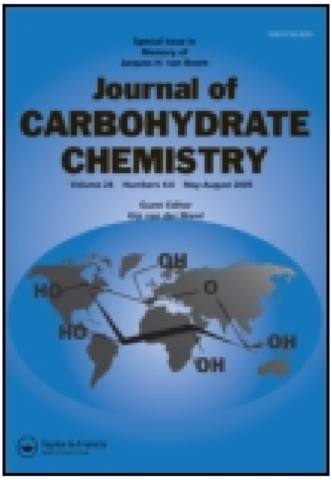

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Synthesis of a Hematoside Analog Containing Phytosphingosine and α-Hydroxyfatty Acid

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SYNTHESIS OF A HEMATOSIDE ANALOG CONTAINING PHYTOSPHINGOSINE AND α-HYDROXYFATTY ACID

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ABSTRACT

The hematoside analog **1** [NeuGcα(2→3)Galβ(1→4)Glcβ(1→1)Cer], which contains a phytosphingosine as a sphingoid base and an α-hydroxyfatty acid, has been synthesized. Coupling of the methyl (methyl 5-benzyloxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosid)onate **5**, prepared from the corresponding 5-acetamido derivative **2**, with a lactose derivative **6** afforded sialolactoside **7**, which was converted to the corresponding trichloroacetimidate **10**. Glycosylation of **10** with the ceramide tribenzoate **12** gave the protected hematoside analog **13**, which was deprotected to the hematoside analog **1**.

INTRODUCTION

The hematoside [NeuGcα(2→3)Galβ(1→4)Glcβ(1→1)Cer] was first isolated from equine erythrocyte by Yamakawa *et al.*,[1] and later identified as the antigen for human Hanganutziu-Deicher (H-D) heterophile antibodies[2] and as the tumor-associated foreign antigen of a Marek's disease lymphoma-derived chicken cell line.[3] Moreover, we have recently isolated a hematoside type ganglioside called GAA-6, which was partially methylated and possessed a phytosphingosine and an α-hydroxyfatty acid, from the starfish *Asterias amurensis versicolor*.[4]

Although a total synthesis of the hematoside was achieved by Ogawa *et al.*,[5] we conducted the total synthesis of the hematoside analog which possessed a phytosphingosine and an α-hydroxyfatty acid for the synthesis of the unique starfish ganglioside GAA-6. Previously, we demonstrated that the methyl 2-thioglycoside of *N*-glycolylneuraminic acid (NeuGc) is useful for producing NeuGc-containing glycoconjugates.[6] In this paper, we report a detailed procedure for the preparation of the NeuGc donor and the facile synthesis of the hematoside analog **1**.

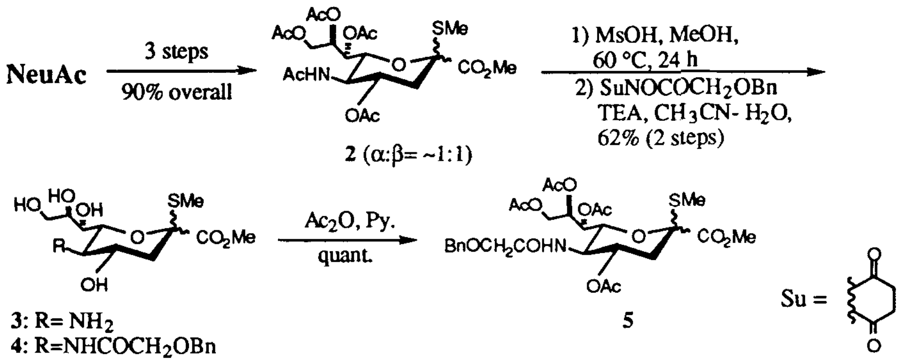
RESULTS AND DISCUSSION

Methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosid)onate **2**, prepared from *N*-acetylneuraminic acid (NeuAc)[7] in 3 steps, is known as one of the most useful NeuAc donors, and the anomeric methylthio group appeared stable in many organic operations. Therefore, we synthesized the thioglycoside of NeuGc as a potentially good NeuGc donor by exchanging the *N*-acetyl group of **2** with the *N*-glycolyl group.

