Lipid A and Related Compounds. XXXIII.[1] Synthesis and Structure–Activity Relationships of *N*-Acylated L-Serine or L-Threonine-Containing D-Glucosamine Derivatives as Mimics of Lipid A Disaccharide

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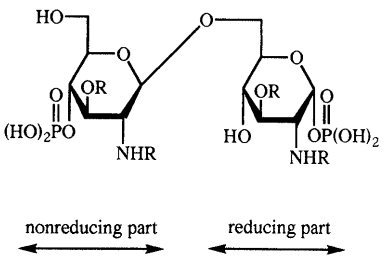
Received November 5, 1996; accepted February 5, 1997

Four novel *N*-acylated L-serine or L-threonine-containing non-phosphorylated D-glucosamine derivatives structurally corresponding to the lipid A disaccharide backbone were synthesized. Compounds 2, 3 and 4 exhibited potent mitogenic activity. Among the threonine-linked lipid A analogs (1—3), serine-linked lipid A analogs (4—6) and homoserine-linked lipid A analogs (7—9), the serine compounds (4, 6) showed the most potent mitogenic activity.

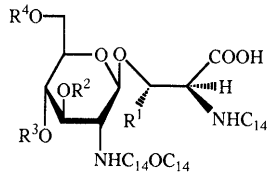
Key words *N*-acylated L-threonine; lipid A analog; structure–activity relationship; mitogenic activity

Lipid A is important in the expression of many of the biological activities, such as endotoxicity, adjuvanticity, antitumor activity and so on, of lipopolysaccharide (LPS) of gram-negative bacteria.[2] It consists of a D-glucosamyl-(1→6)-β-D-glucosamine carrying two phosphates and several fatty acids residues,[3] as indicated in Chart 1. Many compounds related to lipid A partial structures have been synthesized with a view to separating unwanted endotoxic properties from potentially beneficial immunostimulatory properties. Among the various synthetic lipid A analogs, D-glucosamine-4-phosphate analogs corresponding to the non-reducing unit of lipid A show many of the biological activities of LPS.[4] Recently, various acyclic analogs related to lipid A partial structure have been synthesized.[5] We have already reported the synthesis and biological activities of *N*-acylated L-serine or L-homoserine-containing D-glucosamine 4-phosphate derivatives structurally similar to the lipid A disaccharide backbone.[6] As a result, it has been found that the mitogenic activity of L-serine-linked lipid A analogs (**5**, **6**) is stronger than that of homoserine-linked lipid A analogs (**7**—**9**); in particular, that of compound **6**

Structure of Lipid A



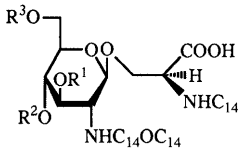
R = (*R*)-3-Hydroxytetradecanoyl or its derivatives



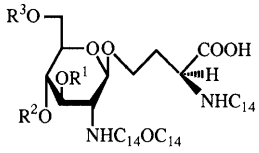
1 : $R^1 = CH_3$, $R^2 = R^3 = R^4 = Ac$
2 : $R^1 = CH_3$, $R^2 = R^3 = R^4 = H$
3 : $R^1 = CH_3$, $R^2 = C_{14}$, $R^3 = R^4 = H$
4 : $R^1 = H$, $R^2 = R^3 = R^4 = H$

C_{14}: $CH_3(CH_2)_{12}C(O)$-
$C_{14}OC_{14}$: $CH_3(CH_2)_{10}\underset{(R)}{C}HCH_2C(O)$-
$CH_3(CH_2)_{12}C(O)O$

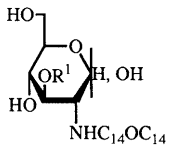
Chart 1



5 : $R^1 = R^2 = R^3 = Ac$
6 : $R^1 = C_{14}$, $R^2 = R^3 = H$



7 : $R^1 = R^2 = R^3 = Ac$
8 : $R^1 = R^2 = R^3 = H$
9 : $R^1 = C_{14}$, $R^2 = R^3 = H$



10 : $R^1 = C_{14}OC_{14}$

Chart 2

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was about twice that of the original acylderivative (**10**)[7] of D-glucosamine-4-phosphate, and the phosphate group was not required in lipid A analogs for mitogenicity. Based on the above results, with the aim of increasing the biological activities and chemical stability, and further making a comparison with the biological activity of *N*-acylated L-serine-linked lipid A analogs (**5**, **6**) and L-homoserine-linked lipid A analogs (**7**—**9**), we designed new non-phosphorylated lipid A analogs (**1**—**3**), in which the reducing unit of lipid A is mimicked by L-threonine instead of L-serine. In addition, we designed a new *N*-acylated L-serine-containing D-glucosamine derivative (**4**) for comparison of its biological activities with those of compound **2**.

