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Synthesis of a Ganglioside GM_3 Analog Containing a Hydroxymethyl Group in Place of the Carboxyl Group in the *N*-Acetylneuraminic Acid Unit†

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A ganglioside GM_3 analog containing a hydroxymethyl group in the place of the carboxyl group in the Neu5Ac unit was synthesized, in order to investigate the relationship between the structure of sialic acid and the functions of gangliosides.

Sialic acids are well known as important constituents of cell-surface glycoproteins and glycolipids, and are involved in their various biological functions.[2-7] In consequence, there has been a great deal of research work done in recent years, not only on the syntheses[8-12] of the derivatives and analogs of *N*-acetylneuraminic acid, but also on the glycosphingolipids containing modified sialic acids. It is of interest to investigate the relationship between the structure of sialic acid and the functions of gangliosides. As part of a program to elucidate the functions of sialic acid containing glycolipids at the molecular level, we describe here the synthesis of a ganglioside GM_3 analog carrying a hydroxymethyl group in place of the carboxyl group in the *N*-acetylneuraminic acid unit to learn the role of the carboxyl group in the functions of GM_3. To synthesize the desired ganglioside GM_3 analog, we chose as the starting material 2-(trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→3)-*O*-(6-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,6-di-*O*-benzoyl-β-D-glucopyranoside (**1**), which was prepared according to our previous report.[14]

Treatment of **1** with sodium borohydride in methanol and subsequent *O*-acetylation gave compound **2** in 83% yield. When treated[15] with boron trifluoride etherate ($BF_3 \cdot OEt_2$) in dichloromethane for 7 hr at 0°C, **2** gave 1-hydroxy compound **3** in 73% yield. Treatment of **3** with trichloroacetonitrile[16,17] in the presence of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) for 2 hr at 0°C afforded trichloroacetimidate **4** as the α anomer in 81% yield after column chromatography. Glycosylation of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[18,19] (**5**) by **4** in the presence of $BF_3 \cdot OEt_2$[16,18] for 4.5 hr at 0°C yielded only expected β-glycoside **6** in 64% yield. Significant signals in the ^{1}H-NMR spectrum of **6** were a one-proton doublet at δ4.51 ($J_{1,2}$=7.7 Hz, H-1) and a one-proton doublet of doublets at δ4.92 ($J_{1,2}$=7.7 Hz, $J_{2,3}$=9.2 Hz, H-2), showing the newly formed β-glycosidic linkage. Other ^{1}H-NMR data are consistent with structure **6**. Selective reduction[18,20] of the azide group in compound **6** with H_2S in 5:1 pyridine–water gave the amine, which, on condensation with octadecanoic acid using 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride (WSC) in dichloromethane, gave the protected ganglioside GM_3 analog **7** in 84% yield. Finally, *O*-deacylation of **7** with sodium methoxide in methanol yielded almost quantitatively expected ganglioside GM_3 analog **8**. Influenza virus A (Aichi 2/68) neuraminidase did not hydrolize[21] the sialic acid analog on **8** unit, indicating that the carboxyl group in Neu5Ac is critically important for recognizing between the sialidase and GM_3.

Experimental

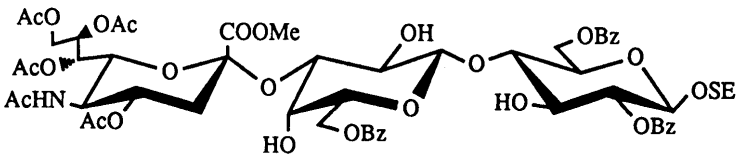
Specific rotation was determined with a Union PM-201 polarimeter, and IR spectra were recorded with a Jasco-A-100 spectrophotometer at 25°C. ^{1}H-NMR spectra were recorded with a Jeol JNM-GX270 spectrometer, using tetramethylsilane as an internal standard. Preparative chromatography on silica gel (Wako Co., 200 mesh) was accomplished with the solvent systems specified. concentration and evaporation procedures were conducted *in vacuo*.

