Poststatin, a New Inhibitor of Prolyl Endopeptidase

VI. Endopeptidase Inhibitory Activity of Poststatin Analogues Containing Pyrrolidine Ring

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Several pyrrolidine-containing analogues of poststatin were synthesized and examined for their inhibitory activity against prolyl endopeptidase and cathepsin B *in vitro*. Replacement of the postine residue with 2-oxo-2-(2-pyrrolidinyl)acetic acid increased the selectivity and inhibitory activity against prolyl endopeptidase. Benzyloxycarbonyl-L-phenylalanyl-(*S*)-2-oxo-2-(2-pyrrolidinyl)acetyl-D-phenylalanine was about 46 times as active to prolyl endopeptidase as natural poststatin.

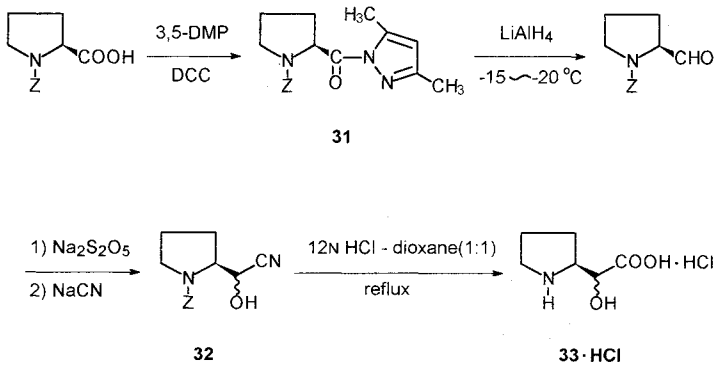
Poststatin (PST), a new inhibitor of prolyl endopeptidase (PEP), with a structure of L-Val-L-Val-(*S*)-3-amino-2-oxovaleryl-D-Leu-L-Val, has been isolated from a culture filtrate of *Streptomyces viridochromogenes* MH534-30F3[1~3].

In the preceding paper[4], we synthesized thirty analogues of PST containing a β-substituted-β-amino-α-oxopropionic acid residue and described the relationship between structure and the inhibitory activity to some serine and cysteine endopeptidases.

Low molecular weight inhibitors of PEP have been widely studied. Proline containing chloromethyl ketone derivatives or peptide aldehyde analogues such as benzyloxycarbonyl (abbreviated as Z)-Gly-Pro-CH_2Cl or Z-Pro-prolinal were designed, synthesized, and found to show strong inhibitory activity to PEP by YOSHIMOTO in 1977[5] and WILK in 1983[6]. Recently, thiazolidine derivatives such as Z-thiopro-thiazolidine and pyrrolidine derivatives such as 1-(*N*-(4-phenylbutyryl)-Pro)-pyrrolidine were reported as another type inhibitor of PEP by TSURU in 1988[7] and SAITO in 1991[8].

In comparison with these structure, PST included a unique β-amino-α-oxocarboxylic acid residue in it and had another two amino acid residues at the P'_1 and P'_2 positions. The latter suggests it is possible to modify or replace with suitable structures the subsite of each

Scheme 1. Synthesis of key intermediate of pyrrolidine analogues.



endopeptidase.

In order to increase the selectivity for PEP we replaced 2-oxo-2-(2-pyrrolidinyl)acetic acid for 3-amino-2-oxovaleric acid (named as postine, abbreviated as Pos) as the derivative of β-amino-α-oxocarboxylic acid. The synthesis of (*RS*)-2-hydroxy-2-((*S*)-2-pyrrolidinyl)acetic acid was shown in Scheme 1.

In this paper, we report the synthesis of a new type of pyrrolidine derivatives as potential inhibitors of PEP, and inhibitory activity against PEP as contrasted with cathepsin B *in vitro*.

Results and Discussion

The results obtained are summarized in Table 1. The effect of introducing a pyrrolidine ring at P_1 was clearly demonstrated. Replacement of Pos with 2-oxo-2-(2-pyrrolidinyl)acetic acid residue (No. **2** *vs* No. **1**) increased the inhibitory activity against PEP significantly, and markedly decreased the inhibitory activity against cathepsin B. The *N*- and *C*-terminal protected derivatives of pyrrolidine analogue **2** (No. **4**) also showed stronger inhibitory activity against PEP than Pos-containing analogue (No. **3**). Therefore, pyrrolidine ring at the P_1

Table 1. Relationship between structure and endopeptidase inhibitory activities.

Compound No.	Structure							IC_{50} (μg/ml)	
	P_4	P_3	P_2	P_1	P'_1	P'_2	P'_3	PEP	Cat-B
1[a]		Val-	Val-	(*S*)Pos-	D-Leu-	Val		0.030	2.1
2		Val-	Val-	(*S*)ProCO-	D-Leu-	Val		0.0015	>100
3	Z-	Val-	Val-	(*S*)Pos-	D-Leu-	Val-	OBzl	1.0	100
4	Z-	Val-	Val-	(*S*)ProCO-	D-Leu-	Val-	OBzl	0.0027	>100
5[b]		Z-	Phe-	(*RS*)Pos-	D-Leu-	Val-	OBu^t	0.11	>100
6[b]		Z-	Phe-	(*RS*)Pos-	D-Leu-	Val		0.0070	0.64
7[b]		Z-	Phe-	(*RS*)Pos-	D-Leu-	OBu^t		0.031	0.48
8[b]		Z-	Phe-	(*RS*)Pos-	D-Leu-			0.12	0.47
9		Z-	Phe-	(*S*)ProCO-	D-Leu-	Val-	OBzl	0.0024	>100
10		Z-	Phe-	(*S*)ProCO-	D-Leu-	OBu^t		0.0020	>100
11		Z-	Phe-	(*S*)ProCO-	D-Leu			0.0013	>100
12[b]		Val-	Val-	(*RS*)Pos-	D-Leu-	Val		0.29	34
13[b]		Val-	Val-	(*RS*)Pos-	Leu-	Val		12	0.040
(10)		Z-	Phe-	(*S*)ProCO-	D-Leu-	OBu^t		0.0020	>100
14		Z-	Phe-	(*S*)ProCO-	Leu-	OBu^t		0.0018	>100
(11)		Z-	Phe-	(*S*)ProCO-	D-Leu			0.0013	>100
15		Z-	Phe-	(*S*)ProCO-	Leu			0.00084	29
(10)		Z-	Phe-	(*S*)ProCO-	D-Leu-	OBu^t		0.0020	>100
16		Z-	Phe-	(*S*)ProCO-	D-Phe-	OBu^t		0.0013	>100
17		Z-	Phe-	(*S*)ProCO-	Gly-	OBu^t		0.00080	>100
(11)		Z-	Phe-	(*S*)ProCO-	D-Leu			0.0013	>100
18		Z-	Phe-	(*S*)ProCO-	D-Phe			0.00065	100
19		Z-	Phe-	(*S*)ProCO-	Gly			0.0011	120
20		Z-	Phe-	(*S*)ProCO-	NHcHx			0.0012	20
(7)		Z-	Phe-	(*RS*)Pos-	D-Leu-	OBu^t		0.031	0.48
21			Z-	(*RS*)Pos-	D-Leu-	OBu^t		2.0	>100
(9)		Z-	Phe-	(*S*)ProCO-	D-Leu-	Val-	OBzl	0.0024	>100
22		Boc-	Val-	(*S*)ProCO-	D-Leu-	Val-	OBzl	0.0024	>100
23		Z-	Hph-	(*S*)ProCO-	D-Leu-	OBu^t		0.0018	>100
(10)		Z-	Phe-	(*S*)ProCO-	D-Leu-	OBu^t		0.0020	>100
24			Dmb-	(*S*)ProCO-	D-Leu-	OBu^t		0.017	>100
25			Z-	(*RS*)ProCO-	D-Leu-	OBu^t		0.046	>100
26		Z-	Phe-	(*S*)ProCO-	D-Leu-	NHBu^t		0.0014	>100
27			cHxCO-	(*S*)ProCO-	D-Leu-	NHBu^t		0.25	>100
28			Bz-	(*S*)ProCO-	D-Leu-	NHBu^t		0.056	>100
29			Bz-	(*S*)ProCO-	Gly-	OBu^t		0.035	>100

[a] Natural poststatin.

