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Design and synthesis of chromogenic thiopeptolide substrates as MetAPs active site probes

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Abstract—Twenty one chromogenic thiopeptolide substrates were designed and synthesized as the active site probes and analyzed with each S1 site of mutant residues and enzymes of wild-type MetAP1s. The preliminary enzymatic experiments indicate that cysteine 70 or 202, at either *Escherichia coli* or human MetAP1, played a crucial role in the methionine hydrolysis.

1. Introduction

The methionine aminopeptidases (MetAPs) represent a unique class of proteases, which are capable of removing the N-terminal methionine residue from nascent polypeptide chains.[1,2] Proteins synthesized in eukaryotic cells are initiated with an N-terminal methionine residue, and proteins synthesized in prokaryotes, mitochondria, and chloroplasts are initiated with an N-terminal formylmethionine residue. The formyl group is removed by peptide deformylase before MetAPs remove the N-terminal methionine in a nonprocessive manner. Removal of the initiator, methionine residue, is often required for post-translational modification to the N-terminus, such as myristoylation and acetylation, which lead to proper localization, targeting, and eventual degradation. The MetAPs were given more attention because antiangiogenic compounds such as fumagillin, ovalicin, TNP-470 and their derivatives, are potent enzyme inhibitors for the type II human MetAP enzyme, and the enzyme inhibition correlated with their anti-proliferation activity on endothelial cells.[3] The presence of MetAPs is essential for cell viability, and disruption of the gene is a lethal event for MetAP in *Escherichia coli* (*Ec*MetAP1) or *Salmonella typhimurium*.[4] Since the yeast *Saccharomyces cerevisiae* has both type I and type II MetAPs (*Sc*MetAP1 and *Sc*MetAP2), both genes need to be blocked to affect cell viability, and disruption of either gene alone will result in only a slow-growth phenotype.[5] Therefore, the MetAP enzymes present good targets for new antibiotic drug discovery, and inhibitors against MetAPs offer hope for a new treatment of bacterial and fungal infections.[4a]

MetAPs have shown remarkable specificity for substrates with a terminal methionine. MetAPs are also very selective for the penultimate residue, with the side chain length limited to <3.68 Å.[6] Usually, small and uncharged amino acids in the penultimate position (Gly, Ala, Ser, Thr, Pro, Val, and Cys) direct MetAPs to cleave the initiator methionine (Fig. 1).

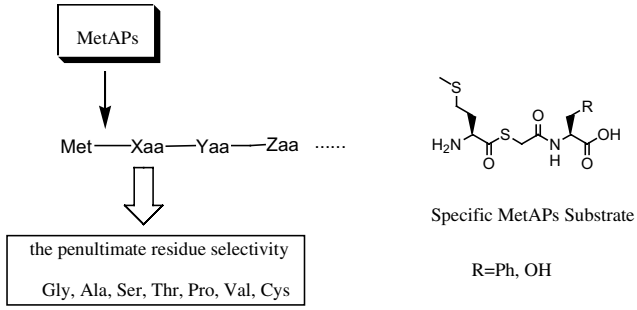

Figure 1. Specificity for substrates of MetAPs.

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A continuous spectrophotometric assay has been developed for detecting MetAP activity,[7] using a thioester substrate (thiopeptolide). Upon enzymatic removal of the N-terminal methionine, a free thiol group is generated. The released thiol is quantitated using Ellman's reagent DTNB. The MetAP reaction is conveniently monitored at 412 nm on a UV–vis spectrophotometer in a continuous fashion, with the addition of an excess of DTNB into the assay reaction. Two tripeptide analogues Met-S-Gly-Phe and Met-S-Gly-Ala were synthesized and found to be excellent substrates for MetAPs. Because of the highly specificity of MetAPs toward its substrate, any change in the P1 position of the tripeptide substrate will result in loss of enzymatic hydrolyzing action. Therefore MetAPs S1 site mutants, the analogues of the thiopeptide substrate Met-S-Gly-Phe, can be used as probe to reveal the importance of mutated residues in interaction with its specific substrates. The information gathering from these investigations will also be important for indicating the interactions between the small molecular inhibitor and MetAPs and useful for new inhibitor design.

For type I MetAPs, most of the residues involved at the S1 site were associated with the MetAP activity toward the same normal substrate. However, whether these mutations change the substrate specificity of the enzyme remains to be revealed.

In the present paper, two types of chromogenic thiopeptolide substrates Xaa-S-Gly-Phe and Met-S-Yaa-Ser (See Fig. 2; Xaa and Yaa indicate any natural or unnatural amino acid) were designed and synthesized as the active site probes and analyzed with each S1 site of mutant residues and enzymes of wild-type MetAP1s (Fig. 2).

The above mentioned are based on the tripeptide Met-Ala-Ser and Met-Gly-Phe, with replacement of the peptide bond between the first and second residues of a cleavable and chromogenic thioester bond, and variations at the first (P1) Xaa residue from methionine to other amino acids (Xaa-S-Gly-Phe), or the second (P1′) Yaa residue from favorable (Gly, Ala) to unfavorable (Phe, Leu, Met) residues (Met-S-Yaa-Ser).

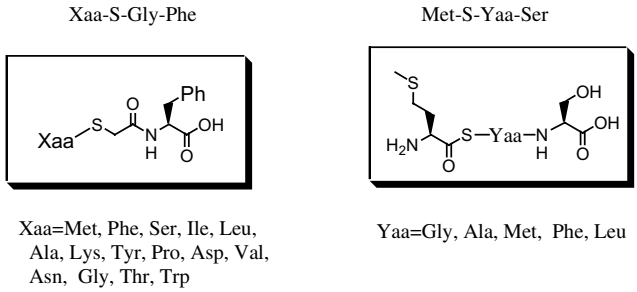


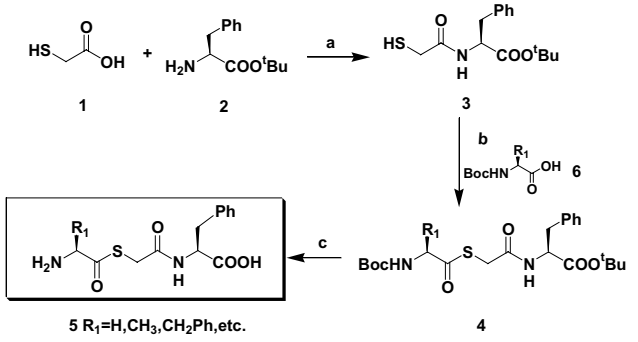
Figure 2. Proposed chromogenic thiopeptolide substrates Xaa-S-Gly-Phe, Met-S-Yaa-Ser.

2. Results and discussion

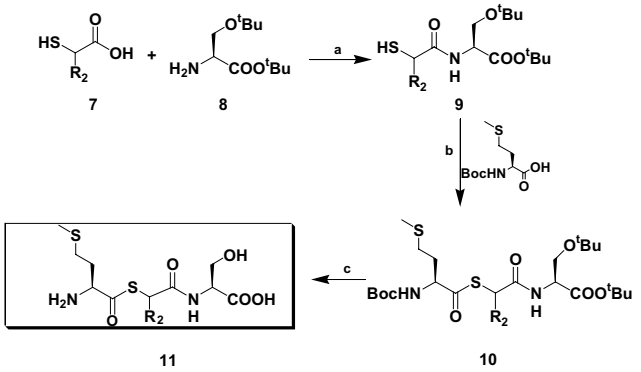
2.1. Chemistry

The synthesis of thiopeptolides Xaa-S-Gly-Phe (**5**, $R_1 = H$, CH_3, CH_2Ph, etc.) is straightforward and is outlined in Scheme 1, starting with the DCC catalyzed coupling of thioglycolic acid (**1**) and *t*-butyl protected phenylalanine (**2**). Further coupling with various protected amino acids (**6**) and removal of protecting groups will generate the desired thiopeptolide substrates (**5**) (Scheme 1).

