BODIPY-based colorimetric/ratiometric fluorescence probes for sulfite in aqueous solution and in living cells

Shanshan Liu,[a]Lun Song,[a] Qian Sun,[a]Zhaoyang Chen,[a] Yu Ge,[b]Weibing Zhang[a] and Junhong Qian[*a]

[a]*Shanghai Key Laboratory of Functional Materials Chemistry, School of Chemistry and Molecular Engineering, East China University of Science and Technology, Shanghai 200237, China*

[b]*Shanghai Institute of Quality Inspection and Technical Shanghai, 200233, China.*

junhongqian@ecust.edu.cn

Supporting Information

Contents

Experiment

Materials and reagents

All the commercial reagents and solvents (Aladdin Corporation) were of analytical grade and used without further purification. Ultra-pure water was prepared through a Sartorius Arium 611DI system. Phosphate salts were used to keep a stable pH and ion strength in detection systems. Absorption spectra were measured with an Evolution 220 UV-Visible spectrophotometer (Thermo Scientific). Fluorescence spectra were conducted on a Lumina Fluorescence Spectrometer (Thermo Scientific). All pH measurements were performed with a model FE20 meter purchased from Mettler Toledo. NMR spectra were recorded using a Bruker AV-400 spectrometer (400MHz). Mass spectra were performed with a MA 1212 Instrument using standard condition (ESI, 70 eV).

Time titration of sulfite-probe systems

3 mM of the stock solutions of **BSP1** and **BSP2** in DMF were prepared ahead. The stock solution of **BSP1** or **BSP2** was diluted with PBS (20 mM, pH 7.4) with or without 1 mM CTAB to acquire 1×10^{-5} M dye aqueous solution. 50 μL of 30 mM freshly prepared Na_2SO_3 in PBS (20 mM, pH 7.4) were added to 3 mL of 1×10^{-5} M dye aqueous solution. Absorption and emission spectra of the above solution were collected at different intervals.

Sulfite titration

0~50 μL of 30 mM Na_2SO_3 in PBS were added into 3 mL of 1×10^{-5} M **BSP1** in CTAB-PBS or **BSP2** in PBS solutions. The spectra were recorded 2 h and 20 min after each addition of sulfite to **BSP1** and **BSP2** solutions, respectively.

HPLC traces

High-performance liquid chromatography (HPLC) spectra were carried out on an Agilent Technologies 1260 Infinity LC system. The mobile phases were degassed with an ultrasonic apparatus for 14 min. Mobile phase: A: water, B: acetonitrile; gradient elution: 2–8 min 10–95% B, 10-12 min 95–10% B; Isocratic elution: 0–2 min 10% B, 8-10 min 95% B, and 12-14 min 10% B. Injection volume: 10 μL; flow rate: 1.0 mL/min; detection wavelength: isosbestic point 512 nm.

Live cell culture and fluorescence imaging

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in an atmosphere of 5% CO_2 and 95% air at 37 °C. The cells were washed with phosphate buffered solution (PBS, 20 mM, pH 7.4) and pre-incubated with 500 μM Na_2SO_3, then incubated with **BSP1** (10 μM) and CTAB (1 mM) in DMEM for 120 min at 37 °C and washed 3 times with PBS. For the control experiment, the cells were only incubated with 10 μM of **BSP1** and CTAB (1 mM) for 120 min. Cell imaging was carried out after washing the cells with PBS. Emission was collected at 500-550 nm for the green channel and at 570–620 nm for the red channel. The excitation wavelength was set at 514 nm for green and red channels.

