

Supporting Information

A Lysosome-targeted Fluorescent Chemodosimeter for Monitoring Endogenous and Exogenous Hydrogen Sulfide in *In-Vivo* Imaging

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Experimental Section

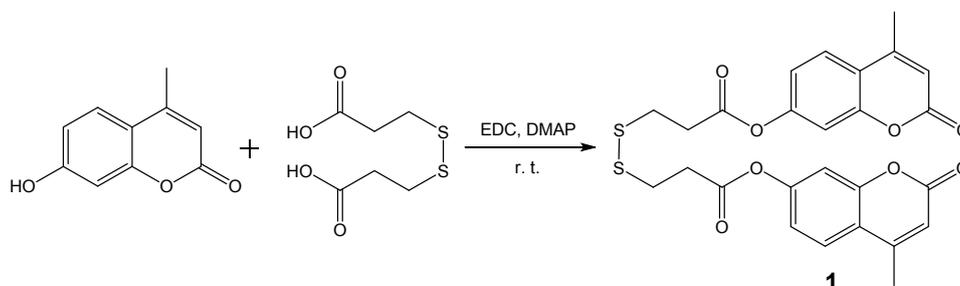
General methods

Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Flash chromatography was carried out on silica gel (230-400 mesh). ^1H NMR spectra were recorded using BRUKER DRX 500 spectrometer; ^{13}C NMR spectra were recorded using BRUKER DRX 500 spectrometer; mass spectrometry was recorded with Agilent LC/MSD TOF mass spectrometer. The UV-Vis spectra were obtained using UV-240IPC spectrophotometer. The fluorescence spectra were obtained with F-4500 FL spectrometer with a 1cm standard quartz cell.

Confocal microscopy imaging

The images were observed with a fluorescence microscope (Olympus FV1000; Olympus, Tokyo, Japan) which was equipped with U-MWU2.

Synthesis



Scheme 1. The synthesis route of compound 1.

3, 3'-ditthiodipropionic acid (210 mg, 1.0 mmol), 4-dimethylaminopyridine (DMAP, 40 mg), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimidehydrochloride (EDC, 400 mg, 2.1 mmol) and 7-hydroxy-4-methylcoumarin (370 mg, 2.1 mmol) was mixed in 30 ml anhydrous dichloromethane. The mixture was stirred at room temperature overnight and then the solvent was removed under reduced pressure. The resulting residue was purified by silica gel column chromatography CH_2Cl_2 to give the target **1** as off-white solids. (410 mg, 78 %). ^1H NMR δ (DMSO- d_6): 2.42 (3H, s), 3.08 (2H, t), 3.11 (2H, t), 6.39 (1H, s), 7.20 (1H, t), 7.23 (1H, s), 7.81 (1H, t). ^{13}C NMR δ (DMSO- d_6): 19.01, 33.24, 34.57, 110.80, 114.66, 118.47, 119.14, 127.32, 153.55, 153.57, 154.36, 160.41, 170.66. HRESIMS: calcd for $\text{C}_{26}\text{H}_{22}\text{O}_8\text{S}_2$ $[\text{M} + \text{Na}]^+ = 549.0648$, found m/z 549.0646; calcd for $\text{C}_{26}\text{H}_{22}\text{O}_8\text{S}_2$ $[\text{M} + \text{K}]^+ = 565.0384$, found m/z 565.0383.

The linear range and detection limit

The detection limit was calculated based on the method reported in the previous literature [1]. The fluorescence emission spectrum of **1** was measured by twenty times and the standard deviation of blank measurement was achieved. The fluorescence intensity at 453 nm was plotted as a concentration of H_2S . The detection limit was calculated by using detection limit $3\sigma/k$: Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity versus H_2S concentration.

Culture of Hela cells and confocal microscope imaging

Hela was cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) supplemented with 10 % FBS (fetal bovine serum) in an atmosphere of 5 % CO₂ and 95 % air at 37 °C. The cells were seeded in 24-well flat-bottomed plates and then incubated for 24 h at 37 °C under 5 % CO₂. Then the cells were incubated with 300 μM NaHS and 10.0 μM Neutral Red (NR) in an atmosphere of 5 % CO₂ and 95 % air for 1h at 37 °C. Neutral Red (NR) (5 PM) was used to costain the cells. Then the cells were incubation with 10.0 μM 1 for 1 h .Wash cells twice with 1 mL PBS at room temperature, and then add 1mL RPMI 1640 culture medium. Fluorescence cell imaging was performed with an (Olympus FV1000) confocal microscopy with a 40× objective lens. Fluorescence images of Hela cells were monitored at 405–440 nm and 585–610 nm for blue and red channels, respectively.

The cells of *C. elegans* and confocal microscope imaging

The *C. elegans* wild type strain N₂ was acquired from the Kunming Institute of Zoology. The larval stage 4 (L4) *C. elegans* was used. To expose the L4 stage nematodes to NaHS, the Petri dish was first filled with 1mL of M9 buffer supplemented with 0.5 mM AO and 0.3 mM NaHS at 20 °C for 6 h. After the incubation, the exposed nematodes were washed three times with M9 buffer by centrifugation at 3000 r/min for 2 minutes. For imaging of accumulations of H₂S in the nematode, the previously exposed worms were incubated in Petri dishes filled with M9 buffer, containing of 1 (10 μM), at 20 °C for 1 h. The images of the mounted nematodes were acquired by a confocal microscopy (Olympus FV1000) with a 20× objective lens. Fluorescence images of *C. elegans* were monitored at 405–440 nm and 510–550 nm for blue and red channels respectively.

The imaging of the *Drosophila* gut system

We cultured *Drosophila melanogaster* (*white eye, w*) into the glass vials with culture cornmeal medium containing NaHS with a concentration of 300 μM. After Three days, transferred *Drosophila melanogaster* which obtained a clouds of H₂S into the glass vials with medium containing 1 (10μM) for 15 h. The gut and ovary were dissected and a fluorescence microscope (Olympus IX71) with a 20×objective lens was used to observe the fluorescence.

