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Supporting Information for

Development of an activatable hydrogen sulfide specific fluorescent probe for bioimaging in the air pouch inflammation

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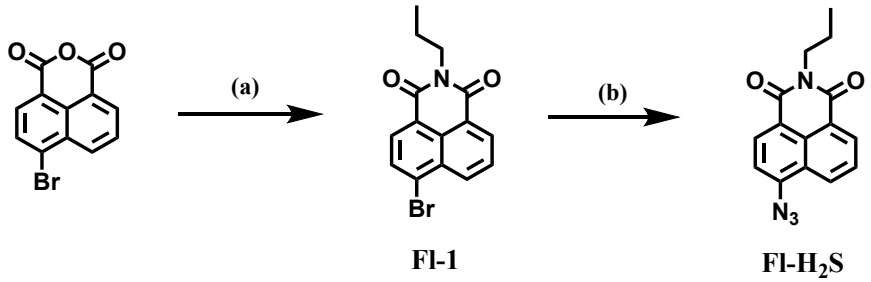
Experimental Section

1. Materials and Instrumentation.

All reagents and solvents were obtained from commercial suppliers without further purification. Solvents were purified by standard methods prior. Aqueous solutions were all prepared using ultrapure water (18.2 MΩ·cm) from a ULPURE water purification system and all glassware were cleaned with ultrapure water, and dried before use.

TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals. The pH measurements were performed with PHS-3E pH meter. UV-vis absorption spectra were obtained on a Shimadzu UV-2700 spectrophotometer, and fluorescence spectra were measured on a HITACHI F4700 fluorescence spectrophotometer (PMT Voltage, 600v; EX Slit,5nm; EM Slit, 5 nm; λ_{ex} =440 nm, λ_{em} = 561 nm). CCK-8 for cell cytotoxicity assays was purchased from TransGen Biotechnology. The imaging of mice was performed with Small Animal Imaging System (IVIS Lumina III). ^{1}H and ^{13}C NMR spectra were measured on a Varian Unity 600 spectrometer. High resolution mass spectrometric (HRMS) analyses were measured on Brooke solanX 70 FT-MS, Agilent 6540T.

2. Synthesis Procedure.



Fl-1 **Fl-H_2S**

Scheme S1 Synthetic route of probe **Fl-H_2S**.

3. Spectroscopic Measurement.

The stock solution of 2 mM the probe was prepared freshly in dry DMSO. For H_2S response experiments, UV-Vis absorption spectroscopy were monitored 60 min after mixing in PBS buffer (10 mM, pH 7.4, 1% DMSO) at 37 °C.

4. Cell culture and cytotoxicity assays.

HeLa cells were cultured in DMEM and 1640 (Dulbecco's modified Eagle's medium or Roswell Park Memorial Institue medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO_2 and 95% air at 37 °C. In vitro cytotoxicity was measured using CCK-8 assay on HeLa cells. The Cells were seeded into a 96-well tissue culture plate in the presence of completed Dulbecco's modified Eagle's medium (DMEM) or Roswell Park Memorial Institue medium (1640) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin

at 37 °C and 5% CO_2 atmosphere overnight followed by incubation for 24 h in the presence of probe **Fl-H_2S** at different concentrations (1, 2, 5, 10, 20, 30, 40, 50 μM). A commercial cell counting kit-8 (CCK-8) (TransGen Biotechnology, China) was used to detecting the cell viability and the assay was run following the manufactures' instructions. The cell viability was determined by assuming 100% cell viability for cells without probe **Fl-H_2S**.

5. Fluorescence Imaging of Cells.

For the fluorescence imaging experiment of exogenous H_2S, the cells were incubated with 5 μM **Fl-H_2S** for 0.5 h. Then, NaHS (0 μM, 100 μM, 200 μM, 300 μM) was added for incubation of another 1 h.

For the fluorescence imaging experiment of endogenous H_2S in the live HeLa cells, we conducted three experiments. For the control experiment group (**Fl-H_2S** group), the HeLa cells were incubated with 10 μM **Fl-H_2S** for 1h. For the experiment group (**Cys+ Fl-H_2S** group), the HeLa cells were pretreated with 250 μM Cys for 1 h and then incubated with 10 μM **Fl-H_2S** for 1 h. For the negative control experiment group (**NEM+Fl-H_2S** group), the HeLa cells were pretreated with 500 μM NEM for 60 min and then incubated with 10 μM **Fl-H_2S** for 1 h. The fluorescence images were further analysed using software Image J.

Finally, the probe imaging under a confocal imaging microscope (Leica TCS SP8 CARS confocal microscope with a 100 × objective lens). The particular observation conditions were as follows: green channel, (One-photon imaging：λ_{ex} = 488 nm, λ_{em} = 500-600 nm; Two-photon imaging：λ_{ex} = 880 nm, λ_{em} = 500-600 nm.)

