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

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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 Ser⁴²–Ser⁴⁹ 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*-carbomethoxy-(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 Boc₂O at –40 °C to give **4** in 67% yield. Coupling of **4** and Z-Pro was carried out by the dicyclohexylcarbodiimide (DCC)-HOBt coupling method⁸⁾ 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 method⁹⁾ and deprotection of Z- with Pd/C–H₂ 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

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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 LiN₃ and reduction with Pd/C-H₂ 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-OBu^t, Z-D-Phe and Z-L-Pro by the water soluble carbodiimide (WSC)-HOBt method gave **34** in 72% yield. After de-

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 (μ g/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	<i>o</i> -(CPF-NH)-PA-	60	>100
25b	<i>m</i> -(CPF-NH)-PA-	100	27
25c	<i>p</i> -(CPF-NH)-PA-	>100	34
32	<i>p</i> -(CPF-NH-C ₃)-PA-	>100	N.D.
37	<i>p</i> -(CPF- β -Ala)-PA-	>100	N.D.

a) Z = benzyloxycarbonyl-, PA = phenoxyacetyl-, CPF = *N*-carbomethoxy-carbonyl-propyl-phenylalanyl-, -C₃ = 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 CH₂Cl₂ 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**; EC₅₀ = 60 μ g/ml, **25b**; EC₅₀ = 100 μ 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. ¹H-NMR spectra were taken on a JEOL JNM-GX 270 (270 MHz) spectrometer. The ¹H chemical shifts (δ) are given in ppm relative to that of Me₄Si (δ = 0) in CDCl₃ or CD₃OD 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-F₂₅₄ (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 CH₂Cl₂, and the mixture was washed with saturated aqueous NaHCO₃, 10% aqueous citric acid and brine, then dried (MgSO₄), 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. [α]_D²² –90.7° (*c* = 1.0, CHCl₃). ¹H-NMR (CDCl₃) δ : 1.30 (s, 9H, *tert*-Bu), 1.60–2.31 (m, 4H, CH₂CH₂), 3.52 (m, 2H, NCH₂), 4.23 (m, 1H, CH), 5.17 (s, 2H, PhCH₂O), 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 Et₂O (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 NaHCO₃ (3.33 g, 39.6 mmol) in water at 0°C. The mixture was stirred for 2 h, then NaHCO₃ (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 Et₂O, and the aqueous layer was acidified with 6*N* HCl, and extracted with EtOAc. The organic layer was dried (MgSO₄), and concentrated *in vacuo* to give a colorless oil (3.40 g, 84%). [α]_D²² –55.8° (*c* = 1.60, MeOH). ¹H-NMR (CDCl₃) δ : 2.09–2.25 (m, 2H, CH₂CHOH), 3.56 (m, 2H, NCH₂), 4.46 (m, 2H, NCH, CHOH), 5.10 (br s, 2H, OCH₂Ph), 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. [α]_D²² –80.5° (*c* = 1.0, CHCl₃). FAB-MS (*m/z*) 321 (M+H)⁺. ¹H-NMR (CDCl₃) δ : 1.29 (s, 9H, *tert*-Bu), 2.05–3.06 (m, 2H, CH₂CHOH), 3.55 (br s, 1H, CHOH), 4.30, 4.46 (each br s, 2H, NCH, CHOH), 5.15 (br s, 2H, OCH₂Ph), 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 CH₂Cl₂ (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 CH₂Cl₂ and the solution was washed with 10% aqueous citric acid. The aqueous layer was alkalinized with 6*N* NaOH and extracted with CH₂Cl₂. The combined organic layers were dried (MgSO₄), and

concentrated *in vacuo* to give a white powder (225 mg, 67%). mp 65–67 °C. $^1\text{H-NMR}$ (CDCl_3) δ : 1.09 (s, 6H, C(CH₃)₂), 1.45 (s, 9H, *tert*-Bu), 3.00 (brd, $J=6.2$ Hz, 2H, CH₂NH).

