Poststatin, a New Inhibitor of Prolyl Endopeptidase

VII. *N*-Cycloalkylamide Analogues

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Poststatin analogues containing (*S*)-2-oxo-2-(2-pyrrolidinyl)acetyl moiety in P_1 were synthesized and examined for their inhibitory activity against prolyl endopeptidase and cathepsin B *in vitro*. Introduction of non-peptidyl cycloalkylamine component in P_1' was effective and P_3-acyl groups must be widely modifiable for prolyl endopeptidase inhibition. Acyl-L-phenylalanyl-(*S*)-2-oxo-2-(2-pyrrolidinyl)acetyl-cycloalkylamide type compounds showed IC_{50} value of nano to subnano g/ml as prolyl endopeptidase inhibitor and were shown no significant inhibitory activities against cathepsin B, a cysteine protease.

Prolyl endopeptidase (PEP) [EC 3.4.21.26] is a serine protease[1] that is highly active in the brain and degrades proline-containing oligopeptides such as oxytocin, neurotensin, substance P, thyrotropin releasing hormone, bradykinin, and angiotensin II[2~7]. PEP also degrades vasopressin which has been suggested to play an important role in learning and memory[8~10]. Moreover, PEP may be involved in processing the *C*-terminal portion of the amyloid precursor protein in the ALZHEIMER's disease[11].

Recently, many potent inhibitors such as benzyloxycarbonyl(Z)-Gly-Pro-CH_2Cl[1], Z-Pro-prolinal[12], 1-(*N*-(4-phenylbutyryl)-Pro)-pyrrolidine[13], and related compounds[13~19] have been studied, and peptidyl aldehydes and pyrrolidine derivatives have been reported to ameliorate the experimental amnesia induced by scopolamine in rats[13,16].

In the course of our study, poststatin (PST) which was a potent inhibitor of PEP with the structure of L-Val-L-Val-(*S*)-3-amino-2-oxovaleryl-D-Leu-L-Val, was isolated from a culture filtrate of *Streptomyces viridochromogenes* MH534-30F3[20~22], and many PST analogues were synthesized for the structure-activity relationships[23]. In the preceding paper we have designed PST analogues containing (*S*)-2-oxo-2-(2-pyrrolidinyl)acetyl (ProCO) moiety in the P_1, which was very effective and selective for PEP inhibitor. We have also found P_1' in the ProCO containing inhibitor was able to substitute the non-peptidyl cyclohexyl (cHx) amine component without significant loss of inhibitory activity[24]. To find more potent analogues for PEP inhibitor, we modified not only P_1' but P_2 and P_3 of the Z-L-Phe-ProCO-NH-cHx as a lead compound. In this paper, we described the synthesis of new cycloalkylamide-containing PEP inhibitors and their inhibitory activity contrasted with cathepsin B *in vitro*.

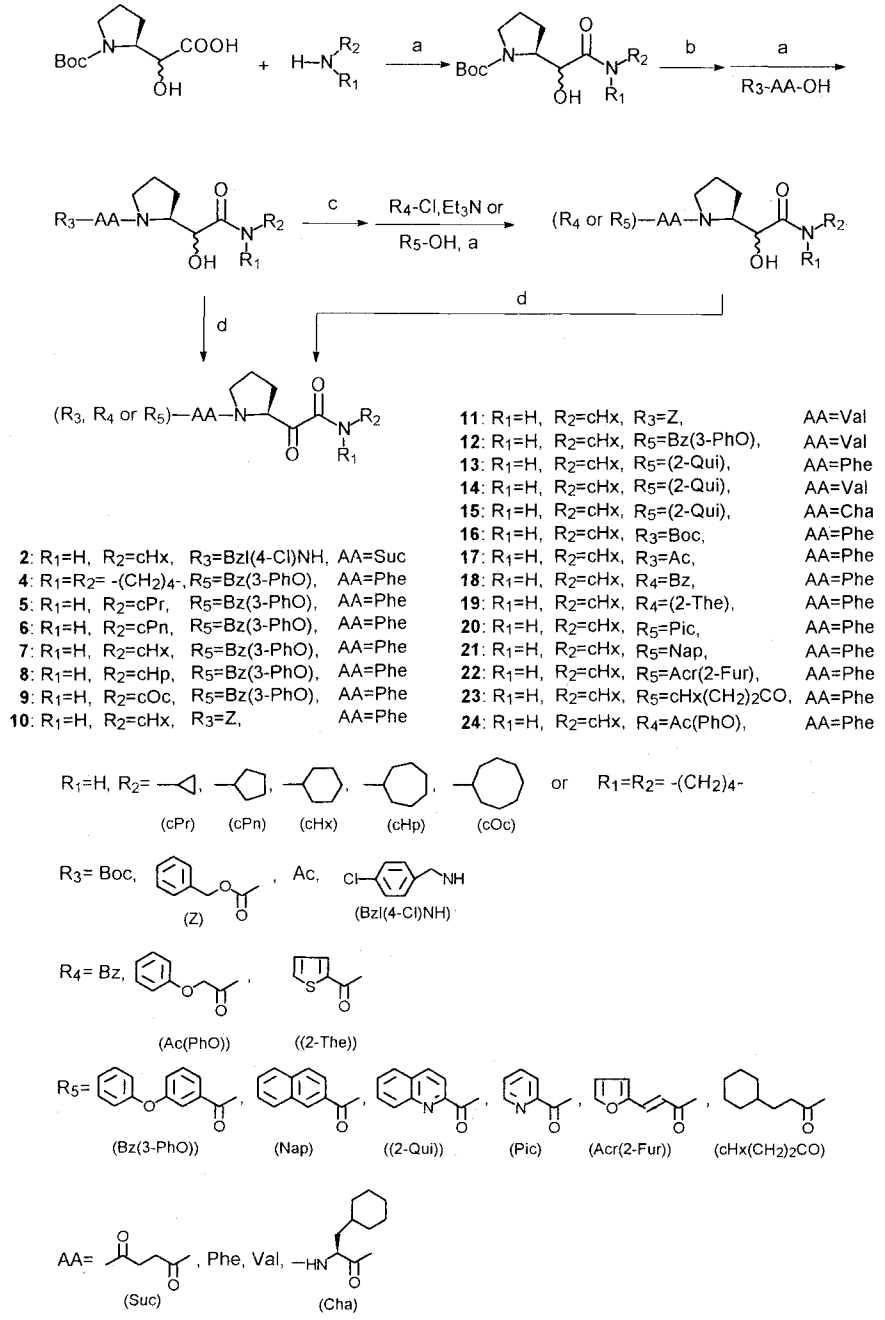
Chemistry

The synthetic route is outlined in Scheme 1. Starting *N*-Boc-(*RS*)-2-hydroxy-2-((*S*)-2-pyrrolidinyl)acetic acid was prepared from Z-L-proline in five steps according to the procedure described in the previous paper[24]. Coupling reaction of acid component with amine component was performed by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide(EDC)-1-hydroxybenzotriazole (HOBt) method or acid chloride method. Deprotection of temporary protective group was performed by acid treatment for Boc-group and hydrogenation for Z-group. All of the epimeric mixture containing hydroxyl group indicated satisfactory FAB-MS and/or NMR spectra. Oxidation of hydroxyl group to ketone was performed by the Pfitzner-Moffatt[25] or the Albright-Goldman[26] method.

Results and Discussion

The results obtained are summarized in Table 1. The influence of P_1' cycloalkylamine component was clearly demonstrated as following in comparison with aldehyde-type inhibitor. Compound **2** showed about 12 times as much active against PEP as compound **1** (ONO-1603,

Scheme 1.



a: EDC·HCl, HOBt, (Et_3N, in case of TFA or HCl salt as an amine component), b: TFA or 4 N HCl-dioxane, c: TFA or H_2, Pd-black, d: EDC·HCl, DMSO, pyridinium trifluoroacetate or Ac_2O, DMSO.

PEP inhibitor which is in phase II clinical trials[27]). Similarly compound **7** showed about 170 times more potent than compound **3** for PEP inhibition.

The systematic change of ring size at P_1' cycloalkylamine component indicated that all these components (cyclopropylamine; **5**, cyclopentylamine; **6**, cyclohexylamine; **7**, cycloheptylamine; **8**, and cyclooctylamine; **9**) were very effective for PEP inhibition in contrast with cyclic amine (pyrrolidine; **4**). Among them cHx ring was most effective.

To enhance the inhibitory activity against PEP, *N*-protected amino acid residue at the P_3-P_2 was widely studied. Although P_2-Val was more potent than Phe or *β*-cyclohexylalanine (**11** *vs.* **10**, **12** *vs.* **7**, and **14** *vs.* **13**, **15**), we selected Phe at the P_2 because it was not found the mammalian protease to digest the Phe-Pro peptidyl bond[28]).

Compounds, in which P_3 were introduced Ac (**17**), Bz

Table 1. Relationship between structure and endopeptidase inhibitory activities.

Compound	Structure[a]				IC_{50} (μg/ml)	
No.	P_3	P_2	P_1	P_1'	PEP	Cat-B[b]
1	Bzl(4-Cl)NH-	Suc-	Pro-H		0.027	>100
2	Bzl(4-Cl)NH-	Suc-	ProCO-	NH-cHx	0.0022	>100
3	Bz(3-PhO)-	Phe-	Pro-H		0.11	7.0
4	Bz(3-PhO)-	Phe-	ProCO-	$N=(CH_2)_4$	0.25	>100
5	Bz(3-PhO)-	Phe-	ProCO-	NH-cPr	0.00070	>100
6	Bz(3-PhO)-	Phe-	ProCO-	NH-cPn	0.00082	>100
7	Bz(3-PhO)-	Phe-	ProCO-	NH-cHx	0.00065	>100
8	Bz(3-PhO)-	Phe-	ProCO-	NH-cHp	0.00080	>100
9	Bz(3-PhO)-	Phe-	ProCO-	NH-cOc	0.00095	>100
10	Z-	Phe-	ProCO-	NH-cHx	0.0012	20
11	Z-	Val-	ProCO-	NH-cHx	0.00050	>100
(7)	Bz(3-PhO)-	Phe-	ProCO-	NH-cHx	0.00065	>100
12	Bz(3-PhO)-	Val-	ProCO-	NH-cHx	0.00050	>100
13	(2-Qui)-	Phe-	ProCO-	NH-cHx	0.0011	>100
14	(2-Qui)-	Val-	ProCO-	NH-cHx	0.00064	>100
15	(2-Qui)-	Cha-	ProCO-	NH-cHx	0.0020	>100
(10)	Z-	Phe-	ProCO-	NH-cHx	0.0012	20
16	Boc-	Phe-	ProCO-	NH-cHx	0.0015	>100
17	Ac-	Phe-	ProCO-	NH-cHx	0.0084	>100
18	Bz-	Phe-	ProCO-	NH-cHx	0.00090	>100
19	(2-The)-	Phe-	ProCO-	NH-cHx	0.0011	100
20	Pic-	Phe-	ProCO-	NH-cHx	0.00085	>100
21	Nap-	Phe-	ProCO-	NH-cHx	0.0017	>100
(13)	(2-Qui)-	Phe-	ProCO-	NH-cHx	0.0011	>100
22	Acr(2-Fur)-	Phe-	ProCO-	NH-cHx	0.0031	>100
(7)	Bz(3-PhO)-	Phe-	ProCO-	NH-cHx	0.00065	>100
23	$cHx(CH_2)_2CO$-	Phe-	ProCO-	NH-cHx	0.00050	>100
24	Ac(PhO)-	Phe-	ProCO-	NH-cHx	0.00080	5.0

a) Abbreviations are defined in scheme 1. b) Cat-B: cathepsin B.

