Supplementary Information

A Dual-Boron-Cored Luminogen Capable for Sensing and Imaging

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1. Experimental Details

Materials and methods. All reagents were purchased from Sigma-Aldrich and Admas-beta. Cyclohexane, dichloromethane chloroform, acetonitrile, dioxane, dimethyl formamide and dimethyl sulfoxide were distilled from calcium hydride. Methanol was distilled from calcium oxide. Diethyl ether, tetrahydrofuran and toluene were distilled from sodium.

Nuclear Magnetic Resonance (NMR). ^{1}H and ^{13}C NMR spectra were recorded on a Mercury Plus 400 (400 MHz for proton, 100 MHz for carbon) spectrometer with tetramethylsilane as the internal reference using $CDCl_3$ or DMSO-d_6 as solvent in all cases. ^{11}B NMR spectra were recorded on a Bruker 400 MHz (128 MHz for boron) spectrometer with an internal standard of saturate boric acid aqueous solution (19.46 ppm) in DMSO-d_6 as solvent.

Mass spectrometry. Mass spectrometry was measured with an Ultra Performance Liquid Chromatography & Quadrupole-Time-of-Flight Mass Spectrometer.

Ultraviolet-Visible Spectrometry (UV-Vis). UV-vis spectra were recorded on a HITACHI U-4100 Spectrophotometer.

Photoluminescence Spectrometry (PL). Fluorescent spectra were obtained with a FluoroMax-4 spectrophotometer.

X-ray diffraction (XRD). XRD analysis was performed on a Bruker D8 Advance powder diffractometer.

Scanning Electron Microscope (SEM). SEM photographs were performed on a FEI Sirion-200 field emission scanning electron microscope.

Transmission Electron Microscope (TEM). TEM photographs were conducted on a JEOL-2100 electron microscope at an operating voltage of 200 kV.

Synthetic procedures

Synthesis of 4,7-dibromobenzo-[2,1,3]thiadiazole (2)[1]: To a 1000 mL three-necked round-bottomed flask containing benzothiadiazole **1** (30.1 g, 221.0 mmol) and HBr (300 mL, 48%), a solution of Br_2 (124.8 g, 780.9 mmol) in HBr (160 mL, 48%) was carefully added dropwise. After complete addition of Br_2, the drop funnel was washed by HBr (100 mL, 48%). Then the mixed solution was stirred at 110 °C

for 12 hours. Much precipitation of dark orange solid appeared during the reaction. The reaction mixture was cooled to room temperature and poured into a sufficient amount of a saturated solution of $NaHSO_3$ to completely consume the excess Br_2. The mixture was then filtered under vacuum and the solid was washed exhaustively with water and cold Et_2O for three times. The light yellow powder was finally dried under vacuum for 48 h to afford dibrominated product in 87% yield (57.5 g, 195.6 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (s, 2H).

Synthesis of 4,7-di(*1H*-pyrazol-1-yl)benzo-[2,1,3]thiadiazole (3)[2]: A mixture of 4,7-dibromobenzo-[2,1,3]thiadiazole **2** (1.5 g, 5.1 mmol), pyrazole (868.0 mg, 12.7 mmol), K_2CO_3 (3.6 g, 26.0 mmol), *N,N'*-dimethylethylenediamine (327.6 mg, 3.7 mmol), and 35 mL of p-xylenes were degassed by bubbling for half an hour. Under a nitrogen blanket, CuI (132.0 mg, 0.7mmol) was added. The mixture was then heated under nitrogen at reflux for 72 h. After cooling to room temperature, 100 mL of H_2O was added to facilitate the workup. The mixture was extracted with CH_2Cl_2 (300 mL, three times). Then all organic solutions were collected and dried over $MgSO_4$ and filtered. The solvent was removed by rotary evaporation to leave solid residues. The residues were recrystallized in CH_2Cl_2:hexanes (1:1). After the following column chromatography by using CH_2Cl_2:hexanes (1:1) as eluent, yellow powder was isolated in 72% yield (990.7 mg, 3.7 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.09 (dd, 1H, J = 2.6 Hz, J = 0.6 Hz), 8.34 (s, 1H), 7.84 (d, 1H, J = 1.7 Hz), 6.59 (dd, 1H, J = 2.5 Hz, J = 1.8 Hz).^{13}C NMR (100 MHz, $CDCl_3$): δ 147.9, 141.8, 131.5, 129.5, 119.6, 109.2. HRMS. ($C_{12}H_8N_6S$, ESI+): calculated for $[M+H]^+$ 269.0610, Found: 269.0609.

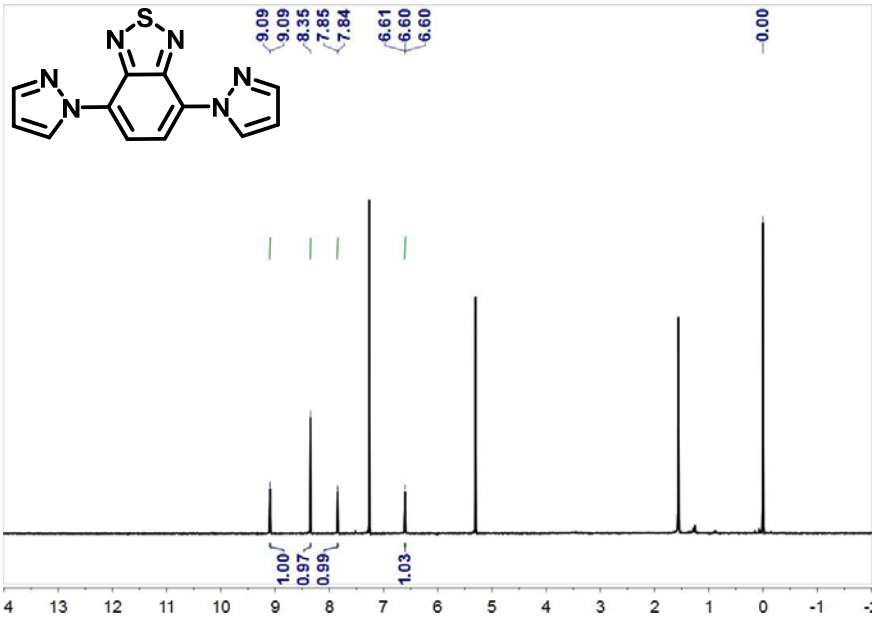


Fig. S1. ^{1}H NMR spectra of compound **3** (400 MHz, $CDCl_3$, ppm).

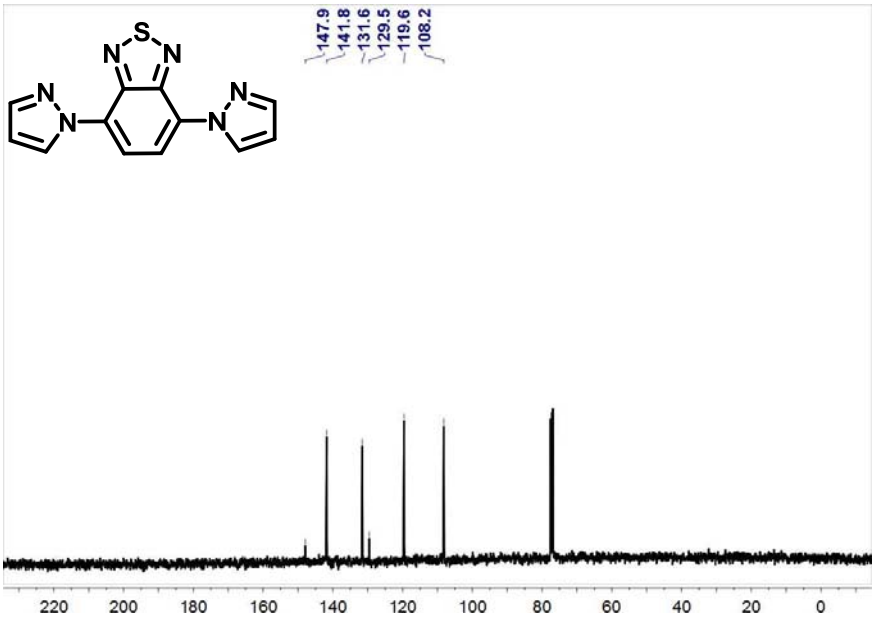


Fig. S2. ^{13}C NMR spectra of compound **3** (100 MHz, $CDCl_3$, ppm).

