A phthalimide-based fluorescent probe for thiol detection with a large Stokes shift

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Table of Contents

Titration experiments

In titration experiment, the stock solutions of probe **1** were prepared at 1 mM in CH_3CN. The solutions of amino acid was prepared from Asp, Ala, Val, Phe, His, Leu, Ser, IIe, Trp, Lys, Arg, Pro, Gly, Met, Tyr, Glu, Thr, Hcy, GSH and Cys in twice-distilled water. The test solution of probe **1** (10.0 μM) in 3 mL HEPES buffer (10 mM, pH = 7.4) containing 20% CH_3CN was prepared by placing 0.03 mL stock solution of probe **1,** 0.57 mL CH_3CN and 2.35 mL HEPES. The resulting solution was shaken well and incubated with 0.05 mL appropriate testing species for 60 min at room temperature and then obtained the results.

pH experiments

In the pH experiments, the aqueous solutions with different pH values (from 2 to 13) were obtained by adding NaOH (0.1 M) or HCl (0.2 M) to HEPES buffer (10.0 mM, pH = 7.4). Next, place 0.03 mL stock solution of probe 1, 0.57 mL CH_3CN into 2.35 mL of the above obtained aqueous solution with a corresponding pH value. Finally, minimum amounts of NaOH (0.1 M) or HCl (0.2 M) was added to the resulting solution to finely adjust pH values and the resulting solution was shaken well and incubated for 60 min at room temperature before recording the spectra. The description of titration experiments and pH experiments was added into the supplementary information.

Detection limit

The detection limit was calculated with the following equation:

$$\text{Detection limit} = 3\sigma/k$$

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity vs Cys, Hcy and GSH concentration. The detection limit for Cys, Hcy and GSH was deduced to be 0.8 nM, 1.1 nM, 0.6 nM.

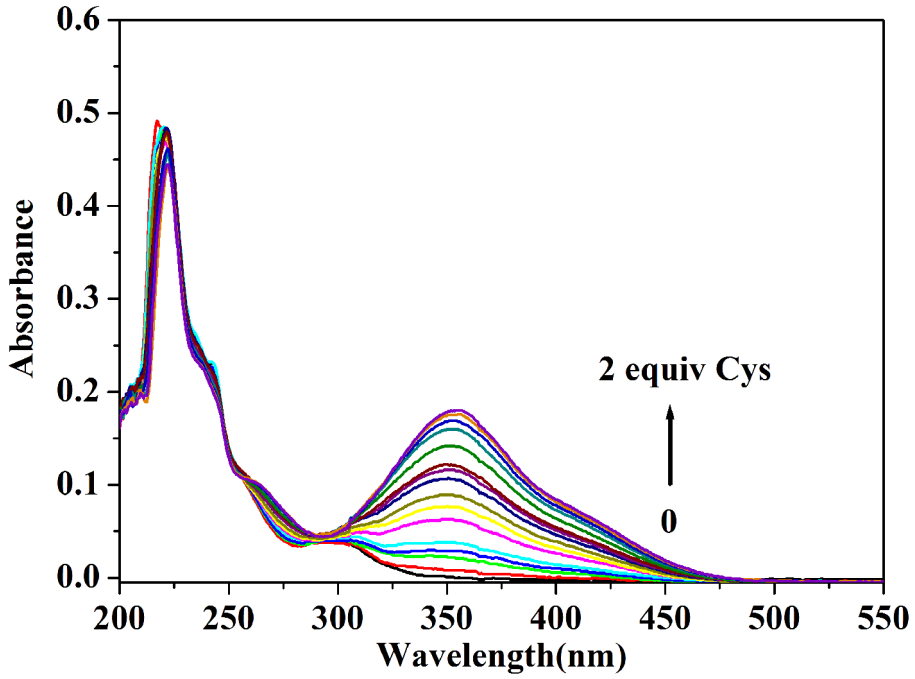

Fig. S1 Absorption spectra of probe **1** (10.0 μM) upon the addition of Cys (0.0-2.0 equiv.) in HEPES buffer (10.0 mM, pH = 7.4) containing 20% CH_3CN.

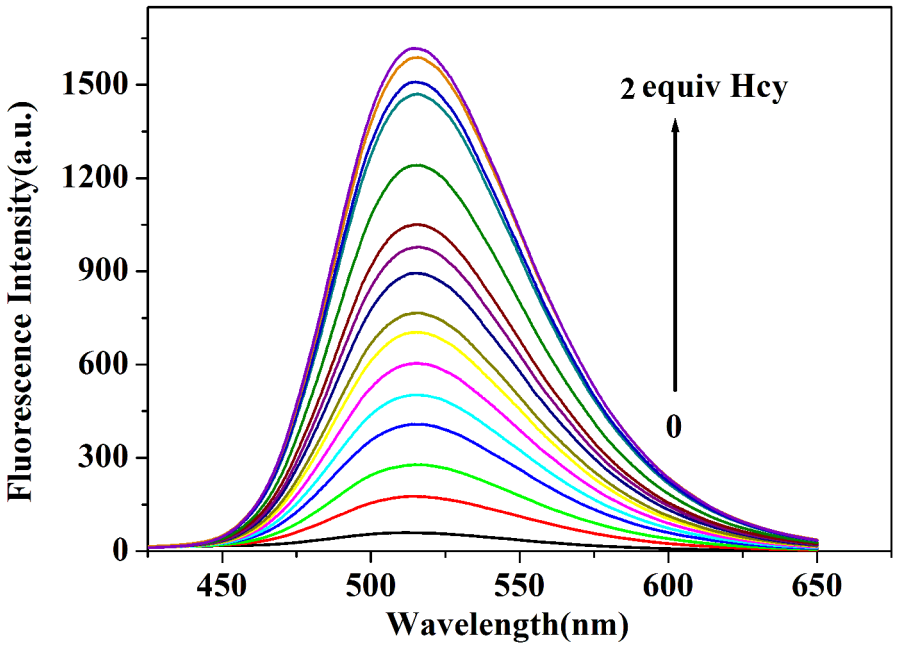

Fig. S2 Emission spectra of probe **1** (10.0 μM) upon the addition of Hcy (0.0-2.0 equiv.) with excitation at 358 nm in HEPES buffer (10.0 mM, pH = 7.4) containing 20% CH_3CN.

