

Highly selective naphthalimide-based fluorescent probe for direct hydrogen sulfide detection in environment

Supporting information

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1. General experimental section

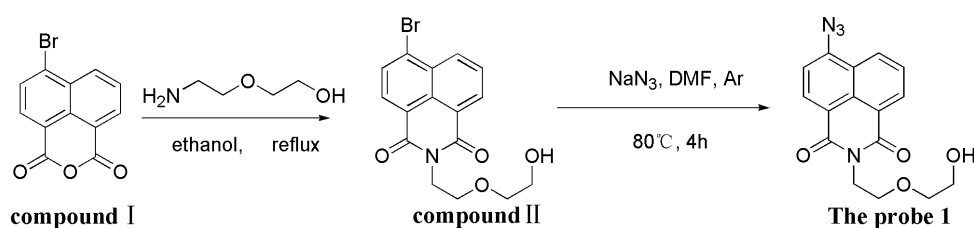
¹H and ¹³C NMR spectra were recorded on a Varian Mercury-Plus 400 NMR instrument (¹H 400 MHz; ¹³C 100MHz). UV-visible absorption spectra and fluorescence spectra was measured by Thermo Scientific Varioskan Flash. The fluorescence detection was carried out by using Corning costar 96 well-coated plates 3925. The pH measurements were made with a Sartorius basic pH meter PB-10. Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents were purified and dried using standard procedures during the procedure of synthesis of the compounds. Ultrapure water was prepared in the

laboratory using a Milli-Q SP reagent water system from Millipore (Milford, MA). Ultrapure water was used throughout. When we started experiment, all the solvents were fresh.

2. Synthesis and characterization of compounds

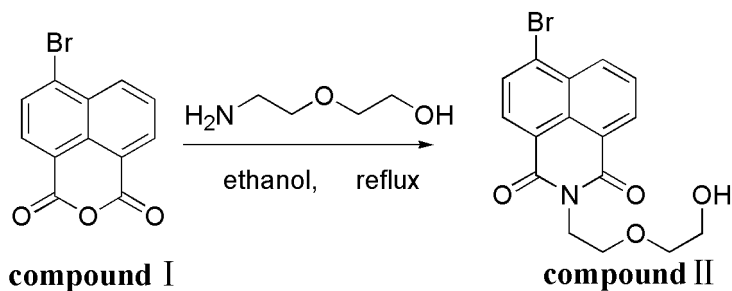
^1H and ^{13}C NMR spectra were recorded on a Varian Mercury-Plus 400 NMR instrument (^1H 400 MHz; ^{13}C 100MHz) in either CDCl_3 or DMSO-d_6 . Abbreviations for data quoted are s, singlet; brs, broad singlet; d, doublet; t, triplet; dd, doublet of doublets; m, multiplet. Mass spectra and high resolution mass spectra were measured on a Finnigan MAT-95 mass spectrometer. Thin-layer chromatography was done on pre-coated silica gel 60 F 254 plates (Merck).

Scheme S1: Synthesis and Characterization of Probe 1



The synthesis of the probe is outlined in Scheme S1. 4-Bromo-N-(2-hydroxyethoxy)phthalimide (compound I), 8-naphthalimide compound II was prepared following a reported procedure.¹ The probe 1 was acquired followed by the sodium azide and protection from Argon.

