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Structure–Activity Relationships of HIV-1 PR Inhibitors Containing AHPBA—II. Modification of Pyrrolidine Ring at P1′ Proline

Tomoaki Komai,[b] Susumu Higashida,[a] Mitsuya Sakurai,[a] Tamayo Nitta,[a] Atsushi Kasuya,[a] Shuichi Miyamaoto,[a] Ryuichi Yagi,[b] Yuji Ozawa,[b] Hiroshi Handa,[c] Hiroshi Mohri,[d] Akira Yasuoka,[d] Shinichi Oka,[d] Takashi Nishigaki,[b,*] Satoshi Kimura,[d] Kaoru Shimada[d] and Yuichiro Yabe[a,*]

[a]*Exploratory Chemistry Research and* [b]*Biological Research Laboratories, Sankyo Co. Ltd, 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140, Japan*

[c]*Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 227, Japan*

[d]*Institute of Medical Science, University of Tokyo, Shiroganedai, Minato-ku, Tokyo 108, Japan*

Abstract—Systematic replacement in the 3- or 4-position of the pyrrolidine ring at P1′ proline was carried out. Compound **26**, which has a Cl atom in the 4(*S*)-position was the most active among inhibitors substituted with other halogen atoms or other substituents. Furthermore, the replacement of the *Z* group in compound **26** with five- or six-membered fused aromatic heterocycle carbonyl groups produced more potent inhibitors. 7-Methoxybenzofuran-2-carbonyl derivative (**44**) was the best of these and showed $K_i = 4.5$ nM against HIV PR and IC_{90}s 0.58 μM and 0.06 μM in chronic and acute infections, respectively. These results suggest that the combination of the 4(*S*)-Cl atom and fused bicyclic heterocycles may be effective in improving their cellular penetration. Copyright © 1996 Elsevier Science Ltd

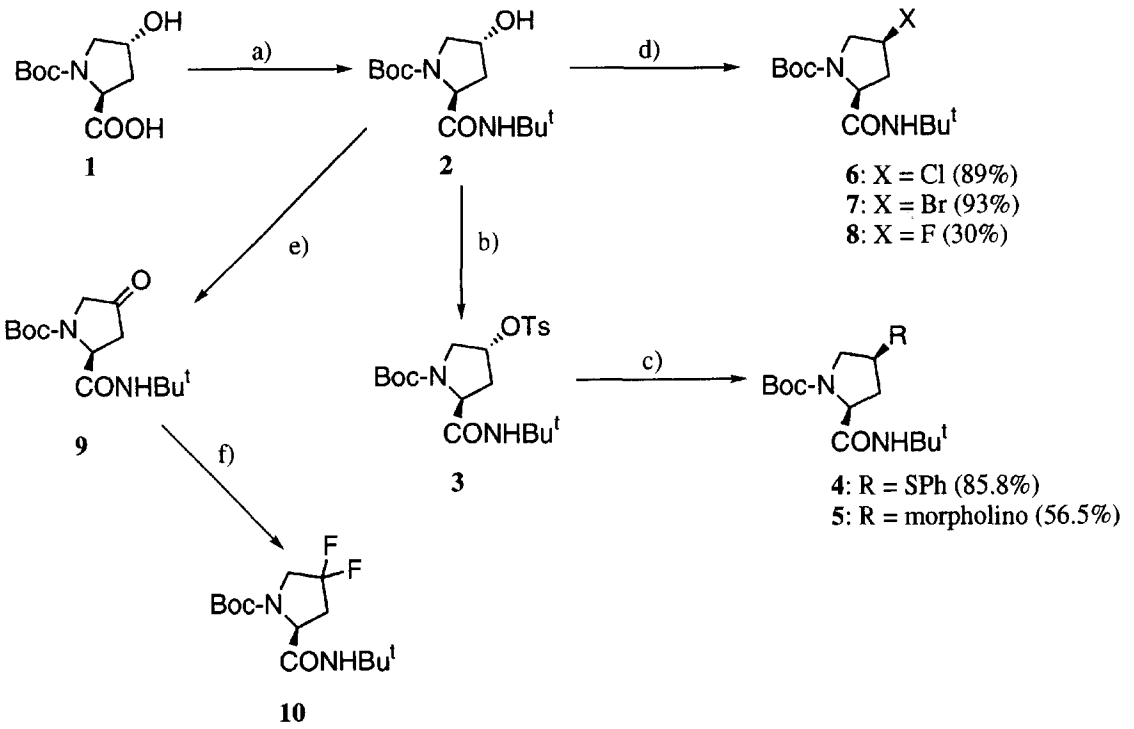
Introduction

The world-wide search for safe and effective therapies for AIDS has prompted an intensive research effort on the structure and biology of the human immunodeficiency virus (HIV), which is the causative agent of this disease. This research had led to the elucidation of a myriad of specific viral targets for drug discovery and design with the result that strategies now exist for targeting virtually every aspect of the viral life cycle.[1] HIV-1 protease (HIV PR) is a virally encoded enzyme that cleaves, or processes, viral *gag* and *gag-pol* protein precursors during virus assembly and maturation.[2] Kramer et al. first suggested in 1986 that inhibition of HIV PR would represent an appropriate strategy for interfering with HIV-1 replication.[3] In 1988 it was observed that deletion mutagenesis of the HIV PR gene resulted in the production of non-infectious, immature virus particles.[4] This experiment demonstrated that HIV PR performs an essential function in the life cycle of HIV-1 and thus makes this enzyme an important target for the design of specific antiviral agents for AIDS. Once it was recognized from pepstatin inhibition and partial-sequence homology that HIV PR was an aspartic protease, many investigators chose to screen known renin inhibitors as possible HIV PR antagonists and to apply the aspartic protease-inhibitors design concept.[5–7]

Our previous report described a series of HIV PR inhibitors that contain 3-amino-2-hydroxy-4-phenylbutanoic acid (AHPBA) at the cleavage site of the substrate.[8] Systematic replacement at the sites from P3 to P2′ showed that some bicyclic heteroarylcarbonyl derivatives had potent and selective inhibitory activities against HIV PR. These compounds also showed potent anti-HIV-1 activities.[9] In this report we describe the HIV PR inhibition and the anti-HIV-1 activity of AHPBA-based inhibitors which were substituted at the 3- or 4-position of pyrroridine ring at P1′ proline.

Chemistry

N-Boc-4(*R*)-hydroxyproline *tert*-butylamide (**2**) was prepared in a good yield from *N*-Boc-L-hydroxyproline (**1**) by using the mixed anhydride method. The reaction of the tosylate **3** derived from **2** with sodium thiophenolate or morpholine gave compounds **4** or **5**. Swern oxidation of **2** gave *N*-Boc-4-ketoproline *tert*-butylamide (**9**) in a good yield. The reaction of **2** with CCl_4 or CBr_4 in the presence of triphenylphosphine (Ph_3P) afforded *N*-Boc-4(*S*)-chloro- (**6**) or *N*-Boc-4(*S*)-bromoproline *tert*-butylamide (**7**) in a moderate yield. On the other hand, the reaction of **2** with diethylaminosulfur trifluoride (DAST) gave *N*-Boc-4(*S*)-fluoroproline *tert*-butylamide (**8**) in a low yield with recovered **2**. *N*-Boc-4,4-difluoroproline *tert*-butylamide (**10**) was prepared in a good yield by the reaction of **9** with DAST (Scheme 1). *N*-*Z*-4(*S*)-hydroxy- (**11**) and *N*-*Z*-4(*R*)-chloroproline *tert*-butylamide (**12**) were obtained in the same way as the preparation of **2** and **6**.



Scheme 1. Reagents and conditions: (a) isobutyl chloroformate, THF, *N*-methylmorpholine, *t*-BuNH$_2$, 84.7%; (b) *p*-tosyl chloride, pyrrolidine, 55.8%; (c) sodium thiophenolate, DMF:AcOEt, 2:1 or morpholine; (d) CCl_4/Ph_3 or CBr_4/Ph_3P or DAST, THF; (e) oxalyl chloride/DMSO, CH_2Cl_2, 73.9%); (f) DAST, THF, 89%.

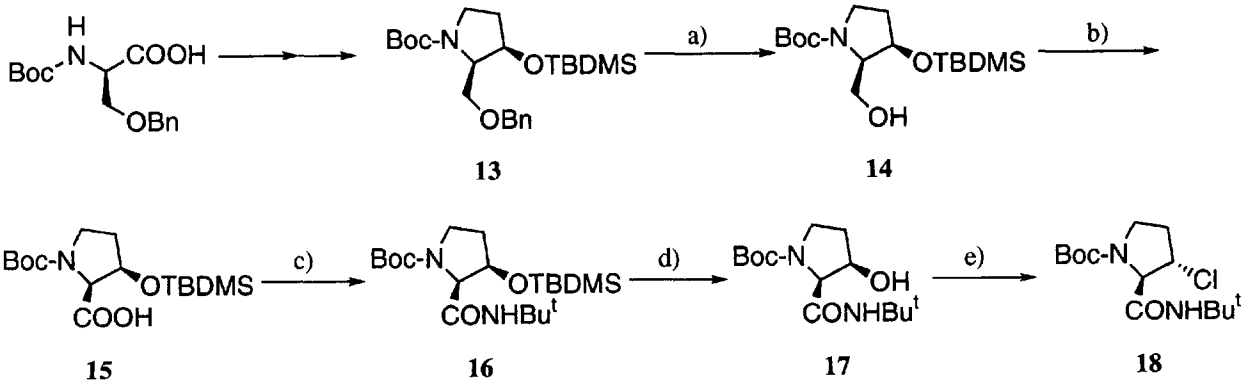
For the synthesis of 3-hydroxyl- or 3-chloroproline derivatives, *N*-Boc-*O*-benzyl-D-serine was used as the starting material instead of *N*-Boc-D-serine[10] (Schemes 2 and 3). The key intermediate (**13**) was prepared in six steps from *N*-Boc-*O*-benzyl-D-serine. Hydrogenolysis, followed by Jones oxidation gave the carboxylic acid (**15**). The spectral data of **14** was identical to the compound reported by Ewing and Joullie'.[10] The reaction of the mixed anhydride of **15** with *tert*-butylamine afforded the *tert*-butylamide (**16**). Desilylation of **16**, followed by the reaction with CCl_4–Ph_3P gave *N*-Boc-3(*S*)-chloroproline *tert*-butylamide (**18**). Desilylation of **13**, followed by the Mitsunobu reaction with benzoic acid, and hydrolysis, oxidation, *tert*-butylamidation and alkali hydrolysis gave *N*-Boc-3(*S*)-hydroxyproline *tert*-butylamide (**23**).