6. Statistical Analysis.

The data were expressed as mean ± SD. Statistical calculation of experimental data was using the one-way ANOVA statistical analysis. The data were classified with p values and denoted by （NS) for $p > 0.05$, (*) for $p < 0.05$ and (**) for $p < 0.01$, (***) for $p < 0.001$.

7. Acquisition and imaging of mouse liver tissue

Tissue sections were obtained from the liver tissue of mice, after cut to 400 mm thickness in 25 mM PBS (pH=7.4) with vibrating-blade. Then they were transferred to a glass bottom plate and observed under two-photon confocal microscope (Leica SP8). Fluorescence images of the slices were obtained from 880 nm excitation and fluorescence emission windows of 500-600 nm.

8. *Visualization of H_2S detection in mice*.

All animal experiments were in accordance with relevant laws and regulations, and approved by the Institutional Animal Protection and Utilization Committee of Guangxi University. Female BALB/C Kunming mice (6-8 weeks of age) were collected from Experimental Animal Center of Guangxi Medical University (Nanning, China). Each group had three mice to in vivo fluorescence imaging. The air balloon inflammation of mice was established according to the following steps,

and sterile air was injected into the same place after 3 h. First, 3 mL of sterile air was injected subcutaneously into the back of female Kunming mice to form an air pouch. Repeat the above operation for 7 days to form air pouches. Each group underwent fluorescence imaging by in situ injection of probes or drugs into the air pouch. Finally, Blank group, fluorescence imaging of blank mice. Control group, fluorescence imaging after 60 min injection of **Fl-H_2S** (30 μM) in live mice in the absence of NaHS. +15 equiv NaHS group, fluorescence imaging 60 min after injection of NaHS (15 equiv) followed by **Fl-H_2S** (30 μM) in live mice. +30 equiv NaHS group, fluorescence imaging 60 min after injection of NaHS (30 equiv) followed by **Fl-H_2S** (30 μM) in live mice. Before in vivo imaging, the hair around the air pouch was removed by an electric shaver and then, the mice were anesthetized by a 4% chloral hydrate aqueous solution (100 μL). Fluorescence imaging was done at Guangxi University. (Small Animal Imaging System, IVIS Lumina III and USA). The excitation wavelength for all fluorescence images was 440 nm and the emission wavelength was 570 nm.

9. Mouse air pouch inflammation model.

All animal experiments were in accordance with relevant laws and regulations, and approved by the Institutional Animal Protection and Utilization Committee of Guangxi University. Female BALB/C Kunming mice (6-8 weeks of age) were collected from Experimental Animal Center of Guangxi Medical University (Nanning, China). Each group had three mice to in vivo fluorescence imaging. The air balloon inflammation of mice was established according to the following steps, and sterile air was injected into the same place after 3 h. First, 3 mL of sterile air was injected subcutaneously into the back of female Kunming mice to form an air pouch. Repeat the above operation for 7 days to form air pouches. Finally, **LPS+Fl-H_2S** group, Mice of air pouches were pretreated with LPS（100 μg/mL,100 μL）to induce inflammatory response to establish a mouse air pouch model, and then incubated with **Fl-H_2S** (30 μM) for 60 min incubation for fluorescence imaging. **SPSS+Fl-H_2S** group, Mice of air pouches were pretreated with stroke-physiological saline solution (SPSS), and then incubated with **Fl-H_2S** (30 μM) for 60 min incubation for fluorescence imaging. **NEM+Fl-H_2S** group, Mice of air pouches were pretreated with LPS（100 μg/mL,100 μL） to induce inflammatory response to establish a mouse air pouch model, then treated with NEM（500 μg/mL,100 μL） for 1 h, and finally added with **Fl-H_2S** (30 μM) for 60 min for fluorescence imaging. The excitation wavelength for all fluorescence images was 440 nm and the emission wavelength was 570 nm.

