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Supporting Information

A NBD-S-rhodamine dyad for dual-color discriminative imaging of biothiols and Cys/Hcy

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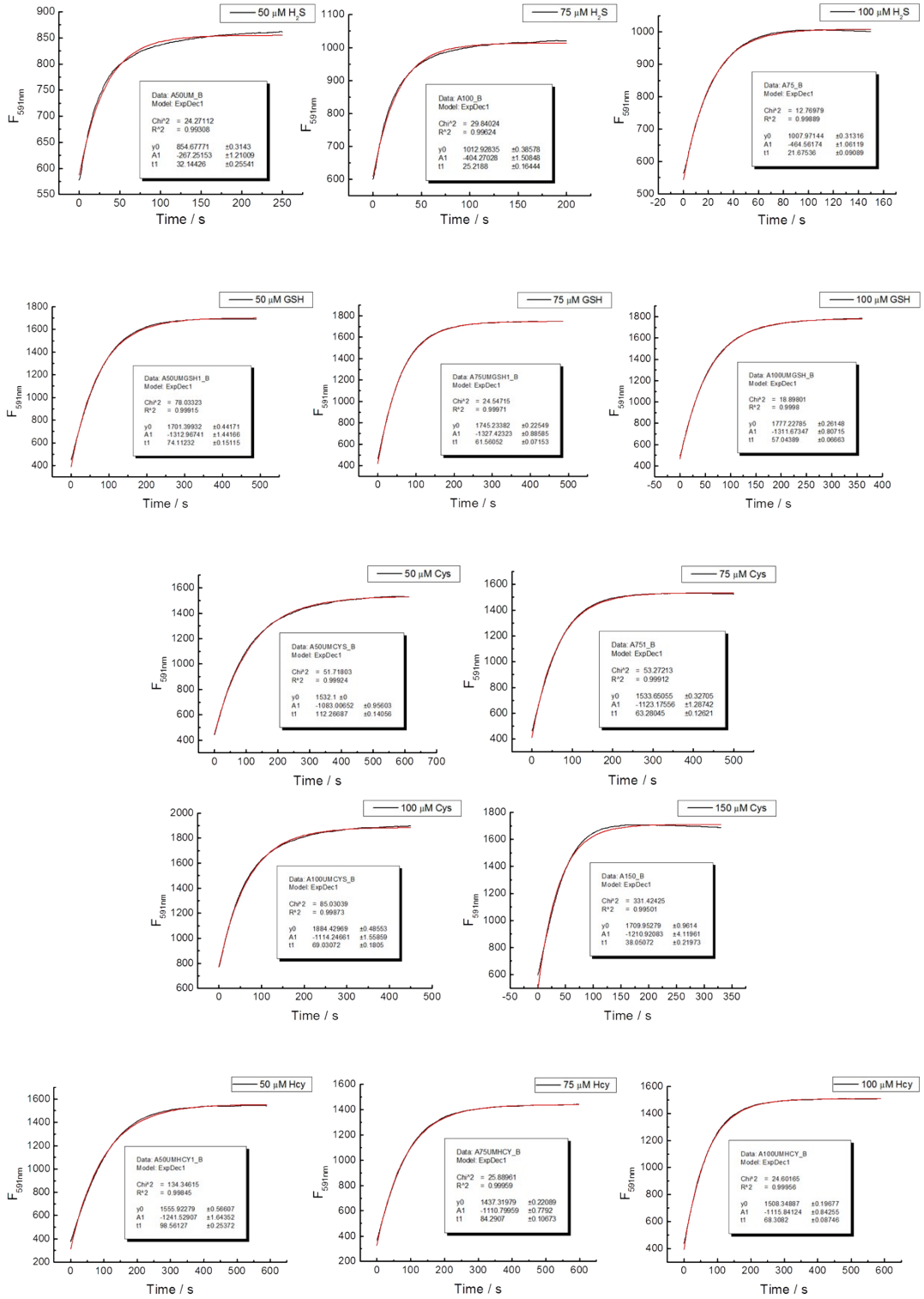
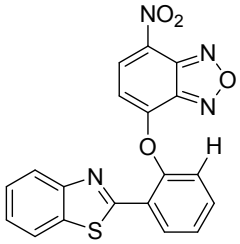
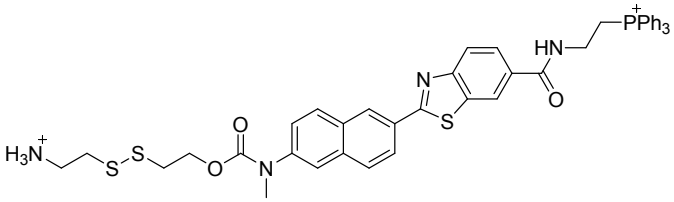
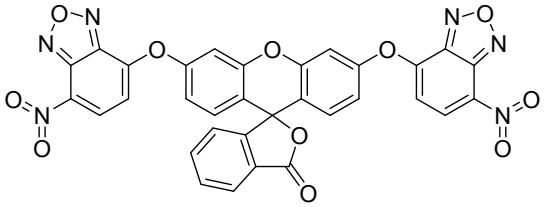
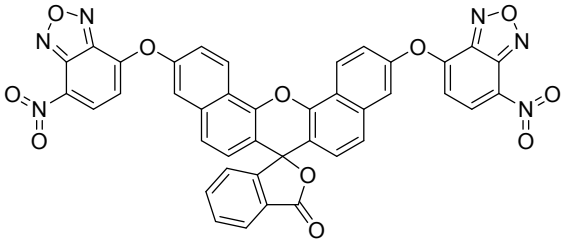
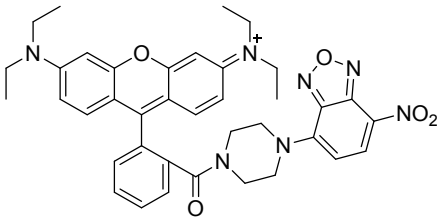
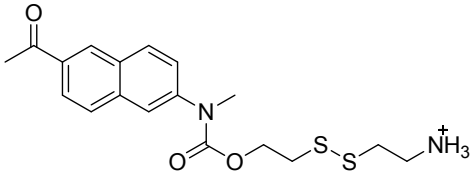
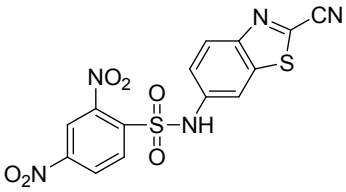
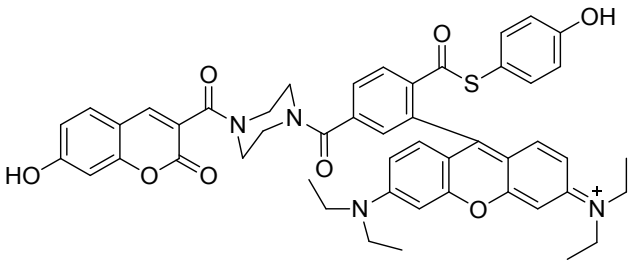
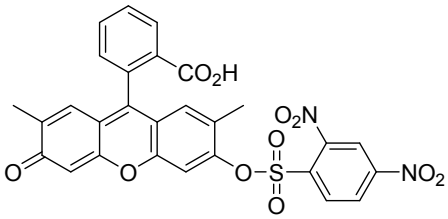
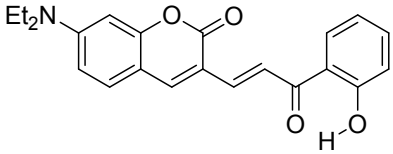


