Selective Mitochondrial Proteins Labeling Enabled by Biocompatible Photocatalytic Reactions inside Live Cells

Supporting Information

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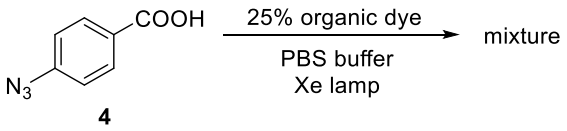
I. Mechanistic Studies

The photocatalytic decomposition of aryl azide

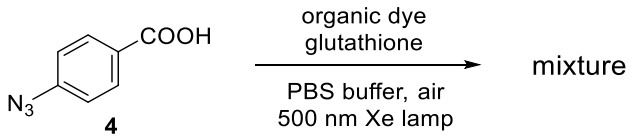
Reactions were conducted in 5 mL clear-colored glass vials equipped with a magnetic stir bar at ambient temperature. 4-azidobenzoic acid **4** (200 μM, 50 mM stock solution in DMSO) and organic dyes (50 μM, 50 mM stock solution in DMSO) were mixed in 2.0 mL PBS buffer (10 mM, pH 7.4) and irradiated with visible light for 1 h. The reactions were carried out with a smart xenon lamp light source CEL-HXF300E/HXUV300E (*CEAULIGHT*) equipped with band-pass filters (15 cm from the Xe lamp, 19.8 mW cm^{-2}). The light intensity was measured with an FZ-A photometer (*BNU Photoelectric Instrument*). After the reaction, a sample of the mixture (50 μL) was directly subjected to HPLC analysis to afford the conversions. HPLC experiments were performed on a Dionex Ultimate 3000 HPLC system (*ThermoFisher scientific*) using a C18 column (250 × 4.6 mm internal diameter, particle size 5 μm).



4

entry	organic dye	light	λ_{max}	E_T (eV)	$E_{1/2}$(red)	conversion (in air)	conversion (in N_2)
1	blank	500 nm				<5%	<5%
2	Acridine Orange	500 nm	495 nm	2.13	-0.92 V	40%	70%
3	Fluorescein	500 nm	491 nm	1.94	-1.22 V	28%	33%
4	Rhodamine 123	500 nm	505 nm	1.90	-0.89 V	54%	50%
5	Rhodamine B	550 nm	550 nm	1.80	-0.96 V	<5%	<5%
6	TMRE	550 nm	550 nm	~1.80	~ -0.96 V	<5%	6%

Table S1. The photocatalytic decomposition of 4-azidobenzoic acid **4** with different organic dyes. Conversions were given by the area changes of **4** (retention time 10.8 min, 210 nm) showed in HPLC. HPLC gradient: 5% to 75% acetonitrile (0-15 min) in phosphate buffer (20 mM, pH 4.0). The wavelengths of the maximum absorption (λ_{max}), triplet energies (E_T), and part of ground state reductant potentials ($E_{1/2}$ (red)) of the organic dyes were adapted from literatures.[1-2] Measured ground state redox potentials ($E_{1/2}$(red)) were shown in Figure S1. The E_T and $E_{1/2}$(red) of TMRE were estimated by those of Rhodamine B. Parts of the results were also shown in Figure 3b.



entry	organic dye	glutathione	conversion
1	Acridine Orange	0.2 mM	16%
2	Acridine Orange	1 mM	12%
3	Acridine Orange	5 mM	11%
4	Rhodamine 123	0.2 mM	25%
5	Rhodamine 123	1 mM	23%
6	Rhodamine 123	5 mM	16%
7	Fluorescein	0.2 mM	24%
8	Fluorescein	1 mM	34%
9	Fluorescein	5 mM	36%

Table S2. The photocatalytic decomposition of 4-azidobenzoic acid **4** in the presence of glutathione. HPLC conditions were identical to those shown in Table S1.

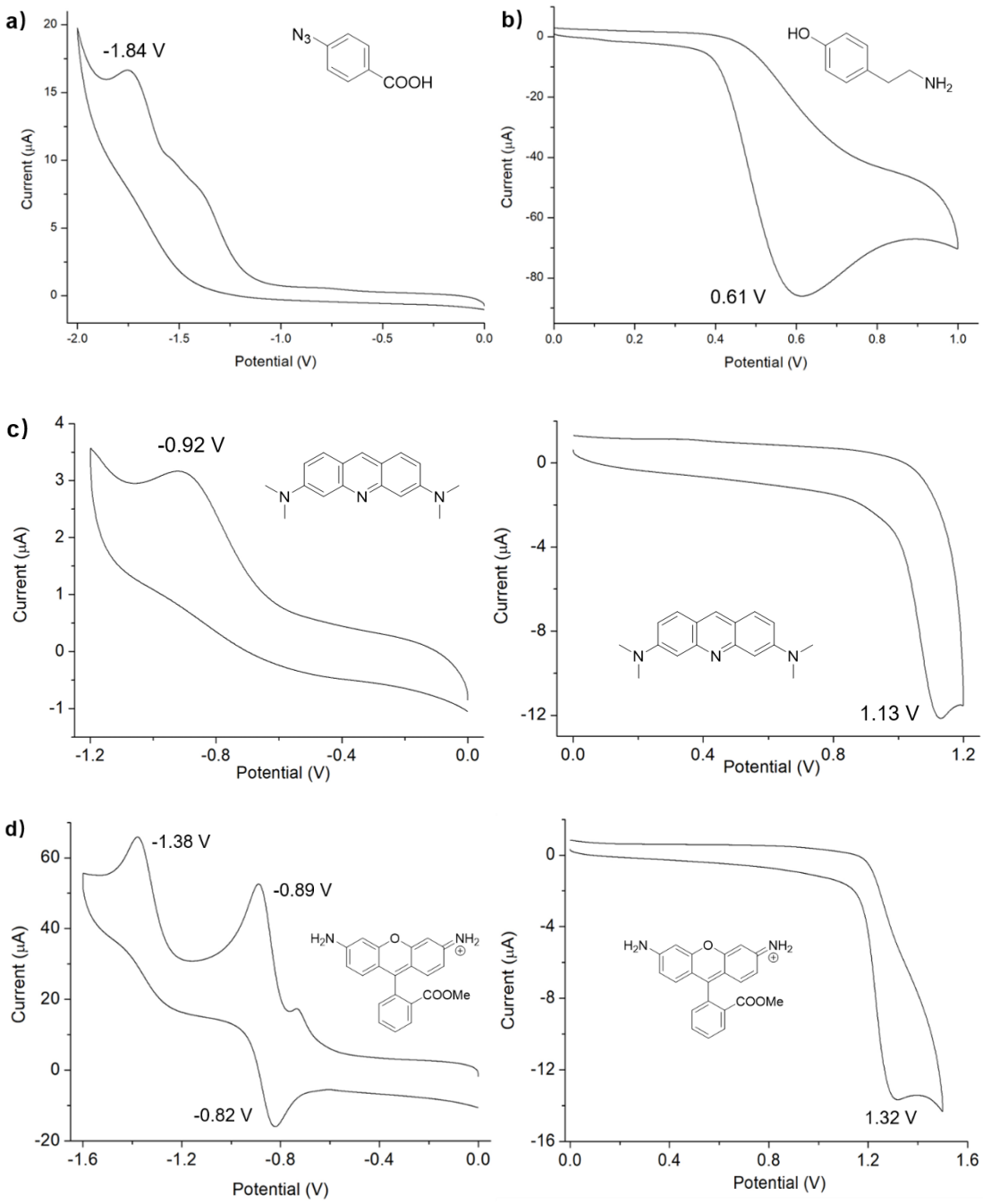


Figure S1. Cyclic voltammogram of the representative compounds and organic dyes Acridine Orange (AO) and Rhodamine 123 (Rh 123). Experiments were performed with a CH Electrochemical Analyzer (*CH Instruments, Inc.*) at room temperature under a nitrogen atmosphere. A solution of 1 mM sample in methanol containing 100 mM tetrabutylammonium hexafluorophosphate (TBAP) as the supporting electrolyte was measured with a three-electrode system: a glassy carbon working electrode, a Pt wire counter electrode, and a saturated calomel reference electrode. The scan rate was set as 0.1 V/s.

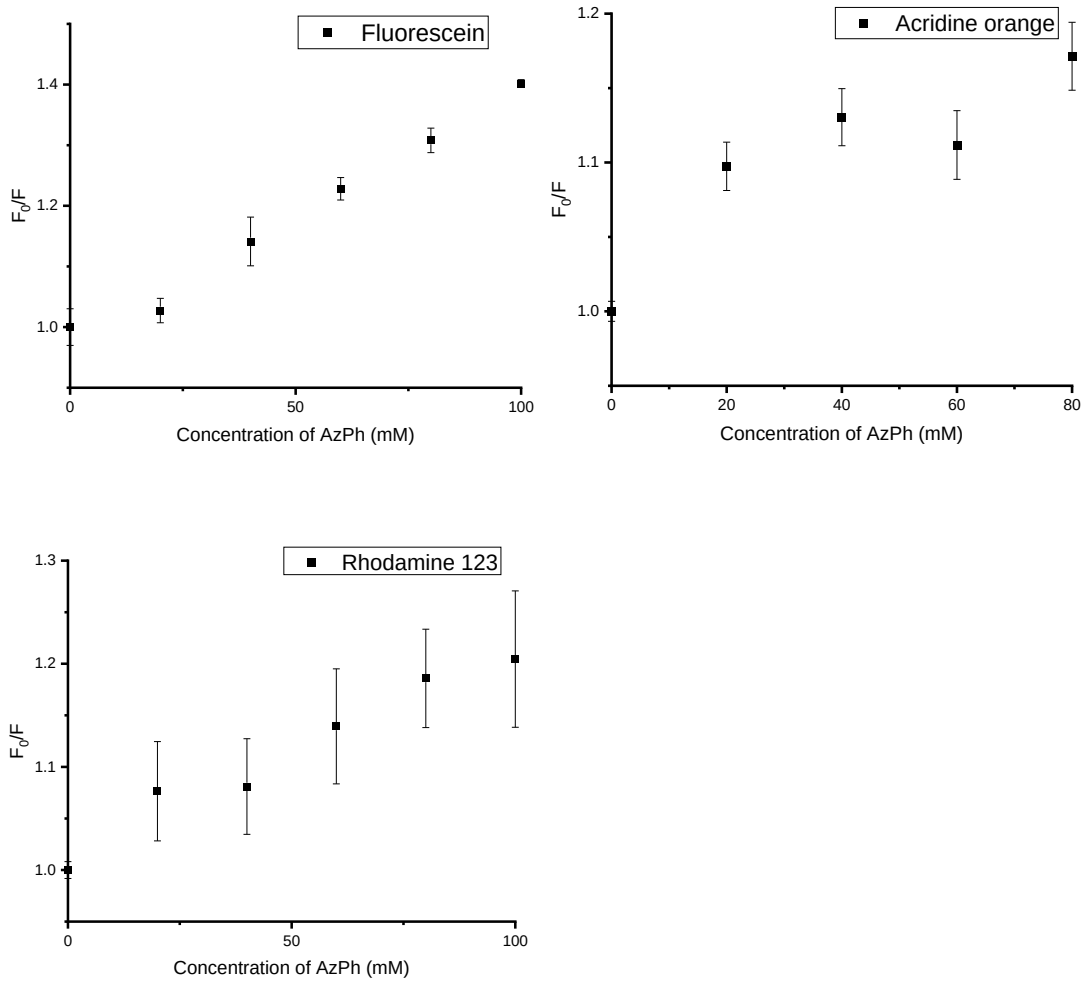


Figure S2. Fluorescence quenching of organic dyes with different concentrations of AzPh **5** (Stern-Volmer analysis). F_0 and F were emission intensity in the absence and presence of quencher in Stern-Volmer equation ($F_0/F = 1 + k[Q]$, where k was the slope and [Q] was the concentration of quencher) respectively. The methanol solution of organic dye (20 μM) was mixed with methyl 4-azidobenzoate **5** under the nitrogen atmosphere, and was added to a 1 cm quartz cuvette. The emission spectra of samples were collected with a HITACHI F-2700 FL Spectrophotometer. The excitation wavelength (Ex) and the wavelength in emission spectra used for calculation (Em) were shown as following: Fluorescein Ex = 490 nm, Em = 519 nm; Acridine Orange Ex = 495 nm, Em = 526 nm; Rhodamine 123 Ex = 500 nm, Em = 536 nm. Error bars represented mean ± SD (n = 3).

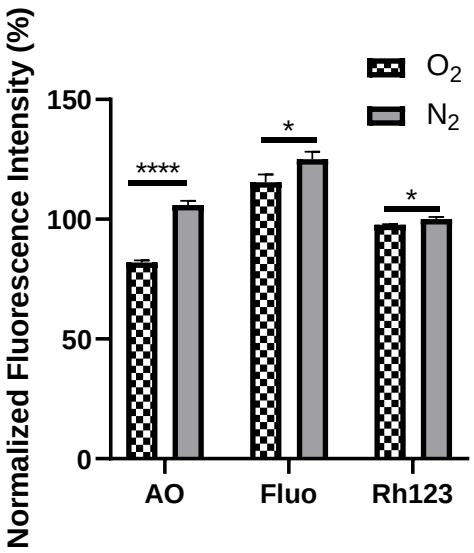


Figure S3. Effect of oxygen on the fluorescence of organic dyes. Fluorescence intensity measured under air atmosphere in each group was set as the 100% standard respectively. Error bars represented mean ± SD (n = 3). The statistical significance of the differences between groups was evaluated with the unpaired Student's t-test. P value of 0.05 and below was considered significant: $p < 0.05$ (*) and $p < 0.0001$ (****).

Determination of singlet oxygen quantum yields ($\Phi_{\triangle}$) of organic dyes

According to the previous literature,[3] singlet oxygen generation by organic dyes involved two main reaction pathways with the quencher A.

$$A + {}^1O_2 \xrightarrow{k_r^A} AO_2 \text{ or other products} \qquad A + {}^1O_2 \xrightarrow{k_q^A} A + {}^3O_2 \text{ (physical quenching by A)}$$

The rate of disappearance of a specific chemical quencher A obeys Supplementary equation (1)

$$-\frac{d[A]}{dt} = I_{abs}\,\Phi_{\triangle}\frac{k_r^A}{k_d}[A] \quad (1)$$

After integration, equation (1) was transformed to Supplementary equation (2)

$$ln\frac{[A]}{[A]_0} = -I_{abs}\,\Phi_{\triangle}\frac{k_r^A}{k_d}t \quad (2)$$

k_d is the constant of deactivation of the singlet oxygen via the solvent, k_r is the rate constant of quenching of singlet oxygen by quencher A, and I_{abs} is intensity of light absorbed by solution.

ADPA is water soluble and used as the chemical quencher of singlet oxygenfor determination of the singlet oxygen quantum yields[4]. ADPA (100 μM, 100 mM stock solution in DMSO) and organic dyes (10 μM, 50 mM stock solution in DMSO) were mixed in 1.0 mL PBS buffer (10 mM, pH 7.4 or 2.0). The solution was placed in a cuvette and irradiated for different time at λ = 500 nm (a smart xenon lamp light source, 19.8 mW /cm^2) under air. The absorbance of the solution was measured at 400 nm. By taking the logarithm of the ratio of the absorption value at each time to the initial absorption value as the ordinate, and time is the abscissa, we fit each point to get the slope (slope $= -\mathrm{I}_{\mathrm{abs}}\,\Phi_{\triangle}\frac{k_r^A}{k_d}$). f is the fraction of light absorbed at λ = 500 nm (equation (4). The absorbance (A) of organic dyes solution (10 μM) at 500 nm was measured. By taking Rose Bengal ($\Phi_{\triangle stan}$ = 0.76) as the standard [5], the singlet oxygen quantum yields was calculated using equation (3), $slope_{meas}$ and f_{meas} belonged to the organic dyes to be tested while $slope_{stan}$ and f_{stan} was obtained from Rose Bengal.

$$\Phi_{\triangle cal} = \frac{slope_{meas} f_{stan}\,\Phi_{\triangle ref}}{slope_{stan} f_{meas}} \quad (3)$$

$$f = 1 - 10^{-A} \quad (4)$$

The singlet oxygen quantum yields of Eosin Y, Fluorescein, Rhodamine 123 and Acridine orange were determined. The singlet oxygen quantum yield of Eosin Y was 0.56, this was in good agreement with that determined by Van de Vorst et al.($\Phi_{\triangle}$=0.57) [4]

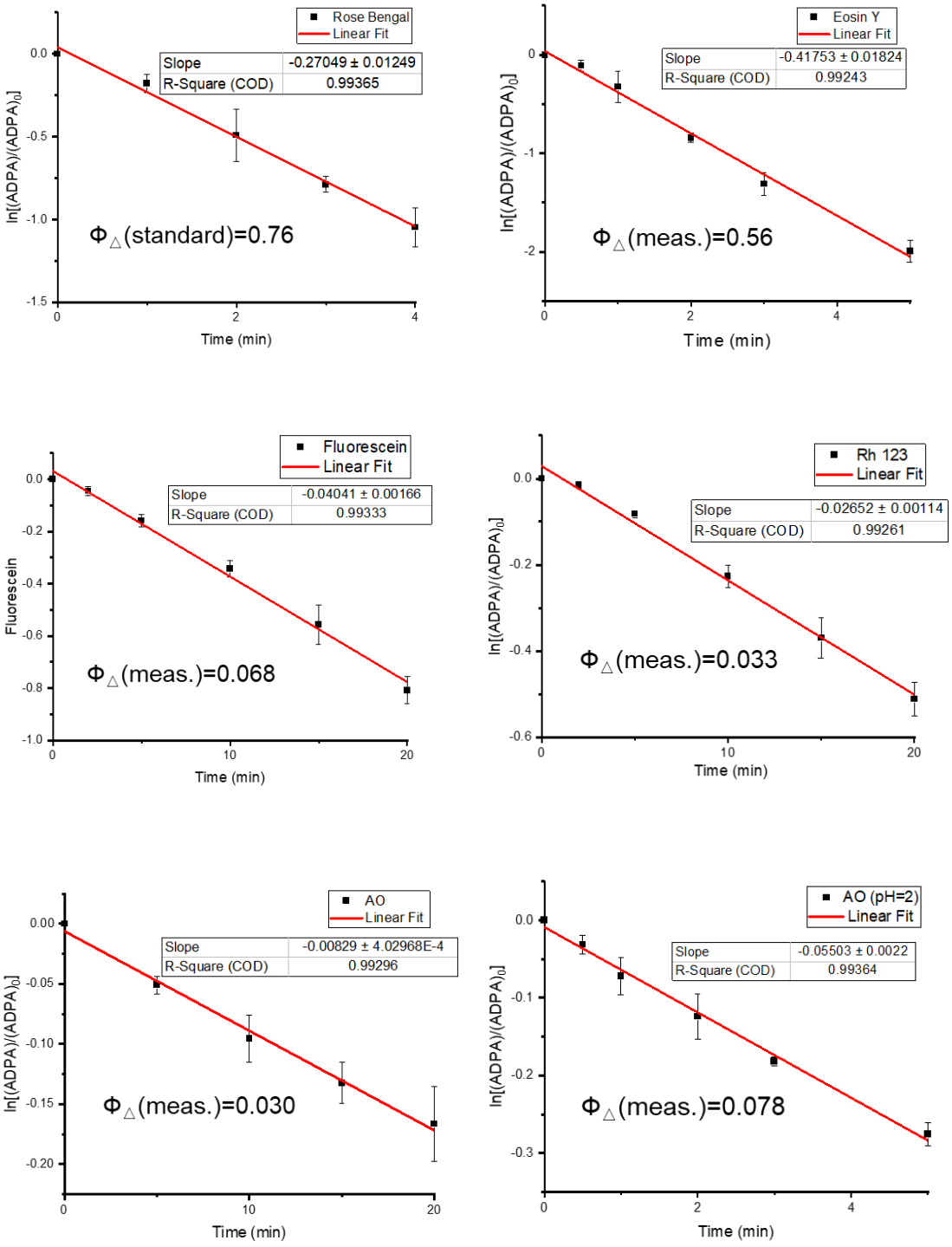


Figure S4. Plots used to calculate singlet oxygen quantum yields of organic dyes in the neutral or acidic buffer solutions (AO, pH = 2). Error bars represented mean ± SD (n = 3).

Time-course of the photocatalytic decomposition of AzPh-biotin probe 1

To evaluate the time course of the photocatalytic decomposition, organic dyes (100 μM, 10 mM stock solution in DMSO) was added to a solution of AzPh-biotin probe **1** with or without BSA in the PBS buffer (10 mM, pH 7.4) and irradiated with blue or green LED for 0, 2, 5, 10, 20, 30, 60 min. The solutions were then subjected to HPLC analysis to afford the conversions. HPLC experiments were performed on a Dionex Ultimate 3000 HPLC system (*ThermoFisher scientific*) using a C18 column (250 × 4.6 mm internal diameter, particle size 5 μm).

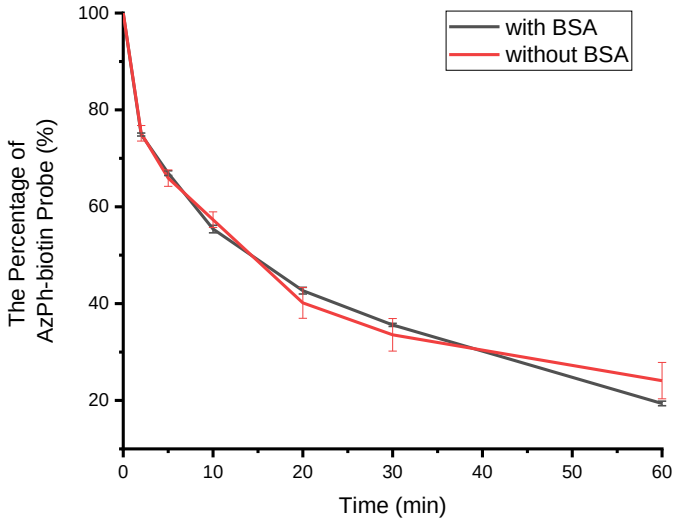


Figure S5. Kinetic study for conversion of AzPh-biotin probe **1** (100 μM) with fluorescein (100 μM) using 468 nm (5.8 mW/cm^2) LED light in the air atmosphere with or without BSA (2 μM). First-order rate constant was 0.0279 min^{-1}, $t_{1/2}$=24.8 min. Error bars represented mean ± SD (n = 3).