The inhibitors were prepared using diethyl phosphorylcyanide (DEPC) or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride/1-hydroxybenzotriazole (EDC/HOBt) as a condensation reagent in a convenient peptide synthesis as shown in Scheme 4.

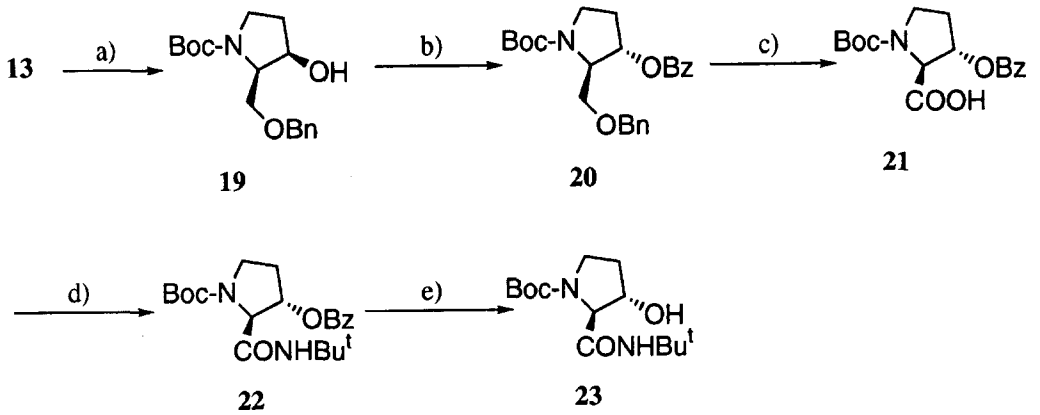
Results and Discussion

Structure–activity relationship

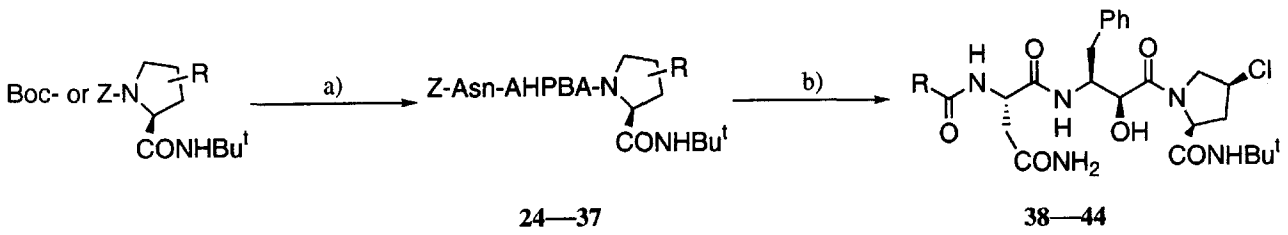
Table 1 shows the inhibitory activity of AHPBA-based HIV PR inhibitors with modifications at P1′ proline. Replacement of the 3- or 4-positions on the pyrrolidine ring at P1′ proline affected their HIV PR inhibitory activities. Although an inhibitor that contains a hydro-



Scheme 2. Reagents and conditions: (a) Pd black, 4.4% HCOOH in MeOH, 98%; (b) Jones Reagent, acetone, 74.7%; (c) isobutyl chloroformate, THF, *N*-methylmorpholine, *t*-BuNH$_2$, 47.5%; (d) (*t*-Bu)$_4$NF, THF: (e) CC_4/Ph_3P, THF.



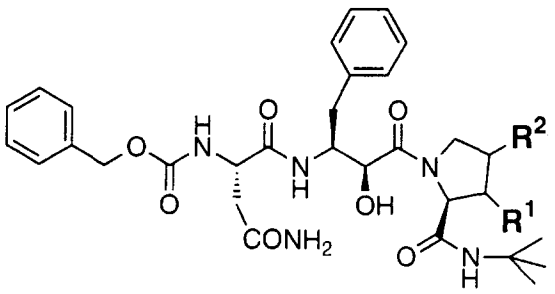
Scheme 3. Reagents and conditions: (a) $(t\text{-Bu})_4NF$, THF, 99%; (b) diethyl azodicarboxylate, Ph_3P, THF, 78.5%; (c) (1) Pd black, 4.4% HCOOH in MeOH; (2) Jones Reagent, acetone; 9d) isobutyl chloroformate, *N*-methylmorpholine, THF, $ButNH_2$; (e) 1 N NaOH, MeOH, 78.5%.



24—37 **38—44**

Scheme 4. Reagents and conditions: (a) (1) 4 N HCl–dioxane or H_2/10%Pd on carbon, MeOH; (2) *Z*-Asn-AHPBA-OH, DEPC, Et_3N, DMF or EDC • HCl/HOBt. DMF; (b) (1) H_2/10%Pd on carbon, MeOH; (2) RCOOH, DEPC, Et_3N, DMF.

Table 1. HIV PR inhibitory activity and anti-HIV-1 activity of AHPBA-based HIV PR inhibitors modified pyrrolidine ring at P1′ proline



No.	R^1	R^2	K_i (nM)[a]	No.	R^1	R^2	K_i (nM)[a]
I	H	H	57.5	**31**	H	—SPh	125
24	H	—OH	(1)[a]	**32**	H	—N⟨ ⟩O	(1)[a]
25	H	···OH	56	**33**	H	=O	12.5
26	H	—Cl	8	**34**	H	$=(OMe)_2$	92
27	H	···Cl	90	**35**	—OH	H	(0.1)[a]
28	H	—Br	22.5	**36**	···OH	H	32
29	H	—F	18	**37**	···Cl	H	14.5
30	H	$=F_2$	35				

[a]The numbers in parentheses indicate the concentration (μM) of the inhibitor which is equipotent with 1 μM pepstatin A (K_i = 1.1 μM).

philic 4(*R*)-hydroxyl group showed the same potency as nonsubstituted compounds, 3(*R*)- and 4(*S*)-hydroxylproline derivatives decreased in potency (**25** versus **35** and **24**). Only replacement by the 3(*S*)-hydroxyl group gave a more active inhibitor (**36**).

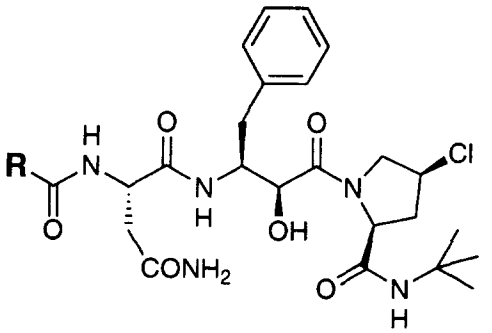
The conversion of the hydroxyl group into hydrophobic halogen atoms increased their inhibitory activities (K_i = 8–27.5 nM), except in the case of 4(*R*)-chloroproline (**27**). Among 4(*S*)-halogenoprolines the chlorine atom is the best. The order of the potency is as follows; Cl > F > Br (**26**, **29**, **28**). The results suggest that the size and the direction of the Cl atom is suitable for interaction with the enzyme. Conversion of the 3(*R*)-hydroxyl group into the 3(*S*)-Cl atom enhanced the protease inhibitory activity. Mimoto et al. also reported that introduction of two methyl groups at the 3-position of the pyrrolidine and thiazolidine increased the potency.[11] These indicate that the hydrophobicity of the substituents is important for the activity. On the other hand, introduction of one more fluorine atom at the 4-position decreased the potency by about one-half (**30**). Inhibitors that were replaced by 4(*S*)-phenylthio or 4(*S*)-morpholinoproline decreased their inhibitory activities (**31**, **32**). In the series of hydroxyethylamine-derived HIV PR inhibitors, it was suggested that the (4a*S*,8a*S*)-decahydroisoquinoline-3(*S*)-carbonyl group at the P1′ site fits into a hydrophobic pocket in the S1′ site. As described in our previous report, however, an AHPBA-based inhibitor containing this group showed only poor activity. Interestingly, oxidation of the 4(*R*)-hydroxyl group to ketone resulted in four times higher potency than the 4(*R*)-hydroxylproline derivative (**33** versus **25**). Conversion of the ketone into methyl ketal decreased the activity (**34**). The results also suggest that the size of the hydrophobic group at the 4-position on the pyrrolidine ring at P1′ proline greatly affects the activity.

The benzyloxycarbonyl (*Z*) group at P3 was replaced by several bicyclic heteroarylcarbonyl groups because of the expected increase in the inhibitory activity (Table 2), as described in our previous report. Conversion of the *Z* group into the quinoxaline-2-carbonyl group (**38**) resulted in an almost two-fold potency enhancement compared with **26**. Unfortunately, some five-membered fused aromatic heterocycles at P3 decreased their inhibitory activities with the exception of 7-methoxybenzofuran (**44**), which was the most potent inhibitor in the series.

Molecular modeling study

To explain the difference in the inhibitory activity between **26** with 4(*S*)-Cl and **27** with 4(*R*)-Cl at P1′ proline, the structures of the protease–inhibitor complexes of **I**, **26** and **27** were modeled based on the crystal structure of the HIV PR–MVT-101 complex.[12,13] As for the **26**–protease complex, the 4(*S*)-Cl atom is in the bottom of the S1′ pocket of the protease and surrounded by hydrophobic amino acids, Pro81, Val82, Ile84 and Ile50′, as shown in Fig. 1. No distinct change in the protease structure was observed compared with the complex with 4H-inhibitor, indicating that the

Table 2. Inhibitory activity of 4(*S*)-chloroproline containing HIV PR inhibitors modified at P3-site



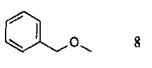
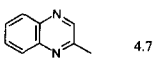
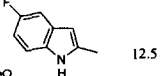
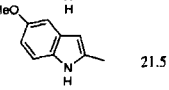
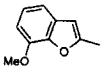
No.	R	HIV PR inhibition K_i (nM)	No.	R	HIV PR inhibition K_i (nM)
26	 8	**8**	**41**		**20**
38	 4.7	**4.7**	**42**		**16**
39	 12.5	**12.5**	**43**		**28**
40	 21.5	**21.5**	**44**		**4.5**

Figure 1. A stereoview of inhibitor **26** (blue line)-HIV-1 PR (white line) complex overlayed with inhibitor **27** (yellow line)-HIV-1 PR (orange line) complex. Cl atom is indicated in green.

