Supporting information

A novel fluorescent probe with a large Stokes shift for colorimetric and selective detection of cysteine in water, milk, cucumber, pear and tomato

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1. Experimental section

1.1 Materials

All the chemical reagents for synthesis in this work were of analytical grade without further purification unless otherwise noted. Tetrahydrofuran for spectrum test, 4-Methoxy-2-methylpyridine, benzene sulfonyl chloride, phenyl magnesium bromide, piperidine, malonditrile, 4-hydroxybenzaldehyde, triethylamine, 2,4-dinitrobenzene sulfonyl chloride, acryloyl chloride, Cysteine (Cys), glutathione (GSH), homocysteine (Hcy), glycine (Gly), alanine (Ala), serine (Ser), threonine (Thr), isoleucine (Ile), leucine (Leu), asparagine (Asp), glutamic acid (Glu), arginine (Arg), histidine (His), phenylalanine (Phe), tryptophan (Trp), potassium iodide (KI), sodium carbonate (Na_2CO_3), potassium chloride (KCl), sodium hydrosulfide (NaHS), sodium chloride (NaCl), sodium acetate (CH_3COONa), potassium bromide (KBr), sodium bicarbonate ($NaHCO_3$), sodium Fluoride (NaF) were purchased from Shanghai Aladdin Co., LTD (Shang hai, China). Twice-distilled water was used to prepare all aqueous solutions.

1.2 Instrumentation

NMR spectra were tested by using a Bruker III-400 MHz NMR spectrometer (^1H NMR at 400 MHz, ^{13}C NMR at 101 MHz) using tetramethyl silane (TMS) as an internal standard. High resolution mass spectroscopy (HRMS) was performed using a Bruker-Esquire 3000 spectrometer. Fluorescence spectra were tested by using a Hitachi F-4500 fluorescence spectrometer. Ultraviolet-visible (UV-vis) absorption spectra were tested by using Agilent 8453 spectrometer. all the pH were tested by using a DAPU PHS-3E pH meter, and the pH meter was calibrated with pH 9.18 and pH 6.86 buffer solution before used.

1.3 Preparation of the test solution and spectroscopic measurements

The stock solution of probe **1** and probe **2** (10 μM) were prepared in pure THF. The stock solutions of various analytes (Amino acids: Cys, GSH, Hcy, Gly, Ala, Ser, Thr, Ile, Leu, Asp, Glu, Arg, His, Phe, Trp; Anion: I^-, Br^-, F^-, CO_3^{2-}, HS^-, Ac^-, HCO_3^-; Cation: K^+, Na^+) were prepared in twice-distilled water. UV-vis (ultraviolet-visible) and fluorescence spectra of probes and various analytes were measured in PBS buffer solution (10 mM, pH=7.4, containing 50% THF, v/v). All the solutions were fully shaken and reacted at room temperature for 60 minutes, then the spectrum was recorded.

1.4 Preparation of food samples

Tap water (in glass container, 500 mL) was collected from the running water network of Zhengzhou. Eight commercial samples were purchased from local supermarkets, including one brand of purified water (bottle, 500 mL), one brand of mineral water (bottle, 500 mL); Three different brands of whole milk (cardboard, 240 mL; pasteurized); Fresh cucumber, pear and tomato. Before Cys concentration was analysised, 60 times twice-distilled water was added to the milk samples. Cucumber, pear and tomato were cleared, washed, squeezed, and filtrated to acquire the juice. 30 μL of the solution was taken from the processed samples to determine the content of Cys. Different amounts of Cys (0.01mM, 0.02 mM, 0.03 mM) were add to the sample and record the maximum fluorescence intensity of each sample. Repeat each test 3 times.

2. Characteristic

2.1 Characteristic of compound 2

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67-7.64 (m, 2 H), 7.56-7.52 (m, 1 H), 7.46-7.40 (m, 2 H), 7.38-7.27 (m, 5 H), 5.96-5.94 (m, 1 H), 5.43 (s, 1 H), 3.17-3.04 (m, 2 H), 1.89 (s, 3 H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.8, 171.7, 155.2, 137.8, 135.9, 132.3, 129.0, 128.9, 128.3, 127.9, 126.6, 115.4, 59.9, 42.1, 24.3. HRMS (positive ESI, m/z): $[M + H]^+$ calcd for $C_{19}H_{18}NO_2$ 292.1338, found 292.1334.

2.2 Characteristic of compound 3

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61-7.55 (m, 3H), 7.46-7.32 (m, 5H), 7.21-7.18 (m, 2H), 6.08 (s, 1H), 5.76-5.74 (m, 1H), 3.60-3.23 (m, 2H), 2.06 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 162.4, 153.1, 136.6, 135.1, 132.9, 129.3, 129.2, 128.5, 128.4, 126.1, 113.3, 112.5, 110.5, 75.2, 57.9, 34.4, 24.9. $[M + H]^+$ calcd for $C_{22}H_{18}N_3O$ 340.1444, found 340.1447.

2.3 Characteristic of compound 4

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65-7.60 (m, 2H), 7.44-7.28 (m, 8H), 6.98 (d, J=8.4 Hz, 2H), 6.90(d, J=16.0 Hz, 1H), 6.79(d, J=8.4 Hz, 2H), 6.45 (s, 1H), 6.15(d, J=15.9 Hz, 1H), 6.00-5.98 (m, 1H), 5.83 (s, 1H), 3.68-3.31 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 162.4, 158.1, 151.3, 138.0, 136.6, 135.0, 132.8, 129.6, 129.2, 128.9, 128.7, 128.4, 127.6, 126.3, 122.6, 116.1, 113.7, 113.2, 107.8, 74.6, 56.8, 34.5. $[M + H]^+$ calcd for $C_{29}H_{22}N_3O_2$ 444.1707, found 444.1710.

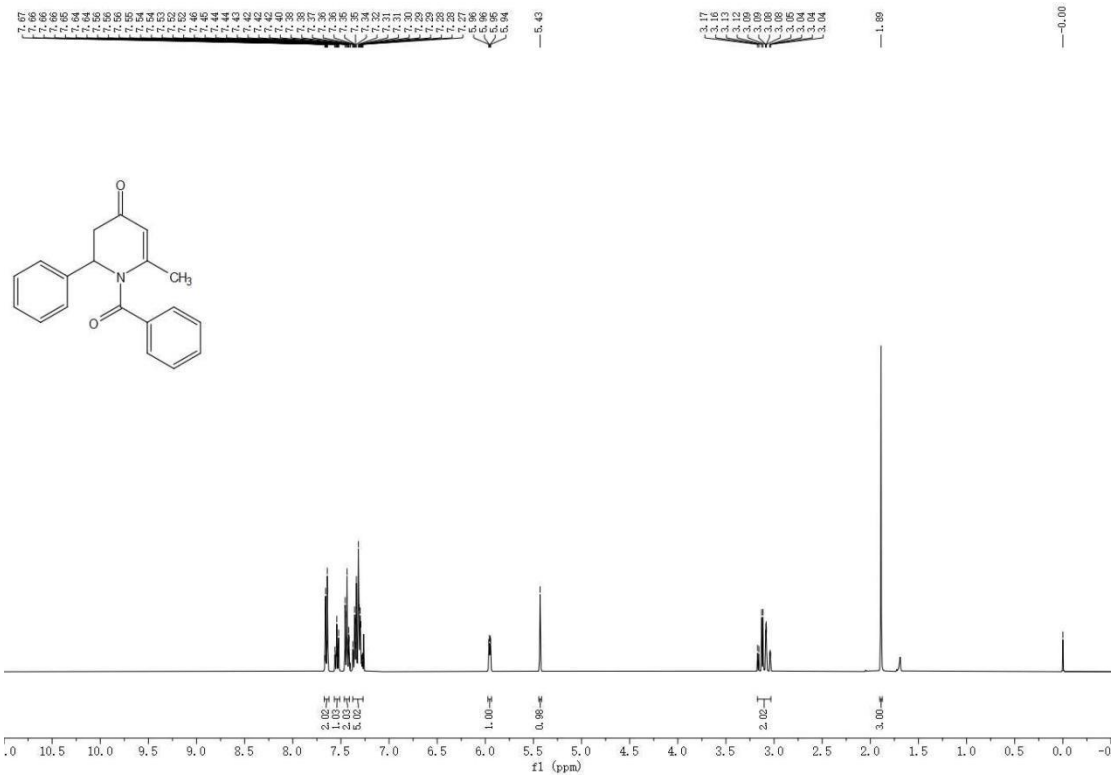
2.4 Characteristic of probe 1

^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.10 (d, *J*= 2.3 Hz, 1H), 8.59 (d, *J*= 8.7 Hz, 1H), 8.22 (d, *J*= 8.7 Hz, 1H), 7.69-7.67 (m, 2H), 7.44-7.28(m, 10H), 7.11-7.08 (m, 2H), 7.02 (s, 1H), 6.84 (d, *J*= 16.2 Hz, 1H), 6.37 (s, 1H), 5.96-5.94 (m, 1H), 3.85-3.54 (m, 2H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 171.8, 164.7, 151.9, 151.6, 149.3, 148.6, 137.8, 136.1, 135.9, 135.7, 134.0, 132.4, 131.1, 130.0, 129.2, 128.9, 128.2, 127.9, 127.8, 126.6, 122.6, 121.6, 114.4, 113.5, 108.3, 73.6, 56.6, 34.42. $[M + H]^+$ calcd for $C_{35}H_{24}N_5O_8S$ 674.1340, found 674.1342.

