Supporting Information for

A water-soluble polymer fluorescent probe polymerized via RAFT polymerization for dynamic monitoring of cellular lipid droplet levels

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1. Materials and instruments

High-resolution electrospray mass spectra (HRMS) were gained from Bruker APEX IV-FTMS 7.0T mass spectrometer; NMR spectra were examined from AVANCE II 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 fluorescent images of the cells and zebrafish were obtained with Leica SP8 inverted fluorescence confocal microscope. The number average molecular weight (Mn) and PDI were determined by Waters 2695 gel permeation chromatography (GPC) at 25°C with THF as eluent (1.0 mL/min). The dynamic diameter of the micelles was measured directly by the Zetasizer Nano ZS (Malvern Instruments). 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.

2. Synthesis of coumarin fluorescent monomer (CMMA)

The synthesis design route of cmma was shown in Scheme S1, and the specific synthesis method was as follows.

Synthesis of compound 1

Diethyl malonate (1.60 g, 10 mmol), 4-(diethylamino)-2-hydroxybenzaldehyde (1.93 g, 10 mmol) and piperidine (0.25 g, 3 mmol) were dissolved in anhydrous ethanol (15 mL) and reacted by heating at reflux for 6 h under nitrogen protection. After the reaction, the remaining solvent was removed by evaporation under reduced pressure. Then, the mixed solution HCl/glacial acetic acid (1:1, v/v, 30 mL) were added to the reaction system at 120°C. The reaction was continued for 7 h. After the reaction, the resulting mixture was poured into deionized water (40 mL). The pH was adjusted to neutral with sodium hydroxide solution (40 %). The dark brown precipitate was

produced, which was filtered and washed several times with water and ethanol to obtain the target product (1.98 g, 91 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, J = 9.3 Hz, 1H), 7.24 (d, *J* = 8.8 Hz, 1H), 6.56 (d, *J* = 8.8 Hz, 1H), 6.46 (s, 1H), 6.02 (d, *J* = 9.3 Hz, 1H), 3.40 (q, *J* = 7.2 Hz, 4H), 1.20 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.18, 156.68, 150.66, 143.81, 128.85, 108.88, 108.71, 108.20, 97.29, 44.74, 12.41. HRMS (ESI) Found: 218.1181 $[M + H]^+$; Molecular formula $C_{13}H_{15}NO_2$ requires $[M+H]^+$ 218.1176 (Fig. S8-S10).

*Synthesis of compound **2***

DMF (1 mL) was added to a dry and N_2-protected reaction bottle, and $POCl_3$ (1.61 g, 10.5 mmol) dissolved in DMF (1 mL) was slowly injected into the reaction bottle and stirred at 50 °C for 45 min. Compound **2** (0.76 g, 3.5 mmol) was dissolved in DMF (5 mL) and added to the above mixed system and stirred for 2 h at 60 °C. After the reaction, the mixture was slowly poured into 100 mL ice water and the pH was adjusted to 7 with NaOH (20%). The precipitate was filtered and washed several times with deionized water and anhydrous ethanol to obtain a yellow solid powder (0.69 g, 81 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.13 (s, 1H), 8.26 (s, 1H), 7.43 (d, *J* = 9.0 Hz, 1H), 6.68 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.53 (d, *J* = 2.7 Hz, 1H), 3.48 (q, *J* = 7.1 Hz, 4H), 1.26 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 187.55, 161.64, 158.80, 153.51, 145.30, 132.53, 113.96, 110.32, 108.11, 96.96, 45.28, 12.45. HRMS (ESI) Found: 246.1130 $[M + H]^+$; Molecular formula $C_{14}H_{15}NO_3$ requires $[M+H]^+$ 246.1125 (Fig. S11-S13).

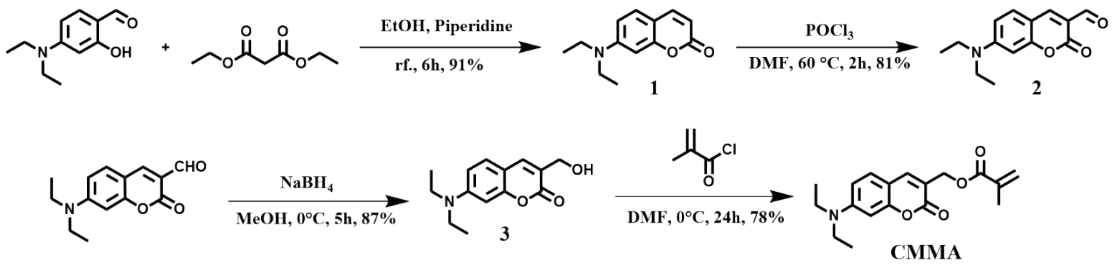
Synthesis of compound 3

Compound 2 (24.5 mg, 0.1 mmol) was dissolved in methanol (3 mL) and added $NaBH_4$ (2.65 mg, 0.07 mmol) slowly at 0 °C for 30min, then transferred to room temperature and continued to react for 3h. The reaction was burst with water, extracted three times with DCM, dried with anhydrous sodium sulfate, filtered, and finally purified by column chromatography (petroleum ether/ethyl acetate = 3/1) to obtain a light yellow solid powder (23.28 mg, 94%).^{1}H NMR (400

MHz, DMSO-*d6)* δ 7.76 (s, 1H), 7.47 (s, 1H), 6.68 (d, J = 10.9 Hz, 1H), 6.53 (s, 1H), 5.23 (t, J = 5.6 Hz, 1H), 4.29 (d, J = 4.7 Hz, 2H), 3.42 (q, J = 7.1 Hz, 4H), 1.12 (t, J = 7.0 Hz, 6H) (Fig. S14).

