Supplemental Information

Overview of the Synthesis of N-Palmitoyl-D-erythro-sphingosyl-[6-^{13}C]GalSph. [6-^{13}C]GalCer (**11**) was prepared as shown in Scheme 1 using β-D-galactose labeled with ^{13}C at carbon 6 as a starting material (Omnicron Biochemicals). A complete description of the synthetic procedures is provided below. Briefly, [6-^{13}C]galactose (**1**) was converted to per-*O*-acetyl-[6-^{13}C]galactose (**2**) in 80% yield by treatment with acetic anhydride in dry pyridine. Regioselective 1-*O*-deacetylation of peracetylated galactose **2** was achieved in 83% yield by treatment with ammonia in acetonitrile (56), affording 2,3,4,6-tetra-*O*-acetyl-D-galactose (**3**). [6-^{13}C]galactosyl α,β-trichloroacetimidate (**4**) (57) was prepared in 78% yield by base-catalyzed (K_2CO_3) reaction of trichloroacetonitrile with **3**.

The galactosyl acceptor was synthesized as follows. D-*erythro*-Sphingosine was converted to 2-azido-D-*erythro*-sphingosine (**5**) by diazo transfer from trifluoromethane-sulfonyl azide (TfN_3) (58) in 86% yield; then the primary hydroxyl group of **5** was protected as a *tert*-butyldiphenylsilyl (TBDPS) ether **6** in the presence of two equivalents of imidazole (59). Protection of the secondary hydroxyl group as a 3-*O*-benzoyl ester afforded **7,** which was desilylated with *n*-Bu_4NF (TBAF) in the presence of imidazole at -23°C to give 2-azido-3-*O*-benzoyl-D-*erythro*-sphingosine (overall yield for the four steps from sphingosine, 60%). Without the addition of imidazole, substantial migration of the benzoyl group took place on desilylation.

The glycosylation step was carried out by treating a mixture of [6-^{13}C]galactosyl trichloroacetimidate (**4**) and 2-azido-3-*O*-benzoyl-sphingosine (**8**) in CH_2Cl_2 with a catalytic amount of $BF_3{\cdot}OEt_2$ in the presence of 300AW molecular sieves, giving the corresponding fully acetylated β-D-galactosyl derivative **9** in 74% yield. The *O*-acetyl and *O*-benzoyl

protecting groups were removed with methanolic sodium methoxide. Reduction of the azido function in **10** with triphenylphosphine in aqueous THF (60), followed by in situ *N*-acylation with 4-nitrophenyl palmitate, provided [6-^{13}C]GalCer (**11**) in 71% yield.

Synthesis and Characterization of the Reaction Intermediates Resulting in [6-^{13}C]GalCer. Melting points were measured on a Hoover capillary melting point apparatus and were uncorrected. NMR spectra were recorded on a Bruker spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C. THF was distilled from Na and benzophenone immediately before use. CH_2Cl_2, DMF, and acetonitrile were distilled over CaH_2. Molecular sieves 300AW were predried for 5 h at 150°C. TLC was carried out using Merck silica gel 60F_{254} (0.25-mm thick) sheets.

*[6-^{13}C]-1,2,3,4,6-Penta-O-acetyl-D-galactose (**2**).* The reaction steps in the preparation of [6-^{13}C]GalCer are numbered as shown in Scheme 1. To a solution of 300 mg (1.66 mmol) of [6-^{13}C]-enriched D-galactose in dry pyridine (2 ml) was added acetic anhydride (1.2 ml, 12.7 mmol) at room temperature. The reaction mixture was stirred at this temperature under nitrogen for 3 h. The solvent was removed in a rotary evaporator. The residue was dissolved in $CHCl_3$, washed with water (2 x 10 ml), saturated aqueous $NaHCO_3$ solution (2 x 5 ml), and brine (2 x 5 ml). The organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (elution with hexane/EtOAc 1:1) to give 520 mg (80%) of 1,2,3,4,6-penta-*O*-acetyl-D-galactose (**2**) as a white solid; mp 140-141°C; R_f 0.42 (1:1 hexane/EtOAc); ^{1}H NMR ($CDCl_3$) δ 5.71 (d, 1H, *J* = 8.3 Hz), 5.44 (d, 1H, *J* = 3.3 Hz), 5.35 (m, 1H), 5.09 (dd, 1H, *J* = 3.4, 3.4 Hz), 4.37-4.33 (m, 1H), 4.09-3.93 (m, 2H), 2.19-2.01 (5s, 15H); ^{13}C NMR ($CDCl_3$) δ 168.8, 168.6, 168.4, 167.4, 90.6, 69.9, 69.3, 66.3, 65.3, 59.9, 19.3, 19.2, 19.1, 19.0.

