Lipid A and Related Compounds. XXIX.[1] Synthesis of Biologically Active *N*-Acylated L-Asparagine-Containing D-Glucosamine Derivatives Structurally Related to Lipid A

Yoshitomo SUHARA, Misa ARAKAWA, Kiyoshi IKEDA, and Kazuo ACHIWA*

School of Pharmaceutical Sciences, University of Shizuoka, Yada 52–1, Shizuoka 422, Japan.
Received May 25, 1994; accepted July 20, 1994

New β-*N*-acylated L-asparagine-linked D-glucosamine derivatives, in which the reducing unit of lipid A has been mimicked by a lipoamino acid, have been synthesized. Compounds 1 and 2 exhibit mitogenicity and nitric oxide (NO) productivity.

Keywords D-glucosamine-asparagine compound; *N*-acylated L-aspartic acid; lipid A analog; glycosylamine; mitogenic activity; nitric oxide productivity

Lipid A of gram-negative bacterial lipopolysaccharides (LPS) is of considerable pharmacological interest, because it is responsible for the expression of many biological activities of LPS, *e.g.*, endotoxicity, adjuvanticity, antitumor activity and so on.[2] Lipid A consists of a D-glucosaminyl-β(1→6)-D-glucosamine disaccharide acylated with several fatty acids, mostly (*R*)-3-hydroxytetradecanoic acid, and bearing two phosphate groups at positions 1 and 4,[3] as indicated in Chart 1. A large number of compounds related to lipid A partial structures have been synthesized with the aim of separating unwanted endotoxic properties from potentially beneficial immunostimulatory properties. Among the various synthetic lipid A analogs, D-glucosamine 4-phosphate analogs of the non-reducing unit of lipid A showed many of the biological activities of LPS.[4]

Recently, numerous acyclic analogs related to lipid A partial structure have been synthesized.[5] We have already reported that *N*-acylated L-serine-containing D-glucosamine 4-phosphate derivatives as mimicks of lipid A disaccharide had remarkable mitogenetic activity.[6] In addition, it is noteworthy that the phosphate group was not required in lipid A analogs for mitogenicity. However, the *O*-linked glycopeptides of glycosyl-serine is complicated by the acid-lability of glycosides in general and the base-sensitivity (retro-Michael reaction) of the serinyl glycosides in particular.[7] *N*-Glycopeptides as well as *O*-glycopeptides are active in the binding and transport of enzymes, hormones and antibodies, and in cell–cell interactions.[8] With a view to increasing the biological and chemical stabilities, we designed *N*-glycoside-type saccharides as new acyclic analogs of lipid A, using L-aspartic acid instead of L-serine to afford stability to acids and bases, in the hope of generating interesting biological activity. We describe here the synthesis of L-asparagine-containing D-glucosamine analogs (**1**, **2**) structurally similar to the lipid A disaccharide backbone, and their biological effects.

The synthetic sequence for the *N*-tetradecanoyl L-asparagine-containing D-glucosamine derivative (**1**) was as follows. The L-aspartic acid derivatives (**7**) were easily prepared from *tert*-butyloxycarbonyl-L-aspartic acid α-benzyl ester (**3**) as shown in Chart 2.

The carboxyl group of **3** was protected using phenacyl bromide and triethylamine (NEt_3) in CH_2Cl_2, and the product was treated with 4 N HCl in AcOEt to afford the hydrochloride (**5**) in almost quantitative yield in two steps. Compound **5** was condensed with tetradecanoic acid using diethyl phosphorocyanidate (DEPC) and NEt_3 in CH_2Cl_2 to give **6** in 83% yield, and this in turn was treated with activated Zn-dust in AcOH to give the lipoamino acid (**7**) in 75% yield. Chart 3 shows the synthesis route to **1**.

Next, the acylation of the amino group of D-glucosamine hydrochloride with *N*-tetradecanoyloxysuccinimide (**8**), prepared from tetradecanoic acid, *N*-hydroxysuccinimide and dicyclohexylcarbodiimide (DCC) in tetrahydrofuran (THF) in quantitative yield, in the presence of NEt_3 afforded the amide compound (**9**) in 85% yield. The per-*O*-acetylation of **9** with Ac_2O–pyridine gave the tetra-*O*-acetyl compound (**10**) in 90% yield. Bromination of **10** with 33% HBr–AcOH,[9] followed by treatment with AgN_3 afforded the β-azide compound (**11**) in 76% yield in two steps. The configuration at C-1 of **11** was assigned on the basis of the proton nuclear magnetic resonance

