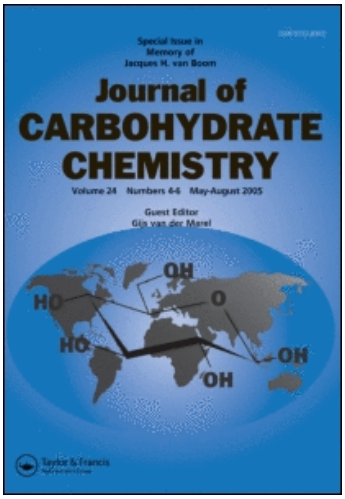

Journal of Carbohydrate Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Studies on the Thioglycosides of *N*-Acetylneuraminic Acid. 6: Synthesis of Ganglioside GM_4 Analogs[1]

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To cite this Article Ito, Yukiyasu , Kiso, Makoto and Hasegawa, Akira(1989) 'Studies on the Thioglycosides of *N*-Acetylneuraminic Acid. 6: Synthesis of Ganglioside GM_4 Analogs[1]', Journal of Carbohydrate Chemistry, 8: 2, 285 — 294
To link to this Article: DOI: 10.1080/07328308908048010
URL: http://dx.doi.org/10.1080/07328308908048010

J. CARBOHYDRATE CHEMISTRY, 8(2), 285-294 (1989)

STUDIES ON THE THIOGLYCOSIDES OF N-ACETYLNEURAMINIC ACID 6: SYNTHESIS OF GANGLIOSIDE GM_4 ANALOGS[1]

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Received August 17, 1988 - Final Form January 17, 1989

ABSTRACT

Two kinds of ganglioside GM_4 thioanalogs having different fatty acyl groups at the ceramide moiety, (2S, 3R, 4E)-1-O-[3-S-(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-3-thio-β-D-galactopyranosyl]-2-octadecanamido (or -tetracosanamido)-4-octadecene-1,3-diol (12, 13), have been synthesized. Condensation of the trichloroacetimidate 7, derived from 1,2,4,6-tetra-O-acetyl-3-S-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-3-thio-β-D-galactopyranose (6) by selective 1-O-deacetylation and subsequent trichloroacetimidation, with (2S, 3R, 4E)-2-azido-3-O-benzoyl-4-octadecene-1,3-diol (4), gave the coupling product (8), which was converted into the title compounds via selective reduction of the azide group, condensation with fatty acids, and removal of the protecting groups.

INTRODUCTION

Gangliosides are present in most mammalian tissues, and have many functions[2] in biological systems. Among the gangliosides, GM_4 has one of the simplest chemical structures. GM_4 has been detected,[3] apart from human and chicken brain, as a major ganglioside of mouse erythrocytes, chicken-embryonic liver, and egg york. In order to study the structure-function relationship of gangliosides, syntheses of a variety of gangliosides and various types of analogs are necessary. Recently, Ogawa et al.[4] reported the first synthesis of ganglioside GM_4.

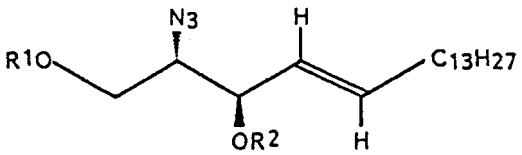
As a part of our project on the synthesis of thioglycosides of N-acetylneuraminic acid, we report here a synthesis of GM_4 thioanalogs.

