Syntheses of HIV-Protease Inhibitors Having a Peptide Moiety Which Binds to GP120

Akira ASAGARASU, Taketo UCHIYAMA, and Kazuo ACHIWA*

School of Pharmaceutical Sciences, University of Shizuoka. 52–1 Yada, Shizuoka 422, Japan.
Received October 13, 1997; accepted December 2, 1997

Some HIV-protease inhibitor derivatives having an *N*-carbomethoxycarbonyl-prolyl-phenylalanine benzyl ester (CPF) moiety as a binding site to gp120 were designed and synthesized. Almost all the compounds bearing CPF on the phenoxyacetyl group showed protease-inhibitory activity. Compounds 25a and 25b, which have the CPF moiety at the *ortho*- and *meta*-positions of the phenoxyacetyl group, respectively, had anti-HIV activity, although the others showed only protease-inhibitory activity. These results suggest that 25b binds to gp120 and inhibits HIV protease.

Key words HIV; protease inhibitor; CPF; gp120

The rapid spread of the acquired immunodeficiency syndrome (AIDS) epidemic has stimulated the search for therapeutic agents to arrest the replication of the causative virus, human immunodeficiency virus (HIV). Infection by HIV was found to be initiated by binding of its envelope protein gp120 to the T-cell surface glycoprotein CD4.[1] The amino acid Ser^{42}–Ser^{49} region of the CD4 V1 domain was reported to be critical for binding to gp120 (Fig.1).[2] We previously synthesized a series of AZT and 2′,3′-dideoxynucleoside (ddN) derivatives bearing *N*-carbomethoxycarbonyl-prolyl-phenylalanine benzyl ester (CPF),[3] which binds selectively to gp120 and inhibits reverse transcriptase.[4] On the other hand, protease is also a key enzyme for viral replication,[5] and it is known to be inhibited by KNI-102 (Fig.2).[6] Thus, we designed and synthesized some peptide derivatives having CPF as a binding site to gp120 linked with KNI-102 as a HIV protease inhibitor.

Results and Discussion

Preparation of Modified Tripeptide Derivatives of HIV Protease Inhibitor and Their Anti-HIV Activities To decide the position at which to introduce CPF, several protease inhibitor derivatives were synthesized. The key compound, *N*-carbobenzoxy-(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid (Z–AHPA) (**1**) (Fig. 2), was prepared from L-Phe according to the reported procedure.[7] The syntheses of the modified protease inhibitors are shown in Chart 1. Compound **10**, which has a hydroxy group on Pro, was synthesized from hydroxyproline in the same way as employed for the preparation of **9**. Compound **11**, bearing a modified *tert*-butyl group, was obtained as follows. Selective protection of the 1-amino group of 1,2-diamino-2-methylpropane was performed with Boc_2O at −40 °C to give **4** in 67% yield. Coupling of **4** and Z–Pro was carried out by the dicyclohexylcarbodiimide (DCC)-HOBt coupling method[8] to give **5** in 73% yield. Stepwise coupling of proline derivative **5**, Z–(2*S*,3*S*)AHPA (**1**) and Z–Asn by the diethyl phosphorocyanidate (DEPC)-triethylamine (TEA) coupling method[9] and deprotection of Z– with Pd/C–H_2 gave **11** in 37% overall yield from **5**. Treatment of **11** with HCl–dioxane gave the amine **12** in a quantitative yield.

The syntheses of protease inhibitor derivatives **13** and **16** modified on the *N*-terminus are summarized in Chart 2. Compound **13** bearing a phenoxyacetyl group was synthesized by deprotection of **9** followed by treatment with phenoxyacetyl chloride and TEA. Nitrophenoxyacetic acids **15a—c** were synthesized by the condensation of *tert*-butyl bromoacetate and the corresponding sodium nitrophenolates, followed by deprotection with trifluoroacetic acid (TFA). They were coupled with deprotected **9** to obtain **16a—c** in 67, 64 and 58% yields, respectively.

Anti-HIV activity was determined in a similar manner to that reported in ref.10 and HIV-protease inhibitory activity was determined by HPLC screening of the enzymatic reaction between HIV-protease expressed from *E. coli* and its specific substrate Ser–Gln–Asn–Tyr/Pro–Ile–Val, which corresponds to p24/p17.[11] Compounds **13** and **16a—c**, which are modified at the *N*-terminus of Asn, were found to have anti-HIV activity as shown in Table 1.

Design and Synthesis of HIV Protease Inhibitor Linked with Binding Unit to gp120 On the basis of the results of the assay (Table 1), we decided to introduce CPF at the

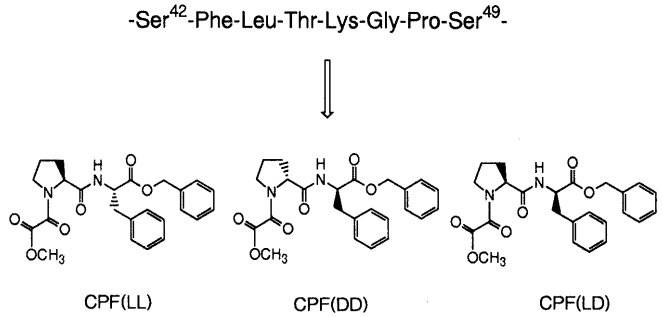


Fig. 1. Structure of CPFs (*N*-Carbomethoxycarbonyl-prolyl-phenylalanine Benzyl Esters)

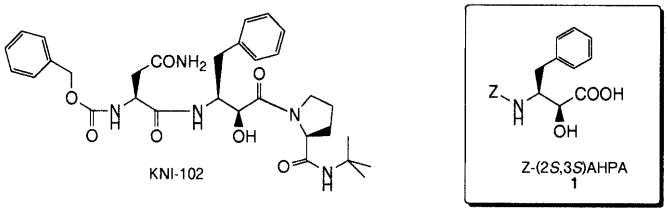


Fig. 2. HIV Protease Inhibitor KNI-102 and the Key Compound Z-(2*S*,3*S*)AHPA

* To whom correspondence should be addressed.

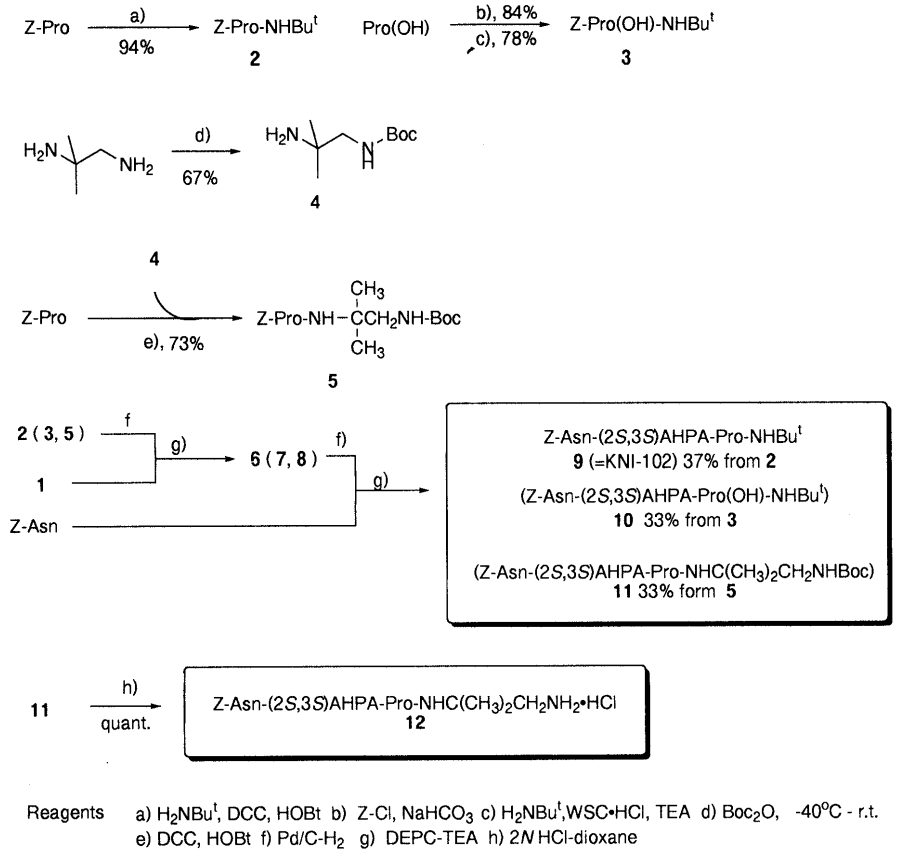


Chart 1. Syntheses of HIV Protease Inhibitor and Its Derivatives

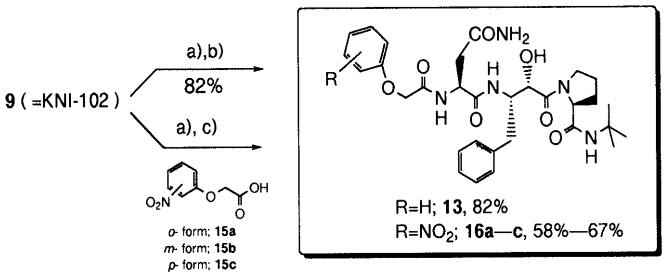
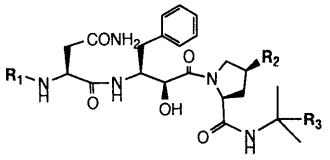