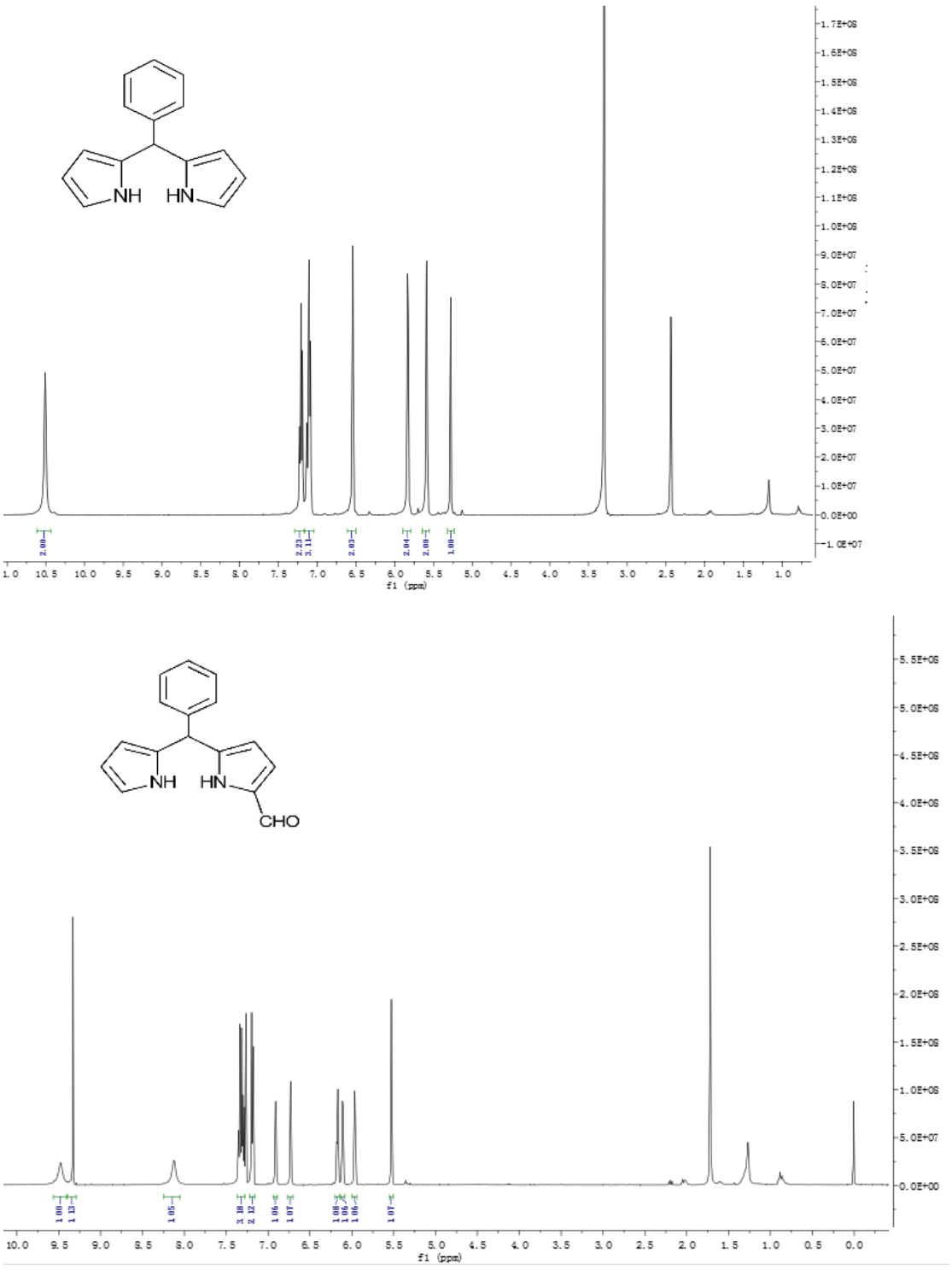
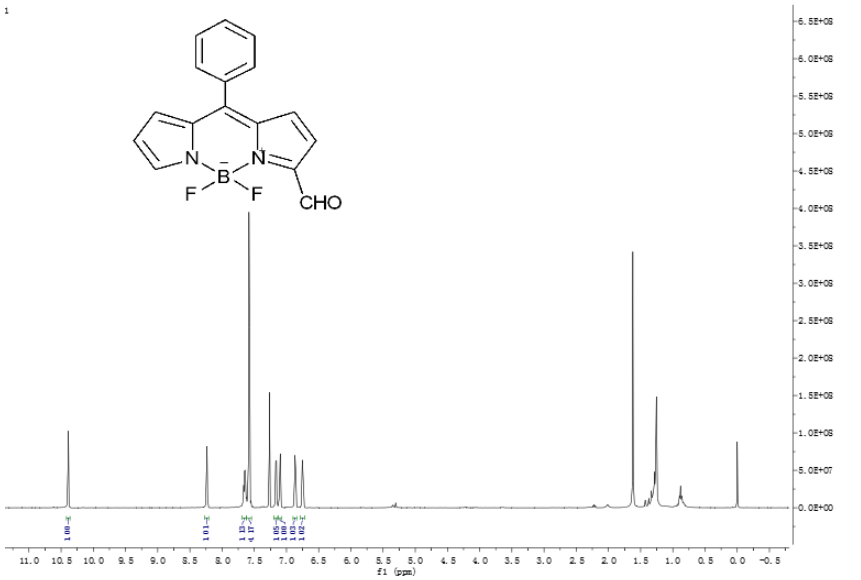
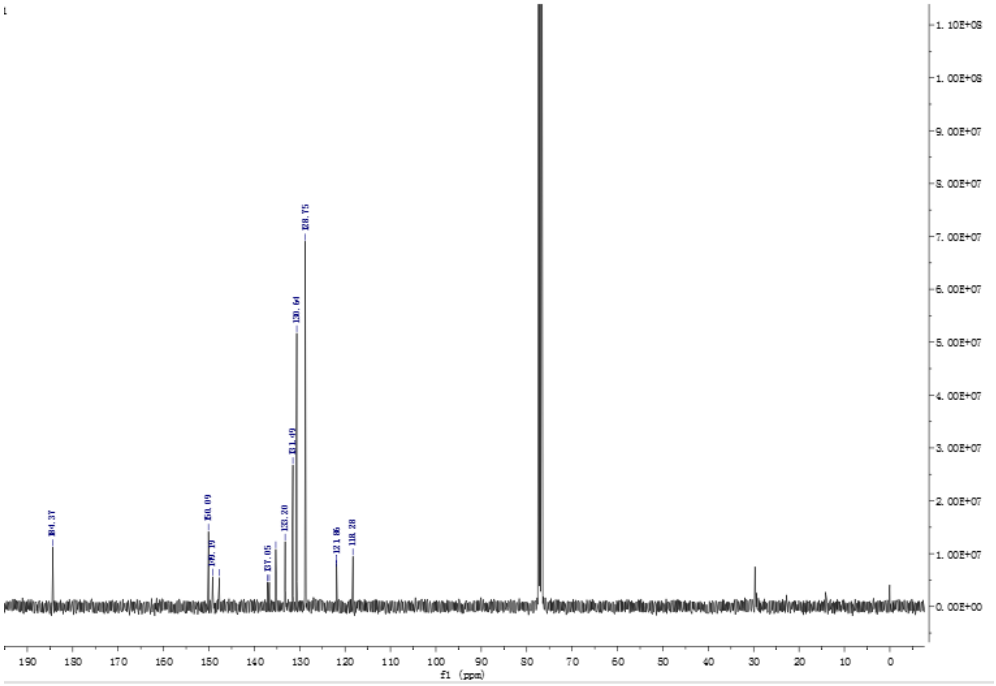


Fig. S1 ^{1}H-NMR spectra of **M1** and **M2**.


F
B
F
N
N
CHO
f1 (ppm)


194.37
150.09
149.79
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131.49
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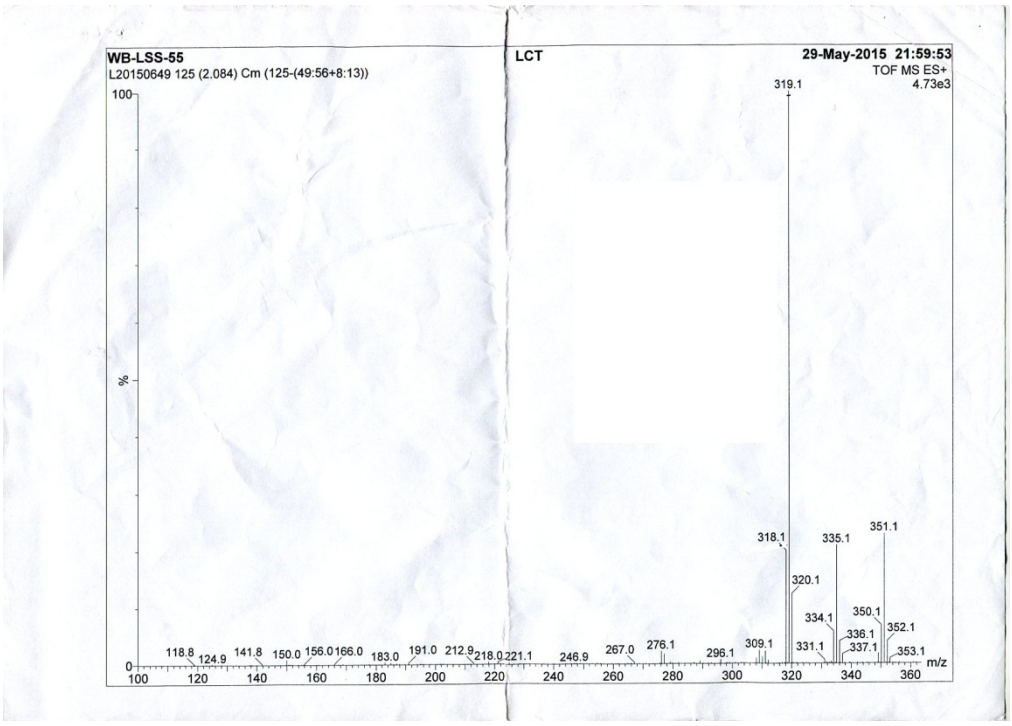
121.86
118.28
f1 (ppm)



