Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting information

Dual-Site Lysosome-Targeted Fluorescent Probe for Separate Detection of Endogenous Biothiols and SO_2 in Living Cells

Ming-Yu Wu*[a], Yue Wang[a], Yan-Hong Liu[b], Xiao-Qi Yu*[b]

a School of Life Science and Engineering, Southwest Jiaotong University, Chengdu 610031, China. E-mail: wumy1050hx@swjtu.edu.cn.

b Key Laboratory of Green Chemistry and Technology, Ministry of Education, College of Chemistry, Sichuan University, Chengdu, 610064, China. E-mail: xqyu@scu.edu.cn.

Table of contents

1. Optical Properties

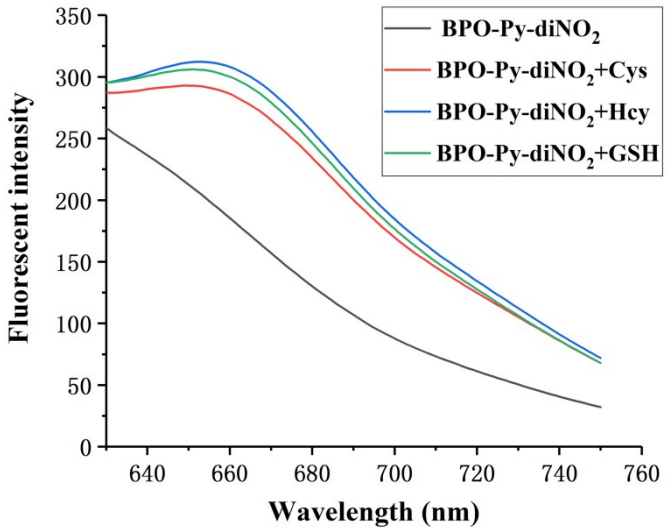


Fig. S1 Fluorescent spectra changes of **BPO-Py-diNO$_2$** (10 μM) interacting with 500 μM biothiols in near-infrared region. λ_{ex} = 556 nm. Slit: 10 nm/10 nm.

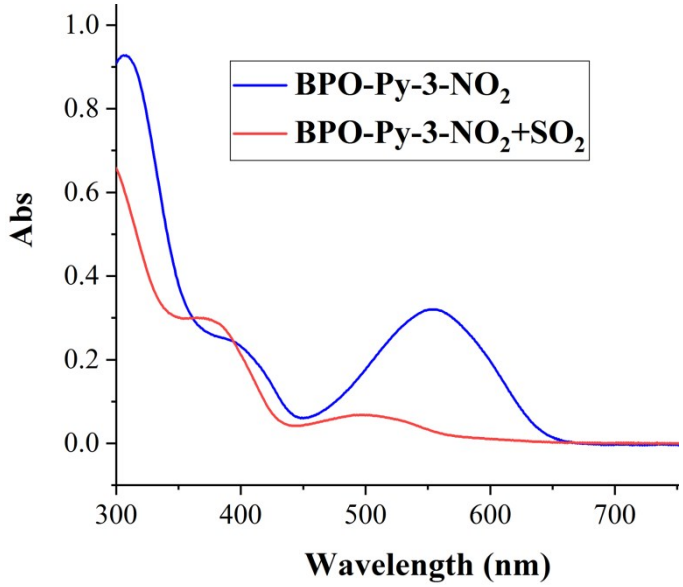


Fig. S2 Absorption spectra of **BPO-Py-3-NO$_2$** (10 μM) before and after intreacting with 500 μM SO_2.

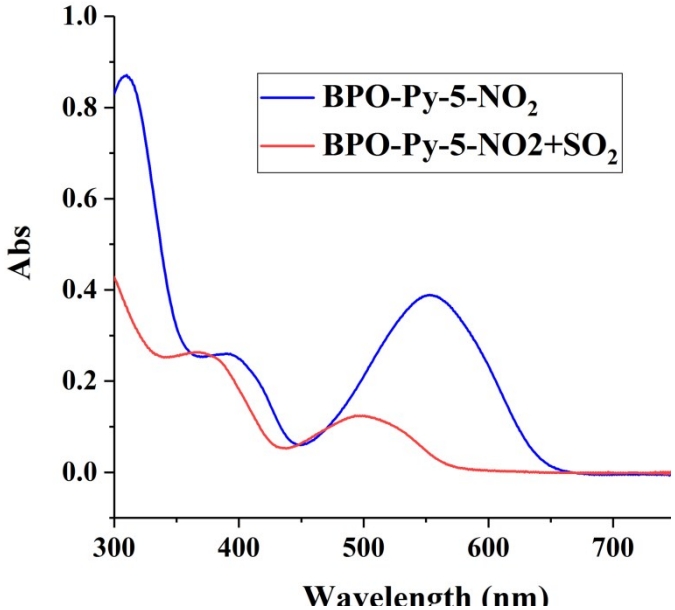


Fig. S3 Absorption spectra of **BPO-Py-5-NO_2** (10 μM) before and after intreacting with 500 μM SO_2.

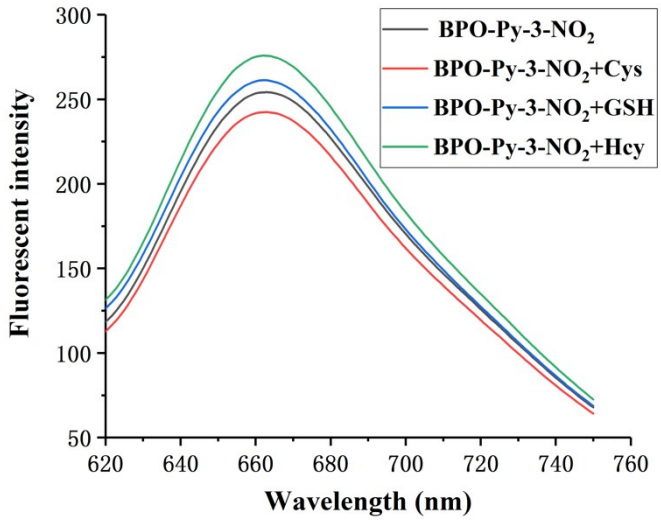


Fig. S4 Fluorescent spectra changes of **BPO-Py-3-NO_2** (10 μM) interacting with 500 μM biothiols in near-infrared region. λ_{ex} = 556 nm. Slit: 10 nm/10 nm.

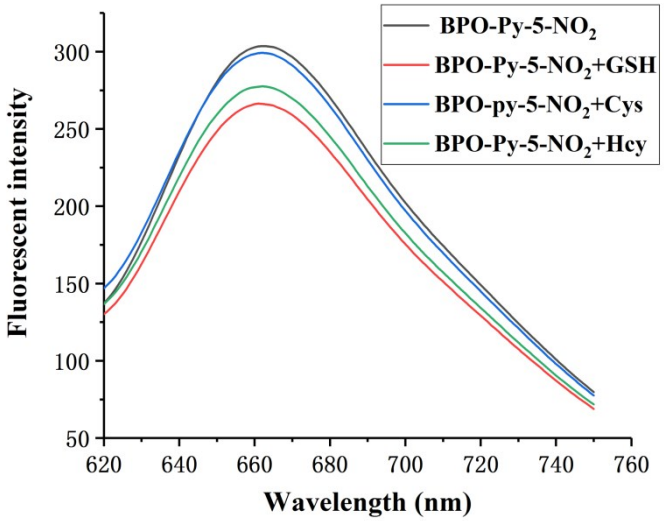


Fig. S5 Fluorescent spectra changes of **BPO-Py-5-NO$_2$** (10 μM) interacting with 500 μM biothiols in near-infrared region. λ_{ex} = 556 nm. Slit: 10 nm/10 nm.

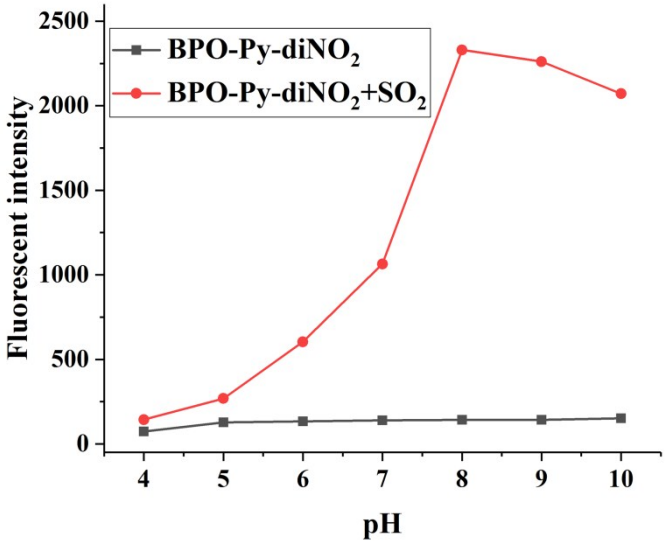


Fig. S6 pH dependent fluorescent changes of **BPO-Py-diNO$_2$** (10 μM) and **BPO-Py-diNO$_2$** (10 μM) interacting with SO_2 (500 μM). λ_{ex} = 390 nm, λ_{em} = 495 nm, Slit: 5 nm/5 nm.