(**18**), 2-naphthoyl (**21**) and 2-furylacryloyl (**22**) instead of urethane-type protective groups (Z; **10** and Boc; **16**) were synthesized. The inhibitory data of these compounds suggest that not only urethane but acyl-type protective groups were preferable for PEP inhibition, and among them Bz was most effective ($IC_{50}=0.9$ ng/ml).

Interestingly, the protecting groups larger than Bz but flexible one such as 3-phenoxybenzoyl (**7**), 3-cyclohexylpropionyl (**23**) and phenoxyacetyl (**24**) indicated strong inhibitory activities against PEP.

Moreover introduction of hetero atom in the P_3-acyl groups showed about the same inhibitory activity against those of parental compounds (**18** *vs.* **19**, **20** and **21** *vs.* **13**). Therefore P_3-protecting group must be widely modifiable, and all these compounds indicated strong IC_{50} value of nano to subnano g/ml as PEP inhibitor and no significant inhibitory activities against cysteine protease, cathepsin B except for **24**. Among them compound **23** showed IC_{50} value of 0.5 ng/ml.

In summary, starting from natural PST ($IC_{50}=0.03$ μg/ml), introduction of pyrrolidine ring in the P_1, exchange of P_1'-P_2' (D-Leu-L-Val) to non-peptidyl cycloalkylamine component, and modification of P_3-P_2 (L-Val-L-Val) to acyl-Phe achieved 10~60 times more enhanced activity than PST for PEP inhibition.

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. ^1H NMR spectra were recorded at 400 MHz, 270 MHz or

90 MHz with a JEOL JNM-GX400, a JNM-EX270 or a Valian EM-390 spectrometer, respectively. FAB-MS spectra were measured on a JEOL JMS-SX102 mass spectrometer. TLC was carried out on Merck precoated silica gel $60F_{254}$ plate. Abbreviations used in the following section were defined in Scheme 1.

Enzyme Assay

Inhibitory activities of PEP and cathepsin B were measured by the procedure described in the previous paper[20].

Synthesis

(*S*)-2-Acetylamino-3-cyclohexylpropionic Acid (**25**)

To a solution of Ac-L-phenylalanine (4.03 g, 19.4 mmol) in MeOH (50 ml) was added 5%Rh-Al_2O_3 (0.41 g). The mixture was hydrogenated at room temperature under 2.5 kg/cm^2 of hydrogen atmosphere in a Parr low-pressure hydrogenator for 23 hours. The catalyst (0.40 g) was added and hydrogenation was continued for 20 hours. After additional hydrogenation (additional catalyst; 0.1 g, for 13 hours) the catalyst was filtered off, and the solvent was evaporated to give **25** as a solid (4.07 g, 98.1%). This solid was recrystallized from EtOH to give needles: Rf 0.64 ($CHCl_3$-MeOH-AcOH, 60:10:3); mp 199~200°C; $[\alpha]_D^{26}$ −4.4° (*c* 1.1, MeOH); FAB-MS *m/z* 212 $(M-H)^-$; ^{1}H NMR (400 MHz, CD_3OD) δ 0.80~1.09 (2H, m, cHx protons), 1.10~1.45 (4H, m, cHx protons), 1.56 (1H, ddd, *J*=4.9, 10.3, 13.7 Hz, β-C*Ha*Hb), *ca.* 1.60~1.85 (6H, m, overlapping, β-CHa*Hb*, cHx protons), 1.97 (1H, s, Ac), 4.42 (1H, dd, *J*=4.9, 10.3 Hz, α-CH).

(*S*)-2-(*t*-Butoxycarbonyl)amino-3-cyclohexylpropionic Acid (Boc-β-cyclohexylalanine (Boc-L-Cha; **26**))

A mixture of **25** (3.82 g, 17.9 mmol) in 7 N HCl (100 ml) was refluxed for 4 hours, and the solvent was evaporated, washed with acetone (20 ml and 10 ml) to give (*S*)-2-amino-3-cyclohexylpropionic acid hydrochloride (**27**) as a solid (3.59 g, 96.6%). This solid was recrystallized from MeOH-EtOAc to give needles: Rf 0.13 ($CHCl_3$-MeOH-AcOH, 60:10:3); mp 235~239°C (dec, transition occured from 196°C); $[\alpha]_D^{26}$ +20.2° (*c* 1.5, MeOH); FAB-MS *m/z* 172 $(M-HCl+H)^+$.

To a solution of **27** (3.00 g, 14.4 mmol) in water (60 ml) and dioxane (90 ml) was added triethylamine (4.25 ml, 30.4 mmol) and di-*t*-butyl dicarbonate (3.47 g, 15.9 mmol) in an ice bath, and stirred at room temperature for 4.5 hours. After evaporation of the solvent, the solid obtained was dissolved in water (30 ml), washed with EtOAc (20 ml), and acidified (pH 2) with 5 N HCl (3.0 ml). The mixture was extracted twice with EtOAc (20 ml), washed with saturated aq NaCl (20 ml), and dried (Na_2SO_4). Evaporation of the solvent gave an amorphous solid of **26**, 3.06 g. Moreover crude **26** (0.92 g) was recovered from the EtOAc layer before acidified, and the crude product was chromatographed on a column of Sephadex LH-20 with MeOH elution to give **26**, 0.86 g (total 3.92 g, 100%): Rf 0.29 ($CHCl_3$-MeOH-AcOH, 95:5:1); mp 40~42°C; $[\alpha]_D^{26}$ −2.7° (*c* 1.1, $CHCl_3$), (**26**·dicyclohexylamine salt was prepared for the specific rotation: $[\alpha]_D^{25}$ +1.9° (*c* 1.0, $CHCl_3$) [lit. $[\alpha]_D^{20}$ +1.58° (*c* 1.01, $CHCl_3$)][29]); FAB-MS *m/z* 270 $(M-H)^-$; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82~1.05 (2H, m, cHx protons), 1.06~1.33 (3H, m, cHx protons), 1.34~1.57 (2H, m, overlapping, β-C*Ha*Hb, cHx protons), 1.45 (9H, s, Boc), 1.58~1.89 (6H, m, β-CHa*Hb*, cHx protons), 4.20 and 4.34 (total 1H, m and br ddd, α-CH (*cis-trans* rotamers of amide bond were observed)), 4.87 and 5.96 (total 1H, d, *J*=7.8 Hz and br s, NH (*cis-trans* rotamers of amide bond were observed)), 8.58 (1H, br, COOH).

(*RS*)-*N*-Cyclohexyl-2-[(*S*)-2-(1-*t*-butoxycarbonylpyrrolidinyl)]-2-hydroxyacetoamide (Boc-H_2ProCO-NH-cHx, **28a**) and its Analogues (**28b**~**28f**)

28a was prepared from (*RS*)-2-[(*S*)-2-(1-*t*-butoxycarbonylpyrrolidinyl)]-2-hydroxyacetic acid and cyclohexylamine in 96.3% yield according to the procedure described in the previous paper[24].

The compounds **28b**~**28f** were prepared by a similar procedure using corresponding amine instead of cyclohexylamine.

(*RS*)-*N*-Cyclopropyl-2-[(*S*)-2-(1-*t*-butoxycarbonylpyrrolidinyl)]-2-hydroxyacetoamide (Boc-H_2ProCO-NH-cPr, **28b**): Yield 83.0%; Rf 0.29, 0.33 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 285 $(M+H)^+$, 229, 211, 185, 170, 114, 70, 57; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.36~0.60 (2H, m, cPr protons), 0.70~0.85 (2H, m, cPr protons), 1.45, 1.48 (4.5H, 4.5H, two s, Boc), 1.59~2.20 (3H, m, CH_2C*Ha*Hb(pyrrolidinyl)), 2.45 (1H, m, CHa*Hb*), 2.68, 2.72 (0.5H, 0.5H, two m, NCH), 3.17~3.57 (2H, m, NCH_2), 3.92, 4.03 (0.5H, m, 0.5H, br t, NC*H*CHOH), 3.92, 4.21 (0.5H, m, 0.5H, d, *J*=7.8 Hz, C*H*OH), 6.11, 6.24 (0.5H, d, *J*=7.8 Hz, 0.5H, br s, OH), 6.96, 7.06 (0.5H, 0.5H, two br s, NH).

(*RS*)-*N*-Cyclopentyl-2-[(*S*)-2-(1-*t*-butoxycarbonylpyrrolidinyl)]-2-hydroxyacetoamide (Boc-H_2ProCO-NH-cPn, **28c**): Yield 93.1%; Rf 0.23, 0.28 (CH_2Cl_2-MeOH, 30:1); FAB-MS *m/z* 313 $(M+H)^+$, 257, 213, 170, 114, 70, 57; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28~2.16 (11H, m, CH_2×4(cPn), CH_2C*Ha*Hb(pyrrolidinyl)), 1.46, 1.48 (4.5H, 4.5H, two s, Boc), 2.48 (1H, m, CHa*Hb*), 3.16~3.47 (2H, m, NCH_2), 3.93, 4.06 (0.5H, m, 0.5H, br t, NC*H*CHOH), 3.93, 4.11~4.27 (0.5H, 1.5H, two m, C*H*OH, NCH), 6.16, 6.20 (0.5H, br d, 0.5H, br s, OH), 6.83, 6.96 (0.5H, 0.5H, two br s, NH).