Synthesis of 3,6-di(*1H*-pyrazol-1-yl)-1,2-diaminobenzene (4)[3]: A mixture of 4,7-di(*1H*-pyrazol-1-yl)benzo-[2,1,3]thiadiazole **3** (510.9 mg, 1.9 mmol), $NaBH_4$ (1.1 g, 29.0 mmol), $Co(CH_3COO)_2{\cdot}4H_2O$ (3.7 mg, 0.01 mmol) and 45 mL of EtOH was refluxed for 4 h. After cooling to room temperature, the

mixture was filtrated to separate the black solid and washed by THF. Then the solvent was evaporated, water (60 mL) was added and the organic product was extracted with Et_2O (3×100 mL). The combined organic extracts were dried over $MgSO_4$ and the solvent was removed, grey powder was finally obtained without further purification in quantitative yield (460.0 mg, 1.9 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, 1H, J = 1.5 Hz), 7.75 (d, 1H, J = 2.3 Hz), 6.79 (s, 1H), 6.47 (d, 1H, J = 2.0 Hz), 4.55(s, 2H).^{13}C NMR (100 MHz, $CDCl_3$): δ 141.1, 133.2, 130.2, 127.2, 113.7, 107.0. HRMS. ($C_{12}H_{12}N_6$, ESI+): calculated for $[M+H]^+$ 241.1202, Found: 241.1196.

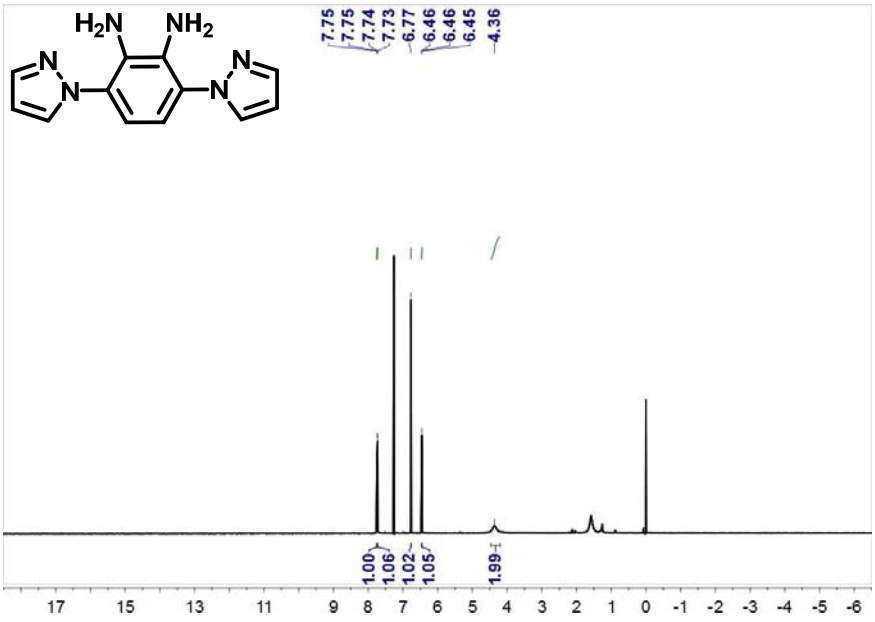


Fig. S3. ^{1}H NMR spectra of compound **4** (400 MHz, $CDCl_3$, ppm).

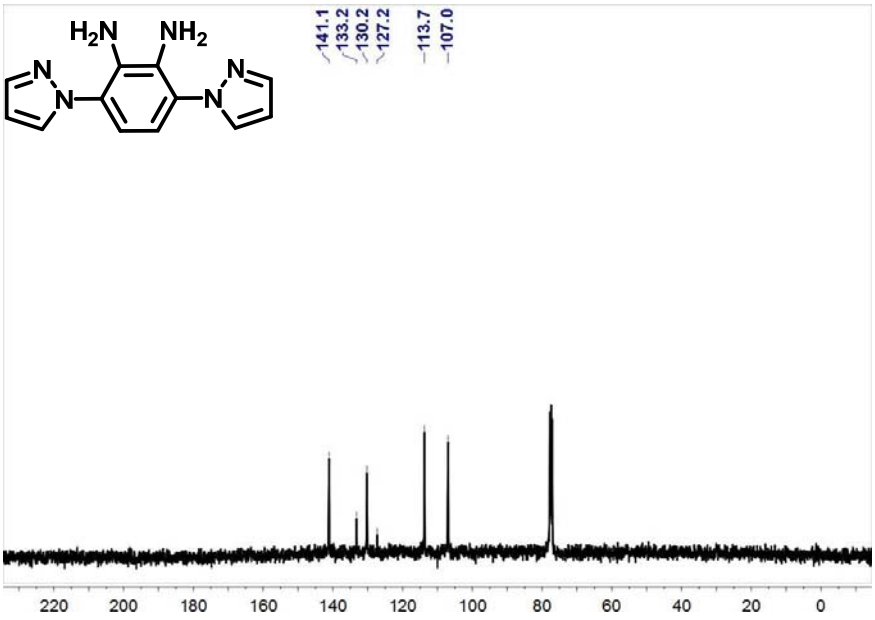


Fig. S4. ^{13}C NMR spectra of compound **4** (100MHz, $CDCl_3$, ppm).

Synthesis of compound 5[2]: A mixture of 3,6-di(*1H*-pyrazol-1-yl)-1,2-diaminobenzene **4** (460.0 mg, 1.9 mmol), BPh_3 (927.3 g, 3.8 mmol), and 45 mL of toluene was refluxed for 24 h. After cooling to room temperature, the mixture was centrifuged to collect the solid and washed by petroleum ether. After removal of the solvent by evaporation, final product was obtained as yellow powder without further purification in 88% yield (962.3 mg, 1.7 mmol). ^{1}H NMR (400 MHz, CD_2Cl_2, ppm): δ 7.91 (dd, 1H, J = 2.8 Hz, J = 0.9 Hz), 7.37 (dd, 1H, J = 2.5 Hz, J = 0.9 Hz), 7.13-7.18 (m, 5H), 7.07-7.12 (m, 5H), 3.97 (s, 1H).^{13}C NMR (100 MHz, CD_2Cl_2, ppm): δ 151.3, 142.6, 136.8, 136.6, 135.2, 133.1, 132.1, 129.1, 129.4, 128.4, 122.6, 110.9, 109.3, 105.7. ^{11}B NMR (126 MHz, DMSO-d_6, ppm): δ -7.9. HRMS. ($C_{36}H_{30}B_2N_6$, ESI+): calculated for $[M+H]^+$ 569.2797, Found: 569.2801.

