Hamdy M. Abdel-Rahman[a], Nawal A. El-Koussi[a], Gamal S. Alkaramany[a], Adel F. Youssef[a], Yoshiaki Kiso[b]

[a] Pharmaceutical Medicinal Chemistry Department, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt
[b] Department of Medicinal Chemistry, Kyoto Pharmaceutical University, Kyoto 607-8412, Japan

A Novel Dipeptide-based HIV Protease Inhibitor Containing Allophenylnorstatine

Dipeptide analogues incorporating allophenylnorstatine [Apns; (2S,3S)-3-amino-2-hydroxy-4-phenylbutyric acid] as a transition state mimic at the scissile bond were designed and synthesized in the hope of obtaining a novel KNI series of HIV protease inhibitors. The precursors, N-P_2'-3-(2S,3S)-3-(tert-butyloxycarbonyl)amino-2-hydroxy-4-phenylbutanoyl)-5,5-dimethylthiazolidine-4-carboxamide (N-Boc-Apns-Dmt-P_2') **4a**–**p** were prepared by deprotection of the synthones N-P_2'-(tert-butyloxycarbonyl)-5,5-dimethylthiazolidine-4-carboxamide (Boc-Dmt-P_2') **2a**–**p**, then coupling with (2S,3S)3-(*tert*-butyloxycarbonyl)amino-2-hydroxy-4-phenylbutanoic acid (N-Boc-Apns-OH) **3**. The deprotected intermediates **4** were coupled with the activated carboxyl groups of the P_2 ligands to afford the target dipeptides. In this work, we fixed at the P_2 site either a 2,6-dimethylphenoxyacetyl or a 3-hydroxy-2-methylbenzoyl group. Substitutes at the P_2' site were varied to afford the members of the series **7** and **8**. Improved activity of most of the members of series **8** relative to their analogues of series **7** can be partially attributed to the differences in the structures of the P_2 moieties. Positional isomerism in the P_2' moieties significantly affected the activity and polarity of the target.

Keywords: Dipeptide; Antiviral Drugs; HIV Protease Inhibitory Activity; SAR

Received: February 25, 2004; accepted: September 10, 2004 [FP882]
DOI 10.1002/ardp.200400882

Introduction

Inhibition of human immunodeficiency virus (HIV) protease is one of the most important and promising approaches for the treatment of an HIV infection.

The design and development of potent HIV protease inhibitors have generated considerable interest among the AIDS research field and HIV-positive patients. A promising class of HIV protease inhibitors containing allophenylnorstatine (Apns) as a transition state mimic yielded a series of KNI derivatives of highly potent inhibitory properties. In this work, we reported the synthesis of dipeptides with different substituents at the P_2 and P_2' positions with respect to the leads KNI-577 and KNI-901 (see Figure 1) and their protease inhibition activities.

The use of the 2,6-dimethylphenoxyacetyl moiety as P_2 ligand was already reported and provided compounds with potent HIV protease inhibitory activity such as the clinically used drug ABT-378 [1, 2] (Figure 2).

On the other hand, incorporation of the 2,6-dimethylphenoxyacetyl moiety into the KNI series of inhibitors led to the development of highly potent compounds, like the dipeptide KNI-901 [3].

Further challenging of the protease inhibitory activity of the KNI series was practiced by introduction of the

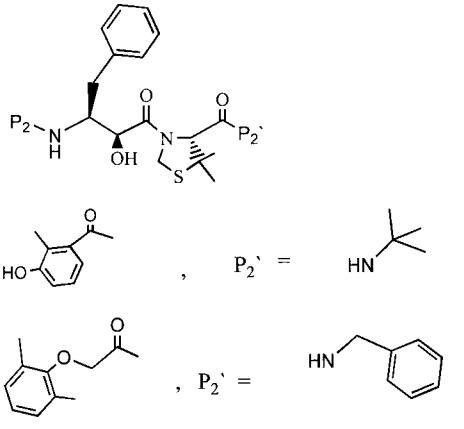


Figure 1. Lead protease inhibition of the KNI series.

Correspondence: Nawal A. El-Koussi, Pharmaceutical Medicinal Chemistry Department, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt; Phone: +20 88 411297, Fax: +20 88 332776, e-mail: nawal-a@acc.aun.edu.eg

Full Paper

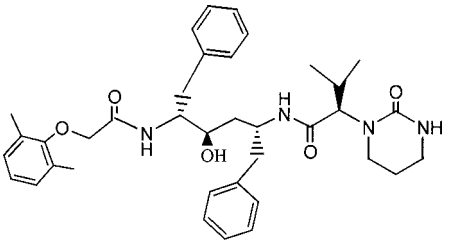


Figure 2. ABT-378 (Lopinavir).

3-hydroxy-2-methylbenzoyl moiety at P_2 sites, which simultaneously provides effective capabilities of hydrophobic and hydrogen bonding interactions with the enzyme relative sites [4].

This approach yielded the highly active KNI-577 [4–7]. The improved activity may be correlated with the combined capabilities of hydrophobic and hydrogen bonding potentialities of their intact moiety.

Guided by the leads KNI-577 and KNI-901, we prepared two series of dipeptide analogues. In one of these series, the P_2 ligand was the 2,6-dimethylphenoxyacetyl moiety as shown by compounds **7** in Table 1. Introduction of the 3-hydroxy-2-methylbenzoyl moiety at the P_2 site provided the second series of dipeptides represented by the compounds **8** in Table 2. In series **7** and **8**, P_2' was changed in the hope to improve protease inhibition potential and enhance polarity. It is clear that the partition coefficient, represented by the Rt value, and solubility are closely related phenomena; therefore, the choice of moieties at P_2' with predominant polar functions was targeted in both series [8, 9].

Results and discussion

Chemistry

The target dipeptide inhibitors were prepared as illustrated in Scheme 1, starting from 5,5-dimethylthiazolidine-3-carboxylic acid (Dmt-OH), which was prepared by cyclization of L-penicillamine with formaldehyde, followed by N-protection by Boc to yield Boc-Dmt-OH **1** according to reported procedures [10]. Coupling of the amino ligands $P_2'NH_2$ with **1** in the presence of 1-hydroxybenzotriazole (HOBt) in DMF [11, 12] or diphenylphosphochloridate (DPPCl), Et_3N and AcOEt [13] afforded the intermediates **2a**–**p** (Table 3). N-elimination of the Boc moiety and coupling with Boc-Apns-OH **3** by either 1-ethyl-3-(3′-dimethylaminopropyl) carbodiimide (EDC) or benzotriazole-1-yloxytris-(dimethylamino) phosphonium hexafluorophosphate (BOP) in the presence of HOBt in DMF [14] yielded the synthones **4a**–**p** (Table 4). These intermediates were used as scaffolds for the synthesis of the targets **7** and **8**. 2,6-Dimethylphenoxyacetic acid **5** was activated by EDC, HOBt in DMF or THF and coupled with the deprotected **4a**–**n** to afford the series **7a**–**n** (Table 1). In series **8**, 3-acetyl-2-methylbenzoic acid **6** was activated by DPPCl in AcOEt and then coupled with the deprotected synthones **4a**–**h**, **o** and **p**. Hydrolytic cleavage of the 3-acetyl group by LiOH yielded **8a**–**h**, **o** and **p** dipeptides in Table 2. The final compounds obtained after crystallization were checked by analytical HPLC; from the resulting data, preparative HPLC was established and carried out. The purity was checked again by analytical HPLC. The fractions were mixed and lyophilized to afford the analytically pure final compounds; yields of the products were determined by reversed-phase HPLC (RP-HPLC). Within each series, the parallelism between Rt of the compounds and the polarity of the P_2' moieties could be easily observed. The effect of the location of the polar groups in the P_2' moieties on the Rt values of the positional isomers **7g**, **h**, **i**, **k**, **l**, **m**, **n** and in **8g**, **h**, **o**, **p** was perceptible. Homogenity of targets **7** and **8** was checked by TLC using two systems of different polarities: chloroform/methanol (10:1) and chloroform/methanol/water (8:3:1).