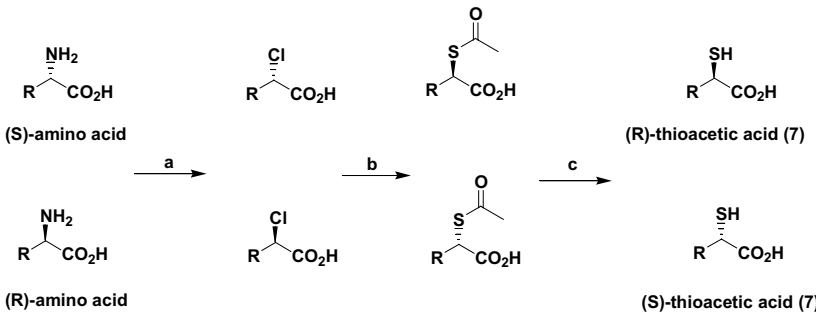
Using the same method, the thiopeptolides, Met-S-Yaa-Ser, were synthesized, starting with the DCC catalyzed coupling of 2-substituted 2-thiol acetic acid (**7**) and *t*-butyl protected serine (**8**). No substitution ($R = H$) at the 2-position corresponds to a Gly at the penultimate position, and substitution with methyl or benzyl ($R = CH_3$, CH_2Ph) will put Ala or Phe at the penultimate position. Further coupling with protected methionine and removal of protecting groups will generate the desired thiopeptolide substrates (**11**) (Scheme 2).



Scheme 1. Reagents and conditions: (a) DCC, HOBT, CH_2Cl_2, 4 Å molecular sieves, rt, overnight; (b) protected amino acid, DCC, DMAP, HOBT, CH_2Cl_2, 4 Å molecular sieves, rt, overnight; (c) TFA/DCM (v/v 1/2), overnight.



Scheme 2. Reagents and conditions: (a) DCC, HOBT, CH_2Cl_2, 4 Å molecular sieves, rt, overnight; (b) N-Boc-methionine, DCC, DMAP, HOBT, CH_2Cl_2, 4 Å molecular sieves, rt, overnight; (c) TFA/DCM (v/v 1/2), overnight.



Scheme 3. Reagents and conditions: (a) $NaNO_2$, 5 N HCl, 0 °C, 5 h, rt, overnight; (b) $CsSCOCH_3$, DMF, rt, overnight; (c) 2 N $NH_3 \cdot H_2O$, DTT, rt, 5 h.

The syntheses of the thioacetic acids (**7**) with 2-substitution were accomplished as shown in Scheme 3, starting with diazotation of α-amino acids to form α-halogeno acids with retention of configuration[8] followed by substitution with a cesium thiolate with inversion of configuration.[9] Removal of the acetyl group was then accomplished with aqueous ammonia to give the thiols (**7**). This reaction was carried out in the presence of dithiothreitol (DTT) to suppress disulfide formation. Using this method, (*S*)-amino acid gave (*R*)-thioacetic acid and (*R*)-amino acid gave (*S*)-thioacetic acid (Scheme 3).

2.2. Catalytic efficiencies in hydrolyzing thiopeptide substrates by wild-type and mutant MetAP1s

Fourteen S1 site mutants of *E. coli* (C59A, Y62A, Y65A, C70A, H79A, F177A, W221A) and human type I enzyme(P191A, Y194A, F197A, C202A, H211A, F308A, W352A) were prepared and assayed to evaluate their catalytic efficiencies in hydrolyzing synthesized thiopeptide substrates as our recent reports.[10,11] In the 15 synthesized thiopeptides Xaa-S-Gly-Phe, 14 of substrates (**5a**–**m**,**o**) can not be cleaved by wild-type *E. coli* and human type I enzyme, only the substrate with the methionine at the N-terminal (**5n**) can be cleaved as previous described,[7] which indicated the high selectivity of the MetAP enzyme for the N-terminal methionine at the substrate. From the mutants assay, it was found that most of the substrates can not be hydrolyzed, but remarkable activity (167.8 ± 28.9, 217.7 ± 43.3, 663.0 ± 36.1 μM/min/μM protein) was observed with leucine located at the N-terminal methionine position substrate by C59A, Y62A, and C70A mutants, with only C70A giving cleavage on the phenylalanine substrates (315.7 ± 21.7 μM/min/μM protein). This suggested that instead of these three residues, the small side chain of alanine of these three residues, especially cysteine 70, relaxed the leucine and phenylalanine as the N-terminal residues.

The similar relaxation of substrate specificity by *Hs*MetAP1 C202A reinforced the idea that cysteine 70 or 202, at either *E. coli* or human MetAP1, played a crucial role in the methionine hydrolysis. This can be favorably explained by the observation that cysteine 70 is one of two conservative residues (C70 and W221) in the hydrophobic S1 subsite of all type I MetAP enzymes (Table 1).

Table 1. Synthesized thiopeptolides

Compound no	Composition
5a	Ala-S-Gly-Phe
5b	Val-S-Gly-Phe
5c	Phe-S-Gly-Phe
5d	Ile-S-Gly-Phe
5e	Ser-S-Gly-Phe
5f	Leu-S-Gly-Phe
5g	Lys-S-Gly-Phe
5h	Tyr-S-Gly-Phe
5i	Asp-S-Gly-Phe
5j	Thr-S-Gly-Phe
5k	Pro-S-Gly-Phe
5l	Gly-S-Gly-Phe
5m	Trp-S-Gly-Phe
5n	Met-S-Gly-Phe
5o	Asn-S-Gly-Phe
11a	Met-S-(*S*)-Ala-Ser
11b	Met-S-Gly-Ser
11c	Met-S-(*S*)-Phe-Ser
11d	Met-S-(*R*)-Phe-Ser
11e	Met-S-(*R*)-Leu-Ser
11f	Met-S-(*R*)-Met-Ser

Evaluation of thiopeptolide substrates Met-S-Yaa-Ser (**11a**–**f**) cleavage by the wild-type *E. coli* and human type I enzyme was consistent with the reported result that only small and uncharged amino acids in the penultimate position of substrate(**11a**,**b**) can be cleaved.

3. Conclusion

In summary, 21 chromogenic thiopeptolide substrates were designed and synthesized as the active site probes and analyzed with each S1 site of mutant residues and enzymes of wild-type MetAP1s. The preliminary enzymatic experiments indicate that cysteine 70 or 202, at either *E. coli* or human MetAP1, played a crucial role in the methionine hydrolysis. More results about the activity of all substrates toward the other mutant in S1 and S1′ sites are in progress.