The negative control experiment with *C. elegans*

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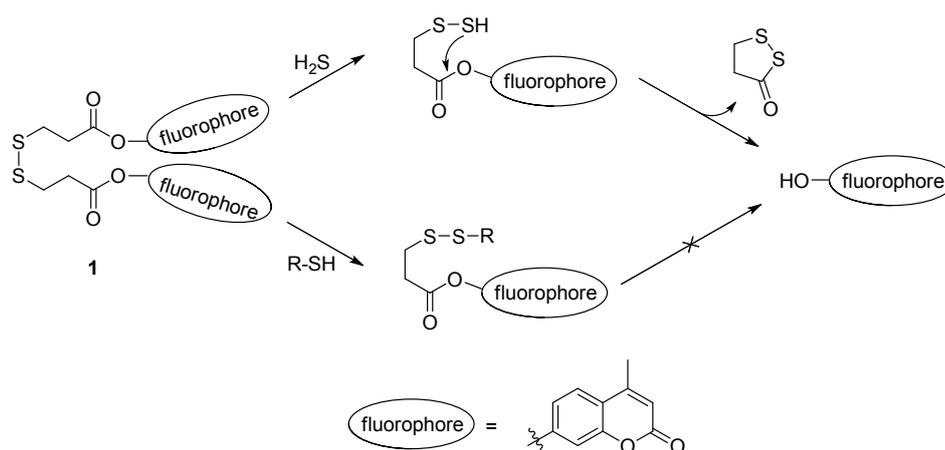
The negative control experiment with *Drosophila*

We cultured *Drosophila melanogaster* (*white eye, w*) into the glass vials with culture cornmeal medium containing 1 (10 μM) for 15 h. The gut and ovary were

dissected and a fluorescence microscope (Olympus IX71) with a 20×objective lens was used to observe the fluorescence.

The imaging of the transgenic nematodes

The young adults *srp-6(ok319)* nematodes was acquired from the Caenorhabditis Genetics Center (CGC). We divided the experiment into two groups. The worms were stained with M9 and H₂O (25 °C) respectively. Then the worms were stained with 1mM Acridine orange for 6h. Then feeding on media containing **1** (10μM) for 1h. After washing with M9 medium for three times, the worms were mounted in M9 onto microscope slides. The red fluorescence of Acridine orange and **1** were monitored using a fluorescence microscope (Olympus IX71)



Scheme S1. The proposed reaction-based binding mechanism of **1** with H₂S.

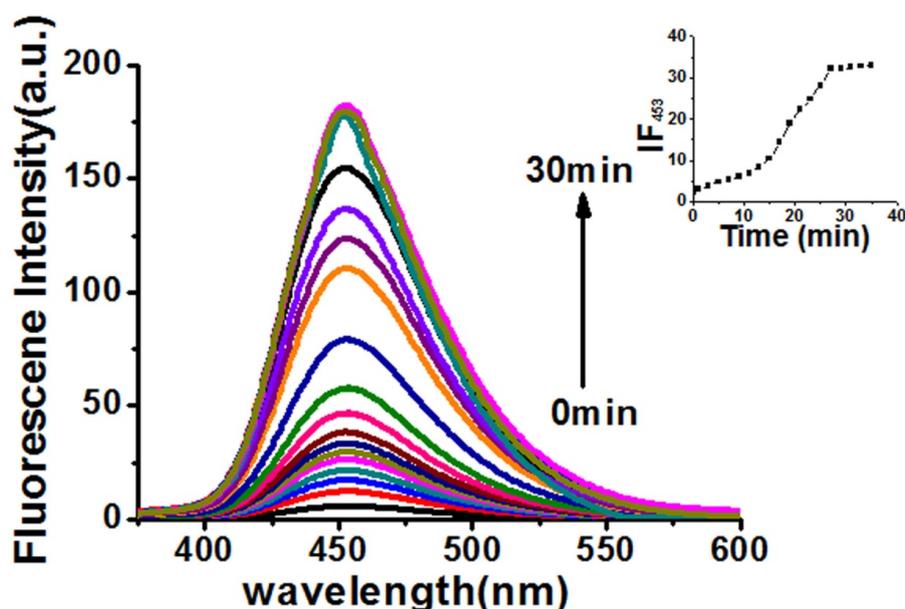


Figure S1. Time dependence of fluorescence intensity of **1** (20 μM) with 30 equiv of H₂S. Inset: Fluorescent emission intensity at 453 nm of **1** (20 μM) with 30 equiv of H₂S as a function of time

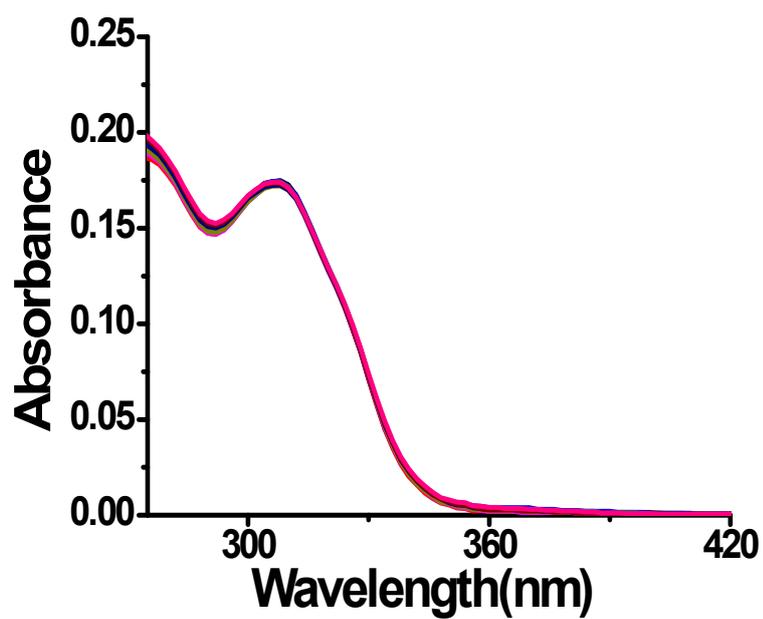


Figure S2. UV-vis absorption spectra of 10 μM probe in the presence of 0–30 equiv of NaHS in an aqueous solution (CTAB 1.0mM, $\text{CH}_3\text{CN}/\text{Tris-HCl} = 3:7$, pH = 7.4).

