Supporting information for

A new fluorescent probe with high selectivity and sensitivity for Cys detection in bovine serum

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Content

1. Experimental

1.1 Synthesis process of the BTAB

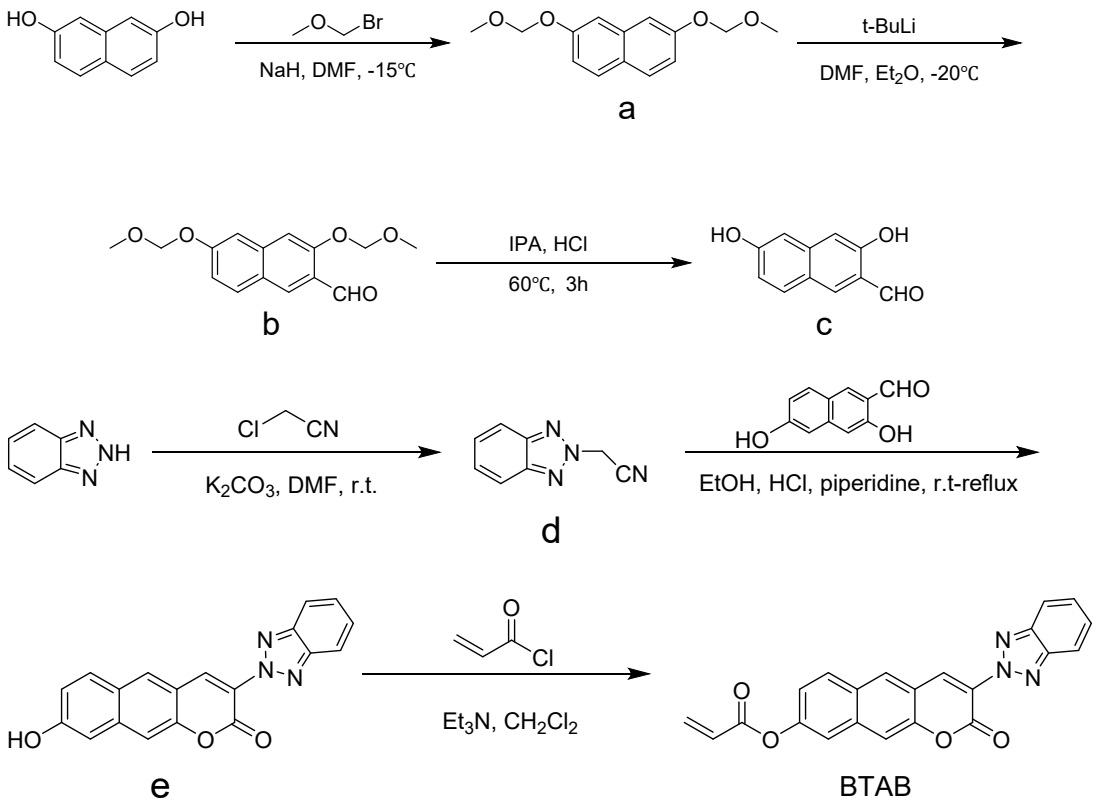


Figure S1 Synthesis of the BTAB

Synthesis of compound a

The compound 2,7-dihydroxynaphthalene (1.0 g, 6.25 mmol, 1.0 equiv.) was dissolved in a solution of anhydrous DMF (10 mL) under an argon atmosphere. NaH (60 %, 299 mg, 7.49 mmol, 1.2 equiv.) was subsequently added to the reaction mixture at -15 degrees Celsius and stirred until the evolved hydrogen gas was completely discharged. Then, bromo-methyl methyl (780.1 mg, 6.25 mmol, 1.0 equiv.) ether was introduced into the system at -15 degrees Celsius and allowed to react at room temperature for 6 hours. After the reaction, EA was used for extraction and PE/EA=10:1 column chromatography to

obtain a (1.41 g, 5.68 mmol, 91%).[1]H NMR (400 MHz, CDCl3) δ 7.70 (d, J = 8.8 Hz, 2H), 7.34 (s, 2H), 7.11 (d, J = 8.8 Hz, 2H), 5.29 (s, 4H), 3.53 (s, 6H).

