Synthesis of Specifically Labelled Ganglioside [1c-^{13}C]-GM_3

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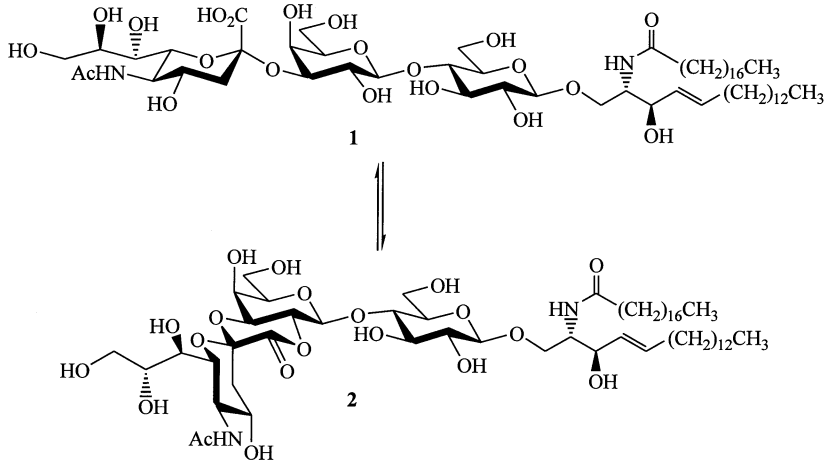
Glycosidation of the lactose derivative **4** with [1-^{13}C]-sialyl xanthate **3**, prepared from enzymatically obtained [1–^{13}C]-sialic acid gave the trisaccharide **5** which was transformed into [1c-^{13}C]-GM_3 ([1c-^{13}C]-**1**).

Gangliosides are sialic acid containing glycosphingolipides which are present in the outer membranes of living cells[1]. They may act as tumor-associated antigens[2] and play an important role in cell-cell and cell-surface interactions. Gangliosides influence the cell growth by interaction with e.g. the epidermal growth factor[3] and they are involved in apoptosis, the molecular regulation of cell death[4]. In addition, transformed gangliosides such as the GM_3-ganglioside lactone **2** are believed to be tumorspecific markers[5], which might be used in the development of vaccines against cancer[6]. The GM_3-lactone **2** was found to occur in the membranes of melanoma and liver carcinoma cells[7] in an equilibrium with GM_3 (**1**)[8].

Scheme 1. Formation of GM_3-lactone **2** from GM_3 (**1**)



However, so far no direct evidence for the lactonisation of **1** on the surface of tumor cells to give **2** was found[9]. A clarification of this important biochemical transformation may be possible by NMR spectroscopy on living tumor cells, using gangliosides with [1-^{13}C]-labelled sialylic acid. Recently, the GM_3-trisaccaride moiety was synthesised enzymatically in [^{13}C]-enriched form[10]. Herein we describe a chemical synthesis[11] of specifically labelled [1c-^{13}C]-GM_3 ([1c-^{13}C]-**1**) containing [1-^{13}C]-labelled *N*-Ac-neuraminic acid[12].

Results and Discussion

The retrosynthesis of [1c-^{13}C]-GM_3 ([1c-^{13}C]-**1**) leads to stearic acid, azidosphingosine **9**, the lactose derivative **4** and the [1-^{13}C]-labelled sialyl donor **3**. For the synthesis of **4**, lactose was transformed into the 3b,4b-*O*-unprotected derivative **4** as previously described[13].

[1-^{13}C]-*N*-acetylneuraminic acid was synthesised enzymatically according to a literature procedure[14] using sodium [1-^{13}C]-pyruvate. Sialylation of **4** was performed with [1-^{13}C]-*N*-acetylneuraminic xanthate **3**[15] as sialyl donor. The reaction was carried out in acetonitrile/dichloromethane (2:1) at −70°C using 1.0 equivalent of phenyl sulfenyltriflate (PST) as promotor[16] to give the trisaccharide **5** in 42% yield exclusively (Scheme 2).