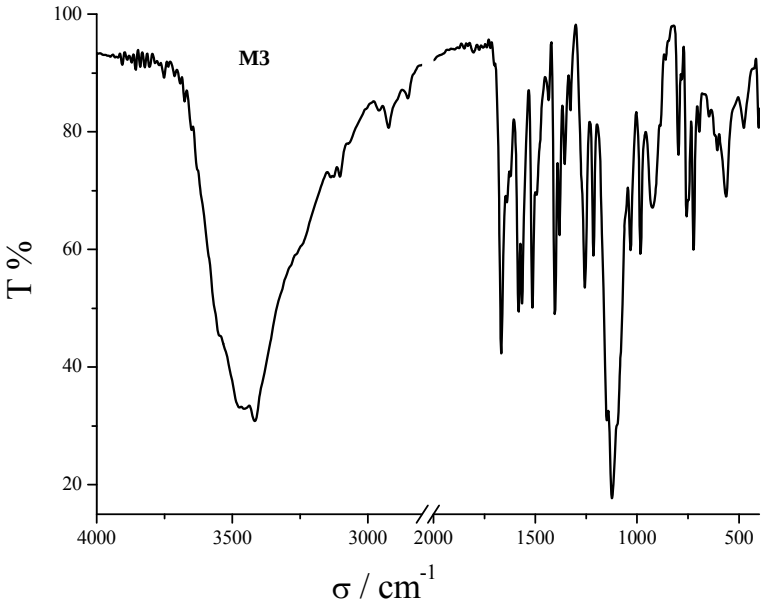
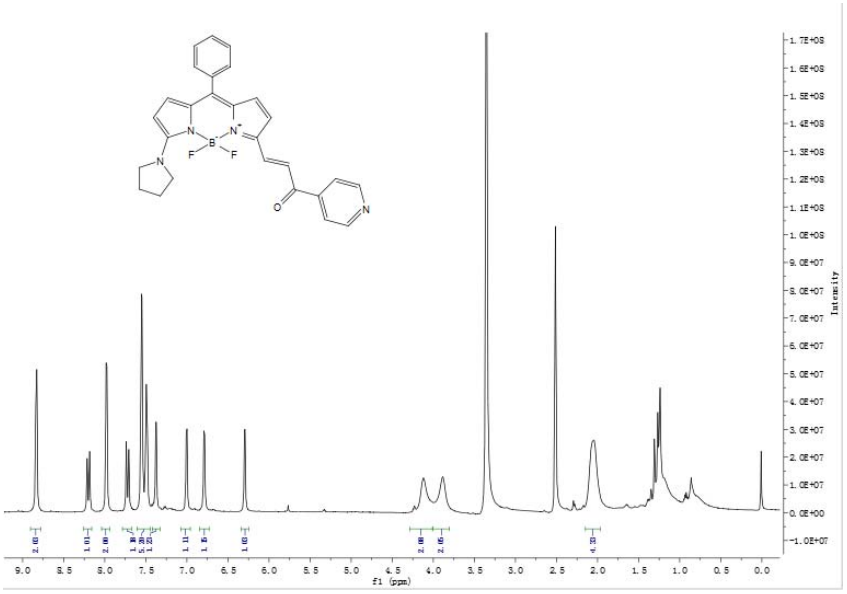


Fig. S2 ^{1}H-NMR, ^{13}C-NMR, MS and IR spectra of **M3**.


f1 (ppm)
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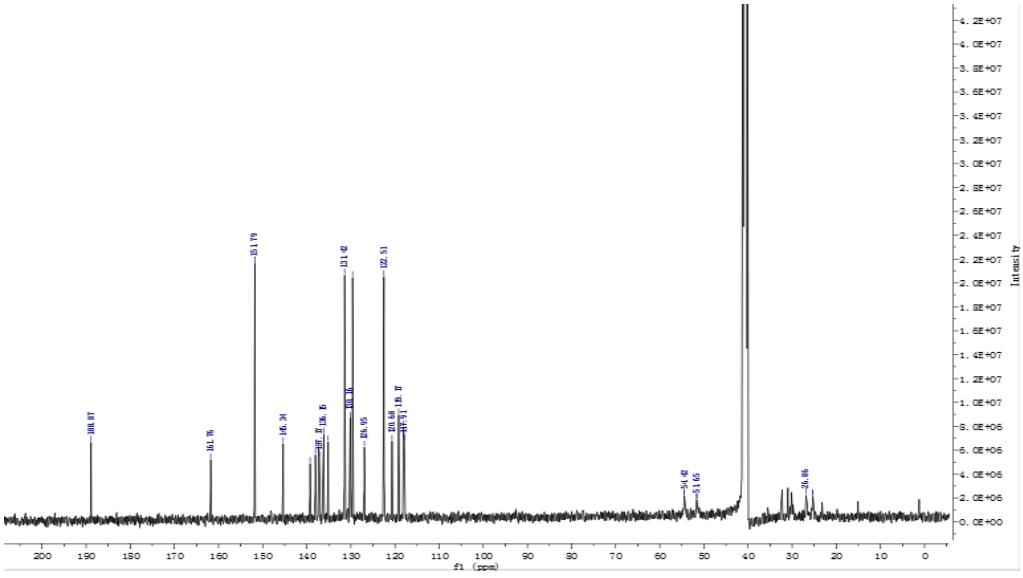
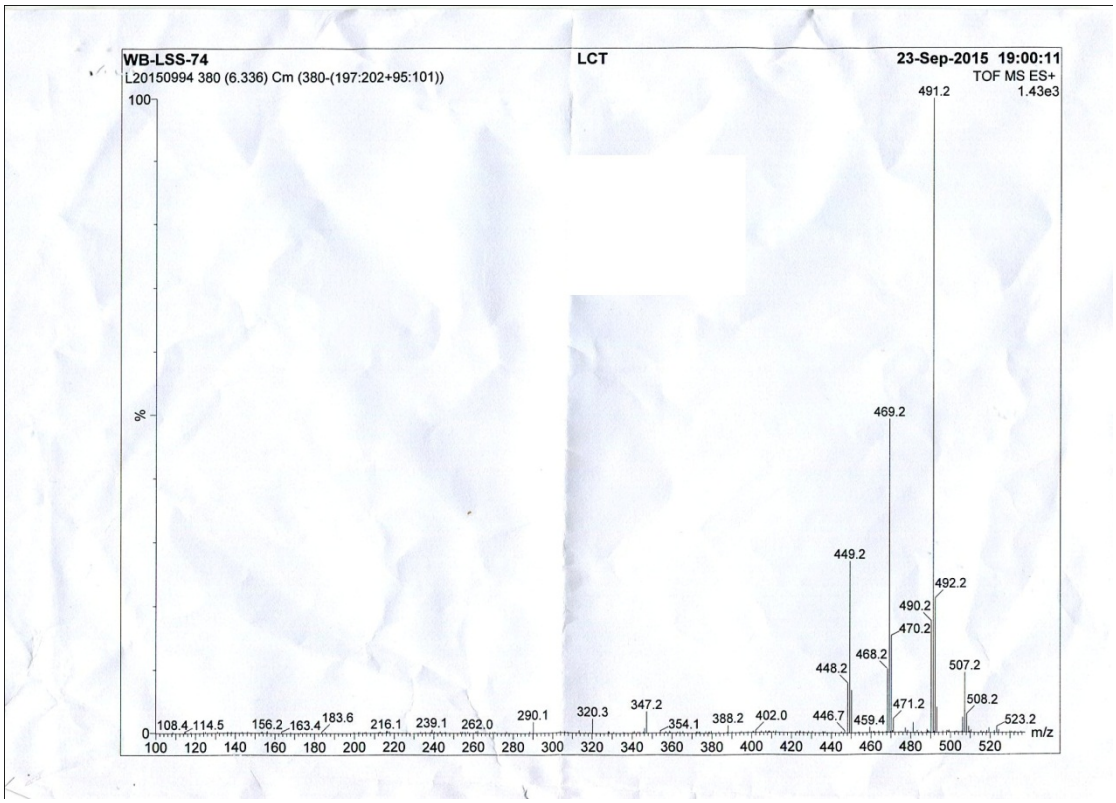