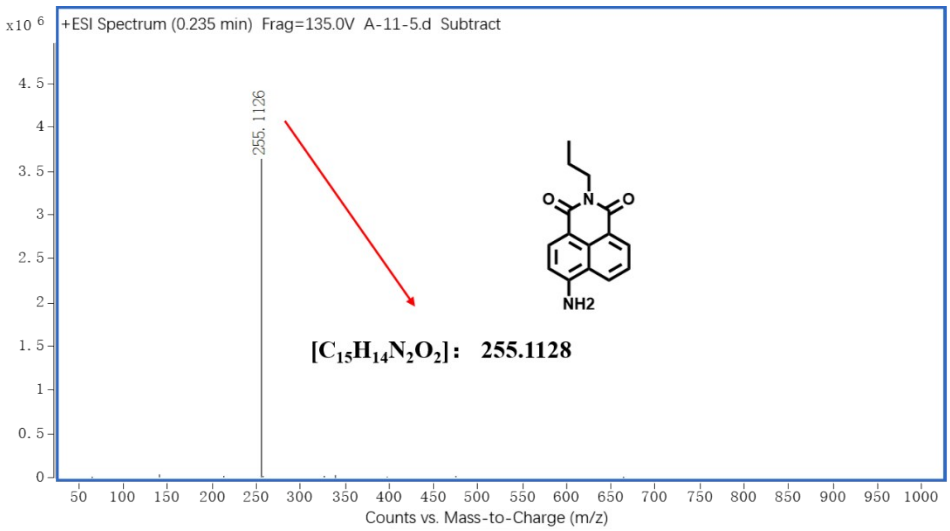


Fig. S1 ESI-MS spectrum of **Fl-H_2S** with the addition of NaHS.

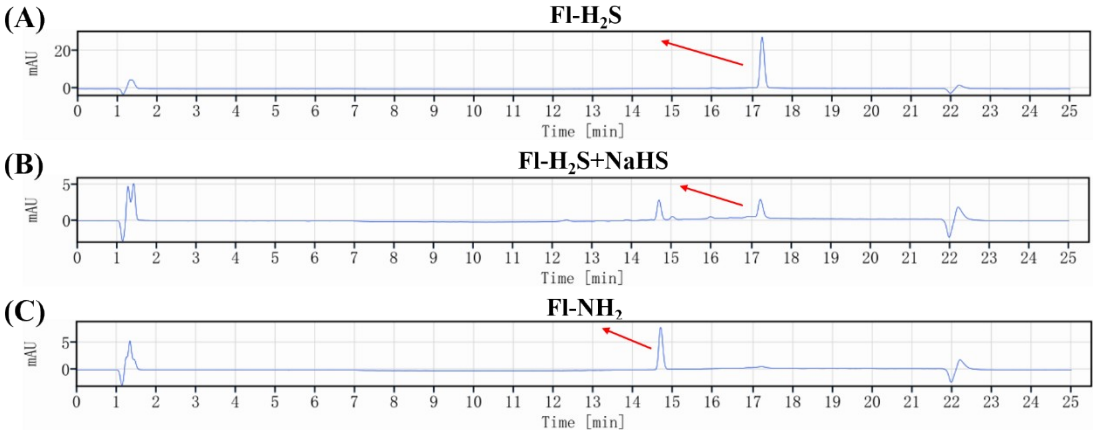


Fig. S2 The response mechanism of **FI-H_2S** to H_2S was verified by HPLC. (A) HPLC data of probe **FI-H_2S**; (B) HPLC data of probe **FI-H_2S** after addition of H_2S; (C) HPLC data of **FI-NH_2**.

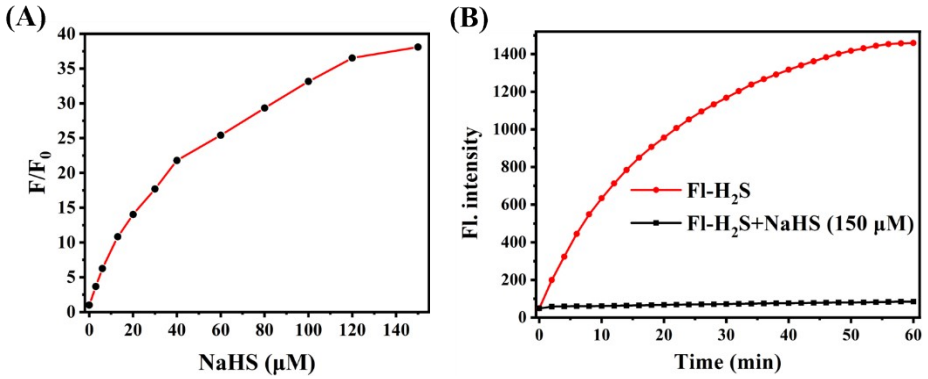


Fig. S3 (A) Fluorescence intensity ratio (F/F_0, F_0 is the fluorescence intensity of **Fl-H_2S** in the absence of NaHS.) changes at 561 nm of **Fl-H_2S** (5 μM) with the amount of NaHS (0-150 μM). (B) Time response profiles of **Fl-H_2S** (5 μM) to 150 μM of NaHS. The fluorescence intensities at 561 nm were continuously monitored at time intervals.

$\lambda_{ex/em}$ = 440/561 nm.