Neither the β-glycoside nor a product formed by a glycosidation at 4b-OH of **4** were found. Hydrogenolytic cleavage of the *O*-benzyl groups in **5** with $Pd(OH)_2$, followed by peracetylation of the crude product led to **6** in 76% yield. Re-

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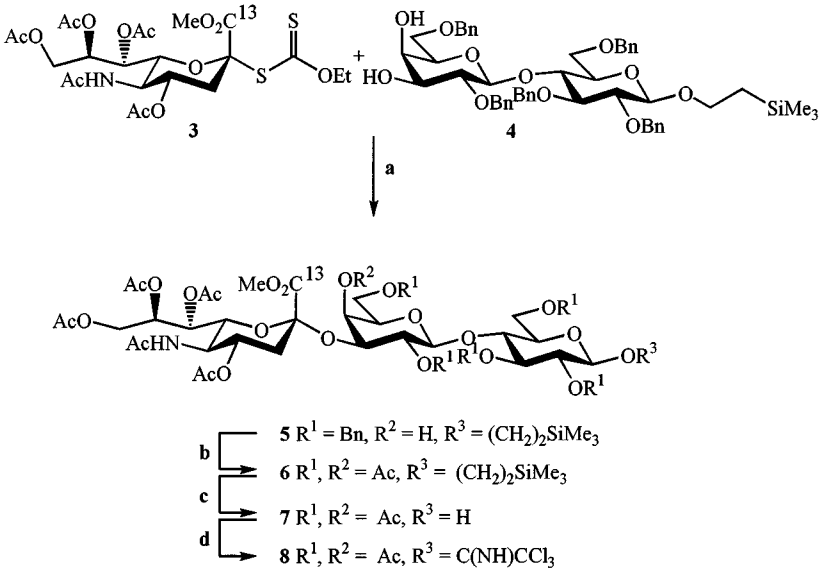
Scheme 2

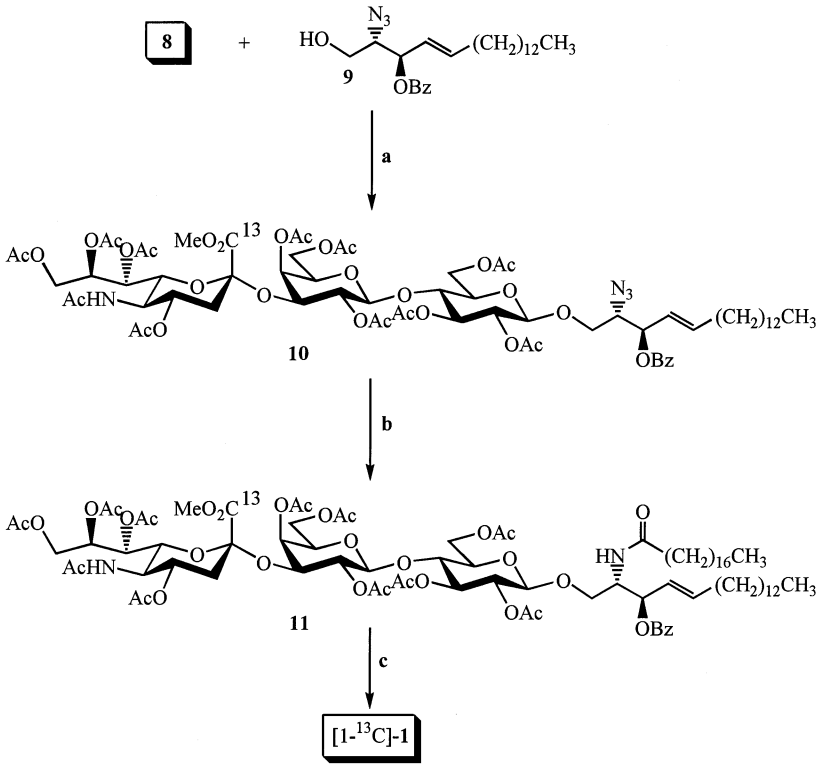


Reagents and conditions: (a) PST, AgOTf, CH_3CN/CH_2Cl_2, −70°C (42%); (b) (i) $Pd(OH)_2/C$, H_2, MeOH, (ii) Ac_2O/Py, 0°C (76%); (c) $BF_3{\cdot}OEt_2$, CH_2Cl_2, 0°C (95%); (d) CCl_3CN, DBU, CH_2Cl_2 (84%).

moval of the protecting group at C-1a with boron trifluoride−diethyl ether gave **7** in 95% yield[17]. For the glycosidation of azidosphingosine **9**, the trisaccharide **7** was transformed into the α-trichloroacetimidate[18] **8** by treatment with trichloroacetonitrile in the presence of DBU to afford **8** in 84% yield.