4(*S*)-substituent is well accommodated in the S1′ pocket.

On the other hand, in the **27**–protease complex, the side chains of Val82 and Pro81 are shifted away from the Cl atom to avoid close contact between the Cl atom and the Val82 side chain. Furthermore, the 4(*R*)-Cl atom, located in the entrance of the S1′ pocket interacting with Leu23, Val82 and Ile84, is accessible to the solvent. These points suggest that the degree of stabilization of **27** when bound to the enzyme is less than that of **26**.

Anti-HIV-1 activity

Table 3 summarizes the anti-HIV-1 activity of eight inhibitors selected. Compound **26** with a 4(*S*)-Cl atom was more potent than compounds with the other substituents on the pyrrolidine ring at P1′ proline (**26** versus **29**, **33** and **37**). As described in our previous report, the replacement of the *Z* group with the quinoxaline-2-carbonyl group at the P3 site increased the potency.[8] Compound **38** showed a great improvement (four times) of anti-HIV-1 activity in acute infection, although the improvement in chronic infection was almost the same as compound **II**. The replacement of the quinoxaline ring with five-membered fused aromatic heterocycles also showed a pronounced antiviral effect. Among those, the 7-methoxybenzofuran derivative (**44**) was the most active inhibitor. These results suggest that both the chlorine atom at the P1′ site and five- or six-membered fused aromatic heterocycles at P3 site are important in improving cellular penetration.

Experimental

Melting points were measured with a Yanagimoto melting point apparatus and are uncorrected. IR spectra were measured with a Nic 5SXC FT IR spectrophotometer. ^{1}H NMR spectra were recorded on a JEOL JNM-GX 270 FT NMR spectrophotometer. Chemical shifts are expressed in δ ppm from the internal standard tetramethylsilane. EI- and FAB-MS were taken on a JEOL JMS-D 300 mass spectrometer and relevant data are tabulated as *m/z*. Column chromatography was carried out on Kieselgel 60 F_{254} (Merck, 70–230 mesh). Preparative TLC were run on Kieselgel 60 F_{254} plates (Merck art. 5717 or art. 5744).

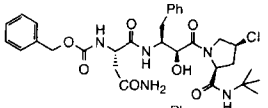
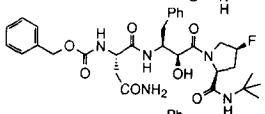
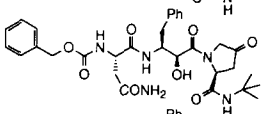
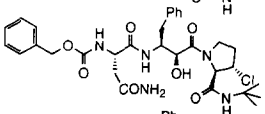
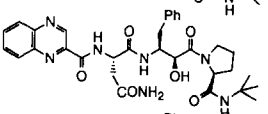
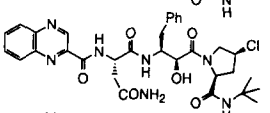
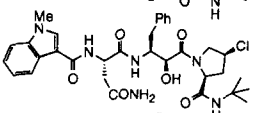
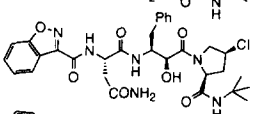
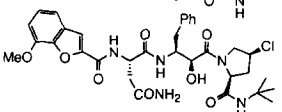
1-*tert*-Butoxycarbonyl-4(*R*)-hydroxy-L-proline *tert*-butylamide (2). To a stirred solution of 1-*tert*-butoxycarbonyl-4(*R*)-hydroxy-L-proline (**1**; 32.9 g, 142 mmol) and *N*-methylmorpholine (17.15 mL, 156 mmol) in tetrahydrofuran (THF, 250 mL) was added isobutyl chloroformate (20.23 mL, 156 mmol) at −10 °C. The mixture was stirred for 30 min at the same temperature. A solution of *tert*-butylamine (12.46 g, 170 mmol) in CH_2Cl_2 (50 mL) was added dropwise to the reaction mixture. The stirring was continued for 16 h at −5 °C and for 8 h at 0 °C. The reaction mixture was poured into AcOEt:ice, the organic solution was separated, washed with 10% aq citric acid soln, 10% aq $NaHCO_3$ soln and brine, and dried (Na_2SO_4). Concentration in

vacuo and recrystallization from AcOEt gave 34.46 g (84.7%) of **2** as a white crystal: mp 192–193 °C. IR (KBr) cm^{-1}: 3504, 1600, 1494, 1361, 1246, 1175, 1047, 997, 975, 929, 822 532. NMR (CD_3OD) δ ppm: 4.39–4.34 (m, 1H), 4.21 (t, 1H, J=8.3, 7.8 Hz), 3.52 (dd, 1H, J=3.9, 7.3 Hz), 3.48–3.43 (m, 1H), 2.22–2.14 (m, 1H), 2.01–1.90 (m, 1H), 1.45 (s, 9H), 1.34 (s, 9H). Anal. calcd for $C_{14}H_{26}N_2O_4$: C, 58.72; H, 9.15; N, 9.78. Found: C, 58.49; H, 9.33; N, 9.59.

1-*tert*-Butoxycarbonyl-4(*R*)-(*p*-toluenesulphonyl)oxy-L-proline *tert*-butylamide (3). To a stirred solution of **2** (10 g, 34.97 mmol) in pyrrolidine (100 mL) cooled to 0 °C was added *p*-toluenesulfonyl chloride (12.0 g, 63.16 mmol). Stirring was continued for 10 h. The mixture was poured into AcOEt:ice, the organic solution was separated, washed with 10% aq citric acid soln, 10% aq $NaHCO_3$ soln and brine, and dried (Na_2SO_4). Concentration in vacuo gave an oily substance. Purification of crude **4** by column chromatography on silica (*n*-hexane:AcOEt, 2:1) gave a white solid. Recrystallization from AcOEt:ethyl ether Et_2O:*n*-hexane gave 8.60 g (55.8%) of **3** as a white crystal: mp 125–127 °C. Anal. calcd for $C_{21}H_{32}N_2O_6S$: C, 57.25; H, 7.32; N, 6.36; S, 7.28. Found: C, 57.41; H, 7.09; N, 6.47; S, 7.31.

1-*tert*-Butoxycarbonyl-4(*S*)-phenylthio-L-proline *tert*-butylamide (4). To a stirred solution of tosylate **3** (1.23 g, 3.08 mmol) in *N,N*-dimethylformamide DMF:AcOEt (2:1, 10 mL) was added sodium thiophenolate (0.45 g, 3.41 mmol) at room temperature. Stirring was continued for 3 h. The reaction mixture was poured into AcOEt:10% aq $NaHCO_3$ soln and the organic layer was separated, washed with brine and dried (Na_2SO_4). Concentration in vacuo and purification by column chromatography on silica (*n*-hexane:AcOEt, 3:1) gave 1.0 g (85.8%) of **4** as a colorless powder: mp 119–121 °C. IR (KBr) cm^{-1}: 3409, 1959, 1731, 1674, 1523, 1247, 1151, 1099, 914, 696, 538. NMR ($CDCl_3$) δ ppm: 7.42–7.37 (m, 2H), 7.34–7.26 (m, 3H), 6.07–6.00 (m, 1H), 4.16 (br.s, 1H), 3.95–3.89 (m, 1H), 3.68 (quintet, 1H, J=6.4, 3.9 Hz), 3.42–3.40 (m, 1H), 2.59 (m, 1H), 2.22 (m, 1H), 1.46 (s, 9H), 1.38 (s, 9H): *m/z* 378 (M^+). Anal. calcd for $C_{20}H_{30}N_2O_3S$: C, 63.46;

Table 3. Anti-HIV-1 activity of AHPBA-based inhibitors **25**, **28**, **32**, **34**, **37**, **40**, **42**, and **43**

No.	Inhibitors	Molt 4/HIV-1$_{IIIB}$ IC_{90} (μM)	CEM/HIV-1$_{IIIB}$ IC_{90} (μM)
26		N.D.	0.5
29		N.D.	5.0
33		N.D.	1.5
37		N.D.	1.5
II		1.0	0.5
38		1.2	0.12
41		2.1	0.20
43		3.2	0.16
44		0.58	0.06

H, 7.99; N, 7.40; S, 8.47. Found: C, 63.31; H, 7.74; N, 7.35; S, 8.77.

1-*tert*-Butoxycarbonyl-4(*S*)-morpholino-L-proline *tert*-butylamide (5). A mixture of tosylate **3** (1.0 g, 2.27 mmol) and morpholine (5 g) was heated for 3 h. The reaction mixture was concentrated in vacuo and the satd aq soln of $NaHCO_3$ was added to the residue. The mixture was extracted with AcOEt. The organic layer was separated, washed with brine and dried (Na_2SO_4). Concentration in vacuo and recrystallization from CH_2Cl_2:Et_2O gave 0.65 g (56.5%) of **5** as a white crystal: mp 172–173 °C. IR (KBr) cm^{-1}: 3338, 2977, 2815, 1683, 1669, 1536, 1427, 1365, 1268, 1228, 1158, 1114, 895. NMR (CD_3OD) δ ppm: 4.09 (dd, 1H, J=9.3, 9.7 Hz), 3.82 (dd, 1H, J=9.3, 9.7 Hz), 3.68 (dd, 1H, J=4.4, 4.9 Hz), 3.19 (dd, 1H, J=9.8 Hz), 2.78–2.67 (m, 1H), 2.57–2.40 (m, 5H), 1.71 (dd, 1H, J=11.2, 10.7 Hz), 1.44 (s, 9H), 1.34 (s, 9H). *m/z* 355 (M^+). Anal. calcd for $C_{18}H_{33}N_3O_4$: C, 60.82; H, 9.36; N, 11.82. Found: C, 60.51; H, 9.28; N, 11.80.

