Tetrahedron 67 (2011) 8879–8886

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Development of UV-responsive catch-and-release system of a cysteine protease model peptide

Akira Shigenaga [a,*], Ko Morishita [a], Keiko Yamaguchi [a], Hao Ding [a], Koji Ebisuno [a], Kohei Sato [a], Jun Yamamoto [a], Kenichi Akaji [b], Akira Otaka [a,*]

[a] Institute of Health Biosciences and Graduate School of Pharmaceutical Sciences, The University of Tokushima, Shomachi, Tokushima 770-8505, Japan
[b] Department of Medicinal Chemistry, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8412, Japan

ARTICLE INFO

Article history:
Received 27 August 2011
Received in revised form 13 September 2011
Accepted 15 September 2011
Available online 22 September 2011

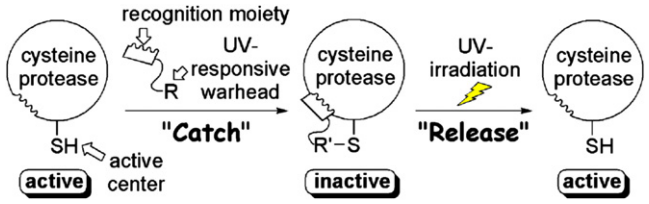
Keywords:
Aromatization
Caged compound
Cysteine protease
Protective group
UV-responsive

ABSTRACT

Cysteine proteases are attractive drug targets due to their involvement in a wide variety of diseases. To evaluate the potential of a particular protease as a drug target, use of a reagent that controls activity of the protease is indispensable. In this context, we have developed a catch-and-release reagent that first forms a covalent bond with the active center thiol of a cysteine protease to suppress its activity and then is removed by UV-irradiation to release the parent active protease. In this paper, the design and synthesis of a catch-and-release reagent of thiols are described. Its application to caging (catch) and UV-induced uncaging (release) of a model peptide derived from an active site of caspase-9 and introduction of a recognition moiety on the reagent are also reported.

1. Introduction

Cysteine proteases represent attractive drug targets due to their involvement in a wide variety of conditions, such as cardiovascular, inflammatory, viral and immunological disorders, and cancer.[1] Methodology for controlling the activity of a disease-related cysteine protease may provide useful means for evaluating the protease as a drug target. In this context, caged cysteine proteases that release the parent proteases after exposure to UV light have been reported.[2] Activity control of the proteases is enabled by UV-induced conversion of the 'inactive' caged proteases to the 'active' parent proteases. Recently, an advanced methodology, which enables three-step activity control, such as 'active (intact enzyme)' to 'inactive (caging in living cells by addition of a caging reagent)' to 'active (uncaging by UV-irradiation),' was developed for enzymes other than cysteine proteases.[3] To our knowledge, its application to cysteine proteases has yet to be reported. Therefore, we designed a catch-and-release system for the advanced activity control of a cysteine protease as shown in Scheme 1. When a catch-and-release reagent possessing a recognition moiety for selective binding to the target protease and a warhead to react with an active center thiol is added to a proteome, the target cysteine protease is selectively caught by the reagent and is inactivated.[4,5] If the catch-and-release reagent can be irreversibly removed by exposure to UV light, photo-induced re-activation of the protease is achieved.



Scheme 1. Catch-and-release strategy for controlling the activity of cysteine proteases.

In this article, the design and synthesis of the UV-responsive catch-and-release reagent of thiols are described. Introduction of the recognition moiety on the reagent is also discussed.

2. Results and discussion

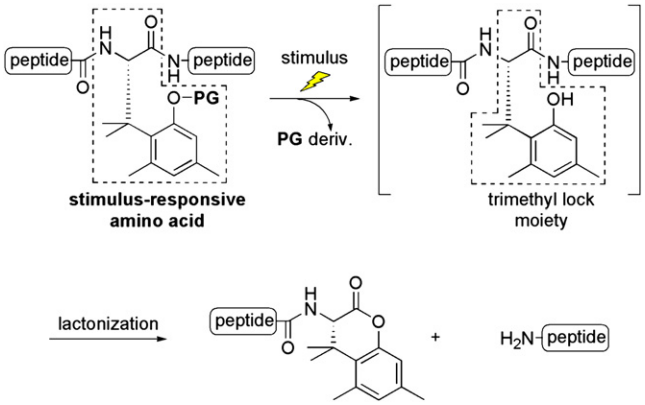
2.1. Design of catch-and-release reagent

We previously reported a stimulus-responsive amino acid[6] that induced peptide bond cleavage after stimulus induced deprotection followed by lactonization of the trimethyl lock moiety (Scheme 2).[7]

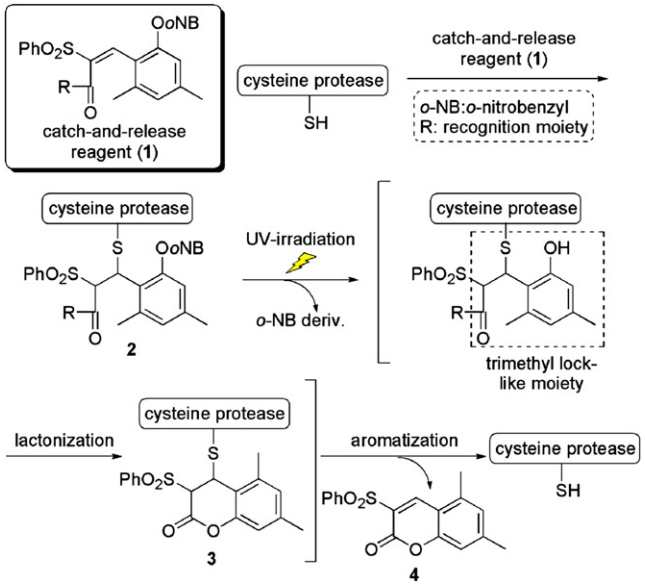
* Corresponding authors. E-mail addresses: ashige@ph.tokushima-u.ac.jp (A. Shigenaga), aotaka@ph.tokushima-u.ac.jp (A. Otaka).

0040-4020/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2011.09.062

With this in mind, UV-responsive catch-and-release reagent **1**, composed of a trimethyl lock-like moiety and the UV-removable *o*-nitrobenzyl (*o*-NB) group, was designed (Scheme 3). Compound **1** possesses an electron-deficient olefin moiety and a recognition moiety (R), therefore, it can recognize a target protease and react via Michael addition to generate the corresponding inactivated cysteine protease **2**. After UV-induced removal of the *o*-nitrobenzyl group followed by lactonization of the trimethyl lock-like moiety, intermediate **3** should easily aromatize to release the parent cysteine protease.



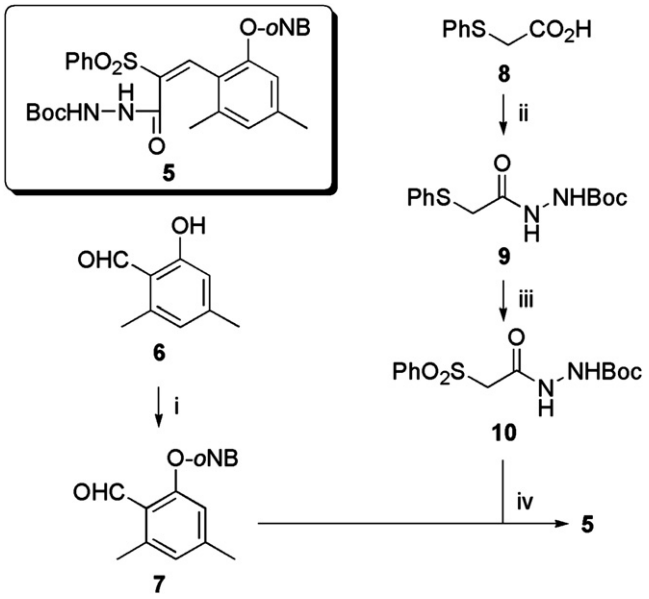
Scheme 2. Stimulus-responsive amino acid based on trimethyl lock system (**PG**: protective group removable by a stimulus).