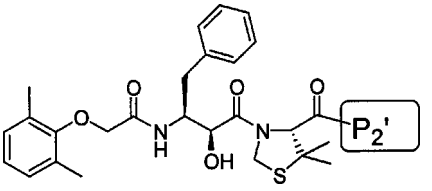
Structure activity relationship against HIV-1 protease

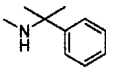
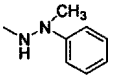
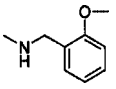
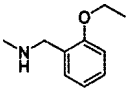
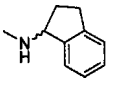
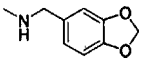
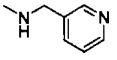
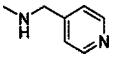
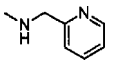
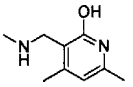
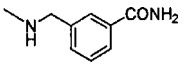
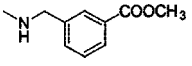
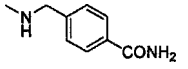
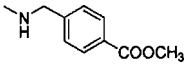
The effect of variation of the P_2' moieties on HIV-1 protease inhibition of the potent leads KNI-901 and KNI-577 was regarded as the main objective of the present study. The benzylamino group at the P_2' site in KNI-901 was the target modified to afford the dipeptides **7**, whereas the t-butyl group at the P_2' site in KNI-577 was replaced by the same modified benzylamino derivatives to afford almost all members of series **8**.

Three approaches were considered for modification of the benzylamine moiety. First, the assumption that the phenyl ring must be distanced by one atom from the amidic NH represents an essential requisite. Groups of different bulkiness and polarity, with one atom bridging the phenyl ring and the amidic NH, were inserted to replace the α-methylene group in benzylamine. This approach was challenged by the compounds **7a**, **b**, **e** and **8a**, **b**, **e**. As shown in Table 5, compounds **7b** and **8b** with inserted α-N (CH_3) were the least active ones. On the other hand, the 2-isopropylene bridge significantly improved the activity of **7a** and **8a**, which are still less active than the corresponding leads. Compound **8e** revealed 91.2% inhibition potential, which is higher than the potential of the lead KNI-577. Matching the activities of **7a**, **e** with their analogues **8a**, **e** dem-

onstrated a boosting effect of the P_2 moiety in series **8**. Secondly, introduction of a polar substitute at the *o*-, *m*-, or *p*-position on the benzene ring was tried by preparing **7c**, **d**, **f**, **k**–**n** and **8c**, **d**, **f** dipeptides. All these isomers were less active than the leads; however, the derivatives of the **8** series still revealed a

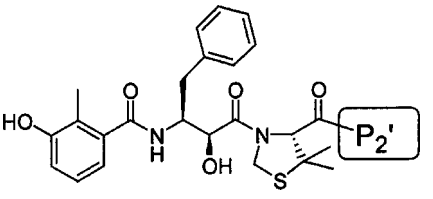
Table 1. Physical data of dipeptide-based HIV protease inhibitors **7**.

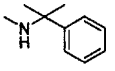
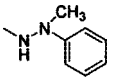
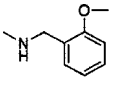
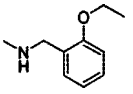
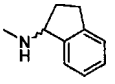
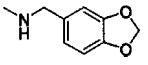
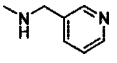
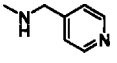
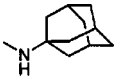
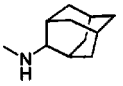


No.	P_2'	mp (°C)	Yield (%)	HPLC[§] Rt (min)	TLC	
					Rf_1[†]	Rf_2[¥]
7a		89–90	85	27.44	0.98	0.96
7b		115–117	54.5	24.81	0.81	0.90
7c		89–91	65	26.37	0.68	0.89
7d		85–88	71	27.12	0.72	0.90
7e[#]		103–105	61	27.28	0.90	0.96
7f		110–113	78	25.08	0.90	0.93
7g		107–108	71	16.92	0.80	0.86
7h		141–143	70	17.34	0.62	0.77
7i		98–100	73	18.10	0.68	0.84
7j		149–151	55	21.10	0.62	0.77
7k		124–126	79	20.38	0.54	0.83
7l		98–100	53	24.70	0.84	0.91
7m		140–142	57	19.81	0.52	0.90
7n		99–100	63	24.38	0.89	0.94

[#] Mixture of diastereomers; [§] 20–80% CH_3CN in 0.1% aqueous TFA over 30 min; [†] $CHCl_3/CH_3OH$ (10:1); [¥] $CHCl_3/CH_3OH/H_2O$ (8:3:1).

Table 2. Physical data of dipeptide-based HIV protease inhibitors **8**.

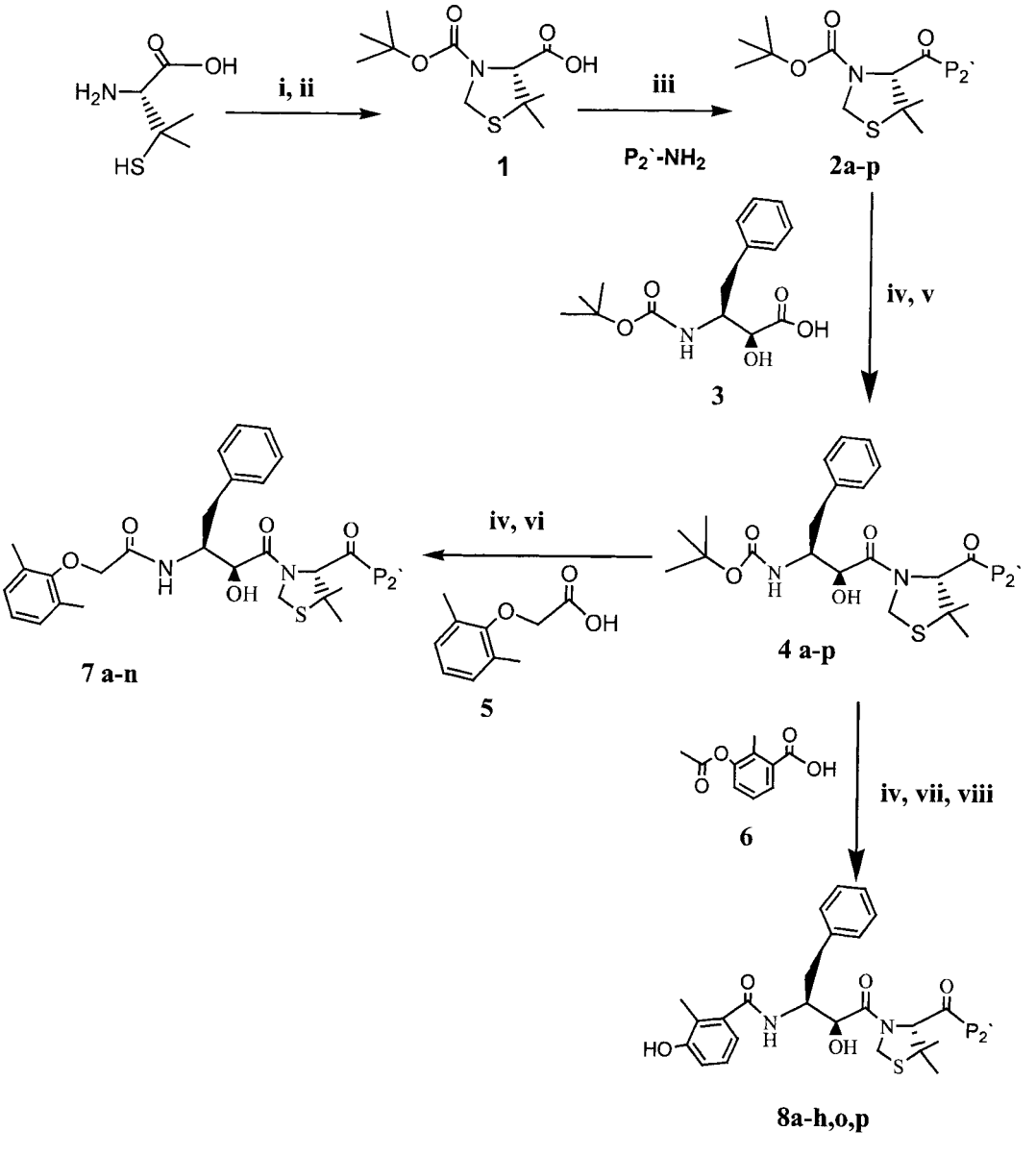


No.	P_2'	mp (°C)	Yield (%)	HPLC[§] Rt (min)	TLC	
					Rf_1[†]	Rf_2[¥]
8a		128–130	65.5	20.86	0.69	0.88
8b		150–152	78	18.08	0.37	0.76
8c		127–129	68	19.45	0.57	0.83
8d		121–123	72	21.10	0.67	0.86
8e[#]		139–141	58	20.42 20.52	0.56	0.76
8f		139–140	71	20.24	0.59	0.67
8g		141–143	35.5	11.47	0.58	0.80
8h		154–156	24	9.94	0.35	0.65
8o		153–155	76	27.15	0.56	0.66
8p		159–161	66	26.64	0.54	0.70

