Supplementary Information (SI)

For

Detection and Differentiation of Cys, Hcy and GSH mixtures by ^{19}F NMR Probe

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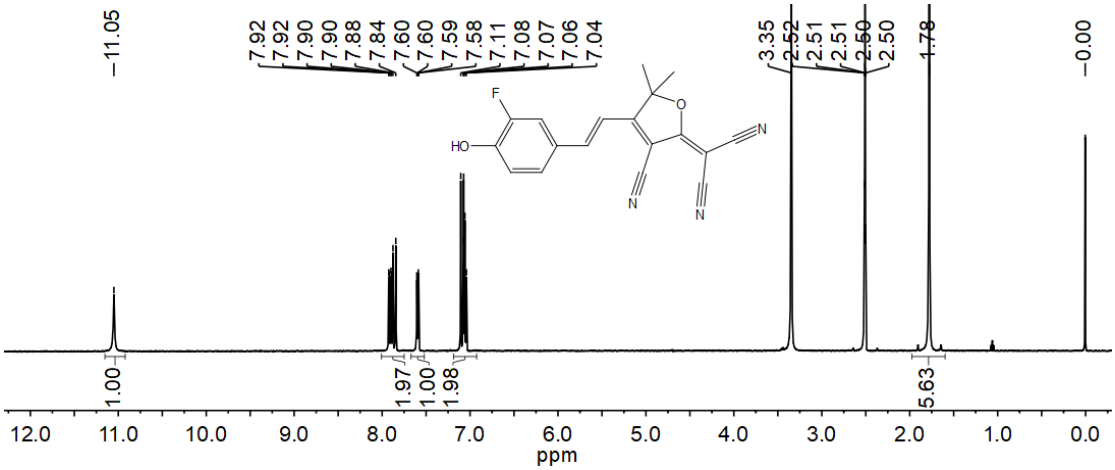
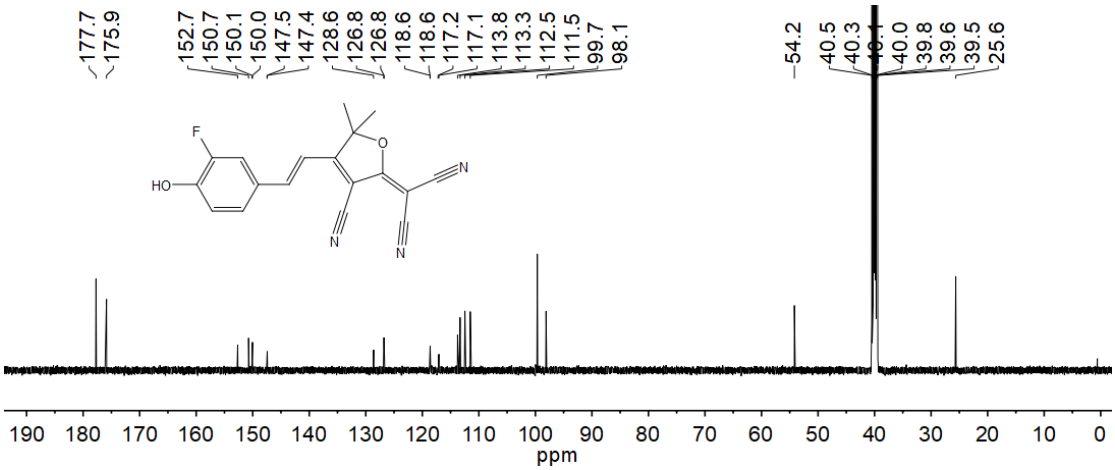
Supporting Information

Contents:

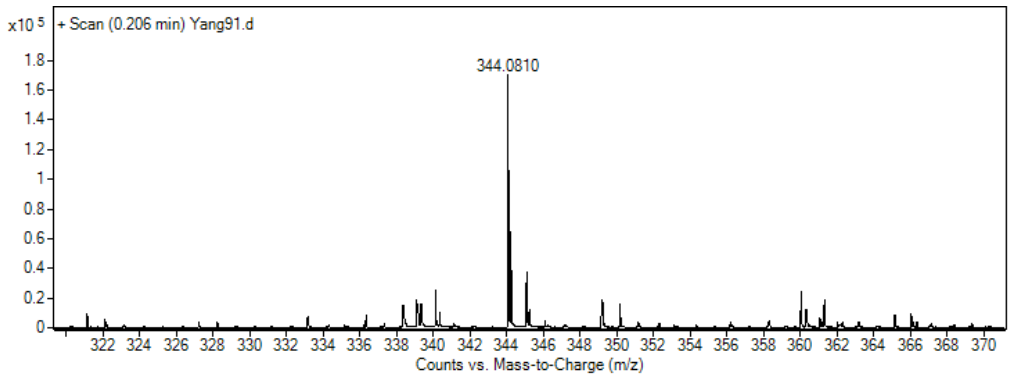
Characterization of compound 2 and 1



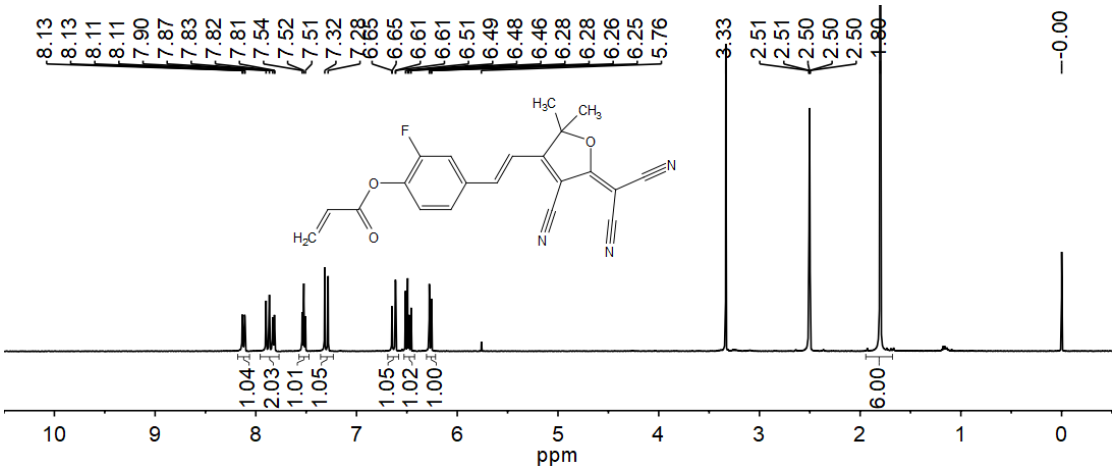
^{1}H NMR of compound 2 recorded in DMSO-d_6.