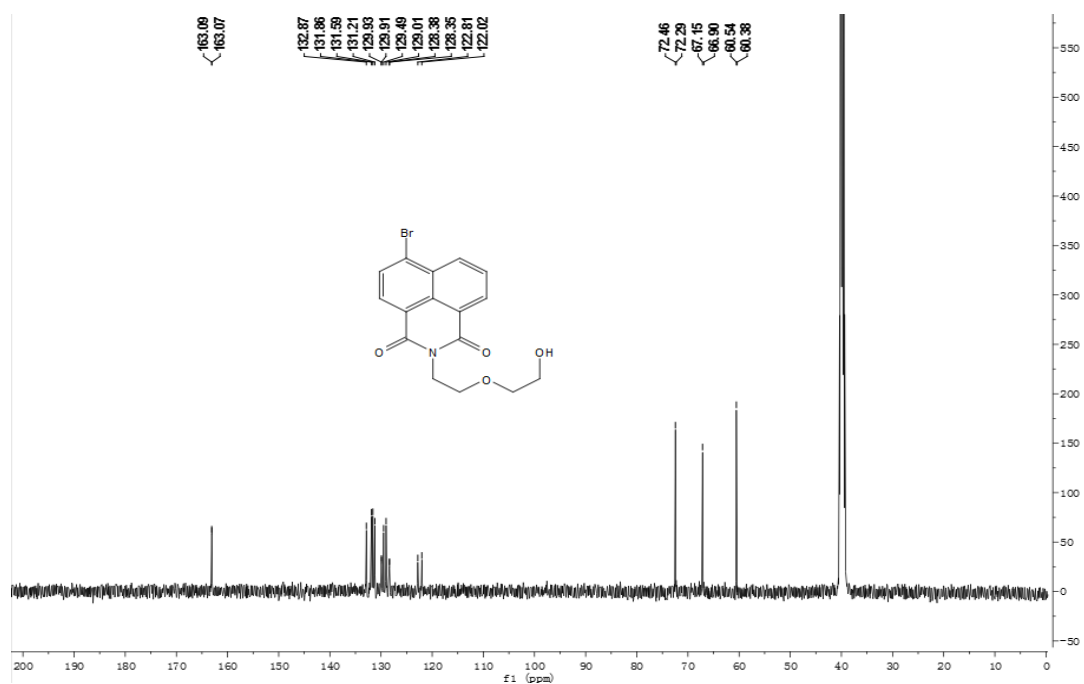
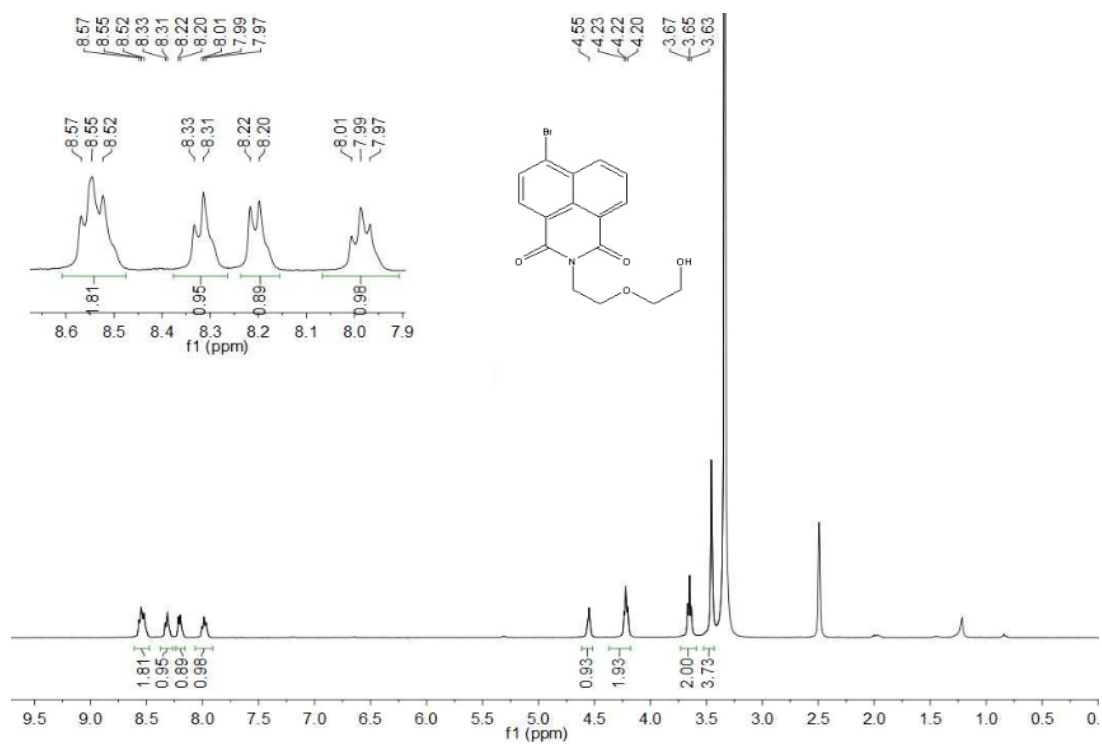
4-Bromo-N-(2-hydroxyethoxy)-1,8-naphthalimide