Synthesis of compound CMMA

Compound 3 (24.7 mg, 0.1 mmol) and triethylamine (0.2 eq.) were dissolved in DMF (3 mL) at 0 °C and slowly added to methacryloyl chloride (12.0 μL, 0.12 mmol) by syringe in N_2 environment for 1h and then moved to room temperature and continued the reaction for 6h. After the reaction was completed, it was extracted three times with water and DCM followed by two extractions with saturated sodium bicarbonate solution, dried over anhydrous sodium sulfate and then filtration. Finally, a yellow solid powder (24.52 mg, 73%) was obtained by column chromatography (petroleum ether/ethyl acetate = 20/1). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (s, 1H), 7.36 (d, *J* = 8.8 Hz, 1H), 6.90 (s, 1H), 6.73 (s, 1H), 6.19 (s, 1H), 5.62 (s, 1H), 5.11 (s, 2H), 3.44 (q, *J* = 7.1 Hz, 4H), 1.98 (s, 3H), 1.23 (t, *J* = 7.2 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.30, 161.66, 156.40, 150.47, 142.54, 136.14, 129.15, 126.06, 116.12, 109.34, 108.52, 97.74, 62.01, 45.19, 18.35, 12.35.(Fig. S15-S16)



Scheme S1. The synthesis route of coumarin fluorescent monomer (CMMA)

3. General information for spectroscopic studies

A certain amount of probe **P(COU-PEG-LD)** was dissolved in dimethyl sulfoxide (DMSO) to obtain the stock solution (1 mg/mL). The 10 μg/mL probe was used in all spectroscopic experiments,

prepared by adding 20 μL of the probe stock solution to the cuvette after dilution to 2 mL with different solvents. Solutions of various interfering substances (10 mM) were prepared in ultrapure water. The PBS buffer solutions with different pH from 1-14 were measured and prepared with a pH meter. The excitation wavelength was 380 nm; Vol. 600 v; both of the excitation and emission slit widths are 5 nm.

4. Cell culture and cytotoxicity assays

Hela cells were provided by Jiangsu Kaiji Biotechnology Co., Ltd. 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. The cytotoxic effects of the probe (**P(COU-PEG-LD)**) were tested by the MTT assay. 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 μg/ml (final concentration) of the probe **P(COU-PEG-LD)** (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 **P(COU-PEG-LD)**.

5. Imaging of HeLa cells and co-localization experiments

The concentration of the probe in the cell imaging experiments was 10 μg/mL, and the cells were incubated at a temperature of 37°C with 5% CO_2 for 30 min. To remove the residual probe, the cells were rinsed three times with PBS buffer solution before imaging. Starvation cell group: obtained by feeding with fetal bovine serum (10%, FBS) only without any added high sugar for 5h under the same culture conditions; Oleic acid cell group: obtained by adding oleic acid at a concentration of 10 μM for 1h under normal culture conditions; Dynamic lipid droplet imaging: starved cultured cell groups were first co-incubated with probe **P(COU-PEG-LD)** (10 μg/mL) for 30 min and then scanned for imaging at different time ranges after adding oleic acid (10 μM) stimulation. Finally, the cells were imaged with a Leica SP8 inverted fluorescence confocal microscope with excitation wavelength of 405 nm and emission wavelengths of 440-480 nm (blue channel) and 500-560 nm (green channel), respectively.

6. Zebrafish imaging experiments

Live zebrafish under different conditions were incubated with 10 μg/mL **P(COU-PEG-LD)** in PBS buffer for 30 min and then transferred to another imaging plate containing a trace of water and placed under a confocal microscope for imaging with an excitation wavelength of 405 nm and emission wavelengths of 440-480 nm.

Table S1. Molecular weight distribution data of polymer probe **P(COU-PEG-LD)**.

Sample	*Mn*	*Mw*	PDI
P(COU-PEG-LD)	5603	7341	1.31

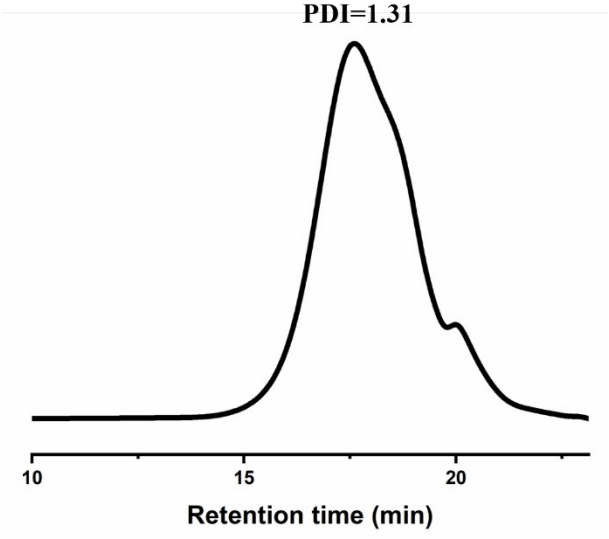


Fig. S1. Polymer probe **P(COU-PEG-LD)** GPC traces using THF as eluent (refractive index response).

