Supporting Information for

A lysosomal targeted fluorescent probe based on coumarin for monitoring hydrazine in living cells with high performance

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Materials and instruments

Without other noted, all the solvents, reagents and materials were obtained from business company and used without other purification. Twice-distilled water was applied to all measurements and experiments. High-resolution electrospray mass spectra (HRMS) were gained from Bruker APEX IV-FTMS 7.0T mass spectrometer; NMR spectra were examined from AVANCE III 400 MHz Digital NMR Spectrometer with TMS as an internal standard; Electronic absorption spectra were recorded on a LabTech UV Power spectrometer; Fluorescence spectra were obtained with a HITACHI F4600 fluorescence spectrophotometer; The fluorescence images of cells and tissues were collected with Nikon A1MP confocal microscopy with a CCD camera; The pH measurements were implemented on a Mettler-Toledo Delta 320 pH meter; analysis was exhibited on silica gel plates and column chromatography was carried out over silica gel (mesh 200-300). Both TLC and silica gel were purchased from the Qingdao Ocean Chemicals.

Cell culture

The living HeLa cells were cultured in the Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine serum (10% FBS) under the atmosphere containing 5% CO_2 and 95% air at 37 °C.

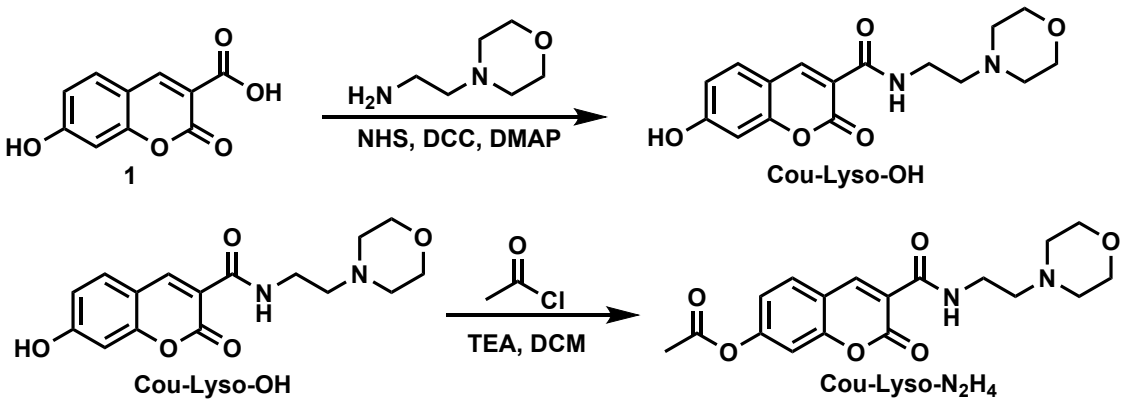
Cytotoxicity assays

The living cells line were treated in DMEM (Dulbecco's Modified Eagle Medium) supplied with fetal bovine serum (10%, FBS), penicillin (100 U/mL) and streptomycin (100 μg/mL) under the atmosphere of CO_2 (5%) and air (95%) at 37 °C. The HeLa cells were then seeded into 96-well plates, and 0, 2, 5, 10, 20, 30 μM (final concentration) of the probe **Cou-Lyso-N_2H_4** (99.9% DMEM and 0.1% DMSO) were added respectively. Subsequently, the cells were cultured at 37 °C in an atmosphere of CO_2 (5%) and air (95%) for 24 hours. Then the HeLa cells were washed with PBS buffer, and DMEM medium (500 μL) was added. Next, MTT (50 μL, 5 mg/mL) was injected to every well and incubated for 4 h. Violet formazan was treated with sodium dodecyl sulfate solution

(500 μL) in the H_2O-DMF mixture. Absorbance of the solution was measured at 570 nm by the way of a microplate reader. The cell viability was determined by assuming 100% cell viability for cells without **Cou-Lyso-N_2H_4**.

Imaging of N_2H_4 in HeLa cells

Before using, the HeLa cells were washed with PBS three times and then incubated with **Cou-Lyso-N_2H_4** (10 μM) for 30 min at an atmosphere of CO_2 (5%) and air (95%) in the condition of 37 °C. After incubating with N_2H_4 (100 μM) for another 0.5 h under the same conditions. Subsequently, the HeLa cells were rinsed by PBS buffers three times. The ideal fluorescence images were obtained by means of Nikon A1MP confocal microscopy with the equipment of a cooled CCD camera.



