Electronic Supplementary Information

Reversible spatial and temporal control of lipid signaling

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Contents

1. Synthesis and characterization of caged lipids

Compound 1

Compound 1 was synthesized from vanillin using the protocol described by Baker et al.[1] Product is pale-yellow sticky solid. NMR data is in good accordance with literature. **^{1}H NMR** ($CDCl_3$, 400 MHz), ppm: 7.76 (s, 1H), 7.28 (s, 1H), 7.21 (br s, 1H, *amide*), 5.00 (s, 2H), 4.88 (br s, 1H, *amide*), 4.58 (s, 2H), 4.03 (s, 3H), 3.53-3.45 (m, 2H), 3.35-3.27 (m, 2H), 1.43 (s, 9H). **^{13}C NMR** ($CDCl_3$, 100 MHz), ppm: 167.98, 154.46, 145.57, 139.49, 134.22, 111.86, 111.45, 79.85, 69.00, 62.61, 56.45, 40.37, 28.30. **LCMS** (m/z): ESI, found $[M+Na]^+$ = 422.2 (calculated for $C_{17}H_{25}N_3O_8$+Na = 422.15).

Compound FAAzo4 was synthesized from 4-butylaniline using protocol reported by Frank et al.[2]

Compound 2

Procedure: ONB alcohol **1** (40 mg, 1 eq) and DMAP (18 mg, 1.2 eq) were dissolved in DCM and stirred to get clear solution. Then EDC (22 mg, 1.2 eq) was added quickly to the reaction mixture. Finally, FAAzo4 (40 mg, 1.0 eq) was added to the reaction mixture and reaction was stired overnight at room temperature in the absence of light. TLC confirmed the completion of reaction. DCM was evaporated under reduced pressure and water was added to the crude mixture. The product was extracted three times using ethyl acetate, dried over sodium sulfate and concentrated under reduced pressure to get yellow viscous liquid. Product was purified by colum chromatography, eluting DCM followed by 25 % ethyl acetate/DCM. Yield = 86%. **^{1}H NMR** ($CDCl_3$, 400MHz, δ): 7.86-7.83 (m, 4H), 7.76 (s, 1H), 7.33-7.31 (m, 4H), 7.03 (s, 1H), 5.52 (s, 2H), 4.58 (s, 2H), 3.96 (s, 3H), 3.50-3.48 (m, 2H), 3.34-3.31 (m, 2H), 2.80-2.78 (m, 2H), 2.70-2.68 (m, 2H), 2.48 (t, J = 8 Hz, 2H), 2.09-2.06 (m, 2H), 1.68-1.64 (m, 2H), 1.43 (s, 9H), 1.40-1.38 (m, 2H), 0.96 (t, J = 6 Hz, 3H) ppm. **^{13}C NMR** ($CDCl_3$, 100 MHz, δ): 172.7, 168.1, 154.2, 151.4, 151, 146.7, 144.4, 129.3, 129.2, 129, 123, 122.9, 112.1, 111.3, 69.1, 63.3, 56.5, 35.7, 33.6, 33.5, 28.4, 26.4, 22.5, 14.1 ppm. **HRMS** (m/z): ESI, found $[M+Na]^+$ = 728.325 (calculated for $C_{37}H_{47}N_5O_9$ Na = 728.327).

Compound 3

Procedure: Compound 2 (40 mg, 1.0 eq) was dissolved in DCM (2.5 mL) and cooled to 0°C. Then TFA (110 µL, 20.0 eq) was added dropwise to the reaction mixture. Next, 10 µL water was added and the reaction mixture was allowed to heat up to room temperature and stirred for several

minutes. TLC indicated the complete consumption of the starting material. DCM was evaporated under reduced pressure. Diethyl ether 500 µL was added to the crude mixture and evaporated on rotavapor to remove TFA. The same step was repeated thrice to get yellowish solid. Yield = 99%. **^{1}H NMR** ($CDCl_3$, 400 MHz, δ): 7.82-7.80 (m, 4H), 7.68 (s, 1H), 7.33-7.31 (m, 4H), 6.96 (s, 1H), 5.43 (s, 2H), 4.60 (s, 2H), 3.89 (s, 3H), 3.66-3.66 (br s, 2H), 3.21 (br s, 2H), 2.76-2.70 (m, 2H), 2.68-2.66 (m, 2H), 2.47 (t, J = 6 Hz, 2H), 2.21 (br s, 5H), 2.06-2.04 (m, 2H), 1.64-1.62 (m, 2H), 1.41-1.37 (m, 2H), 0.94 (t, J = 8 Hz, 3H) ppm. **^{13}C NMR** ($CDCl_3$, 100 MHz, δ): 172.8, 170.6, 154.1, 151.4, 151.1, 146.6, 145.8, 144.4, 139.7, 129.3, 129.2, 129.1, 123, 122.9, 63, 56.5, 35.7, 35, 26.4, 22.5, 14.1 ppm. **HRMS** (m/z): ESI, found $[M+Na]^+$ = 628.273 (calculated for $C_{32}H_{39}N_5O_7$ Na = 628.274).

Compound IM-FA

Procedure: Compound 3 (50 mg, 1.0 eq) and DIEA (16 µL, 3.0 eq) were mixed in DCM (5 mL). Then, Sulforhodamine B acid chloride (26 mg, 1.5 eq as solution in DCM) was added, and the reaction mixture was stirred at RT overnight. The red homogeneous solution was formed. Organic solvent was evaporated under vacuum. The obtained residue was purified by column chromatography (eluent DCM/MeOH = 98/2, then 96/4) to give the final product as a dark red solid. Yield = 48%. **^{1}H NMR** ($CDCl_3$, 400 MHz), ppm: 8.77 (s, 1H), 7.98-7.95 (m, 1H), 7.85-7.83 (m, 4H), 7.76-7.73 (m, 1H), 7.66 (s, 1H), 7.39 (d, J = 8 Hz, 2H), 7.34-7.32 (m, 4H), 7.21 (d, J = 8 Hz, 1H), 6.99 (s, 1H), 6.95-6.92 (m, 2H), 6.69-6.67 (m, 2H), 5.48 (s, 2H), 4.54 (s, 2H), 3.97 (s, 3H), 3.60-3.51 (m, 8H), 3.44-3.42 (m, 2H), 3.23-3.19 (m, 2H), 2.78 (t, J = 8 Hz, 2H), 2.70 (t, J = 8 Hz, 2H), 2.48 (t, J = 8 Hz, 2H), 2.19 (s, 3H), 2.10-2.03 (m, 2H), 1.69-1.63 (m, 2H), 1.30-1.26 (m, 14H), 0.95 (t, J = 8.0 Hz, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz, δ): 173.1, 168.4, 158.9, 158.2, 156, 154.7, 151.4, 147.8, 147.1, 144.8, 142.2, 140.2, 134.1, 130.2, 129.6, 128.4, 127.7, 123.3, 123.2, 114.8, 114.4, 111.8, 111.6, 95.9, 68.9, 63.7, 56.9, 46.3, 43.3, 39.5, 36, 35.4, 33.9, 31.9, 30.7, 30.2, 26.8, 22.8, 14.4, 13.1 ppm. **HRMS** (m/z): ESI, found $[M+1]^+$ = 1075.5131 (calculated for $C_{57}H_{78}N_4O_{12}S_2$+H = 1075.5136).

