Electronic Supplementary Material (ESI) for RSC Advances.

Construction of a lysosome-targetable ratiometric fluorescent probe for H_2O_2 tracing and imaging in living cells and inflamed model

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Materials and instruments

All chemical reagents were obtained from commercial suppliers and used without further purification. In all experiments, double distilled water was obtained from a Millipore Milli-Q system (USA). LCQ advantage ion trap mass spectrometer (Thermo Finnigan) was used to record the mass spectra. All chemical reactions were uniform stirred by a magnetic stirrer and monitored by thin-layer chromatography (TLC). Flash column chromatography was carried out using silica gel (200-300 mesh). A Bruker DRX-400 spectrometer using TMS as an internal standard to give NMR spectra. A Hitachi U-4100 UV-vis spectrophotometer (Japan) with 1.0 cm path length quartz cuvette was used to collect UV-vis absorption spectra. A PHS-3C pH meter was used to measure pH values. All fluorescence measurements with a G9800A fluorescence spectrometer with both excitation and emission slits fixed at 2.5 nm, respectively. Fluorescence imaging experiment was conducted on a confocal laser scanning microscope (Olympus, Japan) with 405, and 553 nm excitation.

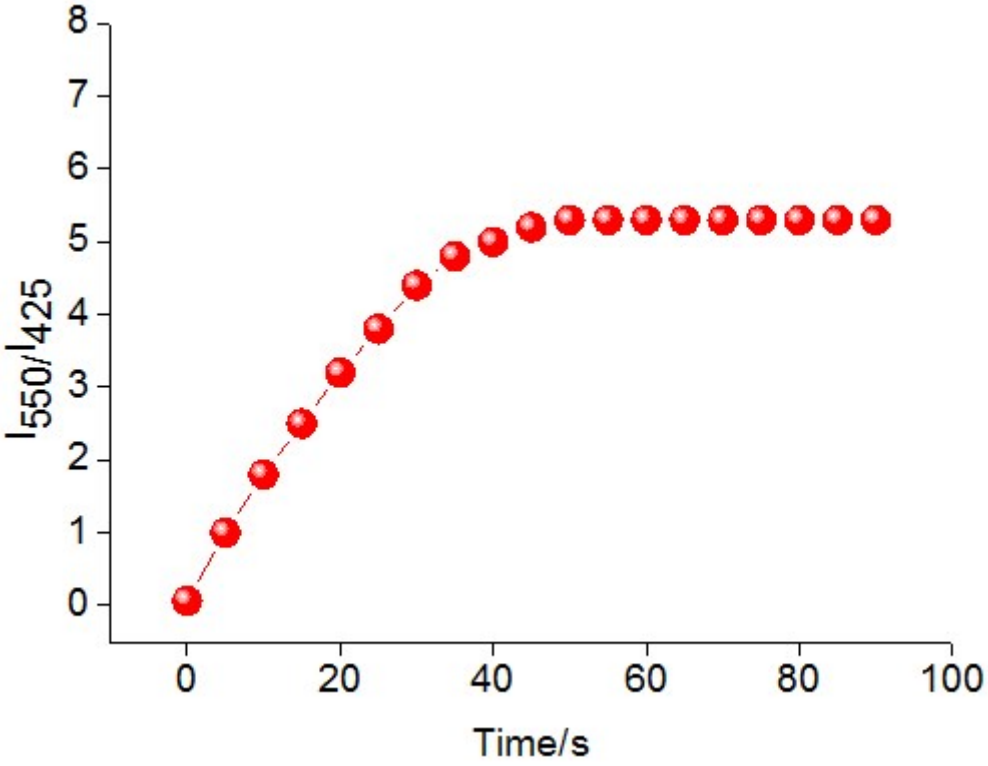


Fig.S1. Time-dependent fluorescence intensity ratios (I_{550}/I_{425}) of **NPT-H_2O_2** upon addition of H_2O_2 (60.0 μM).