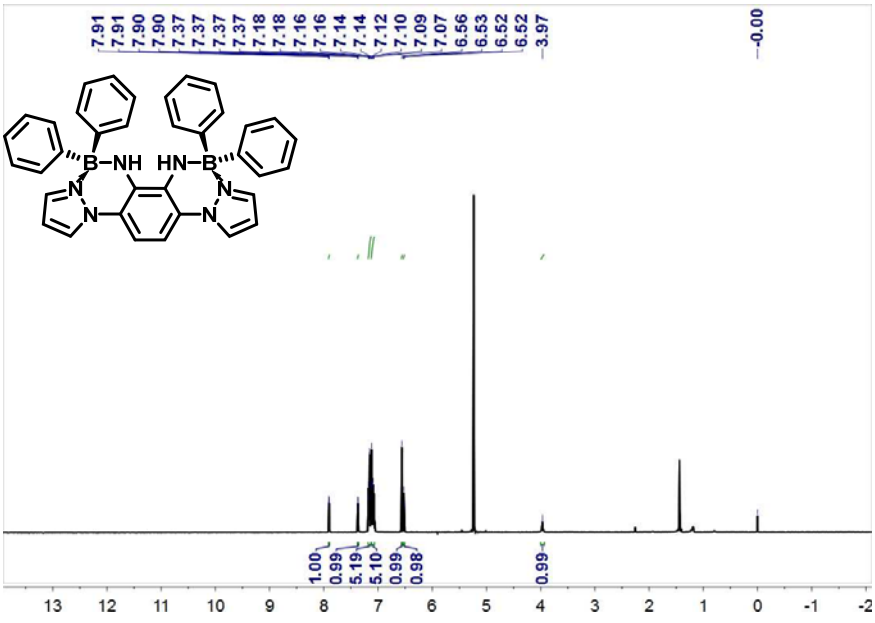


Fig. S5. ^{1}H NMR spectra of compound **5** (400MHz, CD_2Cl_2, ppm).

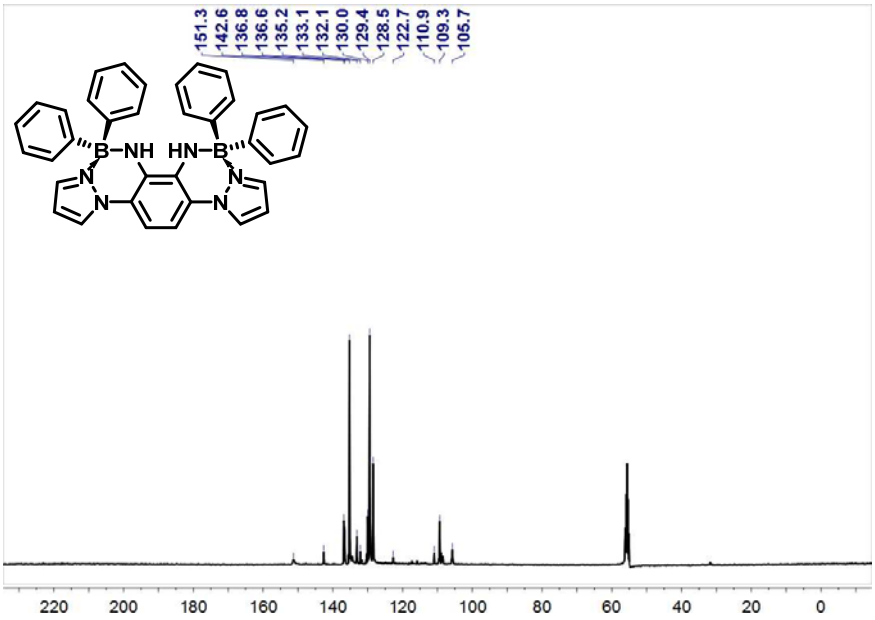


Fig. S6. ^{13}C NMR spectra of compound **5** (100MHz, CD_2Cl_2, ppm).

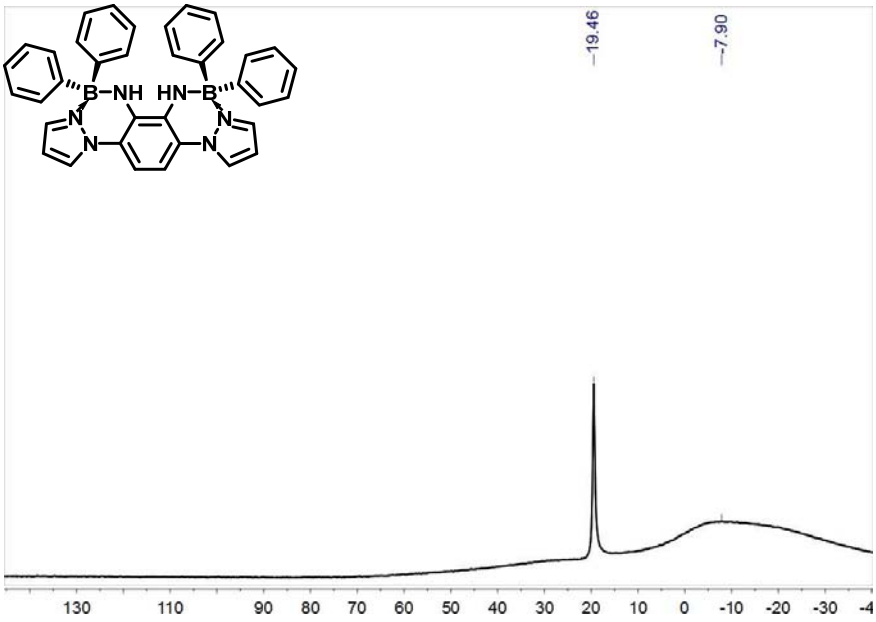


Fig. S7. ^{11}B NMR spectra of compound **5** (128 MHz, DMSO-*d*$_6$, ppm)

Aggregate of compound 5. Compound **5** (40 mg) dissolved in DMSO (4 mL) was stirred uniformly before use. Under gentle stirring, deionized water was added dropwise into DMSO solution. Then the DMSO was removed by dialyzing against deionized water for 24 h (MWCO = 2000), during which the water was renewed every 4 h. The final concentration of the aggregate in the resultant solution was diluted with deionized water. All procedures were performed at room temperature.

Fluorescent photobleaching. The fluorescent photostability of compound **5** in aqueous solution was measured continuously under a ZF-1 UV lamp with 30W power. With different exposed time, the fluorescent spectra were recorded on a FluoroMax-4 spectrophotometer.

Cell Cultures. HeLa cells (a human uterine cervix carcinoma cell line) and NIH/3T3 normal cells (a mouse embryonic fibroblast cell line) were cultured in DMEM supplied with 10% FBS, and antibiotics (50 units mL^{-1} penicillin and 50 units mL^{-1} streptomycin) at 37 °C under a humidified atmosphere containing 5% CO_2.

***In vitro* cytotoxicity measurements of compound 5.** The relative cytotoxicity of compound 5 against NIH/3T3 cells was estimated by MTT viability assay. In the MTT assay, NIH/3T3 cells were seeded into 96-well plates with a density of 1.0×10^4 cells per well in 200 μL of medium. After 24 h of incubation, the culture medium was removed and replaced with 200 μL of a medium containing serial dilutions of

hybrid nanoparticles. The cells were grown for another 48 h. Then, 20 μL of 2.5 mg/mL MTT assays stock solution in phosphate buffered solution (PBS) was added to each well. After incubating the cells for 4 h, the medium containing unreacted dye was removed carefully. The obtained blue formazan crystals were dissolved in 200 μL per well DMSO and the absorbance was measured in a BioTek Elx800 at a wavelength of 490 nm.