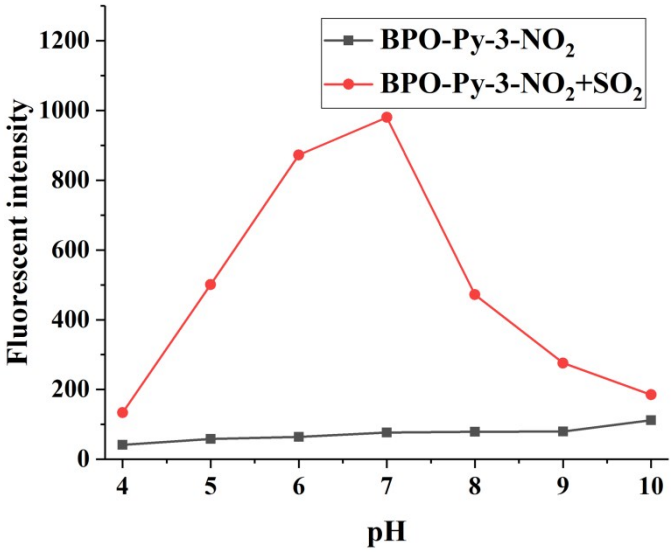


Fig. S7 pH dependent fluorescent changes of **BPO-Py-3-NO_2** (10 μM) and **BPO-Py-3-NO_2** (10 μM) interacting with SO_2 (500 μM). λ_{ex} = 390 nm, λ_{em} = 495 nm, Slit: 5 nm/5 nm.

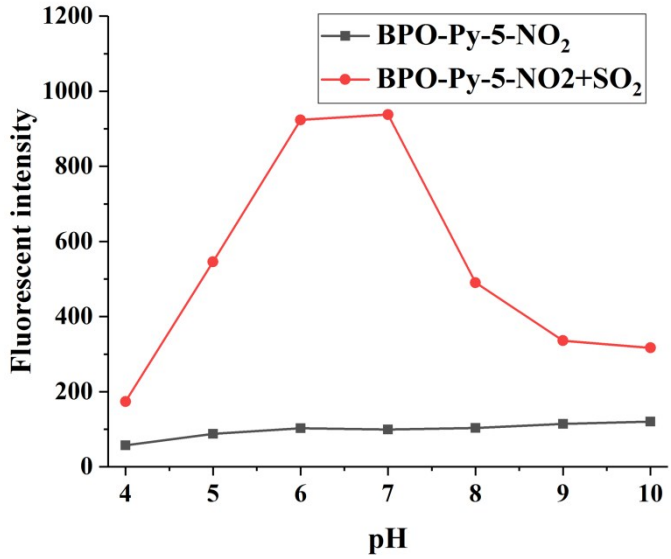


Fig. S8 pH dependent fluorescent changes of **BPO-Py-5-NO_2** (10 μM) and **BPO-Py-5-NO_2** (10 μM) interacting with SO_2 (500 μM). λ_{ex} = 390 nm, λ_{em} = 495 nm, Slit: 5 nm/5 nm.

Table S1 The quantum yields.

Content	Quantum yields	Detection limit (nM)
BPO-Py-diNO$_2$ (green channel)	0.003	150
BPO-Py-diNO$_2$+SO_2 (green channel)	0.130	
BPO-Py-3-NO$_2$ (green channel)	0.002	66
BPO-Py-3-NO$_2$+SO_2 (green channel)	0.055	
BPO-Py-5-NO$_2$ (green channel)	0.006	298
BPO-Py-5-NO$_2$+SO_2 (green channel)	0.05	
BPO-Py-diNO$_2$ (near-infrared channel)	0.0004	202
BPO-Py-diNO$_2$+GSH (near-infrared channel)	0.0015	

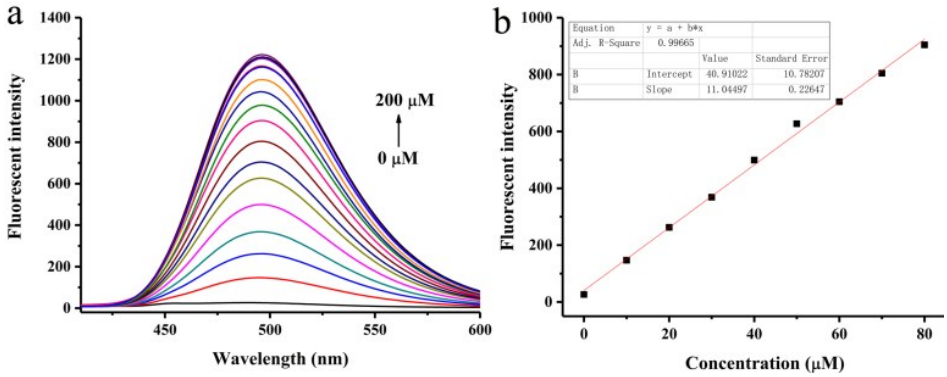


Fig. S9 (a) Fluorescence titration of **BPO-Py-3-NO$_2$** (10 μM) with SO_3^{2-} (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200 μM) (λ_{ex} = 390 nm, Slit: 5 nm/5 nm) in a 10 mM PBS:DMSO = 8:2 pH 7.0 buffer solution. (b) Linear fit of fluorescence intensity changes with Na_2SO_3.

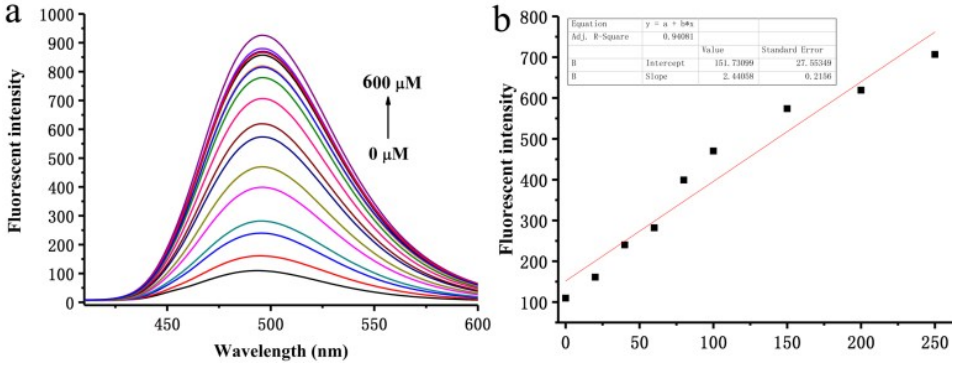


Fig. S10 (a) Fluorescence titration of **BPO-Py-5-NO$_2$** (10 μM) with SO_3^{2-} (0, 20, 40, 60, 80, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600 μM) (λ_{ex} = 390nm, Slit: 5 nm/5 nm) in a 10 mM PBS:DMSO = 8:2 pH 7.0 buffer solution. (b) Linear fit of fluorescence intensity changes with Na_2SO_3.

