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Supporting information

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

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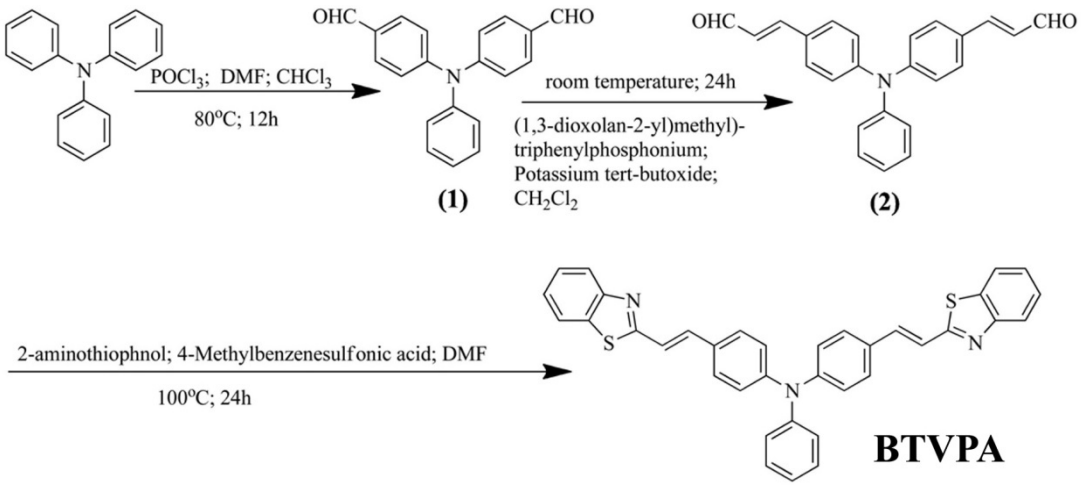
Experiment details of synthesis:



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 ℃ 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 ℃ 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 CH_2Cl_2. The combined organic phase was washed with water and saturated brine, dried over anhydrous magnesium sulfate overnight. After CH_2Cl_2 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%. ^{1}H 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).

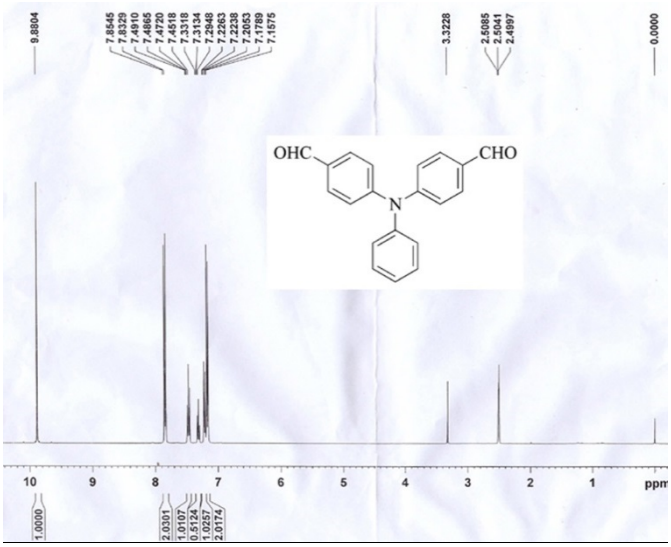


Fig. S1 ^{1}H NMR spectrum of (1) in DMSO.

***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 distilled to remove solvent, then poured into H_2O (500 mL) and extracted with CH_2Cl_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-*d6*): δ (ppm): 9.64 (d, J = 7.8 Hz, 2H), 7.7 (t, J = 9.08 Hz, 6H), 7.44 (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).

