Syntheses of Lactosyl Ceramide Analogues Carrying Novel Bifunctional BODIPY Dyes Directed towards the Differential Analysis of Multiplexed Glycosphingolipids by MS/MS using iTRAQ

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Supplementary Information

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General Procedures: All chemicals were purchased as reagent grade and used without further purification. Reactions were monitored using thin-layer chromatography (TLC) on a pre-coated plate of silica gel $60F_{254}$ (layer thickness, 0.25 mm; E. Merck, Germany). Spots were detected under UV (254 nm) and/or by staining with *p*-methoxybenzaldehyde–H_2SO_4–MeOH (1:2:17, v/v/v) or 0.5% ninhydrin *n*BuOH solution followed by heating for a few minutes. Flash column chromatography was performed using pre-packed disposable silica gel columns (normal phase, particle size 30 μm or 60 μm) from SHOKO SCIENCE, Inc. Open column chromatography was performed using silica gel 60 with 40–50 μm (Cica-Reagent). Preparative TLC was performed on Merck Art. 5745, Kieselgel 60 F254/0.50 nm thick plates. Size exclusion column chromatography was performed on Sephadex LH-20 (GE Healthcare Little Chalfont. UK) and MeOH as the eluent. The medium pressure liquid column chromatography (MPLC) purifications were performed on a Combi Flash® R_f.

IR spectra were obtained with a HORIBA FT-720 FREEXACT-II spectrometer with attenuated total reflection (ATR) method. ^{1}H NMR (500 MHz) spectra were recorded (Advance 500 spectrometer, Bruker Biospin Inc.) in a deuterated solvent using $(CH_3)_4Si$ (0.00 ppm) or the solvent peak (CD_3OD: 3.31 ppm) as the internal standard. ^{13}C NMR spectra were obtained by using the same NMR spectrometers and were calibrated with CD_3OD (δ = 49.00 ppm). Splitting pattern are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; brs, broad singlet for ^{1}H NMR data. Electrospray ionization high resolution (ESI-HR) mass spectra were recorded on Waters Syanpt G2 mass spectrometer equipped with a positive-mode.

Clog P (logarithm of the partition coefficient between water and octanol) values were calculated by ChemOffice Ultra 2001 (CambridgeSoft Corp., Cambrige, MA).

Synthesis of two novel bifunctional azido-BODIPY acid derivatives: 4,4-difluoro-3-(5-azido pentyl)-5-(2-carboxylethyl)-4-bora-3a,4a-diaza-s-indacene (9) and 4,4-difluoro-3-(5-azido pentyl)-5,7-dimethyl-6-(2-carboxylethyl)-4-bora-3a,4a-diaza-s-indacene (12)

3-(5-Formyl-1*H*-pyrrol-2-yl)-propionic acid methyl ester (**4**)

To a *N*,*N*-dimethylformamide (DMF, 2 g, 28 mmol) at 0 °C was added slowly phosphorous oxylchloride ($POCl_3$, 4.3 g, 28 mmol) while stirring under an argon atmosphere. The reaction mixture was stirred for 15 min at same temperature and 1,2-dichloroethane (8 mL) was added. To this mixture was added slowly a solution of 3-(1*H*-pyrrol-2-yl)-propionic acid methyl ester **3** (5.3 g, 25.3 mmol) in 1,2-dichloroethane (15 mL) at 0 °C under an argon atmosphere. The reaction mixture was then reflexed for 15 min, during which time there was copious evolution of hydrogen chloride gas. After cooling to room temperature, a solution of sodium acetate

trihydrate (38.1g, 280 mmol) in water (30 mL) was added to the reaction mixture. After refluxed for 15 min, then reaction mixture was cooled and the organic layer was separated. The aqueous layer was extracted with chloroform. The combined organic layer was washed with a saturated aqueous (sat. aq.) $NaHCO_3$, brine, dried over $MgSO_4$, and concentration. The resulting residue was purified using open silica gel column chromatography with hexane–EtOAc (5 : 1 to 3 : 1, v/v), to afford the title compound **4** (3.8 g, 83%) as white solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 10.23 (bs, 1H), 9.42 (s, 1H), 6.89 (d, 1H, J = 6.1 Hz), 6.09 (d, 1H, J = 6.1 Hz), 3.73 (s, 3H), 3.21 (t, 2H, J = 6.7 Hz), 2.27 (t, 2H, J = 6.8 Hz).