Fig. S1. Time-dependent emission of **1** (5 μM) toward different concentration of biothiols (50, 75, 100, 150 μM).

Table S1 Rates of reported probes for biothiols

Probe	Selectivity	Reaction rate k_2	Reference
	Cys	74.4 $M^{-1}S^{-1}$	1
	GSH	2.3×10^{-2} $M^{-1}S^{-1}$	2
	H_2S	1.78 $M^{-1}S^{-1}$	3
	H_2S	0.56 $M^{-1}S^{-1}$	3
	H_2S	113 $M^{-1}S^{-1}$	4
	2-AET	2.2×10^{-5} $M^{-1}S^{-1}$	5
	Hcy/Cys	13.6 $M^{-1}S^{-1}$/ 26.8 $M^{-1}S^{-1}$	6

	Hcy/Cys	11.17 $M^{-1}S^{-1}$/ 4.97 $M^{-1}S^{-1}$	7
	GSH	140 $M^{-1}S^{-1}$	8
	GSH	6.98×10^{-2} $M^{-1}S^{-1}$	9

1. Z. Zhu, L. Wei, L. Cheng, Z. Li, Z. Xi and L. Yi, *Tetrahedron Lett.*, 2015, **56**, 3909;
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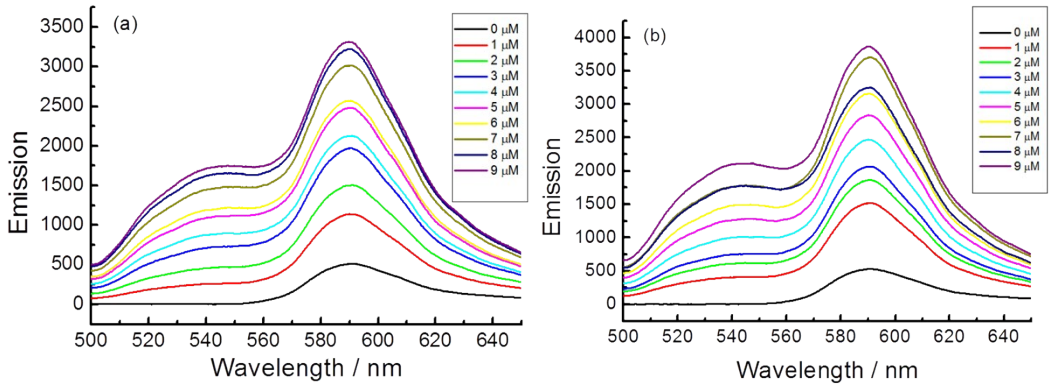


Fig. S2. The fluorescence spectra of probe 1 on addition of different concentrations of Cys (a) or Hcy (b). Excitation: 470 nm.

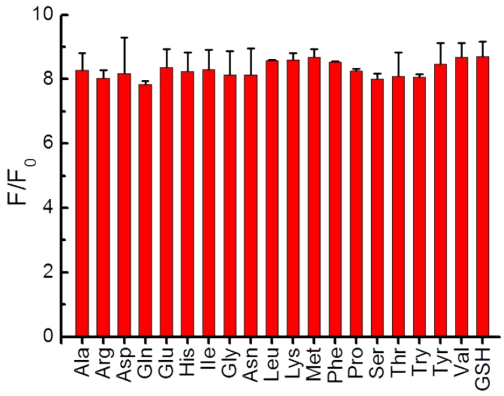


Fig. S3. Relative fluorescence of 1 (5 μM) with 470 nm excitation toward GSH (100μM) and other amino acids (1mM) in PBS buffer (pH 7.4, 50 mM, containing 10% DMSO). Emission for 591 nm. All reactions were performed for 20 min.

