Partial Synthesis of a Sea Cucumber Ganglioside Analogue from a Starfish Cerebroside

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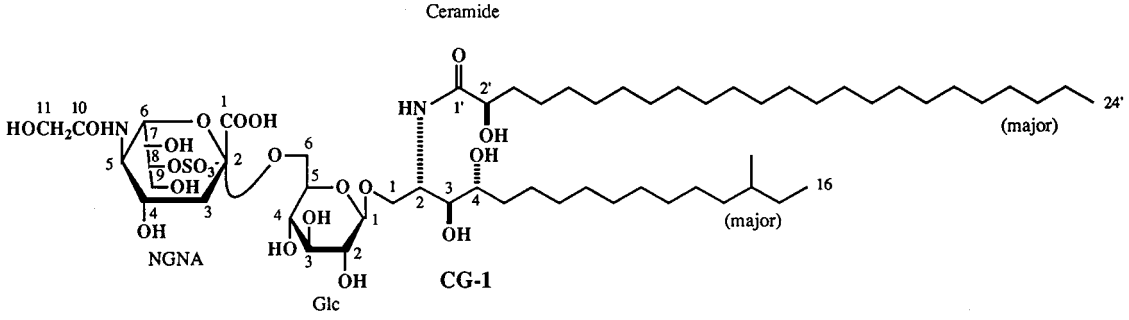
A sea cucumber ganglioside analogue **7** (NGNAα2→6Glcβ1→1Cer), which contains a phytosphingosine as a long-chain base and an α-hydroxy fatty acid, has been synthesized. Coupling of the methyl 2-thioglycoside derivative **5** of *N*-glycolylneuraminic acid with a cerebroside derivative **3**, prepared from acanthacerebroside A (**1**), afforded protected ganglioside analogue **6**, which was deprotected to give the corresponding ganglioside **7**.

Gangliosides, sialic acid containing glycosphingolipids, have received much attention owing to their biological functions.[1] Meanwhile, it is known that the gangliosides present in echinoderms possess unique structures[2] and biological activities,[3] and therefore they can be expected to represent components of pharmacological interest. However, they have usually been treated as a mixture with heterogeneous ceramide moieties. In view of the considerable importance of synthesizing the unique gangliosides in a pure state, a series of studies on the synthesis of gangliosides from echinoderms have been performed in our laboratory.[4] In continuation of the preceding study,[4b] we carried out the partial synthesis of a desulfated analogue of the unique ganglioside **CG-1**,[5] which was obtained from the sea cucumber *Cucumaria echinata*, and possesses an NGNA(*N*-glycolylneuraminic acid)α2→6Glcβ1→1Cer moiety (Scheme 1). In this paper, we report the partial synthesis of the ganglioside analogue **7** starting from the pure starfish cerebroside **1** (acanthacerebroside A)[6] and the methyl 2-thioglycoside derivative **5** of *N*-glycolylneuraminic acid.[4]



Scheme 1

The receptor **3** was synthesized in 62% overall yield from the known glucocerebroside **1** (acanthacerebroside A),[6] which has been obtained from the starfish *Acanthaster planci* and was found to consist of a (2*S*,3*S*,4*R*)-C_{16}-phytosphingosine base, (2*R*)-2-hydroxytetracosanoic acid, and D-glucose. This was achieved as follows: Tritylation (TrCl, Py, DMAP) followed by benzoylation (BzCl) of **1** afforded **2**, and subsequent detritylation (*p*TsOH) of **2** yielded **3**. The structure of **3** was confirmed by acetylation to give the corresponding monoacetate **4**. ^{1}H-NMR data showed that the Glc 6-H_2 (δ = 3.33 and 3.51) of **3** were deshielded and gave a signal at δ = 4.04 in **4**, thus indicating the presence of a hydroxy group at C-6 of Glc in **3**.

Based on the Hasegawa method,[7] glycosylation of **3** with the NGNA donor **5**, which was prepared[4] from *N*-acetylneuraminic acid (NANA), in EtCN/CH_2Cl_2 in the presence of *N*-iodosuccinimide (NIS), trifluoromethanesulfonic acid (TfOH) and 4-Å molecular sieves for 2 h at −40°C, gave the α-sialoside **6** in 31% yield.[8] The configuration of the sialic acid moiety of **6** was established on the basis of the large $J_{H(7)-H(8)}$ coupling constant (J = 9.2 Hz, α configuration).[9] **6** was deprotected (Pd/C/H_2, NaOMe) to give the ganglioside analogue **7** in 93% yield (Scheme 2).

