Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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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.

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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 photomuliplier 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_2Cl_2 and triethylamine (Et_3N) 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)

($Pd(PPh_3)_2Cl_2$) [5] and Methyl 3,4,5-tris(2-(2-(2-methoxyethoxy)ethoxy)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), Na_2CO_3 (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 (SiO_2, 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 Na_2SO_4, 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 $C_{29}H_{42}INO_2$: C 61.81 H 7.51 N 2.49. Found: C 61.73 H 7.3 N 2.45. ^{1}H NMR ($CDCl_3$): 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). ^{13}C NMR ($CDCl_3$): 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 18hours. 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 $C_{45}H_{48}IN_3O_4.H_2O$: C 64.36 H 6.00 N 5.00. Found: C 64.40 H 6.09 N 5.29. ^{1}H NMR ($CDCl_3$): 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 ($CDCl_3$): δ 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 hydrogencarbonate 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 $C_{39}H_{70}ClN_3O_2.0.3H_2O$: C 71.64 H 10.88 N 6.43. Found: C 71.66 H 10.91 N 6.52. ^{1}H NMR (CDCl3): δ 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 (CDCl3): δ 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

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saturated $NaHCO_3$ aqueous solution. The aqueous phase was extracted with diethyl ether (3x30mL). The combiend organic phases were washed with with saturated $NaHCO_3$ solution, half saturated $Na_2S_2O_5$, 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 (SiO_2, pentane-ether: 15-5 v/v): 0.6. Anal. Calcd. for $C_{39}H_{70}IN_3O_2$:C 63.31 H 9.54 N 5.68. Found: C 63.59 H 9.83 N 5.61. ^{1}H 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). ^{13}C 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 Et_3N (160mL) and the solution degassed by bubbling argon. CuI (257mg, 1.35mmol) and $Pd(PPh_3)_2Cl_2$ (470mg, 0.6mmol) were added, the tube closed and the mixture stirred at room temperature for 4 days. The mixture was filtered trough a plough of Celite, which was thoroughly washed with ether. The filtrate was washed with saturated NH_4Cl aqueous solution (2x100mL), brine (50ml), was dried over Na_2SO_4, filtered and the solvent evaporated. The crude product was purified by column chromatography (eluent CH_2Cl_2) to yield 4.18g of yellow oil (82%). Rf (SiO_2, CH_2Cl_2-2.5% diethyl ether v/v): 0.45. Anal. Calcd. for $C_{34}H_{79}N_3O_2Si$: C 74.41 H 11.21 N 5.92. Found: C 74.01 H 11.43 N 5.63. ^{1}H 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). ^{13}C 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_2CO_3 (2.10g, 15.3mmol, 5eq.) was added to a solution of 4-(trimethylsilylethynyl)-2,6-bis(di-*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_2SO_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 $C_{41}H_{71}N_3O_2$: C 77.18 H 11.22 N 6.59. Found: C 77.13 H 11.22 N 6.59. ^{1}H NMR (CDCl3): δ 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_3N (30mL) and the solution degassed by bubbling argon. CuI (81mg, 0.43mmol) and $Pd(PPh_3)_2Cl_2$ (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 trough a plough of Celite, which was then thoroughly washed with ether. The filtrate was washed with saturated NH_4Cl aqueous solution (2x100mL), brine (50ml). It was dried over Na_2SO_4, filtered and the solvent evaporated. The crude product was purified by column chromatography (eluent CH_2Cl_2 to 20% Ethyl Acetate in CH_2Cl_2 v/v) to yield 1.72g of **13a** as yellow solid (65%). Rf (SiO_2, CH_2Cl_2-ethyl acetate 4/1 v/v): 0.2. Anal. Calcd. for $C_{62}H_{70}N_6O_6$: C 73,75 H 7,15 N 8,32. Found: C 73,74 H 7,44 N 8,38. ^{1}H NMR ($CDCl_3$): δ 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 ($CDCl_3$): δ 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 $Pd(PPh_3)_2Cl_2$ (60mg) using the same experimental procedure as for **13a**. Purification by column chromatography (eluent CH_2Cl_2 to 5% Ethyl Acetate in CH_2Cl_2 v/v) yielded 1.39g of yellow oil (60%). Rf (SiO_2, 5% Ethyl Acetate in CH_2Cl_2): 0.56. Anal. Calcd. for $C_{86}H_{118}N_6O_6$: C 77.35 H 8.93 N 6.31. Found: C 77.39 H 9.14 N 6.17. ^{1}H NMR ($CDCl_3$): 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). ^{13}C NMR ($CDCl_3$): 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 $LiOH.H_2O$ (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 CH_2Cl_2 (10mL) and acidified with concentrated HCl until a troubled mixture was obtained (pH=1). The solution was then extracted with CH_2Cl_2 (2x10mL) and the combined extracts dried over Na_2SO_4, filtered and evaporated to afford colorless oil (2.36g, 100%) that was used without further purification. Rf (SiO_2, CH_2Cl_2 – Acetone – Methanol - Water 56-20-20-4 v/v): 0.66. ^{1}H NMR ($CDCl_3$): δ 7.35 (s, 2H), 4.26-4.18 (m, 6H), 3.89-3.56 (m, 30H), 3.37 (s, 3H), (s, 6H), ^{13}C NMR ($CDCl_3$): 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 Crotyl 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% Et_3N in acetone-water 19/1 v/v). Yield: 350 mg (95%), bright yellow solid. Rf (SiO_2, 5% Et_3N in Acetone - Water 19/1 v/v): 0.22. m.p. 236°C. Anal. Calcd for $C_{46}H_{66}N_6O_2.2H_2O$: C 74.75 H 9.55 N 11.37. Found: C 73.35 H 9.03 N 9.96. ^{1}H NMR ($CDCl_3$): 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). ^{13}C NMR ($CDCl_3$): 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% Et_3N in Acetone - Water 19/1 v/v) Rf (SiO_2, 2% Et_3N in Acetone - Water 19/1 v/v): 0.25. HRMS (ESI): Calcd for $C_{70}H_{114}N_6O_2$: 1071.9081. Found 1071.9089. ^{1}H 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). ^{13}C NMR ($CDCl_3$): 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 CH_2Cl_2 was added DiC (35µL, 0.23mmol, 2.3eq) and HOBt (30mg, 0.22mmol 2.3eq).The reaction was monitored by tlc (SiO_2, eluent: Acetone) and the solution was stirred at room temperature until completion. A white precipitate was filtered off, washed with CH_2Cl_2. The filtrate was washed with 2M NaOH (5mL), 2M HCl (5mL), saturated aqueous $NaHCO_3$, dried over Na_2SO_4, filtered and evaporated. The crude yellow oil was purified by column chromatography (eluent: CH_2Cl_2 to 10% MeOH in CH_2Cl_2 v/v) to yield 51% (110mg) of a yellow oil (51%). Rf SiO_2, 2% MeOH in CH_2Cl_2):