4. Experimental section

4.1. General methods

^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Varian Mercury-Vx300 Fourier transform spectrometer, using $CDCl_3$ or D_2O as solvent. ^{1}H chemical shifts were reported with $CHCl_3$ ($\delta = 7.26$ ppm) as internal standards, and ^{13}C chemical shifts with MeOH ($\delta = 49.15$ ppm, D_2O as solvent) as internal standards. 2-thioacetic acids **7** with substitution were synthesized according to Scheme 3.[8,9]

4.2. (2*S*)-2-(2-Mercapto-acetylamino)-3-phenyl-propionic acid *tert*-butyl ester (3)

A solution of *t*-butyl phenylalanine hydrochloride **2** (1.289 g, 5 mmol), some 4 Å molecular sieves, and triethylamine (0.84 mL, 6 mmol) in 16 mL of dry CH_2Cl_2 was stirred for 30 min at room temperature. HOBT (811 mg, 6 mmol) and 2-mercaptoacetic acid **1** (0.42 mL, 6 mmol) was then added, and the resulting mixture was stirred for 10 min at room temperature. Then DCC (1.238 g, 6 mmol) was added and the mixture was stirred overnight at room temperature under a nitrogen atmosphere. The white precipitate formed was removed by filtration and the filtrate was diluted into 100 mL ethyl acetate and washed with 5% HCl, 5% $NaHCO_3$, and saturated NaCl solution. The crude material was purified by flash chromatography on a silica gel column using petroleum ether/ethyl acetate (3:1) as eluent to give 1.253 g of a colorless oil (85% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32–7.14 (m, 5H), 7.04 (d, $J = 7.5$ Hz, 1H), 4.74 (dt, $J = 7.5$, 6.3 Hz, 1H), 3.22 (d, $J = 9.0$ Hz, 2H), 3.12 (d, $J = 6.3$ Hz, 2H), 1.81 (t, $J = 9.0$ Hz, 1H), 1.42 (s, 9H).

4.3. Preparation of compounds 4a–o

To compound **3** (0.5 mmol), some 4 Å molecular sieves, and DMAP (~7 mg) in 10 mL dry CH_2Cl_2 was added HOBT (81 mg, 0.6 mmol) and various protected amino acids **6a**–**o** (0.6 mmol), and the resulting mixture was stirred for 20 min at room temperature. Then DCC (124 mg, 0.6 mmol) was added and the mixture was stirred overnight at room temperature. The white precipitate formed was removed by filtration and the filtrate was diluted into 30 mL ethyl acetate and washed with saturated NaCl solution (30 mL). The crude material was purified by silica gel chromatography using petroleum ether/ethyl acetate as eluent to give **4a**–**o** as colorless oil.

4.3.1. (2*S*)-2-{2-[(2*S*)-2-*tert*-Butoxycarbonylamino-propionylsulfanyl]-acetylamino}-3-phenyl-propionic acid *tert*-butyl ester (4a). Yield 90%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31–7.12 (m, 5H), 6.59 (d, $J = 7.5$ Hz, 1H), 4.93 (br, 1H), 4.67 (m, 1H), 4.41 (m, 1H), 3.59, 3.49 (dd of AB system, $J = 15.0$ Hz, 2H), 3.06 (d, $J = 6.3$ Hz, 2H), 1.46 (s, 9H), 1.39 (s, 9H), 1.35 (d, $J = 7.2$ Hz, 3H).

4.3.2. (2*S*)-2-{2-[(2*S*)-2-*tert*-Butoxycarbonylamino-3-methyl-butyrylsulfanyl]-acetylamino}-3-phenyl-propionic acid *tert*-butyl ester (4b). Yield 74%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31–7.12 (m, 5H), 6.62 (d, $J = 7.5$ Hz, 1H), 4.96 (d, $J = 9.0$ Hz, 1H), 4.66 (dt, $J = 7.5$, 6.3 Hz, 1H), 4.32 (m, 1H), 3.60, 3.50 (dd of AB system, $J = 15.0$ Hz, 2H), 3.06 (d, $J = 6.3$ Hz, 2H), 2.29 (m, 1H), 1.46 (s, 9H), 1.38 (s, 9H), 0.97 (d, $J = 6.9$ Hz, 3H), 0.80 (d, $J = 6.9$ Hz, 3H).

4.3.3. (2*S*)-2-{2-[(2*S*)-2-*tert*-Butoxycarbonylamino-3-phenyl-propionylsulfanyl]-acetylamino}-3-phenyl-propionic acid *tert*-butyl ester (4c). Yield 72%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.35–7.12 (m, 10H), 6.65 (d, $J = 7.2$ Hz, 1H), 4.89 (d, $J = 8.4$ Hz, 1H), 4.67 (m, 2H), 3.60, 3.50 (dd of AB system, $J = 15.0$ Hz, 2H), 3.15 (dd of ABX system, $J = 14.7$, 5.4 Hz, 1H), 3.08 (d, $J = 6.0$ Hz, 2H), 2.98 (dd of ABX system, $J = 14.7$, 6.0 Hz, 1H), 1.40 (s, 18H).

4.3.4. (2*S*)-2-{2-[(2*S*,3*S*)-2-*tert*-Butoxycarbonylamino-3-methyl-pentanoylsulfanyl]-acetylamino}-3-phenyl-propionic acid *tert*-butyl ester (4d). Yield 91%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31–7.13 (m, 5H), 6.62 (d, $J = 6.9$ Hz, 1H), 4.96 (d, $J = 9.3$ Hz, 1H), 4.66 (m, 1H), 4.36 (m, 1H), 3.60, 3.50 (dd of AB system, $J = 15.0$ Hz, 2H), 3.06 (d, $J = 6.0$ Hz, 2H), 2.04 (m, 1H), 1.46 (s, 9H), 1.38 (s, 9H), 1.15 (m, 2H), 0.95 (d, $J = 6.6$ Hz, 3H), 0.88 (t, $J = 7.2$ Hz, 3H).

4.3.5. (2*S*)-2-{2-[(2*S*)-3-*tert*-Butoxy-2-*tert*-butoxycarbonylamino-propionylsulfanyl]-acetylamino}-3-phenyl-propionic acid *tert*-butyl ester (4e). Yield 71%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31–7.12 (m, 5H), 6.67 (d, $J = 7.5$ Hz, 1H), 5.49 (d, $J = 8.7$ Hz, 1H), 4.65 (dt, $J = 7.5$, 6.0 Hz, 1H), 4.43 (m, 1H), 3.86 (dd of ABX system, $J = 9.0$, 2.7 Hz, 1H), 3.54 (m, 2H), 3.51 (dd of ABX system, $J = 9.0$, 3.3 Hz, 1H), 3.05 (d, $J = 6.0$ Hz, 2H), 1.48 (s, 9H), 1.37 (s, 9H), 1.11 (s, 9H).

4.3.6. (2*S*)-2-{2-[(2*S*)-2-*tert*-Butoxycarbonylamino-4-methyl-pentanoylsulfanyl]-acetylamino}-3-phenyl-propionic acid *tert*-butyl ester (4f). Yield 93%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31–7.12 (m, 5H), 6.59 (d, $J = 7.5$ Hz, 1H), 4.82 (d, $J = 8.4$ Hz, 1H), 4.67 (dt, $J = 7.5$, 6.0 Hz, 1H), 4.38 (m, 1H), 3.60, 3.48 (dd of AB system, $J = 15.0$ Hz, 2H), 3.06 (d, $J = 6.0$ Hz, 2H), 1.64 (m, 3H), 1.46 (s, 9H), 1.39 (s, 9H), 0.93 (d, $J = 6.0$ Hz, 6H).