A related ganglioside possessing an NGNAα2→6Glc moiety has been synthesized by following an alternative strategy.[10] We believe that the partial synthesis of the sea cucumber ganglioside analogue using a natural cerebroside as reported herein constitutes a notable new approach. The biological activities of **7** will be examined in due course.

Experimental Section

General: Melting points: Micro melting point apparatus (Yanaco MP-3); uncorrected values. − Optical rotations: Jasco DIP-370

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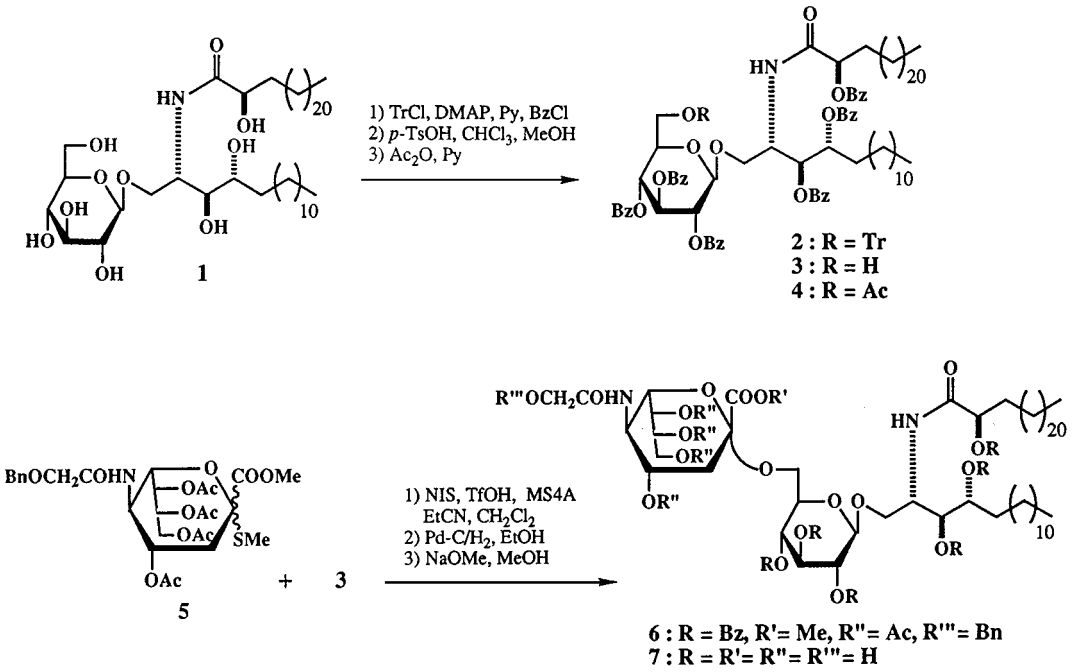

Scheme 2

digital polarimeter at 25°C. − ^{1}H-NMR spectra: Jeol GX-270 spectrometer (270 MHz), Varian Unity-500 spectrometer (500 MHz), Varian Unity-600 spectrometer (600 MHz). − FAB mass spectra: Jeol SX102A (xenon atom beam); matrix: HMPA/TEG (negative-ion mode) and *m*-nitrobenzyl alcohol (positive-ion mode). − Abbreviations: Glc: glucose; NA: neuraminic acid; Cer: ceramide; eq: equatorial.

1-*O*-(β-D-Glucopyranosyl)-(2*S*,3*S*,4*R*)-2-[(2*R*)-2-hydroxytetracosanoylamino]-1,3,4-hexadecanetriol (Acanthacerebroside A) (1): According to the previous paper[6a], 80 mg of **1** was isolated from the cerebroside mixture A-1 (425 mg) obtained from the starfish *Acanthaster planci*.