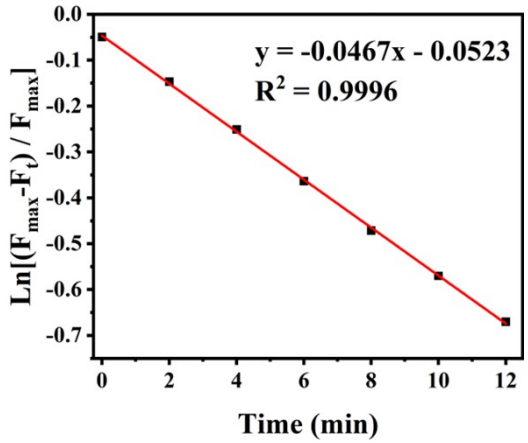


Fig. S4 Pseudo-first-order kinetic plot of the reaction of **Fl-H_2S** (5 μM) with NaHS (150 μM). Slope = 0.05 min^{-1}.

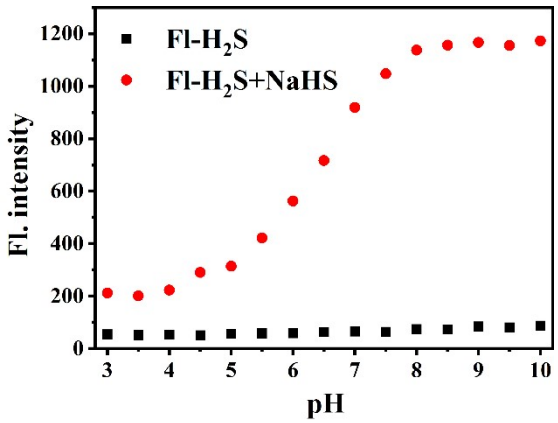


Fig. S5 Fluorescence intensity changes of **Fl-H_2S** (5 μM) at different pH values in the absence (■) or presence (●) of NaHS (150 μM).

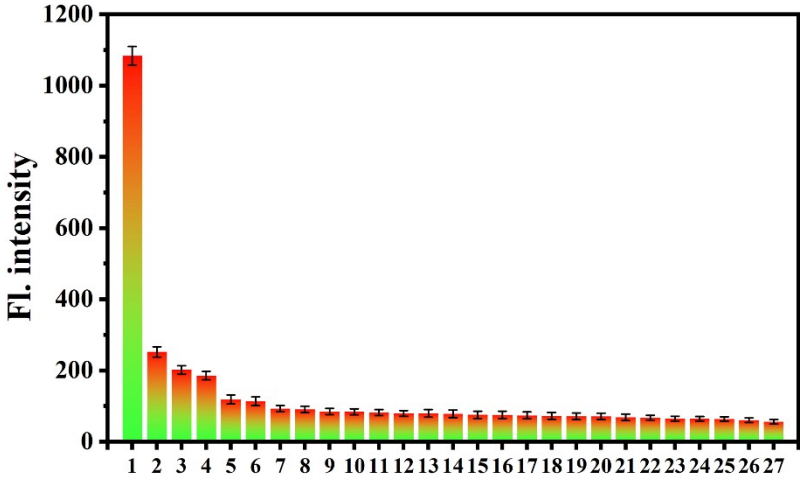


Fig. S6 Fluorescence responses of **Fl-H_2S** (5μM) in the presence of various relevant analytes. After the incubation of **Fl-H_2S** with the analytes for 60 min, the data were obtained. (1) NaHS (100 μM), (2) DL-Hcy (1 mM), (3) L-Glutathione (1 mM), (4) L-Cys (1 mM), (5) methylglyoxal (200 μM), (6) NaSN (200 μM), (7) NaClO (200 μM),

(8) Na_2SO_3 (200 μM), (9) L-Serine (2 mM), (10) glyoxal (200 μM), (11) pyruvic acid (200 μM), (12) acetaldehyde (200 μM), (13) KCl (2 mM), (14) chloral hydrate (200 μM), (15) sodium pyruvate (200 μM), (16) $CaCl_2$ (2 mM), (17) Formaldehyde (200 μM), (18) H_2O_2 (200 μM), (19) acetone (200 μM), (20) $MgCl_2$ (2 mM), (21) L-Phenylalanine (2 mM), (22) L-Arg (2 mM), (23) Tert-Butylhydroperoxid (200 μM), (24) L-Glysine (2 mM), (25) L-Histidine (2 mM), (26) $NaNO_2$ (200 μM), (27) Glucose (2 mM). $\lambda_{ex/em}$ = 440/561 nm. Error bars represent standard deviation (± S.D.), n = 3.

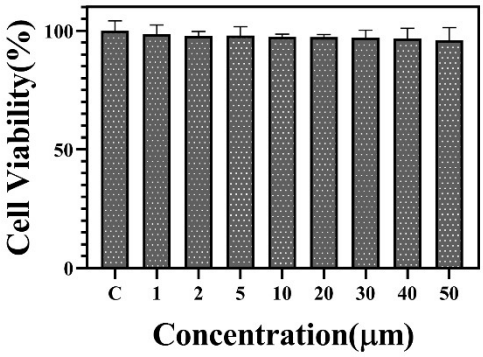


Fig. S7 Survival rate of HeLa cells after 24 h of incubation with different concentrations of **Fl-H_2S** by CCK-8 assays.

