Supporting information for:

Neutral Push-Pull Chromophores for Nonlinear Optical Imaging of Cell Membrane.

Cyril Barsu, Rouba Cheaib, Stéphane Chambert, Yves Queneau, Olivier Maury, Davy Cottet, Hartmut Wege, Julien Douady, Yann Bretonnière and Chantal Andraud.
SUPPORTING INFORMATION PARAGRAPH

General

$^1$H and $^{13}$C NMR spectra were recorded at room temperature on Bruker AC 200, ALS300, DRX300 or DRX500 spectrometers. $^{13}$C NMR spectra were recorded with complete proton decoupling. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as internal standard.$^1$ For proton, data are reported as follows: chemical shift, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, b=broad), coupling constants in Hz. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 20°C. Microanalyses and high-resolution mass spectra were performed at the Service Central d’Analyse du CNRS (Vernaison, France). UV/vis absorption measurements were recorded on a JASCO V550 spectrometer. Fluorescence spectra were measured using a Horiba-Jobin Yvon Fluorolog-3 spectrofluorimeter, equipped with a red-sensitive Hamamatsu R928 photomultiplier tube. Spectra were reference corrected for both the excitation source light intensity variation (lamp and grating) and the emission spectral response (detector and grating). All solvents were of spectrophotometric grade. Coumarin 153 laser grade was purchased from Acros.

All air- or moisture-sensitive reactions were carried out in flame-dried glassware under Ar atmosphere. Tetrahydrofuran (THF) was freshly distilled over sodium/benzophenone ketyl, CH$_2$Cl$_2$ and triethylamine (Et$_3$N) over CaH$_2$. Anhydrous DMF was purchased from Acros. Thin-layer chromatography (tlc) was performed with Merck 60F254 precoated silica gel plates. Column chromatography was carried out using Merck silica gel 60 (70-230 mesh). 4-ethynyl-2,6-bis(diethylcarbamoyl)pyridine (12a), 4-ethynyl-2,6-bis(di-n-octylcarbamoyl)pyridine (12b) and 2-amino-7-iodo-9,9-di-n-hexylfluorene (6)$^2$, carboxymethyl-3,4,6-tri-O-acetyl-α-D-glucopyranoside-2-O-lactone (15)$^3$ and carboxymethyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-acetyl-α-D-glucopyranoside-2-O-lactone (16)$^4$, Dichlorobis(triphenylphosphine)-palladium(II)
(Pd(PPh₃)₂Cl₂)⁵ and Methyl 3,4,5-tris(2-(2-methoxyethoxy)ethoxy)benzoate ⁶ were obtained according to published protocols.

**Synthesis of 7.**

1-bromoethanol (4.73mL, 50.48mmol) was added under argon to a solution of 6 (6.0g, 12.62mmol), Na₂CO₃ (2.68g, 25.24mmol) and NaI (7.57, 50.47mmol) in anhydrous DMF (35mL). The mixture was heated at 95°C until completion (48h) as shown by tlc (SiO₂, eluent: Pentane - Ethyl Acetate 2/1 v/v). After cooling to room temperature, the mixture was poured into water (40mL) and extracted with diethyl ether (3x40mL). The combined organic phases were dried with brine, over Na₂SO₄, filtered and evaporated to afford a brown oil that was purified by column chromatography (eluent Pentane - Ethyl Acetate 2/1 v/v). Yield: greenish oil 4.7g, 66%. Anal. Calcd. for C₂₉H₄₂INO₂: C 61.81 H 7.51 N 2.49. Found: C 61.73 H 7.3 N 2.45. ¹H NMR (CDCl₃): δ 7.58-7.43 (m, 2H), 7.29-7.26 (m, 1H), 6.63 (d, 1H, J = 8.5 Hz), 6.57 (s, 1H), 3.87 (t, 4H, J = 4.6 Hz), 3.62 (t, 4H, J = 4.6 Hz), 3.52 (s, 2H), 1.87-1.71 (m, 4H), 1.19-0.97 (m, 12H), 0.84-0.76 (m, 6H), 0.73-0.64 (m, 4H). ¹³C NMR (CDCl₃): δ 152.3, 151.9, 148.0, 141.1, 135.6, 131.6, 129.8, 120.6, 120.0, 111.8, 106.6, 89.9, 60.8, 55.7, 55.1, 40.4, 31.4, 29.6, 23.6, 22.6, 14.0.