(*RS*)-*N*-Cycloheptyl-2-[(*S*)-2-(1-*t*-butoxycarbonylpyrrolidinyl)]-2-hydroxyacetoamide (Boc-H_2ProCO-NH-cHp, **28d**): Yield 96.7%; Rf 0.32, 0.41 (CH_2Cl_2-MeOH, 30:1); FAB-MS *m/z* 341 $(M+H)^+$, 241, 170, 114, 70, 57; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32~2.15 (15H, m, CH_2×6(cHp), CH_2C*Ha*Hb(pyrrolidinyl)), 1.46, 1.49 (4.5H, 4.5H, two s, Boc), 2.48(1H, m, CHa*Hb*), 3.17~3.55 (2H, m, NCH_2), 3.83~4.00 (2H, m, NCH, NC*H*C*H*OH (each 0.5H)), 4.07 (0.5H, br t, NC*H*CHOH), 4.20 (0.5H, br d, CHOH), 6.15, 6.16 (0.5H, 0.5H, two

br s, OH), 6.82, 6.96 (0.5H, 0.5H, two br s, NH).

(*RS*)-*N*-Cyclooctyl-2-[(*S*)-2-(1-*t*-butoxycarbonylpyrrolidinyl)]-2-hydroxyacetoamide (Boc-H_2ProCO-NH-cOc, **28e**): Yield 96.3%; Rf 0.41, 0.48 (CH_2Cl_2-MeOH, 30:1); FAB-MS *m/z* 355 $(M+H)^+$, 299, 255, 170, 114, 70, 57; ^1H NMR (400 MHz, $CDCl_3$) δ 1.32~2.15 (17H, m, $CH_2 \times 7$(cOc), CH_2CHa*Hb*(pyrrolidinyl)), 1.46, 1.48 (4.5H, 4.5H, two s, Boc), 2.47 (1H, m, CHa*Hb*), 3.18~3.57 (2H, m, NCH_2), 3.93 (1H, m, NCH), 3.93, 4.06 (0.5H, m, 0.5H, br t, NC*H*CHOH), 3.93, 4.21 (0.5H, m, 0.5H, br d, C*H*OH), 6.12, 6.17 (0.5H, br d, 0.5H, br s, OH), 6.84, 6.96 (0.5H, 0.5H, two br s, NH).

1-{(*RS*)-2-[(*S*)-2-(1-*t*-butoxycarbonylpyrrolidinyl)]-2-hydroxyacetyl}pyrrolidine (Boc-H_2ProCO-N=$(CH_2)_4$, **28f**): Yield 85.9%; Rf 0.36 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 299 $(M+H)^+$, 243, 255, 199, 197, 170, 114, 70, 57.

Boc-L-Phe-H_2ProCO-NH-cHx (**29a**) and its Analogues (**29b~29j**)

To a **28a** (2.157 g, 6.61 mmol) was added 4 N HCl-dioxane (40 ml) in an ice bath, and stirred at room temperature for 1 hour. The solution was evaporated, and the solid obtained was washed with ether (30 ml), and dried to give H_2ProCO-NH-cHx·HCl (**30**), 1.711 g (98.5%).

To the **30** (720.9 mg, 2.74 mmol) was added Boc-L-phenylalanine (763.3 mg, 2.88 mmol) and HOBt (740.6 mg, 5.48 mmol) in DMF (6 ml). Triethylamine (0.403 ml, 2.88 mmol) and EDC·HCl (735.3 mg, 3.84 mmol) was added under ice cooling, and the mixture was stirred in an ice bath for 2 hours and at room temperature for 4 hours. The mixture was diluted with EtOAc (60 ml), and was washed with 4% aq $NaHCO_3$, saturated aq NaCl, 1% aq citric acid and saturated aq NaCl (each 40 ml), and dried (Na_2SO_4). After removal of the solvent, the product was purified by silica gel column chromatography with CH_2Cl_2-MeOH (80:1) to give **29a** as a solid, 1.225 g (94.3%): Rf 0.31, 0.38 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 474 $(M+H)^+$, 418, 374, 247, 227, 192, 164, 100, 70, 57; ^1H NMR (270 MHz, $CDCl_3$) δ 1.02~*ca.* 1.49 (5H, m, overlapping, $CH_2 \times 2$, C*Ha*Hb-(cHx)), 1.37, 1.42 (4.5H, 4.5H, two s, Boc), 1.50~2.49 (9H, m, $CH_2 \times 2$, CHa*Hb*(cHx), $CH_2 \times 2$(pyrrolidinyl)), 2.68, 3.52 (0.5H, 0.5H, two m, NCHaHb), 2.82, 2.91~3.10 (0.5H, dd, J=8.3, 13.5 Hz, 1.5H, m, β-CH_2(Phe)), 3.26 (0.5H, m, NC*Ha*Hb), 3.60~3.86 (1.5H, m, NCHa*Hb*, NCH), 3.79, 4.10~4.50 (0.5H, d, J=8.6 Hz, 1.5H, m, NCHCHCO), 4.68 (1H, m, α-CH(Phe)), 5.22, 5.28 (0.5H, 0.5H, two br d, NH(Phe)), 6.80, 6.92 (0.5H, br d, J=8.3 Hz, 0.5H, br d, J=8.2 Hz, NH), 7.16~7.38 (5H, m, Ph).

The compound **29b** and **29d~29f** were prepared from **28b** and **28d~28f** by a similar procedure. The compound **29c** was prepared from **28c** by a similar procedure except for the deprotection of Boc-group by TFA treatment. The compound **29g~29j** were prepared by a similar procedure using Z-L-Phe, Ac-L-Phe, Z-L-Val and Boc-L-Cha respectively instead of Boc-L-Phe.

Boc-L-Phe-H_2ProCO-NH-cPr (**29b**): Yield 86.6%; Rf 0.18, 0.22 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 432 $(M+H)^+$, 376, 332, 247, 185, 183, 70, 57.

Boc-L-Phe-H_2ProCO-NH-cPn (**29c**): Yield 62.1%; Rf 0.49 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 460 $(M+H)^+$, 404, 360, 247, 211, 192, 164, 70, 57.

Boc-L-Phe-H_2ProCO-NH-cHp (**29d**): Yield 91.3%; Rf 0.46, 0.52 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 488 $(M+H)^+$, 388, 247, 241, 239, 192, 164, 70, 57.

Boc-L-Phe-H_2ProCO-NH-cOc (**29e**): Yield 93.8%; Rf 0.26 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 502 $(M+H)^+$, 446, 402, 255, 253, 247, 239, 192, 164, 70, 57.

Boc-L-Phe-H_2ProCO-N=$(CH_2)_4$ (**29f**): Yield 82.5%; Rf 0.30, 0.34 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 446 $(M+H)^+$, 390, 375, 372, 346, 291, 247, 199, 197, 70, 57.

Z-L-Phe-H_2ProCO-NH-cHx (**29g**): Yield 92.9%; **29g** was subjected to the next step without FAB-MS analysis.

Ac-L-Phe-H_2ProCO-NH-cHx (**29h**): Yield 84.7%; Rf 0.49 (CH_2Cl_2-MeOH, 10:1); FAB-MS *m/z* 416 $(M+H)^+$, 227, 225, 190, 100, 70.

Z-L-Val-H_2ProCO-NH-cHx (**29i**): Yield 86.7%; Rf 0.39, 0.43 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 460 $(M+H)^+$, 361, 333, 227, 91, 70.

Boc-L-Cha-H_2ProCO-NH-cHx (**29j**): Yield 95.8%; Rf 0.31, 0.37 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 480 $(M+H)^+$, 424, 380, 325, 281, 253, 227, 225, 198, 170, 126, 100, 70, 57.

Bzl(4-Cl)NH-Suc-H_2ProCO-NH-cHx (**29k**)

To a solution of succinic anhydride (1.00 g, 9.99 mmol) in dry THF (8 ml) was added triethylamine (1.40 ml, 10.0 mmol), and the solution was treated dropwise with 4-chlorobenzylamine (1.22 ml, 10.0 mmol) in dry THF (8 ml) under ice cooling over a period of 30 minutes. The mixture was stirred for additional 3 hours at room temperature, and the solvent was evaporated. To the mixture was added 0.5 N HCl (50 ml), and the mixture was extracted with EtOAc (40 ml and 20 ml × 2). The combined extracts were washed with 10% aq NaCl (50 ml), and dried (Na_2SO_4). Evaporation of the solvent gave a solid of *N*-(4-chlorobenzyl)succinamic acid (**29ka**) 2.29 g (94.7%): Rf 0.44 ($CHCl_3$-MeOH-AcOH, 90:10:5); mp 145~146.5°C; FAB-MS *m/z* 240 $(M-H)^-$; ^1H NMR (90 MHz, DMSO-d_6) δ 2.43 (4H, t, J=3.2 Hz, $CH_2 \times 2$), 4.26 (2H, d, J=6.0 Hz, Ph(4-Cl)CH_2), 7.20~7.50 (4H, m, aromatic protons), 8.40 (1H, br t, J=6.0 Hz, NH), 12.08 (1H, br s, COOH).

The compound **29k** was prepared from **29ka** (107.7 mg, 0.446 mmol) and **30** (110.6 mg, 0.421 mmol) according to the procedure described for the preparation of **29a**: Yield 89.5%; Rf 0.53 (CH_2Cl_2-MeOH, 10:1); FAB-MS *m/z* 450 $(M+H)^+$, 416, 351, 323, 309, 227, 224, 182, 125, 100, 70.

