Electronic Supplementary Information

A dual emission fluorescent probe enables simultaneous detection of glutathione and cysteine/homocysteine

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CONTENTS:

Materials and Instruments

All chemicals were purchased from Sigma-Aldrich and Aladdin reagent Co. (Shanghai, China) and used without further purification. Column chromatography was conducted over silica gel (200 - 300 mesh) obtained from the Qingdao Ocean Chemicals. Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents were purified and dried by standard methods prior to use. Double-distilled water was used throughout the experiments. In all experiments enantiomerically pure natural amino acids were used except for Hcy which was used as the racemate.

The fluorescence spectra and relative fluorescence intensity were measured with a Shimadzu RF-5301 spectrofluorimeter with a 10 mm quartz cuvette. Absorption spectra were recorded using a Shimadzu UV-2550 spectrophometer. High-resolution mass spectra were collected using a Bruker micrOTOF-Q II mass spectrometer (Bruker Daltonics Corp.,USA) in electrospray ionization (ESI) mode. ^{1}H and ^{13}C NMR spectra were recorded on an INOVA-400 spectrometer (Varian Unity), using tetramethylsilane (TMS) as the internal standard. The pH measurements were carried out on a Sartorius PB-10 pH meter.

General procedure for the spectral measurements

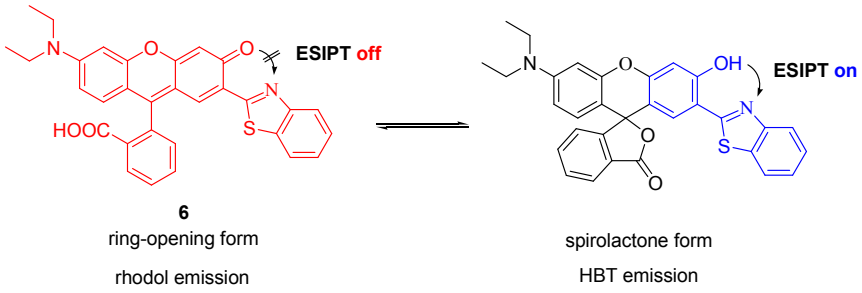
In a set of 10 mL volumetric tubes containing 1.0 mL phosphate buffer (0.2 M, pH 7.4), 3.0 mL of DMF and 50 μL of probe **1** (1.6 mM), different concentrations of analytes were added and the reaction mixture was diluted to 10.0 mL with H_2O. The resulting solution was well-mixed and kept at 37 ^{o}C for 10 min, and then the absorption or fluorescence spectra were recorded. The fluorescence emission intensity at 454 or 587 nm was measured at the excitation wavelength of 305 nm.

Detection of GSH and Cys in MDA-MB-231 human breast cancer cells

MDA-MB-231 human breast cancer cells were cultured to 50% density and collected after trypsin digestion. The collected cells were washed three times by PBS buffer and then suspended in PBS buffer. The cells were lysed with sonication to release biothiols from the cells, and the lysed cells were then analyzed by using the procedure described above.

Cell Cultures and fluorescence imaging

HepG2 cells were seeded in 6-well culture plates containing sterile coverslips and were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS), penicillin (100 U mL^{-1}) and streptomycin (100 μg mL^{-1}) at 37 °C in a 95% humidity atmosphere under 5% CO_2 for 24 h. Before imaging, the cells were washed with PBS (pH 7.4) three times and then incubated with probe **1** (8.0 μM) in PBS (pH 7.4) at 37 °C for 30 min. After that, the samples were rinsed three times with PBS (pH 7.4) to remove the remaining probe. For the control experiment, the cells were pretreated with NEM (1 mM) at 37 °C for 40 min. After washing with PBS (pH 7.4) three times, the cells were further incubated with probe **1** (8 μM) in a DMSO / PBS (5 : 95, v/v) solution at 37 °C for 30 min. Fluorescence imaging was performed by an Olympus FV1000 confocal laser scanning microscope (Japan). Emission was collected at 425-475 nm for the blue channel (excitation at 405 nm) and at 560-660 nm for the red channel (excitation at 543 nm).



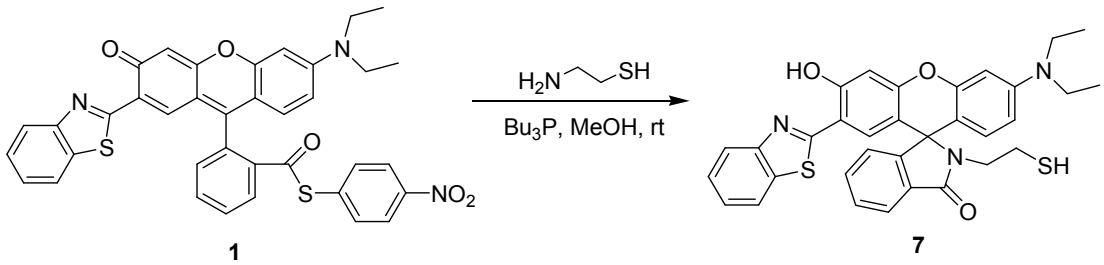
Scheme S1. The equilibrium between an "open" quinoid form and a "closed" spirolactone form of rhodol **6**.

Synthesis of probe 1

Rhodol **6** was obtained according to the method we reported previously [S1]. Probe **1** was prepared by 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC)/4-(N,*N*-dimethylamino)- pyridine (DMAP) coupling of compound **6** with 4-nitrothiophenol. Briefly, to a suspension of **6** (0.104 g, 0.2 mmol) in anhydrous dichloromethane (10 mL) was added EDC (0.058 g, 0.3 mmol), DMAP (0.037 g, 0.3 mmol) and 4-nitrothiophenol (0.031 g, 0.2 mmol). The mixture was stirred at room temperature overnight and then the solvent was removed under reduced pressure. The

resulting residue was purified by silica gel column chromatography (methanol/ethyl acetate, 1:10, v/v) to give the target compound **1** as purple solids (0.056 g, 43%). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.27 (d, J = 7.2 Hz, 2H), 8.06 (d, J = 8.8 Hz, 2H), 7.92 (d, J = 8.4 Hz, 2H), 7.87-7.78 (m, 2H), 7.50 (d, J = 7.2 Hz, 1H), 7.39 (t, J = 7.6 Hz, 1H), 7.31 (d, J = 8.8 Hz, 3H), 6.89 (d, J = 9.2 Hz, 1H), 6.60 (s, 1H), 6.57–6.52 (m, 2H), 3.45 (q, J = 7.1 Hz, 4H), 1.23 ppm (t, J = 7.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 188.12, 180.98, 162.55, 158.40, 156.03, 153.13, 153.09, 151.88, 148.20, 137.39, 136.88, 135.49, 135.21, 133.47, 132.46, 131,60, 130.22, 129.91, 129.22, 128.92, 128.32, 126.38, 125.61, 124.48, 124.26, 123.98, 122.39, 121.66, 114.25, 111.62, 110.74, 105.70, 96.76, 45.40, 12.68 ppm. HRMS (ESI): *m/z* calcd for $C_{37}H_{28}N_3O_5S_2$ $[M + H]^+$ 658.1470; found: 658.1473.

Synthesis of compound 7



Scheme S2. Synthesis of compound **7** from probe **1**.