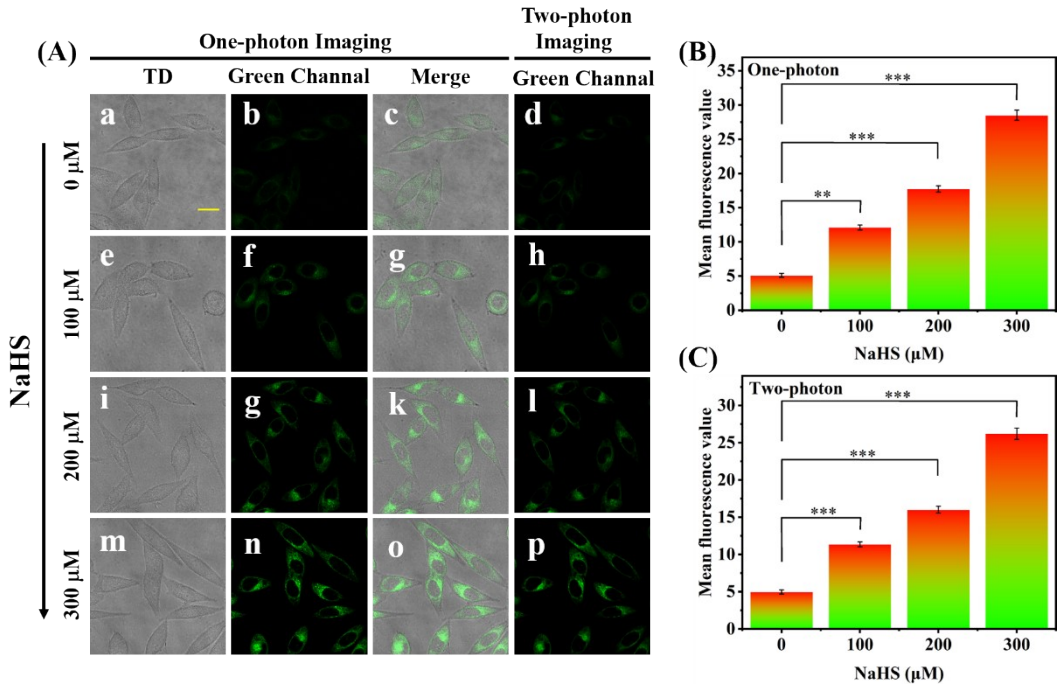


Fig. S8 (A) Fluorescence imaging of exogenous H_2S in living HeLa cells with **Fl-H_2S** in one-photon mode and two-photon mode. (a-d) HeLa cells were added with **Fl-H_2S** (10 μM) and stained for 60 min. (e-h) HeLa cells were treated with NaHS (100 μM) for 30 min, finally incubated by **Fl-H_2S** (10 μM) 60 min. (i-l) HeLa cells were pretreated with NaHS (200 μM) for 30 min, and then treated with **Fl-H_2S** (10 μM) for 60 min. (m-p) HeLa cells were pretreated with H_2S (300 μM) for 30 min, and then treated with **Fl-H_2S** (10 μM) for 60 min.(B) Fluorescence intensities of b, f, g and n channels at one-photon mode. (C) Fluorescence intensities of d, h, l and p channels at

two-photon mode. Scale bar: 20 μm. Error bars represent standard deviation (± S.D.), n = 3.