Compound PM-FA

Procedure: Atto-532 (1 mg, 1.0 eq) was dissolved in 1 mL DCM/Acetonitrile (1:1). DIPEA (3eq) was added to the mixture and the mixture was stirred for few minutes. HBTU (2eq) was added to this mixture and allowed to stir for next 15 min. Then solution of Compound 3 (1.2 eq) in DCM was transferred to the reaction mixture and stirred at room temperature for overnight. After 24 h,

monitoring of reaction progress using TLC indicated the > 65% conversion of atto-532. Further, Compound 3 (0.2 eq) was added to the mixture and allowed to to stir for overnight. TLC confirmed the completion of reaction. Solvent was evaporated under high vacuum. The crude mixture was loaded on a small column containing silica saturated with triethyl amine and eluted with 2.5% Methanol/DCM to obtain pure dark red sticky compound. Yield = 42%. **HRMS** (m/z): ESI, found $[M-2H]^-$ = 1231.411 (calculated for $C_{61}H_{67}N_8O_{16}S_2$ = 1231.410).

2. Photophysical properties of caged lipids

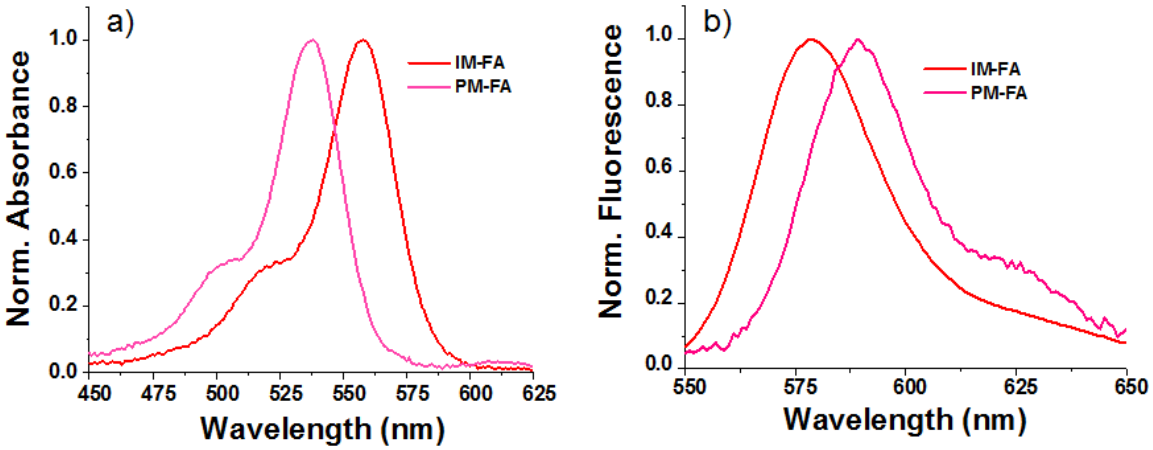


Fig. S1 Normalized absorption (a) and emission (b) spectra of caged lipids IM-FA and PM-FA. Spectral measurements were made in methanol.

Absorption and emission spectra were recorded on Cary-100 spectrophotometer (Varian) and FluoroMax-4 spectrofluorometer (Horiba Scientific), respectively. Stock solutions of caged lipids (1 mM concentration) were prepared in DMSO. Measurements were performed in a 1 cm quartz cuvette (3.5 mL). The excitation wavelength was 520 nm, slits were 2 nm. Fluorescence quantum yield of the caged lipids was determined using Rhodamine B (ϕ_R = 0.31 in water) as the standard.[3]

$$\phi_S = \phi_R \left(\frac{A_R}{A_S}\right)\left(\frac{D_S}{D_R}\right)$$

Where ϕ_S and ϕ_R are the quantum yields of the sample and reference; A_S and A_R are absorbance of the sample and reference at the excitation wavelength; and D_S and D_R are the integral fluorescence intensities.

Table S1 Photophysical properties of caged lipids.

Compound	λ_{abs} (nm)	λ_{em} (nm)	Quantum yield, ϕ
IM-FA	561	589	0.10
PM-FA	536	578	0.11

3. Determination of photoreaction quantum yield

The quantum yields of uncaging of PM-FA and IM-FA were determined using 2-nitrobenzyl pivalate as a reference (uncaging QY_{254} = 0.13).[4] Solutions of IM-FA, PM-FA, and the 2-nitrobenzyl pivalate in acetonitrile with OD ≈ 0.02 were irradiated with 254 nm (± 5 nm). The uncaging was monitored by absorption at 254 nm every 5 s during 1000 s. The kinetic curves were fitted to equation: $A=A_0\exp(-k\varepsilon QYt)$ where k is a constant depending on the lamp intensity (the same for all measurements), ε is the absorption coefficient of a compound at 254 nm, QY is the photoreaction quantum yield and t is the illumination time. All 3 compounds are based on o-nitrobenzyl (ONB), therefore we assumed that their absorption coefficients are very close and the observed rate constants are proportional to the QY. The error of QY was calculated as a sum of relative errors in observed reaction rates for the reference and PM-FA or IM-FA, respectively.

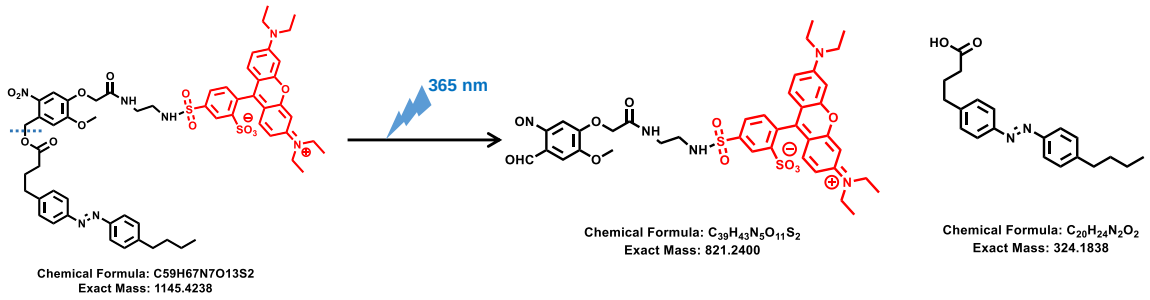
In the case of IM-FA the photoreaction QY is almost the same as for unmodified ONB. For IM-FA, QY was three-fold lower, which can be a result of absorption of Rhodamine at the excitation wavelength, or energy transfer from ONB unit to Rhodamine occurring parallel to uncaging.

Table S2 Photoreaction quantum yield of caged lipids.

Compound	Observed rate constant, s^{-1}	QY
2-nitrobenzyl pivalate	28±1	0.13 [4]
PM-FA	33±3	0.15±0.02
IM-FA	9.7±1	0.045±0.006

4. Uncaging study of caged lipids in solution

A stock solution 5 mM of IM-FA was prepared in a mixture of solvents CH_3CN/CH_3OH (1:1). A six set of solutions in CH_3CN (1 mM, 50 µL each) were exposed under LED-365B (350 mW) lamp in regular time intervals (2, 5, 7 and 10 min) to monitor the dynamics of uncaging. The progress of uncaging reaction was monitored by TLC (ALUGRAM Xtra SIL G/UV254). Further, the samples of unexposed and exposed IM-FA were submitted for mass analysis to detect the appearance of photoreleased FAAzo4.