*2,3,4,6-Tetra-O-acetyl-D-galactose (**3**).* 1,2,3,4,6-Penta-*O*-acetyl-D-galactose (520 mg, 1.33 mmol) was dissolved in a solution of ammonia in acetonitrile at 0 °C (prepared by bubbling ammonia gas through 50 ml of acetonitrile at 0 °C for 30 min). The mixture was stirred for 3 h at 0 °C, and then allowed to warm to room temperature, and stirring was continued for 14 h. The solution was concentrated, and the residue was purified by chromatography (elution with hexane/EtOAc, 1:1), giving 387 mg (83%) of 2,3,4,6-tetra-*O*-acetyl-D-galactose (**3**) as a white solid; mp 114-117°C; R_f 0.30 (hexane/EtOAc, 1:1); ^{1}H NMR ($CDCl_3$) δ 5.46-5.33 (m, 2H), 5.11-5.00 (m, 1H), 4.40-4.02 (m, 2H) 3.90-3.84 (m, 2H), 3.25 (s, 1H) 2.12-1.93 (4s, 12 H); ^{13}C NMR ($CDCl_3$) δ 169.6, 169.4, 169.3, 169.1, 95.1, 89.8, 70.2, 70.1, 69.3, 67.3, 67.2, 66.3, 66.2, 65.4, 60.9, 60.5, 19.9, 19.8, 19.7, 19.6.

*2,3,4,6-Tetra-O-acetyl-**a**- and **b**-D-galactopyranosyl Trichloroacetimidate (**4**).* A mixture of 2,3,4,6-tetra-*O*-acetyl-D-galactose (385 mg, 1.11 mmol), trichloroacetonitrile (300 μL, 3.0 mmol), and anhydrous K_2CO_3 (400 mg, 2.89 mmol) in dry CH_2Cl_2 (10 ml) was stirred at room temperature for 24 h. After the disappearance of the starting material, the reaction mixture was filtered through Celite, which was washed with CH_2Cl_2 (10 ml). The combined filtrate was evaporated to dryness. The residue was purified by column chromatography (elution with hexane/EtOAc 3:1) to give 423 mg (78%) of a mixture of α- and β-trichloroacetimidates **4**; R_f 0.64 and 0.53 (hexane/EtOAc, 1:1); ^{1}H NMR ($CDCl_3$) δ 8.65, 8.60 (s, 1H, α and β NH), 6.54 (d, 0.5H, *J* = 3.4 Hz, anomeric H-1), 5.77 (d, 0.5H, *J* = 8.2 Hz, anomeric H-1), 5.49-5.28 (m, 2H), 5.05 (dd, 1H, *J* = 3.4, 3.4 Hz), 4.38-3.85 (2m, 3H), 2.16-2.00 (4s, 12H, 4 Ac); ^{13}C NMR ($CDCl_3$) δ 171.2, 171.03, 171.01, 170.9, 170.7, 161.8, 126.8, 98.3, 94.4, 91.6, 74.7, 72.3, 70.0, 69.9, 68.4, 68.3, 67.8, 66.9, 62.3, 62.2, 59.3, 24.7, 21.7, 21.6, 21.55, 21.52, 19.3, 16.1.

