Supporting Information for

A lysosome-targeted turn-on fluorescent probe for endogenous formaldehyde in living cells

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Materials and instrumentation

Unless otherwise stated, all solvents and reagents were commercially available and used without further purification. Solvents used were purified by standard methods prior to use. Doubly distilled water was used throughout all experiments. Thin-layer chromatography (TLC) analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200-300), both of which were obtained from the Qingdao Ocean Chemicals. MTT (3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was from Sigma. LysoTracker Deep Red was purchased from Life Technologies. High-resolution mass spectra (HRMS) were recorded on a Bruker Apex Ultra 7.0 T FTMS mass spectrometer in electrospray ionization (ESI) mode. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 400 MHz Digital NMR Spectrometer, and using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal reference respectively. UV-vis absorption spectra were recorded on a Shimadzu UV-2600 UV-vis spectrophotometer and fluorescent spectra were measured on a Hitachi F-4600 luminescence spectrophotometer with a 1 cm standard quartz cell. The fluorescence imaging of cells was performed using a Nikon A1MP confocal microscope. The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter.

Determination of the fluorescence quantum yield:

The fluorescence quantum yields (Φ) were calculated by equation (1):

$$\Phi_s=\Phi_r(n_s/n_r)^2(A_r/A_s)(F_s/F_r) \quad (1)$$

In equation, A_s and A_r are the absorbance of the sample and the reference, respectively, at the same excitation wavelength, F_s and F_r are the corresponding relative integrated fluorescence intensities, and n is the refractive index of the solvent. Φ was quantum yield.

General procedure for the spectrum measurement

The stock solution of the probe **Na-FA-Lyso** was prepared at 1 mM in DMSO. The solutions of the various testing species were prepared from the representative amino acids, Na_2SO_3, $NaNO_2$, $MgCl_2$, KNO_3, H_2O_2, NaClO, $CaCl_2$, Na_2S, $NaHSO_3$, NO, and *tert*-Butyl hydroperoxide in the doubly distilled water, and ketones and aldehydes in the DMSO. The test solution of the probe **Na-FA-Lyso** (5.0 μM) in 10 mM PBS buffer (pH 7.4) with 1 % DMSO was prepared by placing the probe **Na-FA-Lyso** stock solution and DMSO in PBS buffer. The resulting solution was shaken well and incubated with the appropriate testing species before recording the spectra. Unless otherwise noted, for all the measurements, the excitation wavelength was 440 nm, the excitation slit widths were 5 nm, and the emission slit widths were 5 nm.

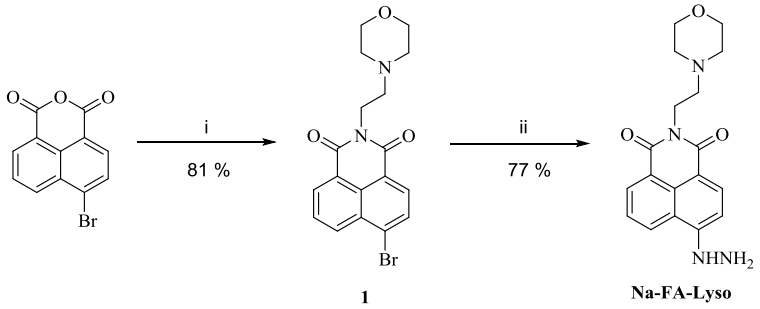
Cell culture and imaging materials and methods.

HeLa cells were cultured in Dulbecco's Modified Eagle Medium media (DMEM, Hyclone) supplemented with 10 % heat-inactivated fetal bovine serum (FBS, Sijiqing) and 1 % antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin, Hyclone) at 37 °C and 5 % CO_2. Before the imaging experiments, 1 mL of HeLa cells were subcultured and seeded in the glass bottom culture dishes (Nest) at a density of 1×10^5. The next day, the cells reached about 70 % confluence for the further experiments.

For the fluorescence imaging studies of added FA in the living HeLa cells, we performed two sets of control experiments: the HeLa cells in the culture medium; the HeLa cells were incubated with 50 μM FA in the culture medium for 30 min. For the experimental group, the culture medium of the cells was changed to a fresh media containing 5 μM probe and further incubated for 30 min. Then, the medium was removed and washed three times with PBS to remove the excess probe. After that, 1mL of the culture medium containing 50 μM FA was added to the cells, and then incubated for 30 min. The residual FA was removed by washing three times using PBS before the imaging by a Nikon A1MP fluorescence microscope equipped with 40×objective lens. For detecting fluorescence imaging, the excited wavelength was set at 488 nm, and emission wavelengths were collected from 500 nm to 550 nm.

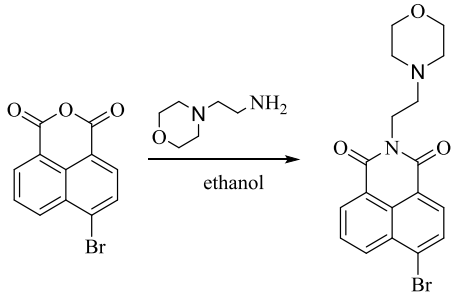
For the fluorescence imaging experiment of the endogenous FA in the living HeLa cells, we first conducted a control experiment, in which the HeLa cells were incubated with 200 μM $NaHSO_3$ for 30 min. For the experimental group, the culture medium of the cells was changed to a fresh media with 5 μM probe, and then incubated for 30 min. Subsequently, the medium was removed and washed three times with PBS to remove the excess probe. Furthermore, we carried out the negative control experiment, in which the culture medium of the cells was changed to a fresh medium with 200 μM $NaHSO_3$, and then incubated for 30 min. Then, the medium was removed and washed three times with PBS to remove the excess $NaHSO_3$. After that, 1mL of the medium containing 5 μM probe was added and then incubated for 30 min. The residual probe was washed three times before the imaging by a Nikon A1MP fluorescence microscope equipped with 40× objective lens. For detecting fluorescence imaging, the excited wavelength was set at 488 nm, and emission wavelengths were collected from 500 nm to 550 nm.