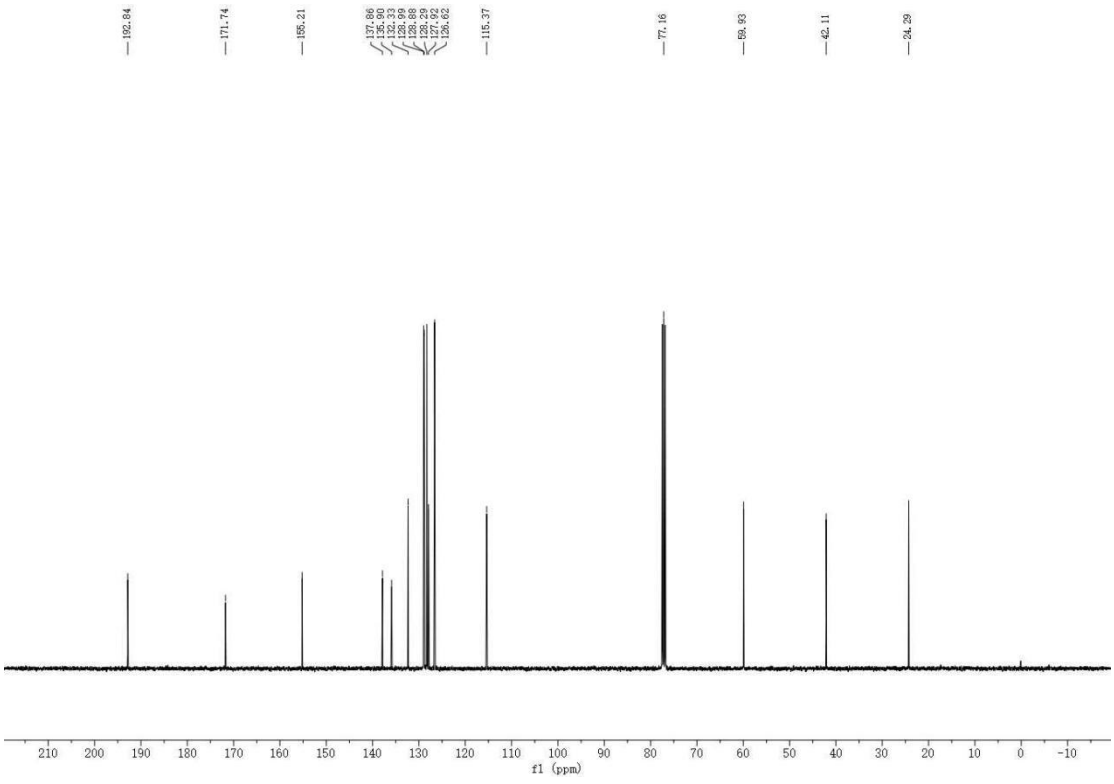
2.5 Characteristic of probe 2

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64-7.61 (m, 2H), 7.45-7.28 (m, 8H), 7.13-7.10 (m, 2H), 7.06-7.03 (m, 2H), 6.93 (d, J=16.0 Hz, 1H), 6.60 (d, J=16.2 Hz, 1H), 6.47 (s, 1H), 6.32-6.23 (m, 2H), 6.04-6.03 (m, 1H), 6.03-6.01 (m, 1H), 3.72-3.32 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.6, 164.3, 162.3, 151.9, 150.5, 136.7, 136.6, 135.0, 133.3, 132.8, 132.6, 129.2, 129.0, 128.8, 128.6, 128.5, 127.7, 126.3, 125.3, 122.2, 113.5, 112.9, 108.3, 75.8, 56.5, 34.4. $[M + H]^+$ calcd for $C_{32}H_{24}N_3O_3$ 498.1812, found 498.1814.

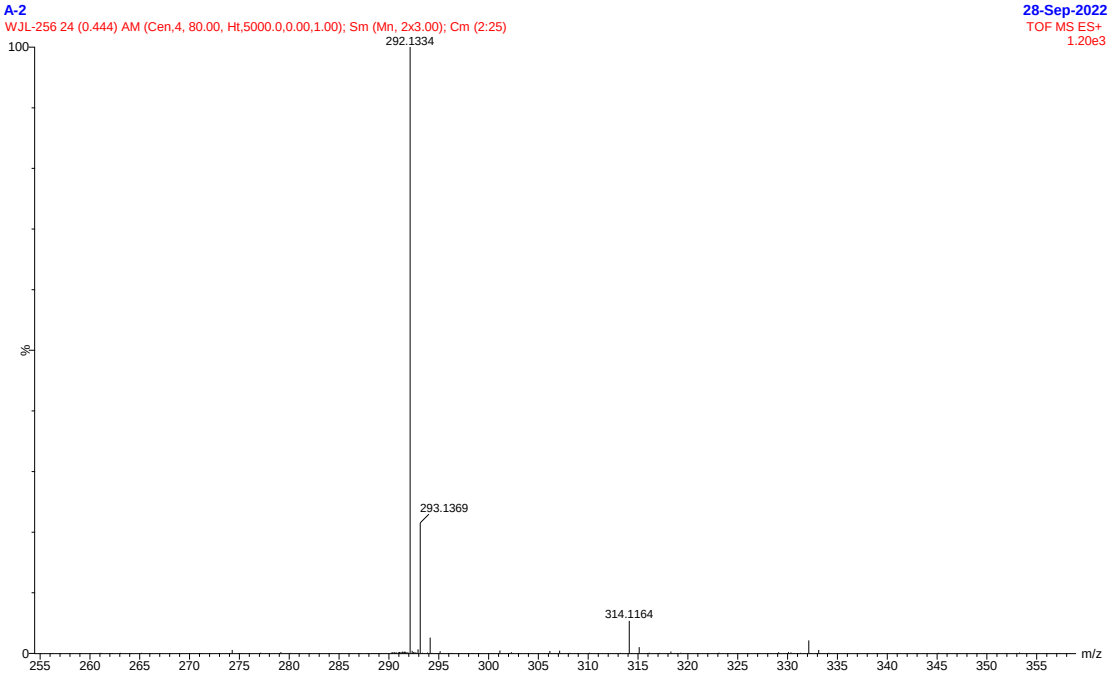
3. NMR and MS spectra



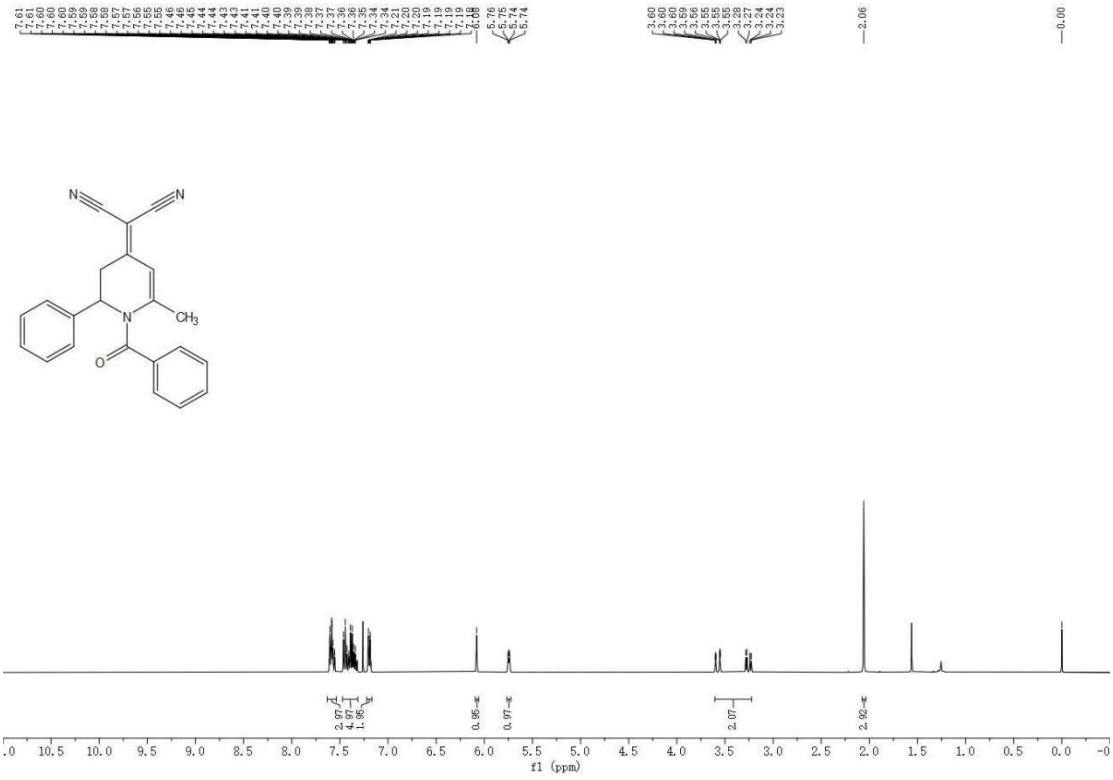
^{1}H NMR spectra of compound **2**.