Synthesis of the probe Cou-Lyso-OH

Compound **1** (2.0 mmol, 412.3 mg), DMAP (9.24 mg, 0.076 mmol) and *N*-Hydroxysuccinimide (261.03 mg, 2.27 mmol) were dissolved in 5.0 mL of anhydrous DMF, and stirred at 0 °C for 30 min. A solution of DCC (652.3 g, 3.16 mmol) in anhydrous DCM (3.0 mL) was added drop wisely and stirred for 1 h at room temperature. Then the resulting mixture was added drop wisely into the solution of 4-(2-Aminoethyl)morpholine (312.5 mg, 2.4 mmol) in anhydrous DCM (7.0 mL) at 0 °C under N_2, and the mixture was stirred at room temperature overnight. After that, the precipitates were filtrated, and the filtrate was concentrated under vacuum and redissolved in dichloromethane. The organic layer was washed by water and brine,

dried over with Na_2SO_4 and evaporated under vacuum. The product was purified by silica column chromatography to give compound **Cou-Lyso-OH** as a white solid (432.9 mg, 68%). ^{1}H NMR (400 MHz, DMSO-d_6) 11.08 (s, 1H), 8.86 (s, 1H), 8.79 (s, 1H), 7.82 (d, J = 8.5 Hz, 1H), 6.88 (d, J = 8.2 Hz, 1H), 6.79 (s, 1H), 3.59 (s, 4H), 3.43 (d, J = 5.6 Hz, 2H), 2.50 – 2.38 (m, 6 H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.19, 161.86, 161.48, 156.77, 148.45, 132.43, 114.80, 113.97, 111.54, 102.24, 66.72, 57.13, 53.54, 36.47.

Synthesis of the probe Cou-Lyso-N_2H_4

Compound **Cou-Lyso-OH** (0.5 mmol, 159.1 mg, 1.0 equiv) was dissolved in DCM (2.0 mL). And the triethylamine (0.6 mmol, 60.7 mg, 1.2 equiv) was added. The reaction was put in an ice bath. Then, the acetyl chloride (0.6 mmol, 47.1 mg, 1.2 equiv) was added drop by drop. The reaction was moved to room temperature for about 5 h and the ice distilled water (10.0 mL) was added for extracting with DCM. The product was purified by silica column chromatography to give the probe **Cou-Lyso-N_2H_4** with 53% yield. ^{1}H NMR (400 MHz, $CDCl_3$) 9.09 (s, 1H), 8.7 (s, 1H), 7.88 (d, J = 6.8 Hz, 1H), 7.21 (d, J = 1.6 Hz, 1H), 7.15 (dd, J_1 = 2.0 Hz, J_2 = 6.8 Hz, 1H), 3.76 (t, J = 3.6 Hz, 4H), 3.59 (dd, J_1 = 4.4, J_2 = 8.8, 2H), 2.61 (s, 2H), 2.53(s, 4H), 2.36(s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.4, 161.3, 161.0, 155.1, 154.9, 147.5, 130.6, 119.4, 118.0, 116.4, 110.1, 67.0, 56.8, 53.4, 36.7, 21.2; HRMS (ESI) m/z calcd for $C_{18}H_{21}N_2O_6^+$ $(M+H)^+$: 361.1670; found 361.1678.

