Supporting Information

Clickable gold-nanoparticles as generic probe precursors for facile application of photoaffinity labeling

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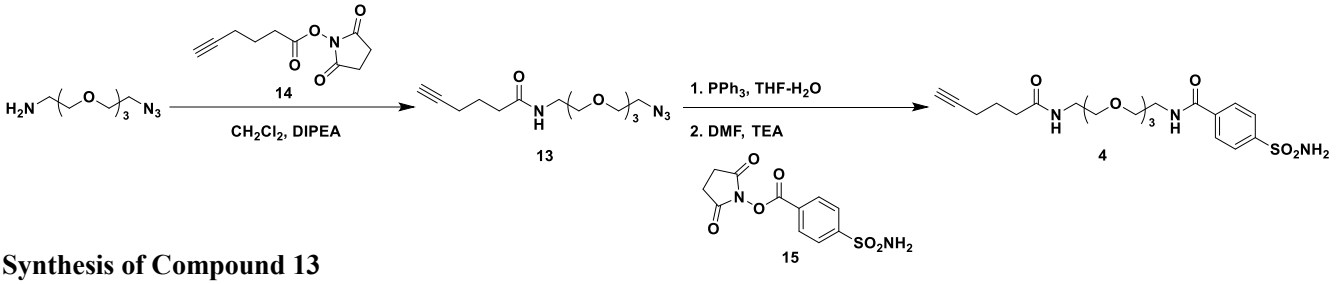
1. General Experimental Methods

NMR experiments were performed at 293 K on JEOL ECX 300, 400 or ECA 500 using 5 mm z-gradient probes and data were processed with Delta software. The spectra are referenced internally according to residual solvent signals of $CDCl_3$ (^{1}H NMR; δ = 7.26 ppm, ^{13}C NMR; δ = 77.0 ppm) and DMSO-d_6 (^{1}H NMR; δ = 2.50 ppm, ^{13}C NMR; δ = 39.5 ppm). Positive ion ESI-TOF-MS data were obtained by JEOL AccuTOF mass spectrometer. MALDI-TOF-MS data were obtained by autoflex speed TOF/TOF (Bruker Daltonics). Unless noted otherwise, all chemical reagents were purchased from Wako Chemicals, TCI and Sigma-Aldrich. Preparative thin-layer chromatography (TLC) was performed using PLC Silica gel 60 F254 pre-coated plates (Merck Millipore). Flash column chromatography was performed using Silica gel 60 (spherical, particle size 40-100 μm; Kanto Chemical). UV-visible spectra were recorded on a NanoDrop ND-1000 spectrophotometer and VARIAN CARY 50 conc UV-visible spectrophotometer. Fluorescence imaging was scanned with Typhoon 8600 (GE Healthcare Science). CBB-stained and silver stained images were scanned with CanoScan LiDE210 (Cannon).

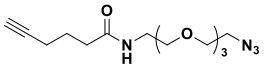
2. Synthesis of azide-PEG3 lipoate 6, diazirin-PEG3 lipoate 7, PEG2 lipoate 8, alkyne-tagged BzSA 4.

Compound **6-8** were synthesized according to the published procedures.[1] The synthesis and structural characterization of compound **5** was reported previously.[2]

2-1. Synthesis of alkyne-tagged BzSA 4



Synthesis of Compound 13



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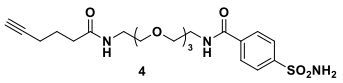
A solution of 5-hexynoic acid (100.8 mg, 0.90 mmol) in dry CH_2Cl_2 (2.7 ml) was added *N*-hydroxysuccinimide (NHS) (108.8 mg, 0.945 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC-HCl, 181.2

mg, 0.945 mmol), and then was stirred at room temperature for 1 h. The mixture was diluted with CH_2Cl_2 and washed with 10% aqueous NH_4Cl three times, then with brine. The organic layer was dried over Na_2SO_4, which was filtered and concentrated *in vacuo* to give the crude product **14** (167.3 mg) as yellow oil.

A solution of 11-azide-3,6,9-trioxaundecanamine (87.3 mg, 0.40 mmol) in dry CH_2Cl_2 (1.2 ml) was added crude compound **14** (167.3 mg) and DIPEA (279 µl, 1.6 mmol), and was stirred at room temperature for 3 h. The mixture was concentrated *in vacuo*. The resultant residue was purified by preparative thin layer chromatography (EtOAc/MeOH = 9/1) to give compound **13** (16.2 mg, 0.05 mmol, 13%) as yellow liquid:^{1}H NMR (300 MHz, $CDCl_3$): δ 6.32 (1H, s), 3.64-3.52 (12H, m), 3.42-3.37 (2H, m), 2.65 (2H, m), 2.32-2.27 (2H, m), 2.26-2.18 (2H, m) 1.97-1.95 (1H, m), 1.84-1.77 (2H, m); ^{13}C NMR (300 MHz, $CDCl_3$): δ 172.5, 83.3, 76.7, 70.2 (×4), 69.6, 69.3, 50.3, 38.9, 34.6, 25.1, 17.5; HRESI-MS calculated for $C_{14}H_{24}N_4NaO_4$ ($M+Na^+$): 335.170; found: 335.170.

Synthesis of alkyne-tagged benzensulfonamide 4



A solution of 4-sulfamoyl benzoic acid (250.0 mg, 1.24 mmol), DIPEA (648 µl, 3.7 mmol) and EDC-HCl (397 µl, 1.9 mmol) in dry DMF (9.5 mL) was stirred for 5 min at room temperature, and was added *N*-hydroxysuccinimide (185.3 mg, 1.6 mmol) to stir at room temperature for 3 h. The mixture was diluted with EtOAc and was washed with 1 M aqueous HCl three times, saturated aqueous $NaHCO_3$ solution, then brine. The organic layer was dried over Na_2SO_4, and filtered and concentrated *in vacuo*. The residue was co-evaporated with toluene three times and purified by flash column chromatography (EtOAc/hexane = 3/7 to 1/0) to give crude product of compound **15** (83.7 mg) and used in the next step.

To a solution of compound **13** (15.8 mg, 0.05 mmol) in THF (250 µl) was added MilliQ water (25 µl) and PPh_3 (13.4 mg, 0.05 mmol), which was stirred at room temperature for 18 h. The mixture was diluted with 1 M aqueous HCl and was washed with EtOAc three times. The aqueous layer was extracted with CH_2Cl_2 ten times and the organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*, which was used in the next step without further purification.

Crude compound **15** (12.7 mg, 0.04 mmol) in dry DMF (400 µl) was added TEA (75 µl) and reduced compound **13** (26.2 mg), and then stirred at room temperature for 16 h. The mixture was concentrated *in vacuo*. The residue was purified by preparative thin layer chromatography (MeOH/EtOAc = 1/3) to give compound **4** (11.5 mg, 0.02 mmol, 56%) as yellow liquid: ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.72 (1H, s), 7.99-7.97 (2H, d), 7.89-7.86 (3H, d), 7.47 (2H, s), 3.53-3.33 (14H, m), 3.16-3.15 (2H, m), 2.77-2.76, 2.77 (1H, t, J = 4.0 Hz), 2.16-2.11 (4H, m), 1.66-1.61 (2H, m); ^{13}C NMR (400 MHz, DMSO-d_6): δ 172.1, 165.8, 146.7, 137.8, 128.4 (×2), 126.1 (×2), 84.7, 72.0, 72.0 (×2),70.1 (×2), 69.6, 69.3, 40.6, 39.0, 34.6, 24.8, 17.9; HRESI-MS calculated for $C_{21}H_{31}N_3NaO_7S$ ($M+Na^+$): 492.178; found: 492.179.

