Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

A dual functional fluorogenic probe for visualization of intracellular pH and formaldehyde with distinct fluorescence signals

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1. Materials and methods

All chemicals were commercially available and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker Avance 500 MHz spectrometers. The spectra were reported in ppm (δ) and referenced to a tetramethylsilane (TMS) standard in $CDCl_3$-d1, DMSO-d6. Thin layer chromatography (TLC) for reaction monitoring was performed on pre-coated silica gel plates (Merck 60 F254 nm) with fluorescent indicator UV254, and the column chromatography was conducted over silica gel (mesh 300-400). The fluorescence and UV–vis spectra were acquired on a SpectraMax M5, Molecular Devices. HPLC was carried out on Thermo Fisher UltiMate 3000 systems equipped with an autosampler, using reverse-phase Phenomenex Luna 5 μm C18 100 Å 50 × 3.0 mm columns, and the flow rate was 0.5 mL/min. The elution method is 8 min 2%-100% ACN, 5 min 100%-2% ACN, in total 13 min. Mass spectra were recorded on a Thermo Fisher LTQ XL spectrometer.

2. MTT assay

To confirm that the toxicity of probe **DPFP**, the viability of HeLa cells in our incubation condition were carried out. HeLa cells (1×10^5 cells per well) were incubated with probe **DPFP** with concentrations (2, 5, 10, 20 and 30 μM, respectively) for 24 h. Subsequently, 50 μL MTT was added into each well, and the cells were incubated for 4 h at 37 °C under 5% CO_2. Then the medium was removed and DMSO (150 μL) was added to each well. OD values were detected at 550 nm.

3. Cell culture and fluorescence imaging for cellular pH

Fresh stock of HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and appropriate amounts of antibiotics (penicillin and streptomycin). Approximately 1×10^5 cells were seeded in a confocal dish (20 mm) with 1 mL of medium at 37 °C. Before probe **DPFP** was added, the cells to be tested were allowed to adhere to the dish for 24 h. They were then incubated with probe **DPFP** (10 μM) at 37 °C for 30 min and washed with fresh medium. Then the medium was replaced with fresh medium containing Lyso-Tracker Red and incubated for 20 min. After that, cells were wished with PBS twice for

confocal imaging using Olympus Fluoview FV 1200 confocal fluorescence microscopy.

4. Cell imaging for exogenous FA

Fresh stock of HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and appropriate amounts of antibiotics (penicillin and streptomycin). Approximately 1×10^5 cells were seeded in a confocal dish (20 mm) with 1 mL of medium at 37 °C. Subsequently, they were then incubated with probe **DPFP** (10 μM) at 37 °C for 30 min and washed with fresh medium. Then FA (0.3, 0.6, 1, 2 mM) was added for another 3 h incubation. After that, cells were wished with PBS twice for confocal imaging.

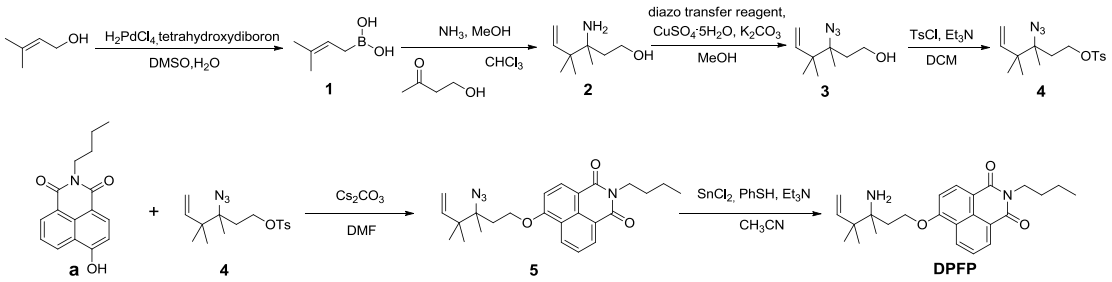
5. Cell imaging for endogenous FA

Fresh stock of HeLa cells was seeded into a confocal dish with a density of 1×10^5 cells per dish, and incubated for 24 h. Subsequently, they were then incubated with inhibitor (NAC or $NaHSO_3$) for 30 min and washed with fresh medium. Then probe **DPFP** was added for another 3 h incubation. After that, cells were wished with PBS twice for confocal imaging.

6. Cell imaging for endogenous ph and FA simultaneously

Fresh stock of HeLa cells was seeded into a confocal dish with a density of 1×10^5 cells per dish, and incubated for 24 h. Subsequently, they were incubated with probe **DPFP** (10 μM) at 37 °C for 30 min and washed with fresh medium. After 3 h, cells were wished with PBS twice for confocal imaging.

Synthesis



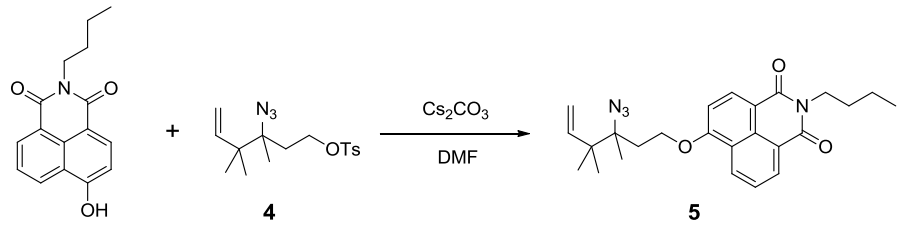
Scheme S1 Synthesis of probe **DPFP**

Compound **4** and compound **a** were prepared following the reported procedures.[1,2]

