pH Switchable and Fluorescent Ratiometric Squarylium Indocyanine Dyes as Extremely Alkaline Sensors

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Experimental section

Material and methods

4-Hydrazinobenzoic acid hydrochlorid (97%), 3,4-dihydroxycyclobut-3-ene-1,2-dione (98%), 4-bromomethylbenzoic acid (97%), 3-methyl-2-butanone (98%), *o*-(7-azabenzo triazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HATU) (99%), and ethyldiisopropylamine (99%) were purchased from Alfa Aesar and used without further purifications. All solvents were dried prior to use with appropriate drying agents. Anhydrous *N,N*-dimethylformamide (DMF) was obtained from Alfa Aesar and used directly. Column chromatography was performed using silica gel 60 (230-400 mesh). Analytical thin layer chromatography (TLC) was carried out on Yantai chemical industry silica gel plates and visualized by UV.

The pH measurements were carried out using a pH meter (Mettler Toledo S40K) which was calibrated with pH 4 and pH 7 buffers before use. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker 400 (400 MHz ^{1}H; 100 MHz ^{13}C) or Bruker 600 (600 MHz ^{1}H; 150 MHz ^{13}C) spectrometer at room temperature. Mass spectra (MS) were measured with a XEVO-G2QTOF (ESI) (Waters, USA). UV-visible spectra were obtained on a spectrometer (Cintra 20, GBC, Australia). Fluorescence spectroscopic studies were performed on a fluorescence spectrophotometer (Horiba Jobin Yvon FluoroMax-4 NIR, NJ, USA).

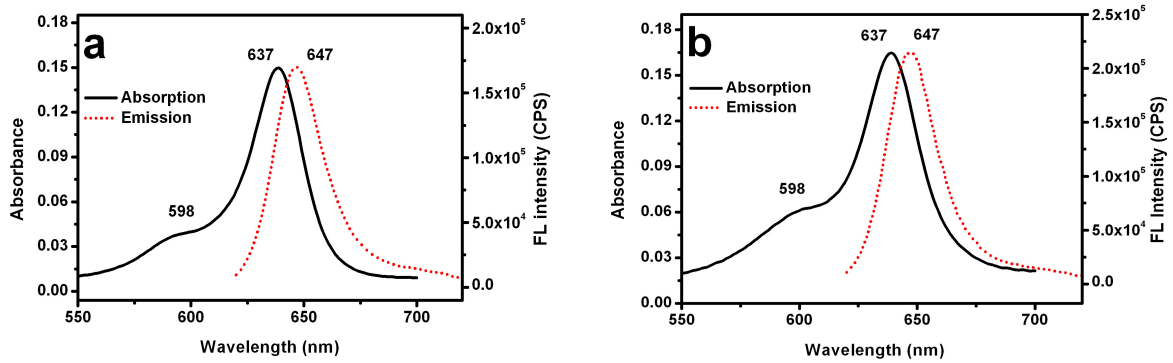


Fig. S1 UV-Vis absorption and fluorescence emission spectra of (a) **D1** and (b) **D2** in Tris-HCl buffer (pH=8). (a) concentration of 1 μM; (b) concentrations of 2 μM for absorption and 1 μM for emission.

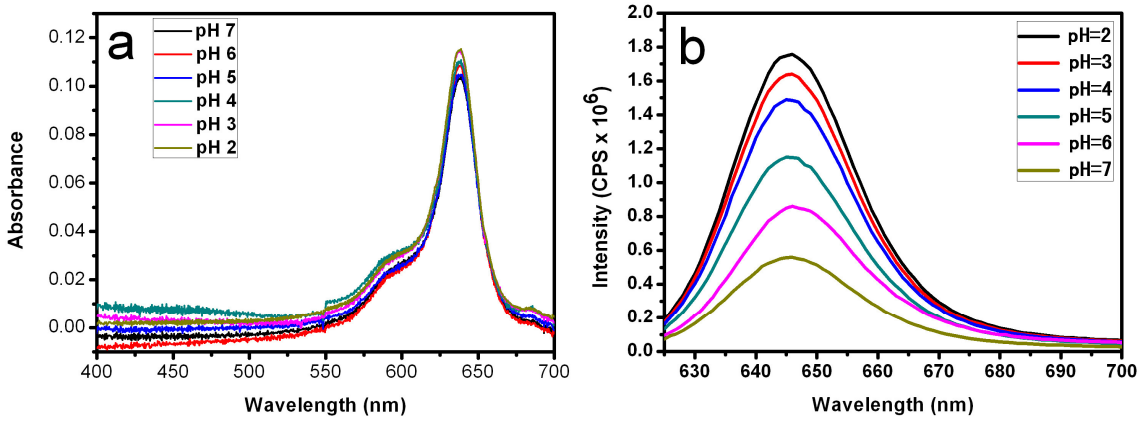


Fig. S2 pH-dependent (a) absorbance and (b) fluorescence of **D2** in Tris-HCl buffer (1 μM, pH=2-7, excitation at 598 nm).

