Aza-BODIPY Dyes With Enhanced Hydrophilicity

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. Dry DMF, (<50 ppm water) was purchased from EMD. Tetrahydrofuran (THF), acetonitrile (MeCN), 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 spectrometer (^1H at 400 MHz and ^{13}C at 100 MHz) at room temperature unless otherwise mentioned. Chemical shifts of ^1H NMR spectra were recorded and reported in ppm from the solvent resonance ($CDCl_3$ 7.26 ppm, CD_3OD 3.30 ppm, DMSO-d_6 2.50 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, DMSO-d_6 39.5 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.

2. Syntheses Of Sulfonated aza-BODIPY Derivatives

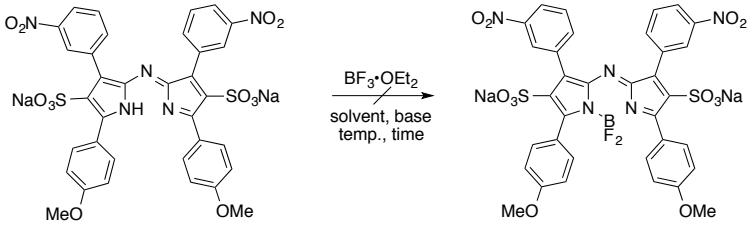
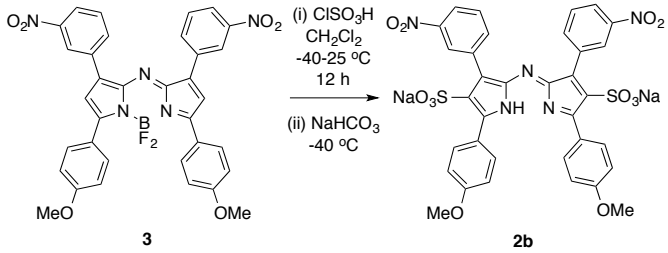


Table S1. Conditions to form BF_2 complex.

Solvent	Base	Temperature (°C)	Time (h)
toluene	$^{i}Pr_2EtN$	80	12
CH_2Cl_2	$^{i}Pr_2EtN$	25	12
CH_2Cl_2	Et_3N	25	12
CH_2Cl_2	NaH	25	12
$CH_2CH_2Cl_2$	$^{i}Pr_2EtN$	60	12



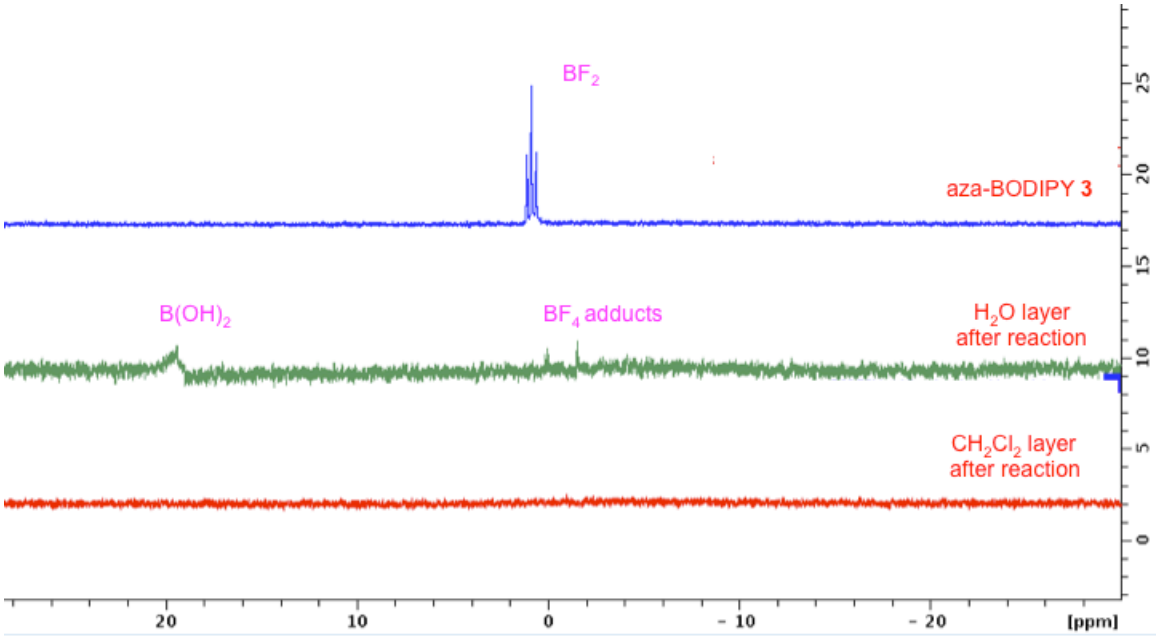
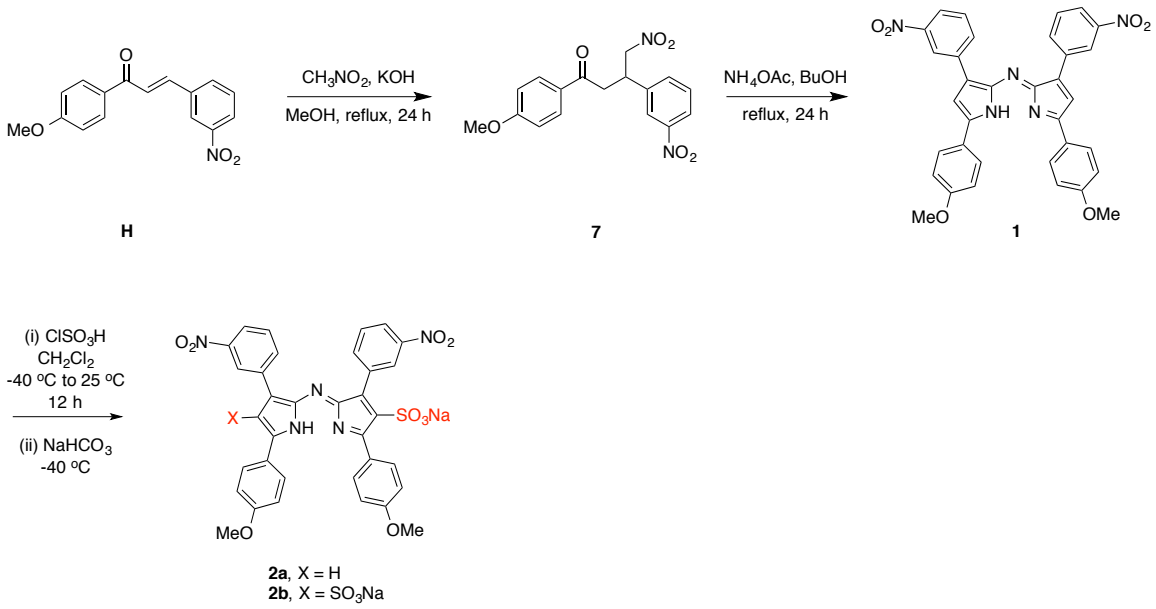


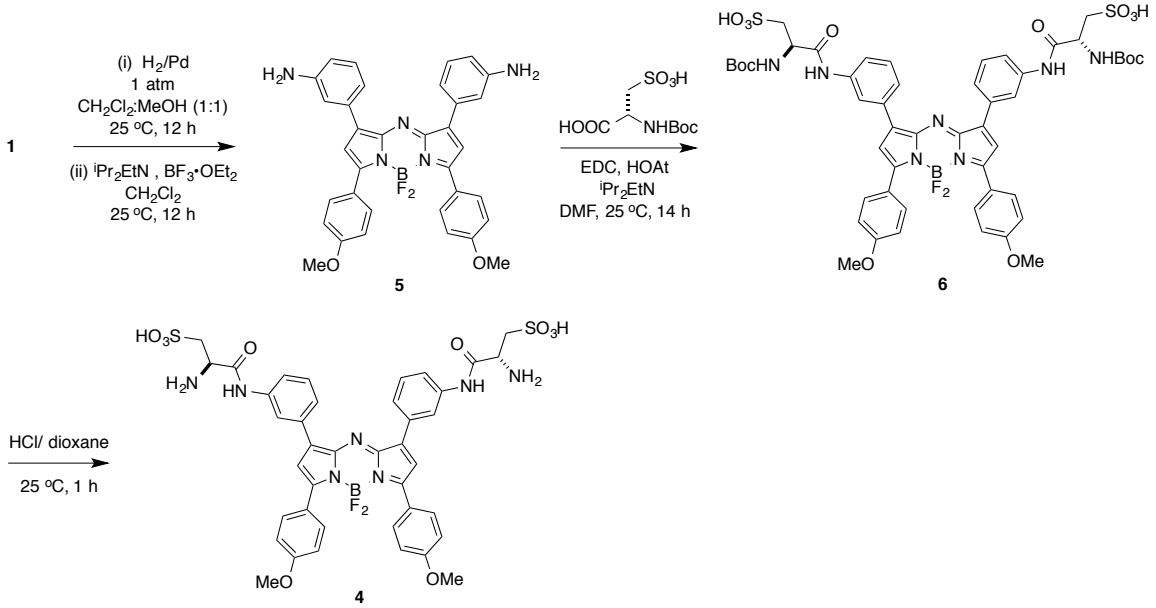
Fig. S1 ^{11}B NMR of the crude product showed the loss of the BF_2 fragment.



