# Supplementary Information

## A red-emitting fluorescence probe for hydrogen sulfide in living cells with a large Stokes shift

Lei Yang,[a] Yuanan Su,[a] Zhankui Sha,[b] Yani Geng,[a] Fengpei Qi [a,c] and Xiangzhi Song[a*]

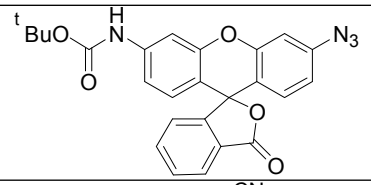
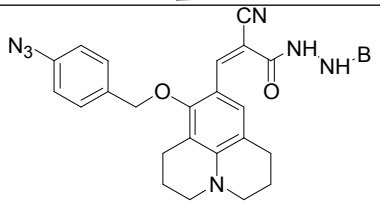
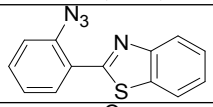
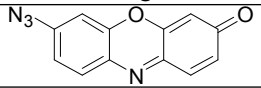
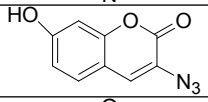
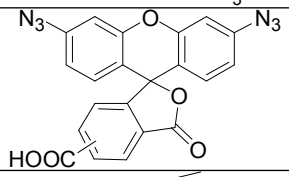
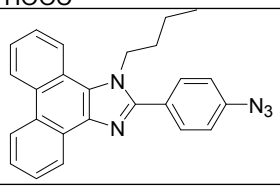
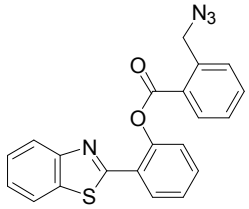
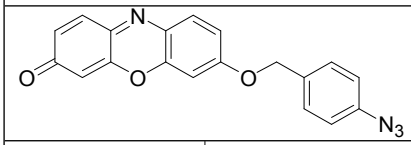
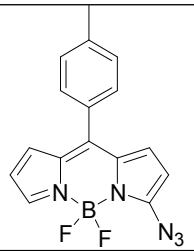
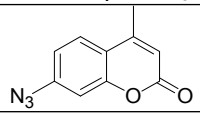
[a] College of Chemistry & Chemical Engineering, Central South University, Changsha, Hunan Province, P. R. China, 410083.

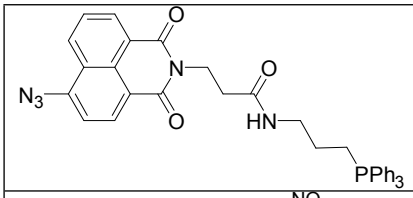
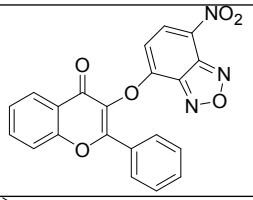
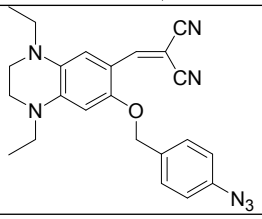
[b] Faculty of Materials Science and Chemistry, China University of Geosciences, Wuhan, Hubei, Province, P.R. China 430074,

[c] Department of Chemistry and Environmental Engineering, Hunan City University, Yiyang, Hunan Province, P. R. China, 413000.

* Corresponding author. Fax: +86-731-88836954; Tel: +86-731-88836954; E-mail: xzsong@csu.edu.cn

**Table S1** Fluorescent probes for Hydrogen sulfide based on the reaction of azido reduction.

| Probe | $\lambda_{ex}/\lambda_{em}$ | Stokes shift | Literature |
|---|---|---|---|
|  | 490 nm/525 nm | 35 nm | J. Am. Chem. Soc. 2011, 133, 10078–10080 |
|  | 440 nm/500 nm | 60 nm | Anal. Chem. 2016, 88, 7873-7877 |
|  | 375 nm/450 nm | 75 nm | Chem. Comm, 2014, 50, 4214-4217 |
|  | 565 nm/590 nm | 25 nm | Anal. Bioanal. Chem., 404, 2012, 1919–1923 |
|  | 390 nm/483 nm | 93 nm | J. Fluoresc, 23, 2013, 181–186 |
|  | 480 nm/525 nm | 45 nm | PNAS, 2013, 18, 7131–7135 |
|  | 350 nm/423 nm | 73 nm | Org. Biomol. Chem., 2012, 10, 9683 |
|  | 381 nm/462 nm | 81 nm | RSC Adv., 2016, 6, 62406–62410 |
|  | 575 nm/600 nm | 25 nm | Chem. Commun., 2017, 53, 2275--2278 |
|  | 444 nm/520 nm | 76 nm | Org. Biomol. Chem., 2013, 11, 8166–8170 |
|  | 340 nm/445 nm | 115 nm | Analyst, 2013, 138, 946–951 |

| | | | |
|---|---|---|---|
|  | 440 nm/540 nm | 100 nm | Sensors and Actuators B, 2017, 248, 50–56 |
|  | 346 nm/516 nm | 170 nm | Tetrahedron, 2016, 72, 3531-3534 |
|  | 485 nm/610 nm | 125 nm | This Work |