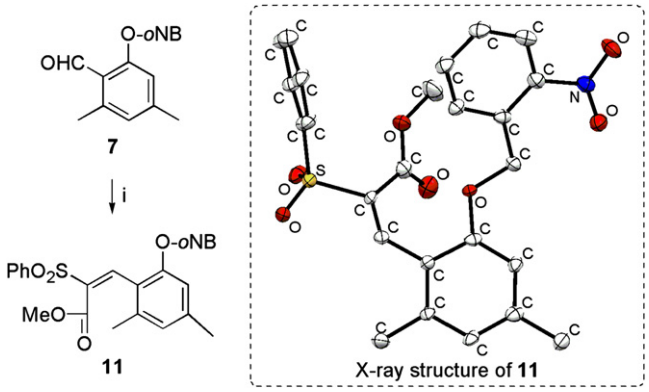
Scheme 3. Design of catch-and-release reagent for control of activity of cysteine protease.

2.2. Synthesis of catch-and-release reagent

Catch-and-release reagent **5** possessing a Boc-protected acyl hydrazine for facile introduction of the recognition moiety was synthesized as shown in Scheme 4. Phenol **6**[8] was alkylated with *o*-nitrobenzyl bromide to afford ether **7**. Knoevenagel condensation of **7** with sulfonylacetamide **10** derived from sulfanylacetic acid **8** was performed and catch-and-release reagent **5** was obtained. Because the geometry of compound **5** could not be determined using NMR and X-ray methods, we synthesized easily crystallizable surrogate **11** and its geometry was determined to be (*E*) using X-ray crystallography (Scheme 5). Therefore, we assumed that the geometry of **5** was also (*E*).



Scheme 4. Reagents and conditions: (i) *o*-Nitrobenzyl bromide, K_2CO_3, CH_2Cl_2, 77%; (ii) H_2N-NHBoc, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU), *N*,*N*-diisopropylethylamine (DIEA), DMAP, CH_2Cl_2, 92%; (iii) Oxone®, MeOH/H_2O, 0 °C to room temperature, 74%; (iv) Piperidine, AcOH, toluene, reflux, 85%. (*o*-NB: *o*-nitrobenzyl).

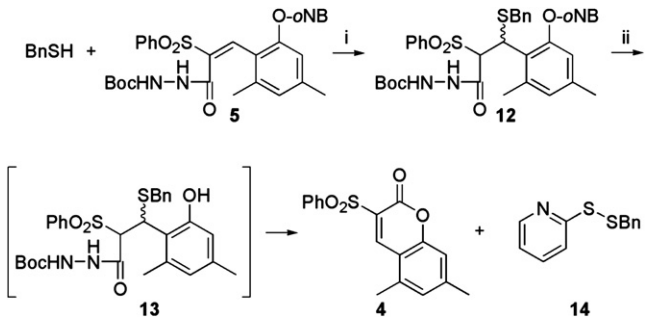


Scheme 5. Reagents and conditions: (i) $PhSO_2CH_2CO_2Me$, piperidine, AcOH, toluene, reflux, 83%. All hydrogen atoms of the X-ray structure of **11** are omitted for clarity.

2.3. Catch-and-release experiment of compound 5

The catch-and-release reaction of a thiol with compound **5** was examined. First, benzyl mercaptan was used as a thiol for simplicity (Scheme 6). Benzyl mercaptan was treated with compound **5** and Et_3N in CH_2Cl_2, affording the Michael addition product **12** in 82% yield. After purification, caged thiol **12** was irradiated by UV (>365 nm) for 5 min, and the reaction mixture was shaken after addition of 2,2′-dithiodipyridine[9] and Et_3N. The reaction progress was monitored by HPLC (Fig. 1). After UV-irradiation, the *o*-nitrobenzyl group of substrate **12** was completely removed to generate phenol **13**. By the addition of 2,2′-dithiodipyridine and Et_3N followed by shaking for 2 h, intermediate **13** was successfully aromatized to release benzyl mercaptan derivative **14** and **4**. The products were identified by ESI-MS and comparison of retention times with those of authentic samples.[10] The synthesis of an authentic sample of compound **4** is depicted in Scheme 7. Briefly, after

protection of phenol **6** with a methoxymethyl group followed by Knoevenagel condensation with commercially available phenylsulfonylacetic acid methyl ester, obtained product **16** was treated with TFA to afford aromatized product **4**.



Scheme 6. Reagents and conditions: (i) Et_3N, CH_2Cl_2, 82%; (ii) UV-irradiation (>365 nm, 5 min) in MeOH followed by addition of 2,2′-dithiodipyridine and Et_3N.

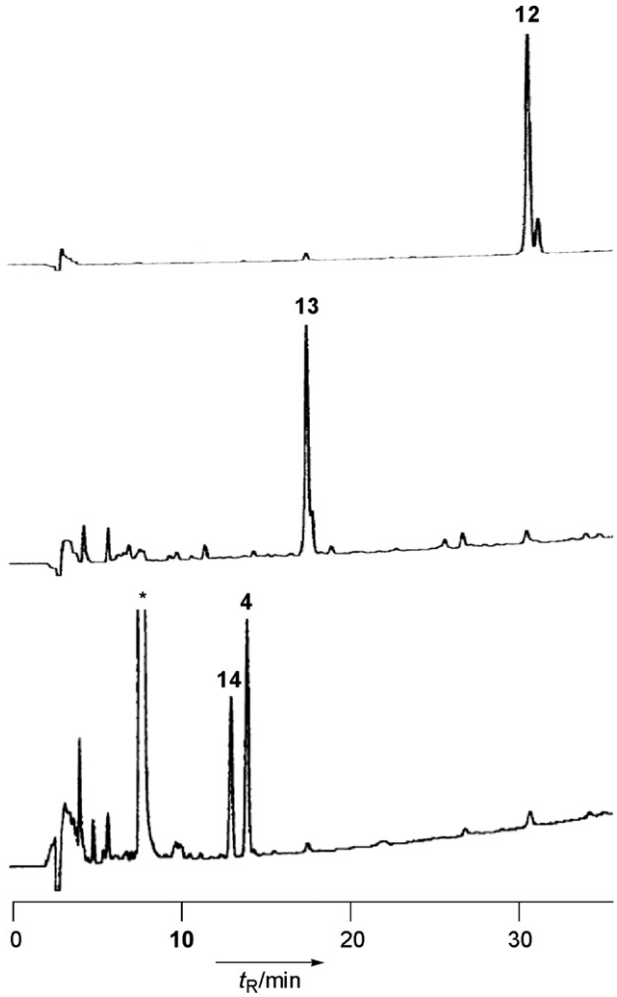
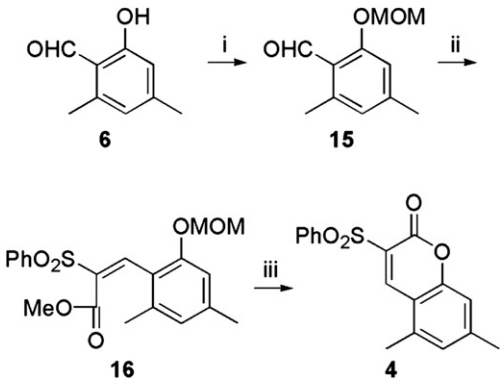


Fig. 1. HPLC profiles of photo-reaction of compound **12**: (a) before UV-irradiation, (b) after UV-irradiation (>365 nm, 5 min), (c) after UV-irradiation (>365 nm, 3 min) followed by addition of 2,2′-dithiodipyridine and Et_3N and shaking for 2 h. Analytical HPLC conditions: linear gradient of solvent B in solvent A, 50–80% over 30 min. Compounds eluted at 3–6 min are derivatives of photo-cleaved *o*-nitrobenzyl group. *2,2′-Dithiodipyridine.