Scheme S1. Synthesis of the fluorescent formaldehyde probe **Na-FA-Lyso.**



Reagents and conditions: (i) 4-(2-Aminoethyl)morpholine, ethanol, reflux, 2h; (ii) Hydrazine hydrate, ethanol, reflux, 4h.

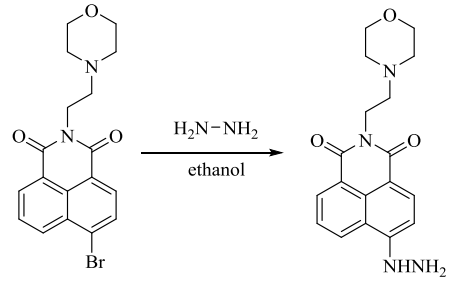
Synthesis of N-(2-morpholinoethyl)-4-bromo-naphthalimide 1.



1

4-Bromo-1,8-naphthalic anhydride 277 mg (1 mmol) and 4-(2-Aminoethyl)-morpholine 169 mg (1.3 mmol) were added to 2 mL of ethanol, and the reaction mixture was heated to reflux. After 2 h, the mixture was cooled to room temperature, filtered, and dried to afford the desired product as a gray solid 315 mg with a yield of 81 %. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (dd, J_1= 7.2 Hz, J_2= 0.8 Hz, 1H), 8.44 (d, J= 8.8 Hz, 1H), 8.24 (d, J = 7.6 Hz, 1H), 8.13 (d, J = 7.6 Hz, 1H), 7.92 (dd, J_1= 8.4 Hz, J_2= 7.2 Hz, 1H), 4.14 (t, J = 7.2 Hz, 2H), 3.54 (t, J = 4.4 Hz, 4H), 2.56 (t, J = 7.2 Hz, 2H), 2.47 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.91, 162.86, 132.70, 131.67, 131.43, 131.05, 129.86, 129.19, 128.88, 128.35, 122.77, 121.99, 66.25, 55.48, 53.42, 36.97; HR-MS calculated for $C_{18}H_{17}BrN_2O_3$ $[M+H]^+$ m/z 389.0495, found 389.0490.

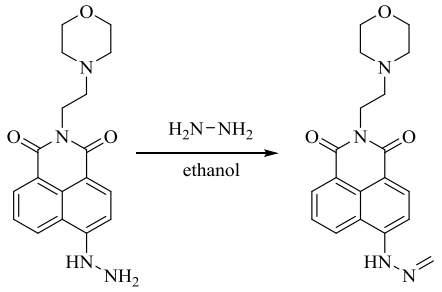
Synthesis of N-(2-morpholinoethyl)-4-hydrazino-naphthalimide Na-FA-Lyso.



1 **Na-FA-Lyso**

A mixture of compound **1** 78 mg (0.2 mmol) and 0.3 mL 80% hydrazine hydrate in 2 mL ethanol was heated under reflux for 4 h. After cooling to room temperature, the precipitated product was filtered, washed with cold-EtOH, and the crude product was purified by flash chromatography on silica gel (DCM/MeOH = 30:1) and re-crystallized from EtOH to afford an orange solid **Na-FA-Lyso** 52 mg with a yield of 77%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (s, 1H), 8.60 (d, J= 8.0 Hz, 1H), 8.40 (d, J = 8.0 Hz, 1H), 8.28 (d, J = 8.0 Hz, 1H), 7.63 (t, J = 8.0 Hz, 1H), 7.24 (d, J = 8.0 Hz, 1H), 4.69 (s, 2H), 4.14 (t, J = 8.0 Hz, 2H), 3.54 (t, J = 4.0 Hz, 4H), 2.53 (t, J = 8.0 Hz, 2H), 2.46 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.21, 163.31, 153.65, 134.66, 131.01, 129.74, 128.70, 124.52, 122.09, 118.84, 107.72, 104.42, 66.67, 56.28, 53.89, 36.81; HR-MS calculated for $C_{18}H_{21}N_4O_3$ $[M+H]^+$ m/z 341.1608, found 341.1612.

Synthesis of the product of the probe with FA (the compound 2).



Na-FA-Lyso **2**

The compound **Na-FA-Lyso** (68 mg, 0.2 mmol) was dissolved in 95 % ethanol (1 mL), and then FA (375 mg, 1 mmol, 40 % solution in H_2O) was added. The suspension was stirred at room temperature for 15 min. Subsequently, the mixture was concentrated under vacuum, and the resulting residue was purified by silica gel column chromatography (DCM/MeOH = 20:1 to 10:1) to afford an orange solid 49 mg with a yield of 70 %. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.27 (s, 1H), 8.70 (d, J = 8.4 Hz, 1H), 8.45 (d, J = 6.4 Hz, 1H), 8.32 (d, J = 8.4 Hz, 1H), 7.75 (dd, J_1 = 8.4 Hz, J_2 = 7.6 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.39 (d, J = 12.0 Hz, 1H), 6.68 (d, J = 12.0 Hz, 1H), 4.14 (t, J = 6.8 Hz, 2H), 3.54 (t, J = 4.4 Hz, 4H), 2.53 (t, J = 7.2 Hz, 2H), 2.46 (s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.03, 163.31, 147.13, 135.68, 133.84, 131.25, 129.45, 128.71, 125.31, 122.31, 118.70, 111.46, 107.05, 66.68, 56.16, 53.88, 36.94. HR-MS calculated for $C_{19}H_{21}N_4O_3$ $[M+H]^+$ m/z 353.1608, found 353.1612.

