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Supporting information

Naked-eye and ratiometric fluorescence probe for fast and sensitive detection of hydrogen sulfide and its application in bioimaging

Jianlong Ma[ac], Feifei Li[bc], Qiang Li[b], Yijing Li[a], Chaoxian Yan[d], Xiaofeng Lu[a]* and Yong Guo[a]*

[a]Key Laboratory of Chemistry of Northwestern Plant Resources and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou Gansu 730000, P. R. China; [b]Key Laboratory of Heavy Ion Radiation Biology and Medicine of Chinese Academy of Sciences, Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou 730000, P. R China; [c] University of the Chinese Academy of Sciences, 100049, Beijing, P. R. China.
[d]State Key Laboratory of Applied Organic Chemistry Lanzhou University

* E-mail: guoyong@licp.cas.cn

* E-mail: luxf@licp.cas.cn

Contents

1. Synthetic procedures in detail

Synthesis of **Compound 1.** The dark green solid with metallic luster was obtained with great yield (88%). ^{1}H NMR (400 MHz, CD3OD) δ (ppm): 8.46 (d, *J* = 14.1 Hz, 2H), 7.54 (d, *J* = 7.4 Hz, 2H), 7.44 (t, *J* = 7.7 Hz, 2H), 7.38 – 7.27 (m, 4H), 6.30 (d, *J* = 14.1 Hz, 2H) , 4.23 (d, *J* =7.2 Hz, 4H), 2.75 (t, *J* = 6.1 Hz, 4H), 2.02 – 1.94 (m, 2H), 1.75 (s, 12H), 1.42 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ (ppm): 173.83, 151.10, 145.63, 143.15, 142.73, 129.94, 127.91, 126.56, 123.57, 112.05, 101.95, 50.65, 40.35, 28.23, 27.35, 22.12, 12.50. ESI-MS calcd for $C_{34}H_{40}ClN_2^+$ (M^+): 511.29. Found: 511.29.

Synthesis of **Compound 2.** In detail, under nitrogen atmosphere, K_2CO_3 (311 mg, 2.50 mmol) was added rapidly to resorcinol (275 mg, 2.50 mmol) in anhydrous acetonitrile (6 mL) at room temperature, and the resulting mixture was stirred for 15 min. Then a solution of compound **1** (590 mg, 1.00 mmol) in 10 mL of anhydrous acetonitrile was introduced to the above mixture via a syringe and the mixture was heated at 50℃ for 4 h. After the reaction was complete, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (dichloromethane: methanol=100:1) on silica gel. Obtaining the desired product (362 mg, yield: 76%) as a blue powder. ^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 8.76 (d, *J* = 14.8 Hz, 1H), 7.65 (d, *J* = 7.6 Hz, 1H), 7.52 (d, *J* = 2.4 Hz, 2H), 7.45 (s, 2H), 7.43 – 7.39 (m, 1H), 6.86 (d, *J* = 6.7 Hz, 2H), 6.46 (d, *J* = 14.8 Hz, 1H), 4.43 – 4.31 (m, 2H), 2.79 (t, *J* = 6.4 Hz, 2H), 2.73 (t, *J* = 6.0 Hz, 2H), 2.00 – 1.91 (m, 2H), 1.82 (s, 6H), 1.48 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ (ppm): 177.94, 164.12, 163.62, 156.29, 146.63, 143.35, 142.65, 136.37, 130.47, 130.20, 128.02, 127.38, 123.80, 116.34, 116.08, 115.58, 113.32, 103.48, 102.97, 51.73, 41.20, 29.96, 28.32, 25.07, 21.74, 12.76. ESI-MS calcd for $C_{27}H_{28}NO_2^+$ (M^+): 398.21. Found: 398.21.

Synthesis of **CyT.** Under nitrogen atmosphere, compound **2** (238 mg, 0.50 mmol) was stirred in 6ml dichloromethane and triethylamine mixed solution (v/v = 1:1) at 0℃ for 10 min. Then, triflic anhydride (423 mg, 1.50 mmol) dissolved in 1.5 mL of methylene chloride was added dropwise with continuously stirring. The solution was stirred for another 60 min at 0℃ and then 25℃ for 1 h. After the reaction was complete, 20 ml water poured into and extracted with 30 ml dichloromethane for 3

times. The water in organic phase was eliminated by adding excessive sodium sulphate anhydrous. Eventually the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (dichloromethane: methanol=50:1) on silica gel. Affording the desired product (192 mg, yield: 63%) as purple sticky solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.78 (d, *J* = 15.3 Hz, 1H), 7.71 (dd, *J* = 15.1, 7.6 Hz, 2H), 7.58 (m, 2H), 7.54 (dd, *J* = 9.1, 4.7 Hz, 2H), 7.28 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.24 (s, 1H), 6.72 (d, *J* = 15.6 Hz, 1H), 4.51 (q, *J* = 7.2 Hz, 2H), 2.77 (dt, *J* = 18.0, 5.8 Hz, 4H), 1.97 (dd, *J* = 17.2, 11.3 Hz, 2H), 1.85 (s, 6H), 1.53 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ (ppm): 180.79, 159.96, 154.35, 151.48, 148.02, 144.36, 142.22, 133.15, 130.45, 130.34, 130.13, 129.59, 124.01, 123.62, 119.13, 116.48, 114.68, 110.82, 107.77, 52.83, 42.25, 30.42, 27.70, 24.97, 21.36, 13.22. ESI-MS calcd for $C_{27}H_{28}F_3NO_4S^+$ (M+): 530.58. Found: 530.16