1-*tert*-Butoxycarbonyl-4(*S*)-chloro-L-proline *tert*-butylamide (6). A mixture of **2** (1.0 g, 3.50 mmol), CCl_4 (30 mL) and Ph_3P (1.56 g, 5.95 mmol) was refluxed for 2 h. The insoluble material was removed by filtration and was washed with Et_2O. The filtrate was concentrated in vacuo and the residue was chromatographed on silica (*n*-hexane:AcOEt, 4:1). Recrystallization from CH_2Cl_2:*n*-hexane gave 0.95 g (89%) of **6** as a white crystal: mp 149–150 °C. IR (KBr) cm^{-1}: 3340, 2971, 1698, 1672, 1544, 1414, 1367, 1160. NMR (CD_3OD) δ ppm: 4.43 (dd, 1H, J=6.4, 5.9 Hz), 4.15 (dd, 1H, J=5.9 Hz), 3.94 (dd, 1H, J=5.9, 6.4 Hz), 3.52 (dd, 1H, J=5.9, 5.4 Hz), 2.77–2.68 (m, 1H), 2.23–2.13 (m, 1H), 1.45 (s, 9H), 1.35 (s, 9H). *m/z* 305 (M^+). Anal. calcd for $C_{14}H_{25}N_2O_3Cl$: C, 55.17; H, 8.27; N, 9.19; Cl, 11.63. Found: C, 55.31; H, 8.30; N, 8.97; Cl, 11.64.

1-*tert*-Butoxycarbonyl-4(*S*)-bromo-L-proline *tert*-butylamide (7). A mixture of **2** (2.86 g, 10.0 mmol), CBr_4 (5.0 g, 15.11 mmol) and triphenylphosphine (Ph_3P; 3.9 g, 14.89 mmol) in THF (60 mL) was refluxed for 1 h. The insoluble material was removed by filtration and was washed with Et_2O. The filtrate was concentrated in vacuo and the oily residue was chromatographed on silica (*n*-hexane:AcOEt, 4:1) to give a colorless solid. Recrystallization from Et_2O:*n*-hexane gave 3.25 g (93%) of **7** as a colorless crystal: mp 159–160 °C. IR (KBr) cm^{-1}: 3339, 2968, 1698, 1686, 1672, 1544, 1412, 1366, 1158. NMR ($CDCl_3$) δ ppm: 6.20 (br s, 1H), 4.39 (m, 1H), 4.22 (t, 1H, J=6.4 Hz), 4.00 (dd, 1H, J=5.4 Hz), 3.77–3.33 (m, 1H), 2.7 (m, 2H), 1.48 (s, 9H), 1.37 (s, 9H). Anal. calcd for $C_{14}H_{25}N_2O_3Br$: C, 48.14; H, 7.21; N, 8.02; Br, 22.88. Found: C, 48.00; H, 7.13; N, 8.03; Br, 23.03.

1-*tert*-Butoxycarbonyl-4(*S*)-fluoro-L-proline *tert*-butylamide (8). To a stirred solution of **2** (2.86 g, 10 mmol) in THF (50 mL) was added diethylammonium sulfur trifluoride (DAST; 3.22 g, 20 mmol) at −50 °C in a nitrogen atmosphere. The temperature was raised to room temperature after 10 min and stirring was continued for 4 h. MeOH (5 mL) and AcOEt were added to the mixture and the mixture was washed with 10% aq citric acid soln, 10% aq $NaHCO_3$ soln and brine, and dried (Na_2SO_4). The solvent was evaporated in vacuo. The residue was chromatographed on silica (*n*-hexane:AcOEt, 3:1) to give 1.2 g (42%) of starting material (**2**) and 0.5 g (29.8%) of compound **8**. Recrystallization of **8** from CH_2Cl_2:*n*-hexane gave a colorless crystal: mp 126–127 °C. *m/z* 289 (M^+). NMR ($CDCl_3$) δ ppm: 5.95 (m, 1H), 5.28 (dd, 1/2H, J=3.4, 3.7 Hz), 5.09 (dd, 1/2H, J=3.4, 3.9 Hz), 4.27–4.24 (m, 1H), 3.84–3.65 (m, 1H), 3.6 (dd, 1/2H, J=3.4 Hz), 3.5 (dd, 1/2H, J=3.4 Hz), 2.61–2.36 (m, 2H), 1.48 (s, 9H), 1.34 (s, 9H).

1-*tert*-Butoxycarbonyl-4-oxo-L-proline *tert*-butylamide (9). To a stirred solution of oxalyl chloride (0.98 g, 7.72 mmol) in CH_2Cl_2 (20 mL) was added a soln of DMSO (1.21 g, 15.51 mmol) in CH_2Cl_2 (5 mL) at −50 to −60 °C. After 5 min, a solution of **2** (2.0 g, 7.00 mmol) in CH_2Cl_2 (40 mL) was added to the mixture at the same temperature and stirring was continued for 15 min. To this mixture was added triethylamine (Et_3N; 3.5 g, 34.6 mmol) and the mixture was stirred for 5 min. The temperature was raised to room temperature and stirring continued for 1 h. The reaction mixture was poured into AcOEt:ice, the organic layer was separated, washed with 10% aq $NaHCO_3$ solution and brine, and dried (Na_2SO_4). Concentration in vacuo gave a colorless solid. Recrystallization from CH_2Cl_2:Et_2O gave 1.47 g (73.9%) of **9** as a colorless crystal: mp 147–149 °C. IR (KBr) cm^{-1}: 3348, 2982, 1773, 1674, 1540, 1410, 1398, 1369, 1158, 1117. NMR ($CDCl_3$) δ ppm: 6.62 (br s, 1H), 4.61 (br s, 1H), 3.81 (q, 2H, J=18.6, 46.5 Hz), 2.92 (m, 1H), 2.66 (m, 1H), 1.49 (s, 9H), 1.34 (s, 9H). *m/z* 284 (M^+). Anal. calcd for $C_{14}H_{24}N_2O_4$: C, 59.14; H, 8.51; N, 9.85. Found: C, 58.90; H, 8.35; N, 9.67.

1-*tert*-Butoxycarbonyl-4,4-difluoro-L-proline *tert*-butylamide (10). The title compound was prepared from the reaction of **9** (1.42 g, 5 mmol) with DAST (2.43 g, 15 mmol), as described for the synthesis of compound **8**. Recrystallization from CH_2Cl_2:*n*-hexane gave 1.36 g (89%) of compound **10** as a colorless crystal: mp 135–136 °C. IR (KBr) cm^{-1}: 3324, 2986, 1702, 1656, 1551, 1399, 1367, 1150, 1104, 938, 769. *m/z* 307 (M^+).

1-Benzyloxycarbonyl-4(*S*)-hydroxy-L-proline *tert*-butylamide (11). To a stirred solution of 1-benzyloxycarbonyl-4(*S*)-hydroxy-L-proline (3.68 g, 1.39 mmol) in DMF (30 mL) cooled to 0 °C were added diphenylphosphoryl azide (DPPA, 4.56 g, 16.58 mmol), *tert*-butylamine (1.50 g, 20.23 mmol) and Et_3N (2.78 g, 27.5 mmol), keeping the temperature below 5 °C. Stirring was continued for 5 h. The reaction mixture was poured into AcOEt:ice, the organic solution was separated, washed with 10% aq citric acid soln, 10% aq $NaHCO_3$ soln and brine, and dried (Na_2SO_4). Concentration in vacuo and recrystallization from CH_2Cl_2:Et_2O gave 1.10 g (24.7%) of amide as a white crystal: mp

129–130 °C. IR (KBr) cm^{-1}: 3281, 2957, 1703, 1647, 1452, 1410, 1212, 1082, 968, 698. NMR (CD_3OD) δ ppm: 7.38–7.27 (m, 5H), 5.17–5.08 (m, 1H), 5.13 (s, 2H), 4.32–4.26 (m, 1H), 3.62 (dd, 1H, J=4.9, 11.2 Hz), 3.53–3.52 (m, 1H), 2.41 (m, 1H), 1.93 (br d, 1H, J=13.2Hz), 1.32, 1.19 (1:2; s, 9H). Anal. calcd for $C_{17}H_{24}N_2O_4$: C, 63.73; H, 7.55; N, 8.34. Found: C, 64.00; H, 7.47; N, 8.80.

1-*tert*-Butoxycarbonyl-3(*R*)-*tert*-butyldimethylsilyloxy-L-prolinol (14). To a solution of (2*R*,3*S*)-2-benzyloxymethyl-1-*tert*-butoxycarbonyl-3-*tert*-butyldimethylsilyloxypyrrolidine (**13**; 800 mg, 1.9 mmol),[10] which was prepared from *N*-*tert*-butoxycarbonyl-*O*-benzyl-D-serine according to Ewing and Joullie',[10] in MeOH (8 mL) were added formic acid (0.4 mL) and Pd black (400 mg) and the mixture stirred for 3 h in a nitrogen atmosphere. The catalyst was filtered off and the filtrate was concentrated in vacuo. The oily residue was chromatographed on silica (*n*-hexane:AcOEt, 6:1) to give 620 mg (98%) of compound **14** as a colorless oil. IR (liquid film) cm^{-1}: 3424, 2955, 2931, 2893, 2859, 1698, 1674, 1408, 1367,1255, 1174, 1134, 836, 776. NMR ($CDCl_3$) δ ppm: 4.44 (m, 1H), 3.89–3.84 (m, 2H), 3.74–3.66 (m, 1H), 3.46–3.35 (m, 2H), 2.03–1.80 (m, 2H), 1.47 (s, 9H), 0.90 (s, 9H), 0.10 (s, 6H). *m/z* 332 (M+H). Anal. calcd for $C_{16}H_{33}NO_4Si$: C, 56.87; H, 9.84; N, 4.12. Found: C, 57.06; H, 9.63; N, 4.16.