RESULTS AND DISCUSSION

For a synthesis of our target compounds, ganglioside GM_4 thioanalogs carrying different fatty acyl groups at the ceramide moiety, and with the oxygen atom of the α(2→3) glycoside linkage between N-acetylneuraminic acid and D-galactose has been replaced by sulfur, 1,2,4,6-tetra-O-acetyl-3-S-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-3-thio-D-galactopyranose[6] (5) and (2S, 3R, 4E)-2-azido-4-octadecene-1,3-diol[7] (1) have served as convenient starting materials. Treatment of compound 1 with tert-butyldimethylsilyl chloride in pyridine gave the 1-O-tert-butyldimethylsilyl (TBDMS) derivative (2) in quantitative yield, which was converted, via 3-O-benzoylation and selective hydrolysis of TBDMS group with boron trifluoride etherate at 0 °C, into (2S, 3R, 4E)-2-azido-3-O-benzoyl-4-octadecene-1,3-diol (4). Selective O-deacetylation at the anomeric position of compound 5 with hydrazine acetate[8] in N,N-dimethylformamide at 50 °C afforded 6 in quantitative yield. When treated with trichloroacetonitrile[9] in the presence of sodium hydride in dichloromethane at 0 °C, compound 6 gave O-[2,4,6-tri-O-acetyl-3-S-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-3-thio-β-D-galactopyranosyl]-trichloroacetimidate (7) in 85% yield; in this reaction, no α-anomer of the trichloroacetimidate was isolated. The significant signals in ^{1}H NMR spectrum were a one-proton doublet of doublets at δ 5.12 ($J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 11.7 Hz, H-2) and a one proton doublet at δ 6.17 (H-1). Other ^{1}H NMR data are given in the Experimental section and are consistent with structure 7.

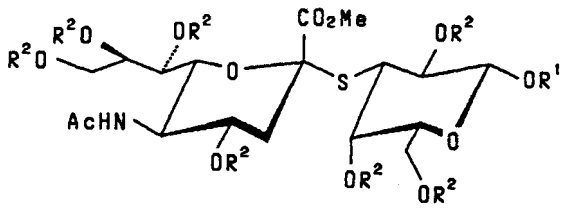
Coupling of the azide sphingosine (4) as a glycosyl acceptor, with the trichloroacetimidate (7) thus obtained, in the presence of boron trifluoride etherate[10] in dichloromethane under a nitrogen atmosphere at 0 °C, yielded the expected β-glycoside 8 in 70% yield. The structure was unambiguously proved by ^{1}H NMR spectroscopy. The observed chemical shift (at δ 4.88) and coupling constant ($J_{1,2}$ = 7.7 Hz) for H-1 of the galactose moiety in 8 are characteristic of β-glycosidic linkage. Other ^{1}H NMR data are consistent with structure 8. Selective reduction[11] of the azide group in 8 with hydrogen sulfide in pyridine-water gave the amine compound 9, which was used in the next reaction without further purification.

Treatment of 9 with octadecanoic acid or tetracosanoic acid in the presence of dicyclohexylcarbodiimide (DCC) in dichloromethane, gave the corresponding acylamino derivatives (10, 11) in almost quantitative yields, respectively. O-Deacetylation and O-debenzoylation of compounds

