Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Visible Light Responsive Photoacids for Subcellular pH and Temperature Correlated Fluorescence Sensing

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Experimental section

Reagents:

Methanol, ethanol, toluene, dimethyl sulfoxide (DMSO), and 5-nitrosalicylaldehyde were obtained from J&k Scientific Ltd in China. 2,3,3-trimethylindolenine, 2-hydroxy-5-methoxybenzaldehye, and propane sultone were obtained from Bidepharmatech in China. Boric acid, critic acid, sodium phosphate monobasic monohydrate, and trifluoroacetic acid (TFA) were obtained from Sigma-Aldrich (Merck). DMSO-d6 was obtained from Cambridge Isotope Laboratories, Inc. The Dulbecco's modified Eagle's medium (DMEM, with 4.5 g/L glucose), sterile phosphate buffered saline (PBS), sterile fetal bovine serum (FBS), penicillin-streptomycin solution, and LysoTracker Deep Red were obtained from Thermal Fisher Scientific in China. Cell Counting Kit-8 (CCK8) was obtained from GLPBIO. Bafilomycin A1, 3-allyl-2-hydroxybenzaldehyde, and sodium hydroxide were obtained from MACKLIN in Shanghai, China. Deionized water was used after purification by Milli-Q Integral 5.

Instrumentation:

Nuclear magnetic resonance spectra (^{1}H and ^{13}C NMR) were recorded on Bruker AVANCE NEO 400 with tetramethylsilane as the internal reference. Mass spectra data were obtained on a Q-Exactive electrospray ionization mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). UV-Vis absorption spectra were measured with a light absorption spectrometer (Thermo Fisher Scientific, Evolution 220). Kinetic absorbance changes were measured on the same spectrometer equipped with a temperature control element (Thermo Fisher Scientific, PCCU1), with an additional LED source (Thorlabs, LED4D232) to provide irradiation at 455 nm through an optical fiber. Fluorescence spectra were measured on a fluorescence spectrometer (Edinburgh, FLS 1000). Photographs of the PAs were captured with a digital camera (Canon EOS 5D Mark IV). Fluorescence imaging of HeLa cells was performed on a confocal microscope (Zeiss, LSM 900) with a 63× objective lens. The stage of confocal microscope was equipped with micro cell incubator composed of Gas mixer CO_2/O_2 (ibidi Gas Incubation System for CO_2/O_2) and temperature controller (ibidi Heating System, Universal Fit for 1 chamber).

Synthesis of PAs:

Intermediate 1: A solution of 2,3,3-trimethylindolenine (2.38 g, 15 mmol) and propane sultone (2.44 g, 20 mmol) in toluene (20 mL) was stirred overnight at 120 °C under a nitrogen atmosphere and reflux. The intermediate 1 in Scheme 1 was washed with petroleum ether under vacuum filtration. The product intermediate 1 was collected as a dry purple powder and used without further purification. Yield: 62%. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.05 (dd, J = 6.3, 2.4 Hz, 1H), 7.86 – 7.78 (m, 1H), 7.68 – 7.57 (m, 2H), 4.70 – 4.61 (m, 2H), 2.83 (s, 3H), 2.62 (t, J = 6.5 Hz, 2H), 2.11 (d, J = 30.2 Hz, 2H), 1.53 (s, 6H).

PA-allyl: A solution of the intermediate 1 (281 mg, 1 mmol) and 3-allyl-2-

hydroxybenzaldehyde (162 mg, 1.5 mmol) in ethanol (10 mL) was stirred overnight at 90 °C under a nitrogen atmosphere. After cooling, the precipitation was filtered and washed with ethanol under vacuum filtration. The product PA-allyl was collected as a sticky tawny solid. Yield: 45%.

