A fluorescence turn-on probe for visualizing lysosome in hypoxic tumor cells

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Materials and methods

1. General information of materials and methods

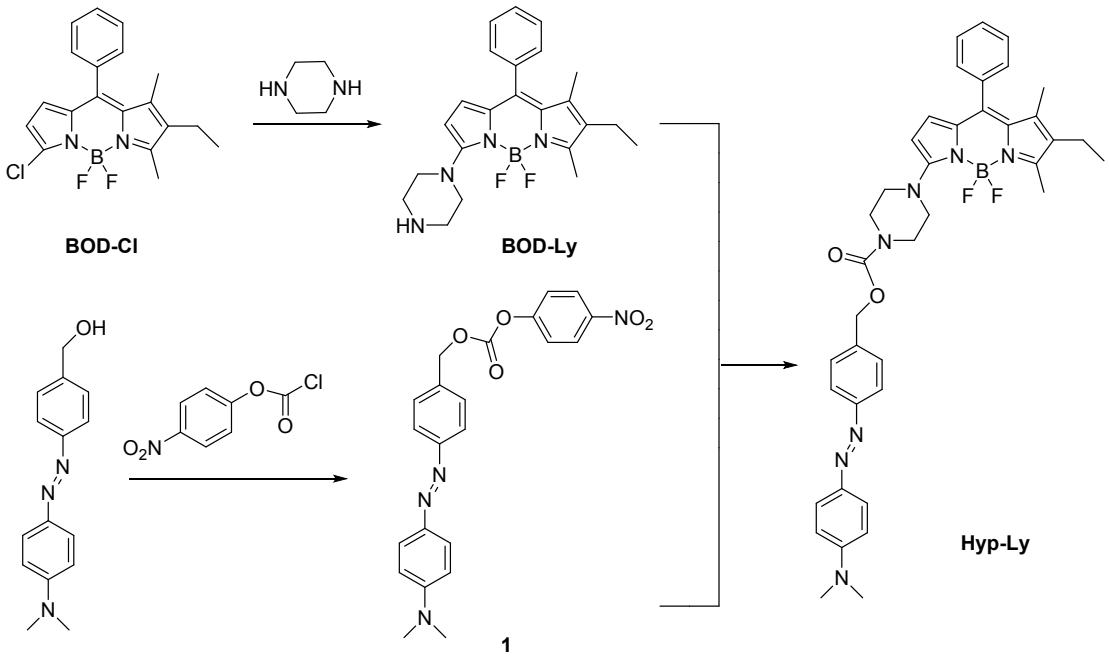
Generally, chemical reagents for synthesis were provided by commercial suppliers and were used without further purification unless otherwise noted. ^{1}H NMR and ^{13}C NMR spectra were carried out to identify the structures of new chemical compounds, which were recorded on a Bruker AV-400 spectrometer at ambient temperature. Mass spectra were further employed to demonstrate the obtained compounds, measured on a HP 1100 LC-MS spectrometer. For testing the optical properties, UV-vis absorption spectra were measured utilizing a Varian Cary 100 spectrophotometer, while fluorescence spectra were obtained on a Varian Cary Eclipse Fluorescence spectrophotometer.

The testing buffer solutions (PB buffer + 1 mM CTAB, pH 7.4) were initially bubbled

with argon gas for 30 minutes. Then rat liver microsomes (200 μg/mL), Hyp-Ly (10 μM) and NADPH (50 μM) were added quickly to 3 mL of the above solution. Time-dependent fluorescent intensity changes were recorded in the Fluorescence spectrophotometer.

2. Cell Cultures. In the experiments of monitoring cellular hypoxia in living cells, HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C under a humidified atmosphere of 20% O_2 with 5% CO_2 for normoxic condition, 1% O_2 with 5% CO_2 for hypoxia condition with varying incubation time.

3. Confocal fluorescence image of cells. Cells were grown in a glass bottom dish for 24 h. Staining experiments with probe, Lyso-Tracker Green or Mito-Tracker Green were performed in the same medium at 37 °C, followed by washing process with PBS buffer. Fluorescence images were collected using Nikor AIR with a 60 × oil objective. The red fluorescence signal of cells incubated with Hyp-Ly was collected at 550-600 nm with 514 nm as excitation wavelength, while the green signal from Lyso-Tracker Green or Mito-Tracker Green was observed at 500-530 nm and λ_{ex} = 488 nm.



Scheme S1. Synthesis of lysosome targetable probe for identification of hypoxic tumor cells.

