

Electronic Supplementary Information

A Ratiometric Near-Infrared pH-Responsive Fluorescent Dye Based on Distyryl BODIPY

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^1H and $^{13}\text{C}\{^1\text{H}\}$ NMR spectra of **2-5**

Experimental Section

General. All reactions were performed under an atmosphere of nitrogen. Tetrahydrofuran (THF) and toluene were distilled from sodium benzophenone ketyl and sodium respectively. Chromatographic purifications were performed on silica gel (Macherey-Nagel, 70-230 mesh) columns with the indicated eluents. All other solvents and reagents were of reagent grade and used as received. Compound **1** was synthesised as described.¹

¹H and ¹³C{¹H} NMR spectra were recorded on a Bruker AVANCE III 400 spectrometer (¹H, 400; ¹³C, 100.6 MHz) in CDCl₃ or CD₂Cl₂ at ambient temperature. Spectra were referenced internally using the residual solvent [¹H: δ 7.26 (for CDCl₃) or 5.32 (for CD₂Cl₂)] or solvent (¹³C: δ 77.2 for CDCl₃) resonances relative to SiMe₄. Electrospray ionisation (ESI) mass spectra were measured on a Thermo Finnigan MAT 95 XL mass spectrometer. Elemental analyses were performed by the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences.

Electronic absorption and fluorescence emission spectra were recorded on a Cary 5G UV-Vis-NIR spectrophotometer and a Hitachi F-7000 spectrofluorometer respectively at ambient temperature. The excitation and emission slit widths were 5 and 10 nm respectively. The fluorescence quantum yields were determined by the equation: $\Phi_{F(\text{sample})} = (F_{\text{sample}}/F_{\text{ref}})(A_{\text{ref}}/A_{\text{sample}})(n_{\text{sample}}^2/n_{\text{ref}}^2)\Phi_{F(\text{ref})}$,² where *F*, *A* and *n* are the measured fluorescence (area under the emission peak), the absorbance at the excitation position and the refractive index of the solvent respectively. The

unsubstituted zinc(II) phthalocyanine in DMF was used as the reference [$\Phi_{F(\text{ref})} = 0.28$].³ The acidity of the Tween 80 aqueous solution was modulated by adding HCl. All pH measurements were made with a pH meter (Irion Model 420A).

Triethylene glycol monomethyl ether substituted BODIPY 2. To a solution of compound **1** (0.81 g, 3.0 mmol) and 2,4-dimethylpyrrole (0.63 g, 6.6 mmol) in THF (90 mL) was added several drops of trifluoroacetic acid (TFA). The mixture was stirred at ambient temperature overnight, then a solution of 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (0.68 g, 3.0 mmol) in THF (120 mL) was added. The mixture was stirred continuously for another 4 h. After the addition of triethylamine (18 mL, 0.13 mol), $\text{BF}_3 \cdot \text{OEt}_2$ (18 mL, 0.15 mol) was added dropwise to the mixture, which was cooled in an ice-water bath. The mixture was kept stirring at ambient temperature overnight, then filtered through a celite pad. The residue was washed with CH_2Cl_2 (50 mL), then the combined filtrate was rotary evaporated to dryness. The residue was redissolved in CH_2Cl_2 (100 mL) and washed with water (100 mL \times 2). The organic portion was dried over anhydrous MgSO_4 , and then evaporated *in vacuo*. The crude product was purified by silica gel column chromatography using CH_2Cl_2 /ethyl acetate (10:1, v/v) as the eluent to give an orange-yellow solid (0.51 g, 35%). ^1H NMR (CDCl_3): δ 7.16 (d, $J = 8.4$ Hz, 2 H, ArH), 7.02 (d, $J = 8.4$ Hz, 2 H, ArH), 5.97 (s, 2 H, pyrrole-H), 4.19 (t, $J = 4.8$ Hz, 2 H, OCH_2), 3.91 (t, $J = 4.8$ Hz, 2 H, OCH_2), 3.76-3.79 (m, 2 H, OCH_2), 3.70-3.72 (m, 2 H, OCH_2), 3.66-3.69 (m, 2 H, OCH_2), 3.56-3.58 (m, 2 H, OCH_2), 3.39 (s, 3 H, OCH_3), 2.55 (s, 6 H, CH_3), 1.42 (s, 6 H, CH_3). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ 159.4, 155.3, 143.2,

141.9, 131.9, 129.2, 127.2, 121.2, 115.2, 72.0, 70.9, 70.7, 70.6, 69.8, 67.5, 59.1, 14.7 (two overlapping signals). MS (ESI): m/z 509 {100%, $[M+Na]^+$ } and 467 {20%, $[M-F]^+$ }. HRMS (ESI): m/z calcd for $C_{26}H_{33}BF_2N_2NaO_4$ $[M+Na]^+$: 509.2394, found: 509.2398. Anal. calcd for $C_{26}H_{33}BF_2N_2O_4$: C, 64.21; H, 6.84; N, 5.76. Found: C, 64.17; H, 6.97; N, 5.77.