Synthesis of compound b

The compound **a** (2.0 g, 8.06 mmol, 1.0 equiv.) was dissolved in anhydrous ether (45 mL) under an argon atmosphere. Once completely dissolved, tert-butyllithium (1.3 M, 9.25 mL, 1.5 equiv.) was gradually added to the system at a temperature of -20 degrees Celsius while stirring for a duration of 2 hours and 30 minutes. Subsequently, anhydrous DMF (21 mL) was added at -20 degrees Celsius and continued to stir for another hour. Following this step, a solution of 4M HCl (10 mL) was vigorously stirred into the reaction system for a period of 30 minutes. The resulting mixture was then extracted using EA and separated by column chromatography with a ratio of PE/EA=10:1 yielding the final product mass (1.78 g, 6.44 mmol, 80%).[1]H NMR (400 MHz, CDCl3) δ 8.31 (s, 1H), 7.80 (d, J = 8.9 Hz, 1H), 7.38 (s, 1H), 7.30 (s, 1H), 7.11 (d, J = 9.0 Hz, 1H), 5.39 (s, 2H), 5.30 (s, 2H), 3.56 (s, 3H), 3.52 (s, 3H).

Synthesis of compound c

Compound **b** (1.0 g, 3.62 mmol, 1.0 equiv.) was dissolved in 40 mL of isopropyl alcohol and subsequently treated with 20 mL of 5M hydrochloric acid at a reaction temperature of 60 °C for a duration of three hours. The resulting mixture was subjected to extraction using EA followed by separation via column chromatography (PE/EA=5:1) to yield the final product c (638 mg, 3.39 mmol, 92%). [1]H NMR (400 MHz, DMSO) δ 10.67 (s, 1H), 10.25 (s, 1H), 10.07 (s, 1H), 8.18 (s, 1H), 7.73 (d, *J* = 8.9 Hz, 1H), 7.51 (s, 1H), 7.19 (d, *J* = 8.9 Hz, 1H), 4.52 (s, 1H).

Synthesis of compound d

The benzotriazole (1 g, 8.39 mmol, 1.0 equiv.) was dissolved in a DMF solution, followed by the addition of K_2CO_3 (2.32 g, 16.8 mmol, 2.0 equiv.) and chloroacetonitrile (697.2 mg, 9.23 mmol, 1.1 equiv.). The resulting solution was stirred at room temperature overnight. Product **d** (1.08 g, 6.88 mmol, 82%) was obtained. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 – 7.80 (m, 2H), 7.49 – 7.36 (m, 2H), 5.66 (s, 2H).

Synthesis of compound e

Compound **c** (500 mg, 2.66 mmol, 1.0 equiv.) and compound **d** (420 mg, 2.66 mmol, 1.0 equiv.) were dissolved in anhydrous ethanol (5 mL) and stirred at room temperature until fully dissolved. Two drops of piperidine were subsequently added to the reaction mixture which was then allowed to react overnight at room temperature. The resulting precipitate was filtered off from the reaction system followed by stirring with 6M hydrochloric acid (6 mL) under reflux conditions overnight. The resulting mixture was extracted using EA and further purified through column chromatography to yield product **e** (438 mg, 1.33 mmol, 50%).^{1}H NMR (400 MHz, DMSO) δ 11.34 (s, 1H), 8.82 (s, 1H), 8.36 (s, 1H), 8.30 (s, 1H), 8.10 – 8.00 (m, 2H), 7.92 (d, *J* = 8.7 Hz, 1H), 7.62 – 7.53 (m, 2H), 7.62 – 7.54 (m, 2H), 7.50 (d, *J* = 8.7 Hz, 1H), 7.50 (d, *J* = 8.7 Hz, 1H), 4.77 (s, 1H), 4.77 (s, 1H). ^{13}C NMR (101 MHz, DMSO) δ 155.96 (s), 155.42 (s), 150.17 (s), 144.80 (s), 140.31 (s), 136.77 (s), 131.81 (s), 130.07 (s), 128.35 (s), 126.21 (s), 125.77 (s), 119.38 (s), 118.68 (s), 115.04 (s), 108.84 (s).

