SUPPORTING INFORMATION

for

Organelle-resolved imaging of formaldehyde reveals its spatiotemporal dynamics

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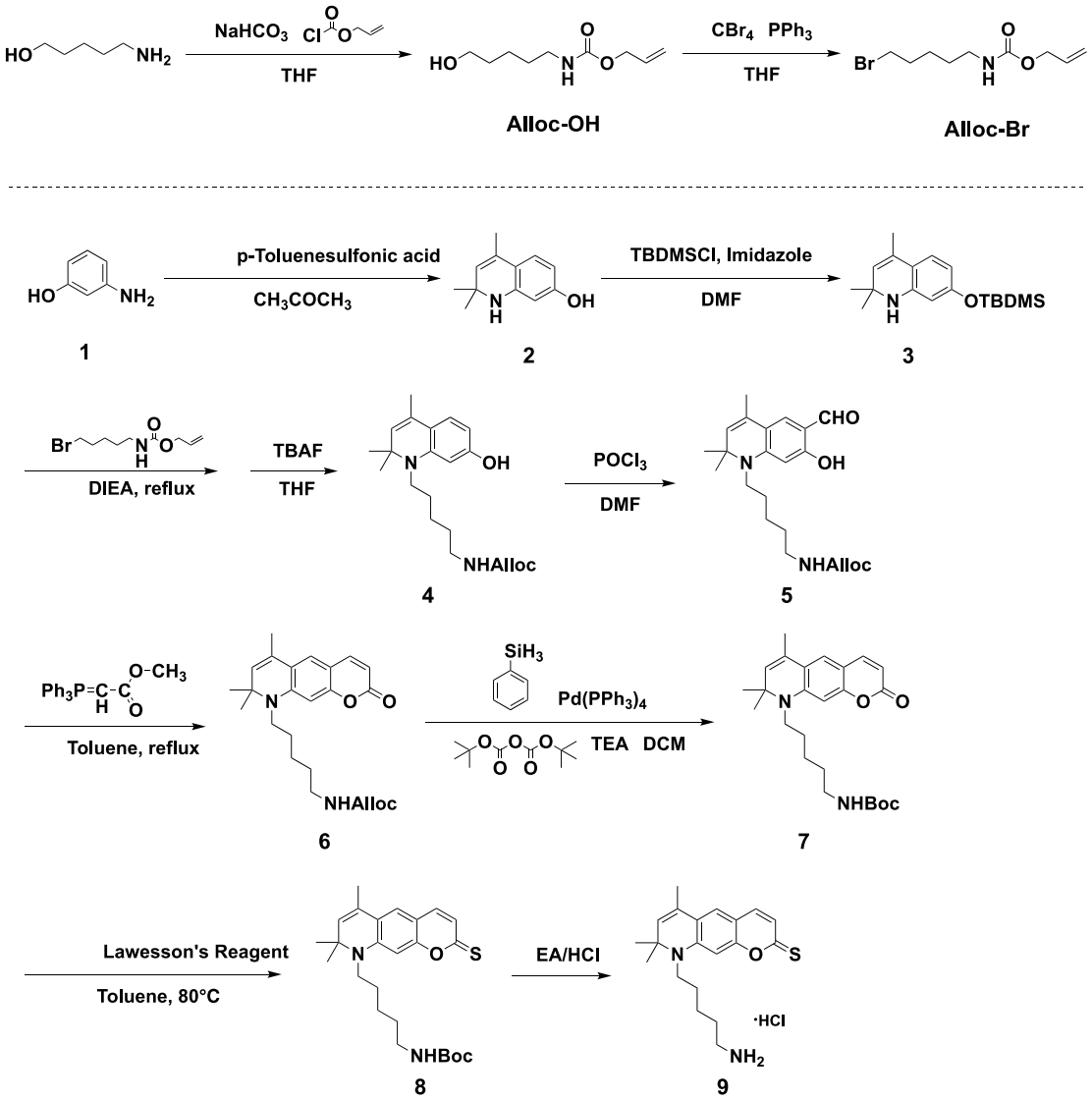
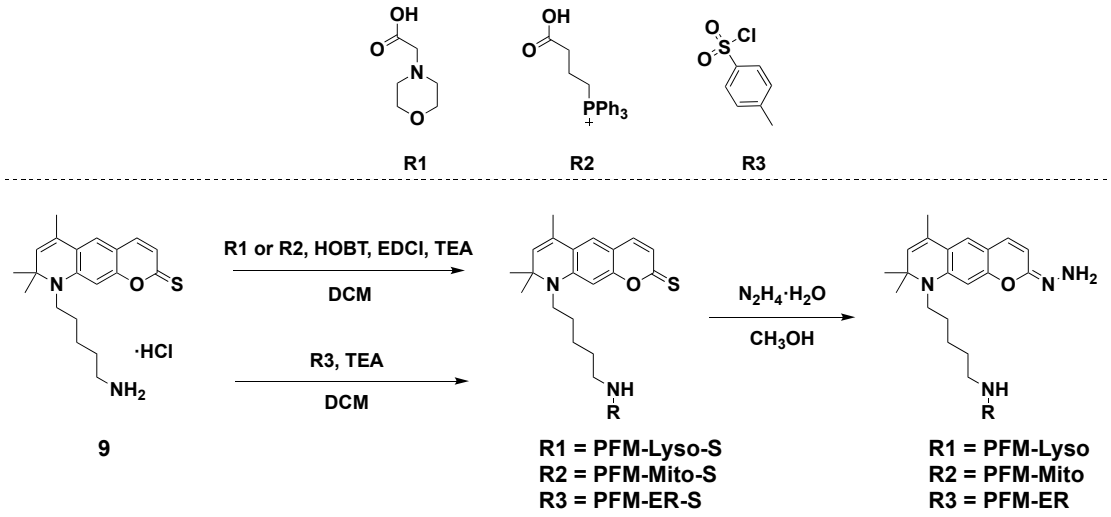
General Experimental for Chemistry

All reagents and solvents were purchased from commercial suppliers and used without further purification unless otherwise indicated. Reactions were monitored by thin-layer chromatography (TLC) carried out on Silica gel 60 F254 plates supplied by Qingdao Puke Separation Material Corporation, and UV light was used as the visualizing agent. Flash column chromatography was performed using 200-300 mesh silica gel supplied by Qingdao Marine Chemical Factory, Qingdao, China. A mixture of petroleum ether (PE, b.p. 60-90 °C) and ethyl acetate (EtOAc) was used as the eluting solvent for both TLC analysis and column chromatography isolation. ^{1}H NMR spectra were obtained on a Bruker Fourier transform spectrometer (500 MHz) at 25 °C. ^{13}C NMR spectra were recorded on a Bruker Fourier transform spectrometer (125 MHz) spectrometer. All NMR spectra were calibrated using residual solvents as internal references (for $CDCl_3$: ^{1}H NMR = 7.26, ^{13}C NMR = 77.16; for DMSO: ^{1}H NMR = 2.50, ^{13}C NMR = 39.52). All chemical shifts were reported in parts per million (ppm) and coupling constants (*J*) in Hz. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Electrospray ionization mass spectroscopy (ESI-MS) spectra were obtained with a Shimadzu LCMS-2020 mass spectrometer with mobile phases as methanol and water containing 0.1% formic acid. High resolution mass spectra (HRMS) were measured on an Agilent 6224 TOF LC/MS spectrometer using ESI-TOF (electrospray ionization-time-of-flight).

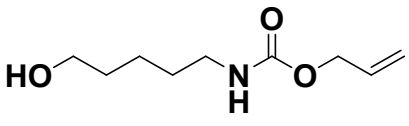
Probe Synthesis and Characterization



Scheme S1. Synthesis of the key intermediate compound 9.



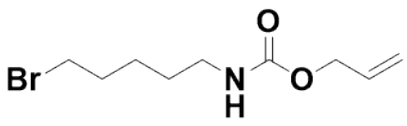
Scheme S2. Synthesis of probes FA-Lyso, FA-Mito, FA-ER.



Alloc-OH

In an ice bath cooled mixture of 5-aminopentan-1-ol (2 mL, 0.02 mol) in THF and Saturated $NaHCO_3$ (aq) (20 mL, 1:8), allyl chloroformate (3 mL, 0.03 mL) was dropwise added. The mixture was then stirred at room temperature 4h. After completion as shown by TLC analysis, the reaction was quenched with H_2O, extracted with EtOAc, washed sequentially with H_2O and brine. The organic layer was then dried over anhydrous sodium sulfate and concentrated by rotary evaporation under reduced pressure. The target compound **Alloc-OH** was isolated as a white oil (1.7 g, 47 % yield) by flash column chromatography on a silica gel column by eluted with PE : EA (1 : 1).

