Electronic Supplementary Material (ESI) for Analyst.

Supporting Information for

A mitochondria-targeted fluorescent probe based on ESIPT phthalimide for the detection of Hg^{2+} with large Stokes shift

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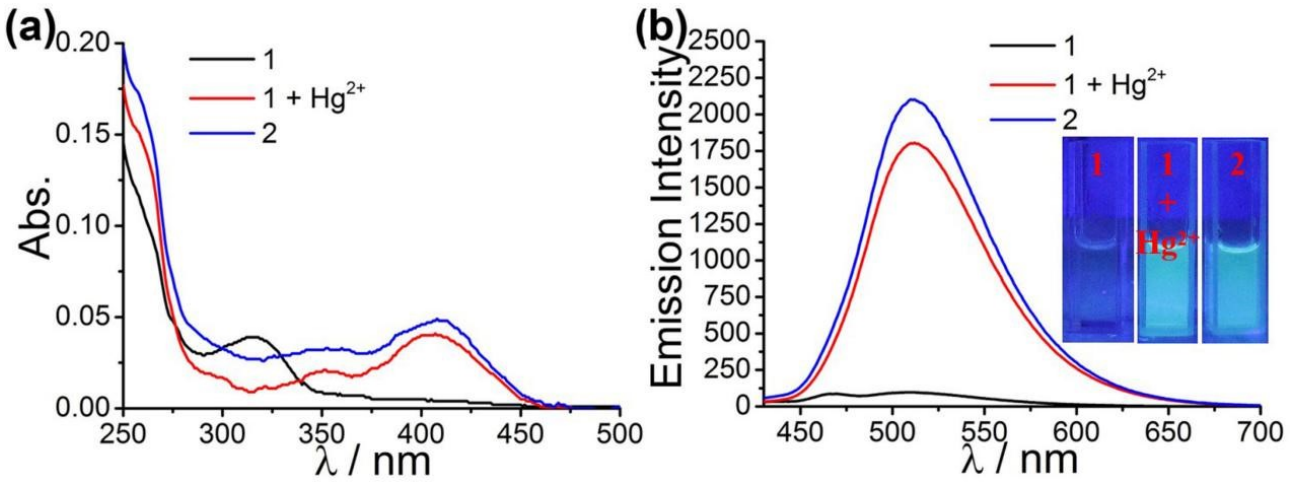


Fig. S1 (a) Absorption spectra of **2** (10 μM) (blue line), **1** (10 μM) before (black line) and after (red line) reaction with Hg^{2+} (25 μM) in HEPES buffer solution (10 mM, H_2O/EtOH, 3:2, v/v, pH = 7.4, 25°C). (b) Fluorescence spectra of **2** (10 μM) (blue line), **1** (10 μM) before (black line) and after (red line) reaction with Hg^{2+} (25 μM) in HEPES buffer solution (10 mM, H_2O/EtOH, 3:2, v/v, pH = 7.4, 25°C, λ_{ex} = 405 nm). Inset: The fluorescence color changes of **1** before and after the reaction and **2** in HEPES buffer solution under illumination with a 365 nm UV lamp.

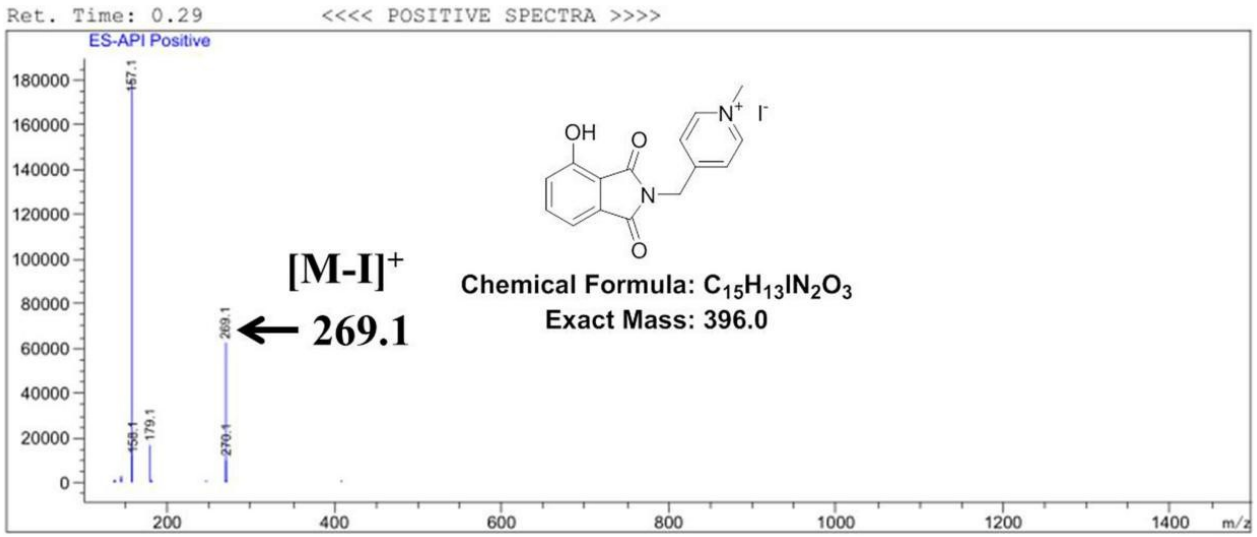


Fig. S2 ESI-MS spectrum of **1** after addition of Hg^{2+}.