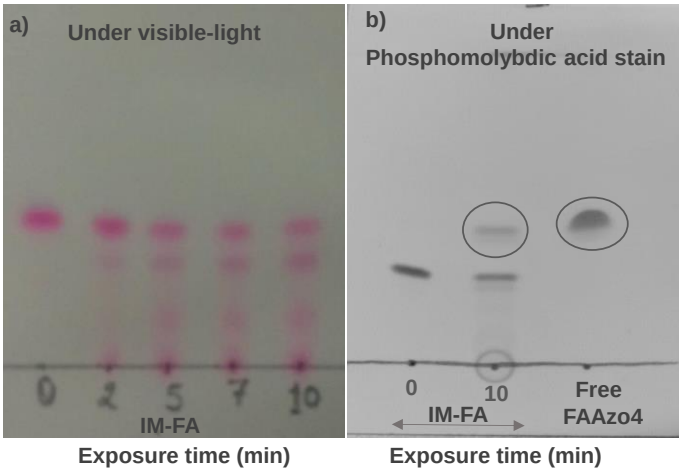


Fig. S2 Uncaging of IM-FA under 365 nm LED lamp (350 mW) monitored by TLC (ALUGRAM Xtra SIL G/UV254). a) TLC of the irradiated samples under visible light; b) TLC of IM-FA with and without irradiation, and free FAAzo4 under phosphomolybdic acid stain. 1 mM solution of IM-FA in MeCN was illuminated. Small aliquots were spotted on ALUGRAM and TLC was run in 1:9 MeOH/DCM.

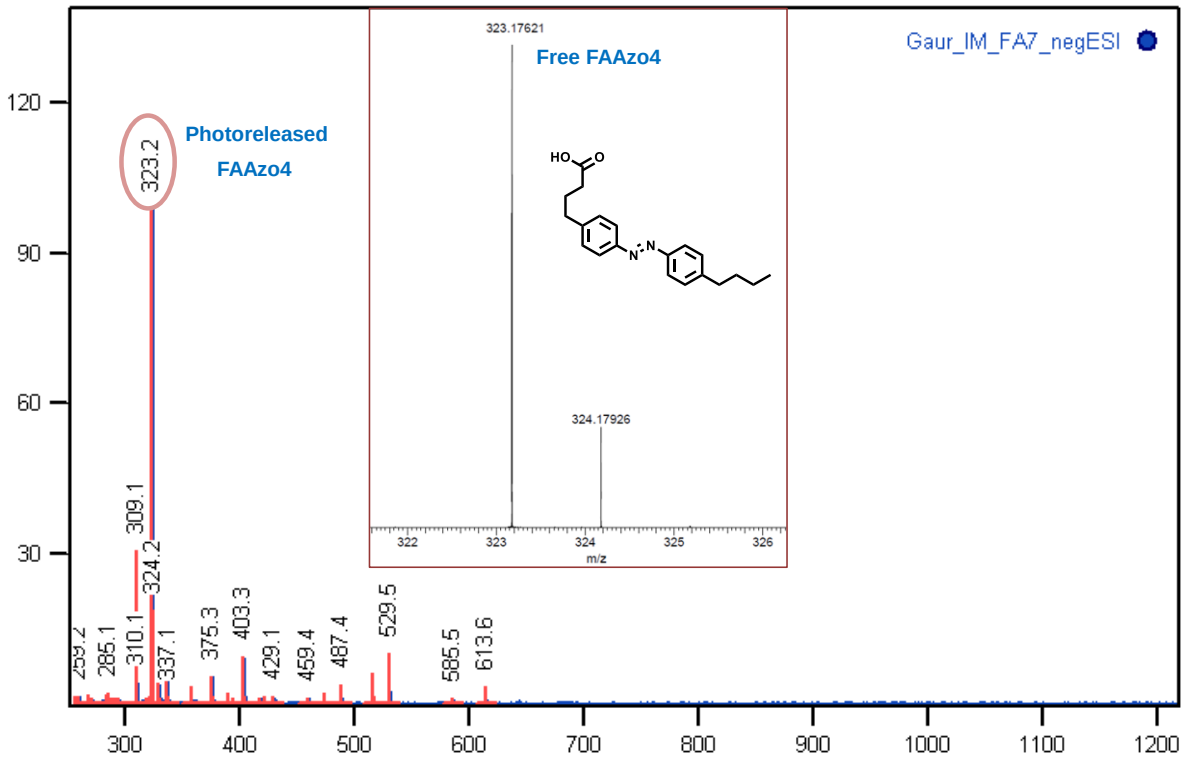


Fig. S3 Mass spectrum of irradiated IM-FA. Inset presents the mass spectrum of pure FAAzo4.

5. Cellular studies

Cell growth

HeLa cells were grown in growth media containing full DMEM (DMEM (Gibco # 11965092), supplemented with 10% FBS (BioWest South America #S1810), 2 mM L-Glutamine (Gibco # 25030081), 20 mM sodium pyruvate (Gibco # 11360070) and antibiotic (Primocin, InvivoGen). First, cells were seeded in an 8-well Lab-Tek chambered coverslip (ThermoScientific #155411) 24 h before transfection at 37°C and 5% CO_2, 300 µL growth media per well.

Transfection protocol

Transfection was carried out with Lipofactamine 2000 in DMEM free of FBS and antibiotics. First, media was aspirated and the cells were washed with PBS. The wells were charged with full DMEM (210 µL per well). Plasmid DNA (0.5 µg of DNA per well, 1:1 plasmid ratio for cotransfection) and Lipofactamine (1.5 µL per well) were diluted in two different eppendorfs (epi 1, 2) containing 20 µL DMEM/epi. Transfection mixture was prepared by mixing the diluted DNA and Lipofactamine. The mixture was pipetted properly and incubated at room temperature for 5-

10 min. The mixture was then added dropwise to each well of the 8-well Lab-Tek (use 200 µL tips). After 10-24 h cells were washed with full DMEM, and 300 µL full DMEM was added into each well.

The cells were incubated at 37 °C and 5% CO_2 for 24 h before the microscopy experiments were performed. Total transfection time before imaging 36 h.

Used Plasmids

Name	**Characterization**
GCaMP6s[5]	Green genetically-encoded fluorescent Ca^{2+} sensor
GPR40	Free fatty acid receptor

Live-cell imaging

The cells seeded in eight-well Lab-Tek chambered coverslip were washed with PBS and charged with DMEM (300 µL). Imaging was performed using confocal microscope Leica SP8, with 63× (oil) objective. Microscope settings were adjusted to generate images displaying background fluorescence values slightly larger than zero in order to capture the complete signal stemming from respective fluorescent dyes.

Image J Fiji was used to analyse the cell imaging data. Fluorescence intensities were measured with respect to time and calculated relative to the maximum detected fluorescence intensity after background substraction (F/F_0). The average of three regions of interest were used for statical analysis. Cells with represenative behavior were selected per condition and their responses were averaged.

Cellular localization of IM-FA and PM-FA

Con A-FITC (Concanavalin A conjugated with FITC, plasma membrane tracker) was used as the counterstain to check the localization of caged oleates. Con A-FITC was excited with 488 nm laser and emitted light was collected at 495-555 nm. IM-FA and PM-FA, were excited with 561 nm lasers and emitted light was collected at 585-645 nm.

Photouncaging of PM-FA and photoswitching of uncaged fatty acid

HeLa cells seeded in eight-well Lab-Tek chambered coverslip were washed with PBS and charged with DMEM (250 µL). Uncaging and photoswitching were performed on a confocal microscope Olympus Fluoview 1200, with a 63× (oil) objective. This microscope houses two independent, fully synchronized laser scanners for simultaneous laser stimulation and confocal observation and permits capturing cellular responses that occur during or immediately following laser stimulation. Uncaging of PM-FA was performed using 375 nm laser (5 frames, 4 sec/frame) at 37 ^{o}C and 5% CO_2 in live HeLa cells expressing GCaMP6s and GPR40. Fluorescence of GCaMP6s was used as the sensor to monitor elevation in Ca^{2+} levels in response to the activation of uncaged fatty acid after removal of 375 nm exposure. λ_{ex} = 488 nm, λ_{em} = 495-555 nm were used for the measuremet of GCaMP6s fluorescence. λ_{ex} = 561 nm, λ_{em} = 585-650 nm were used to measure the fluorescene of PM-FA.