Cell cytotoxicity assay by MTT methods

1×10^4 HeLa cells were seeded into a 96-well plate. The next day the medium was changed into a medium containing a series of concentrations of the probe (1, 5, 10, 20 and 50 μM). After 24 h, 10 μL of MTT (5 mg/mL in PBS) was added and incubated for 4 h. Then, the culture medium was removed, and 100μL DMSO was added into the dishes to dissolve the formazan crystal product. The plate was shaken for 10 min, and then absorbance at 570 nm was measured by the microplate reader. The cell viability (%) was calculated by equation (2):

$$\text{The cell viability (\%)} = (OD_{570\ sample}-OD_{570\ blank})/(OD_{570\ control}-OD_{570\ blank})\times100\%. \quad (2)$$

In equation, $OD_{570\ sample}$ denotes the cells incubated with various of concentrations of the probe, $OD_{570\ control}$ denotes the cells without the probe, $OD_{570\ blank}$ denotes the wells containing only the culture medium.

Absorption spectrum of the probe Na-FA-Lyso: The absorption profile of compound **Na-FA-Lyso** in 10 mM PBS buffer (pH 7.4, 1 % DMSO) is shown in Figure S1. The probe displays a maximal absorption at around 440 nm (ε = 26,800 M^{-1} cm^{-1}) in 10 mM PBS buffer (pH 7.4, 1 % DMSO).

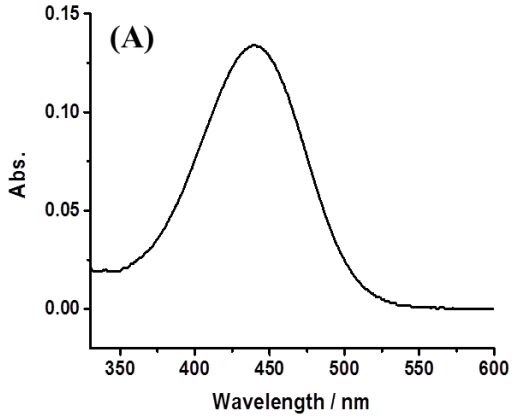


Figure S1. Absorption spectrum of the probe **Na-FA-Lyso** (5 μM) in 10 mM PBS buffer (pH 7.4, 1 % DMSO).

Detection limit: The detection limit was determined from the fluorescence titration data based on a reported method.[1] The probe **Na-FA-Lyso** (5 μM) was titrated with FA (0-200 μM) for 30 min. The fluorescent intensity data at 543 nm were normalized between the minimum intensity and the maximum intensity. A linear regression curve was then fitted to the normalized fluorescent intensity data and the point at which this line crossed the axis was considered as the detection limit (5.02×10^{-6} M).

[1] Shortreed M.; Kopelman R.; Kuhn M.; Hoyland B.; *Anal. Chem.*, **1996**, *68*, 1414-1418.

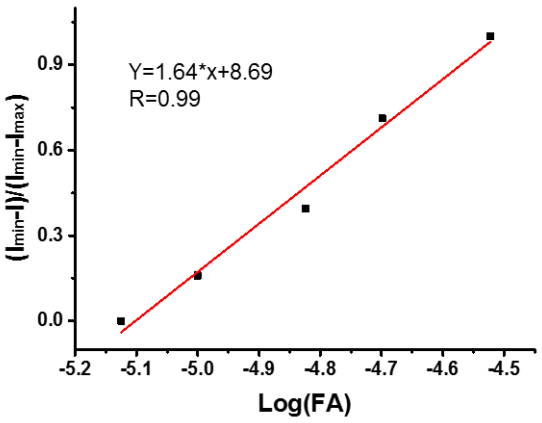


Figure S2. Normalized response of the fluorescence signal by changing the concentration of FA.

