[Chem. Pharm. Bull. 35(11)4436—4441(1987)]

Lipid A and Related Compounds. XIV.[1] A New Synthesis of Lipid Y, the Reducing Sugar Moiety of *Salmonella*- and *Proteus*-Type Lipid A

KIYOSHI IKEDA, SHIN-ICHI NAKAMOTO, TOSHIO TAKAHASHI, and KAZUO ACHIWA*

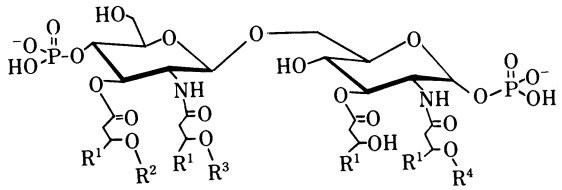
School of Pharmaceutical Science, University of Shizuoka, Oshika 2-2-1, Shizuoka 422, Japan

(Received March 26, 1987)

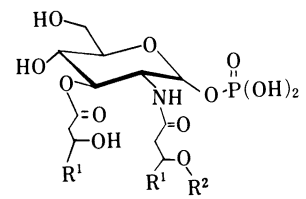
An efficient synthesis of lipid Y by the use of chemoselective debenzylation as a key step is described.

Keywords——lipid A; lipid Y; glucosamine derivative; chemoselective debenzylation; lipid Y synthesis

Lipid A, a constituent of lipopolysaccharide (LPS) of gram-negative bacteria, has been shown to play an important role in the various biological activities[2] of LPS, such as pyrogenicity, lethal toxicity, adjuvant activity and so on. The chemical structure of lipid A consists of a $\beta(1\rightarrow6)$ linked D-glucosamine disaccharide which carries phosphate residues at positions 1 and 4′ as well as amide-bound and ester-bound D-3-hydroxy and/or acyloxy fatty acids,[3] as indicated in Chart 1.



1a: $R^1=CH_3(CH_2)_{10}-$, $R^2=R^3=R^4=H$ (*Salmonella* mutant)
1b: $R^1=CH_3(CH_2)_{10}-$, $R^2=CH_3(CH_2)_{12}CO-$, $R^3=CH_3(CH_2)_{10}CO-$, $R^4=H$ (*Escherichia coli*)
1c: $R^1=CH_3(CH_2)_{10}-$, $R^2=CH_3(CH_2)_{12}CO-$, $R^3=CH_3(CH_2)_{10}CO-$, $R^4=CH_3(CH_2)_{14}CO-$ (*Salmonella minnesota*)
1d: $R^1=CH_3(CH_2)_{10}-$, $R^2=CH_3(CH_2)_{12}CO-$, $R^3=CH_3(CH_2)_{12}CO-$, $R^4=CH_3(CH_2)_{14}CO-$ (*Proteus mirabilis*)



lipid Y (**2**): $R^1=CH_3(CH_2)_{10}-$, $R^2=CH_3(CH_2)_{14}CO-$

Chart 1

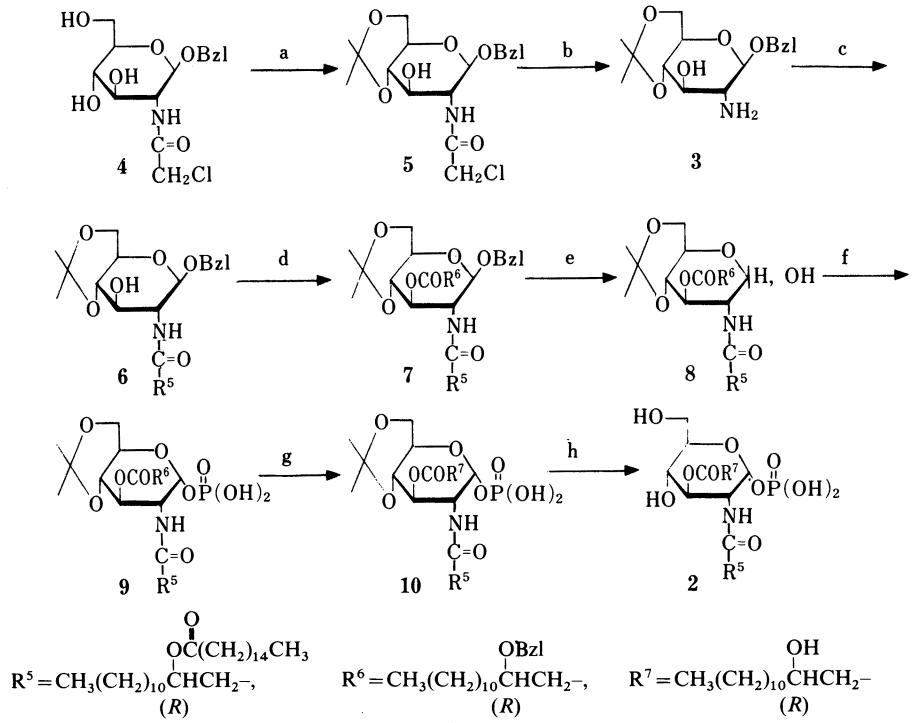
Although synthetic procedures have been developed, improved methods for chemoselective protection and deprotection of 2-amino-2-deoxy-D-glucose derivatives remain important, especially in the field of lipid A synthesis.[1,4]