f1 (ppm)
Intensity



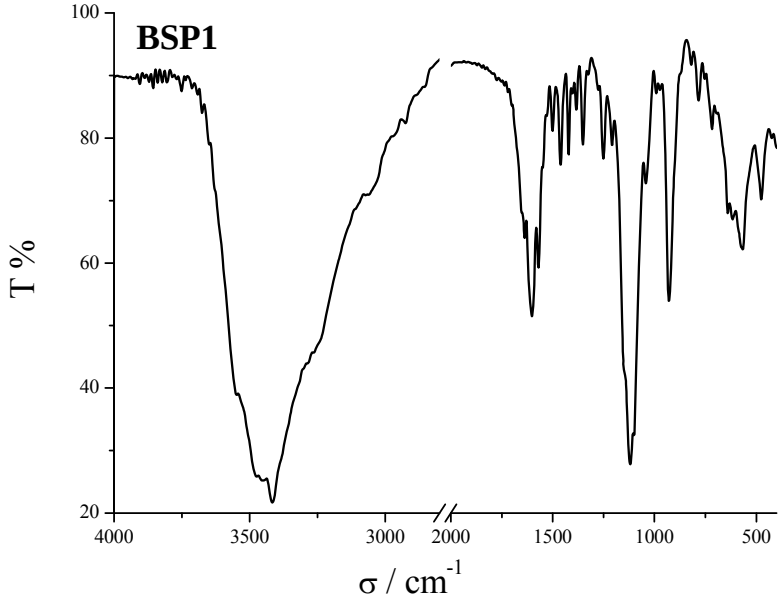
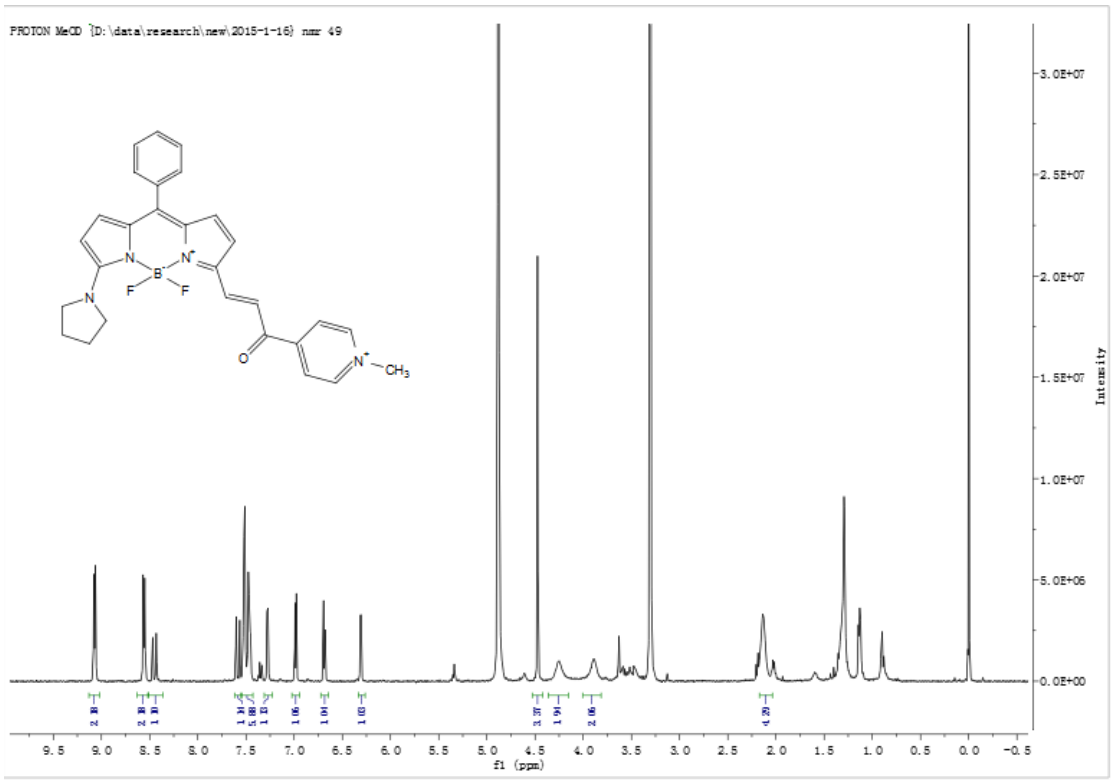
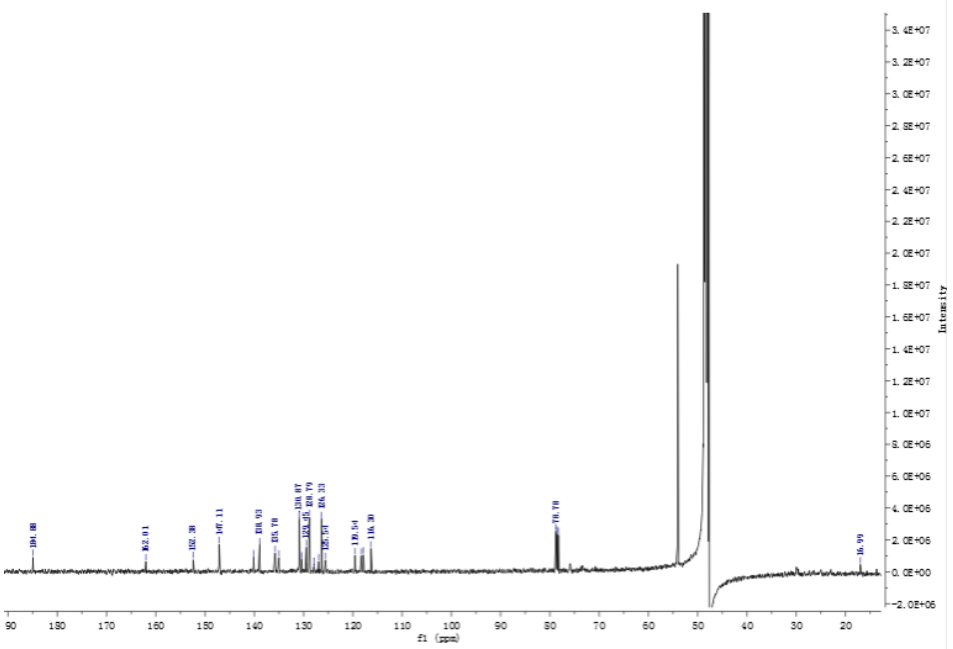


Fig. S3 ^{1}H-NMR,^{13}C-NMR, MS and IR spectra of **BSP1**.