Compound **4**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.3 Hz, 2H), 7.34 (d, *J* = 8.1 Hz, 2H), 5.85 (dd, *J* = 17.5, 10.9 Hz, 1H), 5.02 (ddd, *J* = 18.5, 14.2, 1.1 Hz, 2H), 4.19 – 4.08 (m, 2H), 2.43 (s, 3H), 1.86 (s, 1H), 1.78 (dd, *J* = 14.5, 7.3 Hz, 1H), 1.25 (s, 3H), 1.00 (d, *J* = 2.1 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 144.80, 143.13, 132.77, 129.77, 127.73, 113.98, 67.26, 66.64, 45.37, 34.50, 22.08, 21.89, 21.44, 17.57. $C_{16}H_{23}N_3O_3S$ (M + H) 337.1, found 337.2.

Compound **a**: ^{1}H NMR (500 MHz, DMSO) δ 8.43 (dd, *J* = 8.3, 0.8 Hz, 1H), 8.40 – 8.33 (m, 1H), 8.26 (d, *J* = 8.2 Hz, 1H), 7.71 – 7.62 (m, 1H), 7.09 (d, *J* = 8.2 Hz, 1H), 4.00 – 3.91 (m, 2H), 1.62 – 1.47 (m, 2H), 1.38 – 1.25 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO) δ 163.54, 162.88, 160.19, 133.36, 130.92, 129.05, 128.72, 125.37, 122.28, 121.68, 112.49, 109.86, 39.04, 29.71, 19.81, 13.68. $C_{16}H_{15}NO_3$ (M + H) 270.3, found 270.2.

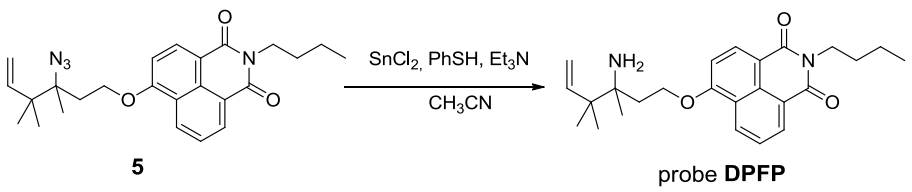
Preparation and Characterization of Compound 5



To a solution of compound **a** (0.65 g, 2.4 mmol) and Cs_2CO_3 (0.78 g, 2.4 mmol) in dry DMF, compound **4** (0.67, 2 mmol) was added dropwise, and the mixture was stirred under N^2 at 40 °C overnight. The solvent was removed under high vacuum, and the resulting residue was purified

by silica column chromatography using EtOAc/PE (v/v, 1:10) to afford a pale yellow solid compound **5** (yield 30%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 – 8.34 (m, 3H), 7.59 (dd, *J* = 8.3, 7.4 Hz, 1H), 6.94 (d, *J* = 8.3 Hz, 1H), 6.00 (dd, *J* = 17.5, 10.9 Hz, 1H), 5.20 – 5.03 (m, 2H), 4.34 (t, *J* = 6.7 Hz, 2H), 4.17 – 4.02 (m, 2H), 2.22 (dd, *J* = 13.8, 6.9 Hz, 1H), 2.10 (dt, *J* = 14.2, 7.0 Hz, 1H), 1.67 (tt, *J* = 7.7, 6.6 Hz, 2H), 1.45 (s, 3H), 1.41 (dt, *J* = 14.2, 7.2 Hz, 2H), 1.13 (s, 6H), 0.95 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.18, 163.58, 159.52, 143.42, 133.07, 131.20, 129.08, 128.22, 125.69, 123.19, 122.23, 114.94, 114.06, 105.61, 67.10, 65.70, 45.53, 39.89, 34.57, 30.13, 22.31, 22.13, 20.29, 18.01, 13.75. $C_{25}H_{30}N_4O_3$ (M + H) 435.2, found 435.2.

Preparation and Characterization of Probe DPFP



5 probe **DPFP**

$SnCl_2$ (0.08 g, 0.35 mM) was added to a round-bottomed flask, then a solution of PhSH (0.1 g, 1 mM) and Et_3N (0.1 g, 1 mM) in MeCN was added and the mixture was stirred for 15 min at room temperature, then compound **5** (0.1 g, 0.23 mM) was added as a solution in MeCN and stirred at room temperature for an additional 12 h. The reaction was concentrated under reduced pressure, Purification by silica chromatography using EtOAc/PE (v/v, 1:3) afforded probe **DPFP** (yield 20%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56 (dd, *J* = 7.3, 1.1 Hz, 1H), 8.53 – 8.47 (m, 2H), 7.66 (dd, *J* = 8.2, 7.4 Hz, 1H), 7.06 (d, *J* = 8.3 Hz, 1H), 6.05 (dd, *J* = 17.5, 10.9 Hz, 1H), 5.13 (ddd, *J* = 18.8, 14.2, 1.3 Hz, 2H), 4.47 (dtd, *J* = 17.0, 9.2, 6.3 Hz, 2H), 4.23 – 4.07 (m, 2H), 2.15 (ddd, *J* = 14.1, 7.8, 6.4 Hz, 1H), 2.05 (dt, *J* = 13.8, 6.8 Hz, 1H), 1.73 – 1.68 (m, 4H), 1.50 – 1.39 (m, 2H), 1.18 (s, 3H), 1.12 (s, 6H), 0.98 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.53, 163.96, 160.20, 144.70, 133.47, 131.39, 129.35, 128.54, 125.76, 123.48, 122.44, 114.83, 113.82, 105.96, 66.69, 55.40, 44.21, 40.06, 35.50, 30.26, 22.11, 22.07, 21.77, 20.40, 13.85. $C_{25}H_{32}N_2O_3$ (M + H) 409.2413, found 409.2492.