[b] These compounds were described in the previous paper[4].

Compounds in parentheses are also listed earlier in the table. Abbreviations; PEP: prolyl endopeptidase, Cat-B: cathepsin B, Pos: postine, 3-amino-2-oxovaleric acid residue, ProCO: 2-oxo-2-(2-pyrrolidinyl)acetic acid residue, Z: benzyloxycarbonyl, Bzl: benzyl, Bu^t: *t*-butyl, cHx: cyclohexyl, Hph: homophenylalanine residue, Dmb: 3,3-dimethylbutyryl, Bz: benzoyl.

is important for PEP inhibition.

As reported in our previous paper[4], replacement of the P_2 and P_3 of poststatin by Z-Phe increased the inhibitory activity against PEP. Therefore pyrrolidine derivatives containing Z-Phe at P_2 and P_3 were synthesized. The Pos-containing analogue (No. **5**), in which the *C*-terminal of P'_2 at active analogue **6** was esterificated, decreased the inhibitory activity against PEP. The Pos-containing analogue (No. **8**), in which P'_2 residue was deleted from active analogue **6** or **7**, also showed weak inhibitory activity. In contrast with Pos-containing analogues, the pyrrolidine analogues (No. **9** and **10**) showed about the same strong inhibitory activity as analogue **11** in spite of the modification at P'_3 or P'_2. Therefore, the influence of P'_3 or P'_2 is smaller in pyrrolidine-containing peptides than in Pos-containing peptides.

To estimate the effect of the stereochemistry at P'_1, we synthesized L-Leu analogues at P'_1 (No. **14** and **15**). In the Pos-containing analogues (No. **12** and **13**), replacement of D-Leu by L-Leu decreased the inhibitory activity against PEP significantly. In contrast, the pyrrolidine analogues (No. **14** and **15**) showed strong inhibitory activity against PEP as well as analogues **10** and **11** in which P'_1 positions were D-Leu. Therefore, the D configuration at the P'_1 is not essential for pyrrolidine-containing peptides.

Analogues, in which P'_1 of analogues **10** and **11** were replaced by D-Phe (No. **16** and **18**) and Gly (No. **17** and **19**) were synthesized. Moreover, analogue **20**, in which P'_1 was not an amino acid residue but a cyclohexylamine component was prepared. All these analogues showed strong inhibitory activity against PEP, and among them analogue **18** showed about 46 times as much active as natural PST (No. **1**). Therefore, the contribution of P'_1 in the pyrrolidine-containing peptides is small though P'_1 in the Pos-containing peptides was important.

In the Pos-containing peptides, deletion of the amino acid residue at P_2 markedly decreased the inhibitory activity against PEP (No. **21**). Pyrrolidine analogues **24**, **25**, **27**~**29**, in which amino acid residues at P_2 were deleted showed lower inhibitory activity against PEP than the analogues having an amino acid residue at P_2 (No. **9**, **22**, **23**, **10** and **26**). Some of the analogues lacking the amino acid residue at P_2 maintained about the same inhibitory activity as natural PST. Among these short pyrrolidine analogues, analogue **24** was about 1.8 times more potent than natural PST in its inhibitory activity. Therefore, the presence of one amino acid residue at P_2 is preferable for strong inhibition against PEP, but P_2-deleted analogues have still inhibitory potency against PEP.

Experimental

General

Melting points were determined on a micro melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. ^{1}H NMR spectra were recorded at 400 MHz, 270 MHz or 200 MHz with a JEOL JNM-GX400, JNM-EX270 or a Varian GEMA-200 spectrometer respectively. FAB-MS spectra were measured on a JEOL JMS-SX102 or VG ZAB-HF mass spectrometer respectively. TLC was carried out on Merck precoated silica gel $60F_{254}$ plates. Abbreviations used in the following section were defined in the above section and Table 1.

Enzyme Assay

Inhibitory activities of PEP and cathepsin B were measured by the procedure described in the previous paper[1].

Synthesis

Synthesis of (*RS*)-2-[(*S*)-2-(1-*t*-Butoxycarbonylpyrrolidinyl)]-2-hydroxyacetic Acid (Boc-H_2ProCO, **30**)

Z-L-Prolyl-3,5-dimethylpyrazolide (**31**)

To a solution of Z-L-proline (37.35 g, 150.0 mmol) in CH_2Cl_2 (150 ml) was added 3,5-dimethylpyrazole (14.45 g, 150.0 mmol) and dicyclohexylcarbodiimide (abbreviated as DCC, 30.95 g, 150.0 mmol) at -15°C, and the resulting solution was chilled in an ice bath for 30 minutes and then left at room temperature overnight. After removal of the undissolved material, the solvent was evaporated, and the residual oil was dissolved in EtOAc (500 ml). The organic layer was washed with saturated aq $NaHCO_3$ (200 ml), 5% aq citric acid (200 ml) and saturated aq NaCl (200 ml), and dried (Na_2SO_4). Evaporation of the solvent gave an oil of **31**, 48.82 g (99.5%): Rf 0.73 ($CHCl_3$-MeOH, 30:1); $[\alpha]_D^{20}$ -41.3° (*c* 1.0, MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.86~2.18 (3H, m, CH_2C*Ha*Hb), 2.22 (3H, s, CH_3), *ca.* 2.30~2.58 (1H, m, overlapping, CHa*Hb*), 2.44, 2.54 (1.5H, 1.5H, two s, CH_3), 3.45~3.85 (2H, m, NCH_2), 5.02, 5.16 and 5.10, 5.21 (1H and 1H, two ABq, each J=12.0 Hz, CH_2Ph), 5.59, 5.64 (0.5H, 0.5H, two t, each, J=3.6 Hz, α-CH(Pro)), 5.95 (1H, br s, olefinic CH), 7.08~7.45 (5H, m, Ph).