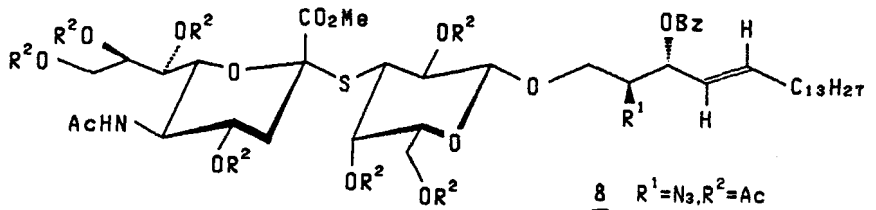


1 $R^1=R^2=H$
2 R^1=TBDMS
$R^2=H$
3 R^1=TBDMS,
R^2=Bz
4 $R^1=H, R^2$=Bz

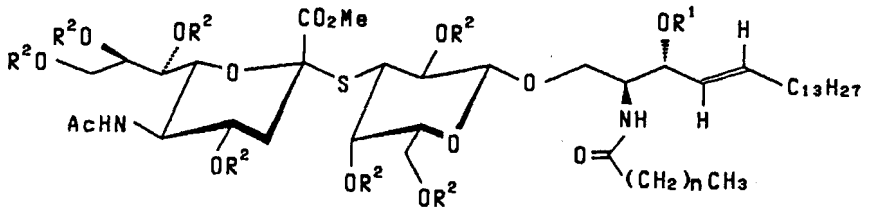
TBDMS=Me_3CMe_2Si
Bz=benzoyl



5 $R^1=R^2$=Ac
6 $R^1=H, R^2$=Ac
7 R^1=a, R^2=Ac
a= $-C(=NH)-CCl_3$



8 $R^1=N_3, R^2$=Ac
9 $R^1=NH_2, R^2$=Ac



10 n=16, R^1=Bz, R^2=Ac, R^3=Me
11 n=22, R^1=Bz, R^2=Ac, R^3=Me
12 n=16, $R^1=R^2=R^3=H$
13 n=22, $R^1=R^2=R^3=H$

10 and 11 with sodium methoxide in methanol, followed by saponification of the methyl ester group with 0.2M potassium hydroxide afforded the desired ganglioside GM_4 thioanalogs 12 and 13 as crystals in high yields, respectively.

In conclusion, the stereoselective glycosylation by using the trichloroacetimidate (7) as the donor and the azido-sphingosine derivative as the glycosyl acceptor, and subsequent convertion into the ganglioside analogs might be useful for the synthesis of a variety of glycosphingolipids and the analogs.

EXPERIMENTAL

General Procedures. Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. Specific rotations were determined with a Union MP-201 polarimeter, and IR spectra were recorded with a JASCO IR-1 spectrophotometer. ^{1}H NMR spectra were recorded with a JEOL JMN-GX 270 spectrometer. Preparative chromatography was performed on silica gel (Waco Co., 200 mesh) with the solvent systems specified. Concentrations and evaporations were conducted in vacuo.

(2S, 3R, 4E)-2-Azido-1-O-tert-butyldimethylsilyl-4-octadecene-1,3-diol (2). To an ice-cooled solution of (2S, 3R, 4E)-2-azido-4-octadecene-1,3-diol[7] (1, 3.29 g, 10 mmol) in dry pyridine (30 mL) was added, with stirring, tert-butyldimethylsilyl chloride (1.8 g, 11.9 mmol), and the mixture was stirred for 4 h at room temperature; the course of the reaction being monitored by TLC. After completion of the reaction, methanol (2 mL) was added, and the mixture was concentrated to a syrup, which was extracted with dichloromethane. The extract was successively washed with 2M hydrochloric acid, M sodium carbonate, and water, dried (sodium sulfate), and evaporated to leave a syrup, which was chromatographed on a column of silica gel (300 g) using 250:1 toluene-ethanol, to give compound 2 quantitatively as a syrup: $[\alpha]_D$ - 0.77° (c 5.2, chloroform); IR (film) 3430 (OH), 2100 (N_3), 970 (C=C), and 840 cm^{-1} (MeSi); ^{1}H NMR ($CDCl_3$) δ 0.83-0.97 (m, 12H, Me_3C, Me), 1.14-1.38 (m, 22H, $11CH_2$), 2.06 (q, 2H, $J_{5,6} = J_{5,6'} = 6.6$ Hz, H-6,6'), 3.42 (q, 1H, $J_{1,2} = J_{2,3} = 5.9$ Hz, H-2), 3.80 (d, 2H, $J_{1,2} = 5.9$ Hz, H-1,1'), 4.21 (t, 1H, $J_{2,3} = J_{3,4} = 5.9$ Hz, H-3), 5.49 (dd, 1H, $J_{3,4} = 7.1$ Hz, $J_{4,5} = 15.4$ Hz, H-4), and 5.78 (ddd, 1H, $J_{4,5} = 15.4$ Hz, $J_{5,6} = J_{5,6'} = 6.6$ Hz, H-5).

Anal. Calcd for $C_{24}H_{49}N_3O_2Si$: C, 65.55; H, 11.23; N, 9.56. Found: C, 65.68; H, 11.35; N, 9.51.