Rf (SiO_2, 10% MeOH in CH_2Cl_2): 0.5. HRMS (ESI): Calcd for $C_{102}H_{158}N_6O_{28}$ 2Na $(M+2Na)^{2+}$: 980.5455. Found: 980.5458. ^1H NMR ($CDCl_3$): 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: CH_2Cl_2 to 10% MeOH in CH_2Cl_2 v/v) afforded 215mg of a yellow oil (Yield: 58%). Rf (SiO_2, 5% MeOH in CH_2Cl_2): 0.2. HRMS (ESI): Calcd for $C_{126}H_{206}N_6O_{28}$ 2Na $(M+2Na)^{2+}$: 1148.7333. Found: 1148.7337. ^1H NMR ($CDCl_3$): 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). ^{13}C NMR ($CDCl_3$): 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 CH_2Cl_2 (1mL) was added 2eq. of carboxymethyl-3,4,6-tri-*O*-acetyl-a-D-glucopyranoside-2-*O*-lactone **15** (21 mg, 60µmol). 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: CH_2Cl_2-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: CH_2Cl_2-acetone-methanol-water 78/10/10/2) to afford compound **4a** (14.4 mg, 12 µmol, 45%). Rf (SiO_2, CH_2Cl_2-acetone-methanol-water 56/20/20/4): 0.67. HRMS (ESI) Calcd. for $C_{62}H_{90}N_6O_{16}$ Na $(M+Na)^+$: 1197.6311. Found: 1197.6304. $[\alpha]_D$ +44 (c = 0.5, CH_3OH). ^1H NMR (300 MHz, CD_3OD-$CDCl_3$ 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* = 7.2 Hz), 0.54 (m, 4H). ^{13}C NMR (125 MHz, CD_3OD-$CDCl_3$ 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 (eluent: CH_2Cl_2-acetone-methanol-water 78/10/10/2) the probe **4b** (89 mg, 59 μmol, 74%). Rf (SiO_2, CH_2Cl_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. $[\alpha]_D$ +37 (c = 1, CH_3OH). ^{1}H NMR (500 MHz, CD_3OD-$CDCl_3$ 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_3OD-$CDCl_3$ 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 (eluent: CH_2Cl_2-acetone-methanol-water 56/20/20/4) the probe **5a** (20 mg, 13 μmol, 30%). Rf (SiO_2, CH_2Cl_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. $[\alpha]_D$ +18 (c = 0.2, CH_3OH). ^{1}H NMR (500 MHz, CD_3OD-$CDCl_3$ 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,

4H). ^{13}C NMR (125 MHz, CD_3OD-$CDCl_3$ 1/1): δ 171.8, 169.0, 154.7, 154.2, 150.8, 149.1, 144.8, 135.6, 132.0, 130.3, 126.8, 125.4, 122.2, 118.9, 117.9, 112.2, 104.6, 100.3, 99.3, 86.0, 80.2, 76.6, 74.4, 72.8, 72.4, 72.2, 72.0, 69.9, 67.7, 62.2, 61.3, 55.9, 51.5, 44.4, 41.2, 40.4, 37.5, 32.2, 30.4, 24.5, 23.2, 14.4, 14.3, 12.9.

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: CH_2Cl_2-acetone-methanol-water 56/20/20/4) the probe **5b** (62 mg, 34 μmol, 63%). Rf (SiO_2, CH_2Cl_2-acetone-methanol-water 56/20/20/4): 0.20. HRMS (ESI) Calcd. for $C_{98}H_{158}O_{26}N_6\ Na_2$ $(M+2Na)^{2+}$: 940.5506. Found: 940.5516. $[\alpha]_D$ +20 (c = 0.2, CH_3OH). ^{1}H NMR (500 MHz, CD_3OD-$CDCl_3$ 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). ^{13}C NMR (125 MHz, CD_3OD-$CDCl_3$ 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.

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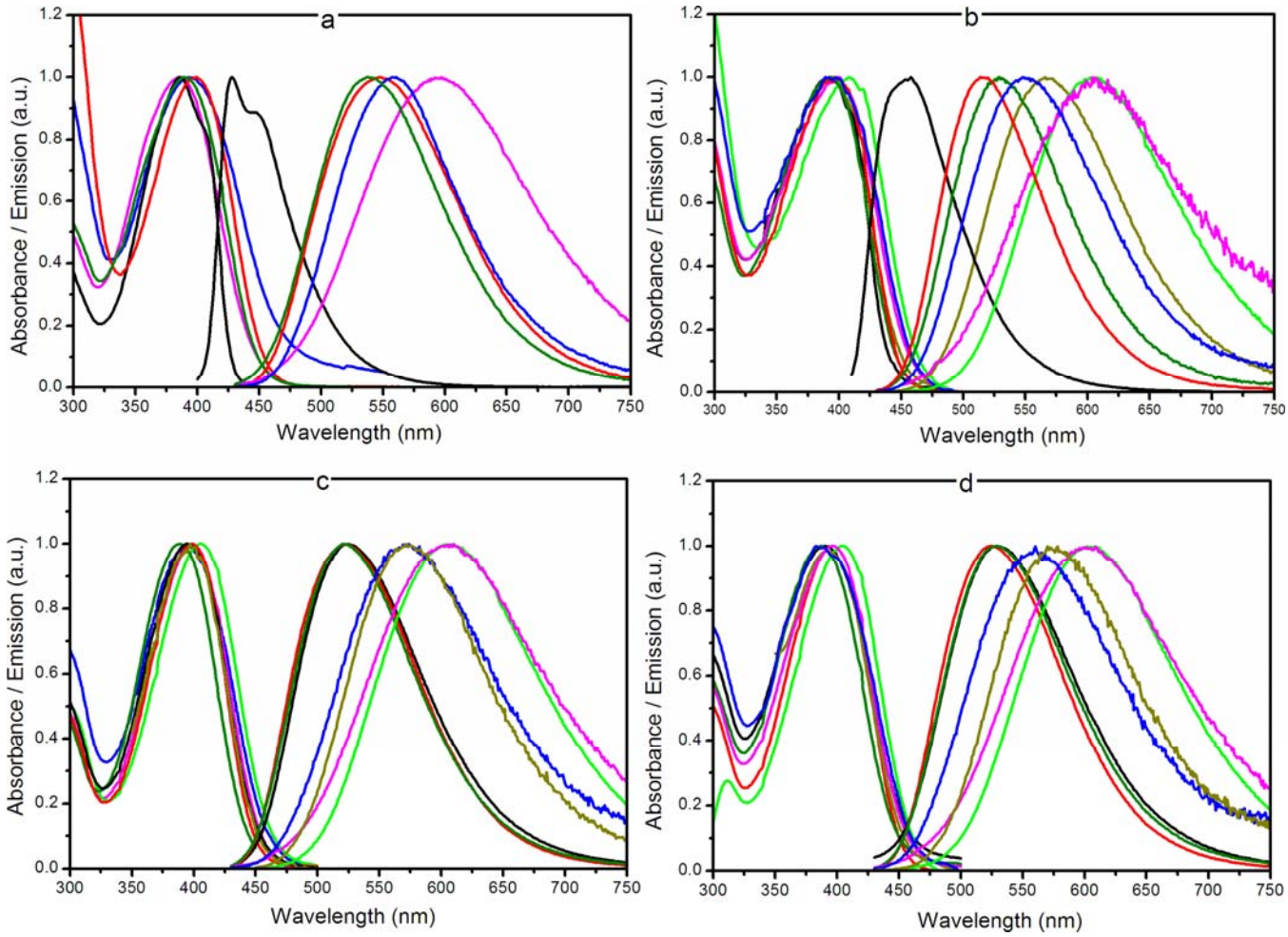

Figure S1. Absorption and emission spectra for compounds **2b** (a), **3b** (b), **4b** (c) and **5b** (d) in different solvents: (—) cyclohexane, (—) THF, (—) CH_2Cl_2, (—) acetone, (—) DMSO, (—) methanol, (—) water.