Chart 2. Syntheses of HIV Protease Inhibitor and Its Derivatives Having a Phenoxyacetyl Group Instead of the Z-group

N-terminus of Asn or on the phenoxyacetyl group to induce selective inhibition of HIV-protease on infected cells. As shown in Chart 3, compounds **21**, **22** and **25a—c**, which are modified KNI-102 linked with CPF, were synthesized. Pro–OBzl·HCl was treated with methyloxalyl chloride in the presence of TEA to obtain the Pro derivative **17**. Compounds **21** and **22** bearing the CPF moiety directly at the *N*-terminus of the protease inhibitor unit were synthesized as follows. Debenzylation of **17** with Pd/C-H_2 gave compound **18** in quantitative yield. Deprotection of KNI-102 (**9**) by Pd/C-H_2, followed by condensation with Z–D-Phe (or Z–L-Phe) and then coupling with **18** by the DEPC–TEA coupling method gave **21** and **22** in 53 and 67% overall yields, respectively, from **9**. Then, we synthesized protease inhibitor derivatives bearing the CPF moiety on the phenoxyacetyl group. Reduction of the nitro group of **14a—c**, and subsequent condensation of Z–D-Phe

Table 1. Anti-HIV and HIV Protease-inhibitory Activity of HIV Protease Inhibitors and Derivatives



Compound	R_1[a]	R_2	R_3	EC_{50} (μg/ml)[b]	IC_{50} (ng/ml)[c,d]
9	Z-	H	CH_3	<1.0	N.D.
10	Z-	OH	CH_3	10—30	N.D.
11	Z-	H	CH_2NHBoc	>100	N.D.
12	Z-	H	$CH_2NH_2 \cdot HCl$	>100	N.D.
13	PA-	H	CH_3	4.5—6.0	22
16a	*o*-NO_2-PA-	H	CH_3	6.0	18
16b	*m*-NO_2-PA-	H	CH_3	6.0—10	32
16c	*p*-NO_2-PA-	H	CH_3	4.5	28

a) Z- = benzyloxycarbonyl-, PA- = phenoxyacetyl-. *b*) Anti HIV-activity was determined as reported in ref. 10. *c*) N.D. = not done. *d*) HIV-protease inhibitory activity was determined by HPLC screening of the enzymatic reaction between HIV-protease expressed from *E. coli* and Ser–Gln–Asn–Tyr/Pro–Ile–Val.[11]

by the WSC-HOBt coupling method[12] gave **23a—c** in 63, 87 and 90% yields, respectively. Deprotection of **23a—c** with Pd/C-H_2, followed by coupling with **18** gave **24a—c** in 77, 48 and 61% yields, respectively. After the treatment of **24a—c** with TFA, the resulting carboxylic acids were coupled with deprotected **9** to obtain modified HIV protease inhibitors **25a—c**, which have a CPF moiety on the phenoxyacetyl group, in 63, 44 and 46% yields, re-

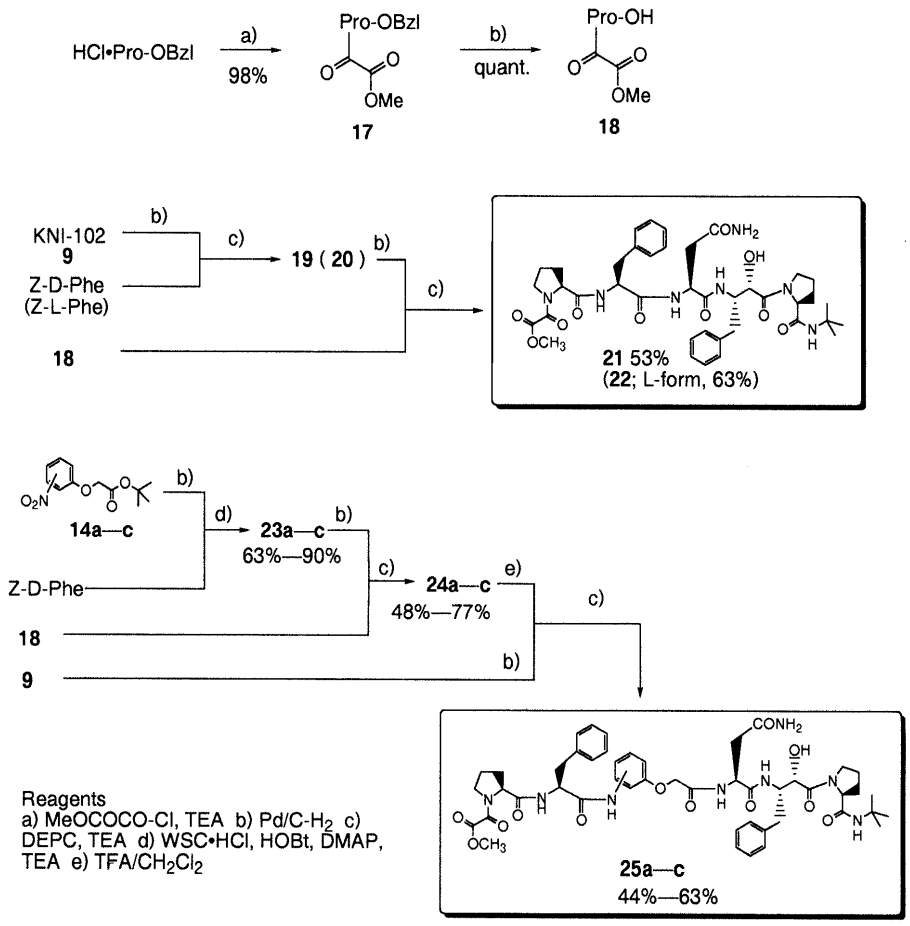


Chart 3. Synthesis of HIV Protease Inhibitor Derivatives Linked with CPF Moiety

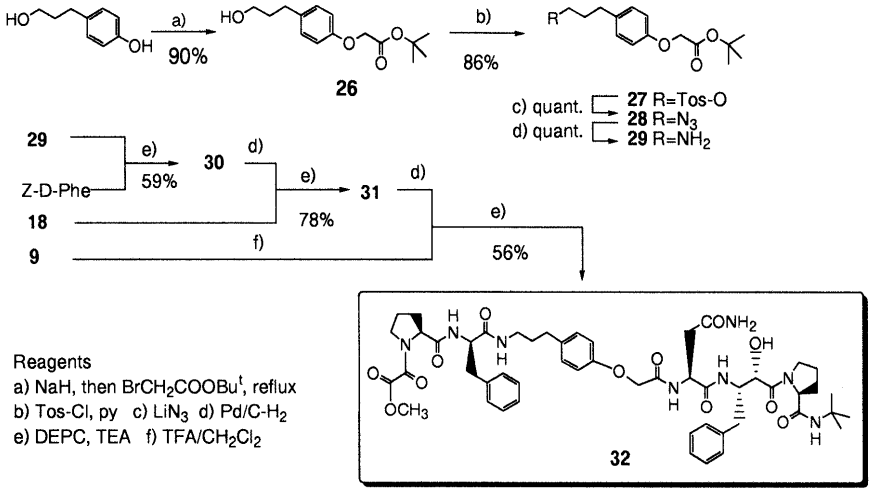
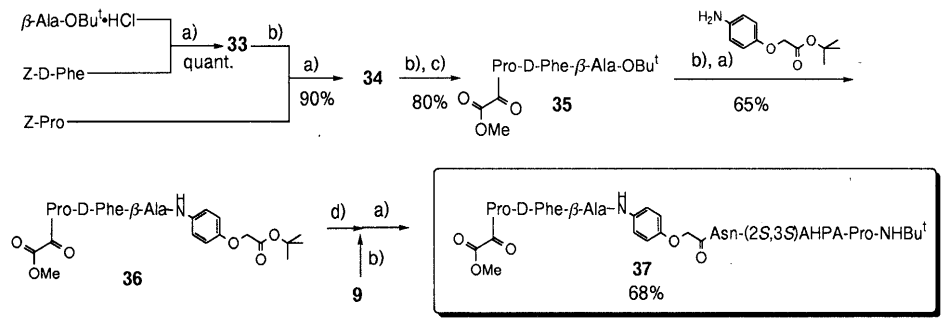


Chart 4. Synthesis of Protease Inhibitor Unit Linked with CPF Derivative *via* a C3 Spacer

spectively.