H **7** **1**

2a, X = H
2b, X = SO_3Na

Scheme S1.



5 **6** **4**

Scheme S2.

1-(4-methoxyphenyl)-4-nitro-3-(3-nitrophenyl)butan-1-one (7).

Potassium hydroxide (33.6 g, 60 mmol) was added to a solution of chalcone **H** (17 g, 60 mmol) in MeOH (250 mL) at 25 °C. Nitromethane (64 mL, 1.2 mol) was added to the reaction mixture, and then the reaction was heated to reflux at 78 °C for 24 h. After the reaction was cooled to 25 °C, HCl (0.2 N) was added to neutralize. Precipitate was filtered out and washed with cold MeOH. The product was obtained as slightly brown

solid (14.5 g, 70 % yield) and used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 (s, 1H), 8.19 (d, *J* = 7.7 Hz, 1H), 7.92 (d, *J* = 8.9 Hz, 2H), 7.69 (d, *J* = 7.7 Hz 1H), 7.54 (dd, *J* = 7.9, 7.9 Hz, 1H), 6.95 (d, *J* = 8.9 Hz, 2H), 4.89 (m, 1H), 4.75 (m, 1H), 4.38 (m, 1 H), 3.90 (S, 3 H), 3.47 (d, *J* = 6.9 Hz, 2H). ^{13}C (100 MHz, $CDCl_3$) δ 194.3, 164.1, 141.5, 134.3, 130.3, 130.2, 130.0, 129.1, 122.9, 122.2, 114.0, 79.0, 55.5, 40.7, 39.0. MS (ESI+) calcd for $C_{17}H_{17}N_2O_6$ $\{M+H\}^+$ 345.1087, found 345.1096.

(*Z*)-5-(4-methoxyphenyl)-*N*-(5-(4-methoxyphenyl)-3-(3-nitrophenyl)-1*H*-pyrrol-2-yl)-3-(3-nitrophenyl)-2*H*-pyrrol-2-imine (1).

7 (10 g, 29 mmol) was dissolved in ^{n}BuOH (300 mL). Ammonium acetate (78 g, 1 mol) was added to the solution. The reaction was heated up to reflux at 120 ºC and stirred for 24 h. The mixture was cooled to 40 ºC, then the solvent was removed. The residue was precipitated in cold EtOH, the solid was filtered to give dark solid **1** (9 g, 52 % yield). The product was used without further purification. NMR spectra cannot be obtained from compound **1** due to solubility. However, after complexation with BF_2 to yield compound **3**, the NMR was obtained nicely. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (s, 2H), 8.37 (d, *J* = 7.2 Hz, 2H), 8.28 (d, *J* = 8.0 Hz, 2H), 8.14 (d, *J* = 8.6 Hz, 4H), 7.70 (t, *J* = 7.8 Hz, 2H), 7.20 (s, 2H), 7.06 (d, *J* = 8.6 Hz, 4H), 3.91 (s, 6H). ^{13}C (100 MHz, CD_2Cl_2) δ 161.6, 158.3, 155.5, 149.4, 148.7, 141.3, 140.8, 135.0, 133.7, 133.3, 132.4, 131.5, 130.7, 130.3, 130.2, 128.9, 124.2, 124.1, 124.0, 122.5, 122.3, 117.6, 116.7, 114.8, 114.6, 55.8. HRMS(ESI-) calcd for $C_{34}H_{24}BClF_2N_5O_6$ $\{M+Cl\}^-$ 682.1476, found 682.1464.

Synthesis of disulfonic acid (2a, 2b)

1 (30 mg, 0.05 mmol) was dissolved in CH_2Cl_2 (5.5 mL) and the solution was cooled to -40 ºC. Solution of chlorosulfonic acid (1 eq. for **2a** and 4 eq. for **2b**) in CH_2Cl_2 (0.5 mL) was slowly added to the solution over 5 min at -40 ºC. The mixture was slowly warmed to 25 ºC and stirred for 5-12 h. The reaction was quenched with sat. $NaHCO_3$ at -40 ºC. Organic layer was separated and the crude product was purified by flash silica chromatography eluting with CH_2Cl_2:MeOH (85:15) to yield 20 mg (60 %) of **2a** as a purple powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (s, 2H), 8.18 (d, *J* = 7.2 Hz, 1H), 8.18 (d, *J* = 7.6 Hz, 1H), 8.13 (d, *J* = 7.6 Hz, 1H), 8.06 (d, *J* = 7.2 Hz, 1H), 7.95 (d, *J* =

7.8 Hz, 2H), 7.91 (d, *J* = 8.1 Hz, 2H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.37 (s, 1H), 7.34-7.8 (m, 1H, overlap with $CDCl_3$) 7.01-6.97 (m, 4H), 3.86 (s, 6H). HRMS (ESI-) calcd for $C_{34}H_{24}N_5O_9S^-$ {M-Na}$^-$ 678.1300, found 678.1321. For **2b**, the product was precipitated from the reaction. The precipitate was filtered to give a purple powder 60 mg, 79 % yield. NMR cannot be obtained due to solubility. HRMS (ESI-) calcd for $C_{34}H_{23}N_5NaO_{12}S_2^-$ {M-Na}$^-$ 780.0676, found 780.0640.

3,3'-(5,5-difluoro-3,7-bis(4-methoxyphenyl)-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,5,2]triazaborinine-1,9-diyl)dianiline (5).

1 (1 g, 1.67 mmol) was dissolved in CH_2Cl_2 : MeOH (1:1, 30 mL). Pd/C (180 mg, 0.17 mmol) was added to the solution. The mixture was stirred under H_2 (1 atm) at 25 ºC for 20 h, the reaction was followed by TLC. The product was filtered through Celite® to give dark blue solid after the solvent was removed. The solid was then dissolved in CH_2Cl_2 (200 mL), *N*,*N*-diisopropylethylamine (2.9 mL, 16.7 mmol) was added to the solution. The mixture was stirred at 25 ºC for 20 min, BF_3•OEt_2 (3.1 mL, 25 mmol) was then added in portions and the mixture was stirred at 25 ºC for 12 h. The reaction was quenched with careful addition of H_2O (20 mL) and the system was stirred vigorously for 15 min. The organic layer was separated and washed with HCl (0.2 *N*, 1 × 10 mL), NaOH (2 *N*, 2 × 10 mL) and brine (10 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 EtOAc:Hexanes (3:1 to 2:1) to yield 883 mg (90 %) of **4** as a dark green solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, *J* = 8.9 Hz, 4H), 7.71 (s, 2H), 7.33 (d, *J* = 7.8 Hz, 2H), 7.24 (d, *J* = 7.8 Hz, 2H), 7.03 (d, *J* = 9.8 Hz, 4H). 6.77-6.74 (m, 2H), 3.91 (s, 6H). ^{13}C (100 MHz, $CDCl_3$) δ 161.9, 158.0, 146.6, 145.3, 143.0, 133.5, 131.6, 129.4, 124.3, 119.3, 118.6, 116.4, 116.2, 114.2, 55.4. ^{11}B NMR (128 MHz, $CDCl_3$) *δ* 0.94 (t, *J* = 31.9 Hz, *B*F2) ppm. ^{19}F NMR (376 MHz, $CDCl_3$) *δ* -131.89 (q, *J* = 31.9 Hz, B*F*2). HRMS (ESI+) calcd for $C_{34}H_{29}BF_2N_5O_2$ {M+H}$^+$ 588.2382, found 588.2359.