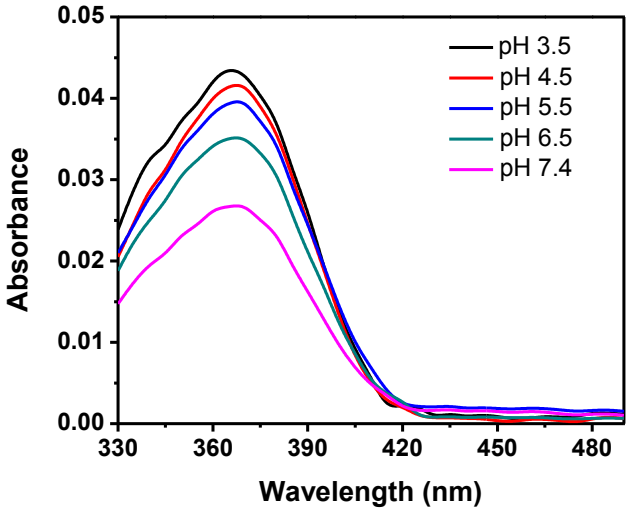


Fig. S1 Absorption spectra of probe **DPFP** (5 μM) only at different pH values.

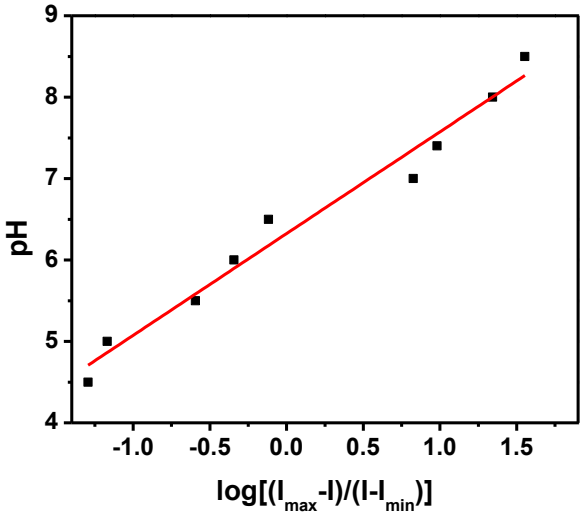


Fig. S2 Plot of pH vs $\log[(I_{max}-I)/(I-I_{min})]$, where I is the relative fluorescence intensity of probe **DPFP**. Ex/Em = 365/455 nm.

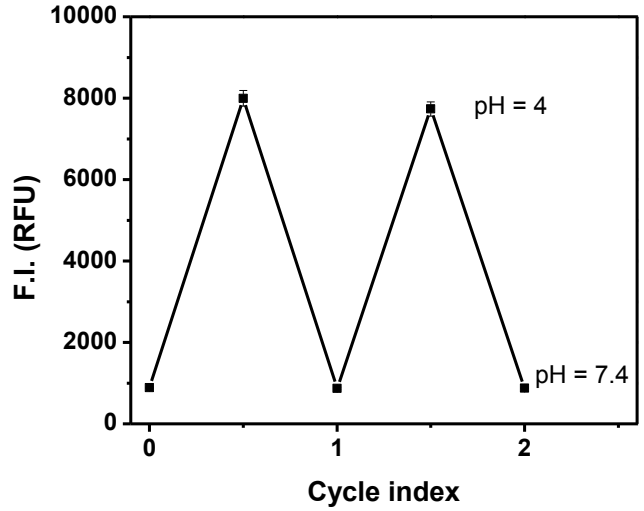


Fig. S3 Reversible fluorescence (455 nm) changes between pH 4 and pH 7.4. All data were obtained from excitation at 365 nm in PBS buffer (0.5% DMSO, 10 mM) at 37 ˚C.

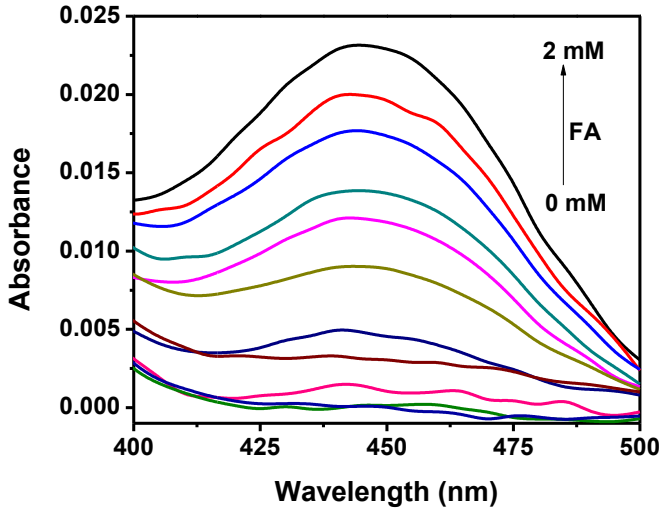


Fig. S4 Absorption spectra of probe **DPFP** (5 μM) with FA (0-2 mM) titration.