1-*tert*-Butoxycarbonyl-3(*R*)-*tert*-butyldimethylsilyloxy-L-proline (15). To a stirred solution of **14** (245 mg, 0.74 mmol) in acetone (10 mL) was added Jones Reagent (0.7 mL) at 0 °C and stirring was continued for 2 h. The reaction mixture was poured into Et_2O:H_2O and the organic layer was separated, washed with brine, and dried (Na_2SO_4). Evaporaton in vacuo gave 191 mg (74.7%) of compound **15** as a colorless crystal which, was crystallized from *n*-hexane: mp 146–147 °C. IR (KBr) cm^{-1}: 2976, 2953, 2930, 2885, 2857, 1752, 1656, 1474, 1436, 1367, 1262, 1253, 1229, 1205, 1180, 1131, 1114, 1053, 935, 836, 779. NMR ($CDCl_3$) δ ppm: 4.58 (q, 1H, J=6.6 Hz), 4.38–4.28 (m, 1H), 3.67–3.59 (m, 1H), 3.45–3.38 (m, 1H), 2.00 (dd, 1H, J=6.6, 7.3 Hz), 1.43 (s, 9H), 0.87 (s, 9H), 0.09 (s, 6H). Anal. calcd for $C_{16}H_{31}NO_5Si$: C, 55.62; H, 9.04; N, 4.05. Found: C, 55.47; H, 8.85; N, 4.05.

1-*tert*-Butoxycarbonyl-3(*R*)-*tert*-butyldimethylsilyloxy-L-proline *tert*-butylamide (16). To a stirred solution of **15** (214 mg, 0.62 mmol) in THF (5 mL) were added Et_3N (95 μL, 0.68 mmol) and isobutyl chloroformate (89 μL, 0.68 mmol) at −40 °C. The temperature was raised to −10 °C and the mixture was stirred for 20 min at the same temperature. The mixture was cooled to −15 °C and *tert*-butylamine (68.3 μL, 0.65 mmol) was added to the mixture. Stirring was continued for 14 h at −5 °C. The reaction mixture was poured into AcOEt:H_2O and the organic layer was separated, washed with brine and dried (Na_2SO_4). The solution was concentrated in vacuo and the residue was chromatographed on silica gel (*n*-hexane:AcOEt, 4:1) to give 118 mg (47.5%) of compound **16** as a colorless crystal: mp 67–69 °C. IR (KBr) cm^{-1}: 3337, 2966, 2930, 2891, 2859, 1701, 1672, 1545, 1416, 1366, 1251, 1174, 1109, 1047, 935, 836, 775. *m/z* 400 (M^+). Anal. calcd for $C_{20}H_{40}N_2O_4Si$: C, 59.96; H, 10.06; N, 6.99. Found: C, 59.68; H, 10.04; N, 7.07.

1-*tert*-Butoxycarbonyl-3(*R*)-hydroxy-L-proline *tert*-butylamide (17). To a stirred solution of **16** (48 mg, 0.12 mmol) in THF (1 mL) cooled to 0 °C was added a solution of tetrabutylammonium fluoride (94 mg, 0.36 mmol) in THF (0.5 mL) and the mixture was stirred for 7 h at room temperature. The reaction mixture was diluted with AcOEt and the solution was washed with 1 N HCl, H_2O, 5% aq $NaHCO_3$ soln and brine, and dried (Na_2SO_4). The solvent was evaporated in vacuo to give 19 mg (55.2%) of compound **17** as an oil. NMR ($CDCl_3$) δ ppm: 4.49 (br s, 1H), 4.16 (d, 1H, J=6.6 Hz), 3.75–3.45 (m, 4H), 2.14–1.93 (m, 2H), 1.44 (s, 9H), 1.35 (s, 9H).

1-*tert*-Butoxycarbonyl-3(*S*)-chloro-L-proline *tert*-butylamide (18). The reaction of **17** (19 mg, 0.066 mmol) with Ph_3P (26.2 mg, 0.1 mmol) in CCl_4 (4 mL) as described for compound **6** gave the title compound **18** as an oil.

2(*R*)-Benzyloxymethyl-1-*tert*-butoxycarbonyl-3(*R*)-hydroxypyrrolidine (19). To a stirred soltution of **13** (2.16 g, 5.12 mmol)[10] in THF (5 mL) cooled to 0 °C was added a solution of tetra-*n*-butylammonium fluoride (2.01 g, 7.68 mmol) in THF (5 mL) and the mixture was stirred for 4 h at room temperature. The reaction mixture was poured into AcOEt:0.2 N HCl and the organic layer was separated, washed with H_2O, 5% aq $NaHCO_3$ soln and brine, and dried (Na_2SO_4). Concentration in vacuo gave an oil. The crude oil was purified by column chromatographed on silica gel (*n*-hexane:AcOEt, 3:1) to give 1.57 g (99.2%) of compound **19** as a colorless oil. IR (liquid film) cm^{-1}: 3443, 2977, 2932, 2893, 1695, 1674, 1399, 1367, 1172, 1119, 738, 698. NMR ($CDCl_3$) δ ppm: 7.37–7.27 (m, 5H), 4.58, 4.53 (ca. 6:1, ss, 2H), 4.45 (q, 1H, J=5.9, 12.5 Hz), 4.00–3.70 (m, 3H), 3.42–3.38 (m, 2H), 3.03 (m, 1H), 2.08–1.87 (m, 2H), 1.43 (s, 9H). *m/z* 307 (M^+). Anal. calcd for $C_{17}H_{25}NO_4 \cdot 0.1H_2O$: C, 66.04; H, 8.22; N, 4.53. Found: C, 65.95; H, 8.20; N, 4.58.

3(*S*)-Benzoyloxy-2(*R*)-benzyloxymethyl-1-*tert*-butoxycarbonylpyrrolidine (20). To a stirred solution of **19** (1.0 g, 3.25 mmol), Ph_3P (0.87 g, 3.31 mmol) and benzoic acid (0.42 g, 3.47 mmol) in THF was slowly added a solution of diethyl azodicarboxylate (0.52 mL, 3.31 mmol) at room temperature and stirring was continued for 14 h. The reaction mixture was poured into AcOEt:5% aq $NaHCO_3$ soln and the organic layer was separated, washed with brine, and dried (Na_2SO_4). The solution was concentrated in vacuo. The crude oil was chromatographed on silica (*n*-hexane:AcOEt, 3:1) to give 1.05 g (78.5%) of compound **20** as a colorless oil. IR (liquid film) cm^{-1}: 2977, 1720, 1697, 1479, 1453, 1395, 1367, 1317, 1270, 1175, 1114, 1026, 738, 714, 698. NMR ($CDCl_3$) δ ppm: 8.02 (d, 2H, J=7.3 Hz),

7.59–7.26 (m, 8H), 5.54 (m, 1H), 4.56 (q, 2H, $J=11.9$, 19.1 Hz), 4.09 (d, 1H, $J=40.3$ Hz), 3.80–3.47(m, 3H), 2.45–2.27 (m, 1H), 2.16–2.05 (m, 1H), 1.48, 1.42 (47:66; s, 9H). *m/z* 307 (M^+). Anal. calcd for $C_{17}H_{25}NO_4 \cdot 0.1H_2O$: C, 66.04; H, 8.22; N, 4.53. Found: C, 65.95; H, 8.20; N, 4.58. *m/z* 411(M^+), 290, 234, 190, 91, 57. Anal: calcd for $C_{24}H_{29}NO_5$: C, 70.05; H, 7.10; N, 3.40. Found: C, 70.14; H, 7.10; N, 3.73.

3(*S*)-Benzoyloxy-1-*tert*-butoxycarbonyl-L-proline *tert*-butylamide (22). Compound **20** was submitted to the same reaction as described in the preparation of compound **16**: mp 132–134 °C. IR (KBr) cm^{-1}: 3342, 2977, 1720, 1676, 1532, 1405, 1269, 1172, 1109, 1087, 712. Anal. calcd for $C_{21}H_{30}N_2O_5 \cdot 1/4H_2O$: C, 63.86; H, 7.78; N, 7.09. Found: C, 64.10; H, 7.63; N, 7.30.

1-*tert*-Butoxycarbonyl-3(*S*)-hydroxy-L-proline *tert*-butylamide (23). To a stirred solution of **22** (63 mg, 0.16 mmol) in MeOH (1 mL) cooled to 0 °C was added 1 N NaOH (0.17 mL, 0.17 mmol) and stirring was continued for 30 min at 0 °C. The reaction mixture was neutralized with 1 N HCl and concentrated in vacuo. The residue was partitioned between AcOEt and 5% aq citric acid soln and the organic layer was separated, washed with H_2O, 5% aq $NaHCO_3$ soln and brine, and dried (Na_2SO_4). The solvent was evaporated in vacuo and the residue was purified by preparative TLC (*n*-hexane:AcOEt, 1:1) to give 36 mg (78.5%) of compound **23** as a colorless crystal: mp 122–124 °C. IR (KBr) cm^{-1}: 3448, 3316, 2976, 1687, 1663, 1405, 1366, 1167. NMR ($CDCl_3$) δ ppm: 4.65–4.45 (m, 1H), 4.17–4.00 (m, 1H), 3.73–3.45 (m, 2H), 2.13–1.86 (m, 2H), 1.47 (s, 9H), 1.33 (s, 9H). Anal. calcd for $C_{14}H_{26}N_2O_6$: C, 58.72; H, H, 9.15; N, 9.78. Found: C, 58.70; H, 9.18; N, 9.65.

Z-Asn-(2*S*,3*S*)-AHPBA-[4(*S*)-hydroxy]Pro *tert*-butylamide (24). To a stirred solution of **11** (77 mg, 0.24 mmol) in MeOH (5 mL) were added 1 N HCl (0.25 mL) and 10% Pd on charcoal (20 mg) and the mixture was hydrogenated for 3 h at room temperature. The catalyst was filtered off and the filtrate was concentrated in vacuo. The residue was dissolved in DMF (3 mL) and *Z*-Asn-(2*S*,3*S*)-AHPBA-OH (100 mg, 0.226 mmol), DEPC (44.2 mg, 0.271 mmol) and Et_3N (68 mg, 0.673 mmol) were added to the solution at 0 °C. The mixture was stirred for 5 h at the same temperature, and was concentrated in vacuo. The residue was partitioned between AcOEt and 10% aq citric acid soln, and the organic solution was separated, washed with H_2O, 5% aq $NaHCO_3$ soln and brine, and dried (Na_2SO_4). The solvent was evaporated in vacuo and the residue was purified by preparative TLC (CH_2Cl_2:MeOH, 10:1). Crystallization from Et_2O gave 70 mg (49%) of compound **24** as a colorless solid: mp 115–120 °C. IR (KBr) cm^{-1}: 3327, 2969, 1660, 1534, 1455, 1266, 1227, 1090, 1054, 749, 699. NMR (CD_3OD) δ ppm: 7.36–7.09 (m, 10H), 5.07 (s, 2H), 4.47–4.31 (m, 5H), 3.89–3.75 (m, 2H), 2.88–2.73 (m, 2H), 2.67–2.57 (m, 1H), 2.48–2.30 (m, 2H), 1.95–1.87 (m, 1H), 1.34, 1.31 (1:14; s, 9H). *m/z* 594 (M-17). Anal. calcd for $C_{31}H_{40}N_5O_8 \cdot 1/2H_2O$: C, 60.08; H, 6.67; N, 11.30. Found: C, 60.01; H, 7.00; N, 11.22.