In a recent communication,[1d] we reported an efficient synthesis of lipid Y,[5] which

corresponds to the reducing glucosamine unit of *Salmonella minnesota* (**1c**)[3c] and *Proteus mirabilis* lipid A (**1d**),[3c] using a chemoselective debenzylation of the glycosidic benzyl group of a 2-amino-2-deoxy-D-glucose derivative that carries another benzyl group protecting a 3-hydroxytetradecanoyl substituent at O-3. In our strategy, the key intermediate (**3**) that we designed is useful for the synthesis of two precursors of lipid A, *i.e.*, the non-reducing unit and the reducing unit. This paper describes in detail the successful application of the key intermediate (**3**) to lipid Y synthesis.

In the initial stage of the synthesis of lipid Y, the key intermediate (**3**) was prepared starting from benzyl 2-chloroacetamido-2-deoxy-β-D-glucopyranoside (**4**)[6] in two steps (Chart 2). The latter compound (**4**) was treated with 2,2-dimethoxypropane in dimethylformamide (DMF) in the presence of a catalytic amount of *p*-toluenesulfonic acid (*p*-TSA) to afford compound (**5**) in 87% yield. Removal of the chloroacetyl group of **5** was effected by forming a pyridinium salt with pyridine as a base, followed by hydrolysis to give the key intermediate (**3**) in 80% yield. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **3** showed the presence of a doublet (J=8.1 Hz) at 4.35 ppm assigned to the anomeric proton and an AB quartet (J=7.9 Hz) at 4.57 and 4.90 ppm due to the methylene protons of the glycosidic benzyl group.

The key intermediate (**3**) thus obtained was applied for the synthesis of lipid Y as follows. The free amino group of **3** was acylated with optically pure (*R*)-3-hexadecanoyloxytetradecanoic acid and dicyclohexylcarbodiimide (DCC) in CH_2Cl_2 to afford the *N*-acylated compound (**6**) in 90% yield. The remaining hydroxyl group of **6** was acylated with optically pure (*R*)-3-benzyloxytetradecanoic acid, DCC, and a catalytic amount



a) $(CH_3)_2C(OCH_3)_2$, *p*-TSA; b) i) pyridine, ii) 5% aq. NaOH–MeOH (1 : 1); c) R^5CO_2H, DCC; d) R^6CO_2H, DCC, 4-dimethylaminopyridine; e) 10% Pd/C, H_2 in THF–EtOH (1 : 1); f) i) *n*-BuLi, $(BzlO)_2POCl$, ii) 10% Pd/C, H_2; g) Pd-black, H_2; h) 90% AcOH

Chart 2

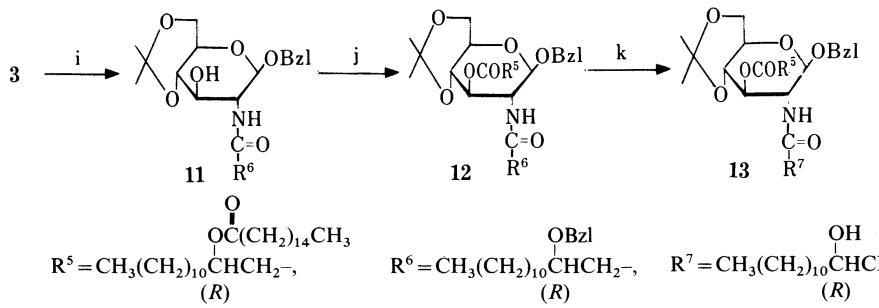
of 4-dimethylaminopyridine (DMAP) in CH_2Cl_2 to afford the triacylated compound (**7**) in 72% yield.