^{1}H NMR (400 MHz, DMSO-d6) δ 7.09 (t, *J* = 7.0 Hz, 2H), 7.05 – 7.01 (m, 1H), 7.01 – 6.94 (m, 2H), 6.77 (d, *J* = 7.6 Hz, 2H), 6.74 (d, *J* = 7.7 Hz, 1H), 6.63 (d, *J* = 7.9 Hz, 1H), 5.75 (d, *J* = 10.1 Hz, 1H), 4.82 (d, *J* = 9.7 Hz, 1H), 4.71 (d, *J* = 17.0 Hz, 1H), 3.25 – 3.04 (m, 2H), 2.99 (t, *J* = 7.1 Hz, 2H), 2.42 (ddd, *J* = 14.7, 9.2, 6.0 Hz, 2H), 1.85 (dd, *J* = 14.6, 7.9 Hz, 2H), 1.20 (s, 3H), 1.10 (s, 3H).

ESI-MS data: Calculated $[M+H]^+$= 426.1734 (z=1) Founded $[M+H]^+$= 426.1720 (z=1)

PA-NO$_2$: A solution of the intermediate 1 (281 mg, 1 mmol) and 5-nitrosalicylaldehyde (167 mg, 1.5 mmol) in ethanol (10 mL) was stirred overnight at 90 °C under a nitrogen atmosphere. After cooling, the precipitate was filtered and washed with ethanol under vacuum filtration. The product PA-NO$_2$ was collected as a sticky tawny solid product. Yield:35%.

^{1}H NMR (400 MHz, DMSO-d6) δ 9.10 (d, *J* = 2.9 Hz, 1H), 8.50 (d, *J* = 16.4 Hz, 1H), 8.31 (dd, *J* = 9.2, 2.8 Hz, 1H), 8.15 – 8.02 (m, 2H), 7.97 – 7.84 (m, 1H), 7.72 – 7.57 (m, 2H), 7.21 (d, *J* = 9.2 Hz, 1H), 4.95 – 4.70 (m, 2H), 2.64 (t, *J* = 6.8 Hz, 2H), 2.20 (p, *J* = 7.0 Hz, 2H), 1.80 (s, 6H).

ESI-MS data: Calculated $[M+H]^+$= 431.1271 (z=1) Founded $[M+H]^+$= 431.1258 (z=1)

PA-OMe: A solution of the intermediate 1 (281 mg, 1 mmol) and 2-hydroxy-5-methoxybenzaldehyde (228 mg, 1.5 mmol) in ethanol (10 mL) was stirred for overnight at 90 °C under a nitrogen atmosphere and reflux. After cooling, the precipitate was filtered and washed with ethanol. The solid was collected and dried to produce PA-OMe as a dry tangerine solid. Yield: 34%.^{1}H NMR (400 MHz, DMSO-d6) δ 10.62 (s, 1H), 8.60 (d, *J* = 16.3 Hz, 1H), 8.00 (dd, *J* = 6.9, 1.8 Hz, 1H), 7.96 (d, *J* = 16.3 Hz, 1H), 7.90 – 7.81 (m, 2H), 7.62 (tt, *J* = 7.4, 6.1 Hz, 2H), 7.10 (dd, *J* = 9.0, 3.0 Hz, 1H), 6.97 (d, *J* = 9.0 Hz, 1H), 4.86 (t, *J* = 7.8 Hz, 2H), 3.87 (s, 3H), 2.69 – 2.62 (m, 2H), 2.26 – 2.15 (m, 2H), 1.77 (s, 6H).

ESI-MS data: Calculated $[M+H]^+$= 416.1526 (z=1) Founded $[M+H]^+$= 416.1532 (z=1)

Cell Culture and Experiments:

The HeLa cell lines were originally obtained from the Procell Life Science&Technology Co. Ltd. and maintained in DMEM supplemented with 10% of FBS and 100 μL/mL penicillin-streptomycin. Cells were incubated in a humidified environment with 5% CO_2 at 37 °C.