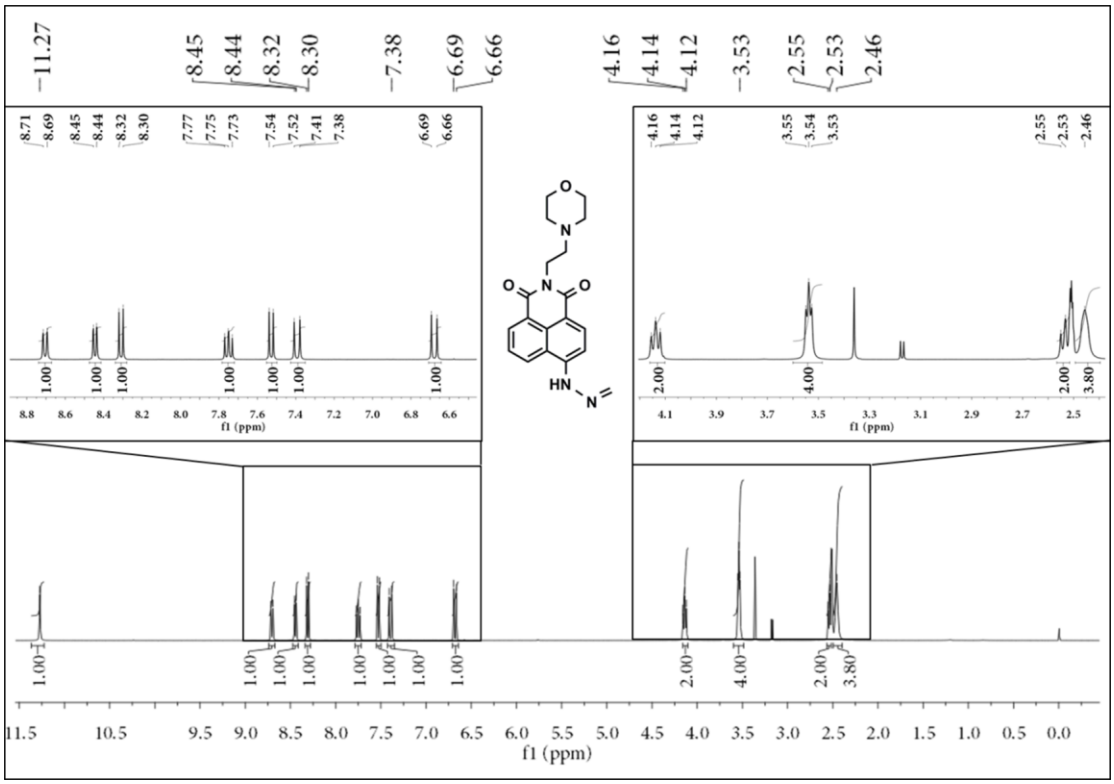


Figure S3. ^{1}H NMR spectrum of the product (the compound **2**) of the probe with FA.

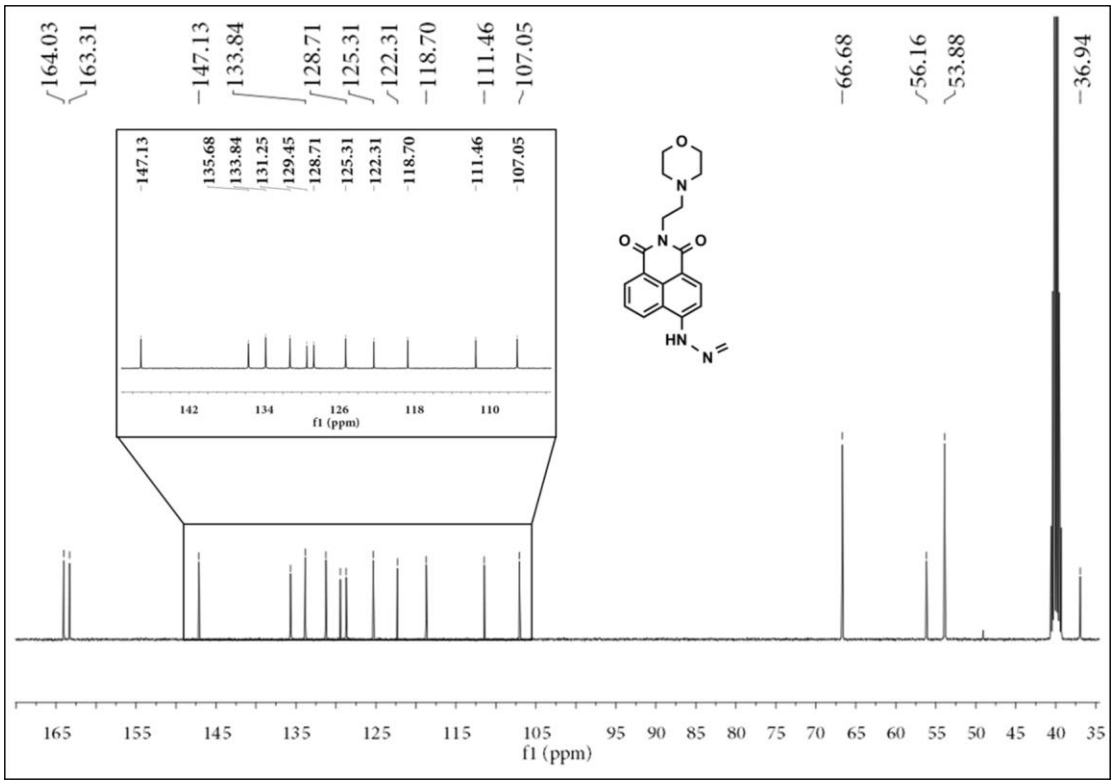


Figure S4. ^{13}C NMR spectrum of the product (the compound **2**) of the probe with FA.

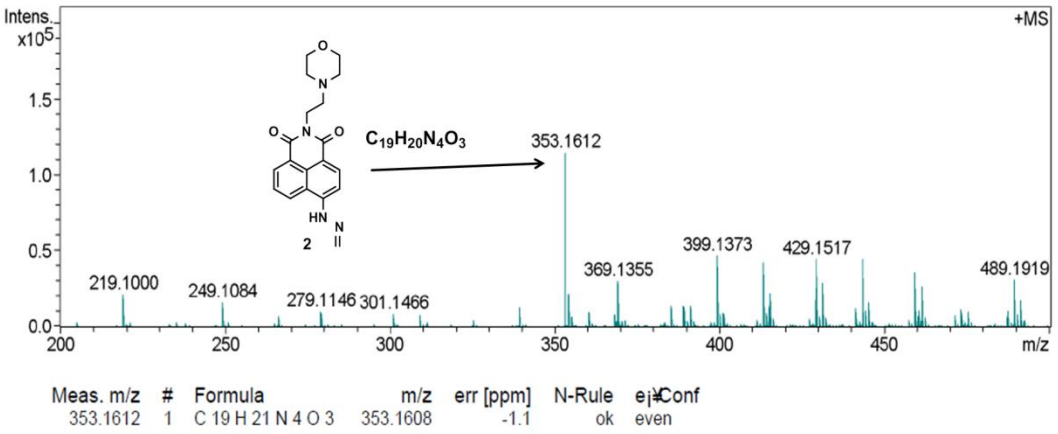


Figure S5. HR-MS spectrum of 50 μM **Na-FA-Lyso** treated with 2 mM FA in 10 mM PBS buffer (pH 7.4, 1 % DMSO).

