Supporting Information

A facile synthesis of novel near-infrared pyrrolopyrrole aza-BODIPY luminogens with aggregation-enhanced emission characteristics

Lanqing Li, Lingyun Wang*, Hao Tang and Derong Cao

Key Laboratory of Functional Molecular Engineering of Guangdong Province, School of Chemistry and Chemical Engineering, South China University of Technology, Guangzhou, 510641, China.

E-mail: lingyun@scut.edu.cn; Fax: +86 20 87110245; Tel: +86 20 87110245

1. General Experimental Section

1.1 Materials

All chemicals used in this study were analytical reagent grade. HeLa cells were obtained from the cell bank of the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China).

1.2 Instruments

The UV-vis absorption spectra were recorded using a Helios Alpha UV-Vis scanning spectrophotometer with a 1 cm quartz cell. Fluorescence spectra were quantitatively measured by FluoroMax-4 spectrofluorometer with a xenon lamp and 0.5 cm quartz cells. High-resolution mass spectra were carried on LCQ Fleet LC-MS System (Thermo Fisher Scientific). $^1$H NMR and $^{13}$C NMR spectra were carried on a Bruker spectrometer. The fluorescence images of HeLa cells were taken by a confocal laser scanning microscope (Japan Olympus Co., Ltd) with an objective lens ($\times$ 60).
The particle size and distribution were determined by dynamic light scattering (DLS) using a Malvern Nano-ZS90 particle size analyzer at a fixed angle of 90° at 25 °C. Elemental analyses were performed with an CHN-analyser Vario EL from Elementar.

1.3 Cell culture

HeLa cells were cultured in high-glucose Dulbecco’s Modified Eagle’s Medium (H-DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin streptomycin at 37 °C in a humidified environment containing 5% CO₂. Before the experiment, the cells were precultured until confluence was reached.

1.4 Cell imaging

HeLa cells were seeded in the 12-well plate and cultured in H-DMEM with 10% FBS at 37 °C in a humidified environment containing 5% CO₂. After 80% confluence, the medium was removed and the adherent cells were rinsed twice with 1 × PBS. PPAB-2 NPs in DMEM medium with FBS at 50 μM was then added to the culture plate. After incubation for 2 hours, the cells were washed three times with 1 × PBS buffer. The nuclei were stained by 4’,6-diamidino-2-phenylindole (DAPI) for 10 min. The cell monolayer was then washed twice with 1 × PBS buffer and imaged using a confocal laser scanning microscope (Japan Olympus Co., Ltd) with an objective lens (× 60). Excitation of HeLa cells at 635 nm was carried out with a HeNe laser and the emission was collected from 700 nm to 800 nm.

1.5 Cytotoxicity

Cell viability was evaluated using the Cell Counting Kit-8 (CCK-8) assay. CCK-8 was just as WST-8 to produce formazan in the presence of an electron mediator, and the amount of the formazan generated in cells was directly proportional to the number
of living cells. The HeLa cells were seeded in 96-well plates at a density of 3000 cells per well. PPAB-2 NPs at 1 μM or 10 μM was added into a 96-well plate. Meanwhile, cells culture with complete medium (H-DME with 10% FBS) were evaluated as a control. The HeLa cells were incubated in the medium under 5% CO₂ in an incubator maintained at 37 °C for 1, 2 and 4 days, respectively. Then, 10 μL of the CCK-8 was added to each well of a 96-well plate incubated for additional 2 h. The absorbance was measured at 450 nm using a microplate reader (Varioskan Flash, Thermo Scientific). The assay was repeated three times.
Scheme S1. Synthetic routes of PPAB-1, PPAB-2 and PPAB-3.

2. Synthesis of PPAB-1, PPAB-2 and PPAB-3

6-Bis(4-octyloxyphenyl)-2,5-dihydropyrrolo[3,4-c]pyrrole-1,4-dione (1),

6-(tert-butyl)benzo[d]thiazol-2-amine (2a) and 4-(1,2,2-triphenylvinyl)aniline (2b')
were prepared as previously described [1, 2].

**Synthesis of 6-(1,2,2-triphenylvinyl)benzod[thiazol-2-amine (2b)**

2b' (100 mg, 0.29 mmol) and NaSCN (93 mg, 1.15 mmol) were resolved in 15 mL glacial acetic acid and stirred under 17 °C for 15 min, then Br₂ (46 mg 0.29 mmol) in 5 mL glacial acetic acid was added to the mixture dropwise. After reaction for 5 h, the mixture was dropped into 200 mL ice water, neutralized by ammonium hydroxide, then extracted with ethyl acetate. The combined organic phases were dried over anhydrous MgSO₄ and concentrated using a rotary evaporator. The residue was purified by column chromatography on silica (petroleum ether / ethyl acetate / CH₂Cl₂, 6/2/3) to afford 99 mg of product 2b as a yellow powder in 85% yield. m.p. 163.1-163.2 °C.