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.91 (ddt, J = 16.4, 10.8, 5.6 Hz, 1H), 5.30 (dd, J = 17.1, 1.8 Hz, 1H), 5.24 – 5.17 (m, 1H), 5.07 (s, 1H), 3.62 (t, J = 6.5 Hz, 2H), 3.18 (q, J = 6.7 Hz, 2H), 2.47 (s, 2H), 1.64 – 1.49 (m, 4H), 1.45 – 1.35 (m, 2H).

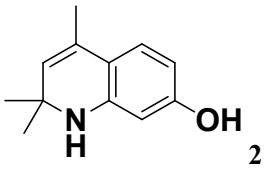


Alloc-Br

To a solution of **Alloc-OH** (1.7g, 0.009 mol) in THF (50 mL) was added CBr_4 (4.5 g, 0.0135 mol) and PPh_3 (3.5 g, 0.0135 mol). The solution was stirred at room temperature. After completion

as shown by TLC analysis, the reaction was added PE (50 mL), a large amount of triphenylphosphine was precipitated and filtered. The organic layer was then dried over anhydrous sodium sulfate and concentrated by rotary evaporation under reduced pressure. The target compound **Alloc-Br** was isolated as a white oil (1.8 g, 84 % yield) by flash column chromatography on a silica gel column by eluted with PE : EA (1 : 1).

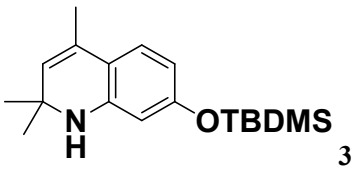
^{1}H NMR (500 MHz, $CDCl_3$) δ 5.92 (ddt, J = 16.4, 10.8, 5.6 Hz, 1H), 5.30 (dq, J = 17.2, 1.7 Hz, 1H), 5.21 (dt, J = 10.4, 1.5 Hz, 1H), 5.07 – 4.87 (m, 1H), 4.56 (d, J = 6.0 Hz, 2H), 3.41 (t, J = 6.7 Hz, 2H), 3.19 (t, J = 6.9 Hz, 2H), 1.88 (p, J = 6.9 Hz, 2H), 1.58 – 1.42 (m, 4H).



2

To a solution of 3-aminophenol (10 g) in acetone (100 mL) was added *p*-toluenesulfonic acid (100 mg). The solution was stirred at room temperature for 24h. The reaction was concentrated under reduced pressure and then purified by silica gel chromatography (3:1:0→3:1:1, PE: DCM: EA). The crude product was dissolved in a bit of dichloromethane, then 3 times volume of petroleum ether was added slowly to yield a white solid (5.8 g, 33 % yield).

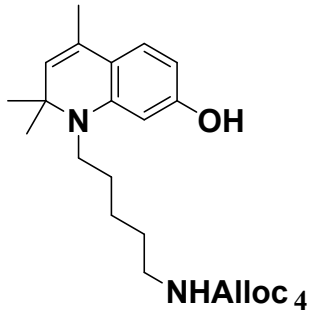
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.91 (d, J = 8.2 Hz, 1 H), 6.10 (dd, J = 8.2, 2.5 Hz, 1 H), 5.93 (d, J = 2.5 Hz, 1 H), 5.19 (q, J = 1.5 Hz, 1 H), 1.95 (d, J = 1.4 Hz, 3 H), 1.25 (s, 6 H). ESI-MS: m/z = $[M+H]^+$ calculated 190.12, found 190.35.



3

To a solution of compound **2**（2 g, 0.0106 mol）in DMF (30 mL) at 0 °C was added imidazole (1.8 g, 0.0265 mol), tert-butyldimethylsilyl chloride (1.9 g, 0.01272 mol). The reaction was then allowed to warm to ambient temperature slowly. After completion as shown by TLC analysis, the reaction was quenched with H_2O, extracted with EtOAc, washed sequentially with H_2O and brine. The organic layer was then dried over anhydrous sodium sulfate and concentrated by rotary evaporation under reduced pressure to give compound **3** as a white solid (3.1 g, 97 % yield) which was used without further purification.

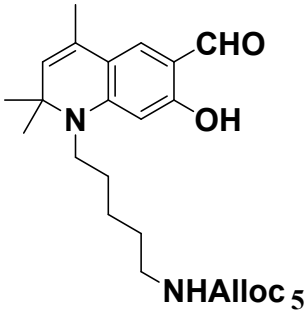
^{1}H NMR (500 MHz, $CDCl_3$) δ 6.91 (d, J = 8.2 Hz, 1H), 6.13 (dd, J = 8.3, 2.4 Hz, 1H), 5.98 (d, J = 2.4 Hz, 1H), 5.19 (q, J = 1.5 Hz, 1H), 1.96 (d, J = 1.5 Hz, 3H), 1.26 (s, 6H), 0.98 (s, 9H), 0.19 (s, 6H). ESI-MS: m/z = $[M+H]^+$ calculated 304.20, found 304.30.



N,N-diisopropyl-N-ethyl amine (DIEA, 1.57 mL, 9.5 mmol) was added to a mixture of **3** (960 mg, 3.17 mmol) and Alloc-Br (1.4 g, 5.6 mmol) placed in a screw-cup bottle, and the reaction mixture was stirred with heating (110 °C) for 3 days. After completion as shown by TLC analysis, the reaction was quenched with H_2O, extracted with EtOAc, washed sequentially with H_2O and brine. The organic layer was then dried over anhydrous sodium sulfate and concentrated by rotary evaporation under reduced pressure. The crude product was isolated as a yellow oil (1.1 g, 64 % yield) by flash column chromatography on a silica gel column by eluted with PE.

Then, to a solution of crude product (500 mg, 1.06 mmol) in THF (15mL) was added TBAF (5 mL, 5 mol/L in THF). The solution was stirred at room temperature. After completion as shown by TLC analysis, the reaction was quenched with H_2O, extracted with EtOAc, washed sequentially with H_2O and brine. The organic layer was then dried over anhydrous sodium sulfate and concentrated by rotary evaporation under reduced pressure. The target compound **4** was isolated as a white oil (370 mg, 98 % yield) by flash column chromatography on a silica gel column by eluted with PE : EA (10 : 1).

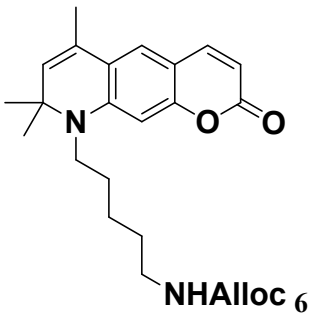
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 – 7.28 (m, 1H), 6.90 (d, J = 8.1 Hz, 1H), 6.18 – 6.13 (s, 1H), 6.14 – 6.10 (m, 1H), 5.91 (ddt, J = 17.2, 10.4, 5.6 Hz, 1H), 5.31 (dq, J = 17.3, 1.6 Hz, 1H), 5.21 (dq, J = 10.4, 1.3 Hz, 1H), 5.04 (d, J = 1.6 Hz, 1H), 5.04 – 4.98 (m, 1H), 4.58 (d, J = 5.7, 2H), 3.23 (m, 2H), 3.15 – 3.08 (m, 2H), 1.92 (d, J = 1.4 Hz, 3H), 1.63 (m, 2H), 1.59 – 1.53 (m, 2H), 1.37 (m, 2H), 1.26 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.30, 157.62, 156.91, 145.15, 132.54, 127.70, 126.11, 124.82, 117.97, 115.53, 98.09, 65.90, 56.68, 44.15, 40.08, 29.00, 28.45, 26.50, 23.00, 21.06. ESI-MS: m/z = $[M+H]^+$ calculated 359.22, found 359.35.



5

$POCl_3$ (1.68 mL, 0.018 mol) was added to DMF (20 mL) at 0°C in anaerobic conditions, and the mixture was allowed to warm to room temperature. After stirring for 15 min at room temperature, it was cooled down (0 °C), and a solution of compound **4** (4.33 g, 0.012 mol) in DMF (20 mL) was added slowly. The cooling bath was removed, and the reaction mixture was allowed to warm up to room temperature, stirred for 5 min. The TLC control of this reaction is difficult, because the product was found to have the same R_f value, as the starting material. After cooling, the reaction was "quenched" by adding 5 mL of sat. aq. $NaHCO_3$, and the product was extracted with dichloromethane. The organic layer was separated, dried $NaSO_4$, after evaporation of solvents, the target compound **5** was isolated as a yellow oil (3.37 g, 72 % yield) by flash column chromatography on a silica gel column by eluted with PE : EA (8 : 1).

