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Lipid A and Related Compounds. XXX.[1] Synthesis of Biologically Active *N*,*N*′-Diacyl Chitobiose Derivatives Structurally Related to Lipid A

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Two new glycolipids, which mimic lipid A disaccharide, were synthesized from *N*,*N*′-diacylchitobiose *via* a key intermediate (2). They showed mitogenicity and nitric oxide (NO) productivity.

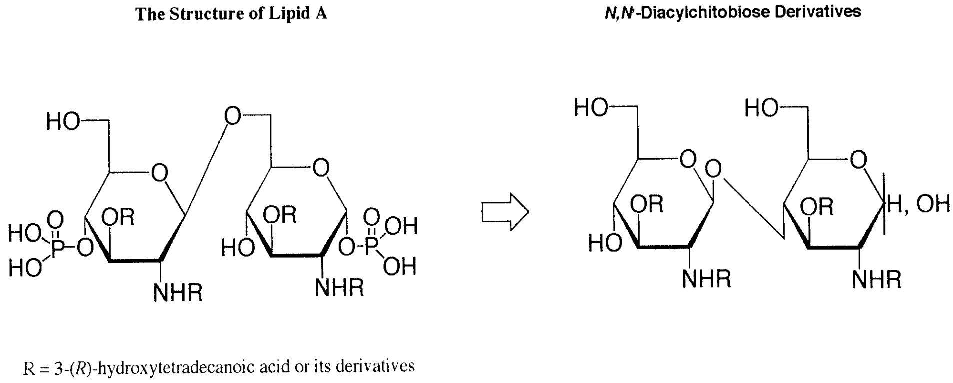
Key words *N*-acylated glycolipid; *N*,*N*′,-diaclychitobiose; lipid A analogue; mitogenic activity; NO production

Lipid A of gram-negative bacterial lipopolysaccharides (LPS) is composed of a 1,4-bisphosphorylated *β*-(1→6)linked D-glucosamine disaccharide[2] with amide- and ester-linked D-3-(*R*)-hydroxy and/or acyloxy fatty acids, as indicated in Chart 1. Lipid A and its synthetic analogues possess a wide variety of biological activities, *e.g.*, endotoxicity, adjuvanticity, mitogenicity, antitumor activity and so on.[3] Many compounds related to lipid A partial structures have been synthesized with the aim of enhancing the potentially beneficial immunostimulatory properties.[4]

Among the amino sugar derivatives, chitooligosaccharides, with a *β*(1→4)linked D-glucosamine skeleton, are known to be biologically active.[5] To create novel analogues of lipid A possessing a good balance between toxicity and beneficial bioactivities, we have synthesized new glycolipids differing from lipid A in the type of glycosidic linkage in the backbone.

The suitably functionalized key intermediate (**2**), carrying two amino and two hydroxyl groups at the C-2, 2′ and C-3, 3′ positions of the D-glucosamine disaccharide backbone, was readily prepared from *N*,*N*′-diacetylchitobiose in 7 steps, as shown in Chart 2. Condensation between oxazoline (**3**)[6] and benzyl alcohol was performed in chloroform with trifluoromethanesulfonic acid as the catalyst to give *β*-benzyl glycoside (**4**) in 51% yield. The nuclear magnetic resonance (^{1}H-NMR) spectrum of **4** showed the presence of the anomeric proton signal as a doublet at δ4.58 (J=7.8 Hz), indicating that the newly formed glycoside bond has the *β* configuration. To protect other hydroxyl groups, *O*-deacetylation of **4** by the Zemple'n procedure with NaOMe in MeOH–CH_2Cl_2 (4 : 1) afforded compound **5** in 96% yield, then treatment of **5** with α,α-dimethoxytoluene in dimethylformamide (DMF) in the presence of a catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH) gave the benzyl acetal (**6**) in 80% yield. Compound **6** was heated at 60 °C for 6 d with trityl chloride in DMF–pyridine (1 : 9) mixture in the presence of 4-dimethylaminopyridine (DMAP), giving the 6-*O*-trityl derivative (**7**) in 49% yield. Cleavage of the *N*-acetyl group of **7** was effected with KOH–EtOH at 110—120 °C to afford the key intermediate (**2**) in 52 % yield. The structure of **2** was confirmed by the disappearance of the *N*-acetyl proton (CH_3CO–) signal in the ^{1}H-NMR spectrum and the amide absorption in the infrared (IR) spectrum. The ^{1}H-NMR spectrum of **2** revealed the C-2′ and C-2 protons at δ2.45 and 2.98, respectively. The key intermediate (**2**) thus obtained was used for the synthesis of the desired compounds (**1**) as follows:

We selected the dodecanoyl and tetradecanoyl groups at C-2, -2′ and C-3, -3′ of the D-glucosamine disaccharide backbone. The free amino and hydroxyl groups of **2** were simultaneously acylated with dodecanoyl chloride and tetradecanoyl chloride in the presence of a catalytic amount of DMAP in DMF–pyridine (1 : 1) to give the diacylates (**8a** and **8b**) in yields of 43% and 30%, respectively. Finally, the protective benzyl acetal and benzyl groups of **8a** and **8b** were removed stepwise by hydrolysis with 80% aqueous AcOH at 60—70 °C, followed by hydrogenolysis of the *O*-benzyl group with H_2, Pd-black in 1 : 1 MeOH–THF at room temperature to afford the target compounds (**1a** and **1b**) in 41% and 30% yield in two steps, respectively, after purification by


The Structure of Lipid A

N,*N*′-Diacylchitobiose Derivatives

R = 3-(*R*)-hydroxytetradecanoic acid or its derivatives

Chart 1

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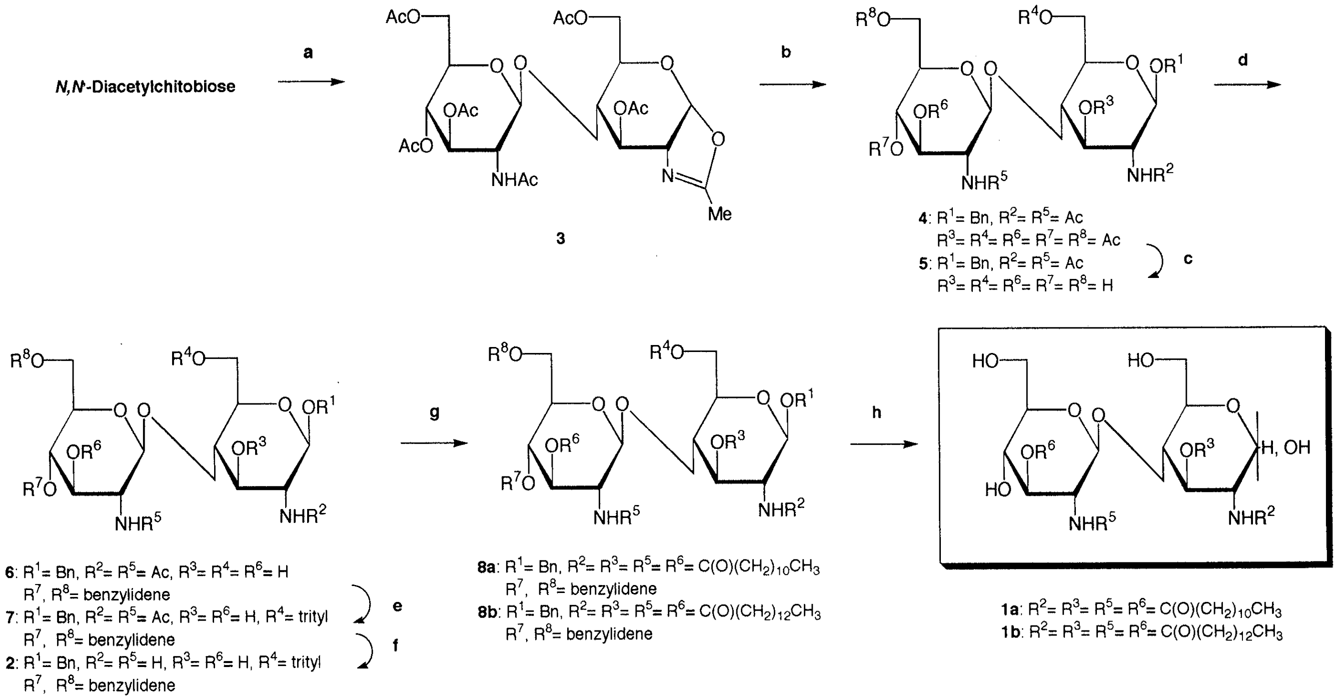