1H NMR (DMSO-d₆, 400 MHz, ppm): δ ppm = 7.47 (s, 2H), 7.20-6.94 (m, 17H), 6.83 (dd, J=4, 1H). 13C NMR (DMSO-d₆, 100 MHz, ppm): δ ppm =170.77, 167.31, 151.97, 143.99, 141.09, 140.38, 136.45, 131.15, 129.03, 128.22, 126.81, 123.37, 117.36.


**Synthesis of PPAB-1 and 3**

A mixture of compound 1 (365 mg, 0.67 mmol) and compound 2a (1.11 g, 5.36 mmol) was dissolved in 40 mL of toluene. This mixture was stirred for 10 min at 50 °C, then trimethylamine (2.24 g, 22.11 mmol), TiCl₄ (1.52 g, 8.04 mmol) was added. Upon compound 1 was consumed by TLC, BF₃·Et₂O (2.63 g, 18.56 mmol) was added into the mixture, the solution was refluxed for 30 min. The reaction mixture was poured into 200 mL of water and extracted with CH₂Cl₂. The crude product was purified by
column chromatography on silica (from petroleum ether: ethyl acetate = 20/1, v/v to CH$_2$Cl$_2$) to give crude PPAB-1 and 3, respectively. Pure PPAB-1 was obtained by recrystallization from CH$_2$Cl$_2$/methanol to give 64 mg dark red solid in 19% yield. Pure 3 was obtained by recrystallization from CH$_2$Cl$_2$/hexane to give 68 mg brown power in 26% yield.

**PPAB-1**: m.p. 227.6-227.7 °C. $^1$H NMR (CDCl$_3$, 400 MHz, ppm): $\delta$= 8.45 (d, $J$=4, 4H), 7.9 (d, $J$=4, 2H), 7.66 (d, $J$=4, 2H), 7.09 (d, $J$=4, 4H), 4.13 (t, $J$=8, 4H), 1.91-1.82 (m, 4H), 1.54-1.51 (m, 4H), 1.39-1.37 (m, 18H), 1.30-1.24 (m, 16H), 0.93-0.89 (t, $J$=8, 6H). $^{13}$C NMR (CDCl$_3$, 100 MHz, ppm): $\delta$= 170.19, 162.61, 154.84, 154.64, 149.10, 138.88, 133.50, 127.57, 125.70, 121.46, 118.60, 118.24, 117.49, 114.09, 68.26, 35.10, 31.87, 31.44, 29.40, 29.30, 29.25, 26.11, 22.72, 14.16. HRMS (ESI): m/z [M + Na]$^+$ calcd. for C$_{56}$H$_{66}$B$_2$F$_4$N$_6$O$_2$S$_2$Na: 1039.8998, found: 1039.4703. Elemental analysis calcd (%) for C$_{56}$H$_{66}$B$_2$F$_4$N$_6$O$_2$S$_2$ [M = 1036.91 g/mol]: C 66.14, H 6.54, N 8.26; found: C 66.34, H 6.37, N 8.46.

3: m.p. 259.1-260.2 °C. $^1$H NMR (CDCl$_3$, 400 MHz, ppm): $\delta$= 9.81 (s, 1H), 8.62 (d, $J$=8, 2H), 8.35 (d, $J$=8, 2H), 7.88 (d, $J$=4, 1H), 7.640 (d, $J$=4, 1H), 7.49 (dd, $J$=4, 1H), 7.01 (q, $J$=8, 4H), 1.85-1.70 (m ,4H), 1.51-1.41 (m, 4H), 1.38 (s, 4H), 1.36-1.22 (m, 16H), 0.91(t, $J$=4, 6H). $^{13}$C NMR (THF-$d_8$, 100 MHz, ppm): $\delta$= 203.39, 164.11, 162.56, 161.02, 153.18, 147.97, 132.78, 131.67, 126.78, 124.74, 122.26, 119.70, 118.37, 116.79, 115.07, 113.44, 97.16, 68.40, 67.70, 53.66, 34.67, 31.82, 30.77, 29.36, 25.95, 22.57, 13.44.
HRMS (ESI): m/z [M+Na]^+ calecd for C_{45}H_{55}BF_{2}N_{4}O_{3}SNa: 803.8058, found: 803.3963. Elemental analysis calecd (%) for C_{45}H_{55}BF_{2}N_{4}O_{3}S [M = 780.82 g/mol]: C 69.22, H 7.10, N 7.18; found: C 69.45, H 7.02, N 7.45.