*(2S,3R,4E)-2-Azido-D-erythro-sphingosine (**5**).* To a solution of D-*erythro*-sphingosine (75 mg, 0.25 mmol) in CH_2Cl_2 (10 ml), DMAP (40.8 mg, 0.33 mmol) was added, followed by dropwise addition of TfN_3 solution in CH_2Cl_2 (2.9 ml, 0.40 M solution, 1.16 mmol). The reaction mixture was stirred at 26°C under nitrogen until the disappearance of D-*erythro*-sphingosine was noticed by TLC (hexane/EtOAc, 1:1). The reaction mixture was concentrated in a rotary evaporator and the residue was purified by chromatography (elution with hexane/EtOAc, 3:2) to give 70 mg (86%) of (2*S*,3*R*,4*E*)-2-azido-D-*erythro*-sphingosine (**5**); R_f 0.50 (hexane/EtOAc 1:1); ^{1}H NMR ($CDCl_3$) δ 5.82 (m, 1H), 5.57 (m, 1H), 4.27 (t, 1H, *J* = 6.3 Hz), 3.81 (m, 2H), 3.52 (m, 1H), 2.12-2.07 (m, 4H), 1.43-1.28 (m, 22H), 0.91 (t, 3H, *J* = 3.3 Hz); ^{13}C NMR ($CDCl_3$) δ 134.2, 126.0, 71.9, 64.7, 60.6, 30.3, 30.0, 27.7, 27.6, 27.5, 27.4, 27.2, 26.9, 20.7, 12.2.

*(2S,3R,4E)-2-Azido-1-O-(tert-butyldiphenylsilyl)-D-erythro-sphingosine (**6**).* A mixture of *tert*-butyldiphenylsilyl chloride (54 mg, 0.19 mmol) and imidazole (27 mg, 0.39 mmol) in CH_2Cl_2 (5 ml) was stirred at room temperature for 1 h. A solution of (2*S*,3*R*,4*E*)-2-azido-D-*erythro*-sphingosine (63 mg, 0.19 mmol) in CH_2Cl_2 (5 ml) was added dropwise, and the reaction mixture was stirred at room temperature for 12 h. The mixture was diluted with CH_2Cl_2 (10 ml) and washed with water (2 x 2 ml) and brine (2 ml), dried (Na_2SO_4), and evaporated under vacuum. The residue was purified by column chromatography (elution with hexane/EtOAc 95:5) to give 97 mg (89%) of (2*S*,3*R*,4*E*)-2-azido-1-*O*-(*tert*-butyldiphenylsilyl)-D-*erythro*-sphingosine (**6**); R_f 0.66 (hexane/EtOAc, 4:1); ^{1}H NMR ($CDCl_3$) δ 7.63 (m, 4H), 7.33 (m, 6H), 5.66 (m, 1H), 5.35 (m, 1H), 4.15 (t, 1H, *J* = 6.0 Hz), 3.72 (m, 2H), 3.43 (m, 1H), 1.94 (m, 3H), 1.25-1.18 (m, 22H), 1.00 (s, 9H), 0.81 (t, 3H, *J* = 6.80 Hz);

^{13}C NMR ($CDCl_3$) δ 136.0, 135.9, 135.5, 135.2, 133.1, 130.3, 130.1, 128.2, 128.1, 73.2, 67.2, 64.5, 32.7, 32.3, 30.1, 30.0, 29.9, 29.8, 29.6, 29.3, 27.1, 26.9, 23.1, 19.5, 19.4, 14.5.