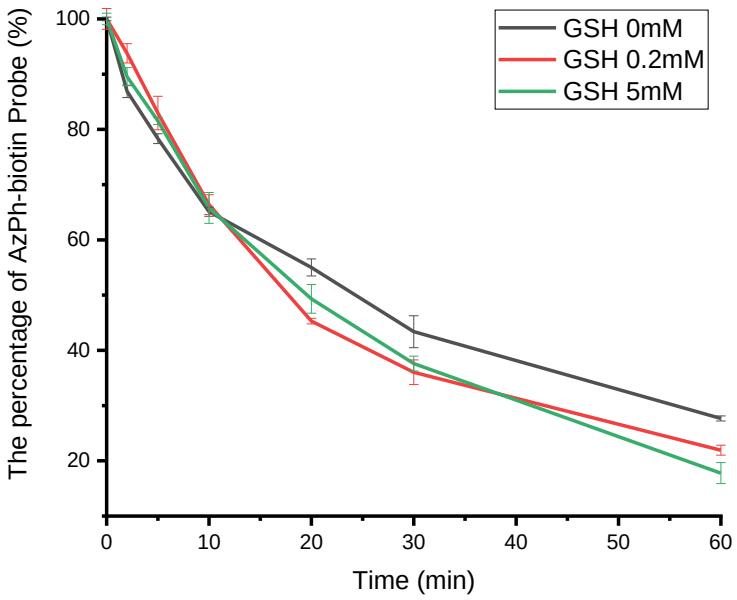


Figure S6. Kinetic study for conversion of AzPh-biotin probe **1** (100 μM) with fluorescein (100 μM) using 468 nm (5.8 mW/cm^2) LED light in the air atmosphere in the presence of GSH at different concentrations (0, 0.2, 5mM, respectively). Error bars represented mean ± SD (n = 3).

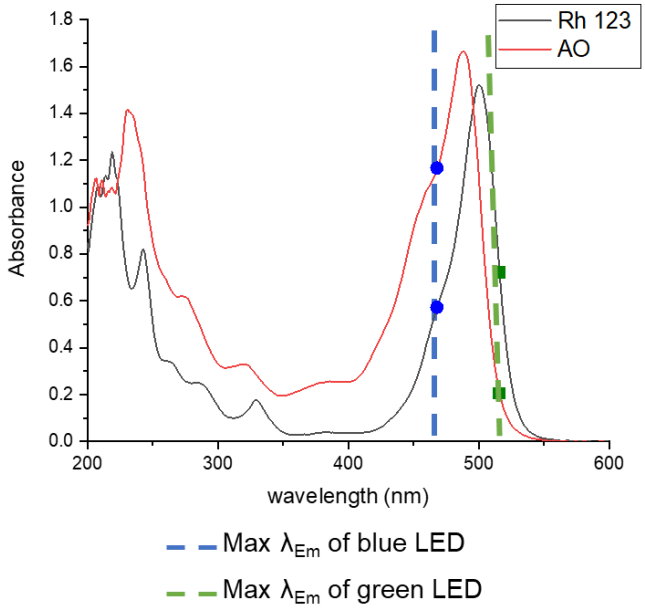


Figure S7. Absorption spectra of Acridine orange (25 μM) and Rhodamine 123 (25 μM) and maximum emission wavelength of blue LED (468 nm) and green LED (515 nm).

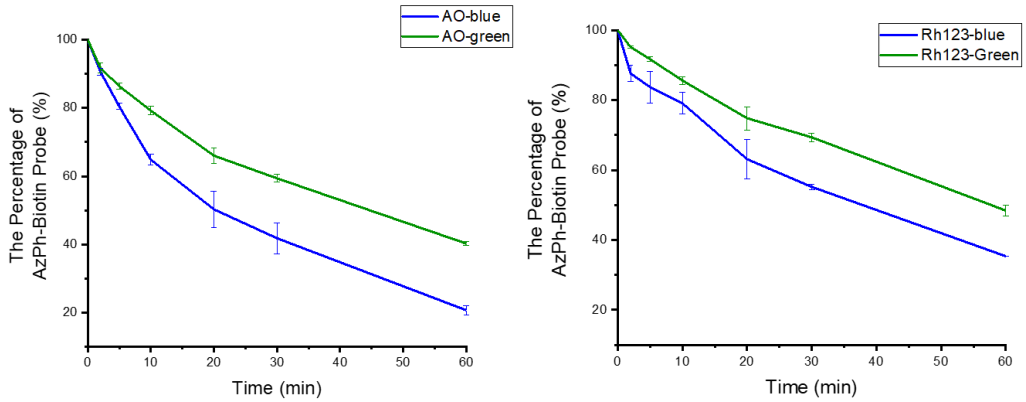


Figure S8. Kinetic study for AzPh-biotin probe **1** (100 μM) with organic dyes (100 μM) using 468 nm blue LED light (5.8 mW/cm^2) or 515 nm green LED light (2.9 mW/cm^2). Error bars represented mean ± SD (n = 3). In the presence of AO, first-order rate constants of blue and green light group were 0.025 and 0.014 min^{-1}, respectively. In the presence of Rh 123, first-order rate constant of blue and green light group were 0.016 and 0.012 min^{-1}, respectively.

Time-course of capturing photocatalytic reaction intermediates by amines

A solution of **5** (18 mg, 0.10 mmol) and Acridine Orange (6.6 mg, 0.020 mmol) in methanol (2.0 mL) was placed in a 5.0 mL clear-colored glass vial equipped with a magnetic stir bar. After the addition of 1.0 mL diethylamine **13**, the reaction was bubbled with nitrogen gas to remove the oxygen. The vial was then sealed and exposed to 2 × 4W 468 nm blue LEDs (10.0 cm from the LEDs, 10.4 mW/cm^2) with stirring at ambient temperature for 1, 3, 6, 12, 24, 48 hours. After irradiation, the conversion and yields were determined by ^{1}H NMR using *p*-nitrobenzyl alcohol as an external standard or HPLC using standard curve.

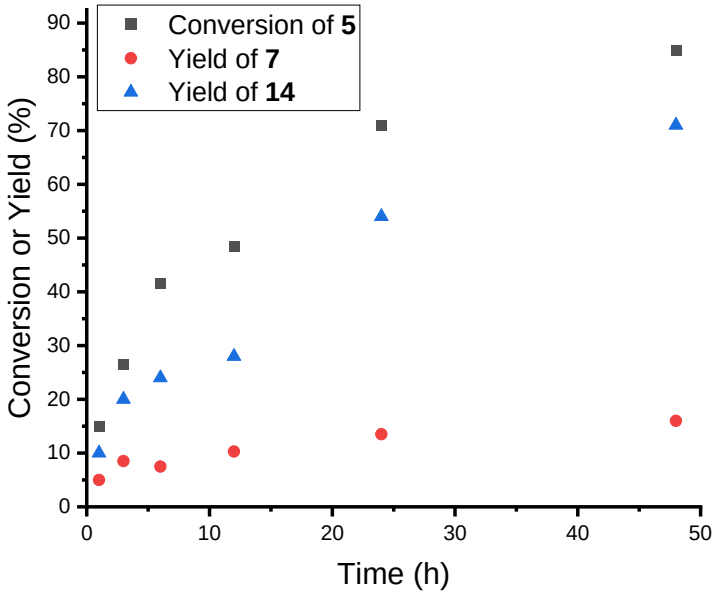
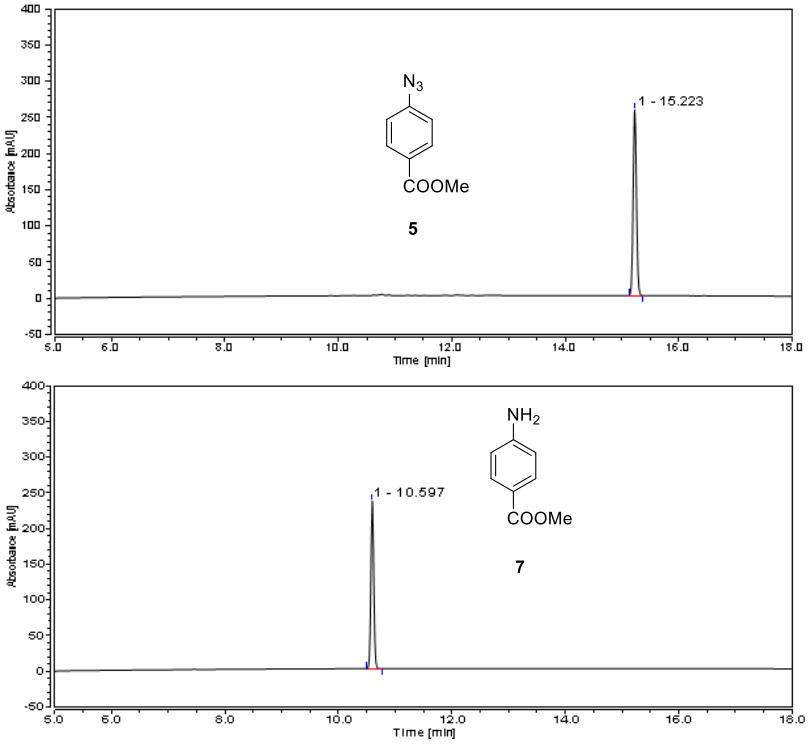


Figure S9. Kinetic study for conversion of compound **5** to **7** and **14** after adding diethylamine **13** under 2 × 4W 468 nm blue LEDs (10.4 mW/cm^2) irradiation at room temperature in methanol. Conversions of **5** and and yields of **7** and **14** were determined by ^{1}H NMR analysis.



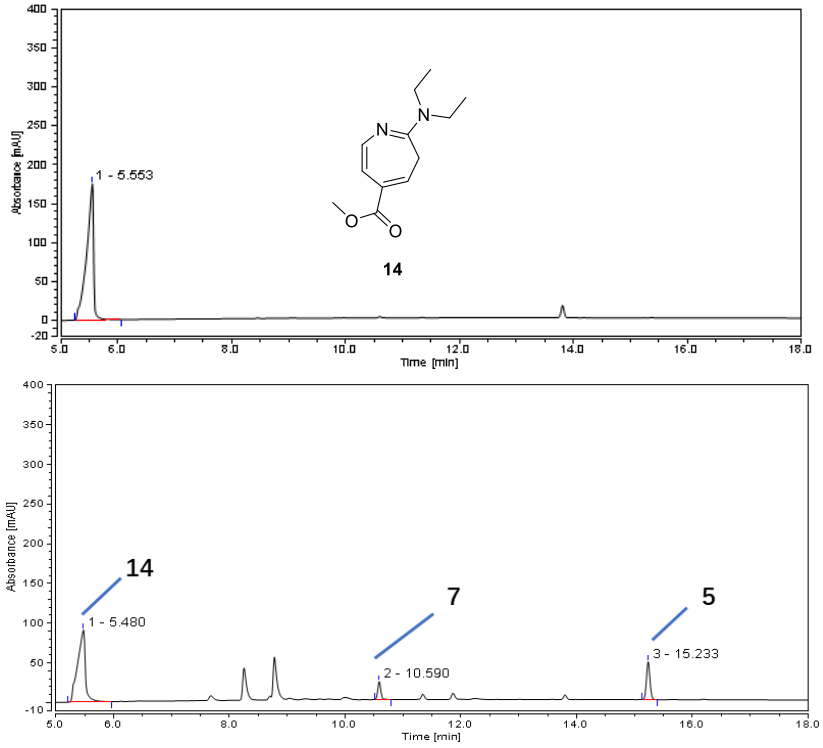


Figure S10. HPLC traces for conversion of compound **5** to **7** and **14** under 2 × 4W 468 nm blue LEDs (10.4 mW/cm^2) (48 h irradiation): the standard compounds **5**, **7** and **14** and the reaction mixtures. The conversion of **5** was 86%, yields of **7** and **14** were 13% and 52%, respectively. HPLC conditions were identical to those shown in Table S1.

II. Protein Photolabeling *in Vitro*

General procedure

To a solution of 2 μM bovine serum albumin (BSA) in PBS buffer (10 mM, pH 7.4) in 1.5 mL colorless EP tubes was added the biotin probe (100 μM, 50 mM stock solution in DMSO), organic dyes (100 μM, 50 mM stock solution in DMSO) and other additives. The solution was mixed and irradiated at 25 ºC with 468 nm blue LEDs (8 cm from the LED, 5.8 mW/cm^2) or a smart xenon lamp light source CEL-HXF300E/HXUV300E (*CEAULIGHT*) equipped with band-pass filters (15 cm from the Xe lamp, 19.8 mW cm^{-2}).

Western blotting analysis

After photolabeling, samples were added with 5× protein loading buffer (*Sangon*

Biotech, containing 313 mmol/L Tris-HCl (pH 6.8), 0.05% bromophenol blue, 50% glycerol, 10% SDS and DTT) and fully mixed, then directly subjected to SDS-polyacrylamide gel (10% acrylamide) electrophoresis (PAGE) at 180 V for 60 min.

The PAGE gels were transferred to the activated PVDF membrane with Trans-Blot Turbo Transfer System (*Bio-Rad*) and blocked with 4% BSA in TBST (0.1% Tween-20 in Tris-buffered saline) at 4 °C overnight. The blots were incubated with 0.2 μg/mL streptavidin-HRP (*Beyotime Biotechnology*) in TBST at room temperature for 60 minutes, then washed with TBST 3×10 minutes before development with SuperSignal West Pico reagent (*Thermo Scientific*) and obtained image with ChemiDoc XRS System and Image Lab Software (*Bio-Rad*). The duplicated PAGE gels were stained with Coomassie brilliant blue (CBB) and obtained image with ChemiDoc XRS System and Image Lab Software.

Effect of the reactive oxygen species on protein labeling

To a solution of 2 μM bovine serum albumin (BSA) in PBS buffer (10 mM, pH 7.4) in 1.5 mL colorless EP tubes was added the AzPh-biotin probe **1** (100 μM, 50 mM stock solution in DMSO), organic dyes (100 μM, 50 mM stock solution in DMSO) and the reactive oxygen species. The solution was mixed and irradiated at 25 ºC with 468 nm blue LEDs. We prepared stock solution of various ROS ($^1O_2$, O_2^-, H_2O_2, OCl^-, t-BuOOH, CuOOH, and •OH) by adding reagents described in the literature[6-7] as following:

1. Preparation of $^1O_2$ (10 mM): 10 mM NaOCl and 10 mM H_2O_2
2. O_2^- (10 mM): 10 mM KO_2
3. H_2O_2 (10 mM): 30% H_2O_2 solution diluted to 10 mM
4. OCl^- (10 mM): 10 mM NaOCl
5. t-BuOOH (10 mM): 10 mM tert-butylhydroperoxide
6. CuOOH (10 mM): 10 mM cumene hydroperoxide
7. •OH (100 μM): 20 mM $FeSO_4$•$7H_2O$ and 200 mM H_2O_2.

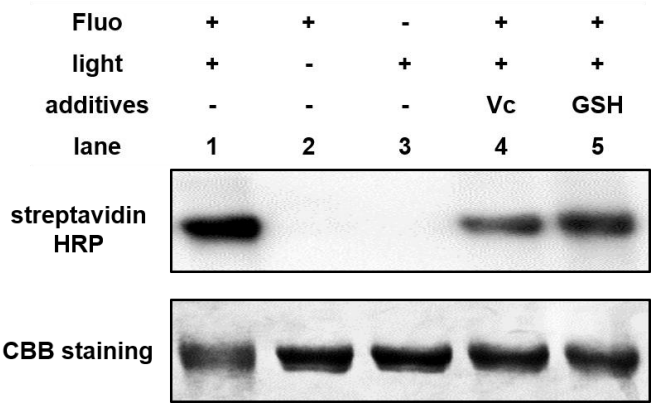


Figure S11. Photocatalytic labeling of BSA with the Phenol-biotin probe **2** and the control experiments. 1 mM ascorbates sodium (Vc) or glutathione (GSH) was added as additives. Samples were irradiated with 468 nm blue LEDs for 1 h in the air.

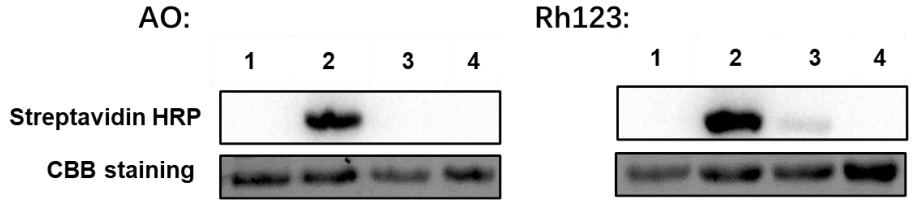


Figure S12. Photocatalytic BSA labeling with biotin probes **1-3** (100 μM) using organic dyes (100 μM) under the blue LED irradiation for 1 h in the air. Lane 1: blank; Lane 2: AzPh-biotin probe **1**; Lane 3: Phenol-biotin probe **2**; Lane 4: AcPh-biotin probe **3**.

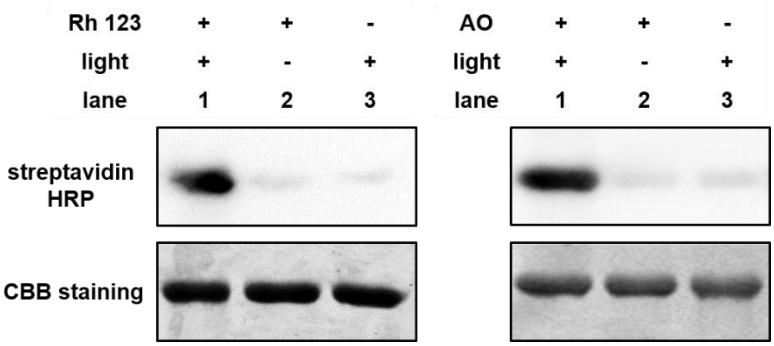


Figure S13. Control experiments of the photocatalytic labeling of BSA using different organic dyes and AzPh-biotin probe **1**. Rhodamine 123 (Rh 123) and Acridine Orange (AO) were used as the photocatalyst respectively. Samples were irradiated with 468 nm blue LEDs (5.8 mW/cm^2)) for 1 h in the air.

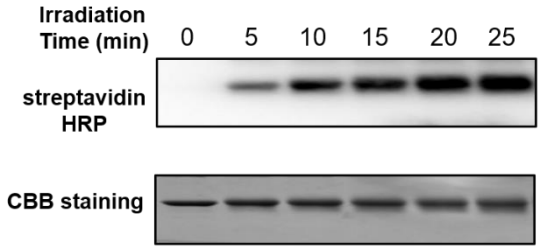


Figure S14. Time course of the photocatalytic BSA labeling by AzPh-biotin probe **1** (100 μM) with fluorescein (100 μM) under 468 nm (5.8 mW/cm^2) LED light irradiation in the air.

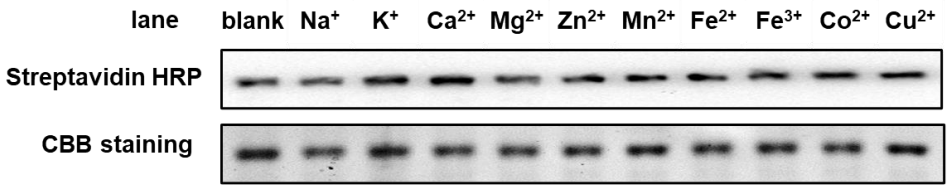


Figure S15. Effects of the metal ions on the photocatalytic BSA labeling by AzPh-biotin probe **1** (100 μM) with fluorescein (100 μM) under 468 nm (5.8 mW/cm^2) LED light irradiation in the air atmosphere (20 min irradiation). The concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+} were 1 mM, and concentrations of Zn^{2+}, Mn^{2+}, Fe^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+} were 100 μM.

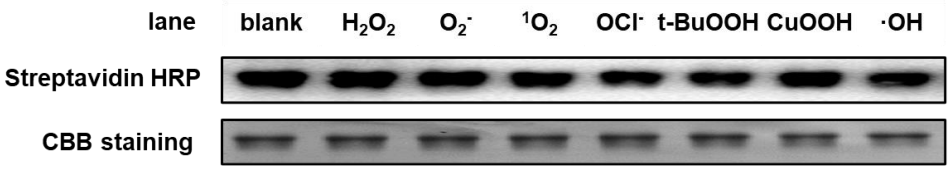


Figure S16. Effects of the reactive oxygen species on the photocatalytic BSA labeling by AzPh-biotin probe **1** (100 μM) with fluorescein (100 μM) under 468 nm (5.8 mW/cm^2) LED light irradiation in the air atmosphere (20 min irradiation). The concentrations of H_2O_2, O_2^-, $^1O_2$, OCl^-, t-BuOOH (tert-butylhydroperoxide) and CuOOH (cumene hydroperoxide) were 100 μM, while the concentration of ·OH was 1 μM.[6-7]

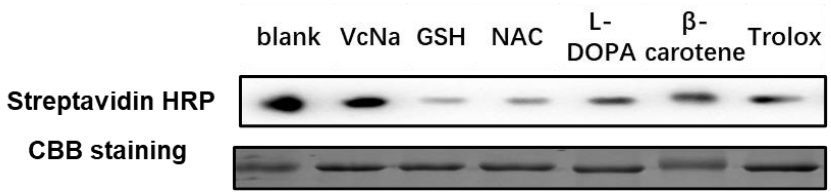


Figure S17. Effects of the radical quenchers or reductants on the photocatalytic BSA labeling by AzPh-biotin probe **1** (100 μM) with fluorescein (100 μM) under 468 nm (5.8 mW/cm^2) LED light irradiation in the air atmosphere (20 min irradiation). The concentrations of additives were 1mM.