In this paper, we describe the synthesis and biological activities of *N*-acylated L-threonine-containing D-glucosamine analogs (**1**—**3**) and the *N*-acylated L-serine-containing D-glucosamine analog (**4**), as well as the structure-activity relationships of *N*-acylated L-serine, L-homoserine or L-threonine-containing D-glucosamine derivatives.

Compounds **1**—**4** were synthesized by a method similar to that employed in our previous synthesis of *N*-acylated L-serine or L-homoserine-linked lipid A analogs.[6a,b] Compounds **1**, **2** and **4** were synthesized by the route shown in Chart 3. Coupling of **11**[6b] and **12** with *N*-bromosuccinimide (NBS),[8] iodine, and molecular sieves 4 Å using tetrabutylammonium trifluoromethanesulfonate (TBAOTf) as a promoter in CH_2Cl_2 gave the β-glycoside (**13**) in 68% yield. The β-configuration of **13** was determined from the coupling constant value (8.2 Hz) of the signal due to the anomeric proton in the proton magnetic resonance (^{1}H-NMR) spectrum of **13**.[9] After cleavage of the 2,2,2-trichloroethoxycarbonyl (Troc) group of **13** with activated zinc powder in acetic acid (AcOH), an (*R*)-3-tetradecanoyloxytetradecanoyl group was introduced onto the amino group by the diethylphosphorocyanidate (DEPC)–triethylamine (TEA) method to give **14** in 65% yield. The benzyl group of **14** was removed by hydrogenolysis over palladium-black at room temperature in MeOH–tetrahydrofuran (THF) to afford the desired compound **1** in 63% yield. Removal of the acetyl groups in **1** by treatment with concentrated NH_4OH in MeOH–THF gave the alcohol **2** in 82% yield after purification followed by lyophilization from dioxane.

Subsequently, as described for **2**, compound **15**[6a] was treated with concentrated NH_4OH to give the desired compound **4** in 91% yield after purification followed by lyophilization from dioxane.

Next, the synthesis of **3** was carried out as follows (Chart 4). Condensation of **16**[6b] and **12** with $HgBr_2$ as a promoter and molecular sieves 4 Å in CH_2Cl_2 gave the β-glycoside **17** in 42% yield. The β-configuration of **17** was determined from the J_{C-H} value of 159.3 Hz in the carbon magnetic resonance (^{13}C-NMR) spectrum of **17**.[10] After removal of the Troc group of **17**, an (*R*)-3-tetradecanoyloxytetradecanoyl group was introduced onto the amino group by the dicyclohexylcarbodiimide (DCC) method to give **18** in 71% yield. The remaining hydroxy group of **18** was acylated with tetradecanoic acid, DCC–4-dimethylaminopyridine (DMAP) to give **19** in 69% yield. Finally, catalytic hydrogenolysis using palladium-black in MeOH– THF gave the desired compound **3** in 74% yield after purification followed by lyophilization from dioxane.

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

In a preliminary examination of mitogenic activity towards the splenocytes of C3H/He mice,[11] compounds **2** and **4** were about 2 and 1.6 times more active, respectively, than compound **10**, while compound **3** was roughly equipotent with **10**, and **1** was only about half as potent.

In conclusion, among these *N*-acylated L-serine, L-homoserine or L-threonine-containing D-glucosamine derivatives, the serine compounds (**4**—**6**) exhibited the most potent mitogenicity, and the mitogenicity of the threonine compounds (**1**—**3**) was stronger than that of