PROTON MeOD [D:\data\research\new\2015-1-16] nmr 49
Intensity
f1 (ppm)


Intensity
f1 (ppm)

Monoisotopic Mass, Even Electron Ions
205 formula(e) evaluated with 15 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-35 H: 0-49 N: 0-4 O: 0-1 B: 0-1 F: 0-2

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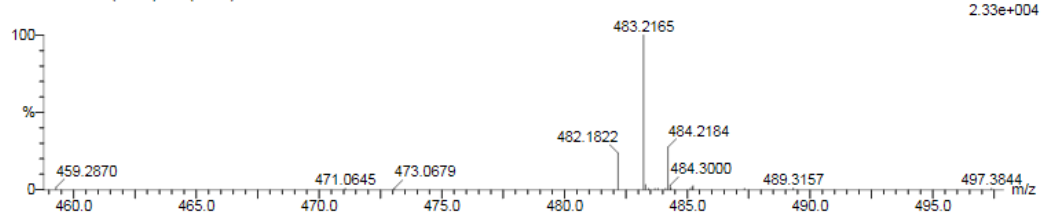
WB-LSS-73 46 (1.504) Cm (42:46) 1: TOF MS ES+ 2.33e+004



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
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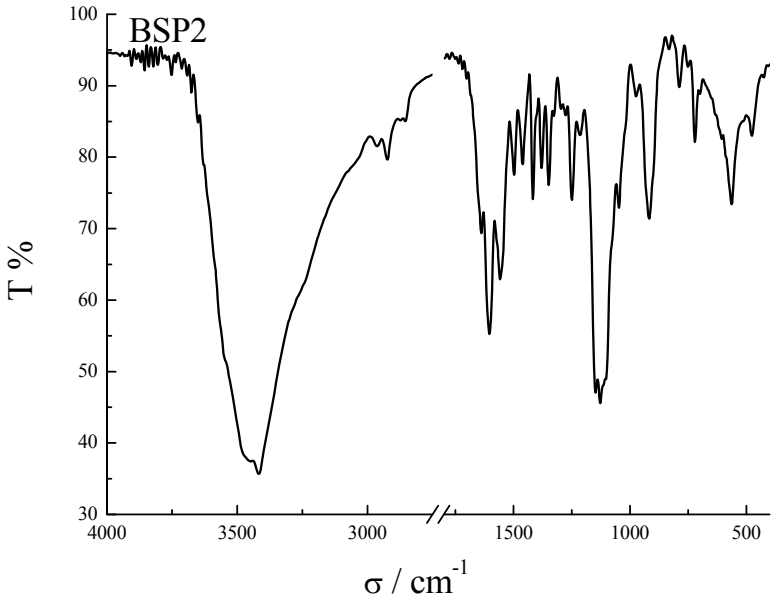

Fig. S4 ^{1}H-NMR, ^{13}C-NMR, MS and IR spectra of **BSP2**.

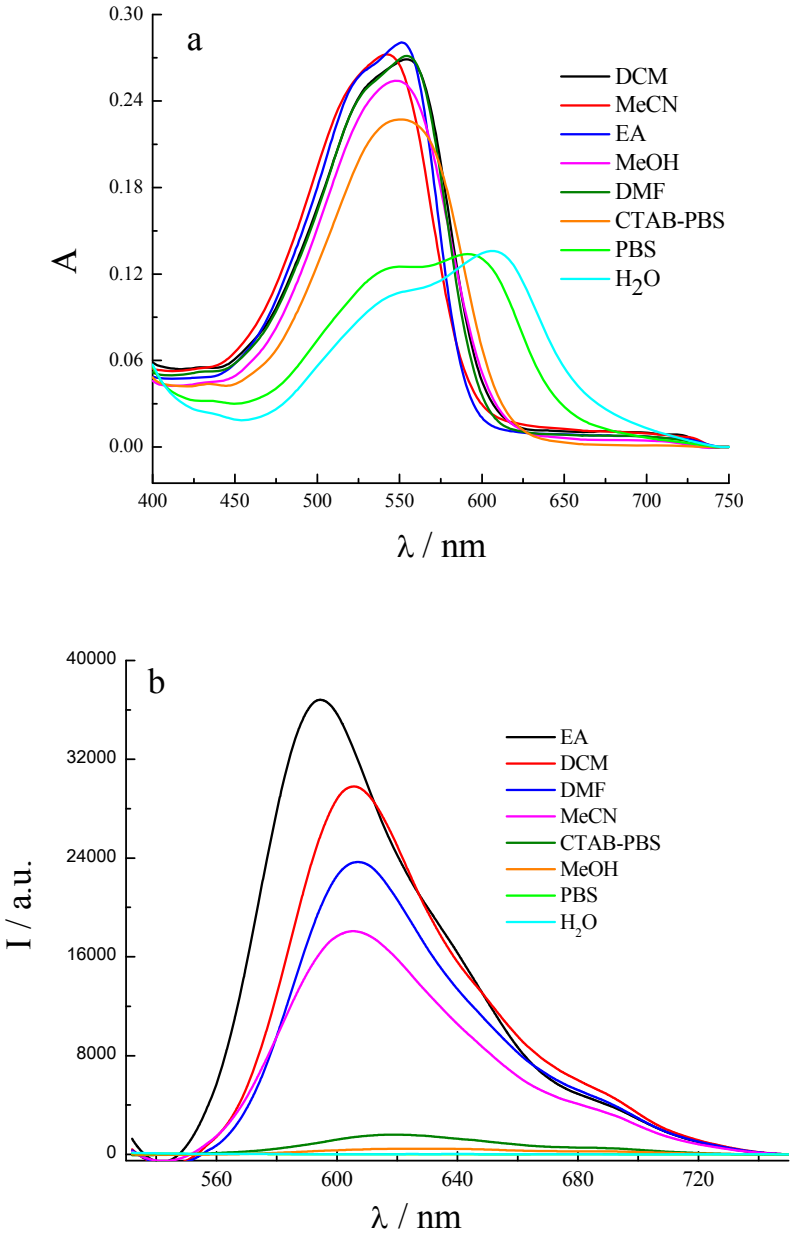

Fig. S5 The absorption (a) and emission (b) spectra of **BSP1** (10 μM) in different solvents.