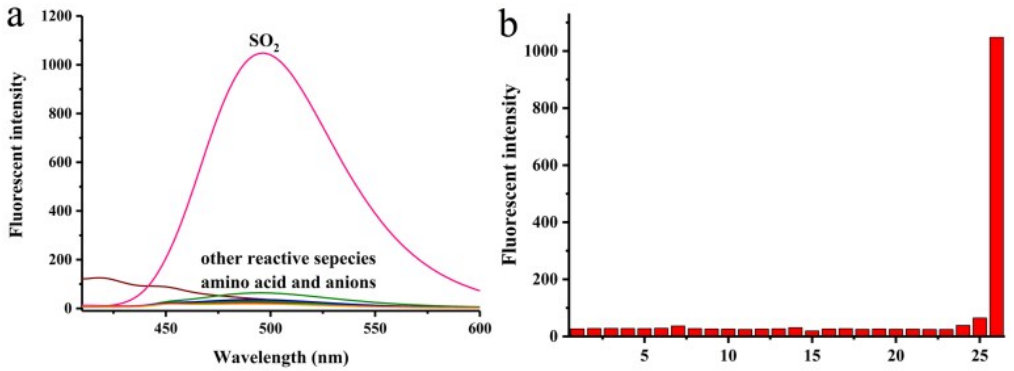


Fig. S11 Fluorescence spectrum (a) and intensity (b) changes of **BPO-Py-3-NO_2** (10 μM) interacting with 500 μM amino acids (Arg, Met, Ser, Asp, Gly, Ala, His, Val, Lys, Leu, Glu, Pro, Ile, Phe), reactive oxygen species (H_2O_2, NaClO, TBHP), reactive nitrogen species (NO_3^-, NO_2^-) and reactive sulfur species (SO_4^{2-}, Cys, Hcy, GSH, H_2S, SO_2) in 10 mM pH 7.0 PBS buffer solution containing 20% DMSO, λ_{ex} = 390 nm. Slit: 5 nm/5 nm. 1. **BPO-Py-3-NO_2**, 2. Ala, 3. Arg. 4. Asp, 5. Glu, 6. Gly, 7. His, 8. Ile, 9. Leu, 10. Lys, 11. Met, 12. Phe, 13. Pro, 14, Ser, 15. Val, 16. H_2O_2, 17, NaClO, 18. TBHP, 19. NO_3^-, 20. NO_2^-, 21. SO_4^{2-}, 22. Cys, 23. Hcy, 24. GSH, 25. H_2S, 26. SO_2.

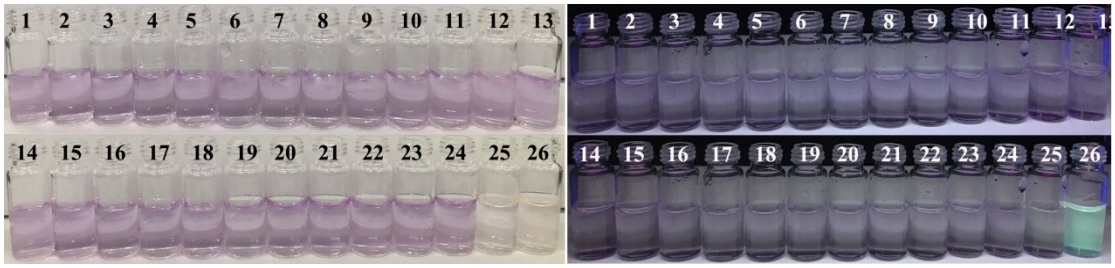


Fig. S12 The colour and fluorescence changes of **BPO-Py-3-NO2** (10 μM) upon addition of 50 equiv. of different species under visible light and UV lamp (E_x = 365 nm). 1. **BPO-Py-3-NO_2**, 2. Ala, 3. Arg. 4. Asp, 5. Glu, 6. Gly, 7. His, 8. Ile, 9. Leu, 10. Lys, 11. Met, 12. Phe, 13. Pro, 14, Ser, 15. Val, 16. H_2O_2, 17, NaClO, 18. TBHP, 19. NO_3^-, 20. NO_2^-, 21. SO_4^{2-}, 22. Cys, 23. Hcy, 24. GSH, 25. H_2S, 26. SO_2.

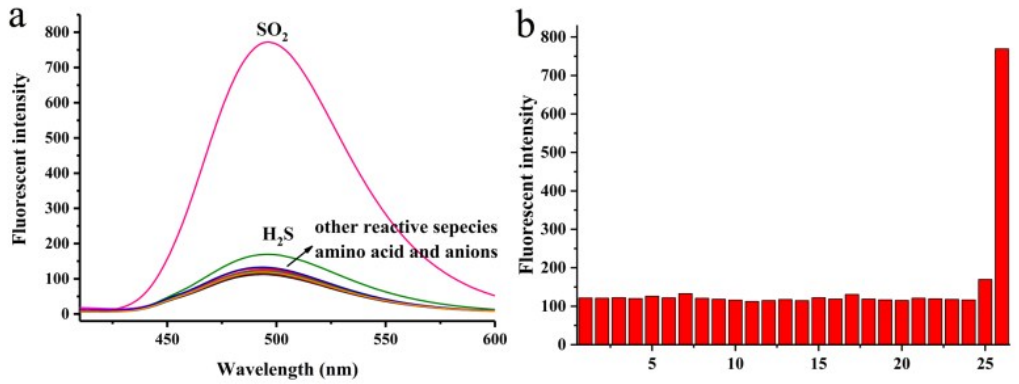


Fig. S13 Fluorescence spectrum (a) and intensity (b) changes of **BPO-Py-5-NO_2** (10 μM) interacting with 500 μM amino acids (Arg, Met, Ser, Asp, Gly, Ala, His, Val, Lys, Leu, Glu, Pro, Ile, Phe), reactive oxygen species (H_2O_2, NaClO, TBHP), reactive nitrogen species (NO_3^-, NO_2^-) and reactive sulfur species (SO_4^{2-}, Cys, Hcy, GSH, H_2S, SO_2) in 10 mM pH 7.0 PBS buffer solution containing 20% DMSO, λ_{ex} = 390 nm. Slit: 5 nm/5 nm. 1. **BPO-Py-5-NO_2**, 2. Ala, 3. Arg. 4. Asp, 5. Glu, 6. Gly, 7. His, 8. Ile, 9. Leu, 10. Lys, 11. Met, 12. Phe, 13. Pro, 14, Ser, 15. Val, 16. H_2O_2, 17, NaClO, 18. TBHP, 19. NO_3^-, 20. NO_2^-, 21. SO_4^{2-}, 22. Cys, 23. Hcy, 24. GSH, 25. H_2S, 26. SO_2.

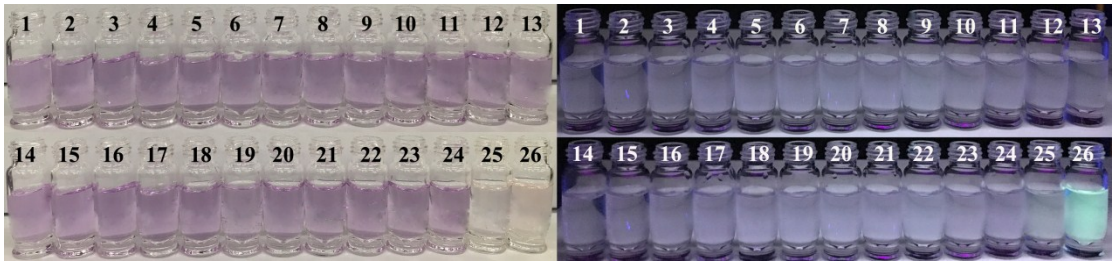


Fig. S14 The colour and fluorescence changes of **BPO-Py-5-NO_2** (10 μM) upon addition of 50 equiv. of different species under visible light and UV lamp (E_x = 365 nm). 1. **BPO-Py-5-NO_2**, 2. Ala, 3. Arg. 4. Asp, 5. Glu, 6. Gly, 7. His, 8. Ile, 9. Leu, 10. Lys, 11. Met, 12. Phe, 13. Pro, 14, Ser, 15. Val, 16. H_2O_2, 17, NaClO, 18. TBHP, 19. NO_3^-, 20. NO_2^-, 21. SO_4^{2-}, 22. Cys, 23. Hcy, 24. GSH, 25. H_2S, 26. SO_2.

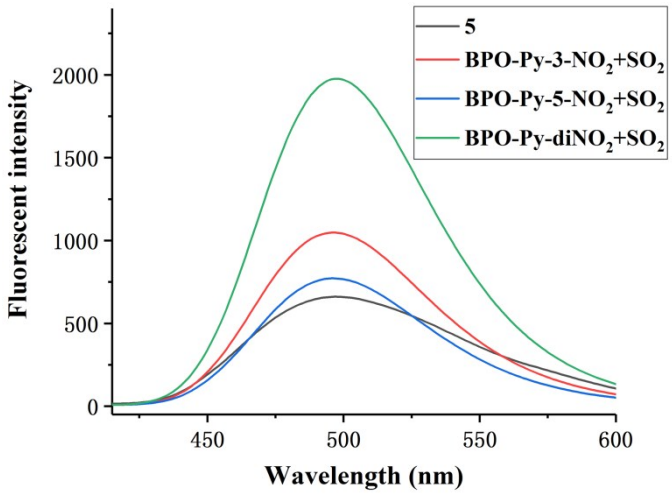


Fig. S15 Fluorescent spectra of compound **5** and probes interacting with SO_2. λ_{ex} = 390 nm. Slit: 5 nm/5 nm.