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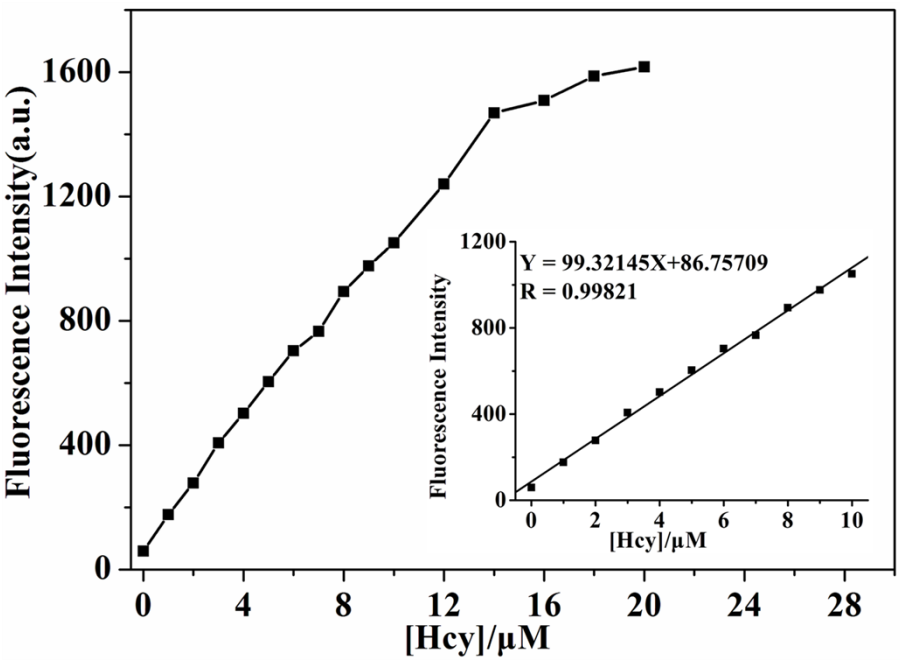

Fig. S3 Plot of emission intensity at 516 nm of probe **1** (10.0 μM) (black dot) upon the addition of Hcy (0.0-2.0 equiv.) in HEPES buffer (10.0 mM, pH = 7.4) containing 20% CH_3CN. Inset: the linear relationship between the emission intensity at 516 nm of probe **1** and the concentration of Hcy.

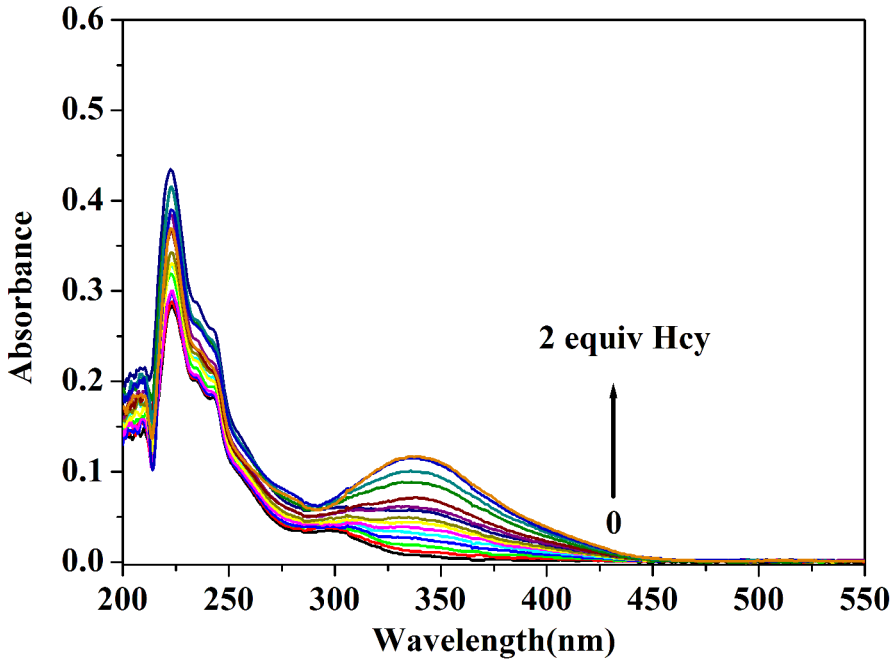

Fig. S4 Absorption spectra of probe **1** (10.0 μM) upon the addition of Hcy (0.0-2.0 equiv.) in HEPES buffer (10.0 mM, pH = 7.4) containing 20% CH_3CN.

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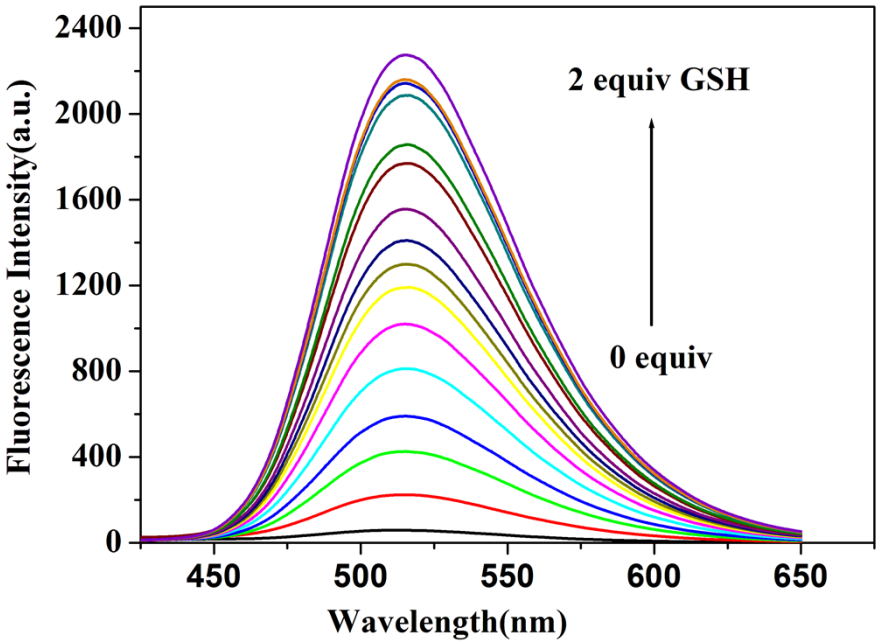

Fig. S5 Emission spectra of probe **1** (10.0 μM) upon the addition of GSH (0.0-2.0 equiv.) with excitation at 358 nm in HEPES buffer (10.0 mM, pH = 7.4) containing 20% CH_3CN.