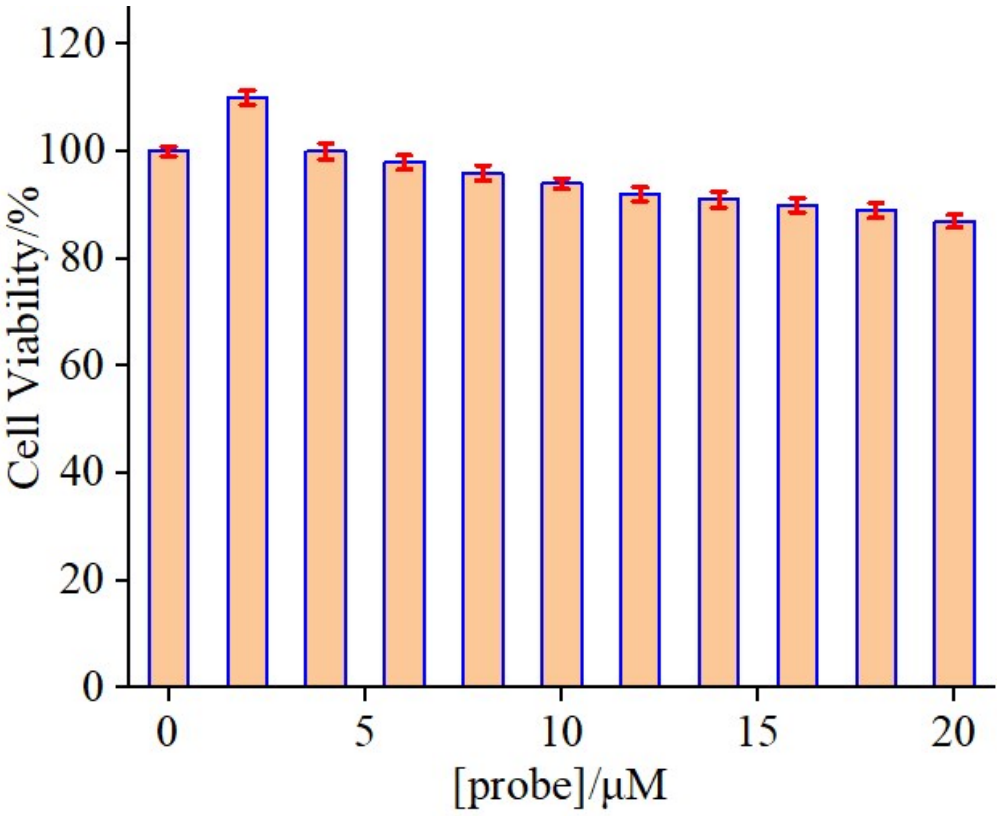


Fig. S2. Viability of HeLa cells treated with various concentrations (0-20.0) μM of **NPT-H_2O_2** for 24 h. Error bars represent mean values ± SD. (n= 3)

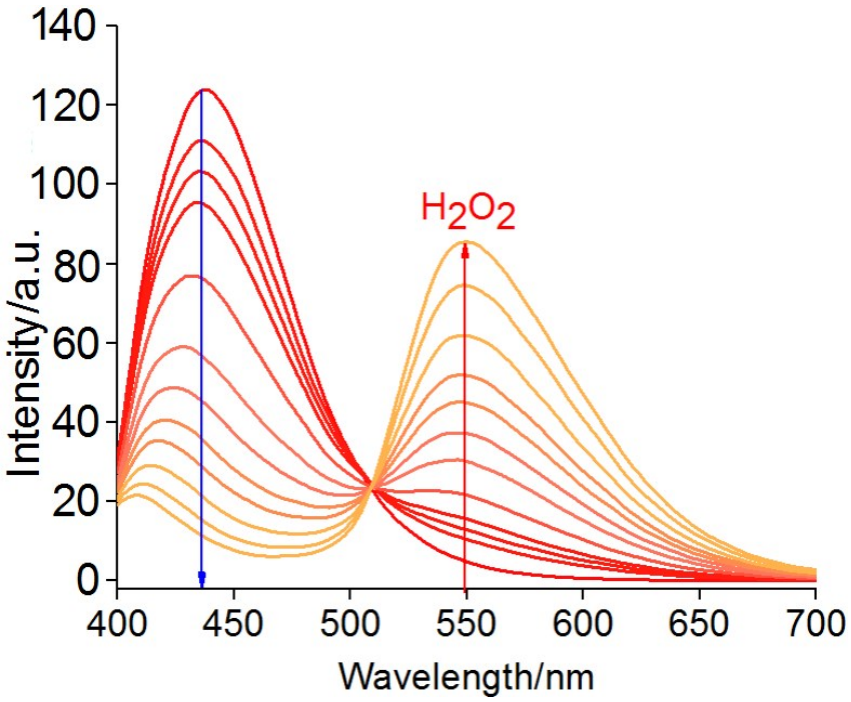


Fig.S3. Fluorescence spectra of 5.0 μM **NPT-H_2O_2** after adding different amount of H_2O_2 (0-60.0) μM in 10 mM PBS solution (pH 7.4), λ_{ex}=375 nm.