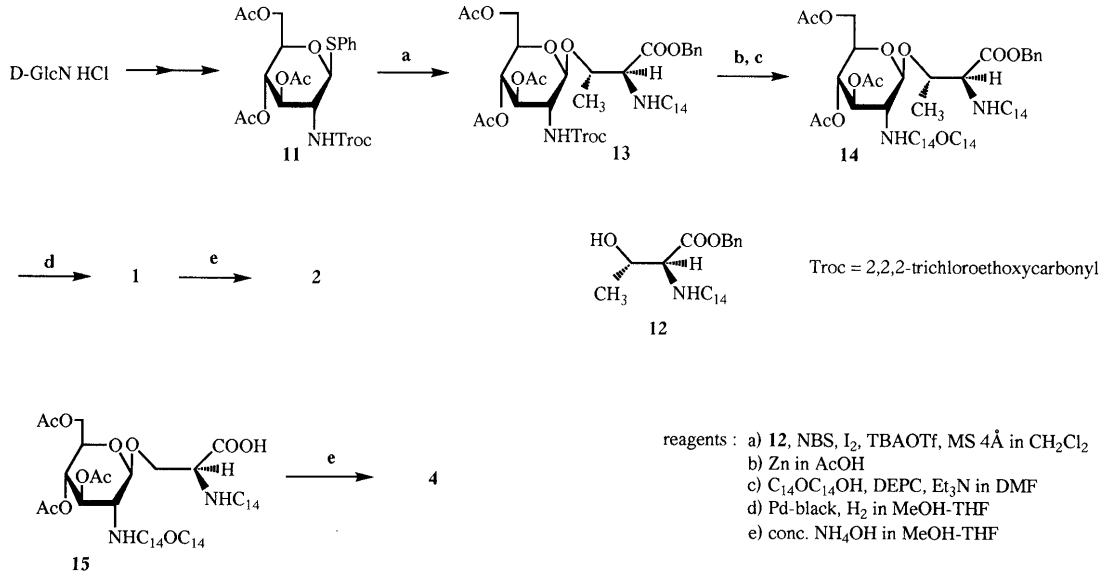

Chart 3

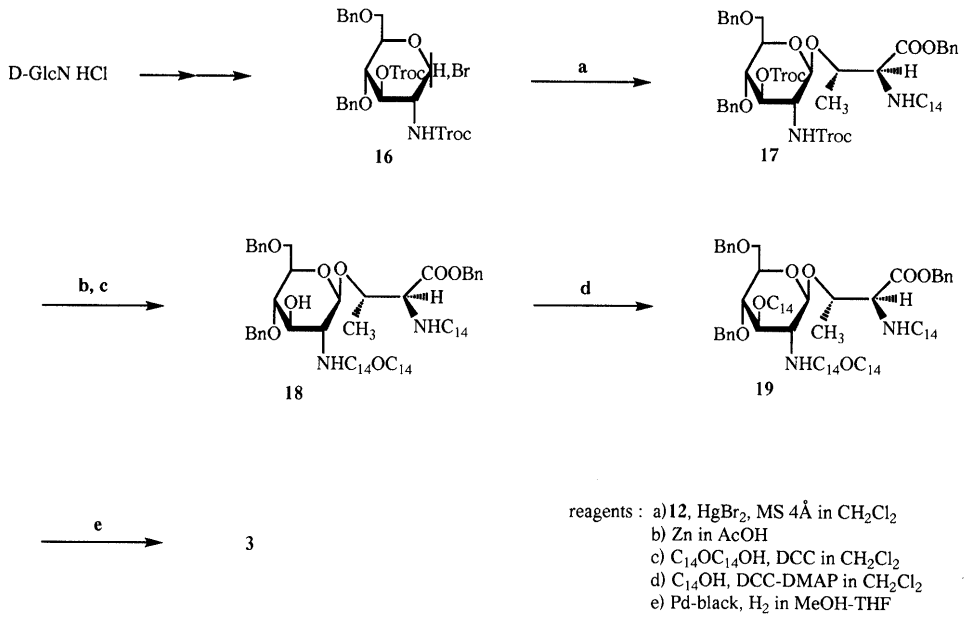

Chart 4

the homoserine compounds (**7**—**9**). The reason why the homoserine compounds showed low mitogenicity in comparison with the serine and threonine compounds is unclear, but the difference in carbon chain length between the homoserine residue and the serine or threonine residue may affect the potency.

