Synthesis of a Ganglioside GM₃ Position Isomer, N-Acetylneuraminosyl-α(2→6)-lactosylβ(1→1)-ceramide†

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Ganglioside GM₃, as well as other gangliosides, is a polymorphous molecule in the sialic acid and ceramide moieties, and exhibits various types of important biological behavior as an influenza A virus receptor, inducer of monocytic differentiation of human myeloid, and enhancing activity. In view of these facts, it is of interest to investigate the functions of GM₃ at the molecular level. Previously, we have synthesized GM₃ and the analogs containing a variety of lipophilic parts in place of ceramide, the carbon 7 and 8 sialic acids, and also the deoxy-sialic acids in order to elucidate the role of the ceramide and sialic acid parts in the function of GM₃. In continuing to investigate the structure–activity relationships of gangliosides, we describe here the synthesis of a positional isomer of GM₃ [Neu5Acα(2→6)Galβ(1→4)Glcβ(1→1)Cer], with regard to substituting the Gal residue by N-acetylneuraminic acid. Catalytic hydrogenolysis of the banzyl group in 2-(trimethylsilyl)ethyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-o-glycero-2-O-galacto-2-nonulopyranosyl(1→4)-2,3,6-tri-O-acetyl-β-D-galactopyranosyl(1→4)-O-acetyl-β-D-glucopyranosyl(1→1)-cerebroside (1) in ethanol-formic acid system in the presence of 10% Pd-C, and subsequent O-acetylation gave compound 3 in a 93% yield. Treatment with 2% Bf₃·OEt₃ in dichloromethane for 4 hr at 0°C gave 1-hydroxy compound 4 in a 96% yield. When treated with trichloroacetonitrile in dichloromethane in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) for 3 hr at 0°C, 4 gave trichloroacetimidate 5 in a good yield. Glycosylation of 5 with 25% HCl Et₂O·2H₂O·3-0-benzoyl-4-octadecene-1,3-diol9·10 in dichloromethane in the presence of BF₃·OEt₃ for 2 hr at 0°C afforded β-glycoside 7 in a 71% yield. Selective reduction of the azide group in 7 with hydrogen sulfide in aqueous pyridine for 48 hr at room temperature gave amine 8, which after condensation with octadecanoc acid by using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide C, 1.5:1 HCl, 6.36% N, 1.24%, O-Deacetylation of 9 with sodium methoxide in methanol, with subsequent saponification of the methyl ester group, yielded desired GM₃ position isomer 10 in an almost quantitative yield. The 1H NMR spectrum of 10 contains two signals (doublets) due to anomeric protons at 4.19 (J = 7.7 Hz, H-1a) and 4.25 (J = 7.7 Hz, H-1b), and one-proton doublet of doublets due to the H-3e-α proton at 3.79 (Jₕ,α = 11.7 Hz, Jₕ,α = 4.6 Hz). The other 1H-NMR data given in the experimental section are consistent with structure 10.

Experimental
Specific rotation values were determined with a Union PM-201 polarimeter at 25°C. IR spectra were recorded with a Jasco A-100 spectrophotometer, and 1H-NMR spectra were recorded with a JEOL JNM-GX 270 spectrometer. Preparative chromatography was performed on silica gel (Wako Co., 200 mesh) with the solvent systems specified. All concentration was determined in vacuo.

2-(Trimethylsilyl)ethyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-o-glycero-2-O-galacto-2-nonulopyranosyl(1→4)-O-acetyl-β-D-galactopyranosyl(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranosyl(2) A solution of 3 (240 mg, 0.2 mmol) in ethanol (10 ml) and formic acid (1 ml) was hydrogenolyzed in the presence of 10% Pd-C (100 mg) overnight at room temperature, filtered, and then concentrated. Column chromatography (70:1 chichloromethane-methanol) of the product on silica gel (40 g) gave 2 (190 mg, 93%) as an amorphous mass; [α]D₂₀ = −29.7° (c 1.9, CHCl₃), IR νmax (KBr cm⁻¹): 3700–3400 (OH, NH), 1750 and 1230 (ester), 1670 and 1540 (amide), and 860 and 840 (TMS); NMR (CDCl₃): δ 0.93 (m, 2 2H, Me₂SiCH₂CH₂), 1.88 (s, 3H, AcN), 2.03 (2), 2.04, 2.05, 2.10, 2.12, 2.13, 2.17 (2) (9s, 27H, 9AcO), 2.55 (dd, 1H, Jₕ,ₗₕ = 12.8, Jₕ,ₗₕ = 4.8 Hz, H-3e-α), 3.83 (s, 3H, MeO), 3.93–4.11 (m, 3H, H-3e, 6c, 9b), 4.20 (dd, 1H, Jₕ,ₗₕ = 5.9, Jₕ,ₗₕ = 11.7 Hz, H-6b), 4.29 (dd, 1H, Jₕ,ₗₕ = 1.8, Jₕ,ₗₕ = 12.8 Hz, H-9b), 4.48 (2d, 2H, Jₕ,ₗₕ = 7.9 Hz, H-1a, b), 4.86 (m, 1H, H-4e), 5.28 (m, 2H, H-7e, 8c), and 5.45 (d, 1H, Jₕ,ₗₕ = 9.5 Hz, NH).