Diiodo BODIPY 3. Iodic acid (0.35 g, 2.0 mmol) dissolved in a minimum amount of water was added dropwise to a mixture of BODIPY **2** (0.51 g, 1.0 mmol) and iodine (0.63 g, 2.5 mmol) in EtOH (150 mL). The mixture was then heated at 60 °C for 2 h. After cooling, the mixture was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography using CH_2Cl_2 /ethyl acetate (25:1, v/v) as the eluent followed by recrystallisation from CH_2Cl_2 and hexane to afford a bright red solid (0.49 g, 66%). 1H NMR ($CDCl_3$): δ 7.12 (d, $J = 8.8$ Hz, 2 H, ArH), 7.05 (d, $J = 8.8$ Hz, 2 H, ArH), 4.20 (t, $J = 4.8$ Hz, 2 H, OCH_2), 3.92 (t, $J = 4.8$ Hz, 2 H, OCH_2), 3.77-3.80 (m, 2 H, OCH_2), 3.71-3.73 (m, 2 H, OCH_2), 3.67-3.69 (m, 2 H, OCH_2), 3.56-3.59 (m, 2 H, OCH_2), 3.40 (s, 3 H, OCH_3), 2.64 (s, 6 H, CH_3), 1.44 (s, 6 H, CH_3). ^{13}C $\{^1H\}$ NMR ($CDCl_3$): δ 159.9, 156.7, 145.5, 141.7, 131.8, 129.1, 126.9, 115.6, 85.7, 72.0, 71.0, 70.8, 70.7, 69.8, 67.7, 59.2, 17.3, 16.1. MS (ESI): m/z 761 {100%, $[M+Na]^+$ }. HRMS (ESI): m/z calcd for $C_{26}H_{31}BF_2I_2N_2NaO_4$ $[M+Na]^+$: 761.0327, found: 761.0324. Anal. calcd for $C_{26}H_{31}BF_2I_2N_2O_4$: C, 42.31; H, 4.23; N, 3.80. Found: C, 42.40; H, 4.50; N, 3.56.

Distyryl BODIPY 4. A mixture of **3** (0.20 g, 0.27 mmol), 4-methoxybenzaldehyde (0.11 g, 0.81 mmol), glacial acetic acid (0.41 mL, 7.2 mmol),

piperidine (0.46 mL, 4.7 mmol) and a small amount of $\text{Mg}(\text{ClO}_4)_2$ in toluene (60 mL) was refluxed for 2 h. The water formed during the reaction was removed azeotropically with a Dean-Stark apparatus. The mixture was concentrated under reduced pressure. The residue was then purified by silica gel column chromatography using ethyl acetate/hexane (1:1, v/v) as the eluent. The green coloured fraction was collected and rotary evaporated, then it was further purified by size-exclusion chromatography with Bio-beads S-X1 beads using THF as the eluent followed by recrystallisation from CH_2Cl_2 and hexane (86 mg, 33%). ^1H NMR (CDCl_3): δ 8.13 (d, $J = 16.8$ Hz, 2 H, CH=CH), 7.62 (d, $J = 8.8$ Hz, 4 H, ArH), 7.59 (d, $J = 16.8$ Hz, 2 H, CH=CH), 7.16 (d, $J = 8.8$ Hz, 2 H, ArH), 7.06 (d, $J = 8.8$ Hz, 2 H, ArH), 6.95 (d, $J = 8.8$ Hz, 4 H, ArH), 4.22 (t, $J = 4.8$ Hz, 2 H, OCH_2), 3.94 (t, $J = 4.8$ Hz, 2 H, OCH_2), 3.87 (s, 6 H, OCH_3), 3.78-3.81 (m, 2 H, OCH_2), 3.72-3.74 (m, 2 H, OCH_2), 3.68-3.70 (m, 2 H, OCH_2), 3.57-3.60 (m, 2 H, OCH_2), 3.40 (s, 3 H, OCH_3), 1.50 (s, 6 H, CH_3). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3): δ 160.8, 159.9, 150.5, 145.9, 139.2, 138.7, 133.4, 129.7, 129.4, 127.6, 116.9, 115.6, 114.4, 82.8, 72.1, 71.1, 70.8, 70.7, 69.9, 67.7, 59.2, 55.6, 17.9. MS (ESI): m/z 997 {100%, $[\text{M}+\text{Na}]^+$ }. HRMS (ESI): m/z calcd for $\text{C}_{42}\text{H}_{43}\text{BF}_2\text{I}_2\text{N}_2\text{NaO}_6$ $[\text{M}+\text{Na}]^+$: 997.1164, found: 997.1164. Anal. calcd for $\text{C}_{42}\text{H}_{43}\text{BF}_2\text{I}_2\text{N}_2\text{O}_6$: C, 51.77; H, 4.45; N, 2.87. Found: C, 51.93; H, 4.84; N, 2.77.