**Synthesis of 8.** To a solution of 7 (4.67g, 8.29mmol), Phthalimide (2.44g, 16.57mmol, 2eq.) and Triphenylphosphine (4.35g, 16.57mmol, 2eq.) in anhydrous THF (90mL) was added dropwise a 40% solution of Diethyl Azodicarboxylate in toluene (7.60mL, 16.57mmol, 2eq.). The mixture was stirred at room temperature for 18 hours. The solvent was removed under reduced pressure and resulting solid suspended in Ethyl Acetate and filtered. The solvent was removed and the crude orange oil purified by column chromatography (eluent: Ethyl Acetate). Yield: 6.50g (95%) orange solid. Anal. Calcd. for C₄₅H₄₈IN₃O₄·H₂O: C 64.36 H 6.00 N 5.00. Found: C 64.40 H 6.09 N 5.29. ¹H NMR (CDCl₃): δ 7.82 (dd, 4H, J = 5.4 Hz, J = 3.0 Hz), 7.67 (dd, 4H, J = 5.3Hz, J = 3.1 Hz), 7.55 (s, 1H), 7.53 (d, 1H, J = 8.0 Hz), 7.41 (d, 1H, J = 7.8 Hz), 7.25 (s, 2H), 7.22 (d, 1H, J = 8.2 Hz), 6.99 (s, 1H), 6.89 (d, 1H, J = 8.4 Hz), 3.93 (t, 4H, J = 7.0 Hz), 3.69 (t, 4H, J = 7.1 Hz), 1.93-1.81 (m, 4H), 1.08-0.98 (m, 12H), 0.81-
0.76 (m, 6H), 0.73-0.66 (m, 4H). $^{13}$C NMR (CDCl$_3$): $\delta$ 168.1, 152.6, 152.4, 147.2, 141.3, 135.4, 133.9, 132.0, 131.6, 129.8, 123.3, 120.7, 120.0, 111.1, 106.8, 89.8, 55.3, 48.8, 40.2, 34.8, 31.4, 29.6, 23.6, 22.6, 14.0.

4-chloro-2,6-bis(di-n-octylcarbamoyl)pyridine 10b.

Anhydrous DMF (0.5mL) is added to a solution of chelidamic acid monohydrate (2.00g, 9.94mmol) in thionyl chloride (20mL) and mixture is heated to reflux for 2 hours. Thionyl chloride was distilled off under reduced pressure. Toluene was added to the resulting solid and removed under reduced pressure to remove any remaining thionyl chloride. The solid was then dissolved in dichloromethane (15mL), the solution cooled to 0°C. Triethylamine (11mL, 78.9mmol, 8eq.) and di-n-octylamine (6.01mL, 19.9mL, 2eq.) were added with caution. The mixture was then heated to reflux for 2 hours. Water was added and the pH brought to 1 with concentrated HCl. The aqueous phase was extracted twice with dichloromethane. The combined organic phases were washed with saturated hydrogen carbonate solution, dried over sodium sulfate, filtered and evaporated. The crude product was dissolved in ethylacetate and filtered through a plug of silica. Yield: 5.82g, 90 %, brown oil. Rf (SiO$_2$, pentane-diethyl ether: 15-1 v/v): 0.63. Anal. Calcd. for C$_{39}$H$_{70}$ClN$_3$O$_2$·0.3H$_2$O: C 71.64 H 10.88 N 6.43. Found: C 71.66 H 10.91 N 6.52. $^1$H NMR (CDCl$_3$): $\delta$ 7.59 (s, 2H), 3.43 (t, 4H, J = 7.6 Hz); 3.24 (t, 4H, J = 7.5 Hz), 1.65-1.47 (m, 8H), 1.30-1.25 (m, 32H), 1.19-1.05 (m, 8H), 0.86-0.80 (m, 12H). $^{13}$C NMR (CDCl$_3$): $\delta$ 166.9, 154.8, 145.9, 124.2, 48.8, 45.9, 31.8, 31.7, 29.3, 29.2, 29.1, 28.9, 27.4, 27.0, 26.6, 22.6, 14.0.

4-iodo-2,6-bis(di-n-octylcarbamoyl)pyridine 11b.

HI (57% aqueous solution, 10.5mL, 79.8mmol, 9eq.) is added dropwise to a mixture of 4-chloro-2,6-bis(di-n-octylcarbamoyl)pyridine 10b (5.82g, 8.97mmol, 1eq.) and phosphorous acid (395mg, 4.8mmol, 1 eq.) cooled at 0°C. After the addition was completed, the reaction mixture was heated to 80°C for 3.5h. After cooling to room temperature, the mixture was neutralized by slow addition of a
saturated NaHCO₃ aqueous solution. The aqueous phase was extracted with diethyl ether (3x30mL). The combined organic phases were washed with saturated NaHCO₃ solution, half saturated Na₂S₂O₅, dried over sodium sulfate, filtered and evaporated. The crude product was purified by column chromatography (eluent petroleum ether-ether 15-5 v/v) to yield 5.10g of 11b as yellow oil (76%). Rf (SiO₂, pentane-ether: 15-5 v/v): 0.6. Anal. Calcd. for C₃₉H₇₀IN₃O₂: C 63.31 H 9.54 N 5.68. Found: C 63.59 H 9.83 N 5.61. ¹H NMR (CDCl₃): δ 7.97 (s, 2H), 3.44 (t, 4H, J = 7.6 Hz); 3.25 (t, 4H, J = 7.5 Hz), 1.64-1.46 (m, 8H), 1.32-1.24 (m, 32H), 1.20-1.05 (m, 8H), 0.87-0.81 (m, 12H). ¹³C NMR (CDCl₃): δ 166.6, 153.7, 132.9, 106.6, 48.8, 45.9, 31.8, 29.2, 28.9, 27.4, 27.0, 26.6, 22.6, 14.0.