Next, the catch-and-release reaction of a model peptide derived from a cysteine protease was examined. In this experiment, model peptide **17**[11] derived from the active site of caspase-9[12] was used,



Scheme 7. Reagents and conditions: (i) Chloromethyl methyl ether, DIEA, CH_2Cl_2, 81%; (ii) $PhSO_2CH_2CO_2Me$, AcOH, piperidine, toluene, reflux, 94%; (iii) TFA, CH_2Cl_2, 96%.

and each step of the catch-and-release reaction was monitored by HPLC (Scheme 8). In MeCN/phosphate buffer (pH 7.8), peptide **17** was treated with excess catch-and-release reagent **5**, and caged peptide **18** was obtained after 24 h of incubation at 37 °C (Fig. 2(a), (b)). Next purified peptide **18** was irradiated by UV (>365 nm, 5 min), and the *o*-nitrobenzyl group of **18** was removed to generate phenol **19** (Fig. 2(c), (d)). After 2 h of incubation at 37 °C in the presence of tris(2-carboxyethyl)phosphine (TCEP), release of caspase-9 model peptide **17** occurred along with generation of aromatized product **4** as major products (Fig. 2(e)).[13]

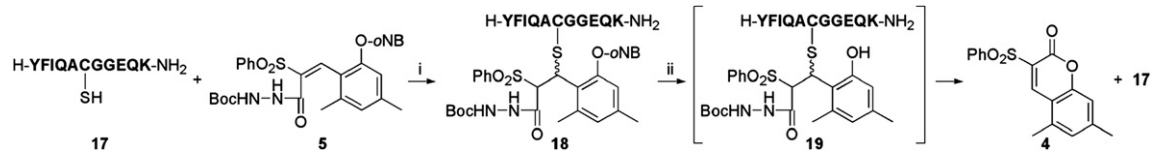
These results suggest that compound **5** can potentially work as a prototype of a catch-and-release reagent for controlling the activity of cysteine proteases.

2.4. Introduction of recognition moiety onto catch-and-release reagent

Introduction of a recognition moiety, such as a substrate peptide sequence of a target protease to the catch-and-release reagent is of value for selective caging of the target protease in a proteome.[4,5] Previously, one of the authors (K.A.) reported that a peptidyl substrate derivative of SARS cysteine protease (SARS $3CL^{pro}$) possessing an aldehyde at the C-terminal position acted as a reversible inhibitor of SARS $3CL^{pro}$.[14] Therefore, we decided to introduce catch-and-release reagent **5** onto the C-terminus of peptidyl substrates to control the activity of SARS $3CL^{pro}$ (Scheme 9).[15] Peptide conjugates **22** and **23** were synthesized as follows. After removal of the Boc group of **5**, the obtained product was coupled with Boc-protected glutamine to generate compound **21**. Then, the N-terminal Boc group was removed by acid treatment, and the obtained product was coupled with protected peptides **24** or **25**, which were synthesized using standard Boc-based solution phase peptide synthesis. Finally, the obtained materials were subjected to global deprotection to generate peptide conjugates **22** and **23**. Application of peptide conjugates **22** and **23** to control activity of SARS $3CL^{pro}$ is underway in our laboratory.

3. Conclusion

In conclusion, a catch-and-release reagent for thiols was developed. By using this reagent, catch and UV-induced release of thiols including an active site model peptide of caspase-9 were achieved. Introduction of a peptidyl recognition moiety onto the reagent was also successful. We believe that these results represent an important step toward the development of catch-and-release systems of cysteine proteases for controlling their activities. Application of the catch-and-release reagent to control activity of SARS $3CL^{pro}$ is underway.



Scheme 8. Reagents and conditions: (i) MeCN/phosphate buffer (pH 7.8)=1:1 (v/v), 37 °C; (ii) UV-irradiation (>365 nm, 5 min) in MeCN/phosphate buffer (pH 7.8)=1:1 (v/v) containing glutathione and EDTA followed by incubation at 37 °C in the presence of TCEP. (**A**: alanine; **C**: cysteine; **E**: glutamic acid; **F**: phenylalanine; **G**: glycine; **I**: isoleucine; **K**: lysine; **Q**: glutamine; **Y**: tyrosine).

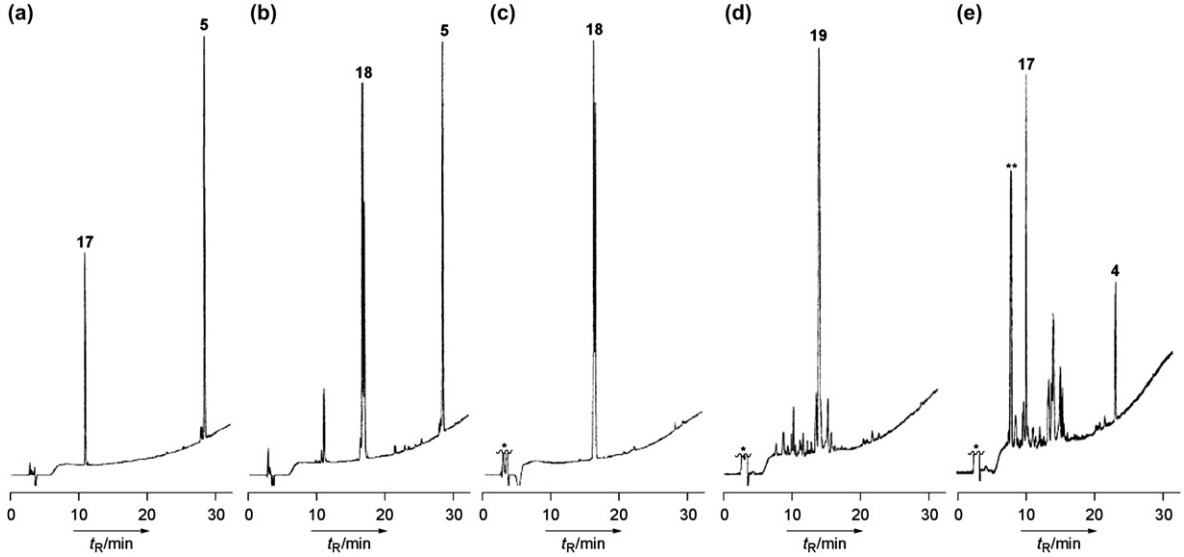
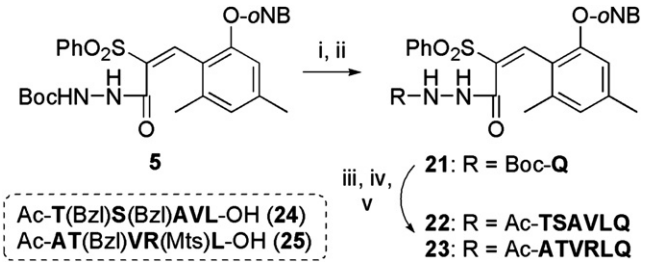


Fig. 2. HPLC profiles of catch-and-release reaction of compound **5**. Michael addition of peptide **17** to compound **5**: (a) 0 h, (b) 24 h. UV-irradiation experiment of compound **18**: (c) before UV-irradiation, (d) after UV-irradiation (>365 nm, 5 min), (e) after UV-irradiation (>365 nm, 5 min) followed by incubation at 37 °C in the presence of TCEP for 2 h. Analytical HPLC conditions: linear gradient of solvent B in solvent A, 10–95% over 30 min *Glutathione. **Non-peptidyl compound.



Scheme 9. Reagents and conditions: (i) TFA then HCl/AcOEt; (ii) Boc-**Q**-ONp, HOBt·H_2O, Et_3N, DMF, 85% (2 steps); (iii) TFA then HCl/AcOEt; (iv) **24** (for **22**) or **25** (for **23**), EDC·HCl, HOBt·H_2O, Et_3N, DMF; (v) TFA, TMSBr, thioanisole, *m*-cresol, 0 °C (**A**: alanine; **L**: leucine; **Q**: glutamine; **R**: arginine; **S**: serine; **T**: threonine; **V**: valine; Np=*p*-nitrophenyl).

