Studies of HIV-1 Protease Inhibitors. I. Incorporation of a Reduced Peptide, Simple Aminoalcohol, and Statine Analog at the Scissile Site of Substrate Sequences

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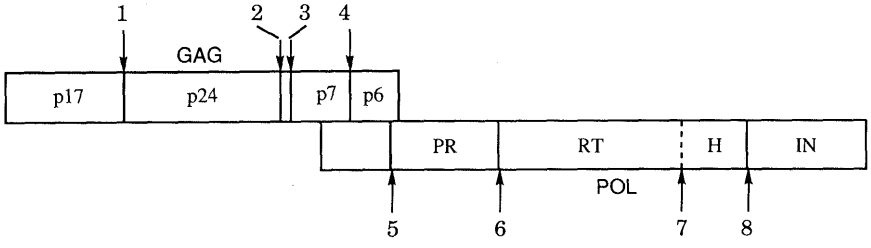
Inhibitors of the protease of human immunodeficiency virus type-1 (HIV-1) were designed and synthesized. A reduced peptide, simple aminoalcohol, and statine analog, 4-amino-3-hydroxy-5-phenylpentanoic acid (AHPPA), were inserted at the scissile site of substrate sequences of HIV-1 protease. While both reduced peptides and simple aminoalcohol derivatives were weak inhibitors, the peptides containing AHPPA demonstrated moderate inhibitory activity. The more potent alcohol configuration of AHPPA is (*R*), which is opposite to the configuration in potent inhibitors of other aspartic proteases. In particular, compound 28 ((3*R*,4*S*)-4-(*N*-*tert*-butoxycarbonyl-L-glutaminyl-L-asparaginyl)amino-3-hydroxy-5-phenylpentanoic acid 2′-methylbutylamide) had a K_i of 0.36 μM and exhibited excellent enzyme specificity.

Keywords AIDS; HIV-1 protease inhibitor; 4-amino-3-hydroxy-5-phenylpentanoic acid; reduced peptide; simple aminoalcohol; pepstatin A

The number of patients with acquired immunodeficiency syndrome (AIDS) has rapidly increased since the disease was first identified in the early 1980s. AIDS is provoked by a defect of the human immune system due to preferential infection of T4 lymphocytes by human immunodeficiency virus type 1 (HIV-1),[1] resulting in death at a high rate owing to opportunistic infections and neurologic and neoplastic diseases. Drugs used against AIDS are, so far, only azidothymidine (AZT) and dideoxyinosine (DDI), which inhibit the reverse transcriptase of HIV-1. However, serious adverse reactions to AZT[2] and the appearance of AZT-resistant strains[3] have been reported. Therefore, new types of drugs with different mechanisms from AZT and with lower toxicity are required.

HIV-1 encodes its own protease which proteolytically processes big precursor *gag* and *pol* proteins to form the mature proteins needed for production of infectious viral particles. This HIV-1 protease had been assumed to be an aspartic protease since the characteristic aspartic protease sequence, Asp–Thr–Gly, is conserved[4] and the protease is inhibited by pepstatin A (Iva–Val–Val–Sta–Ala–Sta–OH; Iva = isovaleryl, Sta = statine),[5] which is a well-known aspartic protease inhibitor. Several X-ray crystal structure analyses have established that HIV-1 protease is a homodimer of two identical peptides with 99 amino acid residues.[6] In addition, several groups have analyzed the complexes between the protease and its inhibitors by X-ray crystallography and have reported their binding modes.[7] Moreover, replacement of the catalytically active residues in protein precursors by site-directed mutagenesis led to the formation of immature, noninfective virions.[8] For this reason, selective inhibition of HIV-1 protease is regarded as a promising approach for treating AIDS and related diseases. Therefore, numerous laboratories are searching for HIV-1 protease inhibitors.[9]

Herein, we report our initial approach to develop HIV-1 protease inhibitors, involving the synthesis of three types of inhibitors: reduced peptide, simple aminoalcohol and 4-amino-3-hydroxy-5-phenylpentanoic acid (AHPPA) derivatives.



1	p17 * p24	Ser-Gln-Asn-**TYR** * **PRO**-Ile-Val-Gln
2	p24 * X	Ala-Arg-Val-**LEU** * **ALA**-Glu-Ala-Met
3	X * p7	Ala-Thr-Ile-**MET** * **MET**-Gln-Arg-Gly
4	p7 * p6	Pro-Gly-Asn-**PHE** * **LEU**-Gln-Ser-Arg
5	* PR	Ser-Phe-Asn-**PHE** * **PRO**-Gln-Ile-Thr
6	PR * RT	Thr-Leu-Asn-**PHE** * **PRO**-Ile-Ser-Pro
7	RT51* RNaseH	Ala-Glu-Thr-**PHE** * **TYR**-Val-Asp-Gly
8	RT * IN	Arg-Lys-Ile-**LEU** * **PHE**-Leu-Asp-Gly

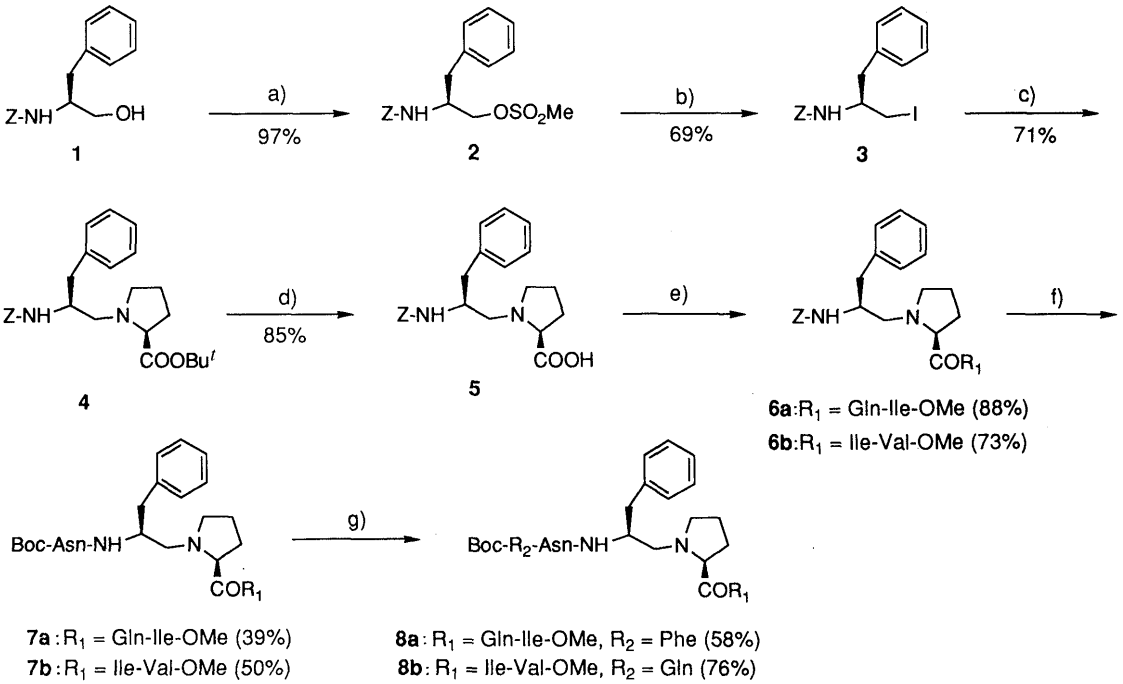
Fig. 1. Sites of Scission of the Substrates of HIV-1 Protease

PR = protease, RT = reverse transcriptase, H = ribonuclease H, IN = integrase.

Chemistry HIV-1 protease is known to cleave eight amide bonds in precursor *gag* and *gag–pol* proteins as indicated in Fig. 1.[10] Initially, we looked at the partial substrate sequences 1, 5 and 6, because 1) few mammalian aspartic proteases cleave the peptide bond in front of a Pro residue,[11] and so the possibility exists of discovering specific HIV-1 protease inhibitors, 2) three of the eight substrate sequences are cleaved at the Tyr–Pro or Phe–Pro bond, and 3) the amino acid residue at the P_2 site, Asn, is constant. However, these three sequences may be distinguished by the amino acid residues at the P_3 and P'_2 sites. Therefore, two reduced peptides shown in Chart 1 were synthesized in order to examine the effect of polarity at the P_3 and P'_2 sites. Mesylation of *N*-benzyloxycarbonyl(Z)-L-phenylalaninol **1** was followed by conversion to the iodide **3** by use of NaI. Alkylation of L-proline *tert*-butylester by using the iodide **3** afforded the reduced Z-phenylalanylproline *tert*-butylester **4** in a good yield. Removal of the *tert*-butyl group by using 4 N hydrogen chloride in dioxane and condensation with two dipeptide esters in the presence of diphenylphosphoryl azide (DPPA)[12] and triethylamine afforded **6a** and **6b**, respectively. After removal of the Z group of **6a**, acylation with *N-tert*-butoxycarbonyl(Boc)–L-Asn–OH yielded **7a**. Acid treatment of **7a** followed by coupling with Boc–L-Phe–OH afforded the objective peptide **8a**. Another reduced peptide **8b** was prepared in the same way (Chart 1).

