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Highly selective fluorescent probe for fast detection of hydrogen sulfide in aqueous solution and living cell

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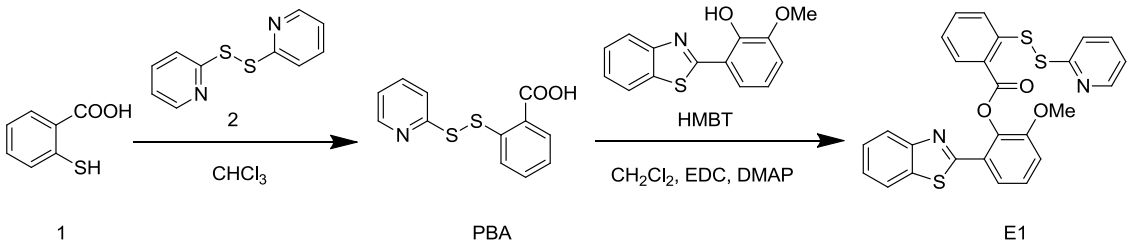
1. General Experimental

Materials and methods: All chemical regents and solvents were purchased from J&K Corporation and used without further purification. Thin-layer chromatography (TCL) was performed on silica gel plates. Column chromatography was performed using silica gel (Hailang, Qingdao) 200-300 mesh. 10 mM NaHS stock solution in Tris-HCl buffer (20 mM pH 7.4). Ultrapure water was used throughout.

Instruments: Fluorescence spectra were determined using a Varian Cary Eclipse fluorescence spectrometer. Absorption spectra were determined by a Varian Cary 100 UV-vis spectrophotometer. All pH measurements were made with a Sartorius basic pH-Meter PB-20. ^{1}H NMR and ^{13}C NMR spectra were recorded employing a Bruker AV-400 spectrometer with chemical shifts expressed in parts per million (in deuteriochloroform, Me_4Si as internal standard). Electrospay ionization (ESI) mass spectrometry was performed in a HP 1100 LC-MS spectrometer.

2. Synthesis and Characterization of Compounds



Scheme S1. Synthetic route of probe **E1**.

Synthesis of 2-(pyridin-2-yldisulfanyl)benzoic acid (PBA)

PBA was prepared according to the literature procedure.[1] To a solution of 1,2-di(pyridin-2-yl)disulfane **1** (1.016 g, 4.61 mmol) in chloroform (30 ml) was added 2-mercaptobenzoic acid **2** (178 mg, 1.15 mmol), the mixture was stirred for 1 hours at room temperature. Then, the solvent was removed under reduced pressure to produce a yellow solid (~320mg). The product was subjected to column chromatography for purification. PBA was obtained as a yellow solid. ^{1}H NMR (DMSO, 400 MHz) δ 8.45-8.44 (m, 1H), 8.00 (dd, 1H, J_1 = 7.6 Hz, J_2 = 1.2 Hz), 7.73 (m, 1H), 7.67 (d, 1H, J = 8.0 Hz), 7.42 (d, 1H, J = 8.0 Hz), 7.39-7.35 (m, 1H), 7.24-7.20 (m, 2H);

Synthesis of E1[1]

To a mixture of compounds HMBT (98 mg, 0.38 mmol), PBA (100 mg, 0.38 mmol), EDC (73mg, 0.38 mmol) and DMAP (5mg, 0.038mmol) was added CH_2Cl_2 (25 mL) at room temperature. The mixture was stirred for 5 hours. Then solvent was evaporated under reduced pressure and resulted residue was subjected to column

chromatography for purification. **E1** was obtained as a white solid (105 mg, 54.97% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.55 (d, 1H, J = 7.6 Hz), 8.48 (d, 1H, J = 3.6 Hz), 8.03 (t, 2H, J = 8.4 Hz), 7.97 (d, 1H, J = 8.0 Hz), 7.85 (d, 1H, J = 7.6 Hz), 7.64-7.35 (m, 8H), 7.18 (d, 1H, J = 8.0 Hz), 7.09 (t, 1H, J = 5.6 Hz), 3.93 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.9, 162.2, 159.3, 152.9, 152.1, 149.5, 141.8, 138.0, 137.4, 135.5, 133.8, 132.7, 127.6, 127.0, 126.5, 126.3, 126.2, 126.0, 125.3, 123.4, 121.5, 121.4, 120.9, 119.6, 114.2, 56.5; HRMS (ES+) calcd for $C_{26}H_{18}N_2O_3S_3$ $[M+H]^+$ 503.0558, found 503.0565.