Selective hydrogenolysis of the glycosidic benzyl group of **7** was examined under various conditions, as follows. At first, when a solution of **7** in benzene–MeOH (5 : 1) was hydrogenated for 30 h at room temperature under slight pressure in the presence of 10% Pd-on-carbon, the starting compound (**7**) was recovered unchanged. When a solution of **7** in tetrahydrofuran (THF)–MeOH (5 : 1) was hydrogenated for 15 h at room temperature under atmospheric pressure in the presence of 10% Pd-on-carbon, the desired product (**8**) was obtained in poor yield, together with a considerable amount of the compound didebenzylated at glycosidic and O-3 protecting groups. A small amount of unchanged starting material (**7**) was recovered. When a solution of **7** in THF was hydrogenated for 72 h at 30 °C under 50 atm in the presence of 10% Pd-on-carbon, the expected 1-debenzylated compound (**8**) was isolated in 59% yield as a single anomer. NMR and thin-layer chromatographic (TLC) analyses of this 1-debenzylated product (**8**) indicated high chemoselectivity. The ^{1}H-NMR spectrum of **8** showed the presence of the methylene proton signal of the benzyl group protecting the 3-(3-benzyloxy)tetradecanoyl substituent at 4.50 ppm (singlet) and the disappearance of the signal attributed to the glycosidic benzyl group. In addition, the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **8** showed a signal at 92.3 ppm (doublet) suggestive of the α-anomeric configuration. As a result of the improvement of chemoselectivity of the debenzylation of **7**, the most promising result was obtained as follows. When a solution of **7** in THF–EtOH (1 : 1) was hydrogenated for 38 h at 30 °C under slight pressure in the presence of 10% Pd-on-carbon, the desired product (**8**) was obtained in an acceptable yield of 64% as a mixture of anomers ($\alpha : \beta = 3 : 1$). This method is satisfactory for practical use. In the ^{1}H-NMR spectrum of **8**, two sets of doublet at 6.14 and 6.54 ppm were assigned to the amide protons of the α-anomer and the β-anomer, respectively.

This assignment was verified by observing the disappearance of the ^{1}H-NMR signal of the β-anomer after treatment with an anomerization reagent. The α/β ratio of **8** was determined by integration of the amide proton signals in the ^{1}H-NMR spectrum. Complete conversion of β- into α-anomer was observed when the mixture was dissolved in THF–AcOH (3 : 1) and stirred for 48 h at 45 °C.

The glycosidic hydroxyl group of **8** was then phosphorylated with dibenzylphosphorochloridate and *n*-butyllithium in THF at −70 °C.[7] The reaction mixture was directly hydrogenolyzed with 10% Pd-on-carbon to afford the 1-phosphate (**9**) in 48% yield after purification by preparative TLC ($CHCl_3$–MeOH = 5 : 1). Deprotection of the remaining benzyl group of **9** was carried out by hydrogenolysis over Pd-black in THF–MeOH (1 : 3) to afford **10** in 63% yield after purification by preparative TLC ($CHCl_3$–MeOH = 5 : 1). Finally, deprotection of **10** by treatment with 90% AcOH gave **2** in 91% yield after purification by preparative TLC ($CHCl_3$–MeOH = 5 : 1). The triacylglucosamine-1-phosphate (**2**) was clearly positive with the specific spray reagent for the phosphoric group.[8] The structure of **2** was assigned on the basis of the positive fast atom bombardment mass spectrometry (FAB MS), which showed an $(M+Na)^+$ ion at *m/z* 973, $(M-H_2PO_4)^+$ at 853, $(M-C_{16}+Na+H)^+$ at 734, and $(M-C_{16}-OH-C_{14}-H_2PO_4+2H)^+$ at 388. The specific rotation of **2** was in good agreement with that of lipid Y reported by Shiba's group.[9] The structures of all compounds were characterized by ^{1}H- and ^{13}C-NMR spectroscopies, as well as infrared (IR) spectroscopy and elemental analyses.

In order to check the limitations of this chemoselective debenzylation, we examined the hydrogenation of **12** in THF at 30 °C for 24 h in the presence of 10% Pd-on-carbon. In the case of **12**, the benzyl group protecting the 2-(3-benzyloxy)tetradecanamido substituent was removed as readily as the glycosidic benzyl group, to give **13** in 68% yield. These results suggested that the order of reactivity of debenzylation is the benzyl group on an amide



i) R^6CO_2H, DCC; j) $R^5=CO_2H$, DCC, 4-dimethylaminopyridine; k) 10% Pd/C, H_2 in THF

Chart 3

substituent ≋ the glycosidic benzyl group > the benzyl group on an ester substituent.

This method of chemoselective removal of the benzyl group should be of great help for the synthesis of natural products such as complex carbohydrates.