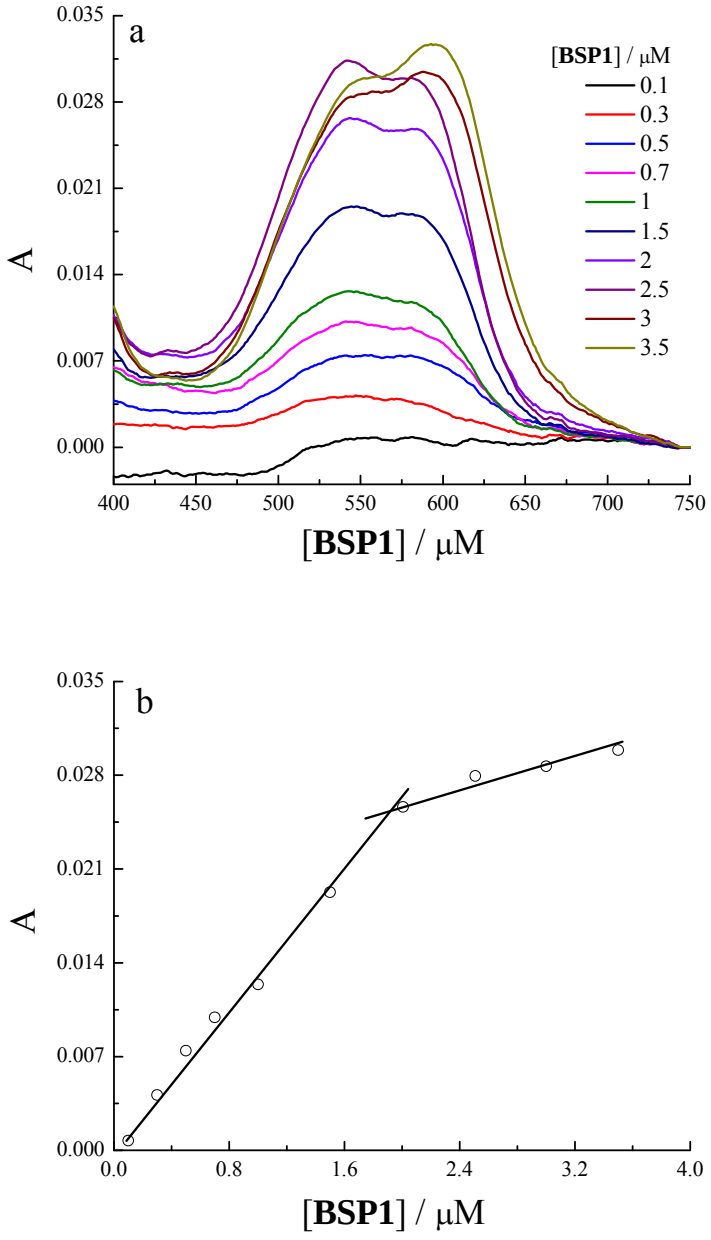

Fig. S6 The absorption spectra of **BSP1** with different concentrations in PBS.

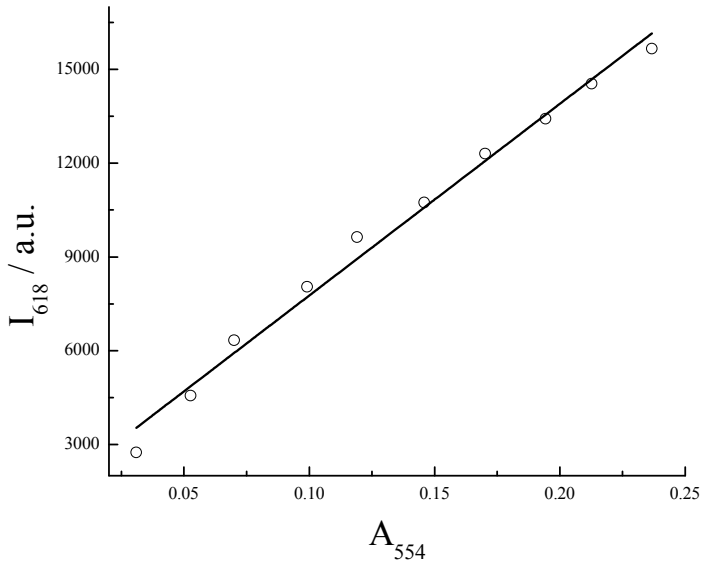

Fig. S7 Plot of fluorescence intensity vs. absorbance of **BSP1**.

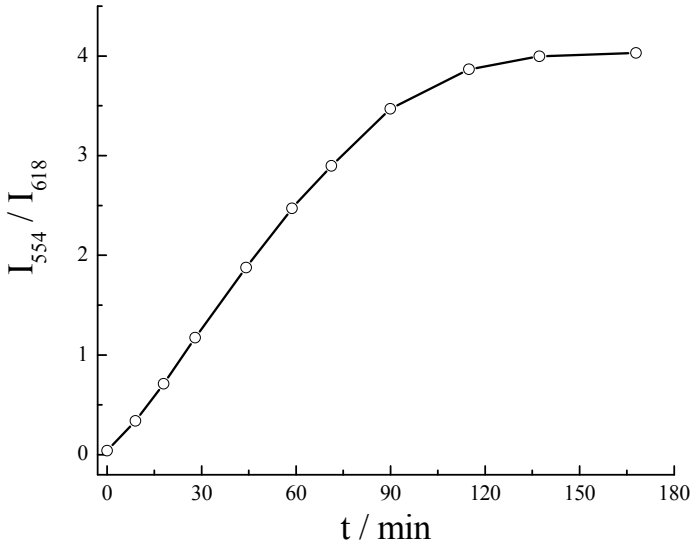

Fig. S8 I_{554}/I_{618} vs. time of **BSP1**-sulfite system. [**BSP1**] =10 μM, [sulfite] = 500 μM, 1 mM CTAB-PBS (20 mM, pH 7.4), λ_{ex}= 512 nm, 25°C.

