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Electronic Supplementary Information for

A fluorogenic probe for dynamically tracking of lipid droplets polarity in the evolution of cancer

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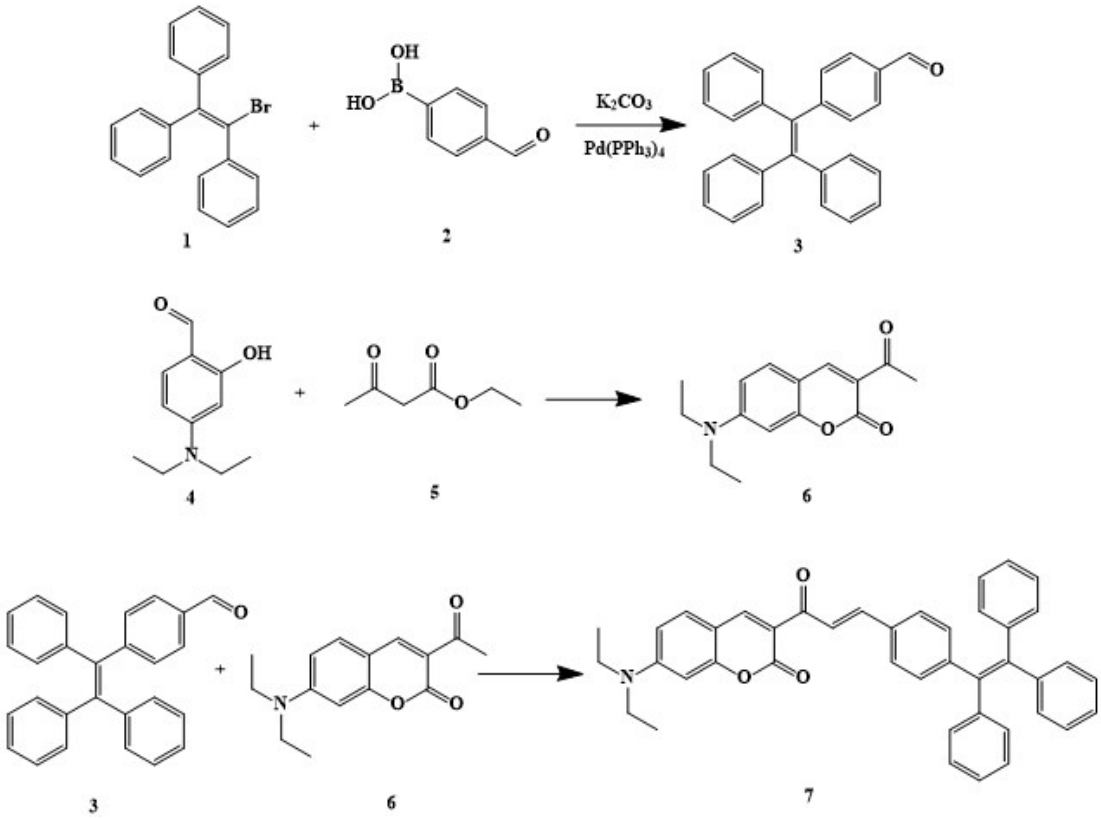
Materials and instruments

UV-vis absorption spectra were obtained on a Shimadzu UV-2700 spectrophotometer and fluorescence spectra were measured on a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell. MTT was purchased from J&K Scientific Ltd. Fluorescence imaging experiments were performed with Nikon A1MP confocal microscopy. TLC analysis carried out on silica gel plates and column chromatography was conducted over silica gel (mesh 200−300), both of them were purchased from the Qingdao Ocean Chemicals. ^{1}H and ^{13}C NMR spectra were measured on an AVANCE III digital NMR spectrometer, using tetramethylsilane (TMS) as internal reference. High resolution mass spectrometric (HRMS) analyses were measured on an Agilent 1100 HPLC/MSD spectrometer. Cell and tissue imaging experiment was performed on Nikon A1 fluorescence Microscopy equipped with a cooled CCD camera. The mice were purchased from School of Pharmaceutical Sciences, Shandong University, and the studies were approved by the Animal Ethical Experimentation Committee of Shandong University. All mice were kindly kept during experiment according to the requirements of the National Act on the use of experimental animals (China).

Synthesis routine of CTPE



Scheme S1. The synthetic routine of **CTPE**.

Synthesis of compound 3

Bromotristyrene (0.335 g, 1 mmol), (4-formylphenyl)boronic acid (0.18 g, 1.2 mmol), K_2CO_3 (0.445 g, 3.23 mmol)and tetrakis(triphenylphosphine) palladium (37 mg) were added into the reaction flask with10 mL of tetrahydrofuran (THF) as the solvent. Then the mixture was reacted at 60°C for 12 hours under the protection of nitrogen. Before completion, the reaction was monitored by TLC plate. The crude product was further purified by passing through a silica gel column (petroleum ether: dichloromethane=1:1).