In order to decrease the influence of CPF on the protease inhibitor unit, we designed some compounds with a spacer between CPF and modified KNI-102. We first synthesized **32** which has a C3 spacer between CPF and the protease inhibitor unit (Chart 4). The reaction of 3-(4-hydroxyphenyl)propanol with NaH and *tert*-butyl bromoacetate gave the phenoxyacetate **26** in 90% yield. Conversion of the alcohol **26** to the amine **29** was easily performed as follows. Tosylation of **26** with Tos-Cl in the presence of TEA, followed by azidation with LiN_3 and reduction with Pd/C-H_2 gave **29** in 84% overall yield from compound **26**. Stepwise coupling of Z–D-Phe and **18** by the method described above, followed by the coupling of **31** and the HIV protease inhibitor unit afforded **32** in 26% yield from **29**.

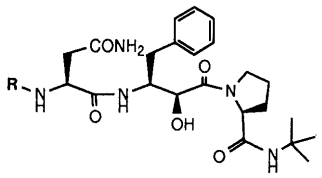
The synthesis of compound **36** having β-Ala as a spacer is illustrated in Chart 5. Stepwise coupling of β-Ala–OBut, Z–D-Phe and Z–L-Pro by the water soluble carbodiimide (WSC)-HOBt method gave **34** in 72% yield. After de-



Reagents a) WSC•HCl, HOBt•H_2O, DMAP, TEA / DMF b) Pd/C-H_2 / MeOH c) MeOCOCO-Cl, TEA / CH_2Cl_2 d) TFA / CH_2Cl_2

Chart 5. Synthesis of HIV-Protease Inhibitor Linked with CPF Moiety *via* a β-Alanine Spacer

Table 2. Anti-HIV and HIV Protease-inhibitory Activity of Protease Inhibitors Having a CPF Derivative as a Binding Unit to gp120



Compound	R[a)]	EC_{50} (μg/ml)[b)]	IC_{50} (ng/ml)[c,d)]
9	Z-	<1.0	N.D.
10	PA-	4.5—6.0	22
21	CPF(LD)-	>100	N.D.
22	CPF(LL)-	>100	N.D.
25a	*o*-(CPF-NH)-PA-	60	>100
25b	*m*-(CPF-NH)-PA-	100	27
25c	*p*-(CPF-NH)-PA-	>100	34
32	*p*-(CPF-NH-C_3)-PA-	>100	N.D.
37	*p*-(CPF-β-Ala)-PA-	>100	N.D.

a) Z- = benzyloxycarbonyl-, PA- = phenoxyacetyl-, CPF- = *N*-carbomethoxy-carbonyl-prolyl-phenylalanyl-, -C_3- = propyl. *b*) Anti HIV-activity was determined as reported in ref. 10. *c*) N.D. = not done. *d*) HIV-protease inhibitory activity was determined by HPLC screening of the enzymatic reaction between HIV-protease expressed from *E. coli* and Ser–Gln–Asn–Tyr/Pro–Ile–Val.[11)]

protection of the tripeptide **34**, the product was treated with methyloxalyl chloride in the presence of TEA in CH_2Cl_2 to give a tripeptide derivative **35** in 80% yield in 2 steps. After deprotection of **35**, the carboxylic acid was coupled with aminophenoxyacetate to afford the CPF derivative **36**. Coupling of KNI-102 and **36** was performed by the described method.

Anti-HIV Activity and Inhibitory Activity against HIV Protease of Modified Protease Inhibitors Linked with CPF The anti-HIV activity and protease inhibitory activity are shown in Table 2. The activities were measured in the same way as described above.[10,11)] The compounds having a CPF moiety, which were modified on the aromatic region of *N*-terminus of Asn, showed relatively low anti-HIV activity. Most of them showed protease-inhibitory activity. The *ortho*- and *meta*- substituted compounds **25a** and **25b** showed anti-HIV activity (**25a**; $EC_{50} = 60\,\mu g/ml$, **25b**; $EC_{50} = 100\,\mu g/ml$), while **25a** had no protease-inhibitory activity. The results suggest that **25a** and **25b** possess anti-HIV activity due to their binding through the CPF moiety.

Experimental

All melting points are uncorrected. Optical rotations were measured with a JASCO DIP-140 digital polarimeter. IR spectra were recorded on a JASCO A-202 infrared spectrophotometer. ^{1}H-NMR spectra were taken on a JEOL JNM-GX 270 (270 MHz) spectrometer. The ^{1}H chemical shifts (δ) are given in ppm relative to that of Me_4Si ($\delta = 0$) in $CDCl_3$ or CD_3OD as an internal standard. Column chromatography was carried out on Silica gel 60 (70—230 mesh, Merck). Thin-layer chromatography (TLC) on Silica gel 60-F_{254} (Merck) was used to monitor the reaction and to ascertain the purity of the reaction products. The spots were visualized by spraying the plates with 10% 12 molybdo(VI)phosphoric acid *n*-hydrate in EtOH solution and then heating. IR spectral data and elemental analysis data support the intermediate structures.

***N*-*tert*-Butyl-1-carbobenzoxy-L-proline Amide (2)** Dicyclohexylcarbodiimide (DCC) (1.99 g, 9.63 mmol) and HOBt (1.30 g, 9.64 mmol) were added to a solution of Z-L-Pro (2.00 g, 8.02 mmol) in *N*,*N*-dimethylformamide (DMF) (20 ml) at 0 °C and then *tert*-butylamine (600 mg, 8.13 mmol) was dropped into the reaction mixture at the same temperature. The whole was stirred overnight at room temperature under an Ar atmosphere. After removal of DMF, the residue was redissolved in CH_2Cl_2, and the mixture was washed with saturated aqueous $NaHCO_3$, 10% aqueous citric acid and brine, then dried ($MgSO_4$), and concentrated *in vacuo*. The residue was chromatographed on silica gel (*n*-hexane : EtOAc = 2 : 1) to give a white powder (2.29 g, 94%). mp 86—88 °C. $[\alpha]_D^{22}$ −90.7° (*c* = 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.30 (s, 9H, *tert*-Bu), 1.60—2.31 (m, 4H, CH_2CH_2), 3.52 (m, 2H, NCH_2), 4.23 (m, 1H, CH), 5.17 (s, 2H, $PhCH_2O$), 7.36 (m, 5H, Ph).

***N*-*tert*-Butyl-1-carbobenzoxy-*trans*-4-hydroxy-L-proline Amide (3)** 1) *N*-Carbobenzoxy-*trans*-4-hydroxy-L-proline: Carbobenzoxy chloride (1.91 g, 11.2 mmol) in Et_2O (50 ml) was added to a solution of *trans*-4-hydroxy-L-proline ((2*S*,4*R*)-(−)-4-hydroxy-2-pyrrolidinecarboxylic acid, 2.00 g, 15.3 mmol) and $NaHCO_3$ (3.33 g, 39.6 mmol) in water at 0 °C. The mixture was stirred for 2 h, then $NaHCO_3$ (1.67 g, 19.9 mmol) and carbobenzoxy chloride (1.91 g, 11.2 mmol) were added at the same temperature and the whole was stirred for 3 h at room temperature. The mixture was washed with Et_2O, and the aqueous layer was acidified with 6 N HCl, and extracted with EtOAc. The organic layer was dried ($MgSO_4$), and concentrated *in vacuo* to give a colorless oil (3.40 g, 84%). $[\alpha]_D^{22}$ −55.8° (*c* = 1.60, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 2.09—2.25 (m, 2H, CH_2CHOH), 3.56 (m, 2H, NCH_2), 4.46 (m, 2H, NCH, CHOH), 5.10 (br s, 2H, OCH_2Ph), 7.26—7.32 (m, 5H, Ar-H).

2) *N*-*tert*-Butyl-1-carbobenzoxy-*trans*-4-hydroxy-L-proline Amide (**3**): White powder. mp 121—123 °C. $[\alpha]_D^{22}$ −80.5° (*c* = 1.0, $CHCl_3$). FAB-MS (*m*/*z*) 321 $(M+H)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (s, 9H, *tert*-Bu), 2.05—3.06 (m, 2H, CH_2CHOH), 3.55 (br s, 1H, CHOH), 4.30, 4.46 (each br s, 2H, NCH, CHOH), 5.15 (br s, 2H, OCH_2Ph), 7.32 (br s, 5H, Ar-H).

1-*N*-*tert*-Butoxycarbonyl-1,2-propyldiamine (4) Di-*tert*-butyl dicarbonate (500 mg, 2.29 mmol) was added to a solution of 1,2-diamino-2-methylpropane (168 mg, 1.91 mmol) in CH_2Cl_2 (20 ml) at −40 °C under a Ar atmosphere. The mixture stirred for 2 h at the same temperature and 1 h at room temperature, then concentrated *in vacuo*. The residue was taken up in CH_2Cl_2 and the solution was washed with 10% aqueous citric acid. The aqueous layer was alkalified with 6 N NaOH and extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), and

concentrated *in vacuo* to give a white powder (225 mg, 67%). mp 65—67 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.09 (s, 6H, C $(CH_3)_2$–), 1.45 (s, 9H, *tert*-Bu), 3.00 (br d, J=6.2 Hz, 2H, $\underline{CH_2}NH$).