Z-Asn-(2*S*,3*S*)-AHPBA-[4(*R*)-hydroxy]Pro *tert*-butylamide (25). Compound **2** (77 mg, 0.24 mmol) was stirred in 4 N HCl/dioxane solution (3 mL) for 30 min at room temperture and the mixture was concentrated in vacuo until dry. The remaining solid was dissolved in a small volume of MeOH and benzene, and the solution was evaporated in vacuo (repeated three times). The residue was dried in vacuo for 2 h, and dissolved in DMF (3 mL). *Z*-Asn-(2*S*,3*S*)-AHPBA-OH (100 mg, 0.226 mmol), DEPC (44.2 mg, 0.271 mmol) and Et_3N (68 mg, 0.673 mmol) were added to the solution at 0 °C. The mixture was stirred for 5 h at the same temperature and was concentrated in vacuo. The residue was partitioned between AcOEt and 10% aq citric acid soln and the organic solution was separated and washed with H_2O, 5% aq $NaHCO_3$ soln and brine, and dried (Na_2SO_4). The solvent was evaporated in vacuo and the residue was purified by preparative TLC (CH_2Cl_2:MeOH, 10:1). Crystallization from Et_2O gave 70 mg (49%) of compound **25** as a colorless solid: mp 115–120 °C. IR (KBr) cm^{-1}: 3337, 2967, 1669, 1536, 1455, 1258, 1227, 748, 699. NMR (CD_3OD) δ ppm: 7.36–7.08 (m, 10H), 5.08 (s, 2H), 4.54–4.43 (m, 3H), 4.33–4.26 (m, 1H), 3.78–3.76 (m, 2H), 2.84–2.78 (m, 2H), 2.66–2.56 (m, 1H), 2.47–2.39 (m, 2H), 2.16–2.02 (m, 2H), 1.32 (s, 9H). *m/z* 594 (M-17). Anal. calcd for $C_{31}H_{40}N_5O_8 \cdot 1/2H_2O$: C, 60.08; H, 6.67; N, 11.30. Found: C, 60.01; H, 7.00; N, 11.22.

Z-Asn-(2*S*,3*S*)-AHPBA-[4(*S*)-chloro]Pro *tert*-butylamide (26). Compound **6** (680 mg, 2.23 mmol) was stirred in 4 N HCl dioxane solution (10 mL) for 30 min at room temperature and the mixture was concentrated in vacuo until dry. The remaining solid was dissolved in a small volume of MeOH and benzene, and the solution was evaporated in vacuo (repeated three times). The residue was dried in vacuo for 2 h and dissolved in DMF (5 mL). To the solution were added *Z*-Asn-(2*S*,3*S*)-AHPBA-OH (880 mg, 1.99 mmol),[8] DEPC (420 mg, 2.58 mmol) and Et_3N (640 mg, 6.4 mmol) under ice-cooling and stirring was continued for 3 h. The reaction mixture was concentrated in vacuo and the residue was partitioned between AcOEt and 10% aq citric acid soln. The organic layer was separated and washed with H_2O, 5% aq $NaHCO_3$ soln and brine, and dried (Na_2SO_4). The solvent was evaporated in vacuo to give an oily substance. The residue was purified by preparative TLC (CH_2Cl_2:MeOH, 10:1). Crystallization from Et_2O gave 850 mg of compound **26** as a colorless crystal: mp 107–109 °C. IR (KBr) cm^{-1}: 3331, 2968, 1668, 1530, 1455, 1267, 1228, 1055, 749, 699. NMR (CD_3OD) δ ppm: 7.36–7.10 (m, 10H), 5.07 (s, 2H), 4.45–4.28 (m, 7H), 3.73 (dd, 1H, $J=10.3$, 7.3 Hz), 2.97–2.90 (m, 1H), 2.81–2.37 (m, 4H), 2.16–2.05 (m, 1H), 1.34, 1.31 (1:14; s, 9H). Anal. calcd for $C_{31}H_{40}N_5O_7Cl$: C, 58.26; H, 6.46; N. 10.95; Cl, 5.55. Found: C, 58.35; H, 6.56; N, 10.93; Cl, 5.46.

Z-Asn-(2S,3S)-AHPBA-[4(R)-chloro]Pro *tert*-butylamide (27). The title compound was prepared as described for **24** using 1-benzyloxycarbonyl-4(*R*)-chloro-L-proline *tert*-butylamide (**12**) instead of 11: mp 107–109 °C. IR (KBr) cm^{-1}: 3328, 1668, 1536, 1455, 1256, 1227, 748, 699. NMR (CD_3OD) δ ppm: 7.35–7.10 (m, 10H), 5.08 (s, 2H), 4.72 (br s, 1H), 4.62 (t, 1H, $J=8$ Hz), 4.47–4.44 (m, 2H), 4.28–4.23 (m, 1H), 4.10–4.01 (m, 1H), 2.86–2.75 (m, 1H), 2.65–2.58 (m, 4H), 2.47–2.34 (m, 3H), 1.32 (s, 9H). Anal. calcd for $C_{31}H_{40}N_5O_7Cl$: C, 58.26; H, 6.46; N. 10.95; Cl, 5.55. Found: C, 58.35; H, 6.56; N 10.93; Cl, 5.46.

The following six compounds were prepared as described for **25**. Their melting points, IR spectral data, 270 MHz ^1H NMR data, MS data and elemental analyses are noted.

Z-Asn-(2S,3S)-AHPBA-[4(S)-bromo]Pro *tert*-butylamide (28). Mp 100–105 °C. IR (KBr) cm^{-1}: 3331, 2968, 1668, 1527, 1455, 750, 699. NMR (CD_3OD) δ ppm: 7.56–7.10 (m, 10H), 5.08 (s, 2H), 4.45–4.25 (m, 5H), 3.78 (dd, 1H, $J=7.8$, 8.3 Hz), 2.96–2.86 (m, 4H), 2.84–2.72 (m, 2H), 2.60 (dd, 1H, $J=5.9$ Hz), 2.40 (d, 1H, $J=7.8$ Hz), 2.24–2.12 (m, 1H), 1.32 (s, 9H), 0.90 (dd, 1H, $J=6.3$, 7.8 Hz). *m/z* 674 (M^+). Anal. calcd for $C_{31}H_{40}N_5O_8Br\cdot 1/2H_2O$: C, 54.49; H, 6.04; N, 10.24; Br, 11.69. Found: C, 54.61; H, 6.10; N, 10.10; Br, 11.31.

Z-Asn-(2S,3S)-AHPBA-[4(S)-fluoro]Pro *tert*-butylamide (28). Mp 95–100 °C. IR (KBr) cm^{-1}: 3328, 1668, 1531, 1455, 1263, 1227, 744, 698. NMR (CD_3OD) δ ppm: 7.35–7.10 (m, 10H), 5.39–5.16 (m, 1H), 5.06 (s, 2H), 4.52 (dd, 1H, $J=2.9$ Hz), 4.49–4.32 (m, 2H), 4.21–3.72 (m, 3H), 3.03–2.83 (m, 1H), 2.65–2.28 (m, 4H), 129 (s, 9H), 0.90 (t, 1H, $J=6.8$ Hz). Anal. calcd for $C_{31}H_{40}N_5O_8F\cdot 1/2H_2O$: C, 54.47; H, 6.64; N, 11.25; F, 3.05. Found: C, 60.08; H, 6.45; N, 7.01; F, 2.67.

Z-Asn-(2S,3S)-AHPBA-(4,4-difluoro)Pro *tert*-butylamide (30). Mp 101–103 °C. IR (KBr) cm^{-1}: 3328, 1668, 1531, 1455, 1368, 1257, 1227, 748, 699. NMR (CD_3OD) δ ppm: 7.35–7.11 (m, 10H), 5.08 (s, 2H), 4.59 (dd, 1H, $J=7.3$, 7.4 Hz), 4.46–4.40 (m, 2H), 4.28–4.05 (m, 3H), 2.94–2.75 (m, 2H), 2.73–2.58 (m, 2H), 2.50–2.34 (m, 2H), 1.32 (s, 9H). *m/z* 632 (M^+). Anal. calcd for $C_{31}H_{39}N_5O_7F_2\cdot 1/2H_2O$: C, 58.12; H, 6.29; N, 10.93; F, 5.93. Found: C, 58.26; H, 6.30; N, 10.87; F, 5.53.

Z-Asn-(2S,3S)-AHPBA-[4(S)-phenylthio]Pro *tert*-butylamide (31). Mp 96–98 °C. IR (KBr) cm^{-1}: 3332, 2968, 1668, 1530, 1454, 1264, 1227, 746, 698. NMR (CD_3OD) δ ppm: 7.89–7.10 (m, 10H), 5.08, 5.06 (3:1; s, 2H), 4.45–4.18 (m, 5H), 3.76–3.68 (m, 1H), 3.51 (t, 1H, $J=10$ Hz), 2.95–2.36 (m, 4H), 1.92–1.80 (m, 1H), 1.33, 1.31 (1:13; s, 9H). *m/z* 686 (M-18). Anal. calcd for $C_{37}H_{45}N_5O_7S\cdot 1/2H_2O$: C, 62.34; H, 6.50; N, 9.82; S, 4.50. Found: C, 62.45; H, 6.45; N, 9.70; S, 4.46.