2-(5-Bromopentyl)-1*H*-pyrrole (**6**)

To a stirred solution of pyrrole **5** (1.26 mL, 18.2 mmol) in freshly diethyl ether (10 mL) under an argon atmosphere was added a solution of BuMgBr (10 mL, 20 mmol, 2.0 M solution in diethyl ether) at 0 °C and stirred at the same temperature for 1 h. A solution of 1,5-dibromopentane (5 mL, 36.4 mmol) in fresh diethyl ether was slowly added to the reaction mixture using a syringe. The resulting reaction mixture was stirred at the same temperature for 3 h and then was poured into a sat. aq. $NaHCO_3$, extracted with diethyl ether, died over $MgSO_4$, and concentrated under reduced pressure. The resulting residue was purified using flash column chromatography on silica gel (hexane–EtOAc = 10 : 1, v/v), to afford the title compound **6** (450 mg, 11%) with a 2-alkylated pyrrole/2,3-dialkylated pyrrole ratio of 3 : 1. The resulting isomeric mixtures were used for the next steps without further purification.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.91 (brs, 1H), 6.67 (dd, 1H, J = 2.5 Hz and J = 8.0 Hz), 6.12 (dd, 1H, J = 2.8 Hz and J = 11.4 Hz), 5.92 (s, 1H), 3.42 (t, 1H, J = 6.8 Hz), 2.26 (d, 2H, J = 7.5 Hz), 1.93–1.83 (m, 2H), 1.71–1.63 (m, 2H), 1.53–1.46 (m, 2H).

2-(5-Azidopentyl) -1*H*-pyrrole (**7**)

To a stirred solution of **6** (440 mg, 2.0 mmol) in DMSO (5 mL) at 50 °C was added slowly sodium azide (NaN_3, 260 mg, 4.0 mmol) and the reaction mixture stirred at the same temperature for overnight. The hot reaction mixture was poured into ice-water and diethyl ether. The organic layer was separated. The aqueous layer was washed with diethyl ether twice. The combined organic layer was successively washed with water, dried ($MgSO_4$), and concentration. The resulting residue was purified using flash column chromatography with hexane–EtOAc (10 : 1, v/v), to afford the title compound **7** (quantitative) with a 2-alkylated pyrrole/2,3-dialkylated pyrrole ratio of 3 : 1. The resulting isomeric mixtures were used for the next steps without further purification.

IR ν_{max} (ATR)/cm^{-1} 2094 (N_3); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.91 (bs, 1H), 6.67 (dd, 1H, J = 2.5 Hz and J = 8.1 Hz), 6.12 (dd, 1H, J = 4.0 Hz and J = 11.4 Hz), 5.92 (s, 1H), 3.30–3.23 (m,

4H), 2.26 (d, 2H, J = 7.5 Hz), 1.70–1.58 (m, 4H), 1.47–1.39 (m, 2H).

4,4-difluoro-3-(5-azido pentyl)-5-(2-methoxycarbonylethyl)-4-bora-3a,4a-diaza-s-indacene (**8**)
5-Azidopentyl pyrrole **7** (135 mg, 0.76 mmol) and carboxylaldehyde pyrrole **4** (125 mg, 0.69 mmol) were dissolved in EtOH (10 mL). The resulting mixture was cooled to 0 °C, followed by addition of hydrobromic acid (HBr,113 µL, 2.1 mmol, 48% solution in water) and was stirred vigorously at the same temperature. After 1 h stirring, TLC analysis showed complete consumption of the starting materials. The crude product was co-evaporation with fresh dichloromethane and stirred in fresh dichloromethane (5 mL) at 0 °C under an argon atmosphere. Triethylamine (Et_3N, 288 µL, 2.1 mmol) and $BF_3{\cdot}Et_2O$ (349 µL, 1.38 mmol) were added slowly. After 3 h, the solution was warmed to room temperature, filtered over short silica gel column and concentrated. The resulting residue was purified using flash column chromatography with hexane–EtOAc (10 : 1, v/v), to afford the title compound **8** (17 mg, 18%) as an orange red solid.
IR ν_{max} (ATR)/cm^{-1} 2094 (N_3); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.10 (s, 1H), 7.00 (d, 1H, J = 4.1 Hz), 6.96 (d, 1H, J = 4.0 Hz), 6.36 (d, 1H, J = 4.1 Hz), 6.32 (d, 1H, J = 4.0 Hz), 3.71 (s, 3H, OCH_3), 3.31 (t, 2H, J = 7.5 Hz), 3.29 (t, 2H, J = 6.9 Hz), 3.01 (t, 2H, J = 7.7 Hz), 2.78 (t, 2H, J = 7.7 Hz), 1.82–1.73 (m, 2H), 1.70–1.63 (m, 2H), 1.55–1.24 (m, 2H): ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.8, 163.6, 159.6, 134.8, 134.4, 130.9, 129.8, 127.6, 118.6, 118.0, 51.8, 51.3, 33.1, 28.7, 28.6, 28.1, 26.6, 24.1; ESI-HRMS (*m/z*) calcd for $C_{18}H_{22}BF_2N_5O_2Na^+$: 412.1732; Found: 412.1730.