2-(Trimethylsilyl)ethyl O-(5-acetamido-1,4,7,8,9-penta-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)-(2→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**2**). To a solution of **1**[14] (250 mg, 0.2 mmol) in methanol (25 ml) was added sodium borohydride (750 mg, 19.8 mmol), and the mixture was stirred for 5 hr at room temperature. After completing the reaction, acetic acid (2 ml) was added to the mixture, and the solution was concentrated. Acetylation of the residue with acetic anhydride (10 ml) in pyridine (15 ml) for 15 hr at room temperature gave compound **2** (200 mg, 83%) as an amorphous mass, after column chromatography (80:1 dichloromethane-methanol) on silica gel (60 g); $[\alpha]_D$ +8.0° (*c* 0.9, chloroform); IR ν_{max} (KBr) cm^{-1}: 3400 (NH), 1750 (ester), 1670 and 1540 (amide), and 860 and 840 (Me_3Si). NMR ($CDCl_3$): lactose unit δ0.90 (m, 2H, $Me_3SiC\underline{H}_2CH_2$), 3.57 (m, 1H, $Me_3SiCH_2C\underline{H}_2$), 4.45 (dd, 1H, $J_{2',3'}$=9.5 Hz, $J_{3',4'}$=3.3 Hz, H-3), 4.47 (d, 1H, $J_{1,2}$=8.1 Hz, H-1), 4.57 (d, 1H, $J_{1',2'}$=7.7 Hz, H-1'), 4.87 (dd, 1H, $J_{2,3}$=9.5 Hz, H-2), 5.00 (dd, 1H, H-2'), 5.17 (t, 1H, $J_{2,3}$=$J_{3,4}$=9.5 Hz, H-3), and 5.31 (broad d, 1H, H-4'); sialic acid analog unit δ1.86 (s, 3H, AcN), 3.86 (dd, 1H, $J_{5,6}$=10.6 Hz, $J_{6,7}$=2.2 Hz, H6), 5.00 (m, 1H, H-4), 5.19 (m, 1H, H-8), 5.37 (dd, 1H, $J_{7,8}$=5.8 Hz, H-7), and 5.63 (d, 1H, NH); *O*-acetyl groups δ2.00, 2.02 (2), 2.04, 2.05, 2.06, 2.08, 2.09, 2.10, 2.12, and 2.20 (11s, 33H, 11AcO).

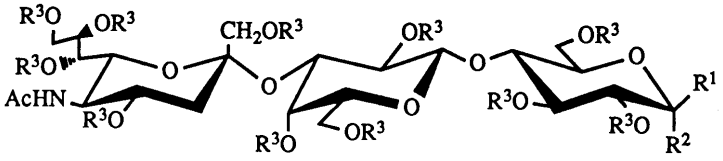
Anal. Found: C, 50.61; H, 6.45; N, 1.15. Calcd. for $C_{50}H_{75}NO_{29}Si$ (1182.2): C, 50.79; H, 6.39; N, 1.18%.

O-(5-Acetamido-1,4,7,8,9-penta-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyl)-(2→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-D-glucopyranose (**3**). To a solution of **2** (180 mg, 0.15 mmol) in dichloromethane (5 ml) cooled to 0°C was added $BF_3 \cdot OEt_2$ (0.2 ml), and the mixture was stirred for 6 hr at 0°C; the course of the reaction was monitored by TLC. Dichloromethane (100 ml) was then added to the mixture, and the solution was successively washed with M Na_2CO_3 and water, dried (Na_2SO_4) and evaporated. Column chromatography (40:1 dichloromethane–methanol) of the residue on silica gel (30 g) gave **3** (120 mg, 73%) as an amorphous mass; $[\alpha]_D$

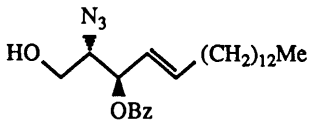
† Synthetic Studies on Sialoglycoconjugates. Part 32. For Part 31, see ref. 1.



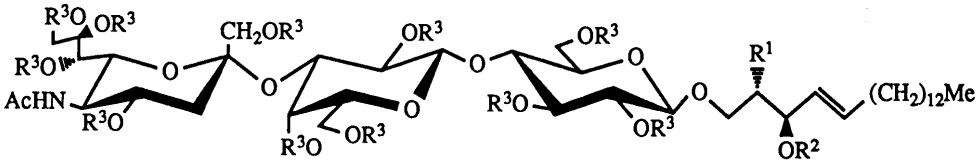
1



2 R^1 = OSE, R^2 = H, R^3 = Ac
3 R^1, R^2 = H, OH, R^3 = Ac
4 R^1 = H, R^2 = OC(=NH)CCl_3



5



6 R^1 = N_3, R^2 = Bz, R^3 = Ac
7 R^1 = $NHCO(CH_2)_{16}Me$, R^2 = Bz, R^3 = Ac
8 R^1 = $NHCO(CH_2)_{16}Me$, R^2 = R^3 = H

SE = 2-(trimethylsilyl)ethyl
Bz = benzoyl

+50.8° (*c* 1.9, chloroform); IR ν_{max} (KBr) cm^{-1}: 3600—3300 (OH, NH), 1730 (ester), 1650 and 1530 (amide).