Synthesis of compound 6

4-(Diethylamino)salicylaldehyde (1.939 g, 0.01 mol), (4-formylphenyl)boronic acid (1.561 g, 0.012 mmol) were added into the reaction flask with three drops of piperidine as a catalyst, and add 20 mL of ethanol as a solvent. Then, the mixture was stirred at room temperature for 12 hours, and the solid product was washed with ethanol after filtering off the filtrate.

Calculation of fluorescence quantum yield of CTPE

The fluorescence quantum yields (Φ_f) were determined by using fluorescein as the reference according to the literature method. Quantum yields were corrected as follows:

$$\emptyset_f = \emptyset_r \left(\frac{A_r {\eta_s}^2 D_s}{A_s {\eta_s}^2 D_r} \right)$$

Where the s and r indices designate the sample and reference samples respectively. A is the absorbance at λ_{ex}, η is the average refractive index of the appropriate solution, and D is the integrated area under the corrected emission spectrum.

Cytotoxicity Assays

The cytotoxicity of the probe **CTPE** to HepG2, Hela and 4T1 cells was studied by standard MTT test. 2×10^4 cells/mL cells were seeded in 96-well plates and then incubated with various concentrations of **CTPE** (0-50 μM) for 24 h. After that, 10 μL MTT (5 mg/mL) was added to each well and incubated for another 4 h. Finally, the media was discharged, and 100 μL of DMSO was loaded to dissolve the formazan crystals. The plate was shaken for about 10 min, and each well was analyzed by the microplate reader and detected at the absorbance of 490 nm.

$$\text{The cell viability (\%)} = (OD_s - OD_b) / (OD_c - OD_b) \times 100\%$$

As it shown in the formula above, s, b and c represent the sample group, the blank group and the control group respectively.

Preparation of mouse tumor slices for imaging experiments

The animals were purchased from School of Pharmaceutical Sciences, Shandong University, and the studies were approved by the Animal Ethical Experimentation Committee of Shandong University. All animals were kindly kept during experiment

according to the requirements of the National Act on the use of experimental animals (China).

4T1 cells were inoculated into normal mice to establish tumor model, and tumor tissues were obtained on 2 days, 5 days and 10 days respectively. Concurrently, The 8 day-tumor mice were treated with 20 μL taxol for 2 days, and the tumor tissue after treatment was obtained. Use a vibrating microtome to cut the above tissue into a thickness of 200mm.The slices were incubated with 10 μM **CTPE** in PBS buffer bubbled with 95% O_2 and 5% CO_2 for 2 h at 37 °C, and then washed three times with PBS, transferred to the glass bottomed dishes, and observed under confocal microscope (Nikon A1MP). The fluorescence images of the slices were acquired using 488nm excitation and fluorescence emission windows of 500-550 nm.

Table S1. The photophysical properties of **CTPE** in the different solvents. $E_T(30)$ is the empirical parameter for solvent polarity. Φ_f is the relative fluorescence quantum yield.

Solvents	$E_T(30)$	λ_{abs}/nm	λ_{em}/nm	Stokes shift/ nm	$\emptyset_{f(\%)}$
Toluene	33.9	456	502	46	35.11
Dioxane	36.0	453	513	60	33.02
DCM	40.7	461	535	74	22.78
Acetone	42.2	457	544	87	14.03
DMF	43.2	464	561	97	10.23