Boc-H_2ProCO

To a suspension of $LiAlH_4$ (10.62 g, 279.8 mmol) in dry THF (300 ml) was added a solution of **31** (114.5 g, 349.7 mmol) in dry THF (120 ml) over a period of 1 hour keeping the temperature at -15 to -20°C in an argon atmosphere. After standing for another 1.5 hours at the same temperature, the reaction mixture was cooled to

$-60°C$ and the mixture was diluted with THF (100 ml). 3 N HCl (900 ml) was added slowly at the temperature below $-40°C$. After removal of $Al(OH)_3$ by filtration, the filtrate was concentrated. The aqueous layer was extracted twice with EtOAc (300 ml). The combined organic layer was washed with saturated aq $NaHCO_3$ (300 ml), saturated aq NaCl (300 ml × 2) and dried (Na_2SO_4). Evaporation of the solvent gave an oil of crude Z-L-prolinal (56.7 g). To the Z-L-prolinal in EtOAc (78 ml) was added aq solution of $Na_2S_2O_5$ (95%; 48.64 g, 243.1 mmol) in water (78 ml) and the mixture was stirring overnight. The aqueous layer was separated, and added water to 300 ml. To the solution of $NaHSO_3$ adduct was added EtOAc (300 ml) and aq solution of NaCN (11.91 g, 243.0 mmol) in water (80 ml) in an ice bath. After standing for 30 minutes at 0°C, the reaction mixture was stirred for 4 hours at room temperature. The organic layer was separated and washed twice with saturated aq NaCl (180 ml) and dried (Na_2SO_4). Evaporation of the solvent gave an oil of (*RS*)-2-[(*S*)-2-(1-benzyloxycarbonylpyrrolidinyl)]-2-hydroxyacetonitrile (**32**; 34.3 g, 131.8 mmol).

To the **32** was added 12 N HCl (70 ml) and dioxane (70 ml), and refluxed for 36 hours. Evaporation of the solvent and decantation twice with ether (100 ml) gave a solid of (*RS*)-2-hydroxy-2-((*S*)-2-pyrrolidinyl)acetic acid hydrochloride (**33**·HCl).

To a solution of **33**·HCl in water (100 ml) and dioxane (100 ml) was added triethylamine (13.8 g, 136.4 mmol) and di-*t*-butyl dicarbonate (28.76 g, 131.8 mmol) in an ice bath, and stirred at room temperature overnight. After concentration of the solvent, the residue was washed with Et_2O and acidified with phosphoric acid. The mixture was extracted twice with EtOAc (200 ml), washed thrice with saturated aq NaCl (150 ml), and dried (Na_2SO_4). Evaporation of the solvent and decantation twice with petroleum ether - ether (4 : 1) gave a white powder of **30**, 19.95 g (23.3%): Rf 0.36, 0.44 ($CHCl_3$ - MeOH - AcOH, 90 : 10 : 5); FAB-MS *m/z* 244 $(M-H)^-$; ^1H NMR (200 MHz, $CDCl_3$) δ 1.46 (9H, s, Boc), 1.60 ~ 2.37 (4H, m, CH_2CH_2), 3.20 ~ 3.60 (2H, m, NCH_2), 4.14, 4.44 (1.5H, 0.5H, two brs, CHCH), 6.53 (2H, br, OH, COOH).

General Procedure A: Deprotection of Boc and *t*-Butyl Ester Groups

A solution of Boc-peptide or peptide *t*-butyl ester in TFA (0.7 ~ 1.4 ml/100 mg of substrate) was stirred at room temperature for 40 minutes (for Boc-peptide) or 120 minutes (for peptide *t*-butyl ester). The solution was evaporated, and the residue was coevaporated twice with toluene.

General Procedure B: Coupling Reaction

To the amine component (1 equiv) was added Boc- or Z-amino acid (acid component, 1 ~ 1.1 equiv), and 1-hydroxybenzotriazole (1.5 ~ 2 equiv) in DMF or CH_2Cl_2. *N*-Methylmorpholine or triethylamine (1 equiv, in case of TFA or HCl salt as a starting material) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (abbreviated as EDC·HCl, 1.4 equiv) was added under ice cooling, and the mixture was stirred in an ice bath for 2 hours and at room temperature for 6 ~ 17 hours. The completion of the reaction was monitored by TLC. The mixture was diluted with EtOAc (no dilution in case of CH_2Cl_2 as a solvent), and the mixture was washed with 4% aq $NaHCO_3$, saturated aq NaCl, 1% aq citric acid, and saturated aq NaCl. The organic layer was dried over Na_2SO_4, and the solvent was evaporated.

Typical Synthetic Procedure of Boc-based Stepwise Elongation

Boc-H_2ProCO-D-Leu-L-Val-OBzl (35)

Boc-D-Leu-L-Val-OBzl[9] (**34**; 605.1 mg, 1.439 mmol) was deprotected according to the general procedure A, and was coupled to **30** (390.1 mg, 1.590 mmol) according to the general procedure B to give crude **35**. The product was purified by silica gel column chromatography with CH_2Cl_2 - MeOH (100 : 1) to give **35** as a solid, 707.1 mg (89.7%): Rf 0.48, 0.59 (CH_2Cl_2 - MeOH, 20 : 1); FAB-MS *m/z* 548 $(M+H)^+$, 448, 358, 321, 213, 208, 114, 91, 70, 57.

Boc-L-Val-H_2ProCO-D-Leu-L-Val-OBzl (36)

Crude **36** was obtained, in a manner similar to that described in the preparation of **35**, by coupling reaction of TFA salt of deprotected **35** (1.290 mmol) with Boc-Val (308.4 mg, 1.419 mmol). The product was purified by silica gel column chromatography with CH_2Cl_2 - MeOH (100 : 1) to give **36** as an amorphous solid, 672.4 mg (80.6%): Rf 0.34 (CH_2Cl_2 - MeOH, 20 : 1); FAB-MS *m/z* 647 $(M+H)^+$, 547, 448, 91, 70, 57.

Z-L-Val-L-Val-H_2ProCO-D-Leu-L-Val-OBzl (37)

Crude **37** was obtained, in a manner similar to that described in the preparation of **35**, by coupling reaction of TFA salt of deprotected **36** (0.729 mmol) with Z-Val (201.4 mg, 0.802 mmol). The product was purified by silica gel column chromatography with CH_2Cl_2 - MeOH (100 : 1 ~ 100 : 2) to give **37** as an amorphous solid, 542.1 mg (95.4%): Rf 0.31 (CH_2Cl_2 - MeOH, 20 : 1); FAB-MS *m/z* 780 $(M+H)^+$, 573, 538, 448, 333, 234, 208, 91, 70.

Z-L-Val-L-Val-(*S*)-ProCO-D-Leu-L-Val-OBzl (4)

A mixture of **37** (386.8 mg, 0.496 mmol), pyridinium trifluoroacetate (48.6 mg, 0.252 mol), EDC·HCl (287.3 mg, 1.499 mmol), anhydrous DMSO (4.0 ml) was stirred at room temperature for 23 hours. The reaction mixture was diluted with EtOAc (40 ml) and washed with water (30 ml), and dried (Na_2SO_4). After evaporation of the solvent, the product was purified by silica gel column chromatography with CH_2Cl_2 - MeCN (20 : 1 ~ 20 : 7) to give **4** as an amorphous solid, 200.8 mg (52.0%): Rf 0.41 (CH_2Cl_2 - MeOH, 20 : 1); mp 81 ~ 83°C; $[\alpha]_D^{27}$ $-31.0°$ (*c*

2.9, $CHCl_3$); FAB-MS *m/z* 778 $(M+H)^+$, 446, 333, 234, 91, 70; ^1H NMR (270 MHz, $CDCl_3$) δ 0.77~1.10 (24H, m, $CH_3 \times 8$), 1.50~2.44 (10H, m, β-CH × 3(Val), β-CH_2(Leu), γ-CH(Leu), CH_2CH_2(pyrrolidinyl)), 3.63 (1H, br ddd, NC*Ha*Hb), 3.84 (1H, br ddd, NCHa*Hb*), 4.05 (1H, br dd, α-CH(Val)), 4.47 (1H, br ddd, α-CH(Leu)), 4.55 (1H, dd, *J*=4.6, 8.6 Hz, α-CH(Val)), 4.60 (1H, br dd, α-CH(Val)), *ca.* 5.11, 5.17 (2H, ABq, overlapping, *J*=12.1 Hz, C*H*$_2$Ph), *ca.* 5.09, *ca.* 5.11 (2H, ABq, overlapping, *J*=13.5Hz, C*H*$_2$Ph), 5.25~5.44 (2H, m, CH(pyrrolidinyl), NH(Val)), 6.55 (2H, br d, NH × 2 (Val)), 7.25~7.45 (11H, m, Ph × 2, NH(Leu)).