3. Effect of pH Values

Fluorescence pH titrations were performed in buffer solution at a probe concentration of 10 μm in 20 mM Tris-HCl with 40% CH_3OH. As is shown in Fig. S1, **E1** is stable during pH range from 2 to 12.

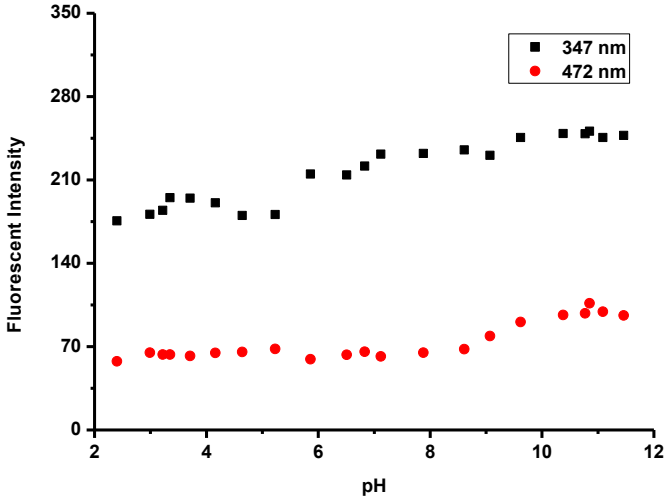
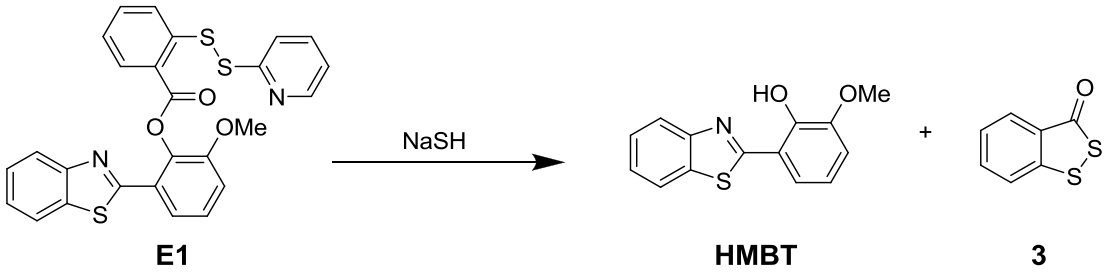


Fig. S1 Fluorescence response of **E1** (10 μm) to various pH in 20 mM Tris-HCl with 40% CH_3OH. pH 2~12. Red line: 472 nm; Black line: 347 nm.

4. Reaction of E1 with H_2S



Scheme S2. Reaction of **E1** with H_2S

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Solution of **E1** (48mg, 0.09 mmol) in DMF (10 mL) was added NaHS (8.9 mg, 0.09 mmol) in Tris-HCl buffer (5 mL, 20 mM, pH = 7.4). The mixture was stirred for 1 hours at room temperature. The color of solution turned to light yellow. Then solvent was evaporated under reduced pressure and resulted residue was subjected to column chromatography for purification. Compound **3** was obtained as a white solid (13 mg, 86% yield). The formation of **3** was confirmed by ^{1}H NMR, ^{13}C NMR and HRMS (EI+).

5. Effect of CTAB to the fluorescent intensity of E1

Test were performed in buffer solution at a probe concentration of 10 μm in 20 mM Tris-HCl. CTAB concentration is 1 mM. As is shown in Fig. S2, CTAB has minimal impact on probe **E1**.