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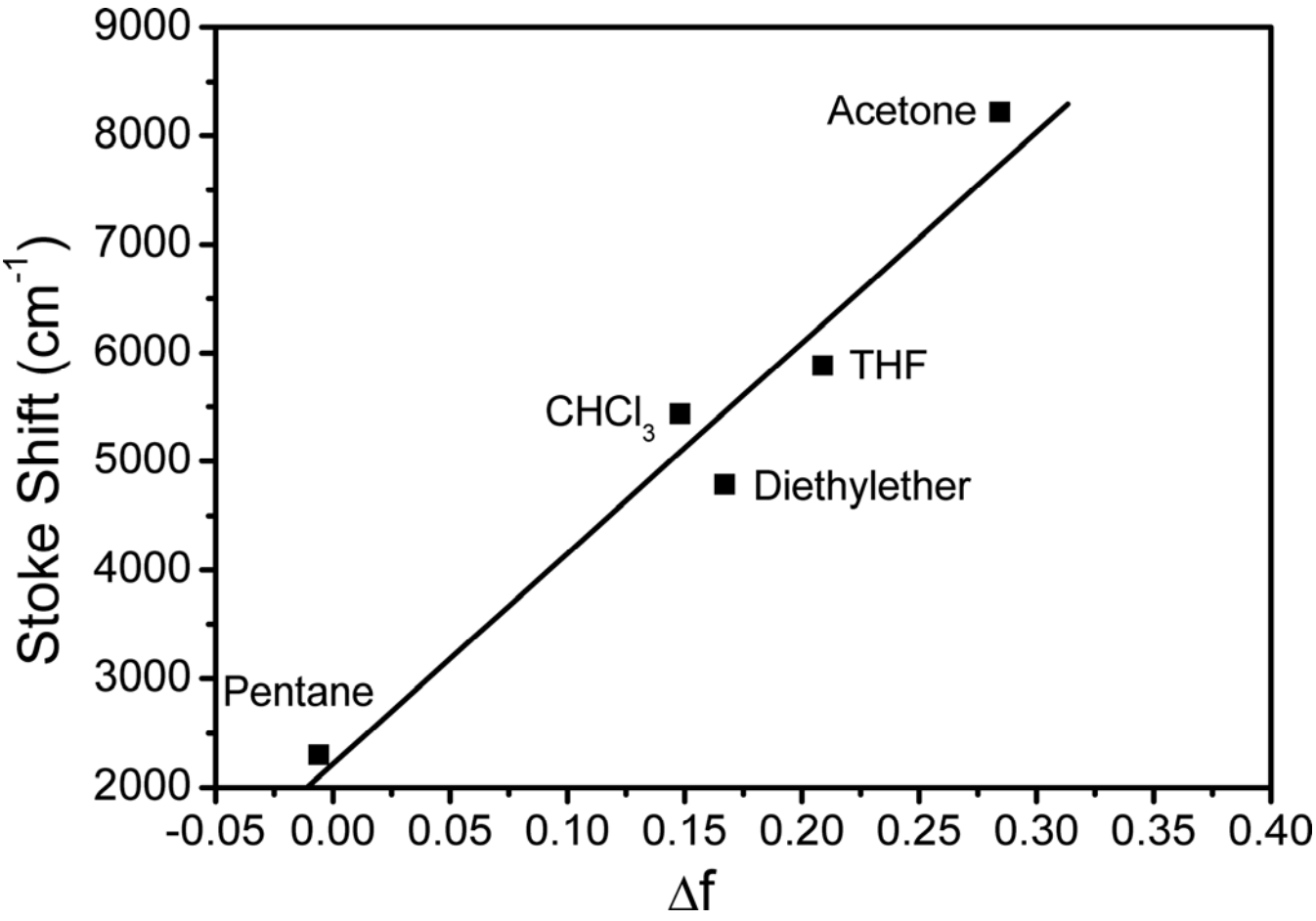

Figure S2. Lippert-Mataga plot of ref. **1** (see main text). Solvent system ranged from pentane ($\Delta f = -0.006$) to acetone ($\Delta f = 0.284$). Equation expressing the collinear approximation was as follow:

$$\Delta\bar{\nu} = 1.0\times10^{4}\,\Delta f + 2.9\times10^{3}\ (R = 0.990)$$

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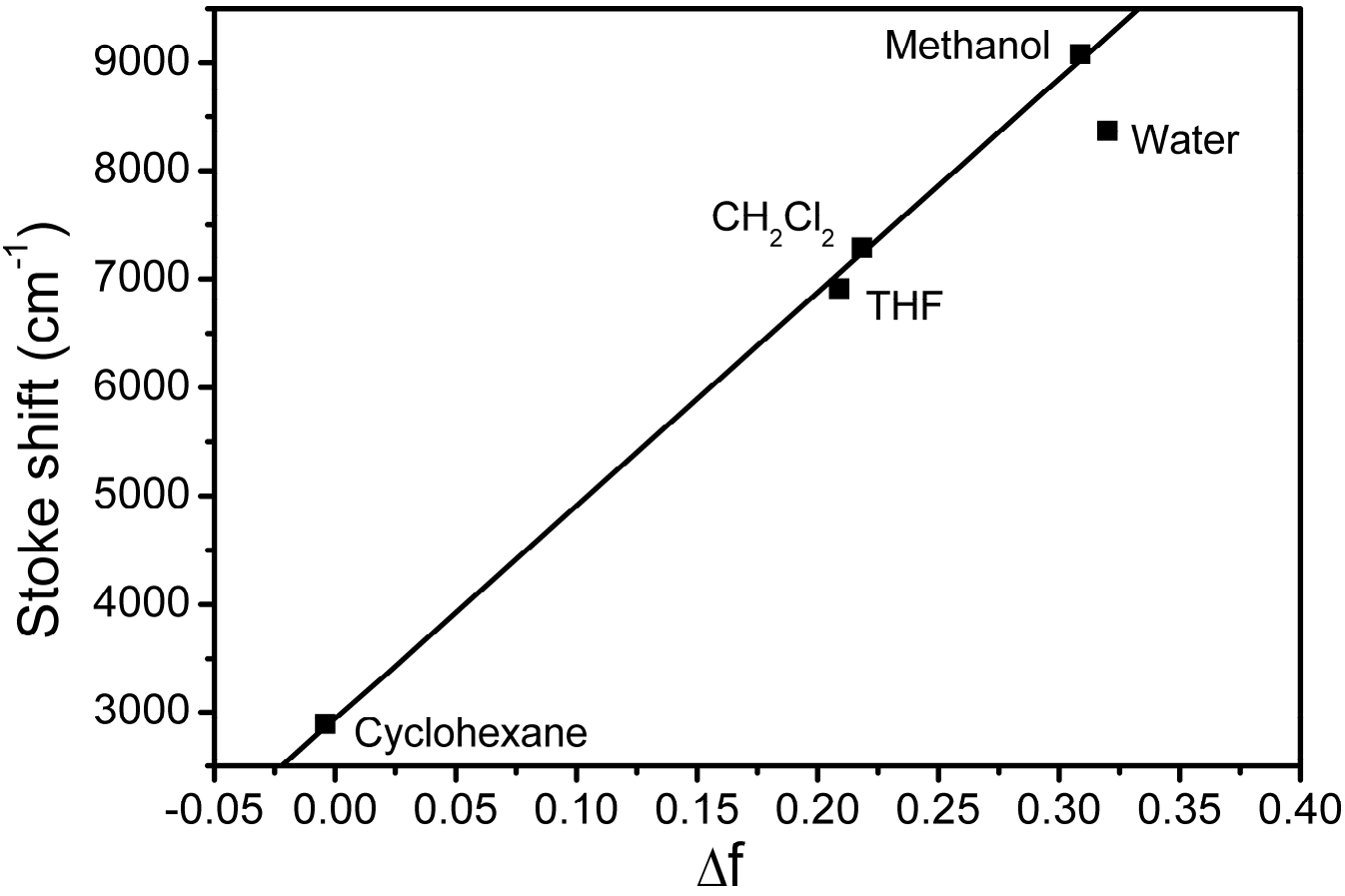

Figure S3. Lippert-Mataga plot of **2a** (see main text). Solvent system ranged from cyclohexane ($\Delta f = -0.004$) to methanol ($\Delta f = 0.30879$). Point for water ($\Delta f = 0.32008$) was excluded from the fit. Equation expressing the collinear approximation was as follow:

$$\Delta \bar{\nu} = 1.97\times10^{4}\,\Delta f + 2.9\times10^{3} \quad (R = 0.99935)$$