(2*S*,3*S*,4*R*)-3,4-Di-*O*-benzoyl-2-[(2*R*)-2-benzyloxytetracosanoylamino]-1-*O*-[β-D-(2,3,4-tri-*O*-benzoyl-6-*O*-triphenylmethyl)glucopyranosyl]hexadecane-1,3,4-triol (2): Compound **1** (75.6 mg, 92.4 μmol), triphenylmethyl chloride (TrCl, 255 mg, 924 μmol), and 4-(dimethylamino)pyridine (DMAP, 11 mg, 90 μmol) were added to pyridine (1.6 mL) and the mixture was stirred for 1 h at 65°C. Then, 132 μL (1.11 mmol) of benzoyl chloride (BzCl) was added and stirring was continued for 16 h at room temperature. The reaction mixture was subsequently diluted with EtOAc, and the resulting solution was washed successively with 2 N HCl, H_2O, and satd. aqueous $NaHCO_3$ solution. The organic layer was dried with Na_2SO_4, filtered, and the filtrate was concentrated. The resulting residue was purified by chromatography on a silica gel column (eluent: *n*-hexane/EtOAc, 6:1) to afford **2** (121.8 mg, 79% yield) as an amorphous powder, m.p. 44−45°C, $[\alpha]_D$ = −4.3 (*c* = 1.0 in $CHCl_3$). − Positive FAB MS: *m/z* = 1706 $[M + Na]^+$. − ^{1}H NMR ($CDCl_3$): δ = 8.05−7.05 (m, 45 H, aromatic H), 6.88 (d, *J* = 8.7 Hz, 1 H, NH), 5.62 (t, *J* = 9.7 Hz, 1 H, Glc 3-H), 5.58 (t, *J* = 5.6 Hz, 1 H, Cer 3-H), 5.46 (m, 1 H, Cer 4-H), 5.42 (t, *J* = 9.7 Hz, 1 H, Glc 4-H), 5.25 (q, *J* = 9.7, 7.9 Hz, 1 H, Glc 2-H), 5.17 (q, *J* = 6.6, 5.4 Hz, 1 H, Cer 2′-H), 4.69 (m, 1 H, Cer 2-H), 4.66 (d, *J* = 7.9 Hz, 1 H, Glc 1-H), 4.10 (q, *J* = 10.5, 5.4 Hz, 1 H, Cer 1-H), 3.85 (q, *J* = 10.5, 4.4 Hz, 1 H, Cer 1-H), 3.59 (m, 1 H, Glc 5-H), 3.21 (q, *J* = 10.9, 2.8 Hz, 1 H, Glc 6-H), 3.07 (q, *J* = 10.9, 4.8 Hz, 1 H, Glc 6-H), 0.88 (t, *J* = 6.9 Hz, 6 H, 2 CH_3). − $C_{107}H_{129}NO_{16}$ (1685.1): calcd. C 76.26, H 7.72, N 0.83; found C 76.47, H 7.76, N 0.81.

(2*S*,3*S*,4*R*)-3,4-Di-*O*-benzoyl-2-[(2*R*)-2-benzyloxytetracosanoylamino]-1-*O*-[β-D-(2,3,4-tri-*O*-benzoyl)glucopyranosyl]hexadecane-1,3,4-triol (3): To a solution of compound **2** (118.3 mg, 70.2 μmol) in MeOH (1.2 mL) and $CHCl_3$ (1.2 mL), *p*-toluenesulfonic acid (*p*TsOH, 10 mg, 53 μmol) was added and the mixture was stirred for 1.5 h at room temperature. The reaction mixture was then diluted with satd. aqueous $NaHCO_3$ solution, extracted with $CHCl_3$, and the combined organic extracts were dried with Na_2SO_4. After filtration and evaporation of the solvent, the residue obtained was purified by chromatography on a silica gel column (eluent: *n*-hexane/EtOAc, 3:1) to give **3** (79.6 mg, 79% yield) as an amorphous powder, m.p. 50−51°C, $[\alpha]_D$ = −0.6 (*c* = 1.0 in $CHCl_3$). − Positive FAB MS: *m/z* = 1464 $[M + Na]^+$. − ^{1}H NMR ($CDCl_3$): δ = 8.15−7.27 (m, 30 H, aromatic H), 7.20 (d, *J* = 9.2 Hz, 1 H, NH), 5.75 (t, *J* = 9.6 Hz, 1 H, Glc 3-H), 5.68 (m, 1 H, Cer 3-H), 5.60 (m, 1 H, Cer 4-H), 5.34 (t, *J* = 9.6 Hz, 1 H, Glc 4-H), 5.33 (q, *J* = 9.6, 7.8 Hz, 1 H, Glc 2-H), 5.34 (m, 1 H, Cer 2′-H), 4.74 (m, 1 H, Cer 2-H), 4.71 (d, *J* = 7.8 Hz, 1 H, Glc 1-H), 3.85 (q, *J* = 11.0, 6.0 Hz, 1 H, Cer 1-H), 3.79 (q, *J* = 11.0, 3.2 Hz, 1 H, Cer 1-H), 3.70 (m, 1 H, Glc 5-H), 3.51 (m, 1 H, Glc 6-H), 3.33 (m, 1 H, Glc 6-H), 0.88 (t, *J* = 6.6 Hz, 6 H, 2 CH_3). − $C_{88}H_{115}NO_{16}$ (1442.8): calcd. C 73.25, H 8.03, N 0.97; found C 73.29, H 8.09, N 0.94.

