Electronic Supplementary Material (ESI) for Analytical Methods.
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Supporting Information for

A turn-on fluorescent formaldehyde probe regulated by combinational PET and ICT mechanisms for bioimaging applications

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Instruments

Mass spectra were performed using an LCQ Advantage ion trap mass spectrometer from Thermo Finnigan; High resolution mass spectrometric (HRMS) analyses were measured on a Finnigan MAT 95 XP spectrometer; NMR spectra were recorded on an INOVA-400 spectrometer, using TMS as an internal standard; Electronic absorption spectra were obtained on a LabTech UV Power spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer; The optical density was measured by a Thermo Scientific Multiskan FC microplate reader in cytotoxicity assay; The fluorescence imaging of cells was performed with OLYMPUS FV1000 (TY1318) confocal microscopy; The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; The melting point was measured by YUHUA (X-5, FCE-3000 serials) melting point detector; 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.

Fluorescence quantum yield calculation

Fluorescence quantum yields of probe **Naph-FA** in the absence or presence were determined by using Fluorescein (Φ_f = 0.95 in 0.1 M NaOH aqueous solution) as fluorescence standard. The quantum yields can be calculated using the following equation.

$$\Phi_{F(X)} = \Phi_{F(S)} (A_S F_X / A_X F_S) (n_X / n_S)^2$$

Where Φ_F is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvent used. Subscripts $_S$ and $_X$ refer to the standard and to the unknown, respectively.

Determination of the detection limit

The detection limit was determined from the fluorescence titration data based on a reported method. **Naph-FA** (10.0 μM) was titrated with different concentrations of FA, the linear relationship between the emission intensity at 516 nm and the concentration of FA was fitted based on the fluorescence titration.

$$\text{Detection limit} = 3\sigma/k$$

Where σ is the standard deviation of the blank sample and 'k' is the slope of the linear regression equation.

Cells culture. HeLa cells were cultured in Dulbecco's Modified Eagle Medium media (DMEM, Hyclone) supplemented with 10 % heat-inactivated fetal bovine serum (FBS, Sijiqing) 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 at a density of 1×10^5. About 36 hours later, the cells reached about 70 % confluence for the further experiments.

Fluorescence imaging of FA in living cells

The cell imaging experiments were divided into control and experimental groups. As the control group, HeLa cells were incubated with **Naph-FA** (5 μM) for 30 min, then washed by PBS buffer before imaging. As the experimental groups, HeLa cells were incubated with **Naph-FA** (5 μM) for 30 min, followed by treatment with formaldehyde (500 or 1000 μM) for 180 min, and then washed by PBS buffer before imaging. The confocal microscopic imaging uses Nikon A1MP confocal microscope with an excitation filter of 405 nm and the collection wavelength range is from 530-575 nm (green channel).

Synthesis of Naph-FA. The starting compound **1** was well prepared via a previous method. (*J. Mater. Chem. B*, 2018, **6**, 580-585) The mixture of compound **1** (254 mg, 1 mmol) and 25% ammonia (756 μL, 10 mmol) in 5 mL of methanol was stirred at 0 °C for 30 min. After warming to room temperature, allylboronic acid pinacol ester (203 mg, 1.2 mmol) was added and continued to stir about 10 hours. The solvent was removed under reduced pressure and the solid residue was purified by flash chromatography column using methanol/dichloromethane (v/v 1:25) to afford a pale yellow solid as compound **Naph-FA** (163 mg, yield 46%). Melting point: 107-114 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 2.70 (4H), 2.70-2.73 (t, *J* = 7.0 Hz, 2H), 2.76-2.96 (m, 2H), 3.71-3.73 (t, *J* = 4.4 Hz, 4H), 4.28-4.31 (t, *J* = 6.8 Hz, 2H), 4.53-4.57 (t, *J* = 7.6 Hz, 1H), 5.14-5.23 (2H), 5.76-5.85 (1H), 7.41-7.45 (t, *J* = 6.8 Hz, 1H), 8.05-8.06 (s, 1H), 8.31-8.32 (d, *J* = 4.4 Hz, 1H), 8.46-8.47 (d, *J* = 4.0 Hz, 1H); ^{1}H NMR (400 MHz, pydine-d_5) δ 2.59 (4H), 2.83-2.86 (t, *J* = 6.6 Hz, 2H), 3.00-3.14 (2H), 3.69 (4H), 4.63-4.66 (t, *J* = 6.8 Hz, 2H), 4.87-4.90 (t, *J* = 6.6 Hz, 1H), 5.09-5.19 (2H), 5.91-6.01 (1H), 7.48-7.51 (t, *J* = 7.0 Hz, 1H), 8.68 (s, 1H), 8.76-8.78 (d, *J* = 7.6 Hz, 1H), 9.03-9.05 (d, *J* = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 37.69, 38.10, 54.72, 54.98, 57.48, 67.70, 103.58, 119.74, 122.34, 123.88, 128.78, 132.36, 132.48, 132.74, 134.41, 135.34, 165.84, 166.76. HRMS (ESI) m/z calcd for $C_{22}H_{25}N_3O_4$ $[M + H]^+$: 396.1918. Found 396.1915.