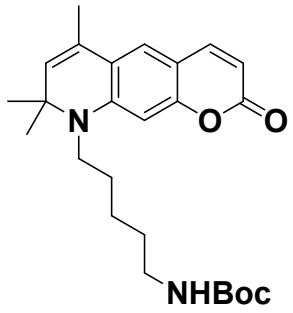
^{1}H NMR (500 MHz, $CDCl_3$) δ 11.79 (s, 1H), 9.44 (s, 1H), 7.00 (s, 1H), 5.90 (ddt, J = 16.4, 10.9, 5.6 Hz, 1H), 5.84 (s, 1H), 5.32 – 5.25 (dd, J = 17.2, 1.4, 1H), 5.19 (dq, J = 10.4, 1.4 Hz, 1H), 5.15 (d, J = 1.6 Hz, 1H), 4.87 (s, 1H), 4.54 (d, J = 5.6 Hz, 2H), 3.27 – 3.20 (m, 2H), 3.21 – 3.15 (m, 2H), 1.93 (d, J = 1.4 Hz, 3H), 1.63 (m, 2H), 1.59 – 1.51 (m, 2H), 1.37 (m, 2H), 1.33 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.76, 164.64, 156.33, 151.09, 132.98, 128.05, 127.30, 126.11, 117.58, 115.82, 110.72, 96.14, 65.46, 58.22, 44.72, 40.89, 36.48, 31.42, 29.83, 27.64, 24.22. ESI-MS: m/z = $[M+H]^+$ calculated 387.22, found 387.35.



6

To a solution of compound **5** (500 mg, 1.29 mmol) in toluene (30 mL) was added methyl (triphenylphosphoranylidene)acetate (649 mg, 1.94 mmol). The reaction requires was refluxed under N_2. After completion as shown by TLC analysis, the target compound **6** was isolated as a yellow oil (387 mg, 73 % yield) by flash column chromatography on a silica gel column by eluted with PE → PE : EA (4 : 1).

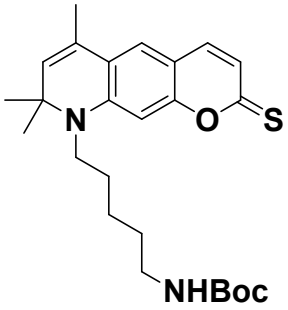
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.51 (d, J = 9.2 Hz, 1H), 6.99 (s, 1H), 6.24 (s, 1H), 6.02 (d, J = 9.2 Hz, 1H), 5.93 (ddt, J = 16.4, 10.9, 5.7 Hz, 1H), 5.31 (dq, J = 17.3, 1.6 Hz, 1H), 5.27 (d, J = 1.5 Hz, 1H), 5.21 (dq, J = 10.4, 1.3 Hz, 1H), 4.78 (s, 1H), 4.57 (d, J = 5.6 Hz, 2H), 3.26 – 3.17 (m, 4H), 1.97 (d, J = 1.4 Hz, 3 H), 1.63 (m, 2H), 1.58 (m, 2H), 1.43 – 1.37 (m, 2H), 1.35 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.31, 156.49, 156.36, 147.47, 143.86, 133.00, 129.66, 126.17, 122.15, 119.97, 117.54, 109.00, 108.11, 96.83, 65.73, 65.44, 57.77, 44.61, 40.93, 29.90, 29.69, 29.23, 27.49, 24.27. ESI-MS: m/z = $[M+H]^+$ calculated 411.22, found 411.30.



7

To a solution of compound **6** (100 mg, 0.2439 mmol) in DCM (15 mL) was added $Pd(PPh_3)_4$ (5.64 mg, 0.00487 mmol). The reaction requires was refluxed under N_2. And then phenylsilane (60μL, 0.4878 mmol) was added by injection. The solution was stirred at room temperature. After completion as shown by TLC analysis, the reaction injected with TEA (103 μL, 0.74 mmol), di-tert-butyl dicarbonate (67 μL,0.29 mmol) at 0 °C. The cooling bath was removed, and the reaction mixture was allowed to warm up to room temperature. After completion as shown by TLC analysis, the reaction was quenched with H_2O, extracted with EtOAc, washed sequentially with H_2O and brine. The organic layer was then dried over anhydrous sodium sulfate and concentrated by rotary evaporation under reduced pressure. The target compound **7** was isolated as a yellow oil (100 mg, 96 % yield) by flash column chromatography on a silica gel column by eluted with PE : EA (6 : 1).

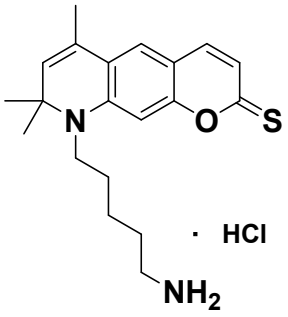
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (d, J = 9.3 Hz, 1H), 6.99 (s, 1H), 6.24 (s, 1H), 6.01 (d, J = 9.3 Hz, 1H), 5.26 (d, J = 1.5 Hz, 1H), 4.60 (s, 1H), 3.24 – 3.18 (m, 2H), 3.14 (q, J = 6.7 Hz, 2H), 1.97 (d, J = 1.4 Hz, 3H), 1.61 (m, 2H), 1.57 – 1.51 (m, 2H), 1.44 (s, 9H), 1.40 – 1.35 (m, 2H), 1.34 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.33, 156.51, 156.04, 147.50, 143.84, 129.67, 126.18, 122.13, 119.97, 109.04, 108.10, 96.88, 79.15, 58.43, 57.78, 44.66, 40.49, 30.02, 29.24, 28.44, 27.52, 24.35, 18.75. ESI-MS: m/z = $[M+H]^+$ calculated 427.25, found 427.30.



8

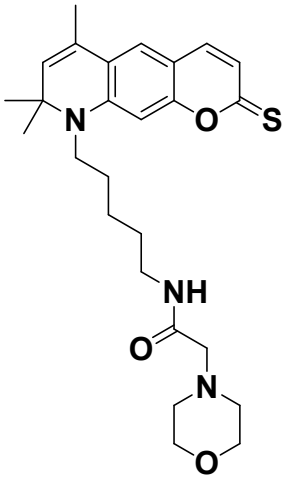
To a solution of compound **7** (800 mg, 1.88 mmol) in toluene (30 mL) was added lawesson's reagent (1.14 g，2.817 mmol). The reaction requires was refluxed at 80 °C under N_2. After completion as shown by TLC analysis, the target compound **8** was isolated as a yellow powder (520 mg, 62 % yield) by flash column chromatography on a silica gel column by eluted with PE : DCM : EA (45 : 5 : 3).

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (d, J = 8.8 Hz, 1H), 7.02 (s, 1H), 6.95 (d, J = 8.9 Hz, 1H), 6.42 (s, 1H), 5.33 (d, J = 1.5 Hz, 1H), 5.29 (s, 1H), 3.27 – 3.20 (m, 2H), 3.15 (t, J = 7.0 Hz, 2H), 1.98 (d, J = 1.3 Hz, 3H), 1.63 (m, 2H), 1.57 – 1.49 (m, 2H), 1.45 (s, 9H), 1.40 (m, 2H), 1.37 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 197.11, 159.87, 148.45, 136.37, 130.85, 126.13, 123.28, 122.04, 121.55, 111.01, 96.59, 58.33, 53.57, 45.07, 30.11, 29.83, 29.50, 28.57, 27.52, 24.44, 18.86. ESI-MS: m/z = $[M+H]^+$ calculated 443.23, found 443.25.



9

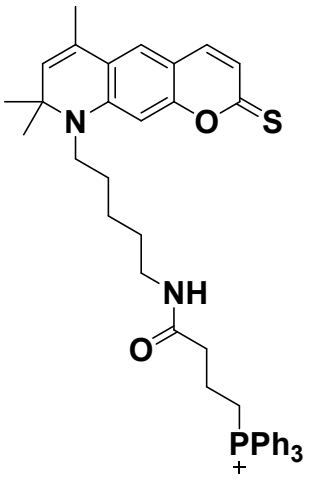
Compound **8** (500 mg, 1.13 mmol) was dissolved in EA/HCl (2 mol/L, 20 mL). After completion as shown by TLC analysis, the solution was concentrated by rotary evaporation under reduced pressure to get target compound **9** as a red powder, 100% yield. ESI-MS: m/z = $[M+H]^+$ calculated 343.17, found 343.30.