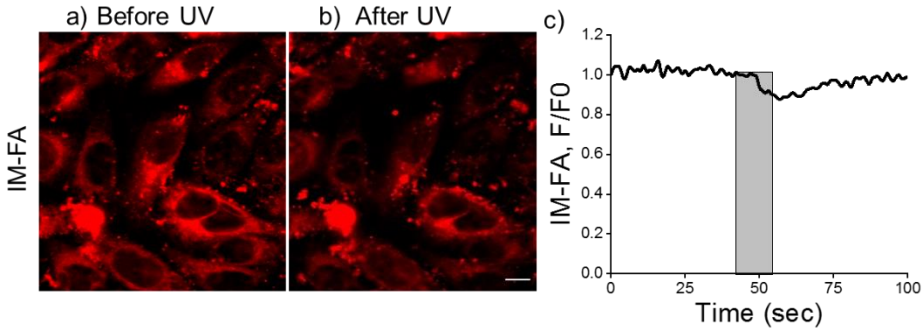


Fig. S4 a, b) Change in fluorescence intensity of IM-FA (λ_{ex} = 561 nm, λ_{em} = 585-650 nm) upon photo-uncaging using 375 nm laser. Scale bar: 20 µm. c) Average intensity profile of IM-FA in three different regions of interest.

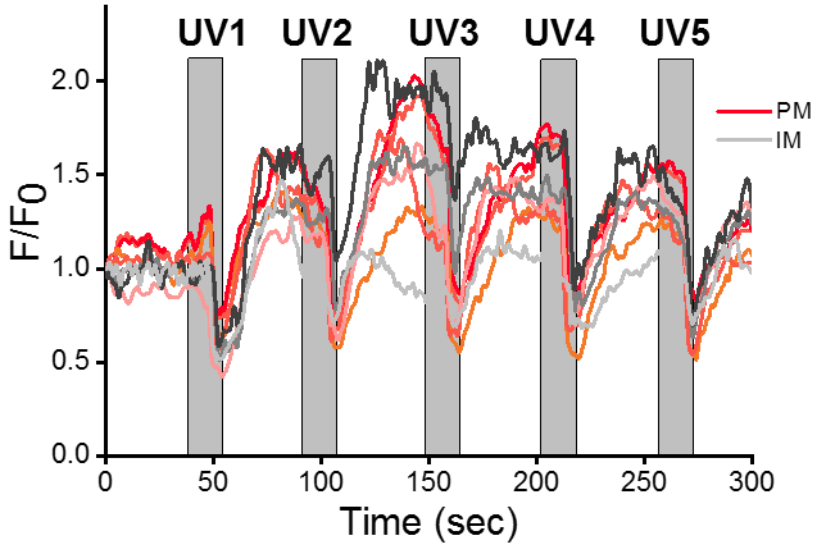


Fig. S5 Change in calcium sensor intensity profiles and the illumination time pattern of individual cells incubated with PM-FA (red) and IM-FA (gray). Gray rectangles show the time frame of exposure.

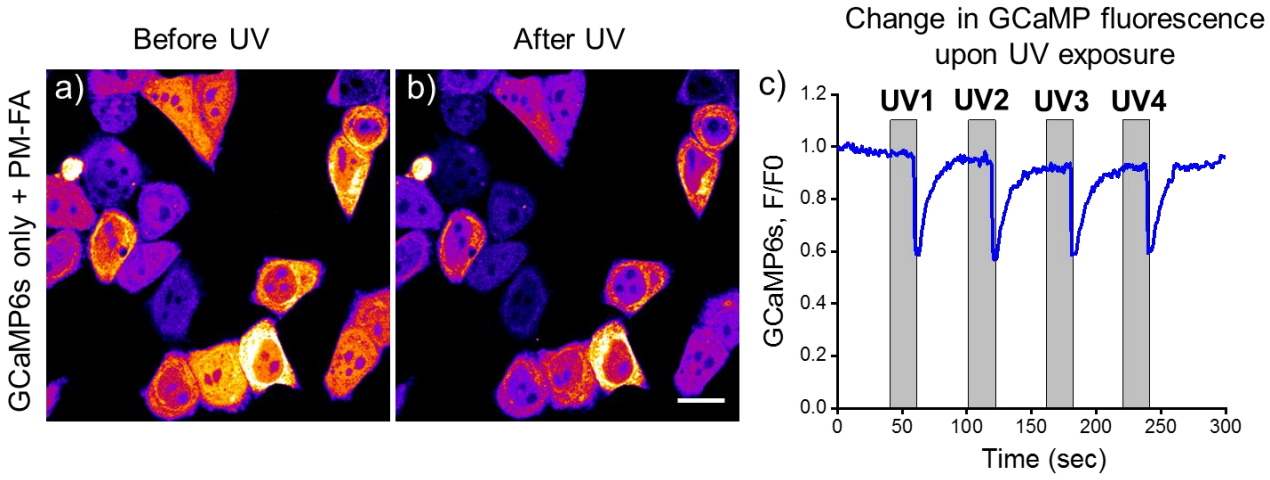


Fig. S6 a, b) Imaging of GCaMP6s in response to uncaging of PM-FA. Magnification 63x, scale bar is 20 µm. c) Change in GCaMP6s fluorescence upon UV-illumination. The uncaging experiment was performed at 37 ºC and 5% CO_2 in live HeLa cells expressing GCaMP6s only (without GPR40).

