Phototriggered Labeling and Crosslinking by 2-Nitrobenzyl Alcohol Derivatives with Amine Selectivity

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1. General materials.

All starting materials were obtained from commercial suppliers and were used without further purification unless otherwise stated. All air- or moisture-sensitive reactions were performed using oven-dried or flame-dried glassware under an inert atmosphere of dry argon. BSA, SA and ConA proteins were purchased from Sigma-Aldrich. Native IgG from mouse was purchased from Abcam. Recombinant SPA from *E.coil* was purchased from Beyotime.

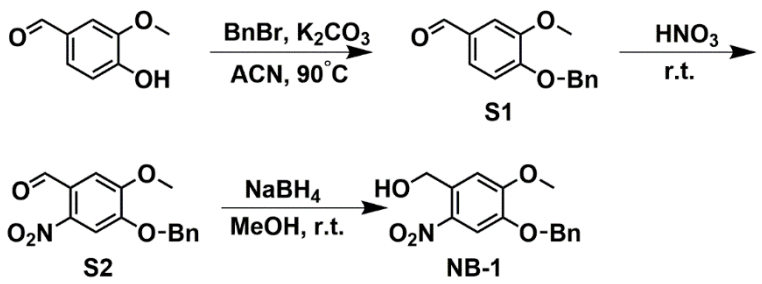
2. Characterizations.

Proton and carbon nuclear magnetic resonance spectra (^{1}H, ^{13}C NMR) were recorded on a Bruker Avance 400 MHz spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from the Me_4Si resonance which was used as the internal standard when recording ^{1}H NMR spectra. Mass spectra were recorded on a Micromass GCTTM and a Micromass LCTTM. Absorption spectra were recorded on a Shimadzu UV-2550 UV-Vis spectrometer. The reversed-phase HPLC was monitored on an Agilent 1200 Series using BetaBasic-18 column. MALDI-TOF was Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry. In-gel fluorescence images were acquired by scanning SDS-PAGE gels with a BioRad PharosFX imager. Proteins (BSA and ConA) were digested with trypsin. Peptide mixtures were analyzed by LC/MS/MS on an UltiMate 3000 RSLC nano-HPLC system coupled to an Orbitrap Q-Exactive mass spectrometer (Thermo Fisher Scientific, Bremen, Germany), both equipped with nano-ESI sources.

3. Synthesis of compounds.

3.1 Synthesis of **NB-1**.

Scheme S1. The synthesis procedure for **NB-1**.



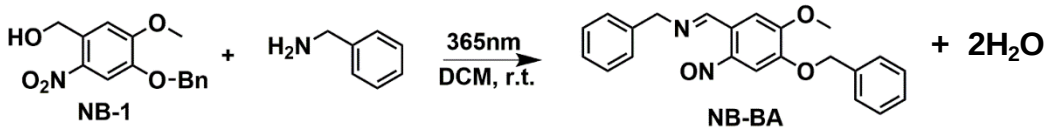
Compound S1: To a solution of 4-hydroxy-3-methoxybenzaldehyde (10.0 g, 66.0 mmol) in ACN (200 mL) was added BnBr (15.8 g, 92.0 mmol) and K_2CO_3 (12.8 g, 92.0 mmol). The mixture was stirred at 90 ℃ for 12 h. Then the mixture was cooled to room temperature and K_2CO_3 was filtered. The solvent was removed under vacuum and colorless oil appeared. The crude product was further purified by recrystallization from ethanol to obtain white solid compound S1 (12.0 g, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 9.81 (s, 1H); 7.45-7.37 (m, 7H); 7.01 (d, J = 8.6 Hz, 1H); 5.24 (s, 2H); 3.94 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 190.9, 153.6, 150.1, 136.0, 130.3, 128.8, 128.2, 127.2, 126.6, 112.4, 109.3, 70.8, 56.1.MS (ESI): m/z: Calcd. for $C_{15}H_{14}O_3Na$ $[M+Na]^+$: 265.2. Found: 265.2.

Compound S2: Compound S1 (10.0 g, 41 mmol) was added into a concentrated nitric acid (30 mL) at 0 ℃. The mixture was stirred at room temperature for 30 min and poured into water. The solid obtained was filtered and dried. The product was further purified by recrystallization from ethanol to obtain yellow solid compound S2 (7.0 g, 60% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 10.44 (s, 1H), 7.67 (s, 1H), 7.45-7.36 (m, 6H), 5.26 (s, 2H), 4.01 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 187.8, 153.7, 151.4, 134.8, 128.8, 127.6, 125.7, 110.0, 108.8, 71.5, 56.7. MS (ESI): m/z: Calcd. for $C_{15}H_{14}NO_5$ $[M+H]^+$: 288.2. Found: 288.2.

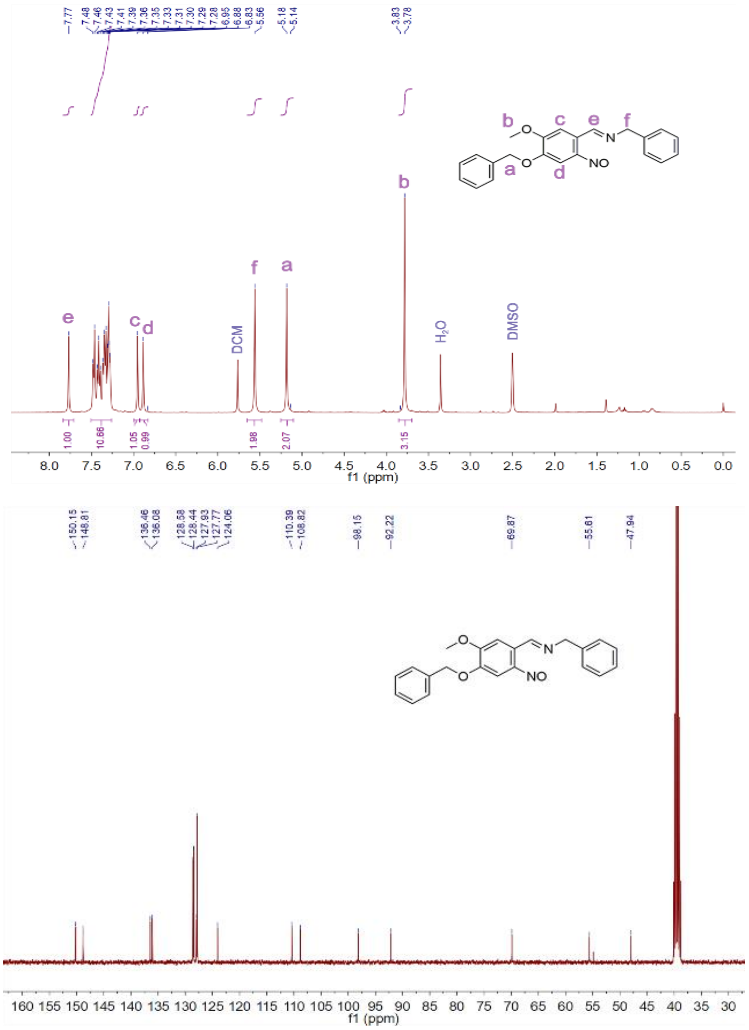
Compound **NB-1**: To a solution of compound S2 (2.0 g, 7 mmol) in methanol (100 mL) was added $NaBH_4$ (0.53 g, 14 mmol). The mixture was stirred at room temperature for 15 min. The volume of the reaction mixture was then reduced to 15 mL under vacuum. The reaction mixture was diluted with brine (100 mL) followed by extraction with DCM (3×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (DCM/MeOH = 1000:1) to afford yellow solid compound **NB-1** (1.9 g, 94% yield). ^{1}H NMR (400 MHz, DMSO), δ (ppm): 7.79 (s, 1H), 7.50 – 7.33 (m, 6H), 5.60 (t, J = 5.4 Hz, 1H), 5.20 (s, 2H), 4.83 (d, J = 4.6 Hz, 2H), 3.92 (s, 3H). ^{13}C NMR (100 MHz, DMSO), δ (ppm): 153.62, 145.57, 138.01, 136.09, 134.27, 128.27, 127.73, 109.52, 109.22, 70.04, 59.89, 55.89. HRMS (ESI): m/z: Calcd. for $C_{15}H_{15}NNaO_5$ $[M+Na]^+$: 312.0848. Found: 312.0849

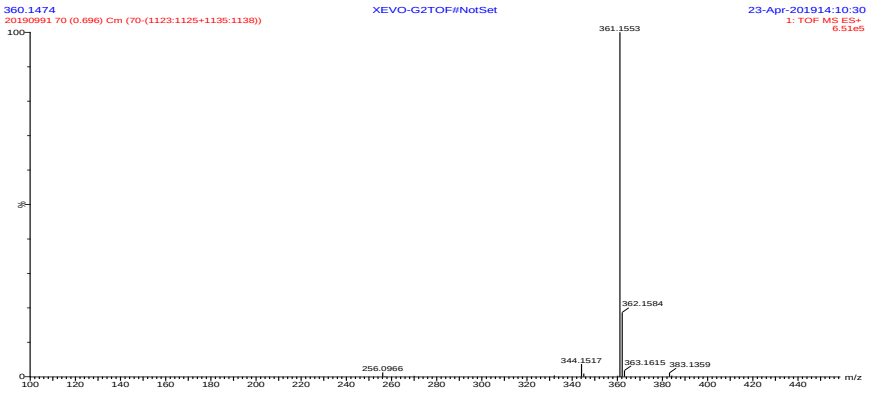
3.2 Synthesis and purification of **NB-BA**.

Scheme S2. The synthesis procedure for **NB-BA**.