Reagents: a) 1) Ac_2O-pyridine, 2) TMSOTf, NEt_3 in $ClCH_2CH_2Cl$; b) BnOH, CF_3SO_3H in $CHCl_3$; c) NaOMe in MeOH-CH_2Cl_2 (4:1); d) $PhCH(OMe)_2$, *p*-TsOH in DMF; e) trityl chloride, DMAP,DMF-pyridine (1:9); f) KOH in EtOH; g) $CH_3(CH_2)_{10}COCl$ or $CH_3(CH_2)_{12}COCl$, DMAP, DMF-pyridine (1:1); h) 1) 80% AcOH, 2) Pd-black, H_2 in MeOH-THF (1:1).

Chart 2

silica gel column chromatography (CH_2Cl_2 : MeOH = 10 : 1), followed by lyophilization from dioxane.

The structures of all compounds were characterized by ^1H- and ^{13}C-NMR spectroscopies, as well as IR spectroscopy, elemental analyses, and fast-atom bombardment (FAB) mass spectroscopy.

Preliminary examination of the biological activity of the two chemically synthesized compounds showed that compounds **1a** and **1b** were weakly mitogenic,[7] and they exhibited significant NO production activity[8] (1.7 and 5.7 nmol/10^6 cells, respectively, at 10 μM), in murine peritoneal macrophages. Furthermore, **1b** had low cytotoxicity. The NO-productivity of **1b** was higher than that of **1a**, suggesting that the bound fatty acid moieties of **1a** and **1b** of the lipid A analogues affect the expression of biological activities.

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. ^1H-NMR spectra were taken on a JEOL JNM-GX 270 (270 MHz) spectrometer. ^{13}C-NMR spectra were recorded with a JEOL JNM-GX 270 (67.5 MHz) spectrometer. The ^1H and ^{13}C chemical shifts (δ) are given in ppm relative to Me_4Si ($\delta = 0$)in $CDCl_3$ or CD_3OD as an internal standard. The abbreviations of signal patterns are as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra (MS) were recorded on a JEOL JMS-SX102 spectrometer. 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.

Benzyl 3,6-Di-*O*-acetyl-2-acetylamino-4-*O*-(3,4,6-tri-*O*-acetyl-2-acetylamino-2-deoxy-*β*-D-glucopyranosyl)-2-deoxy-*β*-D-glucopyranoside (4) A solution of the oxazoline **3** (0.59 g, 0.93 mmol), benzyl alcohol (1.0 g, 9.3 mmol) and molecular sieves 4 Å (0.5 g) in anhydrous chloroform (20 ml) containing trifluoromethanesulfonic acid (14 mg, 0.093 mmol) was stirred under an argon atmosphere for 15 h at 80—90 °C. The reaction mixture was filtered through Celite 545 and the filtrate was washed with aqueous sodium hydrogencarbonate and water, then dried, and evaporated. The syrupy residue was subjected to silica gel chromatography with CH_2Cl_2–MeOH (50 : 1) to give **4** (0.35 g, 51%), as white prisms, mp 278—279 °C. $[\alpha]_D$ −55.1° ($c = 0.98$, $CHCl_3$). IR (KBr): 3280 (NH), 1747 (ester), 1654, 1546 (amide), 722, 696 (Ph) cm^{-1}. ^1H-NMR ($CDCl_3$) δ: 1.78, 1.93, 2.00, 2.03, 2.06, 2.16 (21H, s, NAc and OAc), 3.64—3.65 (2H, m, H-5 and H-5′), 3.75 (1H, t, $J = 8.5$ Hz, H-4), 3.86 (1H, ddd, $J = 7.8$, 8.9, 10.3 Hz, H-2′), 4.03 (1H, dd, $J = 12.5$, 2.0 Hz, H-6′a), 4.09 (1H, ddd, $J = 7.8$, 9.2, 9.5 Hz, H-2), 4.32 (1H, dd, $J = 12.7$, 4.6 Hz, H-6a), 4.40 (1H, d, $J = 12.7$, 3.0 Hz, H-6b), 4.47 (1H, d, $J = 7.8$ Hz, H-1′), 4.58 (1H, d, $J = 7.8$ Hz, H-1), 4.57, 4.86 (each 1H, d, $J = 12.4$ Hz, $C\underline{H}_2Ph$), 5.04 (1H, dd, $J = 9.7$ Hz, H-4′), 5.05 (1H, dd, $J = 9.5$, 8.5 Hz, H-3), 5.19 (1H, dd, $J = 10.3$, 9.7 Hz, H-3′), 7.27—7.37 (5H, m, Ar). ^{13}C-NMR ($CDCl_3$) δ: 20.6, 20.7, 21.0, 23.2, 53.4, 54.7, 61.8, 62.5, 68.1, 70.4, 71.9, 72.4, 73.0, 75.9, 99.5, 101.1, 127.9. 128.0, 128.5, 136.9, 169.4, 170.2, 170.5, 170.6, 170.9, 171.1. *Anal.* Calcd for $C_{33}H_{44}N_2O_{16}$: C, 54.69; H, 6.12; N, 3.37. Found: C, 54.83; H, 6.23; N, 3.71.

