Supporting Information

Investigation of Thiolysis of NBD Amines for the Development of H_2S Probes and Evaluating the Stability of NBD dyes

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1. Supporting figures

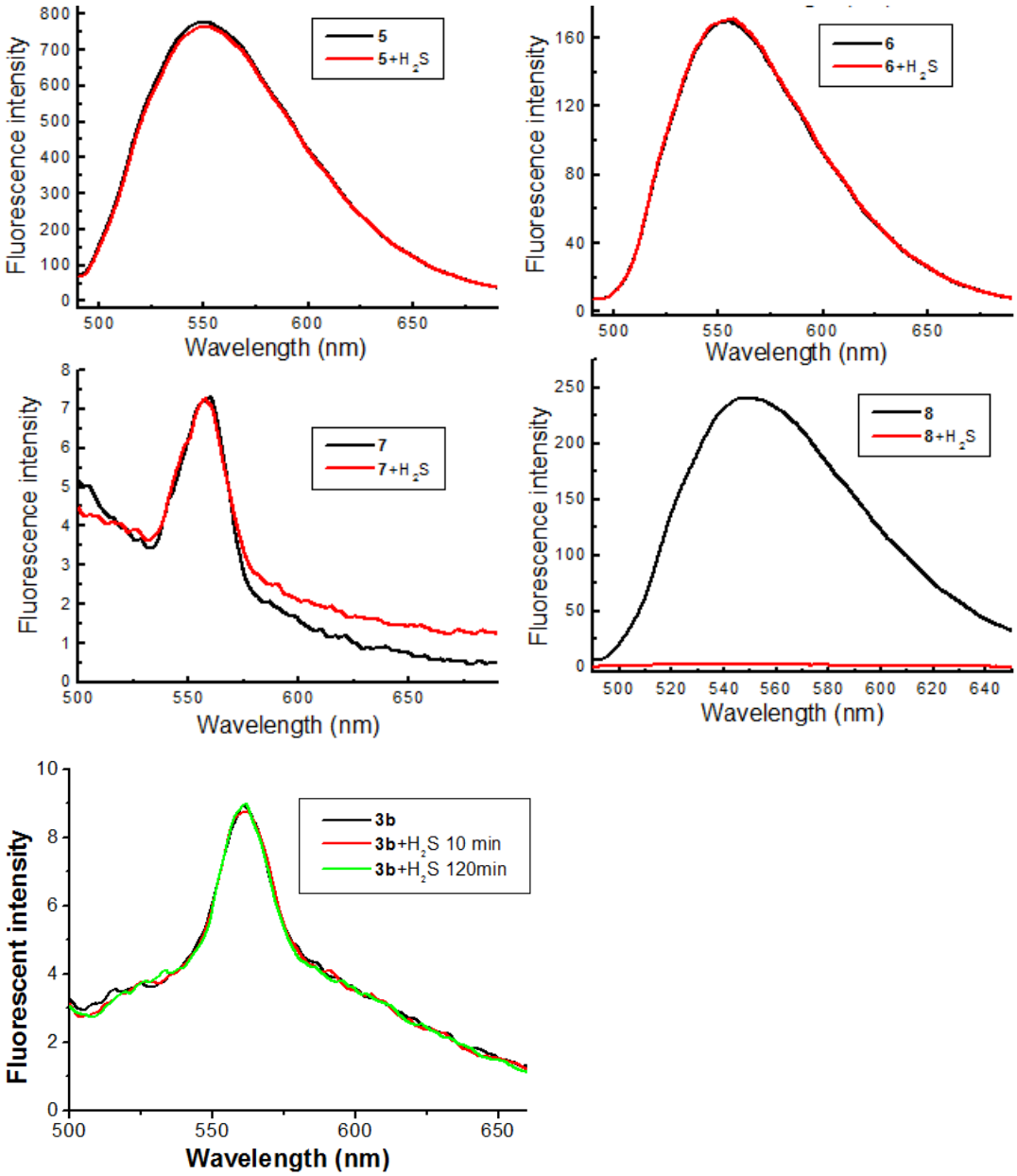


Figure S1. Emission spectra of NBD-based probes (**5-8** or **3b** 1 μM) in the absence (black line) or presence (red line) of 100 μM H_2S. Excitation is 470 nm for all tests in PBS buffer (50 mM, pH 7.4, containing 20% DMSO).

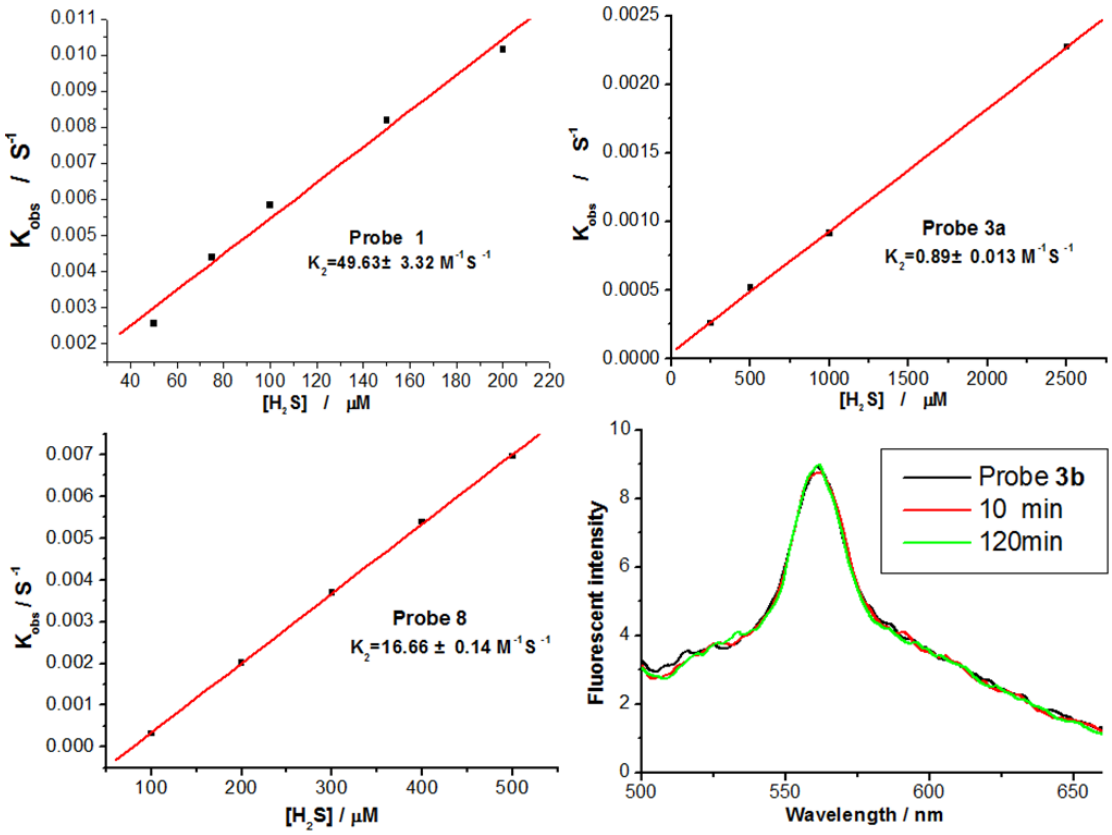


Figure S2. Kinetic studies for thiolysis of NBD amines in 1% DMSO-containing PBS. Time-dependent fluorescence intensity at 560 nm (excitation = 470 nm) of probe **1, 3a, 8** (1 μM) upon reaction with different concentrations of H_2S in PBS buffer (pH 7.4). The linear relationship of k_{obs} versus H_2S concentrations gives reaction kinetics constant k_2 for **1**, **3a**, **8** and H_2S. The fluorescence spectra of probe **3b** with 200 μM H_2S at different reaction time.

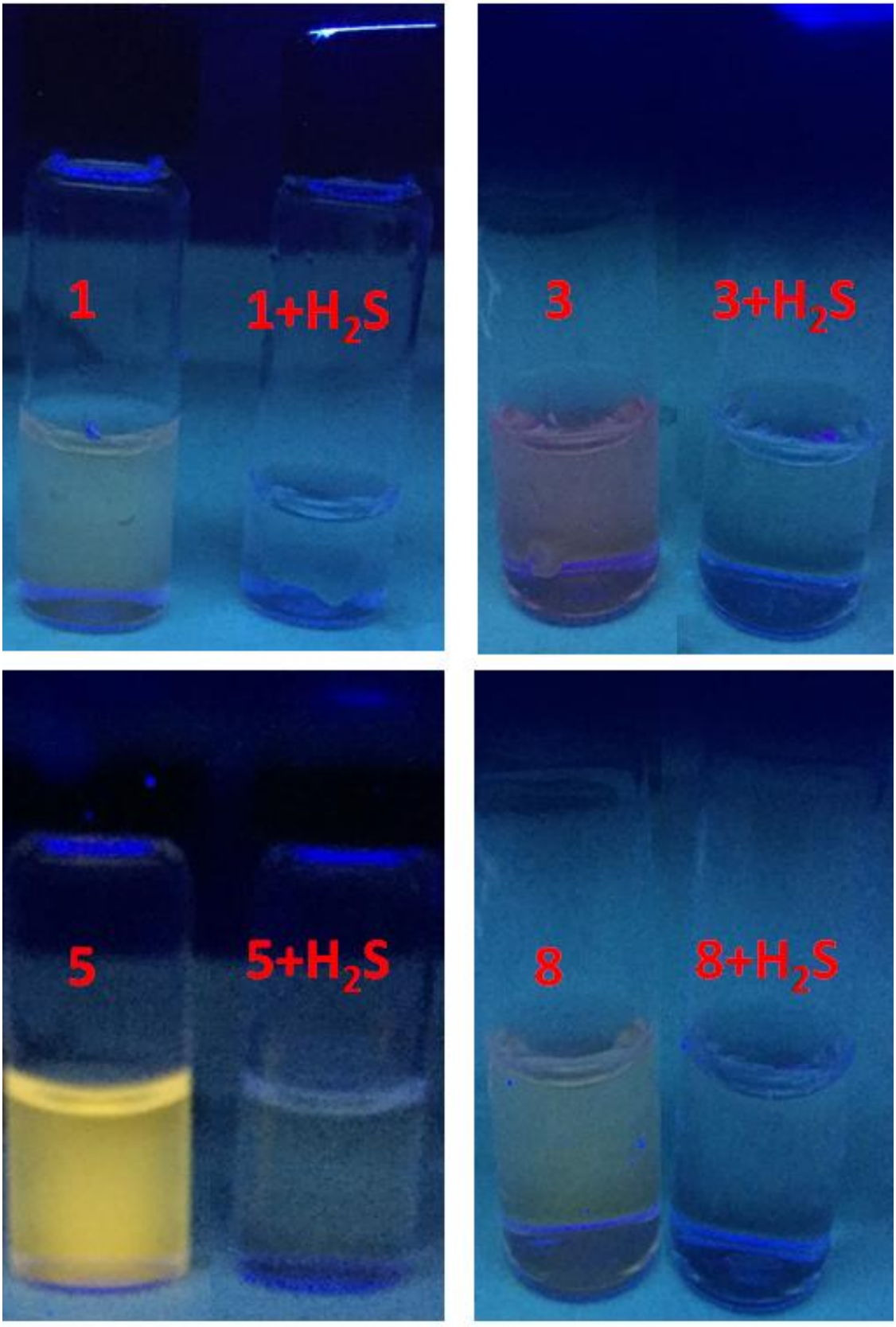


Figure S3. Photographs of probes (10 μM) and their reaction with H_2S (100 μM for **1** or **8**; 2 mM for **3** or **5**) under 365 nm UV lamp.

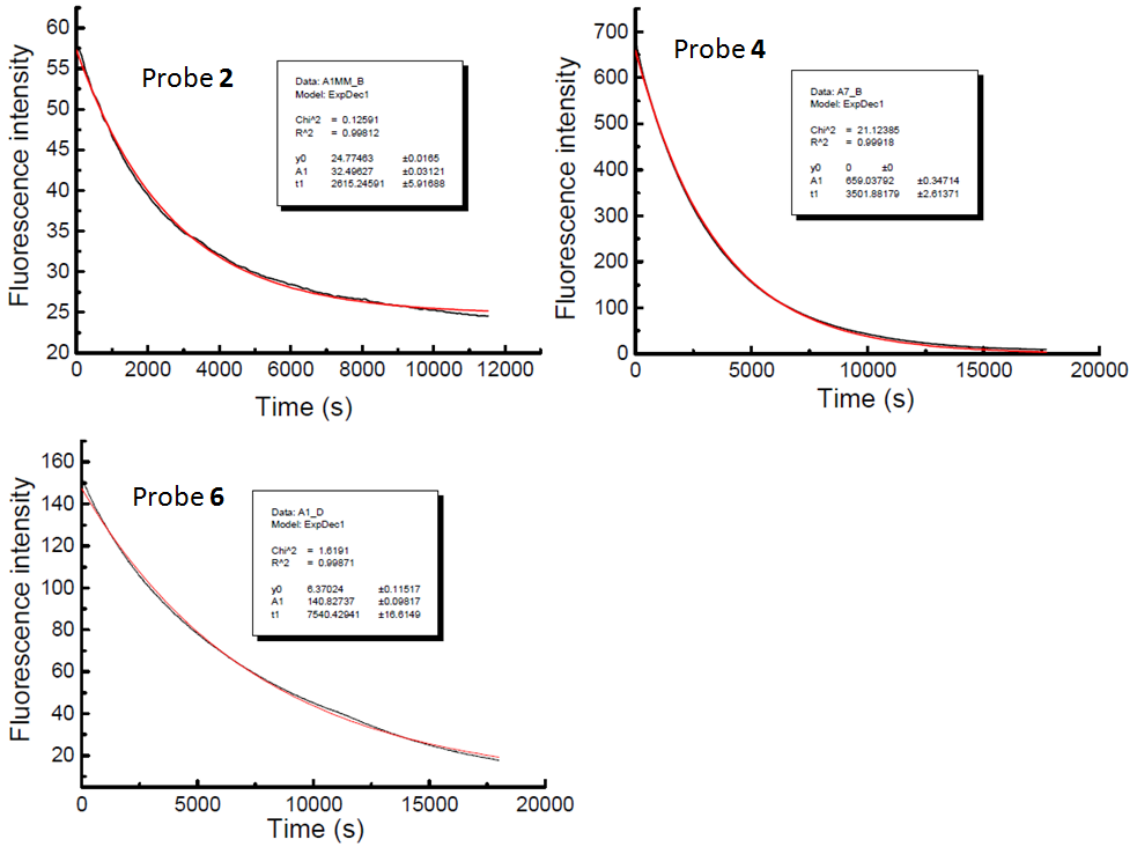


Figure S4. Time-dependent emission intensity of probe **2**, **4** or **6** (1 μM) in the presence of millimolar H_2S in PBS buffer (50 mM, pH 7.4, containing 20% DMSO). The red line represents the best fitting for first-order reaction. (a) 2 mM H_2S, k_{obs}, $3.8*10^{-4}$ s^{-1}. (b) 7 mM H_2S, $k_{obs,}$ $2.9*10^{-4}$ s^{-1}. (c) 7 mM H_2S, k_{obs}, $1.3*10^{-4}$ s^{-1}.