2. ^{1}H NMR, ^{13}C NMR and Mass spectra

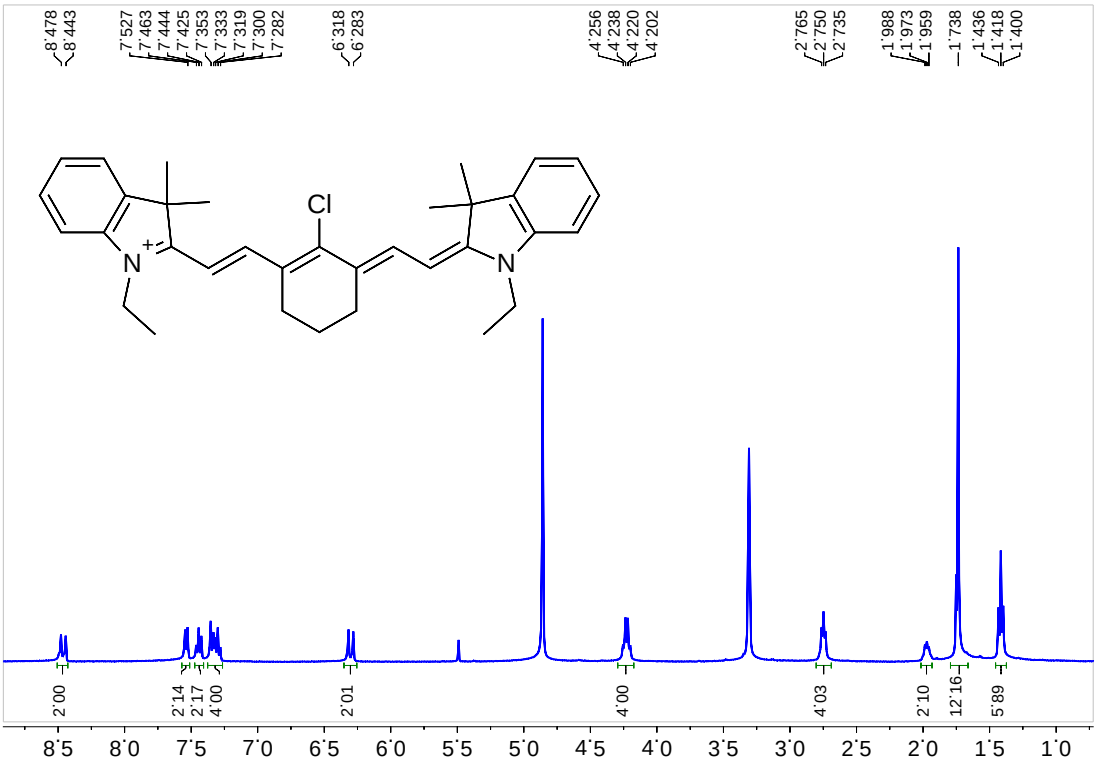

Fig. S1 ^{1}H NMR (400 MHz) spectrum of **compound 1** in CD_3OD

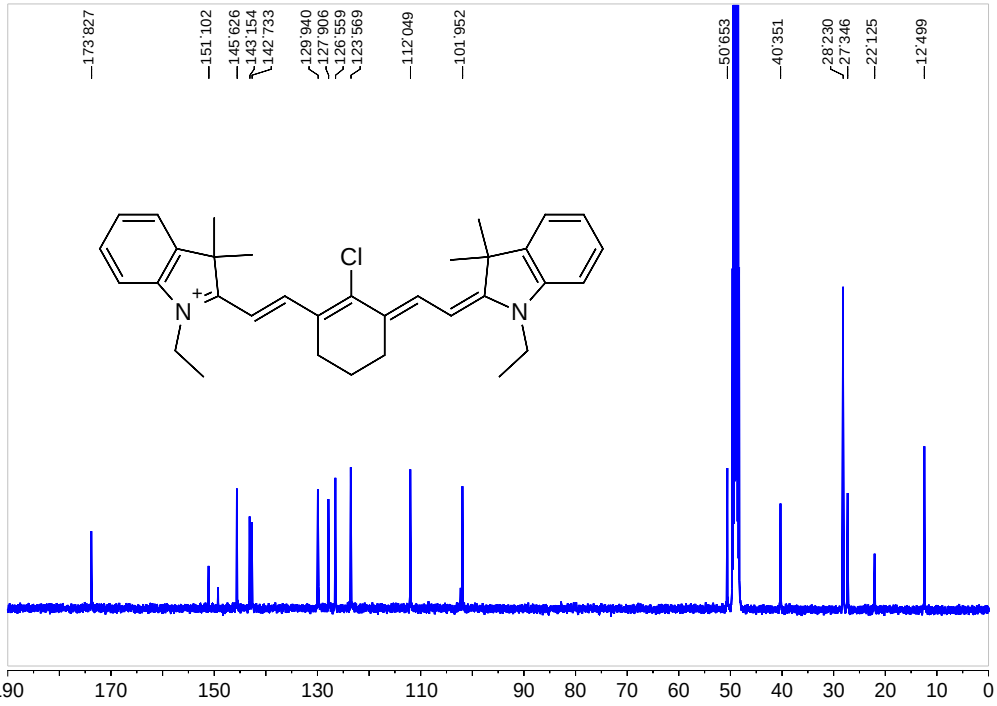

Fig. S2 ^{13}C NMR (100 MHz) spectrum of **compound 1** in CD_3OD

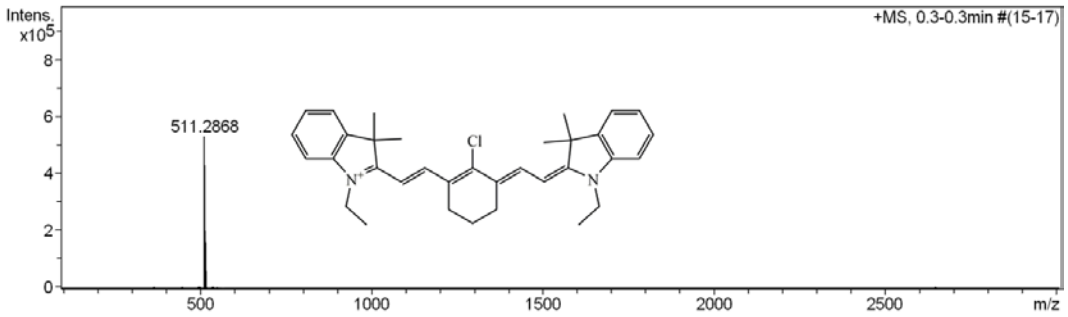

Fig. S3 Mass spectrum of **compound 1**

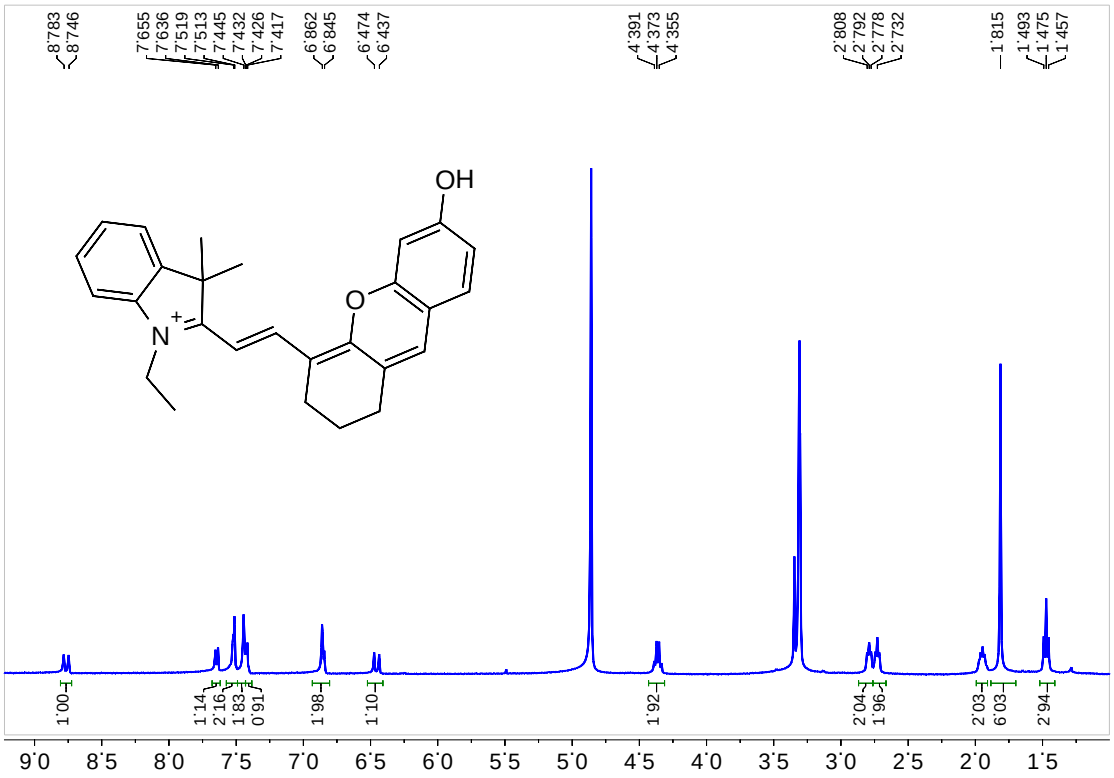

Fig. S4 ^{1}H NMR (400 MHz) spectrum of **compound 2** in CD_3OD

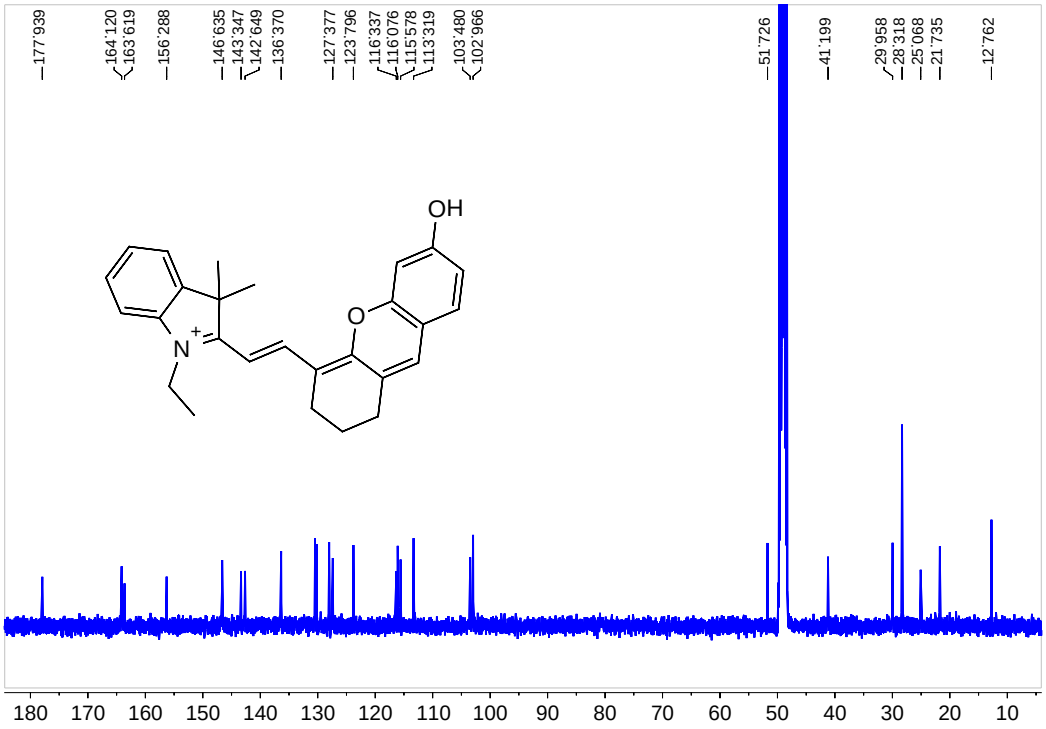

Fig. S5 ^{13}C NMR (100 MHz) spectrum of **compound 2** in CD_3OD