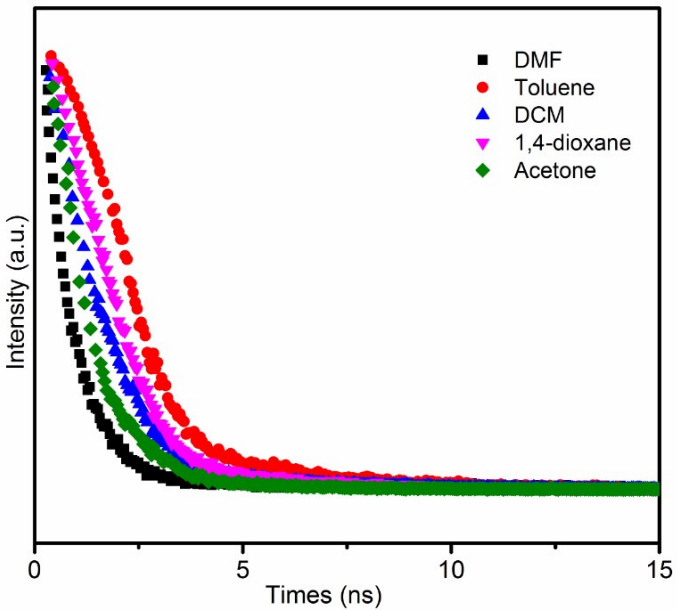


Fig. S1 The fluorescent lifetime of **CTPE** in various polarity solvents

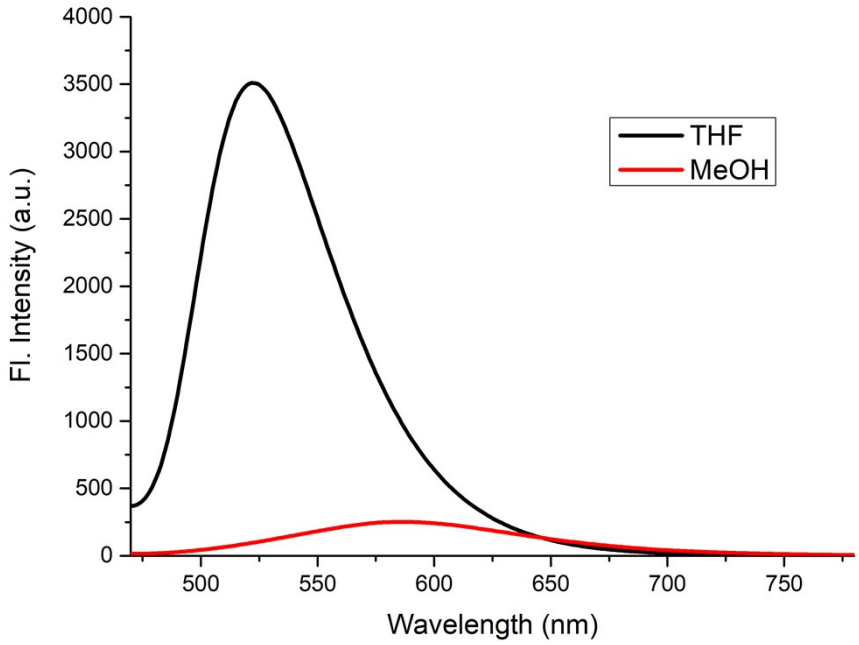


Fig. S2 The fluorescence spectra of **CTPE** in THF and methanol. THF and methanol have almost the same viscosity (0.53 cP vs 0.60 cP) but different polarity ($E_T(30)$ = 37.4 vs 55.4).

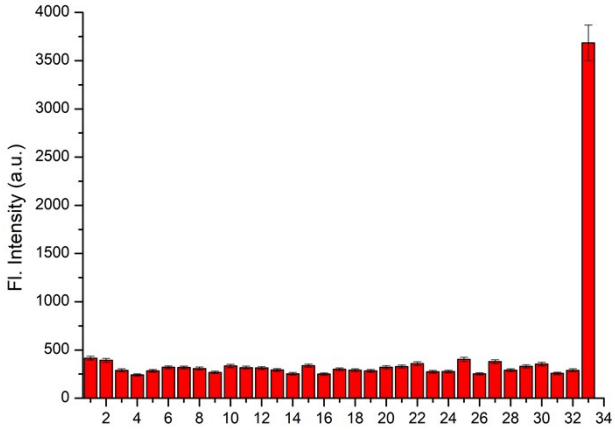


Fig. S3 The Relative fluorescence intensity of **CTPE** (10 μM) to various relevant analytes in phosphate buffer (pH 7.4, 10 mM, 5% DMSO). 1, $AlCl_3$; 2, $BaCl_2$; 3, $CaCl_2$; 4, $CuCl_2$; 5, $FeCl_3$; 6, KCl; 7, $MgCl_2$; 8, NaCl; 9, $SnCl_2$; 10,$ZnCl_2$; 11,$CuSO_4$; 12, Na_2SO_4; 13, Na_2SO_3; 14, KNO_3; 15, $NaNO_3$; 16, $NaNO_2$, 17, NaBr; 18, NaF; 19, NaI; 20, NaSCN; 21, Phe; 22, Ser; 23, Gln; 24, Thr; 25, Asn; 26, Val; 27, His; 28, Cys; 29, Gly; 30, Glu; 31, Arg; 32, Ile; 33, Toluene (500μM)

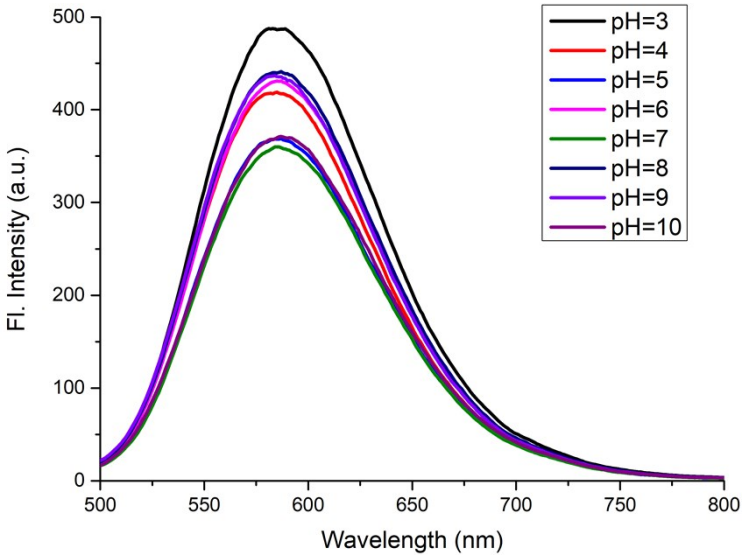


Fig. S4 The fluorescence spectra of **CTPE** in different pH values.