***N*-[2-[(*tert*-Butoxycarbonyl)amino]-1,1-dimethylethyl]-1-carbobenzoyl-L-proline Amide (5)** White powder. $[\alpha]_D^{22} -29.4^\circ$ ($c=1.4$, CHCl₃). $^1\text{H-NMR}$ (CDCl_3) δ : 1.26, 1.27 (each brs, 6H, C(CH₃)₂), 1.43 (s, 9H, *tert*-Bu), 1.78–2.17 (m, 4H, CH₂CH₂), 3.15–3.54 (m, 4H, CH₂NH, Pro; NCH₂), 4.11 (m, 1H, NCHCO), 5.18 (brs, 2H, OCH₂Ph), 7.27–7.36 (m, 5H, Ar-H).

***N*-Carbobenzoyl-L-asparagyl-[(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 H₂ 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. $[\alpha]_D^{22} -30.3^\circ$ ($c=1.0$, CHCl₃). FAB-MS (m/z): 482 (M+H)⁺, 504 (M+Na)⁺. $^1\text{H-NMR}$ (CDCl_3) δ : 1.26 (brs, 9H, *tert*-Bu), 1.87–2.33 (m, 4H, CH₂CH₂), 2.70 (m, 2H, CH₂Ph), 3.76 (m, 2H, Pro; NCH₂), 4.16–4.59 (m, 3H, CHCH, Pro; CH), 5.00 (brs, 2H, OCH₂Ph), 5.49 (brd, $J=8.8$ Hz, 1H, NH*tert*-Bu), 6.47 (s, 1H, NHCH-), 7.10–7.32 (m, 10H, Ar-H).

7 (hydroxyproline type compound), white powder. mp 88–91 °C. $[\alpha]_D^{22} -17.1^\circ$ ($c=1.0$, CHCl₃). $^1\text{H-NMR}$ (CDCl_3) δ : 1.29 (brs, 9H, *tert*-Bu), 2.03–2.35 (m, 2H, Pro(OH); CH₂CHOH), 2.68 (m, 2H, CH₂Ph), 3.68–3.88 (m, 2H, Pro(OH); NCH₂), 4.10 (m, 1H, Pro(OH); NCHCO), 4.43–4.54 (m, 3H, NHCHCHCO, Pro(OH); CHOH), 4.96 (brs, 2H, OCH₂Ph), 5.42 (brs, 1H, NHCO), 6.48 (s, 1H, NH*tert*-Bu), 7.12–7.31 (m, 10H, Ar-H).

***N*-Carbobenzoyl-L-asparagyl-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-*N*-[2-[(*tert*-butoxycarbonyl)amino]-1,1-dimethylethyl]-L-proline Amide (8)** Colorless oil. $[\alpha]_D^{22} +32.1^\circ$ ($c=1.0$, CHCl₃). $^1\text{H-NMR}$ (CDCl_3) δ : 1.24, 1.30 (each brs, 6H, C(CH₃)₂), 1.43 (s, 9H, *tert*-Bu), 1.78–2.17 (m, 4H, CH₂CH₂), 2.69 (m, 2H, CH₂Ph), 3.28 (m, 2H, CCH₂NH), 3.76 (m, 2H, Pro; NCH₂), 4.08–4.31 (m, 2H, NHCHCHCO), 4.58 (m, 1H, Pro; CH), 4.99 (brs, 2H, OCH₂Ph), 7.17–7.34 (m, 10H, Ar-H).

***N*-Carbobenzoyl-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. $[\alpha]_D^{22} -37.6^\circ$ ($c=1.0$, CHCl₃). FAB-MS (m/z): 596 (M+H)⁺, 618 (M+Na)⁺. IR (KBr) cm^{-1} : 3340, 1700, 1660. $^1\text{H-NMR}$ (CDCl_3) δ : 1.29 (s, 9H, *tert*-Bu), 1.86–2.24 (m, 4H, Pro; CH₂CH₂), 2.54–2.79 (m, 4H, Asn; CH₂CONH₂, CHCH₂Ph), 3.60 (brs, 2H, Pro; NCH₂), 4.23–4.42 (m, 4H, Pro; CH, Asn; CH, AHPA; CHCH), 5.07 (s, 2H, OCH₂Ph), 7.17–7.33 (m, 10H, Ar-H).