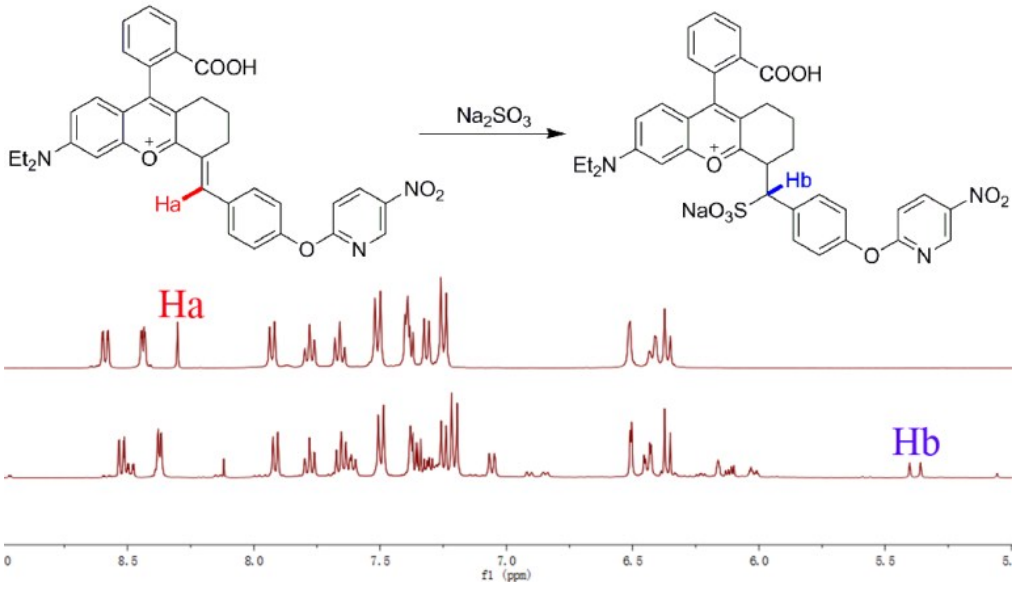


Fig. S16 ^{1}H NMR spectra of 5 mM **BPO-Py-5-NO$_2$** and 5 mM **BPO-Py-5-NO$_2$** with 10 equiv. sulfite in the mixture solvent of DMSO-d_6: D_2O = 3:2

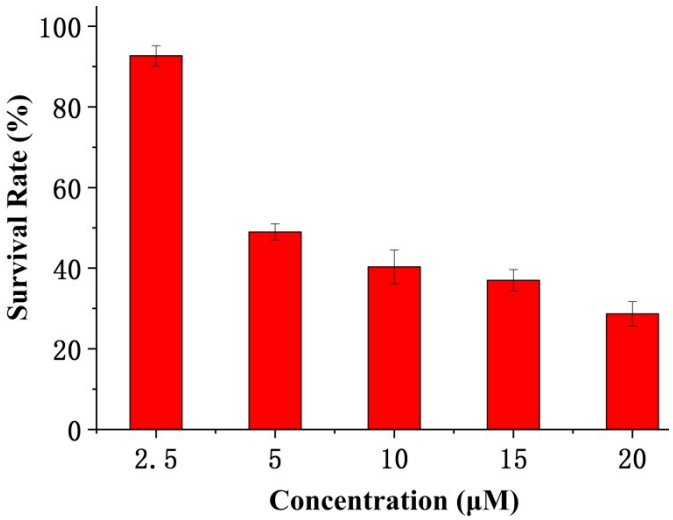


Fig. S17 Cell viability of **BPO-Py-3-NO_2** by a standard CCK-8 assay in living Hela cells for 24 h. The experiment was repeated three times (±S.D.).

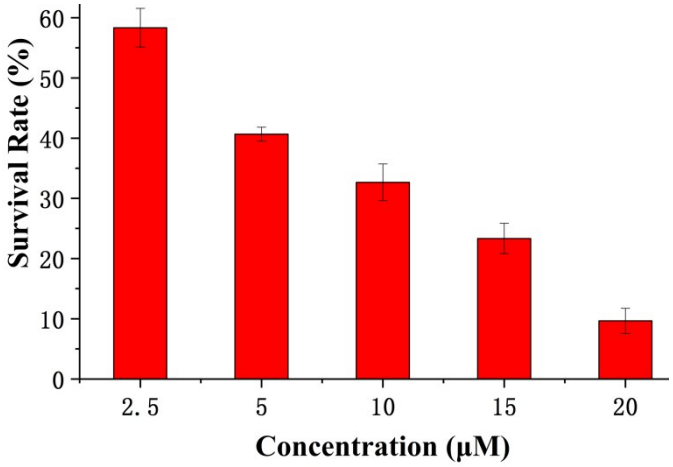


Fig. S18 Cell viability of **BPO-Py-5-NO_2** by a standard CCK-8 assay in living Hela cells for 24 h. The experiment was repeated three times (±S.D.).

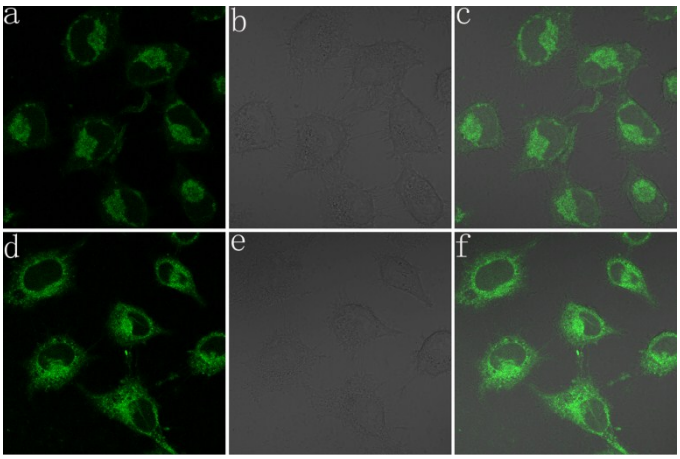


Fig. S19 Confocal images of SO_2 in living Hela cells. (a-c) Hela cells incubated with 10 μM **BPO-Py-3-NO_2** for 30 min; (d-f) Hela pretreated with 10 μM **BPO-Py-3-NO_2** for 30 min, and then further incubated with 500 μM Na_2SO_3 for another 90 min. The fluorescence imaging was collected from 450−470 nm with excitation at 405 nm.