For co-localization, HeLa cells were seeded in culture dishes with glass bottom for 24 h followed by incubation with 1 μL of PA-allyl solution dissolved in DMSO (10 mM) for 8 min. Then the cells were washed with PBS solution and further incubated with LysoTracker Deep Red for 5 min. After washing with PBS three times, the samples were imaged under the confocal microscope (LSM 900) with excitation at 488 and 640 nm.

For experiments in Fig. 5, HeLa cells were seeded in culture dishes with glass bottom for 24 h followed by incubation with 1 μL of PA-allyl solution (10 mM) dissolved in DMSO for 8 min

and rinsed by PBS solution three times.

- To monitor the pH changes, 50 nM bafilomycin A1 was incubated with cells for 20 min under the same condition. The emission signals from 500-600 nm and 600-700 nm were collected under 488 nm and 561 nm excitation, respectively.
- To monitor the temperature changes, the ibidi Temperature Controller was used on the stage of the confocal microscope to adjust the temperature. The HeLa cells were first irradiated at 488 nm for 10 s. The laser was then switched off and Time-lapse images were captured with different time delays and the excitation light at 488 nm was just temporarily switched on to bring minimum perturbation. Fluorescent signals from 500-620 nm were collected.

CCK-8 assay was used to measure the cellular metabolic potential of treated cells after different concentrations of PA-allyl solution treatment.

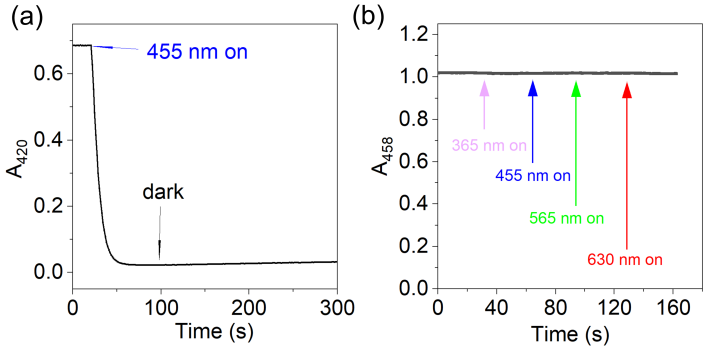


Fig. S1 (a) Evolution of absorbance at 420 nm of the PA-NO_2 at different light condition illumination. (25.0 ℃) (b) Evolution of absorbance at 458 nm of the PA-OMe at different light condition illumination. (25.0 ℃)

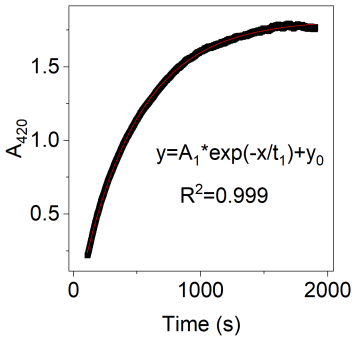


Fig. S2 The mono-exponential fitting of the kinetic absorbance evolution at 420 nm of PA-allyl (0.3 mM in universal buffer) solution at 10 ℃.

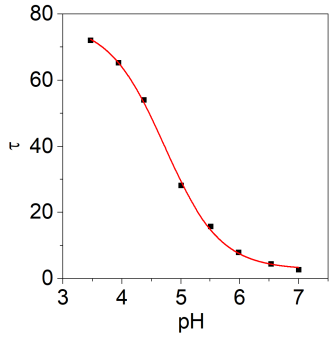


Fig. S3 The lifetime of PA-allyl (0.3 mM in universal buffer) solution determined at different pH values. (25.0 ℃)

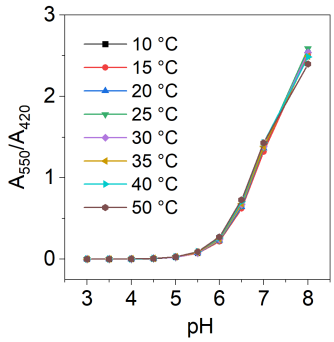


Fig. S4 Evaluation of the influence of temperature on the pH response of PA-allyl (0.2 mM in universal buffer) solution. The absorbance ratio of 550 nm over 420 nm was plotted against pH.