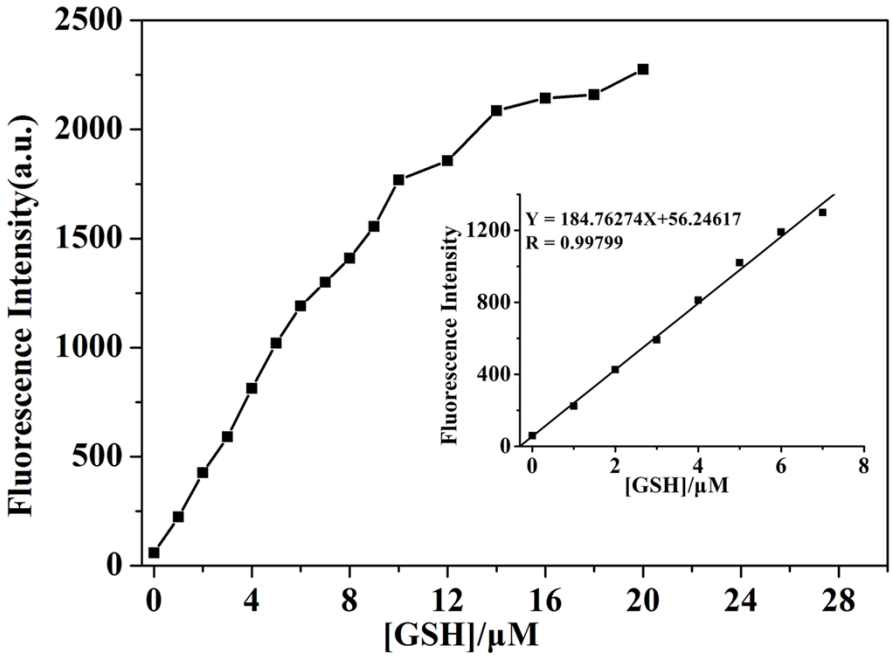

Fig. S6 Plot of emission intensity at 516 nm of probe **1** (10.0 μM) (black dot) upon the addition of GSH (0.0-2.0 equiv.) in HEPES buffer (10.0 mM, pH = 7.4) containing 20% CH_3CN. Inset: the linear relationship between the emission intensity at 516 nm of probe **1** and the concentration of GSH.

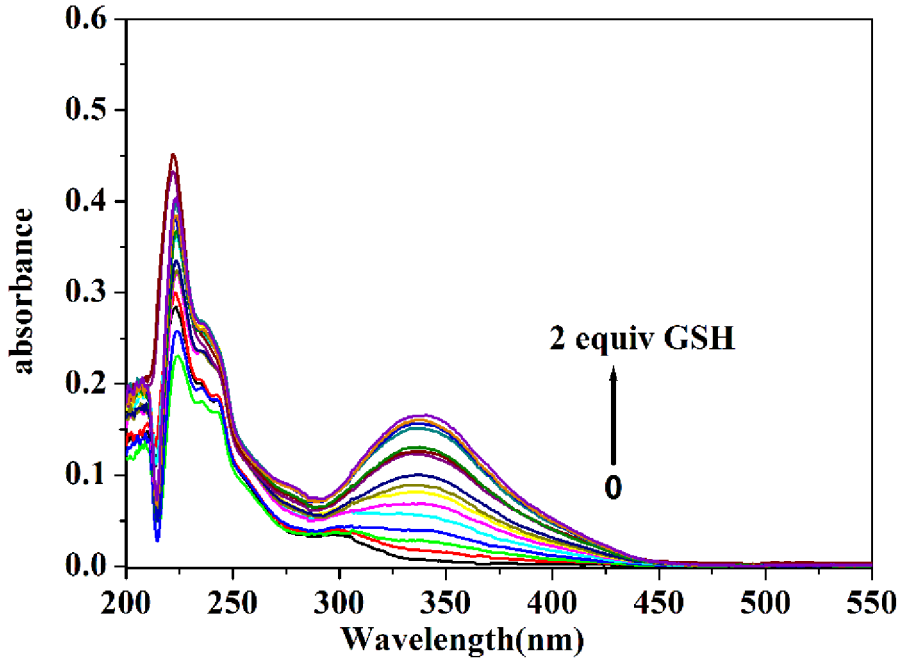

Fig. S7 Absorption spectra of probe **1** (10.0 μM) upon the addition of GSH (0.0-2.0 equiv.) in HEPES buffer (10.0 mM, pH = 7.4) containing 20% CH_3CN.

