

Synthesis of *N*-Acetylglucosaminyl- and *N*-Acetylgalactosaminylceramides as Cerebroside Analogs and Their Anti-human Immunodeficiency Virus Type 1 Activities

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Monoglycosylceramide derivatives containing mimicks of ceramide were synthesized as cerebroside analogs from D-glucosamine or D-galactosamine derivatives and *N*-benzyloxycarbonyl-L-serine myristylamide by using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a promoter. The synthesized sulfated glycolipids show moderate anti-HIV-1 activities.

Key words sulfated glycosylceramide; cerebroside analog; anti-HIV-1 activity

Galactosylceramide is an essential component of neural receptor for type 1 human immunodeficiency virus (HIV) surface glycoprotein gp120.¹⁾ Various galactosylceramide analogs have been synthesized and their biological activities were examined.²⁾ In preceding papers, we reported the synthesis of sulfated gangliosides³⁾ and sulfated cerebroside analogs⁴⁾ containing L-serine diamide derivatives as mimicks of the ceramide moieties of gangliosides showing anti-influenza virus activities and anti-HIV type 1 activities. *N*-Acetyl D-glucosamine and *N*-acetyl D-galactosamine are often found as constituents of glycoconjugates,⁵⁾ and they have various biological activities and functions.

As a part of our synthetic studies on biologically active new compounds designed by modifying natural glycoconjugates, we describe here the synthesis of sulfated monoglycosylceramides, as indicated in Chart 1, together with some results of biological testing.

Chart 2 shows the synthetic route to **5a** and **5b**. First, the neighboring-group-assisted coupling of 2-chloroacetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl- β -D-glucopyranose and 2-chloroacetamido-2-deoxy-1,3,4,6-tetra-*O*-acetyl- β -D-galactopyranose with an *N*-benzyloxycarbonyl-L-serine myristylamide derivative in the presence of Me₃SiOSO₂CF₃ (TMSOTf) and molecular sieves 4 Å in ClCH₂CH₂Cl gave the desired glycosides (**1a** and **1b**) in yields of 69 and 74%, respectively. The ¹H-NMR data for the anomeric proton H-1 [δ 4.71 ($J_{1,2}$ = 6.8 Hz) in **1a**, δ 4.69 ($J_{1,2}$ = 7.0 Hz) in **1b**] indicated the stereochemistry of the newly formed glycosidic bond to be β . Reduction of the benzyloxycarbonyl and chloroacetyl groups in **1a** and **1b** by hydrogenolysis over Pd-C in MeOH gave the alcohols **2a** and **2b** (quantitative and 93% yield, respectively). De-*O*-acetylation of **2a** and **2b** had to be done under mild conditions, due to base-sensitivity (*retro*-Michael reaction) of the *O*-serinyl glycosyl portion in particular.⁶⁾ The best results were achieved by treatment of **2a** and **2b** with triethylamine (TEA)-MeOH (1 : 10)⁷⁾ at room temperature to give the triols **3a** and **3b** (quantitative and 88% yield, respectively). During the de-*O*-acetylation, no β -elimination product was detected. Acylation of the free amino groups of **3a** and **3b** with stearoyl chloride and aqueous NaHCO₃ gave diacylated compounds **4a** and **4b** (87%

yield and quantitative, respectively). Finally, *O*-sulfation of **4a** and **4b** was achieved with sulfur trioxide-tri-pyridine complex in *N,N*-dimethylformamide (DMF). Removal of pyridine was readily accomplished by brief treatment with trifluoromethanesulfonic acid (TFA) in dichloromethane and the products were purified by chromatography on Sephadex LH-20 (CHCl₃:MeOH:H₂O = 6:6:1) and lyophilized to afford the 3,4,6-tri-*O*-sulfated glycosides **5a** and **5b** (42 and 46% yields, respectively). Absorptions at 1215–1268 cm⁻¹ (due to S=O stretching) and 756–834 cm⁻¹ (due to C–O–S vibration) were observed in the infrared (IR) spectra of **5a** and **5b**, indicating the presence of sulfate esters. Furthermore, **5a** and **5b** gave a positive test with a specific spray-reagent (azure A reagent) for sulfated glycolipids.⁸⁾

The structures of all compounds were characterized by ¹H-NMR spectroscopy, as well as IR spectroscopy, elemental analyses, and positive FAB-mass spectrometry.

