Supporting Information for

A mitochondria-targeted and deep-red emission ratiometric fluorescent probe for real-time visualization of SO_2 in living cells, zebrafish and living mice

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Materials and instruments

The pH measurements were performed with a Mettler-Toledo Delta 320 pH meter. UV−vis absorption spectra were measured on a Shimadzu UV-2700 spectrophotometer and fluorescence spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer. Viscosity value were carried out on a NDJ-8 rotational viscometer. MTT was purchased from *J&K Scientific Ltd*. Fluorescence imaging experiments were performed with Nikon A1MP confocal microscopy. TLC analysis carried out on silica gel plates and column chromatography was conducted over silica gel (mesh 200−300), both of them were purchased from the *Qingdao Ocean Chemicals*. ^{1}H and ^{13}C NMR spectra were performed on an AVANCE III 400 MHz digital NMR spectrometer. High resolution mass spectrometric (HRMS) analyses were carried out on an Agilent 1100 HPLC/MSD spectrometer.

Detection limit

The detection limit was based on a reported method. [S1] According to the result of titrating experiment, the fluorescence ratio intensities (I_{458}/I_{569}) of **CSP** treated with different $NaHSO_3$ were normalized between the minimum intensity and the maximum intensity. A linear regression curve was then fitted to the normalized fluorescent intensity data and the point at which this line crossed the axis was considered as the detection limit.

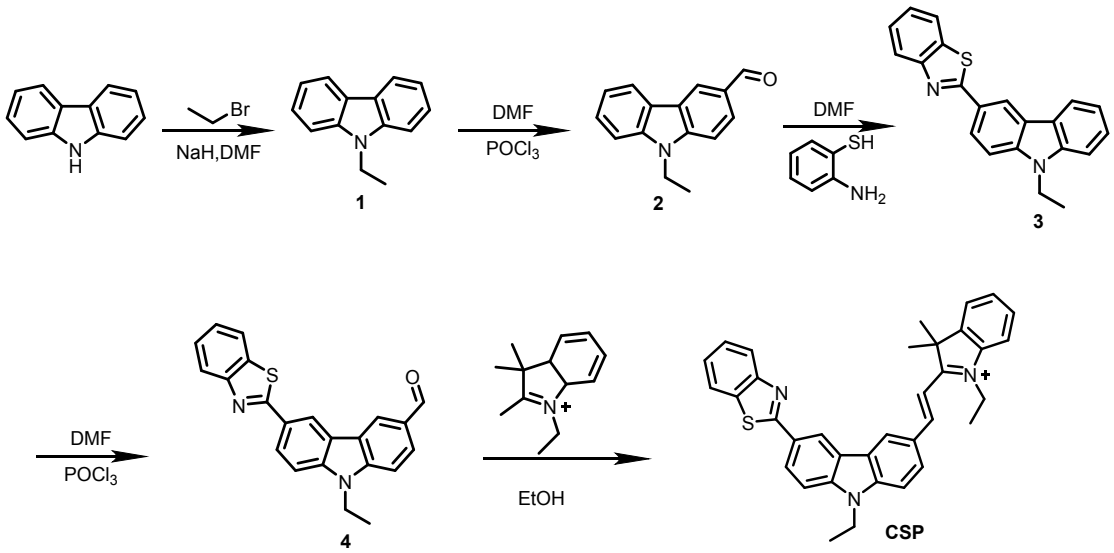
Cell culture and cytotoxicity assays

HeLa and A549 cells were cultured in Dulbecco's Modified Eagle Medium media (DMEM, Hyclone) supplemented with 10 % fetal bovine serum (FBS, Sijiqing), penicillin (100 U/ml, Hyclone) and streptomycin sulfate (100 U/ml, Hyclone) under an atmosphere of 5% CO_2 and 95% air at 37 °C.