10 (hydroxyproline type compound), white powder. mp 118–121 °C. $[\alpha]_D^{22} +13.1^\circ$ ($c=1.0$, CHCl₃). FAB-MS (m/z): 612 (M+H)⁺, 634 (M+Na)⁺. IR (KBr) cm^{-1} : 3410, 1700, 1660. $^1\text{H-NMR}$ (CDCl_3) δ : 1.35 (s, 9H, *tert*-Bu), 1.78–2.04 (m, 2H, Pro(OH); CH₂CHOH), 2.38–2.78 (m, 4H, Asn; CH₂CONH₂, CHCH₂Ph), 3.48–3.73 (brs, 3H, Pro(OH); NCH₂, CH(OH)), 4.29–4.48 (m, 3H, Asn; CH, AHPA; CHCH), 4.85–5.16 (m, 3H, Pro(OH); CH, OCH₂Ph), 7.11–7.37 (m, 10H, Ar-H).

***N*-Carbobenzoyl-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. $[\alpha]_D^{22} +15.6^\circ$ ($c=1.0$, CHCl₃). FAB-MS (m/z): 711 (M+H)⁺, 733 (M+Na)⁺. IR (KBr) cm^{-1} : 3500–3200, 2980, 1660. $^1\text{H-NMR}$ (CDCl_3) δ : 1.24–1.30 (m, 6H, CH₃), 1.42 (s, 9H, *tert*-Bu), 1.85–2.17 (m, 4H, Pro; CH₂CH₂), 2.60–2.80 (m, 4H, Asn; CH₂CONH₂, CHCH₂Ph), 3.28 (m, 2H, CCH₂NH-), 3.69 (m, 2H, Pro; NCH₂), 4.16–4.37 (m, 4H, Asn; CH, AHPA; CHCH, Pro; CH), 5.07 (brs, 2H, OCH₂Ph), 7.13–7.33 (m, 10H, Ar-H).

***N*-Carbobenzoyl-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. $[\alpha]_D^{22} -16.8^\circ$ ($c=0.8$, CHCl₃). IR (KBr) cm^{-1} : 3320, 1670. $^1\text{H-NMR}$ ($\text{CDCl}_3\text{-CD}_3\text{OD}$) δ : 1.37, 1.44 (each brs, 6H, CH₃), 1.90–2.20 (m, 4H, Pro; CH₂CH₂), 2.60 (brs, 2H, CHCH₂Ph), 2.86 (brs, 2H, Asn; CH₂), 3.28 (m, 2H, CCH₂NH-), 3.69 (m, 2H, Pro; NCH₂), 4.16–4.37 (m, 4H, Asn; CH, AHPA; CHCH, Pro; CH), 5.07 (brs, 2H, OCH₂Ph), 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 H₂ atmosphere. After filtration and evaporation, the residue was redissolved in CH₂Cl₂ (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 (CH₂Cl₂:MeOH=15:1) to give a white powder (156 mg, 82%). mp 94–97 °C. $[\alpha]_D^{20} +36.2^\circ$ ($c=1.0$, CHCl₃). FAB-MS (m/z): 596 (M+H)⁺, 618 (M+Na)⁺. IR (KBr) cm^{-1} : 3340, 1690, 1660. $^1\text{H-NMR}$ (CDCl_3) δ : 1.28 (s, 9H, *tert*-Bu), 1.90–2.28 (m, 4H, Pro; CH₂CH₂), 2.54–2.78 (m, 4H, Asn; CH₂, AHPA; CH₂Ph), 3.64 (m, 2H, Pro; NCH₂), 4.15–4.49 (m, Asn; CH, AHPA; CHCH, Pro; CH), 4.74 (s, OCH₂CO), 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 CH₂Cl₂, and the mixture was washed with saturated aqueous NaHCO₃, 10% aqueous citric acid and brine, dried (MgSO₄), 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. $^1\text{H-NMR}$ (CDCl_3) δ : 1.47 (s, 9H, *tert*-Bu), 4.67 (s, 2H, OCH₂CO), 6.98 (d, $J=8.3$ Hz, 1H, Ar-H₆), 7.11 (m, 1H, Ar-H₄), 7.55 (m, 1H, Ar-H₅), 8.88 (d, $J=7.9$ Hz, 1H, Ar-H₃).