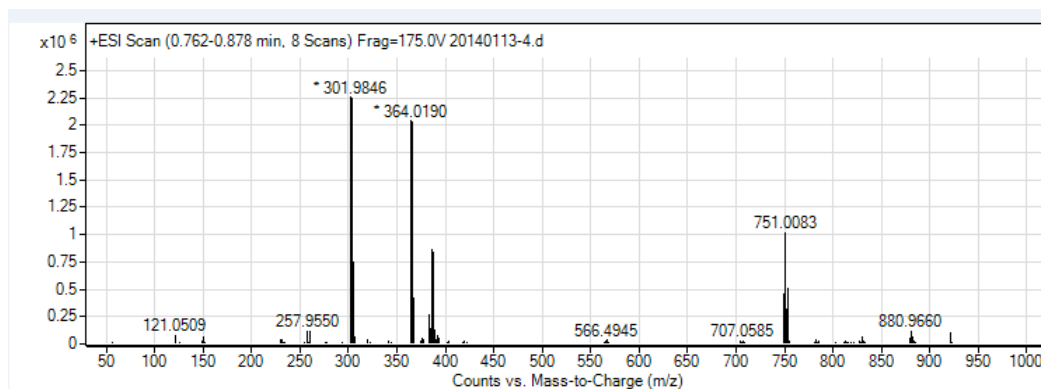


To a solution of 4-bromo-1,8-naphthalic anhydride compound I (5.54 g, 20.0 mmol) in 100 mL ethanol was added 1-amino-2-(2-hydroxyethoxy)ethane (4.18 g, 40.0 mmol) and the mixed solution was refluxed at 78.5°C for 20 h, TLC (PE:EA, 8:2) indicated the formation of the product ($R_f = 0.5$) with the complete consumption of starting material ($R_f = 0.2$). After cooling to room temperature, the brown color suspension was put into water. The resulting precipitate was filtered and washed with water, and dried to yield a light yellow product II (4.59g, 63%). M.p. $128.5\sim 130.0^\circ\text{C}$.

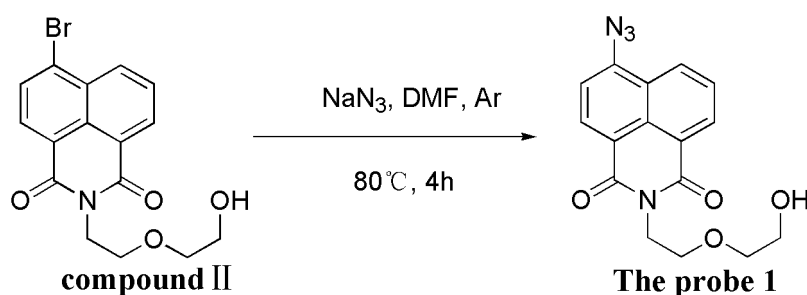
^1H NMR (500 MHz, DMSO-d_6): $\delta = 8.60\text{--}8.49$ (m, 2H, Ar-H), 8.32 (d, $J = 7.6$ Hz, 1H, Ar-H), 8.21 (d, $J = 7.6$ Hz, 1H, Ar-H), 7.99 (t, $J = 7.6$ Hz, 1H, Ar-H), 4.55 (s, 1H, -OH), 4.22 (t, $J = 6.3$ Hz, 2H, -CH₂-), 3.65 (t, $J = 6.4$

Hz, 2H, -CH₂-), 3.46 (s, 4H, -CH₂-). ¹³C NMR (500 MHz, DMSO-d₆): δ = 163.09, 163.07, 143.21, 131.98, 131.88, 128.74, 128.58, 127.63, 123.86, 122.35, 118.35, 116.29, 72.45, 67.23, 60.53. ESI-MS: m/z 364.0190 [M+H]⁺.