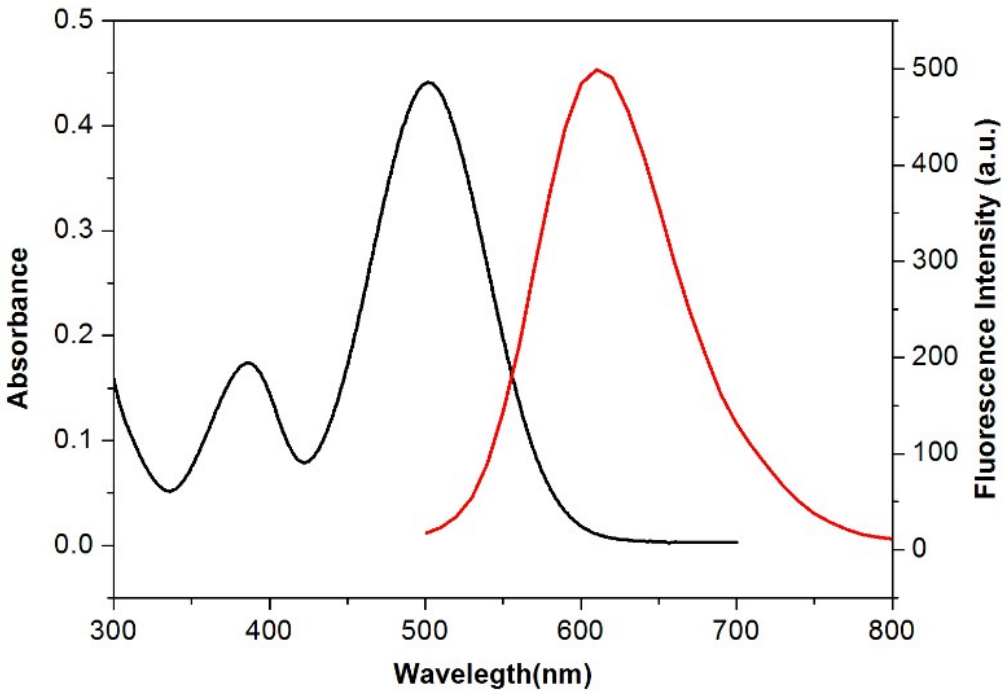



**Fig. S1** Absorption (black) and emission (red) spectra of dye **5** in HEPES buffer (20.0 mM, 1.0 mM CTAB, pH=7.4).

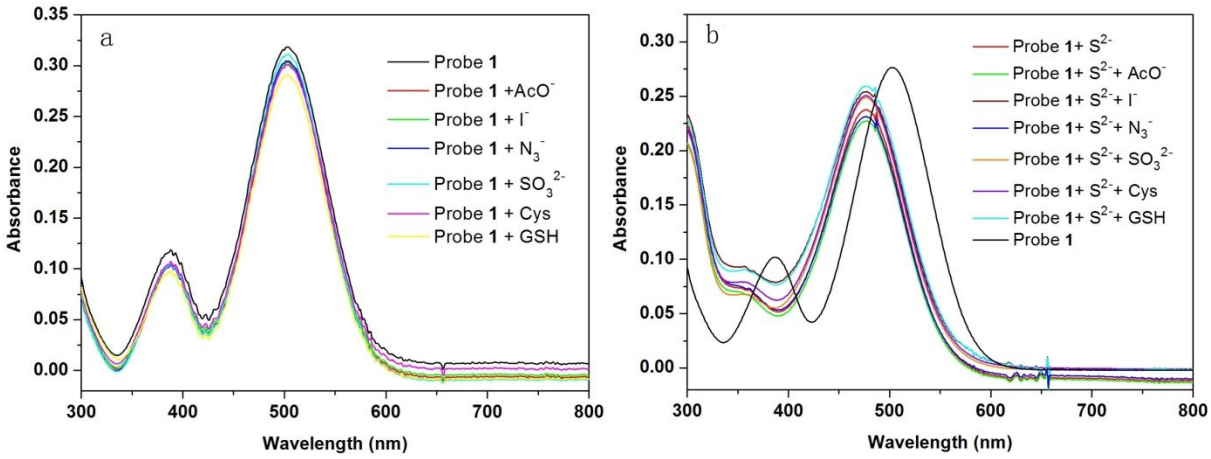



**Fig. S2** (a) Absorption spectra of Probe **1** (10.0 $\mu$M) upon the treatment with electron donors in HEPES buffer (20.0 mM, 1.0 mM CTAB, pH = 7.4). (b) Absorption spectra of Probe **1** (10.0 $\mu$M in response to $Na_2S$ (5.0 equiv.) with the co-existence of electron donors in HEPES buffer (20.0 mM, 1.0 mM CTAB, pH = 7.4).