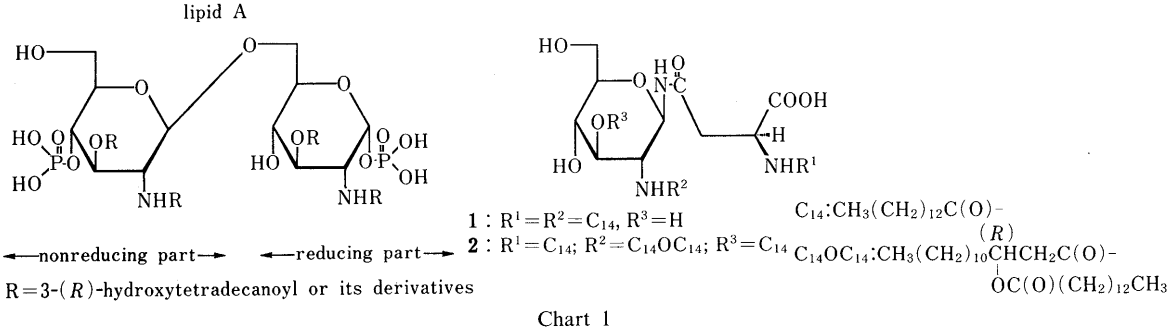


Chart 1

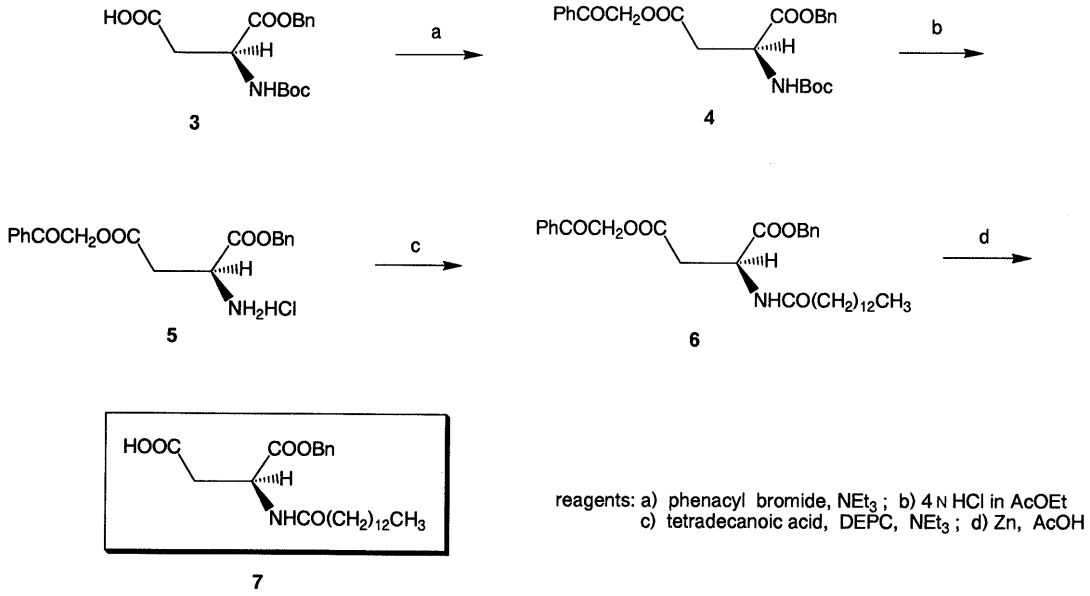


Chart 2

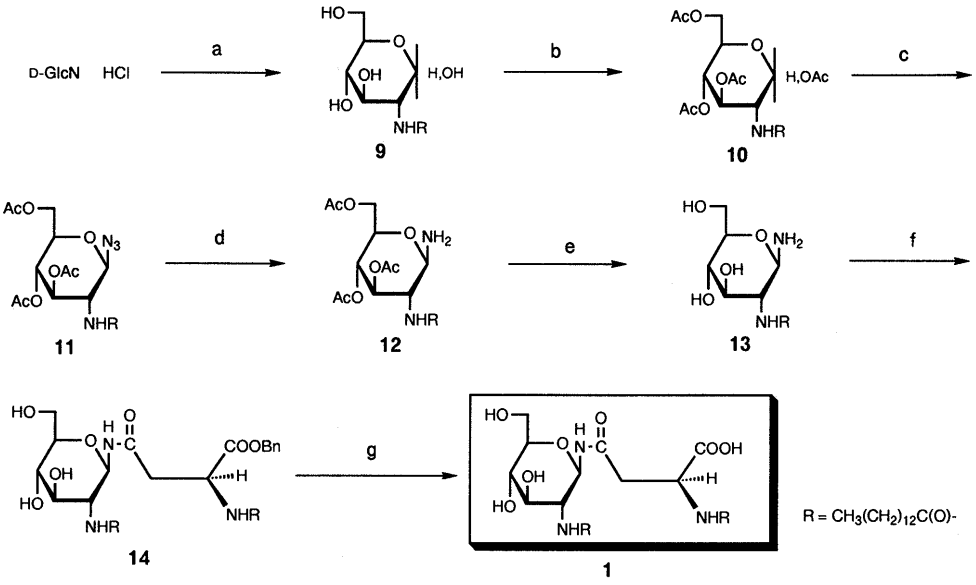
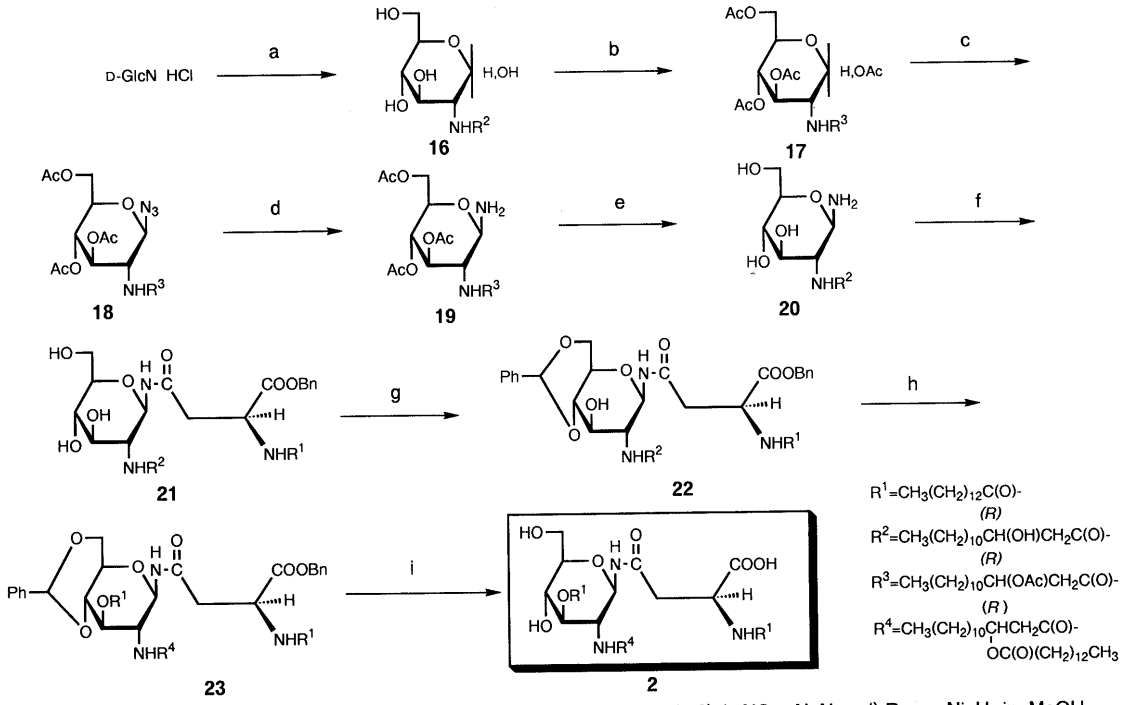


Chart 3

(^{1}H-NMR) spectrum of **11**, which showed the H-1 signal at δ 5.97 as a doublet with $J_{1,2} = 7.6$ Hz. The absorption at 2112 cm^{-1} in the infrared (IR) spectrum of **11** indicated the presence of the azide group. The β-glycosyl azide (**11**) was reduced using atmospheric hydrogen over Raney Ni in MeOH to provide the β-glycosylamine (**12**) in 64% yield. Zemplén deacetylation of **12** with NaOMe in MeOH gave the triol compound (**13**) in 55% yield. Condensation with **13** and **7** in the presence of diisopropylethylamine, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) and a catalytic amount of 1-hydroxy-1*H*-benzotriazole (HOBt) in *N*,*N*-dimethylformamide (DMF) afforded the asparagine-linked compound (**14**) in a low yield (20%), together with the starting material. Finally, the benzyl group of **14** was removed by hydrogenolysis over 10% palladium-on-carbon at room temperature in MeOH to afford the expected product (**1**) in quantitative yield after purification followed by lyophilization from H_2O.

Next, we planned to synthesize *N*-acylated L-asparagine-containing D-glucosamine analogs (**2**) bearing the (*R*)-3-tetradecanoyloxytetradecanoyl group at *N*-2 and the tetradecanoyl group at *O*-3 of the D-glucosamine skeleton of the GLA-27 type,[4j] as indicated in Chart 4.

The introduction of an (*R*)-3-hydroxytetradecanoyl group into the amino group of D-glucosamine hydrochloride with *N*-[(*R*)-3-hydroxytetradecanoyloxy]succinimide (**15**) and NEt_3 gave the amide compound (**16**) in



reagents: a) **15**, NEt_3 in DMF; b) Ac_2O, pyridine; c) 1) HBr in AcOH, 2) $AgNO_3$, NaN_3; d) Raney Ni, H_2 in MeOH
e) MeONa in MeOH; f) **7**, DIEA, BOP, HOBt, in DMF; g) $PhCH(OMe)_2$, *p*-TsOH in DMF
h) tetradecanoic acid, DCC, DMAP, NEt_3; i) Pd-C, H_2 in MeOH

Chart 4

quantitative yield in a similar way to that described for **9**. The acetylation of **16** with pyridine and acetic anhydride afforded **17** in 83% yield. Bromination of **17** and subsequent replacement of bromide by azide using AgN_3 gave the glycosyl azide (**18**) in 82% yield in two steps. The ^{1}H-NMR spectrum of **18** showed a doublet due to H-1 at δ 6.08 ($J_{1,2}$ = 8.5 Hz), indicating the stereochemistry of the glycosidic bond formed to be β. The azide group of **18** was hydrogenated in the presence of Raney Ni to afford the β-1-amino compound (**19**) in 64% yield. The cleavage of the protective acetyl group of **19** with MeOH in the presence of a catalytic amount of NaOMe gave **20** in 60% yield. Compound **20** was condensed with the activated ester (**7**) in the same way as described for **13** to give the coupling product (**21**) in 20% yield. Then compound **21** was protected with a benzylidene group using benzaldehyde dimethyl acetal and a catalytic amount of *p*-toluenesulfonic acid monohydrate (*p*-TsOH) to afford the 4,6-*O*-benzylidene compound (**22**) in 50% yield. Compound **22** was acylated with tetradecanoic acid by treatment with DCC, NEt_3 and a catalytic amount of DMAP in DMF to give compound **23** in 43% yield. Finally, the protective benzyl and benzylidene groups of **23** were removed by hydrogenolysis catalyzed by 10% palladium-on-carbon to afford the desired product (**2**) in 40% yield after lyophilization from H_2O.

