Supporting Information for

A Two-Photon Fluorescent Probe for Bio-imaging of Formaldehyde in Living Cells and Tissues

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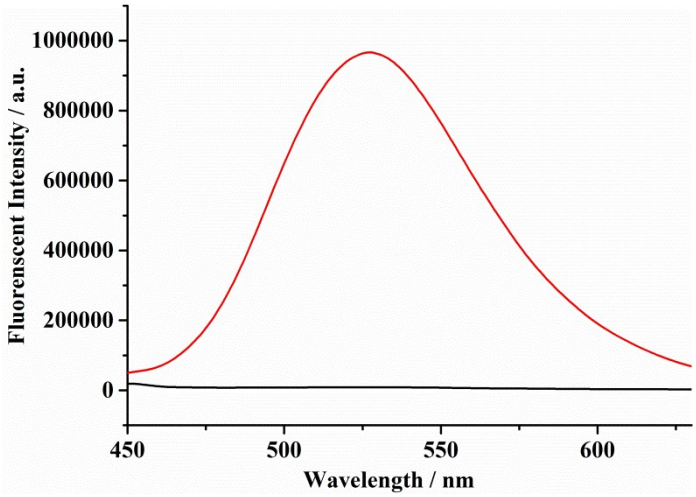


Figure S1. Fluorescence spectra of **FATP1** (10 μM) and compound **1** (10 μM). Data were acquired at 37 °C in PBS buffered (20 mM, pH 7.4) aqueous solution (H_2O/DMSO = 19:1, v/v, λ_{ex} = 390 nm). The black and red lines represent **FATP1** and compound **1**, respectively.

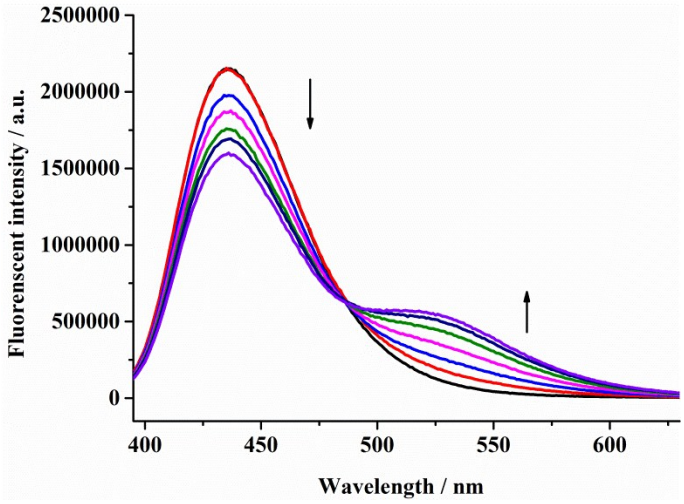


Figure S2. Fluorescence response of 10 μM compound **2** to 200 μM FA. Data were acquired at 37 °C in PBS buffered (20 mM, pH 7.4) aqueous solution (H_2O/DMSO = 19:1, v/v, λ_{ex} = 380 nm). Time points represent 0, 30, 60, 90, 120, 150, and 180 min after addition of 200 μM FA.

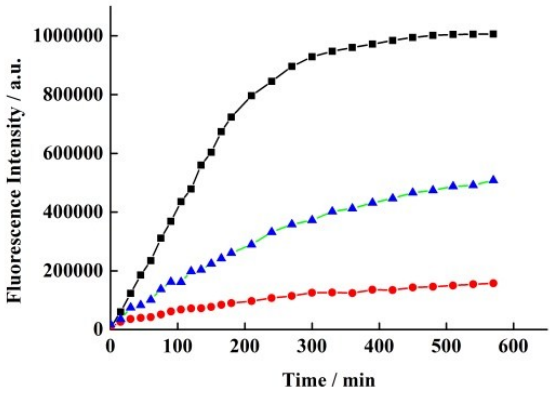


Figure S3. A plot of fluorescence intensity of **FATP1** (10 μM) vs the reaction time in the presence of varied concentrations of FA, (from bottom to top): 100, 200 and 500 μM. The measurements were performed at 37 °C in PBS buffered (20 mM, pH 7.4) aqueous solution (H_2O/DMSO = 19:1, v/v) with λ_{ex} = 390 nm, λ_{em} = 526 nm.

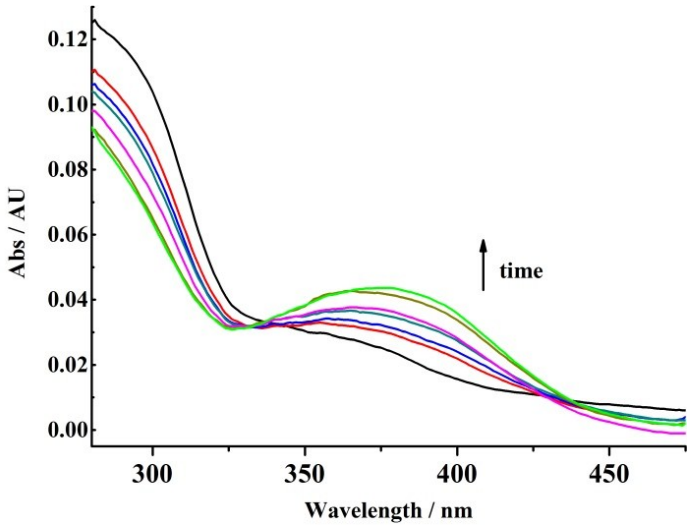


Figure S4. UV-vis response of 10 μM **FATP1** to 200 μM FA. Time points represent 0, 30, 60, 90, 120, 150 and 180 minutes after addition of 200 μM FA. Data were acquired at 37 °C in PBS buffered (20 mM, pH 7.4) aqueous solution (H_2O/DMSO = 19:1, v/v).

