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SUPPORTING INFORMATION

Title: New GM1 Ganglioside Derivatives for Selective Single and Double Labelling of the Natural Glycosphingolipid Skeleton

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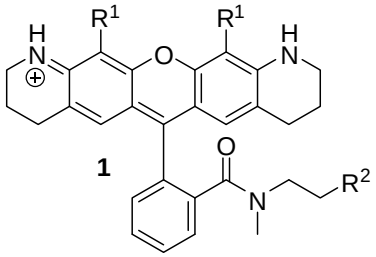
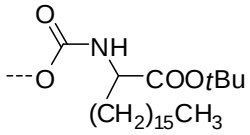
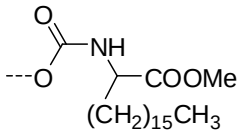
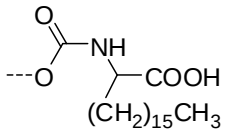
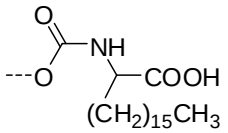
Ref. No.: O200900645

Experimental Procedures

Table 1S. Model fluorescent dye **1** and its conjugates with 2-aminostearic acid.

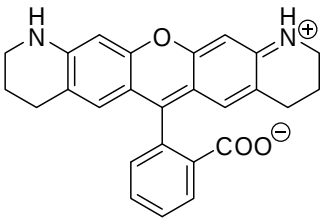


1

	R^1	R^2		R^1	R^2
1a	H	OH	**1b**	H	COOMe
1i	SO_3H		**1k**	SO_3H	
1j	H		**1l**	SO_3H	

Compound 1a: To a solution of rhodamine Q (0.50 g, 1.2 mmol) and 2-(*N*-methylamino)ethanol (0.44 g, 0.47 mL, 5.9 mmol) in *N,N*-dimethylacetamide (10 mL) was added HATU (0.61 g, 1.6 mmol), and the mixture was stirred at room temperature for 19 h, followed by the addition of HATU (0.30 g, 0.79 mmol). After 17 h, the solvent was evaporated in vacuo at ambient

temperature, and the product was isolated by chromatography on SiO_2 (130 g) eluting with CH_2Cl_2/MeOH (10:1, v/v), yield 0.54 g (96%) as a bordeaux solid. ^{1}H NMR (300 MHz, [D_6]-DMSO, ppm): δ = 1.80 (br. s, $(CH_2)\underline{CH_2}(CH_2)$, 4 H), 2.67 (br. s, $ArCH_2$, 4 H), 2.84 (m, 2 rotamers, NMe, 3 H), 3.18 (m, $\underline{CH_2}NCH_3$, 2 H), 3.41 (br. s, CH_2N, 4 H), 3.47 (m, $\underline{CH_2}OH$, 2 H), 6.65 (s, H-4/5, 2 H), 6.79 (m, H-1/8, 2 H), 7.39–7.46 (m, H-3', 1 H), 7.59–7.76 (m, H-4'/5'/6', 3 H), 8.77 (br. s, NH, 2 H). ESI-MS, positive mode, *m/z* ($C_{29}H_{29}N_3O_3$): 468.5 $[M + H]^+$.



Rhodamine Q

Compound 1b: To a solution of rhodamine Q (0.50 g, 1.2 mmol) and methyl 3-(*N*-methylamino)propionate (0.36 g, 0.34 mL, 3.1 mmol) in *N,N*-dimethylacetamide (10 mL) was added HATU (0.61 g, 1.6 mmol), and the mixture was stirred at room temperature for 2 days. The solvent was evaporated in vacuo at ambient temperature, and the product was isolated by chromatography on SiO_2 (150 g) eluting with CH_2Cl_2/MeOH (10:1, v/v), yield 0.34 g (56%) as a bordeaux solid. ^{1}H NMR (300 MHz, [D_6]-DMSO, ppm): δ = 1.79 (br. s, $(CH_2)\underline{CH_2}(CH_2)$, 4 H), 2.04 (t, $^3J_{H,H}$ = 6.9 Hz, $(CH_2)\underline{CH_2}(CO_2CH_3)$, 2 H), 2.65 (br. s, $ArCH_2$, 4 H), 2.86 (s, NMe, 3 H), 3.31 (t, $^3J_{H,H}$ = 6.9 Hz, $\underline{CH_2}NCH_3$, 2 H), 3.39 (br. s, CH_2N, 4 H), 3.48 (m, OMe, 3 H), 6.65 (s, H-4/5, 2 H), 6.78 (br. s, H-1/8, 2 H), 7.44–7.50 (m, H-3', 1 H), 7.58–7.64 (m, H-6', 1 H), 7.66–7.74 (m, H-4'/5', 2 H), 8.79 (br. s, NH, 2 H). ESI-MS, positive mode, *m/z* ($C_{31}H_{31}N_3O_4$): 510.5 $[M + H]^+$. HR-MS (ESI, positive mode): calcd: 510.2387; found 510.2384 $[M + H]^+$.