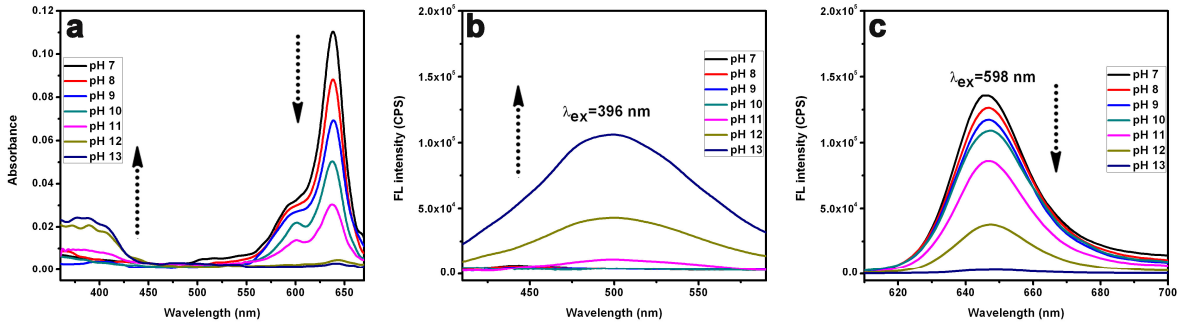


Fig. S3 Variations of (a) absorption and (b, c) emission spectra of **D2** with pH in aqueous buffer solutions. [**D2**]=2 μM; Note: (b) excitation wavelength of 396 nm; (c) excitation wavelength of 598 nm.

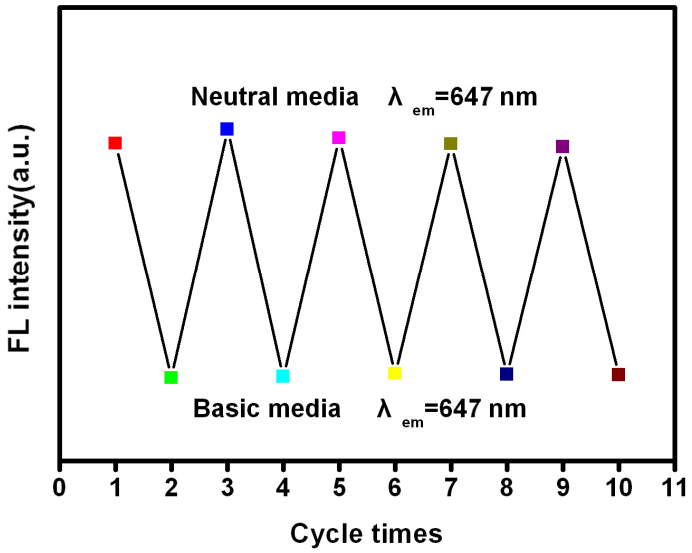
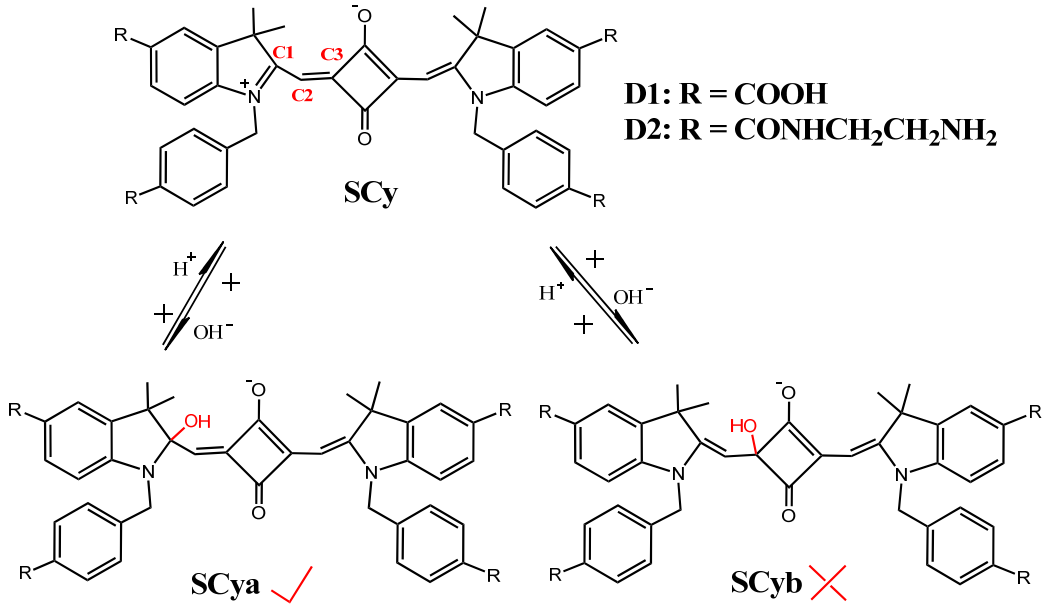


Fig. S4 Reversible switching of the emission of **D2** by repeated adjustment of its solution to pH 7 and pH 13 environments, excitation wavelength of 598 nm.



Scheme S1 Switching mechanism of the SCy dyes under the pH value of 7 and 13.