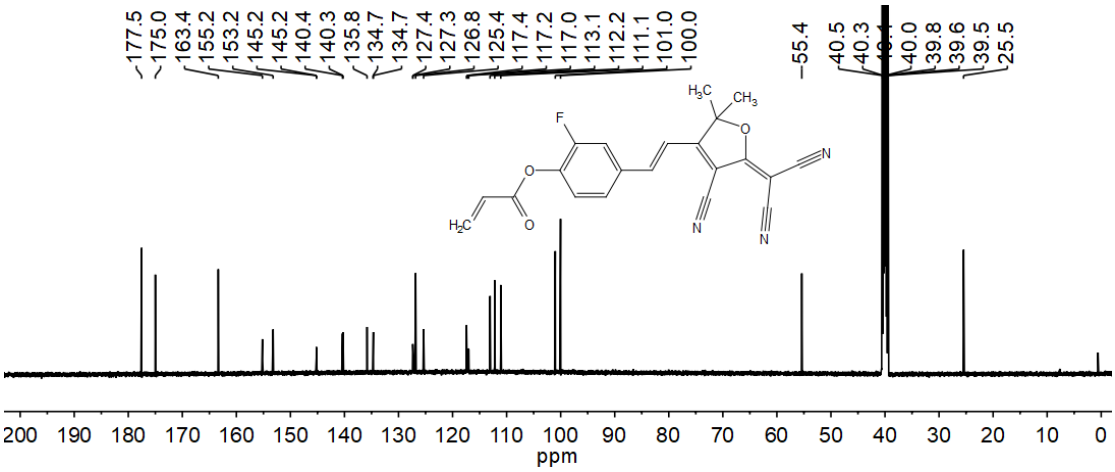
^{13}C NMR of compound 2 recorded in DMSO-d_6.



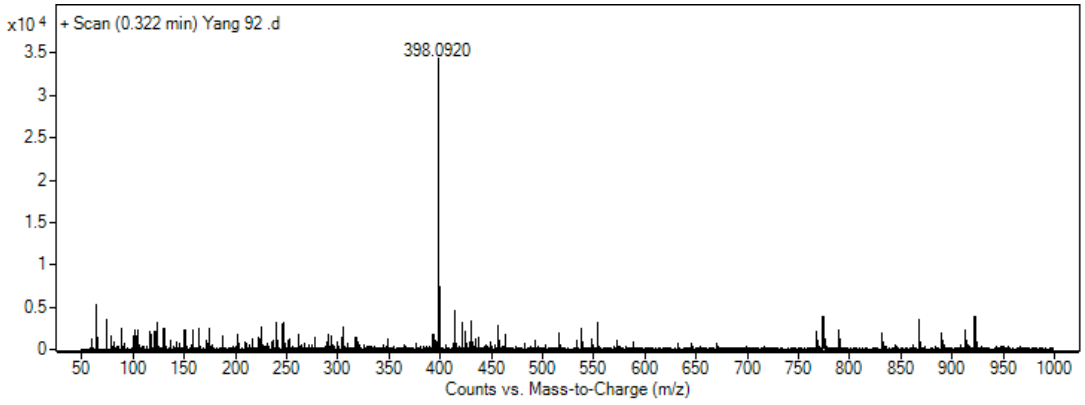
HR MS-ESI of compound 2.



^{1}H NMR of probe 1 recorded in DMSO-d$_6$.



^{13}C NMR of probe 1 recorded in DMSO-d$_6$.



HR MS-ESI of probe 1.

Color changes of probe 1 with Cys, Hcy and GSH:

Figure S1. Color changes of probe 1 (10 μM) in 20 mM HEPES buffer (pH 7.4) solution with 30% acetonitrile and 5% D_2O as cosolvent at 25 °C. The solutions were incubated with 10 equiv. Cys, 10 equiv. Hcy and 10 equiv. GSH for 90 min, respectively.

Fluorescence scan kinetics of probe 1 with Cys:

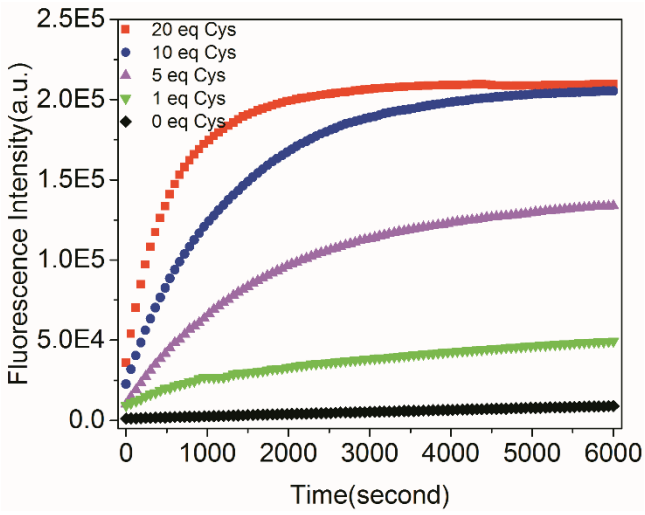


Figure S2. Time-dependent of fluorescence kinetics spectra of probe 1 (10 μM) upon addition of different concentrations of Cys in HEPES (20 mM, pH 7.4) solution with 30% acetonitrile and 5% D_2O at 25 °C. The reactions were monitored at 615 nm.

Uv-vis spectra changes of probe 1 upon addition of Hcy and GSH:

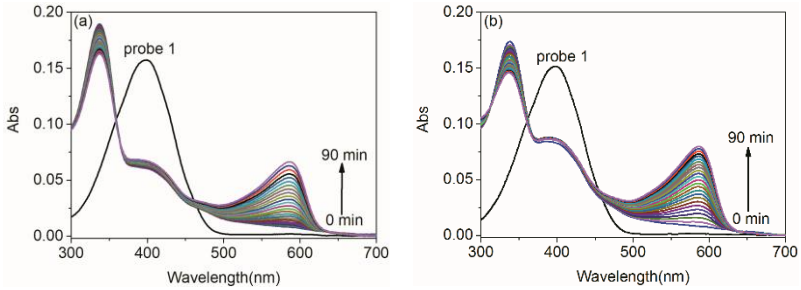


Figure S3. Uv-vis spectra changes of probe 1 (10 μM) upon addition of 10 equiv. of (a) Hcy and (b) GSH. The Spectra were collected from 0 to 90 min. All the spectra were carried out in 20 mM HEPES buffer (pH 7.4) with 30% acetonitrile and 5% D_2O at 25 °C.

Fluorescence spectra changes of probe 1 upon addition of Hcy and GSH:

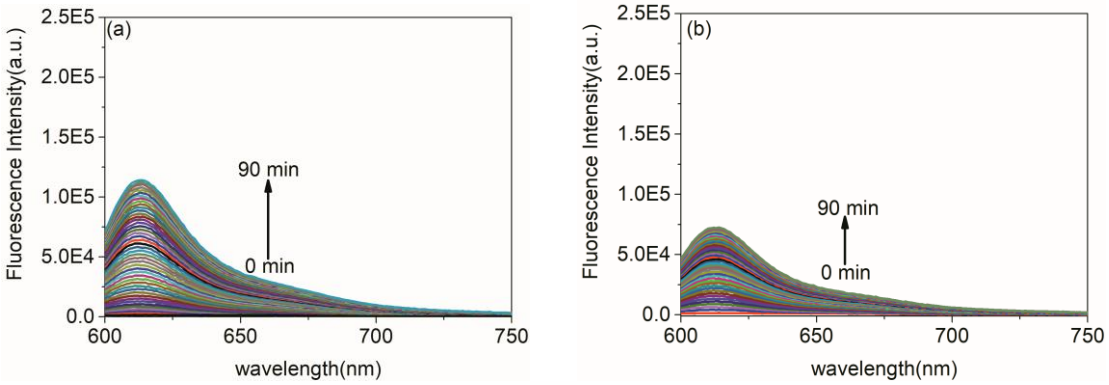


Figure S4. Fluorescence spectra changes of probe 1 (10 μM) in the presence of (a) 10 equiv. Hcy and (b) 10 equiv. GSH. The Spectra were collected from 0 to 90 min. All the experiments were carried out in HEPES buffer solution (pH 7.4, 20 mM) with 30% acetonitrile and 5% D_2O at 25 °C.