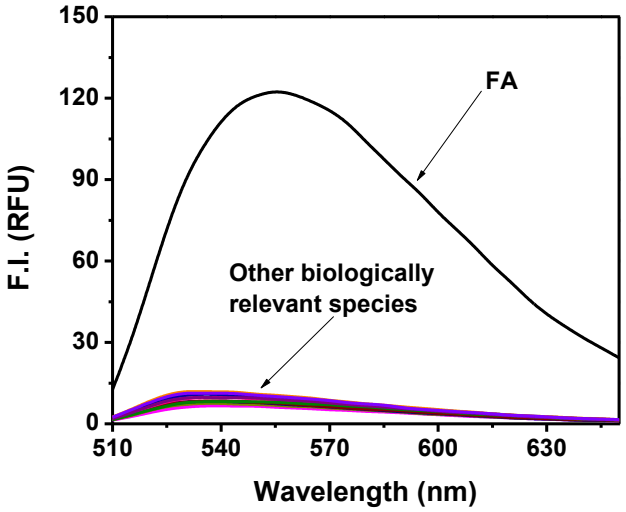


Fig. S5 Fluorescence spectra of probe **DPFP** (5 μM) in the presence of FA and other various biologically relevant species in PBS buffer (0.5% DMSO, 10 mM, pH 7.4). Ex/Em = 455/555 nm.

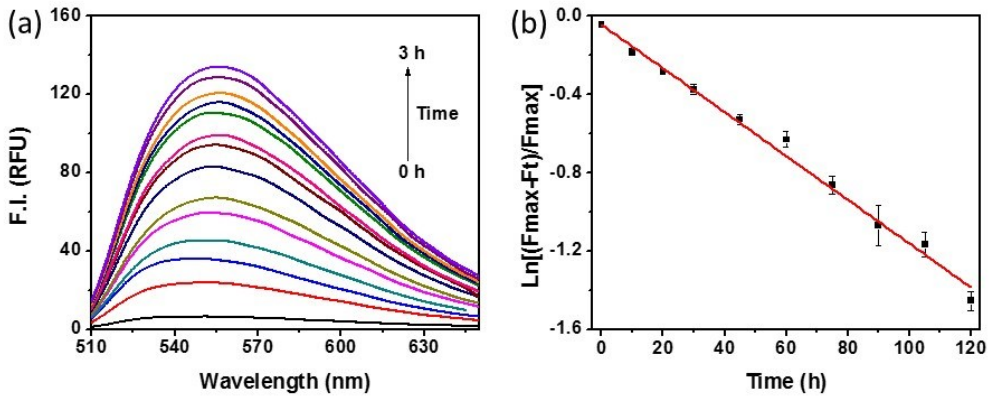


Fig. S6 (a) Fluorescence spectrum of probe **DPFP** (5 μM) in the presence of 0.5 mM FA; (b) Pseudo-first-order kinetic plot of the reaction of probe **DPFP** (5 μM with 0.5 mM FA. Slope = -0.01. Data were acquired in PBS buffer (0.5% DMSO, 10 mM, pH 7.4) for 3 h at 37 °C at Ex/Em = 455 nm/555 nm.[3]

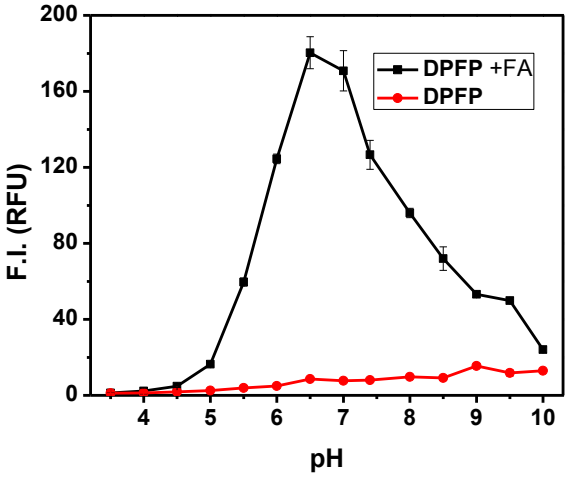


Fig. S7 pH-Fluorescence profile of probe **DPFP** (5 μM) in the absence and in the presence of FA (0.5 mM) in PBS buffer (0.5% DMSO, 10 mM) followed by a 3 h incubation period. Ex/Em = 455/ 555 nm. Red points represent free probe **DPFP**, black points represent probe **DPFP** with FA.

