Supporting Information

An ICT-Based Colorimetric and Ratiometric Fluorescent Probe for Hydrogen Sulfide and Its Application in Live-Cell Imaging

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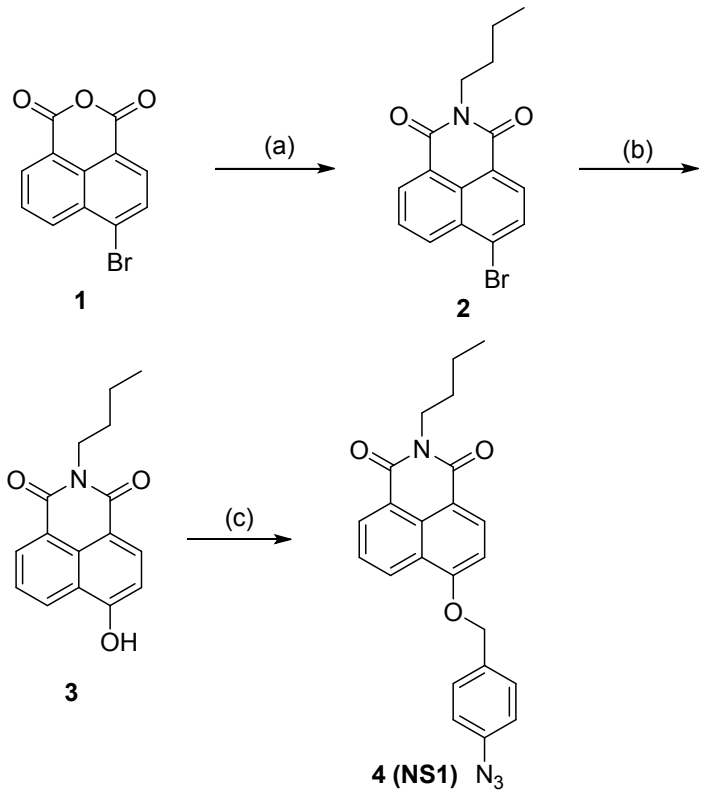
Contents

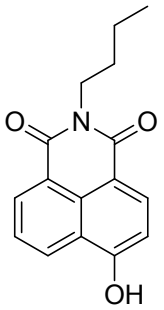
Materials and methods

All the solvents were of analytic grade. NMR experiments were carried out on a Bruker AV-400 NMR spectrometer with chemical shifts reported in ppm (in $CDCl_3$, *d*-DMSO, D_2O, or TMS as an internal standard). Mass spectra were measured on an Agilent 1290 LC-MS spectrometer. All pH measurements were made with a Sartorius basic pH-Meter PB-10. Fluorescence spectra were determined on a PerkinElmer LS55 Fluorescence spectrophotometer. Absorption spectra were collected on a Shimadzu UV 2501(PC)S UV-Visible spectrophotometer. The excitation and emission widths for measurements were all 3 nm. All the cation solutions were prepared from GSH, Cys, $NaHSO_3$, $Na_2S_2O_4$, $Na_2S_2O_3$, Na_2SO_3, NaClO, KI, $CuCl_2$, $FeCl_3$, and NaHS in distilled water, with a concentration of 1 mM~~, respectively~~.

Synthesis and characterization

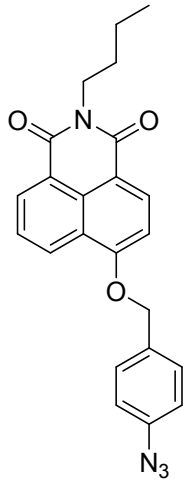


Scheme S1 Synthesis of **NS1**: (a) *n*-butylamine, ethanol, reflux, 8 h, 93%; (b) i) NaOMe, MeOH, reflux, 12 h; ii) HI (57%), H_2O, reflux, 12 h, 65% over two steps; (c) 1-azido-4-(chloro-methyl) benzene, K_2CO_3, acetone, reflux, 12 h, 72%.



3

N-butyl-4-hydroxy-1, 8-naphthalimide (3), was obtained according to a published procedure.[1] ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.81 (brs, 1H), 8.48 (d, J = 8.3 Hz, 1H), 8.41 (d, J = 7.2 Hz, 1H), 8.28 (d, J = 8.0 Hz, 1H). 7.70 (t, J = 7.6 Hz, 1H), 7.11 (d, J = 8.0 Hz, 1H), 3.98 (t, J = 7.3 Hz, 2H), 1.57 (p, J = 7.5 Hz, 2H), 1.32 (h, J = 7.3 Hz, 2H), 0.90 (t, J = 7.3 Hz, 3H).



4 (NS1)

4-(4′-azidobenzyloxy)-N-butyl-1, 8-naphthalimide (4, NS1): compound **3** (100 mg, 0.37 mmol), K_2CO_3 (103 mg, 0.74 mmol), KI (62 mg, 0.37 mmol), and 1-azido-4-(chloromethyl) benzene (68 mg, 0.41 mmol) were dissolved in acetone (10 mL), and the mixture solution was refluxed for 12 h until all the starting material was consumed, which was monitored by TLC analysis. Then water (50 mL) was added, and the mixture solution was extracted with dichloromethane (3 × 20 mL). The extract was dried over sodium sulfate and then concentrated under vacuum. The product was purified by flash chromatography using pure dichloromethane as the eluant to give **4** as a pale white solid (107 mg, 72%).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.53 (m, 3H), 7.66 (t, J = 7.7 Hz, 1H), 7.51 (d, J = 8.0 Hz, 2H), 7.08 (d, J = 8.0 Hz, 2H), 7.06 (d, J = 7.6 Hz, 1H), 5.31 (s, 2H), 4.15 (t, J = 7.3 Hz, 2H), 1.71 (p, J = 7.0 Hz, 2H), 1.45 (h, J = 15.1, 7.4 Hz, 2H), 0.97 (t, J = 7.3 Hz, 3H).

^{13}C NMR (100 MHz, Chloroform-*d*) δ 164.34, 163.76, 159.45, 140.40, 133.10, 132.13, 131.47, 129.31, 129.20, 128.45, 125.97, 123.49, 122.44, 119.37, 115.42, 106.27, 70.21, 40.05, 30.21, 20.36, 13.82.

HR-MS (TOF-ESI): *Calcd*. for $([M])^+$, 401.1614; Found, 401.1614.