4.3.7. (2*S*)-2-{2-[(2*S*)-2,6-Bis-*tert*-butoxycarbonylamino-hexanoylsulfanyl]-acetylamino}-3-phenyl-propionic acid *tert*-butyl ester (4g). Yield 92%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.30–7.12 (m, 5H), 6.60 (d, $J = 7.5$ Hz, 1H), 5.18 (br, 1H), 4.66 (m, 1H), 4.60 (br, 1H), 4.32 (m, 1H), 3.60, 3.49 (dd of AB system, $J = 15.0$ Hz, 2H), 3.09 (m, 4H), 1.81–1.62 (m, 6H), 1.45 (s, 9H), 1.44 (s, 9H), 1.38 (s, 9H).

4.3.8. (2*S*)-2-{2-[(2*S*)-2-*tert*-Butoxycarbonylamino-3-(4-hydroxy-phenyl)-propionylsulfanyl]-acetylamino}-3-phenyl-propionic acid *tert*-butyl ester (4h). Yield 51%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32–7.13 (m, 5H), 6.97 (d, $J = 8.7$ Hz, 2H), 6.74 (d, $J = 8.7$ Hz, 2H), 6.66 (d, $J = 7.5$ Hz, 1H), 4.91 (d, $J = 9.3$ Hz, 1H), 4.69 (dt, $J = 7.5$, 6.0 Hz, 1H), 4.57 (m, 1H), 3.58, 3.49 (dd of AB system, $J = 15.0$ Hz, 2H), 3.08 (d, $J = 6.0$ Hz, 2H), 2.97 (m, 2H), 1.41 (s, 9H), 1.40 (s, 9H).

4.3.9. (2*S*)-2-{2-[(2*S*)-3-*tert*-Butoxycarbonyl-2-*tert*-butoxycarbonylamino-propionylsulfanyl]-acetylamino}-3-phenyl-propionic acid *tert*-butyl ester (4i). Yield 50%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32–7.12 (m, 5H), 6.62 (d, $J = 7.8$ Hz, 1H), 5.64 (d, $J = 9.9$ Hz, 1H), 4.64 (m, 2H), 3.54 (m, 2H), 3.04 (m, 2H), 2.97 (dd of ABX system, $J = 17.1$, 5.4 Hz, 1H), 2.67 (dd of ABX system, $J = 17.1$, 4.5 Hz, 1H), 1.48 (s, 9H), 1.42 (s, 9H), 1.39 (s, 9H).

4.3.10. (2*S*)-2-{2-[(2*S*,3*R*)-2-*tert*-Butoxycarbonylamino-3-hydroxy-butyrylsulfanyl]-acetylamino}-3-phenyl-propionic acid *tert*-butyl ester (4j). Yield 51%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.34–7.16 (m, 5H), 6.68 (d, $J = 8.4$ Hz, 1H), 5.56 (d, $J = 9.0$ Hz, 1H), 4.69 (dt, $J = 8.4$, 6.0 Hz, 1H), 4.36 (m, 1H), 4.19 (m, 1H), 3.79, 3.20 (dd of AB system, $J = 14.1$ Hz, 2H), 3.04 (m, 2H), 1.47 (s, 9H), 1.39 (s, 9H), 1.21 (d, $J = 6.6$ Hz, 3H).

4.3.11. (2*S*)-2-{[(1*S*)-1-*tert*-Butoxycarbonyl-2-phenyl-ethylcarbamoyl]-methylsulfanylcarbonyl}-pyrrolidine-1-carboxylic acid *tert*-butyl ester (4k). Yield 70%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.30–7.13 (m, 5H), 6.58 (br, 1H), 4.68 (m, 1H), 4.45 (m, 1H), 3.66–3.33 (m, 4H), 3.06 (d, $J = 6.0$ Hz, 2H), 2.17–1.85 (m, 4H), 1.41 (s, 9H), 1.38 (s, 9H).

4.3.12. (2*S*)-2-[2-(2-*tert*-Butoxycarbonylamino-acetylsulfanyl)-acetylamino]-3-phenyl-propionic acid *tert*-butyl ester (4l). Yield 85%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.29–7.11 (m, 5H), 6.62 (d, $J = 7.5$ Hz, 1H), 5.19 (br, 1H), 4.67 (m, 1H), 4.02 (d, $J = 6.0$ Hz, 2H), 3.59, 3.52 (dd of AB system, $J = 15.0$ Hz, 2H), 3.06 (m, 2H), 1.46 (s, 9H), 1.39 (s, 9H).

4.3.13. (2*S*)-2-{2-[(2*S*)-2-*tert*-Butoxycarbonylamino-3-(1*H*-indol-3-yl)-propionylsulfanyl]-acetylamino}-3-phenyl-propionic acid *tert*-butyl ester (4m). Yield 91%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.16 (br, 1H), 7.36–7.11 (m, 9H), 6.89 (s, 1H), 6.60 (d, $J = 7.2$ Hz, 1H), 5.01 (d, $J = 8.4$ Hz, 1H), 4.69 (m, 2H), 3.59, 3.47 (dd of AB system, $J = 15.0$ Hz, 2H), 3.28 (m, 2H), 3.08 (m, 2H), 1.42 (s, 9H), 1.41 (s, 9H).

4.3.14. (2*S*)-2-{2-[(2*S*)-2-*tert*-Butoxycarbonylamino-4-methylsulfanyl-butyrylsulfanyl]-acetylamino}-3-phenyl-propionic acid *tert*-butyl ester (4n). Yield 65%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.30–7.12 (m, 5H), 6.63 (d, $J = 7.2$ Hz, 1H), 5.24 (d, $J = 8.7$ Hz, 1H), 4.66 (m, 1H), 4.51 (m, 1H), 3.60, 3.49 (dd of AB system, $J = 15.0$ Hz, 2H), 3.06 (d, $J = 6.0$ Hz, 2H), 2.53 (m, 2H), 2.13 (m, 1H), 2.08 (s, 3H), 1.88 (m, 1H), 1.45 (s, 9H), 1.38 (s, 9H).

4.3.15. (2*S*)-2-{2-[(2*S*)-2-*tert*-Butoxycarbonylamino-3-carbamoyl-propionylsulfanyl]-acetylamino}-3-phenyl-propionic acid *tert*-butyl ester (4o). Yield 63%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31–7.12 (m, 5H), 6.79 (d, $J = 7.8$ Hz, 1H), 6.20 (d, $J = 9.3$ Hz, 1H), 5.86 (br, 1H), 5.73 (br, 1H), 4.66 (m, 1H), 4.57 (m, 1H), 3.60, 3.49 (dd of AB system, $J = 15.3$ Hz, 2H), 3.09–2.93 (m, 3H), 2.60 (dd of ABX system, $J = 15.6$, 4.2 Hz, 1H), 1.46 (s, 9H), 1.38 (s, 9H).

4.4. Preparation of compounds 5a–o

To compound **4a**–**o** (0.1 mmol) dissolved in 4 mL CH_2Cl_2 at about 0 °C was added TFA (2 mL), then the mixture was stirred overnight with gradual warming to room temperature. The solvents were removed under vacuum. The residue was dissolved in water (5 mL) and lyophilized to give **5a**–**o** as a white solid.