Apparent rate constant for the reaction of probe 1 with Cys, Hcy and GSH:

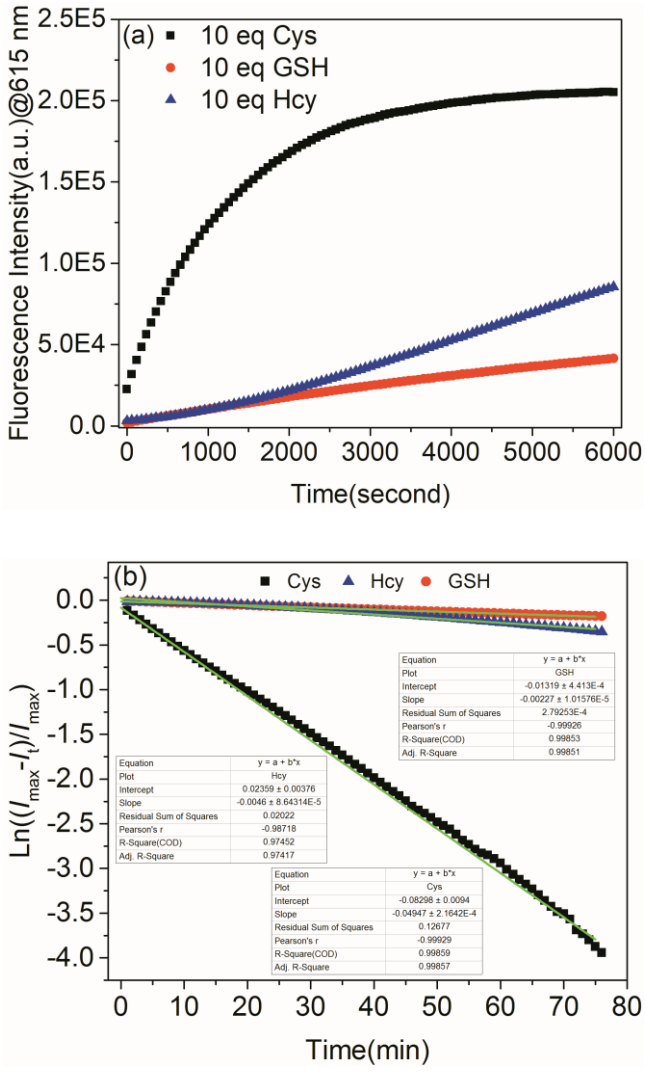


Figure S5. (a) Fluorescence scan kinetics spectra of probe 1 (10 μM) in the presence of 10 equiv. Cys, 10 equiv. Hcy and 10 equiv. GSH, respectively. (b) The apparent rate constant for the reaction of probe 1 with Cys, Hcy and GSH

were calculated by fitting (green solid line) the scanning kinetics data to the pseudo first-order equation: Ln((I_{max}-I_t)/I_{max}) = -kt, where I_{max} and I_t are the final fluorescence intensity and the fluorescence intensity at the maximum emission wavelength at time t, and k is the apparent rate constant. All the experiments were carried out in HEPES buffer solution (pH 7.4, 20 mM) with 30% acetonitrile and 5% D_2O at 25 °C.

Absorption and fluorescence selectivity experiments of probe 1 towards other species:

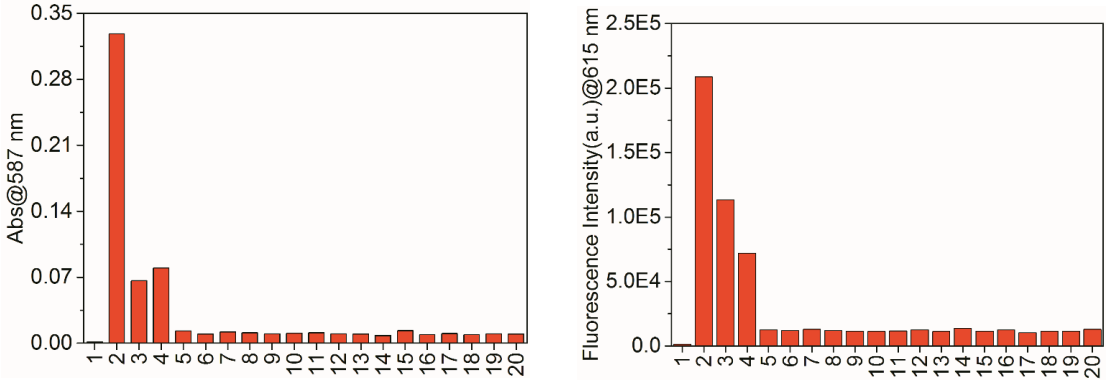


Figure S6. The response of absorption and fluorescence intensity of probe 1 (10 μM) toward various other species. Bars represent the relative intensity after addition of 10 equiv of a single species (from left to right: 1. probe 1, 2. Cys, 3. Hcy, 4. GSH, 5. $Na_2S_2O_3$, 6. Threonine, 7. Serine, 8. Leucine, 9. Sodium Erythorbate, 10. K_2SO_8, 11. Glutamine, 12. Tyrosine, 13. Lysine, 14. Glycine, 15. Methionine, 16. Aspartic acid, 17. Tryptophan, 18. Pyroglutamic acid, 19. Phenylalanine, 20. Phenylglycine). All experiments were performed in HEPES buffer solution (pH 7.4) with 30% acetonitrile and 5% D_2O at 25 °C and each spectrum was obtained at 90 min after addition of analyte.