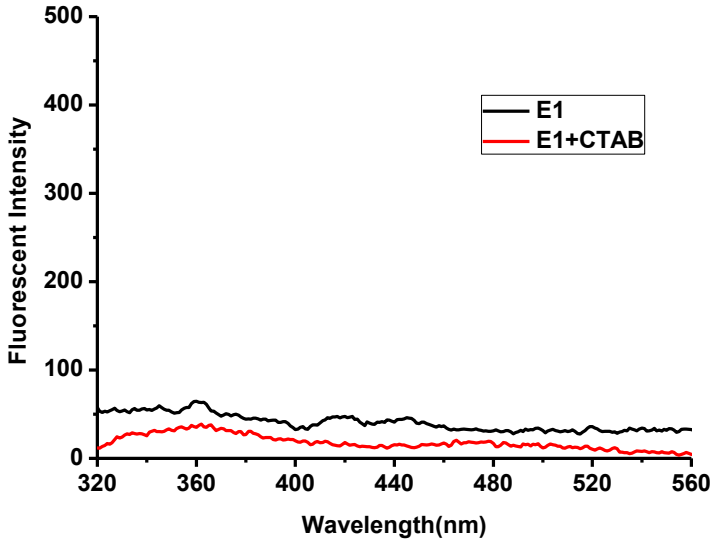

Fig. S2 Fluorescence response of **E1** (10 μm) with and without 1mM CTAB in 20 mM Tris-HCl. pH = 7.4. Red line: E1 + CTAB; Black line: E1. Slite: 10, 5.

6. Selectivity of E1

Test were performed in buffer solution at a probe concentration of 10 μm in 20 mM Tris-HCl. CTAB concentration is 1 mM. As is shown in Fig. S3, probe **E1** has a good selectivity.

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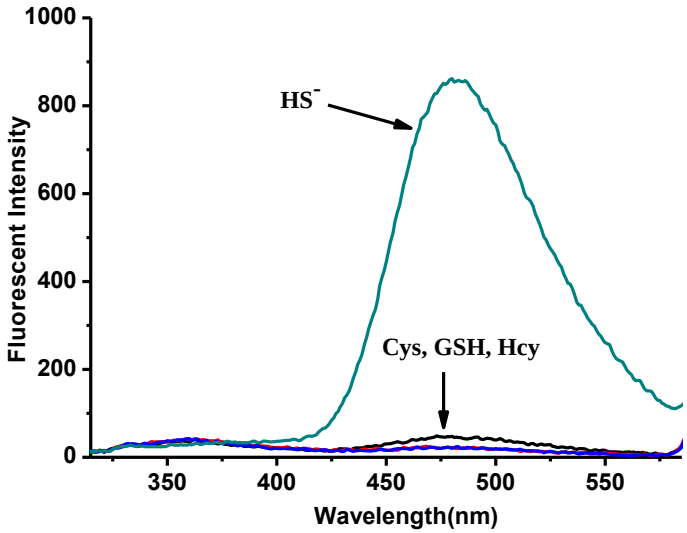

Fig. S3 Fluorescent intensity change after addition of NaHS and other anions. **E1** (10 μM) + amino acid (100 μM) in 20 mM Tris-HCl buffer with 1 mM CTAB (pH 7.4). (λex = 295 nm). Slite: 10, 5.

7. Fluorescence spectral changes of E1 with H_2S in EtOH/Tris-HCl buffer

Test were performed in EtOH/Tris-HCl buffer (20 mM, pH 7.4, 2:8 v/v) at a probe concentration of 10 μm. As is shown in Fig. S4, probe **E1** produced 1.3-fold turn-on response in this buffer solution.