FA-Lyso-S

To a solution of compound **9** (110 mg, 0.337 mmol) and 2-morpholinoacetic acid (59 mg, 0.404 mmol) in DCM (15 mL) was added HOBT (68 mg, 0.505 mmol), EDCI (164 mg, 0.842 mmol). The solution was stirred at room temperature. After completion as shown by TLC analysis, the reaction was quenched with H_2O, extracted with DCM, washed sequentially with H_2O and brine. The organic layer was then dried over anhydrous sodium sulfate and concentrated by rotary evaporation under reduced pressure. The target compound **FA-Lyso-S** was isolated as a yellow oil (80 mg, 50 % yield) by flash column chromatography on a silica gel column by eluted with DCM : EA (1 : 3).

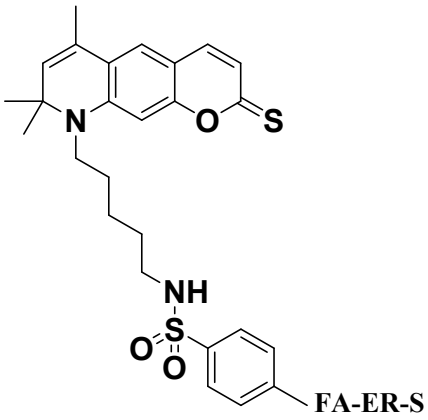
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (d, J = 8.9 Hz, 1H), 7.02 (s, 1H), 6.95 (d, J = 8.9 Hz, 1H), 6.40 (s, 1H), 5.33 (d, J = 1.5 Hz, 1H), 3.76 (t, J = 4.6 Hz, 4H), 3.66 (s, 7H), 3.32 (q, J = 6.8 Hz, 2H), 3.27 – 3.21 (m, 2H), 3.11 (s, 2H), 2.63 (d, J = 5.5 Hz, 4H), 2.35 – 2.29 (m, 4H), 1.98 (d, J = 1.4 Hz, 3H), 1.69 – 1.64 (m, 6H), 1.64 – 1.55 (m, 4H), 1.41 (m, 2H), 1.38 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 196.96, 173.80, 159.72, 148.28, 136.26, 130.71, 126.00, 123.19, 121.93, 121.43, 110.90, 96.43, 66.69, 61.71, 58.22, 51.58, 44.89, 38.97, 33.69, 29.71, 27.32, 24.38, 18.74. ESI-MS: m/z = $[M+H]^+$ calculated 470.24, found 470.26.



FA-Mito-S

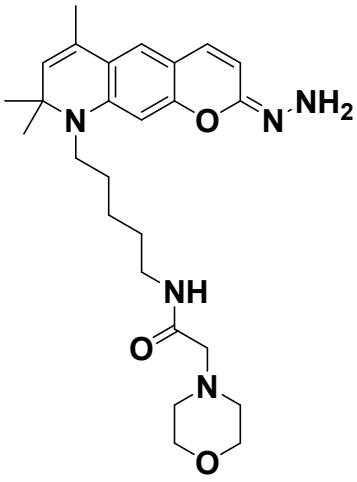
The synthesis method is the same as that for compound **FA-Lyso-S**. The target compound **FA-Mito-S** was isolated as a yellow oil (115 mg, 44 % yield) by flash column chromatography on a silica gel column by eluted with DCM : MeOH (10 : 1).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.52 (s, 1 H), 7.82 – 7.72 (m, 9 H), 7.69 (td, J = 7.6, 3.5 Hz, 6 H), 7.27 (s, 1 H), 7.00 (s, 1 H), 6.92 (d, J = 8.9 Hz, 1 H), 6.38 (s, 1 H), 5.32 (d, J = 1.5 Hz, 1 H), 3.65 (s, 2 H), 3.34 – 3.23 (m, 4 H), 2.83 (s, 2 H), 2.04 – 1.98 (m, 2H)1.97 (d, J = 1.3 Hz, 3 H), 1.71 – 1.59 (m, 4H), 1.45 (m, 2 H), 1.38 (s, 6 H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.11, 159.82, 148.51, 136.31, 135.18, 135.15, 133.63, 133.55, 130.99, 130.64, 130.54, 125.78, 122.88, 121.79, 121.47, 118.49, 117.80, 110.78, 96.41, 58.32, 45.05, 39.31, 29.70, 29.50, 29.10, 27.23, 24.53, 18.74. ESI-MS: m/z = $[M]^+$ calculated 673.3, found 673.26.



To a solution of compound **8** (95 mg, 0.25 mmol) in DCM (10 mL) was added TEA (104 μL, 0.75 mmol) and tosyl chloride (48 mg, 0.25 mmol). The solution was stirred at room temperature. After completion as shown by TLC analysis, the reaction was quenched with H_2O, extracted with DCM, washed sequentially with H_2O and brine. The organic layer was then dried over anhydrous sodium sulfate and concentrated by rotary evaporation under reduced pressure. The target compound **FA-ER-S** was isolated as a yellow oil (80 mg, 64 % yield) by flash column chromatography on a silica gel column by eluted with PE : EA (8 : 1).

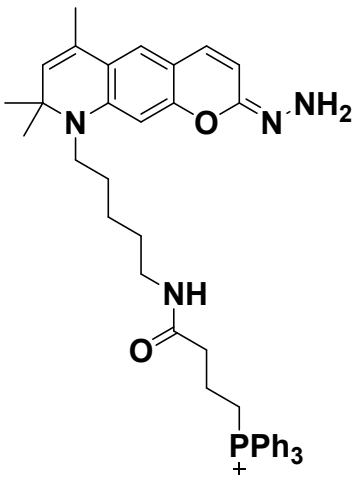
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 – 7.75 (m, 2H), 7.33 (d, J = 8.0 Hz, 2H), 7.28 (d, J = 8.9 Hz, 1H), 7.02 (s, 1H), 6.96 (d, J = 8.9 Hz, 1H), 6.37 (s, 1H), 5.34 (d, J = 1.5 Hz, 1H), 4.49 (t, J = 6.3 Hz, 1H), 3.25 – 3.17 (m, 2H), 2.98 (q, J = 6.8 Hz, 2H), 2.43 (s, 3H), 1.98 (d, J = 1.4 Hz, 3H), 1.60 (t, J = 4.1 Hz, 2H), 1.57 – 1.52 (m, 2H), 1.43 – 1.37 (m, 2H), 1.37 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 196.96, 159.70, 148.25, 143.48, 136.89, 136.24, 130.72, 129.79, 127.10, 125.99, 123.21, 121.93, 121.43, 110.90, 96.41, 58.21, 44.78, 43.10, 29.52, 29.37, 27.18, 24.02, 21.56, 18.74. ESI-MS: m/z = $[M+H]^+$ calculated 496.18, found 496.18.



FA-Lyso

To a solution of compound **FA-Lyso-S** (50 mg, 0.106 mmol) in CH_3OH (5 mL) was added hydrazine hydrate (36.6 μL, 0.53 mmol, 85%). The solution was stirred at room temperature. After completion as shown by TLC analysis, the reaction was concentrated by rotary evaporation under reduced pressure. the reaction was extracted with DCM, washed sequentially with H_2O and brine. The organic layer was then dried over anhydrous sodium sulfate and concentrated by rotary evaporation under reduced pressure to give target compound **FA-Lyso** as a yellow oil (42 mg, 84 % yield) which was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.72 (s, 1H), 6.56 (d, *J* = 9.7 Hz, 1H), 6.08 (s, 1H), 5.90 (d, *J* = 9.7 Hz, 1H), 5.12 (s, 1H), 3.65 (q, *J* = 4.5 Hz, 4H), 3.25 (q, *J* = 6.8 Hz, 2H), 3.16 – 3.07 (m, 2H), 2.94 (s, 2H), 2.46 (m, 2H), 2.26 (m, 2H), 1.88 (s, 3H), 1.53-1.54 (m, 4H), 1.34-1.36 (m, 2H), 1.24 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.91, 169.93, 154.40, 146.31, 145.74, 128.71, 128.51, 126.93, 122.02, 113.74, 108.77, 97.39, 67.12, 62.16, 57.40, 51.70, 44.46, 39.02, 29.86, 28.90, 28.10, 24.50, 18.89. HRMS: m/z = $[M+H]^+$ calculated 468.2896, found 468.2973.