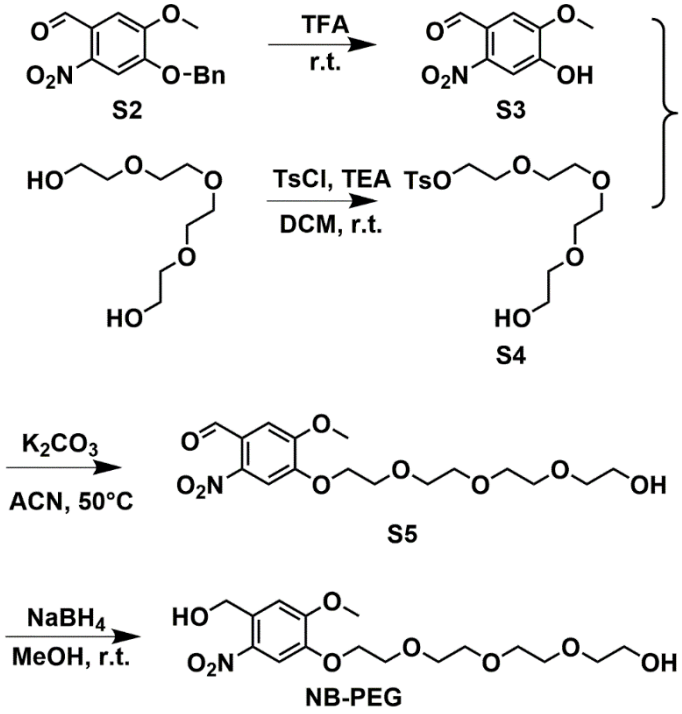
Compound **NB-BA**: To a solution of compound **NB-1** (300 mg, 1 mmol) in DCM (20 mL) was added benzylamine (107 mg, 1 mmol). Then the mixture was stirred and irradiated by a LED 365 light at 10 mW cm^{-2} for 2.5 h. The solvent was removed under vacuum. The product was purified by silica gel flash column chromatography (DCM/MeOH=100:1) to afford brown powder compound **NB-BA** (168 mg, 45% yield). ^{1}H NMR (400 MHz, DMSO), δ (ppm): 7.77 (s, 1H), 7.54 – 7.23 (m, 10H), 6.95 (s, 1H), 6.88 (s, 1H), 5.56 (s, 2H), 5.18 (s, 2H), 3.78 (s, 3H). ^{13}C NMR (100 MHz, DMSO), δ (ppm): 150.15, 148.81, 136.46, 136.08, 128.58, 128.44, 127.93, 127.77, 124.06, 110.39, 108.82, 98.15, 92.22, 69.87, 55.61, 47.94. HRMS (ESI): m/z: Calcd. for $C_{22}H_{21}N_2O_3$ $[M+H]^+$: 361.1552. Found: 361.1553.





3.3 Synthesis and purification of **NB-PEG**.

Scheme S3. The synthesis procedure for **NB-PEG** and **NB-F**.



Compound S3: Compound S2 (10 g, 35 mmol) was dissolved in 100 mL trifluoroacetic acid and the resultant solution was stirred at room temperature for overnight. Trifluoroacetic acid was then evaporated by vacuum and the resulting crude was diluted with ethyl acetate and then basified by aqueous NaOH solution. The pH of the solution was adjusted to 5 by addition of HCl aqueous solution. The whole mixture was extracted with ethyl acetate and the combined organic layers were dried over Na_2SO_4 and concentrated. The product was purified by silica gel flash column chromatography (PE/EA=1:1) to afford yellow solid compound S3 (6 g, 87% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 11.12 (s, 1H), 10.16 (s, 1H), 7.51 (s, 1H), 7.36 (s, 1H), 3.95 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 188.37, 151.75, 150.94, 143.74, 123.38, 111.04, 110.52, 56.31. MS (ESI): m/z: Calcd. for $C_8H_7NO_5Na$ $[M+Na]^+$: 220.0. Found: 220.0.

Compound S4: To a solution of tetraethylene glycol (5 g, 26 mmol) in DCM (250

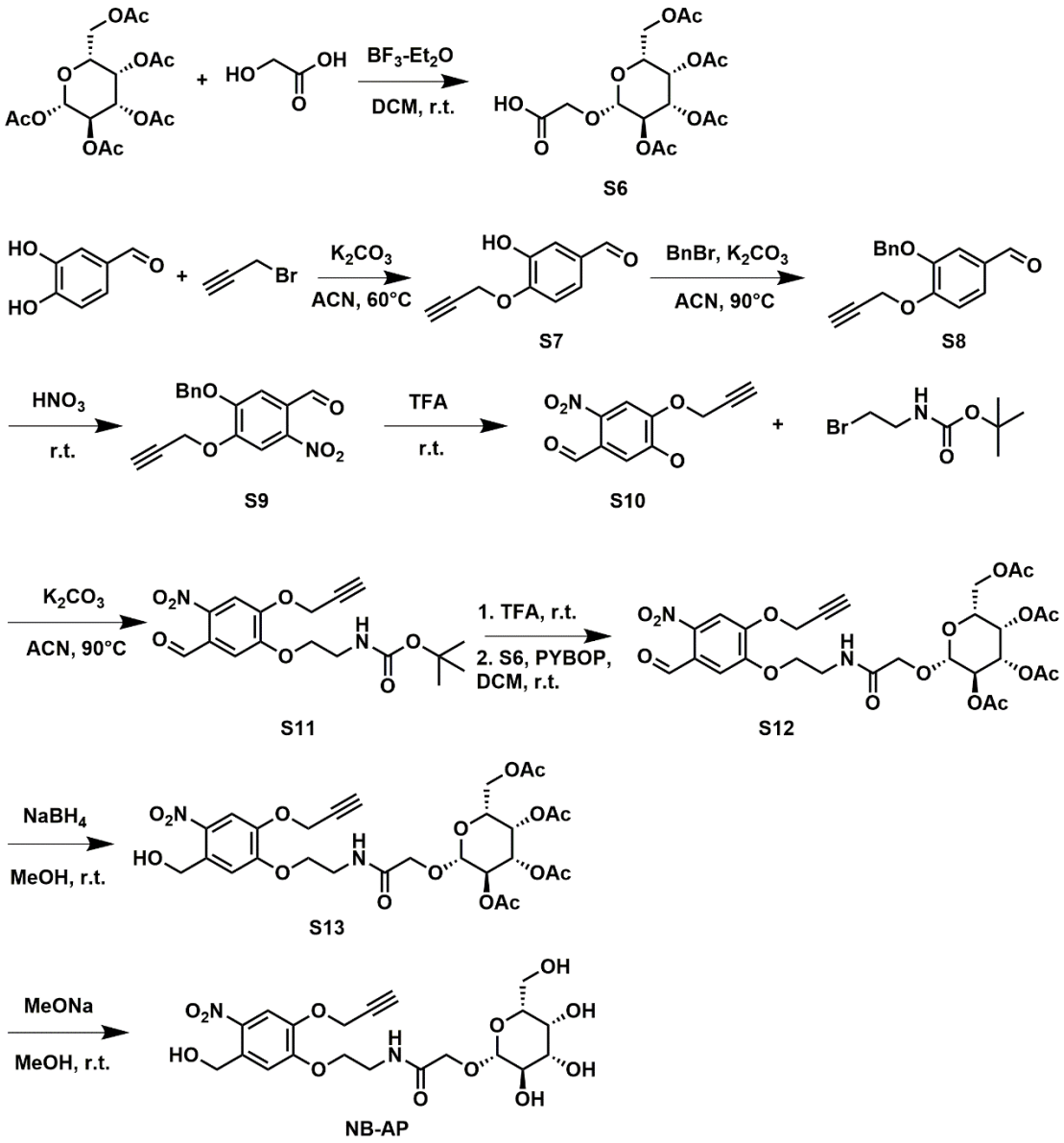
mL) was added tosyl chloride (4.9 g, 26 mmol) and Triethylamine (3 g, 30 mmol). The mixture was stirred at room temperature for 12 h. The whole mixture was extracted with DCM and the combined organic layers were dried over Na_2SO_4 and concentrated. The product was purified by silica gel flash column chromatography (DCM/PE=5:1) to afford colorless oil compound S4 (7 g, 77% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.81 (s, 2H), 7.36 (s, 2H), 5.31 (s, 1H), 4.17 (t, *J*=4.79Hz, 2H), 3.65 (m, 14H), 2.45 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 144.88, 132.89, 129.86, 127.99, 72.40, 70.68, 70.64, 70.42, 70.23, 69.28, 68.70, 61.70. MS (ESI): m/z: Calcd. for $C_{15}H_{24}O_7SNa$ $[M+Na]^+$: 371.1. Found: 371.1.

Compound S5: To a solution of compound S3 (2 g, 10 mmol) in ACN (100 mL) was added K_2CO_3 (2.8 g, 20 mmol) and stirred at room temperature for 15 min. Then compound S4 (5.2 g, 15 mmol) was added and stirred at 50 ℃ for 12 h. Then the mixture was cooled to room temperature and K_2CO_3 was filtered. The solvent was removed under vacuum. The product was purified by silica gel flash column chromatography (DCM/MeOH=100:1) to afford yellow solid compound S5 (3 g, 80% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 10.44 (s, 1H), 7.72 (s, 1H), 7.41 (s, 1H), 5.31 (s, 1H), 4.39 – 4.32 (m, 2H), 4.01 (s, 3H), 3.98 – 3.92 (m, 2H), 3.77 – 3.68 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 188.24, 151.45, 150.74, 143.53, 123.44, 111.34, 110.12, 70.68, 70.64, 70.42, 70.23, 69.28, 68.70, 61.70, 56.44. MS (ESI): m/z: Calcd. for $C_{16}H_{24}NO_9$ $[M+H]^+$: 374.2. Found: 374.1.

Compound **NB-PEG**: To a solution of compound S5 (2 g, 5 mmol) in methanol (100 mL) was added $NaBH_4$ (0.53 g, 14 mmol). The mixture was stirred at room temperature for 15 min. The volume of the reaction mixture was then reduced to 15 mL under vacuum. The reaction mixture was diluted with brine (100 mL) followed by extraction with DCM (3 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The product was purified by silica gel flash column chromatography (DCM/MeOH = 100:2) to afford yellow solid compound **NB-PEG** (1.5 g, 75% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.71 (s, 1H), 7.20 (s, 1H), 5.31 (s, 1H), 4.96 (s, 2H), 4.26 – 4.19 (m, 2H), 3.94 (s, 3H), 3.98 – 3.87 (m, 2H), 3.78 – 3.62 (m, 10H), 3.60 (dd, *J* = 5.4, 3.6 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 154.24, 146.79, 139.00, 133.38, 110.38, 109.74, 72.57, 70.79, 70.65, 70.20, 69.46, 68.85, 62.23, 61.63, 56.31, 53.50. MS (ESI): m/z: Calcd. for $C_{16}H_{25}O_9NNa$ $[M+Na]^+$: 398.1427. Found: 398.1428

3.4 Synthesis and purification of **NB-AP** and **F-N$_3$**.

Scheme S4. The synthesis procedure for **NB-AP**.



Compound S6: To a solution of peracetylated mannose (3 g, 7.7 mmol) in DCM (50 mL) was added glycolic acid (1 g, 13.2 mmol). Then the mixture was stirred at 0 ℃ and BF_3-diethyl etherate (12 mL, 45 mmol, 48% solution) was added dropwise. After 12 h, 50 mL of saturated $NaHCO_3$ solution was added and extracted. The organic layer was extracted with saturated $NaHCO_3$ (3 × 50 mL). Then the combined aqueous layers were acidified with dilute hydrochloric acid and extracted with DCM (3 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to afford colorless oil compound S6 (2 g, 64% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 5.37 (dd, J = 3.5, 1.3 Hz, 1H), 5.25 (dd, J = 11.0, 3.4 Hz, 1H), 5.15 (d, J = 3.7 Hz, 1H), 4.96 (dd, J = 10.9, 3.6 Hz, 1H), 4.40 – 4.32 (m, 1H), 4.17 (s, 2H), 4.03 (tdd, J = 11.2, 7.9, 4.7 Hz, 2H), 2.12 (s, 3H), 2.04 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 170.68, 169.88, 169.54, 67.16, 66.59, 63.95, 61.35, 20.36. MS (ESI): m/z: Calcd. for $C_{16}H_{21}O_{12}$ $[M\text{-}H]^-$: 405.1. Found: 405.1.

