Electronic Supplementary Material (ESI) for Analytical Methods.

Supplementary Information

A Fluorescent Probe Based on Reversible Michael Addition–elimination Reaction for the Cycle Between Cysteine and H_2O_2

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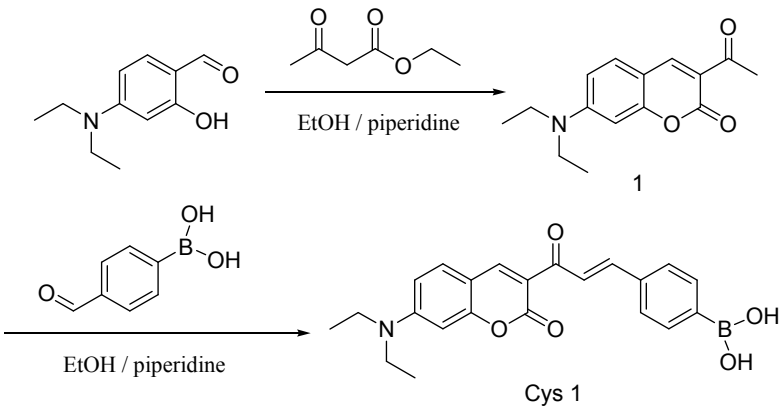
Materials and instrumentation

All chemical reagents and materials used were purchased from commercial companies and used without further purification. Doubly distilled water was used throughout the experiments. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co.) was used for column chromatography. NMR spectra were measured on Bruker Avance III at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR with chemical shifts reported as ppm (in DMSO-d6, TMS as internal standard). Mass spectra (MS) were recorded with Bruker Apex IV FTMS using electrospray ionization (ESI). Absorption spectra were recorded by a Purkinje TU-1901 spectrophotometer. Fluorescence data were taken through a Hitachi F-7000 fluorescence spectrometer with a 1 cm standard quartz cell. pH measurements were carried out with a pH acidometer (Mettler Toledo FE-30). Cell imaging was performed using a confocal laser scanning microscope (OLYPUS) with a 60× objective lens.



Scheme S1. Synthesis of **Cys 1**.

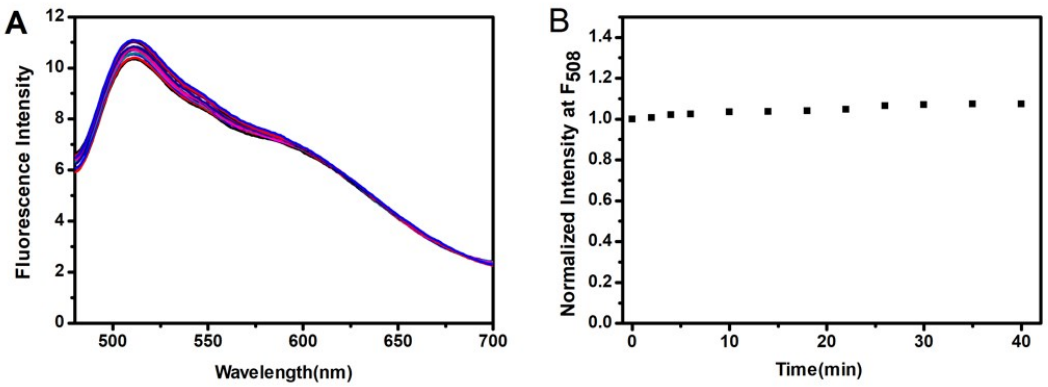


Figure S1. (A)Fluorescent spectra changes of the probe (10 μM **Cys1**) and (b)fluorescence intensity of the probe (10 μM **Cys1**) in 508 nm, recorded over 40 min. Each data was recorded in PBS buffer (10 mM, pH = 7.4, with 10% DMSO),λ_{ex} = 460 nm, slit width: $d_{ex} = d_{em}$ = 5 nm.