Absorption and fluorescence spectra changes of probe 1 towards the mixture of Cys, Hcy and GSH:

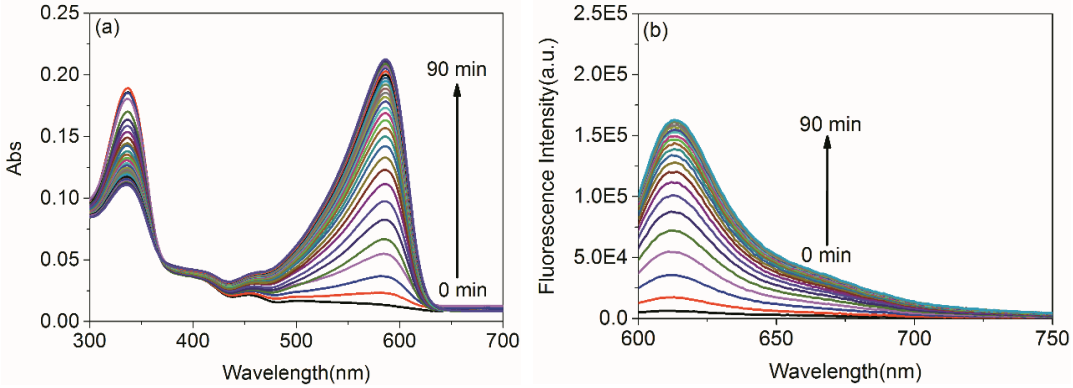


Figure S7. (a) Absorption and (b) fluorescence spectra changes of probe 1 (10 μM) upon addition of the mixture of Cys (100 μM), Hcy (200 μM) and GSH (200 μM). All the experiments were carried out in HEPES buffer solution (pH 7.4, 20 mM) with 30% acetonitrile and 5% D_2O at 25 °C.

^{19}F NMR spectra changes of probe 1 upon addition of 3 and 1 equivalents Cys:

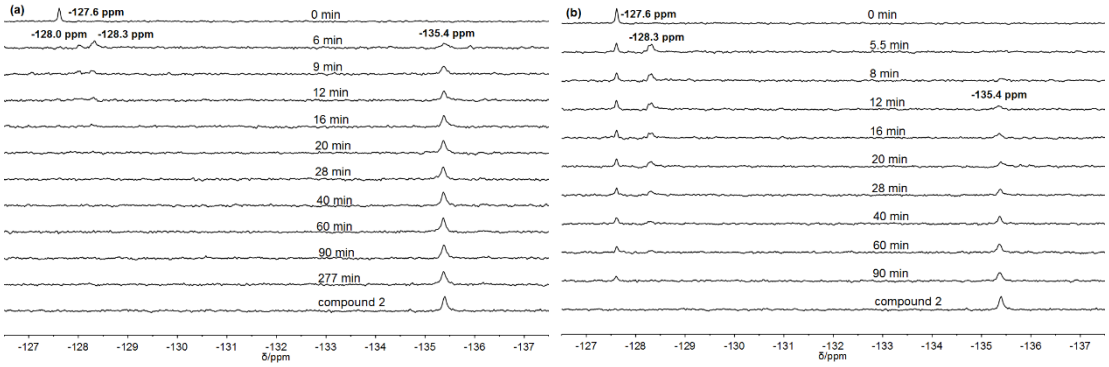


Figure S8. ^{19}F NMR spectra changes of probe 1 (200 μM) upon addition of (a) 3 equiv. and (b) 1 equiv. Cys. All the spectra were measured in HEPES buffer solution (pH 7.4) with 30% acetonitrile and 5% D_2O at 25 °C by 24 scans.

^{19}F NMR spectra changes of probe 1 upon addition of 5 equivalents Hcy and GSH:

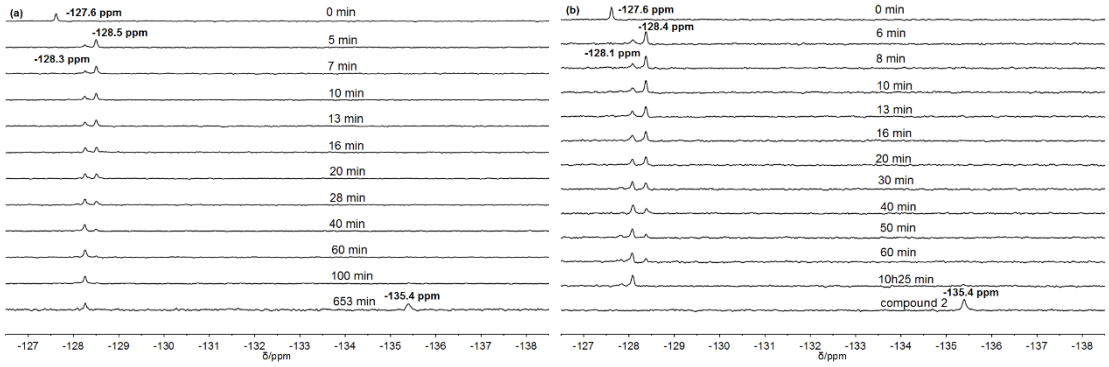


Figure S9. ^{19}F NMR spectra changes of probe 1 (200 μM) upon addition of 5 equiv. (a) Hcy and (b) GSH. All the spectra were measured in HEPES buffer solution (pH 7.4) with 30% acetonitrile and 5% D_2O at 25 °C by 24 scans.

^{19}F NMR spectra changes of probe 1 upon addition of 10 and 20 equivalents Hcy:

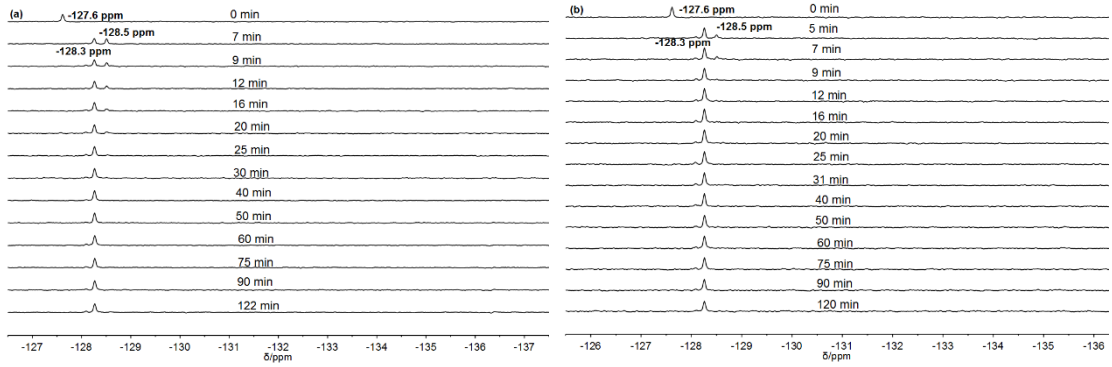


Figure S10. ^{19}F NMR spectra changes of probe 1 (200 μM) upon addition of (a) 10 equiv. and (b) 20 equiv. Hcy. All the spectra were measured in HEPES buffer solution (pH 7.4) with 30% acetonitrile and 5% D_2O at 25 °C by 24 scans.