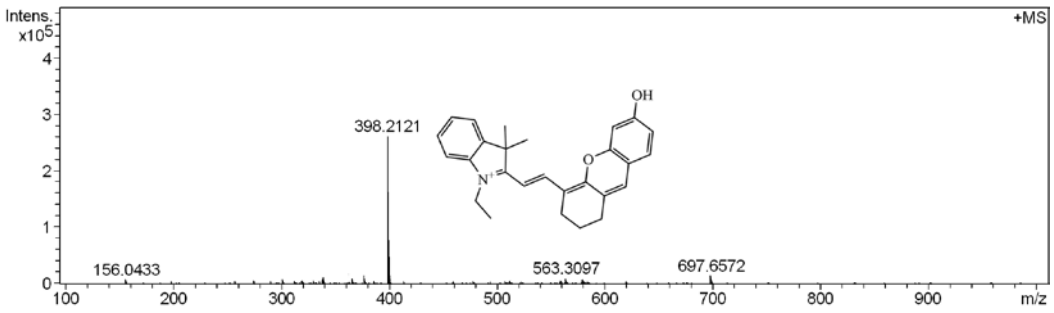

Fig. S6 Mass spectrum of **compound 2**

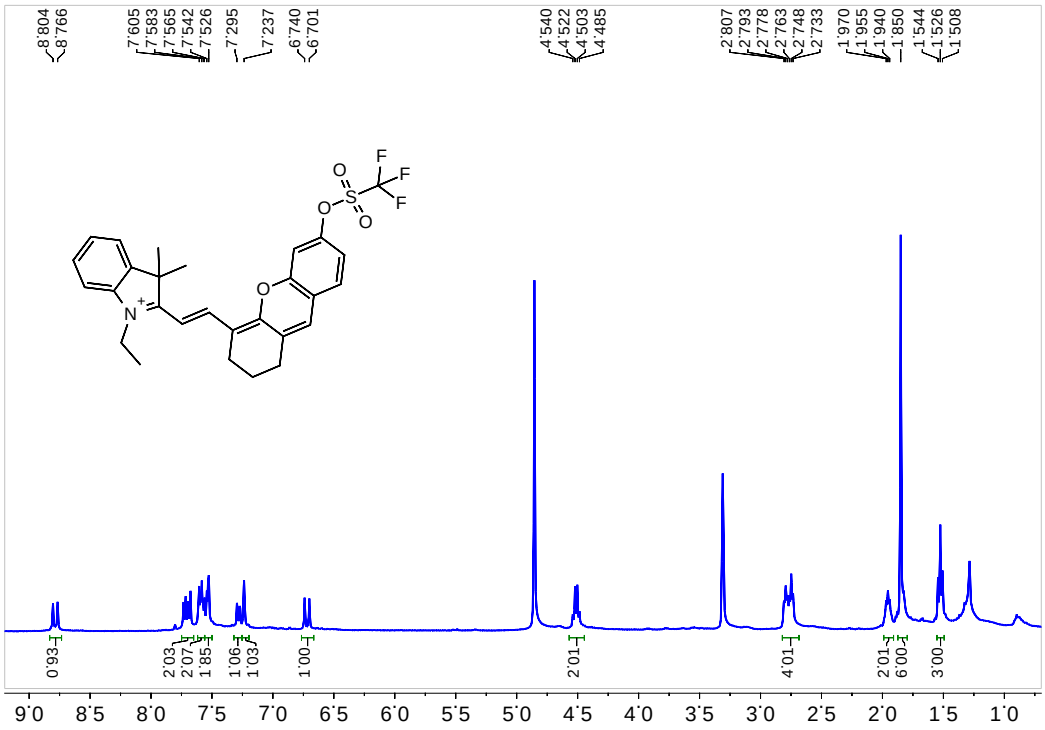

Fig. S7 ^{1}H NMR (400 MHz) spectrum of **CyT** in CD_3OD

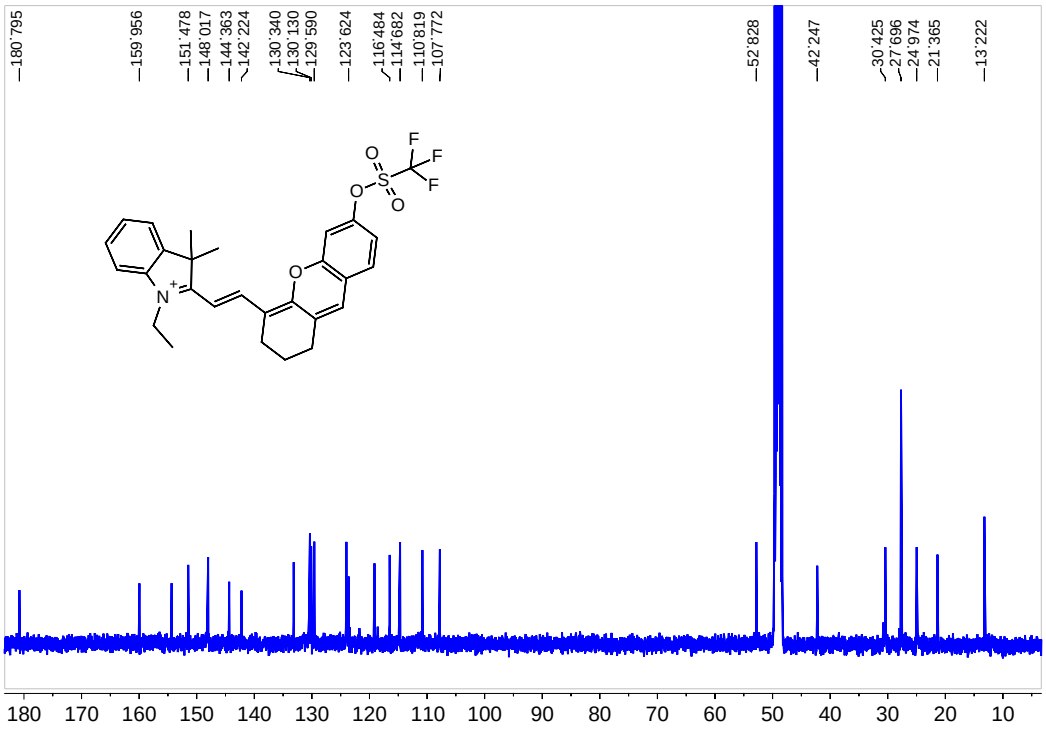

Fig. S8 ^{13}C NMR (100 MHz) spectrum of **CyT** in CD_3OD

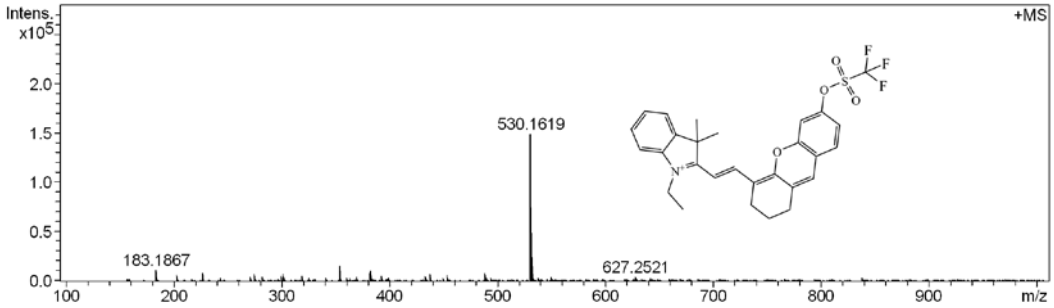

Fig. S9 Mass spectrum of **CyT**

3. Supporting figures

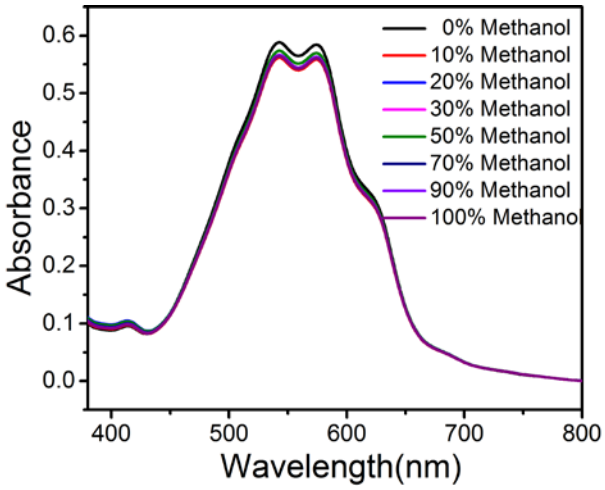

Fig. S10 The absorption spectra of **CyT** in methanol/PBS mixtures with different methanol fractions.