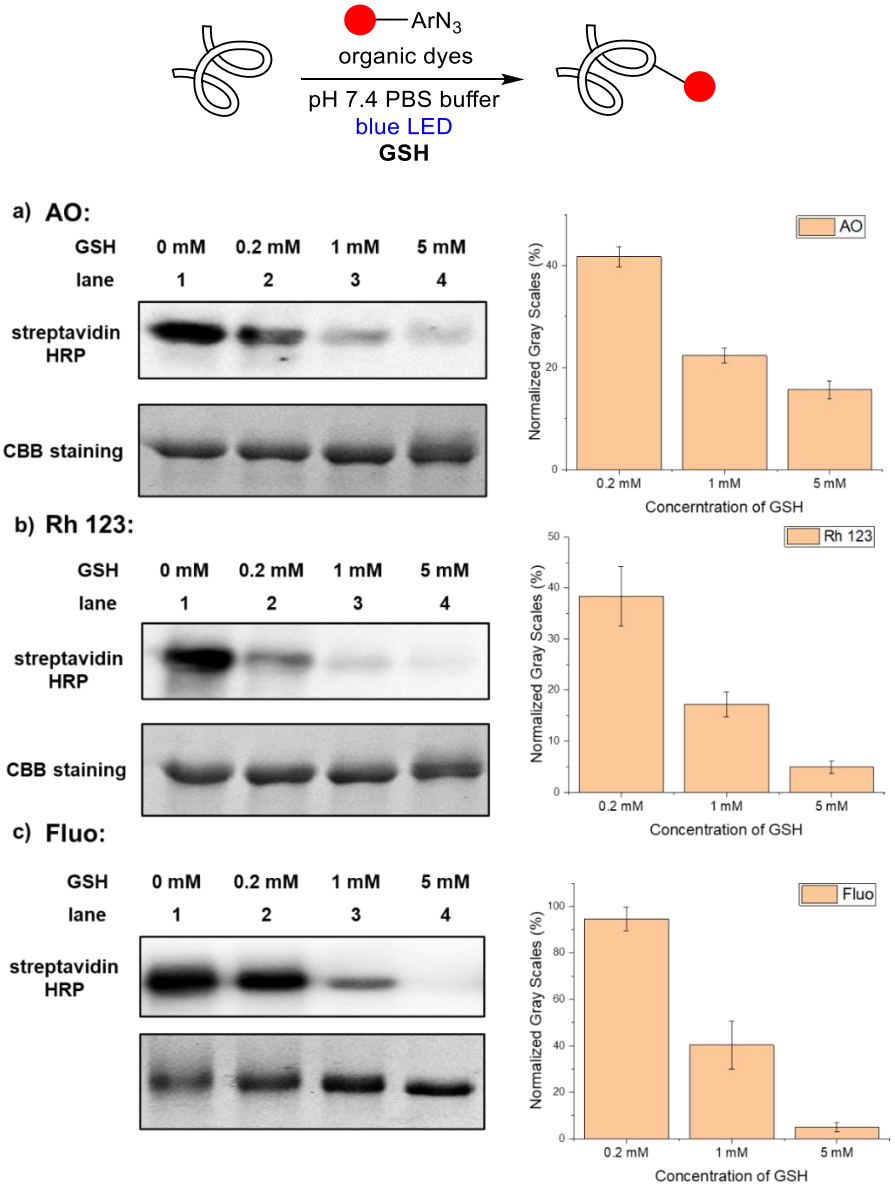


Figure S18. Photocatalytic labeling of BSA with AzPh-biotin probe **1** in the presence of different concentrations of glutathione (GSH) under 468 nm (5.8 mW/cm^2) LED

light irradiation for 20 min. Acridine Orange (AO), Rhodamine 123 (Rh 123) and Fluorescein (Fluo) were used as the photocatalyst respectively **(a-c)**. Left: the western blotting assay. Right: Normalized gray scale of the western blotting assay. The gray scales of lanes were calculated with the Image Lab Software (*Bio-Rad*), and lane 1 in each group (without GSH addition) was set as the 100% standard respectively. Data were expressed as mean and SD, n=3.

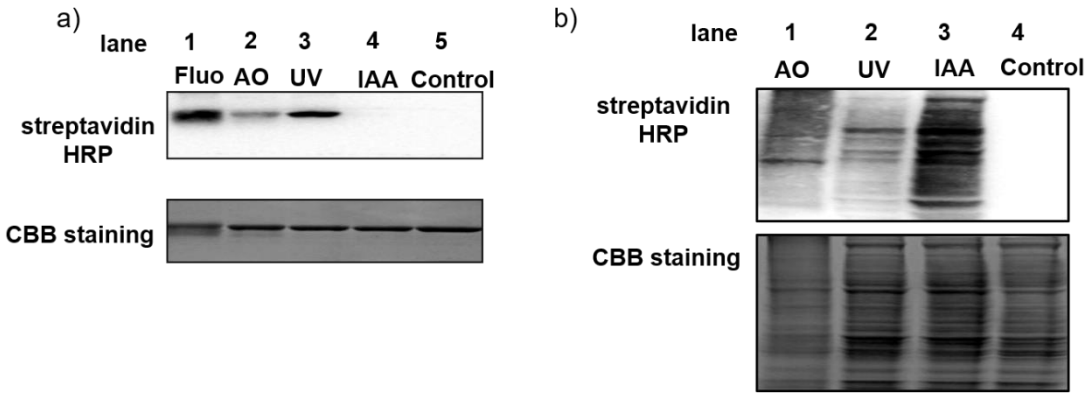


Figure S19. Evaluation of the photocatalytic labeling reactivity on BSA (a) and MCF-7 cell lysates (b). Photocatalytic labeling with AzPh-biotin probe **1** (100 μM) and Fluo / AO (100 μM) was carried out under 468 nm (2.95 mW/cm^2) LED light irradiation on BSA (20 min irradiation) and MCF-7 lysate (10 min irradiation). UV photolabeling with AzPh-biotin probe **1** (100 μM) was carried out under 365 nm UV lamp (7.92 mW/cm^2) on BSA (20 min irradiation) and MCF-7 lysate (10 min irradiation); IAA Protein labeling with Biotin-iodoacetamide (IAA) (100 μM) was carried out under 37 ^{o}C for 1.5 h. Control group were protein or cell lysates treated without light and AzPh-biotin probe **1**.

III. Mammalian Cell Studies

Mammalian cell culture

MCF-7 cells and HeLa cells used in the experiments were purchased from ATCC. Cells were grown in the Dulbecco's modified Eagle's medium (DMEM, *Hyclone*) supplemented with 10% fetal bovine serum (FBS, *Gibco*) and 100 U/mL Penicillin-Streptomycin (Pen strep, *Gibco*) under a humidified atmosphere of 5% CO_2 at 37 ºC. Dulbecco's phosphate buffered saline solution (DPBS) was purchased from Hyclone. Ultra-pure dimethylsulfoxide (DMSO) was purchased from Amresco.

General procedure of photolabeling in live cells

Cells were seeded in the appropriated dishes or plates in the culture media. At 80-90% confluence, 10 μM organic dyes was added to the media and incubated for 20 min. Cells were washed with DPBS for three times, then incubated in the fresh DMEM (no phenol red) containing 200 μM AzPh-biotin probe **1** for 30 min. The cell culture was irradiated with the 515 nm green LED (10 cm from the top of the cell culture dish, 2.9 mW/cm^2) for 30 min (Acridine Orange) or 60 min (Rhodamine 123) at 37 ºC under air atmosphere. For control experiments, cells were added with the same amount of DMSO instead of organic dyes (no photocatalyst group), or kept in the dark during light irradiation (dark group). For UV-light induced photolabeling, the culture media was changed to fresh DMEM (no phenol red) containing 200 μM AzPh-biotin probe **1** at 80-90% confluence. After incubation for 30 min, cells were irradiated with a handy 365 nm UV lamp (1 cm from the top of cell culture dish, 11.8 mW/cm^2) at 37 ºC. The light intensity of the UV lamp was measured with a UV-B photometer (*BNU Photoelectric Instrument*).

Confocal imaging analysis

Cells were seeded in a 12-well plate with glass coverslip in the culture media. After photolabeling, cells were washed three times with DPBS, then fixed with 4% paraformaldehyde in PBS at room temperature for 15 minutes. Cells were washed three times with PBS before permeabilized with 0.2% Triton X-100 in PBS at room temperature for 10 minutes. After PBS washing, the fixed cells were blocked with 4%

BSA in PBS at room temperature for 30 minutes, then incubated with 1 μg/mL Cy5-streptavidin (*Jackson ImmunoResearch*) in PBS at room temperature for 60 minutes. Samples were washed 4×5 minutes with PBS before mounted with Fluorescent Mounting Medium (*Dako*) and sealed with the nail polish. Images were acquired on a Leica TCS SP8 confocal laser scanning microscopy platform with 40× dry objective lens or 63× oil-immersion objective lens by using the following regular settings: 488 nm laser with FICT filter (Em 495-530 nm), 561 nm laser with TRITC filter (Em 575-615 nm), and 633 nm laser with Cy5 filter (Em 650-690 nm). For mitochondria-specific labeling, 5 μM mitochondria indicator TMRE was added to the media together with Rhodamine 123. Digital pictures of different samples in each group were taken under identical conditions of gain and exposure, and the pictures in different channels were merged using ImageJ. The colors of TRITC channel (TMRE) and Cy5 channel (Cy5-streptavidin) were set as green and red respectively. Pearson's correlation coefficient (R) was calculated using Coloc 2 plugin in ImageJ.

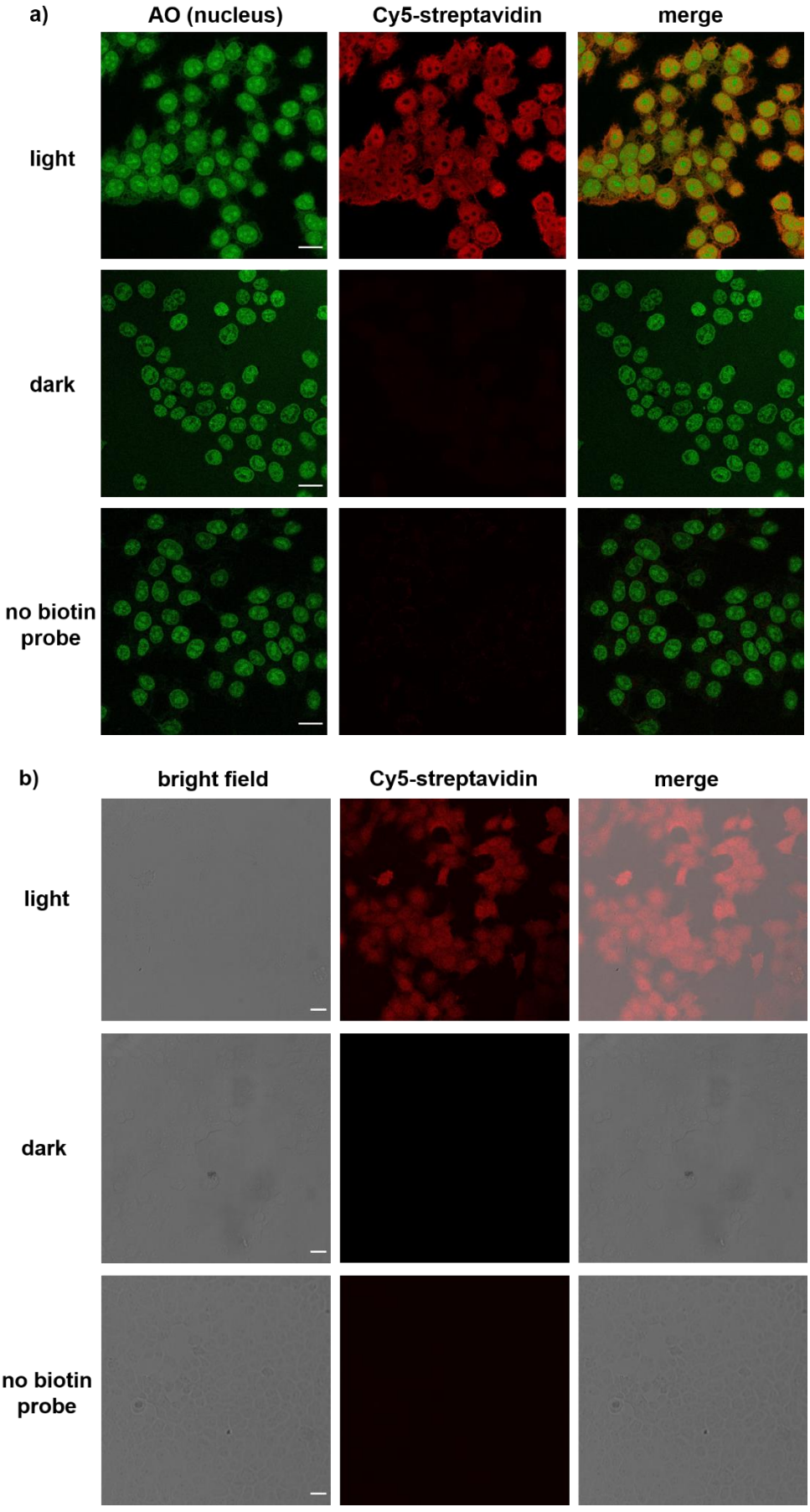

a)
AO (nucleus)
Cy5-streptavidin
merge
light
dark
no biotin probe
b)
bright field
Cy5-streptavidin
merge
light
dark
no biotin probe

Figure S20. Confocal imaging analysis of AO promoted photocatalytic labeling with AzPh-biotin probe **1** (200 μM) and the control experiments in MCF-7 cells. The cell culture of light and no biotin probe group was irradiated with the 515 nm green LED (2.9 mW/cm^2) for 30 min **a)** Images of Cy5 channel were merged with FITC channel (AO); **b)** Images of Cy5 channel were merged with bright field. Samples kept in dark instead of light irradiation (dark) and without the addition of AzPh-biotin probe **1** (no biotin probe) were set as the control experiments. Images in panel **a** and **b** were obtained from different samples. Cells were fixed with paraformaldehyde after photolabeling, and the biotin labeling signals were detected with Cy5-streptavidin. Images were merged with ImageJ, and the color of Cy5 channel was set as red. Scale bar: 20 μm.

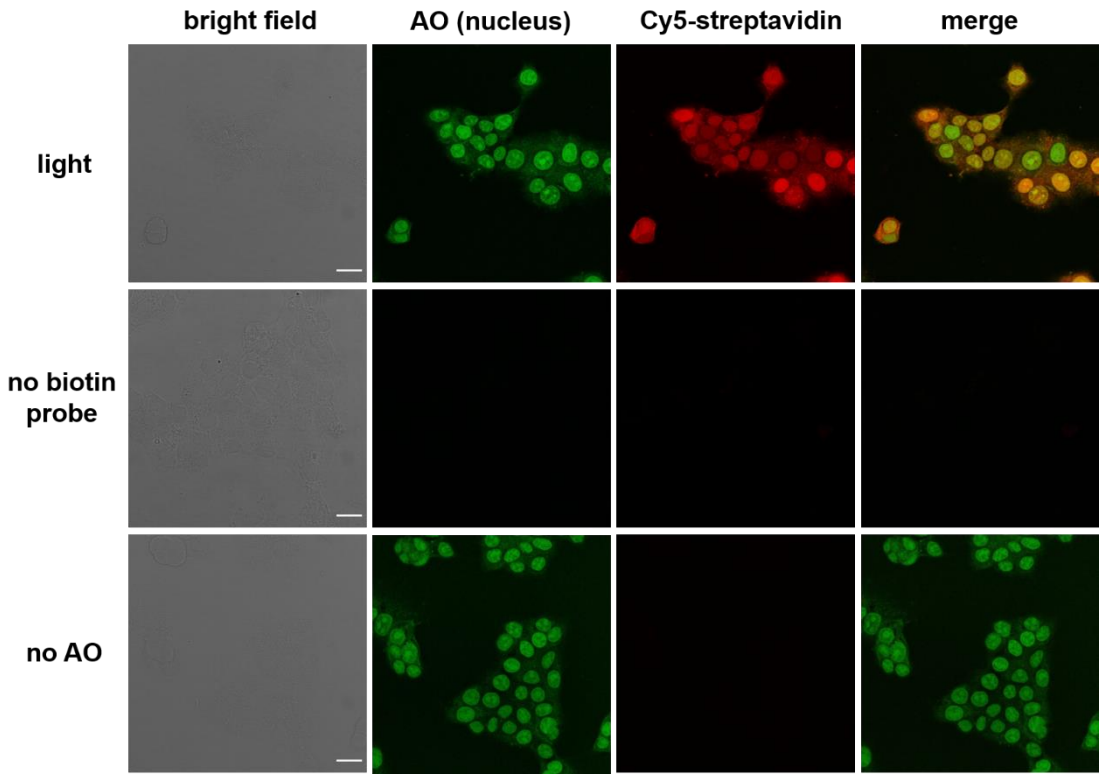


Figure S21. Confocal imaging analysis of AO promoted photocatalytic labeling with AzPh-biotin probe **1** (100 μM) after a 520 nm (95 mW/cm^2) LED light irradiation for 1 min. Cells were fixed with paraformaldehyde after photolabeling, and teh biotin labeling signals were detected with Cy5-streptavidin. Images were merged with ImageJ, and the color of Cy5 channel was set as red. Scale bar: 20 μm.

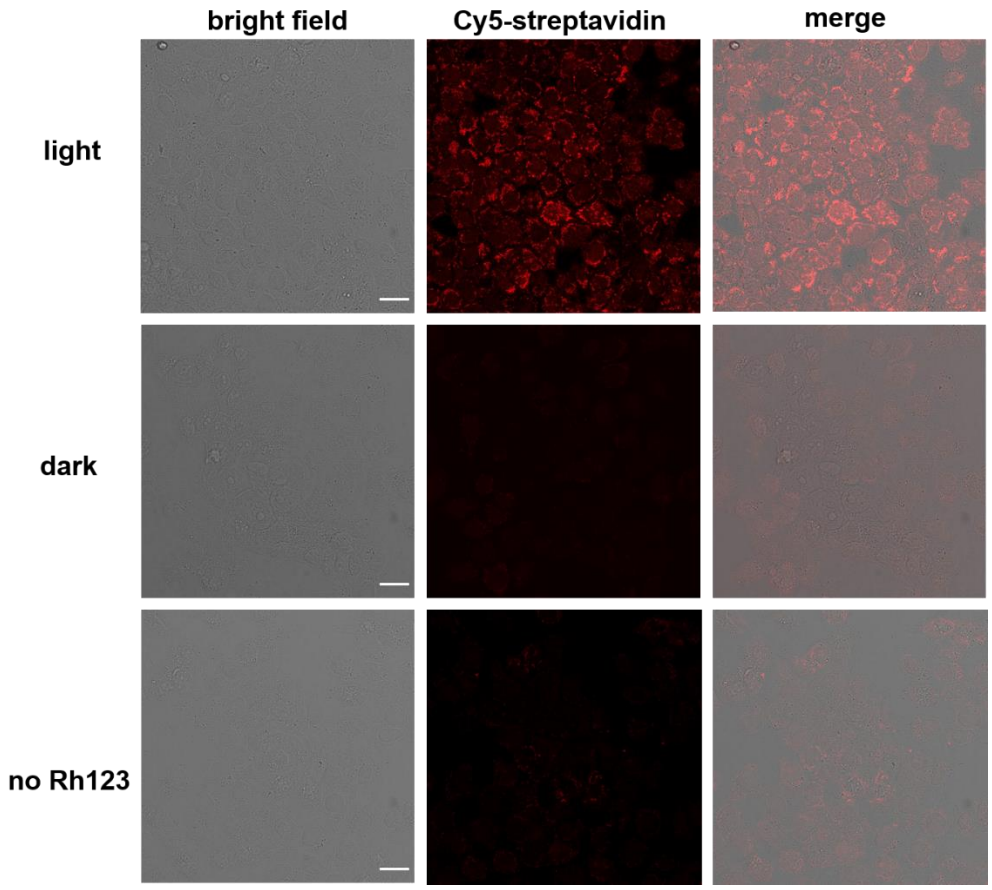


Figure S22. Confocal imaging analysis of Rh 123 promoted photocatalytic labeling with AzPh-biotin probe **1** (200 μM) and the control experiments in MCF-7 cells. The cell culture of light and no biotin probe group was irradiated with the 515 nm green LED (2.9 mW/cm^2) for 60 min Images of Cy5 channel were merged with the bright field and shown as the merge. Samples kept in the dark instead of light irradiation (dark) and without the addition of Rh 123 (no Rh 123) were set as the control experiments. Cells were fixed with paraformaldehyde after photolabeling, and biotin labeling signals were detected with Cy5-streptavidin. Images were merged with ImageJ and the color of Cy5 channel was set as red. Scale bar: 20 μm.