[#], [§], [†] and [¥] as in Table 1.

higher pattern of activity that can only be attributed to the different P_2 moieties. Finally, the benzyl moiety was replaced by the bioisosteric α-, β-, and γ-picolines. This approach yielded the most active derivative **7g**, which is equally active as the lead KNI-901, with an apparently enhanced polarity. Improvement of polarity presumably may advantageously affect its absorption and distribution properties. Furthermore, the presence of a basic pyridine center allows for formation of salts.

The significantly reduced activity of the α-picolinyl derivative **7i** may be attributed to the possible competition for intramolecular hydrogen bond formation between the pyridine N and the amidic NH, which participates in a crucial hydrogen bonding with the water molecule bridging Ala28 and Asp29 in the S_2' site of the enzyme [15]. In the absence of such interaction, higher activity of the β- and γ-picoline **7g** and **7h** was observed. On the other hand, the lowered activity of the alkoxy **7c**, **d**, **8c**, **d** and 4,6-dimethyl-1,2-dihydro-3-



(i) aq 37% HCHO (ii) $(Boc)_2O$ (iii) HOBt in DMF or DPPCl, Et_3N, AcOEt(iv) N HCl/dioxane

(v) EDC, HOBt in DMFor BOP, HOBt in DMF (vi) EDC, HOBt in DMFor THF

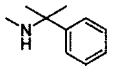
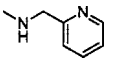
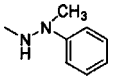
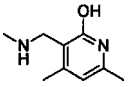
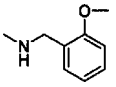
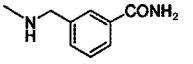
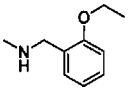
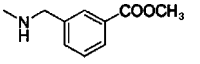
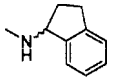
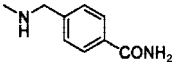
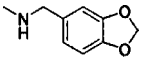
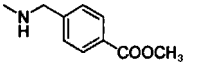
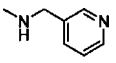
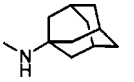
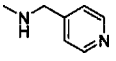
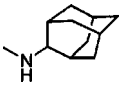
(vii) DPPCl in AcOEt, (viii) LiOH

Scheme 1. Synthesis of dipeptides **7** and **8**.

picolinyl-2- one **7j** can be equally attributed to possible intramolecular hydrogen bonding between the unshared pair of electrons on the *o*-oxygen and the amidic NH.

Positional isomers of the substituted benzyl amines and of the picolines revealed a significant impact on activity. The β-picolinyl **7g**, and **8g** and the *m*-substituted benzyl derivatives **7k**, **l** on the one hand exerted

Table 3. Physical data of intermediate compounds N-Boc-Dmt-P_2' **2**.

No.	P_2'	mp (°C)	HPLC[§] Rt (min)	No.	P_2'	mp (°C)	HPLC[§] Rt (min)
2a		102–104	23.24	**2i**		116–119	10.29
2b		185–188	19.71	**2j**		128–130	13.30
2c		112–113	23.34	**2k**		88–89	13.48
2d		113–114	22.92	**2l**		‡	20.14
2e[#]		60–62	23.92 24.16	**2m**		107–109	13.92
2f		124–126	21.68	**2n**		115–116	20.96
2g		‡	7.68	**2o**		155–156	30.56
2h		162–164	9.86	**2p**		178–179	30.10

[#] Mixture of diastereomers; [§] 20–80% CH_3CN in 0.1% aqueous TFA over 30 min; [‡] not determined, sticky semisolid material.

a relatively higher activity when matched with the γ-picolines **7h**, and **8h** and the *p*-substituted benzyls **7m**, **n** on the other hand. A common feature of these isomers is the availability of an atom or group on the aromatic ring that carries an unshared pair of electrons. It seems feasible to correlate the observed differences in activity with the unshared pair of electrons that should be suitably oriented at a critical distance from the amidic NH. Deviation from this critical distance might lead to decreased activity. Thus, a location of the unshared pair of electrons separated by three carbons from the amidic NH seems to give the optimum activity, as in **7g**. Shifting of the unshared pair of electrons on N towards α- or γ-positions either gets it involved in intramolecular hydrogen bonding, as in **7i**, or diminishes its supporting role of interaction with the enzyme, as in **7h**. By analogy, the differences in activities of the *m*- and *p*-isomers of benzylcarboxamides **7k** and **7m** and the methyl benzylcarboxylates **7l** and **7n** were found to parallel the distance separating the carbonyl oxygen from the amidic NH.

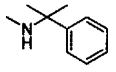
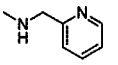
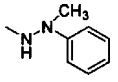
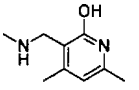
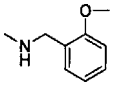
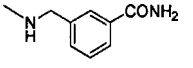
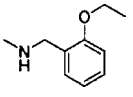
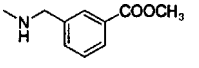
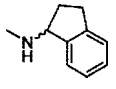
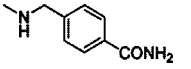
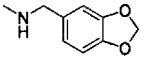
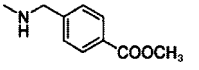
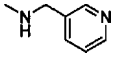
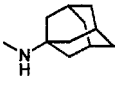
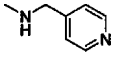
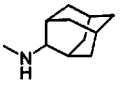
The tolerance of the S_2' site to accommodate a bulky substituent was challenged by the attachment of 1- or 2- adamantyl groups, which can be regarded as the constrained analogues of the tert-butyl moiety at the P_2' position in the lead KNI-577. The yielded dipeptides **8o** and **8p** were found to be the least active derivatives.

Conclusions

KNI-577 and KNI-901 leads are substrate-based HIV protease inhibitors. The two leads are members of the KNI series containing allophenylnorstatine (Apns) with hydroxymethylcarbonyl isostere as a transition state mimic at the scissile peptide bond.

The activity of the prepared series of P_2-Apns-Dmt-P_2' strongly depends on the simultaneous balance between electronic and steric properties of both the P_2 and P_2' moieties. As shown from Table 5, the ability of interaction of P_2 and P_2' with the relevant enzyme

Table 4. Physical data of intermediate compounds N-Boc-Dmt-P_2' **4**.

No.	P_2'	mp (°C)	HPLC[§] Rt (min)	No.	P_2'	mp (°C)	HPLC[§] Rt (min)
4a		102–106	25.56	**4i**		94–98	15.52
4b		106–109	22.72	**4j**		147–150	18.87
4c		99–102	23.70	**4k**		132–135	18.80
4d		100–103	25.57	**4l**		117–120	25.44
4e[#]		95–98	25.06 25.23	**4m**		96–99	17.78
4f		75–78	25.66	**4n**		110–112	22.66
4g		96–100	16.19	**4o**		103–106	32.75
4h		105–109	14.92	**4p**		107–109	31.94

[#] and [§] as in Table 3.

sites S_2 and S_2' would significantly affect the fitting of Apns to the enzyme catalytic site, which reflects the HIV protease inhibitory activity.

Two derivatives were found to be equally or more active than the leads KNI-577 and KNI-901 and present promising candidates for further investigations.