4,4-difluoro-3-(5-azido pentyl)-5-(2-carboxylethyl)-4-bora-3a,4a-diaza-s-indacene (**9**)
A suspension of the BODIPY **8** (16 mg, 41.1µmol) and Lipase PS Amano (160 mg) in DMF (3 mL) and potassium phosphate buffer (KPB,1 mL, 50 mM, pH 6.7) at room temperature. After TLC (toluene–EtOAc, 1 : 1, v/v) analysis to check that the starting material was consumed and then was poured into water and diethyl ether. The organic layer was separated. The aqueous layer was washed with diethyl ether twice. The combined organic layer was successively washed with water, dried ($MgSO_4$), and concentration. The resulting residue was purified using open column chromatography with toluene–EtOAc (1 : 1, v/v), to afford the title compound **9** (14 mg, 91%) as an orange solid.
IR ν_{max} (ATR)/cm^{-1} 2094 (N_3); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.11 (s, 1H), 7.01 (d, 1H, J = 4.2 Hz), 6.96 (d, 1H, J = 4.1 Hz), 6.36 (d, 1H, J = 4.2 Hz), 6.34 (d, 1H, J = 4.1 Hz), 3.32 (t, 2H, J = 7.5 Hz), 3.29 (t, 2H, J = 7.1 Hz), 3.01 (t, 2H, J = 7.8 Hz), 2.84 (t, 2H, J = 7.6 Hz), 1.82–1.74 (m, 2H), 1.71–1.62 (m, 2H), 1.55–1.47 (m, 2H): ^{13}C NMR (125 MHz, $CDCl_3$): δ 177.9, 163.6, 159.4, 134.8, 134.4, 130.9, 129.9, 127.6, 118.6, 118.0, 51.3, 33.0, 28.7, 28.6, 28.1, 26.7, 24.7; λ_{Abs} =

508 nm, λ_{em} = 515 nm and extinction coefficient (ε) = 38,000 $cm^{-1}M^{-1}$ in water.

4,4-difluoro-3-(5-azido pentyl)-5,7-dimethyl-6-(2-methoxycarbonylethyl)-4-bora-3a,4a-diaza-s-indacene (**11**)

5-Azidopentyl pyrrole **7** (84 mg, 0.4 mmol) and carboxylaldehyde pyrrole **10** (78.4 mg, 0.44 mmol) were dissolved in EtOH (3 mL). The resulting mixture was cooled to 0 °C, followed by addition of hydrobromic acid (HBr, 65 μL, 1.2 mmol, 48% solution in water) and was stirred vigorously at the same temperature. After 1 h stirring, TLC analysis showed complete consumption of the starting materials. The crude product was coevaporation with fresh dichloromethane and stirred in fresh dichloromethane (5 mL) at 0 °C under an argon atmosphere. Triethylamine (Et_3N, 166 μL, 1.2 mmol) and $BF_3{\cdot}Et_2O$ (202 μL, 0.8 mmol) were added slowly. After 2 h, the solution was warmed to room temperature, filtered over short silica gel column and concentrated. The resulting residue was purified using PTLC with hexane–EtOAc (2 : 1, v/v), to afford the title compound **11** (105 mg, 63%) as an orange red solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.03 (s, 1H), 6.87 (d, 1H, J = 4.0 Hz), 6.25 (d, 1H, J = 4.0 Hz), 3.68 (s, 3H, OCH_3), 3.28 (t, 2H, J = 6.9 Hz), 2.97 (t, 2H, J = 7.8 Hz), 2.72 (t, 2H, J = 8.0 Hz), 2.54 (s, 3H, CH_3), 2.45 (t, 2H, J = 8.0 Hz), 2.19 (s, 3H, CH_3), 1.80–1.72 (m, 2H), 1.70–1.62 (m, 2H), 1.54–1.46 (m, 2H): ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.9, 159.4, 158.4, 139.8, 134.1, 133.1, 129.4, 128.0, 123.1, 116.4, 51.8, 51.3, 33.9, 29.7, 28.6, 28.4, 28.3, 26.7, 19.4, 12.9, 9.6; ESI-HRMS (*m/z*) calcd for $C_{20}H_{26}BF_2N_5O_2Na^+$: 440.2045; Found: 440.2045.