Table S1. The details of fluorescent quantum yields

pH 5.0 + 0 μM H_2O_2		pH 5.0+60 μM H_2O_2	
0.51 (425 nm)	0.032 (550 nm)	0.051 (425 nm)	0.23 (550 nm)

The quantum yields of **NPT-H_2O_2** was calculated by comparison with rhodamine 6G (R=0.95 in ethanol) as a reference using the following equation: $\Phi_F=IA_R(n/n_R)^2\Phi_{FR}/I_RA$. Where F is the quantum yield, I is the integrated area under the fluorescence spectra, A is the absorbance, n is the refractive index of the solvent, and R refers to the reference rhodamine 6G.

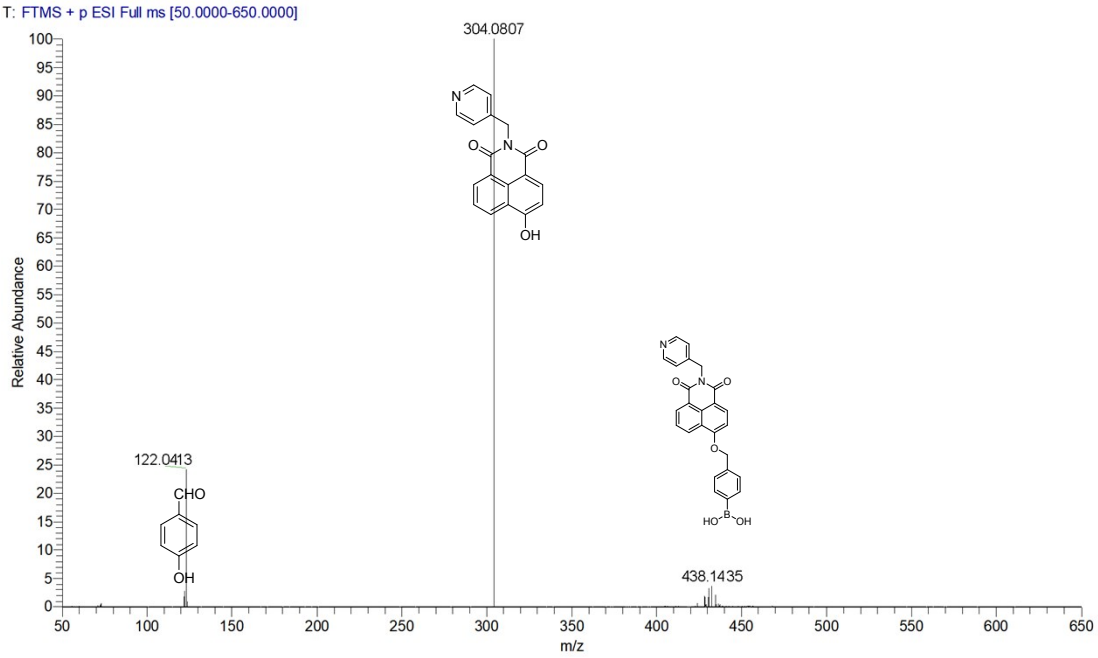
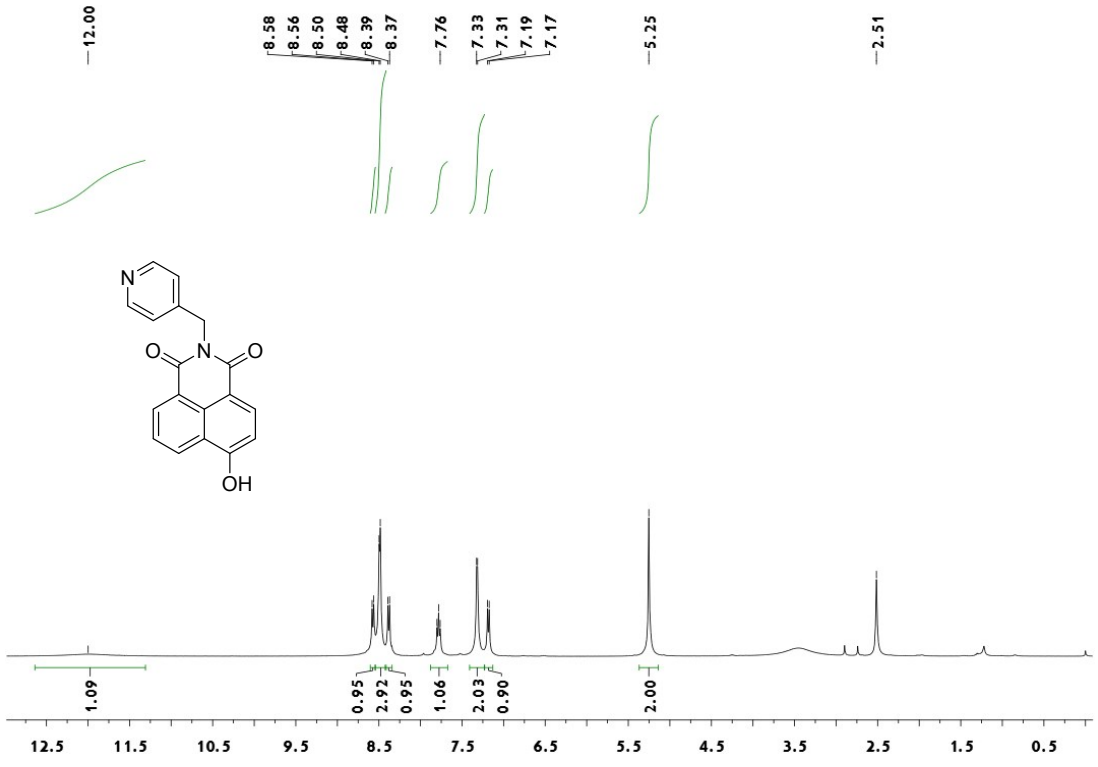
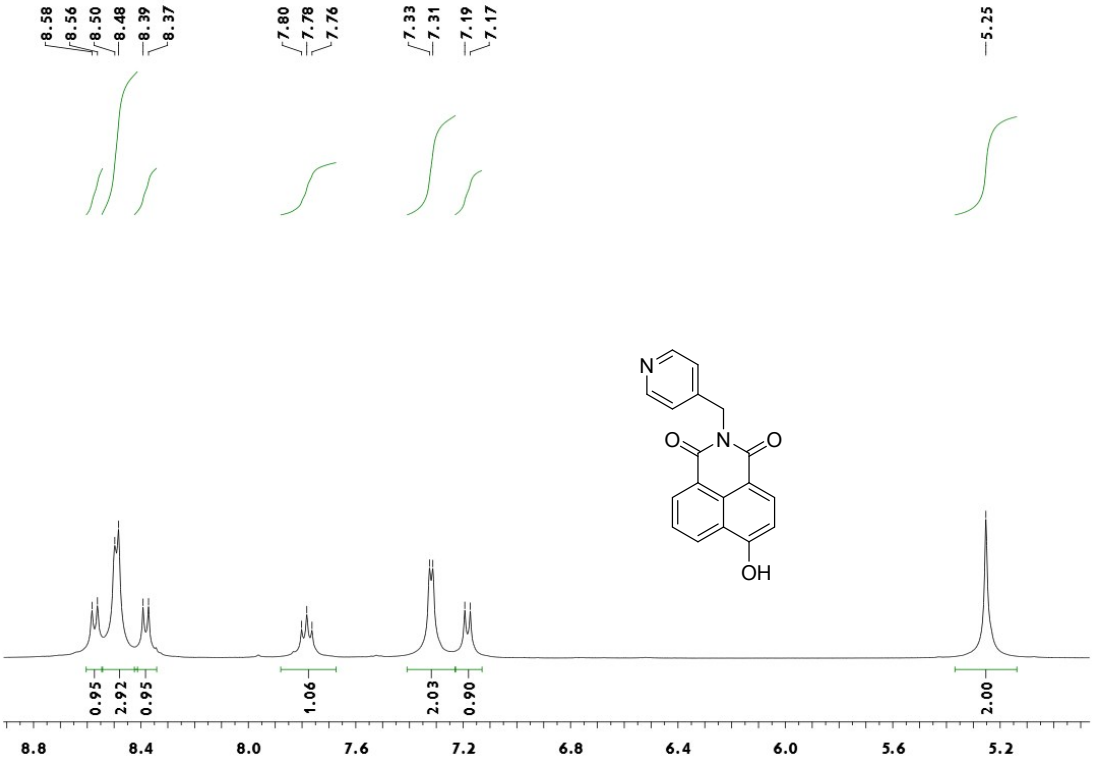


Fig. S4. The response mechanism of 5.0 μM **NPT-H_2O_2** and 60.0 μM hydrogen peroxide in 10 mM PBS.