Compounds **1** (0.132 g, 0.2 mmol), cysteamine hydrochloride (0.034 g, 0.3 mmol) and tributylphosphine (0.075 mL, 0.3 mmol) were dissolved in MeOH (15 mL) and the reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate, 1:1, v/v) to give the desired compound **7** as a light pink solid (0.03 g, 25.9%). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 12.77(s, 1H), 7.99 (dd, J_1 = 6.0 Hz, J_2 = 2.4 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H), 7.48-7.51 (m, 2H), 7.45 (d, J = 8.0 Hz, 1H), 7.35 (t, J = 7.4 Hz, 1H), 7.09 (dd, J_1 = 6.0 Hz, J_2 = 2.0 Hz, 1H), 7.01 (s, 1H), 6.89 (s, 1H), 6.45 (d, J = 9.2 Hz, 2H), 6.32 (d, J = 7.6 Hz,1H), 3.28–3.46 (m, 6H), 2.26–2.37 (m, 2H), 1.67 (s, 1H), 1.19 ppm (t, J = 7.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ= 168.38, 168.21,

159.12, 155.07, 153.124, 152.11, 151.60, 149.15, 133.08, 132.33, 130.51, 128.72, 126.82, 125.66, 123.76, 123.38, 122.06, 121.57, 114.24, 112.25, 108.90, 104.71, 104.45, 97.95, 64.38, 44.52, 43.93, 23.06, 12.66 ppm. HRMS (ESI): *m/z* calcd for $C_{33}H_{30}N_3O_3S_2$ $[M + H]^+$ 580.1729; found: 580.1728.

References

[S1] H. Wen, Q. Huang, X.-F. Yang, H. Li, *Chem. Commun.,* 2013, **49**, 4956–4958.

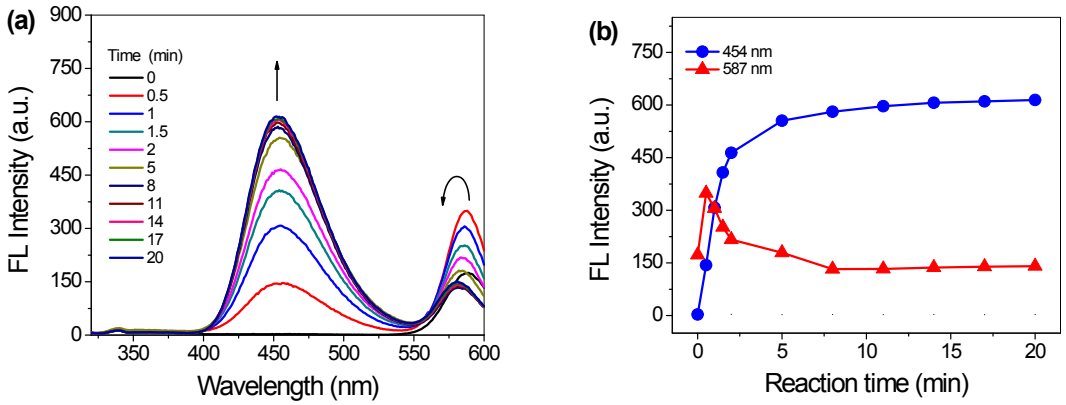
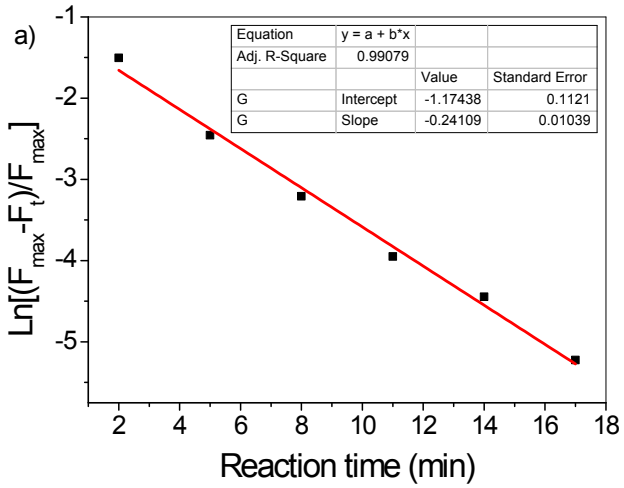
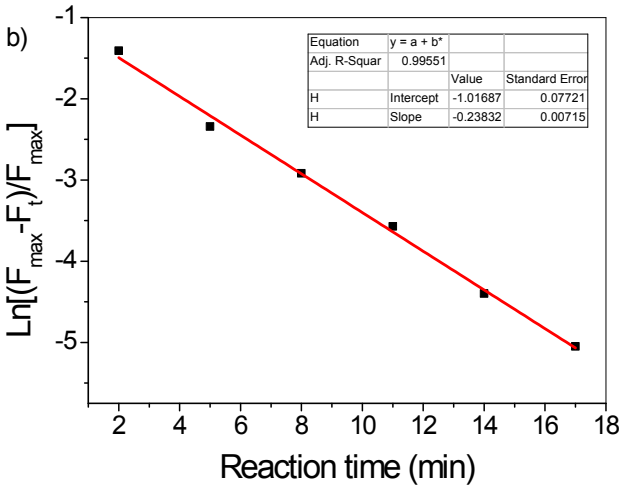


Figure S1. (a) Time-dependent fluorescence spectral changes of **1** (8 μM) with 10 equiv of Hcy in DMF/phosphate buffer (3:7 v/v, 20 mM, pH 7.4). Each spectrum was acquired at 37 °C with excitation at 305 nm. (b) Time-dependent fluorescence intensity changes of probe **1** (8 μM) in the presence of Hcy (10 equiv).





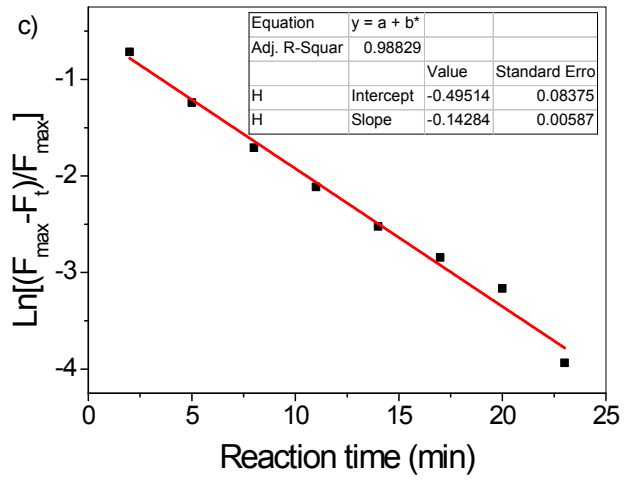


Figure S2. Pseudo first-order kinetic plot of the reaction of probe **1** (8 μM) with 10 equiv of Cys (a), Hcy (b) and GSH (c) in DMF/phosphate buffer (3:7 v/v, 20 mM, pH

7.4) at 37 °C.

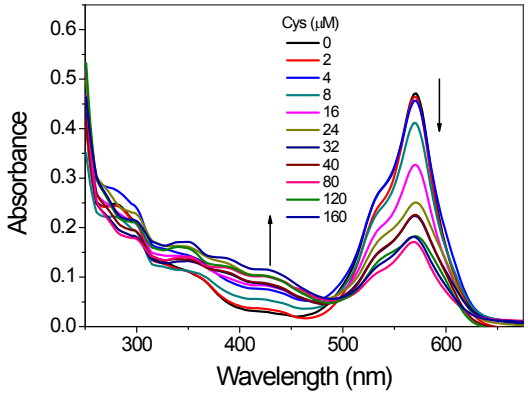


Figure S3. Absorption of probe **1** (8 μM) upon addition of increasing concentrations of Cys in DMF/phosphate buffer (3:7 v/v, 20 mM, pH 7.4) at 37 °C for 10 min. The absorption around 425 nm is mainly contributed by 4-nitrothiolphenol generated via transthioesterification of **1** with Cys.