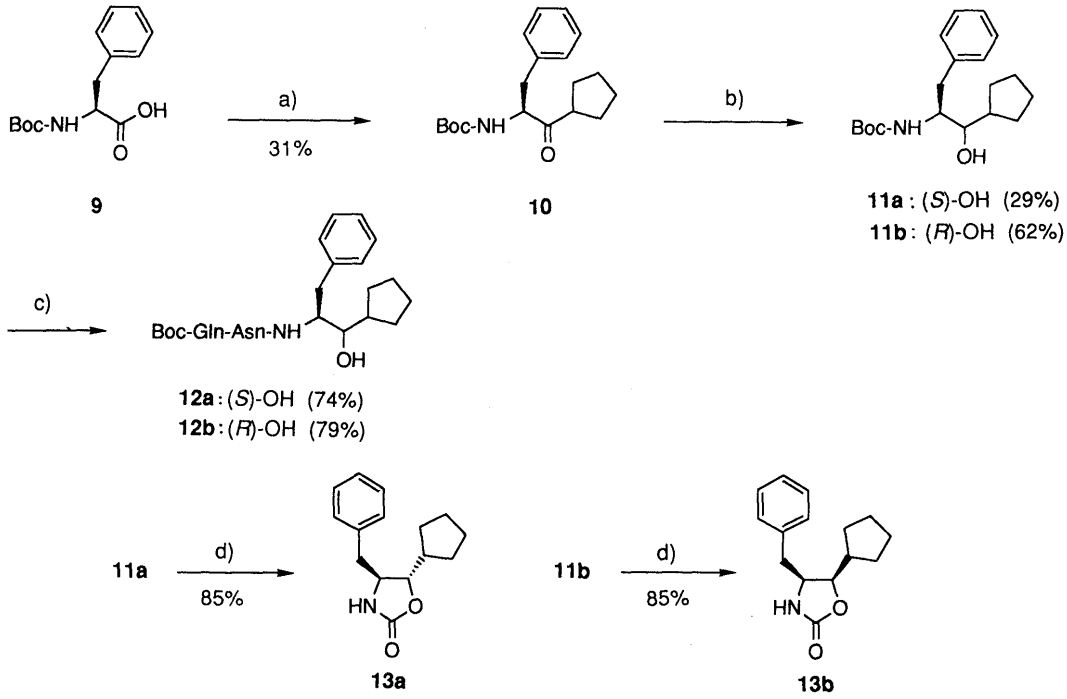
Secondly, a simple aminoalcohol, which mimics the simplest form of the transition state of the hydrolyzed Phe–Pro moiety,[13] was incorporated at the scissile site (Chart 2), since the peptides containing a simple aminoalcohol similar to compound **11a** inhibited renin, an aspartic protease like HIV-1 protease.[14] Treatment of Boc–L-Phe–OH with carbonyldiimidazole followed by the addition of cyclopentylmagnesium chloride afforded the *N*-Boc amino ketone **10** in a moderate yield.[15] Reduction of this ketone with sodium borohydride gave a mixture of α-hydroxyl and β-hydroxyl compounds (**11a** : **11b** = 1 : 2). After conversion of the alcohols **11a** and **11b** to the respective 2-oxazolidinones **13a** and **13b** by base treatment, the configuration at the chiral carbon bearing the hydroxyl group of the alcohols **11a** and **11b** could be determined from the 270 MHz ^{1}H-NMR signals of **13a** and **13b**. The coupling constant between 4H and 5H of **13a** (4.9 Hz) or **13b** (6.8 Hz) was compared with the reported data for the 4,5-*trans* (4—5 Hz) and 4,5-*cis*-oxazolidinone (7—8 Hz).[16] Removal of the Boc group and condensation of Boc–L-Gln–L-Asn–OH with DPPA and triethylamine afforded **12a** and **12b**, respectively (Chart 2).

Lastly, some peptides containing AHPPA as an analog of statine, which is an unnatural amino acid contained in pepstatin homologs, were synthesized, because a statine analog is often used as a transition state mimic. AHPPA was introduced at the scissile site of the substrate sequences 1, 5 and 6 shown in Fig. 1 because of the enzyme specificity, as mentioned above. In Chart 3, the general synthetic pathway is shown, and the synthetic procedure for compound **28** is described as an example of this series in the experimental section. Both (3*S*,4*S*)-AHPPA and (3*R*,4*S*)-AHPPA could be synthesized *via* stereoselective reduction of the corresponding ketone using 2,2′-bis-(diphenylphosphino)-1,1′-binaphthyl (BINAP)–Ru complex by the method of Nishi *et al.*[17] Boc–AHPPA–OH **14** was condensed with alcohol or amine using dicyclohexyl-



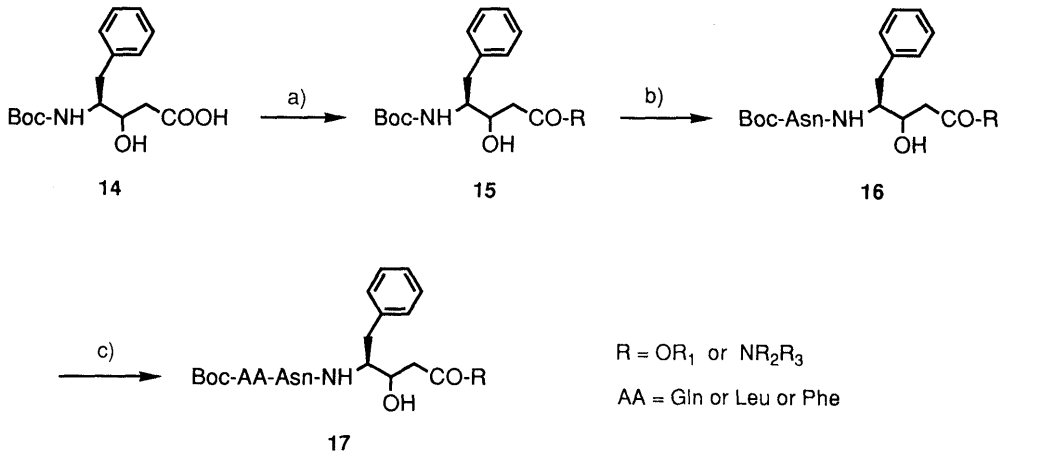
a) $MeSO_2Cl$, pyridine, 0°C b) NaI, acetone, reflux c) L-proline *tert*-butylester, Na_2CO_3, DMF, 80°C d) 4 N HCl / dioxane, r.t. e) HCl•H-Gln-Ile-OMe or HCl•H-Ile-Val-OMe, DPPA, Et_3N, DMF, 0°C f) i) Pd / C, H_2, MeOH ii) Boc-Asn-OH, DEPC, Et_3N, DMF, 4°C g) i) 4 N HCl / dioxane, r.t. ii) Boc-Phe-OH or Boc-Gln-OH, DEPC, Et_3N, DMF, 4°C

Chart 1



a) i) carbonyldiimidazole, THF, r.t. ii) cyclopentylmagnesium chloride, THF, -60°C→-20°C
b) $NaBH_4$, THF–EtOH, 0°C c) i) 4 N HCl / dioxane, r.t. ii) Boc-Gln-Asn-OH, DPPA, Et_3N, DMF, r.t.
d) NaH, DMF, r.t.

Chart 2



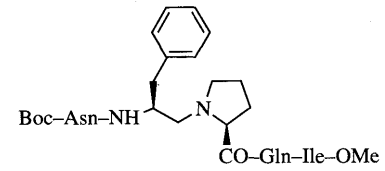
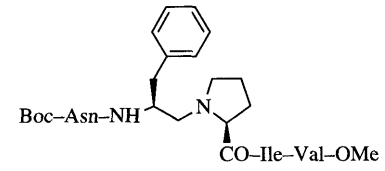
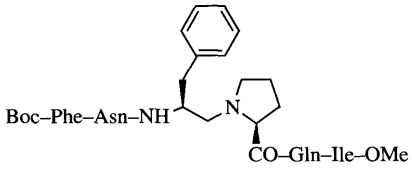
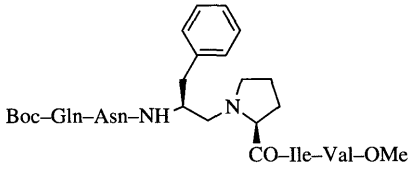
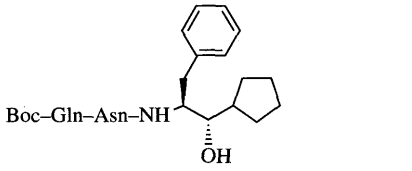
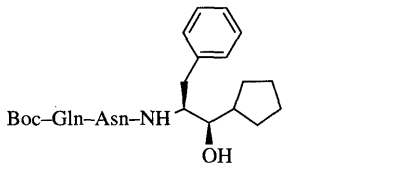
a) HO-R_1, DCC, DMAP, or HNR_2R_3, DEPC, Et_3N b) i) 4 N HCl / dioxane, r.t. ii) Boc-Asn-OH, DEPC, Et_3N, DMF, 0°C c) i) 4 N HCl / dioxane, r.t. ii) Boc-Gln-OH or Boc-Leu-OH or Boc-Phe-OH, DEPC, Et_3N, DMF, 0°C

Chart 3

carbodiimide (DCC) and 4-dimethylaminopyridine (DMAP), or diethylphosphoryl cyanide (DEPC)[18] and triethylamine, respectively. Removal of the Boc group of **15** followed by coupling with Boc–L-Asn–OH and DEPC afforded **16**. The desired compound **17** was obtained by deprotection of **16** and acylation with Boc-amino acid (Chart 3).