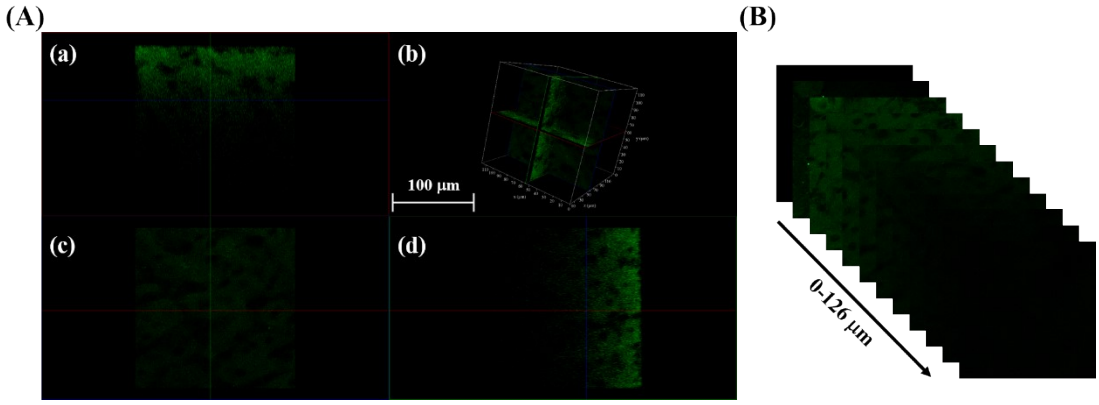


Fig. S9 (A) 3D Image of mouse tissue liver sections pre-treated with **Fl-H_2S** (30 μM) at a depth of 0-126 μm in two-photon mode. (a)The change of the fluorescence signal intensity of **Fl-H_2S** in the Y-axis section of the liver tissue; (b) 3D imaging of **Fl-H_2S** in liver tissue; (c) The change of the fluorescence signal intensity of **Fl-H_2S** in the X-axis section of the liver tissue; (d) The change of the fluorescence signal intensity of **Fl-H_2S** in the Z-axis section of the liver tissue; (B) Penetrating depth of mouse tissue liver sections pre-treated and **Fl-H_2S** (30 μM) at a depth of 0-126 μm in two-photon mode. λ_{em} = 500-600 nm, λ_{ex} =880 nm.

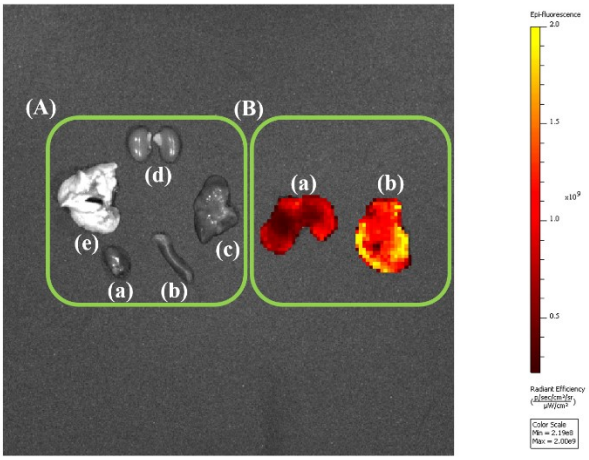


Fig. S10 The fluorescence image of the tissue and the fluorescence image of the tissue pretreated with **Fl-H_2S** and H2S. (A) Fluorescence imaging of blank tissue. (a), heart; (b) spleen; (c), liver; (d), kidney; (e), lung. (B) Fluorescence imaging of liver tissue with **Fl-H_2S** in the presence and absence of H_2S. (a) Fluorescence imaging of liver tissue pretreated only with **Fl-H_2S** (30 μM). (b) Fluorescence imaging of liver tissue pretreated with **Fl-H_2S** (30 μM) and H_2S (30 equiv).

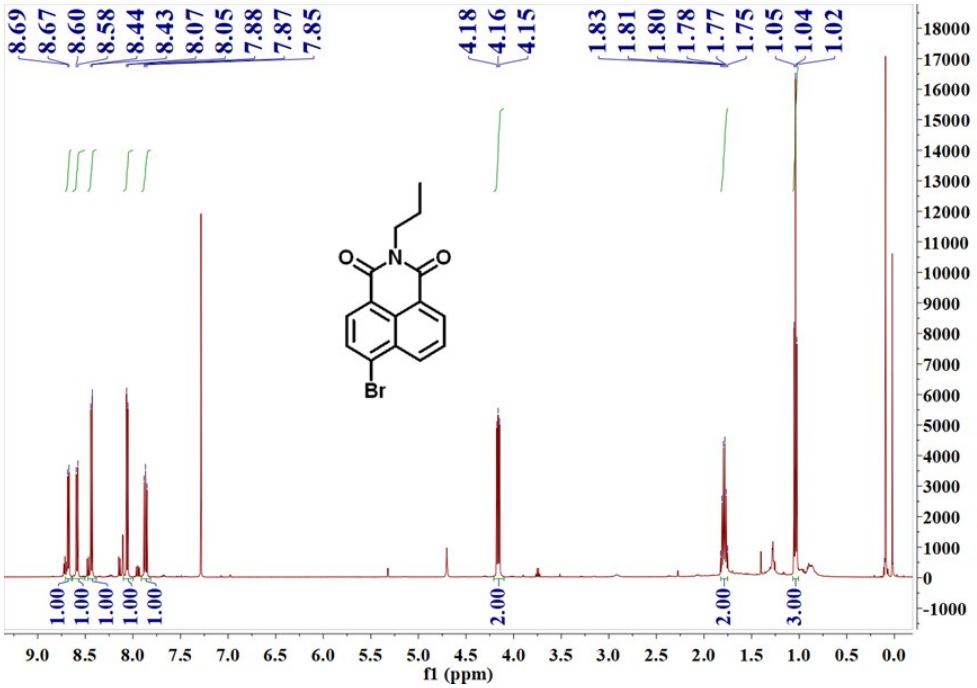


Fig. S11 ^{1}H NMR of Compound **Fl-1**.