Distyryl BODIPY 5. A mixture of **4** (0.12 g, 0.12 mmol), $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (9 mg, 13 μmol), CuI (6 mg, 0.03 mmol), 4-(dimethylamino)phenylethyne (52 mg, 0.36 mmol) and triethylamine (5 mL) in THF (30 mL) was stirred at room temperature overnight. The volatiles were removed *in vacuo*, then the dark green residue was extracted with

CH₂Cl₂ (100 mL). The extract was washed with water (30 mL × 2), dried over anhydrous MgSO₄, then concentrated under reduced pressure. The mixture was chromatographed using CH₂Cl₂/ethyl acetate (25:1, v/v) as the eluent to give a dark green solid (52 mg, 42%). ¹H NMR (CD₂Cl₂): δ 8.49 (d, *J* = 16.4 Hz, 2 H, CH=CH), 7.69 (d, *J* = 16.4 Hz, 2 H, CH=CH), 7.64 (d, *J* = 8.8 Hz, 4 H, ArH), 7.39 (d, *J* = 8.8 Hz, 4 H, ArH), 7.26 (d, *J* = 8.8 Hz, 2 H, ArH), 7.10 (d, *J* = 8.8 Hz, 2 H, ArH), 6.98 (d, *J* = 8.8 Hz, 4 H, ArH), 6.69 (d, *J* = 8.8 Hz, 4 H, ArH), 4.21 (t, *J* = 4.8 Hz, 2 H, OCH₂), 3.88 (t, *J* = 4.8 Hz, 2 H, OCH₂), 3.87 (s, 6 H, OCH₃), 3.71-3.73 (m, 2 H, OCH₂), 3.64-3.66 (m, 2 H, OCH₂), 3.61-3.63 (m, 2 H, OCH₂), 3.52-3.54 (m, 2 H, OCH₂), 3.35 (s, 3 H, OCH₃), 3.00 (s, 12 H, NCH₃), 1.64 (s, 6 H, CH₃). ¹³C{¹H} NMR (CDCl₃): δ 160.6, 159.6, 151.9, 150.1, 143.7, 138.5, 138.3, 133.2, 132.2, 130.2, 129.9, 129.3, 127.5, 117.2, 115.2, 114.5, 114.4, 112.0, 110.5, 99.5, 82.0, 72.0, 71.0, 70.7, 70.6, 69.8, 67.6, 59.2, 55.5, 40.3, 13.5. MS (ESI): *m/z* 1009 {100%, [M+H]⁺}. HRMS (ESI): *m/z* calcd for C₆₂H₆₄BF₂N₄O₆ [M+H]⁺: 1009.4881, found:1009.4897. Anal. calcd for C₆₂H₆₅BF₂N₄O₇ (5·H₂O): C, 72.51; H, 6.38; N, 5.46. Found: C, 72.19; H, 6.00; N, 5.19.

References

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- 2 D. F. Eaton, *Pure Appl. Chem.*, 1988, **60**, 1107.
- 3 I. Scalise and E. N. Durantini, *Bioorg. Med. Chem.*, 2005, **13**, 3037.

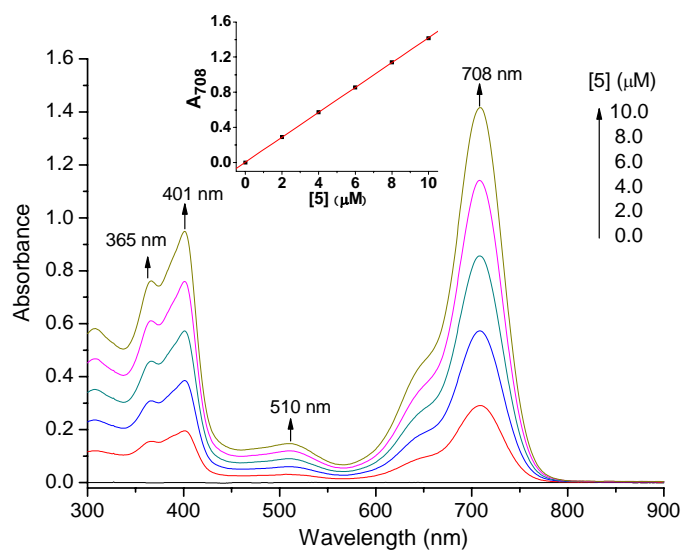


Fig. S1 Electronic absorption spectra of **5** in chloroform at various concentrations.

The inset plots the Q-band absorbance at 708 nm vs. the concentration of **5**.

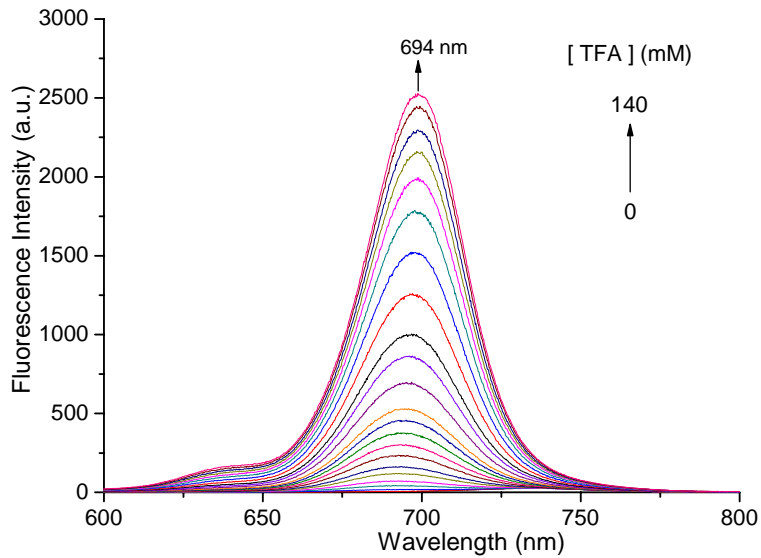


Fig. S2 Change in fluorescence emission spectrum of **5** in chloroform (1 μM) upon addition of TFA (excited at 409 nm).