4.4.1. (2*S*)-2-{2-[(2*S*)-2-Amino-propionylsulfanyl]-acetylamino}-3-phenyl-propionic acid (5a). Yield 91%. ^{1}H NMR (D_2O, 300 MHz): δ 7.37–7.23 (m, 5H), 4.65 (m, 1H), 4.35 (q, $J = 6.3$ Hz, 1H), 3.74 (br, 2H), 3.20 (dd of ABX system, $J = 13.5$, 5.4 Hz, 1H), 3.01 (dd of ABX system, $J = 13.5$, 8.4 Hz, 1H), 1.57 (d, $J = 6.3$ Hz, 3H).

^{13}C NMR (D_2O, 75 MHz): δ 197.61, 174.62, 169.77, 136.60, 129.45, 128.95, 127.39, 55.11, 54.63, 36.75, 32.29, 16.58.

4.4.2. (2*S*)-2-{2-[(2*S*)-2-Amino-3-methyl-butyrylsulfanyl]-acetylamino}-3-phenyl-propionic acid (5b). Yield 100%. ^{1}H NMR (D_2O, 300 MHz): δ 7.39–7.26 (m, 5H), 4.67 (m, 1H), 4.23 (d, $J = 4.8$ Hz, 1H), 3.82, 3.73 (dd of AB system, $J = 15.3$ Hz, 2H), 3.22 (dd of ABX system, $J = 14.1$, 5.7 system, 1H), 3.04 (dd of ABX Hz, $J = 14.1$, 8.7 Hz, 1H), 2.36 (m, 1H), 1.04 (d, $J = 6.9$ Hz, 3H), 0.97 (d, $J = 6.9$ Hz, 3H).

^{13}C NMR (D_2O, 75 MHz): δ 196.70, 174.62, 169.71, 136.59, 129.45, 128.98, 127.42, 64.30, 54.67, 36.78, 32.46, 30.42, 17.90, 16.43.

4.4.3. (2*S*)-2-{2-[(2*S*)-2-Amino-3-phenyl-propionylsulfanyl]-acetylamino}-3-phenyl-propionic acid (5c). Yield 92%. ^{1}H NMR (D_2O, 300 MHz): δ 7.39–7.22 (m, 10H), 4.67 (m, 2H), 3.80, 3.70 (dd of AB system, $J = 15.3$ Hz, 2H), 3.31 (dd of ABX system, $J = 14.1$, 6.6 Hz, 1H), 3.18 (m, 2H), 3.04 (dd of ABX system, $J = 14.1$, 9.0 Hz, 1H).

^{13}C NMR (D_2O, 75 MHz): δ 196.42, 174.74, 169.67, 136.70, 133.36, 129.81, 129.54, 129.49, 129.05, 128.45, 127.47, 60.00, 54.81, 37.11, 36.81, 32.45.

4.4.4. (2*S*)-2-{2-(2*S*,3*S*)-2-Amino-3-methyl-pentanoylsulfanyl]-acetylamino}-3-phenyl-propionic acid (5d). Yield 66%. ^{1}H NMR (D_2O, 300 MHz): δ 7.47–7.35 (m, 5H), 4.75 (m, 1H), 4.36 (d, $J = 3.3$ Hz, 1H), 3.90, 3.81 (dd of AB system, $J = 15.6$ Hz, 2H), 3.31 (dd of ABX system, $J = 13.8$, 5.1 Hz, 1H), 3.12 (dd of ABX system, $J = 13.8$, 9.0 Hz, 1H), 2.15 (m, 1H), 1.52 (m, 1H), 1.33 (m, 1H), 1.07 (d, $J = 6.6$ Hz, 3H), 1.01 (t, $J = 7.5$ Hz, 3H).

4.4.5. (2*S*)-2-{2-[(2*S*)-2-Amino-3-hydroxy-propionylsulfanyl]-acetylamino}-3-phenyl-propionic acid (5e). Yield 100%. ^{1}H NMR (D_2O, 300 MHz): δ 7.46–7.33 (m, 5H), 4.73 (m, 1H), 4.55 (m, 1H), 4.15 (d, $J = 3.3$ Hz, 2H), 3.89, 3.83 (dd of AB system, $J = 15.3$ Hz, 2H), 3.30 (dd of ABX system, $J = 13.8$, 5.1 Hz, 1H), 3.10 (dd of ABX system, $J = 13.8$, 8.7 Hz, 1H).

^{13}C NMR (D_2O, 75 MHz): δ 194.92, 174.73, 169.72, 136.67, 129.50, 128.99, 127.42, 60.86, 60.39, 54.76, 36.81, 32.39.

4.4.6. (2*S*)-2-{2-[(2*S*)-2-Amino-4-methyl-pentanoylsulfanyl]-acetylamino}-3-phenyl-propionic acid (5f). Yield 88%. ^{1}H NMR (D_2O, 300 MHz): δ 7.35–7.21 (m, 5H), 4.62 (m, 1H), 4.27 (m, 1H), 3.74, 3.68 (dd of AB system, $J = 15.6$ Hz, 2H), 3.18 (dd of ABX system, $J = 13.8$, 4.5 Hz, 1H), 2.98 (dd of ABX system, $J = 13.8$, 8.7 Hz, 1H), 1.70 (m, 3H), 0.90 (d, $J = 5.4$ Hz, 6H).

^{13}C NMR (D_2O, 75 MHz): δ 197.53, 174.70, 169.79, 136.66, 129.51, 129.02, 127.47, 57.65, 54.82, 40.22, 36.85, 32.46, 24.38, 22.03, 21.10.

4.4.7. (2*S*)-2-{2-[(2*S*)-2,6-Diamino-hexanoylsulfanyl]-acetylamino}-3-phenyl-propionic acid (5g). Yield 100%. ^{1}H NMR (D_2O, 300 MHz): δ 7.30–7.12 (m, 5H), 4.53 (m, 1H), 4.24 (m, 1H), 3.70, 3.62 (dd of AB system, $J = 15.6$ Hz, 2H), 3.10 (dd of ABX system, $J = 13.5$, 4.2 Hz, 1H), 2.92 (dd of ABX system, $J = 13.5$, 8.7 Hz, 1H), 2.85 (m, 2H), 1.85 (m, 2H), 1.56 (m, 2H), 1.35 (m, 2H).

^{13}C NMR (D_2O, 75 MHz): δ 196.79, 174.74, 169.73, 136.63, 129.46, 128.95, 127.38, 58.69, 54.83, 39.15, 36.77, 32.38, 30.57, 26.52, 21.30.

4.4.8. (2*S*)-2-{2-[(2*S*)-2-Amino-3-(4-hydroxy-phenyl)-propionylsulfanyl]-acetylamino}-3-phenyl-propionic acid (5h). Yield 100%. ^{1}H NMR (D_2O, 300 MHz): δ 7.35–7.15 (m, 5H), 7.02 (d, $J = 7.2$ Hz, 2H), 6.81 (d, $J = 7.2$ Hz, 2H), 4.60 (m, 1H), 4.49 (t, $J = 6.0$ Hz, 1H), 3.74, 3.63 (dd of AB system, $J = 15.0$ Hz, 2H), 3.19–2.99 (m, 4H).

4.4.9. (2*S*)-2-{2-[(2*S*)-2-Amino-3-carboxy-propionylsulfanyl]-acetylamino}-3-phenyl-propionic acid (5i). Yield 100%. ^{1}H NMR (D_2O, 300 MHz): δ 7.29–7.15 (m, 5H), 4.57 (m, 2H), 3.70 (br, 2H), 3.17–3.02 (m, 3H), 2.96 (dd of ABX system, $J = 13.8$, 8.4 Hz, 1H).

