Acyl-caged rhodamines: Photo-controlled and self-calibrated generation of acetyl radical for neural function recovery in early AD mouse

Xiao Luo[1, ‡, *], Zhonghui Zhang[1, ‡], Jie Wang[3, ‡], Xueli Wang[4], Yani Zhang[5], Jinquan Chen[4], Guangbo Ge[5], Wen Yang[3,*], Xuhong Qian[1,2,*], Yang Tian[1,*], Youjun Yang[2,*]

[‡] These authors contributed equally to this work

Table of Contents

Experimental Procedures

General methods

Chemicals were purchased from major venders based in China and used without further purification. Analytical grade solvents were from Titan Scientific, China. THF was dried over sodium/benzoketyl still. The progresses of reactions were monitored by thin layer chromatography (TLC). Column chromatography was performed with silica gel (300-400 mesh). The ^{1}H-NMR and ^{13}C-NMR spectra were collected on a Bruker AV-400/Ascend 600 spectrometer. Chemicals shifts are referenced to the residue solvent peaks and given in parts per million (ppm). HR-MS spectra were acquired on a Micromass GCT spectrometer. Absorption spectra were collected by a SHIMADZU UV-2600 UV-vis spectrophotometer. Fluorescence excitation and emission spectra were collected in a PTI-QM4 steady-stead fluorimeter with a 75 W Xenon arc-lamp and a model 810 PMT, the excitation and emission slits are both 2 nm. The voltage of PMT was 950 V. The excitation and emission slits were set to 2 nm and all emission spectra were corrected. The pH values were measured by a pH-meter (FE20-FiveEasy Plus) from METTLER TOLEDO.

ACRs refers to acyl-caged rhodamines, i.e. **ACR575a**, **ACR575p**, **ACR670a** and **ACR670b**, in which "***a***" is for acetyl radical and "***p***" is for pentanoyl radical. And 575/670 refer to the fluorescent emission maximum (nm) of rhodamine **8** or Si-rhodamine.

Photoirradiation of ACRs in cuvettes

The stock DMF solution of **ACRs** at 1 mM were prepared. The **ACRs** solution of various concentrations were prepared by diluting this stock solution into various solvents (MeCN, EtOH, DMSO, AcOH, phosphate buffer (pH = 7.4, 50 mM) with 10% MeCN as co-solvent). A 375 ± 25 nm LED (20 mW cm^{-2} at 375 nm) was used as the irradiation light source. The prepared solution in a cuvette was placed on a magnetic stirrer. After each irradiation, the absorption spectrum and the emission spectrum of the prepared solution were then acquired. The irradiation was terminated until the absorption and fluorescence spectra stop increasing. The maximum absorption or maximum fluorescence intensity of each acquired spectrum was plotted against the irradiation time (min).

Uncaging quantum efficiency determination

The uncaging quantum efficiency was determined using a previously reported method[1] with BisCMNB-FL as the reference. **ACRs** (1 μM) or BisCMNB-FL (1 μM) in sodium phosphate buffer (50 mM, pH = 7.4) with 10% MeCN was irradiated at 290 nm (0.6 mW/cm^2 at 290 nm) with stirring for 5 min and 10 min. Then the absorption spectrum of the solution was acquired. The remaining caged concentration was calculated by the following equation:

$$\text{Remaining concentration of } \textbf{ACR575a/p} \ (\mu M) = 1\ (\mu M) - \text{Abs@556}/\varepsilon_{556\,nm}^{rhodamine\ 8}\ (\mu M)$$

$$\text{Remaining concentration of } \textbf{ACR670a/p} \ (\mu M) = 1\ (\mu M) - \text{Abs@655}/\varepsilon_{655\,nm}^{Si-rhodamine}\ (\mu M)$$

Remaining concentration of fluorescein (μM) = 1 (μM) – Abs@490/ $\varepsilon^{fluorescein}_{490\,nm}$ (μM)

$\varepsilon^{rhodamine\,8}_{556\,nm}$ = 1.003 x 10^5 (cm^{-1}•M^{-1})

$\varepsilon^{Si-rhodamine}_{655\,nm}$ = 1.163 x 10^5 (cm^{-1}•M^{-1})

$\varepsilon^{fluorescein}_{490\,nm}$ = 0.833 x 10^5 (cm^{-1}•M^{-1})

The remaining caged percent was then plotted with irradiation time (min) and fitted to a linear equation with the slope (k^{ACR} and $k^{BisCMNB\text{-}FL}$) calculated. The uncaging quantum efficiency of **ACR** was calculated according to the equation in Figure S5.

Transient absorption measurement

Femtosecond transient absorption spectra of **ACRs** were obtained by a transient absorption spectrometer (Helios fire, Ultrafast System). The pump beam was the generated 330 nm pulses with a power of 0.1 mW.

Electron paramagnetic resonance (EPR) studies

EPR spectra were recorded on a on Bruker EMX instrument EMXPLUS-10/12 (Bruker Analystische Messtechnick GMBH, Germany). The spectra were measured at 298 K. Typical instrumental conditions were as follows: central field, 3506.7 G; modulation frequency, 100 kHz; modulation amplitude, 1.00 G; sweep width, 100 G; microwave power, 2 mW; sweep time, 60.0 s; conversion time, 58.60 ms; time constant, 0 ms. A solution of 1 mM **ACRs** and 10 mM DMPO (5,5-Dimethyl-1-pyrroline N-oxide) was prepared. The sample solution was encapsulated in a 0.9 mm × 80.0 mm capillary tube before being placed in a EPR sample tube with air and irradiated *in situ* with a UV light (LOT-Quantum Design GmbH) for 8 s or 16 s before data acquisition.

Measurement of ROS released by photoirradiation of ACR575a

The highly oxidative species released from **ACR575a** under irradiation with a 405 nm laser (10 mw cm^{-2}) for different time was measured by a previously reported ratiometric nanosensor[2]. The ROS concentration was determined by the fluorescence intensity ratio at 615 nm and 800 nm.

Cell culture

HeLa cell culture.

HeLa cells were purchased from Cell Bank of Type Culture Collection of Chinese Academy of Sciences. HeLa cells were all maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Hyclone) and 1% penicillin-streptomycin. The cells

were cultured in a humidified atmosphere of 5% CO_2/95% air at 37 °C and grown on 25 mm cover slips (Fisherbrand, 12-545-102) for 1–2 days to reach 70–90% confluency before use.

Neural stem cell (NSC) isolation and culture.

The embryos of pregnant C57/BL-6 wild mice on embryonic day 13 were dissected and put into precooling HBSS buffer. The brain was dissected under the microscope. After stripping the meningeal and blood vessels, the tissue was cut into small pieces and filtered. The filtered cells were collected and dispersed in the proliferation medium and cultured in 37 °C under 5% CO_2. Observe and culture until about five days.

Fluorescent imaging of HeLa cells

The partially photo-activated confocal fluorescent images of **ACR575a** incubated HeLa cells were recorded with a Leica SP5 confocal microscope (Figure 4a). HeLa cells were incubated with **ACR575a** (5 μM) and Hoechst 33342 (50 μg/mL) for 30 min sequentially, and were washed with PBS for three times. Cells were activated with the built-in 405 nm laser for 10 s before the fluorescence images from the two channels were collected (**ACR575a**: λ_{ex}, 543 nm, λ_{em}, 580 -650 nm; Hoechst 33342: λ_{ex},405 nm, λ_{em}, 460 -490 nm). For colocalization imaging, HeLa cells were incubated with **ACR575a** (5 μM) and MitoTracker Green FM (200 nM) for 30 min and washed with PBS for three times. Cells was irradiated with 405 nm laser for 10 s before the fluorescence images from the two channels were collected (MitoTracker Green: λ_{ex}, 488 nm, λ_{em}, 500 – 525 nm). The Pearson correlation coefficient was calculated to be 0.80.

Photoactivation of **ACRs** in HeLa cells by UV light were performed by a widefield microscopy (Leica, DMi8) with a live-cell imaging chamber (INU Series). Images were acquired with a 100x/1.40-0.70 oil lens. UV-portion (325-375 nm) of the emission spectrum of a mercury lamp through a band-pass filter was employed to photo-trigger cellular **ACRs** (5 μM). The following protocol was used for activation and imaging of HeLa cells incubated with **ACRs**, *i.e.* UV-light irradiation for 1 ms (325 - 375 nm), a fluorescence image acquisition with an exposure time of 25 ms, and sitting without photo-irradiation for 2 s. For **ACR575a/p**, λ_{ex}: 535 - 555 nm and λ_{em}: 575 - 635 nm. For **ACR670a/p**, λ_{ex}: 550 - 600 nm and λ_{em}: 665 - 715 nm. For colocalization imaging studies of **ACRs**, MitoTracker Green (200 nM) was used as a commercial mitochondria specific fluorophore (λ_{ex}: 460 - 500 nm, λ_{em}: 512 – 542 nm).

Fluo-4 AM (Thermo Fisher Scientific) was used for cytosolic Ca^{2+} detection. HeLa cells were incubated with Fluo-4 AM (2.5 μM) for 1 h followed by **ACR575a** (5 μM) for 30 min. The following protocol was used for activation and imaging of HeLa cells. *i.e.* UV-light irradiation for 1 ms (325 - 375 nm) to induce acetyl radical release, a fluorescence image acquisition with an exposure time of 25 ms for **ACR575a** (λ_{ex}: 535 -555 nm, λ_{em}: 575 – 635 nm), followed by a fluorescence image acquisition with an exposure time of 25 ms for Fluo-4 AM (λ_{ex}: 460 - 500 nm, λ_{em}: 512 – 542 nm), and sitting without photo-irradiation for 5 s. When sensing the oxidative stress in HeLa cells induce by photoactivation of **ACR575a**, a CellROX Deep Red (Thermo Fisher Scientific) was introduced with its working concentration as 5 μM (λ_{ex}: 590 - 650 nm, λ_{em}: 662 – 738 nm).

Evaluation of rhodamine **8** and Si-rhodamine as a mitochondrial membrane potential (MMP) probe. HeLa cells were incubated with 20 nM rhodamine **8**/Si-rhodamine and 200 nM MitoTracker Green for

30 min. The fluorescent signals from the two channels were collected (rhodamine **8**: λ_{ex}: 535 -555 nm, λ_{em}: 575 – 635 nm; MitoTracker Green: λ_{ex}: 460 - 500 nm, λ_{em}: 512 – 542 nm; Si-rhodamine: λ_{ex}: 550 - 600 nm and λ_{em}: 665 - 715 nm). The ionophore uncoupler carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP, 1 μM) was added as an inducer of the collapse of MMP.

Cytotoxicity assay

Dark cytotoxicity studies of **ACRs** toward HeLa cells for 24 h was performed using MTT assay. HeLa cells were grown to 70%~80% confluency before they were passaged and seeded into 96-well cell culture plate at 104/well, with 100 μL complete media for 24 h. A 5 mM stock solution of **ACRs** was diluted with complete medium to obtain different concentrations (0, 5, 10, 20, 40 μM). The culture medium was carefully removed, and different concentrations of **ACRs** were added into each well. After incubation at 37 °C for 24 h, MTT assay were performed.

Photo cytotoxicity study of **ACR575a** toward NSC was performed with MTT assay. Different concentrations of **ACR575a** (0, 5, 10, 20, 30 and 50 μM) were added to 96-well plates pre-incubated with NSCs, respectively. After being stimulated by 405 nm light (10 mw cm^{-2}) for different times, the cells were cultured for 48 h. Subsequently, 20 μL 3- (4,5-dimethyl-2-thiazolyl) -2,5-diphenyl-2-H-tetrazolium bromide (MTT) was added to each well under dark conditions. After 4 h, the mixed solution was removed and 80 μL dimethyl sulfoxide (DMSO) was added. After shaking for 5 min, the absorbance at 490 nm was measured. The cell viability values were determined according to the following formula: cell viability (%) = the absorbance of experimental group/the absorbance of the blank control group × 100%.

Evaluation of the stability and biosafety of ACR575a

To explore the stability of the **ACR575a** in NSCs, NSCs were incubated with **ACR575a** (20 μM) for 25 min, and cultured for different time (0.5, 2, 4, 6, 8, 10, 15, 20 h). Meanwhile, **ACR575a** (5 μL, 20 μM) was injected into the SGZ of the mouse brain through a micro-syringe (Hamilton), then the brains and vital organs of mice (heart, liver, spleen, lung, and kidney) were removed after various time intervals (0.3, 6, 12, 18, 22, 26, 30, 34 h). The brain slices (~300 μm) were obtained by a vibratory microtome (Leica VT1000 S). The NSCs or brain slices obtained at different time intervals were then irradiated by a 405 nm laser (10 mw cm^{-2}) for 4 min before being imaged with a fluorescence confocal microscope (Leica SP8, λ_{ex}: 532 nm, λ_{em}: 575 – 635 nm;). Organs of mice (heart, liver, spleen, lung, and kidney) were also imaged through a small animal irradiator (Rad source, RS2000Pro-225) after the irradiation of a 405 nm laser (10 mw cm^{-2}) for 4 min.

Inhibition of Aβ-plague formation of ACR575a

The Aβ aqueous solution (1 mg/ml) was ultrasonically broken for 4 seconds. After 9 minutes of circulation operation, the bacteria were removed by a 0.22 mm filter. Then 20 μM **ACR575a** were added to the Aβ solution. In order to study the effect of acetyl radical on the growth of Aβ, 10 mU/ml superoxide dismutase (SOD) was added as a superoxide scavenger. After irradiated by a 405 nm laser for 4 min,

the treated Aβ solution was incubated at 37 °C for different time (4, 8, 16, 24 h). AFM (Bruker) was used to observe the growth of Aβ-plague.

ACR575a treatment for AD mouse

C57/BL-6 wild type mice (14 weeks and 26 weeks) and APPswe/PS1dE9 double transgenic mice (2×Tg-AD, 14 and 26 weeks old) were used for *in vivo* experiments. All animal experiments were performed according to the guidelines of the Care and Use of Laboratory Animals formulated by the Ministry of Science and Technology of China and were approved by the Animal Care and Use Committee of East China Normal University (approval no, m+ R20190304. Shanghai. China). The mice were anesthetized with gas isoflurane (ABS small animal gas anesthesia machine. Shanghai Yuvan Scientific Instrument Co. Ltd.), in which the induction anesthesia concentration of isoflurane was 5 mg/ml, and the continuous anesthesia concentration was 2 mg/mL. During surgery, the mouse was wrapped in a heating pad to maintain body temperature at 37 ^{o}C. Then the optical fiber (100 μm, NA 0.22, Thorlabs) was implanted into the subgranular zone (SGZ: AP = -1.30 mm, L= 0.25 mm anterior to bregma, and V=1.80 mm from the surface of skull). After that, the anesthesia system was removed to wake up the mice and acclimate to the holder for 5 days. Then the mice were anesthetized with 2% isoflurane again and body temperature was maintained at 37 °C, a total of 5 μL (20 μM) **ACR575a** were injected into SGZ. A 405 nm (10 mw cm^{-2}) laser was used to irradiate the **ACR575a** for 4 min. This treatment was repeated for six times during a period of 28 days before the mice were sacrificed and evaluated.

Immunofluorescence staining of neural stem cells and brain slices

The NSCs and brain slices treated with **ACR575a** were washed five times with PBS, and 4% paraformaldehyde was added to fix the cells at 37 ^{o}C for 30 min. After that, the sample was washed with PBS for 3 x 5 min, and then added with 2 % PBST for cell membrane permeability for 15 min. After being washed with PBS for 3 x 5 min, the sample was added with 10 % goat serum and shaken at 25 ^{o}C for 1 h. Subsequently, it was washed with PBS for three times before Anti-GFAP antibody (abcam, ab7260) and Anti-Tuj1 antibody (abcam, ab14545) (1: 200) were added and incubated at 4 ^{o}C shaker overnight. The samples were then washed with PBS for 3 x 5 min. The Cy3-AffiniPure Goat Anti-Mouse IgG (H+L) (Yeasen, 33208ES60) and FITC-AffiniPure Goat Anti-Rabbit IgG (H+L) (Yeasen, 33107ES60) (1:500) was then added and incubated at 37 ^{o}C to avoid light for 1 h. After staining, the sample was washed with PBS three times. Then DAPI solution was added and stained for 5 min. After washing with PBS three times, the samples were analyzed by confocal fluorescence imaging and flow cytometry.

Aβ staining and polarized light microscopic imaging

The brains of **ACR575a**-stimulated mice were extracted and sliced with a thickness of 30 μm using freezing-microtome (Leica CM1950), and washed with PBS for 3 x 5 min. Then the brain slices were stained in Congo red staining solution for 10 min. Subsequently, the sample was fractionated with

Bennhold differentiation solution for 10-30 s, then rinsed with deionized water for 5 x 5 min. The samples were then subjected to polarized light microscopic imaging and confocal fluorescence imaging.

Morris water maze experiments

Morris water maze (MWM) test is mainly used to evaluate the learning and memory ability of experimental animals as described before[3] with slight modifications. Briefly, put the treated mouse head towards the wall of the pond into the water. Record the time animals found the underwater platform. In the early stage of trainings, if this time exceeds 150 s, the animals are guided to the platform and let animals stay on the platform for 10 seconds. And then remove and dry the animals. Each animal was trained five times a day, and the interval between the two trainings was 20 - 30 min for six consecutive days. On the second day after the last acquired training, the platform was removed and the exploration training for 60 s was started. The time spent by animals in the target quadrant (the quadrant where the platform was originally placed) and the number of times they entered the quadrant were recorded as the detection indexes of spatial memory. For 26-week-old AD mice, since they could not reach the platform within the typical 60-s exploration window, the time when they first reached the platform within 150 s were recorded in Figure 7n in order to minimize experimental accidents.