Cell lysis

After photolabeling, cells were washed three times with DPBS, then detached with a cell scraper and lysed in 1 mL RIPA lysis buffer (*Beyotime Biotechnology*, containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1× protease cocktail and 1 mM PMSF) at 4 °C. Lysates were collected by centrifugation at 13,000 rpm for 10 minutes at 4 °C. The supernatants were collected, and the concentrations of solubilized proteins in each group were normalized with BCA assay kit (*Beyotime Biotechnology*) for immunoprecipitation and western blotting analysis.

Western blotting analysis

Cell lysates were added with 5× protein loading buffer and heated at 98 °C for 5 min, then subjected to SDS-polyacrylamide gel (10% acrylamide) electrophoresis (PAGE) at the condition of 180 V for 60 min. The PAGE gels were transferred to the activated PVDF membrane with Trans-Blot Turbo Transfer System (*Bio-Rad*) and blocked with 4% BSA in TBST (0.1% Tween-20 in Tris-buffered saline) at 4 °C overnight. The blots were incubated with 0.2 μg/mL streptavidin-HRP (*Beyotime Biotechnology*) in TBST at room temperature for 60 minutes, then washed with TBST 3×10 minutes before development with SuperSignal West Pico reagent (*Thermo Scientific*) and obtained image with ChemiDoc XRS System and Image Lab Software (*Bio-Rad*). The duplicated PAGE gels were stained with Coomassie brilliant blue (CBB) and obtained image with ChemiDoc XRS System and Image Lab Software.

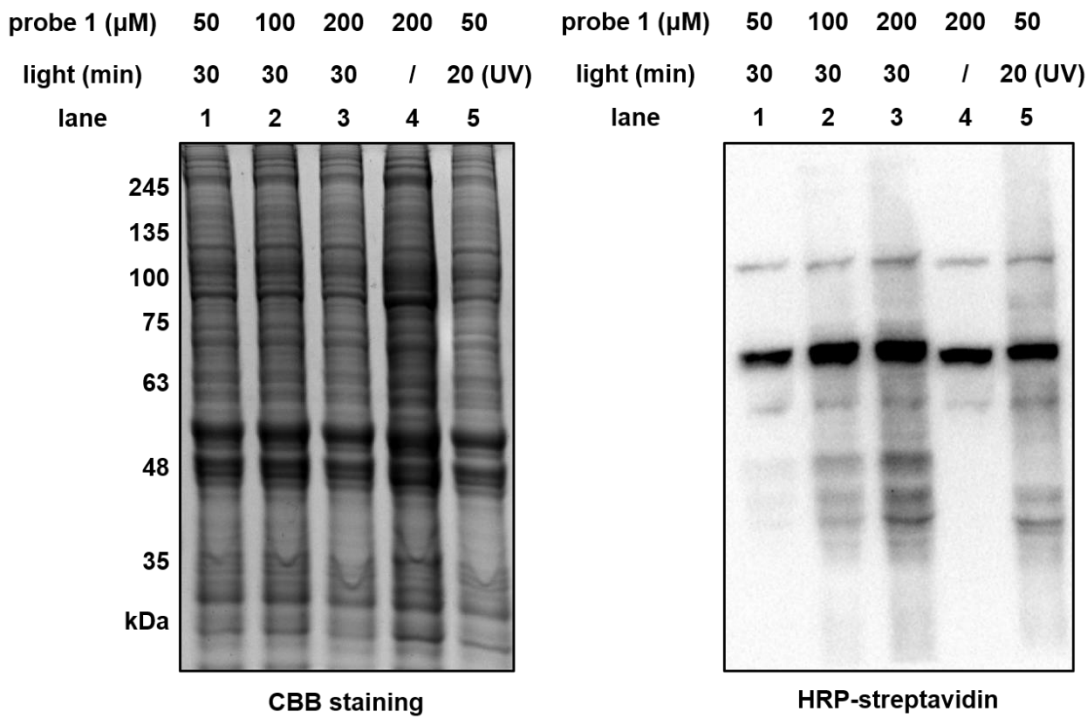


Figure S23. Western blotting analysis of photocatalytic biotinylation in MCF-7 cells with different concentrations of AzPh-biotin probe **1**. Cells were labeled with Rhodamine 123 under the irradiation with 515 nm green LEDs (2.9 mW/cm^2). For UV-induced labeling, cells were irradiated with 365 nm UV-light (11.8 mW/cm^2) for 20 min. Biotin signals in lane 4 indicated the endogenous biotinylated proteins.[8]

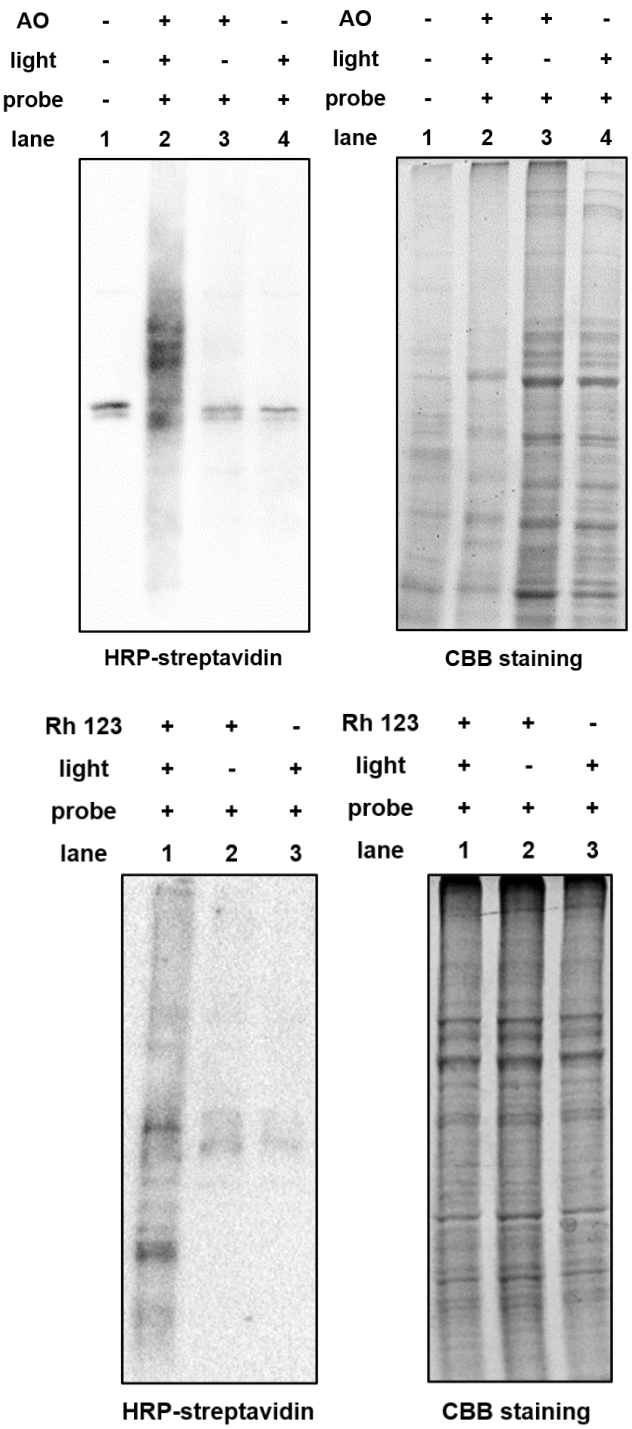


Figure S24. Western blotting analysis of photocatalytic biotinylation with the AzPh-biotin probe **1** (200 μM) in MCF-7 cells and the control experiments. Cells were labeled with Acridine Orange (AO, top) or Rhodamine 123 (Rh 123, bottom) under the irradiation with 515 nm green LEDs (2.9 mW/cm^2) for 30 min and 60 min, respectively.

Photocytotoxicity assay in MCF-7 cells

MCF-7 cells were seeded in a 24-well plate at a density of 2×10^5 cells/mL under a humidified atmosphere of 5% CO_2 in air at 37 °C for 24 h in DMEM. The Cells were then incubated with 20 μM Rhodamine 123 or 10 μM Acridine Orange for 20 min. After washing with DPBS for three times, the cells were incubated in fresh DMEM (no phenol red) containing 100 μM AzPh-biotin probe **1** for another 30 min. The cell culture was irradiated with the 515 nm green LED (10 cm from the top of cell culture dish, 2.9 mW/cm^2) for 30 min (Acridine Orange) or 60 min (Rhodamine 123) at 37 ºC under air atmosphere. Control experiments were carried out as cells cultured with or without organic dyes or AzPh-biotin probe **1** under light irradiation or not. After the photocatalytic labeling reaction, MTT reagent were added to each well and incubated for 4 h. The produced formazan was dissolved in 0.5 mL of DMSO and the absorbance at 570 nm was measured with a Spectramax Microwell plate reader (Molecular Devices). The background absorbance was measured and subtracted at 690 nm. The cell viability was calculated as Abs_{570}-Abs_{690}. The cell viability of cells dealt without small molecules and light irradiation was used as a 100% standard in normalization.

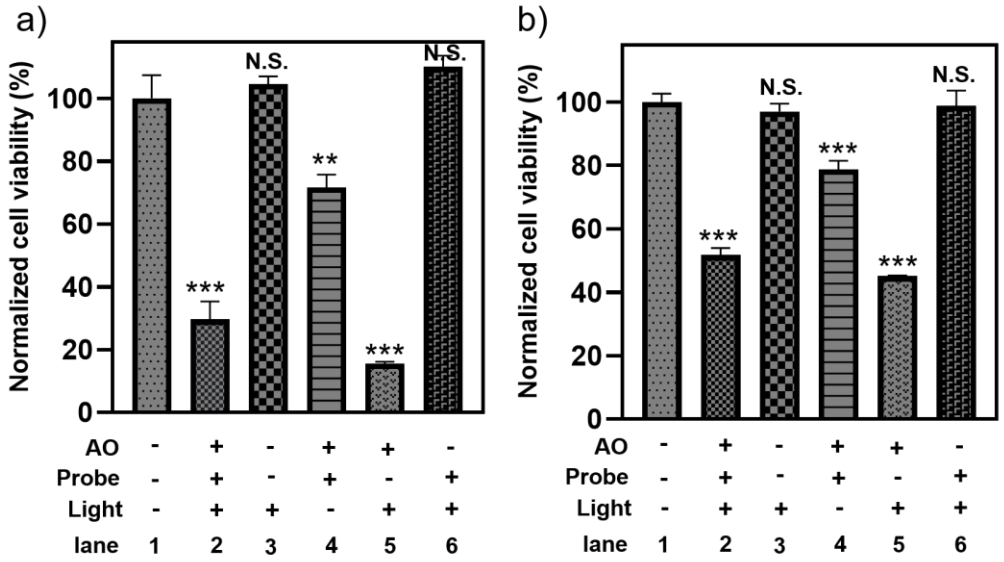


Figure S25. Phototoxicity of photocatalytic reactions in live MCF-7 cells with AzPh-biotin probe **1** (100 μM) and AO (10 μM) under a 515 nm (2.9 mW/cm^2) LED light for 30 min **(a)** or 520 nm (95 mW/cm^2) LED light for 1 min **(b)**. The statistical significance of the differences between groups was evaluated with the unpaired t test. P value of 0.05 and below was considered significant: $p < 0.01$ (**), $p < 0.001$ (***), N.S. is not statistically significant. All p-values were calculated with control cells that treated without light and small molecules. Error bars represented mean ± SD (n = 3).

Photolabeling of mammalian cells after rotenone treatment

HeLa cells were seeded in appropriated dishes or plates in culture media. At 60-70% confluence, Rotenone was added to each group in different concentrations. The control group with the same amount of DMSO was set as the blank. Cells were incubated for 24 h, then underwent the photolabeling procedure as described above.

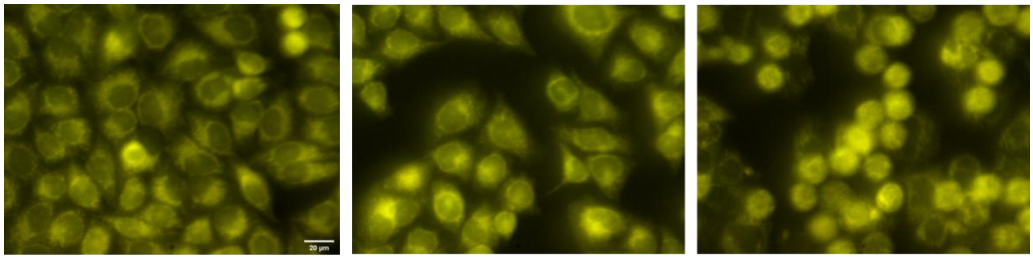


Figure S26. TMRE-staining analysis of Rotenone-treated HeLa cells. At 60-70% cell confluence, Rotenone was added to each group in different concentrations and the control group added with the same amount of DMSO instead of Rotenone was set as the blank. After 24 h incubation, 5 μM TMRE was added to each cell media for mitochondria staining. Cells were incubated for 20 min, then washed with PBS for three times before fluorescent imaging. Images were acquired on a DMi8 Leica fluorescence microscopy platform with 40× objective lens and TRITC filter. Digital pictures of different samples in each group were taken under identical conditions of gain and exposure. Positively-charged TMRE localizes in the negatively-charged mitochondrial inner membrane, and can be used as the fluorescent indicator of mitochondrial membrane potential. Significant changes of TMRE location in most of cells were observed compared with the control (blank) after 2 μM Rotenone treatment, indicating the loss of membrane potential induced by high concentration of Rotenone. Scale bar: 20 μm.

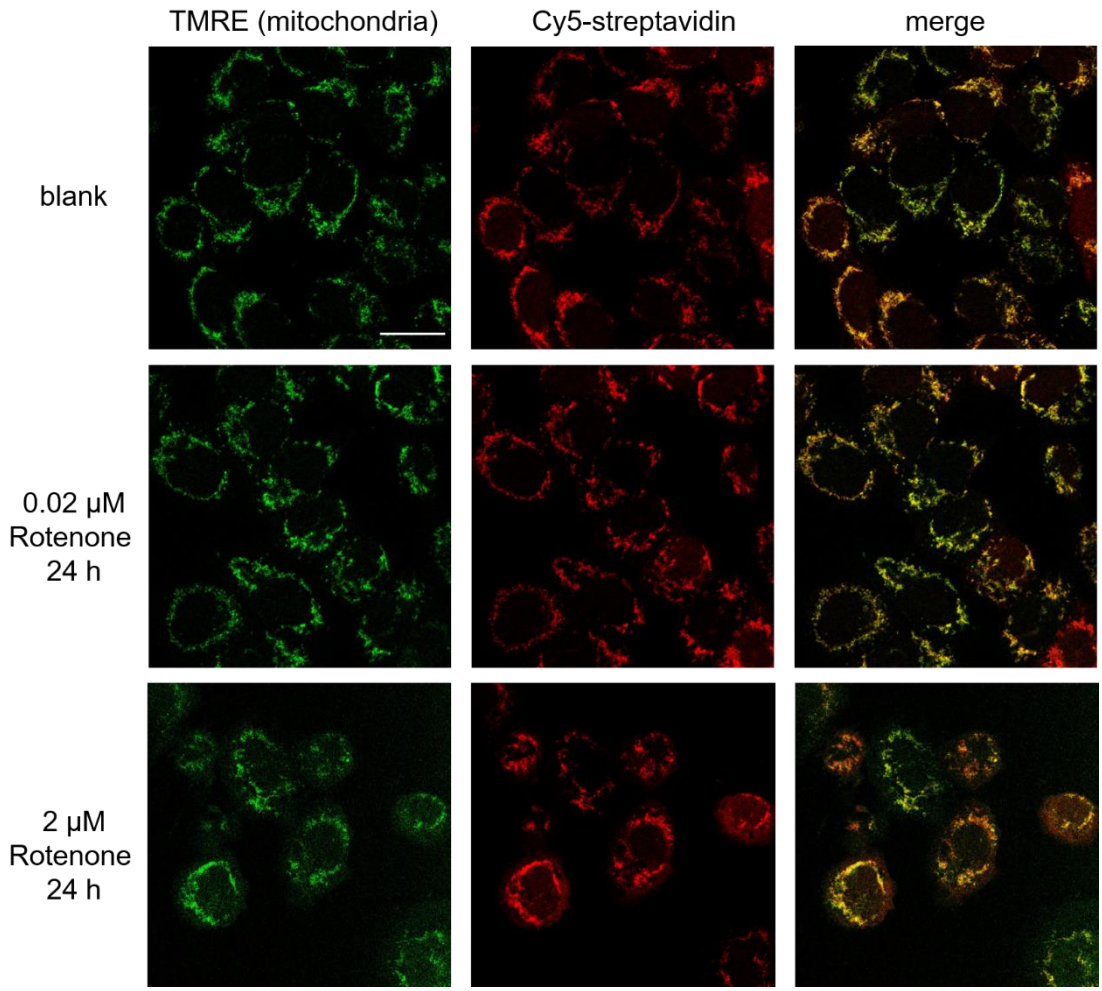


Figure S27. Confocal imaging analysis of the photolabeling of Rotenone-treated HeLa cells with AzPh probe **1** (200 μM). The cell culture was irradiated with the 515 nm green LED (2.9 mW/cm^2) for 30 min. Cells were fixed with paraformaldehyde after photolabeling, and biotin labeling signals were detected with Cy5-streptavidin. Images of Cy5 channel were merged with TRITC channel using ImageJ and the color of TRITC channel (TMRE) and Cy5 channel were set as green and red respectively. Scale bar: 20 μm.

IV. Protein and peptide analysis by LC-MS/MS

LC-MS/MS sample preparation for identification the modification sites

The photocatalytic labeling on BSA protein was carried out with AzPh-biotin probe **1** (100 μM) and Acridine Orange (100 μM) under the 468 nm blue LED (10 cm from the top of the protein samples, 5.8 mW/cm^2) irradiation for 20 min. The photocatalytic labeling in MCF-7 cells was carried out with AzPh-biotin probe **1** (100 μM) and Acridine Orange (10 μM) under the 515 nm green LED (1 cm from the top of the cell plate, 95 mW/cm^2) irradiation for 20 min. The labeled cells were lysed with RIPA buffer. All labeled proteins were precipitated with the chilled acetone, and then resolubilized in RIPA. The samples were diluted with 8 M urea (final conc. 4 M), and reduced with 20 mM DTT at 65 °C for 5 min, and alkylated with 40 mM iodoacetamide at 37 °C for 30 min. The mixtures were then precipitated with 20% methanol containing acetone. The collected pellet of proteins was solubilized in urea buffer (2 M urea, 100 mM Tris-HCl, pH 8.0 and incubated with trypsin (*w*: *w* = 1: 100) for 16 h at 37 °C. After boiling at 65°C for 5 min, the resulting solutions were diluted with Tris-HCl, pH 8.0 and incubated with magnetic streptavidin beads (MyOne^{TM} Streptavidin T1, Invitrogen) for 2 h with continuous rotation. The beads were successively washed with PBS buffer containing 1% NP40, PBS, water and 10% CH_3CN/water. The labeled peptides were eluted with 50% CH_3CN/water (2% TFA). These eluents were collected, freeze-dried and redissolved in water for further LC-MS/MS analysis.

Site-specific LC-MS/MS analysis of labeled peptide

The biotinylated peptides were desalted, and analyzed on a nano HPLC coupled to a timsTOF Pro mass spectrometer (Bruker Daltonics). Peptides were separated on a 25cm C_{18} UHPLC column (IonOpticks) through a 120-min gradient. PASEF mode was chosen for the timsTOF Pro. The values for mobility-dependent collision energy ramping were set to 59 eV at 1.6 Vs/cm^2 and 20 eV at 0.6 Vs/cm^2. Target intensity per individual PASEF precursor was set to 20,000. The scan range was set between 100 m/z and 1700 m/z. Active exclusion was enabled for 0.4 min (mass width 0.015 m/z, 1/k0

width 0.015 Vs/cm^2).