Experimental

All melting points are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL JNM-FX (90 MHz) FT-NMR spectrometer with tetramethylsilane (in $CDCl_3$) as an internal standard. ^{13}C-NMR spectra were recorded on a JEOL JNM-FX90Q (22.5 MHz) FT-NMR spectrometer with tetramethylsilane (in $CDCl_3$) as an internal standard reference. Abbreviations are as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. IR spectra were recorded on a JASCO A-202 infrared spectrophotometer. Optical rotations were determined with a JASCO DIP-140 digital polarimeter.

Column chromatography was carried out on silica gel (Kiesel gel-60, 70—230 mesh, Merck). TLC on Kiesel gel 60-F (Merck) was used to monitor the reaction and to ascertain the purity of reaction products. The spots were visualized by spraying with aqueous sulfuric acid and then heating.

Benzyl 2-Chloroacetamido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (5)——2,2-Dimethoxypropane (24.9 g, 240 mmol) was added to a stirred solution of benzyl 2-chloroacetamido-2-deoxy-β-D-glucopyranoside (**4**) (24.4 g, 71 mmol), prepared as described in the literature,[6] and *p*-TSA (1.38 g, 8 mmol) in DMF (150 ml) at room temperature under nitrogen. After 12 h, the reaction mixture was poured into a solution of water (1200 ml) and 5% aqueous sodium hydroxide (13 ml). The whole was extracted with three 150 ml portions of ethyl acetate. The organic extracts were combined, washed with water (100 ml), and dried ($MgSO_4$). After removal of the solvent, the residue was purified by crystallization from *n*-hexane to furnish **5** (23.9 g, 87%) as a white powder, mp 173—176 °C. $[\alpha]_D^{22}$ −91.4° ($c=1.15$, $CHCl_3$). IR (KBr): 3456 (OH), 3330 (NH), 1694 (amide), 853 (Me_2C), 700 (Ph) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 and 1.52 (each 3H, s, Me_2C), 3.99 (2H, s, $COCH_2$), 4.44—4.98 (3H, m, CH_2Ph and H-1), 7.31 (5H, s, Ph). *Anal.* Calcd for $C_{18}H_{24}ClNO_6$: C, 56.03; H, 6.27; N, 3.63. Found: C, 55.95; H, 6.28; N, 3.68.

Benzyl 2-Amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (3)——A solution of **5** (23.9 g, 62 mmol) in pyridine (120 ml) was heated at 85—90 °C for 1.5 h, then allowed to cool. The solvent was evaporated off and the residue was dissolved in a solution (152 ml) of 5% aqueous sodium hydroxide and methanol (1 : 1), then stirred at 30 °C for 16 h. After removal of the solvent, the residue was dissolved in chloroform (180 ml), washed with water, and dried ($MgSO_4$). After removal of the solvent, the residue was purified by crystallization from MeOH to furnish **3** (15.3 g, 80%) as a white powder, mp 132—133 °C. $[\alpha]_D^{22}$ −98.0° ($c=1.25$, $CHCl_3$). IR (KBr): 3572 (OH), 3376 (NH), 854 (Me_2C), 692 (Ph) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 and 1.50 (each 3H, s, Me_2C), 4.35 (1H, d, $J=8.1$ Hz, H-1), 4.57 and 4.90 (each 1H, d, $J=7.9$ Hz, CH_2Ph), 7.33 (5H, s, Ph). *Anal.* Calcd for $C_{16}H_{23}NO_5$: C, 62.12; H, 7.49; N, 4.52. Found: C, 61.94; H, 7.44; N, 4.55.

Benzyl 2-Deoxy-2-[(*R*)-3-hexadecanoyloxytetradecanamido]-4,6-*O*-isopropylidene-β-D-glucopyranoside (6)——DCC (0.297 g, 1.44 mmol) was added to a stirred solution of **5** (0.371 g, 1.20 mmol) and (*R*)-3-hexadecanoyloxytetradecanoic acid (0.695 g, 1.44 mmol) in dry dichloromethane (8 ml) at 0 °C under nitrogen. The mixture was stirred for 5 h at 0 °C, then at room temperature for 12 h. The resulting suspension was filtered through Celite and evaporated. The residue was chromatographed on silica gel with chloroform–ether (20 : 1) to give **6** (0.840 g, 90%), mp 72—74 °C. $[\alpha]_D^{20}$ −41.1° ($c=1.13$, $CHCl_3$). IR (KBr): 3440 (OH), 3376 (NH), 1731 (ester), 1673 (amide), 853 (Me_2C), 697 (Ph) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (6H, t, $J=5.4$ Hz, $(CH_2)_{10}CH_3$ and $(CH_2)_{14}CH_3$), 1.25 (48H, br s, $(CH_2)_{10}CH_3$ and $(CH_2)_{14}CH_3$), 1.44 and 1.53 (each 3H, s, Me_2C), 2.13—2.52 (4H, m, $COCH_2\times2$), 4.61 and 4.89