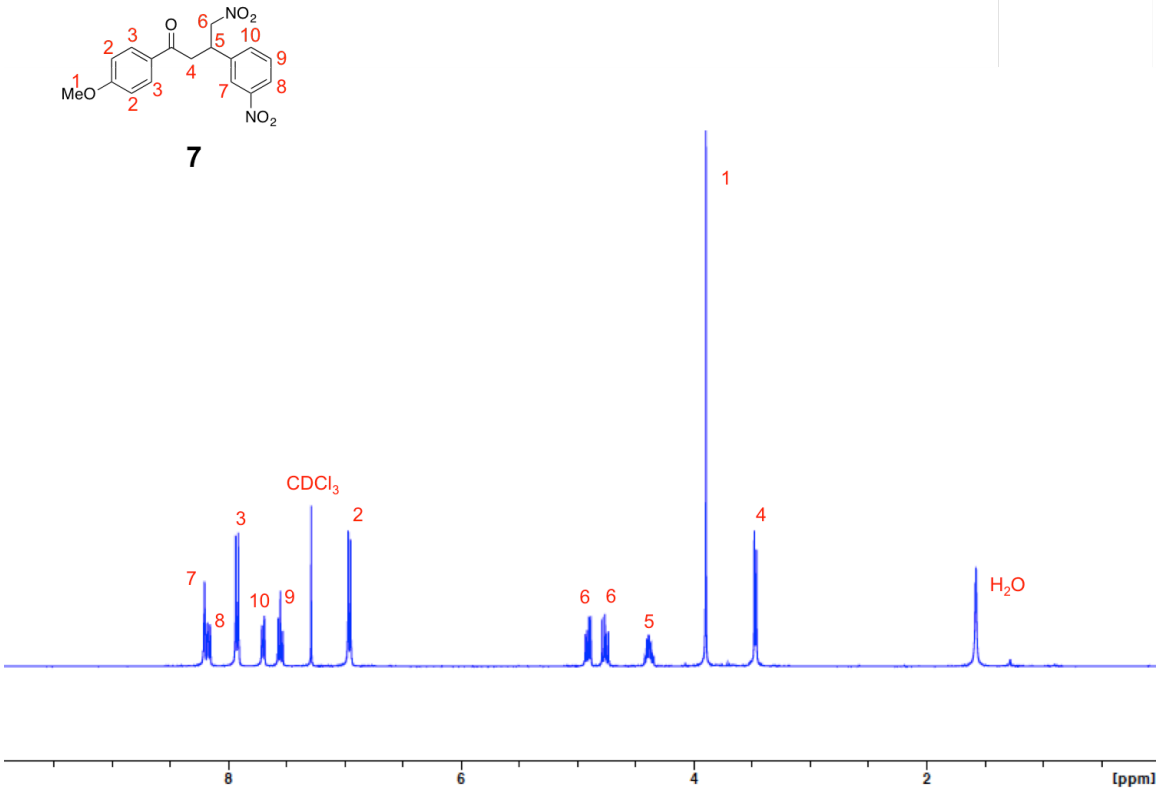
(2*R*,2'*R*)-3,3'-(((5,5-difluoro-3,7-bis(4-methoxyphenyl)-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,5,2]triazaborinine-1,9-diyl)bis(3,1-phenylene))bis(azanediyl))bis(2-

((*tert*-butoxycarbonyl)amino)-3-oxopropane-1-sulfonic acid) (6).

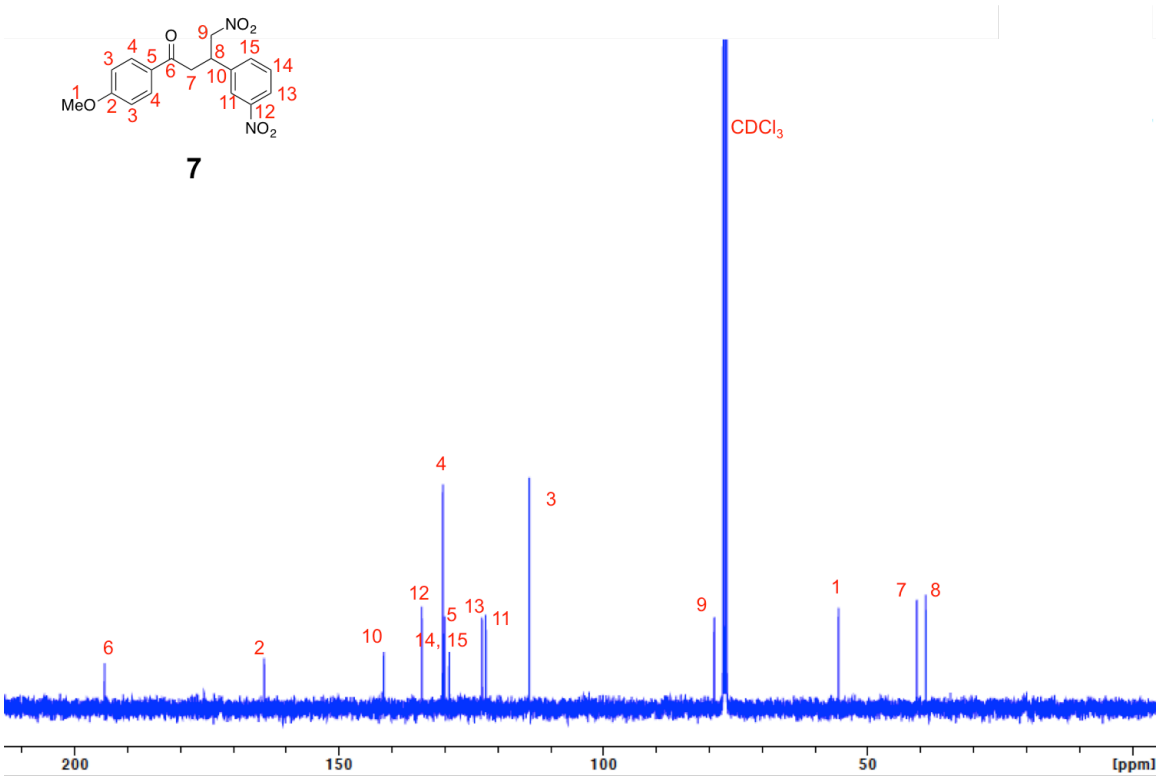
Boc-protected Cysteic acid (367 mg, 1.28 mmol) was dissolved in DMF and cooled to 0 ºC. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, EDC (274 mg, 1.43 mmol) and 1-hydroxy-7-azabenzotriazole, HOAt (198 mg, 1.46 mmol) were added to the solution. After stirring for 30 min, compound **5** (150 mg, 0.26 mmol) was added to the reaction mixture. *N*,*N*-Diisopropylethylamine (0.45 mL, 2.6 mmol) was then added in one portion. The reaction was stirred at 25 ºC for 14 h. The solvent was removed and the product was purified by reverse phase MPLC eluting with water and acetonitrile to yield 212 mg (75 %) as a greenish powder. ^{1}H NMR (400 MHz, CD_3OD) δ 8.14 (s, 2H), 8.08 (d, *J* = 8.6 Hz, 4H), 7.90 (d, *J* = 6.0 Hz, 2H), 7.53 (m, 2H), 7.33 (m, 2H), 7.15 (s, 2H), 6.98 (d, *J* = 8.6 Hz, 4H), 3.86 (s, 6H), 3.62-3.59 (m, 2H), 3.17-3.11 (m, 4H), 1.47 (s, 18H). ^{13}C (100 MHz, CD_3OD) δ 170.3, 162.2, 160.1, 157.7, 156.2, 144.9, 142.2, 138.4, 132.7, 131.7, 128.7, 125.7, 123.6, 120.4, 118.9, 79.7, 55.2, 54.8, 42.1, 27.4. HRMS (MALDI-) calcd for $C_{50}H_{53}BF_2N_7O_{14}S_2$ {M-H}$^-$ 1088.3156, found 1088.3108.

(*R*)-2-amino-3-((3-(9-(3-((*R*)-2-amino-3-sulfopropanamido)phenyl)-5,5-difluoro-3,7-bis(4-methoxyphenyl)-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-*c*:2',1'-*f*][1,3,5,2]triazaborinin-1-yl)phenyl)amino)-3-oxopropane-1-sulfonic acid (4).