Compound S7: To a solution of 3,4-dihydroxybenzaldehyde (5 g, 36 mmol) in ACN

(200 mL) was added propargyl bromide (4.3 g, 36 mmol) and K_2CO_3 (5 g, 36 mmol). The mixture was stirred at 60 ℃ for 12 h. Then the mixture was cooled to room temperature and K_2CO_3 was filtered. The solvent was removed under vacuum and colorless oil appeared. The product was further purified by silica gel flash column chromatography (DCM/PE=1:1) to afford colorless oil compound S7 (2 g, 31% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.79 (s, 1H), 7.41 (dd, J = 8.3, 2.0 Hz, 1H), 7.29 (d, J = 2.0 Hz, 1H), 7.19 (d, J = 8.3 Hz, 1H), 4.93 (d, J = 2.4 Hz, 2H), 3.64 (t, J = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 191.52, 150.97, 147.35, 130.45, 123.76, 114.01, 113.44, 78.75, 56.00. MS (ESI): m/z: Calcd. for $C_{10}H_9O_3$ $[M+H]^+$: 177.1. Found: 177.1

Compound S8: To a solution of compound S7 (2 g, 11 mmol) in ACN (200 mL) was added BnBr (3 g, 17 mmol) and K_2CO_3 (3.2 g, 22 mmol). The mixture was stirred at 90 ℃ for 12 h. Then the mixture was cooled to room temperature and K_2CO_3 was filtered. The solvent was removed under vacuum and colorless oil appeared. The product was further purified by silica gel flash column chromatography (DCM/PE=1:2) to afford white powder compound S8 (2.8 g, 93% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.85 (s, 1H), 7.61 – 7.25 (m, 8H), 5.19 (s, 2H), 4.97 (d, J = 2.5 Hz, 2H), 3.65 (t, J = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 191.37, 152.09, 148.41, 136.56, 130.21, 128.44, 127.97, 127.81, 125.76, 113.28, 111.58, 78.96, 78.60, 69.89, 56.12. MS (ESI): m/z: Calcd. for $C_{17}H_{15}O_3$ $[M+H]^+$: 267.1. Found: 267.1

Compound S9: Compound S8 (2.8 g, 10 mmol) was added into a concentrated nitric acid (30 mL) at 0 ℃. The mixture was stirred at room temperature for 30 min and poured into water. The whole solution was extracted with DCM and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The product was further purified by silica gel flash column chromatography (DCM/PE=1:2) to afford yellow solid compound S9 (2.8 g, 85% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 10.21 (s, 1H), 7.86 (s, 1H), 7.54 – 7.33 (m, 6H), 5.34 (s, 2H), 5.11 (d, J = 2.5 Hz, 2H), 3.73 (t, J = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 188.57, 151.98, 149.62, 143.16, 135.70, 128.55, 128.27, 127.96, 125.50, 111.70, 109.51, 79.73, 77.92, 70.60, 56.86. MS (ESI): m/z: Calcd. for $C_{17}H_{14}NO_5$ $[M+H]^+$: 312.1. Found: 312.2

Compound S10: Compound S9 (2.5 g, 8 mmol) was dissolved in 100 mL trifluoroacetic acid and the resultant solution was stirred at room temperature overnight. Trifluoroacetic acid was then evaporated by vacuum and the resulting crude was diluted with ethyl acetate and then basified by aqueous NaOH solution. The pH of the solution was adjusted to 5 by addition of HCl aqueous solution. The whole mixture was extracted with ethyl acetate and the combined organic layers were dried over Na_2SO_4 and concentrated. The product was purified by silica gel flash column chromatography (PE/EA=1:1) to afford yellow solid compound S10 (0.9 g, 50% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 11.24 (s, 1H), 10.19 (s, 1H), 7.82 (s, 1H), 7.24 (s, 1H), 5.06 (d, J = 2.5 Hz, 2H), 3.72 (t, J = 2.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 188.87, 152.31, 148.25, 141.50, 126.48, 114.24, 110.20, 79.51, 78.07, 56.70. MS (ESI):

m/z: Calcd. for $C_{10}H_6NO_5$ $[M-H]^-$: 220.0. Found: 220.1

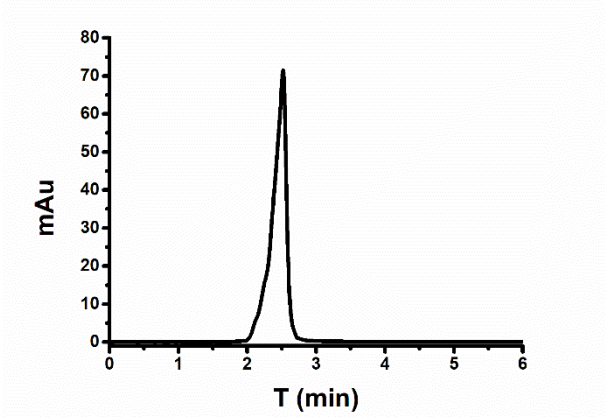
Compound S11: To a solution of compound S10 (0.82 g, 3.7 mmol) in ACN (100 mL) was added K_2CO_3 (1 g, 5.6 mmol) and stirred at room temperature for 15 min. Then tert-butyl (2-bromoethyl)carbamate (0.9 g, 4.4 mmol) was added and stirred at 60 ℃ for 12 h. Then the mixture was cooled to room temperature and K_2CO_3 was filtered. The solvent was removed under vacuum. The product was purified by silica gel flash column chromatography (DCM/MeOH=100:1) to afford yellow solid compound S11 (0.34 g, 25% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 10.20 (s, 1H), 7.83 (s, 1H), 7.40 (s, 1H), 7.03 (t, J = 5.6 Hz, 1H), 5.08 (d, J = 2.4 Hz, 2H), 4.19 (t, J = 5.7 Hz, 2H), 3.73 (t, J = 2.3 Hz, 1H), 1.38 (s, 9H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 189.64, 160.87, 157.40, 154.70, 148.21, 130.90, 114.90, 84.96, 83.11, 73.11, 67.39, 62.10, 33.42. MS (ESI): m/z: Calcd. for $C_{17}H_{21}N_2O_7$ $[M+H]^+$: 365.1. Found: 365.0

Compound S12: To a solution of S11 (330 mg, 0.9 mmol) in DCM/TFA (20 mL, 4:1) was stirred at room temperature for 1 h. The solvent was removed under vacuum and dissolved in DCM (50 mL). Then compound S6 (420 mg, 1 mmol) and PYBOP (730 mg, 1.4 mmol) were added and stirred at room temperature for 8 h. The solvent was removed under vacuum. The product was purified by silica gel flash column chromatography (DCM/MeOH=100:1) to afford yellow solid compound S12 (300 mg, 54% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 10.20 (s, 1H), 8.12 (t, J = 5.7 Hz, 1H), 7.84 (s, 1H), 7.43 (s, 1H), 5.40 – 5.35 (m, 3H), 5.14 – 5.06 (m, 4H), 4.26 (t, J = 6.0 Hz, 2H), 4.08 – 3.96 (m, 4H), 3.75 – 3.71 (m, 1H), 3.55 (q, J = 5.9 Hz, 2H), 2.12 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 188.59, 169.97, 169.53, 168.56, 152.04, 149.43, 143.08, 125.58, 111.59, 109.67, 95.62, 77.87, 67.70, 67.09, 66.28, 56.84, 20.39. MS (ESI): m/z: Calcd. for $C_{28}H_{33}N_2O_{16}$ $[M+H]^+$: 653.2. Found:653.2

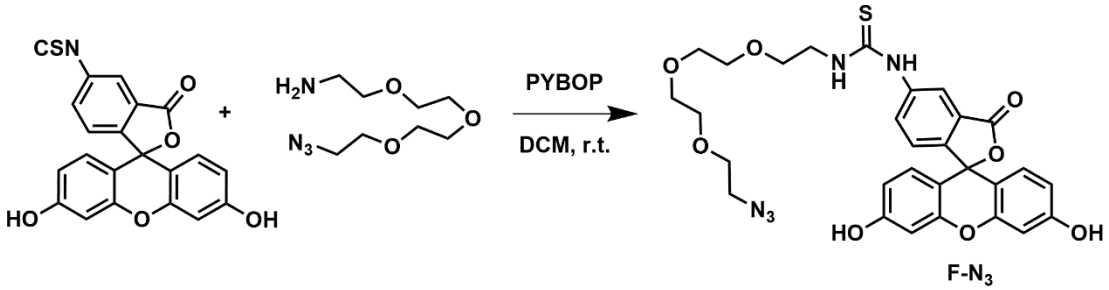
Compound S13: To a solution of compound S12 (250 mg, 0.4 mmol) in methanol (15 mL) was added $NaBH_4$ (20 mg, 0.5 mmol). The mixture was stirred at room temperature for 15 min. The volume of the reaction mixture was then reduced to 5 mL under vacuum. The reaction mixture was diluted with brine (50 mL) followed by extraction with DCM (3 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The product was purified by silica gel flash column chromatography (DCM/MeOH = 100:5) to afford yellow solid compound S13 (150 mg, 57% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 8.14 (t, J = 5.6 Hz, 1H), 7.83 (s, 1H), 7.42 (s, 1H), 5.60 (t, J = 5.4 Hz, 1H), 5.38 (d, J = 9.2 Hz, 2H), 5.13 (d, J = 3.7 Hz, 1H), 5.00 (ddd, J = 10.2, 3.6, 1.8 Hz, 1H), 4.94 (d, J = 2.4 Hz, 2H), 4.83 (d, J = 5.5 Hz, 2H), 4.37 (t, J = 6.4 Hz, 1H), 4.20 (t, J = 6.0 Hz, 2H), 4.03 (ddd, J = 19.7, 10.0, 4.6 Hz, 4H), 3.64 (t, J = 2.3 Hz, 1H), 3.57 (dt, J = 7.3, 5.0 Hz, 2H), 2.12 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 169.98, 169.54, 168.59, 153.07, 144.45, 138.32, 135.06, 111.00, 78.48, 67.09, 60.04, 20.43. MS (ESI): m/z: Calcd. for $C_{28}H_{35}N_2O_{16}$ $[M+H]^+$: 655.2. Found:655.1

Compound **NB-AP**: To a solution of compound S13 (120 mg, 0.18 mmol) in

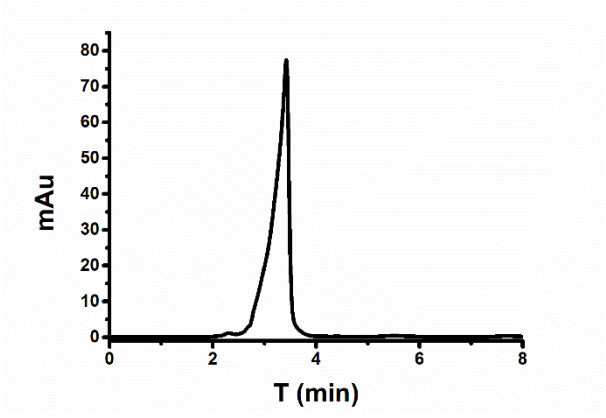
methanol (10 mL) was added sodium methoxide (20 mg), the solution was kept stirring at room temperature for 2 h. Then the reaction was quenched by ion-exchange resin Dowex 50WX8-400, filtered and concentrated to afford compound **NB-AP** (77 mg, 88% yield). HRMS (ESI): m/z: Calcd. for $C_{20}H_{26}N_2O_{12}Na$ $[M+Na]^+$: 509.1383. Found: 509.1384. The product purity was verified by analytical HPLC as shown below.



