An Unexpected Sialylation: Total Syntheses of G_{M4} and a Positional Isomer

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The use of glycals 3 and 4 as precursors to 1,2-anhydrosugars greatly simplifies the installation of a β-linked ceramide glycoside. By permuting the introduction of the ceramide and the sialic acid, one can access the ganglioside G_{M4} (1) and, by an unexpected regioselective glycosylation, its NeuAc (2→2) Gal positional isomer 10.

Gangliosides are cell surface glycolipids containing at least one sialic acid residue. They are anchored into the cell membrane via a ceramide unit. The "ceramide" section of gangliosides obtained by isolation is not a homogeneous moiety but is composed of closely related compounds varying in homologous chain lengths which also incorporate different levels of unsaturation. This microheterogeneity seriously complicates isolation of gangliosides as truly single entities.

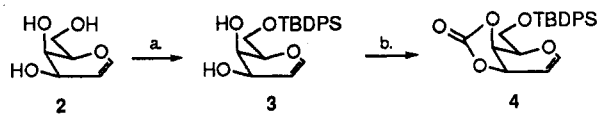
Structurally, G_{M4} (1) is the simplest of the gangliosides. It has been isolated as a minor component from brain, rat kidney, mouse erythrocytes, and chicken egg yolk.[1] It has been shown to prevent experimental allergic encephalomyelitis in guinea pigs.[2] In addition, it has been shown to possess marked immunosuppressive activity in vitro.[3] Because of its interesting biological activities and its very low availability from natural sources, the study of an efficient route for the preparation of 1 seemed to be particularly appropriate.[4]

The most challenging considerations in designing a synthesis of G_{M4} are regio- and stereoselective sialic acid incorporation and β-stereoselectivity in the introduction of the ceramide. In the past, this goal had been achieved by participation of a properly selected C-2 protecting group. Such strategies required that the C-2 hydroxyl function be differentiated from the C-6 and/or the C-3 and C-4 hydroxyls and required recourse to a number of protection/deprotection steps. A series of reports from these laboratories has delineated a route to β-linked saccharides which involves Lewis acid-catalyzed opening of 1,2-anhydrosugars by suitable glycosyl acceptors.[5,6] The anhydrosugars are in turn generated by direct epoxidation of glycals using dimethyl dioxirane.[7] This method obviates the need for a unique directing group at C-2. By implementing this strategy on a suitably protected galactal derivative, we hoped to develop a concise synthesis of G_{M4}.

Galactal derivative 4 seemed to be a possible precursor to G_{M4}.[8] It was envisioned that epoxidation and introduction of a ceramide synthon would be followed by removal of the cyclic carbonate providing a triol ready for coupling with a sialic acid donor. Appropriate functional group manipulations followed by deprotection would provide G_{M4}.

Reaction of D-galactal (2) with *tert*-butyldiphenylsilyl chloride provided the monoprotected derivative 3 in 90% yield (Scheme I). Treatment of 3 with carbonyldiimidazole

Scheme I[a]



2 3 4

[a] (a) 1.1 equiv of TBDPSCl, 3.0 equiv of TEA, DMF, rt, 3 h, 90%; (b) 1.2 equiv of $CO(imid)_2$, THF, rt, 12 h, 74%.

afforded the cyclic carbonate 4 in 74% yield. Epoxidation of 4 generated the 1α,2α-anhydrosugar which upon reaction with the pre-ceramide synthon 5[9] in the presence of zinc chloride gave the β-glycoside 6 in 62% yield (Scheme II). The product arising from addition of the C-3 hydroxyl of 5 was only a minor component. Compound 6 was readied for sialylation by removal of the carbonate functionality with catalytic sodium methoxide in methanol providing tetraol 7 in quantitative yield.

Galactosyl triols similar to 7, but with different blocking groups at C-6 and smaller groups at the anomeric carbon, have been reported to react with sialic acid donors specifically at the O-3′ position.[10] *Contrary to these precedents, sialylation of 7 with the sialyl chloride 8*[11] *occurred at the O-2′ position providing the sialoside 9 in 48% yield.*[12] The regiochemical outcome was evident in that the COSY spectrum of the product showed the H-3′ and H-4′ coupled to hydroxyl protons. Additionally, upon acetylation of 9, H-4′ shifted from δ 4.00 to δ 5.78 and H-3′ shifted from δ 3.68 to δ 4.72 whereas H-2′ shifted from δ 4.21 to δ 4.30.

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(12) Ninety percent of the unreacted 5 was recovered from this reaction.