Synthesis of compound Nap-CHO. Compound **Naph-FA** (40 mg, 0.1 mmol) was dissolved absolutely in methanol (5 mL), and FA (150 μL, 40% w%) diluted in 1 mL methanol was added and stirred for 4 hour at room temperature. After complete reaction, 50 mL water was added into the flask and the mixture was extracted with dichloromethane (3×25 mL). The organic layer was collected, and treated with anhydrous Na_2SO_4, then the solvent was evaporated under the reduced pressure affording the crude product, which was purified by flash chromatography column using dichloromethane/ethanol (v/v 25:1) to afford deep yellow solid as compound **Nap-CHO** (12 mg, yield 34%). Melting point: 112-117 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 2.64 (4H), 2.69-2.73 (t, *J* = 7.0 Hz, 2H), 3.69-3.71 (t, *J* = 4.6 Hz, 4H), 4.31-4.35 (t, *J* = 7.0 Hz, 2H), 7.54-7.58 (t, *J* = 7.6 Hz, 1H), 8.46-8.48 (d, *J* = 7.2 Hz, 1H), 8.68-8.70 (d, *J* = 7.6 Hz, 1H), 8.73 (s, 1H), 10.34 (s, 1H).

Table S1. Summary of the properties of representative fluorescent probes for detecting FA.

Probes	Chemical structures	Emission wavelength/nm	Limit of detection/M	Sensing mechanism	Samples of bioimaging application	References
FP1		649	1×10^{-5}	d-PET	HEK293TN and NS1 cells	1
FAP-1		662	5×10^{-6}	Ring-opened	HEK293T and MCF7 cells	2
6		518	1×10^{-5}	ICT	HEK293T and HAP1 cells	3
RFAP-2		420/470 (excitation wavelength)	3×10^{-7}	Not mentioned	HEK293T, HeLa, MCF-7, MCF-10A, RKO, SHSY5Y, and U-2OS and HAP1 cells	4
RRFP		359/451	6.0×10^{-5}	ICT	HeLa cells	5
FATP1		526	2×10^{-7}	d-PET	HEK-293 and MCF-7 cells and rat liver tissues	6
PIPBA		440	8.4×10^{-7}	ICT	HeLa cells and zebrafish	7
1		438/533	1×10^{-5}	ICT	MCF7 cells and rat organ tissues	8
HBT-FA		462/541	4.1×10^{-4}	ICT/ESIPT	Not mentioned	9

Na-FA		543	7.1×10^{-7}	PET	HeLa cells and rat liver tissues	10
R6-FA		560	7.7×10^{-7}	Ring-opened	HeLa cells	11
Naph-FA		518	5.5×10^{-6}	PET and ICT	HeLa cells	This work

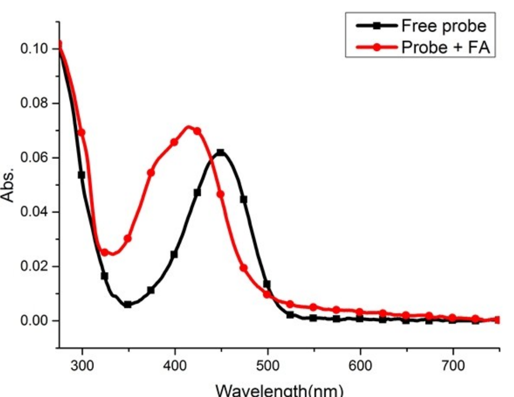

Fig. S1. Absorption spectra of **Naph-FA** (10 μM) with FA (200.0 equiv) in PBS buffer (25 mM, pH 7.4, containing 20 % DMSO).