Synthesis of the BTAB

The compound **e** (200 mg, 0.61 mmol, 1.0 equiv.) was dissolved in 10 mL of anhydrous dichloride. Subsequently, the system was supplemented with 5 drops of triethylamine and acrylyl chloride (82.5 mg,

0.91 mmol, 1.5 equiv.), followed by overnight reaction at room temperature. The solvent in the reaction mixture was then removed and purified using column chromatography with a PE/EA ratio of 5:1. This process yielded the resulting probe **BTAB** (151.3 mg, 0.4 mmol, 65%).^{1}H NMR (400 MHz, DMSO) δ 9.06 (s, 1H), 8.69 (d, *J* = 8.1 Hz, 1H), 8.29 (d, *J* = 8.9 Hz, 1H), 8.15 (s, 4H), 8.01 (s, 1H), 7.64 (t, *J* = 10.2 Hz, 3H), 7.55 (d, *J* = 9.0 Hz, 1H), 6.62 (dt, *J* = 17.3, 13.8 Hz, 3H), 6.31 (d, *J* = 10.2 Hz, 1H).^{13}C NMR (101 MHz, DMSO) δ 164.59 (s), 150.81 (s), 150.27 (s), 144.93 (s), 140.11 (s), 135.97 (s), 134.75 (s), 131.39 (s), 131.16 (s), 128.51 (d, *J* = 8.5 Hz), 127.96 (s), 122.62 (s), 118.82 (d, *J* = 9.3 Hz), 112.61 (s), 55.42 (s), 29.51 (s).

