Electronic Supplementary Information (ESI)

Lighting up fluoride ions in cellular mitochondria with a highly selective and sensitive fluorescent probe

Shiling Zhang,[a] Jiangli Fan,[a] Shuangzhe Zhang,[a] Jingyun Wang,[b] Xianwu Wang,[b] Jianjun Du[a] and Xiaojun Peng*[a]

[a] *State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian, P.R. China*

[b] *School of Life Science and Biotechnology, Dalian University of Technology, Dalian, P.R. China*

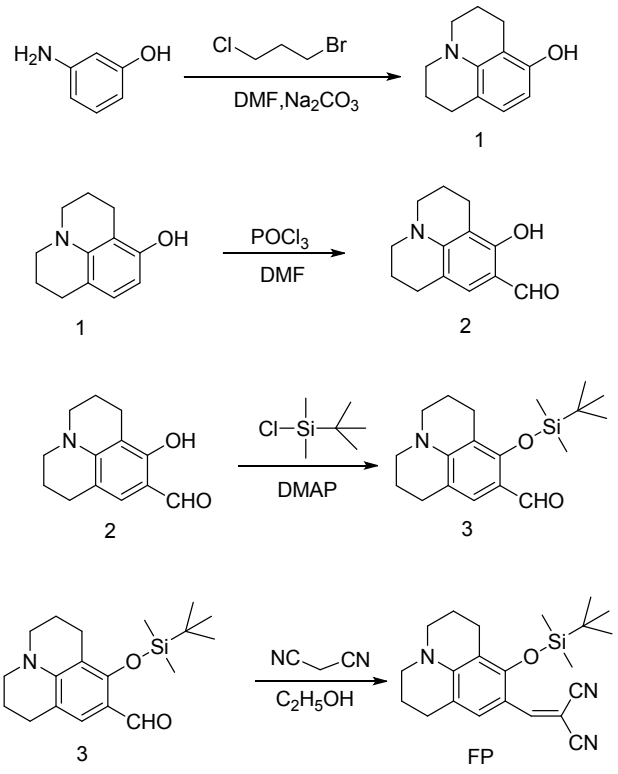
* Corresponding Author Email: pengxj@dlut.edu.cn Tel/Fax: +86-411-84986306

Contents:

1. Reagents and instruments

All solvents and reagents were of reagent grade. The stock solution of FP was prepared in CH_2Cl_2 for the *in vitro* test and in CH_3CN for the vivo experiment. Analyte solutions of TBAF, TBACl, TBABr, TBAI, $TBAHSO_4$, TBAAcO, $TBANO_3$, $TBAH_2PO_4$, TBAOH, NaF, NaCl, NaBr, NaI, $NaHCO_3$, NaH_2PO_4, NaAcO were prepared by dissolving the salts in distilled water to final concentrations of 10.0 mM for the TBAF and 25 mM for the other anions. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a VARIAN INOVA-400 spectrometer with chemical shifts (δ) reported in ppm (in $CDCl_3$, TMS as the internal standard). Mass spectrometry data were obtained using an HP1100LC/MSD mass spectrometer and an LC/Q-TOF MS spectrometer. Absorption spectra were measured using a Lambda 35 UV/VIS spectrophotometer (Perkin Elmer). UV-visible spectra were collected on an Agilent 8453 UV-Visible spectrophotometer. Fluorescence measurements were performed on a VAEIAN CARY Eclipse Fluorescence Spectrophotometer (Serial No. FL1109-M018) with slit widths set at 2.5 nm and 2.5 nm for excitation and emission, respectively.

2. Synthesis



Scheme S1

Synthesis of **1**

1 was synthesized from 3-aminophenol by the procedure published in literature [S1].

Synthesis of **2**

Compound **2** was synthesized from compound **1** by the procedure published in literature [S1].

Synthesis of **3**

Compound **2** (65 mg, 0.3 mmol), 4-dimethylaminopyridine (DMAP, 37 mg, 0.3 mmol) and several drops Et_3N were combined in anhydrous dichloromethane (10 mL) at 0 °C, under a nitrogen atmosphere. After being stirred for 10 min, to the mixture was added t-butyldimethylsilyl chloride (53 mg, 0.35 mmol) in dichloromethane (5 mL) dropwise at the same temperature. The mixture was stirred at room temperature for 12 h, and then treated with a saturated $NaHCO_3$ solution (10 mL). The two layers were separated, and the aqueous layer was extracted with dichloromethane (20 mL × 3). The combined organic extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (eluent EtOAc/hexane = 1:20) to afford compound 3 as a yellow solid (79 mg, 80%).

^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ (ppm) 9.95 (s, 1H), 7.32 (s, 1H), 3.24–3.26 (t, *J* = 4.00 Hz, 4H), 2.68 (t, *J* = 4.00 Hz, 2H), 2.63 (t, *J* = 4.00 Hz, 2H), 1.90 (m, *J* = 4.00 Hz, 4H), 1.04 (s, 9H), 0.16 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 187.74, 155.85, 149.18, 131.26, 126.18, 116.59, 115.68, 50.29, 49.94, 27.44, 26.11, 25.83, 22.21, 21.72, 21.19. MS (TOF MS ES+) calculated for: $C_{19}H_{29}NO_2Si$: 331.1968, measured: $[C_{19}H_{29}NO_2Si + H]^+$ 332.2039.

Synthesis of **FP**

To a stirred solution of alcohol **3** (74 mg, 0.2 mmol) and malononitrile (15.2 mg, 0.23 mmol) in ethanol (5 mL) at room temperature under nitrogen was added piperidine (0.1 mL, 2 mmol). The reaction mixture was allowed to stir at room temperature for 1 h. The reaction process was monitored by TLC. After the reaction was completed, The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent EtOAc/hexane = 1:10) to afford **FP** as a orange solid (25 mg, 31%).

^{1}H NMR (400 MHz, $CDCl_3$, Me_4Si) δ (ppm) 7.83 (s, 1H), 7.69 (s, 1H), 3.34–3.30 (m, *J* = 4.00 Hz, 4H), 2.69 (t, *J* = 8.00 Hz, 2H), 2.60 (t, *J* = 8.00 Hz, 2H,), 1.94 (t, *J* = 4.00 Hz, 2H), 1.89 (t, *J* = 4.00 Hz, 2H), 1.05 (s, 9H), 0.15 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 154.19, 152.19, 150.20, 126.39, 117.25, 116.11, 112.02, 110.40, 77.36, 77.04, 76.72, 66.62, 50.43, 49.96, 29.70, 29.71, 27.40, 25.92, 22.21, 21.25, 20.70, 18.62. MS (TOF MS ES+) calculated for: $C_{22}H_{29}N_3OSi$: 379.2080, measured: $[C_{22}H_{29}N_3OSi + H]^+$ 380.2157.