Synthesis of PPAB-2

Compound PPAB-2 was synthesized under similar reaction conditions to PPAB-1 from 3 (100 mg, 0.13 mmol), 2b (316 mg, 0.78 mmol), trimethylamine (4346 mg, 4.29 mmol), TiCl_{4} (296 mg, 1.56 mmol) and BF_{3}Et_{2}O (5116 mg, 3.60 mmol). The reaction mixture was purified similarly to PPAB-1, to give PPAB-2 as a green solid in 15% yield. m.p. 160.1-160.3 °C.

^{1}H NMR (CDCl_{3}, 400 MHz, ppm): δ= 8.48-8.35 (m, 4H), 7.90 (d, J=4, 1H), 7.70-7.63 (m, 2H), 7.52 (dd, J=2, 1H), 7.30 (d, J=2, 1H), 7.20-6.95 (m, 20H), 4.11 (t, J=0.8, 4H), 1.91-1.80 (m, 4H), 1.54-1.45 (m, 5H), 1.37 (s, 9H), 1.36-1.22 (m, 5H), 0.89 (t, J=6, 6H). ^{13}C NMR (CDCl_{3}, 100 MHz, ppm): δ= 170.21, 162.67, 162.60, 154.89, 154.67, 149.16, 143.19, 143.12, 141.39, 139.40, 138.88, 133.48, 131.38, 131.24, 128.03, 127.90, 127.72, 127.17, 126.94, 126.82, 126.70, 125.71, 121.42, 118.58, 118.22, 117.52, 117.01, 114.07, 68.27, 35.09, 31.84, 31.42, 29.36, 29.26, 29.23, 26.08, 22.68, 14.11.

HRMS (ESI): m/z [M + Na] ^+ calecd for C_{72}H_{72}B_{2}F_{4}N_{6}O_{2}S_{2} Na: 1237.1198, found: 1237.5173. Elemental analysis calecd (%) for C_{72}H_{72}B_{2}F_{4}N_{6}O_{2}S_{2} [M = 1215.13 g/mol]: C 71.17, H 5.97, N 6.92; found: C 71.38, H 6.05, N 6.70.
Synthesis of PPAB-3

Compound PPAB-3 was synthesized under similar reaction conditions to PPAB-1 from 1 (200 mg, 0.45 mmol), 2b (725 mg, 1.79 mmol), trimethylamine (1.50 g, 14.78 mmol), TiCl₄ (1.02 g, 5.38 mmol) and BF₃·Et₂O (1.78 g, 12.54 mmol). The reaction mixture was purified similarly to PPAB-1, to give PPAB-3 as a green solid in 10% yield. m. p. 178.1-179.0°C.

¹H NMR (CDCl₃, 400 MHz, ppm): δ= 8.38 (d, J=8.8, 4H), 7.66 (d, J=8.4, 2H), 7.30 (d, J=16, 2H), 7.16-6.99 (m, 36H), 4.074 (t, J=6.8, 4H), 1.89-1.79 (m, 4H), 1.40-1.25 (m, 21H), 0.89 (t, J=6.4, 6H). ¹³C NMR (CDCl₃, 100 MHz, ppm): δ=170.21, 162.66, 154.68, 143.33, 143.18, 142.43, 141.43, 139.37, 133.45, 131.44, 131.24, 128.03, 127.91, 127.72, 126.95, 126.83, 124.41, 121.27, 117.01, 114.04, 68.26, 31.83, 29.35, 29.25, 29.20, 26.07, 22.68, 14.12.


