Supporting information

A membrane-permeable dye to living cells with large two-photon excited fluorescence action cross-sections for bioimaging

Ruiqing Feng, a Yuming Sun, b Minggang Tian, a Ge Zhang, a Ruoyao Zhang, a Lifang Guo, a Xuechen Li, a Xiaoqiang Yu*, a and Ning Zhao*, c

a Center of Bio & Micro/Nano Functional Materials, State Key Laboratory of Crystal Materials, Shandong University, Jinan 250100, P. R. China. Tel: +8653188366418; E-mail: yuxq@sdu.edu.cn

b School of Information Science and Engineering, Shandong University, P. R. China.

c Key Laboratory for Adhesion & Sealing Materials of Shandong Province, New Material Institute of Shandong Academy of Sciences, Jinan 250014, Shandong, P. R. China; E-mail: zhaon@sdas.org
Experiment details of synthesis:

**Scheme S1: The synthesis of BTVPA.**

*Synthesis of 4, 4'-diformyltriphenylamine (1):*

To a mixture of triphenylamine (5.0 g, 20.4 mmol) and dry dimethylformamide (35 mL) at 0 °C was dropwisely added phosphoryl chloride (19 mL, 203.8 mmol) under stirring. The reaction mixture was stirred at room temperature for 1 h and then mixture was warmed at 80 °C under nitrogen for 12 h. After being cooled to room temperature, the reaction mixture was poured into ice-water, neutralized with NaOH solution and then extracted with CH₂Cl₂. The combined organic phase was washed with water and saturated brine, dried over anhydrous magnesium sulfate overnight. After CH₂Cl₂ was removed, the crude product was purified by column chromatography with ethyl acetate/petroleum ether (1:8, v/v) as eluent, and finally the light-yellow solid was obtained with a yield of 55%. ¹H NMR (400 MHz, DMSO-d6): δ (ppm) 9.88 (s, 2H), 7.85 (d, \( J = 8.64 \) Hz, 4H), 7.49 (t, \( J = 7.84 \) Hz, 2H), 7.33 (t, \( J = 7.4 \) Hz, 1H), 7.22 (d, \( J = 8.4 \) Hz, 2H), 7.17 (d, \( J = 8.56 \) Hz, 4H).
Synthesis of (2E, 2'E)-3, 3'-(phenylazanediyl) bis (4, 1-phenylene) diacrylaldehyde (2):

Compound 1 (2.41 g, 8 mmol) and (1, 3-dioxolan-2-yl)methyl)-Triphenylphosphonium (8.24g, 19.2 mmol) were dissolved in 100 mL of chloroform, then added into a flask and bubbled with nitrogen for 30 min. A solution of potassium tert-butoxide (12.68g, 112 mmol) in chloroform (40 ml) was added into the system. The mixture was then bubbled with nitrogen for 30 min and then at room temperature for 24 h under the protection of nitrogen and a brownish yellow suspension was obtained. The mixture was then bubbled with nitrogen for 30 min and then at room temperature for 24 h under the protection of nitrogen and a brownish yellow suspension was obtained. The mixture was distilled to remove solvent, then poured into H$_2$O (500 mL) and extracted with CH$_2$Cl$_2$ after the resulting mixture was cooled to room temperature. The organic phase was separated, dried with MgSO$_4$ and removed by vacuum distillation. Yellow powder product was obtained after the residue was purified by column chromatography with ethyl acetate/petroleum ether (1:4, v/v) as eluent with a yield of 57%. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ (ppm): 9.64 (d, $J = 7.8$ Hz, 2H), 7.7 (t, $J = 9.08$ Hz, 6H), 7.4 (t, $J = 7.78$ Hz, 2H), 7.26 (t, $J = 7.36$ Hz, 1H), 7.17 (d, $J = 7.75$ Hz, 2H), 7.06 (d, $J = 8.56$ Hz, 4H), 6.77(dd, $J_1 = J_2 = 7.8$ Hz, 2H).