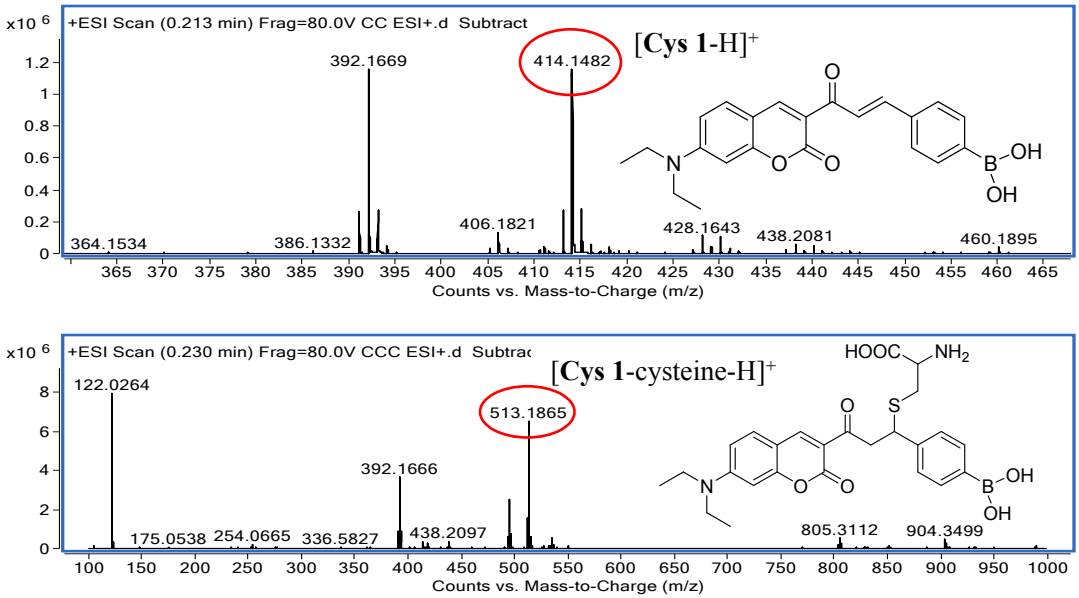


Figure S2. MS Spectra of **Cys 1** (upper) and the reaction addictive product (lower).

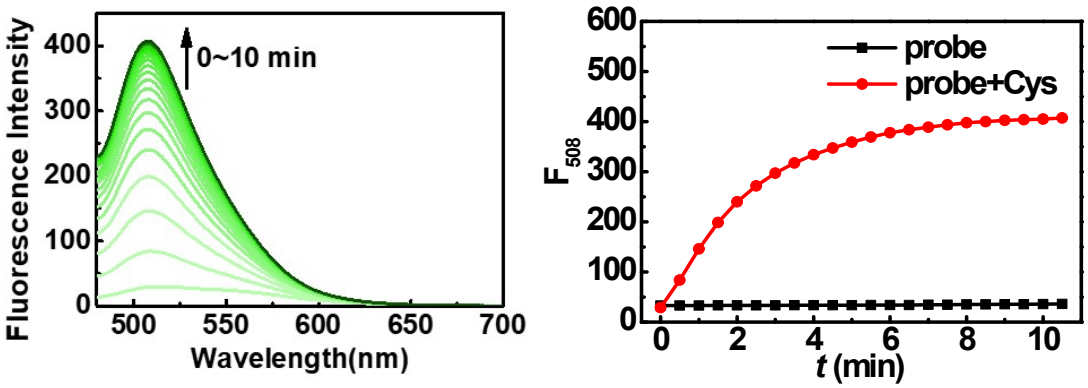


Figure S3. Fluorescence kinetics ratio intensity (F_{508}) responses of probe (10 μM) upon addition of 100 μM Cysteine in 10 mM, pH 7.4 PBS with 10% DMSO at room temperature. λ_{ex} = 460 nm, slit width: d_{ex} = d_{em} = 5 nm.

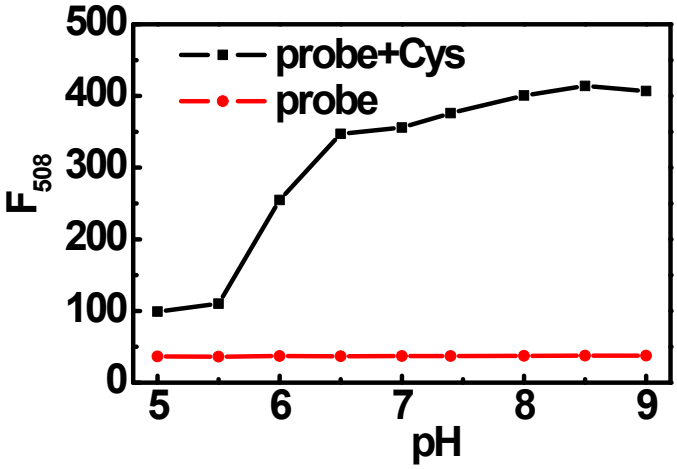


Figure S4. Fluorescence responses of probe (10 μM) in the absence and presence of Cysteine (100 μM) under different pHs with 10% DMSO at room temperature. λ_{ex} = 460 nm, slit width: $d_{ex} = d_{em}$ = 5 nm.