***N*-{2-[(*tert*-Butoxycarbonyl)amino]-1,1-dimethylethyl}-1-carbobenzoxy-L-proline Amide (5)** White powder. $[\alpha]_D^{22}$ −29.4° (c=1.4, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.26, 1.27 (each br s, 6H, –C$(CH_3)_2$–), 1.43 (s, 9H, *tert*-Bu), 1.78—2.17 (m, 4H, $CH_2\underline{CH_2}$), 3.15—3.54 (m, 4H, $\underline{CH_2}NH$, Pro; NCH_2), 4.11 (m, 1H, NCHCO), 5.18 (br s, 2H, $O\underline{CH_2}Ph$), 7.27—7.36 (m, 5H, Ar-H).

***N*-Carbobenzoxy-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-*N*-*tert*-butyl-L-proline Amide (6)** A solution of **2** (608 mg, 2.00 mmol) and Pd/C (300 mg) in MeOH (50 ml) was stirred for 6 h under an H_2 atmosphere. After filtration and evaporation, the residue was dissolved in DMF (20 ml). To this solution, **1** (530 mg, 1.61 mmol), DEPC (326 mg, 1.98 mmol) and TEA (202 mg, 2.00 mmol) were added at 0 °C under an Ar atmosphere. The mixture was stirred for 16 h at room temperature. After usual work-up, the residue was chromatographed on silica gel (*n*-hexane : EtOAc = 1 : 2) to give a white powder (519 mg, 67%). mp 73—75 °C. $[\alpha]_D^{22}$ −30.3° (c=1.0, $CHCl_3$). FAB-MS (m/z): 482 $(M+H)^+$, 504 $(M+Na)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (br s, 9H, *tert*-Bu), 1.87—2.33 (m, 4H, $CH_2\underline{CH_2}$), 2.70 (m, 2H, $\underline{CH_2}Ph$), 3.76 (m, 2H, Pro; NCH_2), 4.16—4.59 (m, 3H, CHCH, Pro; CH), 5.00 (br s, 2H, $O\underline{CH_2}Ph$), 5.49 (br d, J=8.8 Hz, 1H, $\underline{NH}$*tert*-Bu), 6.47 (s, 1H, $\underline{NH}$CH–), 7.10—7.32 (m, 10H, Ar-H).

7 (hydroxyproline type compound), white powder. mp 88—91 °C. $[\alpha]_D^{22}$ −17.1° (c=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.29 (br s, 9H, *tert*-Bu), 2.03—2.35 (m, 2H, Pro(OH); $\underline{CH_2}$CHOH), 2.68 (m, 2H, $\underline{CH_2}Ph$), 3.68—3.88 (m, 2H, Pro(OH); NCH_2), 4.10 (m, 1H, Pro(OH); NCHCO), 4.43—4.54 (m, 3H, NHCHCHCO, Pro(OH); CHOH), 4.96 (br s, 2H, $O\underline{CH_2}Ph$), 5.42 (br s, 1H, NHCO), 6.48 (s, 1H, $\underline{NH}$*tert*-Bu), 7.12—7.31 (m, 10H, Ar-H).

***N*-Carbobenzoxy-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-*N*-{2-[(*tert*-butoxycarbonyl)amino]-1,1-dimethylethyl}-L-proline Amide (8)** Colorless oil. $[\alpha]_D^{22}$ +32.1° (c=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.24, 1.30 (each br s, 6H, C$(CH_3)_2$), 1.43 (s, 9H, *tert*-Bu), 1.78—2.17 (m, 4H, $CH_2\underline{CH_2}$), 2.69 (m, 2H, $\underline{CH_2}Ph$), 3.28 (m, 2H, C$\underline{CH_2}$NH), 3.76 (m, 2H, Pro; NCH_2), 4.08—4.31 (m, 2H, NHCHCHCO), 4.58 (m, 1H, Pro; CH), 4.99 (br s, 2H, $O\underline{CH_2}Ph$), 7.17—7.34 (m, 10H, Ar-H).

***N*-Carbobenzoxy-L-asparagyl-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-*N*-*tert*-butyl-L-proline Amide (9)** White powder. mp 107—109 °C. $[\alpha]_D^{22}$ −37.6° (c=1.0, $CHCl_3$). FAB-MS (m/z): 596 $(M+H)^+$, 618 $(M+Na)^+$. IR (KBr) cm^{-1}: 3340, 1700, 1660. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (s, 9H, *tert*-Bu), 1.86—2.24 (m, 4H, Pro; $CH_2\underline{CH_2}$), 2.54—2.79 (m, 4H, Asn; $\underline{CH_2}CONH_2$, CH$\underline{CH_2}$Ph), 3.60 (br s, 2H, Pro; NCH_2), 4.23—4.42 (m, 4H, Pro; CH, Asn; CH, AHPA; CHCH), 5.07 (s, 2H, $O\underline{CH_2}Ph$), 7.17—7.33 (m, 10H, Ar-H).

10 (hydroxyproline type compound), white powder. mp 118—121 °C. $[\alpha]_D^{22}$ +13.1° (c=1.0, $CHCl_3$). FAB-MS (m/z): 612 $(M+H)^+$, 634 $(M+Na)^+$. IR (KBr) cm^{-1}: 3410, 1700, 1660. ^{1}H-NMR ($CDCl_3$) δ: 1.35 (s, 9H, *tert*-Bu), 1.78—2.04 (m, 2H, Pro(OH); $\underline{CH_2}$CHOH), 2.38—2.78 (m, 4H, Asn; $\underline{CH_2}CONH_2$, CH$\underline{CH_2}$Ph), 3.48—3.73 (br s, 3H, Pro(OH); NCH_2, $\underline{CH}$(OH)), 4.29—4.48 (m, 3H, Asn; CH, AHPA; CHCH), 4.85—5.16 (m, 3H, Pro(OH); CH, $O\underline{CH_2}Ph$), 7.11—7.37 (m, 10H, Ar-H).

***N*-Carbobenzoxy-L-asparagyl-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-*N*-{2-[(*tert*-butoxycarbonyl)amino]-1,1-dimethylethyl}-L-proline Amide (11)** White powder. mp 100—103 °C. $[\alpha]_D^{22}$ +15.6° (c=1.0, $CHCl_3$). FAB-MS (m/z): 711 $(M+H)^+$, 733 $(M+Na)^+$. IR (KBr) cm^{-1}: 3500—3200, 2980, 1660. ^{1}H-NMR ($CDCl_3$) δ: 1.24—1.30 (m, 6H, CH_3), 1.42 (s, 9H, *tert*-Bu), 1.85—2.17 (m, 4H, Pro; $CH_2\underline{CH_2}$), 2.60—2.80 (m, 4H, Asn; $\underline{CH_2}CONH_2$, CH$\underline{CH_2}$Ph), 3.28 (m, 2H, C$\underline{CH_2}$NH–), 3.69 (m, 2H, Pro; NCH_2), 4.16—4.37 (m, 4H, Asn; CH, AHPA; CHCH, Pro; CH), 5.07 (br s, 2H, $O\underline{CH_2}Ph$), 7.13—7.33 (m, 10H, Ar-H).

***N*-Carbobenzoxy-L-asparagyl-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-*N*-(2-amino-1,1-dimethylethyl)-L-proline Amide Hydrochloride (12)** Compound **11** (70 mg, 0.10 mmol) was dissolved in 2 N HCl in dioxane (10 ml) under an Ar atmosphere. The mixture was stirred for 2 h at room temperature, then concentrated to give a white powder (62 mg, 98%). mp 114—117 °C. $[\alpha]_D^{22}$ −16.8° (c=0.8, $CHCl_3$). IR (KBr) cm^{-1}: 3320, 1670. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 1.37, 1.44 (each br s, 6H, CH_3), 1.90—2.20 (m, 4H, Pro; $CH_2\underline{CH_2}$), 2.60 (br s, 2H, CH$\underline{CH_2}$Ph), 2.86 (br s, 2H, Asn; CH_2), 3.28 (m, 2H, C$\underline{CH_2}$NH–), 3.69 (m, 2H, Pro; NCH_2), 4.16—4.37 (m, 4H, Asn; CH, AHPA; CHCH, Pro; CH), 5.07 (br s, 2H, $O\underline{CH_2}Ph$), 7.17—7.38 (m, 10H, Ar-H).