Anal. Found: C, 49.79; H, 5.78; N, 1.33. Calcd. for $C_{45}H_{63}NO_{29}$ (1082.0): C, 49.95; H, 5.87; N, 1.29%.

*O-(5-Acetamido-1,4,7,8,9-penta-O-acetyl-3,5-dideoxy-*D*-glycero-*α*-*D*-galacto-2-nonulopyranosyl)-(2→3)-O-(2,4,6-tri-O-acetyl-*β*-*D*-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-*α*-*D*-glucopyranosyl trichloroacetimidate* (**4**). To a solution of **3** (90 mg, 0.08 mmol) in dichloromethane (1.5 ml) cooled to 0°C were added trichloroacetonitrile (0.3 ml) and 1.8-diazabicyclo[5,4,0]undec-7-ene (DBU; 10 mg). The mixture was stirred for 2 hr at 0°C and then concentrated. Column chromatography (55:1 dichloromethane–methanol) of the residue on silica gel (30 g) afforded **4** (83 mg, 81%) as an amorphous mass; $[\alpha]_D$ +55.0° (*c* 1.2, chloroform). NMR ($CDCl_3$): lactose unit δ 4.60 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-1'), 4.90 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 5.03 (dd, 1H, $J_{2',3'}$ = 10.3 Hz, H-2'), 5.32 (broad d, 1H, H-4), 5.55 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, H-3), 6.48 (d, 1H, H-1), and 8.66 (s, 1H, C=NH); sialic acid analog unit δ 1.87 (s, 3H, AcN), 5.02 (m, 1H, H-4), 5.25 (m, 1H, H-8), 5.37 (m, 1H, H-7), and 5.62 (d, 1H, $J_{5,NH}$ = 9.9 Hz, H-5); *O*-acetyl groups δ 2.00, 2.01, 2.03, 2.05, 2.06 (2), 2.07, 2.10 (2), 2.13, 2.21 (11s, 33H, 11AcO).

Anal. Found: C, 46.11; H, 5.26; N, 2.24. Calcd. for $C_{47}H_{63}N_2O_{29}Cl_3$ (1226.4): C, 46.03; H, 5.18; N, 2.28%.

*O-(5-Acetamido-1,4,7,8,9-penta-O-acetyl-3,5-dideoxy-*D*-glycero-*α*-*D*-galacto-2-nonulopyranosyl)-(2→3)-O-(2,4,6-tri-O-acetyl-*β*-*D*-galactopyranosyl)-(1→4)-O-(2,3,6-tri-O-acetyl-*β*-*D*-glucopyranosyl-(1→1)-(2S,3R,4E)-2-azido 3-O-benzoyl-4-octadecene-1,3-diol* (**6**). To a solution of **4** (32.5 mg, 0.03 mmol) and (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol[18] (**5**; 23 mg, 0.05 mmol) in dry dichloromethane (2 ml) was added 4 A° molecular sieves (type AW 300; 2.0 g), and the mixture was stirred for 30 min at room temperature, before being cooled to 0°C. Boron trifluoride etherate (0.04 ml) was added to the mixture, and this was stirred for 4 hr at 0°C. The precipitate was filtered off and washed thoroughly with dichloromethane. The solution was washed with M Na_2CO_3 and water, dried (Na_2SO_4) and evaporated to syrup. This syrup

was chromatographed on a column of silica gel (20 g) with 60:1 dichloromethane–methanol to give **6** (25.5 mg, 64%) as an amorphous mass; $[\alpha]_D$ +11.5° (*c* 0.25, chloroform); IR ν_{max} (KBr) cm^{-1}: 3400 (NH), 2940 and 2850 (Me, methylene), 2100 (N_3), 1730 (ester), 1670 and 1520 (amide), and 710 (Ph). NMR ($CDCl_3$): lactose unit δ 4.45 (dd, 1H, $J_{2',3'}$ = 9.9 Hz, $J_{3',4'}$ = 3.0 Hz, H-3'), 4.51 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.58 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-1'), 4.92 (dd, 1H, $J_{2,3}$ = 9.2 Hz, H-2), 5.00 (dd, 1H, H-2'), 5.18 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3), and 5.31 (broad d, 1H, H-4'); sialic acid analog unit δ 1.87 (s, 3H, AcN), 4.97 (m, 1H, H-4), 5.19 (m, 1H, H-8), 5.37 (dd, 1H, $J_{6,7}$ = 2.2 Hz, $J_{7,8}$ = 4.5 Hz, H-7), and 5.45 (d, 1H, $J_{5,NH}$ = 9.5 Hz, NH); sphingosine unit δ 0.87 (t, 3H, J_{Me,CH_2} = 7.2 Hz, $\underline{Me}CH_2$), 1.24 (s, 22H, $11CH_2$), and 5.94 (dt, 1H, $J_{4,5}$ = 15.0 Hz, $J_{5,6}$ = $J_{5,6'}$ = 6.6 Hz, H-5); *O*-acyl groups δ 2.00 (2), 2.05 (2), 2.07 (2), 2.09 (2), 2.10 (2), 2.19 (11s, 33H, 11AcO), and 7.42—8.06 (m, 5H, Ph).

