Synthesis of Dipeptide-Type Human Immunodeficiency Virus (HIV) Protease Inhibitors with a Binding Unit to GP120

Akira ASAGARASU, Nao TAKAYANAGI, and Kazuo ACHIWA*

School of Pharmaceutical Sciences, University of Shizuoka, 52–1 Yada, Shizuoka 422–8526, Japan.
Received November 4, 1997; accepted December 27, 1997

Some dipeptide-type human immunodeficiency virus (HIV) protease inhibitors derived from KNI-102, with a *N*-carbomethoxycarbonylprolyl-phenylalanine benzyl ester (CPF) moiety as a binding site to gp120, were synthesized. Compounds 11a showed 7—100 times higher HIV protease-inhibitory activity (11a; $IC_{50}=0.90$ μg/ml, 1.1 μM) than the standard compound 3 or 4 (3; $IC_{50}=3.7$ μg/ml, 7.7 μM, 4; $IC_{50}=75$ μg/ml, 155 μM). Generally, the compounds substituted at the *o*-position of the phenoxyacetyl group 7a, 11a, 16a and 21a showed several times higher inhibitory activity than 3.

Key words HIV; protease inhibitor; CPF; gp120; hybrid compound

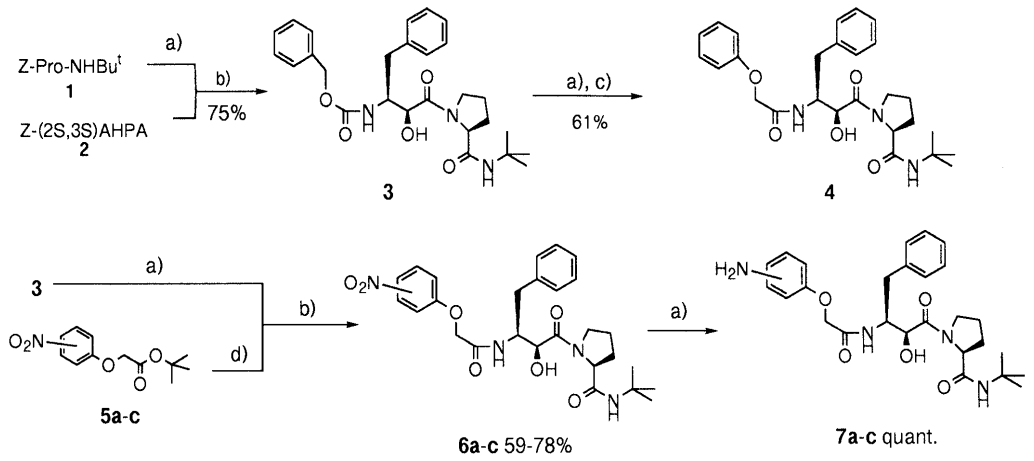
Human immunodeficiency virus (HIV) is the etiological agent of acquired immunodeficiency syndrome (AIDS). The viral-encoded protease has emerged as one of the most interesting targets for therapeutic agents.[1] Infection by HIV is initiated when its envelope protein gp120 binds to the T-cell surface glycoprotein CD4.[2] The amino acid Ser^{42}—Ser^{49} region of the CD4 V1 domain is important in binding to gp120.[3] It was also reported that the CPFs (*N*-carbomethoxycarbonyl-prolyl-phenylalanine benzyl esters) bind to gp120.[4]

In this paper, we designed and synthesized some dipeptide-type HIV protease inhibitor derivatives of KNI-102[5] which were modified by linking them to a CPF moiety. We expected that the modified compounds would bind selectively to infected cells, owing to the interaction of CPF and gp120, and inhibit the HIV protease.

Results and Discussion

The synthesis of the dipeptide-type HIV protease inhibitors is summarized in Chart 1. The key compound Z-(2*S*,3*S*) AHPA (**2**; AHPA = 3-amino-2-hydroxy-4-phenylbutyric acid) was synthesized from Z-Phe.[6] Compounds **3** and **4** were synthesized in reasonable yields. The compounds modified on the phenoxyacetyl group were obtained as follows. Nitrophenoxyacetates **5a—c** were prepared from the corresponding nitrophenols and *tert*-butyl bromoacetate. They were deprotected with trifluoroacetic acid (TFA) to give carboxylic acids. Coupling of the carboxylic acids and deprotected **3** by the water soluble carbodiimide (WSC)-1-hydroxy-1*H*-benzotriazol (HOBt) coupling method[7] in the presence of 4-dimethylaminopyridine (DMAP) and triethylamine (TEA) in *N*,*N*-dimethylformamide (DMF) gave the modified inhibitors with a nitrophenoxyacetyl group at *N*-terminus (**6a—c**). Reduction then gave the aminophenoxy type compounds **7a—c**.