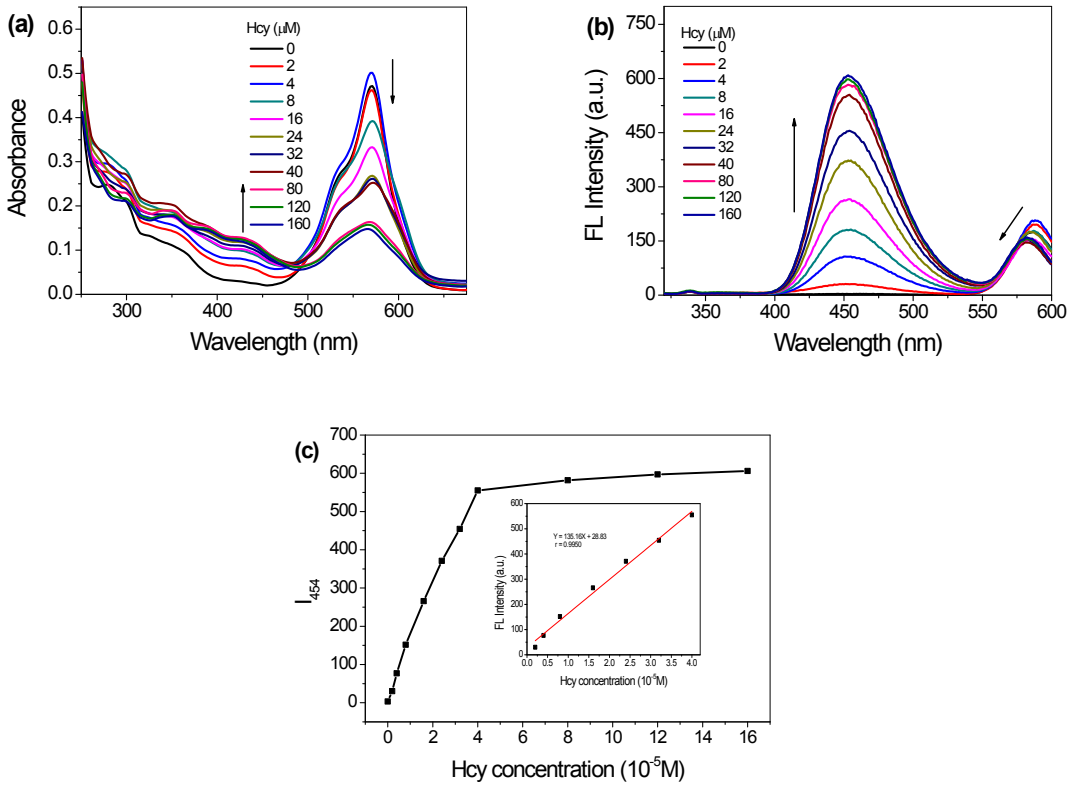


Figure S4. Absorption (a) and emission spectra (b) of probe **1** (8 μM) upon addition of increasing concentrations of Hcy in DMF/phosphate buffer (3:7 v/v, 20 mM, pH 7.4) for 10 min. Each spectrum was acquired at 37 °C with excitation at 305 nm. (c) Plot of the fluorescence intensity as a function of Hcy concentration. Inset: the linear

relationship between maximum fluorescence intensity and the concentration of Hcy.

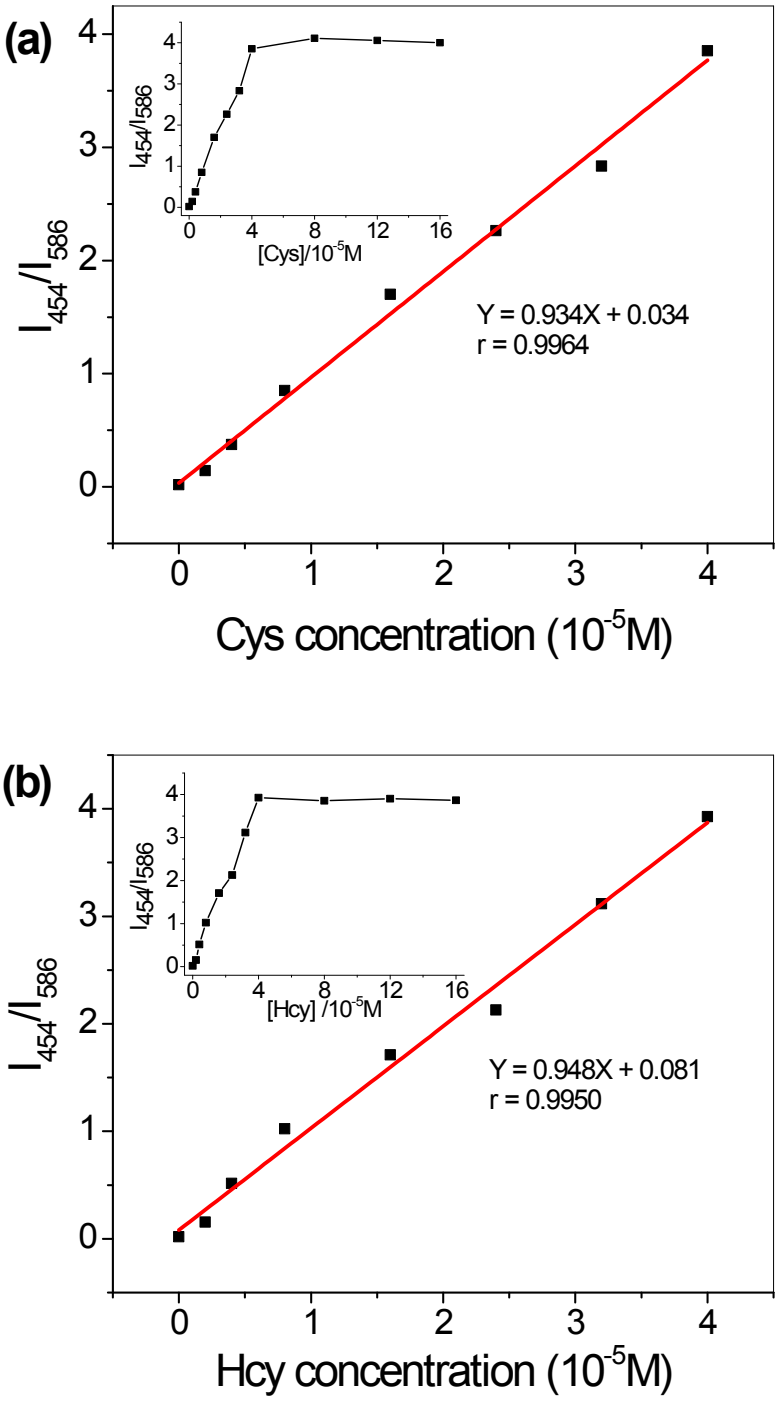


Figure S5. Plot of the fluorescent intensity ratio (I_{454}/I_{586}) as a function of Cys (a) or Hcy (b) concentration.

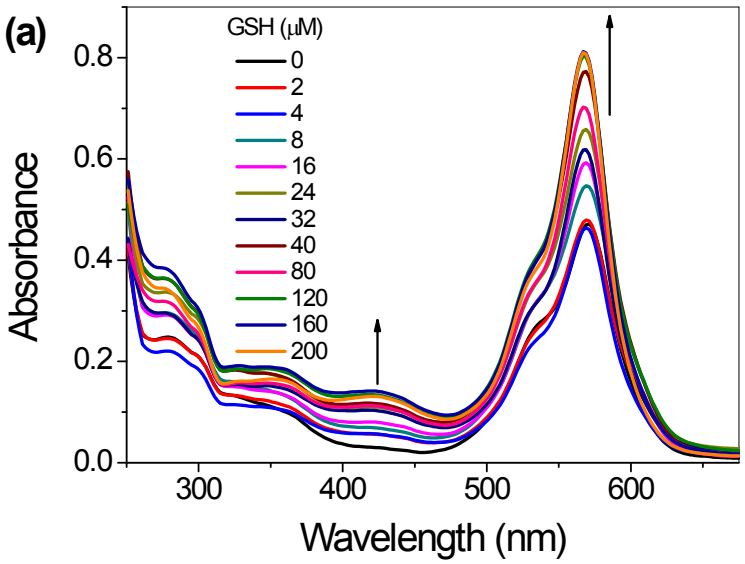


Figure S6. Absorption of probe **1** (8 μM) upon addition of increasing concentrations of GSH in DMF/phosphate buffer (3:7 v/v, 20 mM, pH 7.4) at 37 °C for 10 min.