Anal. Found: C, 50.21; H, 6.48; N, 1.29. Calcd. for C₂₄H₂₁NO₄S₂ (197 mg, quantitatively), 50.20 C, 6.36 H, 1.27 N. A sample of compound 2 (190 mg) was acetylated with acetic anhydride (1 ml) in pyridine (3 ml) in the usual way to give 3 (197 mg, quantitative). After column chromatography (80:1 dichloromethane-methanol) on silica gel (50 g): [α]D₂₀ = −24.5° (c 1.5, CHCl₃), NMR (CDCl₃): δ 0.93 (m, 2H, Me₂SiCH₂CH₂), 1.89 (s, 3H, AcN), 1.95 (2.3) (2), 2.04, 2.06, 2.08, 2.10, 2.15, 2.14, 2.18 (10s, 30H, 10AcO), 2.54 (dd, 1H, Jₕ,ₗₕ = 12.8, Jₕ,ₗₕ = 4.4 Hz, H-3e-α), 4.29 (m, 1H, H-9b), 4.48 (d, 1H, Jₕ,ₗₕ = 1.8 Hz, H-1a), 4.56 (d, 1H, Jₕ,ₗₕ = 7.7 Hz, H-1b), 4.89 (m, 1H, H-4e), 4.98 (dd, 1H, Jₕ,ₗₕ = 10.3, Jₕ,ₗₕ = 3.3 Hz, H-3b), 5.08 (dd, 1H, H-2b), and 5.30–5.41 (m, 1H, H-7e, 8c, NH).

Anal. Found: C, 50.21; H, 6.50; N, 1.18. Calcd. for C₂₄H₂₁NO₄S₂ (160.2 mg) 50.33 C, 6.38 H, 1.20 N.

O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-o-glycero-a-
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O-(5-acetoxy-4,7,8,9-tetra-o-acetyl-3,5-dioxyo-o-galacto-α-D-galactopyranosyl)-(2→6)-O-(2,3,4-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-galactopyranoside (4). To a solution of 3 (270 mg, 0.23 mmol) in dichloromethane (5 mL) was added BF_3·OEt_2 (0.3 mL) at 0°C, and the mixture was stirred for 4 hr at 0°C, before being diluted with dichloromethane (50 mL). The mixture was washed with M Na_2CO_3 and water, dried (Na_2SO_4), and concentrated. Column chromatography (50:1 dichromel-methanol) of the residue on silica gel (50 g) gave 4 (236 mg, 96%) as an amorphous mass. [α_D]_20 (c 1.4, CHCl_3) 18.5° ± 0.2°. IR ν_mαx (KBr cm⁻¹) 3600-3300 (OH), 1750 and 1220 (ester), 1660 and 1540 (amide). NMR (CDCl_3): δ 1.89 (s, 3H, AcN), 1.92, 2.03, 2.04 (2), 2.07, 2.08, 2.09, 2.12, 2.15, 2.17 (10s, 30H, 10AcO). 2.55 (dd, 1H, J_H, J_ac = 12.8, J_ac = 4.4 Hz, H-3-εq), 3.79 (s, 3H, MeO). 4.39 (d, 1H, J_H, J_ac = 8.1 Hz, H-1b), 4.82 (dd, 1H, J_H, J_ac = 8.1 Hz, J_ac = 3.3 Hz, H-3b), 4.99 (t, 1H, J_H, J_ac = 5.0 Hz, J_ac = 3.3 Hz, H-3a). Anal. Found: C, 49.45; H, 5.88; N, 1.30. Calcd. for C_{55}H_{93}NO_{29} (1067.9): C, 49.48; H, 5.76; N, 1.31%.

O-(5-Methyl-5-acetoxy-4,7,8,9-tetra-o-acetyl-3,5-dioxyo-o-galacto-o-D-galactopyranosyl)-(2→6)-O-(2,3,4-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-galactopyranosyl-(1→1)-(2S,3R,4E)-2-azido-3-O-benzoyl-4-octadecene-1,3-diol (10). To a solution of 9 (63 mg, 48 µmol) in methanol (5 mL) was added sodium methoxide (20 mg), the mixture was stirred for 5 hr at room temperature, and water (0.5 mL) was added. The solution was stirred overnight at room temperature, neutralized with Amberlite IR-120 (H⁺) resin, and then filtered. The resin was washed with methanol, and the combined filtrate and washings were concentrated. Column chromatography (methanol) of the residue on Sephadex LH-20 (30 g) gave 10 (quantitative) as an amorphous mass; [α_D]_20: +8.9° (0.18, 1:1 CHCl_3-MeOH). NMR (1 CDOD-CDCl_3): δ 0.89 (s, 6H, 2MeCH_3), 1.26 (s, 50H, 25CH_2), 1.99 (s, 3H, AcN), 2.16 (t, 1H, J_H, J_ac = 11.7 Hz, H-3-εx), 2.79 (dd, 1H, J_H, J_ac = 4.4 Hz, H-3-εq), 5.43 (dd, 1H, J_H, J_ac = 7.0 Hz, H-5, H-sp), and 5.68 (dt, 1H, J_H, J_ac = 7.0 Hz, H-5, H-sp). Anal. Found: C, 59.83; H, 9.40; N, 2.35. Calcd. for C_{82}H_{108}N_{23}O_{31} (1181.5): C, 59.98; H, 9.21; N, 2.37%.

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References