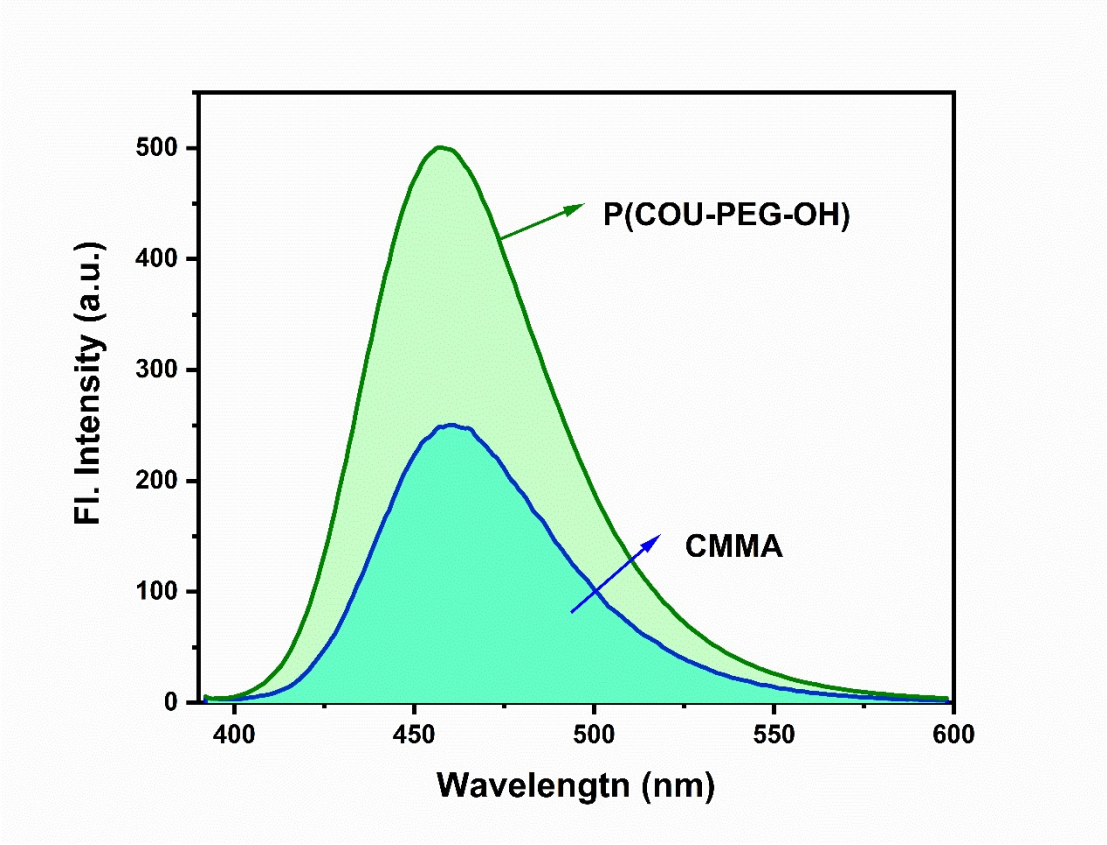


Fig. S2. Fluorescence emission spectra of polymeric probe **P(COU-PEG-OH)** (10 µg/mL) and fluorescent monomer CMMA (1.25µM) in DMSO. λ_{ex}=380 nm, Vol.=600 V, Slit =5 nm.

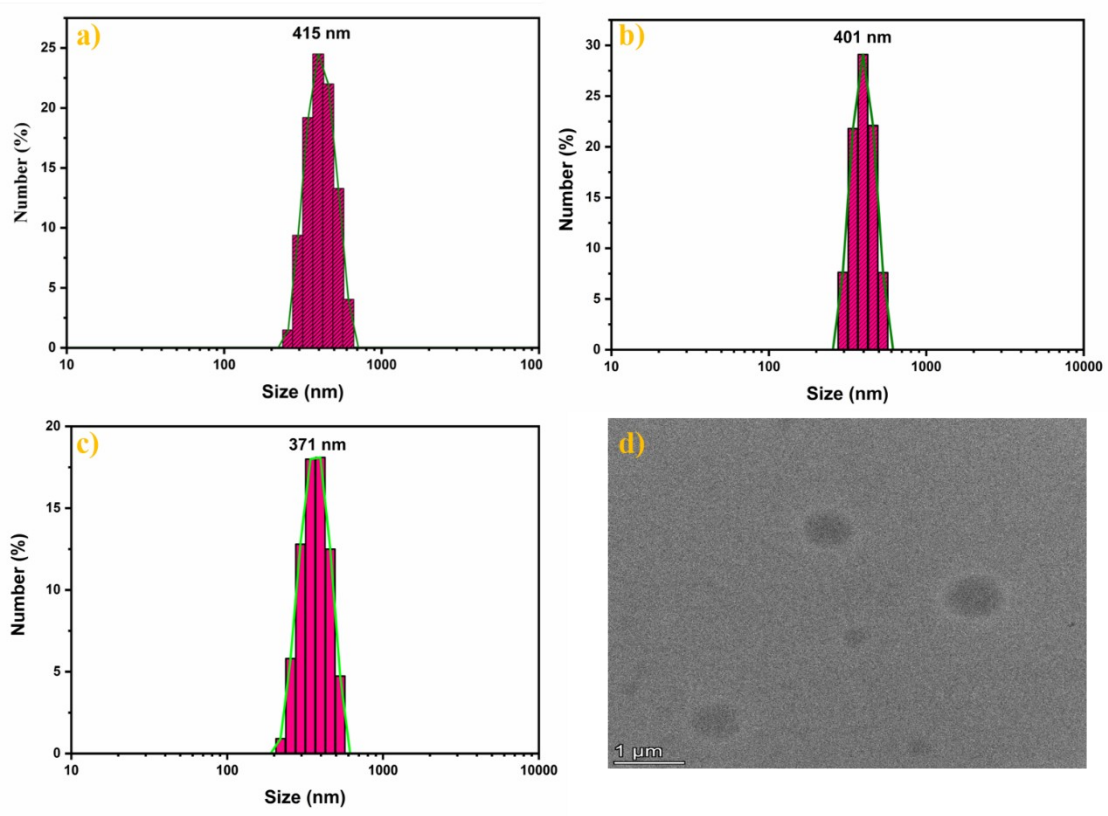


Fig. S3. a) Particle size distribution of polymeric probe **P(COU-PEG-OH)** (10 μg/mL) in PBS. **b)** Particle size distribution of polymeric probe **P(COU-PEG-OH)** (20 μg/mL) in PBS. **c)** Particle size distribution of polymeric probe **P(COU-PEG-OH)** (30 μg/mL) in PBS. **d)** TEM image of polymeric probe **P(COU-PEG-OH)** dispersed in PBS, scale bar = 1 μm.

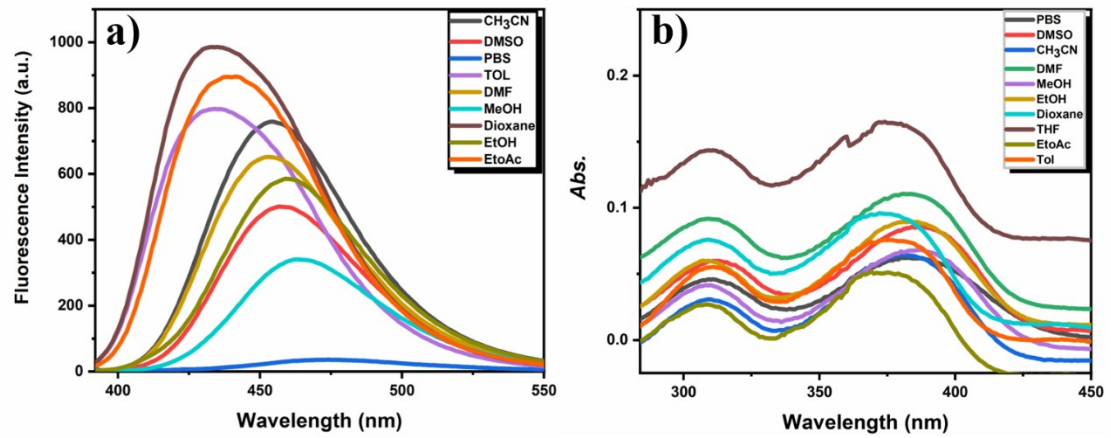


Fig. S4. a) Fluorescence emission spectra of probe **P(COU-PEG-LD)** (10 μg/mL) in different solvents. **b)** UV-absorption spectra of probe **P(COU-PEG-LD)** (10 μg/mL) in different solvents.