^{1}HNMR, ^{13}CNMR and MS

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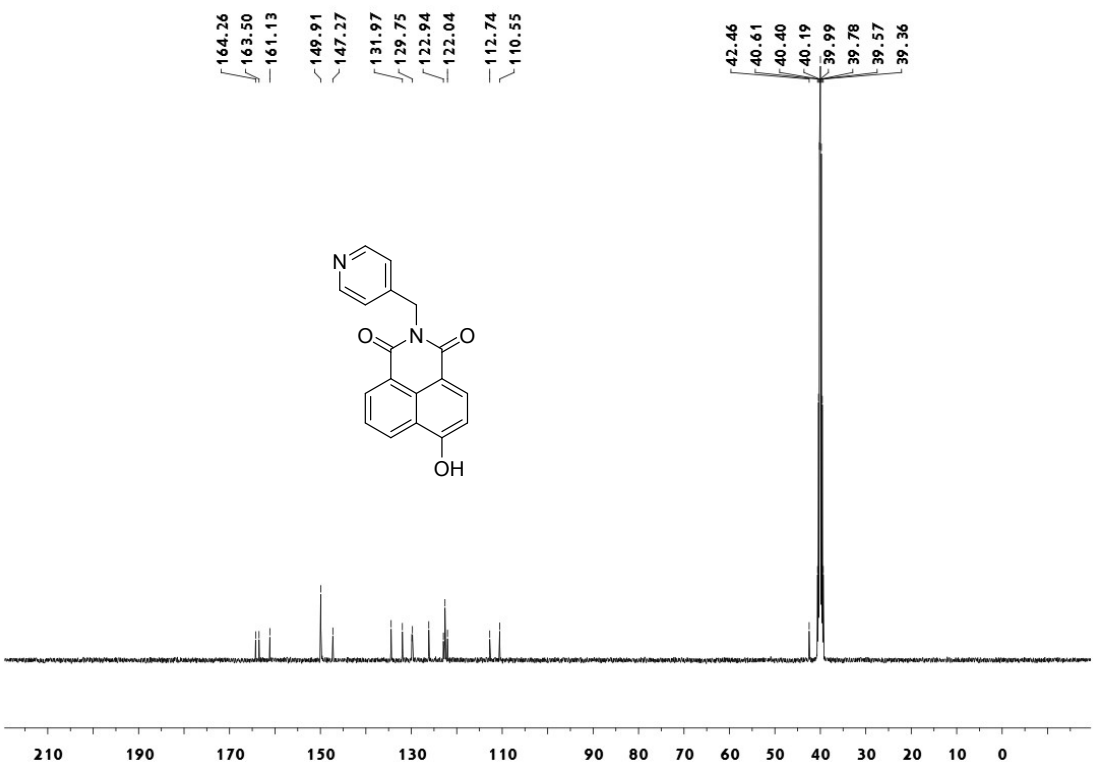
8.56
8.50
8.48
8.39
8.37
7.80
7.78
7.76
7.33
7.31
7.19
7.17
5.25
N
O
N
O
OH
0.95
2.92
0.95
1.06
2.03
0.90
2.00
8.8
8.4
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2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163.50
161.13
149.91
147.27
131.97
129.75
122.94