MS data were processed by the PEAKS Studio 10.6 software (Bioinformatics Solutions Inc) for both de novo sequencing and database search. For de novo and database search, precursor mass tolerance was set to 15 ppm, and fragment mass tolerance was set to 0.05 Da. Carbamidomethylation of Cys (+57.0125) was set as the fixed modification. Variable modifications were analyzed in three independent searches. Biotinylation of Lys (+226.0776), oxidation of Met (+15.9949) and photocatalytic Azph-Biotin probe 1 modification (+491.2212) of Lys, Cys, Ser (1st search); Thr, Tyr, Asp (2nd search); and His, Gln, Glu, Gly (3rd search) were set as variable modifications. The false discovery rates for peptides and proteins were controlled at no larger than 1%. The minimum PTM AScore was set at 13.[9] The peptides with the highest scores were annotated as the identifications.

```
  1 MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA
 51 FSQYLQQCPF DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK
101 VASLRETYGD MADCCEKQEP ERNECFLSHK DDSPDLPKLK PDPNTLCDEF
151 KADEKKFWGK YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC
201 LLPKIETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA RLSQKFPKAE
251 FVEVTKLVTD LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE
301 CCDKPLLEKS HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL
351 GSFLYEYSRR HPEYAVSVLL RLAKEYEATL EECCAKDDPH ACYSTVFDKL
401 KHLVDEPQNL IKQNCDQFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS
451 RSLGKVGTRC CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC
501 TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT
551 ALVELLKHKP KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV
601 STQTALA ↵
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Figure S28. Labeled residues on BSA. Modified residues were labeled in yellow

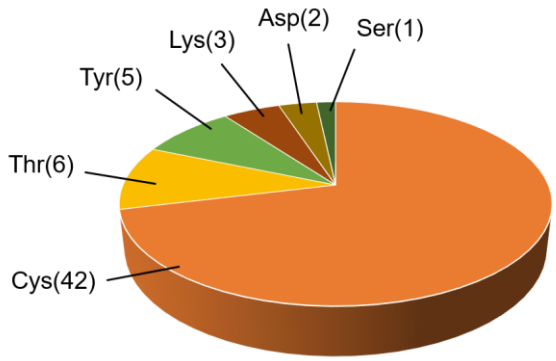


Figure S29. Modified amino acid residues after photocatalytic labeling in MCF-7 cells

Table S3. List of modified peptides and sites on photocatalytic BSA modification.[a]

Annotated Sequence
DTHKSEIAHR
ECCHGDLLECADDRADLAKYICDNQDTISSK
ECCHGDLLECADDRADLAK
LSQKFPK
YNGVFQECCQAEDK
LKHLVDEPQNLIK
CASIQKFGER
VHKECCHGDLLECADDRADLAK
KQTALVELLKHKPK
ALKAWSVAR
RHPEYAVSVLLR
KVPQVSTPTLVEVSR
KQTALVELLK

KFWGKYLYEIAR
YLYEIAR
KQTALVELLK
FWGKYLYEIAR
SHCIAEVEKDAIPENLPPLTADFAEDKDVCK
SHCIAEVEKDAIPENLPPLTADFAEDK
SHCIAEVEKDAIPENLPPLTADFAEDKDVCK
QNCDQFEKLGEYGFQNALIVR
GACLLPKIETMR
GACLLPKIETMR
LGEYGFQNALIVR
CCTKPESERMPCTEDYLSLILNR
MPCTEDYLSLILNR
MPCTEDYLSLILNR
GLVLIAFSQYLQQCPFDEHVK
DAFLGSFLYEYSR
VGTRCCTKPESERMPCTEDYLSLILNR
TVMENFVAFVDKCCAADDKEACFAVEGPK[b]
TVMENFVAFVDKCCAADDKEACFAVEGPK
MPCTEDYLSLILNR
GLVLIAFSQYLQQCPFDEHVK

[a] The labeled sites were shown in red.

[b] It was considered as a false positive data after manual comparing the LC-MS/MS spectra in de novo sequencing and BSA database.

Table S4. List of modified peptides and sites on photocatalytic MCF-7 modification.[a]

Annotated Sequence
LCAAAASILGKPADR
LLDLVQQSCNYK
GLCAIAQAESLR
SFFLGQGLSALLPCVLALVQGVGR
SLLINAVEASCIR
GIDQCIPLFVEAALER
LICLVTGSPSIR
FSFCCSPEPEAEAEAAAGPGPCER
NTGIICTIGPASR
TIAECLADELINAAK
LYVYNTDTDNCR
LILDVFCGSQMHFVR
TKDDIIICEIGDVFK

LCEEWPVDETKR
QLSSSVTGLTNIEEENCQR
GPSGCVESLEVTCR
SYCYVSKEELK
VAVTEGCQPSR
DVVICPDASLEDAKK
IEYNDQNDGSCDVK
ENVNVEEMFNCITELVLR
SDCKEFSSEAR
TNCNVAVINVGAPAAGMNAAVR
ALNALCDGLIDELNQALK
IECDDKGDGSCDVR
VPLDVACAR
SLHDALCVLAQTVK
TNCNVAVINVGAPAAGMNAAVR
ALENDPDCR
TTGLVGLAVCNTPHER
ALVDGPCTQVR
LLCGLLAER
LLVGNKCDLTTK
LVDVICEK
MDCQECPEGYR
CITDPQTGLCLLPLKEK
SGSQAHEQR
ICPVETLVEEAIQCAEK
GSDFDCELR
ICPVETLVEEAIQCAEK
YEELECLYAAVGK
GCAVVEFK
LAPIPPK
IGEIVAEMDVPLHCR
LLPALLILRAFKPHR
AECMLQQAER
YYYDGKDYIEFNK
GYSFTTTAER
KLTGRLMLAVGGAVLGSLQFGYNTGVINAPQK
KQTALVELLK
SYELPDGQVITIGNER
YEELECLYAAVGK
EYQELMNTK
YMACCMLYR
TAALGLLLLCAAAAGAGK
DVNVAIAAIK
VTLLEGDHVR

AVTLECVSAGEPR
EKHPDGKILIIGGSIANFTNVAATFK

[a] The labeled sites were shown in red.

LC-MS/MS sample preparation for identification of the labeled proteins

150 μL streptavidin agarose resins (*G-Bioscience*) were washed three times with PBS, then the cell lysate samples (described above) were mixed with the streptavidin beads and incubated at room temperature for 2 h with gentle rotation. The beads were washed with 1 mL RIPA lysis buffer twice, then washed with 1 mL PBS five times. The biotinylated proteins binding on streptavidin beads were resuspended in 8 M urea with 100 mM Tris buffer (pH 8.5). 5 mM TCEP was added and incubated at room temperature for 20 min. Subsequently, 10 mM iodoacetamide was added and incubated at room temperature for 15 min in the dark. Samples were diluted with 100 mM Tris buffer (pH 8.5) by a factor of 4 with 1 mM $CaCl_2$, followed by the addition of 10 μg/mL sequencing-grade modified trypsin (0.5 mg/mL, *Promega*), and were incubated under gentle mixing over night at 37 °C. The digestion was stopped by the addition of 5% formic acid, then the resulting peptides were desalted and concentrated with HC-C18 SPE cartridges (*CNW*). Samples of each group contained three replicates were treated with the identical condition.

LC-MS/MS analysis of the labeled proteins

Peptides were analyzed by an HPLC EASY-nL-LC 1000 coupled with an online Q Exactive HF mass spectrometer (*Thermo Scientific*). Samples were loaded directly on a 100 μm I.D. 150 mm analytic column (in-house packed, 1.9 μm, C18 resin, *Dr.Maisch*). Mobile phase A consisted of 0.1% FA, 2% ACN and 98% H_2O, mobile phase B consisted of 0.1% FA, 2% H_2O and 98% ACN. A 60 min gradient (4% B at 0 min, 8% B at 4 min, 25% B at 45 min, 37% B at 53 min, 95% B at 57 min, at 95% B at 60 min) was used at a static flow rate of 300 nL/min. For MS1, the scan range of MS was 350 to 1500 m/z at a resolution of 60,000, and the AGC target was 3e6. The maximum injection time for the precursor ion was 20 ms. For MS2, the resolution was

set to 30,000 and the AGC target was set to 1e5 with the maximum injection time of 45ms. High-energy collisional dissociation (HCD) was used to fragment the precursor peptides, normalized collision energy was 27.

MS data analysis of the labeled proteins

MaxQuant software (version 1.5.3.30) was used for the protein identification and label-free quantitation. “Match between runs” was applied, and the match time window was set within 0.7 min. The MS data were searched against the UniProt human protein database (downloaded September 2016, #70,630 entries). Trypsin was set as the enzyme, and the maximum missed cleavage was set to 2. Carbamidomethyl (C) (+57.02 Da) was set as a fixed modification, and the variable modifications were oxidation of methionine (+15.99 Da) and protein N-terminal acetylation (+42.01 Da). The false-discovery rate (FDR) for peptides and proteins was set to 1%, and the minimum peptide length was set to 7. Proteins identified only by site modification or found in the decoy database were not included.

Subcellular proteome

For identification of the subcellular-localization of photolabeling, the samples included one experiment group and two control groups (no photocatalyst group and dark group). The proteins quantified less than twice in three replicates of experiment group were removed. *P*-values of the experiment group to two control groups were calculated respectively with unpaired Student's t-test using label-free quantitation (LFQ) intensity. Average LFQs for each group were used for calculating the fold-change (FC) of experiment group comparing to the two control groups. Proteins with FC>2 and $P<0.05$ were defined as the enriched proteins.

For identification of the photolabeling of Rotenone-treated cells, the samples included one experiment group and one control group (added with DMSO as the blank). The proteins quantified at least twice in three replicates of both experiment group and the blank were reserved. Average LFQs for each group were used for calculating the fold-change (FC) of experiment group comparing to the blank. Proteins with $P<0.05$ were screened, and those with FC>2 or FC<0.5 were defined as increased measurements and decreased measurements proteins respectively.

The biological process (BP) and cellular component (CC) of screened proteins were annotated using DAVID Bioinformatics Resources (v6.8) and UniProt database.[10-11] MitoCarta 2.0 database was also used for annotating mitochondrial proteins.[12]

Statistical analysis

All biological experiments were repeated 3 times independently unless otherwise noted. *P* values in unpaired Student's t-test were calculated with Excel and error bars represent mean ± SD.

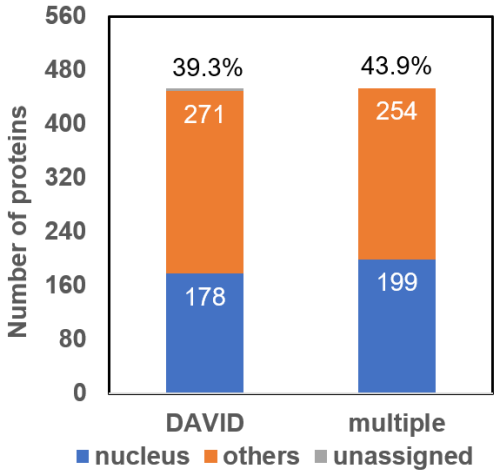


Figure S30. Counts of the proteins in AO promoted photolabeling of MCF-7 cells. Nucleic proteins were annotated only by DAVID (178/453, 39.3%, DAVID) or by the combination of DAVID and Uniprot database (199/453, 43.9%, multiple).

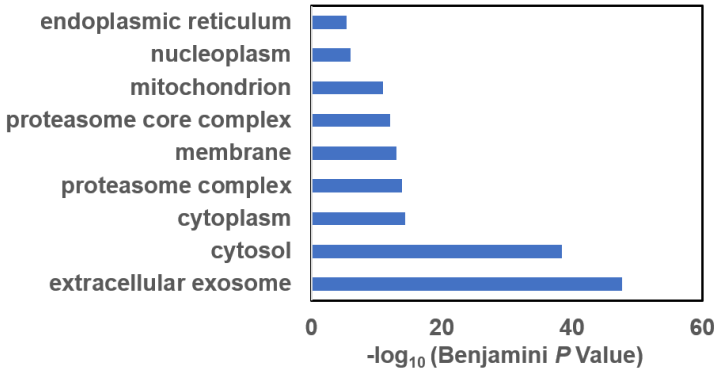


Figure S31. Classification of the identified proteins with cellular components in AO promoted photolabeling of MCF-7 cells. Proteins were annotated by DAVID.

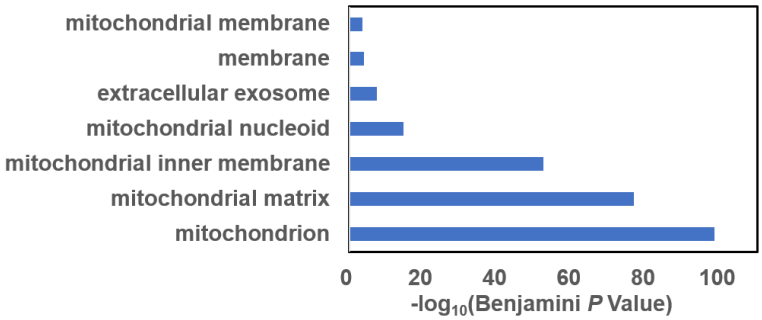


Figure S32. Classification of the identified proteins with cellular components in Rh 123 promoted photolabeling of MCF-7 cells. Proteins were annotated by DAVID.