Experimental

Melting points were determined on a micro hot plate of a Yanaco micro melting point apparatus and were uncorrected. The optical rotations were measured on a Horiba model SEPA-300 digital polarimeter. TLC was performed on precoated Merck silica gel 60 F_{254} sheets. Column chromatography was carried out on Merck silica gel 60 (particle size 0.063–0.200 mm).

Analytical RP-HPLC was performed with a Hitachi L-7100 pump and an L-7400 UV detector utilizing YMC Pack ODS-AM AM 302.

Preparative RP-HPLC was performed with a Shimadzu LC-4A liquid chromatograph utilizing a YMC Pack ODS-AM type SH-343-5AM column (250 × 20 mm i.d., S-5 μm, 120 A).

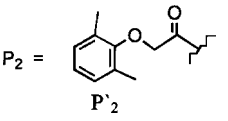
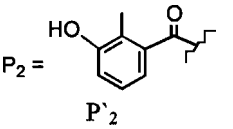
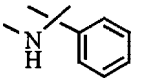
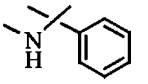
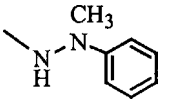
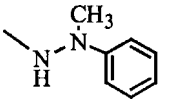
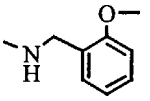
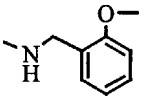
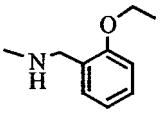
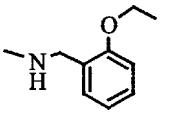
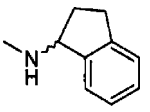
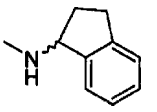
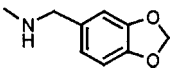
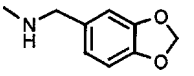
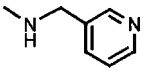
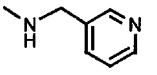
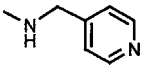
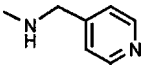
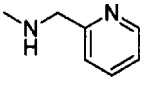
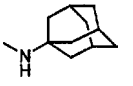
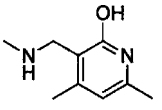
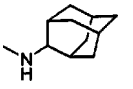
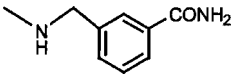
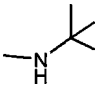
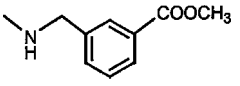
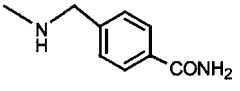
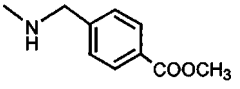
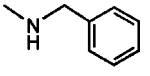
^{1}H NMR spectra were recorded on a JEOL JNM-EX 270 (270 MHz) spectrometer. Chemical shifts are given in (δ ppm) relative to tetramethylsilane (TMS) as an internal standard. 13C NMR spectra were recorded on a JEOL JNM-EX 270 (67.5 MHz) using solvents as internal standard. FAB mass spectra (FAB-MS) and high-resolution FAB-MS (HRFAB-MS) were recorded on a JEOL JMS-SX102 AQQ/MS-HYB10 mass spectrometer using glycerol, thioglycerol or Magic Bullet as internal references. MALDI TOF mass spectra were measured at Voyager-DE™RP Biospectrometry™ Workstation (PerSeptive Biosystems). Commercially available chemicals were purchased from Nacalai tesque, Waku Chemicals or Tokyo Chemical Industries, Japan, and were used without further purification. Commercially non-available chemicals were prepared according to standard methods described in [16] and showed 1H NMR and 13C NMR spectra in accordance with the assigned structures.

*(tert-Butyloxycarbonyl)-5,5-dimethylthiazolidine-4-carboxylic acid Boc-Dmt-OH (**1**) [10]*

Yield 81%, mp 124–127°C.

*N-P_2'-(tert-Butyloxycarbonyl)-5,5-dimethylthiazolidine-4-carboxamide Boc-Dmt-P_2' (**2a**–**p**)*

Table 5. HIV protease inhibitory activity of targeted dipeptides P_2-Apns-Dmt-P_2' **7** and **8**.

No.	P_2 =  P'_2	% HIV protease inhibition (50 nM)	No.	P_2 =  P'_2	% HIV protease inhibition (50 nM)
7a		72.5	**8a**		82.2
7b		15.3	**8b**		12.9
7c		24.0	**8c**		65.8
7d		41.7	**8d**		49.7
7e[#]		64.5	**8e**[#]		91.2
7f		27.6	**8f**		67.6
7g		86.8	**8g**		62.6
7h		70.7	**8h**		34.5
7i		57.8	**8o**		7.1
7j		20.9	**8p**		5.5
7k		67.7	KNI-577		87.6
7l		45.0			
7m		3.3			
7n		28.6			
KNI-901		86			

[#] mixture of diasteromers.

Boc-Dmt-OH (1.5 g, 5.74 mmol) was dissolved and stirred in AcOEt (20 mL); Et_3N (0.88 mL, 6.33 mmol) and DPPCl (1.31 mL, 6.33 mmol) were added at 0 °C. The mixture was stirred at room temperature for 1 h, then appropriate amine (6.33 mmol) and Et_3N (0.88 mL, 6.33 mmol) were added. Stirring was continued at room temperature for 6 h. The mixture was washed twice with 10% citric acid, 5% $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated under reduced pressure, crystallized from *n*-hexane and dried in a dessicator [16, 17].

*(2S,3S)3-(tert-Butyloxycarbonyl)amino-2-hydroxy-4-phenylbutanoic acid N-Boc-Apns-OH (**3**) [18]*

Yield 8%, mp147–148 °C.

*N-P_2'-3-(2S,3S)-3-(tert-butyloxycarbonyl)amino-2-hydroxy-4-phenylbutanoyl)-5,5-dimethylthiazolidine-4-carboxamide Boc-Apns-Dmt-P_2' (**4a**–**p**)*

To the appropriate Boc-Dmt-P_2' **2a**–**p** (0.5 mmol) in 4 N HCl/dioxane solution (2 mL), anisol (108 μL, 1 mmol) was added at 0 °C. This solution was stirred for 2 h at room temperature. The solvent was evaporated *in vacuo*, ether was added, the mixture was centrifuged, and the residue was dissolved in DMF (5 mL). Boc-Apns-OH **3** (134 mg, 0.45 mmol), HOBt.H_2O (76.6 mg, 0.5 mmol), BOP (211 mg, 0.5 mmol) and Et_3N (139 mL, 1 mmol) were added at 0 °C. The mixture was stirred overnight at room temperature. The solvent was then removed under vacuum and the residue was extracted with AcOEt. The organic layer was washed with 10% citric acid, 5% $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated under reduced pressure. The residue was purified by column chromatography, and the appropriate fractions were pooled and evaporated to yield the coupled peptide (Boc-Apns-Dmt-P_2'), which was dried in a desiccator [16, 17].

*2,6-Dimethylphenoxyacetic acid (**5**)*

Compound **5** was synthesized starting from 2,6-dimethyl phenol by alkylation with ethyl-2-bromoacetate, then hydrolysis. Yield 37%, mp138–139 °C.

3-Hydroxy-2-methylbenzoic acid [16]

Yield 86.5%, mp142–144 °C.

*3-Acetyloxy-2-methylbenzoic acid (**6**)*

Acetylation of 3-hydroxy-2-methylbenzoic acid yielded 3-acetyloxy-2-methylbenzoic acid **6** (quantitative, mp 147–148 °C).

