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

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ABSTRACT

The hematoside analog **1** [NeuGc α (2 \rightarrow 3)Gal β (1 \rightarrow 4)Glc β (1 \rightarrow 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 [NeuGc α (2 \rightarrow 3)Gal β (1 \rightarrow 4)Glc β (1 \rightarrow 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 antibodies² 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**.

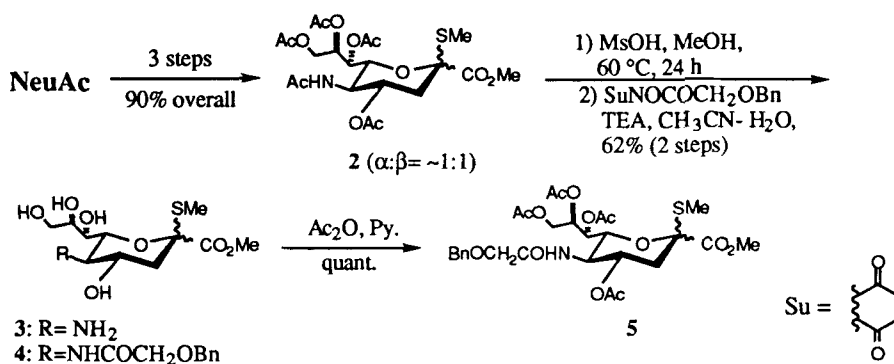
RESULTS AND DISCUSSION

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.

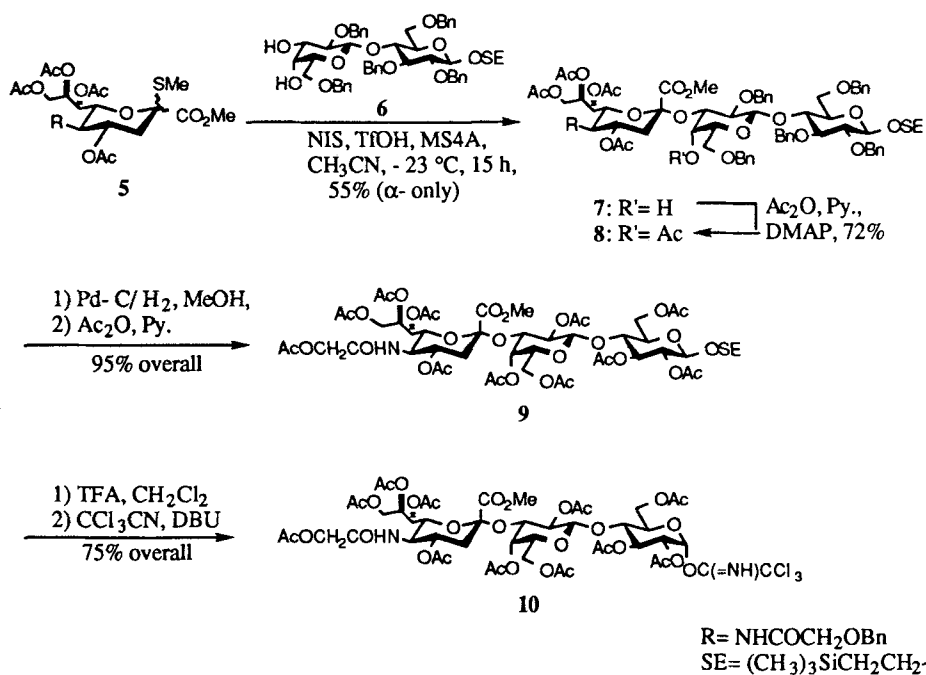
The thioglycoside **2** was *N,O*-deacetylated with methanesulfonic acid (MsOH) in MeOH for 24 h at 60 °C (Scheme 1). The resulting amine **3** with the benzylglycolic acid *N*-hydroxysuccinimide ester in the presence of triethylamine in CHCl₃-H₂O gave the *N*-glycolyl derivative **4** in 62% yield in 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 CH₃CN 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 $J_{C(1)-H(3ax)}$ coupling constant ($J=7.1$ Hz, α -configuration).⁹ The regiochemistry of **7** was confirmed by acetylation to the corresponding pentaacetate **8**. ¹H 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/H₂ 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