Photophysical properties of NS1

Table S1 Photophysical properties of the probe.

entry	λ_{ab} (nm)	λ_{em} (nm)	Φ^a	ε / M^{-1} cm^{-1}
NS1	370	444	0.11	9623
NS1+NaHS	455	539	0.20[b]	14912

(a) The quantum yield (Φ) of **NS1** and **NS1**-NaHS systems ~~were~~ was determined according to the literature.[2] (b) Φ was determined in the present of 100.0 equiv. of NaHS.

$$\Phi_{Sample} = \frac{\Phi_{QS} \cdot A_{QS} \cdot F_{Sample} \cdot \lambda_{exQS} \cdot \eta^2_{Sample}}{A_{Sample} \cdot F_{QS} \cdot \lambda_{exSample} \cdot \eta^2_{QS}}$$

Where Φ is quantum yield; A is absorbance at the excitation wavelength; F is integrated area under the corrected emission spectra; λ_{ex} is the excitation wavelength; η is the refractive index of the solution; the Sample and QS refer to the sample and the standard, respectively. We chose fluorescein in 0.1 M NaOH as standard, which has the quantum yield of 0.95.[3]

Additional spectroscopic data

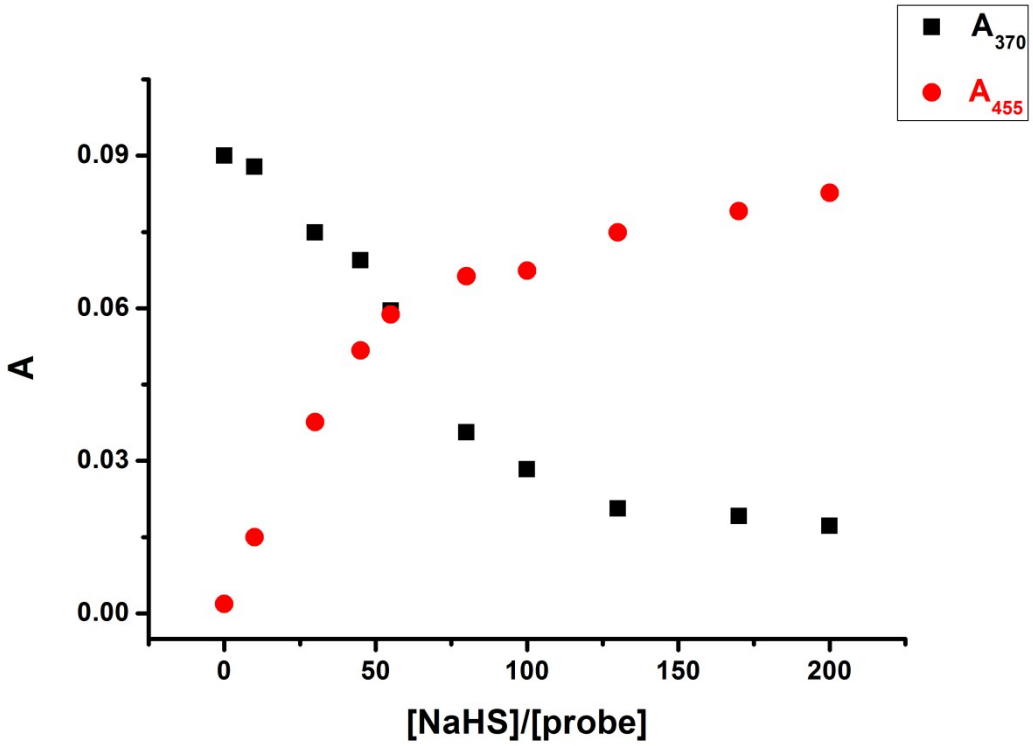


Fig. S1 The UV-vis absorption of **NS1** (10.0 μM) at 455 and 370 nm, respectively, as a function of NaHS concentration (0-200.0 equiv.) in HEPES buffer solution (10 mM, pH 7.4, containing 50% EtOH).

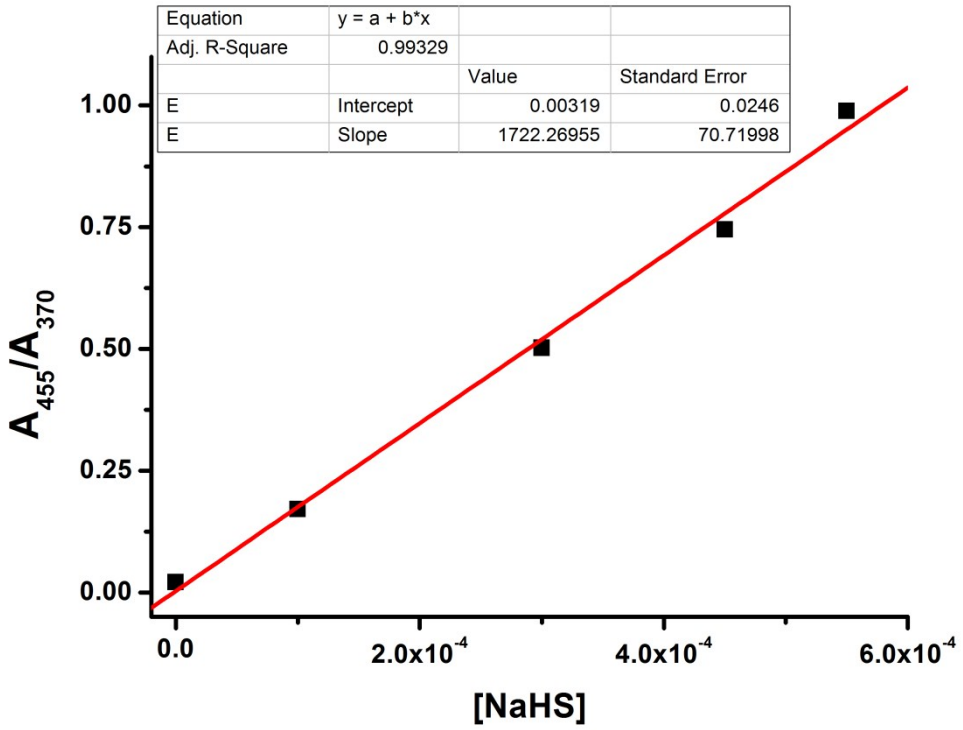


Fig. S2 The ratio of UV-vis absorption of **NS1** (10.0 μM) at 455 and 370 nm ($A_{455\ nm}/A_{370\ nm}$) as a function of NaHS concentration (0-60.0 equiv.) in HEPES buffer solution (10 mM, pH 7.4, containing 50% EtOH).