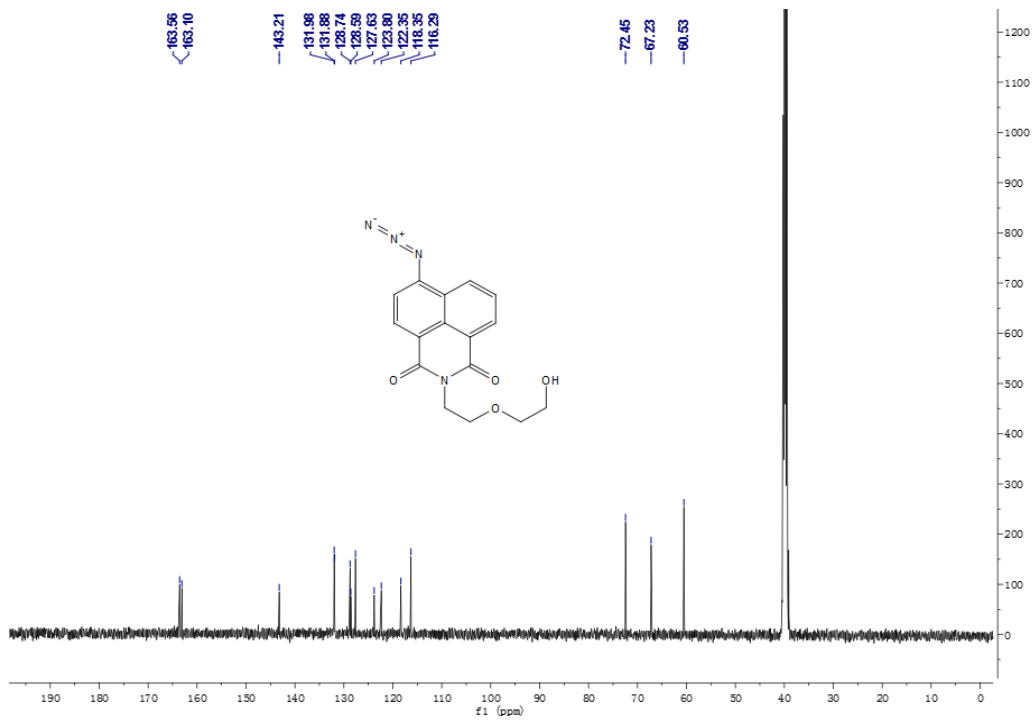
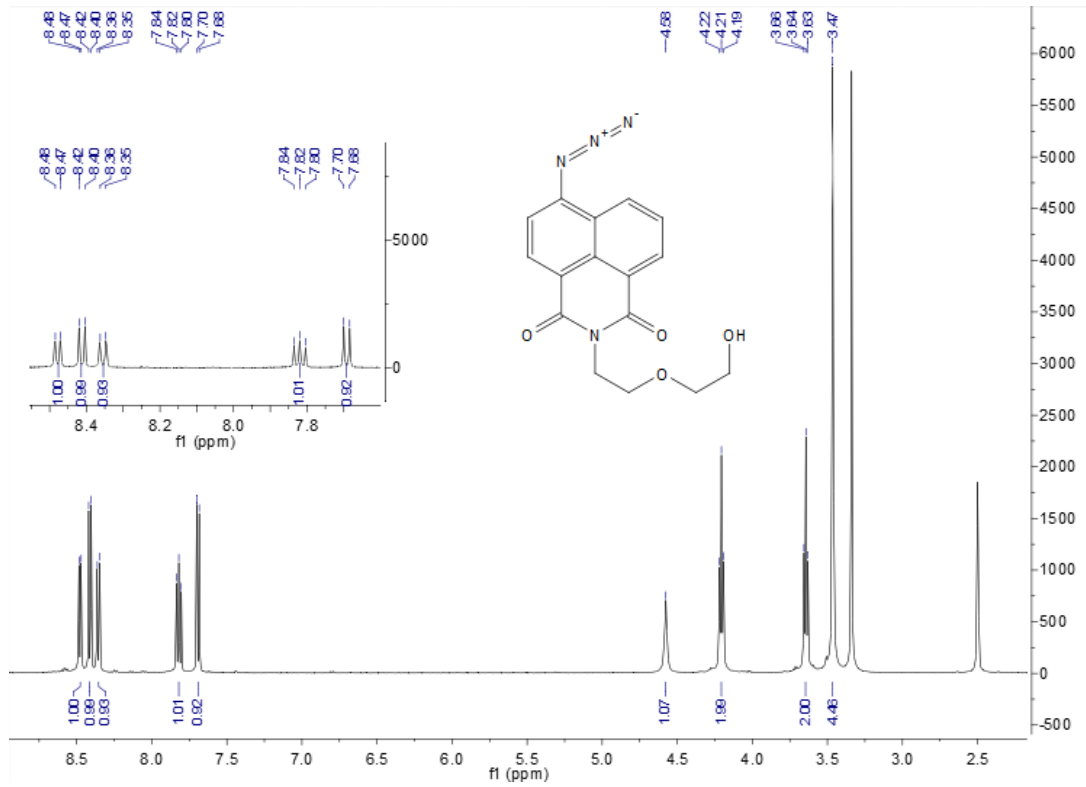