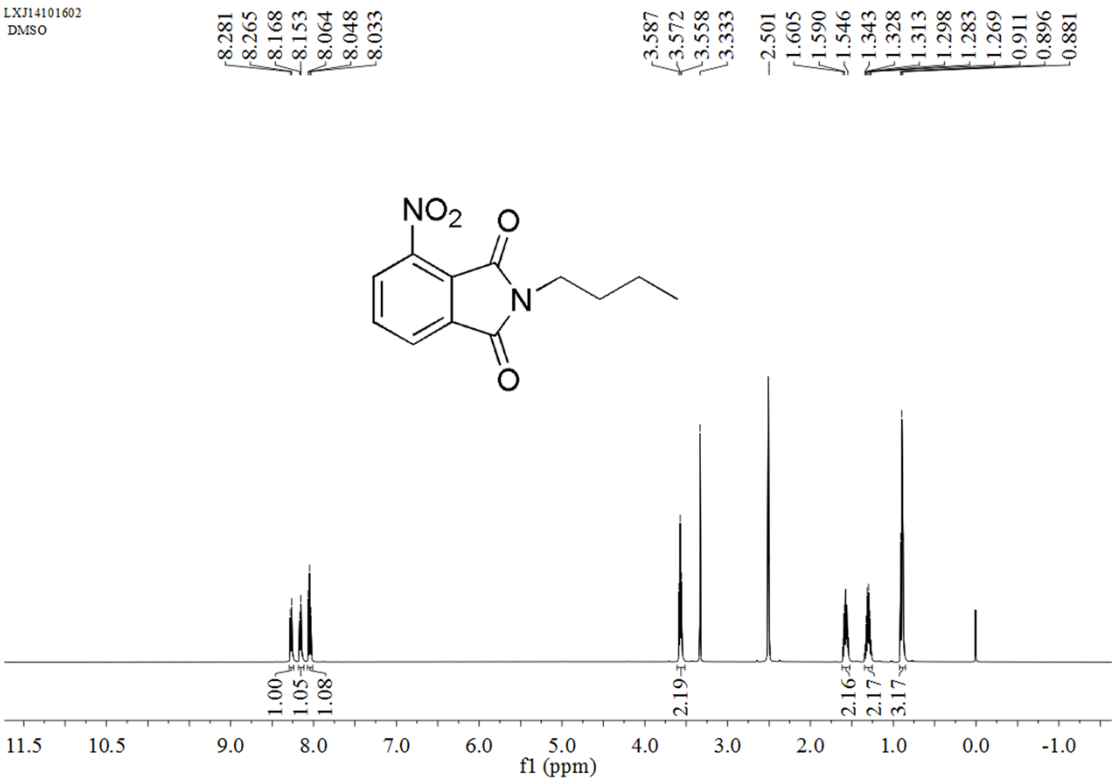

Fig. S8 ^{1}H NMR spectrum (500 MHz, DMSO-d_6, 298 K) of compound **4**.

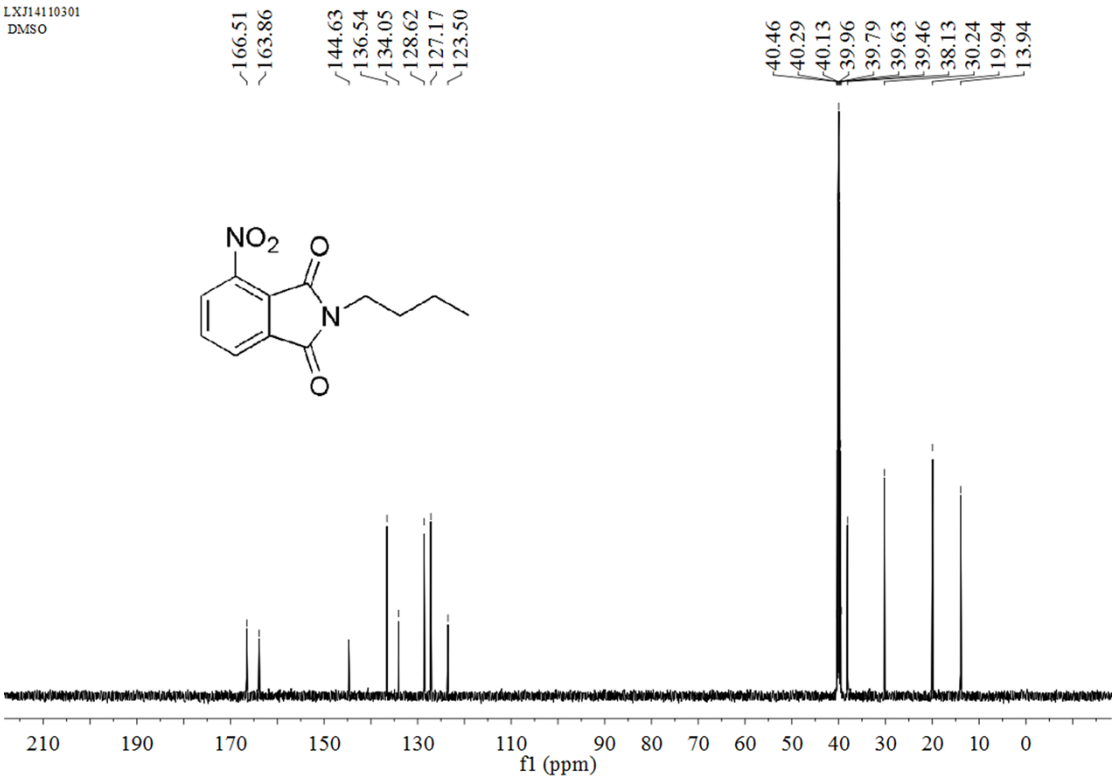

Fig. S9 ^{13}C NMR spectrum (125 MHz, DMSO-d_6, 298 K) of compound **4**.