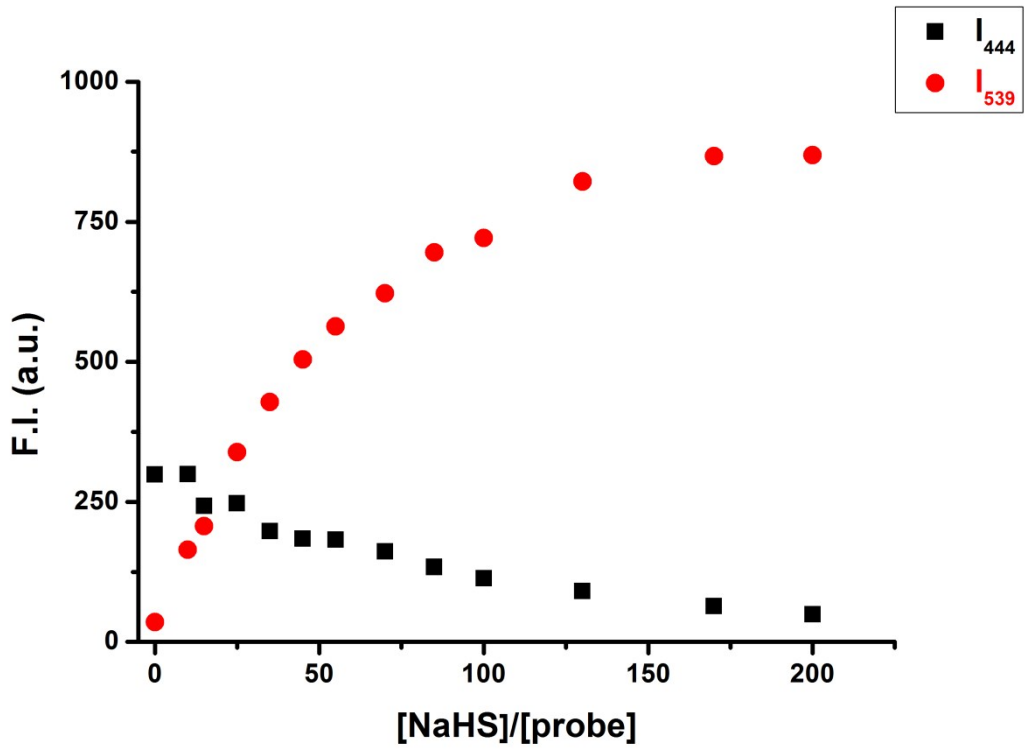


Fig. S3 The fluorescent intensity of **NS1** (10.0 μM) at 539 and 444 nm, respectively, as a function of NaHS concentration (0-200.0 equiv.) in HEPES buffer solution (10 mM, pH 7.4, containing 50% EtOH) (λ_{ex} = 410 nm).

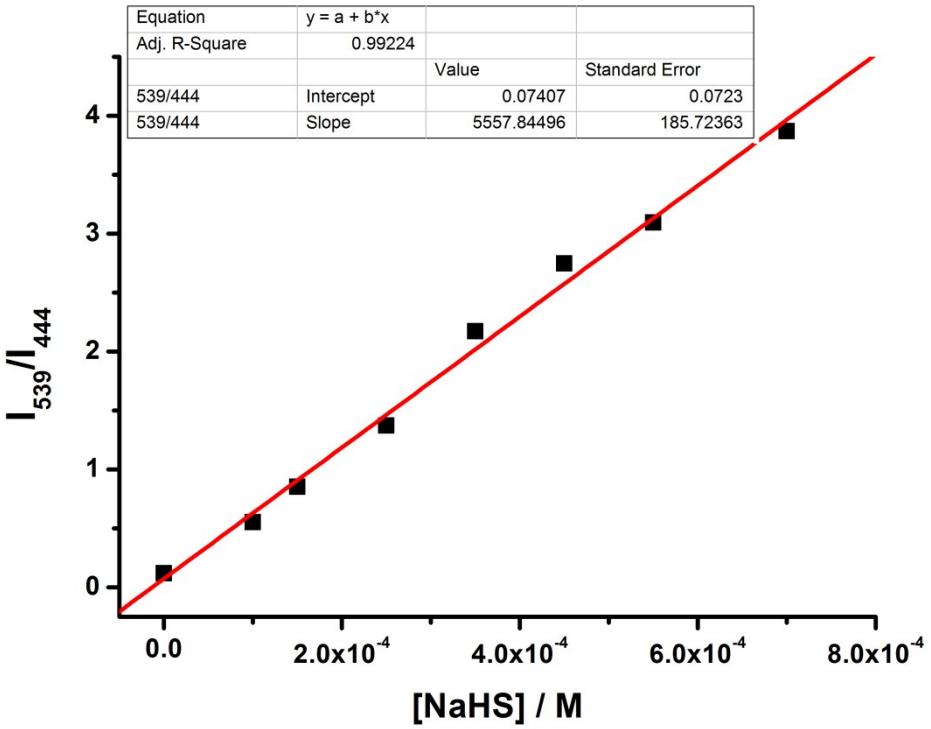


Fig. S4 The ratio of fluorescent intensity of **NS1** (10.0 μM) at 539 and 444 nm ($I_{539\ nm}/I_{444\ nm}$) as a function of NaHS concentration (0-80.0 equiv.) in HEPES buffer solution (10 mM, pH 7.4, containing 50% EtOH) (λ_{ex} = 410 nm).

The detection limit (DL) of H_2S using **NS1** was determined from the following equation: [4]

$$DL = 3*\sigma/K$$

Where σ is the standard deviation of the blank solution; K is the slope of the calibration curve.

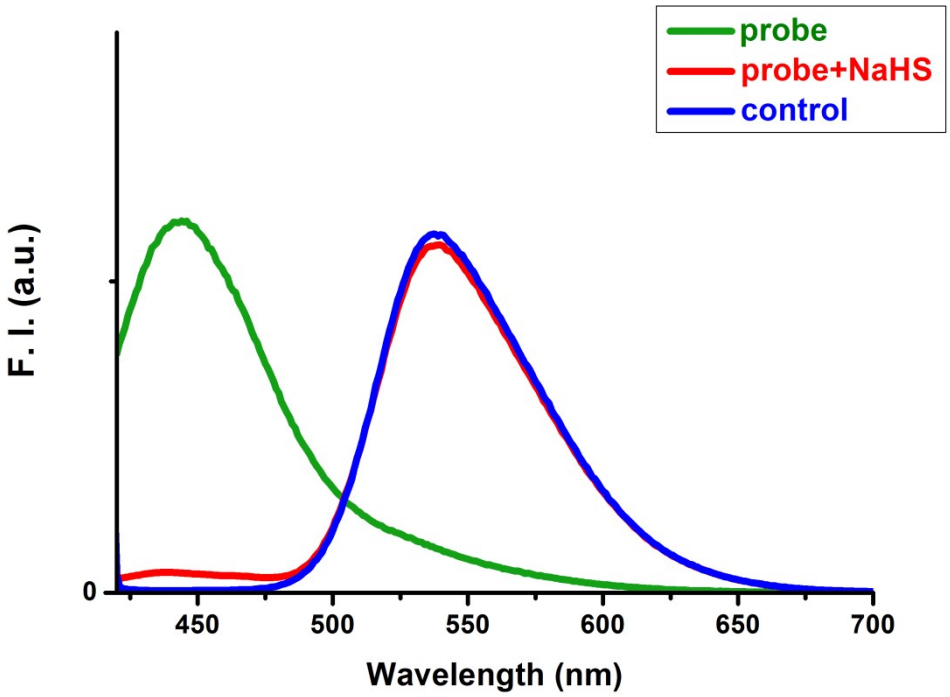


Fig. S5 The comparison of fluorescence spectra of probe (**NS1**), control (compound **3**), and the probe-NaHS mixture solution (**NS1**-NaHS mixture solution) in HEPES buffer solution (10 mM, pH 7.4, containing 50% EtOH) (λ_{ex} = 410 nm).