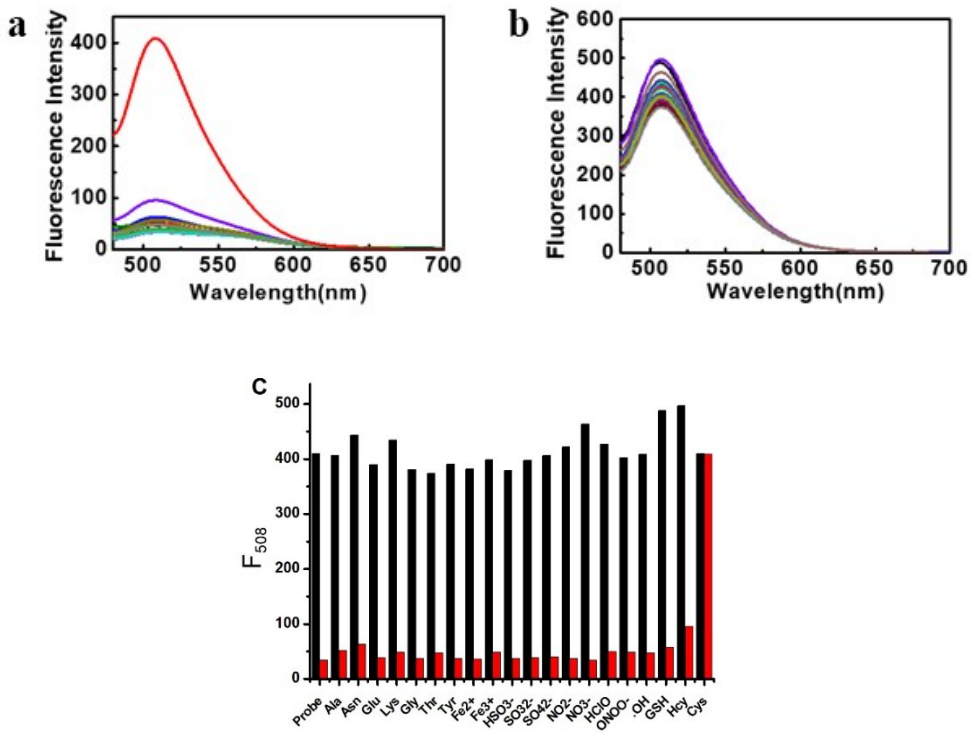


Figure S5. (a) Fluorescent spectra responses of **Cys 1** (10 μM) in the absence various analytes (100 μM);(b) Fluorescent spectra responses of **Cys 1** (10 μM) in the absence various analytes (100 μM) and Cysteine (100 μM);(c)Red column: Fluorescent intensity at 508nm of **Cys 1** (10 μM) in the absence various analytes (100 μM); Black column: Fluorescent intensity at 508nm of **Cys 1** (10 μM) in the absence various analytes (100 μM) and Cysteine (100 μM). 0. Probe itself, 1. Ala, 2. Asn, 3. Glu, 4. Lys, 5. Gly, 6. Thr, 7. Tyr, 8. Fe^{2+}, 9. Fe^{3+}, 10. $HSO3^-$, 11. $SO3^-$, 12. $SO4^{2-}$, 13. NO_2^-, 14. NO_3^- , 15. HClO, 16. $ONOO^-$, 17.·OH, 18. GSH, 19. Hcy, 20. Cys.