Z-Asn-(2S,3S)-AHPBA-[4(S)-morpholino]Pro *tert*-butylamide (32). Mp 115–117 °C. IR (KBr) cm^{-1}: 3330, 2965, 1672, 1536, 1455, 1268, 1118, 747, 699. NMR (CD_3OD) δ ppm: 7.36–7.09 (m, 10H), 5.08 (s, 2H), 4.46–4.41 (m, 2H), 4.37–4.29 (m, 2H), 4.18–4.11 (m, 1H), 3.44 (dd, 1H, $J=10.3$, 9.8 Hz), 2.90–2.80 (m, 2H), 2.76–2.37 (m, 5H), 1.78 (q, 1H, $J=10.7$, 2107 Hz), 1.33, 1.31 (1:14; s, 9H). *m/z* 680 (M^+). Anal. calcd for $C_{35}H_{48}N_6O_8\cdot 3/4H_2O$: C, 60.55; H, 7.18; N, 12.10. Found: C, 60.59; H, 7.14; N, 12.14.

Z-Asn-(2S,3S)-AHPBA-(4-oxo)Pro *tert*-butylamide (33). Mp 116–120 °C. IR (KBr) cm^{-1}: 3338, 2968, 1767, 1665, 1535, 1455, 1261, 1226, 748, 698. NMR (CD_3OD) δ ppm: 7.36–7.09 (m, 10H), 5.12 (s, 2H), 4.46–4.19 m, 2H), 2.92–2.73 (m, 3H), 2.66–2.52 (m, 1H), 2.48–2.38 (m, 2H), 1.31 (s, 9H). *m/z* 609 (M^+). Anal. calcd for $C_{31}H_{39}N_5O_8\cdot 1/2H_2O$: C, 60.18; H, 6.52; N, 11.32. Found: C, 59.92; H, 6.63; N, 11.27.

Z-Asn-(2S,3S)-AHPBA-(4,4-dimethoxy)Pro *tert*-butylamide (34). To a stirred solution of **9** (210 mg, 0.743 mmol) in MeOH (2 mL) was added 4 N HCl dioxane soln (2 mL) and stirring was continued for 15 min. A small volume of benzene was added to the mixture and the solution was concentrated in vacuo. This procedure was repeated three times. (4,4-Dimethoxy)Pro *tert*-butylamide was obtained during this procedure. The residue and Z-Asn-(2S,3S)AHPBA-OH (329 mg, 0.743 mmol) were dissolved in DMF (10 mL) and the mixture was cooled to 0 °C. To the solution were added DEPC (149 mg, 0.829 mmol) and Et_3N (225 mg, 2.23 mmol). The stirring was continued for 3 h at the same temperature. The mixture was concentrated in vacuo and the residue was partitioned between AcOEt and 10% aq citric acid soln. The organic solution was separated, washed with H_2O, 5% aq $NaHCO_3$ soln and brine, and dried (Na_2SO_4). The solvent was evaporated in vacuo and the residue was purified by preparative TLC (CH_2Cl_2:MeOH, 10:1) to give 270 mg (54.7%) of compound **34** as a colorless solid: mp 100–105 °C. IR (KBr) cm^{-1}: 3328, 2966, 1668, 1535, 1455, 1051, 748, 699. NMR (CD_3OD) δ ppm: 7.36–7.11 (m, 10H), 5.11 (s, 2H), 4.46–4.36 (m, 3H), 4.31–4.24 (m, 1H), 4.04 (d, 1H, $J=11$ Hz), 3.66 (d, 1H, $J=11$ Hz), 3.29 (s, 3H), 3.25 (s, 3H), 2.88–2.76 (m, 2H), 2.64–2.54 (m, 1H), 2.49–2.41 (m, 2H), 2.03 (dd, 1H, $J=8.6$, 8.7 Hz), 1.33, 1.31 (1:10, s, 9H). Anal. calcd for $C_{33}H_{45}N_5O_9\cdot 1/2H_2O$: C, 59.63; H, 6.97; N, 10.54. Found: C, 59.68; H, 6.68; N, 10.52.

Z-Asn-(2S,3S)-AHPBA-[3(R)-hydroxy]Pro *tert*-butylamide (35). To a stirred solution of compound **17** (50 mg, 0.125 mmol) in MeOH (0.5 mL) was added 4 N HCl:dioxane solution (1 mL) at room temperature, and the mixture was stirred for 30 min. The mixture was concentrated in vacuo until dry. The remaining solid was dissolved in a small volume of MeOH and benzene and the solution was evaporated in vacuo (repeated three times). The residue was dried in vacuo for 2 h, and dissolved in DMF (1 mL). To the solution were added Z-Asn-(2S,3S)-AHPBA-OH (55.4 mg, 0.125 mmol),[8] HOBt monohydrate (203 mg, 0.15 mmol), EDC·HCl (28.8 mg, 0.15 mmol) and Et_3N (38.2 μL, 0.275 mmol) under ice-cooling and the stirring was continued for 5 h. The reaction mixture was concen-

trated in vacuo and the residue was partitioned between AcOEt and 10% aq citric acid soln. The organic layer was separated and was washed with H_2O, 5% aq $NaHCO_3$ soln and brine, and dried (Na_2SO_4). The solvent was evaporated in vacuo to give an oily substance. The residue was purified by preparative TLC (CH_2Cl_2:MeOH, 10:1). Crystallization from Et_2O gave 33 mg (43%) of compound **35** as a colorless solid: mp 109–112 °C. NMR (CD_3OD:$CDCl_3$, 1:2) δ ppm: 7.66 (d, 1H, J=7.9 Hz), 7.41–7.13 (m, 10H), 5.08 s, 2H), 4.48–4.31 (m, 4H), 3.88–3.70 (m, 1H), 3.40–3.36 (m, 1H), 2.87–2.80 (m, 2H), 2.75–2.65 (m, 2H), 2.14–2.03 (m, 2H), 1.32 (s, 9H). Anal. calcd for $C_{31}H_{41}N_5O_8 \cdot 1/2H_2O$: C, 59.98; H, 6.82; N, 11.28. Found: C, 60.15; H, 7.02; N, 11.05.

The following two compounds were prepared as described for **35**. Their melting points, IR spectral data, 270 MHz ^{1}H NMR data, MS data and elemental analyses are noted.

***Z*-Asn-(2*S*,3*S*)-AHPBA-[3(*S*)-hydroxy]Pro *tert*-butylamide (36)**. Mp 111–113 °C. IR (KBr) cm^{-1}: 3332, 2968, 1668, 1530, 1454, 1264, 1227, 746, 698. NMR (CD_3OD) δ ppm: 7.89–7.10 (m, 10H), 5.08, 5.06 (3:1; s, 2H), 4.45–4.18 (m, 5H), 3.76–3.68 (m, 1H), 3.51 (t, 1H, J=10 Hz), 2.95–2.36 (m, 4H), 1.92–1.80 (m, 1H), 1.33, 1.31 (1:13; s, 9H). *m/z* 686 (M-18). Anal. calcd for $C_{31}H_{41}N_5O_8 \cdot 3/4H_2O$: C, 59.55; H, 6.85; N, 11.20. Found: C, 59.73; H, 6.86; N, 10.82.

***Z*-Asn-(2*S*,3*S*)-AHPBA-[3(*S*)-chloro]Pro *tert*-butylamide (37)**. Mp 102–104 °C. IR (KBr) cm^{-1}: 3328, 1668, 1536, 1455, 1256, 1227, 748, 699. NMR (CD_3OD) δ ppm: 7.35–7.10 (m, 10H), 5.08 (s, 2H), 4.72 (br s, 1H), 4.62 (t, 1H, J=8 Hz), 4.47–4.44 (m, 2H), 4.28–4.23 (m, 1H), 4.10–4.01 (m, 1H), 2.86–2.75 (m, 1H), 2.65–2.58 (m, 4H), 2.47–2.34 (m, 3H), 1.32 (s, 9H). Anal. calcd for $C_{31}H_{40}N_5O_7Cl \cdot H_2O$: C, 57.44; H, 6.53; N. 10.81. Found: C, 57.44; H, 6.36; N, 10.43.

(Quinoxaline-2-carbonyl)-Asn-(2*S*,3*S*)-AHPBA-[4(*S*)-chloro]Pro *tert*-butylamide (38). To a stirred solution of **26** (300 mg, 0.48 mmol) in MeOH (10 mL) were added 1 N HCl (0.6 mL) and 10% Pd on charcoal (60 mg), and the mixture was hydrogenerated for 3 h at room temperature. The catalyst was filtered off and the filtrate was concentrated in vacuo. The residue was dissolved in DMF (5 mL), and quinoxaline-2-carboxylic acid (99 mg, 0.57 mmol), DEPC (93 mg, 0.57 mmol) and Et_3N (105 mg, 1.05 mmol) were added to the solution at 0 °C. The mixture was stirred for 5 h at the same temperature, and was concentrated in vacuo. The residue was partitioned between AcOEt and 10% aq $NaHCO_3$ soln, and the organic solution was separated and washed with H_2O and brine, and dried (Na_2SO_4). The solvent was evaporated in vacuo and the residue was purified by preparative TLC (CH_2Cl_2:MeOH, 10:1). Crystallization from Et_2O gave 140 mg (44%) of compound **38** as a colorless solid: mp 132–134 °C. NMR (CD_3OD) δ ppm: 9.49, 9.48 (ca. 6:1; s, 1H), 8.25–8.18 (m, 2H), 8.01–7.93 (m, 2H), 7.24 (d, 2H, J=7.3 Hz), 7.02 (dd, 2H, J=7.3, 7.8 Hz), 6.86 (t, 1H, J=7.3 Hz), 4.89 (dd, 1H, J=6.4, 5.9 Hz), 4.45–4.34 (m, 5H), 3.78–3.72 (m, 1H), 2.96–2.89 (m, 1H), 2.83–2.70 (m, 1H), 2.13–2.08 (m, 1H), 1.33, 1.27 (1:94; s, 9H). *m/z* 594 (M-17). Anal. calcd for $C_{32}H_{38}N_7O_6Cl \cdot 1/2H_2O$: C, 58.13; H, 5.94; N, 14.83; Cl, 5.36. Found: C, 58.25; H, 6.07; N, 14.67; Cl, 5.31.

The following six compounds were prepared as described for **38**. Their melting points, IR spectral data, 270 MHz ^{1}H NMR data, MS data and elemental analyses are noted.