Table S1. Some typical spectroscopic data for the reaction product (the compound **2**) of the probe **Na-FA-Lyso** with FA.

Maximum absorption wavelength (λ)	Molar absorption coefficient (ε)	maximum emission wavelength (λ)	Fluorescence quantum yield (Φ)
440 nm	20,800M^{-1} cm^{-1}	543 nm	0.31

Kinetic studies: The rate constant was determined from the fluorescence titration data based on a reported method.[2] The reaction of the probe **Na-FA-Lyso** (5 μM) with FA in PBS (10 mM, pH 7.4, 1 % DMSO) was monitored using the fluorescence intensity at 543 nm. The reaction was carried out at room temperature. The *pseudo*-first-order rate constant for the reaction was determined by fitting the fluorescence intensities of the samples to the *pseudo*-first-order equation (3):

$$\mathrm{Ln}[(F_{max} - F_t) / F_{max}] = - k't \qquad (3)$$

Where F_t and F_{max} are the fluorescence intensities at 543 nm at time *t* and the maximum value obtained after the reaction was complete. k' is the *pseudo*-first order rate constant. The *pseudo*-first-order plots for the reaction of **Na-FA-Lyso** with 40 equiv. of FA is shown in Figure S6, The negative slope of the line provides the *pseudo*-first-order rate constant for FA.

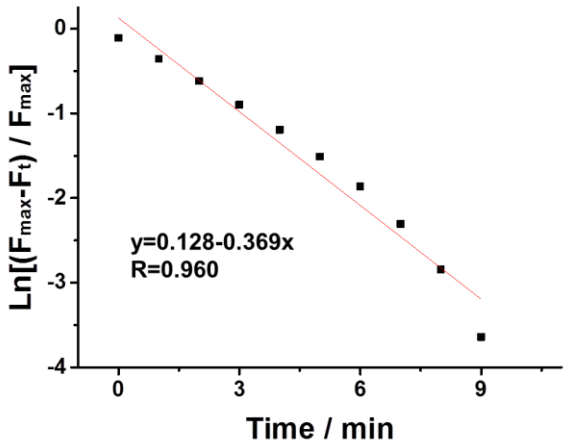


Figure S6. Pseudo-first-order kinetic plot of the reaction of **Na-FA-Lyso** (5 μM) with FA (40 equiv.) in PBS (10 mM, pH 7.4, 1% DMSO). Slope = 0.37 min^{-1}.

[2] Dale T. J.; Rebek J. Jr. *J. Am. Chem. Soc.*, **2006**, *128*, 4500–4501.

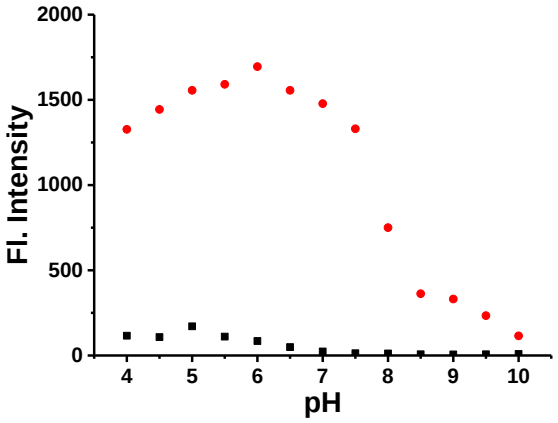


Figure S7. Fluorescence intensity changes of the probe **Na-FA-Lyso** (5 μM) at different pH values in the absence (■) or presence (●) of FA (10 equiv.) for 30 min.

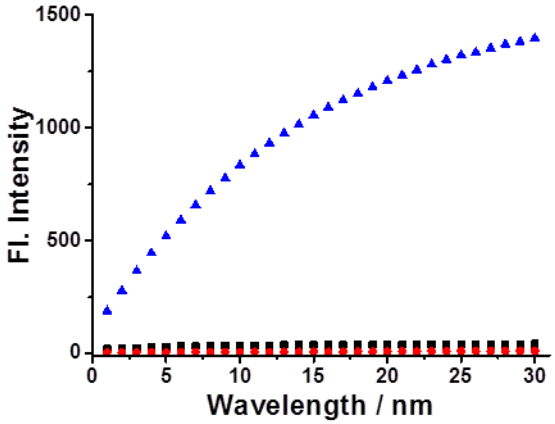


Figure S8. Reaction-time profiles of the probe treated with FA [▲], free probe [●], or FA pre-incubated with $NaHSO_3$ then treated with the probe [■]. The fluorescence intensities at 543 nm were continuously monitored at time intervals in PBS (10 mM, pH 7.4, 1 % DMSO). The concentrations for the probe, FA, and $NaHSO_3$ are 5, 50, and 200 μM, respectively. λ_{ex} = 440 nm.

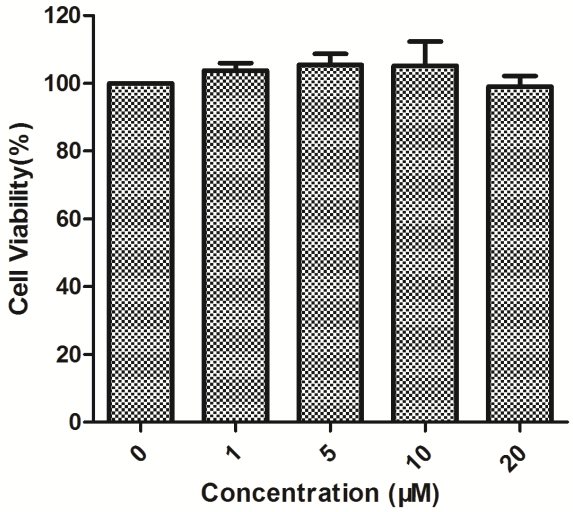


Figure S9. Effects of the probe **Na-FA-Lyso** with varied concentrations (0-20 μM) on the viability of the Hela cells. The probe with varied concentrations was incubated with the cells for 24 h. The viability of the cells in the absence of the probe is defined as 100 %, and the data are the mean standard deviation of five separate measurements.