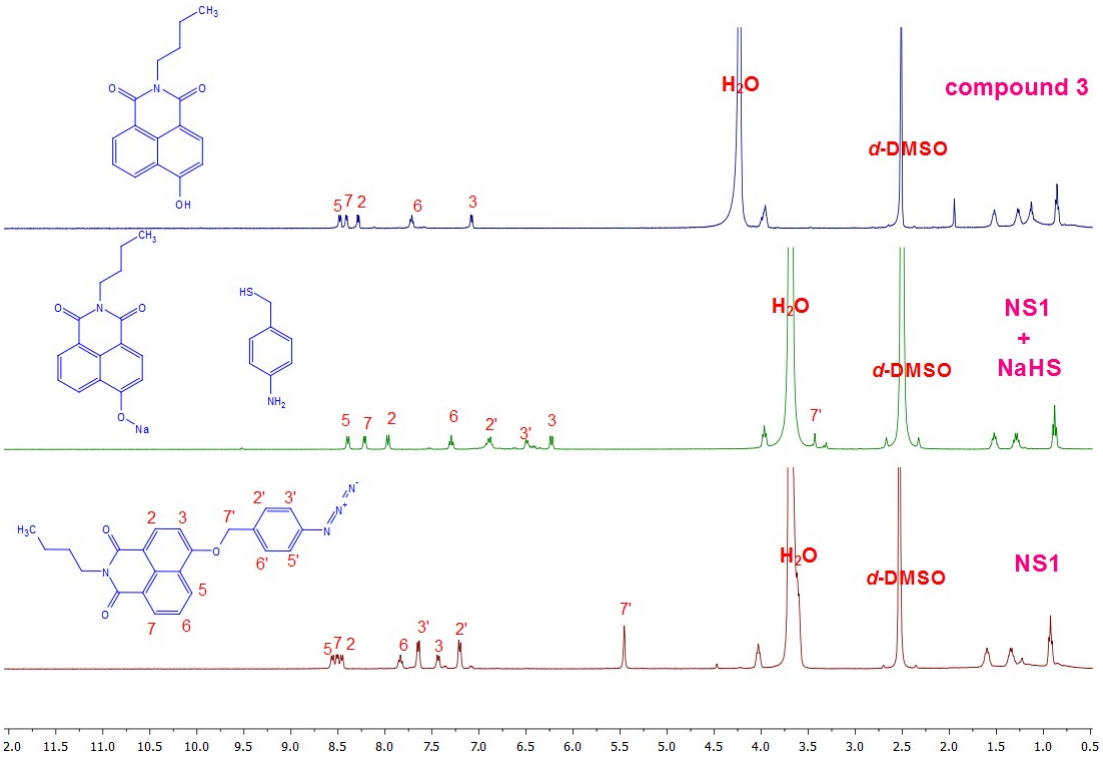
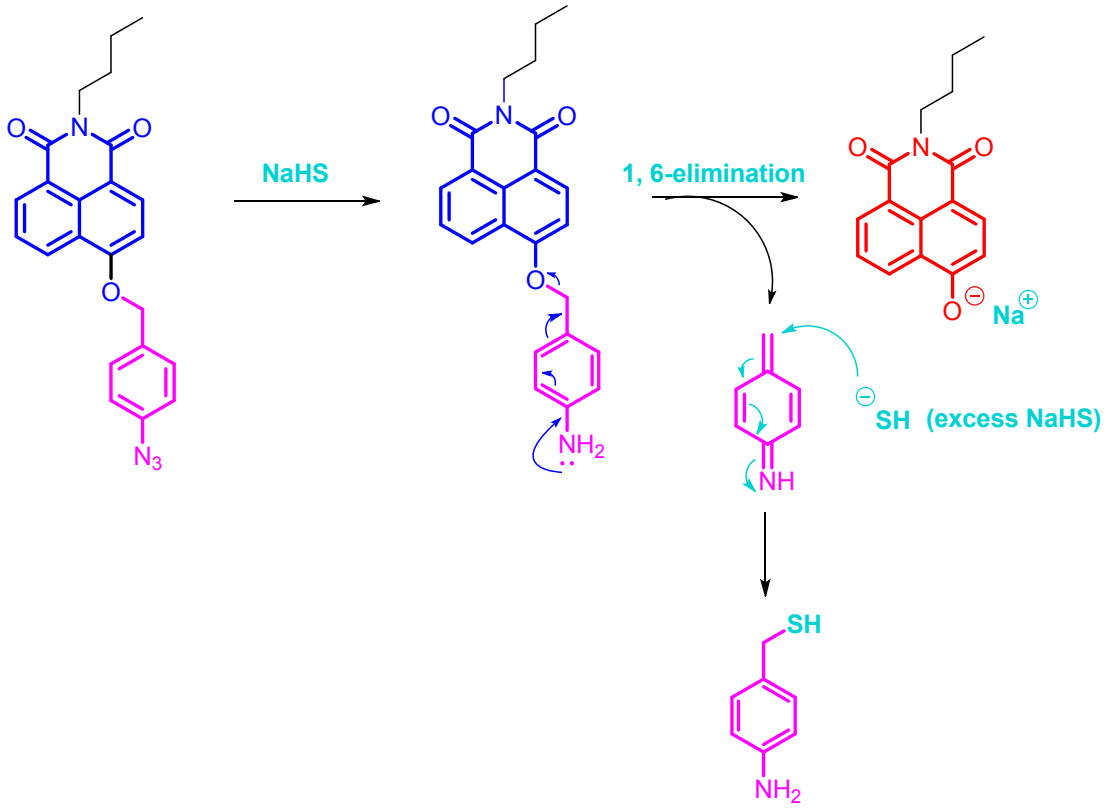


Fig. S6 The ^{1}H NMR spectra of **NS1** (4 mg in the mixture of 0.25 mL *d*-DMSO and 0.25 mL D_2O), **NS1**-NaHS mixture (4 mg of **NS1** with the addition of 20 equiv. of NaHS in 0.25 mL D_2O), and compound **3** (in *d*-DMSO-D_2O 1:1).



Scheme S2 The proposed mechanism of **NS1**-NaHS interactions.