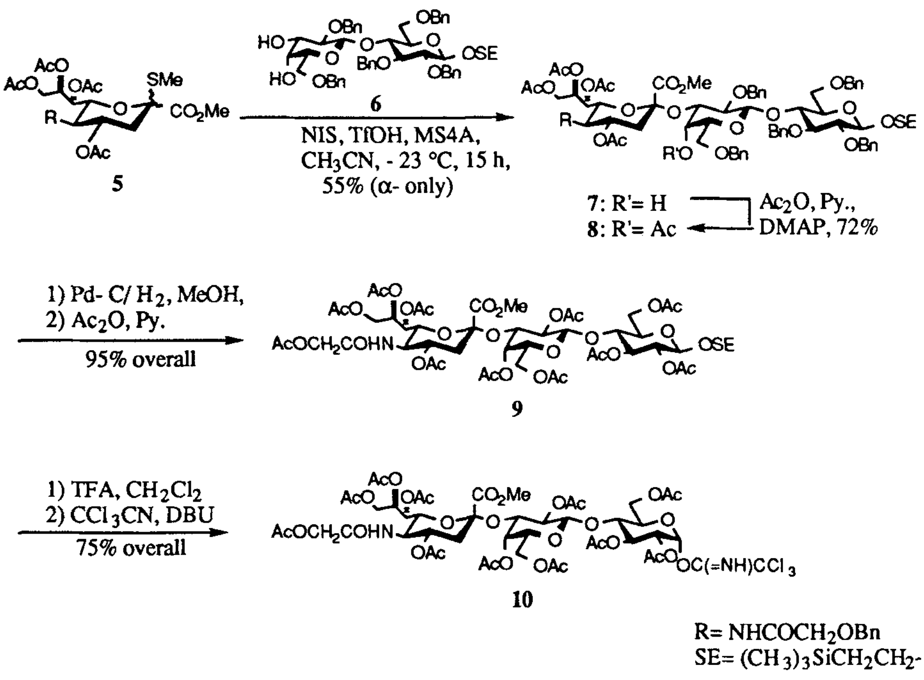
The thioglycoside **2** was *N,O*-deacetylated with methanesulfonic acid (MsOH) in MeOH for 24 h at 60 °C (Scheme 1). The resulting amine **3** with the benzylglycolic acid *N*-hydroxysuccinimide ester in the presence of triethylamine in $CHCl_3$-H_2O gave the *N*-glycolyl derivative **4** in 62% yield in 2 steps. Compound **4** was acetylated with pyridine and acetic anhydride to the methyl 2-thioglycoside of NeuGc **5** in quantitative yield as a 1:1 anomeric mixture. Thus, **5** was prepared in 56% overall yield in 6 steps from NeuAc.

Glycosylation of **6** with **5**[8] in CH_3CN in the presence of *N*-iodosuccinimide (NIS), trifluoromethanesulfonic acid (TfOH) and powdered 4A-molecular sieves for 15 h at -23 °C gave the α-sialoside **7** in 55% yield and neither the β-glycoside nor any positional isomers (Scheme 2). The configuration of **7** was determined by a large long-range $J_{C(1)\text{-}H(3ax)}$ coupling constant (J=7.1Hz, α-configuration).[9] The regiochemistry of **7** was confirmed by acetylation to the corresponding pentaacetate **8**. ^{1}H NMR data showed that the Gal H-4 (δ 3.82 ppm) of **7** was deshielded and gave a signal at δ 5.04, thus showing that the new glycosidic linkage was introduced at Gal-3.