3. Preparation of PPABs nanoparticles

The NPs formed by PPABs were prepared through nanoprecipitation in the presence of Pluronics 127 as the stabilizer. The preparation was carried out as follows, 50 μL of 10⁻³ M PPABs and 5 mg Pluronic F127 was completely dissolved in 1 mL of THF, then the mixture was quickly injected into the 5 mL deionized water under sonication for 15 min at room temperature. Then the mixture was evaporated to remove THF.
completely on a rotary evaporator at 40 °C. The resulting mixture was cooled to room temperature. The concentration of F127 in the analyzed systems is 0.2 mM, and the concentration of PPABs was 10⁻⁵M. When the nanoparticles formed, the dynamic light scattering (DLS) experiment has been carried out to determine the size distribution of PPABs NPs.
## Table S1 Spectroscopic data of PPAB-1~ PPAB-3

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\lambda_{ab}$/nm</th>
<th>$\varepsilon$ (M$^{-1}$cm$^{-1}$)</th>
<th>$\lambda_{em,sol}$/nm</th>
<th>$\varphi_{F,,sol}$/%$^a$</th>
<th>$\varphi_{F,,sol}$/%$^b$ $f_h=0$</th>
<th>$f_h=90%$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPAB-1</td>
<td>659</td>
<td>9.78*10$^4$</td>
<td>672</td>
<td>58.5</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>PPAB-2</td>
<td>668</td>
<td>1.28*10$^5$</td>
<td>683</td>
<td>0.8</td>
<td>0.7</td>
<td>21.9</td>
</tr>
<tr>
<td>PPAB-3</td>
<td>677</td>
<td>1.15*10$^5$</td>
<td>697</td>
<td>1.1</td>
<td>0.4</td>
<td>18.8</td>
</tr>
</tbody>
</table>

$^a$ $\varphi$ was measured using Hamamatsu Quantaurus-QY C11347 spectrometer, in THF.

$^b$ in CH$_2$Cl$_2$/hexane mixtures with $f_h=0$ and $f_h=90\%$. Solution concentration: 1*10$^{-5}$ M.
Table S2 Spectroscopic data of **PPAB-1~ PPAB-3** (10 μM) in different organic solvents.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solvent</th>
<th>$\lambda_{\text{max, abs}}$(nm)</th>
<th>$\lambda_{\text{max, em}}$(nm)</th>
<th>Stokes shift (nm)</th>
<th>$\varepsilon$ (M$^{-1}$ cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hexane</td>
<td>654</td>
<td>671</td>
<td>17</td>
<td>1.97*10$^5$</td>
</tr>
<tr>
<td></td>
<td>Toluene</td>
<td>665</td>
<td>680</td>
<td>15</td>
<td>1.82*10$^5$</td>
</tr>
<tr>
<td>PPAB-1</td>
<td>THF</td>
<td>660</td>
<td>676</td>
<td>16</td>
<td>1.94*10$^5$</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>661</td>
<td>680</td>
<td>19</td>
<td>2.07*10$^5$</td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td>665</td>
<td>682</td>
<td>17</td>
<td>0.78*10$^5$</td>
</tr>
<tr>
<td></td>
<td>Toluene</td>
<td>673</td>
<td>690</td>
<td>17</td>
<td>1.44*10$^5$</td>
</tr>
<tr>
<td></td>
<td>CH$_2$Cl$_2$</td>
<td>667</td>
<td>693</td>
<td>26</td>
<td>1.64*10$^5$</td>
</tr>
<tr>
<td>PPAB-2</td>
<td>THF</td>
<td>668</td>
<td>687</td>
<td>19</td>
<td>1.39*10$^5$</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>670</td>
<td>685</td>
<td>15</td>
<td>1.35*10$^5$</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>670</td>
<td>696</td>
<td>26</td>
<td>1.36*10$^5$</td>
</tr>
<tr>
<td></td>
<td>Toluene</td>
<td>682</td>
<td>702</td>
<td>20</td>
<td>1.52*10$^5$</td>
</tr>
<tr>
<td></td>
<td>CH$_2$Cl$_2$</td>
<td>677</td>
<td>699</td>
<td>22</td>
<td>0.58*10$^5$</td>
</tr>
<tr>
<td>PPAB-3</td>
<td>THF</td>
<td>677</td>
<td>695</td>
<td>18</td>
<td>1.51*10$^5$</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>671</td>
<td>690</td>
<td>19</td>
<td>0.69*10$^5$</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>680</td>
<td>705</td>
<td>10</td>
<td>1.49*10$^5$</td>
</tr>
<tr>
<td></td>
<td>$E_{\text{HOMO}}^a$</td>
<td>$E_{\text{LUMO}}^a$</td>
<td>$E_{\text{g,calc}}^a$</td>
<td>$E_{\text{OX}}^b$</td>
<td>$E_{\text{red}}^b$</td>
</tr>
<tr>
<td>--------</td>
<td>------------------</td>
<td>------------------</td>
<td>------------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>PPAB-1</td>
<td>-5.493</td>
<td>-3.445</td>
<td>2.049</td>
<td>1.020</td>
<td>-0.517</td>
</tr>
<tr>
<td>PPAB-2</td>
<td>-5.214</td>
<td>-3.227</td>
<td>1.987</td>
<td>0.990</td>
<td>-0.534</td>
</tr>
<tr>
<td>PPAB-3</td>
<td>-5.173</td>
<td>-3.233</td>
<td>1.940</td>
<td>1.016</td>
<td>-0.500</td>
</tr>
</tbody>
</table>