^{13}C NMR (D_2O, 75 MHz): δ 195.45, 174.73, 172.41, 169.62, 136.66, 129.52, 129.00, 127.46, 55.56, 54.47, 36.81, 34.83, 32.52.

4.4.10. (2*S*)-2-{2-[(2*S*,3*R*)-2-Amino-3-hydroxy-butyrylsulfanyl]-acetylamino}-3-phenyl-propionic acid (5j). Yield 100%. ^{1}H NMR (D_2O, 300 MHz): δ 7.33–7.23 (m, 5H), 4.65 (m, 1H), 4.34 (m, 1H), 4.26 (m, 1H), 3.75 (m, 2H), 3.20 (dd of ABX system, $J = 14.1$, 5.4 Hz, 1H), 3.00 (dd of ABX system, $J = 14.1$, 9.0 Hz, 1H), 1.29 (d, $J = 6.6$ Hz, 3H).

^{13}C NMR (D_2O, 75 MHz): δ 195.39, 174.78, 169.79, 136.69, 129.52, 129.02, 127.45, 66.34, 64.32, 54.88, 36.83, 32.54, 19.12.

4.4.11. (2*S*)-3-Phenyl-2-{2-[(2*S*)-pyrrolidine-2-carbonylsulfanyl]-acetylamino}-propionic acid (5k). Yield 100%. ^{1}H NMR (D_2O, 300 MHz): δ 7.32–7.21 (m, 5H), 4.63 (m, 2H), 3.74, 3.68 (dd of AB system, $J = 15.9$ Hz, 2H), 3.33 (m, 2H), 3.19 (dd of ABX system, $J = 14.1$, 5.4 Hz, 1H), 2.99 (dd of ABX system, $J = 14.1$, 8.4 Hz, 1H), 2.38 (m, 1H), 1.99 (m, 3H).

^{13}C NMR (D_2O, 75 MHz): δ 196.12, 174.70, 169.82, 136.64, 129.48, 128.98, 127.39, 65.74, 54.64, 46.59, 36.74, 32.38, 29.91, 23.59.

4.4.12. (2*S*)-2-[2-(2-Amino-acetylsulfanyl)-acetylamino]-3-phenyl-propionic acid (5l). Yield 100%. ^{1}H NMR (D_2O, 300 MHz): δ 7.34–7.20 (m, 5H), 4.62 (m, 1H), 4.13 (s, 2H), 3.72 (m, 2H), 3.18 (dd of ABX system, $J = 13.8$, 5.1 Hz, 1H), 2.97 (dd of ABX system, $J = 13.8$, 8.7 Hz, 1H).

^{13}C NMR (D_2O, 75 MHz): δ 193.53, 174.72, 169.87, 136.61, 129.45, 128.92, 127.37, 54.69, 47.02, 36.74, 32.09.

4.4.13. (2*S*)-2-{2-[(2*S*)-2-Amino-3-(1*H*-indol-3-yl)-propionylsulfanyl]-acetylamino}-3-phenyl-propionic acid (5m). Yield 67%. ^{1}H NMR (D_2O, 300 MHz): δ 7.29–7.09 (m, 10H), 4.60 (m, 2H), 3.71, 3.63 (dd of AB system, $J = 15.3$ Hz, 2H), 3.45 (dd of ABX system, $J = 15.3$, 6.6 Hz, 1H), 3.35 (dd of ABX system, $J = 15.3$, 7.2 Hz, 1H), 3.16 (dd of ABX system, $J = 13.5$, 4.5 Hz, 1H), 2.96 (dd of ABX system, $J = 13.5$, 9.0 Hz, 1H).

4.4.14. (2*S*)-2-{2-[(2*S*)-2-Amino-4-methylsulfanyl-butyrylsulfanyl]-acetylamino}-3-phenyl-propionic acid (5n). Yield 99%. ^{1}H NMR (D_2O, 300 MHz): δ 7.39–7.26 (m, 5H), 4.65 (m, 1H), 4.49 (t, $J = 6.3$ Hz, 1H), 3.81, 3.75

(dd of AB system, $J = 15.6$ Hz, 2H), 3.23 (dd of ABX system, $J = 13.8$, 5.1 Hz, 1H), 3.02 (dd of ABX system, $J = 13.8$, 8.7 Hz, 1H), 2.62 (t, $J = 7.2$ Hz, 2H), 2.22 (m, 2H), 2.10 (s, 3H).

^{13}C NMR (D_2O, 75 MHz): δ 196.54, 174.79, 169.58, 136.72, 129.49, 128.99, 127.42, 57.99, 54.82, 36.88, 32.52, 30.26, 28.45, 14.20.

4.4.15. (2*S*)-2-{2-[(2*S*)-2-Amino-3-carbamoyl-propionylsulfanyl]-acetylamino}-3-phenyl-propionic acid (5o). Yield 100%. ^{1}H NMR (D_2O, 300 MHz): δ 7.36–7.28 (m, 5H), 4.65 (m, 2H), 3.77 (m, 2H), 3.35–3.00 (m, 4H).

^{13}C NMR (D_2O, 75 MHz): δ 195.59, 175.26, 174.68, 169.58, 136.61, 129.46, 128.94, 127.38, 55.79, 54.72, 36.76, 34.79, 32.44.

4.5. Preparation of compounds 9a–f

A solution of H-Ser(Bu^t)-OBu^t·HCl **8** (254 mg, 1.0 mmmol), some 4 Å molecular sieves, and DIEA (0.21 mL, 1.2 mmol) in 10 mL of dry CH_2Cl_2 was stirred for 30 min at room temperature. HOBT (162 mg, 1.2 mmol) and 2-thioacetic acids **7** with or without substitution (1.2 mmol) was then added, and the resulting mixture was stirred for 10 min at room temperature. Then DCC (247 mg, 1.2 mmol) was added and the mixture was stirred overnight at room temperature _under a nitrogen atmosphere. The white precipitate formed was removed by filtration and the filtrate was diluted into 30 mL ethyl acetate and washed with 5% HCl, 5% $NaHCO_3$, and saturated NaCl solution (30 mL each). The crude material was purified by flash chromatography on a silica gel column using petroleum ether/ethyl acetate as eluent to give **9a**–**f** as colorless oil.

4.5.1. (2*S*)-3-*tert*-Butoxy-2-[(2*S*)-2-mercapto-propionyl-amino]-propionic acid *tert*-butyl ester (9a). Yield 37%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.05 (d, $J = 8.1$ Hz, 1H), 4.54 (ddd, $J = 8.1$, 3.0, 2.7 Hz, 1H), 3.77 (dd of ABX system, $J = 8.7$, 2.7 Hz, 1H), 3.53 (dd of ABX system, $J = 8.7$, 3.0 Hz, 1H), 3.47 (m, 1H), 2.09 (d, $J = 8.4$ Hz, 1H), 1.54 (d, $J = 7.2$ Hz, 3H), 1.45 (s, 9H), 1.14 (s, 9H).