4. **Synthesis of BOD-Ly.**

To a solution of piperazine (145 mg, 1.68 mmol) and Et3N (one drop) in 10 mL acetonitrile was added a solution of BOD-Cl (100 mg, 0.28 mmol) in 15 mL acetonitrile slowly. The reaction mixture was stirred for 12 hours at room temperature. After the reaction, acetonitrile was evaporated and CH_2Cl_2 was added to the residue, the organic layer was washed with 100 mL water dried over anhydrous Na_2SO_4, followed by evaporation under vacuum. Purification by chromatography afforded 82 mg solid product (71.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (dd, 3H), 7.30 (dd, 3H), 6.42 (d, 1H), 5.97 (d, 1H), 3.87 – 3.78 (m, 4H), 3.12 – 3.03 (m, 4H), 2.49 (s, 3H), 2.33 (q, 2H), 1.36 (s, 3H), 1.00 (t, 3H). ^{13}C NMR (101 MHz, DMSO-D_6) δ 135.01, 133.46, 132.80, 131.22, 129.53, 128.48, 128.21, 111.80, 50.46, 45.43, 16.62, 15.05, 11.73, 10.90. HRMS (ESI, m/z): calc. for $C_{23}H_{27}BF_2N_4$: 409.2375, found: 409.2372 $[M+H]^+$.

5. **Synthesis of Hyp-Ly.**

Compound **1** (210 mg, 0.5 mmol), BOD-Ly (205 mg, 0.5 mmol) and DMAP (92 mg, 0.75 mmol) were dissolved in 30 mL anhydrous CH_2Cl_2. The resulted solution was stirred for 10 hours at room temperature. After reaction, the mixture was washed with 300 mL water and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to remove organic solvent. The residual solid was purified by chromatography to give 138 mg product (40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 2H), 7.98 (d, 2H), 7.50 (d, 2H), 7.44 (dd, 3.3 Hz, 3H), 7.32 (dd, 2H), 6.98 (s, 2H), 6.44 (d, 1H), 5.96 (d, 1H), 5.24 (s, 2H), 3.75 (m, 8H), 3.20 (s, 6H), 2.52 (s, 3H), 2.35 (q, 2H), 1.39 (s, 3H), 1.02 (t, 3H). ^{13}C NMR (101 MHz, DMSO-D_6) δ 160.77, 154.36, 152.52, 152.01, 143.29, 142.51, 138.08, 134.85, 134.59, 129.47, 128.35, 128.26, 124.75, 121.82, 117.69, 112.56, 111.52, 66.04, 59.72, 54.89, 49.02, 14.98, 13.92, 13.36, 10.96. HRMS (ESI, m/z): calc. for $C_{39}H_{42}BF_2N_7O_2$: 690.3539, found: 690.3536 $[M+H]^+$.