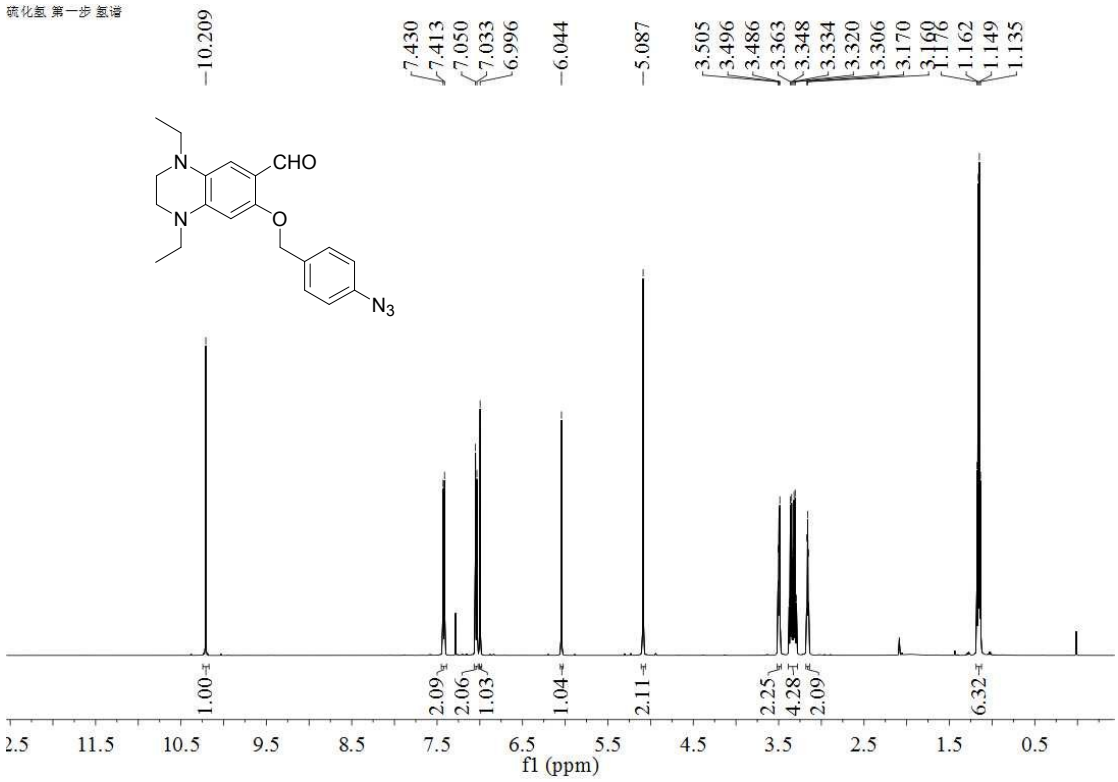



**Fig. S3** $^1$H NMR spectrum of compound **4** in $CDCl_3$.

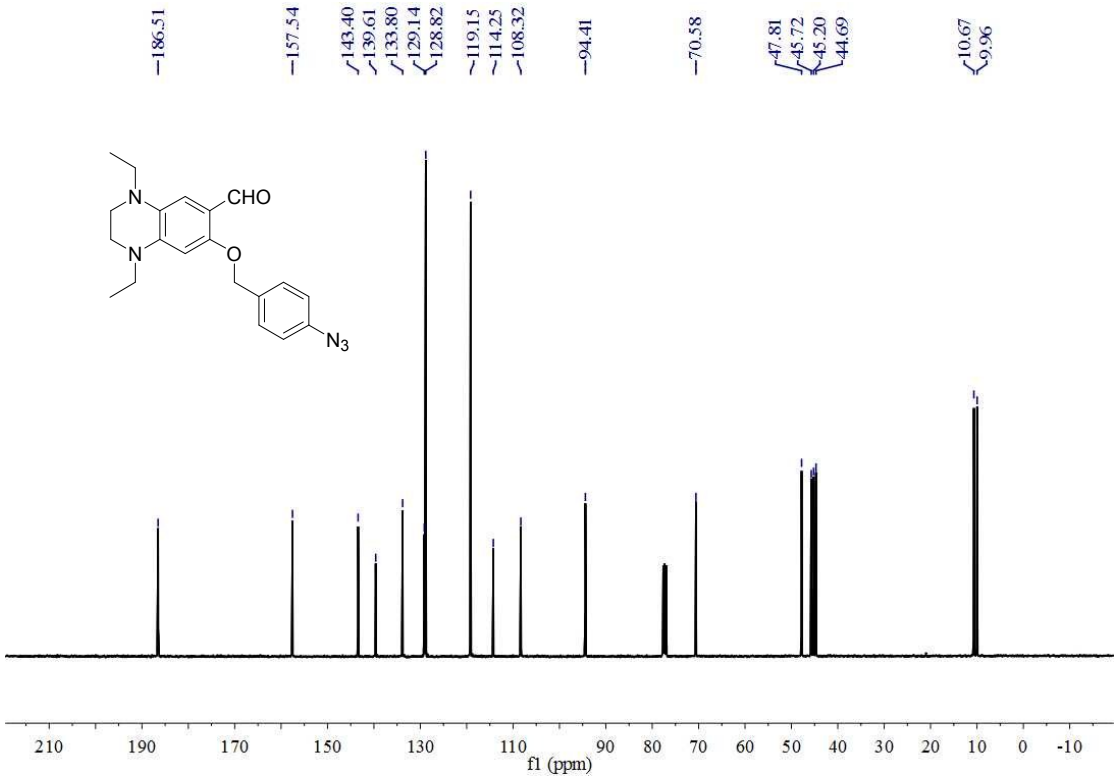



**Fig. S4** $^{13}C$ NMR spectrum of compound **4** in $CDCl_3$.

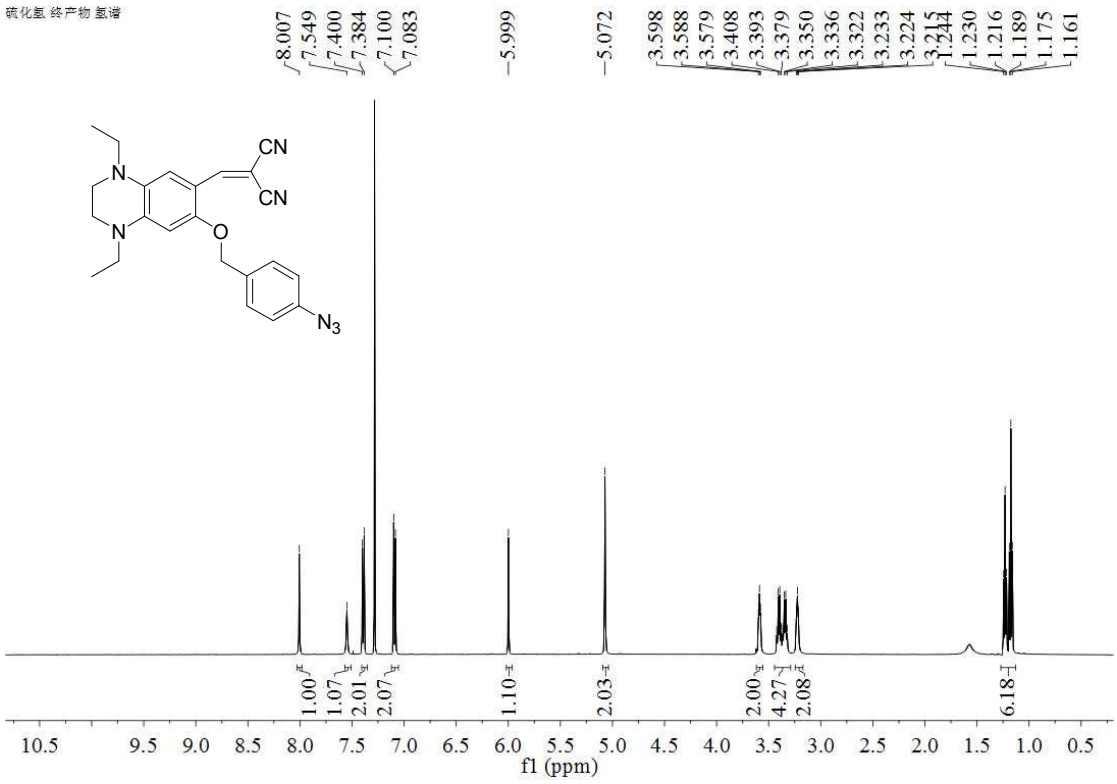



**Fig. S5** $^{1}H$ NMR spectrum of Probe **1** in $CDCl_3$.

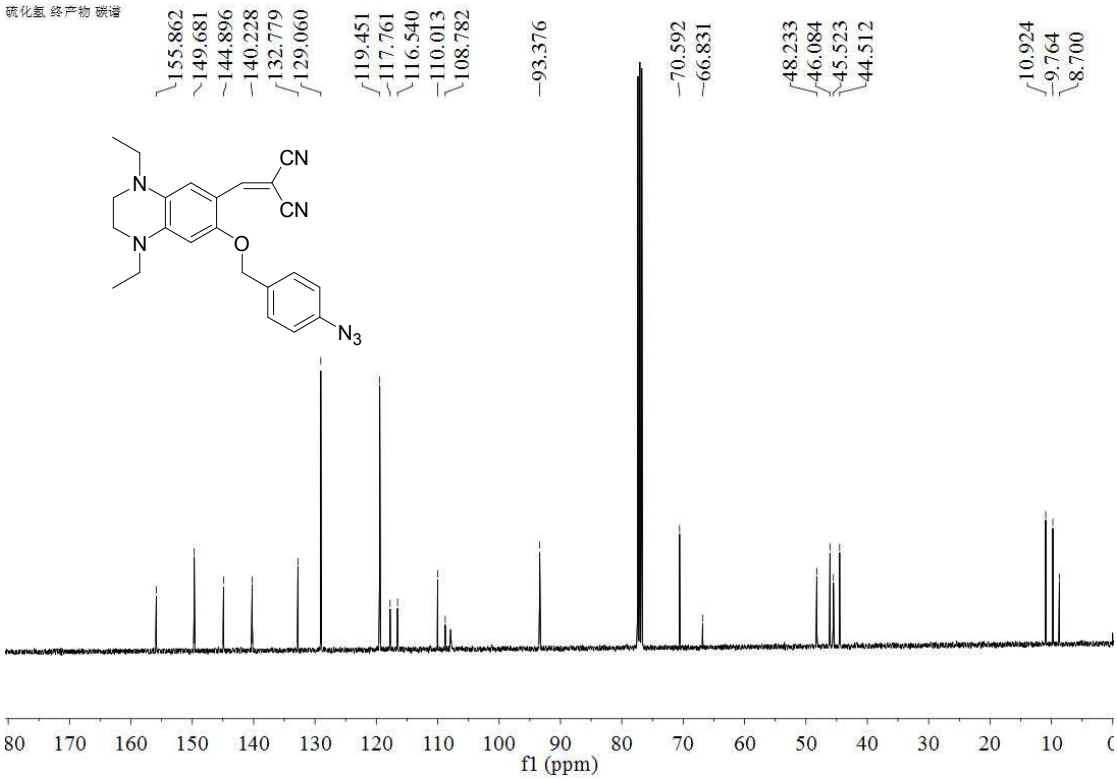



**Fig. S6** $^{13}C$ NMR spectrum of Probe **1** in $CDCl_3$.

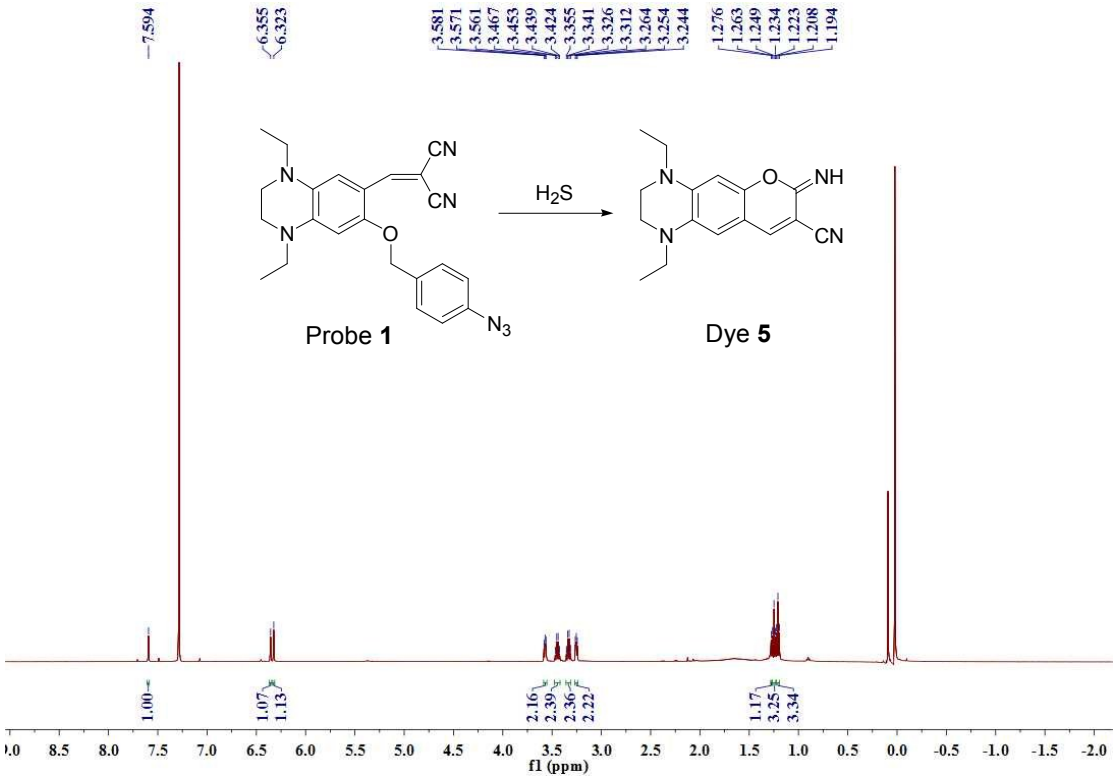



**Fig. S7** $^{1}H$ NMR spectrum of the reaction product of Probe **1** with $Na_2S$ in $CDCl_3$.

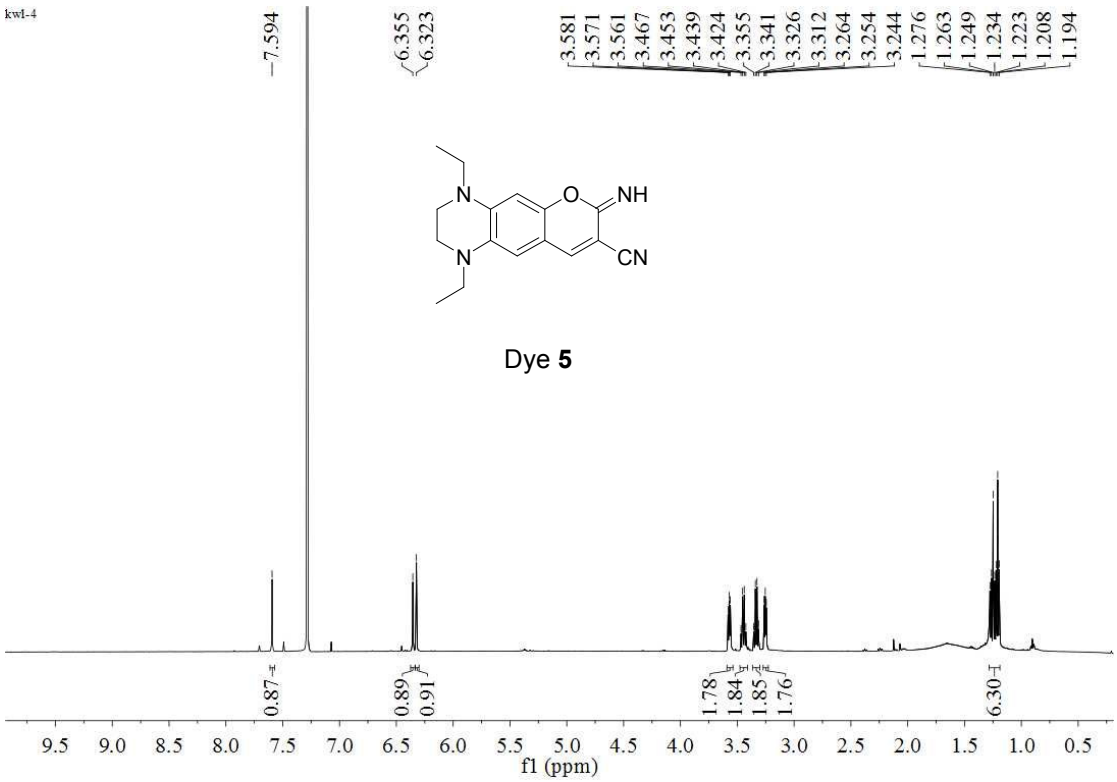