3. Preperation of functionalized gold nanoparticles

3-1. Synthesis of citrate-stabilized gold nanoparticles

Citrate-stabilized gold nanoparticles were synthesized according to the reported procedure.[1] Briefly, to a boiling

solution of $HAuCl_4 \cdot 3H_2O$ (1 mM) was quickly added a hot aqueous solution of 2% trisodium citrate dihydrate, which was stirred at 100 ℃ for 10 minutes. After 10 minutes, the color of the mixture changed from violet to dark red. The mixture was diluted up to 40 mL with Milli Q water.

3-2. Synthesis of clickable photoreactive gold nanoparticles 1-3.

Bis (*p*-sulfonatophenyl) phenylphosphine (BSPP) dihydrate dipotassium salt (0.5 mg/mL in MilliQ water) was added to 1 mL solution of citrate-stabilized gold nanoparticles prepared in 3-1 and the mixture was stirred on an orbital shaker at 50 ℃ for 1 h. The resulting mixture was centrifuged at 18,000 × *g* at 10 ℃ for 1 h and the supernatant was carefully removed. The BSPP-stabilized gold nanoparticles were washed with Milli Q water (0.5 mL× 3) and were diluted in 0.5 mL MeOH. The stock solutions of compound **6-8** in MeOH (10 mM) were mixed at the desired ratios to be added to a solution of the BSPP-stabilized gold nanoparticles at a molar ratio of 10000:1. The mixture was stirred on a plate shaker at room temperature for 24 h. The functionalized gold nanoparticles were washed with Milli Q water (0.5 mL× 3). The concentration of the functionalized gold nanoparticles was determined by the visible absorbance at 520 nm according to the published procedure.[3]

3-3. Synthesis of ligand clicked photoaffinity probes 9-12.

The mixed solution of clickable photoreactive gold nanoparticles (50 nM, **1-3**) prepared in 3-2, alkyne-tagged ligand **4** or **5** (125 μM), ascorbic acid (1.25 mM), tris[[1-(3-hydroxypropyl)-1*H*-1,2,3-triazol-4-yl] methyl] amine (THPTA, 2.5 mM) and $CuSO_4 \cdot 5H_2O$ (1.25 mM) in DMSO/*t*BuOH/6.5% PEG8000 aq. = 1/1/1 was stirred on a plate shaker at room temperature for 18 h. The reaction was quenched by addition of 100 mM EDTA-3% PEG8000 aq. solution and the reaction mixture was centrifuged at 18,000 × *g* at 10 ℃ for 1.5 h to separate the gold nanoparticles from the unreacted alkyne-tagged ligand. The pellet fraction containing gold nanoparticles was washed once with Milli Q water.

4. UV-VIS spectrometric analysis[3]

The solution of variously functionalized gold nanoparticles (2 μL) was each recorded on a NanoDrop ND-1000 spectrophotometer.

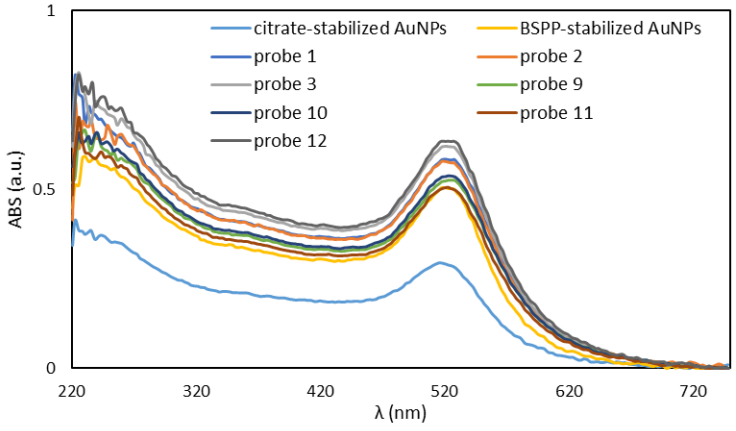


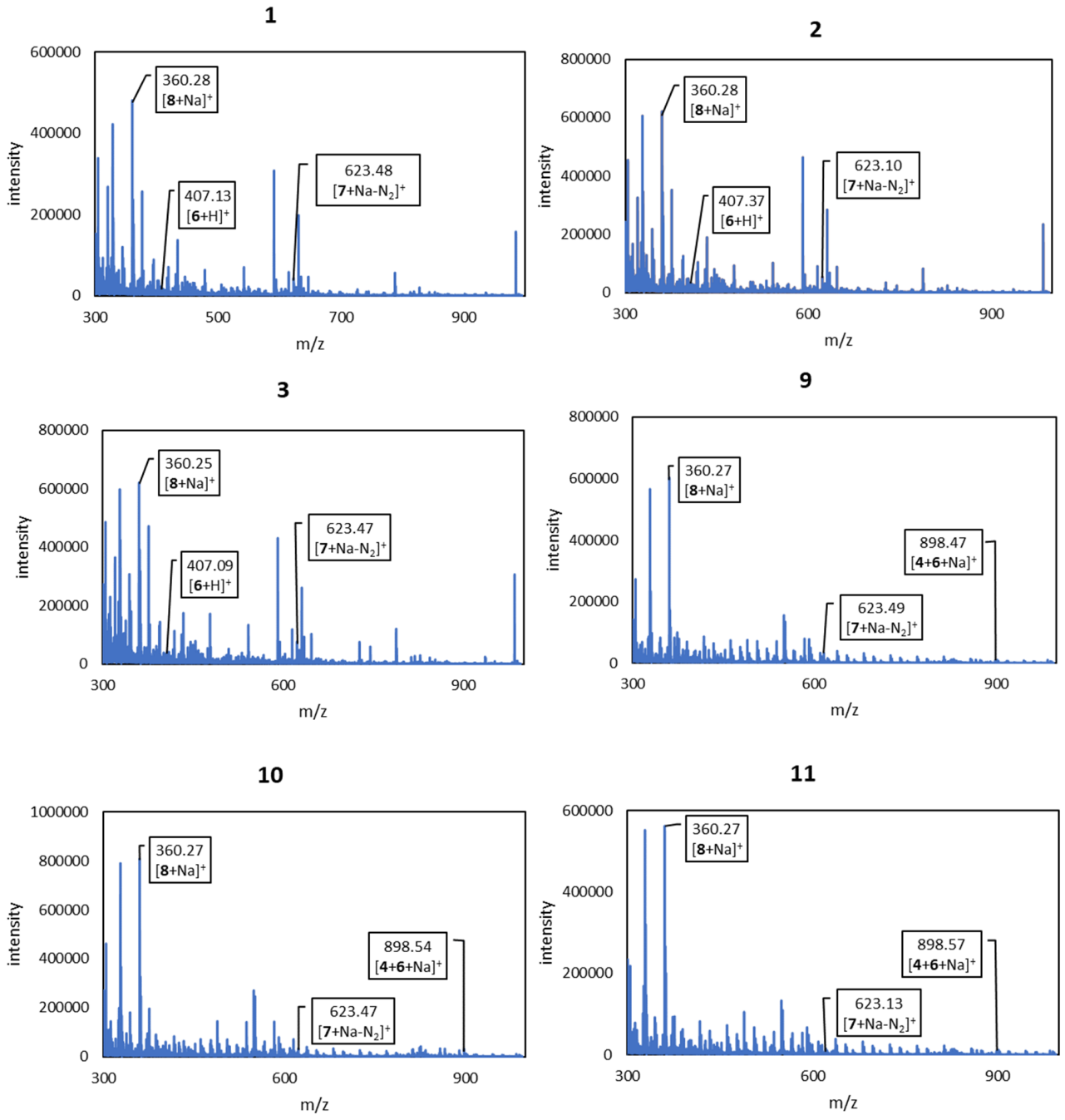
Figure S1. UV-VIS spectra of citrate-stabilized gold nanoparticles (0.03 pmol), BSPP-stabilized gold nanoparticles (0.004 pmol), clickable photoreactive gold nanoparticles **1-3** (0.04 pmol) and ligand-clicked photoaffinity probes **9-12**(0.05 pmol).