The anti-HIV-1 activities of the two nonsulfated glycosylceramides (**4a** and **4b**) and the two sulfated glycosylceramides (**5a** and **5b**) are shown in Table 1. The anti-HIV-1 activity was tested by the syncytium-formation

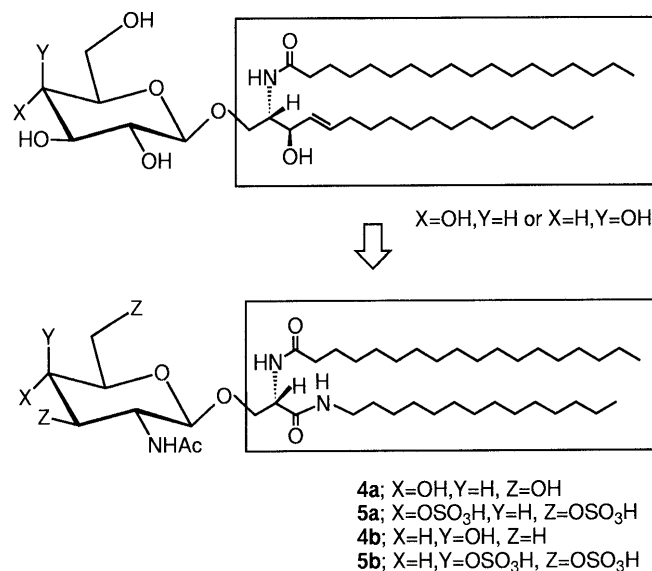
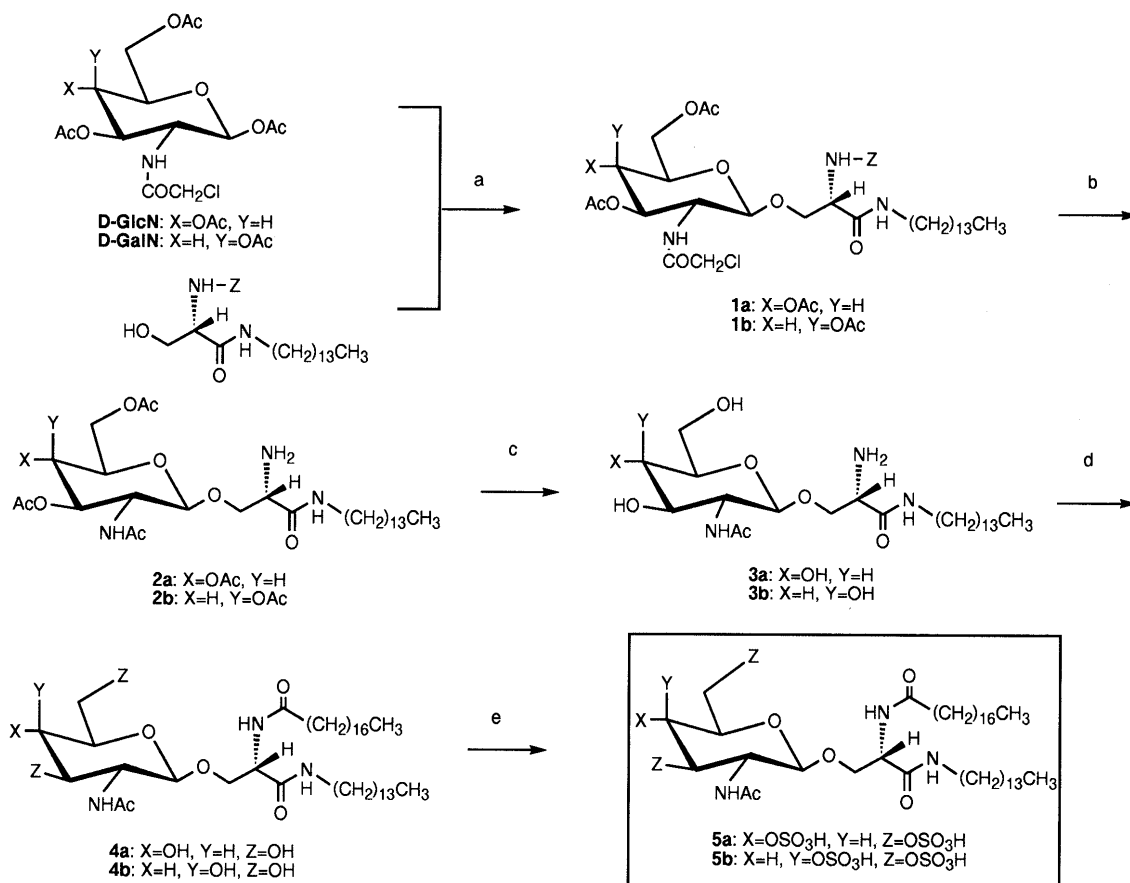