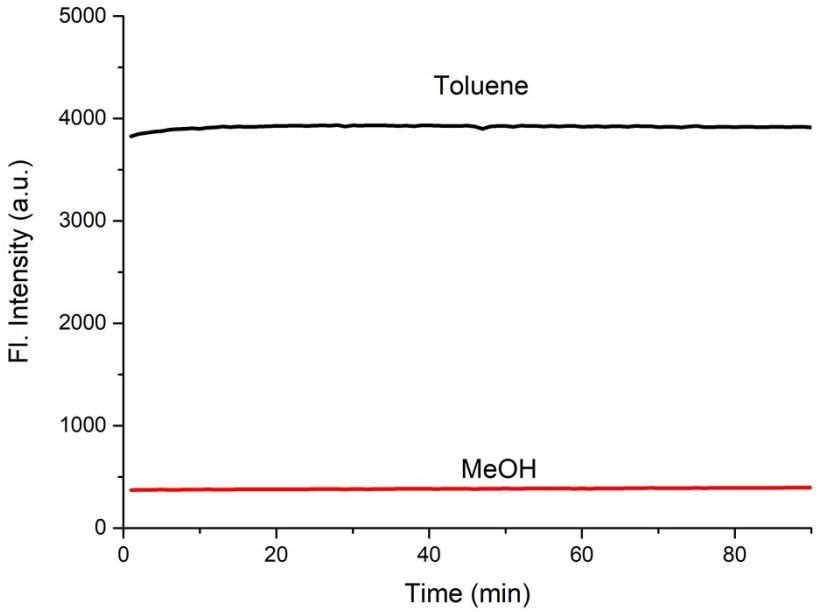


Fig. S5 The photostability experiments of **CTPE** under different polarity condition with continuous irradiation by laser light for 90 min. Conditions: λ_{em}= 460 nm; $\lambda_{ex\ (Toluene)}$= 502 nm; $\lambda_{ex\ (MeOH)}$= 573 nm.

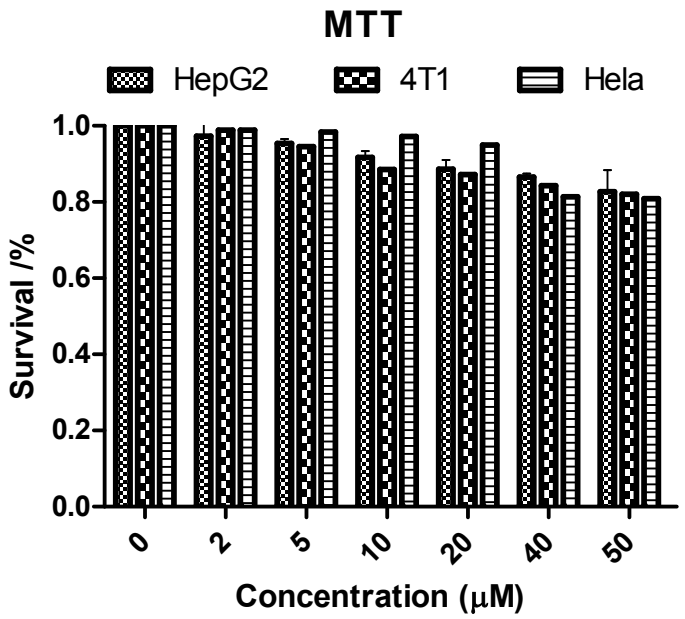


Fig. S6 (A) The MTT experiments of **CTPE** under different concentrations for HepG2 cells; 4T1 cells; Hela cells.

