Electronic Supplementary Information

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

Hui He and Dennis K. P. Ng*

Contents

Experimental Section

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

Fig. S2  Change in fluorescence emission spectrum of 5 in chloroform (1 μM) upon addition of TFA.

Fig. S3  Electronic absorption spectra of 5 in different solvents.

Fig. S4  Electronic absorption spectra of 5 in water with 0.05% (v/v) Tween 80 at various concentrations.

Fig. S5  Fluorescence emission spectra of 5 in different solvents.

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

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

$^1$H and $^{13}$C{$^1$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: \[ \Phi_{F,{\text{sample}}} = \left( \frac{F_{{\text{sample}}}}{F_{{\text{ref}}}} \right) \left( \frac{A_{{\text{ref}}}}{A_{{\text{sample}}}} \right) \left( \frac{n_{{\text{sample}}}}{n_{{\text{ref}}}} \right)^2 \Phi_{F,{\text{ref}}}^2 \] 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), BF$_3$·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$_2$Cl$_2$ (50 mL), then the combined filtrate was rotary evaporated to dryness. The residue was redissolved in CH$_2$Cl$_2$ (100 mL) and washed with water (100 mL × 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$_2$Cl$_2$/ethyl acetate (10:1, v/v) as the eluent to give an orange-yellow solid (0.51 g, 35%). $^1$H NMR (CDCl$_3$): $\delta$ 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$_3$). $^{13}$C{ $^1$H} NMR (CDCl$_3$): $\delta$ 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_{2}N_{2}NaO_{4} [M+Na]^+: 509.2394, found: 509.2398. Anal. calcd for C_{26}H_{33}BF_{2}N_{2}O_{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_{2}Cl_{2}/ethyl acetate (25:1, v/v) as the eluent followed by recrystallisation from CH_{2}Cl_{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}). ^13C{^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_{2}I_{2}N_{2}NaO_{4} [M+Na]^+: 761.0327, found: 761.0324. Anal. calcd for C_{26}H_{31}BF_{2}I_{2}N_{2}O_{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 Mg(ClO₄)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 CH₂Cl₂ and hexane (86 mg, 33%). ¹H NMR (CDCl₃): 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, OCH₂), 3.94 (t, J = 4.8 Hz, 2 H, OCH₂), 3.87 (s, 6 H, OCH₃), 3.78-3.81 (m, 2 H, OCH₂), 3.72-3.74 (m, 2 H, OCH₂), 3.68-3.70 (m, 2 H, OCH₂), 3.57-3.60 (m, 2 H, OCH₂), 3.40 (s, 3 H, OCH₃), 1.50 (s, 6 H, CH₃).

¹³C{¹H} NMR (CDCl₃): 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%, [M+Na]⁺}. HRMS (ESI): m/z calcld for C₄₂H₄₃BF₂I₂N₂NaO₆ [M+Na]⁺: 997.1164, found: 997.1164. Anal. calcld for C₄₂H₄₃BF₂I₂N₂O₆: 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), Pd(PPh₃)₂Cl₂ (9 mg, 13 µmol), Cul (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
CH$_2$Cl$_2$ (100 mL). The extract was washed with water (30 mL × 2), dried over anhydrous MgSO$_4$, then concentrated under reduced pressure. The mixture was chromatographed using CH$_2$Cl$_2$/ethyl acetate (25:1, v/v) as the eluent to give a dark green solid (52 mg, 42%). $^1$H NMR (CD$_2$Cl$_2$): $\delta$ 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), 6.69 (d, $J = 8.8$ Hz, 4 H, ArH), 4.21 (t, $J = 4.8$ Hz, 2 H, OCH$_2$), 3.88 (t, $J = 4.8$ Hz, 2 H, OCH$_2$), 3.87 (s, 6 H, OCH$_3$), 3.71-3.73 (m, 2 H, OCH$_2$), 3.64-3.66 (m, 2 H, OCH$_2$), 3.61-3.63 (m, 2 H, OCH$_2$), 3.52-3.54 (m, 2 H, OCH$_2$), 3.35 (s, 3 H, OCH$_3$), 3.00 (s, 12 H, NCH$_3$), 1.64 (s, 6 H, CH$_3$). $^{13}$C$^1$H NMR (CDCl$_3$): $\delta$ 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 C$_{62}$H$_{64}$BF$_2$N$_4$O$_6$ [M+H]$^+$: 1009.4881, found:1009.4897. Anal. calcd for C$_{62}$H$_{65}$BF$_2$N$_4$O$_7$ (5-H$_2$O): C, 72.51; H, 6.38; N, 5.46. Found: C, 72.19; H, 6.00; N, 5.19.

References


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 µM).

**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 μM) (excited at 640 nm).

**Fig. S6** The colour change of a solution of 5 in water with 0.05% (v/v) Tween 80 (10 μM) 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 µM) at pH = 5.95 (left) and 0.38 (right).
In all of the following spectra, (residual) solvent signals are marked with asterisks.

**H NMR spectrum of compound 2 in CDCl₃**

**¹³C{¹H} NMR spectrum of compound 2 in CDCl₃**
$^1$H NMR spectrum of compound 3 in CDCl$_3$

$^{13}$C\{$^1$H\} NMR spectrum of compound 3 in CDCl$_3$
\( ^1H \) NMR spectrum of compound 4 in CDCl₃

\( ^{13}C\{^1H\} \) NMR spectrum of compound 4 in CDCl₃
$^1\text{H}$ NMR spectrum of compound 5 in CD$_2$Cl$_2$

$^{13}\text{C}^{'1\text{H}}$ NMR spectrum of compound 5 in CDCl$_3$