4.5.2. (2*S*)-3-*tert*-Butoxy-2-(2-mercapto-acetylamino)-propionic acid *tert*-butyl ester (9b). Yield 33%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.29 (d, $J = 8.1$ Hz, 1H), 4.51 (ddd, $J = 8.1$, 3.0, 3.0 Hz, 1H), 3.73 (dd of ABX system, $J = 8.7$, 3.0 Hz, 1H), 3.47 (dd of ABX system, $J = 8.7$, 3.0 Hz, 1H), 3.21 (d, $J = 8.4$ Hz, 2H), 1.90 (t, $J = 8.4$ Hz, 1H), 1.40 (s, 9H), 1.08 (s, 9H).

4.5.3. (2*S*)-3-*tert*-Butoxy-2-[(2*S*)-2-mercapto-3-phenyl-propionylamino]-propionic acid *tert*-butyl ester (9c). Yield 6%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.38–7.23 (m, 5H), 7.03 (d, $J = 7.8$ Hz, 1H), 4.57 (m, 1H), 3.79 (dd of ABX system, $J = 8.7$, 3.0 Hz, 1H), 3.65 (m, 1H), 3.53 (dd of ABX system, $J = 8.7$, 3.0 Hz, 1H), 3.35 (dd of ABX system, $J = 13.8$, 6.0 Hz, 1H), 3.09 (dd of ABX system, $J = 13.8$, 7.8 Hz, 1H), 2.00 (d, $J = 8.4$ Hz, 1H), 1.48 (s, 9H), 1.15 (s, 9H).

4.5.4. (2*S*)-3-*tert*-Butoxy-2-[(2*R*)-2-mercapto-3-phenyl-propionylamino]-propionic acid *tert*-butyl ester (9d). Yield 11%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.34–7.21 (m, 5H), 6.84 (d, $J = 6.9$ Hz, 1H), 4.54 (m, 1H), 3.66 (dd of ABX system, $J = 8.7$, 3.0 Hz, 1H), 3.54 (m, 1H), 3.38–3.31 (m, 2H), 3.02 (dd of ABX system, $J = 13.8$, 7.2 Hz, 1H), 2.06 (d, $J = 8.7$ Hz, 1H), 1.46 (s, 9H), 1.08 (s, 9H).

4.5.5. (2*S*)-3-*tert*-Butoxy-2-[(2*R*)-2-mercapto-4-methyl-pentanoylamino]-propionic acid *tert*-butyl ester (9e). Yield 7%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.91 (d, $J = 8.1$ Hz, 1H), 4.57 (ddd, $J = 8.1$, 3.0, 2.7 Hz, 1H), 3.78 (dd of ABX system, $J = 8.4$, 2.7 Hz, 1H), 3.54 (dd of ABX system, $J = 8.4$, 3.0 Hz, 1H), 3.35 (m, 1H), 1.99 (d, $J = 8.1$ Hz, 1H), 1.93–1.75 (m, 3H), 1.47 (s, 9H), 1.14 (s, 9H), 0.94 (d, $J = 6.3$ Hz, 3H), 0.91 (d, $J = 6.3$ Hz, 3H).

4.5.6. (2*S*)-3-*tert*-Butoxy-2-[(2*R*)-2-mercapto-4-methyl-sulfanyl-butyrylamino]-propionic acid *tert*-butyl ester (9f). Yield 18%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.01 (d, $J = 9.6$ Hz, 1H), 4.56 (m, 1H), 3.79 (dd of ABX system, $J = 9.0$, 3.0 Hz, 1H), 3.55 (dd of ABX system, $J = 9.0$, 3.0 Hz, 1H), 3.49 (m, 1H), 2.64 (t, $J = 6.9$ Hz, 2H), 2.37–2.20 (m, 1H), 2.10 (s, 3H), 2.05 (d, $J = 9.3$ Hz, 1H), 2.01–1.91 (m, 1H), 1.47 (s, 9H), 1.15 (s, 9H).

4.6. Preparation of compounds 10a–f

To compound **9a**–**f** (0.5 mmol), some 4 Å molecular sieves, and DMAP (~7 mg) in 10 mL dry CH_2Cl_2 was added HOBT (81 mg, 0.6 mmol) and N-Boc-methionine (150 mg, 0.6 mmol), and the resulting mixture was stirred for 20 min at room temperature. Then DCC (124 mg, 0.6 mmol) was added and the mixture was stirred overnight at room temperature. The white precipitate formed was removed by filtration and the filtrate was diluted into 30 mL ethyl acetate and washed with saturated NaCl solution (30 mL). The crude material was purified by silica gel chromatography using petroleum ether/ethyl acetate as eluent to give **10a**–**f** as colorless oil.

4.6.1. (2*S*)-3-*tert*-Butoxy-2-{(2*S*)-2-[(2*S*)-2-*tert*-butoxy-carbonylamino-4-methylsulfanyl-butyrylsulfanyl]-propionylamino}-propionic acid *tert*-butyl ester (10a). Yield 84%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.81 (d, $J = 8.1$ Hz, 1H), 5.10 (d, $J = 8.7$ Hz, 1H), 4.52 (m, 2H), 4.10 (m, 1H), 3.74 (dd of ABX system, $J = 8.4$, 3.0 Hz, 1H), 3.45 (dd of ABX system, $J = 8.4$, 3.0 Hz, 1H), 2.54 (m, 2H), 2.15 (m, 1H), 2.03 (s, 3H), 1.90

(m, 1H), 1.47 (d, $J = 7.2$ Hz, 3H), 1.44 (s, 18H), 1.13 (s, 9H).

4.6.2. (2*S*)-3-*tert*-Butoxy-2-{2-[(2*S*)-2-*tert*-butoxycarbonylamino-4-methylsulfanyl-butyrylsulfanyl]-acetylamino}-propionic acid *tert*-butyl ester (10b). Yield 76%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.86 (d, $J = 8.1$ Hz, 1H), 5.32 (d, $J = 8.7$ Hz, 1H), 4.48 (m, 2H), 3.72 (dd of ABX system, $J = 9.0$, 3.0 Hz, 1H), 3.66, 3.50 (dd of AB system, $J = 15.0$ Hz, 2H), 3.47 (dd of ABX system, $J = 9.0$, 3.0 Hz, 1H), 2.60 (m, 2H), 2.16 (m, 1H), 2.06 (s, 3H), 1.94 (m, 1H), 1.42 (s, 18H), 1.11 (s, 9H).

4.6.3. (2*S*)-3-*tert*-Butoxy-2-{(2*S*)-2-[(2*S*)-2-*tert*-butoxycarbonylamino-4-methylsulfanyl-butyrylsulfanyl]-3-phenyl-propionylamino}-propionic acid *tert*-butyl ester (10c). Yield 99%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32–7.17 (m, 5H), 6.73 (d, $J = 7.8$ Hz, 1H), 5.04 (d, $J = 9.3$ Hz, 1H), 4.48 (m, 2H), 4.26 (m, 1H), 3.71 (dd of ABX system, $J = 9.0$, 2.7 Hz, 1H), 3.45 (dd of ABX system, $J = 9.0$, 3.0 Hz, 1H), 3.37 (dd of ABX system, $J = 14.1$, 6.9 Hz, 1H), 2.98 (dd of ABX system, $J = 14.1$, 8.4 Hz, 1H), 2.41 (m, 2H), 2.04 (s, 3H), 1.82 (m, 2H), 1.43 (s, 18H), 1.15 (s, 9H).