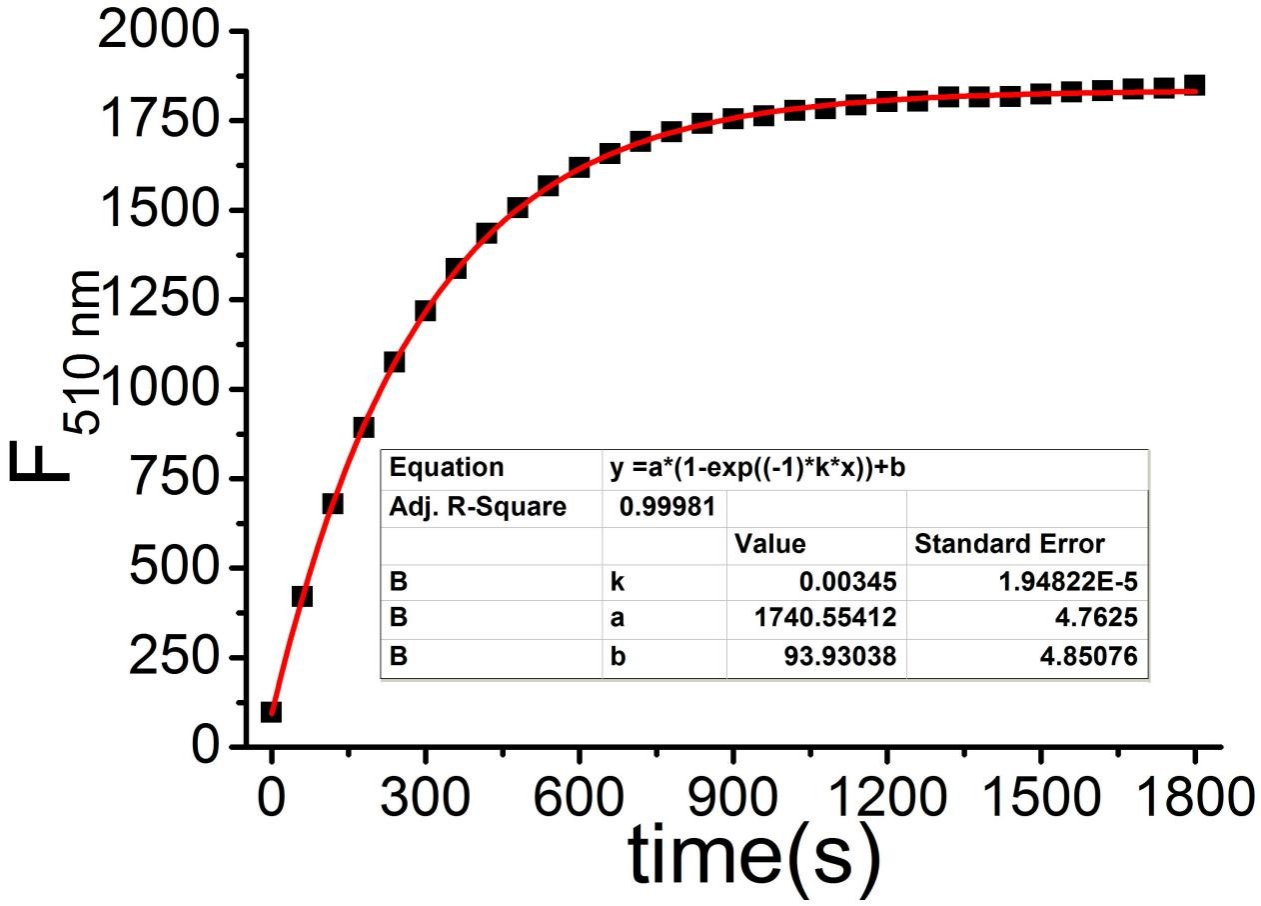


Fig. S3 Kinetic plot of fluorescence intensity at 510 nm of the pseudo-first order reaction of **1** (10 μM) to Hg^{2+} (25 μM), using excitation wavelength at 405 nm. The slope of the plot corresponds to the observed reaction rate of **3.45 × 10^{-3} s^{-1}**.