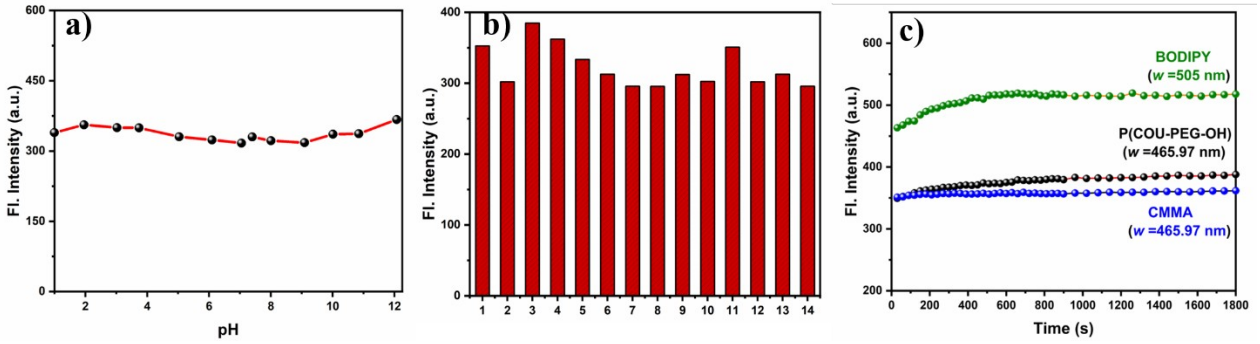


Fig. S5. **a)** Fluorescence emission intensity of the probe **P(Cou-PEG-LD)** (10 μg/mL) in different pH Dioxane/PBS buffers (v/v = 1/1). **b)** Fluorescence intensity of the probe **P(Cou-PEG-LD)** (10 μg/mL) in the presence of different analytes (10 μM) in dioxane/PBS buffers (v/v = 1/1). Where 1-14 represent blank, HS^-, H_2O_2, TBHP, NO_2, SO_4^{2-}, Cl^-, HSO_3^-, ClO^-, HCO_3^-, Cys, Na^+, Mg^{2+}, Ca^{2+} respectively. **c)** BODIPY **(1.25 μM)** and CMMA **(1.25 μM)** were used as references to detect the kinetic stability fluorescence spectra of probe **P(Cou-PEG-LD)** (10 μg/mL) in dioxane/PBS buffer (v/v=1/1). For **P(COU-PEG-OH)** and **CMMA**: λ_{ex}=380 nm, Vol.=600 V, Slit =5 nm. For **BODIPY**: λ_{ex}=430 nm, Vol.=600 V, Slit =5 nm.

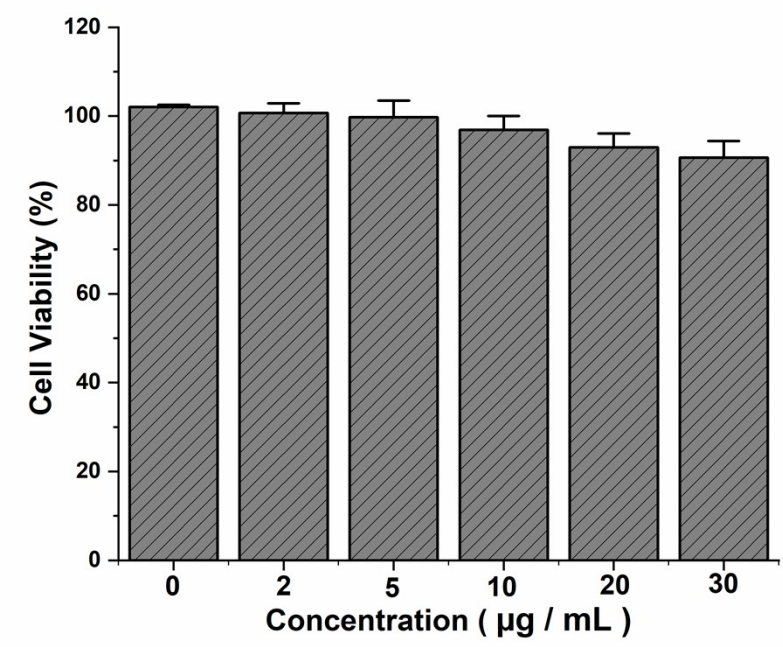


Fig. S6. Cytotoxicity assays of probe **P(COU-PEG-LD)** at different concentrations (0 μg/mL; 2 μg/mL; 5 μg/mL; 10 μg/mL; 20 μg/mL; 30 μg/mL) for HeLa cells.