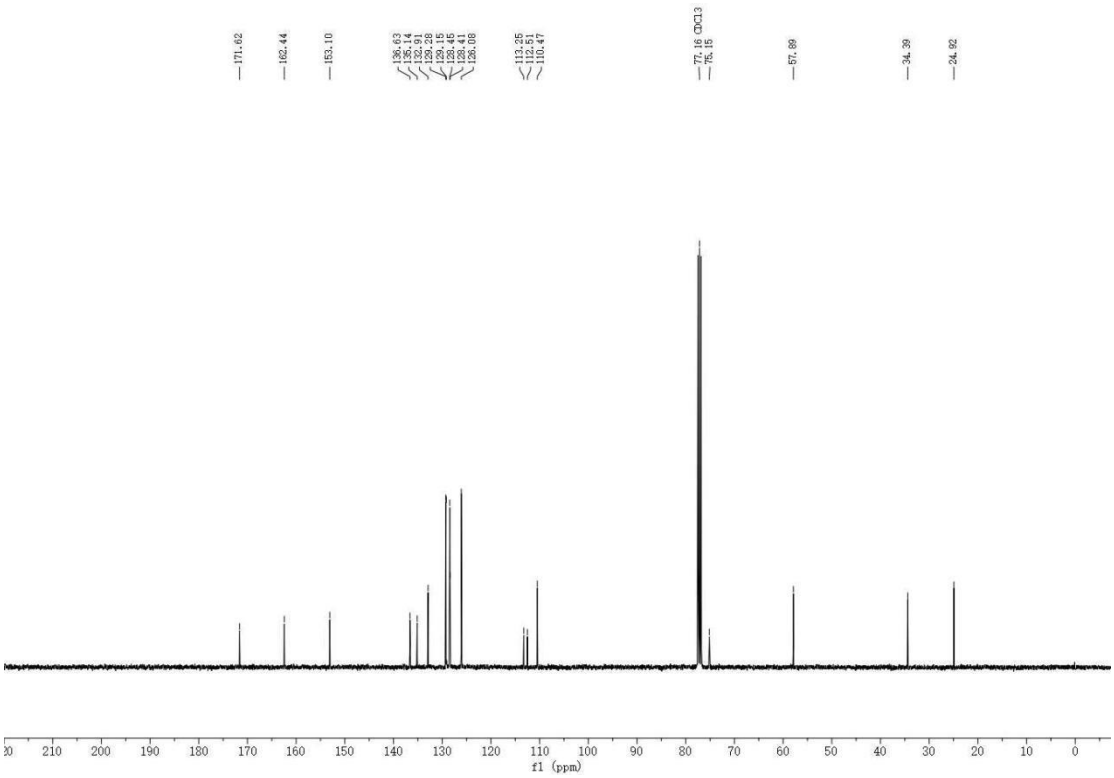
^{13}C NMR spectra of compound **2**.



HRMS of compound **2**.



^{1}H NMR spectra of compound **3**.



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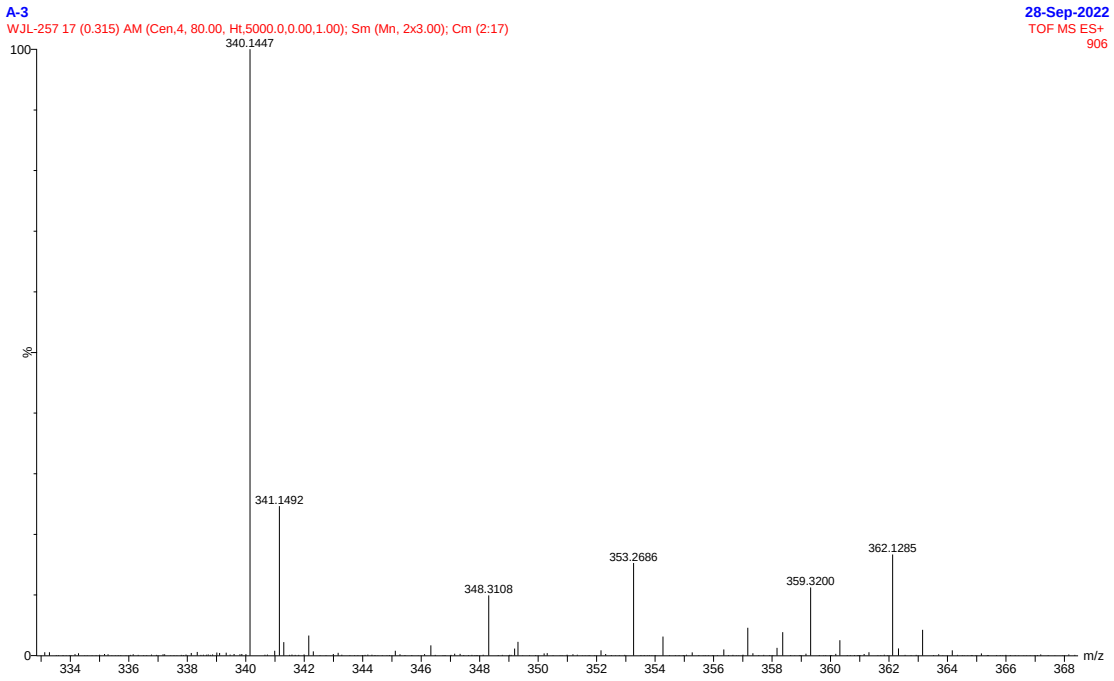