122.04
112.74
110.55
42.46
40.61
40.40
40.19
39.99
39.78
39.57
39.36
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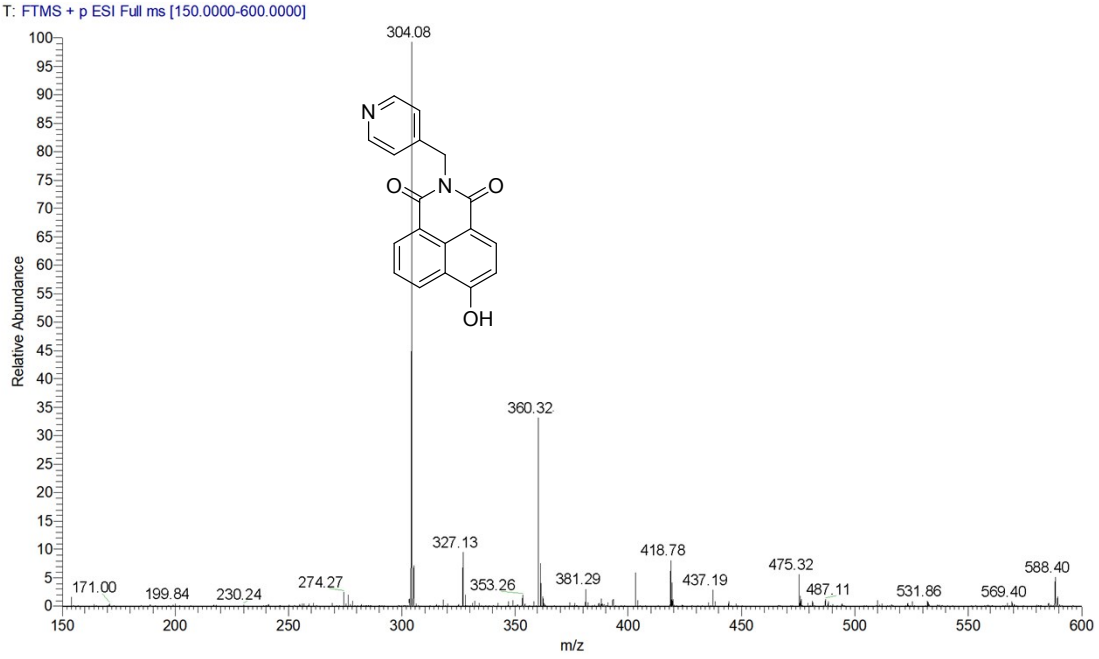
90
80
70
60
50
40
30
20
10
0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N
O
N
O
OH
Relative Abundance
360.32
327.13
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475.32
588.40