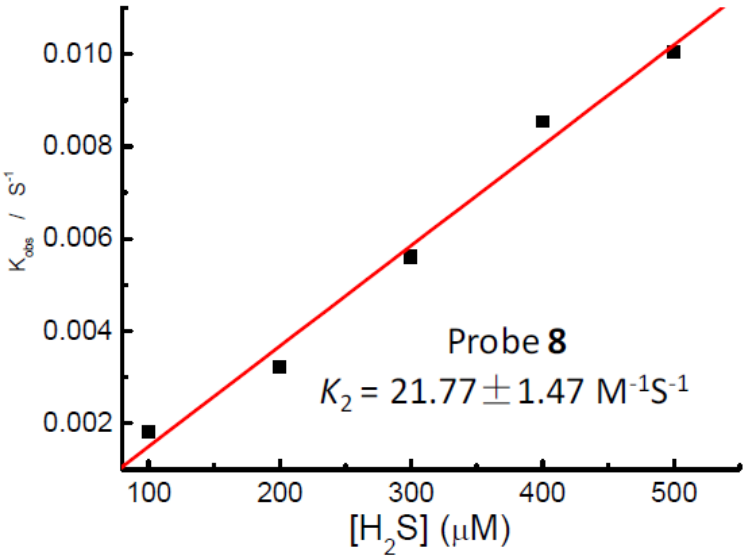


Figure S5. Linear relationship between k_{obs} and H_2S concentration gives the reaction kinetics of 21.77 $M^{-1}s^{-1}$ for probe **8**.

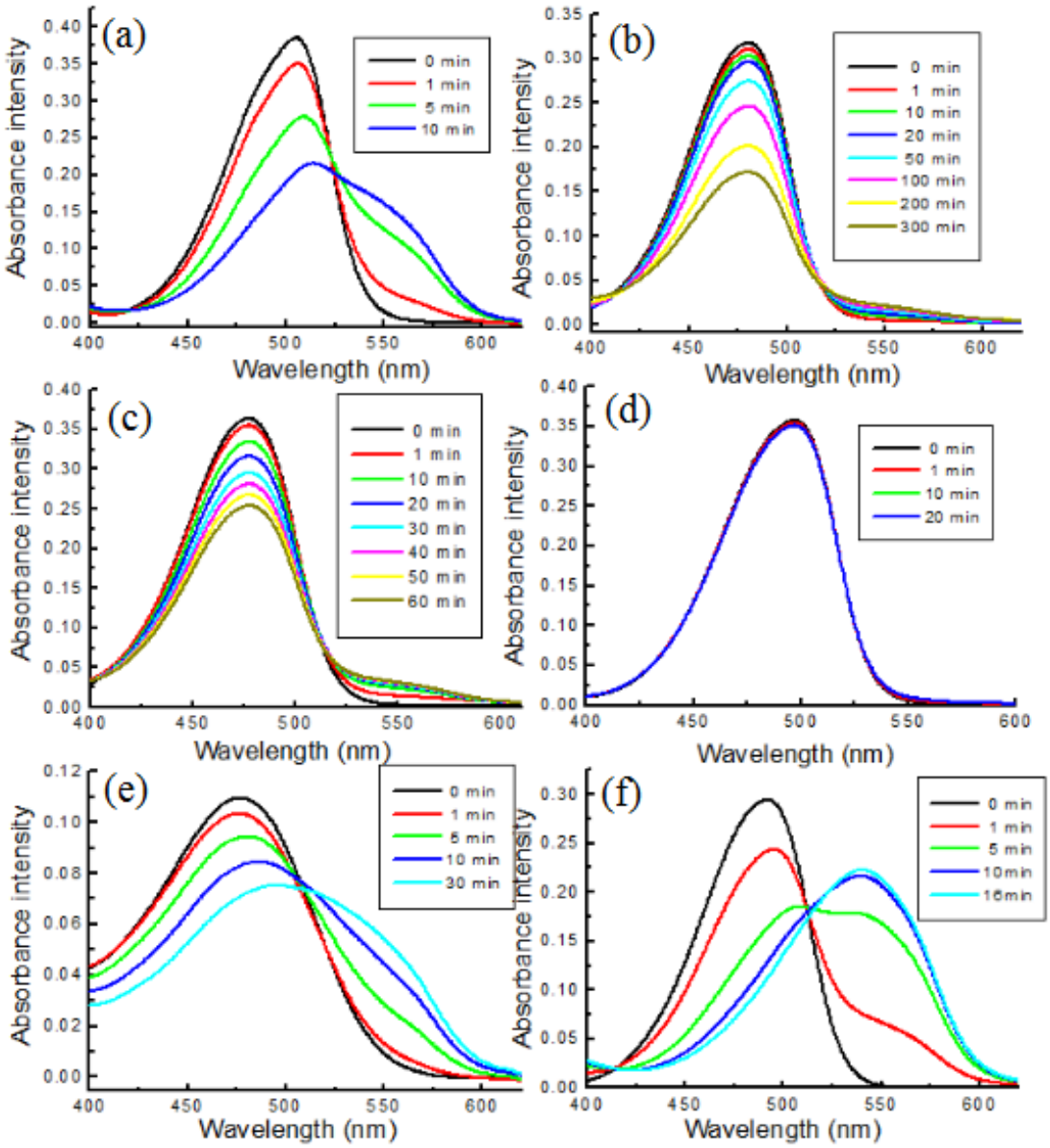


Figure S6. The absorbance spectra of probes **3a-8** (10 μM) in the presence of 100 μM (for **3a** or **8**) or 2 mM H_2S (for **4-7**) in PBS buffer (50 mM, pH 7.4, containing 20% DMSO). (a-f) for probes **3-8**, respectively.

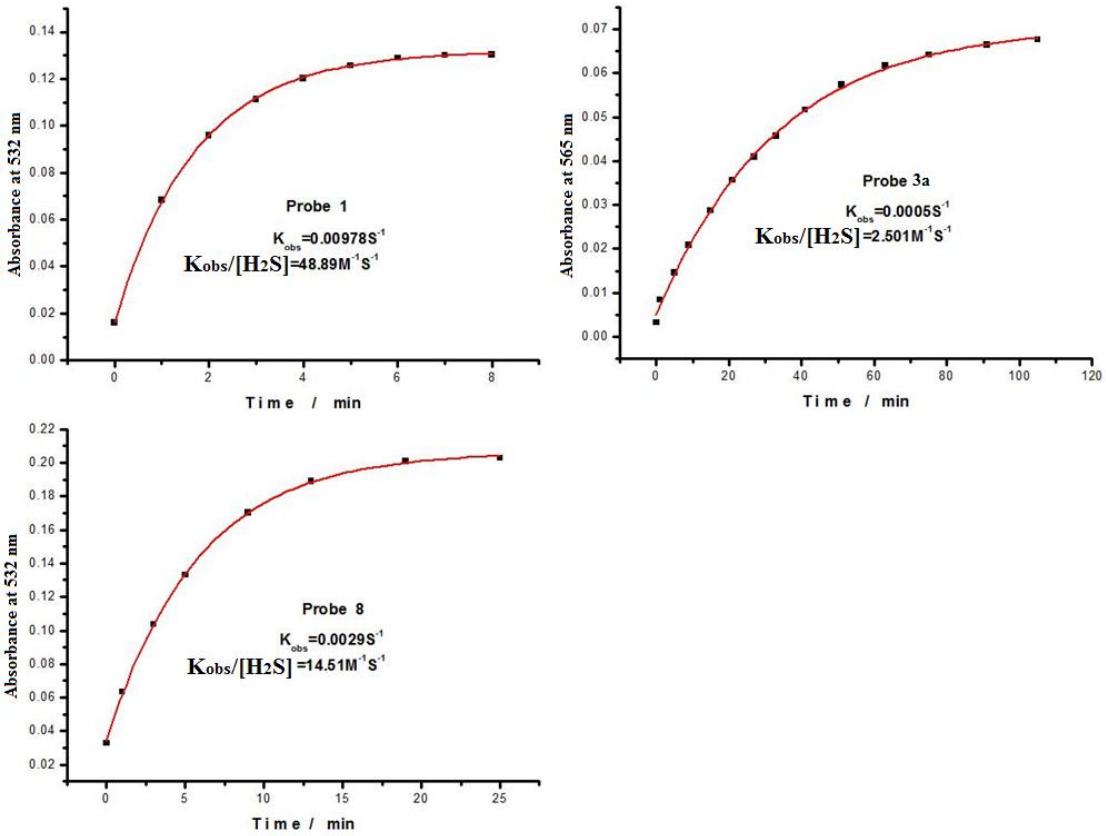


Figure S7. The time-dependent absorbance intensity of probes (10 μM) in the presence of 200 μM in PBS buffer (50 mM, pH 7.4, containing 1% DMSO). The red line represents the best fitting for first-order reaction.

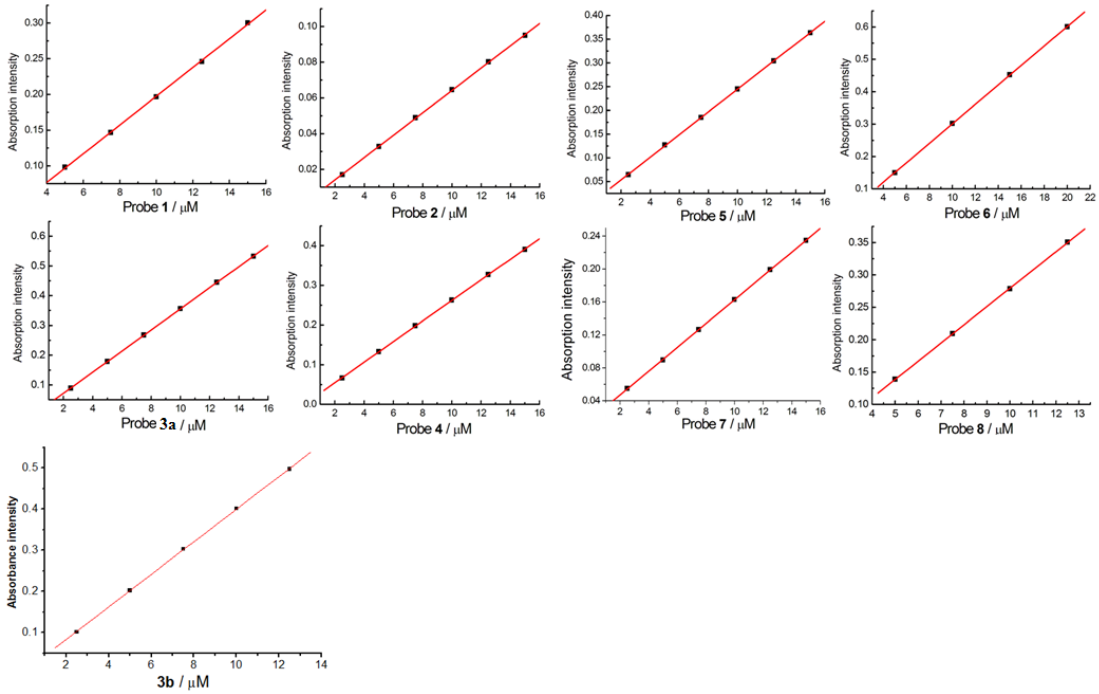


Figure S8. Solubility tests of probes **1**-**8** in PBS buffer (50 mM, pH 7.4, containing 20% DMSO).

The linear relationship between absorption intensity and probe concentrations indicated the good solubility for all probes up to at least 16 μM.