4. Experimental section

4.1. General methods

All reactions were carried out under a positive pressure of argon except for UV-irradiation experiments and solid phase peptide synthesis. For column chromatography, Silica Gel 60 N (Kanto Chemical Co., Inc., spherical, neutral, particle size 63–210 μm) was employed. Exact mass spectra were recorded on a Waters Micromass® LCT Premier™ (ESI-TOF) or a Bruker Esquire200T (ESI-Ion Trap) spectrometer. NMR spectra were measured using a Bruker AV400N, a JEOL GSX400 or a JEOL GSX300 spectrometer. For HPLC separation, a Cosmosil 5C_{18}-AR-II analytical column (Nacalai Tesque, 4.6×250 mm, flow rate 1 mL/min), a Cosmosil 5C_{18}-AR-II semi-preparative column (Nacalai Tesque, 10×250 mm, flow rate 2.5 mL/min) or a Cosmosil 5C_{18}-AR-II preparative column (Nacalai Tesque, 20×250 mm, flow rate 10 mL/min) was employed, and eluting products were detected by UV at 220 nm. A solvent system consisting of 0.1% (v/v) TFA aqueous solution (solvent A) and 0.1% (v/v) TFA in MeCN (solvent B) was used for HPLC elution. Photolysis by UV-irradiation was performed using a Moritex MUV-202U with the filtered output (>365 nm) of a 3000 mW/cm^2 Hg–Xe lamp. Optical rotation was determined on a JASCO P-2200 polarimeter (concentration in g/100 mL).

4.2. Synthesis of catch-and-release reagent 5

*4.2.1. 2,4-Dimethyl-6-(2-nitrobenzyloxy)benzaldehyde (**7**).* To a stirred solution of phenol **6**[8] (400 mg, 2.66 mmol) in DMF (13.0 mL) were added K_2CO_3 (1.44 g, 10.4 mmol) and *o*-nitrobenzyl bromide (1.00 g, 4.60 mmol), and the resulting suspension was stirred at room temperature for 4 h. After addition of saturated aqueous solution of NH_4Cl followed by stirring for 1 h, the mixture was extracted with AcOEt. The organic layer was washed with saturated

aqueous solution of NH_4Cl and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (hexane/AcOEt=10:1 (v/v)) and 585 mg of benzaldehyde **7** (2.05 mmol, 77%) was obtained as pale yellow needles; mp: 129–130 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ=2.36 (3H, s), 2.58 (3H, s), 5.57 (2H, s), 6.71 (1H, s), 6.73 (1H, s), 7.54 (1H, t, J=8.0 Hz), 7.74 (1H, t, J=8.0 Hz), 7.94 (1H, d, J=8.0 Hz), 8.21 (1H, d, J=8.0 Hz), 10.75 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ=21.5, 22.2, 67.3, 110.9, 121.1, 125.0, 125.8, 128.2, 128.5, 133.1, 134.2, 1423, 145.9, 146.6, 161.4, 190.9; HRMS (ESI-TOF) m/z calcd for $C_{16}H_{16}NO_4$ ($[M+H]^+$) 286.1079, found 286.1090. Anal. calcd for $C_{16}H_{15}NO_4$ C, 67.36; H, 5.30; N, 4.91, found C, 67.20; H, 5.24; N, 5.00.

*4.2.2. N′-(2-Phenylsulfanylacetyl)hydrazinecarboxylic acid tert-butyl ester (**9**).* To a solution of phenylthioacetic acid **8** (7.00 g, 41.6 mmol) in CH_2Cl_2 (100 mL) were added DMAP (460 mg, 3.70 mmol), HBTU (15.0 g, 39.5 mmol), and DIEA (6.88 mL, 39.5 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 20 min, and *tert*-butyl carbazate (2.50 g, 37.8 mmol) was added to the reaction mixture at 0 °C. After being stirred at room temperature for 11 h, the resulting mixture was quenched by addition of H_2O and extracted with CH_2Cl_2. The organic layer was washed with 5% (w/v) aqueous solution of citric acid, 5% (w/v) aqueous solution of $NaHCO_3$ and brine, and was dried over Na_2SO_4. After concentration in vacuo, the crude product was purified by column chromatography (hexane/AcOEt=2:1 (v/v)) and 9.83 g of sulfide **9** (34.8 mmol, 92%) was obtained as colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ=1.43 (9H, s), 3.69 (2H, s), 6.40 (1H, br s), 7.22–7.26 (1H, m), 7.29–7.35 (2H, m), 7.35–7.40 (2H, m), 8.27 (1H, br s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ=28.1, 36.2, 82.0, 127.0, 129.0, 129.3, 134.4, 155.1, 167.5; HRMS (ESI-TOF) m/z calcd for $C_{13}H_{18}N_2NaO_3S$ ($[M+Na]^+$) 305.0936, found 305.0945.

*4.2.3. N′-(2-Benzenesulfonylacetyl)hydrazinecarboxylic acid tert-butyl ester (**10**).* To a solution of sulfide **9** (2.13 g, 7.56 mmol) in MeOH/H_2O (1:1 (v/v), 4.0 mL) was slowly added Oxone® (5.11 g, 8.31 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 15 h. The reaction mixture was extracted with CH_2Cl_2, and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography (hexane/AcOEt=1:2 (v/v)) and 1.75 g of sulfone **10** (5.57 mmol, 74%) was obtained as white powder; ^{1}H NMR ($CDCl_3$, 400 MHz) δ=1.47 (9H, s), 4.12 (2H, s), 6.73 (1H, br s), 7.58 (2H, dd, J=7.6 and 7.5 Hz), 7.69 (1H, tt, J=7.5 and 1.1 Hz), 7.99 (2H, dd, J=7.6 and 1.1 Hz), 8.71 (1H, br s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ=28.1, 60.5, 82.4, 128.5, 129.4, 134.5, 138.0, 155.1, 160.7; HRMS (ESI-TOF) m/z calcd for $C_{13}H_{18}N_2NaO_5S$ ($[M+Na]^+$) 337.0834, found 337.0833. Anal. calcd for $C_{13}H_{18}N_2O_5S$ C, 49.67; H, 5.77; N, 8.91, found C, 49.39; H, 5.55; N, 8.85.

*4.2.4. N′-{2-Benzenesulfonyl-3-[2,4-dimethyl-6-(2-nitrobenzyloxy)phenyl]-(E)-acryloyl}hydrazinecarboxylic acid tert-butyl ester (**5**).* Piperidine (52.0 μL, 531 μmol) and AcOH (60.0 μL, 1.06 mmol) were added to a solution of sulfone **10** (1.84 g, 5.85 mmol) and aldehyde **7** (1.50 g, 5.31 mmol) in toluene (60.0 mL). After refluxing for 18 h, H_2O was added to the reaction mixture at room temperature. The mixture was extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (hexane/AcOEt=3:1 (v/v)) and 2.62 g of catch-and-release reagent **5** (4.50 mmol, 85%) was obtained as pale yellow amorphousness; ^{1}H NMR ($CDCl_3$, 400 MHz) δ=1.35 (9H, s), 2.30 (3H, s), 2.78 (3H, s), 5.45 (2H, s), 6.30 (1H, br s), 6.60 (1H, s), 6.71 (1H, s), 7.45 (1H, td, J=7.6 and 1.2 Hz), 7.49–7.59 (3H, m), 7.62 (1H, tt, J=7.6 and 1.2 Hz), 7.71 (1H, d, J=7.6 Hz), 8.03 (2H, dd, J=7.6 and 1.2 Hz), 8.23 (1H, s), 8.27 (1H, d, J=4.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ=20.1, 21.8, 27.9, 67.2, 81.8, 110.8, 118.1, 124.4, 124.8, 128.3, 128.4, 128.5, 129.2, 133.4, 133.7, 134.1, 136.7, 138.4, 139.4, 141.9, 142.8, 146.6, 154.1, 154.9, 159.7; HRMS (ESI-TOF) m/z calcd for $C_{29}H_{31}N_3NaO_8S$ ($[M+Na]^+$) 604.1730, found 604.1746.