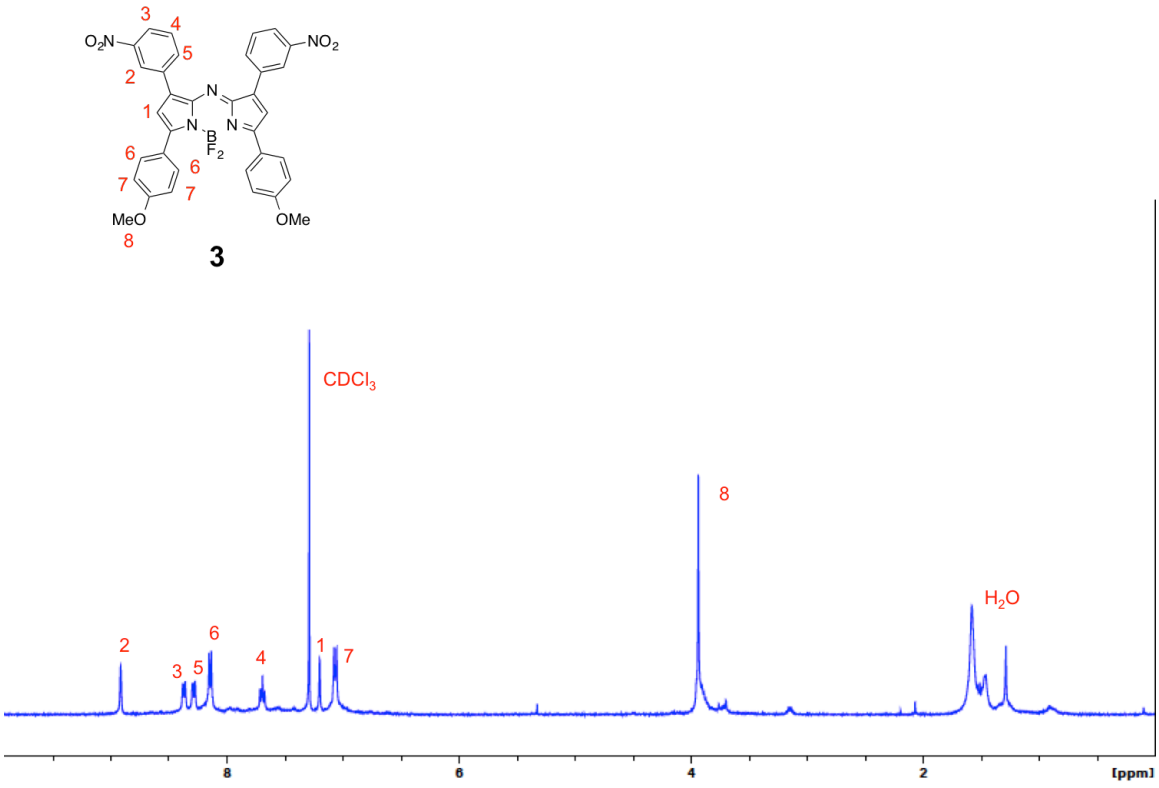
Compound **5** (40 mg, 0.37 mmol) was dissolved in dioxane (5 mL). Solution of HCl in dioxane (2 M, 5 mL) was added into the solution. The reaction was stirred at 25 ºC for 1 h. The solvent was removed to yield **4** 33 mg, quantitative yield as a green solid. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 10.81 (s, 2H), 8.25 (s, 2H), 8.17 (d, *J* = 8.9 Hz, 4H), 7.95 (d, *J* = 7.7 Hz, 2H), 7.74 (d, *J* = 7.2 Hz, 2H), 7.51 (t, *J* = 8.0 Hz, 2H), 7.44 (s, 2H), 7.16 (d, *J* = 8.9 Hz, 4H), 4.19 (br, 2H), 3.90 (s, 6H), 3.21-3.18 (m, 2H), 2.94 (dd, *J* = 9.8 Hz, 4H). ^{13}C (100 MHz, DMSO-*d6*) δ 166.5, 162.6, 158.0, 145.0, 142.7, 139.0, 132.8, 132.3, 129.8 125.9, 123.6, 121.6, 120.5, 120.3, 115.0, 56.1, 51.5, 50.7. ^{11}B NMR (128 MHz, DMSO-*d6*) *δ*: 0.88 (t, *J* = 32.0 Hz, *B*F2) ppm. ^{19}F NMR (376 MHz, DMSO-*d6*) *δ*: -130.52 (q, *J* = 32.0 Hz, B*F*2). HRMS (MALDI-) calcd for $C_{40}H_{36}BF_2N_7NaO_{10}S_2$ {M-Na}$^-$ 910.1925, found 910.1968.