Hydrogenolysis of the trisaccharide **7** with Pd-C/H_2 in methanol followed by acetylation with pyridine and acetic anhydride gave the peracetate **9** in 95% yield. The treatment of **9** with trifluoroacetic acid in dichloromethane for 30 min at room temperature gave the 1-hydroxy derivative, which was subsequently treated with



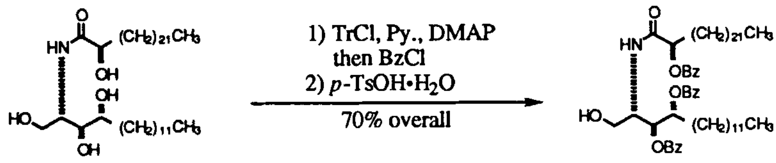
Scheme 1



Scheme 2

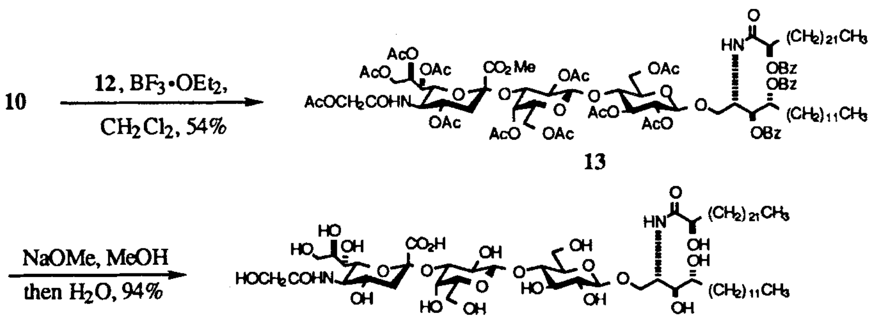
trichloroacetonitrile and DBU in dichloromethane for 2 h at 0 °C to produce the α-trichloroacetimidate **10** in 75% yield.

The ceramide acceptor **12** was synthesized in 70% overall yield from the ceramide **11**[10] (Scheme 3) which consisted of a C_{16}-phytosphingosine base and α-hydroxy tetra-cosanoic acid as follows: tritylation (TrCl, Py., DMAP), benzoylation (BzCl) and



11 12

Scheme 3



1

Scheme 4

detritylation (p-TsOH•H_2O). Coupling of trichloroacetimidate **10** with tribenzoate **12** (Scheme 4) in the presence of boron trifluoride etherate and AW-300 molecular sieves in dichloromethane afforded the glycosylated product **13** in 54 % yield. The newly formed glycosidic linkage of **13** was assigned as β based on the ^{1}H NMR data, which contained a signal for Glc-1 at δ 4.33 ppm as a doublet with a coupling constant of 8.0 Hz. **13** was deprotected to give the hematoside analog **1** in 94% yield.

EXPERIMENTAL

General Procedures. - Optical rotations were determined with a JASCO LR-700 polarimeter for solutions in $CHCl_3$ unless noted otherwise. ^{1}H NMR spectra were performed using either a JEOL GX-270 or a Varian Unity-500 spectrometer, and the spectra were recorded for solutions in deuterochloroform, unless otherwise stated, using tetramethylsilane as the internal standard. Negative FABMS and high-resolution negative FABMS were measured with a JEOL SX-102 mass spectrometer using a xenon atom beam source (10 kV accelating potential), and the spectra were obtained from the triethylene glycol matrix. Column chromatography was performed on columns of either Silica Gel 60 (Merck, 70-230 mesh) or Silica Gel BW-300 (200-400 mesh, Fuji Davison Co., Ltd.). TLC and HPTLC were performed on Silica Gel 60 F_{254} (Merck).