The cytotoxicity of **CSP** to living cells was performed by standard MTT assays. 2×10^4 cells/mL living cells were seeded in 96-well plates and then incubated with different concentrations of **CSP** (0-10 μM) for 12 h. Subsequently, HeLa cells were incubated with 5 mg/mL MTT (10 μL per well) and treated for 4 h. After that the supernatants were aspirated and 100 μL DMSO was added to per well. The absorbance of the solution at 570 nm was recorded using microplate reader. The cell viability (%) = $(OD_{sample}-OD_{blank}) / (OD_{control}-OD_{blank}) \times 100$ %.

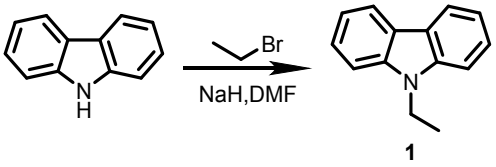
OD_{sample} denotes cells treated with various concentrations of **CSP**; OD_{blank} denotes the plates with DMEM; $OD_{control}$ denotes cells without treated with **CSP**. Each concentration was conducted with three parallel samples, and the results were expressed as mean ± standard deviation (SD).

Synthesis



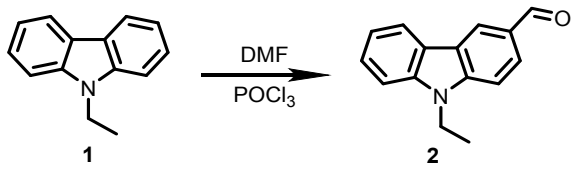
Scheme S1. Synthesis route of the fluorescent probe **CSP**.

Synthesis of compound 1.



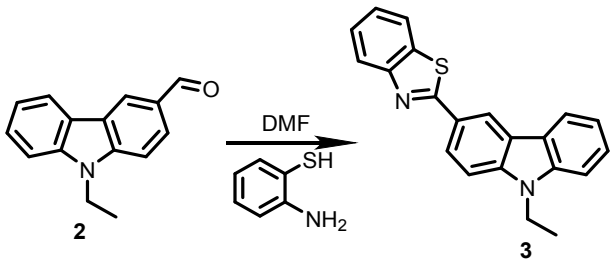
At room temperature, carbazole (1.67 g, 10 mmol) and sodium hydride (240 mg, 10 mmol) were added to a flask containing 6 mL of DMF. After stirring for 1.5 hours, ethyl bromide (5.4 g, 50 mmol) was added dropwise. The mixture was stirred at room temperature for 2 hours. After that the reaction mixture was poured into 150 mL of ice water. The product was extracted with ethyl acetate, and then dried with anhydrous sodium sulfate. The ethyl acetate removed by rotary evaporation and the white acicular compound 9-ethyl-9h-carbazole was obtained, the yield was 87%. The product does not need to be purified.

Synthesis of compound 2.



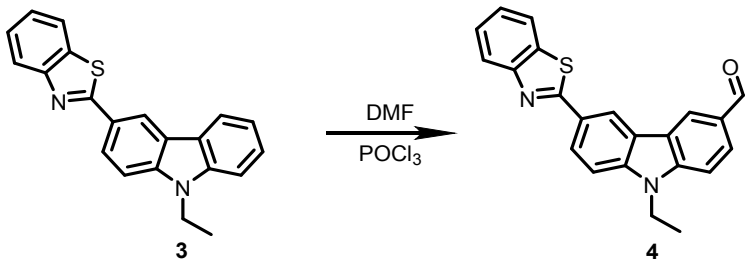
DMF (1.80 mL, 25.3 mmol) was added to a three-necked flask and $POCl_3$ was slowly added into DMF solution, and the mixture was stirred in an ice bath for 30 min under the protection of N_2. Compound **1** (5 g, 25 mmol) dissolved in dichloromethane was added to the reaction mixture, stirred at room temperature for 0.5 h, and then refluxed for 12 h. After the reaction was completed, the reaction solution was added to 200 mL ice water and extracted with ethyl acetate. The ethyl acetate removed by rotary evaporation. The crude product was purified by chromatography on silica gel eluting to afford light yellow solid **2** (yield: 83%).

Synthesis of compound 3.