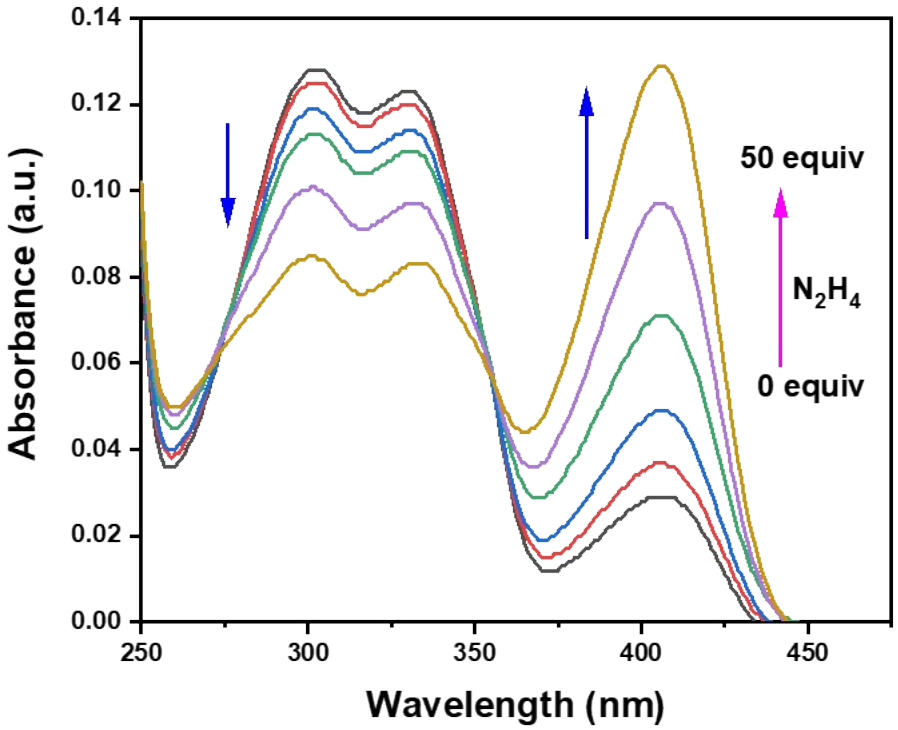


Fig. S1. The absorption spectra of **Cou-Lyso-N_2H_4** (10 μM) in pH 7.4 PBS/DMSO (v/v = 4/1) in the absence or presence of N_2H_4.

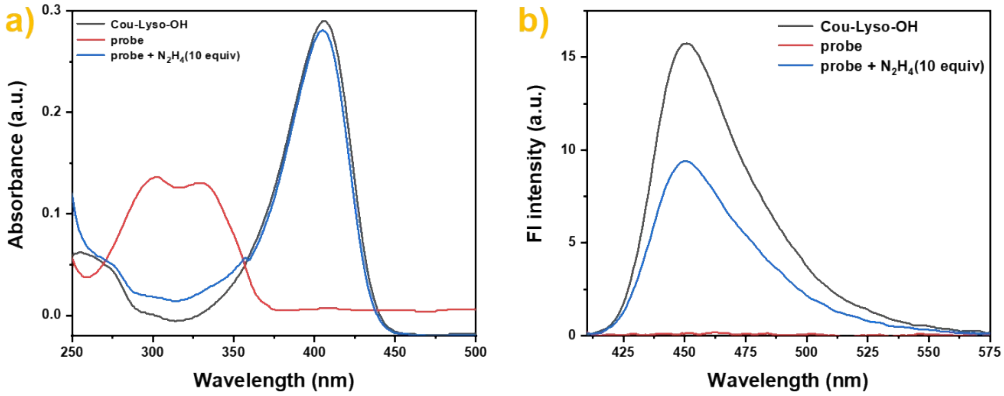


Fig. S2. a) The absorption spectra of **Cou-Lyso-OH** (10 μM) and **Cou-Lyso-N_2H_4** (10 μM) in pH 7.4 PBS/DMSO (v/v = 4/1) in the absence or presence of N_2H_4; b) The fluorescence spectra of **Cou-Lyso-OH** (10 μM) and **Cou-Lyso-N_2H_4** (10 μM) in pH 7.4 PBS/DMSO (v/v = 4/1) in the absence or presence of N_2H_4.