*Synthesis of dipeptides containing 2,6-dimethyl phenoxyacetic acid as P_2 ligand (2,6-dimethylphenoxyacetyl-Apns-Dmt-P_2') (**7a**–**n**)*

The titled compounds were prepared, starting from N-Boc-Apns-Dmt-P_2' **4a**–**n** (20.8 mmol) in 4 N HCl/dioxane (40 mL); anisole (4.5 mL, 41.67 mmol) was added at 0 °C. The reaction mixture was stirred for 1 h at room temperature, and the solvent was then removed *in vacuo* at room temperature; ether was added, and the mixture was centrifuged. The formed precipitate was dissolved in DMF (40 mL), and then 2,6-dimethylphenoxyacetic acid **5** (22.85 mmol), HOBt.H_2O (3.5 g, 22.85 mmol), EDC.HCl (4.3 g, 22.43 mmol) and Et_3N (5.78 mL, 41.6 mmol) were added at 0 °C. The reaction mixture was stirred overnight at room temperature, and the solvent was removed under reduced pressure. The residue was extracted with AcOEt. The organic layer was washed with 10% citric acid, 5% $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was crystallized from *n*-hexane. Physical data are listed in Table 1.

*2,6-Dimethylphenoxyacetyl-Apns-Dmt-NH-cumyl (**7a**)*

$[a]_D^{28}$ −1.60 (c = 0.218, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.43 (s, 3H), 1.49 (s, 1H), 1.54 (s, 3H), 1.65 (s, 3H), 2.16 (s, 6H), 2.70–2.73 (m, 2H), 3.99–4.05 (d, *J* = 14.51 Hz, 1H), 4.14–4.20 (d, *J* = 13.85 Hz, 1H), 4.28 (bs, 1H), 4.42 (s, 1H), 4.66 (s, 1H), 4.84–4.93 (m, 2H), 6.92–7.02 (m, 3H), 7.15–7.24 (m, 10H), 7.41–7.44 (d, *J* = 7.69 Hz, 1H), 8.24–8.27 (d, *J* = 8.58 Hz, 1H), HRFAB-MS: *m/z* 618.3004 for $[M+H]^+$ (calcd. 618.3002 for $C_{35}H_{44}N_3O_5S$).

*2,6-Dimethylphenoxyacetyl-Apns-Dmt-NH-MPH (**7b**)*

$[a]_D^{24.9}$ −11.42 (c = 0.07, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.49 (s, 3H), 1.58 (s, 3H), 2.16 (s, 6H), 2.76–2.82 (m, 2H), 3.97–4.02 (d, *J* = 13.53 Hz, 1H), 4.15–4.20 (d, *J* = 13.52 Hz, 1H), 4.26–4.48 (m, 3H), 4.97–5.00 (m, 2H), 6.68–6.71 (t, *J* = 7.26 Hz, 1H), 6.81–7.03 (m, 4H), 7.12–7.25 (m, 8H), 8.17–8.20 (d, *J* = 8.91 Hz, 1H), 10.17 (s, 1H), HRFAB-MS: *m/z* 605.2804 for $[M+H]^+$ (calcd. 605.2798 for $C_{33}H_{41}N_4O_5S$).

*2,6-Dimethylphenoxyacetyl-Apns-Dmt-NH-(2-OCH_3)Bz (**7c**)*

$[a]_D^{24}$ *−14.85 (c = 0.202, CH_3OH)*, ^1H NMR (DMSO-d_6) δ: 1.34 (s, 3H), 1.50 (s, 3H), 2.14 (s, 6H), 2.77–2.80 (m, 2H), 3.77 (s, 3H), 3.97–4.02 (d, *J* = 14.19 Hz, 1H), 4.13–4.18 (d, *J* = 14.18 Hz, 1H), 4.25–4.27 (d, J = 5.28 Hz, 2H), 4.37–4.40 (m, 1H), 4.46–4.49 (m, 2H), 4.91–5.00 (m, 2H), 6.79–6.85 (t, *J* = 7.59 Hz, 1H), 6.89–7.01 (m, 2H), 7.15–7.30 (m, 9H), 8.12–8.15 (d, *J* = 8.57 Hz, 1H), 8.27–8.35 (t, *J* = 5.6 Hz, 1H), HRFAB-MS: *m/z* 620.2854 for $[M+H]^+$ (calcd. 620.2794 for $C_{34}H_{42}N_3O_6S$).

*2,6-Dimethylphenoxyacetyl-Apns-Dmt-NH-(2-OC_2H_5)Bz (**7d**)*

$[a]_D^{27.3}$ *−9.96 (c = 0.158, CH_3OH)*, ^1H NMR (DMSO-d_6) δ: 1.31–1.35 (m, 6H), 1.51 (s, 3H), 2.14 (s, 6H), 2.77–2.80 (m, 2H), 3.93–4.05 (m, 3H), 4.12–4.18 (m, 1H), 4.26–4.28 (d, *J* = 4.29 Hz, 2H), 4.39 (s, 1H), 4.47–4.50 (m, 2H), 4.92–5.00 (m, 2H), 6.78–6.83 (t, *J* = 6.27 Hz, 1H), 6.90–7.01 (m, 3H), 7.12–7.30 (m, 7H), 8.13–8.15 (d, *J* = 7.91 Hz, 1H), 8.30 (t, *J* = 5.6 Hz, 1H), HRFAB-MS: *m/z* 634.2969 for $[M+H]^+$ (calcd. 634.2951 for $C_{35}H_{44}N_3O_6S$).

*2,6-Dimethylphenoxyacetyl-Apns-Dmt-NH-Ind (**7e**)*

$[a]_D^{25}$ +19.86 (c = 0.448, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.44 (s, 3H), 1.51 (s, 3H), 1.79–1.84 (m, 1H), 2.15 (s, 6H), 2.34–2.40 (m, 1H), 2.81–2.91 (m, 4H), 3.95–4.02 (m, 1H), 4.16–4.22 (m, 1H), 4.39–4.50 (m, 3H), 4.98–4.99 (m, 2H), 5.32–5.35 (m, 1H), 6.90–7.08 (m, 4H), 7.17–7.38 (m, 8H), 8.14–8.17 (d, *J* = 8.24 Hz, 1H), 8.35–8.45 (dd, *J* = 8.24 Hz, 1H), HRFAB-MS: *m/z* 616.2878 for $[M+H]^+$ (calcd. 616.2845 for $C_{35}H_{42}N_3O_5S$), TOF-MS 616.727 for $[M+H]^+$.

*2,6-Dimethylphenoxyacetyl-Apns-Dmt-NH-Pip (**7f**)*

$[a]_D^{27}$ −2.00 (c = 0.15, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.34 (s, 3H), 1.51 (s, 3H), 2.15 (s, 6H), 2.76–2.80 (m, 2H), 3.99–4.02 (m, 1H), 4.14–4.29 (m, 3H), 4.34–4.48 (m, 3H),

4.97 (s, 2H), 5.88–5.92 (m, 2H), 6.77 (s, 2H), 6.85 (s, 1H), 6.95–7.02 (m, 3H), 7.19–7.30 (m, 4H), 8.09–8.13 (d, *J* = 9.17 Hz, 1H), 8.43–8.48 (t, *J* = 6.11 Hz, 1H), HRFAB-MS: *m/z* 634.2583 for $[M+H]^+$ (calcd. 634.2587 for $C_{34}H_{40}N_3O_7S$).

*2,6-Dimethylphenoxyacetyl-Apns-Dmt-NH-3-picolyl (**7g**)*

$[a]_D^{23.8}$ +47.14 (c = 0.28, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.34 (s, 3H), 1.53 (s, 3H), 2.12 (s, 6H), 2.76–2.84 (m, 2H), 3.93–3.98 (d, *J* = 14.19 Hz, 1H), 4.11–4.17 (d, *J* = 14.19 Hz, 1H), 4.19–4.72 (m, 5H), 5.00 (m, 2H), 6.89–7.01 (m, 3H), 7.16–7.24 (m, 5H), 7.63–7.68 (m, 1H), 8.07–8.11 (d, *J* = 8.91 Hz, 1H), 8.14–8.17 (d, *J* = 7.59 Hz, 1H), 8.57–8.58 (d, *J* = 3.96 Hz, 1H), 8.67 (s, 1H), 8.72–8.80 (t, *J* = 5.6 Hz, 1H), HRFAB-MS: *m/z* 591.2639 for $[M+H]^+$ (calcd. 591.2641 for $C_{32}H_{39}N_4O_5S$). TOF-MS 591.543 for $[M+H]^+$.