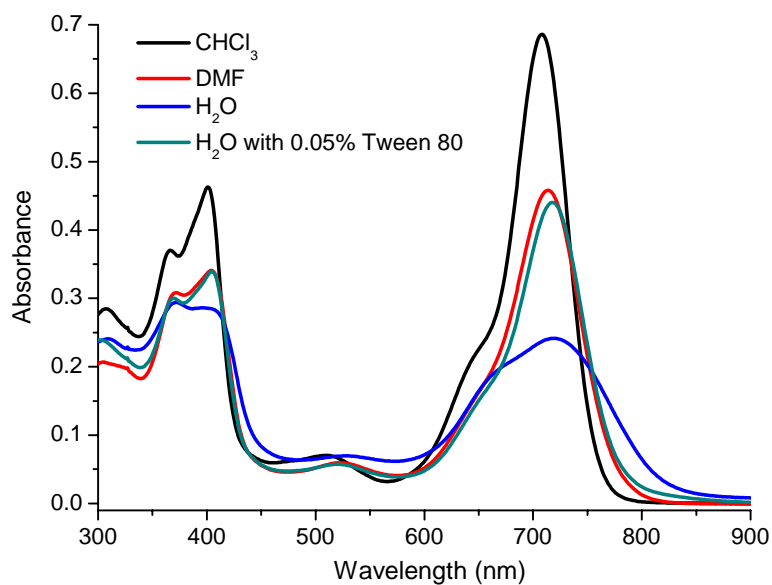


Fig. S3 Electronic absorption spectra of **5** in different solvents (5 μ M).

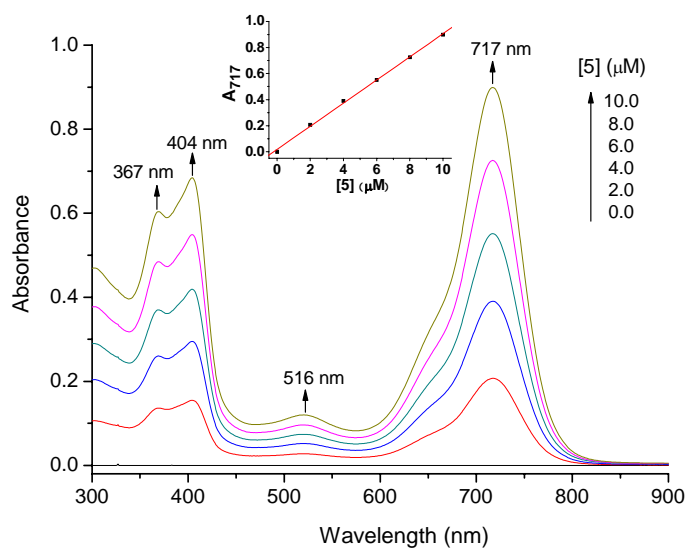


Fig. S4 Electronic absorption spectra of **5** in water with 0.05% (v/v) Tween 80 at various concentrations. The inset plots the Q-band absorbance at 717 nm vs. the concentration of **5**.

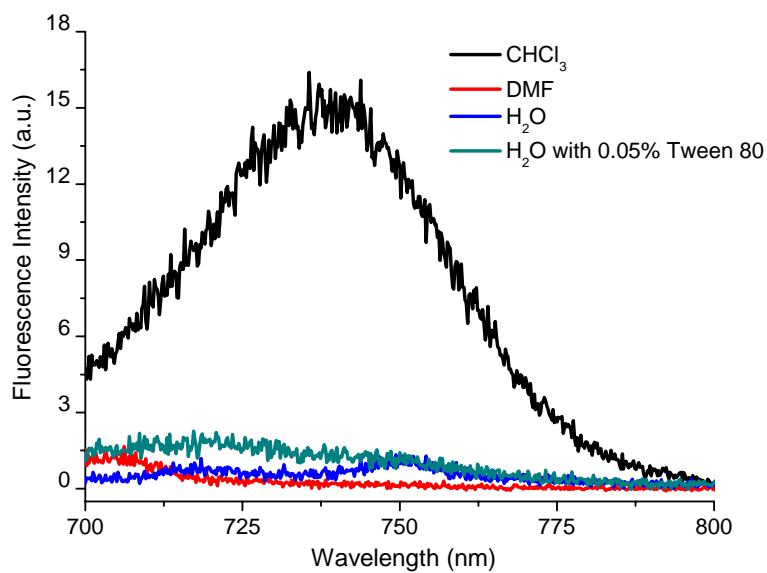


Fig. S5 Fluorescence emission spectra of **5** in different solvents (5 μ M) (excited at 640 nm).