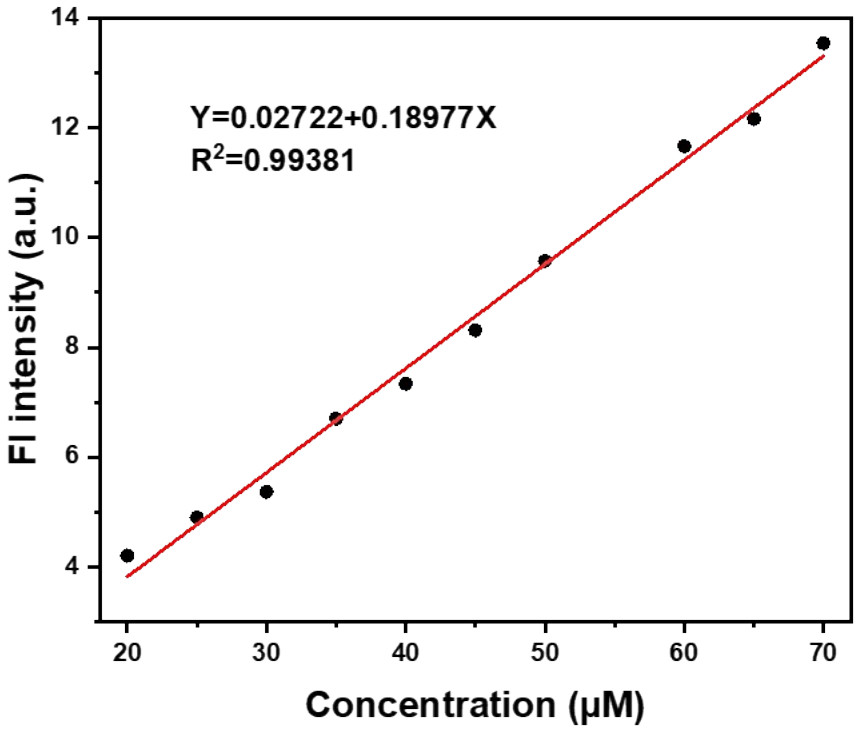


Fig. S3. The linear fit of **Cou-Lyso-N_2H_4** (10 μM) in pH 7.4 PBS/DMSO (v/v = 4/1) in the absence or presence of N_2H_4 (2-7 equiv).

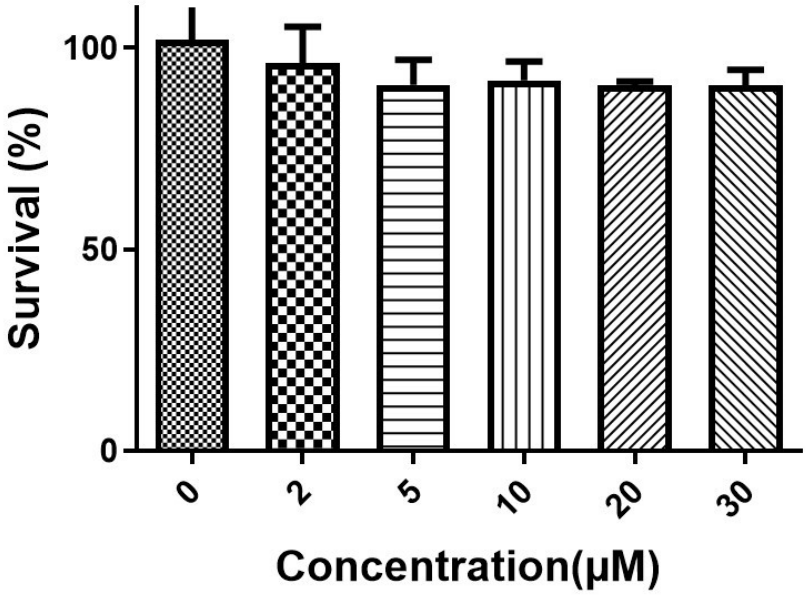


Fig. S4. Cytotoxicity assays of **Cou-Lyso-N_2H_4** at different concentrations (0 μM; 1μM; 5 μM; 10 μM; 20 μM; 30 μM) for HeLa cells.