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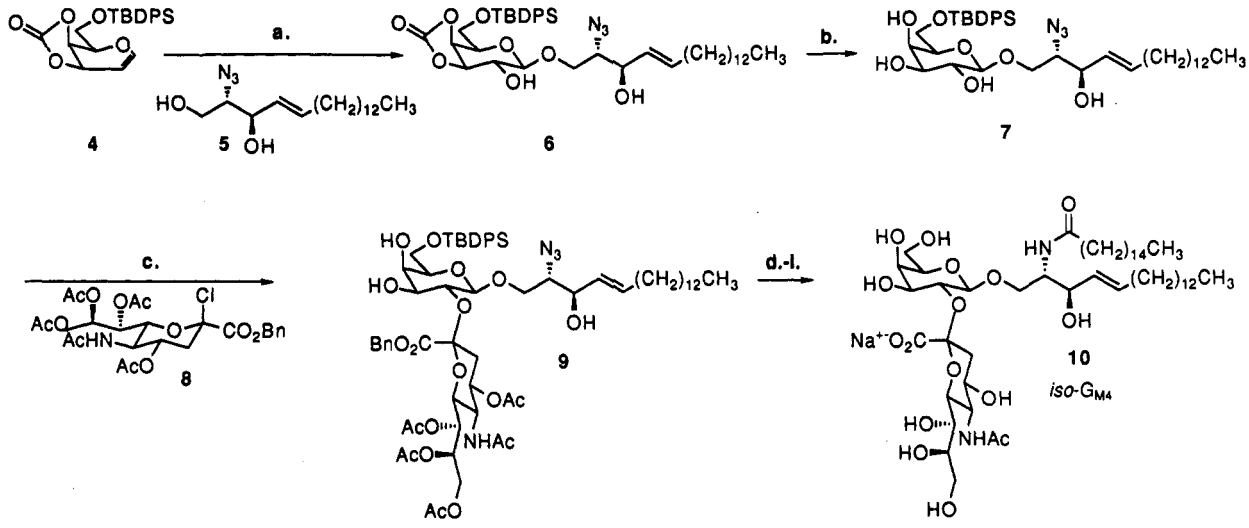
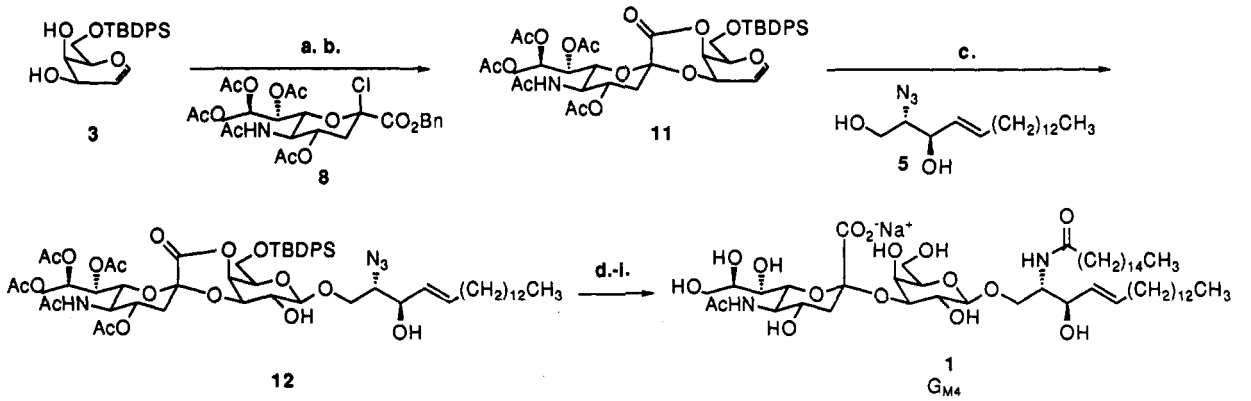
Scheme II[a]



[a] (a) 1.0 equiv of dimethyldioxirane and then 2.0 equiv of 5, 1.2 equiv of $ZnCl_2$, 62%; (b) catalytic NaOMe in MeOH, 100%; (c) 2.0 equiv of AgOTf, 2.0 equiv of 2,6-di-*tert*-butylpyridine, 2.0 equiv of 8, $CaSO_4$, THF, −78 °C → −10 °C, 48%; (d) 20 equiv of BzCl, 20 equiv of pyridine, catalytic DMAP, CH_2Cl_2, rt; (e) H_2S, pyridine/H_2O 5:1, rt, 24 h; (f) 3.0 equiv of EDCI, 1.5 equiv of palmitic acid, CH_2Cl_2, rt, 12 h; (g) 10.0 equiv of TBAF, THF, rt, 6 h; (h) catalytic NaOMe in MeOH and then H_2O; (i) LiChroprep RP-18 purification using 5% H_2O in MeOH as eluent, 42% for d-l.

Scheme III[a]



[a] (a) 2.0 equiv of AgOTf, 2.0 equiv of 2,6-di-*tert*-butylpyridine, 2.0 equiv of 8, $CaSO_4$, THF, −78 °C → −10 °C, 12 h; (b) 1.0 equiv of DBU, CH_2Cl_2, 0 °C, 55% for two steps; (c) 1.0 equiv of dimethyldioxirane, CH_2Cl_2, 0 °C and then 10.0 equiv of 5, 1.5 equiv of $ZnCl_2$, THF, 0 °C, 55%; (d) 5.0 equiv of 1,3 propanedithiol, 5.0 equiv of triethylamine, MeOH, rt, 48 h; (e) 3.0 equiv of EDCI, 1.5 equiv of palmitic acid, CH_2Cl_2, rt, 12 h; (f) 5.0 equiv of TBAF, THF, 0 °C → rt, 12 h; (g) catalytic NaOMe in MeOH and then H_2O, rt; (h) LiChroprep RP-18 purification using 5% H_2O in MeOH as eluent, 52% for d-h.