4,4-difluoro-3-(5-azido pentyl)-5,7-dimethyl-6-(2-carboxylethyl)-4-bora-3a,4a-diaza-s-indacene (**12**)

A suspension of the BODIPY **11** (113 mg, 0.24 mmol) and Lipase PS Amano (565 mg) in DMF (5 mL)–potassium phosphate buffer (KPB, 1.5 mL, 50 mM, pH 6.7) at room temperature. After TLC (toluene–EtOAc, 1 : 1, v/v) analysis to check that the starting material was consumed and then was poured into water and diethyl ether. The organic layer was separated. The aqueous layer was washed with diethyl ether twice. The combined organic layer was successively washed with water, dried ($MgSO_4$), and concentration. The resulting residue was purified using open column chromatography with toluene–EtOAc (1 : 1, v/v), to afford the title compound **12** (87 mg, 90%) as an orange solid.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.04 (s, 1H), 6.88 (d, 1H, J = 4.0 Hz), 6.26 (d, 1H, J = 4.0 Hz), 3.28 (t, 2H, J = 6.9 Hz), 2.97 (t, 2H, J = 7.8 Hz), 2.74 (t, 2H, J = 7.5 Hz), 2.55 (s, 3H, CH_3), 2.50 (t, 2H, J = 7.5 Hz), 2.20 (s, 3H, CH_3), 1.80–1.72 (m, 2H), 1.70–1.62 (m, 2H), 1.52–1.46 (m, 2H): ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.8, 159.7, 158.3, 139.8, 134.0, 133.2, 129.0, 128.2,

123.2, 116.5, 51.4, 33.6, 29.7, 28.6, 28.4, 28.3, 26.6, 19.2, 13.0, 9.6; λ_{Abs} = 513 nm, λ_{em} = 524 nm and extinction coefficient (ε) = 56,000 $cm^{-1}M^{-1}$ in water.

Synthesis of LacSphBODIPY analogues 1 and 2

LacSphBODIPY analogues (**1**)

D-Lactosyl-β-1-1′-D-*erythro*-sphingosine (5 mg, 8 μmol) and BODIPY **9** (3.6 mg, 9.6 μmol) were dissolved in THF (1 mL) and water (0.25 mL). To the solution was added DMT-MM (8 mg, 28 μmol), then the mixture was stirred for 16 h at room temperature. TLC (EtOAc–MeOH–H_2O = 8.5 : 1.5 : 1.0, v/v) confirmed complete disappearance of the stating material. The resulting residue was purified using PTLC (EtOAc–MeOH–H_2O = 8.5 : 1.5 : 1.0, v/v), to afford the title compound **1** (6.4 mg, 82%).