*2,6-Dimethylphenoxyacetyl-Apns-Dmt-NH-4-picolyl (**7h**)*

$[a]_D^{26}$ +36.36 (c = 0.55, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.37 (s, 3H), 1.55 (s, 3H), 2.12 (s, 6H), 2.71–2.89 (m, 2H), 3.93–3.98 (d, *J* = 14.19 Hz, 1H), 4.09–4.15 (d, *J* = 14.19, 1H), 4.32–4.61 (m, 5H), 5.00 (s, 2H), 6.89–7.01 (m, 3H), 7.12–7.26 (m, 5H), 7.63–7.66 (d, *J* = 5.74 Hz, 2H), 8.09–8.13 (d, *J* = 9.24 Hz, 1H), 8.58–8.61 (d, *J* = 6.26 Hz, 2H), 8.77–8.81 (t, *J* = 5.94 Hz, 1H), HRFAB-MS: *m/z* 591.2628 for $[M+H]^+$ (calcd. 591.2641 for $C_{32}H_{39}N_4O_5S$).

*2,6-Dimethylphenoxyacetyl-Apns-Dmt-NH-2-picolyl (**7i**)*

$[a]_D^{25}$ +21.80 (c = 0.61, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.37 (s, 3H), 1.54 (s, 3H), 2.13 (s, 6H), 2.76–2.85 (m, 2H), 3.95–4.00 (d, *J* = 14.19 Hz, 1H), 4.12–4.17 (d, *J* = 14.19 Hz, 1H), 4.26–4.56 (m, 5H), 4.94–5.02 (q, *J* = 9.24 Hz, 2H), 6.89–7.01 (m, 3H), 7.12–7.26 (m, 5H), 7.32–7.37 (t, *J* = 6.6 Hz, 1H), 7.53–7.56 (d, *J* = 7.59 Hz, 1H), 7.81–7.87 (t, *J* = 7.58 Hz, 1H), 8.11–8.14 (d, *J* = 8.57 Hz, 1H), 8.53–8.54 (d, *J* = 4.62 Hz, 1H), 8.71–8.75 (t, *J* = 5.94 Hz, 1H), HRFAB-MS: *m/z* 591.2631 for $[M+H]^+$ (calcd. 591.2641 for $C_{32}H_{39}N_4O_5S$).

*2,6-Dimethylphenoxyacetyl-Apns-Dmt-NH-(2-hydroxy-4,6-dimethyl)-3-picolyl (**7j**)*

$[a]_D^{26.3}$ −4.28 (c = 0.07, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.31 (s, 3H), 1.45 (s, 3H), 2.05–2.24 (m, 12H), 2.73–2.80 (m, 2H), 3.98–4.20 (m, 4H), 4.29–4.40 (bs, 1H), 4.44–4.50 (m, 2H), 4.93 (m, 2H), 5.74 (s, 1H), 6.91–7.06 (m, 3H), 7.13–7.37 (m, 5H), 7.98–8.02 (t, *J* = 5.6 Hz, 1H), 8.13–8.16 (d, *J* = 7.71 Hz, 1H), HRFAB-MS: *m/z* 635.2897 for $[M+H]^+$ (calcd. 635.2903 for $C_{34}H_{43}N_4O_6S$).

*2,6-Dimethylphenoxyacetyl-Apns-Dmt-NH-Bz(3-$CONH_2$) (**7k**)*

$[a]_D^{26}$ +42.66 (c = 0.075, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.34 (s, 3H), 1.51 (s, 3H), 2.14 (s, 6H), 2.71–2.80 (m, 2H), 3.96–4.02 (d, *J* = 14.19 Hz, 1H), 4.14–4.18 (d, *J* = 14.19 Hz, 1H), 4.24–4.49 (m, 5H), 4.94–5.03 (m, 2H), 6.90–7.05 (m, 3H), 7.15–7.34 (m, 6H), 7.44–7.48 (d, *J* = 6.6 Hz, 1H), 7.68–7.70 (d, *J* = 7.58 Hz, 1H), 7.81 (s, 1H), 7.93 (s, 2H), 8.09–8.13 (d, *J* = 9.24 Hz, 1H), 8.55–8.62 (t, *J* = 5.8 Hz, 1H), HRFAB-MS: *m/z* 633.2756 for $[M+H]^+$ (calcd. 633.2747 for $C_{34}H_{41}N_4O_6S$).

*2,6-Dimethylphenoxyacetyl-Apns-Dmt-NH-Bz(3-$COOCH_3$) (**7l**)*

$[a]_D^{24}$ −13.33 (c = 0.03, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.33 (s, 3H), 1.51 (s, 3H), 2.13 (s, 6H), 2.75–2.79 (m, 2H), 3.81 (s, 3H), 3.96–4.02 (d, *J* = 14.19 Hz, 1H), 4.13–4.17 (d, *J* = 14.19 Hz, 1H), 4.30–4.52 (m, 5H), 4.96–4.98 (m, 2H), 5.50–5.53 (d, *J* = 8.7 Hz, 1H), 6.92–7.01 (m, 3H), 7.19–7.26 (m, 5H), 7.36–7.42 (t, *J* = 7.2 Hz, 1H), 7.57–7.61 (d, *J* = 6.6 Hz, 1H), 7.75–7.78 (d, *J* = 7.58 Hz, 1H), 7.91 (s, 1H), 8.09–8.12 (d, *J* = 8.25 Hz, 1H), 8.58–8.62 (t, *J* = 5.8 Hz, 1H), HRFAB-MS: *m/z* 648.2753 for $[M+H]^+$ (calcd. 648.2743 for $C_{35}H_{42}N_3O_7S$).

*2,6-Dimethylphenoxyacetyl-Apns-Dmt-NH-Bz(4-$CONH_2$) (**7m**)*

$[a]_D^{25}$ +22.00 (c = 0.05, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.35 (s, 3H), 1.52 (s, 3H), 2.14 (s, 6H), 2.77–2.89 (m, 2H), 3.96–4.03 (m, 1H), 4.15–4.20 (m, 1H), 4.26–4.52 (m, 5H), 5.00 (s, 2H), 6.90–7.03 (m, 3H), 7.15–7.33 (m, 5H), 7.35–7.40 (d, *J* = 7.2 Hz, 2H), 7.76–7.81 (d, *J* = 7.2 Hz, 2H), 7.89 (s, 2H), 8.10–8.15 (d, *J* = 8.1 Hz, 1H), 8.55–8.60 (t, *J* = 5.4 Hz, 1H), HRFAB-MS: *m/z* 633.2763 for $[M+H]^+$ (calcd. 633.2747 for $C_{34}H_{41}N_4O_6S$).

*2,6-Dimethylphenoxyacetyl-Apns-Dmt-NH-Bz(4-$COOCH_3$) (**7n**)*

$[a]_D^{26}$ +16.66 (c = 0.202, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.35 (s, 3H), 1.52 (s, 3H), 2.14 (s, 6H), 2.76–2.79 (m, 2H), 3.81 (s, 3H), 3.96–4.01 (d, *J* = 14.19 Hz, 1H), 4.13–4.19 (d, *J* = 14.51 Hz, 1H), 4.29–4.48 (m, 5H), 4.98 (s, 2H), 6.90–7.01 (m, 3H), 7.18–7.24 (m, 5H), 7.43–7.46 (d, *J* = 7.26 Hz, 2H), 7.82–7.85 (d, *J* = 8.24 Hz, 2H), 8.11–8.15 (d, *J* = 8.75 Hz, 1H), 8.63 (t, *J* = 5.4 Hz, 1H), HRFAB-MS: *m/z* 648.2753 for $[M+H]^+$ (calcd. 648.2743 for $C_{35}H_{42}N_3O_7S$).