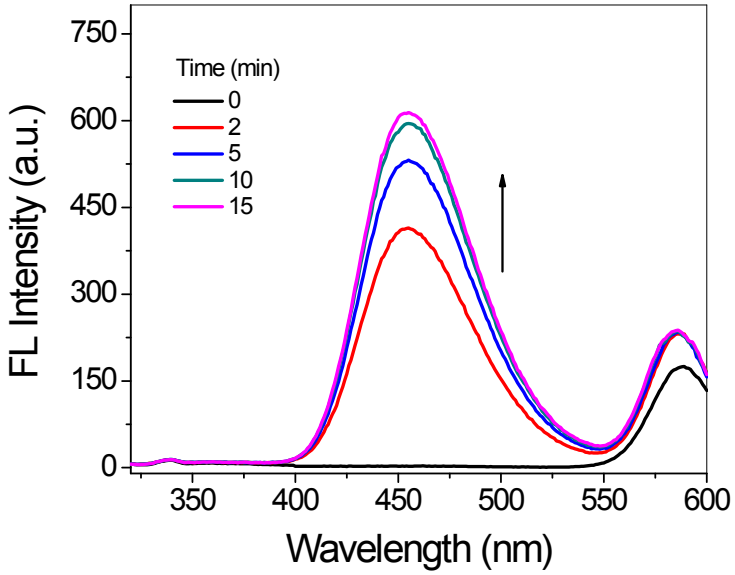


Figure S7. Time-dependent fluorescence spectral changes of **1** (8.0 μM) with 5 equiv of cysteamine in phosphate buffer (20 mM, pH 7.4, containing 30% DMF as a cosolvent) at 37 °C. λ_{ex} = 305 nm.

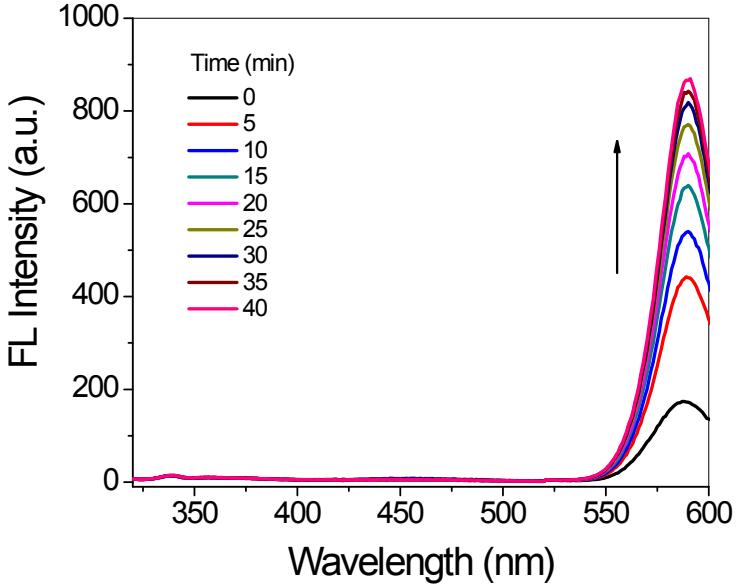


Figure S8. Time-dependent fluorescence spectral changes of **1** (8.0 μM) with 5 equiv of NAC in phosphate buffer (20 mM, pH 7.4, containing 30% DMF as a cosolvent) at 37 °C. λ_{ex} = 305 nm.

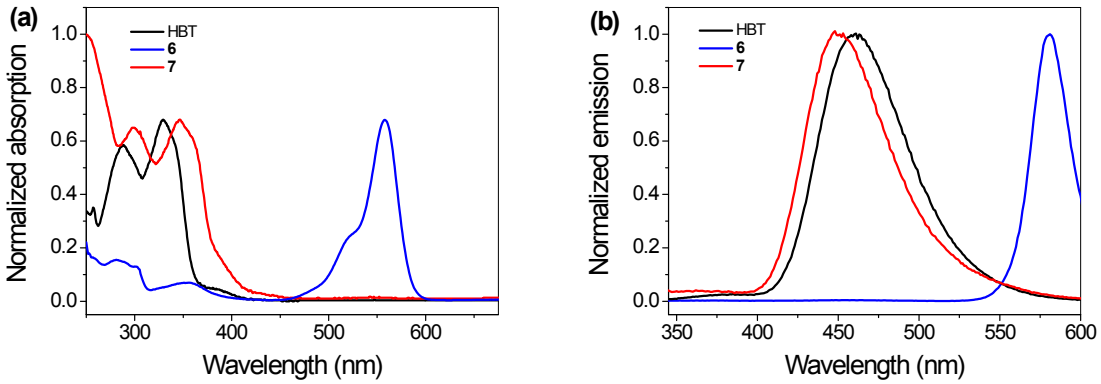


Figure S9. Normalized absorption (a) and emission spectra (b) of HBT, rhodol **6** and compound **7**. All absorption were recorded in phosphate buffer (20 mM, pH 7.4, containing 30% DMF as a cosolvent).

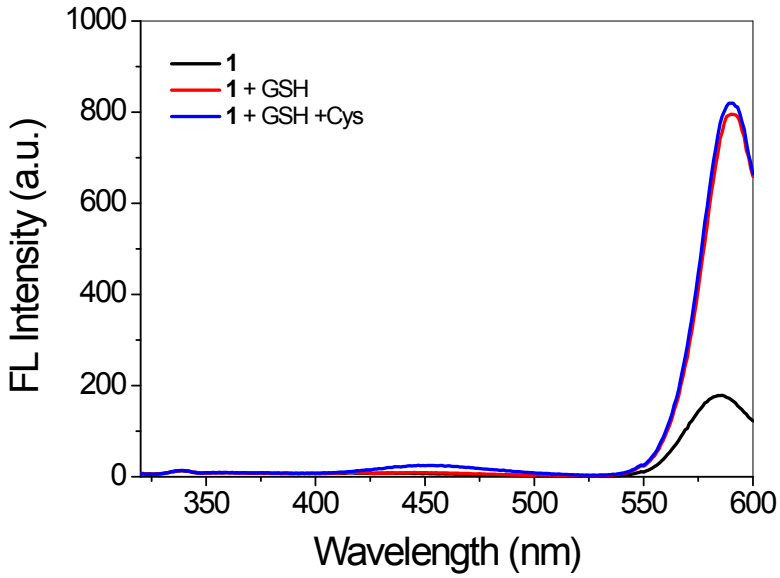


Figure S10. Fluorescence spectra of **1** (8 μM) upon addition of GSH (120 μM) in DMF/phosphate buffer (3:7 v/v, 20 mM, pH 7.4) for 20 min, and then introduction of Cys (120 μM) for 20 min. Each spectrum was acquired at 37 °C with excitation at 305 nm.