3. Determination of quantum yields

The fluorescence quantum yields of probe **FP** and **FP** + F^- were determined using the equation [S2]:

$$\emptyset_u = \frac{(\emptyset_s)(FA_u)(A_s)(\lambda_{exs})(\eta_u^2)}{(FA_s)(A_u)(\lambda_{exu})(\eta_s^2)}$$

Where Φ is the fluorescence quantum yield; FA is integrated area under the corrected emission spectrum; A is the absorbance at the excitation wavelength; λ_{ex} is the excitation wavelength; η is the refractive index of the solution; and the subscripts 'u' and 's' refer to the unknown and the standard, respectively. We chose rhodamine B as the standard, which has a fluorescence quantum yield of 0.49 in ethanol. [S3]

4. The response time of probe FP

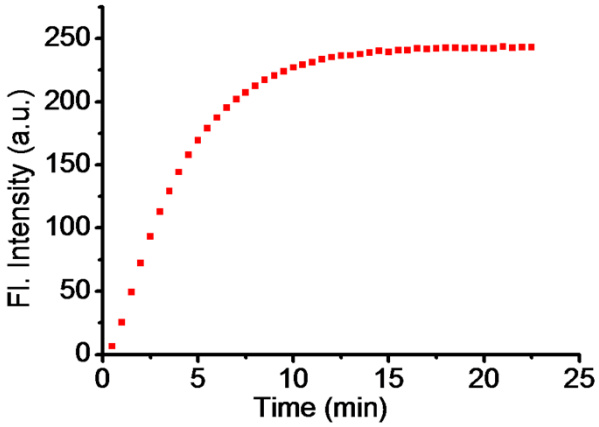


Fig. S1 The response time of probe **FP** (5 μM) upon the addition of TBAF（50 μM) in acetonitrile at rt.

5. The absorbance spectra

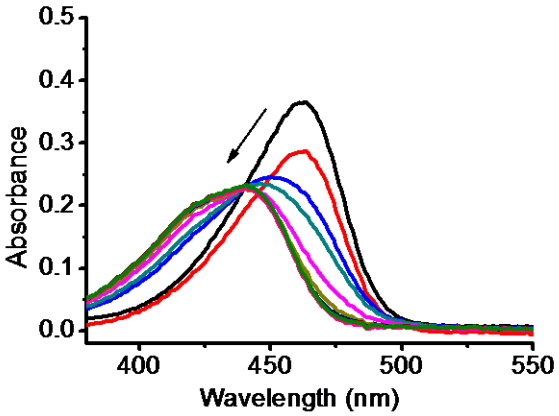


Fig. S2 The absorbance spectrum of probe **FP** (5 μM) upon the addition of F^- from *n*-Bu_4N^+ salts (0, 5, 10, 15, 20, 25, 30, 40, 60, and 100 μM) in acetonitrile after 7 min at rt.

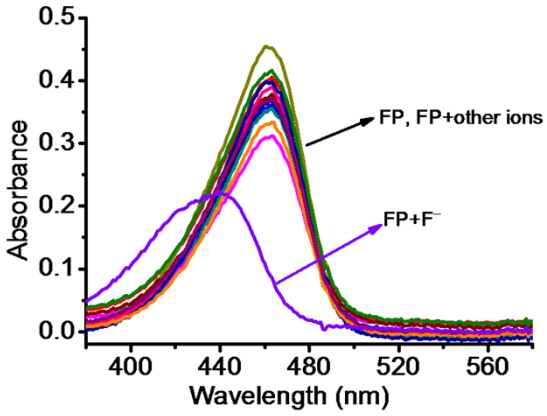


Fig. S3 The absorbance spectrum of probe **FP** (5 μM) upon the addition of F^-, Cl^-, Br^-, I^-, HSO_4^-, AcO^-, $H_2PO_4^-$, NO_3^-, and OH^- from *n*-Bu_4N^+ salts in acetonitrile after 7 min at rt.

6. Calculation of the detection limit

The limit of detection (LOD) was calculated based on the fluorescence titration curve of **FP** in the presence of F^- (0–5 μM). The fluorescence emission spectrum of **FP** was measured three times to get the standard division of the fluorescence intensity at 485 nm, and the result was set as the blank measurement. The detection limit was calculated with the following equation: LOD = 3σ/k, where σ is the standard division of blank measurement mentioned above and k is the slope of the linear regression equation between the log (Fl. Intensity) and concentrations of F^-.

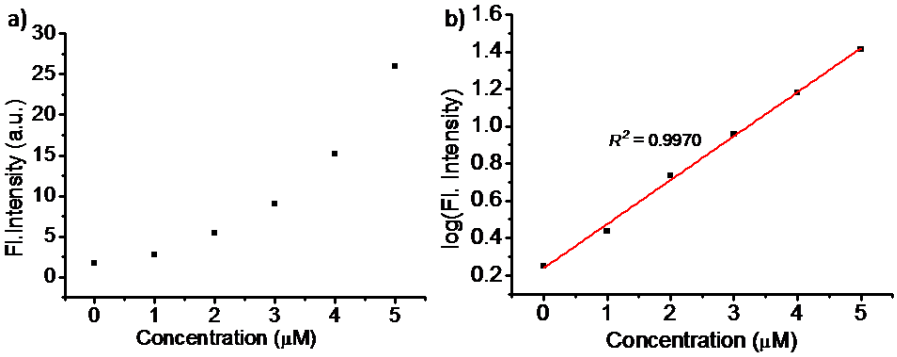


Fig. S4 The fluorescence intensity of probe **FP** (5 μM) upon the addition of F^- from *n*-Bu_4N^+ salts (0, 1, 2, 3, 4, and 5 μM) in acetonitrile after 7 min at rt.

7. Determination of cytotoxicity

Toxicities of **FP** towards MCF-7 and COS-7 cells were assessed by the standard MTT cytotoxicity assay. It involves the reduction of MTT tetrazolium to MTT formazan pigment by the metabolic activity of living cells. Briefly, MCF-7 cells were seeded in 96-well microplates (Nunc, Denmark) at a density of 1×10^5 cells mL^{-1} in 100 μL of medium containing 10% FBS. After 24 h of cell attachment, MCF-7 cells were treated

with **FP** at serial concentrations (1.0, 2.5, and 5.0 μM) for 12 h. Six replicate wells were used for each control and tested concentrations. After incubation for 12 h, the medium was removed and cells were washed with PBS twice. MTT tetrazolium solution (100 μL of 0.5 mg/mL in PBS) was added to each well, and the cells were further incubated at 37 ºC for 4 h in a 5% CO_2 humidified atmosphere. The medium was carefully removed, and the purple products were lysed in 200 μL DMSO. The plate was shaken for 10 min, and the absorbance was measured at 570 and 630 nm using a microplate reader (Thermo Fisher Scientific). Cell viability was expressed as a percentage of the control culture value.