The structures of all compounds were characterized by ^{1}H-NMR spectroscopy, as well as infrared (IR) spectroscopy, elemental analyses, and fast-atom bombardment (FAB) mass spectroscopy.

Preliminary examination of the biological activity of these chemically synthesized compounds showed that compound **1** had only weak mitogenicity and *N*-tetradecanoyl L-asparagine-linked 3-*N*,*O*-acylated D-glucosamine (**2**) possessed a weaker mitogenicity than that of the corresponding L-serine-linked D-glucosamine. However, compound **2** as well as the corresponding L-serine-linked D-glucosamine generated nitric oxide (NO) in murine peritoneal macrophages.

Experimental

All melting points are uncorrected. Optical rotations were measured with a JASCO DIP-140 digital polarimeter. IR spectra were recorded on a JASCO A-202 infrared spectrophotometer ^{1}H-NMR spectra were taken on a JEOL JNM-GX 270 (270 MHz) spectrometer with tetramethylsilane (in $CDCl_3$) as an internal standard, and the chemical shifts are given in δ values. The abbreviations of signal patterns are as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Column chromatography was carried out on Silica gel 60 (70—230 mesh, Merck). Thin-layer chromatography (TLC) on Silica gel 60-F_{254} (Merck) was used to monitor the reaction and to ascertain the purity of the reaction products. The spots were visualized by spraying the plates with 5% aqueous sulfuric acid and then heating.

***N*-*tert*-Butoxycarbonyl-L-aspartic Acid α-Benzyl β-Phenacyl Ester (4)** Triethylamine (121 mg, 1.2 mmol) was added to a stirred solution of *tert*-butoxycarbonyl-L-aspartic acid α-benzyl ester (**3**) (323 mg, 1.0 mmol) and phenacyl bromide (239 mg, 1.2 mmol) in ethyl acetate (AcOEt) (10 ml) at room temperature. The mixture was stirred for 12 h at room temperature. After evaporation of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH, 10:1) to give **4** (quantitative) as a white powder. $[\alpha]_D$ −1.3° (*c* = 1.00, $CHCl_3$). IR (KBr): 1735 (ester), 1699, 1675, 1596 (amide), 755 (Ph) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (9H, t, $C(CH_3)_3$), 3.00, 3.21 (each 1H, dd, *J* = 8.4, 4.6 Hz, CH_2CH), 4.69—4.72 (1H, m, CH_2CH), 5.10—5.33 (4H, m, $PhCH_2$ and $PhCOCH_2$), 5.91 (1H, d, *J* = 8.9 Hz, NH), 7.27—7.85 (10H, m, Ph). ^{13}C-NMR ($CDCl_3$) δ: 36.4 (t, CH_2CH), 49.9 (d, CH_2CH), 66.0 (t, $PhCH_2$), 67.1 (t, $PhCOCH_2$), 79.7 (s, $C(CH_3)_3$), 127.5, 127.7, 128.0, 128.2, 128.6, 133.7 (d, Ph), 133.6, 135.2 (s, Ph), 155.3, 169.8, 170.5, 191.3 (s, C=O). *Anal.* Calcd for $C_{24}H_{27}NO_7$: C, 65.29; H, 6.16; N, 3.17. Found: C, 65.44; H, 6.24; N, 3.06.

L-Aspartic Acid α-Benzyl β-Phenacyl Ester Hydrochloride (5) A

solution of 4 N HCl in AcOEt (1 : 1) (10 ml) was added to a stirred solution of compound **4** (514 mg, 1.0 mmol) in AcOEt (10 ml) at room temperature. The mixture was stirred at room temperature for 3 h. After evaporation of the solvent, the residue was washed with ether to give **5** (quantitative) as a white powder, $[\alpha]_D$ −12.4° (c = 1.00, MeOH). IR (KBr): 1746, 1728 (ester), 1696, 1595 (amide), 754 (Ph) cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 3.25 (2H, dd, J = 5.0, 2.0 Hz, CH_2CH), 4.52 (1H, t, J = 5.0 Hz, CH_2CH), 5.44, 5.52 (each 1H, d, J = 12.2 Hz, $PhCH_2$), 5.34, 5.29 (each 1H, d, J = 15.8 Hz, $PhCOCH_2$), 7.33—7.98 (10H, m, Ph). ^{13}C-NMR (CD_3OD) δ: 36.4 (t, CH_2CH), 50.0 (d, CH_2CH), 68.3 (t, $PhCH_2$), 69.6 (t, $PhCOCH_2$), 129.0, 129.7, 129.8, 129.9, 130.1, 135.4 (d, Ph), 135.2, 136.3 (s, Ph), 169.0, 170.3 (s, C=O). *Anal.* Calcd for $C_{19}H_{20}ClNO_5$: C, 60.40; H, 5.34; N, 3.71. Found: C, 60.31; H, 5.21; N, 3.65.

***N*-Tetradecanoyl-L-aspartic Acid α-Benzyl β-Phenacyl Ester (6)** DEPC (206 mg, 1.2 mmol) was added to a stirred solution of compound **5** (341 mg, 1.0 mmol), tetradecanoic acid (283 mg, 1.2 mmol) and NEt_3 (121 mg, 1.2 mmol) in DMF (1.0 ml) at 0 °C under argon. The mixture was stirred at 0 °C for 1 h, and then at room temperature for 12 h. The reaction mixture was diluted with AcOEt, and then washed successively with water, and 10% aqueous citric acid, water, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous $MgSO_4$, and concentrated to dryness *in vacuo*. The residue was chromatographed on silica gel using CH_2Cl_2–MeOH (20 : 1) to give **6** (458 mg, 83%) as a colorless oil, $[\alpha]_D$ +2.1° (c = 1.00, $CHCl_3$). IR (neat): 1745, 1725 (ester), 1700 (ketone), 1640, 1537 (amide), 751 (Ph) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 6.9 Hz, $-CH_3$), 1.24 (20H, br s, $-CH_2-$), 1.66 (2H, br s, $NHCOCH_2CH_2$), 2.33 (2H, t, J = 7.3 Hz, $NHCOCH_2$), 2.98 (2H, dd, J = 16.7, 5.0 Hz, CH_2CH), 3.28 (1H, dd, J = 16.5, 4.1 Hz, CH_2CH), 5.15, 5.23 (each 1H, d, J = 16.5 Hz, $PhCH_2$), 4.98, 5.42 (each 1H, d, J = 18.3 Hz, $PhCOCH_2$), 7.25—7.88 (10H, m, Ph). ^{13}C-NMR ($CDCl_3$) δ: 14.1 (q, $-CH_3$), 22.7, 25.6, 29.2, 29.4, 29.5, 29.7, 32.0, 36.5 (t, $-CH_2-$), 36.7 (t, CH_2CH), 48.4 (d, CH_2CH), 66.1, 67.4 (t, $PhCH_2$), 127.8, 128.4, 128.4, 128.5, 128.6, 129.0, 134.3 (d, Ph), 133.7, 135.4 (s, Ph), 169.8, 170.6, 173.6, 192.1 (s, C=O). *Anal.* Calcd for $C_{33}H_{45}NO_6$: C, 71.84; H, 8.22; N, 2.54. Found: C, 72.08; H, 8.37; N, 2.70.