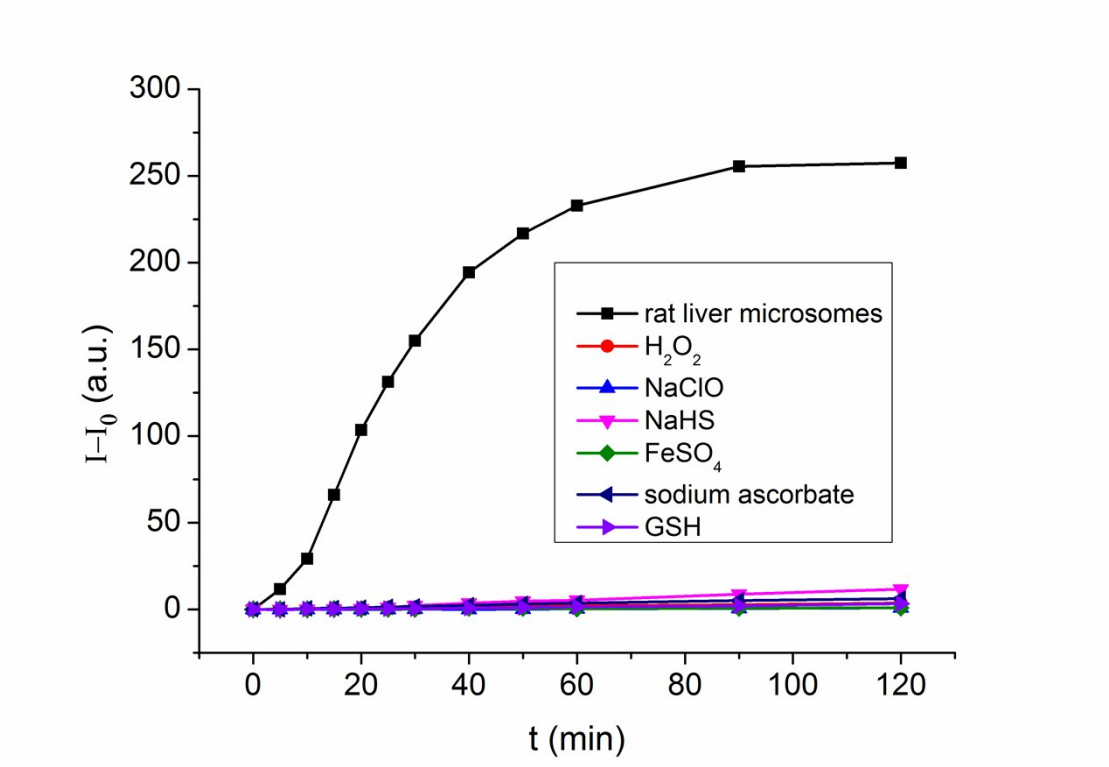


Figure S1. Time-dependent fluorescence changes of Hyp-Ly (10 μM) in the presence of rat liver microsomes and other species, including sodium ascorbate (10 mM), H_2O_2 (10 mM), ClO^- (10 mM), Fe^{2+} (5 mM), HS^- (10 mM), and GSH (10 mM) in a testing mode (PB buffer + 1 mM CTAB, pH 7.4) at 37 ^{0}C. I represents the fluorescence intensity of Hyp-Ly in the presence of analytes, I_0 represents the fluorescence intensity of Hyp-Ly in the absence of analytes.

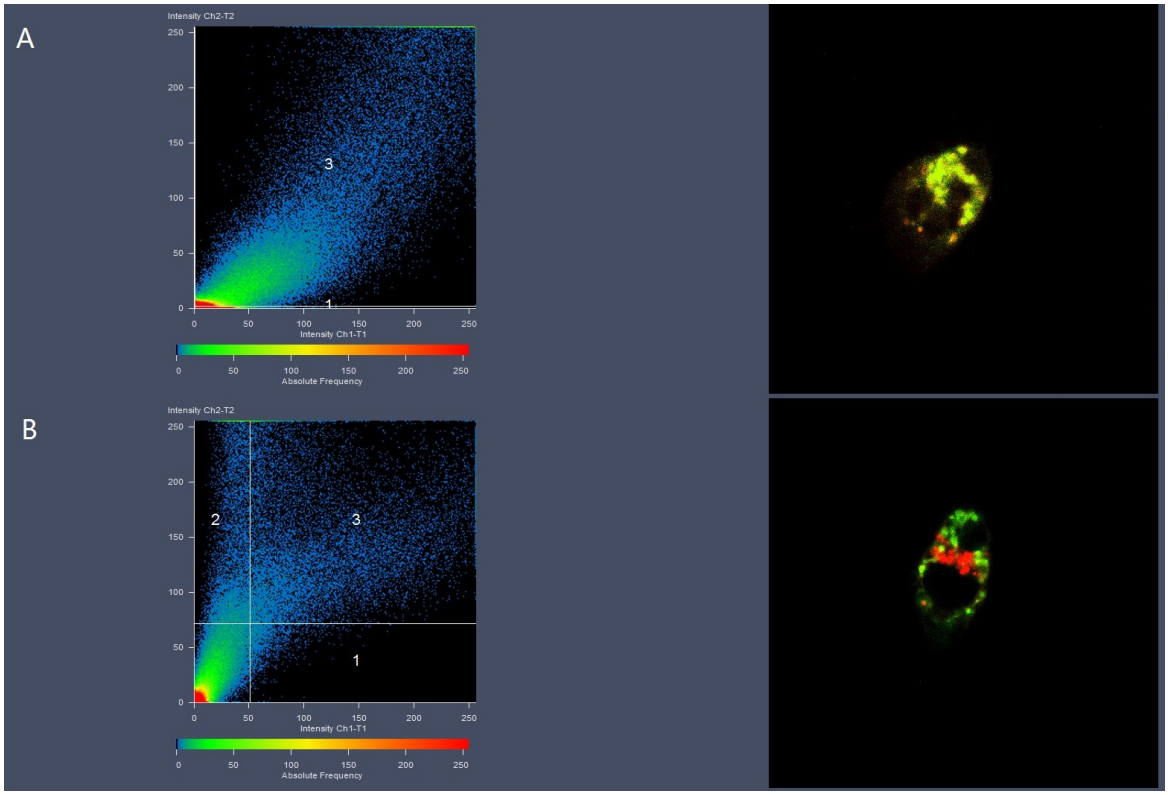


Figure S2. (A) Colocalization images of HepG2 cells under hypoxic condition (1% oxygen) stained with Hyp-Ly and Lyso-Tracker Green, and the correlation of Hyp-Ly and Lyso-Tracker Green intensities. (B) Colocalization images of HepG2 cells under hypoxic condition (1% oxygen) stained with Hyp-Ly and Mito-Tracker Green, and the correlation of Hyp-Ly and Mito-Tracker Green intensities.

