Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Oligoethylene Glycol-substituted Aza-BODIPY Dyes As Red Emitting ER-Probes

Supporting Information

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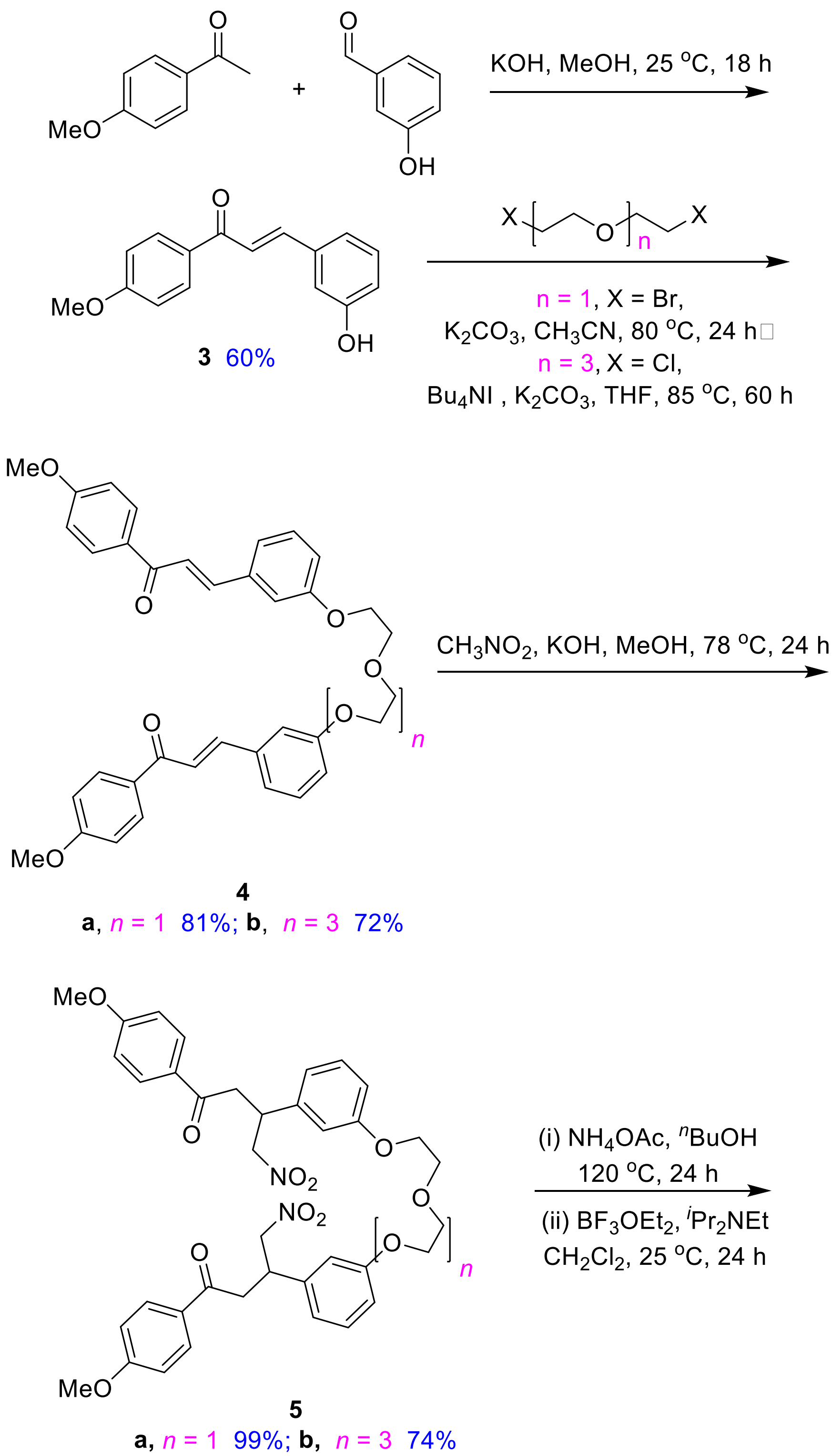
1. General Procedures

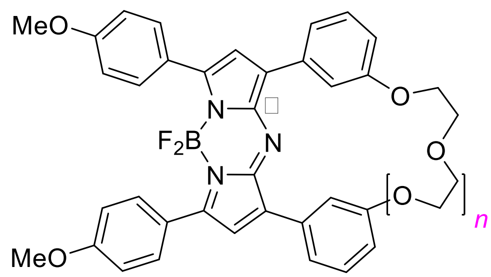
All reactions were carried out under an atmosphere of dry argon. Glassware was oven-dried prior to use. Unless otherwise indicated, common reagents or materials were obtained from commercial source and used without further purification. Tetrahydrofuran (THF), dichloromethane (CH_2Cl_2), and methanol (MeOH) were dried by Mbraun solvent drying system. Other solvents and reagents were used as received.

NMR spectra were recorded on a Bruker-400 MHz spectrometers (^{1}H at 400 MHz and ^{13}C at 100 MHz) at room temperature unless other mentioned. Chemical shifts of ^{1}H NMR spectra were recorded and chemical shifts are reported in ppm from the solvent resonance ($CDCl_3$ 7.26 ppm, CD_3OD 3.30 ppm, acetone-d_6 2.05 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, br = broad, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants, and number of protons. Proton decoupled ^{13}C NMR spectra were also recorded in ppm from tetramethylsilane (TMS) resonance ($CDCl_3$ 77.0, CD_3OD 49.1, acetone-d_6 206.26 and 29.84 ppm). Analytical thin layer chromatography (TLC) was performed on EM Reagents 0.25 mm silica-gel 60-F plates, and visualized with UV light. Flash chromatography was performed using silica gel 60 (230–400 mesh). MS were measured under ESI or MALDI conditions.

Analytical HPLC analyses were carried out on 150 x 4.6 mm C-18 column using gradient conditions (80 – 95% B, flow rate = 0.75 mL/min).

2. Syntheses Of Oligoethylene Glycol-strapped Aza-BODIPY





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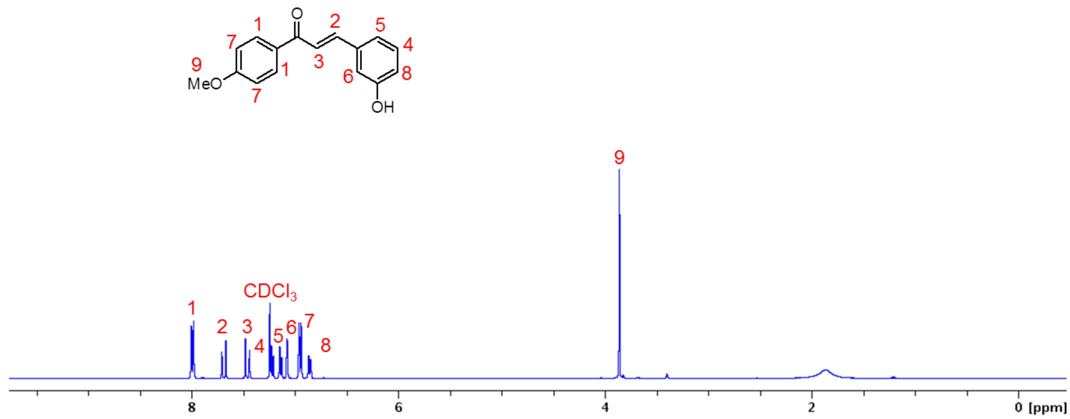
a, $n = 1$ 14%; **b**, $n = 3$ 3%

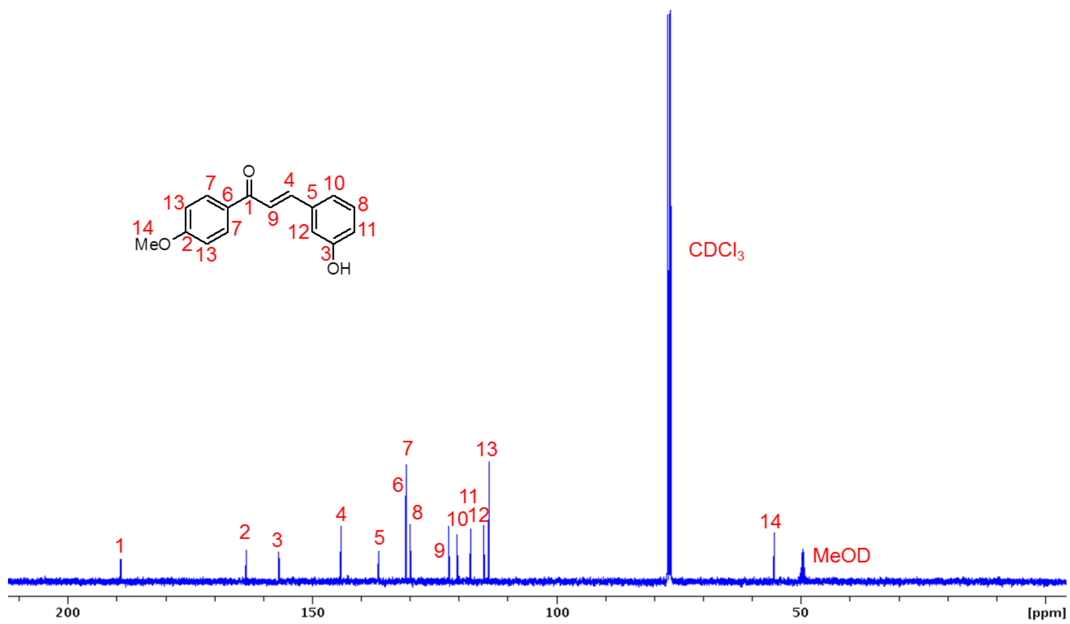
3. Experimental Procedures And Characterization Of Oligoethylene Glycol-strapped Aza-BODIPY

(*E*)-3-(3-hydroxyphenyl)-1-(4-methoxyphenyl)prop-2-en-1-one (3).

An aqueous solution of sodium hydroxide (60%, 16 mL) was added to a solution of 4-methoxyacetophenone (5 g, 33 mmol) and 3-hydroxybenzaldehyde (4 g, 33 mmol) in methanol (20 mL) and stirred at room temperature for a period of 24 h. The reaction mixture was poured into ice water and adjusted pH to 2 (using HCl). The obtained solid was filtered and recrystallized from ethanol to yield 5 g (60%) of **3** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.4 Hz, 2H), 7.69 (d, *J* = 15.6 Hz, 1H), 7.46 (d, *J* = 15.6 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 7.13 (d, *J* = 7.6 Hz, 1H), 7.07 (s, 1H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.85 (d, *J* = 8.0 Hz, 1H), 3.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.2, 163.5, 156.8, 144.2, 136.5, 131.0, 130.9, 130.0, 122.1, 120.4, 117.6, 114.9, 113.9, 55.5. HRMS (ESI) calcd for $C_{16}H_{14}O_3$ $\{M+Li\}^+$ 261.1103, found 261.1076.

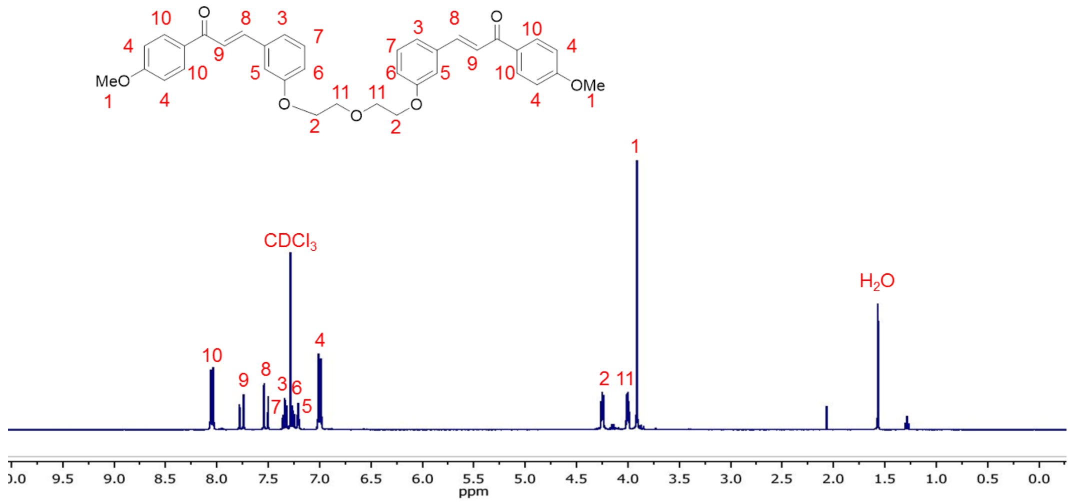


^{1}H-NMR of compound 3

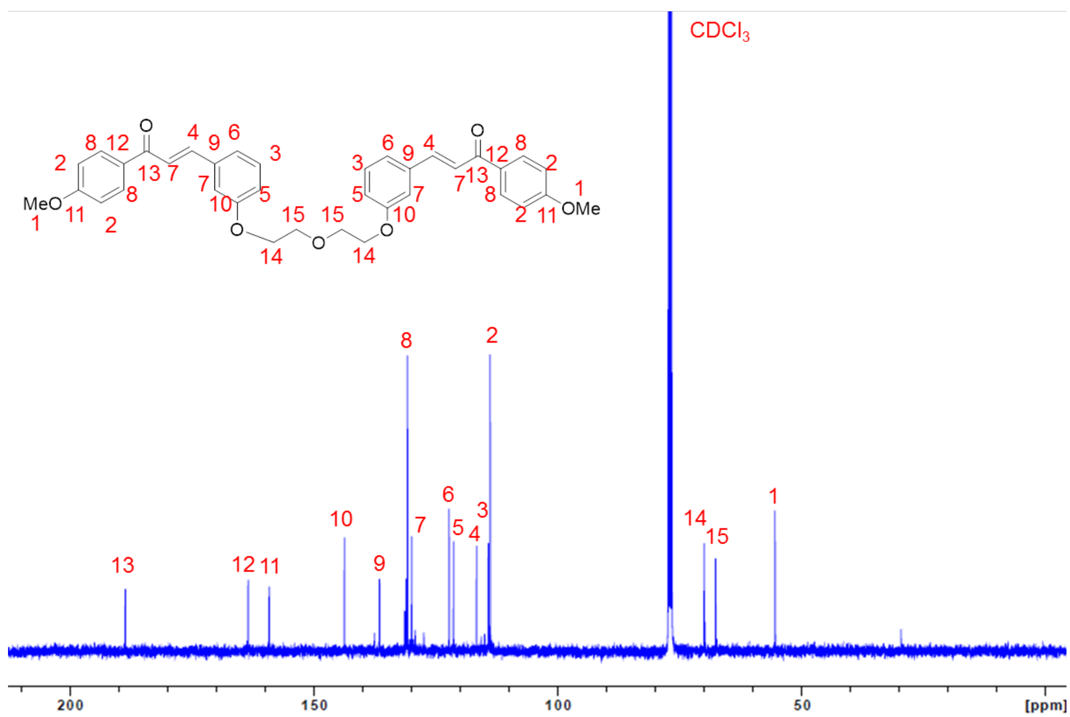


^{13}C-NMR of compound 3

(2*E*,2'*E*)-3,3'-(((oxybis(ethane-2,1-diyl))bis(oxy))bis(3,1-phenylene))bis(1-(4-methoxy phenyl)prop-2-en-1-one) (4a). Anhydrous potassium carbonate (0.95 g, 6.90 mmol) was added to a solution of **3** (1.00 g, 3.93 mmol) and bis(2-bromoethyl)ether (0.24 mL, 1.97 mmol) in acetonitrile (20 mL). The reaction mixture was heated at reflux for 24 h. Then solvent was removed under vacuum. The residue was redissolved in ethyl acetate and extracted with 0.2 N NaOH (2 × 20 mL) and H_2O (20 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The residue was purified by flash silica column chromatography eluting with hexanes:EtOAc (2:1) to yield 875 mg (81 %) of **4a** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 8 Hz, 4H), 7.76 (d, *J* = 8.0 Hz, 2H), 7.52 (d, *J* = 8 Hz, 2H), 7.34 (t, *J* = 8 Hz, 2H), 7.26 (t, *J* = 4 Hz, 2H), 7.21 (s, 2H), 7.00 (d, *J* = 8 Hz, 6H), 4.25 (t, *J* = 4 , 4H), 4.00 (t, *J* = 4 Hz, 4H), 3.91 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.6, 163.5, 159.1, 143.8, 136.5, 131.2, 131.1, 130.8, 129.9, 122.3, 121.3, 116.7, 114.2, 113.9, 113.8, 70.0, 67.7, 55.5. MS (ESI) calcd for $C_{36}H_{34}O_7$ $\{M+Li\}^+$ 585.2465, found 585.2548.