***N*-Tetradecanoyl-L-aspartic Acid α-Benzyl Ester (7)** Activated zinc powder (2.5 g) was added to a stirred solution of compound **6** (551 mg, 1.0 mmol) in acetic acid (20 ml) at room temperature. The reaction mixture was stirred at 50 °C for 2 h, then the insoluble materials were filtered off and the filtrate was concentrated to dryness *in vacuo*. The residue was chromatographed on silica gel using CH_2Cl_2–MeOH (10 : 1) to give **7** (166 mg, 75%) as a white powder, $[\alpha]_D$ +9.1° (c = 1.00, $CHCl_3$). IR (KBr): 1723 (ester), 1673, 1560 (amide), 732 (Ph) cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 0.88 (3H, t, J = 6.3 Hz, $-CH_3$), 1.24 (20H, br s, $-CH_2-$), 1.66 (2H, br s, $NHCOCH_2CH_2$), 2.33 (2H, t, J = 7.6 Hz, $NHCOCH_2$), 2.98, 3.28 (each 1H, dd, J = 16.5, 4.1 Hz, CH_2CH), 4.95—4.89 (1H, m, CH_2CH), 6.62 (1H, d, J = 8.2 Hz, NH), 7.22—7.37 (5H, m, Ph). ^{13}C-NMR (CD_3OD) δ: 14.1 (q, $-CH_3$), 22.7, 25.6, 29.2, 29.3, 29.4, 29.5, 29.6, 31.9, 36.1 (t, $-CH_2-$), 36.4 (t, CH_2CH), 48.4 (d, CH_2CH), 67.6 (t, $PhCH_2$), 127.2, 128.2, 128.5, 128.6 (d, Ph), 135.1 (s, Ph), 170.6, 173.7, 174.9 (s, C=O). *Anal.* Calcd for $C_{25}H_{39}NO_5$: C, 69.25; H, 9.07; N, 3.23. Found: C, 69.17; H, 8.97; N, 3.15.

***N*-Tetradecanoyloxysuccinimide (8)** DCC (495 mg, 2.4 mmol) was added to a stirred solution of tetradecanoic acid (456 mg, 2.0 mmol) and *N*-hydroxysuccinimide (276 mg, 2.4 mmol) in THF (10 ml) at 0 °C. The reaction mixture was stirred at the same temperature and then at room temperature for 12 h. After evaporation of the solvent, the residue was dissolved in AcOEt, the insoluble materials were filtered off and the filtrate was evaporated to dryness *in vacuo* to give **8** (quantitative) as a white powder. IR (KBr): 1654 (C=O) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 6.9 Hz, $-CH_3$), 1.26 (20H, br s, $-CH_2-$), 1.72 (2H, br s, $CH_2CH_2C_{11}H_{23}$), 2.60 (2H, t, J = 7.6 Hz, $CH_2C_{12}H_{25}$), 2.83 (4H, s, CH_2). ^{13}C-NMR ($CDCl_3$) δ: 14.0 (q, $-CH_3$), 22.6, 24.5, 24.6, 24.9, 25.4, 25.5, 28.7, 29.0, 29.3, 29.5, 30.9, 31.8, 33.9, 34.8 (t, $-CH_2-$), 168.6, 169.2 (s, C=O). *Anal.* Calcd for $C_{18}H_{31}NO_4$: C, 66.43; H, 9.60; N, 4.30. Found: C, 65.98 ; H, 9.73 ; N, 4.45.

2-Deoxy-2-tetradecanoylamino-D-glucopyranose (9) Triethylamine (202 mg, 2.0 mmol) was added to a stirred solution of compound **8** (606 mg, 2.0 mmol) and D-(+)-glucosamine hydrochloride (431 mg, 2.0 mmol) in DMF (10 ml) at room temperature under argon. The reaction mixture was stirred for 12 h at room temperature. After evaporation of the solvent, the residual product was successively washed with AcOEt and water to give **9** (331 mg, 85%) as a white powder, $[\alpha]_D$ +43.9° (c = 0.50, DMSO). IR (KBr): 3306 (OH), 1641, 1544 (amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.78 (3H, t, J = 6.6 Hz, $-CH_3$), 1.16 (20H, br s, $-CH_2-$), 1.38 (2H, s, $CH_2CH_2C_{11}H_{23}$), 2.01 (2H, t, J = 7.6 Hz, $CH_2C_{12}H_{25}$), 3.03 (1H, dd, J = 12.9, 9.2 Hz, H-2), 3.23—3.56 (4H, m, H-3, H-5, H-6, H-6'), 4.35 (1H, t, J = 11.5, 5.6 Hz, H-4), 4.83 (1H, d, J = 3.6 Hz, H-1). ^{13}C-NMR (DMSO-d_6) δ: 13.9 (q, $-CH_3$), 22.0, 28.6, 28.8, 28.9, 29.0, 31.2, 31.5, 35.2 (t, $-CH_2-$), 54.2 (d, C-2), 61.1 (t, C-6), 70.8 (d, C-3), 71.1 (d, C-5), 72.1 (d, C-4), 90.3 (d, C-1), 172.1 (s, C=O). *Anal.* Calcd for $C_{20}H_{39}NO_6 \cdot 1/2H_2O$: C, 60.28; H, 10.12; N, 3.51. Found: C, 59.83; H, 10.04; N, 3.55.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-tetradecanoylamino-D-glucopyranose (10) Acetic anhydride (2.04 g, 20 mmol) was added to a stirred solution of compound **9** (425 mg, 1.0 mmol) in pyridine (3.16 g, 40 mmol) on an ice bath. The mixture was stirred for 1 h at the same temperature, and then at room temperature for 12 h. The reaction mixture was poured into ice-cold water (200ml), then the precipitates were collected and washed with ether. The resulting solid was purified by column chromatography using CH_2Cl_2–MeOH (20 : 1) to afford **10** (502 mg, 90%) as a white powder, $[\alpha]_D$ +62.5° (c = 1.00, $CHCl_3$). IR (KBr): 1744 (ester), 1648, 1522 (amide) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 6.9 Hz, $-CH_3$), 1.25 (20H, br s, $-CH_2-$), 1.55 (2H, br s, $CH_2CH_2C_{11}H_{25}$), 2.04—2.19 (14H, m, CH_3CO and $CH_2C_{12}H_{25}$), 4.04—4.09 (2H, m, H-5 and H-6'), 4.26 (1H, dd, J = 4.0, 6.4 Hz, H-6), 4.50 (1H, dd, J = 9.9, 3.3 Hz, H-2), 5.17—5.33 (2H, m, H-3 and H-4), 5.88 (1H, d, J = 8.9 Hz, NH), 6.18 (1H, d, J = 3.3 Hz, H-1). ^{13}C-NMR ($CDCl_3$) : 13.9 (q, $-CH_3$), 20.3, 20.4, 20.6 (t, CH_3CO), 22.4, 24.7, 25.3, 25.5, 28.6, 28.9, 29.1, 29.2, 29.4, 31.6, 36.2, 36.2 (t, CH_2), 50.6 (d, C-2), 61.4 (t, C-6), 67.4 (d, C-4), 69.5 (d, C-5), 70.3 (d, C-3), 90.4 (d, C-1), 168.4, 168.9, 170.4, 171.2, 173.0 (s, C=O). *Anal.* Calcd for $C_{28}H_{47}NO_{10}$: C, 60.30; H, 8.49; N, 2.51. Found: C, 59.92; H, 8.60; N, 2.49.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-tetradecanoylamino-D-glucopyranosyl Azide (11) A freshly prepared, dried solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-tetradecanoylamino-D-glucopyranosyl bromide, prepared from **10** (558 mg, 1.0 mmol) and HBr–AcOH (33%, 30 ml), in chloroform (10 ml) was added to a suspension of the silver azide in chloroform (30 ml). The suspension had been prepared by mixing aqueous solutions of silver nitrate (510 mg, 3.0 mmol) and sodium azide (195 mg, 3.0 mmol) and washing the precipitate by decantation with water, ethanol, ether, and chloroform. The mixture was refluxed for 3 h, then it was cooled and filtered. The filtrate was concentrated to dryness *in vacuo*. The residual product was purified by column chromatography (CH_2Cl_2–MeOH, 10 : 1) to give compound **11** (411 mg, 76% in two steps from **10**) as a yellow oil, $[\alpha]_D$ +9.7° (c = 1.40, $CHCl_3$). IR (neat): 2112 (azide), 1747 (ester), 1663, 1558 (amide) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 6.3 Hz, $-CH_3$), 1.26 (20H, br s, $-CH_2-$), 1.59—1.67 (2H, m, $CH_2CH_2C_{11}H_{23}$), 2.03—2.12 (12H, m, CH_3CO), 2.37 (2H, t, J = 6.9 Hz, $CH_2C_{12}H_{24}$), 3.57—3.63 (1H, m, H-5), 4.08—4.30 (3H, m, H-2, H-6, H-6'), 4.92 (1H, d, J = 9.2 Hz, H-4), 5.30 (1H, t, J = 2.3 Hz, H-3), 5.97 (1H, d, J = 7.6 Hz, H-1). ^{13}C-NMR ($CDCl_3$) δ: 14.1 (q, $-CH_3$), 20.6, 20.8, 20.9 (t, CH_3CO), 22.7, 25.6, 25.7, 28.1, 29.2, 29.3, 29.4, 29.5, 29.6, 31.9 (t, $-CH_2-$), 63.5 (t, C-6), 64.7 (d, C-2), 67.5 (d, C-5), 68.4 (d, C-4), 70.2 (d, C-3), 99.1 (d, C-1), 169.6, 169.8, 170.6, 173.5 (s, C=O). *Anal.* Calcd for $C_{26}H_{44}N_4O_8$: C, 57.76; H, 8.20; N, 10.36. Found: C, 57.21; H, 8.06; N, 10.11.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-tetradecanoylamino-β-D-glucopyranosylamine (12) A solution of compound **11** (541 mg, 1.0 mmol) in MeOH (20 ml) was hydrogenated under atmospheric pressure of H_2 in the presence of Raney Ni (30 mg) at room temperature for 12 h. The catalyst was filtered off and the filtrate was evaporated to dryness *in vacuo*. The residue was chromatographed on a column of silica gel (CH_2Cl_2–MeOH, 10 : 1) to give **12** (330 mg, 64%) as a white powder, $[\alpha]_D$ +37.9° (c = 1.00, $CHCl_3$). IR (KBr): 1744 (ester), 1648, 1522 (amide) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 6.9 Hz, $-CH_3$), 1.25 (20H, br s, $-CH_2-$), 1.57 (2H, d, J = 2.0 Hz, $CH_2CH_2C_{11}H_{23}$), 1.98—2.19 (14H, m, CH_3CO, $CH_2CH_2C_{11}H_{23}$), 4.11—4.35 (4H, m, H-2, H-5, H-6, H-6'), 5.10—5.35 (2H, m, H-3, H-4), 5.86 (1H, d, J = 7.6 Hz, H-1). ^{13}C-NMR ($CDCl_3$) δ: 14.1 (q, $-CH_3$), 20.6, 20.8, 20.9 (q, CH_3CO), 22.7, 25.7, 29.2, 29.4, 29.5, 29.6, 31.9, 36.7 (t, $-CH_2-$), 52.1 (d, C-2), 62.1 (t, C-6), 68.3 (d, C-5), 70.9 (d, C-3), 91.7 (d, C-1), 169.4, 170.9, 171.4, 173.4 (s, C=O). *Anal.* Calcd for $C_{26}H_{46}N_2O_8$: C, 60.68; H, 9.01; N, 5.44. Found: C, 60.52; H, 9.01; N, 5.44.