2-aminothiophenol (24 mmol, 2.52 mL) and Compound **2** (12 mmol, 2.78 g) were dissolved in 50 mL DMF. After the reaction mixture was refluxed for 4 h, it was cooled to room temperature. Then 200 mL ice water was added to the above solution, and the precipitate was filtered, and the crude product was recrystallized by methanol to give a dark yellow compound **3** (yield: 91%).

Synthesis of compound 4.



DMF (1.80 mL, 25.3 mmol) was added to a three-necked flask and $POCl_3$ was slowly added into DMF solution, and the mixture was stirred in an ice bath for 30 min under the protection of N_2. Then, Compound **3** (8.2g, 25 mmol) dissolved in dichloromethane was added to the reaction mixture, stirred at room temperature for 0.5 h, and refluxed for 12 h. After the reaction was completed, the reaction system was added dropwise to 200 mL of ice water and extracted with ethyl acetate. The ethyl acetate removed by rotary evaporation. The crude product was purified by chromatography on silica gel eluting to afford brown solid **4** (yield: 70%). ^{1}H NMR (400 MHz, DMSO) δ 8.93 (d, J = 1.6 Hz, 1H), 8.39 (d, J = 7.6 Hz, 1H), 8.21 (dd, J_1 = 8.6 Hz, J_2 = 1.8 Hz, 1H), 8.15 (d, J = 8.0 Hz, 1H), 8.05 (d, J = 8.0 Hz, 1H), 7.80 (d, J = 8.8 Hz, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.55 (m, 2H), 7.44 (t, J = 7.2 Hz, 1H), 7.30 (t, J = 7.4 Hz, 1H), 4.52 (q, J = 7.1 Hz, 2H), 1.36 (t, J = 7.2 Hz, 3H).

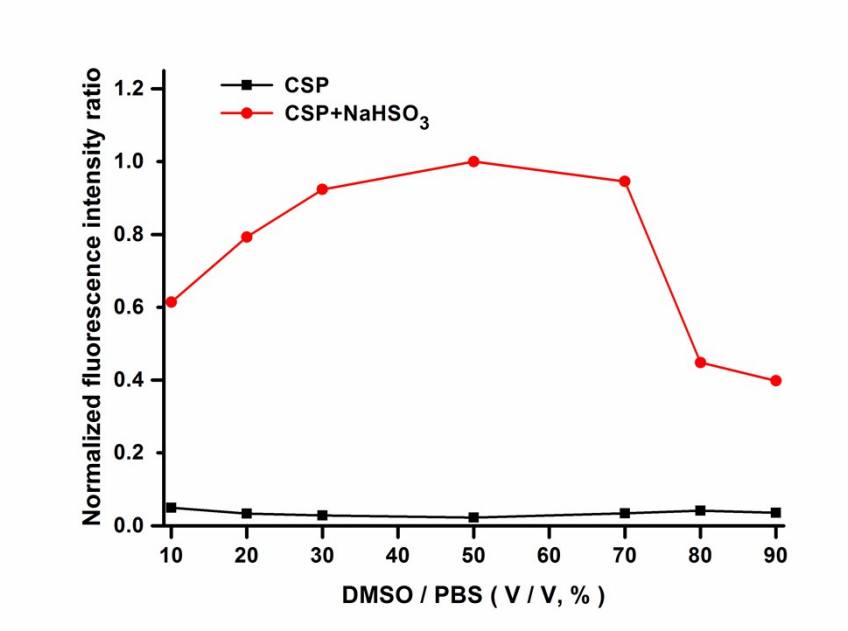


Fig. S1. Normalized fluorescence intensity ratio of **CSP** in the absence or presence of $NaHSO_3$ with different ratios of DMSO and PBS.