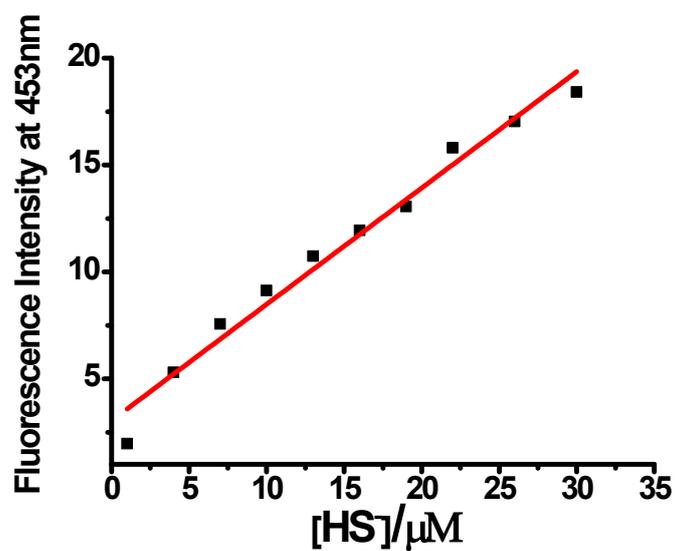


Figure S3. Fluorescence intensity of **1** (1 μM) versus increasing concentrations of H_2S . Excitation wavelength is 345 nm.

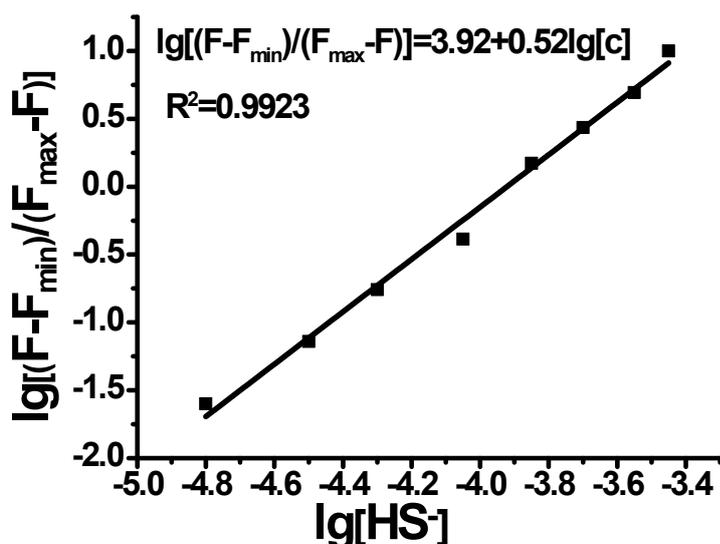


Figure S4. The equation plot of **1** with HS⁻.

$$\text{Log} \left(\frac{F - F_{\text{min}}}{F_{\text{max}} - F} \right) = 3.92 + 0.52 \lg[c]$$

$$\text{Log } k = 3.92, k = 8.3 \times 10^3$$

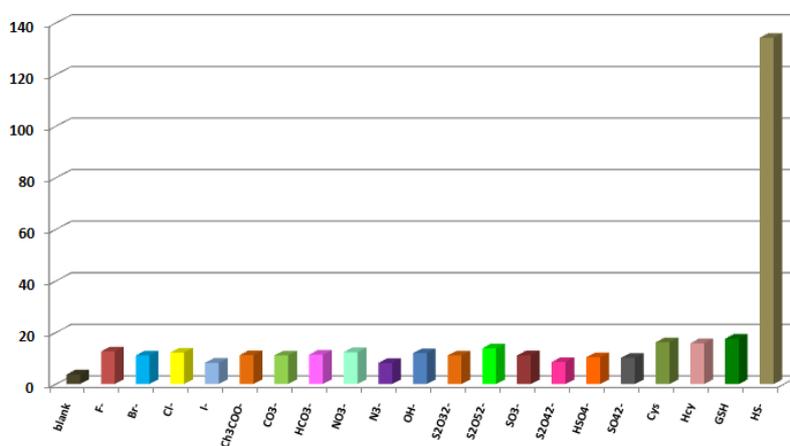


Figure S5. Fluorescence responses of 10 μM **1** in bovine serum to various analytes in aqueous solution (CTAB 1.0 mM, CH₃CN/Tris-HCl = 3:7, pH = 7.4, 37 °C). Excitation at 345 nm. Bars represent the final fluorescence intensity of **1** with 0.3 mM analytes over the original emission of free **1**. 1) blank; 2) K⁺; 3) Mg²⁺; 4) Na⁺; 5) Br⁻; 6) Cl⁻; 7) F⁻; 8) I⁻; 9) CH₃COO⁻; 10) CO₃²⁻; 11) HCO₃⁻; 12) NO₃⁻; 13) N₃⁻; 14) OH⁻; 15) S₂O₃²⁻; 16) S₂O₅²⁻; 17) SO₃⁻; 18) SO₃²⁻; 19) HSO₄⁻; 20) SO₄²⁻; 21) cysteine; 22) homocysteine; 23) glutathione; 24) NaHS.