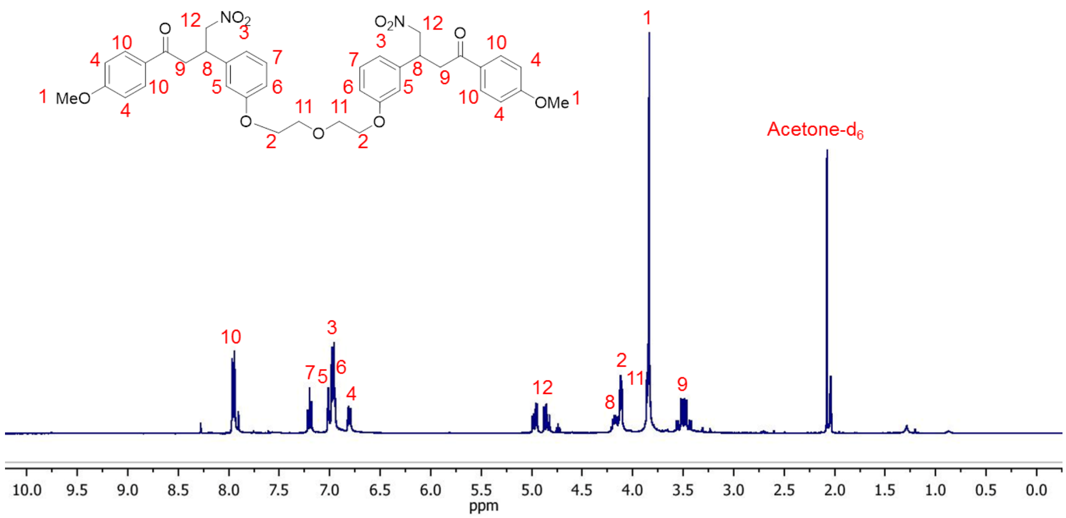


^{1}H-NMR of compound 4a

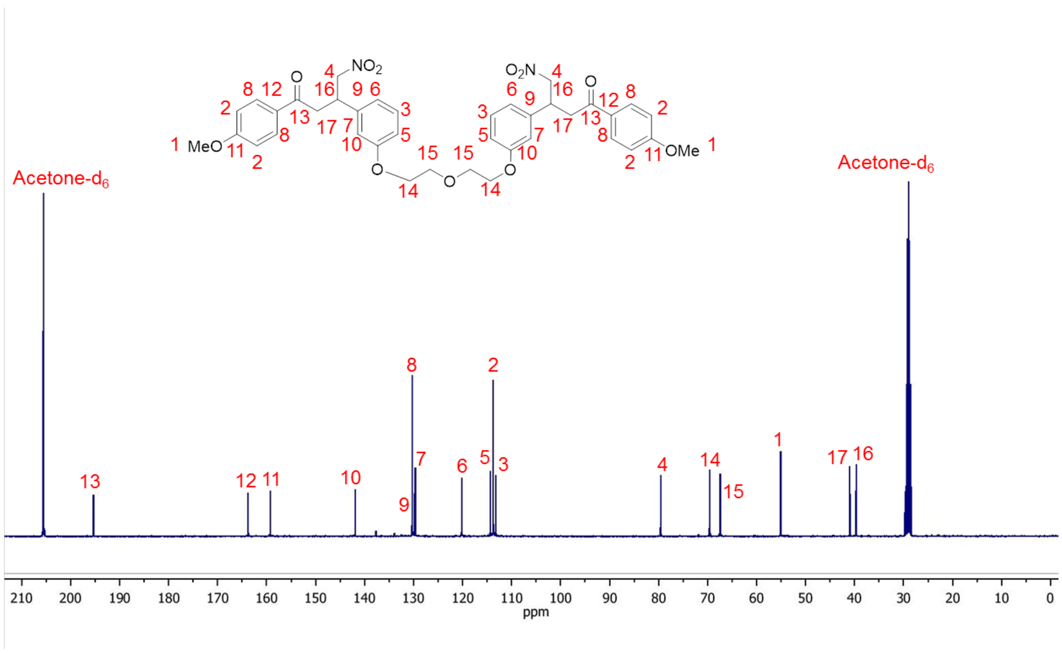


^{13}C-NMR of compound 4a

3,3'-(((Oxybis(ethane-2,1-diyl))bis(oxy))bis(3,1-phenylene))bis(1-(4-methoxyphenyl)-4-nitrobutan-1-one) (5a). **4a** (875 mg, 2.70 mmol), nitromethane (5.80 mL, 108 mmol) and potassium hydroxide (330 mg, 5.90 mmol) were dissolved in dry methanol (15 mL) and heated under reflux for 24 h. The reaction mixture was cooled and solvent was removed. The residue was dissolved in ethyl acetate and extracted with 0.2 M HCl (2 × 20 mL). The aqueous phase was extracted with EtOAc. The combined organic phase was extracted with 0.2 M HCl (20 mL), H_2O (20 mL) and brine (20 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The product was used in the next step without further purification. ^{1}H NMR (400 MHz, acetone-d_6) δ 7.95 (d, J = 8.9 Hz, 4H), 7.20 (t, J = 7.9 Hz, 2H), 7.05-6.91 (m, 8H), 6.81 (dd, J_1 = 8.3 Hz, J_2 = 1.9 Hz, 2H), 4.91 (ddd, J_1 = 22.1 Hz, J_2 = 12.8 Hz, J_3 = 7.6 Hz, 4H), 4.25-4.13 (m, 2H), 4.13 (dd, J_1 = 12.4 Hz, J_2 = 7.8 Hz, 4H), 3.85 (d, J = 4.8 Hz, 4H), 3.84 (s, 6H), 3.49 (qd, J_1 = 17.7 Hz, J_2 = 7.0 Hz, 4H). ^{13}C NMR (100 MHz, acetone-d_6) δ 195.3, 163.8, 159.2, 141.9, 130.3, 129.9, 129.7, 120.2, 114.3, 113.8, 113.2, 79.6, 69.6, 67.4, 55.1, 41.0, 39.7, 29.0. HRMS (ESI) calcd for $C_{38}H_{40}N_2O_{11}$ $\{M+H\}^+$ 701.2710, found 701.2683.

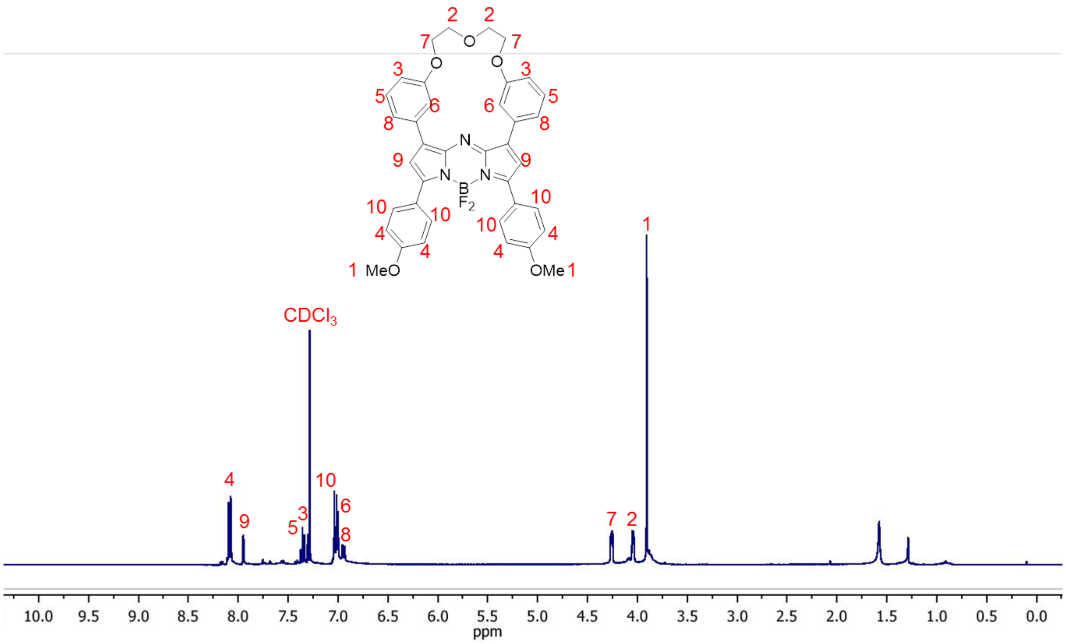


[1]H-NMR of compound 5a

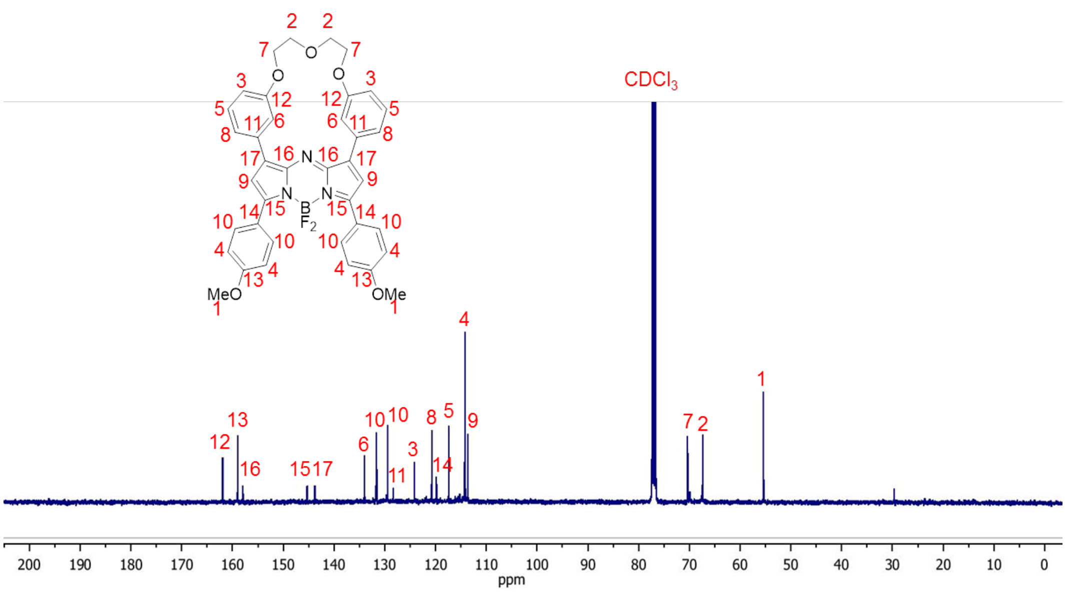


[13]C-NMR of compound 5a

Target Strapped aza-BODIPY 1a (n = 1). **5a** (1.20 g, 2.69 mmol), ammonium acetate (4.10 g, 53.8 mmol) and ^{n}BuOH (20 mL) were heated under reflux for 24 h. The reaction was allowed to cool to room temperature and solvent was removed. Cold methanol was added to the residue and product was filtrated to give dark blue solid. The dark blue solid (300 mg, 0.49 mmol) and diisopropylethylamine (0.63 mL, 4.90 mmol) were dissolved in dry CH_2Cl_2 (20 mL) and stirred at room temperature for 30 min. BF_3•OEt_2 (0.89 mL, 7.35 mmol) was added and the mixture was stirred at room temperature under argon for 24 h. The mixture was washed with water and the organic layer was separated, dried over $MgSO_4$ and evaporated to dryness. The residue was purified by flash silica column chromatography eluting with hexanes:EtOAc (2:1) to give a green solid to yield 43.6 mg (14%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, *J* = 8.9 Hz, 4H), 7.95 (s, 2H), 7.25-7.40 (m, 4H), 7.07 – 6.97 (m, 6H), 6.95 (dd, J_1 = 8.0 Hz, J_2 = 1.8 Hz, 2H), 4.26 (t, *J* = 4 Hz, 4H), 4.05 (t, *J* = 4 Hz, 4H), 3.91 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.0, 159.0, 158.0, 145.3, 143.8, 134.0, 131.7, 129.4, 128.4, 124.2, 120.7, 119.9, 117.4, 114.3, 114.2, 113.6, 70.4, 67.4, 55.4. Hi-Res MALDI-MS: m/z calcd for $C_{38}H_{32}BF_2N_3O_5$ $\{M+H\}^+$ 660.2482, found 660.2487.

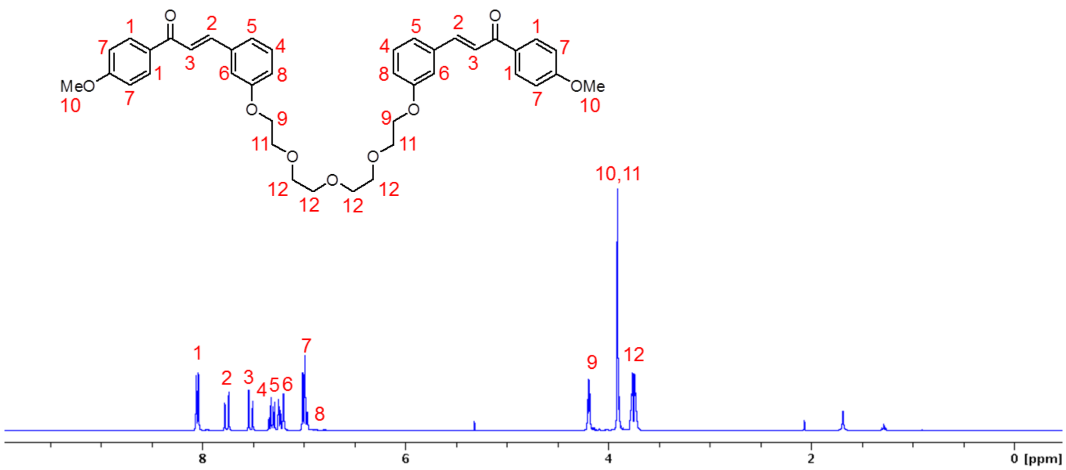


^{1}H-NMR of compound 1a

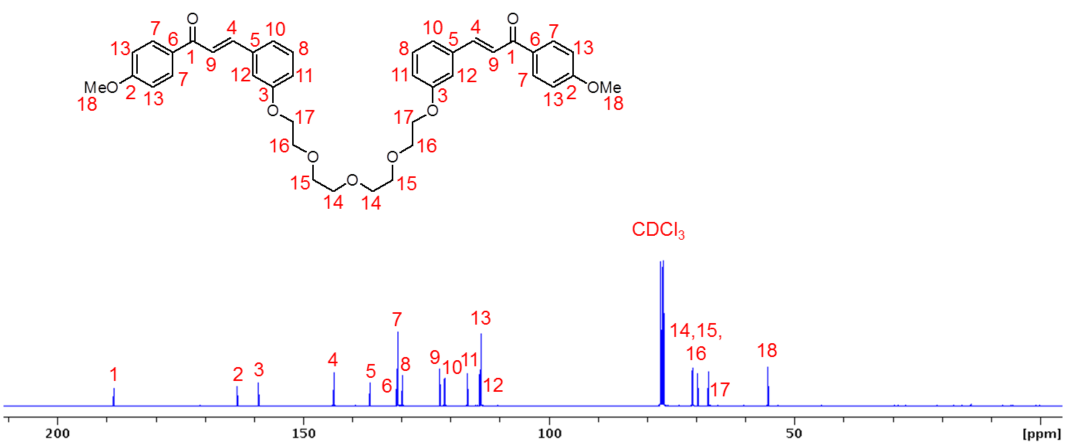


^{13}C-NMR of compound 1a

Linked Dichalcone (4b). Potassium carbonate (970 mg, 7.03 mmol) was added to a solution of **3** (1.36 g, 5.35 mmol) and bis[2-(2-chloroethoxy)ethyl] ether (0.4 mL, 1.97 mmol) in tetrahydrofuran (15 mL). Tetrabutylammonium iodide was added in small amount to the mixture solution. The reaction mixture was refluxed at 85 °C for 60 h. Then solvent was removed under vacuum. The residue was redissolved in ethyl acetate and extracted with 0.2 N NaOH (2 x 30 mL) and H_2O (2 x 30 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The residue was purified by flash silica chromatography eluting with hexanes:EtOAc (2:1 to 1:3) to yield 980 mg (72 %) of **4b** as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 8.8 Hz, 4H), 7.76 (d, *J* = 16.0 Hz, 2H), 7.52 (d, *J* = 16.0 Hz, 2H), 7.31 (dd, J_1 = 16.0 Hz, J_2 = 8.0 Hz, 2H), 7.24 (d, *J* = 7.6 Hz, 2H), 7.20 (s, 2H), 7.02-6.95 (m, 6H), 4.19 (t, *J* = 4.8 , 4H), 3.92-3.88 (m, 10H), 3.80-3.70 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.6, 163.5, 159.2, 143.3, 136.5, 131.1, 130.8, 129.9, 122.3, 121.3, 116.7, 114.2, 113.9, 70.9, 70.7, 69.7, 67.6, 55.5. HRMS (ESI) calcd for $C_{40}H_{42}O_9$ $\{M+H\}^+$ 667.2907, found 667.2920.