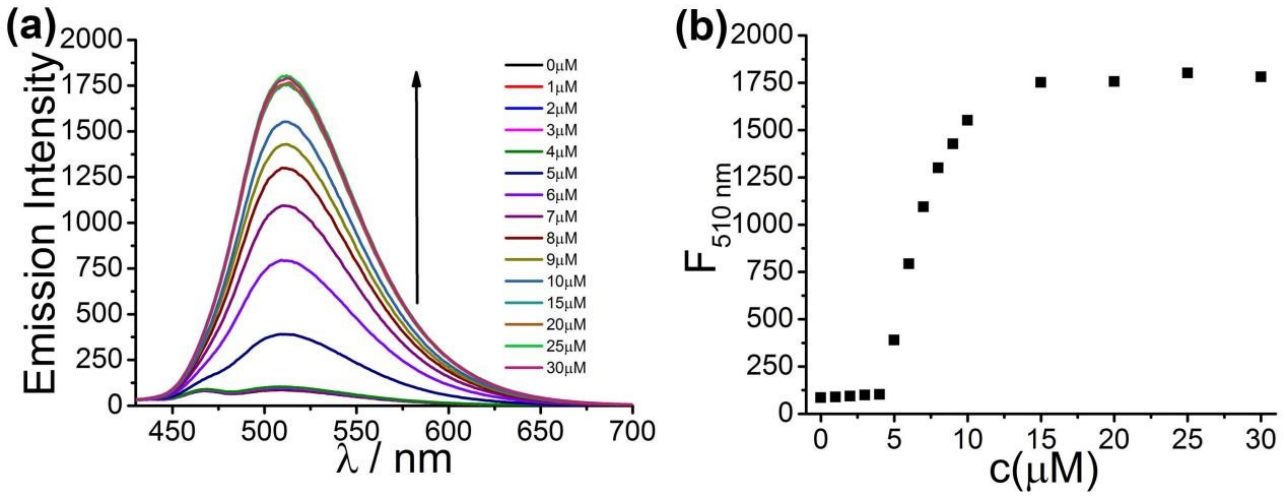


Fig. S4 (a) Fluorescence spectra of **1** (10 μM) after reaction with varied concentrations of Hg^{2+} (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25 and 30 μM). (b) The fluorescence intensity at 510 nm as a function of the concentrations of Hg^{2+}. Data were acquired at 20 min in HEPES buffer solution (10 mM, H_2O/EtOH, 3:2, v/v, pH = 7.4, 25°C, λ_{ex} = 405 nm).

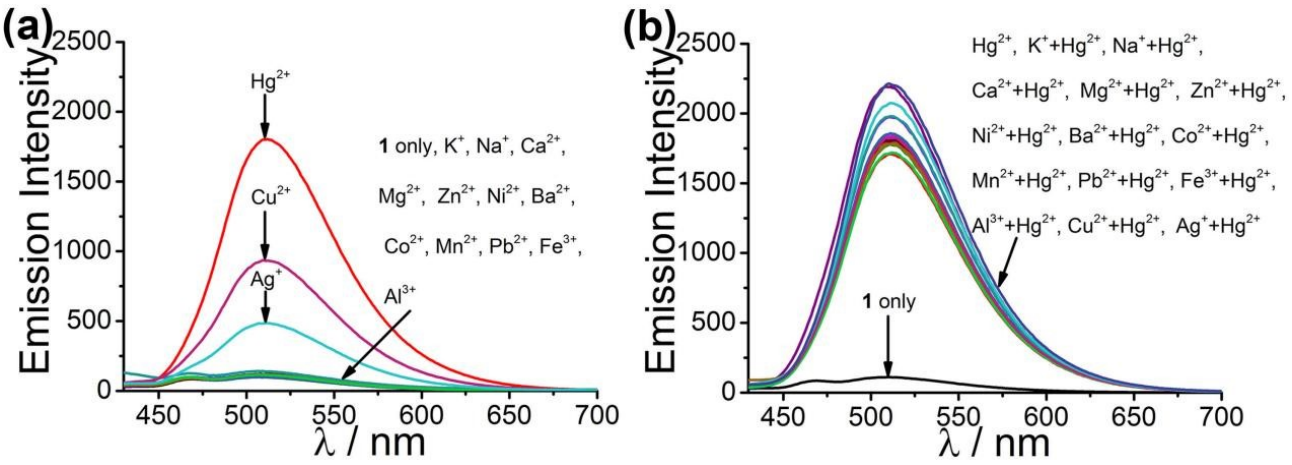


Fig. S5 Fluorescence spectra of **1** (10 μM) after addition of various metal ions (25 μM). Data were acquired at 20 min after addition of various metal ions in HEPES buffer solution (10 mM, H_2O/EtOH, 3:2, v/v, pH = 7.4, 25°C, λ_{ex} = 405 nm).

