Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

A Rhodamine-Formaldehyde Probe Fluorescently Discriminates H_2S from Biothiols

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1. General Experimental Section

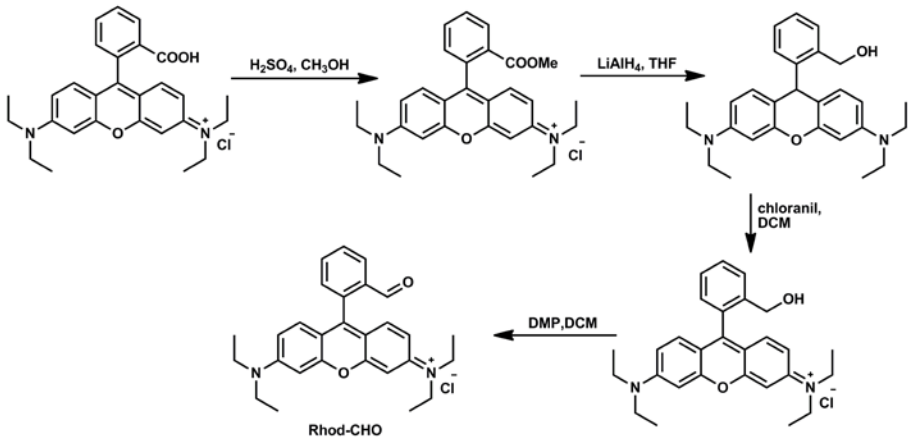
Materials: All the reagents were purchased from commercial suppliers (Sinopharm Chemical Reagent Co. ltd., China; Alfa Aesar Chemical Co. ltd.) and were of AR grade, and MeCN were of HPLC grade. Solvents were dried according to standard procedures. All reactions were magnetically stirred and monitored by t hin-layer chromatography (TLC). Flash chromatography (FC) was performed using silica gel 60 (200-300 mesh). Doubly distilled water was used throughout the experiments.

Instruments: UV absorption spectra were obtained on Shimadzu UV-2350 Spectrophotometer. Fluorescence emission spectra were obtained on Varian Cary Eclipse Fluorescence spectrophotometer. ^{1}H NMR and ^{13}C NMR were measured on a Bruker AV400 Nuclear Magnetic Resonance Spectrometer. Bruker MALDI-TOF-TOF Mass Spectrometer was used for high resolution mass spectrometer. Fluorescence images were acquired on Zeiss LSM710 confocal laser-scanning microscope.

General detection procedures: Compound **Rhod-CHO** was dissolved in CH_3CN to make a 1.0×10^{-3} M stock solution, which was

further diluted to required concentration for measurement. Silver ion was prepared from its nitrate, and the other ions were prepared from their chloride salts. The cysteine (Cys), homocysteine (Hcy), glutathione (GSH) ， hydrogen sulfide (H_2S) and metal ions stock solution of 1.0×10^{-2} M was diluted to 1.0×10^{-3} M with deionized water for spectra titration studies. Spectra data were recorded in an indicated time after the addition.

2. Synthesis Section



Scheme 1. Synthesis of fluorescent rhodamine-formaldehyde probe (**Rhod-CHO**)

Synthesis of *N*-(6-(diethylamino)-9-(2-hydroxymethylphenyl)-3*H*-xanthen-3-ylidene)-*N*-ethylethanaminium

Rhodamine B 479 mg (1 mmol, 1 eq.) was dissolved in MeOH (20 mL). The solution was acidified with H_2SO_4, and then stirred overnight at 80 °C under N_2