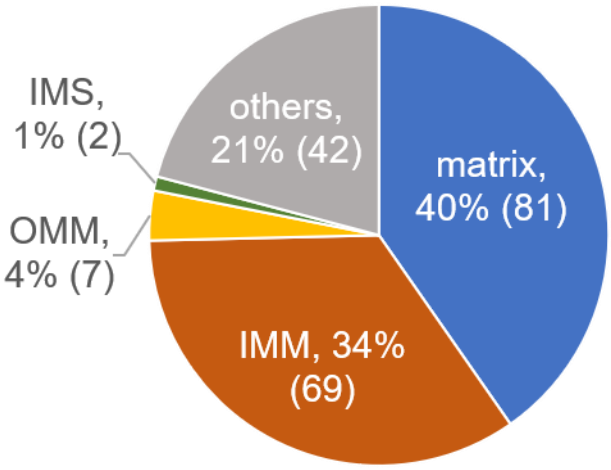


Figure S33. The sub-mitochondrial localization of the photocatalytic labeled proteome. The numbers of proteins in different sub-mitochondrial compartments are shown in parenthesis. Matrix: mitochondrial matrix; IMM: inner mitochondrial membrane; IMS: intermembrane mitochondrial space; OMM: outer mitochondrial membrane.

Table S5. List of the mitochondrial complex proteins in Rh 123 promoted photolabeling of MCF-7 cells.

Protein/Gene Name	Category	Localization
ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit(ATP5O)	ATP synthase	IMM
ATP synthase, H+ transporting, mitochondrial Fo complex subunit B1(ATP5F1)	ATP synthase	matrix
ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide(ATP5B)	ATP synthase	matrix
ATP synthase, H+ transporting, mitochondrial Fo complex subunit D(ATP5H)	ATP synthase	IMM
NADH:ubiquinone oxidoreductase core subunit S1(NDUFS1)	complex I	matrix
NADH:ubiquinone oxidoreductase core subunit V2(NDUFV2)	complex I	IMM
NADH dehydrogenase, subunit 4 (complex I)(ND4)	complex I	IMM
NADH:ubiquinone oxidoreductase core subunit S3(NDUFS3)	complex I	IMM
NADH:ubiquinone oxidoreductase core subunit S2(NDUFS2)	complex I	IMM
succinate dehydrogenase complex flavoprotein subunit A(SDHA)	complex II	IMM
succinate dehydrogenase complex iron sulfur subunit B(SDHB)	complex II	IMM
ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1(UQCRFS1)	complex III	IMM
ubiquinol-cytochrome c reductase core protein I(UQCRC1)	complex III	IMM
cytochrome c oxidase subunit 4I1(COX4I1)	complex IV	IMM
cytochrome c oxidase subunit 5B(COX5B)	complex IV	IMM
cytochrome c oxidase subunit 6C(COX6C)	complex IV	IMM

cytochrome c oxidase subunit II(COX2)	complex IV	IMM
GrpE like 1, mitochondrial(GRPEL1)	PAM complex	matrix
peptidase, mitochondrial processing alpha subunit(PMPCA)	PAM complex	matrix
heat shock protein family D (Hsp60) member 1(HSPD1)	PAM complex	matrix
peptidase, mitochondrial processing beta subunit(PMPCB)	PAM complex	matrix
translocase of inner mitochondrial membrane 44(TIMM44)	PAM complex	matrix
translocase of outer mitochondrial membrane 70(TOMM70)	TOM complex	OMM

Note: mitochondrial matrix (matrix); inner mitochondrial membrane (IMM); intermembrane mitochondrial space (IMS); outer mitochondrial membrane (OMM).

Table S6. List of 31 proteins with <0.5-fold change (FC) in HeLa cells treated by high concentration (2 μM) of Rotenone (severe toxicity).

Protein/Gene Name	$\log_2$ (FC)	$-\log_{10}$ (*P*-value)	mitochondrial
NADH:ubiquinone oxidoreductase subunit S6(NDUFS6)	-1.753	1.952	Yes
cytochrome c oxidase subunit 4I1(COX4I1)	-1.732	1.641	Yes
chondroitin sulfate proteoglycan 4(CSPG4)	-1.707	2.933	No
isovaleryl-CoA dehydrogenase(IVD)	-1.564	2.458	Yes
carnitine palmitoyltransferase 2(CPT2)	-1.537	5.148	Yes
leucine zipper and EF-hand containing transmembrane protein 1(LETM1)	-1.528	1.317	Yes
dihydrolipoamide dehydrogenase(DLD)	-1.457	4.373	Yes
flavin adenine dinucleotide synthetase 1(FLAD1)	-1.387	1.346	No
Mov10 RISC complex RNA helicase(MOV10)	-1.379	1.306	No
succinate dehydrogenase complex flavoprotein subunit A(SDHA)	-1.378	3.302	Yes
tRNA methyltransferase 61B(TRMT61B)	-1.345	2.363	Yes
integrin subunit alpha 6(ITGA6)	-1.325	3.378	No
cytidine deaminase(CDA)	-1.295	1.338	No
aldehyde dehydrogenase 5 family member A1(ALDH5A1)	-1.276	2.469	Yes
pitrilysin metallopeptidase 1(PITRM1)	-1.263	3.379	Yes
complement C1q binding protein(C1QBP)	-1.260	1.806	Yes

ETHE1, persulfide dioxygenase(ETHE1)	-1.250	2.250	Yes
pyrophosphatase (inorganic) 2(PPA2)	-1.215	3.492	Yes
hydroxysteroid 17-beta dehydrogenase 10(HSD17B10)	-1.180	2.639	Yes
NAD kinase 2, mitochondrial(NADK2)	-1.169	1.407	Yes
transmembrane protein 65(TMEM65)	-1.161	2.485	Yes
heat shock protein family E (Hsp10) member 1(HSPE1)	-1.136	2.577	Yes
acyl-CoA dehydrogenase, very long chain(ACADVL)	-1.131	3.554	Yes
hydroxyacyl-CoA dehydrogenase(HADH)	-1.120	2.817	Yes
enoyl-CoA hydratase, short chain 1(ECHS1)	-1.115	3.730	Yes
thioredoxin reductase 2(TXNRD2)	-1.109	2.621	Yes
Enoyl-CoA delta isomerase 1, mitochondrial (ECI1)	-1.060	2.272	Yes
cytochrome c oxidase subunit 5B(COX5B)	-1.048	1.697	Yes

Table S7. List of 19 proteins with >2-fold change (FC) in HeLa cells treated by high concentration (2 μM) of Rotenone (severe toxicity).

Protein/Gene Name	log_2(FC)	-log_{10} (*P*-value)	mitochondrial
GAR1 ribonucleoprotein(GAR1)	1.902	1.614	No
histone cluster 2 H3 pseudogene 2(HIST2H3PS2)	1.717	1.565	No
epiplakin 1(EPPK1)	1.538	1.691	No
arginine and glutamate rich 1(ARGLU1)	1.478	1.349	Yes
early endosome antigen 1(EEA1)	1.456	1.507	No
methylcrotonoyl-CoA carboxylase 1(MCCC1)	1.355	3.791	Yes
cysteinyl-tRNA synthetase(CARS)	1.327	1.314	No
TSR1, ribosome maturation factor(TSR1)	1.301	1.324	No
histone cluster 1 H4 family member i(HIST1H4I)	1.287	2.115	No
S100 calcium binding protein A6(S100A6)	1.236	2.204	No
serine and arginine rich splicing factor 11(SRSF11)	1.200	1.995	No
topoisomerase (DNA) I(TOP1)	1.198	6.682	No
solute carrier family 38 member 2(SLC38A2)	1.164	2.761	No
propionyl-CoA carboxylase beta subunit(PCCB)	1.087	3.061	Yes

Table S8. List of 7 proteins with <0.5-fold change (FC) in HeLa cells treated by low concentration (0.02 μM) of Rotenone (mild stress).

Protein/Gene Name	$\log_2$(FC)	$-\log_{10}$ (*P*-value)	mitochondrial
myoferlin (MYOF)	-2.516	2.606	No
spastic paraplegia 20 (Troyer syndrome) (SPG20)	-1.543	1.499	Yes
histone acetyltransferase 1 (HAT1)	-1.249	3.218	No
histidyl-tRNA synthetase (HARS)	-1.226	2.581	No
La ribonucleoprotein domain family member 4 (LARP4)	-1.118	2.935	No
SUMO1 activating enzyme subunit 1 (SAE1)	-1.113	2.764	No
BUD31 homolog (BUD31)	-1.094	3.120	No

Table S9. List of 13 proteins with >2-fold change (FC) in HeLa cells treated by low concentration (0.02 μM) of Rotenone (mild stress).

Protein/Gene Name	$\log_2$(FC)	$-\log_{10}$ (*P*-value)	mitochondrial
high mobility group nucleosomal binding domain 2(HMGN2)	2.186	1.411	No
H3 histone family member 3B(H3F3B)	1.867	1.934	No
BCS1 homolog, ubiquinol-cytochrome c reductase complex chaperone(BCS1L)	1.781	2.049	Yes
coiled-coil domain containing 51(CCDC51)	1.692	1.500	Yes
ATPase family, AAA domain containing 3A(ATAD3A)	1.586	1.568	Yes
zinc metallopeptidase STE24(ZMPSTE24)	1.390	2.383	No
mitochondrial carrier 2(MTCH2)	1.183	3.041	Yes
calumenin(CALU)	1.166	3.450	No
reticulocalbin 1(RCN1)	1.121	2.878	No
isovaleryl-CoA dehydrogenase(IVD)	1.119	2.669	Yes
heat shock protein family E (Hsp10) member 1(HSPE1)	1.105	2.514	Yes
thioredoxin reductase 1(TXNRD1)	1.104	2.762	Yes
pitrilysin metallopeptidase 1(PITRM1)	1.014	3.193	Yes

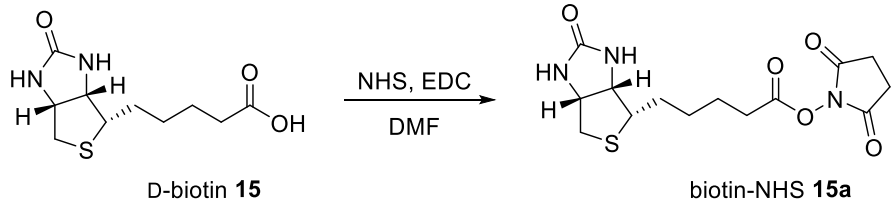
V. Substrate Preparations and Characterizations

General procedures for substrate preparation

Unless otherwise noted, all reactions of substrates preparation were conducted in flame-dried glassware under a nitrogen atmosphere using anhydrous solvent passed through an activated alumina column (*Innovative Technology*). Commercially available reagents were used without further purification. Thin layer chromatography (TLC) was performed using Jiangyou TLC silica gel plates HSG F_{254} and visualized using UV light and potassium permanganate. Flash chromatography was performed on Lisure science EZ purification system using the Santai technologies silica gel cartridge. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-400 MHz or an Agilent 500 MHz spectrometer. Chemical shifts in ^{1}H NMR spectra were reported in parts per million (ppm) on the δ scale from an internal standard of residual $CDCl_3$ (7.26 ppm), CD_3OD (3.31 ppm) or DMSO-d_6 (2.50 ppm). Data for ^{1}H NMR were reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, ddd = doublet of double doublets, m = multiplet, br = broad), coupling constant in Herts (Hz) and integration. Data for ^{13}C NMR spectra were reported in terms of chemical shift in ppm from the central peak of $CDCl_3$ (77.16 ppm), CD_3OD (49.00 ppm) or DMSO-d_6 (39.52 ppm). IR spectra were recorded on a Thermo Scientific Nicolet 380 FT-IR spectrometer. MS experiments were performed on a Bruker maXis 4G instrument for HRMS-ESI.

Synthesis of D-biotin NHS ester **15a**

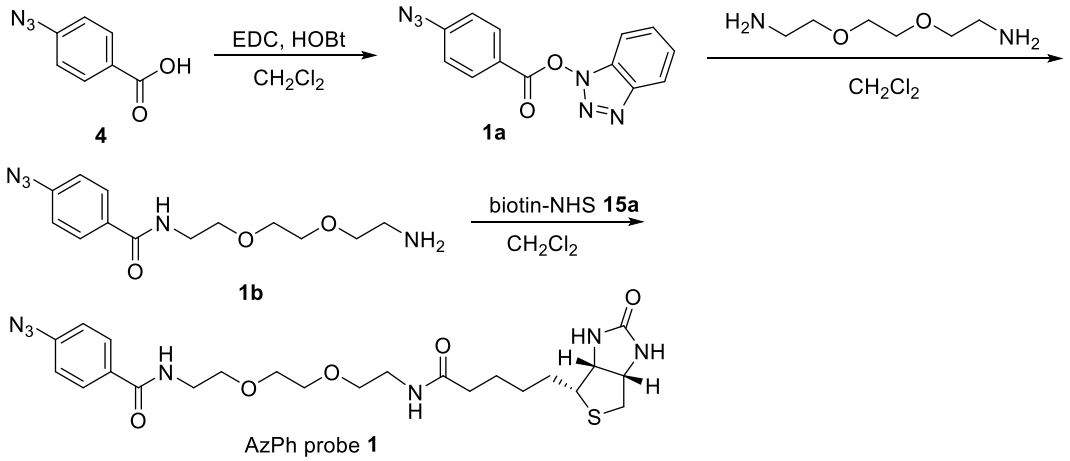
This compound was synthesized according to the literature methods.[13]



D-biotin **15** biotin-NHS **15a**

N-hydroxysuccinimide (NHS, 1.38 g, 12.0 mmol) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, 2.30 g, 12.0 mmol) were added to a solution of D-biotin **15** (2.44 g, 10.0 mmol) in 50 mL DMF. The reaction was stirred at room temperature for 18 h, then concentrated in vacuum. The residue was washed with EtOH/acetic acid/ H_2O (95: 1: 4) to give **15a** (3.11 g, 95%) as a white solid, which was directly used for the next step. ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.42 (s, 1H), 6.36 (s, 1H), 4.30 (dd, J = 8.0, 5.0 Hz, 1H), 4.15 (ddd, J = 7.5, 4.5, 1.5 Hz, 1H), 3.32 (s, 1H), 3.11 (ddd, J = 8.5, 6.5, 4.5 Hz, 1H), 2.81 (s, 4H), 2.67 (t, J = 7.5 Hz, 2H), 2.58 (d, J = 12.0 Hz, 1H), 1.71 – 1.58 (m, 3H), 1.56 – 1.32 (m, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 170.24, 168.91, 162.65, 60.98, 59.16, 55.22, 39.90, 29.99, 27.82, 27.56, 25.44, 24.30.

Synthesis of AzPh-biotin probe **1**