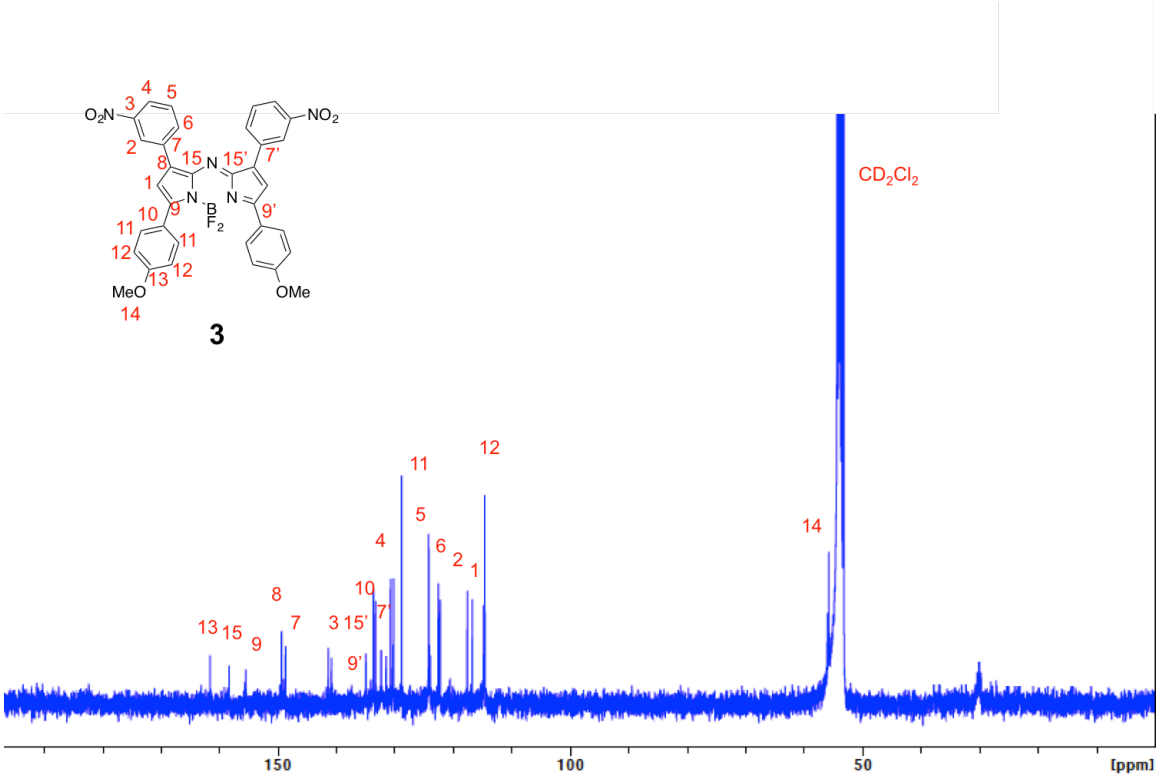
^{1}H-NMR of compound 7



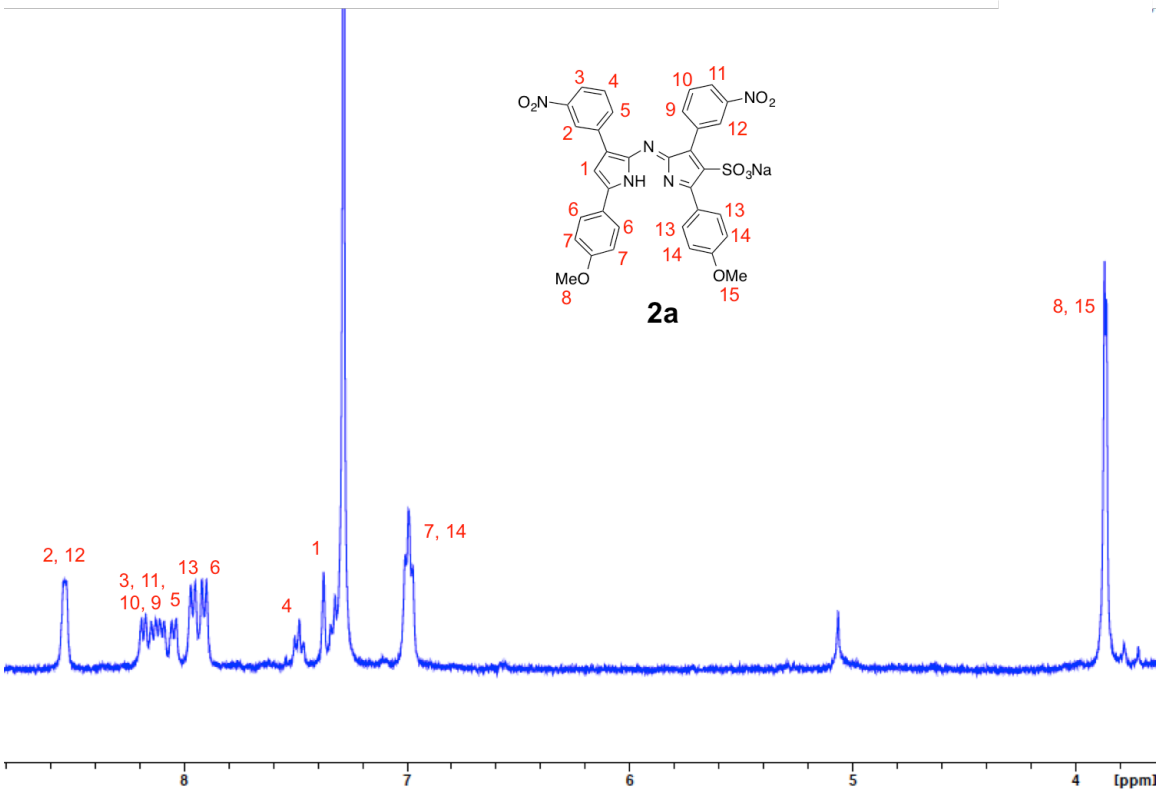
^{13}C-NMR of compound 7



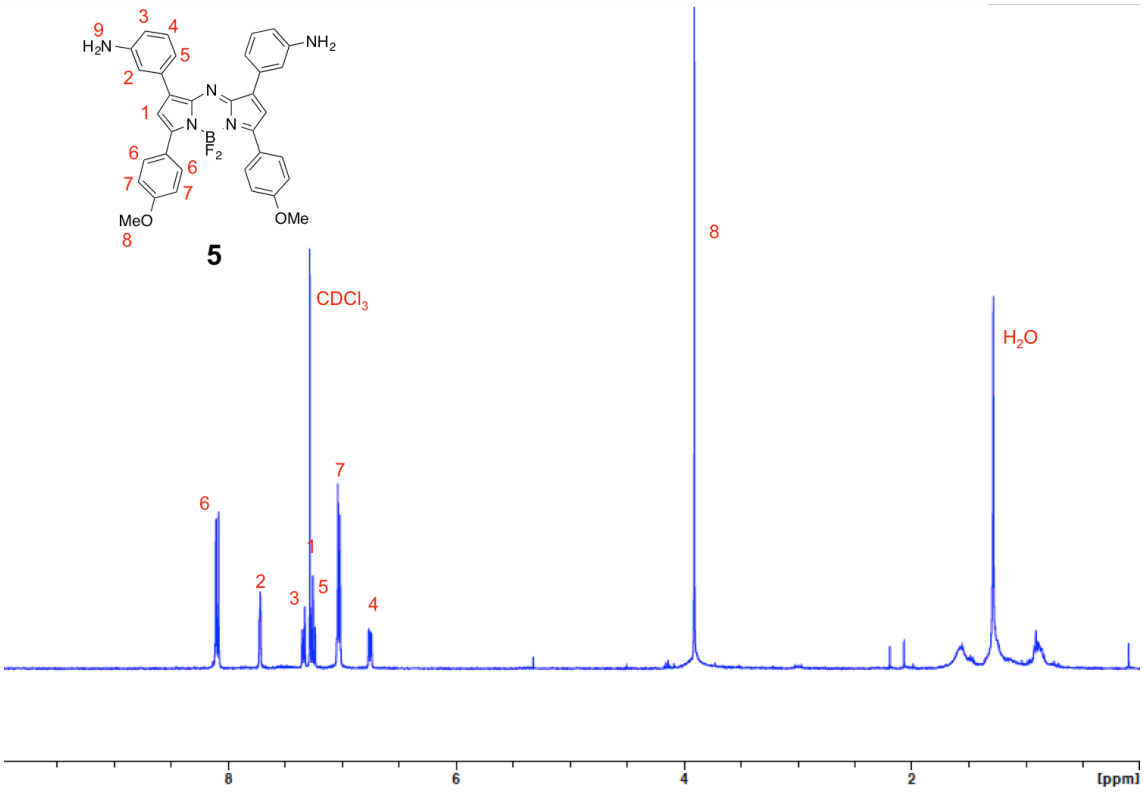
^{1}H-NMR of compound 3



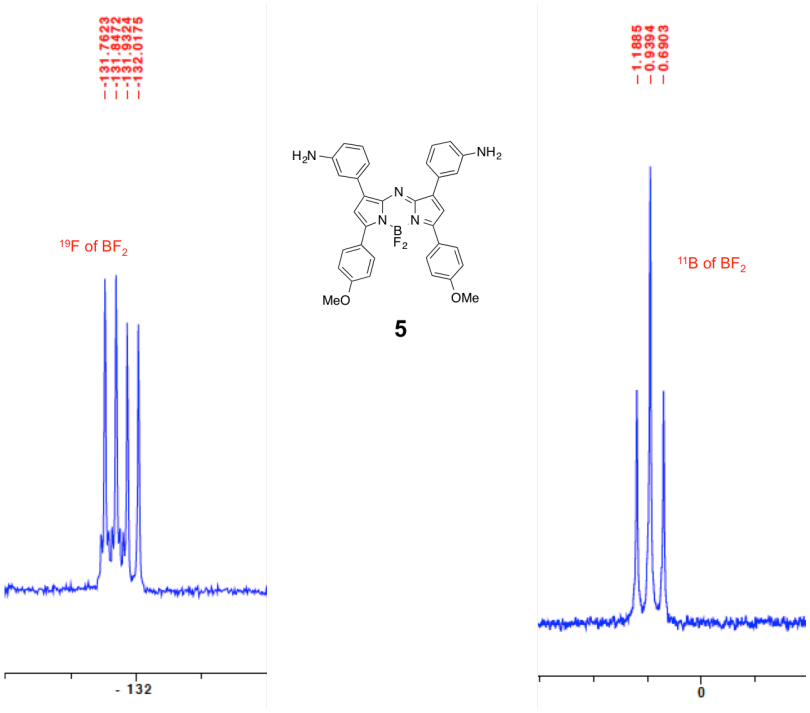
^{13}C-NMR of compound 3



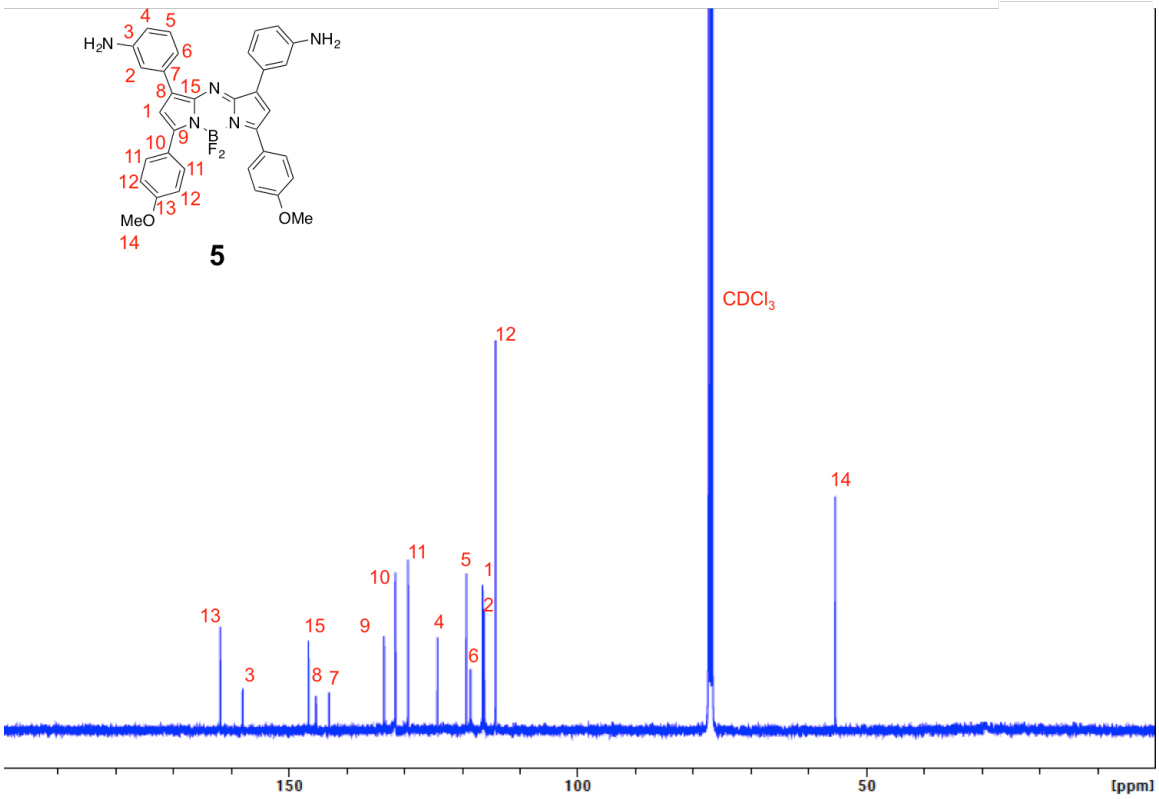
^{1}H-NMR of compound 2a



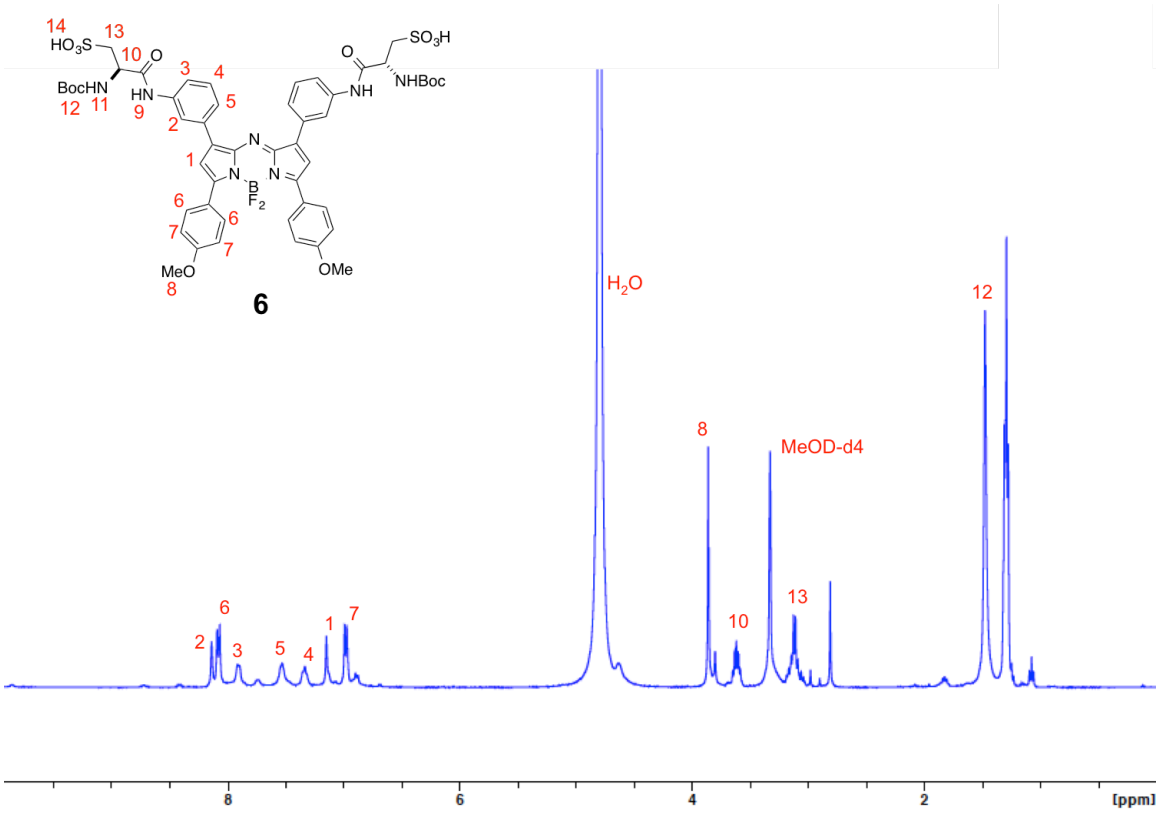
^{1}H-NMR of compound 5



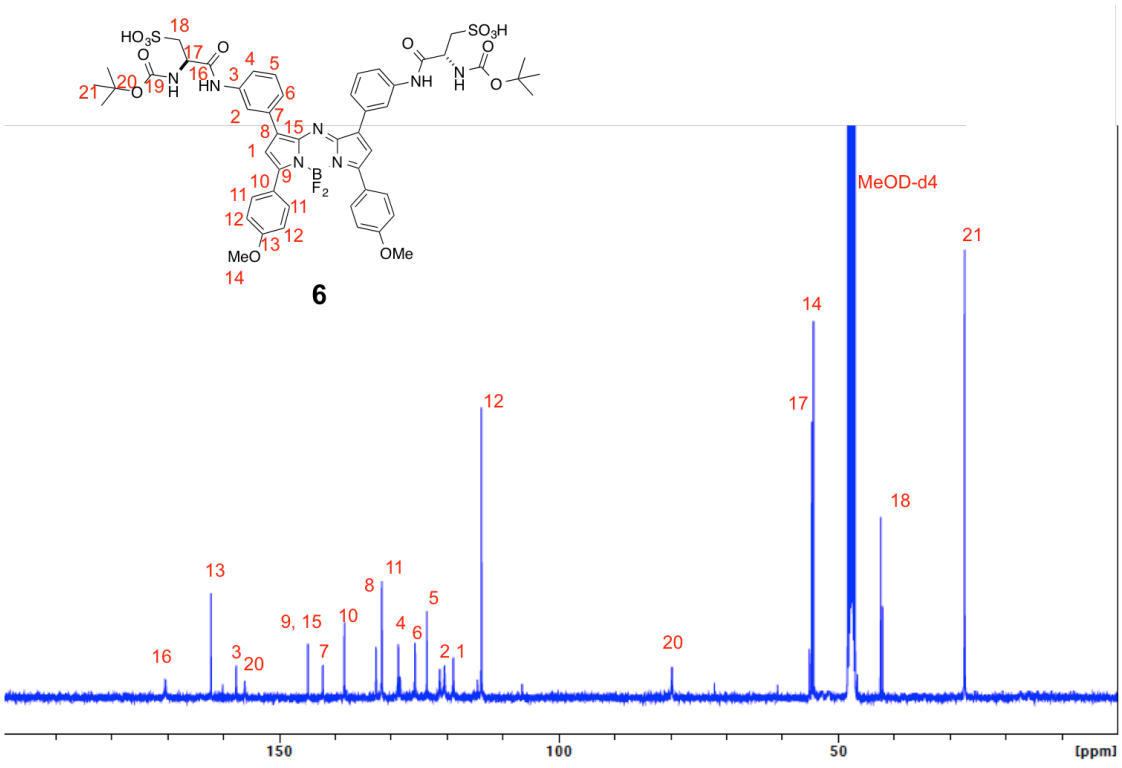
^{11}B, ^{19}F-NMR of compound 5



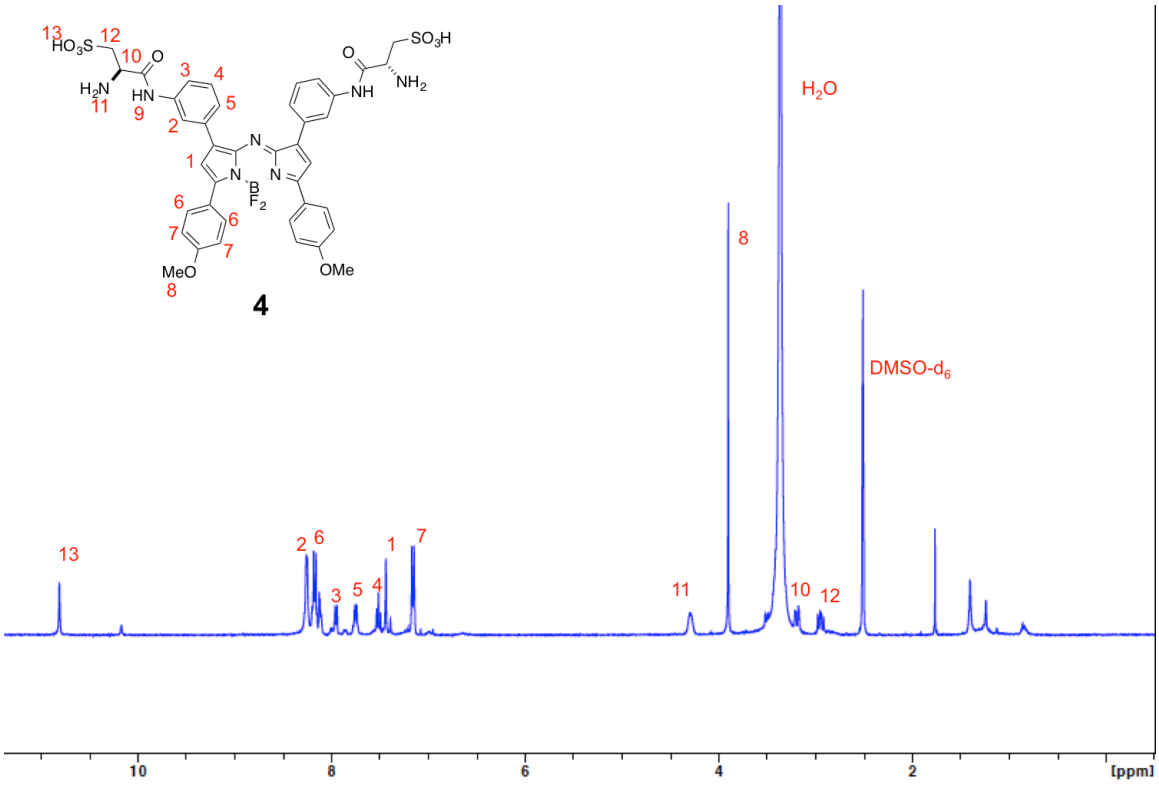
^{13}C-NMR of compound 5



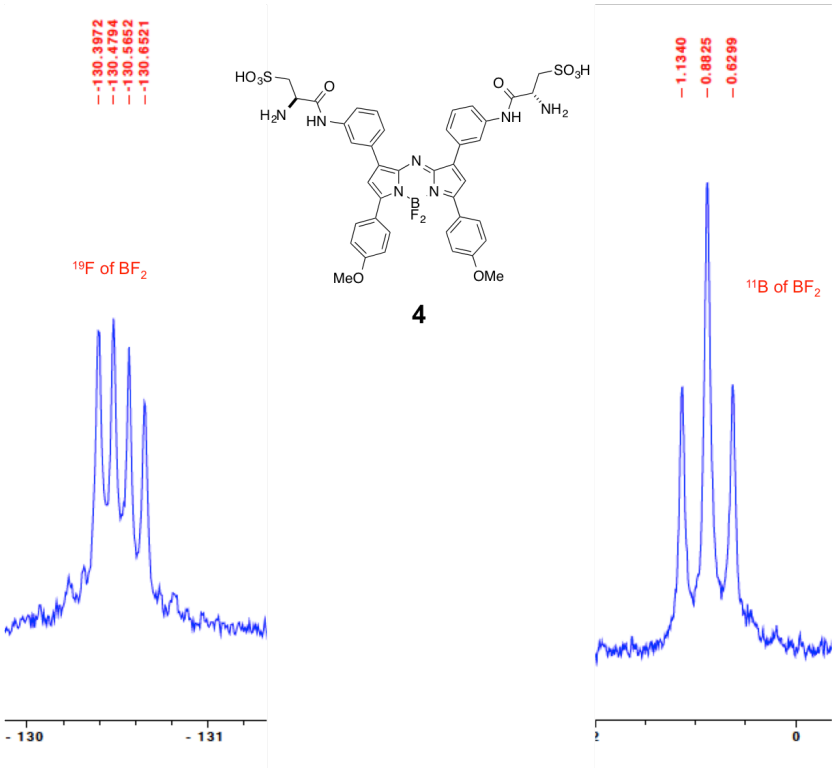
^{1}H-NMR of compound 6



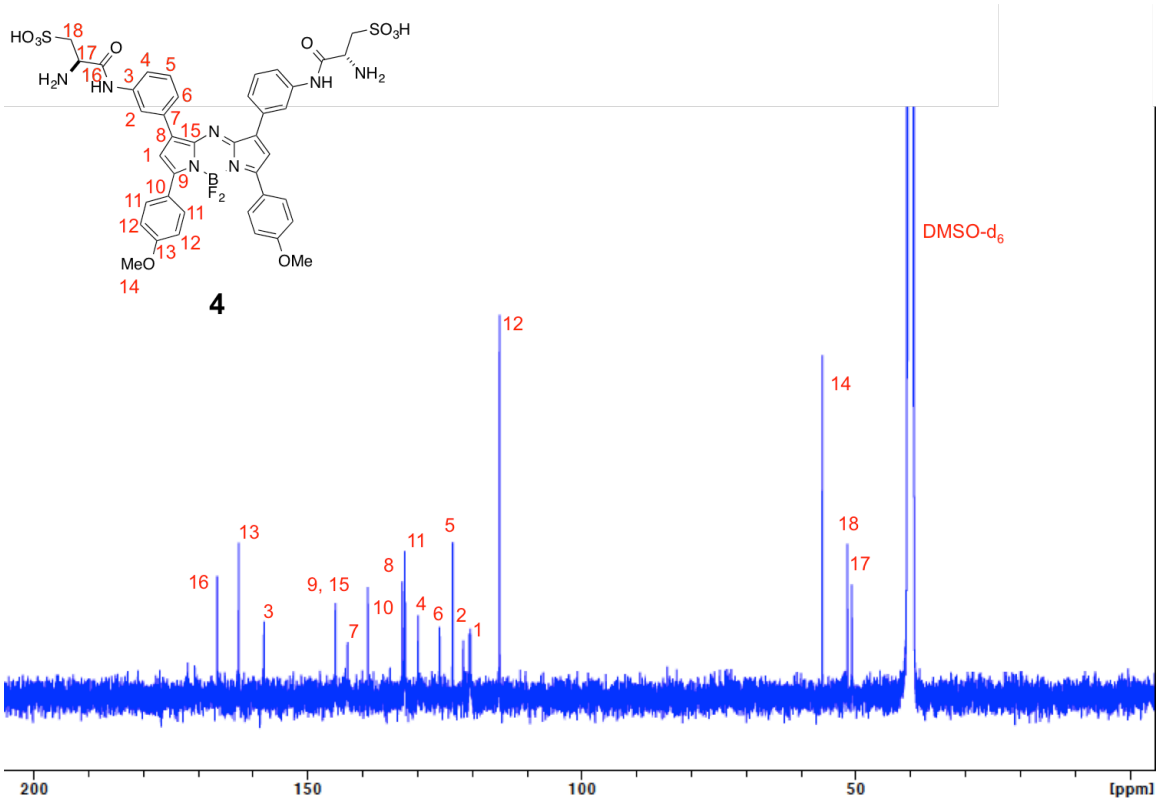
^{13}C-NMR of compound 6



^{1}H-NMR of compound 4



^{11}B, ^{19}F-NMR of compound 4



^{13}C-NMR of compound 4

3. Spectroscopic Data Of Compound 4.

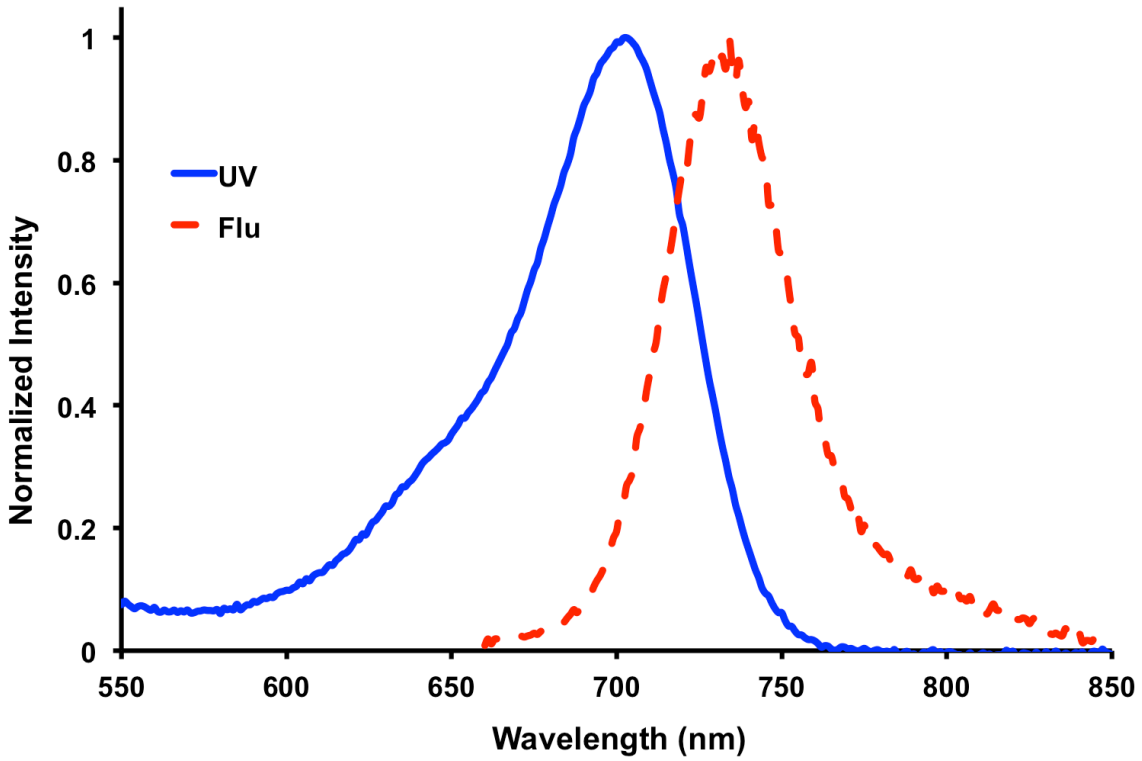