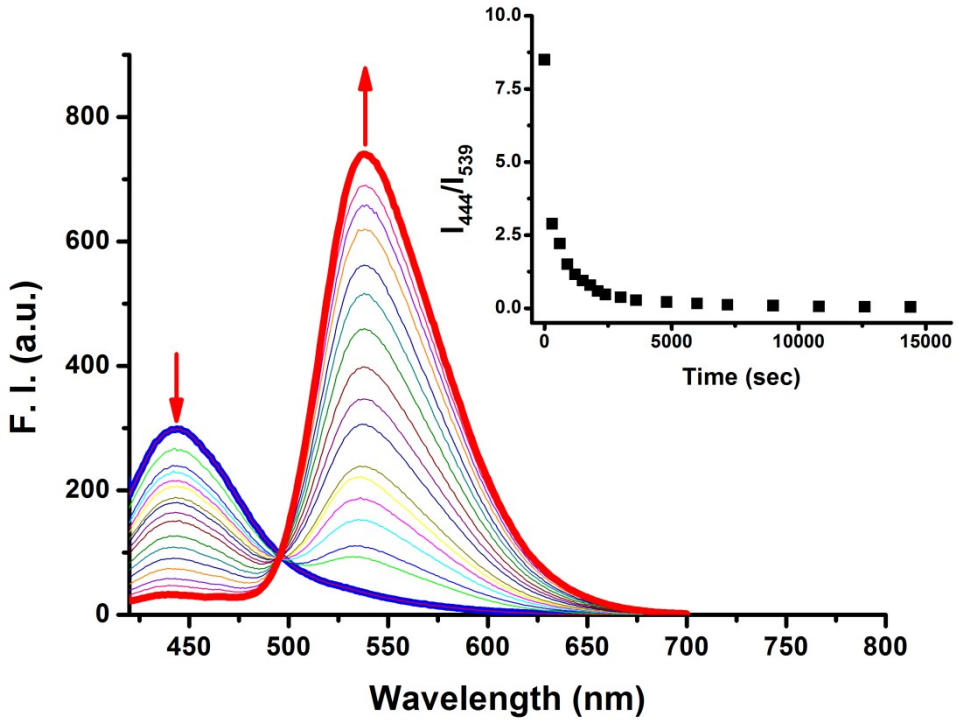


Fig. S7 Time-dependent fluorescence spectra of **NS1** (10.0 μM) upon addition of NaHS (100.0 equiv., 1 mM) in HEPES buffer solution (10 mM, pH 7.4, containing 50% EtOH) (λ_{ex} = 410 nm). Inset: The ratio of fluorescent intensity of **NS1** (10.0 μM) at 539 and 444 nm ($I_{444\ nm}/I_{539\ nm}$) as a function of reaction times (0-270 min).

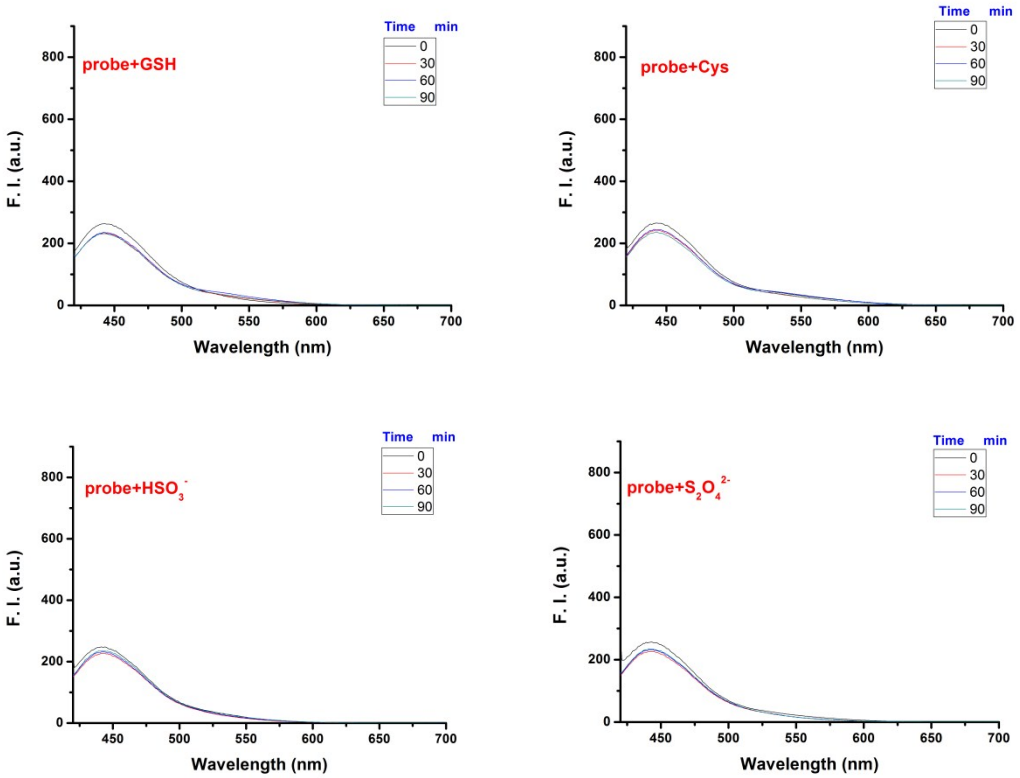


Fig. S8 Fluorescence responses of **NS1** (10.0 μM) in the presence of 100.0 equiv. of GSH, Cys, HSO_3^-, $S_2O_4^{2-}$, respectively, at 0, 30, 60, and 90 min after addition of each analytes in HEPES buffer solution (10 mM, pH 7.4, containing 50% EtOH) (λ_{ex} = 410 nm).