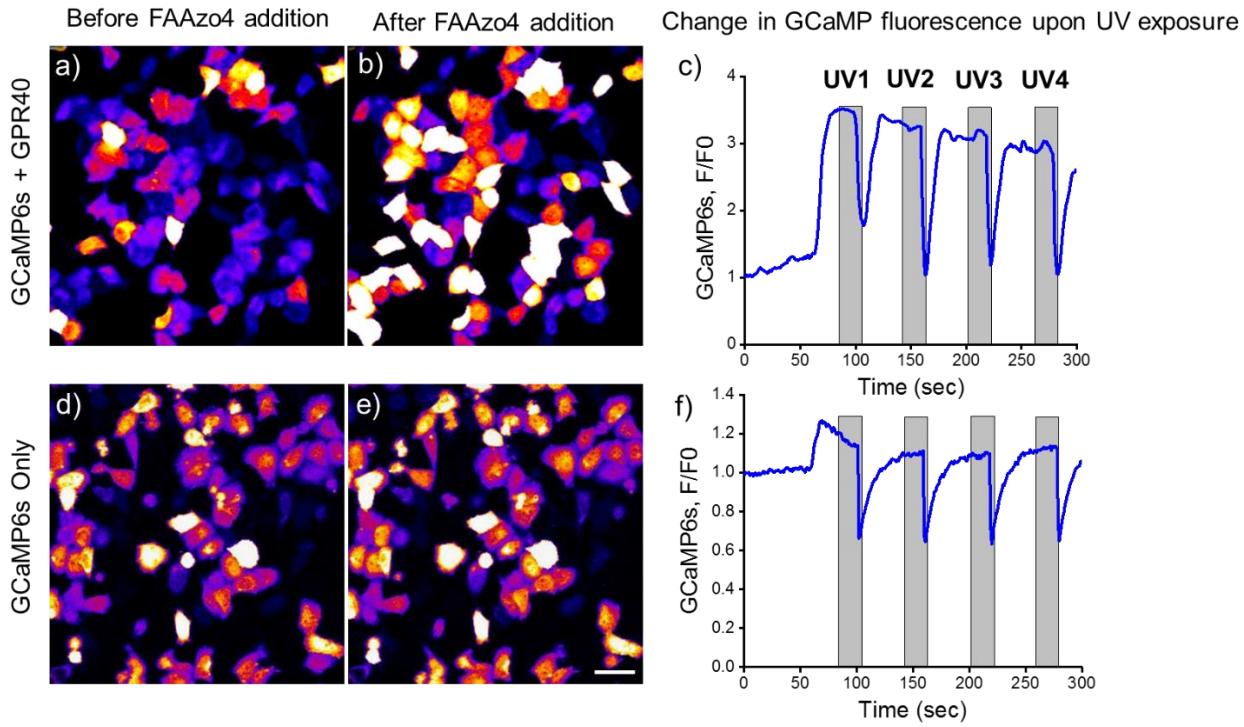


Fig. S7 a, b and d, e) Imaging of GCaMP6s with simultaneous contolled UV-illumination upon addition of free FAAzo4 (10 µM) in live cells expressing GCaMP6s and GPR40, and cell expressing GCaMP6s respectively. Magnification 63x, scale bar is 20 µm. c, f) Change in GCaMP6s fluorescence upon UV-illumination of free FAAzo4 loaded cells expressing GPR40 (c) and not (f). Gray rectangles show the time frame of exposure.

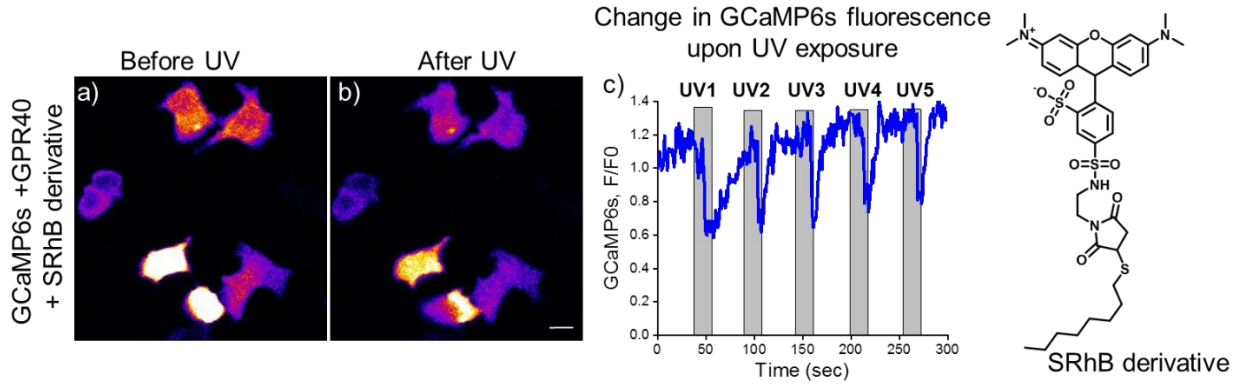


Fig. S8 a, b) Imaging of GCaMP6s with simultaneous contolled UV-illumination in live HeLa cells incubated with SRhB derivative (30 µM). Magnification 63x, scale bar is 20 µm. c) Normalized fluorescence intensity of GCaMP6s in response to photoactivation of SRhB derivative. Imaging was performed in live HeLa cells at 37 ^{o}C and 5% CO_2. Gray rectangles show the time frame of exposure.

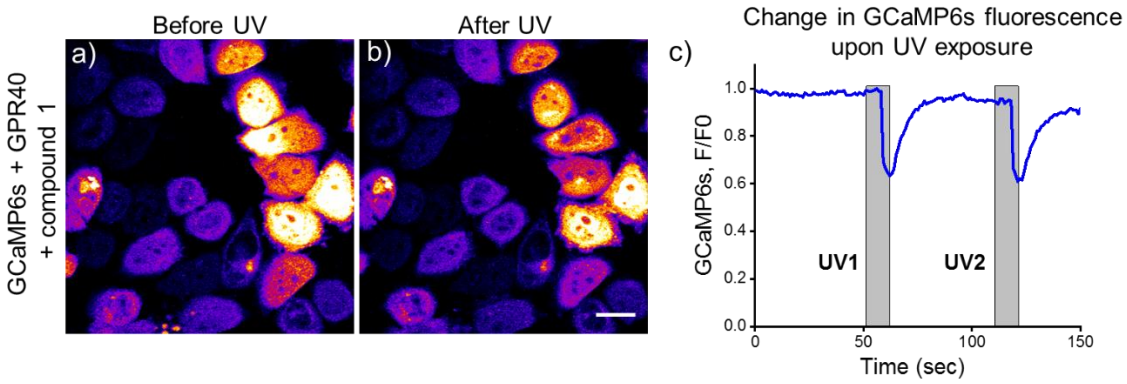


Fig. S9 a, b) Imaging of GCaMP6s with simultaneous contolled UV-illumination in live HeLa cells incubated with compound 1 (30 µM). Magnification 63x, scale bar is 20 µm. c) Normalized fluorescence intensity of GCaMP6s in response to photoactivation of compound 1. Imaging was performed in live HeLa cells at 37 ºC and 5% CO_2. Gray rectangles show the time frame of exposure.