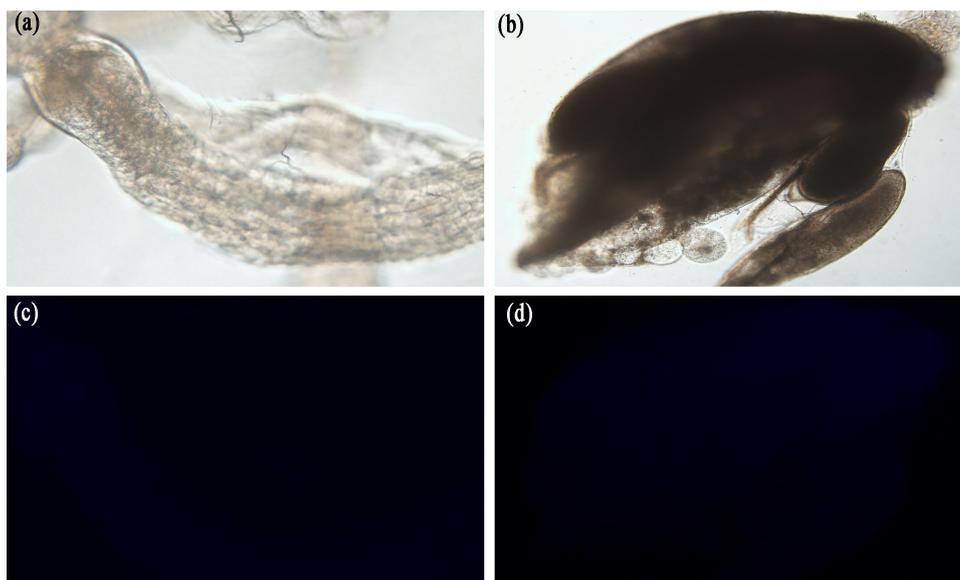


Figure S6. Detection of *D. melanogaster* only in the presence of **1** ($10\mu\text{M}$). (a) and (b): Bright-field images. (c) Fluorescence image of mid gut. (d) Fluorescence image of ovary.

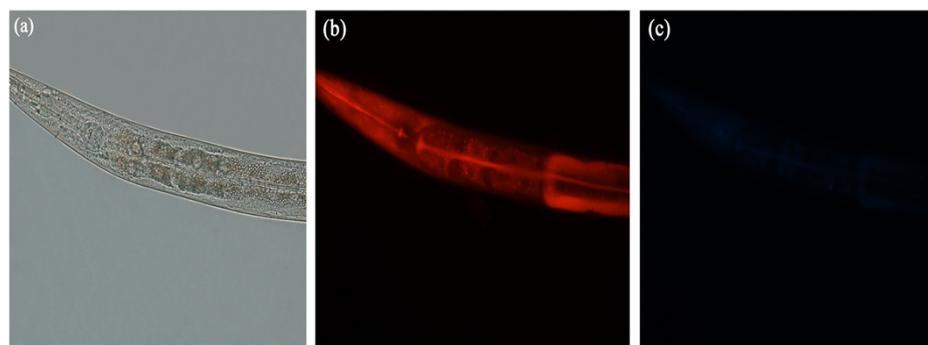


Figure S7. The negative control experiment with *C. elegans* only in the presence of **1** ($10\mu\text{M}$). (a) Bright field image. (b) $500\ \mu\text{M}$ AO incubated for 4 h at $20\ ^\circ\text{C}$ (Channel 2: $\lambda_{\text{ex}} = 485\ \text{nm}$, $\lambda_{\text{em}} = 510\text{--}550\ \text{nm}$). (c) $10.0\ \mu\text{M}$ **1** incubated for 30 min at $20\ ^\circ\text{C}$ (Channel 1: $\lambda_{\text{ex}} = 385\ \text{nm}$, $\lambda_{\text{em}} = 405\text{--}440\ \text{nm}$).

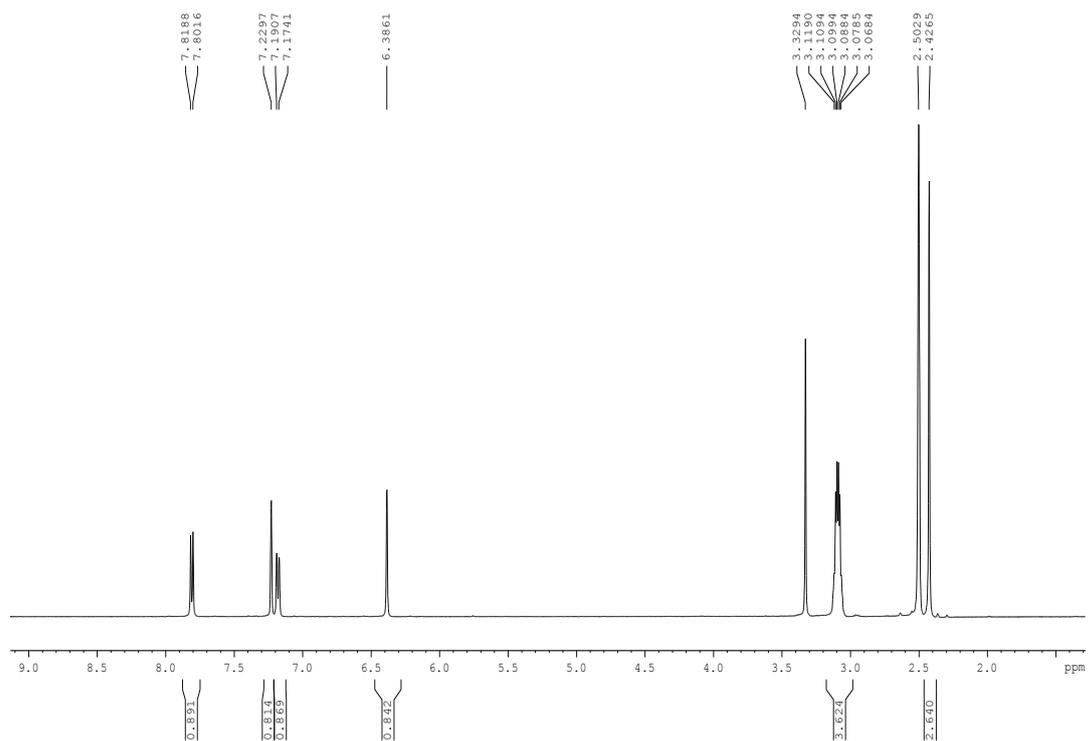


Figure S8. ^1H NMR (DMSO,500 MHz) spectra of compound **1**.