2-Deoxy-2-tetradecanoylamino-β-D-glucopyranosylamine (13) A mixture of compound **12** (515 mg, 1.0 mmol), MeOH (20 ml), and sodium methoxide (50 mg, 0.93 mmol) was stirred at 0 °C under argon. The

mixture was stirred at room temperature for 12 h, and adjusted to pH 7.0 with IRC-50 (1.0 g). The resin was filtered off, and the filtrate was concentrated to dryness *in vacuo* to give **13** (0.214g, 55%) as a colorless oil, $[\alpha]_D$ +21.0° (c=1.00, MeOH). IR (neat): 3480 (NH, OH), 1651, 1560 (amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.78 (3H, t, J=6.9 Hz, $-CH_3$), 1.27 (20H, br s, $-CH_2-$), 1.62 (2H, br s, $CH_2CH_2C_{11}H_{23}$), 2.20—2.35 (2H, m, $CH_2C_{12}H_{25}$), 3.33—3.94 (6H, m, H-2, H-3, H-4, H-5, H-6, H-6'), 4.35 (1H, d, J=8.1 Hz, H-1). ^{13}C-NMR (DMSO-d_6) δ: 13.8 (q, $-CH_3$), 22.0, 25.0, 25.2, 25.3, 28.4, 28.6, 28.8, 28.9, 30.0, 35.4 (t, $-CH_2-$), 54.2 (d, C-2), 61.1 (t, C-6), 71.3 (d, C-3), 72.1 (d, C-5), 72.6 (d, C-4), 91.4 (d, C-1), 173.0 (s, C=O). *Anal.* Calcd for $C_{20}H_{40}N_2O_5 \cdot 1/2H_2O$: C, 60.42; H, 10.39; N, 7.05. Found: C, 60.53; H, 10.22; N, 7.01.

Benzyl N^2-Tetradecanoyl-N^4-(2-deoxy-2-tetradecanoylamino-β-D-glucopyranosyl)-L-asparaginate (14) Compound **7** (433 mg, 1.0 mmol), diisopropylethylamine (DIEA, 129 mg, 1.0 mmol), BOP (663 mg, 1.5 mmol), and HOBt (77 mg, 0.5 mmol) were added to a solution of compound **13** (338 mg, 1.0 mmol) in DMF (10 ml) at room temperature under argon. The reaction mixture was stirred at room temperature for 36 h, then concentrated to dryness, and the residual product was successively washed with AcOEt and water to give **14** (150 mg, 20%) as a yellow oil, $[\alpha]_D$ +34.0° (c=1.00, DMSO). IR (neat): 3280 (OH), 1720 (ester), 1651, 1560 (amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.88 (6H, t, J=6.9 Hz, $-CH_3$), 1.25 (40H, br s, $-CH_2-$), 1.60 (4H, br s, $CH_2CH_2C_{11}H_{23}$), 2.11 (4H, br s, $CH_2C_{12}H_{25}$), 2.28—2.35 (2H, m, CH_2CH), 3.21—3.67 (7H, m, CH_2CH, H-2, H-3, H-4, H-5, H-6, H-6'), 5.14 (1H, t, J=8.3 Hz, H-1), 5.45 (2H, br s, $PhCH_2$), 7.33—7.38 (5H, m, Ph). ^{13}C-NMR (DMSO-d_6) δ: 14.1 (q, $-CH_3$), 22.7, 25.0, 29.1, 29.2, 29.3, 29.4, 29.5, 29.6, 31.9, 34.1 (t, $-CH_2-$), 37.5 (t, CH_2CH), 48.6 (d, CH_2CH), 54.5 (d, C-2), 60.8 (d, C-6), 70.4 (d, C-4), 74.4 (d, C-3), 78.7 (d, C-5), 78.8 (d, C-1), 127.4, 127.8, 128.2 (d, Ph), 135.9 (s, Ph), 169.2, 169.7, 171.2, 172.1 (s, C=O). *Anal.* Calcd for $C_{45}H_{77}N_3O_9$: C, 50.79; H, 9.65; N, 5.23. Found: C, 51.20; H, 9.23; N, 5.14.