Inhibitory Activity and Discussion All of the HIV-1 protease inhibitors prepared here were evaluated with the recombinant *gag* substrate and HIV-1 protease. The 55 kDa *gag* protein was chosen as the substrate for the enzyme reaction, because it contains four cleavage sites (substrate sequences 1—4) as shown in Fig. 1. A typical enzyme assay is shown in Fig. 2. If an inhibitor examined is effective, the inhibition of the cleavage of 55 kDa *gag* protein is easily detected on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). In this assay, pepstatin A, which has an inhibition constant (K_i) of 1.1 μM in our assay system using the partially purified protease and synthetic substrate (Ac–Ser–Gln–Asn–Tyr–Pro–Ile–Val–NH_2), was employed as an active control. Inhibitory activity of each inhibitor in Tables I and II is given as the concentration

TABLE I. Inhibitory Activities of Reduced Dipeptide and Simple Aminoalcohol Derivatives

No.	Compound	Inhibitory activity[a] (μM)
7a		100
7b		100
8a		100
8b		10
12a		100
12b		100

a) Inhibitory activity is given as the concentration which is equipotent with 1 μM pepstatin A.

which is nearly equipotent with 1 μM pepstatin A. Although accurate comparison among the inhibitors examined is difficult in this SDS-PAGE assay, the value is close to the K_i value, as shown in this and following reports.[19]

The inhibitory activities of the reduced peptides and the simple aminoalcohol derivatives are shown in Table I. The reduced peptides possessing the P_2 to P'_3 amino acids showed some inhibitory activity (**7a** and **7b**), but the shorter peptides that lack Asn at the P_2 site were inactive (**6a** and **6b**). Compound **8a**, which contains hydrophobic Phe and hydrophilic Gln at the P_3 and P'_2 sites, respectively, was 10-fold less potent than compound **8b**, which contains hydrophilic Gln and hydrophobic Ile at the P_3 and P'_2 sites, respectively. The result revealed that the reduced peptide inhibitor derived from substrate sequence 1 was preferable to that derived from sequence 5. However, both reduced peptides were less potent than pepstatin A. This result suggested that further study of reduced peptides as HIV-1 protease inhibitors would not be worthwhile. The weak inhibitory activity is probably due to the absence of a hydroxyl group, which is crucial for the interaction with the catalytic aspartic acids in the protease.

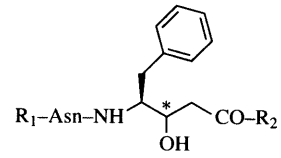
In the case of the simple aminoalcohol derivatives, both stereoisomers, compounds **12a** and **12b**, were 100-fold less potent than pepstatin A, in contrast with the successful renin inhibitors.[14] These aminoalcohol derivatives possess the important hydroxyl group, but lack the carbonyl group at the P'_1 site. This result supports the suggestion that the carbonyl group at the P'_1 site plays a crucial role in the interactions between HIV-1 protease and inhibitors bound at the active site.[7]

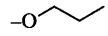
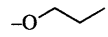
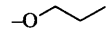
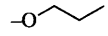
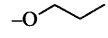
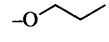
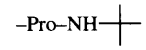
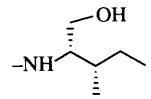
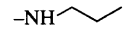
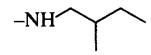
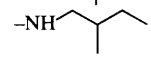
Although the peptides containing the reduced peptide and simple aminoalcohol were only weak inhibitors, we were intrigued by the result with the AHPPA derivatives (Table II). Concerning the alcohol configuration of statine analogs, (*S*)-configuration is always preferred to (*R*)-configuration among inhibitors of general aspartic proteases such as renin, pepsin and so on.[20] Moreover, Raju and Deshpande recently reported that a compound analogous to substrate sequence 2 in Fig. 1 containing (3*S*,4*S*)-AHPPA was the most potent HIV-1 protease inhibitor among the four possible stereoisomers.[9o] However, our result was inconsistent with these observations. Compound **21** with a (3*R*)-hydroxyl group was found to be 10-fold more potent than compound **19** with a (3*S*)-hydroxyl group, although both compounds **18** and **20**, which lack the P_3 amino acid, did not show inhibitory activity. Compounds **22** and **23**, which correspond to substrate sequences 5 and 6 in Fig. 1, were completely inactive. This result clearly suggests that the hydrophilic amino acid Gln is preferred to hydrophobic amino acids such as Leu and Phe at the P_3 site in this series. While introduction of Ile–Val–NH_2 or proline *tert*-butyl amide at the carboxyl terminus of AHPPA led to a decrease in potency, introduction of isoleucinol retained the inhibitory activity (**24**, **25** and **26**). This fact indicates that large groups at the carboxyl terminus of AHPPA are not appropriate for potent inhibitors. Surprisingly, conversion of the ester to an amide group improved the inhibitory activity (**21** and **27**). During further transformation at the P'_2 site, compound **28** with a 2-methylbutyl group ($K_i = 0.36$ μM) was found to have 10-fold and 3-fold greater potency than pepstatin A based on SDS-PAGE assay and K_i values, respectively. The importance of the hydroxyl configuration was confirmed by the fact that compound **29** with a (3*S*)-hydroxyl group was much less potent than compound **28**. Although there are related reports that the inhibitors containing *erythro*-hydroxyethylamine[9e,21] and -hydroxymethylcarbonyl[9m,n,p,r] isosteres corresponding to (3*R*,4*S*)-AHPPA were more potent than the respective inhibitors with the *threo* relationship, our result is the first case of its type among derivatives of statine-type peptide isosteres.

Since compound **28** has different alcohol stereochemistry from inhibitors of other aspartic proteases, it was expected to be a specific inhibitor of HIV-1 protease. In fact, IC_{50}s of compound **28** against pepsin and cathepsin D were higher than 1 mM, whereas those of compound **29**, which has the usual (*S*)-alcohol configuration, were lower than 200 μM. Moreover, 10 μM **28** gave only 8.7% inhibition of renin activity (Table III).

In conclusion, we have synthesized three different types

TABLE II. Inhibitory Activities of AHPPA Derivatives



No.	R_1	Absolute configuration at *	R_2	Inhibitory activity[a] (μM)	Formula	Analysis (%) Calcd (Found) C	H	N	mp (°C)
18	Boc–	*S*		10000	$C_{23}H_{35}N_4O_7 \cdot 0.5H_2O$	58.21 (58.63)	7.65 7.42	8.85 8.81)	156—158
19	Boc–Gln–	*S*		100	$C_{28}H_{43}N_5O_9 \cdot 0.5H_2O$	55.80 (55.85)	7.36 7.05	11.62 11.72)	223—224
20	Boc–	*R*		10000	$C_{23}H_{35}N_3O_7$	59.34 (59.10)	7.58 7.47	9.03 9.10)	198—201
21	Boc–Gln–	*R*		10	$C_{28}H_{43}N_5O_9 \cdot 0.5H_2O$	55.80 (55.96)	7.36 7.08	11.62 11.67)	234—236
22	Boc–Leu–	*R*		10000	$C_{29}H_{46}N_4O_8$	60.19 (59.78)	8.01 7.87	9.68 9.57)	203—205
23	Boc–Phe–	*R*		10000	$C_{32}H_{44}N_4O_8 \cdot 0.5H_2O$	61.82 (61.96)	7.30 7.09	9.01 9.03)	208—210
24	Boc–Gln–	*R*	–Ile–Val–NH_2	100	$C_{36}H_{58}N_8O_{10} \cdot 2H_2O$	54.12 (54.01)	7.82 7.37	14.03 13.64)	220—223
25	Boc–Gln–	*R*		100	$C_{34}H_{53}N_7O_9 \cdot 1.5H_2O$	55.87 (56.07)	7.72 7.47	13.42 13.65)	128—130
26	Boc–Gln–	*R*		10	$C_{31}H_{50}N_6O_9 \cdot 0.5H_2O$	56.43 (56.71)	7.79 7.54	12.74 12.50)	245—248
27	Boc–Gln–	*R*		1	$C_{28}H_{44}N_6O_8 \cdot 0.5H_2O$	55.89 (56.01)	7.54 7.32	13.97 13.81)	242—245
28	Boc–Gln–	*R*		0.1	$C_{30}H_{48}N_6O_8 \cdot 0.5H_2O$	57.21 (57.33)	7.84 7.54	13.35 13.20)	219—222
29	Boc–Gln–	*S*		>100	$C_{30}H_{48}N_6O_8 \cdot 0.25H_2O$	57.63 (57.60)	7.82 7.89	13.44 13.38)	224—226

a) Inhibitory activity is given as the concentration which is equipotent with 1 μM pepstatin A.