*4.2.5. 2-Benzenesulfonyl-3-[2,4-dimethyl-6-(2-nitrobenzyloxy)phenyl]-(E)-acrylic acid methyl ester (**11**).* Piperidine (5.0 μL, 40 μmol) and AcOH (5.0 μL, 87 μmol) were added to a solution of aldehyde **7** (110 mg, 0.396 mmol) and phenylsulfonylacetic acid methyl ester (99.0 mg, 0.462 μmol) in toluene (4.00 mL). The reaction mixture was refluxed for 17 h and was quenched by addition of H_2O at room temperature. The resulting mixture was extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (hexane/AcOEt=4:1 (v/v)) and 152 mg of compound **11** (0.320 mmol, 83%) was obtained as colorless prisms. Recrystallized material from Et_2O was brought to X-ray analysis; mp: 129–130 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ=2.32 (6H, s), 3.40 (3H, s), 5.33 (2H, s), 6.63 (1H, s), 6.75 (1H, s), 7.30 (1H, t, J=7.6 Hz), 7.40 (1H, t, J=7.6 Hz), 7.49 (1H, d, J=7.6 Hz), 7.51 (2H, t, J=7.6 Hz), 7.65 (1H, t, J=7.6 Hz), 8.01 (2H, d, J=7.6 Hz), 8.10 (1H, d, J=7.6 Hz), 8.33 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ=20.1, 21.8, 52.0, 67.4, 110.9, 118.7, 124.5, 124.7, 128.1, 128.2, 128.5, 128.8, 132.7, 133.2, 133.9, 136.1, 138.6, 140.5, 1419, 143.5, 146.2, 155.0, 162.3; HRMS (ESI-TOF) m/z calcd for $C_{25}H_{24}NO_7S$ ($[M+H]^+$) 482.1273, found 482.1273. Anal. calcd for $C_{25}H_{23}NO_7S$ C, 62.36; H, 4.81; N, 2.91, found C, 62.13; H, 4.79; N, 2.99.

4.3. Catch-and-release experiment of compound 5

4.3.1. Michael addition of benzyl mercaptan. To a solution of catch-and-release reagent **5** (40.0 mg, 69.7 μmol) in CH_2Cl_2 (700 μL) were added benzyl mercaptan (60.0 μL, 510 μmol) and Et_3N (71.0 μL, 510 μmol) with additional stirring at room temperature for 6 h. After addition of H_2O, the mixture was extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography (hexane/AcOEt=4:1 (v/v)) and 40.0 mg of diastereomeric mixture **12** (56.6 μmol, 82%) was obtained as colorless amorphousness. ^{1}H NMR ($CDCl_3$, 400 MHz, peaks derived form a major isomer was depicted) δ=1.50 (9H, s), 1.83 (3H, s), 2.09 (3H, s), 3.78 (1H, d, J=13.1 Hz), 3.94 (1H, d, J=13.1 Hz), 4.74 (1H, d, J=11.2 Hz), 5.02 (2H, s), 5.30 (1H, d, J=11.2 Hz), 5.76 (1H, s), 6.46 (1H, s), 7.00 (1H, br s), 7.13–7.20 (2H, m), 7.20–7.30 (6H, m), 7.38 (2H, d, J=7.3 Hz), 7.48 (1H, tt, J=7.6 and 1.2 Hz), 7.72 (1H, t, J=7.6 Hz), 7.95 (1H, d, J=7.6 Hz), 8.15 (1H, dd, J=8.0 and 1.0 Hz), 8.25 (1H, br s); HRMS (ESI-TOF) m/z calcd for $C_{36}H_{39}N_3NaO_8S_2$ ($[M+Na]^+$) 728.2076, found 728.2053.

*4.3.2. UV-irradiation experiment of compound **12**.* Compound **12** (33 μg, 0.47 μmol) in MeOH (100 μL) was irradiated by UV (>365 nm) for 5 min. To the reaction mixture were added 2,2′-dithiodipyridine (200 μg, 0.90 μmol) and Et_3N (5.0 μL, 35 μmol) at 0 °C, and the obtained mixture was shaken at room temperature for 2 h. Progress of the reaction was monitored by analytical HPLC. Analytical HPLC conditions: linear gradient of solvent B in solvent A, 50–80% over 30 min. Retention time: **12**, 29.3 and 29.8 min; **13**, 16.8 and 17.1 min; **4**, 13.4 min; **14**, 12.5 min. Intermediate **13** was identified by LRMS (ESI-TOF) m/z calcd for $[M+H]^+$ 571.2, found 571.2 (diastereomeric mixture). Compounds **4** and **14** were identified by RLMS and comparison of retention time with that of authentic samples. Compound **4**: LRMS (ESI-TOF) m/z calcd for $[M+H]^+$ 315.1, found 315.2. Compound **14**:[10] LRMS (ESI-TOF) m/z calcd for $[M+H]^+$ 234.0, found 234.2.

*4.3.3. Caspase-9 model peptide (**17**).* The protected peptidyl resin was manually constructed using Fmoc-based solid phase peptide

synthesis on NovaSyn® TGR resin (loading: 0.25 mmol/g). Fmoc-protected amino acid derivatives (3 equiv) were successively condensed using *N*,*N*′-diisopropylcarbodiimide (3 equiv) in the presence of HOBt·H_2O (3 equiv) in DMF for 2 h. The following side-chain protective groups were used: Trt for cysteine and glutamine, ^{t}Bu for glutamic acid, and Boc for lysine. Fmoc group was removed by treatment of the resin with 20% (v/v) piperidine in DMF for 10 min. The completed resin (41.0 mg) was treated with TFA/thioanisole/*m*-cresol/1,2-ethanedithiol/Et_3SiH/H_2O (80:5:5:2.5:2.5:5 (v/v), 2.0 mL) at room temperature for 2 h. After filtration, cooled Et_2O was added to the filtrate, and the resulting precipitate was collected by centrifugation. The obtained precipitate was washed with Et_2O and purified by preparative HPLC to give model peptide **17** (1.0 mg, 10%). Analytical HPLC conditions: linear gradient of solvent B in solvent A, 1–80% over 30 min. Retention time: 13.0 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 0–30% over 60 min. LRMS (ESI-Ion Trap) *m*/*z* calcd for $[M+H]^+$ 1242.6, found 1242.8.

*4.3.4. Michael addition of model peptide **17**.* To a solution of model peptide **17** (2.00 mg, 1.60 μmol) in sodium phosphate buffer (pH 7.8, 50 mM phosphate, 2.0 mL) was added catch-and-release reagent **5** (4.00 mg, 6.87 μmol) in MeCN (2.0 mL). After incubation at 37 °C followed by HPLC purification, peptide conjugate **18** (0.78 mg, 28%) was obtained as a diastereomeric mixture. Analytical HPLC conditions: linear gradient of solvent B in solvent A, 10–95% over 30 min. Retention time: 16.9, 17.2 or 17.4 min, respectively, for each diastereomer. Semi-preparative HPLC conditions: linear gradient of solvent B in solvent A, 29–39% over 30 min. LRMS (ESI-TOF) *m*/*z* calcd for $[M+2H]^{2+}$ 912.4, found 912.1, 912.1 or 912.1, respectively.