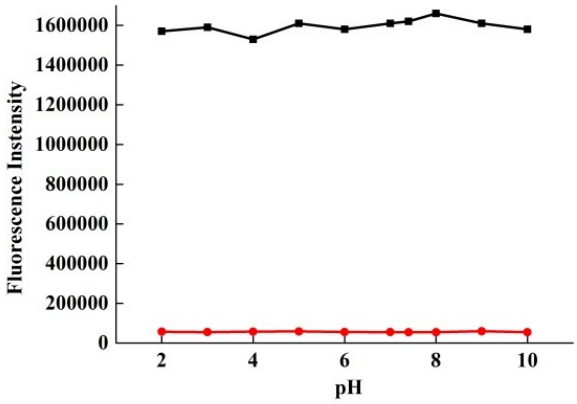


Figure S5. pH-Fluorescence profile of **FATP1** (10 μM) and compound **1** (10 μM) in PBS buffered (20 mM, pH 2.0-10.0) aqueous solution (H_2O/DMSO = 19:1, v/v), the pH were adjusted by NaOH (aq, 1 M) or HCl (aq, 1 M), λ_{ex} = 390 nm. Fluorescence responses are shown **FATP1** (red line) and compound **1** (black line) at 526 nm, respectively. All the reactions were performed at 37 °C for 3 h.

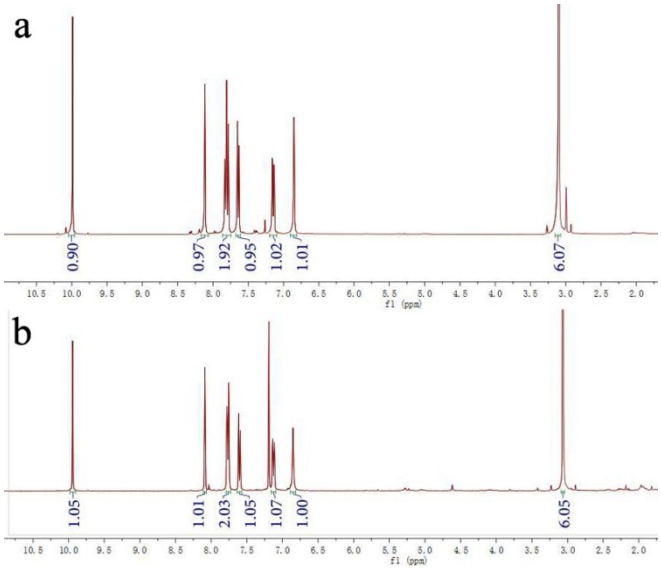


Figure S6. (a) ^{1}H NMR spectrum of the compound **1**. (b) ^{1}H NMR spectrum of the isolated product of the probe **FATP1** reacted with FA.

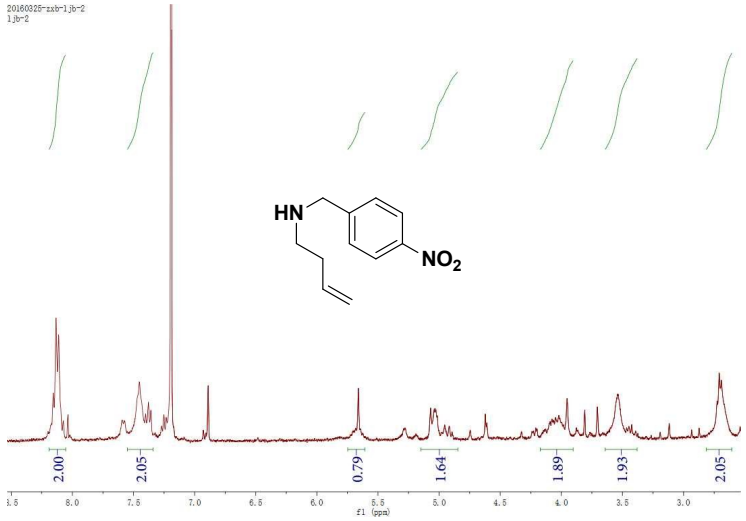


Figure S7. ^{1}H NMR spectrum of the nitrobenzyl derivative.