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, FAB-MS on a JEOL JMS-SX 102 spectrometer, ^{1}H-NMR spectra on a JEOL JNM-GX 270 (270 MHz) spectrometer, and ^{13}C-NMR spectra on a JEOL JNM-GX 270 (67.5 MHz) spectrometer. The ^{1}H and ^{13}C chemical shifts (δ) are given in ppm relative to that of 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. 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*-Tetradecanoyl-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-L-threonine Benzyl Ester (13)** A solution of **11** (859 mg, 1.5 mmol) and *N*-tetradecanoyl-L-threonine benzyl ester **12** (755 mg, 1.80 mmol) in anhydrous CH_2Cl_2 (20 ml) was stirred for 1 h at room temperature under argon in the presence of 4 Å powdered molecular sieves (1.0 g). The mixture was cooled to 0 °C, then NBS (1.07 g, 6 mmol), iodine (1.53 g, 6 mmol), and TBAOTf (115 mg, 0.3 mmol) were added. The mixture was stirred at the same temperature for 1 h. After removal of the insoluble materials by filtration, the filtrate was washed successively with 10% aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography using CH_2Cl_2–CH_3COCH_3 (20 : 1) to give **13** (898 mg, 68%) as a syrup, $[\alpha]_D$ − 2.5° ($c=0.84$, $CHCl_3$). IR (Nujol): 3338, 1744, 1659 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, $J=6.9$ Hz, CH_3), 1.15 (3H, d, $J=6.3$ Hz, $CHCH_3$), 1.26 (20H, br s, $-CH_2-$), 1.65 (2H, br s, $CH_2CH_2C_{11}H_{23}$), 2.01, 2.02, 2.03 (each 3H, s, $OCOCH_3$), 2.27 (2H, t, $J=8.3$ Hz, $CH_2C_{12}H_{25}$), 3.40—3.56 (2H, m, H-2, H-5), 4.03 (1H, dd, $J=2.3$, 12.5 Hz, H-6), 4.18 (1H, dd, $J=4.6$, 12.5 Hz, H-6), 4.21—4.43 (1H, m, $CHCH_3$), 4.49 (1H, d, $J=8.2$ Hz, H-1), 4.65—4.78 (3H, m, CH_2CCl_3, $OCH(CH_3)CHNH$), 4.99 (1H, t, $J=9.6$ Hz, H-4), 5.11—5.23 (3H, m, H-3, OCH_2Ph), 6.39 (1H, d, $J=8.9$ Hz, NH), 7.37 (5H, s, Ph). ^{13}C-NMR ($CDCl_3$) δ: 14.1 (q, CH_3), 17.3 (q, $CHCH_3$), 20.6, 20.7 (q, $OCOCH_3$), 22.6, 25.6, 29.3, 29.5, 29.6 31.8, 36.5 (t, CH_2), 56.2 (d, $OCH(CH_3)CHNH$), 56.3 (d, C-2), 61.6 (t, C-6), 67.2 (t, CH_2Ph), 68.2 (d, C-4), 71.5 (d, C-3), 71.6 (d, C-5), 74.6 (t, CH_2CCl_3), 74.6 (d, $CHCH_3$), 95.7 (s, CH_2CCl_3), 98.7 (d, C-1), 128.3, 128.5, 128.6 (d, Ph), 135.5 (s, Ph), 154.0, 169.3, 170.0, 170.5, 170.7, 173.8 (s, C=O). Positive FAB-MS *m/z*: 881 $[(M+H)^+$ for $C_{40}H_{59}{}^{35}Cl_3N_2O_{13}]$.

***N*-Tetradecanoyl-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-[(*R*)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranosyl]-L-threonine Benzyl Ester (14)** Activated zinc powder (300 mg, 4.6 mmol) was added to a solution of **13** (309 mg, 0.35 mmol) in AcOH (20 ml), and the mixture was vigorously stirred at 40—50 °C for 20 h. After removal of the insoluble materials by filtration, the solvent was evaporated *in vacuo*. The residue was dissolved in CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated *in vacuo*. The resulting oily product was dissolved in *N*,*N*-dimethylformamide (DMF) (10 ml), and (*R*)-3-tetradecanoyloxytetradecanoic acid (160 mg, 0.35 mmol), DEPC (57 mg, 0.35 mmol) and TEA (35 mg, 0.35 mmol) were added to the solution with ice cooling under argon. After stirring for 16 h, the reaction mixture was diluted with CH_2Cl_2, and then washed successively with saturated aqueous $NaHCO_3$ and brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography using CH_2Cl_2–CH_3COCH_3 (20 : 1) to give **14** (260 mg, 65%) $[\alpha]_D$ −7.8° ($c=0.68$, $CHCl_3$). IR (Nujol): 3286, 1742, 1645, 1565 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (9H, t, $J=6.9$ Hz, CH_3), 1.17 (d, $J=6.9$ Hz, $CHCH_3$), 1.25 (58H, br s, $-CH_2-$), 1.49—1.61 (6H, m, $-CH_2-$), 2.01, 2.02, 2.03 (each 3H, s, $OCOCH_3$), 2.17—2.36 (5H, m, $-CH_2-$), 2.45 (1H, dd, $J=5.6$, 14.5 Hz, $NHCOCH_2CH(OCO)$), 3.37—3.41 (1H, m, H-5), 3.68—3.72 (1H, m, H-2), 4.00—4.05 (1H, m, H-6), 4.17 (1H, dd, $J=4.3$, 12.5 Hz, H-6), 4.41—4.44 (1H, m, $CHCH_3$), 4.58 (1H, d, $J=8.3$ Hz, H-1), 4.63—4.70 (1H, m, $OCH(CH_3)CHNH$), 4.94—5.01 (2H, m, H-4, $NHCOCH_2CH(OCO)$), 5.11—5.14 (1H, m, H-3), 5.15 (2H, br s, CH_2Ph), 6.10 (1H, d, $J=8.6$ Hz, NH), 6.54 (1H, d, $J=8.6$ Hz, NH), 7.37 (5H, s, Ph). Positive FAB-MS *m/z*: 1144 $(M+H)^+$.