Chart 1

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Reagents: a) TMSOTf, ClCH₂CH₂Cl; b) H₂, Pd-C, MeOH; c) TEA-MeOH (1:10); d) CH₃(CH₂)₁₆COCl, NaHCO₃, ether-H₂O; e) i) SO₃-pyridine, DMF-pyridine (1:1); ii) TFA, CH₂Cl₂, then LH-20 (CHCl₃-MeOH-H₂O= 6:6:1)

Chart 2

Table 1. Results of Anti-HIV Assay by IFA Using MT-4 Cells

Compd. No.	HIV-1 infection (IC ₅₀) (μg/ml) ^a	CT ^b
4a	> 100	(+ +)
4b	> 100	(+ +)
5a	30—100	(-)
5b	30—100	(-)

^a Concentrations (μg/ml) of compounds at which 50% of MT-4 cells expressed HIV-1 antigens. ^b CT: cytotoxic (- to ++).

assay method using MT-4 cells according to our previously reported method.^{3b)} Among the synthesized compounds, the sulfated compounds (**5a** and **5b**) showed moderate activities with 50%-inhibitory concentration (IC₅₀) values of 30—100 μM, and they were noncytotoxic. The non-sulfated compounds (**4a** and **4b**) were practically inactive (IC₅₀ > 100 μM) against HIV-1 and were cytotoxic.

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 with tetramethylsilane (in CDCl₃) as an internal standard, and the chemical shifts are given in δ values. The abbreviations of signal patterns are as follows: s, singlet; brs, 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-F₂₅₄ (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. Sulfated glycolipids were visualized with azure A reagent. The bands of lipids containing sulfate esters were stained blue.

N-Benzyloxycarbonyl-O-(3,4,6-tri-O-acetyl-2-chloroacetyl-amino-2-deoxy-β-D-glucopyranosyl)-L-serine Myristylamide (1a) A solution of 2-chloroacetamido-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose (0.85 g, 2 mmol) and *N*-benzyloxycarbonyl-L-serine myristylamide (0.90 g, 2 mmol) in anhydrous ClCH₂CH₂Cl (20 ml) was stirred for 1 h at room temperature under argon in the presence of powdered molecular sieves 4 Å. The mixture was cooled to 0 °C, then TMSOTf (0.45 g, 2 mmol) was added. The mixture was stirred for 15 h at room temperature, and the reaction mixture was filtered through Celite 545. The filtrate was washed with aqueous NaHCO₃ and H₂O, dried (MgSO₄), and concentrated *in vacuo*. The residual product was chromatographed on SiO₂ with CH₂Cl₂-CH₃COCH₃ (20:1) to give **1a** (1.10 g, 69%) as an amorphous powder. [α]_D²⁰ +1.1° (c=0.90, CHCl₃). IR (Nujol): 1744 (ester), 1657, 1535 (amide) cm⁻¹.