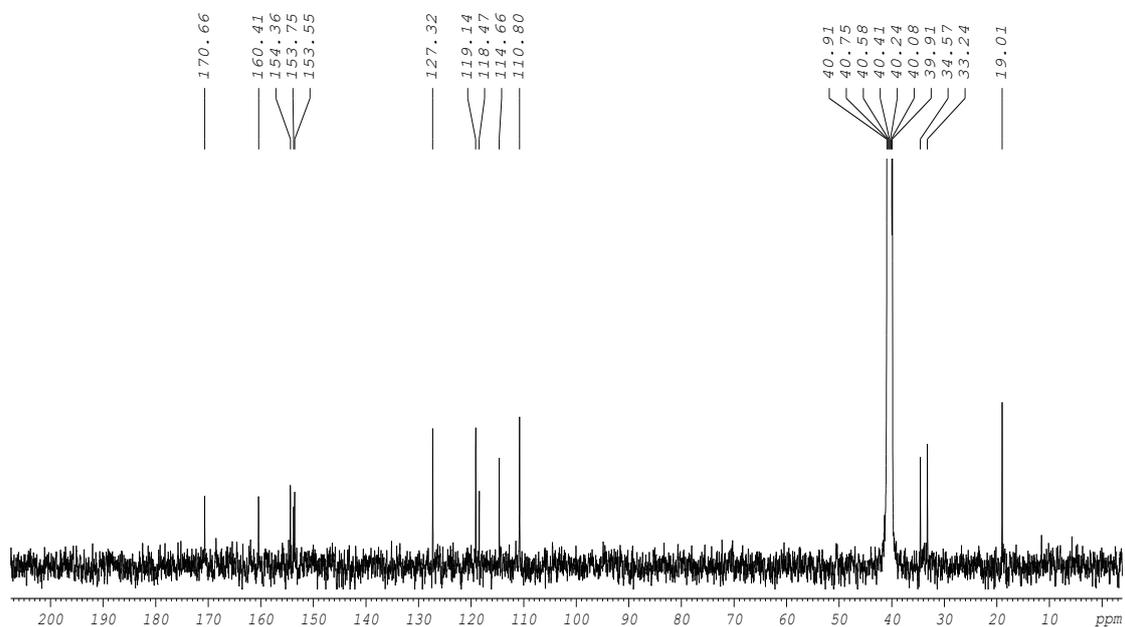


Figure S9. ^{13}C NMR (DMSO, 500 MHz) spectra of compound **1**.

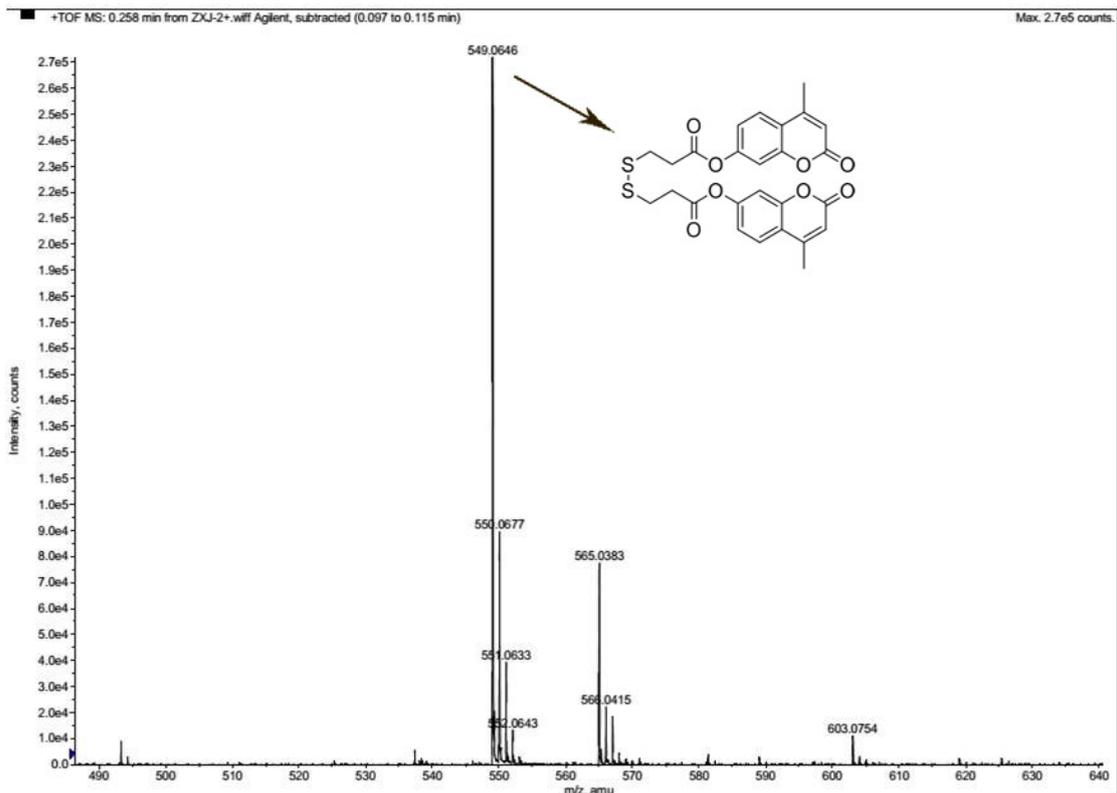


Figure S10. FAB mass of compound 1.