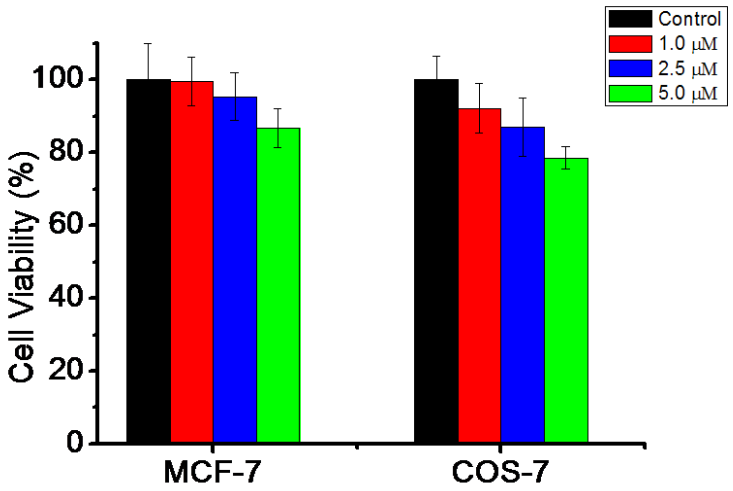


Fig. S5 The cytotoxicity of probe **FP** at various concentrations (1.0, 2.5, and 5.0 μM) in living MCF-7 and COS-7 cells for 12 h, cell viabilities were examined using Thermo Fisher Scientific.

8. Cell incubation and imaging in living cells

MCF-7 and COS-7 cells were selected as cell imaging objects. They were cultured in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen). Cells were seeded in a glass bottom dish (MatTek, 35 mm dish with a 20 mm bottom well) and incubated for 24 h at 37 °C under 5% CO_2. **FP** (2.5 μM, CH_3CN, 5 mM), TBAF (100 μM, distilled water, 50 mM) or NaF (100 μM, distilled water, 50 mM) were added and cells were further incubated for 15 min under 5% CO_2 and washed three times with PBS buffer solution. Fluorescence imaging was performed using an OLYMPUS FV-1000 inverted fluorescence microscope with a 60× objective lens. Fluorescence images collected the emissions at 445–510 nm (λ_{ex} = 405 nm).

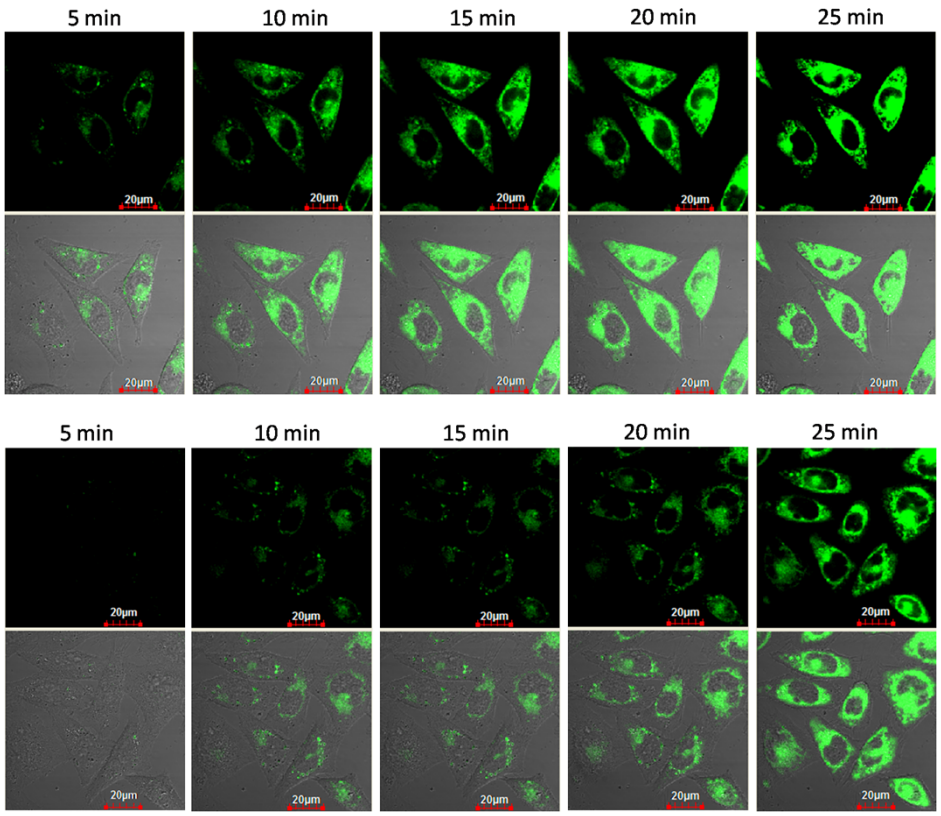


Fig. S6 Fluorescence imaging of MCF-7 cells incubated with NaF (top) and TBAF (bottom) (200 μM) for 30 min at 37 °C under 5% CO_2, then washed the cells with PBS buffered solution three times, and treated with probe **FP** (2.5 μM) for different time. The excitation wavelength was 405 nm. The fluorescence was collected at 445–510 nm. Scale bar: 20 μm.

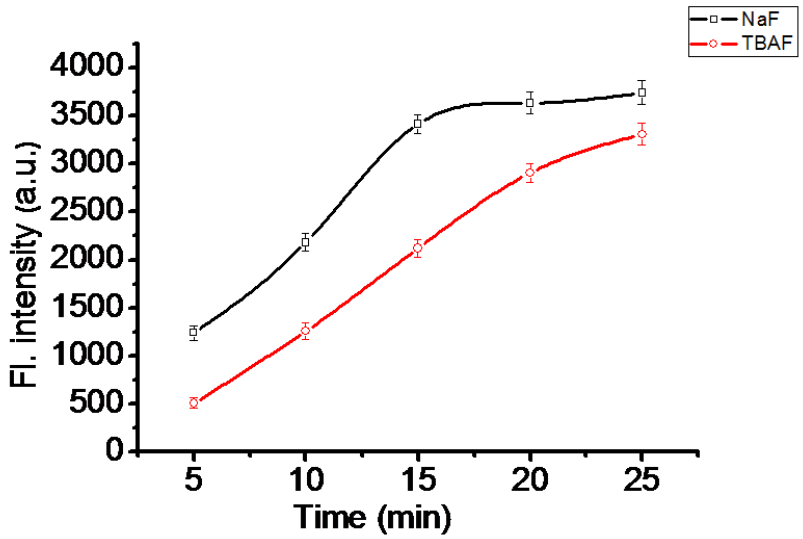


Fig. S7 Fluorescent imaging intensity curve of MCF-7 cells incubated with NaF, TBAF (200 μM) for 30 min, respectively, then washed the cells with PBS solution three times, and treated with probe **FP** (2.5 μM) for different time. The excitation wavelength was 405 nm. The fluorescence was collected at 445–510 nm.