2. Characterization of organic molecules

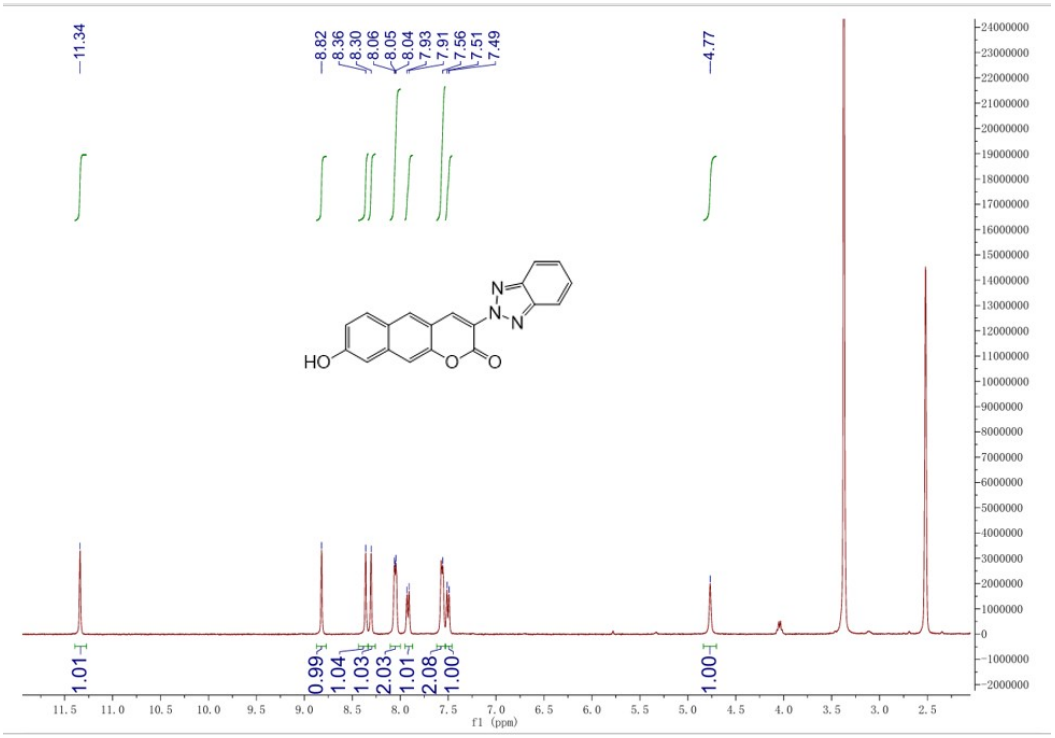


Figure S2 [1]H NMR spectrum of the compound e

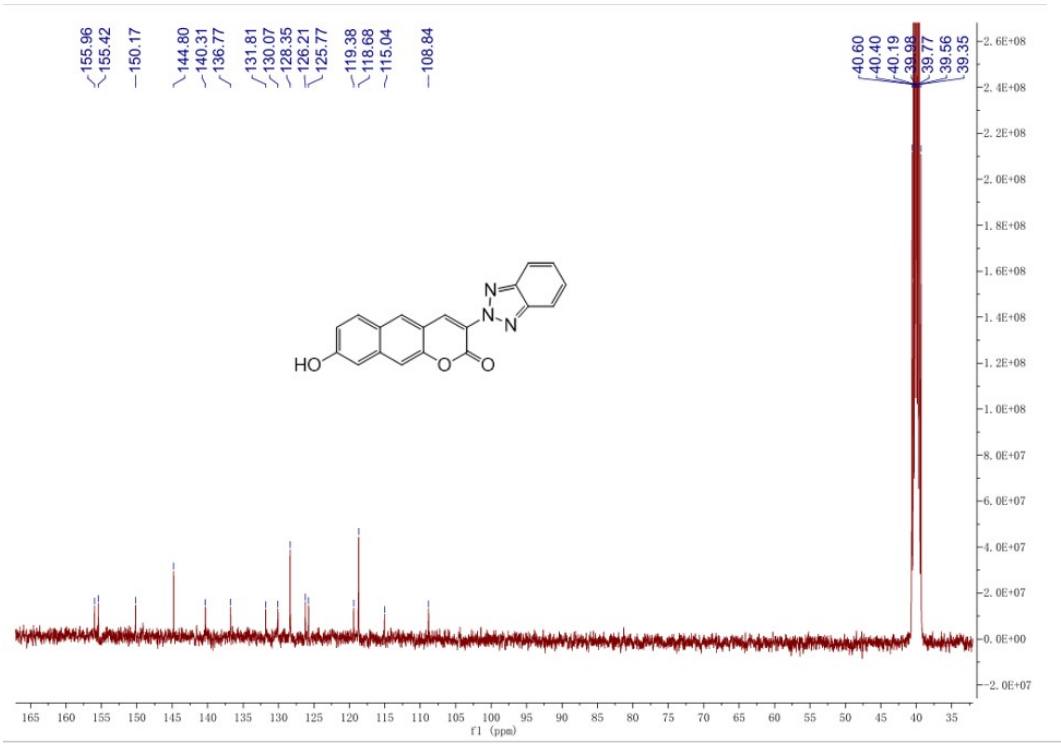


Figure S3 [13]C NMR spectrum of the compound e