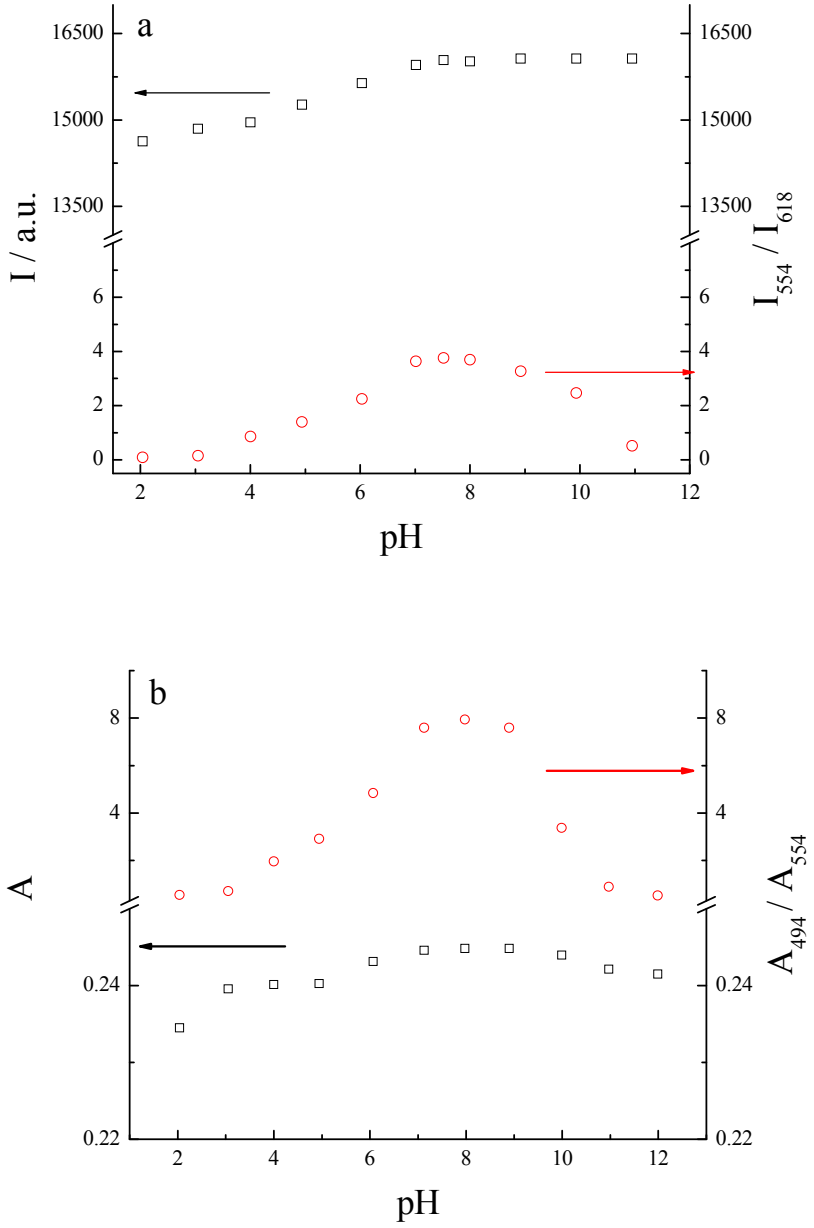

Fig. S9 The effect of pH value on the fluorescence intensity of **BSP1** at 618 nm and I_{554}/I_{618} of **BSP1**-SO_3H (a), and the absorbance of **BSP1** at 554 nm and A_{494}/A_{554} of **BSP1**-SO_3H (b), 1 mM CTAB-PBS (20 mM).

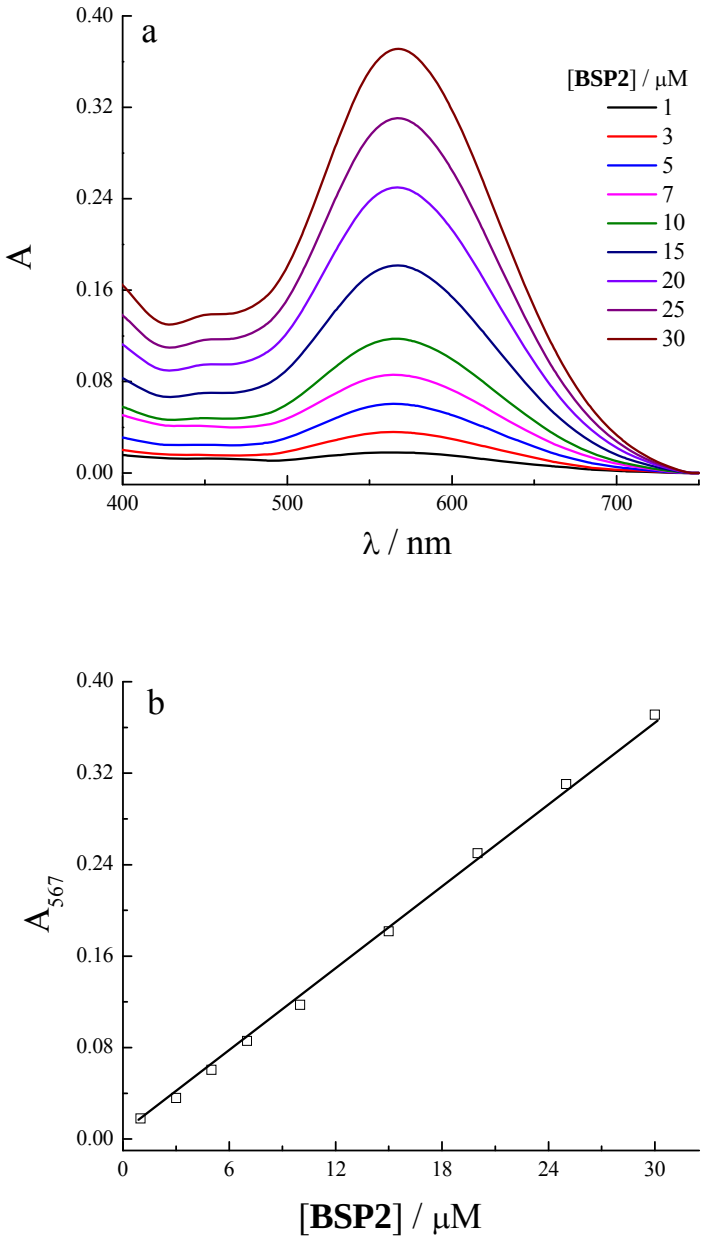

Fig. S10 The absorption spectra of **BSP2** with different concentrations (1~30μM) in PBS.

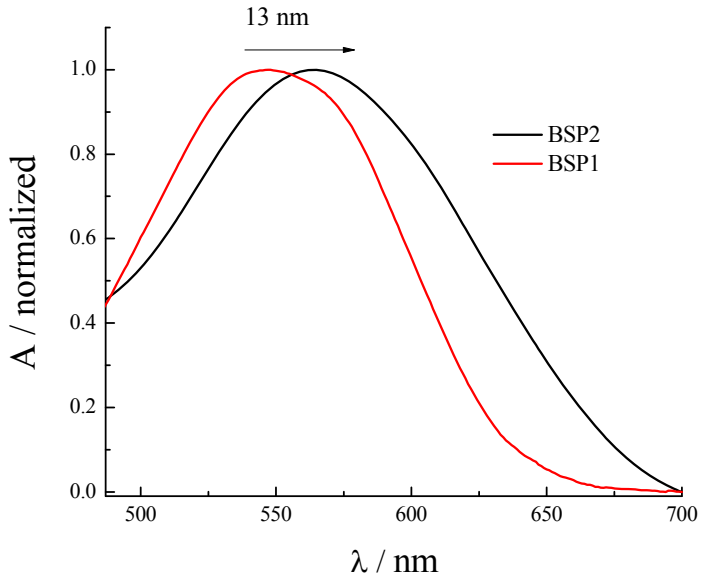

Fig. S11 The normalized absorption spectra of **BSP1** and **BSP2**.