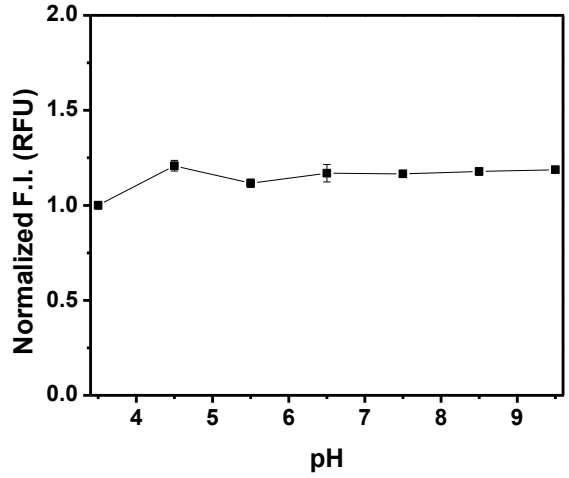
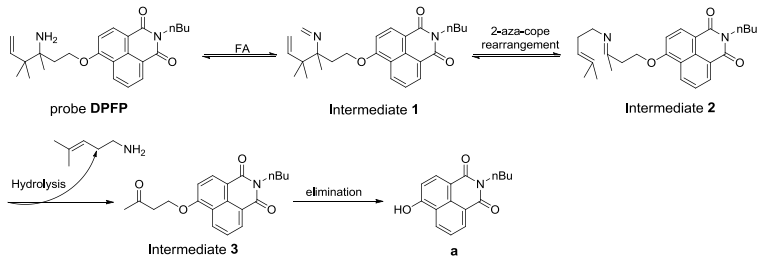
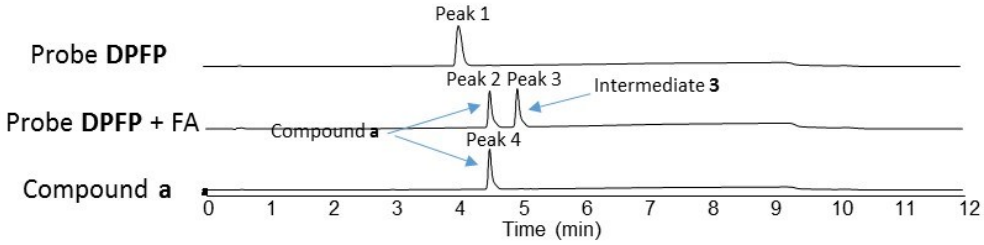


Fig. S8 pH-fluorescence profile of compound **5** (5 μM) in PBS buffer (0.5% DMSO, 10 mM) at different pH values. Ex/Em = 365/455 nm.

(a)



(b)



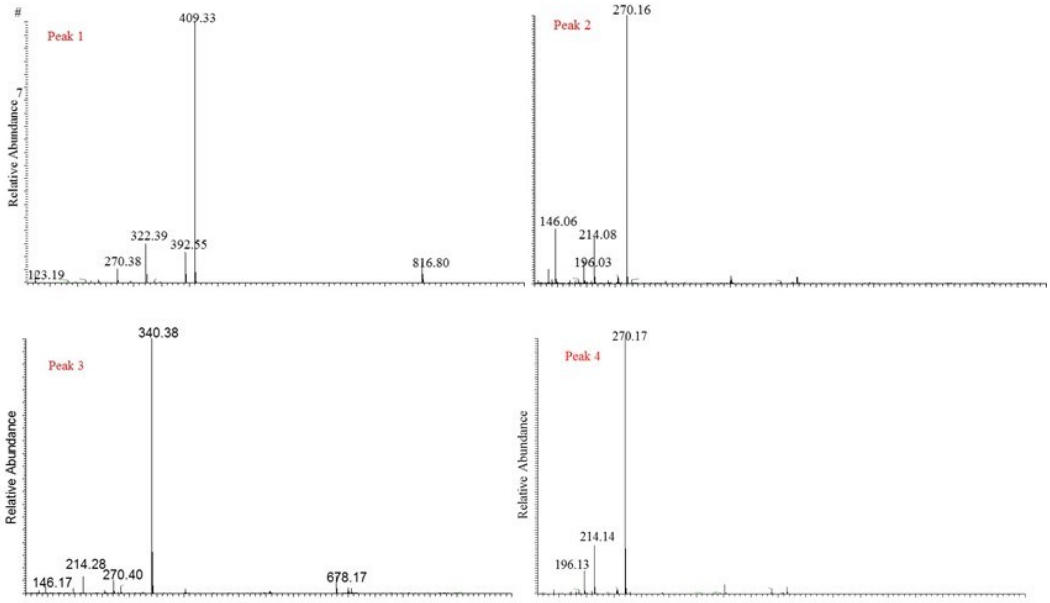


Fig. S9 (a) The mechanism of Self-Immolative Aza-Cope Strategy and (b)LC-MS data of reaction between probe **DPFP** in the absent and present of FA and compound **a**.

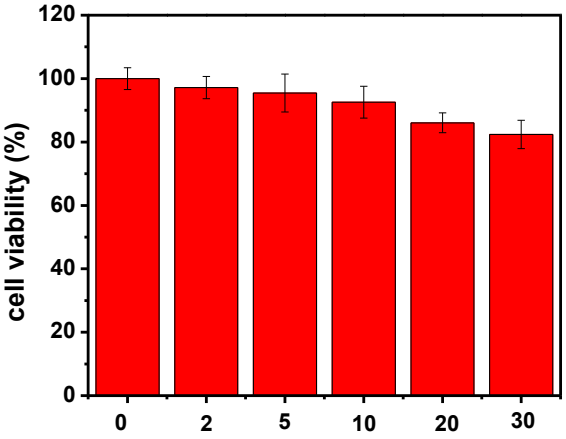


Fig. S10 MTT assay with different concentrations of probe **DPFP**. HeLa cells were incubated with various concentrations of probe **DPFP** (2, 5, 10, 20 and 30 μM) for 24 h.