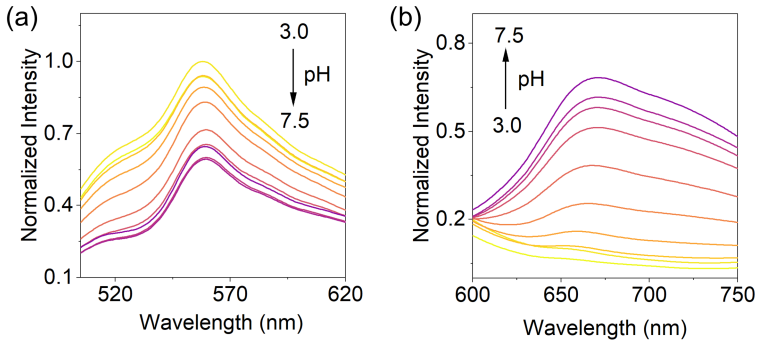


Fig. S5 The fluorescence emission spectra of PA-allyl (0.3 mM in universal buffer) adjusted to different pH values, excited by 488 nm(b) and 561 nm (c), respectively. (25.0 ℃)

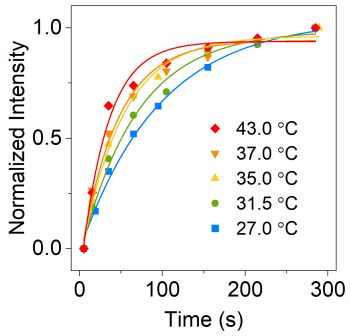


Fig. S6 The fluorescence thermal relaxation curves of PA-allyl in HeLa cells at 27.0 ℃to 43.0 ℃, fitting to mono-exponential decay function.