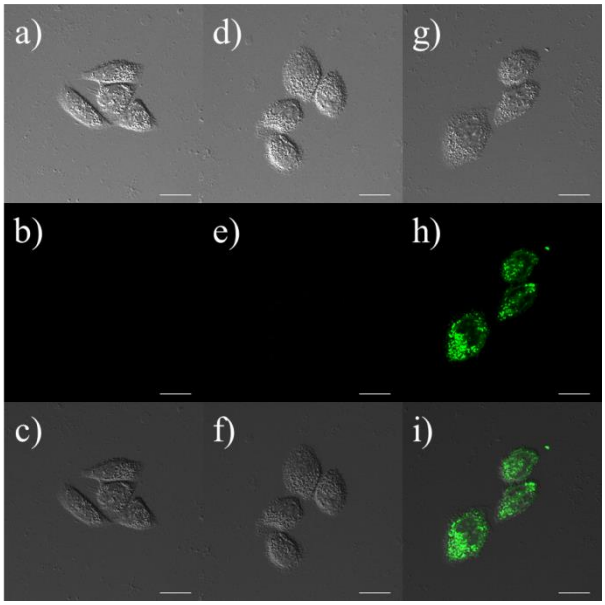


Figure S10. Fluorescence imaging of added FA in the living HeLa cells. a) Bright-field image of the living HeLa cells; b) The fluorescence image of a; c) The merge image of a and b); d) Bright-field image of the HeLa cells treated with FA (50 μM); e) The fluorescence image of d; f) The merged image of d and e; j) Bright-field image of the HeLa cells treated with **Na-FA-Lyso** (5 μM) and FA (50 μM); k) The fluorescence image of j; l) The merged image of j and k. Excitation was at 488nm and emission collection was from 500 - 550 nm. Scale bar: 20 μm.

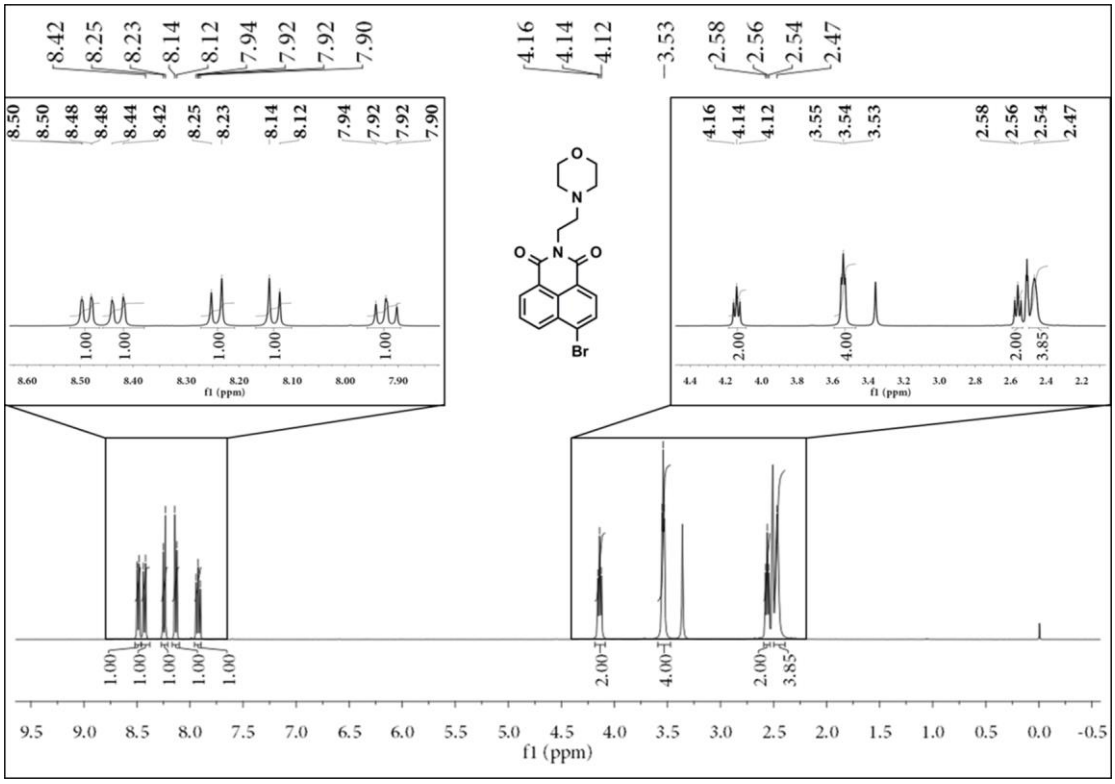


Figure S11. ^{1}H NMR spectrum of the compound **1**.