Phenoxyacetyl-L-asparagyl-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-*N*-*tert*-butyl-L-proline Amide (13) A solution of **9** (190 mg, 0.32 mmol) and Pd/C (110 mg) in MeOH (20 ml) was stirred for 14 h under an H_2 atmosphere. After filtration and evaporation, the residue was redissolved in CH_2Cl_2 (15 ml), then TEA (32 mg, 0.32 mmol) and phenoxyacetyl chloride (54 mg, 0.32 mmol) were added at 0 °C. The reaction mixture was stirred for 1.5 h at the same temperature. After usual work-up, the residue was chromatographed on silica gel (CH_2Cl_2 : MeOH = 15 : 1) to give a white powder (156 mg, 82%). mp 94—97 °C. $[\alpha]_D^{20}$ +36.2° (c=1.0, $CHCl_3$). FAB-MS (m/z): 596 $(M+H)^+$, 618 $(M+Na)^+$. IR (KBr) cm^{-1}: 3340, 1690, 1660. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (s, 9H, *tert*-Bu), 1.90—2.28 (m, 4H, Pro; $CH_2\underline{CH_2}$), 2.54—2.78 (m, 4H, Asn; CH_2, AHPA; $\underline{CH_2}Ph$), 3.64 (m, 2H, Pro; NCH_2), 4.15—4.49 (m, Asn; CH, AHPA; CHCH, Pro; CH), 4.74 (s, OCH_2CO), 6.92—7.48 (m, 10H, Ar-H).

***tert*-Butyl (2-Nitrophenoxy)acetate (14a)** A solution of 2-nitrophenol (2.00 g, 14.38 mmol) in tetrahydrofuran (THF) (10 ml) was added to a solution of NaH (414 mg, 17.3 mmol) in THF (10 ml), and the mixture was stirred for 0.5 h at room temperature under an Ar atmosphere. *tert*-Butyl bromoacetate (3.37 g, 17.3 mmol) was added, and the reaction mixture was heated under reflux for 5—7 d. After filtration, the filtrate was concentrated *in vacuo*. The residue was redissolved in CH_2Cl_2, and the mixture was washed with saturated aqueous $NaHCO_3$, 10% aqueous citric acid and brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was chromatographed on silica gel (*n*-hexane : EtOAc = 3 : 1) to give yellow plates (3.50 g, 96%). mp 47 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (s, 9H, *tert*-Bu), 4.67 (s, 2H, OCH_2CO), 6.98 (d, J=8.3 Hz, 1H, Ar-H_6), 7.11 (m, 1H, Ar-H_4), 7.55 (m, 1H, Ar-H_5), 8.88 (d, J=7.9 Hz, 1H, Ar-H_3).

14b (*m*-form compound), white needles. mp 47—49 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.50 (s, 9H, *tert*-Bu), 4.61 (s, 2H, OCH_2CO), 7.24—7.89 (m, 4H, Ar-H).

14c (*p*-form compound), white needles. mp 84 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.49 (s, 9H, *tert*-Bu), 4.62 (s, 2H, OCH_2CO), 6.69 (d, J=9.2 Hz, 2H, Ar-$H_{2,6}$), 8.22 (d, J=9.2 Hz, 2H, Ar-$H_{3,5}$).

(2-Nitrophenoxy)acetic Acid (15a) A solution of **14a** (1.00 g, 3.95 mmol) in CH_2Cl_2 (12 ml) was treated with TFA (4 ml) and the mixture was stirred for 6 h. Evaporation and recrystallization gave colorless plates (760 mg, 98%). mp 156 °C. ^{1}H-NMR ($CDCl_3$) δ: 4.83(s, 2H, OCH_2CO), 7.08 (d, J=8.2 Hz, 1H, Ar-H_6), 7.19 (t, J=8.3 Hz, 1H, Ar-H_4), 7.61 (t, J=8.3 Hz, 1H, Ar-H_5), 8.01 (d, J=8.2 Hz, 1H, Ar-H_3).

15b (*m*-form compound), colorless needles. mp 152 °C. ^{1}H-NMR ($CDCl_3$) δ: 4.79 (s, 2H, OCH_2CO), 7.30 (d, J=6.3 Hz, 1H, Ar-H_6), 7.49 (t, J=8.3 Hz, 1H, Ar-H_5), 7.76 (s, 1H, Ar-H_2), 7.92 (d, J=7.9 Hz, 1H, Ar-H_4).

15c (*p*-form compound), colorless prisms. mp 189 °C. ^{1}H-NMR ($CDCl_3$) δ: 4.80 (s, 2H, OCH_2CO), 7.02 (d, J=9.2 Hz, 2H, Ar-$H_{2,6}$), 8.26 (d, J=9.2 Hz, 2H, Ar-$H_{3,5}$).

(2-Nitrophenoxy)acetyl-L-asparagyl-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-*N*-*tert*-butyl-L-proline Amide (16a) Yellow powder. mp 104—107 °C. $[\alpha]_D^{24}$ −41.5° (c=1.0, $CHCl_3$). FAB-MS (m/z): 641 $(M+H)^+$, 663 $(M+Na)^+$. IR (KBr) cm^{-1}: 3330, 1662. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (s, 9H, *tert*-Bu), 1.87—2.26 (m, 4H, Pro; $CH_2\underline{CH_2}$), 2.61—2.80 (m, 4H, Asn; CH_2, AHPA; $\underline{CH_2}Ph$), 3.66 (t, J=6.7 Hz, 2H, Pro; NCH_2), 4.43—4.50 (m, 4H, Asn; CH, AHPA; CHCH, Pro; CH), 4.65 (s, 2H, OCH_2CO), 7.08—8.84 (m, 9H, Ar-H).

16b (*m*-form compound), yellow powder. mp 104—107 °C. $[\alpha]_D^{24}$ −30.9° (c=1.0, $CHCl_3$). FAB-MS (m/z): 641 $(M+H)^+$, 663 $(M+Na)^+$. IR (KBr) cm^{-1}: 3332, 1664, 1528, 1350. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (s, 9H, *tert*-Bu), 1.77—2.20 (m, 4H, Pro; $CH_2\underline{CH_2}$), 2.64 (m, 2H, Asn; CH_2), 2.82 (m, 2H, AHPA; $\underline{CH_2}Ph$), 3.70 (m, 2H, Pro; NCH_2), 4.27—4.79 (m, 4H, Asn; CH, AHPA; CHCH, Pro; CH), 4.61 (s, 2H, OCH_2CO), 7.00—7.86 (m, 9H, Ar-H).

16c (*p*-form compound), white powder. mp 106—108 °C. $[\alpha]_D^{24}$ −23.3° (c=1.0, $CHCl_3$). FAB-MS (m/z): 641 $(M+H)^+$, 663 $(M+Na)^+$. IR (KBr) cm^{-1}: 3330, 1660, 1520. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (s, 9H, *tert*-Bu), 1.85—2.30 (m, 4H, Pro; $CH_2\underline{CH_2}$), 2.61 (m, 2H, Asn; CH_2), 2.90 (m, 2H, AHPA; $\underline{CH_2}Ph$), 3.70 (m, 2H, Pro; NCH_2), 4.35—4.50 (m, 4H, Asn; CH, AHPA; CHCH, Pro; CH), 4.61 (s, 2H, OCH_2CO), 6.94—8.23 (m, 9H, Ar-H).

***N*-Methoxalyl-L-proline Benzyl Ester (17)** TEA (500 mg, 4.96 mmol) and methyloxalyl chloride (669 mg, 5.46 mmol) were added to a solution of H–L-Pro–OBzl · HCl (1.20 g, 4.96 mmol) in CH_2Cl_2 (30 ml) at 0 °C and the mixture was stirred for 0.5 h at 0 °C and 1 h at room temperature under an Ar atmosphere. After usual work-up, the residue was

chromatographed on silica gel (CH_2Cl_2 : MeOH = 15 : 1) to give a colorless oil (1.42 g, 98%). $[\alpha]_D^{22}$ +59.8° (c = 1.02, $CHCl_3$). FAB-MS (m/z): 292 $(M+H)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.85—2.35 (m, 4H, CH_2CH_2), 3.87 (s, 3H, OCH_3), 3.64—3.90 (m, 2H, NCH_2), 4.96 (dd, J = 8.5, 3.3 Hz, 1H, NCHCO), 5.18 (br s, 2H, $O\underline{CH_2}Ph$), 7.30—7.40 (m, 5H, Ar-H).

***N*-Methoxalyl-L-proline (18)** A solution of **17** (300 mg, 1.03 mmol) and Pd/C (200 mg) in MeOH (20 ml) was stirred for 4 h under an H_2 atmosphere. Filtration and evaporation gave a white powder (202 mg, 98%).

***N*-Carbobenzoxy-D-phenylalanyl-L-asparagyl-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-*N*-*tert*-butyl-L-proline Amide (19)** White powder. mp 93—95 °C. $[\alpha]_D^{22}$ −27.5° (c = 1.0, $CHCl_3$). FAB-MS (m/z): 743 $(M+H)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (s, 9H, *tert*-Bu), 1.84—2.12 (m, 4H, Pro; CH_2CH_2), 2.24 (m, 2H, Asn; CH_2), 2.57—3.07 (m, 4H, Phe; $\underline{CH_2}Ph$, AHPA; $\underline{CH_2}Ph$), 3.61 (m, 2H, Pro, NCH_2), 4.08—4.57 (m, 5H, AHPA; CHCH, Phe; CH, Asn; CH, Pro; CH), 5.06 (br s, 2H, $O\underline{CH_2}Ph$), 7.33 (m, 15H, Ar-H).