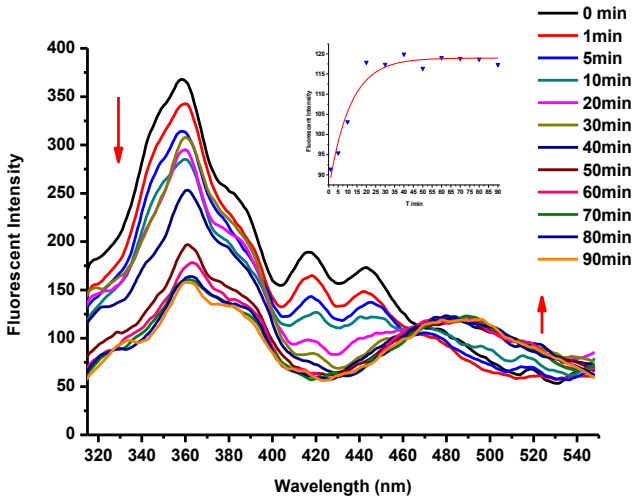

Fig. S4 Time-dependent fluorescence spectral changes of **E1** with H_2S (**E1** 10 μM, NaHS 50 μM) in EtOH/Tris-HCl buffer (20 mM, pH 7.4, 2:8 v/v). Time points represent 0, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80 and 90 min. Insert: Reaction time profile of E1 and H_2S. Slite 10, 10.

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8. Fluorescent Microscopy Imaging for E1 in Hela Cells

Hela cells were obtained from American Type Culture collection and grown in Dulbecco's modification of Eagle's medium Dulbecco (DMEM/high: with 4500 mg/L Glucose, 4.0 mM L-Glutamine, and 110 mg/L Sodium Pyruvate), supplemented with 10% foetal bovine serum (FBS). Cells were incubated in a 5% CO_2 humidified incubator at 37 ^{o}C and typically passaged with sub-cultivation ratio of 1:4 for two days.

Hela cells were seeded in 12-well culture plate for one night. Stocks solution of **E1** (1 mM) was prepared in DMSO at the same day of experiment, which was diluted into the cell culture media at 100 μM. The Hela cells were preloaded with the 100 μM **E1** for 30 min in 5% CO_2 incubator at 37 ^{o}C , and washed with phosphate buffer (pH = 7.4) one time. Then cells were treated without 100 μM NaSH, with 100 μM NaSH and 100 μM NaSH (1 mM CTAB) as indicated. Afterwards the Hela cells were also incubated in 5% CO_2 at 37 ^{o}C for 30 min, then rinsed with phosphate buffer (pH = 7.4) three times. Fluorescence imaging was performed with Nikon Ti-s with Xenon lamp and camera. Exposure time is 300 ms for green emission.

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9. ^{1}H NMR, ^{13}C NMR and ESI of E1 and 3