(each 1H, d, J=8.1 Hz, CH_2Ph), 6.19 (1H, br d, J=7.8 Hz, NH), 7.32 (5H, s, Ph). ^{13}C-NMR ($CDCl_3$) δ: 99.9 (d, C-1 and s, $Me_2\underline{C}$), 171.3, 174.0 (s, $>C=O \times 2$). *Anal.* Calcd for $C_{46}H_{79}NO_8 \cdot 1/2H_2O$: C, 70.55; H, 10.30; N, 1.79. Found: C, 70.83; H, 10.22; N, 2.17.

Benzyl 3-*O*-[(*R*)-3-Benzyloxytetradecanoyl]-2-deoxy-2-[(*R*)-3-hexadecanoyloxytetradecanamido]-4,6-*O*-isopropylidene-β-D-glucopyranoside (7)——DCC (0.161 g, 0.78 mmol) was added to a stirred solution of **6** (0.503 g, 0.65 mmol), (*R*)-3-benzyloxytetradecanoic acid (0.261 g, 0.78 mmol) and 4-dimethylaminopyridine (0.032 g, 0.26 mmol) in dry dichloromethane (5 ml) at 0 °C under nitrogen. The mixture was stirred for 5 h at 0 °C, then at room temperature for 12 h. The resulting suspension was filtered through Celite and evaporated. The residue was chromatographed on silica gel with chloroform–isopropyl ether (20 : 1) to give **7** (0.508 g, 72%) as a syrup. $[\alpha]_D^{20}$ −21.1° (c=1.80, $CHCl_3$). IR (neat): 3332 (NH), 1732 (ester), 1659 (amide), 860 (Me_2C), 696 (Ph) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, J=4.9 Hz, $(CH_2)_{10}C\underline{H}_3 \times 2$ and $(CH_2)_{14}CH_3$), 1.26 (68H, br s, $(C\underline{H}_2)_{10}CH_3 \times 2$ and $(C\underline{H}_2)_{14}CH_3$), 1.46 and 1.58 (each 3H, s, $\underline{Me}_2C$), 2.05—2.70 (6H, m, $COCH_2 \times 3$), 4.49 (2H, s, $-C\underline{H}_2-CH-OC\underline{H}_2Ph$), 4.57 and 4.87 (each 1H, d, J=7.6 Hz, $-O-CH-OC\underline{H}_2Ph$), 5.89 (1H, br d, J=9.0 Hz, NH), 7.29 (10H, s, Ph × 2). ^{13}C-NMR ($CDCl_3$) δ: 99.7 (d, C-1), 100.7 (s, Me_2C), 169.6, 171.5, 173.5 (s, $>C=O \times 3$). *Anal.* Calcd for $C_{67}H_{111}NO_{10}$: C, 73.79; H, 10.26; N, 1.28. Found: C, 74.05; H, 10.12; N, 1.30.

3-*O*-[(*R*)-3-Benzyloxytetradecanoyl]-2-deoxy-2-[(*R*)-3-hexadecanoyloxytetradecanamido]-4,6-*O*-isopropylidene-α-D-glucopyranose (8)——Compound **7** (0.105 g, 0.105 mmol) was dissolved in THF–EtOH (1 : 1) (10 ml) and the solution was stirred under atmospheric pressure of H_2 in the presence of 10% Pd-on-carbon (87 mg) for 38 h at 30 °C. The catalyst was filtered off and the filtrate was evaporated *in vacuo*. The residue was again dissolved in THF–AcOH (3 : 1) (2.5 ml) and the mixture was kept at 45 °C for 48 h. After removal of the solvent, the residue was chromatographed on neutral aluminium oxide W200 with ether–hexane (10 : 1) to give **8** (0.082 g, 64%) as a syrup. $[\alpha]_D^{23}$ +12.7° (c=1.07, $CHCl_3$). IR (neat): 3320 (OH, NH), 1739 (ester), 1659 (amide), 858 (Me_2C), 694 (Ph) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, J=6.0 Hz, $(CH_2)_{10}C\underline{H}_3 \times 2$ and $(CH_2)_{14}C\underline{H}_3$), 1.25 (68H, br s, $(C\underline{H}_2)_{10}CH_3 \times 2$ and $(C\underline{H}_2)_{14}CH_3$), 1.43 (6H, s, Me_2C), 2.17—2.73 (6H, m, $COCH_2 \times 3$), 4.50 (2H, s, $-CH_2-CH-OC\underline{H}_2Ph$), 6.14 (1H, br d, J=9.0 Hz, NH), 7.31 (5H, s, Ph). ^{13}C-NMR ($CDCl_3$) δ: 92.3 (d, C-1), 99.8 (s, $Me_2\underline{C}$), 170.1, 172.2, 173.4 (s, $>C=O \times 3$). *Anal.* Calcd for $C_{60}H_{105}NO_{10}$: C, 72.03; H, 10.58; N, 1.40. Found: C, 71.83; H, 10.51; N, 1.48.