Bz(3-PhO)-L-Phe-H_2ProCO-NH-cHx (**31a**) and its Analogues (**31b~31l**)

A solution of **29a** (168.9 mg, 0.357 mmol) in TFA

(1.6 ml) was stirred at room temperature for 40 minutes. The solution was evaporated, and the residue was coevaporated twice with toluene (each 2 ml). To the residue was added 3-phenoxybenzoic acid (80.5 mg, 0.376 mmol) and HOBt (96.4 mg, 0.713 mmol) in DMF (2 ml). Triethylamine (60 μl, 0.429 mmol) and EDC·HCl (95.7 mg, 0.499 mmol) was added under ice cooling, and the mixture was stirred in an ice bath for 2 hours and at room temperature for 21 hours. The mixture was diluted with EtOAc (20 ml), and was washed with 4% aq $NaHCO_3$, 1% aq citric acid (this operation was not performed for **31g**, **31h** and **31l**) and saturated aq NaCl (each 10 ml), and dried (Na_2SO_4). After removal of the solvent, the product was purified by silica gel column chromatography with CH_2Cl_2-MeOH (120:1 ~ 100:1) to give **31a** as an amorphous solid, 172.2 mg (84.8%): Rf 0.45, 0.51 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 570 $(M+H)^+$, 344, 316, 227, 197, 70.

The compound **31b** ~ **31f** were prepared from **29b** ~ **29f** by a similar procedure. The compound **31g** ~ **31k** were prepared from **29a** by a similar procedure using quinaldic ((2-Qui)) acid, picolinic (Pic) acid, 2-naphthoic (Nap) acid, 3-(2-furyl)acrylic (Acr(2-Fur)) acid, and 3-cyclohexyl propionic (cHx$(CH_2)_2$CO) acid respectively instead of 3-phenoxybenzoic acid. The compound **31l** were prepared from **29j** by a similar procedure using quinaldic ((2-Qui)) acid instead of 3-phenoxybenzoic acid.

Bz(3-PhO)-L-Phe-H_2ProCO-NH-cPr (**31b**): Yield 79.5%; Rf 0.27, 0.31 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 528 $(M+H)^+$, 344, 316, 197, 185.

Bz(3-PhO)-L-Phe-H_2ProCO-NH-cPn (**31c**): Yield 94.5%; Rf 0.20, 0.25 (CH_2Cl_2-MeOH, 30:1); FAB-MS *m/z* 556 $(M+H)^+$, 344, 316, 213, 197, 70.

Bz(3-PhO)-L-Phe-H_2ProCO-NH-cHp (**31d**): Yield 87.0%; Rf 0.23, 0.28 (CH_2Cl_2-MeOH, 30:1); FAB-MS *m/z* 584 $(M+H)^+$, 344, 316, 241, 197, 70.

Bz(3-PhO)-L-Phe-H_2ProCO-NH-cOc (**31e**): Yield 94.7%; Rf 0.31, 0.37 (CH_2Cl_2-MeOH, 30:1); FAB-MS *m/z* 598 $(M+H)^+$, 344, 316, 255, 253, 197, 70.

Bz(3-PhO)-L-Phe-H_2ProCO-N=$(CH_2)_4$ (**31f**): Yield 69.6%; Rf 0.38, 0.46 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 542 $(M+H)^+$, 344, 316, 199, 197.

(2-Qui)-L-Phe-H_2ProCO-NH-cHx (**31g**): Yield 94.6%; Rf 0.43, 0.46 (CH_2Cl_2-MeOH-Et_3N, 20:1:0.5); FAB-MS *m/z* 529 $(M+H)^+$, 303, 275, 227, 225, 128, 70.

Pic-L-Phe-H_2ProCO-NH-cHx (**31h**): Yield 94.6%; Rf 0.47 (CH_2Cl_2-MeOH-Et_3N, 20:1:0.5); FAB-MS *m/z* 479 $(M+H)^+$, 380, 322, 253, 227, 225.

Nap-L-Phe-H_2ProCO-NH-cHx (**31i**): Yield 93.2%; Rf 0.23 (CH_2Cl_2-MeOH, 40:1); FAB-MS *m/z* 528 $(M+H)^+$, 371, 302, 227, 155, 127, 70.

Acr(2-Fur)-L-Phe-H_2ProCO-NH-cHx (**31j**): Yield 90.7%; Rf 0.40, 0.45 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 494 $(M+H)^+$, 268, 240, 227, 225, 121, 70.

cHx$(CH_2)_2$CO-L-Phe-H_2ProCO-NH-cHx (**31k**): Yield 91.6%; Rf 0.30, 0.36 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 512 $(M+H)^+$, 286, 227, 225, 70.

(2-Qui)-L-Cha-H_2ProCO-NH-cHx (**31l**): Yield 90.7%; Rf 0.50, 0.54 (CH_2Cl_2-MeOH-Et_3N, 20:1:0.5); FAB-MS *m/z* 535 $(M+H)^+$, 309, 281, 227, 225, 156, 128, 70.

(2-Qui)-L-Val-H_2ProCO-NH-cHx (**31m**)

To a solution of **29i** (364.4 mg, 0.793 mmol) in MeOH (4 ml) was added palladium-black catalyst (9.3 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 an amorphous solid, 258.0 mg (L-Val-H_2ProCO-NH-cHx; **29ia**). To the product (130.3 mg, 0.400 mmol) was added quinaldic acid (73.6 mg, 0.425 mmol) and HOBt (108.2 mg, 0.800 mmol) in DMF (2 ml). EDC·HCl (107.5 mg, 0.561 mmol) was added under ice cooling, and the mixture was stirred in an ice bath for 2 hours and at room temperature for 14 hours. The mixture was diluted with EtOAc (20 ml), and was washed with 4% aq $NaHCO_3$ and saturated aq NaCl (each 15 ml), and dried (Na_2SO_4). After removal of the solvent, the product was purified by silica gel column chromatography with CH_2Cl_2-MeOH-Et_3N (120:1:1) to give **31m** as an amorphous solid, 188.4 mg (97.9%): Rf 0.43, 0.46 (CH_2Cl_2-MeOH-Et_3N, 20:1:0.5); FAB-MS *m/z* 481 $(M+H)^+$, 382, 354, 255, 227, 128, 70.

Bz(3-PhO)-L-Val-H_2ProCO-NH-cHx (**31n**)

To the **29ia** (127.7 mg, 0.392 mmol) was added 3-phenoxybenzoic acid (90.7 mg, 0.423 mmol) and HOBt (108.7 mg, 0.804 mmol) in DMF (2 ml). EDC·HCl (107.9 mg, 0.563 mmol) was added under ice cooling, and the mixture was stirred in an ice bath for 2 hours and at room temperature for 5 hours. The mixture was diluted with EtOAc (20 ml), and was washed with 4% aq $NaHCO_3$, 1% aq citric acid and saturated aq NaCl (each 10 ml), and dried (Na_2SO_4). After removal of the solvent, the product was purified by silica gel column chromatography with CH_2Cl_2-MeOH (100:1) to give **31n** as an amorphous solid, 187.9mg (91.8%): Rf 0.42, 0.46 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 522 $(M+H)^+$, 395, 296, 268, 227, 197, 70.

Bz-L-Phe-H_2ProCO-NH-cHx (**31o**) and its Analogues (**31p** and **31q**)

To a **29a** (613.6 mg, 1.30 mmol) was added 4 N HCl-dioxane (10 ml) in an ice bath, and stirred at room temperature for 1 hour. The solution was evaporated, and the solid obtained was washed with ether (10 ml), and dried to give L-Phe-H_2ProCO-NH-cHx·HCl (**29aa**), 521.4 mg (98.2%).

To a solution of **29aa** (138.9 mg, 0.339 mmol) in dry THF (1.4 ml) was added triethylamine (105 μl, 0.750 mmol), and the mixture was treated dropwise with benzoyl chloride (44 μl, 0.379 mmol) in dry THF (3 ml) at room temperature over a period of 30 minutes. The mixture was stirred for additional 3 hours at room temperature, and the solvent was evaporated. To the mixture was added 1 N HCl (6 ml), and the mixture was

extracted with EtOAc (8 ml and 4 ml × 2). The combined extracts were washed with saturated aq $NaHCO_3$ and saturated aq NaCl (each 12 ml), and dried (Na_2SO_4). After removal of the solvent, the product was purified by silica gel column chromatography with CH_2Cl_2-MeOH (100:1~80:1) to give **31o** as an amorphous solid, 150.1 mg (92.8%): Rf 0.50, 0.54 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 478 $(M+H)^+$, 252, 227, 225, 224, 105, 70.

The compound **31p** and **31q** were prepared by a similar procedure using 2-thenoyl (2-The) chloride and phenoxyacetyl (Ac(PhO)) chloride respectively instead of benzoyl chloride.

(2-The)-L-Phe-H_2ProCO-NH-cHx (**31p**): Yield 98.4%; Rf 0.35 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 484 $(M+H)^+$, 258, 230, 227, 225, 111.

Ac(PhO)-L-Phe-H_2ProCO-NH-cHx (**31q**): Yield 96.4%; Rf 0.32, 0.38 (CH_2Cl_2-MeOH, 20:1); FAB-MS *m/z* 508 $(M+H)^+$, 409, 381, 254, 227, 225, 100, 70.

Pfitzner-Moffatt Oxidation (Boc-L-Phe-(*S*)-ProCO-NH-cHx (**16**) and its Analogues (**2**, **10**, **11** and **15**))

A mixture of **29a** (214.5 mg, 0.453 mmol), pyridinium trifluoroacetate (43.8 mg, 0.227 mmol), EDC·HCl (260.5 mg, 1.359 mmol), anhydrous DMSO (2 ml) was stirred at room temperature for 9 hours. The reaction mixture was diluted with EtOAc (20 ml), and the mixture was washed with water (10 ml), and dried (Na_2SO_4). After removal of the solvent, the product was purified by silica gel column chromatography with CH_2Cl_2-MeCN (20:1~10:1) to give an amorphous solid of **16**, 171.3 mg (80.2%): Rf 0.50 ($CHCl_3$-MeOH, 40:1); mp 65~67°C; $[\alpha]_D^{24}$ −26.6° (*c* 1.0, $CHCl_3$); FAB-MS *m/z* 472 $(M+H)^+$, 416, 398, 372, 345, 225, 223, 192, 164, 70, 57; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.08~1.48 (5H, m, $CH_2\times2$, C*Ha*Hb(cHx)), 1.37 (9H, s, Boc), 1.55~2.06 (8H, m, $CH_2\times2$, CHa*Hb*(cHx), CH_2C*Ha*Hb(ProCO)), 2.32 (1H, m, CHa*Hb*(ProCO)), 2.88 (1H, dd, *J*=6.8, 13.7 Hz, β-C*Ha*Hb(Phe)), 3.06 (1H, dd, *J*=7.1, 13.7 Hz, β-CHa*Hb*(Phe)), *ca.* 3.09 (1H, m, overlapping, NC*Ha*Hb), 3.64 (1H, m, NCHa*Hb*), 3.74 (1H, m, NCH(cHx)), 4.64 (1H, ddd, *J*=6.8, 7.1, 8.6 Hz, α-CH(Phe)), 5.23 (1H, d, *J*=8.6 Hz, NH(Phe)), 5.32 (1H, dd, *J*=5.8, 8.4 Hz, NCHCOCO), 6.79 (1H, d, *J*=8.2 Hz, NH), 7.17~7.37 (5H, m, Ph).