5. MALDI-TOF MS analysis

The solution of functionalized gold nanoparticles (0.1 pmol, **1-3**, **9-12**) and total of 4 μL of a matrix solution (10 mg/mL 2,5-dihydroxybenzoic acid in MeCN/H_2O = 1/1) were alternately spotted onto the sample plate for direct MALDI-TOF MS analysis. MALDI-TOF MS spectra were acquired in the linear mode by Autoflex speed TOF/TOF (Bruker Daltonics). All the MS scans were acquired over the mass range 300-1000 m/z in the linear mode. The MS spectra were externally calibrated using tetraethylene glycol (calc. for $C_8H_{18}O_5$ 194.23) and dodecaethylene glycol (calc. for $C_{24}H_{50}O_{13}$ 546.65).

Probe number	Compound	Chemical formula	Calculated mass	Observed mass
1	**6**	$C_{16}H_{31}N_4O_4S_2$ $(M+H)^+$	407.57	407.13
	7	$C_{28}H_{48}N_4NaO_6S_2$ $(M+Na-N_2)^+$	623.82	623.48
	8	$C_{14}H_{27}NNaO_4S_2$ $(M+Na)^+$	360.48	360.28
2	**6**	$C_{16}H_{31}N_4O_4S_2$ $(M+H)^+$	407.57	407.37
	7	$C_{28}H_{48}N_4NaO_6S_2$ $(M+Na-N_2)^+$	623.82	623.10
	8	$C_{14}H_{27}NNaO_4S_2$ $(M+Na)^+$	360.48	360.28
3	**6**	$C_{16}H_{31}N_4O_4S_2$ $(M+H)^+$	407.57	407.09
	7	$C_{28}H_{48}N_4NaO_6S_2$ $(M+Na-N_2)^+$	623.82	623.47
	8	$C_{14}H_{27}NNaO_4S_2$ $(M+Na)^+$	360.48	360.25
9	**4+6**	$C_{37}H_{61}N_7NaO_{11}S_3$ $(M+Na)^+$	899.10	898.47
	7	$C_{28}H_{48}N_4NaO_6S_2$ $(M+Na-N_2)^+$	623.82	623.49
	8	$C_{14}H_{27}NNaO_4S_2$ $(M+Na)^+$	360.48	360.27
10	**4+6**	$C_{37}H_{61}N_7NaO_{11}S_3$ $(M+Na)^+$	899.10	898.54
	7	$C_{28}H_{48}N_4NaO_6S_2$ $(M+Na-N_2)^+$	623.82	623.47
	8	$C_{14}H_{27}NNaO_4S_2$ $(M+Na)^+$	360.48	360.27
11	**4+6**	$C_{37}H_{61}N_7NaO_{11}S_3$ $(M+Na)^+$	899.10	898.57
	7	$C_{28}H_{48}N_4NaO_6S_2$ $(M+Na-N_2)^+$	623.82	623.13
	8	$C_{14}H_{27}NNaO_4S_2$ $(M+Na)^+$	360.48	360.26
12	**5+6**	$C_{47}H_{77}N_5NaO_6S_2$ $(M+Na)^+$	883.26	883.59
	7	$C_{28}H_{48}N_4NaO_6S_2$ $(M+Na-N_2)^+$	623.82	623.32
	8	$C_{14}H_{27}NNaO_4S_2$ $(M+Na)^+$	360.48	360.15

Table S1. MALDI-TOF MS peaks detected for clickable photoreactive gold nanoparticles (**1-3**) and ligand-clicked photoaffinity probes (**9-12**).



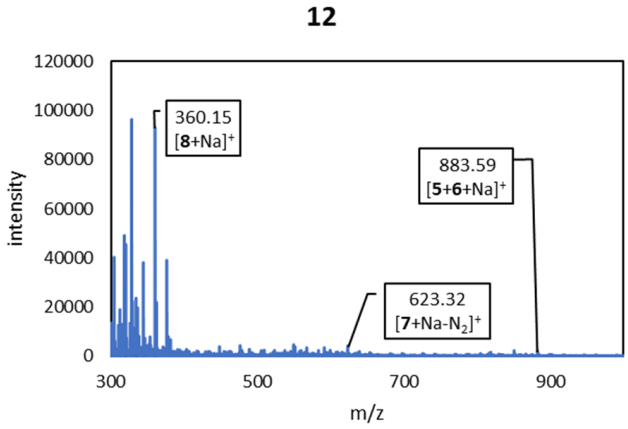


Figure S2. MALDI-TOF MS spectra detected for clickable photoreactive gold nanoparticles (**1-3**) and ligand-clicked photoaffinity probes (**9-12**).

6. Agarose gel analysis

0.2 pmol aliquots of clickable photoreactive gold nanoparticles (**1-3)** and ligand-clicked photoaffinity probes (**9-12**) were suspended in 50% glycerol/H_2O and were electrophoresed in 0.5×TBE buffer (44.5 mM Tris, 44.5 mM boric acid, 1 mM EDTA) for 30 min at 100 mV using a 0.5% agarose gel.