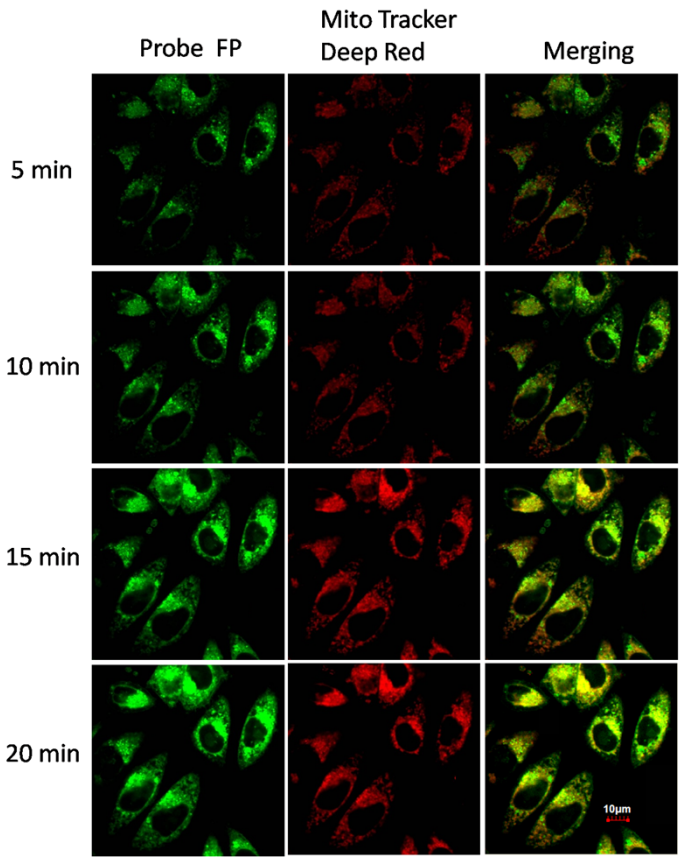


Fig. S8 Fluorescence imaging of MCF-7 cells incubated with NaF (100 μM) for 30 min at 37 °C under 5% CO_2, then washed the cells with PBS buffered solution three times, treated with probe **FP** (2.5 μM, green channel) and Mito Tracker Deep Red FM(0.5 μM, red channel) for different time. The excitation wavelength was 405 nm for probe FP and 635 nm for Mito Tracker Deep Red FM. The fluorescence was collected at 445–510 nm and 660–700 nm.

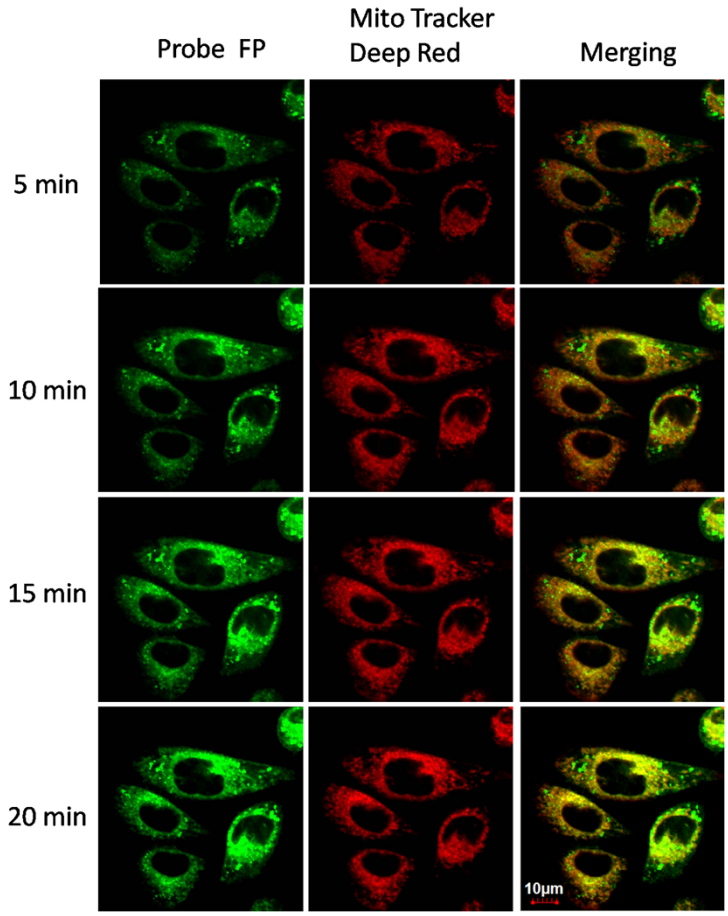


Fig. S9 Fluorescence imaging of MCF-7 cells incubated with probe **FP** (2.5 μM, green channel) and Mito Tracker Deep Red FM (0.5 μM, red channel) for 15 min at 37 °C under 5% CO_2, washed the cells with PBS buffered solution three times, then treated with NaF (100 μM) for different time. The excitation wavelength was 405 nm for probe FP and 635 nm for Mito Tracker Deep Red FM. The fluorescence was collected at 445–510 nm and 660–700 nm.

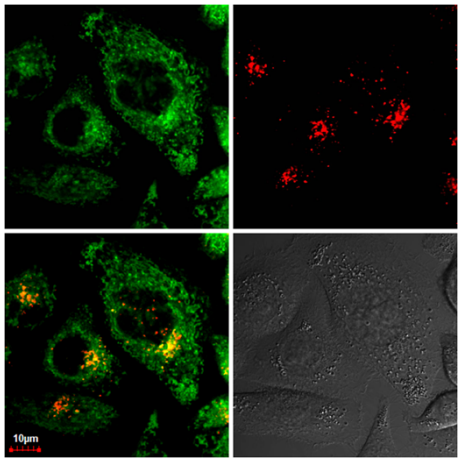


Fig. S10 Probe **FP** colocalises to lysosomes in MCF-7 cells. Cells were stained with 100 μM NaF for 30 min at 37 °C under 5% CO_2, then washed the cells with PBS solution three times, added 2.5 μM probe **FP** (green channel) and 0.2 μM Lyso-Tracker Red (red channel) for another 20 min at 37 °C under 5% CO_2, washed the cells with PBS solution three times again; The excitation wavelengths

were 405 nm (probe **FP**) and 559 nm (Lyso-Tracker Red). The fluorescence was collected at 445–510 nm (probe **FP**) and 570–620 nm (Lyso-Tracker Red). Pearson's Coefficient is 0.62.

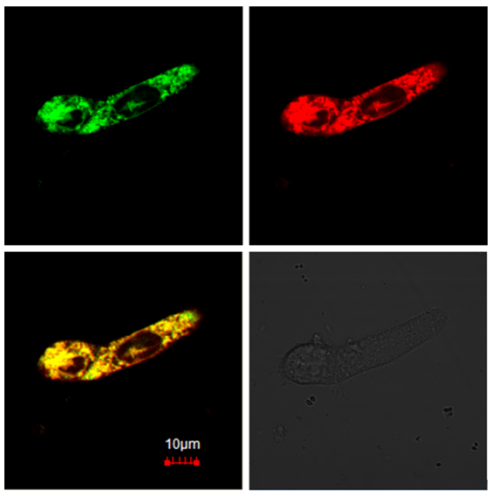


Fig. S11 Probe FP co-localises to mitochondria in COS-7 cells. Cells were stained with 100 μM NaF for 30 min at 37 °C under 5% CO2, then washed the cells with PBS solution three times, added 2.5 μM probe FP (green channel) and 0.5 μM Mito Tracker Deep Red FM (red channel) for another 20 min at 37 °C under 5% CO_2, washed the cells with PBS solution three times again. The excitation wavelengths were 405 nm (probe FP) and 635 nm (Mito Tracker Deep Red FM). The fluorescence was collected at 445–510 nm (probe FP) and 660–700 nm (Mito Tracker Deep Red FM). Pearson's Coefficient is 0.93.