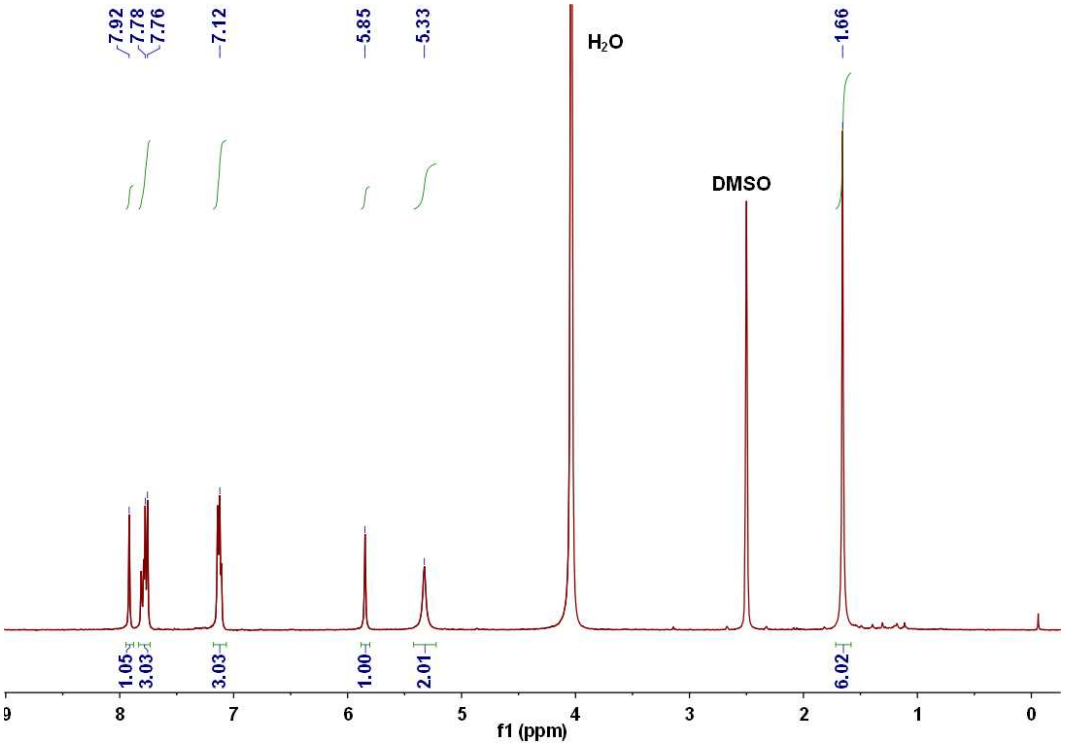


Fig. S5 ^{1}H NMR spectrum of **D1** in 0.5 mL of DMSO/D_2O (4:1 v/v), pH=7.

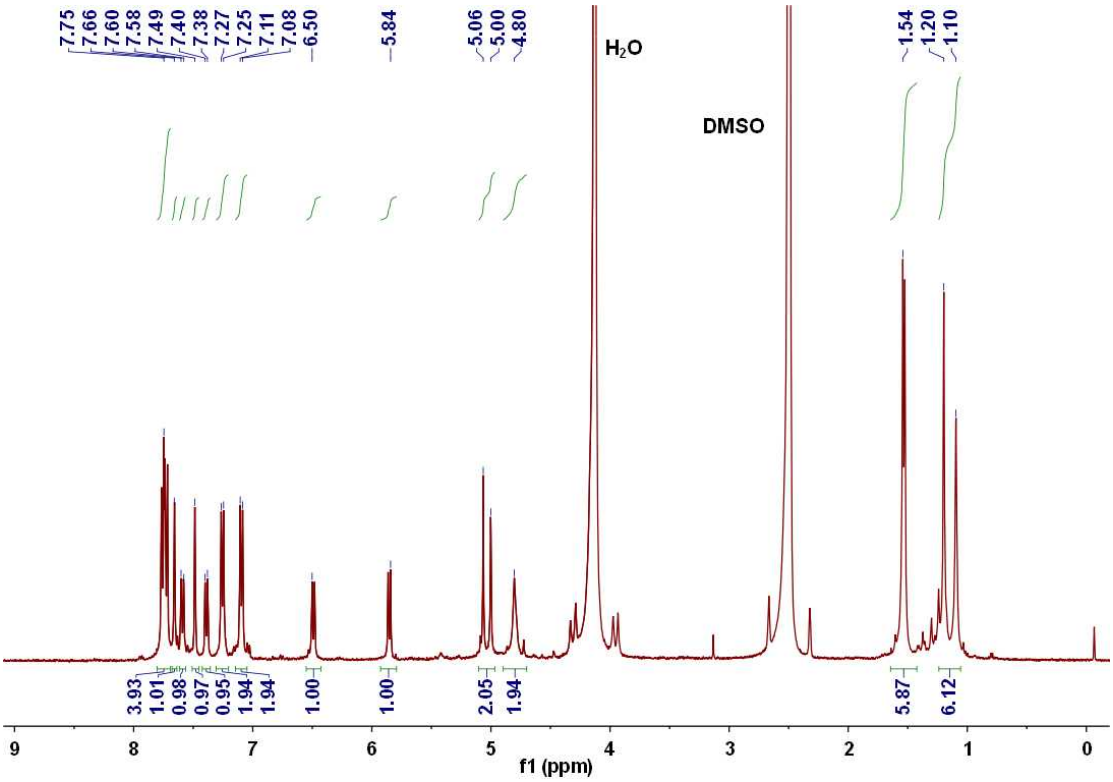


Fig. S6 ^{1}H NMR spectrum of **D1** in 0.5 mL of DMSO/D_2O (4:1 v/v) after addition of 0.05 mL of 2 M NaOH in D_2O, pH=13.