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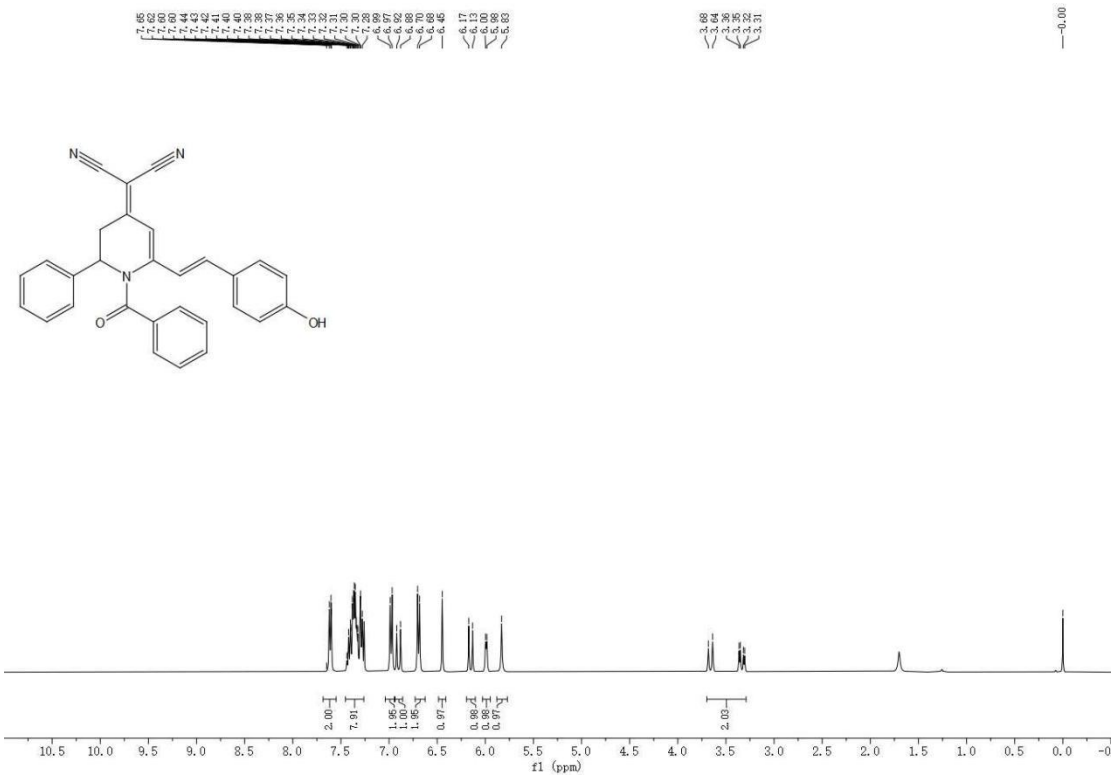
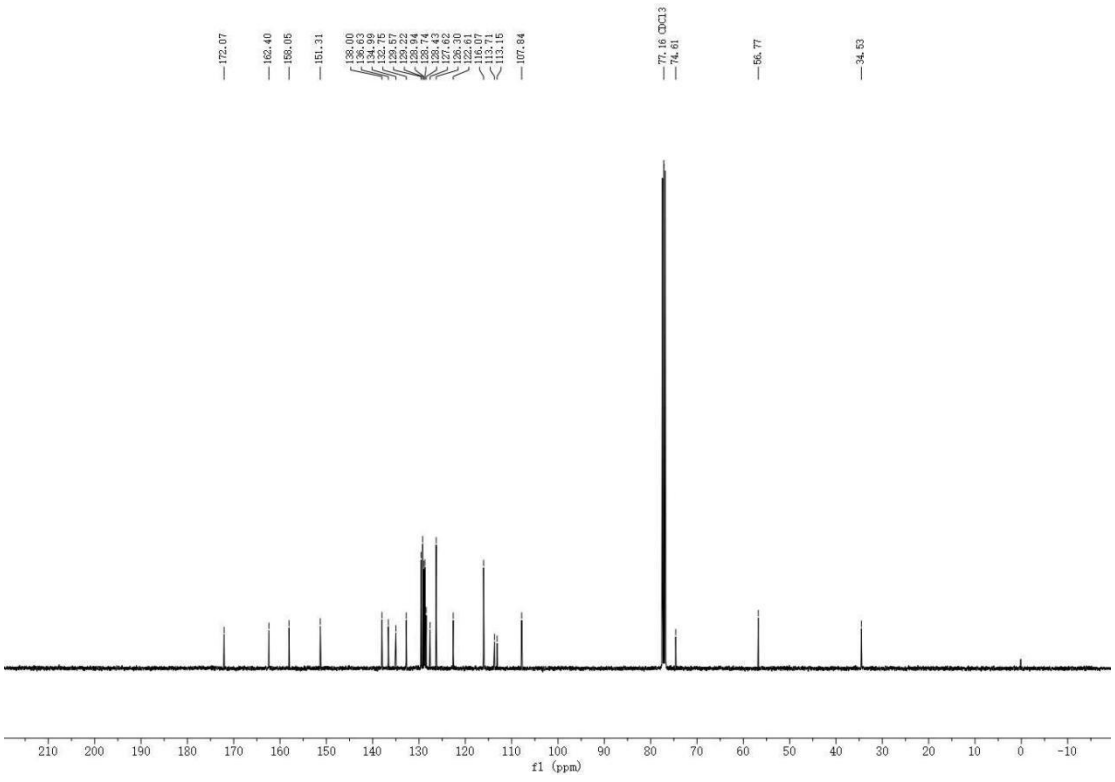
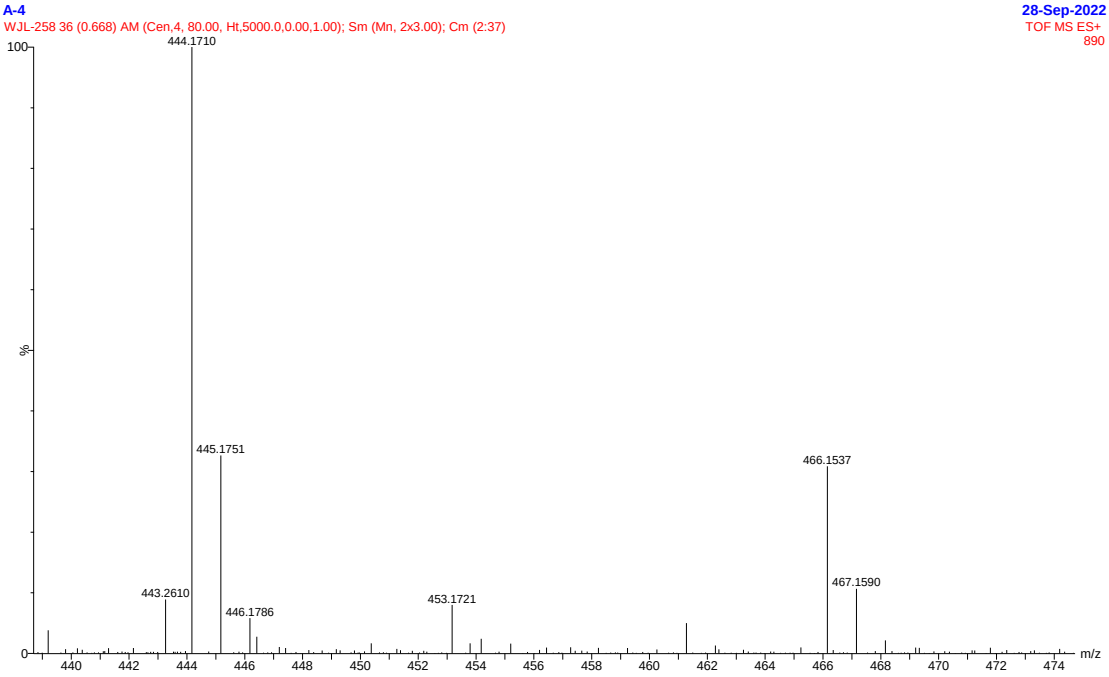


^{1}H NMR spectra of compound **4**.



^{13}C NMR spectra of compound **4**.



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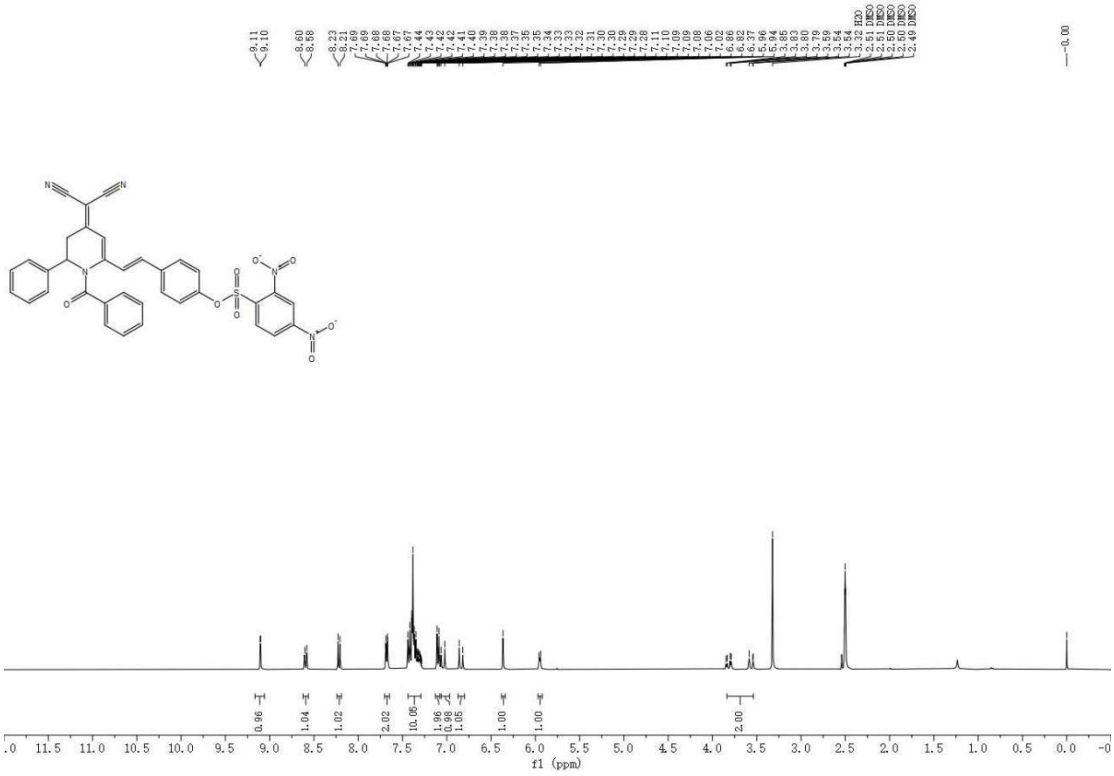
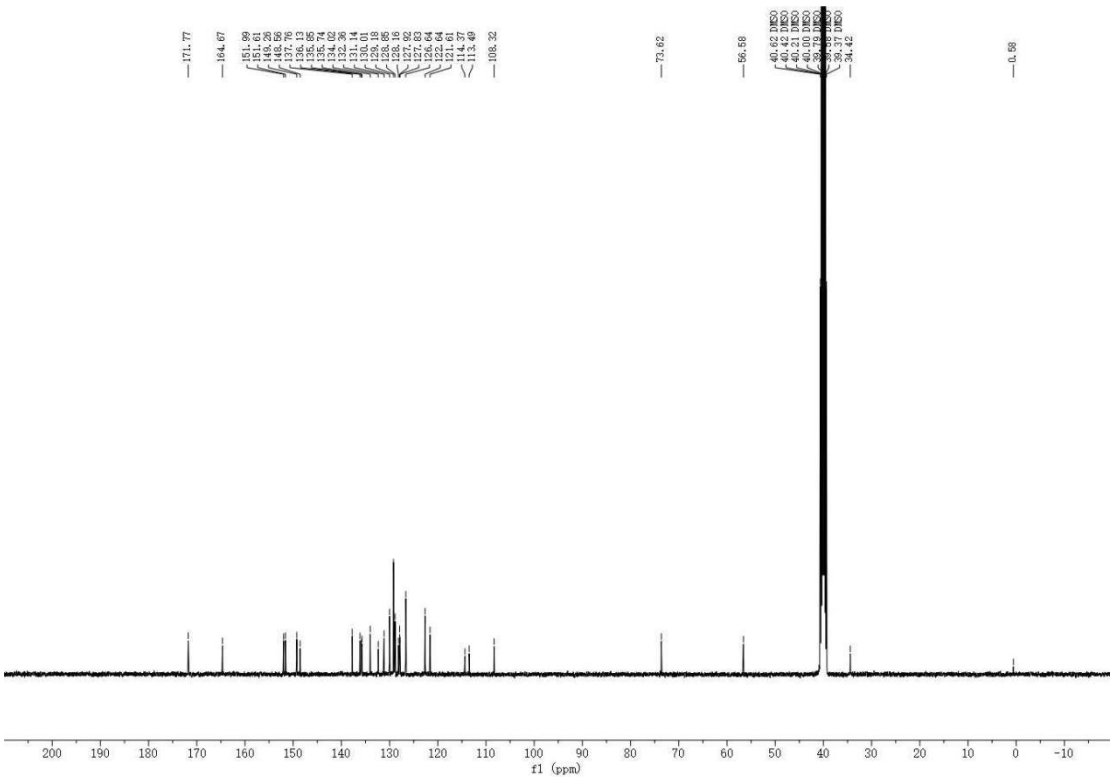
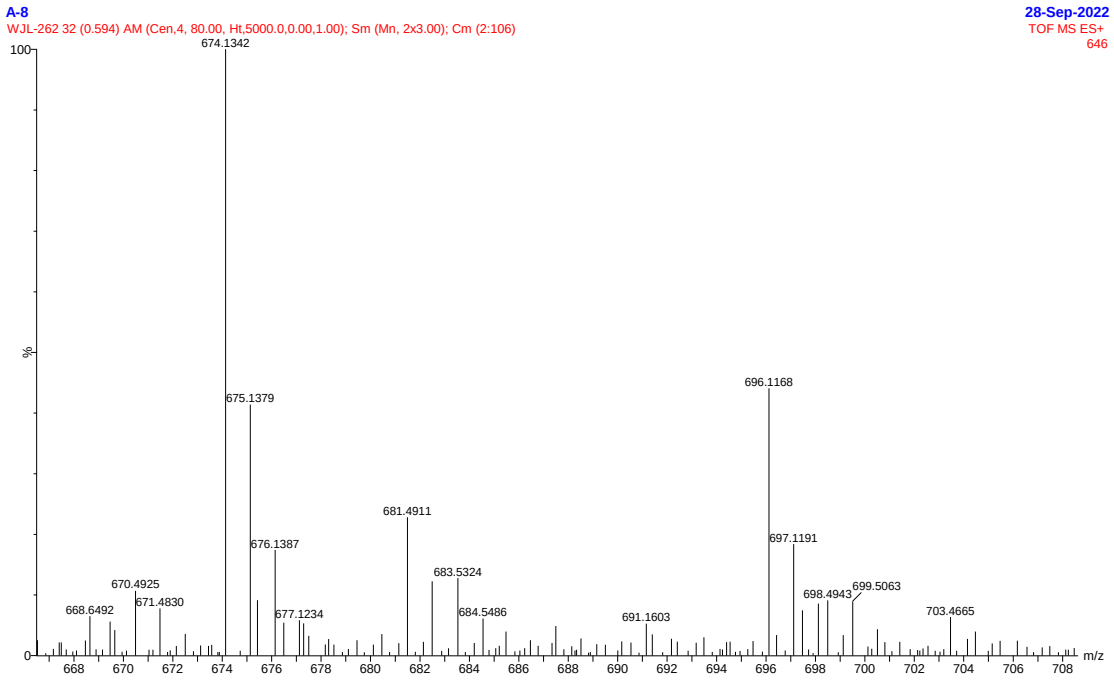