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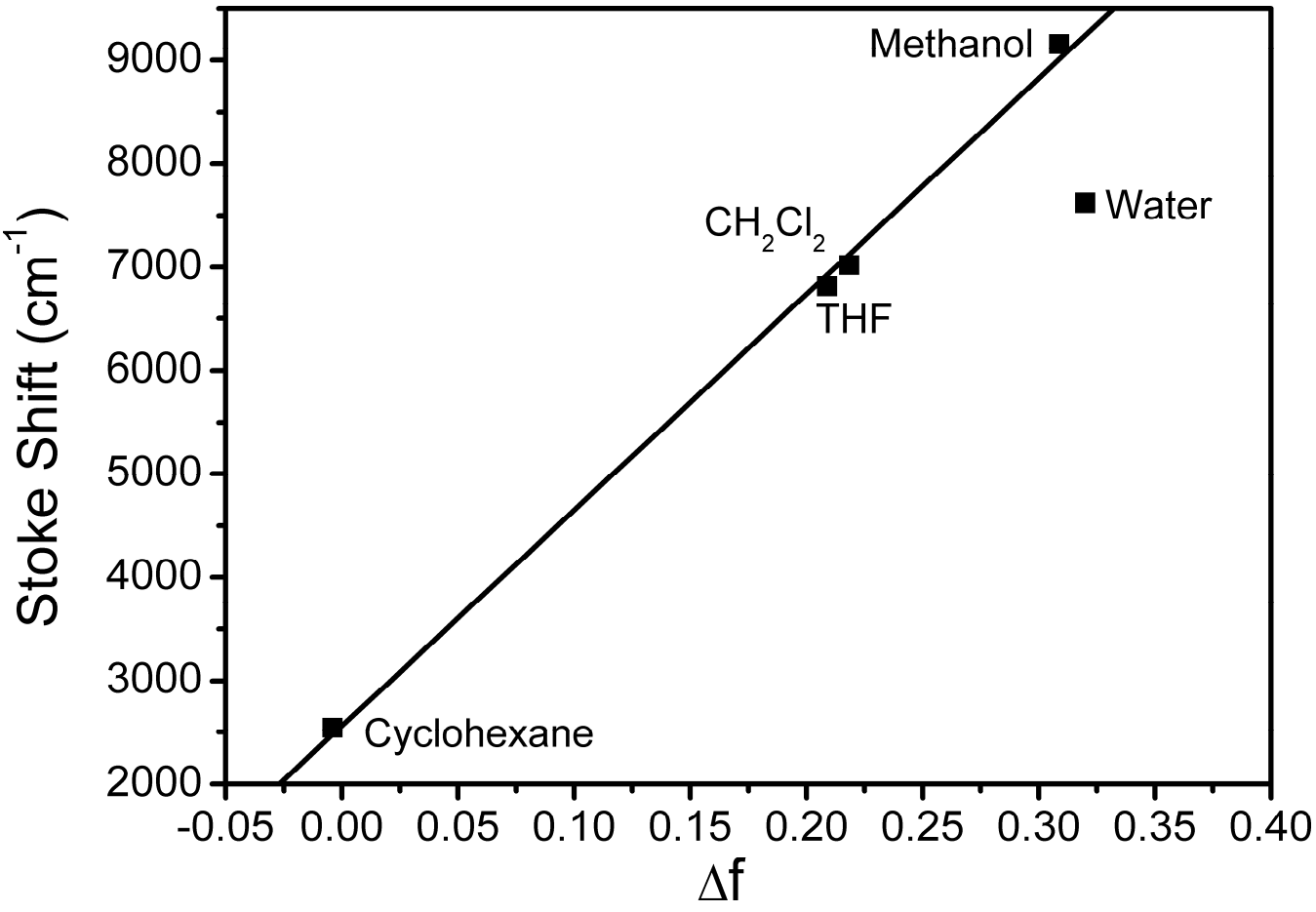

Figure S4. Lippert-Mataga plot of **2b** (see main text). Solvent system ranged from cyclohexane ($\Delta f = -0.004$) to methanol ($\Delta f = 0.30879$). Point water ($\Delta f = 0.32008$) was excluded from the fit. Equation expressing the collinear approximation was as follow:

$$\Delta\bar{\nu} = 2.08\times10^{4}\,\Delta f + 2.5\times10^{3}\ (R = 0.99896)$$

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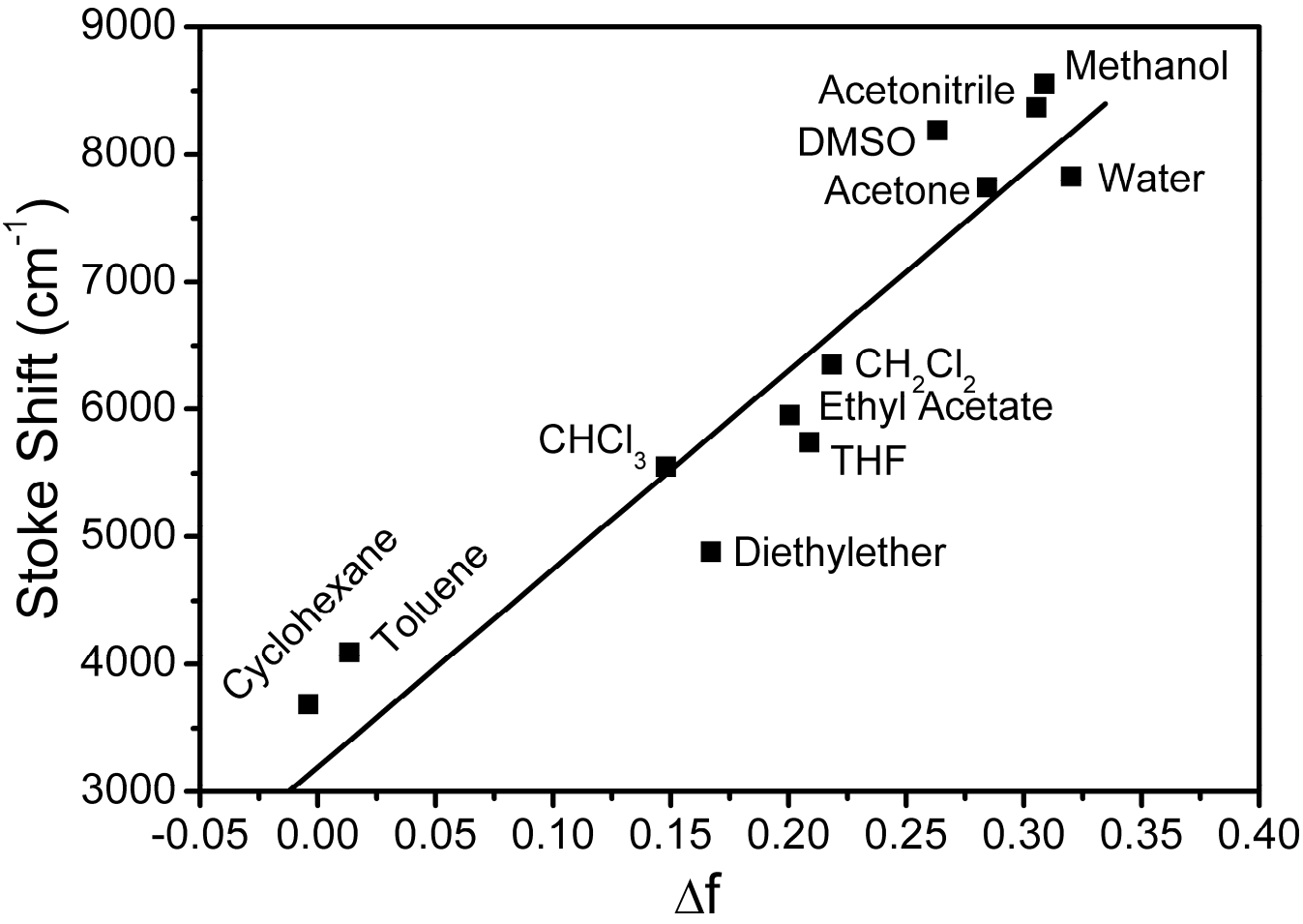