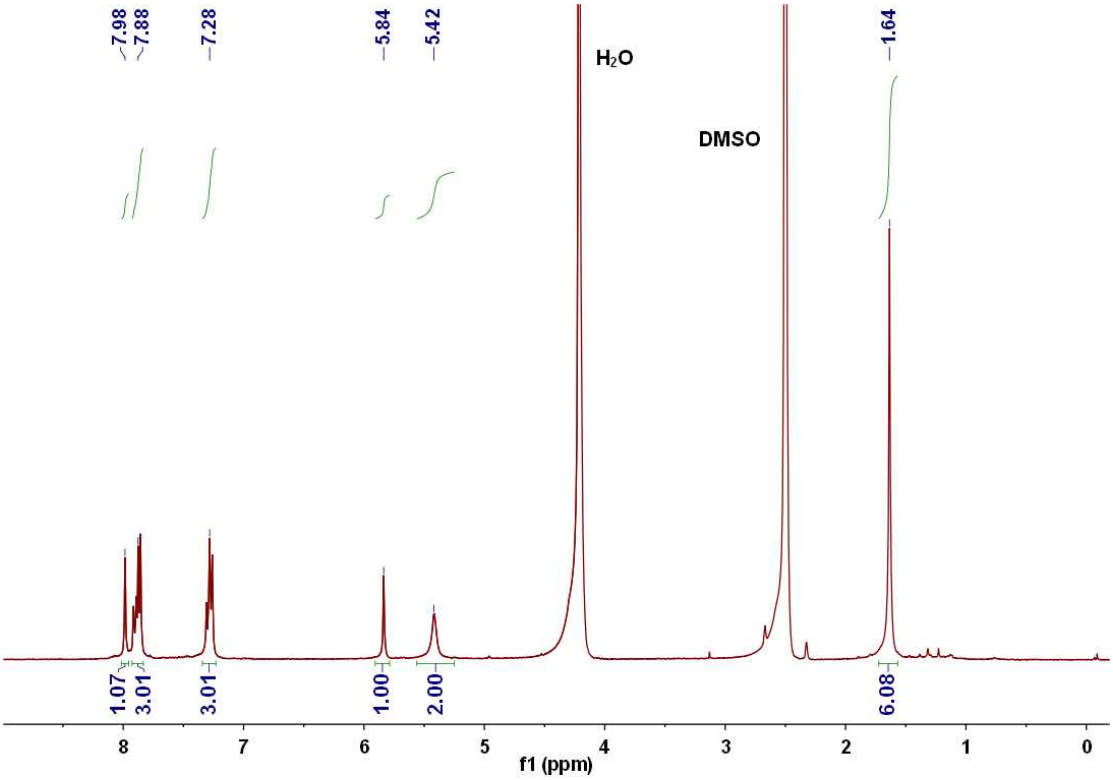


Fig. S7 ^{1}H NMR spectrum of **D1** in 0.5 mL of DMSO/D_2O (4:1 v/v) after addition of 0.05 mL of 2 M NaOH in D_2O, and the system was treated with 0.05 mL of 2 M HCl in D_2O again, pH=7.