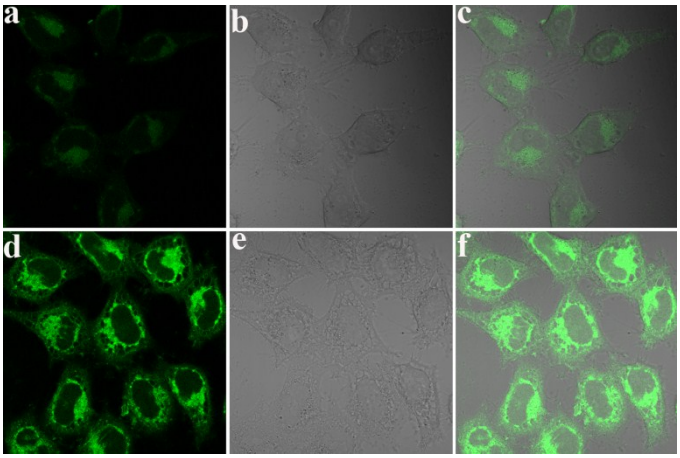
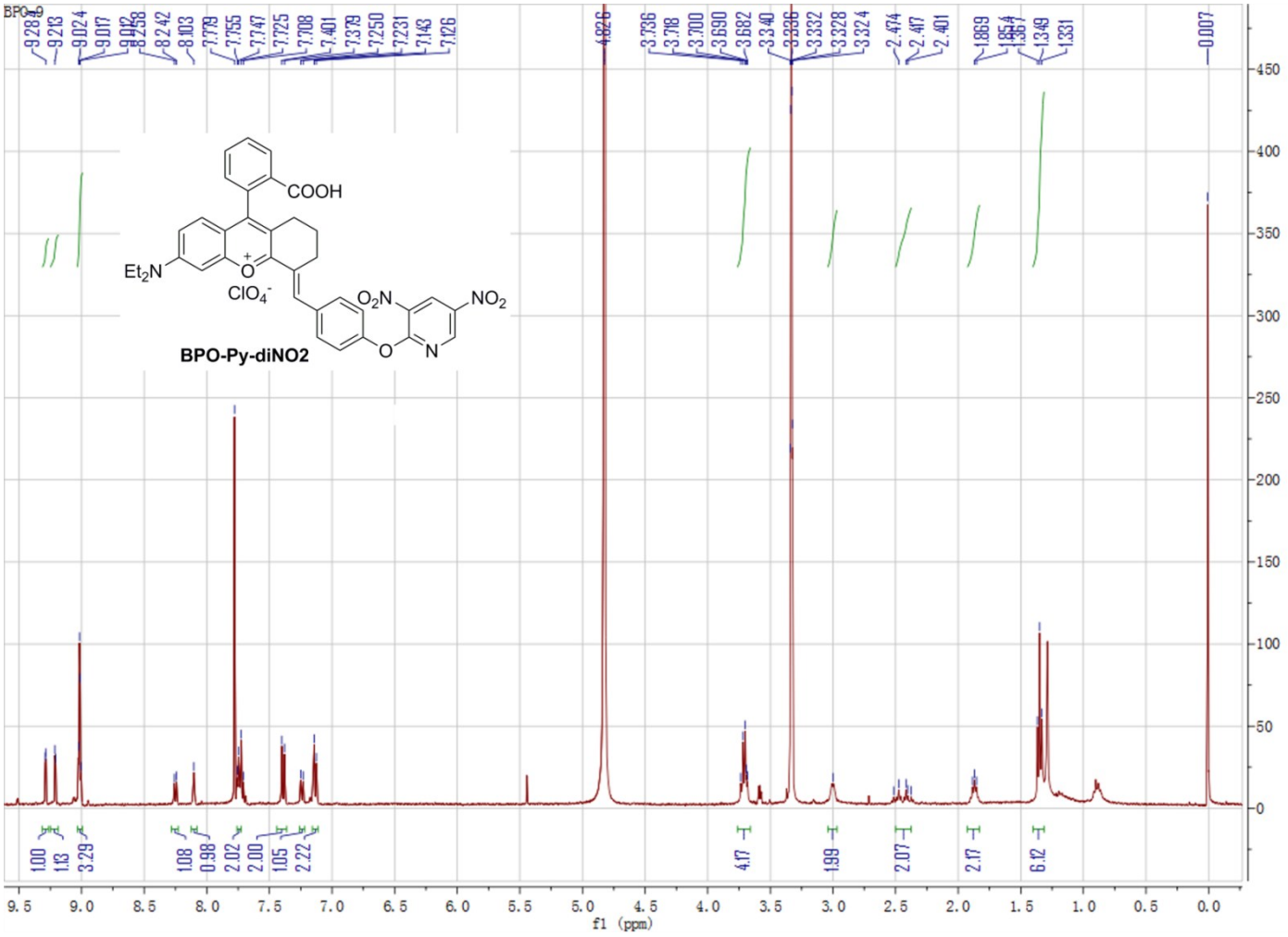
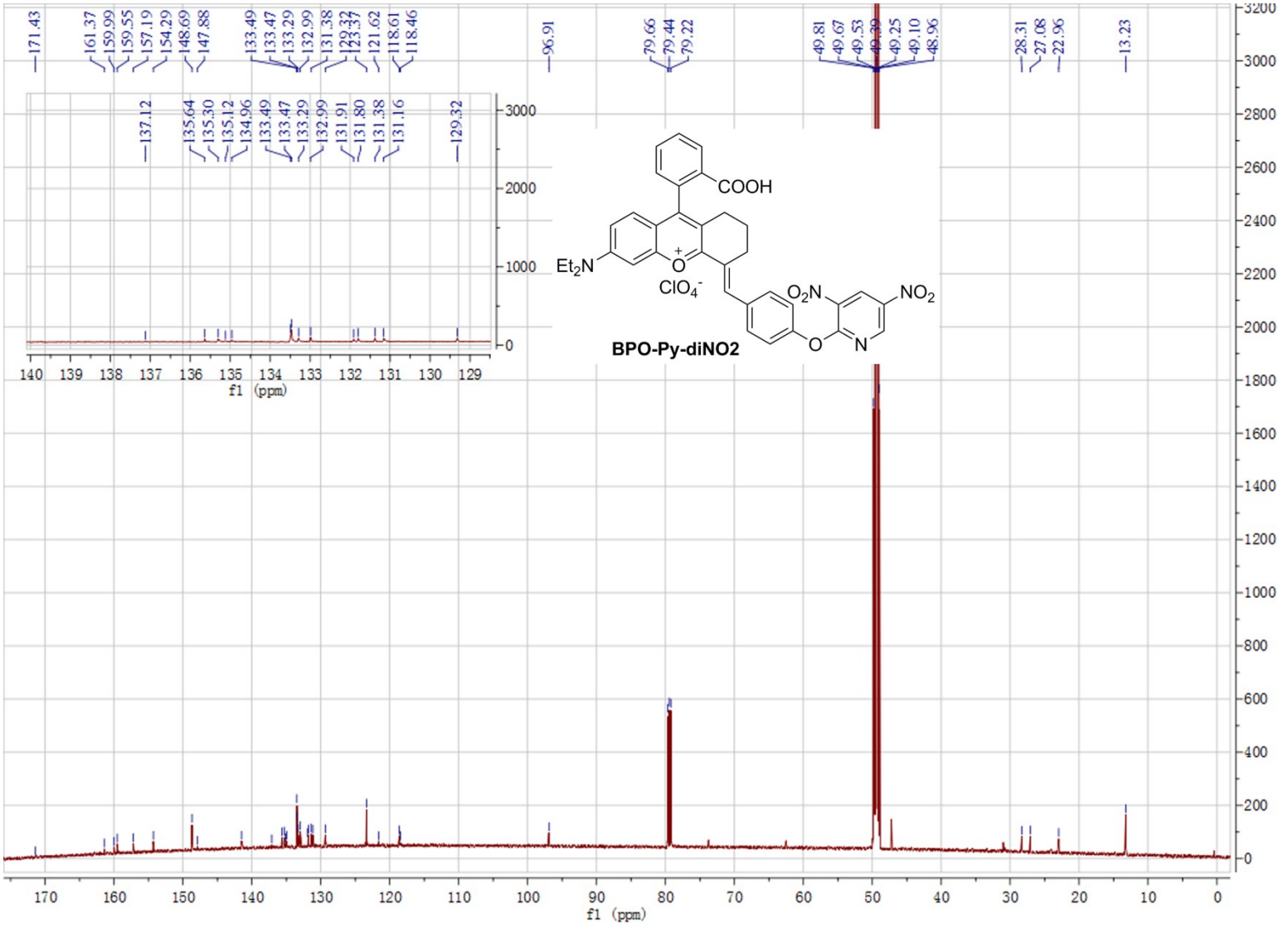