L-Val-L-Val-(*S*)-ProCO-D-Leu-L-Val (**2**)

To a solution of **4** (184.0 mg, 0.237 mmol) in AcOH - MeOH - H_2O (6:3:1, v/v (5 ml)) was added palladium-black catalyst (18.3 mg). The mixture was hydrogenated at room temperature in a hydrogen atmosphere for 8 hours. The catalyst was filtered off, evaporation of the solvent gave an amorphous solid. This solid was subjected to Sephadex LH-20 column chromatography with 0.3% AcOH - MeOH to give **2**, 127.4 mg (97.3%): Rf 0.42 ($CHCl_3$ - MeOH - AcOH, 75:25:3); mp 166~168°C; $[\alpha]_D^{23}$ −46.5° (*c* 0.84, AcOH); FAB-MS *m/z* 554 $(M+H)^+$, 356, 199, 171, 72, 70; ^1H NMR (270 MHz, DMSO-d_6) δ 0.65~1.05 (24H, m, $CH_3 \times 8$), 1.35~2.30 (10H, m, β-CH × 3(Val), β-CH_2(Leu), γ-CH(Leu), CH_2CH_2(pyrrolidinyl)), 3.15 (1H, d, *J*=4.6 Hz, α-CH(Val)), 3.61, 3.81 (1H, 1H, m, m, NCH_2), 4.05 (1H, dd, *J*=5.3, 8.6 Hz, α-CH(Val)), 4.37 (1H, dd, *J*=3.3, 8.6 Hz, α-CH(Val)), 4.46 (1H, m, α-CH(Leu)), 5.12 (1H, dd, *J*=5.9, 8.9 Hz, NCHCOCO), 8.06 (1H, d, *J*=8.6 Hz, NH(Val)), 8.17 (1H, d, *J*=8.6 Hz, NH(Val)), 8.54 (1H, d, *J*=8.6 Hz, NH(Leu)).

The compounds **9** and **22** were obtained under analogous Boc-based stepwise elongation method.

9: FAB-MS *m/z* 727 $(M+H)^+$, 446; ^1H NMR (200 MHz, $CDCl_3$) δ 0.86, 0.92 (3H, 3H, two d, each *J*=6.9 Hz, $CH_3 \times 2$(Val)), 0.96 (6H, d, *J*=5.9 Hz, $CH_3 \times 2$(Leu)), 1.50~2.04 (7H, m, β-CH(Val), β-CH_2(Leu), γ-CH(Leu), CH_2C*Ha*Hb(pyrrolidinyl)), 2.21 (1H, m, CHa*Hb*(pyrrolidinyl)), 2.93 (1H, dd, *J*=6.5, 13.8 Hz, β-C*Ha*Hb(Phe)), 3.08 (1H, dd, *J*=7.0, 13.8 Hz, β-CHa*Hb*(Phe)), 3.48 (1H, m, NC*Ha*Hb), 3.61 (1H, m, NCHa*Hb*), *ca.* 4.50 (1H, m, overlapping, α-CH(Leu)), 4.56 (1H, dd, *J*=4.6, 8.7 Hz, α-CH(Val)), 4.69 (1H, br ddd, α-CH(Phe)), 5.05, 5.06 (2H, ABq, overlapping, C*H*$_2$Ph), 5.10, 5.18 (2H, ABq, *J*=12.3 Hz, C*H*$_2$Ph), 5.34 (1H, dd, *J*=5.2, 8.1 Hz, NCHCOCO), 5.55 (1H, d, *J*=8.6 Hz, NH(Phe)), 6.56 (1H, d, *J*=8.7 Hz, NH(Val)), 7.12~7.45 (16H, m, Ph × 3, NH(Leu)).

22: FAB-MS *m/z* 645 $(M+H)^+$, 545, 269, 241, 208, 91, 70, 57; ^1H NMR (270 MHz, $CDCl_3$) δ 0.85, 0.88 (3H, 3H, two d, each *J*=6.9 Hz, $CH_3 \times 2$(Val)), 0.93, 0.95 (3H, 3H, two d, each *J*=6.9 Hz, $CH_3 \times 2$(Leu)), 0.95, 1.03 (3H, 3H, two d, each *J*=6.6 Hz, $CH_3 \times 2$(Val)), 1.42 (9H, s, Boc), 1.53~2.10 (7H, m, β-CH(Val), β-CH_2(Leu), γ-CH(Leu), C*H*$_2$C*Ha*Hb(pyrrolidinyl)), 2.19 (1H, m, β-CH(Val)), 2.33 (1H, m, CHa*Hb*(pyrrolidinyl)), 3.63 (1H, m, NC*Ha*Hb), 3.83 (1H, m, NCHa*Hb*), 4.28 (1H, dd, *J*=6.4, 9.4 Hz, α-CH(Val)), 4.47 (1H, α-CH(Leu)), 4.55 (1H, dd, *J*=4.6, 8.8 Hz, α-CH(Val)), 5.11, 5.18 (2H, ABq, *J*=12.2 Hz, C*H*$_2$Ph), 5.38 (1H, dd, *J*=6.3, 8.9 Hz, NCHCOCO), 6.55 (1H, d, *J*=8.8 Hz, NH(Val)), 7.26 (1H, d, *J*=8.3 Hz, NH(Leu)), *ca.* 7.24~7.45 (5H, m, overlapping, Ph).

Typical Synthetic Procedure of Z-based Stepwise Elongation

(*RS*)-2-[(*S*)-2-(1-Benzyloxycarbonylpyrrolidinyl)]-2-hydroxyacetic Acid (Z-H_2ProCO, **38**)

To the cyanohydrin (**32**) prepared from **31** (20.07 g, 61.3 mmol) as described for the preparation of **30** was added 12 N HCl (45 ml) and dioxane (45 ml), and refluxed for 6 hours. After evaporation of the solvent, the residue (6.36 g) was dissolved in distilled water and deionized through a column of strong acidic ion-exchange resin (Dowex50W-X4 (350 ml), H^+ form, 2 N aq NH_3 as an eluent). Concentration of the ninhydrin positive eluate gave solid of **33**, 2.14 g (24.0%): FAB-MS *m/z* 146 $(M+H)^+$.