Compound 9 was converted into what we call "iso-G_{M4}". Benzoylation followed by azide reduction and acylation with palmitic acid in the presence of EDCI provided a mixture of products.[13] This mixture was directly reacted with TBAF followed by sodium methoxide/water hydrolysis to provide the sodium salt of iso-G_{M4} (10)which was purified on LiChroprep RP-18 resin.

While the reasons for the unexpected sialylation have not yet been sorted out, it was possible to revise our synthetic plan such as to lead to G_{M4}. Sialylation at the stage of 6-*O*-monoprotected galactal 3 would eliminate the possibility of reaction at O-2′ and would confine the regiochemical issue to selectivity between an allylic equatorial hydroxyl at C-3 and an axial hydroxyl at C-4. In addition, lactone 11 was expected to approximate the steric and electronic properties of 4 in terms of favoring β-glycoside formation.

In the event, reaction of 3 with sialyl chloride 8 generated an inseparable mixture of α and β 2→3 glycosylation products (Scheme III). No 2→4 product could be detected. Treatment of the mixture with DBU generated the desired lactone 11 which was purified in 55% overall yield. Epoxidation of 11 followed by treatment with azido diol 5 in the presence of zinc chloride provided β-glycoside 12 in 55% isolated yield.[14] Again, the product arising from addition of the pre-ceramide synthon at the C-3 hydroxyl was only a minor component. Reduction of the azide followed by acylation with palmitic acid in the presence of EDCI generated a mixture containing the expected product as well as products apparently arising from deacetylation and lactone opening. Processing of this mixture was accomplished using the following sequence. Treatment with TBAF effected removal of the C-6 protecting group. Hydrolysis with sodium methoxide in methanol followed by aqueous sodium hydroxide generated fully synthetic G_{M4} (1) which was purified on LiChroprep RP-18 resin in 52% overall yield. The NMR spectrum of

(13) A mixture of products arising from C3′ lactonization in addition to the expected product was obtained in this sequence.

(14) The α-linked glycosylation product was obtained in 8% yield.

this material closely resembled those of two G_{M4} homologs prepared by Hasegawa.[4b]

In conclusion, the stereoselective glycosylation of an undifferentiated ceramide precursor 3 by a glycal epoxide constitutes a major simplification in ganglioside synthesis in that it avoids the need for a multistep installation of a directing group at C-2. By permuting the timing of sialylation and ceramide introduction, this chemistry could be exploited to provide highly concise syntheses of G_{M4} (1) and, surprisingly, its 2→2 isomer 11. Further extensions of these findings will be the subject of future reports.

Experimental Section

1,5-Anhydro-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-D-*lyxo*-hex-1-enopyranose (3): D-Galactal (2.4 g, 16.4 mmol) was diluted in dimethylformamide with triethylamine (7 mL, 50 mmol), and *tert*-butyldiphenylsilyl chloride (4.7 mL, 18 mmol) was added. The reaction was stirred at room temperature for 3 h and then quenched by the addition of ethyl acetate and extraction with water. After concentration, the oil was subjected to flash chromatography using 3:1 hexanes/ethyl acetate as eluent to afford 5.7 g of 3 as a colorless oil (90% yield): $[\alpha]^{20}_D$ +1.59° (c = 2.1, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 7.71–7.65 (m, 4H), 7.44–7.35 (6, m), 6.35 (d, J = 6.2 Hz, 1H, H-1), 4.69 (dd, J = 6.2, 1.6 Hz, 1H, H-2), 4.34 (broad, 1H), 4.12 (broad, 1H), 3.99–3.87 (m, 3H), 2.99 (d, J = 5.2 Hz, 1H), 2.68 (d, J = 8.9 Hz, 1H), 1.07 (s, 9H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 144.4, 135.6, 135.5, 129.9, 127.8, 103.4, 76.1, 65.8, 64.5, 63.7, 26.8, 19.2; FTIR (neat) 3396, 3070, 3044, 2953, 2927, 2888, 2855, 1642, 1472, 1427, 1388, 1225, 1101, 1068, 1029, 742, 690 cm^{-1}; HRMS (FAB) calcd for $C_{22}H_{28}NaO_4Si$ (M + Na) 407.1655, found 407.1668.