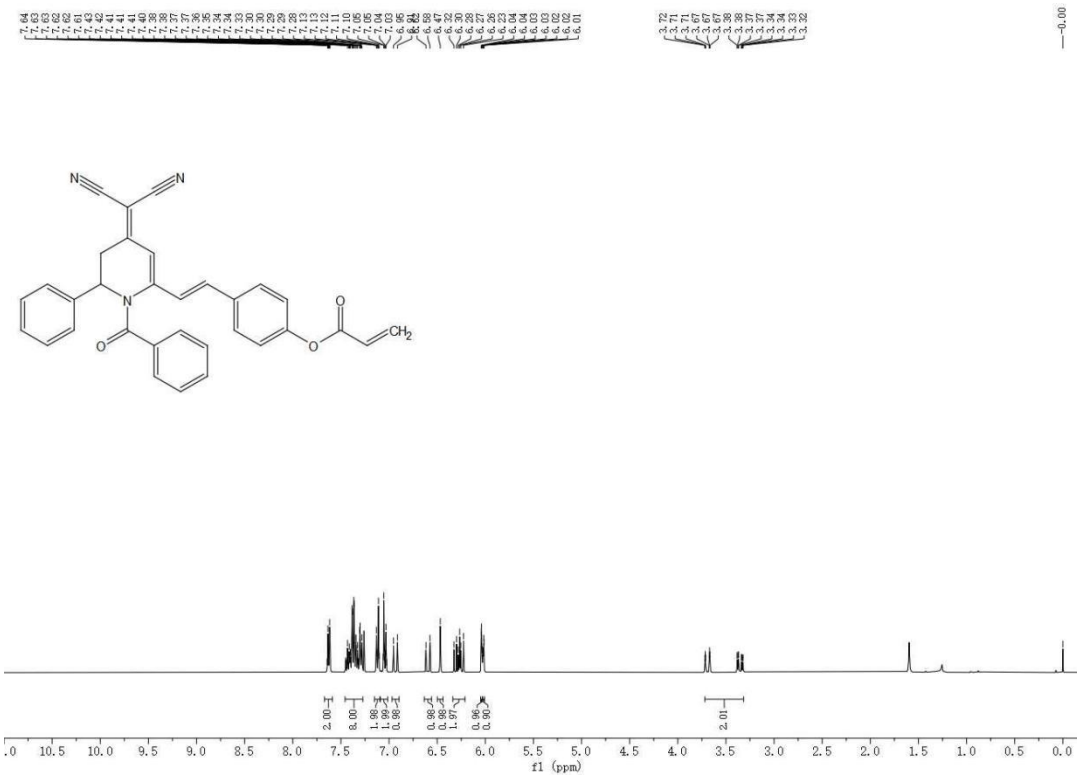
^{1}H NMR spectra of probe **1**.



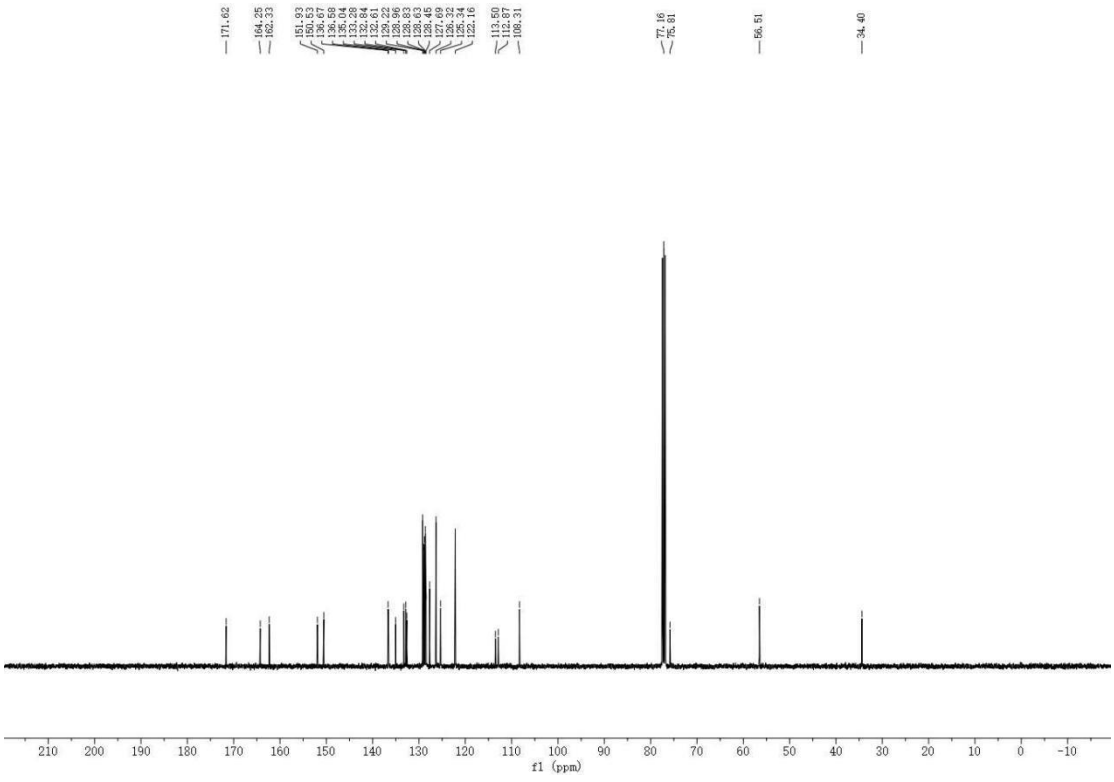
^{13}C NMR spectra of probe **1**.