*(2S,3R,4E)-2-Azido-3-O-benzoyl-1-O-(tert-butyldiphenylsilyl)-D-erythro-sphingosine (**7**).* A solution of **6** (97 mg, 0.17 mmol), benzoic anhydride (135 mg, 0.60 mmol), and DMAP (50 mg, 0.41 mmol) in CH_2Cl_2 (8 ml) was stirred at room temperature for 14 h. After solvent was removed under vacuum, the product was purified by column chromatography (elution with 5% EtOAc in hexane) to give 101 mg (88%) of (2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-1-*O*-(*tert*-butyldiphenylsilyl)-D-*erythro*-sphingosine (**7**); R_f 0.78 (hexane/EtOAc 9:1);^{1}H NMR ($CDCl_3$) δ 8.02 (m, 2H), 7.69 (m, 4H), 7.66 (m, 1H), 7.48-7.34 (m, 8H), 5.90 (m, 1H), 5.69 (m, 1H), 5.51 (m, 1H), 3.85 (m, 1H), 3.75 (d, 2H, *J* = 5.6 Hz), 2.04 (q, 2H, *J* = 7.0 Hz), 1.37-1.22 (m, 22H), 1.1 (s, 9H), 0.90 (t, 3H, *J* = 6.6 Hz); ^{13}C NMR ($CDCl_3$) δ 164.6, 138.0, 135.0, 132.5, 132.3, 132.1, 129.5, 129.31, 129.27, 129.2, 127.9, 127.3, 127.2, 122.7, 73.8, 65.2, 62.8, 31.8, 31.4, 29.1, 29.0, 28.9, 28.8, 28.6, 28.2, 26.1, 22.2, 18.6, 13.6.

*(2S,3R,4E)-2-Azido-3-O-benzoyl-D-erythro-sphingosine (**8**).* A solution of **7** (90 mg, 0.15 mmol) and imidazole (70 mg, 1.03 mmol) in dry THF (3 ml) was stirred over a 30-min period at -23 °C. A 1-M tetra-*n*-butylammonium fluoride THF solution (250 µL, 0.25 mmol) was added, and the resulting mixture was stirred at -23 °C for 4 h. The solvent was evaporated in a rotary evaporator and the crude product was purified by chromatography (elution with hexane/EtOAc 55:45) to give 48 mg (89%) of **8** as an oil; R_f 0.16 (hexane/-EtOAc, 9:1); ^{1}H NMR ($CDCl_3$) δ 8.0 (m, 2H), 7.52 (m, 1H), 7.39 (m, 2H), 5.88 (m, 1H), 5.55 (m, 2H), 3.74-3.67 (m, 2H), 3.57 (m, 1H), 2.01 (m, 2H), 1.52 (m, 4H), 1.25-1.00 (m, 22H),

0.78 (t, 3H, *J* = 4.0 Hz); ^{13}C NMR ($CDCl_3$) δ 181.0, 139.2, 133.7, 130.2, 128.9, 123.7, 75.0, 66.6, 62.4, 32.8, 32.3, 32.0, 30.0, 29.8, 29.7, 29.5, 29.1, 23.1, 23.0, 14.5.

*(2S,3R,4E)-1-O-(2',3',4',6'-Tetra-O-acetyl-**b**-D-galactopyranosyl)-2-azido-3-O-benzoyl-D-erythro-sphingosine (**9**).* A mixture of trichloroacetimidate (**4**, 45 mg, 0.090 mmol), 2-azido-3-*O*-benzoyl-D-*erythro*-sphingosine (**8**, 45 mg, 0.12 mmol), and 190 mg of dry 300AW molecular sieves in 2.5 ml of CH_2Cl_2 was stirred for 30 min. After the mixture was cooled to -23 °C a solution of $BF_3{\cdot}OEt_2$ (5 µl, 0.041 µmol) in CH_2Cl_2 (1 ml) was added dropwise over 10 min. The resulting mixture was stirred for 4 h at room temperature. The reaction was quenched by adding 2 ml of saturated aqueous $NaHCO_3$ solution and 2 ml of CH_2Cl_2. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 x 3 ml). The combined organic layers were dried (Na_2SO_4) and concentrated. The crude product was chromatographed (elution with hexane/EtOAc 2:1) to give (2*S*,3*R*,4*E*)-1-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-2-azido-3-*O*-benzoyl-D-*erythro*-sphingosine (51 mg, 74%) as an oil; R_f 0.10 (hexane/EtOAc 4:1); ^{1}H NMR ($CDCl_3$) δ 7.83 (dd, *J* = 5.4, 1.4 Hz, 2H), 7.38 (m, 1H), 7.24 (m, 2H), 5.70 (m, 1H), 5.40-5.29 (m, 2H), 5.15 (dd, 1H, *J* = 3.3, 3.2 Hz), 5.03-5.00 (m, 1H), 4.79 (d, 1H, *J* = 3.4, 3.5 Hz), 4.28 (d, 1H, *J* = 7.9 Hz), 4.20-4.07 (m, 2H), 3.75-3.66 (m, 3H), 3.39-3.37 (m, 1H), 1.96-1.77 (4s and m, 14H), 1.32 (s, 6H), 1.17-1.02 (m, 16H), 0.66 (t, 3H, *J* = 5.1 Hz); ^{13}C NMR ($CDCl_3$) δ 227.1, 168.6, 168.5, 167.7, 163.5, 137.5, 131.6, 128.3, 128.1, 126.8, 121.0, 99.3, 73.1, 69.2, 66.9, 66.3, 65.7, 65.3, 61.9, 60.9, 60.2, 60.0, 59.7, 59.6, 59.5, 30.7, 30.3, 28.0, 27.9, 27.8, 27.7, 27.5, 27.1, 21.0, 19.1, 19.0, 18.9, 12.5.