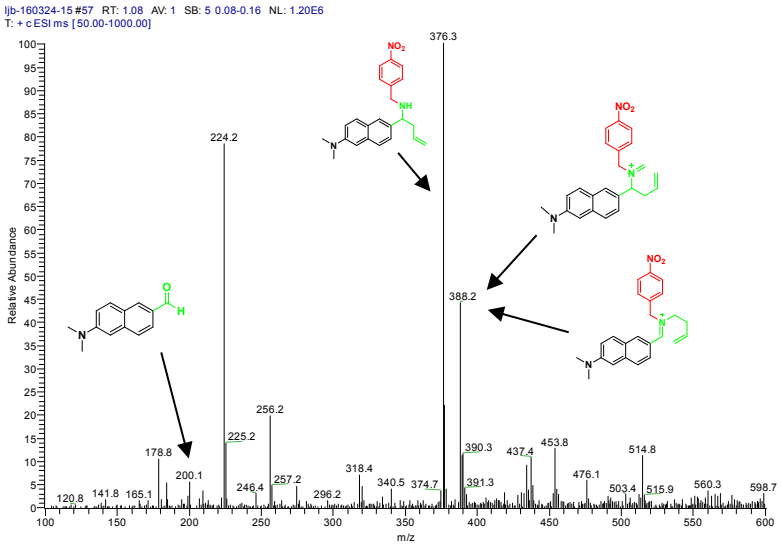


Figure S8. Mass spectrum (ESI) of the reaction mixture of the probe **FATP1** reacted with FA.

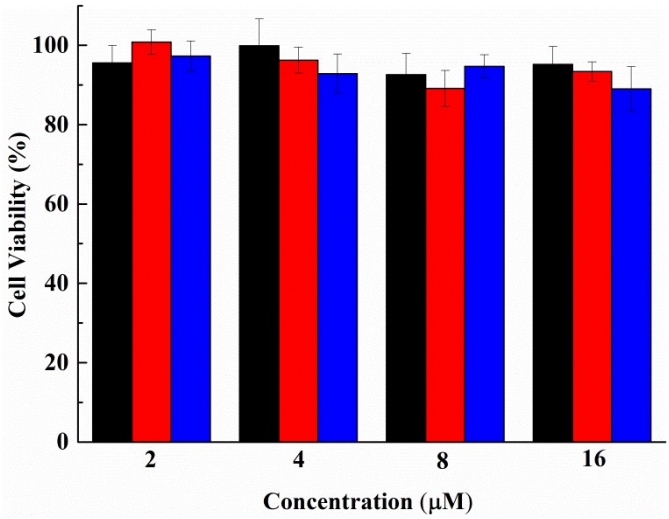


Figure S9. Cytotoxicity of **FATP1**, compound **1** and nitrobenzyl derivative against HEK-293 cells as determined by MTT assay. HEK-293 cells were treated with **FATP1** or compound **1** (2-16 μM). Black bar represents cytotoxicity of **FATP1**, red bar represents cytotoxicity of compound **1** and the blue bar represents cytotoxicity of nitrobenzyl derivative. The cells were incubated for 24 h. The results are the mean ± standard deviation of five separate measurements.

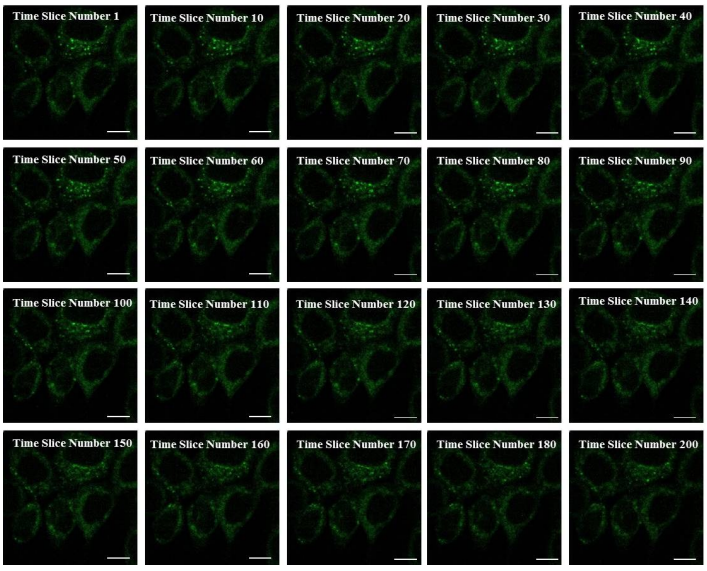


Figure S10. Photostablility analysis of **FATP1** in HEK-293 cells. Images of HEK-293 cells stained with **FATP1** (10 μM) were acquired with 200 scans. λ_{ex} = 780 nm, emission window (500-560 nm). Scale bar: 10 μm.