The compound **2**, **10**, **11** and **15** were prepared from **29k**, **29g**, **29i** and **31l** by a similar procedure, respectively.

Bzl(4-Cl)-NH-Suc-(*S*)-ProCO-NH-cHx (**2**): Yield 67.9%; Rf 0.35 (CH_2Cl_2-MeOH, 20:1); mp 168~170°C (crystal); $[\alpha]_D^{27}$ −7.4° (*c* 1.0, $CHCl_3$); FAB-MS *m/z* 448 $(M+H)^+$, 321, 225, 125, 70; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.07~1.50 (5H, m, $CH_2\times2$, C*Ha*Hb(cHx)), 1.54~2.11 (8H, m, $CH_2\times2$, CHa*Hb*(cHx), CH_2C*Ha*Hb (ProCO)), 2.33 (1H, m, CHa*Hb*(ProCO)), 2.42~2.82 (4H, m, $CH_2\times2$(Suc)), 3.50~*ca.* 3.70 (2H, m, overlapping, NCH_2), 3.72 (1H, m, N-CH), 4.34 (1H, dd, *J*=5.9, 15.2 Hz, Ph(4-Cl)C*Ha*HbNH), 4.40 (1H, dd, *J*=5.9, 15.2 Hz, Ph(4-Cl)CHa*Hb*NH), 5.28 (1H, dd, *J*=5.1, 9.1 Hz, NCHCOCO), 6.58 (1H, br dd, CH_2N*H*), 6.72 (1H, d, *J*=8.3 Hz, NH), 7.19 (2H, m, aromatic protons), 7.29 (2H, m, aromatic protons).

Z-L-Phe-(*S*)-ProCO-NH-cHx (**10**): Yield 81.2%; FAB-MS *m/z* 506 $(M+H)^+$, 225, 91, 70; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.05~1.50 (5H, m, $CH_2\times2$, C*Ha*Hb(cHx)), 1.54~2.05 (8H, m, $CH_2\times2$, CHa*Hb*(cHx), CH_2C*Ha*Hb (ProCO)), 2.32 (1H, m, CHa*Hb*(ProCO)), 2.91 (1H, dd, *J*=6.9, 13.9 Hz, β-C*Ha*Hb(Phe)), 3.00~3.20 (2H, m, NC*Ha*Hb, β-CHa*Hb*(Phe)), 3.55~3.85 (2H, m, NCHa*Hb*, NCH (cHx)), 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_2OCO), 5.32 (1H, dd, *J*=5.4, 8.1 Hz, NCHCO-CO), 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).

Z-L-Val-(*S*)-ProCO-NH-cHx (**11**): Yield 74.0%; Rf 0.40 (CH_2Cl_2-MeOH, 30:1); mp 55~57°C (amorphous solid); $[\alpha]_D^{24}$ −80.7° (*c* 1.0, $CHCl_3$); FAB-MS *m/z* 458 $(M+H)^+$, 331, 225, 223, 91, 70; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93, 1.04 (6H, two d, each *J*=6.8 Hz, $CH_3\times2$(Val), 1.10~1.45 (5H, m, $CH_2\times2$, C*Ha*Hb (cHx)), 1.56~1.78 (3H, m, CH_2, CHa*Hb*(cHx)), 1.83~2.12 (6H, m, CH_2(cHx), CH_2C*Ha*Hb(ProCO), β-CH(Val)), 2.39 (1H, m, CHa*Hb*(ProCO)), 3.66 (1H, m, NC*Ha*Hb), 3.72 (1H, m, N-CH), 3.85 (1H, m, NCHa*Hb*), 4.34(1H, dd, *J*=6.3, 9.3 Hz, α-CH(Val)), 5.06, 5.09 (2H, ABq, *J*=12.5 Hz, PhCH_2OCO), 5.30 (1H, dd, *J*=7.1, 8.5 Hz, NCHCOCO), 5.40 (1H, d, *J*=9.3 Hz, NH(Val)), 6.73 (1H, br d, *J*=8.3 Hz, NH), 7.24~7.43 (5H, m, Ph).

(2-Qui)-L-Cha-(*S*)-ProCO-NH-cHx (**15**): Yield 74.9%; Rf 0.44 (CH_2Cl_2-MeOH, 30:1); mp 82~84°C (amorphous solid); $[\alpha]_D^{26}$ −49.3° (*c* 1.2, $CHCl_3$); FAB-MS *m/z* 533 $(M+H)^+$, 309, 281, 225, 156, 128, 70; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85~1.56 (11H, m, cHx protons), 1.57~2.17 (15H, m, β-CH_2(Cha), CH_2C*Ha*Hb(ProCO), cHx protons), 2.39 (1H, m, CHa*Hb*(ProCO)), 3.66~3.80 (2H, m, NC*Ha*Hb, N-CH), 3.98 (1H, dt, *J*=6.4, 9.8 Hz, NCHa*Hb*), 5.14 (1H, ddd, *J*=5.4, 9.3, 9.3 Hz, α-CH(Cha)), 5.28 (1H, dd, *J*=6.6, 8.5 Hz, NCHCOCO), 6.75 (1H, d, *J*=8.3 Hz, NH), 7.60, 7.76 (2H, two m, aromatic protons), 7.86, 8.13, 8.25, 8.29 (4H, four d, aromatic protons), 8.71 (1H, d, *J*=9.3 Hz, NH(Cha)).

Albright-Goldman Oxidation (Bz(3-PhO)-L-Phe-(*S*)-ProCO-NH-cHx (**7**) and its Analogues (**4**~**6**, **8**, **9**, **12**~**14** and **17**~**24**))

A mixture of **31a** (155.3 mg, 0.273 mmol), anhydrous DMSO (0.5 ml) and Ac_2O (0.52 ml, 5.50 mmol) was stirred at room temperature for 24 hours. The reaction mixture was diluted with H_2O (15 ml) and stirred for 30 minutes. The mixture was extracted with EtOAc (10 ml × 2), and the mixture was dried (Na_2SO_4). After removal of the solvent, the product was purified by silica gel column chromatography with CH_2Cl_2-MeCN (100:3~50:4) to give an amorphous solid of **7**, 125.1 mg (80.8%): Rf 0.65 (CH_2Cl_2-MeOH, 40:1); mp 73~75°C; $[\alpha]_D^{22}$ −43.6° (*c* 1.1, $CHCl_3$); FAB-MS *m/z* 568

$(M+H)^+$, 441, 344, 316, 225, 197, 70; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.05~1.50 (5H, m, $CH_2\times2$, C*Ha*Hb(cHx)), 1.52~2.04 (8H, m, $CH_2\times2$, CHa*Hb* (cHx), CH_2CHaHb(ProCO)), 2.33 (1H, m, CHa*Hb*(ProCO)), 3.07 (1H, dd, J=6.1, 13.7 Hz, β-C*Ha*Hb(Phe)), *ca.* 3.12 (1H, m, overlapping, NC*Ha*Hb), 3.19 (1H, dd, J=7.1, 13.7 Hz, β-CHa*Hb*(Phe)), 3.64~3.84 (2H, m, NCHa*Hb*, NCH(cHx)), 5.12 (1H, br ddd, α-CH(Phe)), 5.34 (1H, dd, J=6.3, 8.6 Hz, NCHCOCO), 6.80 (1H, d, J=8.6 Hz, NH), 6.90~7.50 (15H, m, Ph×2, phenylene, NH(Phe)).

The compound **4~6**, **8**, **9**, **12~14** and **17~24** were prepared from **31f**, **31b~31e**, **31n**, **31g**, **31m**, **29h**, **31o**, **31p**, **31h~31k** and **31q** by a similar procedure, respectively.

Bz(3-PhO)-L-Phe-(*S*)-ProCO-N=$(CH_2)_4$ (**4**): Yield 32.5%; Rf 0.42 (CH_2Cl_2-MeOH, 40:1); mp 62~64°C (amorphous solid); $[\alpha]_D^{24}$ −54.8° (*c* 0.58, $CHCl_3$); FAB-MS *m/z* 540 $(M+H)^+$, 344, 316, 197, 70; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65~2.19 (7H, m, 4-CH_2(ProCO), 3-C*Ha*Hb(ProCO), $CH_2\times2$(pyrrolidinyl)), 2.38 (1H, m, 3-CHa*Hb*(ProCO)), 3.01 (1H, dd, J=5.9, 13.7 Hz, β-C*Ha*Hb(Phe)), 3.20 (1H, dd, J=6.3, 13.7Hz, β-CHa*Hb*(Phe)), 3.36 (1H, m, NC*Ha*Hb(ProCO)), 3.50, 3.67 (2H, 2H, two m, H_2C-NCH_2), 3.78 (1H, m, NCHa*Hb*(ProCO)), 4.88 (1H, dd, J=6.8, 7.8 Hz, NCHCOCO), 5.13 (1H, ddd, J=5.9, 6.3, 7.8 Hz, α-CH(Phe)), 6.82 (1H, d, J=7.8 Hz, NH), 6.99 (2H, m, aromatic protons), 7.12 (2H, m, aromatic protons), 7.18~7.44 (10H, m, aromatic protons).

Bz(3-PhO)-L-Phe-(*S*)-ProCO-NH-cPr (**5**): Yield 90.4%; Rf 0.44 (CH_2Cl_2-MeOH, 30:1); mp 70~72°C (amorphous solid); $[\alpha]_D^{26}$ −48.6° (*c* 1.5, $CHCl_3$); FAB-MS *m/z* 526 $(M+H)^+$, 441, 344, 316, 197, 183, 70; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.61, 0.84 (4H (each 2H), two m, $CH_2\times2$(cPr)), 1.78~2.05 (3H, m, CH_2CHaHb (ProCO)), 2.32 (1H, m, CHa*Hb*(ProCO)), 2.78 (1H, m, N-CH), 3.06 (1H, dd, J=5.9, 13.7 Hz, β-C*Ha*Hb(Phe)), *ca.* 3.15 (1H, m, overlapping, NC*Ha*Hb), 3.16 (1H, dd, J=7.1, 13.7 Hz, β-CHa*Hb*(Phe)), 3.72 (1H, m, NCHa*Hb*), 5.10 (1H, ddd, J=5.9, 7.1, 7.8 Hz, α-CH(Phe)), 5.30 (1H, dd, J=6.3, 8.3 Hz, NCHCOCO), 6.93 (1H, d, J=2.9 Hz, NH), 6.96~7.05 (3H, m, aromatic protons, NH(Phe)), 7.12 (2H, m, aromatic protons), 7.17~7.46 (10H, m, aromatic protons).