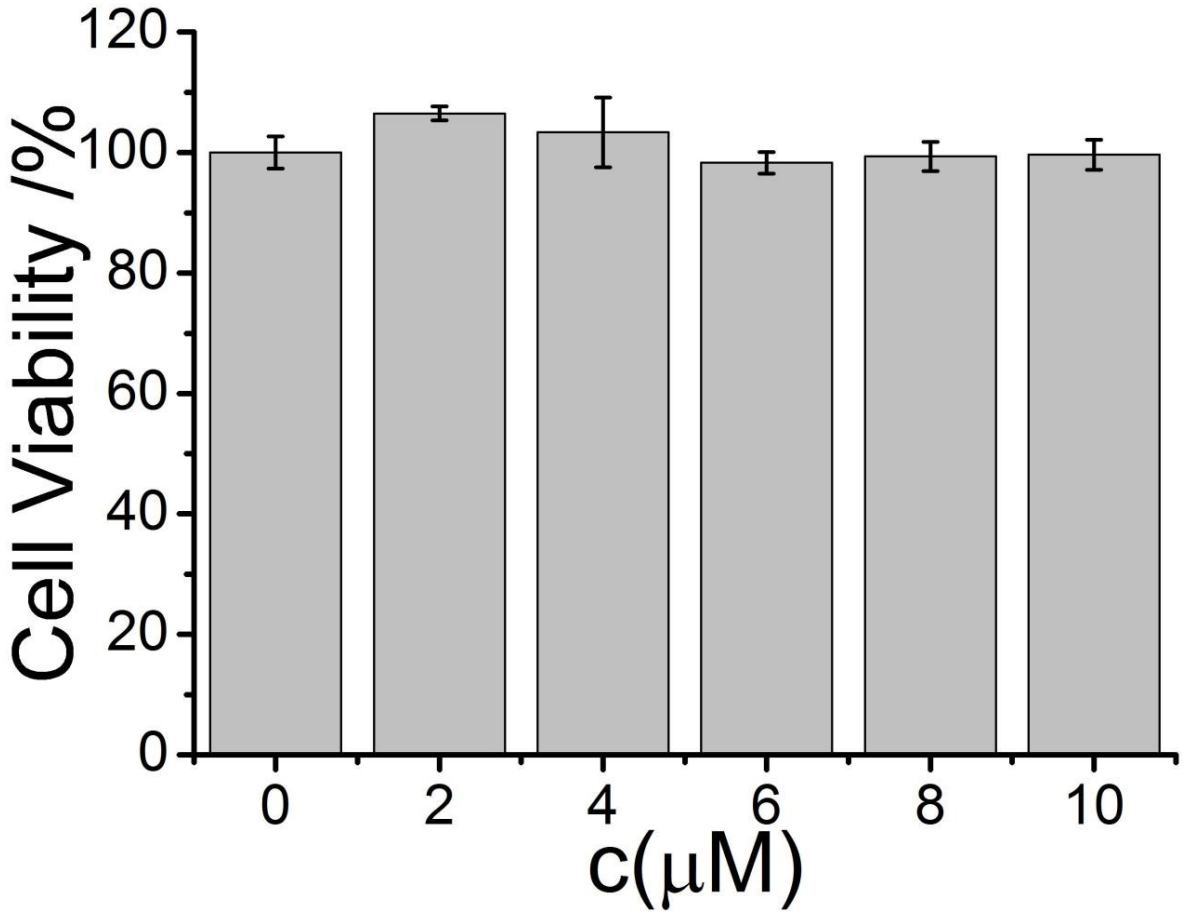


Fig. S6 CCK-8 assay of HeLa cells in the presence of various concentrations of **1** (0, 2, 4, 6, 8, 10 μM) for 24 h at 37 °C.

Determination of the detection limit (LOD)

The detection limit was calculated based on the fluorescence titration. A linear regression curve was fitted according to the fluorescence intensity at 510 nm of **1** as a function of Hg^{2+} concentration, and the slope (k) of the curve was obtained. The emission spectrum of **1** (10 μM) in HEPES buffer solution(10 mM, H_2O/EtOH, 3:2, v/v, pH = 7.4, 25°C, λ_{ex} = 405 nm) was collected for 30 times and the standard deviation of blank measurements (δ) was determined. The detection limit was calculated using the equation.

Detection limit (LOD) = 3δ/k

Where δ is the standard deviation of the blank measurements; k is the slope of the fluorescence intensity at 510 nm of **1** versus Hg^{2+} concentration.