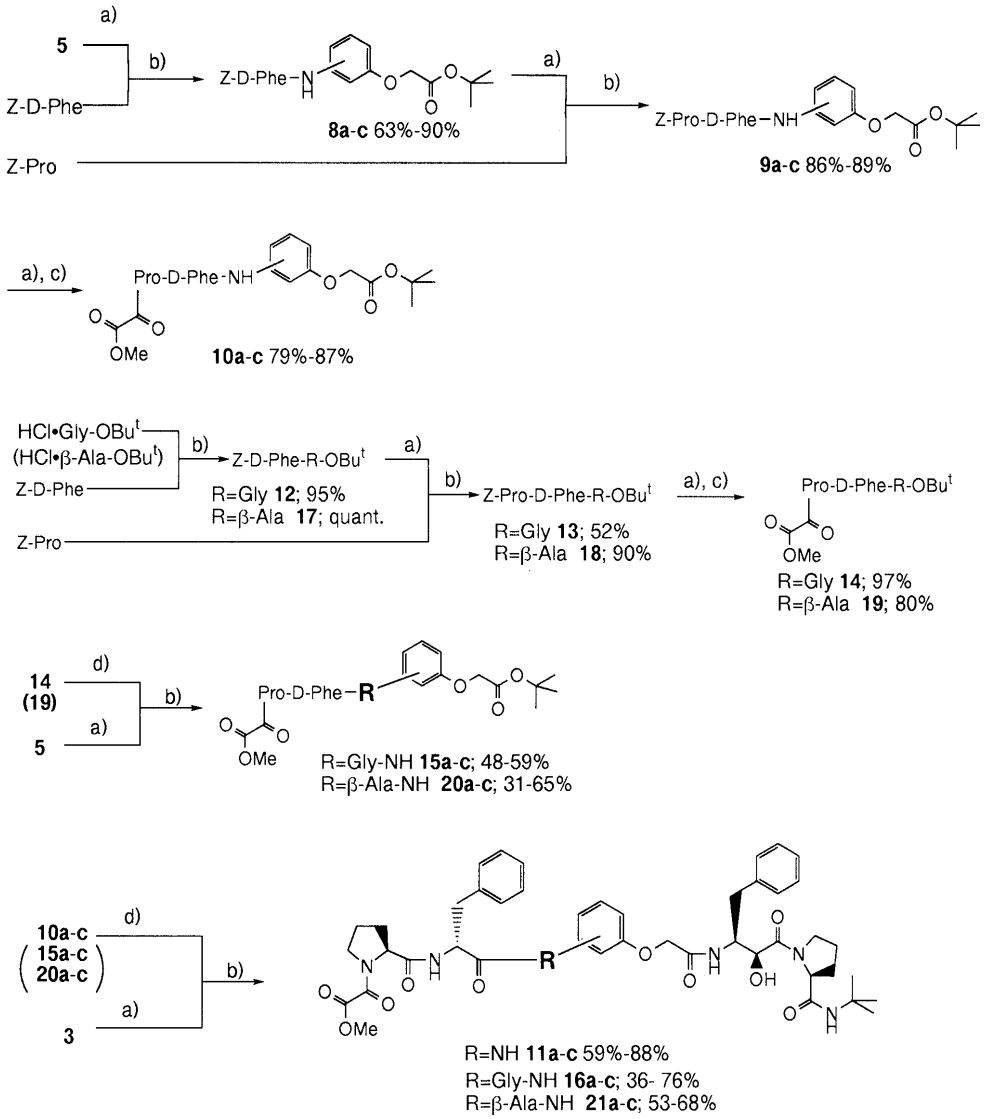
As shown in Chart 2, compounds **11a—c**, **16a—c** and **21a—c**, which are modified dipeptide-type HIV protease inhibitors linked with a binding site to gp120, were synthesized. Nitrophenoxy acetates **5** were reduced with Pd/C–H_2 followed by the coupling with Z-D-Phe, Z-L-Pro and methyloxalyl chloride to give the CPF derivatives **10a—c** which have no spacer between Phe and phenoxyacetate. They were treated with TFA and coupled with deprotected dipeptide **3** to obtain **11a—c** bearing CPF on the phenoxyacetyl group.



