Design and Synthesis of Sulfur Based Inhibitors of Matrix Metalloproteinase-1

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Fibroblast collagenase (MMP-1), a member of the matrix metalloproteinases family, is believed to be a pathogenesis of arthritis, by cleaving triple-helical type II collagen in cartilage. From the similarity of the active site zinc binding mode with hydroxamate, we designed and synthesized α-mercaptocarbonyl possessing compounds (3—5), which incorporated various peptide sequences as enzyme recognition sites. The P_4–P_1 peptide incorporating compound (3) exhibited as potent inhibition as the hydroxamate (1) and the carboxylate (2) type inhibitors, with an IC_{50} of 10^{-6} M order against MMP-1. But the inhibitor (3) related compounds (6—8) displayed decreased or no inhibitory potencies. These results suggest that the existence of both the carbonyl and thiol groups might be critical for the inhibition, and the distance between the two functional groups is important for inhibitory potency. For P_n' peptide incorporating compounds (4a—k), except for 4h and 4k, all compounds showed IC_{50} values under sub-nanomolar. Among them, for potent inhibition, Leu was better than Phe and Val as the P_1' amino acid, and the P_2' position amino acid was necessary, and preferentially Phe. Insertion of the P_n peptide into 4d or 4k, giving compounds 5a—c, did not increase the activities of 4d and 4k. Substitution of the mercapto group with other functional groups lost the activity of compound 4a. The stereochemical preference at the thiol-attached position was also determined by preparation of both isomers of 4a. It was found that the *S* configuration compound (36b) is approximately 100 times more potent than the corresponding *R*-isomer (36a).

Key words matrix metalloproteinase; inhibitor; α-mercaptocarbonyl; isoserine; *in vitro* activity

Matrix metalloproteinases (MMPs) are zinc endometallopeptidases that are involved in the degradation and remodeling of connective tissues. This family of enzymes shows proteolytic activity towards virtually all of the constituents of the extracellular matrix. The members of this family, currently numbering 20, can be classified into four groups, which are: the collagenases that cleave triple-helical interstitial collagen; the gelatinases that cleave denatured collagen, elastin, and type IV and V collagen; the stromelysins that mainly cleave proteoglycans; and the membrane-type MMPs that have a C-terminal transmembrane domain for anchoring to the cell membrane. The MMPs are involved in crucial physiological and physiopathological events, such as wound healing, nerve growth, angiogenesis, and pregnancy. In these physiological processes, MMP activity is tightly regulated.[1—5] However, excessive MMP synthesis and release can lead to connective tissue degradation and destruction, which occurs in tumor invasion, metastasis,[6] corneal ulceration,[7] arthritis,[8a] periodontal disease,[9] and multiple sclerosis.[10]

A number of MMP inhibitors have progressed into clinical trials mainly for cancer and rheumatoid arthritis. In the structures of those MMPs inhibitors, a hydroxamate group is utilized as a zinc-chelating group (ZCG) predominantly because of its superior zinc-chelating potency. Previously, we explored MMP-1 inhibitors that had a hydroxamate group (**1**)[11] and a carboxylate group (**2**).[12] These compounds contain a P_4–P_1 peptide sequence and exhibited inhibitory activity for MMP-1 in the order of 10^{-6} M for IC_{50} (Fig. 1). Our objective is to explore the usefulness of novel ZCGs as inhibitors of MMPs, and in particular of MMP-1. A further aim is to compare the MMP-1 inhibitory potencies of compounds that incorporate various enzyme recognition moieties.

Mercapto groups are good ZCGs, as found in Captopril, which is a well-known angiotensin converting enzyme (ACE) inhibitor. In this study, we explored mercapto group based compounds that incorporated various peptide sequences as enzyme recognition functionalities.

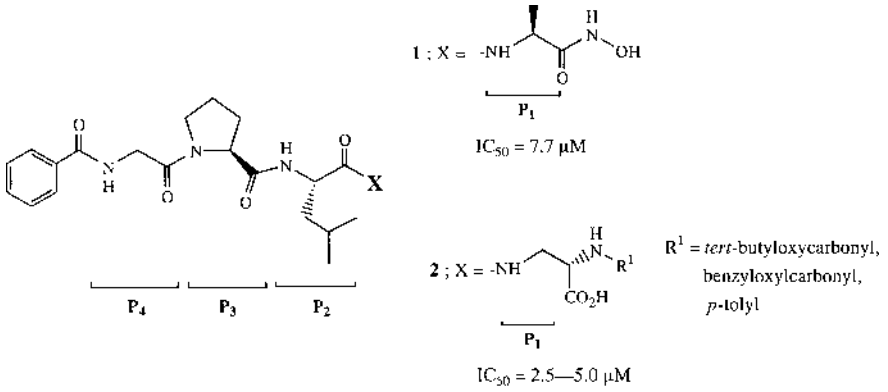

Fig. 1. Structures of the P_n Peptide Based Inhibitors that Possess a Hydroxamate or a Carboxyate as ZCG and of Its MMP-1 Inhibitory Potencies

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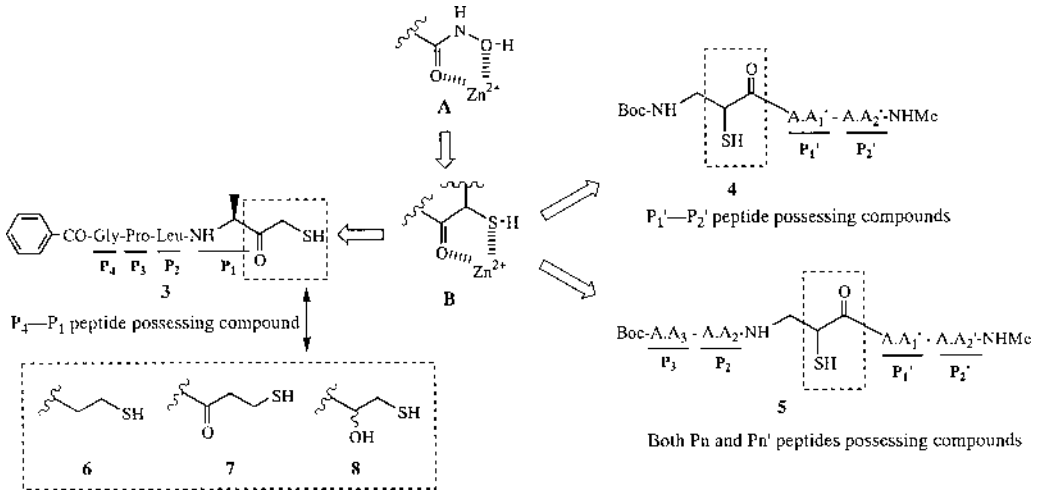


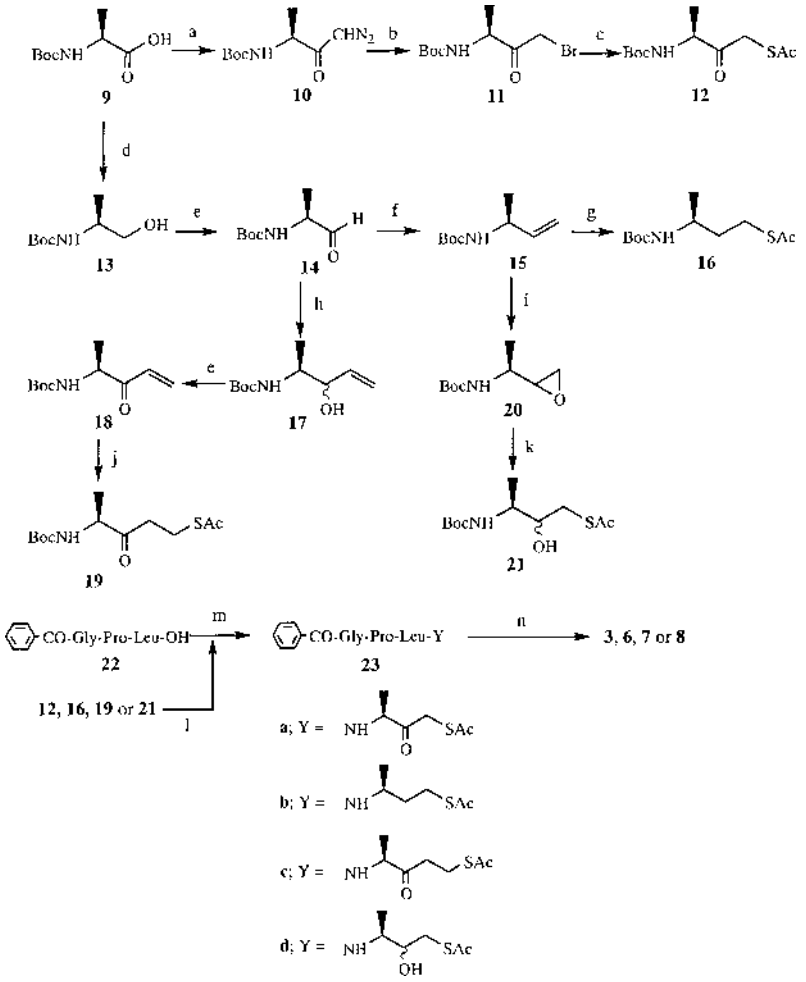
Fig. 2. The Design of an α-Mercaptocarbonyl Moiety Containing Compounds (**3**—**5**) and Related Compounds (**6**—**8**)

Design The X-ray crystal structures of MMP-1 with bound inhibitors reveal that the hydroxamate acts as a bidentate ligand with each oxygen at an optimal distance (2.1—2.2 Å) from the active-site zinc ion (structure A, Fig. 2).[13] From this structural analysis data, we reasoned that the α-mercaptocarbonyl group (structure B) at the cleavage site would chelate at the active-site zinc ion by both carbonyl and mercapto groups in a similar manner as the hydroxamate (Fig. 2). In comparison with the hydroxamate possessing MMPs inhibitors, little is known about thiol group including compounds. But, previously Donald and colleagues developed a β-mercapto carbonyl group possessing compound that exhibited 10^{-7} M order of IC_{50} against MMP-1.[14] The compound differs in the distance between the carbonyl and mercapto groups from the structure B. In addition, Baxter and colleagues have prepared the thiol containing inhibitors utilizing computer-aided drug design, expecting new interactions with MMP-8 by hydrogen bonds, *e.g.* Ser172 of MMP-8.[15] These compounds exhibited potent inhibitions with an IC_{50} of 10^{-7}—10^{-9} M against MMP-3, -8, -9, but were not tested on MMP-1. We designed three different compounds containing an α-mercaptocarbonyl group, based on the enzyme recognition sites. The P_4–P_1 peptide incorporating compound (**3**), the P_1'–P_2' peptide incorporating compound (**4**), and both P_n and P_n' peptides incorporating compound (**5**) were designed. Also we designed three different thiol compounds based on (**3**) to confirm the necessity of the carbonyl group and the propriety of the distance between the carbonyl and thiol group: one lacking the carbonyl group (**6**); insertion of a methylene spacer between the carbonyl and the mercapto group (**7**); and replacement of the carbonyl by a hydroxy group (**8**).

To verify the usefulness of the α-mercaptocarbonyl group for MMP-1 inhibitory potency, substitutions of the mercapto group with other functional groups (alkylsulfanyl, alkylsulfinyl, alkyldisulfanyl, hydroxyl, amino, carboxyl, or hydroxamate) were conducted and compared with the mercapto group substituted compound against MMP-1 inhibitory potencies.

Synthesis The preparations of P_n peptide possessing compounds (**3**, **6**—**8**) are summarized in Chart 1. The key intermediates (**12**), (**16**), (**19**) and (**21**) were prepared from *N*-*tert*-butyloxycarbonyl alanine (Boc–Ala–OH) (**9**). A mixed anhydride, which was prepared from the reaction of **9** with ethyl chloroformate ($ClCO_2Et$)/Et_3N, was reacted with excess diazomethane to give a diazomethyl ketone (**10**). This was converted to the bromomethyl ketone (**11**) by reaction with HBr in ether.[16] The acetylsulfanylmethylketone (**12**) was obtained by reaction of **11** with potassium thioacetate followed by purification by silica gel column chromatography.

The deoxygenated compound of **12** depicted as **16** was synthesized as follows. The aldehyde (**14**), obtained *via* the aminoalcohol (**13**)[17] from **9**, was treated with the ylide derived from potassium bis(trimethylsilyl)amide [$[(CH_3)_3Si]_2NK$] and methyltriphenylphosphonium bromide (CH_3PPh_3Br), to give the olefin (**15**).[18] Then an addition of thioacetic acid to compound **15** was carried out under ultraviolet light catalyzed conditions[19] to afford **16**. The acetylsulfanylethylketone (**19**) that differed from intermediate (**12**) by the distance between the carbonyl and mercapto groups, was prepared *via* the α,β-unsaturated compound (**18**).[20] Thus the aminoaldehyde (**14**) was transformed into the vinyl alcohol (**17**), which on Swern oxidation gave the corresponding α,β-unsaturated ketone (**18**). Compound **19** was obtained by an 1,4-addition of thioacetic acid to **18**.[21] The alcohol derivative (**21**) was prepared by an epoxide ring opening with thioacetic acid.[22] Thus the olefin (**15**) was treated with *m*-chloroperbenzoic acid (MCPBA), which was allowed to react with thioacetic acid to give the desired alcohol (**21**). After deprotection of the Boc group from the intermediates (**12**), we initially utilized *N*-ethyl *N*′-(3-dimethylaminopropyl)carbodiimide (EDC)/1-hydroxybenzotriazol (HOBt) as a coupling reagent for the condensation with *N*-benzoyl–glycyl–prolyl–leucine (Bz–Gly–Pro–Leu–OH, **22**). But unexpectedly, the desired compound (**23a**) was not obtained owing to the instability of the free amino compound. So we selected a mixed anhydride procedure [iso-butylchloroformate/*N*-ethylmorpholine (NEM)], by which an amide bond could be formed before degradation of the free amine compound and succeeded in obtaining compound **23a**. Compounds **23b** and **23c** were also prepared by the same method. In the case of compound **23d**, BOP Reagent [benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate] was utilized as the coupling reagent to avoid the side reaction caused by the free hydroxy



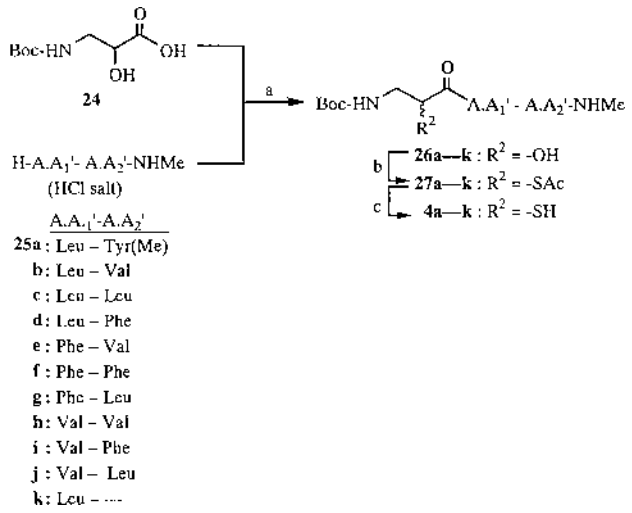
Reagents: (a) (1) $ClCO_2Et/Et_3N$ (2) CH_2N_2; (b) 0.65 M HBr; (c) AcSK; (d) (1) HOSu/EDC (2) $NaBH_4$; (e) $(COCl)_2$/DMSO; (f) Ph_3P^+-$MeBr^-$/$(TMS)_2NK$; (g) AcSH/UV; (h) vinylmagnesium bromide; (i) MCPBA; (j) AcSH/$Bu_4N^+F^-$; (k) AcSH; (l) TFA or 4 N HCl/AcOEt; (m) $ClCO_2i$-Bu/Et_3N or BOP reagent; (n) conc.NH_4OH/MeOH.

Chart 1. Syntheses of the P_4–P_1 Peptide Based Compounds

group. Finally, hydrolysis of the thioester (**23a**—**d**) gave the free thiol compounds (**3**, **6**—**8**).