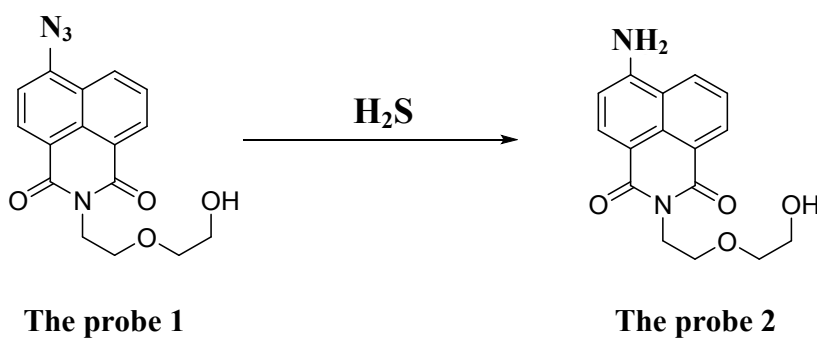
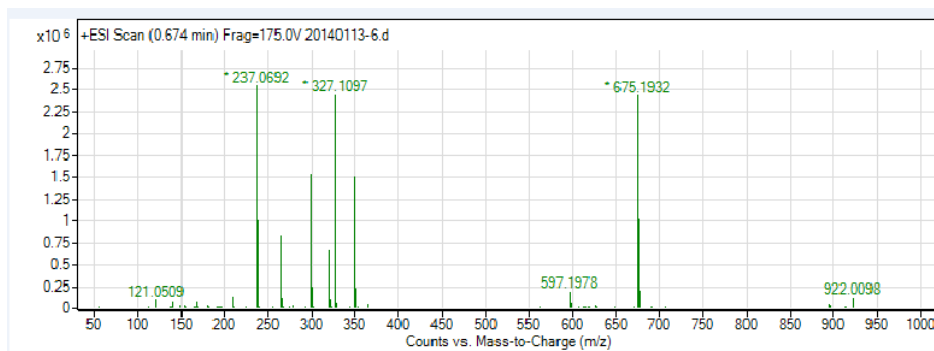
4-azido-N-hydroxydiethyl ether-1,8-naphthalimide