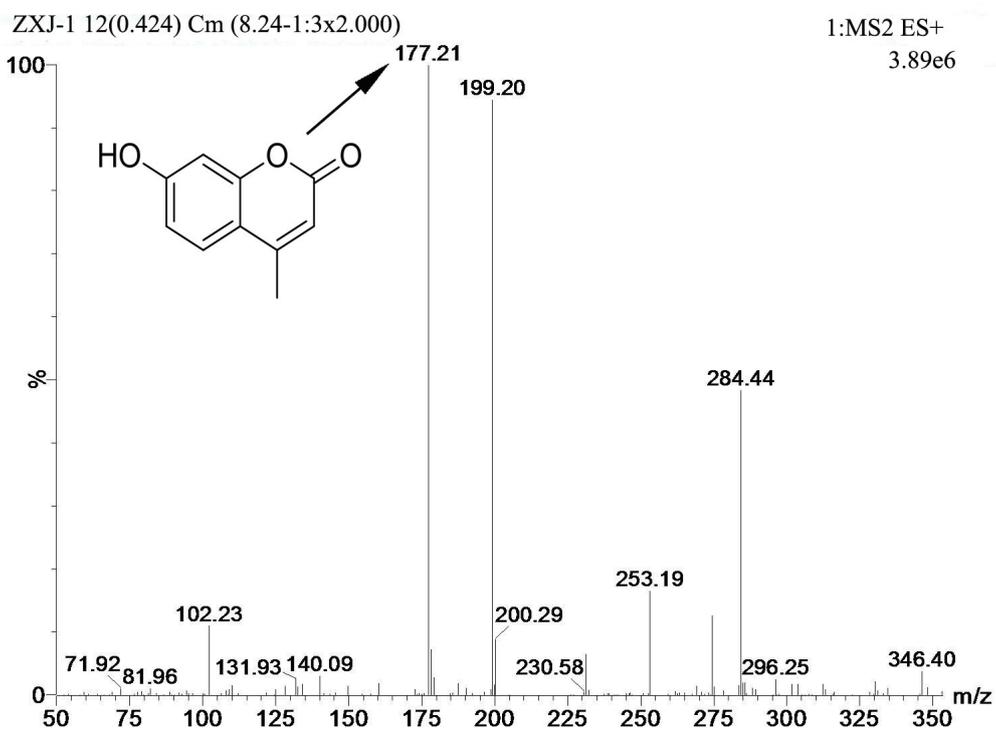


Figure S11. FAB mass of compound.

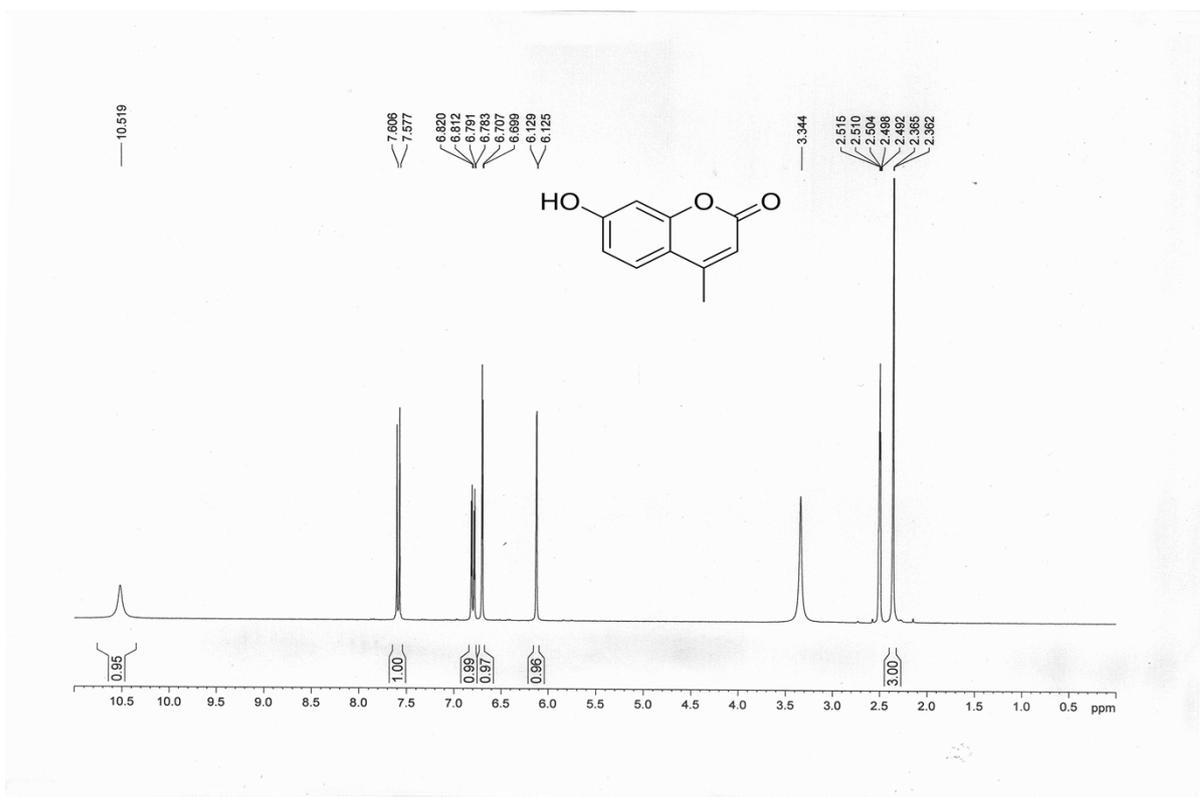


Figure S12. ¹H NMR (DMSO, 500 MHz) spectra of fluorophore.