Reagents a) Pd/C-H_2/MeOH; b) WSC•HCl, HOBt•H_2O, DMAP, TEA/DMF; c) $PhOCH_2CO$-Cl, TEA/CH_2Cl_2; d) TFA/CH_2Cl_2;

Chart 1. Synthesis of Dipeptide-Type HIV Protease Inhibitor Derivatives

* To whom correspondence should be addressed.



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

Chart 2. Synthesis of Dipeptide-Type HIV Protease Inhibitor Linked with a CPF Moiety

Coupling of Gly–OBu^t or β-Ala–OBu^t with Z-D-Phe and Z-L-Pro was performed by the method described above to give the tripeptide **14** or **19**. Compound **14** or **19** was treated with methyloxalyl chloride after removal of the Z-group to obtain **15** or **20**. Coupling of the CPF moiety **15** or **20** and dipeptide **3** was carried out according to the described method to give **16** or **21** with a Gly or β-Ala residue as a spacer between CPF and the protease inhibitor.

HIV Protease Inhibitory Activities of the Derivatives with a Binding Unit to gp120 HIV protease-inhibitory activities were 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.[8,9] The standard compounds **3** and **4** showed moderate inhibitory activities (**3**; $IC_{50}=3.7\,\mu g/ml$, $7.7\,\mu M$), while some of the modified compounds were more potent (Table 1). The compounds bearing an amino group on the phenoxyacetyl moiety generally showed higher inhibitory activity than standard type **3** and **4**. Compound **11a** showed 7 times higher inhibitory activity ($IC_{50}=0.9\,\mu g/ml$, $1.1\,\mu M$) than **3**. The other *ortho*-substituted compounds **7a**, **16a** and **21a** also showed several times higher inhibitory activities than **3**. In conclusion, among these dipeptide-type HIV protease inhibitor derivatives bearing the CPF moiety, the *ortho*-substituted amine or amide compounds exhibited the most potent inhibitory activities against HIV protease. The reason why compound **11a** showed higher inhibitory activity is unclear, but the CPF moiety on the phenoxyacetyl group might act in part as a HIV protease inhibitor.

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 IR 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). Precoated thin-layer chromatography (TLC) plates (Silica gel 60-F_{254}) (Merck) were used to

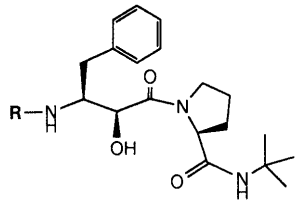
monitor the reaction and to ascertain the purity of the reaction products. The spots were detected by spraying the plates with 5% aqueous sulfuric acid and 10% 12-molybdo(VI) phosphoric acid *n*-hydrate in EtOH solution and then heating. Physical data for all intermediates are shown in Table 2. IR spectra data, ^{1}H-NMR spectra data and elemental analysis data support the structures.

General Procedure for Synthesis of Peptide by WSC Coupling Method. Synthesis of *N*-Carbobenzoxy-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-L-proline *tert*-Butyl Amide (3) A solution of Z-ProNHBu^t (**1**) (1.52 g, 5.01 mmol) and Pd/C (300 mg) in MeOH was stirred for 10 h under an H_2 atmosphere. After filtration and evaporation, the residue was redissolved in DMF (30 ml). To this solution, Z-(2*S*,3*S*) AHPA (**2**) (1.50 g, 4.55 mmol), WSC·HCl (960 mg, 5.01 mmol), HOBt·H_2O (770 mg, 5.01 mmol), DMAP (100 mg) and TEA (0.70 ml, 5.01 mmol) were added at 0 °C. The reaction mixture was stirred for 8 h at room temperature under an argon atmosphere. After removal of DMF, the residue was redissolved in CH_2Cl_2, and the mixture was washed with saturated aqueous $NaHCO_3$, and 10% aqueous citric acid and saturated aqueous NaCl, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel (*n*-hexane : EtOAc = 1 : 1) to give a white powder (1.82 g, 75%). mp 64—65 °C. $[\alpha]_D^{28}$ −33.0° (*c* = 1.0, $CHCl_3$). *Anal.* Calcd for $C_{27}H_{35}N_3O_5$: C, 67.33; H, 7.32; N, 8.72. Found: C, 66.37; H, 7.42; N, 8.64. FAB-MS (*m/z*): 482 $(M+H)^+$, 504 $(M+Na)^+$. IR (KBr) cm^{-1}: 3336 (OH), 2964 (CH_3), 1683 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.29 (s, 9H, *tert*-Bu), 1.96—2.36(m, 4H, CH_2CH_2), 2.68 (2H, d, *J* = 7.9 Hz, AHPA; C<u>H</u>$_2$Ph), 3.86 (2H, m, NCH_2), 4.16 (1H, m, NHCH), 4.51 (1H, m, Pro; CH), 4.99 (2H, s, OC<u>H</u>$_2$Ph), 5.20 (1H, d, *J* = 8.9 Hz, C<u>H</u>OH), 6.44 (1H, br s, N<u>H</u>*tert*-Bu), 7.10—7.38 (10H, m, aromatic-H).