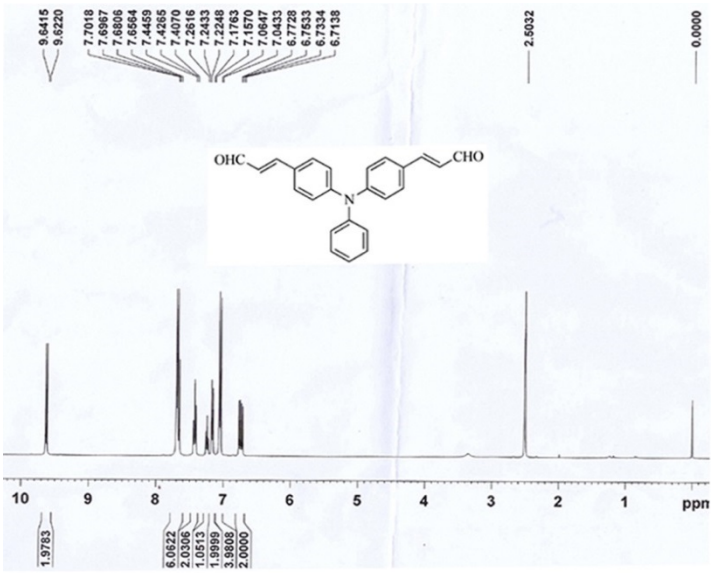


Fig. S2 ^{1}H NMR spectrum of (1) in DMSO.

Synthesis of 4-((E)-2-(benzo[d]thiazol-2-yl) vinyl)-N-(4-((E)-2-(benzo[d]thiazol-2-yl) vinyl) phenyl)-N-phenylaniline **(BTVPA):**

A mixture of **2** (0.71 g, 2 mmol), 2-aminothiophnol (0.63 g, 5 mmol) and p-toluenesulfonic acid monohydrate (0.136g, 0.8 mmol) in 20 mL DMF was stirred at 120 ℃ 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%. ^{1}H NMR (400 MHz, DMSO-*d6*): δ (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 = 8.08Hz, 2H), 7.51 (t, J = 8.04Hz, 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 = 8.48Hz, 4H). ^{13}C NMR (400 MHz, DMSO- d6): δ (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^+).

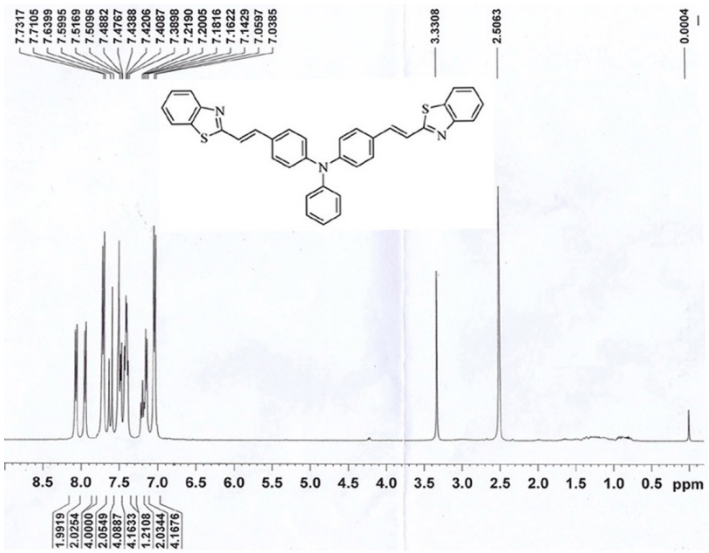


Fig. S3 ^{1}H NMR spectrum of BTVPA in DMSO.