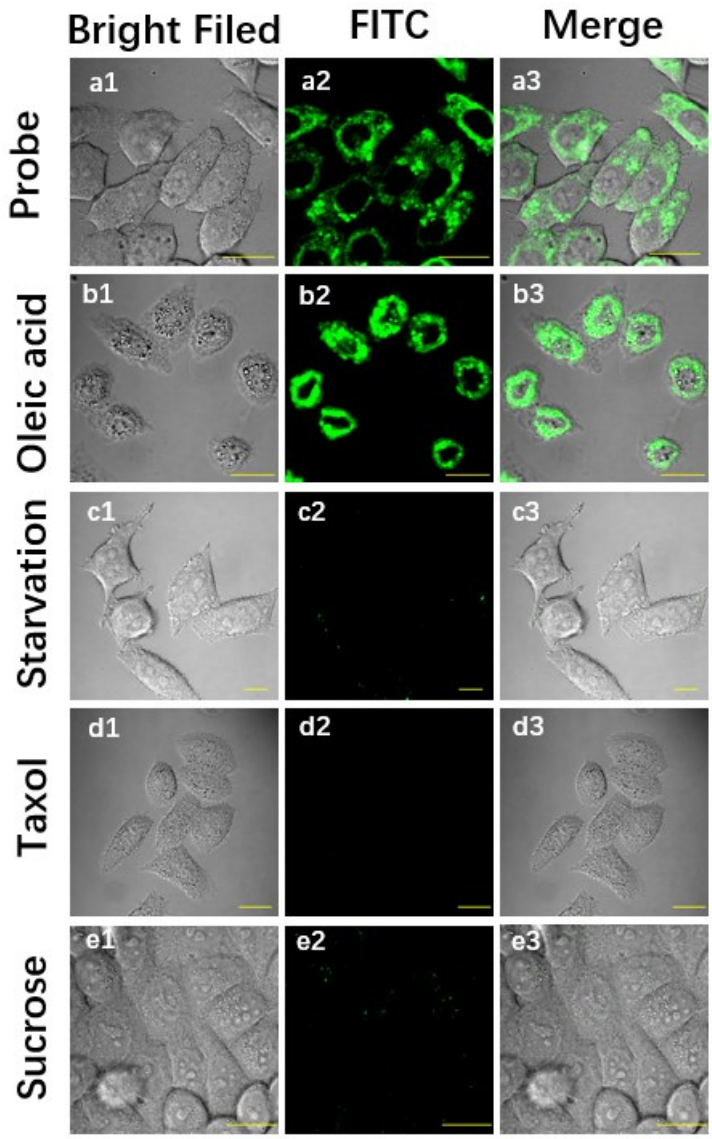


Fig. S7 Fluorescence imaging of Hela cells. (a1−a3) Hela cells incubated with 10 μM probe **CTPE** for 30 min; (b1-b3) Hela cells incubated with 20 μM oleic acid for 1 h and 10 μM **CTPE** for another 30 min;(c1−c3) Hela cells were incubated with PBS for 24 hours and 10 μM **CTPE** for another 30 min; (d1−d3) Hela cells incubated with 20 μM taxol for 30 min and 10 μM **CTPE** for another 30 min.(e1−e3) Hela cells incubated with 20 μM sucrose solution for 30 min and 10 μM **CTPE** for another 30 min. Conditions: λ_{em}= 500−550 nm; λ_{ex}= 488 nm. Scale bar: 20 μm.