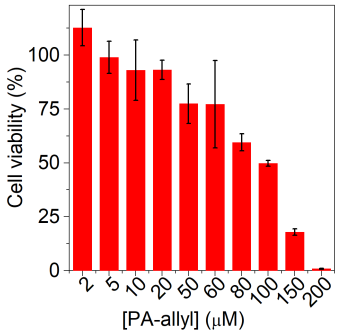
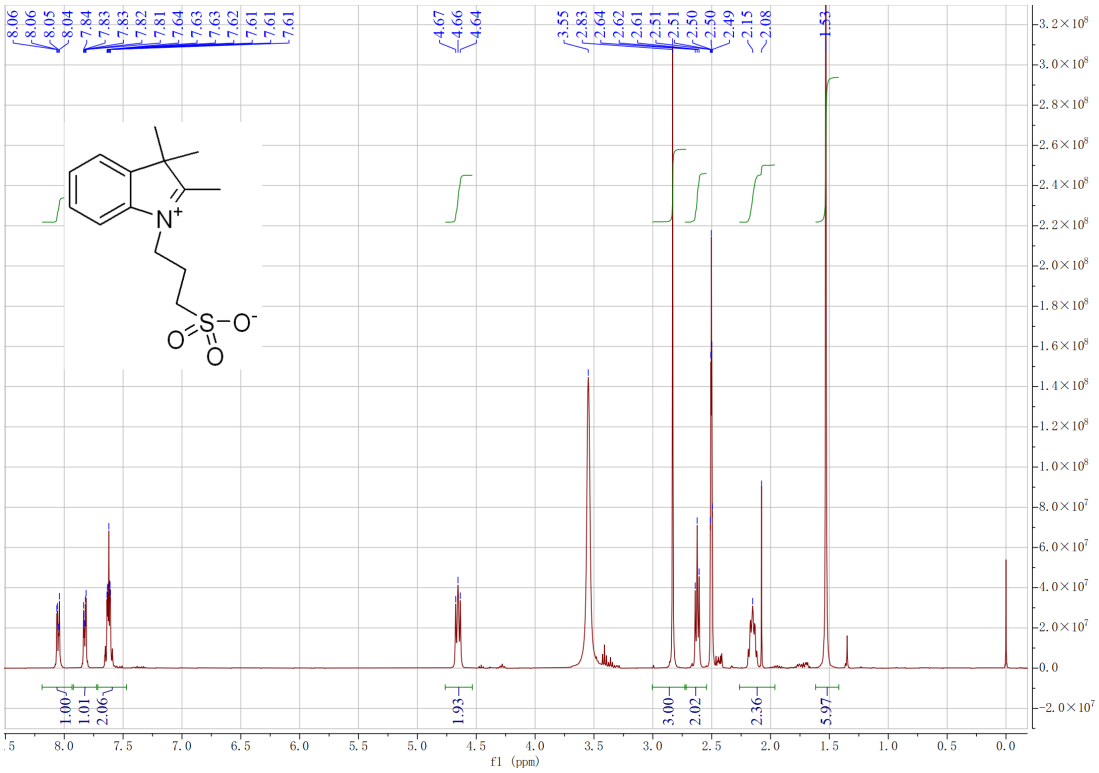
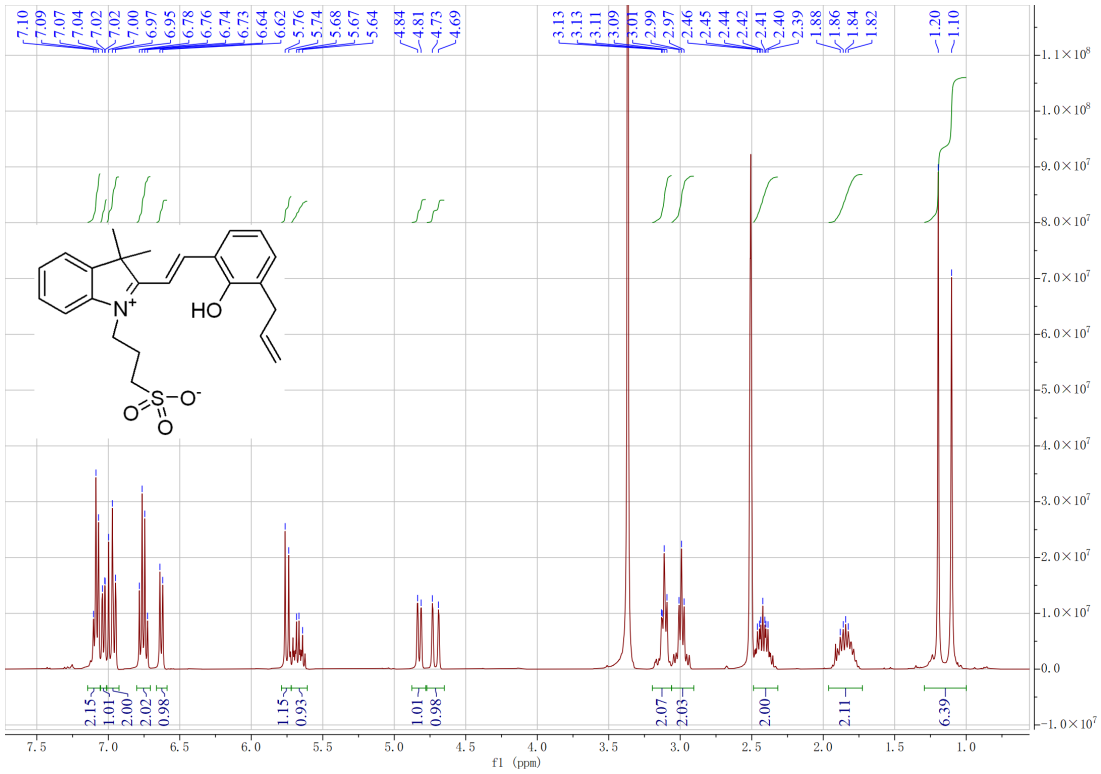


Fig. S7 The viability of HeLa cells after incubation with the indicated concentrations of PA-allyl in DMEM at room temperature.