Scheme S5. The synthesis procedure for **F-N3**.

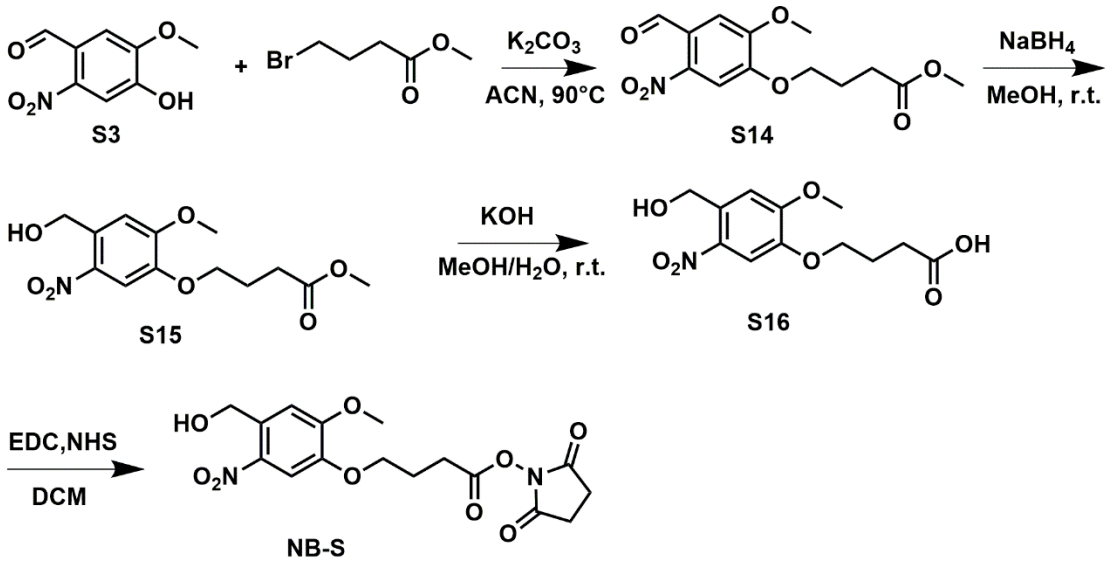


Compound **F-N3**: To a solution of fluorescein isothiocyanate (0.8 g, 2 mmol) in DCM (100 mL) was added 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-amine (0.5 g, 2.3 mmol) and PYBOP (1.5 g, 3 mmol). After stirring at room temperature for 12 h, the solvent was removed under vacuum. The product was purified by silica gel flash column chromatography (DCM/MeOH = 100:10) to afford yellow solid compound **F-N3** (300 mg, 33% yield). HRMS (ESI): m/z: Calcd. for $C_{29}H_{30}N_5O_8S$ $[M+H]^+$: 608.1815. Found: 608.1816. The product purity was verified by analytical HPLC as shown below.



3.5 Synthesis and purification of crosslinkers **NB-S**, **PA-S** and **BP-S**.

Scheme S6. The synthesis procedure for **NB-S**.



Compound S14: To a solution of compound S3 (5 g, 25 mmol) in ACN (250 mL) was added K_2CO_3 (5.6 g, 40 mmol), the mixture was stirred at room temperature for 15 min. Then methyl 4-bromobutyrate (5.4 g, 30 mmol) was added and the reaction was kept stirring at 90 ℃ for 12 h. After cooling to room temperature, K_2CO_3 was filtered and the solvent was removed under vacuum. The product was purified by silica gel flash column chromatography (DCM/PE=10:1) to afford yellow solid compound S14 (5.9 g, 80% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 10.16 (s, 1H), 7.51 (s, 1H), 7.36 (s, 1H), 4.13 (t, J = 6.2 Hz, 2H), 3.98 (s, 3H), 3.71 (s, 3H), 2.57 (t, J = 7.2 Hz, 2H), 2.27–2.11 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 188.43, 173.67, 154.89, 147.53, 139.36, 132.67, 111.50, 109.40, 68.26, 56.43, 51.79, 30.54, 24.67. MS (ESI): m/z: Calcd. for $C_{13}H_{16}NO_7$ $[M+H]^+$: 298.1. Found: 298.1

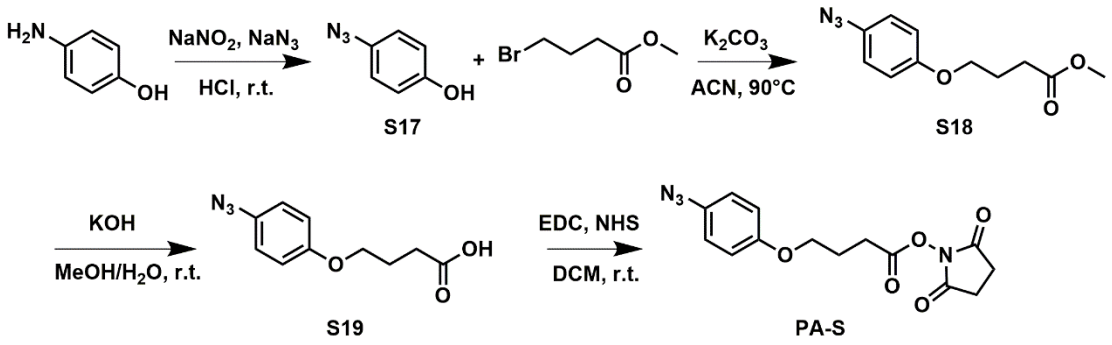
Compound S15: To a solution of compound S14 (4.5 g, 15 mmol) in methanol (100 mL) was added $NaBH_4$ (1.1 g, 30 mmol). The mixture was stirred at room temperature for 15 min. The volume of the reaction mixture was then reduced to 15 mL under vacuum. The reaction mixture was diluted with brine (100 mL) followed by extraction with DCM (3 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The product was purified by silica gel flash column chromatography (DCM/MeOH = 100:1) to afford yellow solid compound S15 (4.1 g, 95% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.70 (s, 1H), 7.18 (s, 1H), 4.96 (s, 2H), 4.13 (t, J = 6.2 Hz, 2H), 3.98 (s, 3H), 3.71 (s, 3H), 2.57 (t, J = 7.2 Hz, 2H), 2.27–2.11 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 173.43, 154.29, 147.13, 139.56, 132.49, 111.10, 109.48, 68.26, 62.77, 56.43, 51.76, 30.38, 24.25. MS (ESI): m/z: Calcd. for $C_{13}H_{17}NO_7Na$ $[M+Na]^+$: 322.1. Found: 322.1.

Compound S16: To a solution of compound S15 (2 g, 6.7 mmol) in MeOH/H_2O (200 mL, 9:1) was added potassium hydroxide (1 g, 18 mmol), and the solution was stirred at room temperature for 2 h. Then the pH of the solution was adjusted to 3 by addition of 6 N HCl aqueous solution. The whole mixture was extracted with DCM and

the combined organic layers were dried over Na_2SO_4 and concentrated to afford yellow solid compound S16 (1.8 g, 94% yield) which was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 12.20 (s, 1H), 7.67 (s, 1H), 7.39 (s, 1H), 4.83 (s, 2H), 4.07 (t, J = 6.5 Hz, 2H), 3.93 (s, 3H), 2.41 (t, J = 7.3 Hz, 2H), 1.97 (p, J = 6.9 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 174.00, 153.68, 145.98, 138.25, 134.23, 109.61, 108.82, 67.83, 60.08, 56.03, 29.91, 24.01. MS (ESI): m/z: Calcd. for $C_{12}H_{15}NO_7K$ $[M+K]^+$: 324.0. Found: 324.1.

Compound **NB-S**: To a solution of compound S16 (1 g, 3.5 mmol) in DCM/MeOH (200 mL, 4:1) was added N-hydroxysuccinimide (0.8 g, 7 mmol) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (1.3 g, 7 mmol). The mixture was stirred at room temperature for 12 h and reduced to 50 mL under vacuum. The reaction mixture was diluted with brine (250 mL) followed by extraction with DCM (3 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (DCM/MeOH = 1000:1) to afford yellow powder compound **NB-S** (0.7 g, 52% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 7.69 (s, 1H), 7.41 (s, 1H), 5.59 (t, J = 5.4 Hz, 1H), 4.83 (d, J = 5.4 Hz, 2H), 4.14 (t, J = 6.3 Hz, 2H), 3.92 (s, 3H), 2.87 (t, J = 7.4 Hz, 2H), 2.82 (s, 4H), 2.10 (p, J = 6.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 170.21, 168.64, 153.77, 145.82, 138.29, 134.50, 109.77, 109.15, 67.17, 60.08, 56.10, 26.89, 25.42, 23.92. HRMS (ESI): m/z: Calcd. for $C_{16}H_{18}N_2O_9Na$ $[M+Na]^+$: 405.0910. Found: 405.0909.

Scheme S7. The synthesis procedure for **PA-S**.