P_n' peptide incorporating compounds **4a**—**k** were synthesized by the route shown in Chart 2. The 3-amino-2-hydroxypropanoic acid (DL-isoserine), which was prepared from 2-aminoethanol by Nishizawa and Saino[23] was a suitable precursor for the construction of an α-mercaptocarbonyl moiety, and the amino group was a good scaffold for the P_n peptide sequence. The amino group in isoserine was protected by a Boc group, followed by condensation with various dipeptides or amino acid *N*-methylamides (**25a**—**k**) using EDC/HOBt as a coupling reagent. Replacement of the hydroxy group with an acetylsulfanyl group (**27a**—**k**) was achieved *via* a mesylate intermediate. The free thiol (**4a**—**k**) was obtained by an alkaline hydrolysis according to a standard procedure. The physicochemical properties of **26a**—**k**, **27a**—**k**, and **4a**—**k** are shown in Tables 1—6.

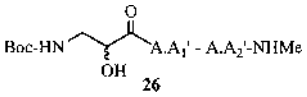
Syntheses of both P_n and P_n' peptides possessing compounds (**5a**—**c**) are summarized in Chart 3. The *N*-Boc group of compounds **27d** and **27k** were removed by treatment with 4 N HCl in EtOAc followed by condensation with *N-tert*-butyloxycarbonyl–prolyl–leucine (Boc–Pro–Leu–OH, **28a**) or *N-tert*-butyloxycarbonyl–leucine (Boc–Leu–OH, **28b**) using a mixed anhydride procedure ($ClCO_2i$-Bu/NEM)



Reagents: (a) Et_3N/HOBt/EDC; (b) (1) methanesulfonyl chloride/Et_3N (2) AcSK; (c) aq. NaOH/MeOH.

Chart 2. Syntheses of the P_1'–P_2' Peptide Incorporating Compounds

Table 1. Physical Data of Compounds **26a**—**k**



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Compd.	$A.A_1'$	$A.A_2'$	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (*c*, MeOH)	Formula (M+H), HR-MS *m/z* Calcd (Found)
26a	Leu	Tyr(Me)	70	172—174	−28.0 (1.15)	$C_{25}H_{41}N_4O_7$ 509.2975 (509.2937)
26b	Leu	Val	69	143—145	−45.3 (1.03)	$C_{20}H_{39}N_4O_6$ 431.2870 (431.2855)
26c	Leu	Leu	93	137—141	−47.6 (1.01)	$C_{21}H_{41}N_4O_6$ 445.3026 (445.3029)
26d	Leu	Phe	86	119—121	−41.1 (1.03)	$C_{24}H_{39}N_4O_6$ 479.2870 (479.2856)
26e	Phe	Val	75	194—197	−29.2 (1.03)	$C_{23}H_{37}N_4O_6$ 465.2713 (465.2712)
26f	Phe	Phe	82	160—163	−13.5 (1.0)	$C_{27}H_{37}N_4O_6$ 513.2713 (513.2740)
26g	Phe	Leu	77	152—154	−15.1 (0.98)	$C_{24}H_{39}N_4O_6$ 479.2870 (479.2877)
26h	Val	Val	62	185—187	−46.6 (0.50)	$C_{19}H_{37}N_4O_6$ 417.2713 (417.2712)
26i	Val	Phe	83	189—193	−31.7 (1.01)	$C_{23}H_{37}N_4O_6$ 465.2713 (465.2731)
26j	Val	Leu	81	171—174	−33.7 (1.0)	$C_{20}H_{39}N_4O_6$ 431.2870 (431.2848)
26k	Leu	—	68	142—146	−22.4 (1.0)	$C_{15}H_{30}N_3O_5$ 332.2185 (332.2198)

to give the compounds (**29a**—**c**). The free thiol groups (**5a**—**c**) were also obtained by an alkaline hydrolysis.

Preparation of the single isomers of **4a** described as compounds **36a**, **b** are shown in Chart 4 and were achieved by utilizing chiral isoserines (**32a**, **b**) reported by Maeda *et al.*[24] Formation of the 2-oxooxazolidine isomers (**31a**, **b**) were conducted by the treatment of malic acid half esters (**30a**, **b**)[25] with diphenylphosphoryl azide (DPPA) and Et_3N. Alkaline hydrolysis of the 2-oxooxazolidine (**31a**, **b**) was followed by protection of the amino group by treating with di-*tert*-butyl dicarbonate giving *N*-protected isoserine (**33a**, **b**). After condensations of the isoserine (**33a**, **b**) with leucyl–*O*-methyl–tyrosine–*N*-methylamide (H–Leu–Tyr(Me)–NHMe, **25a**) utilizing EDC/HOBt as a coupling reagent, subsequent conversions of the hydroxy group to the mercapto group were carried out by the same method as that described in the preparation of **4a**.

The preparations of compounds where the mercapto group was replaced with other functional groups are summarized in Chart 5. The alkylsulfanyl compounds (**37a**—**c**) were obtained by the same method described in the conversion of **26** to **27** utilizing the corresponding potassium salt of alkylmercaptane instead of potassium thioacetate. Sequentially, **37a**—**c** were oxidized by treatment with equimolar amounts of MCPBA to give the sulfoxides (**38a**—**c**). The sulfonyl disulfide (**39**) was synthesized from **4a** using diethyl *N*-ethylsulfenylhydrazodicarboxylate.[26] The amino compound (**41**) was also prepared *via* the mesylate followed by treating with sodium azide, and a hydrogenation.[27] The malonyl compound **44** was synthesized from the 3-*tert*-butyloxycarbonylamino-2(*R*,*S*)-benzyloxycarbonylpropanoic acid (**42**),[28] which was coupled to H–Leu–Tyr(Me)–NHMe (**25a**) using EDC/HOBt followed by catalytic hydrogenation in the presence of palladium on charcoal to give the malonyl compound (**44**). We also prepared the hydroxamate compound **46**, which was constructed by the condensation of **44** and *O*-benzylhydroxylamine using EDC/HOBt as the coupling reagent and subsequent removal of the benzyl group by means of a catalytic hydrogenation.

Results and Discussion

P_n Peptide Incorporating Compounds As expected, compound **3** exhibited strong MMP-1 inhibitory activity (3.5 μM IC_{50}), as potent as the hydroxamate (**1**) and the carboxylate (**2**). But modified compounds (**6**) and (**7**) were two magnitudes less potent than **3** (530 and 220 μM IC_{50}, respectively). The alcohol compound (**8**) had no inhibitory potency (0% inhibition at 10^{-3} M). These results clearly suggest that the existence of both the carbonyl and the thiol groups might be critical for the MMP-1 inhibition, and the distance between the two functional groups is important for inhibitory potency. We aimed to find new mercapto groups in the form of α-mercaptocarbonyl moiety as a ZCG, which showed no decrease in inhibitory activity, compared with the hydroxamate (**1**) or the carboxylate (**2**).

P_1'–P_2' Peptide Incorporating Compounds Regarding the P_1' and P_2' amino acids, Van Walt reported the substrate specificity of MMP-1 by measuring the rate of hydrolysis of various synthetic oligopeptide substrates.[29] Of those studied, Leu or Met are preferred at $A.A_1'$ and the aromatic amino acids Trp or Phe are preferred at $A.A_2'$. Based on this information, various P_1'–P_2' site peptides were incorporated into α-mercaptocarbonyl moiety and their MMP-1 inhibitory activities are summarized in Table 7. Although compounds that have Val at $A.A_1'$ (**4i**, **4j**) exhibited sub-nanomolar potencies against MMP-1, substitution of Val at $A.A_2'$ (**4h**) led to a loss of inhibitory potencies. The decreased affinity for MMP-1 is probably due to the repulsion induced by adjacent isopropyl groups in **4h**, which makes the molecule distort. In the case of $A.A_1'$=Phe (**4e**—**g**), the nature of $A.A_2'$ does affect the inhibitory potencies and these compounds all showed sub-nanomolar potencies. In a limited number of these compounds, $A.A_1'$=Leu (**4a**—**d**) provided the best result with respect to the MMP-1 inhibitory potencies. That is, the combinations of Leu at P_1' and hydrophobic amino acid residues at P_2', such as branched alkyls or an aromatic ring, showed nanomolar inhibitory potencies and these results are identical to the substrate specificity study by Van Walt and coworker[29] A comparison of compound **4** with well-explored P_1–P_2' mimic hydroxamate inhibitors, such as batimastat,[30] reveals that the preference at P_2' is a little different. In succinyl hydroxamate type inhibitors, the P_2' site preferred aromatic substituents to an alkyl group for *in vitro* activity,[5] but for the α-mercaptocarbonyl series (**4**), branched alkyl residues

Table 2. ^{1}H-NMR Data for Compounds **26a**—**k**

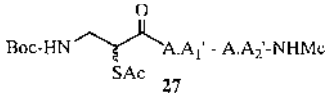
Compd.	A.A$_1$′	A.A$_2$′	^{1}H-NMR, ($CDCl_3$, δ, ppm)
26a	Leu	Tyr(Me)	0.80—1.00 (6H, m), 1.34—1.78 (13H, m), 2.68 (1.5H, d, J=5.0 Hz), 2.71 (1.5H, d, J=4.8 Hz), 2.88—3.09 (2H, m), 3.32—3.51 (1H, m), 3.56—3.74 (1H, m), 3.77 (1.5H, s), 3.78 (1.5H, s), 4.03—4.12 (0.5H, m), 4.18—4.34 (1.5H, m), 4.46—4.60 (1H, m), 5.42 (0.5H, d, J=4.2 Hz), 5.53 (0.5H, d, J=4.6 Hz), 5.87 (0.5H, d, J=7.9 Hz), 5.98 (0.5H, d, J=8.2 Hz), 6.55—6.66 (0.5H, m), 6.70—6.87 (2.5H, m), 7.02—7.15 (2H, m), 7.20—7.24 (1H, m)
26b	Leu	Val	0.81—1.02 (12H, m), 1.35—1.80 (13H, m), 2.08—2.20 (1H, m), 2.82 (3H, d, J=4.9 Hz), 3.40—3.78 (2H, m), 4.07—4.28 (2H, m), 4.28—4.35 (1H, m), 5.40—5.56 (0.5H, m), 5.60—5.68 (0.5H, m), 6.06—6.18 (0.5H, m), 6.20—6.31 (0.5H, m), 6.71—6.81 (0.5H, m), 6.81—6.95 (0.5H, m), 7.21—7.34 (0.5H, m), 7.45—7.53 (0.5H, m)
26c	Leu	Leu	0.80—1.01 (12H, m), 1.30—1.75 (16H, m), 2.74 (1.5H, d, J=5.0 Hz), 2.76 (1.5H, d, J=4.7 Hz), 3.41—3.62 (2H, m), 4.04—4.12 (0.5H, m), 4.23—4.35 (2.5H, m), 5.40—5.47 (0.5H, m), 5.51—5.62 (0.5H, m), 6.05—6.16 (1H, m), 6.62—6.71 (0.5H, m), 6.72—6.80 (0.5H, m), 7.31—7.48 (1H, m)
26d	Leu	Phe	0.78—0.92 (6H, m), 1.37—1.80 (13H, m), 2.67 (1.5H, d, J=4.9 Hz), 2.70 (1.5H, d, J=4.8 Hz), 2.94—3.05 (2H, m), 3.32—3.54 (2H, m), 4.01 (0.5H, m), 4.10—4.21 (1.5H, m), 4.50—4.60 (1H, m), 5.60—5.72 (0.5H, m), 5.70—5.81 (0.5H, m), 6.20—6.34 (0.5H, m), 6.45—6.53 (0.5H, m), 7.00—7.42 (7H, m)
26e	Phe	Val	0.71—0.93 (6H, m), 1.36 (4.5H, s), 1.44 (4.5H, s), 1.59—1.84 (1H, m), 1.95—2.23 (1H, m), 2.74 (1.5H, d, J=4.8 Hz), 2.75 (1.5H, d, J=5.0 Hz), 3.03—3.28 (2H, m), 3.32—3.75 (2H, m), 4.07—4.21 (2H, m), 4.51—4.60 (0.5H, m), 4.60—4.72 (0.5H, m), 5.18—5.33 (0.5H, m), 5.92—6.06 (0.5H, m), 6.11—6.21 (0.5H, m), 6.50 (0.5H, d, J=8.1 Hz), 6.70—6.80 (0.5H, m), 6.84 (0.5H, d, J=8.4 Hz), 7.12—7.34 (5H, m), 7.48 (0.5H, d, J=6.8 Hz), 7.54 (0.5H, d, J=7.5 Hz)
26f	Phe	Phe	1.40 (4.5H, s), 1.44 (4.5H, s), 1.72—2.21 (1H, m), 2.60 (1.5H, d, J=4.8 Hz), 2.65 (1.5H, d, J=4.8 Hz), 2.85—3.18 (4H, m), 3.18—3.38 (1H, m), 3.42—3.67 (1H, m), 3.97—4.05 (0.5H, m), 4.10—4.18 (0.5H, m), 4.47—4.65 (2H, m), 5.11 (0.5H, t, J=6.1 Hz), 5.59—5.68 (0.5H, m), 5.74—5.84 (0.5H, m), 6.31—6.42 (0.5H, m), 6.52 (0.5H, d, J=8.0 Hz), 6.55 (0.5H, d, J=8.3 Hz), 7.02—7.48 (10H, m), 7.72 (0.5H, d, J=7.7 Hz), 7.84 (0.5H, d, J=8.4 Hz)
26g	Phe	Leu	0.82—0.97 (6H, m), 1.41—1.84 (13H, m), 2.71 (1.5H, d, J=4.9 Hz), 2.75 (1.5H, d, J=4.9 Hz), 2.94—3.13 (2H, m), 3.33—3.76 (2H, m), 3.98—4.14 (0.5H, m), 4.14—4.26 (1.5H, m), 4.41—4.56 (1H, m), 5.21—5.34 (0.5H, m), 5.40—5.47 (0.5H, m), 5.47—5.56 (0.5H, m), 5.81—5.93 (0.5H, m), 5.93—6.06 (0.5H, m), 6.60—6.78 (0.5H, m), 7.01—7.42 (5H, m), 7.49—7.51 (0.5H, m), 7.59 (0.5H, d, J=7.9 Hz)
26h	Val	Val	0.82—1.08 (12H, m), 1.43 (4.5H, s), 1.45 (4.5H, s), 1.52—1.71 (1H, m), 2.07—2.21 (1H, m), 2.25—2.42 (1H, m), 2.80 (3H, d, J=4.8 Hz), 3.39—3.57 (1H, m), 3.60—3.82 (1H, m), 4.08—4.20 (2H, m), 4.20—4.29 (1H, m), 5.28—5.39 (0.5H, m), 5.47—5.57 (0.5H, m), 5.64—5.71 (0.5H, m), 6.04—6.19 (0.5H, m), 6.83 (0.5H, d, J=9.0 Hz), 6.89—6.94 (0.5H, m), 7.37 (0.5H, d, J=7.7 Hz), 7.52 (0.5H, d, J=5.9 Hz)
26i	Val	Phe	0.78 (1.5H, d, J=6.8 Hz), 0.81—0.92 (3H, m), 0.94 (1.5H, d, J=7.0 Hz), 1.43 (4.5H, s), 1.48 (4.5H, s), 1.52—1.72 (1H, m), 2.16—2.36 (1H, m), 2.67 (1.5H, d, J=5.0 Hz), 2.71 (1.5H, d, J=4.8 Hz), 2.98—3.13 (2H, m), 3.31—3.53 (1H, m), 3.53—3.76 (1H, m), 4.04—4.18 (1.5H, m), 4.21—4.28 (0.5H, m), 4.52—4.68 (1H, m), 5.14—5.25 (0.5H, m), 5.46—5.62 (0.5H, m), 5.68—5.93 (0.5H, m), 6.46—6.54 (0.5H, m), 6.56—6.69 (1H, m), 7.13—7.41 (6H, m)
26j	Val	Leu	0.82—1.04 (12H, m), 1.41—1.83 (13H, m), 2.09—2.21 (1H, m), 2.76 (1.5H, d, J=5.0 Hz), 2.78 (1.5H, d, J=4.8 Hz), 3.38—3.68 (2H, m), 4.06—4.25 (2H, m), 4.30—4.47 (1H, m), 5.42—5.56 (0.5H, m), 5.64—5.78 (0.5H, m), 6.02—6.23 (1H, m), 6.56—6.72 (0.5H, m), 6.71—6.84 (0.5H, m), 7.30—7.34 (0.5H, m), 7.48—7.59 (0.5H, m)
26k	Leu	—	0.77—0.98 (6H, m), 1.36—1.86 (13H, m), 2.71 (1.5H, d, J=4.9 Hz), 2.74 (1.5H, d, J=5.0 Hz), 3.34—3.51 (2H, m), 4.03 (0.5H, m), 4.12—4.28 (1.5H, m), 4.34—4.47 (1H, m), 6.18—6.32 (1H, m), 6.61—6.83 (1H, m)

also gave rise to inhibitory potency. Deletion of the P_2' site as shown for **4k** abolished the MMP-1 inhibitory activity. All of these compounds (**4a**—**k**) had been prepared in a racemic form and thus 1 : 1 mixtures of diastereoisomers because we used racemic isoserine as the starting material. To determine the stereochemical preference at the thiol attached carbon, we prepared both single isomers of **4a** described as **36a**, **b**[31] starting from chiral malic acids. As a result, the *S*-isomer (**36b**) was found to be approximately 100 times more potent than the corresponding *R*-isomer (Table 7).