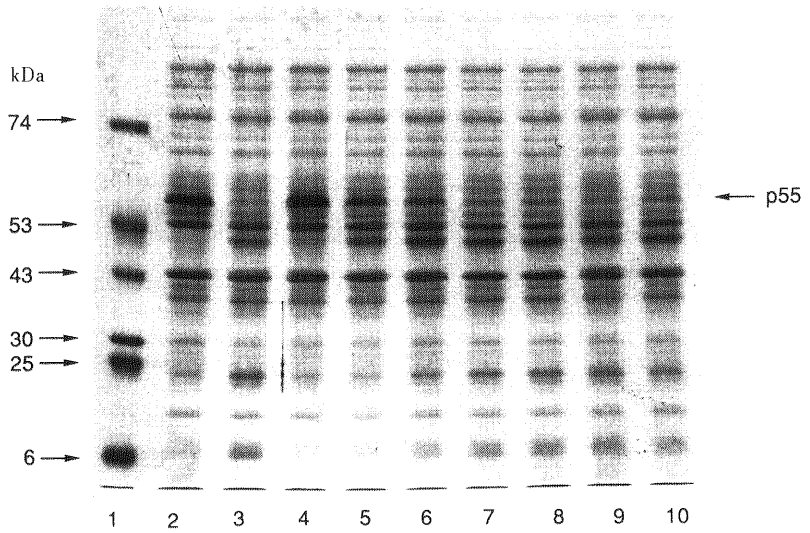


Fig. 2. The SDS-PAGE Assay

Compound **28** and pepstatin A inhibited the processing of 55 kDa *gag* protein by HIV-1 protease. The remaining amount of of p55 in lane 7 (0.1 μM **28**) was visually judged to be nearly equal to that of p55 in lane 10 (1 μM pepstatin A). Lane 1; marker, lane 2; substrate + protease, 37 °C, 0 min, lane 3; substrate + protease, 37 °C, 30 min, lanes 4, 5 and 10; substrate + protease + 100 μM (lane 4), 10 μM (lane 5), and 1 μM (lane 10) of pepstatin A, 37 °C, 30 min, lanes 6—9; substrate + protease + 1 μM (lane 6), 0.1 μM (lane 7), 0.03 μM (lane 8), and 0.01 μM (lane 9) of compound **28**, 37 °C, 30 min.

of peptides, which contain reduced peptide, simple aminoalcohol and AHPPA, as inhibitors of HIV-1 protease. In contrast with compounds containing the two former dipeptide units, the AHPPA derivatives with the unusual (*R*)-configuration at the carbon bearing the hydroxyl group demonstrated moderate inhibitory activity against HIV-1

TABLE III. Enzyme Specificity

No.	HIV-1 Protease Inhibitory activity (M)[a]	HIV-1 Protease K_i (M)	Pepsin IC_{50} (M)[b]	Cathepsin D IC_{50} (M)[c]	Renin % inhibition (M)[d]
28	1×10^{-7}	3.6×10^{-7}	1.4×10^{-3}	4.1×10^{-3}	8.7% at 10^{-5}
29	$>1\times10^{-4}$	—	6.2×10^{-5}	1.9×10^{-4}	—

a) Inhibitory activity is given as the concentration which is equipotent with 1 μM pepstatin A. *b*) Ref. 22. *c*) Ref. 23. *d*) Ref. 24.

protease. In particular, compound **28** exhibited a K_i of 0.36 μM, and showed excellent enzyme specificity. Based on our observation that (*R*)-alcohols are preferred to (*S*)-alcohols among statine-type HIV-1 protease inhibitors in some cases, we are undertaking further structural optimization in order to develop yet stronger specific HIV-1 protease inhibitors.

Experimental

Melting points were determined with a Yanagimoto melting point apparatus and are uncorrected. Infrared (IR) spectra were measured with a Nic 5SXC FT IR spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded with a JEOL JNM-GX 270 FT NMR. Chemical shifts are expressed in ppm relative to tetramethylsilane with tetramethylsilane as an internal reference. Mass spectra (MS) were obtained with a JEOL JMS-D 300 mass spectrometer. Column chromatography was carried out on Kieselgel 60 F_{254} (Merck, 70—230 mesh). Preparative thin-layer chromatographies (PTLC) were run on Kieselgel 60 F_{254} plates (Merck art. 5717 or art. 5744). The organic solutions were dried over Na_2SO_4 before vacuum evaporation.

(*S*)-2-Benzyloxycarbonylamino-1-mesyloxy-3-phenylpropane (2) Mesyl chloride (1.50 ml, 19.4 mmol) was added to a solution of benzyloxycarbonyl-L-phenylalaninol (5.0 g, 17.6 mmol) in pyridine (25 ml) at 0 °C. The mixture was stirred for 5 h at the same temperature, then the solvent was removed *in vacuo*. The residue was extracted with ethyl acetate (AcOEt) and the organic layer was washed with 5% citric acid, 5% $NaHCO_3$, and brine. Drying followed by evaporation and purification by crystallization from *n*-hexane afforded **2** (6.19 g, 97%) as colorless crystals. mp 100—102 °C. $[\alpha]_D^{25}$ −24.5° (*c*=0.86, $CHCl_3$). *Anal.* Calcd for $C_{18}H_{21}NO_5S$: C, 59.46; H, 5.82; N, 3.85; S, 8.82. Found: C, 59.66; H, 5.81; N, 4.01; S, 8.41. IR (KBr) 3342, 1694, 1349, 1187 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.84—2.95 (m, 2H), 2.96 (s, 3H), 4.11—4.28 (m, 3H), 4.97—4.99 (m, 1H), 5.09 (s, 2H), 7.17—7.39 (m, 10H). MS *m/z*: 363 (M^+), 272, 228, 176, 91.

(*S*)-2-Benzyloxycarbonylamino-1-iodo-3-phenylpropane (3) A mixture of **2** (5.50 g, 15.1 mmol) and sodium iodide (2.50 g, 16.6 mmol) in acetone (50 ml) was refluxed for 6 h, and the solvent was removed *in vacuo*. The residue was extracted with AcOEt and the organic layer was washed with 5% $Na_2S_2O_3$, 5% citric acid, and water. Drying followed by evaporation, purification by silica gel column chromatography (AcOEt : *n*-hexane = 1 : 3) and crystallization from *n*-hexane afforded **3** (4.12 g, 69%) as colorless crystals. mp 86—87 °C. $[\alpha]_D^{25}$ +9.7° (*c*=0.28, $CHCl_3$). *Anal.* Calcd for $C_{17}H_{18}INO_2$: C, 51.66; H, 4.59; I, 32.11; N, 3.54. Found: C, 51.74; H, 4.60; I, 32.02; N, 3.58. IR (KBr) 3323, 1690, 1545, 1264 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.80 (dd, 1H, *J*=7.8, 13.7 Hz), 2.93 (dd, 1H, *J*=5.9, 13.7 Hz), 3.18 (dd, 1H, *J*=3.4, 10.3 Hz), 3.41 (dd, 1H, *J*=4.4, 10.3 Hz), 3.63—3.74 (m, 1H), 4.92 (br d, 1H, *J*=7.8 Hz), 5.10 (s, 2H), 7.21—7.40 (m, 10H). MS *m/z*: 396, 304, 260, 177, 91.

(*S*)-*N*-(2-Benzyloxycarbonylamino-3-phenylpropyl)-L-proline *tert*-Butyl Ester (4) A mixture of **3** (1.50 g, 3.80 mmol), L-proline *tert*-butyl ester (0.72 g, 4.17 mmol), and sodium carbonate (0.44 g, 4.17 mmol) in *N*,*N*-dimethylformamide (DMF, 20 ml) was stirred at 80 °C for 2 h, and the solvent was removed *in vacuo*. The residue was extracted with AcOEt and the organic layer was washed with 5% citric acid, 5% $NaHCO_3$, and brine. Drying followed by evaporation and purification by silica gel column chromatography (AcOEt : *n*-hexane = 1 : 3) afforded **4** (1.06 g, 71%) as a colorless oil. $[\alpha]_D^{25}$ −45.1° (*c*=0.85, $CHCl_3$). *Anal.* Calcd for $C_{26}H_{34}N_2O_4$: C, 71.20; H, 7.81; N, 6.39. Found: C, 70.95; H, 7.78; N, 6.27. IR (film) 2976, 1722, 1515 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (s, 9H), 1.81—2.08 (m, 4H), 2.53—2.75 (m, 3H), 2.92—2.96 (m, 2H), 3.10—3.21 (m, 2H), 3.89—3.97 (m, 1H), 5.06 (ABq, 2H, *J*=12.5 Hz, Δ=0.03 ppm), 7.18—7.39 (m, 10H). MS *m/z*: 439 (M^++1), 381, 337, 128, 91.

(*S*)-*N*-(2-Benzyloxycarbonylamino-3-phenylpropyl)-L-proline Hydrochloride (5) The ester **4** (1.00 g, 2.28 mmol) was added to 4 N HCl/dioxane solution (20 ml), and this solution was stirred for 1 h at room temperature. The solvent was removed *in vacuo*, and the residue was evaporated with benzene. Crystallization from diethylether afforded **5** (810 mg, 85%) as colorless crystals. mp 161—164 °C. $[\alpha]_D^{25}$ −70.0° (*c*=0.49, DMF). *Anal.* Calcd for $C_{22}H_{26}N_2O_4\cdot HCl\cdot 0.5H_2O$: C, 61.74; H, 6.60; N, 6.55. Found: C, 61.81; H, 6.39; N, 6.56. IR (KBr) 3030, 1715 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 1.93—2.15 (m, 3H), 2.45—2.58 (m, 1H), 2.77 (dd, 1H, *J*=8.6, 13.9 Hz), 2.92 (dd, 1H, *J*=5.3, 13.9 Hz), 3.23—3.35 (m, 2H), 3.55 (dd, 1H, *J*=3.3, 13.2 Hz), 3.73—3.83 (m, 1H), 4.18—4.28 (m, 1H), 4.40—4.48 (m, 1H), 4.99 (s, 2H), 7.17—7.33 (m, 10H). MS *m/z*: 383 (M^++1), 337, 291, 128, 91.

