Aza-BODIPY Dyes With Enhanced Hydrophilicity

Supporting Information

Anyanee Kamkaew\textsuperscript{a} and Kevin Burgess\textsuperscript{a,b}

\textsuperscript{a} Department of Chemistry, Texas A & M University, Box 30012, College Station, TX 77842.
\textsuperscript{b} Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.

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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$_2$Cl$_2$), 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 ($^1$H at 400 MHz and $^{13}$C at 100 MHz) at room temperature unless otherwise mentioned. Chemical shifts of $^1$H NMR spectra were recorded and reported in ppm from the solvent resonance (CDCl$_3$ 7.26 ppm, CD$_3$OD 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$_3$OD 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.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Base</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>toluene</td>
<td>'Pr$_2$EtN</td>
<td>80</td>
<td>12</td>
</tr>
<tr>
<td>CH$_2$Cl$_2$</td>
<td>'Pr$_2$EtN</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>CH$_2$Cl$_2$</td>
<td>Et$_3$N</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>CH$_2$Cl$_2$</td>
<td>NaH</td>
<td>25</td>
<td>12</td>
</tr>
<tr>
<td>CH$_2$CH$_2$Cl$_2$</td>
<td>'Pr$_2$EtN</td>
<td>60</td>
<td>12</td>
</tr>
</tbody>
</table>

Fig. S1 $^{11}$B NMR of the crude product showed the loss of the BF$_2$ fragment.
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. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 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, 1H), 3.90 (S, 3 H), 3.47 (d, \(J = 6.9\) Hz, 2H). \textsuperscript{13}C (100 MHz, CDCl\textsubscript{3}) \(\delta\) 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\textsubscript{17}H\textsubscript{17}N\textsubscript{2}O\textsubscript{6} \{M+H\}\textsuperscript{+} 345.1087, found 345.1096.

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

7 (10 g, 29 mmol) was dissolved in \textsuperscript{6}BuOH (300 mL). Ammonium acetate (78 g, 1 mol) was added to the solution. The reaction was heated up to reflux at 120 °C and stirred for 24 h. The mixture was cooled to 40 °C, 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\textsubscript{2} to yield compound 3, the NMR was obtained nicely. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 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). \textsuperscript{13}C (100 MHz, CD\textsubscript{2}Cl\textsubscript{2}) \(\delta\) 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\textsubscript{14}H\textsubscript{24}BCIF\textsubscript{2}N\textsubscript{6}O\textsubscript{6} \{M+Cl\}\textsuperscript{-} 682.1476, found 682.1464.

Synthesis of disulfonic acid (2a, 2b)

1 (30 mg, 0.05 mmol) was dissolved in CH\textsubscript{2}Cl\textsubscript{2} (5.5 mL) and the solution was cooled to -40 °C. Solution of chlorosulfonic acid (1 eq. for 2a and 4 eq. for 2b) in CH\textsubscript{2}Cl\textsubscript{2} (0.5 mL) was slowly added to the solution over 5 min at -40 °C. The mixture was slowly warmed to 25 °C and stirred for 5-12 h. The reaction was quenched with sat. NaHCO\textsubscript{3} at -40 °C. Organic layer was separated and the crude product was purified by flash silica chromatography eluting with CH\textsubscript{2}Cl\textsubscript{2}:MeOH (85:15) to yield 20 mg (60 %) of 2a as a purple powder. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 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, \(J = 7.6\) Hz, 1H), 7.28 (d, \(J = 7.6\) Hz, 1H), 7.18 (d, \(J = 7.6\) Hz, 1H), 6.06 (d, \(J = 7.2\) Hz, 1H), 5.75 (d, \(J = 7.6\) Hz, 1H), 5.65 (d, \(J = 7.6\) Hz, 1H), 4.70 (s, 2H), 4.30 (s, 2H), 3.98 (s, 6H), 3.80 (s, 6H). MS (ESI+) calcd for C\textsubscript{34}H\textsubscript{24}BCIF\textsubscript{2}N\textsubscript{6}O\textsubscript{6} \{M+H\}\textsuperscript{+} 882.1476, found 882.1464.
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 CDCl₃) 7.01-6.97 (m, 4H), 3.86 (s, 6H). HRMS (ESI-) calcd for C₃₄H₂₄N₅O₉-{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 C₃₄H₂₃N₄NaO₁₂S₂⁻ {M-Na}⁻ 780.0676, found 780.0640.