Anal. Found: C, 56.18; H, 6.84; N, 3.71. Calcd. for $C_{70}H_{100}N_4O_{31}$ (1493.6): C, 56.29; H, 6.75; N, 3.75%.

*O-(5-Acetamido-1,4,7,8,9-penta-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosyl)-(2→3)-O-(2,4,6-tri-O-acetyl-β-*D*-galactopyranosyl)-(1→4)-O-(2,3,6-tri-O-acetyl-β-*D*-glucopyranosyl)-(1→1)-(2S,3R,4E)-3-O-benzoyl-2-octadecanamido-4-octadecene-1,3-diol* (**7**). Hydrogen sulfide was bubbled through a solution of **6** (20 mg, 0.01 mmol) in pyridine (2.5 ml) and water (0.5 ml) for 36 hr while the solution was stirred at room temperature, the course of the reaction being monitored by TLC. The mixture was concentrated and dissolved in dichloromethane (6 ml). To the stirred solution was added octadecanoic acid (10 mg, 0.035 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC; 15 mg), and the mixture was stirred overnight at room temperature and then concentrated. Column chromatography (65:1 dichloromethane–methanol) of the residue on silica gel (20 g) afforded **7** (19.5 mg, 84%) as an amorphous mass; $[\alpha]_D$ +23.5° (*c* 0.4, chloroform). NMR ($CDCl_3$): lactose unit δ 4.36 (dd, 1H, $J_{2',3'}$ = 10.3 Hz, $J_{3',4'}$ = 3.0 Hz, H-3'), 4.45 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 4.54 (d, 1H, $J_{1',2'}$ = 7.7 Hz, H-1'), 4.89 (dd, 1H, $J_{2,3}$ = 9.2 Hz, H-2), 4.98 (dd, 1H, H-2'), 5.16 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.2 Hz, H-3), and 5.31 (broad d, 1H, H-4'); sialic acid analog unit δ 1.87 (s, 3H, AcN), 4.95 (m, 1H, H-4), 5.18 (m, 1H, H-8), 5.36 (dd, 1H, $J_{6,7}$ = 2.2 Hz, $J_{7,8}$ = 4.4 Hz, H-7), and 5.66 (d, 1H, $J_{5,NH}$ = 9.9 Hz, NH); Cer unit δ 0.88 (near t, 6H, $2\underline{Me}CH_2$), 1.25 (s, 50H, $25CH_2$), 5.84 (dt, 1H, $J_{4,5}$ = 14.5 Hz, $J_{5,6}$ = $J_{5,6'}$ = 6.6 Hz, H-5); *O*-acyl groups δ 1.89, 2.00, 2.02, 2.04, 2.06, 2.08, 2.09 (2), 2.10, 2.11, 2.16 (11s, 33H, 11AcO), and 7.41—8.02 (m, 5H, Ph).

Anal. Found: C, 60.79; H, 7.84; N, 1.58. Calcd. for $C_{88}H_{135}N_2O_{32}$ (1733.0): C, 60.99; H, 7.85; N, 1.62%.