A mixture of **33** (1.60 g, 11.0 mmol), benzyl *S*-4,6-dimethylpyrimidin-2-ylthiocarbonate (3.63 g, 13.2 mmol), water (6 ml), dioxane (6 ml) and triethylamine (2.30 ml, 16.5 mmol) was stirred for 3 hours at room temperature. To a reaction mixture was added water (16 ml), and unreacted carbonate was extracted twice with EtOAc (each 20 ml). The aqueous layer was cooled to 0°C and adjusted to pH 2 by addition of a 3 N HCl, and extracted with EtOAc (once 16 ml, twice 8 ml). The combined organic layer was washed thrice with 1 N HCl (each 10 ml), and twice with saturated aq NaCl (each 10 ml) and dried (Na_2SO_4). Evaporation of the solvent gave crude syrup (2.44 g). The product (0.78 g) was subjected to Sephadex LH-20 column chromatography with MeOH to give **38**, 0.76 g (77.0%): Rf 0.35, 0.46 ($CHCl_3$ - MeOH - AcOH, 18:2:1); FAB-MS *m/z* 278 $(M-H)^-$, 170, 144; ^1H NMR (270 MHz, $CDCl_3$) δ 1.64~2.33 (4H, m, CH_2CH_2), 3.28~3.69 (2H, m, NCH_2), 4.06~4.35, 4.58 (1.5H, m, 0.5H, br s, CHCH), 4.99~5.29 (2H, m, PhC*H*$_2$OCO), 6.28 (2H, br, COOH, OH), 7.35 (5H, s, Ph).

Z-H_2ProCO-L-Leu-OBu^t (**39**)

38 (201.7 mg, 0.722 mmol) was coupled to L-leucine *t*-butyl ester hydrochloride (161.7 mg, 0.723 mmol) according to the general procedure B to give crude **39**. The product was purified by Sephadex LH-20 column chromatography with MeOH to give **39** as needle crystals, 309.7 mg (95.6%): Rf 0.47, 0.59 (CH_2Cl_2 - MeOH, 30:1); FAB-MS *m/z* 449 $(M+H)^+$, 393, 349, 315, 259, 204, 160, 91, 70, 57; ^1H NMR (400 MHz, $CDCl_3$) δ 0.82~1.00 (6H, m, $CH_3 \times 2$), 1.36~2.16 (15H, m, $CH_3 \times 3$, β-CH_2(Leu), γ-CH(Leu), C*H*$_2$C*Ha*Hb(pyrrolidinyl)), 2.40 (1H, m, CHa*Hb*), 3.30, 3.42, 3.33, 3.61 (total

2H(each 0.5H), br dt, m, m, m, NCH_2), 4.05, 4.17, 4.38 (total 2H, br s, br t, br s, NCHCHCO), 4.40~4.53 (1H, m, α-CH(Leu)), 5.04, 5.11 and 5.17 (1H, ABq, J=12.8 Hz, and 1H, s, PhCH_2OCO), 5.62, 6.09 (0.5H, 0.5H, two br, OH), 7.20 (1H, br, NH), 7.28~7.48 (5H, m, Ph).

Z-L-Phe-H_2ProCO-L-Leu-OBu^t (**40**)

To a solution of **39** (309.6 mg, 0.690 mmol) in MeOH (10 ml) was added palladium-black catalyst (13.4 mg). The mixture was hydrogenated at room temperature in a hydrogen atmosphere for 24 hours. The catalyst was filtered off, evaporation of the solvent gave amorphous solid of H_2ProCO-L-Leu-OBu^t (**41**; 216.9 mg). **41** (216.9 mg, 0.690 mmol) was coupled to Z-L-Phe (216.9 mg, 0.725 mmol) according to the general procedure B to give crude **40**. The product was purified by silica gel column chromatography with CH_2Cl_2 - MeOH (100 : 1) to give **40** as an amorphous solid, 393.3 mg (95.7%): Rf 0.41 (CH_2Cl_2 - MeOH, 20 : 1); FAB-MS m/z 596 $(M+H)^+$, 540, 506, 462, 406, 315, 259, 91, 70, 57; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90, 0.92 and 0.94, 0.96 (3H, two d, each J=6.9 Hz and 3H two d, each J=6.0 Hz $CH_3\times2$), 1.25~2.34 (16H, m, OBu^t, β-CH_2(Leu), γ-CH(Leu), CH_2CH_2(pyrrolidinyl)), 2.82~3.10 (2H, m, β-CH_2(Phe)), 2.67, 3.17, 3.52, 3.84 (total 2H(each 0.5H), br, br t, br t, br, NCH_2), 3.86 and 4.15~4.85 (total 4H, br d, J=8.0 Hz and m, NCHCHCO, α-CH(Phe), α-CH(Leu)), 4.93~5.17 (2H, m, PhCH_2OCO), 5.65, 5.71 (0.5H, 0.5H, two br, NH), 6.36 (0.5H, br, OH), 7.10~7.44 (11H, m, Ph×2, NH).

Z-L-Phe-(*S*)-ProCO-L-Leu-OBu^t (**14**)

A mixture of **40** (316.9 mg, 0.532 mmol), pyridinium trifluoroacetate (51.4 mg, 0.266 mmol), DCC (329.6 mg, 1.597 mmol), anhydrous DMSO (4.0 ml) and benzene (2.0 ml) was stirred at room temperature for 9 hours. The reaction mixture was diluted with EtOAc (25 ml), and the undissolved material was removed by filtration. The filtrate was washed with water (20 ml), and dried (Na_2SO_4). After evaporation of the solvent, the product was purified by silica gel column chromatography with CH_2Cl_2~CH_2Cl_2 - MeOH (100 : 1) to give **14** as an amorphous solid. This solid was chromatographed on a column of Sephadex LH-20 with 0.3% AcOH - MeOH elution. Evaporation of the active eluate gave **14** as an amorphous solid, 264.9 mg (83.9%): Rf 0.30 (CH_2Cl_2 - MeOH, 30 : 1); mp 43~46°C; $[\alpha]_D^{26}$ −16.5° (c 0.57, $CHCl_3$); FAB-MS m/z 594 $(M+H)^+$, 538, 504, 460, 404, 257, 91, 70, 57; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95, 0.96 (3H, 3H, two d, each J=7.2 Hz, $CH_3\times2$), 1.48 (9H, s, OBu^t), 1.53~2.05 (6H, m, β-CH_2(Leu), γ-CH(Leu), CH_2CHa*Hb*(pyrrolidinyl)), 2.29 (1H, m, CHa*Hb*(pyrrolidinyl)), 2.90 (1H, dd, J=7.0, 14.0 Hz, β-C*Ha*Hb(Phe)), 3.03~3.18 (2H, m, β-CHa*Hb*(Phe), NC*Ha*Hb), 3.65 (1H, m, NCHa*Hb*), 4.46 (1H, m, α-CH(Leu)), 4.69 (1H, dt, J=7.0, 8.8 Hz, α-CH(Phe)), 5.02, 5.06 (2H, ABq, J=11.0 Hz, PhCH_2OCO), 5.29 (1H, dd, J=5.6, 9.0 Hz, NCHCOCO), 5.48 (1H, br d, J=8.8 Hz, NH(Phe)), 7.12~7.42 (11H, m, Ph×2, NH(Leu)).