atmosphere. The solvent was removed by evaporation, and the residue was dissolved in CH_2Cl_2 and the solution was washed with water and sat. $NaHCO_3$. The organic layer was dried over Na_2SO_4 and evaporated to dryness. A solution of the residue in THF (20 mL) was sealed in a two-necked flask under N_2 atmosphere. Then, 380 mg (10 mmol, 10 eq.) of $LiAlH_4$ was added at 0 °C. The mixture was brought to room temperature and stirred for 3 h. The reaction was quenched by addition of sat. NH_4Cl (10 mL), and the solvent was removed by evaporation. The residue was dissolved in CH_2Cl_2 and the solution was washed with sat. $NaHCO_3$. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude intermediate was dissolved in 20 mL of CH_2Cl_2, and *p*-chloranil 246 mg (1 mmol, 1 eq) was then added. The reaction mixture was stirred for 1 h. The residue was separated on a neutral alumina column packed with CH_2Cl_2. The organic solvents were removed by evaporation to give a white solid (210 mg, 45.3%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.46-7.33 (1 H, m), 7.24 (1 H, dt, J = 4.8, 3.6 Hz), 6.67 (1 H, d, J = 8.6 Hz), 6.37 (1 H, d, J = 2.5 Hz), 6.26 (1 H, dd, J = 8.6, 2.6 Hz), 5.33 (1 H, s), 4.55 (1 H, s), 3.31 (1 H, q, J = 7.1 Hz), 1.14 (1 H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 151.65, 147.76, 138.60, 131.43, 129.96, 129.62, 128.01, 126.94, 111.39, 107.40, 98.75, 63.03, 44.40, 12.64.

Synthesis of Rhod-CHO

To a solution of compound **1** (5 g) in dichloromethane (30 mL), DMP was slowly added. The mixture was stirred at r.t. for 1 h. The organic layer was

washed with NaS_2O_3 and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by neutral alumina column using ethyl acetate-hexane (10 : 1) as the eluent. The solvent was evaporatedand the residue was dissolved in 10 mL of CH_2Cl_2, washed with 10% HCl, and removal of solvent to give the desired compound **Rhod-CHO** (yield, 72%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 9.88 (1 H, s), 8.22 (1 H, d, J = 7.4 Hz), 7.91 (2 H, dt, J = 20.4, 7.5 Hz), 7.43 (1 H, d, J = 7.0 Hz), 7.07 (2 H, d, J = 9.5 Hz), 6.99-6.91 (2 H, m), 6.88 (2 H, s), 3.67 (8 H, q, J = 7.2 Hz), 1.35 (12 H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 190.34, 157.68, 156.06, 155.72, 134.70, 134.60, 132.99, 132.66, 131.21, 131.06, 130.59, 114.62, 113.86, 96.53, 46.28, 12.69. HRMS: calcd for [M^+]: 427.2380, found: 427.2387.

Synthesis of RS

To a solution of compound **Rhod-CHO** (25 mg) in dichloromethane (5mL), 0.1 g of NaHS was added. The mixture was stirred at r.t. for 30 min. The solution was poured into water (10 mL) and then extracted using dichloromethane/water (10 mL). The organic layer was collected, dried over sodium sulfate and then evaporated in vacuo. The residue was purified by neutral alumina column using DCM as the eluent to afford **RS** (yield, 83%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.56 (1 H, d, J = 7.6 Hz), 7.36 (1 H, dd, J = 10.8 Hz, 4.1 Hz), 7.30 (1 H, t, J =7.0 Hz), 6.97 (1 H, d, J = 7.6 Hz), 6.90 (1 H, d, J = 8.8 Hz), 6.79-6.70 (2 H, m), 6.38-6.27 (4 H, m), 3.32 (8 H, q, J = 7.1 Hz), 1.15 (12 H, t, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 152.30, 151.29, 149.59, 148.00, 147.97, 142.62, 130.91,

130.33, 129.83, 127.75, 126.94, 124.76, 114.27, 112.63, 108.29, 108.12, 97.44, 83.87, 44.38, 12.71. HRMS: calcd for [M+H]: 461.2257, found: 461.2246.