6. NMR Spectra

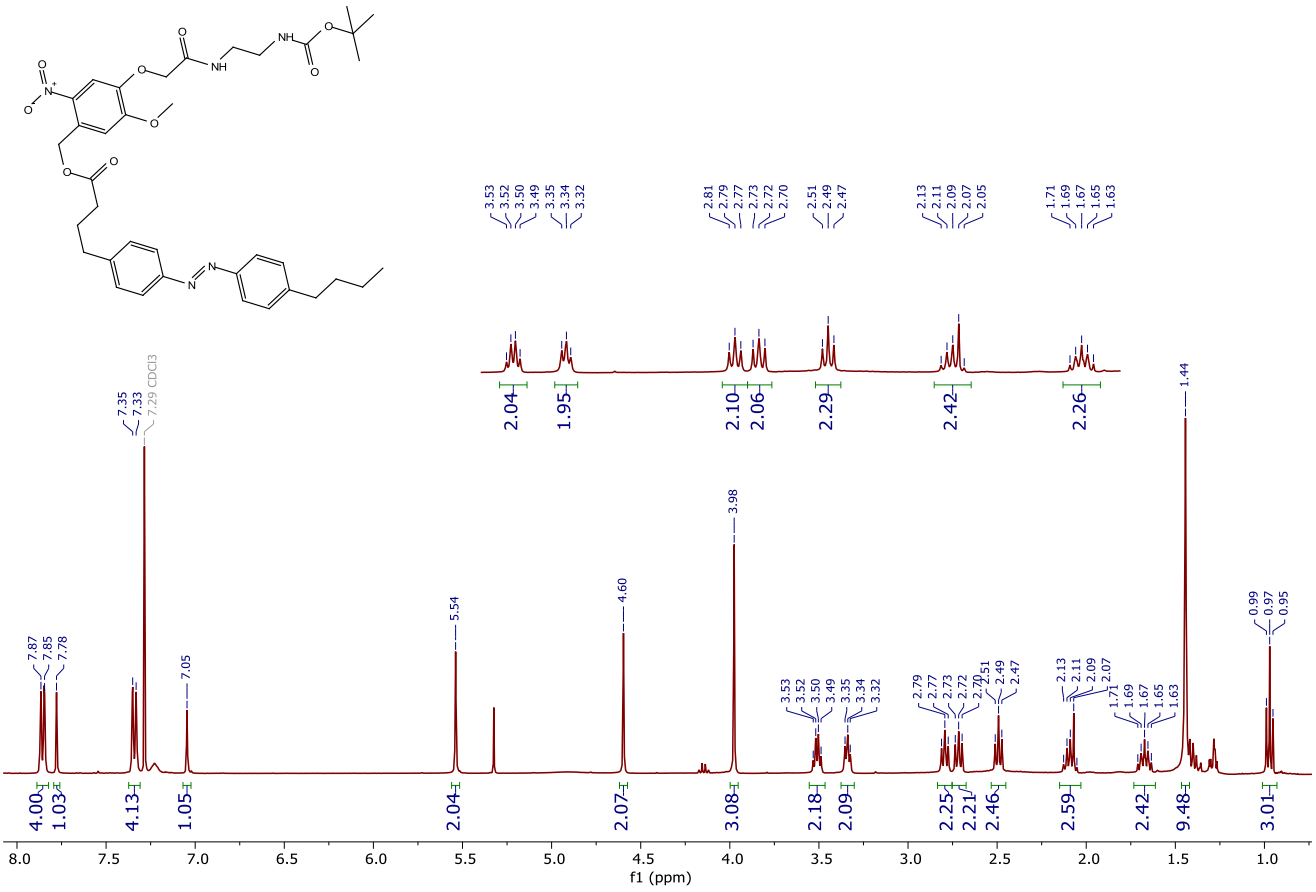


Fig. S10 ^{1}H NMR spectrum of Compound **2** in $CDCl_3$

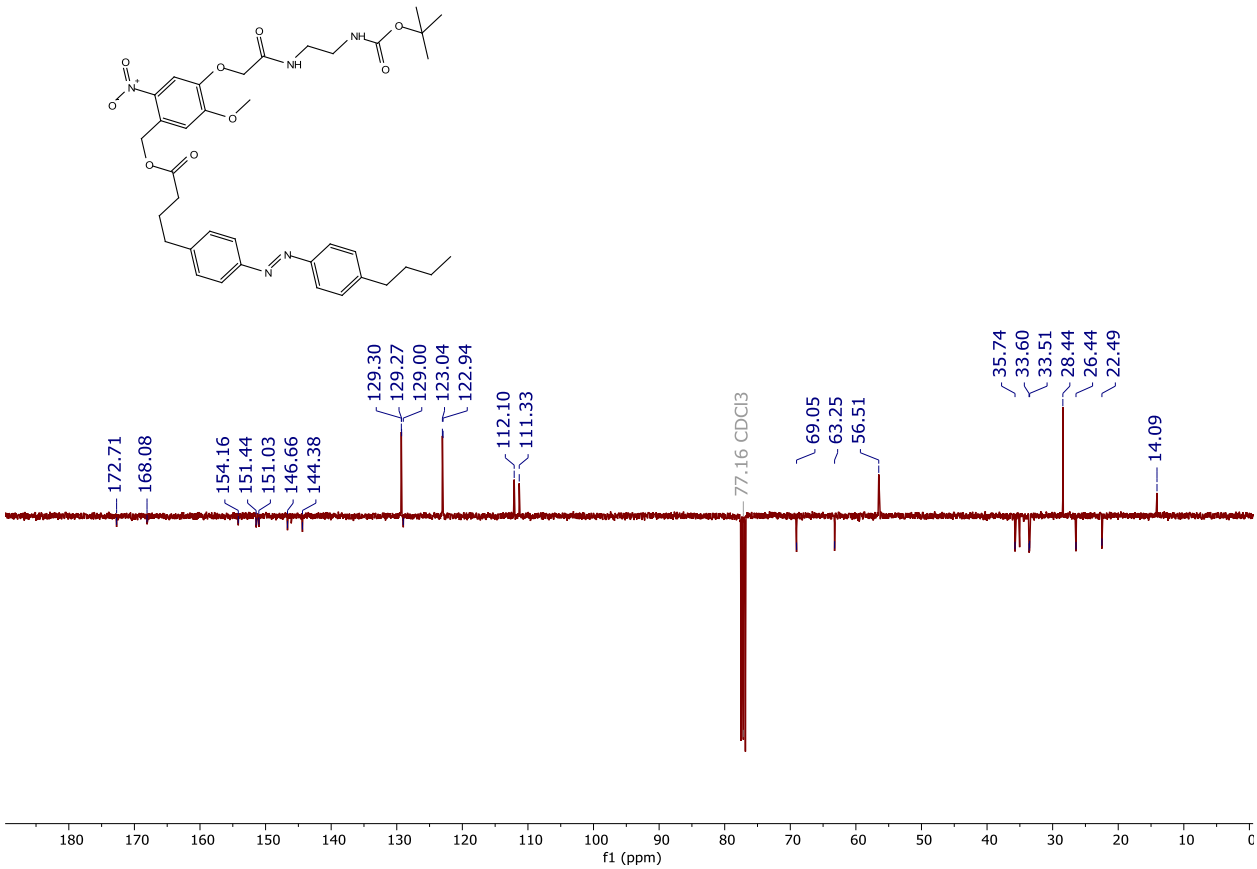


Fig. S11 APT spectrum of Compound 2 in $CDCl_3$

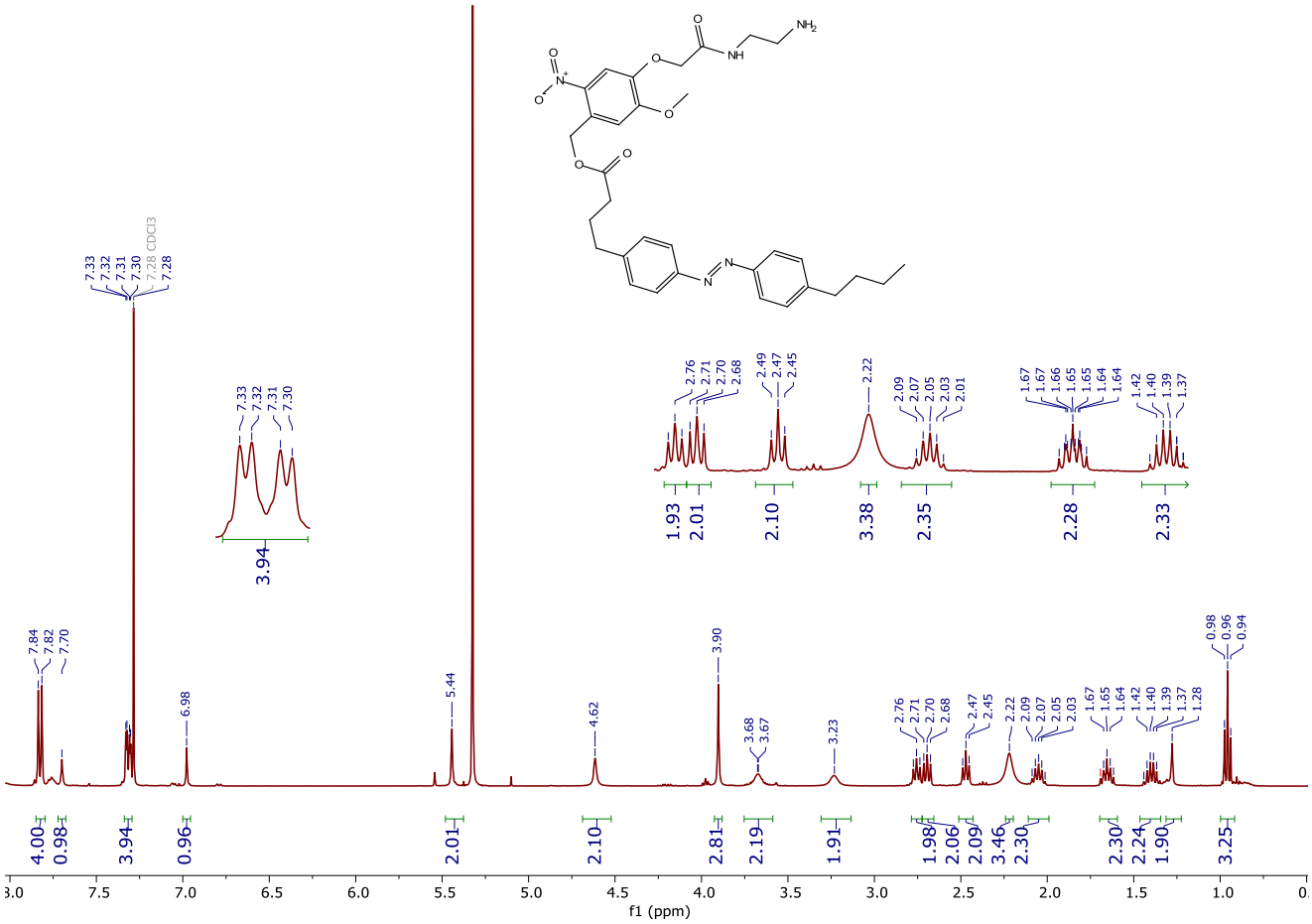


Fig. S12 ^{1}H NMR spectrum of Compound **3** in $CDCl_3$