***N*-Tetradecanoyl-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-[(*R*)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranosyl]-L-threonine (1)** Palladium–black (100 mg) was added to a solution of **14** (100 mg, 0.087 mmol) in THF–MeOH (1 : 1) (10 ml), and the mixture was stirred under a hydrogen atmosphere for 16 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–CH_3COCH_3 (10 : 1) to give **1** (58 mg, 63%) as an amorphous powder, $[\alpha]_D$ +3.1° ($c=1.16$, $CHCl_3$–MeOH = 1 : 1). IR (Nujol): 1744, 1652, 1541 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (9H, t, $J=6.9$ Hz, CH_3), 1.14 (3H, br s, $CHCH_3$), 1.25 (58H, br s, $-CH_2-$), 1.53—1.69 (6H, m, $-CH_2-$), 2.00, 2.01, 2.03 (each 3H, s, $OCOCH_3$), 2.17—2.36 (5H, m, $-CH_2-$), 2.49 (1H, dd, $J=5.6$, 14.5 Hz, $NHCOCH_2CH(OCO)$), 3.72—3.89 (2H, m, H-2, H-5), 4.14—4.42 (4H, m, H-6,

CHCH$_3$, OCH(CH$_3$)CHNH), 4.73 (1H, d, J=8.3 Hz, H-1), 4.92—5.07 (2H, m, H-4, NHCOCH$_2$CH(OCO)), 5.22 (1H, t, J=9.6 Hz, H-3). ^{13}C-NMR (CDCl$_3$–CD$_3$OD) δ: 14.5 (q, CH$_3$), 17.8 (q, CHCH$_3$), 20.9, 21.0 (q, OCOCH$_3$), 23.1, 25.7, 25.4, 26.1, 29.6, 29.7, 29.8, 29.9, 30.7, 30.2, 32.3, 34.2, 34.9, 36.7, 41.7 (t, CH$_2$), 54.3 (d, C-2), 58.2 (d, OCH(CH$_3$)CHNH), 62.2 (t, C-6), 69.1 (d, C-4), 71.9 (d, NHCOCH$_2$CH(OCO)), 72.8 (d, C-3), 73.6 (d, C-5), 76.5 (d, CHCH$_3$), 99.9 (d, C-1), 169.6, 170.0, 171.1, 171.8, 174.2, 175.3, 175.4 (s, C=O). Positive FAB-MS m/z: 1054 (M+H)$^+$.

***N*-Tetradecanoyl-*O*-[2-deoxy-2-[(*R*)-3-tetradecanoyloxy-tetradecanoylamino]-β-D-glucopyranosyl]-L-threonine (2)** Compound **1** (34 mg, 0.033 mmol) was dissolved in a solution of concentrated NH$_4$OH (1 ml) in MeOH–THF (1 : 3) (8 ml). The mixture was stirred for 16 h, and the solvent was removed by evaporation. The residue was purified by silica gel column chromatography using CH$_2$Cl$_2$–MeOH–H$_2$O (12 : 8 : 1) to give **2** (25 mg, 82%) as an amorphous powder, after lyophilization from dioxane, $[\alpha]_D$ −4.7° (c=0.70, CHCl$_3$–MeOH=3 : 2). IR (Nujol): 3284, 1739, 1641 cm^{-1}. ^{1}H-NMR (CDCl$_3$–CD$_3$OD) δ: 0.88 (9H, t, J=6.9 Hz, CH$_3$), 1.15 (3H, br s, CHCH$_3$), 1.25 (58H, br s, –CH$_2$–), 1.61—1.78 (6H, m, –CH$_2$–), 2.28—2.50 (6H, m, –CH$_2$–), 3.36—4.34 (9H, m, H-1, 2, 3, 4, 5, 6, OCH(CH$_3$)CHNH), 5.09—5.14 (1H, m, NHCOCH$_2$CH(OCO)). ^{13}C-NMR (CDCl$_3$–CD$_3$OD) δ: 13.6 (q, CH$_3$), 16.5 (q, CHCH$_3$), 22.3, 24.7, 24.9, 25.5, 28.9, 29.0, 29.1, 29.2, 29.3, 29.4, 30.1, 31.6, 33.9, 34.3, 36.1, 41.1 (t, CH$_2$), 55.6 (d, C-2), 58.0 (d, OCH(CH$_3$)CHNH), 60.2 (t, C-6), 69.9 (d, C-4), 71.0 (d, NHCOCH$_2$CH(OCO)), 73.5 (d, C-5), 75.6 (d, C-3), 75.8 (d, CHCH$_3$), 100.1 (d, C-1), 171.8, 173.1, 174.7, 176.8 (s, C=O). Positive FAB-MS m/z: 950 (M+Na)$^+$.