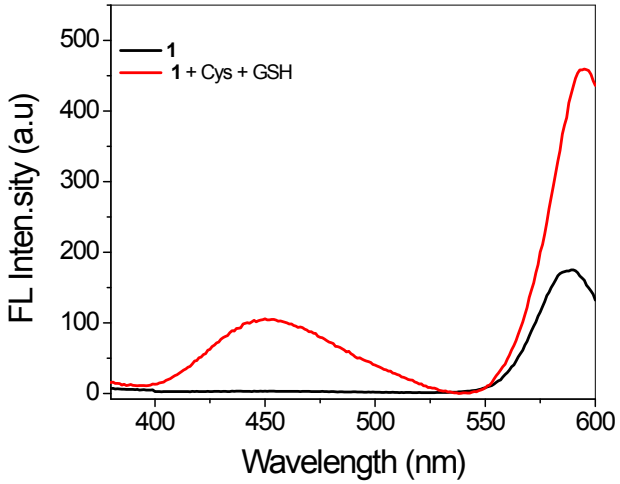


Figure S11. Fluorescence spectra (λ_{ex} = 305 nm) of **1** upon addition of the mixture of Cys (8 μM) and GSH (32 μM) in DMF/phosphate buffer (3:7 v/v, 20 mM, pH 7.4) at 37 °C for 10 min.

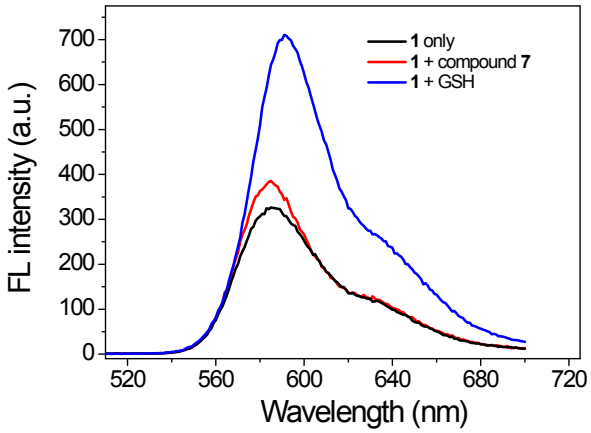


Figure S12. Fluorescence sensing behavior of **1** (8 μM) towards GSH and compound **7** (both 2 equiv) in DMF/phosphate buffer (3:7 v/v, 20 mM, pH 7.4) for 10 min. Each spectrum was acquired at 37 °C with excitation at 500 nm.

Figure S13. Color changes of the solution of probe **1** (8 μM) with the addition of 10 equiv of Cys, Hcy and GSH in DMF/phosphate buffer (3:7 v/v, 20 mM, pH 7.4) for 10 min.

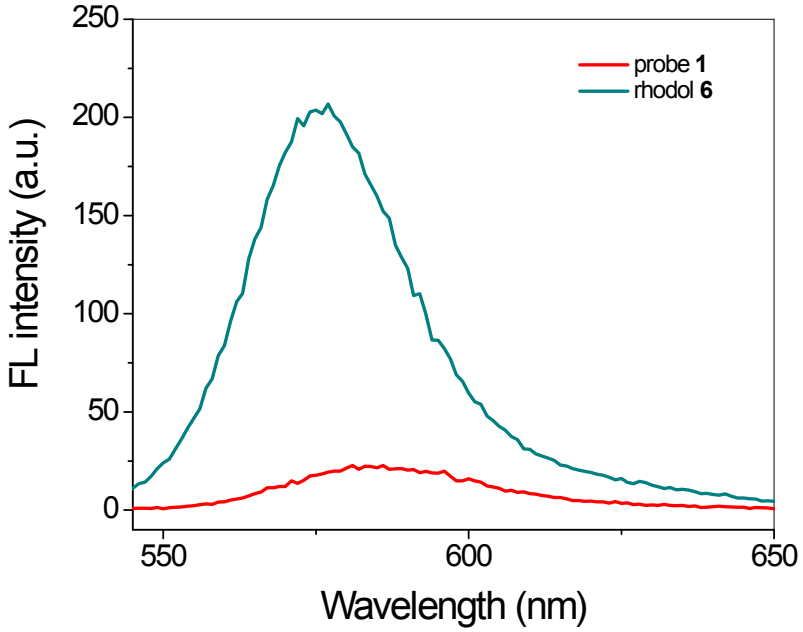


Figure S14. Fluorescence emission spectra (λ_{ex} = 540 nm) of probe **1** and rhodol **6** (both 1.6 μM) in DMF/H_2O (3:7 v/v,).

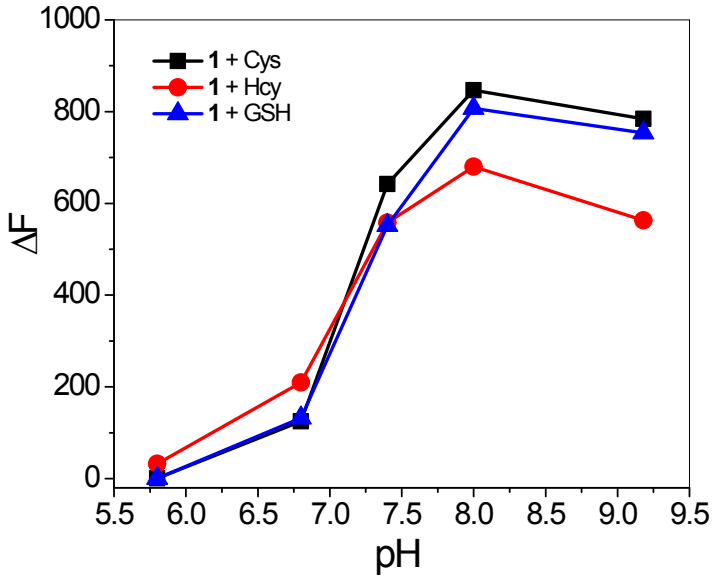


Figure S15. The fluorescence increment of probe **1** (8 μM) in the presence of Cys, Hcy, or GSH (both 5 equiv) at various pH values (5.8-9.18). The fluorescence intensity were measured at 454 nm (for Cys and Hcy) and 587 nm (for GSH), respectively.