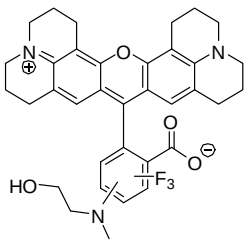
Compound 1i: To compound **1e**[1] (0.68 M solution in *N,N*-dimethylacetamide, 44 µL, 30 µmol) was added Et_3N (4.0 µL, 29 µmol) followed by **4**-*t*BuO,NH_2[2] (10 mg, 28 µmol), and the mixture was stirred at room temperature for 2 days. The solvent was evaporated in vacuo at ambient temperature, and the title product was isolated by chromatography on SiO_2 (30 g) eluting with CH_2Cl_2/MeOH (8:1, v/v), yield 17 mg (58%). ESI-MS, positive mode, *m/z* ($C_{52}H_{72}N_4O_{12}S_2$): 1053.4 $[M - H + 2Na]^+$, negative mode: 1007.5 $[M - H]^-$, 1029.5 $[M - 2H + Na]^-$.

Compound 1j: To a solution of **1i** (17 mg, 17 µmol) in trifluoroacetic acid (5.0 mL), was added at 0 ºC triethylsilane (1.0 g, 1.38 mL, 8.7 mmol), and the mixture was stirred at room temperature for 1 day. The solvent was evaporated in vacuo at ambient temperature, and the title product was isolated by chromatography on SiO_2 (20 g) eluting with CH_2Cl_2/MeOH (8:1, v/v), yield 7 mg (52%) of a red-purple solid. ESI-MS, positive mode, *m/z* ($C_{48}H_{64}N_4O_6$): 793.6 $[M + H]^+$, 815.5 $[M + Na]^+$, negative mode: 791.5 $[M - H]^-$. HR-MS (ESI, positive mode): calcd: 793.4899; found 793.4896 $[M + H]^+$.

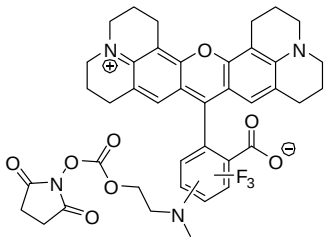
Methyl Ester 1k: To compound **1g** (50 mg, 0.08 mmol) dissolved in anhydrous DMAA (5 mL), di(*N*-succinimidyl) carbonate (45 mg, 0.17 mmol) and Et_3N (0.17 mL, 1.20 mmol) were added under N_2 with stirring. The reaction mixture was left at room temperature for 1 h. Then **4**-MeO,NH_2*HCl (84 mg, 0.24 mmol) was added, and the reaction mixture was stirred at room temperature for 48 h. The volatile materials were removed under reduced pressure, and the residue was purified by column chromatography on SiO_2 (50 mL), eluting with CH_2Cl_2/MeOH (5:1) to yield the title compound (70 mg, 91%) as a red powder. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.85 (t, 3H, Me), 1.22 (m, 28 H, 14×CH_2, H-4‴ to H-17‴), 1.70 (m, 2 H, CH_2-3‴), 1.85 (s, 4 H, 2×CH_2, H-3/9), 2.65 (m, 9 H, NMe and 3×CH_2, H-1″ and H-4/8), 3.10–3.65 (m, 6 H, 3×CH_2, H-2″ and H-