*4.3.5. UV-irradiation experiment of compound **18**.* A solution of glutathione (reduced form, 0.25 M, 8.0 μL) in MeCN (100 μL) was added to compound **18** (0.10 mg, 10 μmol) in sodium phosphate buffer (pH 7.8, 50 mM phosphate, 2 mM EDTA, 92 μL), and the resulting mixture was irradiated by UV (>365 nm) for 5 min. After addition of an aqueous solution of TCEP·HCl (0.10 M, 30 μL), the reaction solution was incubated at 37 °C and the reaction progress was monitored by analytical HPLC. Analytical HPLC conditions: linear gradient of solvent B in solvent A, 10–95% over 30 min. Retention time: **19**, 17.1 or 17.3 min, respectively, for each diastereomer; **4**, 25.0 min; **17**, 10.9 min. LRMS (ESI-Ion Trap) of diastereomeric mixture of intermediate **19**: *m*/*z* calcd for $[M+H]^+$ 1688.7, found 1688.5.

*4.3.6. 2-Methoxymethoxy-4,6-dimethylbenzaldehyde (**15**).* To a stirred solution of phenol **6**[8] (95.0 mg, 0.633 mmol) in CH_2Cl_2 (5.00 mL) were added chloromethyl methyl ether (127 mg, 1.58 mmol) and DIEA (0.550 mL, 3.15 mmol), and the resulting mixture was stirred at room temperature for 23 h. The reaction was quenched with 5% (w/v) aqueous solution of $NaHCO_3$ at 0 °C and the resulting mixture was extracted with Et_2O. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (hexane/AcOEt=4:1 (v/v)) and 99.0 mg of aldehyde **15** (0.510 mmol, 81%) was obtained as colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ=2.34 (3H, s), 2.55 (3H, s), 3.52 (3H, s), 5.26 (2H, s), 6.69 (1H, s), 6.86 (1H, s), 10.61 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ=21.4, 22.0, 56.3, 94.5, 112.8, 121.4, 126.0, 141.6, 145.5, 160.9, 191.4; HRMS (ESI-TOF) *m*/*z* calcd for $C_{11}H_{14}NaO_3$ ($[M+Na]^+$) 217.0841, found 217.0835.

*4.3.7. 2-Benzenesulfonyl-3-(2-methoxymethoxy-4,6-dimethylphenyl)acrylic acid methyl ester (**16**).* Piperidine (2.0 μL, 200 μmol) and AcOH (2.0 μL, 35 μmol) were added to a solution of aldehyde **15** (90.0 mg, 463 μmol) and phenylsulfonylacetic acid methyl ester (97.0 mg, 453 μmol) in toluene (5.00 mL). The resulting mixture was refluxed for 22 h and was quenched by addition of H_2O at room temperature. The mixture was extracted with AcOEt, and the organic layer was washed with H_2O and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (hexane/AcOEt=4:1 (v/v)) and 170 mg of acrylate **16** (435 μmol, 94%) was obtained as pale yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ=2.31 (3H, s), 2.32 (3H, s), 3.19 (3H, s), 3.57 (3H, s), 4.93 (2H, s), 6.73 (1H, s), 6.74 (1H, s), 7.54 (2H, t, *J*=7.2 Hz), 7.61 (1H, t, *J*=7.2 Hz), 8.01 (2H, d, *J*=7.2 Hz), 8.17 (1H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ=20.1, 21.6, 51.9, 56.1, 94.5, 112.8, 119.1, 124.7, 128.2, 128.6, 133.1, 135.8, 138.6, 140.4, 141.8, 142.3, 154.1, 162.7; HRMS (ESI-TOF) *m*/*z* calcd for $C_{20}H_{22}NaO_6S$ ($[M+Na]^+$) 413.1035, found 413.1049.

*4.3.8. 3-Benzenesulfonyl-5,7-dimethylchromen-2-one (**4**).* To a solution of acrylate **16** (49.0 mg, 125 μmol) in CH_2Cl_2 (1.00 mL) was added TFA (1.00 mL), and the resulting mixture was stirred at room temperature for 2 h. After addition of H_2O, the mixture was extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated in vacuo, and 39.0 mg of compound **4** (120 μmol, 96%) was obtained as yellow solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ=2.43 (3H, s), 2.61 (3H, s), 6.99 (1H, s), 7.03 (1H, s), 7.55 (2H, t, *J*=7.6 Hz), 7.63 (1H, t, *J*=7.6 Hz), 8.14 (2H, d, *J*=7.6 Hz), 8.93 (1H, s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ=18.4, 22.1, 114.1, 115.1, 128.0, 128.9, 129.0, 129.3, 134.0, 138.8, 144.3, 144.4, 147.3, 155.1, 156.4; HRMS (ESI-TOF) *m*/*z* calcd for $C_{17}H_{15}O_4S$ ($[M+H]^+$) 315.0691, found 315.0698.

4.4. Introduction of recognition moiety

4.4.1. General procedures for Boc-based solution phase peptide synthesis. Deprotection procedure A: A substrate was treated with TFA (5–10 mL/g substrate) for 1.5 h at 0 °C. After evaporation, HCl/AcOEt (4 M, 4 equiv) was added to the residues and the mixture was stirred for 3 min and concentrated in vacuo. The obtained residue was dried over KOH pellets under reduced pressure.

Deprotection procedure B: A substrate was treated with TFA (5–10 mL/g substrate) for 1.5 h at 0 °C. After evaporation, the obtained residue was dried over KOH pellets under reduced pressure.

Purification procedure A: A material soluble in AcOEt was extracted with AcOEt and the extract was washed successively with 5% (w/v) aqueous solution of $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated under reduced pressure.

Purification procedure B: A material insoluble in AcOEt was precipitated by the addition of an appropriate solvent. The resulting solid was filtered, washed successively with 5% (w/v) aqueous solution of citric acid, 5% (w/v) aqueous solution of $NaHCO_3$ and H_2O, and dried in vacuo.

*4.4.2. [(1S)-1-(N′-{2-Benzenesulfonyl-3-[2,4-dimethyl-6-(2-nitrobenzyloxy)phenyl]-(E)-acryloyl}hydrazinocarbonyl)-3-carbamoylpropyl]carbamic acid tert-butyl ester (**21**).* Compound **5** (2.00 g, 3.43 mmol) was subjected to deprotection procedure A. To solution of the resulting residue in DMF (10 mL), Et_3N (947 μL, 6.80 mmol), Boc-**Q**-ONp (1.25 g, 3.43 mmol), and HOBt·H_2O (701 mg, 3.43 mmol) were added at 0 °C. The reaction mixture was stirred at room temperature for 4.5 h. After concentration in vacuo, the resulting residue was subjected to purification procedure A and the obtained material was purified by column chromatography ($CHCl_3$/MeOH=19:1 (v/v)) and 2.06 g of compound **21** (2.90 mmol, 85%) was obtained as colorless amorphousness; $[\alpha]_D^{17}$ −16.7 (*c* 1.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ=1.37 (9H, s), 1.70–1.90 (2H, m), 2.06–2.18 (2H, m), 2.67 (6H, s), 4.02–4.16 (1H, br m), 5.38 (1H, d, *J*=15.4 Hz), 5.42 (1H, d, *J*=15.4 Hz), 5.50 (1H, d, *J*=7.6 Hz), 6.00 (1H, br s), 6.37 (1H, br s), 6.60 (1H, s), 6.68 (1H, s), 7.44 (1H, t, *J*=8.0 Hz), 7.46–7.55 (3H, m), 7.58 (1H, tt, *J*=7.2 and 1.2 Hz), 7.67 (1H, d, *J*=7.6 Hz), 7.97 (2H, d, *J*=7.6 Hz), 8.08 (1H, dd, *J*=8.0 and 1.2 Hz), 8.21

(1H, s), 8.74 (1H, br s), 9.96 (1H, br s); ^{13}C NMR ($CDCl_3$, 75 MHz) δ=20.0, 21.6, 28.2, 29.2, 31.3, 51.7, 66.9, 80.0, 110.7, 117.8, 124.2, 124.6, 128.1, 128.3, 128.4, 129.1, 133.2, 133.6, 133.9, 136.4, 138.6, 139.4, 142.0, 146.6, 155.1, 155.7, 159.1, 169.6, 175.6; HRMS (ESI-TOF) *m*/*z* calcd for $C_{34}H_{39}N_5NaO_{10}S$ ([M+Na]$^+$) 732.2315, found 732.2339.