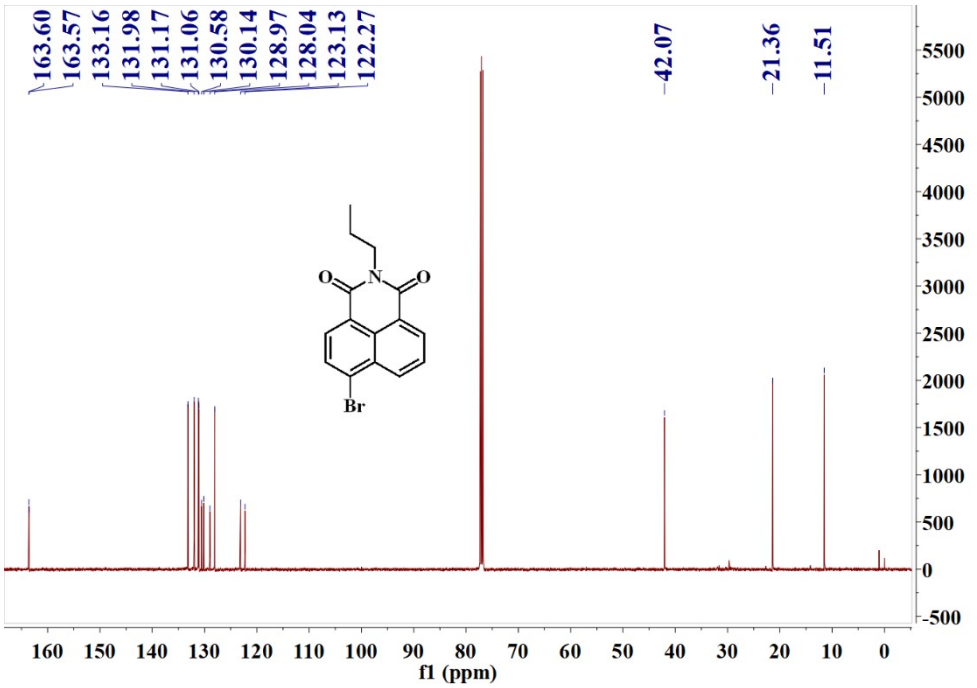


Fig. S12 ^{13}C NMR of Compound **Fl-1**.

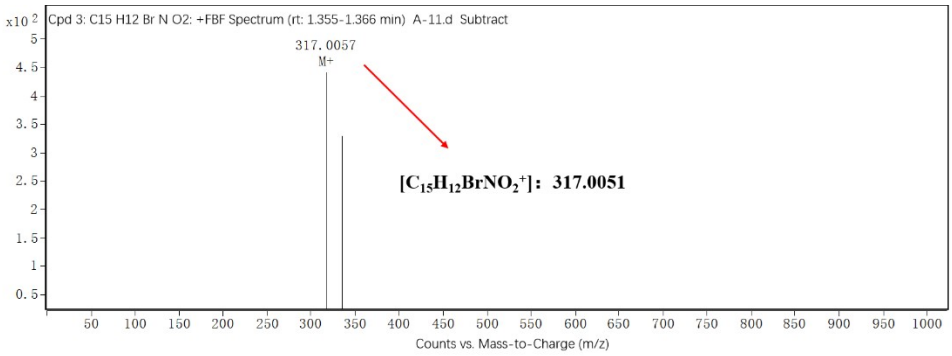


Fig. S13 ESI-MS spectrum of **Fl-1**.