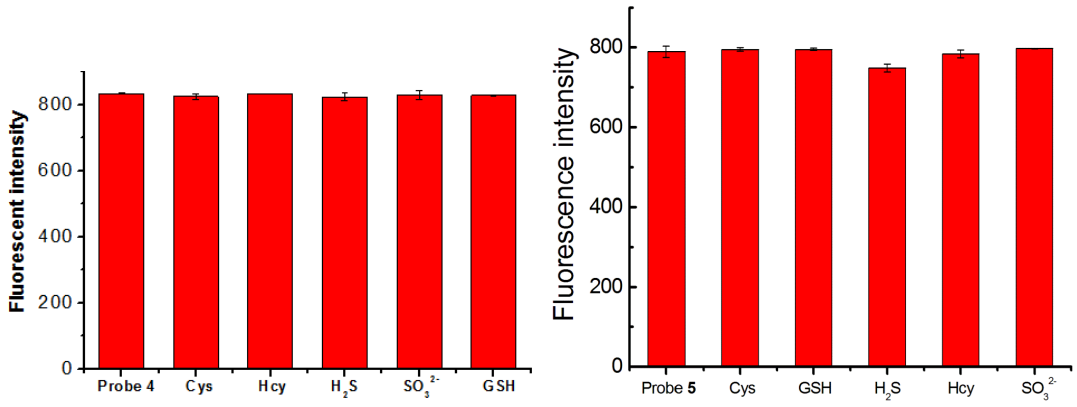
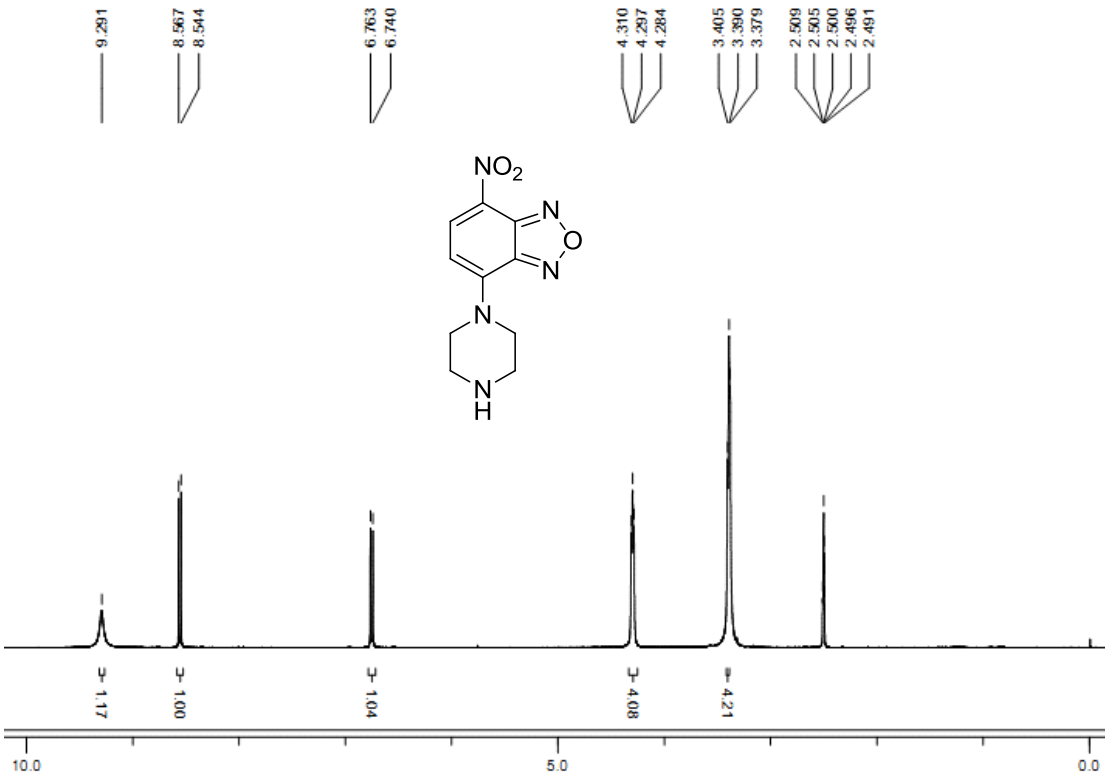
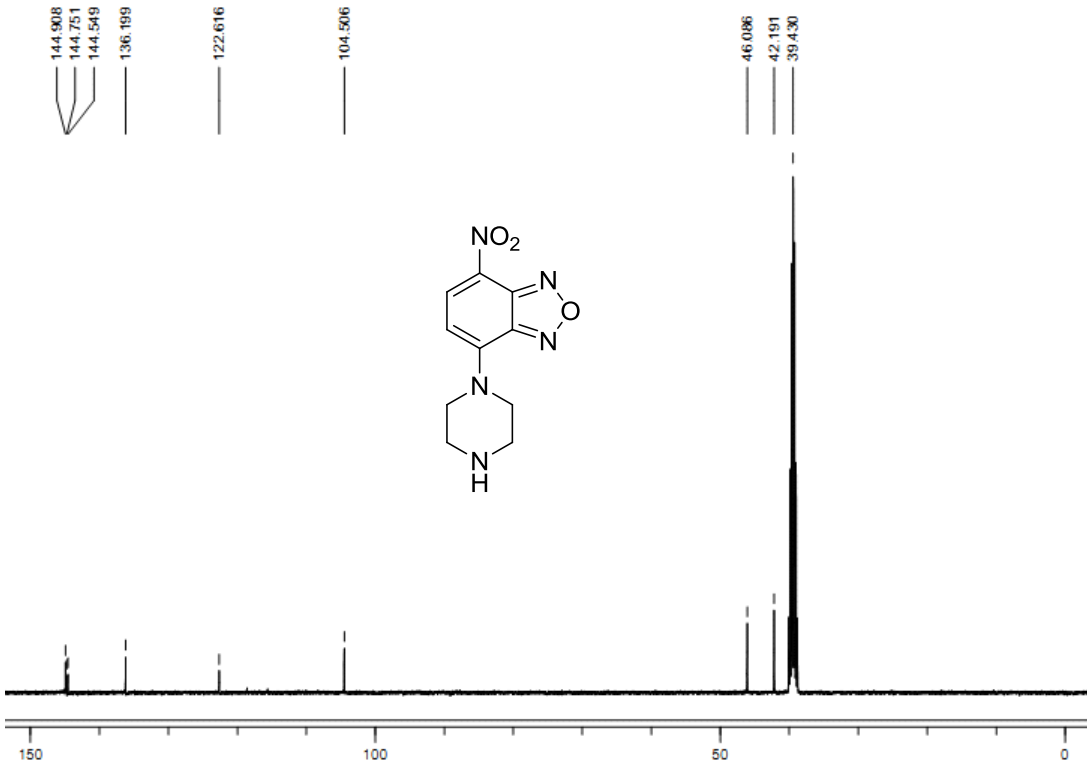
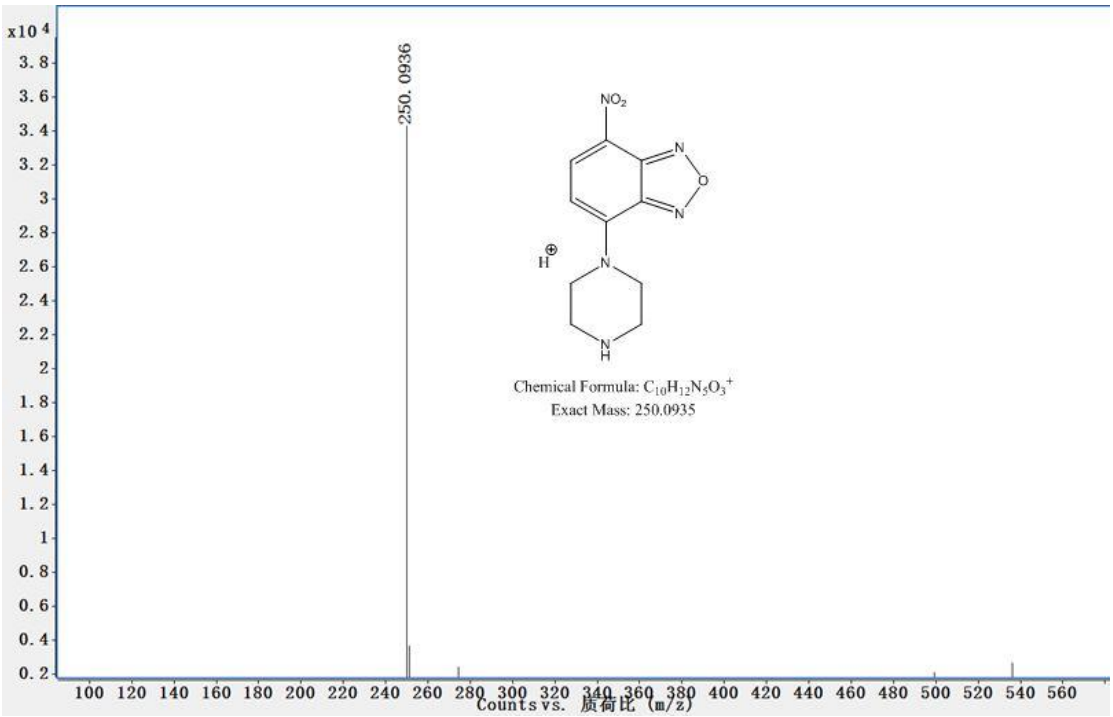


Figure S9. Fluorescent intensity at 560 nm for probes (1 μM) toward different biothiols in PBS buffer. H_2S or SO_3^{2-}, 100 μM; Cys, GSH or Hcy, 1 mM. Reactions were performed for 60 min at room temperature.

2. Supporting NMR and MS spectra



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3.6
3.4
3.2
3
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
250.0936
NO2
N
O
N
H
N
N
H
Chemical Formula: C10H12N5O3+
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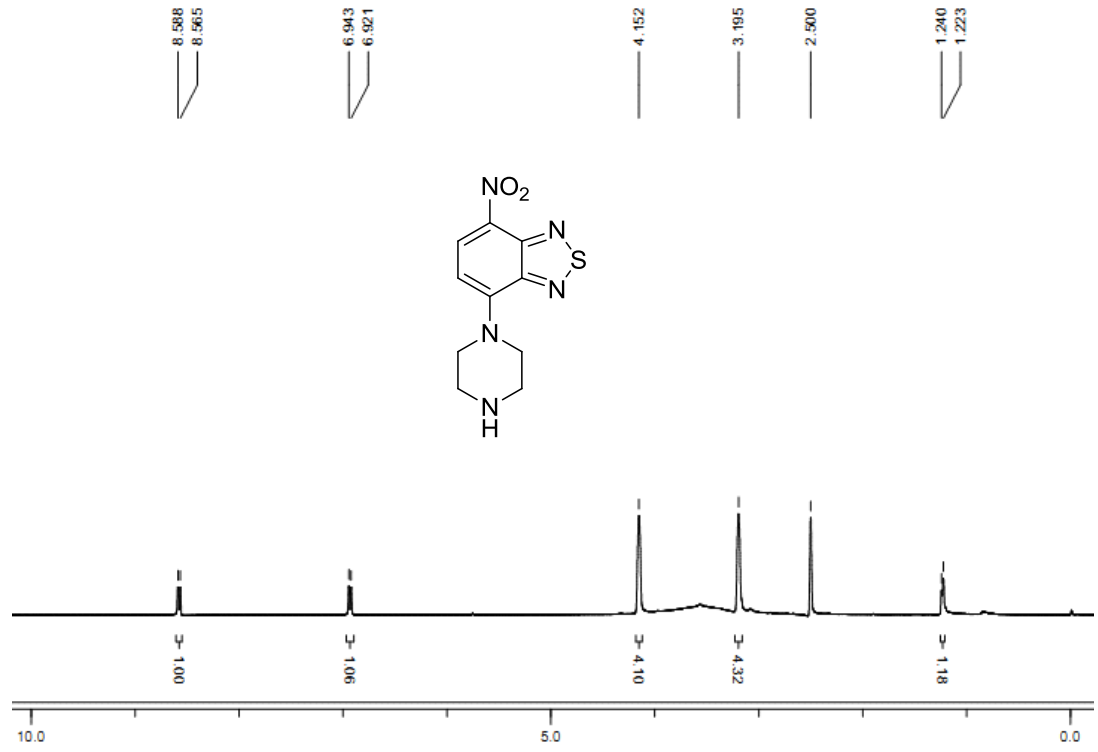
Counts vs. 质荷比 (m/z)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N
N
H
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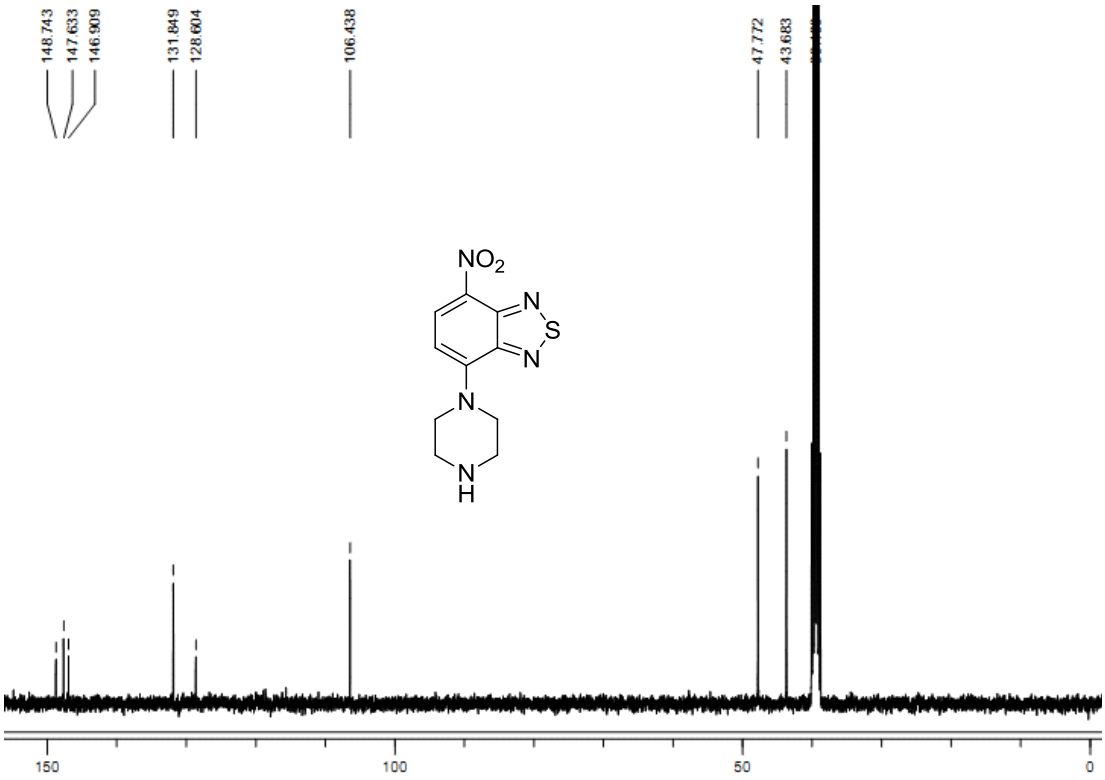
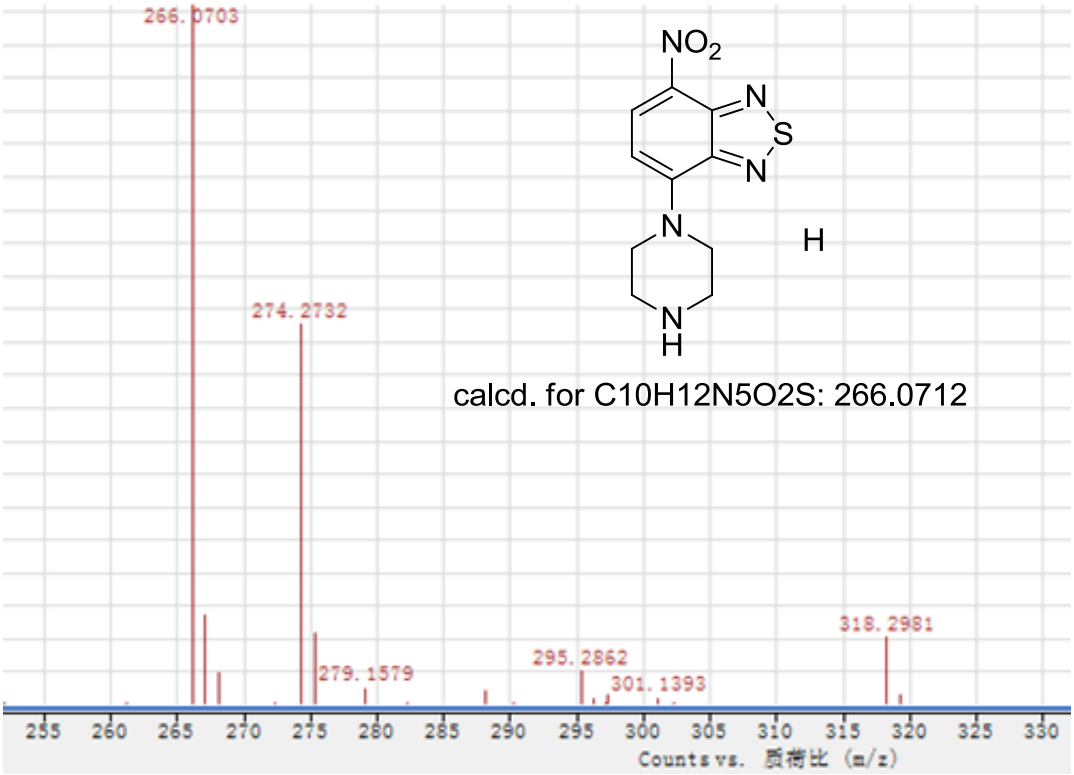
1.18
10.0
5.0
0.0