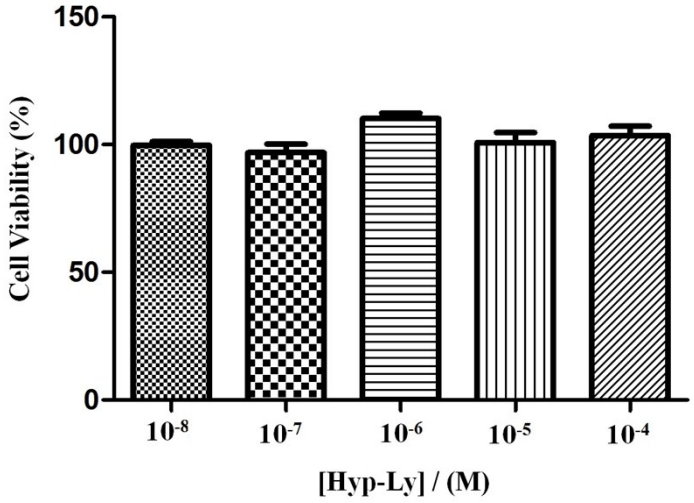


Figure S3. HepG2 cells viability after incubation with various concentrations of Hyp-Ly for 12 h.

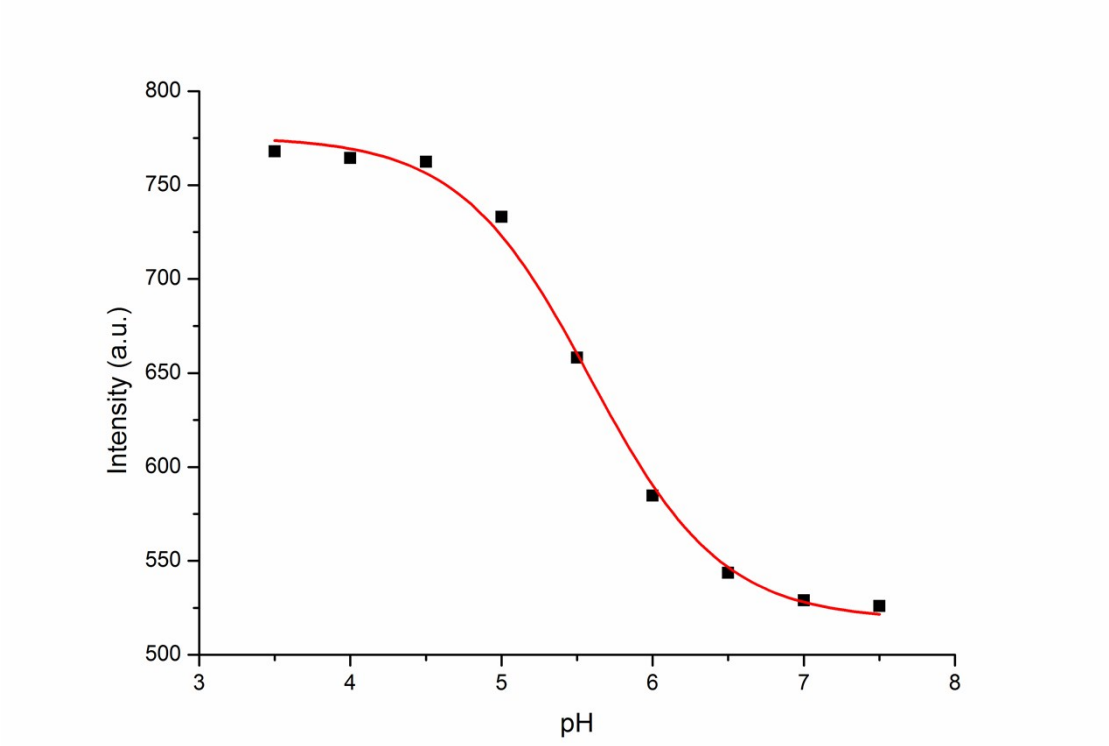
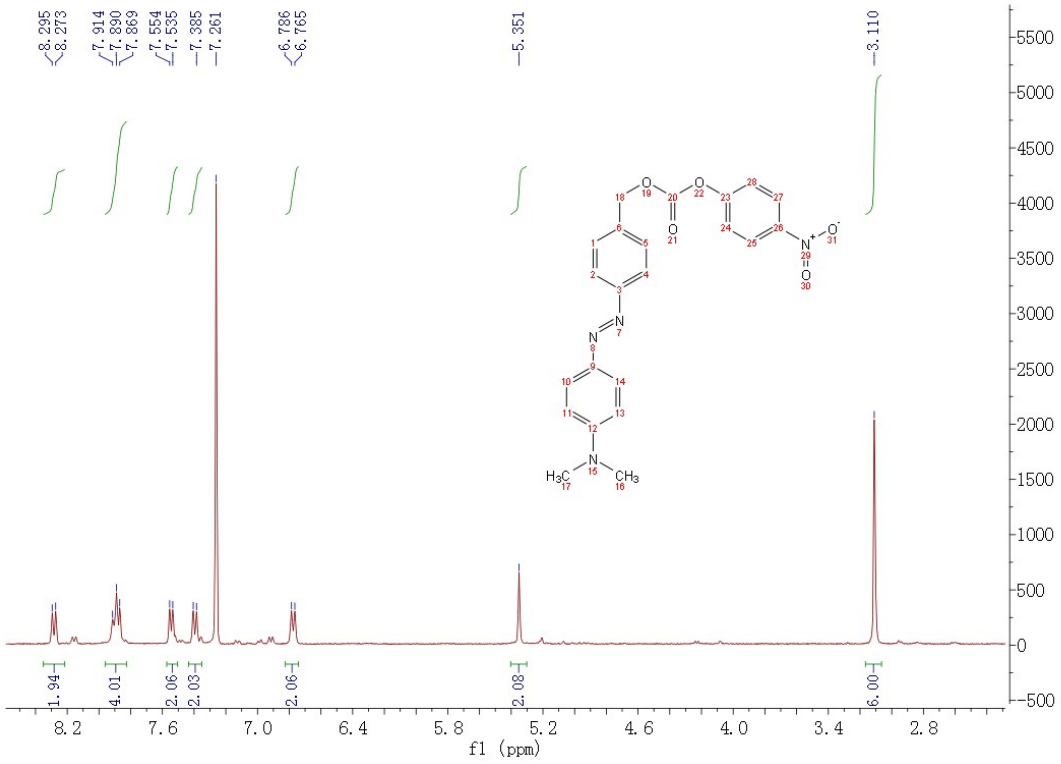
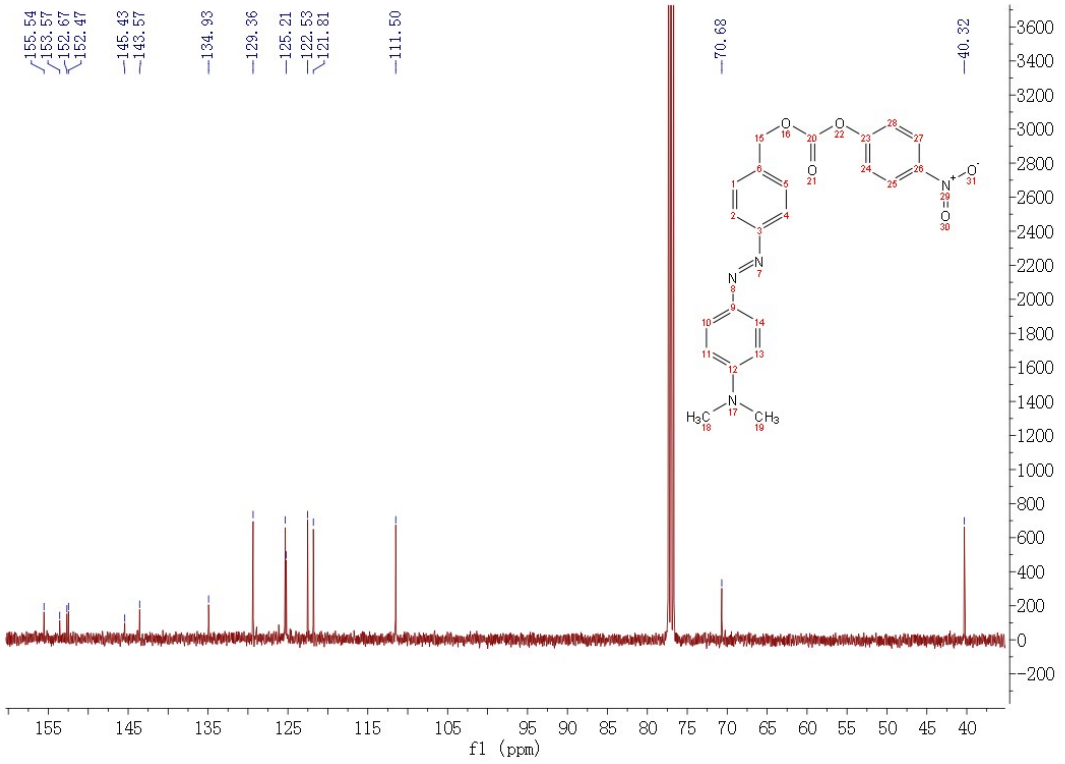