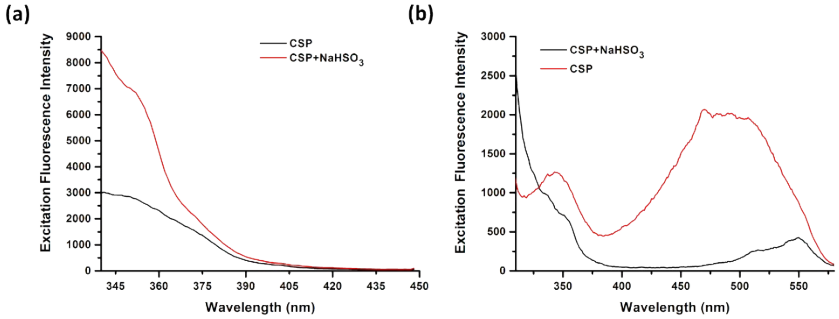


Fig. S2. Fluorescent excitation spectra of **CSP** in the absence or presence of $NaHSO_3$ with 458 nm emission wavelength (a) and 596 nm emission wavelength (b) in DMSO/PBS (1/1, v/v, PBS 10 mM, pH 7.4) solution.

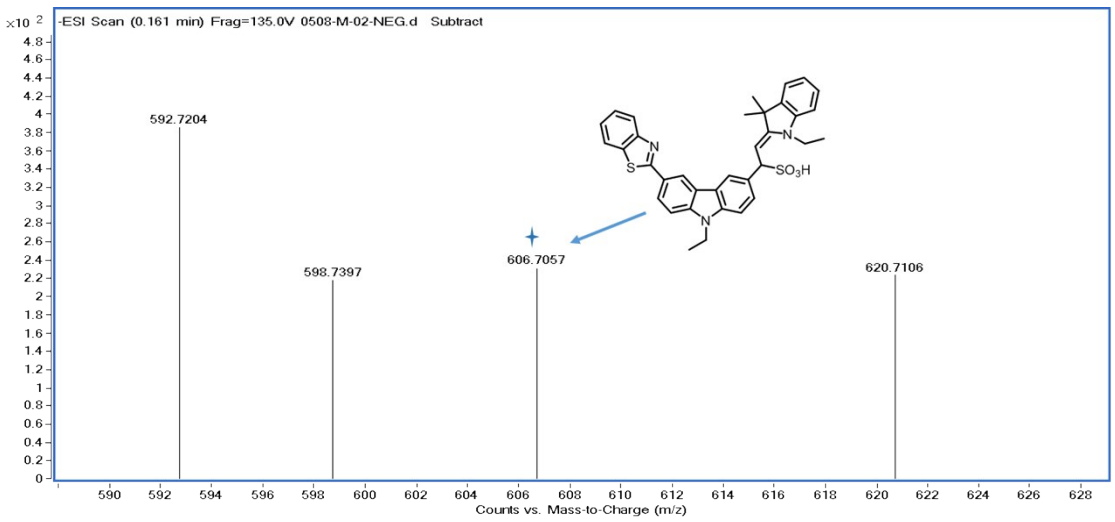


Fig. S3. HRMS spectrum of detection mechanism (**CSP**+HSO_3^-).

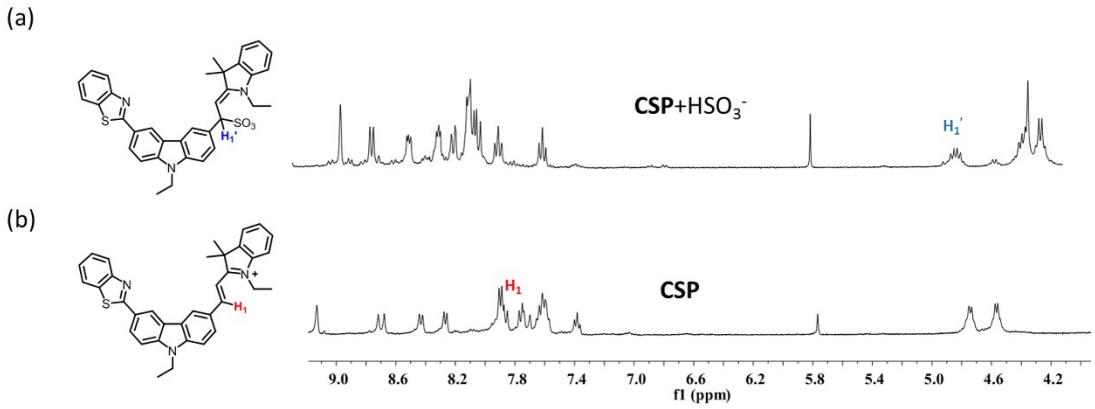


Fig. S4. Partial ^{1}H NMR spectra of **CSP** in the presence (a) or absence (b) of $NaHSO_3$ in DMSO-d_6/D_2O (v/v, 4:1) solution.