Abbreviation: Glc, glucose; Gal, galactose; Neu, neuraminic acid; Sph, phytosphingosine; FA, α-hydroxyfatty acid; e, equatorial; a, axial

Methyl (methyl 5-benzyloxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosid)onate (5). To a stirred solution of methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α- and -β-D-*galacto*-2-nonulopyranosid)onate **2** (500 mg, 0.959 mmol)[7] in dry MeOH (20 mL) was added methanesulfonic acid (620 μL, 9.59 mmol). The mixture was stirred for 24 h at 60 °C, then neutralized with Dowex 1x8 (OH^-) resin, after which the suspension was filtered. The filtrate was concentrated *in vacuo*. To a solution of the residue in CH_3CN-H_2O (15:1, 9 mL) was added benzylglycolic acid *N*-hydroxysuccinimide ester **3** (252 mg, 0.959 mmol) and triethylamine (200 μL). After being stirred for 7 h at room temperature, the mixture was concentrated *in vacuo*. Column chromatography (10:1 $CHCl_3$-MeOH) of the residue on SiO_2 gave **4** (269 mg, 62%). A solution of compound **4** (269 mg) in pyridine (3 mL)-Ac_2O (1.5 mL) was stirred overnight at room temperature, and concentrated *in vacuo*. Column chromatography (10:1 $CHCl_3$:acetone) of the residue on SiO_2 afforded **5** as a 1:1 anomeric mixture (371 mg, quant.): α-SMe: NMR data: δ_H 7.43-7.31 (m, 5H, aromatic-H), 6.33 (d, 1H, *J* =10.6Hz, NH), 5.41 (ddd, 1H, *J* =2.6, 4.6, 8.9Hz, H-8), 5.34 (dd, 1H, *J* =1.7Hz, 8.9Hz, H-7), 4.89 (ddd, 1H, *J* =4.6, 10.4, 11.7Hz, H-4), 4.61, 4.55 (2d, 2H, Ph<u>CH2</u>), 4.30 (dd, 1H, *J* =12.4, 2.6Hz, H-9a), 4.14 (m, 1H, H-5), 4.11 (dd, 1H, *J* =4.6, 12.4 Hz, H-9b), 3.98-3.83 (m, 3H, H-6, CO<u>CH2</u>), 3.82 (s, 3H, CO_2Me), 2.77 (dd, 1H, *J* =4.6, 12.7Hz, H-3e), 2.18, 2.13, 2.12, 2.01, 2.00 (5s, 15H, 4Ac, SMe), 1.99 (m, 1H, H-3a); β-SMe: NMR data: δ_H 7.42-7.32 (m, 5H, aromatic-H), 6.45 (d, 1H, *J* =10.6Hz, NH), 5.46 (dd, 1H, *J* = 2.3, 4.0Hz, H-7), 5.29 (m, 1H, H-4), 5.20 (ddd, 1H, *J* = 2.6, 4.0, 7.6Hz, H-8), 4.77 (dd, 1H, *J* = 12.5, 2.6Hz, H-9a), 4.61, 4.53 (2d, 2H, Ph<u>CH2</u>), 4.36 (dd, 1H, *J* = 2.3, 10.6Hz, H-6), 4.17 (dd, 1H, *J* = 12.5, 7.6Hz, H-9b), 4.10 (nt, 1H, H-5), 3.94- 3.84 (m, 2H, CO<u>CH2</u>), 3.82 (s, 3H, CO_2Me), 2.58 (dd, 1H, *J* = 13.9, 5.0Hz, H-3e), 2.13. 2.07, 2.04, 2.02, 1.99 (5s, 15H, 4Ac, SMe), 2.18- 2.00 (m, 1H, H-3a).