Determination of quantum yields

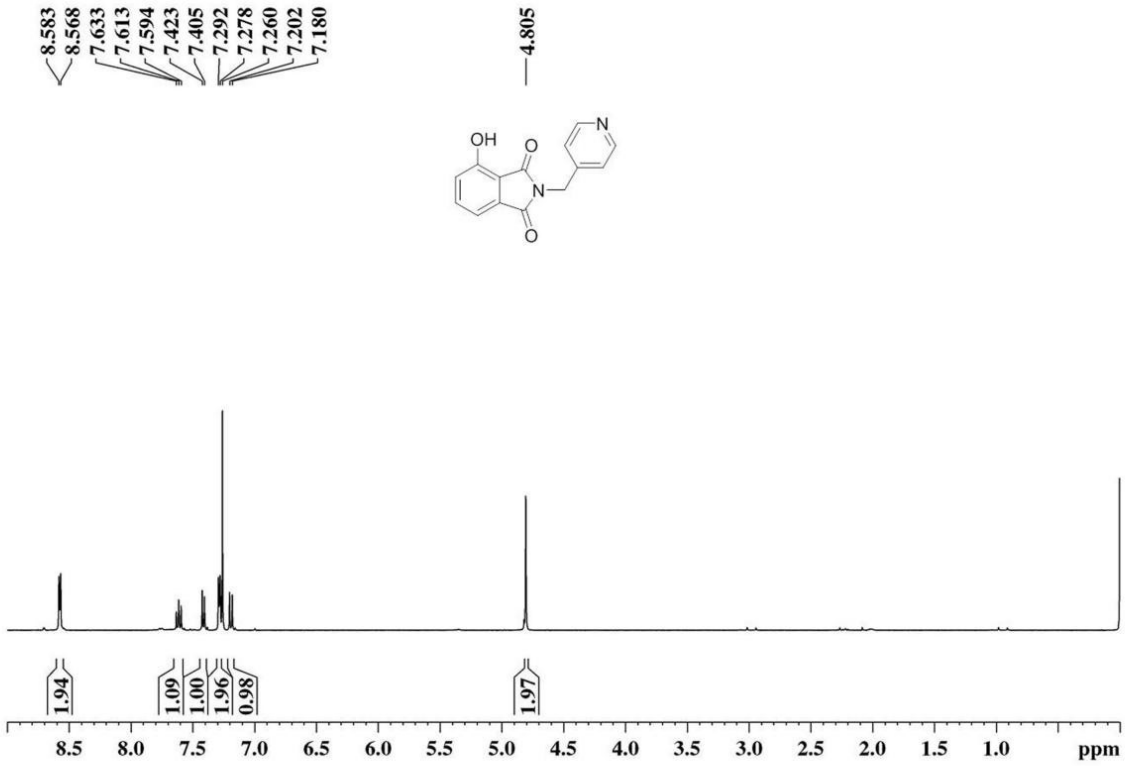
Fluorescence quantum yield was estimated using fluorescein (Φ_f = 0.85 in 1 N NaOH) as the reference [S1]. The quantum yields of **1** and **2** are calculated according to following equation.

$\Phi_x = \Phi_s(A_s S_x \eta_x^2)/(A_x S_s \eta_s^2)$

where Φ is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, S is the area under the corrected emission curve, and η is the refractive index of the solvent used. Subscripts x and s refer to the unknown and the standard, respectively.

[S1] T. Wang, E. F. Douglass Jr, K. J. Fitzgerald and D. A. Spiegel, *J. Am. Chem. Soc.*, 2013, **135**, 12429-12433.