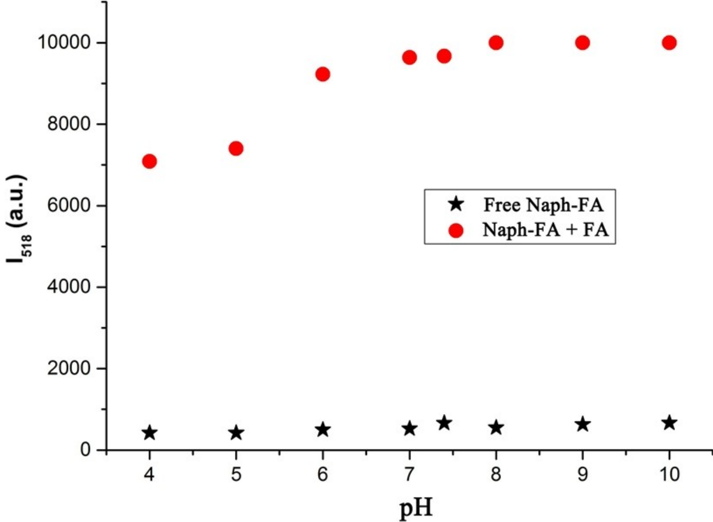

Fig. S2 The pH influence on the fluorescence intensity of **Naph-FA** (10 μM) at 518 nm in the absence or presence of FA (2 mM).

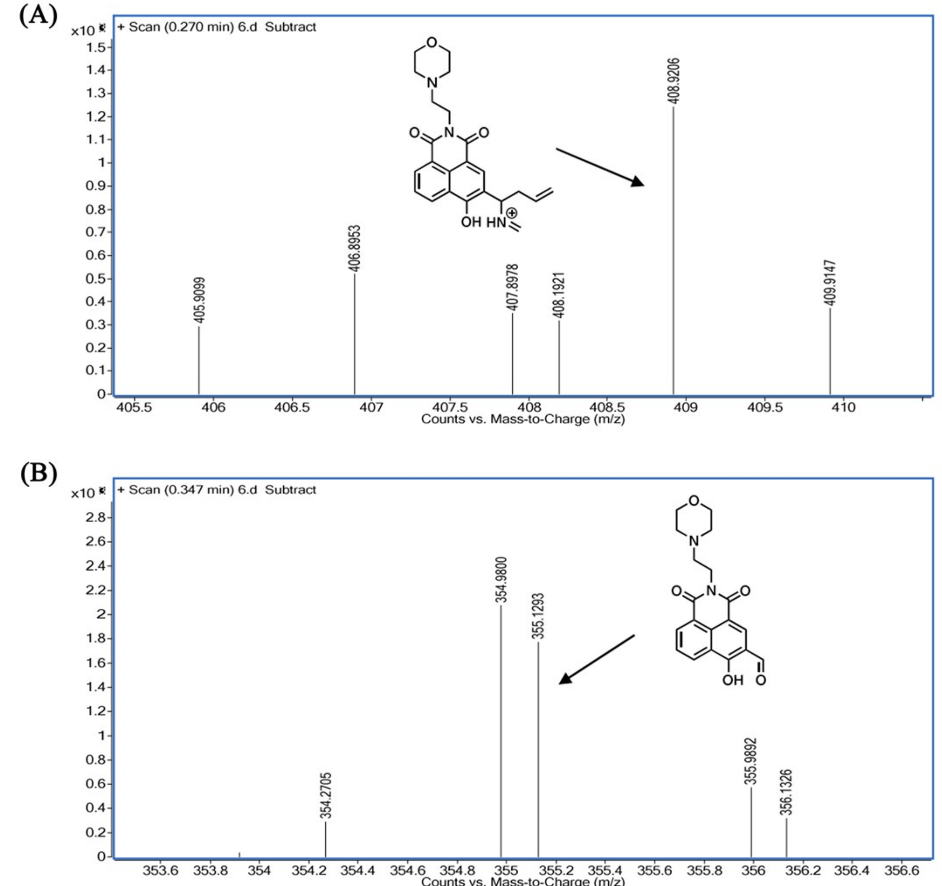

Fig. S3 Mass spectra (ESI) of **Naph-FA** in the presence of FA in aqueous solution. (A) The peak of the imine intermediate (*m/z* 408.9206); (B) The peak of the aldehyde product (*m/z* 355.1293).