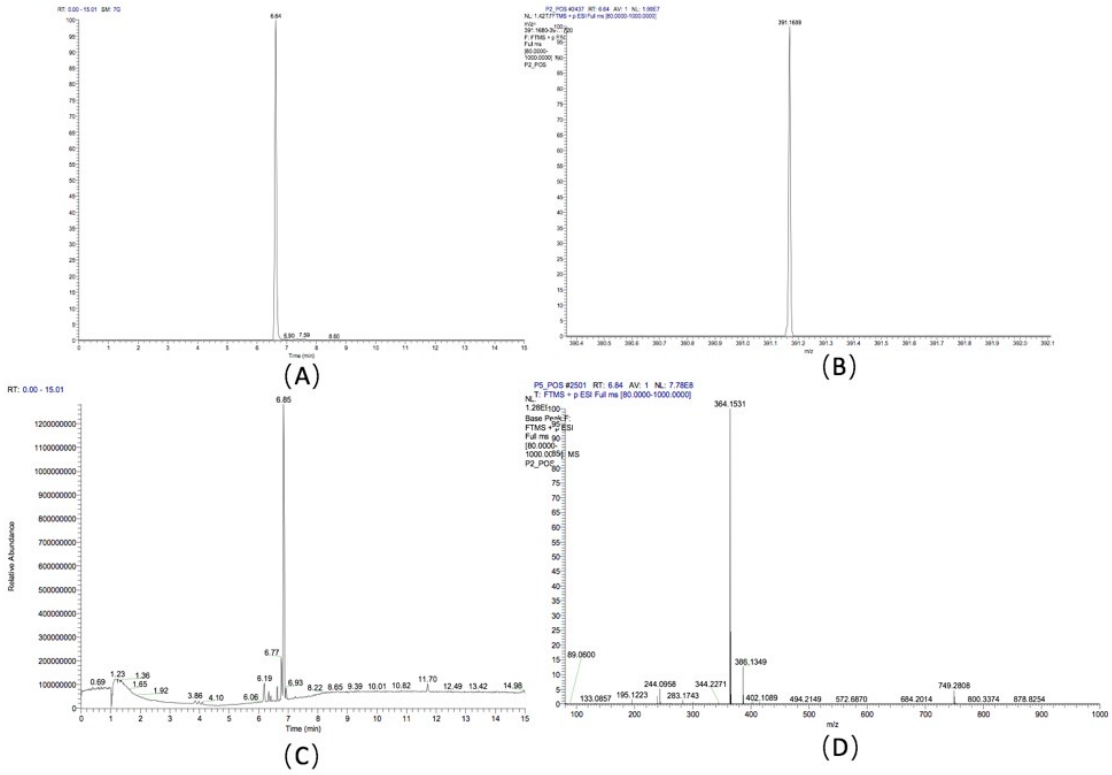


Figure S6. LC-MS analysis of the H_2O_2 reaction product. The LC spectrum(A) and mass spectrum(B) of **Cys 1**, the LC spectrum(C) and mass spectrum(D) of H_2O_2 reaction product.

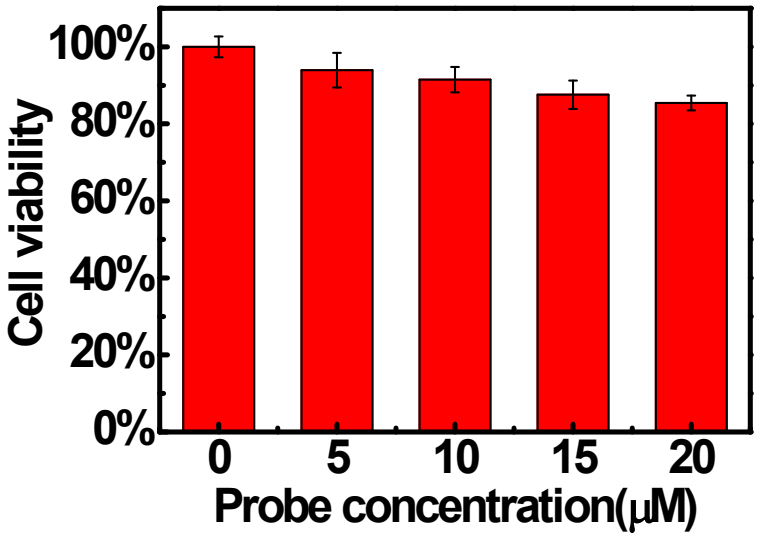


Figure S7. Cell viability with Hela cells, which were cultured in the presence of 0−20 μM **Cys 1** for 24 h.