Glycosidation of 3-*O*-benzoylazidosphingosine **9**[19] as acceptor with **8** in the presence of boron trifluoride−diethyl

Scheme 3



Reagents and conditions: (a) **9**, $BF_3{\cdot}OEt_2$, 0°C (75%); Lindlar catalyst, H_2, $C_{36}H_{70}O_3$ (76%); (c) (i) NaOMe/MeOH, (ii) H_2O, (iii) IRA 120 (88%).

ether gave the β-glycoside **10** in 75% yield. Reduction with Lindlar's catalyst and acylation of the corresponding amine with stearoyl anhydride[20] afforded the ceramide derivative **11** in 76% yield. The removal of the *O*-acyl groups was performed under Zemplén conditions to give [1c-^{13}C]-labelled GM_3 ([1c-^{13}C]-**1**) in 88% yield (Scheme 3). The NMR data of **1** are in full agreement with those reported in the literature[21].

At the moment NMR spectroscopic investigations with [1c-^{13}C]-GM_3 ([1c-^{13}C]-**1**) on cultured cells are performed with the aim to prove the formation of the GM_3-lactone **2** from **1** on the cell surface.

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Experimental Section

General: ^{1}H-NMR and ^{13}C-NMR spectra were recorded with a Varian XL-500 or a Varian XL-300 spectrometer; multiplicities were determined with APT pulse sequence. − Melting points were determined with a Mettler FP 61 apparatus and are uncorrected. − TLC was performed on foil plates (Macherey-Nagel & Co., Polygram SIL G/UV$_{254}$). − Column chromatography was performed using SiO_2 (Merck). − Sodium [1-^{13}C]-pyruvate was purchased from Deuterochem and was used without further purification.

O-Ethyl S-(5-Acetamido-4,7,8,9-tetra-O-acetyl-1-methyl-[1−^{13}C]-α-neuraminosyl) Dithiocarbonate (**3**): To a solution of [1-^{13}C]-sialyl chloride (0.88 g, 1.72 mmol) in ethyl acetate (11 ml) was added a sodium carbonate solution (11 ml, 2 M), then tetrabutylammonium hydrogen sulfate (585 mg, 3.64 mmol) and *O*-ethylxhantic acid potassium salt (320 mg, 1.99 mmol) at 0°C. The two-phase reaction mixture was vigorously stirred at room temp. for 20 min. Ethyl acetate (50 ml) was added, the organic phase was separated and washed with saturated sodium hydrogen carbonate, water and brine. The combined organic extracts were dried with Na_2SO_4, concentrated under reduced pressure and the residue was purified by column chromatography on silica gel, using ethyl acetate as eluant to afford **3** (0.91 g, 1.51 mmol, 88%), m.p 87.8°C. − $[\alpha]^{20} = +82.2$ (c = 1, $CHCl_3$). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.34 (t, J = 7.1 Hz, 3 H), 1.84 (s, 3 H), 1.97 (t, J = 12.9 Hz, 1 H, 3-Hax), 2.03−2.08 (4 s, 12 H), 2.55 (dd, J = 12.9, 4.7 Hz, 1 H, 3-H$_{eq}$), 3.49−3.53 (m, 1 H), 3.81 [d, J(^{13}C-H) = 4.1 Hz, 3 H, CO_2CH_3], 4.01 (q, J = 11.5 Hz, 1 H, 5-H),4.18 (dd, J = 11.0, 2.5 Hz, 1 H, 9-Ha), 4.50−4.58 (m, 1 H, OCH_2Me), 4.56 (dd, J = 11.0, 2.5 Hz, 1 H, 6-H), 4.76−4.83 (m, 1 H, OCH_2Me), 4.84−4.91 (m, 1 H, 4-H), 5.25−5.34 (m, 3 H). − ^{13}C NMR (125.7 MHz, $CDCl_3$): δ = 13.34 (OCH$_2$$CH_3$), 20.75, 20.78, 20.82, 21.07, 23.17 (5 CH$_3$CO, 37.16 (C-3), 49.26 (C-5), 53.30 (d, J = 2.7 Hz, CH_3O), 62.05 (C-9), 67.76 (C-8), 68.85 (C-4), 70.21 (C-7), 70.46 (OCH$_2$CH$_3$), 75.15 (C-6), 86.55 (d, J = 67.7 Hz, C-2), 168.72 (C-1), 170.11, 170.23, 170.56, 170.84 (5 CH$_3$$C$O), 207.19 (CS). − MS (DCI); *m/z* (%): 614.6 (100) [$M^+ + NH_4^+$].