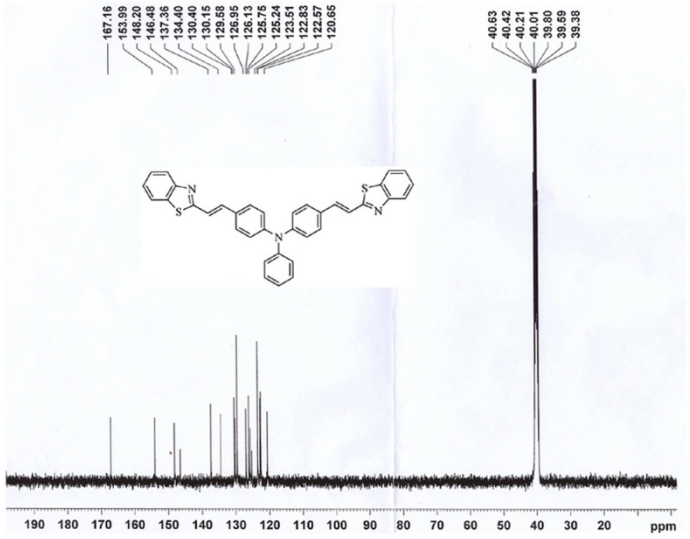


Fig. S4 ^{13}C NMR spectrum of BTVPA in DMSO.

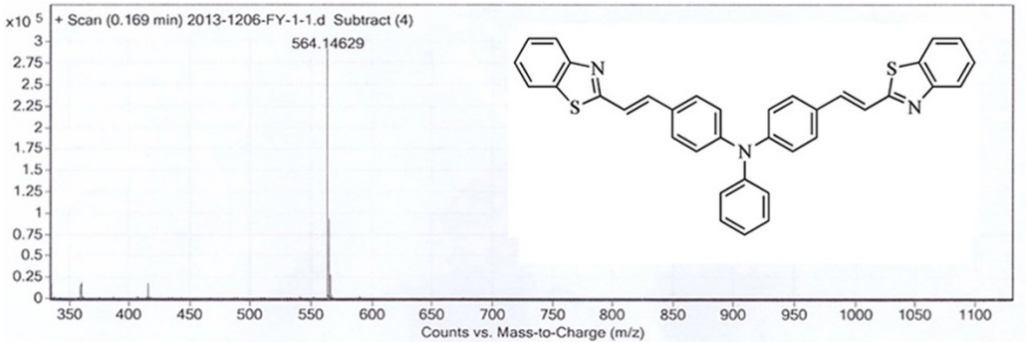


Fig. S5 HRMS spectra of BTVPA.

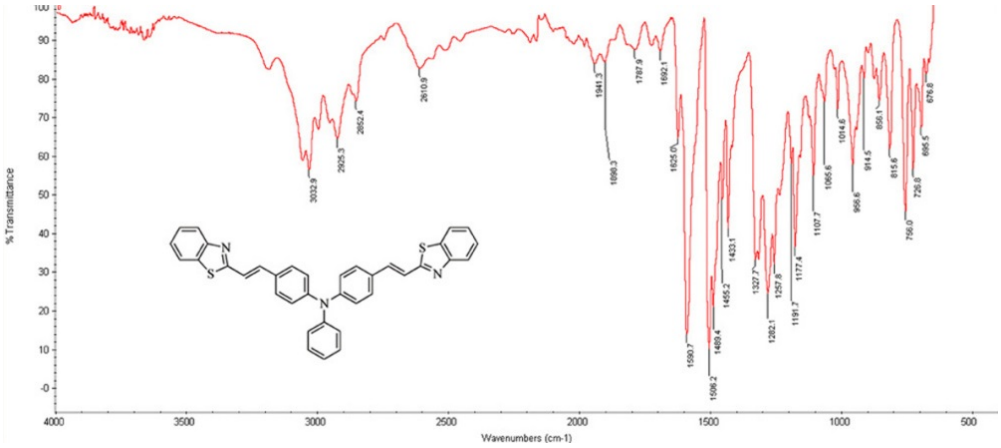


Fig. S6 IR spectra of BTVPA.