Bz(3-PhO)-L-Phe-(*S*)-ProCO-NH-cPn (**6**): Yield 97.5%; Rf 0.42 (CH_2Cl_2-MeOH, 40:1); mp 70~72°C (amorphous solid); $[\alpha]_D^{28}$ −45.1° (*c* 1.0, $CHCl_3$); FAB-MS *m/z* 554 $(M+H)^+$, 441, 344, 316, 211, 197, 70; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.35~1.80 (6H, m, $CH_2\times3$(cPn)), 1.81~2.15 (5H, m, CH_2(cPn), CH_2CHa-Hb(ProCO)), 2.33 (1H, m, C*Ha*Hb(ProCO)), 3.07 (1H, dd, J=5.9, 13.7 Hz, β-C*Ha*Hb(Phe)), *ca.* 3.12 (1H, m, overlapping, NC*Ha*Hb), 3.18 (1H, dd, J=7.3, 13.7 Hz, β-CHa*Hb*(Phe)), 3.70 (1H, m, NCHa*Hb*), 4.18 (1H, sestet, J=7.0 Hz, N-CH), 5.11 (1H, ddd, J=5.9, 7.3, 8.3 Hz, α-CH(Phe)), 5.33 (1H, dd, J=6.1, 8.5 Hz, NCHCOCO), 6.84 (1H, d, J=7.8 Hz, NH), 6.93 (1H, d, J=8.3 Hz, NH(Phe)), 6.98~7.50 (14H, m, Ph×2, phenylene).

Bz(3-PhO)-L-Phe-(*S*)-ProCO-NH-cHp (**8**): Yield 82.7%; Rf 0.53 (CH_2Cl_2-MeOH, 40:1); mp 70~72°C (amorphous solid); $[\alpha]_D^{24}$ −45.0° (*c* 1.0, $CHCl_3$); FAB-MS *m/z* 582 $(M+H)^+$, 441, 344, 316, 239, 197, 70; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40~1.74 (11H, m, $CH_2\times5$, C*Ha*Hb(cHp)), 1.80~2.06 (4H, m, CHa*Hb*(cHp), CH_2CHaHb(ProCO)), 2.32 (1H, m, CHa*Hb*(ProCO)), 3.07 (1H, dd, J=5.9, 13.7 Hz, β-C*Ha*Hb(Phe)), *ca.* 3.12 (1H, m, overlapping, NC*Ha*Hb), 3.18 (1H, dd, J=7.3, 13.7 Hz, β-CHa*Hb*(Phe)), 3.70 (1H, m, NCHa*Hb*), 3.92 (1H, m, N-CH), 5.11 (1H, ddd, J=5.9, 7.3, 7.6 Hz, α-CH(Phe)), 5.33 (1H, dd, J=5.9, 8.8 Hz, NCHCOCO), 6.84 (1H, d, J=8.3 Hz, NH), 6.93 (1H, br d, NH(Phe)), 6.99 (2H, m, aromatic protons), 7.12 (2H, m, aromatic protons), 7.18~7.44 (10H, m, aromatic protons).

Bz(3-PhO)-L-Phe-(*S*)-ProCO-NH-cOc (**9**): Yield 91.7%; Rf 0.53 (CH_2Cl_2-MeOH, 40:1); mp 67.5~69.5°C (amorphous solid); $[\alpha]_D^{26}$ −45.1° (*c* 1.3, $CHCl_3$); FAB-MS *m/z* 596 $(M+H)^+$, 441, 344, 316, 253, 197, 70; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40~2.05 (17H, m, $CH_2\times7$(cOc), CH_2CHaHb(ProCO)), 2.33 (1H, m, CHa*Hb*(ProCO)), 3.07 (1H, dd, J=5.9, 13.7 Hz, β-C*Ha*Hb(Phe)), *ca.* 3.14 (1H, m, overlapping, NC*Ha*Hb), 3.18 (1H, dd, J=7.3, 13.7 Hz, β-CHa*Hb*(Phe)), 3.70 (1H, m, NCHa*Hb*), 3.96 (1H, m, N-CH), 5.11 (1H, ddd, J=5.9, 7.3, 7.8 Hz, α-CH(Phe)), 5.33 (1H, dd, J=5.9, 8.3 Hz, NCHCOCO), 6.85 (1H, d, J=8.3 Hz, NH), 6.99 (3H, m, aromatic protons, NH(Phe)), 7.12 (2H, m, aromatic protons), 7.18~7.49 (10H, m, aromatic protons).

Bz(3-PhO)-L-Val-(*S*)-ProCO-NH-cHx (**12**): Yield 90.8%; Rf 0.59 (CH_2Cl_2-MeOH, 40:1); mp 70~72°C (amorphous solid); $[\alpha]_D^{27}$ −83.7° (*c* 1.0, $CHCl_3$); FAB-MS *m/z* 520 $(M+H)^+$, 393, 296, 268, 225, 197, 70; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00, 1.09 (6H, two d, each J=6.4 Hz, $CH_3\times2$(Val), *ca.* 1.14~1.46 (5H, m, overlapping, $CH_2\times2$, C*Ha*Hb(cHx)), 1.54~1.82 (3H, m, CH_2, CHa*Hb*(cHx)), 1.83~2.11 (5H, m, CH_2(cHx), CH_2CHaHb(ProCO)), 2.18 (1H, m, β-CH(Val)), 2.40 (1H, m, CHa*Hb*(ProCO)), 3.64~3.81 (2H, m, N-CH, NC*Ha*Hb), 3.96 (1H, dt, J=6.1, 10.3 Hz, NCHa*Hb*), 4.83 (1H, dd, J=6.3, 8.8 Hz, α-CH(Val)), 5.31 (1H, dd, J=7.1, 8.5 Hz, NCHCOCO), 6.75 (1H, d, J=8.3 Hz, NH), 6.93 (1H, d, J=8.8 Hz, NH(Val)), 7.01 (2H, m, aromatic protons), 7.12 (2H, m, aromatic protons), 7.28~7.56 (5H, m, aromatic protons).

(2-Qui)-L-Phe-(*S*)-ProCO-NH-cHx (**13**): Yield 72.4%; Rf 0.37 (CH_2Cl_2-MeOH, 30:1); mp 83~84°C (crystal); $[\alpha]_D^{26}$ −42.1° (*c* 0.86, $CHCl_3$); FAB-MS *m/z* 527 $(M+H)^+$, 303, 275, 225, 128; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12~1.48 (5H, m, $CH_2\times2$, C*Ha*Hb(cHx)), 1.64 (1H, m, CHa*Hb*(cHx)), 1.70~2.06 (7H, m, $CH_2\times2$(cHx), CH_2CHaHb(ProCO)), 2.32 (1H, m, CHa*Hb*(ProCO)), *ca.* 3.14 (1H, m, overlapping, NC*Ha*Hb), 3.15 (1H, dd, J=6.8, 13.7 Hz, β-C*Ha*Hb

(Phe)), 3.28 (1H, dd, J=7.3, 13.7 Hz, β-CHa*Hb*(Phe)), 3.70~3.83 (2H, m, NCHa*Hb*, N-CH), 5.22 (1H, ddd, J=6.8, 7.3, 8.8 Hz, α-CH(Phe)), 5.36 (1H, dd, J=6.1, 8.5 Hz, NCHCOCO), 6.82 (1H, d, J=8.3 Hz, NH), 7.20~7.44 (5H, m, Ph), 7.61, 7.76 (2H, two m, aromatic protons), 7.86, 8.12, 8.20, 8.27 (4H, four d, aromatic protons), 8.89 (1H, d, J=8.8 Hz, NH(Phe)).

(2-Qui)-L-Val-(*S*)-ProCO-NH-cHx (**14**): Yield 90.8%; Rf 0.43 (CH_2Cl_2-MeOH, 30:1); mp 145.5~146.5°C (crystal); $[\alpha]_D^{26}$ −35.6° (*c* 1.0, $CHCl_3$); FAB-MS *m/z* 479 $(M+H)^+$, 352, 255, 227, 225, 128, 70; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07, 1.14 (6H, two d, J=6.4, 6.8 Hz, $CH_3\times2$(Val)), *ca.* 1.15~1.47 (5H, m, overlapping, $CH_2\times2$, C*Ha*Hb(cHx)), 1.60~1.83 (3H, m, CH_2, CHa*Hb*(cHx)), 1.86~2.14 (5H, m, CH_2(cHx), CH_2C*Ha*Hb(ProCO)), 2.30 (1H, m, β-CH(Val)), 2.40 (1H, m, C*Ha*Hb(ProCO)), 3.67~3.85 (2H, m, N-CH, NC*Ha*Hb), 4.01 (1H, dt, J=6.3, 9.8 Hz, NCHa*Hb*), 4.88 (1H, dd, J=7.3, 9.8 Hz, α-CH(Val)), 5.33 (1H, dd, J=6.8, 8.3 Hz, NCHCOCO), 6.78 (1H, d, J=8.3 Hz, NH), 7.60, 7.75 (2H, two m, aromatic protons), 7.86, 8.14, 8.25, 8.29 (4H, four d, aromatic protons), 8.78 (1H, d, J=9.8 Hz, NH(Val)).