NMR Spectra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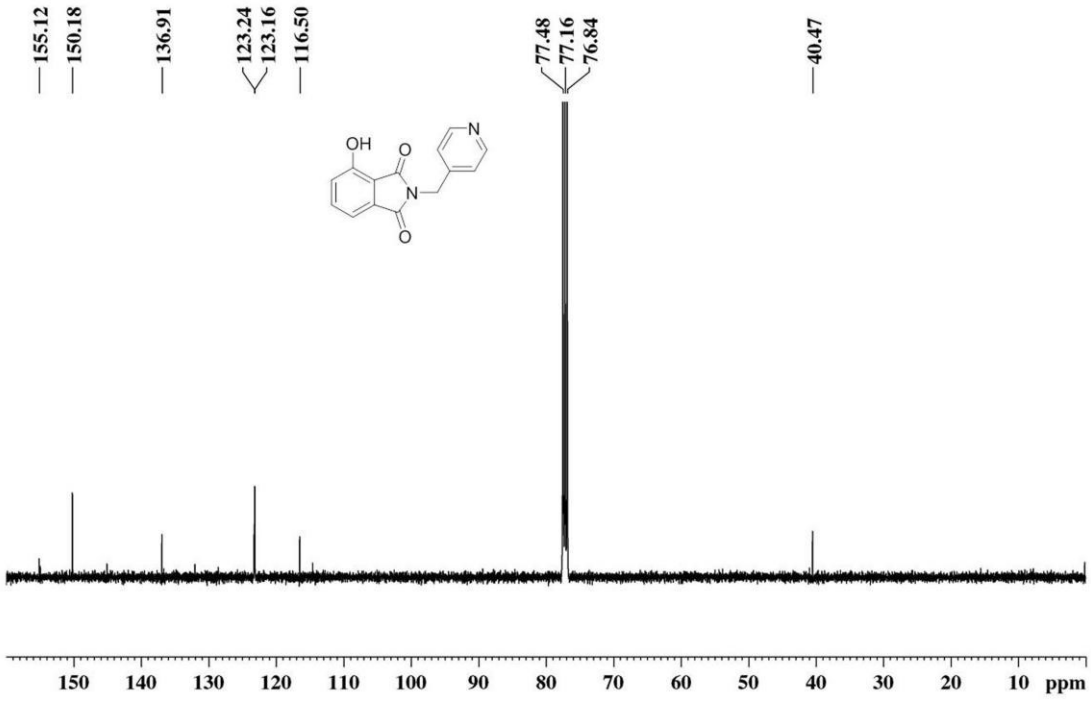
8.568
7.633
7.613
7.594
7.423
7.405
7.292
7.278
7.260
7.202
7.180
4.805
OH
O
N
O
N
1.94
1.09
1.00
1.96
0.98
1.97
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm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150.18
136.91
123.24
123.16
116.50
77.48
77.16
76.84
40.47
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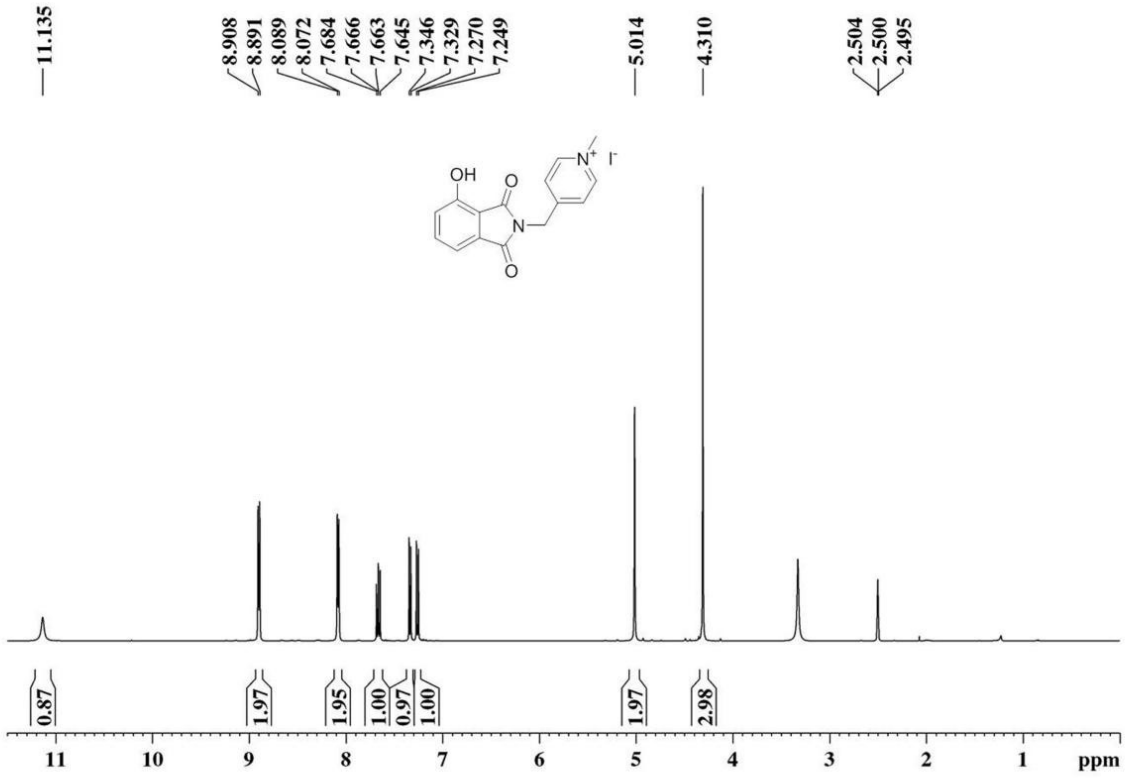
O
N
O
N
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm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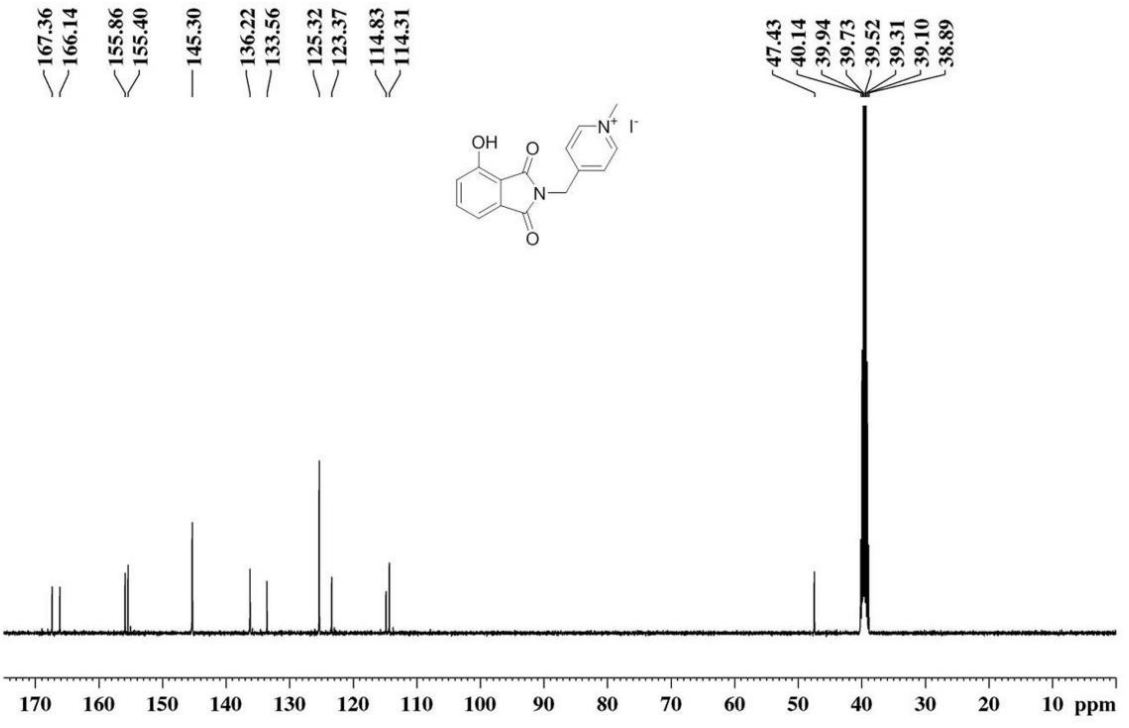
8.891