Spectrum spectrogram

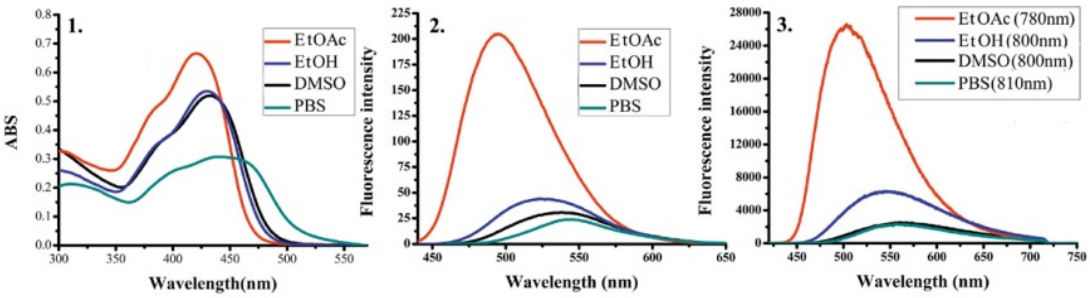


Fig. S7 The absorption and fluorescence spectra of BTVPA in various solvents. 1: The absorption spectra. 2: Single-photon fluorescence spectra (excitation wavelength at the corresponding maximum absorption wavelengths). 3: Two-photon fluorescence spectra. Concentration of samples: 5×10^{-6} mol/L.

Table S1: The photophysical properties of BTVPA

λ_1 (nm)	λ_2[a] (nm)	λ_3 (nm)	ε, (M^{-1} cm^{-1})	Φ_f	δ(GM)	solvents
422	502	506	128130	0.88	1738(780nm)	EtOAc
435	546	559	93800	0.109	595(800nm)	DMSO
438	527	545	89400	0.146	761(800nm)	EtOH
438	550	553	61000	0.141	409(810nm)	PBS[b]

[a]: Sample was excited at λ_{max}; [b]: pH = 7.4.

λ_1: Linear absorption maximum peak. λ_2: Single-photon fluorescent maximum peak. λ_3: Two-photon fluorescent maximum peak. ε: Molar absorptivity. Φ_f: Single-photon

fluorescence quantum yield. δ: Two-photon absorption cross-sections.

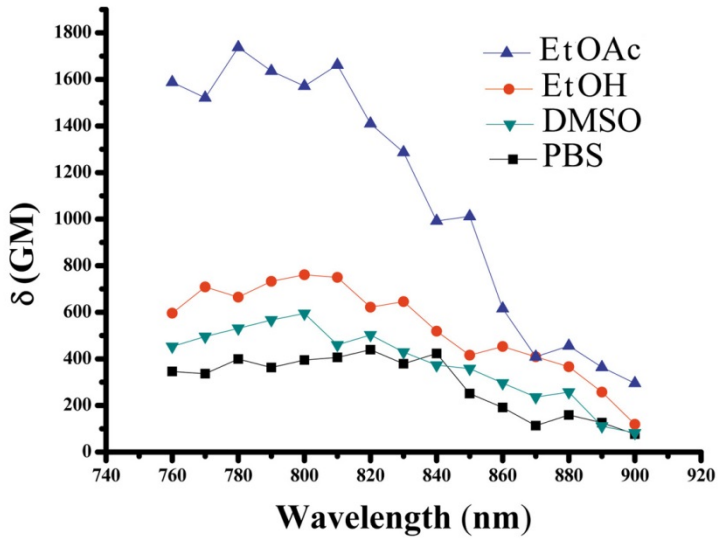


Fig. S8 TPA cross-sections of BTVPA at different wavelength in EtOAc, DMSO, EtOH, and PBS buffer Solution. Concentration of samples: 1×10^{-5} mol/L.