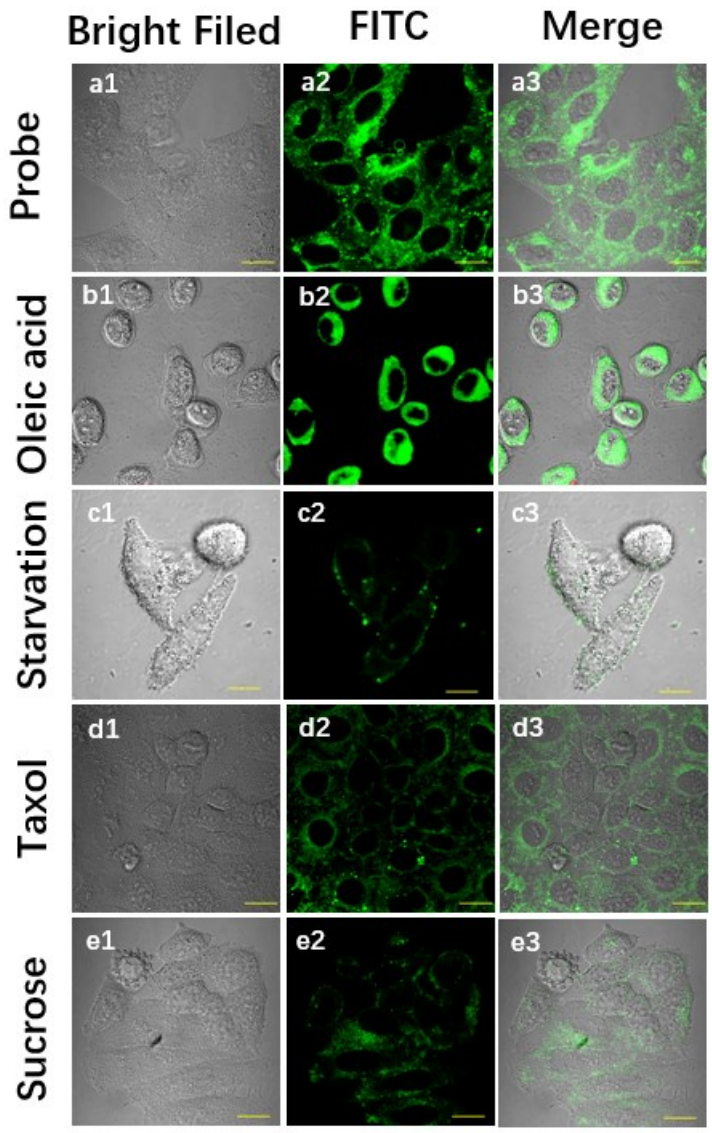


Fig. S8 Fluorescence imaging of HepG2 cells. (a1−a3) HepG2 cells incubated with 10 μM probe **CTPE** for 30 min; (b1-b3) HepG2 cells incubated with 20 μM oleic acid for 1 h and 10 μM **CTPE** for another 30 min; (c1−c3) HepG2 cells were incubated with PBS for 24 hours and 10 μM **CTPE** for another 30 min; (d1−d3) HepG2 cells incubated with 20 μM taxol for 30 min and 10 μM **CTPE** for another 30 min.(e1−e3) HepG2 cells incubated with 20 μM sucrose solution for 30 min and 10 μM **CTPE** for another 30 min. Conditions: λ_{em}= 500−550 nm; λ_{ex}= 488 nm. Scale bar: 20 μm.

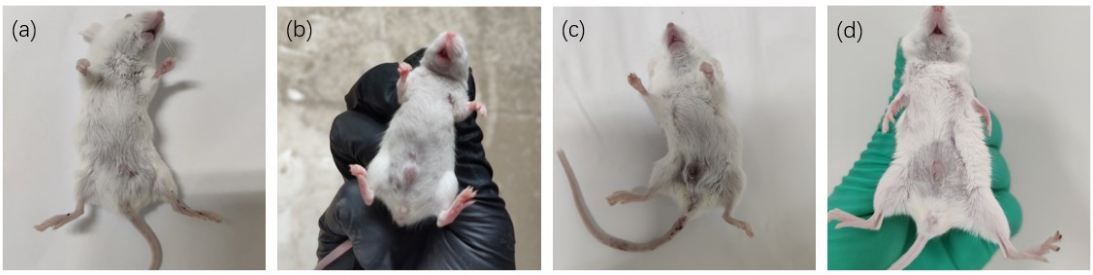


Fig. S9 Pictures of mice inoculated with 4T1 cells after 2 days (a), 5 days (b) and 10 days (c), and pictures of mice after inoculated with 4T1 cells for 8 days and 2 days after treatment with taxol (d).