R_f 0.38 (EtOAc–MeOH–H_2O = 8.5 : 1.5 : 1.0); IR ν_{max} (ATR)/cm^{-1} 2094 (N_3); ^{1}H NMR (500 MHz, CD_3OD): δ 7.45 (S, 1H, BODIPY), 7.17 (d, 1H, J = 4.0 Hz, BODIPY), 7.12 (d, 1H, J = 3.9 Hz, BODIPY), 6.48 (d, 1H, J = 4.0 Hz, BODIPY), 6.46 (d, 1H, J = 3.9 Hz, BODIPY), 5.73 (dt, 1H, J = 6.7 Hz and J = 14.6 Hz, olefinic), 5.48 (dd, 1H, J = 7.7 Hz and J = 15.3 Hz, olefinic), 4.37 (d, 1H, $J_{1', 2'}$ = 7.6 Hz, H-1′), 4.33 (d, 1H, $J_{1, 2}$ = 7.8 Hz, H-1), 4.18 (dd, 1H, J = 4.8 Hz and J = 10.1 Hz, OCH_2-), 4.12 (t, 1H, J = 7.9 Hz, C*H*OH), 4.07–4.01 (m, 1H, -C*H*NH-), 3.96 (dd, 1H, 1H, $J_{5, 6a}$ = 2.4 Hz and $J_{6a, 6b}$ = 12.2 Hz, H-6a), 3.86 (dd, 1H, $J_{5, 6b}$ = 4.1 Hz, H-6b), 3.84 (d, 1H, $J_{3', 4'}$ = 4.2 Hz, H-4′), 3.81 (dd, 1H, $J_{5', 6'a}$ = 7.5 Hz and $J_{6'a, 6'b}$ = 11.4 Hz H-6′a), 3.74 (dd, 1H, $J_{5', 6'b}$ = 11.3 Hz H-6′b), 3.60–3.53 (m, 5H, H-4, 2′, 5′, OCH_2), 3.56 (t, 1H, $J_{2, 3}$ = 8.8 Hz, H-3), 3.47 (dd, 1H, $J_{2', 3'}$ = 9.7 Hz, H-3′), 3.49–3.42 (m, 1H, H-5), 3.38–3.29 (m, 1H, H-2), 3.28 (t, 2H, J = 7.6 Hz, -CH_2- BODIPY), 3.01 (t, 2H, J = 7.7 Hz, -CH_2- BODIPY), 2.66 (t, 2H, J = 7.6 Hz, -CH_2- BODIPY), 2.19–2.03 (m, 2H, -C$H_2$$(CH_2)_{11}CH_3$), 1.87–1.77 (m, 2H, -C$H_2$- BODIPY), 1.74–1.67 (m, 2H, -CH_2- BODIPY), 1.58–1.49 (m, 2H, -CH_2- BODIPY), 1.38–1.21 (m, 22H, -CH_2(CH_2)$_{11}CH_3$), 0.94–0.85 (m, 3H, -$CH_2(CH_2)_{11}CH_3$): ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.2, 164.5, 161.2, 136.3, 135.9, 135.2, 132.3, 131.3, 131.1, 129.3, 119.7, 119.0, 105.1, 104.5, 80.6, 77.1, 76.5, 76.3, 74.8, 73.1, 72.5, 70.3, 69.8, 62.5, 61.8, 54.9, 52.3, 35.8, 33.3, 33.1, 30.8, 30.8, 30.8, 30.7, 30.5, 30.4, 30.4, 29.7, 29.4, 27.7, 25.7, 23.7, 14.5; ESI-HRMS (*m/z*) calcd for $C_{47}H_{75}BF_2N_6O_{13}$: 1003.5351; Found: 1003.5353.

LacSphBODIPY analogues (**2**)

D-Lactosyl-β-1-1′-D-*erythro*-sphingosine (5 mg, 8 μmol) and BODIPY **12** (4.8 mg, 12 μmol) were dissolved in THF (1 mL) and water (0.25 mL). To the solution was added DMT-MM (8 mg, 28 μmol), then the mixture was stirred for 1 d at room temperature. TLC (EtOAc–MeOH–H_2O = 8.5 : 1.5 : 1.0, v/v) confirmed complete disappearance of the stating material. The resulting residue was purified using PTLC (EtOAc–MeOH–H_2O = 8.5 : 1.5 : 1.0,

v/v) to afford the title compound **2** (6.8 mg, 84%).