***N*-Tetradecanoyl-*O*-[2-deoxy-2-[(*R*)-3-tetradecanoyloxy-tetradecanoylamino]-β-D-glucopyranosyl]-L-serine (4)** In the same manner as described for **2**, compound **15** (25 mg, 0.024 mmol) was treated with concentrated NH$_4$OH (2 ml) in MeOH–THF (1 : 1) (10 ml), and the resulting syrup was purified by silica gel column chromatography (CH$_2$Cl$_2$–MeOH–H$_2$O, 12 : 8 : 1) to give **4** (20 mg, 91%) as an amorphous powder, after lyophilization from dioxane, $[\alpha]_D$ −2.3° (c=0.40, CHCl$_3$–MeOH=1 : 1). IR (Nujol): 3299, 1742, 1639 cm^{-1}. ^{1}H-NMR (CDCl$_3$–CD$_3$OD) δ: 0.88 (9H, t, J=6.9 Hz, CH$_3$), 1.25 (58H, br s, –CH$_2$–), 1.60—1.73 (6H, m, –CH$_2$–), 2.20—2.50 (6H, m, –CH$_2$–), 3.30—4.44 (9H, m, H-1, 2, 3, 4, 5, 6, OCH(CH$_3$)CHNH), 5.11—5.20 (1H, m, NHCOCH$_2$CH(OCO)). ^{13}C-NMR (CDCl$_3$–CD$_3$OD) δ: 13.6 (q, CH$_3$), 22.3, 24.7, 24.9, 25.5, 28.9, 29.0, 29.1, 29.2, 29.3, 29.4, 29.9, 31.6, 34.1, 34.3, 36.1, 41.2 (t, CH$_2$), 54.4 (d, OCH$_2$CHNH), 55.5 (d, C-2), 61.6 (t, C-6), 69.7 (d, C-4), 70.3 (t, OCH$_2$CHNH), 71.2 (d, NHCOCH$_2$CH(OCO)), 74.3 (d, C-5), 75.8 (d, C-3), 75.9 (d, CHCH$_3$), 100.9 (d, C-1), 172.3, 173.9, 174.1, 175.2 (s, C=O). Positive FAB-MS m/z: 936 (M+Na)$^+$.

***N*-Tetradecanoyl-*O*-[4,6-di-*O*-benzyl-2-deoxy-3-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-L-threonine Benzyl Ester (17)** A solution of **16** (216 mg, 0.28 mmol) and **12** (96 mg, 0.23 mmol) in anhydrous CH$_2$Cl$_2$ (5 ml) was stirred for 1 h at room temperature under argon in the presence of 4 Å powdered molecular sieves (500 mg).