^{19}F NMR spectra changes of probe 1 upon addition of 10 and 20 equivalents GSH:

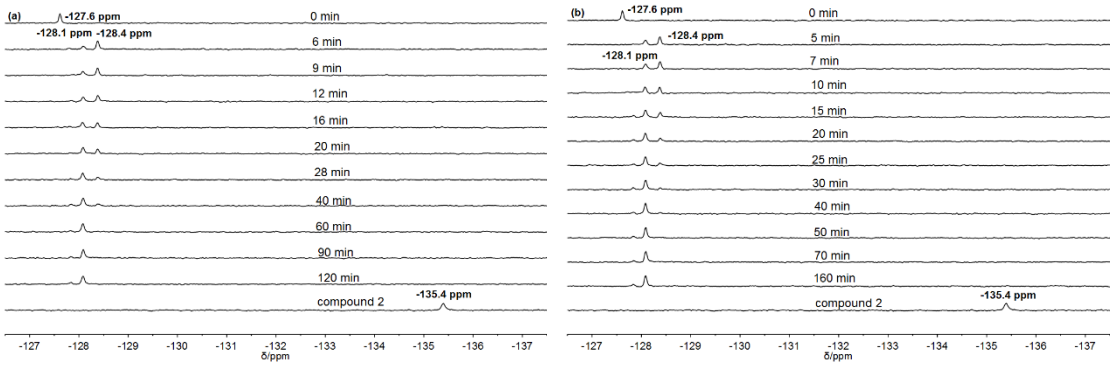


Figure S11. ^{19}F NMR spectra changes of probe 1 (200 μM) upon addition of (a) 10 equiv. and (b) 20 equiv. GSH. All the spectra were measured in HEPES buffer solution (pH 7.4) with 30% acetonitrile and 5% D_2O at 25 °C by 24 scans.

Data of ^{19}F NMR chemical shift changes of probe 1 with Cys, Hcy and GSH:

Analyte	Chemical shift (ppm)				Apparent rate constant (min^{-1})[a]
	before addition	upon addition	after 90 min	Δ δ	
Cys	-127.6	-128.0, -128.3, -135.4	-135.4	7.8	0.049
Hcy	-127.6	-128.5, -128.3	-128.3	0.7	0.0046
GSH	-127.6	-128.4, -128.1	-128.1	0.5	0.0023

Table S1. The ^{19}F NMR chemical shift changes data of probe 1 when respond to Cys, Hcy and GSH. [a] calculated from the data of fluorescence scan kinetics.

HPLC spectra of the reaction between probe 1 and Cys:

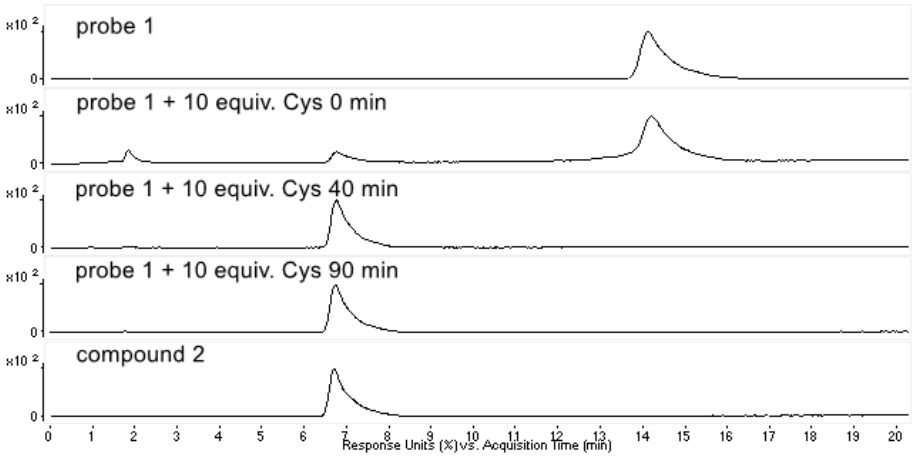


Figure S12. (a) Time course (0-90 min) of HPLC-UV profile of the reaction of probe 1 with 10 equiv. Cys in 20 mM HEPES buffer solution (pH 7.4) with 30% acetonitrile and 5% D_2O at 25 °C. The spectra were monitored at 398 nm.

HR-MS spectra of reaction mixture between probe 1 and 10 equiv. of Cys:

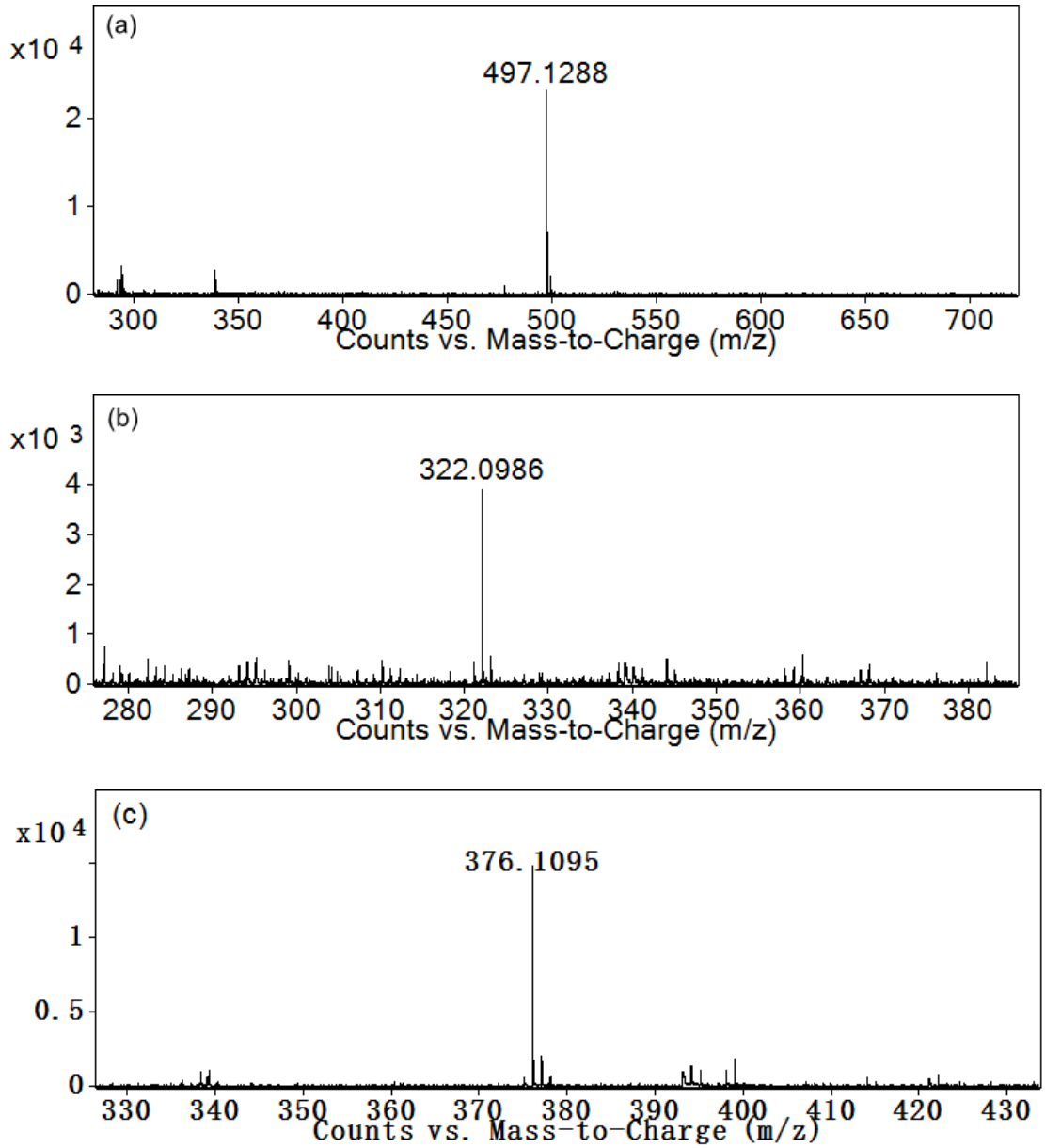


Figure S13. HR MS spectrum of reaction mixture between probe 1 and 10 equiv. of Cys in HEPES buffer solution (pH 7.4, 20 mM) with 30% acetonitrile and 5% D_2O at 25 °C. The peaks were consistent with the molecular weight of (a) [1-Cys+H^+] intermediate (calcd 497.1289), (b) compound [2 + H^+] (calcd 322.0986) and (c) probe [1 + H^+].