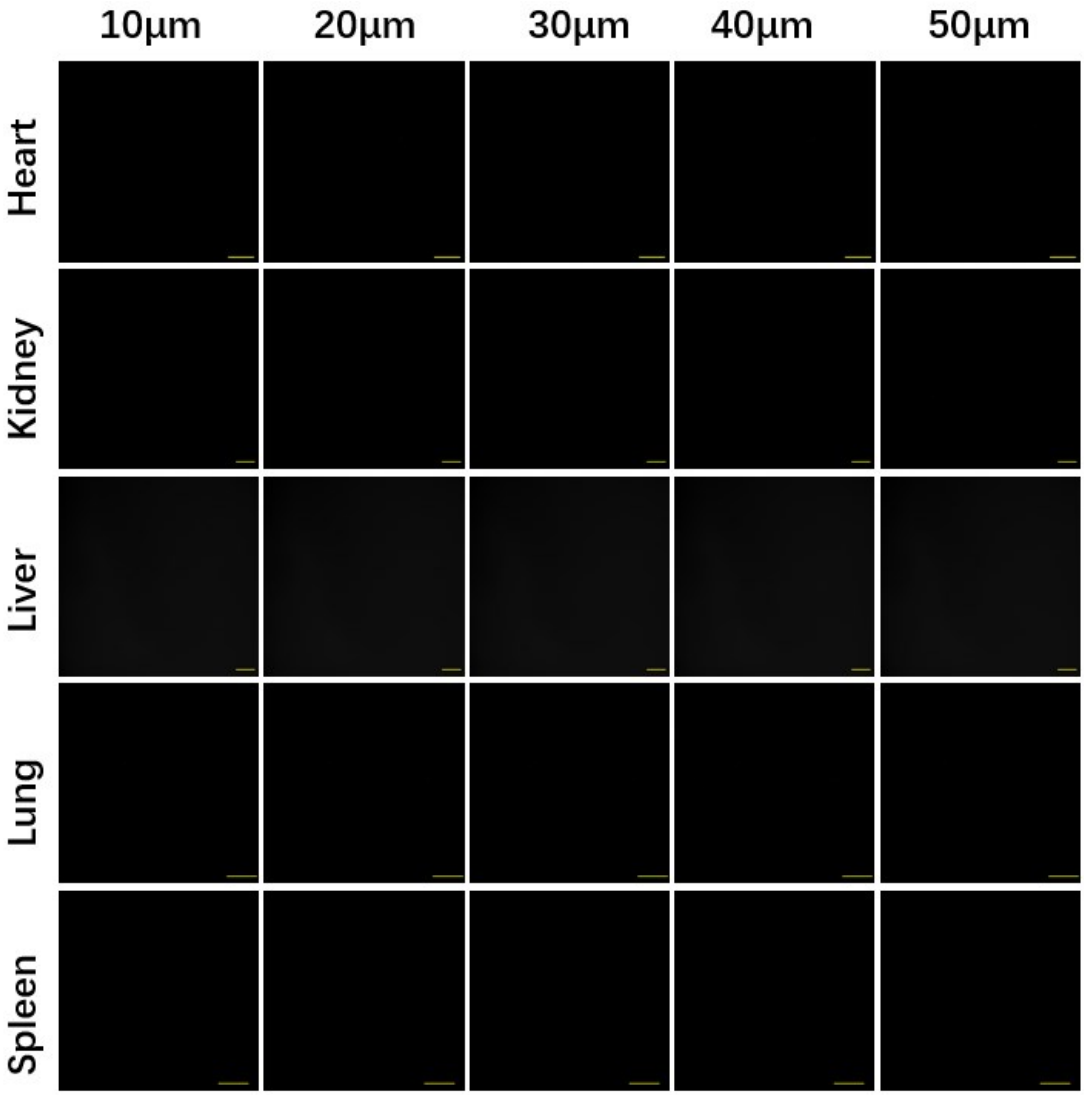


Fig. S10 Fluorescence images of the normal organs slices without any treatment of the probe **CTPE**. The imaging was collected at 500-550 nm with 488 nm excitation.

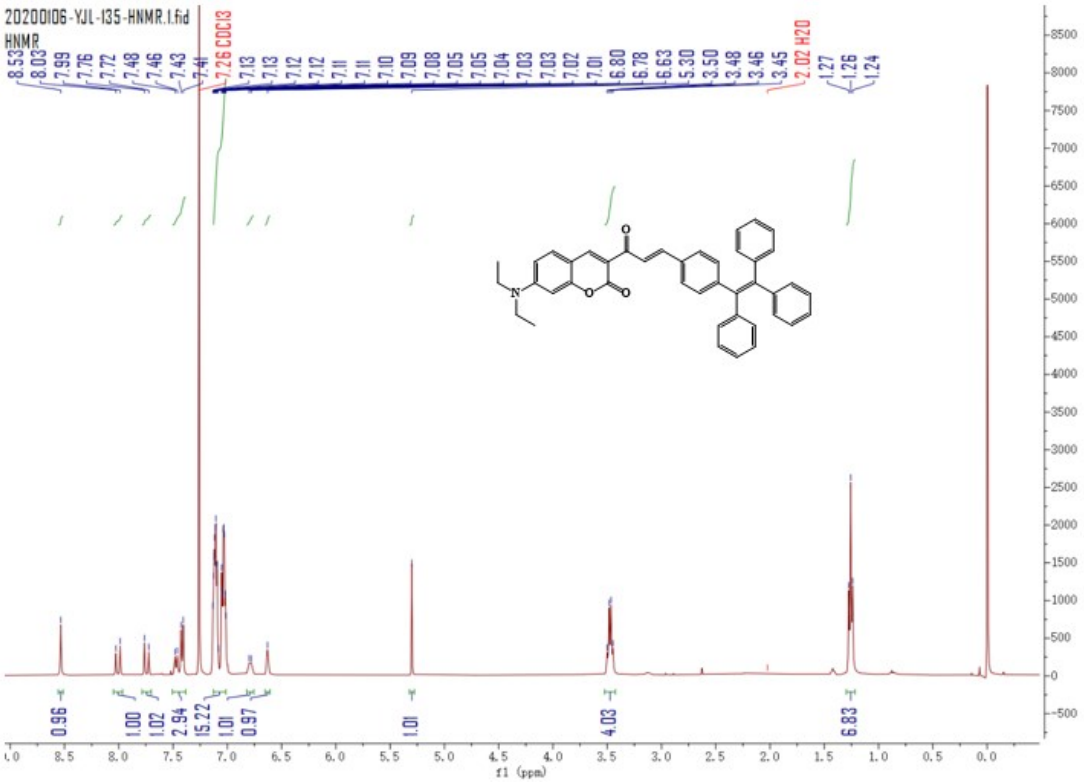


Fig. S11 The ^{1}H NMR spectrum of **CTPE** in chloroform-d.