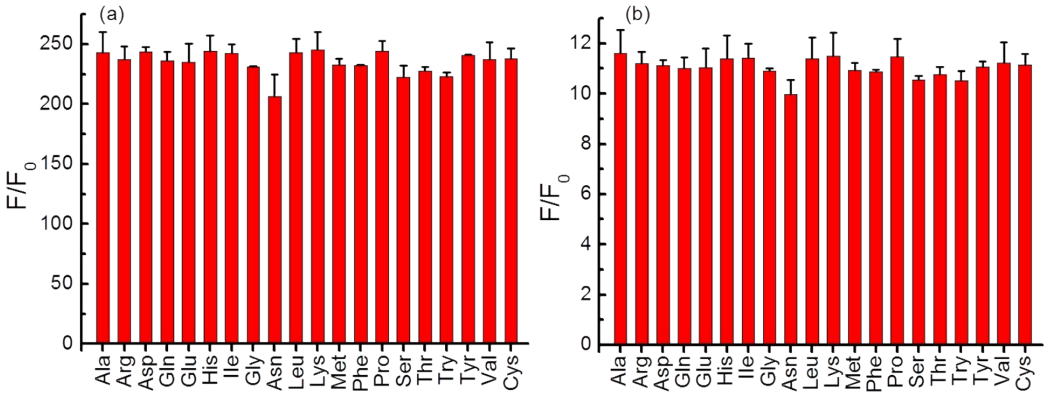


Fig. S4. Relative fluorescence of 1 (5 μM) with 470 nm excitation toward Cys(100μM) and other amino acids (1mM) in PBS buffer (pH 7.4, 50 mM, containing 10% DMSO). Emission for (a) 547 nm; (b) 591 nm. All reactions were performed for 20 min.

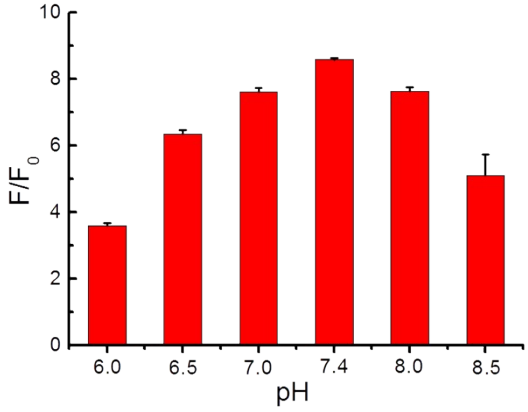


Fig. S5. Relative fluorescence of 1 (5 μM) with 470 nm excitation toward GSH (100 μM) in PBS buffer (pH 7.4, 50 mM, containing 10% DMSO). Emission for 591 nm. All reactions were performed for 20 min.

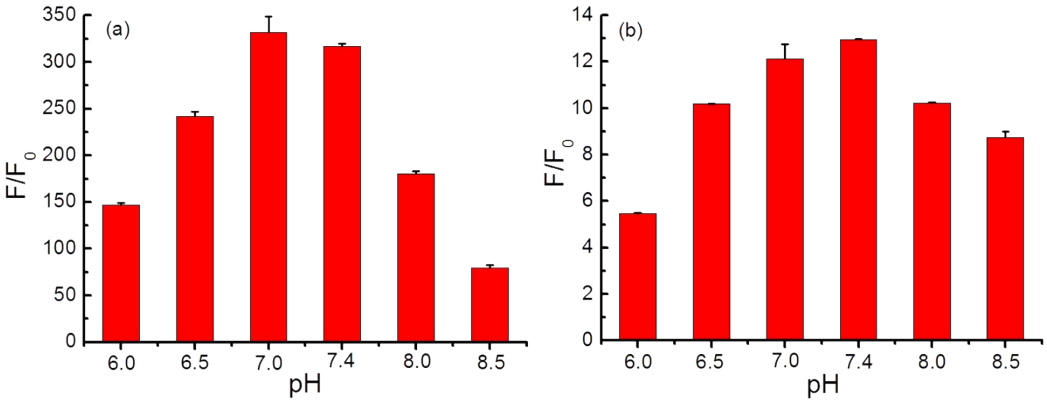


Fig. S6. Relative fluorescence of 1 (5 μM) with 470 nm excitation toward Cys (100 μM) in PBS buffer (pH 7.4, 50 mM, containing 10% DMSO). Emission for (a) 547 nm; (b) 591 nm. All reactions were performed for 20 min.

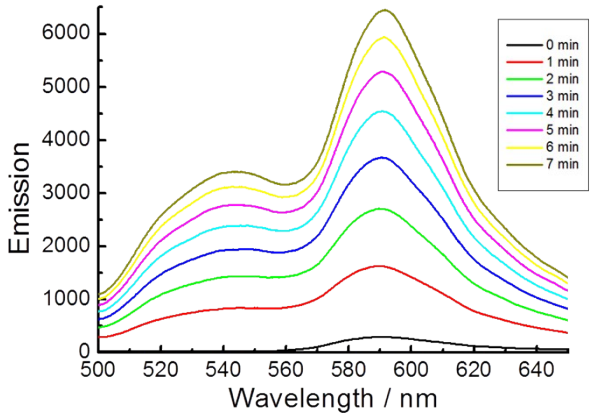


Fig. S7. Time-dependent fluorescence spectra of 1 (5 μM) toward Cys (100 μM) (pH 7.4, 50 mM, containing 2% DMSO). Excitation is 470 nm.

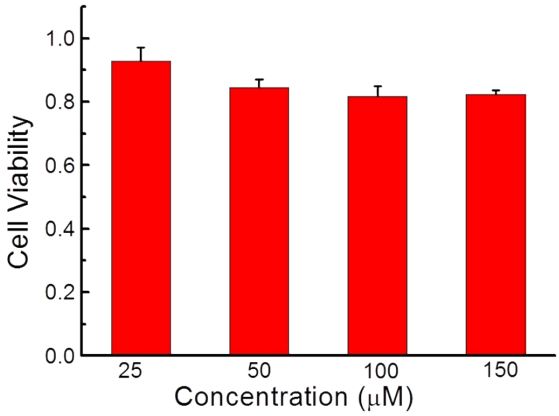


Fig. S8. Toxicity assessment of Probe 1. Cell viability studies with HUVEC cells.