4-(trimethylsilylethynyl)-2,6-bis(di-n-octylcarbamoyl)pyridine.

In a Schlenck tube, 4-iodo-2,6-bis(di-n-octylcarbamoyl)pyridine 11b (5.0g, 6.75mmol) and trimethylsilylacetylene (1.43ml, 10.1mmol, 1.5eq.) were dissolved in a 1/1 v/v mixture of THF and Et₃N (160mL) and the solution degassed by bubbling argon. CuI (257mg, 1.35mmol) and Pd(PPh₃)₂Cl₂ (470mg, 0.6mmol) were added, the tube closed and the mixture stirred at room temperature for 4 days. The mixture was filtered through a plough of Celite, which was thoroughly washed with ether. The filtrate was washed with saturated NH₄Cl aqueous solution (2x100mL), brine (50ml), was dried over Na₂SO₄, filtered and the solvent evaporated. The crude product was purified by column chromatography (eluent CH₂Cl₂) to yield 4.18g of yellow oil (82%). Rf (SiO₂, CH₂Cl₂-2.5% diethyl ether v/v): 0.45. Anal. Calcd. for C₃₄H₇₉N₃O₂Si: C 74.41 H 11.21 N 5.92. Found: C 74.01 H 11.43 N 5.63. ¹H NMR (CDCl₃): δ 7.59 (s, 2H), 3.44 (t, 4H, J = 7.6 Hz), 3.25 (t, 4H, J = 7.5 Hz), 1.64-1.53 (m, 8H), 1.28-1.13 (m, 32H), 0.88-0.83 (m, 8H), 0.24-0.18 (m, 12H). ¹³C NMR (CDCl₃): δ 166.6, 153.7, 132.9, 106.6, 48.8, 45.9, 31.8, 29.2, 28.9, 27.4, 27.0, 26.6, 22.6, 14.0.

4-(ethynyl)-2,6-bis(di-n-octylcarbamoyl)pyridine 12b.
Oven-dried K$_2$CO$_3$ (2.10g, 15.3mmol, 5eq.) was added to a solution of 4-(trimethylsilyl-ethynyl)-2,6-bis(di-\textit{n}-octylcarbamoyl)pyridine (2.18g, 3.07mmol.) in methanol (10mL). The mixture was stirred under argon for 2 hours. Water (50mL) was added and the solution was extracted with pentane (3x20mL). The combined organic phases were dried over Na$_2$SO$_4$, filtered and evaporated to afford a brown oil that was purified by column chromatography (eluent pentane-diethyl ether 2/1 v/v) and used immediately. Yield: 1.47g, 75%. Rf (SiO$_2$, pentane-diethyl ether 2/1 v/v): 0.52. Anal. Calcd. for C$_{41}$H$_{71}$N$_3$O$_2$: C 77.18 H 11.22 N 6.59. Found: C 77.13 H 11.22 N 6.59. $^1$H NMR (CDCl$_3$): $\delta$ 7.59 (s, 2H), 3.44 (t, 4H, $J$ = 7.6 Hz), 3.32 (s, 1H), 3.21 (t, 4H, $J$ = 7.5 Hz), 1.64-1.46 (m, 8H), 1.09-1.27 (m, 32H), 0.81 (t, 12H).