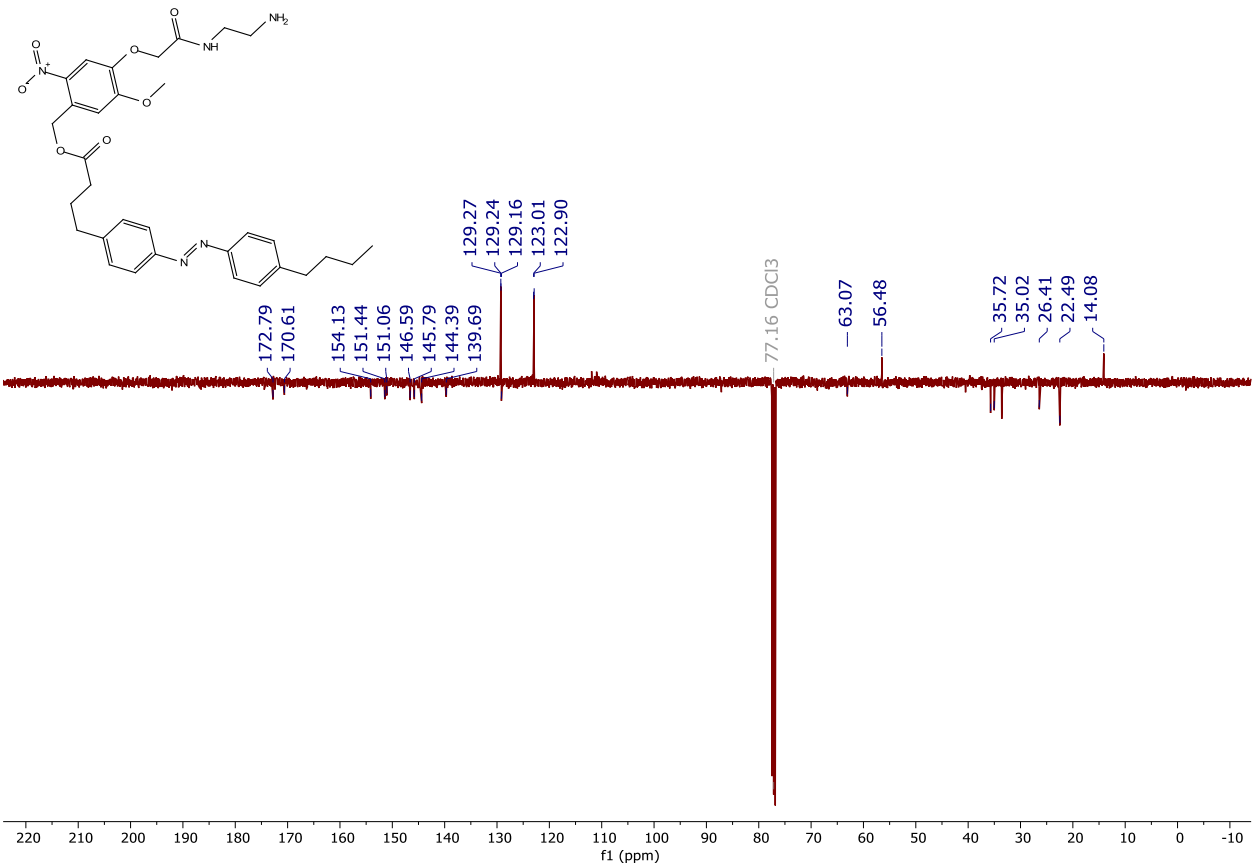


Fig. S13 APT spectrum of Compound 3 in $CDCl_3$

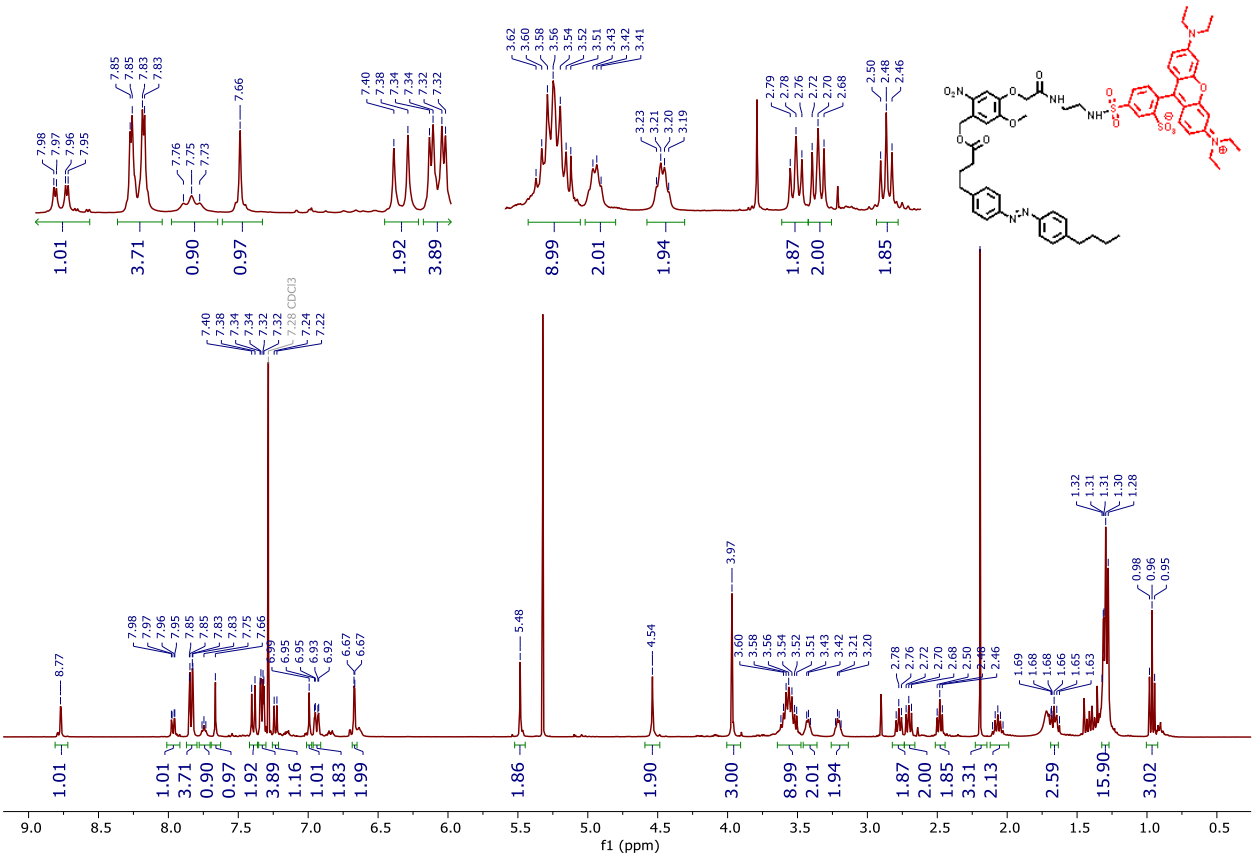


Fig. S14 ^{1}H NMR spectrum of Compound IM-FA in $CDCl_3$

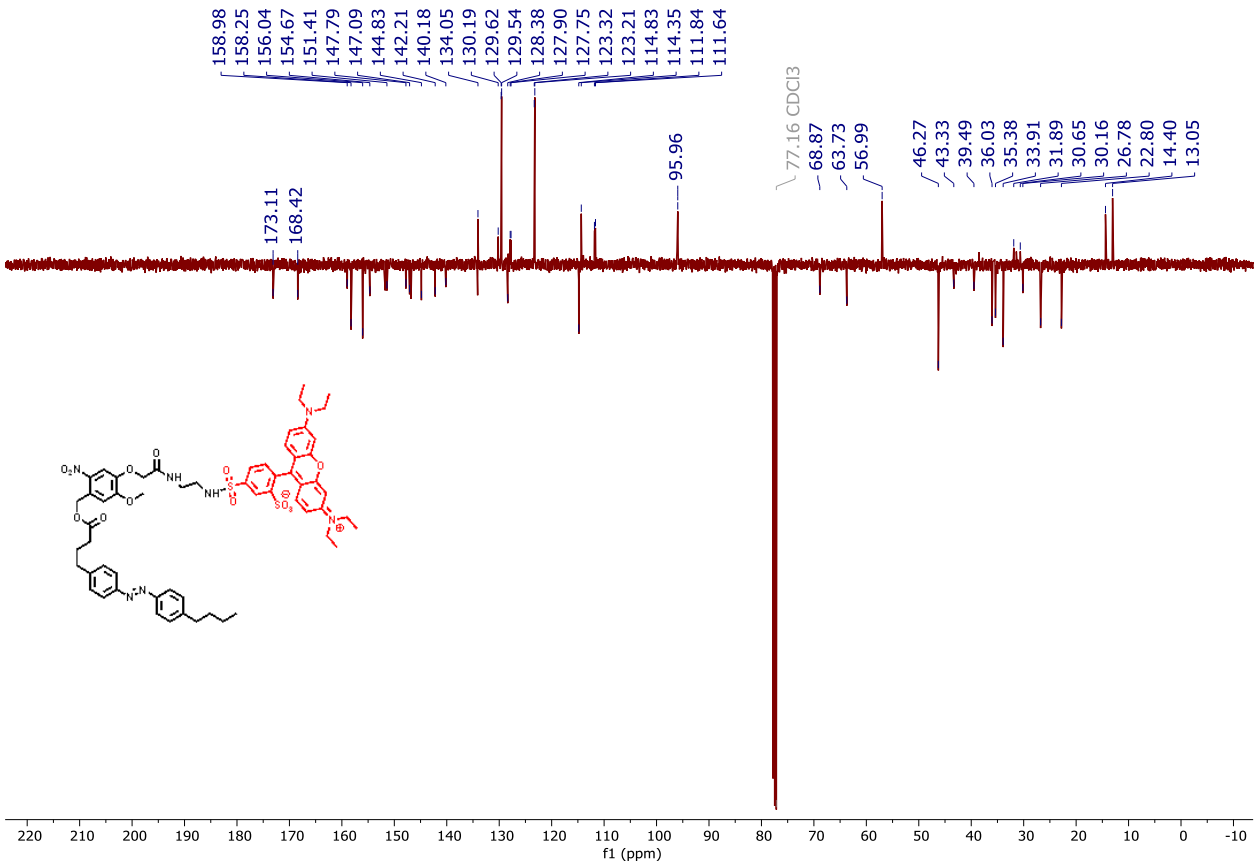


Fig. S15 APT spectrum of Compound IM-FA in $CDCl_3$

7. LC-MS data of PM-FA