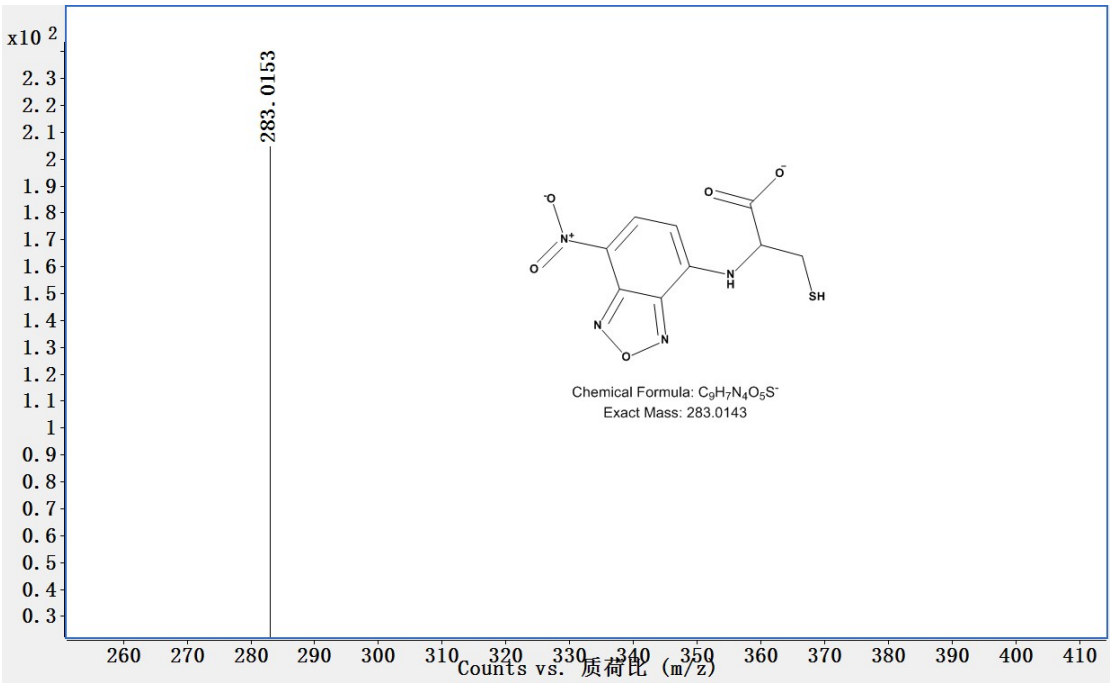
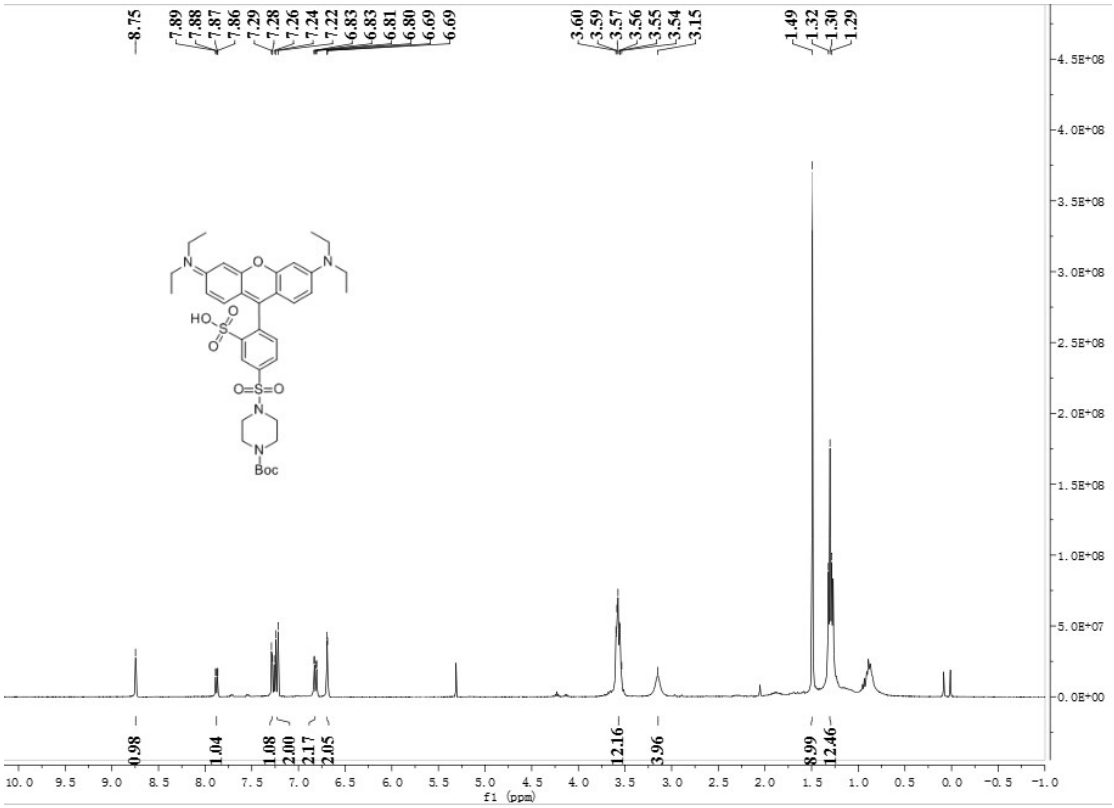
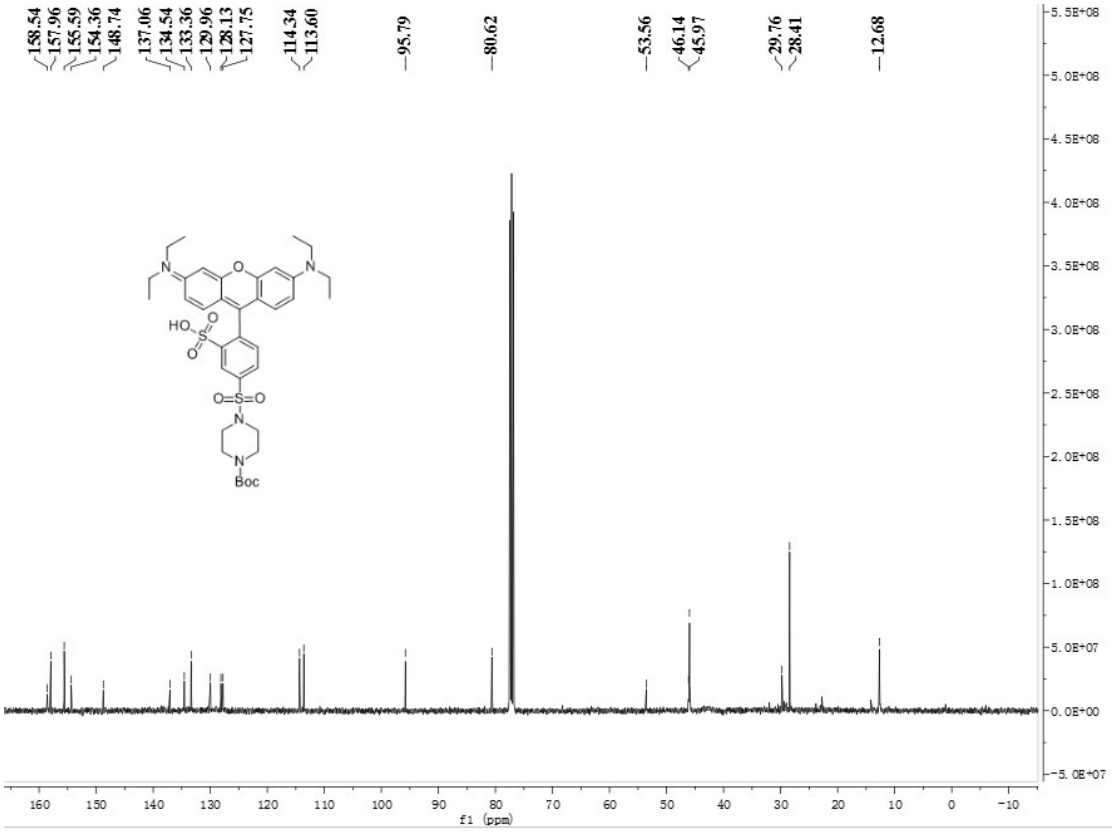