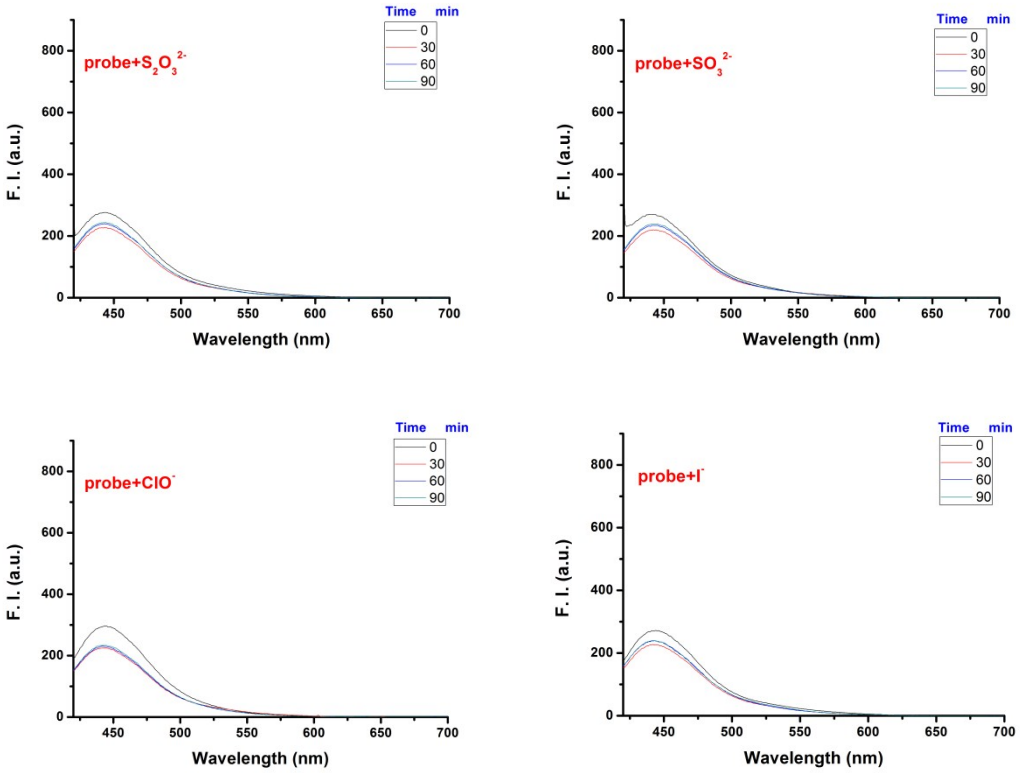


Fig. S9 Fluorescence responses of **NS1** (10.0 μM) in the presence of 100.0 equiv. of $S_2O_3^{2-}$, SO_3^{2-}, ClO^-, I^-, respectively, at 0, 30, 60, and 90 min after addition of each analytes in HEPES buffer solution (10 mM, pH 7.4, containing 50% EtOH) (λ_{ex} = 410 nm).

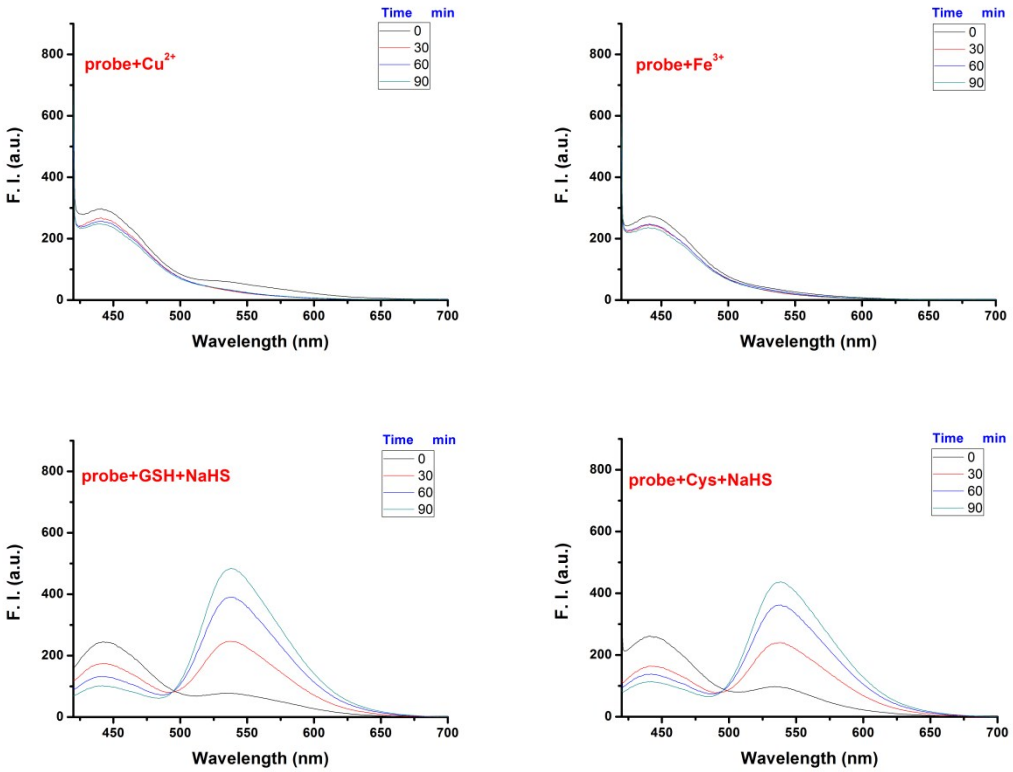


Fig. S10 Fluorescence responses of **NS1** (10.0 μM), in the presence of 100.0 equiv. of Cu^{2+}, Fe^{3+}, and in the presence of 100.0 equiv. of GSH, or Cys followed by 100.0 equiv. of NaHS, respectively, at 0, 30, 60, and 90 min after addition of each analytes in HEPES buffer solution (10 mM, pH 7.4, containing 50% EtOH) (λ_{ex} = 410 nm).

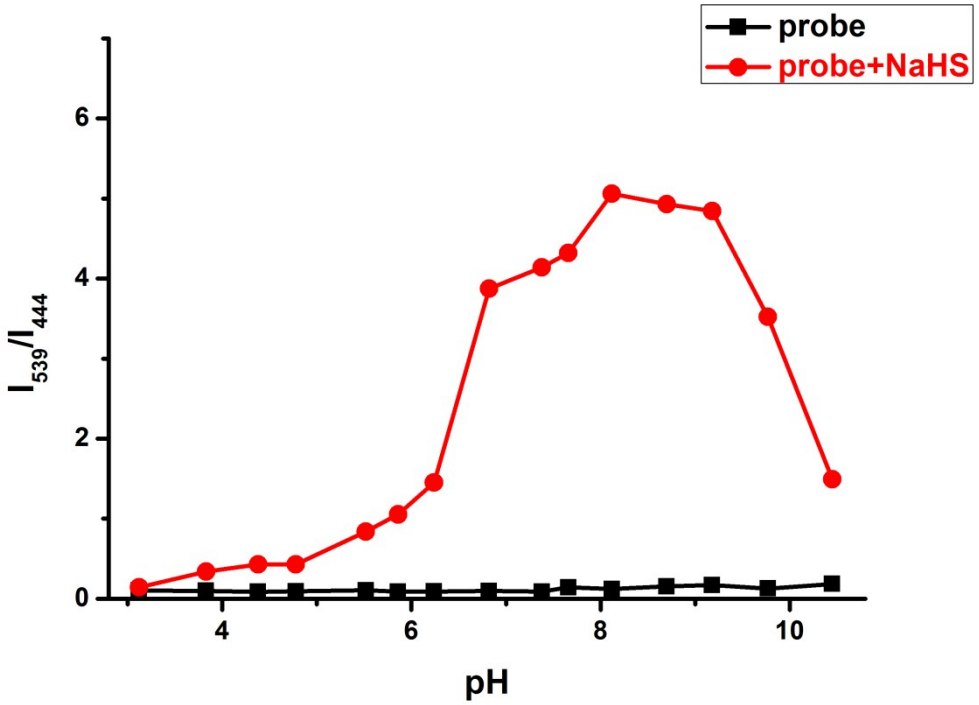


Fig. S11 Effect of the pH on the ratio of fluorescent intensity of **NS1** (10.0 μM) alone and **NS1**-NaHS system (in the addition of 100.0 equiv. of NaHS) at 539 and 444 nm ($I_{539\ nm}/I_{444\ nm}$) (λ_{ex} = 410 nm).