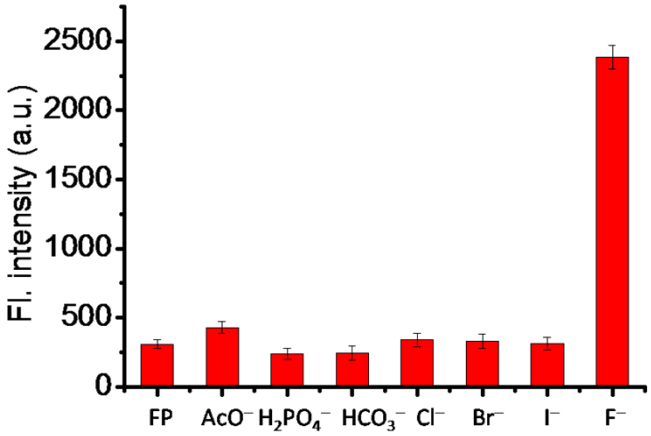


Fig. S12 Fluorescent imaging intensity of MCF-7 cells incubated with AcO^-, $H_2PO_4^-$, HCO_3^-, Cl^-, Br^-, I^-, and F^- for 30 min at 37 °C under 5% CO_2, then washed the cells with PBS solution three times, and treated with probe **FP** (2.5 μM) for 15 min at 37 °C under 5% CO_2, respectively, washed the cells with PBS solution three times again. The excitation wavelength was 405 nm. The fluorescence was collected at 445–510 nm.

9. Colocalisation experiments

Colocalisation experiments were conducted by costaining the MCF-7 cells with combinations of TBAF (100 μM, distilled water, 50 mM) for 30 min firstly at 37 °C under 5% CO_2, probe FP (2.5 μM, CH_3CN, 5 mM) and Mito Tracker Deep Red FM (0.5 μM, Invitrogen) for another 20 min at 37 °C under 5% CO_2. Cofocal fluorescence images collected the emissions at 445–510 nm (FP, λ_{ex} = 405 nm) and 660–700 nm (Mito Tracker Deep Red FM, λ_{ex} = 635 nm), respectively.

10. Preparation of test papers

A test paper of **FP** was prepared by putting a filter paper (3 × 0.5 cm^2) into dichloromethane solution of **FP** (1 mM) for 5 minutes and then dried in the air. For detecting fluoride in aqueous media, the test paper was immersed into a fluoride-containing aqueous solution for several seconds then air-dried.

11. Partition Coefficient.

The partition coefficient (*P*) is defined as the ratio of the equilibrium concentrations of a dissolved substance in a two-phase system consisting of two largely immiscible solvents. In the case of n-octanol and water: *P*ow = cn-octanol/c-water

Pow values in the range log *Pow* between -2 and 4 (occasionally up to 5) can be experimentally determined by the shake flask method, as described in the literature. [S4]

12. Mass spectra and NMR spectra

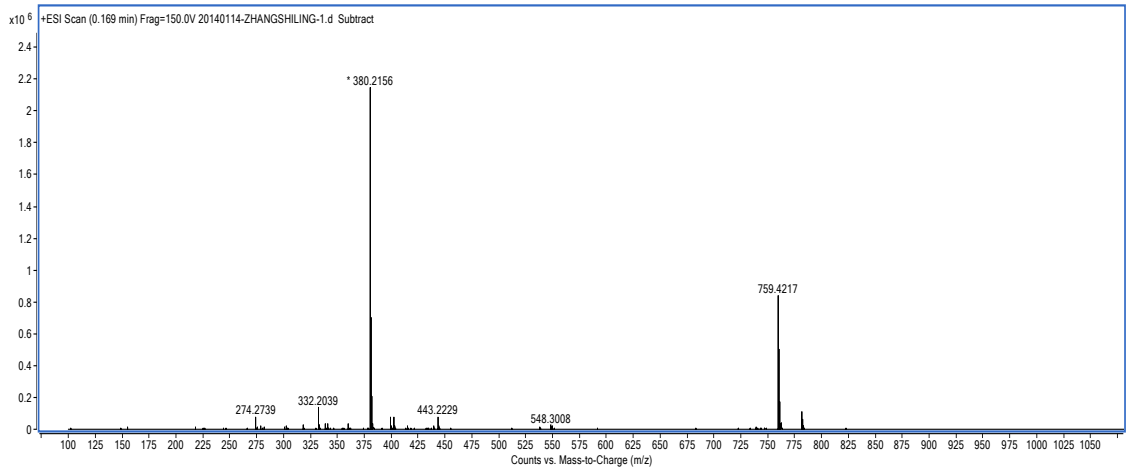
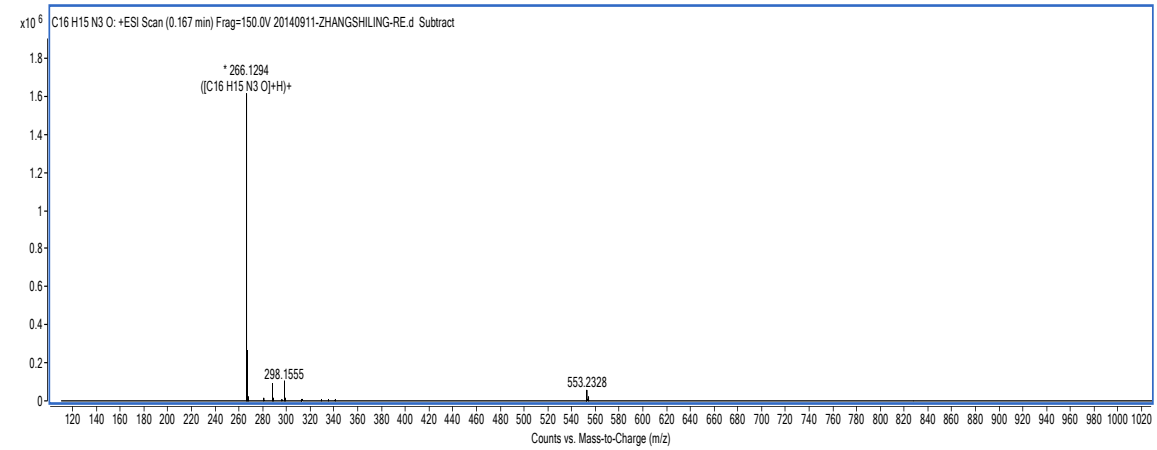
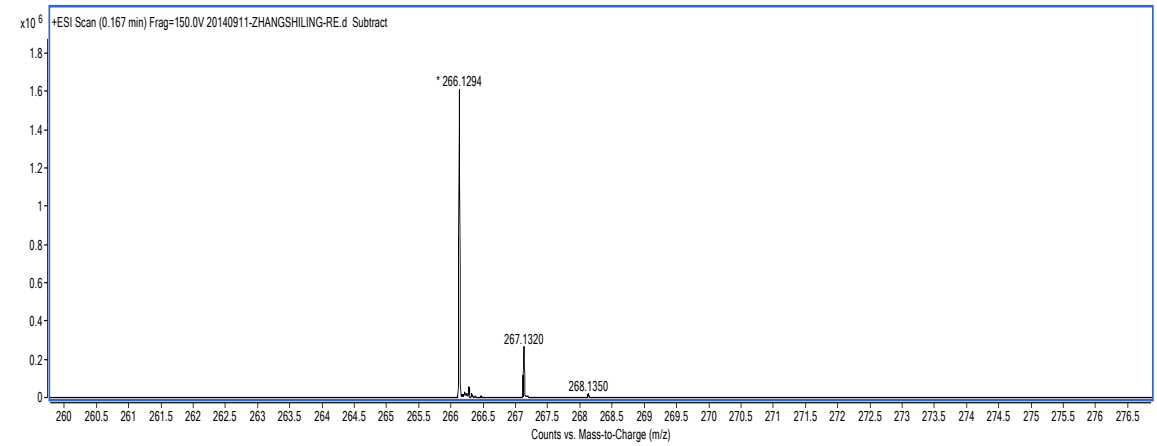