Figure S5. Lippert-Mataga plot of **3a** (see main text). Solvent system ranged from cyclohexane ($\Delta f = -0.004$) to acetonitrile ($\Delta f = 0.30542$). Points for methanol ($\Delta f = 0.30879$) and water ($\Delta f = 0.32008$) were excluded from the fit. Equation expressing the collinear approximation was as follow:

$$\Delta\bar{\nu} = 1.55\times10^{4}\,\Delta f + 3.2\times10^{3} \quad (R = 0.91203)$$

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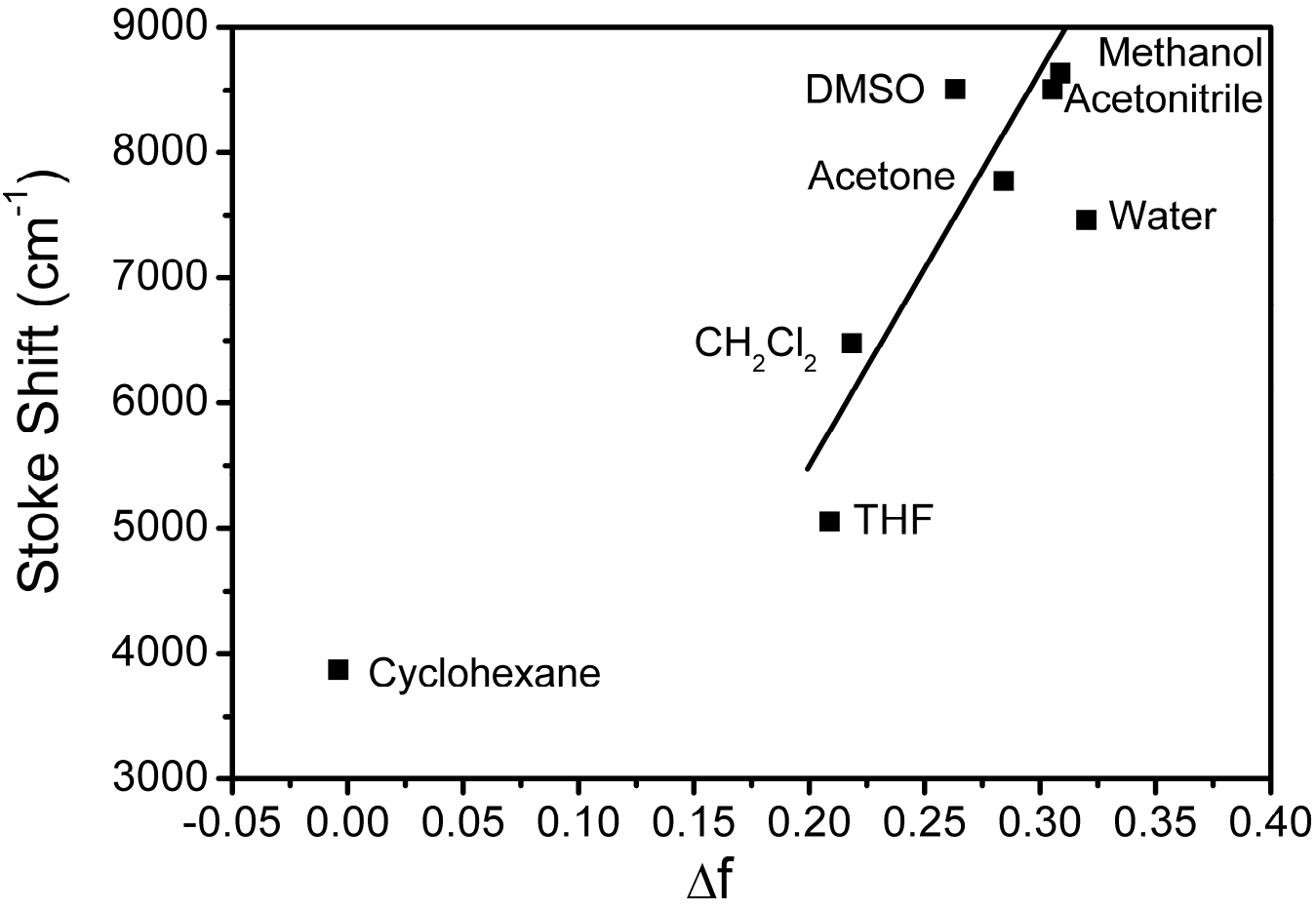

Figure S6. Lippert-Mataga plot of **3b** (see main text). Solvent system ranged from cyclohexane ($\Delta f = -0.004$) to acetonitrile ($\Delta f = 0.30542$). Points for methanol ($\Delta f = 0.30879$) and water ($\Delta f = 0.32008$) were excluded from the fit. Equation expressing the collinear approximation was as follow:

$$\Delta\bar{\nu} = 3.15\times10^{4}\,\Delta f - 0.8\times10^{3}\ (R = 0.88219)$$

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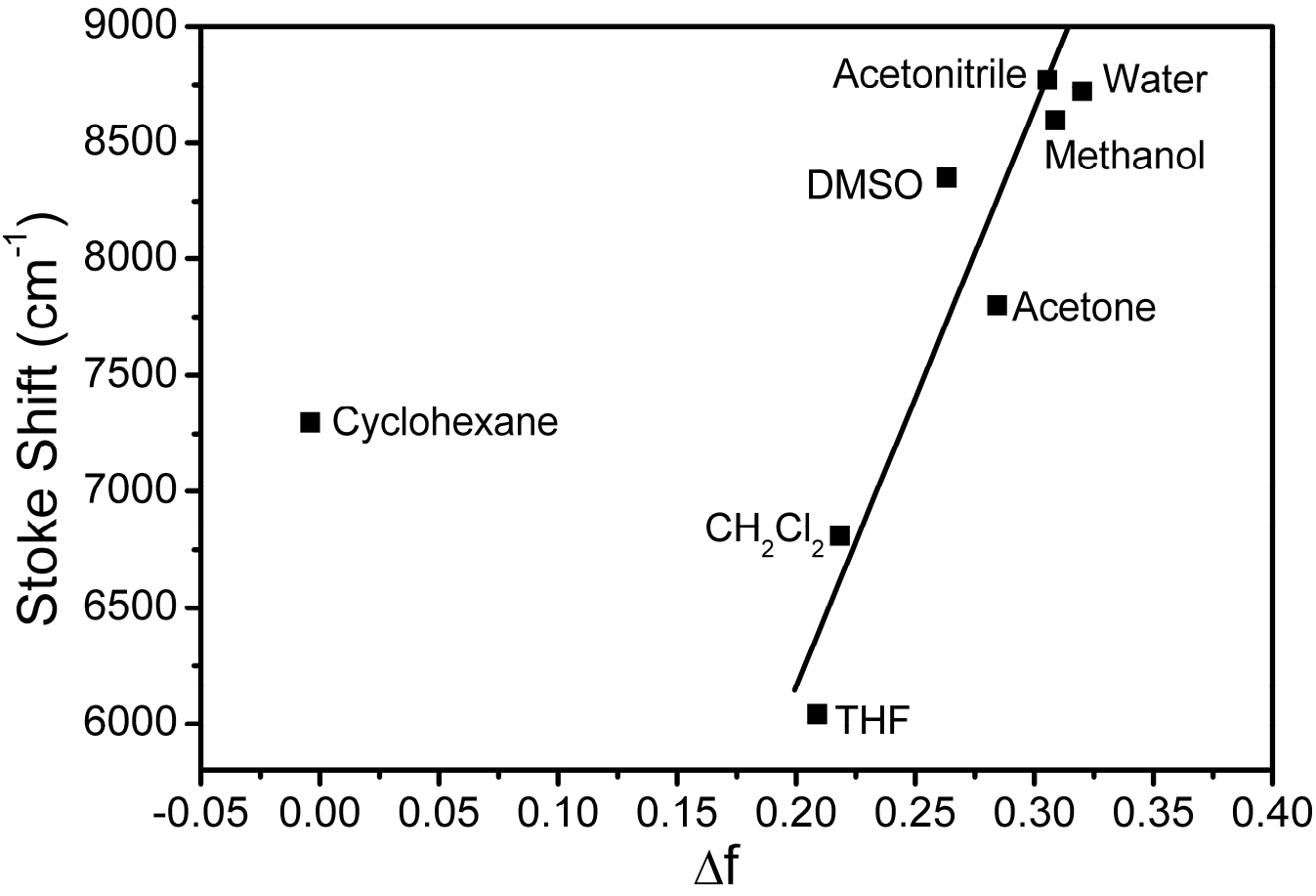