**Synthesis of 13a.** In a Schlenck tube, 8 (2.18g, 2.65mmol, 1eq.) and 13a (800mg, 2.65mmol, 1eq.) were dissolved in a 1/1 v/v mixture of THF and Et$_3$N (30mL) and the solution degassed by bubbling argon. Cul (81mg, 0.43mmol) and Pd(PPh$_3$)$_2$Cl$_2$ (149mg, 0.21mmol) were added, the tube closed and the mixture stirred at 50°C for 2 days. After cooling to room temperature, the mixture was filtered through a plough of Celite, which was then thoroughly washed with ether. The filtrate was washed with saturated NH$_4$Cl aqueous solution (2x100mL), brine (50ml). It was dried over Na$_2$SO$_4$, filtered and the solvent evaporated. The crude product was purified by column chromatography (eluent CH$_2$Cl$_2$ to 20% Ethyl Acetate in CH$_2$Cl$_2$ v/v) to yield 1.72g of 13a as yellow solid (65%). Rf (SiO$_2$, CH$_2$Cl$_2$-ethyl acetate 4/1 v/v): 0.2. Anal. Calcd. for C$_{62}$H$_{70}$N$_6$O$_6$: C 73.75 H 7.15 N 8.32. Found: C 73.74 H 7.44 N 8.38. $^1$H NMR (CDCl$_3$): $\delta$ 7.83 (dd, 4H, $J$ = 5.4 Hz, $J$ = 3.0 Hz), 7.69 (dd, 4H, $J$ = 5.3Hz, $J$ = 3.1 Hz), 7.68 (s, 2H), 7.48-7.41 (m 4H), 7.02 (s, 1H), 6.91 (d, 1H, $J$ = 8.5 Hz), 3.94-3.91 (m, 4H), 3.74-3.70 (m, 4H), 3.55 (q, 4H, $J$ = 7.1 Hz), 3.34 (q, 4H, $J$ = 7.1 Hz), 2.03-1.97 (m, 4H), 1.25 (t, 6H, $J$ = 7.1 Hz), 1.15 (t, 6H, $J$ = 7.1 Hz), 1.07-0.98 (m, 12H), 0.75-0.62 (m, 10H). $^{13}$C NMR (CDCl$_3$): $\delta$ 168.1, 167.7, 153.7, 150.2, 147.4, 143.4, 134.2, 134.0, 132.0, 131.1, 129.8, 126.1, 125.1, 123.3, 121.2, 118.2, 117.4, 111.1, 106.8, 97.8, 85.7, 55.1, 48.7, 43.2, 40.3, 40.1, 34.8, 31.5, 29.7, 23.7, 22.6, 14.3, 14.0, 12.8.
Synthesis of 13b. Compound 13b was obtained from 8 (1.42g, 1.73mmol, 1eq.) and 12b (1.10g, 1.73g, 1eq.), CuI (33mg) and Pd(PPh₃)₂Cl₂ (60mg) using the same experimental procedure as for 13a. Purification by column chromatography (eluent CH₂Cl₂ to 5% Ethyl Acetate in CH₂Cl₂ v/v) yielded 1.39g of yellow oil (60%). Rf (SiO₂, 5% Ethyl Acetate in CH₂Cl₂): 0.56. Anal. Calcd. for C₈₆H₁₁₈N₆O₆: C 77.35 H 8.93 N 6.31. Found: C 77.39 H 9.14 N 6.17. ¹H NMR (CDCl₃): δ 7.85-7.79 (m, 4 H), 7.72-7.66 (m, 6H), 7.49-7.40 (m, 4H), 7.03 (s, 1H), 6.90 (d, 1H, J = 8.5 Hz), 3.94-3.90 (m, 4H), 3.74-3.71 (m, 4H), 3.47 (t, 2H, J = 6.9 Hz), 3.29 (t, 2H, J = 6.9 Hz), 2.00 (bm, 4H), 1.66-1.55 (m, 8H), 1.32-1.06 (m, 60H), 0.88-0.81 (m, 12H), 0.69 (t, 6H), 0.63 (m, 4H). ¹³C NMR (CDCl₃): δ 168.1, 167.7, 153.7, 133.9, 132.0, 123.7, 77.6, 77.0, 76.3, 55.13, 45.8, 31.8, 31.7, 31.4, 29.6, 29.3, 29.2, 29.1, 28.9, 27.4, 27.0, 26.6, 22.6, 22.5, 14.0, 13.9.

3,4,5-tris(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)benzoic acid 14.

A solution of LiOH.H₂O (820mg, 19.49mmol.) in Water (10mL) was added dropwise to a solution of Methyl 3,4,5-tris(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)benzoate (2.43g, 3.9mmol) in Methanol (30mL). The mixture was stirred at room temperature for 3h. Methanol was evaporated and Water (20mL) was added. The solution was washed with CH₂Cl₂ (10mL) and acidified with concentrated HCl until a troubled mixture was obtained (pH=1). The solution was then extracted with CH₂Cl₂ (2x10mL) and the combined extracts dried over Na₂SO₄, filtered and evaporated to afford colorless oil (2.36g, 100%) that was used without further purification. Rf (SiO₂, CH₂Cl₂ – Acetone – Methanol - Water 56-20-20-4 v/v): 0.66. ¹H NMR (CDCl₃): δ 7.35 (s, 2H), 4.26-4.18 (m, 6H), 3.89-3.56 (m, 30H), 3.37 (s, 3H), (s, 6H), ¹³C NMR (CDCl₃): δ 170.4, 152.5, 143.5, 124.1, 109.8, 72.6, 72.1, 71.0, 70.9, 70.7, 69.8, 69.1, 59.2.

Probe 2a. To a solution of 13a (500mg, 0.50mmol) and Crotty Alcohol (2-buten-1-ol, 643μL, 7.54mmol) in anhydrous THF (9mL) was added Hydrazine Hydrate (244μL, 5.02mmol). The solution was heated under reflux for 18 hours. The white precipitate was filtered off and the filtrate evaporated to dryness at 90°C under vacuum. The yellow solid obtained was purified by column chromatography.
eluent: acetone-water 19/1 v/v to 5% Et3N in acetone-water 19/1 v/v). Yield: 350 mg (95%), bright yellow solid. Rf (SiO2, 5% Et3N in Acetone - Water 19/1 v/v): 0.22. m.p. 236°C. Anal. Calcd for C46H66N6O2.2H2O: C 74.75 H 9.55 N 11.37. Found: C 73.35 H 9.03 N 9.96. 1H NMR (CDCl3): δ 7.68 (s, 2H), 7.53-7.38 (m, 4H), 6.89-6.65 (m, 2H), 3.91-3.86 (m, 1H), 3.59-3.57 (m, 2H), 3.54 (q, 4H, J = 7.1 Hz), 3.33 (q, 4H, J = 7.1 Hz), 3.24-3.21 (m, 2H), 2.96 (t, 3H, J = 6.5 Hz), 1.95-1.83 (m, 4H), 1.24 (t, 6H, J = 7.1 Hz), 1.14 (t, 6H, J = 7.1 Hz), 1.07-0.98 (m, 12H), 0.77-0.61 (m, 10H). 13C NMR (CDCl3): δ 167.7, 153.7, 149.9, 148.8, 143.4, 134.2, 131.2, 129.2, 126.1, 125.1, 121.1, 118.7, 117.3, 111.6, 109.9, 106.5, 97.7, 85.7, 66.9, 54.9, 43.2, 40.4, 40.1, 39.7, 31.4, 29.6, 23.6, 22.5, 14.2, 14.0, 12.7.