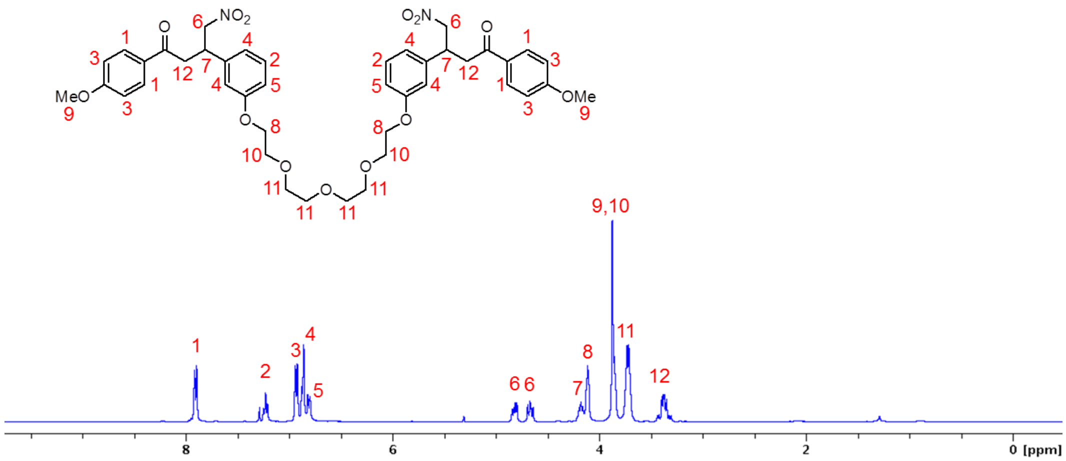


^{1}H-NMR of compound 4b

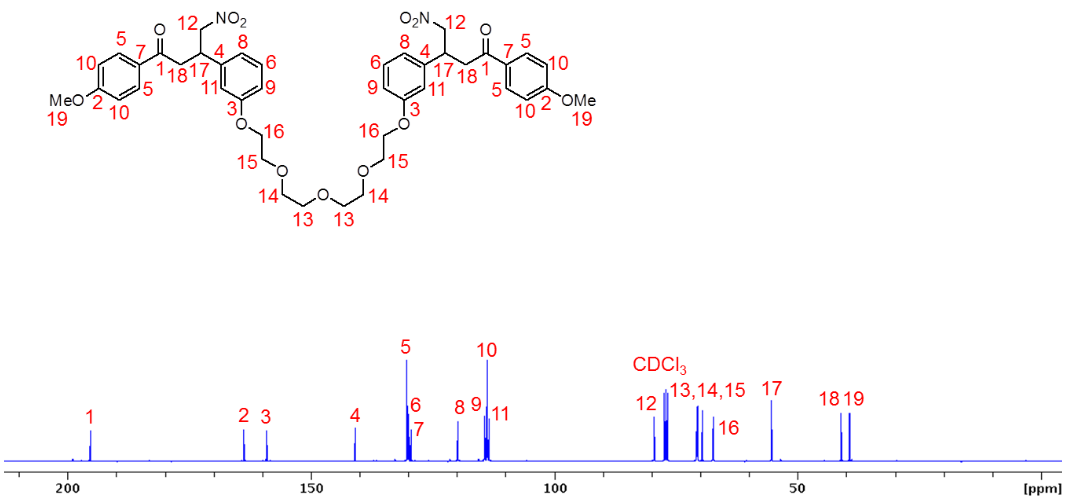


^{13}C-NMR of compound 4b

Conjugate Addition Product 5b. **4b** (650 mg, 0.98 mmol), nitromethane (1.80 mL, 33 mmol) and potassium hydroxide (0.16 g, 2.86 mmol) were dissolved in methanol (10 mL) and heated under reflux for 24 h. The reaction mixture was cooled and solvent was removed. The residue was dissolved in ethyl acetate and extracted with 0.2 M HCl (2 × 30 mL). The aqueous phase was extracted with EtOAc. The combined organic phase was extracted with 0.2 M HCl (30 mL), H_2O (30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The residue was purified by flash silica column chromatography eluting with hexanes:EtOAc (1:3) to yield 775 mg (72 %) of **5b** as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, J = 8.8 Hz, 4H), 7.19 (t, J = 7.8 Hz, 2H), 6.92-6.74 (m, 10H), 4.78 (dd, J_1 = 12.4 Hz, J_2 = 6.2 Hz, 2H), 4.64 (dd, J_1 = 12.8 Hz, J_2 = 8.0 Hz, 2H), 4.17-4.03 (m, 6H), 3.86-3.78 (m, 10H), 3.71-3.62 (m, 8H), 3.38-3.26 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.3, 163.8, 159.2, 141.0, 130.3, 130.0, 129.5, 119.9, 114.4, 113.9, 113.5, 79.5, 70.8, 70.6, 69.7, 67.4, 55.5, 41.1, 39.4. HRMS (ESI) calcd for $C_{42}H_{48}N_2O_{13}$ $\{M+H\}^+$ 789.3235, found 789.3254 .

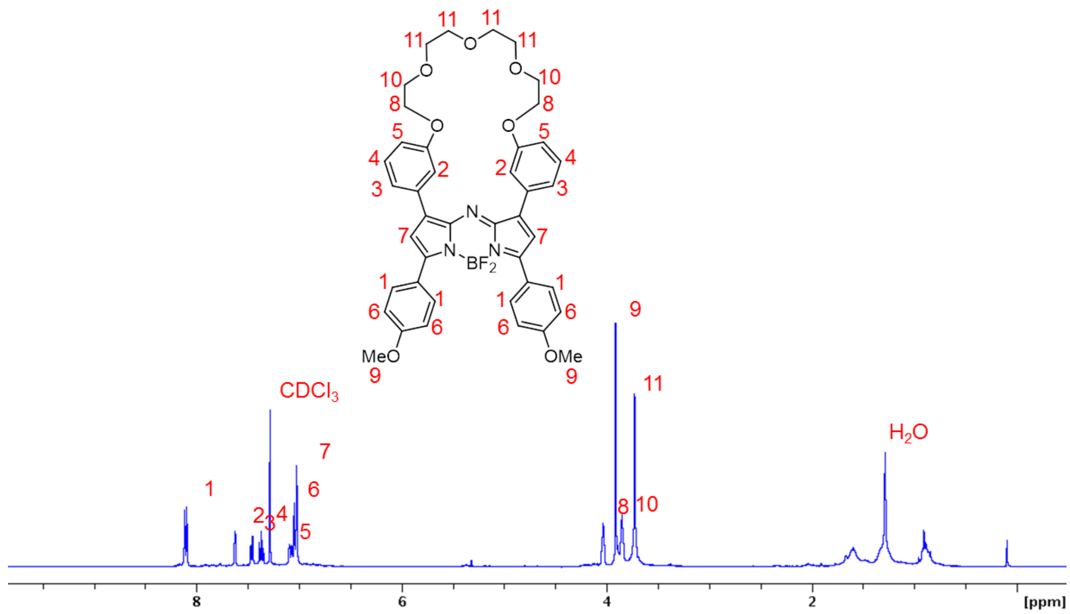


^{1}H-NMR of compound 5b

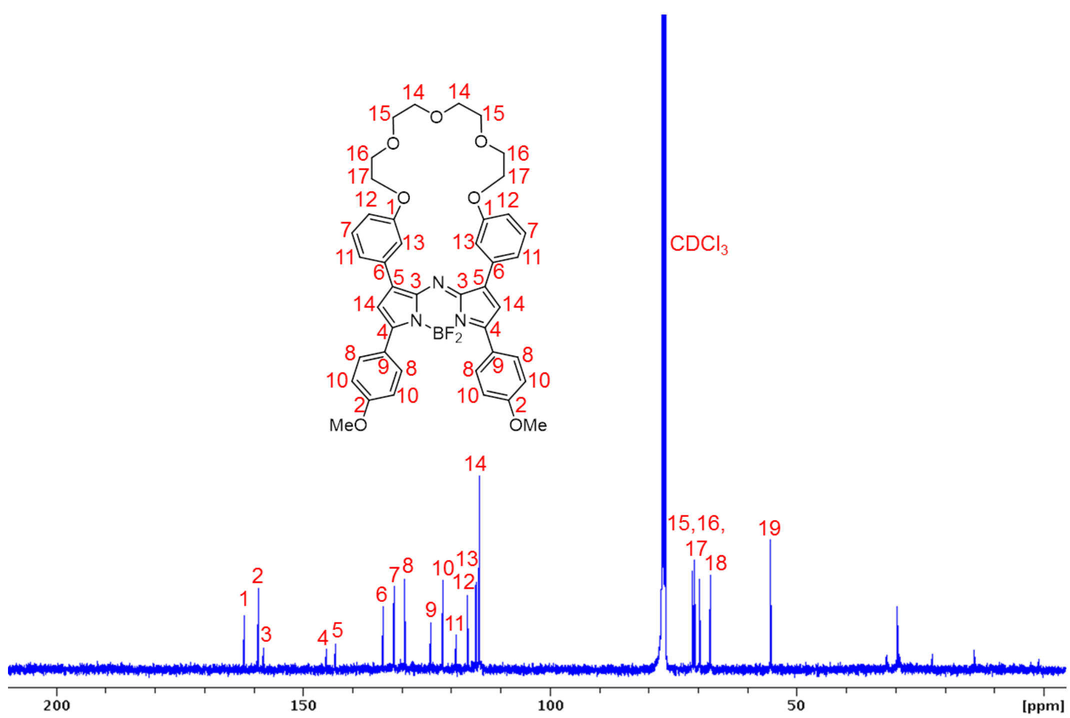


^{13}C-NMR of compound 5b

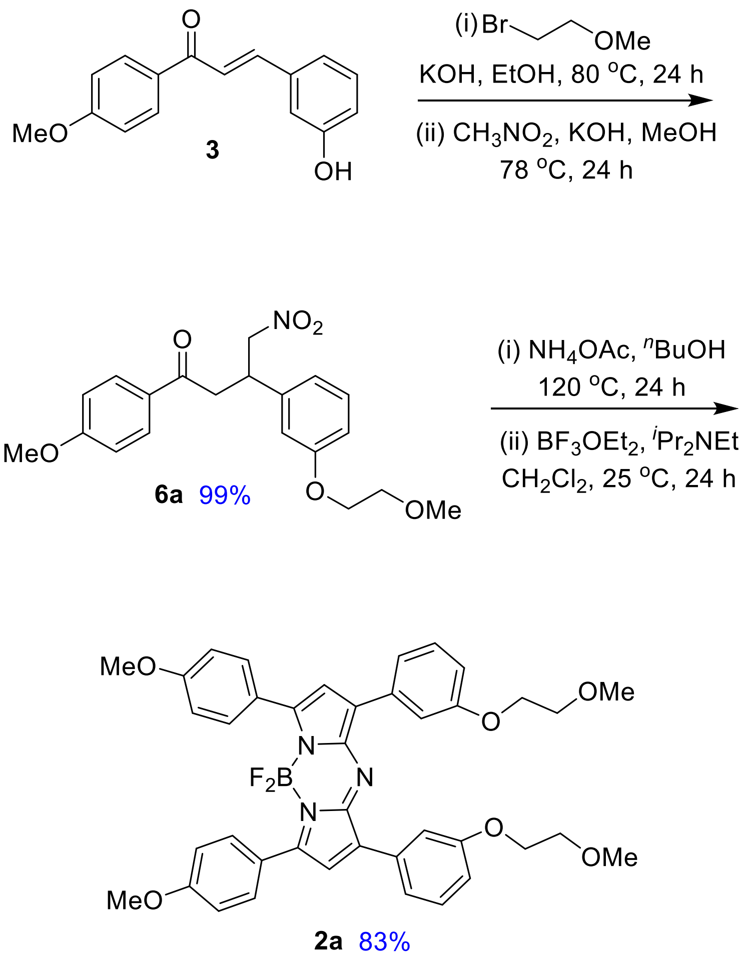
Target Strapped aza-BODIPY 1b (n = 3). **5b** (307 mg, 0.39 mmol), ammonium acetate (1.19 g, 15.44 mmol) and ^{n}BuOH (15 mL) were heated under reflux for 24 h. The reaction was allowed to cool to room temperature and solvent was removed. The residue was dissolved in dichloromethane and extracted with H_2O (2 × 30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure to give dark blue solid and was used in the next step without further purification. The dark blue solid and diisopropylethylamine (0.68 mL, 3.90 mmol) were dissolved in dry CH_2Cl_2 (10 mL) and stirred at room temperature for 30 min. BF_3•OEt_2 (0.74 mL, 5.88 mmol) was added and the mixture was stirred at room temperature under argon for 24 h. The organic layer was extracted with 0.2 M HCl (2 × 30 mL), sat. $NaHCO_3$ (2 × 30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The residue was purified by flash silica column chromatography eluting with hexanes:EtOAc (3:1) to give a green solid to yield 8 mg (3%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, *J* = 8.8 Hz, 4H), 7.63 (s, 2H), 7.46 (d, *J* = 7.6, 2H), 7.37 (t, *J* = 7.8 Hz, 2H), 7.10-7.00 (m, 8H), 4.04 (t, J_1 = 4.6 Hz, 4H), 3.91 (s, 6H), 3.85 (t, J_1 = 4.6 Hz, 4H), 3.73 (br, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.0, 159.1, 158.1, 145.4, 143.5, 133.8, 131.6, 129.5, 124.2, 121.8, 119.1, 116.7, 115.0, 114.3, 71.1, 70.9, 69.8, 67.6, 55.4. Hi-Res MALDI-MS: m/z calcd for $C_{42}H_{40}BF_2N_3O_7$ $\{M+H\}^+$ 748.3000, found 748.3035.



^{1}H-NMR of compound 1b

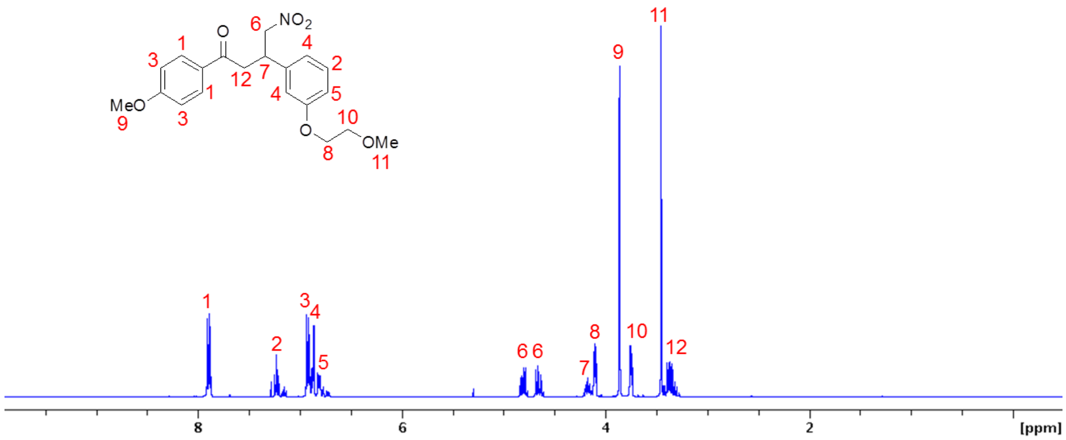


^{13}C-NMR of compound 1b

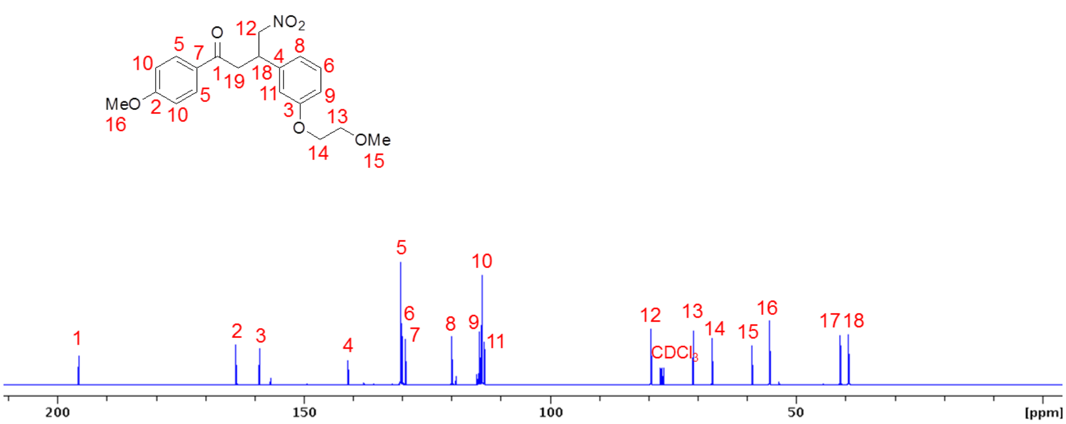


Scheme S1. Syntheses of the non-macrocyclic control molecule **2a**.