Figure S7. Lippert-Mataga plot of **4a** (see main text). Solvent system ranged from THF ($\Delta f = 0.20888$) to acetonitrile ($\Delta f = 0.30542$). Points for cyclohexane ($\Delta f = -0.004$), methanol ($\Delta f = 0.30879$) and water ($\Delta f = 0.32008$) were excluded from the fit. Equation expressing the collinear approximation was as follow:

$\Delta\bar{\nu} = 2.48\times10^{4}\,\Delta f + 1.2\times10^{3}$ (R = 0.9229)

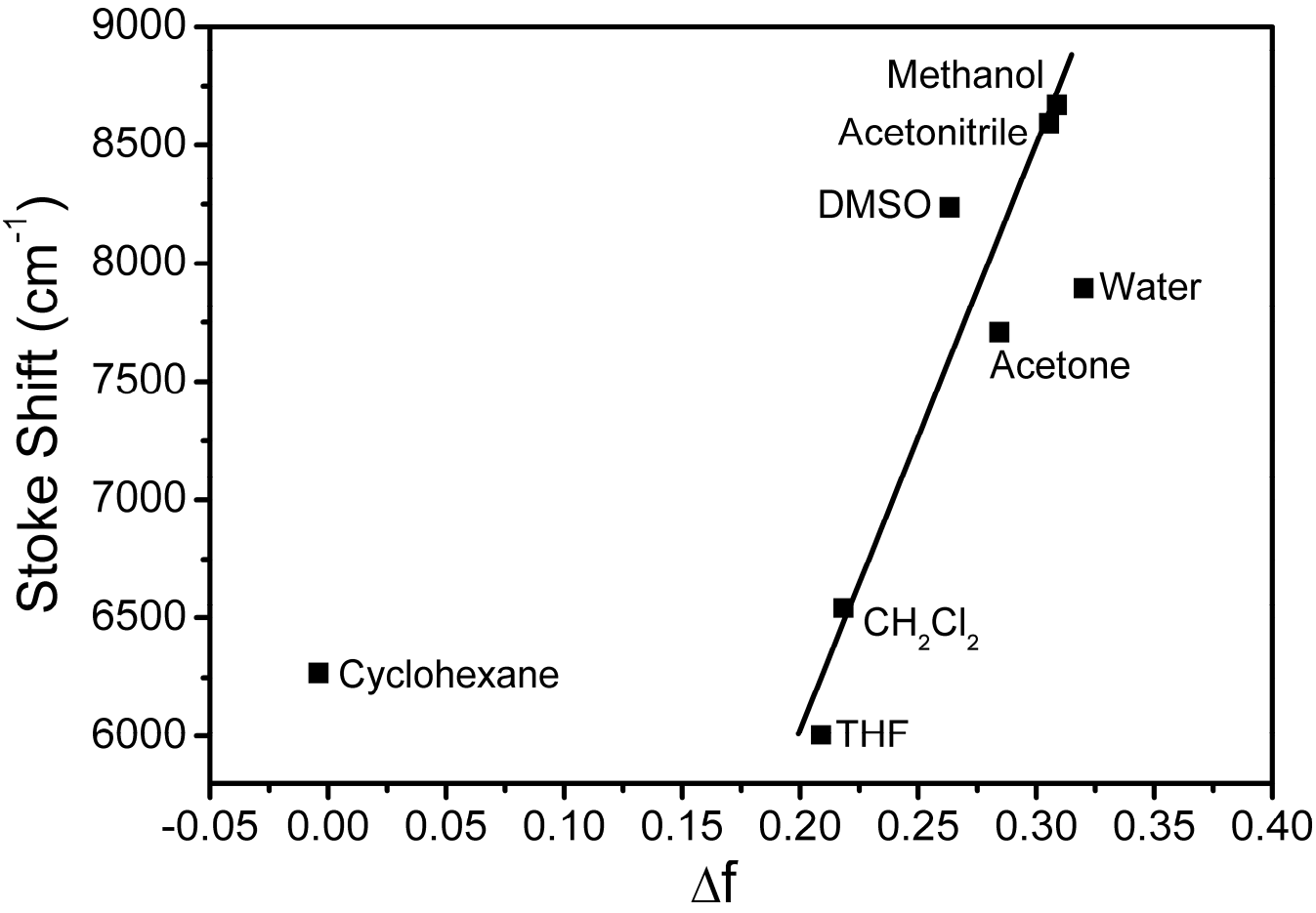

Figure S8. Lippert-Mataga plot of **4b** (see main text). Solvent system ranged from THF ($\Delta f = 0.20888$) to acetonitrile ($\Delta f = 0.30542$). Points for cyclohexane ($\Delta f = -0.004$), methanol ($\Delta f = 0.30879$) and water ($\Delta f = 0.32008$) were excluded from the fit. Equation expressing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

$$\Delta \bar{\nu} = 2.47\times10^{4}\,\Delta f + 1.0\times10^{3} \quad (R = 0.93203)$$