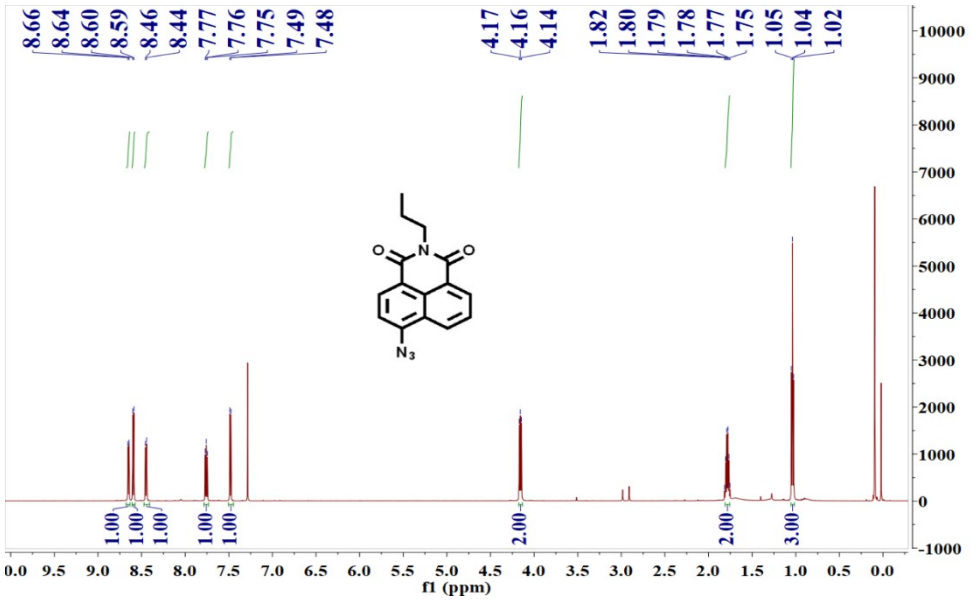


Fig. S14 ^{1}H NMR of dye platform **Fl-H_2S**.

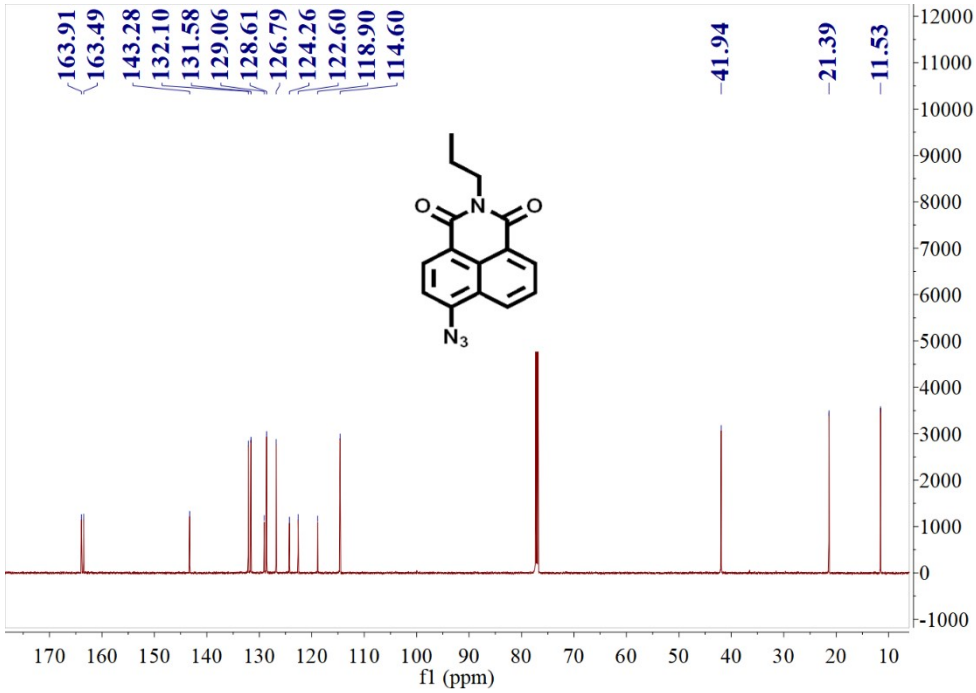


Fig. S15 ^{13}C NMR of **Fl-H_2S**.

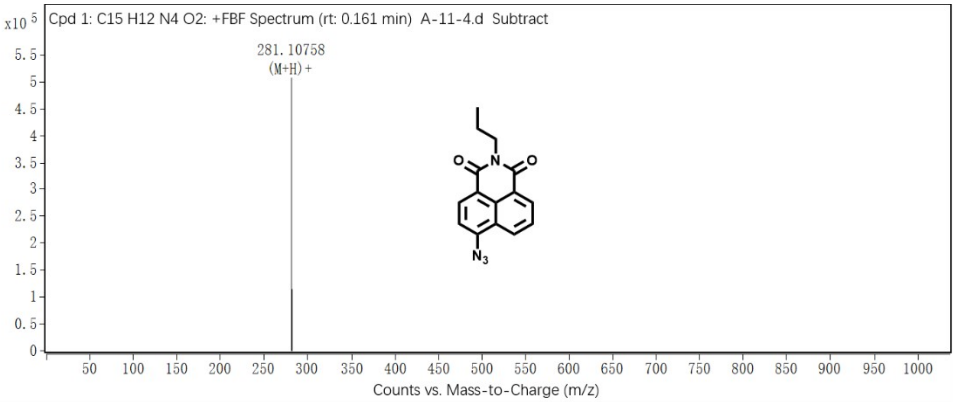


Fig. S16 ESI-MS spectrum of **Fl-H_2S**.