Statistical analysis.

Data are expressed as means ± S.D. of 3-5 samples in each experimental group. Statistical significance was determined by two-sided Welch's ANOVA. The value 0.05 (*), 0.01 (**), and 0.001 (***) was assumed as the level of significance for the statistic tests (n =20; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

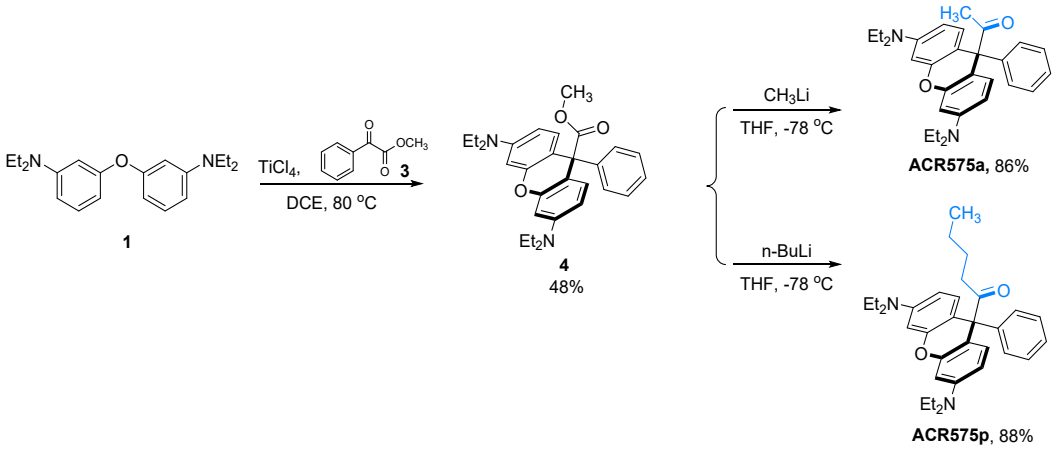
Mass Spectrometry

NSCs were incubated with **ACR575a** (20 μM) for 25 min, and then without or with the irradiation of a 405 nm light (10 mw cm^{-2}) for 4 min. NSCs underwent these treatments were further cultured for 3 days before being lysed in a total volume of 300 μL RIPA buffer (supplemented with 1 mM PMSF and complete EDTA-free protease inhibitor cocktail). Proteins in supernatant were separated using centrifugation (4 °C, 12,000 ×g) for 20 min. The concentration of supernatant fractions was quantified with BCA assay. 100 μg cell lysate was then incubated with 10 mM DTT for 30 min at 37 °C for reduction and then for 1 h at 25 °C with 30 mM acrylamide for alkylation. Salt was removed with methanol-chloroform sedimentation and then resuspended with 100 μL 100 mM TEAB (pH = 7.5). Trypsin/Lys-C Mix (Promegar, V5072) was added at the ratio of enzyme : protein as 1:50, and the digestion was carried out at 37 °C overnight. The tryptic peptides were treated with 1% trifluoroacetic acid (TFA) and purified using the C18 Ziptips and eluted with 0.1% TFA in 50~70% acetonitrile. Peptides were quantified using Pierce Quantitative Colorimetric Peptide Assay (Pierce 23275) and normalized to be compared to equal concentration and volume in 50 mM TEAB pH 8 final volume 100 μL for one reagent vial. The TMT labeling reagents were applied (TMT Mass Tagging Kits and Reagents, Thermo, 90066)

and then the peptides fractionation was conducted with High pH Reversed-Phase Peptide Fractionation Kit (Thermo, 84868).

Fractions were pre-processed and injected to a Orbitrap Fusion Lumos Mass Spectrometry (Thermo Scientific, Waltham, MA, USA) coupled with EASY-nLC 1000 Liquid Chromatograph Instrument (Thermo Scientific, Waltham, MA, USA). Bioinformatics and statistical analysis of original mass spectrometric data were performed using PEAKS Studio version 8.5 using the Mouse Uniprot database (reviewed only; updated November 2020). Only the hits with FDR ≤ 0.01 and the P-value ≤ 0.05 were accepted for discussion.

Synthetic procedures and compound characterization



Scheme S1. The synthetic pathway of **ACR575a** and **ACR575p**.

Synthesis of methyl 3,6-bis(diethylamino)-9-phenyl-9H-xanthene-9-carboxylate (4).

3,3'-oxybis(*N*,*N*-diethylaniline) (**1**) was synthesized via our previously reported method[4]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (t, *J* = 8.3 Hz, 2H), 6.41 (dd, *J* = 8.1, 1.5 Hz, 4H), 6.32 – 6.22 (m, 2H), 3.33 (q, *J* = 7.0 Hz, 8H), 1.15 (t, *J* = 7.1 Hz, 12H).

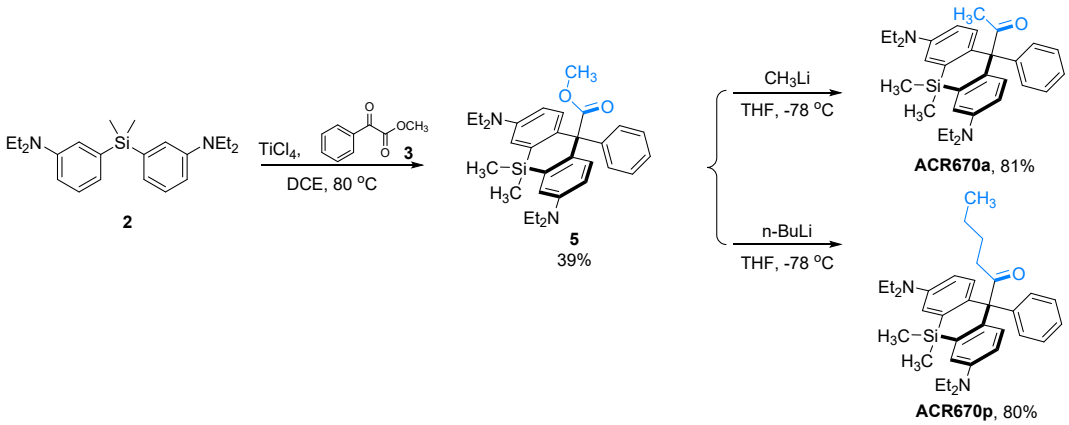
In a solution of compound **1** (2 g, 1 equiv., 6.40 mmol) and methyl 2-oxo-2-phenylacetate (**3**, 1.16 g, 1.05 equiv., 7.04 mmol) in DCE (1,2-dichloroethane, 50 mL) was added $TiCl_4$ (243 mg, 0.2 equiv., 1.28 mmol), and the resulting mixture was vigorously stirred for 12 hrs at 80 °C. The reaction mixture was allowed to cool to room temperature before saturated $NaHCO_3$ solution (20 mL) was poured into the reaction flask. The reaction mixture was extracted with DCM repeatedly. The combined organic layer was dried with anhydrous $MgSO_4$ powder and filtered. All organic solvent was removed under reduced pressure to give a viscous residue, which was purified by a flash column with a mixture of petroleum ether and EtOAc [20:1, v/v] as an eluent to afford **4** (1.42 g) as a transparent solid in a 49% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.18 (m, 5H), 6.84 (d, *J* = 8.7 Hz, 2H), 6.38-6.29 (m, 4H), 3.71 (s, 3H), 3.33 (q, *J* = 7.1 Hz, 8H), 1.16 (t, *J* = 7.1 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.7, 152.1, 148.2, 146.1, 131.7, 129.3, 127.9, 126.4, 110.9, 107.2, 97.9, 55.1, 52.8, 44.4, 12.8. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{29}H_{35}N_2O_3$, 459.2648; found 459.2649.

Synthesis of 1-(3,6-bis(diethylamino)-9-phenyl-9*H*-xanthen-9-yl)ethan-1-one (**ACR575a**).

1.31 mL CH_3Li (1 M in hexane, 2 equiv., 1.31 mmol) was syringed dropwise into a solution of compound **3** (300 mg, 1 equiv., 0.65 mmol) in dry THF (30 mL) at −78 °C under nitrogen, and the resulting mixture was stirred for another 2 hrs before 10 mL saturated solution of NH_4Cl was added. The reaction mixture was extracted with DCM repeatedly. The combined organic layer was dried with anhydrous $MgSO_4$ powder and filtered. Both DCM and THF was removed under reduced pressure to yield a viscous residue, which was purified by a flash column using a mixture of petroleum ether and EtOAc [30:1, v/v] as an eluent to afford **ACR575a** (248 mg) as a white solid in an 86% yield. All above workup procedures were operated in the dark to avoid the photolysis of the product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.22 (m, 5H), 7.17 – 7.15 (m, 1H), 6.79 (d, *J* = 8.7 Hz, 2H), 6.37 (d, *J* = 2.6 Hz, 2H), 6.34 (dd, *J* = 8.8, 2.7 Hz, 2H), 3.34 (q, *J* = 7.1 Hz, 8H), 2.11 (s, 3H), 1.17 (t, *J* = 7.1 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.2, 152.4, 148.5, 145.3, 130.7, 129.7, 127.9, 126.3, 110.4, 107.6, 98.6, 60.2, 44.5, 28.7, 12.8. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{29}H_{35}N_2O_2$, 443.2699; found 443.2700.

Synthesis of 1-(3,6-bis(diethylamino)-9-phenyl-9*H*-xanthen-9-yl)ethan-1-one (**ACR575p**).

0.52 mL n-BuLi (2.5 M in hexane, 2 equiv., 1.31 mmol) was syringed dropwise into a solution of compound **3** (300 mg, 1 equiv., 0.65 mmol) in dry THF (30 mL) at −78 °C under nitrogen, and the resulting mixture was stirred for another 2 hrs before 10 mL saturated solution of NH_4Cl was added. The reaction mixture was extracted with DCM repeatedly. The combined organic layer was dried with anhydrous $MgSO_4$ powder and filtered. Both DCM and THF was removed under reduced pressure to yield a viscous residue, which was purified by a flash column using a mixture of petroleum ether and EtOAc [30:1, v/v] as an eluent to afford **ACR575p** (277 mg) as a white solid in an 88% yield. All above workup procedures were operated in the dark to avoid the photolysis of the product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.13 (m, 5H), 6.76 (d, *J* = 8.7 Hz, 2H), 6.36 (d, *J* = 2.4 Hz, 2H), 6.32 (dd, *J* = 8.8, 2.5 Hz, 2H), 3.34 (q, *J* = 7.0 Hz, 8H), 2.39 (t, *J* = 7.3 Hz, 2H), 1.47 – 1.38 (m, 2H), 1.17 (t, *J* = 7.0 Hz, 12H), 1.10 (dd, *J* = 15.1, 7.5 Hz, 2H), 0.73 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 209.4, 152.4, 148.4, 145.7, 130.9, 129.9, 127.8, 126.2, 110.5, 107.6, 98.5, 60.1, 44.5, 40.0, 26.9, 22.3, 14.0, 12.8, 12.8. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{32}H_{41}N_2O_2$, 485.3168; found 485.3167.



Scheme S2. The synthetic pathway of **ACR670a** and **ACR670p**.

Synthesis of methyl 3,7-bis(diethylamino)-5,5-dimethyl-10-phenyl-5,10-dihydrodibenzo[*b,e*]siline-10-carboxylate (5).

3,3'-(dimethylsilanediyl)bis(*N*,*N*-diethylaniline) (**2**) was prepared following previously reported method[5]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25-7.17 (m, 2H), 6.85 (dd, *J* = 10.3, 4.9 Hz, 4H), 6.70 (dd, J = 8.3, 2.6 Hz, 2H), 3.33 (q, J = 7.0 Hz, 8H), 1.13 (t, J = 7.0 Hz, 12H), 0.53 (s, 6H).

In a solution of compound **2** (2 g, 1 equiv., 5.64 mmol) and methyl 2-oxo-2-phenylacetate (**3**, 1.02 g, 1.1 equiv., 6.20 mmol) in DCE (1,2-dichloroethane, 50 mL) was added $TiCl_4$ (214 mg, 0.2 equiv., 1.13 mmol), and the resulting mixture was vigorously stirred for 14 hrs at 80 °C. The reaction mixture was allowed to cool to room temperature before saturated $NaHCO_3$ solution (20 mL) was poured into the reaction flask. The reaction mixture was extracted with DCM repeatedly. The combined organic layer was dried with anhydrous $MgSO_4$ powder and filtered. All organic solvent was removed under reduced pressure to give a viscous residue, which was purified by a flash column with a mixture of petroleum ether and EtOAc [20:1, v/v] as an eluent to afford **5** (1.09 g) as a transparent solid in a 39% yield. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.15 (t, *J* = 7.4 Hz, 2H), 7.11 (d, *J* = 7.1 Hz, 1H), 7.01 (d, *J* = 7.4 Hz, 2H), 6.95 (d, *J* = 8.8 Hz, 2H), 6.85 (d, *J* = 2.9 Hz, 2H), 6.65 (dd, *J* = 8.8, 2.9 Hz, 2H), 3.71 (s, 3H), 3.37 (qd, *J* = 7.2, 2.8 Hz, 8H), 1.17 (t, *J* = 7.0 Hz, 12H), 0.43 (s, 3H), -0.01 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 176.8, 147.4, 145.6, 136.0, 135.7, 131.1, 130.0, 127.5, 126.0, 115.8, 112.8, 64.9, 52.4, 44.3, 12.8, 0.1, -1.4, -1.8. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{31}H_{41}N_2O_2Si$, 501.2937; found 501.2940.

Synthesis of 1-(3,7-bis(diethylamino)-5,5-dimethyl-10-phenyl-5,10-dihydrodibenzo[*b,e*]silin-10-yl)ethan-1-one (ACR670a). 0.80 mL CH_3Li (1 M in hexane, 2 equiv., 0.80 mmol) was syringed dropwise into a solution of compound **5** (200 mg, 1 equiv., 0.40 mmol) in dry THF (30 mL) at −78 °C under nitrogen, and the resulting mixture was stirred for another 2 hrs before 10 mL saturated solution of NH_4Cl was added. The reaction mixture was extracted with DCM repeatedly. The combined organic layer was dried with anhydrous $MgSO_4$ powder and filtered. Both DCM and THF was removed under reduced pressure to yield a viscous residue, which was purified by a flash column using a mixture of petroleum ether and EtOAc [25:1, v/v] as an eluent to afford **ACR670a** (157 mg) as a white solid in an 81% yield. All above workup procedures were operated in the dark to avoid the photolysis of the product. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.11 (dt, *J* = 8.5, 7.6 Hz, 4H), 7.05 (t, *J* = 7.0 Hz, 1H), 7.00 (d, *J* = 8.8 Hz, 2H), 6.88 (d, *J* = 2.9 Hz, 2H), 6.66 (dd, *J* = 8.9, 3.0 Hz, 2H), 3.38 (q, *J* = 7.1 Hz, 8H), 1.99 (s, 3H), 1.19 (t, *J* = 7.1 Hz, 12H), 0.52 (s, 3H), 0.16 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 208.5, 146.8, 145.7, 135.7, 133.9, 131.2, 130.0, 127.4, 125.6, 115.7, 113.3, 69.3, 44.3, 29.5, 12.8, 0.1, -0.2, -1.9. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{31}H_{41}N_2OSi$, 4485.2988; found 485.2989.

Synthesis of 1-(3,7-bis(diethylamino)-5,5-dimethyl-10-phenyl-5,10-dihydrodibenzo[*b,e*]silin-10-yl)pentan-1-one (ACR670p). 0.32 mL n-BuLi (2.5 M in hexane, 2 equiv., 0.80 mmol) was syringed dropwise into a solution of compound **5** (200 mg, 1 equiv., 0.40 mmol) in dry THF (30 mL) at −78 °C under nitrogen, and the resulting mixture was stirred for another 2 hrs before 10 mL saturated solution of NH_4Cl was added. The reaction mixture was extracted with DCM repeatedly. The combined organic layer was dried with anhydrous $MgSO_4$ powder and filtered. Both DCM and THF was removed under reduced pressure to yield a viscous residue, which was purified by a flash column using a mixture of petroleum ether and EtOAc [30:1, v/v] as an eluent to afford **ACR670p** (169 mg) as a white solid in an 80% yield. All above workup procedures were operated in the dark to avoid the photolysis of the product. All above workup procedures were operated in the dark to avoid the photolysis of the product. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.22 – 7.19 (m, 2H), 7.15 (t, *J* = 7.8 Hz, 2H), 7.06 (t, *J* = 7.2 Hz, 1H), 6.95 (d, *J* = 8.9 Hz, 2H), 6.88 (d, *J* = 2.9 Hz, 2H), 6.65 (dd, *J* = 8.9, 3.0 Hz, 2H), 3.38 (q, *J* = 7.1 Hz, 8H), 2.24 – 2.20

(m, 2H), 1.45 – 1.40 (m, 3H), 1.07 (dd, *J* = 15.0, 7.5 Hz, 2H), 0.71 (t, *J* = 7.3 Hz, 3H), 0.52 (s, 3H), 0.29 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 210.5, 147.6, 145.6, 135.3, 134.2, 131.8, 130.1, 127.4, 125.4, 115.5, 113.5, 68.8, 44.3, 40.6, 27.6, 22.4, 13.9, 12.8, -0.0, -1.5. ESI-HRMS (m/z): $[M+H]^+$ calcd. for $C_{34}H_{47}N_2OSi$, 527.3458; found 527.3459.