3. Absorption and Emission Spectra of Rhod-CHO with NaHS

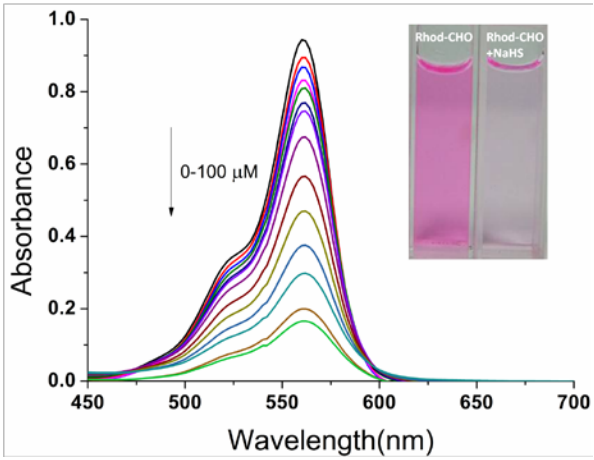


Fig. S1 Absorption and emission spectra of **Rhod-CHO** (10 μM) in PBS (20 mM, pH 7.4, 1% CH_3CN, v/v) upon titration with 100 μM NaHS. Inset: The visual light color change of **Rhod-CHO** (10 μM) before (left) and after (right) addition of 10 equiv. of NaHS.

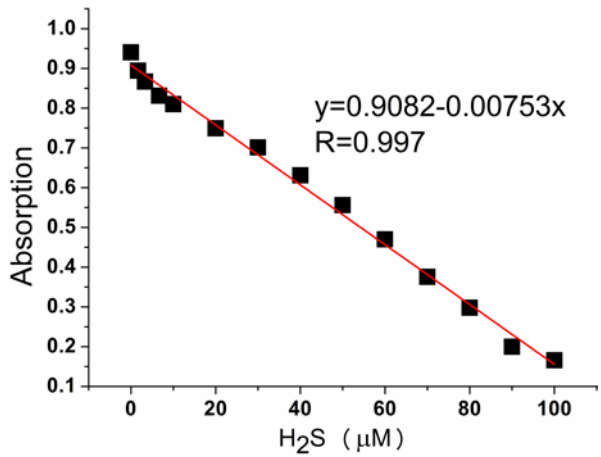


Fig. S2 The linear relationship between the absorption and the concentration of NaHS (0-100 μM)

4. Time dependence of fluorescence profile of Rhod-CHO upon addition of NaHS in PBS buffer

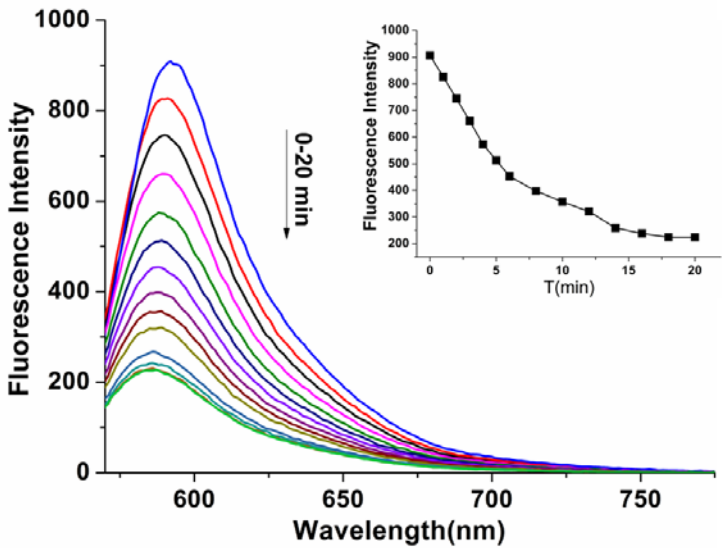


Fig. S3 Time dependence of fluorescence profile of **Rhod-CHO** (10 μM) with 10 equiv. of NaHS in PBS (20 mM, pH 7.4, 1% CH_3CN, v/v). λ_{ex}: 560 nm. Slits: 5/5 nm. Inset: Fluorescence intensity changes of **Rhod-CHO** upon addition 10 equiv. NaHS from 0 to 20 min.