Figure S4. Fluorescence intensity Changes of BOD-Ly (10 μM) at 570 nm as function of pH values in PB buffer + 1 mM CTAB. The pKa of BOD-ly was determined to be 5.59 ± 0.04.





Single Mass Analysis

Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions

130 formula(e) evaluated with 9 results within limits (up to 1 closest results for each mass)

Elements Used:

C: 0-58 H: 0-49 N: 0-4 O: 0-5

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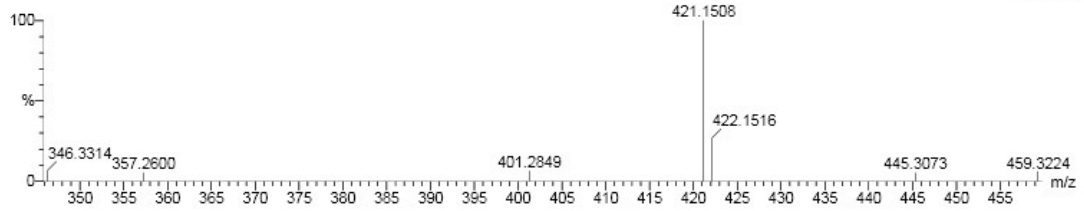

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Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
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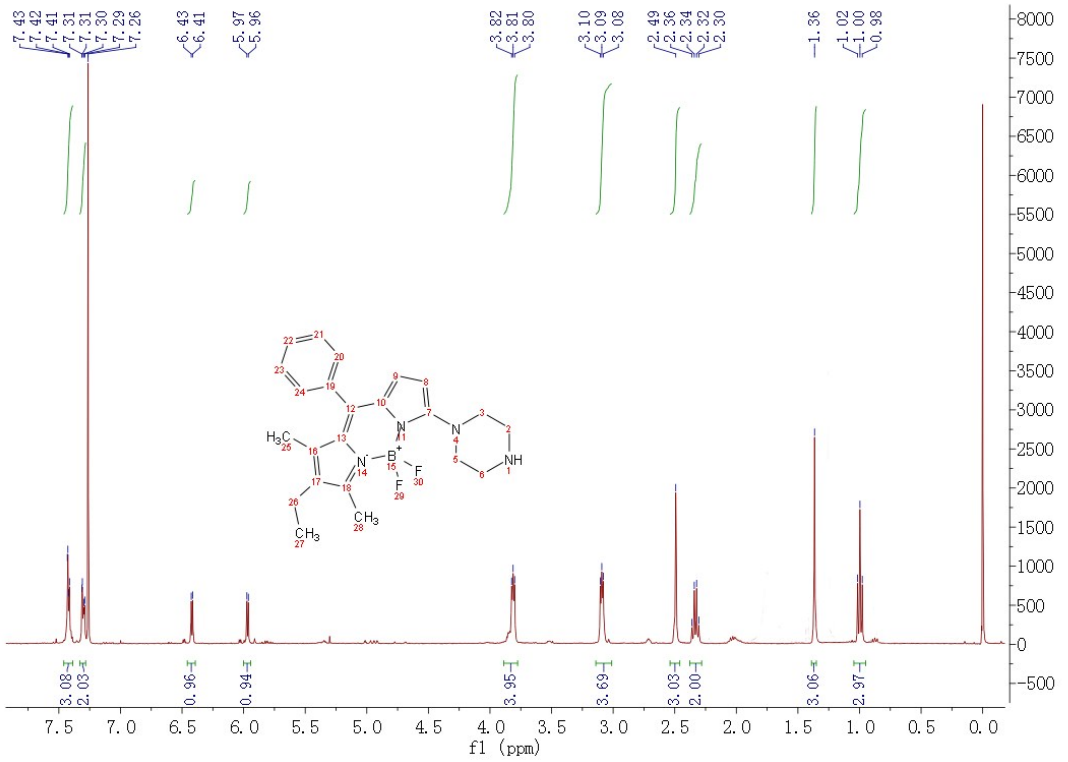
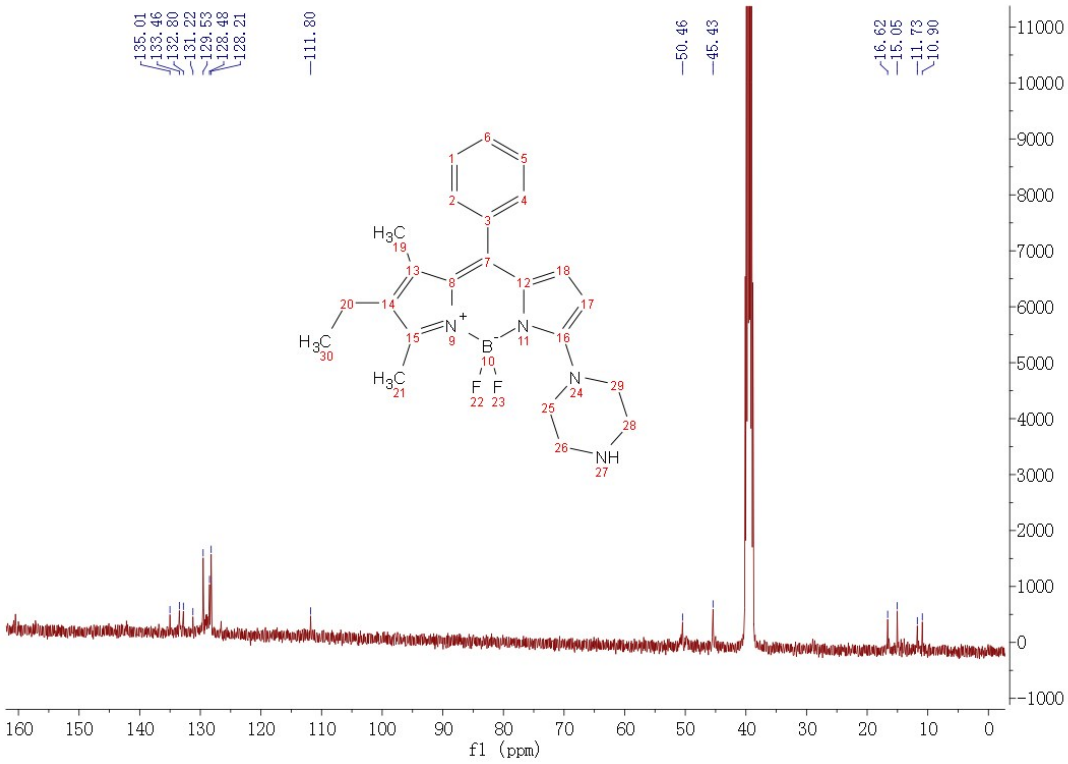
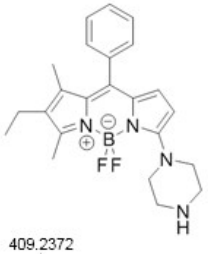