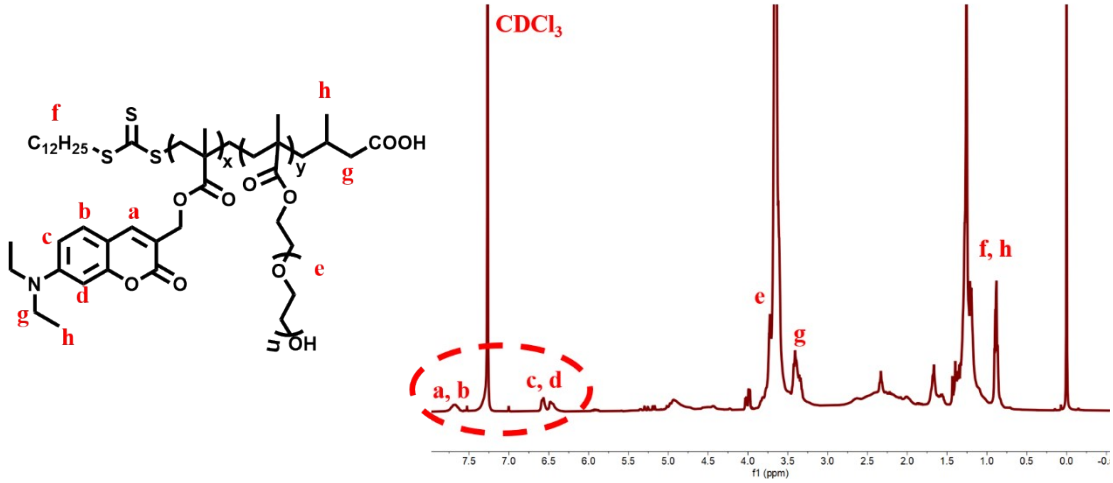


Fig. S7. ^{1}H NMR spectrum of polymeric probe **P(COU-PEG-LD)** in $CDCl_3$

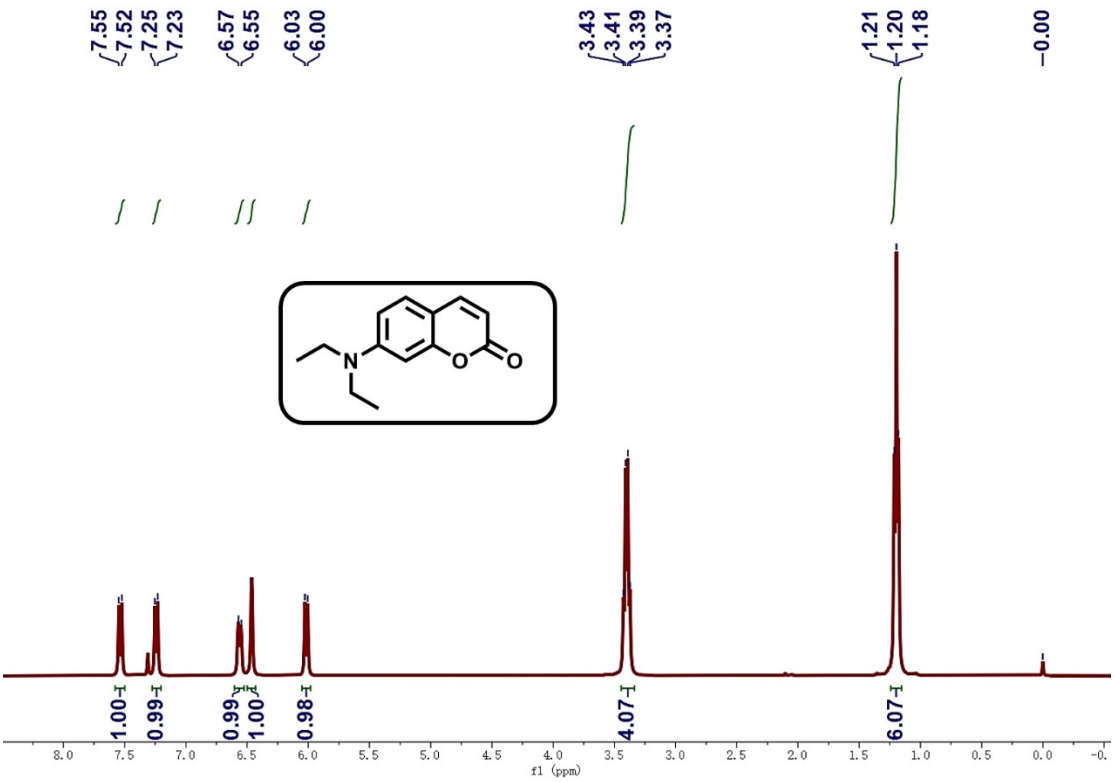


Fig. S8. ^{1}H NMR spectrum of compound **1** in $CDCl_3$.

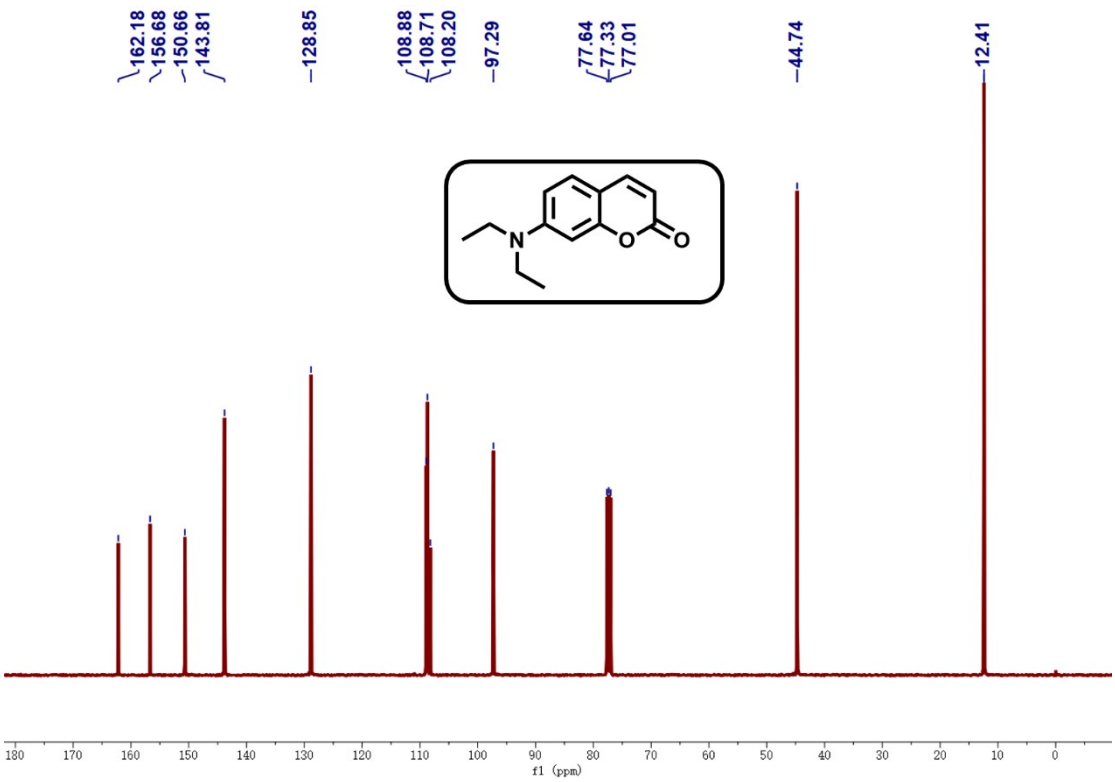


Fig. S9. ^{13}C NMR spectrum of compound **1** in $CDCl_3$.