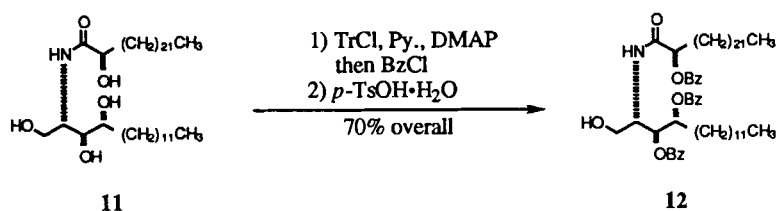
Scheme 1



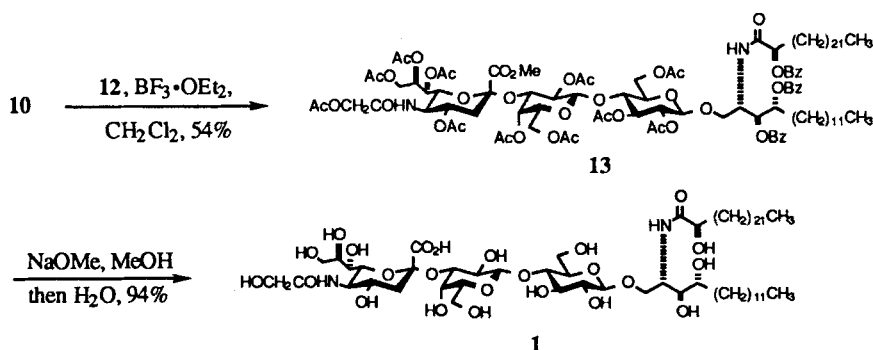
Scheme 2

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 C₁₆-phytosphingosine base and α -hydroxy tetra-cosanoic acid as follows: tritylation (TrCl, Py., DMAP), benzoylation (BzCl) and



Scheme 3



Scheme 4

detritylation (*p*-TsOH·H₂O). 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 ¹H 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 CHCl₃ unless noted otherwise. ¹H NMR spectra were performed using either a JEOL GX-270 or a Varian Unity-500 spectrometer, and the spectra were recorded for solutions in deuteriochloroform, 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 accelerating 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 F₂₅₄ (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 μ L, 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 CH₃CN-H₂O (15:1, 9 mL) was added benzylglycolic acid *N*-hydroxysuccinimide ester **3** (252 mg, 0.959 mmol) and triethylamine (200 μ L). After being stirred for 7 h at room temperature, the mixture was concentrated *in vacuo*. Column chromatography (10:1 CHCl₃-MeOH) of the residue on SiO₂ gave **4** (269 mg, 62%). A solution of compound **4** (269 mg) in pyridine (3 mL)-Ac₂O (1.5 mL) was stirred overnight at room temperature, and concentrated *in vacuo*. Column chromatography (10:1 CHCl₃:acetone) of the residue on SiO₂ 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, PhCH₂), 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, COCH₂), 3.82 (s, 3H, CO₂Me), 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, PhCH₂), 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, COCH₂), 3.82 (s, 3H, CO₂Me), 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-nonulopyranosyl)onate]-(2 \rightarrow 3)-*O*-(2,6-di-*O*-benzyl- β -*D*-galactopyranosyl)-(1 \rightarrow 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 CH₃CN (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 CH₂Cl₂. The filtrate and washings were combined, and the solution was successively washed with 1M Na₂CO₃, 1M Na₂S₂O₃ and H₂O, dried (Na₂SO₄) and concentrated. Column chromatography (6:4 *n*-hexane:AcOEt) of the residue on SiO₂ gave **7** (90.4 mg, 55%): [α]_D +9° (*c* 1.0, CHCl₃); 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 CH₂CH₂Si), 3.95 (nt, 1H, *J* = 9.5Hz, Glc-4), 3.90, 3.84 (2d, 2H, COCH₂), 3.82 (bs, 1H, Gal-4), 3.80-3.76 (m, 1H, Glc-6), 3.77 (s, 3H, CO₂Me), 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 CH₂CH₂Si), 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, CH₂Si), 0.02 (s, 9H, Me₃Si)

Anal. Calcd for C₇₉H₉₇NO₂₄Si: 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 Ac₂O (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 NaHCO₃, dried (Na₂SO₄) and concentrated. Column chromatography (6:4 *n*-hexane:AcOEt) of the residue on SiO₂ 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, CO₂Me), 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, CH₂Si), 0.01 (s, 9H, SiMe₃)