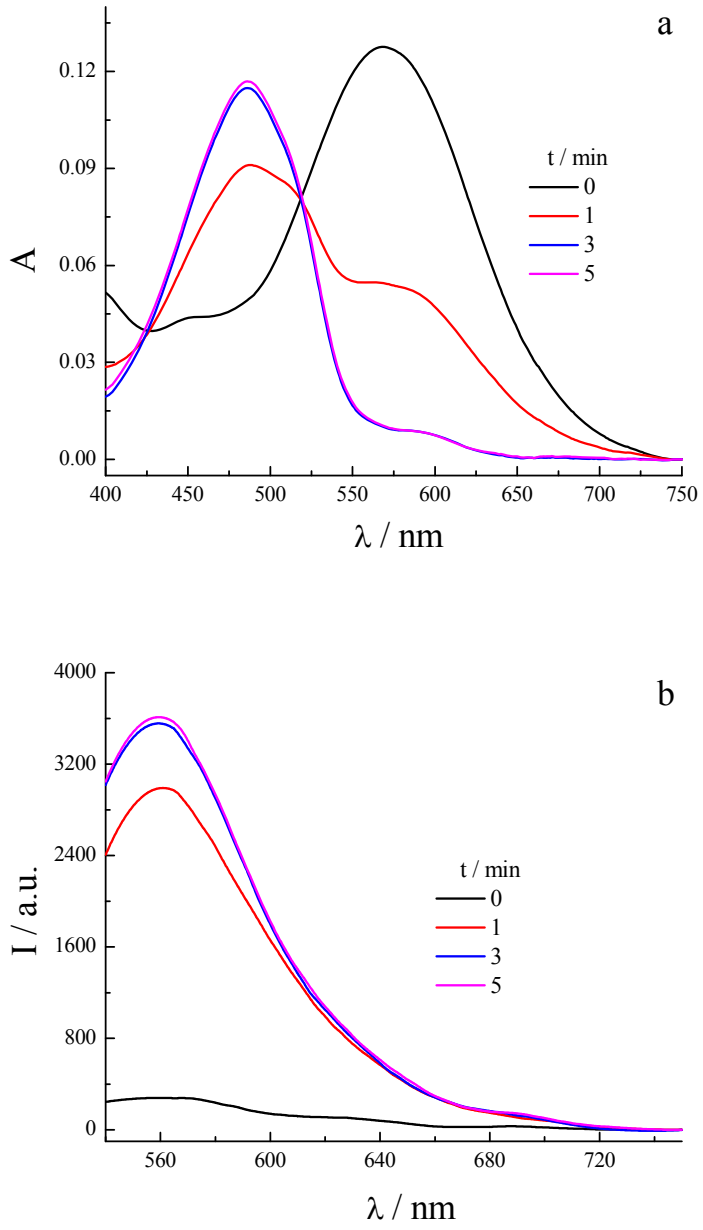

Fig. S12 Time-dependent absorption (a) and emission (b) spectra of **BSP2** (10μM) in the presence of 500 μM of sulfite in 1 mM CTAB-PBS (20 mM, pH 7.4) system, λ_{ex}= 520 nm.

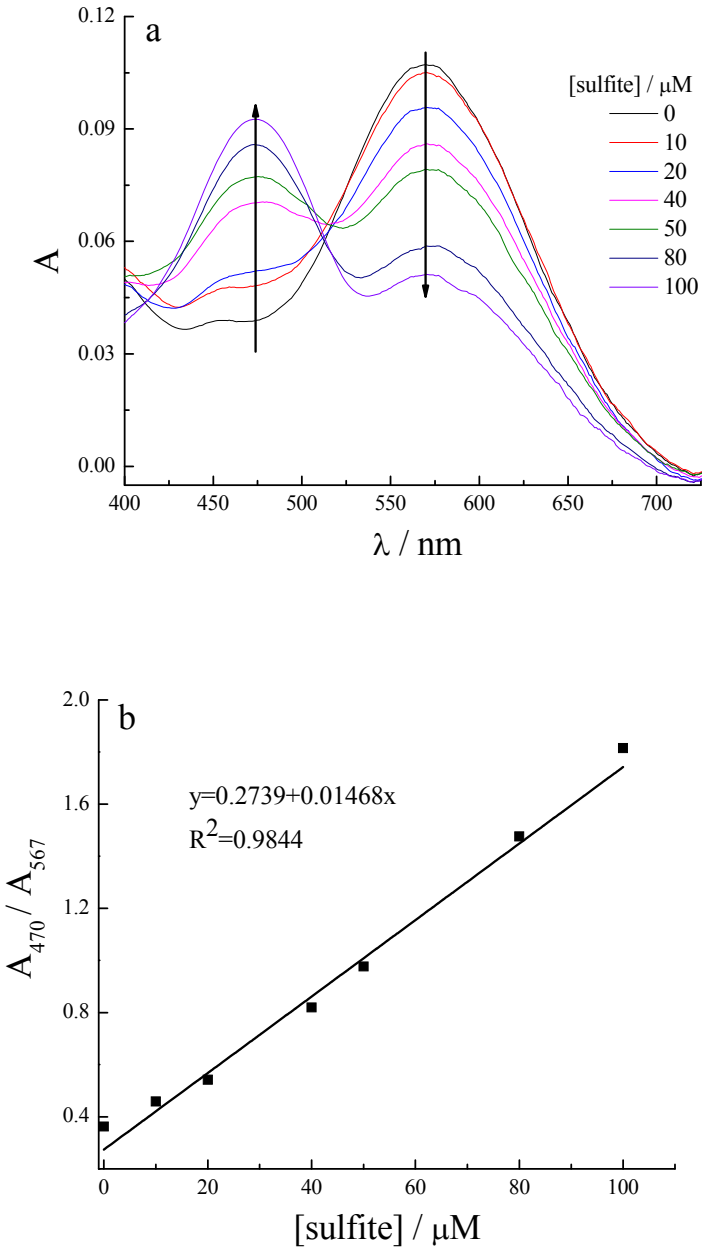

Fig. S13 (a) The absorption spectra of **BSP2** with different concentrations of sulfite; (b) the absorbance ratio at 470 nm and 567 nm (A_{470}/ A_{567}) as a function of sulfite concentration. 20 mM PBS, pH 7.4, 25°C, [**BSP2**] = 10 μM, recorded 20 min after each addition.