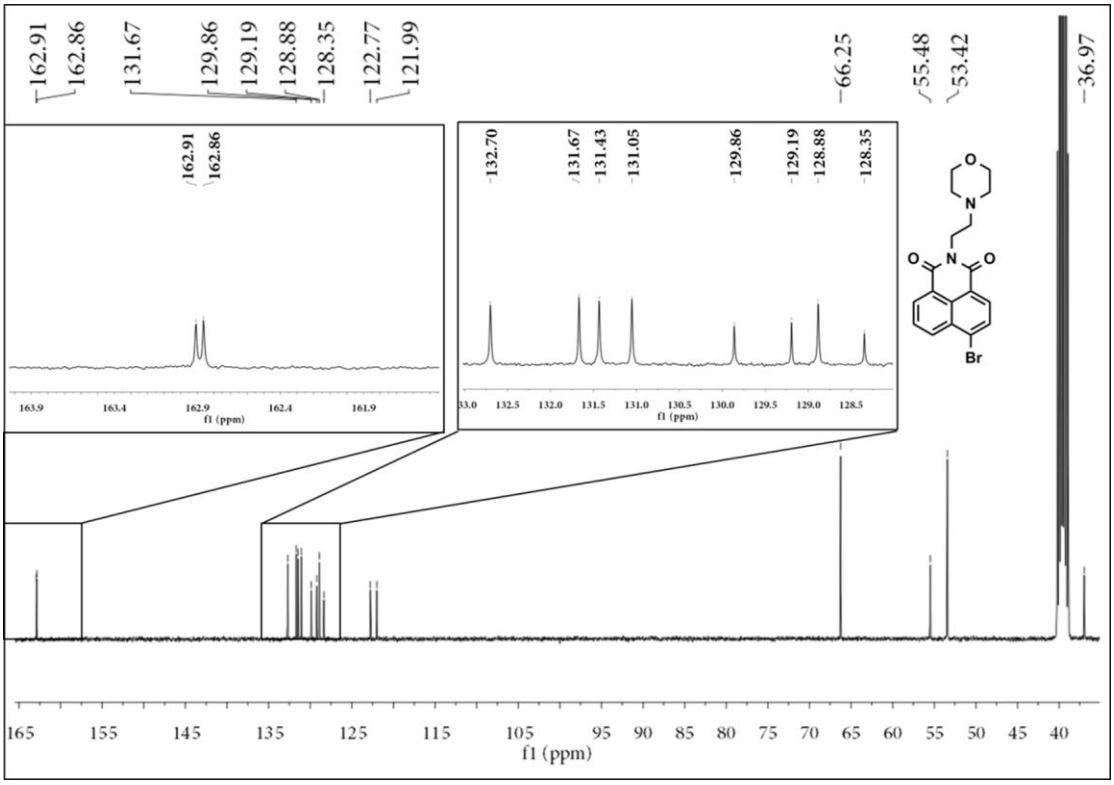


Figure S12. ^{13}C NMR spectrum of the compound **1**.

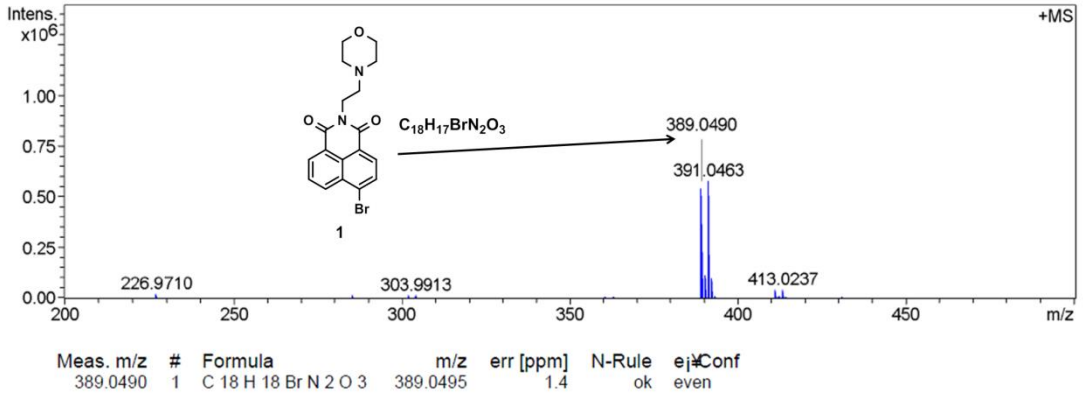


Figure S13. HR-MS spectrum of the compound **1**.