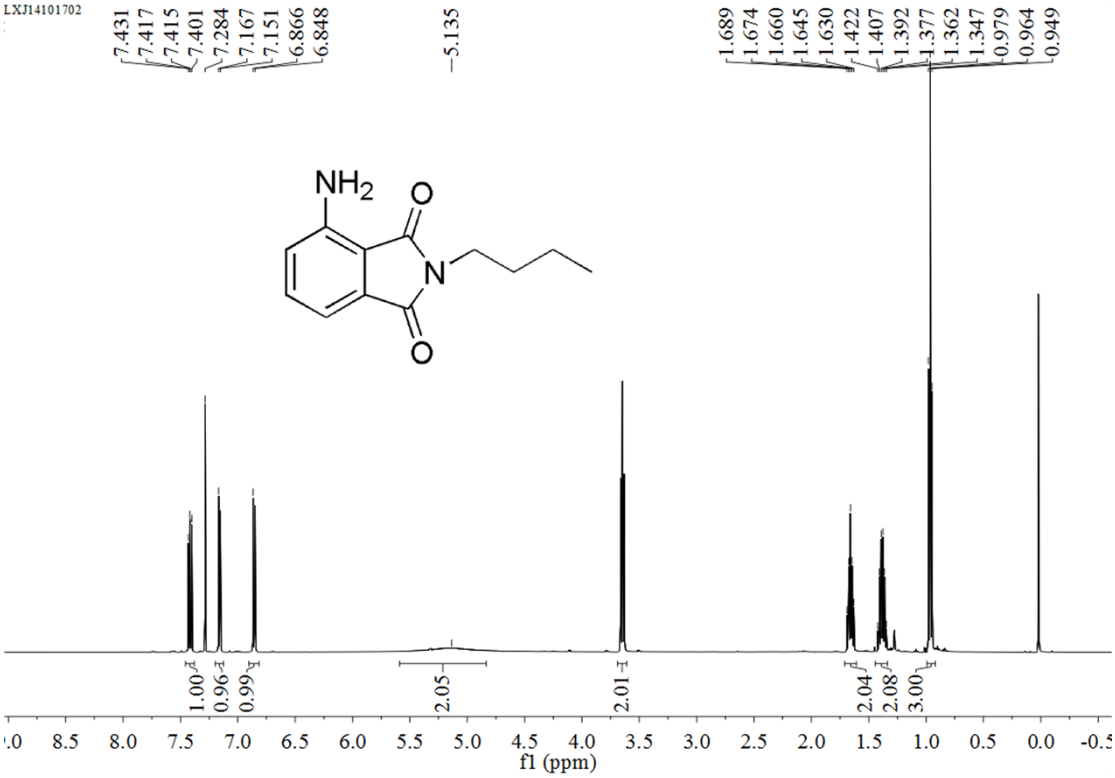

Fig. S10 ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) of compound **3**.

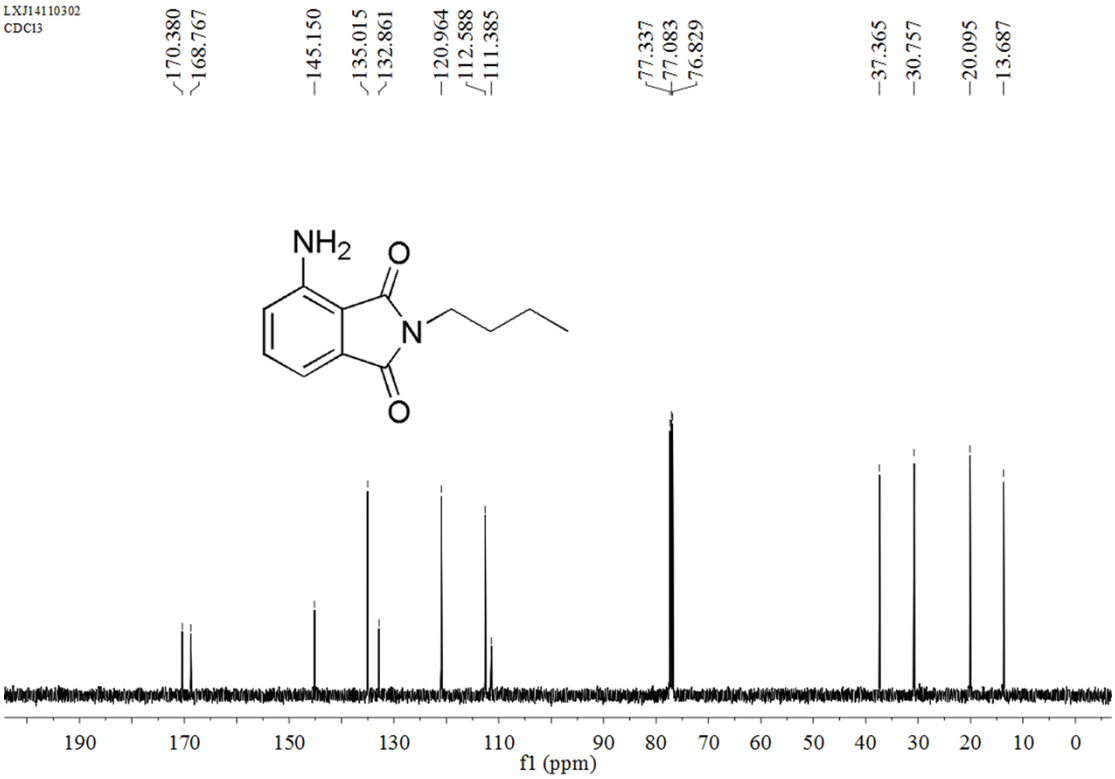

Fig. S11 ^{13}C NMR spectrum (125 MHz, $CDCl_3$, 298 K) of compound **3**.