Compound S17: To a solution of 4-aminophenol (5 g, 46 mmol) in 1N HCl aqueous solution (150 mL) was added $NaNO_2$ (3.4 g, 50 mmol), and the solution was stirred at 0 ℃ for 30 min. Then NaN_3 was added and stirred at room temperature for 3 h. The whole mixture was extracted with ethyl acetate and the combined organic layers were dried over Na_2SO_4 and concentrated to afford brown liquid compound S17 (5 g, 80% yield) which was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 9.52 (s, 1H), 6.99 – 6.88 (m, 2H), 6.85 – 6.72 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 155.92, 131.42, 120.14, 115.85. MS (ESI): m/z: Calcd. for $C_6H_6N_3O$ $[M+H]^+$: 136.1. Found: 136.1

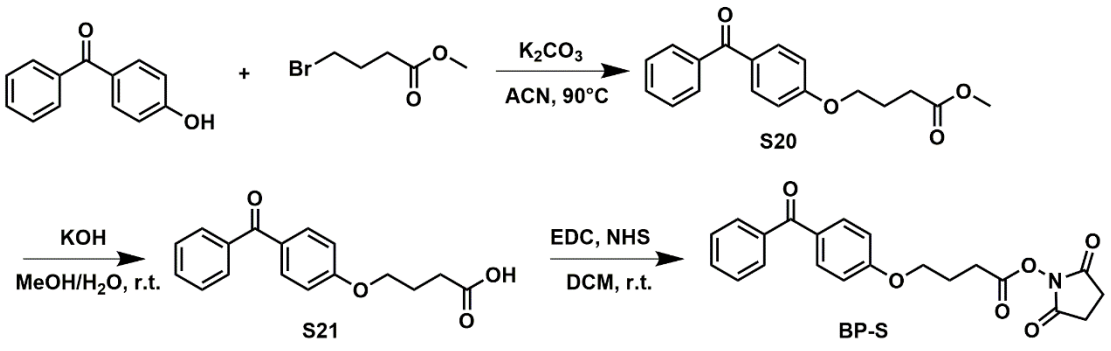
Compound S18: To a solution of compound S17 (2 g, 15 mmol) in ACN (200 mL) was added K_2CO_3 (2.8 g, 20 mmol), and the reaction was stirred at room temperature

for 15 min. Then methyl 4-bromobutyrate (3.6 g, 20 mmol) was added and stirred at 90 ℃ for 12 h. After cooling to room temperature, K_2CO_3 was filtered and the solvent was removed under vacuum. The product was purified by silica gel flash column chromatography (DCM/PE=3:1) to afford brown oil compound S18 (2.4 g, 68% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 7.08 – 7.01 (m, 2H), 7.00 – 6.93 (m, 2H), 3.97 (t, J = 6.3 Hz, 2H), 3.60 (s, 3H), 2.47 (t, J = 7.3 Hz, 2H), 2.03 – 1.88 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 172.98, 155.92, 131.42, 120.14, 115.85, 66.80, 51.31, 29.85, 24.12. MS (ESI): m/z: Calcd. for $C_{11}H_{14}N_3O_3$ $[M+H]^+$: 236.1. Found: 236.1

Compound S19: To a solution of compound S18 (1 g, 4 mmol) in MeOH/H_2O (100 mL, 9:1) was added potassium hydroxide (1 g, 18 mmol), and the solution was stirred at room temperature for 2 h. Then the pH of the solution was adjusted to 3 by addition of 6 N HCl aqueous solution. The whole mixture was extracted with DCM and the combined organic layers were dried over Na_2SO_4 and concentrated to afford yellow powder compound S19 (0.6 g, 68% yield) which was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 12.16 (s, 1H), 7.07 – 7.01 (m, 2H), 7.00 – 6.94 (m, 2H), 3.96 (t, J = 6.4 Hz, 2H), 2.38 (t, J = 7.3 Hz, 2H), 2.03 – 1.77 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 174.05, 155.99, 131.38, 120.13, 115.86, 66.91, 30.02, 24.15. MS (ESI): m/z: Calcd. for $C_{10}H_{12}N_3O_3$ $[M+H]^+$: 222.1. Found: 222.1

Compound **PA-S**: To a solution of compound S19 (1 g, 4.5 mmol) in DCM/MeOH (200 mL, 4:1) was added N-hydroxysuccinimide (0.8 g, 7 mmol) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (1.3 g, 7 mmol). The mixture was stirred at room temperature for 12 h and reduced to 50 mL under vacuum. The reaction mixture was diluted with brine (100 mL) followed by extraction with DCM (3 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (DCM/MeOH = 1000:1) to afford yellow powder compound **PA-S** (1 g, 70% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 7.07 – 7.03 (m, 2H), 7.02 – 6.97 (m, 2H), 4.04 (t, J = 6.3 Hz, 2H), 2.88 – 2.79 (m, 6H), 2.07 (p, J = 6.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 170.21, 168.73, 155.82, 131.56, 120.14, 115.91, 66.17, 27.02, 25.42, 23.96. HRMS (ESI): m/z: Calcd. for $C_{14}H_{14}N_4O_5Na$ $[M+Na]^+$: 341.0862. Found: 341.0861.

Scheme S8. The synthesis procedure for **BP-S**.



Compound S20: To a solution of 4-hydroxybenzophenone (5 g, 25 mmol) in ACN (250 mL) was added K_2CO_3 (5.6 g, 40 mmol). The solution was stirred at room temperature for 15 min. Then methyl 4-bromobutyrate (7.2 g, 40 mmol) was added and stirred at 90 ℃ for 12 h. Then the mixture was cooled to room temperature and K_2CO_3 was filtered. The solvent was removed under vacuum. The product was purified by silica gel flash column chromatography (DCM/PE=5:1) to afford colorless oil compound S20 (5.2 g, 70% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.85 – 7.79 (m, 2H), 7.77 – 7.72 (m, 2H), 7.60 – 7.53 (m, 1H), 7.51 – 7.43 (m, 2H), 6.98 – 6.91 (m, 2H), 4.10 (t, J = 6.1 Hz, 2H), 3.70 (s, 3H), 2.56 (t, J = 7.2 Hz, 2H), 2.20 – 2.11 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 195.57, 173.52, 162.47, 138.27, 132.58, 131.91, 130.18, 129.73, 128.20, 114.00, 66.94, 51.73, 30.40, 24.46. MS (ESI): m/z: Calcd. for $C_{18}H_{19}O_4$ $[M+H]^+$: 299.1. Found: 299.2

Compound S21: To a solution of compound S20 (1 g, 3.3 mmol) in MeOH/H_2O (100 mL, 9:1) was added potassium hydroxide (1 g, 18 mmol), and the solution was stirred at room temperature for 2 h. Then the pH of the solution was adjusted to 3 by addition of 6 N HCl aqueous solution. The whole mixture was extracted with DCM and the combined organic layers were dried over Na_2SO_4 and concentrated to afford colorless oil compound S21 (0.7 g, 75% yield) which was used without further purification.

Compound **BP-S**: To a solution of compound S21 (1.3 g, 4.5 mmol) in DCM/MeOH (200 mL, 4:1) was added N-hydroxysuccinimide (0.8 g, 7 mmol) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (1.3 g, 7 mmol). The mixture was stirred at room temperature for 12 h and reduced to 50 mL under vacuum. The reaction mixture was diluted with brine (100 mL) followed by extraction with DCM (3 × 50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (DCM/MeOH = 1000:1) to afford whiter powder compound **BP-S** (1 g, 60% yield). ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm): 7.79 – 7.72 (m, 2H), 7.72 – 7.67 (m, 2H), 7.67 – 7.62 (m, 1H), 7.55 (t, J = 7.5 Hz, 2H), 7.15 – 7.05 (m, 2H), 4.18 (t, J = 6.3 Hz, 2H), 2.89 (t, J = 7.3 Hz, 2H), 2.83 (s, 4H), 2.14 (p, J = 6.8 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6), δ (ppm): 194.90, 170.70, 169.19, 162.55, 138.24, 132.63, 132.55, 129.96, 129.72, 128.90, 114.83, 66.79, 27.53, 25.93, 24.39. HRMS (ESI): m/z: Calcd. for $C_{21}H_{20}NO_6$ $[M+H]^+$: 382.1291. Found: 382.1293.

4. Photochemical characterizations.

4.1 UV-vis spectra for the photolysis of **NB-1**.

NB-1 solution (1×10^{-4} M in ACN) was irradiated by a LED 365 light at 10 mW cm^{-2} in a cuvette, and at specific time intervals, the solution was analyzed by UV–vis absorption spectra.

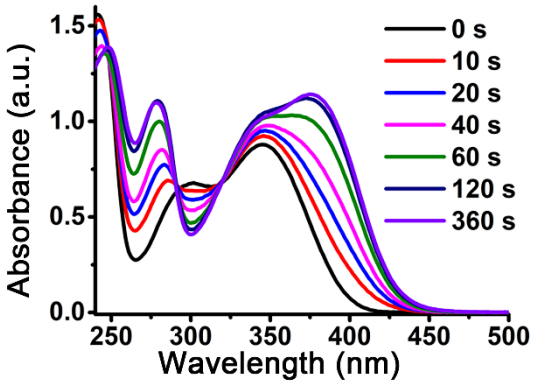


Figure S1. UV-vis spectra of **NB-1** (1×10^{-4} M in ACN).

4.2 NMR spectra for the photo-induced imine-ligation of **NB-1**.

a) **NB-1** (2.9 mg, 1×10^{-2} M) and benzylamine (1.1 mg, 1×10^{-2} M) were dissolved in CD_3CN (1 mL), then, the mixture was placed in a 5 mm NMR sample tube to monitor the process of photo-induced imine ligation by a LED 365 light at 10 mW cm^{-2}.

b) The stability of **NB-BA**: **NB-BA** was dissolved in CD_3CN/D_2O (9:1) to monitor the stability, the concentration was 1 mM.

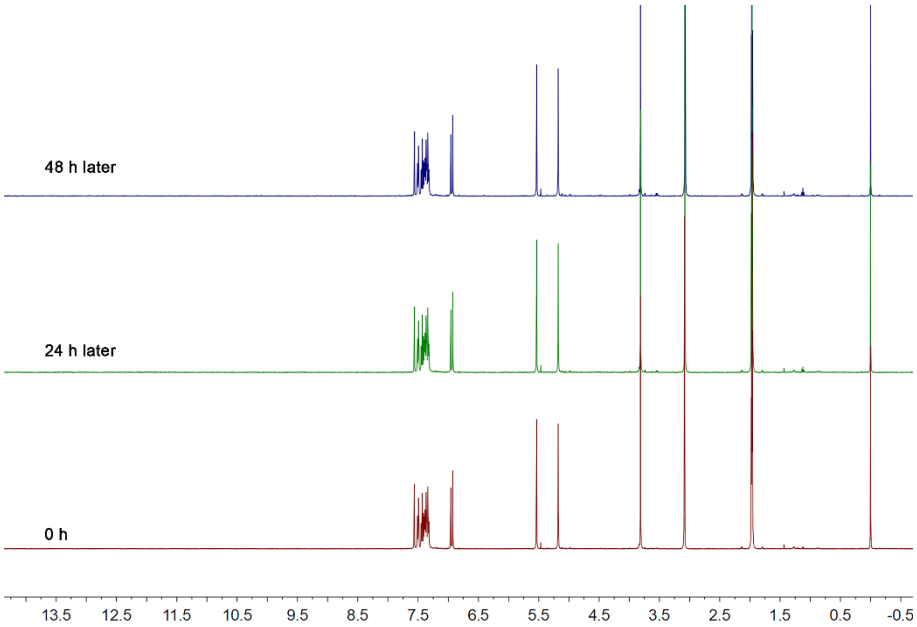


Figure S2. Stability of **NB-BA** (1 mM) in CD_3CN/D_2O (9:1). After 48 h storage, the integral value of characteristic H of **NB-BA** almost unchanged.