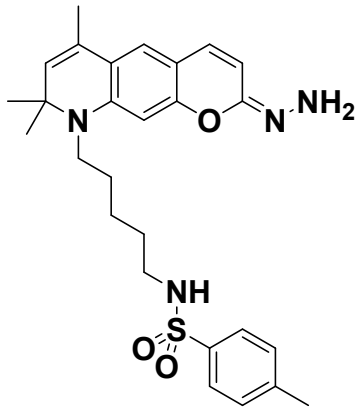


FA-Mito

The synthesis method is the same as that for compound **FA-Lyso**. The target compound **FA-Mito**

was concentrated by rotary evaporation under reduced pressure as a yellow oil (77 mg, 91 % yield) which was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (s, 1 H), 7.82 – 7.66 (m, 15 H), 6.76 (s, 1 H), 6.59 (d, J = 9.7 Hz, 1 H), 6.20 (s, 1 H), 5.93 (d, J = 9.6 Hz, 1 H), 5.16 (q, J = 1.5 Hz, 1 H), 3.69 – 3.56 (m, 2 H), 3.30 (q, J = 6.7 Hz, 2 H), 3.21 – 3.14 (m, 2 H), 2.81 (dt, J = 6.5, 3.3 Hz, 2 H), 2.02 – 1.97 (m, 2 H), 1.94 (d, J = 1.4 Hz, 3 H), 1.66 (m, 2 H), 1.61 (m, 2 H), 1.46 (m, 2 H), 1.30 (s, 6 H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.22, 154.51, 145.90, 135.28, 135.26, 133.68, 133.60, 130.73, 130.63, 128.54, 126.78, 121.82, 118.61, 118.48, 117.92, 113.51, 108.52, 97.51, 60.54, 57.39, 44.68, 39.44, 35.84, 29.26, 29.01, 28.03, 24.65, 21.84, 21.44, 19.50, 18.91, 14.33. HRMS: m/z = $[M]^+$ calculated 671.3509, found 671.3536.



FA-ER

The synthesis method is the same as that for compound **FA-Lyso**. The target compound **FA-ER** was concentrated by rotary evaporation under reduced pressure as a yellow oil (80 mg, 88 % yield) which was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 – 7.71 (m, 2H), 7.35 – 7.28 (m, 2H), 6.78 (s, 1H), 6.62 (d, J = 9.7 Hz, 1H), 6.11 (s, 1H), 5.96 (d, J = 9.7 Hz, 1H), 5.18 (q, J = 1.5 Hz, 1H), 4.53 (t, J = 6.3 Hz, 1H), 3.20 – 3.11 (m, 2H), 2.97 (q, J = 6.7 Hz, 2H), 2.42 (s, 3H), 1.94 (d, J = 1.4 Hz, 3H), 1.60 – 1.52 (m, 4H), 1.45 – 1.36 (m, 2H), 1.29 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.40, 146.29, 145.69, 143.58, 137.11, 129.89, 128.68, 128.52, 127.21, 126.92, 122.02, 118.74, 113.77, 108.78, 97.36, 57.40, 44.32, 43.19, 29.84, 29.63, 28.90, 27.86, 24.16, 21.66, 18.90. HRMS: m/z = $[M+H]^+$ calculated 494.2352, found 494.2429.

Probe Selectivity Test

Species including (1) black, (2) formaldehyde, (3) H_2O_2, (4) HClO, (5) acetaldehyde, (6) glyoxal, (7) methylglyoxal, (8) p-methoxybenzaldehyde, (9) L-(-)-malic acid, (10) ascorbic acid, (11) chloral hydrate, (12) succinic acid, (13) oxalic acid, (14) acetone, (15) 2-Ketoglutaric acid, (16) 4-Nitrobenzaldehyde, (17) GSH

Formaldehyde, H_2O_2, HClO, acetaldehyde, glyoxal, methylglyoxal, L-(-)-malic acid, ascorbic acid, chloral hydrate, succinic acid, oxalic acid, acetone, 2-Ketoglutaric acid, 4-Nitrobenzaldehyde, GSH were prepared by directly dissolving in deionized water to make 20 mM stock solutions. p-methoxybenzaldehyde was dissolved in DMSO (20 mM) to make a stock solution.

Probe (10 μM) was first treated with various biologically relevant analytes (200 μM, 30 min; except for H_2O_2, HClO (50μM), 30min). The fluorescence spectra were recorded after incubation of 30 min. Then, the solutions were treated with formaldehyde (200 μM). The fluorescence spectra were then recorded after a further incubation of 30 min.

Cytotoxicity Assay

HeLa cells were seeded in 96-well plates at a density of 5×10^3 cells/well at 37 °C in a 95% humidified atmosphere with 5% CO_2 for 24 h. After washing with PBS twice, PFM-Mito, PFM-ER and PFM-Lyso with concentration of 1 μM, 5 μM, 10 μM, 20 μM and 50 μM were added to the cells, which were allowed an incubation period of 24 h. After introducing 10 μL of CCK8 solution for 1 h, the absorption at 450 nm was measured by Microplate Spectrophotometer (MD I3X). Each experiment was repeated three times, and the average values were taken in analyses.

Supplementary Figures

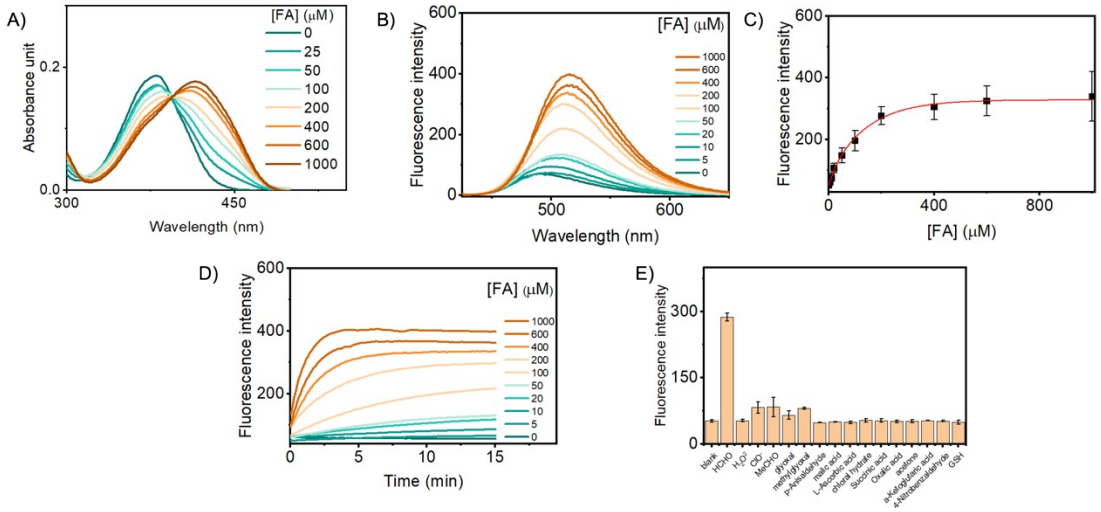


Fig. S1. Spectroscopic responses of PFM-Mito to FA. A) The UV−vis spectra of the probe (20 μM) after being treated with FA for 15 min. B) Fluorescence spectra of the probe (5 μM) after being treated with FA for 15 min. C) The plot of the probe (5 μM) emission intensity at 515 nm versus the surrounding FA concentrations. D) Reaction time courses as indicated by changes of λem 515 nm intensity of the probe (5 μM) with FA of indicated concentrations. E) Fluorescent responses of the probe (5 μM) at 515 nm toward various analytes (200 μM) after a reaction time of 15 min. Data were collected in PBS (pH 7.4, 10 mM) at ambient temperature with λex 420 nm.

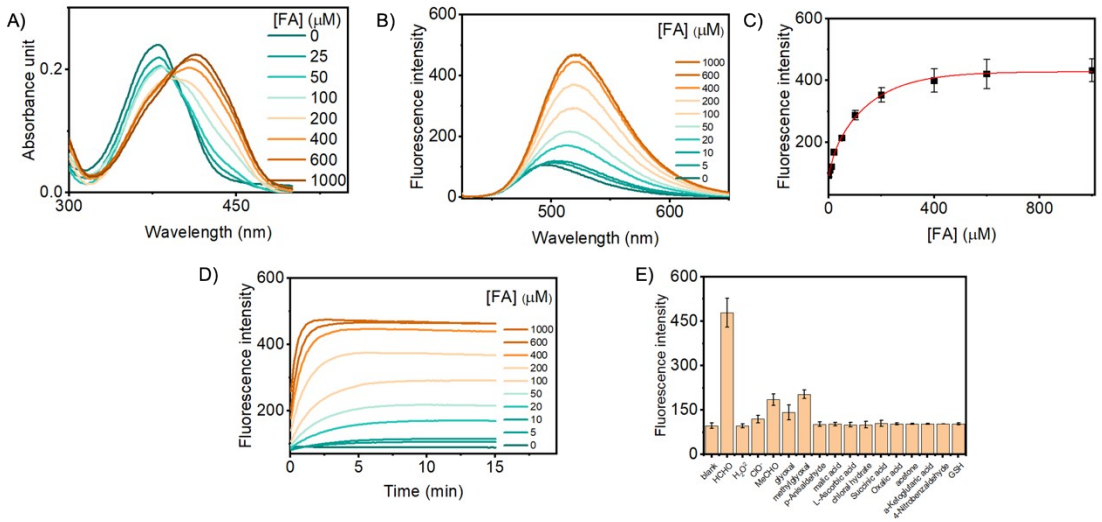


Fig. S2. Spectroscopic responses of PFM-Lyso to FA. A) The UV−vis spectra of the probe (20 μM) after being treated with FA for 15 min. B) Fluorescence spectra of the probe (5 μM) after being treated with FA for 15 min. C) The plot of the probe (5 μM) emission intensity at 515 nm versus the surrounding FA concentrations. D) Reaction time courses as indicated by changes of λem 515 nm intensity of the probe (5 μM) with FA of indicated concentrations. E) Fluorescent responses of the probe (5 μM) at 515 nm toward various analytes (200 μM) after a reaction time of 15 min. Data were collected in PBS (pH 7.4, 10 mM) at ambient temperature with λex 420 nm.