Z-L-Phe-(*S*)-ProCO-L-Leu (**15**)

14 (202.9 mg, 0.342 mmol) was deprotected according to the general procedure A to give crude **15**. The product was purified by silica gel column chromatography with $CHCl_3$ - MeOH - AcOH (200 : 2 : 1) to give **15** as an amorphous solid (130.5 mg). This solid was chromatographed on a column of Sephadex LH-20 with 0.3% AcOH - MeOH elution. Evaporation of the active eluate gave **15** as a white powder, 111.7 mg (62.7%): Rf 0.34 ($CHCl_3$ - MeOH - AcOH, 95 : 5 : 1); mp 70~71°C; $[\alpha]_D^{23}$ −41.0° (c 1.9, $CHCl_3$); FAB-MS m/z 538 $(M+H)^+$, 494, 404, 257, 91, 70; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94, 0.95 (3H, 3H, two d, each J=6.0 Hz, $CH_3\times2$), 1.55~2.03 (6H, m, β-CH_2(Leu), γ-CH(Leu), CH_2CHa*Hb*(pyrrolidinyl)), 2.32 (1H, m, CHa*Hb*(pyrrolidinyl)), 2.90 (1H, dd, J=7.4, 13.6 Hz, β-C*Ha*Hb(Phe)), 3.03 (1H, dd, J=7.4, 13.6 Hz, β-CHa*Hb*(Phe)), 3.09 (1H, overlapping, NC*Ha*Hb), 3.73 (1H, m, NCHa*Hb*), 4.62 (1H, m, α-CH(Leu)), 4.72 (1H, dt, J=7.4, 9.4 Hz, α-CH(Phe)), 5.01, 5.07 (2H, ABq, J=12.4 Hz, PhCH_2OCO), 5.30 (1H, dd, J=5.8, 9.0 Hz, NCHCOCO), 6.30 (1H, d, J=9.4 Hz, NH(Phe)), 7.10~7.43 (11H, m, Ph×2, NH(Leu)).

The compounds **10**, **11**, **16**~**19**, **23** and **25** were obtained under analogous Z-based stepwise elongation methods.

10: FAB-MS m/z 594 $(M+H)^+$, 538, 494, 257; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.96 (6H, d, J=5.9 Hz, $CH_3\times2$(Leu)), 1.47 (9H, s, OBu^t), *ca.* 1.40~2.05 (6H, m, overlapping, β-CH_2(Leu), γ-CH(Leu), CH_2CHa*Hb*(pyrrolidinyl)), 2.28 (1H, m, CHa*Hb*(pyrrolidinyl)), 2.81~3.20 (3H, m, β-CH_2(Phe), NC*Ha*Hb), 3.61 (1H, m, NCHa*Hb*), 4.48 (1H, m, α-CH(Leu)), 4.70 (1H, br ddd, α-CH(Phe)), 5.03, 5.07 (2H, ABq, J=12.4 Hz, PhCH_2OCO), 5.35 (1H, dd, J=5.4, 8.6 Hz, NCHCOCO), 5.52 (1H, d, J=8.7 Hz, NH(Phe)), 7.10~7.49 (11H, m, Ph×2, NH(Leu)).

11: FAB-MS m/z 538 $(M+H)^+$, 257; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.95 (6H, d, J=5.4 Hz, $CH_3\times2$ (Leu)), 1.48~2.09 (6H, m, β-CH_2(Leu), γ-CH(Leu), CH_2CHa*Hb*(pyrrolidinyl)), 2.26 (1H, m, CHa*Hb*(pyrrolidinyl)), 2.87 (1H, dd, J=7.5, 13.9 Hz, β-C*Ha*Hb(Phe)), 3.05 (1H, dd, J=6.4, 13.9 Hz, β-CHa*Hb*(Phe)), 3.25 (1H, m, NC*Ha*Hb), 3.75 (1H, m, NCHa*Hb*), 4.56 (1H, m, α-CH(Leu)), 4.69 (1H, ddd, overlapping, α-CH(Phe)), 5.00, 5.04 (2H, ABq, J=12.6 Hz, PhCH_2OCO), 5.16 (1H, dd, J=6.2, 7.3 Hz, NCHCOCO), 6.02 (1H, d, J=8.7 Hz, NH(Phe)), 7.10~7.47 (11H, m, Ph×2, NH(Leu)).

16: FAB-MS m/z 628 $(M+H)^+$, 572, 494, 438, 291, 91, 70, 57; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.39 (9H, s, OBu^t), 1.70~2.00 (3H, m, CH_2CHa*Hb*(pyrrolidinyl)), 2.25 (1H, m, CHa*Hb*(pyrrolidinyl)), 2.94 (1H, dd, J=6.3, 13.9 Hz, β-C*Ha*Hb(Phe)), *ca.* 2.98~3.25 (4H, m, overlapping, β-CH_2(Phe), β-CHa*Hb*(Phe), NC*Ha*Hb), 3.62 (1H, m, NCHa*Hb*), 4.70 (2H, m, α-CH×2(Phe)), 5.04, 5.08 (2H, ABq, J=12.4 Hz, PhCH_2OCO), 5.34 (1H,

dd, J=5.8, 8.4 Hz, NCHCOCO), 5.52 (1H, d, J=8.9 Hz, NH), 7.10~7.45 (16H, m, Ph×3, NH).

17: FAB-MS m/z 538 $(M+H)^+$, 482, 438; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.49 (9H, s, OBu^t), 1.70~2.05 (3H, m, CH_2CH*a*Hb(pyrrolidinyl)), 2.25 (1H, m, CHa*Hb*(pyrrolidinyl)), 2.91 (1H, dd, J=6.5, 13.6 Hz, β-CH*a*Hb (Phe)), *ca.* 2.97~3.17 (2H, m, overlapping, β-CHa*Hb* (Phe), NCH*a*Hb), 3.63 (1H, m, NCHa*Hb*), 3.90 (1H, dd, J=4.9, 18.4 Hz, CH*a*Hb(Gly)), 4.07 (1H, dd, J=5.9, 18.4 Hz, CHa*Hb*(Gly)), 4.69 (1H, br ddd, α-CH(Phe)), 5.03, 5.06 (2H, ABq, J=12.5 Hz, PhCH_2OCO), 5.32 (1H, br dd, NCHCOCO), 5.50 (1H, d, J=9.1 Hz, NH(Phe)), 7.12~7.48 (11H, m, Ph×2, NH(Gly)).

18: FAB-MS m/z 572 $(M+H)^+$, 528, 379, 291, 91; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.50~2.00 (3H, m, CH_2CH*a*Hb(pyrrolidinyl)), 2.21 (1H, m, CHa*Hb*(pyrrolidinyl)), 2.73~3.31 (5H, m, β-CH_2×2(Phe), NCH*a*Hb), 3.73 (1H, m, NCHa*Hb*), 4.69 (1H, br dt, α-CH(Phe)), 4.80 (1H, br dt, α-CH(Phe)), 5.02, 5.06 (2H, ABq, J=12.4 Hz, PhCH_2OCO), *ca.* 5.11 (1H, m, overlapping, NCHCOCO), 6.05 (1H, d, J=8.6 Hz, NH), 7.05~7.45 (16H, m, Ph×3, NH).

19: FAB-MS m/z 482 $(M+H)^+$, 279, 201; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.50~2.10 (3H, m, CH_2CH*a*Hb (pyrrolidinyl)), 2.28 (1H, m, CHa*Hb*(pyrrolidinyl)), 2.75~3.26 (3H, m, β-CH_2(Phe), NCH*a*Hb), 3.75 (1H, m, NCHa*Hb*), 4.05 (1H, dd, J=6.3, 18.0 Hz, CH*a*Hb (Gly)), 4.14 (1H, dd, J=5.9, 18.0 Hz, CHa*Hb*(Gly)), 4.70 (1H, br ddd, α-CH(Phe)), 5.01, 5.06 (2H, ABq, J=12.5 Hz, PhCH_2OCO), 5.27 (1H, dd, J=6.9, 8.2 Hz, NCHCOCO), 5.75 (1H, d, J=8.8 Hz, NH(Phe)), 7.10~7.44 (10H, m, Ph×2), 7.51 (1H, br dd, NH(Gly)).