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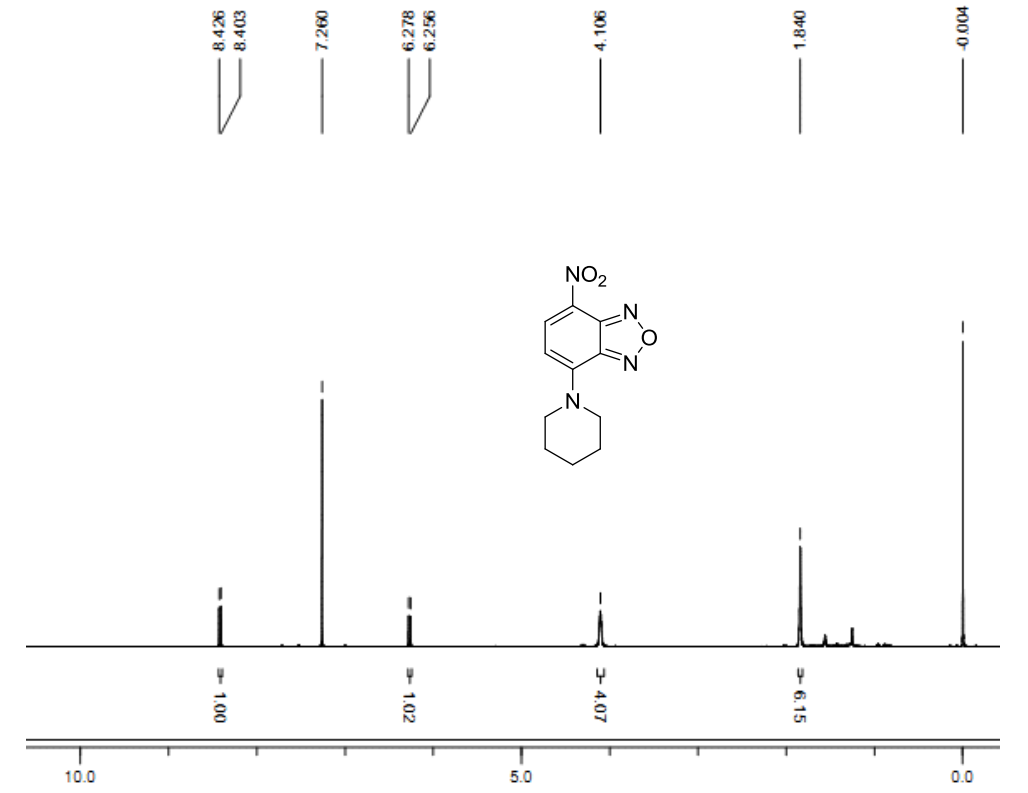
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O
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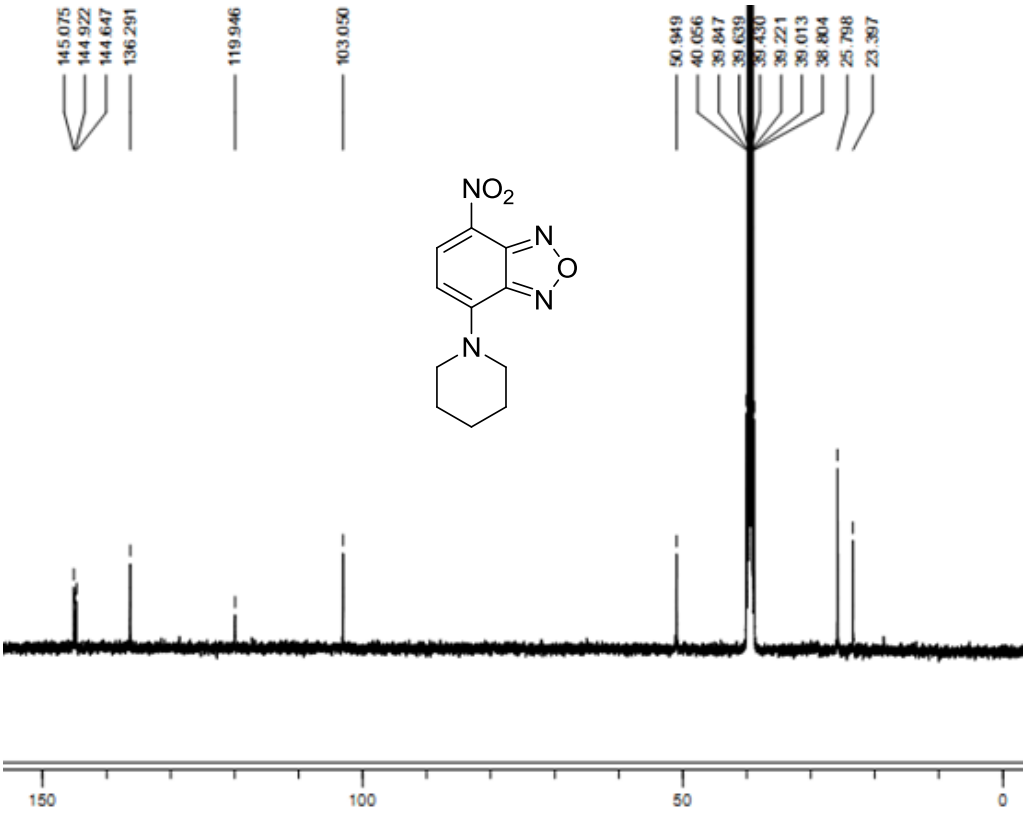
4.07
6.15
10.0
5.0
0.0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144.647
136.291
119.946
103.050
50.949
40.056
39.847
39.639
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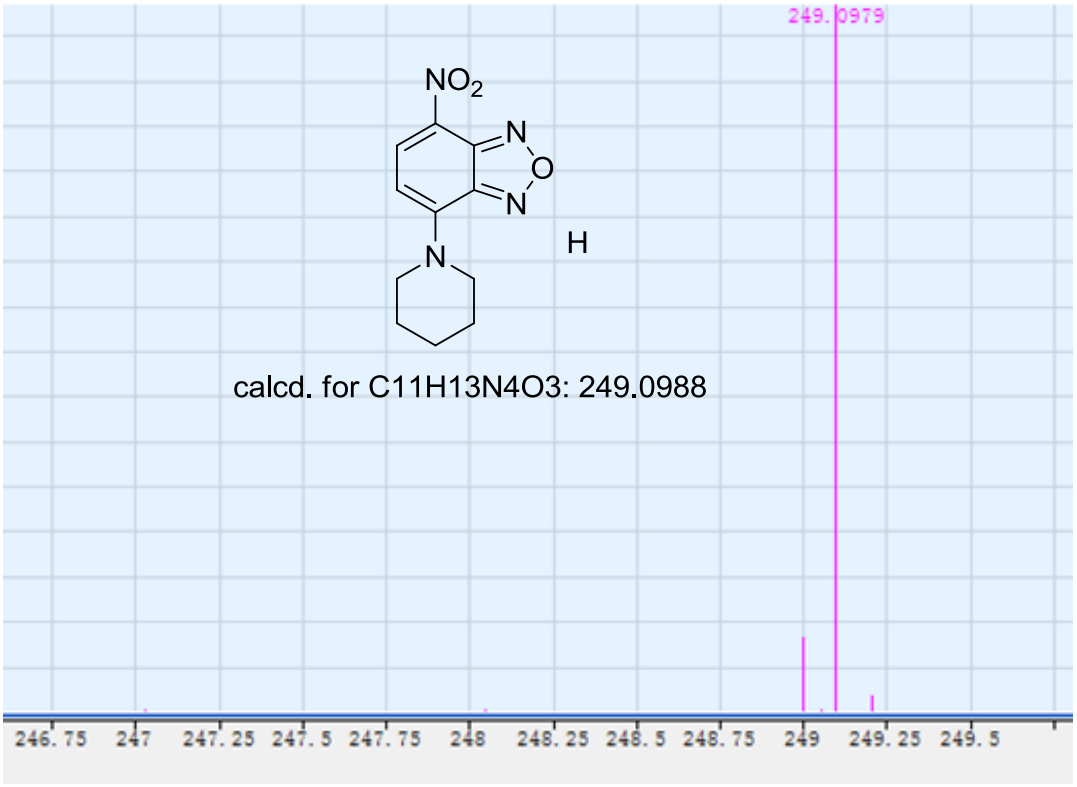
O
N
N
150
100
50
0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O
N
N
H
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247
247.25
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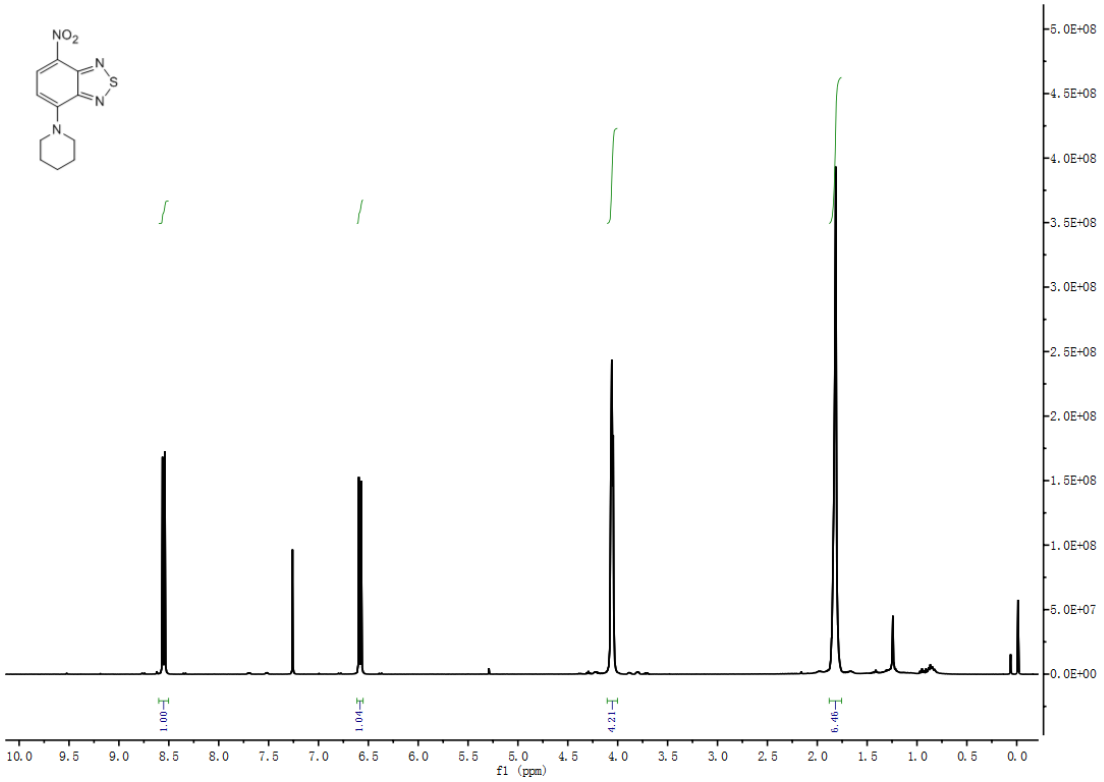
248.25
248.5
248.75
249
249.25
249.5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N
S
N
N
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4.5E+08
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2.0E+08
1.5E+08
1.0E+08
5.0E+07
0.0E+00
1.00
1.04
4.21
6.46
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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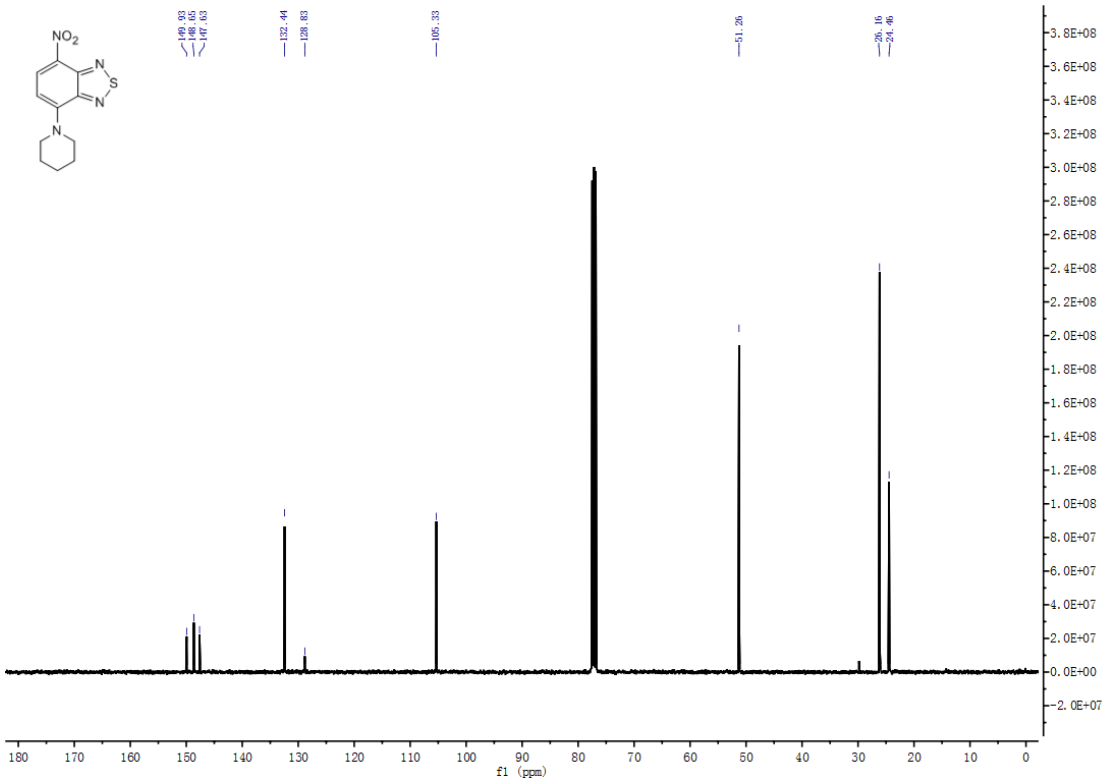
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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N
S
N
N
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148.65
147.63
132.44
128.83
105.33
51.26
26.16
24.46
3.8E+08
3.6E+08
3.4E+08
3.2E+08
3.0E+08
2.8E+08
2.6E+08
2.4E+08
2.2E+08
2.0E+08
1.8E+08
1.6E+08
1.4E+08
1.2E+08