(2S, 3R, 4E)-2-Azido-3-O-benzoyl-1-O-tert-butyldimethylsilyl-4-octadecene-1,3-diol (3). To a stirred solution of 2 (3.96 g, 9.0 mmol) in dry pyridine (40 mL) was added benzoyl chloride (2 mL) at 0 °C. The mixture was stirred for one h at room temperature, and methanol (2 mL) was added, and then concentrated to a syrup, which was extracted with dichloromethane. The extract was successively washed with 2M hydrochloric acid, M sodium carbonate, and water, dried (sodium sulfate), and concentrated. The product was purified by chromatography on a column of silica gel (300 g) with toluene, to give 3 (quantitatively) as a syrup: $[\alpha]_D$ - 24.0° (c 4.8, chloroform); IR (film) 2100 (N_3), 1730 and 1280 (ester), 970 (C=C), 840 (MeSi), and 720 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.90-0.92 (m, 12H, Me_3C, Me), 1.25 (m, 22H, $11CH_2$), 2.08 (m, 2H, H-6,6'), 3.65-3.73 (m, 2H, H-1,1'), 3.79 (m, 1H, H-2), 5.52-5.65 (m, 2H, H-3,4), 5.93 (ddd, 1H, $J_{5,6} = J_{5,6'} = 6.8$ Hz, H-5), and 7.41-8.05 (m, 5H, Ph).

Anal. Calcd for $C_{31}H_{53}N_3O_3Si$: C, 68.46; H, 9.82; N, 7.73. Found: C, 68.60; H, 9.95; N, 7.71.

(2S, 3R, 4E)-2-Azido-3-O-benzoyl-4-octadecene-1,3-diol (4). To a solution of 3 (4.32 g, 7.94 mmol) in dichloromethane (50 mL) was added boron trifluoride etherate (1.96 mL; 16 mmol) at 0 °C, and the mixture was stirred for 4 h at 0 °C. The solution was poured into ice water, and extracted with dichloromethane. The extract was successively washed with M sodium carbonate and water, dried (sodium sulfate), and concentrated to leave a syrup, which was purified by chromatography on a column of silica gel (400 g) with 250:1 toluene-ethanol. Compound 4 (3.3 g, 98%) was obtained as a syrup: $[\alpha]_D$ - 45° (c 5.2, chloroform); IR (film) 3450 (OH), 2100 (N_3), 1720 and 1260 (ester), 960 (C=C), and 700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3H, Me), 1.25 (m, 22H, $11CH_2$), 2.08 (q, 2H, H-6,6'), 2.63 (dd, 1H, $J_{gem} = 11.2$ Hz, $J_{1',2} = 6.8$ Hz, H-1'), 3.76 (dd, 1H, $J_{gem} = 11.2$ Hz, $J_{1,2} = 4.2$ Hz, H-1), 3.81 (m, 1H, H-2), 5.56-5.65 (m, 2H, H-3,4), 5.96 (ddd, 1H, $H_{4,5} = 15.4$ Hz, $J_{5,6'} = 6.6$ Hz, H-5), and 7.42-8.07 (m, 5H, Ph).

Anal. Calcd for $C_{25}H_{39}N_3O_3$: C, 69.89; H, 9.14; N, 9.78. Found: C, 69.82; H, 9.33; N, 9.69.

2,4,6-Tri-O-acetyl-3-S-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-3-thio-D-galactopyranose (6). To a solution of compound 5[6] (73 mg, 0.09 mmol) in dry N,N-dimethylformamide (DMF, 1 mL) was added hydrazine acetate (8.3 mg), and the mixture was heated, with stirring, for 7 h at 50 °C; the progress of the reaction being monitored by TLC. The mixture was concentrated, the residue extracted with dichloromethane, and the extract successively