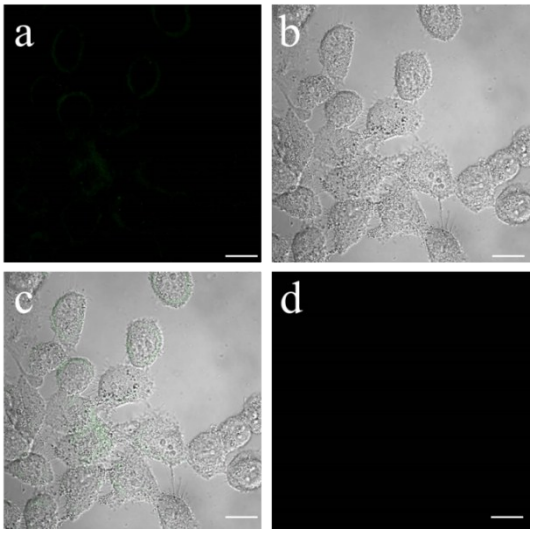
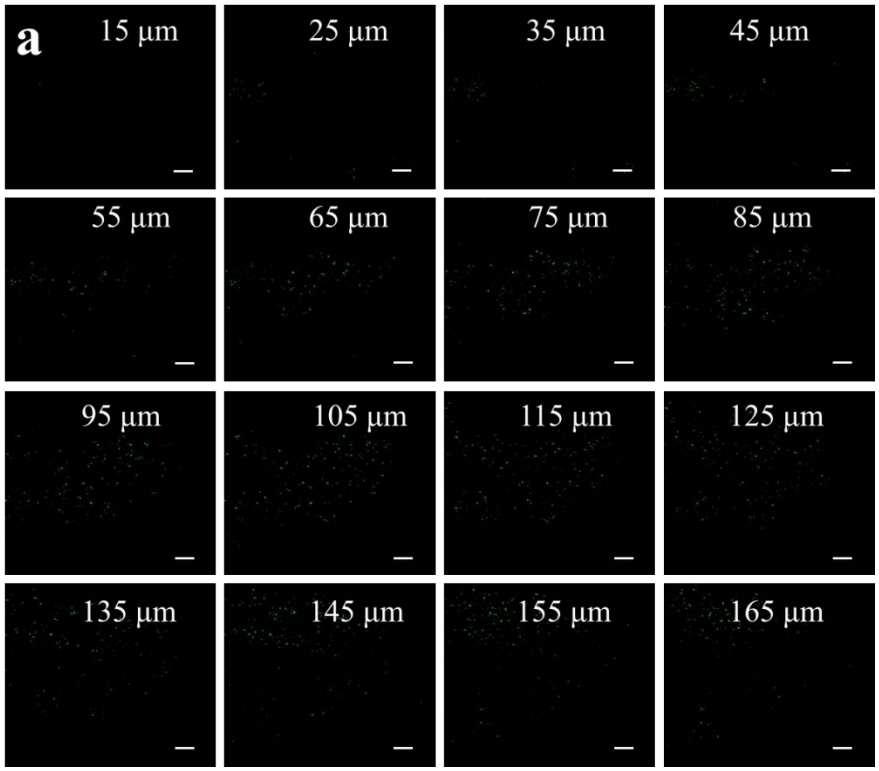


Figure S11. Confocal images of HEK-293 cells without **FATP1**. (a) Confocal fluorescence images of one-photon, (b) the differential interference contrast (DIC) images, (c) merge of fluorescence images and DIC, and (d) the fluorescence images of two-photon channel. Scale bar: 20 μm



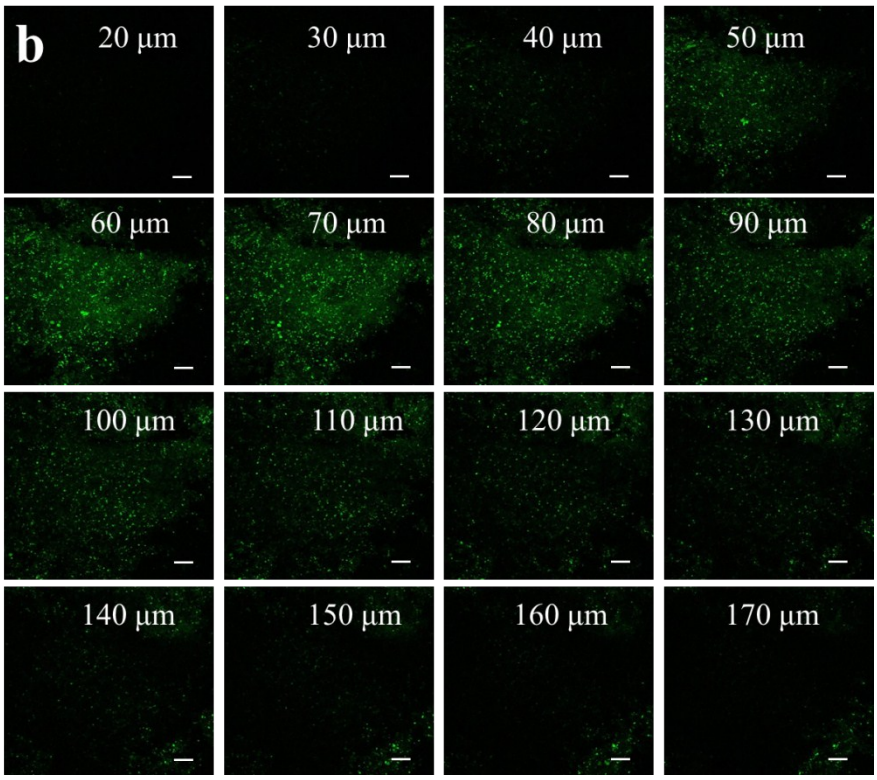
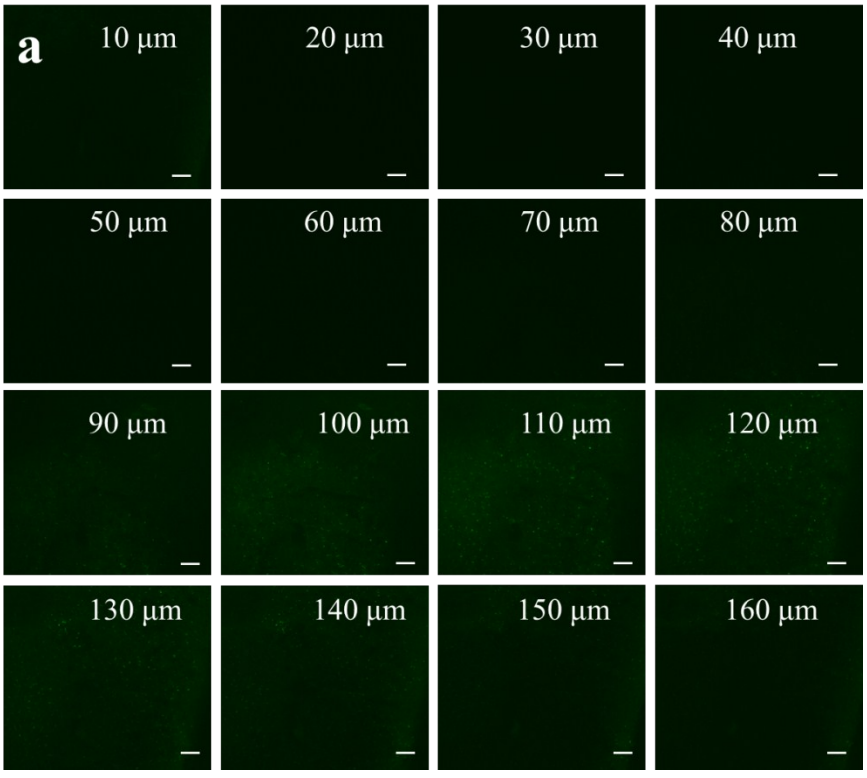