R_f 0.33 (EtOAc:MeOH:H_2O = 8.5:1.5:1.0); IR ν_{max} (ATR)/cm^{-1} 2094 (N_3); ^{1}H NMR (500 MHz, CD_3OD): δ 7.39 (S, 1H, BODIPY), 7.03 (d, 1H, J = 3.8 Hz, BODIPY), 6.36 (d, 1H, J = 3.9 Hz, BODIPY), 5.67 (dt, 1H, J = 6.8 Hz and J = 14.2 Hz, olefinic), 5.43 (dd, 1H, J = 7.8 Hz and J = 15.3 Hz, olefinic), 4.35 (d, 1H, $J_{1',2'}$ = 7.7 Hz, H-1′), 4.18 (dd, 1H, J = 4.8 Hz and J = 10.1 Hz, OCH_2-), 4.12 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.07 (t, 1H, J = 7.9 Hz, CHOH), 3.97–3.93 (m, 1H, -CHNH-), 3.86–3.77 (m, 4H, H-4′, 6′a, OCH_2), 3.74 (dd, 1H, $J_{2',3'}$ = 11.3 Hz, H-3′), 3.63–3.58 (m, 3H, H-6a, 6b, 2′), 3.53–3.48 (m, 2H, H-5, 6′b), 3.44 (t, 1H, $J_{3,4}$ = 9.1 Hz, H-4), 3.42 (t, 1H, $J_{2,3}$ = 8.8 Hz, H-3), 3.26 (t, 1H, $J_{2,3}$ = 8.3 Hz, H-2), 3.22–3.17 (m, 1H, H-5′), 2.95 (t, 2H, J = 7.7 Hz, -CH_2- BODIPY), 2.89–2.71 (m, 2H, -CH_2- BODIPY), 2.53 (s, 3H, CH_3 BODIPY), 2.36 (t, 2H, J = 7.3 Hz, -CH_2- BODIPY), 2.27 (s, 3H, CH_3 BODIPY), 2.09–1.94 (m, 2H, -CH_2(CH_2)$_{11}$$CH_3$), 1.83–1.74 (m, 2H, -C$H_2$- BODIPY), 1.74–1.64 (m, 2H, -CH_2- BODIPY), 1.59–1.49 (m, 2H, -CH_2- BODIPY), 1.42–1.21 (m, 22H, -CH_2(CH_2)$_{11}$$CH_3$), 0.94–0.85 (m, 3H, -$CH_2$($CH_2$)$_{11}CH_3$): ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.5, 160.3, 159.8, 141.9, 135.5, 135.1, 134.6, 131.2, 131.1, 129.6, 125.1, 117.6, 105.1, 104.5, 80.6, 77.1, 76.2, 74.9, 72.9, 72.6, 70.3, 69.9, 62.5, 61.7, 56.4, 56.3, 54.8, 52.4, 37.0, 33.4, 33.1, 30.8, 30.8, 30.8, 30.7, 30.6, 30.5, 30.3, 29.6, 29.5, 29.4, 27.7, 23.7, 21.3, 14.5, 13.2, 9.7; ESI-HRMS (*m/z*) calcd for $C_{49}H_{79}BF_2N_6O_{13}$: 1031.5664; Found: 1031.5680.

Preparation of alkyne-iTRAQ (113 *m/z*) reagent

2-(4-Methyl-piperazin-1-yl)-*N*-propyn-2-yl-acetamide

(4-Methyl-piperazin-1-yl) acetic acid (20 mg, 0.126 mmol) and propynyl amine (17 µL, 0.126 mmol) were dissolved in THF (1 mL). To the solution was added DMT-MM (40 mg, 0.14 mmol), then the mixture was stirred for 3 h at room temperature. The solvent was removed in vacuo and the crude product was purified by open chromatography on silica gel (CH_3Cl–MeOH = 4 : 1, v/v) to afford the title compound (15 mg, 61%).

ESI-Q-TOF $[M + H]^+$ = *m/z* 196.14, MS/MS of $[M + H]^+$ produced *m/z* 113.1 fragment; ^{1}H NMR (500 MHz, CD_3OD): δ 4.01 (S, 2H), 3.27 (S, 4H), 3.17 (S, 2H), 2.83 (s, 3H), 2.81 (s, 4H), 2.51 (s, 1H).

Synthesis of LacSphBODIPY (LacCer analogue)2 labeled with iTRAQ (*m/z* = 113)

To a stirred solution of LacSphBODIPY **2** (2.4 mg, 2.38 µmol) in *t*BuOH (0.25 mL) were added aqueous solution of $CuSO_4 \cdot 5H_2O$ (4.0 mM, 60 µL, 10 mol%) and sodium ascorbate (4.0 mM, 120 µL, 20 mol%). The reaction mixture was stirred at room temperature. The reaction was monitored by TLC and MALDI-TOF MS until the starting material was completely consumed.