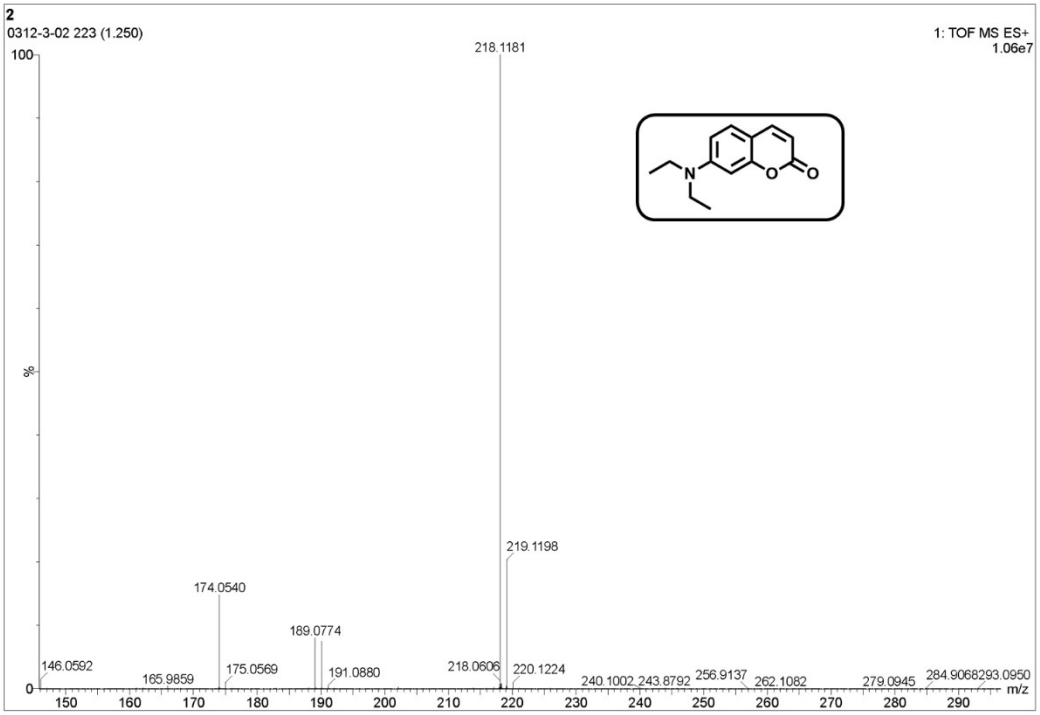


Fig. S10. HR-MS spectrum of compound **1** in CH_3OH.

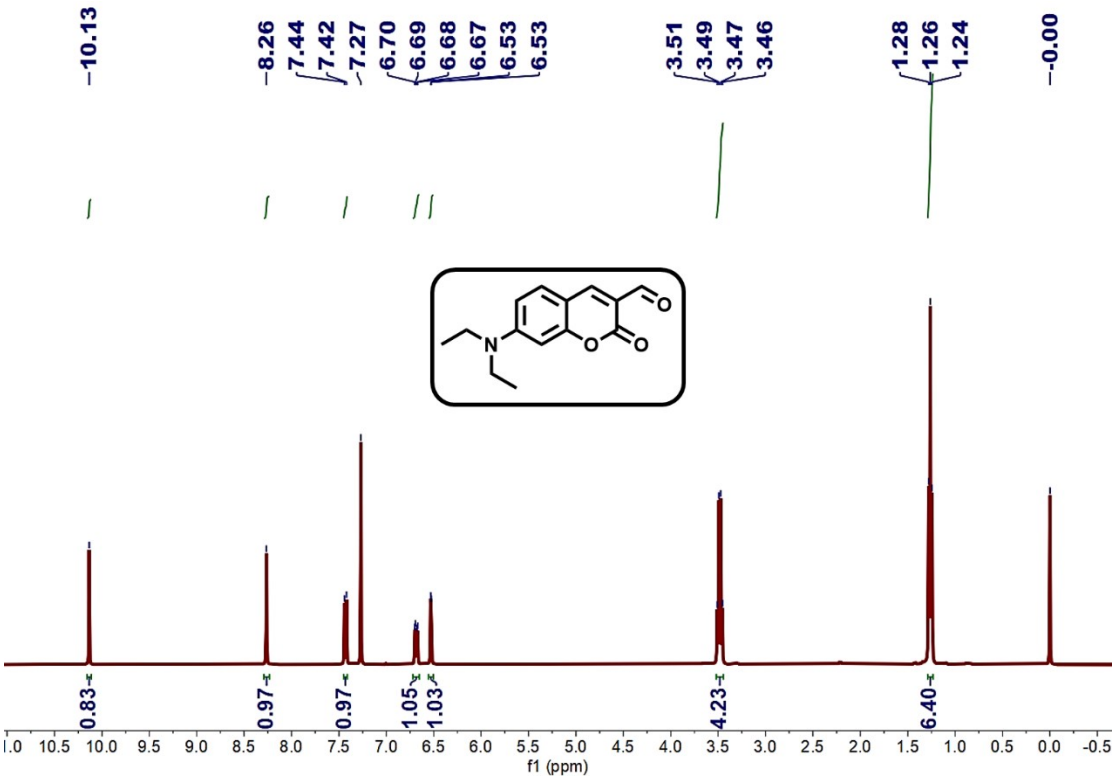


Fig. S11. ^{1}H NMR spectrum of compound **2** in $CDCl_3$.