3,3'-((5,5-difluoro-3,7-bis(4-methoxyphenyl)-5H-4λ⁴,5λ⁴-dipyrrrolo[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 CH₂Cl₂ : MeOH (1:1, 30 mL). Pd/C (180 mg, 0.17 mmol) was added to the solution. The mixture was stirred under H₂ (1 atm) at 25 °C 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 CH₂Cl₂ (200 mL), N,N-diisopropylethylamine (2.9 mL, 16.7 mmol) was added to the solution. The mixture was stirred at 25 °C for 20 min, BF₃•OEt₂ (3.1 mL, 25 mmol) was then added in portions and the mixture was stirred at 25 °C for 12 h. The reaction was quenched with careful addition of H₂O (20 mL) and the system was stirred vigorously for 15 min. The organic layer was separated and washed with HCl (0.2 N, 1 x 10 mL), NaOH (2 N, 2 x 10 mL) and brine (10 mL). The organic layer was dried over MgSO₄, 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. ¹H NMR (400 MHz, CDCl₃) 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). ¹³C (100 MHz, CDCl₃) 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. ¹¹B NMR (128 MHz, CDCl₃) δ 0.94 (t, J = 31.9 Hz, BF₂) ppm. ¹⁹F NMR (376 MHz, CDCl₃) δ -131.89 (q, J = 31.9 Hz, BF₂). HRMS (ESI+) calcd for C₃₄H₂₉BF₂N₅O₂ {M+H}⁺ 588.2382, found 588.2359.

(2R,2'R)-3,3'(((5,5-difluoro-3,7-bis(4-methoxyphenyl)-5H-4λ⁴,5λ⁴-dipyrrrolo[1,2-c:2',1'-f][1,3,5,2]triazaborinine-1,9-diyl)bis(3,1-phenylene))bis(azanediyl))bis(2-
Boc-protected Cysteic acid (367 mg, 1.28 mmol) was dissolved in DMF and cooled to 0 °C. 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 °C 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. 

\( ^1H \) NMR (400 MHz, CD\(_3\)OD) \( \delta \) 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).

\( ^13C \) (100 MHz, CD\(_3\)OD) \( \delta \) 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\(_2\)N\(_7\)O\(_{14}\)S\(_2\) \({\text{[M-H]}}^-\) 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)-5H-4i,4i,5i,5i-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 (2 M, 5 mL). Solution of HCl in dioxane (5 mL) was added into the solution. The reaction was stirred at 25 °C for 1 h. The solvent was removed to yield 4 33 mg, quantitative yield as a green solid. 

\( ^1H \) NMR (400 MHz, DMSO-d6) \( \delta \) 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). 

\( ^13C \) (100 MHz, DMSO-d6) \( \delta \) 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) \( \delta \) : 0.88 (t, \( J = 32.0 \) Hz, BF2) ppm. 

\( ^{19}F \) NMR (376 MHz, DMSO-d6) \( \delta \) : -130.52 (q, \( J = 32.0 \) Hz, BF2). HRMS (MALDI-) calcd for C\(_{40}\)H\(_{36}\)BF\(_2\)N\(_7\)NaO\(_{10}\)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
$^{11}\text{B, }^{19}\text{F-NMR of compound 5}$

$^{13}\text{C-NMR of compound 5}$
S13

\[ ^1\text{H-NMR of compound 4} \]

\[ ^{11}\text{B}, ^{19}\text{F-NMR of compound 4} \]
Fig. S2 UV and fluorescent spectra of 4 in DMSO.

Table S2. Spectroscopic Data of Compound 4.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>λ_{abs max} (nm)</th>
<th>ε (M^{-1} cm^{-1}) \times 10^4</th>
<th>λ_{emis max} (nm)^a</th>
<th>Φ_F^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>703</td>
<td>6.12</td>
<td>730</td>
<td>0.17 ± 0.003</td>
</tr>
<tr>
<td>PBS^c</td>
<td>701</td>
<td>4.72</td>
<td>729</td>
<td>0.34 ± 0.010</td>
</tr>
</tbody>
</table>

^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.
**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 °C 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, whereas 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 cm² 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 % CO₂ and 95 % air.

Subcellular localization was measured on living 4T1 cells using a Olympus FV1000 Confocal 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 °C. After the cells were washed with PBS (2X), LysoTracker Green was added and the cells were incubated for 30 min at 37 °C. The cells were washed again with PBS before imaging.