2-(Trimethylsilyl)ethyl *O*-[methyl (5-benzyloxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonuropyranosyl)onate]-(2→3)-*O*-(2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (7). A mixture of **6** (100 mg, 0.120 mmol), **5** (211 mg, 0.336 mmol) and powdered 4A-molecular sieves (200 mg) in dry CH_3CN (1 mL) was stirred for 3 h at room temperature then cooled to -23 °C. To the cooled mixture was added, with stirring, *N*-iodosuccinimide (NIS; 100.8 mg, 0.448 mmol) and trifluoromethanesulfonic acid (TfOH, 4 μL), and the stirring was then continued for 15 more hours at - 23 °C. The precipitate was filtered off and thoroughly washed with CH_2Cl_2. The filtrate and washings were combined, and the solution was successively washed with 1M Na_2CO_3, 1M $Na_2S_2O_3$ and H_2O, dried (Na_2SO_4) and concentrated. Column chromatography (6:4 *n*-hexane: AcOEt) of the residue on SiO_2 gave **7** (90.4 mg, 55%): $[\alpha]_D$ +9° (*c* 1.0, $CHCl_3$); NMR data: δ_H 7.40-7.17 (m, 30H, aromatic-H), 6.31 (d, 1H, 10.3Hz, Neu-NH), 5.42 (ddd, 1H, *J* = 2.5, 5.7, 8.2Hz, Neu-8), 5.30 (dd, 1H, *J* = 2.1, 8.2Hz, Neu-7), 4.87 (m, 1H, Neu-4), 4.59 (d, 1H, *J* = 7.6Hz, Gal-1), 4.36 (d, 1H, *J* = 7.8Hz, Glc-1), 4.26 (dd, 1H, *J* = 2.5, 12.6Hz, Neu-9a), 4.15-3.96 (m, 5H, Neu-5,6,9, Gal-3, one of $\underline{CH_2}CH_2Si$), 3.95 (nt, 1H, *J* = 9.5Hz, Glc-4), 3.90, 3.84 (2d, 2H, $CO\underline{CH_2}$), 3.82 (bs, 1H, Gal-4), 3.80-3,76 (m, 1H, Glc-6), 3.77 (s, 3H, CO_2Me), 3.71 (dd, 1H, *J* = 4.8, 10.8Hz, Glc-6), 3.67 (m, 1H, Gal-5), 3.62-3.46 (m, 5H, Glc-3, Gal-2, 6a, 6b, one of $\underline{CH_2}CH_2Si$), 3.38 (nt, 1H, Glc-2), 3.38 (m, 1H, Glc-5), 2.55 (dd, 1H, *J* = 4.6, 13.1Hz, Neu-3e), 2.09, 1.97, 1.96, 1.88 (4s, 12H, 4Ac), 2.03 (nt, 1H, Neu-3a), 1.02 (m, 2H, CH_2Si), 0.02 (s, 9H, Me_3Si)

Anal. Calcd for $C_{79}H_{97}NO_{24}Si$: C, 64.43; H, 6.64; N, 0.95. Found: C, 64.01, H, 6.63; N, 0.96.

2-(Trimethylsilyl)ethyl *O*-[methyl (5-benzyloxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonuropyranosyl)onate]-(2→3)-*O*-(4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (8). A solution of compound **7** (2.7 mg) in Ac_2O (100 μL)-pyridine (300 μL) containing a catalytic amount of DMAP was stirred for 4 h at room temperature, and the solution was then successively washed with 10%-HCl and satd. aqueous $NaHCO_3$, dried (Na_2SO_4) and concentrated. Column chromatography (6:4 *n*-hexane:AcOEt) of the residue on SiO_2 afforded **8** (2.0 mg, 72%): NMR data: δ_H 7.42-7.17 (m, 30H, aromatic-H), 6.28 (d, 1H, *J*=10.6Hz, Neu-NH), 5.62 (m, 1H, Neu-8), 5.31 (dd, 1H, *J*=2.3, 8.9Hz, Neu-7), 5.04 (d, 1H, *J*=3.3Hz, Gal-4), 4.97 (m, 1H, Neu-4), 4.79 (d, 1H, *J*=7.3Hz, Gal-1), 4.34 (d, 1H, *J*=7.9Hz, Glc-1), 3.85 (s, 3H, CO_2Me), 2.64 (dd, 1H, *J*=4.9, 12.9Hz, Neu-3e), 2.10, 2.00, 1.98, 1.95, 1.72 (5s, 15H, 5Ac), 1.84 (t, 1H, *J*=12.5Hz, Neu-3a), 1.02 (m, 2H, CH_2Si), 0.01 (s, 9H, $SiMe_3$)