N^2-Tetradecanoyl-N^4-(2-deoxy-2-tetradecanoylamino-β-D-glucopyranosyl)-L-asparagine (1) A mixture of compound **14** (200 mg, 0.25 mmol), palladium-on-carbon (20 mg), and MeOH (10 ml) was stirred under a hydrogen atmosphere. The mixture was stirred at room temperature for 12 h, and the catalyst was filtered off with the aid of Celite 545. The filtrate was concentrated to dryness and the residual product was washed with ether to give compound **1** (quantitative) as a white powder, $[\alpha]_D$ +5.5° (c=1.00, DMSO). IR (KBr): 3280 (OH), 1720 (carbonyl), 1655, 1541 (amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.86 (6H, t, J=6.9 Hz, $-CH_3$), 1.25 (40H, br s, $-CH_2-$), 1.60 (4H, br s, $CH_2CH_2C_{11}H_{23}$), 2.11 (4H, br s, $CH_2C_{12}H_{25}$), 2.31—2.35 (2H, m, CH_2CH), 3.21—3.67 (7H, m, CH_2CH, H-2, H-3, H-4, H-5, H-6, H-6'), 5.14 (1H, t, J=8.3 Hz, H-1). ^{13}C-NMR (DMSO-d_6) δ: 14.1 (q, $-CH_3$), 22.7, 25.0, 29.1, 29.2, 29.3, 29.4, 29.5, 29.6, 31.9, 34.1 (t, $-CH_2-$), 40.3 (t, CH_2CH), 50.1 (d, CH_2CH), 76.7 (d, C-3), 77.1 (d, C-5), 77.6 (d, C-1), 170.5, 171.2, 172.0, 173.1 (s, C=O). *Anal.* Calcd for $C_{38}H_{71}N_3O_9 \cdot 2H_2O$: C, 60.85; H, 9.54; N, 5.60. Found: C, 61.14; H, 9.83; N, 6.16.

***N*-[(*R*)-3-Hydroxytetradecanoyloxy]succinimide (15)** In the same manner as described for **8**, (*R*)-3-hydroxytetradecanoic acid (490 mg, 2.0 mmol) was treated with *N*-hydroxysuccinimide (276 mg, 2.4 mmol) and DCC (495 mg, 2.4 mmol) to give **15** (quantitative) as a white powder. IR (KBr): 1654 (carbonyl) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz, $-CH_3$), 1.31 (18H, br s, $-CH_2-$), 1.55—1.60 (2H, m, $CH_2CH(OH)CH_2C_{10}H_{21}$), 1.71—1.80 (1H, m, $CH_2CH(OH)C_{11}H_{25}$), 1.86—1.95 (2H, m, $CH_2CH(OH)C_{11}H_{25}$), 3.14—3.25 (4H, m, CH_2). ^{13}C-NMR ($CDCl_3$) δ: 14.0 (q, $-CH_3$), 22.5, 24.5, 24.8, 25.1, 25.4, 29.0, 29.3, 29.6, 31.2, 31.7, 33.7, 34.8 (t, $-CH_2-$), 68.0 (d, $CH_2CH(OH)C_{11}H_{25}$), 167.2, 169.1 (s, C=O). *Anal.* Calcd for $C_{18}H_{31}NO_5$: C, 63.32; H, 9.15; N, 4.10. Found: C, 63.15; H, 8.90; N, 3.95.

2-Deoxy-2-[(*R*)-3-hydroxytetradecanoylamino]-D-glucopyranose (16) As described for **9**, D-(+)-glucosamine hydrochloride (431 mg, 2.0 mmol) was reacted with compound **15** (640 mg, 2.0 mmol) and NEt_3 (200 mg, 2.0 mmol) to give **16** (quantitative) as a white powder, $[\alpha]_D$ +63.4° (c=1.20, DMSO). IR (KBr): 3302 (OH), 1641, 1545 (amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.86 (3H, t, J=6.6 Hz, $-CH_3$), 1.25 (18H, br s, $-CH_2-$), 1.59—1.69 (1H, br s, $CH_2CH(OH)C_{11}H_{23}$), 2.20 (2H, d, J=5.9 Hz, $CH_2CH(OH)C_{11}H_{23}$), 3.07—3.18 (1H, m, H-2), 3.38—3.77 (3H, m, H-5, H-6, H-6'), 4.43 (1H, t, J=4.4 Hz, H-3), 4.59—4.64 (1H, m, H-4), 4.91 (1H, d, J=3.6 Hz, H-1). ^{13}C-NMR (DMSO-d_6) δ: 13.9 (q, $-CH_3$), 22.0, 24.4, 25.0, 25.1, 28.6, 28.8, 29.0, 29.1, 31.2, 33.3, 36.7, 35.2, 43.3 (t, $-CH_2-$), 67.4 (d, $CH_2CH(OH)C_{11}H_{23}$), 54.2 (d, C-2), 61.0 (t, C-6), 71.0 (d, C-3, C-5), 72.0 (d, C-4), 90.3 (d, C-1), 171.2 (s, C=O). *Anal.* Calcd for $C_{20}H_{39}NO_7$: C, 59.24; H, 9.69; N, 3.45. Found: C, 59.61; H, 10.02; N, 3.51.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-[(*R*)-3-acetoxytetradecanoylamino]-D-glucopyranose (17) As described for **10**, compound **16** (1.20 g, 3.1 mmol) was treated with pyridine (4.90 g, 62 mmol) and acetic anhydride (3.20 g, 31 mmol) to afford **17** (1.40 g, 83%) as a white powder, $[\alpha]_D$ +81.5° (c=1.00, $CHCl_3$). IR (KBr): 1745 (carbonyl), 1645, 1521 (amide) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz, $-CH_3$), 1.26 (20H, br s, $-CH_2-$), 1.55 (2H, br s, $-CH_2-$), 2.04—2.19 (3H, m, CH_3CO), 2.36—2.48 (2H, m, $-CH_2-$), 4.05—4.16 (2H, m, $CH_2CH(OAc)C_{11}H_{23}$, H-6), 4.27 (1H, dd, J=4.3, 8.9 Hz, H-6'), 4.44—4.50 (1H, m, H-2), 5.05—5.36 (3H, m, H-3, H-4, H-5), 6.12 (1H, d, J=3.6 Hz, H-1α), 6.14 (1H, d, J=9.3 Hz, H-1β). ^{13}C-NMR ($CDCl_3$) δ: 13.5 (q, $-CH_3$), 19.9, 20.1, 20.2, 20.4 (q, CH_3CO), 22.2, 23.4, 24.5, 24.7, 25.2, 28.7, 28.9, 29.0, 29.1, 31.4, 33.3, 33.5, 33.6, 40.5 (t, $-CH_2-$), 50.2 (d, C-2), 61.3 (t, C-6), 67.7 (d, C-4), 69.2 (d, $CH_2CH(OAc)C_{11}H_{23}$), 70.2 (d, C-5), 72.0 (d, C-3), 90.4 (d, C-1), 168.8, 169.2, 170.3, 170.4, 171.3 (s, C=O). *Anal.* Calcd for $C_{30}H_{49}NO_{12} \cdot 1/3C_6H_5N$: C, 59.49; H, 7.90; N, 2.89. Found: C, 59.21; H, 8.20; N, 2.15.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-[(*R*)-3-acetoxytetradecanoylamino]-D-glucopyranosyl Azide (18) As described for **11**, compound **17** (2.00 g, 3.1 mmol) was reacted with 33% hydrogen bromide solution in acetic acid, followed by treatment with AgN_3 to give **18** (1.60 g, 82% in two steps from **19**) as a yellow oil, $[\alpha]_D$ +12.4° (c=1.20, $CHCl_3$). IR (neat): 2102 (azide), 1745 (ester), 1653, 1557 (amide) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz, $-CH_3$), 1.26 (20H, br s, $-CH_2-$), 1.59—1.68 (2H, m, $-CH_2-$), 2.00—2.16 (12H, m, CH_3CO), 2.37—2.52 (2H, m, $-CH_2-$), 3.85—3.99 (1H, m, H-2), 4.08—4.32 (3H, m, H-5, H-6, H-6'), 4.78—5.35 (2H, m, H-3, H-4), 6.08 (1H, d, J=8.5 Hz, H-1). ^{13}C-NMR ($CDCl_3$) δ: 14.2 (q, $-CH_3$), 20.6, 20.7, 21.1 (q, CH_3CO), 22.8, 25.1, 25.3, 25.4, 25.8, 29.3, 29.5, 29.6, 29.7, 32.0, 33.9, 34.1, 41.3 (t, $-CH_2-$), 53.7 (t, C-2), 62.2 (t, C-6), 68.7 (d, C-5), 71.5 (d, $CH_2CH(OH)C_{11}H_{23}$), 72.5 (d, C-4), 73.8 (d, C-3), 88.4 (d, C-1), 169.6, 169.8, 170.6, 173.6 (s, C=O). *Anal.* Calcd for $C_{28}H_{46}N_4O_{10}$: C, 56.17; H, 7.74; N, 9.36. Found: C, 55.97; H, 7.69; N, 9.32.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-[(*R*)-3-acetoxytetradecanoylamino]-D-glucopyranosylamine (19) As described for **12**, compound **18** (1.60 g, 2.96 mmol) was treated with Raney Ni (30 mg) under hydrogen to give **19** (0.90 g, 59%) as a white powder, $[\alpha]_D$ +58.9° (c=1.00, $CHCl_3$). IR (KBr): 1745 (ester), 1645, 1522 (amide) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.9 Hz, $-CH_3$), 1.25 (20H, br s, $-CH_2-$), 1.57 (2H, br s, $-CH_2-$), 2.02—2.11 (12H, m, CH_3CO), 4.09—4.27 (4H, m, H-2, H-5, H-6, H-6'), 5.06—5.56 (3H, m, H-1, H-3, H-4). ^{13}C-NMR ($CDCl_3$) δ: 14.1 (q, $-CH_3$), 20.6, 20.7, 21.0, 21.1 (q, CH_3CO), 22.8, 25.1, 25.3, 25.8, 29.2, 29.5, 29.7, 32.0, 33.9, 34.2, 41.3 (t, $-CH_2-$), 52.3 (d, C-2), 62.5 (t, C-6), 67.1 (d, C-4), 68.9 (d, C-5), 71.4 (d, C-3), 91.4 (d, C-1), 170.1, 170.6, 171.0, 171.4, 171.6 (C=O). *Anal.* Calcd for $C_{28}H_{48}N_2O_{10}$: C, 58.72; H, 8.45; N, 4.89. Found: C, 58.24; H, 8.36; N, 5.01.