3-(3-(2-Methoxyethoxy)phenyl)-1-(4-methoxyphenyl)-4-nitrobutan-1-one (6a). A potassium hydroxide in ethanol (10%, 2 mL) was slowly added to a solution of **3** (1.00 g, 3.93 mmol) and 2-bromo ethyl methyl ether (0.45 mL, 4.79 mmol) in ethanol (6 mL). The reaction mixture was heated at refluxed for 24 h. Then solvent was removed under vacuum. The residue was dissolved in ethyl acetate and extracted with H_2O (2 × 30 mL). The organic phase was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was used in the next step without further purification. The yellow oil, nitromethane (3.90 mL, 72.84 mmol) and potassium hydroxide (230 mg, 4.10 mmol) were dissolved in methanol (20 mL) and heated under reflux for 24 h. The reaction mixture was cooled and solvent was removed. The residue was dissolved in ethyl acetate and extracted with 0.2 M HCl (2 × 30 mL). The aqueous phase was extracted with EtOAc. The combined organic phase was extracted with 0.2 M HCl (30 mL), and brine (30 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure to afford 1.47 g (99 %) of **6a** as an brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 9.2 Hz, 2H), 7.23 (t, J = 8.2 Hz, 1H), 6.95-6.78 (m, 5H), 4.81 (dd, J_1 = 12.4 Hz, J_2 = 6.4 Hz, 1H), 4.66 (dd, J_1 = 12.6 Hz, J_2 = 8.2 Hz, 1H), 4.22-4.08 (m, 3H), 3.86 (s, 3H), 3.75 (t, J = 4.6, 2H), 3.46 (s, 3H), 3.44-3.27 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.7, 163.9, 159.1, 141.0, 130.4, 130.0, 129.4, 120.0, 114.4, 113.9, 113.4, 79.6, 71.0, 67.1, 59.1, 55.5, 41.1, 39.4. HRMS (ESI) calcd for $C_{20}H_{23}NO_6$ {M+H}$^+$ 374.1604, found 374.1617.

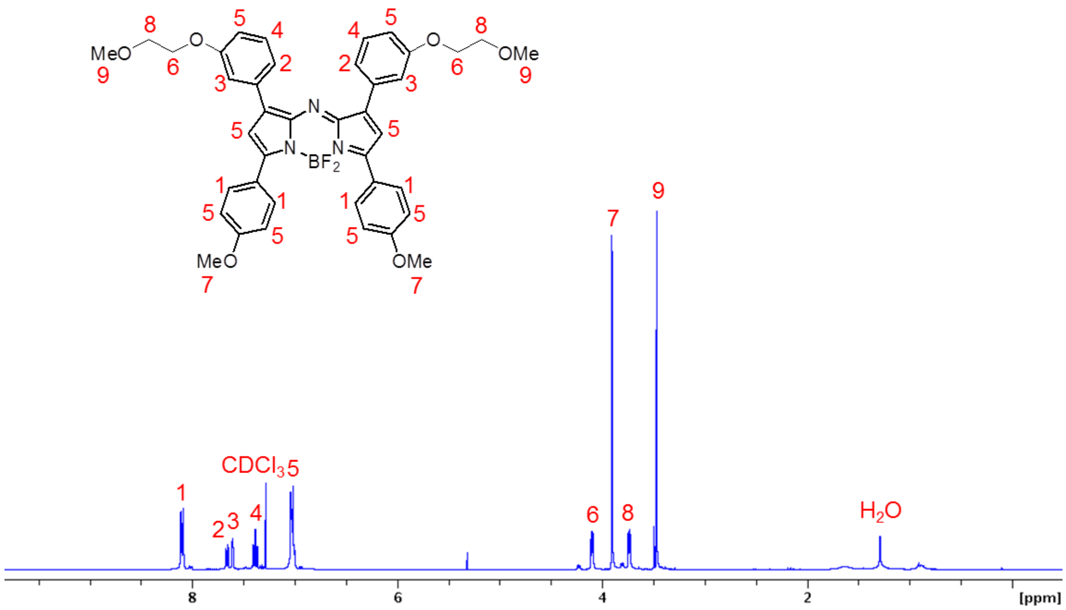


^{1}H-NMR of compound 6a

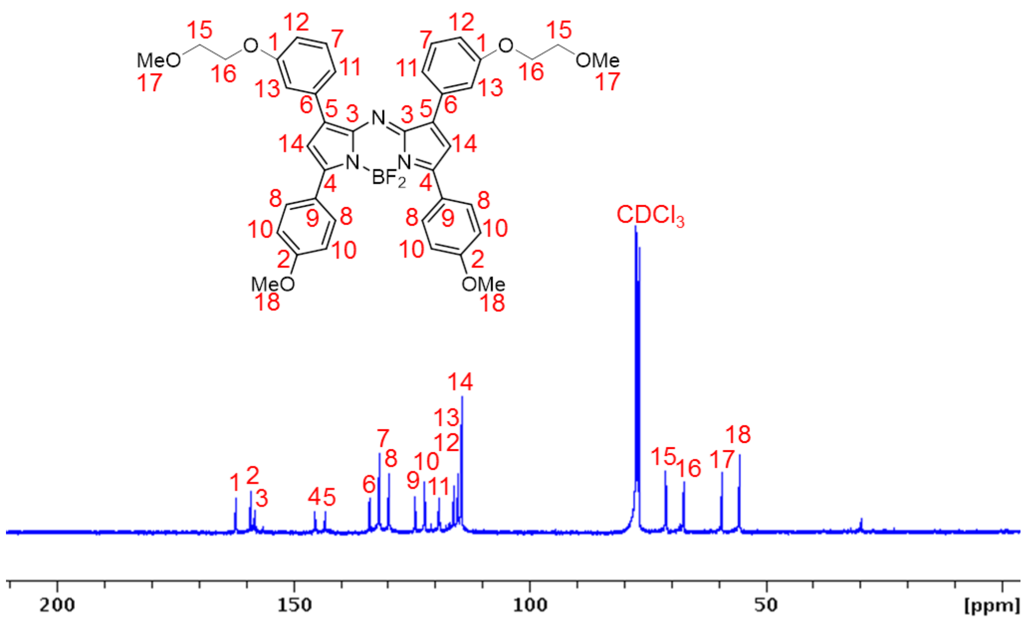


^{13}C-NMR of compound 6a

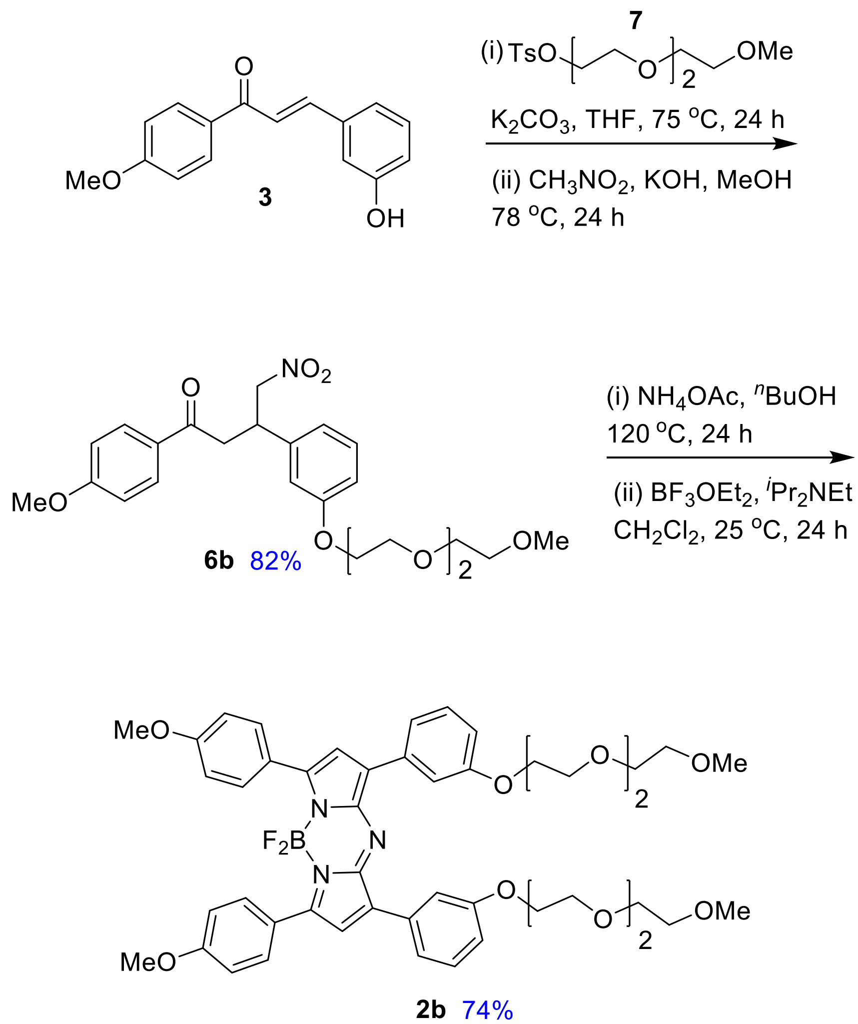
Control PEG-Substituted aza-BODIPY 2a. **6a** (500 mg, 1.27 mmol), ammonium acetate (2.70 g, 35.0 mmol) and ^{n}BuOH (15 mL) were heated under reflux for 24 h. The reaction was allowed to cool to room temperature and solvent was removed. Cold methanol was added to the residue and product was filtrated to yield 190 mg (45%) as a dark blue solid. Then dark blue solid (50 mg, 0.076 mmol) and diisopropylethylamine (0.13 mL, 0.76 mmol) were dissolved in dry CH_2Cl_2 (6 mL) and stirred at room temperature for 30 min. BF_3•OEt_2 (0.14 mL, 1.14 mmol) was then added in portions and the mixture was stirred at 25 ºC for 24 h. The organic layer was extracted with 0.2 M HCl (2 × 30 mL), sat. $NaHCO_3$ (2 × 30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The residue was purified by flash silica column chromatography eluting with hexanes:EtOAc (1:1) to give a green solid to yield 44.3 mg (83%). ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.10 (d, *J* = 8.8 Hz, 4H), 7.66 (d, *J* = 8.0 Hz, 2H), 7.61 (s, 2H), 7.39 (t, *J* = 8.0, Hz, 2H), 7.08 – 6.97 (m, 8H), 4.10 (t, *J* = 4.6 Hz, 4H), 3.91 (s, 6H), 3.74 (t, *J* = 2.4 Hz, 4H), 3.47 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.0, 159.0, 158.1, 145.3, 143.1, 133.8, 131.7, 129.6, 124.1, 122.1, 119.0, 115.9, 115.0, 114.3, 71.0, 67.3, 59.2, 55.4. HRMS (ESI) calcd for $C_{40}H_{38}BF_2N_3O_6$ $\{M+H\}^+$ 706.2900, found 706.2875.



^{1}H-NMR of compound 2a



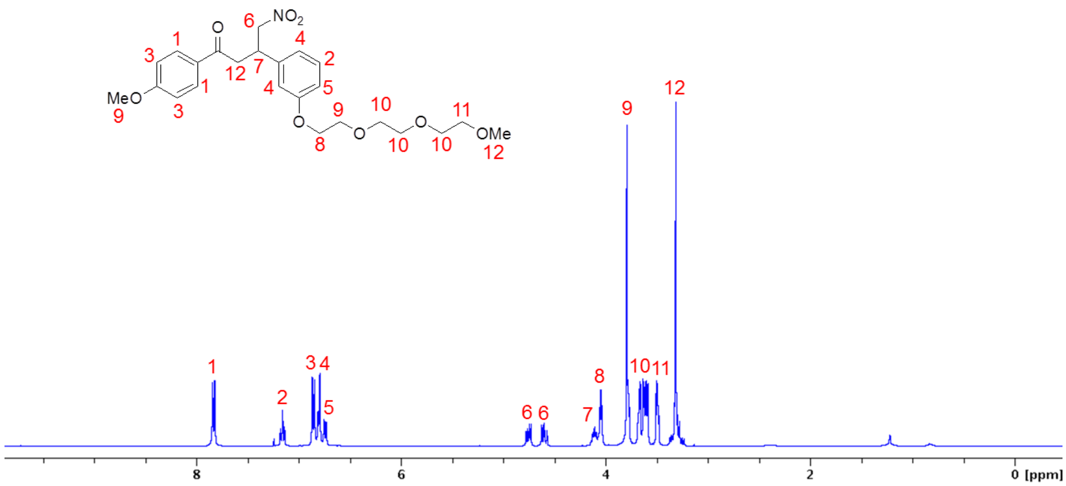
^{13}C-NMR of compound 2a



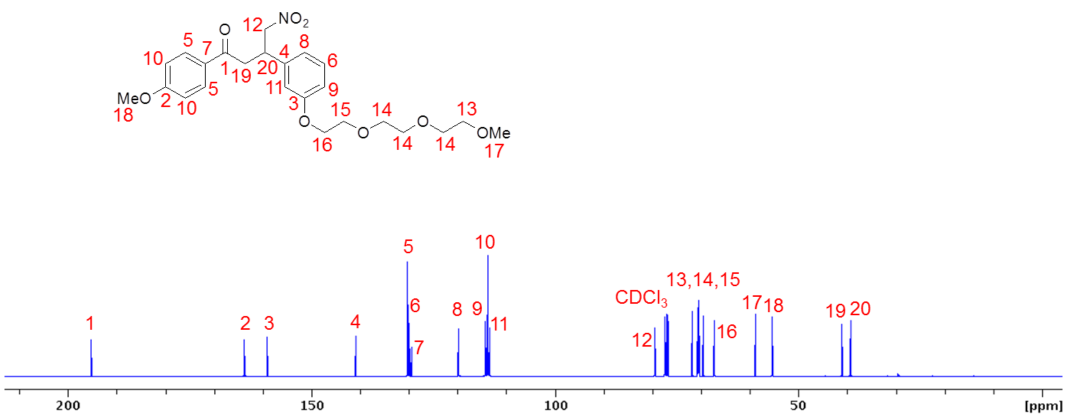
Scheme S2. Syntheses of the non-macrocyclic control molecule **2b.**

Conjugate Addition Product 6b. Potassium carbonate (260 mg, 1.88 mmol) was added to a solution of **3** (173 mg, 0.68 mmol) and compound **7**[1] (200 mg, 0.64 mmol) in tetrahydrofuran (15 mL). The reaction mixture was heated at refluxed for 24 h. Then solvent was removed under vacuum. The residue was redissolved in ethyl acetate and extracted with 0.2 M NaOH (2 x 30 mL), 0.2 M HCl (2 x 30 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude product was used in the next step without further purification. The yellow oil, nitromethane (1.0 mL, 18.7 mmol) and potassium hydroxide (160 mg, 2.86 mmol) were dissolved in methanol (8 mL) and heated under reflux for 24 h. The reaction mixture was cooled and solvent was removed. The residue was dissolved in ethyl acetate and extracted with 0.2 M HCl (2 × 30 mL). The aqueous phase was extracted with EtOAc. The combined organic phase was extracted with 0.2 M HCl (30 mL), H_2O (30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The residue was purified by flash silica column chromatography eluting with hexanes:EtOAc (1:1 to 1:2) to yield 240 mg (82 %) of **6b** as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, J = 8.8 Hz, 2H), 7.16 (t, J = 8.0 Hz, 1H), 6.89-6.71 (m, 5H), 4.75 (dd, J_1 = 12.4 Hz, J_2 = 6.4 Hz, 1H), 4.59 (dd, J_1 = 12.2 Hz, J_2 = 8.4 Hz, 1H), 4.14-4.01 (m, 3H), 3.81-3.74 (m, 5H), 3.69-3.56 (m, 6H), 3.52-3.46 (m, 2H), 3.36-3.21 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.3, 163.8, 159.2, 141.0, 130.3, 130.0 (2), 129.5, 119.9, 114.4, 113.9, 113.4, 79.5, 71.9, 70.8, 70.6, 70.5, 69.7, 67.4, 58.9, 55.5, 41.1, 39.4. HRMS (ESI) calcd for $C_{24}H_{31}NO_8$ {M+H}$^+$ 462.2128, found 462.2141.

[1] K. M. Bonger *et al*. *Bioorg. Med. Chem*. *15* (2007) 4841-4856.