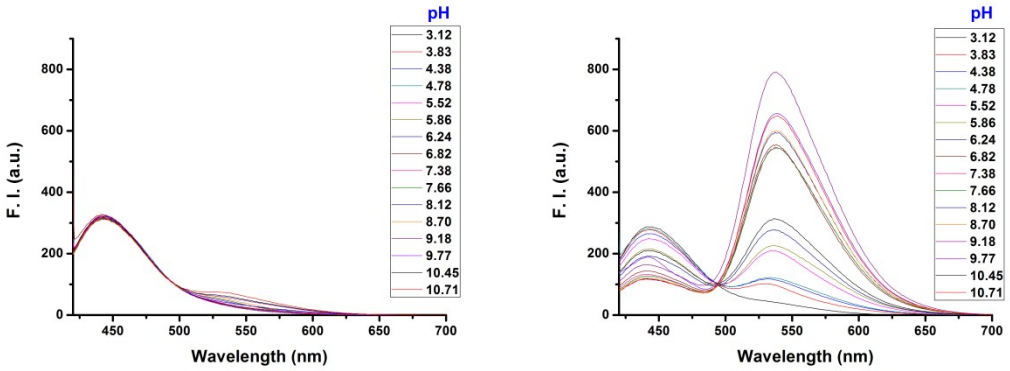


Fig. S12 Effect of the pH on the fluorescent spectra of **NS1** (10.0 μM), and **NS1**-NaHS system (in the addition of 100.0 equiv. of NaHS) (λ_{ex} = 410 nm).

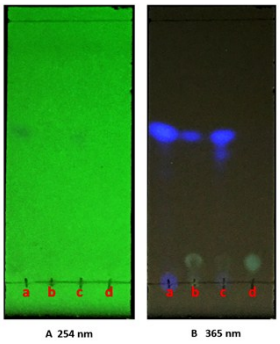


Fig. S13 Comparison of the TLC analysis of NS1, NS1-NaHS mixture in pH 7.4 solutions, NS1-NaHS mixture in pH 11.0 solutions, and compound 3 (control).

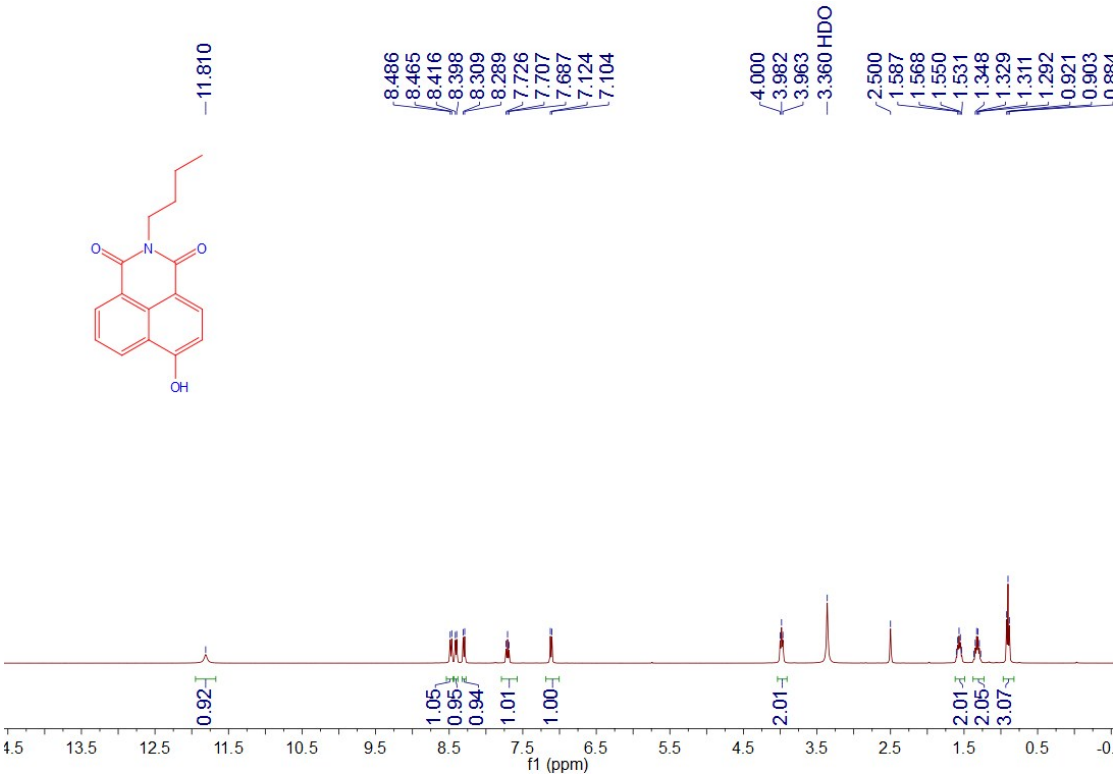
The pictures are the TLC spots of NS1 (a), NS1-H_2S mixture in pH 7.4 solutions (b), NS1-H_2S mixture in pH 11.0 solutions (c), and compound 3 (detection reaction product, d), with the left picture representing the results observed under 254 nm and the right one under 365 nm (The eluent for TLC: hexane:ethyl acetate = 6:1 (v/v)). As shown in the picture, the detection reaction is more favorable in pH 7.4 medium than that in pH 11.0. In the pH 7.4 medium, the reaction goes more rapid to give neatly compound 3. But it seems that some other reactions also take place in the pH 11.0 medium to form a compound with similar fluorescent properties to the probe itself.