Compounds Incorporating Both P_n and P_n' Peptides The MMP-1 inhibitory activities of compounds incorporating both P_n and P_n' peptides (**5a**—**c**) are also summarized in Table 7. Deletion of the P_2' residue described for **5c** also induced a significant loss of MMP-1 inhibitory activity. This result indicated that the P_2' site amino acid plays an important role in recognition for MMP-1. Compounds **5a** and **5b** were expected to have stronger affinity against the enzyme than **4d** or **4k**, which have only the P_1'–P_2' peptide as an enzyme recognition site. But insertion of the P_n peptide into **4d** or **4k** led to loss of activities. By comparing the backbones of collagen substrate and inhibitors **5a**—**c**, it was found that the β-alanine structure in the α-mercaptocarbonyl compounds is longer than the natural peptide sequence, which might introduce a gap between enzyme subsites and side chains of inhibitors **5a**, **b**, and caused the decreasing affinity for MMP-1.

Substitution of the Mercapto Group with Other Functional Groups Using compound **4** as the basic structure, neither the hydroxy intermediate (**26a**) nor sulfur atom containing and relatively stable derivatives such as the alkylsulfanyl compounds (**37a**—**c**), nor the oxidized compounds (**38a**—**c**) showed inhibitory potencies (0% at 10^{-6} M). The unsymmetrical disulfide of the ethyldisulfanyl compound (**39**) and the amino compound (**41**) exhibited little inhibitory potency (18 and 11% at 10^{-6} M respectively). Then we introduced a carboxylate and a hydroxamate that have the potential to chelate a zinc ion. But both the carboxylate (**44**) and hydroxamate (**46**) also showed no inhibitory potencies (0% at

Table 3. Physical Data for Compounds **27a**—**k**



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Compd.	$A.A_1'$	$A.A_2'$	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (*c*) solvent	Formula (M+H), HR-MS *m/z* Calcd (Found)
27a	Leu	Tyr(Me)	31	148—150	−39.2 (1.08) MeOH	$C_{27}H_{43}N_4O_7S$ 567.2852 (567.2845)
27b	Leu	Val	46	179—181	−78.5 (0.45) $CHCl_3$	$C_{22}H_{41}N_4O_6S$ 489.2747 (489.2748)
27c	Leu	Leu	50	174—177	−69.5 (1.07) $CHCl_3$	$C_{23}H_{43}N_4NaO_6S$ 527.2879 (527.2884)
27d	Leu	Phe	50	145—148	−45.7 (1.08) $CHCl_3$	$C_{26}H_{41}N_4O_6S$ 537.2747 (537.2741)
27e	Phe	Val	49	178—179	−39.7 (0.74) $CHCl_3$	$C_{25}H_{39}N_4O_6S$ 523.2590 (523.2606)
27f	Phe	Phe	52	161—163	−35.8 (1.04) $CHCl_3$	$C_{29}H_{39}N_4O_6S$ 571.2590 (571.2613)
27g	Phe	Leu	37	159—163	−40.6 (1.06) $CHCl_3$	$C_{26}H_{41}N_4O_6S$ 537.2747 (537.2756)
27h	Val	Val	42	201—203	−37.6 (0.98) $CHCl_3$	$C_{21}H_{39}N_4O_6S$ 475.2590 (475.2636)
27i	Val	Phe	47	211—213	−42.2 (1.02) $CHCl_3$	$C_{25}H_{39}N_4O_6S$ 523.2590 (523.2583)
27j	Val	Leu	45	183—185	−45.3 (1.06) $CHCl_3$	$C_{22}H_{41}N_4O_6S$ 489.2747 (489.2706)
27k	Leu	—	34	153—156	−35.2 (1.03)	$C_{17}H_{31}N_3O_5S$ 390.2063 (390.2070)

10^{-6} M). On the basis of these facts, it is speculated that only the mercapto group incorporating compound (**4**) provided a sufficient three dimensional distance between the active site zinc ion and P_1'–P_2' subsites.

Conclusion

From the evaluation of the P_4–P_1 peptide incorporating compounds **3**, **6**—**8** against MMP-1 inhibitory potency, we confirmed that the α-mercaptocarbonyl moiety plays a crucial role for chelation of the zinc ion in the MMP-1 active site. For the P_n' peptide incorporating compounds (**4a**—**j**), the α-mercaptocarbonyl unit was also effective against MMP-1 inhibition and Leu at the P_1' position exhibited the best results (**4a**—**d**) by which we achieved MMP-1 inhibitory potency at the nanomolar level. This is as potent as the inhibition of batimastat. But the intrinsic good chelation property of the α-mercaptocarbonyl unit was reduced by the existence of the P_n peptide in **5a**, **b**. Syntheses of both isomers of **4a** revealed that the *S* configuration at the thiol attached carbon was preferred for MMP-1 inhibition and only the thiol group provided a satisfactory inhibitory result. Further evaluation of the α-mercaptocarbonyl moiety will proceed against not only other MMPs but also general metalloenzymes (*e.g.*, angiotensin converting enzyme, tissue necrosis factor-α converting enzyme, aminopeptidase, and carboxypeptidase) to investigate the selectivity for MMP-1.

Experimental

Melting points were determined on a Yanagimoto melting point apparatus without correction. Column chromatography was performed on Silica gel BW-200 (Fuji Silysia Chemical Ltd.). TLC was performed on silica gel (Kiesel-gel 60 F_{254}, Merck). *Rf* values refer to the following v/v solvent system: Rf_1, $CHCl_3$–MeOH–AcOH (5 : 2 : 1); Rf_2, $CHCl_3$–MeOH–AcOH (80 : 10 : 5); Rf_3, $CHCl_3$–MeOH (10 : 1); Rf_4, $CHCl_3$–MeOH (20 : 1). ^{1}H-NMR was recorded on a JEOL JMN-AL300 spectrometer, and chemical shifts are given in ppm (δ) from tetramethylsilane (TMS), which was used as the internal standard. Mass spectra were obtained on a JEOL JMS 700 spectrometer by fast atom bombardment (FAB) ionization techniques. Optical rotations were measured in a JASCO DIP-140 apparatus.

1-Bromo-3(*S*)-*tert*-butyloxycarbonylamino-2-oxobutane (11) A mixed anhydride [prepared from Boc–Ala–OH (8.84 g, 46.7 mmol), ethyl chloroformate (5.58 g, 51.4 mmol) and Et_3N (7.41 ml, 51.4 mmol) as usual] in THF (150 ml) was added to an ice-cold solution of 0.47 N diazomethane in Et_2O (150 ml, 70 mmol). Then the reaction mixture was stirred for 2 h at the same temperature, after which it was evaporated *in vacuo* and the residue was partitioned between EtOAc and H_2O. The organic layer was washed successively with H_2O, sat. aq. $NaHCO_3$, and sat. aq. NaCl, and then was dried over $MgSO_4$. The EtOAc solution was concentrated to give crude Boc–Ala–CHN_2 (**10**) (11.6 g). A HBr solution [20% HBr in AcOH (15.9 ml, 49.0 mmol) diluted with Et_2O (30 ml)] was added dropwise to a solution of crude (**10**) in anhydrous THF (30 ml) at −15 °C over a period of 30 min. under a nitrogen atmosphere, and was stirred for a further 10 min at the same temperature (the final HBr concentration was 0.65 M). The reaction mixture was diluted with EtOAc, washed with successively H_2O, sat. aq. $NaHCO_3$, and sat. aq. NaCl. Then it was dried over $MgSO_4$, and concentrated to give **11** as a yellow solid (10.9 g, 88% yield): mp 52—56 °C, $[\alpha]_D^{23}$ −45.2° (*c*=0.522, MeOH), Rf_4 0.69. ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.36 (3H, d, *J*=6.7 Hz), 1.41 (9H, s), 4.08 (2H, s), 4.53 (1H, m), 5.08 (1H, m). FAB-MS *m/z*: 268 (M+H for ^{81}Br)$^+$, 266 (M+H for ^{79}Br)$^+$.

1-Acetylsulfanyl-3(*S*)-*tert*-butyloxycarbonylamino-2-oxobutane (12) Potassium thioacetate (90% min, 97.2 mg, 0.767 mmol) was added to a solution of **11** (200 mg, 0.693 mmol) in anhydrous acetone (15 ml), after which the mixture was stirred for 30 min and then concentrated. After dilution with EtOAc and H_2O, the organic layer was washed with H_2O and sat. aq. NaCl. Then it was dried over $MgSO_4$ and concentrated to give a yellow oil, which was purified by silica gel column chromatography (eluent; *n*-hexane : Et_2O= 2 : 1) to give **12** as a colorless solid (164 mg, 98%). mp 65.5—69.0 °C, Rf_4 0.66. ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.38 (3H, d, *J*=6.7 Hz), 1.43 (9H, s), 2.36 (3H, s), 3.85 (2H, s), 4.41 (1H, m), 5.10 (1H, m). FAB-MS *m/z*: 262 (M+H)$^+$.

2(*S*)-*tert*-Butyloxycarbonylamino-propanol (13) To a solution of Boc–Ala–OH (**9**) (20.0 g, 106 mmol) and *N*-hydroxysuccinimide (13.4 g, 112 mmol) in CH_2Cl_2 (150 ml), EDC (24.3 g, 127 mmol) was added at −8 °C, followed by stirring for 1 h at the same temperature and then at 4 °C for 15 h. The reaction mixture was washed with H_2O and sat. aq. NaCl, and then dried over $MgSO_4$. The CH_2Cl_2 solution was concentrated under reduced pressure, precipitated from 1-propanol, and collected by filtration to give an activated ester (23.3 g 77% yield). To a solution of this activated ester (23.3 g, 81.4 mmol) in THF (600 ml), $NaBH_4$ (8.01 g, 212 mmol) was added, followed by stirring at room temperature for 1 h. Additional $NaBH_4$ (4.00 g, 106 mmol) was added to the reaction mixture and stirring was continued for 1.5 h. Then the reaction mixture was poured into cold 10% aq. citric acid and the organic material was extracted with EtOAc. Next the organic layer was dried over $MgSO_4$ and evaporated *in vacuo* to give crude **13** (9.89 g, 78%) as a colorless oil. ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.13 (3H, d, *J*=6.4 Hz), 1.42 (9H, s), 2.94 (1H, m), 3.58 (2H, m), 3.72 (1H, m), 4.80 (1H, br d, *J*= 6.7 Hz). FAB-MS *m/z*: 176 (M+H)$^+$.

2(*S*)-*tert*-Butyloxycarbonylamino-propanal (14) To a solution of oxalyl chloride (5.77 ml, 67.7 mmol) in CH_2Cl_2 (50 ml), a solution of dimethylsulfoxide (10.6 ml, 149 mmol) in CH_2Cl_2 (60 ml) was added dropwise at a temperature below −68 °C. To the reaction mixture, a solution of the crude alcohol (**13**) (9.89 g, 56.4 mmol) in CH_2Cl_2 (400 ml) was added dropwise over a period of 15 min. After stirring for 20 min at the same temperature, Et_3N (41.4 ml, 298 mmol) was added to the mixture dropwise over a period of 30 min. Then the reaction mixture was poured into water and extracted with *n*-hexane. The organic layer was washed with 0.5 N aq. HCl and sat. aq.

Table 4. ^{1}H-NMR Data for Compounds **27a**—**k**

Compd.	A.A$_1$′	A.A$_2$′	^{1}H-NMR, (CD_3OD, δ, ppm)
27a	Leu	Tyr(Me)	0.81—0.97 (6H, m), 1.33—1.74 (12H, m), 2.35 (1.5H, s), 2.36 (1.5H, s), 2.66 (3H, s), 2.82—3.01 (1H, m), 3.01—3.18 (1H, m), 3.32—3.44 (m), 3.44—3.58 (1H, m), 3.75 (3H, s), 4.10—4.32 (2H, m), 4.48—4.64 (1H, m), 6.89—7.43 (1H, m)
27b	Leu	Val	0.81—1.01 (12H, m), 1.33—1.78 (12H, m), 1.90—2.08 (1H, m), 2.30 (1.5H, s), 2.34 (1.5H, s), 2.72 (3H, s), 3.20—3.46 (m), 3.46—3.58 (1H, m), 4.11—4.24 (1H, m), 4.30—4.48 (2H, m)
27c	Leu	Leu	0.79—1.04 (12H, m), 1.43 (9H, m), 1.52—1.80 (6H, m), 2.34 (1.5H, s), 2.35 (1.5H, s), 2.70 (3H, s), 3.20—3.60 (m), 4.22—4.41 (1H, m)
27d	Leu	Phe	0.83 (1.5H, d, J=4.6 Hz), 0.85 (1.5H, d, J=4.6 Hz), 0.89 (3H, d, J=6.4 Hz), 1.34—1.74 (12H, m), 2.34 (1.5H, s), 2.37 (1.5H, s), 2.66 (3H, s), 2.81—3.00 (1H, m), 3.03—3.24 (1H, m), 3.24—3.55 (m), 4.14—4.35 (2H, m), 4.43—4.58 (1H, m), 7.12—7.33 (5H, m)
27e	Phe	Val	0.78—0.97 (6H, m), 1.41 (9H, s), 1.93—2.12 (1H, m), 2.28 (1.5H, s), 2.31 (1.5H, s), 2.70 (3H, s), 2.82—2.98 (1H, m), 3.04—3.20 (1H, m), 3.20—3.51 (m), 3.99—4.12 (1H, m), 4.23 (1H, t, J=7.1 Hz), 4.56—4.73 (1H, m), 7.08—7.31 (5H, m)
27f	Phe	Phe	1.42 (9H, s), 2.28 (1.5H, s), 2.33 (1.5H, s), 2.63 (3H, s), 2.75—2.96 (2H, m), 2.96—3.19 (2H, m), 3.25—3.58 (m), 4.14—4.28 (1H, m), 4.42—4.63 (2H, m), 7.07—7.33 (10H, m)
27g	Phe	Leu	0.79 (1.5H, d, J=4.6 Hz), 0.81 (1.5H, d, J=5.0 Hz), 0.84 (3H, d, J=6.2 Hz), 1.43 (9H, s), 1.52—1.79 (3H, m), 2.30 (1.5H, s), 2.32 (1.5H, s), 2.68 (3H, s), 2.86—3.01 (1H, m), 3.17—3.23 (1H, m), 3.25—3.57 (m), 4.24 (1H, t, J=6.9 Hz), 4.28—4.40 (1H, m), 4.52—4.68 (1H, m), 7.15—7.35 (5H, m)
27h	Val	Val	0.81—1.02 (12H, m), 1.42 (9H, s), 1.93—2.19 (2H, m), 2.34 (1.5H, s), 2.36 (1.5H, s), 2.71 (3H, s), 3.23—3.40 (m), 3.40—3.55 (1H, m), 4.02—4.13 (1H, m), 4.17 (1H, d, J=7.3 Hz), 4.35 (1H, dd, J=6.9 and 13.9 Hz), 4.30—4.41 (1H, m)
27i	Val	Phe	0.71—0.93 (6H, m), 1.43 (9H, s), 1.90—2.09 (1H, m), 2.37 (3H, s), 2.66 (3H, s), 2.81—3.00 (1H, m), 3.04—3.23 (1H, m), 3.23—3.41 (m), 3.41—3.58 (1H, m), 4.03 (0.5H, d, J=6.2 Hz), 4.09 (0.5H, d, J=6.6 Hz), 4.26—4.41 (1H, m), 4.48—4.63 (1H, m), 7.12—7.33 (5H, m)
27j	Val	Leu	0.82—1.05 (12H, m), 1.32—1.73 (12H, m), 1.98—2.21 (1H, m), 2.34 (1.5H, s), 2.36 (1.5H, s), 2.71 (3H, s), 3.21—3.98 (m), 3.40—3.59 (1H, m), 4.13 (0.5H, d, J=6.8 Hz), 4.14 (0.5H, d, J=6.6 Hz), 4.27—4.44 (2H, m)
27k	Leu	—	0.81—1.02 (6H, m), 1.31—1.72 (12H, m), 2.34 (3H, s), 2.68 (3H, s), 3.32—3.54 (2H, m), 4.12—4.35 (2H, m)

NaCl, dried over $MgSO_4$, and then concentrated to give **14** (8.00 g, 82%) as a colorless solid. ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.32 (3H, d, J=6.9 Hz), 1.47 (9H, s), 4.18 (1H, m), 5.09 (1H, m), 9.50 (1H, s). FAB-MS m/z: 174 $(M+H)^+$. HR-MS Calcd for $C_8H_{16}NO_3$: 174.1130 $(M+H)^+$. Found: 174.1145.