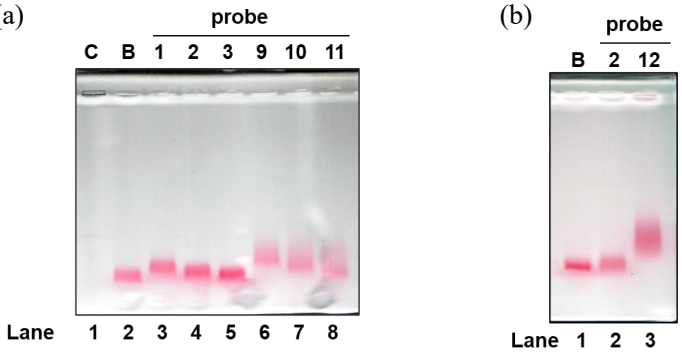


Figure S3. Agarose gel analysis of (a) clickable photoreactive gold nanoparticles **1-3** and ligand-clicked photoaffinity probes **9-11**, and (b) **2** and **12**, using 0.5% gel. C: citrate-stabilized gold nanoparticles; B: BSPP-stabilized gold nanoparticles.

7. General procedures for photoaffinity labeling experiments using photoaffinity probes 9-12.

7-1. Photoaffinity labeling reactions of 9-11 with purified proteins.

bCAII (1.5 μg, 1 μM) and the photoaffinity probe (1 pmol) were mixed in a total volume of 50 μL (20 nM) of 6.5% PEG-HEPES buffer (6.5% PEG8000, 10 mM HEPES pH 7.5, 150 mM NaCl),[6] which was incubated on a rotary mixer at 4 °C for 2 h. For a negative control experiment under the competitive binding condition, 5 mM DMSO stock solution of 4-sulfamoyl benzoic acid (BzSA, final concentration of 100 μM) was added to the reaction mixture. For a selective PAL reaction by probe **9-11** in the presence of excess BSA, BSA (17 μg) was included in the reaction mixture. The

reaction mixture was irradiated at 365 nm at a distance of 5 cm on ice for 20 min using a UV lamp (15W, UVP XX Series) then was diluted in 500 μL of 6.5% PEG-PBS (for reactions with bCAII only) or 500 μL of 1% CHAPS-PBS (for reactions with a mixture of bCAII and BSA) to be transferred to a new microcentrifuge tube. It was centrifuged at 18,000 × *g* at 10 °C for 1 h to remove the supernatant and the pellet was resuspended in 500 μL of 1% CHAPS-PBS by sonicating briefly and agitating on a vortex mixer. The mixture was centrifuged at 18,000 × *g* for 1 h to remove the supernatant, then washed once with 500 μL of 1% CHAPS-1% OG-PBS and finally with 500 μL of 1% CHAPS-PBS. The resultant pellet was suspended in 6× Lammaeli sample buffer (187.5 mM Tris, 3.8% SDS, 2.0 M glycerol, 0.28 mM bromophenol blue, pH 6.8) containing 10% 2-mercaptoethanol and 9% dithiothreitol (DTT) to elute the labeled proteins by heating at 95 °C for 10 min × 3. The eluate was centrifuged at 18,000 × *g* at room temperature for 10 min and the supernatant sample was resolved by SDS-PAGE using 10% gel. The resultant gel was stained by Flamingo Fluorescent Gel stain (Biorad) and analyzed by fluorescence imaging on Typhoon 8600 (GE Healthcare Science). The crosslinking yields (%) were calculated based on the fluorescence intensity of the protein used for a given reaction.

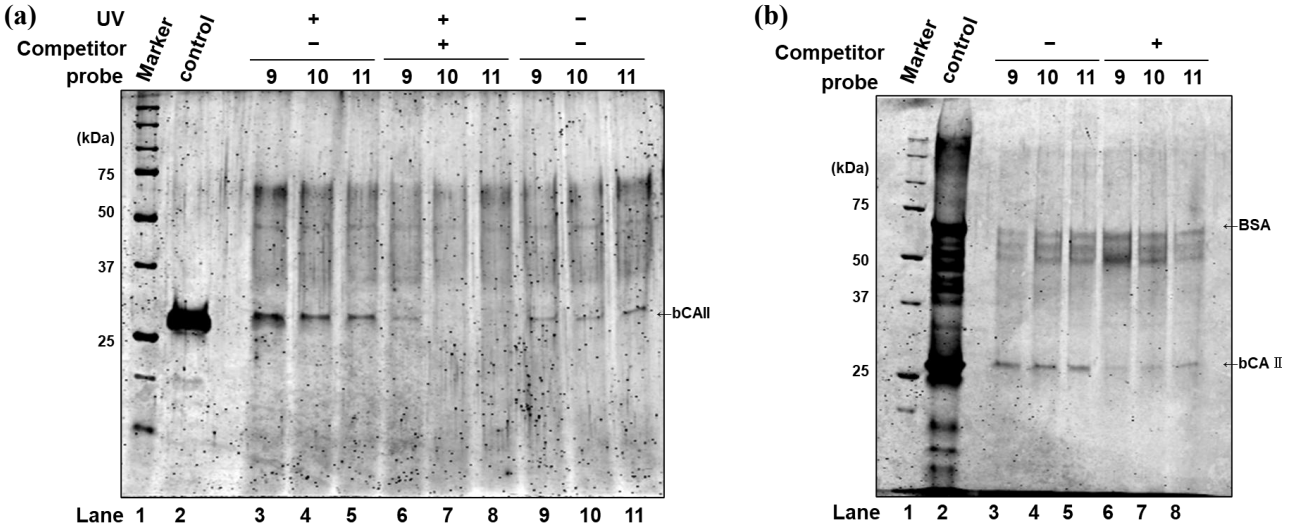


Figure S4. (a) SDS-PAGE analysis of PAL reaction using **9-11** (20 nM) and bCAII (1.5 μg) in 6.5% PEG-HEPES (pH 7.5) visualized by fluorescent Flamingo Pink stain. Lane 1: molecular weight maker, lane 2: bCAII input (1.5 μg), lane 3-5: photocrosslinked bCAII by **9-11**, lane 6-8: negative control experiment using **9-11** with excess BzSA (100 μM), lane 9-11: negative control experiment with no UV irradiation using **9-11**. (b) Selectivity of PAL between **9-11** (20 nM) and a mixture of bCAII (1.5 μg) and BSA (17 μg) in 1% CHAPS-HEPES buffer (pH 7.5) analyzed by SDS-PAGE and fluorescence imaging. Lane 1: molecular weight maker, lane 2: protein mixture input including bCAII (1.5 μg) and BSA (3.3 μg), lane 3-5: photocrosslinked bCAII by **9-11**, lane 6-8: negative control experiment using **9-11** in the presence of excess benzenesulfonamide (100 μM) as a competitor.

7-2. Time course analysis of photoaffinity labeling by probe 10.

bCAII (1.5 μg, 1 μM) and probe **10** (1 pmol, 20 nM) were mixed in 50 μL of 6.5% PEG-HEPES buffer, which was incubated on a rotary mixer at 4 °C for 2 h. The mixture was irradiated at 365 nm at a distance of 5 cm on ice for 1, 5, 10, 20, 40, 60 min using a UV lamp (15W, UVP XX Series). The reaction mixture was diluted in 500 μL of 6.5% PEG-

PBS to be transferred to a new microcentrifuge tube and was centrifuged at 18,000 × g at 10 °C for 1 h to remove the supernatant. The pellet was resuspended in 500 µL of 1% CHAPS-PBS by sonicating briefly and agitating on a vortex mixer and the mixture was centrifuged at 18,000 × g for 1 h to remove the supernatant. The same cycle was repeated once more. The resultant pellet was suspended in 6×Lammaeli sample buffer containing 10% 2-mercaptoethanol to elute the labeled proteins by heating at 95 °C for 10 min × 3. The eluate was centrifuged at 18,000×g at room temperature for 10 minutes. The crosslinked-bCAII was analyzed as in 7-1.