2-(Trimethylsilyl)ethyl [Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-[1−^{13}C]-2-nonulopyranosyl)onate]-(2→3)-(2,6-di-O-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (**5**): A mixture of the lactose derivative **4** (1.09 g, 1.0 mmol), *N*-acetylneuraminic acid derivative **3** (0.90 g, 1.5 mmol), molecular sieves (2.0 g, 4 Å), in a mixture of dry acetonitrile (10 ml) and dry dichloromethane (5 ml) was stirred under argon for 1 h. Silver triflate (427 mg, 1.66 mmol) and 2,6-di-*tert*-butyl-4-methylpyridine (372 mg, 1.81 mmol) were added, and the mixture was cooled to −70°C and kept protected from light. Phenylsulfenyl chloride (190 µl, 1.59 mmol) in dry dichloromethane (300 µl) was added slowly by running down the cold wall of the reaction flask, and then stirring was continued for 3 h at −70°C. The mixture was diluted with ethyl acetate (10 ml), filtered (Celite), washed with saturated aqueous sodium hydrogen sulfate, water and brine, dried with Na_2SO_4 and concentrated in vacuo. The residue was purified by chromatography (SiO_2; ethyl acetate/pentane, 10:1) to give **5** as a foam (482 mg, 0.35 mol, 42%). − ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.09 [s, 9 H, Si(CH$_3$)$_3$], 1.01]m, 2 H, CH_2Si(CH$_3$)$_3$], 1.92−2.10 (m, 15 H, CH$_3$), 2.11 (t, J = 10.8 Hz, 1 H, 3c-H$_{ax}$), 2.48 (dd, J = 13.2, 6.5 Hz, 1 H, 3c-H$_{eq}$), 2.76 (s$_{br.}$, 1 H, 4b-OH), 3.35 (t, J = 6.8 Hz, 1 H, 3-H), 3.42−3.69 (m, 7 H), 3.76 [d, J(^{13}C-H) = 3.1 Hz, 3 H, CO_2CH_3], 3.81 (t, J = 7.2 Hz, 1 H), 3.86−4.15 (m, 6 H), 4.56 (d, J = 8.7 Hz, 1 H, 1a-H), 4.23−4.47 (m, 6 H), 4.65−4.78 (m, 4 H), 4.80 (dd, J = 6.2, 2.9 Hz, 1 H), 4.87 (d, J = 7.4 Hz, 1 H), 4.94 (d, J = 6.1 Hz, 1 H), 5.15 (d, J = 10.1 Hz, 1 H, 1b-H), 5.32 (dd, J = 7.8, 2.3 Hz, 1 H, 7c-H), 5.40 (dt, J = 7.1, 2.6 Hz, 1 H, 8c-H), 7.20−7.45 (m, 25 H, aromatic). − ^{13}C NMR (125 MHz): δ = −1.48 [Si(CH$_3$)$_3$], 18.40 [CH$_2$Si(CH$_3$)$_3$], 20.44, 20.64, 20.75, 21.04, 23.04 (NHAc), 36.34 (C-3c), 49.09 (C-5c), 52.95 (CO_2-CH$_3$), 62.21 [O-CH$_2$-CH$_2$-Si(CH$_3$)$_3$], 67.11, 67.81, 68.32, 68.49, 68.82, 69.05, 72.35, 72.63, 72.93, 73.20, 74.81, 74.99, 75.26, 76.25, 76.57, 78.33, 81.91, 82.91, 98.33 (d, J = 68 Hz, 2c-C), 102.30, (1b-C), 102.97 (1a-C), 127.05, 127.18, 127.21, 127.30, 127.37, 127.39, 127.40, 127.42, 127.92, 127.94, 127.99, 128.02, 128.10, 128.15, 128.16, 138.30, 138.45, 138.70, 138.86, 139.07, 168.28 (1c-C), 169.84, 169.95, 170.27, 170.50, 170.72. − MS (DCI); *m/z* (%): 1384.6 (100) [$M^+ + NH_4^+$].