(*S*)-*N*-(2-Benzyloxycarbonylamino-3-phenylpropyl)-L-prolyl-L-glutaminyl-L-isoleucine Methyl Ester (6a) (i) *N-tert*-Butoxycarbonyl-L-glutaminyl-L-isoleucine Methyl Ester (Boc–Gln–Ile–OMe): Triethylamine (4.50 ml, 32.4 mmol) was added to a mixture of Boc–Gln–OH (3.80 g, 15.4 mmol), HCl·H–Ile–OMe (3.08 g, 17.0 mmol), and 93% DEPC (2.77 ml, 17.0 mmol) in DMF (60 ml) at 0 °C. The mixture was stirred for 2 h, and the solvent was removed *in vacuo*. The residue was extracted with AcOEt and the organic layer was washed with 5% citric acid, 5% $NaHCO_3$, and brine. Drying followed by evaporation and crystallization from *n*-hexane afforded Boc–Gln–Ile–OMe (5.46 g, 95%) as colorless crystals. mp 109—111 °C. $[\alpha]_D^{25}$ +7.0° (*c*=0.57, $CHCl_3$). *Anal.* Calcd for $C_{17}H_{31}N_3O_6$: C, 54.67; H, 8.37; N, 11.25. Found: C, 54.57; H, 8.14, N, 11.21. IR (KBr) 3316, 1738, 1688, 1652 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88—0.94 (m, 6H), 1.12—1.31 (m, 1H), 1.37—1.48 (m, 10H), 1.88—2.11 (m, 3H), 2.40—2.47 (m, 2H), 3.73 (s, 3H), 4.18—4.28 (m, 1H), 4.54 (dd, 1H, *J*=4.9, 8.3 Hz), 5.56 (br s, 1H), 5.85 (br s, 1H), 6.42 (br s, 1H), 7.46 (br d, 1H, *J*=7.3 Hz). MS *m/z*: 373 (M^+), 201, 145, 101, 84.

(ii) (*S*)-*N*-(2-Benzyloxycarbonylamino-3-phenylpropyl)-L-prolyl-L-glutaminyl-L-isoleucine Methyl Ester: Boc–Gln–Ile–OMe (470 mg, 1.26 mmol) was added to 4 N HCl/dioxane solution (5 ml), and this solution was stirred for 30 min at room temperature. The solvent was removed *in vacuo*, and the remaining solid was evaporated with benzene. The residue was dried *in vacuo* for 2 h. This solid was dissolved in DMF (2 ml), and then the acid **5** (405 mg, 0.97 mmol), DPPA (271 mg, 1.26 mmol), and triethylamine (0.44 ml, 3.19 mmol) were added at 0 °C. The mixture was stirred for 5 h at the same temperature, then the solvent was removed *in vacuo*. The residue was extracted with AcOEt, and the organic layer was washed with 5% citric acid, 5% $NaHCO_3$, and brine. Drying followed by evaporation and crystallization from the mixture of *n*-hexane–diethylether (3 : 1, v/v) afforded **6a** (540 mg, 88%) as a white solid. mp 160—163 °C. $[\alpha]_D^{25}$ −38.4° (*c*=0.32, $CHCl_3$). *Anal.* Calcd for $C_{34}H_{47}N_5O_6\cdot 0.5H_2O$: C, 63.14; H, 7.48; N, 10.83. Found: C, 63.16; H, 7.27; N, 10.84. IR (KBr) 3292, 1743, 1693, 1662, 1638 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.85—0.90 (m, 6H), 1.12—1.27 (m, 1H), 1.28—1.43 (m, 1H), 1.60—2.22 (m, 9H), 2.23—3.28 (m, 7H), 3.68—3.78 (m, 4H), 4.37—4.46 (m, 1H), 4.53 (dd, 1H, *J*=4.9, 8.8 Hz), 5.04 (ABq, 2H, *J*=12.2 Hz, Δ=0.05 ppm), 5.51 (br s, 1H), 5.74 (br s, 1H), 6.31 (br s, 1H), 7.16—7.38 (m, 10H), 8.18 (br s, 1H). MS *m/z*: 637 (M^+), 486, 337, 229, 91, 84.

(*S*)-*N*-(2-Benzyloxycarbonylamino-3-phenylpropyl)-L-prolyl-L-isoleucyl-L-valine Methyl Ester (6b) (i) *N-tert*-Butoxycarbonyl-L-isoleucyl-L-valine Methyl Ester (Boc–Ile–Val–OMe): Triethylamine (5.84 ml, 42.0 mmol) was added to a mixture of Boc–Ile–OH (4.63 g, 20.0 mmol), HCl·H–Val–OMe (3.52 g, 21.0 mmol), and 93% DEPC (3.43 ml, 21.0 mmol) in DMF (60 ml) at 0 °C. The mixture was stirred for 14 h, and the solvent was removed *in vacuo*. The residue was extracted with AcOEt and the organic layer was washed with 5% citric acid, 5% $NaHCO_3$, and brine. Drying followed by evaporation and crystallization from *n*-hexane afforded Boc–Ile–Val–OMe (4.32 g, 63%) as colorless crystals. mp 169—170 °C. $[\alpha]_D^{25}$ −15.4° (*c*=0.57, $CHCl_3$). *Anal.* Calcd for $C_{17}H_{32}N_2O_5$: C, 59.28; H, 9.36; N, 8.13. Found: C, 59.18; H, 9.14; N, 8.13. IR (KBr) 3324, 1751, 1684, 1647 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.90—0.95 (m, 12H), 1.12—1.20 (m, 1H), 1.44 (s, 9H), 1.46—1.64 (m, 1H), 1.83—1.93 (m, 1H), 2.14—2.23 (m, 1H), 3.74 (s, 3H), 3.91—3.97 (m, 1H), 4.55 (dd, 1H, *J*=5.1, 8.8 Hz), 5.03 (br d, 1H, *J*=7.3 Hz), 6.34 (br d, 1H, *J*=8.1 Hz). MS *m/z*: 344 (M^+), 186, 130, 86.

(ii) (*S*)-*N*-(2-Benzyloxycarbonylamino-3-phenylpropyl)-L-prolyl-L-isoleucyl-L-valine Methyl Ester: The title compound **6b** was prepared as described above for **6a** using Boc–Ile–Val–OMe instead of Boc–Gln–Ile–OMe, to yield a white foam (350 mg, 73%). $[\alpha]_D^{25}$ −48.2° (*c*=0.16, $CHCl_3$). *Anal.* Calcd for $C_{34}H_{48}N_4O_6\cdot 0.25H_2O$: C, 66.59; H, 7.97; N, 9.14. Found: C, 66.58; H, 7.74; N, 9.20. IR (KBr) 3303, 1744, 1720, 1655 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.81—0.95 (m, 12H), 1.09—1.19 (m, 1H), 1.41—1.93 (m, 5H), 2.05—2.21 (m, 2H), 2.44—2.56 (m, 1H), 2.62

(dd, 1H, J=5.4, 12.7 Hz), 2.90—3.21 (m, 5H), 3.52—3.65 (m, 1H), 3.72 (s, 3H), 4.17 (t, 1H, J=8.3 Hz), 4.51 (dd, 1H, J=4.9, 8.8 Hz), 5.05 (ABq, 2H, J=12.2 Hz, Δ=0.08 ppm), 6.04 (br d, 1H, J=7.3 Hz), 6.43 (br d, 1H, J=8.8 Hz), 7.13—7.38 (m, 10H), 8.06 (br d, 1H, J=8.3 Hz). MS m/z: 608 (M^+), 354, 337, 229, 128, 91.

(*S*)-*N*-{2-(*N'*-*tert*-Butoxycarbonyl-L-asparaginyl)amino-3-phenylpropyl}-L-prolyl-L-glutaminyl-L-isoleucine Methyl Ester (7a) A mixture of **6a** (408 mg, 0.64 mmol), 1 N HCl (1.6 ml, 1.60 mmol), and 10% Pd/C (400 mg) in MeOH (4 ml) was stirred under a hydrogen atmosphere for 1 h at room temperature. The catalyst was filtered off and the filtrate was concentrated. The residue was dissolved in DMF (3 ml), and then Boc–Asn–OH (164 mg, 0.71 mmol), 93% DEPC (0.12 ml, 0.71 mmol), and triethylamine (0.28 ml, 1.99 mmol) were added at 0 °C. The reaction mixture was stirred at 4 °C for 15 h, and precipitated with 5% $NaHCO_3$. The precipitate was washed with 5% $NaHCO_3$ and water. Purification by reprecipitation from AcOEt afforded **7a** (180 mg, 39%) as a white solid. mp 173—175 °C. $[\alpha]_D^{25}$ −39.1° (c=0.64, MeOH). *Anal.* Calcd for $C_{35}H_{55}N_7O_9 \cdot 2H_2O$: C, 55.76; H, 7.89; N, 13.01. Found: C, 55.86; H, 7.55; N, 12.84. IR (KBr): 3292, 1741, 1689, 1667, 1640 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 0.85—0.92 (m, 6H), 1.17—1.28 (m, 1H), 1.35—1.50 (m, 10H), 1.77—1.89 (m, 4H), 2.07—2.15 (m, 3H), 2.30—2.55 (m, 5H), 2.73—2.84 (m, 3H), 2.94—3.11 (m, 2H), 3.24—3.30 (m, 1H), 3.71 (s, 3H), 4.05—4.15 (m, 1H), 4.31—4.47 (m, 3H), 7.13—7.24 (m, 5H). Amino acid ratios in 6 N HCl hydrolysate: Asp 0.76, Glu 1.00, Ile 1.12 (recovery of Glu 100%).