(2*S*,3*S*,4*R*)-1-*O*-[β-D-(6-*O*-Acetyl-2,3,4-tri-*O*-benzoyl)glucopyranosyl]-3,4-di-*O*-benzoyl-2-[(2*R*)-2-benzyloxytetracosanoylamino]-hexadecane-1,3,4-triol (4): 13.6 mg (9.4 μmol) of compound **3** was stirred with pyridine (0.5 mL) and acetic anhydride (Ac_2O, 0.5 mL) for 1.5 h at room temperature. The reaction mixture was then diluted with EtOAc, washed successively with 2 N HCl, H_2O, and satd. aqueous $NaHCO_3$ solution, and the organic layer was dried with Na_2SO_4. After filtration and evaporation of the solvent, the residue obtained was purified by chromatography on a silica gel column (eluent: *n*-hexane/EtOAc, 4:1) to afford **4** (9.1 mg, 65% yield) as an amorphous powder. − ^{1}H NMR ($CDCl_3$): δ =

8.16−7.23 (m, 30 H, aromatic H), 7.08 (d, *J* = 9.3 Hz, 1 H, NH), 5.71 (t, *J* = 9.9 Hz, 1 H, Glc 3-H), 5.69 (q, *J* = 7.3, 4.0 Hz, 1 H, Cer 3-H), 5.41 (t, *J* = 9.9 Hz, 1 H, Glc 4-H), 5.43 (m, 1 H, Cer 4-H), 5.27 (m, 1 H, Cer 2′-H), 5.19 (q, *J* = 9.9, 7.9 Hz, 1 H, Glc 2-H), 4.68 (d, *J* = 7.9 Hz, 1 H, Glc 1-H), 4.68 (m, 1 H, Cer 2-H), 4.04 (m, 2 H, Glc 6-H_2), 3.94 (q, *J* = 10.9, 4.3 Hz, 1 H, Cer 1-H), 3.83 (m, 2 H, Glc 5-H and Cer 1-H), 1.91 (s, 3 H, CH_3CO), 0.88 (t, *J* = 6.6 Hz, 6 H, 2 CH_3).

Nonulopyranoside Methyl Ester 5: See ref.[4]