**Fig. S8** $^{1}$H NMR spectrum of dye **5** in $CDCl_3$.

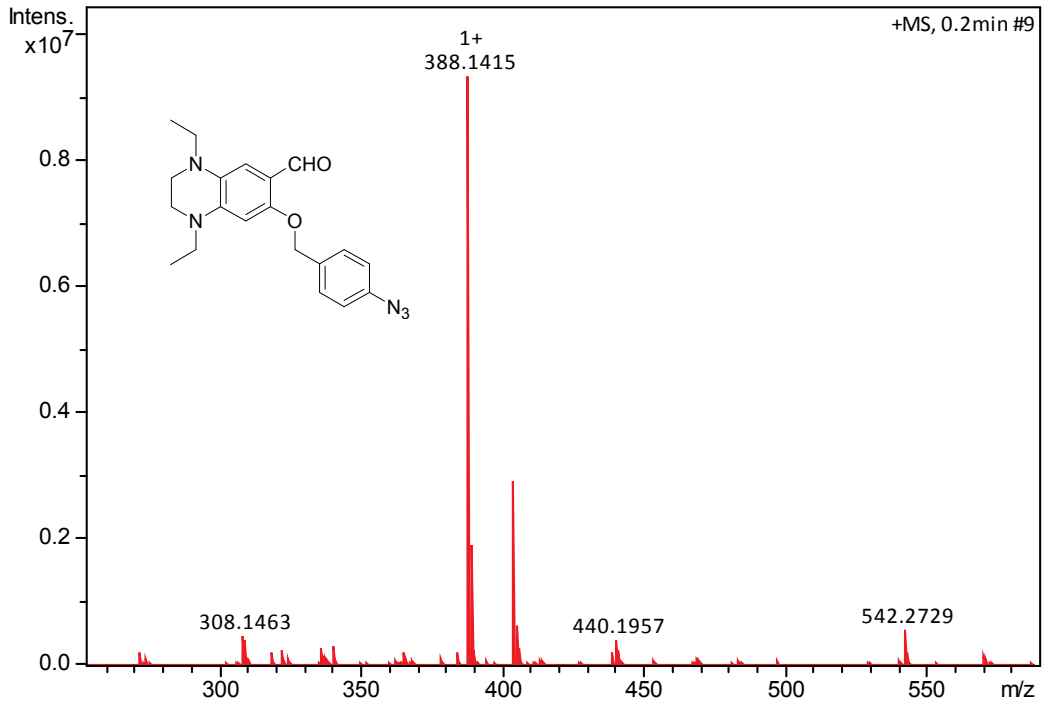



**Fig. S9** HRMS spectrum of compound **4**.

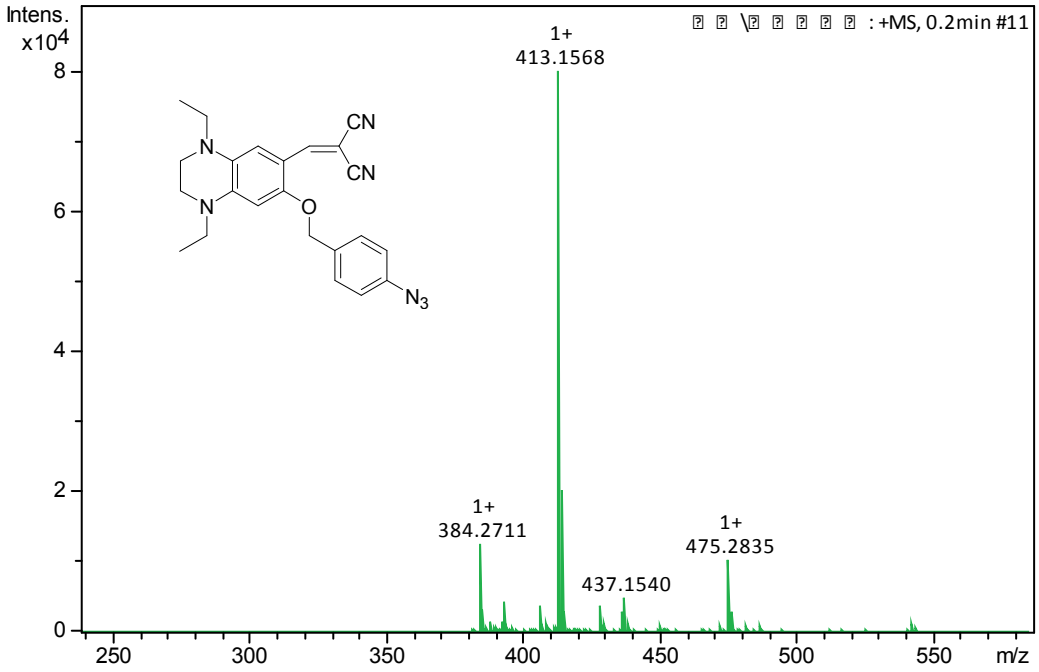



**Fig. S10** HRMS spectrum of Probe **1**.

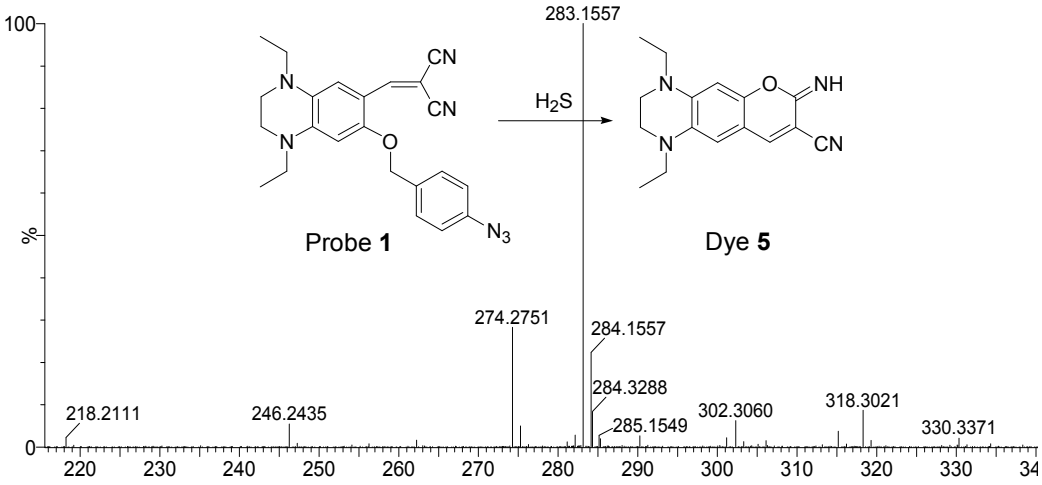



**Fig. S11** HRMS spectrum of the reaction product from Probe **1** with $Na_2S$.

**Comparison of Probe 1 and AcHS in response $H_2S$**

**AcHS** in the literature (J. Org. Chem. 2014, 79, 9481−9489) was