1.0E+08
8.0E+07
6.0E+07
4.0E+07
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-2.0E+07
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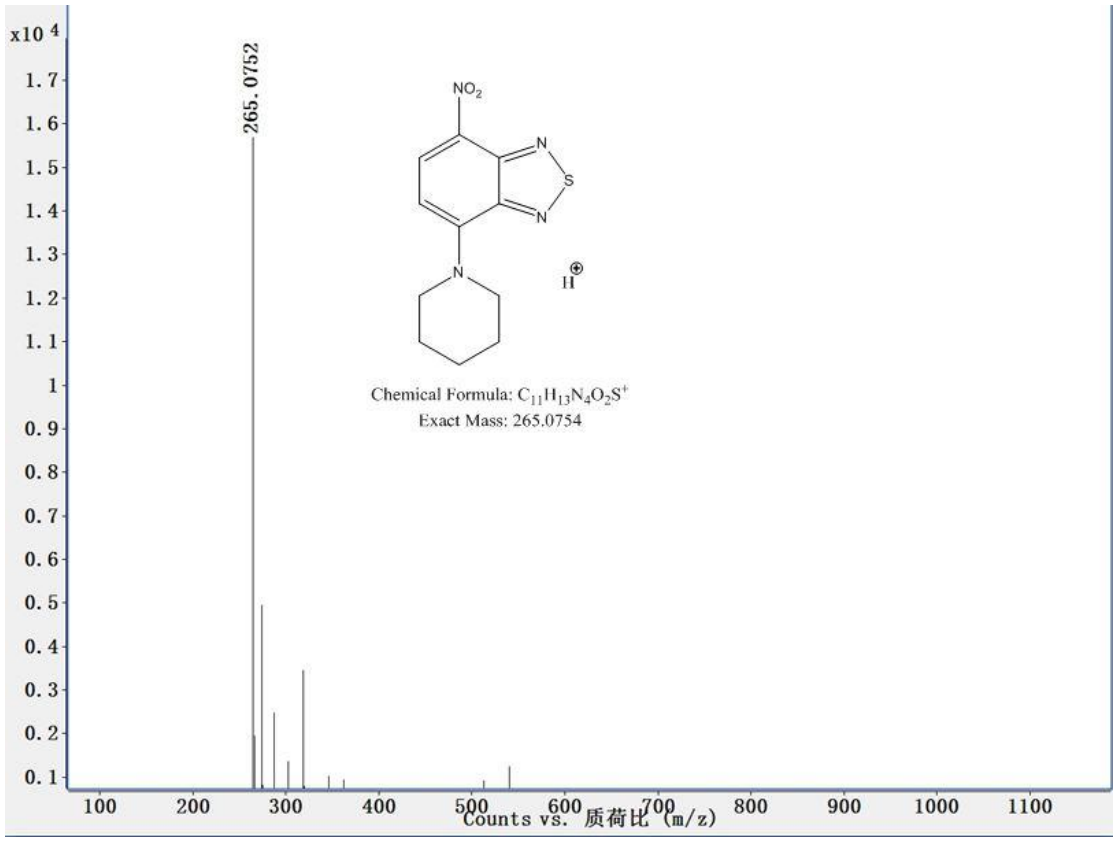
f1 (ppm)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1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
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0.6
0.5
0.4
0.3
0.2
0.1
265.0752
NO2
N
S
N
N
H
Chemical Formula: C11H13N4O2S+
Exact Mass: 265.0754
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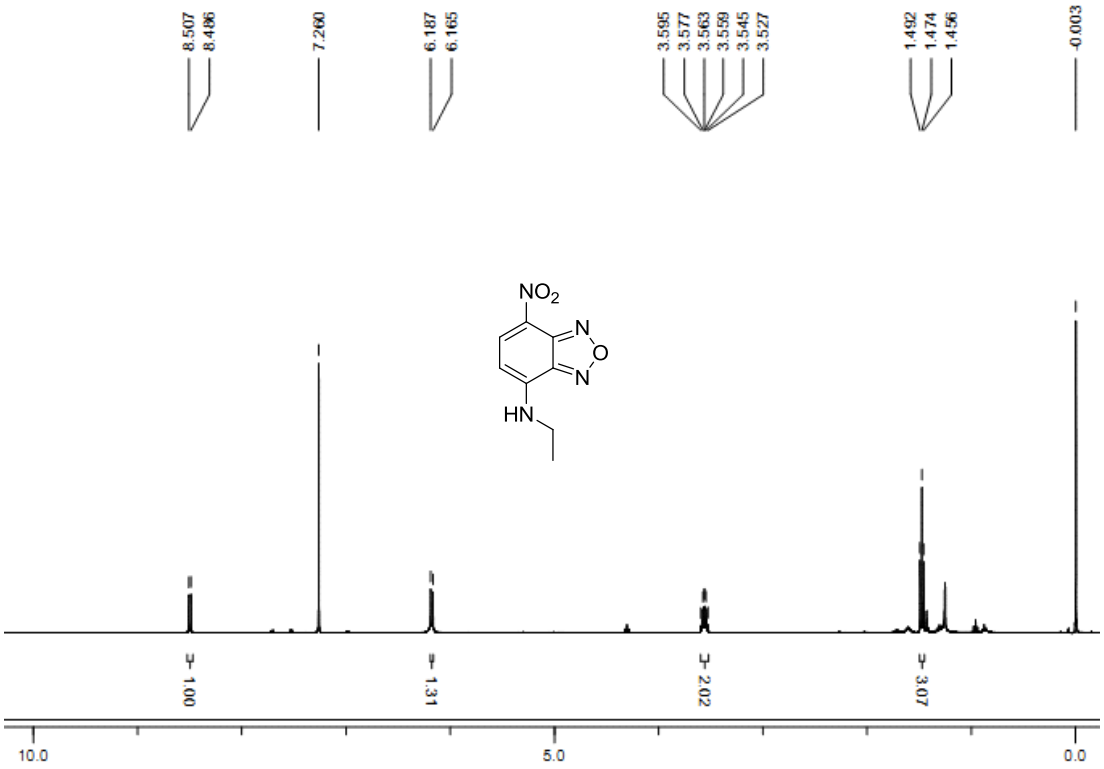
Counts vs. 质荷比 (m/z)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6.187
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O
N
HN
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2.02
3.07
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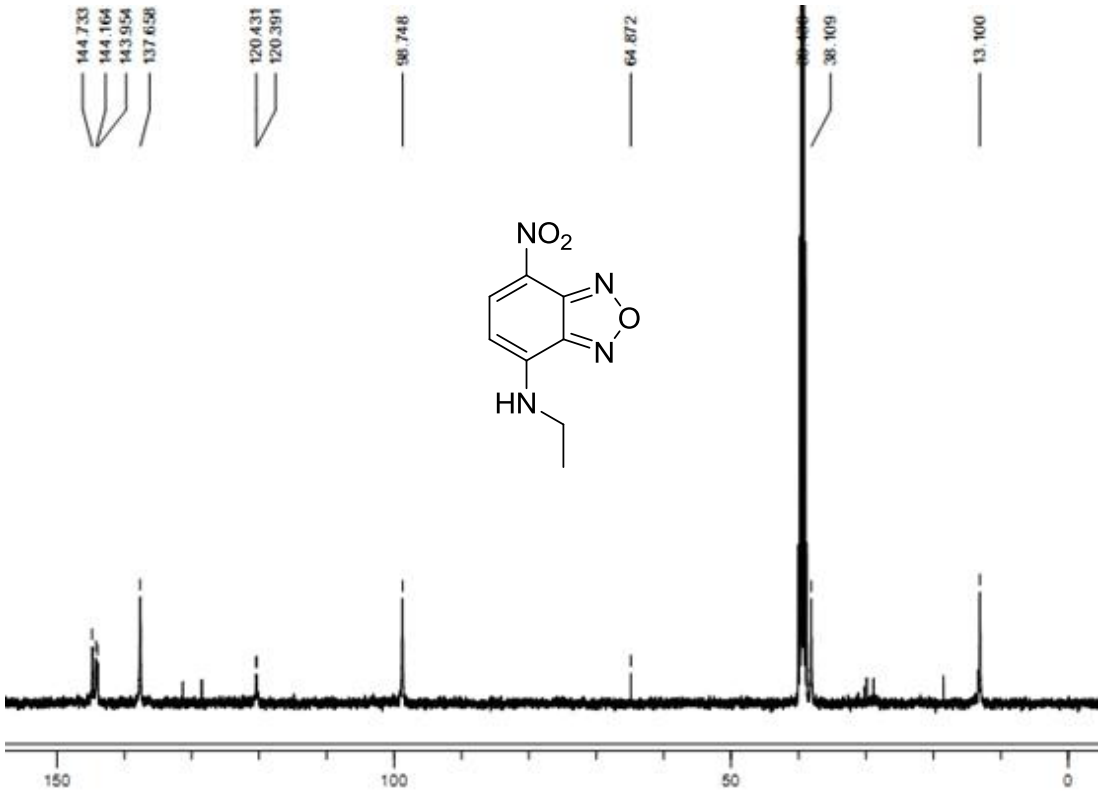
5.0
0.0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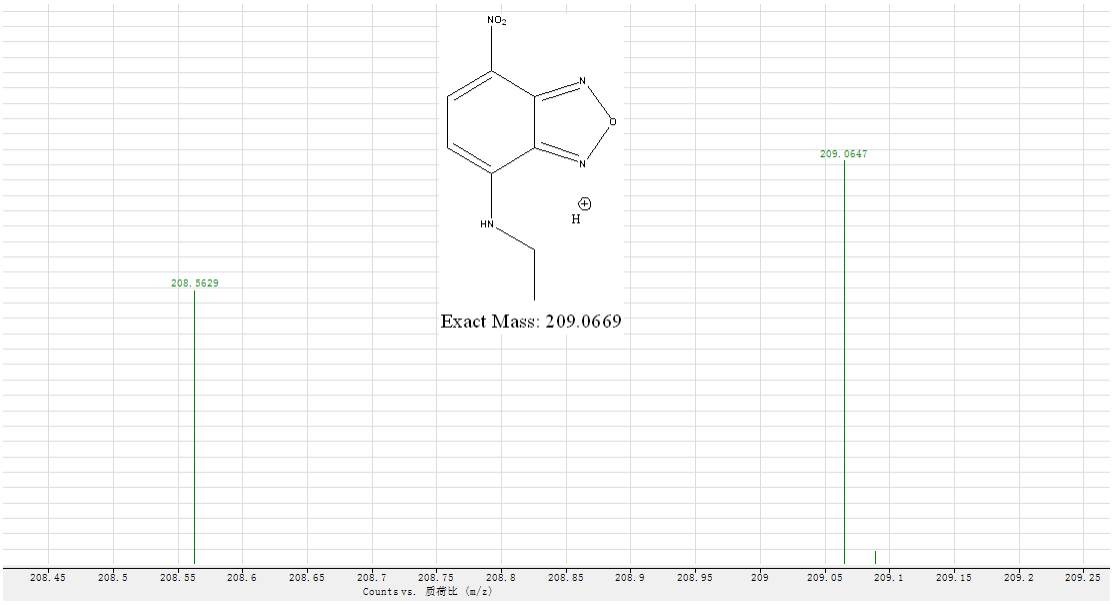
O
N
HN
150
100
50
0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Exact Mass: 209.0669
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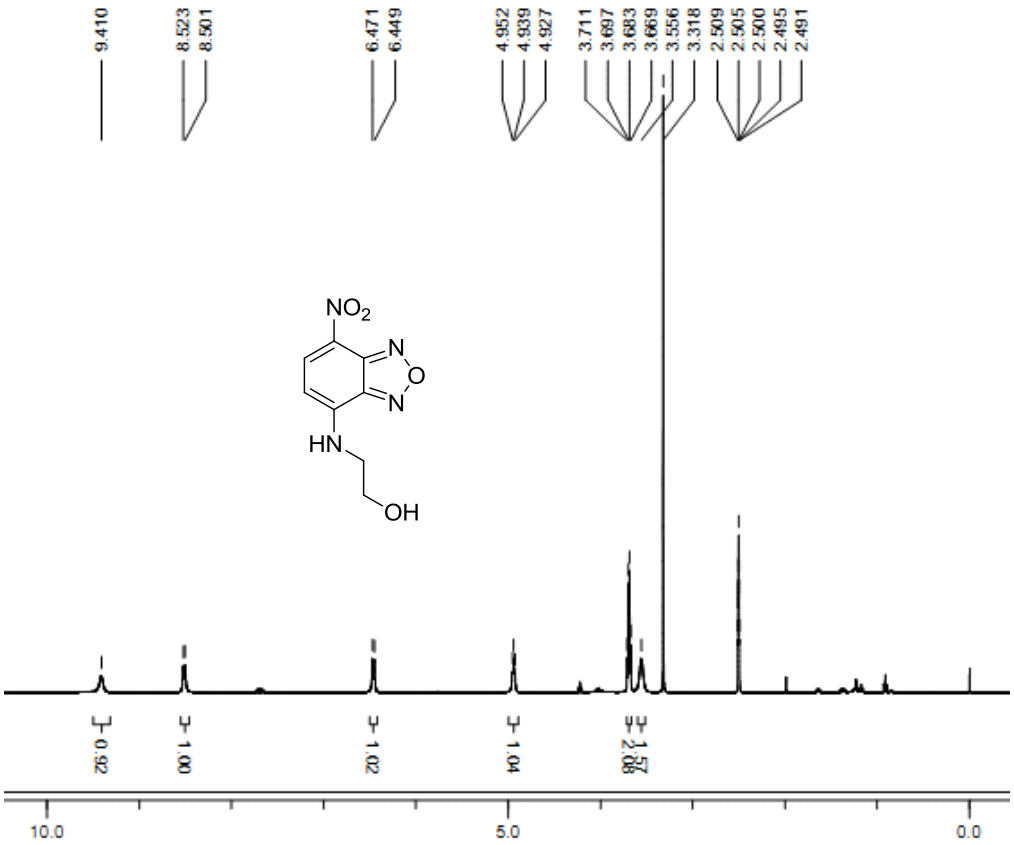
Counts vs. 质荷比 (m/z)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6.449
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3.697
3.683
3.669
3.556
3.318
2.509
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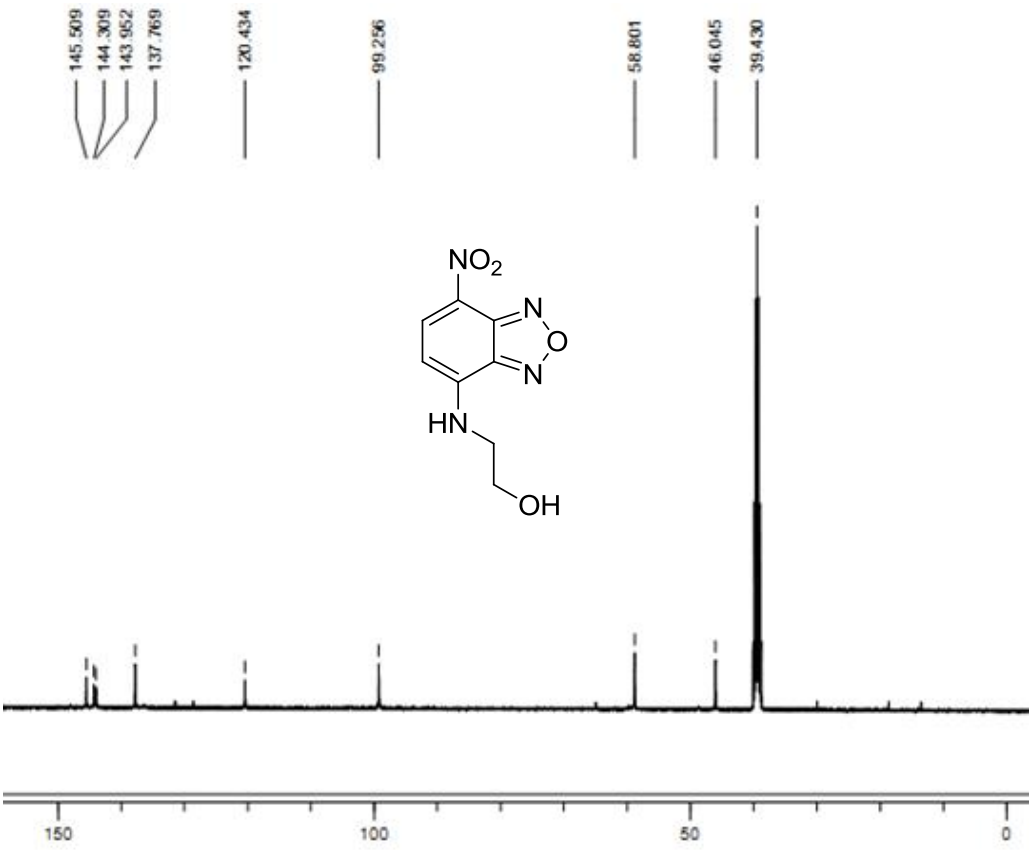
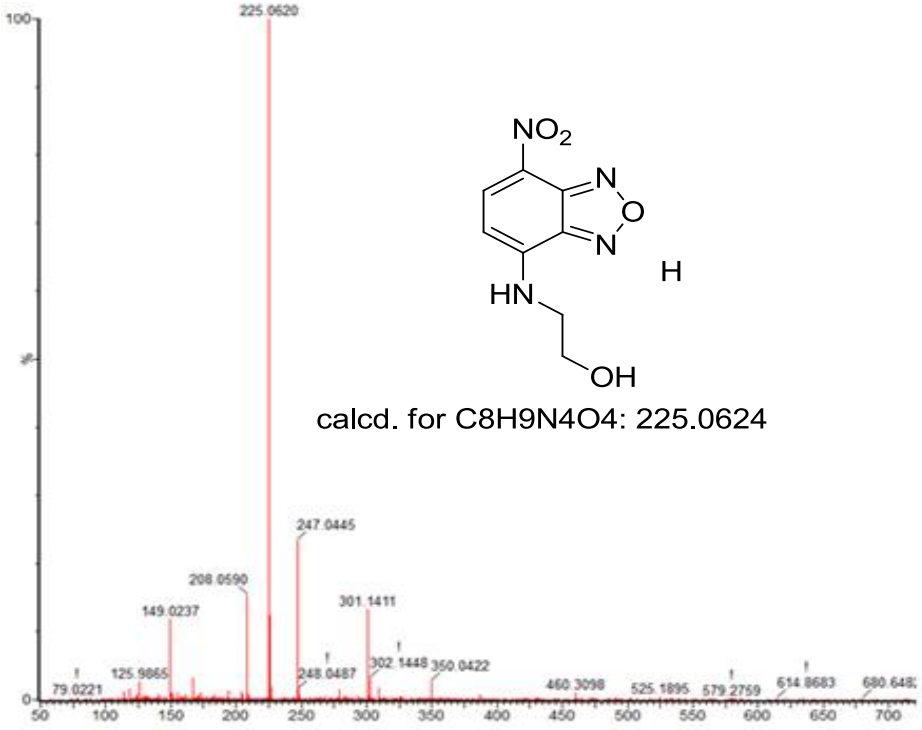
1.04
2.08
10.0
5.0
0.0