Figure S12. Depth TP fluorescence images of: (a) **FATP1** (10 μM) in tissues (0-200 μm) and (b) **FATP1** (10 μM) in tissues (0-200 μm) with FA (200 μM). Step size: 3.5 μm. Scale bars: 100 μm. λ_{ex} = 780 nm, emission window (500-560 nm).



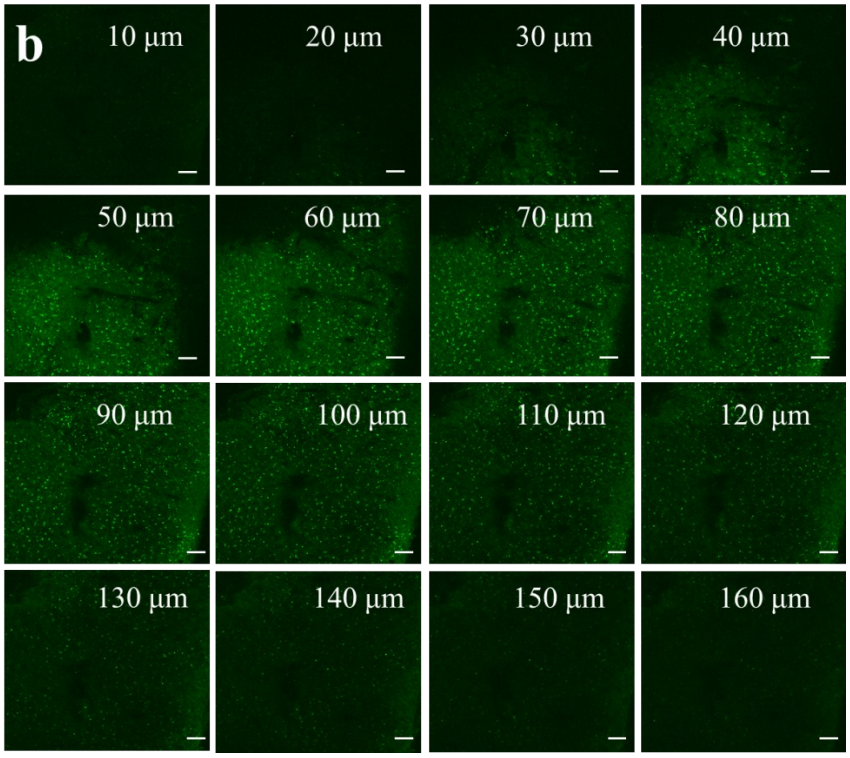
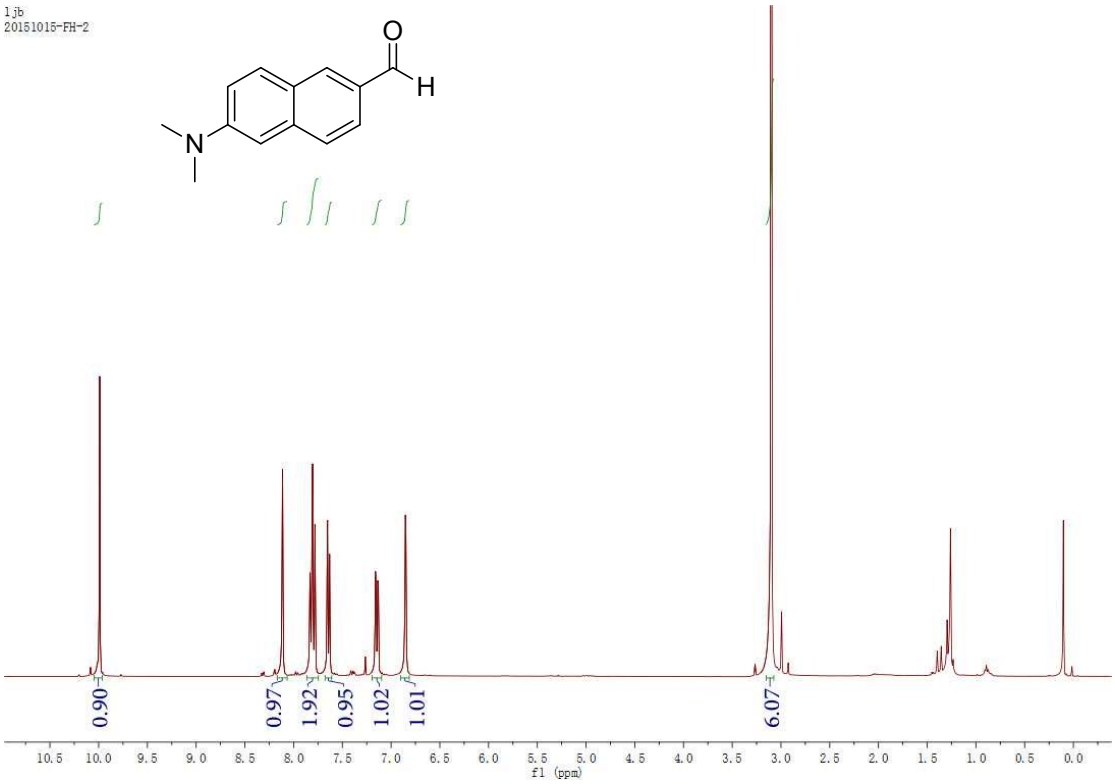