14b (*m*-form compound), white needles. mp 47–49 °C. $^1\text{H-NMR}$ (CDCl_3) δ : 1.50 (s, 9H, *tert*-Bu), 4.61 (s, 2H, OCH₂CO), 7.24–7.89 (m, 4H, Ar-H).

14c (*p*-form compound), white needles. mp 84 °C. $^1\text{H-NMR}$ (CDCl_3) δ : 1.49 (s, 9H, *tert*-Bu), 4.62 (s, 2H, OCH₂CO), 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 CH₂Cl₂ (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. $^1\text{H-NMR}$ (CDCl_3) δ : 4.83 (s, 2H, OCH₂CO), 7.08 (d, $J=8.2$ Hz, 1H, Ar-H₆), 7.19 (t, $J=8.3$ Hz, 1H, Ar-H₄), 7.61 (t, $J=8.3$ Hz, 1H, Ar-H₅), 8.01 (d, $J=8.2$ Hz, 1H, Ar-H₃).

15b (*m*-form compound), colorless needles. mp 152 °C. $^1\text{H-NMR}$ (CDCl_3) δ : 4.79 (s, 2H, OCH₂CO), 7.30 (d, $J=6.3$ Hz, 1H, Ar-H₆), 7.49 (t, $J=8.3$ Hz, 1H, Ar-H₅), 7.76 (s, 1H, Ar-H₂), 7.92 (d, $J=7.9$ Hz, 1H, Ar-H₄).

15c (*p*-form compound), colorless prisms. mp 189 °C. $^1\text{H-NMR}$ (CDCl_3) δ : 4.80 (s, 2H, OCH₂CO), 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. $[\alpha]_D^{24} -41.5^\circ$ ($c=1.0$, CHCl₃). FAB-MS (m/z): 641 (M+H)⁺, 663 (M+Na)⁺. IR (KBr) cm^{-1} : 3330, 1662. $^1\text{H-NMR}$ (CDCl_3) δ : 1.28 (s, 9H, *tert*-Bu), 1.87–2.26 (m, 4H, Pro; CH₂CH₂), 2.61–2.80 (m, 4H, Asn; CH₂, AHPA; CH₂Ph), 3.66 (t, $J=6.7$ Hz, 2H, Pro; NCH₂), 4.43–4.50 (m, 4H, Asn; CH, AHPA; CHCH, Pro; CH), 4.65 (s, 2H, OCH₂CO), 7.08–8.84 (m, 9H, Ar-H).

16b (*m*-form compound), yellow powder. mp 104–107 °C. $[\alpha]_D^{24} -30.9^\circ$ ($c=1.0$, CHCl₃). FAB-MS (m/z): 641 (M+H)⁺, 663 (M+Na)⁺. IR (KBr) cm^{-1} : 3332, 1664, 1528, 1350. $^1\text{H-NMR}$ (CDCl_3) δ : 1.28 (s, 9H, *tert*-Bu), 1.77–2.20 (m, 4H, Pro; CH₂CH₂), 2.64 (m, 2H, Asn; CH₂), 2.82 (m, 2H, AHPA; CH₂Ph), 3.70 (m, 2H, Pro; NCH₂), 4.27–4.79 (m, 4H, Asn; CH, AHPA; CHCH, Pro; CH), 4.61 (s, 2H, OCH₂CO), 7.00–7.86 (m, 9H, Ar-H).