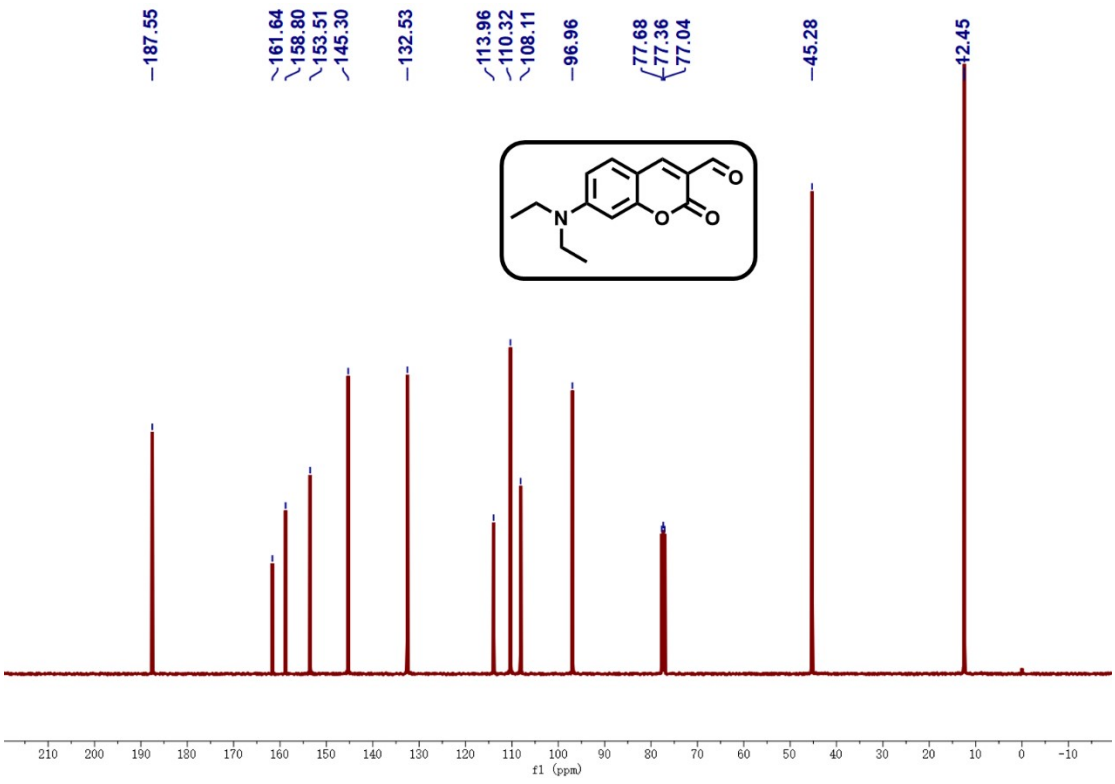


Fig. S12. ^{13}C NMR spectrum of compound **2** in $CDCl_3$.

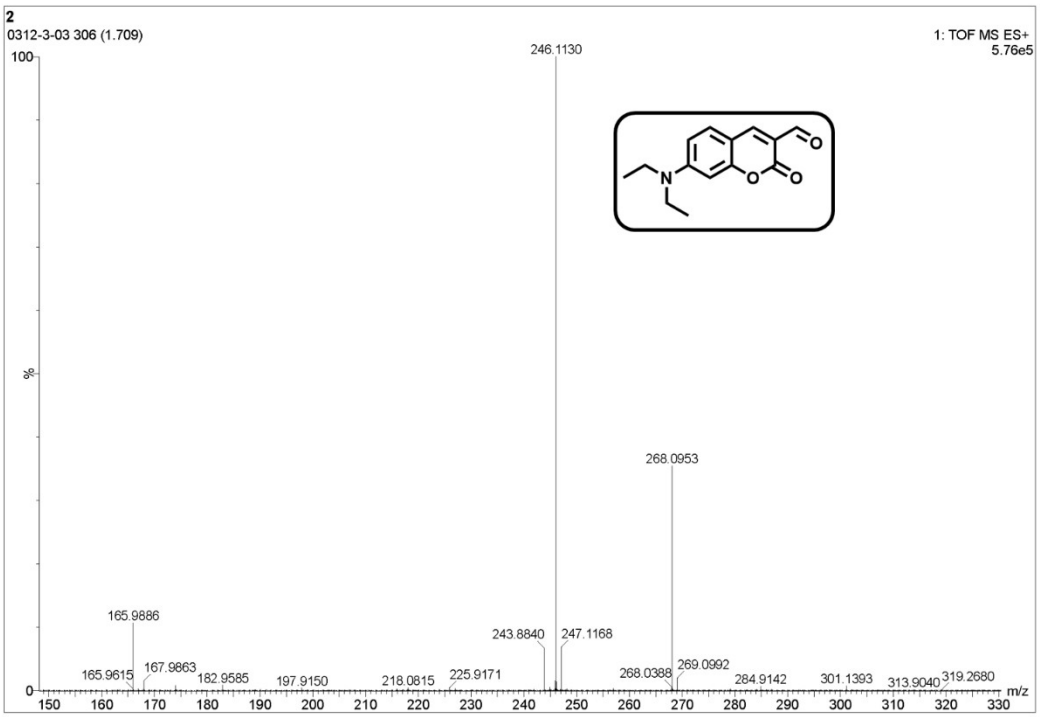


Fig. S13. HR-MS spectrum of compound **1** in CH_3OH.

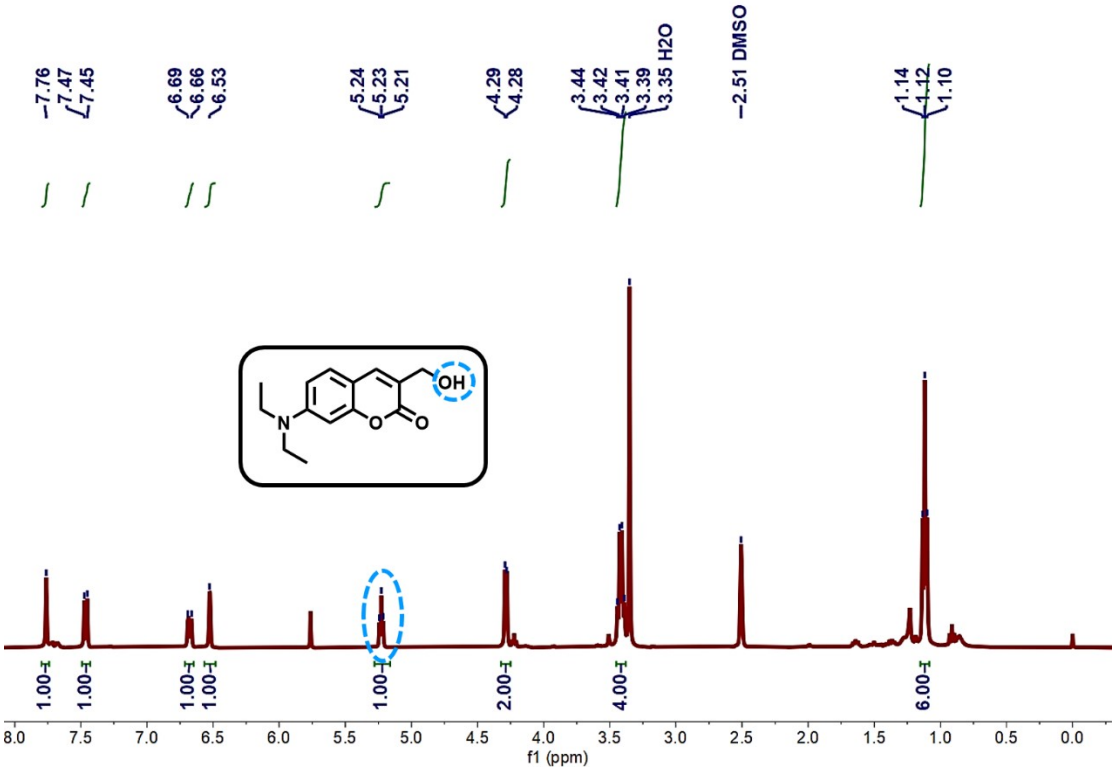


Fig. S14. ^{1}H NMR spectrum of compound **3** in DMSO- *d6*.