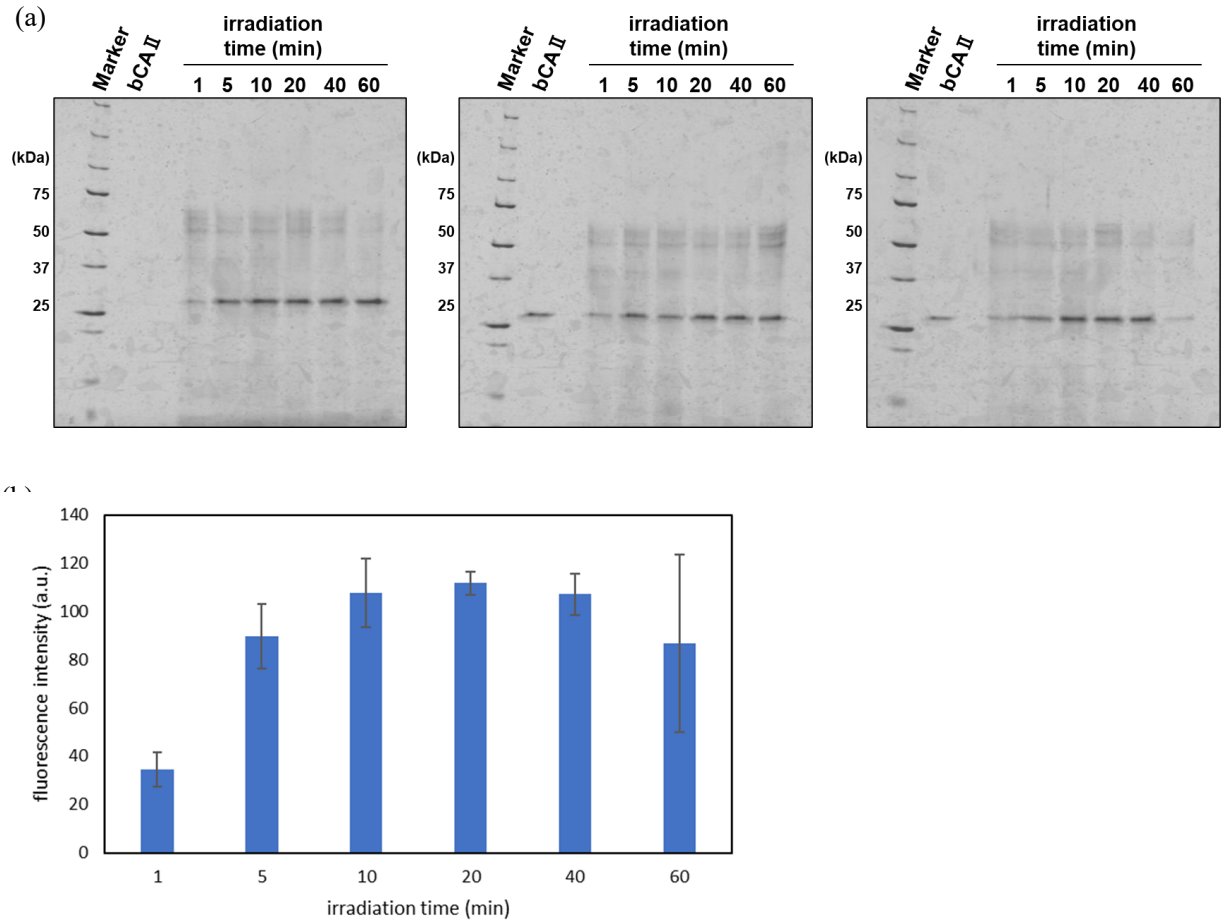


Figure S5. (a) SDS-PAGE analysis of the time course of PAL reaction between **10** (20 nM) and bCAII (1.5 µg, 1 µM) in 6.5% PEG-HEPES buffer (pH 7.5) performed in triplicate. (b) The bar graph showing the averaged fluorescence intensity calculated for each time point in (a). Error bars show standard errors (n=3)

7-3. MALDI-TOF-MS analysis of bCAII obtained by PAL reaction with probe 10.

The PAL reaction of probe **10** with a mixture of bCAII and BSA was conducted in 6.5% PEG-HEPES buffer as described in the section 7.1 was diluted in 300 µL of 6.5% PEG-PBS to be transferred to a new microcentrifuge tube and was centrifuged at 18,000 × g at 10 °C for 1 h to remove the supernatant. The pellet was resuspended in 300 µL of MilliQ water by sonicating briefly and agitating on a vortex mixer and the mixture was centrifuged at 18,000 × g at 10 °C for 1

h to remove the supernatant, which was repeated once more. The pellets were suspended with 6 μL of MALDI matrix solution (10 mg/mL sinapinic acid in 50% MeCN/MilliQ water). This mixture was spotted onto the sample plate for direct MALDI-TOF MS analysis. MALDI-TOF-mass spectra were acquired in the linear mode by Autoflex speed TOF/TOF (Bruker Daltonics).

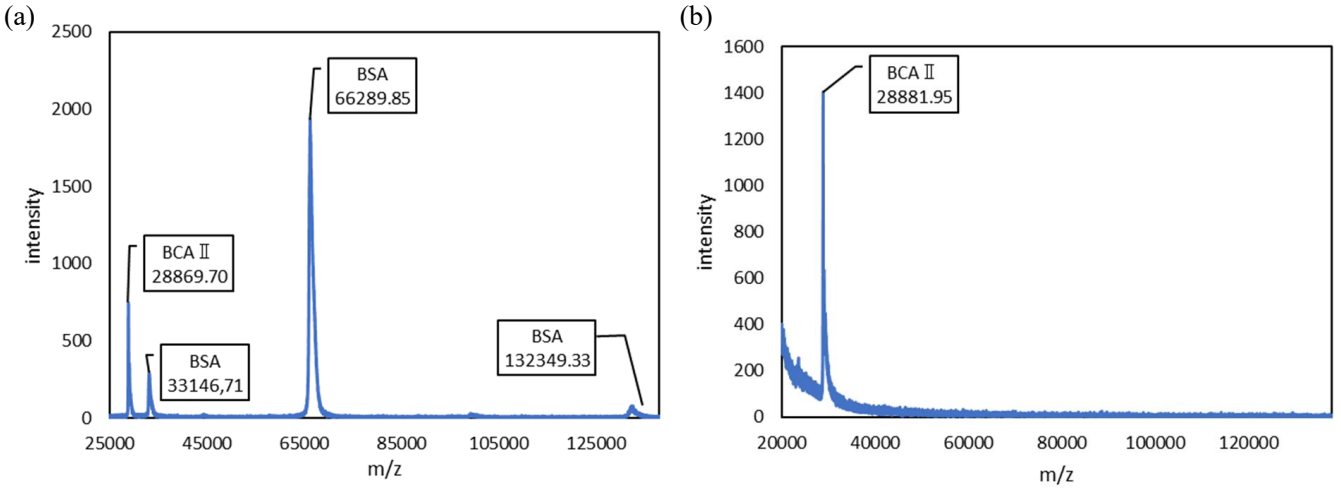


Figure S6. MALDI-TOF mass spectra of (a) a mixture of bCAII (calculated for 29,114)[4] and BSA (calculated for 66430).[5] (b) bCAII captured by probe **10** in the presence of BSA.