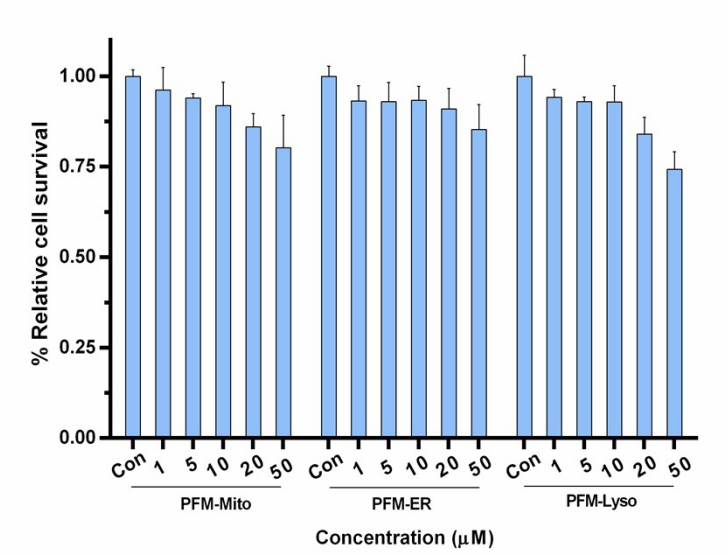


Fig. S3. Cytotoxicity of PFM-Mito, PFM-ER and PFM-Lyso was assessed in Hela cells by CCK8 assays. PFM-Mito, PFM-ER or PFM-Lyso (1 μM, 5 μM, 10 μM, 20 μM and 50 μM) were added to cells, and incubated for 24 h. After introducing CCK8 (10 μL) solution in a 95% humidified atmosphere with 5% CO_2 (37 °C) for 1 h, the absorption at 450 nm was measured by SpectraMax i3x (MD).

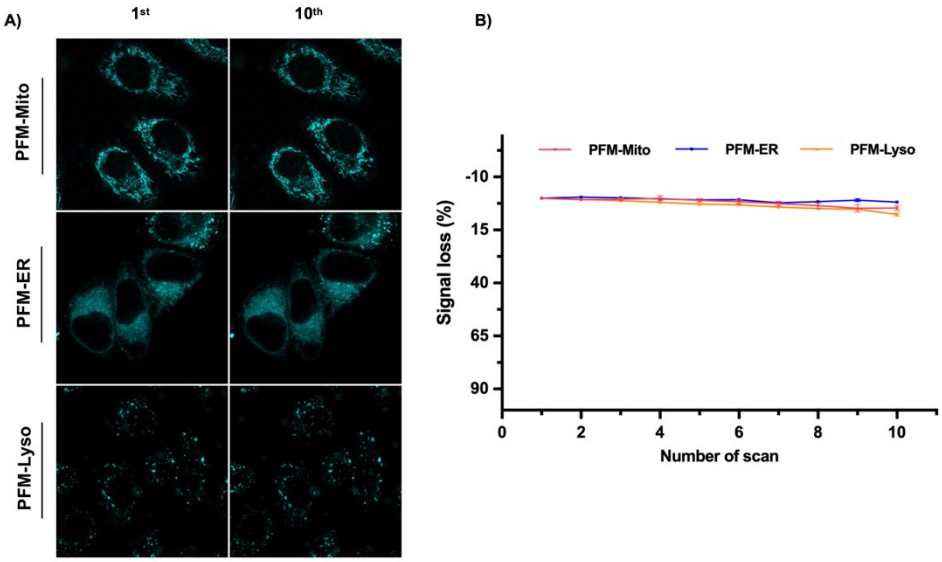
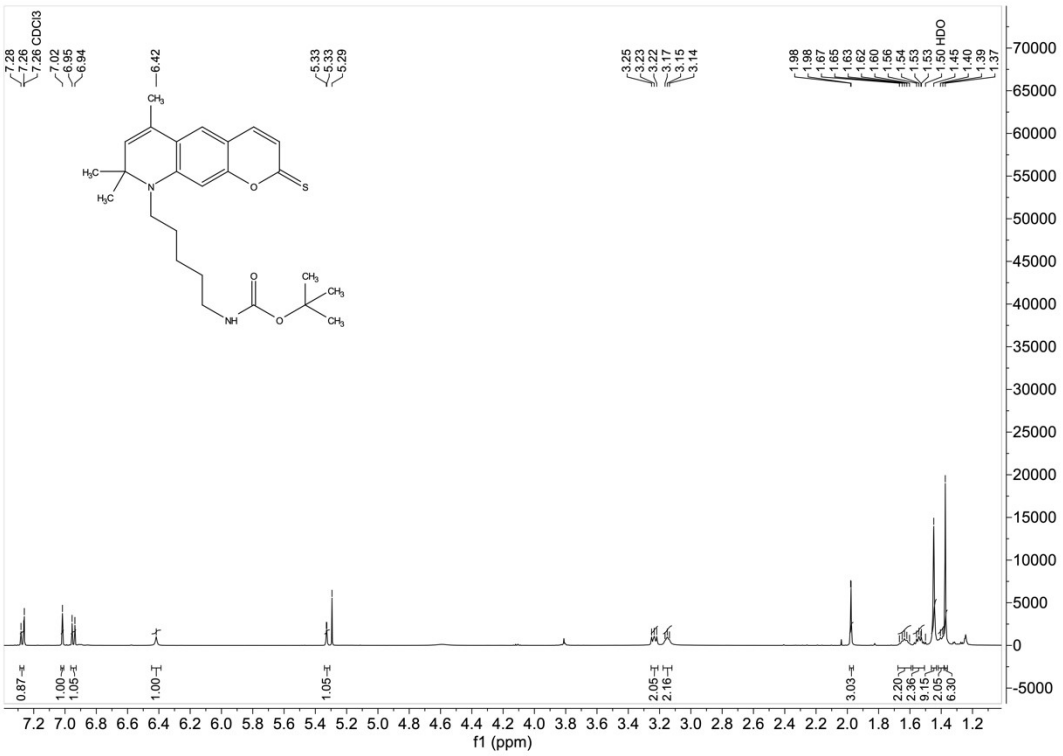
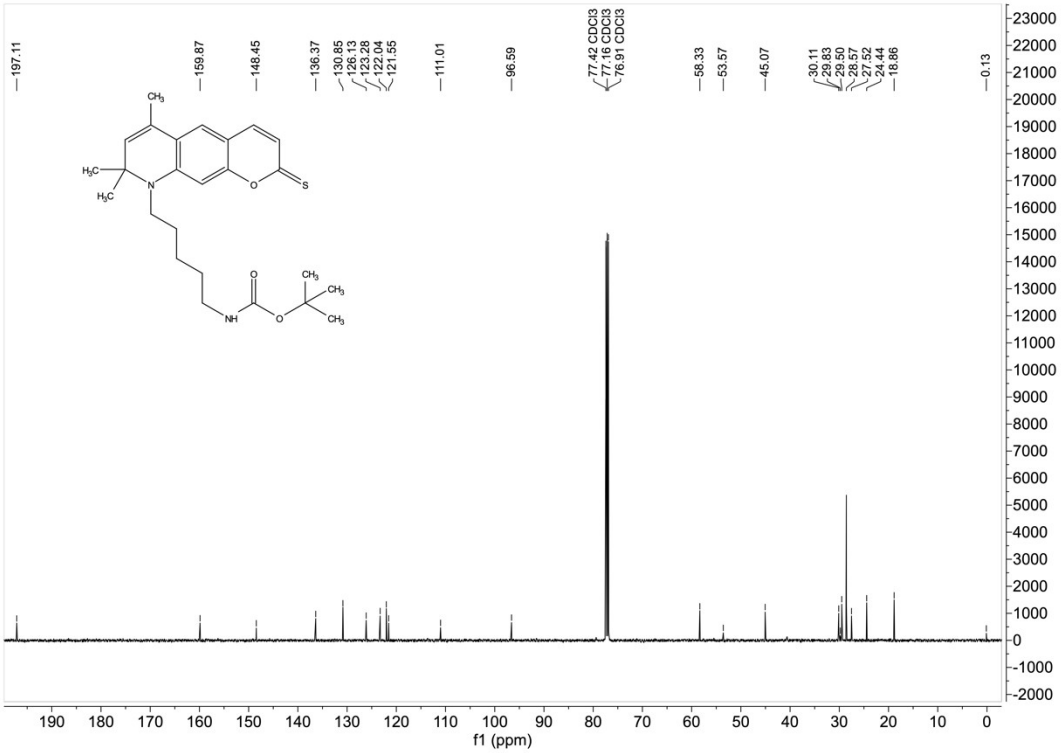