3-*O*-[(*R*)-Benzyloxytetradecanoyl]-2-deoxy-2-[(*R*)-3-hexadecanoyloxytetradecanamido]-4,6-*O*-isopropylidene-α-D-glucopyranosyl-1-phosphate (9)——*n*-Butyllithium (1.6 M in *n*-hexane) (0.13 ml, 0.21 mmol) was added to a stirred solution of **8** (0.172 g, 0.17 mmol) in dry THF (4.0 ml) at −65 °C under nitrogen. After 2 min, dibenzylphosphorochloridate (0.072 g, 0.26 mmol) in dry THF (0.5 ml) was added at the same temperature and then the mixture was stirred for a further 10 min at −50 °C. The whole mixture was immediately subjected to hydrogenolysis over 10% Pd-on-carbon (80 mg) for 20 h at 30—40 °C under 50 atm to give **9** (0.089 g, 48%) as a syrup after purification by preparative TLC ($CHCl_3$–CH_3OH = 5 : 1). $[\alpha]_D^{22}$ +17.4° (c=0.76, $CHCl_3$). IR (neat): 3450 (OH, NH), 1733 (ester), 1660 (amide), 1260 (>P=O), 865 (Me_2C) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (9H, t, J=6.1 Hz, $(CH_2)_{10}C\underline{H}_3 \times 2$ and $(CH_2)_{14}C\underline{H}_3$), 1.25 (68H, br s, $(C\underline{H}_2)_{10}CH_3 \times 2$ and $(C\underline{H}_2)_{14}CH_3$), 1.43 (6H, s, $\underline{Me}_2C$), 2.11—2.61 (6H, m, $COCH_2 \times 3$), 7.30 (5H, br s, Ph). *Anal.* Calcd for $C_{60}H_{106}NO_{13}P$: C, 66.70; H, 9.89; N, 1.30. Found: C, 66.39; H, 9.43; N, 1.09.

2-Deoxy-2-[(*R*)-3-hexadecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-4,6-*O*-isopropylidene-α-D-glucopyranosyl-1-phosphate (10)——Compound **9** (0.038 g, 0.035 mmol) was dissolved in THF–MeOH (1 : 3) (5 ml) and hydrogenolyzed at 30—35 °C under slight pressure in the presence of Pd-black (22 mg) for 48 h. The catalyst was filtered off and the filtrate was evaporated *in vacuo*. The residue was purified by preparative TLC ($CHCl_3$–MeOH = 5 : 1) to afford **10** (0.022 g, 63%) as a colorless powder, mp 89—92 °C. $[\alpha]_D^{24}$ +15.0° (c=0.44, $CHCl_3$). IR (KBr): 3450 (OH, NH), 1733 (ester) (amide), 1260 (>P=O), 865 (Me_2C) cm^{-1}. *Anal.* Calcd for $C_{53}H_{100}NO_{13}P \cdot 2H_2O$: C, 62.02; H, 10.21; N, 1.36. Found: C, 61.77; H, 10.23; N, 1.67.

2-Deoxy-2-[(*R*)-3-hexadecanoyloxytetradecanamido]-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-deoxy-α-D-glucopyranosyl-1-phosphate (2)——A solution of **10** (0.022 g, 0.022 mmol) in acetic acid and water (9 : 1) (0.5 ml) was heated at 85 °C for 15 min. After removal of the solvent the residue was subjected to preparative TLC with $CHCl_3$–CH_3OH (5 : 1) to give **2** (0.019 g, 91%) after lyophilization from dioxane, mp 88—90 °C. $[\alpha]_D^{26}$ +11.7° (c=0.24, $CHCl_3$). [lit.[9] $[\alpha]_D^{13}$ +10.0° (c=0.53, $CHCl_3$)]. IR (KBr): 3400 (OH, NH), 1735 (ester), 1658 (amide) cm^{-1}. Positive FAB MS $(M+Na)^+$ at m/z 973, $(M-H_2PO_4)^+$ at m/z 853, $(M-C_{16}+Na+H)^+$ at m/z 734, $(M-C_{16}-H_2PO_4+H)^+$ at m/z 614, $(M-C_{16}-OH-C_{14}-H_2PO_4+2H)^+$ at m/z 388. *Anal.* Calcd for $C_{50}H_{96}NO_{13}P \cdot 3H_2O$: C, 59.80; H, 10.24; N, 1.39. Found: C, 59.70; H, 9.86; N, 1.35.