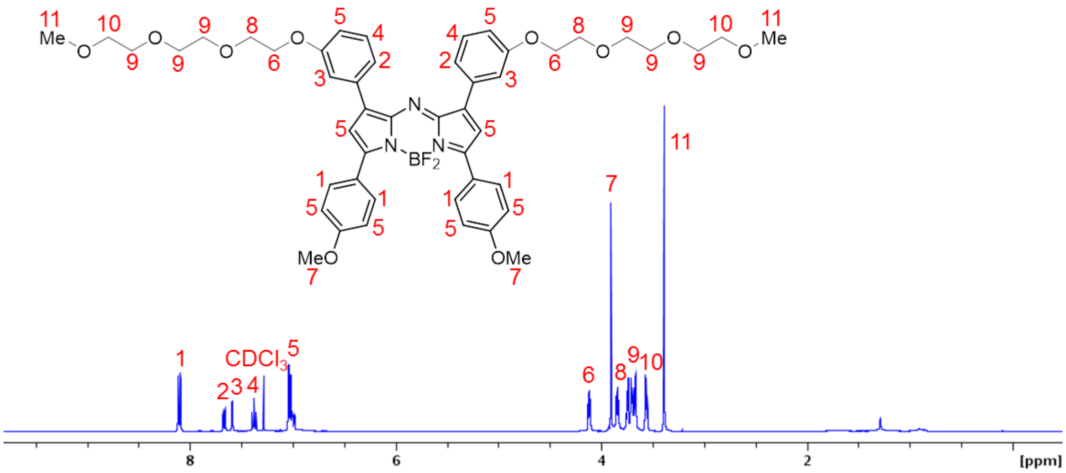


[1]H-NMR of compound 6b

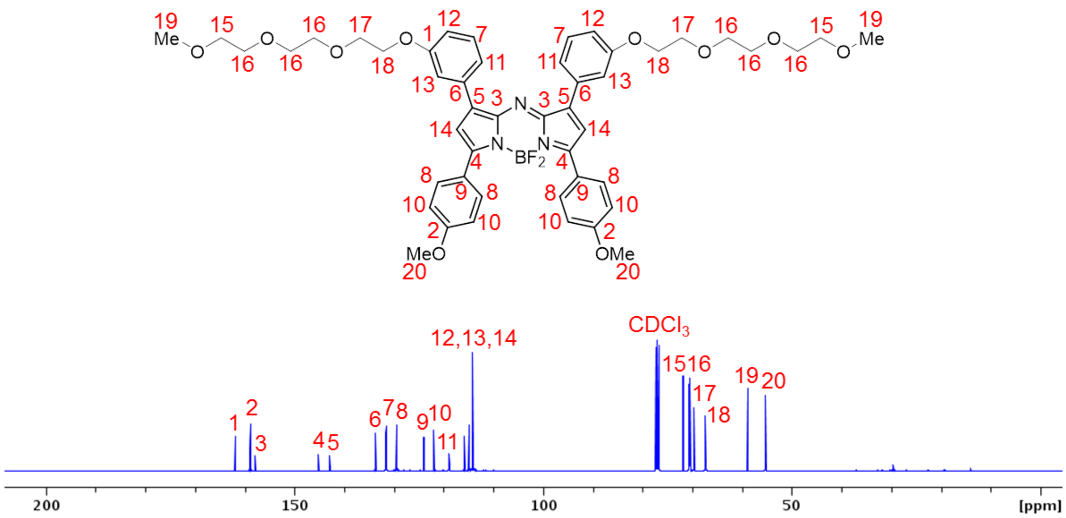


[13]C-NMR of compound 6b

Control PEG-substituted Compound 2b. **6b** (24 mg, 0.052 mmol), ammonium acetate (190 mg, 2.46 mmol) and ^{n}BuOH (2 mL) were heated under reflux for 24 h. The reaction was allowed to cool to room temperature and solvent was removed. The residue was dissolved in dichloromethane and extracted with H_2O (2 × 30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica) with hexanes:EtOAc (1:3 to 1:7) to give a dark blue solid to yield 12.5 mg (58%). The dark blue solid (9.8 mg, 0.012 mmol) and diisopropylethylamine (0.1 mL, 0.57 mmol) were dissolved in dry CH_2Cl_2 (1 mL) and stirred at room temperature for 30 min. BF_3•OEt_2 (0.1 mL, 0.79 mmol) was added and the mixture was stirred at room temperature under argon for 24 h. The organic layer was extracted with 0.2 M HCl (2 × 30 mL), sat. $NaHCO_3$ (2 × 30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica) with MeOH:CH_2Cl_2 (1:99) to give a green solid to yield 7.7 mg (74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (d, J = 8.8 Hz, 4H), 7.62 (d, J = 8.0 Hz, 2H), 7.55 (s, 2H), 7.34 (t, J = 7.8, 2H), 7.02-6.93 (m, 8H), 4.08 (t, J_1 = 4.8 Hz, 4H), 3.86 (s, 6H), 3.80 (t, J_1 = 4.8 Hz, 4H), 3.72-3.61 (m, 12H), 3.54-3.50 (m, 4H), 3.35 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.0, 159.0, 158.0, 145.3, 143.0, 133.8, 131.7, 129.6, 124.1, 122.1, 119.0, 115.9, 115.0, 114.3, 71.9, 70.8, 70.6(2), 69.7, 67.5, 60.0, 55.4. HRMS (ESI) calcd for $C_{48}H_{54}BF_2N_3O_{10}$ {M+H}$^+$ 882.3949, found 882.3972.

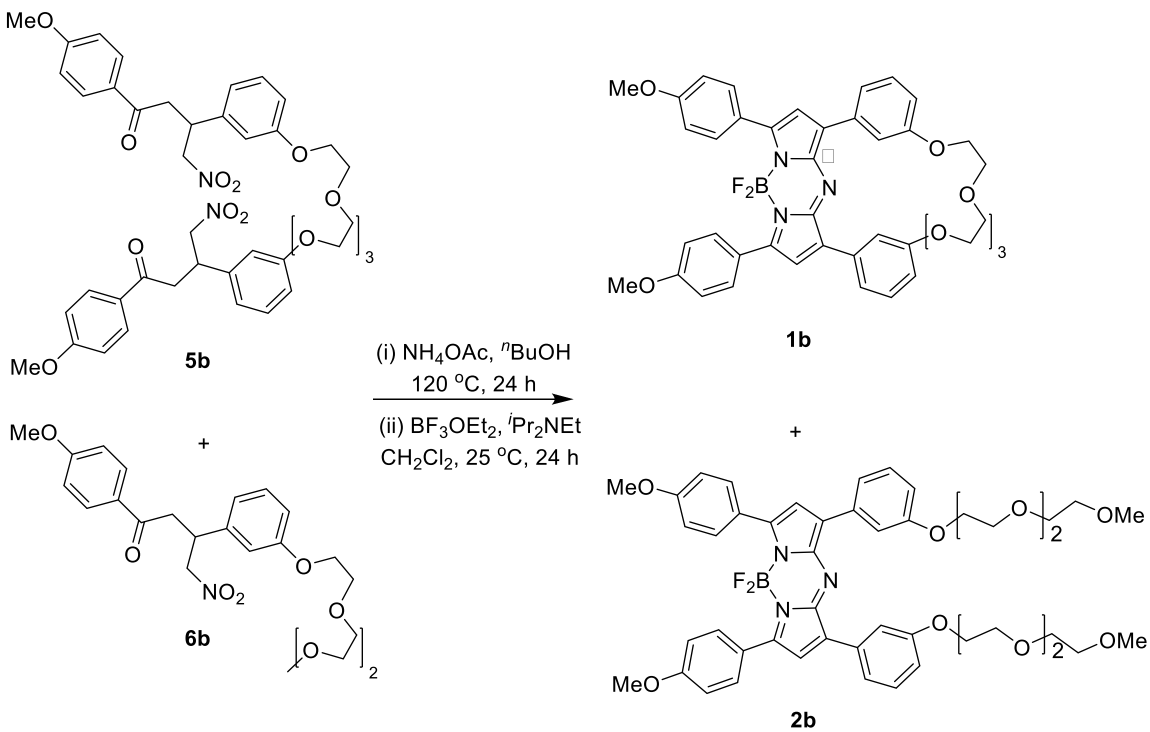


^{1}H-NMR of compound 2b



^{13}C-NMR of compound 2b

4. Relative Yield Experiments



The following procedure was used to determine the relative yields of aza-dipyrromethene precursors to **1b** and **2b.** In step (i) **5b** (76 mg, 0.096 mmol), **6b** (89 mg, 0.193 mmol), ammonium acetate (445 mg, 5.77 mmol) and ^{n}BuOH (15 mL) were heated under reflux for 24 h and the crude product was monitored by analytical HPLC. The reaction was allowed to cool to room temperature and solvent was removed. The residue was dissolved in dichloromethane and extracted with H_2O (2 × 30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure to give dark blue solid and was used in the next step without further purification. In step (ii) the dark blue solid and diisopropylethylamine (0.46 mL, 2.64 mmol) were dissolved in dry CH_2Cl_2 (10 mL) and stirred at room temperature for 30 min. BF_3•OEt_2 (0.5 mL, 3.97 mmol) was added and the mixture was stirred at room temperature under argon for 24 h and the crude product was monitored by analytical HPLC. The organic layer was extracted with 0.2 M HCl (2 × 30 mL), sat. $NaHCO_3$ (2 × 30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. This reaction was not purified because of complication.

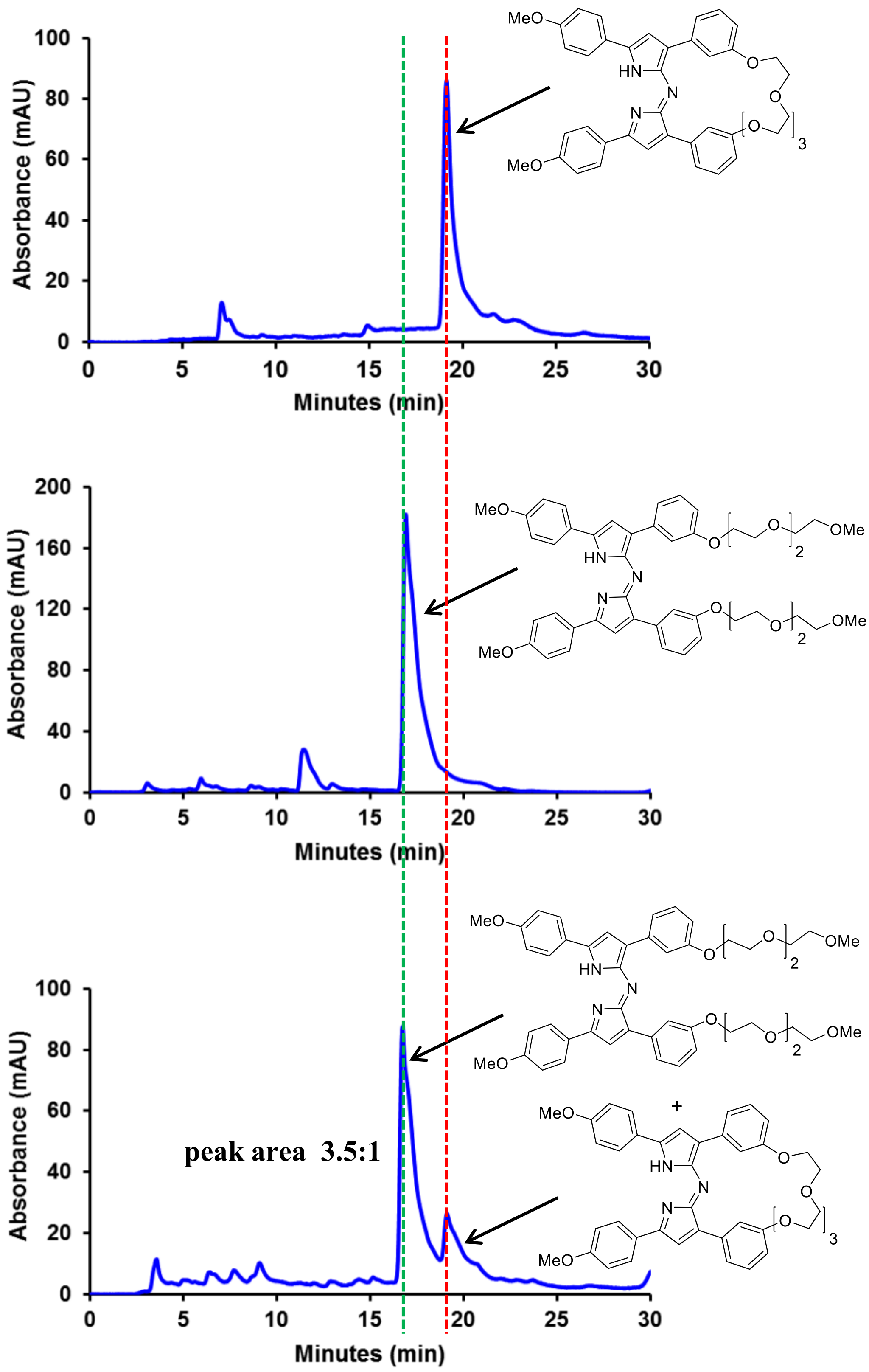


Figure S1. HPLC results in step (i)

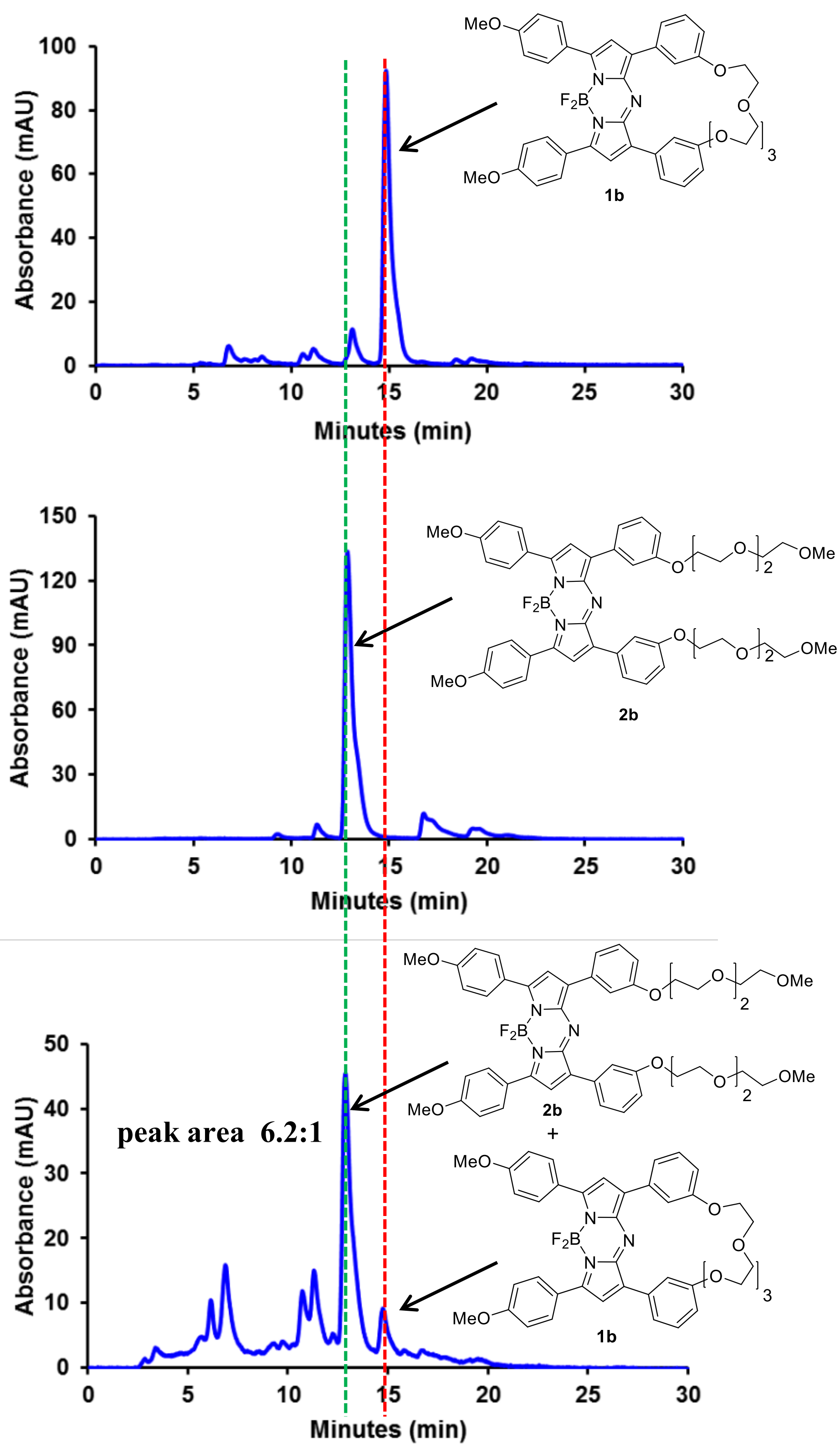


Figure S2. HPLC results in step (ii)

5. Fluorescence Spectoscopy

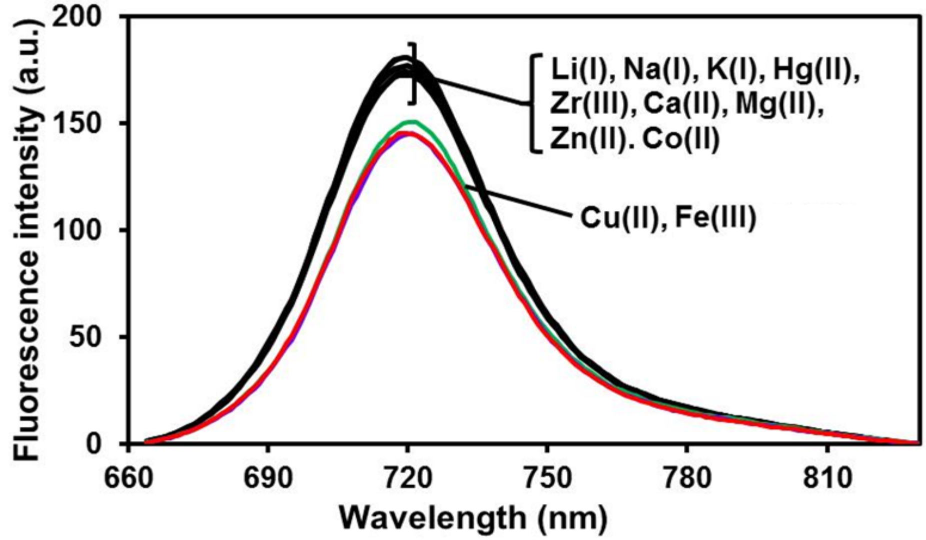


Figure S3. Fluorescence spectra (excited at 650 nm) of **1b** (1.38 μM) in acetonitrile with addition of chloride salts of Li^{+}, Na^{+}, K^{+}, Zr^{3+}, Hg^{2+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+} and Fe^{3+} (33 μM) in water.