HPLC spectra of the reaction between probe 1 with Hcy, GSH:

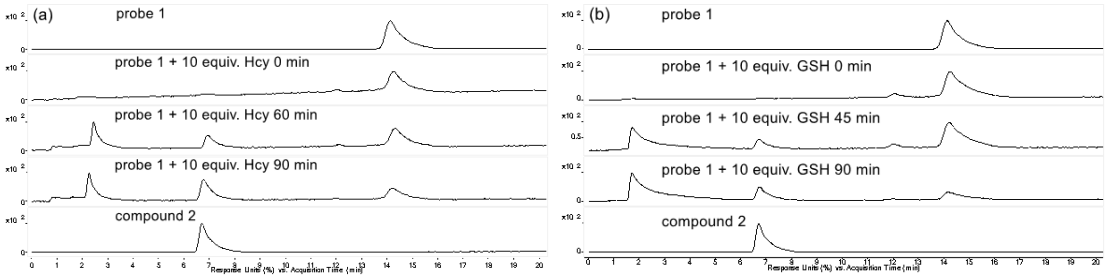


Figure S14. (a) HPLC spectra of the reaction of probe 1 with 10 equiv. Hcy and GSH in HEPES buffer solution (pH 7.4, 20 mM) with 30% acetonitrile and 5% D_2O at 25 °C.

HR-MS spectra of reaction mixture between probe 1 with Hcy, GSH:

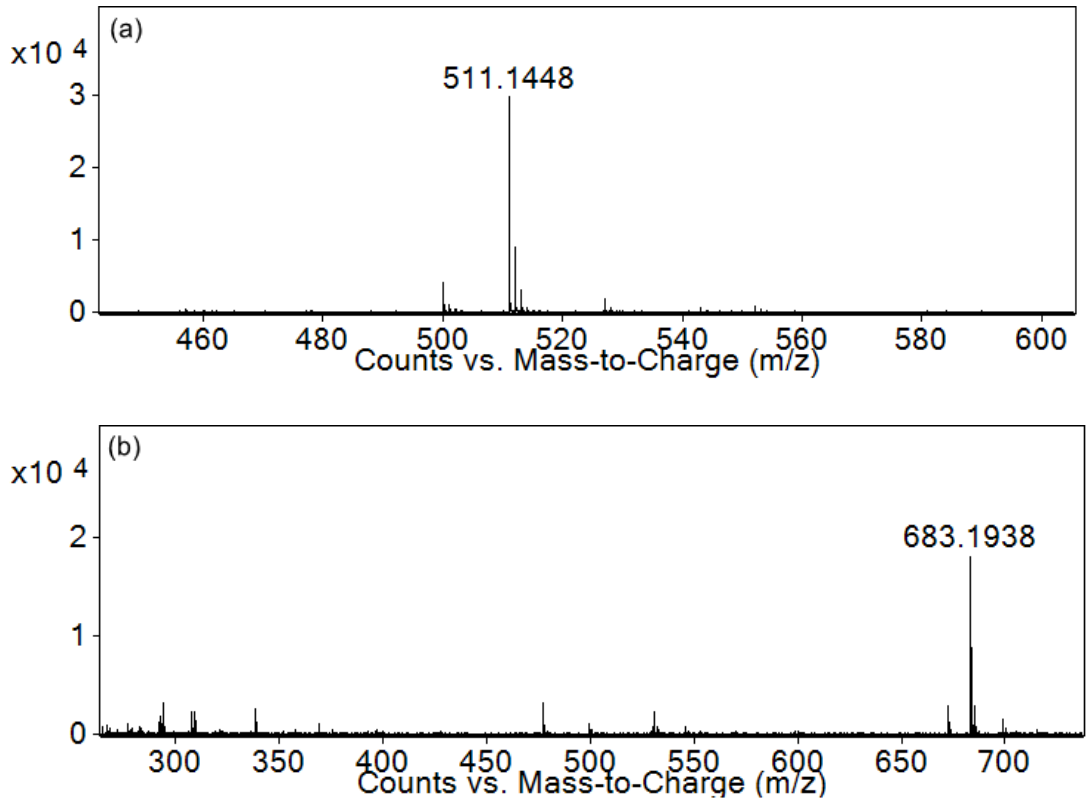


Figure S15. HR MS spectrum of reaction mixtures of probe 1 with 10 equiv. of (a) Hcy and (b) GSH in HEPES buffer solution (pH 7.4, 20 mM) with 30% acetonitrile and 5% D_2O at 25 °C. The peaks were consistent with the molecular weight of [1-Hcy+H^+] intermediate (calcd 511.1446) and compound [1-GSH+H^+] (calcd 683.1930), respectively.

Sensitivity of probe 1:

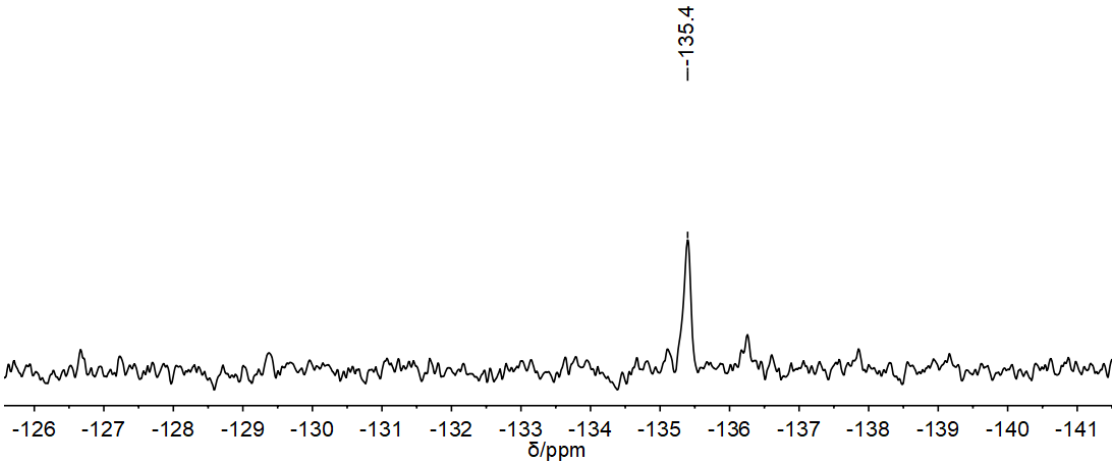


Figure S16. 4800 scans ^{19}F NMR spectrum of 10 μM probe 1 after treatment with 50 μM Cys in 20 mM HEPES buffer solution (pH 7.4) with 30% acetonitrile and 5% D_2O as cosolvent.