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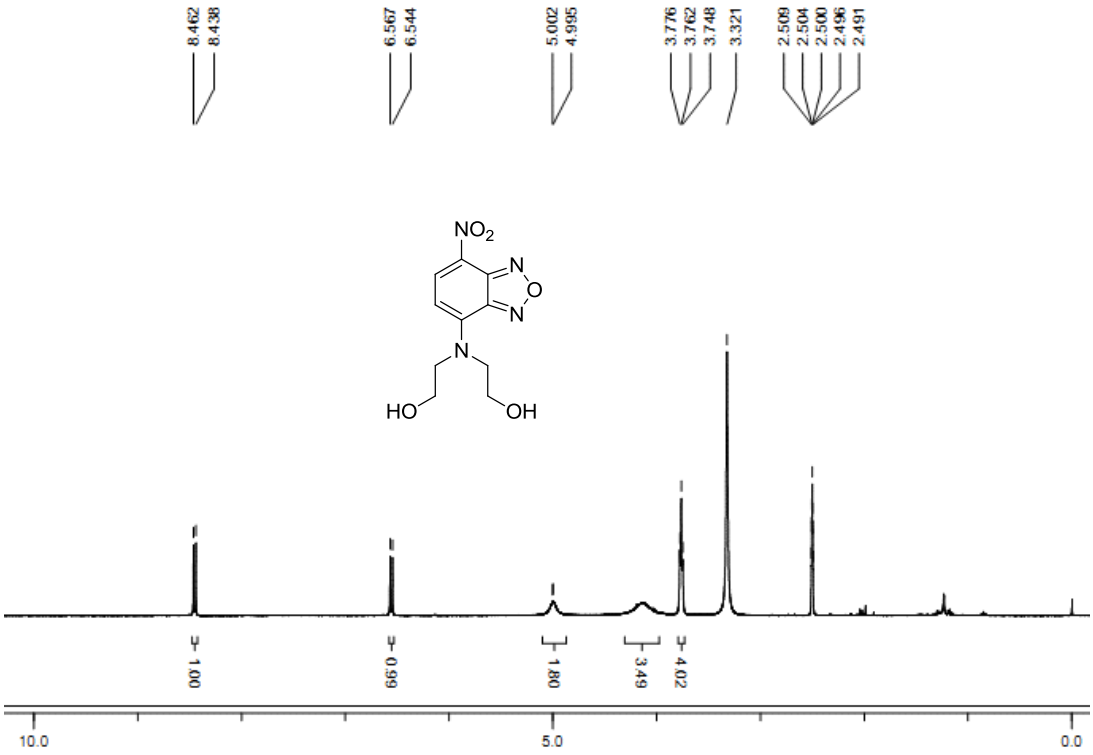
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6.544
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3.762
3.748
3.321
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2.504
2.500
2.496
2.491
NO2
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O
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N
HO
OH
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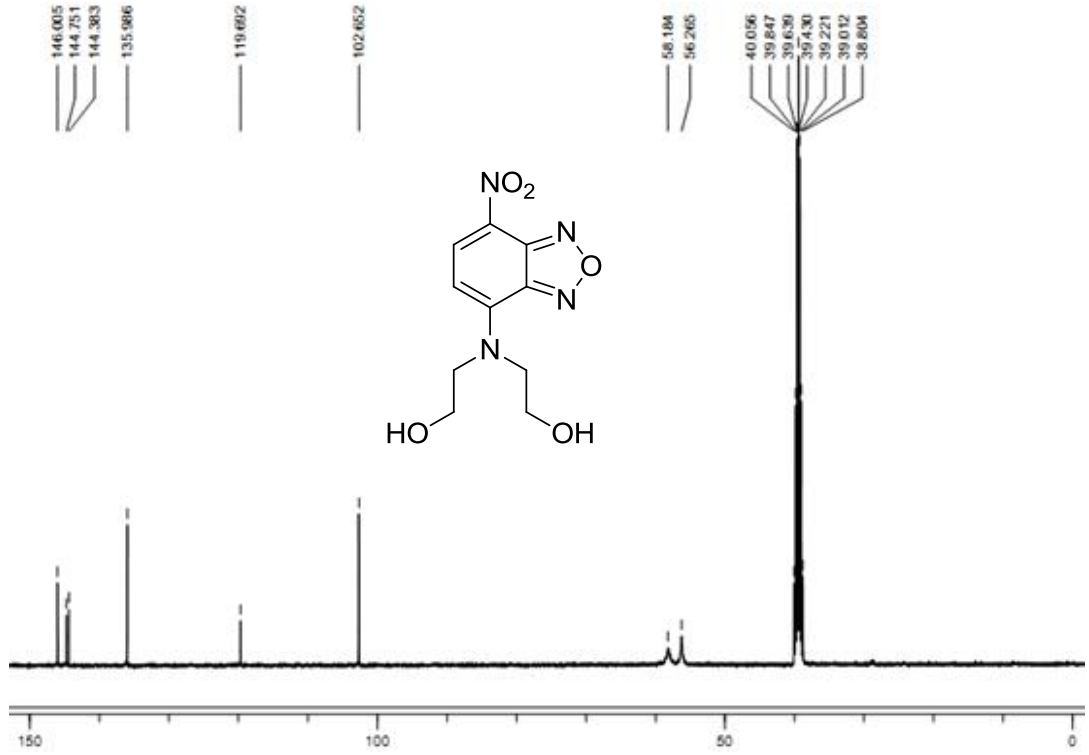
3.49
4.02
10.0
5.0
0.0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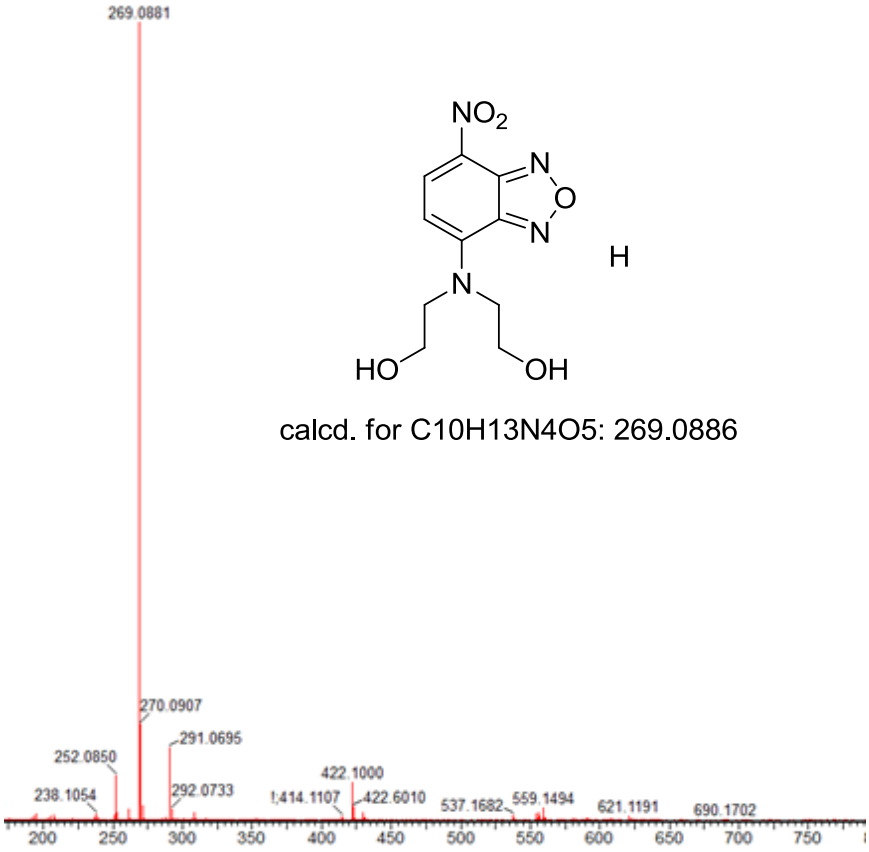
O
N
N
HO
OH
150
100
50
0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292.0733
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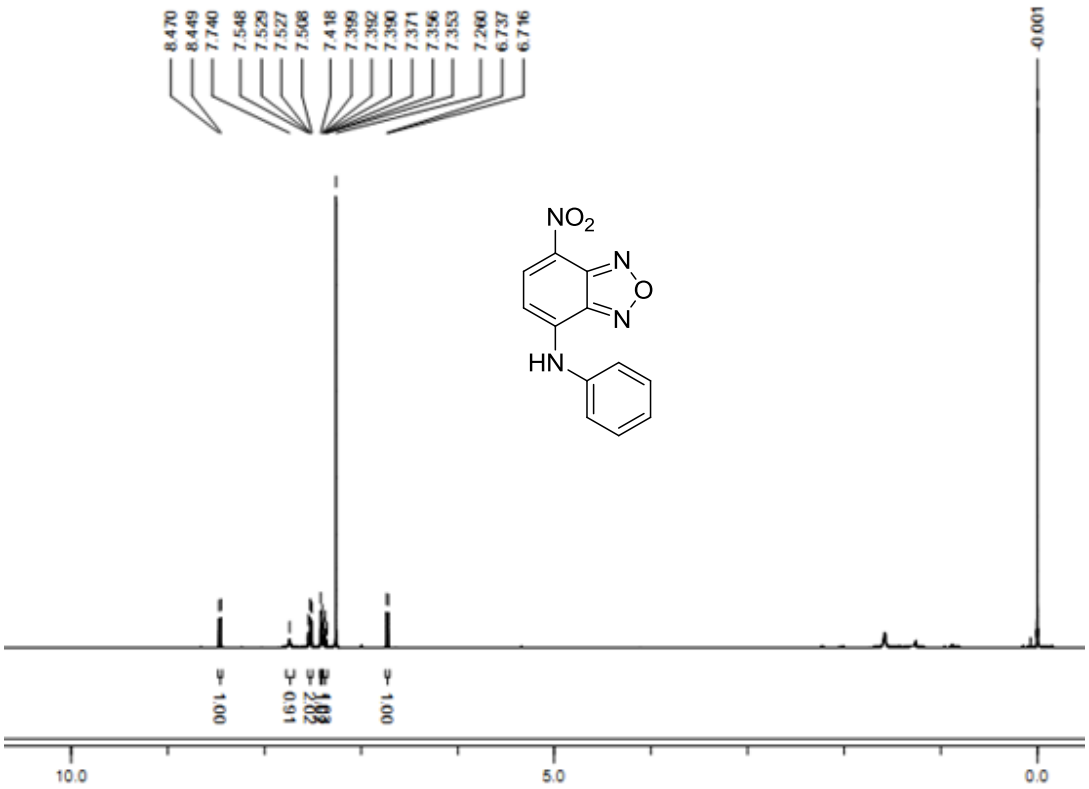
300
350
400
450
500
550
600
650
700
750