1,5-Anhydro-6-*O*-(tert-butyldiphenylsilyl)-2-deoxy-D-*lyxo*-hex-1-enopyranose 3,4-carbonate (4): 6-*O*-(*tert*-butyldiphenylsilyl)galactal (3) (1.2 g, 3.12 mmol) was treated with carbonyldiimidazole (660 mg, 4.1 mmol) in dry tetrahydrofuran (THF) at 27 °C for 12 h. The solvent was removed *in vacuo* and the residue subjected to flash column chromatography using 3:1 hexanes/ethyl acetate as eluent. The desired product 4 was obtained as a clear oil in 74% yield: $[\alpha]^{20}_D$ -38.9° (c = 0.94, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 7.67–7.62 (m, 4H), 7.44–7.38 (m, 6H), 6.60 (d, J = 6.3 Hz, 1H, H-1), 5.17 (dd, J = 7.7, 3.1 Hz, 1H), 5.01 (d, J = 7.7 Hz, 1H), 4.92 (dd, J = 6.3, 3.1 Hz, 1H, H-2), 3.97–3.91 (m, 3H), 1.06 (s, m); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 153.9, 149.2, 135.5, 132.7, 130.0, 127.9, 98.1, 74.0, 72.9, 68.8, 61.9, 26.9, 19.3; IR (neat) 3072, 2952, 2929, 2884, 2850, 1796, 1648, 1472, 1426, 1232, 1147, 1113, 1005, 691 cm^{-1}; HRMS (FAB) calcd for $C_{23}H_{26}NaO_5Si$ (M + Na) 433.1448, found 433.1480.

***O*-[[6-*O*-(*tert*-Butyldiphenylsilyl)-3,4-oxocarbonyl-β-D-galactopyranosyl]-(1→1)]-(2*S*,3*R*,4*E*)-2-azido-4-octadecene-1,3-diol (6):** The galactal carbonate 4 (170 mg, 0.4 mmol) was diluted in 14 mL of dry dichloromethane and cooled to 0 °C. Dimethyldioxirane (0.07 M, 0.4 mmol) was added dropwise and the reaction warmed to room temperature. After 1 h the solvent was removed *in vacuo* leaving a white foam. The pre-ceramide synthon 5 (270 mg, 0.8 mmol) was added to the 1,2-anhydrosugar in 2 mL of THF. The reaction was cooled to -78 °C and $ZnCl_2$ (500 μL of 1 M solution in diethyl ether, 0.5 mmol) was added. The reaction was slowly warmed to room temperature and stirred overnight. The reaction was quenched by filtration through celite, concentrated, and subjected to flash chromatography using 2.5:1 hexanes/ethyl acetate as eluent to afford 192 mg (62% yield) of the pure product: $[\alpha]^{20}_D$ -22.1° (c = 1.12, $CHCl_3$); ^1H NMR (490 MHz, $CDCl_3$) δ 7.66–7.63 (4H, m), 7.45–7.37 (6H, m), 5.78 (dt, J = 15.0, 6.8 Hz, 1H), 5.47 (dd, J = 15.0, 7.3 Hz, 1H), 4.88 (dd, J = 7.6, 1.5 Hz, 1H, H-4 Gal), 4.69 (dd, J = 7.6, 5.6 Hz, 1H, H-3 Gal), 4.46 (d, J = 6.2 Hz, 1H, H-1 Gal), 4.21 (m, 1H, H-3 ceramide), 3.99 (dd, J = 10.5, 5.1 Hz, 1H), 3.95 (m, 1H), 3.90 (m, 2H), 3.79 (m, 1H), 3.63 (dd, J = 10.5, 3.8 Hz, 1H), 3.38 (m, 1H), 3.05 (1H, broad s), 2.34 (d J = 4.3, 1H), 2.02 (q, J = 7.3, 2H), 1.35–1.24 (22H), 1.05 (s, 9H), 0.87 (t, J = 6.4 Hz, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 154.1, 135.9, 135.5, 135.4, 132.6, 129.9, 127.9, 100.8, 77.4, 74.2, 71.9, 71.8, 70.7, 68.4, 64.6, 62.0, 32.3, 31.9, 29.7, 29.5, 29.3, 29.2, 28.9, 26.8, 22.7, 19.2, 14.1; FTIR (neat) 3402, 2923, 2854, 2094, 1795, 1166, 1110, 1079, 1023, 693 cm^{-1}; HRMS (FAB) calcd for $C_{41}H_{61}N_3NaO_8Si$ 774.4128, found 774.4083. Anal. Calcd for $C_{41}H_{61}N_3O_8Si$: C, 65.48; H, 8.18. Found: C, 65.71; H, 8.39.