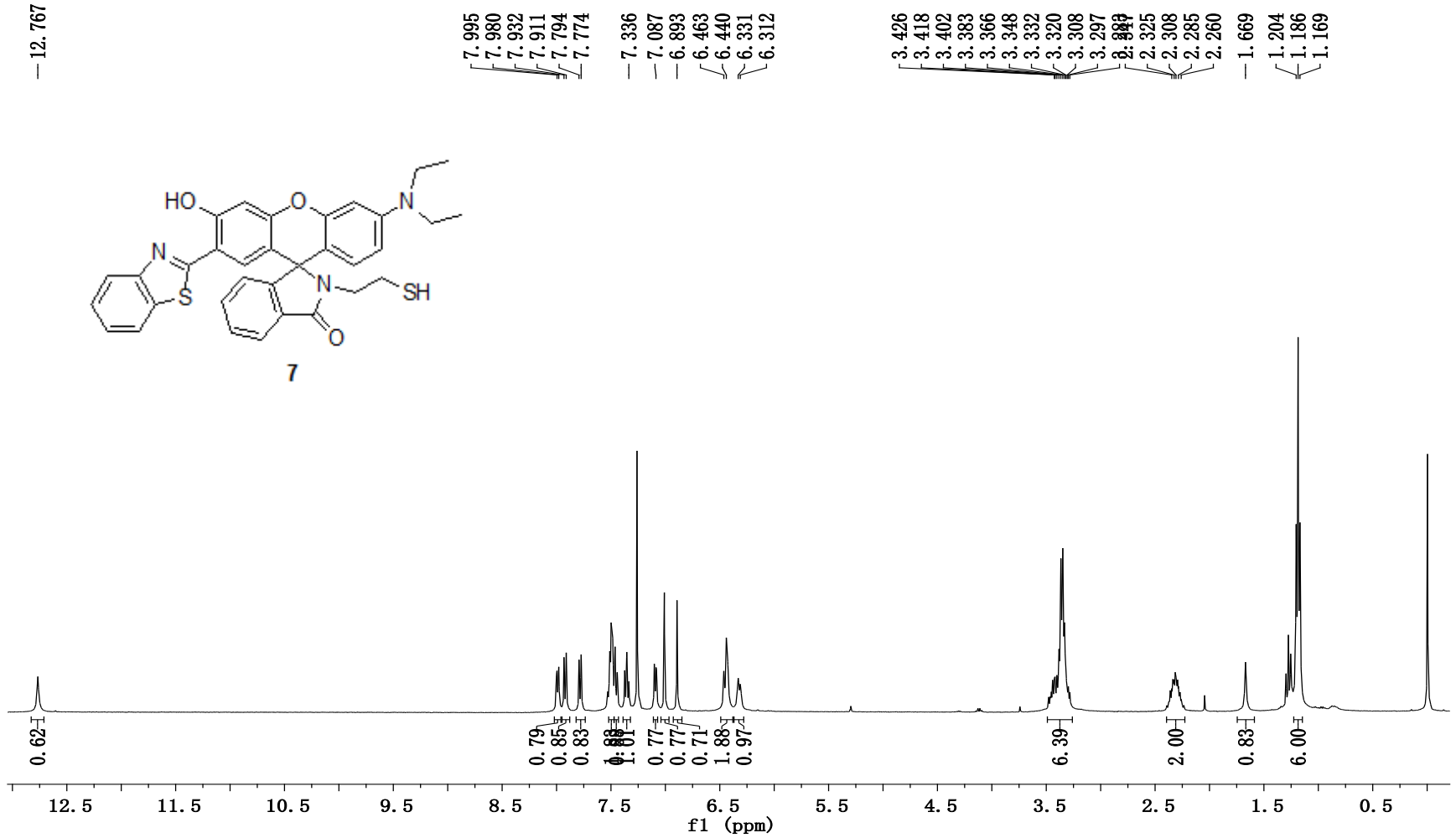


Figure S16. ^{1}H NMR spectrum of **7** in $CDCl_3$

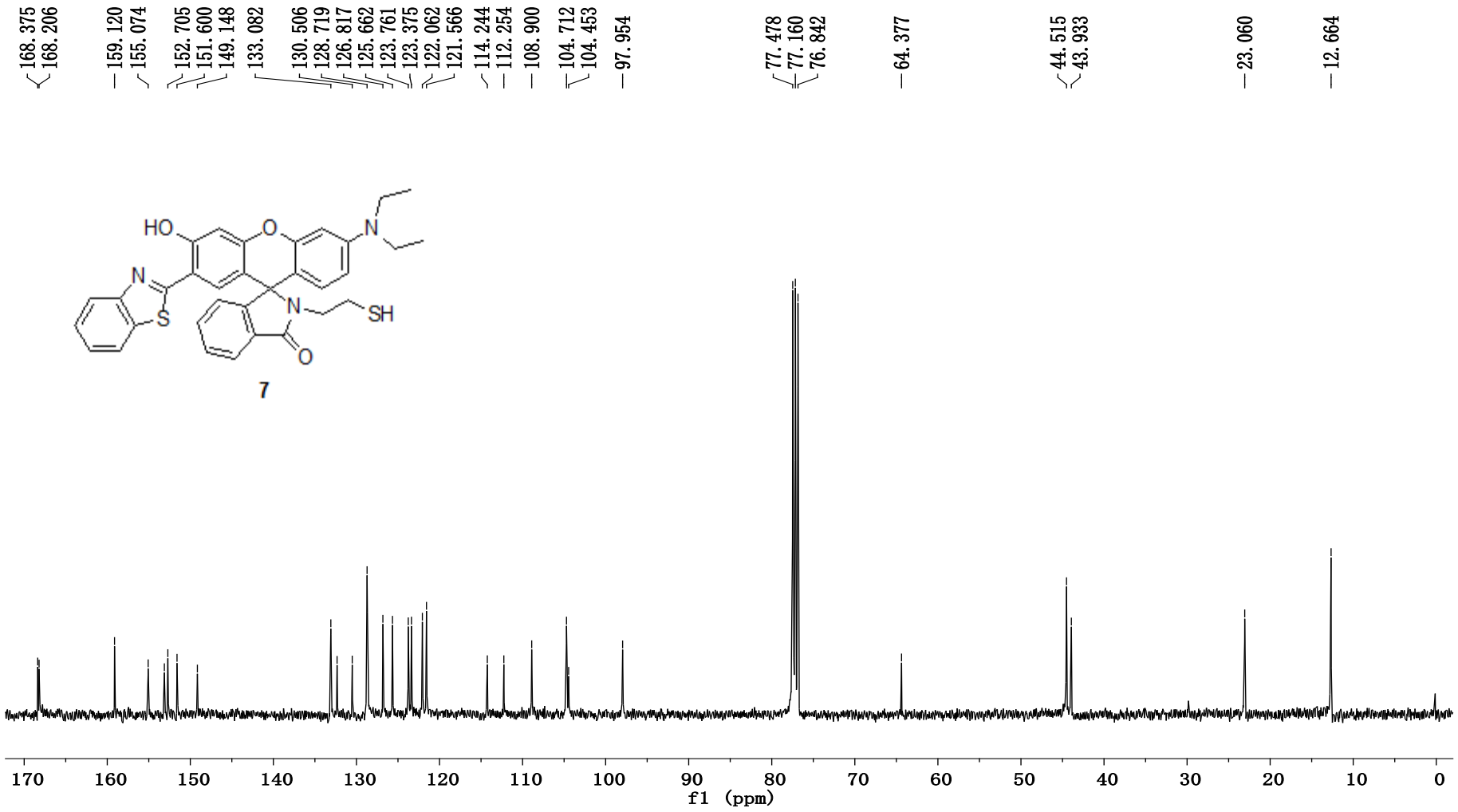


Figure S17. ^{13}C NMR spectrum of **7** in $CDCl_3$

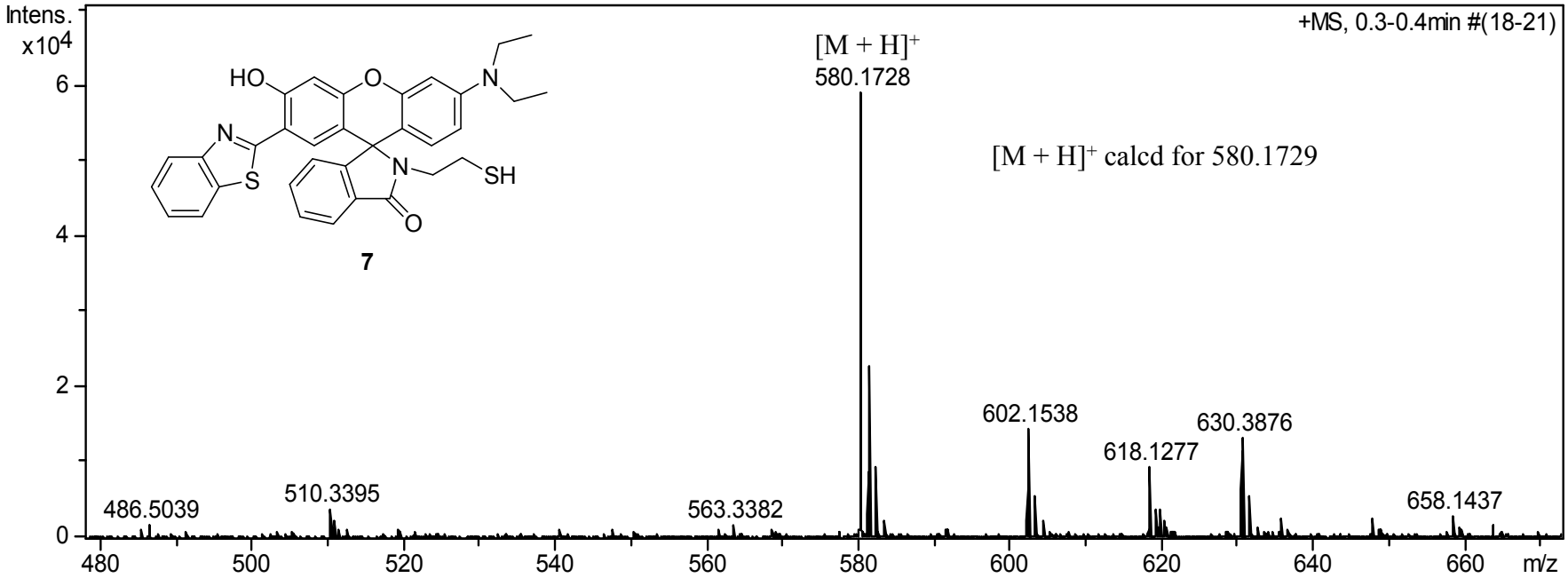


Figure S18. HRMS of **7**