¹H-NMR (CDCl₃) δ: 0.88 (3H, t, *J*=7.0 Hz, -CH₃), 1.26 (22H, brs, -CH₂-), 1.42—1.49 (2H, m, NCH₂CH₂), 2.03, 2.04, 2.06 (each 3H, s, OAc), 3.23 (2H, t, *J*=6.5 Hz, NCH₂CH₂), 3.77 (1H, dd, *J*=10.5, 7.3 Hz, OCH₂H₃CHN), 3.86—3.98 (2H, m, H-2,5), 3.94 (2H, brs, NHCOCH₂Cl), 4.08 (1H, dd, *J*=10.5, 4.3 Hz, OCH₂H₆CHN), 4.15 (1H, dd, *J*=11.7, 2.2 Hz, H-6b), 4.24 (1H, dd, *J*=11.7, 4.9 Hz, H-6a), 4.34—4.36 (1H, m, OCH₂CHN), 4.71 (1H, d, *J*=6.8 Hz, H-1), 5.05 (1H, dd, *J*=9.5 Hz, H-4), 5.11 (2H, brs, CH₂Ph), 5.25 (1H, t, *J*=9.5 Hz, H-3), 5.68 (1H, brs, OCH₂CHN), 6.37 (1H, brs, Ph). *Anal.* Calcd for C₃₉H₆₀ClN₃O₁₂: C, 58.67; H, 7.58; N, 5.26. Found: C, 58.32; H, 7.61; N, 5.05.

O-(3,4,6-Tri-O-acetyl-2-acetyl-amino-2-deoxy- β -D-glucopyranosyl)-L-serine Myristylamide (2a) A mixture of **1a** (0.98 g, 0.84 mmol) and 10% Pd-C (0.24 g) in MeOH (40 ml) was stirred under H₂ overnight at room temperature. The catalyst was removed by filtration and the filtrate was concentrated to dryness. The residue was chromatographed on SiO₂ with CH₂Cl₂-MeOH (10:1) to give **2a** (0.529 g, quant.), mp 125–128 °C. $[\alpha]_D^{25}$ -14.3° (*c* = 0.46, MeOH). IR (KBr): 3300 (NH), 1744 (ester), 1657, 1535 (amide) cm⁻¹. ¹H-NMR (CDCl₃) δ : 0.88 (3H, t, *J* = 7.3 Hz, -CH₃), 1.26 (22H, brs, -CH₂-), 1.50 (2H, brs, NCH₂CH₂), 1.95, 2.03, 2.09 (12H, s, OAc, NAc), 3.18–3.26 (2H, m, NCH₂CH₂), 3.54 (1H, dd, *J* = 7.6, 4.9 Hz, OCH₂CHNH), 3.64–3.70 (1H, m, H-5), 3.82 (1H, dd, *J* = 10.3, 7.6 Hz, OCH₂H_bCHN), 3.90 (1H, dd, *J* = 10.3, 4.9 Hz, OCH₂H_bCHN), 3.98 (1H, dd, *J* = 8.4, 8.9 Hz, H-2), 4.13 (1H, dd, *J* = 12.2, 2.2 Hz, H-6a), 4.25 (1H, dd, *J* = 12.2, 4.6 Hz, H-6b), 4.59 (1H, d, *J* = 8.4 Hz, H-1), 5.07 (1H, t, *J* = 9.2 Hz, H-4), 5.17 (1H, t, *J* = 9.2 Hz, H-3), 5.71 (1H, d, *J* = 8.9 Hz, AcNH). *Anal.* Calcd for C₃₁H₅₅N₃O₁₀: C, 59.12; H, 8.80; N, 6.67. Found: C, 59.10; H, 8.90; N, 6.70.