**Probe 2b.** See the procedure for 2a. Compound 2b was obtained from 13b (1.37mg, 1.03mmol) as bright yellow gummy solid in 90% yield (1.01g) after column chromatography (eluent: Acetone - Water 19/1 v/v to 2% Et3N in Acetone - Water 19/1 v/v): Rf (SiO2, 2% Et3N in Acetone - Water 19/1 v/v): 0.25. HRMS (ESI): Calcd for C70H114N6O2: 1071.9081. Found 1071.9089. 1H NMR (CDCl3): δ 7.68 (s, 2H), 7.52-7.39 (m, 4H), 6.68 (d, 1H, J = 7.1 Hz), 6.65 (s, 1H), 3.67 (bs, 4H), 3.48 (m, 8H), 3.28 and 2.97 (bm, 8H), 1.91 (m, 4H), 1.54-1.05 (m, 52H), 0.80-0.72 (m, 22H). 13C NMR (CDCl3): δ 167.7, 153.7, 153.1, 149.8, 148.6, 143.2, 134.0, 131.1, 129.3, 126.0, 125.2, 121.1, 118.1, 117.4, 111.6, 106.5, 97.5, 85.7, 54.9, 48.8, 45.8, 40.4, 39.6, 31.8, 31.7, 31.4, 30.2, 29.6, 29.3, 29.2, 29.1, 28.9, 27.4, 27.0, 26.6, 23.6, 22.6, 22.5, 14.0, 13.9.

**Probe 3a.** To a solution of 2a (71mg, 0.096mmol) and 14 (137mg, 0.22mmol, 2.3eq) in dry CH2Cl2 was added DiC (35µL, 0.23mmol, 2.3eq) and HOBt (30mg, 0.22mmol 2.3eq).The reaction was monitored by tlc (SiO2, eluent: Acetone) and the solution was stirred at room temperature until completion. A white precipitate was filtered off, washed with CH2Cl2. The filtrate was washed with 2M NaOH (5mL), 2M HCl (5mL), saturated aqueous NaHCO3, dried over Na2SO4, filtered and evaporated. The crude yellow oil was purified by column chromatography (eluent: CH2Cl2 to 10% MeOH in CH2Cl2 v/v) to yield 51% (110mg) of a yellow oil (51%). Rf SiO2, 2% MeOH in CH2Cl2: S 8
Rf (SiO₂, 10% MeOH in CH₂Cl₂): 0.5. HRMS (ESI): Calcd for C₁₀₂H₁₅₈N₆O₂₈ 2Na (M+2Na)²⁺: 980.5455. Found: 980.5458. ¹H NMR (CDCl₃): δ 7.68 (s, 2H), 7.56-7.40 (m, 4H), 6.96 (d, 1H, J=8 Hz), 6.84 (s, 1H), 4.15 (m, 10H), 3.78 (m, 6H), 3.66-3.53 (m, 72H), 3.33 (m, 18H), 1.94 (m, 4H), 1.25 and 1.15 (t and t, 6H and 6H), 1.02 (bm, 6H), 0.72 (t, 6H, J=), 0.62 (bm, 4H).