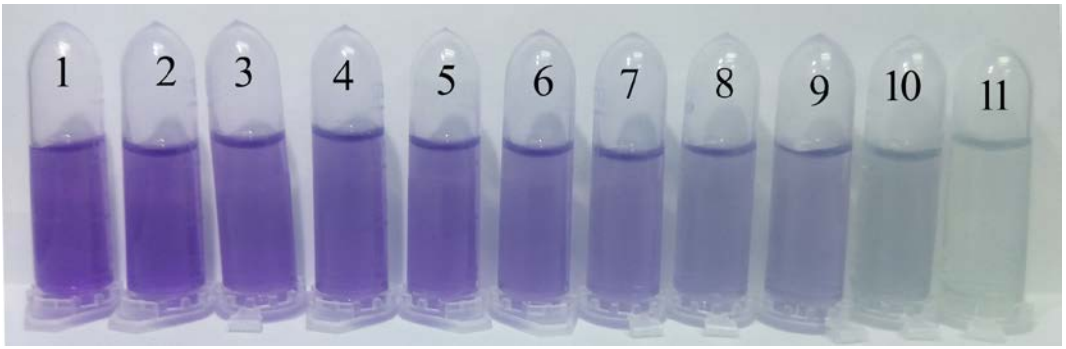

Fig. S11 The color change of **CyT** (10 μM) upon addition of S^{2-}/HS^{-} in PBS buffer (10 mM, pH 7.40, 2% methanol, v/v). Each picture was recorded at 5 min after the addition of S^{2-}/HS^{-} (1-11: 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0 μM).

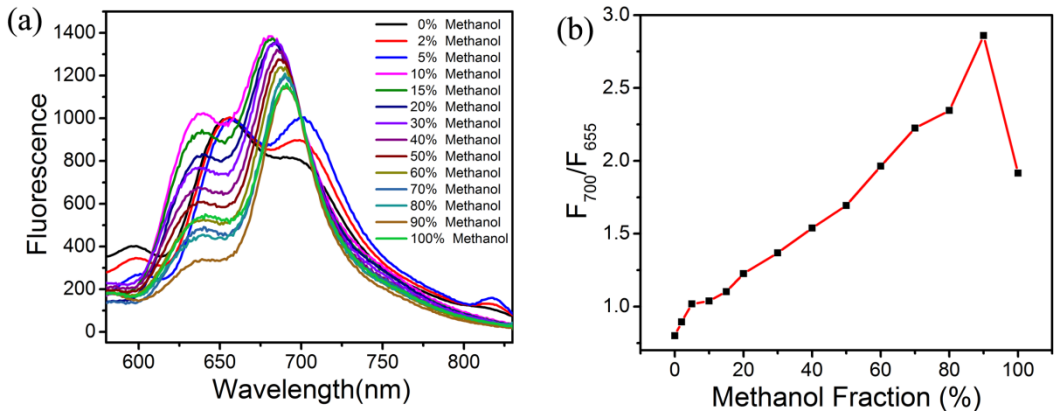

Fig. S12 (a) Normalized fluorescence spectra of CyT in methanol /PBS mixtures with different methanol fractions. (b) Effect of methanol volume on the fluorescence intensity ratio of 700 nm and 655nm (F_{700}/F_{655}), λ_{ex}, 575 nm.

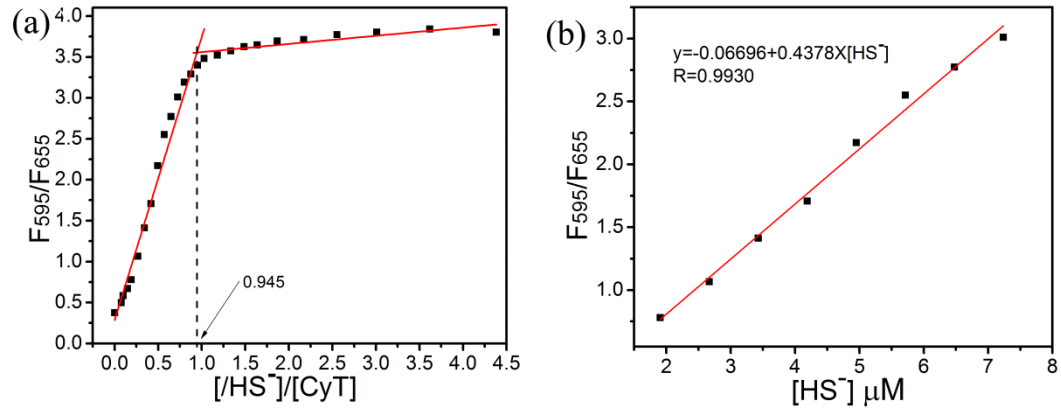

Fig. S13 (a) Fluorescence emission ratio F_{595}/F_{655} of **CyT** (10 μM) in PBS buffer (10 mM, pH 7.40, 2% methanol, v/v) as a function of HS^-/**CyT** in the range of 0~4 upon excitation at 575 nm. (b) Fluorescence emission ratio F_{595}/F_{655} of **CyT** (10 μM) in PBS buffer (10 mM, pH 7.40, 2% methanol, v/v) as a function of HS^- concentration in the range of 2~7.5 μM upon excitation at 575 nm.

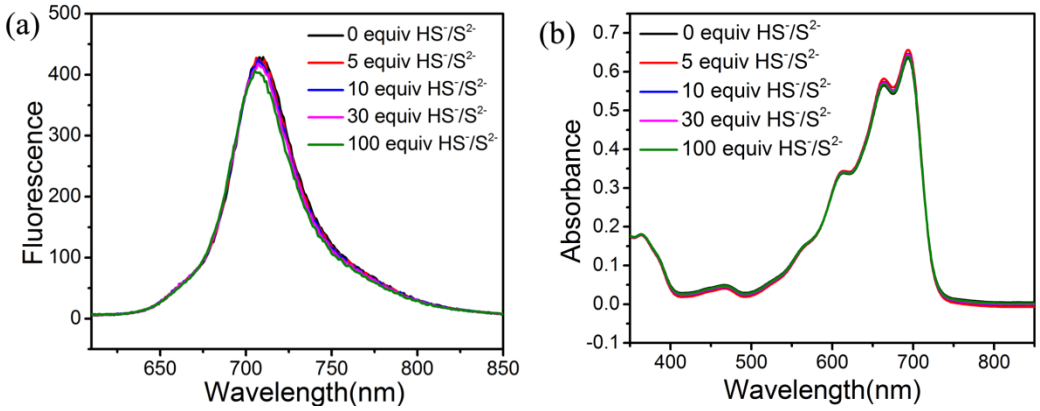

Fig. S14 (a) Fluorescence emission spectra of **compound 2** (10 μM) in PBS (10 mm, pH 7.40, 2% methanol, v/v) obtained upon different amount of S^{2-}/HS^{-}. λ_{ex}, 575 nm. (b) Absorption spectra of **compound 2** (10 μM) in PBS (10.0 mM, pH 7.40, 2% methanol, v/v) obtained upon different amount of S^{2-}/HS^{-}.

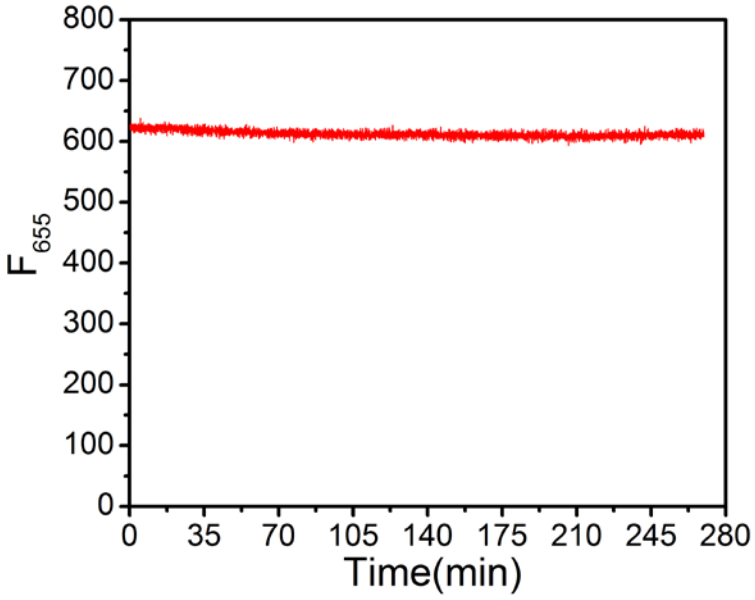

Fig. S15 Fluorescence intensity of **CyT** at 655nm as a function of time, λ_{ex}, 575 nm.