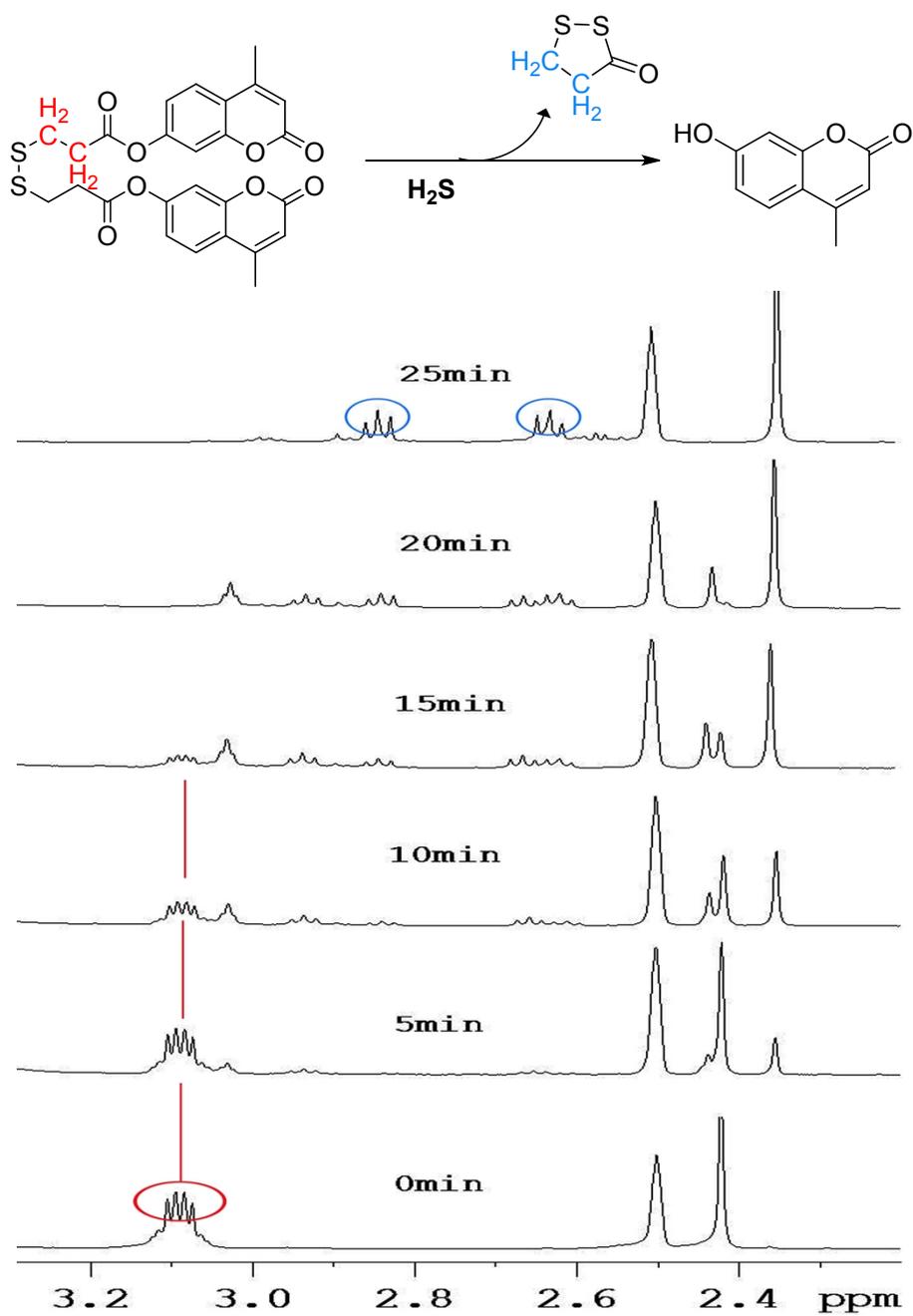


Figure S13. The proposed reaction-based binding mechanism of **1** with H₂S, and partial ¹H NMR spectra of **1** in DMSO-d₆ with H₂S (5, 10, 15, 20 and 25 min); (NaHS were dissolved in D₂O as stock solution.)