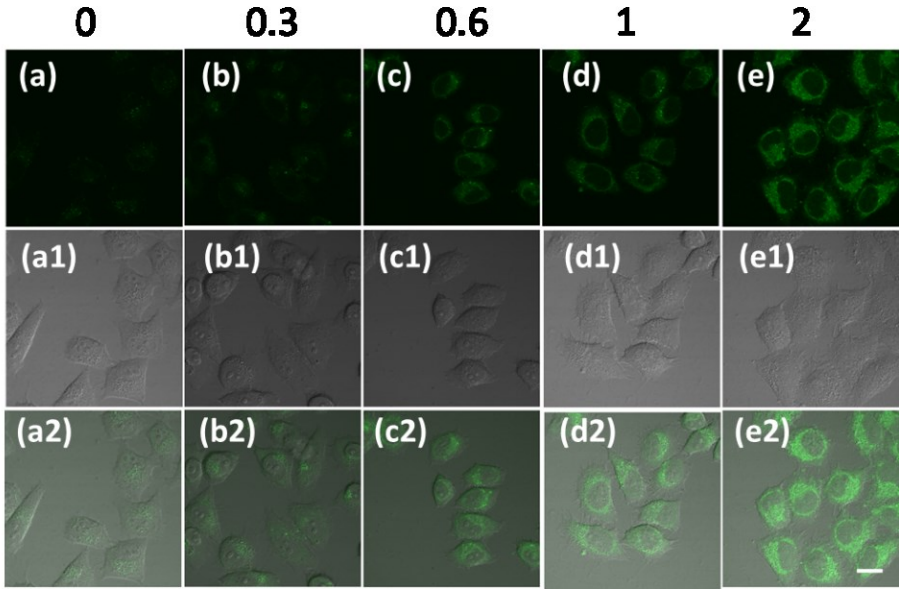


Fig. S11 Confocal fluorescence imaging of exogenous FA in HeLa cells with probe **DPFP** (10 μM). Cells were treated with probe **DPFP** in DMEM for 30 min, exchanged into fresh DMEM, and then FA (0.3, 0.6, 1, 2 mM) was added for another 3 h incubation. After that, cells were wished with fresh medium twice for confocal imaging. Excitation was at 488 nm and emissions were collected from 500–550 nm. Scale bar: 20 μm.

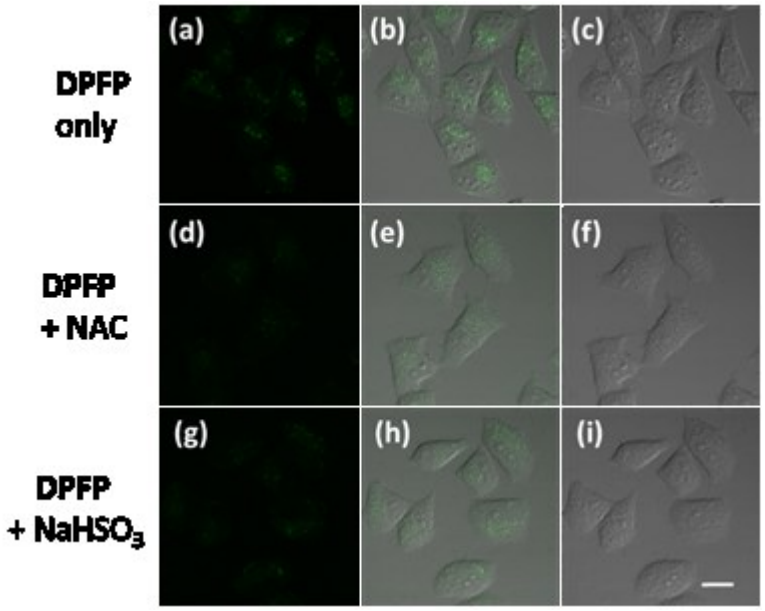


Fig. S12 Confocal fluorescence imaging of endogenous FA in HeLa cells with probe **DPFP** (10 μM). (a) Fluorescent image of HeLa cells stained with probe **DPFP**; (c) Bright-field image of (a); (b) The merged image of (a) and (c); (d) Fluorescent image of HeLa cells stained with NAC (2 mM) and followed by incubation with probe **DPFP**; (f) Bright-field image of (d); (e) The merged image of (d) and (f); (g) Fluorescent image of HeLa cells stained with $NaHSO_3$ (0.2 mM) and followed by incubation with probe **DPFP**; (i) Bright-field image of (g); (h) The merged image of (g) and (i). Excitation was at 488 nm and emissions were collected from 500–550 nm. Scale bar: 20 μm.

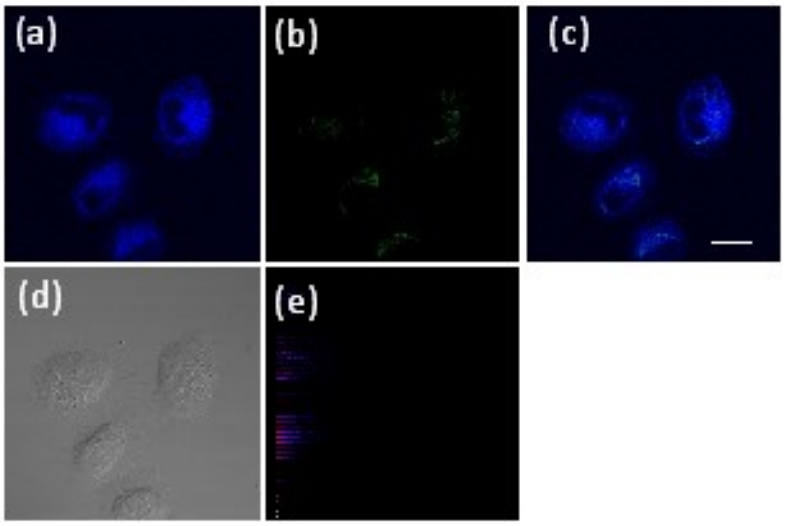
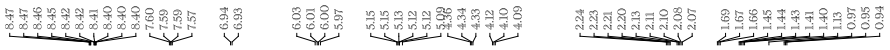


Fig. S13 Confocal fluorescence imaging of endogenous FA in HeLa cells with probe **DPFP** (10 μM). Cells were incubated with probe **DPFP** for 30 min at 37 ◦C and washed with fresh medium. After 3 h, cells were wished with PBS twice for confocal imaging. (a) Blue channel: Ex = 405 nm, collected in the range of 430–480 nm; (b) Green channel: Ex = 488 nm, collected in the range of 500–550 nm; (c) The merged image of (a), (b); (d) Bright field; (e) Intensity scatter plot of blue and green channel. Scale bar: 20 μm.