***O*-[[6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranosyl]-(1→1)]-(2*S*,3*R*,4*E*)-2-azido-4-octadecene-1,3-diol (7):** The cyclic carbonate 6 (150 mg) was reacted with a catalytic amount of sodium methoxide in methanol to provide the tetraol 7 in quantitative yield after flash chromatography using 7:3 benzene/acetone: $[\alpha]^{20}_D$ -10.5° (c = 1.09, $CHCl_3$); ^1H NMR (490 MHz, $CDCl_3$) δ 7.68–7.64 (m, 4H), 7.40–7.36 (m, 6H), 5.77 (dt, J = 15.1, 6.8 Hz, 1H), 5.47 (dd, J = 15.1, 7.4 Hz, 1H), 4.21 (m, 2H), 4.03 (m, 2H), 3.89 (m, 2H), 3.67 (m, 2H), 3.54–3.44 (m, 3H), 2.02 (q, J = 7.4 Hz, 2H), 1.37–1.23 (m, 22H), 1.04 (s, 9H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 135.6, 135.5, 135.5, 133.0, 132.9, 129.8, 127.9, 127.7, 103.0, 74.7, 73.6, 72.2, 71.6, 68.7, 68.6, 65.0, 62.9, 32.4, 31.9, 29.7, 29.5, 29.3, 29.2, 28.9, 26.8, 22.7, 19.1, 14.1; FTIR (neat) 3400, 2954, 2918, 2851, 2093, 1458, 1256, 1104, 1073, 811, 694 cm^{-1}; HRMS (FAB) calcd for $C_{40}H_{63}N_3NaO_7Si$ 748.4336, found, 748.4318. Anal. Calcd for $C_{40}H_{63}N_3O7Si$: C, 66.17; H, 8.75. Found: C, 66.10; H, 8.72.

***O*-(Phenylmethyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→2)-*O*-[6-*O*-(*tert*-butyldiphenylsilyl)-β-D-galactopyranosyl]-(1→1)-(2*S*,3*R*,4*E*)-2-azido-4-octadecene-1,3-diol (9):** Galactosyl azido sphingosine 7 (210 mg, 0.28 mmol) was added to a flask containing AgOTf (72 mg, 0.28 mmol), 2,6-di-*tert*-butylpyridine (63 μL, 0.28 mmol), and $CaSO_4$ (70 mg) in 2 mL of dry THF and stirred for 30 min at room temperature under an argon atmosphere. The reaction mixture was cooled to -78 °C and a solution of the sialyl donor 8 (150 mg, 0.28 mmol) in 1 mL of THF was added dropwise over 1 h. After the addition was complete the reaction was warmed to -55 °C for 1 h. The reaction temperature was again cooled to -78 °C and AgOTf (72 mg, 0.28 mmol) and 2,6-di-*tert*-butyl pyridine (63 μL, 0.28 mmol) were added then 6 (150 mg, 0.28 mmol) in 1 mL of THF was added dropwise over 1 h. The temperature was warmed to -55 °C for 1 h then allowed to slowly warm to -10 °C overnight. The reaction was quenched by dilution with dichloromethane, filtration through Celite, concentration, and FCC using 7:3 benzene/acetone as eluent. The sialoglycoconjugate 9 was isolated as a pure white solid in 48% yield (92% based on recovered 7): $[\alpha]^{20}_D$ -16.3° (c = 1.74, $CHCl_3$); ^1H NMR (490 MHz, $CDCl_3$) δ 7.67–7.65 (m, 4H), 7.41–7.33 (m, 13H), 5.73 (dt, J = 14.5, 6.7 Hz, 1H, H-5), 5.46 (dd, J = 14.5, 7.3 Hz, 1H, H-4), 5.42 (d, J = 10.5 Hz, 1H), 5.24 (s, 2H), 5.22 (m, 1H), 5.17 (dd, J = 12.0, 2.8 Hz, 1H), 5.08 (d, J = 10.1 Hz, 1H), 4.84 (m, 1H), 4.23–4.09 (m, 4H), 4.03–3.89 (m, 3H), 3.80 (dd, J = 11.8, 5.4 Hz, 1H), 3.72–3.63 (m, 2H), 3.39 (m, 3H), 3.18 (s, 1H), 2.91 (dd, J = 12.6, 4.6 Hz, 1H, H-3 NeuAc), 2.59 (d, J = 4.8 Hz, 1H), 2.11 (s, 3H), 2.07 (s, 3H), 2.01 (s, 3H), 2.00 (m, 2H), 1.97 (s, 3H), 1.85 (s, 3H), 1.60–1.23 (m, 23H), 1.03 (s, 9H), 0.86 (t, J = 6.1 Hz, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 171.0, 170.6, 170.3, 170.2, 169.8, 165.7, 135.6, 135.5, 134.9, 133.4, 129.7, 128.8, 128.6, 128.5, 127.9, 127.7, 101.9, 98.6, 76.7, 76.6, 76.5, 75.8, 75.4, 73.2, 73.1, 71.9, 69.2, 69.1, 68.5, 67.8, 67.3, 65.3, 62.8, 62.6, 49.5, 38.4, 32.4, 31.9, 29.7, 29.6, 29.5, 29.3, 29.2, 29.0, 26.8, 23.1, 22.7, 21.0, 20.8, 20.7, 14.1; FTIR (neat) 3489, 3271, 2918, 2853, 2094, 1747, 1700, 1658, 1552, 1535, 1370, 1229, 1105, 1070, 1034, 693 cm^{-1}; HRMS (FAB) calcd for $C_{66}H_{94}O_{19}N_4NaSi$ (M + Na) 1297.6179, found 1297.6246.