2-(Trimethylsilyl)ethyl [Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-[1−^{13}C]-2-nonulopyranosyl)onate]-(2→3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**6**): A mixture of **5** (390 mg, 0.29 mmol) and palladium(II) hydroxide on carbon (100 mg, 10% Pd) in methanol (10 ml) was shaken under hydrogen (3 bar) for 6 h. Then the mixture was filtered through Celite. After washing with methanol (30 ml), the combined filtrates were concentrated in vacuo. The residue was treated with acetic anhydride (16 ml) in pyridine (26 ml) at 0°C. After stirring for 8 h at room temp., the mixture was concentrated in vacuo. Flash chromatography (SiO_2; ethyl acetate/methanol, 30:1) of the residue yielded **6** (260 mg, 0.22 mmol, 76%). − ^{1}H NMR (300 MHz, $CDCl_3$): δ = −0.02 [Si(CH$_3$)$_3$], 0.96 [m, 2 H, CH_2Si(CH$_3$)$_3$], 1.88−2.14 (10 s, 30 H, Ac), 2.23 (s, 3 H, NHAc), 2.14 (t, J = 12.5 Hz, 1 H, 3c-H$_{ax}$), 2.55 (dd, J = 12.5, 4.5 Hz, 1 H, 3c-H$_{eq}$), 3.50−3.62 (m, 5 H), 3.78 [d, J(^{13}C-H) = 4.8 Hz, 3 H, CO_2CH_3], 3.96, (dd, J = 6.1, 1.1 Hz, 1 H, 9c-H), 4.01−4.18 (m, 9 H), 4.29 (dd, J = 11.8, 3.0 Hz, 1 H), 4.45 (d, J = 8.9 Hz, 1 H, 1a-H), 4.52 (dd, J = 12.2, 3.2 Hz, 1 H, 3b-H), 4.67 (d, J = 8.8 Hz, 1 H, 1b-H), 4.78−4.92 (m, 4 H), 5.12 (dd, J = 10.2, 9.8 Hz, 1 H), 5.54 (m$_c$, 1 H, 8c-H). − ^{13}C NMR (75.48 MHz): δ = −1.45 [Si(CH$_3$)$_3$], 18.38 [CH$_2$-Si(CH$_3$)$_3$], 20.77, 20.82, 20.92, 21.11, 21.51, 23.16 (NHAc), 37.33 (C-3c), 49.06 (C-5c), 53.10 (d, J = 4.5 Hz, $CO_2$$CH_3$), 62.20, 62.34, 66.85, 67.27, 67.43, 67.70, 69.29, 69.88, 70.35, 71.26, 71.80, 71.89, 72.45, 73.51, 76.33, 96.26 (d, J = 68.8 Hz, C-2c), 99.90, 100.97 (C-1a, C-1b), 162.03, 166.16, 167.19, 167.26, 167.66, 167.89 (C-1c), 168.25, 168.52, 168.84, 168.98. − MS (DCI); *m/z* (%): 1186.2 (100) [$M^+ + NH_4^+$].

[Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-[1-^{13}C]-2-nonulopyranosyl)onate]-(2→3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-β-D-glucopyranoside (**7**): To a solution of **6** (240 mg, 0.20 mmol) in dichloromethane (20 ml), boron trifluoride−diethyl ether (222 µl)

was added at 0°C, and the mixture was stirred for 2 h at 0°C and then 1 h at room temp. The reaction mixture was diluted with dichloromethane (30 ml), washed with saturated sodium hydrogen carbonate, water and brine and dried with Na_2SO_4. The mixture was concentrated in vacuo and the residue was chromatographed (SiO_2; ethyl acetate/methanol, 30:1) to give **7** (205 mg, 0.19 mmol, 95%). – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.91–2.25 (m, 30 H, 10 Ac), 2.23 (s, 3 H, NHAc), 2.59 (dd, *J* = 12.5, 4.5 Hz, 1 H, 3c-H$_{eq}$), 3.79 [d, *J*(^{13}C-H) = 3.2 Hz, 3 H, CO_2CH_3], 3.96–4.20 (m, 10 H), 4.62 (dd, *J* = 10.0, 3.0 Hz, 1 H, 3b-H), 4.75 (d, *J* = 7.8 Hz, 1 H, 1b-H). – ^{13}C NMR (75.48 MHz): δ = 20.48, 20.61, 20.69, 20.74, 20.82, 20.90, 23.11 (NHAc), 37.30 (C-3c), 49.12 (C-5c), 52.98 (CO_2CH_3), 61.32, 62.07, 62.14, 67.15, 67.67, 67.93, 68.20, 69.30, 69.91, 69.97, 70.29, 71.36, 71.48, 71.54, 71.90, 73.46, 96.69 (d, *J* = 68.6 Hz, C-2c), 99.86, 100.90 (C-1a, C-1b), 161.90, 166.09, 167.16, 167.25, 167.63, 167.87 (C-1c), 168.27, 168.49, 168.84, 168.99.

[Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-[1–^{13}C]-2-nonulopyranosyl)onate]-(2→3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-α-D-glucopyranosyl Trichloracetimidate (**8**): To a solution of **7** (205 mg, 0.19 mmol) and trichloroacetonitrile (570 µl, 5.7 mmol) in dry dichloromethane (10 ml) was added DBU (14 mg, 0.08 mmol) at –5°C. After stirring for 3 h at 0°C, the mixture was concentrated in vacuo and purified by chromatography (ethyl acetate/methanol, 30:1) to give **8** (192 mg, 0.16 mmol, 84%). – ^{1}H NMR (500 MHz): δ = 1.65 (t, *J* = 12.2 Hz, 1 H, 3c-H$_{ax}$), 1.84–2.20 (10 s, 30 H, Ac), 2.22 (s, 3 H, NHAc), 2.61 (dd, *J* = 12.6, 4.5 Hz, 1 H, 3c-H$_{eq}$), 3.61 (dd, *J* = 10.9, 2.9 Hz, 1 H), 3.78 (d, *J* = 4.0 Hz, 1 H), 3.83 [d, *J*(^{13}C-H) = 4.0 Hz, 3 H, CO_2CH_3], 4.21 (dd, *J* = 12.0, 4.5 Hz, 1 H), 4.34 (dd, *J* = 12.5, 2.5 Hz, 1 H), 4.41 (dt, *J* = 12.5, 2.5 Hz, 1 H), 4.56 (dd, *J* = 10.0, 3.5 Hz, 1 H), 4.68 (d, *J* = 8.0 Hz, 1 H, 1b-H), 5.18 (d, *J* = 10.0 Hz, 1 H), 5.35 (dd, *J* = 8.5, 2.5 Hz, 1 H, 7c-H), 6.49 (d, *J* = 4.2 Hz, 1 H, 1a-H), 8.64 (s, 1 H, C=NH). – MS (DCI); *m/z* (%): 1231.3 (100) [$M^+ + NH_4^+$].