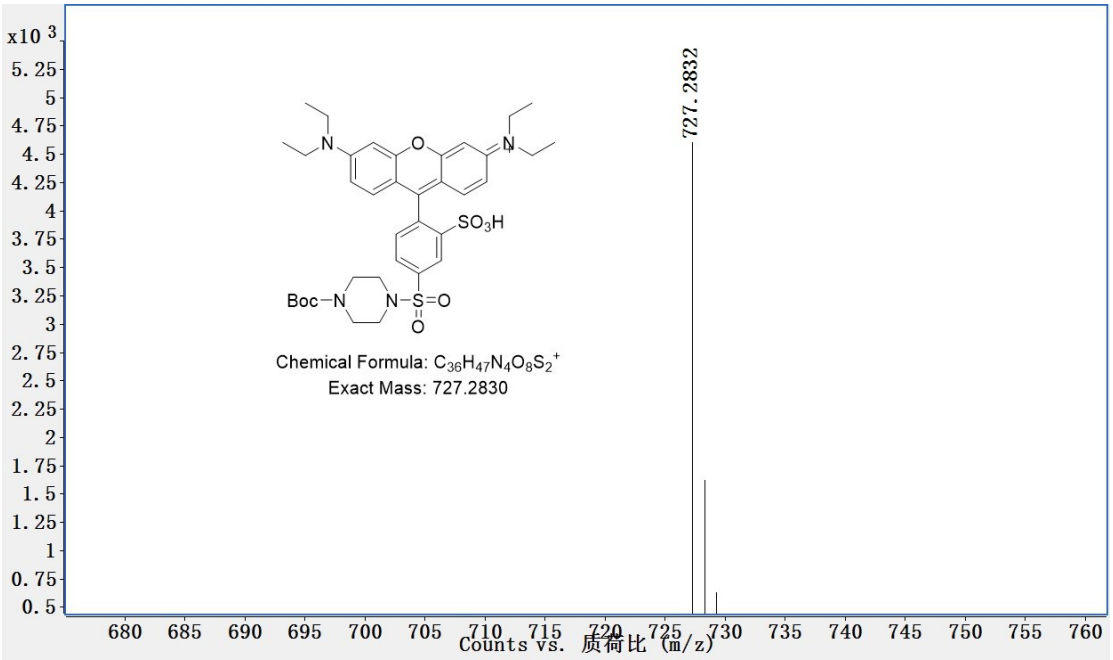
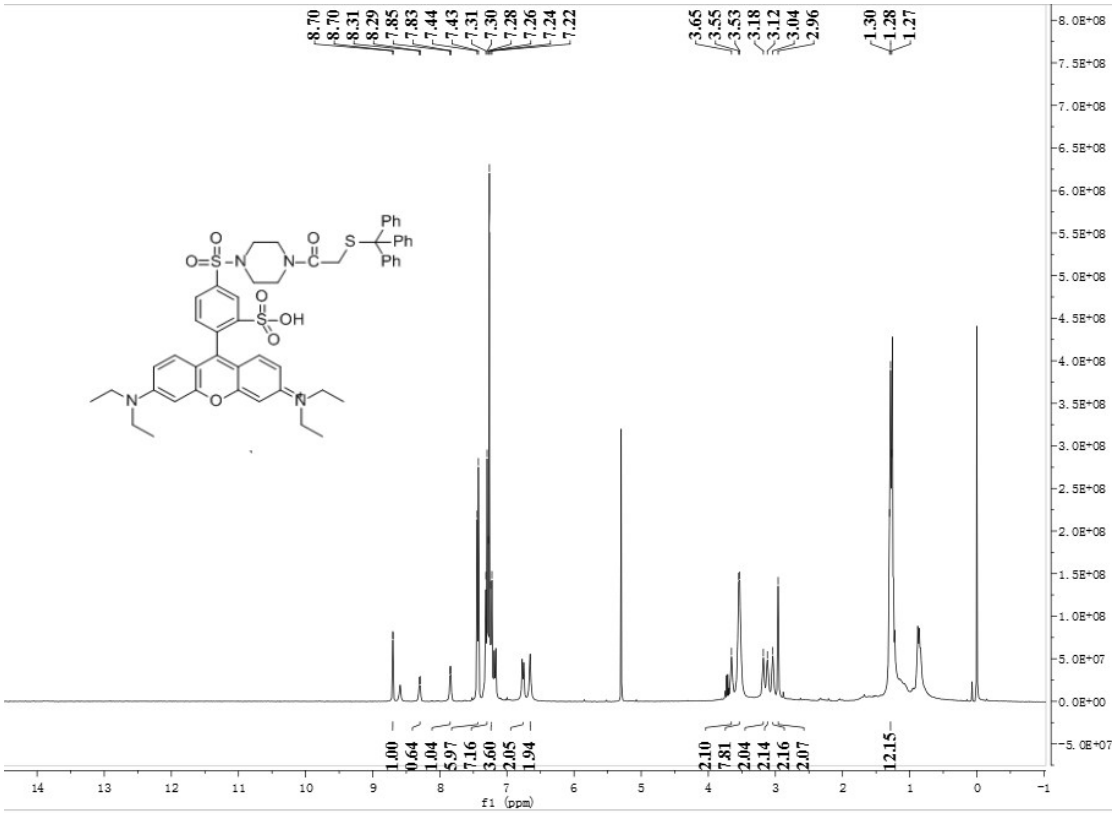