Benzyl 2-[(*R*)-3-Benzyloxytetradecanoyloxytetradecanamido]-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (11)——DCC (0.149 g, 0.72 mmol) was added to a stirred solution of **3** (0.185 g, 0.60 mmol) and (*R*)-3-benzyloxytetradecanoic acid (0.241 g 0.72 mmol) in dry dichloromethane (2 ml) at 0 °C under nitrogen. The mixture was stirred for 5 h at 0 °C, then at room temperature for 18 h. The resulting suspension was filtered through Celite and the filtrate was evaporated. The residue was chromatographed on silica gel with chloroform–acetone (40 : 1) to give **10** (0.273 g, 73%), mp 60—63 °C. IR (neat): 3440 (OH), 3284 (NH), 1652 (amide), 861 (Me_2C), 692 (Ph) cm^{-1}. *Anal.* Calcd for $C_{37}H_{55}NO_7$: C, 71.01; H, 8.86; N, 2.24. Found: C, 70.88; H, 8.75; N, 2.34.

Benzyl 2-[(*R*)-3-Benzyloxytetradecanamido]-2-deoxy-3-*O*-[(*R*)-3-hexadecanoyloxytetradecanoyl]-4,6-*O*-iso-

propylidene-β-D-glucopyranoside (12)——DCC (0.064 g, 0.31 mmol) was added to a stirred solution of **11** (0.162 g, 0.26 mmol), (*R*)-3-hexadecanoyloxytetradecanoic acid (0.150 g, 0.31 mmol) and DMAP (0.013 g, 0.11 mmol) in dry dichloromethane (2 ml) at 0 °C under nitrogen. The mixture was stirred for 5 h at 0 °C, then at room temperature for 12 h. The resulting suspension was filtered through Celite and the filtrate was evaporated. The residue was chromatographed on silica gel with chloroform–isopropyl ether (10 : 1) to give **11** (0.253 g, 90%) as a syrup. $[\alpha]_D^{22}$ −25.6° (*c* = 2.05, $CHCl_3$). *Anal.* Calcd for $C_{67}H_{111}NO_{10}$: C, 73.79; H, 10.26; N, 1.28. Found: C, 73.63; H, 10.30; N, 1.18.

Benzyl 2-Deoxy-3-*O*-[(*R*)-3-hexadecanoyloxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanamido]-4,6-*O*-isopropylidene-β-D-glucopyranoside (13)——Compound **12** (0.090 g, 0.083 mmol) was dissolved in THF (6 ml) and stirred under 50 atm of H_2 in the presence of 10% Pd-on-carbon (65 mg) for 24 h at 30 °C. The catalyst was filtered off and the filtrate was evaporated *in vacuo*. The residue was chromatographed on neutral aluminium oxide W200 with ether–hexane (2 : 1) to give **13** (0.060 g, 68%), mp 79—83 °C. $[\alpha]_D^{20}$ −27.5° (*c* = 0.36, $CHCl_3$). IR (neat): 3296 (OH, NH), 1739 (ester), 1655 (amide), 860 (Me_2C) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (9H, t, *J* = 4.4 Hz, $(CH_2)_{10}CH_3 \times 2$ and $(CH_2)_{14}CH_3$), 1.25 (68H, br s, $(CH_2)_{10}CH_3 \times 2$ and $(CH_2)_{14}CH_3$), 1.43 (6H, s, Me_2C), 2.50—2.66 (6H, m, $COCH_2 \times 3$), 4.58 and 4.87 (each 1H, d, *J* = 11.7 Hz, –O–CH–OCH_2Ph), 6.13 (1H, br d, *J* = 8.6 Hz, NH), 7.30 (5H, br s, Ph). ^{13}C-NMR ($CDCl_3$) δ: 99.7 (d, C-1), 100.5 (s, Me_2C). *Anal.* Calcd for $C_{60}H_{105}NO_{10} \cdot 5H_2O$: C, 66.08; H, 10.63; N, 1.28. Found: C, 66.09; H, 10.72; N, 1.63.