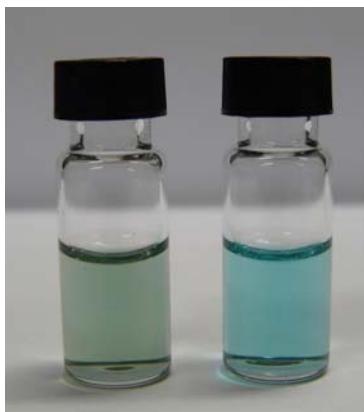
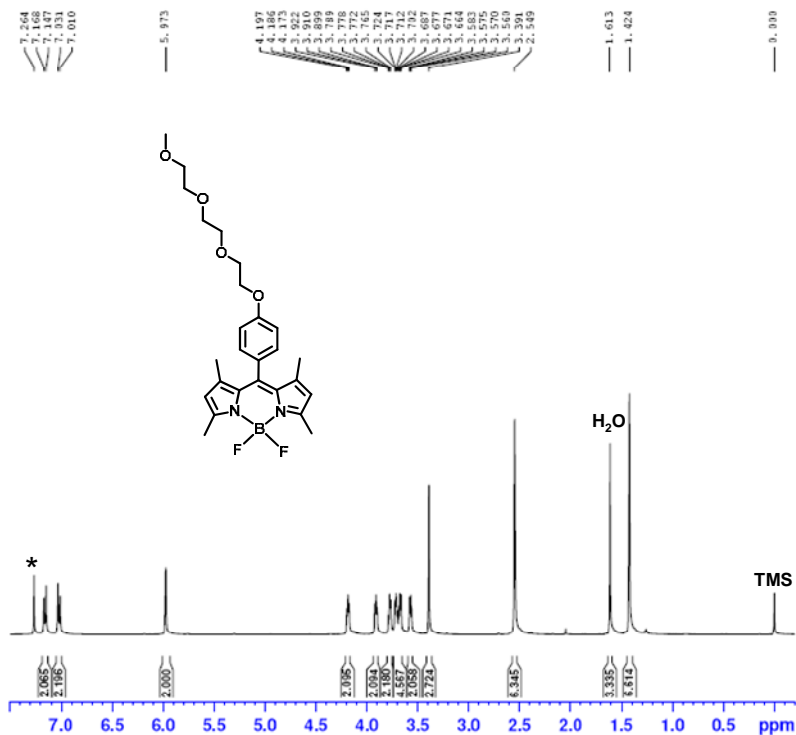


Fig. S6 The colour change of a solution of **5** in water with 0.05% (v/v) Tween 80 (10 μ M) as the pH changes from 5.95 (left) to 0.38 (right).

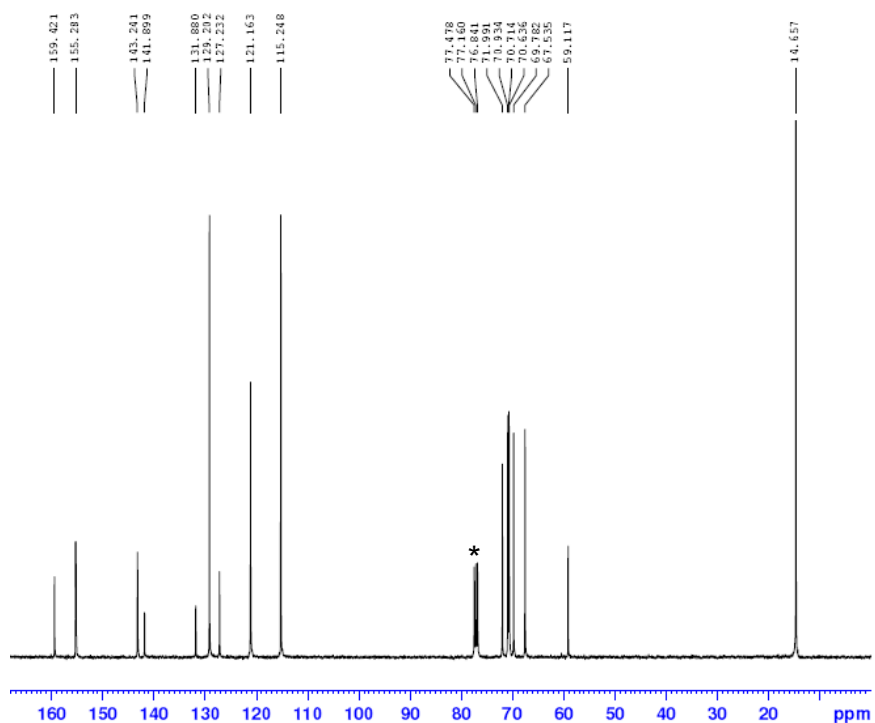


Fig. S7 The fluorescence observed in a solution of **5** in water with 0.05% (v/v) Tween 80 (10 μ M) at pH = 5.95 (left) and 0.38 (right).