5. Fluorescence response of Rhod-CHO upon addition of various species in PBS buffer

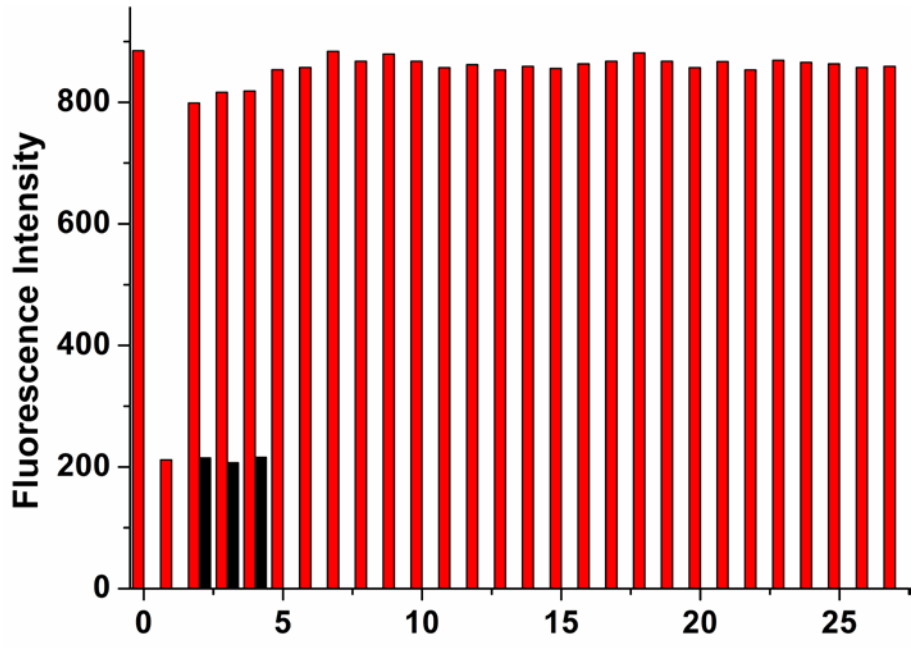


Fig. S4 Fluorescence response of **Rhod-CHO** (10 μM) upon addition of various species in PBS buffer (20 μM, pH 7.4). λex = 560 nm, λem = 592 nm. Slits: 5/5 nm. (0) Rhod-CHO; (1)100 μM NaHS; (2) 200 μM GSH left red bar, 200 μM GSH +100 μM NaHS right black bar; (3) 200 μM Cys left red bar, 200 μM Cys+100 μM NaHS right black bar; (4) 200 μM Hcy left red bar, 200 μM Hcy +100 μM NaHS right black bar; (5) 200 μM F^-; (6) 200 μM HSO_3^-; (7) 200 μM CN^-; (8) 200 μM AcO^-; (9) 200 μM $S_2O_3^{2-}$; (10) 200 μM CO_3^{2-}; (11) 100 μM Cu^{2+}; (12) 200 μM Zn^{2+};(13) 200 μM Mn^{2+}; (14) 200 μM Hg^{2+}; (15) 200 μM SO_4^{2-}; (16) 200 μM NO_3^{2-}; (17) 200 μM lipoic acid; (18) 200 μM SCN^-; (19) 200 μM NO^{2-}; (20) 200 μM NO; (21) 200 μM H_2O_2; (22) 200 μM $O_2^{\cdot-}$; (23) 200 μM t-BuOOH; (24) 200 μM HOCl; (25) 200 μM Ca^{2+}; (26) 200 μM Fe^{3+}; (27) 200 μM Mg^{2+}.