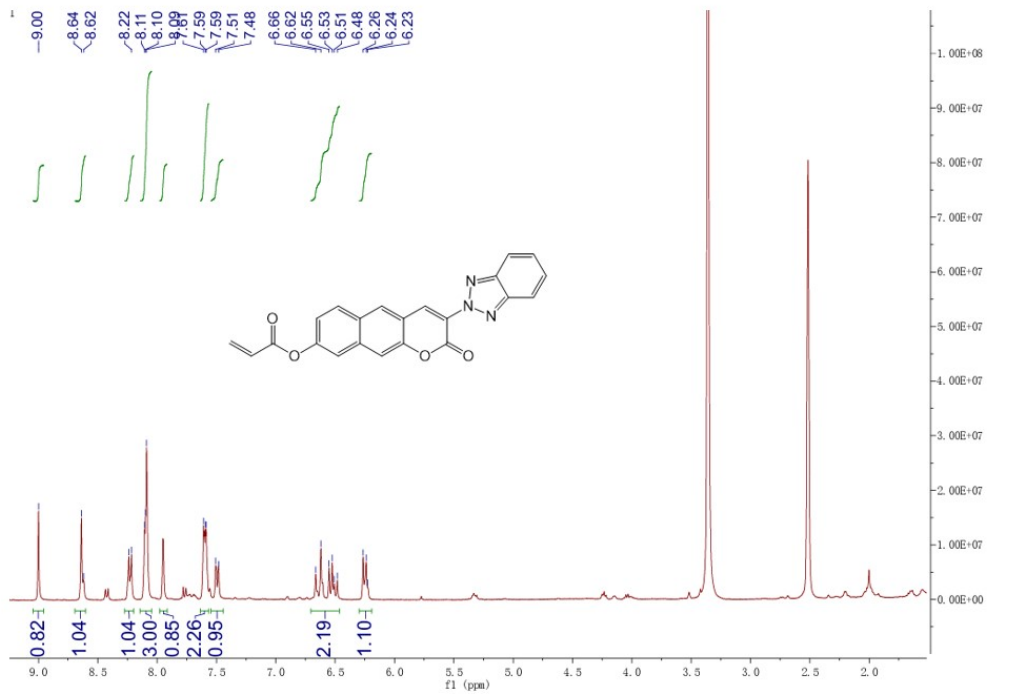


Figure S4 [1]H NMR spectrum of the compound BTAB

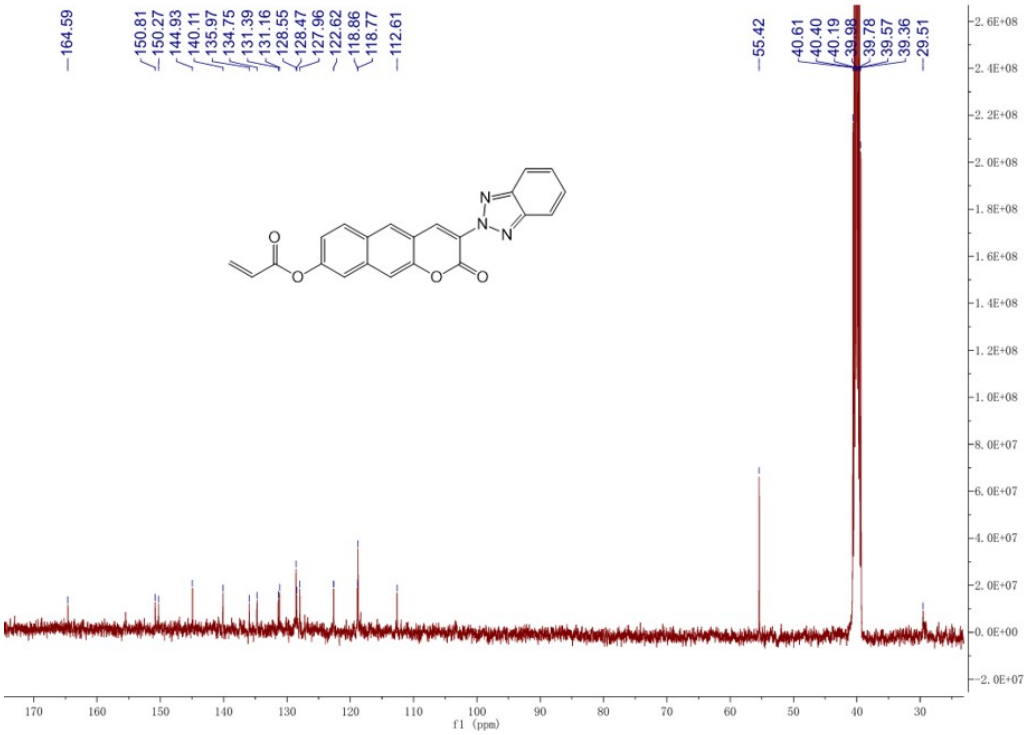
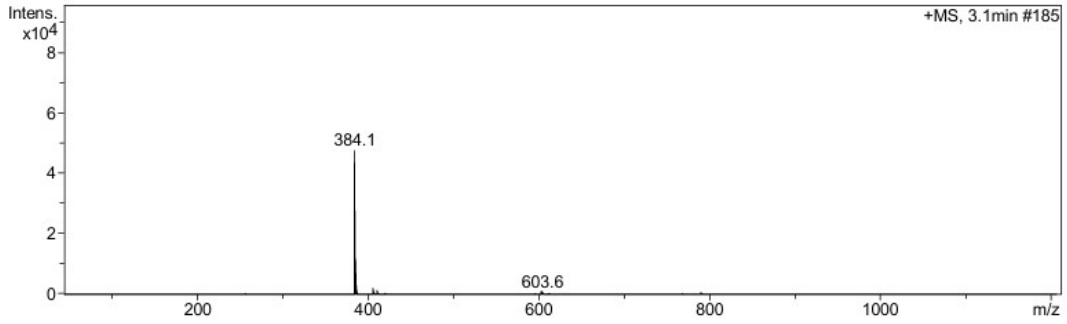