*4.4.3. Synthesis of peptide conjugate **22** and **23**.* Typical procedure. Compound **21** (94.3 mg, 133 μmol) was treated with TFA (1 mL) at 0 °C for 1.5 h. After evaporation, HCl/AcOEt (4 M, 200 μL) was added to the residue at 0 °C. The mixture was stirred at same temperature for 3 min, and then evaporated. The obtained residue was dissolved in DMF (1 mL), and to the solution were added Et_3N (38 μL, 273 μmol), peptide **24** (100 mg, 140 μmol), HOBt·H_2O (29 mg, 140 μmol), and EDC·HCl (27 mg, 140 μmol) at 0 °C. The reaction mixture was stirred at room temperature overnight. After concentration in vacuo, the resulting residue was subjected to purification procedure B and reprecipitation from DMF/AcOEt to give 119 mg of the protected catch-and-release reagent (91.2 mmol, 70%) as white powder. Then, the product (50.0 mg, 383 μmol) was treated with TMSBr (215 μL), thioanisole (192 μL), *m*-cresol (81.1 μL), and TFA (1.21 mL) at 0 °C for 1.5 h. To the resulting mixture was added cooled Et_2O and the generated precipitate was collected by centrifugation. The precipitate was washed with Et_2O and purified by preparative HPLC to give 12.3 mg of peptide conjugate **22** (10.9 μmol, 29%). Protected precursor of compound **22**: LRMS (ESI-Ion Trap) *m*/*z* calcd for [M+K]$^+$ 1341.5, found 1341.6. Protected precursor of compound **23**: LRMS (ESI-Ion Trap) *m*/*z* calcd for [M+H]$^+$ 1464.6, found 1464.6. Compound **22**: analytical HPLC conditions: linear gradient of solvent B in solvent A, 30–80% over 30 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 40–50%. LRMS (ESI-Ion Trap) *m*/*z* calcd for [M+H]$^+$ 1123.5, found 1123.8. Compound **23**: analytical HPLC conditions: linear gradient of solvent B in solvent A, 30–80% over 30 min. Preparative HPLC conditions: linear gradient of solvent B in solvent A, 37–47%. LRMS (ESI-Ion Trap) *m*/*z* calcd for [M+2H]$^+$ 596.8, found 597.0.

*4.4.4. Synthesis of protected peptide **24**.* To a solution of H-**L**-OPac (prepared from Boc-L-OPac[16] (2.00 g, 5.72 mmol) according to deprotection procedure A. Pac: phenacyl), Et_3N (1.67 mL, 12.0 mmol), Boc-**V**-OH (1.37 g, 6.29 mmol) and HOBt·H_2O (1.30 g, 6.29 mmol) in DMF (20 mL), EDC·HCl (1.21 g, 6.29 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature overnight. After concentration in vacuo, the resulting residue was subjected to purification procedure A, and reprecipitation form hexane to give 1.30 g of Boc-**VL**-OPac (2.89 mmo, 46%).

Boc protected amino acids were coupled onto Boc-**VL**-OPac (1.30 g, 2.89 mmol) as similar to that described above. Procedure and yield are summarized below.

Boc-**A**-OH: Deprotection of substrate=procedure A; purification=procedure B; reprecipitation form AcOEt; 85% yield.

Boc-**S**(Bzl)-OH: deprotection of substrate=procedure A; purification=procedure B; reprecipitation from $CHCl_3$/Et_2O; 89% yield.

Boc-**T**(Bzl)-OH: deprotection of substrate=procedure A; purification=procedure B; reprecipitation form $CHCl_3$/Et_2O; 80% yield.

To a solution of TFA·H-**T**(Bzl)**S**(Bzl)**AVL**-OPac (prepared from Boc-**T**(Bzl)**S**(Bzl)**AVL**-OPac (350 mg, 338 μmol) according to deprotection procedure B) in pyridine/DMF (1:1 (v/v), 4 mL) was added Ac_2O (319 μL, 3.38 mmol). The reaction mixture was stirred at room temperature overnight. After concentration in vacuo, the resulting residue was subjected to purification procedure B and reprecipitation from DMF/AcOEt to give 226 mg of Ac-**T**(Bzl)**S**(Bzl)**AVL**-OPac (272 μmol, 81%). It was suspended in AcOH/DMF (9:1 (v/v), 3.50 mL), and Zn dust (356 mg, 5.44 mmol) was added to the suspension. After stirring the mixture at room temperature for 3 h followed by filtration, the filtrate was concentrated in vacuo. The residue was subjected to purification procedure B and reprecipitation form DMF/AcOEt to give 170 mg of peptide **24** (238 μmol, 88%) as white powder; LRMS (ESI-Ion Trap) *m*/*z* calcd for [M+H]$^+$ 712.4, found 712.4.

*4.4.5. Synthesis of protected peptide **25**.* To a solution of Boc-**R**(Mts)-OH (3.15 g, 6.29 mmol) in THF (20 mL) were added isobutyl chloroformate (816 μL, 6.29 mmol) and Et_3N (1.67 mL, 12.0 mmol) at −21 °C, and the reaction mixture was stirred at same temperature for 20 min. The resulting mixture was added to a solution of TFA·H-**L**-OPac (prepared from Boc-**L**-OPac[16] (2.00 g, 5.72 mmol) according to deprotection procedure B) in DMF at 0 °C. After overnight reaction at room temperature, the solution was concentrated in vacuo. The product was subjected to purification procedure A and reprecipitation from Et_2O to give 2.51 g of Boc-**R**(Mts)**L**-OPac (3.64 mmol, 64%).

Boc protected amino acids were coupled onto Boc-**R**(Mts)**L**-OPac (2.40 g, 3.49 mmol) as similar to that described in Section 4.4.4. Procedure and yield are summarized below.

Boc-**V**-OH: deprotection of substrate=procedure A; purification=procedure A; reprecipitation form hexane; 97% yield.

Boc-**T**(Bzl)-OH: deprotection of substrate=procedure A; purification=procedure B; reprecipitation from AcOEt/hexane; 84% yield.

Boc-**A**-OH: deprotection of substrate=procedure A; purification=procedure B; reprecipitation form Et_2O; 95% yield.

Acetyl capping and removal of Pac group were performed as similar to that described in Section 4.4.4. Acetyl cap: deprotection procedure B; purification procedure B; reprecipitation from DMF/AcOEt; 93% yield. Removal of Pac group: purification procedure B; reprecipitation from DMF/AcOEt; 82% yield; LRMS (ESI-Ion Trap) *m*/*z* calcd for [M+H]$^+$ 873.5, found 873.5.

Acknowledgements

We thank Prof. Dr. K. Miyamoto, The University of Tokushima, for his support in X-ray analysis. This research was supported in part by a Grant-in-Aid for Scientific Research on Innovative Areas 'Fusion Materials: Creative Development of Materials and Exploration of Their Function through Molecular Control' (No. 2206) from the Ministry of Education, Culture, Sports, Science and Technology, Japan (MEXT), Young Scientists (B), Scientific Research (B), and Challenging Exploratory Research from the Japanese Society for the Promotion of Science (JSPS). The Takeda Science Foundation and the Astellas Foundation for Research on Metabolic Disorders are also acknowledged. J.Y. is grateful for a SUNBOR Scholarship.

Supplementary data

Crystallographic data of **11** has been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC 839726.

Supplementary data related to this article can be found online at doi:10.1016/j.tet.2011.09.062.