Fig. S13 High-resolution mass spectrum of probe **FP**.

(a)



(b)



(c)

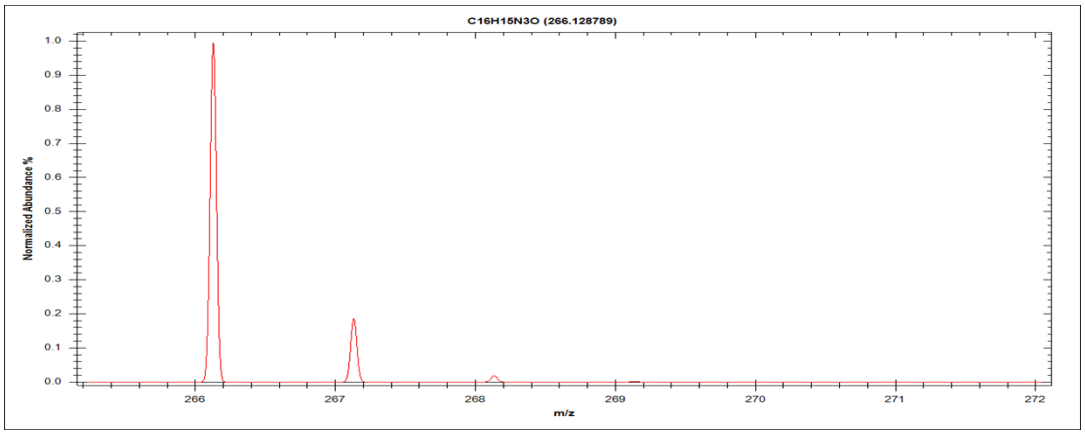


Fig. S14 High-resolution mass spectrum (a and b) and the calculated isotope patterns (c) of the reaction product (**YG**) of probe **FP** with F^-.

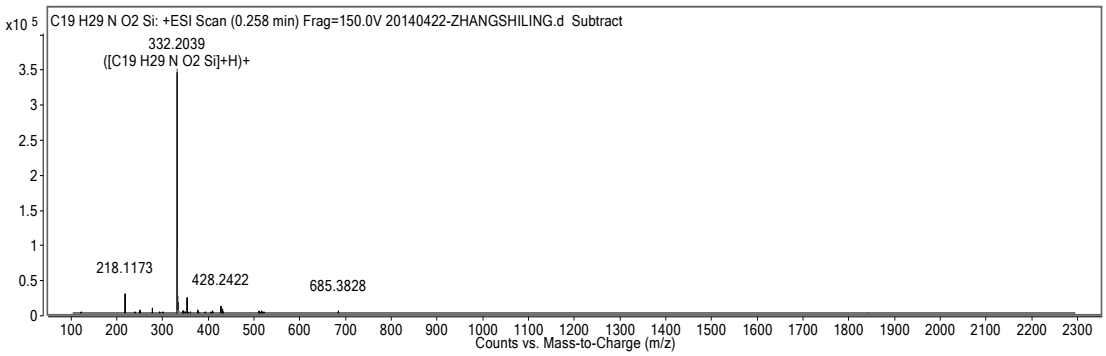


Fig. S15 High-resolution mass spectrum of compound **3**.

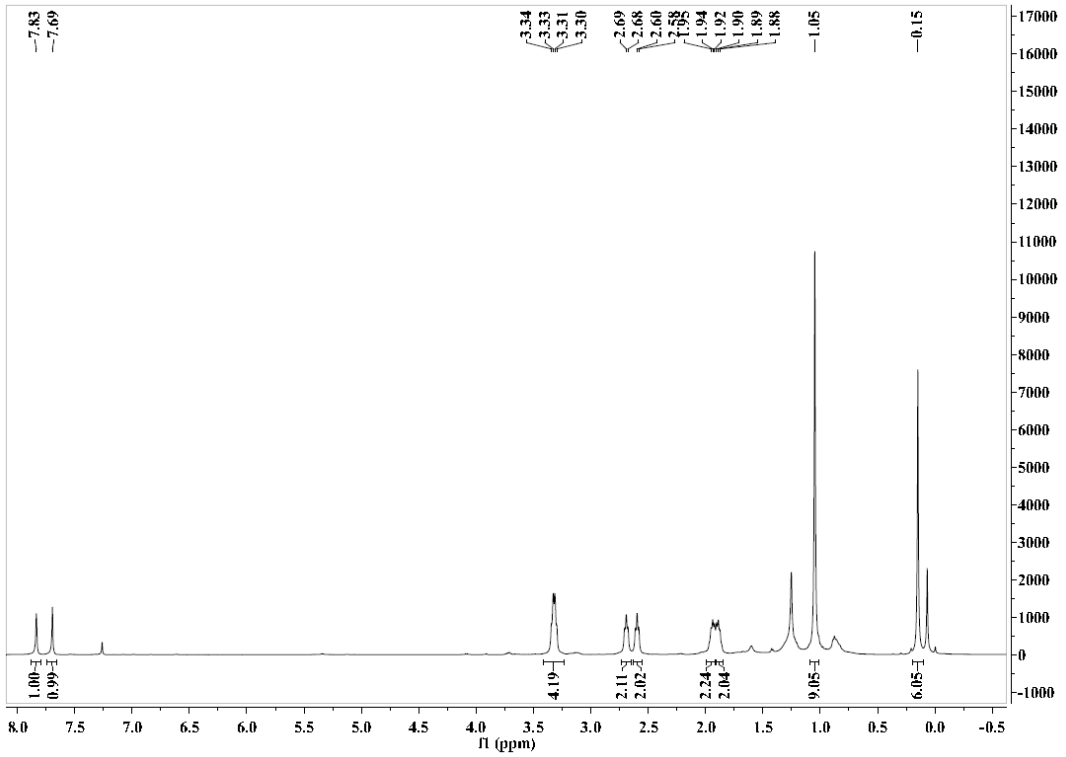


Fig. S16 ^{1}H NMR spectrum of probe **FP** in $CDCl_3$.