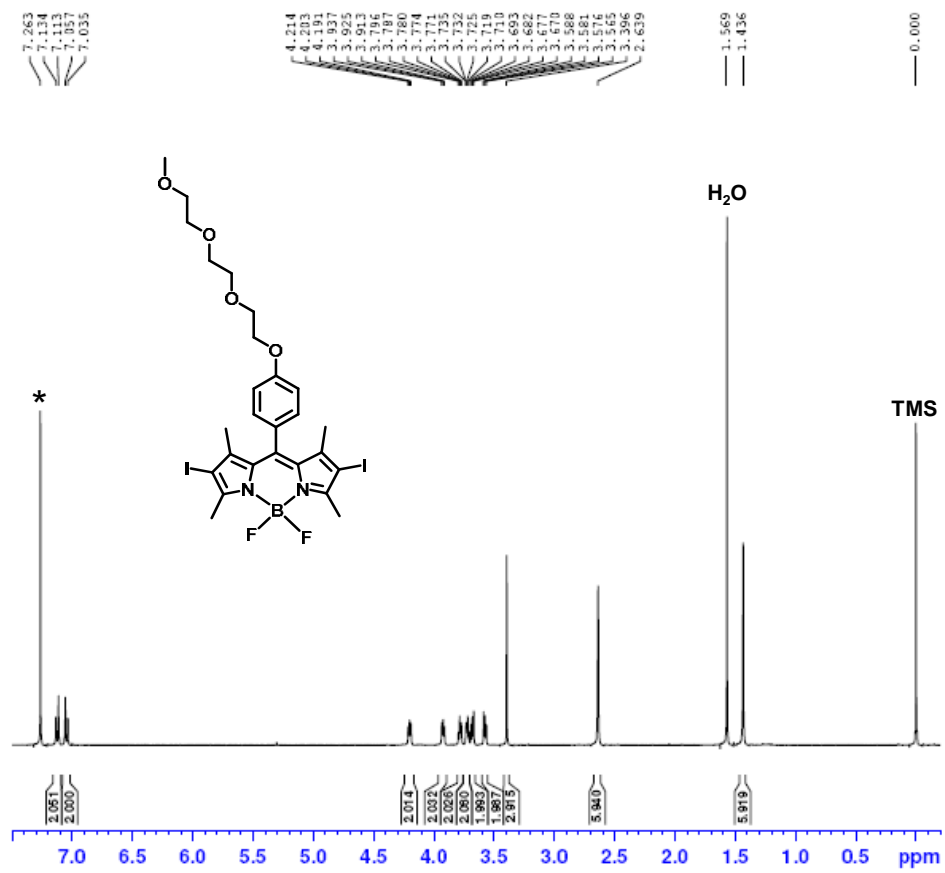
In all of the following spectra, (residual) solvent signals are marked with asterisks.