Cell internalization. HeLa cells (1.0×10^5 cells per well) were seeded on coverslips in a 6-well tissue culture plate and cultured for 24 h. Followed by removing culture medium, the nanoparticles of compound **5** dissolved in DMEM culture medium with a concentration of 9.2 μM was added. The cells were incubated at 37 °C for 15, 30, 60, 120, and 240 min, respectively. After being washed with PBS, the cells were fixed with 4% formaldehyde for 30 min at room temperature, and the slides were rinsed with PBS three times. Finally, the slides were mounted and observed with Leica DMI6000B inverted fluorescence microscope and Leica TCS SP5-II confocal laser scanning microscopy.

2. X-ray Crystallographic Analysis of Compound 5

Crystal data for $C_{39}H_{36}B_2N_6O$, a= 10.9203(9) Å, b = 12.3994(10) Å, c = 13.6076(12) Å, α = 94.079 (3)°, β = 99.826(3)°, γ = 110.183(3)°, V = 1687.11Å^3, Space group *P-1*, *R-Factor* = 0.0529.

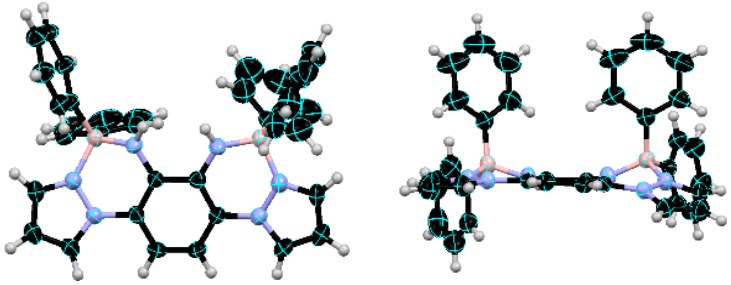


Fig. S8. Molecular structure of compound **5** (left: top view; right: side view, solvent molecules were omitted for clarity).

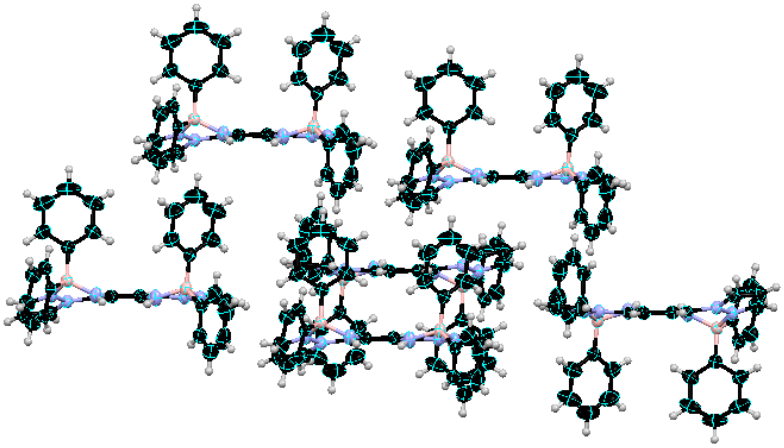


Fig. S9. Packing diagram of compound **5.**

3. Solvent-dependent UV-absorption and Fluorescence spectra

Table S1. Photophysical properties of **5** in different solvents.

Solvent	Cyclohexane	Et_2O	THF	CH_2Cl_2	$CHCl_3$	Toluene	MeCN	Dioxane	MeOH	DMF	DMSO
λ_{Abs}(nm)	408	413	413	394	397	404	402	404	314	415	418
λ_{em}(nm)	510	524	519	486	482	512	486	455	351	520	466

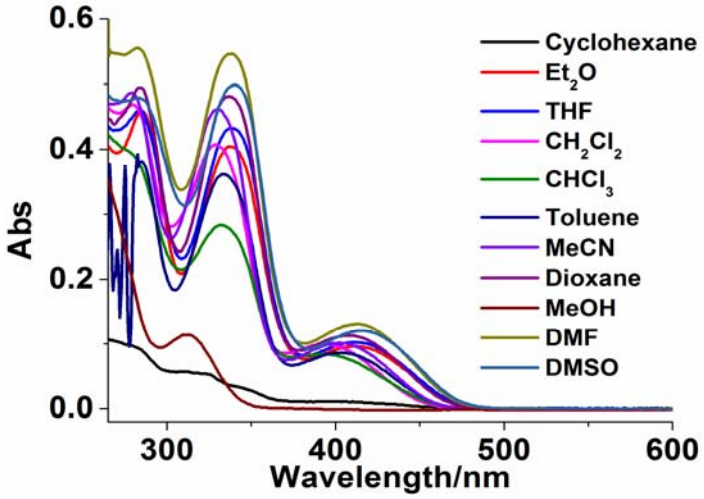


Fig. S10. UV-vis absorption spectra of **5** (5×10^{-5} M in different solvents).

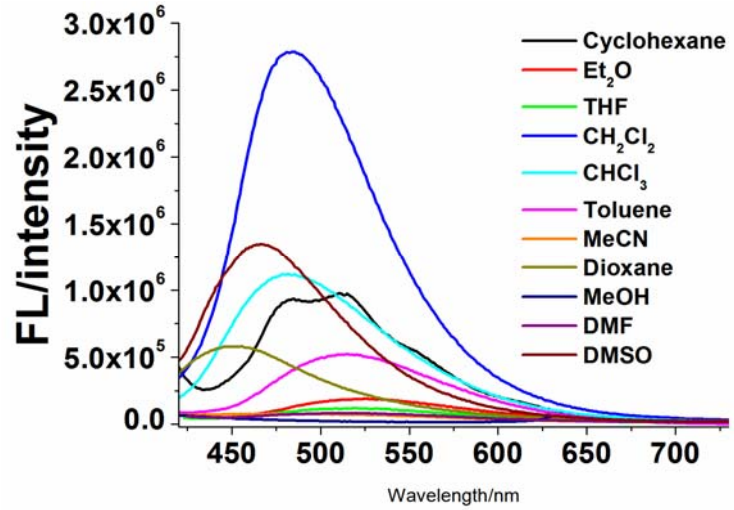


Fig. S11. Fluorescence spectra of **5** (5×10^{-5} M in different solvents).

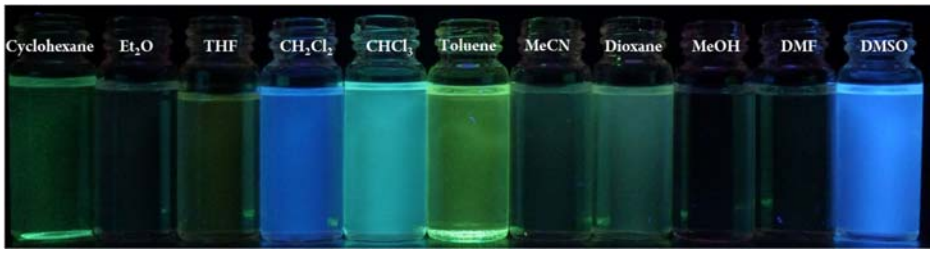


Fig. S12. Digital photos of **5** in different solvents under irradiation of UV lamp at 365 nm.