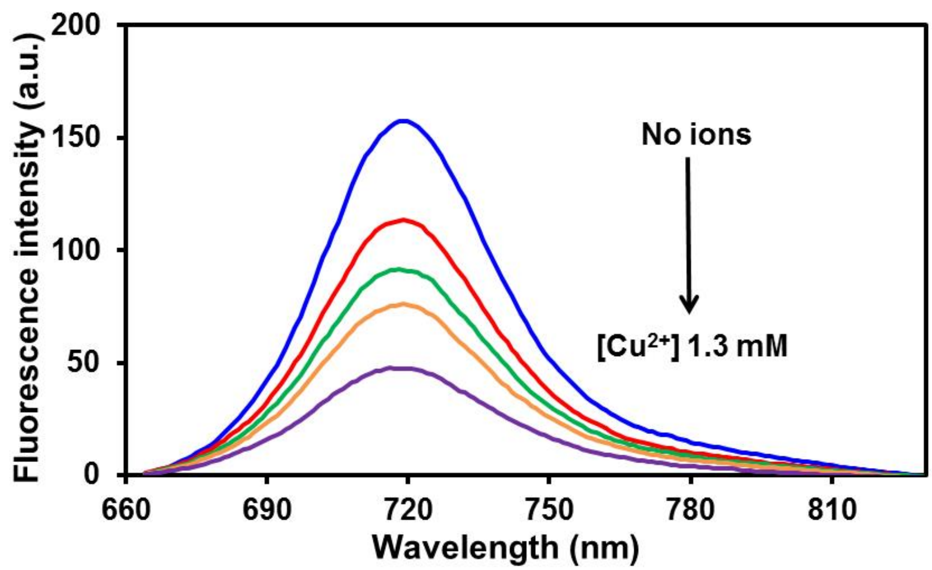


Figure S4. Fluorescence spectra (excited at 650 nm) of **1b** (1.38 μM) in acetonitrile with $CuCl_2$ in water.

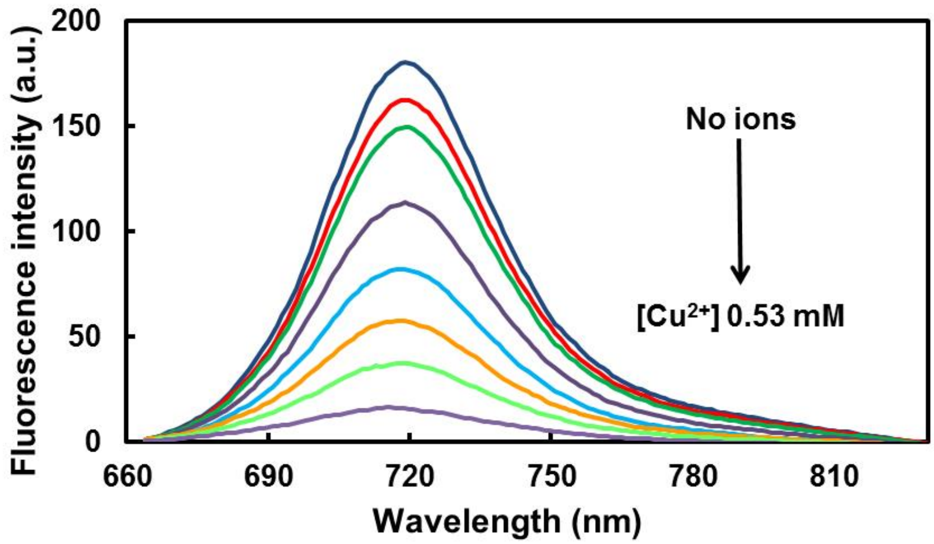


Figure S5. Fluorescence (excited at 650 nm) of **1b** (1.38 μM) in acetonitrile with $Cu(ClO_4)_2$ in water.

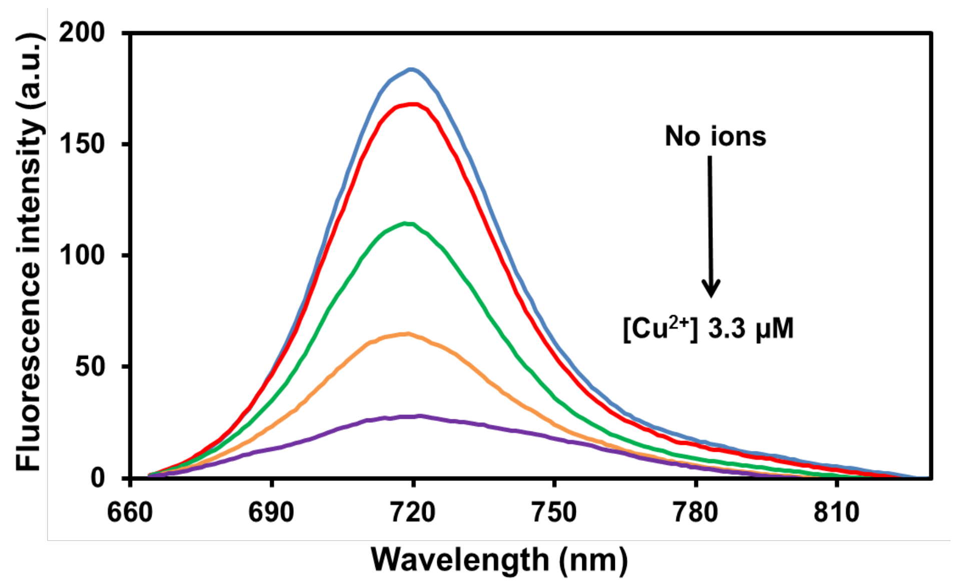


Figure S6. Fluorescence spectra (excited at 650 nm) of **1b** (1.38 μM) in acetonitrile with $Cu(ClO_4)_2$ in acetonitrile.

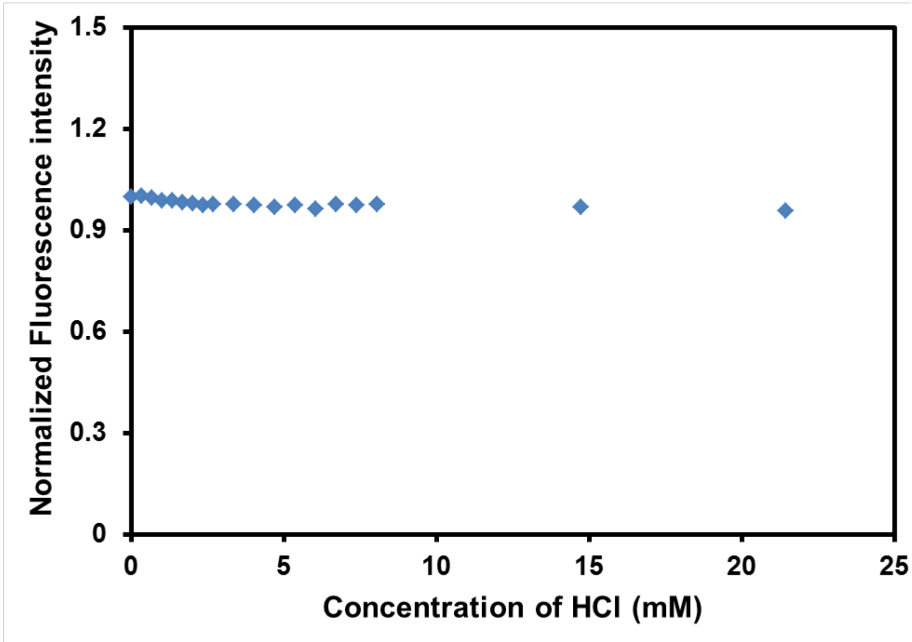


Figure S7. Normalized fluorescence intensity (excited at 650 nm) of **1b** (1.38 μM) in acetonitrile with HCl in water.

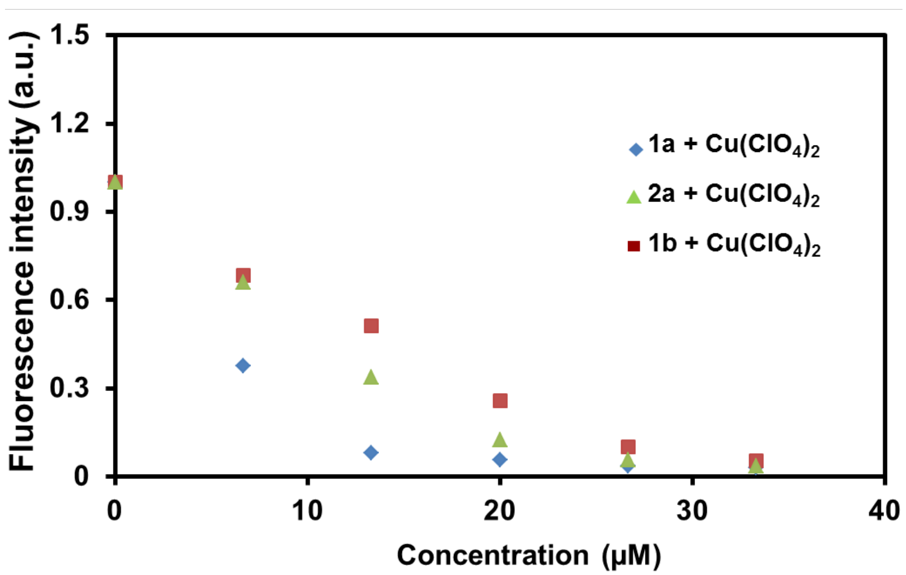


Figure S8. Normalized fluorescence intensity (excited at 650 nm) of **1a, 2a and 1b** (2.0 μM) in acetonitrile with. $Cu(ClO_4)_2$ in acetonitrile.

6. Structural Optimization by DFT Calculations and Cartesian Coordinates

Throughout, the initial geometry was firstly minimized by molecular mechanics with UFF force field with PCM water solvation in Gaussian 09. The final geometry optimization was performed at the B3LYP/6-31G(d') level with PCM water solvation using Gaussian 09.

As a control experiment, 15-crown-5 and Na^{+}•15-crown-5 were studied. The energy-minimized structures are shown in Figure S9.

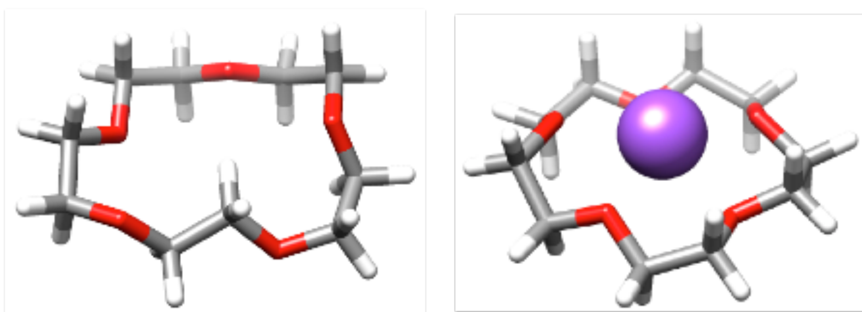


Figure S9. Optimized geometry for 15-crown-5 and Na^{+}□15-crown-5.

Cartesian coordinates of 15-crown-5, Na^{+}•15-crown-5, **1a**, Na^{+}•**1a**, **1b**, Na^{+}•**1b** are given in Angstrom.

15-crown-5

C	1.96357900	2.05088200	-0.30103500
H	1.99312200	1.62249200	-1.31713600
H	2.31757200	3.09464600	-0.37656500
C	2.88297900	1.28740800	0.64572600
H	3.92633200	1.40007100	0.30841200
H	2.80143400	1.72173000	1.64958600
C	-0.31194000	2.63532300	-0.61631200
H	-0.14358400	3.72587200	-0.63935300
H	-0.23720800	2.26194400	-1.65151100
C	-1.69711600	2.34916600	-0.06955000
H	-1.71117300	2.55639100	1.01324800
H	-2.43154400	3.01816100	-0.54990600
C	3.00063600	-0.92276400	-0.26274600
H	2.78388800	-0.48828500	-1.25064600
H	4.09474100	-1.05525700	-0.18603000
O	0.64511800	1.99975600	0.21435500
O	2.53918400	-0.08418200	0.78857500
O	-2.02441700	0.99135500	-0.31993000
C	-3.16052700	0.54856800	0.40264500
H	-4.05280300	1.14391200	0.13691000
H	-2.99075200	0.66555000	1.48669300
C	-3.42797500	-0.91500000	0.08159500
H	-4.35165900	-1.21500900	0.59456300
H	-3.58800500	-1.03944100	-0.99966000
O	-2.40075400	-1.78279300	0.54302000
C	2.31506500	-2.28006700	-0.16548500
H	2.86812400	-2.99155100	-0.79199100
H	2.35405800	-2.63847200	0.87664200
C	-0.00713700	-1.78759900	0.24355200
H	0.06943700	-2.29236400	1.22199700
H	0.10593200	-0.70854700	0.39943800
C	-1.36366500	-2.08982300	-0.38624500
H	-1.41639100	-3.15928100	-0.64374900
H	-1.47772300	-1.51111700	-1.31095400
O	0.98121900	-2.29513700	-0.65137900

Na^+•15-crown-5

C	-2.66910000	-1.32298800	-0.42172700
H	-2.46079500	-0.98805500	-1.45044500
H	-3.43092900	-2.11667700	-0.46891500
C	-3.18661200	-0.17839600	0.44037200
H	-4.12355000	0.21254200	0.02038900
H	-3.38848100	-0.54564000	1.45289000
C	-0.83213800	-2.86278000	-0.54695600
H	-1.39081300	-3.80353200	-0.43149400
H	-0.79253200	-2.60954600	-1.61763500
C	0.57729800	-3.02367700	-0.00602400
H	0.55369200	-3.22992200	1.07606300
H	1.07659800	-3.86475700	-0.51004200
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H	-2.21104800	1.48009800	-1.41011500
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O	-2.22263300	0.87014800	0.59069200
O	1.26892900	-1.80240900	-0.25879700
C	2.58248900	-1.71853500	0.29066500
H	3.21981500	-2.52977700	-0.09198200
H	2.53783900	-1.80062300	1.38879800
C	3.16216400	-0.37985800	-0.13232900
H	4.18263200	-0.26953900	0.26387600
H	3.20869600	-0.33208200	-1.23052200
O	2.31377100	0.65979100	0.35667600
C	-1.11335300	2.84495600	-0.15241800
H	-1.18218800	3.70876100	-0.83050000
H	-1.13859800	3.21267200	0.88537000
C	1.28236200	2.79557000	0.02949800
H	1.38636600	3.75916400	-0.49137500
H	1.24179500	2.98957000	1.11337200
C	2.46546800	1.90953700	-0.31624400
H	3.40028000	2.40561700	-0.01446400
H	2.49699700	1.74167600	-1.40349600
O	0.09453500	2.12153100	-0.38617300
Na	0.01904600	0.00014800	0.68161800

1a

C	-4.88085400	-1.00846900	-1.47743300
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H	-5.36032400	-1.97385600	-1.28700000
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H	-2.80330200	1.40169700	2.69480300
H	-2.50096500	0.33617000	1.29394400
C	-2.21456300	2.81706600	0.35812100
C	-2.43696000	4.03155900	-0.31234300
C	-0.92009000	2.30030100	0.45724500
C	-1.36489400	4.71415100	-0.88182400
H	-3.45108000	4.41818800	-0.37288900
C	0.16243000	2.98236800	-0.13655600
H	-0.72620600	1.37506600	0.98309500
C	-0.06924700	4.20074200	-0.79942600
H	-1.54250100	5.65272400	-1.40208300
H	0.75591400	4.72524400	-1.27337600
C	-2.75173700	-2.06574200	-0.90412800
C	-3.28751400	-3.22825200	-0.33173900
C	-1.37423500	-1.81966300	-0.81664300
C	-2.43521600	-4.12720600	0.31676500
H	-4.34925100	-3.44382800	-0.37720700
C	-0.51722600	-2.72264800	-0.16468700
H	-0.98011900	-0.91698300	-1.27062500
C	-1.06657900	-3.89151600	0.40267300
H	-2.85656600	-5.02467100	0.76459700
H	-0.42599800	-4.59110600	0.93229800
O	-3.33193600	2.21139400	0.86072500
O	-3.45982900	-1.14017100	-1.62287300
C	1.52048200	2.43703300	-0.06893500
C	1.89777900	1.04525600	-0.02392900
C	2.72084300	3.14986800	-0.06881600
C	3.78624900	2.22608300	-0.00648500
H	2.84090500	4.22591200	-0.07663300
C	0.92787600	-2.49150400	-0.07672500
C	1.62404700	-1.22711400	-0.05591800
C	1.92355400	-3.46514800	0.02706600