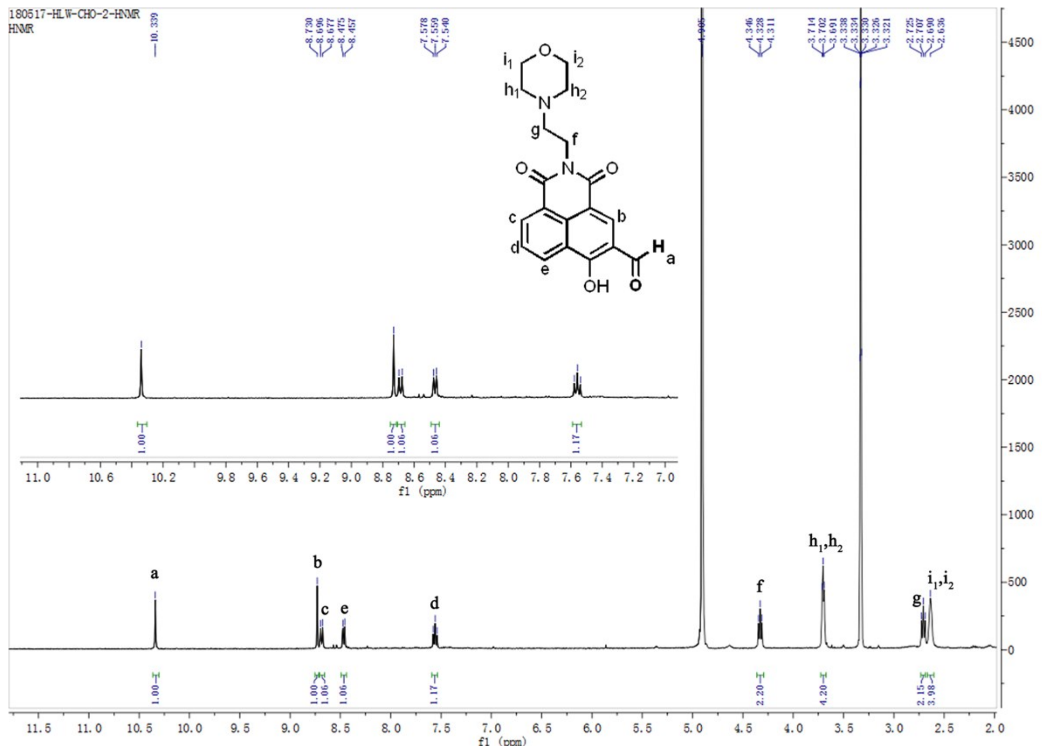

Fig. S4. ^{1}H NMR of the product isolated from the reaction of probe **Naph-FA** with FA in CD_3OD.

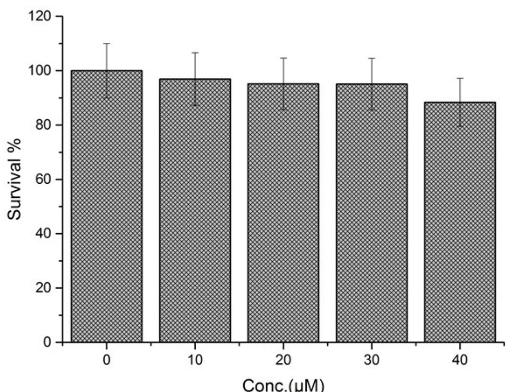

Fig. S5. Cell viability of HeLa cells incubated with probe **Naph-FA** of different concentration (0, 10, 20, 30, or 40 μM) for **24** h.