Benzyl 2-Acetylamino-4-*O*-(2-acetylamino-2-deoxy-*β*-D-glucopyranosyl)-2-deoxy-*β*-D-glucopyranoside (5) A solution of sodium (18 mg) in MeOH (10 ml) was added to a solution of the glycoside **4** (0.54 g, 0.75 mmol) in dry MeOH–CH_2Cl_2 (4 : 1) (50 ml) at 0 °C, and the mixture was stirred for 4 h at room temperature, then treated with an ion exchange resin Amberlite IRC-50 (1.0 g). The suspension was filtered and the filtrate was evaporated to dryness *in vacuo*. The residue was subjected to silica gel chromatography with CH_2Cl_2–MeOH–H_2O (12 : 6 : 0.5) to give **5** (0.37 g, 96%), as a white powder, mp 333—336 °C. $[\alpha]_D$ −34.0° ($c = 0.54$, $CHCl_3$–MeOH (1 : 1)). IR (KBr): 3263 (OH), 1650, 1623, 1553 (amide), 718 (Ph) cm^{-1}. ^1H-NMR ($CDCl_3$–CD_3OD) δ: 1.94, 2.00 (each 3H, br s, NHAc), 4.54, 4.80 (each 1H, d, $J = 12.4$ Hz, $PhC\underline{H}_2$), 7.35—7.51 (5H, m, Ar). *Anal.* Calcd for $C_{23}H_{34}N_2O_{11} \cdot 2H_2O$: C, 50.18; H, 6.96; N, 5.09. Found: C, 49.66; H, 6.30; N, 5.03.

Benzyl 2-Acetylamino-4-*O*-(2-acetylamino-4,6-*O*-benzylidene-2-deoxy-*β*-D-glucopyranosyl)-2-deoxy-*β*-D-glucopyranoside (6) A solution of **5** (0.40 g, 0.78 mmol), α,α-dimethoxytoluene (0.42 g, 2.3 mmol) and *p*-TsOH monohydrate (15 mg, 0.078 mmol) in DMF (10 ml) was heated at

