2920, 2850, 2520, 1730, 1640 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (18H, t, *J*=6.4-6.8 Hz), 1.26 (114H, bs), 1.45-1.71 (14H, m), 2.10-2.24 (4H, m), 2.24-2.32 (4H, m), 2.52-2.68 (4H, m), 3.54 (1H, m), 3.74 (1H, m), 3.91 (2H, m), 4.00 (1H, m), 4.13 (1H, m), 4.35-4.51 (3H, m), 4.60 (1H, d, *J*=6.0), 4.69 (1H, d,

J=6.0), 4.93 (1H, m), 5.12 (2H, m), 5.22 (1H, m), 5.37 (1H, m), 6.71 (1H, broad, NH), 6.83 (1H, broad, NH); MS (FAB, positive) *m/z* 1791 $(M+Na)^+$; High resolution MS (FAB, positive) *m/z*: Calcd. for $C_{98}H_{181}N_2O_{22}PNa$; 1792.2741. Found: 1792.2732. *Anal.* Calcd. for $C_{110}H_{189}N_2O_{22}P$ (1770.5): C, 66.48; H, 10.31; N, 1.58; P, 1.75. Found: C, 66.72; H, 10.74; N, 1.75; P, 1.90.

Diphenylmethyl 2,6-Anhydro-7-*O*-[2,6-anhydro-3,7-dideoxy-5-*O*-diphenylphosphono-7-fluoro-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyl-oxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonyl]-3-deoxy-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonate (22). To a solution of carboxylic acid **16** (96 mg, 0.088 mmol) and diol **8a** (25 mg, 0.024 mmol) in CH_2Cl_2 (5 mL), were added DMAP (11 mg, 0.090 mmol) and DCC (22 mg, 0.107 mmol). The mixture was stirred for 16 h at 24 °C under nitrogen, filtered, and concentrated in vacuo to give a mixture, which was chromatographed on a preparative TLC plate. Development with cyclohexane-EtOAc (3:1) gave **22** (21 mg, 11%, R_f=0.500) as an amorphous solid. IR ν_{max}(Nujol) 3360, 1737, 1725 (shoulder), 1657, 1632 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (18H, t, *J*=6.3-6.9 Hz), 1.25 (112H, bs), 1.40-1.70 (16H, bm),1.91 (2H, t, *J*=7.3-7.8 Hz), 2.08 (2H, t, *J*=7.3-8.2 Hz), 2.17 (2H, t, *J*=7.4-7.8 Hz), 2.29 (2H, t, *J*=7.5 Hz), 2.45-2.54 (4H, m), 3.65-3.74 (2H, m), 4.10 (1H, dm, *J*=25 Hz), 4.25 (1H, dd, *J*=2.0, 10.0 Hz), 4.31-4.60 (5H, m), 4.66-4.76 (3H, m), 5.05-5.22 (3H, m), 5.49 (1H, dd, *J*=8.2, 9.4 Hz), 6.22 (1H, d, *J*=8.4 Hz, NH), 6.38 (1H, d, J=8.9 Hz, NH), 6.94 (1H, s), 7.16-7.21 (5H, m), 7.28-7.38 (15H, m); MS (FAB, positive) *m/z* 2090 $(M+H)^+$; High resolution MS (FAB, positive) *m/z*: Calcd. for $C_{123}H_{199}N_2O_{21}FP$; 2090.4287. Found: 2090.4287.

2,6-Anhydro-7-*O*-[2,6-anhydro-3,7-dideoxy-5-*O*-diphenylphosphono-7-fluoro-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonyl]-3-deoxy-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonic Acid (23). Compound **22** (21 mg) was treated as described in the formation of **19** from **20** to give **23** (21 mg, quantitatively) as a gum. IR ν_{max}(film) 3380, 2920, 2850, 1737, 1675 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.88 (18H, t, *J*=5.9-7.2 Hz), 1.25 (112H, bs), 1.40-1.70 (16H, m), 2.10-2.23 (6H, m), 2.30 (2H, t, *J*=7.3-8.7 Hz), 2.43-2.62 (4H, m), 3.49 (1H, t, *J*=9.3 Hz), 3.70-3.92 (1H, m), 4.01-4.78 (10H, m), 5.06-5.19 (3H, m), 5.47 (1H, dd, *J*=8.6, 10.1 Hz), 6.60 (1H, d, *J*=8.7 Hz, NH), 6.66 (1H, d, *J*=8.5 Hz, D_2O exchanged, NH), 7.14-7.34 (10H, m); MS (FAB, positive) *m/z* 1946 $(M+Na)^+$; High resolution MS (FAB, positive) *m/z*: Calcd. for $C_{110}H_{188}N_2O_{21}FPNa$; 1946.3308. Found: 1946.3290.