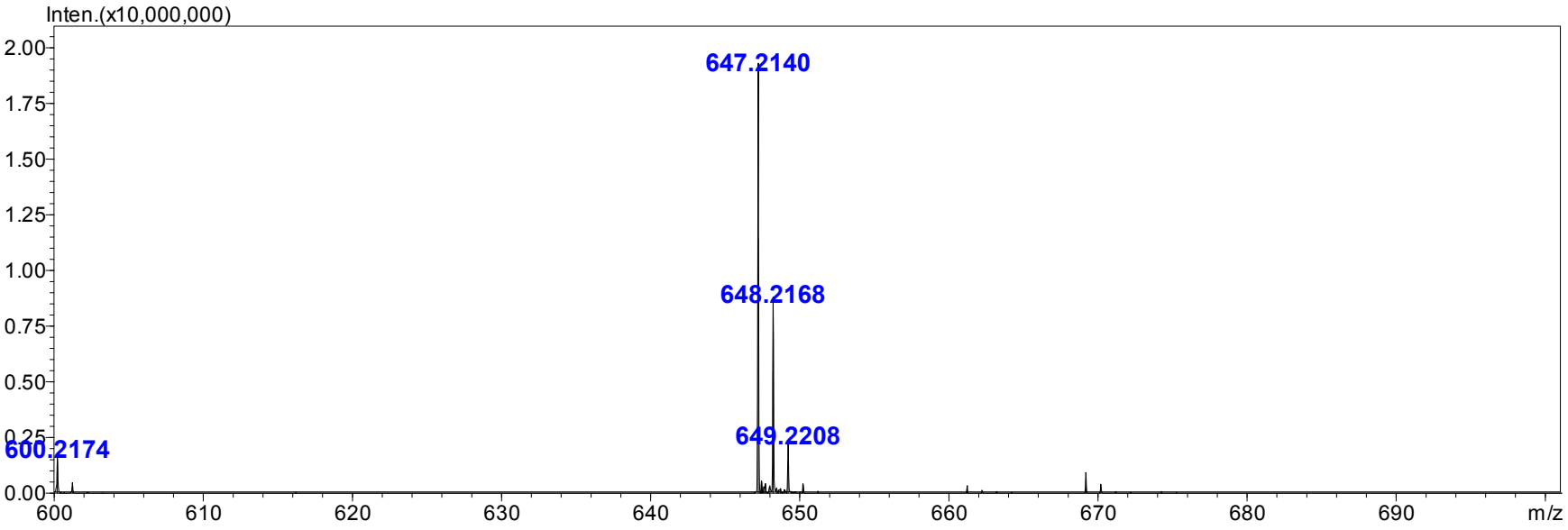
Fig. S20 Confocal images of SO_2 in living Hela cells. (a-c) Hela cells incubated with 10 μM **BPO-Py-5-NO_2** for 30 min; (d-f) Hela pretreated with 10 μM **BPO-Py-5-NO_2** for 30 min, and then further incubated with 500 μM Na_2SO_3 for another 90 min. The fluorescence imaging was collected from 450−470 nm with excitation at 405 nm.

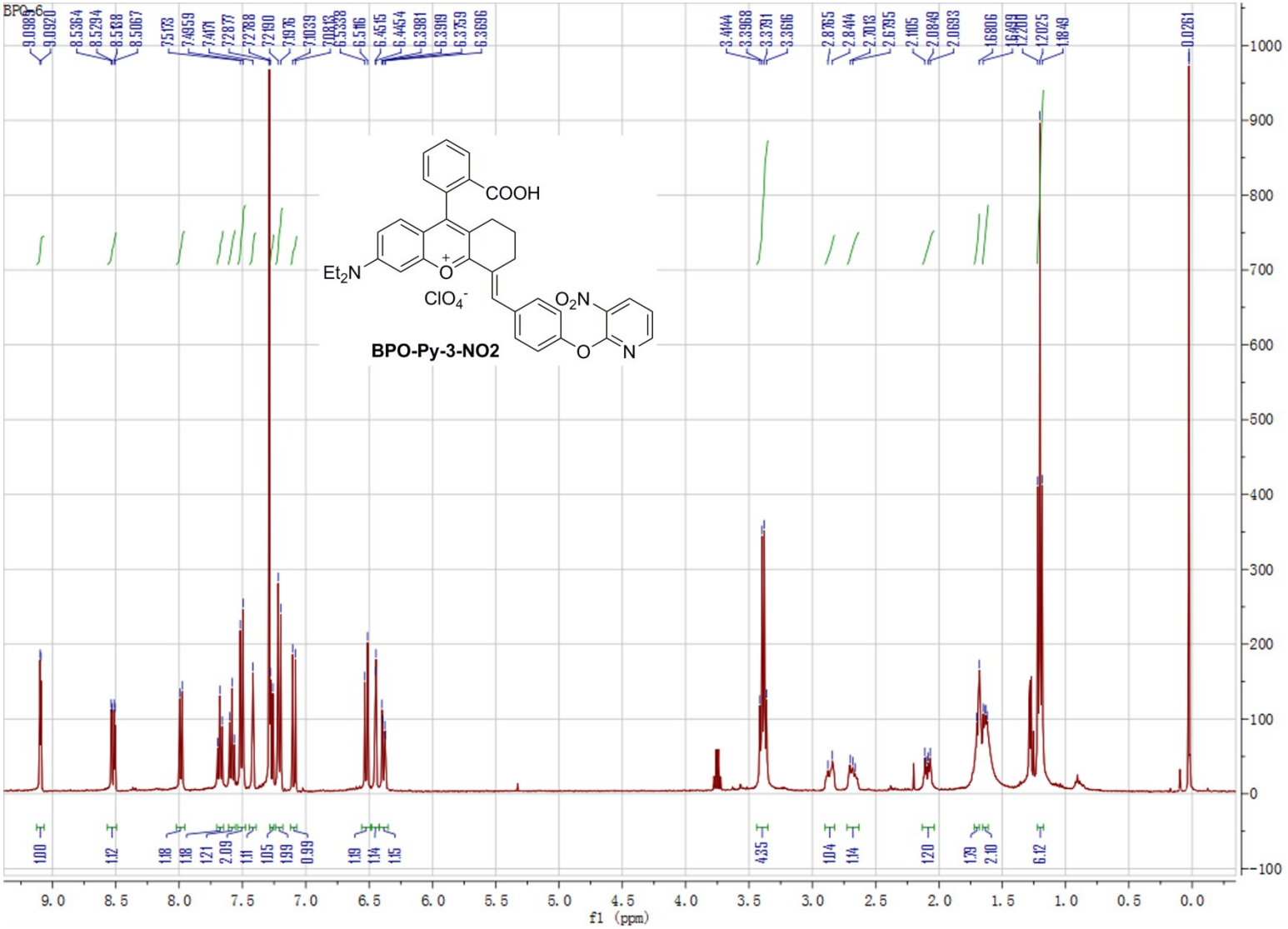
2. ^{1}H NMR, ^{13}C NMR, and HRMS spectrum