**Probe 3b.** See the procedure for 3a. Compound 3b was obtained as yellow oil from 2b (177mg, 0.16mmol) and 14 (235mg, 0.38mmol, 2.3eq), DiC (60µL, 0.38mmol, 2.3eq) and HOBt (51mg, 0.38mmol 2.3eq). Column chromatography (eluent: CH₂Cl₂ to 10% MeOH in CH₂Cl₂ v/v) afforded 215mg of a yellow oil (Yield: 58%). Rf (SiO₂, 5% MeOH in CH₂Cl₂): 0.2. HRMS (ESI): Calcd for C₁₂₆H₂₀₆N₆O₂₈ 2Na (M+2Na)²⁺: 1148.7333. Found: 1148.7337. ¹H NMR (CDCl₃): δ 7.66 (s, 2H), 7.53 (d, 1H, J = 8.1 Hz), 7.45 (d, 1H, J = 8.1 Hz), 7.39 (s, 1H), 7.09 (s, 4H), 6.96 (d, 1H, J=7.1 Hz), 4.13 (m, 12H), 3.77-3.33 (3m, 76H), 3.33 (s, 6H), 3.32 (s, 12H), 1.89 (m, 4H), 1.53 (2m, 8H), 1.26-1.01 (m, 54H), 0.79 (2t, 12H), 0.71 (t, 6H), 0.68 (m, 4H). ¹³C NMR (CDCl₃): δ 167.7, 167.3, 153.7, 153.5, 152.4, 149.9, 148.4, 143.2, 141.4, 134.0, 131.1, 129.5, 129.3, 126.0, 125.2, 121.3, 118.1, 117.5, 111.4, 107.2, 106.2, 97.5, 85.8, 72.3, 71.8, 70.5, 70.4, 70.3, 69.6, 69.0, 58.9, 58.8, 55.0, 50.8, 48.8, 45.8, 40.3, 38.1, 31.8, 31.7, 31.4, 29.6, 29.3, 29.2, 29.1, 28.9, 27.4, 27.0, 26.6, 23.7, 22.6, 22.5, 14.0, 13.9.

**Probe 4a.** To a solution of diamine 2a (20 mg, 27 µmol) in anhydrous CH₂Cl₂ (1mL) was added 2eq. of carboxymethyl-3,4,6-tri-O-acetyl-a-D-glucopyranoside-2-O-lactone 15 (21 mg, 60 µmol). The solution was stirred at room temperature for 12 hours. The solvent was evaporated and the obtained residue was subjected to a short silica gel column chromatography (eluent: CH₂Cl₂-MeOH 20/1). The obtained product was then deacetylated in anhydrous methanol (1mL) using a catalytic amount of sodium methoxide. The mixture was stirred at room temperature for 16 hours, the solvent was removed and the residue was subjected to a flash silica gel chromatography (eluent: CH₂Cl₂-acetone-methanol-water 78/10/10/2) to afford compound 4a (14.4 mg, 12 µmol, 45%). Rf (SiO₂, CH₂Cl₂-acetone-methanol-water 56/20/20/4): 0.67. HRMS (ESI) Calcd. for C₆₂H₉₀N₆O₁₆ Na (M+Na)⁺: 1197.6311. Found: 1197.6304. [α]D +44 (c = 0.5, CH₃OH). ¹H NMR (300 MHz, CD₃OD-CDCl₃ 2/1): δ 7.59 (s, 2H), 7.51-7.37 (m, 4H), 6.76 (m, 2H), 4.76 (d, 2H, J = 3.6 Hz), 4.15 (d, 2H, J = 15.5 Hz), 3.93 (d, 2H, J = 15.5 Hz), 3.72-3.62 (m, 6H), 3.50-3.43 (m, 16H), 3.34-3.25 (m, 8H), 1.97 (m, 4H), 1.20 (t,
6H, J = 7.1 Hz), 1.13 (t, 6H, J = 7.1 Hz), 0.98 (m, 12H), 0.67 (t, 6H, J = 7.1 Hz), 0.54 (m, 4H). $^{13}$C NMR (125 MHz, CD$_3$OD-CDCl$_3$ 2/1): δ 171.8, 168.9, 154.5, 154.2, 150.8, 148.9, 144.7, 135.5, 131.9, 126.8, 125.4, 125.4, 122.1, 118.9, 117.9, 111.9, 106.8, 100.3, 99.3, 85.9, 74.3, 73.6, 72.6, 70.9, 67.5, 62.0, 55.8, 51.4, 44.3, 41.1, 37.4, 32.6, 32.2, 30.0, 24.4, 23.2, 14.5, 14.3, 12.9.

Probe 4b. See the procedure for 4a. Diamine 2b (80 mg, 75 μmol) and carboxymethyl-3,4,6-tri-O-acetyl-a-D-glucopyranoside-2-O-lactone 15 (57 mg, 165 μmol) gave, after flash silica gel chromatography (elucent: CH$_2$Cl$_2$-acetone-methanol-water 78/10/10/2) the probe 4b (89 mg, 59 μmol, 74%). Rf (SiO$_2$, CH$_2$Cl$_2$-acetone-methanol-water 56/20/20/4): 0.65. HRMS (ESI) Calcd. for C$_{86}$H$_{138}$O$_{16}$N$_{6}$ H (M+H)$^+$: 1512.0248. Found: 1512.0252. $^{[α]}$D +37 (c = 1, CH$_3$OH). $^1$H NMR (500 MHz, CD$_3$OD-CDCl$_3$ 9/1): δ 7.59 (s, 2H), 7.51 (m, 2H), 7.37 (m, 2H), 6.87 (s, 1H), 6.82 (d, 1H, J = 8.4 Hz), 4.76 (2H, under water peak), 4.14 (d, 2H, J = 15.6 Hz), 3.94 (d, 2H, J = 15.6 Hz), 3.63 (dd, 2H, J = 12.0 Hz, J = 2.9 Hz), 3.61 (m, 4H), 3.51 (m, 4H), 3.43 (m, 12H), 3.24 (m, 6H), 1.97 (m, 4H), 1.75 (m, 4H), 1.64 (m, 4H), 1.30-0.97 (m, 54H), 0.81 (t, 6H, J = 7.1 Hz), 0.77 (t, 6H, J = 7.1 Hz), 0.67 (t, 6H, J = 7.2 Hz), 0.57 (m, 4H). $^{13}$C NMR (125 MHz, CD$_3$OD-CDCl$_3$ 9/1): δ 172.2, 169.5, 154.9, 154.4, 151.0, 149.4, 145.2, 135.7, 132.2, 130.4, 127.0, 125.8, 122.3, 119.1, 118.2, 112.3, 107.1, 100.7, 99.4, 86.1, 74.6, 74.0, 73.0, 71.2, 67.7, 62.3, 56.1, 51.5, 50.1, 46.9, 41.4, 37.5, 32.8, 32.4, 30.5, 30.3, 30.2, 29.7, 28.3, 27.9, 27.5, 23.6, 23.4, 14.5, 14.3.