2(*S*)-*tert*-Butyloxycarbonylaminobut-3-ene (15) To a suspension of methyltriphenylphosphonium bromide (6.94 g, 19.1 mmol) in anhydrous THF (50 ml), bis(trimethylsilyl)potassium amide (0.5 M solution in toluene, 38 ml, 19.0 mmol) was added dropwise at −69 °C under a nitrogen atmosphere, and the mixture was stirred at the same temperature for 11 h. To this mixture, a solution of aldehyde (**14**) (1.57 g, 9.07 mmol) in anhydrous THF (30 ml) was added and stirred at −65 °C for 15 min. Then the reaction mixture was warmed to room temperature and stirred for 1 h, after which it was stirred at 40 °C for 11 h. The reaction was quenched with MeOH, after which the mixture was washed with aq. Rochelle salt solution, dried over $MgSO_4$ and evaporated to give a brown residue, which was purified by silica gel column chromatography (eluent; *n*-hexane : Et_2O=6 : 1) to give **15** as a colorless oil (1.08 g, 70%). $[\alpha]_D^{25}$ −0.89° (c=1.0, MeOH), Rf_4 0.78. ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.19 (3H, d, J=6.7 Hz), 1.45 (9H, s), 4.03—4.61 (2H, m), 4.95—5.30 (2H, m), 5.66—6.07 (1H, m). FAB-MS m/z: 172 $(M+H)^+$.

1-Acetylsulfanyl-3(*S*)-*tert*-butyloxycarbonylaminobutane (16) A mixture of **15** (200 mg, 1.17 mmol) and thiolacetic acid (AcSH, 99 ml, 1.40 mmol) was irradiated with ultraviolet light (below 3000 Å) for 20 min. Then the reaction mixture was purified by silica gel column chromatography (eluent; *n*-hexane : Et_2O=4 : 1) to give **16** as colorless solid (253 mg, 87%). mp 54.5—55.0 °C, $[\alpha]_D^{25}$ −110° (c=1.0, MeOH), Rf_4 0.23. ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.11 (3H, d, J=6.7 Hz), 1.42 (9H, s), 1.66 (2H, t, J=7.3 Hz), 2.27 (3H, s), 2.82 (2H, dt, J=3.1, 7.3 Hz), 3.64 (1H, m), 4.09 (1H, br d, J=7.8 Hz). FAB-MS m/z: 248 $(M+H)^+$.

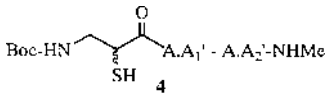
2(*S*)-*tert*-Butyloxycarbonylamino-3(*R*,*S*)-hydroxypent-4-ene (17) To a solution of vinylmagnesium bromide (1.0 M in THF, 36 ml), a solution of **14** (2.00 g, 11.5 mmol) in anhydrous THF (20 ml) was added at 0 °C and stirred for 2 h, followed by stirring at room temperature for 1 h. The reaction was quenched with sat. aq. ammonium chloride and the mixture was extracted with EtOAc. Then the organic layer was washed with sat. aq. NaCl, dried over $MgSO_4$, and evaporated to give an oil, which was purified by silica gel column chromatography (eluent; *n*-hexane : EtOAc=4 : 1) to give **17** as a colorless oil (1.47 g, 63%). ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.15 (3H, d, J=7.1 Hz), 1.42 (9H, s), 2.86 (1H, m), 3.66 (1H, m), 4.01 (1H, m), 4.72 (1H, br d, J=8.5 Hz), 5.12 (1H, m), 5.31 (1H, m), 5.60—5.98 (1H, m). FAB-MS m/z: 202 $(M+H)^+$.

2(*S*)-*tert*-Butyloxycarbonylamino-3-oxopent-4-ene (18) To a solution of oxalyl chloride (0.71 ml, 8.32 mmol) and dimethylsulfoxide (1.10 ml, 15.5 mmol) in CH_2Cl_2 (10 ml), a solution of **17** (1.47 g, 7.30 mmol) in CH_2Cl_2 (20 ml) was added dropwise over a period of 15 min. at −40 °C, followed by stirring at −40 °C for 1 h. After adding Et_3N (5.13 ml, 36.9 mmol), stirring was continued for 6 h at room temperature and then the reaction was quenched with water. The organic layer was separated and the aqueous layer was co-extracted with CH_2Cl_2. Subsequently, the combined organic layer was washed with sat. aq. NaCl, dried over $MgSO_4$, and then evaporated *in vacuo* to give a brown oil, which was purified by silica gel column chromatography (eluent; *n*-hexane : EtOAc=10 : 1) to give **18** as a colorless solid (617 mg, 42%). mp 68.5—72.0 °C, $[\alpha]_D^{25}$ +4.16° (c=1.0, MeOH), Rf_4 0.72. ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.32 (3H, d, J=6.7 Hz), 1.42 (9H, s), 4.55 (1H, m), 5.45 (1H, m), 5.80 (1H, dd, J=3.7, 7.4 Hz), 6.34 (2H, dd, J=3.7, 7.4 Hz). FAB-MS m/z : 200 $(M+H)^+$.

1-Acetylsulfanyl-4(*S*)-*tert*-butyloxycarbonylamino-3-oxopentane (19) To a mixture of thioacetic acid (37.3 μl, 0.527 mmol), **18** (100 mg, 0.502 mmol) and anhydrous THF (0.5 ml), tetrabutylammonium fluoride (50 mg) were added under a nitrogen atmosphere and the mixture was stirred at room temperature for 2 d. Then the reaction mixture was evaporated and purified by silica gel column chromatography (eluent; *n*-hexane : EtOAc=6 : 1) to give **19** as slightly yellow oil (80 mg, 58%). ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.28 (3H, d, J=6.7 Hz), 1.42 (9H, s), 2.27 (3H, s), 2.80 (2H, m), 2.99 (2H, m), 4.18 (1H, m), 5.07 (1H, m). FAB-MS m/z: 276 $(M+H)^+$.

1(*R*,*S*)-[1′(*S*)-*tert*-Butyloxycarbonylaminoethyl]oxirane (20) To a solution of **15** (500 mg, 2.92 mmol) in CH_2Cl_2 (25 ml), MCPBA (3.61 g, 14.6 mmol) was added and the mixture was stirred at room temperature for 1 h. Then the mixture was diluted with Et_2O, and washed successively with cold 10% aq. Na_2CO_3, sat. $NaHCO_3$, and sat. aq. NaCl. Subsequently, the mixture was dried over $MgSO_4$, and then evaporated *in vacuo* to give crude **20**, which was purified by silica gel column chromatography (eluent; *n*-hexane : Et_2O=2 : 1) to give **20** as a colorless oil (388 mg, 71%). $[\alpha]_D^{25}$ −10.5° (c=1.0, MeOH), Rf_4 0.71. ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.13 (1.1H, d, J=6.7

Table 5. Physical Data for Compounds **4a**—**k**



4

Compd.	A.A$_1$′	A.A$_2$′	Yield (%)	mp (°C)	$[\alpha]_D^{25}$ (c) solvent	Formula (M+H), HR-MS m/z Calcd (Found)
4a	Leu	Tyr(Me)	81	185—191	−31.0 (0.92) $CHCl_3$	$C_{25}H_{41}N_4O_6S$ 525.2747 (525.2750)
4b	Leu	Val	72	192—194	−50.7 (0.98) $CHCl_3$	$C_{20}H_{39}N_4O_5S$ 447.2641 (447.2606)
4c	Leu	Leu	60	176—178	−52.4 (1.10) $CHCl_3$	$C_{21}H_{41}N_4O_5S$ 461.2798 (461.2829)
4d	Leu	Phe	84	183—185	−48.5 (1.03) MeOH	$C_{24}H_{39}N_4O_5S$ 495.2641 (495.2625)
4e	Phe	Val	79	223—225	−15.2 (1.06) $CHCl_3$	$C_{23}H_{37}N_4O_5S$ 481.2485 (481.2462)
4f	Phe	Phe	82	179—181	−18.1 (0.99) $CHCl_3$	$C_{27}H_{37}N_4O_5S$ 529.2485 (529.2496)
4g	Phe	Leu	56	184—187	−19.4 (1.02) $CHCl_3$	$C_{24}H_{39}N_4O_5S$ 495.2641 (495.2647)
4h	Val	Val	69	201—205	−10.4 (0.36) DMF	$C_{19}H_{37}N_4O_5S$ 433.2485 (433.2473)
4i	Val	Phe	74	200—202	−14.1 (0.96) DMF	$C_{23}H_{37}N_4O_5S$ 481.2485 (481.2508)
4j	Val	Leu	66	228—230	−13.8 (0.43) DMF	$C_{20}H_{39}N_4O_5S$ 447.2641 (447.2628)
4k	Leu	—	92	165—168	−46.6 (0.42) MeOH	$C_{15}H_{30}N_3O_4S$ 348.1957 (348.1992)

Hz), 1.24 (1.9H, d, J=6.4 Hz), 1.42, 1.43 (9H, s each), 2.52—3.05 (3H, m), 3.94 (1H, m), 4.40 (1H, m). FAB-MS m/z: 188 $(M+H)^+$.

1-Acetylsulfanyl-3(*S*)-*tert*-butyloxycabonylamino-2(*R*,*S*)-hydroxybutane (21) Compound **20** (649 mg, 3.47 mmol) was dissolved in thioacetic acid (2.0 ml) and stirred at room temperature for 15 h. The excess thioacetic acid was removed by evaporation, and then the residue was purified by silica gel column chromatography (eluent; *n*-hexane : EtOAc=4 : 1) to give **21** as a slightly yellow oil (293 mg, 32%). $[\alpha]_D^{25}$ −36.2° (c=1.0, MeOH) Rf_4 0.35. ^{1}H-NMR ($CDCl_3$, TMS) δ: 1.19 (3H, d, J=6.4 Hz), 1.44 (9H, s), 2.05 (1H, d, J=6.4 Hz), 2.36 (3H, s), 3.02 (2H, m), 3.44—4.27 (2H, m), 4.85 (1H, br d, J=8.5 Hz). FAB-MS m/z: 264 $(M+H)^+$.

General Procedure for the Preparation of Compounds 23a—c A mixed anhydride [prepared from Bz–Gly–Pro–Leu–OH (**22**), ethyl chloroformate (1.1 eq of **22**), and *N*-methylmorpholine (NMM, 1.1 eq of **22**) as usual] in *N*,*N*-dimethylformamide (DMF) (4% w/v) was successively added to an ice-cold solution of *N*-deprotected **12**, **16**, or **19** [prepared by treatment with TFA] in DMF (4% w/v) and NMM (1 eq of *N*-deprotected **12**, **16**, or **19**). The reaction mixture was stirred at −15 °C for 1 h, and then was evaporated and the residue was purified by silica gel column chromatography (eluent; $CHCl_3$: MeOH=40 : 1) to give **23a**—**c** as a colorless solid.

1-Acetylsulfanyl-3(*S*)-(benzoyl-glycyl-prolyl-leucyl)amino-2-oxobutane (**23a**): Yield: 76%. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.91 (6H, d, J=6.8 Hz), 1.10—1.53 (6H, m), 1.62 (2H, m), 2.08 (2H, m), 2.33 (3H, s), 3.25—4.05 (6H, m), 4.21—4.89 (3H, m), 5.40 (1H, br t, J=5.1 Hz), 6.92—7.81 (7H, m). FAB-MS m/z: 533 $(M+H)^+$. HR-MS Calcd for $C_{26}H_{37}N_4O_6S$: 533.2434 $(M+H)^+$. Found: 533.2449.

1-Acetylsulfanyl-3(*S*)-(benzoyl-glycyl-prolyl-leucyl)aminobutane (**23b**): Yield: 50%. mp 121—123 °C, $[\alpha]_D^{25}$ −115° (c=1.0, MeOH), Rf_2 0.69. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.77—1.16 (9H, m, J=6.7 Hz), 1.42—1.91 (7H, m), 1.93—2.43 (5H, m), 2.75 (2H, dt, J=3.0, 7.7 Hz), 3.41—4.20 (4H, m), 4.20—4.71 (3H, m), 6.67 (1H, d, J=8.1 Hz), 7.32—7.70 (4H, m), 7.82 (2H, m). FAB-MS m/z: 519 $(M+H)^+$. HR-MS Calcd for $C_{26}H_{38}N_4O_5S$: 519.2641$(M+H)^+$. Found: 519.2621.

1-Acetylsulfanyl-4(*S*)-(benzoyl-glycyl-prolyl-leucyl)amino-3-oxopentane (**23c**): Yield: 58%. mp 100—102 °C, $[\alpha]_D^{25}$ −172° (c=1.0, MeOH), Rf_2 0.57. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.82—1.14 (9H, m, J=6.7 Hz), 1.41—1.92 (3H, m), 1.93—2.41 (7H, m), 2.61—3.24 (4H, m), 3.43—4.62 (7H, m), 7.11 (2H, m), 7.30—7.72 (4H, m), 7.80 (2H, m). FAB-MS m/z: 547 $(M+H)^+$. HR-MS Calcd for $C_{27}H_{39}N_4O_6S$: 547.2590 $(M+H)^+$. Found: 547.2608.

1-Acetylsulfanyl-3(*S*)-(benzoyl-glycyl-prolyl-leucyl)amino-2(*R*,*S*)-hydroxybutane (**23d**): To a solution of 1-acetylsulfanyl-3(*S*)-amino-2(*R*,*S*)-hydroxybutane hydrochloride [prepared from **21** (497 mg, 1.28 mmol) and 4 N HCl in EtOAc (15 ml)] in DMF (20 ml), benzotriazole-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent, 594 mg, 1.34 mmol), Bz-Gly-Pro-Leu-OH (**22**) and Et3N (406 μl, 2.92 mmol) were added successively. The mixture was stirred at room temperature for 15 h, then evaporated under reduced pressure. The residue was diluted with EtOAc, washed successively with 1 N aq. HCl, 10% aq. Na_2CO_3, and sat. aq. NaCl, dried over $MgSO_4$, and then evaporated *in vacuo*. The crude product was purified by silica gel column chromatography (eluent; $CHCl_3$: MeOH= 20 : 1) to give **23d** as colorless powder (385 mg, 56%). mp 110—114 °C, $[\alpha]_D^{25}$ −206° (c=1.0, MeOH), Rf_2 0.46. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.85—1.32 (9H, m), 1.41—1.89 (3H, m), 1.90—2.56 (7H, m), 2.98 (2H, m), 3.22—4.87 (9H, m), 6.72 (1H, m), 7.03—7.65 (5H, m), 7.87 (2H, m). FAB-MS m/z: 535 $(M+H)^+$. HR-MS Calcd for $C_{26}H_{39}N_4O_6S$: 535.2590 $(M+H)^+$. Found: 535.2582.