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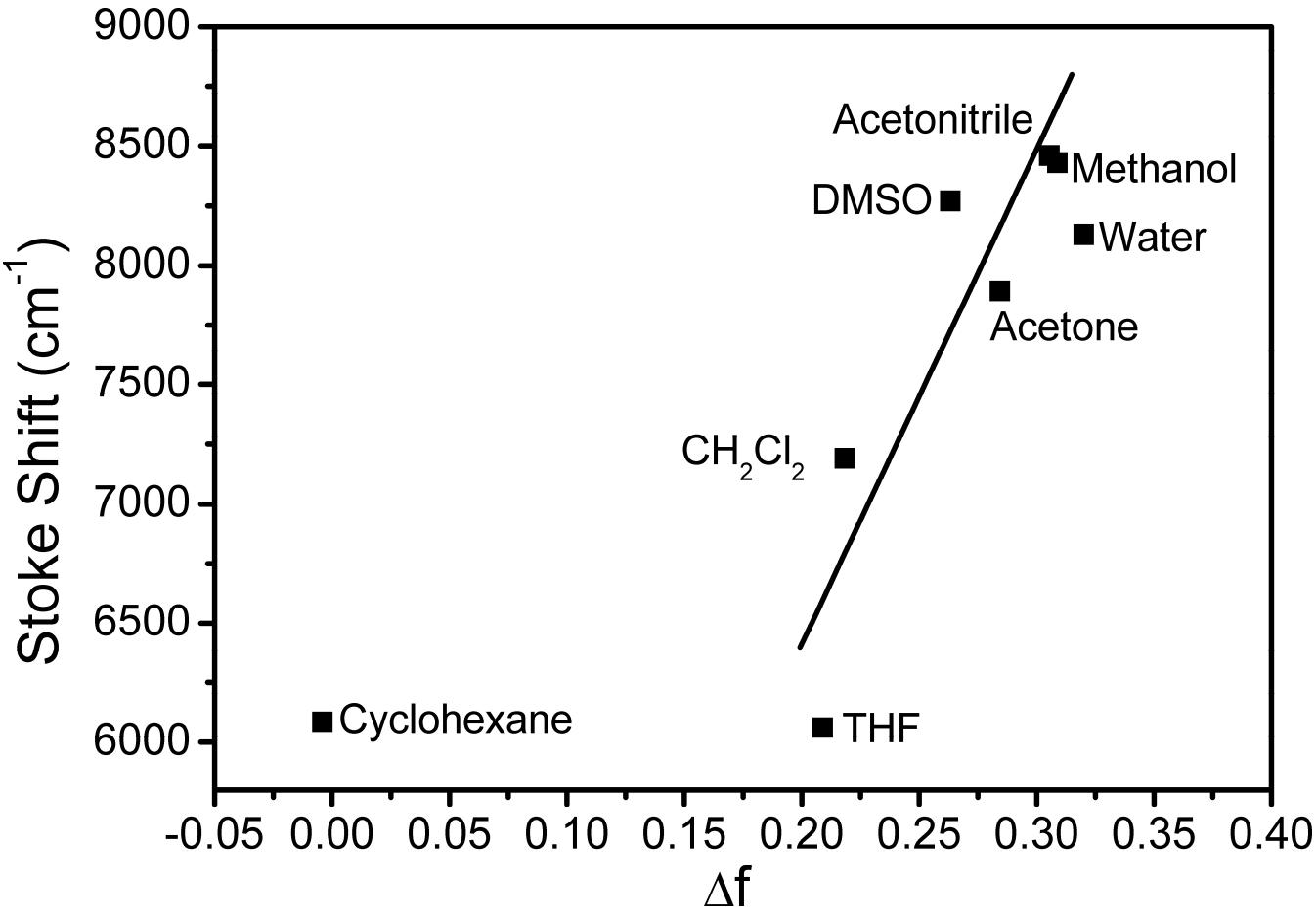

Figure S9. Lippert-Mataga plot of **5a** (see main text). Solvent system ranged from THF ($\Delta f = 0.20888$) to acetonitrile ($\Delta f = 0.30542$). Points for cyclohexane ($\Delta f = -0.004$), methanol ($\Delta f = 0.30879$) and water ($\Delta f = 0.32008$) were excluded from the fit. Equation expressing the collinear approximation was as follow:

$$\Delta \bar{\nu} = 2.07\times10^{4}\,\Delta f + 2.2\times10^{3} \quad (R = 0.88376)$$

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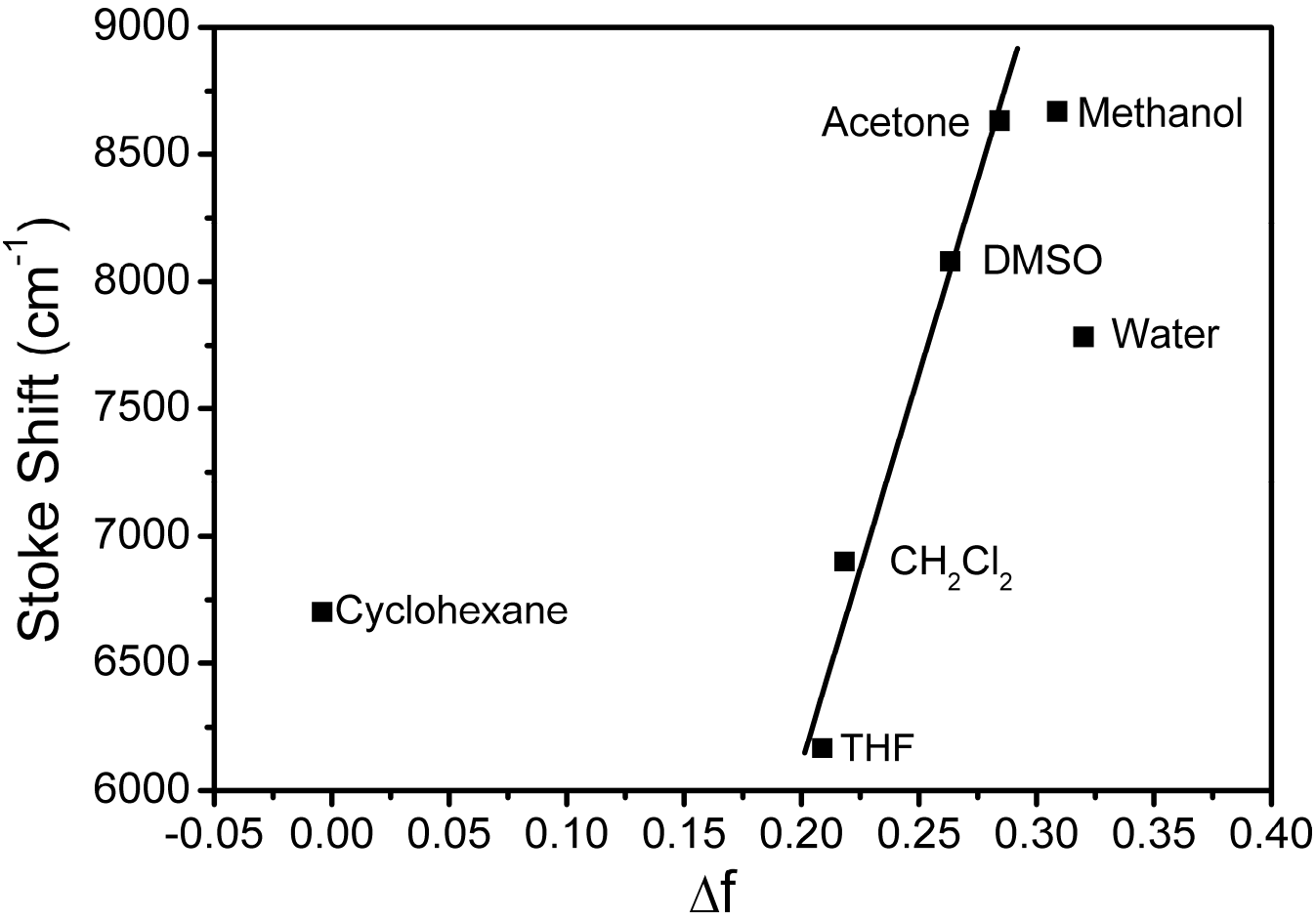

Figure S10. Lippert-Mataga plot of **5b** (see main text). Solvent system ranged from THF ($\Delta f = 0.20888$) to acetonitrile ($\Delta f = 0.30542$). Points for cyclohexane ($\Delta f = -0.004$), methanol ($\Delta f = 0.30879$) and water ($\Delta f = 0.32008$) were excluded from the fit. Equation expressing the collinear approximation was as follow:

$$\Delta\bar{\nu} = 3.05\times10^{4}\,\Delta f - 9.5\times10^{3}\ (R = 0.98628)$$