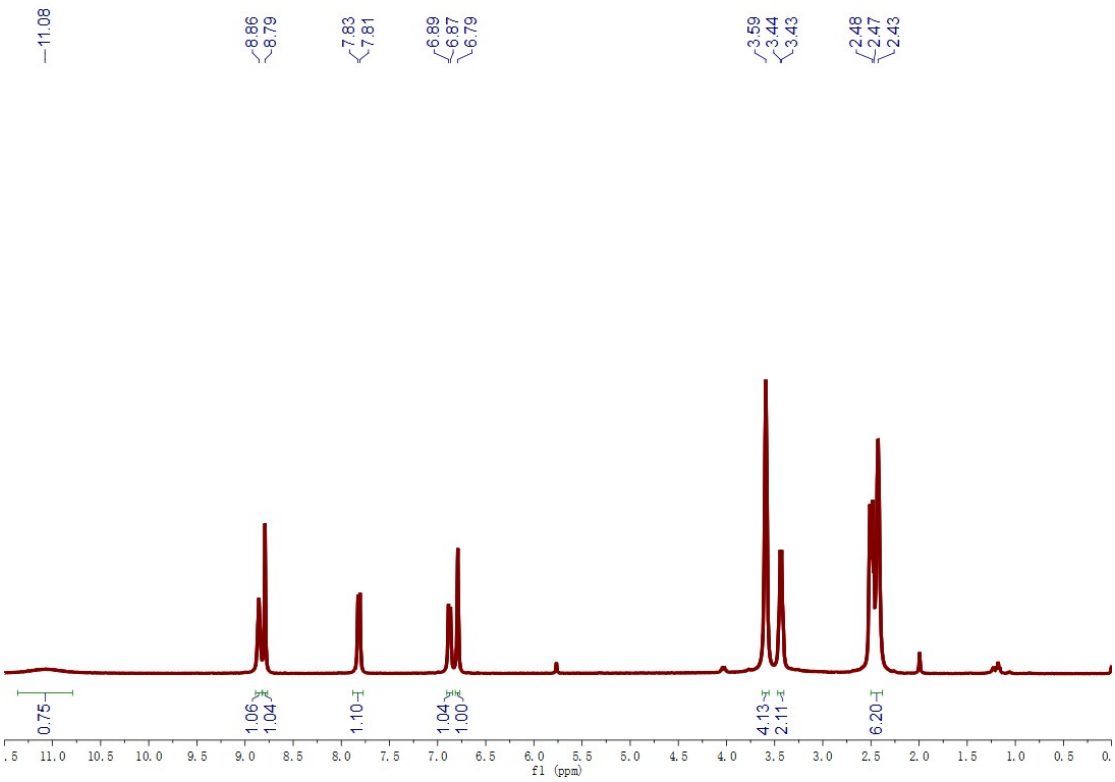


Fig. S5. ^{1}H NMR (DMSO-d_6) spectrum of **Cou-Lyso-OH.**

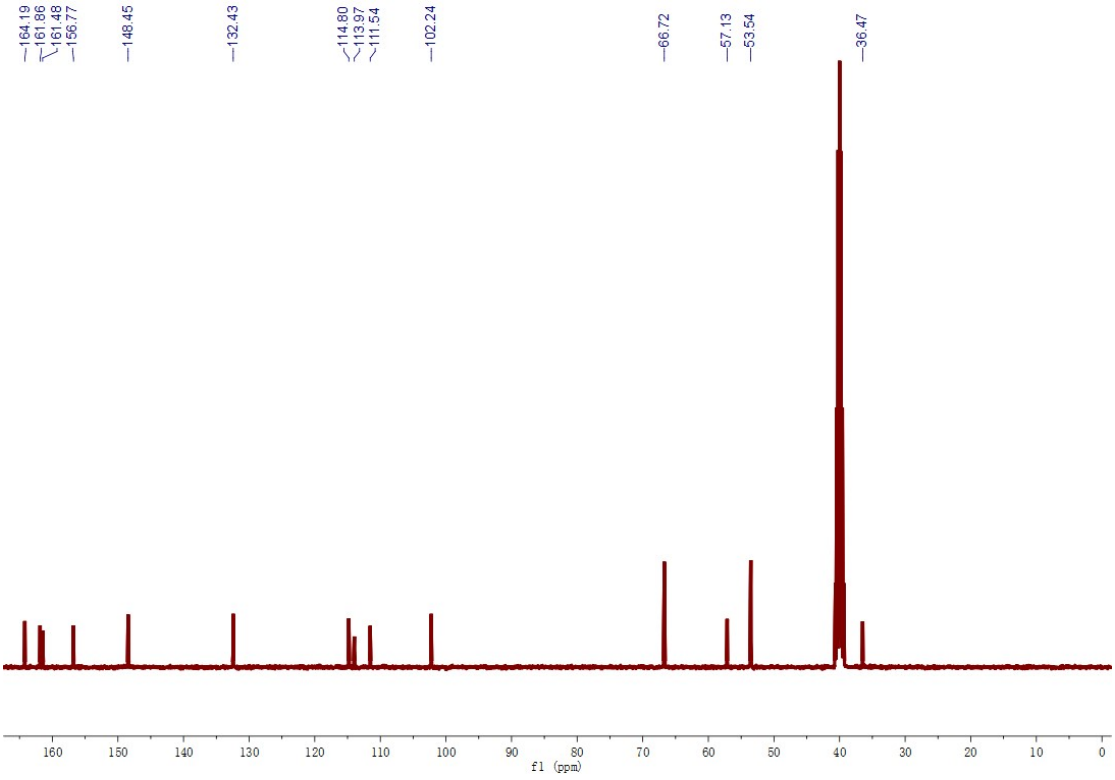


Fig. S6. ^{13}C-NMR (DMSO-d_6) spectrum of **Cou-Lyso-OH.**