The mixture was cooled to 0 °C for 1 h, then HgBr$_2$ (83 mg, 0.23 mmol) was added. The mixture was stirred at room temperature for 20 h. The insoluble materials were filtered off, and the filtrate was washed successively with 10% aqueous KI, saturated aqueous NaHCO$_3$ and brine, dried (MgSO$_4$), and evaporated *in vacuo*. The residue was chromatographed on silica gel using hexane–AcOEt (3 : 1) to give **17** (131 mg, 42%) as an amorphous powder, $[\alpha]_D$ −4.2° (c=1.08, CHCl$_3$). IR (Nujol): 1745, 1730, 1644, 1536 cm^{-1}. ^{1}H-NMR (CDCl$_3$) δ: 0.88 (3H, t, J=6.9 Hz, CH$_3$), 1.14 (3H, d, J=6.6 Hz, CHCH$_3$), 1.25 (20H, br s, –CH$_2$–), 1.60—1.79 (2H, m, CH$_2$CH$_2$C$_{11}$H$_{23}$), 2.22 (2H, t, J=7.3 Hz, CH$_2$C$_{12}$H$_{25}$), 3.31—3.93 (5H, m, H-2, 4, 5, 6), 4.36—4.80 (11H, m, CH$_2$CCl$_3$×2, OCH$_2$Ph×2, CHCH$_3$, OCH(CH$_3$)CHNH, H-1), 5.00—5.08 (1H, m, H-3), 5.13, 5.20 (each 1H, d, J=12.5 Hz, COOCH$_2$Ph), 5.29 (1H, d, J=7.9 Hz, NH), 6.45 (1H, d, J=8.6 Hz, NH), 7.19—7.39 (10H, m, Ph). ^{13}C-NMR (CDCl$_3$) δ: 14.1 (q, CH$_3$), 17.4 (q, CHCH$_3$), 22.6, 25.6, 29.3, 29.5, 29.6, 31.8, 36.5 (t, CH$_2$), 56.2 (d, OCH(CH$_3$)CHNH), 56.6 (d, C-2), 67.1 (t, COOCH$_2$Ph), 68.1 (t, C-6), 73.5 (t, OCH$_2$Ph), 74.4 (d, C-4), 74.6 (t, CH$_2$CCl$_3$), 74.8 (d, CHCH$_3$), 75.0 (t, OCH$_2$Ph), 75.4 (d, C-5), 76.9 (t, CH$_2$CCl$_3$), 79.5 (d, C-3), 94.2 (s, CH$_2$CCl$_3$), 95.4 (s, CH$_2$CCl$_3$), 98.7 (d, C-1), 127.6, 127.7, 127.8, 127.9, 128.2, 128.3, 128.5, 128.6 (d, Ph), 135.5, 137.4, 137.7 (s, Ph), 154.0, 154.3, 170.0, 173.8 (s, C=O). Positive FAB-MS m/z: 1109 [(M+H)$^+$ for C$_{51}$H$_{66}$^{35}Cl$_6$N$_2$O$_{12}$].

***N*-Tetradecanoyl-*O*-[4,6-di-*O*-benzyl-2-deoxy-2-[(*R*)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranosyl]-L-serine Benzyl Ester (18)** Activated zinc powder (200 mg, 3.1 mmol) was added to a solution of **17** (100 mg, 0.09 mmol) in AcOH (5 ml), and the mixture was vigorously stirred at 40—50 °C for 16 h. After removal of the insoluble materials by filtration, the solvent was evaporated *in vacuo*. The residue was dissolved in CH$_2$Cl$_2$, washed with saturated aqueous NaHCO$_3$ and brine, dried (MgSO$_4$), and evaporated *in vacuo*. The resulting oily product and (*R*)-3-tetradecanoyloxytetradecanoic acid (41 mg, 0.09 mmol) were dissolved in CH$_2$Cl$_2$ (15 ml), then DCC (18 mg, 0.09 mmol) was added to the solution with ice cooling under argon. The mixture was stirred for 16 h at room temperature. The precipitated dicyclohexylurea was filtered off, and the filtrate was concentrated by evaporation. The residue was dissolved with AcOEt, and then washed successively with saturated aqueous NaHCO$_3$ and brine, dried (MgSO$_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography using CH$_2$Cl$_2$–CH$_3$COCH$_3$ (50 : 1) to give **18** (76 mg, 71%) as an amorphous powder, $[\alpha]_D$ −8.4°(c=0.66, CHCl$_3$). IR (Nujol): 3320, 1741, 1645, 1554 cm^{-1}. ^{1}H-NMR (CDCl$_3$) δ: 0.88 (9H, t, J=6.9 Hz, CH$_3$), 1.17 (3H, d, J=6.6 Hz, CHCH$_3$), 1.25 (58H, br s, –CH$_2$–), 1.49—1.74 (6H, m, –CH$_2$–), 2.14—2.38 (4H, m, –CH$_2$–), 2.45 (1H, dd, J=5.9, 10.6 Hz, COCH$_2$CH(OCO)), 2.55 (1H, dd, J=5.9, 14.9 Hz, COCH$_2$CH(OCO)), 3.34—3.64 (6H, m, H-2, 3, 4, 5, 6), 4.28—4.63 (7H, m, H-1, OCH$_2$Ph×2, CH–CH$_3$, OCH(CH$_3$)CHNH), 5.01—5.10 (1H, m, COCH$_2$CH(OCO), 5.11, 5.16 (each 1H, d, J=12.2 Hz, COOCH$_2$Ph), 6.21 (1H, d, J=6.3 Hz, NH), 6.68 (1H, d, J=8.3 Hz, NH), 7.18—7.35 (15H, m, Ph). Positive FAB-MS m/z: 1198 (M+H)$^+$.