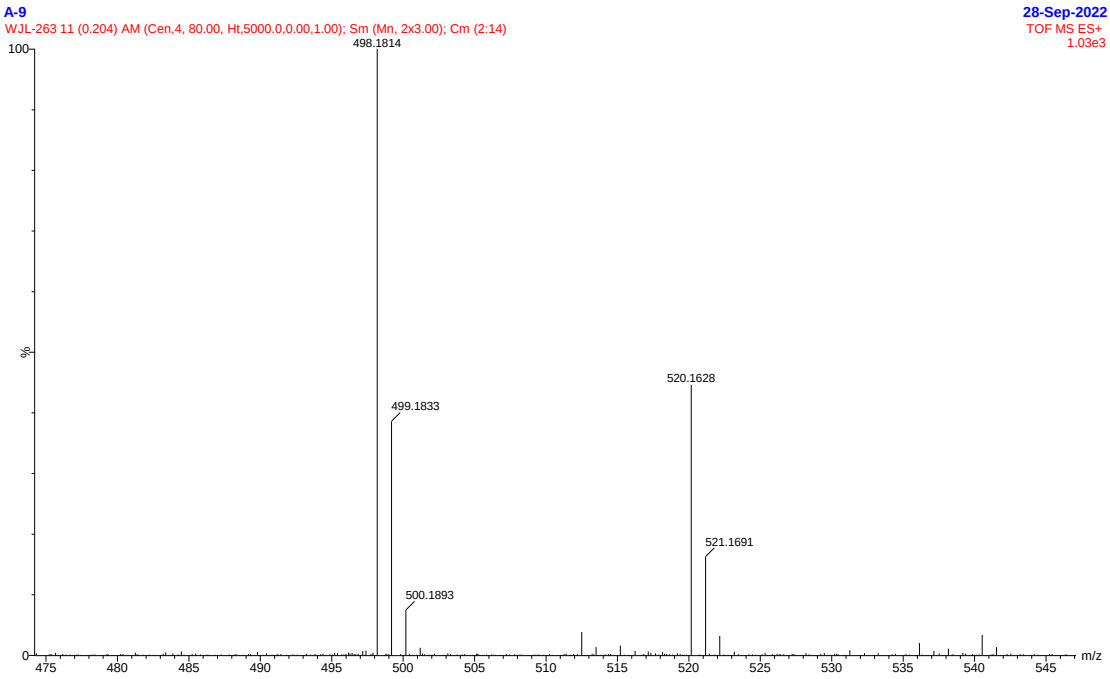
HRMS of probe **1**.



^{1}H NMR spectra of probe **2**.

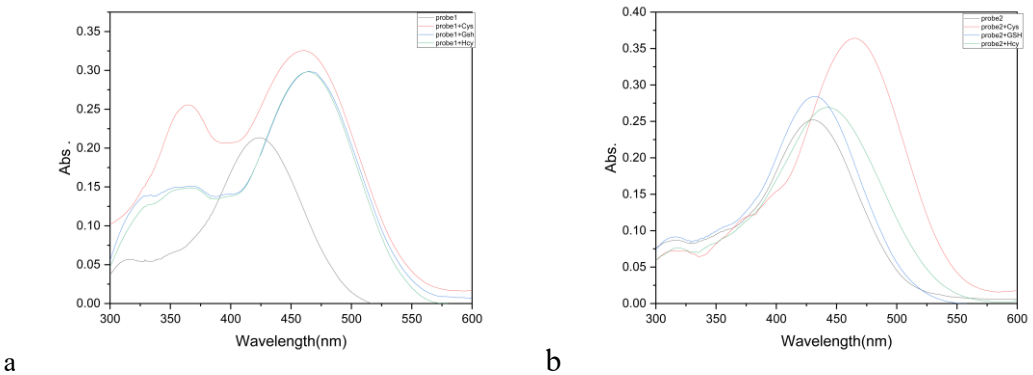


^{13}C NMR spectra of probe **2**.



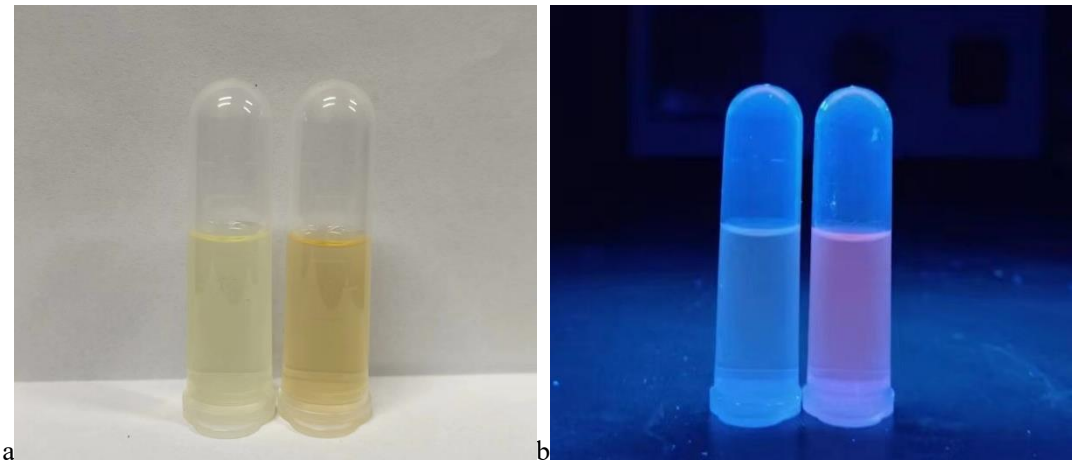
HRMS of probe **2**.

4. Analytical Data



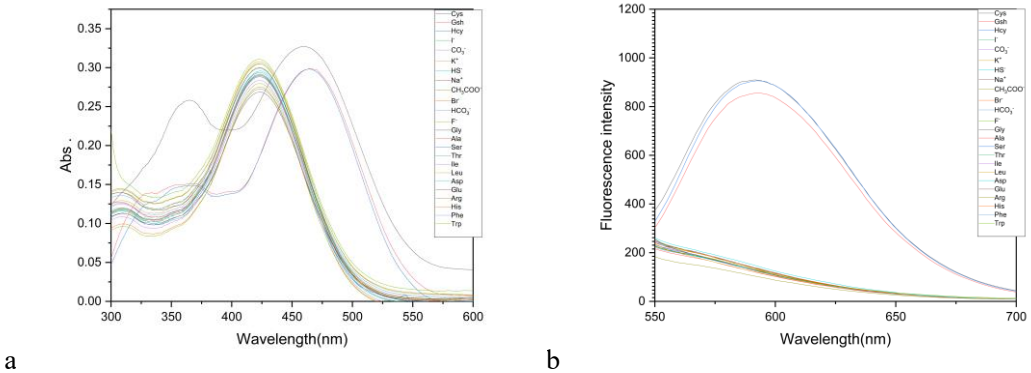
a b

Fig. S1. UV–vis absorption spectra of probe **1** (a) and 2 (b) towards Cys/Hcy and GSH in the PBS buffer (10 mM, pH=7.4, containing 50% THF, v/v).



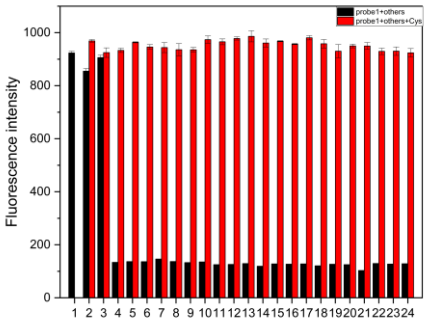
a b

Fig. S2. Photographs observed after the addition of Cys to the solutions of probe **2** (10 μM) under ambient light (a) and under 365 nm uv light (b).