23: FAB-MS m/z 608 $(M+H)^+$, 552, 508, 474, 444, 418, 257, 91, 70, 57; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.95, 0.96 (3H, 3H, two d, each J=5.9 Hz, CH_3×2), 1.46 (9H, s, OBu^t), 1.52~2.20 (8H, m, β-CH_2(Leu), γ-CH(Leu), CH_2CH*a*Hb(pyrrolidinyl), β-CH_2(Phe)), 2.35 (1H, m, CHa*Hb*(pyrrolidinyl)), 2.73 (2H, t, J=7.8 Hz, γ-CH_2(Hph)), 3.37 (1H, m, NCH*a*Hb), 3.62 (1H, m, NCHa*Hb*), 4.47 (1H, ddd, J=5.6, 8.6, 8.6 Hz, α-CH(Leu)), 4.54 (1H, br ddd, α-CH(Hph)), 5.09, 5.12 (2H, ABq, J=12.2 Hz, PhCH_2OCO), 5.35 (1H, dd, J=5.4, 8.4 Hz, NCHCOCO), 5.58 (1H, d, J=8.6 Hz, NH(Hph)), 7.12~7.42 (11H, m, Ph×2, NH(Leu)).

25: FAB-MS m/z 447$(M+H)^+$, 391, 347, 284; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.83~1.03 (6H, m, CH_3×2), 1.47 (9H, s, OBu^t), *ca.* 1.35~2.09 (6H, m, overlapping, β-CH_2(Leu), γ-CH(Leu), CH_2CH*a*Hb(pyrrolidinyl)), 2.38 (1H, m, CHa*Hb*(pyrrolidinyl)), 3.58 (2H, m, NCH_2), 4.45 (1H, m, α-CH(Leu)), 5.02, 5.17 and 5.03, 5.16 (2H, two ABq, J=12.4 Hz and J=10.2 Hz, PhCH_2OCO), 5.25, 5.29 (1H, two dd, J=4.6, 9.8 Hz, J=4.6, 9.4 Hz, NCHCOCO), 7.10~7.42 (6H, m, Ph, NH).

(*RS*)-*N*-Cyclohexyl-2-[(*S*)-2-(1-*t*-butoxycarbonylpyrrolidinyl)]-2-hydroxyacetoamide (Boc-H_2ProCO-NHcHx, **42**)

30 (1.64 g, 6.69 mmol) was coupled to cyclohexylamine (0.922 ml, 8.04 mmol) according to the general procedure B to give crude **42**. The product was purified by Sephadex LH-20 column chromatography with MeOH to give **42** as a solid, 2.11 g (96.6%): Rf 0.39, 0.47 (CH_2Cl_2-MeOH, 30:1); FAB-MS m/z 327 $(M+H)^+$, 271, 227, 114, 70, 57; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.00~*ca.* 1.50 (5H, m, overlapping, CH_2×2, CH*a*Hb(cyclohexyl)), 1.46, 1.49 (4.5H, 4.5H, two s, Boc), 1.53~2.29 (8H, m, CH_2×2, CHa*Hb*(cyclohexyl), CH_2CH*a*Hb(pyrrolidinyl)), 2.46 (1H, m, CHa*Hb*), 3.12~3.57 (2H, m, NCH_2), 3.73 (1H, m, NCH), 3.94, 4.08 (0.5H, 0.5H, br s, br t, NCHCHOH), 3.94, 4.23 (0.5H, 0.5H, br s, br d, CHOH), 6.14, 6.19 (0.5H, 0.5H, br d, br, OH), 6.81, 6.91 (0.5H, 0.5H, br d, br, NH).

The compound **20** was prepared from **42** under Boc-based stepwise elongation methods.

20: FAB-MS m/z 506 $(M+H)^+$, 225, 91, 70; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.05~1.50 (5H, m, CH_2×2, CH*a*Hb(cyclohexyl)), 1.54~2.05 (8H, m, CH_2×2, CHa*Hb*(cyclohexyl), CH_2CH*a*Hb (pyrrolidinyl)), 2.32 (1H, m, CHa*Hb*(pyrrolidinyl)), 2.91 (1H, dd, J=6.9, 13.9 Hz, β-CH*a*Hb(Phe)), 3.00~3.20 (2H, m, NCH*a*Hb, β-CHa*Hb*(Phe)), 3.55~3.85 (2H, m, NCHa*Hb*, NCH (cyclohexyl)), 4.70 (1H, ddd, J=6.9, 7.1, 8.9 Hz, α-CH(Phe)), 5.03, 5.06 (2H, ABq, J=12.4 Hz, PhCH_2-OCO), 5.32 (1H, dd, J=5.4, 8.1 Hz, NCHCOCO), 5.50 (1H, d, J=8.9 Hz, NH(Phe)), 6.78 (1H, d, J=8.2 Hz, NH), 7.10~7.46 (10H, m, Ph×2).

Boc-D-Leucine-*t*-butylamide(Boc-D-Leu-NHBu^t, **43**)

Boc-D-leucine hydrate (623.6 mg, 2.50 mmol) was coupled to *t*-butylamine (190.2 mg, 2.60 mmol) according to the general procedure B to give crude **43**. The product was purified by silica gel column chromatography with hexane-EtOAc (4:1) to give **43** as a solid, 635.4 mg (88.7%): Rf 0.56 (CH_2Cl_2-MeOH, 30:1); mp 138~143°C; $[\alpha]_D^{25}$ +44.2° (*c* 1.7, $CHCl_3$); FAB-MS m/z 287 $(M+H)^+$, 231, 187, 131, 57; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.93, 0.94 (3H, 3H, two d, each J=6.1 Hz, CH_3×2), 1.34 (9H, s, NBu^t), 1.44 (9H, s, Boc), *ca.* 1.30~1.80 (3H, m, overlapping, β-CH_2(Leu), γ-CH(Leu)), 3.94 (1H, br ddd, α-CH(Leu)), 4.90 (1H, br d, NH), 5.92 (1H, br s, NH).

Z-H_2ProCO-D-Leu-NHBu^t (**44**)

43 (588.0 mg, 2.053 mmol) was deprotected according to the general procedure A, and was coupled to **38** (573.9 mg, 2.05 mmol) according to the general procedure B to give crude **44**. The product was purified by silica gel column chromatography with CH_2Cl_2-MeOH (100:1) to give **44** as an amorphous solid, 659.8 mg (71.9%): Rf 0.48 (CH_2Cl_2-MeOH, 20:1); FAB-MS m/z 448 $(M+H)^+$, 375, 314, 262, 204, 160, 91, 70, 57; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.91, 0.94 (3H, 3H, two d, each J=5.9 Hz, CH_3×2), 1.33 (9H, s, NBu^t), 1.40~2.04 (6H, m, β-CH_2(Leu), γ-CH(Leu), CH_2CH*a*Hb(pyrrolidinyl)), 2.33 (1H, m, CHa*Hb*(pyrrolidinyl)), 3.36~3.63 (2H, m, NCH_2), 3.92~4.36 (3H, m, CHCHOH,

α-CH(Leu)), 5.17 (2H, s, PhC*H*$_2$OCO), 5.86 (1H, br, OH), 5.93 (1H, br s, NH), 7.19 (1H, br d, NH(Leu)), 7.30~7.44(5H, m, Ph).