washed with 2M hydrochloric acid and water, dried (sodium sulfate), and concentrated to a syrup, which was chromatographed on a column of silica gel (7 g) using 80:1 and 60:1 dichloromethane-methanol as the eluents. The latter eluate afforded compound 6 in quantitative yield as a syrup: $[\alpha]_D$ + 33.0° (c 0.7, chloroform); IR (film) 3400-3300 (OH, NH), 1740 and 1210 (ester), and 1660 and 1540 cm^{-1} (amide); ^1H NMR ($CDCl_3$) Neu5Ac unit δ 1.86 (s, 3H, AcN), 2.64 (dd, 1H, $J_{3a,3e}$ = 12.5 Hz, $J_{3e,4}$ = 4.4 Hz, H-3e), 3.67 (dd, 1H, $J_{5,6}$ = 10.6 Hz, $J_{6,7}$ = 2.2 Hz, H-6), 3.86 (s, 3H, MeO), 3.95 (dd, 1H, $J_{8,9'}$ = 5.9 Hz, $J_{9,9'}$ = 12.5 Hz, H-9'), 4.05 (q, 1H, $J_{4,5}$ = $J_{5,6}$ = $J_{5,NH}$ = 10.6 Hz, H-5), 4.28 (dd, 1H, $J_{8,9}$ = 2.6 Hz, $J_{9,9'}$ = 12.5 Hz, H-9), 4.78 (ddd, 1H, $J_{3a,4}$ = $J_{4,5}$ = 10.6 Hz, $J_{3e,4}$ = 4.4 Hz, H-4), 5.10 (d, 1H, $J_{NH,5}$ = 10.6 Hz, NH), 5.25 (dd, 1H, $J_{6,7}$ = 2.2 Hz, $J_{7,8}$ = 10.3 Hz, H-7), and 5.59 (ddd, 1H, $J_{7,8}$ = 10.3 Hz, $J_{8,9}$ = 2,6 Hz, $J_{8,9'}$ = 5.9 Hz, H-8); Gal unit δ 3.69 (dd, 1H, $J_{2,3}$ = 11.7 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 4.15 (t, 1H, $J_{5,6}$ = $J_{5,6'}$ = 5.5 Hz, H-5), 4.62 (dd, 1H, $J_{1,2}$ = 7.7 Hz, $J_{2,3}$ = 11.7 Hz, H-2), 4.87 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4), and 4.98 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1); other groups δ 2.02, 2.04, 2.05, 2.08, 2.10, 2.20, 2.22 (7s, 21H, 6AcO, AcN).

Anal. Calcd for $C_{32}H_{45}NO_{20}S$: C, 48.30; H, 5.70; N, 1.76. Found: C, 48.15; H, 5.83; N, 1.75.

O-[2,4,6-Tri-O-acetyl-3-S-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-3-thio-β-D-galactopyranosyl]-trichloroacetimidate (7). A suspension of 6 (79.5 mg, 0.1 mmol) and powdered molecular sieves 4A (100 mg) in dry dichloromethane (3 mL) and dry trichloroacetonitrile (0.18 mL) was stirred for 30 min at room temperature, and cooled to 0 °C, sodium hydride in oil suspension (4.2 mg; 60% of sodium hydride by weight) was added, and the mixture was stirred for 10 h at 0 °C; the course of the reaction being monitored by TLC. Silica gel (50 mg) was added, and the mixture was filtered, and then the solid was washed with acetone. The filtrate and washings were combined, and concentrated to a syrup, which was chromatographed on a column of silica gel (10 g) using 100:1 and 70:1 dichloromethane-methanol. The latter eluate gave compound 7 (79.5 mg, 85%) as a syrup: $[\alpha]_D$ + 27.5° (c 1.1, chloroform); IR (film) 3350 (NH), 1750 and 1220 (ester), and 1680 and 1540 cm^{-1} (amide); ^1H NMR ($CDCl_3$) Neu5Ac unit δ 1.88 (s, 3H, Ac N, 2.66 (dd, 1H, $J_{3a,3e}$ = 12.6 Hz, $J_{3e,4}$ = 4.4 Hz, H-3e), 3.72 (dd, 1H, $J_{5,6}$ = 10.6 Hz, $J_{6,7}$ = 2.2 Hz, H-6), 3.86 (s, 3H, MeO), 3.95 (dd, 1H, $J_{8,9'}$ = 6.2 Hz, $J_{9,9'}$ = 12.3 Hz, H-9'), 4.05 (q, 1H, $J_{4,5}$ = $J_{5,6}$ = $J_{5,NH}$= 10.6 Hz, H-5), 4.33 (dd, 1H, $J_{8,9}$ = 2.4 Hz, $J_{9,9'}$ = 12.3 Hz, H-9), 4.85 ddd, 1H, $J_{3a,4}$ = $J_{4,5}$ = 10.6 Hz, $J_{3e,4}$ = 4.4 Hz, H-4), 5.26 (d, 1H,