4.3 HPLC and LC-MS detection for the photolysis and imine ligation.

HPLC profiles were performed on a reversed-phase HPLC using a BetaBasic-18 column. For the photolysis, the concentration of **NB-1** was 1×10^{-3} M in ACN, and a mixture of 60% ACN and 40% water was used as the eluent at a flow rate of 1 mL min^{-1}. The detection wavelength was 320 nm. The photolysis products were further confirmed by LC-MS. The photochemical quantum yield Φ_{chem} for the intermediate

NB-CHO, defined as the ratio of the yield of photochemical products to the total number of quanta absorbed, were calculated at 0.184.[1]

For the imine ligation, a solution of **NB-1** (1×10^{-2} M) and benzylamine (1×10^{-2} M) in ACN were intermittently irradiated by a LED 365 at 10 mW cm^{-2}. After different intervals, aliquots of the reaction solution were taken out and diluted to 1 mM for HPLC analysis. The test conditions were the same as mentioned above. The conversion yield was determined by external standard method, since pure NB-BA can be purified from the reaction system.

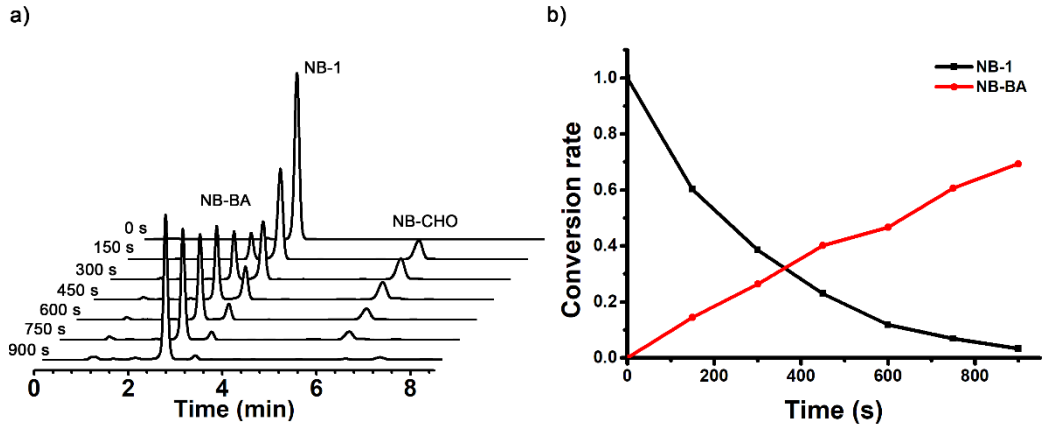


Figure S3. The kinetics of photo-induced imine-ligation. a) time-resolved HPLC analysis of photo reaction. b) **NB-BA** yields determined from a) by external standard method. The concentrations for **NB-BA** and benzyl amine were 10 mM in ACN.

4.4 Amine selectivity of NB.

A solution of **NB-1** (1×10^{-2} M), benzylamine (1×10^{-2} M), benzyl alcohol (1×10^{-2} M) and methylphenylacetic acid (1×10^{-2} M) in ACN were intermittently irradiated by a LED 365 light at 10 mW cm^{-2}. After different intervals, aliquots of the reaction solution were taken out and diluted to 1 mM for HPLC analysis. The test conditions were the same as mentioned above.

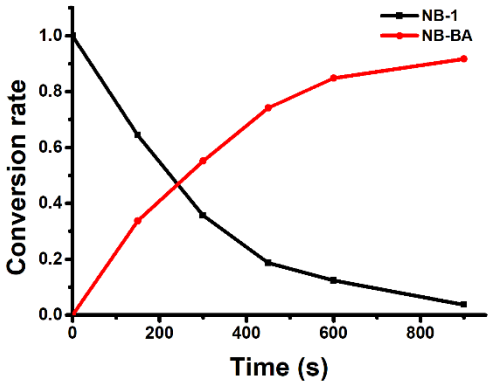


Figure S4. Time-resolved HPLC analysis of photo-conjugation between **NB-1** and benzyl amine in the existence of equimolar benzyl alcohol and methylphenylacetic acid (the selected data was corresponding to Fig. 1d).

To further confirm the selectivity of NB, NMR spectra were carried out. As illustrated in Figure S5-7, the integral values for benzyl alcohol and methylphenylacetic acid almost didn't changed during the 10 min photoreaction. And only benzyl amine was consumed.

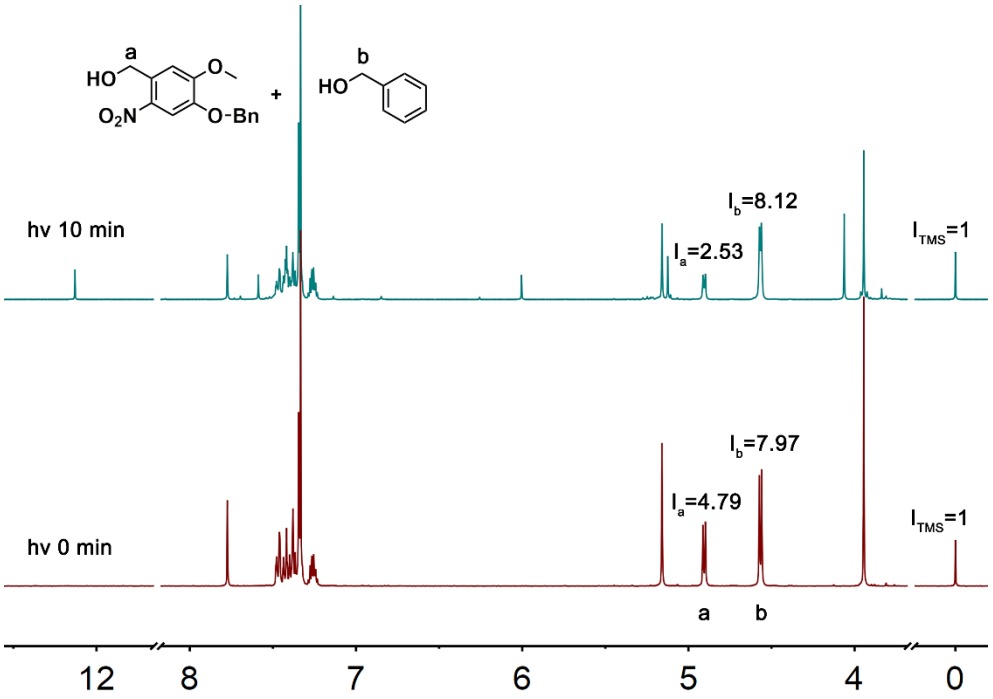


Figure S5. NMR spectra evolution of **NB-1** and benzyl alcohol. After 10 min irradiation, peak a, assigned to H_a of **NB-1**, decreased for photolysis. Peak b, assigned to H_b of benzyl alcohol, changed little, which means that **NB-1** cannot react with benzyl alcohol.

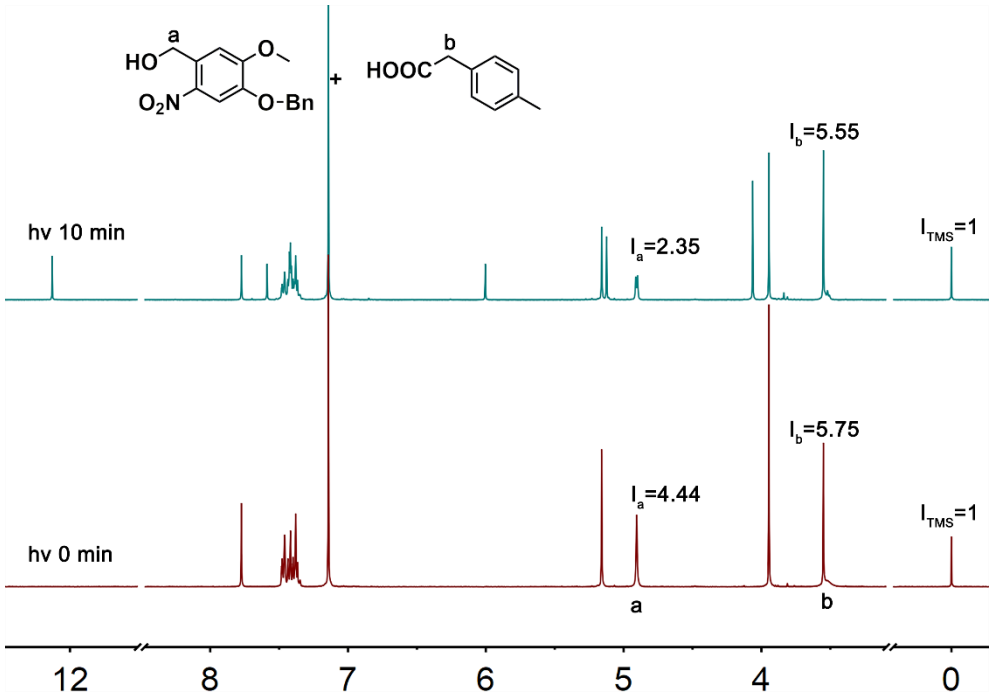


Figure S6. NMR spectra evolution of **NB-1** and methylphenylacetic acid. After 10 min irradiation, peak a, assigned to H_a of NB-1, decreased for photolysis. Peak b, assigned

to H_b of methylphenylacetic acid, changed little, which means that **NB-1** cannot react with methylphenylacetic acid.