4-Bromo-N-hydroxydiethylether-1, 8-naphthalimide compound II (3.64 g, 10.0 mmol), was dissolved in DMF (dry, 40 mL) under nitrogen atmosphere. Sodium azide (0.98g, 15.0mmol) was added and the mixture was stirred at room temperature for 30 min, then heated to 85 °C, TLC (PE:EA, 8:2) indicated the formation of the expected product ($R_f = 0.5$) with the complete consumption of the compound II. After cooling to room temperature, the complex were poured into ice and then put into ethyl acetate. The organic layer was washed with salt aqueous NaCl(4×100mL) and dried over anhydrous MgSO₄. The solvent was removed and obtained the light yellow probe 1 (1.14 g, yield: 35%). M.p. 112.5~114.5 °C.

¹H NMR (500 MHz, DMSO-d₆): $\delta = 8.48$ (d, $J = 7.3$ Hz, 1H, Ar-H), 8.41 (d, $J = 8.0$ Hz, 1H, Ar-H), 8.35 (d, $J = 7.7$ Hz, 1H, Ar-H), 7.87-7.75 (m, 1H, Ar-H), 7.69 (d, $J = 8.0$ Hz, 1H, Ar-H), 4.58 (s, 1H, -OH), 4.21 (t, $J = 6.6$ Hz, 2H, HOCH₂-), 3.64 (t, $J = 6.6$ Hz, 2H, -NCH₂-), 3.47 (s, 4H, -CH₂OCH₂-). ¹³C NMR (500 MHz, DMSO-d₆): $\delta = 163.56, 163.10, 143.21, 131.98, 131.88, 128.74, 128.59, 127.63, 123.80, 122.35, 118.35, 116.29, 72.45, 67.23, 60.53$. ESI-MS: m/z 327.1097 [M+H]⁺.

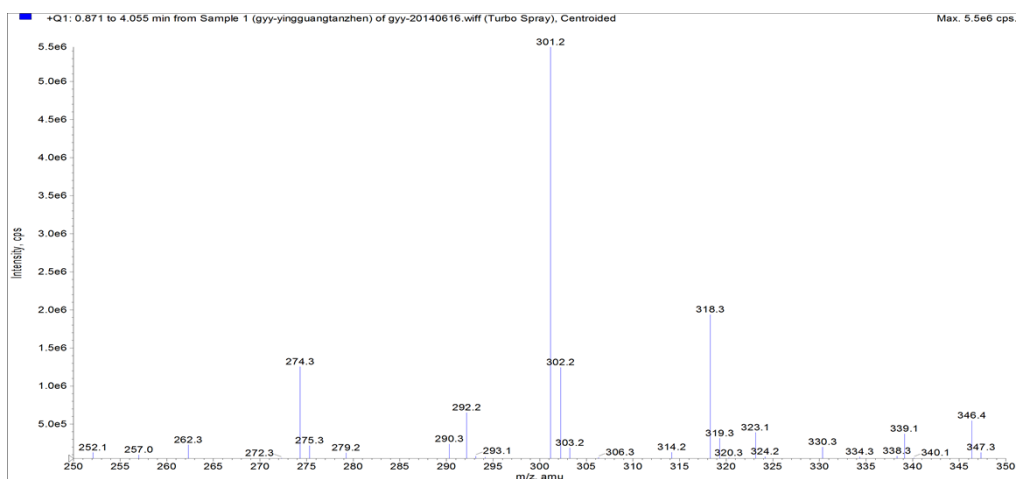




Scheme 2 Design of H₂S “off-on” fluorescent probe based on H₂S reductive property

4-amido-N-hydroxydiethyl ether-1,8-naphthalimide

¹H NMR (DMSO-d₆, 300MHz) δ: 8.63 (d, J = 8.4 Hz, 1H), 8.43(d, J = 7.2 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 7.66 (dd, J = 8.4, 7.2 Hz, 1H), 7.48 (brs, 2H), 6.86 (d, J = 8.4 Hz, 1H), 4.60 (brs, 1H), 4.22 (t, J = 6.6 Hz, 2H), 3.64 (t, J = 6.6 Hz, 2H), 3.49 (brs, 4H); ¹³C NMR (DMSO-d₆, 75MHz) δ: 163.97, 163.03, 152.89, 134.12, 131.18, 129.87, 129.49, 124.09, 121.84, 119.51, 108.33, 107.61, 72.23, 67.28, 60.35. ESI-MS: m/z 301.1110 [M+H]⁺.