O-(2-Acetyl-amino-2-deoxy- β -D-glucopyranosyl)-L-serine Myristylamide (3a) A solution of **1b** (0.55 g, 0.87 mmol) in NEt₃-MeOH (1:10) (30 ml) was stirred at room temperature for 5 h, then concentrated to dryness under reduced pressure. The residue was purified by column chromatography with CH₂Cl₂-MeOH (3:1) to give **3a** (0.44 g, quant.), mp 188–191 °C. $[\alpha]_D^{25}$ -14.5° (*c* = 0.36, CHCl₃-MeOH (1:1)). IR (KBr): 3300 (NH), 1646, 1557 (amide) cm⁻¹. ¹H-NMR (CDCl₃:CD₃OD = 10:1) δ : 0.88 (3H, t, *J* = 7.0 Hz, -CH₃), 1.26 (22H, brs, -CH₂-), 1.44–1.51 (2H, m, NCH₂CH₂), 2.01 (3H, s, AcN), 3.20 (2H, t, *J* = 6.8 Hz, NCH₂CH₂), 3.29–3.41 (4H, m, NCH₂CHN, H-5, 6), 3.44 (1H, t, *J* = 8.4 Hz, H-3), 3.61 (1H, t, *J* = 8.4 Hz, H-2), 3.73 (1H, dd, *J* = 10.3, 4.9 Hz, OCH₂H_bCHN), 3.87 (1H, dd, *J* = 10.3, 2.7 Hz, OCH₂H_bCHN), 4.40 (1H, d, *J* = 8.4 Hz, H-1). *Anal.* Calcd for C₂₅H₄₉N₃O₇·H₂O: C, 57.56; H, 9.85; N, 8.05. Found: C, 58.04; H, 9.58; N, 7.55. Positive FAB-MS *m/z*: 503 (M+1)⁺.

N-Stearoyl-O-(2-acetyl-amino-2-deoxy- β -D-glucopyranosyl)-L-serine Myristylamide (4a) A solution of stearoyl chloride (0.112 g, 0.36 mmol) in ether (10 ml) was added to a solution of **3a** (0.171 g, 0.36 mmol) and saturated aqueous NaHCO₃ (50 ml) at 0 °C. The mixture was stirred for 4 h. The resulting precipitates was washed with ether and dried *in vacuo* to give **4a** (0.24 g, 87%), mp 229–231 °C, as an amorphous powder. IR (KBr): 3280 (OH, NH), 1654, 1542 (amide) cm⁻¹. ¹H-NMR (DMSO-*d*₆) δ : 0.86 (6H, t, *J* = 6.5 Hz, -CH₃), 1.24 (52H, brs, -CH₂-), 1.40 (2H, brs, COCH₂CH₂), 1.49 (2H, brs, NCH₂CH₂), 1.83 (3H, s, AcNH), 4.34 (1H, d, *J* = 7.3 Hz, H-1), 4.48 (1H, brs, NH), 4.89 (1H, brs, NH). *Anal.* Calcd for C₄₃H₈₃N₃O₈·5H₂O: C, 60.04; H, 10.90; N, 4.88. Found: C, 60.20; H, 10.96; N, 4.11. Positive FAB-MS *m/z*: 771 (M+1)⁺, 793 (M+Na)⁺.

N-Stearoyl-O-(2-acetyl-amino-2-deoxy-3,4,6-tri-O-sulfo- β -D-glucopyranosyl)-L-serine Myristylamide (5a) A solution of **4a** (0.093 g, 0.12 mmol) in DMF (4 ml) was stirred for 20 h at 40–50 °C in the presence of sulfur trioxide-pyridine complex (0.086 g, 0.54 mmol). The mixture was cooled and chromatographed on a column of Sephadex LH-20 equilibrated in 1:1 (v/v) CHCl₃-MeOH. Elution with the same solvent gave a residue that was dissolved in CH₂Cl₂ (4 ml). The solution was treated with CF₃SO₃H (0.062 g, 0.54 mmol) for 3 h under ice-cooling, then the solvent was evaporated *in vacuo*. The residue was dissolved in H₂O (1 ml) and chromatographed on a column of Sephadex LH-20. Elution with 6:6:1 (v/v/v) CHCl₃-MeOH-H₂O afforded **5a** (0.052 g, 42%) as an amorphous powder, after lyophilization from H₂O, mp 165–167 °C. IR (KBr): 1652, 1542 (amide), 1251 (S=O), 816 cm⁻¹ (C-O-S). *Anal.* Calcd for C₄₈H₈₃N₃O₁₇S₃·2H₂O: C, 52.11; H, 7.93; N, 3.80. Found: C, 52.33; H, 7.71; N, 3.75.