Figure S13. Depth one-photon (a) and two-photon (b) fluorescence image of a rat liver frozen slice (0-200 μm) pretreated with **FATP1** and then incubated FA. Step size: 3.5 μm. Scale bars: 100 μm. λ_{ex} = 780 nm, emission window (500-560 nm).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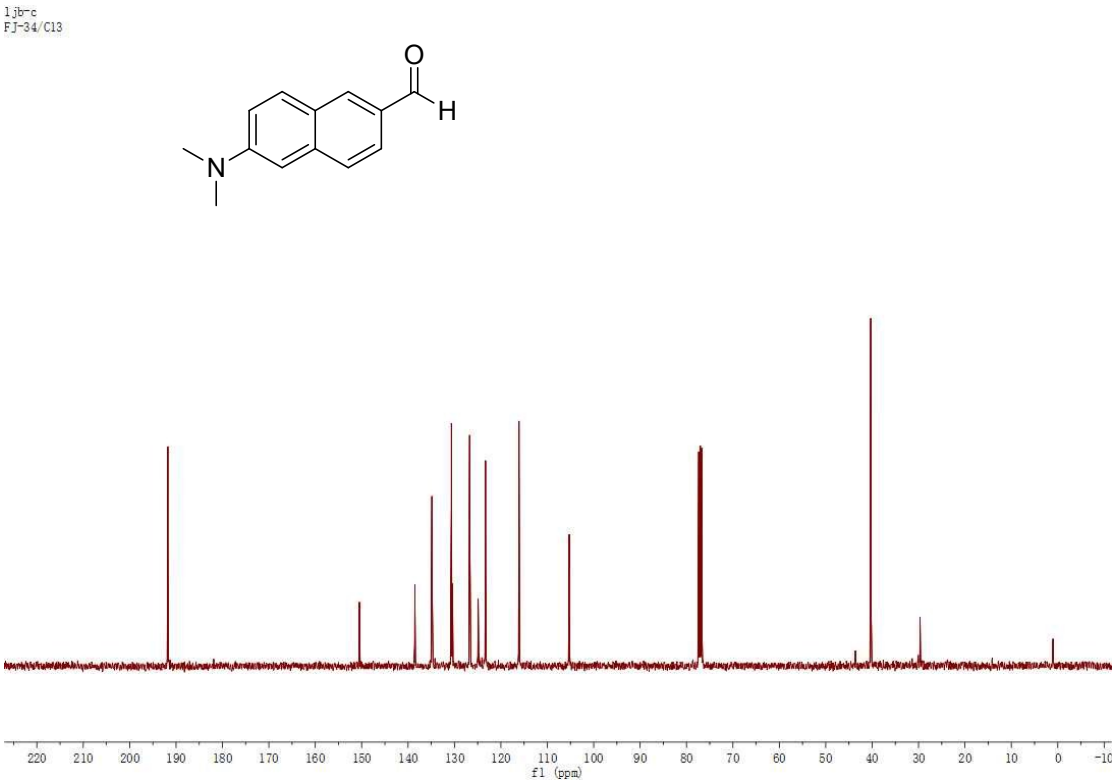
0.95
1.02
1.01
6.07
f1 (ppm)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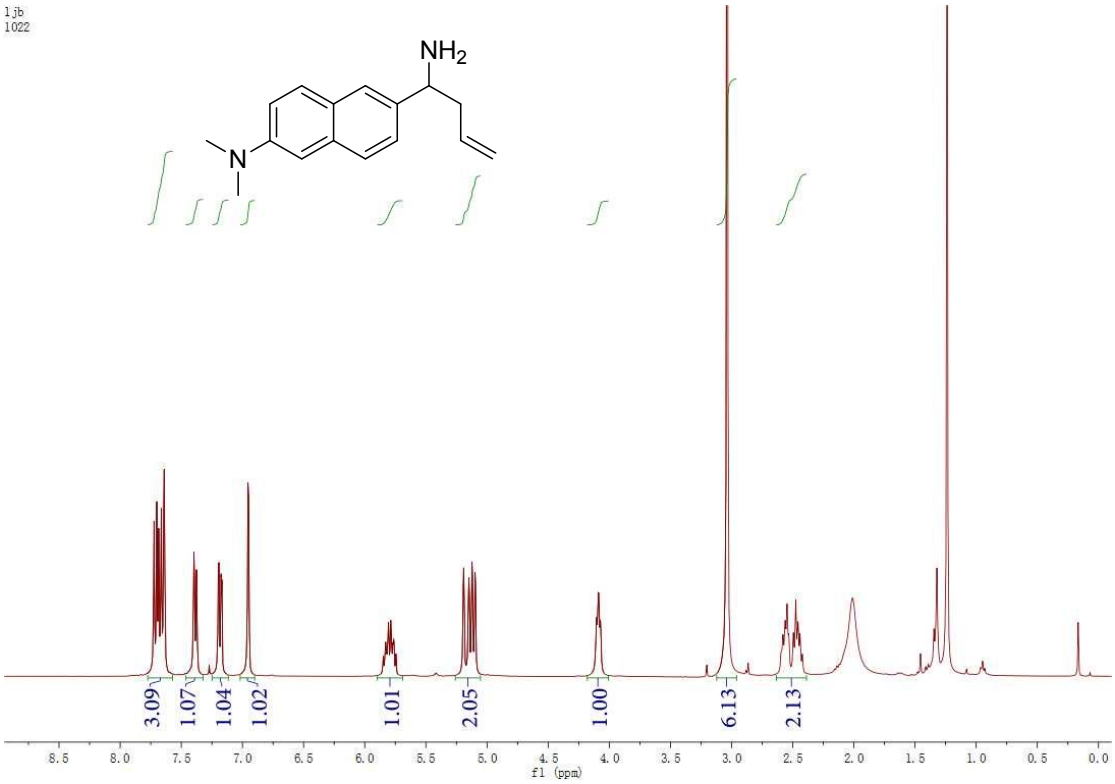
FJ-34/C13
O
H
N
f1 (ppm)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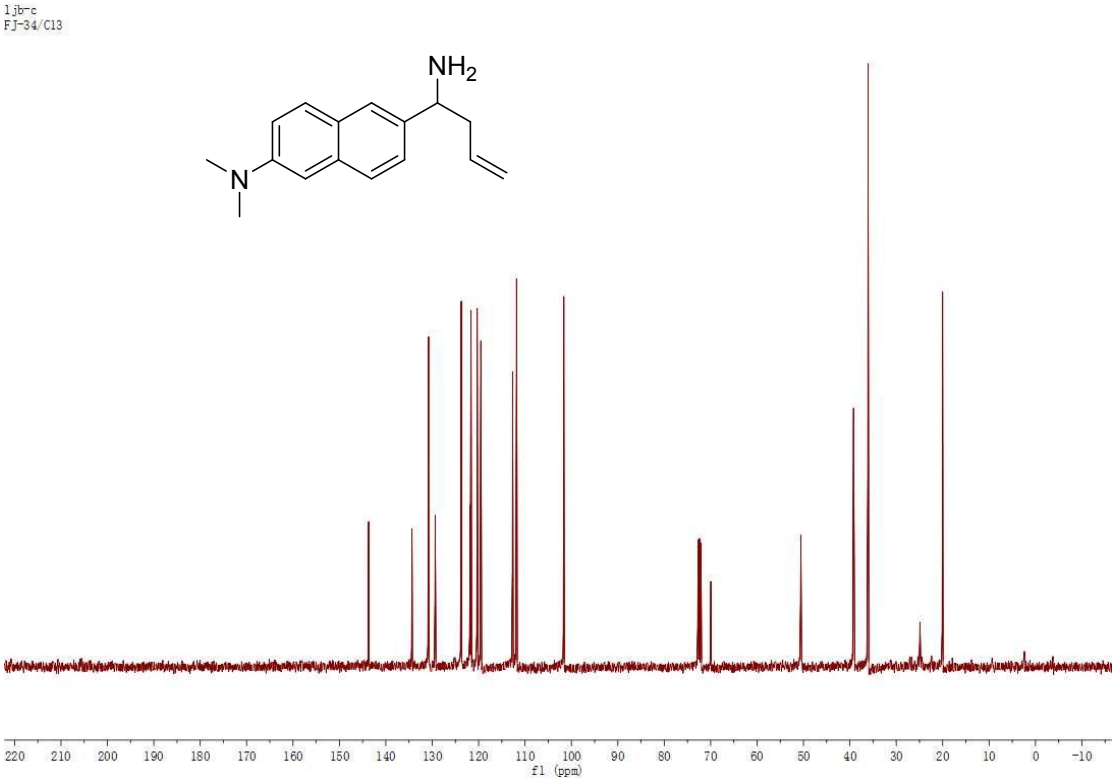