Fig. S2 UV and fluorescent spectra of **4** in DMSO.

Table S2. Spectroscopic Data of Compound **4**.

Solvent	$\lambda_{abs\ max}$ (nm)	$\varepsilon(M^{-1}\,cm^{-1}) \times 10^4$	$\lambda_{emis\ max}$ (nm)[a]	Φ_F[b]
DMSO	703	6.12	730	0.17 ± 0.003
PBS[c]	701	4.72	729	0.34 ± 0.010

[a]Excited at 650 nm. [b]Relative to Zn-pthalocyanine in 1% pyridine/toluene (Φ_F = 0.30). [c]Contained 0.1% CrEL, pH 7.4.

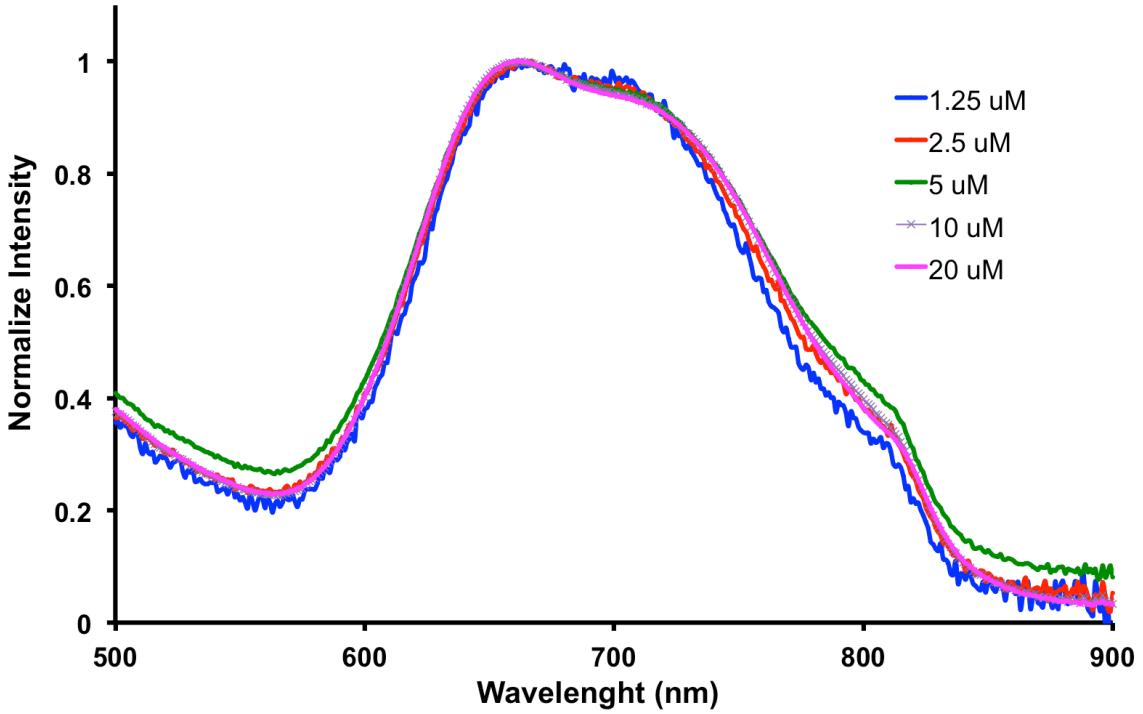


Fig. S3 UV absorptions of **4** at different concentrations. No evidence of aggregation was observed when concentration dependence of **4** in PBS buffer (without additives) was used.

4. Thermodynamic Equilibrium Solubility Measurement

Stock solutions (0.1 M) of test compounds were prepared in DMSO then diluted with aqueous media (PBS pH 7.4 or carbonate buffer pH 9 or acetate buffer