2/10), 3.70 (m, 3 H, MeO), 4.20 (m, 1 H, CH-2‴), 5.94 (m, 1 H, NH-CO), 6.72–6.87 (m, 2 H, CH-5/7), 7.36 (m, 1 H, CH-6′), 7.50–7.70 (m, 3 H, 3×CH, H-3′/4′/5′), 9.80–10.00 (m, 2 H, 2×NH) ppm. ^{13}C NMR ($CDCl_3$, 125.7 MHz): δ = 14.0 (Me), 19.0 (2×CH_2, C-3/9), 23.0 (CH_2), 25.5 (CH_2), 28.0 (2×CH_2, C-4/8), 29.5 (CH_2), 32.0 (CH_2), 39.5 (NMe), 42.6 (2×CH_2, C-2/10), 47.0 (CH_2-1″), 51.3 (OMe), 53.0 (CH-2‴), 62.5 (CH_2-2″), 111.5 (C), 111.7 (C), 112.3 (C), 112.7 (C), 124.3 (C), 127.9 (CH), 129.2 (CH), 129.6 (CH), 130.0 (CH), 130.3 (CH), 130.6 (CH), 136.5 (C), 151.6 (C), 152.2 (C), 152.7 (C), 154.7 (C), 156.4 (CO), 168.0 (CO), 174.0 (CO) ppm. ESI-MS, negative mode, *m/z* (rel. int., %): 965 (100) $[M - H]^-$, positive mode, *m/z* (rel. int., %): 1011 (100) $[M - H + 2Na]^+$. HR-MS (ESI, positive mode): found: 967.4185, 984.4452, and 989.4002; calcd. for $C_{49}H_{66}N_4O_{12}S_2$: 967.4191 $[M + H]^+$, 984.4457 $[M + NH_4]^+$, and 989.4011 $[M + Na]^+$.

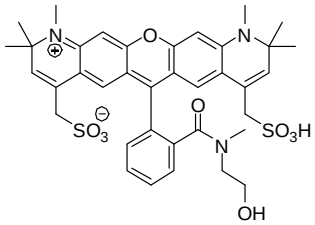
Acid 1l: To a suspension of the methyl ester **1k** (50 mg, 0.05 mmol) in anhydrous THF (20 ml), was added dry LiI (60 mg, 0.45 mmol), and the mixture was heated under reflux in a closed tube (ALDRICH, "Ace Glass" polymerization tube) at 100 °C (bath) for 48 h and cooled to room temperature. TFA (4 µL, 0.05 mmol) was then added, the solvent was removed under reduced pressure, and the residue was purified by preparative TLC (silica gel) eluting with CH_2Cl_2/MeOH (3:1) to yield the title compound (28 mg, 57%) as a red powder. ^{1}H NMR ([D_6]-DMSO, 300 MHz): δ = 0.85 (t, 3 H, Me), 1.23 (m, 28 H, 14×CH_2, H-4‴ to H-17‴), 1.41–1.70 (m, 4 H, 2×CH_2, H-3/9), 1.78 (m, 2 H, CH_2-3‴), 2.59 (s, 1 H, NMe), 2.60–2.81 (m, 4 H, 2×CH_2, H-4/8), 2.88–3.03 (m, 2 H, CH_2-1″), 3.10–3.45 (m, 2 H, CH_2-2″), 3.48–3.63 (m, 4 H, 2×CH_2, H-2/10), 3.90 (m, 1 H, CH-2‴), 5.35 (m, 1 H, NH-CO), 6.68–6.84 (m, 2 H, CH-5/7), 7.28–7.52 (m, 3 H, 2×CH, H-4′/5′), 7.59–7.74 (m, 2 H, 2×CH, H-3′/6′), 10.16–10.28 (m, 2 H, 2×NH) ppm. ^{13}C NMR ([D_6]-DMSO, 125.7 MHz): δ = 13.8 (Me), 19.0 (2×CH_2, C-3/9), 22.0 (CH_2), 25.1 (CH_2), 27.2 (2×CH_2, C-4/8), 28.9 (CH_2), 31.2 (CH_2), 41.6 (2×CH_2, C-2/10), 54.7 (CH-2‴), 59.2 (CH_2-2″), 112.1 (C), 112.8 (C), 116.1 (C), 118.4 (C), 124.1 (C), 128.6 (CH), 128.7 (CH), 129.4 (CH), 130.3 (CH), 145.5 (C), 151.3 (C), 151.6 (C), 157.5 (C), 157.8 (CO), 167.5 (CO), 174.0 (CO) ppm. ESI-MS, negative mode, *m/z* (rel. int., %): 951 (100) $[M - H]^-$, positive mode, *m/z* (rel. int., %): 997 (100) $[M - H + 2Na]^+$. HR-MS (ESI, positive mode): found: 953.4031, 975.3854; calcd. for $C_{48}H_{64}N_4O_{12}S_2$: 953.4035 $[M + H]^+$, 975.3854 $[M + Na]^+$.