Results and Discussion

Table S1. Crystal data and structure refinement for **ACR575a**.

Identification code	mo_20200630e_0m
Empirical formula	$C_{29}H_{34}N_2O_2$
Formula weight	442.58
Temperature/K	298
Crystal system	triclinic
SACR575a group	P-1
a/Å	7.6372(5)
b/Å	9.3281(6)
c/Å	18.7886(12)
α/°	84.927(2)
β/°	82.985(2)
γ/°	69.699(2)
Volume/Å^3	1244.44(14)
Z	2
ρ_{calc}g/cm^3	1.181
μ/mm^{-1}	0.074
F(000)	476.0
Crystal size/mm^3	0.180 x 0.150 x 0.120 mm^3
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	5.05 to 55.428
Index ranges	-9 ≤ h ≤ 9, -11 ≤ k ≤ 12, -24 ≤ l ≤ 24
Reflections collected	16776
Independent reflections	5564 [R_{int} = 0.1785, R_{sigma} = 0.1467]
Data/restraints/parameters	5564/1/303
Goodness-of-fit on F^2	1.134
Final R indexes [I>=2σ (I)]	R_1 = 0.1090, wR_2 = 0.2096
Final R indexes [all data]	R_1 = 0.2011, wR_2 = 0.2440
Largest diff. peak/hole / e Å^{-3}	0.46/-0.37

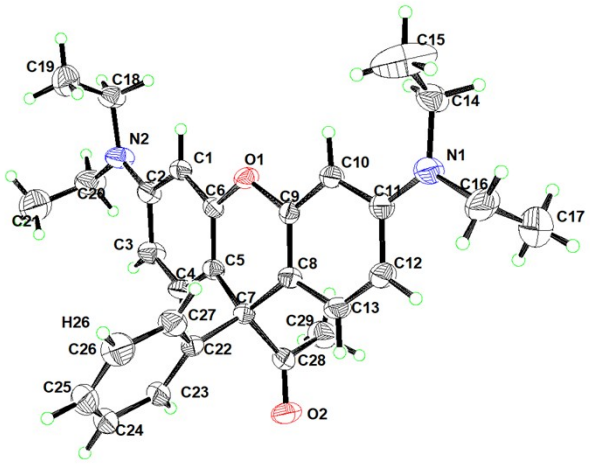


Figure S1. Ortep drawings of **ACR575a** in its solid state.

Table S2. Crystal data and structure refinement for **ACR575p**.

Identification code	mo_d8v191184_0m	
Empirical formula	C32 H40 N2 O2	
Formula weight	484.66	
Temperature	130(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
SACR575a group	P -1	
Unit cell dimensions	a = 10.8013(6) Å	a= 84.885(2)°.
	b = 11.3081(7) Å	b= 74.870(2)°.
	c = 12.0889(7) Å	g = 72.103(2)°.
Volume	1356.34(14) $Å^3$	
Z	2	
Density (calculated)	1.187 Mg/m^3	
Absorption coefficient	0.073 mm^{-1}	
F(000)	524	
Crystal size	0.180 x 0.150 x 0.120 mm^3	
Theta range for data collection	2.338 to 25.998°.	
Index ranges	-13<=h<=13, -13<=k<=13, -14<=l<=14	
Reflections collected	33642	
Independent reflections	5311 [R(int) = 0.0326]	
Completeness to theta = 25.242°	99.7 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7456 and 0.6997	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5311 / 124 / 378	
Goodness-of-fit on F^2	1.038	
Final R indices [I>2sigma(I)]	R1 = 0.0494, wR2 = 0.1293	
R indices (all data)	R1 = 0.0569, wR2 = 0.1363	
Extinction coefficient	0.015(4)	
Largest diff. peak and hole	0.366 and -0.215 $e.Å^{-3}$	

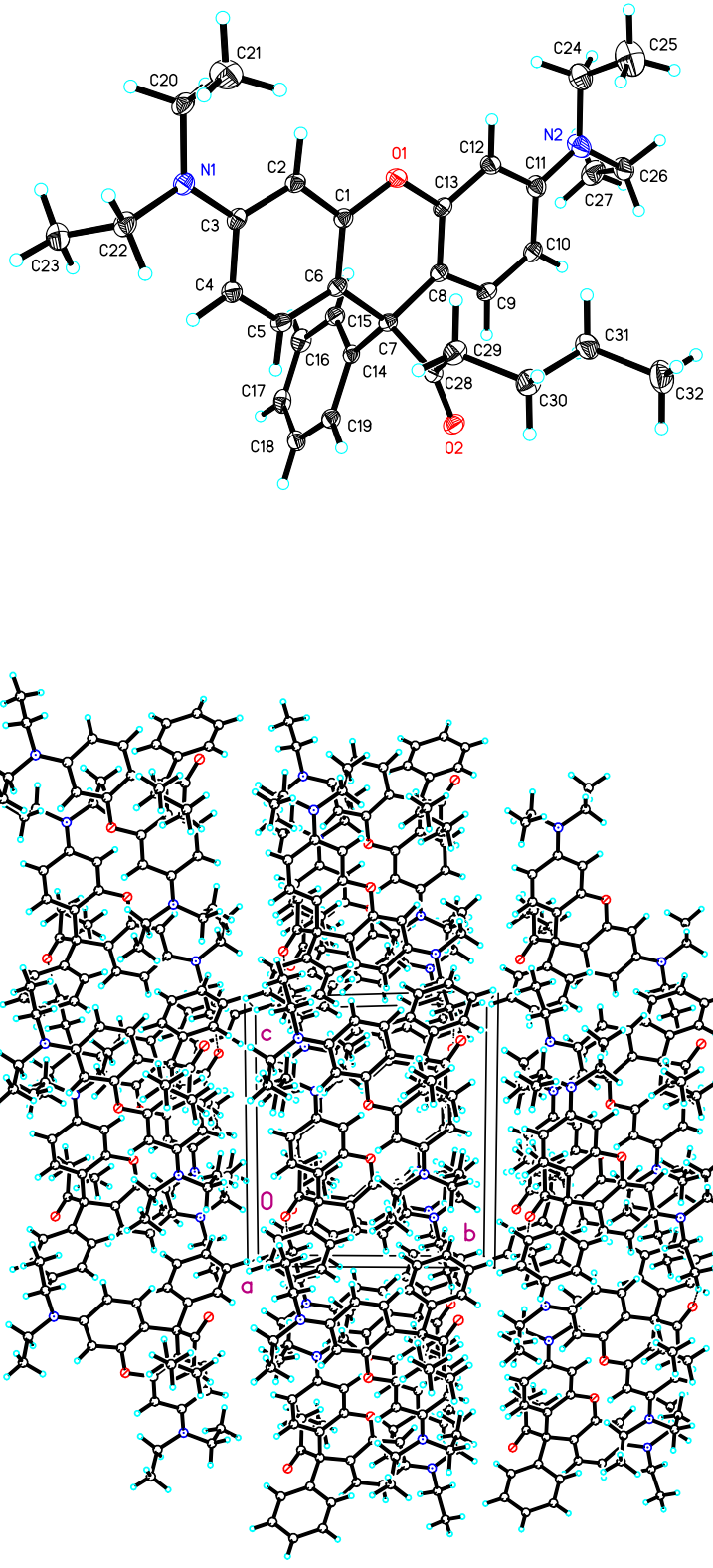


Figure S2. Ortep drawings and the packing mode of **ACR575p** in its solid state.

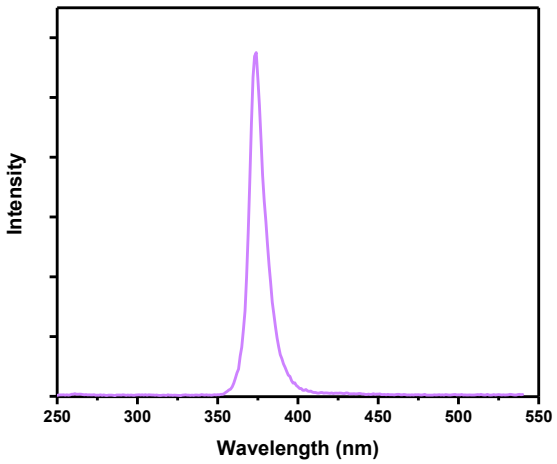


Figure S3. The spectrum of the UV LED light (375 ± 25 nm) used in the photoirradiation experiments.

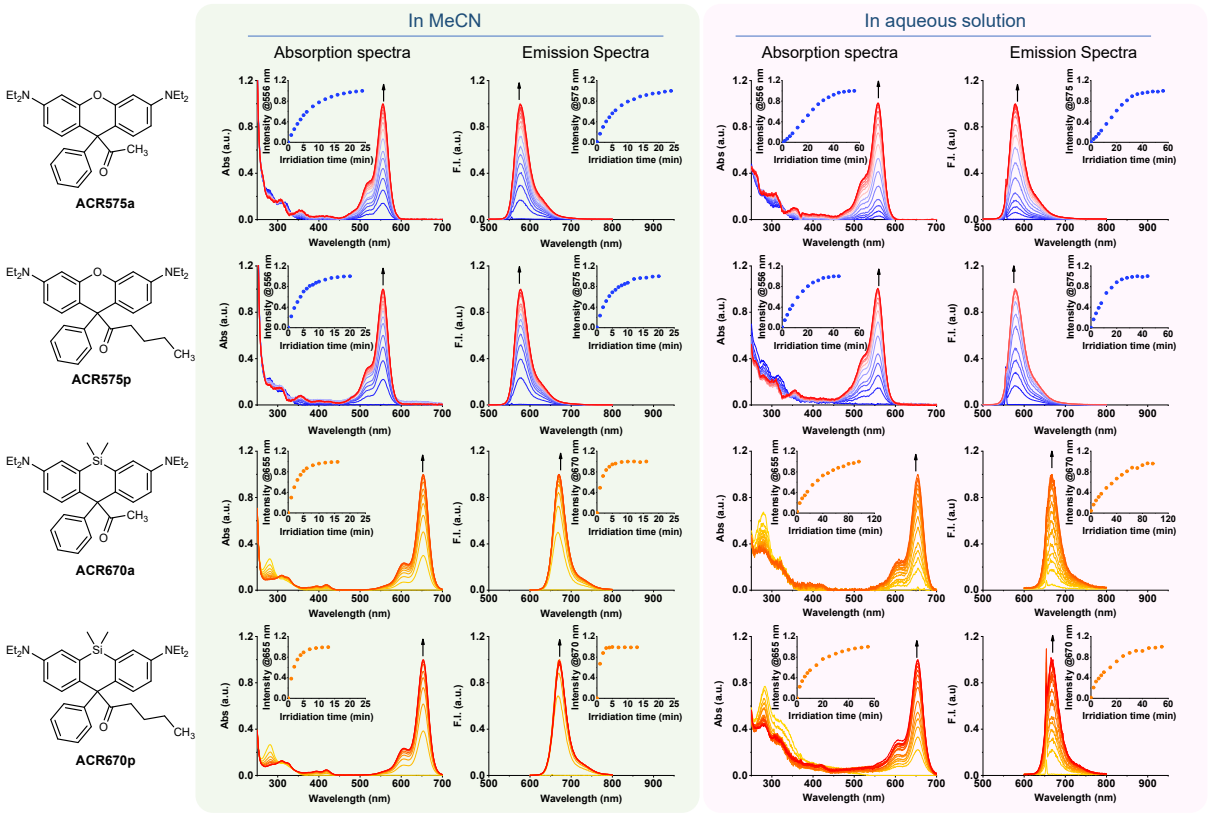
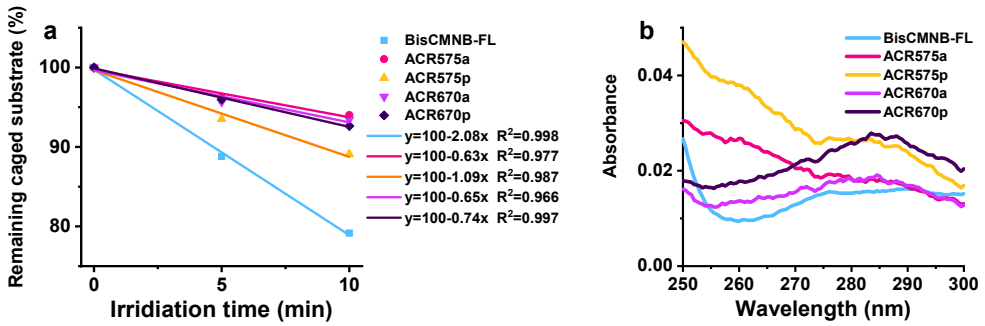


Figure S4. Photoactivation of acyl radical donors (**ACRs**) in solution. **a**, The UV-Vis absorption and fluorescence emission spectra changes of the **ACR575a**, **ACR575**, **ACR670a**, **ACR670b** (2 μM) in MeCN or in phosphate buffer (50 mM, pH = 7.4, with 10% MeCN) upon irradiation by a LED UV light (375 ± 25 nm).



$$\Phi_{uncage}^{ACR} = \frac{\varepsilon_{290\,nm}^{BisCMNB-FL}}{\varepsilon_{290\,nm}^{ACR}} * \frac{k^{ACR}}{k^{BisCMNB-FL}} * \Phi_{uncage}^{BisCMNB-FL}$$

c

	k	ε@290 nm	Φ
BisCMNB-FL	2.08	16200	0.13
ACR575a	0.63	16600	0.037
ACR575p	1.09	23900	0.047
ACR670a	0.65	16900	0.041
ACR670p	0.74	25700	0.029

Figure S5. Determination of the uncaging quantum efficiency of **ACRs** in phosphate buffer (50 mM, pH = 7.4, with 10% MeCN).

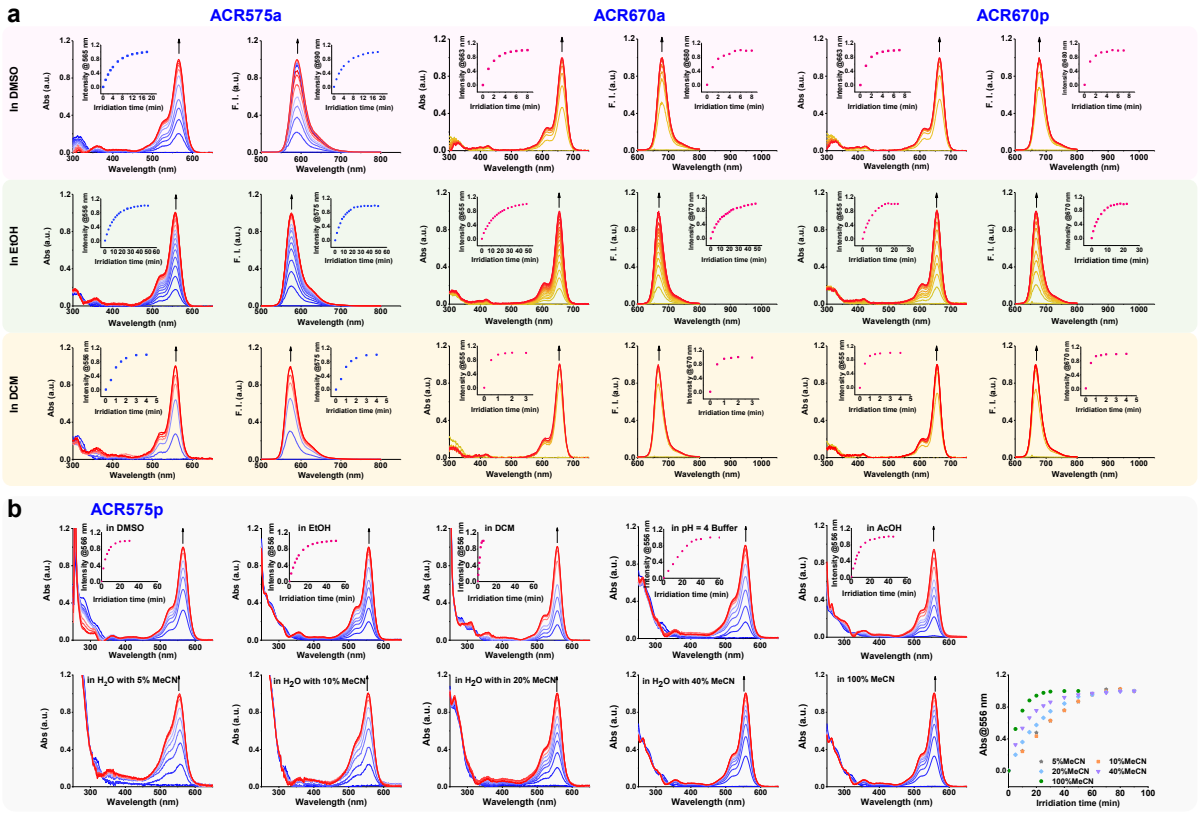


Figure S6. Spectral changes of **ACRs** during UV irradiation. **a**, The UV-Vis absorption and fluorescence emission spectra changes of **ACR575a/ACR670a/ACR670b** (1 μM) in DMSO/EtOH/DCM. **b**, The UV-Vis absorption spectra of **ACR575** (1 μM) in various solvents upon irradiation. Light source: a 375 ± 25 nm LED with a light density of 20 mW/cm^2.