20 (using L-phenylalanine), white powder. mp 103—106 °C. $[\alpha]_D^{22}$ −32.3° (c = 1.0, $CHCl_3$). FAB-MS (m/z): 743 $(M+H)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (s, 9H, *tert*-Bu), 1.91 (m, 4H, Pro; CH_2CH_2), 2.10—2.25 (m, 2H, Asn; CH_2), 2.54—3.10 (m, 4H, Phe; $\underline{CH_2}Ph$, AHPA; $\underline{CH_2}Ph$), 3.60 (m, 2H, Pro, NCH_2), 4.39—4.63 (m, 5H, AHPA; CHCH, Phe; CH, Asn; CH, Pro; CH), 5.00 (br s, 2H, OCH_2Ph), 7.11—7.29 (m, 15H, Ar-H).

***N*-Methoxalyl-L-prolyl-D-phenylalanyl-L-asparagyl-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-*N*-*tert*-butyl-L-proline Amide (21)** White powder. mp 117—119 °C. $[\alpha]_D^{22}$ −27.7° (c = 1.0, $CHCl_3$). FAB-MS (m/z): 792 $(M+H)^+$. IR (KBr) cm^{-1}: 3380, 1740, 1670, 1650. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (s, 9H, *tert*-Bu), 1.82—2.26 (m, 8H, Pro; CH_2CH_2), 3.56, 3.76 (m, 4H, Pro; NCH_2), 3.83 (br s, 3H, OCH_3), 4.26—4.60 (m, 6H, AHPA; CHCH, Phe; CH, Asn; CH, Pro; CH), 7.15—7.27 (m, 10H, Ar-H).

22 (using L-phenylalanine), white powder. mp 112—114 °C. $[\alpha]_D^{22}$ −68.7° (c = 1.0, $CHCl_3$). FAB-MS (m/z): 792 $(M+H)^+$. IR (KBr) cm^{-1}: 3360, 1740, 1660, 1650. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (s, 9H, *tert*-Bu), 1.87—2.32 (m, 8H, Pro; CH_2CH_2), 3.68 (m, 4H, Pro; NCH_2), 3.87 (s, 3H, OCH_3), 4.25—4.68 (m, 6H, AHPA; CHCH, Phe; CH, Asn; CH, Pro; CH), 7.14—7.26 (m, 10H, Ar-H).

***tert*-Butyl [2-(*N*-Carbobenzoxy-D-phenylalaninamido)phenoxy]acetate (23a)** A solution of **14a** (3.01 g, 11.89 mmol) and Pd/C (600 mg) in MeOH (100 ml) was stirred for 2 d under an H_2 atmosphere. After filtration and evaporation, the resulting oil was redissolved in DMF (10 ml), and then Z-D-Phe (3.91 g, 13.07 mmol), WSC·HCl (2.51 g, 13.07 mmol), $HOBt \cdot H_2O$ (2.00 g, 13.07 mmol), dimethylaminopyridine (DMAP) (70 mg) and TEA (1.80 ml, 13.07 mmol) were added at 0 °C. The mixture was stirred for 8 h at room temperature under an Ar atmosphere. After usual work-up, the residue was chromatographed on silica gel (n-hexane : EtOAc c = 5 : 1) to give a yellow amorphous powder (3.75 g, 63%). mp 35—37 °C. $[\alpha]_D^{24}$ +3.1° (c = 1.0, $CHCl_3$). FAB-MS (m/z): 505 $(M+H)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (s, 9H, *tert*-Bu), 3.26 (m, 2H, Phe; $\underline{CH_2}Ph$), 4.45 (s, 2H, OCH_2CO), 4.61 (m, 1H, CH), 5.10 (s, 2H, $O\underline{CH_2}Ph$), 5.51 (br s, 1H, $\underline{NH}CH$), 7.01—7.44 (m, 14H, Ar-H), 8.32 (m, 1H, $\underline{NH}Ph$).

23b (m-form compound), amorphous powder. mp 70—73 °C. $[\alpha]_D^{20}$ +5.3° (c = 1.3, $CHCl_3$). FAB-MS (m/z): 505 $(M+H)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.49 (s, 9H, *tert*-Bu), 3.48 (s, 2H, $\underline{CH_2}Ph$), 4.46 (m, 1H, CH), 4.49 (s, 2H, OCH_2CO), 5.11 (s, 2H, $Ph\underline{CH_2}O$), 6.63—7.49 (m, 14H, Ar-H).

23c (p-form compound), white powder. mp 107 °C. $[\alpha]_D^{26}$ −3.0° (c = 1.0, $CHCl_3$). FAB-MS (m/z): 505 $(M+H)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (s, 9H, *tert*-Bu), 3.17 (m, 2H, $\underline{CH_2}Ph$), 4.47 (s, 2H, OCH_2CO), 4.59 (m, 1H, CH), 5.11 (s, 2H, $Ph\underline{CH_2}O$), 5.40 (br s, 1H, $\underline{NH}CH$), 6.83—7.45 (m, 14H, Ar-H), 8.61 (br s, 1H, $\underline{NH}Ph$).

***tert*-Butyl [2-(*N*-Methoxalyl-L-prolyl-D-phenylalaninamido)phenoxy]-acetate (24a)** Amorphous powder. mp 58—60 °C. $[\alpha]_D^{28}$ −11.2° (c = 1.0, $CHCl_3$). FAB-MS (m/z): 554 $(M+H)^+$, 576 $(M+Na)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (s, 9H, *tert*-Bu), 1.78—2.11 (m, 4H, Pro; CH_2CH_2), 3.17 (m, 2H, Pro; NCH_2), 3.35 (dd, J = 14.2, 5.9 Hz, Phe; $\underline{CH_2}Ph$), 3.72 (s, 1H, Pro; CH), 3.83 (s, 3H, OCH_3), 4.52 (s, 2H, OCH_2CO), 4.94 (m, 1H, Phe; CH), 7.12—8.24 (m, 9H, Ar-H).

24b (m-form compound), amorphous powder. mp 63—65 °C. $[\alpha]_D^{28}$ +79.7° (c = 1.0, $CHCl_3$). FAB-MS (m/z): 554 $(M+H)^+$, 576 $(M+Na)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (s, 9H, *tert*-Bu), 1.67—2.21 (m, 4H, Pro; CH_2CH_2), 3.11 (m, 4H, Pro; NCH_2, Phe; $\underline{CH_2}Ph$), 3.65 (s, 1H, Pro; CH), 3.88 (s, 3H, OCH_3), 4.48 (s, 2H, OCH_2CO), 4.92 (m, 1H, Phe; CH), 7.14—8.41 (m, 9H, Ar-H).

24c (p-form compound), white powder. mp 65—66 °C. $[\alpha]_D^{28}$ +78.5° (c = 1.0, $CHCl_3$). FAB-MS (m/z): 554 $(M+H)^+$, 576 $(M+Na)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (s, 9H, *tert*-Bu), 1.79—2.14 (m, 4H, Pro; CH_2CH_2), 3.18 (m, 2H, Pro; NCH_2), 3.36 (m, 2H Phe; $\underline{CH_2}Ph$), 3.73 (s, 1H, Pro; CH), 3.84 (s, 3H, OCH_3), 4.50 (s, 2H, OCH_2CO), 4.95 (m, 1H, Phe; CH), 6.75—8.27 (m, 9H, Ar-H).

[2-(*N*-Methoxalyl-L-prolyl-D-phenylalaninamido)phenoxy]acetyl-L-asparagyl-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-*N*-*tert*-butyl-L-proline Amide (25a) A solution of **24a** (134 mg, 0.24 mmol) and TFA (3 ml) in CH_2Cl_2 (12 ml) was stirred for 6 h. Evaporation of the mixture gave the deprotected carboxylic acid as an amorphous powder. A solution of **9** (120 mg, 0.20 mmol) and Pd/C (100 mg) in MeOH (20 ml) was stirred for 16 h under an H_2 atmosphere. Filtration and evaporation gave the deprotected amine. These residues were redissolved in DMF (10 ml), then DEPC (39 mg, 0.24 mmol) and TEA (24 mg, 0.24 mmol) were added at 0 °C. The mixture was stirred for 12 h at room temperature under an Ar atmosphere. After usual work-up, the residue was chromatographed on silica gel (CH_2Cl_2 : MeOH = 20 : 1) to give a white powder (120 mg, 63%). mp 121—124 °C. $[\alpha]_D^{24}$ +7.0° (c = 1.0, $CHCl_3$). FAB-MS (m/z): 941 $(M+H)^+$. IR (KBr) cm^{-1}: 3340, 1748. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (s, 9H, *tert*-Bu), 1.77—2.13 (m, 8H, Pro; CH_2CH_2), 2.64—2.83 (m, 6H, AHPA; $\underline{CH_2}Ph$, Phe; $\underline{CH_2}Ph$, Asn; CH_2), 3.33—3.81 (m, 4H, Pro; NCH_2), 3.71 (s, 3H, OCH_3), 4.27—4.79 (m, 5H, AHPA; CHCH, Pro; CH, Phe; CH), 4.45 (s, 2H, OCH_2CO), 6.82—7.27 (m, 14H, Ar-H).