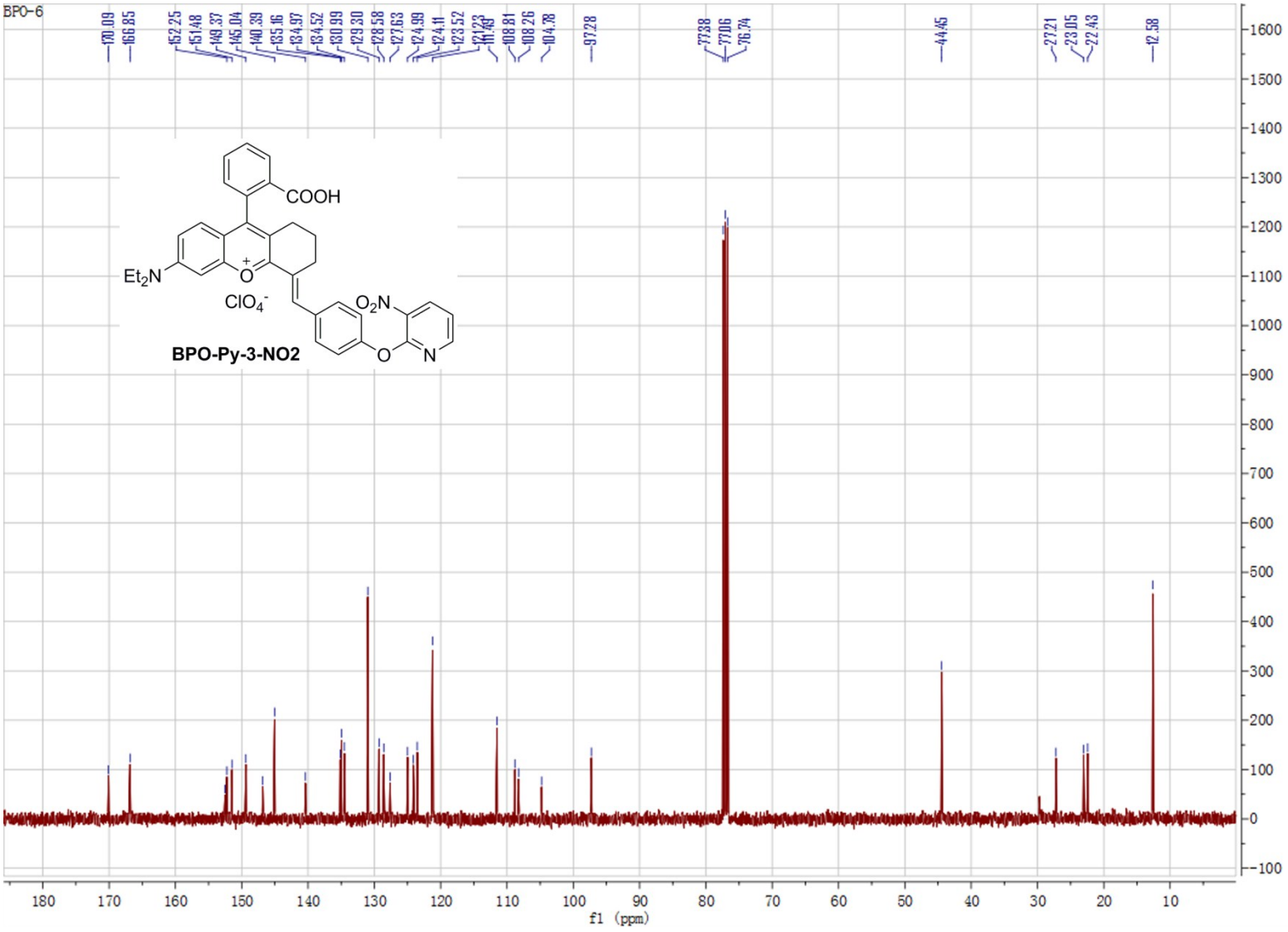



171.43
161.37
159.99
159.55
157.19
154.29
148.69
147.88
133.49
133.47
133.29
132.99
131.38
123.37
121.62
118.61
118.46
96.91
79.66
79.44
79.22
49.81
49.67
49.53
49.39
49.25
49.10
48.96
28.31
27.08
22.96
13.23
137.12
135.64
135.30
135.12
134.96
133.49
133.47
133.29
132.99
131.91
131.80
131.38
131.16
129.32
COOH
Et2N
O
ClO4-
O2N
NO2
O
N
BPO-Py-diNO2
f1 (ppm)
f1 (ppm)

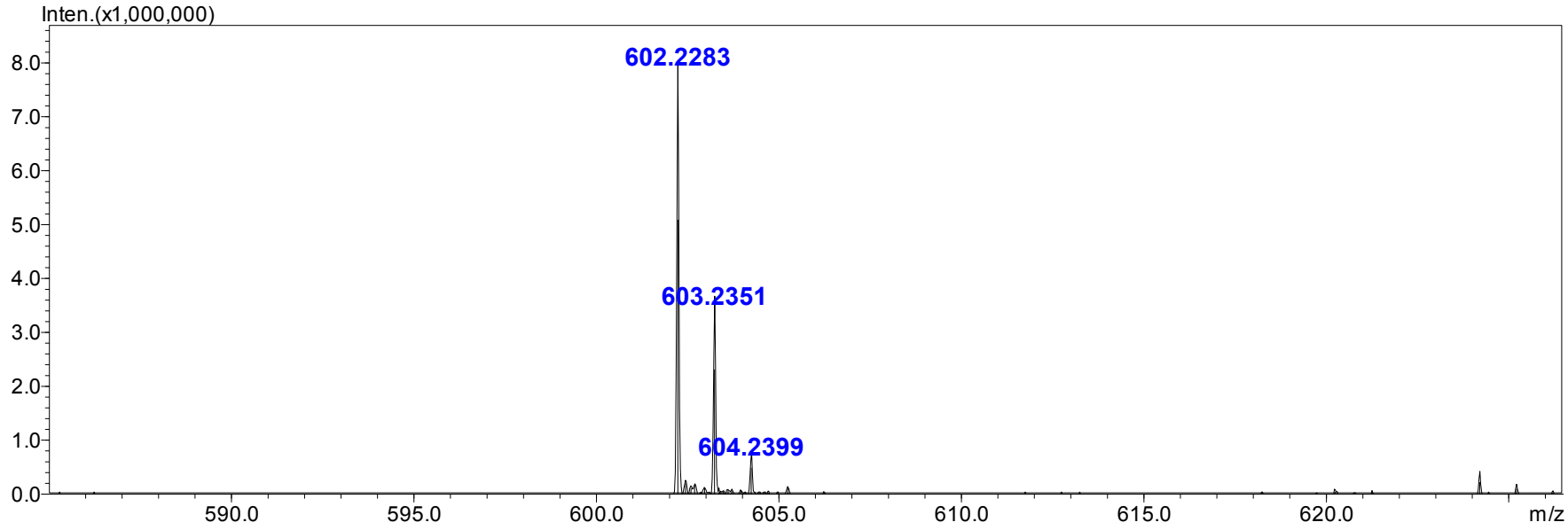
MS(E+)

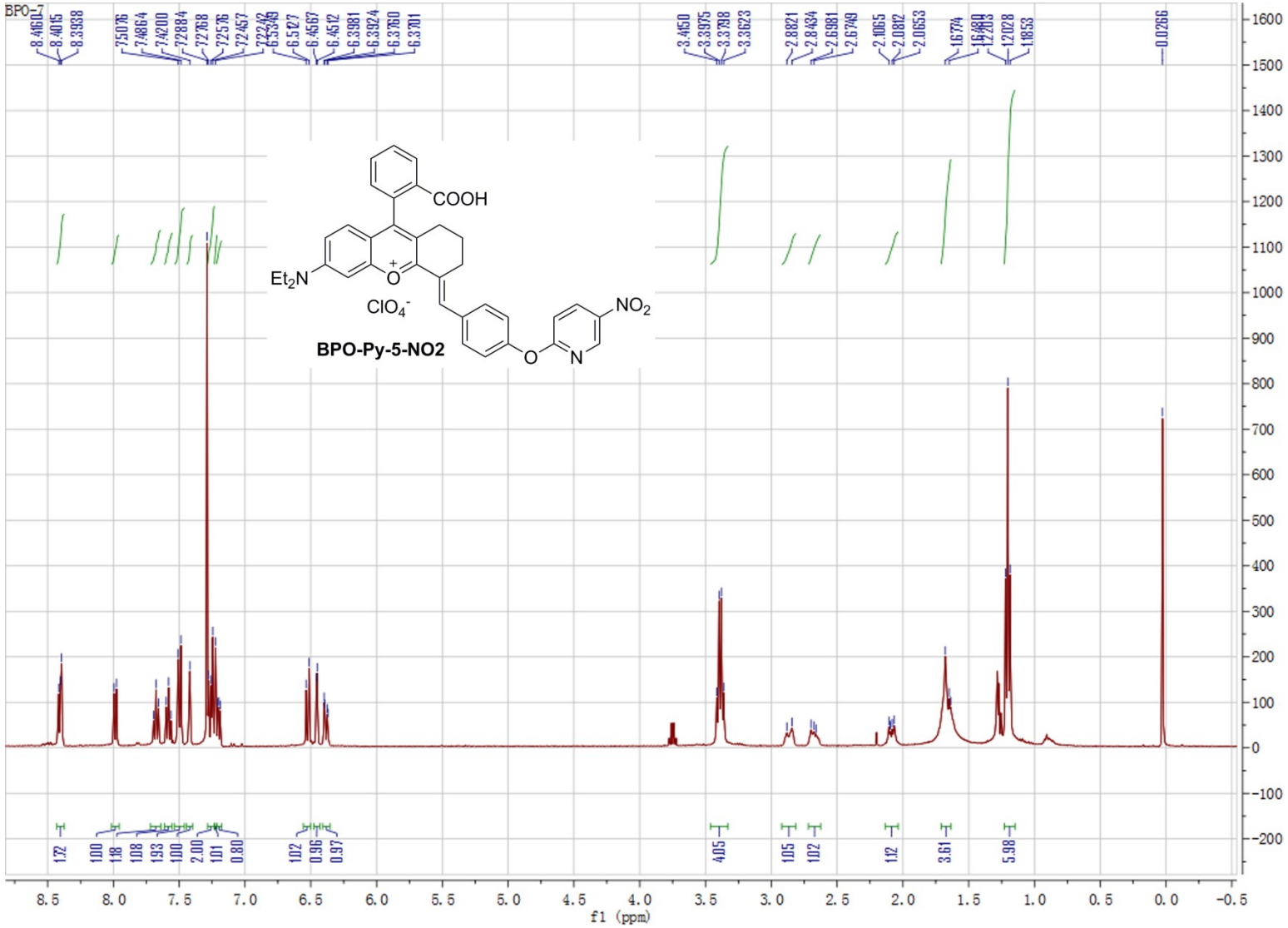

Inten.(x10,000,000)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
647.2140
648.2168
649.2208
600.2174
600
610
620
630
640
650
660
670
680
690
m/z