7-4. Photoaffinity labeling reactions in cell lysate.

Probe **10** (5 pmol, final concentration 50 nM) or **12** (5 pmol, final concentration 50 nM) and HL-60 cell lysate (100 μg) or SNU-1 cell lysate (180 μg) in 2% CHAPS-RIPA lysis buffer (50 mM Tris HCl pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EDTA, 10 mM NaF, 1 mM Na_3VO_4, 1 mM DTT, 1 mM cOmplete Protease Inhibitor Cooktail/EDTA-free (Roche)) were mixed and incubated on a rotary mixer at 4 °C for 2 h. For a negative control experiment under the competitive binding condition for probe **10**, 1 mM DMSO stock solution of BzSA (final concentration of 100 μM against probe **10**) was added to the reaction mixture. For a negative control experiment against probe **12**, clickable gold nanoparticles **2** (50 nM) was used. The mixture was irradiated at 365 nm at a distance of 5 cm on ice for 20 min using a UV lamp (15W, UVP XX Series), which was then diluted in 2 mL of 1% CHAPS-PBS to be transferred to a new microcentrifuge tube. The sample was centrifuged at 18,000 × g at 10 °C for 1 h to remove the supernatant, washed twice with 500 μL of 1% CHAPS-1% octylglucoside (OG)-PBS. The pellet was resuspended in 500 μL of 1% CHAPS-PBS by sonicating briefly and agitating on a vortex mixer. This washing step was repeated once more. The resultant pellet was further washed with 1% CHAPS-PBS, which was centrifuged at 18,000×g at 10 °C for 1 h and the supernatant was removed. The pellet was suspended in 6× Lammaeli sample buffer (375 mM Tris, 12% LDS, 50% glycerol, 0.03% bromophenol blue, pH 6.8) containing 10% 2-mercaptoethanol 9% DTT to elute the labeled proteins by heating at 95 °C for 10 min × 3 and by centrifugation at 18,000 × g at room temperature for 10 minutes. The supernatant sample was split into two batches, which were separately resolved by SDS-PAGE using 5-20% gradient gels. One of the resultant gels was visualized by Flamingo Pink stain and analyzed by fluorescence imaging on Typhoon 8600.

For western blot analysis, the SDS-PAGE gel was blotted to a PDVF membrane by Trans-Blot® Turbo™ Transfer System (Biorad). The membrane was blocked by 0.3% skim milk in TBST (50 mM Tris-Buffered Saline pH 8.2, 138 mM NaCl, 2.7 mM KCl, 0.1% Tween 20), probed with rabbit anti-hCAII (Rockland, 1:15000), and mouse monoclonal anti-OSBP (Santa Cruz Biotechnology, 1:500) followed by anti-rabbit IgG conjugated with horse radish peroxidase (HRP, Proteintech, 1:10000) or anti-mouse IgG (GE Healthcare, 1:5000) and the protein bands were visualized by chemiluminescence using Typhoon 8600. The membrane detected by anti-hCAII or anti-OSBP was reprobed by rabbit anti-GAPDH (Sigma-Aldrich, 1:500) after stripping using a stripping buffer (62.5 mM Tris-HCl, pH 6.8, 2 % SDS, 100 mM 2-mercaptoethanol).