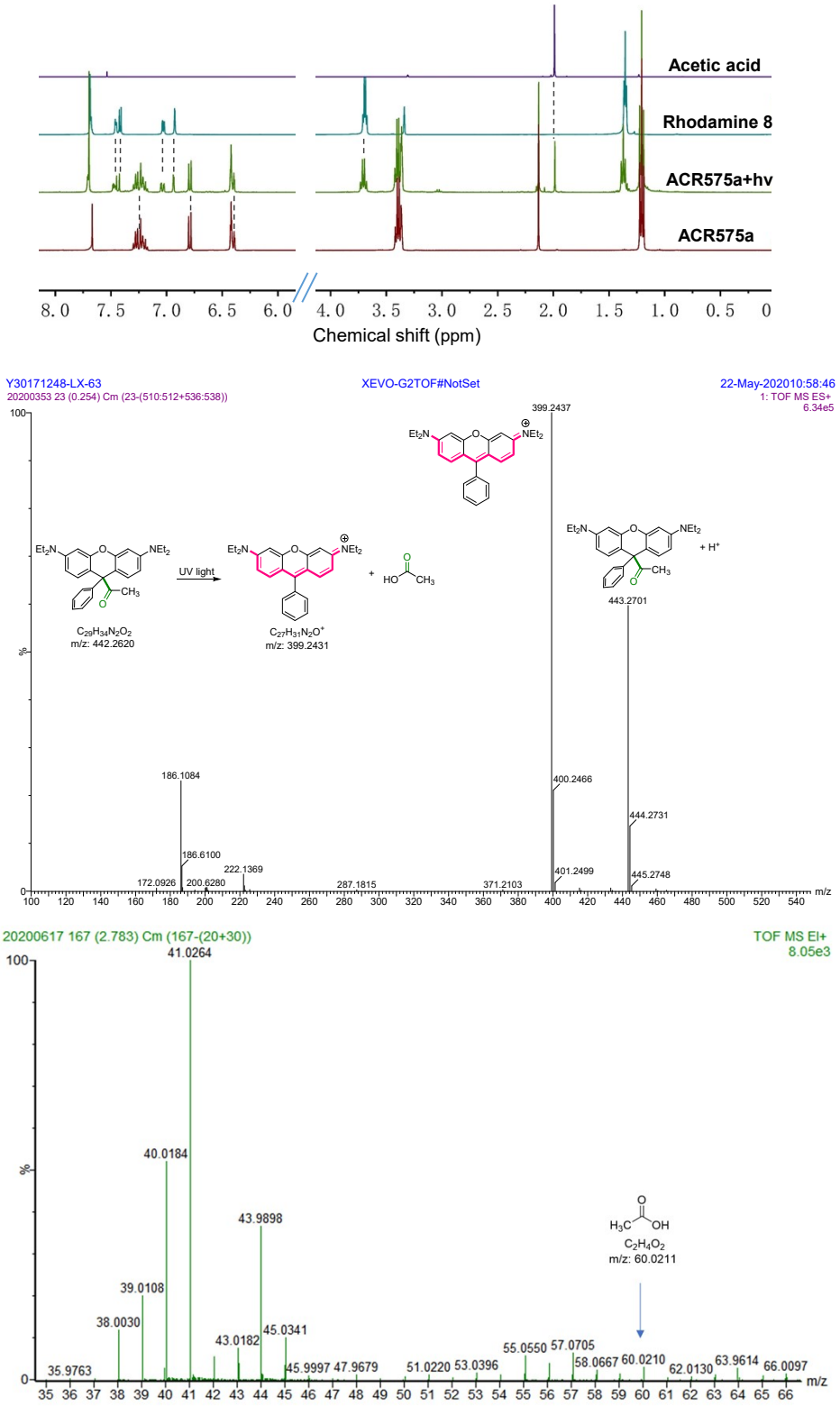


Figure S7. The NMR and HR-MS studies verifying the photolysis products of **ACR575a** to be rhodamine **8** and acetic acid. NMR data of **ACR575a** was acquired in a mixture of CD_3OD and $CDCl_3$. The NMR tube was irradiated for 30 min with a 400 nm laser.

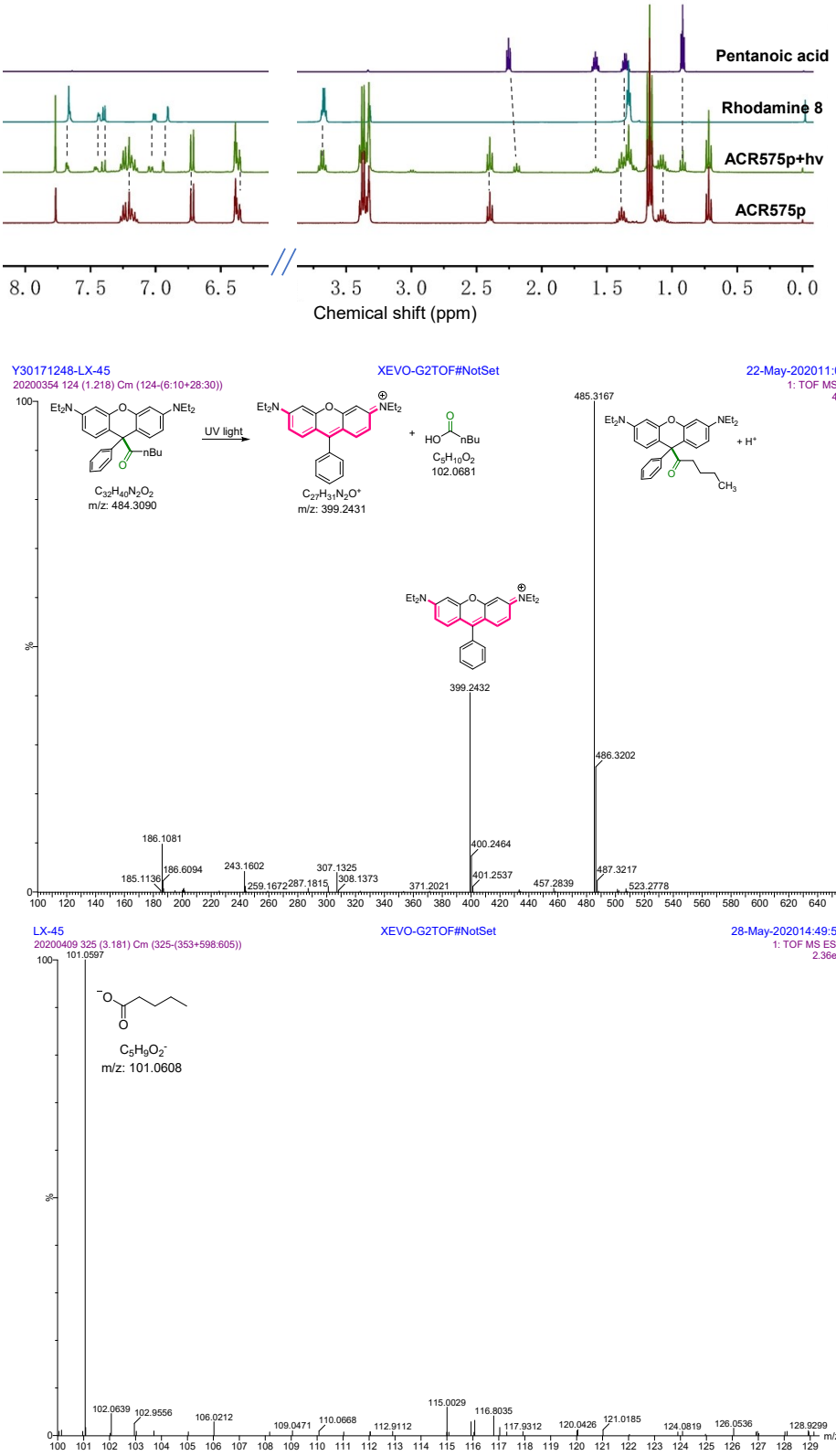


Figure S8. The NMR and HR-MS studies verifying the photolysis products of **ACR575p** to be rhodamine **8** and pentanoic acid. NMR data of **ACR575p** was acquired in a mixture of CD_3OD and $CDCl_3$. The NMR tube was irradiated for 30 min with a 400 nm laser.

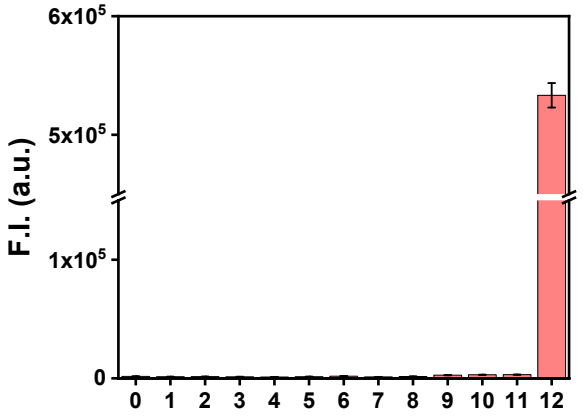


Figure S9. Dark stability of **ACR575a** (1 μM) at the presence of biorelevant species in phosphate buffer (50 mM, pH = 7.4) with 10% MeCN as a co-solvent. 0, Blank; 1, Ascorbic acid (10 mM), 2, Vitamin E (1 mM); 3, GSH (10 mM); 4, Cysteine (10 mM); 5, Na_2S (10 mM); 6, H_2O_2 (10 mM); 7, NaClO (1 mM); 8,·OH (100 μM); 9, KO_2 (100 μM); 10, $^1O_2$; 11, ^-OONO (100 μM); 12, Irradiated with 375 nm LED for 30 mins. The fluorescence intensity is collected at 578 nm with the excitation performed at 556 nm.

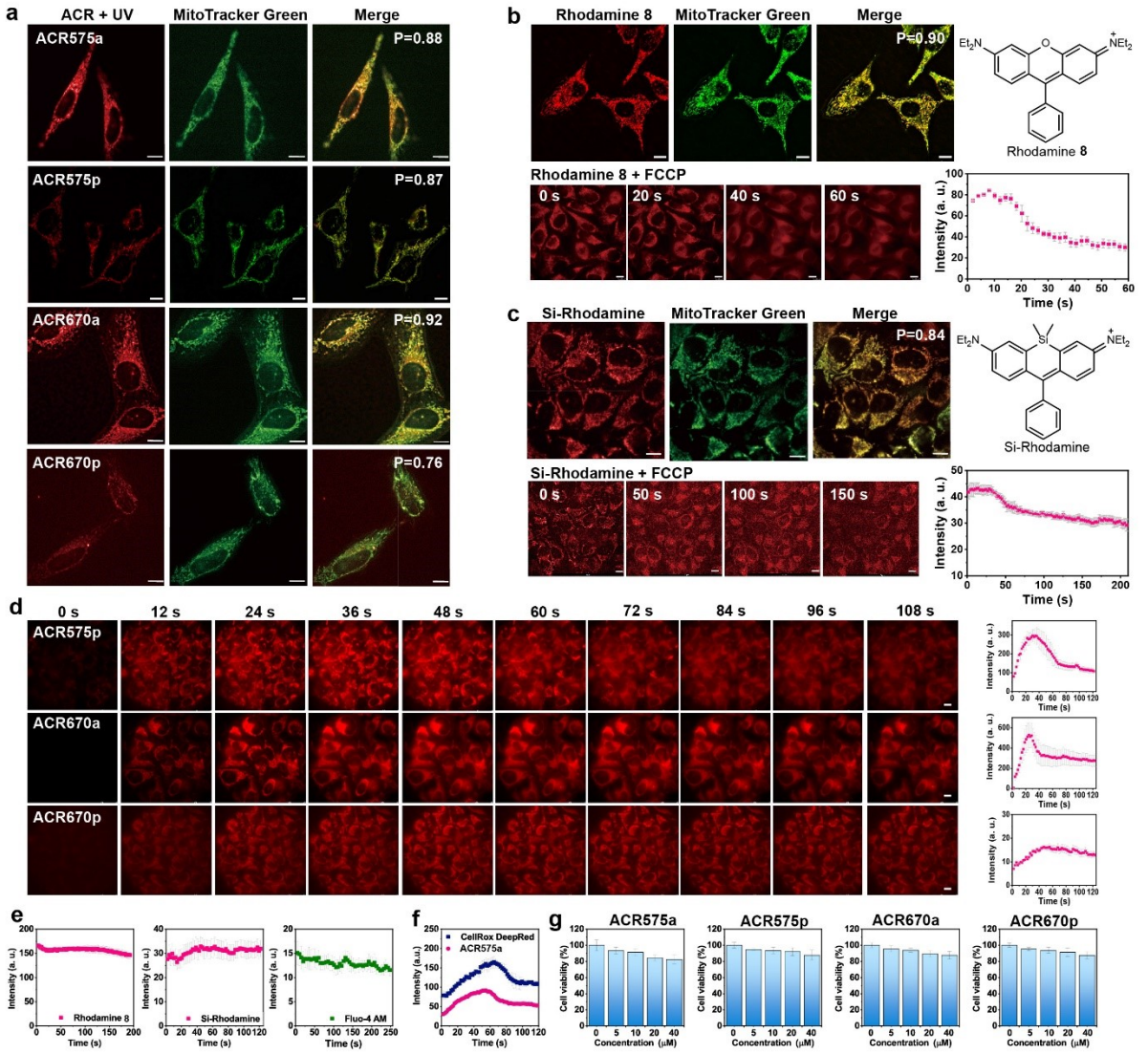


Figure S10. Photoactivation of **ACRs** in live HeLa cells by an epi-fluorescence microscope. **a,** Colocalization of the photolysis products of **ACRs** (5 μM) with MitoTracker Green. Evaluation of the mito-specificity of **b,** Rhodamine **8** and **c,** Si-rhodamine and their potential as indicators of MMP. **d,** Cellular photoactivation processes of **ACRs**. **e,** Photostability of rhodamine **8**, Si-rhodamine and Fluo-4 AM in HeLa cells. **f,** The photo-induced ROS release of **ACR575a** monitored by CellROX Deep Red. **g,** Cell viability of HeLa cells toward **ACRs** for 24 h by MTT assay. Error bars: standard deviation (SD), experimental times: n = 3. Scale bar: 10 μm.

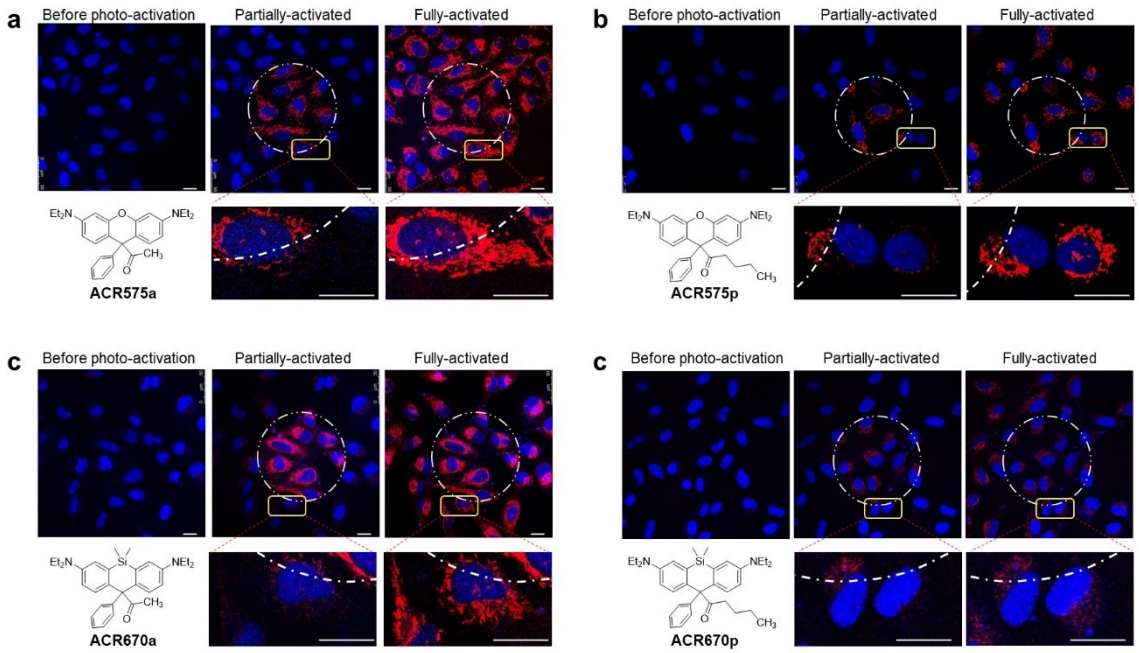


Figure S11. The selective photoactivation of **ACRs** incubated HeLa cells. The confocal images of HeLa cells incubated with **ACRs** (red, 5 μM) upon laser-irradiation (405 nm) and the nuclei were counterstained by Hoechst 33342 (blue, 50 μg/mL). Scale bar: 10 μm.