***N*-Tetradecanoyl-*O*-[4,6-di-*O*-benzyl-2-deoxy-3-*O*-tetradecanoyl-2-[(*R*)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranosyl]-L-serine Benzyl Ester (19)** DCC (17 mg, 0.08 mmol) was added to a solution of **18** (74 mg, 0.06 mmol), tetradecanoic acid (18 mg, 0.08 mmol) and DMAP (10 mg, 0.08 mmol) in CH$_2$Cl$_2$ (5 ml) at 0°C under argon. The mixture was stirred for 18 h at room temperature. The precipitated dicyclohexylurea was filtered off, and the filtrate was concentrated by evaporation. The residue was dissolved with AcOEt, and the solution was washed successively with saturated aqueous NaHCO$_3$ and brine, dried (MgSO$_4$), and evaporated *in vacuo*. The residue was purified by silica gel column chromatography using CH$_2$Cl$_2$–CH$_3$COCH$_3$ (50 : 1) to give **19** (50 mg, 69%) as an amorphous powder, $[\alpha]_D$ −14.3° (c=0.5, CHCl$_3$). IR (Nujol): 1743, 1647, 1555 cm^{-1}. ^{1}H-NMR (CDCl$_3$) δ: 0.88 (12H, t, J=6.9 Hz, CH$_3$), 1.13 (3H, d, J=6.6 Hz, CHCH$_3$), 1.25 (78H, br s, –CH$_2$–), 1.45—1.84 (8H, m, –CH$_2$–), 2.04—2.33 (7H, m, –CH$_2$–), 2.43 (1H, dd, J=5.9, 14.5 Hz, COCH$_2$CH(OCO)), 3.27—3.92 (5H, m, H-2, 4, 5, 6), 4.33 (1H, d, J=8.2 Hz, H-1), 4.37—4.58 (5H, m, OCH$_2$Ph×2, CH–CH$_3$), 4.59—4.64 (1H, m, OCH(CH$_3$)CHNH), 4.99—5.11 (2H, m, H-3, COCH$_2$CH(OCO)), 5.12, 5.17 (each 1H, d, J=12.4 Hz, COOCH$_2$Ph), 5.96 (1H, d, J=8.9 Hz, NH), 6.66 (1H, d, J=8.9 Hz, NH), 7.11—7.39 (15H, m, Ph). Positive FAB-MS m/z: 1409 (M+H)$^+$.

***N*-Tetradecanoyl-*O*-[2-deoxy-3-*O*-tetradecanoyl-2-[(*R*)-3-tetradecanoyloxytetradecanoylamino]-β-D-glucopyranosyl]-L-threonine (3)** Palladium–black (100 mg) was added to a solution of **19** (50 mg, 0.036 mmol) in MeOH–THF (1 : 1) (4 ml), and the mixture was stirred under a hydrogen atmosphere for 24 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$_2$Cl$_2$–MeOH (7 : 1) to give **3** (30 mg, 74%) as a white powder, after lyophilization from dioxane, $[\alpha]_D$ −5.4°(C=0.5, CHCl$_3$). IR (Nujol): 3289, 1741, 1658 cm^{-1}. ^{1}H-NMR (CDCl$_3$–CD$_3$OD) δ: 0.88 (12H, t, J=6.9 Hz, CH$_3$), 1.09 (3H, d, J=6.3 Hz, CH–CH$_3$), 1.25 (78H, br s, –CH$_2$–), 1.46—1.59 (8H, m, –CH$_2$–), 2.26—2.40 (7H, m, –CH$_2$–). 2.48 (1H, dd, J=6.3, 14.5 Hz, COCH$_2$CH(OCO)), 3.35—4.30 (7H, m, H-2, 4, 5, 6, OCH(CH$_3$)CHNH, CHCH$_3$), 4.49 (1H, d, J=8.3 Hz, H-1), 4.94 (1H, t, d, J=10.5 Hz, H-3), 5.13 (1H, m, COCH$_2$CH(OCO)). ^{13}C-NMR (CDCl$_3$–CD$_3$OD) δ: 13.7 (q, CH$_3$), 17.3 (q, CHCH$_3$), 22.4, 24.8, 25.1, 25.6, 28.7, 29.1, 29.2, 29.3, 29.5, 29.6, 31.7, 33.7, 33.9, 34.0, 34.3, 36.2, 40.8 (t, CH$_2$), 53.7 (d, OCH(CH$_3$)CHNH), 58.3 (d, C-2), 60.5 (t, C-6), 68.1 (d, C-4), 70.7 (d, COCH$_2$CH(OCO)), 74.3 (d, CHCH$_3$), 75.8 (d, C-5), 75.9 (d, C-3), 100.7 (d, C-1), 170.9, 173.5, 174.1, 174.8, 176.8 (s, C=O). Positive FAB-MS m/z: 1138 (M+H)$^+$, 1160 (M+Na)$^+$.

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