6. ^{1}H NMR,^{13}C NMR and HRMS of Rhod-CHO

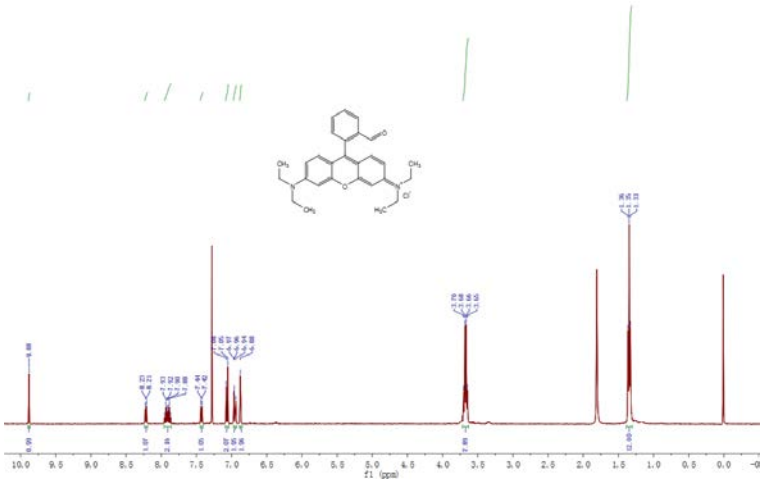


Fig. S5 ^{1}H-NMR spectra of **Rhod-CHO**

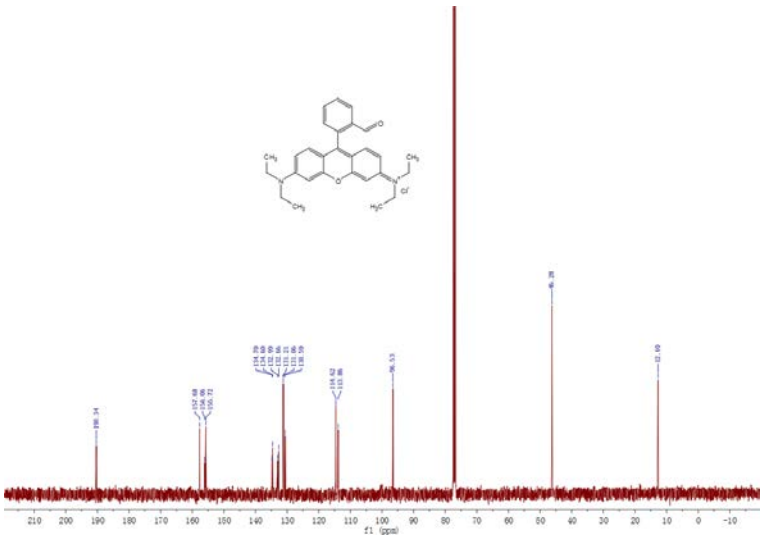


Fig. S6 ^{13}C-NMR spectra of **Rhod-CHO**

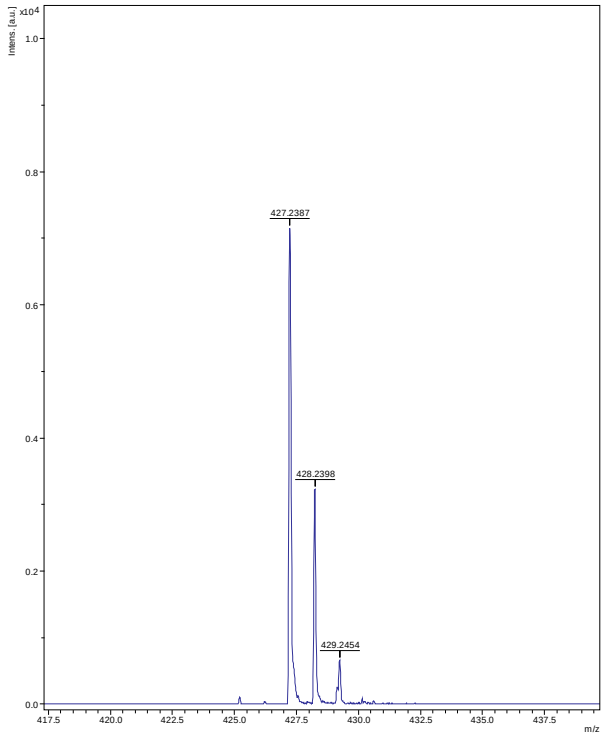


Fig. S7 HRMS of **Rhod-CHO**