2-(Trimethylsilyl)ethyl *O*-[methyl (5-acetoxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonuropyranosyl)onate]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (9). A solution of **7** (28.5 mg, 0.019mmol) in MeOH (3 mL) was hydrogenolyzed in the presence of 10% Pd-C (20 mg) for 3 h at room temperature, then filtered and concentrated. The residue was treated with acetic anhydride (1 mL) and pyridine (2 mL) overnight at room temperature. Column chromatography (9:1 $CHCl_3$:acetone) of the product on SiO_2 gave **9** (22.5 mg, 95%) as an amorphous mass: $[\alpha]_D$ -8° (*c* 0.9, $CHCl_3$); NMR data: δ_H 5.78 (d, 1H, *J*=10.1Hz, Neu-NH), 5.53(m, 1H, Neu-8), 5.34 (dd, 1H, *J*=2.7, 9.2Hz, Neu-7), 5.17 (t, 1H, *J*=9.5Hz, Glc-3), 4.95 (m, 1H, Neu-4), 4.94 (dd, 1H, *J*=8.0, 10.1Hz, Gal-2), 4.89 (d, 1H, *J*=3.2Hz, Gal-4), 4.87 (dd, 1H, *J*=8.0, 9.5Hz, Glc-2), 4.67 (d, 1H, *J*=8.0Hz, Gal-1), 4.56 (d, 1H, *J*=15.3Hz, one of CO$\underline{CH_2}$), 4.53 (dd, 1H, *J*=3.2, 10.1Hz, Gal-3), 4.48 (d, 1H, *J*=8.0Hz, Glc-1), 4.45 (dd, 1H, *J*=2.1, 11.8Hz, Glc-6), 4.43 (dd, 1H, *J*=2.8, 12.6Hz, Neu-9a), 4.28 (d, 1H, *J*=15.1Hz, one of CO$\underline{CH_2}$), 4.18 (dd, 1H, *J*=5.7, 11.9Hz, Glc-6), 4.05-3.98 (m, 4H, Neu-5,9, Gal-6a,6b), 3.94 (m, 1H, one of $\underline{CH_2}CH_2Si$), 3.89-3.83 (m, 2H, Glc-4, Gal-5), 3.86 (s, 3H, CO_2Me), 3.71 (dd, 1H, *J*=2.7, 10.6Hz, Neu-6), 3.61 (m, 1H, Glc-5), 3.56 (m, 1H, one of $\underline{CH_2}CH_2Si$), 2.60 (dd, 1H, *J*=4.6, 12.6Hz, Neu-3e), 2.24, 2.17, 2.17, 2.08, 2.08, 2.08, 2.07, 2.06, 2.03, 2.02, 1.99 (11s, 33H, 11Ac), 1.68 (nt, 1H, Neu-3a), 0.94 (m, 2H, CH_2Si), 0.00 (s, 9H, $SiMe_3$)

Anal. Calcd for $C_{51}H_{75}NO_{31}Si$: C, 49.96; H, 6.17; N, 1.14. Found: C, 49.95, H, 6.17, N, 1.14.