40—50 °C for 17 h under argon, and treated with an ion exchange resin Amberlyst A-21 (0.5 g). The suspension was filtered and the filtrate was evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel with CH_2Cl_2–MeOH (9 : 1) to give **6** (0.44 g, 94%), as a white powder, mp 316—319 °C. $[\alpha]_D$ −38.4° (c=0.76, MeOH). IR (film): 3276 (OH, NH), 1653, 1625, 1550 (amide), 720, 697 (Ph) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.98, 2.04 (each 3H, s, NHAc), 3.36—3.37 (2H, m, H-6 and H-6′), 3.62—3.67 (1H, m, H-5), 3.74 (1H, dd, J=8.1, 9.2 Hz, H-2′), 3.88 (1H, dd, J=8.4, 9.9 Hz, H-2), 4.47 (1H, d, J=8.1 Hz, H-1′), 4.58, 4.85 (each 1H, d, J=12.2 Hz, $PhC\underline{H}_2$), 4.61 (1H, d, J=8.4 Hz, H-1), 5.55 (1H, s, $PhC\underline{H}$), 7.30—7.48 (10H, m, Ar). ^{13}C-NMR ($CDCl_3$) δ: 22.4, 22.5, 55.0, 56.0, 66.1, 67.9, 70.6, 71.3, 71.8, 74.3, 80.2, 80.8, 99.9, 101.6, 102.3, 125.5, 126.0, 127.5, 127.6, 127.9, 128.0, 128.1, 128.7, 129.0, 136.6, 137.0, 171.8, 172.4. *Anal.* Calcd for $C_{30}H_{38}N_2O_{11}\cdot 2H_2O$: C, 56.42; H, 6.63; N, 4.39. Found: C, 56.58; H, 6.09; N, 4.29.

Benzyl 2-Acetylamino-4-*O*-(2-acetylamino-4,6-*O*-benzylidene-2-deoxy-*β*-D-glucopyranosyl)-2-deoxy-6-*O*-trityl-*β*-D-glucopyranoside (7) A solution of **6** (0.72 g, 1.2 mmol) and DMAP (0.15 g, 0.15 mmol) in DMF–pyridine (1 : 9) (12 ml) was treated with trityl chloride (0.84 g, 3.0 mmol). The mixture was stirred at 50—60 °C for 6 d under argon, then cooled and concentrated. The residue was chromatographed on silica gel with CH_2Cl_2–MeOH (50 : 1) to give **7** (0.50 g, 49%), as white prisms, mp 166—168 °C. $[\alpha]_D$ −39.0° (c=1.0, $CHCl_3$). IR (film): 3399, 3303 (OH, NH), 1651, 1537 (amide), 743, 702 (Ph) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.60, 2.02 (each 3H, s, NHAc), 3.01 (1H, br d, J=10 Hz, H-6a), 3.16 (1H, br s, OH), 3.25 (1H, dt, J=4.6, 9.7 Hz, H-5′), 3.36—3.46 (3H, m, H-3′, H-4′, H-5), 3.52 (1H, br s, OH), 3.71 (1H, dd, J=4.6, 10 Hz, H-6′a), 3.79 (1H, br d, J=10 Hz, H-6b), 3.86 (1H, t, J=9.2 Hz, H-3), 3.97 (1H, dd, J=8.1, 9.2 Hz, H-2), 4.10 (1H, dd, J=9.2 Hz, H-4), 4.29 (1H, d, J=8.1 Hz, H-1′), 4.41 (1H, dd, J=4.9, 10 Hz, H-6′b), 4.58 (1H, d, J=8.1 Hz, H-1), 4.67, 4.99 (each 1H, d, J=11.9 Hz, $PhC\underline{H}_2$), 5.50 (1H, s, $PhC\underline{H}$), 5.76 (1H, d, J=6.8 Hz, NH′), 7.29—7.61 (25H, m, Ar). ^{13}C-NMR ($CDCl_3$) δ: 23.2, 23.6, 55.4, 56.1, 61.0, 66.4, 68.4, 69.9, 71.7, 71.9, 73.5, 81.2, 86.2, 99.3, 100.9, 101.8, 126.2, 127.5, 127.9, 128.2, 128.3, 128.5, 128.8, 129.3, 136.7, 137.3, 143.6, 170.8, 170.9. *Anal.* Calcd for $C_{49}H_{52}N_2O_{11}\cdot 2/3H_2O$: C, 67.49; H, 6.36; N, 3.21. Found: C, 67.30; H, 6.11; N, 3.08.