Phenoxyacetyl-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-L-proline *tert*-Butyl Amide (4) A solution of **3** (150 mg, 0.31 mmol) and Pd/C (70 mg) in MeOH was stirred for 10 h under an H_2 stream. After filtration and evaporation, the residue was redissolved in CH_2Cl_2 (30 ml), and to the solution, TEA (35 mg, 0.34 mmol) and phenoxyacetyl chloride (58 mg, 0.34 mmol) were added at 0 °C and the reaction mixture was stirred for 8 h at room temperature under an argon atmosphere. The reaction mixture was washed with saturated aqueous $NaHCO_3$, and 10% aqueous citric acid and saturated aqueous NaCl, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel (*n*-hexane : EtOAc = 1 : 3) to give a white powder (92 mg, 61%). mp 59—62 °C. $[\alpha]_D^{31}$ −46.4° (*c* = 0.5, $CHCl_3$). FAB-MS (*m/z*): 482 $(M+H)^+$. IR (KBr) cm^{-1}: 3600—3100 (OH), 2958 (CH_3), 1680, 1666 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.30 (9H, s, *tert*-Bu), 1.96—2.36 (4H, m, CH_2CH_2), 2.74 (2H, m, AHPA; C<u>H</u>$_2$Ph), 3.73 (1H, m, AHPA; NHC<u>H</u>), 3.81 (2H, m, NCH_2), 4.48 (3H, m, OCH_2, AHPA; C<u>H</u>OH), 4.58 (1H, m, Pro; CH), 6.45(1H, br s, N<u>H</u>*tert*-Bu), 6.80—7.30 (10H, m, aromatic-H).

2-Nitrophenoxyacetyl-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-L-proline *tert*-Butyl Amide (6a) A solution of **5a** (116 mg, 0.46 mmol) and TFA (2.5 ml) in CH_2Cl_2 (10 ml) was stirred for 4 h. Evaporation of the mixture gave the carboxylic acid as a white powder. A solution of **3** (200 mg, 0.42 mmol) in MeOH containing Pd/C (100 mg) was stirred for 5 h under an H_2 atmosphere. Filtration and evaporation gave the deprotected amine. The carboxylic acid and amine were dissolved in DMF, and to this solution, WSC·HCl (88 mg, 0.46 mmol), HOBt·H_2O (70 mg, 0.46 mmol), DMAP (10 mg) and TEA (50 mg 5.00 mmol) were

Table 1. HIV Protease-inhibitory Activity of Dipeptide-Type Protease Inhibitors Having a CPF Derivative as a Binding Unit to gp120



Compound	R[a]	IC_{50} (μg/ml)[b,c]	IC_{50} (μM)[b,c]
3	Z-	3.7	7.7
4	PA-	74.8	155
6a	*o*-NO_2-PA-	40	76
6b	*m*-NO_2-PA-	28	43
6c	*p*-NO_2-PA-	24	56
7a	*o*-NH_2-PA-	2.7	5.4
7b	*m*-NH_2-PA-	13	26
7c	*p*-NH_2-PA-	1.8	3.6
11a	*O*-CPF-NH-PA	0.90	1.1
11b	*m*-CPF-NH-PA	28	34
11c	*p*-CPF-NH-PA	n.d.	n.d.
16a	*O*-CPF-Gly-NH-PA	2.0	2.3
16b	*m*-CPF-Gly-NH-PA	22	25
16c	*p*-CPF-Gly-NH-PA	17	19
21a	*o*-CPF-β-Ala-NH-PA	1.2	1.3
21b	*m*-CPF-β-Ala-NH-PA	10	11
21c	*p*-CPF-β-Ala-NH-PA	30	33

a) Z- = benzyloxycarbonyl-, PA- = phenoxyacetyl-, CPF- = *N*-carbomethoxy carbonyl-L-prolyl-D-phenylalanyl-; *b*) n.d. = not determined; *c*) HIV-protease inhibitory activity was determined by HPLC screening of the enzymatic reaction between HIV protease and Ser–Gln–Asn–Tyr/Pro–Ile–Val.[8]