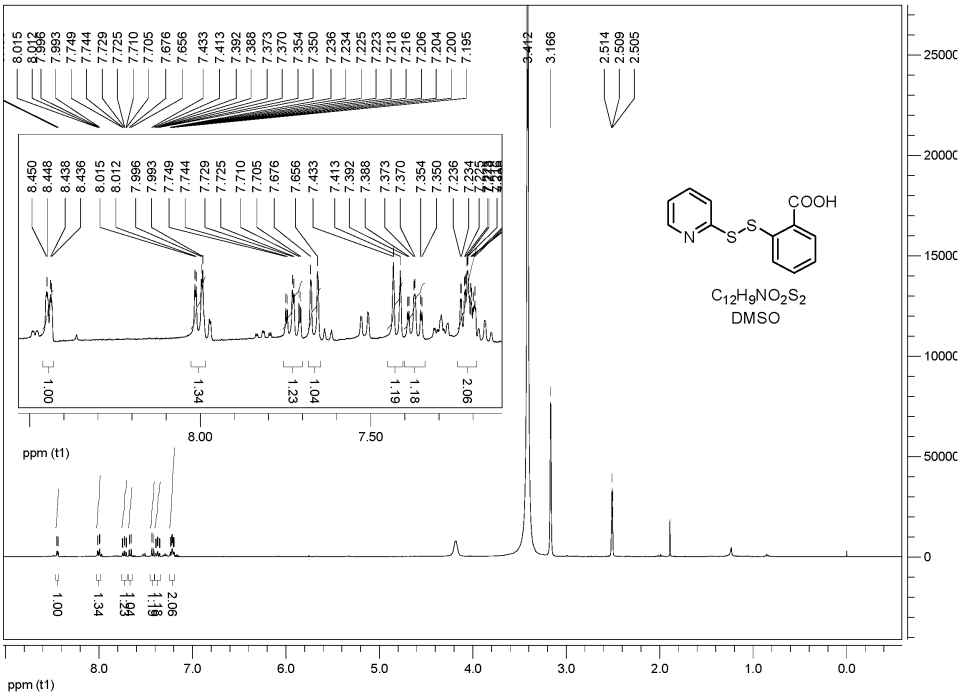

Fig. S5 ^{1}H NMR of **PBA**

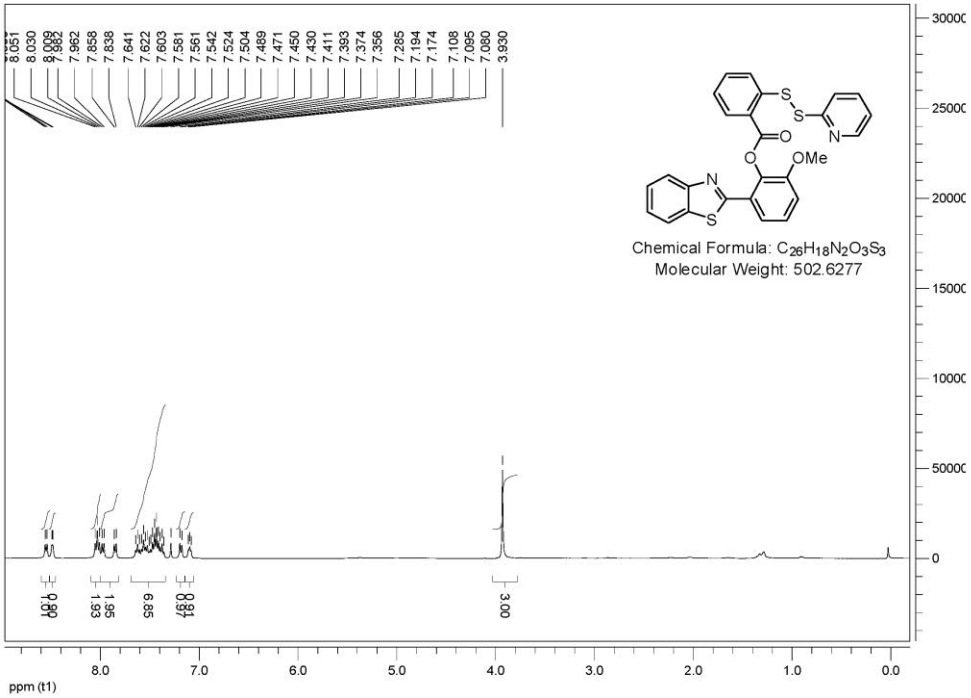

Fig. S6 ^{1}H NMR of **E1**

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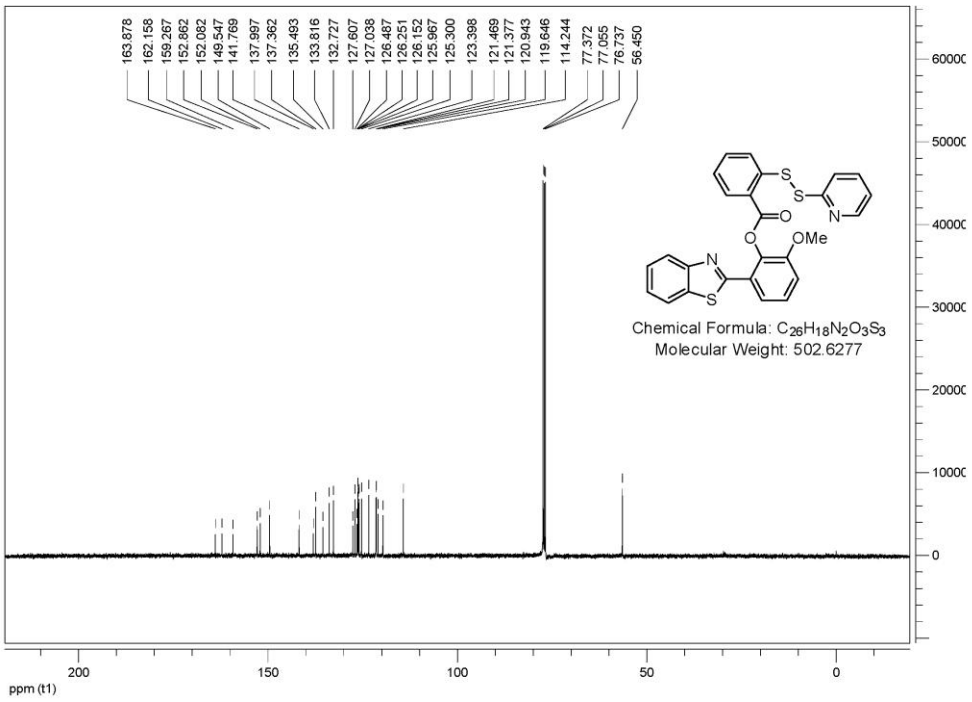