***O*-[Methyl (5-acetoxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonuropyranosyl)onate]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate (10).** To a solution of **9** (26.8 mg, 0.022 mmol) in CH_2Cl_2 (200 μL) was added trifluoroacetic acid (300 μL), and the mixture was stirred for 30 min at room temperature then concentrated. To a solution of the residue in CH_2Cl_2 (500 μL) and trichloroacetonitrile (9 μL) cooled to 0 °C was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 4 μL), and the mixture was stirred for 2 h at 0 °C. Column chromatography (4:1 $CHCl_3$: acetone) of the mixture on SiO_2 afforded **10** (20.7 mg, 75%) as an amorphous mass: $[\alpha]_D$ +27° (*c* 1.0, $CHCl_3$); NMR data: δ_H 8.64 (s, 1H, C=NH), 6.49 (d, 1H, *J*=3.9Hz, Glc-1), 5.79 (d, 1H, *J*=10.1Hz, Neu-NH), 5.55 (t, 1H, *J*=9.7Hz, Glc-3), 5.50 (ddd, 1H, *J*=2.9, 4.5, 9.2Hz, Neu-8), 5.36 (dd, 1H, *J*=2.5Hz, Neu-7), 5.08 (dd, 1H, *J*=10.2Hz, Glc-2), 4.97 (m, 1H, Neu-4), 4.96 (dd, 1H, *J*=8.0, 10.1Hz, Gal-2), 4.91 (d, 1H, *J*=3.2Hz, Gal-4), 4.67 (d, 1H, Gal-1), 4.56 (d, 1H, *J*=15.3Hz, one of CO$\underline{CH_2}$), 4.52 (dd, 1H, Gal-3), 4.44 (m, 2H, Glc-6, Neu-9a), 4.28 (d, 1H, one of CO$\underline{CH_2}$), 4.24 (dd, 1H, *J*=4.7, 12.2Hz, Glc-6), 4.12 (m, 1H, Glc-5), 4.04-3.98 (m, 4H, Neu-5,9, Gal-6a,6b), 3.94 (t, 1H, Glc-4), 3.87 (s, 3H, CO_2Me), 3.86 (m, 1H, Gal-5), 3.71 (dd, 1H, *J*=10.5Hz, Neu-6), 2.60 (dd, 1H, *J*=4.6, 12.8Hz, Neu-3e), 2.24, 2.17, 2.17, 2.09, 2.08, 2.07, 2.07, 2.07, 2.05, 2.00, 1.99 (11s, 33H, 11Ac), 1.70 (t, 1H, Neu-3a)

Anal. Calcd for $C_{48}H_{63}Cl_3N_2O_{31}$: C, 45.38; H, 5.00; N, 2.21. Found: C, 45.17, H, 5.11, N, 2.02.

(2*S*,3*S*,4*R*,2'*R*)-3,4-*O*-Dibenzoyloxy-2-(2'-benzoyloxytetracosanoylamido)hexadecan-1-ol (12). A solution of ceramide **11** (40 mg, 0.061 mmol)[10] in pyridine (3 mL) was added to trityl chloride (180 mg) and *N,N*-dimethylaminopyridine (8 mg) and stirred for 2 h at 65 °C. To the reaction mixture cooled to room temperature was added benzoyl chloride (155 μL), and the stirring was continued overnight at room temperature and then MeOH was added. After stirring for 1 h, the reaction mixture was diluted with $CHCl_3$, washed with H_2O, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was dissolved in $CHCl_3$-MeOH (1:1, 4 mL) containing *p*-TsOH•H_2O (30 mg) and stirred for 10 h at room temperature. The reaction mixture was poured into satd aqueous $NaHCO_3$ and extracted with $CHCl_3$. The extract was washed with satd. aqueous NaCl, dried (Na_2SO_4) and evaporated *in vacuo*. Column chromatography (7:3 *n*-hexane:AcOEt) of the residue on SiO_2 gave **12** (41 mg, 70%): NMR data: δ_H 8.16-7.95 (m, 6H, aromatic-H), 7.62-7.36 (m, 9H, aromatic-H), 7.09 (d, 1H, *J*=9.2 Hz, NH), 5.48-5.44 (m, 3H, Sph-3, 4, FA-2), 4.41 (m, 1H, Sph-2), 3.63 (br, 2H, SPh-1), 0.86 (m, 6H, terminal-CH_3)