N-Benzoyloxycarbonyl-O-(3,4,6-tri-O-acetyl-2-chloroacetyl-amino-2-deoxy- β -D-galactopyranosyl)-L-serine Myristylamide (1b) The same procedure as described for the preparation of **1a** provided a crude product from 2-chloroacetamido-2-deoxy-1,3,4,6-tetra-O-acetyl- β -D-galactopyranose (0.425 g, 1.0 mmol), N-benzoyloxycarbonyl-L-serine myristylamide (0.455 g, 1.0 mmol) and TMSOTf (0.222 g, 1.0 mmol) and this was purified by column chromatography (elution with 20:1 CHCl₃-CH₃COCH₃) to give **1b** (0.59 g, 74%) as an amorphous powder. $[\alpha]_D^{25}$ +2.3° (*c* = 1.30, CHCl₃). IR (KBr): 1744 (ester), 1657, 1535 (amide) cm⁻¹. ¹H-NMR (CDCl₃) δ : 0.88 (3H, t, *J* = 6.8 Hz, -CH₃), 1.25 (22H, brs, -CH₂-), 1.42–1.49 (2H, m, NCH₂CH₂), 2.03, 2.04, 2.14 (each 3H, s, AcO), 3.23 (2H, t, *J* = 5.9 Hz, NCH₂CH₂), 3.78 (1H, dd, *J* = 10.5, 7.0 Hz, OCH₂H_bCHN), 3.95 (2H, brs, NHCOCH₂Cl), 4.01–4.18 (3H, m, H-2, 5, OCH₂H_bCHN), 4.34 (1H, dd, *J* = 4.9, 7.0 Hz, OCH₂CHNH), 4.69 (1H, d, *J* = 7.0 Hz, H-1), 5.11 (2H, s, OCH₂Ph), 5.20 (1H, dd, *J* = 3.0, 8.1 Hz,

H-3), 5.36 (1H, brd, *J* = 3.0 Hz, H-4), 5.71 (1H, brs, OCH₂CHNH), 6.38 (1H, brs, CONH), 6.54 (1H, d, *J* = 8.6 Hz, NHCOCH₂Cl), 7.35 (5H, brs, Ph). *Anal.* Calcd for C₃₉H₆₀ClN₃O₁₂: C, 58.67; H, 7.58; N, 5.26. Found: C, 58.75; H, 7.77; N, 5.06.

O-(3,4,6-Tri-O-acetyl-2-acetyl-amino-2-deoxy- β -D-glucopyranosyl)-L-serine Myristylamide (2b) The same procedure as described for the preparation of **2a** provided a crude product from **1b** (0.59 g, 0.74 mmol), Pd-on-charcoal (0.25 g) and MeOH (15 ml), and this was purified by column chromatography (elution with 10:1 CH₂Cl₂-MeOH) to give **2b** (0.471 g, 93%) as an amorphous powder, mp 110–113 °C. $[\alpha]_D^{25}$ -12.0° (*c* = 0.99, MeOH). IR (KBr): 3354 (NH, OH), 1745 (ester), 1656, 1527 (amide) cm⁻¹. ¹H-NMR (CDCl₃) δ : 0.88 (3H, t, *J* = 7.0 Hz, -CH₃), 1.26 (22H, brs, -CH₂-), 1.51 (2H, brs, NCH₂CH₂), 1.96, 2.01, 2.05, 2.15 (each 3H, s, OAc, NAc), 3.18–3.27 (2H, m, NCH₂CH₂), 3.56 (1H, dd, *J* = 7.6, 4.6 Hz, OCH₂CHNH), 3.83 (1H, dd, *J* = 10.0, 7.6 Hz, H-6a), 3.90 (1H, dd, *J* = 10.0, 4.9 Hz, H-6b), 4.04–4.08 (1H, m, H-5), 4.12–4.22 (1H, m, H-2), 4.62 (1H, d, *J* = 8.4 Hz, H-1), 5.15 (1H, dd, *J* = 11.3, 3.5 Hz, H-3), 5.35 (1H, brd, *J* = 3.5 Hz, H-4), 5.70 (1H, brd, *J* = 8.6 Hz, NHAc). *Anal.* Calcd for C₃₁H₅₅N₃O₁₀: C, 59.12; H, 8.80; N, 6.67. Found: C, 58.67; H, 8.78; N, 6.65.