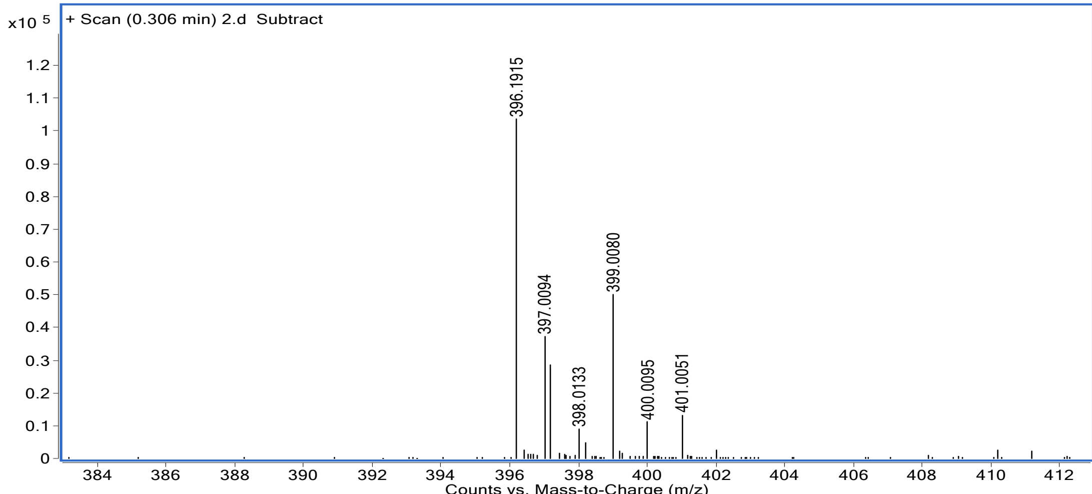

Fig. S6 HRMS (ESI) of compound **Naph-FA**. m/z calcd for $C_{22}H_{25}N_3O_4$ $[M + H]^+$: 396.1918. Found 396.1915.

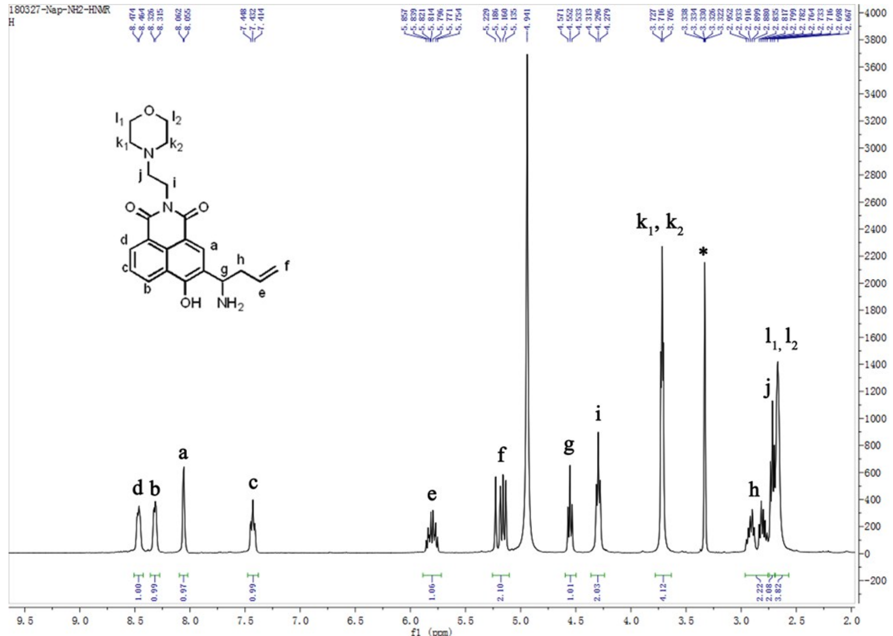

Fig. S7 ^1H NMR spectrum of **Naph-FA** in CD_3OD. Symbol * represents the solvent peak of CD_3OD.