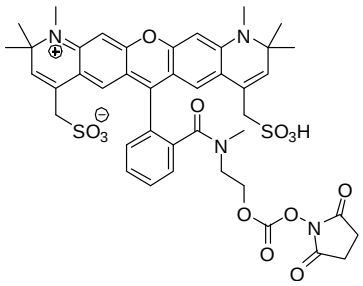
Compound 2-OH: To a stirred solution of **20** (5.6 mg, 0.01 mmol) in anhydrous DMF (0.2 mL), was added *N*-metylaminoethanol (20 µL), and the mixture was stirred at room temperature for 12 h. The solvent was evaporated under reduced pressure, and the title product was isolated by chromatography on SiO_2 (20 mL) eluting with CH_2Cl_2/MeOH (10:1). Yield: 5.7 mg (92%) of a violet solid. ^{1}H NMR (300 MHz, CD_3OD): δ = 1.90–2.02 (m, 4 H, CH_2), 2.03–2.15 (m, 4 H, CH_2), 2.66–2.88 (m, 4 H, CH_2C), 2.98–3.12 (m, 7 H, CH_3N and CH_2C), 3.25–3.34 (m, 4 H, CH_2O, CH_2N), 3.46–3.58 (m, 6 H, CH_2N), 3.72 (t, J = 5.8, 2 H, CH_2N), 6.88 (s, 2 H, CH) ppm. ^{19}F NMR (282.4 MHz, CD_3OD): δ = –120.1 (t, J = 10, 1F), –143.1 (dd, J = 5 and 20, 1F), –144.8 (dd, J = 12 and 20, 1F) ppm. ESI-MS, positive mode, *m/z* (rel. int., %): 618.3 $[M]^+$ (100). HR-MS (ESI, positive mode): found: 618.2580; calcd. for $C_{35}H_{34}F_3N_3O_4$: 618.2574 $[M + H]^+$.



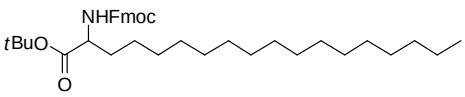
Compound 2-OCONHS: The compound **2**-OH (6.2 mg, 0.01 µmol) was stirred with di(*N*-succinimidyl) carbonate (25.6 mg, 0.10 µmol) and NEt_3 (25 µL) in anhydrous DMF (1 mL) at room temperature for 3 h. The title product was isolated by preparative HPLC (A/B: 60/40 → 100/0 in 25 min, detection at 254 nm, t_R = 6.5 min). ESI-MS, positive mode, *m/z* (rel. int., %): 759.3 $[M]^+$ (100). HR-MS (ESI, positive mode): found: 759.2634; calcd. for $C_{40}H_{38}F_3N_4O_8^+$: 759.2636 $[M]^+$.



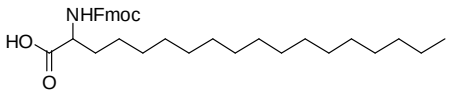
Compound 3-OH: To a solution of compound **21** (65.4 mg, 0.1 mmol) and NEt_3 (50 µL) in anhydrous DMF (1 mL), was added HATU (76.0 mg, 0.2 mmol) followed by *N*-methylaminoethanol (80 µL, 1.0 mmol), and the mixture was stirred at 50 ºC for 24 h. After evaporation of the solvent under reduced pressure, the title compound was isolated by chromatography on SiO_2 (70 mL) eluting with MeOH/Et_2O (1:1 + 0.5% NEt_3). Yield: 45 mg (61%) of a violet powder. ^{1}H NMR (300 MHz, D_2O): δ = 1.62 (s, 6 H, CH_3), 1.68 (s, 6 H, CH_3), 2.71/2.79 (s, 3 H, NCH_3), 3.05–3.11 (m, 2 H, NCH_2), 3.26 (s, 6 H, NCH_3), 3.38 (m, 2 H, CH_2O), 3.80–3.91 (m, 2 H, CH_2S), 3.99–4.10 (m, 2 H, CH_2S), 6.07 (s, 2 H, CH=), 6.85 (s, 2 H, CH), 7.28 (s, 2 H, CH), 7.62–7.70 (s, 1 H, CH), 7.76–7.83 (s, 1 H, CH), 7.90–8.00 (s, 2 H, CH) ppm. ESI-MS, negative mode, *m/z* (rel. int., %): 734.4 $[M - H]^-$ (100). HR-MS (ESI, positive mode): found: 736.2356; calcd. for $C_{37}H_{41}N_3O_9S_2$: 736.2357 $[M + H]^+$.