8.089
8.072
7.684
7.666
7.663
7.645
7.346
7.329
7.270
7.249
5.014
4.310
2.504
2.500
2.495
OH
O
N
O
N+
I-
0.87
1.97
1.95
1.00
0.97
1.00
1.97
2.98
11
10
9
8
7
6
5
4
3
2
1
ppm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155.86
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136.22
133.56
125.32
123.37
114.83
114.31
47.43
40.14
39.94
39.73
39.52
39.31
39.10
38.89
OH
O
N
O
N+
I-
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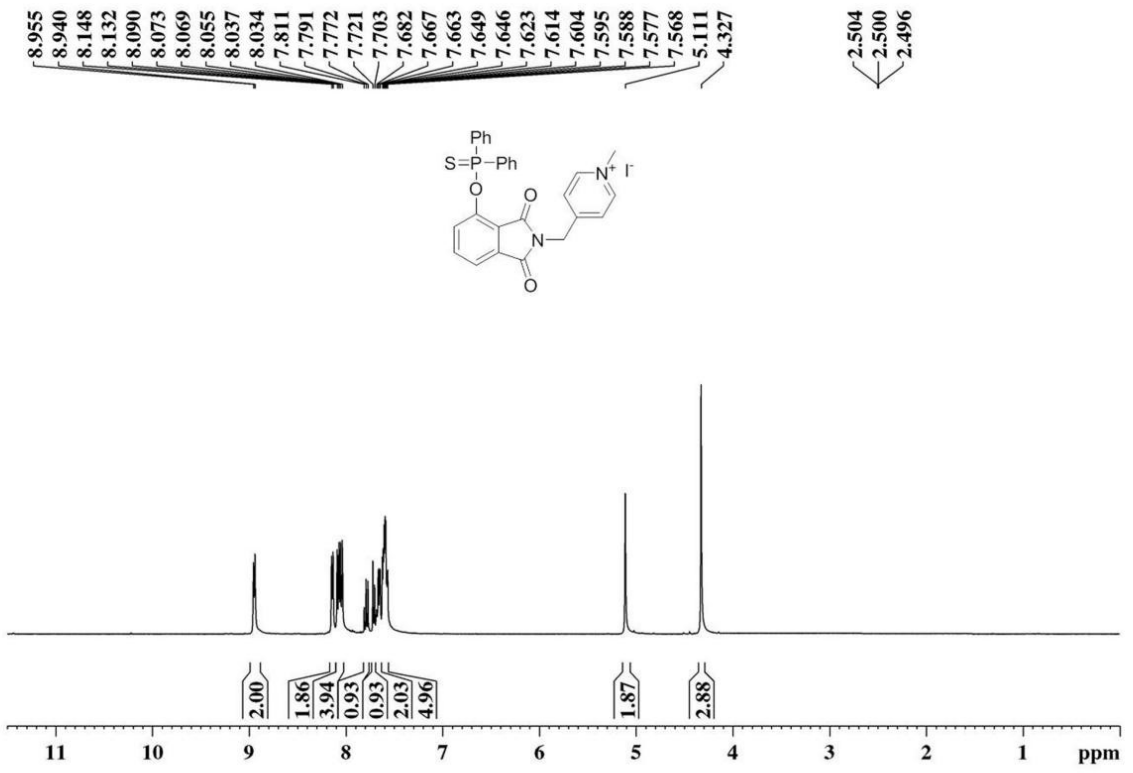
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm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8.132
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8.055