Table S1. The comparison of new probe herein with other representative probes reported.

Probe	Spectral change	λ_{em}	Response time or rate constant	The detection limit.	Solution
F-3[1]	Fluorescence turn-on	510nm	0.0141 s^{-1}	24 nM	DMSO:HEPES = 1:100
BH-HS[2]	Fluorescence turn-on	553 nm	160 s	1.7 μM	DMF:PBS = 1:1
CPC[3]	Fluorescence ratiometric	587 nm/474 nm	600 s	40 nM	DMF:PBS = 4:6
FEPO[4]	Fluorescence turn-off	520 nm	0.00411 s^{-1}	—	Methanol:PBS = 1:99
NR-HS[5]	Fluorescence turn-on	655 nm	20 min	0.27 μM	DMSO: PBS = 1:9
1[6]	Fluorescence ratiometric	566 nm/620 nm	60 min	0.1 μM	PBS
CouMC[7]	Fluorescence ratiometric	652 nm/510 nm	10 s	1 μM	DMSO: PBS = 1:49
Cy–N_3[8]	Fluorescence ratiometric	710 nm/750 nm	20 min	—	HEPES
AzMB-coumarin[9]	Fluorescence turn-on	450 nm	20 min	10 μM	Acetonitrile:PBS = 1:4
Cy–NO_2[10]	Fluorescence turn-on	809 nm	70 min	—	HEPES
HSN2[11]	Fluorescence turn-on	542 nm	45 min	—	PIPES
NIR-H_2S[12]	Fluorescence turn-on	708 nm	0.006 s^{-1}	50 nM	—
HSip-1[13]	Fluorescence turn-on	516 nm	<1 s	—	PIPES
DNS-Az[14]	Fluorescence turn-on	525 nm	<1 s	—	Tween:PBS = 1:200
BOD-PhSe[15]	Fluorescence turn-off	610 nm	20 min	2.5 nM	DMSO:HEPES = 1:9
probe 1[16]	Fluorescence turn-on	532 nm	0.00204 s^{-1}	2.46 μM	Acetonitrile:PBS = 1:9
Azidoluciferin[17]	Fluorescence turn-on	—	60 min	0.1 μM	PBS
P1[18]	Fluorescence turn-on	524 nm	15 min	50 nM	Acetonitrile: HEPES = 1:99
SulpHensor[19]	Fluorescence turn-on	555 nm	0.00049 s^{-1}	0.5 μM	DMF:PBS = 1:9
PAC[20]	Fluorescence	605 nm	<1 s	16 nM	BBS

	turn-on				
Lyso-NHS[21]	Fluorescence turn-on	555 nm	20 min	0.48 μM	Acetonitrile:PBS = 1:9
1-H_2S[22]	Fluorescence turn-on	635 nm	15 min	50 μM	DMSO: PBS = 1:4
This work	Fluorescence ratiometric	655 nm/595 nm	0.1464 s^{-1}	7.33 nM	Methanol:PBS = 1:49

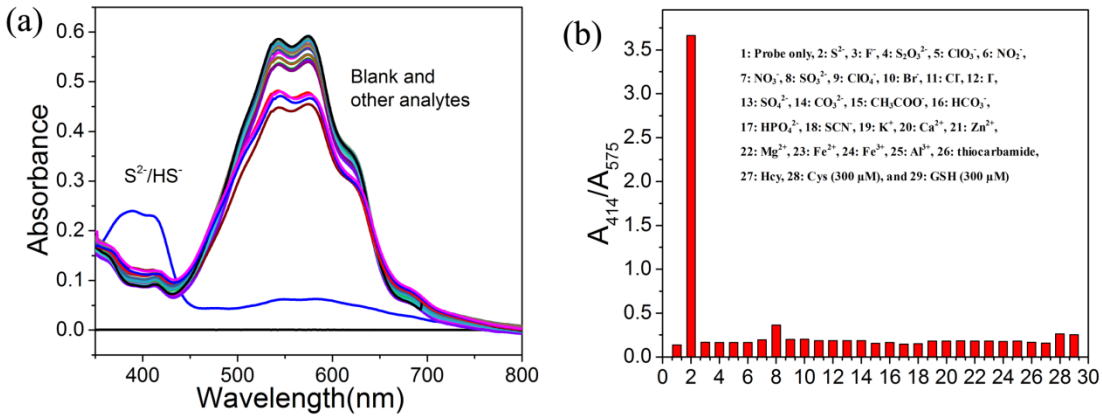

Fig. S16 (a) Absorption spectra and (b) absorbance ratio (A_{414}/A_{575}) of **CyT** (10 μM) in PBS (10 mm, pH 7.40, 2% methanol, v/v) in the presence of (1 equiv) S^{2-}/HS^- and (15~30 equiv) various biologically relevant species (F^-, $S_2O_3^{2-}$, ClO_3^-, NO_2^-, NO_3^-, HSO_3^-, ClO_4^-, Br^-, Cl^-, I^-, SO_4^{2-}, CO_3^{2-}, CH_3COO^-, HCO_3^-, HPO_4^{2-}, SCN^-, K^+, Ca^{2+}, Zn^{2+}, Mg^{2+}, Fe^{2+}, Fe^{3+}, Al^{3+}, thiocarbamide, Cys, Hcy and GSH).