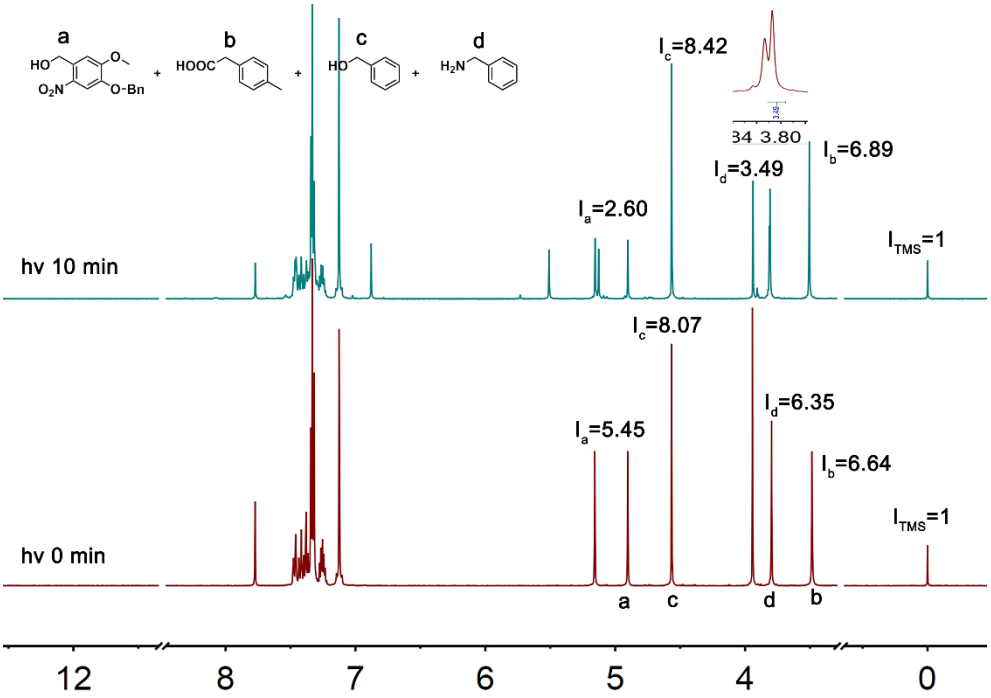


Figure S7. NMR evolution spectra of **NB-1** in the presence of benzyl amine, benzyl alcohol and methylphenylacetic acid. After 10 min irradiation, peak a, assigned to H_a of **NB-1**, decreased for photolysis. Peak b and c changed little, while peak d, assigned to H_d of benzyl amine, changed obviously for reacting with **NB-1**.

5. NB photoreactivity to protein amine.

To a solution of 1 mL BSA (1 mg mL^{-1} in DPBS, 15.2 μM) was added 10 μL **NB-PEG** (28 mg mL^{-1} in ACN, 75 mM), and the mixture was irradiated by a LED 365 light at 10 mW cm^{-2} for 1 min. After incubating at 37 ℃ for 2 h, the sample was analyzed by MALDI-TOF and LC-MS/MS.

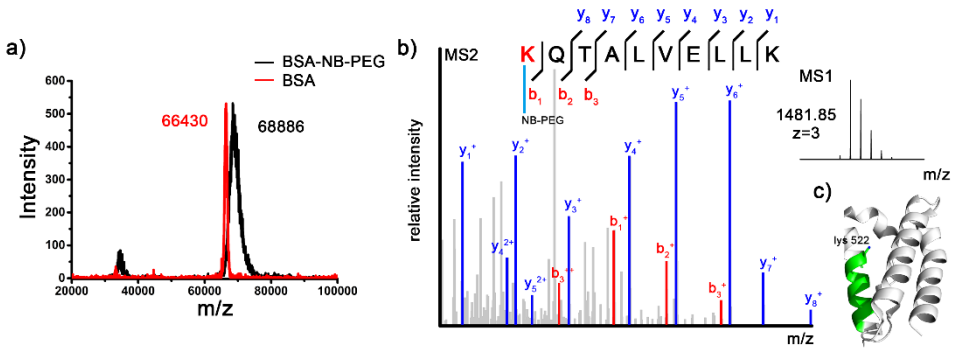


Figure S8. a) MOLDI-TOF analysis of BSA (1 mg mL^{-1}) labeled with **NB-PEG** (75 mM). b) LC-MS/MS spectrum for **NB-PEG** modified peptide fragment, KQTALVELLK, with the fragment ions annotated on the structure. c) Site of **NB-PEG** bound to BSA (PDB code: 3V03).

6. Photoaffinity labeling of NB-AP to ConA

6.1 In-gel fluorescence analysis of ConA.[2]

a) Affinity labeling of ConA in the presence of SA: To a solution (1 mL) of ConA (0.5 mg mL^{-1} in DPBS, 5 μM) and SA (0.5 mg mL^{-1} in DPBS, 8.3 μM) were added 10 μL of **NB-AP** (in ACN, final concentration 10, 50, 100, 200, 500 μM). The mixture was incubated at 4 ℃ in a shaker for 1 h. Then the protein mixture was irradiated by a LED 365 light at 10 mW cm^{-2} for 1 min. Then, 6 μL **F-N_3** (10 mM in DMSO), 2 μL $CuSO_4$ (10 mM in water) and 2 μL NaVC (10 mM in water) was added for click reaction. The obtained mixture was further incubated at 37 ℃ for 10 min subsequently.

b) Affinity labeling of ConA in the presence of cell lysate: HeLa cells were selected and cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), penicillin (100 units mL^{-1}), and streptomycin (100 μg mL^{-1}) at 37 ℃ in a 5 % CO_2 incubator for 3 days. After washing with PBS three times, Cell lysis buffer from Western and IP™ (purchased from Beyotime, P0013) was added according to manufacturer's instructions. The residue was collected and centrifuged for 3 min at 1000 rpm. The supernatant was used as HeLa cell lysate directly and the concentration was determined at 5 mg $mL^{-1}$by Bradford protein assay kit (Bio-Rad).

A click solution was prepared by mixing 100 μL **NB-AP** (10 mM in ACN), 60 μL **F-N_3** (20 mM in ACN), 20 μL $CuSO_4$ (10 mM in water) and 20 μL NaVC (10 mM in water). Then, to a 1 mL solution of HeLa cell lysate was added ConA (1 mg mL^{-1}, 10 μM) and the prepared click solution (20 μL). The resulting mixture was incubated at 37 ℃ for 1 h. Finally, the resulting solution was irradiated by a LED 365 light at 10 mW cm^{-2} for 1 min and further incubated at 37 ℃ for 10 min subsequently.

c) Ligand competitive experiment: To a 1 mL solution of ConA (1 mg mL^{-1} in DPBS, 10 μM) and mannose (Ma) (final concentrations: 0, 0.1, 1, 10, 100 mM for each sample) were added the click solution (20 μL, final concentration of **NB-AP** was 100 μM) and irradiated for 1 min by a LED 365 light at 10 mW cm^{-2}. The obtained protein mixture was further incubated at 37 ℃ for 10 min subsequently.

These protein samples were further analyzed by gel electrophoresis using polyacrylamide gels and imaged with IVIS Lumina XR III small animal imaging system using corresponding filters. Protein loading was assessed by staining with Coomassie Brilliant Blue according to manufacturer's instructions.

6.2 LC-MS/MS analysis of **NB-AP** labeled ConA.

To a solution of 1 mL ConA (1 mg mL^{-1} in DPBS, 10 μM) was added 10 μL **NB-AP** in ACN (final concentration 50 μM), and the mixture was irradiated by a LED 365 light at 10 mW cm^{-2} for 1 min. After incubating at 37 ℃ for 2 h, the sample was digested with trypsin and analyzed by LC-MS/MS.

7. Crosslinking of CMCH in situ.

General procedures: For **NB-S** crosslinking, to a solution of 1 mL CMCH (50 mg

mL^{-1} in dd H_2O) was added 50 µL **NB-S** (51.7 mg mL^{-1} in DMSO, final concentration 6.8 mM), and the obtained mixture was incubated at 37 ℃ in shaker for 1 h. Then the mixture was added into a 5 mL sample vial and irradiated by a LED 365 light at 10 mW cm^{-2} to make a hydrogel.

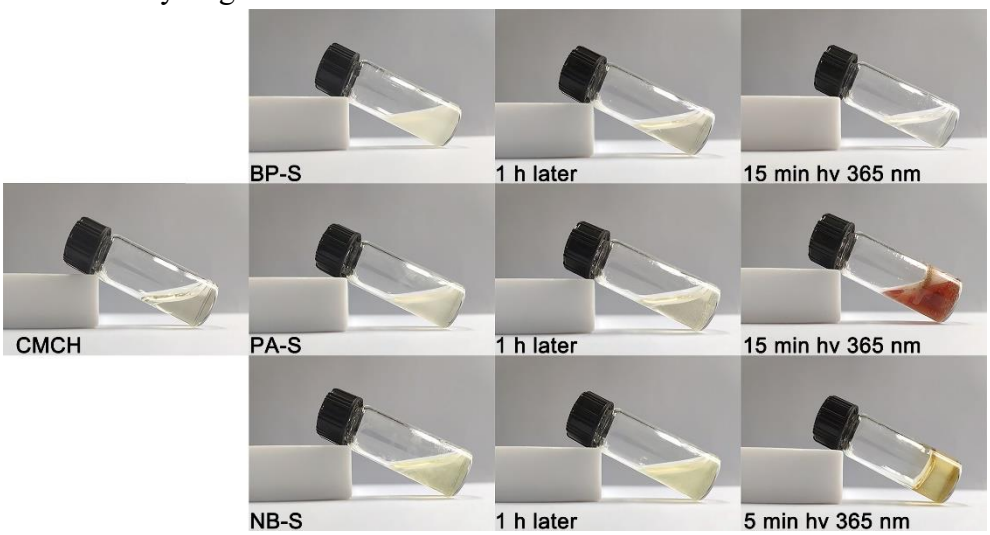


Figure S9. Preparation CMCH hydrogels with NB-S, PA-S and BP-S.

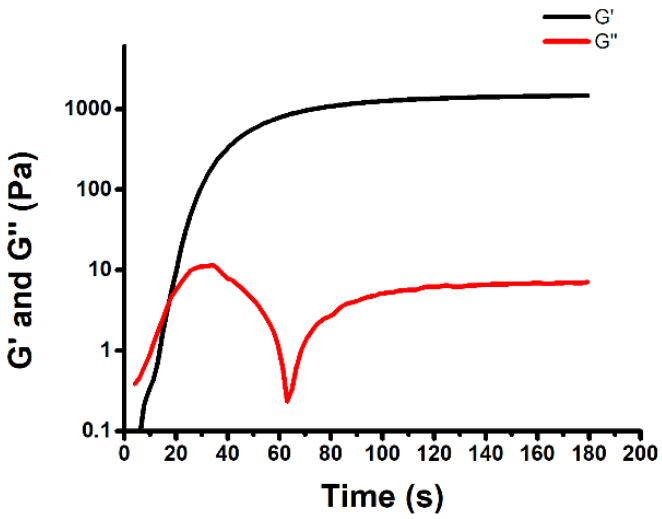


Figure S10. Rheology data of CMCH+**NB-S**. The storage modulus G' is 1400 Pa for **NB-S** crosslinked hydrogel and the gel point is 16 s.