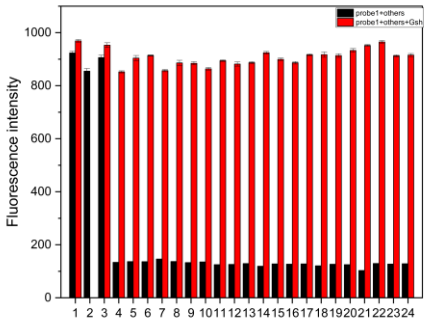


a b

Fig. S3. UV–vis absorption spectra (a) and fluorescence spectra (b) of probe **1** (10 μM) towards various analytes (300 μM) (1-Cys、2-Gsh、3-Hcy、4-I^-、5-CO_3^{2-}、6-K^+、7-HS^-、8-Na^+、9-Ac^-、10-Br^-、11-HCO_3^-、12-F^-、13-Gly、14-Ala、15-Ser、16-Thr、17-Ile、18-Leu、19-Asp、20-Glu、21-Arg、22-His、23-Phe and 24-Trp).



a



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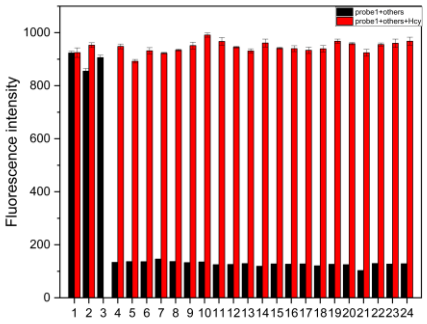
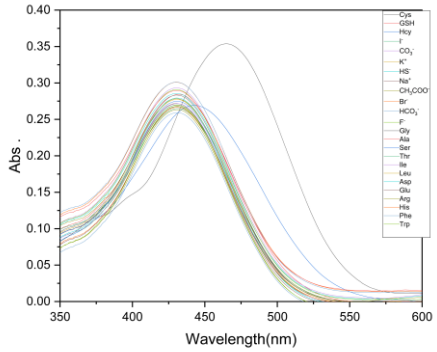


c

Fig. S4. The fluorescence intensity of probe **1** (10 μM) in the presence of different analytes (300 μM). 1-Cys、2-Gsh、3-Hcy、4-I^-、5-CO_3^{2-}、6-K^+、7-HS^-、8-Na^+、9-Ac^-、10-Br^-、11-HCO_3^-、12-F^-、13-Gly、14-Ala、15-Ser、16-Thr、17-Ile、18-Leu、19-Asp、20-Glu、21-Arg、22-His、23-Phe and 24-Trp



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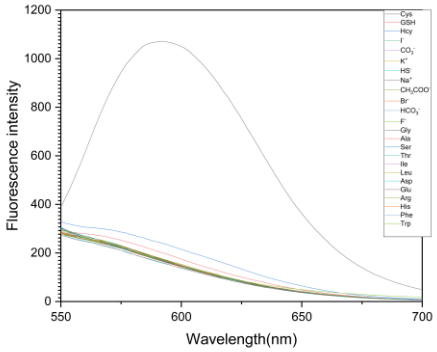
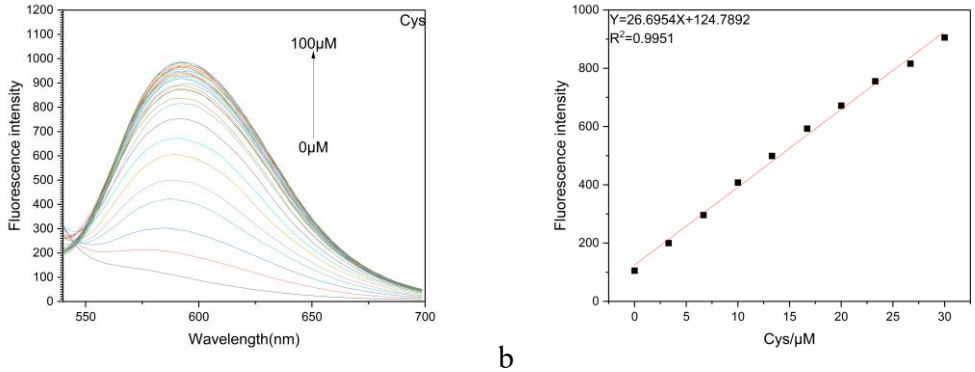


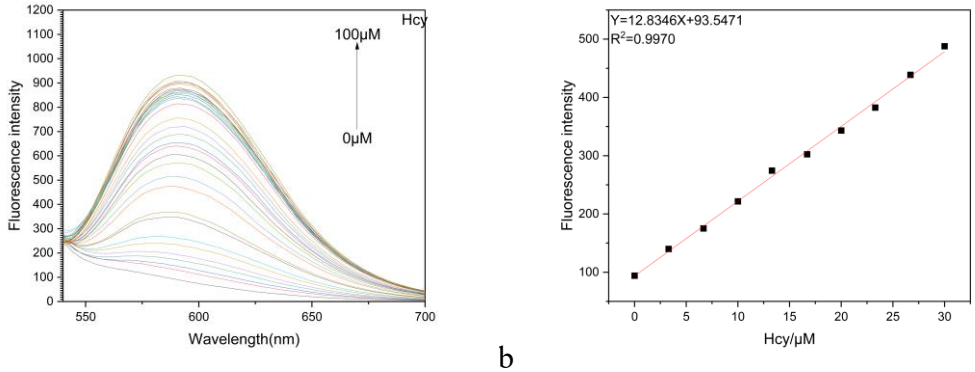
b

Fig. S5. UV–vis absorption spectra (a) and fluorescence spectra (b) of probe **2** (10 μM) towards various analytes (300 μM) (1-Cys、2-Gsh、3-Hcy、4-I^-、5-CO_3^{2-}、6-K^+、7-HS^-、8-Na^+、9-Ac^-、10-Br^-、11-HCO_3^-、12-F^-、13-Gly、14-Ala、15-Ser、16-Thr、17-Ile、18-Leu、19-Asp、20-Glu、21-Arg、22-His、23-Phe and 24-Trp).



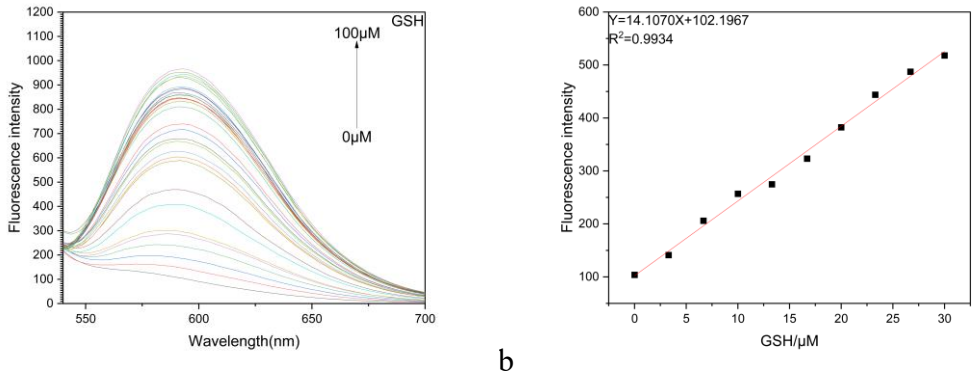
a b

Fig. S6. (a) Fluorescence spectra of probe **1** with Cys (0-100 μM) in the PBS buffer (10 mM, pH=7.4, containing 50% THF, v/v). (b) Linear relationship (R^2 = 0.9951) of the concentrations of Cys and fluorescence intensity. (λex = 490 nm).



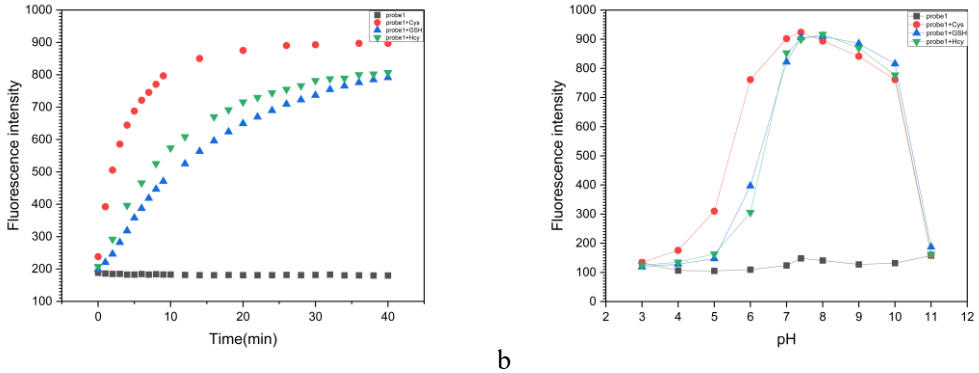
a b

Fig. S7. (a) Fluorescence spectra of probe **1** with Hcy (0-100 μM) in the PBS buffer (10 mM, pH=7.4, containing 50% THF, v/v). (b) Linear relationship (R^2 = 0.9970) of the concentrations of Hcy and fluorescence intensity. (λex = 490 nm).