7.43
7.42
7.41
7.31
7.31
7.30
7.29
7.26
6.43
6.41
5.97
5.96
3.82
3.81
3.80
3.10
3.09
3.08
2.49
2.36
2.34
2.32
2.30
1.36
1.02
1.00
0.98
3.08
2.03
0.96
0.94
3.95
3.69
3.03
2.00
3.06
2.97
f1 (ppm)


135.01
133.46
132.80
131.22
129.53
128.48
128.21
111.80
50.46
45.43
16.62
15.05
11.73
10.90
f1 (ppm)

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
23 formula(e) evaluated with 1 results within limits (up to 1 closest results for each mass)
Elements Used:
C: 0-23 H: 0-28 B: 0-1 N: 0-4 F: 0-2

CC-ZHAO ECUST institute of Fine Chem



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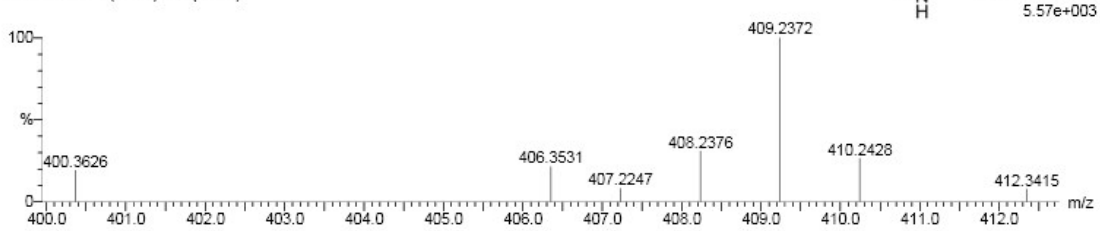
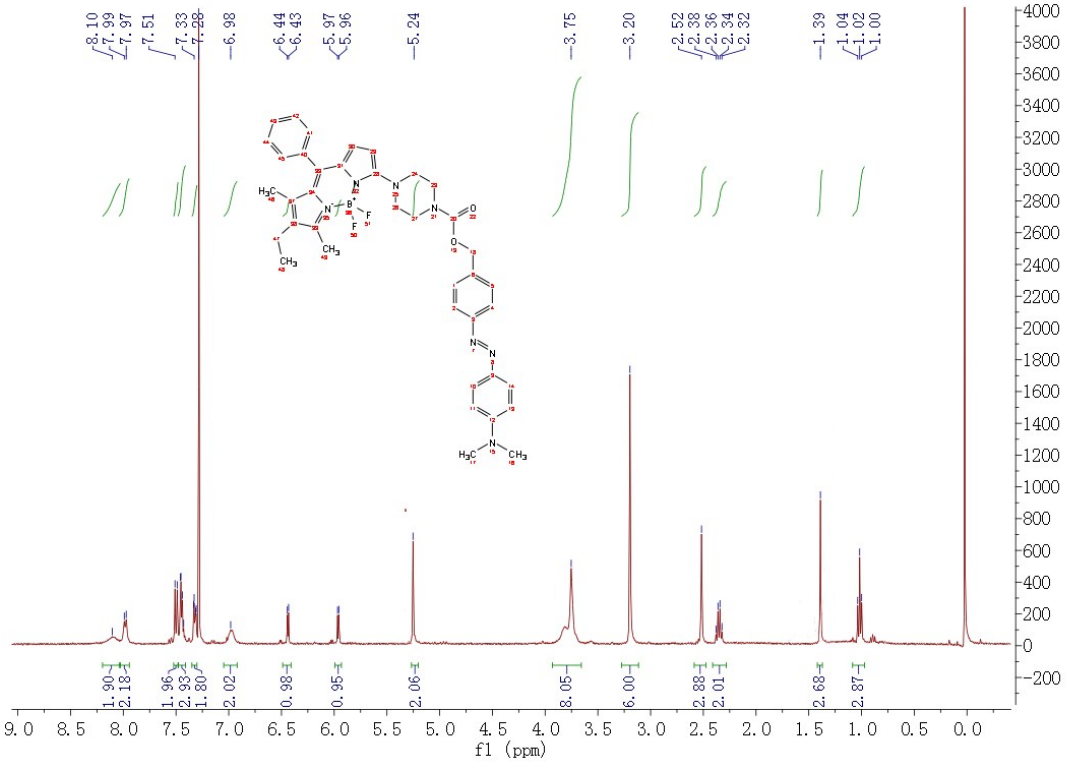
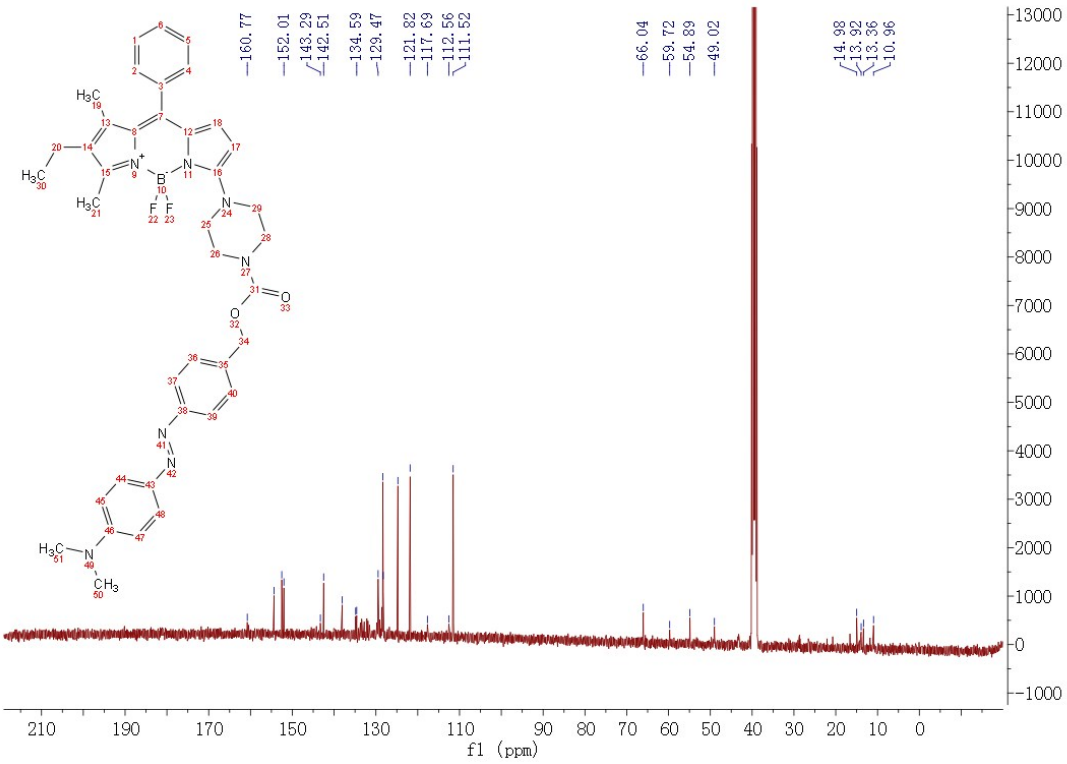
ZC-LSH-01 49 (0.396) Cm (47:53)



Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
409.2372	409.2375	-0.3	-0.7	11.5	22.2	0.0	C23 H28 B N4 F2



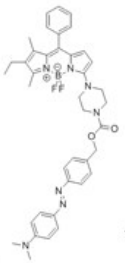


Single Mass Analysis

Tolerance = 50.0 PPM / DBE: min = -1.5, max = 100.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 2



Monoisotopic Mass, Even Electron Ions

103 formula(e) evaluated with 2 results within limits (up to 1 closest results for each mass)

Elements Used:

C: 0-40 H: 0-43 B: 0-1 N: 0-7 O: 0-2 F: 0-2

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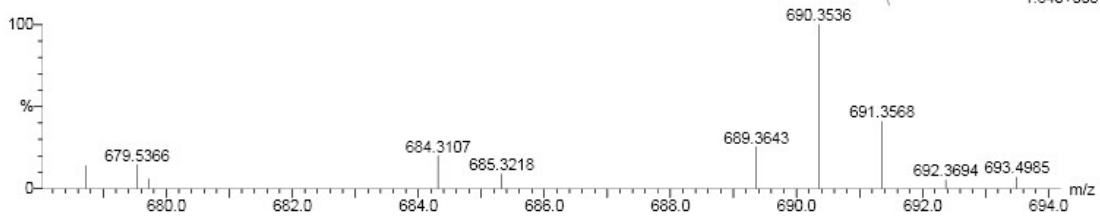

Minimum:				-1.5			
Maximum:		300.0	50.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
690.3536	690.3539	-0.3	-0.4	21.5	21.2	0.0	C39 H43 B N7 O2 F2