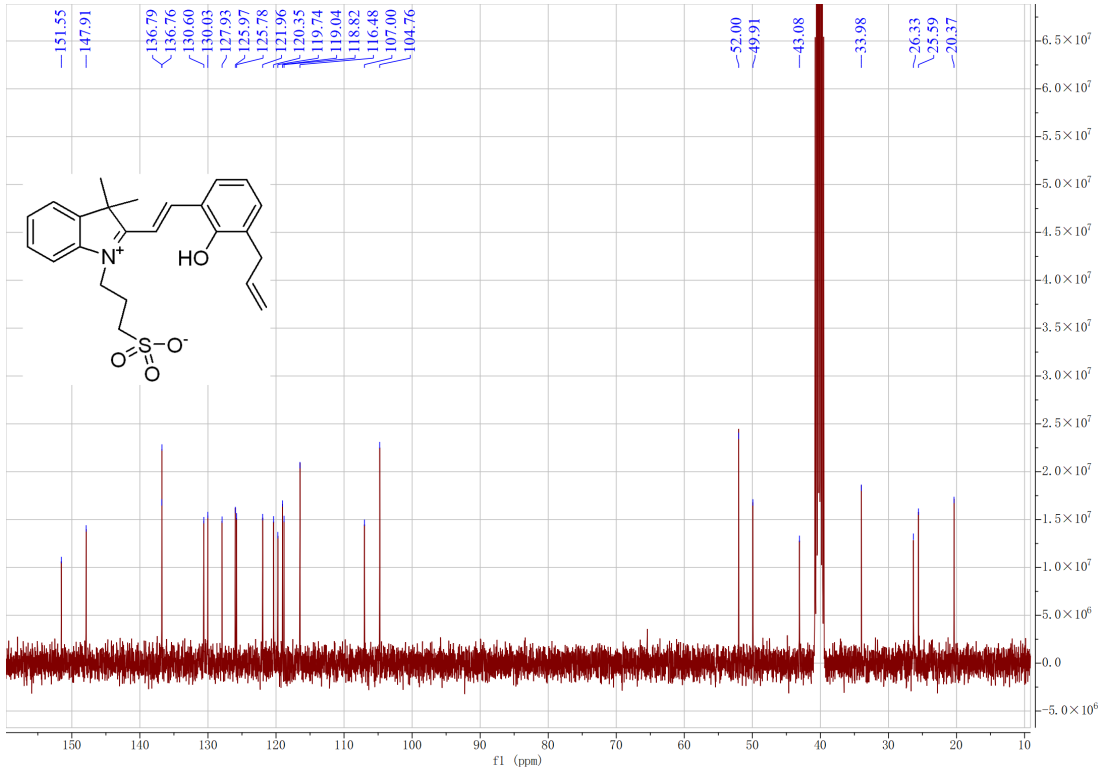
^{1}H NMR spectrum of the intermediate 1.