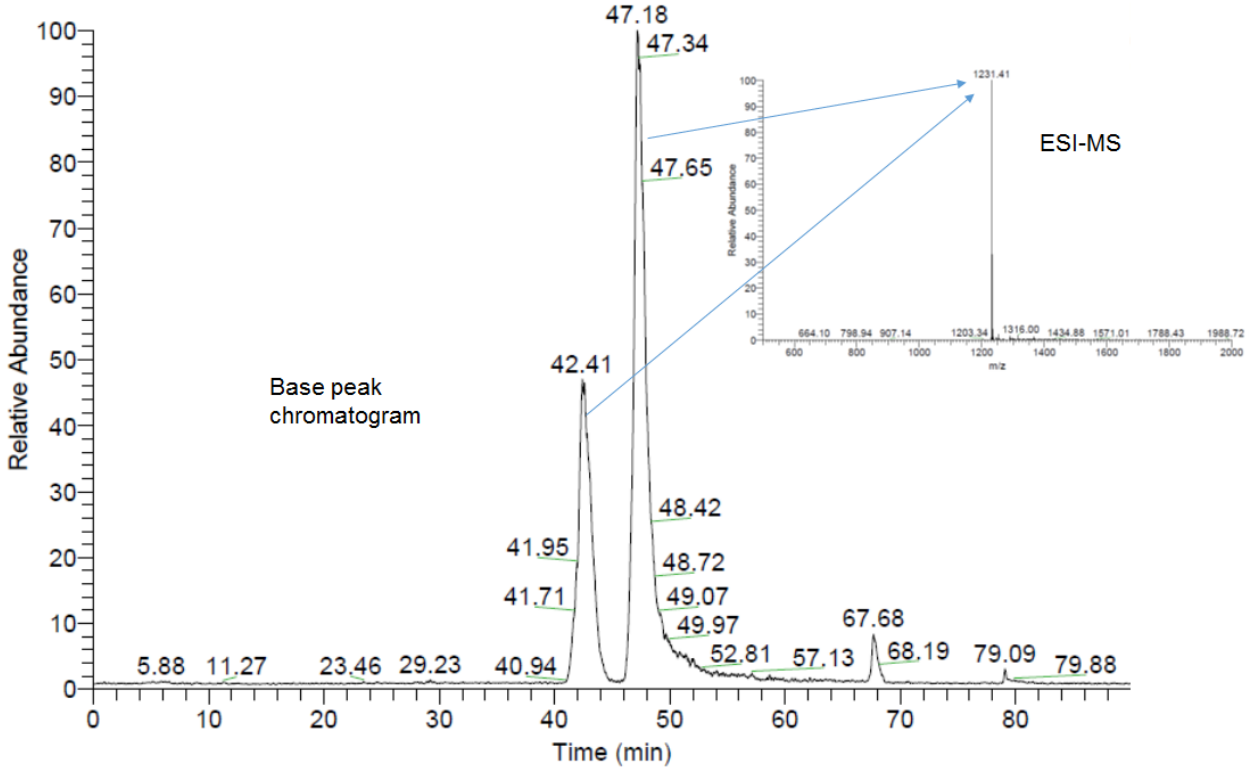


Fig. S16 LC-MS chromatogram and product ion mass spectrum of PM-FA. Existance of two peaks at 42.41 min and 47.31 min correspond to m/z = 1231.41 indicate the presence of two isomeric forms of PM-FA.

8. References

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