(5-Fluoroindole-2-carbonyl)-Asn-(2*S*,3*S*)-AHPBA-[4(*S*)-chloro]Pro *tert*-butylamide (39). Mp 141–143 °C. NMR (DMSO-d_6) δ ppm: 11.77 (d, 1H, J=1.3 Hz), 8.56 (d, 1H, J=8.1 Hz), 7.93 (d, 1H, J=8.7 Hz), 7.56 (s, 1H), 7.47–7.41 (m, 2H), 7.30–7.28 (m, 2H), 7.15–7.13 (m, 1H), 7.09–7.03 (m, 1H), 7.02–6.96 (m, 3H), 6.88 (br s, 1H), 5.05 (d, 1H, J=9.1 Hz), 4.84–4.79 (m, 1H), 4.53–4.46 (m, 1H), 4.39–4.26 (m, 3H), 4.11–4.07 (m, 1H), 3.57 (dd, 1H, J=8.4 Hz), 2.75–2.50 (m, 1H), 1.27, 1.24 (ca. 1:10; s, 9H). Anal. calcd for $C_{32}H_{39}N_6O_6ClF \cdot H_2O$: C, 56.86; H, 6.11; N, 12.43; Cl, 5.24; F, 2.81. Found: C, 57.08; H, 6.04; N, 12.27; Cl, 5.28; F, 2.66.

(5-Methoxyindole-2-carbonyl)-Asn-(2*S*,3*S*)-AHPBA-[4(*S*)-chloro]Pro *tert*-butylamide (40). Mp 144–151 °C. IR (KBr) cm^{-1}: 3338, 2966, 2475, 2424, 1655, 1531, 1452, 1433, 1209, 1173, 1032, 701. NMR (CD_3OD) δ ppm: 7.47 (br s, 1H), 7.35 (d, 1H, J=9.2 Hz), 7.33–7.20 (m, 2H), 7.08 (s, 1H), 7.01–6.89 (m, 4H), 4.91 (t, 1H, J=8.6 Hz), 2.15–2.08 (m, 1H), 1.35, 1.30 (1:6; s, 9H). *m/z* 653 (M-15). Anal. calcd for $C_{33}H_{41}N_6O_7Cl \cdot 1/2H_2O$: C, 58.44; H, 6.24; N, 12.39; Cl, 5.23. Found: C, 58.27; H, 6.11; N, 12.15; Cl, 5.32.

(1-Methylindole-3-carbonyl)-Asn-(2*S*,3*S*)-AHPBA-[4(*S*)-chloro]Pro *tert*-butylamide (41). Mp 129–137 °C. IR (KBr) cm^{-1}: 3343, 2967, 2483, 2419, 1696, 1536, 1468, 1432, 748, 701. NMR (CD_3OD) δ ppm: 8.18–8.06 (m, 1H), 7.78 (s, 1H), 7.48–7.40 (m, 1H), 7.31–7.12 (m, 4H), 7.00–6.90 (m, 3H), 4.92–4.84 (m, H), 4.41–4.32 (m, 5H), 3.86 (s, 3H), 3.82–3.73 (m, 5H), 2.93–2.62 (m, 5H), 2.16–2.08 (m, 1H), 1.32, 1.29 (1:5; s, 9H). *m/z* 637 (M-16). Anal. calcd for $C_{33}H_{41}N_6O_6Cl \cdot 1/2H_2O$: C, 59.58; H, 6.42; N, 12.64; Cl, 5.33. Found: C, 59.43; H, 6.26; N, 12.45; Cl, 5.36.

(1-Methylindazole-3-carbonyl)-Asn-(2*S*,3*S*)-AHPBA-[4(*S*)-chloro]Pro *tert*-butylamide (42). Mp 129–134 °C. IR (KBr) cm^{-1}: 3343, 2969, 2486, 2417 1600, 1527, 1452, 1487, 1433 1395, 1365, 751, 701. NMR (CD_3OD) δ ppm: 8.23 (d, 1H, J=8.6 Hz), 7.62 (d, 1H, J=8.6 Hz), 7.50–7.40 (m, 1H), 7.33–7.13 (m, 3H), 7.06–6.92 (m, 3H), 4.93–4.87 (m, 1H), 4.47–4.33 (m, 5H), 2.94–2.85 (m, 1H), 2.81–2.63 (m, 5H), 2.15–2.06 (m, 1H), 1.34, 1.29 (1:5; s, 9H). *m/z* 653 (M^+). Anal. calcd for $C_{32}H_{40}N_7O_6Cl \cdot 2/3H_2O$: C, 57.69; H, 6.25; N, 14.72; Cl, 5.32. Found: C, 57.79; H, 6.69; N, 14.71; Cl, 5.38.

(Isobenzoxazole-3-carbonyl)-Asn-(2*S*,3*S*)-AHPBA-[4(*S*)-chloro]Pro *tert*-butylamide (43). Mp 118–127 °C. IR

(KBr) cm^{-1}: 3340, 2970, 2491, 2421, 1665, 1530, 1498, 1430, 1393, 1366, 1224, 754, 701. NMR (CD_3OD) δ ppm: 8.19 (d, 1H, J=7.9 Hz), 7.76–7.67 (m, 2H), 7.52–7.46 (m, 1H), 7.30–7.15 (m, 3H), 7.10–6.92 (m, 1H), 4.95–4.88 (m, 1H), 4.43–4.33 (m, 5H), 3.78–3.72 (m, 1H), 3.00–2.63 (m, 5H), 2.17–2.06 (m, 1H), 1.35, 1.32, 1.30 (11:19:90; s, 9H). *m/z* 625 (M-15). Anal. calcd for $C_{31}H_{37}N_6O_7Cl \cdot H_2O$: C, 56.49; H, 5.96; N, 12.75; Cl, 5.38. Found: C, 56.37; H, 5.80; N, 12.75; Cl, 5.82.

(7-Methoxybenzofuran-2-carbonyl)-Asn-(2*S*,3*S*)-AHPBA-[4(*S*)-chloro]Pro *tert*-butylamide (44). Mp 125–136 °C. IR (KBr) cm^{-1}: 3339, 2967, 2482, 2414, 1656, 1590, 1492, 1428, 1274, 1207, 1098, 731, 702. NMR (CD_3OD) δ ppm: 7.47 (s, 1H), 7.30–7.23 (m, 3H), 7.17–6.92 (m, 5H), 4.91 (t, 1H, J=6.6 Hz), 4.43–4.34 (m, 5H), 4.01 (s, 3H), 3.80–3.65 (m, 1H), 3.32–3.28 (m, 1H), 2.96–2.90 (m, 1H), 2.83–2.60 (m, 4H), 2.17–2.06 (m, 1H), 1.35, 1.29 (1:5; s, 9H). *m/z* 655 (M-14). Anal. calcd for $C_{33}H_{40}N_5O_8Cl \cdot 1/2H_2O$: C, 58.37; H, 6.09; N, 10.31; Cl, 5.22. Found: C, 58.59; H, 6.11; N, 10.09; Cl, 5.14.

Molecular modeling study

The atomic coordinates of HIV PR were obtained from the crystal structure of the HIV PR–MVT-101 complex[12] in the Brookhaven Protein Data Bank.[13] The bound structure of each **26** and **27** were modeled on the binding site of the protease using the main chain positions of MVT-101. Solvent water molecules were added at the positions where water molecules are commonly found in crystal structures of the HIV PR–inhibitor complex. The side chain carboxyl group of Asp25′ of the protease active site was modeled in the protonated form. Geometry optimization of each complex structure was performed with the molecular mechanics calculation program CHARMm.[14] First, only the structure of the inhibitor molecule was optimized in the fixed protease binding site and then the whole structure was optimized with the exception of the side chains of Asp25 and Asp25′, which were torsionally restrained in a coplanar orientation during the minimization.

HIV PR inhibition

The inhibitory activities of the compounds toward HIV-1 PR were determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) assay and kinetic study as described in a previous report.[8] Initially, inhibitory activity relative to pepstatin A (Iva-Val-Val-Sta-Ala-Sta-OH; Iva = isovaleryl, Sta = statine, K_i = 1.1 μM) was determined using recombinant 55 kDa *gag* substrate and protease. Table 1 indicates the concentrations that are approximately equipotent with 1 μM pepstatin A. The recombinant 55 kDa *gag* protein includes three scission sites (p17/p24, *C* terminus of p24, and *N* terminus of p7). Therefore, this assay system can simulate the actual enzymatic process: the recognition and cleavage of the proteinic substrates. However, it is difficult to evaluate the inhibitors quantitatively since we visually judge the extent of the disappearance of the 55 kDa substrate. Thus, K_i values were determined for active compounds using the partially purified protease and the synthetic substrate Ac-Ser-Gln-Asn-Tyr-Pro-Ile-Val-NH_2.

Anti-HIV activity

Viruses. HIV-1IIIB was obtained from HIV-lIIIB chronically infected Molt-4 cells (Molt-4/HIV-1IIIB) as a supernatant fluid. The 50% tissue culture infectious dose (TC ID_{50}) was determined by the endpoint titration method.[15]

Measurement of anti HIV-1IIIB activities in acute infection

CEM cells (5×10^4 cells/mL) were exposed to HIV-1IIIB fluid at a multiplicity of infection (m.o.i.) 0.001 TC ID_{50} (mL). Aliquots (0.2 mL) of cells were placed in 96 well microliter plates with 2 μL of the appropriate concentration of test samples dissolved in DMSO. After incubation for 5 days in RPMI-1640 medium containing 10% FCS, the p24 antigen of HIV in the supernatant was determined by ELISA assay kit (RETRO-TEK™, Cellular Products Inc., Buffalo, New York). The IC_{90} value was determined as the concentration of the compound that inhibited virus p24 antigen production by 90% relative to untreated control cultures.

Anti HIV-1IIIB activity against chronic infection.

Molt-4/HIV-1IIIB chronically infected cells (1×10^5 cells/mL) were cultured in 0.2 mL of RPMI-1640 medium containing 10% FCS for 48 h with 2 μL of the appropriate concentration of test samples dissolved in DMSO. The p24 antigen of HIV in the supenatant was determined by ELISA as described above.

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