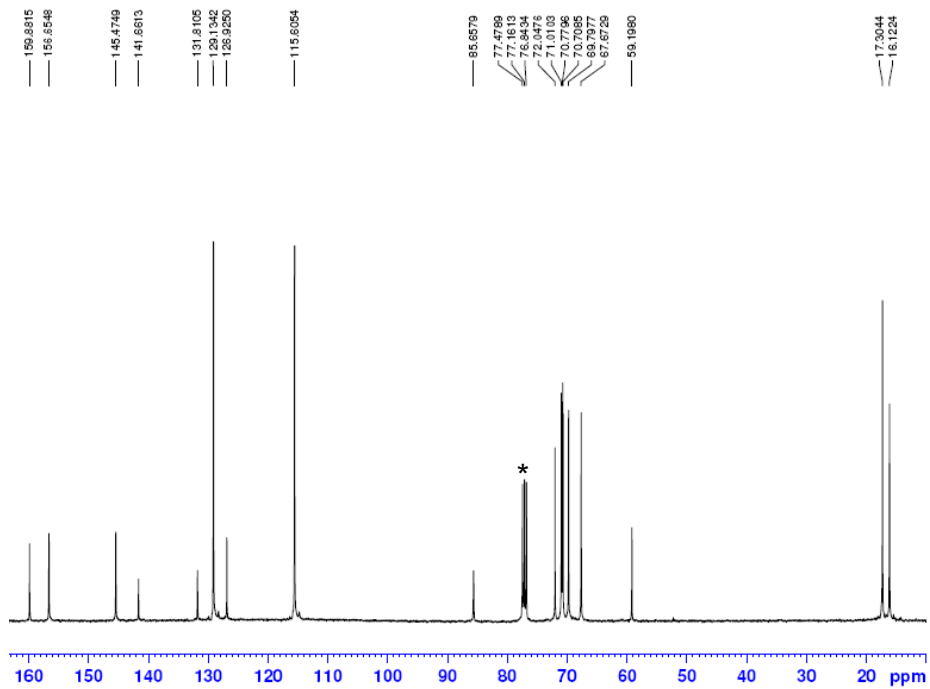
¹H NMR spectrum of compound 2 in CDCl₃



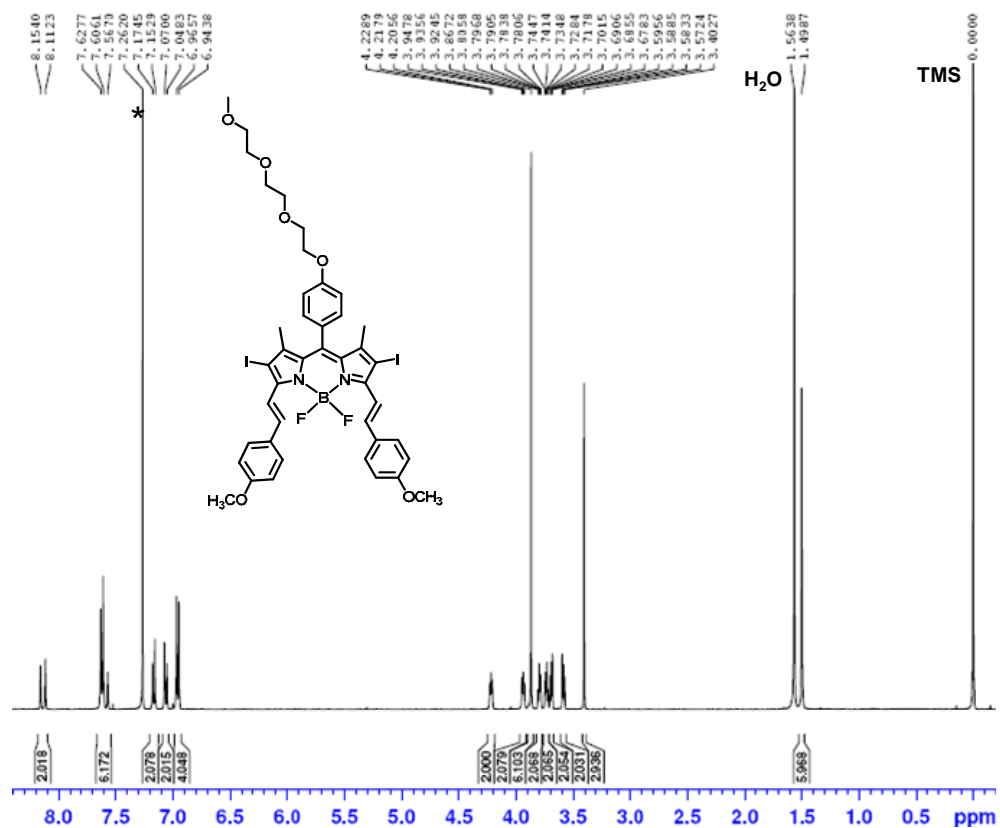
¹³C{¹H} NMR spectrum of compound 2 in CDCl₃



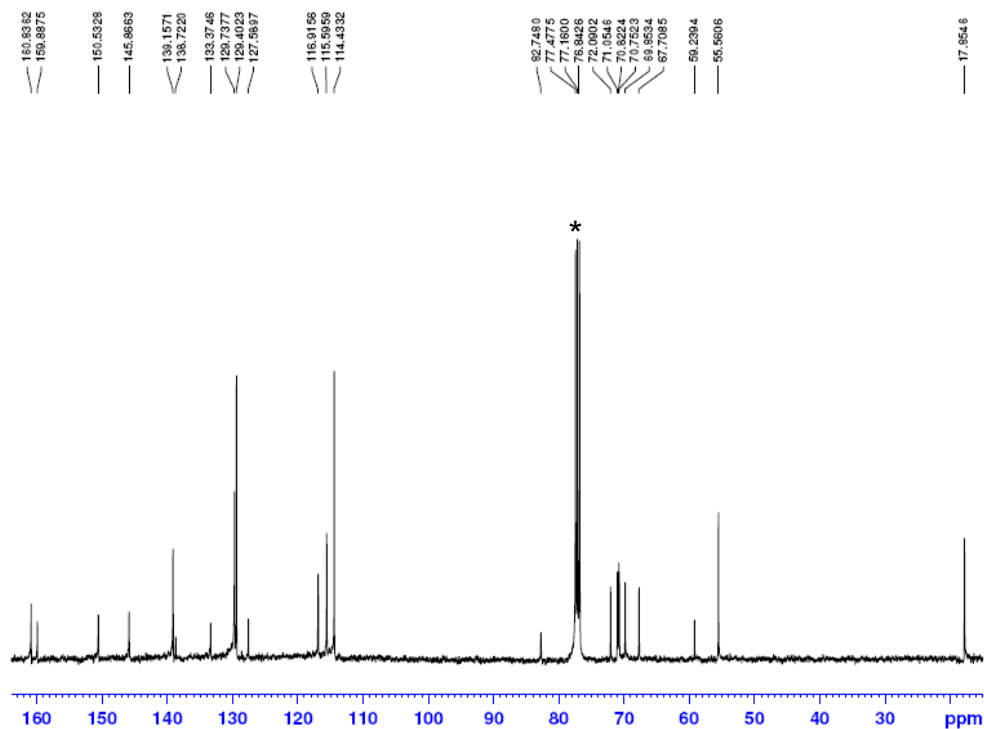
^1H NMR spectrum of compound 3 in CDCl_3