Probe 5a. See the procedure for 4a. Diamine 2a (32 mg, 44 μmol) and lactone 16 (61 mg, 96 μmol) gave, after flash silica gel chromatography (elucent: CH$_2$Cl$_2$-acetone-methanol-water 56/20/20/4) the probe 5a (20 mg, 13 μmol, 30%). Rf (SiO$_2$, CH$_2$Cl$_2$-acetone-methanol-water 56/20/20/4): 0.10. HRMS (ESI) Calcd. for C$_{74}$H$_{110}$O$_{26}$N$_{6}$ Na (M+Na)$^+$: 1521.7367. Found: 1521.7373. $^{[α]}$D +18 (c = 0.2, CH$_3$OH). $^1$H NMR (500 MHz, CD$_3$OD-CDCl$_3$ 1/1): δ 7.69 (s, 2H), 7.60 (m, 2H), 7.49 (m, 2H), 6.92 (s, 1H), 6.90 (m, 1H), 4.85 (d, 2H, J = 3.6 Hz), 4.38 (d, 2H, J = 7.7 Hz), 4.22 (d, 2H, J = 15.6 Hz), 4.02 (d, 2H, J = 15.6 Hz), 3.91-3.81 (m, 10H), 3.76-3.69 (m, 4H), 3.63-3.51 (m, 22H), 3.37 (q, 4H, J = 7.0 Hz), 2.01 (m, 4H), 1.29 (t, 6H, J = 7.1 Hz), 1.20 (t, 6H, J = 7.1 Hz), 0.77 (t, 6H, J = 7.1 Hz), 0.61 (m,
Probe 5b. See the procedure for 4a. Diamine 2b (58 mg, 54 μmol) and lactone 16 (75 mg, 119 μmol) gave, after flash silica gel chromatography (eluent: CH2Cl2-acetone-methanol-water 56/20/20/4) the probe 5b (62 mg, 34 μmol, 63%). Rf (SiO2, CH2Cl2-acetone-methanol-water 56/20/20/4): 0.20. HRMS (ESI) Calcd. for C98H158O26N6 Na2 (M+2Na)2+: 940.5506. Found: 940.5516. [α]D +20 (c = 0.2, CH3OH). 

1H NMR (500 MHz, CD3OD-CDCl3 1/1): δ 7.69 (s, 2H), 7.62 (m, 2H), 7.51 (m, 2H), 7.46 (s, 1H), 6.93 (m, 2H), 4.84 (2H, under water peak), 4.39 (d, 2H, J = 7.7 Hz), 4.22 (d, 2H, J = 15.6 Hz), 4.03 (d, 2H, J = 15.6 Hz), 3.88 (m, 10H), 3.74 (m, 4H), 3.62 (m, 12H), 3.53 (m, 10H), 3.33 (m, 4H, under methanol peak), 2.02 (m, 4H), 1.73 (m, 4H), 1.63 (m, 4H), 1.41-1.08 (m, 52H), 0.92 (t, 6H, J = 6.9 Hz), 0.87 (t, 6H, J = 7.1 Hz), 0.78 (t, 6H, J = 7.2 Hz), 0.63 (m, 4H). 

13C NMR (125 MHz, CD3OD-CDCl3 1/1): δ 170.6, 167.9, 153.5, 153.3, 149.8, 147.8, 143.5, 140.3, 140.1, 139.6, 134.3, 129.3, 126.1, 124.9, 121.2, 118.0, 117.7, 117.0, 106.0, 103.4, 99.1, 98.1, 85.3, 79.0, 75.3, 73.1, 71.8, 71.3, 71.0, 70.9, 68.9, 66.7, 61.4, 60.4, 54.9, 50.8, 45.8, 40.3, 36.7, 31.6, 31.5, 31.3, 29.4, 29.2, 29.0, 28.9, 28.7, 27.2, 26.8, 26.4, 23.5, 22.4, 22.3, 18.5, 13.7, 13.7, 13.6.
REFERENCES