***O*-(Sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→2)-*O*-(β-D-galactopyranosyl)-(1→1)-(2*S*,3*R*,4*E*)-2-hexadecanamido-4-octadecene-1,3-diol (10):** The sialoglycoconjugate 9 (18 mg, 0.015 mmol) was dissolved in 0.5 mL of CH_2Cl_2 at room temperature. A catalytic amount of DMAP was added followed by pyridine (24 μL, 0.3 mmol) followed by benzoyl chloride (35 μL, 0.3 mmol). The reaction mixture was stirred at room temperature for 8 h. Standard workup followed by flash chromatography provided a mixture of sialyl lactones. This mixture was diluted in 2 mL of a 5:1 pyridine/water solution, saturated with hydrogen sulfide, and sealed in a pressure flask. The reaction was stirred for 24 h at room temperature before being quenched by evaporation *in vacuo*. The residue was diluted in dichloromethane. Palmitic acid (8 mg, 0.03 mmol) and EDCI (8 mg, 0.04 mmol) were added. The reaction was stirred overnight under an argon atmosphere. The reaction mixture was concentrated and subjected to flash

chromatography yielding a mixture of sialyl lactones which were directly reacted with TBAF (150 μL, of a 1 M solution in THF) in 1 mL of THF and then with catalytic sodium methoxide in methanol and finally with NaOH in THF/H_2O to yield crude iso-G_{M4}. The product was purified with LiChroprep RP-18 liquid chromatography using 5% H_2O in MeOH as eluent to give 6 mg (42% overall yield) of a pure white foam after lyophilization: $[\alpha]^{20}_D$ +7.9° (c = 0.6, MeOH); ^{1}H NMR (490 MHz, CD_3OD) δ 5.79 (dt, J = 15.4, 6.1 Hz, H-5), 5.38 (dd, J = 15.4, 7.2 Hz, 1H, H-4), 4.32 (apparent dt, J = 7.7, 4.6 Hz, 1H, H-2 Gal), 4.28 (d, J = 9.0 Hz, 1H, H-1a), 4.24 (apparent t, J = 9.0 Hz, 1H, H-3), 4.16 (d, J = 7.7 Hz, 1H, H-1 Gal), 3.90 (ddd, J = 8.8, 5.8, 2.5 Hz, 1H, H-8 NeuAc), 3.80 (d, J = 2.8 Hz, 1H, H-4 Gal), 3.77 (2H, H-2 and H-9 NeuAc), 3.71–3.61 (4H, H-6,6′ Gal, H-4 NeuAc, H-5 NeuAc), 3.53 (dd, J = 11.4, 5.8 Hz, 1H, H-9 NeuAc), 3.47 (dd, J = 10.0 Hz), 3.44–3.38 (3H, H-5 Gal, H-7 NeuAc, H-3 Gal), 3.20 (dd, J = 9.0, 2.5 Hz, 1H), 2.94 (dd, J = 12.5, 4.6 Hz, 1H, H-3eq NeuAc), 2.25 (m, 2H, CH_2 of palmitic amide), 1.95 (s, 3H, NAc), 1.94 (q, J = 6.7 Hz, 2H), 1.40–1.23 (49H), 0.83 (t, J = 6.7 Hz, 6H); ^{13}C NMR (62.5 MHz, CD_3OD) δ 175.6, 173.6, 173.2, 134.1, 131.7, 104.1, 101.2, 76.2, 75.2, 75.0, 74.7, 72.7, 71.3, 69.9, 69.6, 64.9, 62.5, 54.6, 54.1, 50.1, 37.3, 33.6, 33.1, 30.9, 30.8, 30.5, 27.3, 23.7, 22.6, 14.4; FTIR (KBr) 3458. 2915, 2852, 1633, 1577, 1450, 1372, 1084, 929, 858, 823, 604 cm^{-1}; HRMS (FAB) calcd for $^{12}C_{51}H_{93}N_2NaO_{16}$ (M^+) 1013.6504, found 1013.6554.