Benzyl 2-Amino-4-*O*-(2-amino-4,6-*O*-benzylidene-2-deoxy-*β*-D-glucopyranosyl)-2-deoxy-6-*O*-trityl-*β*-D-glucopyranoside (2) Pulverized KOH (0.20 g) was added to a solution of **7** (0.185 g, 0.22 mmol) in EtOH (5 ml). The mixture was stirred at 110—120 °C for 12 h under argon, then treated with an ion exchange resin Amberlite IRC-50 (0.5 g). The suspension was filtered and the filtrate was evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel with CH_2Cl_2–MeOH (10 : 1) to give **2** (0.122 g, 73%) as an amorphous powder. $[\alpha]_D$ +28.3° (c=0.28, $CHCl_3$). IR (film): 3255, 3148 (OH, NH), 738, 719, 697 (Ph) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.45 (1H, ddd, J=7.8, 8.9, 9.2 Hz, H-2,), 2.98 (1H, ddd, J=7.8, 8.4, 9.2 Hz, H-2), 3.24 (1H, dd, J=10.5, 3.8 Hz, H-6′a), 3.34 (1H, t, J=9.2 Hz, H-3′), 3.48 (1H, t, J=9.2 Hz, H-3), 3.73 (1H, t, J=9.2 Hz, H-4′), 3.85 (1H, t, J=9.2 Hz, H-4), 4.09 (1H, d, J=7.8 Hz, H-1′), 4.34 (1H, dd, J=10.5, 4.9 Hz, H-6′b), 4.42 (1H, d, J=7.8 Hz, H-1), 4.70, 5.02 (each 1H, d, J=11.3 Hz, $PhC\underline{H}_2$), 5.50 (1H, s, $PhC\underline{H}$), 7.26—7.52 (25H, m, Ar). ^{13}C-NMR ($CDCl_3$) δ: 30.0, 57.1, 58.1, 62.0, 66.5, 68.3, 70.8, 72.5, 73.9, 74.4, 79.3, 80.9, 86.4, 101.8, 102.0, 104.1, 126.3, 127.3, 127.9, 128.0, 128.2, 128.3, 128.5, 128.8, 129.3, 136.8, 137.1, 143.6. FAB-MS m/z: 761 $(M+1)^+$; 783 $(M+Na)^+$.

Benzyl 4-*O*-(4,6-*O*-Benzylidene-2-deoxy-3-*O*-dodecanoyl-2-dodecanoylamino-*β*-D-glucopyranosyl)-2-deoxy-3-*O*-dodecanoyl-2-dodecanoylamino-6-*O*-trityl-*β*-D-glucopyranoside (8a) Dodecanoyl chloride (0.119 g, 0.48 mmol) was added to a solution of **2** (0.046 g, 0.06 mmol) and DMAP (0.058 g, 0.48 mmol) in DMF–pyridine (3 ml) (1 : 1) under ice-cooling. The mixture was stirred at 40—50 °C for 20 h under argon, then evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel with CH_2Cl_2–MeOH (50 : 1) to give **8a** (0.041 g, 43%) as a wax. $[\alpha]_D$ −5.2° (c=0.30, $CHCl_3$). IR (film): 3398, 3298 (NH), 2925, 2851 (CH), 1743 (ester), 1655, 1532 (amide), 744, 701, 635 (Ph) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (12H, t, J=6.8 Hz, $-CH_3$), 1.26 (64H, br s, $-(CH_2)_8-$), 1.50—1.65 (8H, m, $COCH_2C\underline{H}_2$), 2.20—2.33 (4H, m, $NCOCH_2-$), 3.27—3.39 (1H, m, H-5′), 3.51 (1H, dd, J=8.9 Hz, H-5), 3.63—3.81 (2H, m, H-6′a and H-6b), 4.13 (1H, d, J=8.9 Hz, H-1′), 4.43 (1H, dd, J=10.3, 5.7 Hz, H-6′b), 4.66, 4.99 (each 1H, d, J=11.9 Hz, $PhC\underline{H}_2$), 4.72—4.93 (2H, m, H-3 and H-3′), 5.47 (1H, s, $PhC\underline{H}$), 7.32—7.58 (25H, m, Ar). Positive FAB-MS m/z: 1624 $(M+Na)^+$.