prepared to compare with our probe in the detection of $H_2S$ in solution. In EtOH/PBS (v/v 1:4, pH=7.0), at least 120 min is needed to reach the maximized fluorescence enhancement when **AcHS** (10 μM) was treated with 100 equiv. of $Na_2S$. In our work, the fluorescence intensity reached a plateau within 30 min when our probe (10 μM) was treated with 5.0 equiv. of $Na_2S$ in HEPES buffer (20.0 mM, pH = 7.4, 1.0 mM CTAB). CTAB (cetyl trimethyl ammonium Bromide) is a cationic surfactant, and forms micelles in aqueous solutions which act as "microreactors" to dramatically enhance the reaction rate between the organic reactant.[1] The presence of CTAB in aqueous solution can significantly accelerate the reaction rate of many reactions at a low concentration.[2] Therefore, we investigated the fluorescence response of **AcHS** (10 μM) toward $Na_2S$ in HEPES buffer (20.0 mM, pH = 7.4 with 1.0 mM CTAB). First, we incubated Probe **AcHS** (10 μM) in HEPES buffer (20.0 mM, pH = 7.4, 1.0 mM CTAB) with different concentrations of $Na_2S$ (0-11 equiv.) for 1 hour. As shown in Fig. S12, the fluorescence signal levelled off when the concentration of $Na_2S$ exceeded 100 μM (10 equiv.). As a result, the time-dependent fluorescence experiment was conducted using 100 μM $Na_2S$ to react with 10 μM **AcHS** in HEPES buffer (20.0 mM, pH = 7.4, 1.0 mM CTAB). It was seen in Fig. S13 that a maximized fluorescence signal was obtained within 10 min. This result, coupled with our work and the work in the literature (J. Org. Chem. 2014, 79, 9481−9489), indicated that CTAB played an important role in accelerating the sensing reaction.