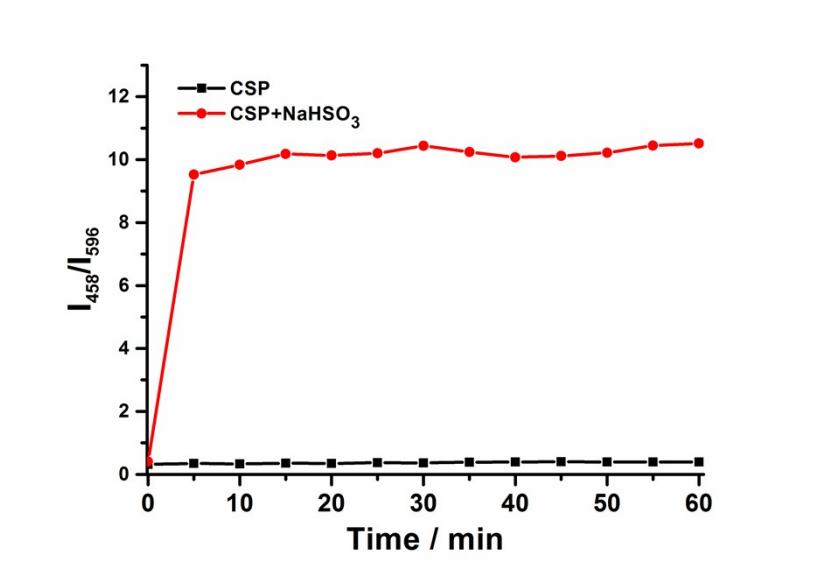


Fig. S5. Photostability profiles of the probe **CSP** in the absence or presence of $NaHSO_3$. λ_{ex} = 350 nm.