Fig. S4. Photostablity analysis of the probes in live HeLa cells. A) Confocal fluorescence images of the HeLa cells incubated with PFM-Mito (1 μM), PFM-ER (1 μM) and PFM-Lyso (1 μM) with increasing number of scans (irradiation time: 5.36 s/scan). For PFM-Mito and PFM-ER, λ_{ex}= 405 nm, λ_{em}= 500-500 nm, For PFM-Lyso, λ_{ex}= 488 nm, λ_{em}= 500-500 nm, 30 % laser power. B) Signal loss (%) of fluorescent emission of probes in HeLa cells with increasing number of scans using confocal microscope. The scan number was shown in the X-axis.

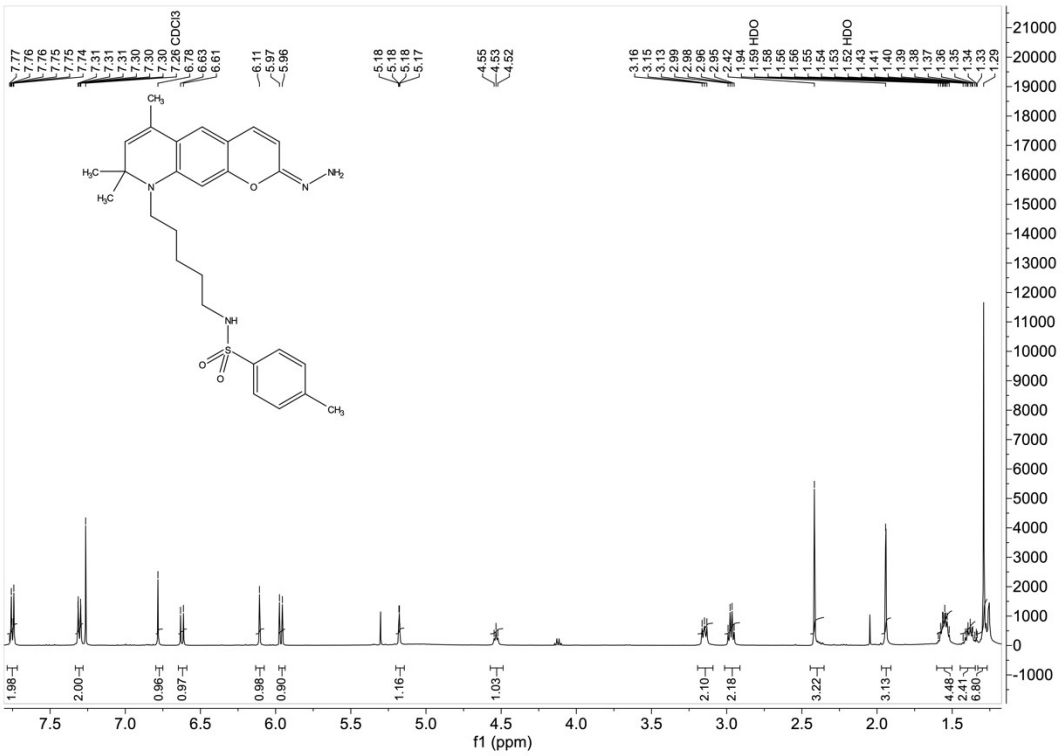
NMR Traces



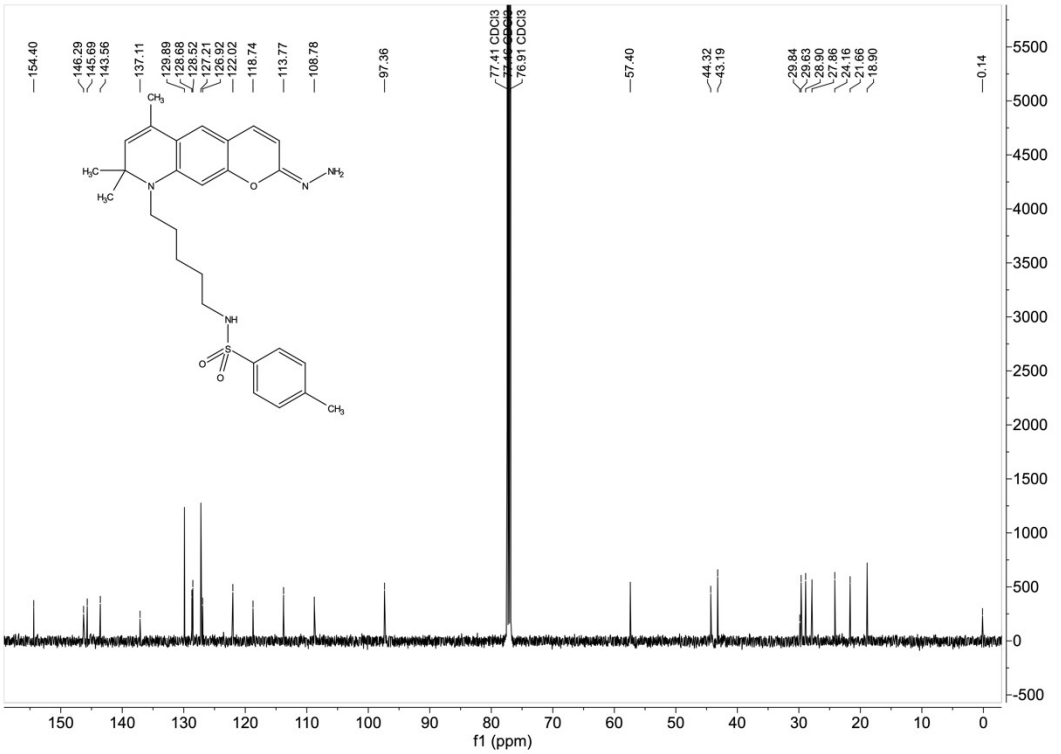
^{1}H NMR trace of the key intermediate 8.



^{13}C NMR trace of the key intermediate 8.



^{1}H NMR trace of probe PFM-ER.