Upon completion of the reaction, the solvent was removed in *vacuo* and the crude product was purified by size-exclusion chromatography (Sephadex LH-20, MeOH) to give the title compound (2.6 mg, 91%) as a red solid. The resulting compounds were analyzed with ESI-Q-TOF mass spectrometer.

Preparation of LacCer analogues 1- and 2-BSA complexes

A complex of LacCer analogues **1** and **2** with defatted BSA (DFBSA, Sigma-Aldrich Corp., Mo, USA) was prepared as described.[1] Briefly, 50 μmol of the desired LacCer analogues in methanol stock solution was completely dried using SpeedVac. The dried lipid was dissolved in 400 μL of ethanol and added dropwise to 600 μL of Dulbecco's phosphate-buffered saline (D-PBS) containing 33 mg BSA while being vortex mixed. The complex was dialyzed overnight at 4 °C against 500 ml of D-PBS, ultracentrifuged (100,000× g) at 4 ^{o}C for 20 min of which supernatant was collected and then frozen at –20 ^{o}C in eppendorf tube for storage. Before use, the complexes were diluted with Dulbecco's modified Eagle's medium (D-MEM) as required.

Live cell imaging and localization analysis of LacCer analogues 1 and 2 using a confocal fluorescence microscopy

PC12D cells were cultured in D-MEM that contained 10% horse serum, 5% fetal bovine serum, and glutamine at 37 ^{o}C with 5% CO_2. Subconfluent cells on collagen-coated glass coverslips were rinsed twice with saline solution, then treated with 2.5 μM of **1**– or **2**–BSA complex and LacSphBODIPY TR-X (Golgi marker) / BSA complex, respectively, in D-MEM, and subsequently processed for cell imaging experiments. Cell imaging was performed with an Olympus Fluoview FV-1000D confocal microscope with 60× oil immersion objective lens (NA = 1.35). The microscope equipped with a controlled environmental chamber that maintains a 5% CO_2/humidified atmosphere at 37 ^{o}C. For LacCer analogues **1** and **2**, excitations were set to 488 nm and emissions were collected 519 nm. For LacSphBODIPY BODIPY TR-X, excitations were set to 559 nm and emissions were collected 612 nm. Images were analyzed using Olympus Fluoview software (version 3.1a).

A time-course quantitative study for the Glogi localization of LacCer analogues 1 and 2

The LacCer analogue (either compound **1** or **2**) and Golgi marker were incorporated into the cell as the BSA conjugates, whereby similar concentration of compounds **1**, **2**, and Golgi marker were added simultaneously to the cell and the cell were monitored for up to 60 min using confocal microscopy. Fluorescence intensity in the Golgi and plasma membrane (PM) of each cell was quantified using Olympus Fluoview software (version 3.1a). Fluorescence intensity

profiles of Golgi and PM taken a line that intersect of each cell. Based on these lines, integrated fluorescence intensities were calculated for the Golgi and the PM. Golgi/PM ratios were calculated for a series of images using five to seven different cells and plotted versus time. All Golgi/PM ratios were expressed as mean ± s.e.m.

MS analysis of iTRAQ-tagged compound 2

ESI-Qq-TOF MS

The samples were analyzed using QSTAR Elite, quadrupole time-of-fight (Qq-TOF) mass spectrometer (AB Sciex, Foster City, CA, USA) equipped with nanoelectrospray ionization interface. The sample was directly infused into electrospray ionization mass spectrometers using a Nanospray Tip (Humanix, Hiroshima, Japan). The general mass spectrometric parameters were as follows: polarity = positive ion mode, ion source gas (nitrogen) pressure = 1 psi, ion spray voltage = 0.8 kV, and scan range = from 0 to 1500 *m/z*. Sample was dissolved in 50% (v/v) aqueous acetonitrile containing 1% (v/v) formic acid. In MS/MS experiment, ion spray voltage was from 0.9 to 1.0 kV and collision energy was adjusted from 40 to 50 V using nitrogen as the collision gas.

References

1. O. C. Martin and R. E. Pagano, *J. Cell. Biol.*, 1994, **125**, 769–781.