1. K. J. Bruemmer, R. R. Walvoord, T. F. Brewer, G. Burgos-Barragan, N. Wit, L. B. Pontel, K. J. Patel and C. J. Chang, *J. Am. Chem. Soc.*, 2017, **139**, 5338.
2. H. Park, S.-K. Chang, *Dyes Pigm*., 2015, **122**, 324.
3. Y. Tang, X. Kong, A. Xu, B. Dong and W. Lin, *Angew. Chem., Int. Ed.*, 2016, **55**, 3356.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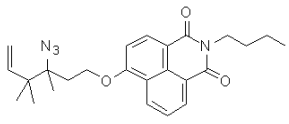
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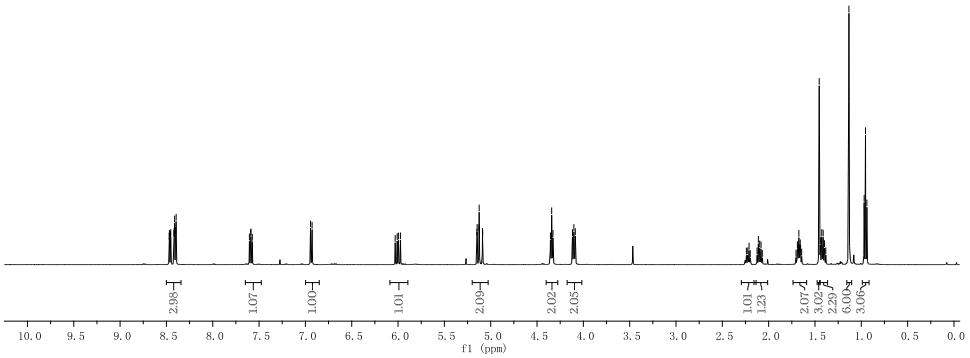
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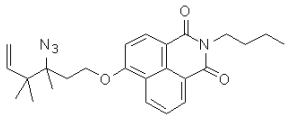
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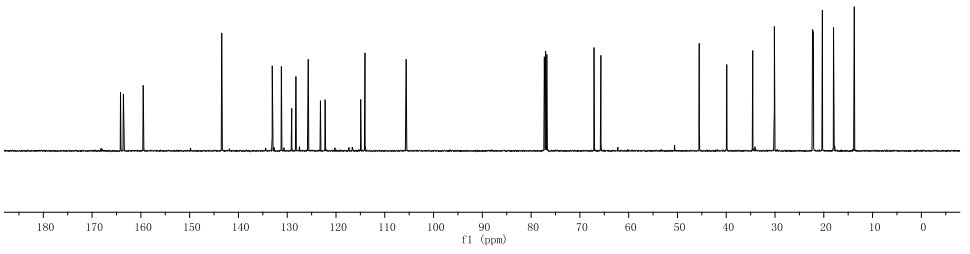