**Figure S1.** Absorption and emission spectra for compounds 2b (a), 3b (b), 4b (c) and 5b (d) in different solvents: (—) cyclohexane, (—) THF, (—) CH2Cl2, (—) acetone, (—) DMSO, (—) methanol, (—) water.
Figure S2. Lippert-Mataga plot of ref. 1 (see main text). Solvent system ranged from pentane ($\Delta f = -0.006$) to acetone ($\Delta f = 0.284$). Equation expressing the collinear approximation was as follow:

$$\Delta \bar{\nu} = 1.0 \times 10^4 \Delta f + 2.9 \times 10^3 (R = 0.990)$$
Figure S3. Lippert-Mataga plot of 2a (see main text). Solvent system ranged from cyclohexane ($\Delta f = -0.004$) to methanol ($\Delta f = 0.30879$). Point for water ($\Delta f = 0.32008$) was excluded from the fit.

Equation expressing the collinear approximation was as follow:

$$\Delta \nu = 1.97 \times 10^4 \Delta f + 2.9 \times 10^3 \ (R = 0.99935)$$
Figure S4. Lippert-Mataga plot of 2b (see main text). Solvent system ranged from cyclohexane ($\Delta f = -0.004$) to methanol ($\Delta f = 0.30879$). Point water ($\Delta f = 0.32008$) was excluded from the fit. Equation expressing the collinear approximation was as follow:

$$\Delta \bar{\nu} = 2.08 \times 10^4 \Delta f + 2.5 \times 10^3$$ (R = 0.99896)
Figure S5. Lippert-Mataga plot of 3a (see main text). Solvent system ranged from cyclohexane ($\Delta f = -0.004$) to acetonitrile ($\Delta f = 0.30542$). Points for methanol ($\Delta f = 0.30879$) and water ($\Delta f = 0.32008$) were excluded from the fit. Equation expressing the collinear approximation was as follow:

$$\Delta \bar{\nu} = 1.55 \times 10^4 \Delta f + 3.2 \times 10^3 \quad (R = 0.91203)$$
**Figure S6.** Lippert-Mataga plot of 3b (see main text). Solvent system ranged from cyclohexane ($\Delta f = -0.004$) to acetonitrile ($\Delta f = 0.30542$). Points for methanol ($\Delta f = 0.30879$) and water ($\Delta f = 0.32008$) were excluded from the fit. Equation expressing the collinear approximation was as follow:

$$\Delta \bar{\nu} = 3.15 \times 10^4 \Delta f - 0.8 \times 10^3 \ (R = 0.88219)$$
Figure S7. Lippert-Mataga plot of 4a (see main text). Solvent system ranged from THF ($\Delta f = 0.2088$) to acetonitrile ($\Delta f = 0.3054$). Points for cyclohexane ($\Delta f = -0.004$), methanol ($\Delta f = 0.3087$) and water ($\Delta f = 0.3200$) were excluded from the fit. Equation expressing the collinear approximation was as follow:

$$
\Delta \nu = 2.48 \times 10^4 \Delta f + 1.2 \times 10^3 \ (R = 0.9229)
$$
Figure S8. Lippert-Mataga plot of 4b (see main text). Solvent system ranged from THF ($\Delta f = 0.20888$) to acetonitrile ($\Delta f = 0.30542$). Points for cyclohexane ($\Delta f = -0.004$), methanol ($\Delta f = 0.30879$) and water ($\Delta f = 0.32008$) were excluded from the fit. Equation expressing the collinear approximation was as follow:

$$\Delta \bar{\nu} = 2.47 \times 10^4 \Delta f + 1.0 \times 10^3 \quad (R = 0.93203)$$
Figure S9. Lippert-Mataga plot of 5a (see main text). Solvent system ranged from THF ($\Delta f = 0.20888$) to acetonitrile ($\Delta f = 0.30542$). Points for cyclohexane ($\Delta f = -0.004$), methanol ($\Delta f = 0.30879$) and water ($\Delta f = 0.32008$) were excluded from the fit. Equation expressing the collinear approximation was as follow:

$$\Delta \tilde{\nu} = 2.07 \times 10^4 \Delta f + 2.2 \times 10^3 \quad (R = 0.88376)$$
Figure S10. Lippert-Mataga plot of 5b (see main text). Solvent system ranged from THF ($\Delta f = 0.20888$) to acetonitrile ($\Delta f = 0.30542$). Points for cyclohexane ($\Delta f = -0.004$), methanol ($\Delta f = 0.30879$) and water ($\Delta f = 0.32008$) were excluded from the fit. Equation expressing the collinear approximation was as follow:

$$
\Delta \tilde{\nu} = 3.05 \times 10^4 \Delta f - 9.5 \times 10^3 \quad (R = 0.98628)
$$
Figure S11. Variations of the Stokes shift (cm$^{-1}$) $\Delta \bar{\nu}$ with the solvent polarity parameter $E_N^T$ for compounds 2a-5a and ref. 1.
Figure S12. Variations of the Stokes shift (cm$^{-1}$) $\Delta \bar{\nu}$ with the solvent polarity parameter $E^T_N$ for compounds 2b-5b and ref. 1.