C	3.17617600	-2.81814200	0.09184700
H	1.78855300	-4.53930900	0.02258700
N	2.99617000	-1.47804100	0.04300100
N	3.29231300	0.96593500	0.01874200
N	1.10043400	-0.01151000	-0.07344200
B	4.08456300	-0.36920500	0.08188500
F	4.94629700	-0.48427100	-1.01767300
F	4.82787300	-0.44828500	1.26758500
C	-5.23034800	0.00051700	-0.39546700
H	-6.31521200	0.20238400	-0.42283200
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H	-4.55230600	-0.16567800	2.81966400
H	-5.34329800	1.17677400	1.96292800
O	-4.85657400	-0.54003800	0.86857600
C	4.52925300	-3.43951300	0.18823000
H	5.15255000	-3.13530600	-0.66202800
H	5.04303200	-3.09836300	1.09572500
H	4.45435300	-4.53017600	0.20686500
C	5.24987500	2.51148300	0.04169100
H	5.69151400	2.09811100	0.95731100
H	5.76180200	2.03189200	-0.80188200
H	5.43545900	3.58854100	0.00907000

Na^{+}•**1a**

C	-4.30426200	-1.74471000	-1.07200300
H	-5.03448800	-1.75391300	-1.88863900
H	-4.57785300	-2.52047500	-0.34893000
C	-3.77892500	1.63634000	2.29337900
H	-4.41034400	2.05202400	3.08693400
H	-2.75498200	1.60826700	2.67919800
C	-2.88555700	2.69299500	0.28395200
C	-3.27835400	3.18922300	-0.96902500
C	-1.52608200	2.45976600	0.54364200
C	-2.32400500	3.44231400	-1.95069000
H	-4.33467700	3.37257300	-1.14699500
C	-0.56715300	2.69412200	-0.46028500
H	-1.18703500	2.16041500	1.53098300
C	-0.97048400	3.19654600	-1.70678200
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H	-0.22802300	3.38232900	-2.47800900
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H	-3.44650200	-4.40053000	-0.41302300
C	0.20774100	-3.01654800	-0.29053000
H	-0.56980200	-1.40688200	-1.52731600
C	-0.12037400	-4.23431100	0.33638900
H	-1.67010900	-5.66524600	0.75619400
H	0.63805900	-4.77885400	0.89196600
O	-3.90409900	2.53223300	1.18502400
O	-3.03053600	-2.02733500	-1.68071900
C	0.87644100	2.47550100	-0.21961700
C	1.57298500	1.22297900	-0.05446400
C	1.85983200	3.45519300	-0.23622900
C	3.11892400	2.82705300	-0.05955900
H	1.70870100	4.52131600	-0.35043000
C	1.55359200	-2.45323000	-0.15342400
C	1.89504800	-1.05122700	-0.07071200

C	2.75712300	-3.14231700	-0.03004700
C	3.79705000	-2.19431600	0.10754100
H	2.90201100	-4.21464500	-0.06546700
N	3.27763900	-0.94536800	0.07544400
N	2.94227200	1.49240600	0.03599200
N	1.07215200	-0.00727800	-0.09071000
B	4.03752900	0.40334900	0.22755700
F	5.02332700	0.52385100	-0.75575300
F	4.61668100	0.50191100	1.49721200
C	-4.33260400	-0.37127900	-0.41366900
H	-5.38161200	-0.10144800	-0.23883400
H	-3.89365700	0.37696100	-1.08590200
C	-4.29540800	0.23773900	1.95239000
H	-4.13984500	-0.41282000	2.82272000
H	-5.37139800	0.27913700	1.74524500
O	-3.61948800	-0.36328000	0.83690900
C	5.25772600	-2.44851500	0.26438000
H	5.81946800	-1.97789100	-0.55174800
H	5.62267800	-2.00377400	1.19901700
H	5.46507800	-3.52175700	0.27377300
C	4.46128200	3.47001100	0.02580800
H	4.94995700	3.21021600	0.97360800
H	5.11326400	3.10581000	-0.77748500
H	4.37384700	4.55728200	-0.04576600
Na	-1.24463300	-0.62448400	1.00215600

1b

C	5.37220800	-1.61501500	2.15854000
H	5.88884000	-2.01038400	3.04955200
H	4.85307900	-2.44441500	1.66298200
C	6.39484100	-1.02081500	1.21110100
H	6.83185900	-0.10917000	1.65316100
H	7.21385700	-1.74572000	1.05981200
C	6.58641100	-0.04204600	-0.93932900
H	7.37029300	-0.71349200	-1.33158300
H	7.08732600	0.81219500	-0.45348500
C	5.73106300	0.46502000	-2.08500700
H	6.38564900	0.90671100	-2.85582700
H	5.17799600	-0.36933400	-2.54857100
C	4.07815500	2.09940100	-2.56726500
H	3.50141100	1.38472100	-3.17740100
H	4.74206400	2.66338400	-3.24499100
C	3.14308600	3.06976200	-1.87375800
H	2.76240300	3.81831400	-2.58303500
H	3.70606100	3.58434600	-1.08509800
C	3.27637400	-1.06913600	3.17903700
H	2.98675200	-0.31717800	3.92530700
H	3.47153800	-2.01250100	3.71056000
C	2.10488200	-1.24669700	2.22183700
H	1.20927200	-1.52421400	2.79751600
H	1.90091900	-0.30976100	1.68392100
C	1.51529600	-2.68597900	0.37471700
C	1.97029900	-3.64043000	-0.55095700
C	0.18668800	-2.24936100	0.33739100
C	1.09152400	-4.16661200	-1.49306200
H	3.00913000	-3.95715500	-0.50649500
C	-0.70886200	-2.80609900	-0.60004900
H	-0.18097300	-1.51645800	1.04565400
C	-0.24534300	-3.76607600	-1.51679500
H	1.45004100	-4.90107200	-2.21079700
H	-0.92730800	-4.18210800	-2.25334900
C	1.12672200	2.99765200	-0.56470000
C	1.35939000	4.23938500	0.04305000

C	-0.11474300	2.36267400	-0.41029400
C	0.35235600	4.81716000	0.82381300
H	2.30463000	4.75744100	-0.07645600
C	-1.13703700	2.95890700	0.34612500
H	-0.28141900	1.41612500	-0.91327700
C	-0.88000200	4.19536400	0.97972400
H	0.54094800	5.77182900	1.30993100
H	-1.64491200	4.65994900	1.59489900
O	2.44118400	-2.27523900	1.28745600
O	2.04598500	2.32942300	-1.32408700
O	4.83194700	1.43338600	-1.57132100
O	5.75309700	-0.71735700	-0.01534800
O	4.44947700	-0.59461400	2.53569100
C	-2.13579200	-2.47149400	-0.57443600
C	-2.75970800	-1.21041800	-0.25595500
C	-3.18422200	-3.35630200	-0.83060300
C	-4.40209000	-2.66952600	-0.64296000
H	-3.10093500	-4.40657600	-1.07973400
C	-2.48704500	2.39082100	0.41794700
C	-2.91808600	1.03028600	0.18837100
C	-3.65631300	3.11233100	0.67256000
C	-4.75476700	2.23472000	0.58033600
H	-3.74033400	4.17473100	0.86068200
N	-4.31404100	0.99095800	0.28551200
N	-4.14702900	-1.38689100	-0.29775500
N	-2.17171700	-0.04087300	-0.04284200
B	-5.17489200	-0.27524600	0.03525300
F	-6.04935900	-0.07036700	-1.04148700
F	-5.91486800	-0.60865600	1.17884600
C	-6.20273900	2.55334900	0.74749300
H	-6.75185800	2.33853400	-0.17788800
H	-6.64491200	1.92610400	1.53137800
H	-6.33948800	3.60615600	1.00913400
C	-5.78918800	-3.20498300	-0.76648300
H	-6.33086900	-3.08779500	0.18067100
H	-6.35086900	-2.64484500	-1.52424700
H	-5.77185800	-4.26313400	-1.04128800

Na$^+$•1b

C	0.00000000	0.00000000	0.00000000
H	0.00000000	0.00000000	1.09966594
H	1.03916103	0.00000000	-0.35388522
C	-0.72536069	1.23747779	-0.48797191
H	-1.77600570	1.22381294	-0.15688435
H	-0.23950287	2.13018872	-0.06602873
C	-1.37658255	2.35024630	-2.50919296
H	-0.88429735	3.30641036	-2.27714174
H	-2.40592367	2.38411125	-2.11929463
C	-1.39159805	2.13044342	-4.00963933
H	-1.89661644	2.97264678	-4.50549382
H	-0.36223154	2.06423088	-4.39521846
C	-2.02134711	0.47067733	-5.62041068
H	-0.97034782	0.37393052	-5.93524927
H	-2.52028736	1.18996897	-6.28741990
C	-2.72737520	-0.86620812	-5.71482877
H	-2.70737739	-1.22820150	-6.75090198
H	-3.77059442	-0.76916814	-5.39000045
C	-0.10776851	-2.40495987	-0.05493122
H	-0.92649460	-3.05628127	0.27466145
H	0.55549472	-2.22808808	0.80055587
C	0.65260377	-3.10440132	-1.17185914
H	1.08498876	-4.04075437	-0.78873968
H	-0.01961927	-3.35672586	-2.00513888
C	2.49015288	-2.58420521	-2.64885281
C	3.47579820	-1.64672942	-2.99962982
C	2.39966484	-3.80103818	-3.33156501
C	4.37150936	-1.93663857	-4.02497849
H	3.52699447	-0.70988945	-2.45053031
C	3.31543100	-4.09585692	-4.36399475
H	1.65356668	-4.53930646	-3.06307586
C	4.30336301	-3.15492841	-4.70302249
H	5.13172703	-1.20756784	-4.29579165
H	4.99941667	-3.37039816	-5.50899039
C	-2.57267399	-3.07678509	-4.77868548
C	-3.84716856	-3.28269646	-4.24012420

C	-1.78404470	-4.14829627	-5.19402285
C	-4.33088912	-4.58861138	-4.12804673
H	-4.44602860	-2.43902326	-3.90663316
C	-2.26853466	-5.46586881	-5.08137063
H	-0.80317404	-3.95447737	-5.61463325
C	-3.55547309	-5.66912143	-4.54368611
H	-5.31725488	-4.76203504	-3.70448567
H	-3.93774471	-6.68021633	-4.43311748
O	1.67849932	-2.21510436	-1.61305456
O	-2.02846343	-1.80001623	-4.86350287
O	-2.08475379	0.90863416	-4.26558846
O	-0.66770211	1.26372296	-1.91345560
O	-0.68177590	-1.16473107	-0.48861361
C	3.29212552	-5.39065552	-5.05061673
C	2.14426274	-6.19743535	-5.38812302
C	4.39981781	-6.10078449	-5.51308934
C	3.94592458	-7.30914658	-6.08584413
H	5.44045037	-5.81731940	-5.41857917
C	-1.49522888	-6.61439428	-5.56146536
C	-0.06154077	-6.75052972	-5.64316991
C	-2.01480707	-7.81070293	-6.05916180
C	-0.93819868	-8.63516118	-6.44936956
H	-3.05925989	-8.06867718	-6.17979965
N	0.22945758	-7.99875495	-6.20192289
N	2.59688468	-7.36554788	-6.01031954
N	0.86419960	-5.89569371	-5.23003244
B	1.66655762	-8.51084822	-6.49384935
F	1.83856101	-8.74088672	-7.86543793
F	1.92532139	-9.69145126	-5.78505119
C	-0.99453730	-9.99585048	-7.05829420
H	-0.48720860	-10.00159967	-8.03100093
H	-0.47116059	-10.72171283	-6.42319153
H	-2.03101101	-10.31783027	-7.19087756
C	4.76070845	-8.40657595	-6.68306480
H	4.57789056	-9.35038875	-6.15392328
H	4.47568129	-8.56938975	-7.72989395
H	5.82656491	-8.16790885	-6.63365210

Na	-1.31311602	-0.81765602	-2.78773432

7. Cellular Imaging

Subcellular localization was measured on living WEHI-13VAR cells using a Olympus FV1000 Confocol Microscope. Throughout, digital images were captured with a 100x / 1.4 oil objective with the following filter sets:

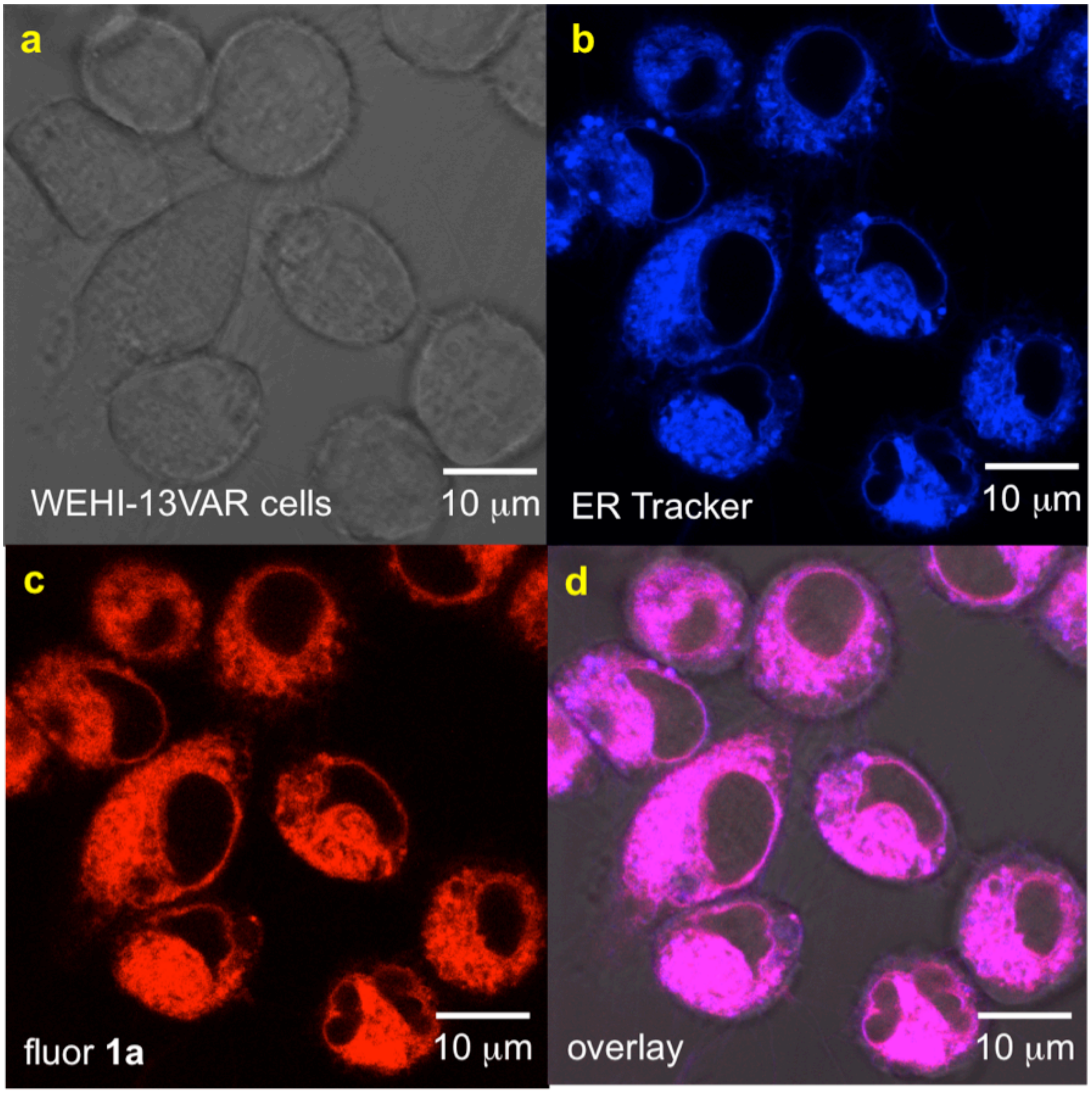
- for ER-Tracker Blue-White DPX: excitation 405 nm
- for aza-BODIPY: excitation 633 nm

Sequential optical sections (Z-stacks) from the basal-to-apical surfaces of the cell were also acquired.