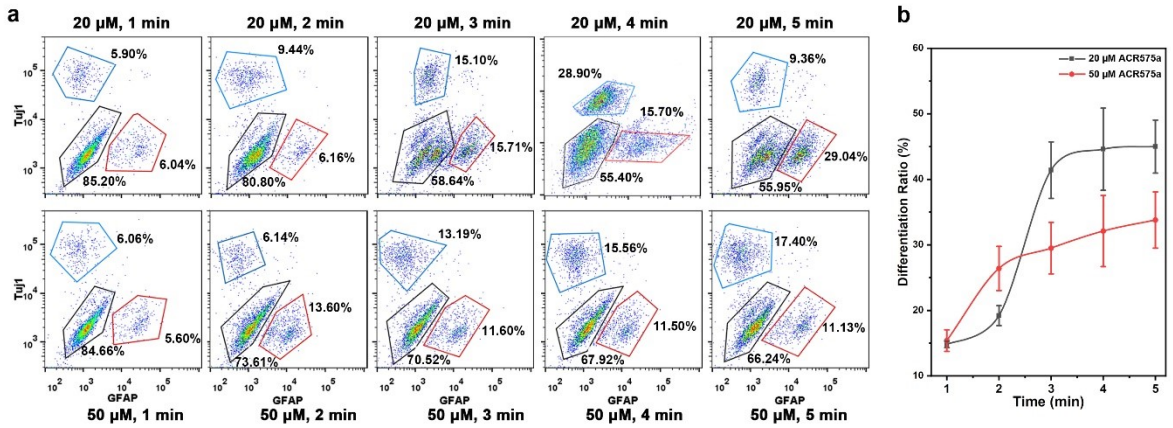


Figure S12. Photoactivation of **ACR575a** induce the differentiation of NSCs. (a) Flow sorting results of NSCs incubated with 20 μM and 50 μM **ACR575a** for different time after photoactivation. (b) Statistics of differentiation rate of NSCs incubated with 20 μM and 50 μM **ACR575a** for different time.

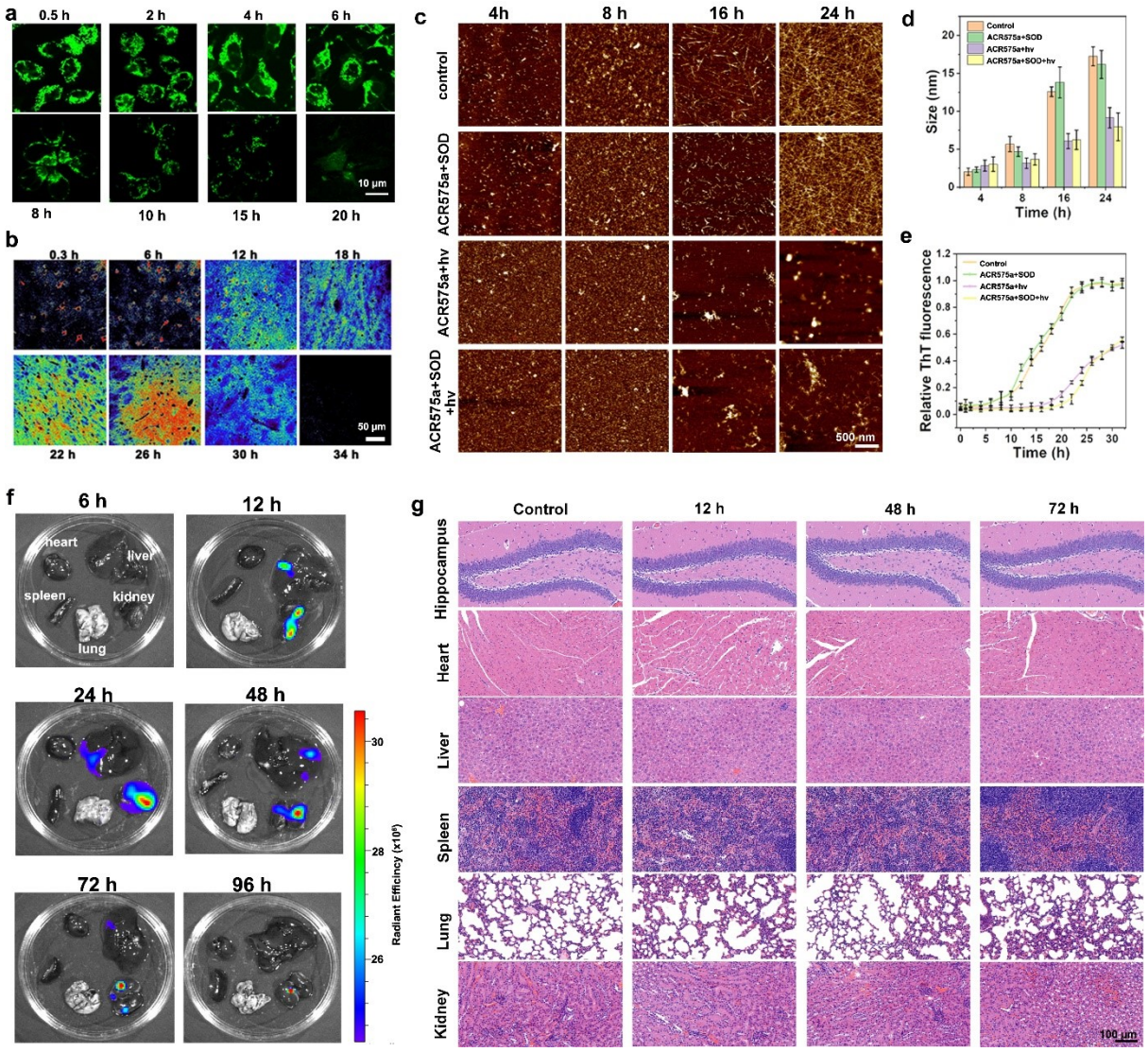


Figure S13. Metabolism of **ACR575a** and its potential for inhibiting the formation of Aβ-plague from Aβ(1-40) amyloid peptide. (a) NSCs incubated with **ACR575a** (20 μM) for 25 min, and the photo-activated fluorescence images were taken after 0.5 h, 2 h, 4 h, 6 h, 8 h, 10 h, 15 h or 20 h, respectively. (b) The photo-activated fluorescence images of cerebral cortex tissue section from 14-week AD mice at different times after the brain injection of **ACR575a** (5 μL, 20 μM). (c) AFM images of Aβ after various treated for different times. (d) Summarized quantification of the diameter of Aβ (e) The fluorescent intensity statistics of Aβ after various treatment for different times. (f) The photo-activated fluorescence images and (g) H&E staining of vital organs of AD mice at various times after the brain injection of **ACR575a** (5 μL, 20 μM). Irradiation light source: a 405 nm laser with a light density of 10 mW/cm^2, λ_{ex} = 532 nm, λ_{em} = 575 – 635 nm.

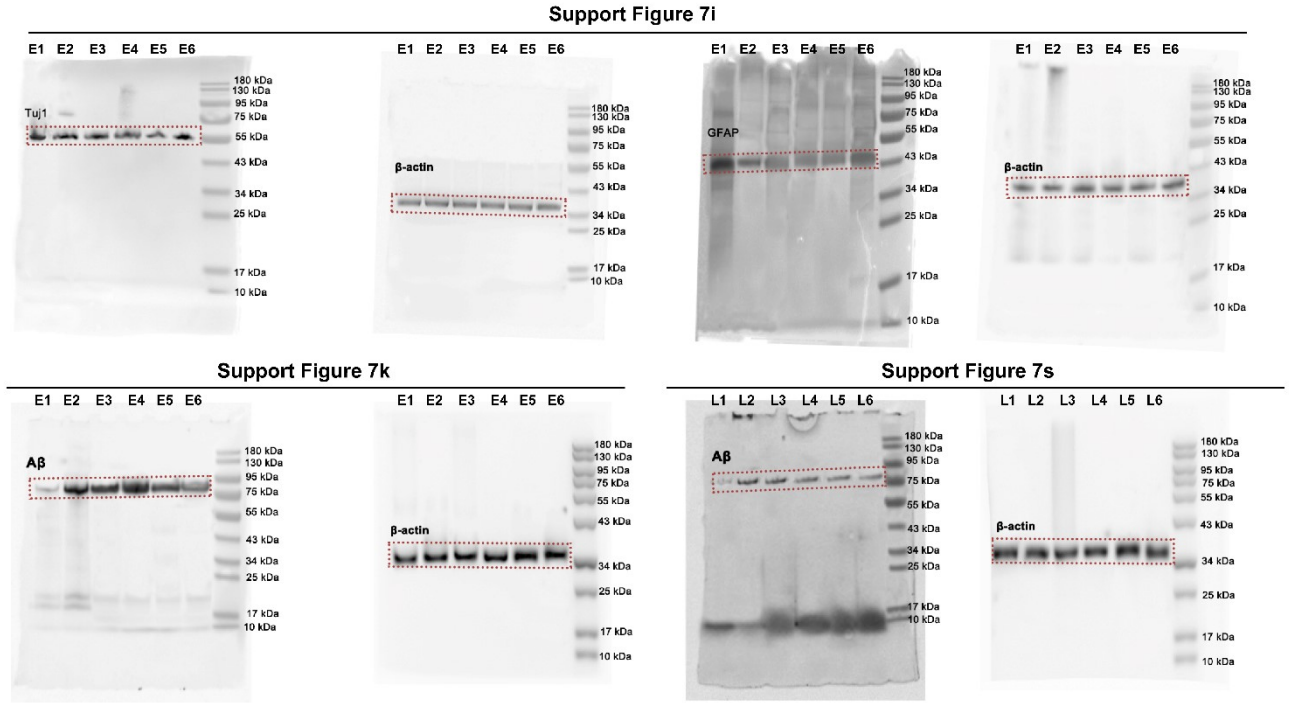


Figure S14. Uncropped western blots for Figures 7i, 7k, 7s. Lanes used are marked with red rectangles.

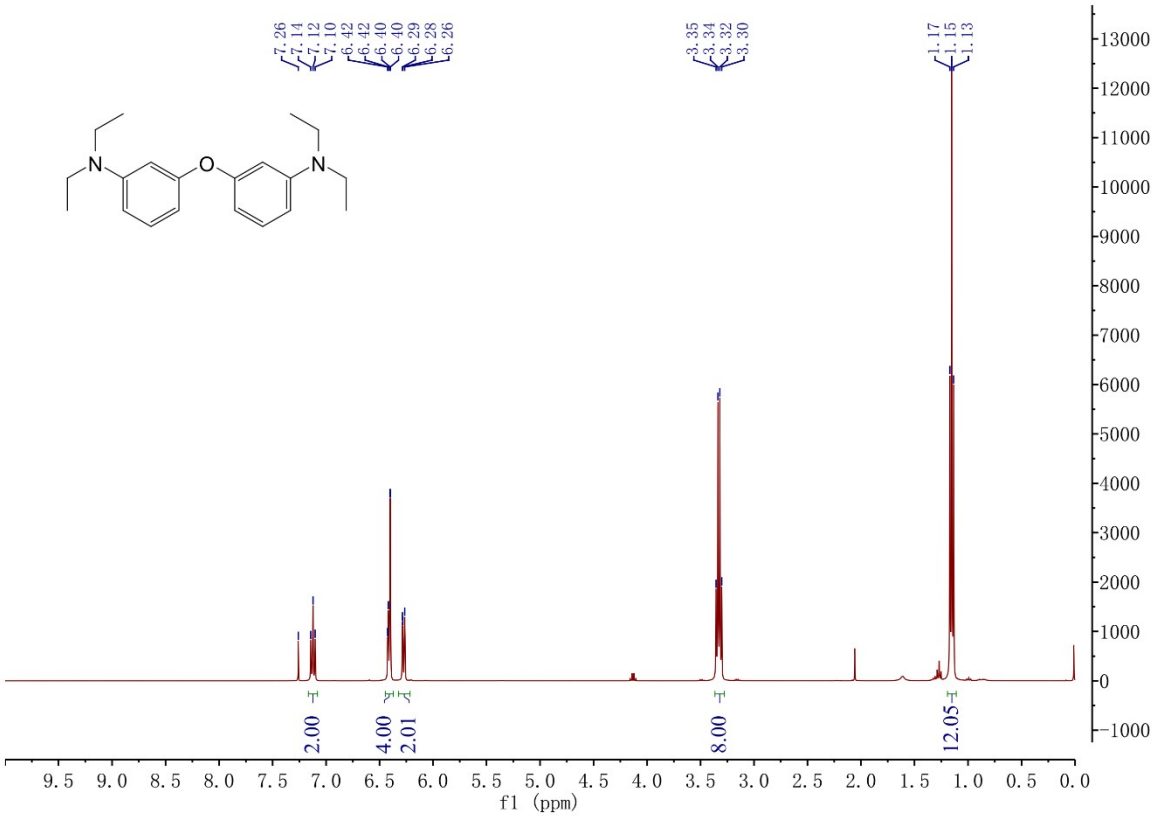


Figure S15. The ^{1}H-NMR of compound **1** in $CDCl_3$ (400 MHz).

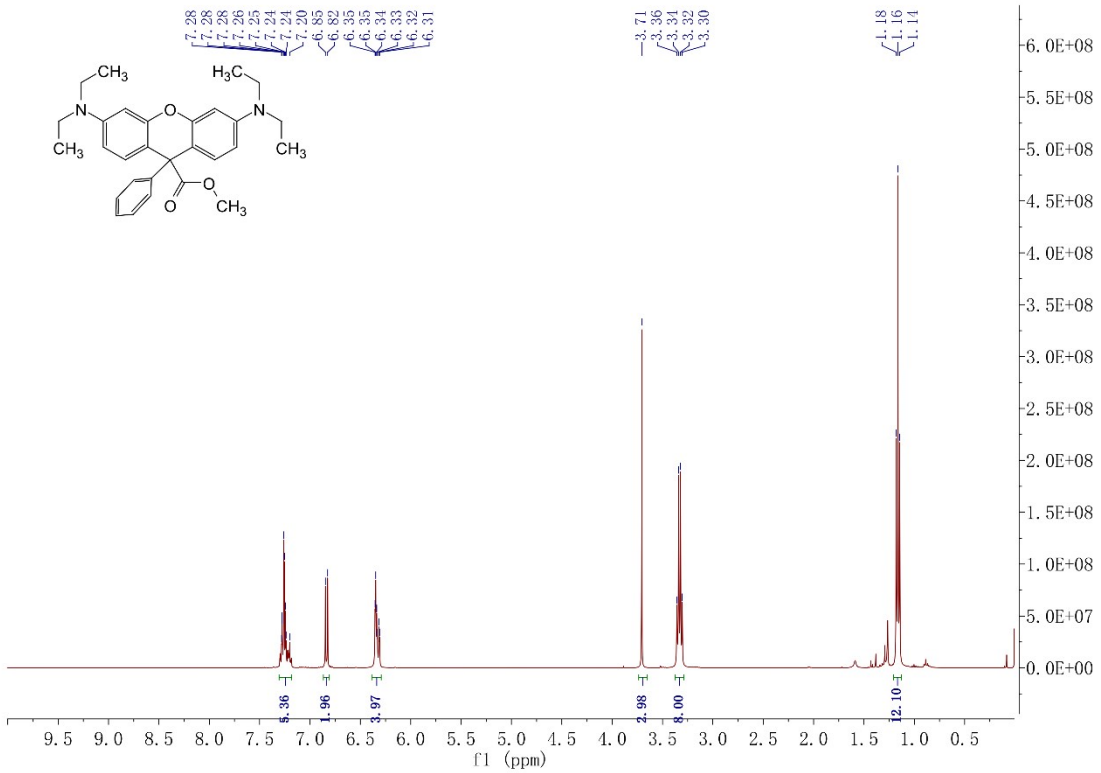


Figure S16. The ^{1}H-NMR of compound **4** in $CDCl_3$ (400 MHz).

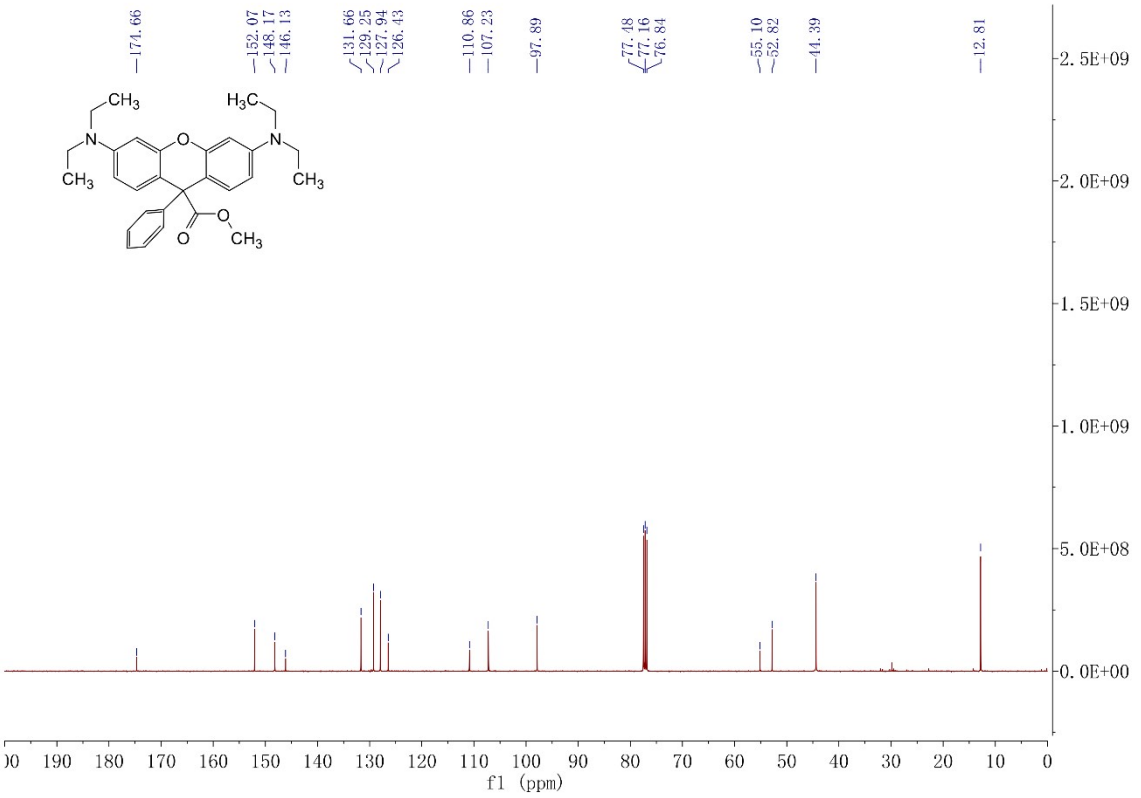


Figure S17. The $^{13}C\{^{1}H\}$-NMR of compound **4** in $CDCl_3$ (101 MHz).