f1 (ppm)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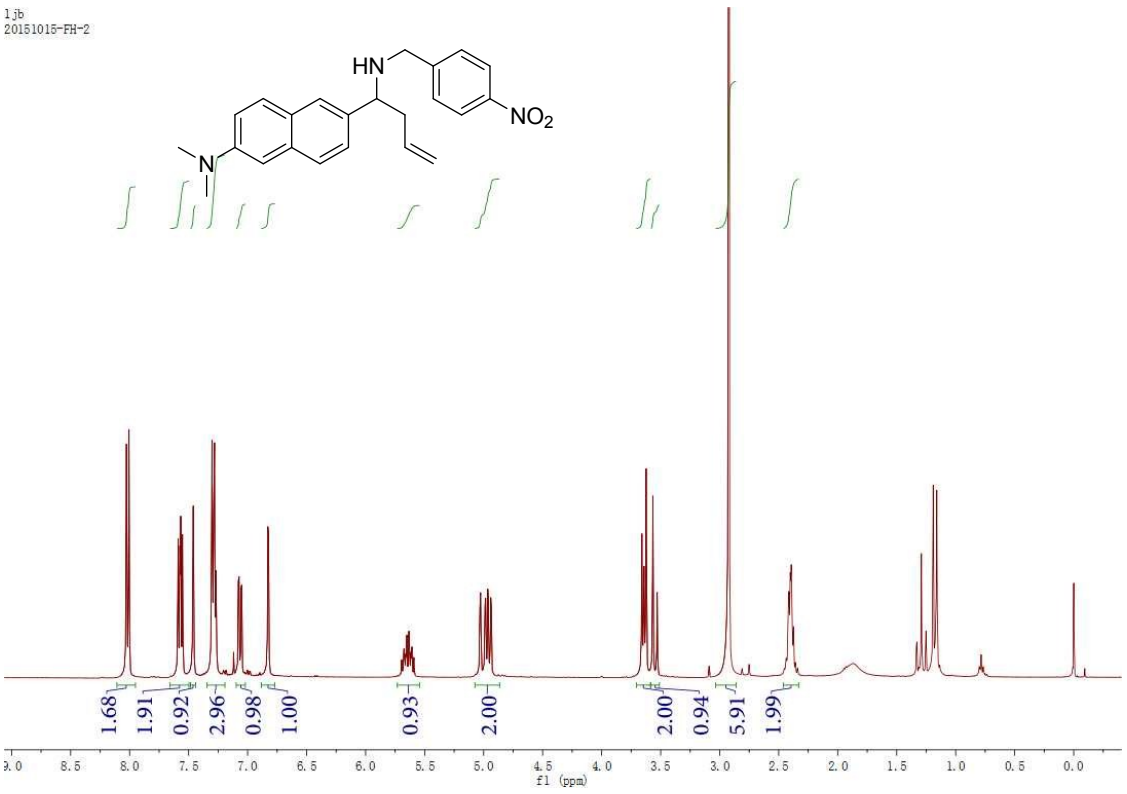
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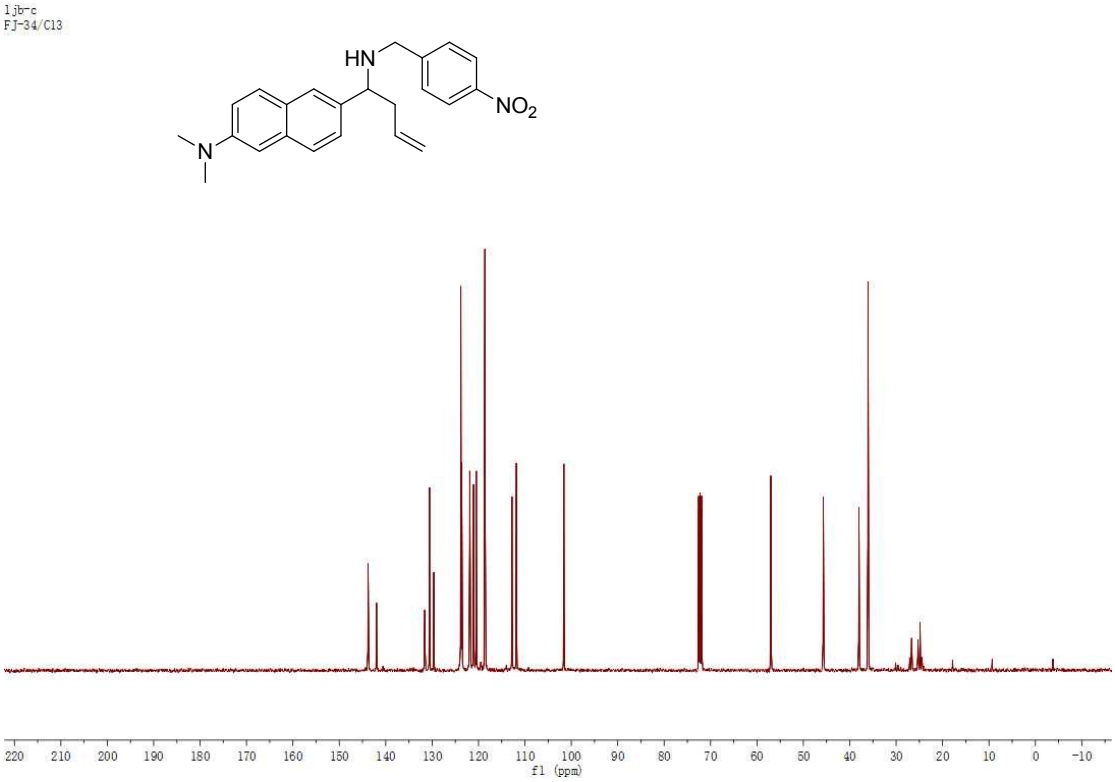
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f1 (ppm)