3. Fluorescence quantum yields

Absolute values are calculated using the standard samples which have a fixed and known fluorescence quantum yield value, according to the following equation:

$$\Phi_X = \Phi_{ST} * (\text{Grad}_X / \text{Grad}_{ST}) * (\eta_X^2 / \eta_{ST}^2)$$

Where the subscripts ST and X denote standard and test respectively, Φ is the fluorescence quantum yield, $Grad$ the gradient from the plot of integrated fluorescence intensity vs absorbance, and η the refractive index of the solvent.

We choose quinine sulfate as the standard sample.

4. General procedure for H₂S detection

Unless otherwise noted, all the measurements were made according to the following procedure: In a 1.5 mL centrifuge tube, 1mL of 20mM pH7.4 HEPES buffer (containing 50%DMSO) and 10 μ M solution of the probe 1 were mixed, followed by addition of an appropriate volume of Na₂S sample solution. All the reaction solution was mixed well. After 10min at room temperature, 150 μ M portion of the reaction solution was transferred to a 96-well assay plate and the fluorescence excitation was at 440nm (both excitation and emission slit widths were set to 2 nm). Meantime, a blank solution containing no Na₂S was prepared and measured under the same conditions for comparison.

5. Spectral properties of the probe 1

The absorption spectra of the probe 1 with the addition of Na₂S solution were recorded. In the test tube, 20mM pH7.4 HEPES buffer (containing 80% tetrahydrofuran) and 10 μ M solution of the probe 1 were mixed, followed by

addition of 100 μ M Na₂S sample solution .The mixture was equilibrated for 60min and the results were shown in Figure S1. It can be seen that the solution of reaction product showed an absorption peak at about 440 nm.

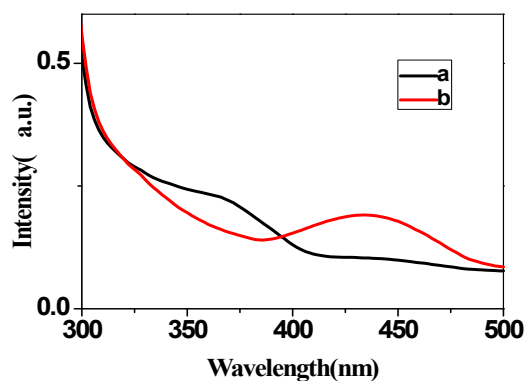


Figure S1. Absorption spectra of the probe 1 (10 μ M) was taken before(a) and after (b) reaction with Na₂S (100 μ M) for 60 min at room temperature.

The fluorescence emission spectra were obtained at the same conditions. The fluorescence intensity was measured at λ_{ex} =440nm and fluorescence emission wavelength was from 460nm to 700nm.

6. Effect of reaction media

We investigated the effect of reaction media and we chose four different organic solvent–water solution, including DMSO-water solution, THF-water solution, IPA-water solution and MEA--water solution. In the test tube, 20mM pH7.4 HEPES buffer (containing 80% organic reagent) and 10 μ M solution of the probe 1 were mixed, followed by addition of 100 μ M Na₂S sample solution. The mixture was equilibrated for 60min and the results were shown in Figure S2. The control group was similar to the experimental group except no Na₂S. Compared with the control, in four organic solvent-water solutions, the fluorescence intensity enhancement of DMSO-water solution was the highest. So we chose DMSO-water solution as the reaction media.