4 **1a** **1b** AzPh probe **1**

1-Hydroxybenzotriazole (HOBt, 244 mg, 1.80 mmol) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, 345 mg, 1.80 mmol) were added to a solution of 4-azidobenzoic acid **4** (245 mg, 1.50 mmol) in 20 mL CH_2Cl_2. The reaction

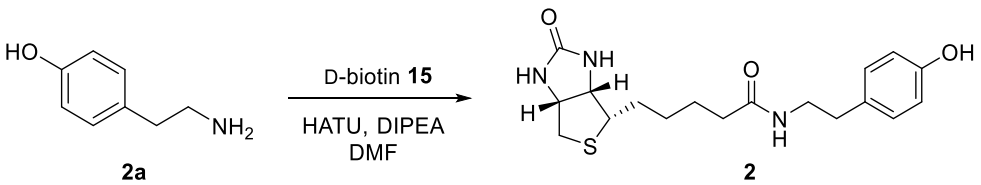
was stirred at room temperature for 12 h, then diluted with 10 mL 1 M Na_2CO_3 aqueous solution. The organic layer was washed with water (20 mL ×3 times) and brine, dried over Na_2SO_4, and concentrated in vacuum to give **1a** as a white solid, which was directly used for the next step.

To a solution of 1,8-diamino-3,6-dioxaoctane (1.33 g, 1.32 mL, 9.0 mmol) in 15 mL CH_2Cl_2, the 30 mL CH_2Cl_2 solution of **1a** was added dropwise. The reaction was stirred at room temperature for 24 h, then diluted with 20 mL water and extracted with CH_2Cl_2 (20 mL ×3 times). The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuum to give **1b**, which was directly used for the next step.

To a solution of **1b** in 20 mL $CHCl_3$, biotin-NHS **15a** (0.49 g, 1.5 mmol) was added. The reaction was stirred at room temperature for 24 h, then concentrated in vacuum. The residue was purified by silica gel column chromatography to give **1** (0.50 g, 64 % for 3 steps) as a light-yellow solid. TLC R_f = 0.33 (EtOAc/MeOH = 4/1); ^{1}H NMR (500 MHz, CD_3OD) δ 7.88 (d, *J* = 8.5 Hz, 2H), 7.15 (d, *J* = 8.5 Hz, 2H), 4.48 (dd, *J* = 8.0, 5.0 Hz, 1H), 4.29 (dd, *J* = 8.0, 4.5 Hz, 1H), 3.70 – 3.61 (m, 6H), 3.58 (t, *J* = 5.5 Hz, 2H), 3.54 (t, *J* = 5.5 Hz, 2H), 3.34 (t, *J* = 5.5 Hz, 2H), 3.19 (ddd, *J* = 9.0, 5.5, 4.5 Hz, 1H), 2.92 (dd, *J* = 13.0, 5.0 Hz, 1H), 2.70 (d, *J* = 13.0 Hz, 1H), 2.68 (s, 1H), 2.19 (t, *J* = 7.5 Hz, 2H), 1.77 – 1.53 (m, 4H), 1.42 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.12, 169.23, 166.09, 144.90, 132.13, 130.24, 120.00, 71.34, 71.30, 70.61, 70.58, 63.36, 61.62, 56.99, 41.04, 40.92, 40.28, 36.72, 29.75, 29.49, 26.82; IR (neat): 3278, 2929, 2866, 2124, 1698, 1646, 1544, 1500, 1284, 1126, 850, 764, 669 cm^{-1}; HRMS-ESI (m/z) [M+Na$^+$] calcd. for $C_{23}H_{33}N_7NaO_5S$ 542.2156, found 542.2158.

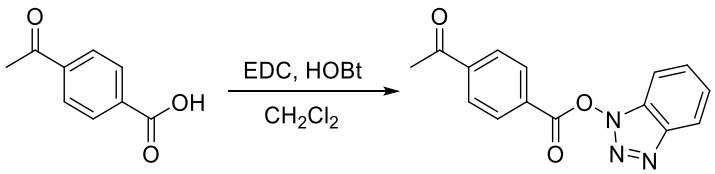
Synthesis of Phenol-biotin probe **2**

This compound was synthesized according to the literature methods.[8]



To a solution of D-biotin **15** (0.12 g, 0.50 mmol) and 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium-3-oxide hexafluorophosphate (HATU, 0.21 g, 0.55 mmol) in 5 mL DMF, *N,N*-diisopropylethylamine (DIPEA, 0.26 mL, 0.19 g, 1.5 mmol) was added dropwise. After stirred at room temperature for 10 minutes, a solution of 4-(2-aminoethyl)phenol **2a** (68 mg, 0.50 mmol) in 3 mL DMF was added dropwise. The reaction was stirred at room temperature for 12 h, then diluted with 20 mL water and 30 mL 1-butanol. The organic layer was collected and washed with water (20 mL ×3 times) and brine, dried over Na_2SO_4, and concentrated in vacuum. The residue was purified by silica gel column chromatography to give **2** (0.11 g, 62%) as a light-yellow solid. ^{1}H NMR (500 MHz, CD_3OD) δ 7.93 (t, *J* = 5.9 Hz, 1H), 7.03 (d, *J* = 8.4 Hz, 2H), 6.70 (d, *J* = 8.4 Hz, 2H), 4.49 (dd, *J* = 7.9, 4.8 Hz, 1H), 4.27 (dd, *J* = 7.9, 4.4 Hz, 1H), 3.36 (m, 2H), 3.16 (ddd, *J* = 8.9, 5.8, 4.4 Hz, 1H), 2.93 (dd, *J* = 12.7, 5.0 Hz, 1H), 2.74 – 2.66 (m, 3H), 2.15 (t, *J* = 7.5 Hz, 2H), 1.76 – 1.50 (m, 4H), 1.42 – 1.27 (m, 2H); ^{13}C NMR (126 MHz, CD_3OD) δ 176.05, 166.08, 156.85, 131.22, 130.75, 116.19, 63.33, 61.61, 56.94, 42.20, 41.04, 36.84, 35.63, 29.65, 29.45, 26.92.

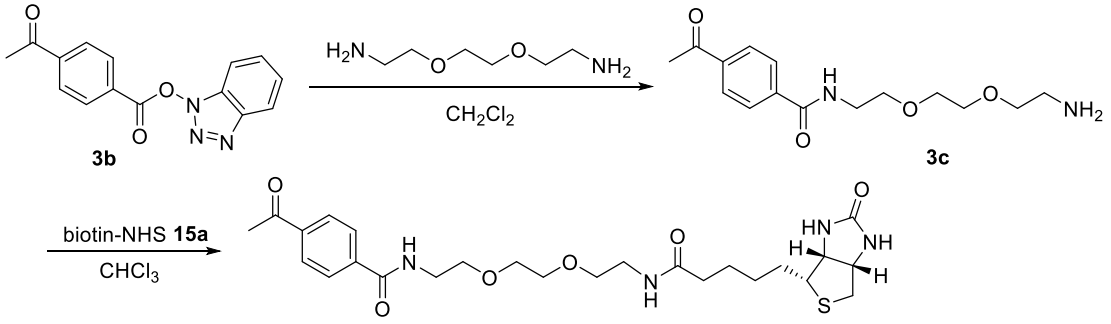
Synthesis of AcPh-biotin probe **3**



3a **3b**

1-Hydroxybenzotriazole (HOBt, 649 mg, 4.80 mmol) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, 920 mg, 4.80 mmol) were added to a solution of 4-acetylbenzoic acid **3a** (657 mg, 4.00 mmol) in 20 mL CH_2Cl_2. The reaction was stirred at room temperature for 12 h, then diluted with 10 mL 1 M Na_2CO_3 aqueous solution. The organic layer was washed with water (20 mL ×2 times) and brine, dried over Na_2SO_4, and concentrated in vacuum to give **3b** (1.08 g, 96%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (d, *J* = 8.2 Hz, 2H), 8.17 (d, *J* = 8.2 Hz, 2H), 8.13 (d,

J = 8.4 Hz, 1H), 7.59 (dd, *J* = 8.4, 6.8 Hz, 1H), 7.51 – 7.44 (m, 2H), 2.72 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.09, 162.22, 143.69, 142.20, 131.22, 129.12, 128.95, 128.81, 128.54, 125.16, 120.83, 108.38, 27.15.

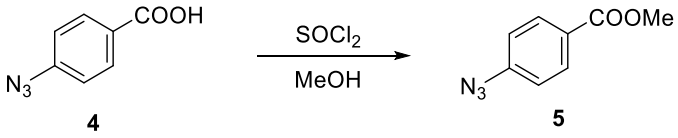


AcPh probe **3**

To a solution of 1,8-diamino-3,6-dioxaoctane (1.33 g, 1.31 mL, 9.0 mmol) in 15 mL CH_2Cl_2, 30 mL CH_2Cl_2 solution of **3b** (0.42 g, 1.5 mmol) was added dropwise. The reaction was stirred at room temperature for 12 h, then diluted with 20 mL water and extracted with CH_2Cl_2 (20 mL ×3 times). The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuum to give **3c** as a light-yellow oil, which was directly used for the next step.

To a solution of **3c** in 20 mL $CHCl_3$, biotin-NHS **15a** (0.49 g, 1.5 mmol) was added. The reaction was stirred at room temperature for 24 h, then concentrated in vacuum. The residue was purified by silica gel column chromatography to give **3** (0.36 g, 46 % for 2 steps) as a white solid. TLC R_f = 0.50 (EtOAc/MeOH = 4/1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.70 (t, *J* = 5.5 Hz, 1H), 8.03 (d, *J* = 8.5 Hz, 2H), 7.96 (d, *J* = 8.5 Hz, 2H), 7.81 (t, *J* = 5.5 Hz, 1H), 6.41 (s, 1H), 6.35 (s, 1H), 4.30 (dd, *J* = 7.5, 5.0 Hz, 1H), 4.12 (ddd, *J* = 7.5, 4.5, 2.0 Hz, 1H), 3.58 – 3.49 (m, 6H), 3.44 (q, *J* = 6.0 Hz, 2H), 3.39 (t, *J* = 6.0 Hz, 2H), 3.17 (q, *J* = 6.0 Hz, 2H), 3.08 (ddd, *J* = 8.5, 6.0, 4.5 Hz, 1H), 2.81 (dd, *J* = 12.5, 5.0 Hz, 1H), 2.62 (s, 3H), 2.57 (d, *J* = 12.5 Hz, 1H), 2.05 (t, *J* = 7.5 Hz, 2H), 1.59 (m, 1H), 1.47 (m, 3H), 1.29 (m, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 197.68, 172.10, 165.50, 162.67, 138.56, 138.16, 128.12, 127.49, 69.56, 69.53, 69.15, 68.77, 61.02, 59.18, 55.40, 39.30, 38.42, 35.08, 28.17, 28.02, 26.95, 25.24, 25.22; IR (neat): 3276, 2921, 2859, 1687, 1647, 1543, 1456, 1266, 1096, 767, 669 cm^{-1}; HRMS-ESI (m/z) [M+Na$^+$] calcd. for $C_{25}H_{36}N_4NaO_6S$ 543.2248, found 543.2249.

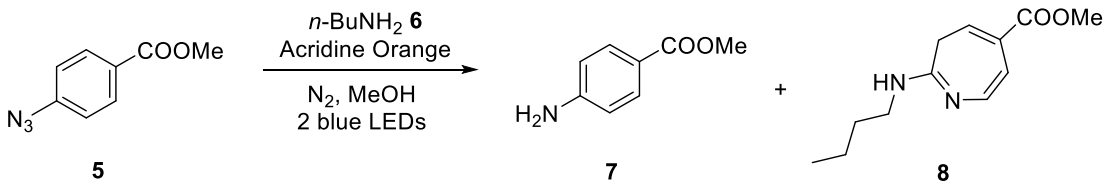
Synthesis of methyl 4-azidobenzoate **5**



4 **5**

To a solution of 4-azidobenzoic acid **4** (1.30 g, 8.0 mmol) in methanol (40 mL), $SOCl_2$ (1.43 g, 0.87 mL, 12.0 mmol) was added dropwise at 0 ºC. The reaction was stirred at rt for 24 h, then concentrated in vacuum. The residue was purified by silica gel column chromatography to give **5** (1.07 g, 75%) as a light-yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, *J* = 8.7 Hz, 2H), 7.06 (d, *J* = 8.7 Hz, 2H), 3.91 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.43, 144.89, 131.55, 126.84, 118.98, 52.30.

Mechanistic studies of the photolabeling intermediates



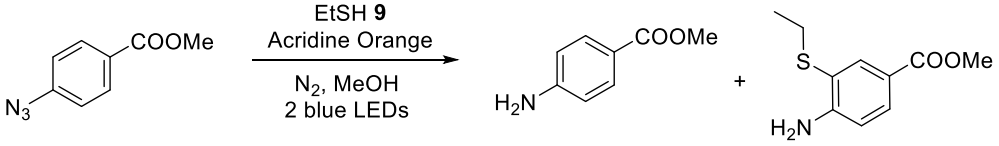
5 **7** **8**

A solution of **5** (18 mg, 0.10 mmol) and Acridine Orange (6.6 mg, 0.020 mmol) in methanol (2.0 mL) was placed in a 5.0 mL clear-colored glass vial equipped with a magnetic stir bar. After the addition of *n*-butylamine **6** (0.49 mL, 5 mmol), the reaction was bubbled with nitrogen gas to remove the oxygen. Then the vial was sealed and exposed to 2 × 4W 468 nm blue LEDs (10.0 cm from the LEDs, 10.4 mW cm^{-2}) with stirring at ambient temperature for 48 hours, then concentrated in vacuum. The residue was purified by silica gel column chromatography to give **8** as a brown liquid (14 mg, 63%). Another isolated product was identified as 4-methoxycarbonyl aniline **7**.

For **7**: TLC R_f = 0.46 (petroleum ether/ethyl acetate = 2/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, *J* = 8.7 Hz, 2H), 6.64 (d, *J* = 8.7 Hz, 2H), 4.05 (br, 2H), 3.85 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.29, 150.91, 131.74, 119.93, 113.95, 51.75.

For **8**: TLC R_f = 0.26 (petroleum ether/ethyl acetate = 1/2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17 (d, *J* = 8.3 Hz, 1H), 6.22 (d, *J* = 8.3 Hz, 1H), 6.19 (t, *J* = 7.4 Hz, 1H), 4.35 (s,

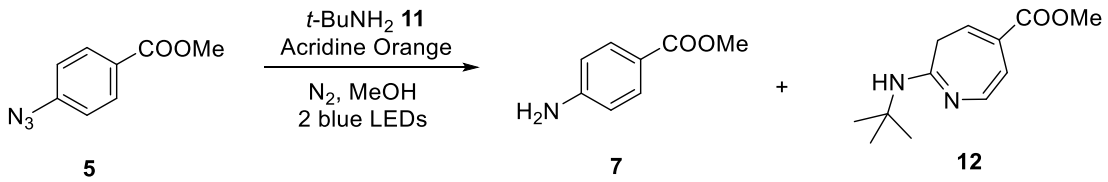
1H), 3.77 (s, 3H), 3.26 (t, J = 7.3 Hz, 2H), 2.71 (d, J = 7.4 Hz, 2H), 1.48 (tt, J = 7.5, 7.3 Hz, 2H), 1.33 (tq, J = 7.5, 7.3 Hz, 2H), 0.90 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.43, 145.92, 142.19, 131.68, 122.90, 108.23, 51.93, 42.13, 36.16, 31.16, 20.12, 13.77. IR (neat): 3381, 3255, 2956, 2932, 2872, 1720, 1588, 1530, 1434, 1378, 1323, 1264, 1193, 1069, 760 cm^{-1}. HRMS-ESI (m/z) [M+H$^+$] calcd. for $C_{12}H_{18}N_2O_2$ 223.1441, found 223.1445.



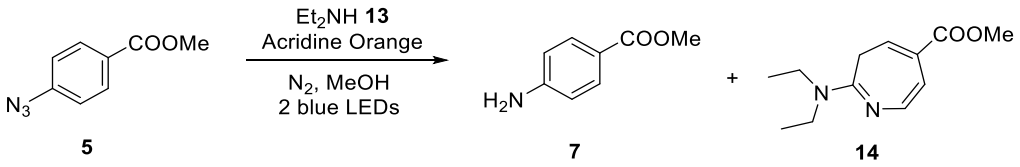
5 **7** **10**

A solution of **5** (18 mg, 0.10 mmol) and Acridine Orange (6.6 mg, 0.020 mmol) in methanol (2.0 mL) was placed in a 5.0 mL clear-colored glass vial equipped with a magnetic stir bar. After the addition of ethanethiol **9** (0.37 mL, 5 mmol), the reaction was bubbled with nitrogen gas to remove the oxygen. Then the vial was sealed and exposed to 2 × 4W 468 nm blue LEDs (10.0 cm from the LEDs, 10.4 mW cm^{-2}) with stirring at ambient temperature for 48 hours, then concentrated in vacuum. The residue was purified by silica gel column chromatography to give **10** as a white solid (3.0 mg, 14%). Another isolated product was identified as 4-methoxycarbonyl aniline **7**.