***O*-(1→4)-(5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-[1,5-anhydro-6-*O*-(*tert*-butyldiphenysilyl)-2-deoxy-D-*lyxo*-hex-1-enopyranose] (11)**: Galactal derivative **3** (92 mg, 0.24 mmol) was added to a flask containing AgOTf (123 mg, 0.48 mmol), 2,6-di-*tert*-butylpyridine (110 μL, 0.48 mmol), and $CaSO_4$ (300 mg) in 2 mL of dry THF and stirred for 30 min at room temperature under an argon atmosphere. The reaction mixture was cooled to −78 °C and a solution of sialyl chloride **8** (280 mg, 0.48 mmol) in 1 mL of THF was added dropwise. After the addition was complete, the reaction was slowly warmed to −10 °C at which temperature it was stirred overnight. The reaction was quenched by dilution with dichloromethane, filtration through Celite, concentration, and flash chromatography using 7:3 benzene/acetone as eluent to provide a mixture of products containing α- and β-disaccharides as well as eliminated sialyl donor. This mixture was dissolved in 2 mL of dichloromethane and cooled to 0 °C. DBU (36 μL, 0.24 mmol) was added and the reaction was stirred fo 5 h at 0 °C. The solvent was removed *in vacuo* and the residue was subjected to flash chromatography using 4:1 benzene/acetone as eluent to afford 114 mg of **11** (55% yield) as a white foam: $[\alpha]^{20}_D$ −26.0° (c = 6.7, $CHCl_3$); ^{1}H NMR (490 MHz, $CDCl_3$) δ 7.68–7.65 (m, 4H), 7.39–7.25 (m, 6H), 6.39 (d, J = 6.2 Hz, 1H, H-1 Gal), 5.46–5.40 (m, 1H), 5.24–5.19 (m, 3H), 4.82–4.80 (m, 1H), 4.78–4.76 (m, 1H), 4.70–4.68 (m, 1H, H-2 Gal), 4.28 (app d, J = 8.3 Hz, 1H, H-3 Gal), 4.19 (dd, J = 12.4, 2.1 Hz, 1H), 4.19 (q, J = 10.4 Hz, 1H), 4.02 (dd, J = 11.5, 8.4 Hz, 1H), 3.94 (dd, J = 12.3, 4.9 Hz, 1H), 3.87 (dd, J = 11.5, 2.6 Hz, 1H), 3.72 (dd, J = 10.5, 1.5 Hz, 1H), 2.12 (s, 3H), 2.04 (dd, J = 13.6, 5.4 Hz, 1H, H-3eq NeuAc), 2.00 (s, 3H), 1.97 (s, 3H), 1.86 (s, 3H), 1.85 (s, 3H), 1.72 (dd, J = 13.5, 11.5 Hz, 1H, H-3ax NeuAc), 1.05 (s, 9H); ^{13}C NMR (122.5 MHz, $CDCl_3$) δ 170.8, 170.3, 170.1, 169.6, 163.6, 146.2, 135.7, 133.3, 133.2, 129.7, 128.3, 127.7, 127.68, 97.9, 95.0, 75.8, 72.2, 72.1, 69.6, 67.9, 66.6, 62.3, 62.0, 61.6, 49.2, 38.1, 29.3, 26.8, 23.2, 20.8, 20.6, 20.4, 19.2; FTIR ($CHCl_3$) 3014, 2965, 2922, 2859, 1739, 1682, 1640, 1506, 1422, 1372, 1281, 1232, 1168, 1112, 1056, 1027, 471 cm^{-1}; HRMS (FAB) calcd for $C_{41}H_{52}O_{15}NSi$ (M + H) 826.3101, found 826.3190.

***O*-(1→4)-(5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-[6-*O*-(*tert*-butyldiphenylsilyl-β-D-galactopyranosyl]-(1→1)-(2*S*,3*R*,4*E*)-2-azido-4-octadecene-1,3-diol (12)**: The sialylgalactal **11** (19.6 mg, 0.025 mmol) was diluted in 0.4 mL of dry dichloromethane and cooled to 0 °C. Dimethyldioxirane (0.08 M, 0.028 mmol) was added dropwise and the reaction was warmed to room temperature. After 1 h the solvent was removed *in vacuo* leaving the 1,2-anhydrosugar as a white foam. The azido sphingosine **5** (114 mg, 0.35 mmol) was added and the reactants were dissolved in 0.1 mL of dry THF. The solution was cooled to −45 °C and $ZnCl_2$ (13 μL of a 1 M solution in diethyl ether, 0.013 mmol) was added. The reaction was allowed to warm to room temperature and was stirred overnight. The reaction was quenched by filtration through Celite, concentrated, and subjected to flash chromatography using 4:1 benzene/acetone as eluent to afford 15.5 mg (55% yield) of pure **12** as a white foam: $[\alpha]^{20}_D$ −31° (c =5.6, $CHCl_3$); ^{1}H NMR (490 MHz, $CDCl_3$) δ 7.71–7.69 (m, 4H), 7.41–7.36 (m, 6H), 5.79 (dt, J = 15.3, 6.8 Hz, 1H, H-4 Cer), 5.50 (dd, J = 15.3, 7.4 Hz, 1H, H-5 Cer), 5.44 (m, 1H), 4.79 (d, J = 4.0 Hz, 1H, H-4 Gal), 4.31 (d, J = 7.7 Hz, 1H, H-1 Gal), 4.26–4.10 (m, 6H), 4.06 (dd, J = 9.6, 4.0 Hz, 1H), 4.01 (dd, J = 11.3, 8.5 Hz, 1H), 3.92 (dd, J = 12.5, 6.0 Hz, 1H), 3.80–3.75 (m, 2H), 3,58–3.55 (m, 2H), 3.51 (m, 1H), 2.76 (d, J = 0.8 Hz, 1H), 2.38 (dd, J = 13.5, 5.4 Hz, 1H, H-2eq NeuAc), 2.12 (s, 3H), 2.02–1.94(m, 2H), 1.99 (s, 3H), 1.95 (s, 3H), 1.86 (s, 3H), 1.67 (s, 3H), 1.27 (m, 20H), 1.04 (s, 9H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 170.9, 170.4, 170.3, 170.0, 169.7, 163.6, 135.8, 135.6, 133.6, 133.2, 129.7, 127.7, 102.1, 95.0, 77.5, 74.9, 73.4, 72.8, 72.5, 72.3, 71.0, 69.6, 68.9, 68.2, 66.8, 65.0, 62.9, 62.2, 49.3, 38.7, 32.2, 31.9, 29.6, 29.57, 29.4, 29.3, 29.2, 28.9, 26.7, 23.1, 22.6, 20.8, 20.7, 20.6, 20.1, 19.2, 14.0; FTIR ($CHCl_3$) 2922, 2852, 2098, 1739, 1682, 1365, 1288, 1246, 1105, 1048, 1020, 464 cm^{-1}; HRMS (FAB) calcd for $C_{59}H_{96}N_4O_{18}SiNa$ (M + Na) 1189.5606, found 1189.5618.