Cell lines and imaging experiments

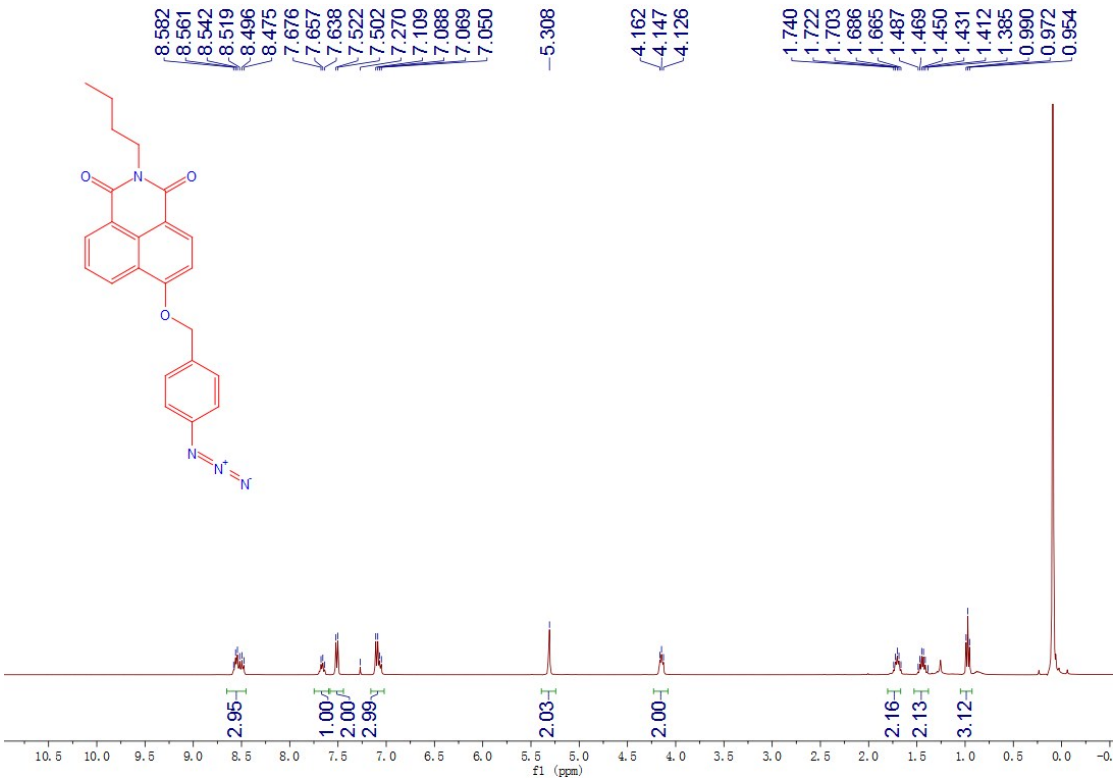
The human breast cancer MCF-7 cell line was selected as a model cell line to test the ability of this probe to image H_2S in live cells. Cells were seeded on glass bottom dishes (35 mm, In Vitro Scientific) and cultured overnight with Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% Fetal bovine serum (Gibco, GlandIsland, NY, USA). Before treatment, the cells were washed with PBS and then incubated with the culture but not the fetal bovine serum (5 μM, in PBS) for 20 min. After probe loading, the cells were again washed quickly with PBS and further incubated with PBS containing NaHS of various concentrations (0, 100, 200 μM) for 30 min. The cells were then observed under confocal microscopy (ZEISS LSM780) with excitation at 405 nm and emission at 507-601 nm.

The characterization data of NS1

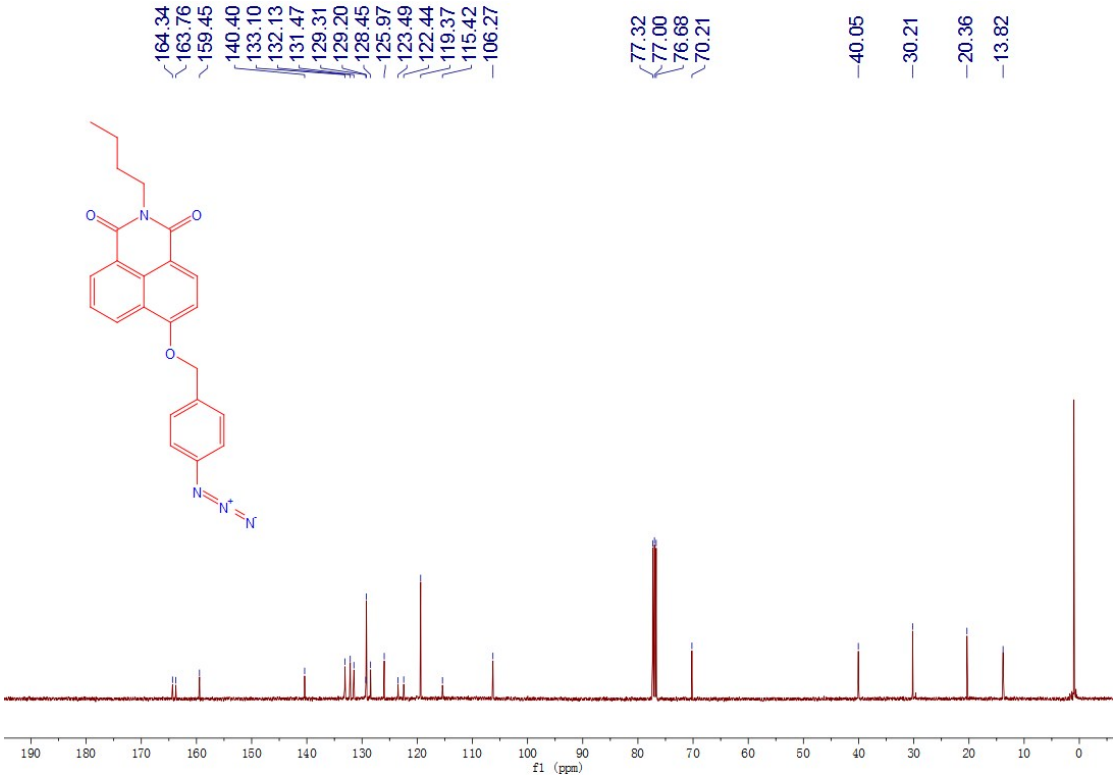
^{1}H NMR of **3**



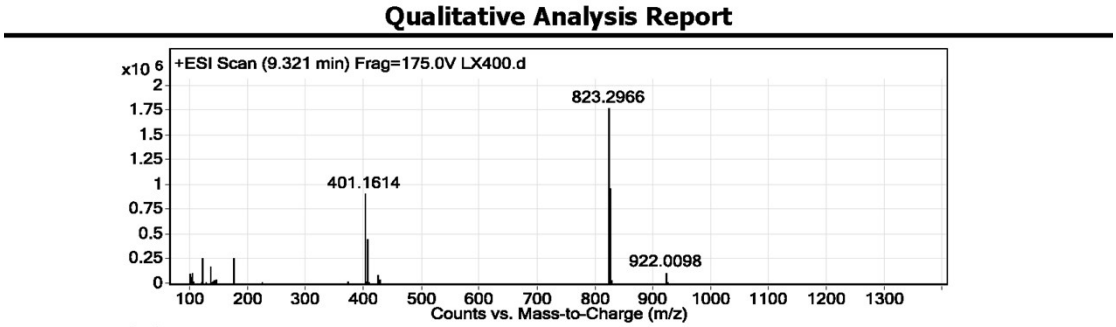
^{1}H NMR of **4 (NS1)**



^{13}C NMR of **4 (NS1)**



HR-MS of **4 (NS1)**



Peak List

m/z	z	Abund
121.0509		270219.3
136.0755		184356.2
174.1124		270050.5
401.1614	1	908991.9
402.1642	1	221798.6
405.1814	1	459418.6
823.2966	1	1774589.4
824.3	1	969955.3
825.3025	1	254950.6
922.0098		119477.1

References

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