*Dipeptides containing the 3-hydroxy-2-methylbenzoyl moiety (3-hydroxy-2-methyl benzoyl-Apns-Dmt-P_2') (**8a**–**h**, **o**, **p**)*

To a solution of the appropriate Boc-Apns-Dmt-P_2' **4a**–**h**, **o**, **p** (0.1 mmol) in 4 N HCl/dioxane solution (1 mL), anisole (22 μL, 0.2 mmol) was added at 0°C. The reaction mixture was stirred for 1 h at room temperature. The solvent was removed in vacuum at room temperature; ether was added. After centrifugation, the residue was suspended in AcOEt (5 mL), then Et_3N (17 μL, 0.12 mmol) was added (solution A). 3-Acetyloxy-2-methylbenzoic acid **6** (21.3 mg, 0.11 mmol) was dissolved in AcOEt (5 mL), then Et_3N (17 μL, 0.12 mmol) and DPPCl (25 μL, 0.12 mmol) were added at 0°C. The mixture was stirred at room temperature for 1 h, then mixed with solution A. The mixture was further stirred at room temperature for 6 h, then washed with 10% citric acid, 5% $NaHCO_3$ and brine. The organic layer was dried and evaporated under reduced pressure. The residue was dissolved in methanol (2 mL), then 1 M LiOH (3 mL) was added. Stirring was continued at room temperature for 5 h, followed by addition of 10% citric acid till pH 3. The mixture was extracted with AcOEt and washed with 10% citric acid and brine. The organic extract was dried, evaporated and crystallized from *n*-hexane. The crude product was purified using analytical, preparative and analytical HPLC. The pure product was lyophilized. Physical data are listed in Table 2.

*3-Hydroxy-2-methylbenzoyl-Apns-Dmt-NH-cumyl (**8a**)*

$[a]_D^{23.5}$ +18.69 (c = 0.23, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.43 (s, 3H), 1.50 (s, 3H), 1.53 (s, 3H), 1.65 (s, 3H), 1.82 (s, 3H), 2.71 (m, 2H), 4.20–4.38 (m, 2H), 4.50–5.51 (d, *J* = 2.97 Hz,

1H), 4.65 (s,1H), 4.93−4.97 (d, *J* = 8.9 Hz, 1H), 5.08−5.12 (d, *J* = 8.9 Hz, 1H), 6.55−6.58 (d, *J* = 7.26 Hz, 1H), 6.76−6.79 (d, *J* = 7.91 Hz, 1H), 6.92−7.04 (m, 2H), 7.11−7.19 (m, 7H), 7.40−7.43 (d, *J* = 8.25 Hz, 2H), 8.19−8.21 (d, *J* = 8.25 Hz, 1H), 9.22−9.32 (bs, 1H), HRFAB-MS: *m/z* 590.2704 for $[M+H]^+$ (calcd. 590.2689 for $C_{33}H_{40}N_3O_5S$).

*3-Hydroxy-2-methylbenzoyl-Apns-Dmt-NH-MPH (**8b**)*

$[a]_D^{26}$ +28.82 (c = 0.17, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.47 (s, 3H), 1.56 (s, 3H), 1.82 (s, 3H), 2.67−2.87 (m, 2H), 3.06 (s, 3H), 4.41 (s, 2H), 4.49 (s, 1H), 5.02−5.06 (d, *J* = 8.91 Hz, 1H), 5.14−5.18 (d, *J* = 9.24 Hz, 1H), 6.54−6.57 (d, *J* = 7.26 Hz, 1H), 6.66−6.72 (t, *J* = 7.26 Hz, 1H), 6.76−6.78 (d, *J* = 7.58 Hz, 1H), 6.87−6.96 (m, 3H), 7.11−7.27 (m, 7H), 8.15−8.19 (d, *J* = 8.25 Hz, 1H), 9.35 (s, 1H), 10.12 (s,1H), HRFAB-MS: *m/z* 577.2492 for $[M+H]^+$ (calcd. 577.2485 for $C_{31}H_{37}N_4O_5S$).

*3-Hydroxy-2-methylbenzoyl-Apns-Dmt-NH-(2-OCH_3)Bz (**8c**)*

$[a]_D^{27.5}$ 0.00 (c = 0.07, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.35 (s, 3H), 1.51 (s, 3H), 1.84 (s, 3H), 2.69−2.88 (m, 2H), 3.79 (s, 3H), 4.20−4.38 (m, 2H), 4.40−4.48 (m, 3H), 4.99−5.03 (d, *J* = 9.24, 1H), 5.12−5.16 (d, *J* = 9.24, 1H), 6.54−6.57 (d, *J* = 7.26 Hz, 1H), 6.76−6.94 (m, 4H), 7.14−7.33 (m, 7H), 8.12−8.15 (d, *J* = 8.24 Hz, 1H), 8.25−8.28 (m, 1H), 9.20−9.50 (bs, 1H), HRFAB-MS: *m/z* 592.2472 for $[M+H]^+$ (calcd. 592.2481 for $C_{32}H_{38}N_3O_6S$).

*3-Hydroxy-2-methylbenzoyl-Apns-Dmt-NH-(2-OC_2H_5)Bz (**8d**)*

$[a]_D^{25.6}$ +20.32 (c = 0.123, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.33−1.38 (m, 6H), 1.51 (s ,3H), 1.83 (s, 3H), 2.76−2.88 (m, 2H), 4.00−4.05 (m, 2H), 4.14−4.49 (m, 5H), 5.00−5.03 (d, *J* = 9.24 Hz, 1H), 5.13−5.16 (d, *J* = 9.24 Hz, 1H), 6.53−6.56 (d, *J* = 7.26 Hz, 1H), 6.75−6.84 (m, 2H), 6.91−7.04 (m, 2H), 7.14−7.35 (m, 7H), 8.12−8.15 (d, *J* = 7.58 Hz, 1H), 8.20−8.30 (m, 1H), 9.35 (s, 1H), HRFAB-MS: *m/z* 606.2652 for $[M+H]^+$ (calcd. 606.2638 for $C_{33}H_{40}N_3O_6S$).

*3-Hydroxy-2-methylbenzoyl-Apns-Dmt-NH-Ind (**8e**)*

$[a]_D^{24.2}$ +35.00 (c = 0.22, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.44 (s, 3H), 1.51 (s, 3H), 1.82 (s, 3H), 2.27−2.49 (m, 2H), 2.75−2.91 (m, 4H), 4.46−4.51 (m, 3H), 5.00−5.04 (m, 1H), 5.06−5.18 (m, 1H), 5.24−5.37 (m, 1H), 6.54−6.57 (d, *J* = 7.26 Hz, 1H), 6.76−6.79 (d, *J* = 7.91 Hz, 1H), 6.84−6.95 (m, 1H), 7.10−7.39 (m, 9H), 8.16−8.19 (t, *J* = 7.59 Hz, 1H), 8.33−8.39 (t, *J* = 8.91 Hz, 1H), 9.37 (bs, 1H), HRFAB-MS: *m/z* 588.2548 for $[M+H]^+$ (calcd. 588.2532 for $C_{33}H_{38}N_3O_5S$).

*3-Hydroxy-2-methylbenzoyl-Apns-Dmt-NH-Pip (**8f**)*

$[a]_D^{24.5}$ +16.36 (c = 0.11, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.34 (s, 3H), 1.50 (s, 3H), 1.83 (s, 3H), 2.73−2.88 (m, 2H), 4.03−4.10 (m, 1H), 4.28−4.36 (m, 1H), 4.42−4.47 (d, *J* = 12.7 Hz, 3H), 4.99−5.03 (d, *J* = 9.24 Hz, 1H), 5.12−5.16 (d, *J* = 8.9 Hz, 1H), 5.90−5.96 (m, 2H), 6.54−6.57 (d, *J* = 7.26 Hz, 1H), 6.73−6.88 (m, 4H), 6.92−6.98 (t, *J* = 7.92 Hz, 1H), 7.16−7.33 (m, 5H), 8.11−8.14 (d, *J* = 7.92 Hz, 1H), 8.37−8.39 (t, *J* = 5.61 Hz, 1H), 9.20−9.60 (bs, 1H), HRFAB-MS: *m/z* 606.2269 for $[M+H]^+$ (calcd. 606.2274 for $C_{32}H_{36}N_3O_7S$).