2-Deoxy-2-[(*R*)-3-hydroxytetradecanoylamino]-D-glycopyranosylamine (20) As described for **13**, compound **19** (900mg, 1.70mmol) was reacted with sodium methoxide (90mg) in MeOH to give **20** (185mg, 60%) as a white powder, $[\alpha]_D$ +41.0° (c=1.00, MeOH). IR (KBr): 3480 (NH, OH), 1650, 1550 (amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.90 (3H, t, J=6.9 Hz, $-CH_3$), 1.29 (20H, br s, $-CH_2-$), 1.48 (2H, br s, $-CH_2-$), 2.31—2.45 (2H, m, $-CH_2-$), 3.40—3.98 (7H, m, H-1, H-2, H-3, H-4, H-5, H-6, H-6'). ^{13}C-NMR (DMSO-d_6) δ: 14.4 (q, $-CH_3$), 23.7, 26.0, 26.6, 26.7, 30.4, 30.7, 33.0, 34.7, 38.2 (t, $-CH_2-$), 54.5 (d, C-2), 62.5 (t, C-6), 69.8 (d, C-3), 72.3 (d, C-5), 72.7 (d, C-4), 90.4 (d, C-1), 170.1 (s, C=O). *Anal.* Calcd for $C_{20}H_{40}N_2O_6$: C, 59.38; H, 9.97; N, 6.92. Found: C, 59.14; H, 10.14; N, 6.81.

Benzyl N^2-Tetradecanoyl-N^4-[2-deoxy-2-[(*R*)-3-hydroxytetradecanoylamino]-β-D-glucopyranosyl]-L-asparaginate (21) As described for **14**, compound **20** (185 mg, 1.0 mmol) was reacted with compound **4** (433 mg, 1.0 mmol), DIEA (129 mg, 1.0 mmol), BOP (663 mg, 1.5 mmol), and HOBt (77 mg, 0.5 mmol) to give **21** (75 mg, 20%) as a white solid, $[\alpha]_D$ +13.0° (c=0.50, DMSO). IR (KBr): 3282 (OH), 1752 (ester), 1645, 1542 (amide) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.88 (6H, t, J=6.9 Hz, $-CH_3$), 1.25 (40H, br s, $-CH_2-$), 1.43 (2H, d, J=6.7 Hz, $-CH_2-$), 1.65 (3H, t, J=6.7 Hz, $-CH_2-$), 2.30 (2H, t, J=7.9 Hz, CH_2CH), 3.26—3.66 (6H, m, H-2, H-3, H-4, H-5, H-6, H-6'), 4.51—4.55 (1H, m, CH_2CH), 4.97 (1H, d, J=8.2 Hz, H-1), 7.25—7.33 (5H, m, Ph). ^{13}C-NMR (DMSO-d_6) δ: 14.1 (q, $-CH_3$), 22.7, 24.9, 29.0, 29.2, 29.5, 29.6, 30.0, 30.3, 31.5, 31.9,

33.9 (t, $-CH_2-$), 36.8 (t, $\underline{C}H_2CH$), 50.6 (d, $CH_2\underline{C}H$), 53.5 (d, C-2), 62.2 (t, C-6), 70.6 (d, C-4), 74.7 (d, C-3), 78.1 (d, C-5), 79.2 (d, C-1), 128.4, 128.9, 129.0, 129.7 (d, Ph), 70.6, 173.7, 174.9 (s, C=O). *Anal.* Calcd for $C_{45}H_{77}N_3O_{10}$: C, 65.90; H, 9.46; N, 5.12. Found: C, 65.32; H, 9.15; N, 5.07.

Benzyl N^2-Tetradecanoyl-N^4-[4,6-*O*-benzylidene-2-deoxy-2-[(*R*)-3-hydroxytetradecanoylamino]-β-D-glucopyranosyl]-L-asparaginate (22) A mixture of compound **21** (75 mg, 0.8 mmol), benzaldehyde dimethylacetal (0.1 ml), and *p*-toluenesulfonic acid (PTSA, 14 mg, 0.8 mmol) in DMF (10 ml) was stirred at room temperature under argon. The mixture was stirred at 50 °C for 12 h, then diluted with AcOEt, and washed with water, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous $MgSO_4$, and concentrated to dryness *in vacuo*. The residue was purified by silica gel chromatography (hexane–AcOEt, 5:1) to afford **22** (42 mg, 51%) as a white solid, $[\alpha]_D$ +9.7° (c=0.20, MeOH). IR (KBr): 1742 (carbonyl), 1689 (carbonyl), 1675, 1597 (amide), 755 (Ph) cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 0.88 (6H, t, J=6.9 Hz, $-CH_3$), 1.25 (40H, br s, $-CH_2-$), 1.60—1.62 (2H, m, $-CH_2-$), 2.30 (2H, t, J=7.9 Hz, $C\underline{H}_2CH$), 4.43—4.50 (1H, m, $CH_2C\underline{H}$), 5.04 (1H, d, J=9.2 Hz, H-1), 5.31 (1H, s, $-C\underline{H}Ph$), 7.47—7.53 (5H, m, $-CH_2\underline{Ph}$), 7.65—7.68 (5H, m, $-CH\underline{Ph}$). ^{13}C-NMR (CD_3OD) δ: 14.1 (q, $-CH_3$), 22.7, 24.9, 29.1, 29.3, 29.5, 29.6, 30.0, 30.3, 31.6, 31.9, 33.9 (t, $-CH_2-$), 36.8 (t, $\underline{C}H_2CH$), 50.6 (d, $CH_2\underline{C}H$), 78.1 (d, C-1), 128.4, 128.9, 129.0, 129.7 (d, $-CH_2\underline{Ph}$), 132.2, 132.8, 133.1, 135.6 (d, $-CH\underline{Ph}$). *Anal.* Calcd for $C_{52}H_{81}N_3O_{10}$: C, 68.77; H, 8.99; N, 4.63. Found: C, 69.02; H, 9.11; N, 4.77.