4. Molecular Orbitals and TD-DFT Calculation

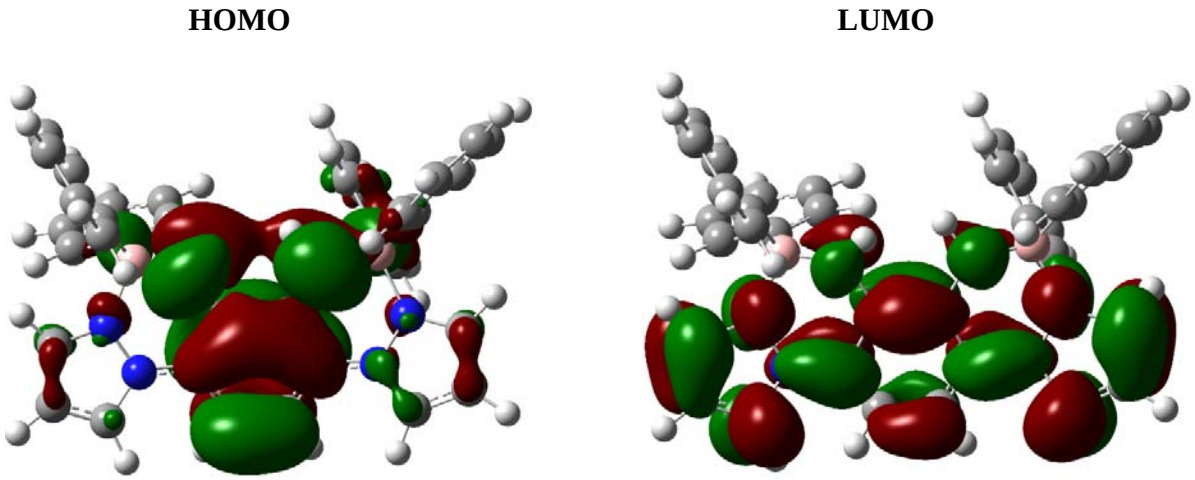


Fig. S13. Calculated molecular orbitals for **5**.

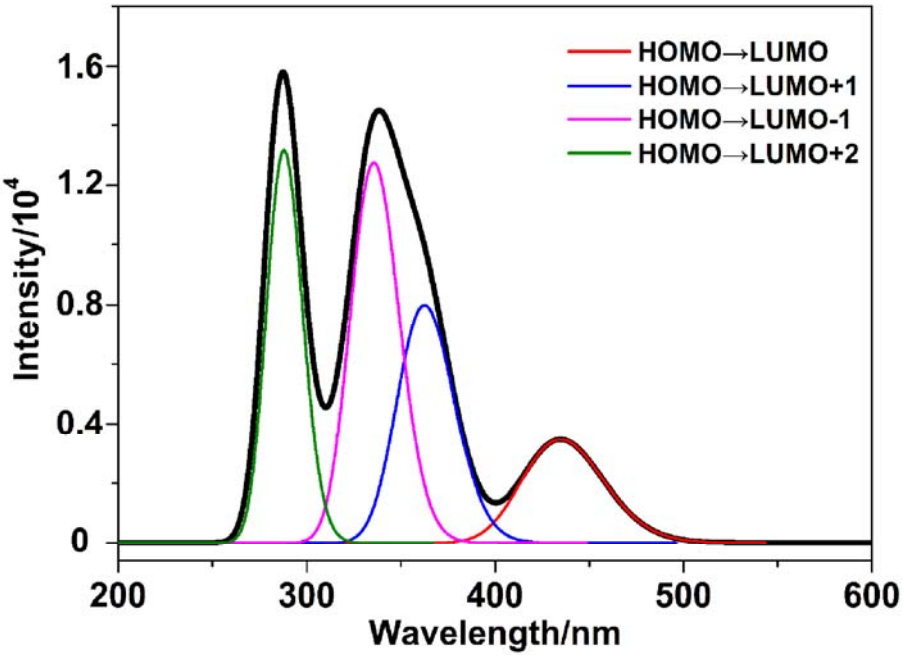


Fig. S14. Calculated UV-vis spectra and four main excited states for **5**.

5. Optical properties and morphologies of compound 5 in the aggregation state

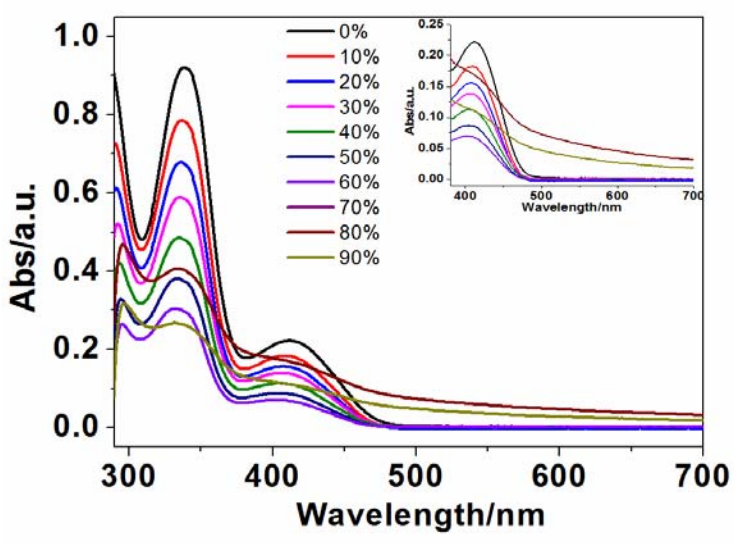


Fig. S15. The UV-vis spectra of compound **5** in THF-H_2O with different H_2O fractions (vol, 0-90%).

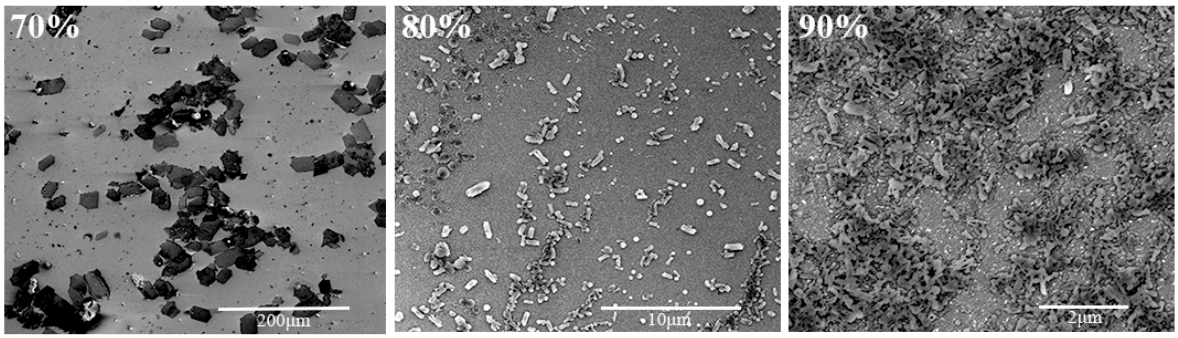


Fig. S16. SEM images of compound **5** in THF-H_2O with 70%, 80% and 90% of H_2O (v/v).