$J_{NH,5}$ = 10.6 Hz, NH), 5.30 (dd, 1H, $J_{6,7}$ = 2.2 Hz, $J_{7,8}$ = 10.1 Hz, H-7), and 5.64 (ddd, 1H, $J_{7,8}$ = 10.1 Hz, $J_{8,9}$ = 2.4 Hz, $J_{8,9'}$ = 6.2 Hz, H-8); Gal unit δ 3.83 (dd, 1H, $J_{2,3}$ = 11.7 Hz, $J_{3,4}$ = 2.9 Hz, H-3), 4.05 (dd, 1H, $J_{5,6}$ = 5.7 Hz, $J_{6,6'}$ = 12.0 Hz, H-6'), 4.11 (dd, $J_{5,6}$ = 5.7 Hz, $J_{6,6'}$ = 12.0 Hz, H-6), 4.36 (t, 1H, $J_{5,6}$ = $J_{5,6'}$ = 5.7 Hz, H-5), 4.97 (near d, 1H, $J_{3,4}$ = 2.9 Hz, H-4), 5.12 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 11.7 Hz, H-2), 6.17 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), and 8.65 (s, 1H, =NH); other groups δ 2.01, 2.02, 2.03 (2), 2.08, 2.17, and 2.22 (7s, 21H, 7AcO).

Anal. Calcd for $C_{34}H_{45}N_2O_{20}Cl_3S$: C, 43.44; H, 4.82; N, 2.98. Found: C, 43.31; H, 4.85; N, 2.79.

(2S, 3R, 4E)-1-O-[2,4,6-Tri-O-acetyl-3-S-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-3-thio-β-D-galactopyranosyl]-2-azido-3-O-benzoyl-4-octadecene-1,3-diol (8). To a stirred suspension of 4 (53.3 mg, 0.12 mmol), the trichloroacetimidate 7 (56.7 mg, 0.06 mmol), and powdered molecular sieves 4A (150 mg) in dry dichloromethane (3 mL) was added boron trifluoride etherate (0.008 mL, 0.065 mmol), and stirring was continued under a nitrogen atmosphere for 2 h at 0 °C. The precipitates were filtered off on celite 545 pad, and washed with dichloromethane. The filtrate and washings were combined, and washed with M sodium hydrogen carbonate and water, dried (sodium sulfate), and the solvent was evaporated to a syrup, which was chromatographed on a column of silica gel (10 g) using dichloromethane and 125:1 dichloromethane-methanol. The latter eluate afforded compound 8 (50 mg, 70%) as a syrup: $[\alpha]_D$ + 3.9° (c 0.48, chloroform); IR (film) 3380 (OH, NH), 2100 (N_3), 1750 and 1210 (ester), 1670 and 1540 (amide), 950 (C=C), and 710 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) Neu5Ac unit δ 1.85 (s, 3H, AcN), 2.65 (dd, 1H, $J_{3a,3e}$ = 12.5 Hz, $J_{3e,4}$ = 4.4 Hz, H-3e), 3.84 (s, 3H, MeO), 5.17 (d, 1H, $J_{NH,5}$ = 9.3 Hz, NH); Gal unit δ 4.88 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1); Sphingosine unit δ 0.88 (t, 3H, MeC), 1.22-1.37 (m, 22H, 11CH_2), 5.93 (ddd, 1H, $J_{4,5}$ = 15.5 Hz, $J_{5,6}$ = $J_{5,6'}$ = 7.0 Hz, H-5), and 7.42-8.07 (m, 5H, Ph); other groups δ 2.00, 2.02 (2), 2.09, 2.10, and 2.21 (2) (7s, 21H, 7AcO).