For **10**: TLC R_f = 0.55 (petroleum ether/ethyl acetate = 4/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (d, J = 2.0 Hz, 1H), 7.79 (dd, J = 8.4, 2.0 Hz, 1H), 6.69 (d, J = 8.4 Hz, 1H), 4.78 (s, 2H), 3.86 (s, 3H), 2.77 (q, J = 7.3 Hz, 2H), 1.23 (t, J = 7.3 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.77, 152.24, 137.91, 131.44, 119.76, 117.01, 113.57, 51.73, 29.01, 14.88. IR (neat): 3462, 3353, 2950, 2923, 1707, 1609, 1497, 1434, 1254, 1121, 765, 750 cm^{-1}. HRMS-ESI (m/z) [M+H$^+$] calcd. for $C_{10}H_{13}NO_2S$ 212.0740, found 212.0742.

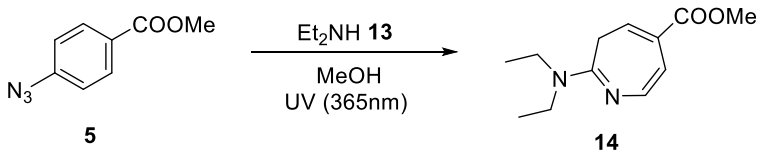


A solution of **5** (18 mg, 0.10 mmol) and Acridine Orange (6.6 mg, 0.020 mmol) in methanol (2.0 mL) was placed in a 5.0 mL clear-colored glass vial equipped with a magnetic stir bar. After the addition of *tert*-butylamine **11** (0.53 mL, 5 mmol), the reaction was bubbled with nitrogen gas to remove the oxygen. Then the vial was sealed and exposed to 2 × 4W 468 nm blue LEDs (10.0 cm from the LEDs, 10.4 mW cm^{-2}) with stirring at ambient temperature for 48 hours, then concentrated in vacuum. The residue was purified by silica gel column chromatography to give **12** as a yellow solid (12 mg, 54%). Another isolated product was identified as 4-methoxycarbonyl aniline **7**. For **12**: TLC R_f = 0.43 (petroleum ether/ethyl acetate = 2/1);^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (dd, *J* = 8.2, 0.7 Hz, 1H), 6.19 (dd, *J* = 8.2, 1.2 Hz, 1H), 6.13 (tt, *J* = 7.5, 0.9 Hz, 1H), 4.19 (s, 1H), 3.78 (s, 3H), 2.61 (d, *J* = 7.5 Hz, 2H), 1.30 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.73, 142.49, 141.93, 131.27, 122.16, 107.25, 62.27, 51.89, 37.66, 28.89. IR (neat): 3385, 2963, 1704, 1590, 1521, 1452, 1434, 1361, 1263, 1221, 1191, 1119, 1080, 1053, 759 cm^{-1}. HRMS-ESI (m/z) [M+H^+] calcd. for $C_{12}H_{18}N_2O_2$ 223.1441, found 223.1444.



A solution of **5** (70 mg, 0.40 mmol) and Acridine Orange (26 mg, 0.080 mmol) in methanol (9 mL) was placed in a 16 mL clear-colored glass vial equipped with a magnetic stir bar. After the addition of diethylamine **13** (1.0 mL, 20 mmol), the reaction was bubbled with nitrogen gas to remove the oxygen. Then the vial was sealed and exposed to 2 × 4W 468 nm blue LEDs (10.0 cm from the LEDs, 10.4 mW cm^{-2}) with stirring at ambient temperature for 40 hours, then concentrated in vacuum. The residue was purified by silica gel column chromatography to give **14** as a yellow solid (18 mg, 40%)[14-15]. Another isolated product was identified as the 4-methoxycarbonyl aniline **7**.

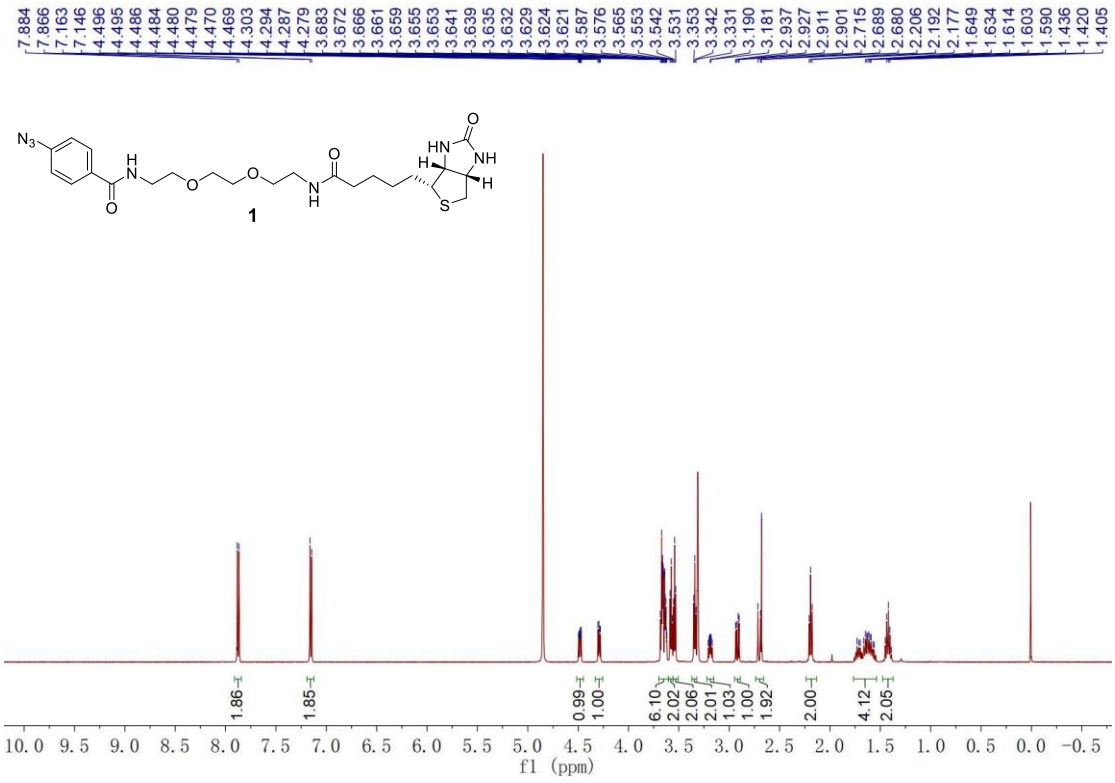
For **14**: TLC R_f = 0.38 (petroleum ether/ethyl acetate = 2/1); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 (d, *J* = 8.0 Hz, 1H), 6.21 (dd, *J* = 8.0, 1.3 Hz, 1H), 6.10 (t, *J* = 7.7 Hz, 1H), 3.77 (s, 3H), 3.37 (d, *J* = 6.8 Hz, 4H), 1.15 (br, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.82, 144.85, 142.05, 132.30, 119.24, 106.93, 51.99, 43.51, 31.80, 13.74; HRMS-ESI (m/z) [M+H$^+$] calcd. for $C_{12}H_{19}N_2O_2$ 223.1441, found 223.1445.[14-15]

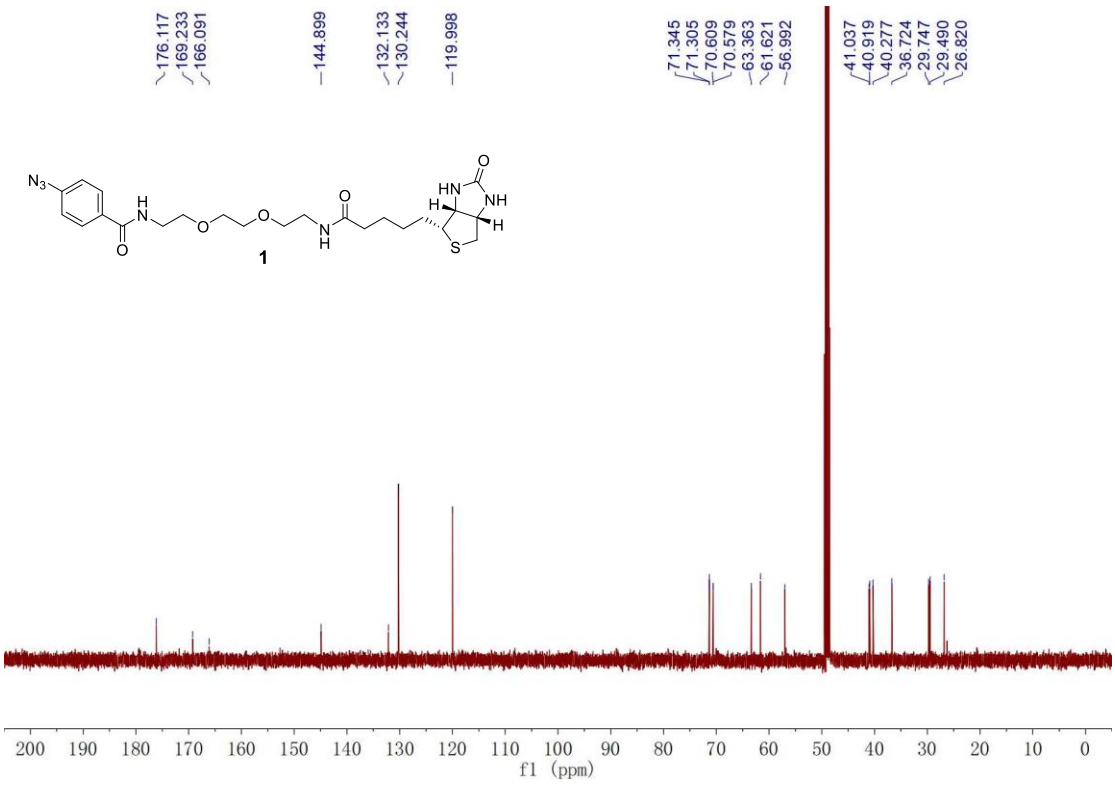


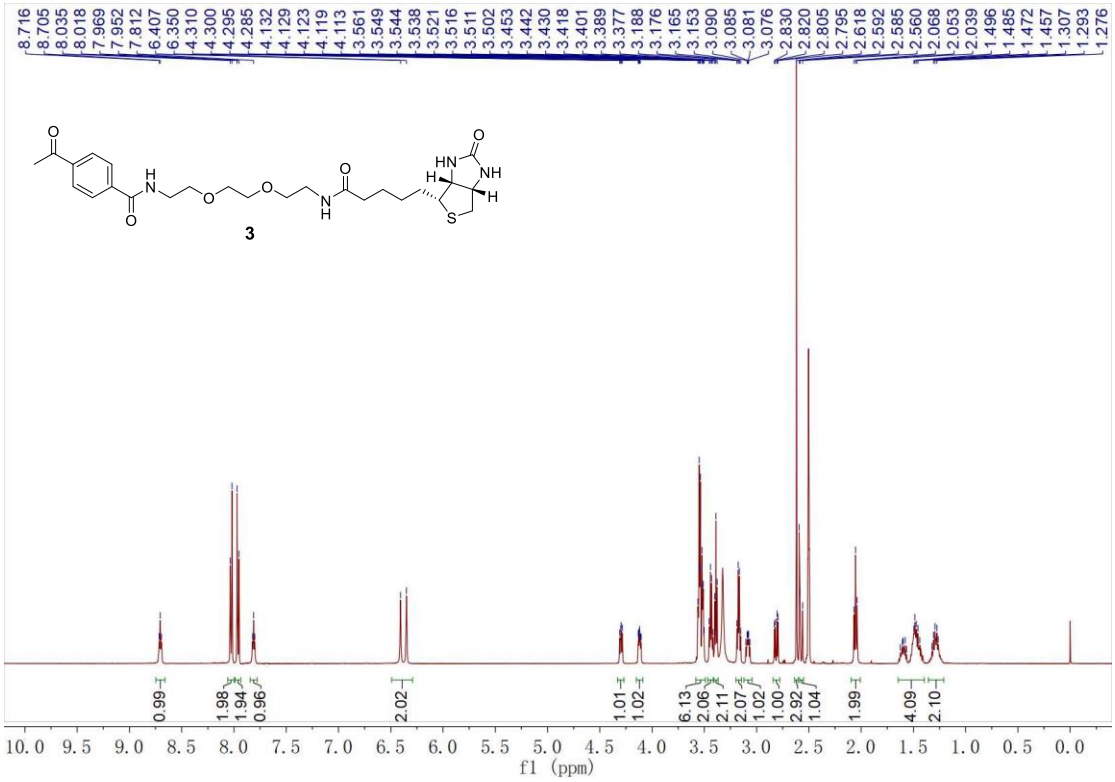
5 **14**

A solution of **5** (18 mg, 0.10 mmol) and diethylamine **13** (0.5 mL, 5 mmol) in methanol (1.5 mL) was placed in a 5.0 mL clear-colored glass vial equipped with a magnetic stir bar. The reaction was exposed to ultraviolet light (15.0 cm from the smart xenon lamp light source equipped with band-pass filters, 19.8 mW cm^{-2}) with stirring at ambient temperature for 12 hours, then concentrated in vacuum. The residue was purified by silica gel column chromatography to give **14** as a yellow solid (2.4 mg, 11%). The TLC and ^{1}H NMR analysis indicated that compound **14** obtained from UV-light induced conditions were identical from the photocatalytic conditions.

VI. NMR Spectra of New Compounds



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8.705
8.035
8.018
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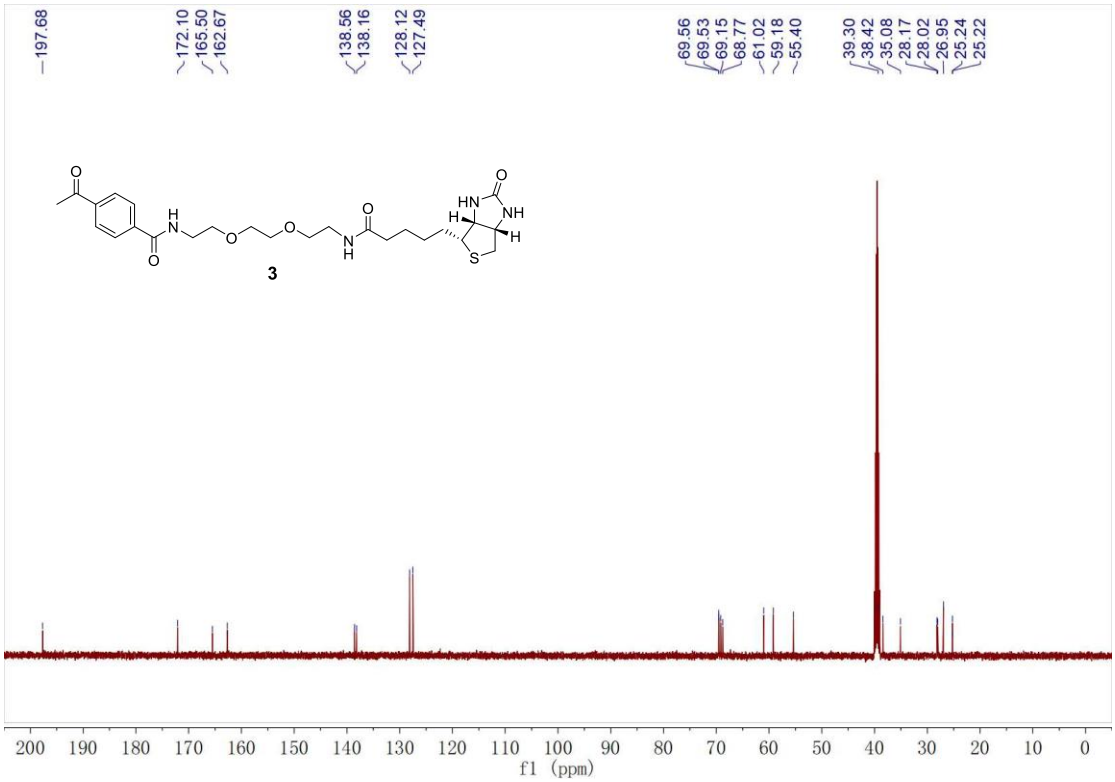
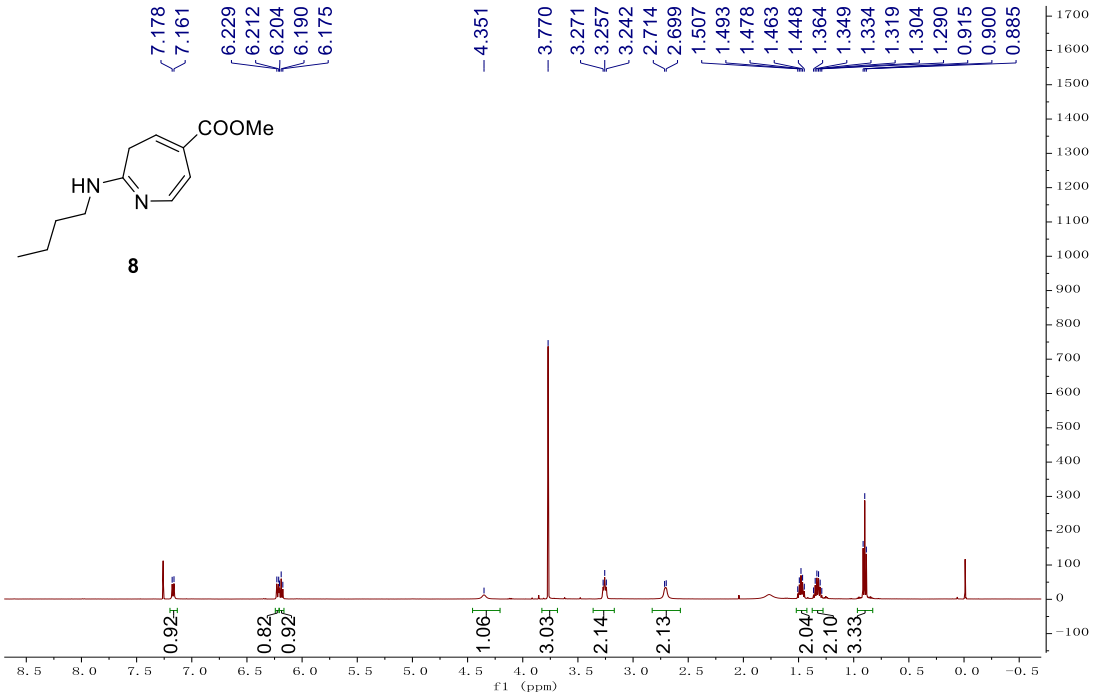
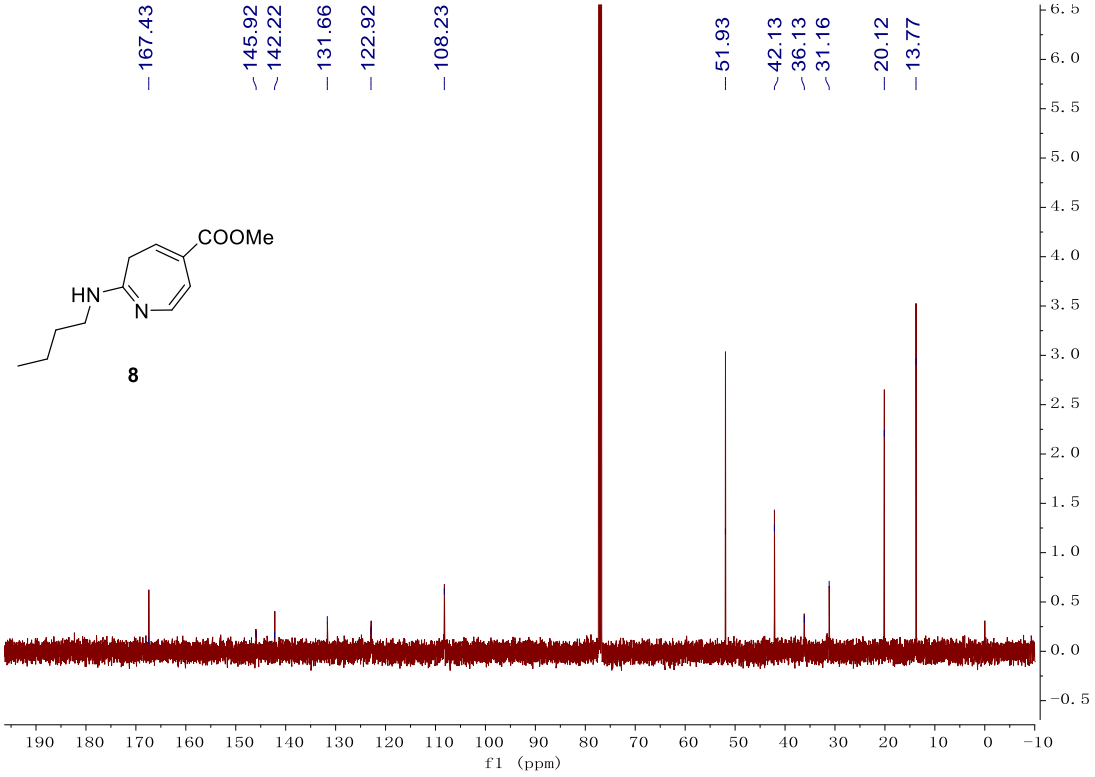
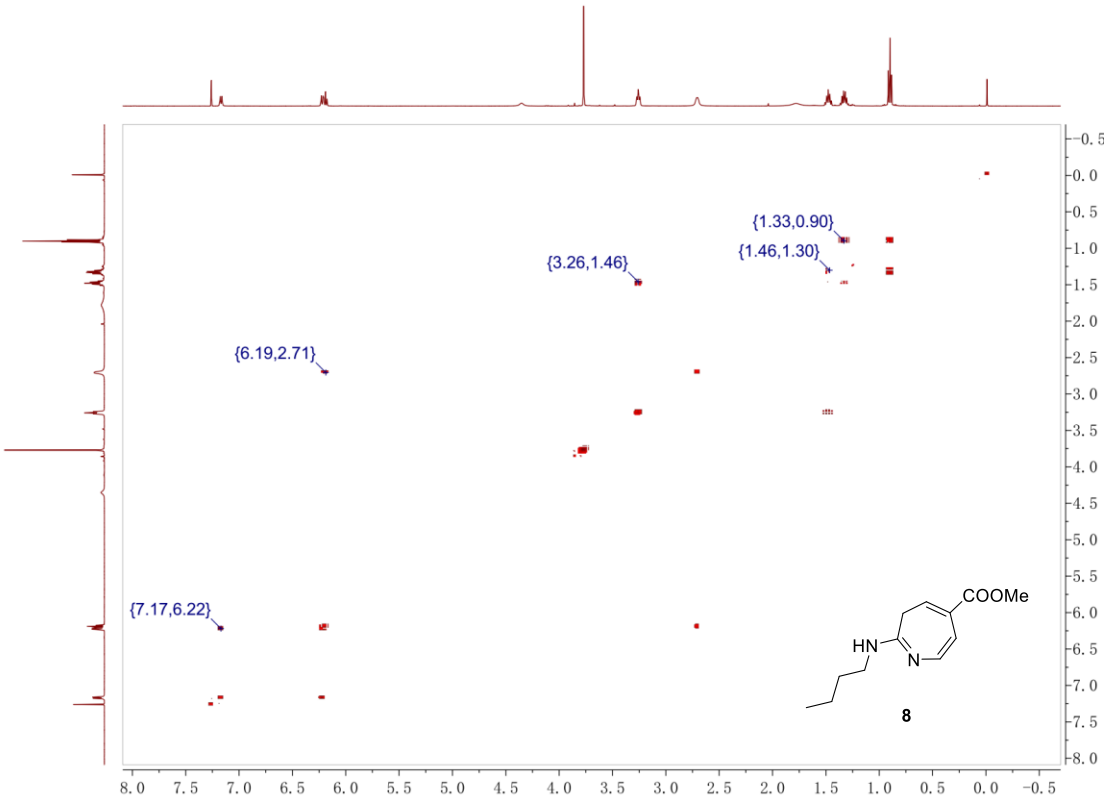
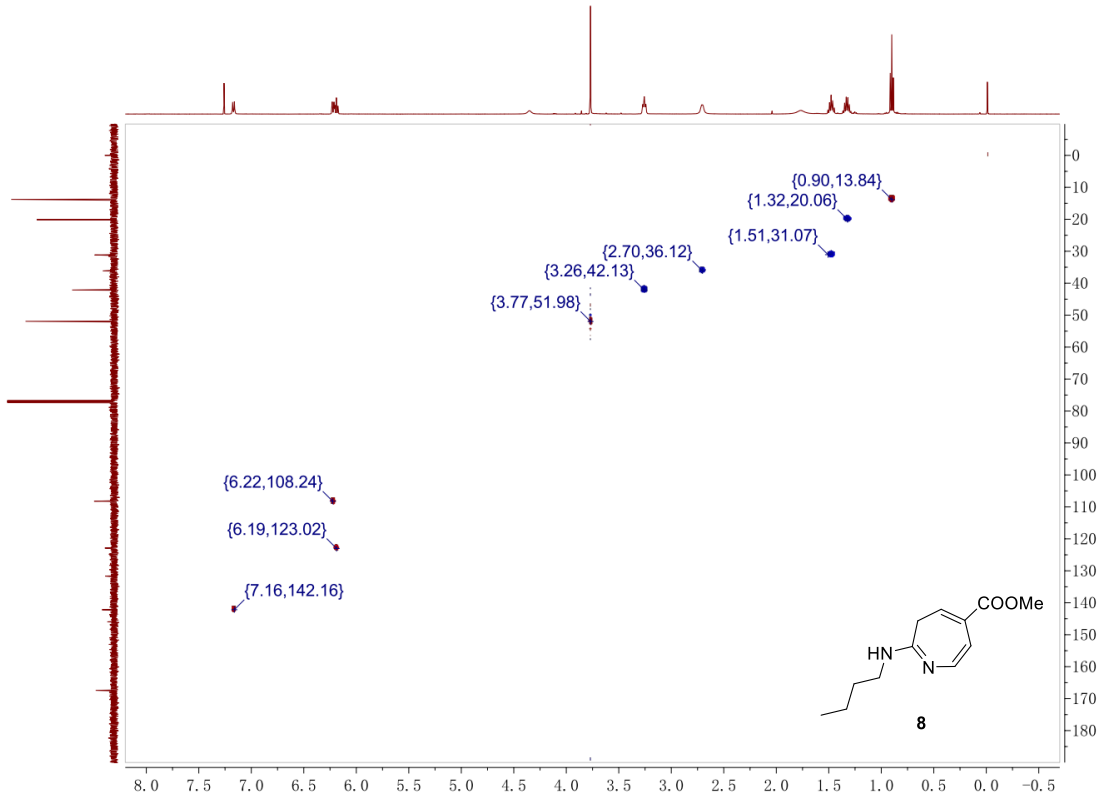
7.952
7.812
6.407
6.350
4.310
4.300
4.295
4.285
4.132
4.129
4.123
4.119
4.113
3.561
3.549
3.544
3.538
3.521
3.516
3.511
3.502
3.453
3.442
3.430
3.418
3.401
3.389
3.377
3.188
3.176
3.165
3.153
3.090
3.085
3.081
3.076
2.830
2.820
2.805
2.795
2.618
2.592
2.585
2.560
2.068
2.053
2.039
1.496
1.485
1.472
1.457
1.307
1.293
1.276
3
0.99
1.98
1.94
0.96
2.02
1.01
1.02
6.13
2.06
2.11
2.07
1.02
1.00
2.92
1.04
1.99
4.09
2.10
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

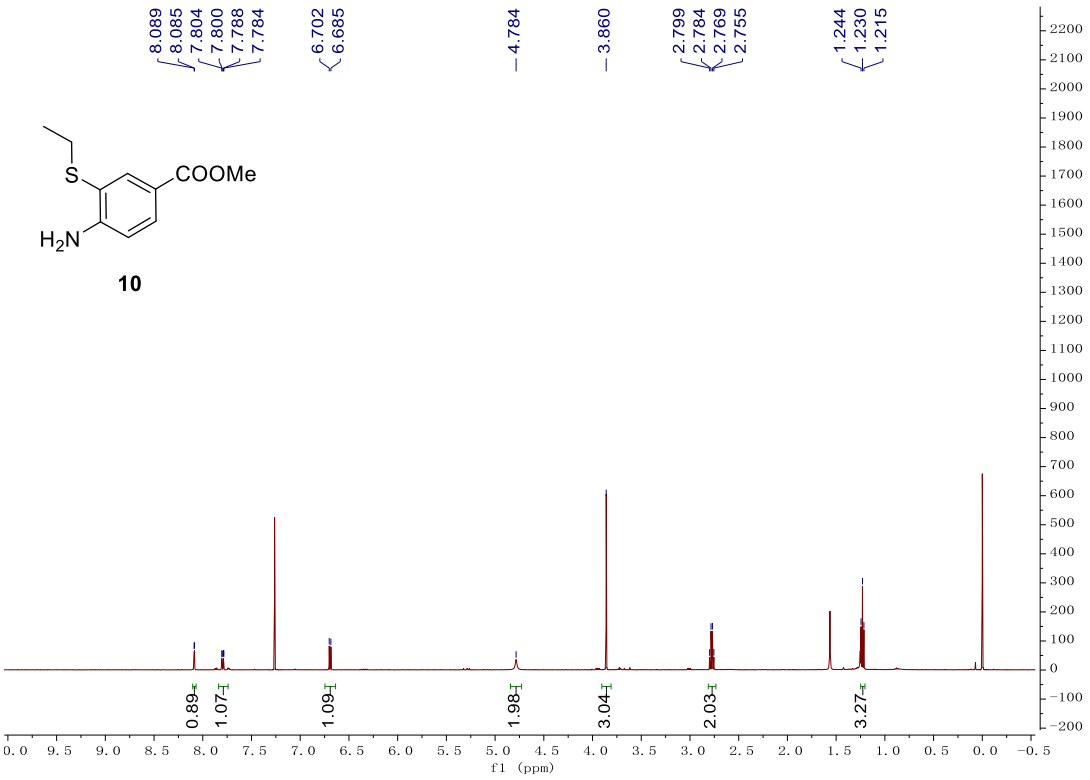

197.68
172.10
165.50
162.67
138.56
138.16
128.12
127.49
69.56
69.53
69.15
68.77
61.02
59.18
55.40
39.30
38.42
35.08
28.17
28.02
26.95
25.24
25.22
3
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

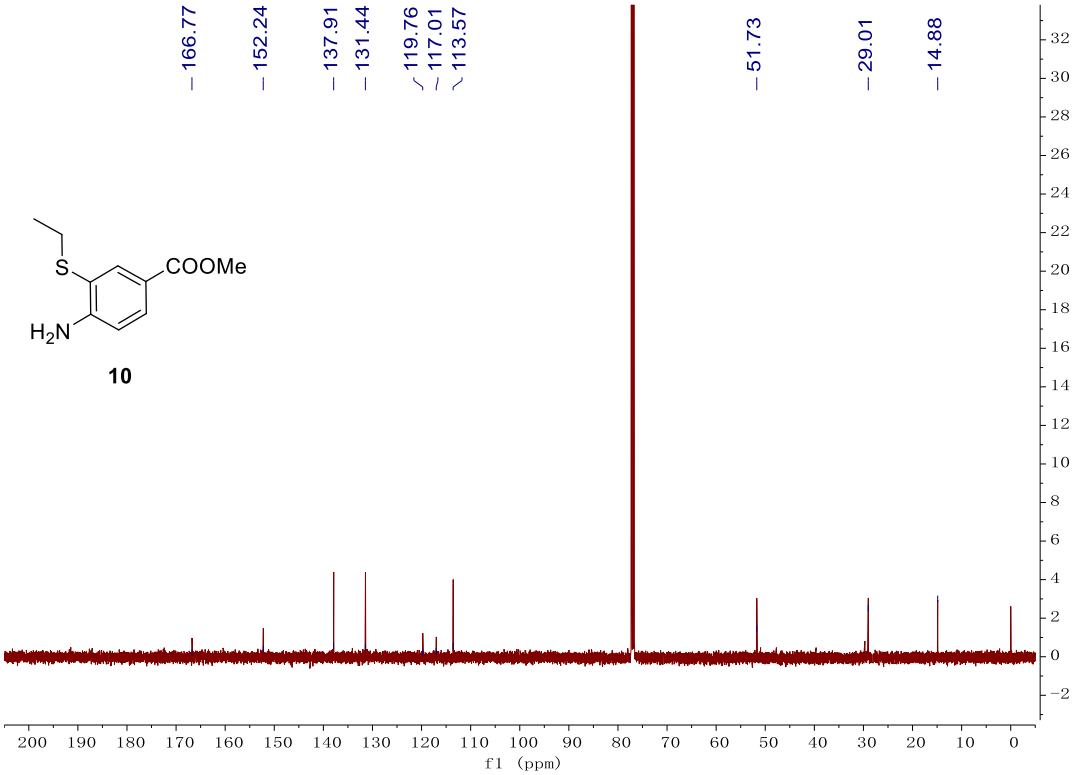

7.178
7.161
6.229
6.212
6.204
6.190
6.175
4.351
3.770
3.271
3.257
3.242
2.714
2.699
1.507
1.493
1.478
1.463
1.448
1.364
1.349
1.334
1.319
1.304
1.290
0.915
0.900
0.885
COOMe
HN
N
8
0.92
0.82
0.92
1.06
3.03
2.14
2.13
2.04
2.10
3.33
f1 (ppm)

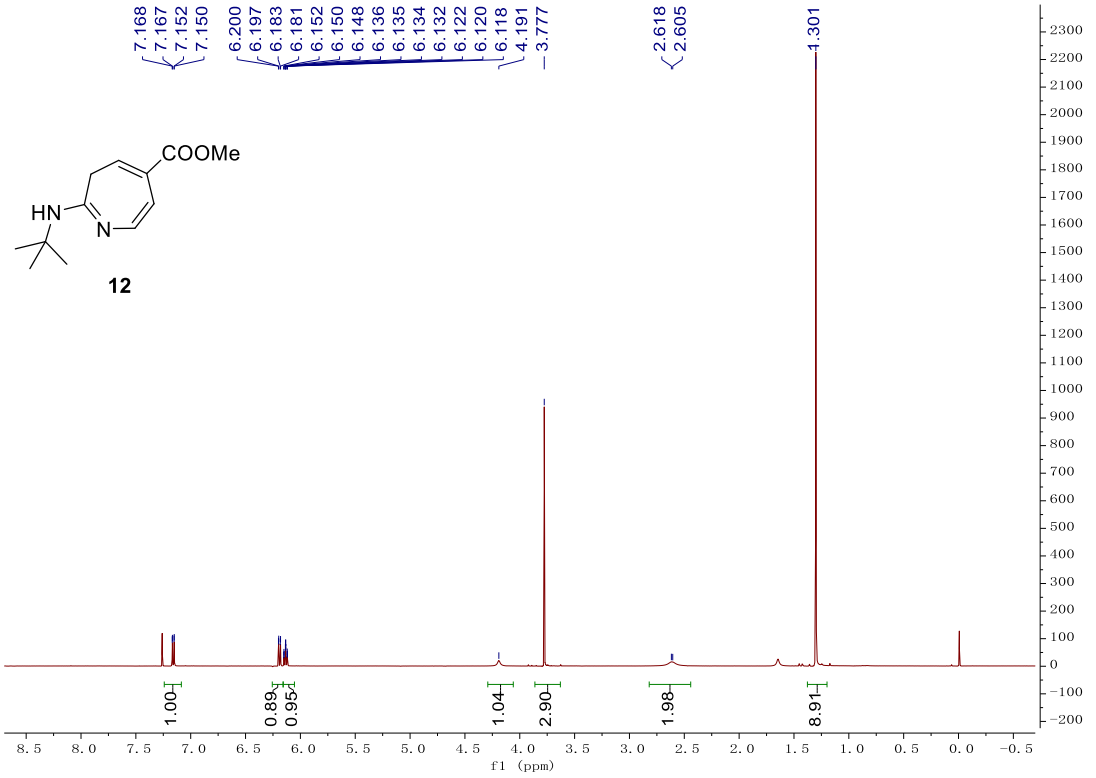

167.43
145.92
142.22
131.66
122.92
108.23
51.93
42.13
36.13
31.16
20.12
13.77
COOMe
HN
N
8
f1 (ppm)

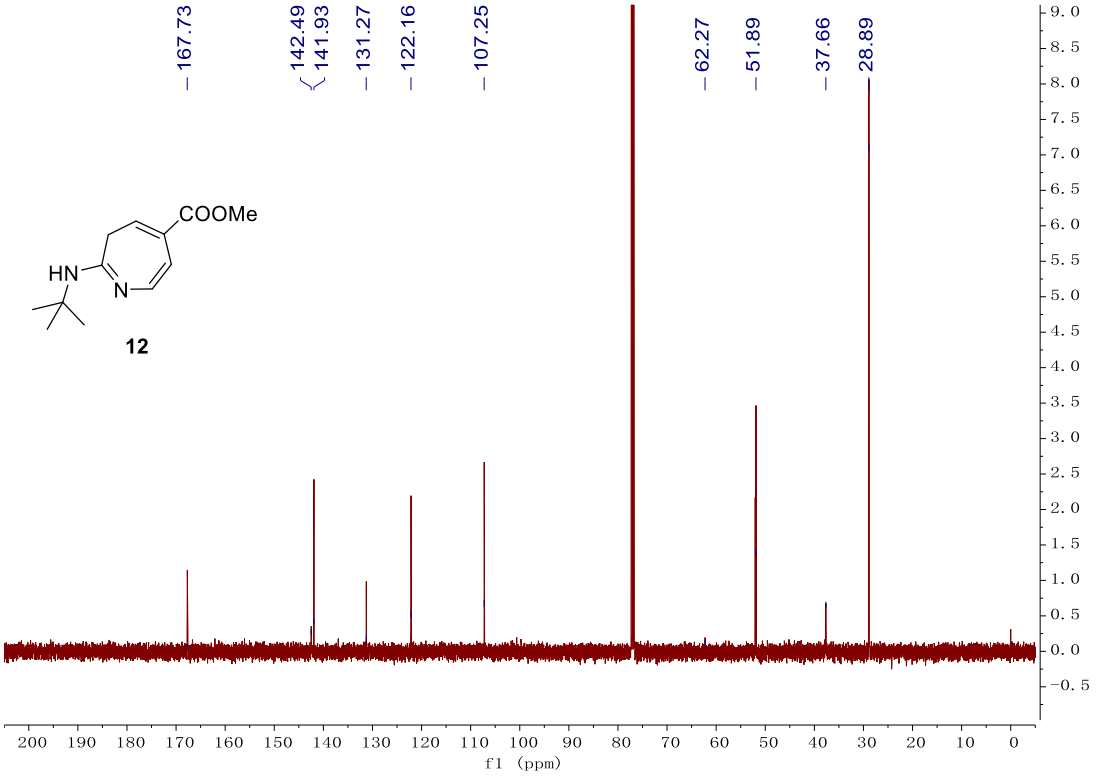

{1.33,0.90}
{1.46,1.30}
{3.26,1.46}
{6.19,2.71}
{7.17,6.22}
COOMe
HN
N
8


{0.90,13.84}
{1.32,20.06}
{1.51,31.07}
{2.70,36.12}
{3.26,42.13}
{3.77,51.98}
{6.22,108.24}
{6.19,123.02}
{7.16,142.16}
COOMe
HN
N
8


8.089
8.085
7.804
7.800
7.788
7.784
6.702
6.685
4.784
3.860
2.799
2.784
2.769
2.755
1.244
1.230
1.215
S
COOMe
H2N
10
0.89
1.07
1.09
1.98
3.04
2.03
3.27
f1 (ppm)


166.77
152.24
137.91
131.44
119.76
117.01
113.57
51.73
29.01
14.88
S
COOMe
H2N
10
f1 (ppm)


7.168
7.167
7.152
7.150
6.200
6.197
6.183
6.181
6.152
6.150
6.148
6.136
6.135
6.134
6.132
6.122
6.120
6.118
4.191
3.777
2.618
2.605
1.301
COOMe
HN
N
12
1.00
0.89
0.95
1.04
2.90
1.98
8.91
f1 (ppm)


167.73
142.49
141.93
131.27
122.16
107.25
62.27
51.89
37.66
28.89
COOMe
HN
N
12
f1 (ppm)

VII. References

1. Pfiffi, D.; Bier, B. A.; Marian, C. M.; Schaper, K.; Seidel, C. A., Diphenylhexatrienes as photoprotective agents for ultrasensitive fluorescence detection. *J. Phys. Chem. A* **2010,** *114* (12), 4099-108.

2. Romero, N. A.; Nicewicz, D. A., Organic Photoredox Catalysis. *Chem. Rev.* **2016,** *116* (17), 10075-166.

3. Wilkinson, F.; Helman, W. P.; Ross, A. B., Quantum Yields for the Photosensitized Formation of the Lowest Electronically Excited Singlet-State of Molecular-Oxygen in Solution. *J. Phys. Chem. Ref. Data* **1993,** *22* (1), 113-262.

4. Gandin, E.; Lion, Y.; Van de Vorst, A., Quantum Yield of Singlet Oxygen Production by Xanthene Derivatives. *Photochem. Photobio.* **1983,** *37* (3), 271-278.

5. Ezquerra Riega, S. D.; Rodriguez, H. B.; San Roman, E., Rose bengal in poly(2-hydroxyethyl methacrylate) thin films: self-quenching by photoactive energy traps. *Methods Appl. Fluoresc.* **2017,** *5* (1), 014010.

6. Kim, M.; Ko, S.-K.; Kim, H.; Shin, I.; Tae, J., Rhodamine cyclic hydrazide as a fluorescent probe for the detection of hydroxyl radicals. *Chem. Commun.* **2013,** *49* (72), 7959-7961.

7. Kim, S.; Tachikawa, T.; Fujitsuka, M.; Majima, T., Far-Red Fluorescence Probe for Monitoring Singlet Oxygen during Photodynamic Therapy. *J. Am. Chem. Soc.* **2014,** *136* (33), 11707-11715.

8. Rhee, H. W.; Zou, P.; Udeshi, N. D.; Martell, J. D.; Mootha, V. K.; Carr, S. A.; Ting, A. Y., Proteomic mapping of mitochondria in living cells via spatially restricted enzymatic tagging. *Science* **2013,** *339* (6125), 1328-1331.

9. Beausoleil, S. A.; Villén, J.; Gerber, S. A.; Rush, J.; Gygi, S. P., A probability-based approach for high-throughput protein phosphorylation analysis and site localization. *Nat. Biotechnol.* **2006,** *24* (10), 1285-1292.

10. Huang da, W.; Sherman, B. T.; Lempicki, R. A., Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* **2009,** *4* (1), 44-57.

11. The UniProt, C., UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res.* **2018,** *47* (D1), D506-D515.

12. Calvo, S. E.; Clauser, K. R.; Mootha, V. K., MitoCarta2.0: an updated inventory of mammalian mitochondrial proteins. *Nucleic Acids Res.* **2015,** *44* (D1), D1251-D1257.

13. Shi, H.; Liu, K.; Xu, A.; Yao, S. Q., Small molecule microarray-facilitated screening of affinity-based probes (AfBPs) for gamma-secretase. *Chem. Commun.* **2009,** (33), 5030-2.

14. Nielsen, P. E.; Buchardt, O., Aryl Azides as Photoaffinity Labels. A Photochemical Study of Some 4-Substituted Aryl Azides. *Photochem. Photobiol.* **1982,** *35* (3), 317-323.

15. Li, Y. Z.; Kirby, J. P.; George, M. W.; Poliakoff, M.; Schuster, G. B., 1,2-Didehydroazepines from the photolysis of substituted aryl azides: analysis of their chemical and physical properties by time-resolved spectroscopic methods. *J. Am. Chem. Soc.* **1988,** *110* (24), 8092-8098.