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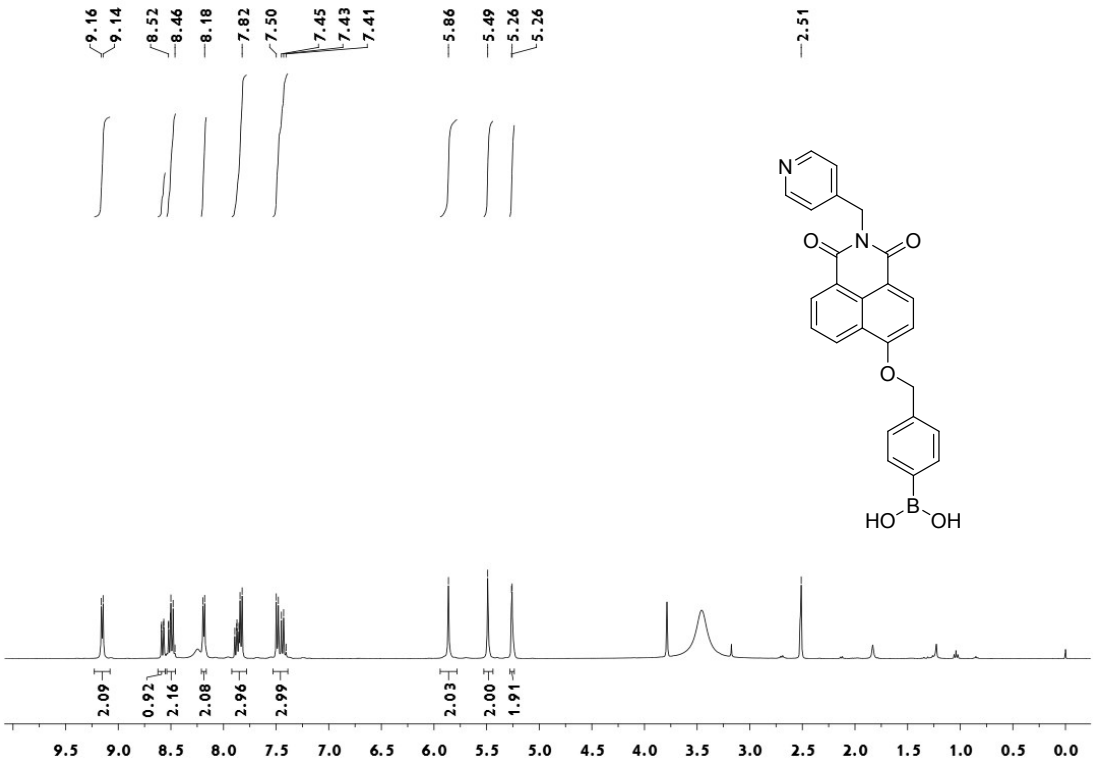
230.24
274.27
353.26
381.29
437.19
487.11
531.86
569.40
m/z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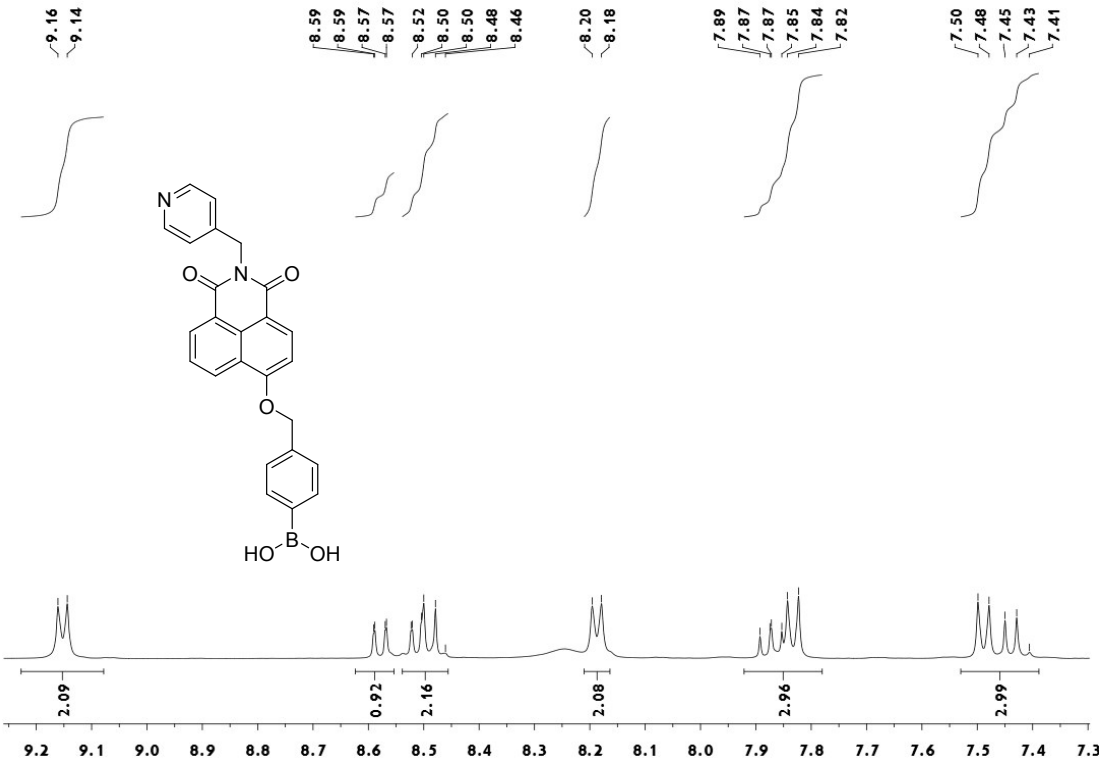
9.14
8.52
8.46
8.18
7.82
7.50
7.45
7.43
7.41
5.86
5.49
5.26
5.26
2.51
N
O
N
O
O
B
HO
OH
2.09
0.92
2.16
2.08
2.96
2.99
2.03
2.00
1.91
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