[Methyl (5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-[1–^{13}C]-2-nonulopyranosyl)onate]-(2→3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→1)-(2S,3R,4E)-3-O-benzoyl-2-octadecaneamido-4-octadecene-1,3-diol (**11**): To a mixture of **8** (175 mg, 0.14 mmol), the azidosphingosine derivative **9** (160 mg, 0.37 mmol), and molecular sieves (0.3 g, 4 Å) in dry dichloromethane (10 ml) at 0°C under argon was added boron trifluoride–diethyl ether (31.4 µl, 0.25 mmol). After 2.5 h, the mixture was washed with saturated aqueous sodium hydrogen carbonate, water and brine, dried with Na_2SO_4, concentrated in vacuo and chromatographed (SiO_2; ethyl acetate/pentane, 5:1) to give **10** (139 mg, 0.09 mmol, 75%). A mixture of the azide **10** (136 mg, 0.09 mmol), Lindlar's catalyst (260 mg) and stearoyl anhydride (125 mg, 0.23 mmol) in ethyl acetate (7 ml) was stirred at room temp. under H_2 for 18 h. The reaction mixture was filtered through a pad of silica gel, washed with ethyl acetate (30 ml) and the combined filtrates were concentrated in vacuo. Chromatography of the crude material (pentane/ethyl acetate, 3:1) provided **11** (120 mg, 0.07 mmol, 76%) as a colorless oil. – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 7.1 Hz, 6 H, 2 CH_3), 1.29 (s, 56 H, CH_2), 1.65 (m$_c$, 1 H, 3c-H*ax*), 1.88–2.17 (10 s, 30 H, Ac), 2.17 (s, 3 H, NHAc), 2.58 (dd, *J* = 12.4, 4.8 Hz, 1 H, 3c-H$_{eq}$), 3.51–3.63 (m, 1 H, 1cer-H), 3.84 [d, *J*(^{13}C-H) = 4.2 Hz, 3 H, CO_2CH_3], 4.32 (dd, *J* = 12.5, 3.1 Hz, 1 H), 4.43 (td, *J* = 10.0, 2.1 Hz, 1 H, 2cer-H), 4.51 (d, *J* = 8.5 Hz, 1 H, 1a-H), 4.69 (d, *J* = 7.9 Hz, 1 H, 1b-H), 4.92 (dd, *J* = 8.5, 3.0 Hz, 3b-H), 5.34 (dd, *J* = 8.0, 2.0 Hz, I H), 5.40 (dd, *J* = 9.1, 3.0 Hz, 1 H, 7c-H), 5.60 (d, *J* = 9.1 Hz, 1 H, NH), 5.92 (dt, *J* = 14.8, 8.1 Hz, 1 H, 5cer-H), 7.43 (t, *J* = 7.0 Hz, 2 H, C_6H_5CO), 7.59 (t, *J* = 7.0 Hz, 1 H, C_6H_5CO), 8.02 (d, *J* = 7.0 Hz, 2 H, C_6H_5CO).

(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-[1–^{13}C]-2-nonulopyranoslylicacid)-(2→3)-O-β-galactopyranosyl-(1→4)-O-β-glucopyranosyl-(1→1)-(2S,3R,4E)-2-octadecanamido-4-octadecene-1,3-diol (**1**): Methanolic sodium methoxide (130 µl, 5.4 M) was added to a solution of **11** (115 mg, 0.07 mmol) in dry methanol (16 ml), and the mixture was stirred overnight at room temp. Then water (900 µl) was added and the solution was stirred for 8 h at room temp. The solution was neutralised with Amberlite IR-120 (H^+), filtered and concentrated in vacuo. Chromatography (SiO_2; chloroform/methanol/water, 155:65:10) of the residue gave **1** (69.9 mg, 0.06 mmol, 88%). – ^{1}H NMR (500 MHz; DMSO/D_2O, 98:2): δ = 0.86 (t, *J* = 7.1 Hz, 6 H, CH_3), 1.24 (s$_{br}$, 54 H, CH_2), 1.31 (m, 1 H, 3c-H*ax*), 1.89 (s, 3 H, NHAc), 1.96 (q, *J* = 7.0 Hz, 2 H, 6cer-H), 2.75 (dd, *J* = 11.5, 5.1 Hz, 1 H, 3c-H$_{eq}$), 3.05 (t, *J* = 9.6 Hz, 1 H, 3a-H), 3.47 (d, *J* = 6.1 Hz, 1 H, 6c-H), 3.89 (dd, *J* = 10.0, 3.2 Hz, 1 H, 1b-H), 4.21 (d, *J* = 8.0 Hz, 1a-H), 5.40 (dd, *J* = 16.0,7.0 Hz, 1 H, 4cer-H), 5.63 (dt, *J* = 16.0, 7.0 Hz, 1 H, 5cer-H).

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