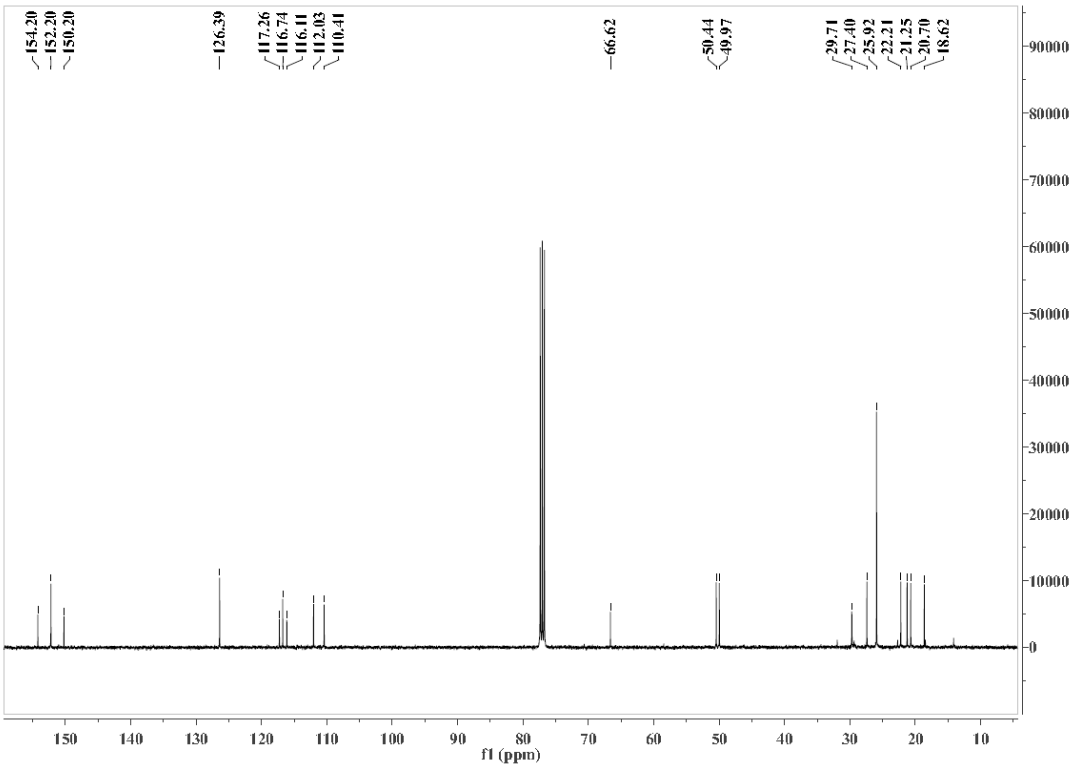


Fig. S17 ^{13}C NMR spectrum of probe **FP** in $CDCl_3$.

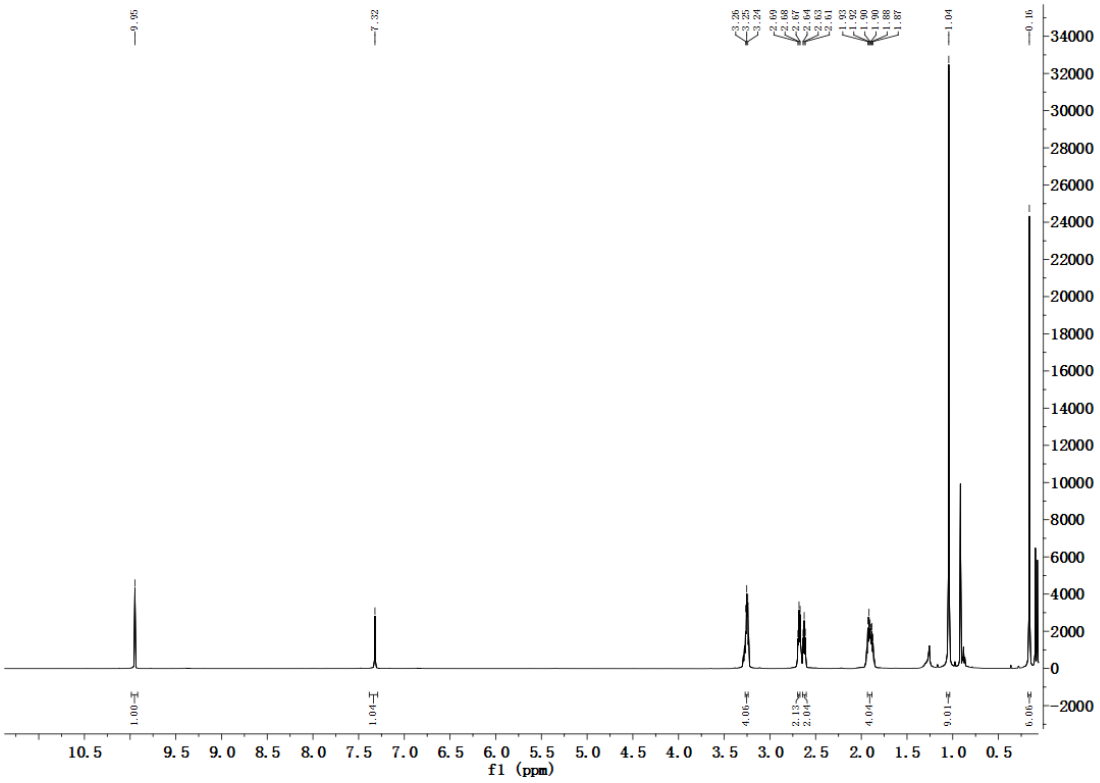


Fig. S18 ^{1}H NMR spectrum of compound **3** in $CDCl_3$.