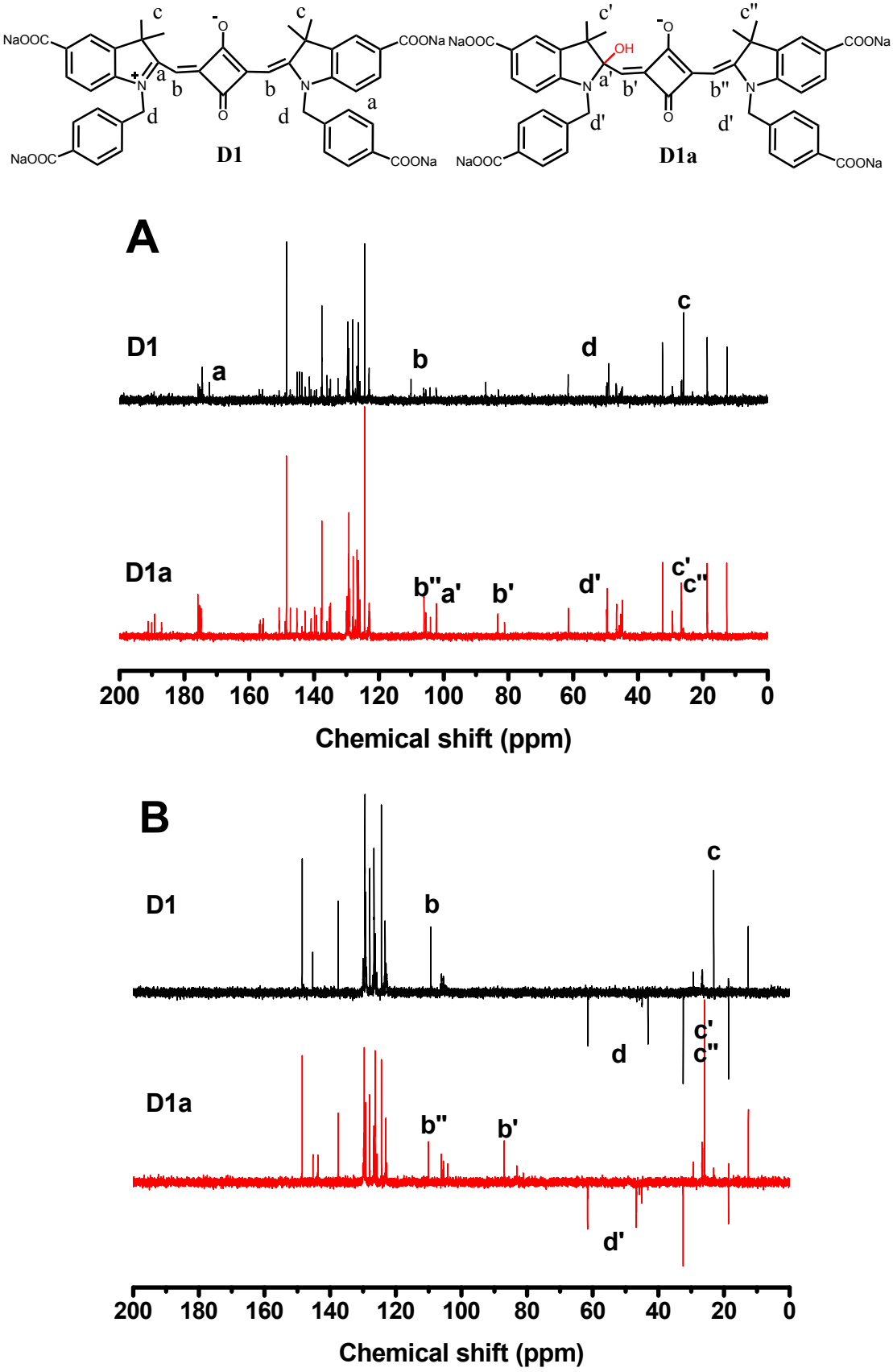


Fig. S8 (A) ^{13}C and (B) DEPT135 NMR spectra of **D1** in neutral (**D1**) and basic (**D1a**) conditions.

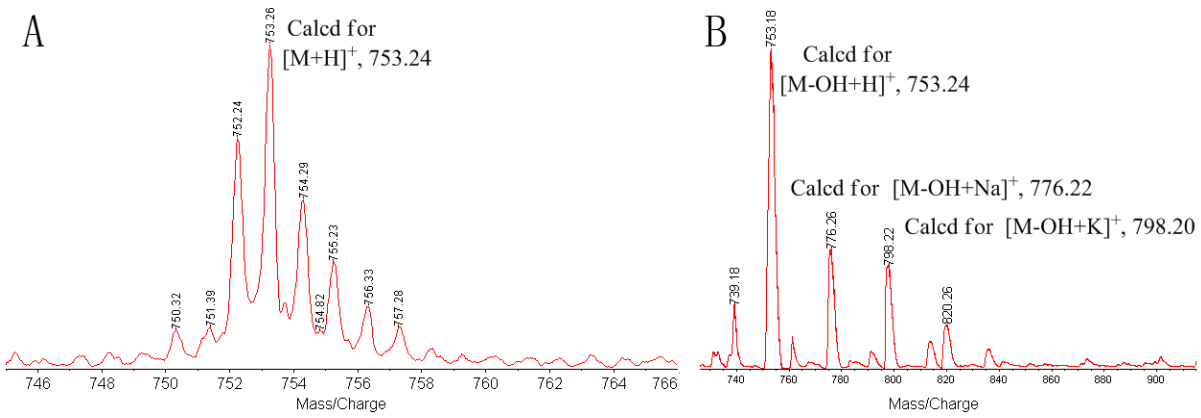


Fig. S9 MALDI-TOF spectrum of **D1** in (A) neutral and (B) basic conditions.