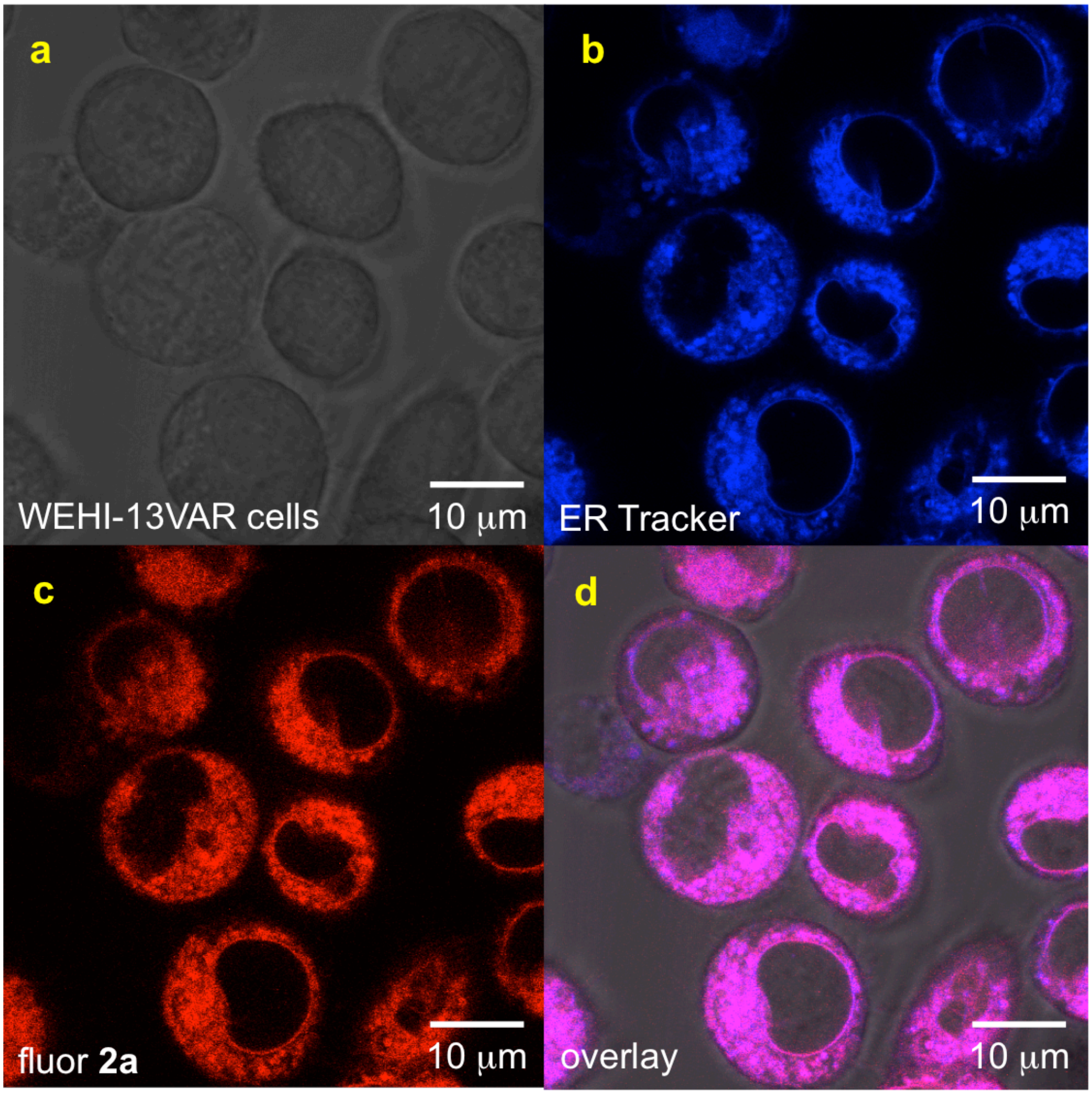
Endoplasmic Reticulum Co-localization

Cells were incubated with fluors for 3 h at 37 ^{o}C. After the cells were washed with PBS (2X), ER-Tracker Blue-White DPX was added and the cells were incubated for 20 min at 37 ^{o}C. The cells were washed again with PBS before imaging.

A


a
WEHI-13VAR cells
10 μm
b
ER Tracker
10 μm
c
fluor **1a**
10 μm
d
overlay
10 μm

B


a
WEHI-13VAR cells
10 μm
b
ER Tracker
10 μm
c
fluor **2a**
10 μm
d
overlay
10 μm

C

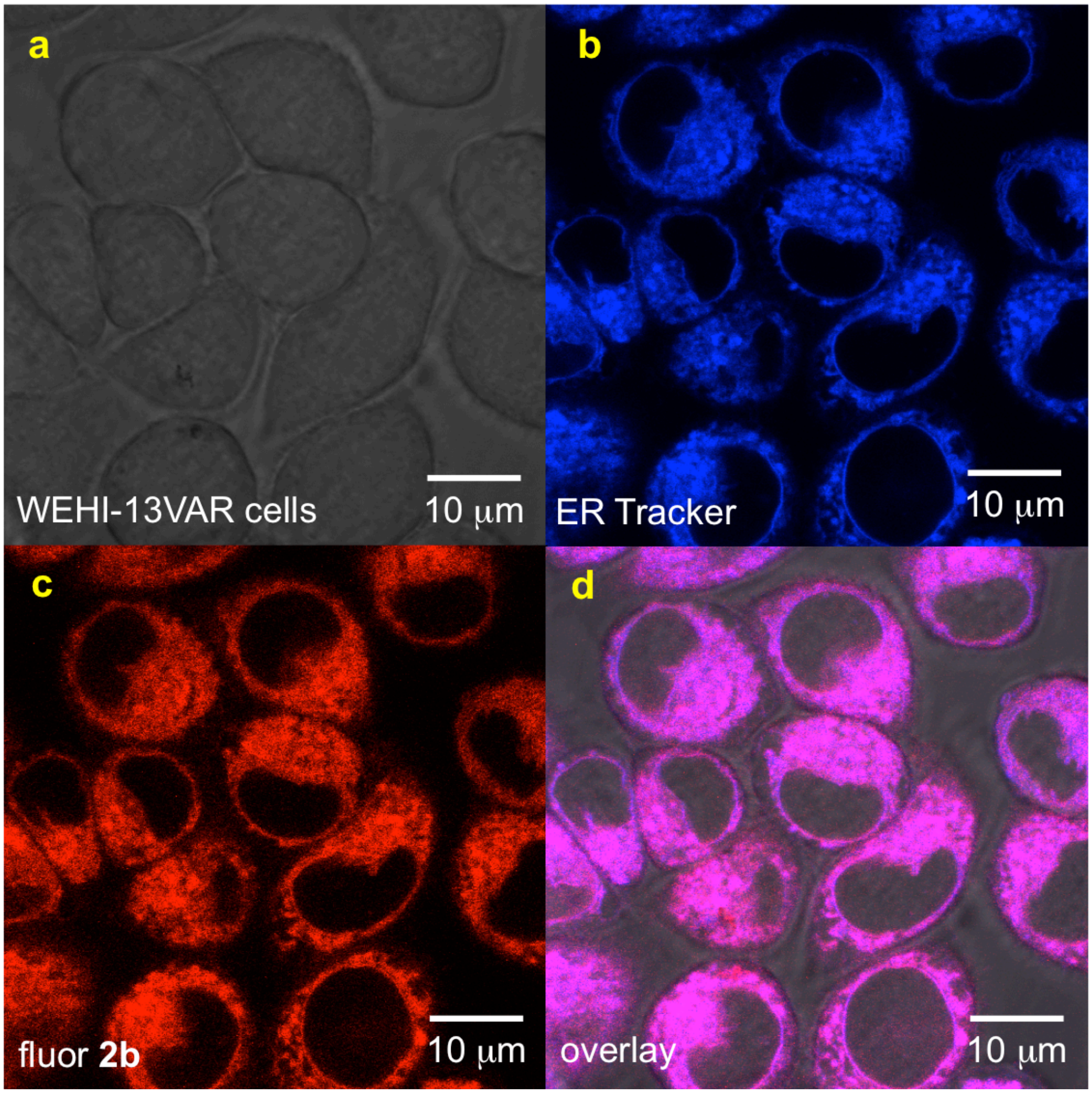
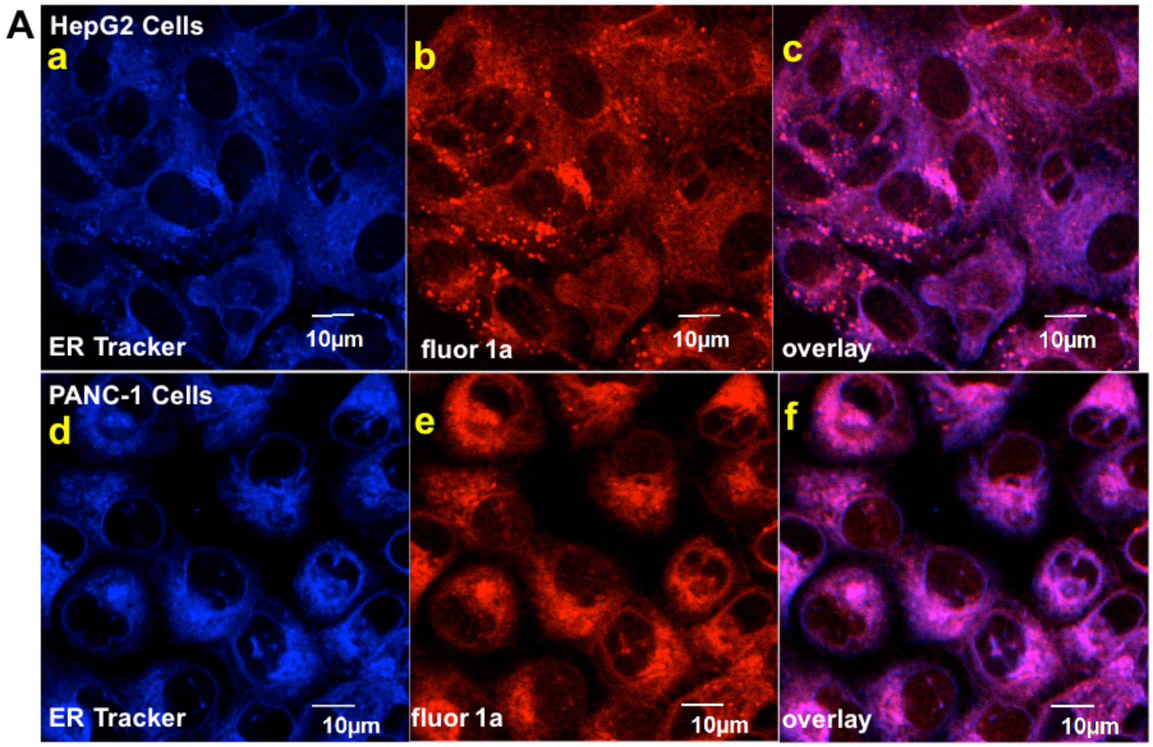
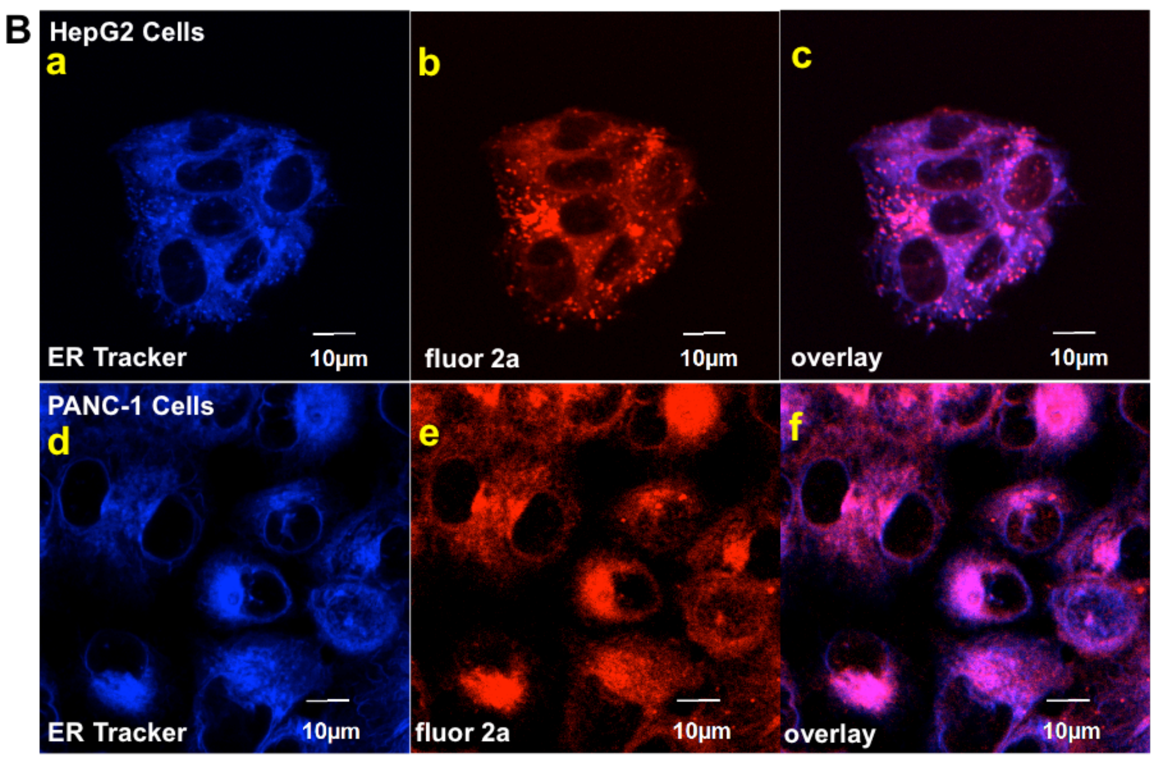


Figure S10. Images of WEHI-13VAR cells. **a** Under a bright-field (DIC image); **b** with ER tracker; **c** with fluor **1a (A)**, **2a (B)** and **2b(C)** ; and, **d** overlay of images **a** – **c**.


A
HepG2 Cells
a
ER Tracker
10μm
b
fluor 1a
10μm
c
overlay
10μm
PANC-1 Cells
d
ER Tracker
10μm
e
fluor 1a
10μm
f
overlay
10μm


B
HepG2 Cells
a
ER Tracker
10μm
b
fluor 2a
10μm
c
overlay
10μm
PANC-1 Cells
d
ER Tracker
10μm
e
fluor 2a
10μm
f
overlay
10μm

C

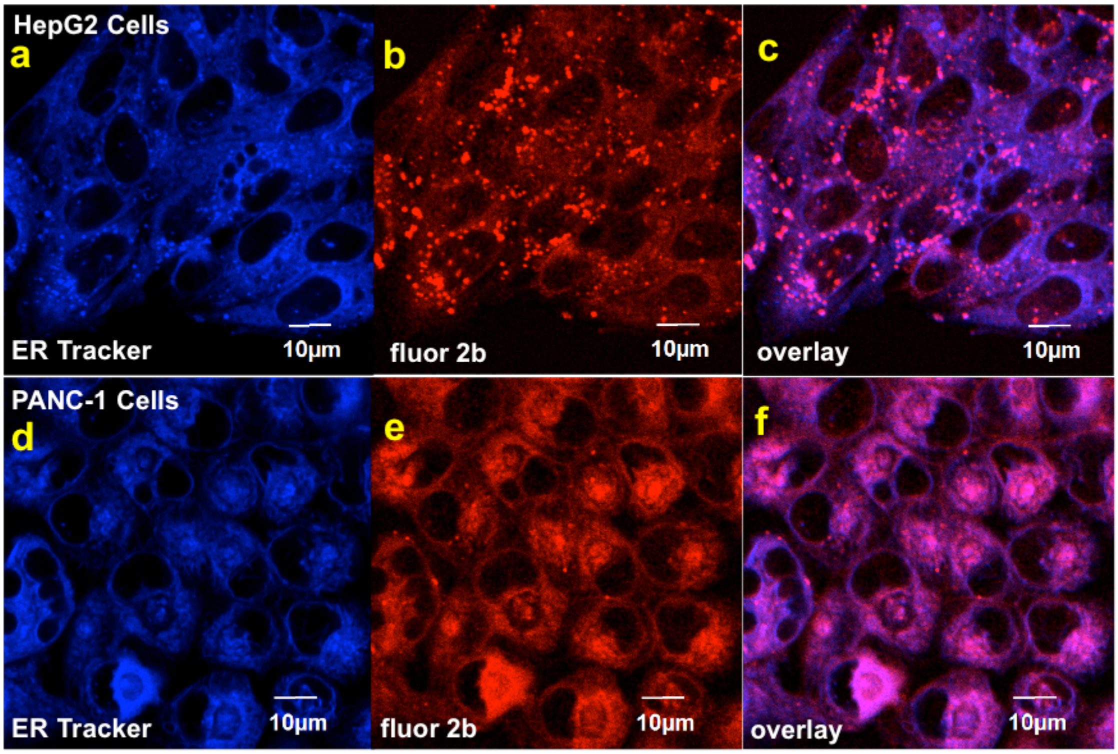


Figure S11. Parallel images for staining HepG2 cells (top) and PANC-1 (bottom) with: **a,d** ER tracker; **b,e** with fluor **1a (A)**, **2a (B)** and **2b(C)**. **c**,**f** Shows the overlay of images for each cell type.