(*S*)-*N*-{2-(*N'*-*tert*-Butoxycarbonyl-L-asparaginyl)amino-3-phenylpropyl}-L-prolyl-L-isoleucyl-L-valine Methyl Ester (7b) The title compound **7b** was prepared as described above for **7a** using **6b** instead of **6a**, to yield a white solid (150 mg, 50%). mp 181—183 °C. $[\alpha]_D^{25}$ −59.5° (c=0.43, MeOH). *Anal.* Calcd for $C_{35}H_{56}N_6O_8 \cdot 1.25H_2O$: C, 59.09; H, 8.29; N, 11.82. Found: C, 59.06; H, 7.97; N, 11.43. IR (KBr): 3329, 1742, 1690, 1668, 1648 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 0.86—0.97 (m, 12H), 1.14—1.24 (m, 1H), 1.42 (s, 9H), 1.45—1.59 (m, 1H), 1.72—1.85 (m, 3H), 1.92—2.21 (m, 3H), 2.41—2.53 (m, 3H), 2.65—2.88 (m, 3H), 2.93—3.11 (m, 2H), 3.20—3.25 (m, 1H), 3.70 (s, 3H), 3.97—4.02 (m, 1H), 4.30—4.39 (m, 3H), 7.13—7.27 (m, 5H). Amino acid ratios in 6 N HCl hydrolysate: Asp 1.04, Val 1.00, Ile 0.94 (recovery of Val 92%).

(*S*)-*N*-{2-(*N'*-*tert*-Butoxycarbonyl-L-phenylalanyl-L-asparaginyl)amino-3-phenylpropyl}-L-prolyl-L-glutaminyl-L-isoleucine Methyl Ester (8a) Compound **7a** (125 mg, 0.17 mmol) was added to 4 N HCl/dioxane solution (1 ml), and this solution was stirred for 30 min at room temperature. The solvent was removed *in vacuo*, and the residue was evaporated with benzene. The resultant residue was dried *in vacuo* for 2 h. This solid was dissolved in DMF (1 ml), and then Boc–Phe–OH (55 mg, 0.21 mmol), 93% DEPC (34 μl, 0.21 mmol), and triethylamine (77 μl, 0.56 mmol) were added at 0 °C. The mixture was stirred at 4 °C for 14 h, then the solvent was removed *in vacuo*. The residue was precipitated with 5% $NaHCO_3$, and the precipitate was washed with 5% $NaHCO_3$ and water. Purification by reprecipitation from AcOEt–diethylether (1 : 2, v/v) afforded **8a** (88 mg, 58%) as a white solid. mp 169—172 °C. $[\alpha]_D^{25}$ −34.7° (c=0.14, DMF). *Anal.* Calcd for $C_{44}H_{64}N_8O_{10} \cdot 1.5H_2O$: C, 59.24; H, 7.57; N, 12.56. Found: C, 59.27; H, 7.24; N, 12.24. ^{1}H-NMR (CD_3OD) δ: 0.85—0.92 (m, 6H), 1.20—1.45 (m, 12H), 1.79—1.93 (m, 4H), 2.05—2.39 (m, 6H), 2.58—3.11 (m, 9H), 3.70 (s, 3H), 4.21—4.28 (m, 2H), 4.36—4.46 (m, 2H), 4.58—4.65 (m, 1H), 7.15—7.31 (m, 10H). Amino acid ratios in 6 N HCl hydrolysate: Asp 0.74, Glu 1.00, Ile 1.10, Phe 1.05 (recovery of Glu 92%).

(*S*)-*N*-{2-(*N'*-*tert*-Butoxycarbonyl-L-glutaminyl-L-asparaginyl)amino-3-phenylpropyl}-L-prolyl-L-isoleucyl-L-valine Methyl Ester (8b) The title compound **8b** was prepared as described above for **8a** using **7b** instead of **7a**, to yield a white solid (90 mg, 76%). mp 179—182 °C. $[\alpha]_D^{25}$ −44.1° (c=0.17, DMF). *Anal.* Calcd for $C_{40}H_{64}N_8O_{10} \cdot 2H_2O$: C, 56.32; H, 7.97; N, 13.14. Found: C, 56.43; H, 7.50; N, 13.03. ^{1}H-NMR (DMSO-d_6) δ: 0.80—0.90 (m, 12H), 0.95—1.08 (m, 1H), 1.35—1.47 (m, 10H), 1.60—1.85 (m, 6H), 1.92—2.10 (m, 4H), 2.26—2.48 (m, 4H), 2.53—2.69 (m, 2H), 2.90—3.12 (m, 3H), 3.60 (s, 3H), 3.81—3.88 (m, 1H), 3.92—4.04 (m, 1H), 4.09—4.18 (m, 1H), 4.35—4.45 (m, 2H), 6.75 (br s, 1H), 6.87 (br s, 1H), 7.00 (br d, 1H, J=7.3 Hz), 7.10—7.29 (m, 7H), 7.67 (br d, 1H, J=8.3 Hz), 7.80 (br d, 1H, J=9.3 Hz), 7.97 (br d, 1H, J=7.8 Hz), 8.30 (br d, 1H, J=7.8 Hz). Amino acid ratios in 6 N HCl hydrolysate: Asp 1.05, Glu 1.05, Val 1.00, Ile 0.94 (recovery of Val 95%).

(*S*)-(2-*tert*-Butoxycarbonylamino-1-oxo-3-phenylpropyl)cyclopentane (10) A mixture of Boc–Phe–OH (1.00 g, 3.77 mmol) and carbonyldiimidazole (0.67 g, 4.15 mmol) in tetrahydrofuran (THF, 10 ml) was stirred under a nitrogen atmosphere for 3 h at room temperature. Then 2 M cyclopentylmagnesium chloride in THF (3.77 ml, 7.54 mmol) was added over 30 min at −60 °C. The reaction mixture was stirred for 4 h at −20 °C and then diluted with AcOEt and 5% citric acid. The organic layer was washed with 5% $NaHCO_3$ and brine. Drying followed by evaporation and purification by silica gel column chromatography (AcOEt : *n*-hexane = 1 : 3) afforded **10** (0.38 g, 31%) as colorless crystals. mp 139—140 °C. $[\alpha]_D^{25}$ +51.7° (c=0.96, $CHCl_3$). *Anal.* Calcd for $C_{19}H_{27}NO_3$: C, 71.89; H, 8.57; N, 4.41. Found: C, 71.88; H, 8.75; N, 4.40. IR (KBr) 3321, 1712, 1681 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (s, 9H), 1.53—1.88 (m, 8H), 2.85—2.99 (m, 2H), 3.07 (dd, 1H, J=6.8, 13.7 Hz), 4.66 (q, 1H, J=6.8 Hz), 5.12 (br d, 1H, J=7.3 Hz), 7.12—7.31 (m, 5H). MS m/z: 317 (M^+), 220, 164, 120, 91.

(1*S*,2*S*)-2-*tert*-Butoxycarbonylamino-1-cyclopentyl-3-phenylpropanol (11a) and (1*R*,2*S*)-2-*tert*-Butoxycarbonylamino-1-cyclopentyl-3-phenylpropanol (11b) A solution of the ketone **10** (610 mg, 1.92 mmol) in THF–EtOH (10 : 1, 4.4 ml) was treated with sodium borohydride (145 mg, 3.84 mmol) at 0 °C. The reaction mixture was stirred for 3 h at the same temperature, and diluted with AcOEt and 5% citric acid. The organic layer was washed with 5% $NaHCO_3$ and brine. Drying followed by evaporation and purification by PTLC (AcOEt : *n*-hexane = 2 : 3) afforded **11a** (180 mg, 29%) and **11b** (380 mg, 62%) as less and more polar colorless crystals, respectively. **11a**: *Rf* 0.53 (AcOEt : *n*-hexane = 2 : 5). mp 120—121 °C. $[\alpha]_D^{25}$ −26.8° (c=0.70, $CHCl_3$). *Anal.* Calcd for $C_{19}H_{29}NO_3$: C, 71.44; H, 9.15; N, 4.38. Found: C, 71.32; H, 9.31; N, 4.49. IR (KBr) 3349, 1685, 1505 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.91—1.02 (m, 1H), 1.21—2.04 (m, 17H), 2.78—2.98 (m, 2H), 3.25 (dd, 1H, J=2.0, 9.3 Hz), 3.78—3.88 (m, 1H), 4.87 (br d, 1H, J=8.8 Hz), 7.17—7.32 (m, 5H). MS m/z: 320 ($M^+ + 1$), 228, 164, 128, 120, 91. **11b**: *Rf* 0.45 (AcOEt : *n*-hexane = 2 : 5). mp 142—142 °C. $[\alpha]_D^{25}$ −22.0° (c=0.96, $CHCl_3$). *Anal.* Calcd for $C_{19}H_{29}NO_3$: C, 71.44; H, 9.15; N, 4.39. Found: C, 71.21; H, 9.10; N, 4.38. IR (KBr) 3356, 1686, 1533 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.27—1.44 (m, 10H), 1.52—1.78 (m, 6H), 1.85—2.06 (m, 2H), 2.68—2.80 (m, 1H), 2.95 (dd, 1H, J=3.9, 14.2 Hz), 3.53—3.58 (m, 1H), 3.78—3.89 (m, 1H), 4.67—4.73 (m, 1H), 7.17—7.31 (m, 5H). MS m/z: 320 ($M^+ + 1$), 228, 164, 128, 120, 91.