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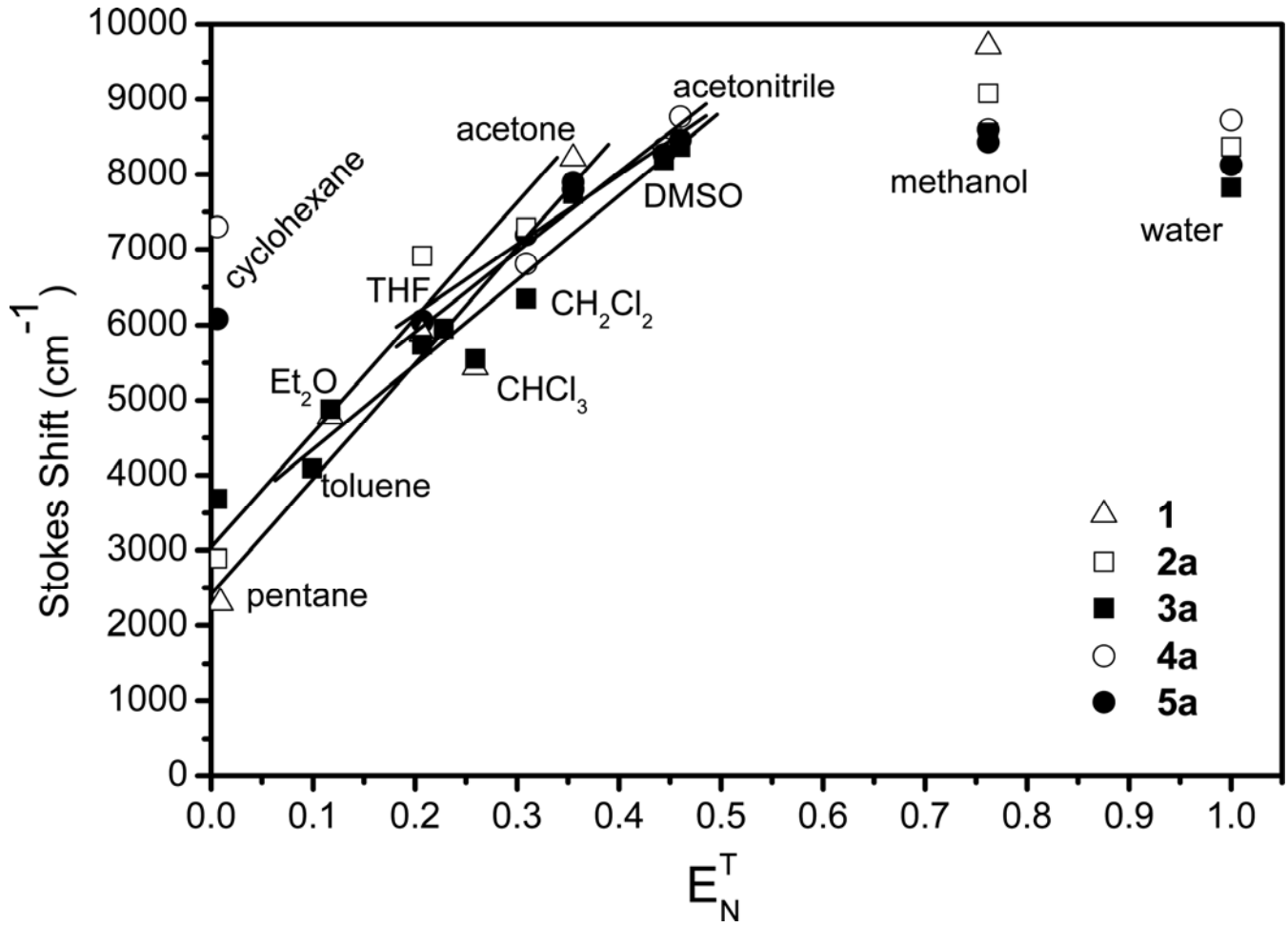

Figure S11. Variations of the Stokes shift (cm^{-1}) $\Delta\bar{\nu}$ with the solvent polarity parameter E_N^T for compounds **2a**-**5a** and ref. **1**.

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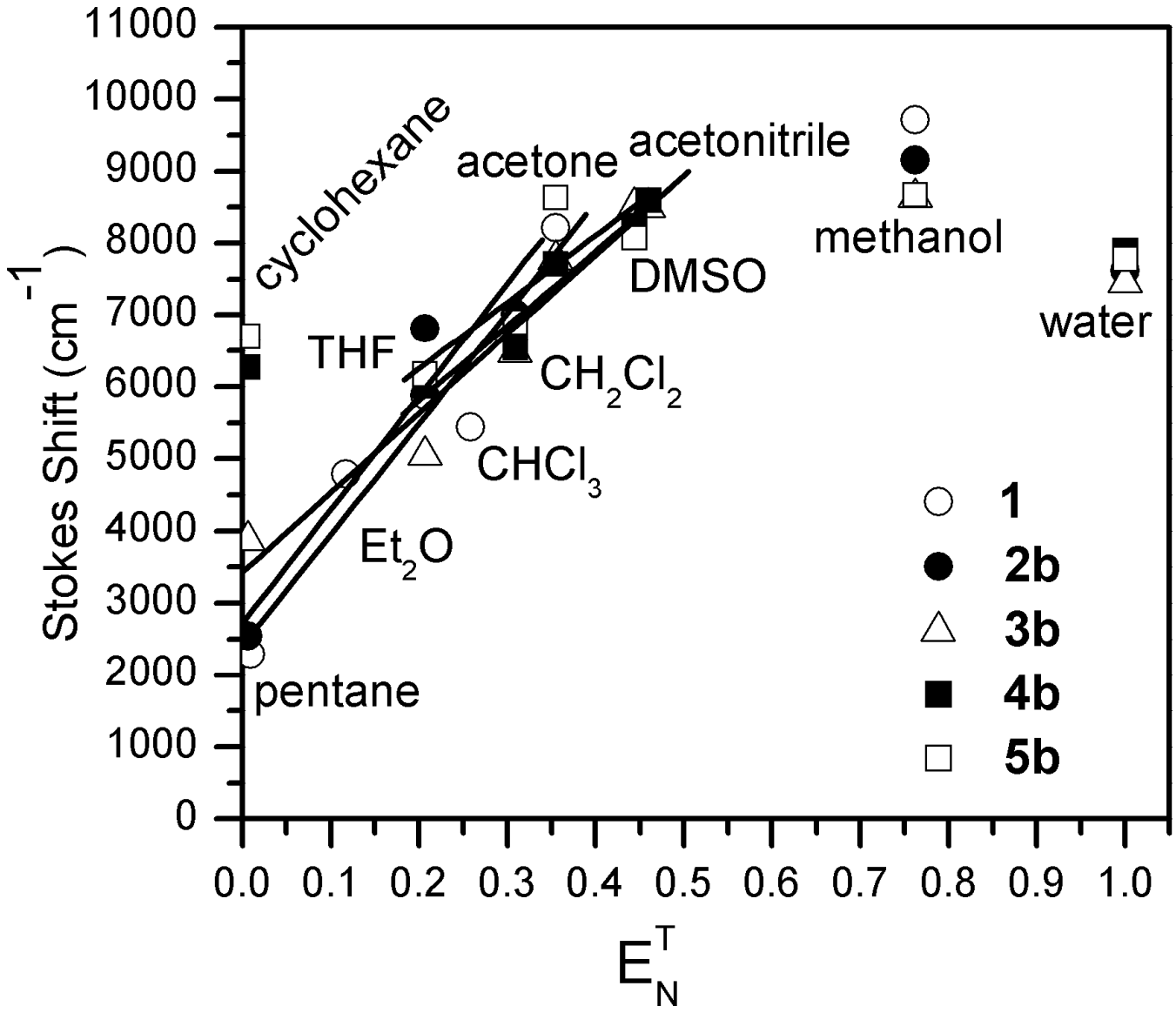

Figure S12. Variations of the Stokes shift (cm^{-1}) $\Delta\bar{\nu}$ with the solvent polarity parameter E_N^T for compounds **2b**-**5b** and ref. **1**.