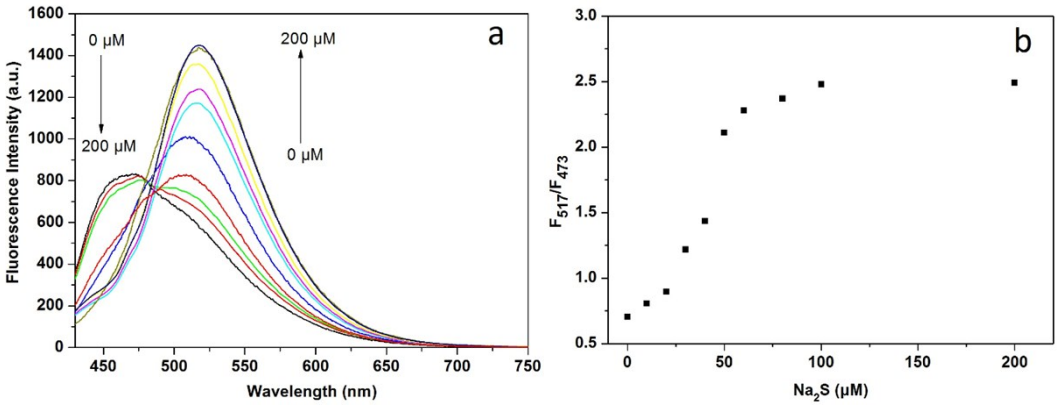



**Fig. S12** (a) Fluorescence spectra of **AcHS** (10 μM) with $Na_2S$ (0-200 μM) for 1 hour in HEPES buffer (20.0 mM, pH = 7.4, 1.0 mM CTAB). (b) Ratio of emission intensity of **AcHS** (10 μM) after incubation with different concentrations of $Na_2S$ for 1 hour in HEPES buffer (20.0 mM, pH = 7.4, 1.0 mM CTAB). Excitation wavelength: 410 nm. EX/EM slits: 5 nm.

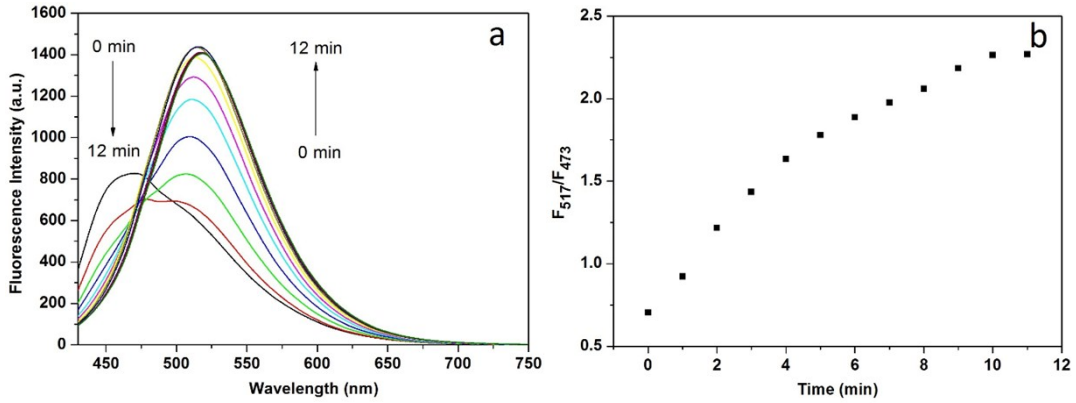



**Fig. S13** (a) Time-dependent fluorescence spectra of **AcHS** (10 μM) with the solution of $Na_2S$ (100 μM) in HEPES buffer (20.0 mM, pH = 7.4, 1.0 mM CTAB). (b) Ratio of emission intensity of **AcHS** (10 μM) with the solution of $Na_2S$ (100 μM) in HEPES buffer (20.0 mM, pH = 7.4, 1.0 mM CTAB) as a function of time. Excitation wavelength: 410 nm. EX/EM slits: 5 nm.

1. C. A. Bunton, F. Nome, F. H. Quina and L. S. Romsted, Ion binding and reactivity at charged aqueous interfaces, Accounts of Chemical Research, 1991, 24, 357-364.

2. B. Samiey, C.-H. Cheng, and J.Wu, Effects of Surfactants on the Rate of Chemical Reactions, Journal of Chemistry, 2014, Article ID 908476, 14 pages.