Anal. Calcd for $C_{57}H_{82}N_4O_{22}S$: C, 56.70; H, 6.85; N,4.64. Found: C, 56.63; H, 6.86; N, 4.48.

(2S, 3R, 4E)-1-O-[2,4,6-Tri-O-acetyl-3-S-(methyl 5-acetamido-4,7,8-9 -tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl-onate)-3-thio-β-D-galactopyranosyl]-3-O-benzoyl-2-octadecanamido-4-octadecene-1,3-diol (10). Hydrogen sulfide was bubbled through a stirred solution of 8 (42 mg, 0.035 mmol) in pyridine (5 mL) and water (1 mL) for

24 h at room temperature. After the completion of the reaction, the mixture was concentrated to a syrup, which was chromatographed on a column of silica gel (5 g) using 125:1 and 50:1 dichloromethane-methanol. The latter eluate gave compound 9 (37 mg, 90%), which was used for the next reaction without further purification. To a solution of the amine compound 9 (37 mg, 0.031 mmol) and octadecanoic acid (18 mg, 0.062 mmol), in dichloromethane (1 mL) was added dicyclohexylcarbodiimide (DCC; 13 mg, 0.062 mmol), and the mixture was stirred for 2 h at room temperature and concentrated. The residue was chromatographed on a column of silica gel (5 g) with 200:1 dichloromethane-methanol to give 10 (44 mg, 97%) as a syrup: $[\alpha]_D$ + 12.0° (c 0.13, chloroform); IR (film) 3280 (NH), 1740 and 1210 (ester), 950 (C=C), and 700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) Neu5Ac unit δ 1.88 (s, 3H, AcN), 2.64 (dd, 1H, $J_{3a,3e}$ = 12.2 Hz, $J_{3e,4}$ = 4.2 Hz, H-3e), 3.84 (s, 3H, MeO), and 5.12 (d, 1H, $J_{NH,5}$ = 10.6 Hz, NH); Gal unit δ 4.79 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1); ceramide unit δ 0.85-0.95 (m, 6H, 2MeC), 1.10-1.72 (m, 52H, $26CH_2$), 5.87 (ddd, 1H, H-5), and 7.40-8.05 (m, 5H, Ph); other groups δ 1.92, 2.01, 2.02, 2.08, 2.09, and 2.18 (2) (7s, 21H, 7AcO).

Anal. Calcd for $C_{75}H_{118}N_2O_{23}S$: C, 62.22; H, 8.21; N, 1.93. Found: C, 62.08; H, 8.25; N, 1.90.

(2S, 3R, 4E)-1-O-[2,4,6-Tri-O-acetyl-3-S-(methyl 5-acetamido-4,7,8,-9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl-onate)-3-thio-β-D-galactopyranosyl]-3-O-benzoyl-2-tetracosanamido-4-O-octadecene-1,3-diol (11). Coupling of 9 (53 mg, 0.045 mmol) with tetracosanoic acid (33 mg, 0.09 mmol) in dry dichloromethane (1 mL) by using DCC (19 mg), as described for the preparation of 10, gave compound 11 as a syrup in quantitative yield: $[\alpha]_D$ + 8.5° (c 0.24, chloroform); IR (film) 3280 (NH), 1740 and 1200 (ester), 950 (C=C), and 700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) Neu5Ac unit δ 1.88 (s, 3H, AcN), 2.64 (dd, 1H, $J_{3a.3e}$ = 12.5 Hz, $J_{3e,4}$ = 4.6 Hz, H-3e), 3.84 (s, 3H, MeO), 5.17 (d, 1H, $J_{NH,5}$ = 10.0 Hz, NH); Gal unit δ 4.79 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1); ceramide unit δ 0.88-0.95 (m, 6H, 2MeC), 1.15-1.71 (m, 64H, $32CH_2$), 5.87 (ddd, 1H, $J_{4,5}$ = 15.4 Hz, $J_{5,6}$ = $J_{5,6'}$ = 6.6 Hz, H-5), and 7.40-8.05 (m, 5H, Ph); other groups δ 1.92, 2.01, 2.02, 2.08, 2.10, and 2.18 (2) (7s, 21H, 7AcO).