Acknowledgment The authors are indebted to Dr. K. Narita and the staff of the Analysis Center of this college for microanalysis. This work was supported in part by a Grant-in-Aid from Tokyo Biochemical Research Foundation.

References

1) *a*) T. Takahashi, C. Shimizu, S. Nakamoto, K. Ikeda, and K. Achiwa, *Chem. Pharm. Bull.*, **33**, 1760 (1985); *b*) S. Nakamoto, T. Takahashi, K. Ikeda, and K. Achiwa, *ibid.*, **33**, 4098 (1985); *c*) T. Shimizu, S. Akiyama, T. Masuzawa, Y. Yanagihara, S. Nakamoto, T. Takahashi, K. Ikeda, and K. Achiwa, *ibid.*, **33**, 4621 (1985); *d*) K. Ikeda, S. Nakamoto, T. Takahashi, and K. Achiwa, *Carbohydr. Res.*, **145**, C5 (1986); *e*) T. Takahashi, S. Nakamoto, K. Ikeda, and K. Achiwa, *Tetrahedron Lett.*, **27**, 1819 (1986); *f*) S. Nakamoto and K. Achiwa, *Chem. Pharm. Bull.*, **34**, 2302 (1986); *g*) T. Shimizu, S. Akiyama, T. Masuzawa, Y. Yanagihara, S. Nakamoto, and K. Achiwa, *ibid.*, **34**, 2310 (1986); *h*) T. Shimizu, S. Akiyama, T. Masuzawa, Y. Yanagihara, S. Nakamoto, T. Takahashi, K. Ikeda, and K. Achiwa, *ibid.*, **34**, 5169 (1986); *i*) T. Shimizu, S. Akiyama, T. Masuzawa, Y. Yanagihara, S. Nakamoto, and K. Achiwa, *ibid.*, **35**, 873 (1987); *j*) K. Ikeda, T. Takahashi, H. Kondo, and K. Achiwa, *ibid.*, **35**, 1311 (1987); *k*) K. Ikeda, T. Takahashi, C. Shimizu, S. Nakamoto, and K. Achiwa, *ibid.*, **35**, 1383 (1987); *l*) T. Shimizu, S. Akiyama, T. Masuzawa, Y. Yanagihara, K. Ikeda, T. Takahashi, H. Kondo, and K. Achiwa, *Microbiol. Immunol.*, **31**, 381 (1987); *m*) T. Shimizu, S. Akiyama, T. Masuzawa, Y. Yanagihara, S. Nakamoto, and K. Achiwa, *Infection and Immunology*, **55**, 2287 (1987).
2) O. Westphal and O. Lüderitz, *Angew. Chem.*, **66**, 407 (1954); O. Lüderitz, C. Galanos, V. Lehmann, H. Mayer, E. T. Rietschel, and J. Weckesser, *Naturwissenschaften*, **65**, 578 (1978).
3) *a*) K. Takayama, N. Qureshi, and P. Mascagni, *J. Biol. Chem.*, 12801 (1983); *b*) M. Imoto, S. Kusumoto, T. Shiba, H. Naoki, T. Iwashita, E. Th. Rietschel, H. W-. Wollenweber, C. Galanos, and O. Lüderitz, *Tetrahedron Lett.*, **24**, 4017 (1983); *c*) U. Seydel, B. Lindner, H. W-. Wollenweber, and E. T. Rietschel, *Eur. J. Biochem.*, **145**, 505 (1984).
4) M. Imoto, H. Yoshimura, M. Yamamoto, S. Kusumoto, and T. Shiba, *Tetrahedron Lett.*, **25**, 2667 (1984); M. Imoto, H. Yoshimura, N. Sakaguchi, S. Kusumoto, and T. Shiba, *ibid.*, **26**, 1545 (1985).
5) K. Takayama, N. Qureshi, P. Mascagni, L. Anderson, and C. R. H. Raetz, *J. Biol. Chem.*, **258**, 14245 (1983).
6) P. H. Gross and R. W. Jeanloz, *J. Org. Chem.*, **32**, 2759 (1967).
7) M. Inage, H. Chaki, S. Kusumoto, and T. Shiba, *Chem. Lett.*, **1982**, 1281.
8) J. C. Dittmer and R. L. Lester, *J. Lipid Res.*, **5**, 126 (1964).
9) S. Kusumoto, M. Yamamoto, and T. Shiba, *Tetrahedron Lett.*, **25**, 3727 (1984).