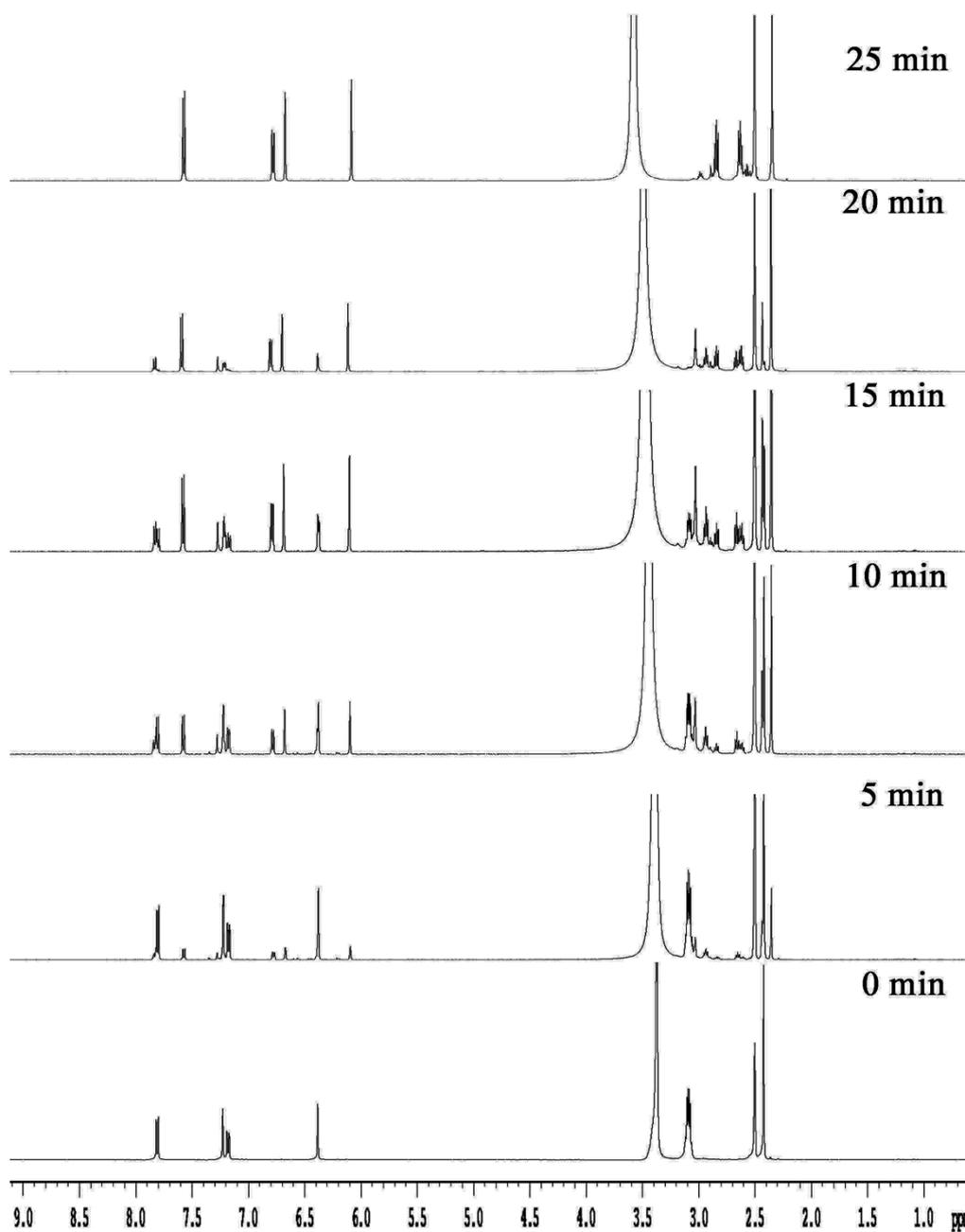


Figure S14. The whole ^1H NMR spectra of **1** in DMSO-d_6 with H_2S (5, 10, 15, 20 and 25 min); (NaHS were dissolved in D_2O as stock solution.)

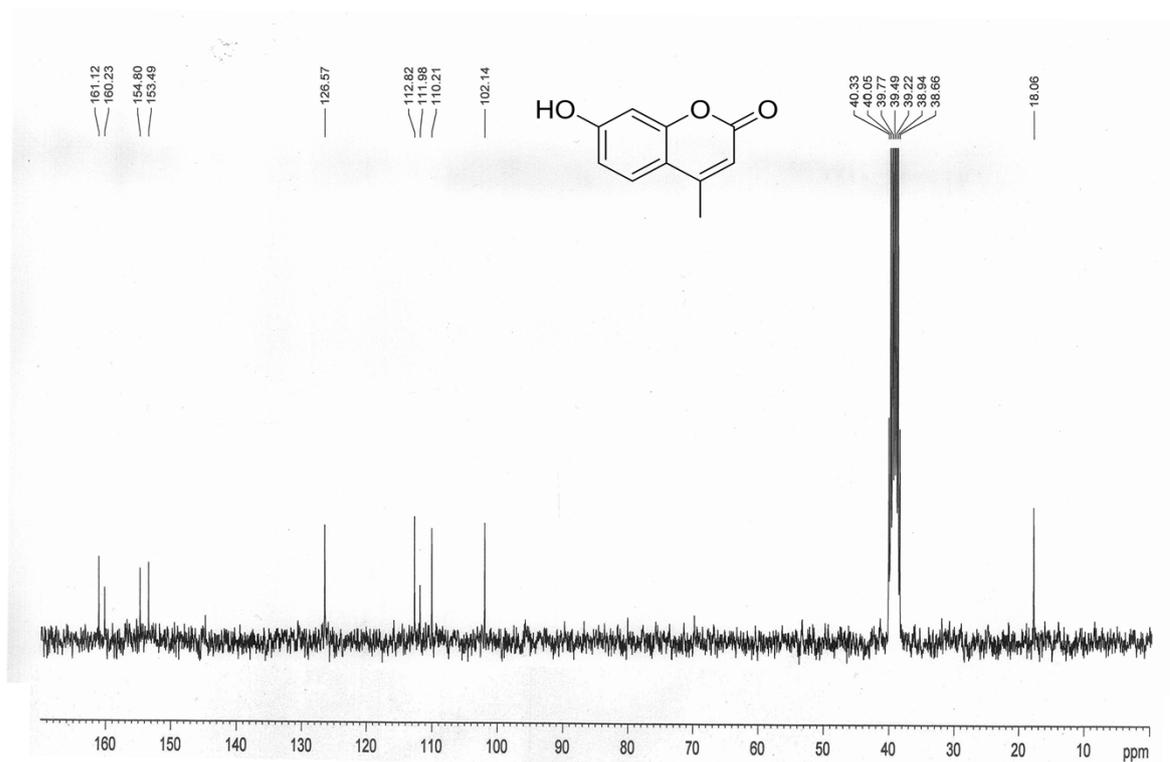


Figure S15. ^{13}C NMR (DMSO, 500 MHz) spectra of fluorophore.

Reference:

[1] B. Zhu, C. Gao, Y. Zhao, C. Liu, Y. Li, Q. Wei, Z. Ma, B. Du and X. Zhang, *Chem. Commun.*, 2011, 47, 8656-8658.