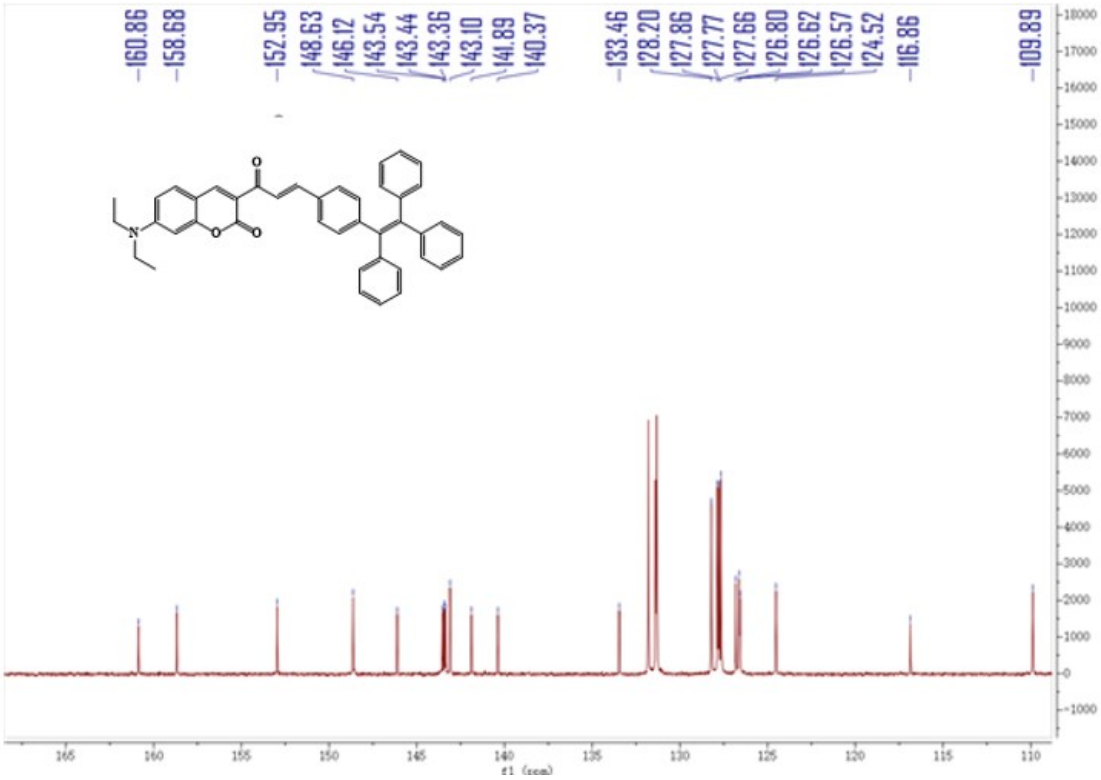


Fig. S12 The ^{13}C NMR spectrum of **CTPE** in chloroform-d.

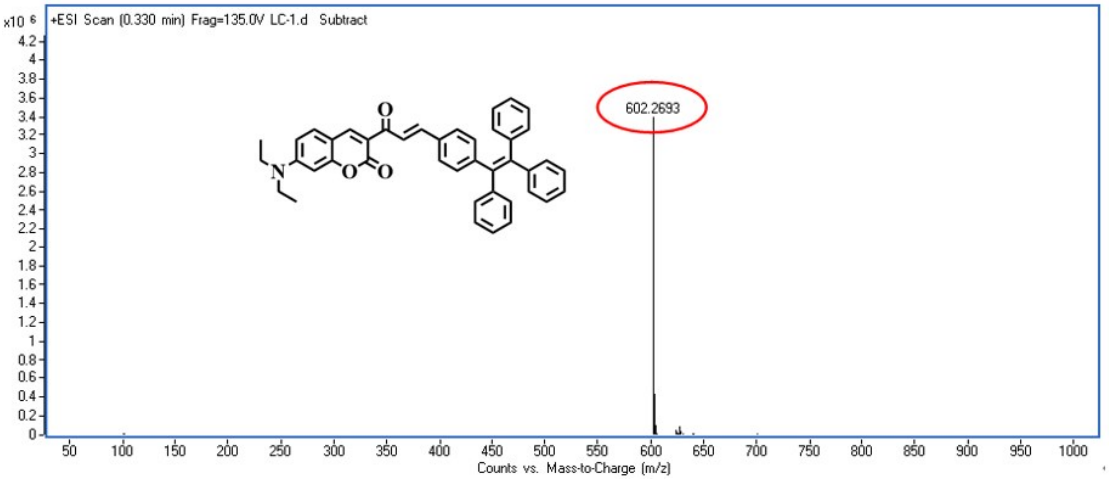


Fig. S13 The HRMS spectrum of **CTPE**.