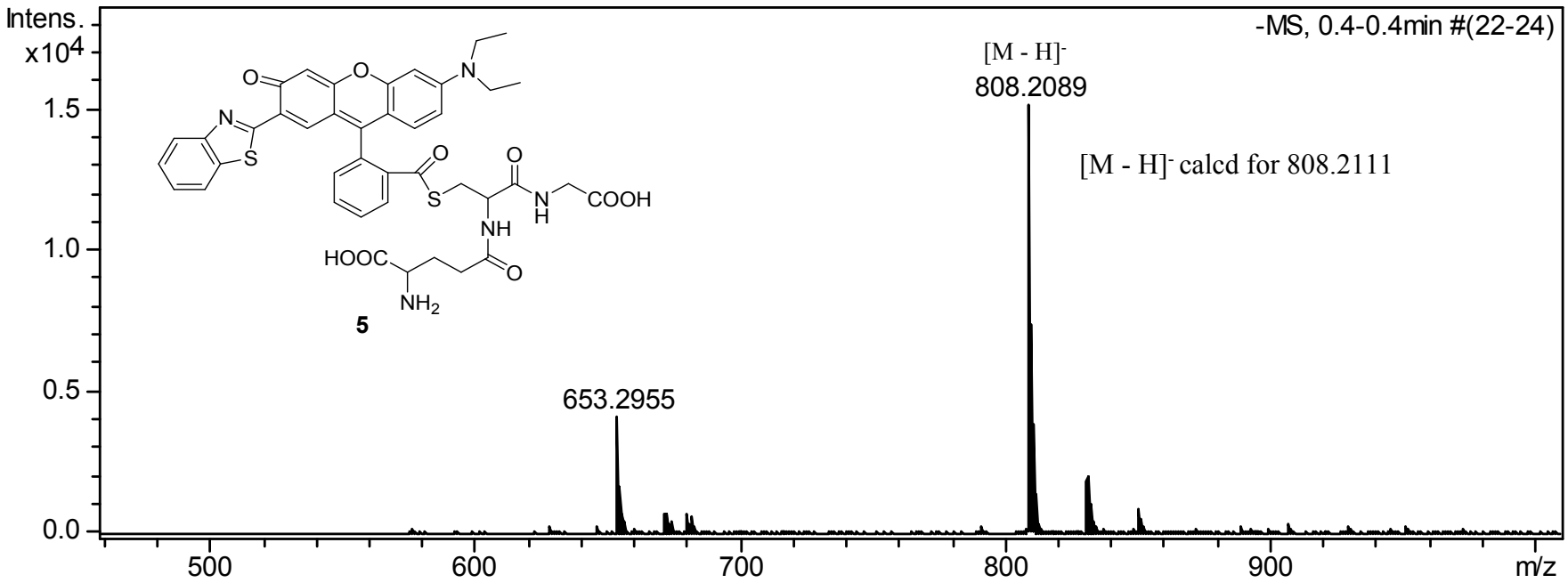


Figure S19. HRMS of **5**. Briefly, GSH (15.4 mg) was dissolved in 0.5 mL H_2O, and then **1** (3.3 mg) dissolved in 5.0 mL CH_3CN was added. The mixture was kept at rt for 2 h and then diluted appropriately with CH_3OH/H_2O (1:1, v:v) for the MS measurement.