Ac-L-Phe-(*S*)-ProCO-NH-cHx (**17**): Yield 69.5%; Rf 0.36 (CH_2Cl_2-MeOH, 20:1); mp 94~96.5°C (amorphous solid); $[\alpha]_D^{25}$ −30.1° (*c* 1.0, $CHCl_3$); FAB-MS *m/z* 414 $(M+H)^+$, 287, 225, 223, 190, 70; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.11~1.48 (5H, m, $CH_2\times2$, C*Ha*Hb(cHx)), 1.55~2.05 (8H, m, $CH_2\times2$, CHa*Hb* (cHx), CH_2C*Ha*Hb(ProCO)), 1.92 (3H, s, Ac), 2.32 (1H, m, CHa*Hb*(ProCO)), 2.94 (1H, dd, J=6.4, 13.7 Hz, β-C*Ha*Hb(Phe)), *ca.* 3.06 (1H, m, overlapping, NC*Ha*-Hb), 3.08 (1H, dd, J=7.3, 13.7 Hz, β-CHa*Hb*(Phe)), 3.46 (1H, m, NCHa*Hb*), 3.75 (1H, m, N-CH), 4.96 (1H, ddd, J=6.4, 7.3, 8.3 Hz, α-CH(Phe)), 5.33 (1H, dd, J=6.1, 8.5 Hz, NCHCOCO), 6.36 (1H, d, J=8.3 Hz, NH(Phe)), 6.78 (1H, d, J=8.3 Hz, NH), 7.15~7.40 (5H, m, Ph).

Bz-L-Phe-(*S*)-ProCO-NH-cHx (**18**): Yield 88.5%; Rf 0.40 (CH_2Cl_2-MeOH, 30:1); mp 78~80°C (amorphous solid); $[\alpha]_D^{26}$ −47.6° (*c* 1.0, $CHCl_3$); FAB-MS *m/z* 476 $(M+H)^+$, 349, 252, 225, 224, 105, 70; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.11~1.48 (5H, m, $CH_2\times2$, C*Ha*Hb (cHx)), 1.55~2.06 (8H, m, $CH_2\times2$, CHa*Hb*(cHx), CH_2C*Ha*Hb(ProCO)), 2.34 (1H, m, CHa*Hb*(ProCO)), 3.10 (1H, dd, J=5.9, 13.7 Hz, β-C*Ha*Hb(Phe)), *ca.* 3.13 (1H, m, overlapping, NC*Ha*Hb), 3.21 (1H, dd, J=7.3, 13.7 Hz, β-CHa*Hb*(Phe)), 3.65~3.83 (2H, m, NCHa*Hb*, N-CH), 5.16 (1H, ddd, J=5.9, 7.3, 7.8 Hz, α-CH(Phe)), 5.35 (1H, dd, J=6.1, 8.5 Hz, NCHCOCO), 6.80 (1H, d, J=8.3 Hz, NH), 6.97 (1H, d, J=7.8 Hz, NH(Phe)), 7.19~7.55 (8H, m, aromatic protons), 7.69 (2H, m, aromatic protons).

(2-The)-L-Phe-(*S*)-ProCO-NH-cHx (**19**): Yield 76.6%; Rf 0.47 (CH_2Cl_2-MeOH, 30:1); mp 88~91°C (amorphous solid); $[\alpha]_D^{27}$ −58.2° (*c* 1.0, $CHCl_3$); FAB-MS *m/z* 482 $(M+H)^+$, 230, 225, 111; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.11~1.48 (5H, m, $CH_2\times2$, C*Ha*Hb(cHx)), 1.55~2.06 (8H, m, $CH_2\times2$, CHa*Hb*(cHx), CH_2C*Ha*Hb (ProCO)), 2.34 (1H, m, CHa*Hb*(ProCO)), 3.10 (1H, dd, J=5.9, 13.7 Hz, β-C*Ha*Hb(Phe)), *ca.* 3.11 (1H, m, overlapping, NC*Ha*Hb), 3.17 (1H, dd, J=7.3, 13.7 Hz, β-CHa*Hb*(Phe)), 3.65~3.81 (2H, m, NCHa*Hb*, N-CH), 5.10 (1H, ddd, J=5.9, 7.3, 8.3 Hz, α-CH(Phe)), 5.38 (1H, dd, J=5.9, 8.8 Hz, NCHCOCO), 6.78 (1H, d, J=8.3 Hz, NH), 6.98 (1H, d, J=8.3 Hz, NH(Phe)), 7.05 (1H, m, aromatic proton), 7.19~7.53 (7H, m, aromatic protons).

Pic-L-Phe-(*S*)-ProCO-NH-cHx (**20**): Yield 68.2%; Rf 0.66 ($CHCl_3$-MeOH-AcOH, 95:5:1); mp 138~139°C (crystal); $[\alpha]_D^{22}$ −53.4° (*c* 1.0, $CHCl_3$); FAB-MS *m/z* 477 $(M+H)^+$, 350, 253, 225; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08~1.50 (5H, m, $CH_2\times2$, C*Ha*Hb(cHx)), 1.52~2.06 (8H, m, $CH_2\times2$, CHa*Hb*(cHx), CH_2C*Ha*Hb(ProCO)), 2.31 (1H, m, CHa*Hb*(ProCO)), 3.08 (1H, dd, J=6.4, 13.7 Hz, β-C*Ha*Hb(Phe)), *ca.* 3.11 (1H, m, overlapping, NC*Ha*Hb), 3.23 (1H, dd, J=7.4, 13.7 Hz, β-CHa*Hb* (Phe)), 3.64~3.84 (2H, m, overlapping, NCHa*Hb*, N-CH), 5.14 (1H, ddd, J=6.4, 7.4, 8.6 Hz, α-CH(Phe)), 5.33 (1H, dd, J=5.9, 8.6 Hz, NCHCOCO), 6.80 (1H, d, J=8.3 Hz, NH), 7.18~7.47 (6H, m, aromatic protons), 7.81 (1H, m, aromatic proton), 8.08 (1H, m, aromatic proton), 8.54 (1H, m, aromatic proton), 8.67 (1H, d, J=8.6 Hz, NH(Phe)).

Nap-L-Phe-(*S*)-ProCO-NH-cHx (**21**): Yield 78.5%; Rf 0.51 (CH_2Cl_2-MeOH, 40:1); mp 181~184°C (crystal); $[\alpha]_D^{23}$ −53.5° (*c* 1.1, $CHCl_3$); FAB-MS *m/z* 526 $(M+H)^+$, 399, 302, 274, 225, 155, 127, 70; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.06~1.50 (5H, m, $CH_2\times2$, C*Ha*Hb(cHx)), 1.54~2.06 (8H, m, $CH_2\times2$, CHa*Hb* (cHx), CH_2C*Ha*Hb(ProCO)), 2.36 (1H, m, CHa*Hb*(Pro-CO)), 3.16 (1H, dd, J=5.6, 13.5 Hz, β-C*Ha*Hb(Phe)), *ca.* 3.17 (1H, m, overlapping, NC*Ha*Hb), 3.26 (1H, dd, J=7.3, 13.5 Hz, β-CHa*Hb*(Phe)), 3.66~3.86 (2H, m, NCHa*Hb*, N-CH), 5.22 (1H, ddd, J=5.6, 7.3, 7.6 Hz, α-CH(Phe)), 5.39 (1H, dd, J=6.3, 8.6 Hz, NCHCOCO), 6.82 (1H, d, J=8.3 Hz, NH), 7.21 (1H, d, J=7.6 Hz, NH(Phe)), *ca.* 7.14~8.31 (12H, m, overlapping, Ph, naphthyl).

Acr(2-Fur)-L-Phe-(*S*)-ProCO-NH-cHx (**22**): Yield 92.4%; Rf 0.40 (CH_2Cl_2-MeOH, 30:1); mp 90~92°C (powder); $[\alpha]_D^{26}$ −53.1° (*c* 1.0, $CHCl_3$); FAB-MS *m/z* 492 $(M+H)^+$, 365, 268, 240, 225, 121, 70; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02~1.46(5H, m, $CH_2\times2$, C*Ha*Hb(cHx)), 1.56~2.04 (8H, m, $CH_2\times2$, CHa*Hb* (cHx), CH_2C*Ha*Hb(ProCO)), 2.32 (1H, m, CHa*Hb*(Pro-CO)), 3.03 (1H, dd, J=5.9, 13.7 Hz, β-C*Ha*Hb(Phe)), *ca.* 3.06 (1H, m, overlapping, NC*Ha*Hb), 3.14(1H, dd, J=7.6, 13.7 Hz, β-CHa*Hb*(Phe)), 3.68 (1H, m, NCHa*Hb*), 3.75 (1H, m, N-CH), 5.08 (1H, ddd, J=5.9, 7.6, 8.3 Hz, α-CH(Phe)), 5.33 (1H, dd, J=5.9, 8.8 Hz, NCHCOCO), 6.25 (1H, d, J=15.6 Hz, olefinic proton), 6.43 (1H, m, aromatic proton), 6.45 (1H, d, J=8.3 Hz, NH(Phe)), 6.52 (1H, m, aromatic proton), 6.79 (1H, d, J=8.3 Hz, NH), 7.20~7.37 (5H, m, aromatic protons), 7.33 (1H, d, J=15.6 Hz, olefinic proton), 7.43 (1H, m, aromatic proton).

cHx$(CH_2)_2$CO-Phe-(*S*)-ProCO-NH-cHx (**23**): Yield

85.9%; Rf 0.35 (CH_2Cl_2 - MeOH, 30 : 1); mp 71~74°C (crystal); $[\alpha]_D^{28}$ −30.0° (*c* 1.0, $CHCl_3$); FAB-MS *m/z* 510 $(M+H)^+$, 383, 286, 258, 225, 223, 70; ^1H NMR (400 MHz, $CDCl_3$) δ 0.77~0.95 (2H, m, cHx protons), 1.05~1.51 (11H, m, cHx protons, CH_2CH_2CONH), 1.55~2.04 (13H, m, cHx protons, CH_2CHa*Hb*(ProCO)), 2.12 (2H, t, CH_2CH_2CONH), 2.32 (1H, m, CHa*Hb*(ProCO)), 2.94 (1H, dd, *J*=6.4, 13.7 Hz, β-C*Ha*Hb(Phe)), *ca.* 3.07 (1H, m, overlapping, NC*Ha*Hb), 3.08 (1H, dd, *J*=7.3, 13.7 Hz, β-CHa*Hb*(Phe)), 3.66 (1H, m, NCHa*Hb*), 3.75 (1H, m, N-CH), 4.97 (1H, ddd, *J*=6.4, 7.3, 7.8 Hz, α-CH(Phe)), 5.32 (1H, dd, *J*=5.9, 8.8 Hz, NCHCOCO), 6.18 (1H, d, *J*=7.8 Hz, NH(Phe)), 6.78 (1H, d, *J*=8.3 Hz, NH), 7.19~7.40 (5H, m, Ph).