O-(2-Acetyl-amino-2-deoxy- β -D-glucopyranosyl)-L-serine Myristylamide (3b) The same procedure as described for the preparation of **3a** provided a crude product from **2b** (0.25 g, 0.50 mmol) and NEt₃-MeOH (1:10) (20 ml), and this was purified by column chromatography (elution with 3:1 CH₂Cl₂-MeOH) to give **3b** (0.15 g, 88%) as an amorphous powder, mp 160–162 °C. $[\alpha]_D^{25}$ -7.6° (*c* = 1:1 CHCl₃-MeOH). IR (KBr): 3282 (NH, OH), 1648, 1560 (amide) cm⁻¹. ¹H-NMR (CDCl₃:CD₃OD = 10:1) δ : 0.88 (3H, t, *J* = 7.0 Hz, -CH₃), 1.27 (22H, brs, -CH₂-), 1.51 (2H, brs, NCH₂CH₂), 2.02 (3H, s, AcN), 3.20 (2H, t, *J* = 7.0 Hz, NCH₂CH₂), 3.49 (1H, dd, *J* = 11.3, 4.9 Hz, H-6a), 3.56 (1H, dd, *J* = 10.5, 3.5 Hz, H-3), 3.71–3.75 (1H, m, H-5), 3.77 (1H, dd, *J* = 4.6, 3.5 Hz, H-4), 3.85 (1H, t, *J* = 10.5 Hz, H-2), 3.84 (1H, dd, *J* = 11.3, 3.2 Hz, H-6b), 4.37 (1H, d, *J* = 8.1 Hz, H-1). *Anal.* Calcd for C₂₅H₄₉N₃O₇: C, 59.62; H, 9.81; N, 8.34. Found: C, 59.32; H, 9.81; N, 8.13.

N-Stearoyl-O-(2-acetyl-amino-2-deoxy- β -D-glucopyranosyl)-L-serine Myristylamide (4b) The same procedure as described for the preparation of **4a** provided a crude product from **3b** (0.12 g, 0.25 mmol), stearoyl chloride (0.078 g, 0.25 mmol) and saturated aqueous NaHCO₃ (25 ml), and this was washed with ether to give **4b** (0.19 g, quant.) as an amorphous powder, mp 219–222 °C. IR (KBr): 3276 (OH), 1639, 1559 (amide) cm⁻¹. ¹H-NMR (DMSO-*d*₆) δ : 0.86 (6H, t, *J* = 5.9 Hz, -CH₃), 1.24 (52H, brs, -CH₂-), 1.40 (2H, brs, COCH₂CH₂), 1.49 (2H, brs, NCH₂CH₂), 1.84 (3H, s, AcNH). *Anal.* Calcd for C₄₃H₈₃N₃O₈·5H₂O: C, 60.04; H, 10.90; N, 4.88. Found: C, 60.07; H, 10.39; N, 4.23.

N-Stearoyl-O-(2-acetyl-amino-2-deoxy-3,4,6-tri-O-sulfo- β -D-glucopyranosyl)-L-serine Myristylamide (5b) The same procedure as described for the preparation of **5a** provided a crude product from **4b** (0.070 g, 0.09 mmol) and sulfur trioxide-pyridine complex (0.065 g, 0.41 mmol), followed by TFA (0.047 g, 0.41 mmol), and this was purified on a column of Sephadex LH-20 equilibrated in CHCl₃-MeOH-H₂O (6:6:1, v/v) to give **5b** (0.042 g, 46%) as an amorphous powder, mp 154–157 °C. IR (KBr): 1653, 1543 (amide), 1250 (S=O), 812 (C-O-C) cm⁻¹. *Anal.* Calcd for C₄₈H₈₃N₃O₁₇S₃·H₂O: C, 52.97; H, 7.87; N, 3.86. Found: C, 53.17; H, 8.26; N, 3.81.

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