2,6-Anhydro-7-*O*-[2,6-anhydro-3,7-dideoxy-7-fluoro-5-*O*-phosphono-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonyl]-3-deoxy-3-tetradecanamido-4-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-D-*glycero*-D-*ido*-heptonic Acid (24). Compound **23** (20 mg) was treated as described in the formation of **21** from **20** to give **24** (18 mg, quantitatively) as a powder. IR ν_{max}(Nujol) 3300-2500, 1733, 1654 cm^{-1}; ^{1}H-NMR ($CDCl_3$ + D_2O) δ 0.88 (18H, t, *J*=6.4-6.8 Hz), 1.26 (112H, bs), 1.45-1.66 (16H, m), 2.10-2.25 (4H, m), 2.25-2.37 (4H, m), 2.45-2.74 (4H, m), 3.50 (1H, m), 3.60 (1H, t, *J*=9.4 Hz), 3.95-4.20 (3H, m), 4.28-4.42 (2H, m), 4.47- 4.52 (1H, m), 4.61-4.64 (2H, m), 4.81 (1H, m), 5.01-5.19 (3H, m), 5.23 (1H, dd, *J*=8.9,11.7 Hz), 5.38 (1H, dd, *J*=8.9, 10.9 Hz), 6.68 (1H, broad before addition of D_2O, NH), 6.92 (1H, broad before

addition of D_2O, NH); MS (FAB, positive) *m/z* 1772 $(M+H)^+$, 1794 $(M+Na)^+$; High resolution MS (FAB, positive) *m/z*: Calcd. for $C_{98}H_{181}N_2O_{21}FP$; 1772.2910. Found; 1772.2928. *Anal.* Calcd. for $C_{98}H_{180}N_2O_{21}FP$ (1772.5): C, 66.41; H, 10.24; N, 1.58; F, 1.07; P, 1.75. Found: C, 65.94; H, 10.05; N, 1.76; F, 1.01; P, 1.72.

Preparation of aqueous solutions of compounds for measurement of the biological activity. Compound **12a** (3.1 mg) obtained above was dissolved in $CHCl_3$ (1 mL), MeOH (2 mL), and 0.1 M HCl (0.8 mL) with stirring at 5-10 °C. Additional $CHCl_3$ (1 mL) and 0.1 M HCl (1 mL) were added to this solution to ensure separation into two phases. The lower chloroform phase was collected, and concentrated to give 3.0 mg of **12a**, which was dissolved in 0.642 mL of aqueous 0.1% Et_3N (v/v) solution for measurement of the biological activity. Aqueous 0.1% Et_3N solutions of compounds **12b**, **17**, **21**, and **24** were prepared as described above in the preparation of the solution of compound **12a**.

Procedures for measurement of the biological activity. The sources of the materials used in the study are as follows: Lipopolysaccharide (LPS) from *E. coli* serotype 026:B6, 12-*O*-tetradecanoylphorbor acetate (TPA) and prednisolone were from SIGMA, St. Louis, MO. PRMI-6140 medium, fetal bovine serum (FBS), and newborn calf serum (NBCS) were from GIBCO, Grand Island, NY. Human tumor necrosis factor-α enzyme-linked immunosorbent assay (TNFα ELISA) kit was from Genzyme, Cambridge, MA.

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

Production of TNFα by U937 cells: U937 cells (1 x 10^4/200 μL/well) were plated in 96-well plates (Corning, Cambridge, MA), and were cultured in the presence of TPA (30 ng/mL) for 72 h at 37° C. After removing of the supernatant, the cell were incubated in 200 μL of fresh RPMI-1640 medium containing 10% of NBCS, 10 ng/mL of LPS and graded concentrations of compounds in a humidified atmosphere of 5% of CO_2 for 4.5 h at 37° C. After incubation, the amounts of TNFα produced in the culture supernatants were determined by the TNFα ELISA kits. As a control, the amount of TNFα produced by U937 cells, which were stimulated with 10 ng/mL of LPS in the absence of compounds, was used. The relative amounts of TNFα were calculated as percentages of the control amounts.

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