General Procedure for the Preparation of Compounds 3, 6—8 To a solution of **23a**—**d** in MeOH–THF (1 : 2, 2% w/v), 0.2 N aq. NaOH (1.2 eq of **23a**—**d**) was added and the mixture was stirred at room temperature for 1 h. Then the reaction mixture was acidified by adding 1 N aq. HCl (pH=2—3) and was evaporated *in vacuo*, followed by extraction with EtOAc, drying over $MgSO_4$, and evaporation *in vacuo*. The residue was precipitated from Et_2O to give **3**, **6**, **7**, or **8** as a colorless solid (the thiol compound was stored under a nitrogen atmosphere to prevent formation of the disulfide).

3(*S*)-(Benzoyl-glycyl-prolyl-leucyl)amino-1-mercapto-2-oxobutane (**3**): Yield: 83%. mp 121—125 °C, $[\alpha]_D^{25}$ −156° (c=1.0, MeOH), Rf_3 0.31. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.89 (9H, m), 1.12—1.51 (3H, m), 1.63 (2H, m), 1.83—2.17 (3H, m), 3.10 (2H, m), 3.34—4.02 (4H, m), 4.21—4.76 (3H, m), 5.41 (1H, br t, J=5.1 Hz), 6.93—7.80 (7H, m). FAB-MS m/z: 491 $(M+H)^+$. HR-MS Calcd for $C_{24}H_{35}N_4O_5S$: 491.2328 $(M+H)^+$. Found: 491.2312.

3(*S*)-(Benzoyl-glycyl-prolyl-leucyl)amino-1-mercaptobutane (**6**): Yield: 86%. mp 164—167 °C, $[\alpha]_D^{25}$ −133° (c=1.0, MeOH), Rf_3 0.57. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.74 (3H, d, J=6.4 Hz), 0.89 (6H, m), 1.32—1.90 (5H, m), 1.90—2.67 (7H, m), 3.42—4.10 (4H, m), 4.10—4.62 (3H, m), 6.52 (1H, br d, J=7.2 Hz), 7.08 (1H, br d, J=7.2 Hz), 7.33—7.69 (4H, m), 7.80 (2H, m). FAB-MS m/z: 477 $(M+H)^+$. HR-MS Calcd for $C_{24}H_{37}N_4O_4S$: 477.2536 $(M+H)^+$. Found: 477.2558.

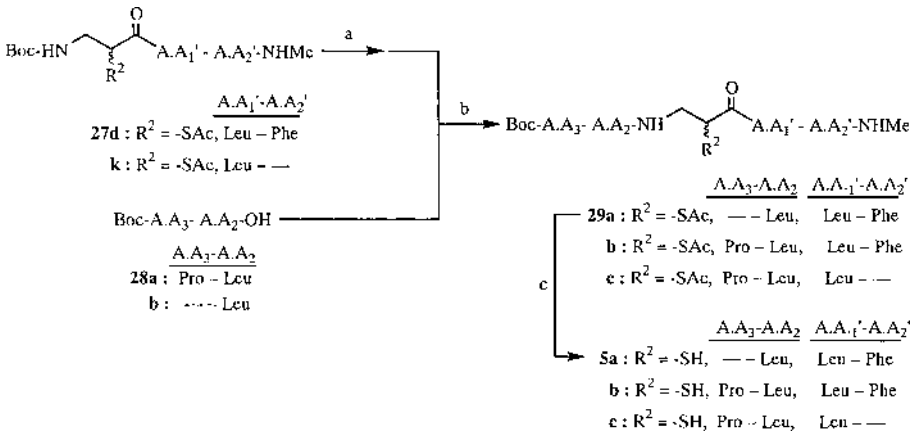
4(*S*)-(Benzoyl-glycyl-prolyl-leucyl)amino-1-mercapto-3-oxopentane (**7**): Yield: 84%. mp 100—102 °C, $[\alpha]_D^{25}$ −181° (c=1.0, MeOH), Rf_2 0.55. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.90 (9H, m), 1.16 (1H, d, J=7.1 Hz), 1.41—1.90 (2H, m), 1.90—2.40 (5H, m), 2.40—3.02 (4H, m), 3.45—4.72 (7H, m), 7.00—7.60 (5H, m), 7.60—8.11 (3H, m). FAB-MS m/z: 505 $(M+H)^+$. HR-MS Calcd for $C_{25}H_{37}N_4O_5S$: 505.2485 $(M+H)^+$. Found: 505.2494.

3(*S*)-(Benzoyl-glycyl-prolyl-leucyl)amino-2(*RS*)-hydroxy-1-mercaptobutane (**8**): Yield: 83%. mp 110—114 °C, $[\alpha]_D^{25}$ −169° (c=1.0, MeOH), Rf_3 0.27. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.69—1.32 (9H, m), 1.32—1.90 (3H, m), 1.90—2.44 (8H, m), 3.31—4.68 (8H, m), 6.78 (1H, m), 6.90—7.68 (4H, m), 7.68—8.13 (3H, m). FAB-MS m/z: 493 $(M+H)^+$. HR-MS Calcd for $C_{24}H_{37}N_4O_5S$: 493.2485 $(M+H)^+$. Found: 493.2476.

3-*tert*-Butyloxycarbonylamino-2(*R*,*S*)-hydroxypropionic Acid (24) To a solution of 2-*tert*-butyloxycarbonylaminoethanol (43.9 g, 272 mmol) and Et_3N (114 ml, 817 mmol) in CH_2Cl_2 (600 ml) cooled to −10 °C, sulfur trioxide-pyridine complex (130 g, 817 mmol) in DMSO (400 ml) was added as a bolus. The mixture was stirred vigorously for 10 min at 35 °C, poured into cold sat. aq. NaCl (1500 ml), and extracted with cooled Et_2O. The organic extracts were washed with cooled 10% aq. citric acid and cooled water, dried over $MgSO_4$, and then evaporated *in vacuo* to give the corresponding aldehyde, which was purified by silica gel column chromatography (eluent; $CHCl_3$: MeOH=300 : 1—100 : 1) to give the aldehyde as a yellow oil (18.2 g, 42%). To a solution of the aldehyde in acetone (50 ml), an ice-cold solution of $NaHSO_3$ (11.9 g, 114 mmol) in water (200 ml) was added, and the mixture was stirred overnight at 5 °C. To the solution of $NaHSO_3$ adduct, a

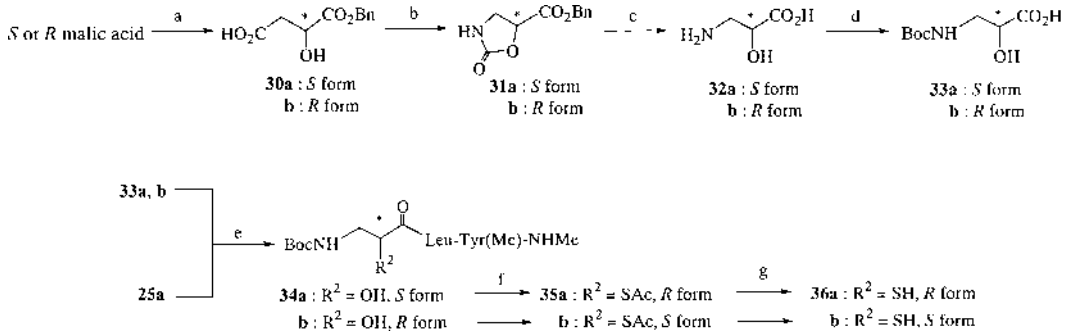
Table 6. ^{1}H-NMR Data for Compounds **4a**—**k**

Compd.	A.A$_1$′	A.A$_2$′	^{1}H-NMR, ($CDCl_3$, δ, ppm)
4a	Leu	Tyr(Me)	0.80—1.01 (6H, m), 1.27—1.70 (12H, m), 2.04 (0.5H, d, *J*=10.3 Hz), 2.11 (0.5H, d, *J*=10.1 Hz), 2.71 (3H, d, *J*=4.8 Hz), 2.90—3.08 (2H, m), 3.28—3.58 (3H, m), 3.78 (3H, s), 4.21—4.38 (1H, m), 4.33—4.50 (1H, m), 5.34 (0.5H, t, *J*=6.5 Hz), 5.60—5.69 (0.5H, m), 6.72—6.93 (3H, m), 7.00—7.18 (3H, m), 7.26—7.34 (0.5H, m), 7.61—7.70 (0.5H, m)
4b	Leu	Val	0.78—1.01 (12H, m), 1.27—1.78 (12H, m), 1.89—1.98 (1H, m), 2.03 (0.5H, d, *J*=10.0 Hz), 2.10 (0.5H, d, *J*=10.3 Hz), 2.70 (1.5H, d, *J*=4.7 Hz), 2.72 (1.5H, d, *J*=4.7 Hz), 3.30—3.61 (3H, m), 4.22—4.50 (2H, m), 5.21 (0.5H, d, *J*=6.4 Hz), 5.32—5.41 (0.5H, m), 5.56—5.64 (0.5H, m), 5.84—5.94 (0.5H, m), 6.40—6.48 (0.5H, m), 6.48—6.58 (0.5H, m), 6.75 (0.5H, d, *J*=7.5 Hz), 6.80—6.92 (0.5H, m)
4c	Leu	Leu	0.78—1.02 (12H, m), 1.41 (4.5H, s), 1.43 (4.5H, s), 1.51—1.82 (6H, m), 2.06 (0.5H, d, *J*=10.3 Hz), 2.12 (0.5H, d, *J*=10.5 Hz), 2.80 (3H, d, *J*=4.8 Hz), 3.26—3.61 (2H, m), 3.61—3.76 (0.5H, m), 3.76—3.91 (0.5H, m), 4.39—4.66 (2H, m), 5.31 (0.5H, t, *J*=6.1 Hz), 5.51—5.68 (0.5H, m), 6.71—6.85 (0.5H, m), 6.93 (0.5H, d, *J*=9.2 Hz), 7.02—7.21 (1H, m), 7.21—7.36 (0.5H, m), 7.67 (0.5H, d, *J*=8.4 Hz)
4d	Leu	Phe	0.78—1.02 (6H, m), 1.31—1.78 (12H, m), 2.01 (0.5H, d, *J*=10.5 Hz), 2.08 (0.5H, d, *J*=10.1 Hz), 2.70 (1.5H, d, *J*=4.8 Hz), 2.71 (1.5H, d, *J*=4.8 Hz), 2.99—3.15 (2H, m), 3.27—3.73 (3H, m), 4.28—4.52 (1H, m), 4.56—4.74 (1H, m), 5.28 (0.5H, t, *J*=6.1 Hz), 5.49—5.60 (0.5H, m), 6.00 (0.5H, d, *J*=6.5 Hz), 6.28—6.40 (0.5H, m), 6.70—6.83 (0.5H, m), 6.93 (0.5H, d, *J*=9.1 Hz), 7.05—7.38 (6H, m)
4e	Phe	Val	0.78—0.97 (6H, m), 1.42 (9H, s), 1.84—1.96 (1H, m), 2.02 (0.5H, d, *J*=10.2 Hz), 2.10 (0.5H, d, *J*=10.4 Hz), 2.70 (1.5H, d, *J*=4.8 Hz), 2.72 (1.5H, d, *J*=4.8 Hz), 2.96—3.12 (2H, m), 3.28—3.67 (3H, m), 4.16—4.21 (1H, m), 4.36—4.51 (1H, m), 5.22 (0.5H, d, *J*=6.1 Hz), 5.37—5.47 (0.5H, m), 5.60—5.74 (0.5H, m), 5.77—5.89 (0.5H, m), 6.41—6.53 (0.5H, m), 6.53—6.65 (0.5H, m), 7.00—7.43 (6H, m)
4f	Phe	Phe	1.43 (9H, s), 1.87 (0.5H, d, *J*=9.9 Hz), 1.89 (0.5H, d, *J*=10.1 Hz), 2.66 (1.5H, d, *J*=4.8 Hz), 2.68 (1.5H, d, *J*=4.8 Hz), 2.86—3.15 (4H, m), 3.26—3.55 (3H, m), 4.47—4.69 (2H, m), 5.07—5.21 (0.5H, m), 5.26—5.39 (0.5H, m), 5.55—5.70 (0.5H, m), 5.77—5.92 (0.5H, m), 6.44 (0.5H, d, *J*=8.3 Hz), 6.49 (0.5H, d, *J*=8.6 Hz), 6.73 (0.5H, d, *J*=6.6 Hz), 6.78 (0.5H, d, *J*=7.0 Hz), 6.97—7.39 (10H, m)
4g	Phe	Leu	0.78—0.97 (6H, m), 1.32—1.78 (12H, m), 2.02 (0.5H, d, *J*=10.2 Hz), 2.09 (0.5H, d, *J*=10.5 Hz), 2.70 (1.5H, d, *J*=4.8 Hz), 2.71 (1.5H, d, *J*=4.8 Hz), 2.92—3.21 (2H, m), 3.32—3.56 (2H, m), 3.60—3.72 (1H, m), 4.32—4.51 (1H, m), 4.51—4.73 (1H, m), 5.30 (0.5H, t, *J*=6.0 Hz), 5.49—5.57 (0.5H, m), 5.92—6.02 (0.5H, m), 6.31—6.43 (0.5H, m), 6.72—6.82 (0.5H, m), 6.85—6.96 (0.5H, m), 7.01—7.46 (6H, m)
4h	Val	Val	0.77—0.97 (12H, m), 1.41 (9H, s), 1.82—2.01 (2H, m), 2.04 (0.5H, d, *J*=10.1 Hz), 2.11 (0.5H, d, *J*=10.5 Hz), 2.70 (1.5H, d, *J*=4.8 Hz), 2.72 (1.5H, d, *J*=4.8 Hz), 3.27—3.81 (3H, m), 4.45—4.78 (2H, m), 5.49—5.56 (0.5H, m), 5.67—5.78 (0.5H, m), 6.16—6.29 (0.5H, m), 6.50—6.60 (0.5H, m), 6.63—6.76 (0.5H, m), 6.80—6.87 (0.5H, m), 6.95—7.08 (0.5H, m), 7.08—7.18 (0.5H, m)
4i	Val	Phe	0.72—0.98 (6H, m), 1.41 (9H, s), 1.89—2.01 (1H, m), 2.03 (0.5H, d, *J*=10.1 Hz), 2.09 (0.5H, d, *J*=10.5 Hz), 2.73 (3H, d, *J*=4.7 Hz), 2.98—3.21 (2H, m), 3.32—3.57 (3H, m), 4.37—4.68 (2H, m), 5.06—5.20 (0.5H, m), 5.31—5.44 (0.5H, m), 5.61—5.73 (0.5H, m), 5.90—6.12 (0.5H, m), 6.41 (0.5H, d, *J*=8.1 Hz), 6.46 (0.5H, d, *J*=8.6 Hz), 6.76 (0.5H, d, *J*=8.1 Hz), 6.82 (0.5H, d, *J*=8.6 Hz), 7.10—7.41 (5H, m)
4j	Val	Leu	0.73—0.97 (12H, m), 1.28—1.62 (12H, m), 1.90—1.98 (1H, m), 2.03 (0.5H, d, *J*=10.1 Hz), 2.08 (0.5H, d, *J*=10.1 Hz), 2.70 (3H, d, *J*=4.8 Hz), 3.37—3.72 (3H, m), 4.30—4.44 (1H, m), 4.51—4.63 (1H, m), 5.34 (0.5H, t, *J*=6.3 Hz), 5.43—5.61 (0.5H, m), 6.29—6.41 (0.5H, m), 6.60—6.73 (0.5H, m), 6.81—6.88 (1H, m), 6.93—7.05 (0.5H, m), 7.12—7.28 (0.5H, m)
4k	Leu	—	0.93 (3H, d, *J*=6.2 Hz), 0.95 (3H, d, *J*=6.4 Hz), 1.36—1.84 (12H, m), 2.07 (1H, d, *J*=9.7 Hz), 2.82 (3H, d, *J*=4.8 Hz), 3.34—3.64 (3H, m), 4.32—4.49 (1H, m), 5.08—5.27 (1H, m), 6.06—6.28 (1H, m), 6.65—6.83 (1H, m)



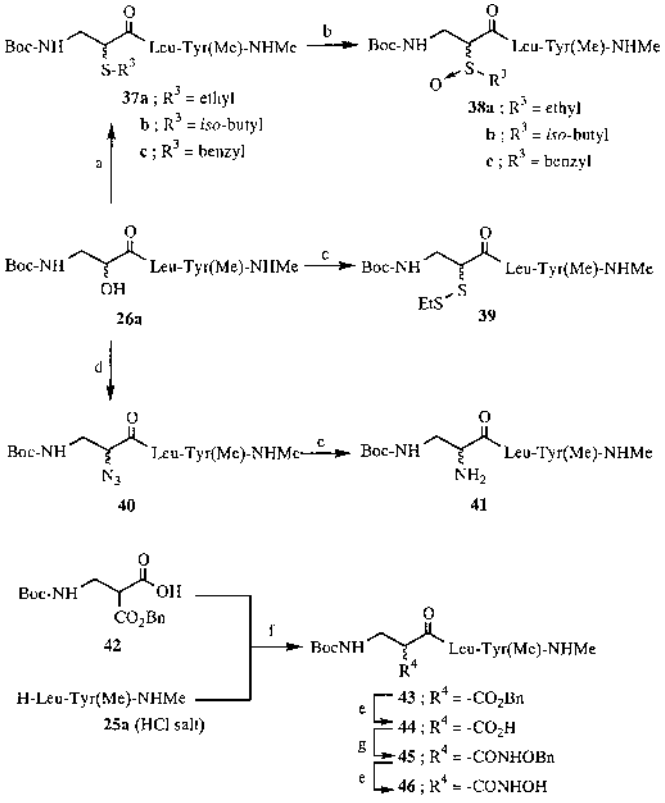
Reagents: (a) 4 N HCl in EtOAc; (b) NEM/$ClCO_2$*i*-Bu; (c) aq. NaOH/MeOH.