Bz-H$_2$ProCO-D-Leu-NHBu^t (**45**)

To a solution of **44** (560.3 mg, 1.251 mmol) in MeOH (6 ml) was added palladium-black catalyst (20 mg). The mixture was hydrogenated at room temperature in a hydrogen atmosphere for 7 hours. The catalyst was filtered off, evaporation of the solvent gave amorphous solid of H$_2$ProCO-D-Leu-NHBu^t (**46**).

To a solution of **46** (99.6 mg, 0.318 mmol) in anhydrous THF (1.0 ml) was added triethylamine (56 μl, 0.397 mmol), and the solution was treated dropwise with benzoyl chloride (46 μl, 0.397 mmol) in anhydrous THF (2 ml) over a period of 30 minutes. The mixture was stirred for additional 2 hours at room temperature, and the solvent was evaporated. To the mixture was added 1 N HCl (3 ml), and the mixture was extracted thrice with EtOAc (each 4 ml). The combined extracts were washed with saturated aq NaHCO$_3$ (8 ml) and saturated aq NaCl (8 ml), and dried (Na$_2$SO$_4$). After removal of the solvent, the product was purified by Sephadex LH-20 column chromatography with MeOH to give **45** as an amorphous solid, 124.9 mg (94.1%): Rf 0.36, 0.41 (CH$_2$Cl$_2$ - MeOH, 20:1); FAB-MS *m/z* 418 $(M+H)^+$, 345, 317, 314, 232, 204, 174, 105, 70, 57.

Bz-(*S*)-ProCO-D-Leu-NHBu^t (**28**)

Crude **28** was prepared from **45** under analogous synthetic procedure as described for the preparation of **14** from **40**. The product was purified by silica gel column chromatography with CH$_2$Cl$_2$ - EtOAc (7:1) to give an amorphous solid. This solid was chromatographed on a column of Sephadex LH-20 with 0.3% AcOH - MeOH elution. Evaporation of the active eluate gave **28** as an amorphous solid, 93.4 mg (75.3%): Rf 0.48 (CH$_2$Cl$_2$ - MeOH, 20:1); mp 66~69°C; $[\alpha]_D^{24}$ +18.2° (*c* 1.2, CHCl$_3$); FAB-MS *m/z* 416 $(M+H)^+$, 343, 315, 312, 230, 202, 187, 174, 105, 70, 57; ^{1}H NMR (270 MHz, CDCl$_3$) δ 0.95 (6H, d, *J*=6.3 Hz, CH$_3$ × 2), 1.24 (9H, s, Bu^t), 1.41~2.17 (6H, m, β-CH$_2$(Leu), γ-CH(Leu), C*H*$_2$C*Ha*Hb(pyrrolidinyl)), 2.40 (1H, m, CHa*Hb*(pyrrolidinyl)), 3.56 (1H, m, NC*Ha*Hb), 3.70 (1H, m, NCHa*Hb*), 4.31 (1H, ddd, *J*=5.2, 8.9, 8.9 Hz, α-CH(Leu)), 5.22 (1H, br dd, NCHCOCO), 5.89 (1H, s, NH), 7.10 (1H, d, *J*=8.9 Hz, NH(Leu)), 7.35~7.64 (5H, m, Ph).

The compound **24** was prepared under an analogous synthetic procedure as described for the preparation of **14** from **40**.

24: FAB-MS *m/z* 441 $(M+H)^+$, 355, 257, 196, 168; ^{1}H NMR (200 MHz, CDCl$_3$) δ 0.95, 0.96 (3H, 3H, two d, each *J*=6.1 Hz, CH$_3$ × 2), 1.05 (9H, s, Bu^t), 1.53~2.09 (6H, m, β-CH$_2$(Leu), γ-CH(Leu), C*H*$_2$C*Ha*Hb(pyrrolidinyl)), 2.18, 2.24 (2H, ABq, *J*=13.6 Hz, COC*H*$_2$Bu^t), 2.30 (1H, m, CHa*Hb*(pyrrolidinyl)), 3.62 (2H, m, NCH$_2$), 4.46 (1H, ddd, *J*=5.8, 8.5, 8.5 Hz, α-CH(Leu)), 5.31 (1H, dd, *J*=5.5, 6.0 Hz, NCHCOCO), 7.21 (1H, d, *J*=8.5 Hz, NH(Leu)).

The compound **26** was prepared from **46** by the general procedure B.

26: FAB-MS *m/z* 593$(M+H)^+$, 312; ^{1}H NMR (200 MHz, CDCl$_3$) δ 0.95 (6H, d, *J*=6.0 Hz, CH$_3$ × 2 (Leu)), 1.35 (9H, s, Bu^t), 1.49~2.05 (6H, m, β-CH$_2$(Leu), γ-CH(Leu), C*H*$_2$C*Ha*Hb(pyrrolidinyl)), 2.28 (1H, m, CHa*Hb*(pyrrolidinyl)), 2.92 (1H, dd, *J*=6.6, 13.6 Hz, β-C*Ha*Hb(Phe)), *ca.* 2.95~3.18 (2H, m, overlapping, β-CHa*Hb*(Phe), NC*Ha*Hb), 3.61 (1H, m, NCHa*Hb*), 4.25 (1H, m, α-CH(Leu)), 4.69 (1H, br ddd, α-CH(Phe)), 5.03, 5.07 (2H, ABq, *J*=12.5 Hz, PhC*H*$_2$OCO), 5.32 (1H, br dd, NCHCOCO), 5.52 (1H, d, *J*=8.7 Hz, NH(Phe)), 5.70 (1H, s, CONH), 7.15~7.45 (11H, m, Ph × 2, NH(Leu)).

The compounds **27** and **29** were prepared under analogous synthetic procedures as described for the preparation of **28** from **45**.

27: FAB-MS *m/z* 422 $(M+H)^+$, 349, 321, 312, 256, 236, 208, 187, 180, 83, 70, 57; ^{1}H NMR (270 MHz, CDCl$_3$) δ 0.93 (6H, d, *J*=6 Hz, CH$_3$ × 2), 1.00~2.14 (16H, m, β-CH$_2$(Leu), γ-CH(Leu), C*H*$_2$C*Ha*Hb(pyrrolidinyl), CH$_2$ × 5(cyclohexyl)), 1.35 (9H, s, Bu^t), 2.17~2.44 (2H, m, CHa*Hb*(pyrrolidinyl), CH(cyclohexyl)), 3.54~3.80 (2H, m, NCH$_2$), 4.26 (1H, ddd, *J*=5.6, 8.9, 8.9 Hz, α-CH(Leu)), 5.10 (1H, dd, *J*=6.3, 8.6 Hz, NHCOCO), 5.85 (1H, br s, NH), 7.11 (1H, d, *J*=8.9 Hz, NH(Leu)).

29: FAB-MS *m/z* 361 $(M+H)^+$, 305, 202, 174, 105, 57; ^{1}H NMR (270 MHz, CDCl$_3$) δ 1.49 (9H, s, OBu^t), 1.91~2.11 (3H, m, C*H*$_2$C*Ha*Hb(pyrrolidinyl)), 2.42 (1H, m, CHa*Hb*(pyrrolidinyl)), 3.54~3.77 (2H, m, NCH$_2$), 3.93 (1H, dd, *J*=4.9, 18.5 Hz, C*Ha*Hb(Gly)), 4.07 (1H, dd, *J*=5.9, 18.5 Hz, CHa*Hb*(Gly)), 5.44 (1H, dd, *J*=5.6, 7.9 Hz, NCHCOCO), 7.30~7.62 (6H, m, Ph, NH(Gly)).

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