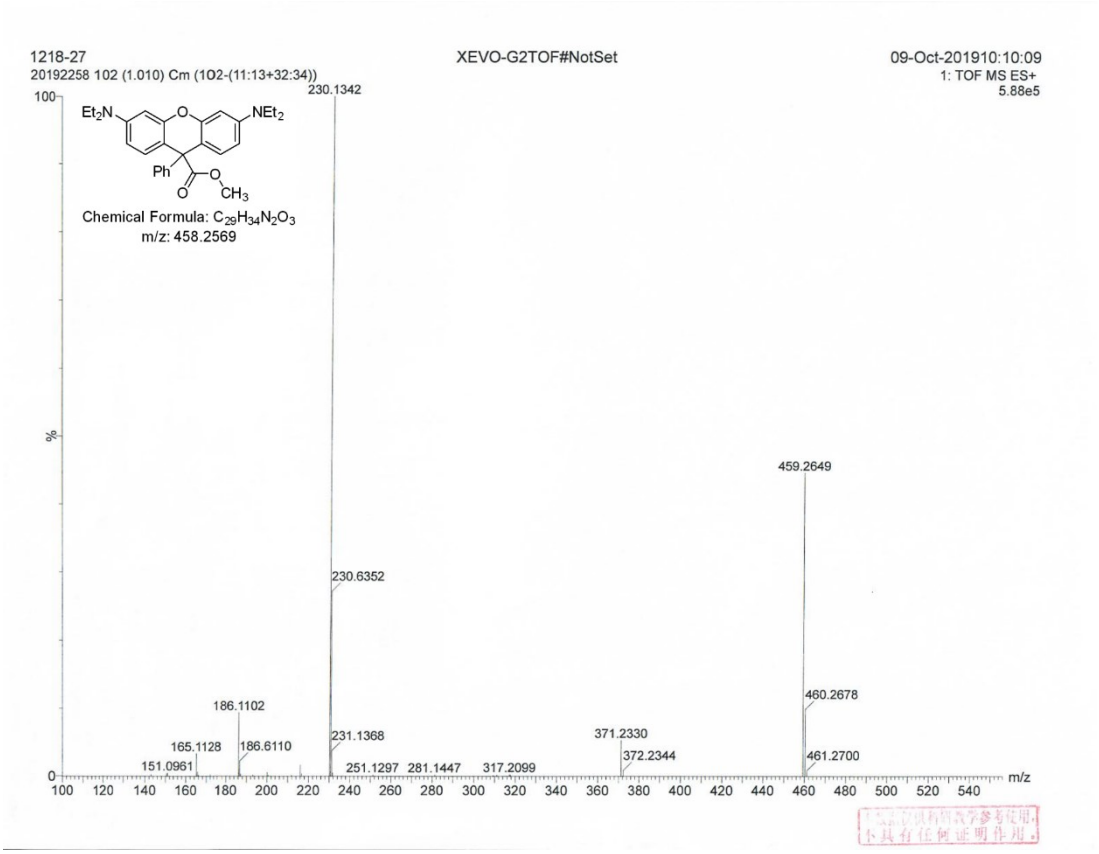


Figure S18. The HR-MS of compound **4**.

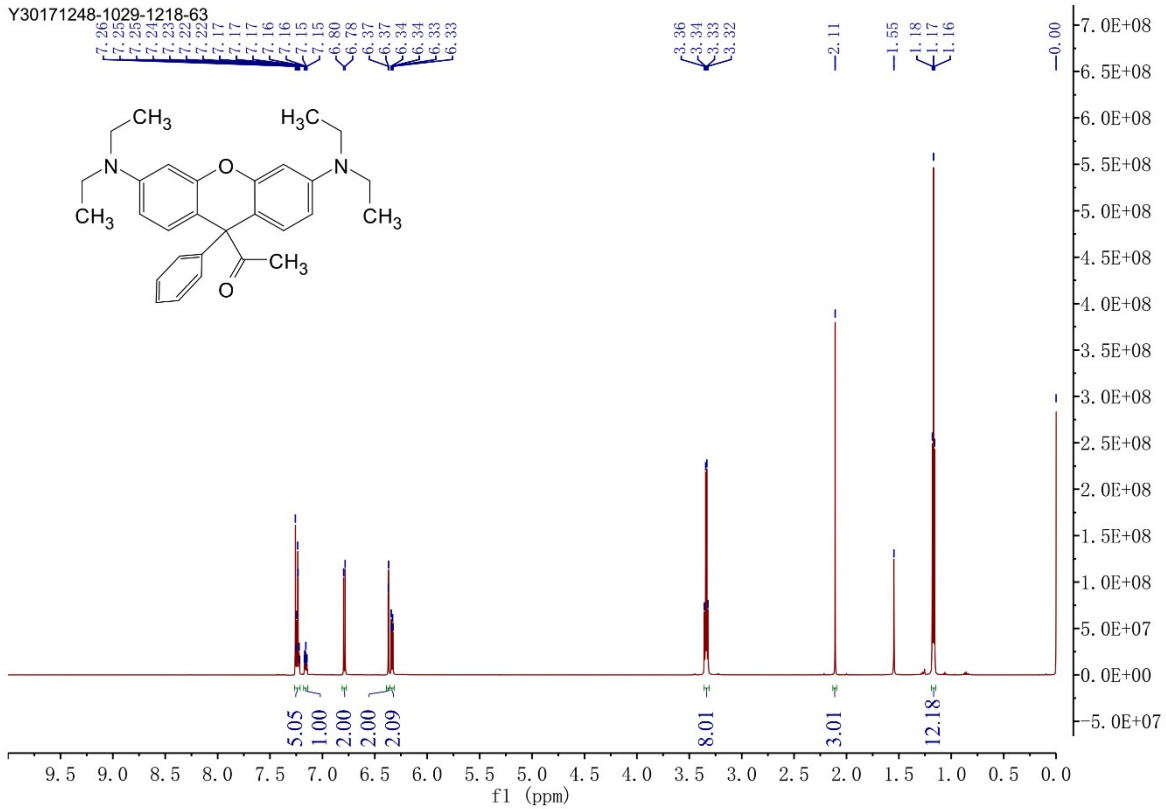


Figure S19. The ^{1}H-NMR of compound **ACR575a** in $CDCl_3$ (400 MHz).

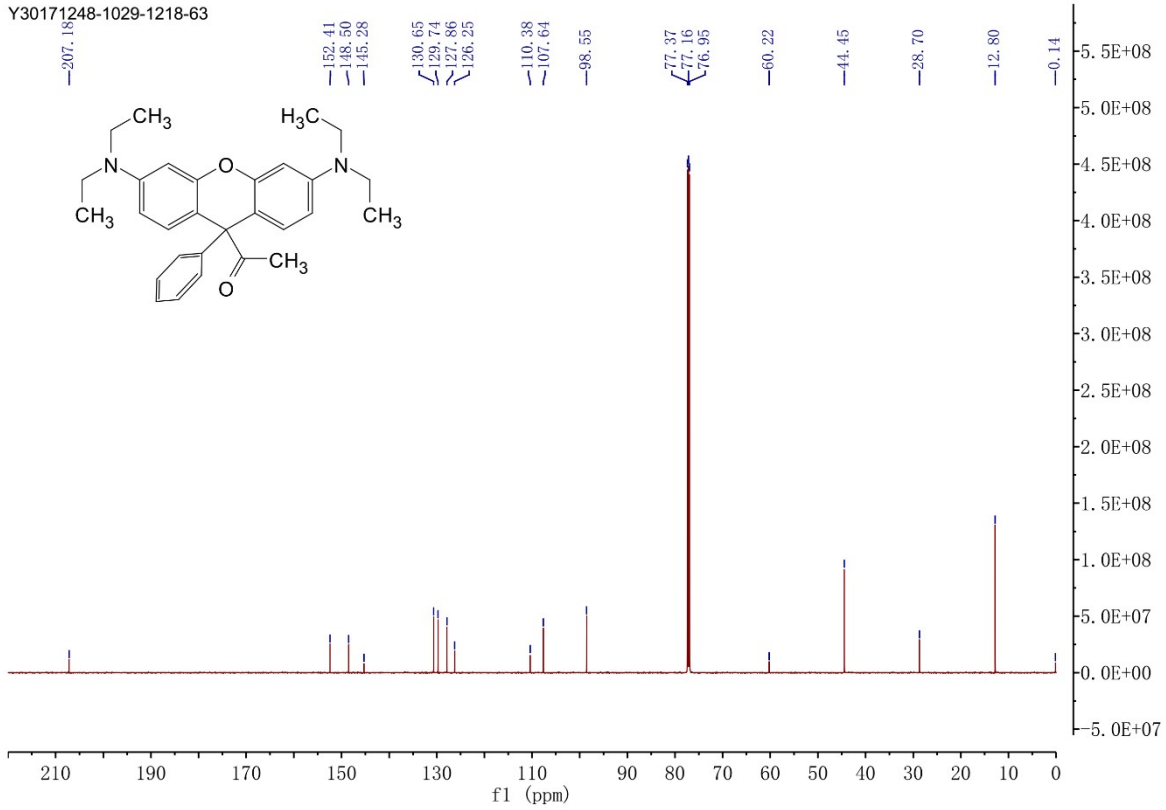


Figure S20. The ^{13}C{^{1}H}-NMR of compound **ACR575a** in $CDCl_3$ (101 MHz).

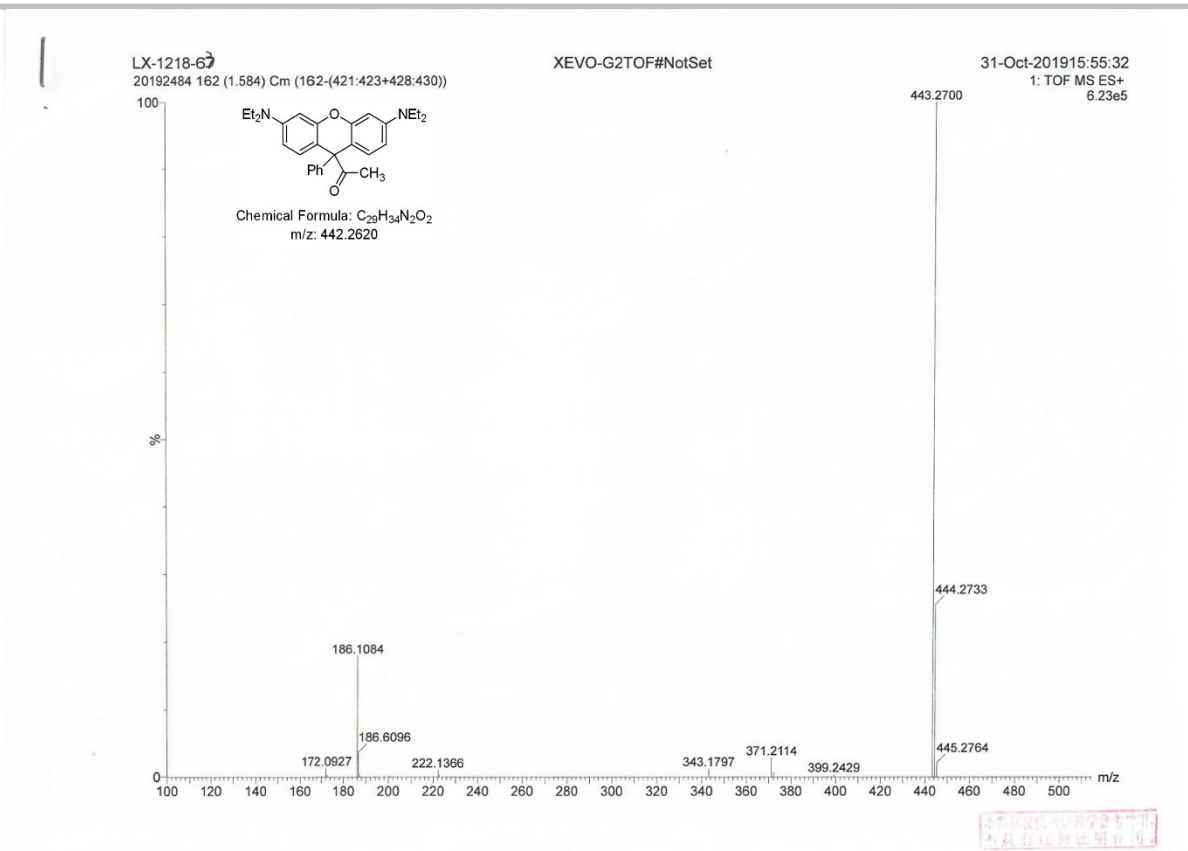


Figure S21. The HR-MS of compound **ACR575a**.

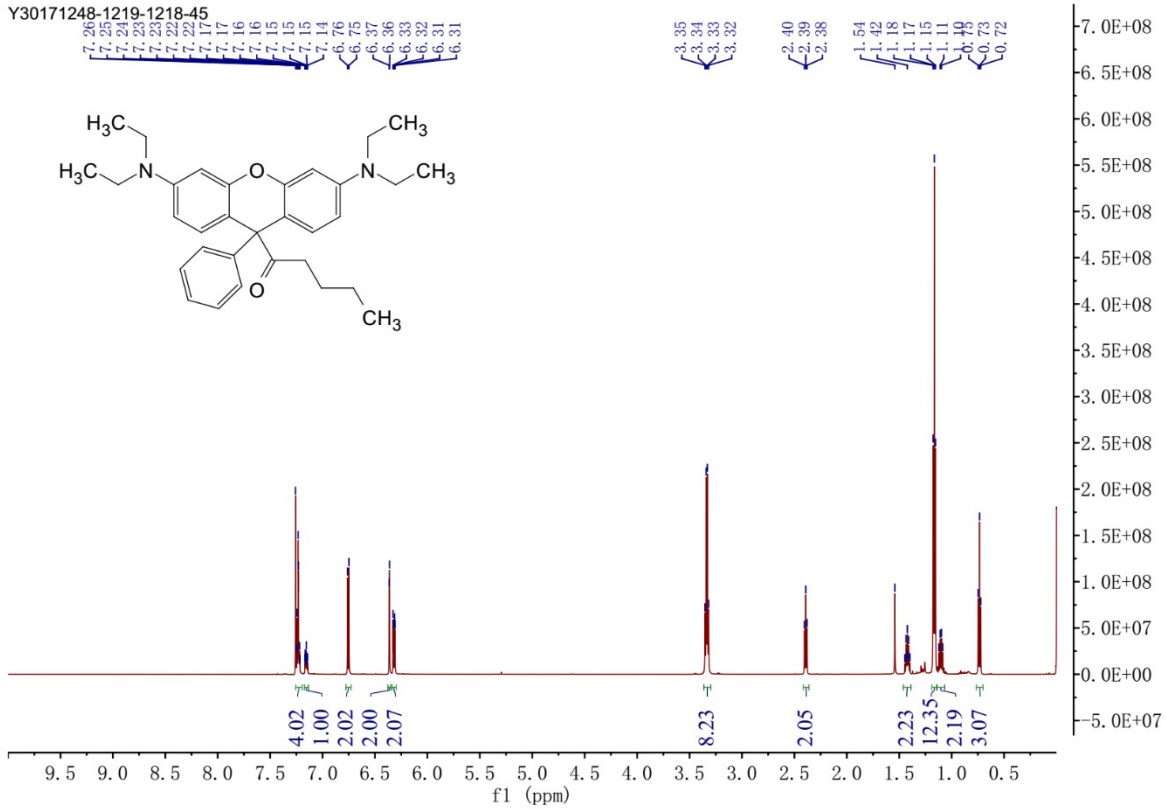


Figure S22. The ^{1}H-NMR of compound **ACR575p** in $CDCl_3$ (400 MHz).