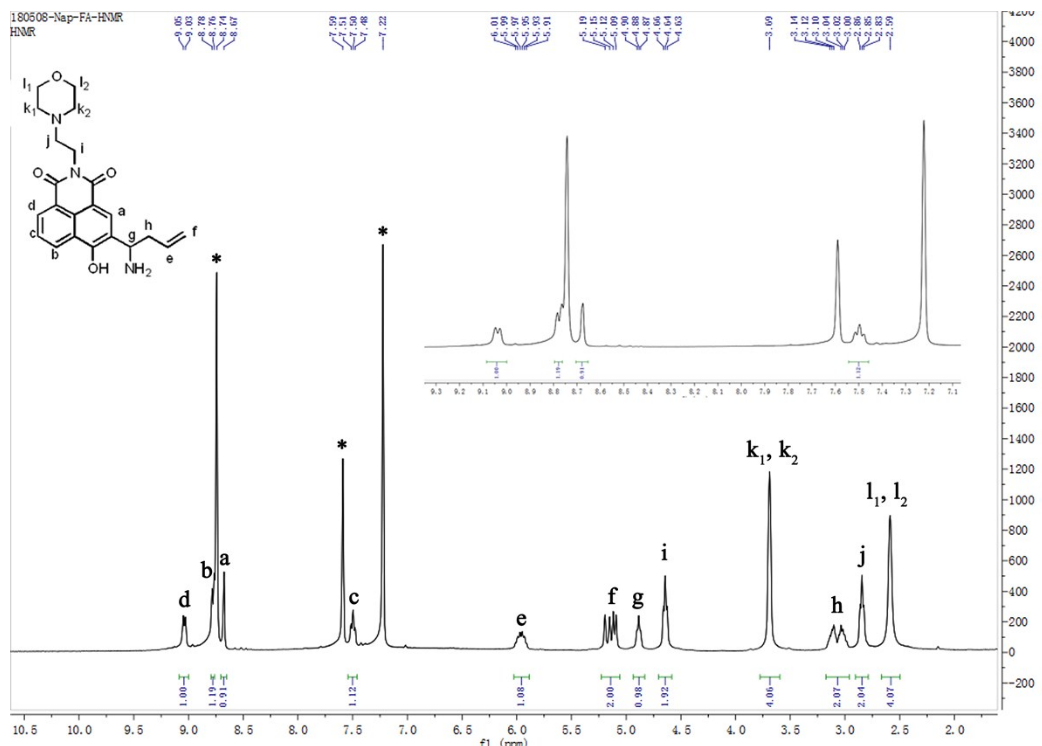

Fig. S8 ^{1}H NMR spectrum of **Naph-FA** in pydine-d_5. Symbol * represents the solvent peak of pydine-d_5.

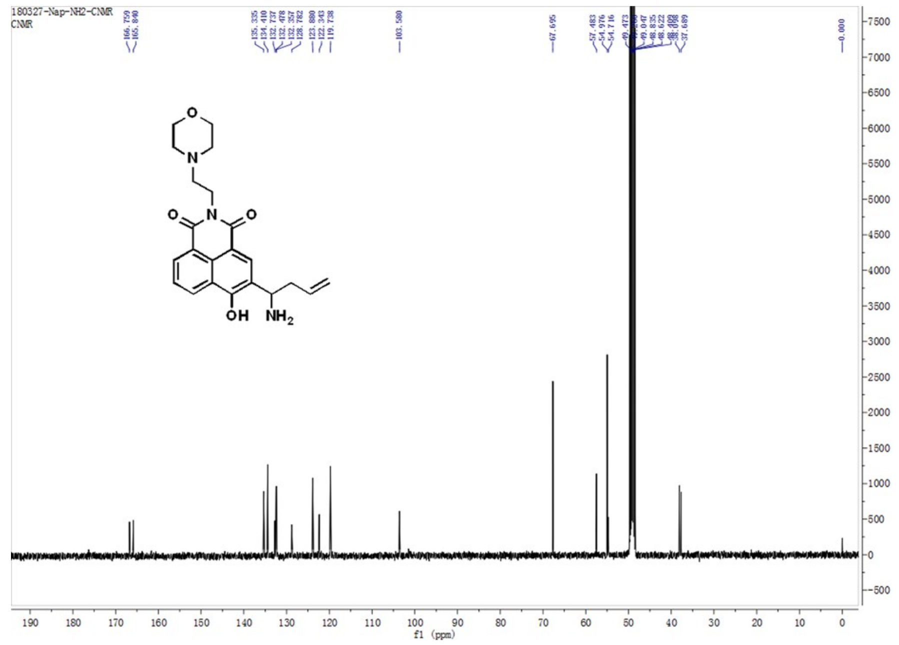

Fig. S9 ^{13}C NMR spectrum of **Naph-FA** in CD_3OD.

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