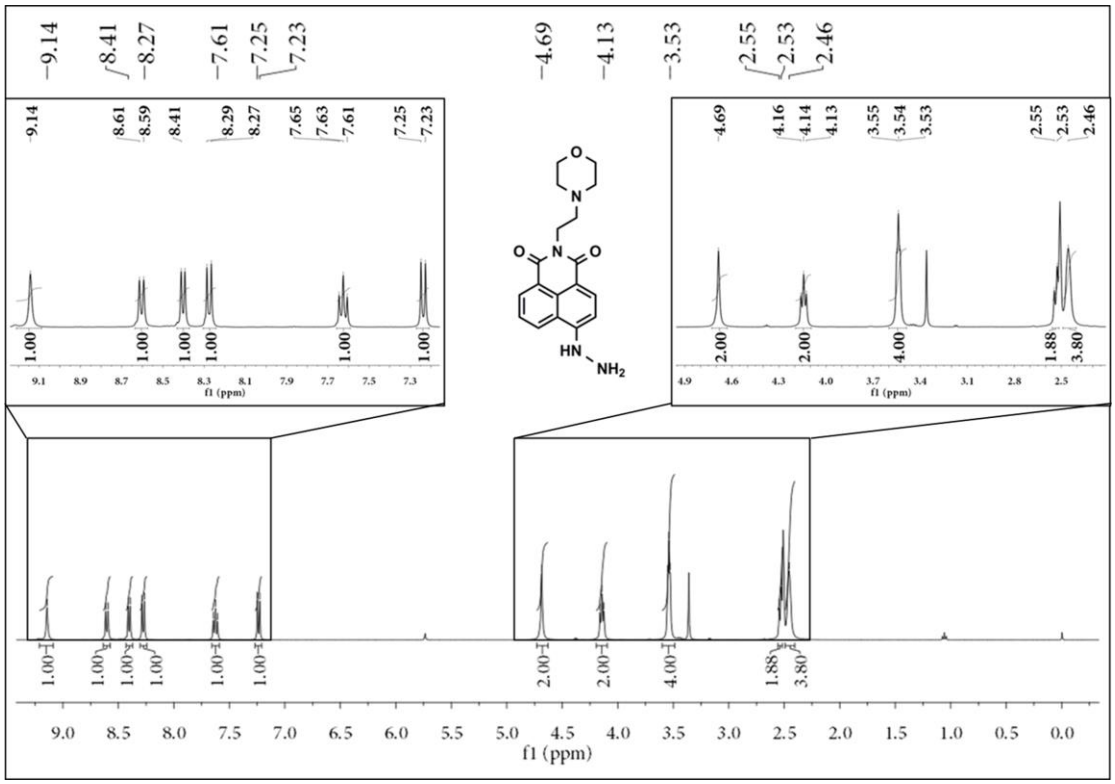


Figure S14. ^{1}H NMR spectrum of the probe **Na-FA-Lyso**.

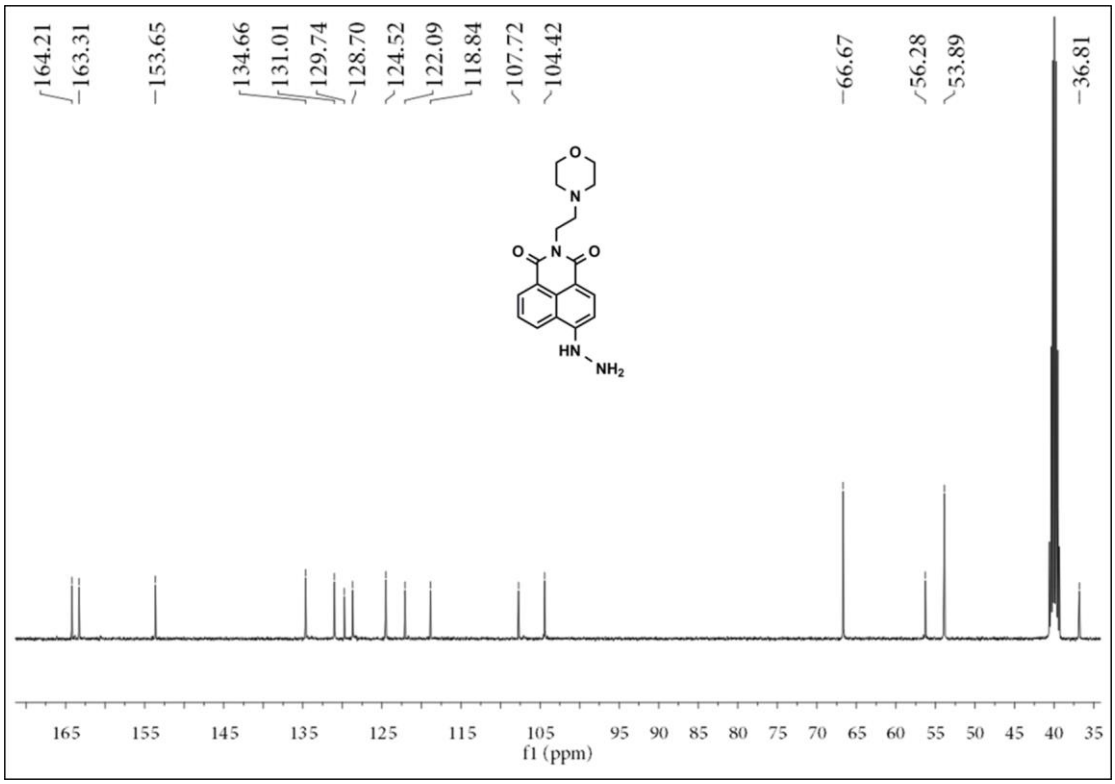


Figure S15. ^{13}C NMR spectrum of the probe **Na-FA-Lyso**.

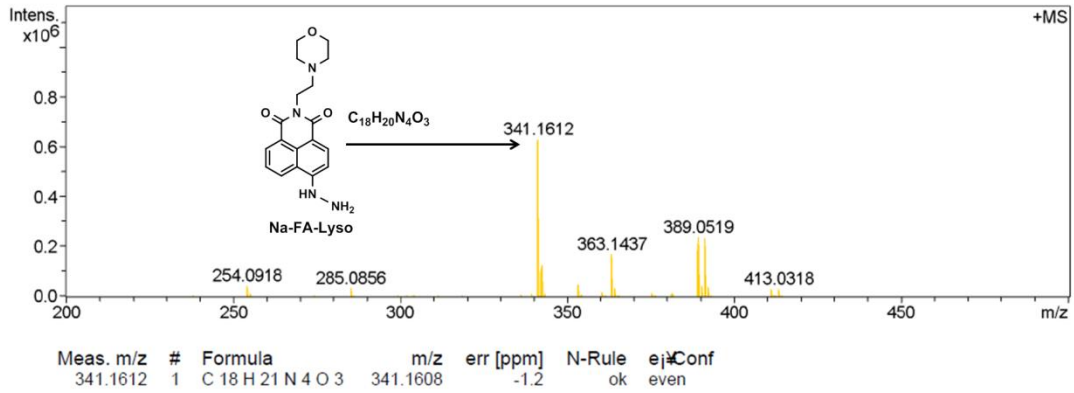


Figure S16. HR-MS spectrum of the probe **Na-FA-Lyso**.