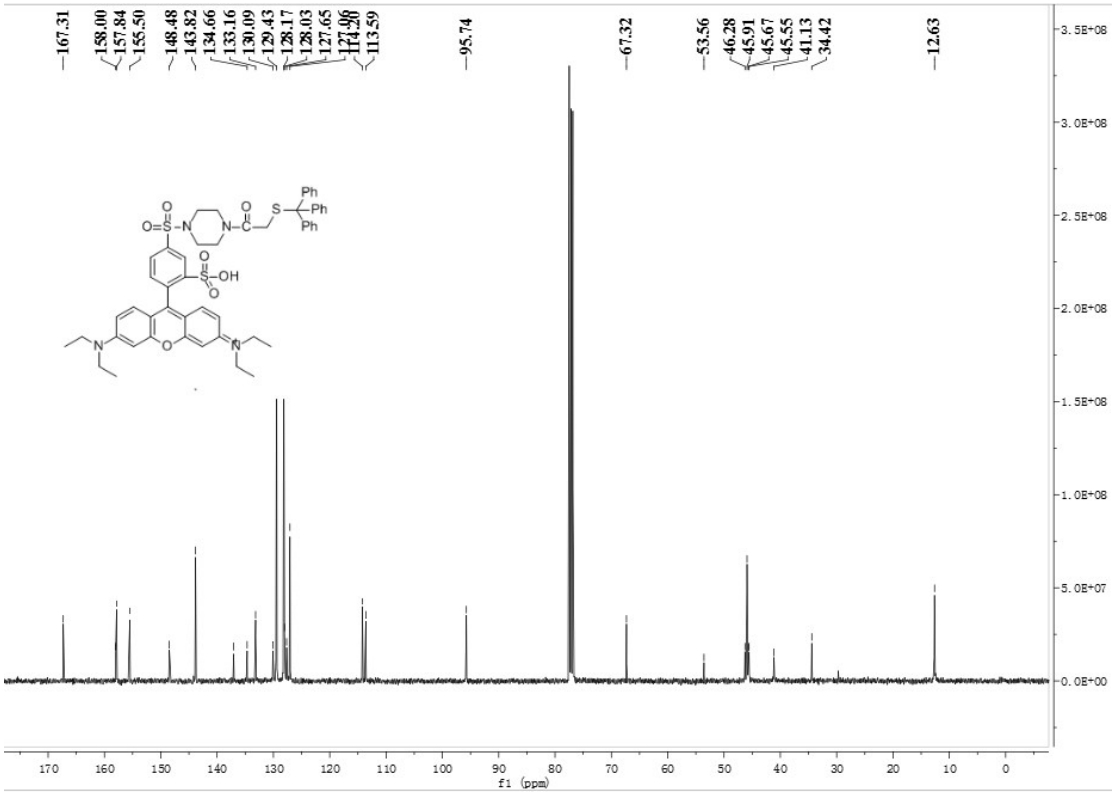
Fig. S9. HRMS spectra of the reaction of 1 and Cys. 1 (100 μM) was incubated with Cys (2.5 mM) in PBS buffer (pH 7.4, 50 mM, 30% CH3CN) at room temperature for 1 h.