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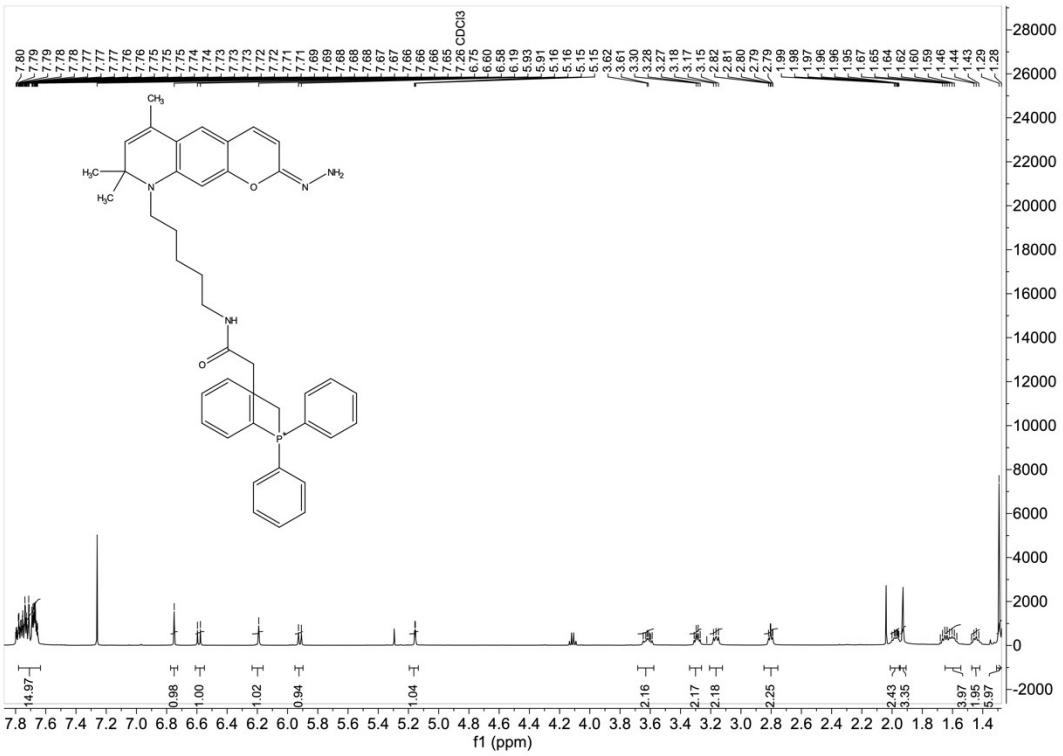
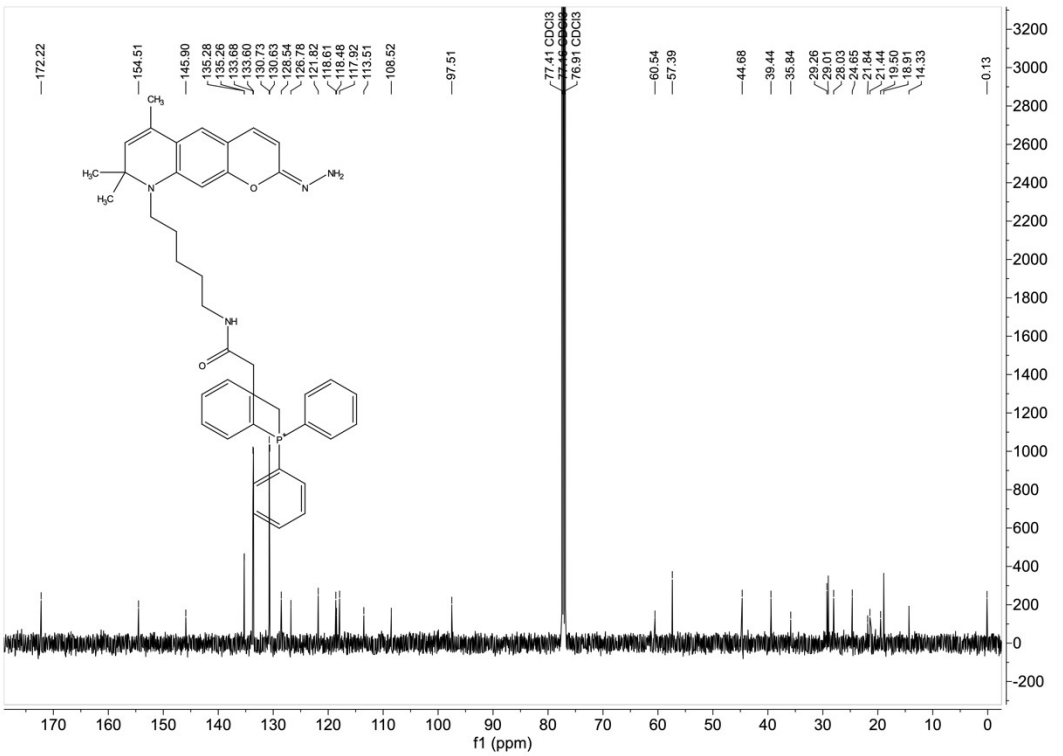


^{1}H NMR trace of probe PFM-Mito.



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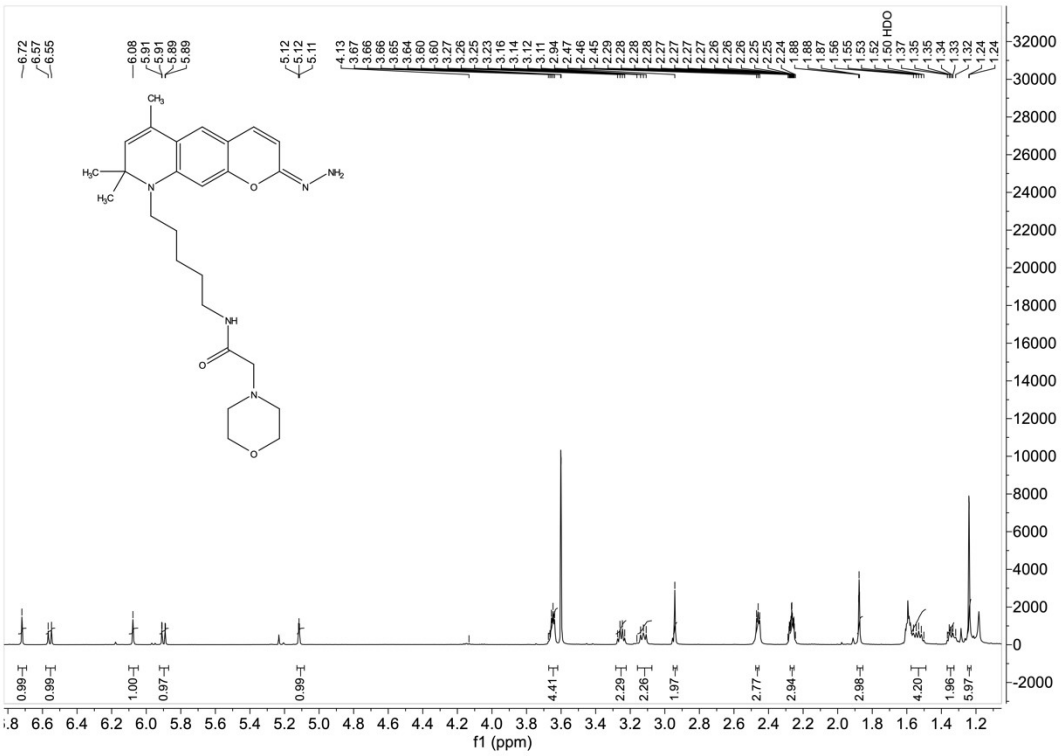
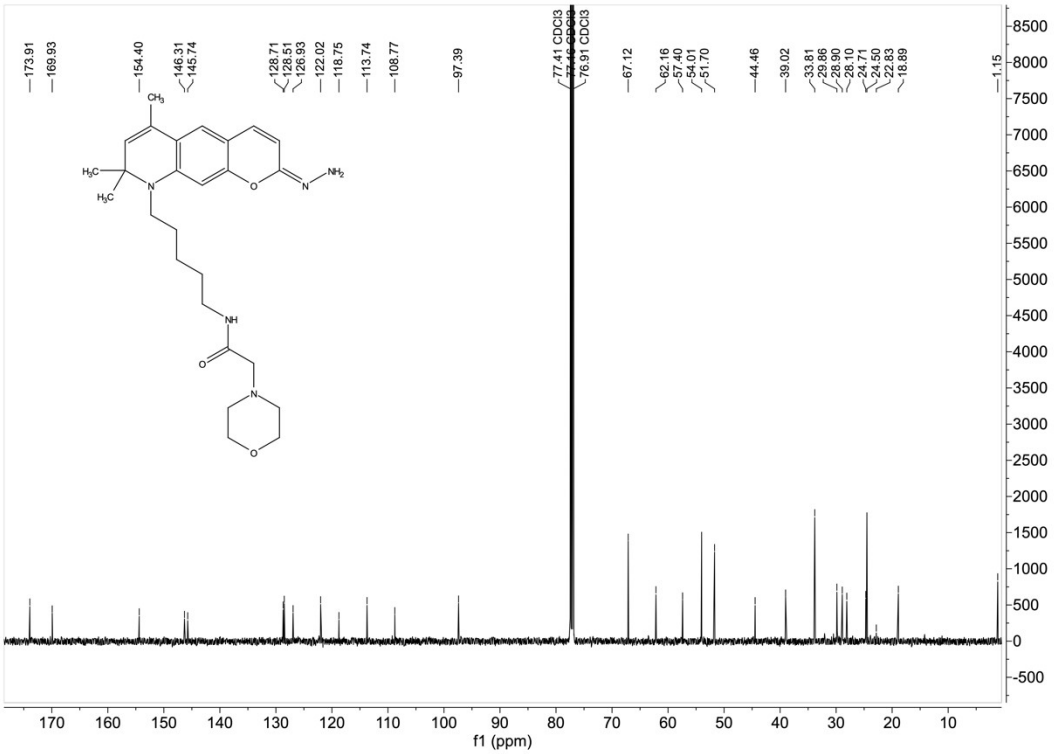


^{1}H NMR trace of probe PFM-Lyso.



^{13}C NMR trace of probe PFM-Lyso.