Anal. Calcd for $C_{81}H_{130}N_2O_{23}S$: C, 63.51; H, 8.55; N, 1.83. Found: C, 63.59; H, 8.67; N, 1.80.

(2S, 3R, 4E)-1-O-[5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-3-thio-β-D-galactopyranosyl]-2-octadecanamido-4-octadecene-1,3-diol (12). To a solution of compound 10 (12.2 mg, 0.0084 mmol) in dry methanol (1 mL) was added sodium methoxide (3 mg), and the

solution was stirred for one h at room temperature, and treated with Amberlite IR-120 (H^+) resin to remove the base. The solution was concentrated, and the residue was dissolved in 1,4-dioxane (2 mL). 0.2M Potassium hydroxide (1 mL) was added to the solution which was then stirred for 10 min at room temperature, and treated with Amberlite IR-120 (H^+) resin. The solution was concentrated to a solid, which was recrystallized from ethanol-ether, to give 12 in quantitative yield: mp 155-157 °C, $[\alpha]_D$ + 11.4° (c 0.14, methanol); IR (KBr) 3500-3350 (OH, NH), 2920 and 2850 (methyl, methylene), 1720 (C=O), 1630 and 1540 (amide), and 950 cm^{-1} (C=C); ^1H NMR (CD_3OD) Neu5Ac δ 2.03 (s, 3H, AcN) and 2.87 (dd, 1H, H-3e); ceramide unit δ 0.87-0.91 (m, 6H, 2MeC), 1.17-1.62 (m, 54H, $27CH_2$), 5.45 (dd, 1H, H-4), and 5.70 (ddd, 1H, H-5).

Anal. Calcd for $C_{53}H_{98}N_2O_{15}S$: C, 61.48; H, 9.54; N, 2.71. Found: C, 61.39; H, 9.58; N, 2.66.

(2S, 3R, 4E)-1-O-[3-S-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-3-thio-β-D-galactopyranosyl]-2-tetracosanamido-4-octadecene-1,3-diol (13). Removal of the protecting groups in compound 11 (13 mg, 0.0085 mmol) with sodium methoxide in methanol, and with 0.2M potassium hydroxide, as described for 12, gave 13 as crystals in quantitative yield: mp 152-155 °C, $[\alpha]_D$ + 11.1° (c 0.2, methanol); IR (KBr) 3500-3300 (OH, NH), 2920 and 2850 (methyl, methylene), 1720 (C=O), 1640 and 1550 (amide), and 950 cm^{-1} (C=C); ^1H NMR (CD_3OD) Neu5Ac unit δ 1.95 (s, 3H, AcN), 2.80 (dd, 1H, H-3e); ceramide unit δ 0.77-0.82 (m, 6H, 2Me), 1.06-1.58 (m, 64H, $32CH_2$), 4.34 (dd, 1H, H-4), and 5.61 (ddd, 1H, H-5).

Anal. Calcd for $C_{59}H_{110}N_2O_{15}S$: C, 63.30; H, 9.90; N, 2.50. Found: C, 63.25; H, 9.98; N, 2.47.

ACKNOWLEDGMENT

This work was supported in part by a Grant-in-Aid (No. 63560122) for the Scientific Research from Ministry of Education, Science and Culture of Japan.

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