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 concent-

rated. The residue was treated with acetic anhydride (1 mL) and pyridine (2 mL) overnight at room temperature. Column chromatography (9:1 CHCl₃:acetone) of the product on SiO₂ gave **9** (22.5 mg, 95%) as an amorphous mass: $[\alpha]_D -8^\circ$ (c 0.9, CHCl₃); NMR data: δ_H 5.78 (d, 1H, $J=10.1$ Hz, Neu-NH), 5.53(m, 1H, Neu-8), 5.34 (dd, 1H, $J=2.7, 9.2$ Hz, Neu-7), 5.17 (t, 1H, $J=9.5$ Hz, Glc-3), 4.95 (m, 1H, Neu-4), 4.94 (dd, 1H, $J=8.0, 10.1$ Hz, Gal-2), 4.89 (d, 1H, $J=3.2$ Hz, Gal-4), 4.87 (dd, 1H, $J=8.0, 9.5$ Hz, Glc-2), 4.67 (d, 1H, $J=8.0$ Hz, Gal-1), 4.56 (d, 1H, $J=15.3$ Hz, one of COCH₂), 4.53 (dd, 1H, $J=3.2, 10.1$ Hz, Gal-3), 4.48 (d, 1H, $J=8.0$ Hz, Glc-1), 4.45 (dd, 1H, $J=2.1, 11.8$ Hz, Glc-6), 4.43 (dd, 1H, $J=2.8, 12.6$ Hz, Neu-9a), 4.28 (d, 1H, $J=15.1$ Hz, one of COCH₂), 4.18 (dd, 1H, $J=5.7, 11.9$ Hz, Glc-6), 4.05-3.98 (m, 4H, Neu-5,9, Gal-6a,6b), 3.94 (m, 1H, one of CH₂CH₂Si), 3.89-3.83 (m, 2H, Glc-4, Gal-5), 3.86 (s, 3H, CO₂Me), 3.71 (dd, 1H, $J=2.7, 10.6$ Hz, Neu-6), 3.61 (m, 1H, Glc-5), 3.56 (m, 1H, one of CH₂CH₂Si), 2.60 (dd, 1H, $J=4.6, 12.6$ Hz, 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, CH₂Si), 0.00 (s, 9H, SiMe₃)

Anal. Calcd for C₅₁H₇₅NO₃₁Si: C, 49.96; H, 6.17; N, 1.14. Found: C, 49.95, H, 6.17, N, 1.14.

O-[Methyl (5-acetoxycetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonuropyranosyl)onate]-(2 \rightarrow 3)-*O*-(2,4,6-tri-*O*-acetyl- β -D-galactopyranosyl)-(1 \rightarrow 4)-2,3,6-tri-*O*-acetyl- β -D-glucopyranosyl trichloroacetimidate (**10**). To a solution of **9** (26.8 mg, 0.022 mmol) in CH₂Cl₂ (200 μ L) was added trifluoroacetic acid (300 μ L), and the mixture was stirred for 30 min at room temperature then concentrated. To a solution of the residue in CH₂Cl₂ (500 μ L) and trichloroacetonitrile (9 μ L) cooled to 0 °C was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 4 μ L), and the mixture was stirred for 2 h at 0 °C. Column chromatography (4:1 CHCl₃: acetone) of the mixture on SiO₂ afforded **10** (20.7 mg, 75%) as an amorphous mass: $[\alpha]_D +27^\circ$ (c 1.0, CHCl₃); NMR data: δ_H 8.64 (s, 1H, C=NH), 6.49 (d, 1H, $J=3.9$ Hz, Glc-1), 5.79 (d, 1H, $J=10.1$ Hz, Neu-NH), 5.55 (t, 1H, $J=9.7$ Hz, Glc-3), 5.50 (ddd, 1H, $J=2.9, 4.5, 9.2$ Hz, Neu-8), 5.36 (dd, 1H, $J=2.5$ Hz, Neu-7), 5.08 (dd, 1H, $J=10.2$ Hz, Glc-2), 4.97 (m, 1H, Neu-4), 4.96 (dd, 1H, $J=8.0, 10.1$ Hz, Gal-2), 4.91 (d, 1H, $J=3.2$ Hz, Gal-4), 4.67 (d, 1H, Gal-1), 4.56 (d, 1H, $J=15.3$ Hz, one of COCH₂), 4.52 (dd, 1H, Gal-3), 4.44 (m, 2H, Glc-6, Neu-9a), 4.28 (d, 1H, one of COCH₂), 4.24 (dd, 1H, $J=4.7, 12.2$ Hz, 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, CO₂Me), 3.86 (m, 1H, Gal-5), 3.71 (dd, 1H, $J=10.5$ Hz, Neu-6), 2.60 (dd, 1H, $J=4.6, 12.8$ Hz, 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 C₄₈H₆₃C₃N₂O₃₁: C, 45.38; H, 5.00; N, 2.21. Found: C, 45.17, H, 5.11, N, 2.02.