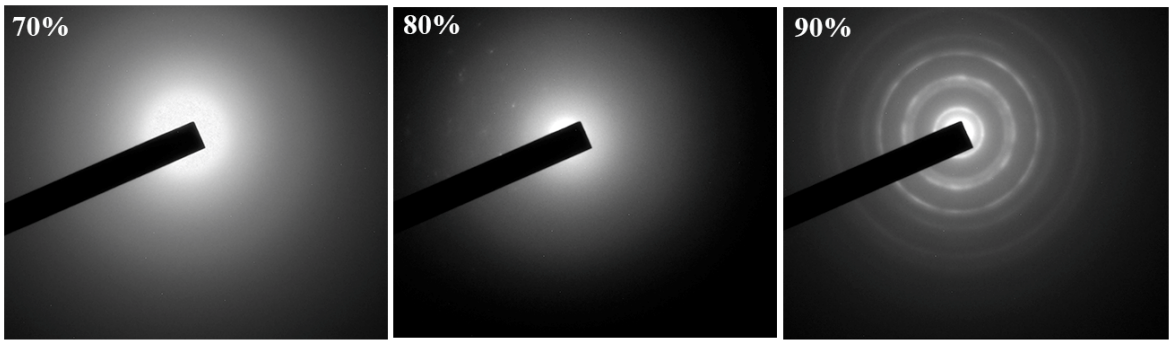


Fig. S17. ED patterns of compound **5** in THF-H_2O with 70%, 80% and 90% of H_2O (v/v).

6. Cell experiment

6.1 Fluorescent photostability of compound 5

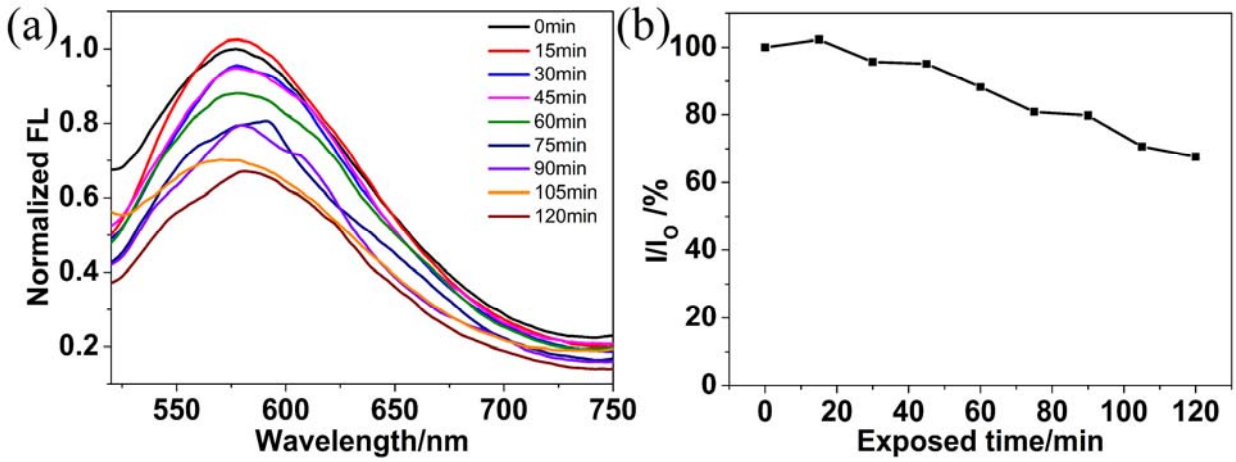


Fig. S18. (a) The photobleaching curves of compound **5** in water/THF under UV lamp with different time; (b) the maximum fluorescence intensity change under UV lamp with different time.

6.2 *In vitro* cytotoxicity of compound 5

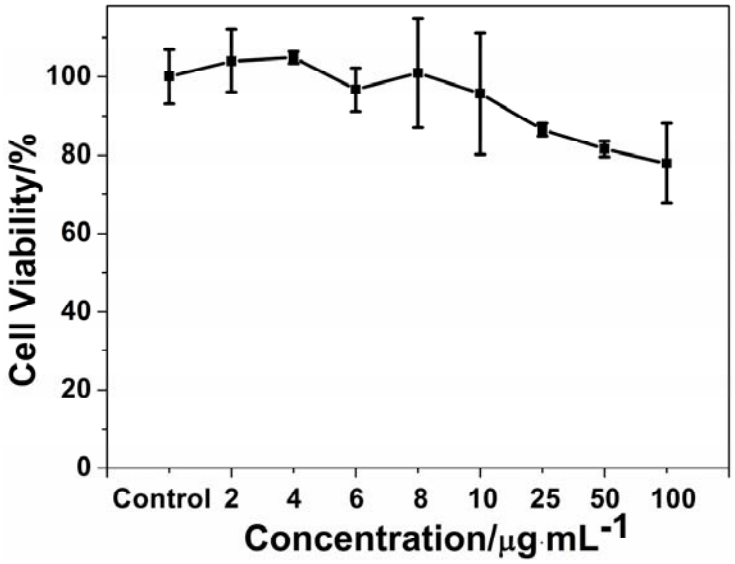
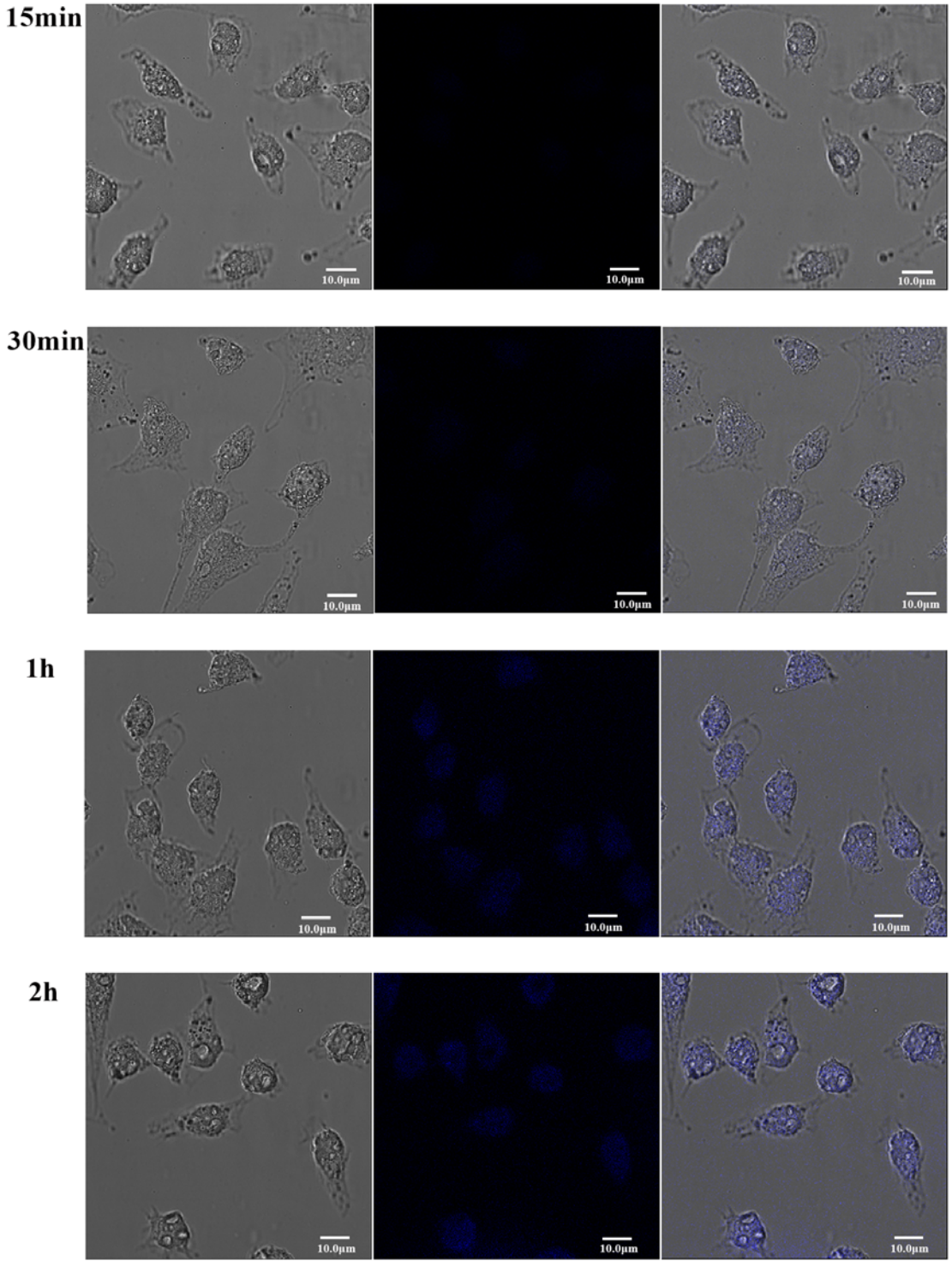


Fig. S19. Cell viability of NIH-3T3 against compound **5** after cultured for 24h with different concentrations.