Table 2. Physical Data for Intermediates

Compound	mp (°C)	$[\alpha]_D$	FAB-MS (*m/z*)
8a	35—37	$[\alpha]_D^{28}$ +6.4° (*c* = 1.1; $CHCl_3$)	505 $(M+H)^+$
8b	70—74	$[\alpha]_D^{26}$ +7.5° (*c* = 1.1; $CHCl_3$)	505 $(M+H)^+$
8c	107	$[\alpha]_D^{26}$ −3.0° (*c* = 1.0; $CHCl_3$)	505 $(M+H)^+$
9a	38—42	$[\alpha]_D^{26}$ −37.2° (*c* = 1.0; $CHCl_3$)	602 $(M+H)^+$
9b	57—60	$[\alpha]_D^{27}$ +25.8° (*c* = 1.1; $CHCl_3$)	602 $(M+H)^+$
9c	105—108	$[\alpha]_D^{27}$ +30.8° (*c* = 1.0; $CHCl_3$)	602 $(M+H)^+$
10a	58—60	$[\alpha]_D^{28}$ −11.2° (*c* = 1.0; $CHCl_3$)	554 $(M+H)^+$, 576 $(M+Na)^+$
10b	63—65	$[\alpha]_D^{28}$ +79.7° (*c* = 1.0; $CHCl_3$)	554 $(M+H)^+$, 576 $(M+Na)^+$
10c	65—66	$[\alpha]_D^{28}$ +78.5° (*c* = 1.0; $CHCl_3$)	554 $(M+H)^+$, 576 $(M+Na)^+$
12	82—84	$[\alpha]_D^{28}$ +1.5° (*c* = 1.0; $CHCl_3$)	413 $(M+H)^+$
13	68—70	$[\alpha]_D^{28}$ −22.0° (*c* = 1.0; $CHCl_3$)	510 $(M+H)^+$
14	83—86	$[\alpha]_D^{28}$ −4.8° (*c* = 1.0; $CHCl_3$)	417 $(M+H)^+$
15a	70—73	$[\alpha]_D^{29}$ +4.3° (*c* = 1.0; $CHCl_3$)	611 $(M+H)^+$
15b	93—95	$[\alpha]_D^{29}$ +3.2° (*c* = 1.0; $CHCl_3$)	611 $(M+H)^+$, 633 $(M+Na)^+$
15c	98—100	$[\alpha]_D^{29}$ +6.9° (*c* = 1.0; $CHCl_3$)	611 $(M+H)^+$, 633 $(M+Na)^+$
17	81—82	$[\alpha]_D^{27}$ −4.4° (*c* = 1.0; $CHCl_3$)	427 $(M+H)^+$
18	104—106	$[\alpha]_D^{29}$ −26.6° (*c* = 1.0; $CHCl_3$)	524 $(M+H)^+$, 546 $(M+Na)^+$
19	50—52	$[\alpha]_D^{30}$ −11.1° (*c* = 1.0; $CHCl_3$)	476 $(M+H)^+$
20a	45—47	$[\alpha]_D^{30}$ −6.1° (*c* = 0.5; $CHCl_3$)	625 $(M+H)^+$
20b	Oil	$[\alpha]_D^{30}$ +6.2° (*c* = 0.5; $CHCl_3$)	625 $(M+H)^+$
20c	178—179	$[\alpha]_D^{30}$ +9.2° (*c* = 1.0; $CHCl_3$)	625 $(M+H)^+$

added at 0 °C. The whole was stirred for 4 h at room temperature under an argon atmosphere. After removal of DMF, the residue was redissolved in CH_2Cl_2, and the mixture was washed with saturated aqueous $NaHCO_3$, and 10% aqueous citric acid and saturated aqueous NaCl, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel (CH_2Cl_2 : MeOH = 40 : 1) to give colorless needles (165 mg, 76%). mp 133—135 °C. $[\alpha]_D^{28}$ −61.3° (c = 0.8, $CHCl_3$). FAB-MS (m/z): 527 $(M+H)^+$. IR (KBr) cm^{-1}: 3382 (OH), 2966 (CH_3), 1671 (C=O), 1523 (N=O). ^{1}H-NMR ($CDCl_3$) δ: 1.30 (9H, s, *tert*-Bu), 1.91—2.43 (4H, m, CH_2CH_2), 2.71—2.96 (2H, m, AHPA; C<u>H</u>$_2$Ph), 3.73—3.91(3H, m, Pro; NCH_2, AHPA; NHC<u>H</u>), 4.43 (3H, m, OCH_2, AHPA; C<u>H</u>OH), 4.54 (1H, m, Pro; CH), 6.50 (1H, br s, N<u>H</u>*tert*-Bu), 6.91—8.06 (9H, m, aromatic-H).