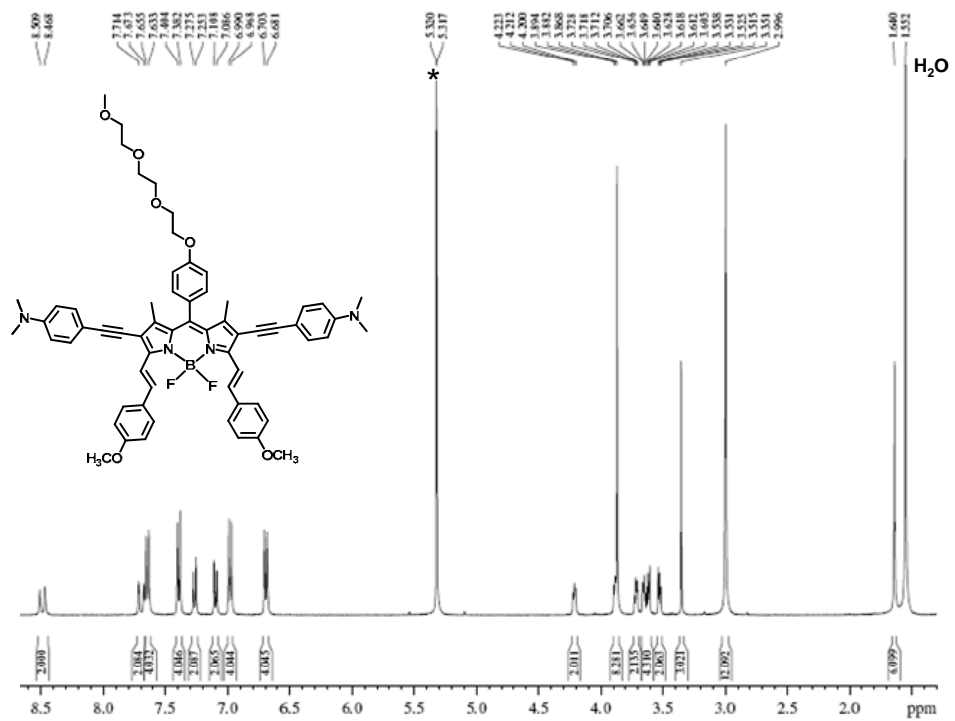
$^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of compound 3 in CDCl_3



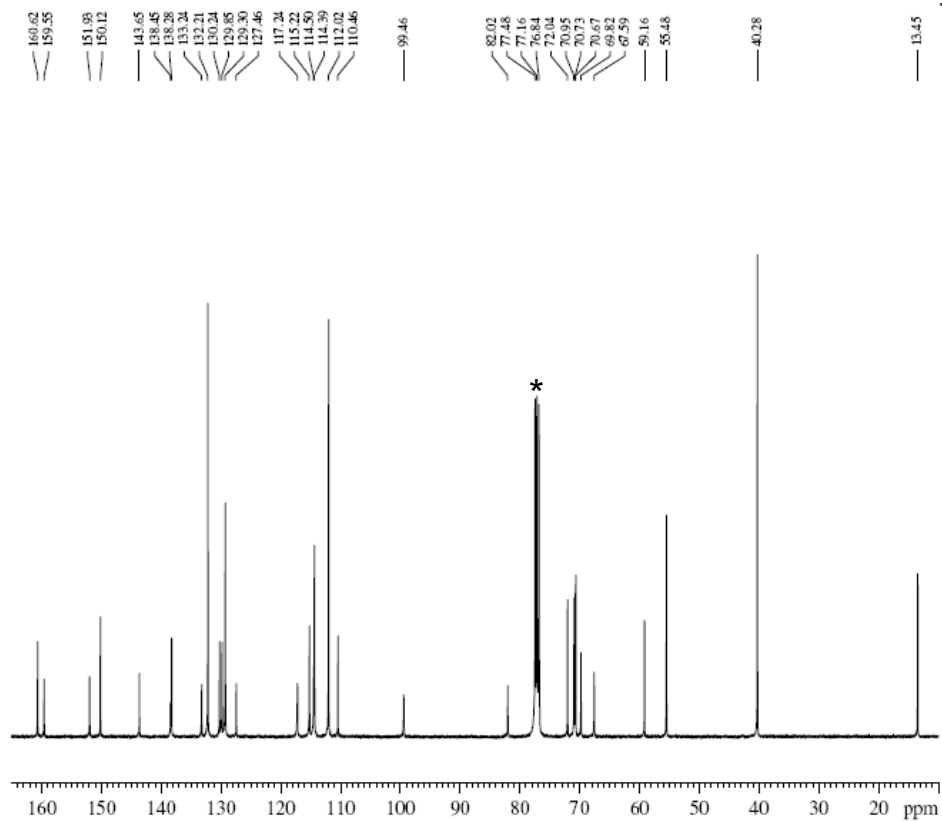
¹H NMR spectrum of compound **4** in CDCl₃



¹³C {¹H} NMR spectrum of compound **4** in CDCl₃



¹H NMR spectrum of compound 5 in CD₂Cl₂



¹³C{¹H} NMR spectrum of compound 5 in CDCl₃