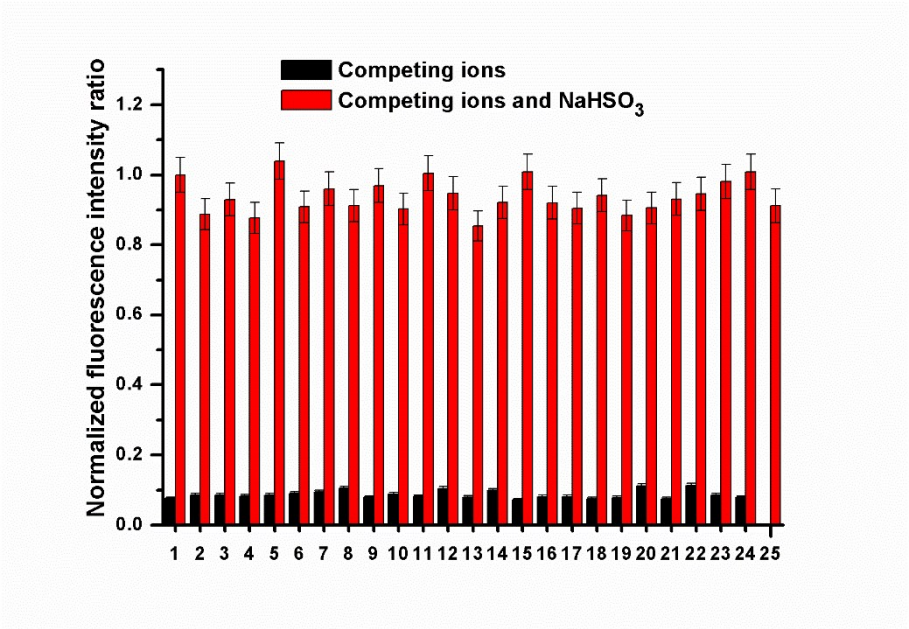


Fig. S6. Fluorescence intensity ratio response of **CSP** towards SO_2 (20 μM) in the presence of representative analytes. 1, $BaCl_2$ (2.5 mM); 2, Na_2S (20 μM); 3, $CaCl_2$ (2.5 mM) ; 4, Cys (500 μM); 5, di-t-butyl peroxide (DTBP) (100 μM); 6, $CuSO_4$ (2.5 mM); 7, $FeSO_4$ (2.5 mM) ; 8, GSH(5 mM); 9, H_2O_2 (100 μM); 10, HClO (100 μM); 11, Hcy (500 μM); 12, KCl (2.5 mM) ; 13, KI (2.5 mM) ; 14, KNO_3 (2.5 mM) ; 15, $MgCl_2$ (2.5 mM); 16, NaCNS (2.5 mM); 17, NaF (2.5 mM); 18, NaBr (2.5 mM); 19, $NaNO_2$ (2.5mM); 20, NH_3PO_4 (2.5mM); 21, tert-butylhydroperoxide (TBHP) (100 μM); 22, glyoxal (2.5 mM); 23, NH_4OAc (2.5 mM); 24, $ZnCl_2$ (2.5 mM); 25, $NaHSO_3$ (20 μM). λ_{ex} = 350 nm.

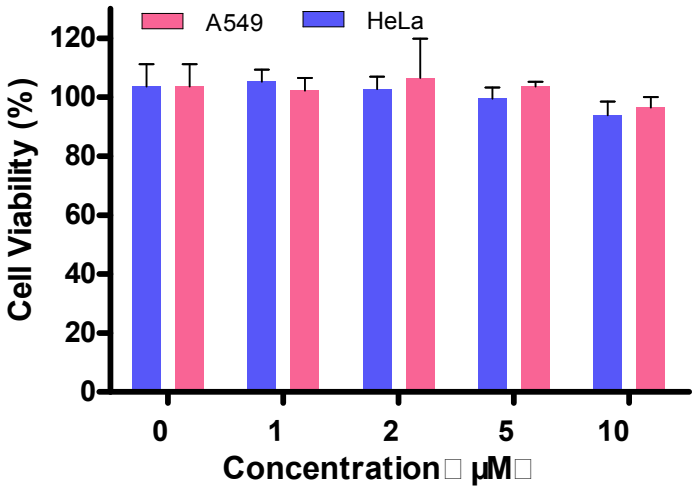


Fig. S7. The viability of HeLa cells and A549 cells was treated with different concentrations (0-10 μM) of **CSP** for 12 hours.