References and notes

1. Leung-Toung, R.; Zhao, Y.; Li, W.; Tam, T. F.; Karimian, K.; Spino, M. *Curr. Med. Chem.* **2006**, *13*, 547–581 and references cited therein.
2. Loudwig, S.; Bayley, H. In *Dynamics Studies in Biology: Phototriggers, Photoswitches and Caged Biomolecules*; Goeldner, M., Givens, R., Eds.; Wiley: Hoboken, NJ, 2005; pp 253–304; and references therein.
3. (a) Loudwig, S.; Nicolet, Y.; Masson, P.; Fontecilla-Camps, J. C.; Bon, S.; Nachon, F.; Goeldner, M. *ChemBioChem* **2003**, *4*, 762–767; (b) Thuring, J. W.; Li, H.; Porter, N. A. *Biochemistry* **2002**, *41*, 2002–2013; (c) Porter, N. A.; Thuring, J. W.; Li, H. *J. Am. Chem. Soc.* **1999**, *121*, 7716–7717; (d) Arabaci, G.; Guo, X.-C.; Beebe, K. D.; Coggeshall, K. M.; Pei, D. *J. Am. Chem. Soc.* **1999**, *121*, 5085–5086; (e) Curley, K.; Lawrence, D. S. *J. Am. Chem. Soc.* **1998**, *120*, 8573–8574.

4. Reviews including target enzyme selective S-alkylation in living cells, see: (a) van der Hoorn, R. In *Chemical Biology*; Waldmann, H., Janning, P., Eds.; Wiley-VCH: Weinheim, 2009; pp 69–78; (b) Evans, M. J.; Cravatt, B. F. *Chem. Rev.* **2006**, *106*, 3279–3301; (c) Powers, J. C.; Asgian, J. L.; Ekici, O. D.; James, K. E. *Chem. Rev.* **2002**, *102*, 4639–4750.
5. Selected examples of target protein specific S-alkylation in living cells or in lysate, see: (a) Perez, D. I.; Palomo, V.; Perez, C.; Gil, C.; Dans, P. D.; Luque, F. J.; Conde, S.; Marinez, A. *J. Med. Chem.* **2011**, *54*, 4042–4056; (b) Lee, H.-M.; Xu, W.; Lawrence, D. S. *J. Am. Chem. Soc.* **2011**, *133*, 2331–2333; (c) Weerapana, E.; Wang, C.; Simon, G. M.; Richter, F.; Khare, S.; Dillon, M. B. D.; Bachovchin, D. A.; Mowen, K.; Baker, D.; Cravatt, B. F. *Nature* **2010**, *468*, 790–795; (d) Gallagher, S. S.; Sable, J. E.; Sheetz, M. P.; Cornish, V. W. *ACS Chem. Biol.* **2009**, *4*, 547–556; (e) Nonaka, H.; Tsukiji, S.; Ojida, A.; Hamachi, I. *J. Am. Chem. Soc.* **2007**, *129*, 15777–15779; (f) Krusemark, C. J.; Belshaw, P. J. *Org. Biomol. Chem.* **2007**, *5*, 2201–2204; (g) Luo, Y.; Knuckley, B.; Bhatia, M.; Pellechia, P. J.; Thompson, P. R. *J. Am. Chem. Soc.* **2006**, *128*, 14468–14469; (h) Keppler, A.; Gendreizig, S.; Gronemeyer, T.; Pick, H.; Vogel, H.; Johnsson, K. *Nat. Biotechnol.* **2003**, *21*, 86–89; (i) Borodovsky, A.; Ovaa, H.; Kolli, N.; Gan-Erdene, T.; Wilkinson, K. D.; Ploegh, H. L.; Kessler, B. M. *Chem. Biol.* **2002**, *9*, 1149–1159; (j) Greenbaum, D.; Baruch, A.; Hayrapetian, L.; Darula, Z.; Burlingame, A.; Medzihradszky, K. F.; Bogyo, M. *Mol. Cell. Proteomics* **2002**, *1*, 60–68; (k) Greenbaum, D.; Medzihradszky, K. F.; Burlingame, A.; Bogyo, M. *Chem. Biol.* **2000**, *7*, 569–581; (l) Fry, D. W.; Bridges, A. J.; Denny, W. A.; Doherty, A.; Greis, K. D.; Hicks, J. L.; Hook, K. E.; Keller, P. R.; Leopold, W. R.; Loo, J. A.; McNamara, D. J.; Nelson, J. M.; Sherwood, V.; Smaill, J. B.; Trumpp-Kallmeyer, S.; Dobrusin, E. M. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12022–12027.
6. (a) Shigenaga, A.; Hirakawa, H.; Yamamoto, J.; Ogura, K.; Denda, M.; Yamaguchi, K.; Otaka, A. *Tetrahedron* **2011**, *67*, 3984–3990; (b) Shigenaga, A.; Yamamoto, J.; Nishioka, N.; Otaka, A. *Tetrahedron* **2010**, *66*, 7367–7372; (c) Shigenaga, A.; Yamamoto, J.; Sumikawa, Y.; Furuta, T.; Otaka, A. *Tetrahedron Lett.* **2010**, *51*, 2868–2871; (d) Shigenaga, A.; Yamamoto, J.; Hirakawa, H.; Ogura, K.; Maeda, N.; Morishita, K.; Otaka, A. *Tetrahedron Lett.* **2010**, *51*, 2525–2528; (e) Shigenaga, A.; Yamamoto, J.; Hirakawa, H.; Yamaguchi, K.; Otaka, A. *Tetrahedron* **2009**, *65*, 2212–2216; (f) Shigenaga, A.; Tsuji, D.; Nishioka, N.; Tsuda, S.; Itoh, K.; Otaka, A. *ChemBioChem* **2007**, *8*, 1929–1931.
7. (a) Jung, M. E.; Piizzi, G. *Chem. Rev.* **2005**, *105*, 1735–1766 and references therein; (b) Amsberry, K. L.; Borchardt, R. T. *J. Org. Chem.* **1990**, *55*, 5867–5877; (c) Caswell, M.; Schmir, G. L. *J. Am. Chem. Soc.* **1980**, *102*, 4815–4821; (d) Milstein, S.; Cohen, L. A. *J. Am. Chem. Soc.* **1972**, *94*, 9158–9165; (e) Borchardt, R. T.; Cohen, L. A. *J. Am. Chem. Soc.* **1972**, *94*, 9166–9174; (f) Milstien, S.; Cohen, L. A. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *67*, 1143–1147.
8. Knight, P. D.; Clarkson, G.; Hammond, M. L.; Kimberley, B. S.; Scott, P. *J. Organomet. Chem.* **2005**, *690*, 5125–5144.
9. For sensitive HPLC detection of released benzyl mercaptan, 2,2′-dithiodipyridine was added to convert the mercaptan to disulfide **14**.
10. Synthesis of disulfide **14**, see: Smith, A. B., III; Savinov, S. N.; Manjappara, U. V.; Chaiken, I. M. *Org. Lett.* **2002**, *4*, 4041–4044.
11. Peptide **17** was synthesized using standard Fmoc solid phase peptide synthesis.
12. Earnshaw, W. C.; Martins, L. M.; Kaufmann, S. H. *Annu. Rev. Biochem.* **1999**, *68*, 383–424 and references therein. For facile detection of the model peptide by UV absorption, one of phenylalanine of the active site of caspase-9 was replaced with tyrosine.
13. Whereas a glutathione was added to sequester photo-generated derivatives of an *o*-nitrobenzyl group and to suppress oxidation of the released peptide, disulfide bond formation of the peptide was observed. Therefore, TCEP was added to reduce them.
14. Akaji, K.; Konno, H.; Onozuka, M.; Makino, A.; Saito, H.; Nosaka, K. *Bioorg. Med. Chem.* **2008**, *16*, 9400–9408.
15. Fan, K.; Wei, P.; Feng, Q.; Chen, S.; Huang, C.; Ma, L.; Lai, B.; Pei, J.; Liu, Y.; Chen, J.; Lai, L. *J. Biol. Chem.* **2004**, *279*, 1637–1642.
16. Neya, M.; Hagiwara, D.; Miyazaki, Y.; Nakamura, T.; Hemmi, K.; Hashimoto, M. *J. Chem. Soc., Perkin Trans. 1* **1989**, 2187–2198.