7. ^{1}H NMR, ^{13}C NMR and HRMS of RS

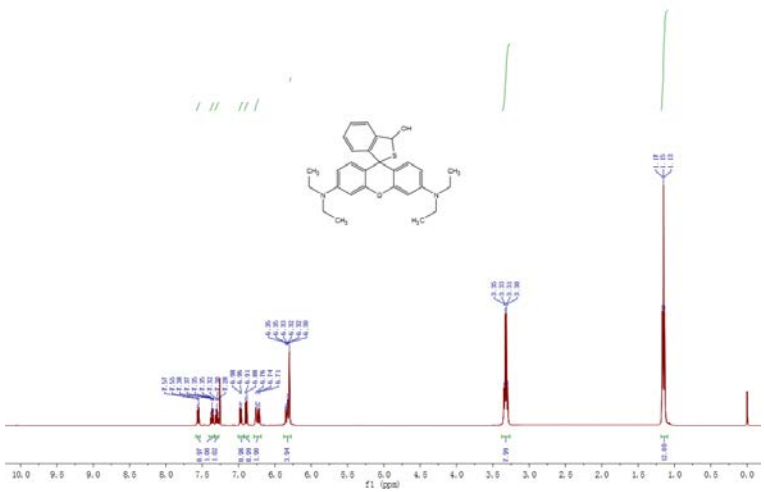


Fig. S8 1H-NMR spectra of **RS**

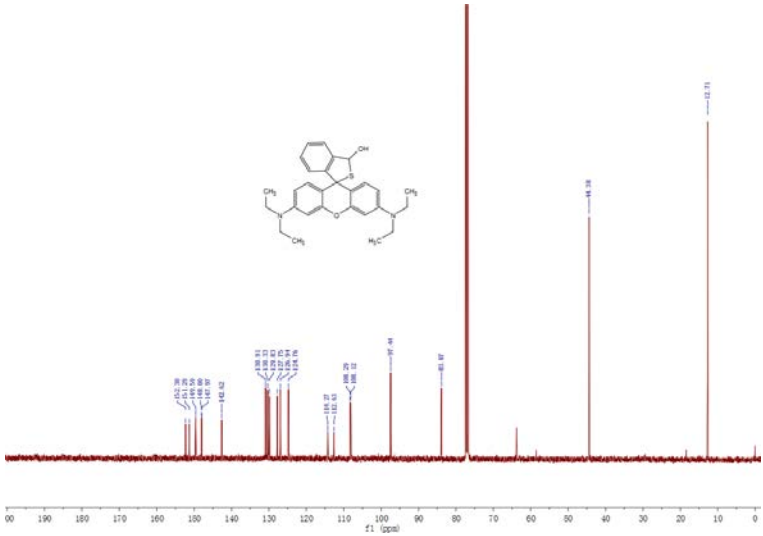


Fig. S9 ^{13}C-NMR spectra of **RS**

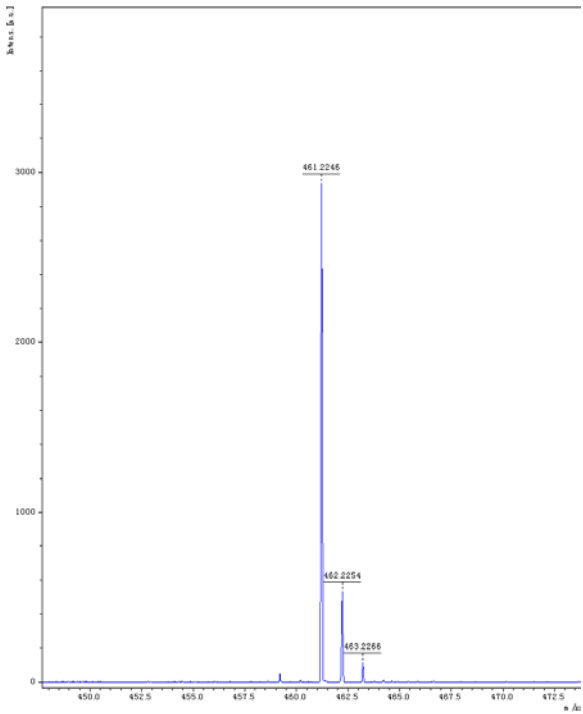


Fig. S10 HRMS spectra of **RS**