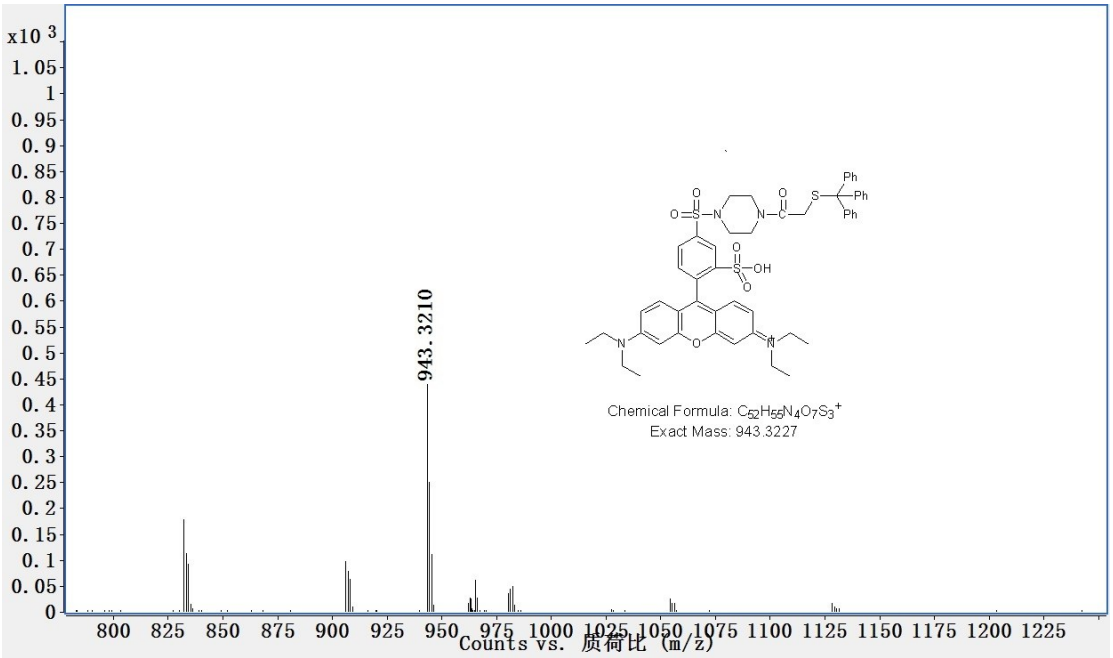

8.75
7.89
7.88
7.87
7.86
7.29
7.28
7.26
7.24
7.22
6.83
6.83
6.81
6.80
6.69
6.69
3.60
3.59
3.57
3.56
3.55
3.54
3.15
1.49
1.32
1.30
1.29
0.98
1.04
1.08
2.00
2.17
2.05
12.16
3.96
8.99
12.46
f1 (ppm)


158.54
157.96
155.59
154.36
148.74
137.06
134.54
133.36
129.96
128.13
127.75
114.34
113.60
95.79
80.62
53.56
46.14
45.97
29.76
28.41
12.68
f1 (ppm)


x10 3
727.2832
Chemical Formula: $C_{36}H_{47}N_4O_8S_2^+$
Exact Mass: 727.2830
Counts vs. 质荷比 (m/z)


f1 (ppm)


167.31
158.00
157.84
155.50
148.48
143.82
134.66
133.16
130.09
129.43
128.17
128.03
127.65
127.06
114.20
113.59
95.74
67.32
53.56
46.28
45.91
45.67
45.55
41.13
34.42
12.63
f1 (ppm)


x10 3
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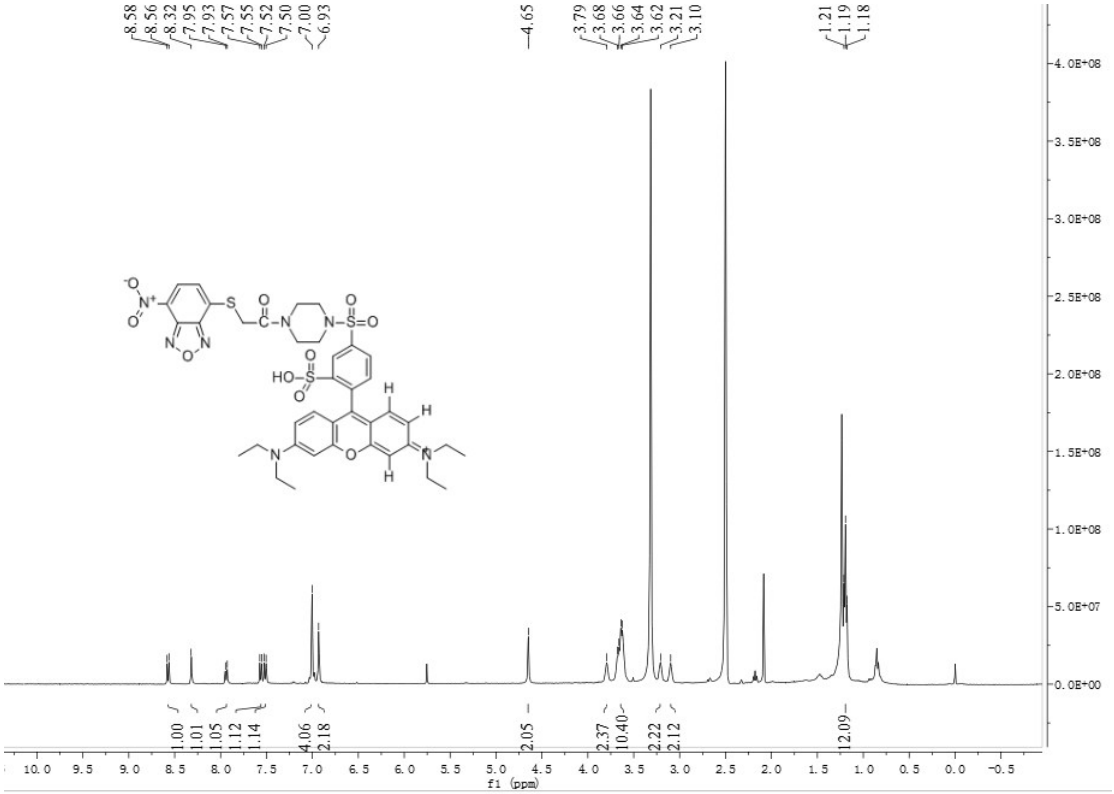
Exact Mass: 943.3227
Counts vs. 质荷比 (m/z)