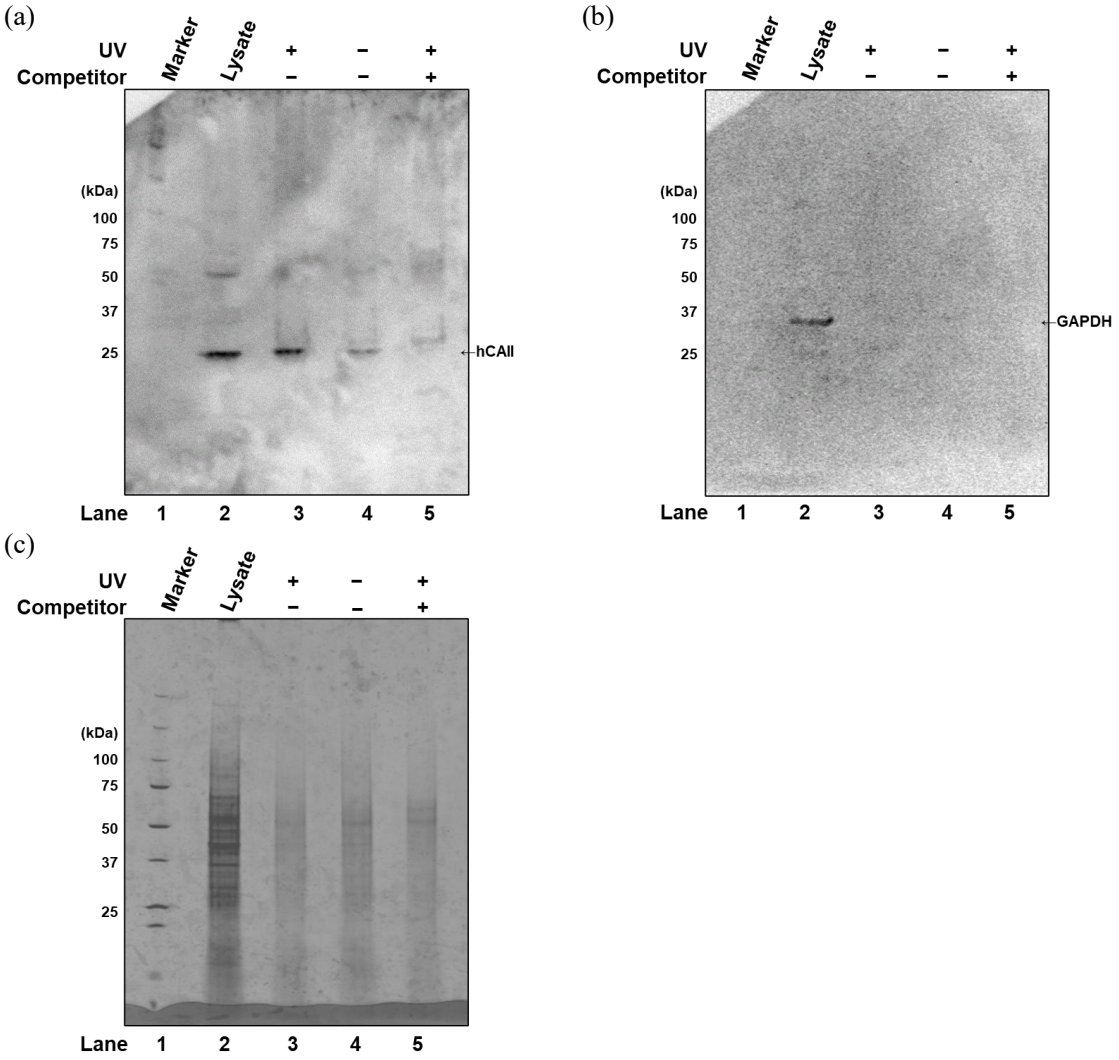


Figure S7. Western blot analysis PAL reaction of probe **10** (50 nM) in cell lysate (100 μg) by (a) anti-hCAII or by (b) anti-GAPDH. Lane 1: molecular weight maker, lane 2: lysate input (10 μg), lane 3: photocrosslinked and enriched hCAII, lane 4: negative control with no UV irradiation, lane 5: negative control experiment in the presence of excess BzSA (100 μM) as a competitor. (c) Corresponding Flamingo Pink stained gel for (a).

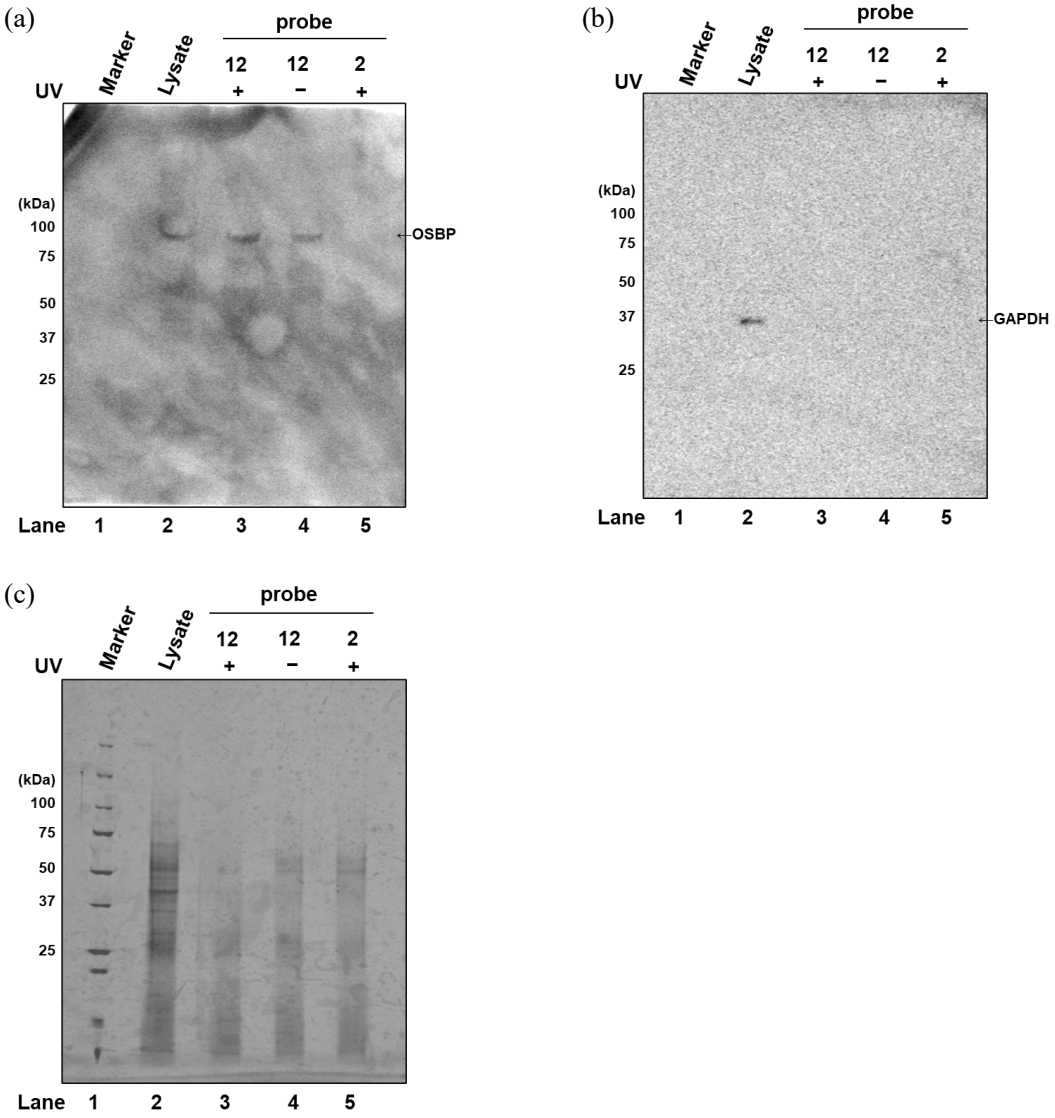


Figure S8. Western blot analysis of PAL reaction using probe **12** (50 nM) in cell lysate (180 μg) by (a) anti-OSBP or (b) anti-GAPDH. Lane 1: molecular weight maker, lane 2: lysate input (10 μg), lane 3: photocrosslinked and enriched OSBP, lane 4: negative control with no UV irradiation, lane 5: negative control experiment with **2** (50 nM). (c) Corresponding Flamingo Pink stained gel for (a).

8. Preparation of HL60 and SNU-1 cell lysate

HL60 cells (RIKEN bioresource center) and SNU-1 cells (RIKEN Bioresource Center) was grown till confluent (1.0-3.0 $\times 10^6$ cell) in RPMI-1640 (Wako) supplemented with 10% FBS and 1% penicillin/streptomycin in humidified 5% CO2 atmosphere at 37 °C. The cells were collected using a cell scraper, pelleted by centrifugation (300 × g, 4°C, 5 minutes) and washed with PBS (0.02 w/v% KCl, 0.8 w/v% NaCl, 0.02 w/v% KH_2PO_4, 0.115 w/v% Na_2HPO_4) two times. The pellet was suspended in RIPA lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% TritonX-100, 0.5% sodium

deoxycholate, 0.1% SDS, 1 mM EDTA, 10 mM NaF, 1 mM Na_3VO_4, 1 mM DTT, 1 mM or 2 mM protease inhibitor cocktail (Roche)). After incubation on ice for 20 minutes, the sample was centrifuged (18000 × g, 4 °C, 30 minutes) to give the supernatant as the cell lysate solution. The concentration of the cell lysate was determined by protein BCA assay (Wako protein assay BCA kit) using a plate reader (Biorad iMark microplate reader).