pH 4) to desired concentrations (0 – 1000 μM, the highest concentration contained 1 % DMSO). Then, the plate covered with aluminium foil was shaken horizontally for 6 h at 25 ºC and kept overnight for equilibration. Thereafter, the plate was centrifuged at 100 rpm for 20 min. Supernatant was pipetted into 96-well UV transparent plate (Corning® 96 Well Clear Flat Bottom UV-Transparent Microplate) and analyzed at λ = 680 nm of compounds against blank using microplate reader (Biotek Synergy H4).

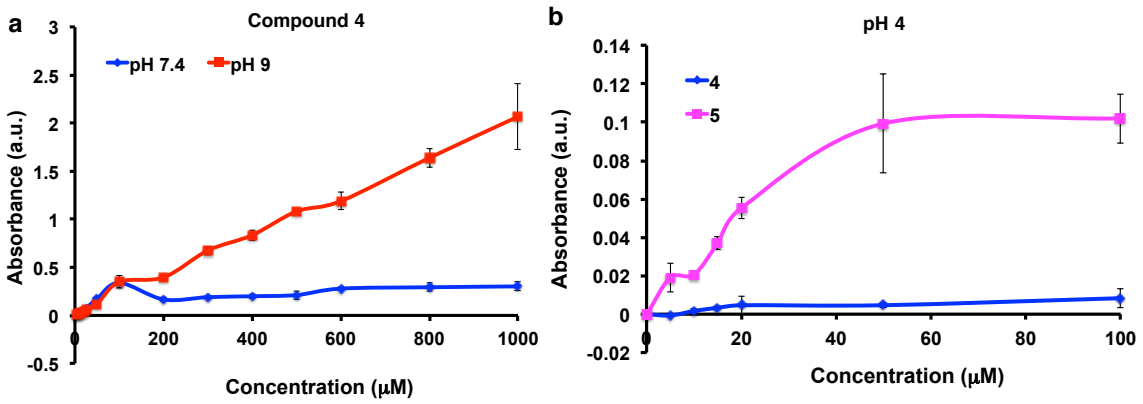


Fig. S4 Solubility Profile. **a** Compound **4** is soluble in PBS pH 7.4 up to 100 μM and more than 1 mM can be dissolved in carbonate buffer pH 9. **b** In acetate buffer, compound 4 is only soluble up to 20 μM, where as 5 can be soluble up to 50 μM. The analysis was performed in triplicate for each compound.

5. Cell Culture And Imaging Studies

4T1 cells were cultured on 75 cm^2 culture flasks in Dulbecco's Modified Eagle Medium/nutrient mixture F-12 (DMEM/F12, Sigma Chemical, St. Louis, MO) supplemented with 10 % FBS. Cells were cultures in a humidified incubator at 37 °C with 5 % CO_2 and 95 % air.

Subcellular localization was measured on living 4T1 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 LysoTracker Green: excitation 488 nm; for aza-BODIPY **4**: excitation 633 nm. Sequential optical sections (Z-stacks) from the basal-to-apical surfaces of the cell were also acquired.

Lysosomal Co-localization

Cells were incubated with fluors for 3 h at 37 ºC. After the cells were washed with PBS (2X), LysoTracker Green was added and the cells were incubated for 30 min at 37 ºC. The cells were washed again with PBS before imaging.