25b (m-form compound), white powder. mp 121—124 °C. $[\alpha]_D^{24}$ +6.9° (c = 1.0, $CHCl_3$). FAB-MS (m/z): 941 $(M+H)^+$, 963 $(M+Na)^+$. IR (KBr) cm^{-1}: 3336, 1750, 1651. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (s, 9H, *tert*-Bu), 1.69—2.18 (m, 8H, Pro; CH_2CH_2), 2.65—2.78 (m, 6H, AHPA; $\underline{CH_2}Ph$, Phe; $\underline{CH_2}Ph$, Asn; CH_2), 3.30—3.75 (m, 4H, Pro; NCH_2), 3.77 (s, 3H, OCH_3), 4.26—4.91 (m, 7H, AHPA; CHCH, Pro; CH, Phe; CH, OCH_2CO), 7.07—7.73 (m, 14H, Ar-H).

25c (p-form compound), white powder. mp 125—127 °C. $[\alpha]_D^{25}$ +9.6° (c = 1.0, $CHCl_3$). FAB-MS (m/z): 941 $(M+H)^+$, 963 $(M+Na)^+$. IR (KBr) cm^{-1}: 3341, 1650. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (s, 9H, *tert*-Bu), 1.70—2.30 (m, 8H, Pro; CH_2CH_2), 2.50—2.80 (m, 6H, AHPA; $\underline{CH_2}Ph$, Phe; $\underline{CH_2}Ph$, Asn; CH_2), 3.65 (m, 4H, Pro; NCH_2), 3.81 (s, 3H, OCH_3), 4.20—4.85 (m, 7H, AHPA; CHCH, Pro; CH, Phe; CH, OCH_2CO), 6.66—7.40, 8.00 (m, 14H, Ar-H).

***tert*-Butyl [4-(3-Hydroxypropyl)phenoxy]acetate (26)** A solution of 3-(4-hydroxyphenyl)-1-propanol (500 mg, 3.29 mmol) in THF (10 ml) was added to a solution of NaH (79 mg, 3.3 mmol) in THF (10 ml), and the mixture was stirred for 0.5 h under an Ar atmosphere. *tert*-Butyl bromoacetate (770 mg, 3.95 mmol) was added dropwise to the mixture and the whole was refluxed for 4 h. After filtration, the filtrate was concentrated *in vacuo*. The residue was redissolved in CH_2Cl_2, washed with saturated aqueous $NaHCO_3$, 10% aqueous citric acid and brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was chromatographed on silica gel (n-hexane : EtOAc = 3 : 1) to give a colorless oil (790 mg, 89%). FAB-MS (m/z): 267 $(M+H)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (s, 9H, *tert*-Bu), 1.71—1.87 (m, 2H, $CH_2\underline{CH_2}CH_2OH$), 2.62 (t, J = 7.3 Hz, 2H, $\underline{CH_2}Ph$), 3.61 (m, 2H, $CH_2CH_2\underline{CH_2}OH$), 4.47 (s, 2H, OCH_2CO), 6.78, 7.10, (each m, 4H, Ar-H).

***tert*-Butyl {4-[3-(*p*-Tolenesulfonyloxy)propyl]phenoxy}acetate (27)** A solution of **26** (1.00 g, 3.76 mmol) in pyridine (10 ml) was treated with Tos-Cl (1.07 g, 5.63 mmol) at −20 °C and the reaction mixture was stirred for 3 h at the same temperature under an Ar atmosphere. After usual work-up, the residue was chromatographed on silica gel (n-hexane : EtOAc = 3 : 1) to give a colorless oil (1.36 g, 86%). ^{1}H-NMR ($CDCl_3$) δ: 1.48 (s, 9H, *tert*-Bu), 1.85—1.96 (m, 2H, $CH_2\underline{CH_2}CH_2OH$), 2.45 (s, 3H, $\underline{CH_3}Ph$), 2.58 (t, J = 7.3 Hz, 2H, $\underline{CH_2}Ph$), 4.01 (t, J = 6.3 Hz, 2H, $CH_2CH_2\underline{CH_2}$–OTos), 4.47 (s, 2H, OCH_2CO), 6.78, 6.99 (each m, 4H, Ar-H), 7.36, 7.80 (each m, 4H, Ar-H).

***tert*-Butyl [4-(3-Azidopropyl)phenoxy]acetate (28)** A solution of **27** (1.00 g, 2.38 mmol) in DMF (10 ml) was treated with LiN_3 (175 mg, 3.57 mmol), and the reaction mixture was refluxed at 80 °C for 1 h under an Ar atmosphere. After evaporation, the residue was dissolved in CH_2Cl_2. The suspension was filtered through Celite 545 and the filtrate was concentrated *in vacuo*. The residue was chromatographed on silica gel (n-hexane : EtOAc = 3 : 1) to give a colorless oil (677 mg, 98%). ^{1}H-NMR ($CDCl_3$) δ: 1.48 (s, 9H, *tert*-Bu), 1.87 (m, 2H, $CH_2\underline{CH_2}$-CH_2OH), 2.64 (t, J = 7.3 Hz, 2H, $\underline{CH_2}Ph$), 3.26 (t, J = 6.6 Hz, 2H, $CH_2CH_2\underline{CH_2}$–N_3), 4.49 (s, 2H, OCH_2CO), 6.84, 7.10 (each m, 4H, Ar-H).

***tert*-Butyl [4-(3-Aminopropyl)phenoxy]acetate (29)** A solution of **28** (390 mg, 1.34 mmol) and Pd/C (120 mg) in MeOH (20 ml) was stirred for 2 h under an H_2 atmosphere. Filtration and evaporation gave a colorless oil (325 mg, 92%).

***tert*-Butyl {4-[3-(*N*-Carbobenzoxy-D-phenylalaninamido)propyl]phenoxy}acetate (30)** Colorless oil. $[\alpha]_D^{22}$ −10.1° (c=1.2, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.49 (s, 9H, *tert*-Bu), 1.72 (m, 2H, $CH_2\underline{CH_2}CH_2Ph$), 2.46 (m, 2H, $CH_2\underline{CH_2}CH_2Ph$), 2.93 (m, 2H, $NH\underline{CH_2}CH_2CH_2Ph$), 4.19 (m, 2H, Phe; $\underline{CH_2}Ph$), 4.29 (m, 1H, Phe; CH), 4.48 (s, 2H, OCH_2CO), 5.08 (s, 2H, OCH_2Ph), 6.79—7.33 (m, 14H, Ar-H).

***tert*-Butyl {4-[3-(*N*-Methoxalyl-L-prolyl-D-phenylalaninamido)propyl]phenoxy}acetate (31)** Colorless oil. $[\alpha]_D^{24}$ −8.1° (c=1.1, $CHCl_3$). FAB-MS (m/z): 596 $(M+H)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.49 (s, 9H, *tert*-Bu), 1.73—2.08 (m, 6H, $CH_2\underline{CH_2}CH_2Ph$, Pro; CH_2CH_2), 2.46—2.52 (m, 2H, $CH_2CH_2\underline{CH_2}Ph$), 3.15—3.66 (m, 6H, $NH\underline{CH_2}CH_2CH_2Ph$, Phe; $Ph\underline{CH_2}$, Pro; NCH_2), 3.75 (s, 3H, OCH_3), 4.19 (m, 1H, Pro; CH), 4.47 (s, 2H, OCH_2CO), 4.75 (m, 1H, Phe; CH), 6.80—7.27 (m, 9H, Ar-H).

{4-[3-(*N*-Methoxalyl-L-prolyl-D-phenylalaninamido)propyl]phenoxy}acetyl-L-asparagyl-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenyl butyryl]-*N*-*tert*-butyl-L-proline Amide (32) White powder. mp 114—117 °C. $[\alpha]_D^{25}$ −10.4° (c=1.0, $CHCl_3$). FAB-MS (m/z): 983 $(M+H)^+$, 1005 $(M+Na)^+$. IR (KBr) cm^{-1}: 3328, 1740, 1657, 1652, 1648. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (s, 9H, *tert*-Bu), 1.71 (m, 2H, $CH_2\underline{CH_2}CH_2Ph$), 1.79—2.25 (m, 8H, Pro; CH_2CH_2), 2.48 (m, 2H, $CH_2CH_2\underline{CH_2}Ph$), 2.59—2.79 (m, 4H, AHPA; $\underline{CH_2}Ph$, Asn; CH_2), 3.04—3.21 (m, 4H, $NH\underline{CH_2}CH_2CH_2Ph$, Phe; $\underline{CH_2}Ph$), 3.50—3.81 (m, 4H, Pro; NCH_2), 3.77 (s, 3H, OCH_3), 4.25—4.82 (m, 5H, AHPA; CHCH, Pro; CH, Phe; CH), 4.40 (s, 2H, OCH_2CO), 6.77—7.27 (m, 14H, Ar-H).