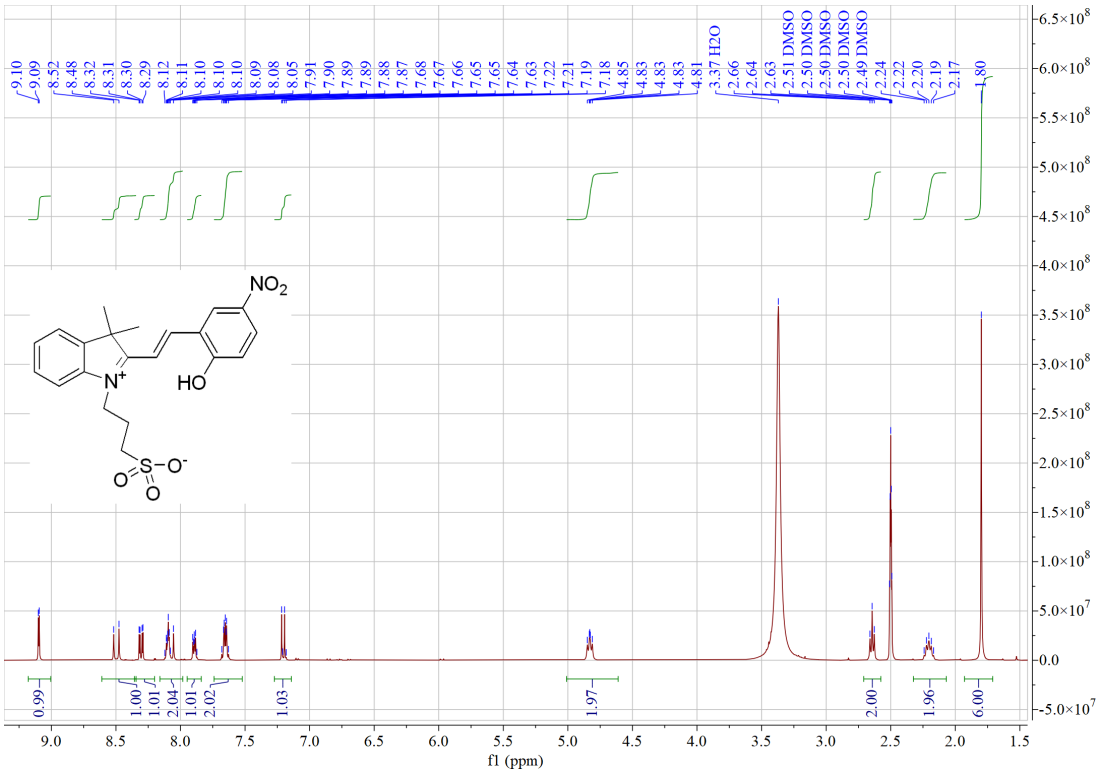
^{1}H NMR spectrum of PA-allyl.



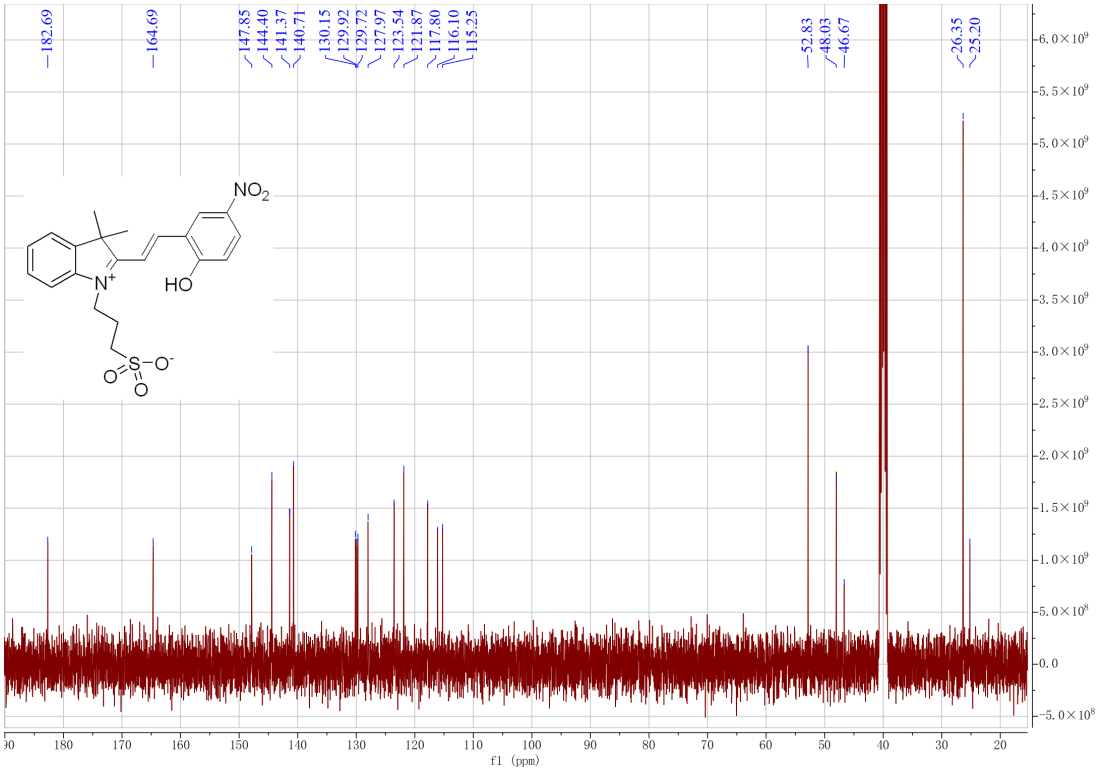
^{13}C NMR spectrum of PA-allyl.