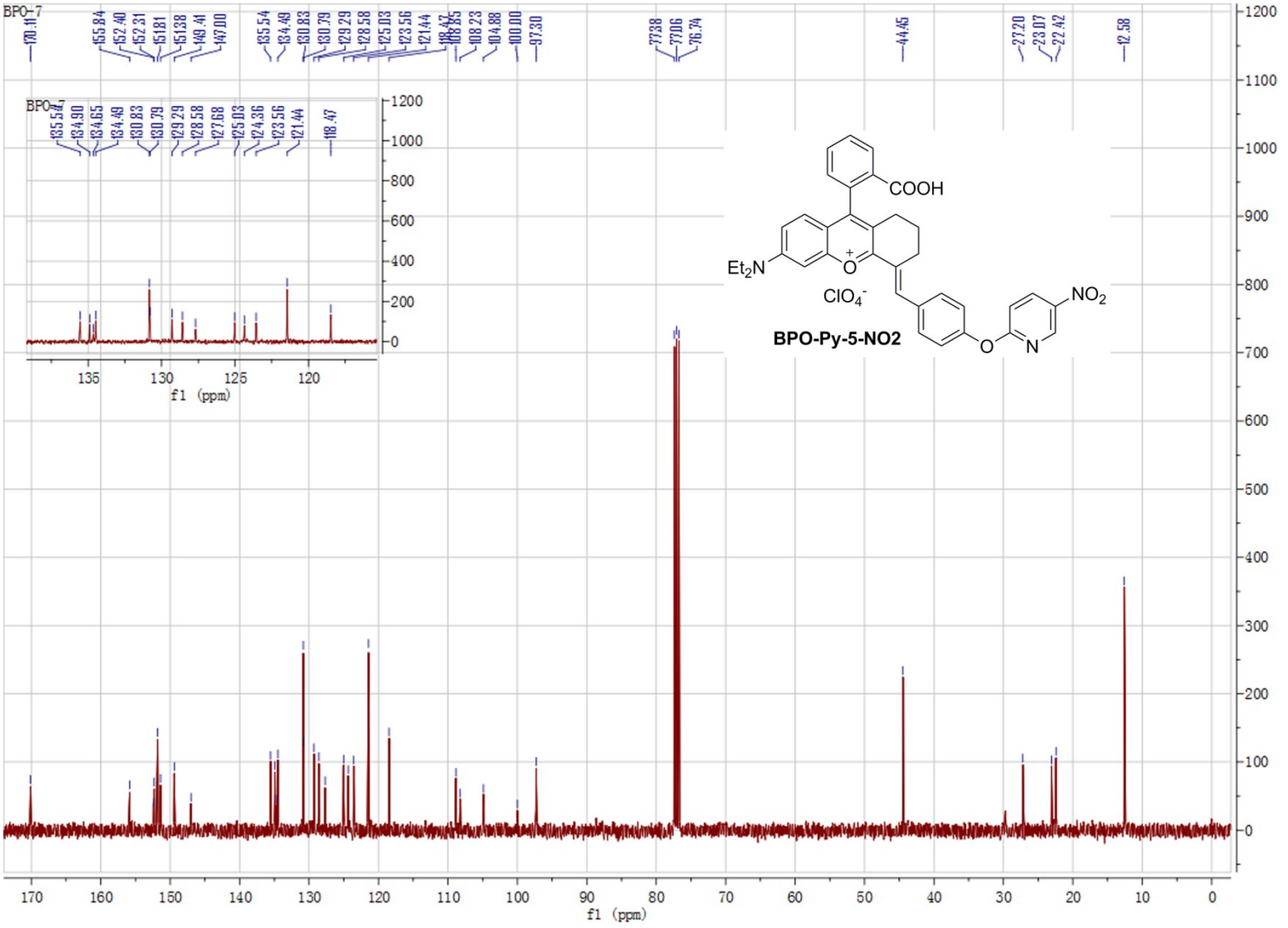

BPO-6
COOH
Et_2N
ClO_4^-
O_2N
O
N
BPO-Py-3-NO2
f1 (ppm)


BPO-6
170.09
166.85
152.25
151.48
149.37
145.04
140.39
135.16
134.97
134.52
130.99
129.30
128.58
127.63
124.99
124.11
123.52
121.23
111.49
108.81
108.26
104.78
97.28
77.38
77.06
76.74
44.45
27.21
23.05
22.43
12.58
COOH
Et2N
O
ClO4-
O2N
O
N
BPO-Py-3-NO2
f1 (ppm)

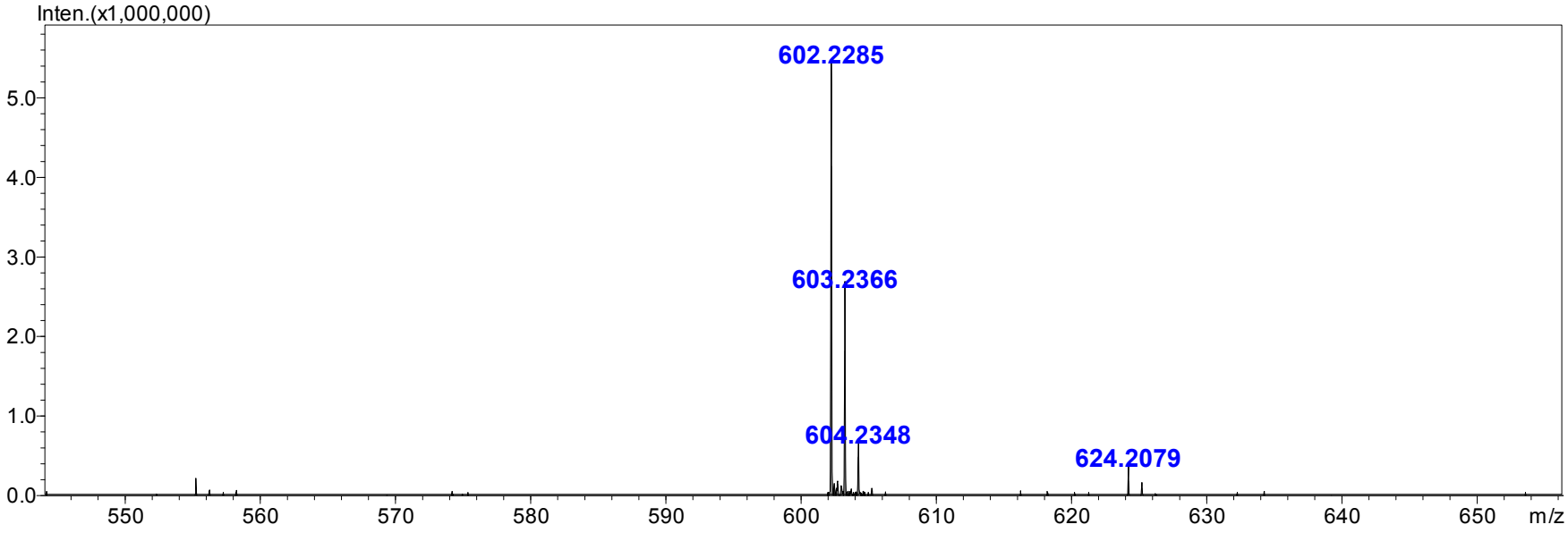
MS(E+)


Inten.(x1,000,000)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
602.2283
603.2351
604.2399
590.0
595.0
600.0
605.0
610.0
615.0
620.0
m/z


BPO-7
8.4610
8.4015
8.3938
7.5076
7.4864
7.4200
7.2884
7.2768
7.2576
7.2457
6.5277
6.4567
6.4512
6.3981
6.3924
6.3760
6.3701
3.4450
3.3975
3.3798
3.3623
2.8821
2.8434
2.6981
2.6749
2.1065
2.0812
2.0653
1.6774
1.2028
1.1853
0.0266
COOH
Et2N
ClO4-
NO2
O
N
BPO-Py-5-NO2
1.72
1.00
1.18
1.08
1.93
1.00
2.00
1.01
0.80
1.02
0.96
0.97
4.05
1.05
1.02
1.12
3.61
5.98
f1 (ppm)


BPO-7
170.11
155.84
152.40
152.31
151.81
151.38
149.41
147.00
135.54
134.49
130.83
130.79
129.29
128.58
125.03
123.56
121.44
118.47
108.23
104.88
100.00
97.30
77.38
77.06
76.74
44.45
27.20
23.07
22.42
12.58
f1 (ppm)
COOH
Et2N
O
ClO4-
NO2
O
N
BPO-Py-5-NO2

MS(E+)


Inten.(x1,000,000)
602.2285
603.2366
604.2348
624.2079
5.0
4.0
3.0
2.0
1.0
0.0
550
560
570
580
590
600
610
620
630
640
650
m/z