Chart 3. Syntheses of the Both P_n and P_n′ Peptides Incorporating Compounds



Reagents: (a) (1) Trifluoroacetic anhydride, (2) BnOH; (b) diphenylphosphoryl azide/Et_3N; (c) aq. NaOH/MeOH; (d) $(Boc)_2O$/aq. NaOH/acetone; (e) Et_3N/EDC/HOBt; (f) (1) methanesulfonyl chloride/Et_3N (2) AcSK; (g) aq. NaOH/MeOH.

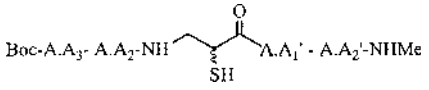
Chart 4. Preparation of the Single Isomers of Compound **4a**



Reagents: (a) (1) methanesulfonyl chloride/Et_3N (2) R^3-SH/KOH; (b) MCPBA; (c) (1) methanesulfonyl chloride/Et_3N (2) AcSK (3) aq. NaOH/MeOH; (4) EtO_2C-N(SEt)-NH-CO_2Et; (d) (1) methanesulfonyl chloride/Et_3N (2) NaN_3; (e) H_2/Pd–C; (f) Et_3N/HOBt/EDC; (g) $BnONH_2$/HOBt/EDC.

Chart 5. Substitution of the Mercapto Group with Other Functional Groups

Table 7. Structure and MMP-1 Inhibitory Activities of α-Mercaptocarbonyl Compounds (**4a**—**k**, **5a**—**c**, and **36a**, **b**)[a]



Compd.	$A.A_3$	$A.A_2$	$A.A_1'$	$A.A_2'$	Inhibitory activity (IC_{50}, nM)
4a	—	—	Leu	Tyr(Me)	6.8
36a[b]	—	—	Leu	Tyr(Me)	240
36b[c]	—	—	Leu	Tyr(Me)	2.5
4b	—	—	Leu	Val	6.7
4c	—	—	Leu	Leu	8.1
4d	—	—	Leu	Phe	1.4
4e	—	—	Phe	Val	48
4f	—	—	Phe	Phe	23
4g	—	—	Phe	Leu	40
4h	—	—	Val	Val	140
4i	—	—	Val	Phe	15
4j	—	—	Val	Leu	24
4k	—	—	Leu	—	8400
5a	—	Leu	Leu	Phe	23
5b	Pro	Leu	Leu	Phe	170
5c	Pro	Leu	Leu	—	>10000

a) Values are averages for 3 experiments. *b*) (*R*)-isomer. *c*) (*S*)-isomer.

solution of KCN (7.61 g, 114 mmol) in water (30 ml) was added. After stirred for 4 h, the mixture was extracted with EtOAc and evaporated to give the cyanohydrin as an oil. Then it was hydrolyzed in dioxane–conc. HCl (1 : 1, 100 ml) by reflux for 12 h. The hydrolyzate was evaporated after washing with Et_2O and the residue was dissolved in H_2O–acetone (1 : 1, 100 ml), which was adjusted to pH 5.5 with NH_4OH. Crystals were precipitated by letting the mixture stand overnight at 4 °C and were filtered and washed with acetone to give 5.66 g of 3-amino-2(*RS*)-hydroxypropionic acid (47% yield from the aldehyde) as a colorless solid. To a mixture of 3-amino-2(*RS*)-hydroxypropionic acid (5.66 g, 53.9 mmol), dioxane–H_2O (1 : 1, 100 ml), and 1 N aq. NaOH (53.9 ml), a solution of $(Boc)_2O$ (12.3 g, 56.6 mmol) in dioxane (50 ml) was added and the mixture was stirred at room temperature for 4 h. After washing with Et_2O, the reaction mixture was adjusted to pH 2 with 1 N aq. HCl, followed by extraction with EtOAc, drying over $MgSO_4$, and evaporation *in vacuo*. Then the residue was precipitated from Et_2O–*n*-hexane to give 8.0 g of **24** (72%) as a colorless solid. mp 92—95 °C, Rf_2 0.59. ^{1}H-NMR (CD_3OD, TMS) δ: 1.43 (9H, s), 3.23—3.35 (m), 3.35—3.47 (1H, m), 4.03—4.27 (1H, dd, J=4.1, 6.8 Hz). FAB-MS m/z : 206 $(M+H)^+$.

General Procedure for the Preparation of Compounds 26a—k EDC (1.1 eq of **24**) was added to a mixture of H–$A.A_1'$–$A.A_2'$–NHMe·HCl (1.05 eq of **24**; prepared from the corresponding Boc–$A.A_1'$–$A.A_2'$–NHMe and 4 N HCl in EtOAc), Et_3N (1.0 eq of H–$A.A_1'$–$A.A_2'$–NHMe·HCl), HOBt (1.05 eq of **24**), compound **24**, and DMF (4—7% w/v) at −12 °C. The mixture was stirred at −3 °C for 6 h and then at 0 °C for 15 h. The reaction mixture was subsequently diluted with EtOAc, washed successively with sat. aq. NaCl, 1 N aq. HCl, sat. aq. NaCl, 10% aq. Na_2CO_3, and sat. aq. NaCl, dried over $MgSO_4$, and evaporated *in vacuo* to give a crude product, which was purified by silica gel column chromatography (eluent; $CHCl_3$: MeOH= 50 : 1—30 : 1) to give **26a**—**k** as a colorless solid. Yield and spectral data for **26a**—**k** are listed in Tables 1 and 2.

General Procedure for the Preparation of Compounds 27a—k To a solution of **26** in CH_2Cl_2–pyridine (3 : 1, 4% w/v), methanesulfonyl chloride (1.5 eq of **26**) was added dropwise at 0 °C, and the mixture was stirred at the same temperature for 2 h. Then the reaction mixture was diluted with EtOAc (0.6% w/v) and poured into cold 1 N aq. HCl (40 eq of **26**). The organic layer was washed with sat. aq. NaCl, dried over $MgSO_4$, and evaporated to give a mesylate. To a solution of the mesylate in DMF (5% w/v), potassium thioacetate (5 eq of mesylate) was added, and the mixture was stirred at room temperature for 2 d. Then the reaction mixture was diluted with EtOAc, washed

with sat. aq. NaCl, dried over $MgSO_4$, and evaporated *in vacuo* to give a slightly brown oil, which was purified by silica gel column chromatography (eluent; $CHCl_3$: MeOH = 60 : 1—30 : 1) to give **27a**—**k** as a colorless solid. Yield and spectral data for **27a**—**k** are listed in Tables 3 and 4.

General Procedure for the Preparation of Compounds 4a—k To a solution of **27** in MeOH (5% w/v), 1 N aq. NaOH (1.05 eq of **27**) was added, and the mixture was stirred at room temperature for 0.5—1 h. Then the reaction mixture was diluted with EtOAc, washed successively with sat. aq. NaCl, 1 N aq. HCl, and sat. aq. NaCl, dried over $MgSO_4$, and evaporated *in vacuo* to give a colorless residue, which was precipitated from Et_2O to give **4a**—**k** as a colorless solid. Yield and spectral data for **4a**—**k** are listed in Tables 5 and 6.

General Procedure for the Preparation of Compounds 29a—c Compound **27d** or **27k** was treated with cold 4 N HCl in EtOAc (4% w/v) for 0.5—1.0 h. The reaction mixture was evaporated *in vacuo* and the residue was precipitated from Et_2O, collected by filtration, and dried under reduced pressure to give the hydrochloride salt in a quantitative yield. A mixed anhydride [prepared from Boc–$A.A_3$–$A.A_2$–OH (**28a** or **b**), ethyl chloroformate (1.1 eq of **28a** or **b**), and NMM (1.1 eq of **28a** or **b**) as usual] in DMF (4% w/v) was added to an ice-cold solution of the amine hydrochloride salt (0.95 eq of **28a** or **b**) in DMF (7% w/v). Then NMM (1 eq of amine hydrochloride salt) was added and the mixture was stirred at 0 °C for 1 h. Subsequently, the reaction mixture was partitioned between EtOAc and H_2O. The organic layer was washed successively with H_2O, sat. aq. $NaHCO_3$, and sat. aq. NaCl, dried over $MgSO_4$, and evaporated *in vacuo* to give a crude product, which was purified by silica gel column chromatography (eluent; $CHCl_3$: MeOH = 80 : 1—40 : 1) to give **29** as a colorless solid.

N^{α}-[3-(*tert*-Butyloxycarbonyl-leucyl-amino)-2(*R*,*S*)-(acetylsulfanyl)propionyl]-leucyl-phenylalanine *N*-Methylamide (**29a**, $A.A_2$=Leu, $A.A_1'$=Leu, $A.A_2'$=Phe): Yield: 92%. mp 126—135 °C, $[\alpha]_D^{25}$ −34.9° (*c*=0.29, $CHCl_3$), Rf_4 0.19 and 0.26. ^{1}H-NMR (CD_3OD, TMS) δ: 0.80—1.01 (12H, m), 1.31—1.72 (15H, m), 2.35 (3H, s), 2.67 (3H, s), 2.85—2.98 (1H, m), 2.98—3.15 (1H, m), 3.45—3.65 (2H, m), 4.10—4.41 (3H, m), 4.41—4.56 (1H, m), 7.12—7.33 (5H, m). FAB-MS *m*/*z*: 650 $(M+H)^+$. HR-MS Calcd for $C_{32}H_{52}N_5O_7S$: 650.3587 $(M+H)^+$. Found: 650.3628.

N^{α}-[3-(*tert*-Butyloxycarbonyl-prolyl-leucyl-amino)-2(*R*,*S*)-(acetylsulfanyl)propionyl]-leucyl-phenylalanine *N*-Methylamide (**29b**, $A.A_3$=Pro, $A.A_2$=Leu, $A.A_1'$=Leu, $A.A_2'$=Phe): Yield: 40%. mp 187—189 °C, $[\alpha]_D^{25}$ −53.8° (*c*=1.02, $CHCl_3$), Rf_2 0.52. ^{1}H-NMR (CD_3OD, TMS) δ: 0.78—1.01 (12H, m), 1.28—1.77 (15H, m), 1.77—2.08 (3H, m), 2.08—2.29 (1H, m), 2.35 (3H, s), 2.67 (3H, s), 2.80—2.95 (1H, m), 3.10—3.23 (1H, m), 3.35—3.68 (4H, m), 4.10—4.42 (4H, m), 4.42—4.57 (1H, m), 7.12—7.31 (5H, m). FAB-MS *m*/*z*: 747 $(M+H)^+$. HR-MS Calcd for $C_{37}H_{59}N_6O_8S$: 747.4115 $(M+H)^+$. Found: 747.4159.

N^{α}-[3-(*tert*-Butyloxycarbonyl-prolyl-leucyl-amino)-2(*R*,*S*)-(acetylsulfanyl)propionyl]-leucine *N*-Methylamide (**29c**, $A.A_3$=Pro, $A.A_2$=Leu, $A.A_1'$=Leu): Yield: 65%. mp 187—193 °C, $[\alpha]_D^{25}$ −41.0° (*c*=0.79, $CHCl_3$), Rf_3 0.36. ^{1}H-NMR (CD_3OD, TMS) δ: 0.80—1.02 (12H, m), 1.30—1.76 (15H, m), 1.76—2.07 (3H, m), 2.07—2.29 (1H, m), 2.34 (3H, s), 2.71 (3H, s), 3.35—3.56 (3H, m), 3.56—3.70 (1H, m), 4.16—4.40 (4H, m). FAB-MS *m*/*z*: 600 $(M+H)^+$. HR-MS Calcd for $C_{28}H_{50}N_5O_7S$: 600.3431 $(M+H)^+$. Found: 600.3435.

General Procedure for the Preparation of Compounds 5a—c To a solution of **29** in MeOH (5% w/v), 1 N aq. NaOH (1.05 eq of **29**) was added and the mixture was stirred at room temperature for 0.5—1 h. Then the reaction mixture was diluted with EtOAc, washed successively with sat. aq. NaCl, 1 N aq. HCl, and sat. aq. NaCl, dried over $MgSO_4$, and evaporated *in vacuo* to give a colorless residue, which was precipitated from EtOAc–Et_2O to give **5a**—**c** as a colorless solid.

N^{α}-[3-(*tert*-Butyloxycarbonyl-leucyl-amino)-2(*R*,*S*)-(mercapto)propionyl]-leucyl-phenylalanine *N*-Methylamide (**5a**, $A.A_2$=Leu, $A.A_1'$=Leu, $A.A_2'$=Phe): Yield: 33%. mp 198—202 °C, $[\alpha]_D^{25}$ −40.2° (*c*=0.53, $CHCl_3$), Rf_2 0.49. ^{1}H-NMR (CD_3OD, TMS) δ: 0.80—1.02 (12H, m), 1.37—1.78 (15H, m), 2.67 (3H, s), 2.87—3.00 (1H, m), 3.03—3.19 (1H, m), 3.45—3.64 (2H, m), 3.95—4.12 (1H, m), 4.18—4.30 (1H, m), 4.45—4.66 (2H, m), 7.14—7.31 (5H, m). FAB-MS *m*/*z*: 608 $(M+H)^+$. HR-MS Calcd for $C_{30}H_{50}N_5O_6S$: 608.3482 $(M+H)^+$. Found: 608.3528.

N^{α}-[3-(*tert*-Butyloxycarbonyl-prolyl-leucyl-amino)-2(*R*,*S*)-(mercapto)propionyl]-leucyl-phenylalanine *N*-Methylamide (**5b**, $A.A_3$=Pro, $A.A_2$=Leu, $A.A_1'$=Leu, $A.A_2'$=Phe): Yield: 58%. mp 201—206 °C, $[\alpha]_D^{25}$ −71.8° (*c*=1.08, MeOH), Rf_3 0.34. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.62—1.01 (12H, m), 1.28—1.80 (15H, m), 1.80—2.29 (4H, m), 2.34—2.57 (1H, m), 2.72 (3H, d, *J*=4.6 Hz), 2.87—3.02 (1H, m), 3.02—3.31 (1H, m), 3.31—3.55 (2H, m), 3.55—3.87 (3H, m), 4.05—4.21 (1H, m), 4.21—4.50 (2H, m), 4.67—4.81 (1H, m), 6.31—6.71 (1H, m), 6.99—7.33 (7H, m), 7.33—7.48 (1H, m), 7.68—7.91 (1H, m). FAB-MS *m*/*z*: 705 $(M+H)^+$. HR-MS Calcd for $C_{35}H_{57}N_6O_7S$: 705.4009 $(M+H)^+$. Found: 705.4031.