(1*S*,2*S*)-2-(*N*-*tert*-Butoxycarbonyl-L-glutaminyl-L-asparaginyl)amino-1-cyclopentyl-3-phenylpropanol (12a) (i) *N*-*tert*-Butoxycarbonyl-L-glutaminyl-L-asparagine Phenacyl Ester (Boc–Gln–Asn–OPac): Boc–Asn–OPac[25] (12.5 g, 35.7 mmol) was added to 4 N HCl/dioxane solution (100 ml), and this solution was stirred for 30 min at room temperature. The solvent was removed *in vacuo*, and the remaining solid was evaporated with benzene. The residue was dried *in vacuo* for 2 h. The solid was dissolved in DMF (100 ml), and then Boc–Gln–OH (9.23 g, 37.5 mmol), 93% DEPC (6.40 ml, 39.3 mmol), and triethylamine (10.4 ml, 74.9 mmol) were added at 0 °C. The reaction mixture was stirred for 14 h at room temperature, then the solvent was removed *in vacuo*. The residue was extracted with AcOEt and the organic layer was washed with 5% citric acid, 5% $NaHCO_3$, and brine. Drying followed by evaporation and purification by crystallization from diethylether afforded Boc–Gln–Asn–OPac (9.8 g, 57%) as colorless crystals. mp 184—186 °C. $[\alpha]_D^{25}$ −26.5° (c=0.73, MeOH). *Anal.* Calcd for $C_{22}H_{30}N_4O_8$: C, 55.22; H, 6.32; N, 11.71. Found: C, 55.22; H, 6.25; N, 11.67. IR (KBr) 3438, 3316, 1746, 1698, 1665 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 1.43 (s, 9H), 1.83—2.14 (m, 2H), 2.28—2.37 (m, 2H), 2.87—2.92 (m, 2H), 4.05—4.13 (m, 1H), 4.95—5.01 (m, 1H), 5.49 (ABq, 2H, J=7.8 Hz, Δ=0.02 ppm), 7.47—7.56 (m, 2H), 7.59—7.68 (m, 1H), 7.93—7.98 (m, 2H).

(ii) *N*-*tert*-Butoxycarbonyl-L-glutaminyl-L-asparagine (Boc–Gln–Asn–OH): A mixture of Boc–Gln–Asn–OPac (3.60 g, 7.52 mmol) and Zn (4.92 g, 75.2 mmol) in 90% acetic acid (18 ml) was stirred for 3 h at 0 °C. Unreacted Zn was filtered off and the filtrate was concentrated. The residue was extracted with *n*-BuOH and the organic layer was washed with 5% ethylenediaminetetraacetic acid (EDTA) and water. Removal of the solvent followed by precipitation from AcOEt afforded crude Boc–Gln–Asn–OH (1.21 g, 45%) as a white solid. ^{1}H-NMR (CD_3OD) δ: 1.44 (s, 9H), 1.83—2.15 (m, 2H), 2.28—2.35 (m, 2H), 2.70—2.81 (m, 2H), 4.03—4.11 (m, 1H), 4.58—4.65 (m, 1H).

(iii) (1*S*,2*S*)-2-(*N*-*tert*-Butoxycarbonyl-L-glutaminyl-L-asparaginyl)-amino-1-cyclopentyl-3-phenylpropanol: Compound **11a** (130 mg, 0.41 mmol) was added to 4 N HCl/dioxane solution (2 ml), and this solution was stirred for 30 min at room temperature. The solvent was removed *in vacuo*, and the remaining solid was evaporated with benzene. The residue was dried *in vacuo* for 2 h. The solid was dissolved in DMF (2 ml), and then Boc–Gln–Asn–OH (178 mg, 0.49 mmol), DPPA (105 mg, 0.49 mmol), and triethylamine (0.14 ml, 1.02 mmol) were added at 0 °C. The reaction mixture was stirred for 14 h at room temperature, then the solvent was removed *in vacuo*. The residue was extracted with AcOEt and the organic layer was washed with 5% citric acid, 5% $NaHCO_3$, and brine. Drying

followed by evaporation and purification by PTLC (CH_2Cl_2 : MeOH = 10 : 1) afforded **12a** (170 mg, 74%) as a white solid. mp 200—202 °C. $[\alpha]_D^{25}$ −36.4° (c = 0.37, MeOH). *Anal.* Calcd for $C_{28}H_{43}N_5O_7 \cdot H_2O$: C, 58.01; H, 7.83; N, 12.08. Found: C, 58.30; H, 7.52; M, 11.93. IR (KBr) 3331, 1666 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 0.81—0.93 (m, 1H), 1.20—1.30 (m, 1H), 1.40—2.08 (m, 18H), 2.25—2.33 (m, 2H), 2.64—2.91 (m, 4H), 3.11—3.17 (m, 1H), 3.98—4.14 (m, 2H), 4.61—4.69 (m, 1H), 7.13—7.30 (m, 5H).

(1*R*,2*S*)-2-(*N*-*tert*-Butoxycarbonyl-L-glutaminyl-L-asparaginyl)amino-1-cyclopentyl-3-phenylpropanol (12b) The title compound **12b** was prepared as described above for **12a** using **11b** instead of **11a**. Purification by crystallization from diethylether afforded **12b** (290 mg, 79%) as colorless crystals. mp 216—218 °C. $[\alpha]_D^{25}$ −31.4° (c = 0.30, DMF). *Anal.* Calcd for $C_{28}H_{43}N_5O_7 \cdot 0.25H_2O$: C, 59.40; H, 7.74; N, 12.37. Found: C, 59.30; H, 7.34; N, 12.31. IR (KBr) 3329, 1656 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.21—2.10 (m, 22H), 2.32—2.37 (m, 2H), 2.57 (dd, 1H, J = 9.8, 13.7 Hz), 2.92 (dd, 1H, J = 2.9, 13.7 Hz), 3.76—3.90 (m, 2H), 4.38—4.45 (m, 1H), 4.67—4.73 (m, 1H), 6.77 (s, 1H), 6.88 (s, 1H), 6.96 (br d, 1H, J = 7.3 Hz), 7.11—7.29 (m, 7H), 7.51 (br d, 1H, J = 8.8 Hz), 7.92 (br d, 1H, J = 7.8 Hz).

(4*S*,5*S*)-4-Benzyl-5-cyclopentyloxazolidin-2-one (13a) A solution of compound **11a** (70 mg, 0.22 mmol) in DMF (1 ml) was treated with 55% sodium hydride (14 mg, 0.33 mmol) at 0 °C. The reaction mixture was stirred for 2 h at room temperature, and diluted with AcOEt and 5% citric acid. The organic layer was washed with 5% $NaHCO_3$ and brine. Drying followed by evaporation and purification by PTLC (CH_2Cl_2 : MeOH = 50 : 1) afforded **13a** (46 mg, 85%) as colorless crystals. mp 82—84 °C. $[\alpha]_D^{25}$ −73.9° (c = 0.34, $CHCl_3$). *Anal.* Calcd for $C_{15}H_{19}NO_2$: C, 73.44; H, 7.81; N, 5.71. Found: C, 73.28; H, 7.65; N, 5.51. IR (KBr) 3324, 1748 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.08—1.88 (m, 8H), 2.09 (sextet, 1H, J = 7.8 Hz), 2.79 (dd, 1H, J = 8.3, 13.2 Hz), 2.88 (dd, 1H, J = 5.4, 13.2 Hz), 3.70 (ddd, 1H, J = 4.9, 5.4, 8.3 Hz), 4.16 (dd, 1H, J = 4.9, 7.8 Hz), 5.22 (br s, 1H), 7.16—7.37 (m, 5H). MS m/z: 246 ($M^+ + 1$), 154, 110, 93, 91.

(4*S*,5*R*)-4-Benzyl-5-cyclopentyloxazolidin-2-one (13b) The title compound **13b** was prepared as described above for **13a** using **11b** instead of **11a**, to yield colorless crystals (46 mg, 85%). mp 115—116 °C. $[\alpha]_D^{25}$ −119.9° (c = 0.81, $CHCl_3$). *Anal.* Calcd for $C_{15}H_{19}NO_2$: C, 73.44; H, 7.81; N, 5.71. Found: C, 73.34; H, 7.83; N, 5.70. IR (KBr) 3281, 1751, 1712 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.25—1.38 (m, 1H), 1.52—1.75 (m, 5H), 1.79—1.92 (m, 1H), 1.94—2.10 (m, 1H), 2.31—2.43 (m, 1H), 2.67 (dd, 1H, J = 11.7, 13.2 Hz), 2.93 (dd, 1H, J = 2.9, 13.2 Hz), 3.86 (ddd, 1H, J = 2.9, 6.8, 11.7 Hz), 4.41 (dd, 1H, J = 6.8, 10.3 Hz), 4.76 (br s, 1H), 7.13—7.19 (m, 2H), 7.23—7.38 (m,3H). MS m/z: 246 ($M^+ + 1$), 154, 110, 93, 91.