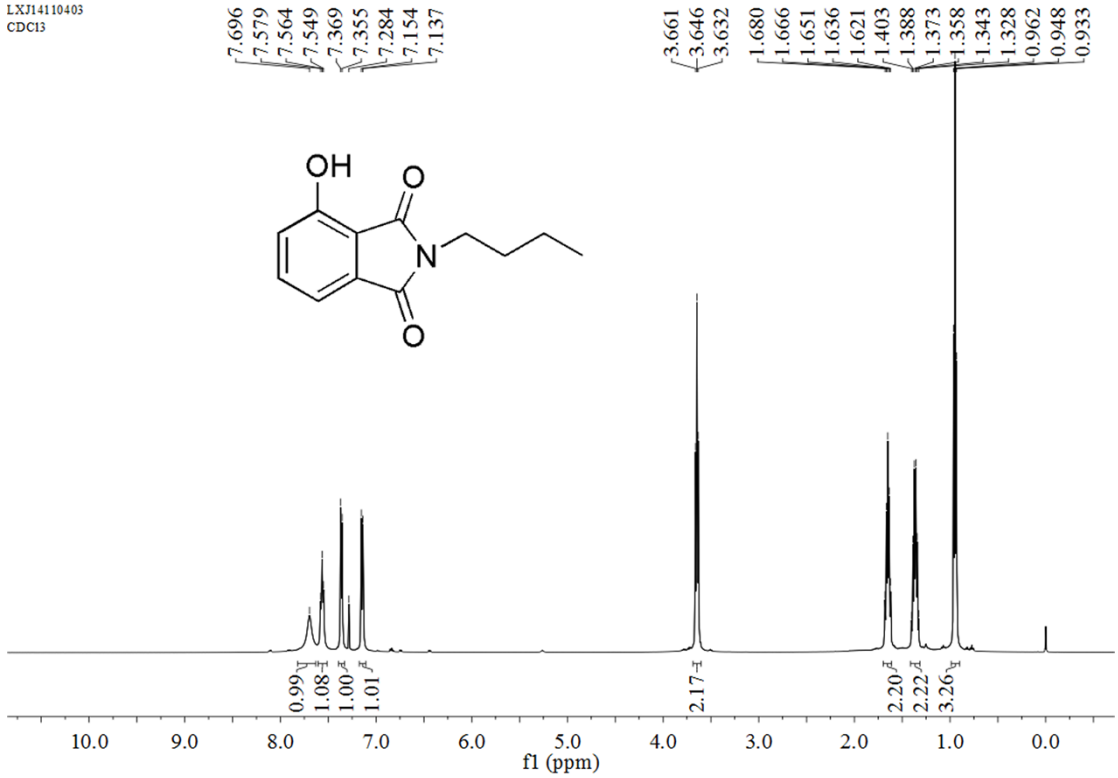

Fig. S12 ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) of compound **2**.

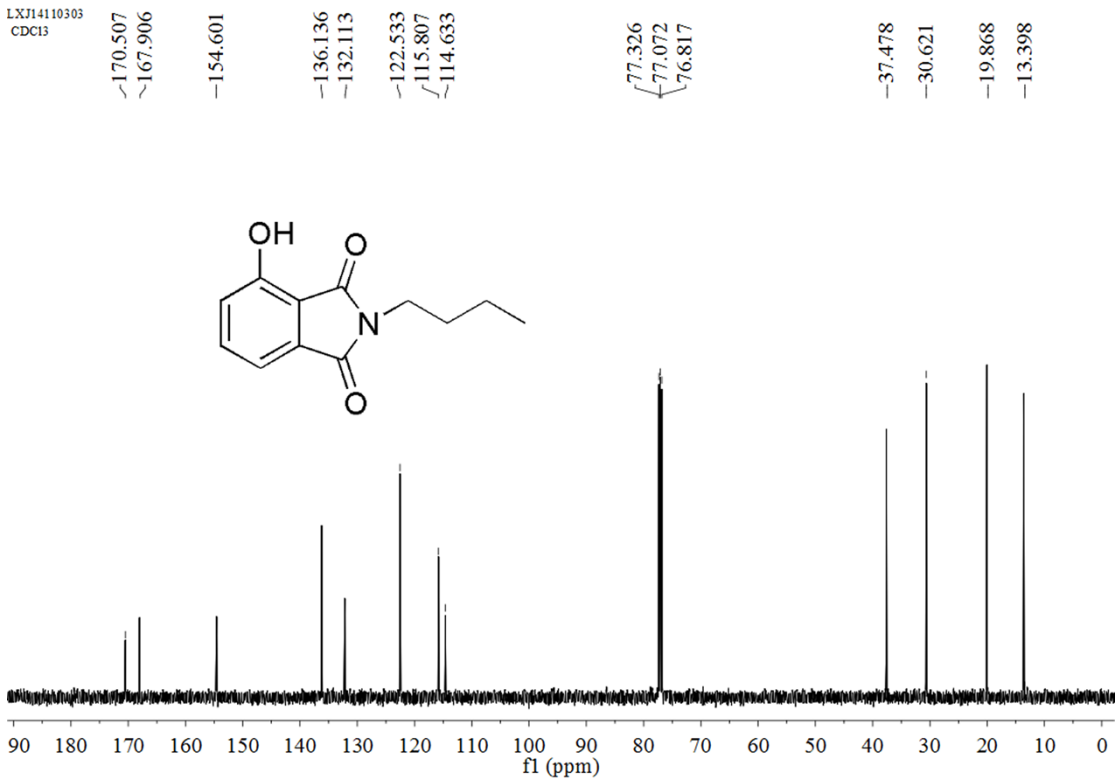

Fig. S13 ^{13}C NMR spectrum (125 MHz, $CDCl_3$, 298 K) of compound **2**.