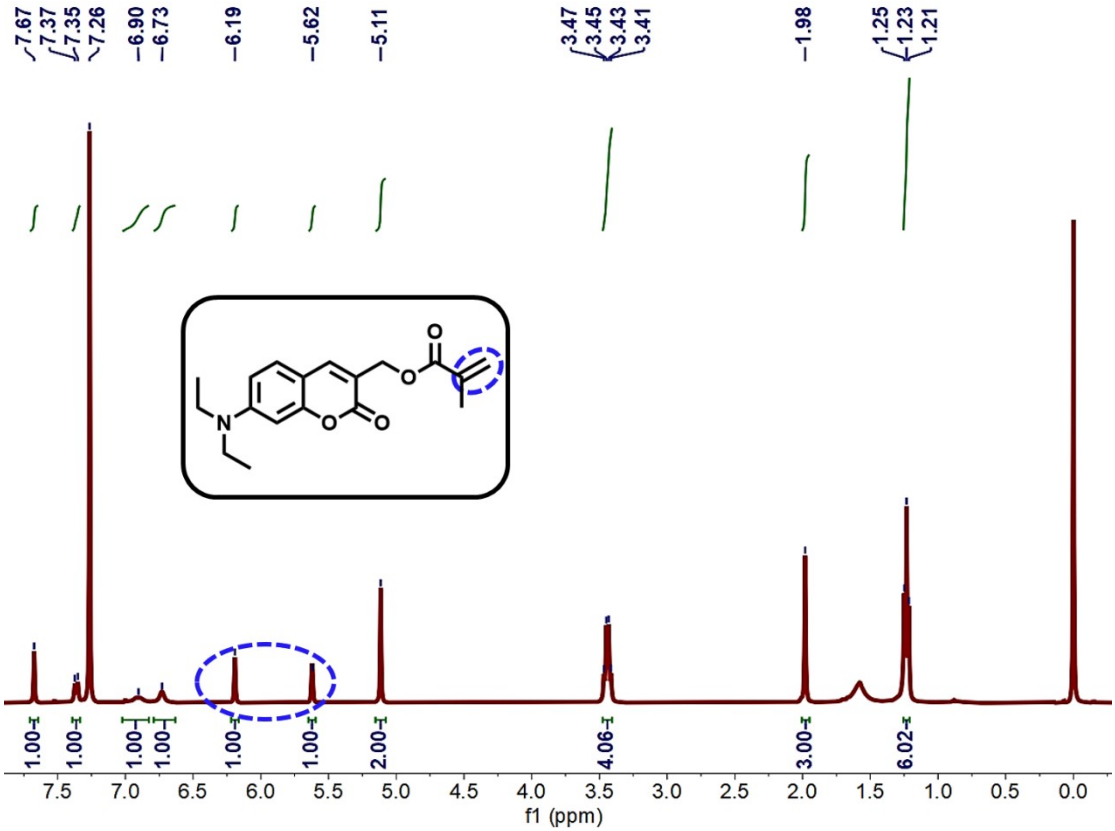


Fig. S15. ^{1}H NMR spectrum of coumarin fluorescent monomer **CMMA** in $CDCl_3$.

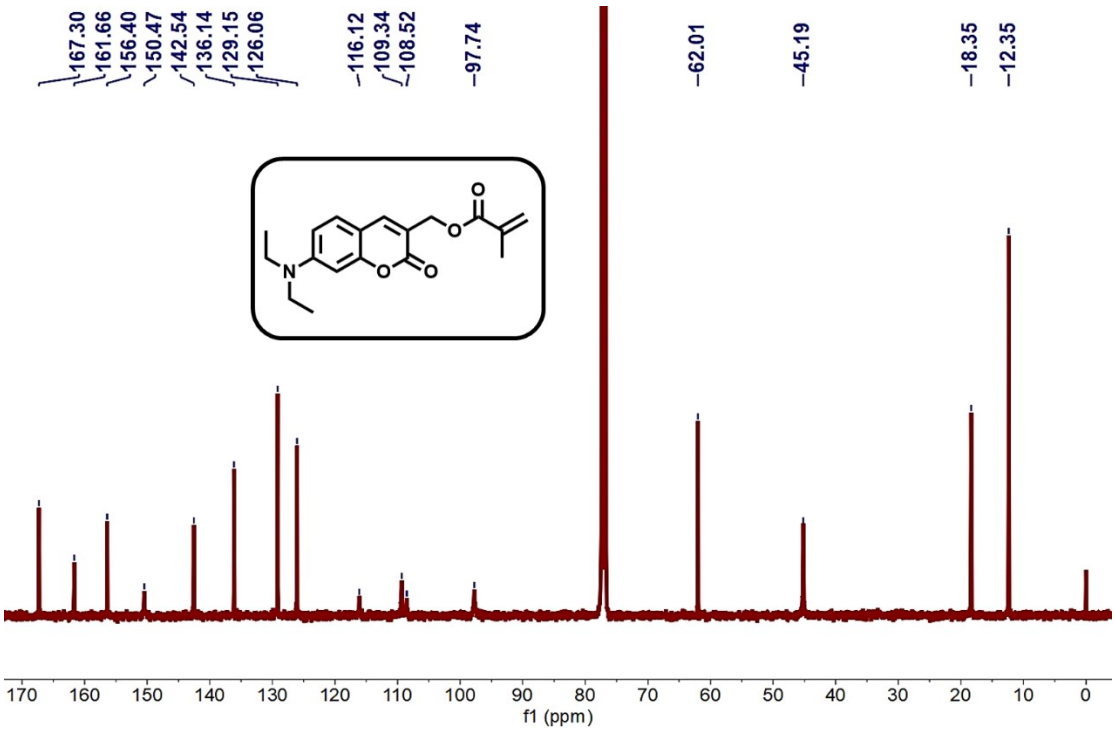


Fig. S16. ^{13}C NMR spectrum of coumarin fluorescent monomer **CMMA** in $CDCl_3$.