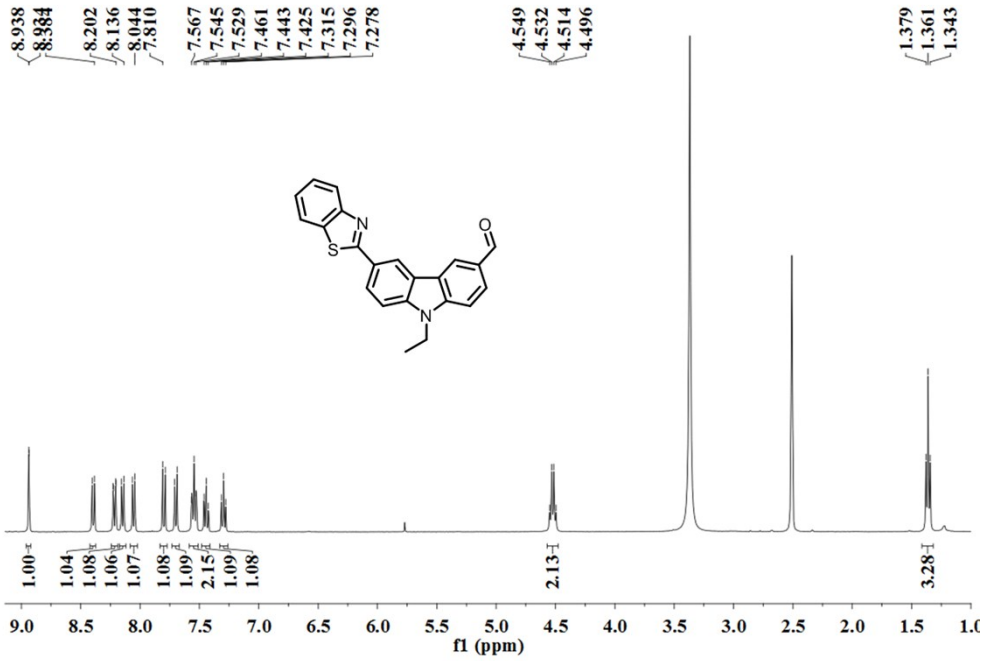


Fig. S8. ^{1}H NMR spectrum of Compound **4** in d_6-DMSO.

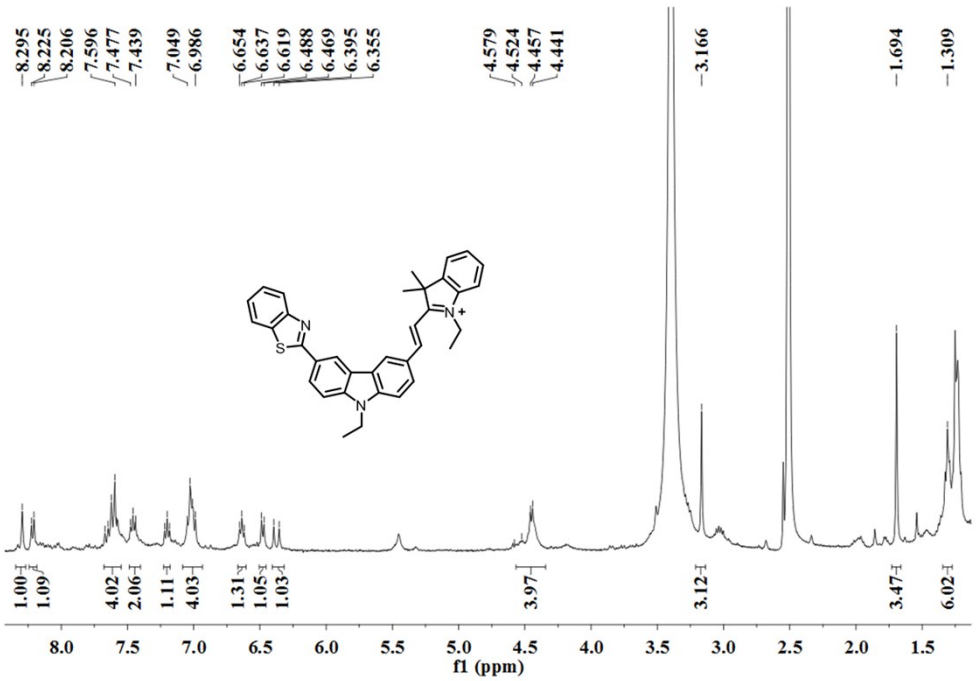


Fig. S9. ^{1}H NMR spectrum of Compound **CSP** in d_6-DMSO.