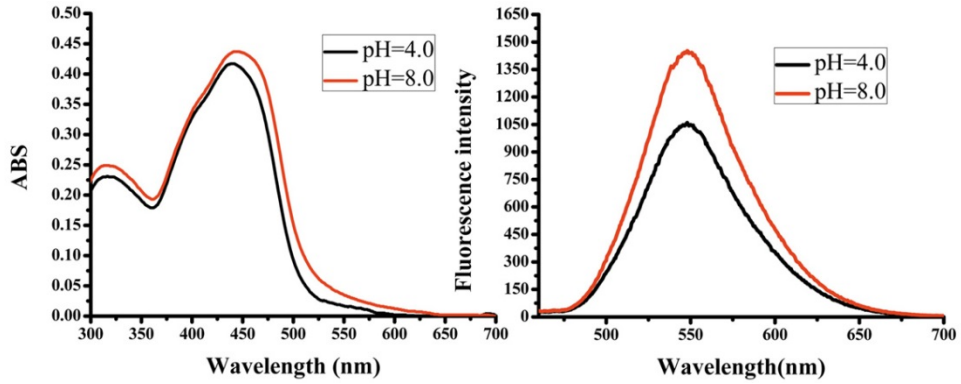


Fig. S9 The absorption and fluorescence spectra of BTVPA in different pH values (pH buffer solution: Britton-Robinson buffer solution). Concentration of samples: 1×10^{-5} mol/L.

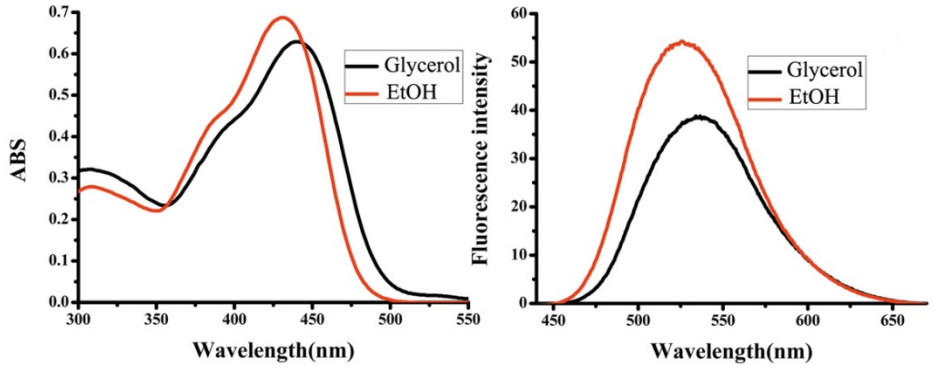


Fig. S10 The absorption and fluorescence spectra of BTVPA in different viscosity at room temperature (20 ℃: EtOH: 1.2 cp, Glycerin: 1412 cp). Concentration of samples: 1×10^{-5} mol/L.

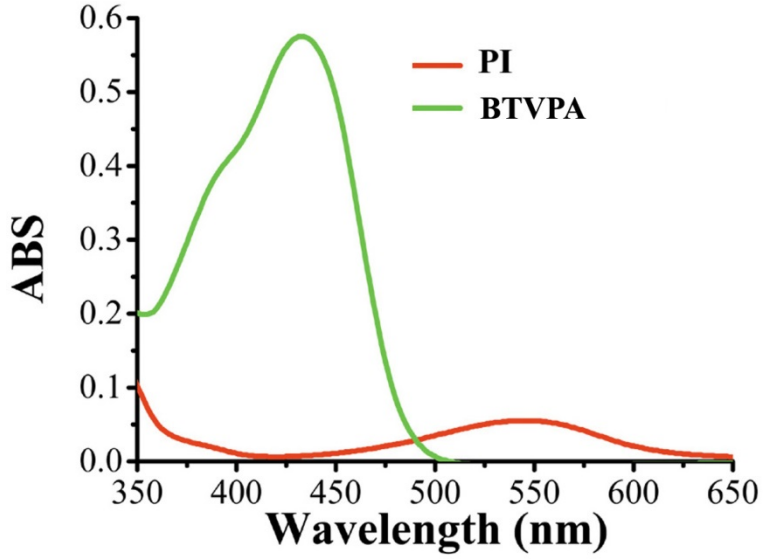


Fig. S11 The absorption spectra of BTVPA and PI in DMSO. Concentration of samples: 5×10^{-6} mol/L for BTVPA and PI.