6b (*m*-Form Compound): Amorphous powder. mp 66—67 °C. $[\alpha]_D^{28}$ −32.0° (c = 1.0, $CHCl_3$). FAB-MS (m/z): 527 $(M+H)^+$.

6c (*p*-Form Compound): White powder. mp 76—79 °C. $[\alpha]_D^{28}$ −35.5° (c = 1.0, $CHCl_3$). FAB-MS m/z): 527 $(M+H)^+$.

2-Aminophenoxyacetyl-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-L-proline *tert*-Butyl Amide (7a) A solution of **6a** (135 mg, 0.26 mmol) and Pd/C (50 mg) in MeOH was stirred for 10 h under an H_2 atmosphere. After filtration and evaporation, the residue was crystallized to give a white powder (130 mg, quant.). mp 70—72 °C. $[\alpha]_D^{28}$ −32.1° (c = 0.5, $CHCl_3$). FAB-MS (m/z): 497 $(M+H)^+$. IR (KBr) cm^{-1}: 3600—3200 (OH, NH_2), 2970 (CH_3), 1653 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.30 (9H, s, *tert*-Bu), 1.92—2.34 (4H, m, CH_2CH_2), 2.73 (2H, m, C<u>H</u>$_2$Ph), 3.79 (2H, br s, NH_2), 3.80 (2H, m, Pro; NCH_2), 4.34 (1H, m, AHPA; C<u>H</u>OH), 4.46 (2H, s, OCH_2), 4.51 (1H, m, AHPA; NHC<u>H</u>), 4.68 (1H, m, Pro; CH), 6.45(1H, br s, N<u>H</u>*tert*-Bu), 6.56—7.26 (9H, m, aromatic-H, AHPA; NH).

7b (*m*-Form Compound): mp 76—79 °C. $[\alpha]_D^{28}$ −47.1° (c = 0.5, $CHCl_3$). FAB-MS (m/z): 497 $(M+H)^+$.

7c (*p*-Form Compound): mp 74—77 °C. $[\alpha]_D^{28}$ −51.3° (c = 0.5, $CHCl_3$). FAB-MS (m/z): 497 $(M+H)^+$.

***tert*-Butyl 2-(*N*-Carbomethoxycarbonyl-L-prolyl-D-phenylalanine amide)phenoxyacetate (10a)** A solution of **9a** (1.80 g, 2.99 mmol) and Pd/C (500 mg) in MeOH was stirred for 4 h under an H_2 atmosphere. After filtration and evaporation, the residue was dissolved in CH_2Cl_2. To this solution, methyloxalyl chloride (0.36 ml, 3.29 mmol) and TEA (0.50 ml, 3.59 mmol) were added at 0 °C and stirred for 3 h at room temperature under an argon atmosphere. The mixture was washed with saturated aqueous $NaHCO_3$, and 10% aqueous citric acid and saturated aqueous NaCl, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel (*n*-hexane : EtOAc = 2 : 3) to give an amorphous powder (1.39 g, 84%). mp 58—60 °C. $[\alpha]_D^{28}$ −11.2° (c = 1.0, $CHCl_3$). FAB-MS (m/z): 554 $(M+H)^+$, 576 $(M+Na)^+$. IR (KBr) cm^{-1}: 3294 (NH), 2972 (CH_3), 1739, 1653 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s, *tert*-Bu), 1.65—2.18 (4H, m, CH_2CH_2), 3.09—3.32 (2H, m, C<u>H</u>$_2$Ph), 3.65 (2H, m, Pro; NCH_2), 3.67, 3.87 (3H, each s, OCH_3), 4.51 (2H, s, OCH_2CO), 4.53 (1H, m, Pro; CH), 4.88 (1H, m, Phe; CH), 6.53 (1H, br s, Phe; NH), 6.79—7.26, 8.26 (9H, m, aromatic-H), 8.78, 8.86 (1H, each br s, N<u>H</u>Ph).