Fig. S7 ^{13}C NMR of **E1**

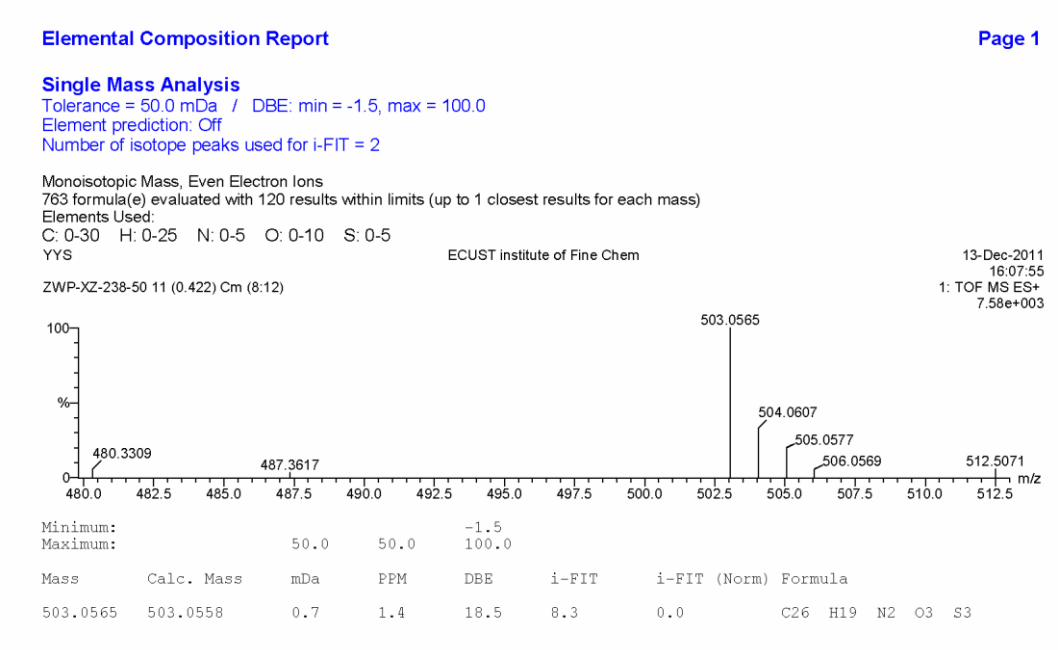

Fig. S8 ESI-Mass spectrum of **E1**

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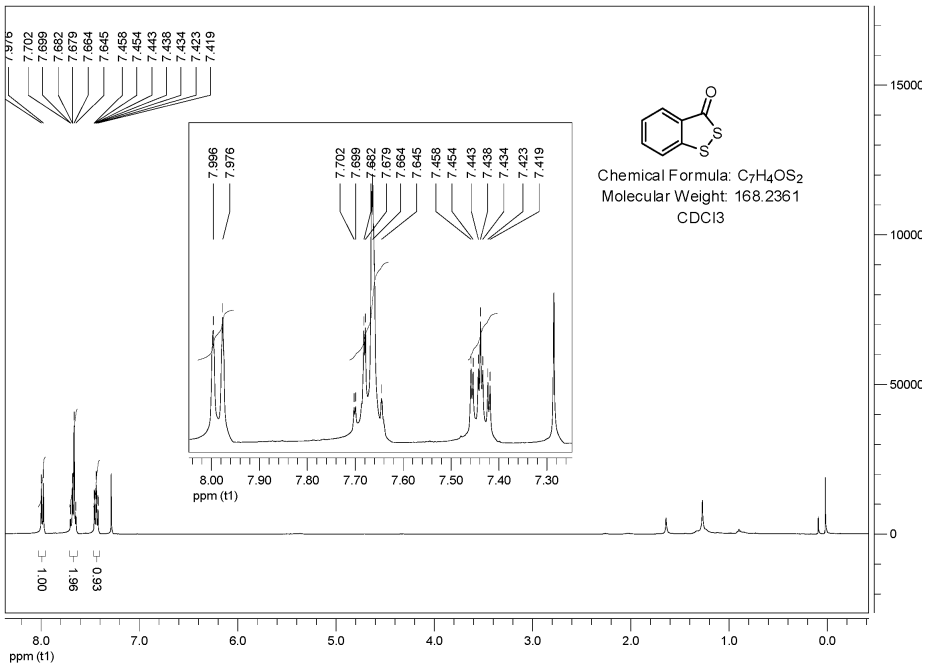

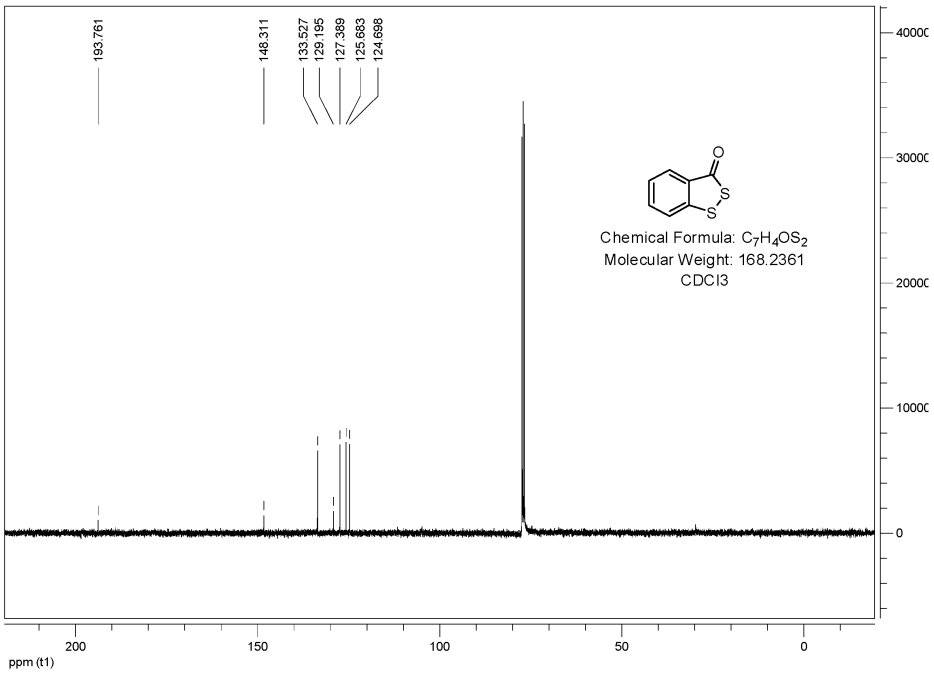

Fig. S9 ^{1}H NMR and ^{13}C NMR of **3**

10. Reference

1. Liu, C.; Pan, J.; Li, S.; Zhao, Y.; Wu, L. Y.; Berkman, C. E.; Whorton, A. R.; Xian, M., Capture and Visualization of Hydrogen Sulfide by a Fluorescent Probe. *Angewandte Chemie International Edition* **2011,** *50* (44), 10327-10329.