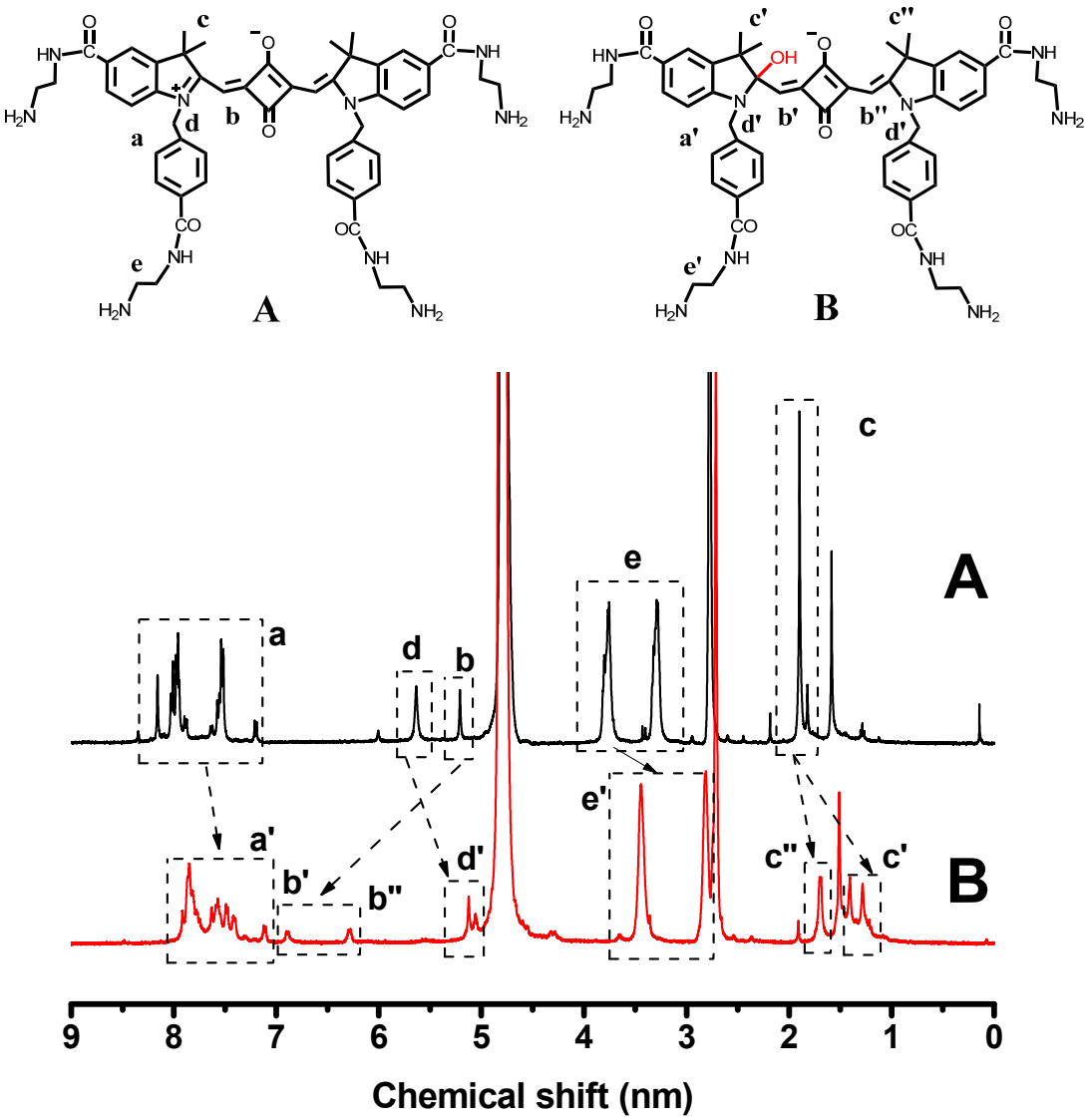


Fig. S10 ^{1}H NMR spectra of **D2** in 0.5 mL of DMSO/D_2O (4:1 v/v): (A) before and (B) after addition of 0.05 mL of 2M NaOH in D_2O.