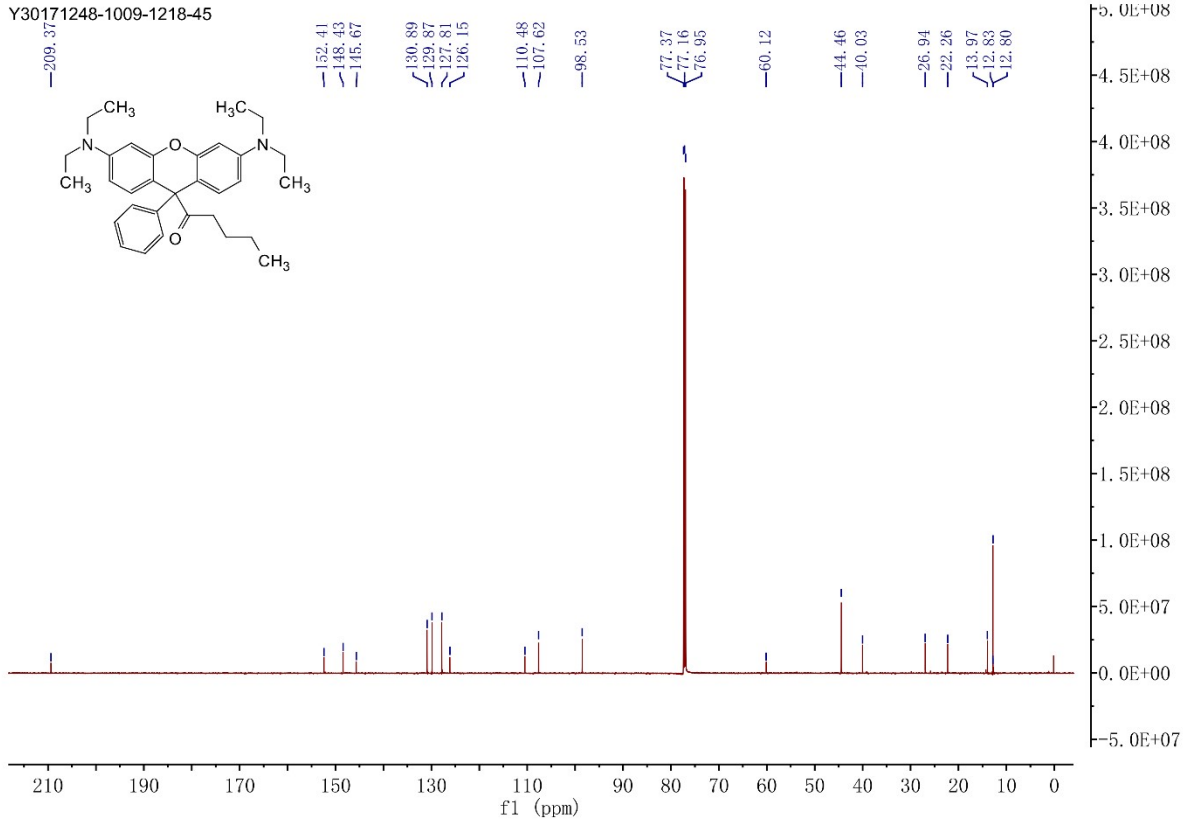


Figure S23. The $^{13}C\{^{1}H\}$-NMR of compound **ACR575p** in $CDCl_3$ (151 MHz).

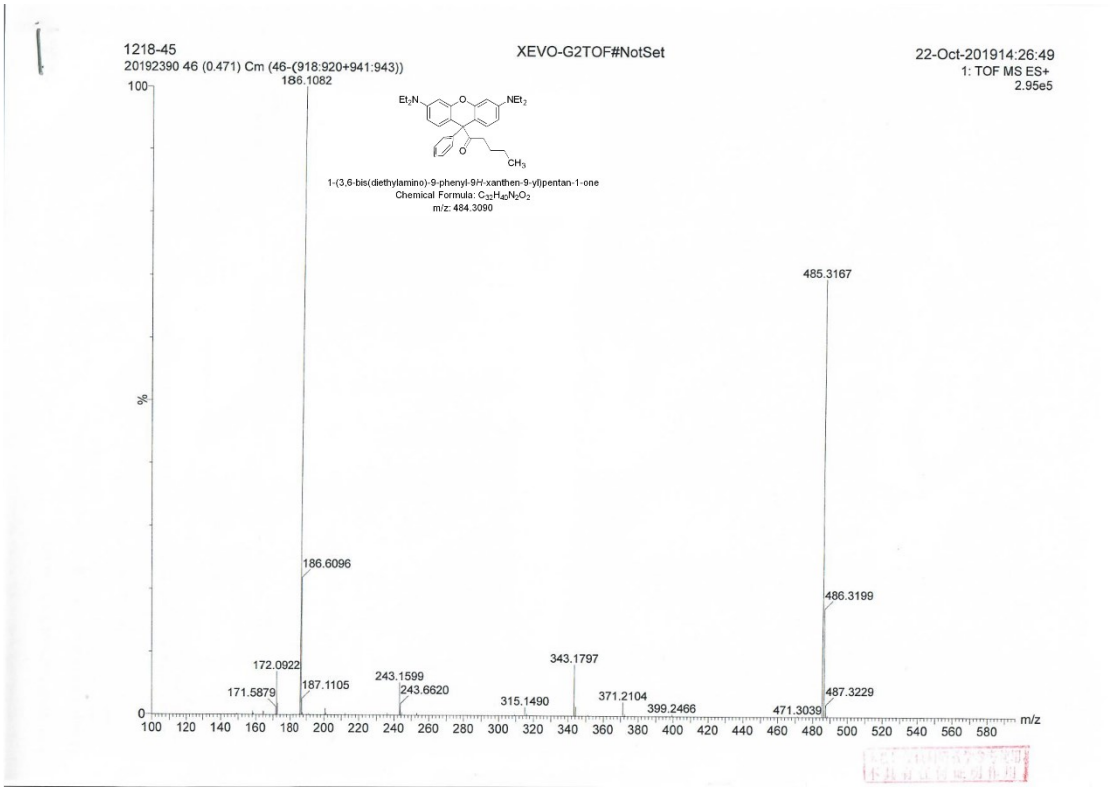


Figure S24. The HR-MS of compound **ACR575p**.

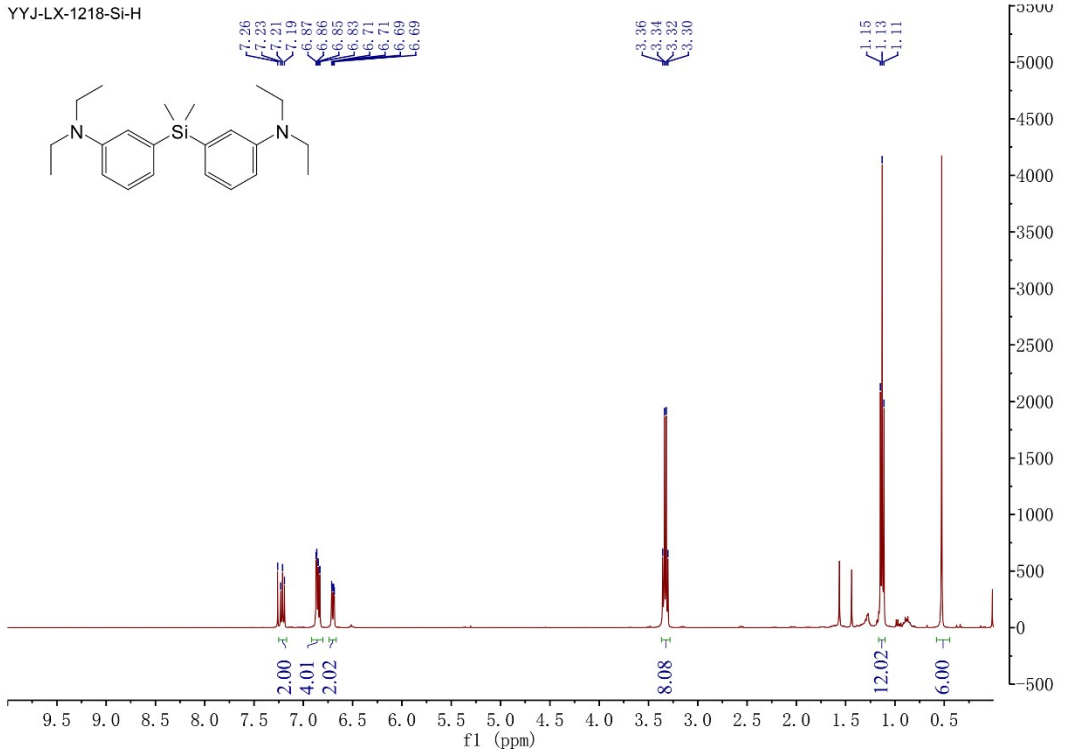


Figure S25. The ^{1}H-NMR of compound **2** in $CDCl_3$ (400 MHz).

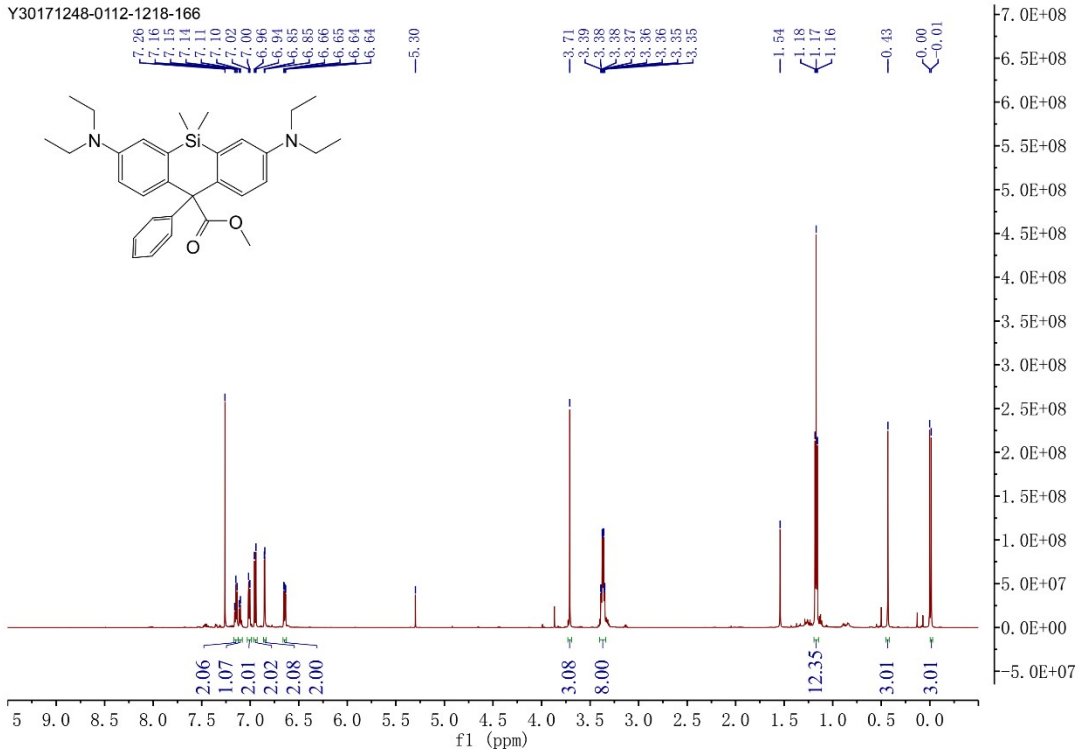


Figure S26. The ^{1}H-NMR of compound **5** in $CDCl_3$ (600 MHz)

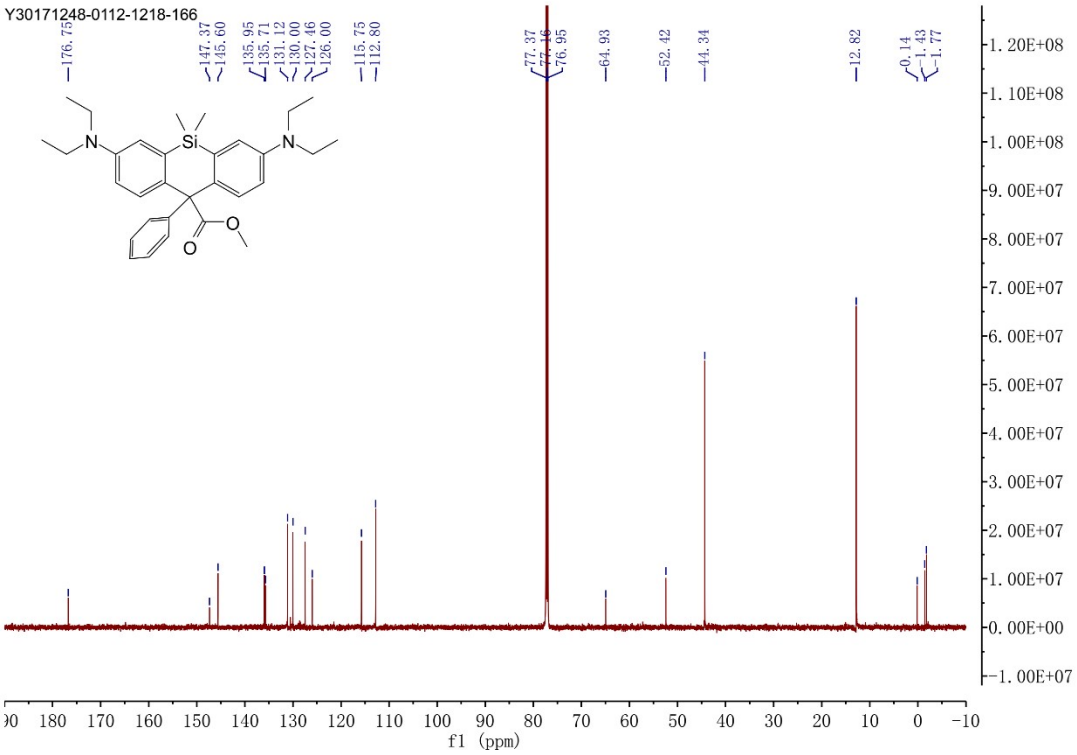


Figure S27. The $^{13}C\{^{1}H\}$-NMR of compound **5** in $CDCl_3$ (151 MHz).

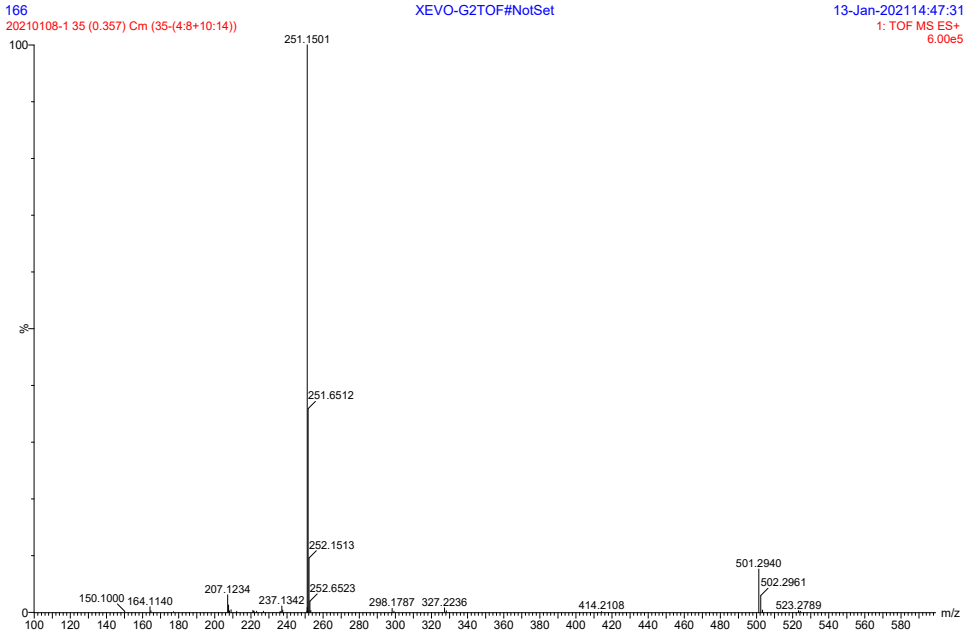


Figure S28. The HR-MS of compound **5**.

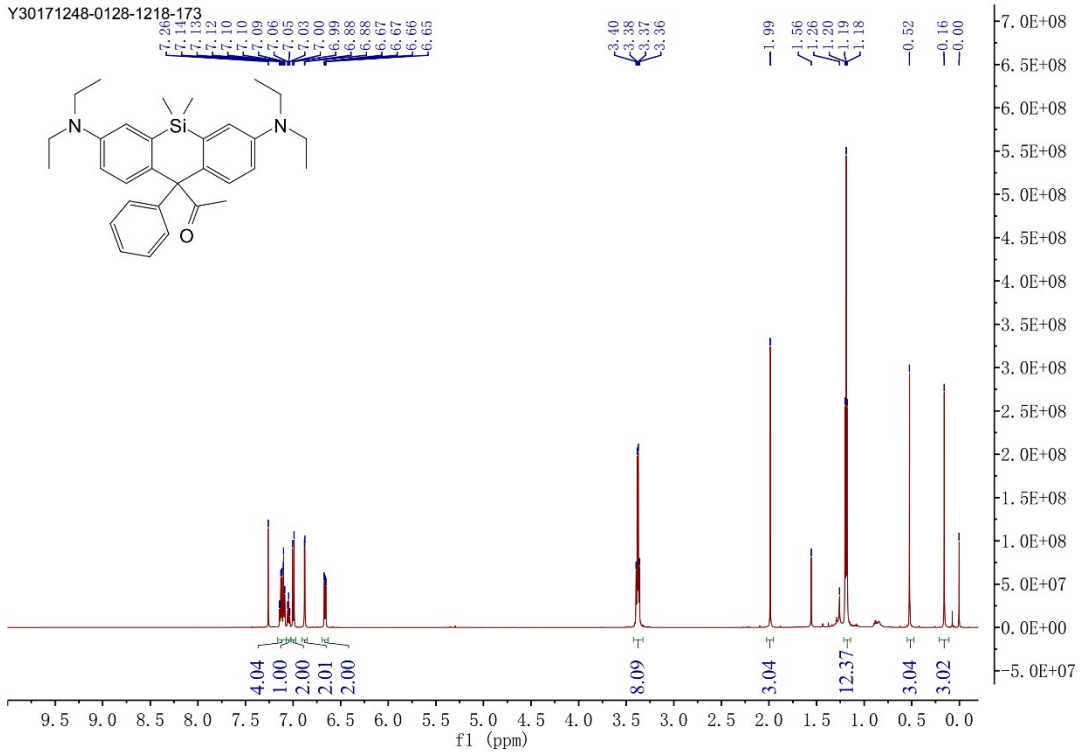


Figure S29. The ^{1}H-NMR of compound **ACR670a** in $CDCl_3$ (600 MHz)