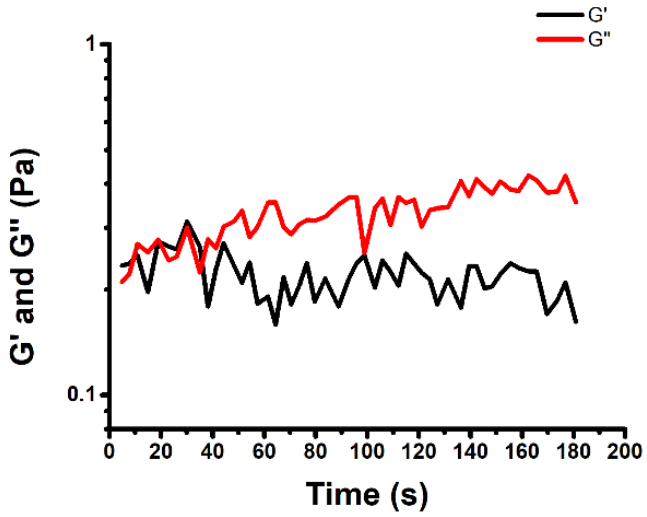


Figure S11. Rheology data of CMCH+**PA-S**.

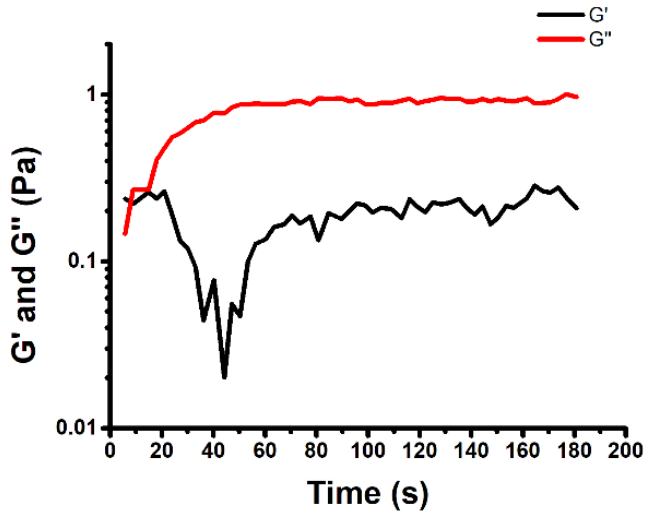


Figure S12. Rheology data of CMCH+**BP-S**.

8. Protein crosslinking.

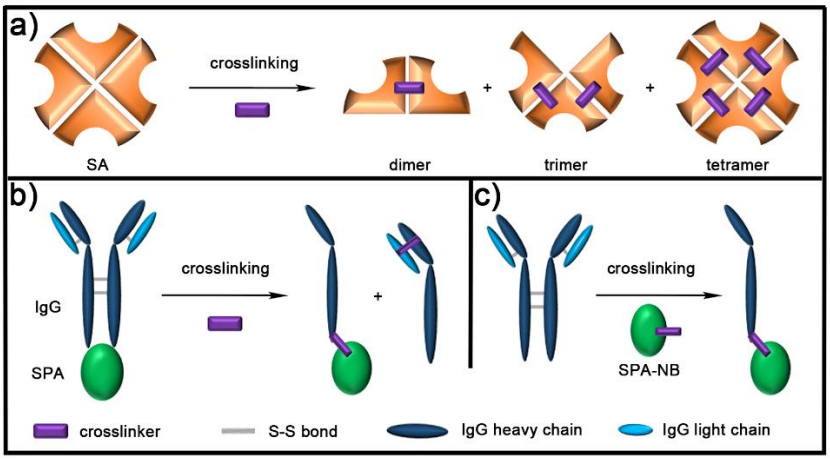


Figure S13. Schematic presentation of possible protein crosslinking. a) Photocrosslinking of SA. As a tetra-subunits protein, SA can be theoretically crosslinked to form dimer, trimer and tetramer. b) Photocrosslinking of IgG and SPA. The crosslinking between IgG and SPA is complicated, and the diagram only shows two kinds of simple crosslinking. c) Photocrosslinking of IgG and **SPA-NB**. Theoretically, **SPA-NB** would selectively crosslinked to the heavy chain of IgG induced by the interaction between IgG and SPA, which would reduce the complexity of crosslinking.

8.1 Crosslinking of SA.

General process: SA was dissolved in DPBS (1 mg mL^{-1}), and **NB-S** were dissolved in ACN on demand. To achieve chemically crosslinking of SA, the crosslinker solution (1 μL) were mixed with SA solution (10 μL) in dark at 37 ℃ for 2 h to complete the NHS-ester reaction, and then irradiated by a LED 365 light source with intensity of 10 mW cm^{-2}. The obtained protein mixtures were incubated at 37 ℃ for 30 min and analyzed by SDS-PAGE subsequently (10 μg protein was uploaded).

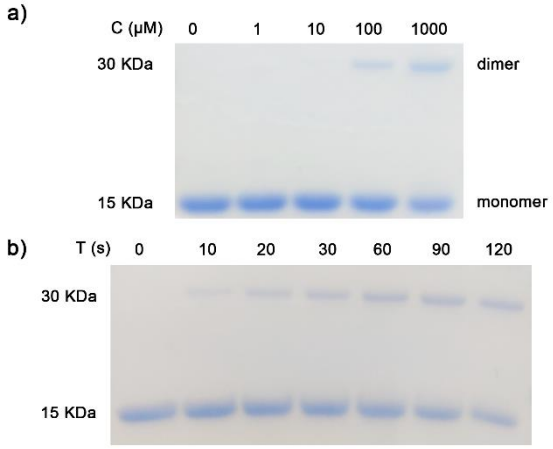


Figure S14. Exploration of photo-crosslinking for SA by **NB-S**. a) Concentration-dependent SA photocrosslinking by **NB-S**, the irradiation time was 60 s. b) Time-dependent SA photocrosslinking by **NB-S**, the concentration of **NB-S** was 1 mM. The protein concentration was 1 mg mL^{-1}.

8.2 Crosslinking comparison between **NB-S**, **PA-S** and **BP-S**.

10 μL SA (1 mg mL^{-1} in DPBS) and 1 μL crosslinkers (10 mM in ACN) were mixed and incubated in dark at 37 ℃ for 2 h to complete the NHS-ester reaction, and then irradiated for 15 min by a LED 365 light at 10 mW cm^{-2}. The obtained protein mixtures were incubated at 37 ℃ for 30 min and analyzed by SDS-PAGE subsequently (10 μg protein was uploaded).

8.3 Crosslinking of SPA and IgG.

a) One-step crosslinking: SPA and IgG protein solutions (1 mg mL^{-1} for each protein in DPBS, 100 μL) were typically incubated with **NB-S** (10 μL,10 mM in ACN,)

in dark at 37 ℃ for 2 h to allow NHS-ester reaction, then the samples were irradiated for 1 min by a LED 365 light at 10 mW cm^{-2}. The obtained protein mixtures were incubated at 37 ℃ for 30 min and analyzed by SDS-PAGE subsequently (35 μg protein was loaded).

b) Two-step crosslinking:

Preparation of **SPA-NB**: SPA (1 mg in 1 mL DPBS) was added 100 μL **NB-S** solution (3.43 mg mL^{-1} in DMSO, 30 eq), and the mixture was incubated at 37 ℃ in shaker for 2 h. Then the mixture was transferred to a dialysis bag (1 kDa regenerated cellulose dialysis membrane) and dialyzed against DPBS for 24 h (2 washes). The content of the dialysis bag was lyophilized and the labeled protein **SPA-NB** was obtained as confirmed by MALDI-TOF.

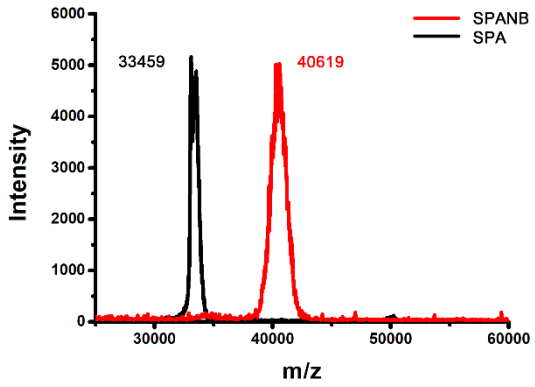


Figure S15. MALDI-TOF of SPA (33.4 K) and **SPA-NB** (40.7 K).

Then, a solution containing of **SPA-NB** and IgG (1 mg mL^{-1} for each protein in DPBS, 30 μL) were irradiated for 1 min by a LED 365 light at 10 mW cm^{-2}. The obtained protein mixture was incubated at 37 ℃ for 30 min and analyzed by SDS-PAGE subsequently (35 μg protein was loaded).

9. Cytotoxicity experiment.

CCK-8 assay was used to evaluate in vitro cytotoxicity of HeLa cells. For the analysis, HeLa cells were seeded in a 96-well plate at an initial density of 5000 cells per well in 200 μL of DMEM medium. After incubating for 24 h, DMEM was replaced with fresh medium containing 1 μL DMSO solution of **NB-S** (final concentration 100 μM). For irradiation operation, the cells were irradiated for 2 min by a LED 365 light at 10 mW cm^{-2} after 1h coculture. After coculture for 6 h, 12 h, 24 h and 48 h respectively, the medium was removed and 100 μL fresh medium containing CCK-8 reagent (10 μL) was added to each well. After incubation for 2 h, the absorption at 450 nm was recorded by a microplate reader, and the data was averaged from at least six trials. For comparison, the cytotoxicity of DMSO and irradiation was also explored.

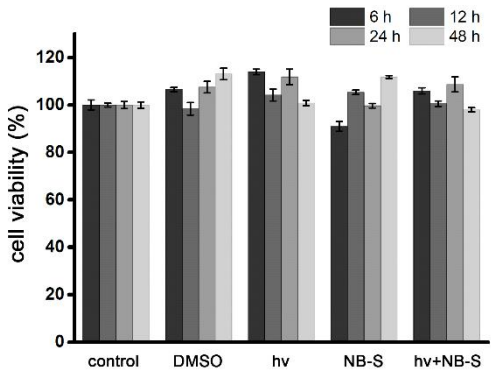


Figure S16. The cytotoxicity analysis of HeLa cells determined by CCK-8 assay. "**NB-S**" means the cells were co-incubated with **NB-S** without irradiation; "hv+**NB-S**" means the cells were co-incubated with **NB-S** and irradiated.

10. Notes and references

1. Q. Lin, L. Yang, Z. Wang, Y. Hua, D. Zhang, B. Bao, C. Bao, X. Gong and L. Zhu, *Angew. Chem. Int. Ed.* 2018, **57**, 3722-3726.
2. P. Kleiner, W. Heydenreuter, M. Stahl, V. S. Korotkov and S. A. Sieber, *Angew. Chem. Int. Ed.* 2017, **56**, 1396-1401.