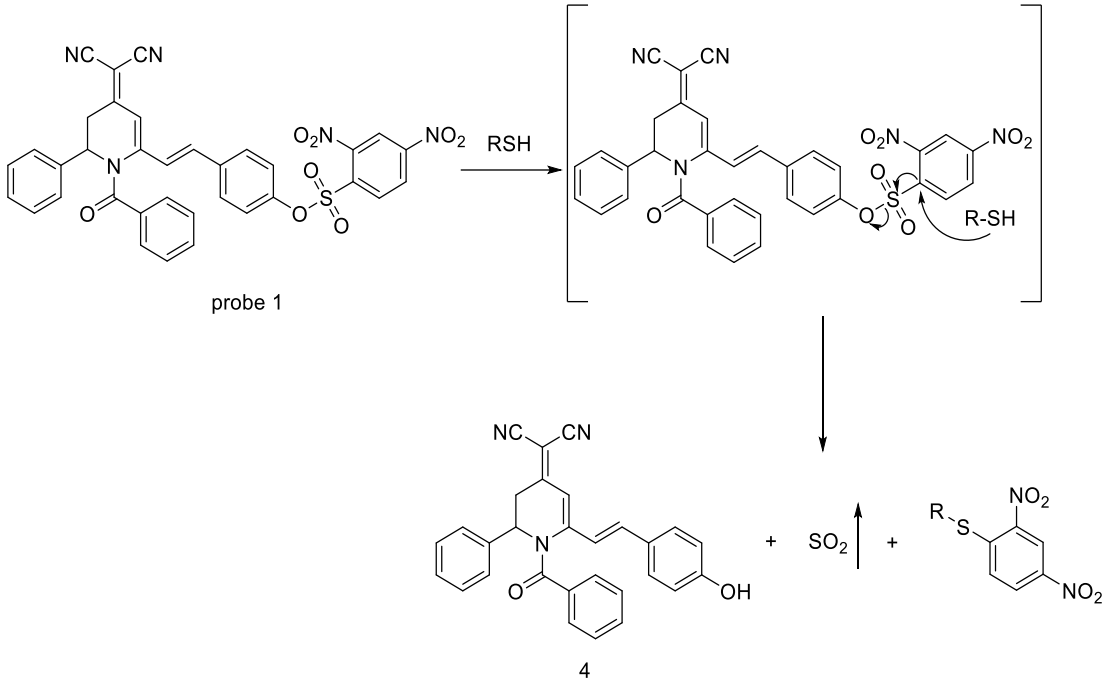
a b

Fig. S8. (a) Fluorescence spectra of probe **1** with GSH (0-100 μM) in the PBS buffer (10 mM, pH=7.4, containing 50% THF, v/v). (b) Linear relationship (R^2 = 0.9934) of the concentrations of GSH and fluorescence intensity. (λex = 490 nm).

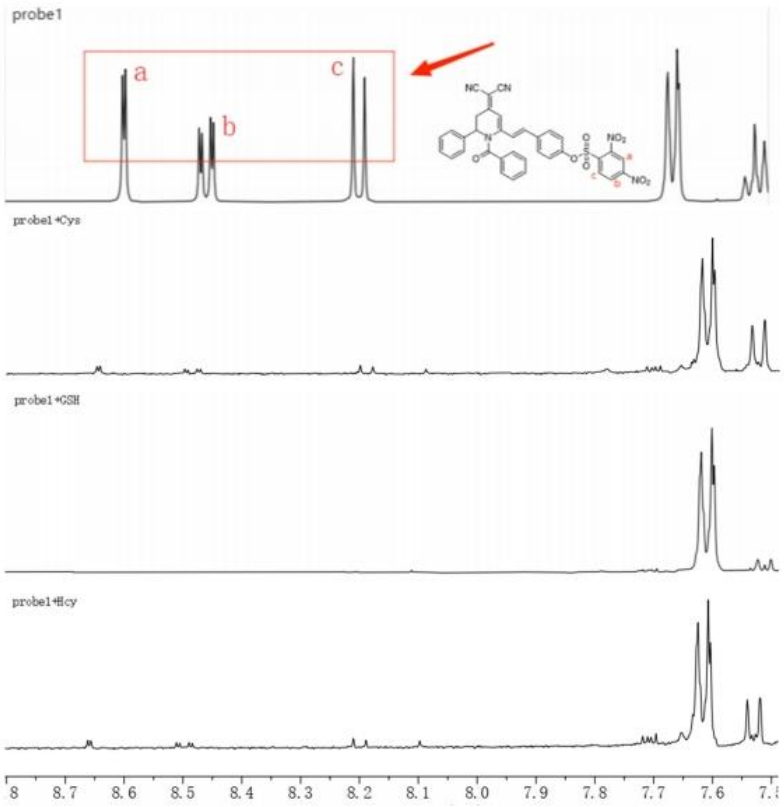


a b

Fig. S9. (a) Fluorescence intensity changes of probe **1** at 590 nm with the times corresponding to the addition of Cys, Hcy and GSH. (b)Fluorescent responses of probe **1** (10 μM) added Cys, Hcy and GSH (100 μM) in different pH buffering solutions (pH 3-11).



Scheme S1 Sensing mechanism of probe **1** toward biothiols



Scheme S2. ^{1}H NMR experiment of probe **1** with Cys, GSH and Hcy in $CDCl_3$

Table S1 Determination of the concentration of Cys in water, milk, cucumber, pear and tomato.

Samples	Cys spiked (mM)	Cys recovered (mM)	Recovery (%)	RSD (%)
Tap water	0	-	-	
	0.0100	0.0095	95%	0.63
	0.0200	0.0194	97%	0.23
	0.0300	0.0291	97%	0.81
Bottled purified water	0	-	-	
	0.0100	0.0106	106%	0.37
	0.0200	0.0212	106%	0.60
	0.0300	0.0292	97%	0.50
Bottled mineral water	0	-	-	
	0.0100	0.0104	104%	0.30
	0.0200	0.0210	105%	1.59
	0.0300	0.0290	97%	0.66
Milk-1	0	-	-	
	0.0100	0.0104	104%	0.43
	0.0200	0.0211	105%	0.23
	0.0300	0.0292	97%	0.32
Milk -2	0	-	-	

	0.0100	0.0105	105%	0.17
	0.0200	0.0205	102%	1.10
	0.0300	0.0293	98%	0.60
Milk -3	0	-	-	
	0.0100	0.0105	105%	1.29
	0.0200	0.0204	102%	0.24
	0.0300	0.0291	97%	0.53
Cucumber	0	0.0025	-	
	0.0100	0.0129	104%	0.49
	0.0200	0.0229	102%	0.23
	0.0300	0.0312	96%	0.19
Pear	0	0.0035	-	
	0.0100	0.0136	101%	0.41
	0.0200	0.0234	99%	0.66
	0.0300	0.0317	94%	0.16
Tomato	0	0.0040	-	
	0.0100	0.0148	108%	0.30
	0.0200	0.0258	109%	0.13
	0.0300	0.0325	95%	0.96

Table S2 Determination of the concentration of Hcy in water, milk, cucumber, pear and tomato.

Samples	Cys spiked (mM)	Cys recovered (mM)	Recovery (%)	RSD (%)
Tap water	0	-	-	
	0.0100	0.0103	103%	0.60
	0.0200	0.0190	95%	0.70
	0.0300	0.0265	88%	1.12
Bottled purified water	0	-	-	
	0.0100	0.0109	109%	2.68
	0.0200	0.0205	102%	0.45
	0.0300	0.0295	98%	0.50
Bottled mineral water	0	-	-	
	0.0100	0.0108	108%	0.79
	0.0200	0.0229	115%	1.15
	0.0300	0.0292	97%	1.04
Milk-1	0	-	-	
	0.0100	0.0108	108%	0.69
	0.0200	0.0222	111%	0.73
	0.0300	0.0302	100%	0.40
Milk -2	0	-	-	
	0.0100	0.0104	104%	0.97
	0.0200	0.0226	113%	2.28
	0.0300	0.0318	106%	1.04

Milk -3	0	-	-	
	0.0100	0.0095	95%	2.13
	0.0200	0.0200	100%	0.87
	0.0300	0.0274	91%	0.52
Cucumber	0	0.0063	-	
	0.0100	0.0173	110%	2.27
	0.0200	0.0272	104%	0.68
	0.0300	0.0353	97%	1.07
Pear	0	0.0072	-	
	0.0100	0.0178	106%	0.88
	0.0200	0.0298	113%	1.23
	0.0300	0.0378	102%	0.61
Tomato	0	0.0085	-	
	0.0100	0.0187	102%	2.29
	0.0200	0.0295	105%	1.16
	0.0300	0.0352	89%	1.81

Table S3 Determination of the concentration of GSH in water, milk, cucumber, pear and tomato.

Samples	Cys spiked (mM)	Cys recovered (mM)	Recovery (%)	RSD (%)
Tap water	0	-	-	
	0.0100	0.0111	111%	1.00
	0.0200	0.0212	106%	0.67
	0.0300	0.0301	100%	0.32
Bottled purified water	0	-	-	
	0.0100	0.0111	111%	0.28
	0.0200	0.0210	105%	1.29
	0.0300	0.0294	98%	1.58
Bottled mineral water	0	-	-	
	0.0100	0.0107	107%	1.34
	0.0200	0.0212	106%	0.12
	0.0300	0.0296	99%	0.56
Milk-1	0	-	-	
	0.0100	0.0111	111%	2.63
	0.0200	0.0216	108%	1.23
	0.0300	0.0298	99%	0.85
Milk -2	0	-	-	
	0.0100	0.0104	104%	1.00
	0.0200	0.0222	111%	1.17
	0.0300	0.0303	101%	0.58
Milk -3	0	-	-	
	0.0100	0.0108	108%	0.93
	0.0200	0.0212	106%	0.84

	0.0300	0.0305	102%	0.94
Cucumber	0	0.0050	-	
	0.0100	0.0158	108%	1.53
	0.0200	0.0248	99%	0.47
	0.0300	0.0322	91%	0.58
Pear	0	0.0066	-	
	0.0100	0.0090	90%	1.13
	0.0200	0.0240	87%	0.67
	0.0300	0.0345	93%	0.76
Tomato	0	0.0066	-	
	0.0100	0.0176	110%	1.32
	0.0200	0.0270	102%	1.07
	0.0300	0.0355	96%	0.42