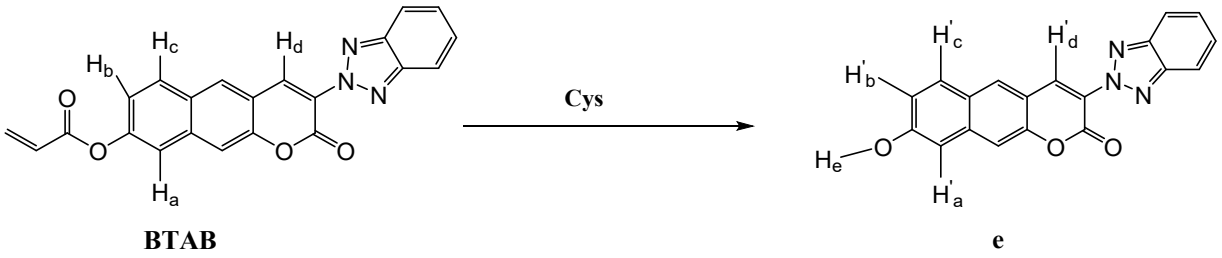
Figure S5 [13]C NMR spectrum of the compound BTAB



Meas. m/z	#	Formula	m/z	err [ppm]	Mean err [ppm]	rdb	N-Rule	e⁻ Conf	mSigma	Std I	Std Mean m/z	Std I VarNorm	Std m/z Diff	Std Comb Dev
384.1	1	C 22 H 14 N 3 O 4	384.1	3.0	3.4	17.5	ok	even	1.94	0.0031	0.0014	0.0013	0.0005	0.8427

Figure S6 The HRMS of BTAB

3. Mechanism of the BTBA detection



BTAB **e**

Figure S7 Reaction of BTAB with Cys

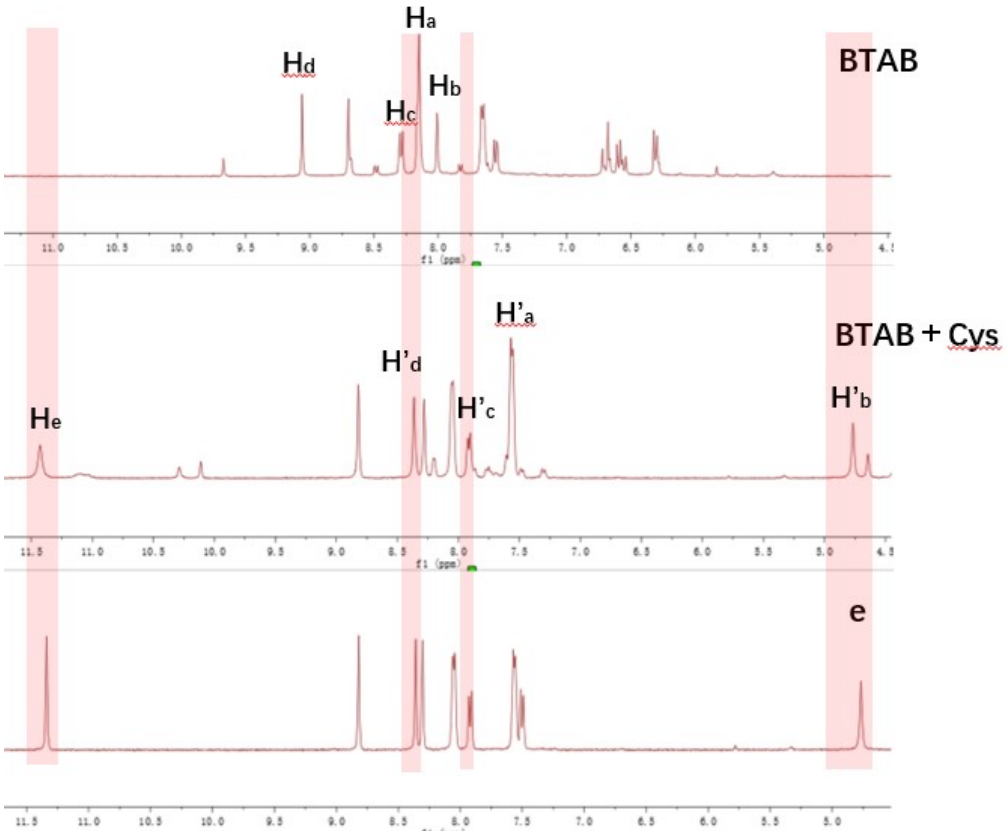
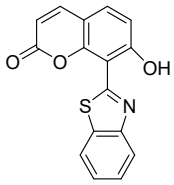
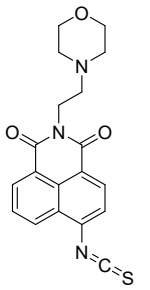
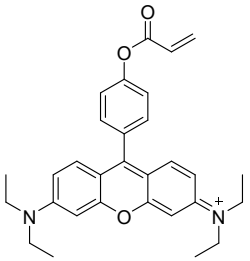
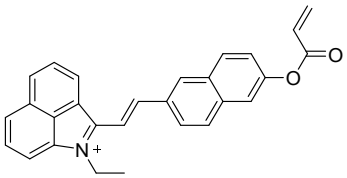
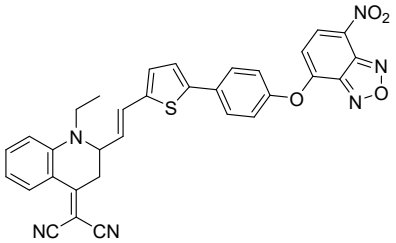
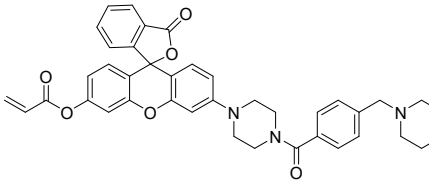
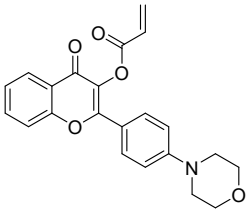
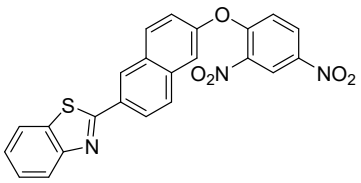
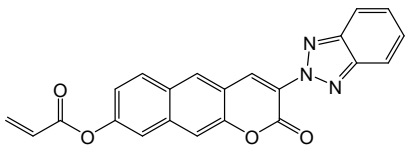