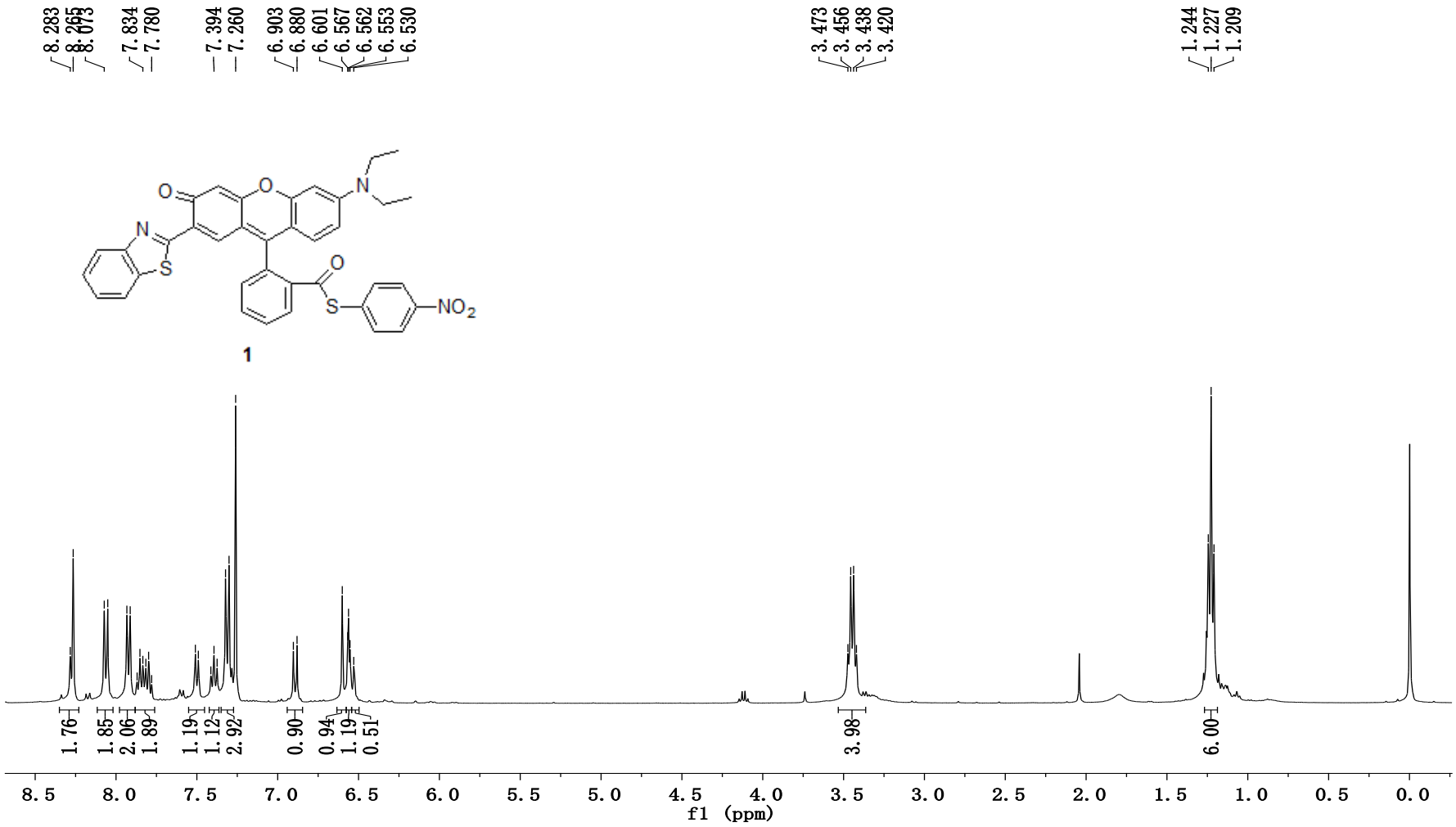


Figure S20. ^{1}H NMR spectrum of **1** in $CDCl_3$

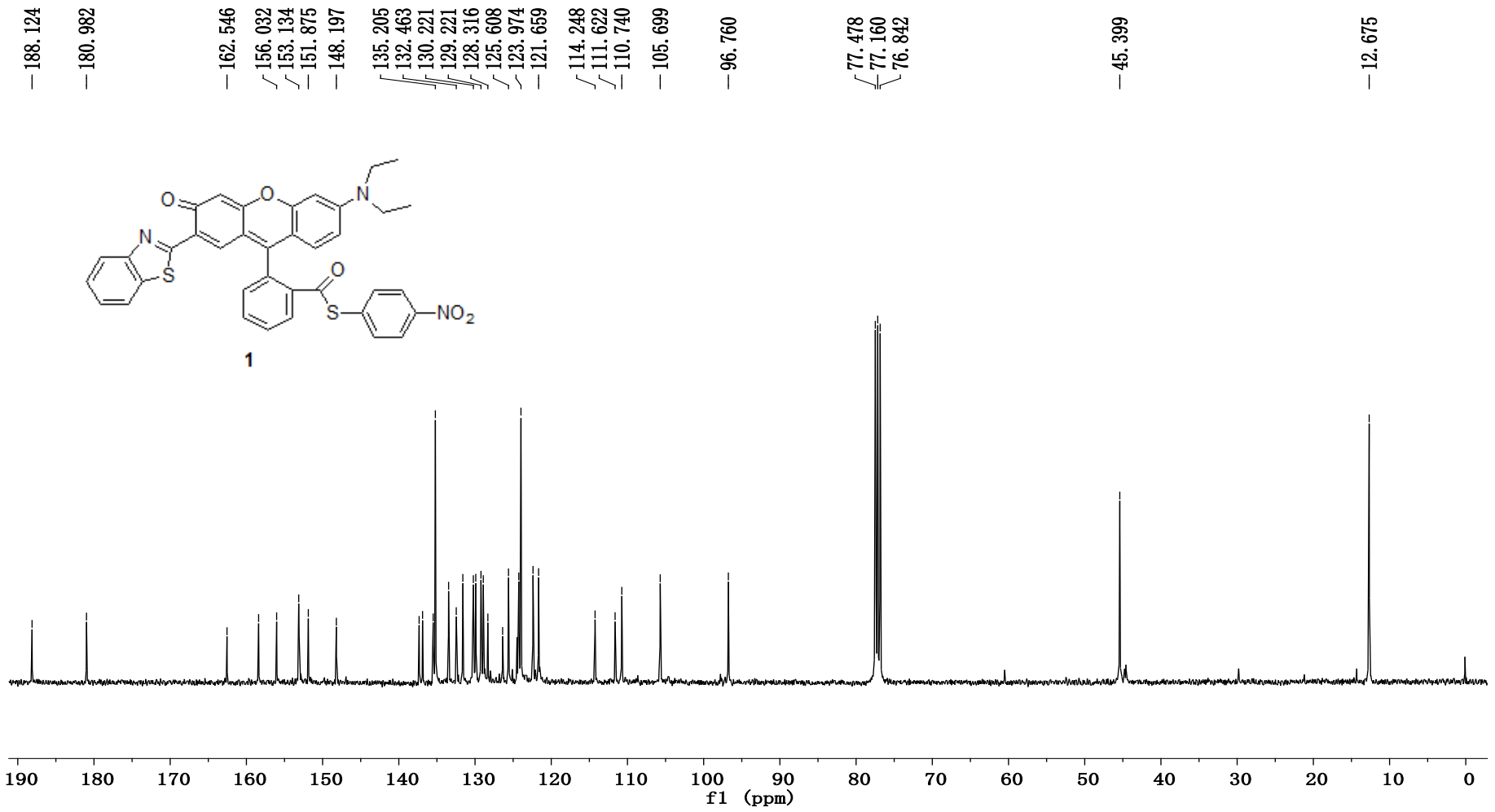


Figure S21. ^{13}C NMR spectrum of **1** in $CDCl_3$

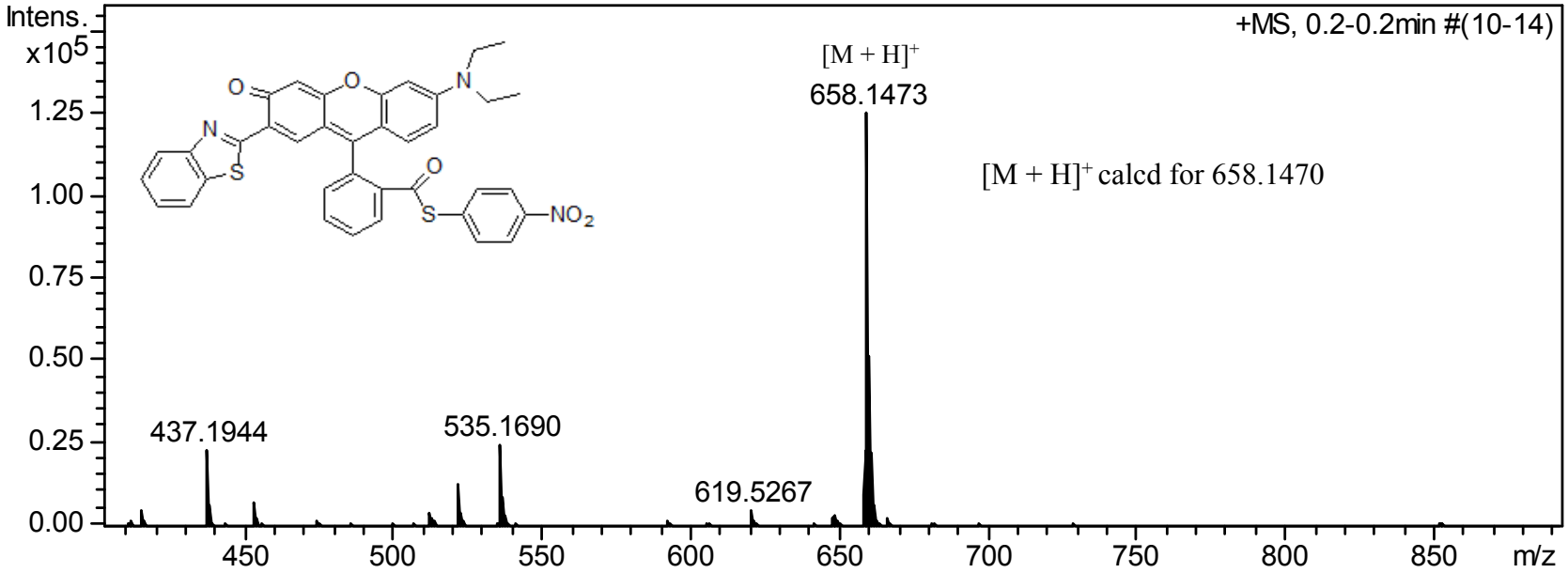


Figure S22. HRMS of **1**.