4.6.4. (2*S*)-3-*tert*-Butoxy-2-{(2*R*)-2-[(2*S*)-2-*tert*-butoxycarbonylamino-4-methylsulfanyl-butyrylsulfanyl]-3-phenyl-propionylamino}-propionic acid *tert*-butyl ester (10d). Yield 56%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.32–7.19 (m, 5H), 6.71 (d, $J = 8.1$ Hz, 1H), 5.23 (d, $J = 9.0$ Hz, 1H), 4.49 (m, 1H), 4.44 (m, 1H), 4.22 (m, 1H), 3.68 (dd of ABX system, $J = 8.7$, 2.7 Hz, 1H), 3.40–3.29 (m, 2H), 2.95 (dd of ABX system, $J = 13.8$, 6.6 Hz, 1H), 2.36 (m, 2H), 2.13 (m, 1H), 2.06 (s, 3H), 1.88 (m, 1H), 1.49 (s, 9H), 1.42 (s, 9H), 1.07 (s, 9H).

4.6.5. (2*S*)-3-*tert*-Butoxy-2-{(2*R*)-2-[(2*S*)-2-*tert*-butoxycarbonylamino-4-methylsulfanyl-butyrylsulfanyl]-4-methyl-pentanoylamino}-propionic acid *tert*-butyl ester (10e). Yield 49%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.85 (d, $J = 8.4$ Hz, 1H), 5.24 (d, $J = 9.0$ Hz, 1H), 4.56–4.46 (m, 2H), 4.05 (t, $J = 7.5$ Hz, 1H), 3.74 (dd of ABX system, $J = 9.0$, 2.7 Hz, 1H), 3.51 (dd of ABX system, $J = 9.0$, 3.0 Hz, 1H), 2.59–2.52 (m, 2H), 2.17 (m, 1H), 2.08 (s, 3H), 1.92 (m, 1H), 1.67 (m, 3H), 1.45 (s, 9H), 1.44 (s, 9H), 1.15 (s, 9H), 0.95 (d, $J = 6.6$ Hz, 3H), 0.89 (d, $J = 6.6$ Hz, 3H).

4.6.6. (2*S*)-3-*tert*-Butoxy-2-{(2*R*)-2-[(2*S*)-2-*tert*-butoxycarbonylamino-4-methylsulfanyl-butyrylsulfanyl]-4-methylsulfanyl-butyrylamino}-propionic acid *tert*-butyl ester (10f). Yield 31%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.90 (d, $J = 8.4$ Hz, 1H), 5.25 (d, $J = 9.3$ Hz, 1H), 4.52 (m, 1H), 4.48 (ddd, $J = 8.4$, 3.0, 3.0 Hz, 1H), 4.22 (m, 1H), 3.75 (dd of ABX system, $J = 9.0$, 3.0 Hz, 1H), 3.51 (dd of ABX system, $J = 9.0$, 3.0 Hz, 1H), 2.56 (t, $J = 6.0$ Hz, 4H), 2.30 (m, 2H), 2.09 (s, 6H), 2.01–1.93 (m, 2H), 1.46 (s, 9H), 1.44 (s, 9H), 1.16 (s, 9H).

4.7. Preparation of compounds 11a–f

To compound **10a–f** (0.1 mmol) dissolved in 4 mL CH_2Cl_2 at about 0 °C was added TFA (2 mL), then the mixture was stirred overnight with gradual warming to room temperature. The solvents were removed under vacuum. The residue was dissolved in water (5 mL) and lyophilized to give **11a–f** as a white solid.

4.7.1. (2*S*)-2-{(2*S*)-2-[(2*S*)-2-Amino-4-methylsulfanyl-butyrylsulfanyl]-propionylamino}-3-hydroxy-propionic acid (11a). Yield 100%. ^{1}H NMR (D_2O, 300 MHz): δ 4.47 (m, 2H), 4.38 (m, 1H), 3.93 (dd of ABX system, $J = 11.7$, 4.5 Hz, 1H), 3.82 (dd of ABX system, $J = 11.7$, 3.9 Hz, 1H), 2.59 (t, $J = 7.2$ Hz, 2H), 2.20 (m, 2H), 2.06 (s, 3H), 1.48 (d, $J = 7.2$ Hz, 3H).

4.7.2. (2*S*)-2-{2-[(2*S*)-2-Amino-4-methylsulfanyl-butyrylsulfanyl]-acetylamino}-3-hydroxy-propionic acid (11b). Yield 89%. ^{1}H NMR (D_2O, 300 MHz): δ 4.52 (m, 2H), 3.96–3.82 (m, 4H), 2.63 (t, $J = 6.9$ Hz, 2H), 2.31–2.17 (m, 2H), 2.08 (s, 3H).

^{13}C NMR (D_2O, 75 MHz): δ 196.64, 173.28, 170.11, 61.22, 58.07, 55.50, 32.71, 30.00, 28.40, 14.17.

4.7.3. (2*S*)-2-{(2*S*)-2-[(2*S*)-2-Amino-4-methylsulfanyl-butyrylsulfanyl]-3-phenyl-propionylamino}-3-hydroxy-propionic acid (11c). Yield 100%. ^{1}H NMR (D_2O, 300 MHz): δ 7.39–7.28 (m, 5H), 4.64 (m, 1H), 4.46 (m, 1H), 4.40 (m, 1H), 3.83 (m, 2H), 3.17 (m, 2H), 2.62 (t, $J = 6.9$ Hz, 2H), 2.24 (m, 2H), 2.10 (s, 3H).

4.7.4. (2*S*)-2-{(2*R*)-2-[(2*S*)-2-Amino-4-methylsulfanyl-butyrylsulfanyl]-3-phenyl-propionylamino}-3-hydroxy-propionic acid (11d). Yield 100%. ^{1}H NMR (D_2O, 300 MHz): δ 7.40–7.29 (m, 5H), 4.67 (m, 1H), 4.46 (m, 1H), 4.39 (m, 1H), 3.85 (dd of ABX system, $J = 11.4$, 5.1 Hz, 1H), 3.68 (dd of ABX system, $J = 11.4$, 7.5 Hz, 1H), 3.30 (dd of ABX system, $J = 13.8$, 7.8 Hz, 1H), 3.11 (dd of ABX system, $J = 13.8$, 8.7 Hz, 1H), 2.45 (m, 2H), 2.14 (m, 2H), 2.08 (s, 3H).

4.7.5. (2*S*)-2-{(2*R*)-2-[(2*S*)-2-Amino-4-methylsulfanyl-butyrylsulfanyl]-4-methyl-pentanoylamino}-3-hydroxy-propionic acid (11e). Yield 66%. ^{1}H NMR (D_2O, 300 MHz): δ 4.51–4.39 (m, 3H), 3.89 (m, 2H), 2.67 (t, $J = 7.2$ Hz, 2H), 2.32–2.22 (m, 2H), 2.12 (s, 3H), 1.82 (m, 1H), 1.64 (m, 2H), 0.94 (d, $J = 4.5$ Hz, 3H), 0.89 (d, $J = 5.4$ Hz, 3H).

4.7.6. (2*S*)-2-{(2*R*)-2-[(2*S*)-2-Amino-4-methylsulfanyl-butyrylsulfanyl]-4-methylsulfanyl-butyrylamino}-3-hydroxy-propionic acid (11f). Yield 100%. ^{1}H NMR (D_2O, 300 MHz): δ 4.51 (m, 2H), 4.41 (m, 1H), 3.90 (m, 2H), 2.65 (m, 4H), 2.25 (m, 4H), 2.12 (s, 3H), 2.09 (s, 3H).

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