Figure S8 The NMR of the reaction mechanism between BTAB and Cys

Table S1 Probes for Cys detection in recent years

Probes	Liner range (μM)	DL (nM)	Response time	Ref.
	0-14	88.2	4 s	[1]
	0-100	16.3	50 min	[2]

	6-20	290	1.5 min	[3]
	0-100	4	30 min	[4]
	0-60	54	30 min	[5]
	0.1-6	7.5	2 s	[6]
	0-20	160	5 min	[7]
	0-50	1300	15min-20 min	[8]
	0.3-10	154	13 min	This work

DL: detection limit

Reference

1. F. Zou, C. Wang, W. Song, L. Shen, R. Xu, M. Wang, M. Wang, T. Sun and J. Wang, Spectrochim. Acta A., **257**, 119775 (2021)
2. Y. Zhang, C. Xu, H. Sun, J. Ai, M. Ren and F. Kong, Spectrochim. Acta A., **304**, 123345 (2024)
3. K. Liu, H. Gu, Y. Sun, C. Xu and B. Zhu, Food. Chem., **356,** 129658-129664 (2021)
4. C. Zheng, X. Zhou, H. Wang, M. Ji and P. Wang, Bioorg. Med. Chem., **127,** 106003-106009 (2022)
5. W. Wang, Z. Peng, M. Ji, J. Chen and P. Wang, Bioorg. Chem., **126**, 105902-105909(2022)
6. L. Li, Q. Liu, R. Cai, Q. Ma, G. Mao, N. Zhu and S. Liu, Microchem. J., **187**, 108449-108456 (2023)
7. H. Tan, Y. Zou, J. Guo, J. Chen and L. Zhou, Spectrochim. Acta A., **280**, 121552-121560 (2022)
8. Z. Lin, X. Zheng, X. Mao, D. Li, R. Hou, and Y. Xia, Chinese J. Anal. Chem., **50**, 33-37 (2022)