8.58
8.56
8.32
7.95
7.93
7.57
7.55
7.52
7.50
7.00
6.93
4.65
3.79
3.68
3.66
3.64
3.62
3.21
3.10
1.21
1.19
1.18
1.00
1.01
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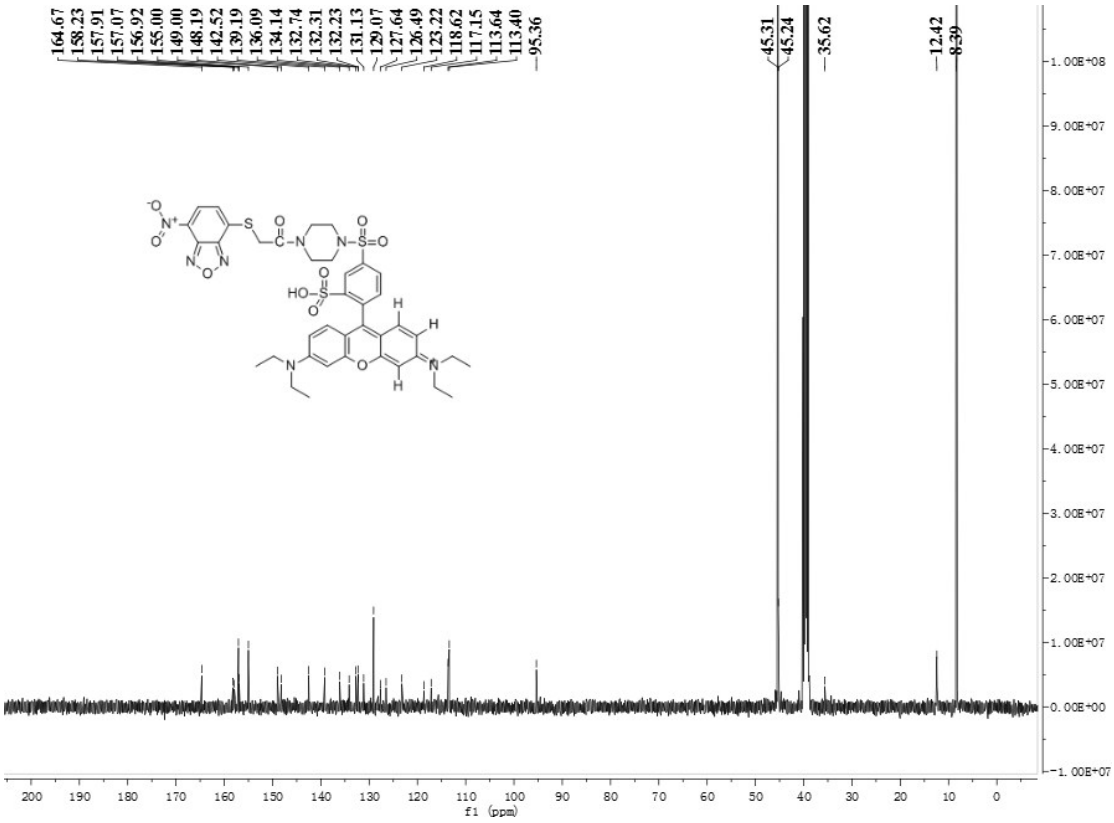
12.09
f1 (ppm)


164.67
158.23
157.91
157.07
156.92
155.00
149.00
148.19
142.52
139.19
136.09
134.14
132.74
132.31
132.23
131.13
129.07
127.64
126.49
123.22
118.62
117.15
113.64
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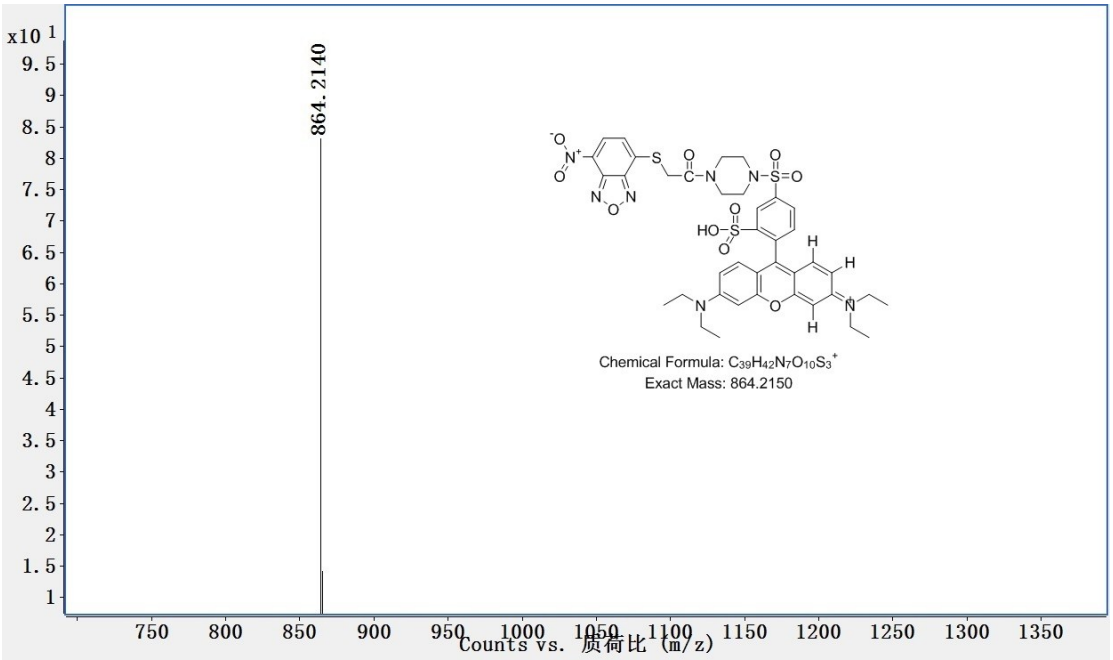
45.31
45.24
35.62
12.42
8.39
f1 (ppm)


x10 1
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
864.2140
750
800
850
900
950
1000
1050
1100
1150
1200
1250
1300
1350
Counts vs. 质荷比 (m/z)
Chemical Formula: C39H42N7O10S3+
Exact Mass: 864.2150