6.3 Cellular internalization of compound 5 by flow cytometry measurement



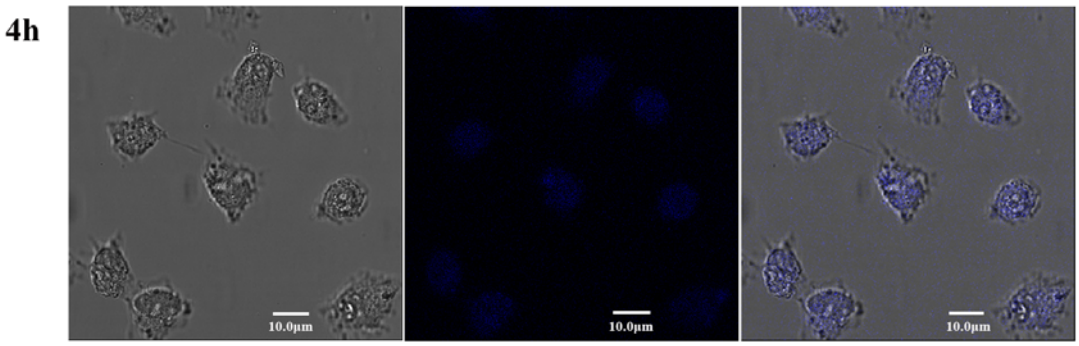


Fig. S20. Fluorescence microscope images of HeLa cells incubated with compound **5** for 15, 30, 60, 120, and 240 min, respectively, left: a bright field image; middle: a fluorescence image; right: a merged image.

7. Photographic Images and Optical Spectra for Sensor

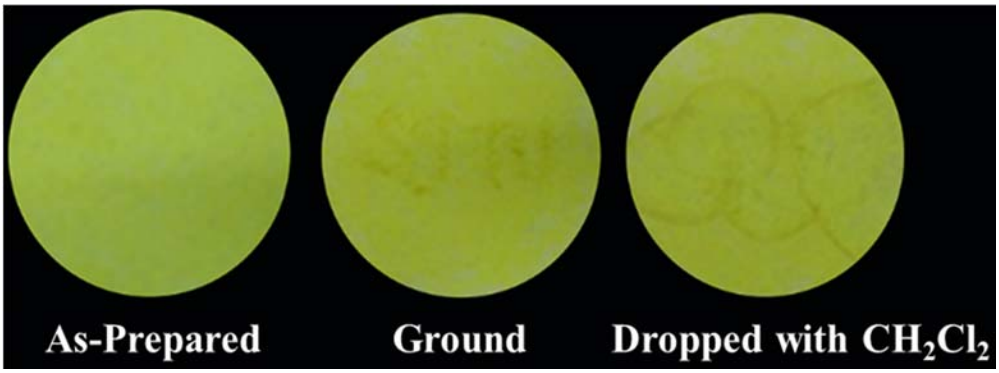


Fig. S21. Photographic images of compound **5** after mechanical grinding under ambient light.

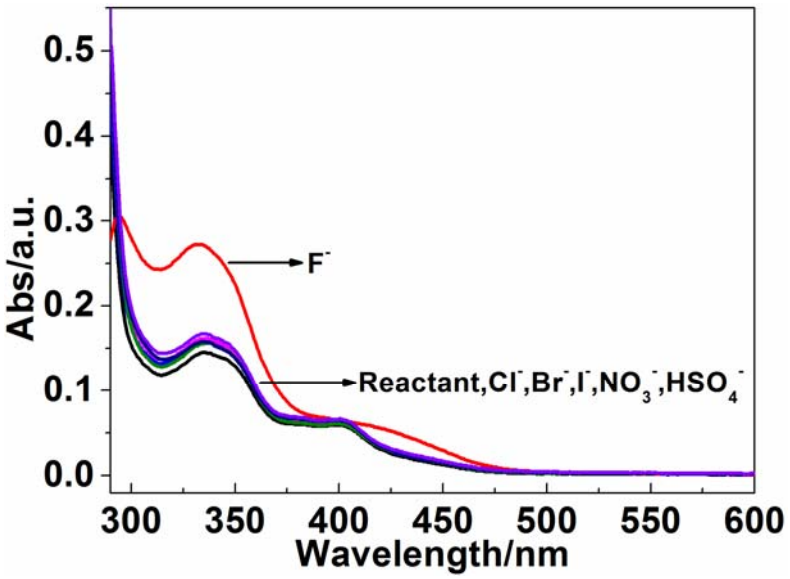


Fig. S22. Absorption spectra of **5** (5×10^{-5} M in DMSO) upon addition of 100 eq of different anions as the TBA salts.

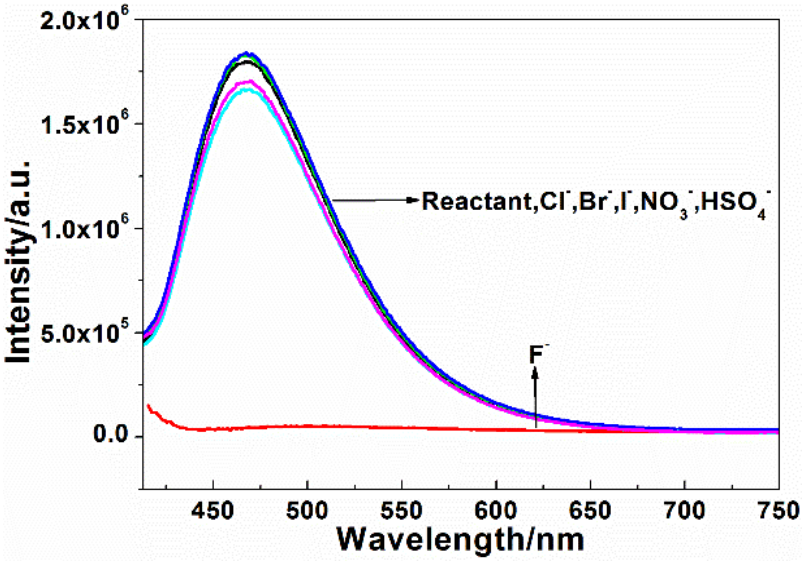


Fig. S23. Fluorescence spectra of **5** (5×10^{-5} M in DMSO) upon addition of 100 eq of different anions as the TBA salts (excited at 402 nm).