NMR and MS Spectra

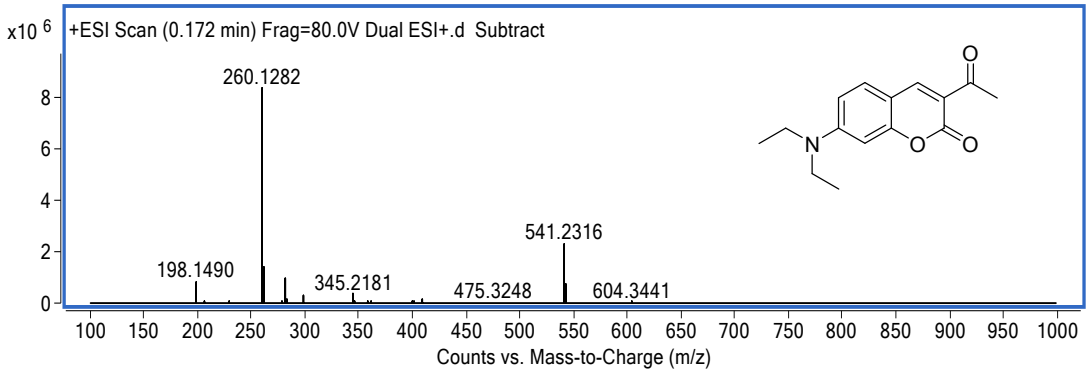


Figure S8. MS Spectra of Compound 1

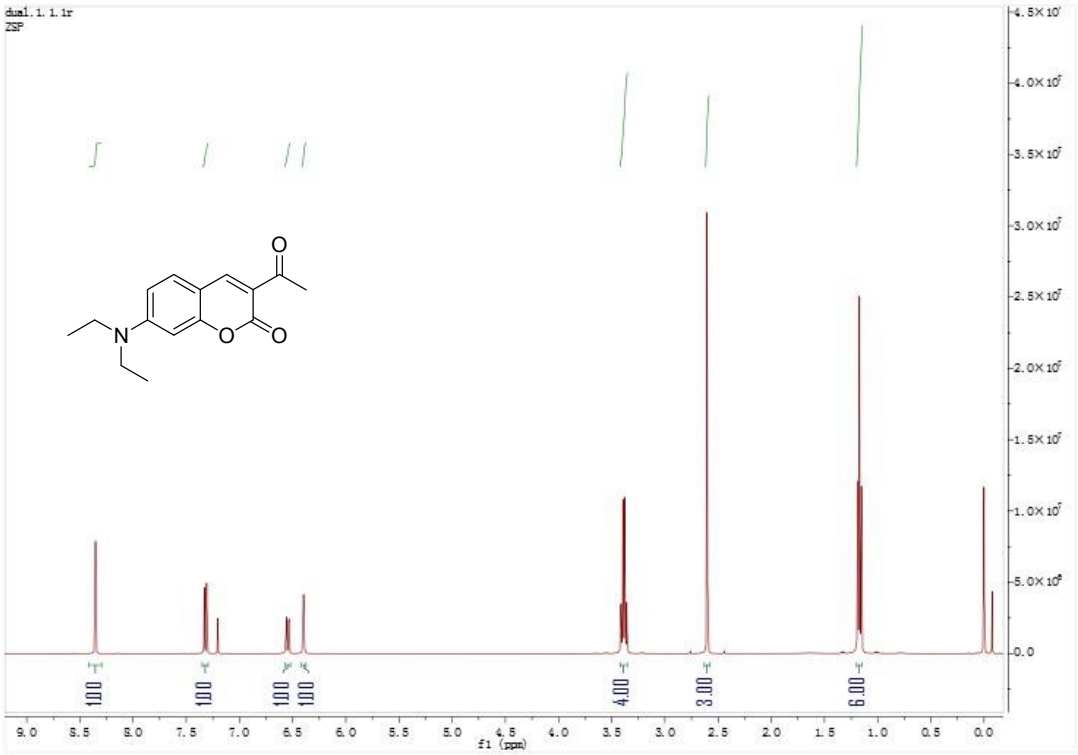


Figure S9. ^{1}H NMR Spectra of Compound 1