2-Deoxy-4-*O*-(2-deoxy-3-*O*-dodecanoyl-2-dodecanoylamino-*β*-D-glucopyranosyl)-3-*O*-dodecanoyl-2-dodecanoylamino-D-glucopyranose (1a) A solution of **8a** (0.45 g, 0.028 mmol) in 80% AcOH (5 ml) was heated at 60—70 °C for 24 h. After evaporation of the solvent, the residue was dissolved in MeOH–THF (1 : 1) (2 ml) containing palladium-black (0.041 g) and the mixture was stirred under a hydrogen atmosphere for 17 h at room temperature. The catalyst was filtered off and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography using CH_2Cl_2–MeOH (50 : 1) to give **1a** (0.27 g, 41%) as an amorphous powder. $[\alpha]_D$ +18.9° (c=0.18, $CHCl_3$). IR (film): 2929, 2851 (CH). 1713 (ester), 1646, 1542 (amide) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (12H, m, $-CH_3$), 1.26 (64H, br s, $-(CH_2)_8-$), 1.50—1.65 (8H, m, $COCH_2C\underline{H}_2$), 2.20—2.33 (4H, m, $NCOCH_2$). *Anal.* Calcd for $C_{68}H_{128}N_2O_{13}$: C, 69.11; H, 10.92; N, 2.37. Found: C, 69.61; H, 11.52; N, 2.69.

Benzyl 4-*O*-(4,6-*O*-Benzylidene-2-deoxy-3-*O*-tetradecanoyl-2-tetradecanoylamino-*β*-D-glucopyranosyl)-2-deoxy-3-*O*-tetradecanoyl-2-tetradecanoylamino-6-*O*-trityl-*β*-D-glucopyranoside (8b) The same procedure as described for the preparation of compound **8a** provided a crude product from **2** (0.049 g, 0.065 mmol) and tetradecanoyl chloride (0.114 g, 0.52 mmol) at 40—50 °C for 17 h and this was purified on a column of silica gel with 50 : 1 CH_2Cl_2–acetone to give **8b** (0.029 g, 30%) as a wax. $[\alpha]_D$ −43.2° (c=0.38, $CHCl_3$). IR (film): 3368 (NH), 2925, 2851 (CH), 1735 (ester), 1684 (amide), 696 (Ph) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (12H, t, J=6.8 Hz, $-CH_3$), 1.26 (80H, br s, $-(CH_2)_{10}-$), 1.45—1.60 (8H, m, $COCH_2C\underline{H}_2-$), 2.05—2.16 (4H, m, $-OCOCH_2-$), 2.19—2.30 (4H, m, $NCOCH_2$), 2.94 (1H, d, J=10.3 Hz, H-6a), 3.11—3.21 (1H, m, H-5′), 3.66—3.75 (2H, m, H-6′a and H-6b), 3.86 (1H, dd, J=8.1, 9.7 Hz, H-2′), 4.24 (1H, d, J=8.1 Hz, H-1′), 4.39—4.45 (1H, m, H-6′b), 4.67, 4.99 (each 1H, d, J=12.2 Hz, $PhC\underline{H}_2$), 4.77 (1H, t, J=9.7 Hz, H-3′), 4.89 (1H, dd, J=10.0 Hz, H-3), 5.46 (1H, s, $PhC\underline{H}$), 7.31—7.58 (25H, m, Ar). Positive FAB-MS m/z: 1512 $(M+Na)^+$.

2-Deoxy-4-*O*-(2-deoxy-3-*O*-tetradecanoyl-2-tetradecanoylamino-*β*-D-glucopyranosyl)-3-*O*-tetradecanoyl-2-tetradecanoylamino-D-glucopyranose (1b) Compound **1b** was obtained from **8b** (0.045 g, 0.028 mmol) by a procedure similar to that described for **1a** and this product was chromatographed on a silica gel with 10 : 1 CH_2Cl_2–MeOH to give **1b** (0.018 g, 30%) as a white amorphous powder. $[\alpha]_D$ −5.7° (c=0.28, $CHCl_3$). IR (film): 3311 (NH, OH), 2916, 2845 (CH), 1743 (ester), 1647, 1540 (amide) cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.81 (12H, m, $-CH_3$), 1.18 (80H, br s, $-(CH_2)_{10}-$), 1.42—1.58 (8H, m, $COCH_2CH_2-$), 2.20—2.29 (4H, m, $NCOCH_2$). *Anal.* Calcd for $C_{60}H_{112}N_2O_{13}$: C, 67.38; H, 10.47; N, 2.62. Found: C, 67.98; H, 11.22; N, 2.80.

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