8.470
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7.740
7.548
7.529
7.527
7.508
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7.399
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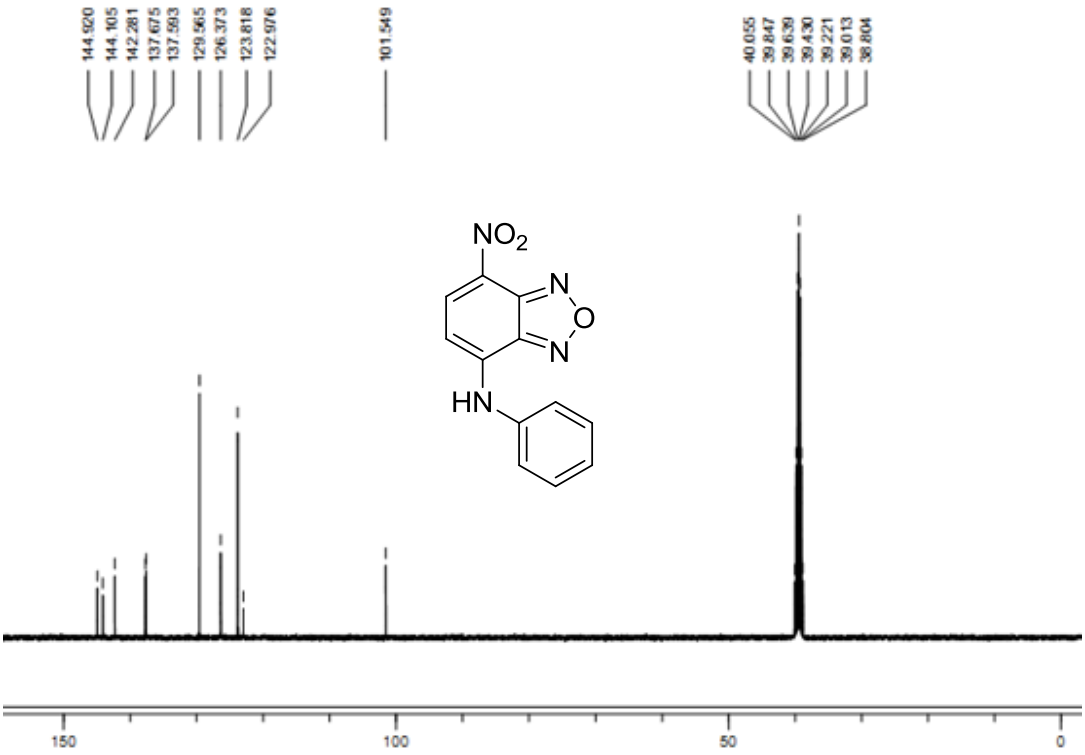
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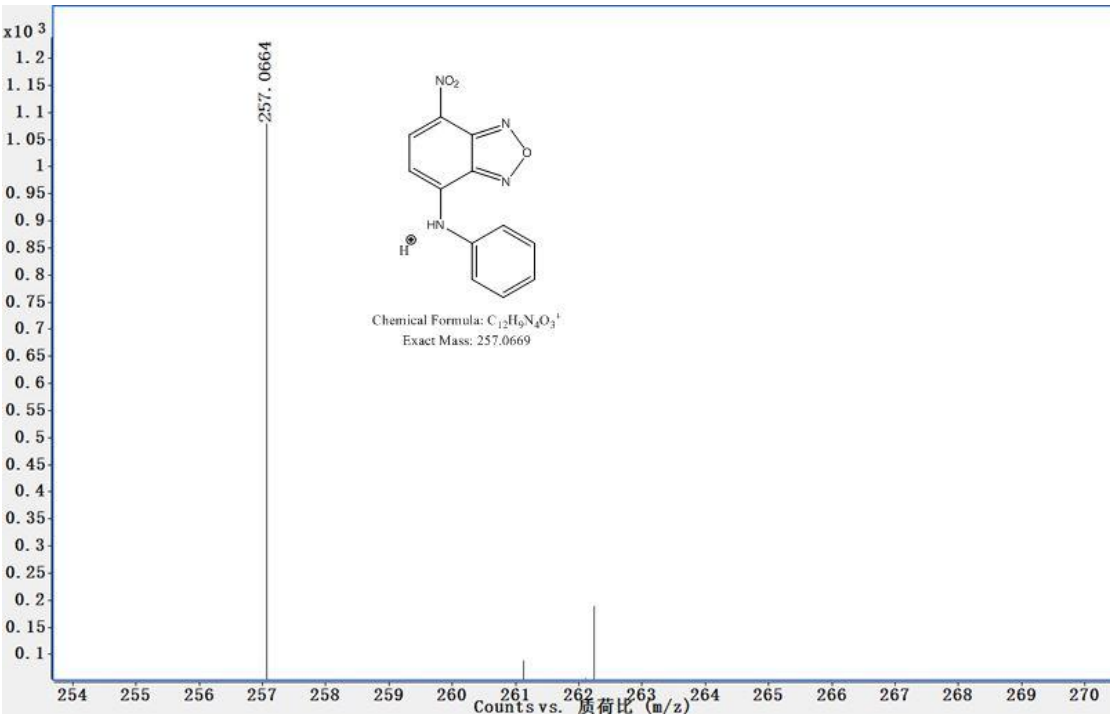
NO2
N
O
N
HN
150
100
50
0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1.15
1.1
1.05
1
0.95
0.9
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
257.0664
NO2
N
O
N
HN
H
Chemical Formula: C12H9N4O3+
Exact Mass: 257.0669
254
255
256
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258
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261
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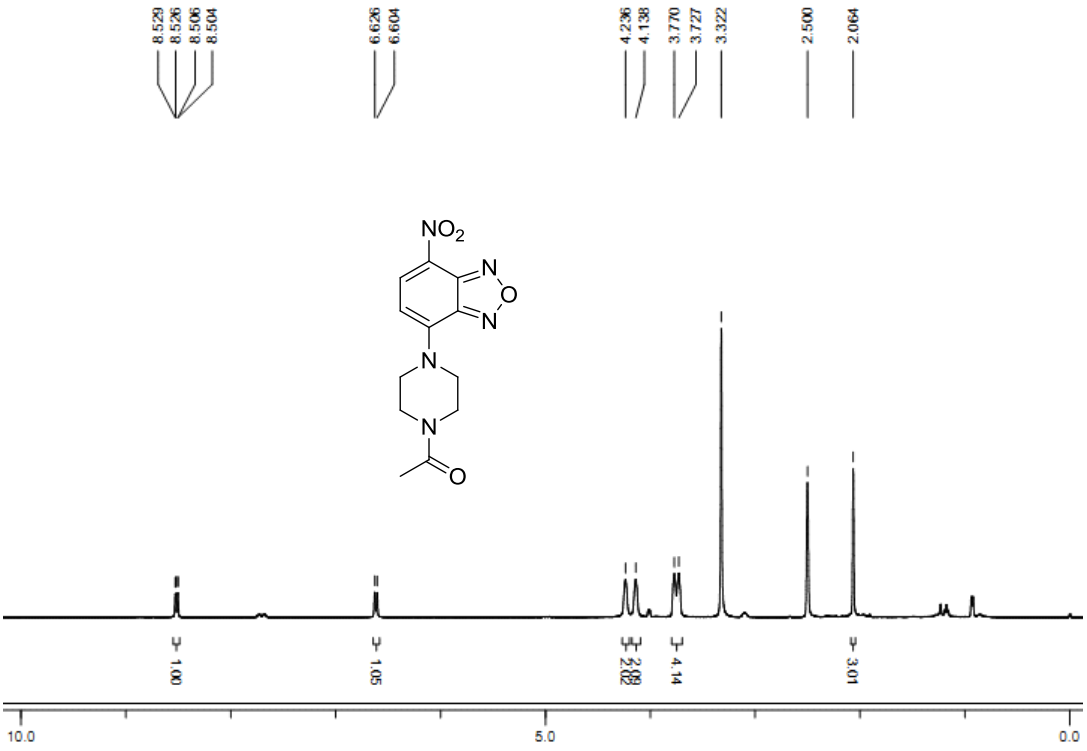
267
268
269
270
Counts vs. 质荷比 (m/z)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3.322
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NO2
N
O
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N
O
1.00
1.05