16c (*p*-form compound), white powder. mp 106–108 °C. $[\alpha]_D^{24} -23.3^\circ$ ($c=1.0$, CHCl₃). FAB-MS (m/z): 641 (M+H)⁺, 663 (M+Na)⁺. IR (KBr) cm^{-1} : 3330, 1660, 1520. $^1\text{H-NMR}$ (CDCl_3) δ : 1.29 (s, 9H, *tert*-Bu), 1.85–2.30 (m, 4H, Pro; CH₂CH₂), 2.61 (m, 2H, Asn; CH₂), 2.90 (m, 2H, AHPA; CH₂Ph), 3.70 (m, 2H, Pro; NCH₂), 4.35–4.50 (m, 4H, Asn; CH, AHPA; CHCH, Pro; CH), 4.61 (s, 2H, OCH₂CO), 6.94–8.23 (m, 9H, Ar-H).

***N*-Methoxalyl-L-proline Benzyl Ester (17)** TEA (500 mg, 4.96 mmol) and methoxalyl chloride (669 mg, 5.46 mmol) were added to a solution of H-L-Pro-OBzl·HCl (1.20 g, 4.96 mmol) in CH₂Cl₂ (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 ($\text{CH}_2\text{Cl}_2:\text{MeOH}=15:1$) to give a colorless oil (1.42 g, 98%). $[\alpha]_D^{22} + 59.8^\circ$ ($c=1.02$, CHCl_3). FAB-MS (m/z): 292 ($\text{M}+\text{H}^+$). $^1\text{H-NMR}$ (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 (brs, 2H, OCH_2Ph), 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. $[\alpha]_D^{22} - 27.5^\circ$ ($c=1.0$, CHCl_3). FAB-MS (m/z): 743 ($\text{M}+\text{H}^+$). $^1\text{H-NMR}$ (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; CH_2Ph , AHPA; CH_2Ph), 3.61 (m, 2H, Pro, NCH_2), 4.08–4.57 (m, 5H, AHPA; CHCH, Phe; CH, Asn; CH, Pro; CH), 5.06 (brs, 2H, OCH_2Ph), 7.33 (m, 15H, Ar-H).

20 (using L-phenylalanine), white powder. mp 103–106 °C. $[\alpha]_D^{22} - 32.3^\circ$ ($c=1.0$, CHCl_3). FAB-MS (m/z): 743 ($\text{M}+\text{H}^+$). $^1\text{H-NMR}$ (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; CH_2Ph , AHPA; CH_2Ph), 3.60 (m, 2H, Pro, NCH_2), 4.39–4.63 (m, 5H, AHPA; CHCH, Phe; CH, Asn; CH, Pro; CH), 5.00 (brs, 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. $[\alpha]_D^{22} - 27.7^\circ$ ($c=1.0$, CHCl_3). FAB-MS (m/z): 792 ($\text{M}+\text{H}^+$). IR (KBr) cm^{-1} : 3380, 1740, 1670, 1650. $^1\text{H-NMR}$ (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 (brs, 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. $[\alpha]_D^{22} - 68.7^\circ$ ($c=1.0$, CHCl_3). FAB-MS (m/z): 792 ($\text{M}+\text{H}^+$). IR (KBr) cm^{-1} : 3360, 1740, 1660, 1650. $^1\text{H-NMR}$ (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·H₂O (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=5:1) to give a yellow amorphous powder (3.75 g, 63%). mp 35–37 °C. $[\alpha]_D^{24} + 3.1^\circ$ ($c=1.0$, CHCl_3). FAB-MS (m/z): 505 ($\text{M}+\text{H}^+$). $^1\text{H-NMR}$ (CDCl_3) δ : 1.45 (s, 9H, *tert*-Bu), 3.26 (m, 2H, Phe; CH_2Ph), 4.45 (s, 2H, OCH_2CO), 4.61 (m, 1H, CH), 5.10 (s, 2H, OCH_2Ph), 5.51 (brs, 1H, NHCH), 7.01–7.44 (m, 14H, Ar-H), 8.32 (m, 1H, NHPh).