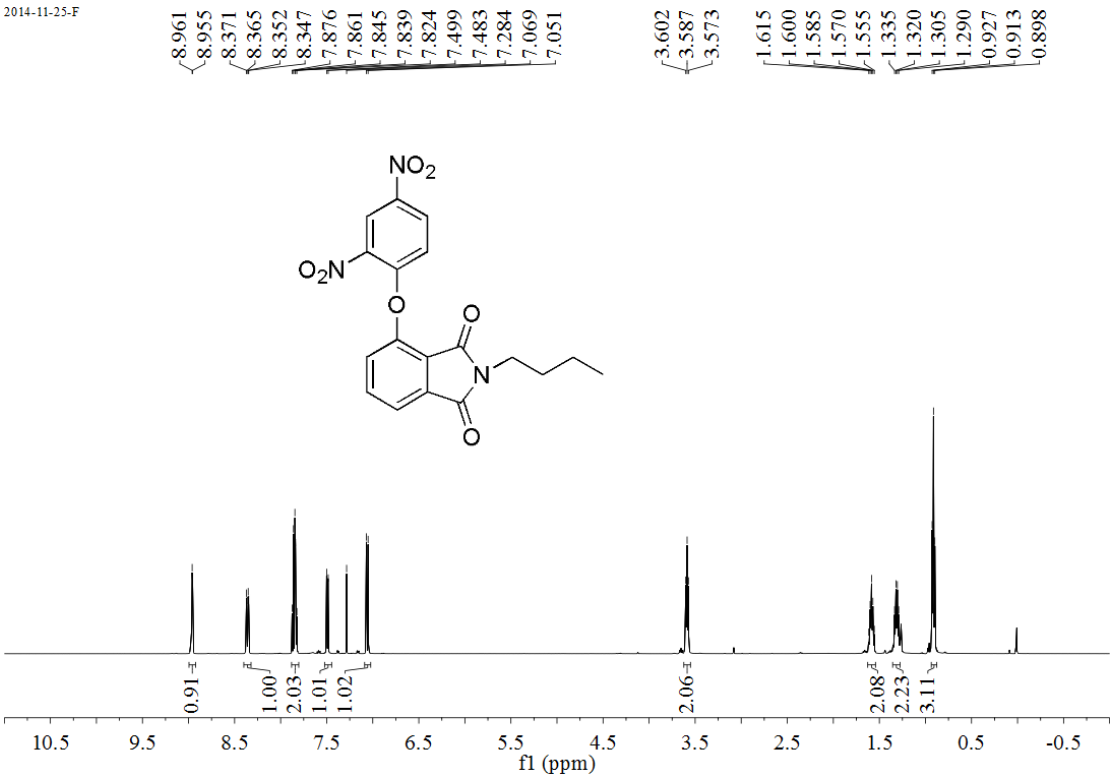

Fig. S14 ^{1}H NMR spectrum (500 MHz, $CDCl_3$, 298 K) of probe **1**.

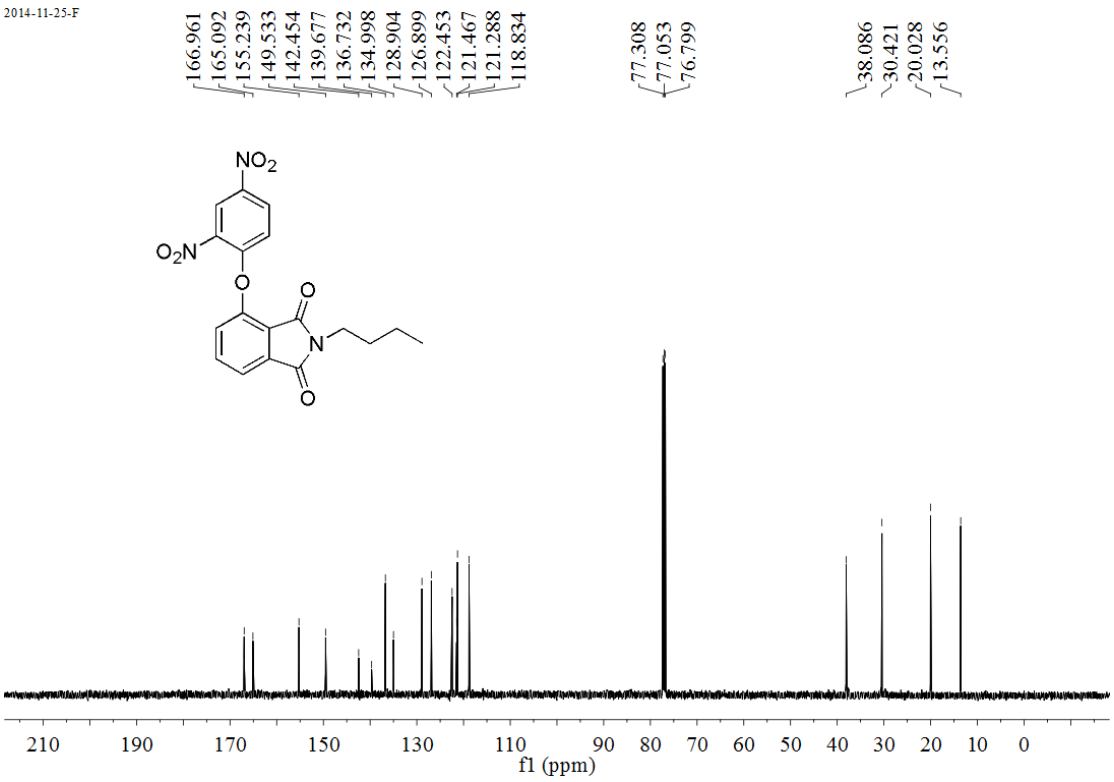

Fig. S15 ^{13}C NMR spectrum (125 MHz, $CDCl_3$, 298 K) of probe **1**.