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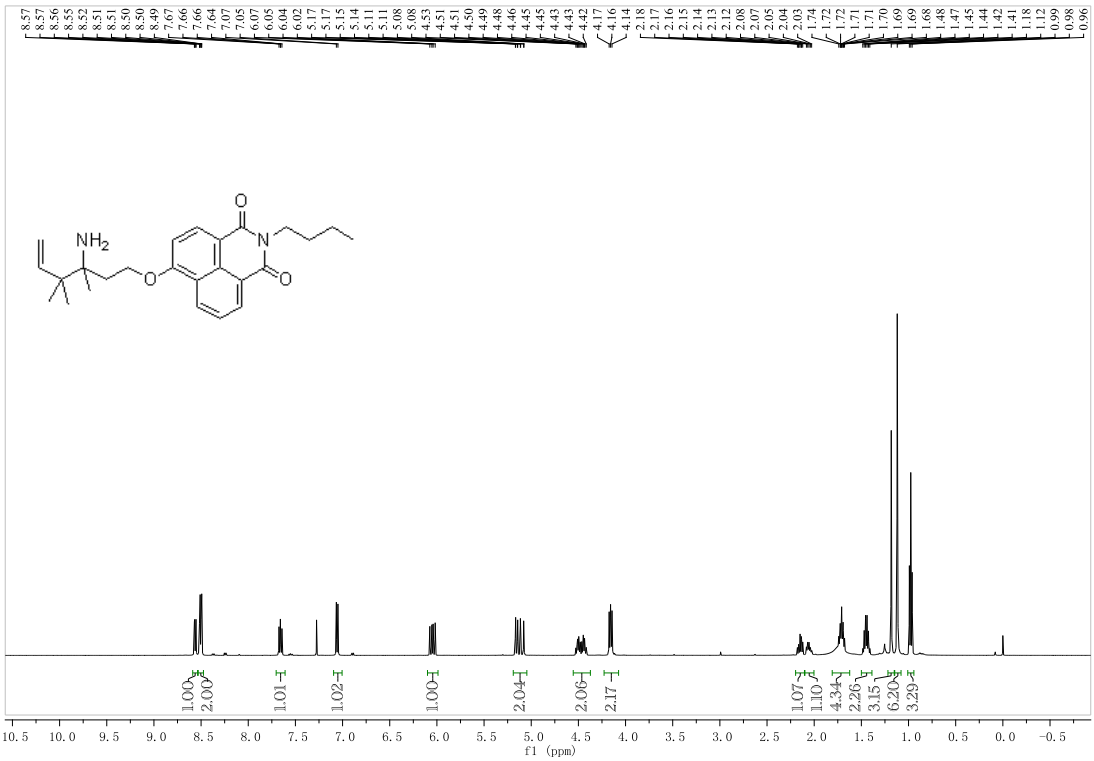
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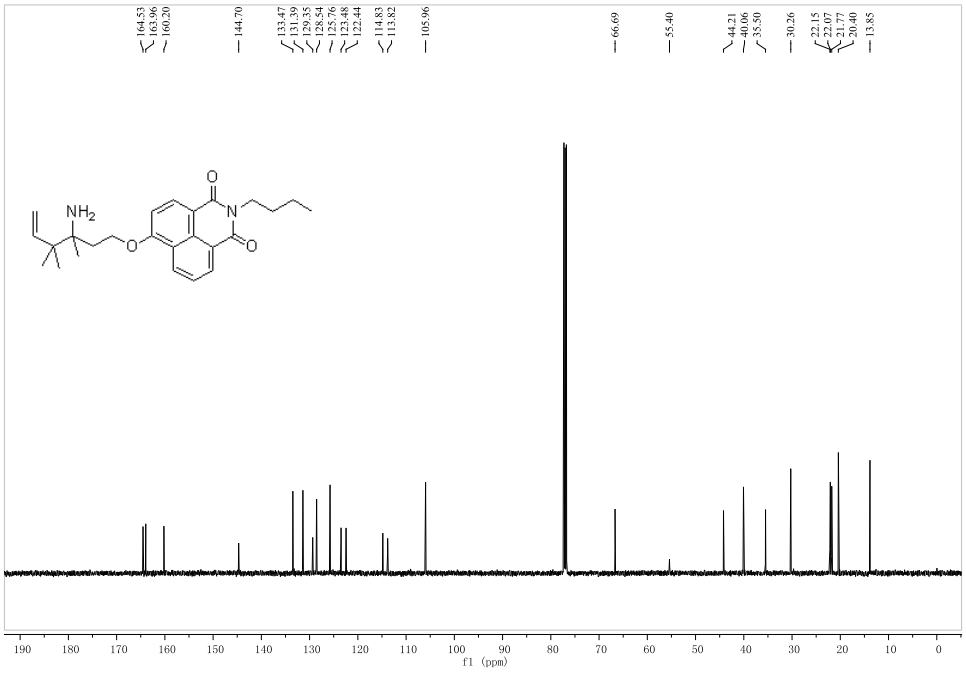
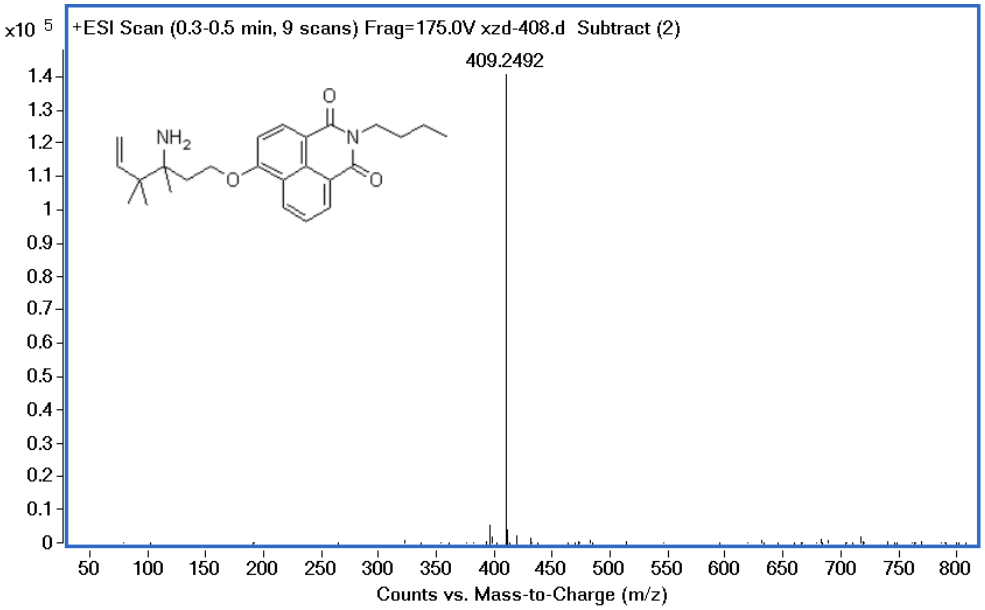
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 −0.5
f1 (ppm)


164.53 163.96 160.20 144.70 133.47 131.39 129.35 128.54 125.76 123.48 122.44 114.83 113.82 105.96 66.69 55.40 44.21 40.06 35.50 30.26 22.15 22.07 21.77 20.40 13.85
NH2
O
N
O
O
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


x10 5
+ESI Scan (0.3-0.5 min, 9 scans) Frag=175.0V xzd-408.d Subtract (2)
409.2492
NH2
O
N
O
O
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
50 100 150 200 250 300 350 400 450 500 550 600 650 700 750 800
Counts vs. Mass-to-Charge (m/z)