*3-Hydroxy-2-methylbenzoyl-Apns-Dmt-NH-3-picolyl (**8g**)*

$[a]_D^{25.8}$ +15.62 (c = 0.224, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.32 (s, 3H), 1.50 (s, 3H), 1.82 (s, 3H), 2.68−2.86 (m, 2H), 4.15−4.23 (m, 1H), 4.28−4.47 (m, 4H), 5.00−5.03 (d, *J* = 8.91, 1H), 5.13−5.17 (d, *J* = 9.23 Hz, 1H), 5.49−5.52 (d, *J* = 6.6 Hz, 1H), 6.53−6.56 (d, *J* = 7.26 Hz, 1H), 6.76−6.79 (d, *J* = 7.91 Hz, 1H), 6.90−6.98 (m, 1H), 7.13−7.31 (m, 6H), 7.67−7.70 (d, *J* = 7.58 Hz, 1H), 8.12−8.15 (d, *J* = 7.91 Hz, 1H), 8.39−8.53 (m, 3H), 9.38 (bs, 1H), HRFAB-MS: *m/z* 563.2341 for $[M+H]^+$ (calcd. 563.2328 for $C_{30}H_{35}N_4O_5S$).

*3-Hydroxy-2-methylbenzoyl-Apns-Dmt-NH-4-picolyl (**8h**)*

$[a]_D^{27.5}$ +25.92 (c = 0.135, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.37 (s, 3H), 1.55 (s, 3H), 1.80 (s, 3H), 2.70−2.90 (m, 2H), 4.20−4.66 (m, 5H), 5.01−5.05 (d, *J* = 8.7 Hz, 1H), 5.15−5.19 (d, *J* = 8.9 Hz, 1H), 6.53−6.55 (d, *J* = 7.26 Hz, 1H), 6.73−6.76 (d, *J* = 7.9 Hz,1H), 6.86−6.92 (t, *J* = 7.83 Hz 1H), 7.05−7.32 (m, 4H), 7.66−7.68 (d, *J* = 5.74 Hz, 2H), 8.08−8.12 (d, *J* = 7.9 Hz, 1H), 8.26 (d, *J* = 1.7 Hz 1H), 8.56−8.60 (d, *J* = 6.26 Hz, 2H), 8.70−8.76 (m, 1H), 9.35−9.45 (bs, 1H), HRFAB-MS: *m/z* 563.2337 for $[M+H]^+$ (calcd. 563.2328 for $C_{30}H_{35}N_4O_5S$).

*3-Hydroxy-2-methylbenzoyl-Apns-Dmt-NH-1-Adam (**8o**)*

$[a]_D^{18.5}$ +7.45 (c = 0.295, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.41 (s, 3H), 1.48 (s, 3H), 1.61 (s, 6H), 1.82−1.96 (m, 12H), 2.69−2.82 (m, 2H), 4.24−4.42 (m, 1H), 4.53 (s, 2H), 4.94−4.97 (d, *J* = 9.23 Hz, 1H), 5.15−5.18 (d, *J* = 8.25 Hz, 1H), 6.55−6.57 (d, *J* = 6.93 Hz, 1H), 6.76−6.79 (d, *J* = 7.58 Hz, 1H), 6.91−6.94 (m, 1H), 7.07−7.23 (m, 3H), 7.37−7.39 (d, *J* = 6.92 Hz, 2H), 7.49 (s, 1H), 8.22−8.25 (d, *J* = 8.25 Hz, 1H), 9.20−9.60 (bs, 1H), HRFAB-MS: *m/z* 606.3011 for $[M+H]^+$ (calcd. 606.3002 for $C_{34}H_{44}N_3O_5S$).

*3-Hydroxy-2-methylbenzoyl-Apns-Dmt-NH-2-Adam (**8p**)*

$[a]_D^{20}$ +11.81 (c = 0.127, CH_3OH), ^1H NMR (DMSO-d_6) δ: 1.38 (s, 3H), 1.48−1.52 (m, 6H), 1.69−2.09 (m, 15H), 2.65−2.81 (m, 2H), 3.89 (s, 1H), 4.31−4.37 (m, 1H), 4.51 (s, 1H), 4.70 (s, 1H), 4.99−5.02 (d, *J* = 8.91 Hz, 1H), 5.13−5.16 (d, *J* = 8.58 Hz, 1H), 6.53−6.56 (d, *J* = 7.59 Hz, 1H), 6.76−6.78 (d, *J* = 7.25 Hz, 4H), 6.91−6.93 (t, *J* = 7.58 Hz, 1H), 7.08−7.32 (m, 5H), 7.81−7.84 (d, *J* = 6.26 Hz, 1H), 8.19−8.22 (d, *J* = 7.92 Hz, 1H), HRFAB-MS: *m/z* 606.3011 for $[M+H]^+$ (calcd. 606.3002 for $C_{34}H_{44}N_3O_5S$).

HIV protease inhibition

HIV protease inhibition was determined by an HPLC method using S10 peptide (H-Lys-Ala-Arg-Val-Tyr*Phe(p-NO_2)-Glu-Ala-Nle-NH_2) as the enzyme substrate. The inhibitory potentials were tested at 50 nM concentration of the inhibitor. The assay protocol was followed as described by Mimoto et al. [15].

References

[1] A.Carrillo, K. D. Stewart, H. L. Sham, D. W. Norbeck, W. E. Kohlbrenner, J. M. Leonard, D. J. Kempf, A. Monna, *J. Virol.* **1998**, *72*, 7532−7541.

[2] L. Tong, S. Pav, S. Mui, D. Lamarre, C. Yoakim, P. Beaulieu, P. C. Anderson, *Structure* **1995**, *3*, 33−40.

[3] A. A. Bekhit, H. Matsumoto, H. M. M. Abdel-Rahman, T. Mimoto, S. Nojima, H. Takaku, T. Kimura, K. Akaji,

Y. Kiso; "Peptide Science – Present and Future", Proceedings of the 1st International Peptide Symposium, Ed. Y. Shimonishi, Kluwer Academic Publishers, Dordrecht, **1999**, pp. 660–661.

[4] R. S. Randad, L. Lubkowska, T. N. Bhat, S. Munshi, S. V. Gulnik, B. Yu, J. W. Erickson, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1707–1712.

[5] R. S. Randad, L. Lubkowska, A. M. Silva, D. M. A. Guerin, S. V. Gulnik, B. Yu, J. W. Erickson, *Bioorg. Med. Chem.* **1996**, *4*, 1471–1480.

[6] D. J. Kempf, C. A. Flentge, N. E. Wideburg, A. Saldivar, S. Vasavanonda, D. W. Norbeck, *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2725–2728.

[7] Y. Kiso, S.Yamaguchi, H. Matsumoto, T. Mimoto, R. Kato, S. Nojime, H. Takaku, T. Fukkazawa, T. Kimura, K. Akaji, *Arch. Pharm. Pharm. Med. Chem.* **1998**, *331*, 87.

[8] M. S. Roger, H. B. Christina, *J. Chromatogr.* **1989**, *475*, 57.

[9] P. Ruelle, W. K. Ulrich, *J. Pharm. Sci.* **1998**, *87*, 987.

[10] M.Schutkowski, C. Mrestani-Klaus, K. Neubert, *Int. J. Peptide Protein Res.* **1995**, *45*, 257–265.

[11] D.Hudson, *J. Org. Chem.* **1988**, *53*, 617–624.

[12] T. Miyazawa, T. Yamada, S. Kuwata, *Bull. Chem. Soc. Jpn.* **1988**, *61*, 606–608.

[13] A. S. Kende, D. Scholz, J. Schneider, *Synth. Commun.* **1978**, *8*, 59–63.

[14] J. Dudash, Jr., J. Jiang, S. C. Mayer, M. M Joullie, *Synth. Commun.* **1993**, *23*, 349–356.

[15] T. Mimoto, R. Kato, H. Takaku, S. Nojima, K. Terashima, S. Misawa, T. Fukazawa, T. Ueno, H.Sato, M. Shintani, Y. Kiso, H. Hayashi, *J. Med. Chem.* **1999**, *42*, 1789–1802.

[16] H. M. M. Abdel-Rahman, Ph.D. Thesis, Assiut University, Egypt, **1999**.

[17] Y. Kiso, *Biopolymers (Peptide Science)* **1996**, *40*, 235–244.

[18] M. M. Sheha, N. M. Mahfouz, H. Y. Hassan, A. F. Youssef, T. Mimoto, Y. Kiso, *Eur. J. Med. Chem.* **2000**, *35*, 887–894.