*O-(5-Acetamido-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosyl)-(2→3)-O-β-*D*-galactopyranosyl-(1→4)-O-β-*D*-glucopyranosyl-(1→1)-(2S,3R,4E)-2-octadecanamido-4-octadecene-1,3-diol* (**8**). To a solution of **7** (18.5 mg, 0.047 mmol) in methanol (2 ml) was added sodium methoxide (10 mg), and the mixture was stirred for 8 hr at room temperature. Water (0.5 ml) was added to the mixture, which was then neutralized with Amberlite IR-120 (H^+) resin, and filtered. The resin was washed with methanol, and the combined filtrate and washings were concentrated. Column chromatography of the residue on Sephadex LH-20 (30 g) with methanol yielded **8** (12 mg, 95%) as an amorphous mass; $[\alpha]_D$ +4.0° (*c* 0.3, 1:1 chloroform–methanol); IR ν_{max} (KBr) cm^{-1}: 3700—3100 (OH, NH), 2930 and 2850 (Me, methylene), and 1640 and 1550 (amide). NMR (1:1 $CDCl_3$–CD_3OD): lactose unit δ 4.19 (dd, 1H, $J_{2',3'}$ = 9.5 Hz, $J_{3',4'}$ = 3.7 Hz, H-3'), 4.30 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), and 4.43 (d, 1H, $J_{1',2'}$ = 7.7 Hz, H-1'); sialic acid analog unit δ 2.00 (s, 3H, AcN); Cer unit δ 0.89 (near t; 6H, $2\underline{Me}CH_2$), 1.28 (s, 50H, $25CH_2$), 5.45 (dd, 1H, $J_{3,4}$ = 7.2 Hz, $J_{4,5}$ = 15.5 Hz, H-4), and 5.68 (dt, 1H, $J_{5,6}$ = $J_{5,6'}$ = 6.6 Hz, H-5).

Anal. Found: C, 60.51; H, 9.73; N, 3.28. Calcd. for $C_{59}H_{110}N_2O_{20}$ (1167.5): C, 60.69; H, 9.50; N, 2.40%.

References

1) A. Hasegawa, K. Adachi, M. Yoshida, and M. Kiso, *J. Carbohydr. Chem.*, in press.
2) S. E. Goelz, C. Hession, D. Goff, B. Griffiths, R. Tizard, B. Newman, G. Chi-Rosso, and R. Lobb, *Cell*, **63**, 1349—1356 (1990).
3) J. B. Lowe, L. M. Stoolman, R. P. Nair, R. D. Larsen, T. L. Berhand, and R. M. Marks, *Cell*, **63**, 475—484 (1990).
4) M. L. Phillips, E. Nudelman, F. C. A. Gaeta, M. Perez, A. K. Singhal, S. Hakomori, and J. C. P. Paulson, *Science*, **250**, 1130—1132 (1990).
5) M. Tiemeyer, S. J. Swiedler, M. Ishihara, M. Moreland, H. Schweingrber, P. Hirtzer, and B. K. Brandley, *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 1138—1142 (1991).
6) S. Tsuji, T. Yamakawa, M. Tanaka, and Y. Nagai, *J. Neurochem.*, **50**, 414—423 (1988).
7) H. Wiegandt (ed.), "Glycolipids, New Comprehensive Biochemistry," Vol. 10, Elsevier, Amsterdam, 1985, pp. 199—260.
8) E. Zbiral, E. Schreiner, R. Christian, R. G. Kleineiden, and R. Schauer, *Liebigs Ann. Chem.*, **1989**, 159—165 and references cited therein.
9) S. J. Danishefsky, M. P. DeNinno, and S. Chen, *J. Am. Chem. Soc.*, **110**, 3929—3940 (1988).
10) F. Baumberger and A. Vasella, *Helv. Chim. Acta*, **71**, 429—445 (1988).
11) T. Nakajima, H. Hori, H. Ohrui, H. Meguro, and T. Ido, *Agric. Biol. Chem.*, **52**, 1209—1215 (1988).
12) A. Hasegawa, T. Murase, M. Ogawa, H. Ishida, and M. Kiso, *J. Carbohydr. Chem.*, **9**, 429—439 (1990) and references cited therein.
13) A. Hasegawa, T. Murase, K. Adachi, M. Morita, H. Ishida, and M. Kiso, *J. Carbohydr. Chem.*, **9**, 181—199 (1990).
14) T. Murase, H. Ishida, M. Kiso, and A. Hasegawa, *Carbohydr. Res.*, **188**, 71—80 (1989).
15) K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, G. Magnusson, J. Dahmen, G. Noori, and K. Stenvall, *J. Org. Chem.*, **53**, 5629—5649 (1988).
16) R. R. Schmidt and G. Grundler, *Synthesis*, **1981**, 885—887 (1981).
17) M. Numata, M. Sugimoto, K. Koike, and T. Ogawa, *Carbohydr. Res.*, **163**, 209—222 (1987).
18) Y. Ito, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, **8**, 285—294 (1989).
19) R. R. Schmidt and P. Zimmermann, *Angew. Chem., Int. Ed. Engl.*, **25**, 725—726 (1986).
20) T. Adachi, Y. Yamada, I. Inoue, and M. Saneyoshi, *Synthesis*, **1977**, 45—46.
21) Y. Suzuki *et al.*, unpublished data.