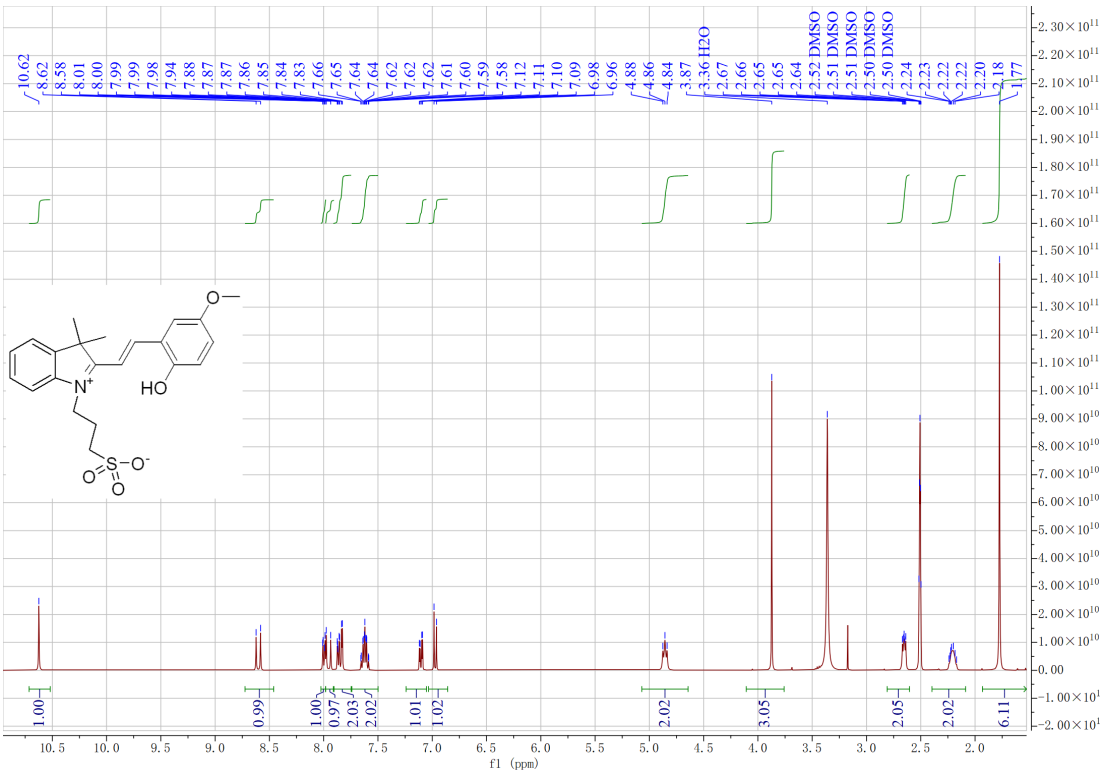
^{1}H NMR spectra of PA-NO_2.



^{13}C NMR spectrum of PA-NO_2.



^{1}H NMR spectra of PA-OMe.



^{13}C NMR spectrum of PA-OMe.