23b (*m*-form compound), amorphous powder. mp 70–73 °C. $[\alpha]_D^{20} + 5.3^\circ$ ($c=1.3$, CHCl_3). FAB-MS (m/z): 505 ($\text{M}+\text{H}^+$). $^1\text{H-NMR}$ (CDCl_3) δ : 1.49 (s, 9H, *tert*-Bu), 3.48 (s, 2H, CH_2Ph), 4.46 (m, 1H, CH), 4.49 (s, 2H, OCH_2CO), 5.11 (s, 2H, PhCH_2O), 6.63–7.49 (m, 14H, Ar-H).

23c (*p*-form compound), white powder. mp 107 °C. $[\alpha]_D^{26} - 3.0^\circ$ ($c=1.0$, CHCl_3). FAB-MS (m/z): 505 ($\text{M}+\text{H}^+$). $^1\text{H-NMR}$ (CDCl_3) δ : 1.48 (s, 9H, *tert*-Bu), 3.17 (m, 2H, CH_2Ph), 4.47 (s, 2H, OCH_2CO), 4.59 (m, 1H, CH), 5.11 (s, 2H, PhCH_2O), 5.40 (brs, 1H, NHCH), 6.83–7.45 (m, 14H, Ar-H), 8.61 (brs, 1H, NHPh).

tert-Butyl [2-(N-Methoxalyl-L-prolyl-D-phenylalaninamido)phenoxy]acetate (24a) Amorphous powder. mp 58–60 °C. $[\alpha]_D^{28} - 11.2^\circ$ ($c=1.0$, CHCl_3). FAB-MS (m/z): 554 ($\text{M}+\text{H}^+$), 576 ($\text{M}+\text{Na}^+$). $^1\text{H-NMR}$ (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; CH_2Ph), 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. $[\alpha]_D^{28} + 79.7^\circ$ ($c=1.0$, CHCl_3). FAB-MS (m/z): 554 ($\text{M}+\text{H}^+$), 576 ($\text{M}+\text{Na}^+$). $^1\text{H-NMR}$ (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; CH_2Ph), 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. $[\alpha]_D^{28} + 78.5^\circ$ ($c=1.0$, CHCl_3). FAB-MS (m/z): 554 ($\text{M}+\text{H}^+$), 576 ($\text{M}+\text{Na}^+$). $^1\text{H-NMR}$ (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; CH_2Ph), 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 ($\text{CH}_2\text{Cl}_2:\text{MeOH}=20:1$) to give a white powder (120 mg, 63%). mp 121–124 °C. $[\alpha]_D^{24} + 7.0^\circ$ ($c=1.0$, CHCl_3). FAB-MS (m/z): 941 ($\text{M}+\text{H}^+$). IR (KBr) cm^{-1} : 3340, 1748. $^1\text{H-NMR}$ (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; CH_2Ph , Phe; CH_2Ph , 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. $[\alpha]_D^{24} + 6.9^\circ$ ($c=1.0$, CHCl_3). FAB-MS (m/z): 941 ($\text{M}+\text{H}^+$), 963 ($\text{M}+\text{Na}^+$). IR (KBr) cm^{-1} : 3336, 1750, 1651. $^1\text{H-NMR}$ (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; CH_2Ph , Phe; CH_2Ph , 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. $[\alpha]_D^{25} + 9.6^\circ$ ($c=1.0$, CHCl_3). FAB-MS (m/z): 941 ($\text{M}+\text{H}^+$), 963 ($\text{M}+\text{Na}^+$). IR (KBr) cm^{-1} : 3341, 1650. $^1\text{H-NMR}$ (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; CH_2Ph , Phe; CH_2Ph , 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 ($\text{M}+\text{H}^+$). $^1\text{H-NMR}$ (CDCl_3) δ : 1.48 (s, 9H, *tert*-Bu), 1.71–1.87 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$), 2.62 (t, $J=7.3$ Hz, 2H, CH_2Ph), 3.61 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$), 4.47 (s, 2H, OCH_2CO), 6.78, 7.10, (each m, 4H, Ar-H).