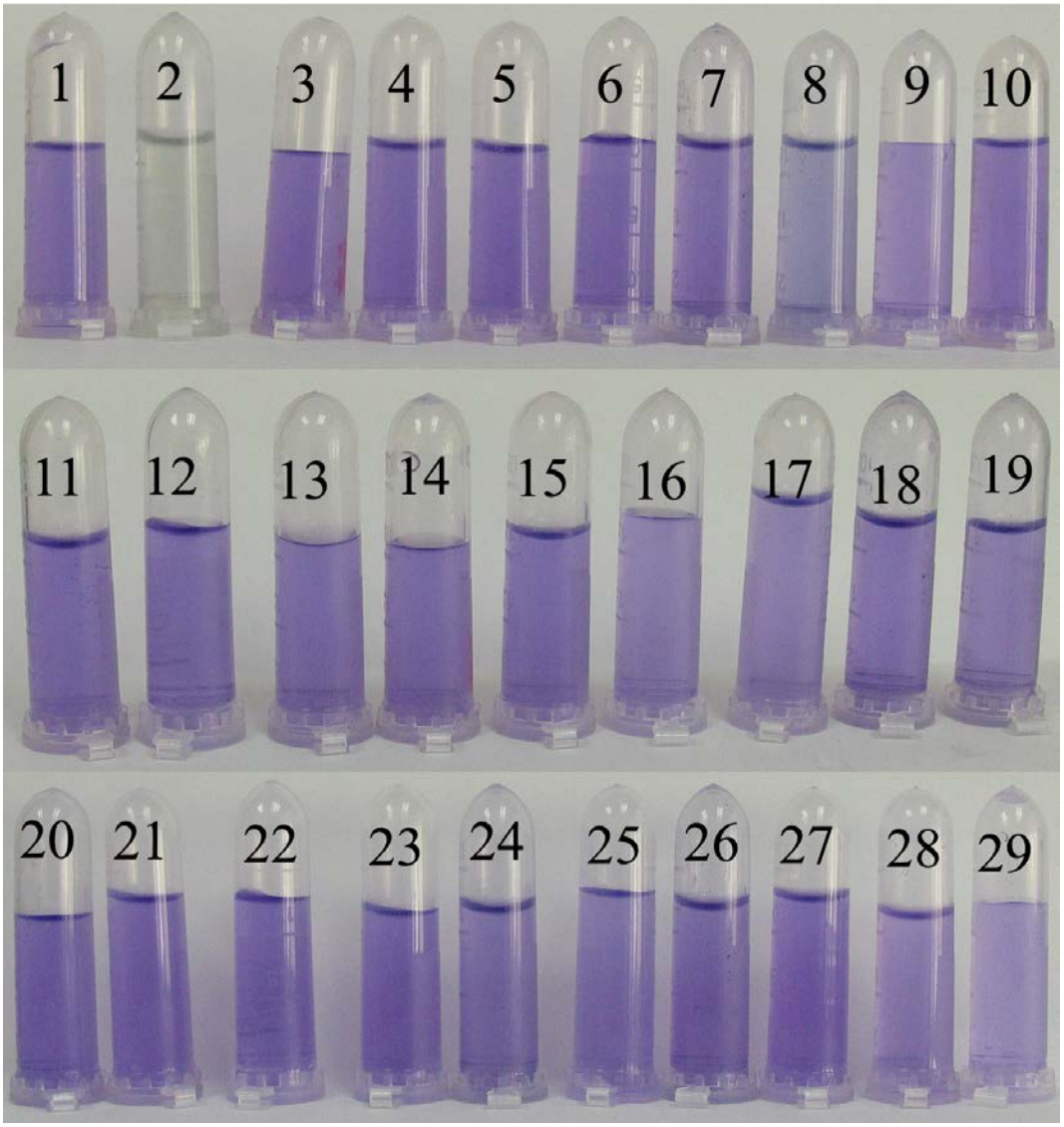

Fig. S17 Color change of **CyT** (10 μM) with Na_2S (10 μM) various analytes (150 μM). The pictures were recorded at 5 min after addition of the analytes (1: black, 2: S^{2-}, 3: F^-, 4: $S_2O_3^{2-}$, 5: ClO_3^-, 6: NO_2^-, 7: NO_3^-, 8: SO_3^{2-}, 9: ClO_4^-, 10: Br^-, 11: Cl^-, 12: I^-, 13: SO_4^{2-}, 14: CO_3^{2-}, 15: CH_3COO^-, 16: HCO_3^-, 17: HPO_4^{2-}, 18: SCN^-, 19: K^+, 20: Ca^{2+}, 21: Zn^{2+}, 22: Mg^{2+}, 23: Fe^{2+}, 24: Fe^{3+}, 25: Al^{3+}, 26: thiocarbamide, 27: Hcy, 28: Cys (300 μM), and 29: GSH (300 μM))

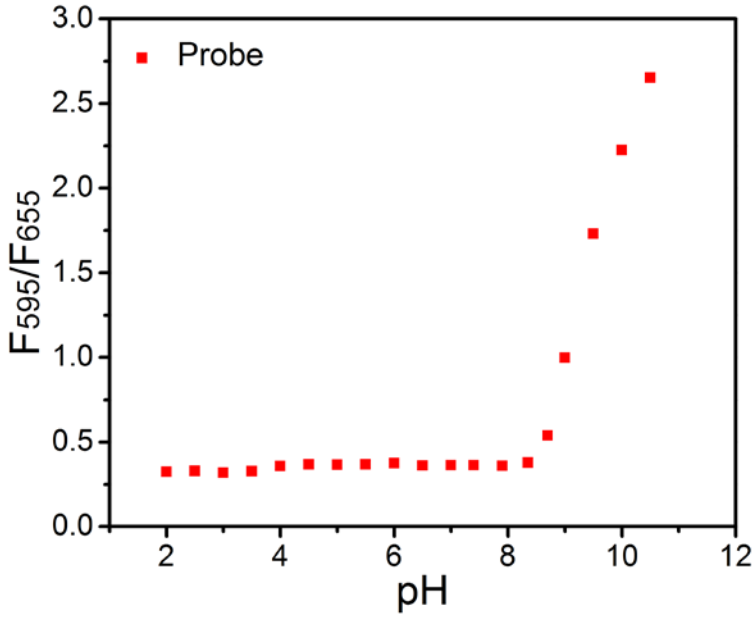

Fig. S18 Emission ratio F_{595}/F_{655} of **CyT** in PBS buffer (10 mM, pH 7.40, 2% methanol, v/v) at different pH values upon excitation at 545 nm.

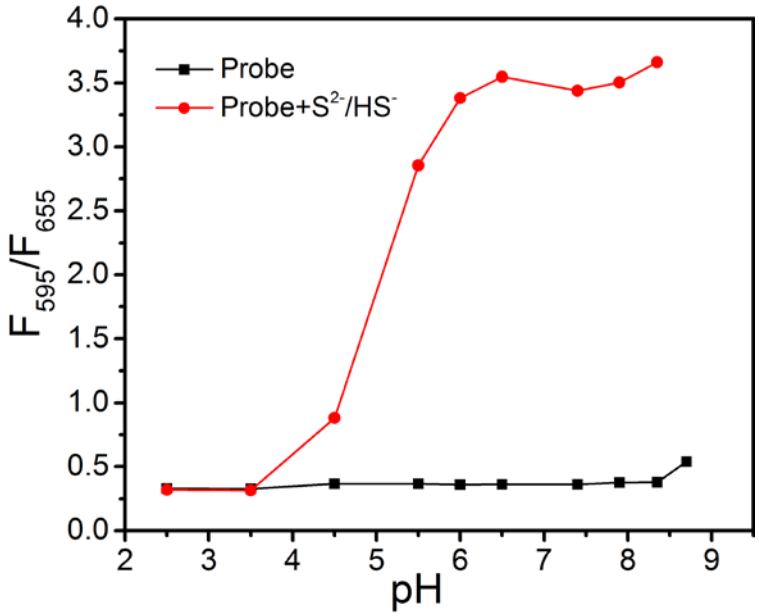

Fig. S19 The pH effect on the fluorescence behavior of CyT (black line) and CyT with S^{2-}/HS^{-} (red line).

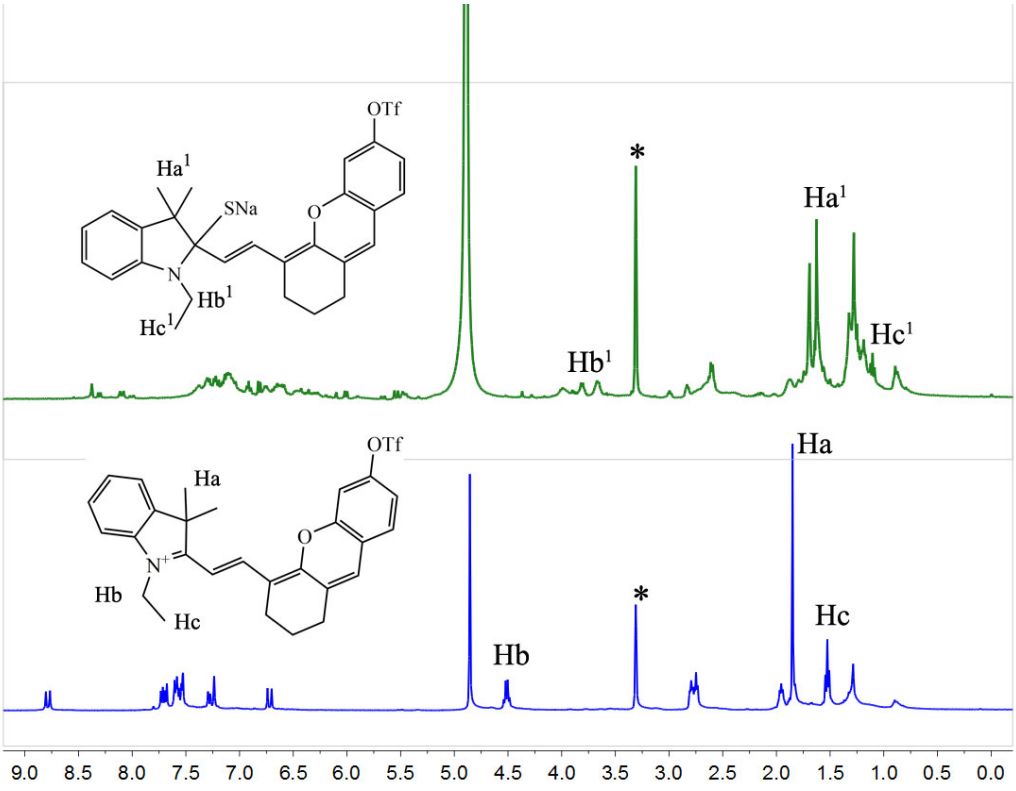

Fig. S20 ^{1}H NMR spectra of **CyT** in the absence (bottom) and presence (top) of 1.5 equiv S^{2-}/HS^{-} in CD_3OD. Signals marked with * are from the solvent.