N^{α}-[3-(*tert*-Butyloxycarbonyl-prolyl-leucyl-amino)-2(*R*,*S*)-(mercapto)propionyl]-leucine *N*-Methylamide (**5c**, $A.A_3$=Pro, $A.A_2$=Leu, $A.A_1'$=Leu): Yield: 74%. mp 202—205 °C, $[\alpha]_D^{25}$ −85.2° (*c*=1.03, MeOH), Rf_3 0.28. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.80—1.01 (12H, m), 1.31—1.78 (15H, m), 1.80—2.17 (3H, m), 2.17—2.29 (2H, m), 2.81 (3H, d, *J*=4.8 Hz), 3.30—3.50 (2H, m), 3.50—3.78 (3H, m), 4.15—4.41 (3H, m), 6.31—6.50 (1H, m), 6.93—7.20 (2H, m), 7.39—7.49 (1H, m). FAB-MS *m*/*z*: 558 $(M+H)^+$. HR-MS Calcd for $C_{26}H_{48}N_5O_6S$: 558.3325 $(M+H)^+$. Found: 558.3322.

(*S*)-Malic Acid 1-Monobenzyl Ester (30a)[25] Trifluoroacetic anhydride (33.6 ml, 238 mmol) was added to L-malic acid (13.4 g, 99.9 mmol) and the mixture was stirred at 0 °C for 2.5 h. Then the reaction mixture was concentrated using a vacuum pump while the flask was kept at 0 °C. To the resulting residue, benzylalcohol (20.7 ml, 200 mmol) was added, after which the mixture was stirred at room temperature for 2 h and then diluted with EtOAc. The reaction mixture was subsequently adjusted to pH 7 by adding 10% aq. Na_2CO_3 to give a colorless suspension, which was collected by filtration and washed with EtOAc. The sodium salt of **30a** was dissolved in 1 N aq. HCl (160 ml), after which it was extracted with EtOAc, washed with sat. aq. NaCl, dried over $MgSO_4$, and evaporated *in vacuo* to give **30a** (13.3 g, 59%) as a colorless oil. $[\alpha]_D^{25}$ −13.2° (*c*=1.0, MeOH). ^{1}H-NMR ($CDCl_3$, TMS) δ: 2.76—2.97 (2H, m), 4.54 (1H, dd, *J*=4.4, 6.1 Hz), 5.12 (2H, s), 6.51 (2H, m), 7.34 (5H, s). FAB-MS *m*/*z*: 225 $(M+H)^+$.

(*R*)-Malic Acid 1-Monobenzyl Ester (30b) Compound **30b** was prepared by the same method as that described for **30a**. $[\alpha]_D^{25}$ +12.7° (*c*=1.0, MeOH).

Benzyl (*S*)-2-Oxooxazolidine-5-carboxylate (31a)[25] A mixture of **30a** (4.06 g, 18.1 mmol), diphenylphosphoryl azide (4.29 ml, 19.9 mmol), Et_3N (2.91 ml, 20.9 mmol), and benzene (60 ml) was heated under reflux for 4 h. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in H_2O (20 ml), which was saturated with NaCl and then extracted with EtOAc. The combined organic layer was washed with sat. aq. $NaHCO_3$ and sat. aq. NaCl, after which it was dried over $MgSO_4$ and evaporated *in vacuo* to leave a yellow solid, which was recrystallized from MeOH–diisopropyl ether to give **31a** (2.50 g, 62%) as slightly yellow crystals. mp 132—134 °C, $[\alpha]_D^{25}$ −1.5° (*c*=1.0, MeOH). ^{1}H-NMR ($CDCl_3$, TMS) δ: 3.69 (1H, dd, *J*=5.5, 9.0 Hz), 3.88 (1H, t, *J*=9.0 Hz), 5.05 (1H, dd, *J*=5.5, 9.5 Hz), 5.24—5.36 (3H, m), 7.37 (5H, s). FAB-MS *m*/*z*: 222 $(M+H)^+$.

Benzyl (*R*)-2-Oxooxazolidine-5-carboxylate (31b) Compound **31b** was prepared by the same method as that described for **31a**. $[\alpha]_D^{25}$ +1.4° (*c*=1.0, MeOH).

***N*-*tert*-Butyloxycarbonyl-(*S*)-isoserine (33a)**[25] A solution of **31a** (4.65 g, 21 mmol) in 3 N aq. NaOH (61.4 ml)–MeOH (15.4 ml) was stirred at 60 °C for 4 h. Then MeOH was evaporated *in vacuo* and washed with Et_2O, neutralized by adding conc. HCl, and concentrated to dryness. To the residue, 1 N aq. NaOH (42 ml) and di-*tert*-butyl dicarbonate [4.82 g (22 mmol) in acetone (45 ml)] were added, and the mixture was stirred for 14 h at 4 °C. Acetone was evaporated, after which the residue was washed with Et_2O (2×80 ml) and then adjusted to pH 2 by adding 1 N aq. HCl. The desired material was extracted with EtOAc, washed with sat. aq. NaCl, dried over $MgSO_4$, and evaporated *in vacuo* to leave a colorless solid, which was recrystallized from EtOAc–Et_2O to give **33a** (3.42 g, 79%) as a colorless solid. mp 86—88 °C, $[\alpha]_D^{25}$ +6.3° (*c*=1.0, MeOH). ^{1}H-NMR (CD_3OD, TMS) δ: 1.43 (9H, s), 3.23—3.35 (m), 3.44 (1H, dd, *J*=4.2, 13.9 Hz), 4.17 (1H, dd, *J*=4.2, 6.7 Hz). FAB-MS *m*/*z*: 206 $(M+H)^+$.

***N*-*tert*-Butyloxycarbonyl-(*R*)-isoserine (33b)** Compound **33b** was prepared by the same method as that described for **33a**. $[\alpha]_D^{25}$ −6.4° (*c*=1.0, MeOH).

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*S*)-hydoxypropionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (34a) Condensation of *N*-protected isoserine (**33a**, 1.44 g, 7.01 mmol) and the dipeptide (**25a**, 2.28 g, 6.37 mmol) was performed by the same method as that described for the preparation of **26**. Yield; 75%, mp 196—198 °C, $[\alpha]_D^{25}$ −48.3° (*c*=1.0, MeOH), ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.87 (3H, d, *J*=6.2 Hz), 0.92 (3H, d, *J*=6.2 Hz), 1.37—1.70 (12H, m), 1.82 (1H, br s), 2.70 (3H, d, *J*=4.8 Hz), 2.94—3.05 (2H, m), 3.38—3.51 (1H, m), 3.77 (3H, s), 4.07—4.15 (1H, m), 4.28—4.38 (1H, m), 4.53 (1H, dd, *J*=7.0, 14.9 Hz), 5.23—5.32 (1H, m), 5.43 (1H, d, *J*=4.6 Hz), 6.05—6.14 (1H, m), 6.79—6.84 (2H, m), 6.87 (1H, d, *J*=8.1 Hz), 7.03—7.12 (2H, m), 7.29 (1H, d, *J*=7.3 Hz). FAB-MS *m*/*z*: 509 $(M+1)^+$.

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*R*)-hydoxypropionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (34b) Compound **34b** was prepared by

the same method as that described for **34a**. Yield; 79%, mp 187—189 °C, $[\alpha]_D^{25}$ −11.8° (*c*=1.0, MeOH). ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.88 (3H, d, *J*=6.1 Hz), 0.93 (3H, d, *J*=6.1 Hz), 1.40—1.54 (10H, m), 1.59—1.85 (2H, m), 2.68 (3H, d, *J*=5.0 Hz), 2.87—3.06 (2H, m), 3.33—3.46 (1H, m), 3.68 (1H, ddd, *J*=3.3, 5.2, 14.9 Hz), 3.77 (3H, s), 4.19—4.31 (2H, m), 4.53 (1H, dd, *J*=8.3, 15.6 Hz), 5.50 (1H, d, *J*=3.5 Hz), 6.00 (1H, d, *J*=4.4 Hz), 6.56—6.65 (1H, m), 6.71—6.84 (3H, m), 7.05—7.14 (2H, m), 7.20—7.30 (1H, m).

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*R*)-(acetylsulfanyl)propionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (35a) Conversion of the hydroxy group (**34a**) to the acetylsulfanyl group (**35a**) was performed by the same method as that described for the preparation of **27**. Yield; 81%, mp 195—197 °C, $[\alpha]_D^{25}$ −6.8° (*c*=1.0, MeOH). ^{1}H-NMR (CD_3OD, TMS) δ: 0.85 (3H, d, *J*=6.4 Hz), 0.90 (3H, d, *J*=6.6 Hz), 1.34—1.51 (11H, m), 1.51—1.67 (1H, m), 2.36 (3H, s), 2.67 (3H, s), 2.81 (1H, dd, *J*=9.4, 13.8 Hz), 3.10 (1H, dd, *J*=5.6, 13.8 Hz), 3.26—3.48 (2H, m), 3.75 (3H, s), 4.21 (1H, dd, *J*=6.4, 8.8 Hz), 4.30 (1H, t, *J*=7.3 Hz), 4.44 (1H, dd, *J*=5.6, 9.4 Hz), 6.78—6.87 (2H, m), 7.09—7.18 (2H, m). FAB-MS *m*/*z*: 567 $(M+1)^+$.

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*S*)-(acetylsulfanyl)propionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (35b) Conversion of the hydroxy group (**34b**) to the acetylsulfanyl group (**35b**) was performed by the same method as that described for the preparation of **27**. Yield; 77%, mp 161—163 °C, $[\alpha]_D^{25}$ −67.7° (*c*=1.0, MeOH). ^{1}H-NMR (CD_3OD, TMS) δ: 0.83 (3H, d, *J*=6.4 Hz), 0.89 (3H, d, *J*=6.4 Hz), 1.36—1.63 (12H, m), 2.35 (3H, s), 2.66 (3H, s), 2.87 (1H, dd, *J*=8.4, 13.8 Hz), 3.05 (1H, dd, *J*=6.4, 13.8 Hz), 3.28—3.38 (m), 3.41—3.54 (1H, m), 3.74 (3H, s), 4.20—4.31 (2H, m), 4.46 (1H, dd, *J*=6.4, 8.4 Hz), 6.79—6.85 (2H, m), 7.08—7.15 (2H, m).

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*R*)-mercaptopropionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (36a) Alkaline hydrolysis of acetylsulfanyl ester (**35a**) was performed by the same method as that described for the preparation of **4**. Yield; 78%, mp 193—196 °C, $[\alpha]_D^{25}$ −21.7° (*c*=0.3, MeOH). ^{1}H-NMR (CD_3OD, TMS) δ: 0.78 (3H, d, *J*=6.4 Hz), 0.82 (3H, d, *J*=6.4 Hz), 1.26—1.60 (12H, m), 2.57 (3H, s), 2.78 (1H, dd, *J*=7.9, 13.9 Hz), 2.93 (1H, dd, *J*=6.6, 13.9 Hz), 3.14—3.30 (m), 3.36—3.50 (1H, m), 3.64 (3H, s), 4.18 (1H, dd, *J*=6.2, 8.8 Hz), 4.25—4.42 (2H, m), 6.67—6.78 (2H, m), 6.96—7.08 (2H, m). HR-MS Calcd for $C_{25}H_{41}N_4O_6S$: 525.2747 $(M+H)^+$. Found: 525.2782.

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*S*)-mercaptopropionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (36b) Alkaline hydrolysis of acetylsulfanyl ester (**35b**) was performed by the same method as that described for the preparation of **4**. Yield; 92%, mp 190—192 °C, $[\alpha]_D^{25}$ −24.4° (*c*=1.0, MeOH). ^{1}H-NMR (CD_3OD, TMS) δ: 0.78 (3H, d, *J*=6.4 Hz), 0.82 (3H, d, *J*=6.4 Hz), 1.27—1.60 (12H, m), 2.56 (3H, s), 2.77 (1H, dd, *J*=8.3, 13.8 Hz), 2.94 (1H, dd, *J*=6.7, 13.8 Hz), 3.17—3.29 (m), 3.34—3.43 (1H, m), 3.65 (3H, s), 4.16 (1H, dd, *J*=6.2, 8.8 Hz), 4.36 (1H, dd, *J*=6.7, 8.3 Hz), 4.48—4.54 (1H, m), 6.69—6.77 (2H, m), 6.97—7.05 (2H, m). HR-MS Calcd for $C_{25}H_{41}N_4O_6S$: 525.2747 $(M+H)^+$. Found: 525.2772.

General Procedure for the Preparation of Compounds 37a—c These compounds were prepared from **26a** and the corresponding R^3-SK [prepared from R^3-SH and KOH in MeOH] by the same method as that described for the preparation of **27**.

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*R*,*S*)-(ethylsulfanyl)propionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (**37a**, R^3=Ethyl): Yield: 79%. mp 160—165 °C, $[\alpha]_D^{25}$ −33.8° (*c*=1.0, MeOH), Rf_3 0.48. ^{1}H-NMR (CD_3OD, TMS) δ: 0.80—1.05 (9H, m), 1.26 (3H, t, *J*=7.8 Hz), 1.36—1.80 (12H, m), 2.31—2.50 (2H, m), 2.67 (3H, s), 2.78—2.96 (1H, m), 2.96—3.12 (1H, m), 3.12—3.60 (m), 3.72 (3H, s), 4.14—4.50 (2H, m), 6.74—6.87 (2H, m), 7.05—7.17 (2H, m). FAB-MS *m*/*z*: 553 $(M+H)^+$. HR-MS Calcd for $C_{27}H_{45}N_4O_6S$: 553.3060 $(M+H)^+$. Found: 553.3049.

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*R*,*S*)-(iso-butylsulfanyl)propionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (**37b**, R^3=Iso-butyl): Yield: 78%. mp 150—152 °C, $[\alpha]_D^{25}$ −33.8° (*c*=1.0, MeOH), Rf_3 0.48. ^{1}H-NMR (CD_3OD, TMS) δ: 0.87—1.10 (12H, m), 1.26—1.88 (13H, m), 2.33—2.52 (2H, m), 2.65 (3H, s), 2.77—2.94 (1H, m), 2.94—3.09 (1H, m), 3.12—3.56 (m), 3.74 (3H, s), 4.18—4.56 (2H, m), 6.68—6.89 (2H, m), 6.98—7.17 (2H, m). FAB-MS *m*/*z*: 581 $(M+H)^+$. HR-MS Calcd for $C_{29}H_{49}N_4O_6S$: 581.3373 $(M+H)^+$. Found: 581.3395.

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*R*,*S*)-(benzylsulfanyl)propionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (**37c**, R^3=Benzyl): Yield: 81%. mp 164—166 °C, $[\alpha]_D^{25}$ −26.8° (*c*=1.0, MeOH), Rf_3 0.49. ^{1}H-NMR (CD_3OD, TMS) δ: 0.76—1.03 (6H, m), 1.27—1.69 (12H, m), 2.65 (3H, s), 2.82—2.94 (1H, m), 2.96—3.08 (1H, m), 3.34—3.60 (3H, m), 3.69 (1.5H, s), 3.73 (1.5H, s), 3.75—3.90 (2H, m), 4.22—4.40 (1H, m), 4.42—4.51 (1H, m), 6.73—6.81 (2H, m), 7.04—7.16 (2H, m), 7.18—7.40 (5H, m). FAB-MS *m*/*z*: 615 $(M+H)^+$. HR-MS Calcd for $C_{32}H_{47}N_4O_6S$: 615.3216 $(M+H)^+$. Found: 615.3251.

General Procedure for the Preparation of Compounds 38a—c To a solution of compound **37a**—**c** in CH_2Cl_2–MeOH (6 : 1, 3% w/v), MCPBA (70% min, 1.06 eq of **37a**—**c**) was added and the mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with EtOAc, washed successively with 5% aq. $NaHCO_3$, and sat. aq. NaCl, dried over $MgSO_4$, and evaporated *in vacuo* to yield a colorless syrup, which was re-precipitated from Et_2O to give **38a**—**c** as a colorless solid.