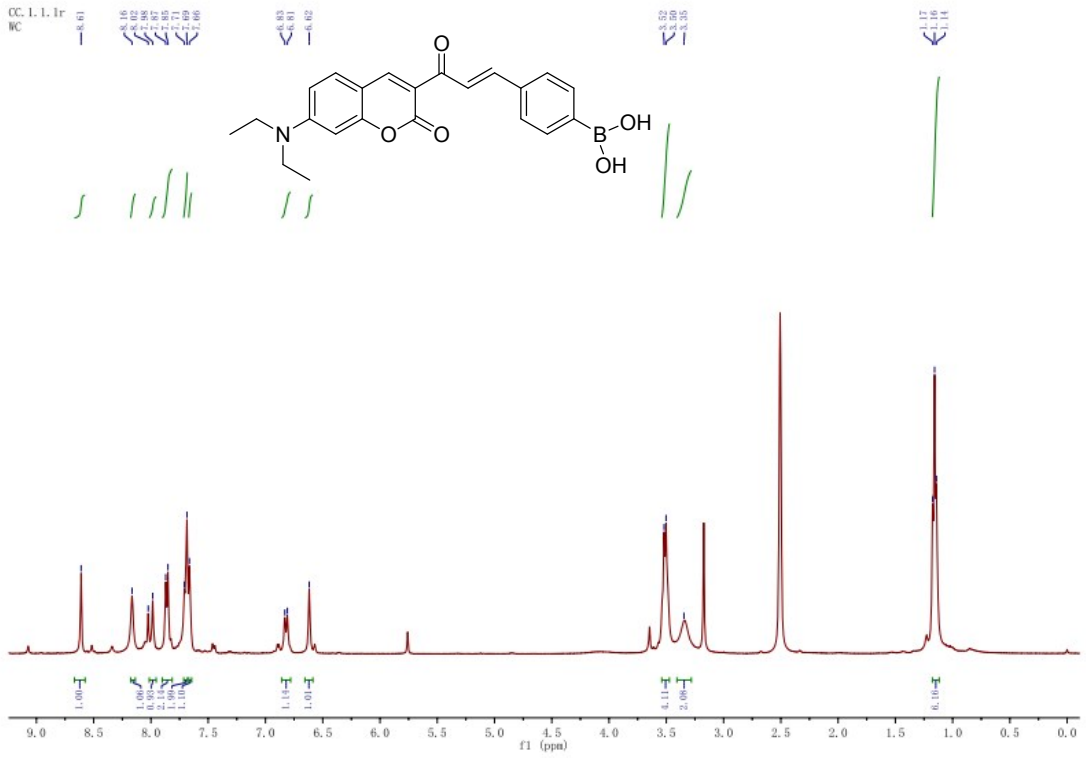


Figure S10. ^{1}H NMR Spectra of **Cys 1**

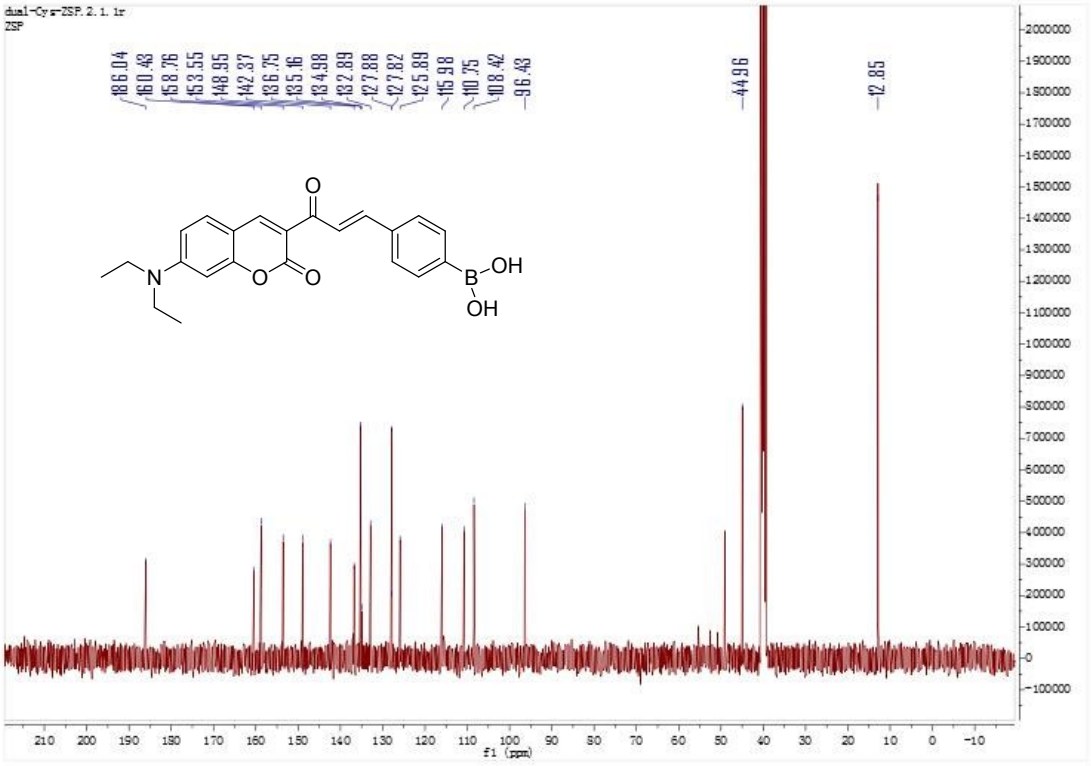


Figure S11. ^{13}C NMR Spectra of **Cys 1**