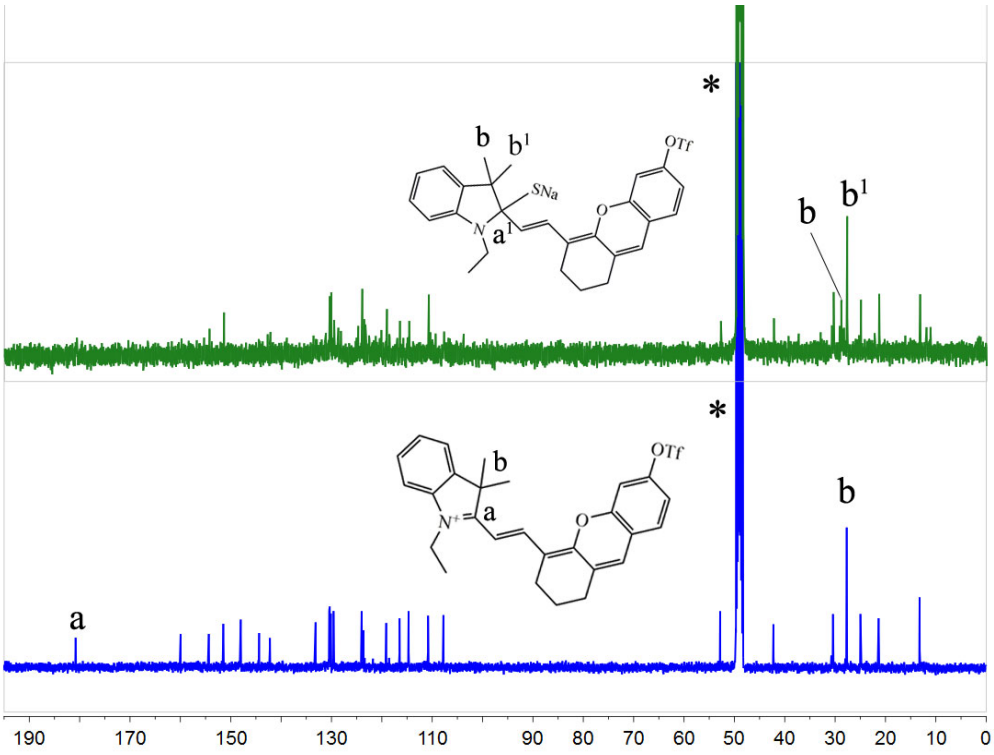

Fig. S21 ^{1}C NMR spectra of **CyT** in the absence (bottom) and presence (top) of 1.5 equiv S^{2-}/HS^{-} in CD_3OD. Signals marked with * are from the solvent.