***O*-[Methyl (5-acetoxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonuropyranosyl)onate]-(2→3)-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*S*,4*R*,2'*R*)-3,4-*O*-dibenzoyloxy-2-(2'-benzoyloxytetracosanoylamido)hexadecane (13).** To a solution of **10** (11.1 mg, 8.74 μmol) and ceramide tribenzoate **12** (12.7 mg, 0.013 mmol) in CH_2Cl_2 (400 μL) was added powdered molecular sieves AW-300 (250 mg). The mixture was stirred for 1 h at room temperature and then cooled to 0 °C. Boron trifluoride etherate (3 μl) was added to the cooled mixture, and stirred for 1 h at 0 °C and then for 21 h at room temperature. The mixture was diluted with CH_2Cl_2, filtered through Celite, washed with satd. aqueous $NaHCO_3$, dried (Na_2SO_4) and concentrated. Column chromatography (5:1 $CHCl_3$:acetone) of the residue on SiO_2 afforded **13** (9.8 mg, 54%): $[\alpha]_D$ +7° (*c* 0.4, CHCl3); NMR data: δ_H 8.14-8.12, 7.96-7.94, 7.60-7.38 (m, 15H, aromatic-H), 6.93 (d, 1H, *J*=8.9Hz, Cer-NH), 5.75 (d, 1H, *J*=10.1Hz, Neu-NH), 5.57 (dd, 1H, *J*=3.9, 7.8Hz, SPh-3), 5.47 (m, 1H, Neu-8), 5.41 (nt, 1H, FA-2), 5.34 (m, 2H, Neu-7, SPh-4), 4.97 (t, 1H, *J*=9.3Hz, Glc-3), 4.93 (m, 1H, Neu-4), 4.85 (m, 2H, Gal-2,4), 4.64 (dd, 1H, *J*=8.1, 9.5Hz, Glc-2), 4.57 (m, 1H, SPh-2), 4.54 (d, 1H, *J*=15.3Hz, one of $COCH_2O$), 4.53 (d, 1H, *J*=8.0Hz, Gal-1), 4.48 (dd, 1H, *J*=3.4, 10.3Hz, Gal-3). 4.40 (dd, 1H, *J*=2.7, 12.8Hz, Neu-9a), 4.33 (d, 1H, *J*=8.0Hz, Glc-1), 4.26 (d, 1H, one of $COCH_2O$), 4.21 (d, 1H, *J*=10.8Hz, Glc-6), 3.87 (dd, 1H, Glc-6), 3.83 (s, 3H, CO_2Me), 3.63 (t, 1H, *J*=9.5Hz, Glc-4), 3.33 (m, 1H, Glc-5), 2.57 (dd, 1H, J=4.7, 12.7Hz, Neu-3e), 2.18, 2.16, 2.14, 2.06, 2.05, 2.04, 2.03, 1.96, 1.95, 1.89, 1.73 (11s, 33H, 11Ac), 0.86 (t, 6H, CH_2CH_3)

Anal. Calcd for $C_{107}H_{154}N_2O_{38}$: C, 61.89; H, 7.48; N, 1.35. Found: C, 61.63, H, 7.62, N, 1.24.

***O*-(5-Acetoxyacetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonuropyranosyloic acid)-(2→3)-(β-D-galactopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→1)-(*2S,3S,4R,2'R*)-3,4-dihydroxy-2-(2'-hydroxytetracosanoylamino)hexadecane (1).** A solution of **13** (4.8 mg, 2.31 μmol) in 0.1M NaOMe-MeOH (3 mL) was stirred for 3 h at room temperature, and then water (0.5 mL) was added to the mixture, and stirred again for 3 h. The solution treated with Dowex-50 (H^+) resin to remove the base, and concentrated *in vacuo*. Column chromatography of the residue on Sephadex LH-20 gave **1** (2.8 mg, 94%): $[\alpha]_D$ -2°(*c* 0.2, pyridine); NMR data: δ_H (49:1 DMSO-d_6, D_2O) 4.18 (d, 1H, *J*=7.8Hz, Gal-1, Glc-1), 2.73 (dd, *J*=11.3, 4.2 Hz, Neu-3e), 0.83 (t, 6H, CH_2CH_3)

Negative FABMS *m/z* : 1285[M-H]$^-$, 978[M-NeuGc]$^-$, 816[M-NeuGc-Gal]$^-$, 654[M-NeuGc-Gal-Glc]$^-$; HR negative FABMS ($C_{63}H_{117}N_2O_{24}$ 1285.7996): 1285.7988 (- 0.8 mmu)

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