Compound 3-OCONHS: The compound **3**-OH (37 mg, 0.05 mmol) was stirred with di(*N*-succinimidyl) carbonate (51 mg, 0.2 mmol) and NEt_3 (0.1 mL) in anhydrous DMF (0.6 mL) at room temperature for 1 h. The product was isolated by preparative HPLC (A/B: 20/80 → 50/50 in 25 min, detection at 254 nm, t_R = 11.5 min). ESI-MS, negative mode, *m/z* (rel. int., %): 875.3 $[M - H]^-$ (100). HR-MS (ESI, positive mode): found: 877.2416; calcd. for $C_{42}H_{44}N_4O_{13}S_2$: 877.2419 $[M + H]^+$.



***tert*-Butyl (2*R*/*S*)-[(9-Fluorenylmethoxycarbonyl)amino]octadecanoate (4-*t*BuO,NHFmoc)**:[3] To a cold solution of *tert*-butyl (2*R*/*S*)-aminostearate (0.142 g, 0.40 mmol) in 1,4-dioxane (8 mL), was added in small portions FmocCl (0.124 g, 0.48 mmol) followed by $NaHCO_3$ (0.2 g). The reaction mixture was stirred at room temperature for 24 h, diluted with EtOAc (50 mL), washed with water (2×20 mL) and dried. Concentration under reduced pressure afforded an oil, which was dissolved in CH_2Cl_2 (5 mL), and the solution was filtered through silica gel (50 mL) to afford the title compound (0.15 g, 65%). HR-MS (ESI, positive mode): found: 578.4202; calcd. for $C_{37}H_{55}NO_4$: 578.4204 $[M + H]^+$.



(2*R*/*S*)-[(9-Fluorenylmethoxycarbonyl)amino]octadecanoic Acid (4-OH,NHFmoc):[3] To a solution of **4**-*t*BuO,NHFmoc (0.145 g, 0.25 mmol) in CH_2Cl_2 (0.5 mL), were added at 0 °C Et_3SiH (50 µL) and TFA (0.5 mL), and the mixture was kept at 4 °C for 20 h. Evaporation in vacuo gave the title compound as an oil (0.125 g, 96%). HR-MS (ESI, positive mode): found: 522.3575; calcd. for $C_{33}H_{47}NO_4$: 522.3578 $[M + H]^+$.

18-Hydroxyoctadecanoic Acid (**10a**): A solution of the acid **9** (1.3 g, 4.4 mmol) in Et_2O (120 mL) was hydrogenated with H_2 (1 atm) over PtO_2 (65 mg) at room temperature for 3 h, filtered through Celite®, and concentrated to give the title compound (1.3 g, 99%) as a colorless solid with m. p. 99–100 ºC (91–93 ºC,[4] 95–96 ºC[5]).

18-Bromooctadecanoic Acid (**10b**): To a solution of **10a** (0.6 g, 2.0 mmol) in AcOH (9.4 mL), was added 48% aqueous HBr (9.4 mL), and the mixture was refluxed for 18 h. Solvents were evaporated in vacuo, and the product was filtered through SiO_2 (100 mL) with hexane/EtOAc (2:1, v/v, + 0.5% AcOH) to give **10b** (0.71 g, 98%) as a colorless solid, m. p. = 80 ºC (74–75.5 ºC[6]).

Chromatographic Behavior:

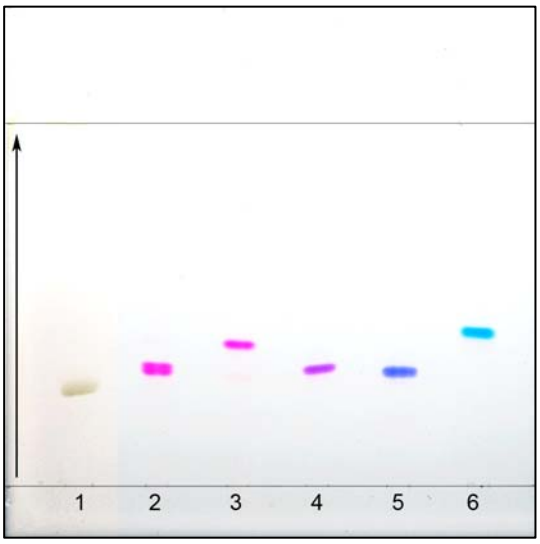


Figure A. High performance thin layer chromatography (HPTLC) of fluorescent labelled gangliosides presented in the Schemes 3, 5, and 8. Lane 1: natural ganglioside GM1; lane 2: product **5**; lane 3: product **23a**; lane 4: product **17b**; lane 5: product **23b**; lane 6: product **17a**. Solvent system: chloroform/methanol/15 mM $CaCl_2$ (60:35:8 v/v). Lane 1 was visualized by treatment with an anisaldehyde reagent followed by heating at 150 °C for 5 min.

Literature

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