α-Sialoside 6: A mixture of **3** (74.2 mg, 51.4 μmol), **5** (80.7 mg, 128.6 μmol) and powdered 4-Å molecular sieves (117 mg) in dry EtCN (0.4 mL) and dry CH_2Cl_2 (0.2 mL) was stirred for 3 h at room temperature, and then cooled to −40°C. To the cooled mixture was added, under stirring, *N*-iodosuccinimide (NIS, 43.0 mg, 192.9 μmol) and trifluoromethanesulfonic acid (TfOH, 3 μL), and stirring was continued for a further 2 h at −40°C. The mixture was then filtered, the collected solid was washed with CH_2Cl_2, and the combined filtrate and washings were successively washed with 5% aqueous $Na_2S_2O_3$ and satd. aqueous $NaHCO_3$ solutions. The organic layer was dried with Na_2SO_4, filtered, and the filtrate was concentrated. The residue was separated by preparative TLC on silica gel (solvent system: $CHCl_3$/acetone, 9:1) to give **6** as an amorphous powder (32.6 mg, 31% yield), m.p. 54−55°C, $[\alpha]_D$ = −3.0 (*c* = 1.0 in $CHCl_3$). − Positive FAB MS: *m/z* = 2043 $[M + Na]^+$. − ^{1}H NMR ($CDCl_3$): δ = 8.11−7.27 (m, 35 H, aromatic H), 6.98 (d, *J* = 8.9 Hz, 1 H, Cer NH), 6.21 (d, *J* = 10.1 Hz, 1 H, NA NH), 5.61 (t, *J* = 9.6 Hz, 1 H, Glc 3-H), 5.55 (q, *J* = 6.6, 5.5 Hz, 1 H, Cer 3-H), 5.54 (t, *J* = 9.6 Hz, 1 H, Glc 4-H), 5.42 (m, 1 H, Cer 4-H), 5.24 (q, *J* = 6.9, 5.0 Hz, 1 H, Cer 2′-H), 5.21−5.17 (m, 2 H, Glc 2-H and NA 7-H), 5.07 (octet, *J* = 9.2, 4.6, 2.8 Hz, 1 H, NA 8-H), 4.77 (m, 1 H, NA 4-H), 4.64 (m, 1 H, Cer 2-H), 4.61 (d, *J* = 8.0 Hz, 1 H, Glc 1-H), 4.56, 4.52 (each d, *J* = 11.7 Hz, 2 H, $PhCH_2O$), 4.03−3.96 (m, 3 H, Cer 1-H, NA 5-H, 6-H), 3.93 (q, *J* = 12.4, 2.5 Hz, 1 H, NA 9-H), 3.83 (m, 1 H, Glc 6-H), 3.86, 3.80 (each d, *J* = 12.8 Hz, 2 H, NA 11-H_2), 3.74 (q, *J* = 11.2, 5.7 Hz, 1 H, Cer 1-H), 3.73 (s, 3 H, $COOCH_3$), 3.68 (m, 1 H, Glc 5-H), 3.63 (q, *J* = 12.6, 4.8 Hz, NA 9-H), 3.52 (q, *J* = 11.2, 3.2 Hz, 1 H, Glc 6-H), 2.48 (q, *J* = 12.8, 4.6 Hz, 1 H, NA 3-H_{eq}), 2.07, 1.95, 1.95, 1.95 (each s, 12 H, 4 CH_3CO), 0.87 (t, *J* = 6.6 Hz, 6 H, 2 CH_3). − $C_{115}H_{148}N_2O_{29}$ (2022.4): calcd. C 68.30, H 7.38, N 1.39; found C 67.95, H 7.38, N 1.39.

Ganglioside Analogue 7: A solution of **6** (27.6 mg, 13.7 μmol) in EtOH (2 mL) was hydrogenated in the presence of 10% Pd/C (20 mg) for 21 h at room temperature, then filtered and concentrated. The residue was dissolved in 0.25 M NaOMe/MeOH (2 mL) and the resulting solution was stirred for 15 min at room temperature. Then, H_2O (1 mL) was added to the mixture, and stirring was continued for a further 1 h. The solution was subsequently treated with Dowex-50 (H^+) resin to remove the base, and then concentrated in vacuo. Column chromatography of the residue on Sephadex LH-20 (eluent: $CHCl_3$/MeOH/H_2O, 5:5:1) gave **7** as an amorphous powder (14.3 mg, 93% yield), m.p. 159−160°C, $[\alpha]_D$ = −11.7 (*c* = 0.3 in C_5H_5N). − Negative FAB MS: *m/z* = 1123 $[M - H]^-$. − ^{1}H NMR (C_5D_5N): δ = 4.68 (m, 2 H, Cer 1-H and NA 8-H), 4.56 (m, 1 H, Cer 1-H), 4.38 (m, 2 H, Glc 6-H_2), 4.23 (m, 2 H, Glc 3-H and 4-H), 4.12 (m, 1 H, Glc 2-H), 3.88 (m, 1 H, Glc 5-H), 3.65 (m, 1 H, NA 3-H), 2.17 (m, 1 H, NA 3-H), 0.84 (t, *J* = 6.6 Hz, 6 H, 2 CH_3); no signals due to aromatic H, methyl ester or acetyl groups were observed. − $C_{57}H_{108}N_2O_{19}\cdot 5H_2O$ (1215.5): calcd. C 56.32, H 9.79, N 2.30; found C 56.25, H 8.84, N 2.36.

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