2-(*N*-Carbomethoxycarbonyl-L-prolyl-D-phenylalanine amide)phenoxyacetyl-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-L-proline *tert*-Butyl Amide (11a) A solution of **10a** (253 mg, 0.46 mmol) and TFA (2 ml) in CH_2Cl_2 (12 ml) was stirred for 3 h. Evaporation of the mixture gave the carboxylic acid as an amorphous powder. A solution of **3** (200 mg, 0.42 mmol) and Pd/C (70 mg) in MeOH was stirred for 5 h under an H_2 atmosphere. Filtration and evaporation gave the deprotected amine. The carboxylic acid and amine were dissolved in DMF (10 ml), and to this solution, WSC·HCl (88 mg, 0.46 mmol), HOBt·H_2O (70 mg, 0.46 mmol), DMAP (10 mg) and TEA (50 mg, 0.50 mmol) were added at 0 °C. The whole was stirred for 4 h at room temperature under an argon atmosphere. After removal of DMF, the mixture was redissolved in CH_2Cl_2, and the mixture was washed with saturated aqueous $NaHCO_3$, and 10% aqueous citric acid and saturated aqueous NaCl, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel (CH_2Cl_2 : MeOH = 30 : 1) to give a white powder (223 mg, 59%). mp 105—107 °C. $[\alpha]_D^{28}$ −41.3° (c = 0.6, $CHCl_3$). FAB-MS (m/z): 827 $(M+H)^+$. IR (KBr) cm^{-1}: 3290 (OH), 2968 (CH_3), 1740, 1654, 1542 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.29 (9H, s, *tert*-Bu), 1.75—2.40 (8H, m, Pro; CH_2CH_2), 2.60—3.37 (4H, m, Phe; AHPA; C<u>H</u>$_2$Ph), 3.70, 3.74 (3H, each s, OCH_3), 3.60—3.85 (4H, m, Pro; NCH_2), 4.05 (1H, m, AHPA; NHC<u>H</u>), 4.34—4.55 (2H, m, Pro; CH), 4.46 (2H, s, OCH_2CO), 4.85 (2H, m, Phe; CH, AHPA; C<u>H</u>OH), 6.43 (1H, br s, N<u>H</u>*tert*-Bu), 6.55 (1H, br s, Phe; NH), 6.74—8.02 (14H, m, aromatic-H), 8.65 (1H, br s, N<u>H</u>Ph).

11b (*m*-Form Compound): White powder. mp 114—117 °C. $[\alpha]_D^{28}$ +12.3° (c = 1.0, $CHCl_3$). FAB-MS (m/z): 827 $(M+H)^+$.

11c (*p*-Form Compound): White powder. mp 119—120 °C. $[\alpha]_D^{27}$ +19.9° (c = 1.0, $CHCl_3$). FAB-MS (m/z): 827 $(M+H)^+$.

***tert*-Butyl 2-(*N*-Carbomethoxycarbonyl-L-prolyl-D-phenylalanylglycine amide)phenoxyacetyl-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl]-L-proline *tert*-butyl Amide (16a)** White powder. mp 120—122 °C. $[\alpha]_D^{30}$ −54.9° (c = 0.5, $CHCl_3$). FAB-MS (m/z): 884 $(M+H)^+$, 906 $(M+Na)^+$. IR (KBr) cm^{-1}: 3356 (NH), 3304 (OH), 3260 (Ph), 2964 (CH_3), 1741, 1657 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.26 (9H, s, *tert*-Bu), 1.70—2.20 (8H, m, Pro; $CH_2CH_2 \times 2$), 2.65—3.25 (4H, m, C<u>H</u>$_2$Ph × 2), 3.61 (2H, m, Gly; CH_2), 3.74 (3H, s, OCH_3), 3.77 (4H, m, Pro; $NCH_2 \times 2$), 3.75—4.30 (3H, m, Pro; CH × 2, AHPA; C<u>H</u>CH_2Ph), 4.46 (2H, m, OCH_2CO), 4.60 (1H, m, AHPA; C<u>H</u>OH), 4.70 (1H, m, Phe; CH), 6.60—7.38 (14H, m, aromatic-H), 8.15 (1H, m, PhN<u>H</u>).

16b (*m*-Form Compound): White powder. mp 118—120 °C. $[\alpha]_D^{30}$ −38.2° (c = 0.5, $CHCl_3$). FAB-MS (m/z): 884 $(M+H)^+$.

16c (*p*-Form Compound): White powder. mp 119—120 °C. $[\alpha]_D^{30}$ −25.4° (c = 0.5, $CHCl_3$). FAB-MS (m/z): 884 $(M+H)^+$.