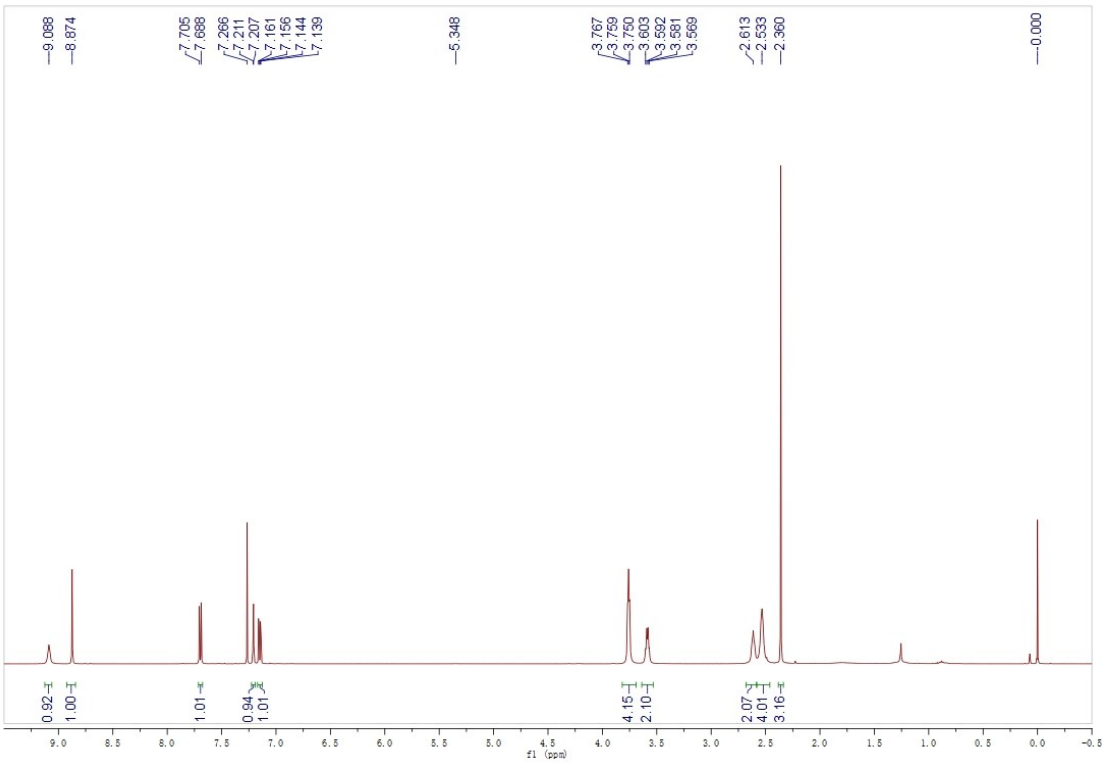


Fig. S7. ^{1}H NMR ($CDCl_3$) spectrum of **Cou-Lyso-N_2H_4.**

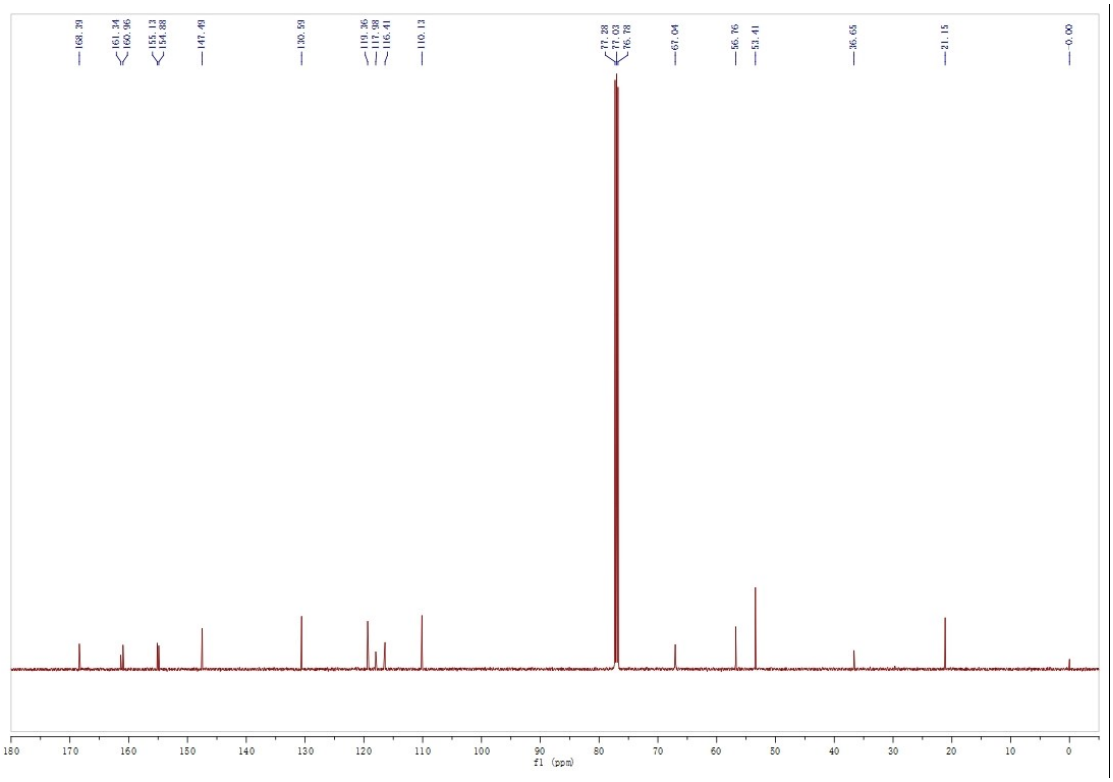


Fig. S8. ^{13}C-NMR ($CDCl_3$) spectrum of **Cou-Lyso-N_2H_4.**

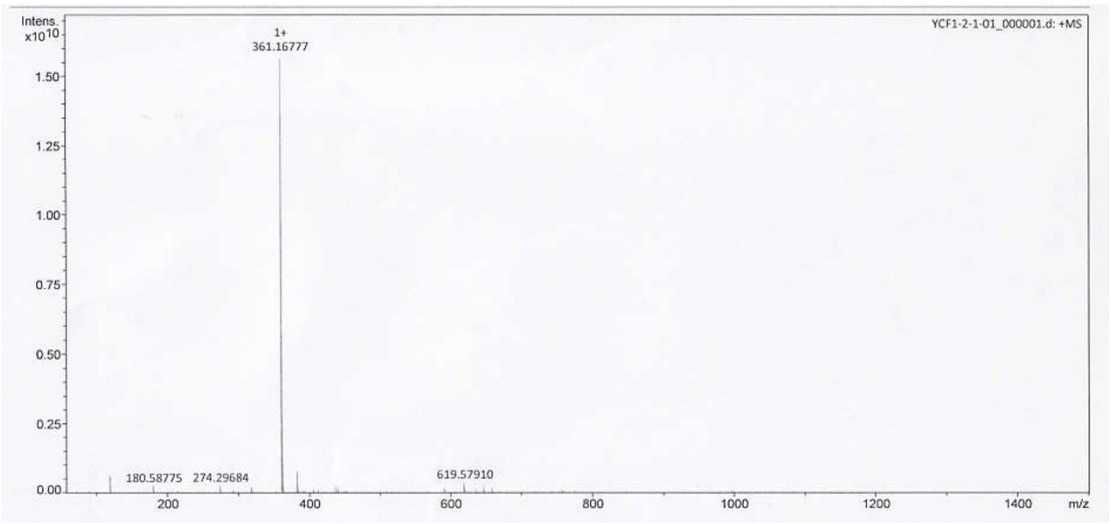


Fig. S9. HRMS (positive ion mode) spectrum of **Cou-Lyso-N_2H_4**.

Fig. S10. HRMS (positive ion mode) spectrum of **Cou-Lyso-N_2H_4**(10 μM) in pH 7.4 PBS/DMSO (v/v = 4/1) in the presence of N_2H_4 (10 equiv).