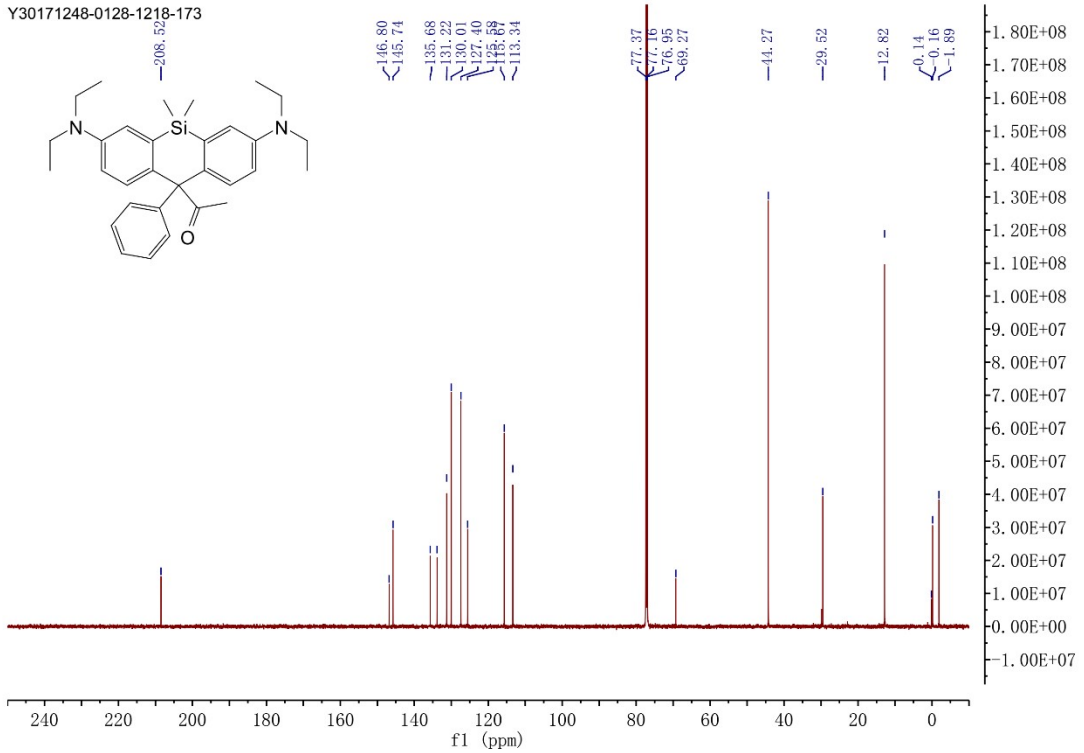


Figure S30. The ^{13}C{^{1}H}-NMR of compound **ACR670a** in $CDCl_3$ (151 MHz).

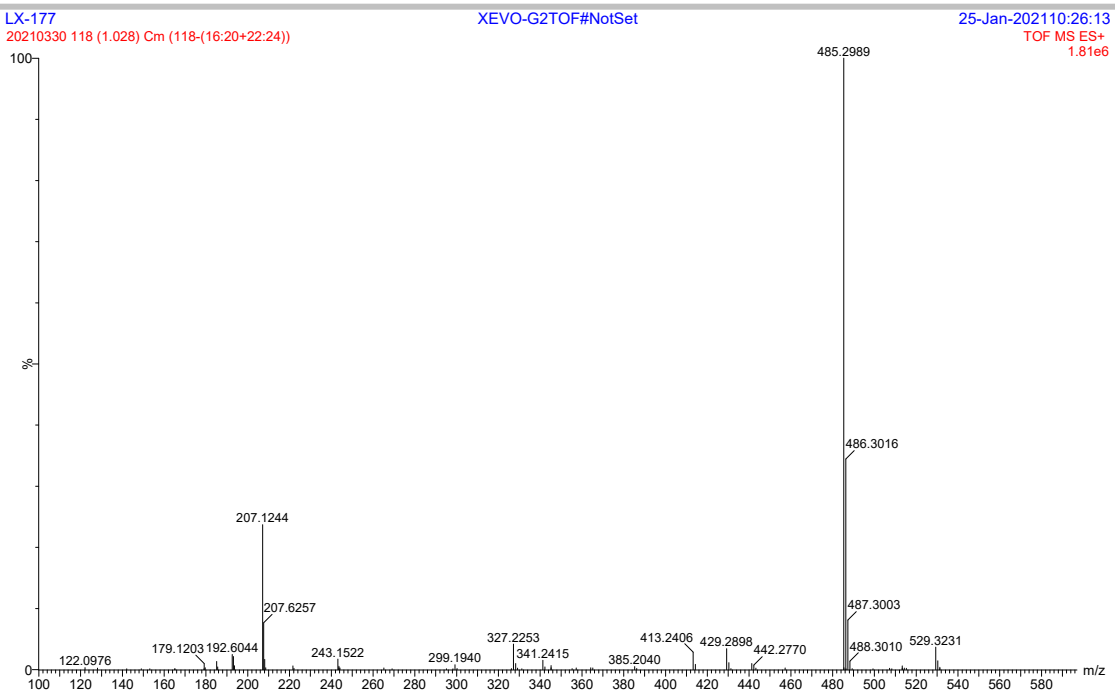


Figure S31. The HR-MS of compound **ACR670a**

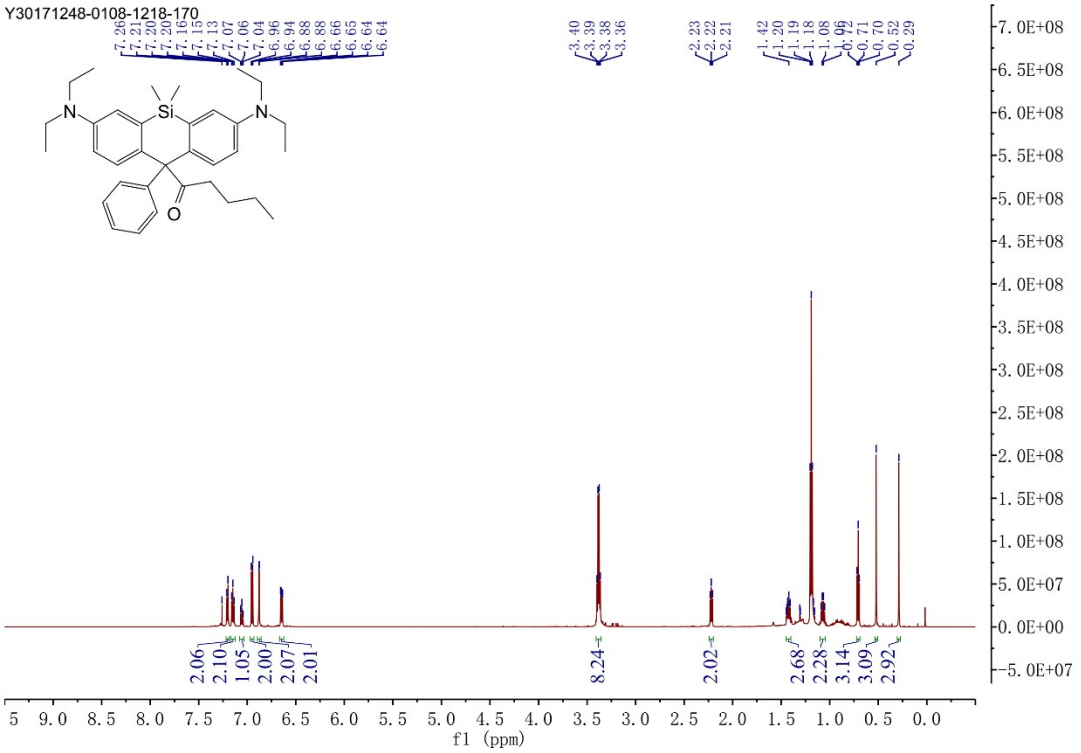


Figure S32. The ^{1}H-NMR of compound **ACR670p** in $CDCl_3$ (600 MHz)

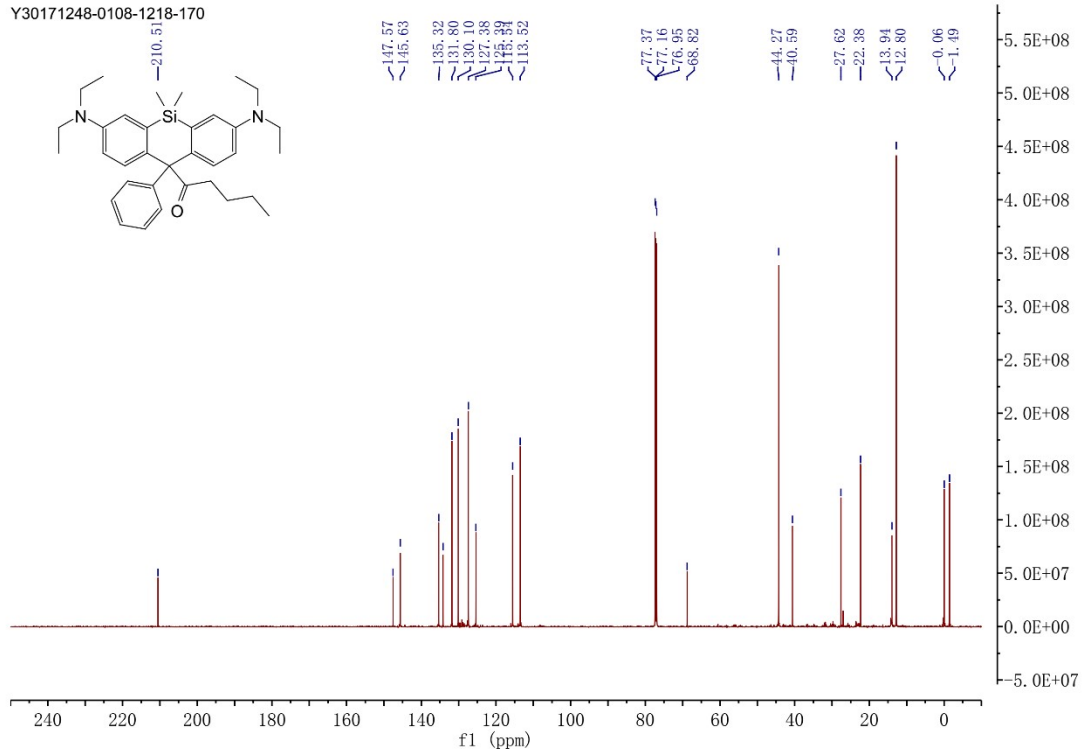


Figure S33. The $^{13}C\{^{1}H\}$-NMR of compound **ACR670p** in $CDCl_3$ (151 MHz).

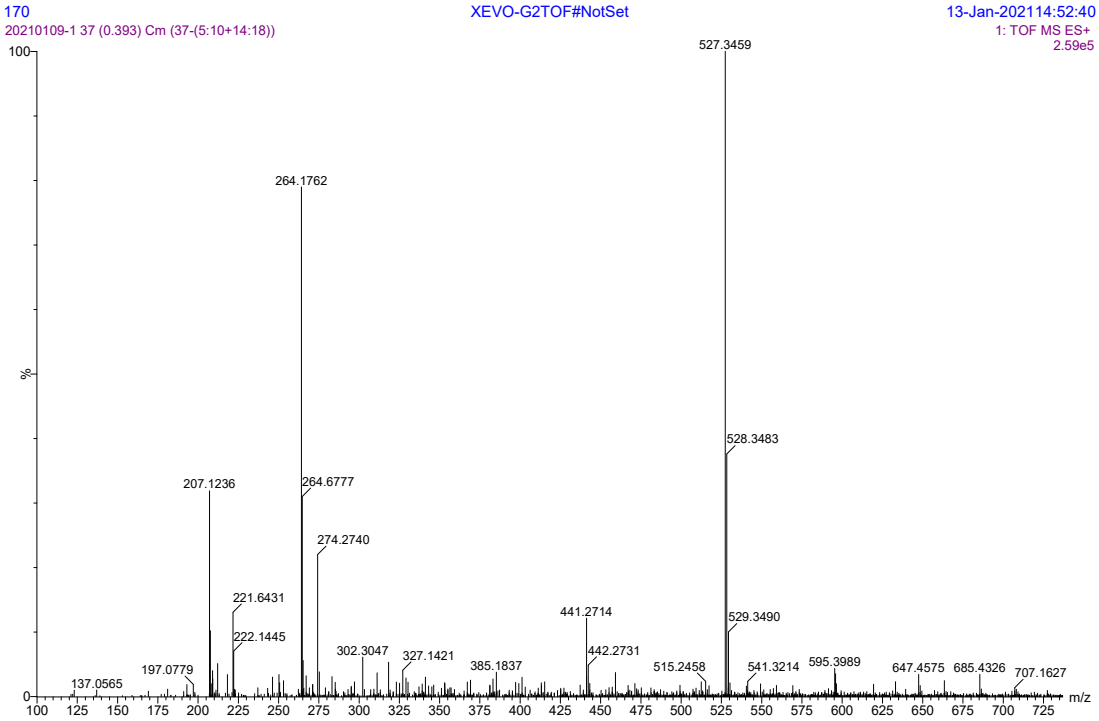
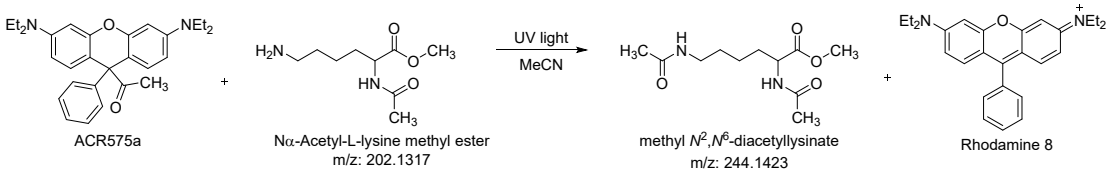


Figure S34. The HR-MS of compound **ACR670p**.



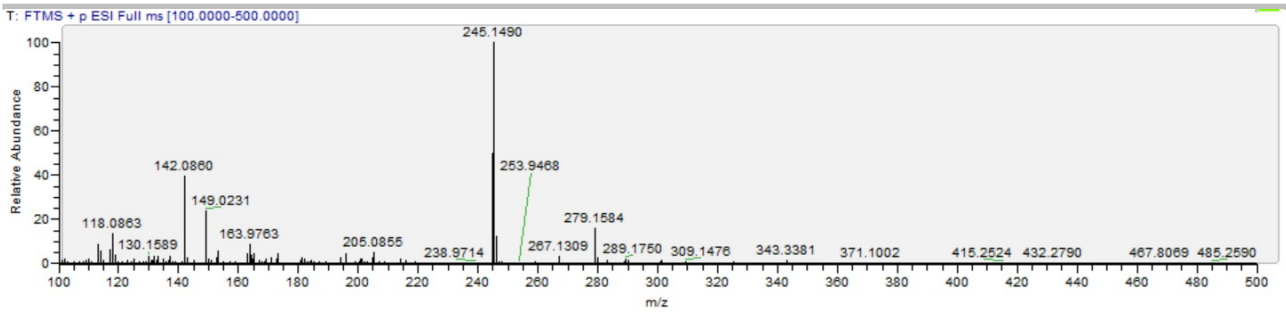


Figure S35. Evaluation of the acetylation potential of **ACR575a** for N_α-Acetyl-L-lysine methyl ester. **ACR575a** (500 μM) and N_α-Acetyl-L-lysine methyl ester hydrochloride (2 mM, CAS: 20911-93-7) neutralized with K_2CO_3 were dissolved in MeCN and the solution was purged with Ar for 15 min to get rid of oxygen before being irradiated with a 375 nm LED light (20 mW/cm^2) for 10 min.

References

1. Kobayashi, T.; Urano, Y.; Kamiya, M.; Ueno, T.; Kojima, H.; Nagano, T. Highly activatable and rapidly releasable caged fluorescein derivatives. *J. Am. Chem. Soc.* **2007**, *129*, 6696-6697.
2. Huang, H.; Dong, F.; Tian, Y. Mitochondria-targeted ratiometric fluorescent nanosensor for simultaneous biosensing and imaging of O2•–and pH in live cells. *Anal. Chem*. **2016**, *88*, 12294-12302.
3. Vorhees, C. V.; Williams, M. T. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat. Protoc*. **2006**, *1*, 848-858.
4. Luo, X.; Yang, H.; Wang, H.; Ye, Z.; Zhou, Z.; Gu, L.; Chen, J.; Xiao, Y.; Liang, X.; Qian, X.; Yang, Y. Highly sensitive hill-type small-molecule pH probe that recognizes the reversed pH gradient of cancer cells. *Anal. Chem*., **2018**, *90*, 5803-5809.
5. Wang, B.; Chai, X.; Zhu, W.; Wang, T.; Wu, Q. A general approach to spirolactonized Si-rhodamines. *Chem. Commun.* **2014**, *50*, 14374-14377.

Author Contributions

Y.Y., Y.T., W.Y., X.Q., and X.L. devised the project and are the corresponding authors. X. L. designed and performed the chemical synthesis, characterization, spectral recording with oversight from Y.Y. and X.Q., along with support from X.W. and J.C. for the TCSPC. Z.Z. performed all NSC associated cell experiments and animal tests under the supervise of Y.T. J.W. performed all Hela cells associated experiments with oversight from W.Y. Principal manuscript writing was by Y.Y., Y.T., X.L and Z.Z. with critical feedback from all authors. Y. Z. performed the protein mass spectrometry with oversight from B. G.. Y.Y. was the submitting author.