***O*-(Sodium 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-*O*-β-D-galactopyranosyl-(1→1)-(2*R*,3*S*,4*E*)-2-hexadecanamido-4-octadecene-1,3-diol (1)**: To a solution of the sialoglycoconjugate **12** (26 mg, 0.022 mmol) in 0.1 mL of MeOH were added triethylamine (12 μL, 0.088 mmol) and 1,3-propanedithiol (9 μL, 0.088 mmol). The mixture was stirred at room temperature under an N_2 atmosphere for 48 h. The solvent was removed *in vacuo*. The residue was dissolved in 0.2 mL of CH_2Cl_2, and palmitic acid (34 mg, 0.132 mmol) was added followed by EDCI (25 mg, 0.132 mmol). The reaction mixture was stirred at room temperature for 12 h after which time the solvent was removed *in vacuo*. The residue was subjected to flash chromatography using 7:3 benzene/acetone as eluent to provide a mixture of products which were treated with TBAF (50 μL of a 1 M solution in THF) in 0.2 mL of THF. After stirring 8 h at room temperature, the solvent was removed and the residue treated with catalytic sodium methoxide in 0.5 mL of MeOH at room temperature for 16 h and finally with NaOH in 0.5 mL of 4:1 THF/H_2O for 16 h to yield crude G_{M4}. The product was purified with LiChroprep RP-18 liquid cromatography using 5% H_2O in MeOH as eluent to give 13 mg of G_{M4} (52%) as a white solid: $[\alpha]^{20}_D$ +2.29° (c = 6.4, CH_3OH); ^{1}H NMR (490 MHz, CD_3OD) δ 5.66 (dt, J = 15.3, 6.7 Hz, 1H, H-5 Cer), 5.43 (dd, J = 15.3, 7.9 Hz, 1H, H-4 Cer), 4.28 (d, J = 7.8 Hz, 1H, H-1 Gal), 4.26 (dd, J = 10.0, 3.3 Hz, 1H, H-1 Cer), 4.11 (t, J = 8.2 Hz, 1H, H-3 Cer), 4.04 (dd, J = 9.7, 3.2 Hz, 1H), 3.93–3.90 (m, 2H), 3.86–3.80 (m, 2H), 3.77–3.67 (m, 4H), 3.66 (dd, J = 11.6, 4.6 Hz, 1H), 3.63–3.57 (m, 3H), 3.53 (dd, J = 10.1, 3.0 Hz, 1H), 3.50–3.48 (m, 2H), 2.87 (dd, J = 12.8, 4.1 Hz, 1H, H-3eq NeuAc), 2.20–2.12 (m, 2H), 2.00 (s, 3H, NAc), 1.73 (apparent t, J = 11.8 Hz, 1H), 1.62–1.52, (m, 2H), 1.39–1.25 (m, 49H), 0.89 (t, J = 6.9 Hz, 6H); ^{13}C NMR (62.5 MHz, CD_3OD) δ 175.9, 175.4, 174.8, 134.7, 131.4, 105.1, 101.0, 77.7, 76.7, 75.0, 72.9, 72.8, 71.1, 70.1, 69.6, 69.4, 69.0, 64.6, 62.8, 59.7, 59.6, 54.9, 54.0, 37.4, 33.4, 33.0, 30.8, 30.7, 30.6, 30.5, 30.4, 27.1, 24.8, 23.7, 22.6, 20.7, 14.4, 13.8; FTIR (KBr) 3352, 2915, 2845, 1739, 1626, 1457, 1365, 1239, 1070, 661, 591 cm^{-1}; HRMS (FAB) calcd for $C_{51}H_{93}N_2Na_2O_{16}$ (M + Na) 1035.6321, found 1035.6346.

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Supplementary Material Available: ^{1}H NMR spectra for compounds **1**, **3**, **4**, **6**, **7** and **9–12** (9 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.