A mixture of 2 (0.71 g, 2 mmol), 2-aminothiophenol (0.63 g, 5 mmol) and p-toluensulfonic acid monohydrate (0.136g, 0.8 mmol) in 20 mL DMF was stirred at 120 °C under nitrogen atmosphere for 16 h. After cooling to room temperature, the mixture was poured into 200 mL water and filtered. The precipitate was washed with distilled water and dried in vacuo. Orange powder product was obtained after the residue was purified by column chromatography with ethyl acetate/petroleum ether (1:2, v/v) as eluent with a yield of 21%. ¹H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) (ppm) = 8.08 (d, \(J = 7.84\) Hz, 2H), 7.96 (d, \(J = 8\) Hz, 2H), 7.73 (d, \(J = 8.48\) Hz, 4H), 7.63 (d, \(J = 8.08\)Hz, 2H), 7.51 (t, \(J = 8.04\)Hz, 4H), 7.43(dd, \(J_1 =7.28\) Hz, \(J_2 =7.56\) Hz, 4H), 7.21(t, \(J = 7.48\) Hz, 1H), 7.16 (d, \(J = 7.72\) Hz, 2H).7.05 (d, \(J = 8.48\)Hz, 4H). ¹³C NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) (ppm) = 167.16, 153.99, 148.20, 146.48, 137.36, 134.40, 130.15, 129.58, 126.95, 126.13, 125.75, 125.24, 123.51, 122.83, 122.57, 120.65ppm; HRMS: m/z calcd for [C36H25N3S2 + H⁺]: 564.1568, found: 564.1462 (M + H⁺).
Fig. S3 $^1$H NMR spectrum of BTVPA in DMSO.

Fig. S4 $^{13}$C NMR spectrum of BTVPA in DMSO.

Fig. S5 HRMS spectra of BTVPA.
Fig. S6 IR spectra of BTVPA.

Spectrum spectrogram

Fig. S7 The absorption and fluorescence spectra of BTVPA in various solvents. 1: The absorption spectra. 2: Single-photon fluorescence spectra (excitation wavelength at the corresponding maximum absorption wavelengths). 3: Two-photon fluorescence spectra. Concentration of samples: \( 5 \times 10^{-6} \) mol/L.

**Table S1:** The photophysical properties of BTVPA

<table>
<thead>
<tr>
<th>( \lambda_1 ) (nm)</th>
<th>( \lambda_2^a ) (nm)</th>
<th>( \lambda_3 ) (nm)</th>
<th>( \varepsilon, \text{M}^{-1} \text{cm}^{-1} )</th>
<th>( \Phi_f )</th>
<th>( \delta ) (GM)</th>
<th>solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td>422</td>
<td>502</td>
<td>506</td>
<td>128130</td>
<td>0.88</td>
<td>1738(780nm)</td>
<td>EtOAc</td>
</tr>
<tr>
<td>435</td>
<td>546</td>
<td>559</td>
<td>93800</td>
<td>0.109</td>
<td>595(800nm)</td>
<td>DMSO</td>
</tr>
<tr>
<td>438</td>
<td>527</td>
<td>545</td>
<td>89400</td>
<td>0.146</td>
<td>761(800nm)</td>
<td>EtOH</td>
</tr>
<tr>
<td>438</td>
<td>550</td>
<td>553</td>
<td>61000</td>
<td>0.141</td>
<td>409(810nm)</td>
<td>PBS(^b)</td>
</tr>
</tbody>
</table>

\(^a\): Sample was excited at \( \lambda_{max} \); \(^b\): pH = 7.4.

\( \lambda_1 \): Linear absorption maximum peak. \( \lambda_2 \): Single-photon fluorescent maximum peak. \( \lambda_3 \): Two-photon fluorescent maximum peak. \( \varepsilon \): Molar absorptivity. \( \Phi_f \): Single-photon
fluorescence quantum yield. δ: Two-photon absorption cross-sections.

**Fig. S8** TPA cross-sections of BTVPA at different wavelength in EtOAc, DMSO, EtOH, and PBS buffer Solution. Concentration of samples: $1 \times 10^{-5}$ mol/L.

**Fig. S9** The absorption and fluorescence spectra of BTVPA in different pH values (pH buffer solution: Britton-Robinson buffer solution). Concentration of samples: $1 \times 10^{-5}$ mol/L.
**Fig. S10** The absorption and fluorescence spectra of BTVPA in different viscosity at room temperature (20 °C: EtOH: 1.2 cp, Glycerin: 1412 cp). Concentration of samples: $1 \times 10^{-5}$ mol/L.

**Fig. S11** The absorption spectra of BTVPA and PI in DMSO. Concentration of samples: $5 \times 10^{-6}$ mol/L for BTVPA and PI.