9.16
9.14
8.59
8.59
8.57
8.57
8.52
8.50
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8.48
8.46
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8.18
7.89
7.87
7.87
7.85
7.84
7.82
7.50
7.48
7.45
7.43
7.41
N
O
N
O
O
O
HO
B
OH
2.09
0.92
2.16
2.08
2.96
2.99
9.2
9.1
9.0
8.9
8.8
8.7
8.6
8.5
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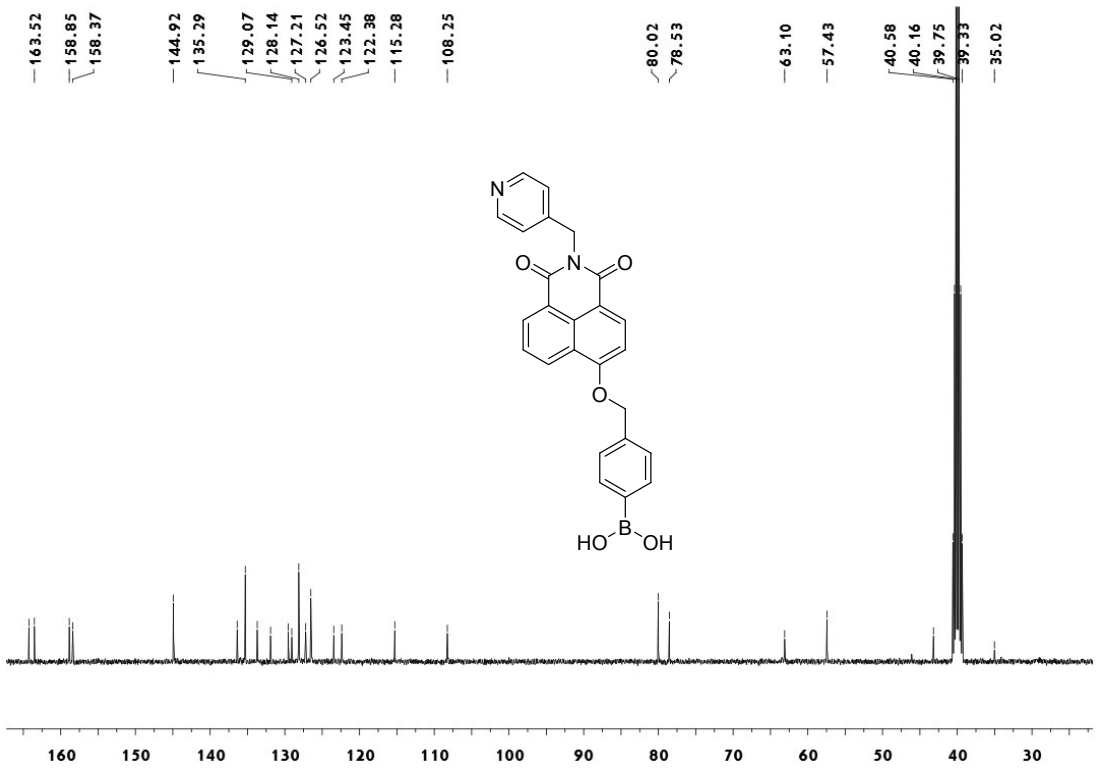
7.7
7.6
7.5
7.4
7.3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158.85
158.37
144.92
135.29
129.07
128.14
127.21
126.52
123.45
122.38
115.28
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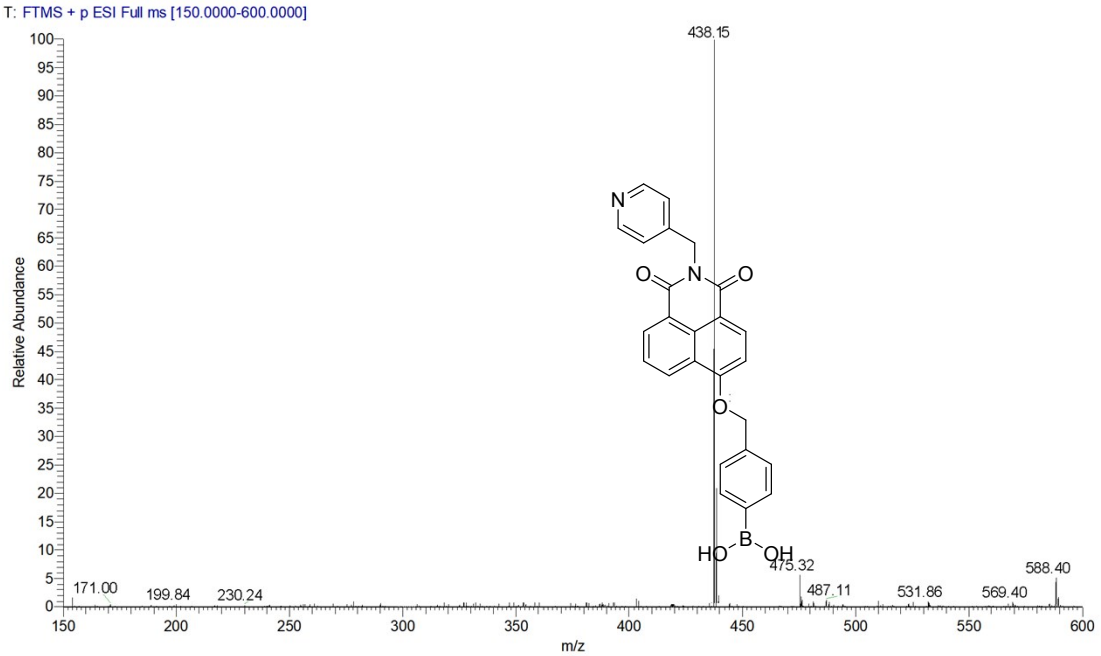
80.02
78.53
63.10
57.43
40.58
40.16
39.75
39.33
35.02
N
O
N
O
O
HO
B
OH
160
150
140
130
120
110
100
90
80
70
60
50
40
30


T: FTMS + p ESI Full ms [150.0000-600.0000]
Relative Abundance
m/z
438.15
475.32
588.40
487.11
531.86
569.40
171.00
199.84
230.24
N
O
N
O
O
HO
B
OH
100
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150
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250
300
350
400
450
500
550
600