2.02
2.09
4.14
3.01
10.0
5.0
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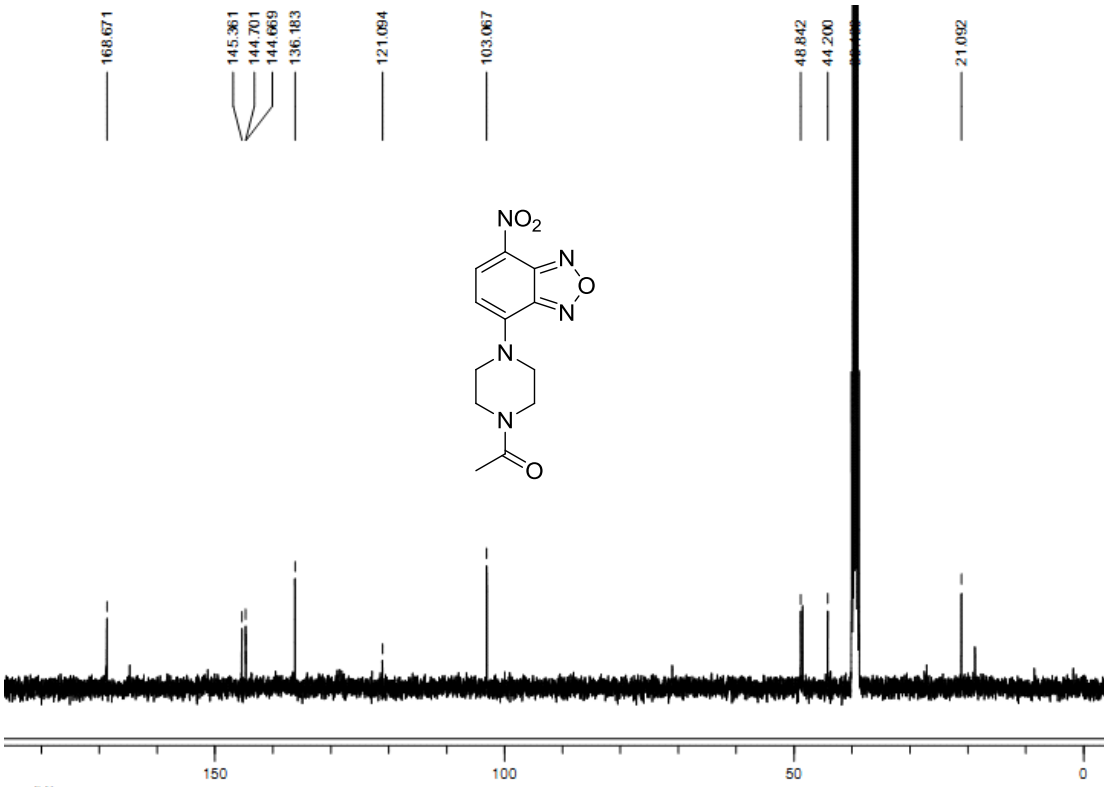
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144.669
136.183
121.094
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NO2
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O
N
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O
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100
50
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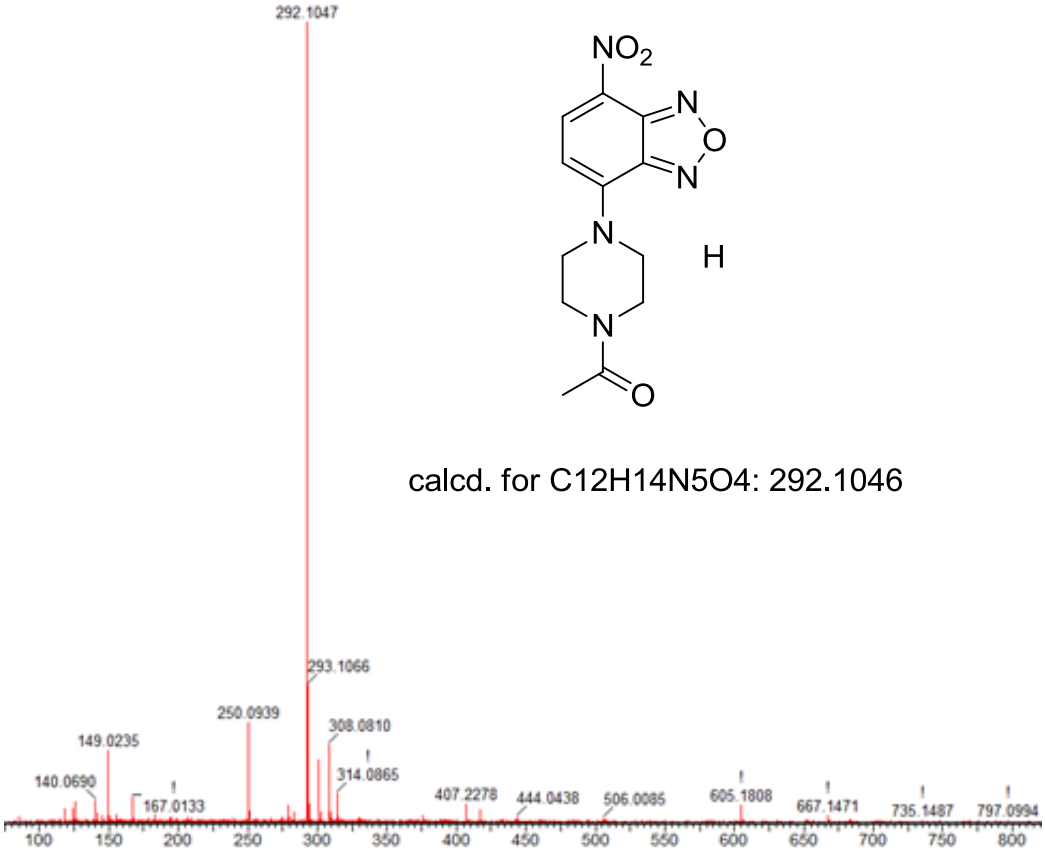
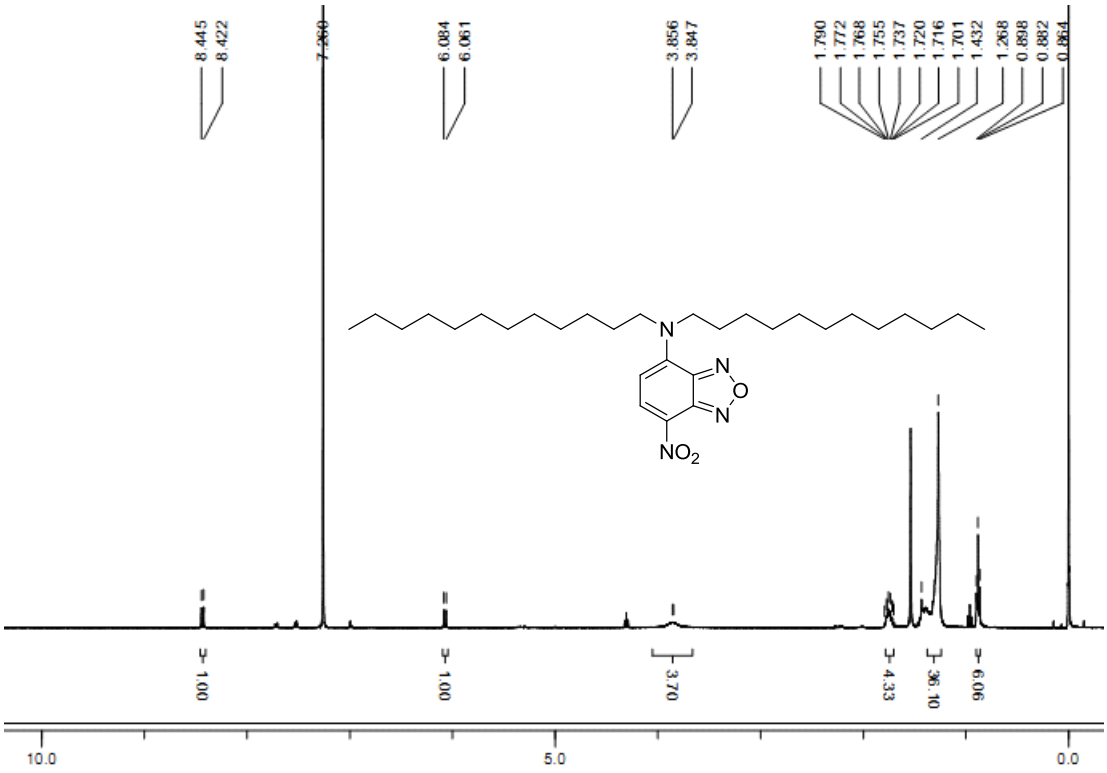
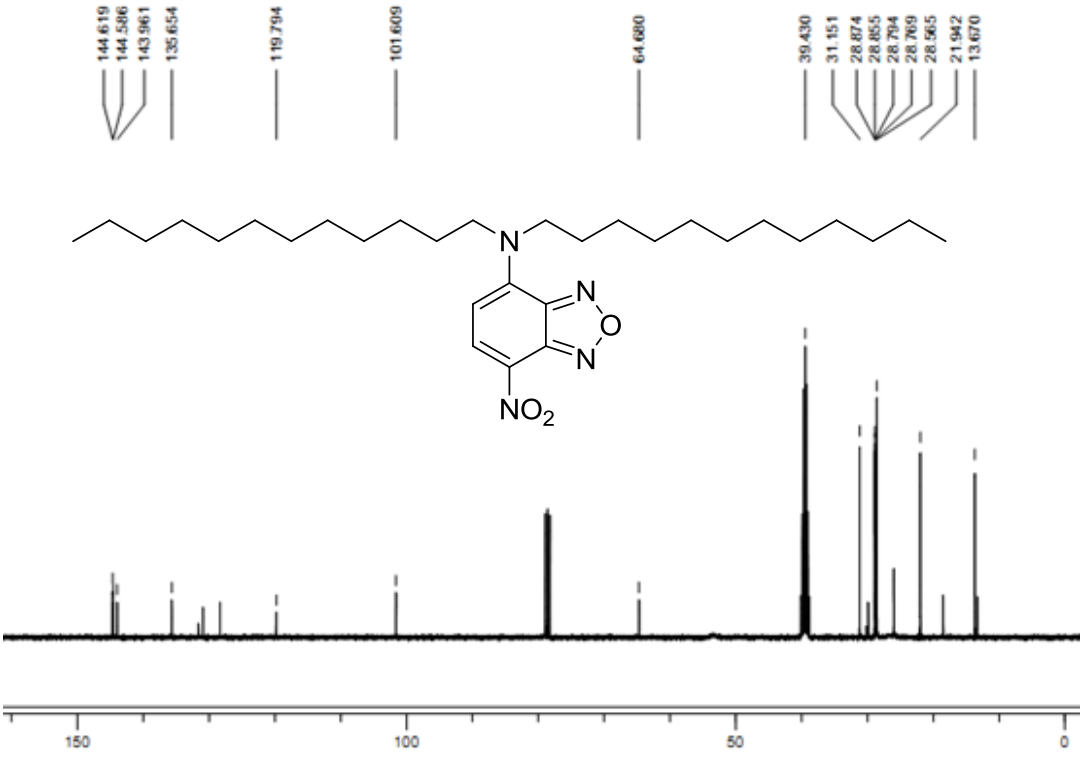
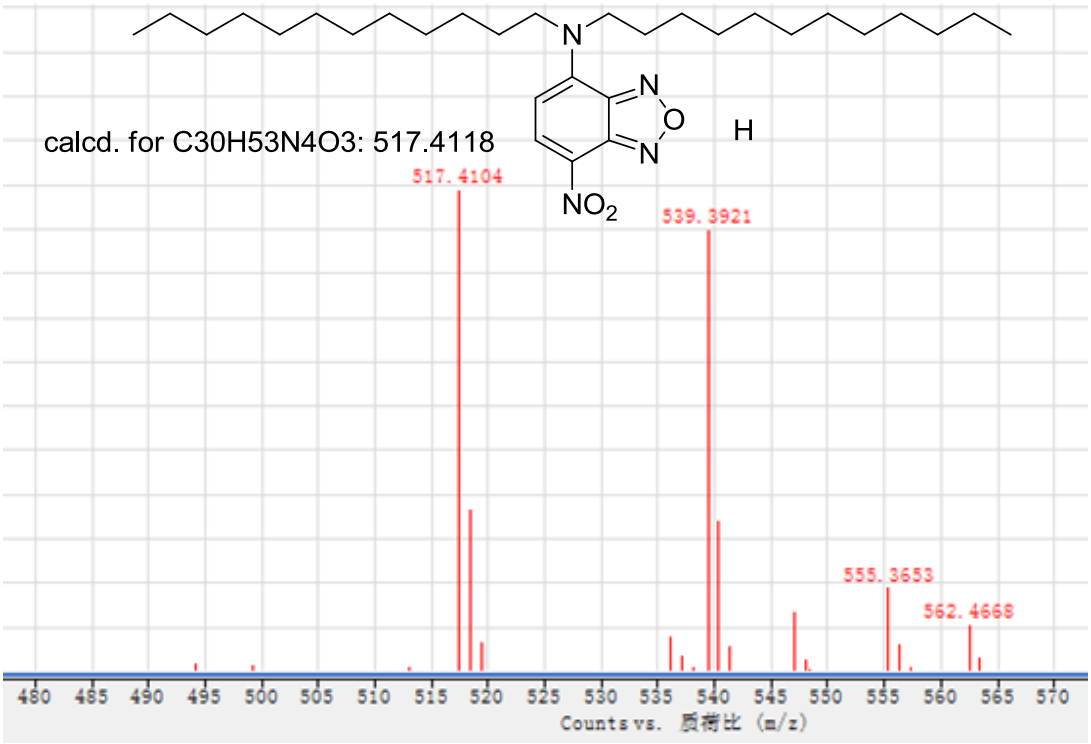




calcd. for C12H14N5O4: 292.1046


144.619
144.586
143.961
135.654
119.794
101.609
64.680
39.430
31.151
28.874
28.855
28.794
28.769
28.565
21.942
13.670
N
N
O
N
NO2
150
100
50
0


N
N
O
N
NO2
H
calcd. for C30H53N4O3: 517.4118
517.4104
539.3921
555.3653
562.4668
480 485 490 495 500 505 510 515 520 525 530 535 540 545 550 555 560 565 570
Counts vs. 质荷比 (m/z)