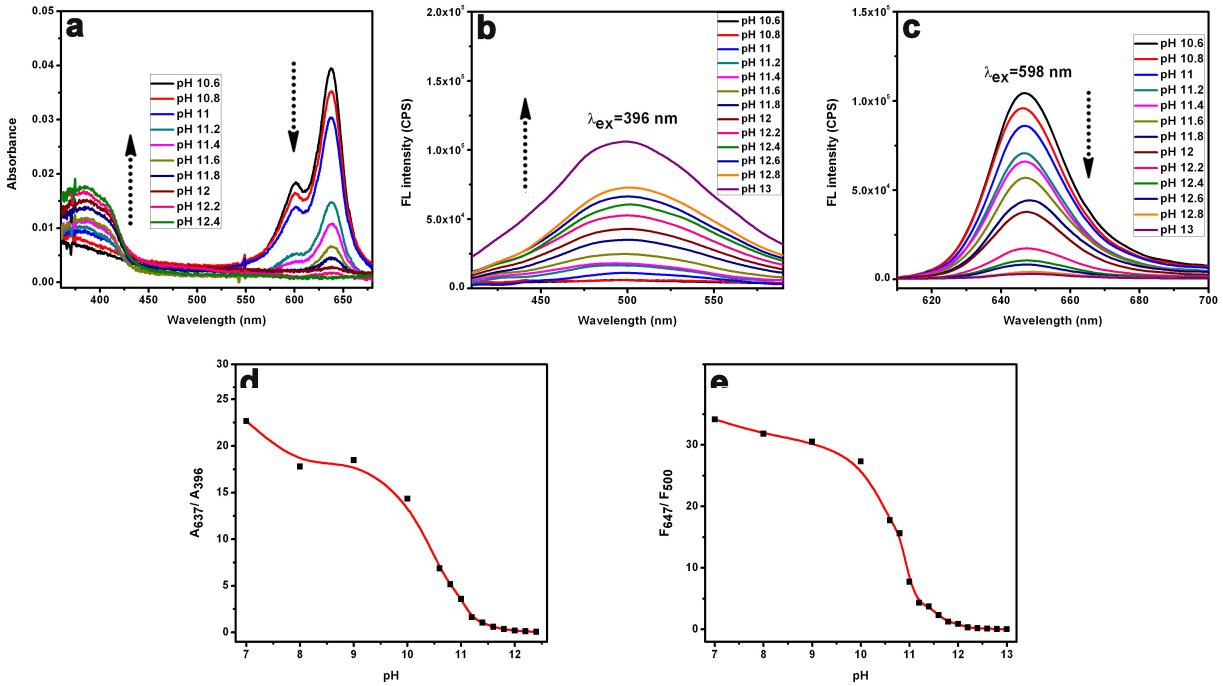
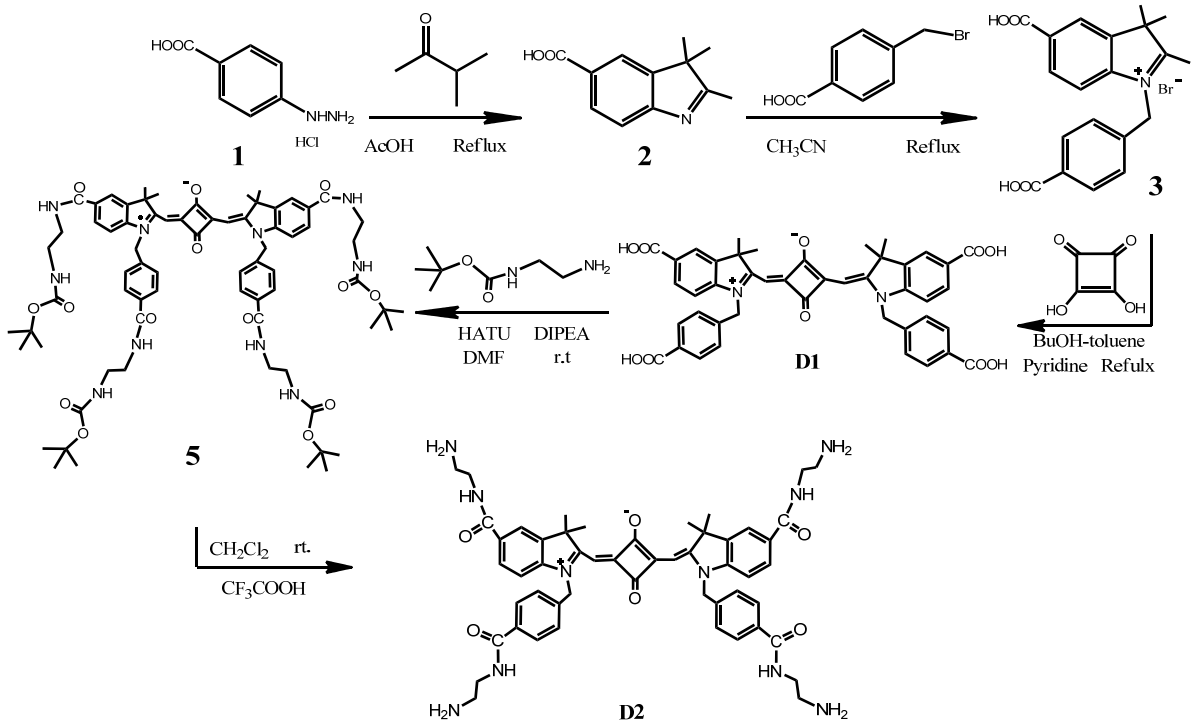


Fig. S11 Variations of (a) absorption and (b, c) emission spectra of **D2** with pH in aqueous buffer solutions; (d) plot of A637/A396 versus pH value, where A637 and A396 are absorption values at wavelengths of 637 and 396 nm, respectively; (e) plot of F647/F500 versus pH value, where F647 and F500 are emission values at wavelengths of 647 and 500 nm, respectively. Note: (b) excitation wavelength of 396 nm; (c) excitation wavelength of 598 nm.

Synthesis and characterization



Scheme S2 Synthesis of SCy dyes **D1** and **D2**.

2,3,3-Trimethylindolenine-5-carboxylic acid (2)

4-Hydrazinobenzoic acid hydrochloride (**1**) (5.66 g, 30 mmol) and 3-methyl-2-butanone (9.6 mL, 90 mmol) were dissolved in acetic acid (20 mL) and then the solution was refluxed for 12 hours. After cooling to room temperature, the solvent was removed under reduced pressure. The resulting residue was dissolved in dichloromethane and then washed with saturated aqueous solution of sodium bicarbonate. The organic extracts were dried over Na_2SO_4, filtered, and evaporated to afford 2,3,3-trimethylindolenine-5-carboxylic acid (**2**) as a khaki solid (4.35 g, yield 71.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ1.38 (s, 6H), 2.40 (s, 3H), 7.70 (s, 1H), 8.07 (s, 1H), 8.15 (s, 1H), 11.74 (s, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 192.52, 171.21, 157.26, 145.53, 130.83, 123.23, 119.65, 53.90, 22.86, 15.51. HRMS (ESI-TOF): *m/z* calcd for $[M-H]^-$, 202.0858; found, 202.0868.

5-Carboxy-1-(p-carboxybenzyl)-2,3,3-trimethylindolenium bromide (3)

Compound **2** (0.82 g, 4 mmol) and 4-bromomethylbenzoic acid (0.86 g, 4 mmol) were dissolved in acetonitrile (10 mL) and then the reaction solution was refluxed for 24 hours.