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*R*,*S*)-(ethylsulfinyl)propionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (**38a**, R^3=Ethyl): Yield: 82%. mp 137—139 °C, $[\alpha]_D^{25}$ −36.6° (*c*=1.0, MeOH), Rf_3 0.44. ^{1}H-NMR (CD_3OD, TMS) δ: 0.76—0.97 (6H, m), 1.24—1.71 (15H, m), 2.66 (3H, s), 2.70—3.03 (2H, m), 3.03—3.26 (2H, m), 3.48—3.70 (2H, m), 3.74 (3H, s), 4.02—4.33 (2H, m), 4.38—4.51 (1H, m), 6.74—6.88 (2H, m), 7.04—7.19 (2H, m). FAB-MS *m*/*z*: 569 $(M+H)^+$. HR-MS Calcd for $C_{27}H_{45}N_4O_7S$: 569.3009 $(M+H)^+$. Found: 569.2977.

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*R*,*S*)-(iso-butylsulfinyl)propionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (**38b**, R^3=Iso-butyl): Yield: 80%. mp 138—142 °C, $[\alpha]_D^{25}$ −39.9° (*c*=1.0, MeOH), Rf_3 0.57. ^{1}H-NMR (CD_3OD, TMS) δ: 0.79—0.96 (6H, m), 1.03—1.22 (6H, m), 1.28—1.71 (12H, m), 2.08—2.48 (1H, m), 2.61—3.25 (7H, m), 3.50—3.70 (2H, m), 3.74 (3H, s), 3.96—4.50 (3H, m), 6.73—6.89 (2H, m), 7.06—7.20 (2H, m). FAB-MS *m*/*z*: 597 $(M+H)^+$. HR-MS Calcd for $C_{29}H_{49}N_4O_7S$: 597.3322 $(M+H)^+$. Found: 597.3323.

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*R*,*S*)-(benzylsulfinyl)propionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (**38c**, R^3=Benzyl): Yield: 82%. mp 152—154 °C, $[\alpha]_D^{25}$ −37.4° (*c*=1.0, MeOH), Rf_3 0.56. ^{1}H-NMR (CD_3OD, TMS) δ: 0.74—1.00 (6H, m), 1.20—1.77 (12H, m), 2.58—2.72 (3H, m), 2.72—2.94 (1H, m), 3.00—3.21 (1H, m), 3.57—3.83 (5H, m), 4.04—4.64 (5H, m), 6.72—6.88 (2H, m), 7.04—7.19 (2H, m), 7.29—7.55 (5H, m). FAB-MS *m*/*z*: 631 $(M+H)^+$. HR-MS Calcd for $C_{32}H_{47}N_4O_7S$: 631.3165 $(M+H)^+$. Found: 631.3146.

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*R*,*S*)-(ethyldisulfanyl)propionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (39) A mixture of diethyl azodicarboxylate (129 μl, 0.787 mmol), ethanethiol (56 μl, 0.75 mmol), and benzene (1.5 ml) was gently refluxed for 2 h under nitrogen atmosphere to give diethyl *N*-ethylsulfenylhydrazodicarboxylates[27] as a yellow solution. To the solution, a suspension of **4a** (375 mg, 0.715 mmol) in benzene (5.0 ml) was added, and the mixture was stirred for 24 h at room temperature under a nitrogen atmosphere. Then the reaction mixture was evaporated to give a yellow residue, which was purified by silica gel column chromatography (eluent; $CHCl_3$: MeOH=80 : 1) and precipitated from Et_2O–*n*-hexane to give **39** as a colorless solid (78 mg, 19 %). mp 119—124 °C, Rf_3 0.74. ^{1}H-NMR ($CDCl_3$, TMS) δ: 0.81—1.01 (6H, m), 1.28 (1.5H, t, *J*=7.2 Hz), 1.32 (1.5H, t, *J*=7.3 Hz), 1.38—1.73 (12H, m), 2.64—2.83 (5H, m), 2.92—3.13 (2H, m), 3.44—3.64 (2H, m), 3.78 (3H, s), 4.16—4.25 (1H, m), 4.25—4.41 (1H, m), 4.46—4.62 (1H, m), 5.20 (0.5H, m), 5.31 (0.5H, m), 5.82 (0.5H, m), 6.00 (0.5H, m), 6.36—6.51 (1H, m), 6.56—6.68 (1H, d, *J*=8.3 Hz), 6.73—6.89 (2H, m), 7.01—7.15 (2H, m). FAB-MS *m*/*z*: 585 $(M+H)^+$. HR-MS Calcd for $C_{27}H_{45}N_4O_6S_2$: 585.2781 $(M+H)^+$. Found: 585.2787.

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*R*,*S*)-azidopropionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (40) To a solution of the mesylate [1.0 g, 1.71 mmol, derived from **26a** in the same manner as that described for the preparation of **27**] in DMF (17 ml), sodium azide (600 mg, 9.23 mmol) was added, and the mixture was stirred under a nitrogen atmosphere for 20 h at room temperature. The reaction mixture was diluted with EtOAc (60 ml), washed successively with sat. aq. NaCl, H_2O, and sat. aq. NaCl, dried over $MgSO_4$, and evaporated *in vacuo* to leave a slightly yellow solid, which was purified by flash chromatography (eluent; *n*-hexane : EtOAc=1 : 2) to give **40** (803 mg, 88%) as a colorless solid. mp 170—172 °C, $[\alpha]_D^{25}$ −30.3° (*c*=1.0, MeOH), ^{1}H-NMR (CD_3OD, TMS) δ: 0.78 (3H, d, *J*=6.2 Hz), 0.82 (3H, d, *J*=6.2 Hz), 1.12—1.26 (1H, m), 1.30—1.57 (11H, m), 2.57 (3H, s), 2.73—2.84 (1H, m), 2.88—3.00 (1H, m), 3.15—3.28 (m), 3.30—3.45 (1H, m), 3.65 (3H, s), 3.88—3.96 (1H, m), 4.17—4.28 (1H, m), 4.32—4.41 (1H, m), 6.70—6.78 (2H, m), 6.99—7.08 (2H, m). FAB-MS *m*/*z*: 534 $(M+H)^+$.

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*R*,*S*)-aminopropionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (41) A solution of **40** (270 mg, 0.506 mmol) in MeOH (15 ml) was hydrogenated on 10% Pd–C (50% wet, 150 mg) for 2.5 h at room temperature and nomal pressure. After removal of the catalyst, the filtrate was evaporated, and the residue was precipitated from Et_2O to give **41** as a colorless solid (226 mg, 88 %). mp 169—171 °C, $[\alpha]_D^{25}$

−31.0° (c=1.0, MeOH). ^{1}H-NMR (CD_3OD, TMS) δ: 0.83—0.96 (6H, m), 1.35—1.67 (12H, m), 2.66 (3H, s), 2.79—2.93 (1H, m), 2.98—3.23 (2H, m), 3.37—3.51 (1H, m), 3.74 (3H, s), 4.08—4.39 (2H, m), 4.42—4.52 (1H, m), 6.78—6.88 (2H, m), 7.05—7.18 (2H, m). FAB-MS m/z: 508 $(M+H)^+$. HR-MS Calcd for $C_{25}H_{42}N_5O_6$: 508.3135 $(M+H)^+$. Found: 508.3109.

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*R*,*S*)-(benzyloxycarbonyl)propionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (43) To a stirred solution of diisopropylamine (2.0 ml, 14.3 mmol) in THF (12 ml), 1.6 M *n*-BuLi in hexane (8.95 ml, 14.3 mmol) was added at −10 °C under a nitrogen atmosphere. After 20 min, a solution of benzyl 3-(*tert*-butyloxycarbonylamino)propionate (2.00 g, 7.16 mmol) in THF (8 ml) was added dropwise over a period of 15 min at −70 °C, and the mixture was stirred at the same temperature for 20 min. Then the reaction mixture (a slightly yellow solution) was poured into dry ice and diluted with 0.1 N aq. H_2SO_4 (225 ml). The organic material was extracted with EtOAc, washed with sat. aq. NaCl, dried over $MgSO_4$, and evaporated *in vacuo* to give crude 3-(*tert*-butyloxycarbonylamino)-2(*R*,*S*)-(benzyloxycarbonyl)propionic acid as a slightly yellow oil, which was used for the subsequent condensation procedure without further purification. EDC (1.51 g, 7.88 mmol) was added to a mixture of H-Leu-Tyr(Me)-NHMe·EHCl (**25a**, 2.68 g, 7.52 mmol), Et_3N (1.05 ml, 7.52 mmol), HOBt (1.02g, 7.52 mmol), crude 3-(*tert*-butyloxycarbonylamino)-2(*R*,*S*)-(benzyloxycarbonyl)propionic acid, and DMF (80 ml) at −12 °C. The mixture was stirred at −3 °C for 6 h and then at 0 °C for 15 h. The reaction mixture was diluted with EtOAc, washed successively with sat. aq. NaCl, 1 N aq. HCl, sat. aq. NaCl, 10% aq. Na_2CO_3, and sat. aq. NaCl, dried over $MgSO_4$, and evaporated *in vacuo* to give crude product, which was purified by silica gel column chromatography (eluent; $CHCl_3$: MeOH=80 : 1—30 : 1) to give **43** as a colorless solid. mp 153—156 °C, Rf_3 0.49. ^{1}H-NMR (CD_3OD, TMS) δ: 0.75 (1.5H, d, J=6.4 Hz), 0.82 (1.5H, d, J=6.4 Hz), 0.85 (1.5H, d, J=6.4 Hz), 0.89 (1.5H, d, J=6.6 Hz), 1.29—1.67 (12H, m), 2.63 (3H, s), 2.73—2.92 (1H, m), 2.98—3.12 (1H, m), 3.40—3.85 (6H, m), 4.19—4.33 (1H, m), 4.39—4.50 (1H, m), 5.14 (1H, m), 5.20 (1H, m), 6.73—6.87 (2H, m), 7.02—7.16 (2H, m), 7.25—7.44 (5H, m). FAB-MS m/z: 627 $(M+H)^+$. HR-MS Calcd for $C_{33}H_{47}N_4O_8$: 627.3394 $(M+H)^+$. Found: 627.3422.

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*R*,*S*)-(hydroxycarbonyl)propionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (44) A solution of **43** (300 mg, 0.479 mmol) in MeOH (20 ml) was hydrogenated on 10% Pd–C (50% wet, 100 mg) for 1 h at room temperature and nomal pressure. After removal of the catalyst, the filtrate was evaporated and the residue was precipitated from Et_2O–EtOAc to give **44** as a colorless solid (234 mg, 91%). mp 201—203 °C, Rf_2 0.31. ^{1}H-NMR (CD_3OD, TMS) δ: 0.84 (3H, d, J=6.6 Hz), 0.87 (1.5H, d, J=6.4 Hz), 0.90 (1.5H, d, J=6.6 Hz), 1.26—1.69 (12H, m), 2.68 (3H, s), 2.73—2.92 (1H, m), 3.01—3.23 (1H, m), 3.41—3.64 (3H, m), 3.74 (3H, s), 4.15—4.29 (1H, m), 4.38—4.52 (1H, m), 6.74—6.89 (2H, m), 7.04—7.20 (2H, m). FAB-MS m/z: 537 $(M+H)^+$. HR-MS Calcd for $C_{26}H_{41}N_4O_8$: 537.2924 $(M+H)^+$. Found: 537.2893.

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*R*,*S*)-(benzyloxycarbamoyl)propionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (45) EDC (371 mg, 1.94 mmol) was added to a mixture of *O*-benzylhydroxylamine hydrochloride (337 mg, 2.11 mmol), Et_3N (293 μl, 2.11 mmol), HOBt (250 mg, 1.85 mmol), **44** (940 mg, 1.76 mmol), and DMF (25 ml) at −12 °C. The mixture was stirred at −3 °C for 6 h and then at 0 °C for 15 h. Then the reaction mixture was poured into ice water (200 ml) and insoluble material was collected by filtration. The residue was washed successively with 1 N aq. HCl, water, 5% aq. $NaHCO_3$, and water, after which it was dried over P_2O_5 under reduced pressure to give **45** as a colorless solid (1.01 g, 90%). mp 236—238 °C, Rf_2 0.69. ^{1}H-NMR (DMSO-d_6, TMS) δ: 0.78 (3H, d, J=6.4 Hz), 0.83 (3H, d, J=6.4 Hz), 1.21—1.58 (12H, m), 2.38—2.60 (m), 2.64—2.80 (1H, s), 2.82—2.97 (1H, m), 3.10—3.50 (m), 3.67 (3H, s), 4.13—4.41 (2H, m), 4.75 (2H, s), 6.67—6.86 (3H, m), 7.00—7.15 (2H, m), 7.36 (5H, m), 7.69—7.86 (2H, m), 7.94 (1H, d, J=7.7 Hz), 8.04 (1H, d, J=8.3 Hz). FAB-MS m/z: 642 $(M+H)^+$. HR-MS Calcd for $C_{33}H_{48}N_5O_8$: 642.3503 $(M+H)^+$. Found: 642.3543.

N^{α}-[3-(*tert*-Butyloxycarbonylamino)-2(*R*,*S*)-(hydroxycarbamoyl)propionyl]-leucyl-*O*-methyl-tyrosine *N*-Methylamide (46) A solution of **45** (710 mg, 1.11 mmol) in MeOH (100 ml) was hydrogenated on 10% Pd–C (50% wet, 200 mg) for 3 h at room temperature and nomal pressure. After removal of the catalyst, the filtrate was concentrated to *ca.* 10 ml and was precipitated from Et_2O to give **46** as a colorless solid (599 mg, 98%). mp 205—209 °C, Rf_2 0.32. ^{1}H-NMR (CD_3OD, TMS) δ: 0.75 (1.5H, d, J=6.4 Hz), 0.76 (1.5H, d, J=6.4 Hz), 0.80 (1.5H, d, J=6.6 Hz), 0.81 (1.5H, d, J=6.4 Hz), 1.21—1.57 (12H, m), 2.59 (3H, s), 2.72—2.86 (1H, m), 2.92—3.08 (1H, m), 3.31—3.49 (3H, m), 3.65 (3H, s), 4.08—4.21 (1H, m), 4.30—4.40 (1H, m), 6.68—6.78 (2H, m), 6.97—7.12 (2H, m). FAB-MS m/z: 552 $(M+H)^+$. HR-MS Calcd for $C_{26}H_{42}N_5O_8$: 552.3034 $(M+H)^+$. Found: 552.3068.

MMP-1 Inhibition Assay The test was carried out essentially as described by Nagai *et al.*[32] In a microtube, 20 μl of proMMP-1 [15 U/ml in an assay buffer [50 mM Tris–HCl (pH 7.5), 0.2 M NaCl, 5 mM $CaCl_2$] containing 0.02% BSA] was mixed with 20 μl of 2 mM 4-aminophenylmercuric acetate (APMA) in assay buffer. Then the mixture was incubated at 35 °C for 2 h to activate the proenzyme. A 20 μl aliquot of inhibitor (at various concentrations) plus 40 μl of assay buffer were added to the mixture and further incubation was done for 15 min at 35 °C. In the case of thiol componds, to ensure that the inhibitor was not oxidised, 1×10^{-4} M β-mercaptoethanol was added to the assay buffer. Then 100 μl of 0.05% FITC-labeled collagen in assay buffer was added, followed by incubation at 35 °C for 2 h, after which the reaction was terminated by adding 10 μl of 80 mM *o*-phenanthroline in 50% ethanol solution. A 200 μl of aliquot of elastase (25 μg/ml assay buffer) was added and the mixture was incubated for 10 min at 35 °C to digest the cleaved FITC-labeled collagen fragments. Next, 400 μl of 70% ethanol in 0.17 M Tris–HCl (pH 9.5)/0.67 M NaCl was added and the mixture was vortexed for 30 s, followed by centrifugation for 20 min at 3000 rpm to obtain the supernatant. The fluoresence intensity of this supernatant was measured (λ_{ex} 495 nm, λ_{em} 520 nm) and the inhibitory activity was calculated as a percentage of the control activity in the absence of any inhibitor.

References and Notes

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