Benzyl N^2-Tetradecanoyl-N^4-[4,6-*O*-benzylidene-2-deoxy-3-*O*-tetradecanoyl-2-[(*R*)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranosylamino]-L-asparaginate (23) DCC (10 mg, 0.03 mmol) was added to a solution of compound **22** (42 mg, 0.03 mmol), NEt_3 (3 mg, 0.03 mmol), and 4-dimethylaminopyridine (DMAP, 0.4 mg, 0.003 mmol) in DMF (10 ml) at 0 °C under argon. The mixture was stirred at room temperature for 36 h. The reaction mixture was diluted with AcOEt, and washed with water, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous $MgSO_4$, and evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel (hexane–AcOEt, 5:1) to afford **23** (26 mg, 43%) as a colorless oil, $[\alpha]_D$ +5.5° (c=0.10, MeOH). IR (neat): 1751 (carbonyl), 1651, 1542 (amide), 752 (Ph) cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 0.88 (9H, t, J=6.9 Hz, $-CH_3$), 1.25 (60H, br s, $-CH_2-$), 4.78 (1H, t, J=8.7 Hz, H-1), 7.22—7.37 (5H, m, $-CH_2\underline{Ph}$), 7.46—7.52 (5H, m, $-CH\underline{Ph}$). ^{13}C-NMR (CD_3OD) δ: 14.1 (q, $-CH_3$), 22.6, 24.9, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 30.0, 31.6, 31.9, 33.9, 35.1 (t, $-CH_2-$), 36.9 (t, $\underline{C}H_2CH$), 50.6 ($CH_2\underline{C}H$), 120.9, 121.4, 122.7, 123.6, 128.4, 129.8, 129.9 (d, Ph). *Anal.* Calcd for $C_{80}H_{133}N_3O_{12}$: C, 72.30; H, 10.09; N, 3.16. Found: C, 72.01; H, 9.87; N, 3.25.

N^2-Tetradecanoyl-N^4-[2-deoxy-3-*O*-tetradecanoyl-2-[(*R*)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranosylamino]-L-asparagine (2) Palladium-on-carbon (10%, 30 mg) was added to a solution of compound **23** (26 mg, 0.02 mmol) in MeOH (5 ml) under hydrogen. The mixture was stirred at room temperature for 12 h, and then filtered through Celite 545. After evaporation to dryness *in vacuo*, the residue was washed with ether to afford compound **2** (9 mg, 40%) as a white powder, $[\alpha]_D$ +4.5° (c=0.10, DMSO). IR (KBr): 3480 (OH), 1750 (carbonyl), 1654, 1542 (amide) cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 0.88 (9H, t, J=6.9 Hz, $-CH_3$), 1.26 (60H, br s, $-CH_2-$), 1.43 (2H, br s, $-CH_2-$), 1.65 (2H, br s, $-CH_2-$). ^{13}C-NMR (CD_3OD) δ: 14.1 (q, $-CH_3$), 22.1, 22.7, 24.9, 29.1, 29.3, 29.5, 29.6, 29.7, 30.0, 31.6, 33.9, 35.1 (t, $-CH_2-$). *Anal.* Calcd for $C_{66}H_{123}N_3O_{12}$: C, 68.89; H, 10.77; N, 3.65. Found: C, 68.23; H, 10.58; N, 3.58. FAB-MS m/z: 1151 $(M+H)^+$.

References

1) Lipid A and Related Compounds. XXIX, Part XXVIII: Reference 6.
2) C. Galanos, O. Lüderitz, E. T. Rietschel, O. Westphal, *Int. Rev. Biochem.*, **14**, 239 (1977); O. Lüderitz, C. Galanos, V. Lehmann, H. Mayer, E. T. Rietschel, J. Wechesser, *Naturwissenschaften*, **65**, 578 (1978).
3) T. Shiba, S. Kusumoto, *Yuki Gosei Kagaku Kyokai Shi*, **42**, 507 (1984); *idem*, *Tampakushitu Kakusan Koso*, **31**, 353 (1986).
4) *a*) S. Kusumoto, M. Yamamoto, T. Shiba, *Tetrahedron Lett.*, **25**, 3727 (1984); *b*) T. Takahashi, C. Shimizu, S. Nakamoto, K. Ikeda, K. Achiwa, *Chem. Pharm. Bull.*, **33**, 1760 (1985); *c*) K. Ikeda, S. Nakamoto, T. Takahashi, K. Achiwa, *Carbohydr. Res.*, **145**, C5 (1986); *d*) S. Nakamoto, T. Takahashi, K. Ikeda, K. Achiwa, *Chem. Pharm. Bull.*, **33**, 4098 (1985); *e*) T. Shimizu, S. Akiyama, T. Masuzawa, Y. Yanagihara, S. Nakamoto, T. Takahashi, K. Ikeda, K. Achiwa, *ibid.*, **33**, 4621 (1985); *f*) *Idem*, *ibid.*, **34**, 5169 (1986); *g*) M. Kiso, H. Ishida, A. Hasegawa, *Agric. Biol. Chem.*, **48**, 251 (1984); *h*) D. Charon, R. Chaby, A. Malinvauld, M. Monage, L. Szabo, *Biochemistry*, **24**, 2736 (1985); *i*) M. Kiso, Y. Ogawa, S. Tanaka, H. Ishida, A. Hasegawa, *J. Carbohydr. Chem.*, **5**, 621 (1986); *j*) M. Kiso, S. Tanaka, M. Tanahashi, Y. Fujishima, Y. Ogawa, A. Hasegawa, *Carbohydr. Res.*, **148**, 221 (1986); *k*) M. Kiso, S. Tanaka, M. Fujita, Y. Fujishima, Y. Ogawa, A. Hasegawa, *ibid.*, **162**, 247 (1987).
5) *a*) T. Pedron, R. Girard, J. Eustache, M. A. R. C. Bulusu, I. Macher, H. Radzyner-Vyplel, P. L. Stütz, R. Chaby, *Int. Immunol.*, **4**, 533 (1992); *b*) M. A. R. C. Bulusu, P. Waldstätten, J. Hildebrandt, E. Schütze, G. Schulz, *J. Med. Chem.*, **35**, 3463 (1992); *c*) M. A. R. C. Bulusu, P. Waldstätten, *Tetrahedron Lett.*, **30**, 1859 (1992); *d*) Y. Kawai, K. Akagawa, *Infect. Immun.*, **57**, 2086 (1989); *e*) L. Brade, W. G. Bessler, H. Brade, *ibid.*, **56**, 1382 (1988); *f*) J. Eustache, A. Grob, H. Retscher, *Carbohydr. Res.*, **251**, 251 (1994).
6) K. Ikeda, T. Asahara, K. Achiwa, *Chem. Pharm. Bull.*, **41**, 1879 (1993).
7) R. Polt, L. Szabo, J. Treiberg, Y. Li, V. J. Hruby, *J. Am. Chem. Soc.*, **114**, 10249 (1992).
8) E. J. Goldstein (ed.), "Carbohydrate Protein Interaction," ACS Symp. Ser. 88, 1979; T. Feizi, *Nature* (London), **314**, 53 (1985); *idem, Biochem. J.*, **245**, 1 (1987).
9) F. Micheel, H. Wulff, *Chem. Ber.*, **89**, 1521 (1956)