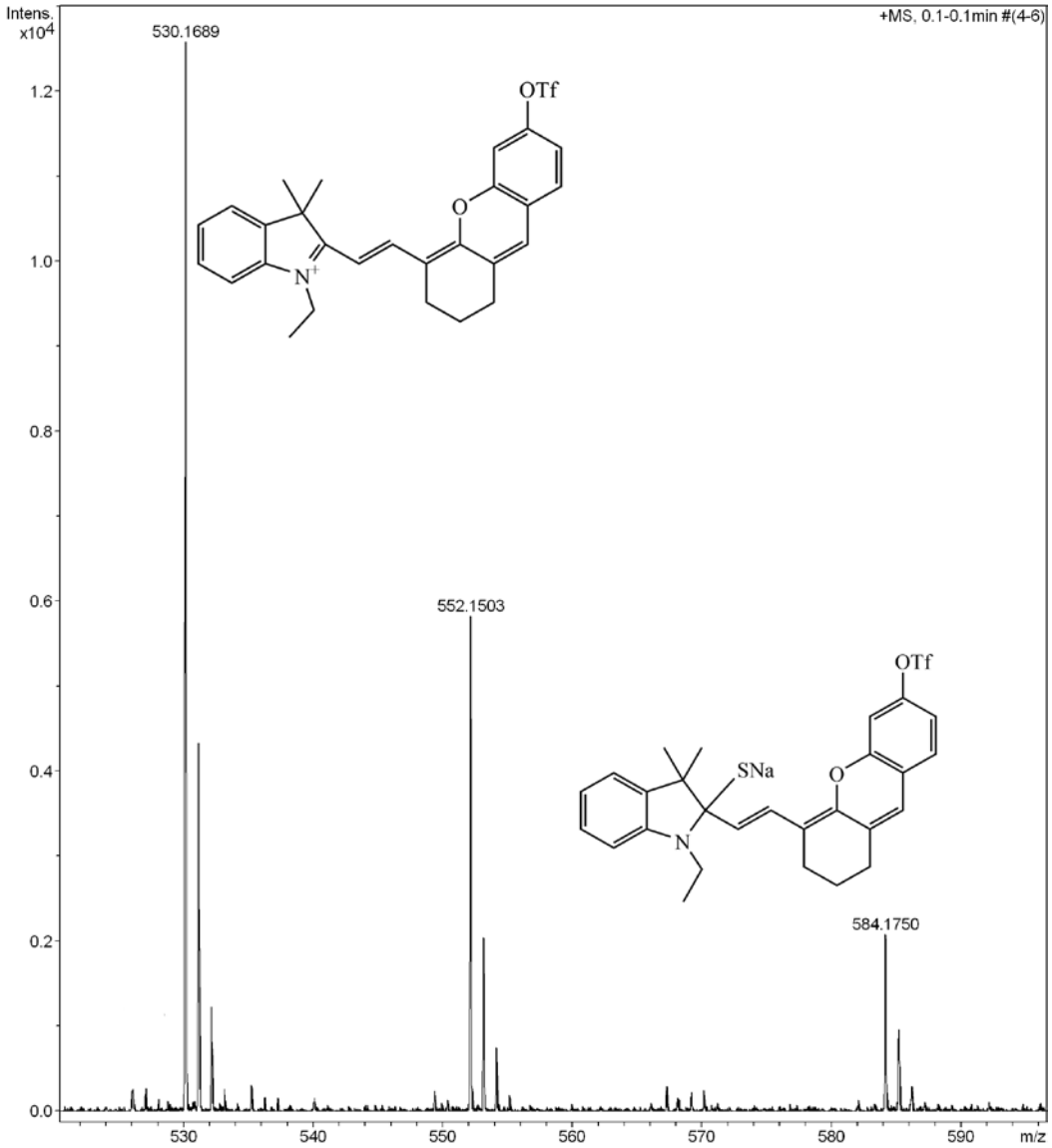

Fig. S22 The ESI-MS of product obtained by reaction of **CyT** and Na_2S

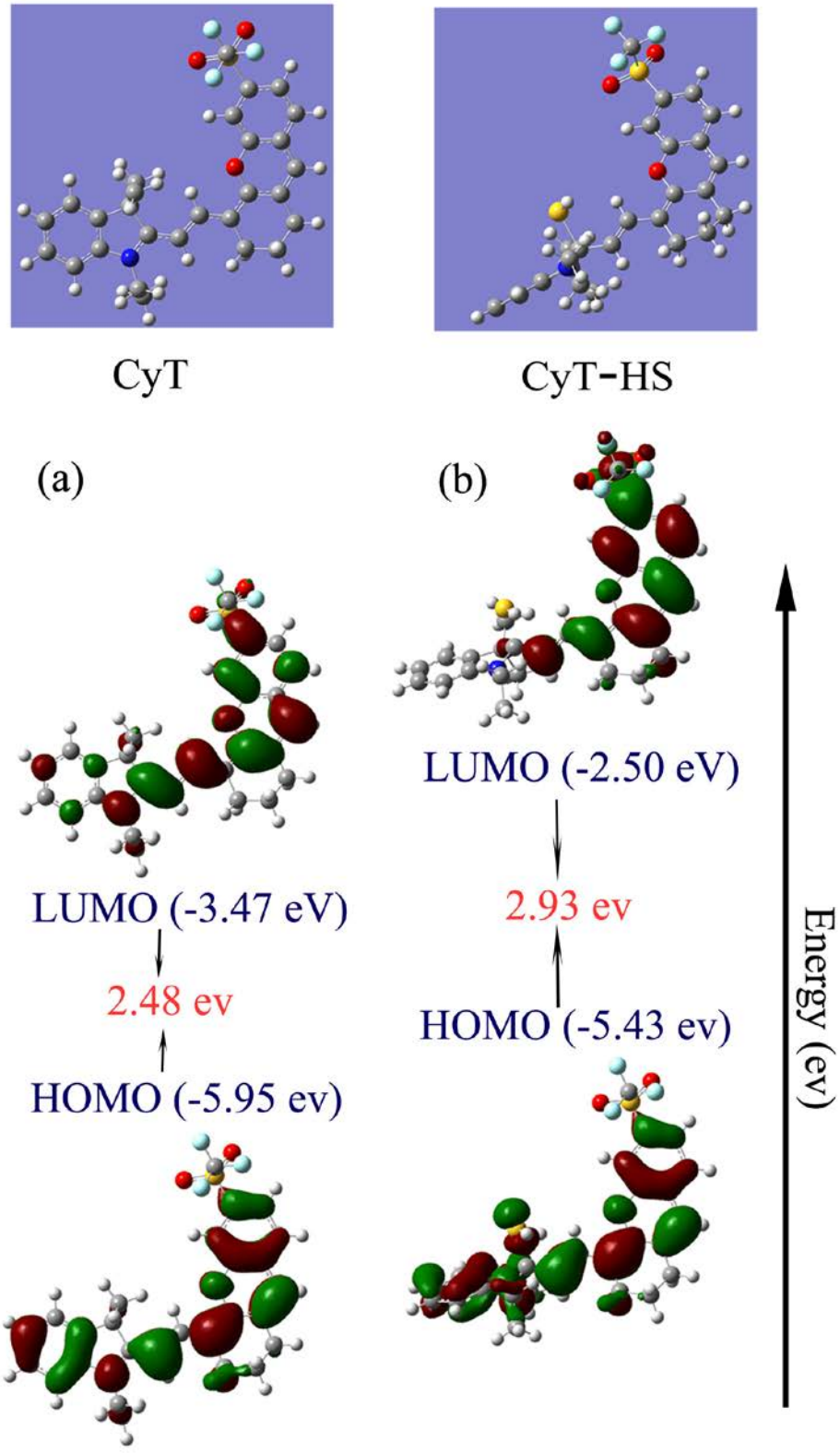

Fig. S23 Optimized structures and frontier molecular orbitals (MOs) of **CyT** before (a) and after (b) reacting with S^{2-}/HS^{-}. Calculations were based on ground state geometry by density functional theory (DFT) at the B3LYP/6-311G (d, p)/level using Gaussian 09 in water. In the ball-and-stick representation, carbon, nitrogen, oxygen, sulfur and fluorine atoms are colored in gray, blue, red, yellow and canal blue, respectively.

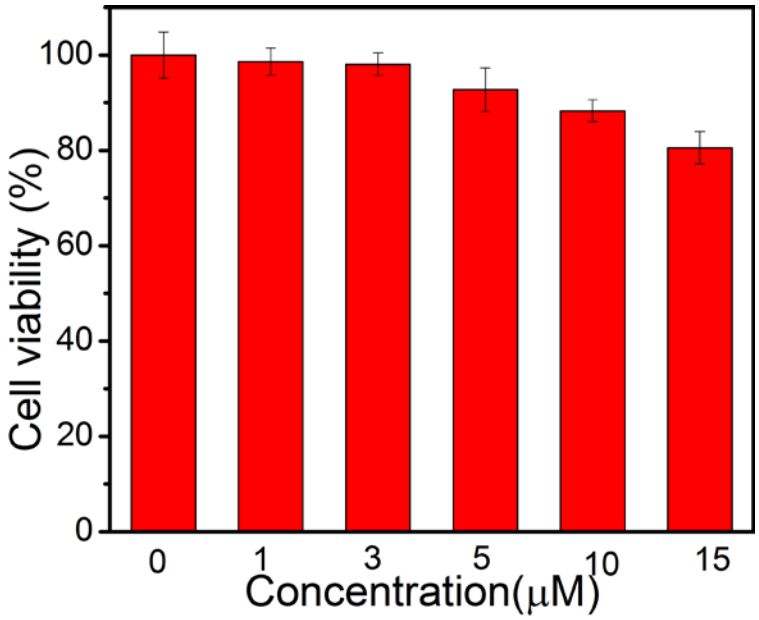

Fig. S24 CCK-8 assay for the survival rate of HeLa cells treated with various concentrations of **CyT** for 24 h. Error bars represent the standard deviations of 6 trials.

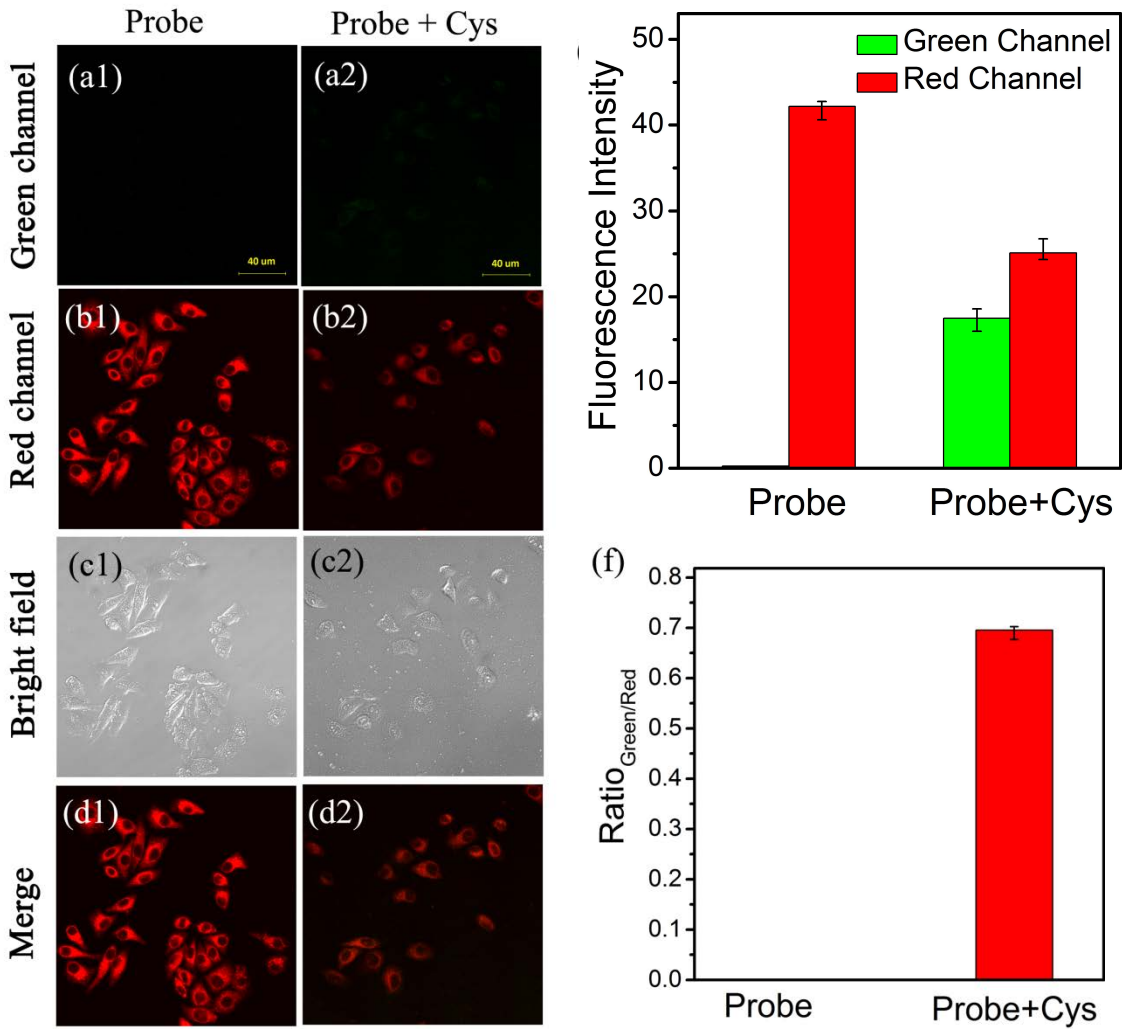

Fig. S25 Confocal images of **CyT** responds to endogenous H_2S in Hela cells. (a1−d1) Hela cells were incubated with **CyT** (10 μM) for 30 min; (a2−d2) Hela cells were stimulated with Cys (100 μM ml^{-1}) for 3 h, then incubated with 10 μM **CyT** for 30 min. (e) Quantitative analysis of fluorescence intensity of green and red channel in Hela cells incubated with **CyT** and Cys. (f) Fluorescence intensity ratios (F_{Green}/F_{Red}) of Hela cells incubated with **CyT** and Cys. Error bars represent standard deviation (SD), n=3. Green channel: λ_{em} = 500 − 550 nm (λ_{ex} = 488 nm); Red channel: λ_{em} = 570 − 620 nm (λ_{ex} = 559 nm); Bright field, Merge; Scale bar, 40 μm.

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