Stability experiment in bovine serum containing solution:

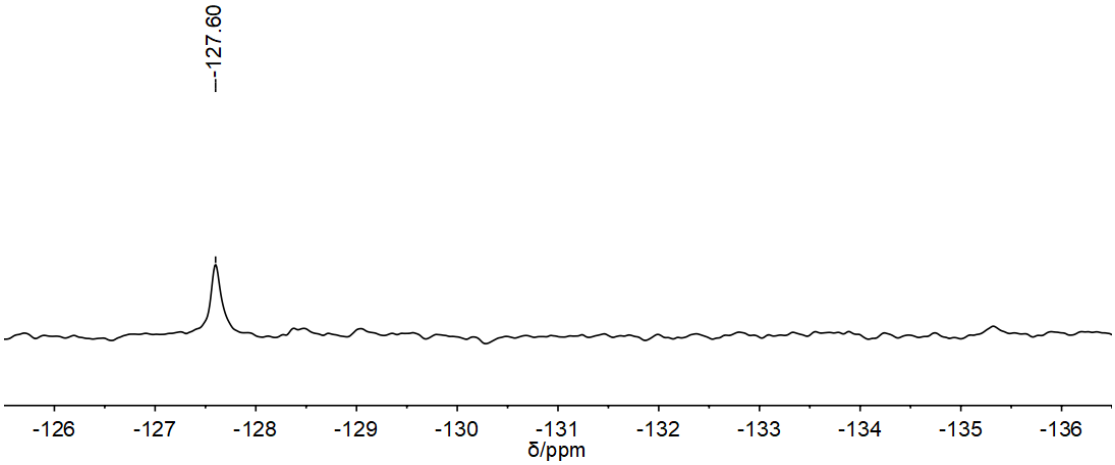
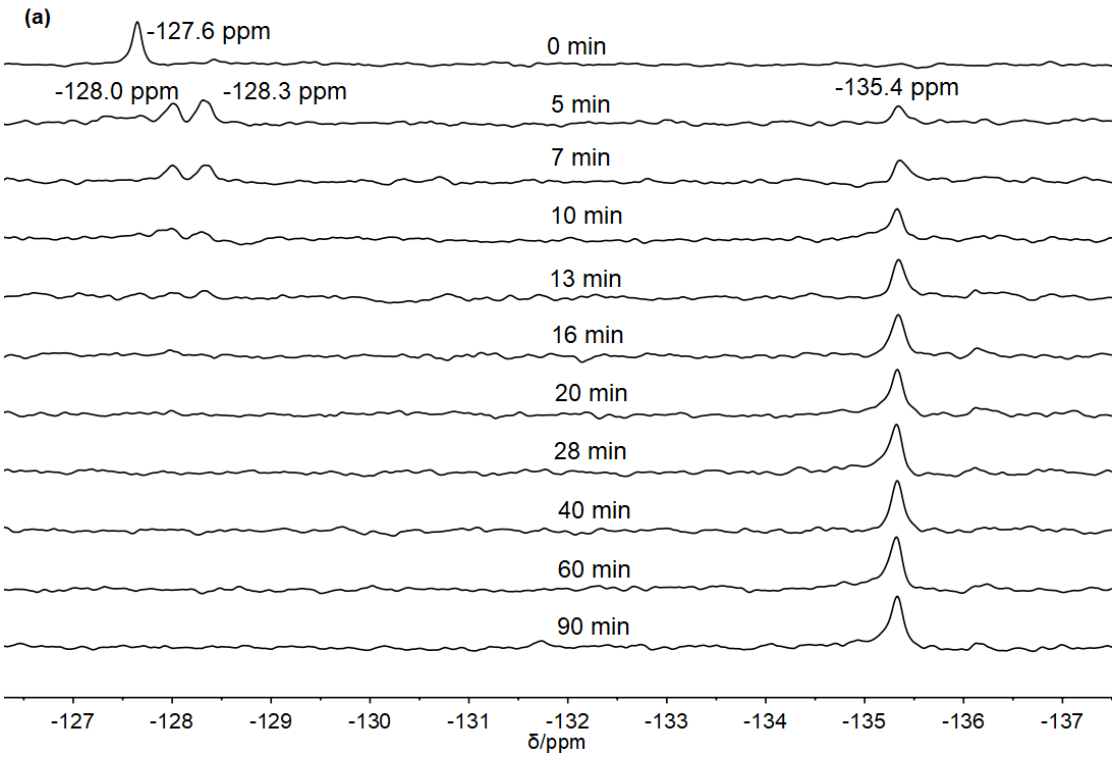
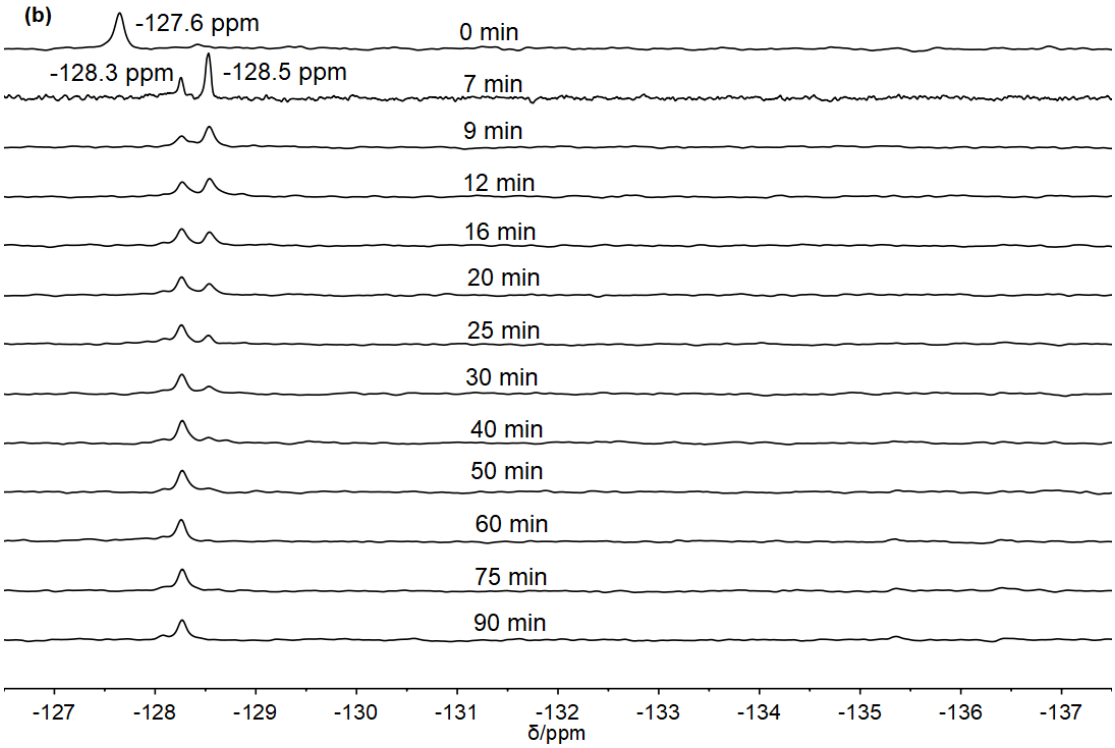


Figure S17. ^{19}F NMR spectrum of probe 1 (200 μM) in 20 mM HEPES buffer solution (pH 7.4) with 30% acetonitrile and 5% D_2O (containing 10% bovine serum) after 90 min.