(3*R*,4*S*)-4-(*N*-*tert*-Butoxycarbonyl-L-glutaminyl-L-asparaginyl)amino-3-hydroxy-5-phenylpentanoic Acid 2′-Methylbutylamide (28) (i) (3*R*,4*S*)-4-*tert*-Butoxycarbonylamino-3-hydroxy-5-phenylpentanoic Acid 2′-Methylbutylamide: Triethylamine (0.24 ml, 1.70 mmol) was added to a mixture of Boc-(3*R*,4*S*)-AHPPA-OH (500 mg, 1.62 mmol; derived from Boc-(3*R*,4*S*)-AHPPA-OEt[17] by alkaline hydrolysis), 2-methylbutylamine (0.23 ml, 1.94 mmol), 93% DEPC (0.30 ml, 1.70 mmol) in THF (5 ml) at 0 °C. The mixture was stirred for 2 h at room temperature, and precipitated with 5% $NaHCO_3$. The precipitate was washed with 5% $NaHCO_3$, 5% citric acid, and water. Purification by reprecipitation from diethylether afforded the title compound (547 mg, 89%) as a white solid. mp 237—238 °C. $[\alpha]_D^{25}$ +16.6° (c = 0.07, DMSO). *Anal.* Calcd for $C_{21}H_{34}N_2O_4 \cdot 0.1H_2O$: C, 66.32; H, 9.06; N, 7.37. Found: C, 66.24; H, 9.06; N, 7.20. IR (KBr) 3341, 1682, 1644 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.80—0.88 (m, 6H), 1.02—1.12 (m, 1H), 1.25 (s, 9H), 1.30—1.51 (m, 2H), 2.15 (dd, 1H, J = 9.3, 14.3 Hz), 2.28 (dd, 1H, J = 3.1, 14.3 Hz), 2.45—2.53 (m, 1H), 2.82—2.91 (m, 1H), 2.95—3.05 (m, 2H), 3.44—3.53 (m, 1H), 3.73—3.81 (m, 1H), 5.00 (d, 1H, J = 5.6 Hz), 6.58 (d, 1H, J = 8.4 Hz), 7.11—7.28 (m, 5H), 7.73—7.81 (m, 1H). MS m/z: 378 (M^+), 287, 231, 187, 158, 120, 88, 57.

(ii) (3*R*,4*S*)-4-(*N*-*tert*-Butoxycarbonyl-L-asparaginyl)amino-3-hydroxy-5-phenylpentanoic Acid 2′-Methylbutylamide: (3*R*,4*S*)-4-*tert*-Butoxycarbonylamino-3-hydroxy-5-phenylpentanoic acid 2′-methylbutylamide (200 mg, 0.53 mmol) was added to 4 N HCl/dioxane solution (3 ml), and this solution was stirred for 30 min at room temperature. The solvent was removed *in vacuo*, and the remaining solid was evaporated with benzene. The residue was dried *in vacuo* for 2 h. This solid was dissolved in DMF (3 ml), and then Boc-Asn-OH (135 mg, 0.58 mmol), 93% DEPC (102 μl, 0.58 mmol), and triethylamine (162 μl, 1.16 mmol) were added at 0 °C. The mixture was stirred for 2 h at the same temperature, and precipitated with 5% $NaHCO_3$. The precipitate was washed with 5% $NaHCO_3$, 5% citric acid, and water. Purification by reprecipitation from diethylether afforded the title compound (229 mg, 88%) as a white solid. mp 242—243 °C. $[\alpha]_D^{25}$ −14.3° (c = 0.10, DMSO). *Anal.* Calcd for $C_{25}H_{40}N_4O_6$: C, 60.95; H, 8.19; N, 11.37. Found: C, 60.79; H, 8.14; N, 11.35. IR (KBr) 3331, 1690, 1655 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.78—0.88 (m, 6H), 0.99—1.10 (m, 1H), 1.25—1.52 (m, 1H), 2.08—2.30 (m, 4H), 2.55—2.68 (m, 1H), 2.78—3.05 (m, 3H), 3.71—3.85 (m, 2H), 4.07—4.15 (m, 1H), 5.02 (d, 1H, J = 5.5 Hz), 6.69 (d, 1H, J = 7.9 Hz), 6.86 (s, 1H), 7.11—7.25 (m, 6H), 7.54 (d, 1H, J = 8.0 Hz), 7.65—7.75 (m, 1H). MS m/z: 493 ($M^+ + 1$), 335, 279, 261, 170, 158, 120.

(iii) (3*R*,4*S*)-4-(*N*-*tert*-Butoxycarbonyl-L-glutaminyl-L-asparaginyl)-amino-3-hydroxy-5-phenylpentanoic Acid 2′-Methylbutylamide: The title compound **28** was prepared as described above for (ii) using Boc-Gln-OH instead of Boc-Asn-OH, to yield a white solid (150 mg, 56%). mp 219—222 °C. $[\alpha]_D^{25}$ −7.1° (c = 0.51, DMSO). *Anal.* Calcd for $C_{30}H_{48}N_6O_8 \cdot 0.5H_2O$: C, 57.21; H, 7.84; N, 13.35. Found: C, 57.33; H, 7.54; N, 13.20. IR (KBr) 3313, 1656 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.78—0.88 (m, 6H), 1.00—1.10 (m, 1H), 1.30—1.48 (m, 11H), 1.61—1.73 (m, 1H), 1.77—1.87 (m, 1H), 2.04—2.17 (m, 3H), 2.28—2.42 (m, 3H), 2.52—2.63 (m, 1H), 2.81—3.03 (m, 3H), 3.71—3.93 (m, 3H), 4.35—4.43 (m, 1H), 4.99 (d, 1H, J = 5.3 Hz), 6.78 (s, 1H), 6.90 (s, 1H), 6.99 (d, 1H, J = 7.5 Hz), 7.10—7.31 (m, 7H), 7.57—7.72 (m, 2H), 7.95 (d, 1H, J = 7.6 Hz).

Recombinant Proteins Recombinant HIV-1 protease was expressed in *E. coli* strain BL-21 (DE3) containing T7 promoter on a chloramphenicol-resistant plasmid, using the pT7HIVGP expression vector derived from pAR3040. Cell extracts were prepared and HIV-1 protease was partially purified by ion exchange chromatography (DEAE Sephadex A25, 50 i.d. × 200 mm; eluent, buffer A (50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) buffer (pH 7.8) containing 1 mM dithiothreitol, 10 μg/ml aprotinin, 5 mM EDTA, 10 μg/ml benzamide, 1 mM fluorophenylmethylsulfonic acid, 10% glycerin)) and gel filtration (TSK G2000SW, 7.5 i.d. × 600 mm; eluent, buffer B (50 mM Tris-HCl buffer (pH 7.5) containing 1 mM dithiothreitol, 1 mM EDTA and 200 mM NaCl)). Recombinant HIV-1 55 kDa *gag* protein, p 55, was also expressed in *E. coli* BL-21 (DE3) and used as a partially purified suspension.

Inhibition Studies Inhibition of p 55 proteolysis was investigated by using SDS-PAGE. Protease, which was confirmed to cleave the substrates (Fig. 2, lane 3), was incubated with 20 μl of a mixture of p 55, various concentrations of a DMSO solution of the synthetic inhibitors, and buffer C (50 mM Tris-HCl buffer (pH 6.0) containing 0.1 mM EDTA, 250 mM NaCl, and 0.1% Triton X-100) at 37 °C for 30 min. Reactions were stopped by the addition of an equal volume of 8 M urea. After the addition of 40 μl of Laemmli buffer[26] to this reaction mixture, it was boiled for 2 min. Ten μl aliquots were subjected to SDS-PAGE on 10% polyacrylamide slab gels (13.8 × 13.8 × 1 mm). Proteins were stained with Coomassie brilliant blue G® (CBB) dye. The concentration that is equipotent with 1 μM pepstatin A was determined by visually judging the remaining amount of p 55 as shown in Fig. 2.

Measurement of the Inhibition Constant (K_i) A reaction mixture containing 1 mM or 1.5 mM synthetic substrate, Ac-Ser-Gln-Asn-Tyr-Pro-Ile-Val-NH_2, 1 μl of the desired concentration of the test compound in a mixture of DMSO-water (1 : 4, v/v), 2 μl of the partially purified enzyme solution and buffer C, to form 10 μl in total, was incubated for 30 min at 37 °C. The reaction was stopped by the addition of 250 μl of a mixture of 0.1% w/v aqueous trifluoroacetic acid-acetonitrile (TFA-CH_3CN, 9 : 1, v/v), and the reaction mixture was passed through a Sep-pak light®. The amount of decomposed peptide, Ac-Ser-Gln-Asn-Tyr-OH, was assayed quantitatively by using HPLC (column, ODS-120T 4.6 i.d. × 250 mm; eluent, 9 : 91 CH_3CN-0.05% TFA mixture). The K_i was calculated according to the Michaelis-Menten method.

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