Ac(PhO)-L-Phe-(*S*)-ProCO-NH-cHx (**24**): Yield 77.5%; Rf 0.53 (CH_2Cl_2 - MeOH, 30 : 1); mp 77~79°C (crystal); $[\alpha]_D^{23}$ −26.3° (*c* 1.1, $CHCl_3$); FAB-MS *m/z* 506 $(M+H)^+$, 379, 282, 254, 225, 70; ^1H NMR (270 MHz, $CDCl_3$) δ 1.10~1.50 (5H, m, $CH_2\times2$, C*Ha*Hb(cHx)), 1.54~2.05 (8H, m, $CH_2\times2$, CHa*Hb*(cHx), CH_2CHa*Hb*(ProCO)), 2.33 (1H, m, CHa*Hb*(ProCO)), 2.96 (1H, dd, *J*=6.6, 13.9 Hz, β-C*Ha*Hb(Phe)), 3.14 (1H, dd, *J*=6.9, 13.9 Hz, β-CHa*Hb*(Phe)), *ca.* 3.14 (1H, m, overlapping, NC*Ha*Hb), 3.61~3.84 (2H, m, NCHa*Hb*, N-CH), 4.41, 4.43 (2H, ABq, *J*=14.9 Hz, OCH_2CO), 5.06 (1H, ddd, *J*=6.6, 6.9, 8.6 Hz, α-CH(Phe)), 5.33 (1H, dd, *J*=5.9, 8.6 Hz, NCHCOCO), 6.80 (1H, d, *J*=8.3 Hz, NH), *ca.* 6.76~7.40 (11H, m, overlapping, Ph×2, NH(Phe)).

N-(4-Chlorobenzyl)succinamoyl-L-prolinol (**32**)

To the **29ka** (1.021 g, 4.23 mmol) was added L-prolinol (0.430 g, 4.25 mmol) and HOBt (1.142 g, 8.45 mmol) in DMF (10 ml). EDC·HCl (1.135 g, 5.92 mmol) was added under ice cooling, and the mixture was stirred in an ice bath for 2 hours and at room temperature for 6.5 hours. The mixture was diluted with EtOAc (100 ml), and was washed with 4% aq $NaHCO_3$, saturated aq NaCl, 1% aq citric acid and saturated aq NaCl (each 100 ml), and dried (Na_2SO_4). Evaporation of the solvent gave **32** as a syrup, 0.941 g (68.6%): Rf 0.58 ($CHCl_3$ - MeOH - AcOH, 18 : 2 : 1); FAB-MS *m/z* 325 $(M+H)^+$, 307, 224, 184, 125, 102, 70; ^1H NMR (270 MHz, $CDCl_3$) δ 1.52~2.13 (3H, m, C*Ha*HbCH_2(pyrrolidinyl)), 2.47~2.77 (5H, m, CHa*Hb*(pyrrolidinyl), $CH_2\times2$(Suc)), 3.40~3.73 (4H, m, NCH_2, CH_2OH), 4.17 (1H, m, N-CH), 4.36 (1H, dd, *J*=5.6, 14.9 Hz, Ph(4-Cl)C*Ha*Hb), 4.43 (1H, dd, *J*=5.6, 14.9 Hz, Ph(4-Cl)CHa*Hb*), 4.86 (1H, br, OH), 6.43 (1H, br t, NH), 7.14~7.39 (4H, m, aromatic protons).

N-(4-Chlorobenzyl)succinamoyl-L-prolinal (**1**)

A mixture of **32** (0.940 g, 2.89 mmol), pyridinium trifluoroacetate (0.279 g, 1.44 mmol), EDC·HCl (1.665 g, 8.69 mmol), anhydrous DMSO (5 ml) and benzene (5 ml) was stirred at room temperature for 16 hours. The reaction mixture was diluted with EtOAc (50 ml), and the mixture was washed with water (50 ml), and dried (Na_2SO_4). After removal of the solvent, the product was purified by silica gel column chromatography with EtOAc - MeCN (50 : 1~5 : 1) to give an amorphous solid of **1**, 0.527 g (56.4%): Rf 0.53 (EtOAc - MeOH, 9 : 1); FAB-MS *m/z* 323 $(M+H)^+$, 289, 224, 182, 125, 100, 70; ^1H NMR (270 MHz, $CDCl_3$) δ 1.80~2.16 (4H, m, $CH_2\times2$(pyrrolidinyl)), 2.44~2.81 (4H, m, $CH_2\times2$ (Suc)), 3.40~3.71 (2H, m, NCH_2), 4.24~4.50 (3H, m, Ph(4-Cl)CH_2, NCHCOCO), 6.78 (1H, br, NH), 7.20 (2H, m, aromatic protons), 7.28 (2H, m, aromatic protons), 9.41 (1H, s, CHO).

Boc-L-Phe-L-prolinol (**33**)

33 was obtained, in a manner similar to that described in the preparation of **32**, by coupling reaction of Boc-L-Phe (444.9 mg, 1.68 mmol) with L-prolinol (169.0 mg, 1.67 mmol). The product was purified by silica gel column chromatography with (CH_2Cl_2 - MeOH - AcOH, 100 : 2 : 0.3~100 : 4 : 0.3) to give **33** as a syrup, 438.5 mg (75.3%): Rf 0.44 ($CHCl_3$ - MeOH - AcOH, 95 : 5 : 1); FAB-MS *m/z* 349 $(M+H)^+$, 297, 275, 249, 102, 70, 57; ^1H NMR (400 MHz, $CDCl_3$) δ 1.43 (9H, s, Boc), *ca.* 1.43 (1H, m, overlapping, 3-C*Ha*Hb(pyrrolidinyl)), 1.66 (2H, m, 4-CH_2(pyrrolidinyl)), 1.94 (1H, m, 3-CHa*Hb*(pyrrolidinyl)), 2.62 (1H, dt, *J*=7.3, 10.3 Hz, NC*Ha*Hb), 2.96 (1H, dd, *J*=9.3, 12.7 Hz, β-C*Ha*Hb(Phe)), 3.05 (1H, dd, *J*=5.4, 12.7 Hz, β-CHa*Hb*(Phe)), 3.26~3.63 (4H, m, NCHa*Hb*, CH_2OH), 4.16 (1H, m, N-CH), 4.65 (1H, ddd, *J*=5.4, 8.3, 9.3 Hz, α-CH(Phe)), 5.37 (1H, d, *J*=8.3 Hz, NH), 7.18~7.34 (5H, m, Ph).

Bz(3-PhO)-L-Phe-L-prolinol (**34**)

34 was obtained, in a manner similar to that described in the preparation of **31a**, by coupling reaction of trifluoroacetate salt of deprotected **33** (0.606 mmol) with 3-phenoxybenzoic acid (137.0 mg, 0.640 mmol). The product was purified by silica gel column chromatography with (CH_2Cl_2 - MeOH, 100 : 1~80 : 1) to give **34** as an amorphous solid, 232.4 mg (86.3%): Rf 0.40 (CH_2Cl_2 - MeOH, 20 : 1); FAB-MS *m/z* 445 $(M+H)^+$, 344, 316, 197, 102; ^1H NMR (400 MHz, $CDCl_3$) δ 1.45 (1H, m, 3-C*Ha*Hb(pyrrolidinyl)), 1.70 (2H, m, 4-CH_2(pyrrolidinyl)), 1.94 (1H, m, 3-CHa*Hb*(pyrrolidinyl)), 2.68 (1H, dt, *J*=7.3, 10.0 Hz, NC*Ha*Hb), 3.13 (1H, dd, *J*=9.3, 12.7 Hz, β-C*Ha*Hb(Phe)), 3.20 (1H, dd, *J*=5.4, 12.7 Hz, β-CHa*Hb*(Phe)), 3.33~3.55 (2H, m, CH_2OH), 3.67 (1H, dt, *J*=6.3, 10.0 Hz, NCHa*Hb*), 4.18 (1H, m, N-CH), 4.22 (1H, br s, overlapping, OH), 5.13 (1H, ddd, *J*=5.4, 9.3, 9.3 Hz, α-CH(Phe)), 7.01 (2H, m, aromatic protons), 7.13 (3H, m, NH, aromatic protons), 7.21~7.41 (8H, m, aromatic protons), 7.46 (2H, m, aromatic protons).

Bz(3-PhO)-L-Phe-L-prolinal (**3**)

A mixture of **34** (216.4 mg, 0.487 mmol), anhydrous DMSO (2.6 ml) and Ac_2O (0.92 ml, 9.74 mmol) was stirred at room temperature for 22 hours. The reaction mixture was diluted with H_2O (40 ml) and stirred for 1 hour. The mixture was extracted with CH_2Cl_2 (20 ml×3), and the combined extracts were dried (Na_2SO_4). After

removal of the solvent, the product was purified by silica gel column chromatography with hexane - EtOAc (2 : 1 ~ 1 : 1) to give an amorphous solid, 85.5 mg. This solid was purified by silica gel column chromatography with CH_2Cl_2 - EtOAc (40 : 1 ~ 5 : 1) to give an amorphous solid of **3**, 46.4 mg (21.5%): Rf 0.51 (CH_2Cl_2 - EtOAc, 2 : 1); FAB-MS *m/z* 443 $(M+H)^+$, 344, 316, 197; ^1H NMR (400 MHz, $CDCl_3$) δ 1.71 (1H, m, 4-C*Ha*Hb(pyrrolidinyl)), 1.77 ~ 1.93 (2H, m, 4-CHa*Hb*, 3-C*Ha*Hb(pyrrolidinyl)), 1.97 (1H, m, 3-CHa*Hb*(pyrrolidinyl)), 2.98 (1H, m, NC*Ha*Hb), 3.17 (1H, dd, J=7.8, 13.2 Hz, β-C*Ha*Hb(Phe)), 3.20 (1H, dd, J=6.4, 13.2 Hz, β-CHa*Hb*(Phe)), 3.70 (1H, dt, J=6.6, 10.3 Hz, NCHa*Hb*), 4.40 (1H, m, N-C*H*CHO), 5.15 (1H, ddd, J=6.4, 7.8, 7.8 Hz, α-CH(Phe)), 7.01 (2H, m, aromatic protons), 7.13 (3H, m, NH, aromatic protons), 7.18 ~ 7.50 (10H, m, aromatic protons), 9.35 (1H, d, J=2.0 Hz, CHO).

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