References

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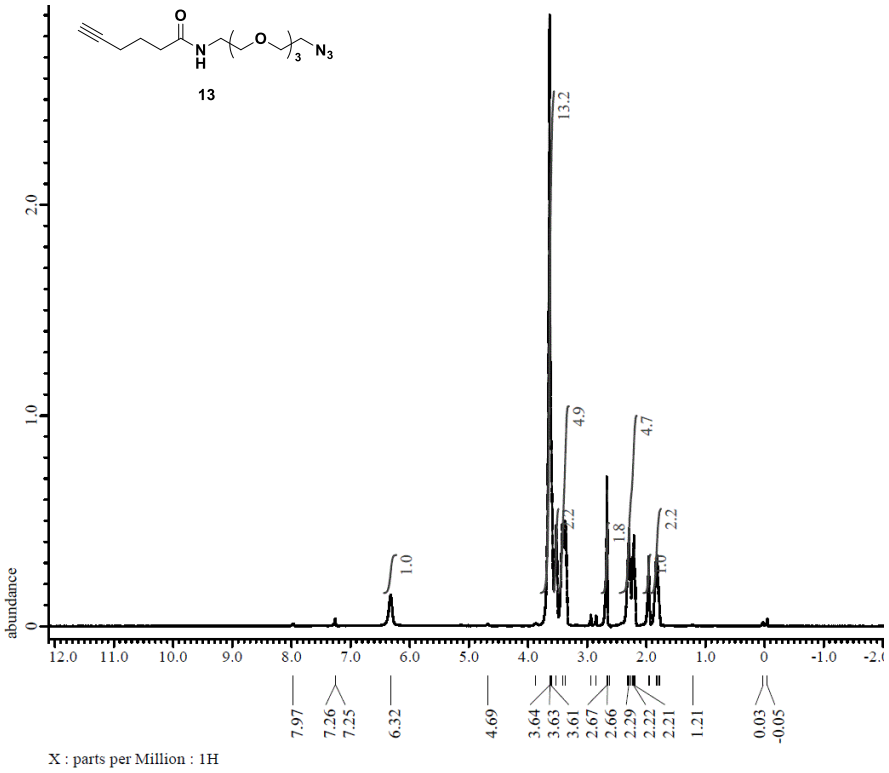
4. The calculated molecular weight is based on the sequence for UniProtKB-P00921 (CAH2_BOVIN).

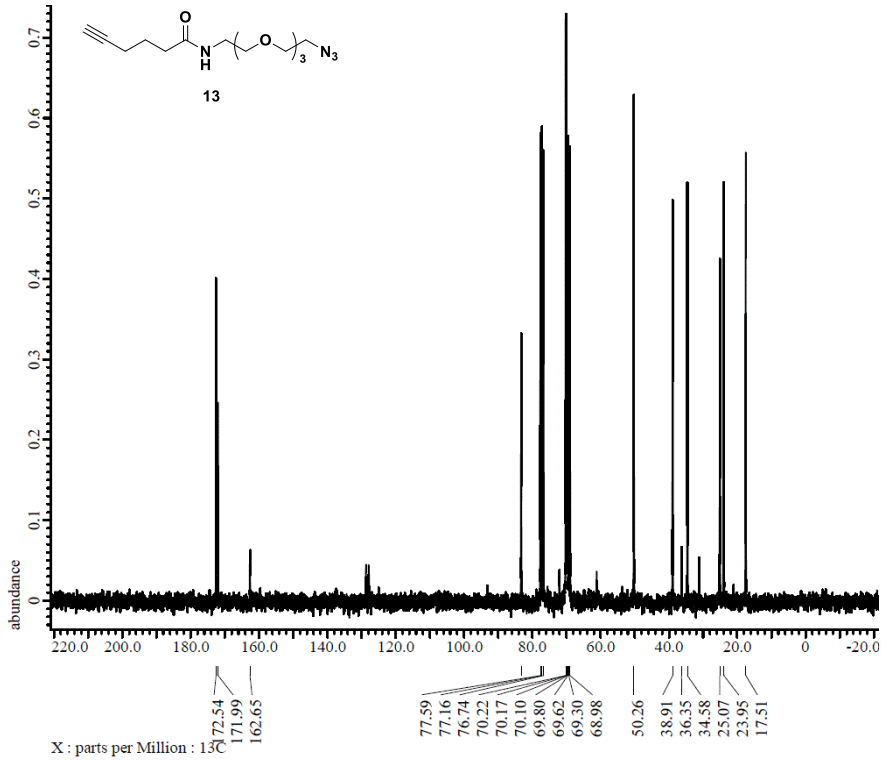
5. The calculated molecular weight is based on the processed sequence for UniProtKB-P02769 (ALBU_BOVIN; Chain PRO_0000001058 25 – 607).

6. In the section 7, 6.5% PEG denotes for 6.5 w/w% aqueous solution of PEG8000.

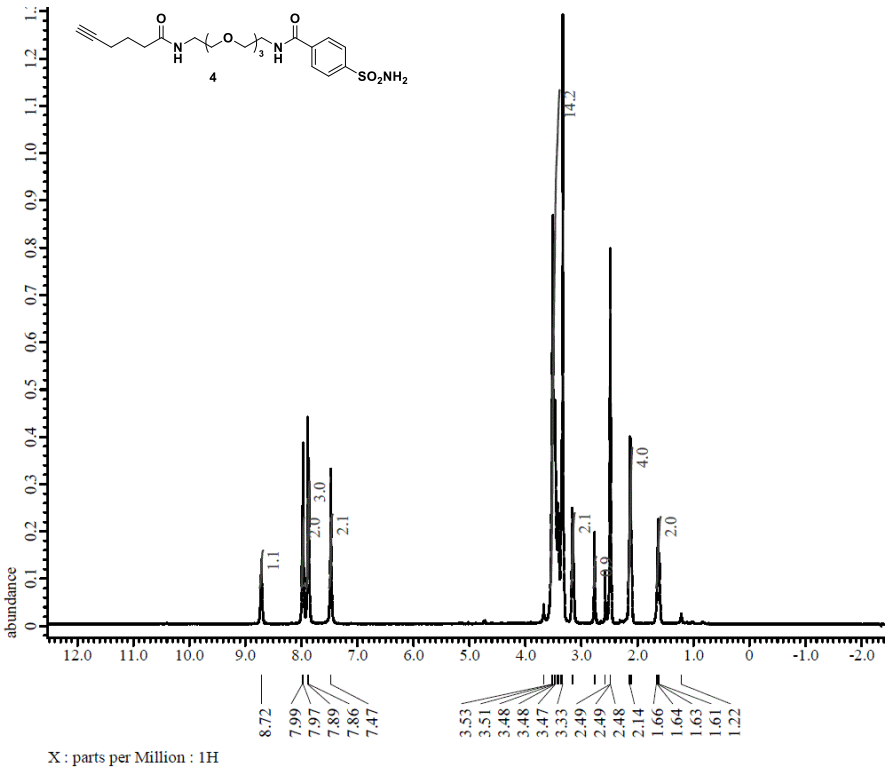
^{1}H NMR ($CDCl_3$, 400 MHz) of compound **13**



^{13}C NMR ($(CD_3)_2SO$, 400 MHz) of compound **13**



^{1}H NMR ($CDCl_3$, 400 MHz) of compound **4**



^{13}C NMR ($(CD_3)_2SO$, 400 MHz) of compound **4**