(*2S,3S,4R,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 μ L), 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 CHCl₃, washed with H₂O, dried (Na₂SO₄) and concentrated *in vacuo*. The residue was dissolved in CHCl₃-MeOH (1:1, 4 mL) containing *p*-TsOH·H₂O (30 mg) and stirred for 10 h at room temperature. The reaction mixture was poured into satd aqueous NaHCO₃ and extracted with CHCl₃. The extract was washed with satd. aqueous NaCl, dried (Na₂SO₄) and evaporated *in vacuo*. Column chromatography (7:3 *n*-hexane:AcOEt) of the residue on SiO₂ 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-CH₃)

O-[Methyl (5-acetoxyacetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonurpyranosyl)onate]-(2 \rightarrow 3)-*O*-(2,4,6-tri-*O*-acetyl- β -D-galactopyranosyl)-(1 \rightarrow 4)-2,3,6-tri-*O*-acetyl- β -D-glucopyranosyl-(1 \rightarrow 1)-(2*S,3S,4R,2'R*)-3,4-*O*-dibenzoyloxy-2-(2'-benzoyloxytetracosanoylamido)hexadecane (**13**). To a solution of **10** (11.1 mg, 8.74 μ mol) and ceramide tribenzoate **12** (12.7 mg, 0.013 mmol) in CH₂Cl₂ (400 μ L) 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 μ l) 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 CH₂Cl₂, filtered through Celite, washed with satd. aqueous NaHCO₃, dried (Na₂SO₄) and concentrated. Column chromatography (5:1 CHCl₃:acetone) of the residue on SiO₂ afforded **13** (9.8 mg, 54%): $[\alpha]_{\text{D}}^{+7}$ (*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 COCH₂O), 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 COCH₂O), 4.21 (d, 1H, *J*=10.8Hz, Glc-6), 3.87 (dd, 1H, Glc-6), 3.83 (s, 3H, CO₂Me), 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, CH₂CH₃)

Anal. Calcd for C₁₀₇H₁₅₄N₂O₃₈: 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 \rightarrow 3)-(β -D-galactopyranosyl)-(1 \rightarrow 4)-(β -D-glucopyranosyl)-(1 \rightarrow 1)-(2*S*, 3*S*, 4*R*, 2'*R*)-3,4-dihydroxy-2-(2'-hydroxytetracosanoylamino)hexadecane (1).** A solution of **13** (4.8 mg, 2.31 μ mol) 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%): $[\alpha]_D^{-20}$ (c 0.2, pyridine); NMR data: δ_H (49:1 DMSO-*d*₆, D₂O) 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, CH₂CH₃)

Negative FABMS *m/z* : 1285[M-H]⁻, 978[M-NeuGc]⁻, 816[M-NeuGc-Gal]⁻, 654[M-NeuGc-Gal-Glc]⁻; HR negative FABMS (C₆₃H₁₁₇N₂O₂₄ 1285.7996): 1285.7988 (- 0.8 mmu)

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REFERENCES AND NOTES

1. T. Yamakawa and S. Suzuki, *J. Biochem.*, **38**, 199 (1951).
2. H. Higashi, M. Naiki, S. Matsuo and K. Okouchi, *Biochem. Biophys. Res. Commun.*, **79**, 388 (1977).
3. H. Higashi, K. Ikuta, S. Ueda, S. Kato, Y. Hirabayashi, M. Matsumoto and M. Naiki, *J. Biochem.*, **95**, 785 (1984).
4. R. Higuchi, K. Inukai, J. X. Zhou, M. Honda, T. Komori, S. Tsuji and Y. Nagai, *Liebigs Ann. Chem.*, 359 (1993).
5. M. Numata, M. Sugimoto, S. Shibayama and T. Ogawa, *Carbohydr. Res.*, **174**, 73 (1988).
6. T. Sugata and R. Higuchi, *Tetrahedron Lett.*, **37**, 2613 (1996).
7. A. Hasegawa, H. Ohki, T. Nagahama, H. Ishida and M. Kiso, *Carbohydr. Res.*, **212**, 277 (1991).
8. K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg and G. Magnusson, *J. Org. Chem.*, **53**, 5629 (1988).
9. H. Hori, T. Nakajima, Y. Nishida, H. Ohruai and H. Meguro, *Tetrahedron Lett.*, **29**, 6317 (1988).
10. A ceramide from this synthetic work was prepared for the cerebroside molecular species derived from the starfish *Acanthaster planci*. This procedure has not yet been published.