After cooling to room temperature, the solvent was removed under reduced pressure. The resulting residue was washed with diethyl ether under stirring for three times. The resulting brown solid was collected by filtration and dried under vacuum to give rise to compound **3** (1.34 g, yield 80.4%). ^{1}H NMR (400 MHz, MeOD) δ 1.76 (s, 6H), 6.00 (s, 2H), 7.51 (s, 2H), 7.88 (s, 1H), 8.09 (s, 2H), 8.25 (s, 1H), 8.44 (s, 1H). ^{13}C NMR (150 MHz, MeOD) δ 201.84, 167.32, 166.48, 144.29, 142.23, 135.98, 132.63, 131.49, 130.74, 130.49-130.11 (m), 127.00, 124.49, 115.79, 55.28, 51.11, 21.42, 16.98. HRMS (ESI-TOF): *m/z* calcd for $[M-2H-Br]^-$, 336.1223; found, 336.1219.

SCy D1

Compound **3** (836 mg, 2 mmol) and 3,4-dihydroxycyclobut-3-ene-1,2-dione (114 mg, 1 mmol) were dissolved in a mixture of n-butyl alcohol (5 mL), toluene (5 mL) and pyridine (5 mL). The solution was refluxed for 24 hours under an argon atmosphere. After the reaction mixture was cooled to room temperature, diethyl ether (100 mL) was added into the reaction solution under stirring. The resulting aquamarine blue precipitate was collected by centrifugation and was washed with ethanol and diethyl ether to give the crude product. The crude product was re-dissolved in DMF (1 mL) and precipitated in diethyl ether (100 mL) under stirring. The resulting solid was collected by centrifugation, washed with diethyl ether, and dried under vacuum to afford the pure aquamarine blue solid **D1** which bears four carboxylic acids (598 mg, yield 79.4%). ^{1}H NMR (400 MHz, D_2O + NaOD) δ 1.72 (s, 12H), 5.43 (s, 4H), 5.91 (s, 2H), 7.23 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 8.1 Hz, 4H), 7.85 (t, J = 8.9 Hz, 6H), 7.99 (s, 2H). ^{13}C NMR (100 MHz, D_2O + NaOD) δ 174.46, 174.29, 172.25, 144.29, 141.40, 137.45, 136.23, 132.59, 129.76, 129.56, 126.62, 122.93, 109.95, 86.81, 48.84, 46.91, 25.73. MALDI-TOF: Calcd for $[M+H]^+$, 753.24; Found, 753.26.

Compound 5

Compound **D1** (301.2 mg, 0.4 mmol), o-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HATU) (1.52 g, 4 mmol) and ethyldiisopropylamine (1.32 mL, 8 mmol) were dissolved in 30 mL anhydrous *N,N*-dimethylformamide (DMF) under stirring at room temperature, followed by the addition of *tert*-butyl *N*-(2-aminoethyl)carbamate (512.6 mg, 3.2 mmol). After stirring for 2 hours, the solvent was removed under reduced pressure. The residue was purified by chromatography

(EtOAc/MeOH 5:2) to afford **5** as a blue powder (416 mg, yield 84.5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (d, J = 13.3 Hz, 36H), 1.82 (s, 12H), 3.31 (m, 8H), 3.47 (m, 8H), 5.34 (s, 4H), 6.03 (s, 2H), 7.02 (d, J = 7.2 Hz, 2H), 7.28 (d, J = 7.5 Hz, 4H), 7.87 – 7.71 (m, 6H), 7.92 (s, 2H). ^{13}C NMR (100 MHz, MeOD) δ 179.77, 173.16, 169.86, 169.58, 158.85, 146.51, 143.17, 139.71, 135.37, 131.63, 129.56, 129.20, 127.79, 122.66, 111.36, 89.36, 80.25, 55.88, 50.68, 43.84, 41.46, 40.82, 28.76, 27.49, 18.76, 17.32. HRMS (ESI-TOF): *m/z* calcd for $[M+H]^+$, 1321.6873; found, 1321.6869; calcd for $[M+Na]^+$, 1343.6692; found, 1343.8243.

Compound SCy D2

Compound **5** (264 mg, 0.2 mmol) was dissolved in 3 mL CH_2Cl_2, followed by the addition of 3 mL CF_3COOH under stirring. After 2 hours of stirring, the resulting blue solid was collected by filtration and washed with diethyl ether, and dried under vacuum to yield the final product **D2** as a blue powder (180 mg, yield 98%). ^{1}H NMR (400 MHz, D_2O) δ 1.85 (s, 12H), 3.17 (m, 10H), 3.66 (m, 10H), 5.52 (s, 4H), 6.08 (s, 2H), 7.32 (m, 2H), 7.42 (m, 4H), 7.88 (m, 6H), 8.05 (s, 2H). ^{13}C NMR (100 MHz, D_2O) δ 175.74 , 171.92, 169.58, 169.50,163.38, 163.02, 162.67, 145.16, 141.70, 138.63, 132.19, 128.72, 128.06, 126.53, 121.45, 117.77, 114.87, 111.96, 110.37, 87.35, 48.84, 46.69, 39.17, 37.30, 25.66. HRMS (ESI-TOF): *m/z* calcd for $[M+H]^+$, 921.4776; found, 921.5265; calcd for $[M+Na]^+$, 943.4595; found, 943.4597.