tert-Butyl [4-[3-(*p*-Toluenesulfonyloxy)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%). $^1\text{H-NMR}$ (CDCl_3) δ : 1.48 (s, 9H, *tert*-Bu), 1.85–1.96 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$), 2.45 (s, 3H, CH_3Ph), 2.58 (t, $J=7.3$ Hz, 2H, CH_2Ph), 4.01 (t, $J=6.3$ Hz, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{OTos}$), 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, 3.76 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%). $^1\text{H-NMR}$ (CDCl_3) δ : 1.48 (s, 9H, *tert*-Bu), 1.87 (m, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{OH}$), 2.64 (t, $J=7.3$ Hz, 2H, CH_2Ph), 3.26 (t, $J=6.6$ Hz, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{N}_3$), 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 H₂ 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. $[\alpha]_D^{22} -10.1^\circ$ ($c=1.2$, CHCl₃). ¹H-NMR (CDCl₃) δ : 1.49 (s, 9H, *tert*-Bu), 1.72 (m, 2H, CH₂CH₂-CH₂Ph), 2.46 (m, 2H, CH₂CH₂CH₂Ph), 2.93 (m, 2H, NHCH₂CH₂-CH₂Ph), 4.19 (m, 2H, Phe; CH₂Ph), 4.29 (m, 1H, Phe; CH), 4.48 (s, 2H, OCH₂CO), 5.08 (s, 2H, OCH₂Ph), 6.79—7.33 (m, 14H, Ar-H).

tert-Butyl [4-[3-(N-Methoxalyl-L-prolyl-D-phenylalaninamido)propyl]phenoxy]acetate (31) Colorless oil. $[\alpha]_D^{24} -8.1^\circ$ ($c=1.1$, CHCl₃). FAB-MS (m/z): 596 (M+H)⁺. ¹H-NMR (CDCl₃) δ : 1.49 (s, 9H, *tert*-Bu), 1.73—2.08 (m, 6H, CH₂CH₂CH₂Ph, Pro; CH₂CH₂), 2.46—2.52 (m, 2H, CH₂CH₂CH₂Ph), 3.15—3.66 (m, 6H, NHCH₂CH₂CH₂Ph, Phe; PhCH₂, Pro; NCH₂), 3.75 (s, 3H, OCH₃), 4.19 (m, 1H, Pro; CH), 4.47 (s, 2H, OCH₂CO), 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-[(2S,3S)-3-amino-2-hydroxy-4-phenylbutyryl]-N-tert-butyl-L-proline Amide (32) White powder. mp 114—117°C. $[\alpha]_D^{25} -10.4^\circ$ ($c=1.0$, CHCl₃). FAB-MS (m/z): 983 (M+H)⁺, 1005 (M+Na)⁺. IR (KBr) cm⁻¹: 3328, 1740, 1657, 1652, 1648. ¹H-NMR (CDCl₃) δ : 1.29 (s, 9H, *tert*-Bu), 1.71 (m, 2H, CH₂CH₂CH₂Ph), 1.79—2.25 (m, 8H, Pro; CH₂CH₂), 2.48 (m, 2H, CH₂CH₂CH₂Ph), 2.59—2.79 (m, 4H, AHPA; CH₂Ph, Asn; CH₂), 3.04—3.21 (m, 4H, NHCH₂CH₂CH₂Ph, Phe; CH₂Ph), 3.50—3.81 (m, 4H, Pro; NCH₂), 3.77 (s, 3H, OCH₃), 4.25—4.82 (m, 5H, AHPA; CHCH, Pro; CH, Phe; CH), 4.40 (s, 2H, OCH₂CO), 6.77—7.27 (m, 14H, Ar-H).