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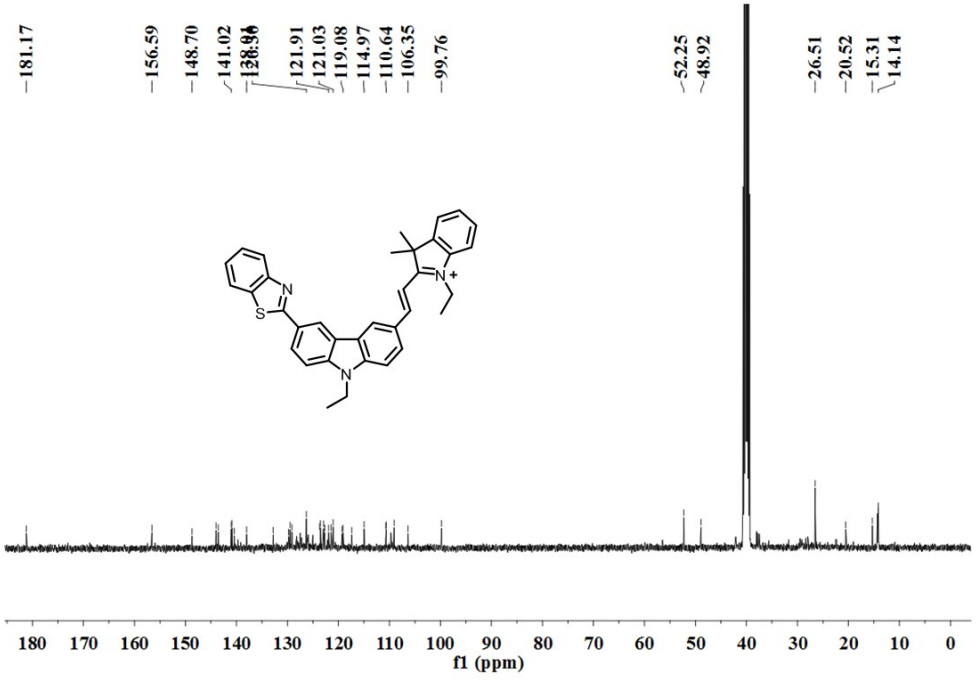

Fig. S10. ^{13}C NMR spectrum of **CSP** in d_6-DMSO.

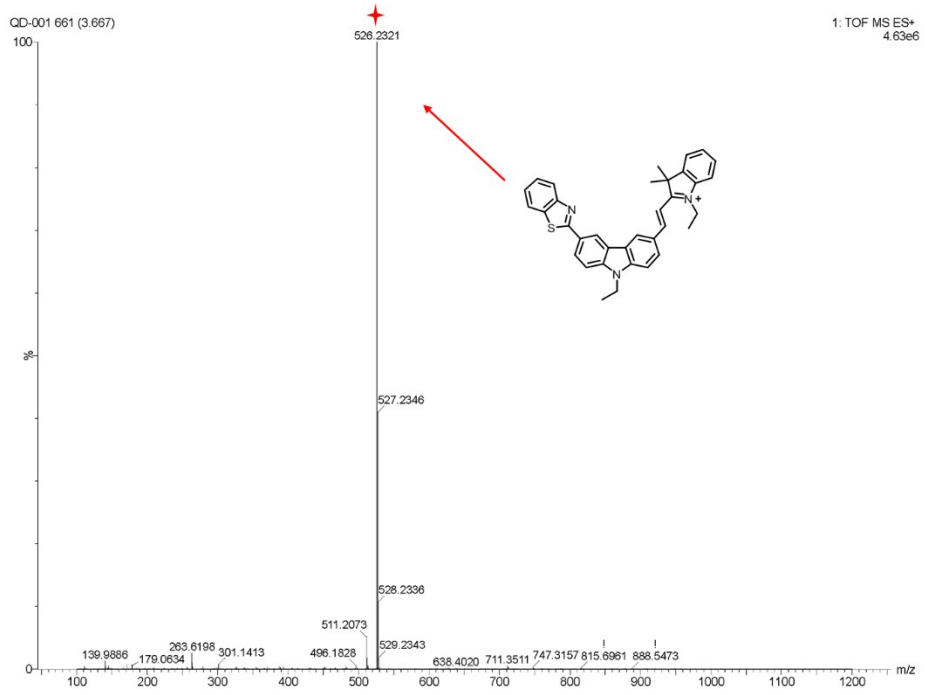

Fig. S11. HR-MS spectrum of **CSP**.

REFERENCES

[S1] Shortreed M.; Kopelman R.; Kuhn M.; Hoyland B.; *Anal. Chem.*, **1996**, *68*, 1414-1418.