*N-Palmitoyl-D-erythro-6-[^{13}C]- **b**-GalSph* (**11**). To a stirred solution of **9** (51 mg, 0.070 mmol) in dry MeOH (1 ml) was added a solution of NaOMe (11 mg, 0.22 mmol) in 1

ml of dry MeOH. After 6 h of stirring, the reaction mixture was neutralized with Dowex 50W-X8 resin, which was removed by filtration through a plug of glass wool. The filtrate was concentrated and the residue was chromatographed (elution with CH_2Cl_2/MeOH 82.5:17.5) to give (2*S*,3*R*,4*E*)-β-D-galactopyranosyl-2-azido-D-*erythro*-sphingosine (**10**, 29 mg, 83%); R_f 0.43 ($CHCl_3$/MeOH 4:1). A solution of **10** (29 mg, 0.059 mmol), 4-nitrophenyl palmitate (55 mg, 0.092 mmol), and PPh_3 (20 mg, 0.079 mmol) in 3 ml of THF/H_2O 9:1 was stirred at room temperature for 72 h. After the disappearance of the starting material, the reaction mixture was concentrated in a rotary evaporator (2-PrOH was used to remove the residual H_2O). The light yellow residue was dissolved in 10 ml of Et_2O and the solution was washed with 1% aqueous Na_2CO_3 solution (2 x 2 ml) to remove the 4-nitrophenol byproduct. The organic phase was dried (Na_2SO_4) and concentrated. The residue was chromatographed (elution with $CHCl_3$/MeOH 4:1) to give 29.6 mg (71%) of [6-^{13}C]-GalCer (**11**) as a semisolid; R_f 0.37 ($CHCl_3$/MeOH 4:1); ^{1}H NMR ($CDCl_3$) δ 5.45-5.34 (m, 1H), 5.20-5.14 (m, 1H), 4.35-4.26 (m, 10H), 3.98-3.83 (m, 2H), 3.72-3.64 (m, 3H), 3.32-3.20 (m, 4H), 3.07 (t, 1H, *J* = 1.6 Hz), 1.97-1.87 (m, 2H), 1.75-1.72 (m, 1H), 1.32-0.89 (m, 42H), 0.60 (t, 6H, *J* = 6.5 Hz); ^{13}C NMR ($CDCl_3$) δ 174.3, 133.6, 128.9, 104.3, 76.7, 73.0, 71.5, 70.9, 68.5, 62.8, 61.88, 60.9, 60.86, 60.8, 60.7, 60.6, 53.0, 36.3, 31.9, 31.4, 29.2, 29.1, 29.0, 28.9, 28.88, 28.8, 25.5, 22.2, 13.4.

Scheme 1. [6-13C]GalCer synthesis.