2-(*N*-Carbomethoxycarbonyl-L-prolyl-D-phenylalanyl-β-alanine amide)-phenoxyacetyl-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenyl butyryl]-L-proline *tert*-Butyl Amide (21a) White powder. mp 116—119 °C. $[\alpha]_D^{30}$ −37.7° (c = 1.0, $CHCl_3$). *Anal.* Calcd for $C_{47}H_{59}N_7O_{11}$: C, 62.86; H, 6.62; N, 10.92. Found: C, 62.77; H, 6.38; N, 11.27. FAB-MS (m/z): 898 $(M+H)^+$. IR (KBr) cm^{-1}: 3600—3200 (OH), 2962 (CH_3), 1650 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 1.29 (9H, s, *tert*-Bu), 1.75—2.35(8H, m, Pro; CH_2CH_2), 2.68 (2H, m, β-Ala; CH_2CO), 2.80—3.15 (4H, m, C<u>H</u>$_2$Ph), 3.60 (2H, m, β-Ala; NHC<u>H</u>$_2$–), 3.72 (4H, m, Pro; NCH_2), 3.77 (3H, s, OCH_3), 4.15 (1H, m, AHPA; NHC<u>H</u>), 4.25 (2H, m, Pro; CH), 4.45 (2H, s, $OC\underline{H}_2CO$), 4.53—4.65 (2H, m, Phe; CH, AHPA; C<u>H</u>OH), 6.45 (1H, br s, N<u>H</u>*tert*-Bu), 6.55 (1H, br s, Phe; NH), 6.65—8.05 (14H, m, aromatic-H), 8.35 (1H, br s, N<u>H</u>Ph).

21b (*m*-Form Compound): White powder. mp 108—111 °C. $[\alpha]_D^{30}$ −30.2° (c = 0.5, $CHCl_3$). *Anal.* Calcd for $C_{47}H_{59}N_7O_{11}$: C, 62.86; H, 6.62; N, 10.92. Found: C, 62.96; H, 6.68; N, 11.05. FAB-MS (m/z): 898 $(M+H)^+$.

21c (*p*-Form Compound): White powder. mp 121—122 °C. $[\alpha]_D^{30}$ −25.7° (c = 0.5, $CHCl_3$). *Anal.* Calcd for $C_{47}H_{59}N_7O_{11}$: C, 62.86; H, 6.62; N, 10.92. Found: C, 62.64; H, 6.50; N, 11.21. FAB-MS (m/z): 898 $(M+H)^+$.

Acknowledgements We are grateful to Dr. K. Dobashi, Central Research Laboratories of Mercian Corporation for measuring the HIV protease-inhibitory activity.

References and Notes

1) Huff J. R., *J. Med. Chem.*, **34**, 2305—2314 (1991).
2) McDougal J. S., Kennedy M. S., Sligh J. M., Cort S. P., Mawle A., Nicholson J. K. A., *Science*, **231**, 382—385 (1986).
3) Bowman M. R., Macferrin K. D., Schreiber S. L., Burakoff S. J., *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 9052—9056 (1990).
4) *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**, 287—291 (1990); *b*) Moore J. P., Simpson G., McKeating J. A., Burakoff S. J., Schreiber S. L., Weiss R. A., *Virology*, **188**, 537—544 (1992); *c*) Venkatramani L., Hossain M. B., Van der Helm D., *Int. J. Pept. Prot. Res.*, **43**, 520—528 (1994).
5) *a*) Mimoto T., Imai J., Tanaka S., Hattori N., Kasanuki S., Akaji K., Kiso Y., *Chem. Pharm. Bull.*, **39**, 3088—3090 (1991); *b*) Mimoto T., Imai J., Kasanuki S., Mimoto H., Hattori N., Akaji K., Kiso Y., *ibid.*, **40**, 2251—2253 (1992); *c*) Kiso Y., *Biopolymers*, **40**, 235—244 (1996).
6) Takita T., Suda H., Aoyagi T., Umezawa H., *J. Med. Chem.*, **20**, 510—515 (1977).
7) *a*) Bures L., Bostik J., Motycka K., Spundova M., Rehak L., *Neoplasma*, **35**, 329—342 (1988); *b*) Bures L., Lichy A., Bostik J., Spundova M., *ibid.*, **37**, 225—231 (1990).
8) The assay was carried out by the Central Research Laboratories of Mercian Corporation.
9) The binding activity to gp120 is under examination.