8.037
8.034
7.811
7.791
7.772
7.721
7.703
7.682
7.667
7.663
7.649
7.646
7.623
7.614
7.604
7.595
7.588
7.577
7.568
5.111
4.327
2.504
2.500
2.496
Ph
S=P–Ph
O
N+ I-
O
N
O
2.00
1.86
3.94
0.93
0.93
2.03
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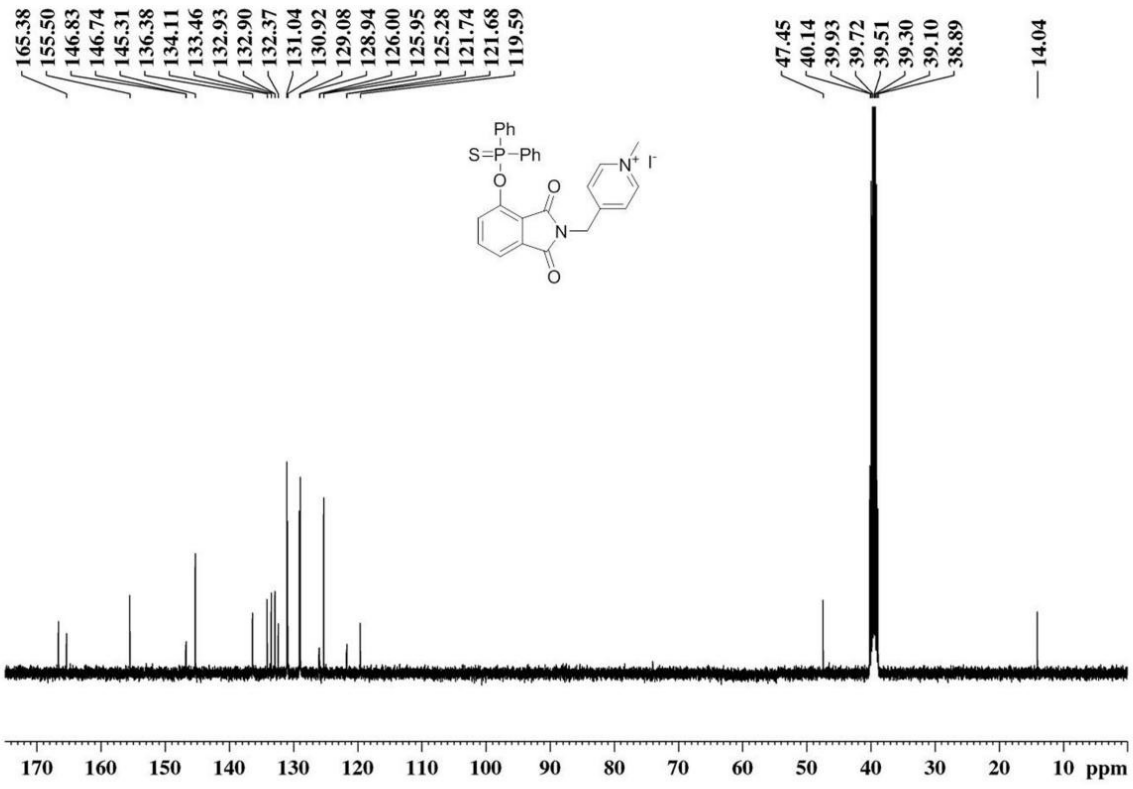
1.87
2.88
11
10
9
8
7
6
5
4
3
2
1
ppm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133.46
132.93
132.90
132.37
131.04
130.92
129.08
128.94
126.00
125.95
125.28
121.74
121.68
119.59
47.45
40.14
39.93
39.72
39.51
39.30
39.10
38.89
14.04
Ph
S=P–Ph
O
N+ I-
O
N
O
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

ESI-MS Spectrum