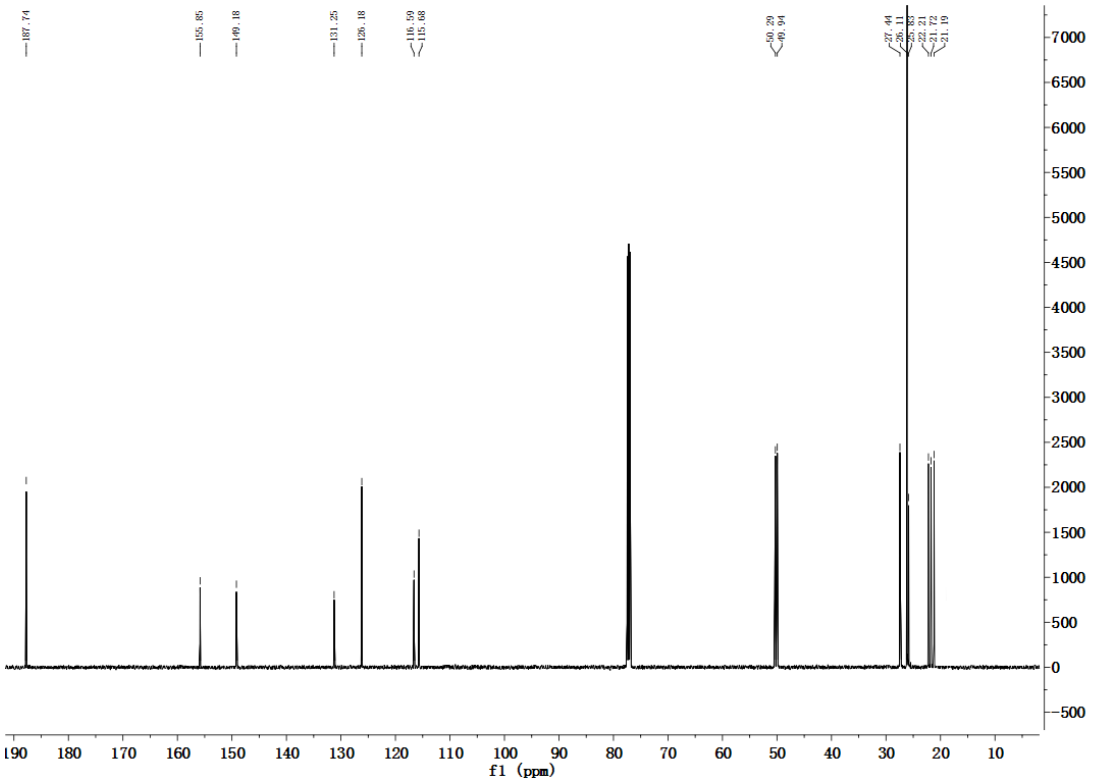


Fig. S19 ^{13}C NMR spectrum of compound **3** in $CDCl_3$.

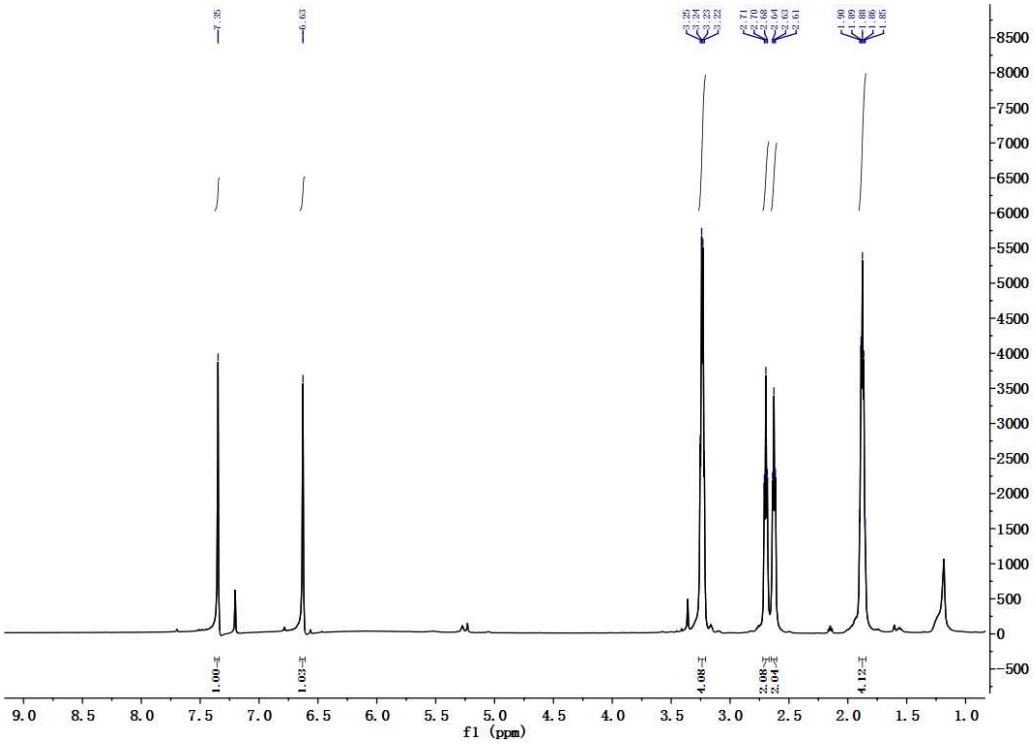


Fig. S20 ^{1}H NMR spectrum of YG in $CDCl_3$.

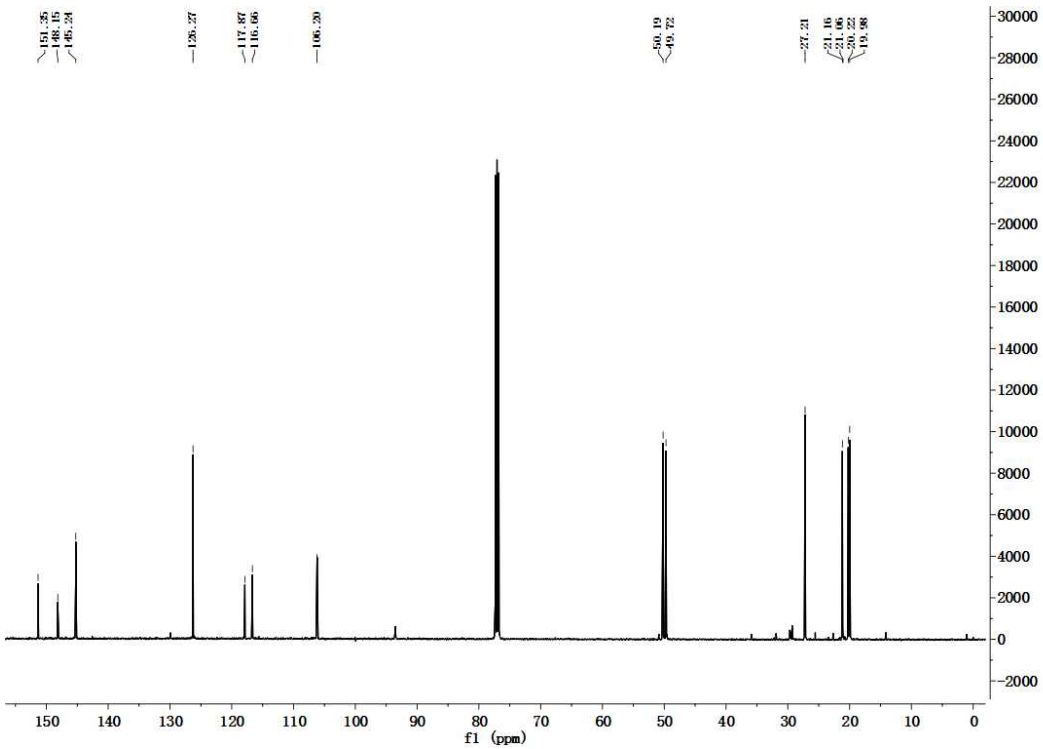


Fig. S21 ^{13}C NMR spectrum of YG in $CDCl_3$.

13. References

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[S4] M. R. Gill, J. Garacia-Lara, S. J. Foster, C. Smythe, G. Battaglia and J. A. Thomas, *Nature Chem.* 2009, **1**,662-667.