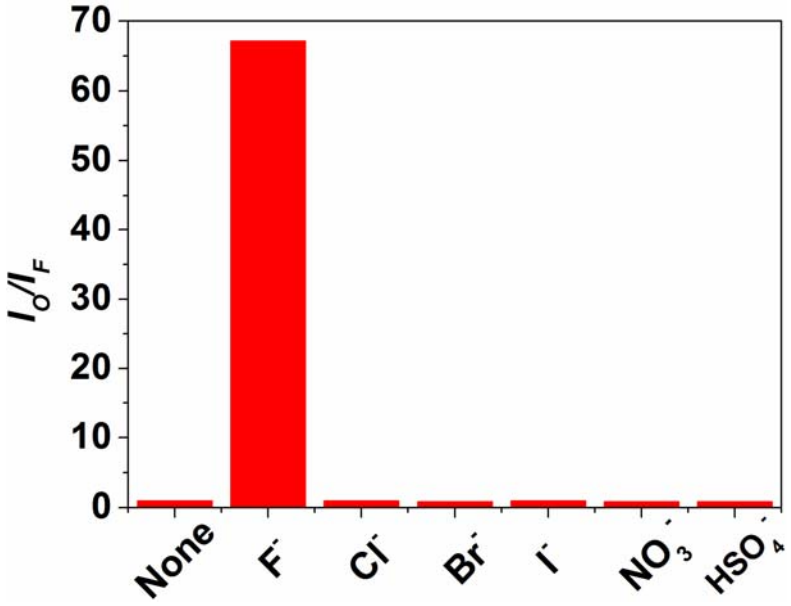


Fig. S24. Photoluminescence responses of compound **5** upon the addition of different anions (I_F and I_O represent the final and original fluorescence intensities, respectively).

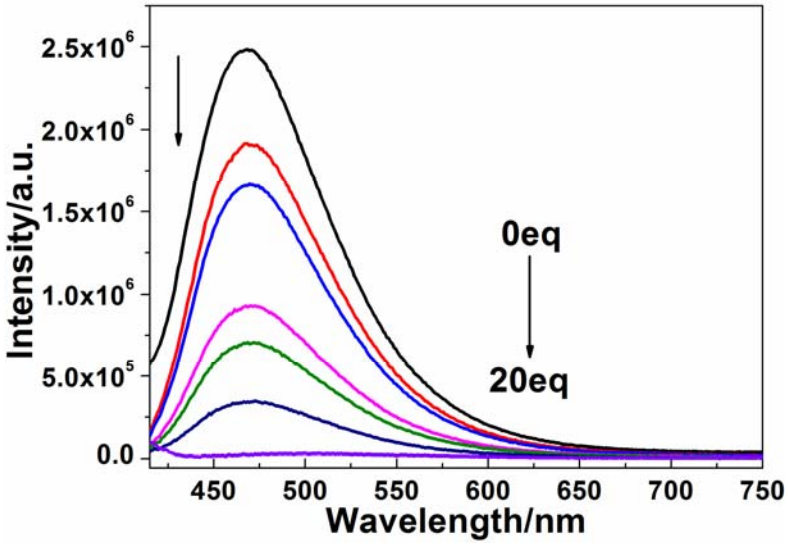


Fig. S25. The fluorescence spectra of **5** (5×10^{-5} M in DMSO) by titration with TBAF (from 0 to 20 eqv).

Detection Limit Calculation for This Method:

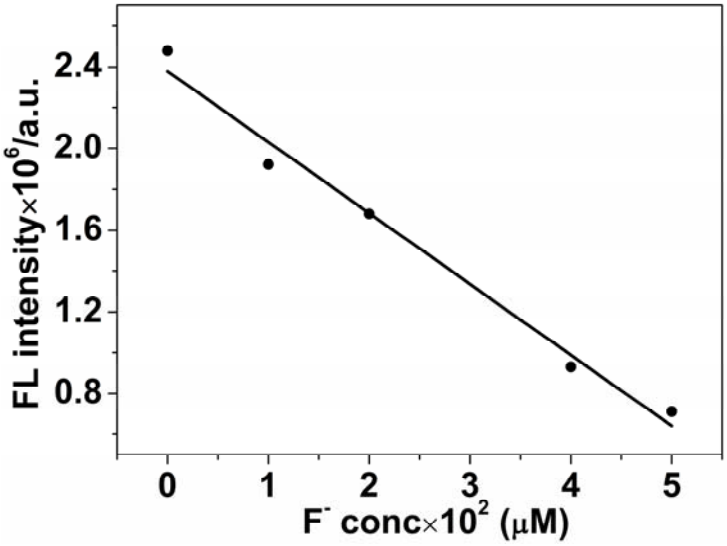


Fig. S26. Linear relationship between fluorescence intensity of 5 (50μM in DMSO) at 402 nm and the concentration of TBAF (0 mM–0.5 mM) in DMSO.

Through fluorometric titrations, the detection limit for F^- was determined. According to the definition, detection limit = $3S_{bi}/k$, where S_{bi} is standard deviation of 6 blank measurements and k is the slope obtained from the calibration curve.[4] In this method, the standard deviation S_{bi} of 1179.186 and the slope from the graph k of 3475.6 are calculated. Therefore, the detection limit = 1.02 μM (namely 19.3 ppb) (R = 0.993) can be obtained.

8. ^{11}B NMR spectra of compound 5 treated with TBAF

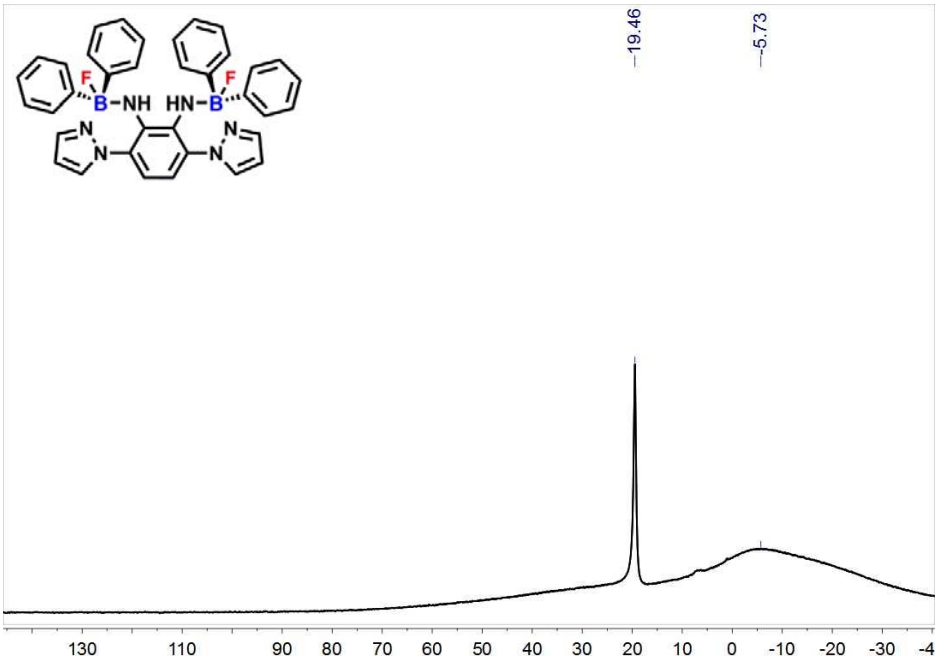


Fig. S27. ^{11}B NMR spectra of compound **5** with 20eq TBAF (128MHz, DMSO-d_6, ppm).

9. References

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4. (a) V. Thomsen, D. Schatzlein, and D. Mercuro, *Spectroscopy*, 2003, **18**, 112; (b) F. Zheng, F. Zeng, C. Yu, X. Hou and S. Wu, *Chem. Eur.J.*, 2013, **19**, 936; (c) A. Roy, D. Kand, T. Saha, P. Talukdar, *Chem. Commun.*, 2014, **50**, 5510.