Probe 1 in response to the individual Cys, Hcy and GSH in bovine serum solution:





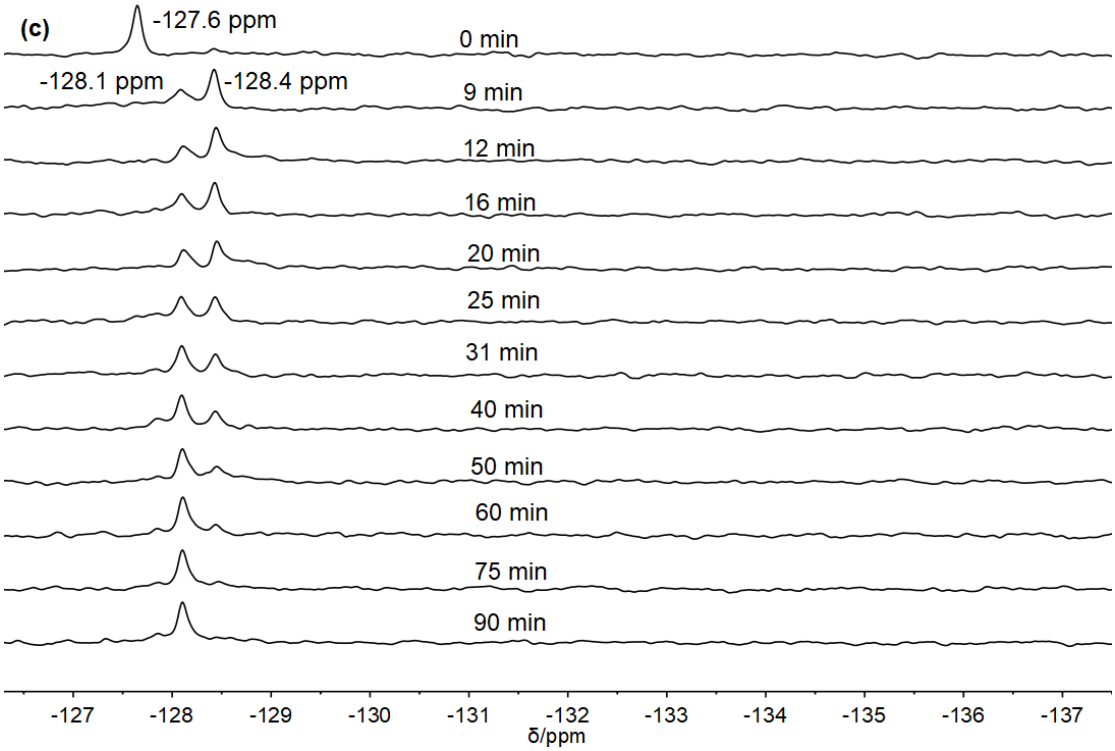


Figure S18. ^{19}F NMR spectra of probe 1 (200 μM) in the presence of (a) 1000 μM Cys, (b) 1000 μM Hcy and (c) 2000 μM GSH in 20 mM HEPES buffer (pH 7.4) solution with 30% acetonitrile and 5% D_2O (containing 10% bovine serum).

Probe 1 in response to the mixture of Cys, Hcy and GSH in bovine serum solution:

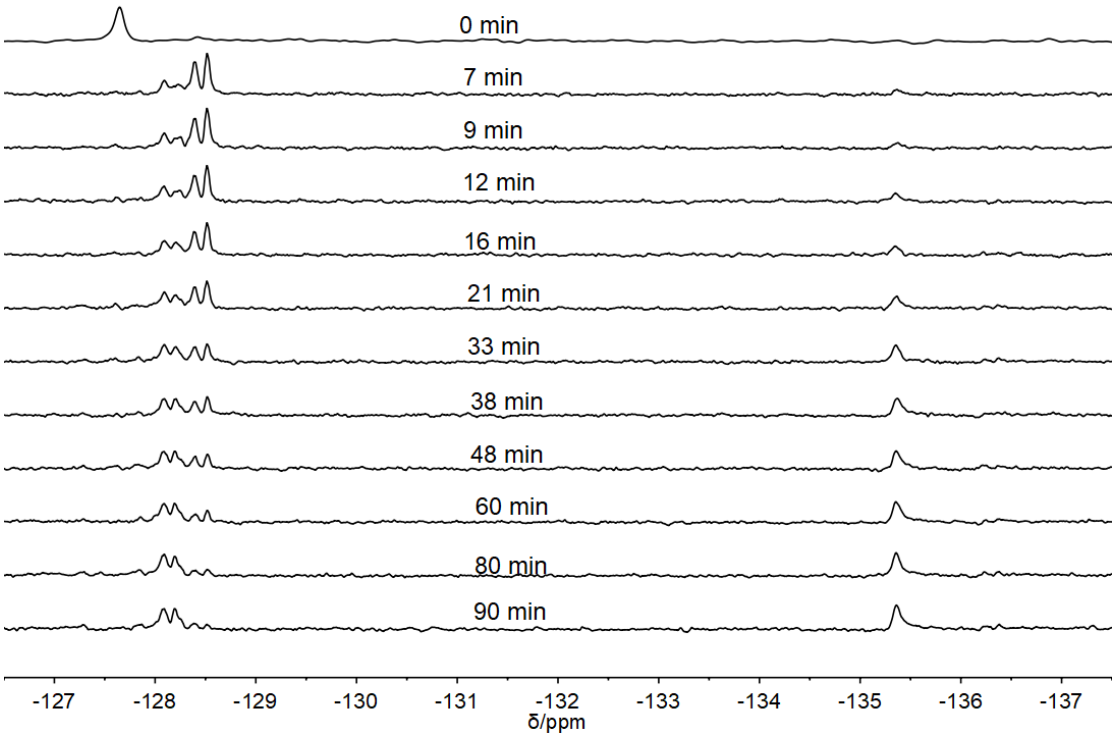


Figure S19. ^{19}F NMR spectra change of probe 1 (600 μM) upon addition of the mixture of Cys (200 μM), Hcy (400 μM) and GSH (400 μM) in 20 mM HEPES buffer (pH 7.4) solution with 30% acetonitrile and 5% D_2O (containing 10% bovine serum) at 25 °C.