N-Carbobenzoxy-D-phenylalanyl-β-alanine tert-Butyl Ester (33) White powder. mp 81—82°C. $[\alpha]_D^{27} -4.4^\circ$ ($c=1.0$, CHCl₃). FAB-MS (m/z): 427 (M+H)⁺. ¹H-NMR (CDCl₃) δ : 1.41 (s, 9H, *tert*-Bu), 2.30 (m, 2H, -CH₂CO), 3.05 (m, 2H, CH₂Ph), 3.39 (m, 2H, NHCH₂-), 4.35 (m, 1H, CH), 5.08 (s, 2H, OCH₂Ph), 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. $[\alpha]_D^{27} -26.6^\circ$ ($c=1.0$, CHCl₃). FAB-MS (m/z): 524 (M+H)⁺. ¹H-NMR (CDCl₃) δ : 1.49 (s, 9H, *tert*-Bu), 1.78—2.10 (m, 4H, Pro; CH₂CH₂), 2.35 (m, 2H, -CH₂CO), 3.13 (m, 2H, CH₂Ph), 3.38 (m, 2H, NHCH₂-), 3.51 (m, 2H, Pro; NCH₂), 4.13 (m, 1H, Pro; CH), 4.61 (m, 1H, Phe; CH), 5.12 (s, 2H, OCH₂Ph), 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. $[\alpha]_D^{30} -11.1^\circ$ ($c=1.0$, CHCl₃). FAB-MS (m/z): 476 (M+H)⁺. ¹H-NMR (CDCl₃) δ : 1.42 (s, 9H, *tert*-Bu), 1.82—2.17 (m, 4H, Pro; CH₂CH₂), 2.37 (m, 2H, -CH₂CO), 3.14 (m, 2H, CH₂Ph), 3.42 (m, 2H, NHCH₂-), 3.63 (m, 2H, Pro; NCH₂), 3.88 (s, 3H, OCH₃), 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. $[\alpha]_D^{30} +9.2^\circ$ ($c=1.0$, CHCl₃). FAB-MS (m/z): 625 (M+H)⁺, 647 (M+Na)⁺. ¹H-NMR (CDCl₃) δ : 1.49 (s, 9H, *tert*-Bu), 1.75—2.14 (m, 4H, Pro; CH₂CH₂), 2.52 (m, 2H, β-Ala; CH₂CO), 3.10—3.18 (m, 2H, CH₂Ph), 3.52 (m, 2H, β-Ala; NHCH₂-), 3.64 (m, 2H, Pro; NCH₂), 3.83 (s, 3H,

OCH₃), 4.18 (m, 1H, Pro; CH), 4.48 (s, 2H, OCH₂CO), 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, NHPh).

[4-(N-Methoxalyl-L-prolyl-D-phenylalanyl-β-alaninamido)phenoxy]-acetyl-L-asparagyl-[(2S,3S)-3-amino-2-hydroxy-4-phenylbutyryl]-N-tert-butyl-L-proline Amide (37) White powder. mp 129—132°C. $[\alpha]_D^{28} -19.3^\circ$ ($c=0.5$, CHCl₃). Anal. Calcd. for C₅₁H₆₅N₉O₁₃·4H₂O: 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. ¹H-NMR (CDCl₃) δ : 1.29 (s, 9H, *tert*-Bu), 1.57—2.20 (m, 8H, Pro; CH₂CH₂), 2.68 (m, 4H, β-Ala; CH₂CO, Asn; CH₂), 3.15 (m, 4H, CH₂Ph), 3.48 (br s, 2H, β-Ala; NHCH₂-), 3.69—3.82 (m, 7H, Pro; NCH₂, OCH₃), 4.25 (m, 2H, Pro; CH), 4.42 (br s, 2H, OCH₂CO), 4.60—4.80 (m, 3H, Asn; CH, Phe; CH, AHPA; CHOH), 6.52 (br s, 1H, NH_{tert}-Bu), 6.55 (br s, 1H, Phe; NH), 6.80—7.38 (m, 14H, Ar-H).

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