$^a$ Calculated by DFT/B3LYP/6-31G(d).  $^b$ Determined by cyclic voltammetry (conditions: Cyclic voltammograms of PPAB-1~3 using 0.1 M tetrabutylammonium hexafluorophosphate (TBAPF$_6$) as electrolyte in CH$_2$Cl$_2$ with platinum button working electrodes, a platinum wire counter electrode, and an SCE reference electrode at a scan rate of 100 mV s$^{-1}$). Potentials are given relative to the ferrocenium/ferrocene couple.
Fig. S1. $^1$H NMR spectrum of 2b in DMSO-$d_6$

Fig. S2. $^{13}$C NMR spectrum of 2b in DMSO-$d_6$
Fig. S3. HRMS spectrum of 2b

Fig. S4. $^1$H NMR spectrum of 3 in CDCl$_3$
Fig. S5. $^{13}$C NMR spectrum of 3 in THF-$d_8$
Fig. S6. HRMS spectrum of 3
Fig. S7. $^1$H NMR spectrum of PPAB-1 in CDCl$_3$

Fig. S8. $^{13}$C NMR spectrum of PPAB-1 in CDCl$_3$
Fig. S9. HRMS spectrum of PPAB-1
Fig. S10. $^1$H NMR spectrum of PPAB-2 in CDCl$_3$

Fig. S11. $^{13}$C NMR spectrum of PPAB-2 in CDCl$_3$
Fig. S12. HRMS spectrum of PPAB-2
Fig. S13. $^1$H NMR spectrum of PPAB-3 in CDCl$_3$

Fig. S14. $^{13}$C NMR spectrum of PPAB-3 in CDCl$_3$
Fig. S15. HRMS spectrum of PPAB-3
Fig. S16. (a) UV-vis and (b) emission spectra of **PPAB-1** (10 μM) in different solvents.

Fig. S17. (a) UV-vis and (b) emission spectra of **PPAB-2** (10 μM) in different solvents.

Fig. S18. (a) UV-vis and (b) emission spectra of **PPAB-3** (10 μM) in different solvents.
Fig. S19. Variation in the PL intensity of PPAB-2 and PPAB-3 in CH$_2$Cl$_2$/hexane mixtures with different hexane fractions.

Fig. S20. NIR absorbance ratio A/A$_0$ of ICG solution and PPAB-1~ PPAB-3 solution as a function of irradiation time. A$_0$ is the initial absorption maximum and A the absorption maximum of the sample at different times after illumination.
Fig. S21. Optimized molecular conformation and molecular orbital amplitude plots of HOMO and LUMO energy levels of PPAB-1~ PPAB-3.

Fig. S22. Cyclic voltammograms of PPAB-1~ PPAB-3 using 0.1 M
tetrabutylammonium hexafluorophosphate (TBAPF$_6$) as electrolyte in CH$_2$Cl$_2$ with platinum button working electrodes, a platinum wire counter electrode, and an SCE reference electrode.

Fig. S23. (a) UV–vis and (b) emission spectra of PPAB-2 NPs (10 μM).

Fig. S24. Dynamic light scattering analysis of PPAB-2 NPs.

Fig. S25. (a) UV–vis and (b) emission spectra of PPAB-3 NPs (10 μM).
Fig. S26. Dynamic light scattering analysis of PPAB-3 NPs.

Fig. S27. Metabolic viability of HeLa cells after incubation with PPAB-2 NPs (1 μM, 10 μM) for 1, 2 and 4 days, respectively.
Fig. S28. Real-time fluorescence images showing PPAB-2 NPs (50 µM) stained HeLa cells at room temperature. “B”: cells nuclei stained by 4’,6-diamidino-2-phenylindole (DAPI), “R”: red fluorescence image of PPAB-2 NPs, “L”: bright field, respectively.

Fig. S29. Fluorescence images of HeLa cells incubated with PPAB-1 NPs (50 µM) for 48 h at room temperature. “B”: cells nuclei stained by 4’,6-diamidino-2-phenylindole (DAPI), “R”: red fluorescence image of PPAB-1 NPs, “L”: bright field, respectively.
References