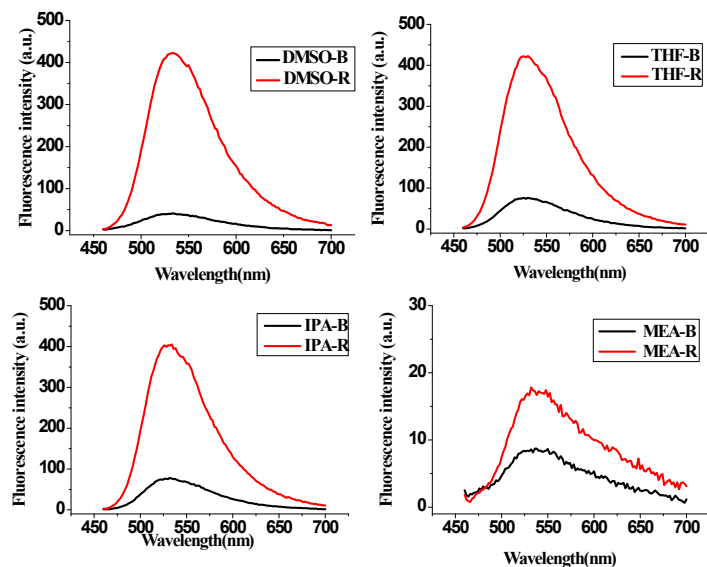


Figure S2. Fluorescence intensity of the solution of probe 1 (10 μM) with addition of Na_2S (100 μM) was in different organic solvent–water solution. It included DMSO-water solution, THF-water solution, IPA-water solution, MEA--water solution. “-B” and “-R” respectively represented before and after reaction of the Na_2S .

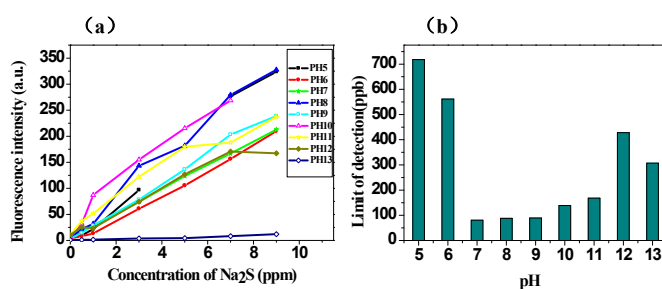


Figure S3. (a) The effect of pH on the fluorescence reaction in different value. (b) Limit of detection in different pH value.

7. Effect of reaction pH

We evaluated the effect of different reaction pH and obtained the suitable conditions for fluorescence measurement. The effect of pH on the fluorescence reaction was studied in the range 5.0-13.0. The properties of the probe 1 in the range of pH 7-9 were similar (Figure S3). Therefore, we chose pH 7.4 HEPES buffer in the following experiment because of the high sensitivity, good linear and well stability.

8. Limit of detection

The fluorescence emission intensity of the probe 1 was measured by six times and the standard deviation of blank measurement was obtained. The detection limit was calculated by using the equation: Detection limit = $3\sigma/k$: Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity versus N_2S concentration.²

9. Acidification-blowing-absorption pretreatment device and working line

100mL sample was in the 250mL conical flask with the addition of 5g zinc granule. Followed by putting in 15mL of concentrated hydrochloric acid, the produced gas was introduced into the bubble absorption tube which contained 10 mL of 2% NaOH solution (mass fraction). The reaction last 60min. Prior to the determination of H_2S by the proposed procedure, the pH was adjusted to 7.4.

1. Y. Zhou, Y. W. Yao, Q. Qi, Y. Fang, J. Y. Li and C. Yao, *Chemical communications*, 2013, **49**, 5924-5926.
2. W. Sun, J. Fan, C. Hu, J. Cao, H. Zhang, X. Xiong, J. Wang, S. Cui, S. Sun and X. Peng, *Chemical communications*, 2013, **49**, 3890-3892.