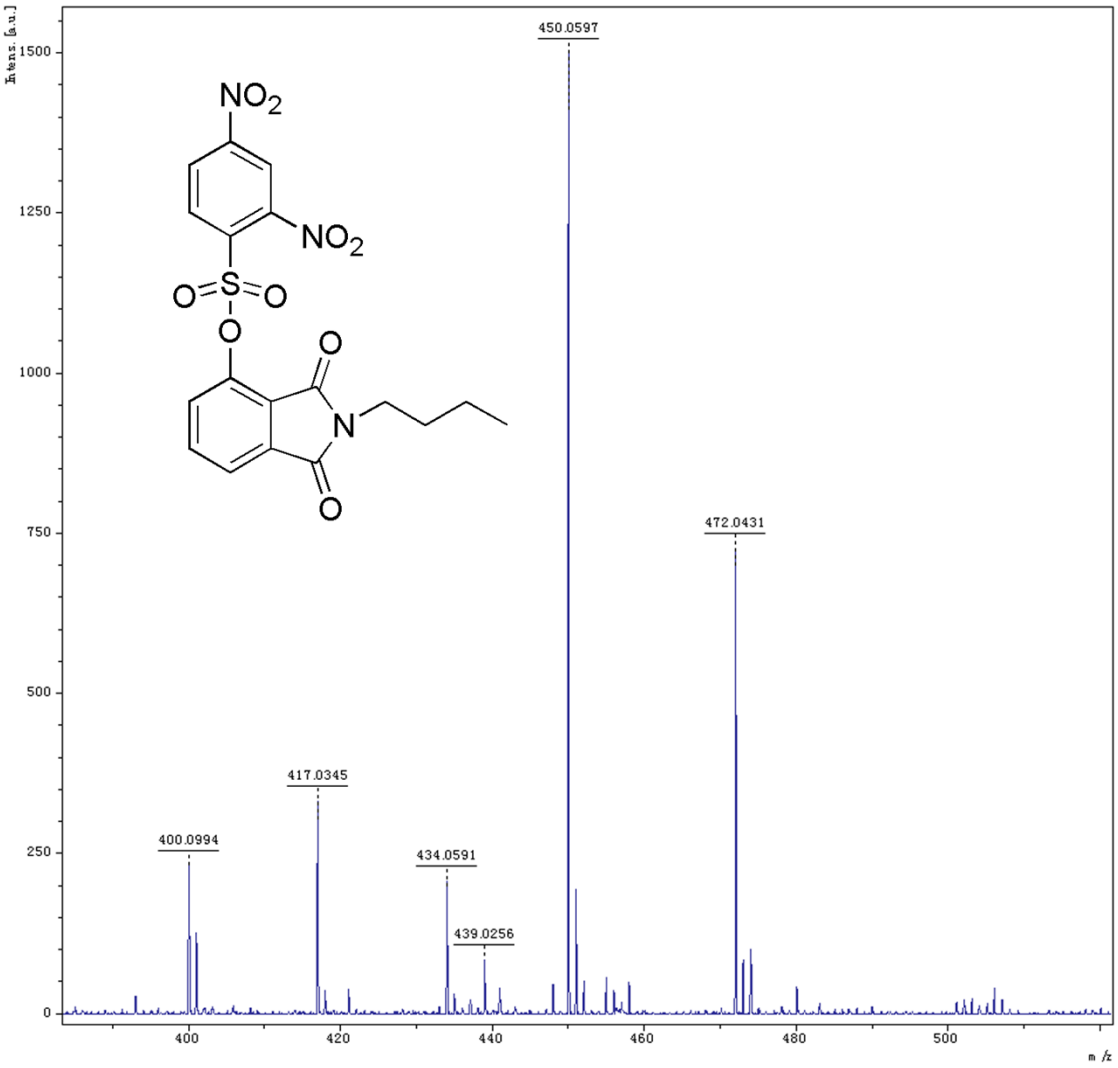

Fig. S16 HRMS spectrum of prob2

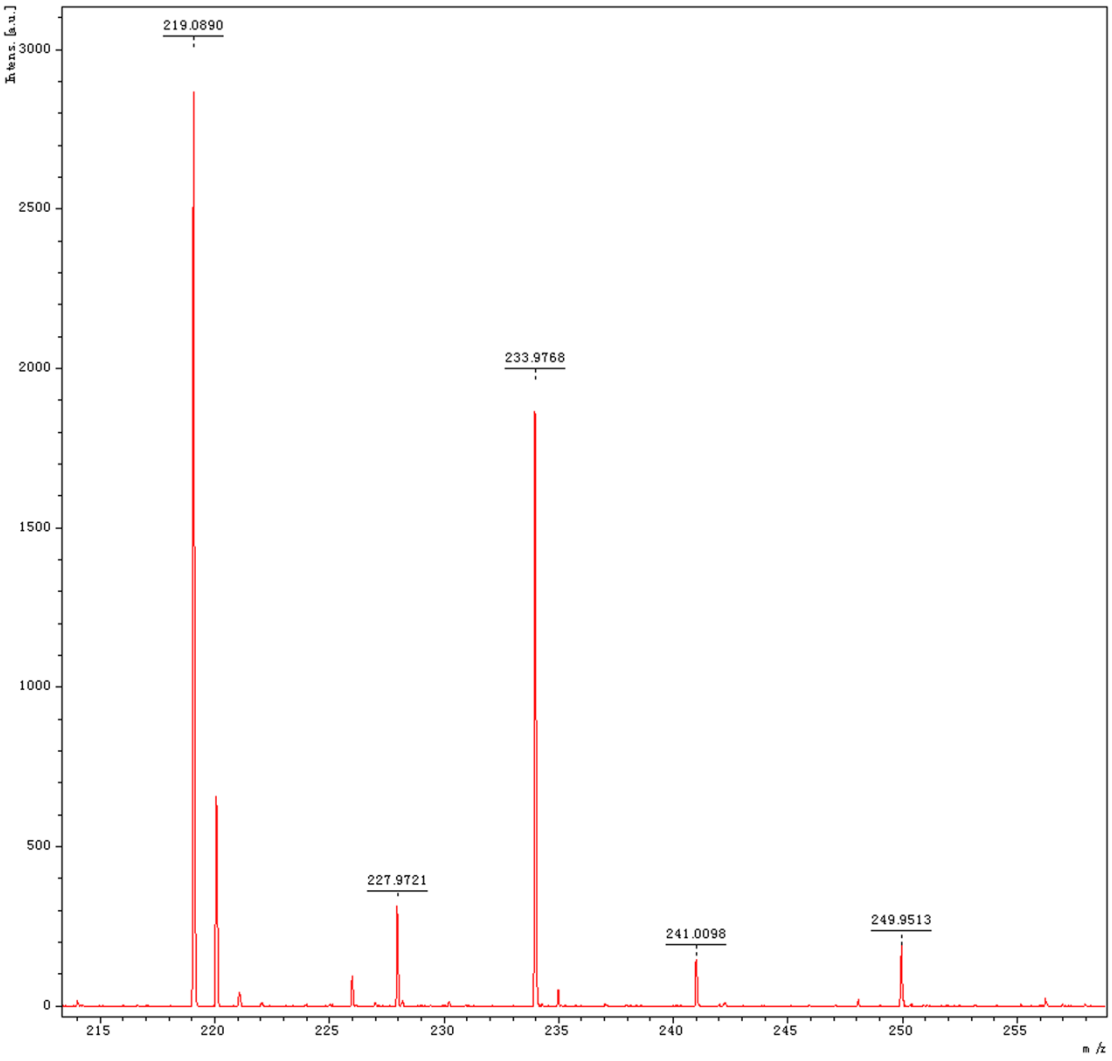

Fig. S17 HRMS spectrum of probe **1** treated with Cys in HEPES buffer (10.0 mM, pH = 7.4) containing 20% CH_3CN.