***N*-Carbobenzoxy-D-phenylalanyl-β-alanine *tert*-Butyl Ester (33)** White powder. mp 81—82 °C. $[\alpha]_D^{27}$ −4.4° (c=1.0, $CHCl_3$). FAB-MS (m/z): 427 $(M+H)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (s, 9H, *tert*-Bu), 2.30 (m, 2H, $-CH_2CO$), 3.05 (m, 2H, $\underline{CH_2}Ph$), 3.39 (m, 2H, $NH\underline{CH_2}-$), 4.35 (m, 1H, CH), 5.08 (s, 2H, $O\underline{CH_2}Ph$), 5.30 (br s, 1H, Phe; NH), 6.19 (br s, 1H, β-Ala; NH), 7.14—7.64 (m, 10H, Ar-H).

***N*-Carbobenzoxy-L-prolyl-D-phenylalanyl-β-alanine *tert*-Butyl Ester (34)** White powder. mp 104—106 °C. $[\alpha]_D^{27}$ −26.6° (c=1.0, $CHCl_3$). FAB-MS (m/z): 524 $(M+H)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.49 (s, 9H, *tert*-Bu), 1.78—2.10 (m, 4H, Pro; CH_2CH_2), 2.35 (m, 2H, $-CH_2CO$), 3.13 (m, 2H, $\underline{CH_2}Ph$), 3.38 (m, 2H, $NH\underline{CH_2}-$), 3.51 (m, 2H, Pro; NCH_2), 4.13 (m, 1H, Pro; CH), 4.61 (m, 1H, Phe; CH), 5.12 (s, 2H, $O\underline{CH_2}Ph$), 6.54 (br s, 1H, Phe; NH), 6.79 (br s, 1H, β-Ala; NH), 7.07—7.46 (m, 10H, Ar-H).

***N*-Methoxalyl-L-prolyl-D-phenylalanyl-β-alanine *tert*-Butyl Ester (35)** Light yellow amorphous powder. mp 50—52 °C. $[\alpha]_D^{30}$ −11.1° (c=1.0, $CHCl_3$). FAB-MS (m/z): 476 $(M+H)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (s, 9H, *tert*-Bu), 1.82—2.17 (m, 4H, Pro; CH_2CH_2), 2.37 (m, 2H, $-CH_2CO$), 3.14 (m, 2H, $\underline{CH_2}Ph$), 3.42 (m, 2H, $NH\underline{CH_2}-$), 3.63 (m, 2H, Pro; NCH_2), 3.88 (s, 3H, OCH_3), 4.32 (m, 1H, Pro; CH), 4.61 (m, 1H, Phe; CH), 6.50 (br d, J=8.3 Hz, 1H, Phe; NH), 6.65 (br s, 1H, β-Ala; NH), 7.14—7.38 (m, 5H, Ar-H).

***tert*-Butyl [4-(*N*-Methoxalyl-L-prolyl-D-phenylalanyl-β-alaninamido)phenoxy]acetate (36)** Colorless needles. mp 178—179 °C. $[\alpha]_D^{30}$ +9.2° (c=1.0, $CHCl_3$). FAB-MS (m/z): 625 $(M+H)^+$, 647 $(M+Na)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.49 (s, 9H, *tert*-Bu), 1.75—2.14 (m, 4H, Pro; CH_2CH_2), 2.52 (m, 2H, β-Ala; CH_2CO), 3.10—3.18 (m, 2H, $\underline{CH_2}Ph$), 3.52 (m, 2H, β-Ala; $NH\underline{CH_2}-$), 3.64 (m, 2H, Pro; NCH_2), 3.83 (s, 3H, OCH_3), 4.18 (m, 1H, Pro; CH), 4.48 (s, 2H, OCH_2CO), 4.65 (m, 1H, Phe; CH), 6.39 (br d, J=7.9 Hz, 1H, Phe; NH), 6.81, 7.14—7.46 (m, 9H, Ar-H), 8.28 (br s, 1H, $\underline{NH}Ph$).

[4-(*N*-Methoxalyl-L-prolyl-D-phenylalanyl-β-alaninamido)phenoxy]acetyl-L-asparagyl-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-*N*-*tert*-butyl-L-proline Amide (37) White powder. mp 129—132 °C. $[\alpha]_D^{28}$ −19.3° (c=0.5, $CHCl_3$). *Anal.* Calcd. for $C_{51}H_{65}N_9O_{13}\cdot 4H_2O$: C, 56.50; H, 6.79; N, 11.63. Found: C, 56.41; H, 6.43; N, 10.57. FAB-MS (m/z): 1012 $(M+H)^+$. IR (KBr) cm^{-1}: 3300—3100, 2954, 1648. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (s, 9H, *tert*-Bu), 1.57—2.20 (m, 8H, Pro; CH_2CH_2), 2.68 (m, 4H, β-Ala; CH_2CO, Asn; CH_2), 3.15 (m, 4H, $\underline{CH_2}Ph$), 3.48 (br s, 2H, β-Ala; $NH\underline{CH_2}-$), 3.69—3.82 (m, 7H, Pro; NCH_2, OCH_3), 4.25 (m, 2H, Pro; CH), 4.42 (br s, 2H, $O\underline{CH_2}CO$), 4.60—4.80 (m, 3H, Asn; CH, Phe; CH, AHPA; $\underline{CH}OH$), 6.52 (br s, 1H, $\underline{NH}$*tert*-Bu), 6.55 (br s, 1H, Phe; NH), 6.80—7.38 (m, 14H, Ar-H).

Acknowledgements We are grateful to Professor H. Hoshino, Gunma University for measuring anti-HIV activity and Dr. K. Dobashi, Central Research Laboratories of Mercian Corporation for measuring the HIV protease inhibitory activity. This work was partly supported by the Japan Health Science Foundation.

References and Notes

1) McDougal J. S., Kennedy M. S., Sligh J. M., Cort S. P., Mawle A., Nicholson J. K. A., *Science*, **231**(4736), 382—385 (1986).
2) Bowman M. R., Macferrin K. D., Schreiber S. L., Burakoff S. J., *Proc. Natl. Acad. Sci. U.S.A.*, **87**(22), 9052—9056 (1990).
3) *a*) Finberg R. W., Diamond D. C., Mitchell D. B., Rosentein Y., Soman G., Norman T. C., Schreiber S. L., Burakoff S. J., *Science*, **249**(4966), 287—291 (1990); *b*) Venkatramani L., Hossain M. B., Van der Helm D., *Int. J. Pept. Prot. Res.*, **43**(6), 520—528 (1994).
4) Uchiyama T., Yoshino H., Takemoto M., Achiwa K., *Chem. Pharm. Bull.*, **39**(11), 3091—3093 (1991).
5) Huff J. R., *J. Med. Chem.*, **34**(8), 2305—2314 (1991).
6) *a*) Mimoto T., Imai J., Tanaka S., Hattori N., Kasanuki S., Akaji K., Kiso Y., *Chem. Pharm. Bull.*, **39**(11), 3088—3090 (1991); *b*) Mimoto T., Imai J., Kasanuki S., Mimoto H., Hattori N., Akaji K., Kiso Y., *ibid.*, **40**(8), 2251—2253 (1992); c) Kiso Y., *Biopolymers*, **40**(2), 235—244 (1996).
7) Takita T., Suda H., Aoyagi T., Umezawa H., *J. Med. Chem.*, **20**(4), 510—515 (1977).
8) Rich D. H., Jasensky R. D., Mueller G. C., Anderson K. E., *J. Med. Chem.*, **24**(5), 567—572 (1981).
9) *a*) Ninomiya K., Shioiri T., Yamada S., *Chem. Pharm. Bull.*, **24**(11), 2711—2715 (1976); *b*) Hamada Y., Rishi S., Shioiri T., Yamada S., *ibid.*, **25**(2), 224—230 (1977).
10) Ikeda K., Asahara T., Achiwa K., Hoshino H., *Chem. Pharm. Bull.*, **45**(2), 402—405 (1997); The biological activities will be reported separately by Prof. Hiroo Hoshino *et al.* (Gunma Univ.).
11) The assay was carried out by Central Research Laboratories of Mercian Corporation.
12) *a*) Bures L., Bostik J., Motycka K., Spundova M., Rehak L., *Neoplasma*, **35**(3), 329—342 (1988); *b*) Bures L., Lichy A., Bostik J., Spundova M., *ibid.*, **37**(3), 225—231 (1990).