Structure–Activity Relationship of Orally Potent Tripeptide-Based HIV Protease Inhibitors Containing Hydroxymethylcarbonyl Isostere

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We designed and synthesized a new class of peptidomimetic human immunodeficiency virus protease inhibitors containing a unique unnatural amino acid, allophenylnorstatine [Apns; (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid], with a hydroxymethylcarbonyl isostere as the active moiety. From a structure–activity relationship study of HIV-1 protease inhibition, enzyme selectivity for other aspartyl proteases, the antiviral activity and pharmacokinetics in rats, 24c (KNI-227) and 24d (KNI-272, our first clinical candidate) were found to be selective and orally potent HIV protease inhibitors. Moreover, an improvement of the pharmacokinetic features of KNI-272 provided two long-lasting and highly bioavailable compounds (24g: JE-2178, 24h: JE-2179).

Key words HIV protease; inhibitor; AIDS; KNI-272; allophenylnorstatine

The alarming spread of human immunodeficiency virus (HIV), the etiologic agent of AIDS, has initiated an urgent pursuit to comprehend and control this disease. Advances in molecular, viral, and cell biology have defined numerous targets for potential drug intervention. The virally encoded homodimeric aspartyl protease, which is responsible for processing the *gag* and *gag/pol* gene products that allow for the organization of core structural proteins and the release of viral enzymes, is one such target.[1)] Inhibition of this enzyme prevents the maturation and replication of the virus in cell culture. Inhibitors of HIV protease are presently being used in therapy for the treatment of AIDS.[2)] The HIV-1 protease is a member of the aspartic acid family of proteases, such as renin, pepsin and cathepsin D. Mammalian aspartyl proteases are well known to have two characteristic Asp–Thr–Gly sequences at the active center of the enzyme, and both side chain carboxyl groups are important in the catalysis of the peptide bond cleavage. In contrast, the retroviral protease has only one Asp–Thr–Gly sequence and has been shown by X-ray crystallography to be a C-2 symmetrical dimer. The HIV-1 protease can recognize Phe–Pro and Tyr–Pro sequences as the retrovirus-specific cleavage site, whereas mammalian aspartyl proteases such as renin, pepsin, and cathepsin D do not have such specificity. These features provided a basis for the rational design of selective HIV protease-targeted drugs for the treatment of AIDS and related diseases. Previously, we[3—5)] and other research groups[6)] reported a series of peptidomimetic HIV protease inhibitors containing allophenylnorstatine [Apns; (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid] with a hydroxymethylcarbonyl (HMC) isostere based on the transition-state mimic concept. Especially the tripeptide compounds containing an Apns–Pro type structure at P1–P1′ showed a potent HIV-1 protease inhibitory activity. Here, we provide the details for the selection of KNI-272 (**24d**, our first clinical candidate, Fig. 1),[5,7)] based on the structure–activity relationship (SAR) of these series of inhibitors, not only in terms of HIV-1 protease inhibitory potency, but also with respect to the enzyme selectivity for other aspartyl proteases, the antiviral activity, and pharmacokinetics in rats. Moreover, an improvement of the pharmacokinetic features of KNI-272, providing two long-lasting and highly bioavailable compounds (**24g**: JE-2178, **24h**: JE-2179, Fig. 1), is reported.

Design of HIV Protease Inhibitor The transition state of amide hydrolysis by an aspartyl protease is proposed as illustrated in Fig. 2. The hydrogen bond between the carboxylic acid of the protease and the hydroxyl group of the substrate transition state is very important in the design of tight binding inhibitors.[8)] The HMC structure (Fig. 2) was reported to be incorporated in renin inhibitors. Therefore, we reasoned that the HMC group at the scissile site would interact at the active site of HIV protease and that the peptide compounds containing this structure would be highly potent HIV-1 protease inhibitors. In order to design substrate-based HIV protease inhibitors, we turned our attention to the *p17/p24* cleavage site region (Table 1).[9)] As described above, Tyr–Pro (or Phe–Pro) is a unique substrate structure for HIV-1 protease, and the synthetic substrates containing this sequence has low *Km* value.[10)] However, the phenolic hydroxyl group of the P1 site and the carboxamide side chain of Gln at the P3 site seem to have no apparent effect on the binding to HIV-1 protease.[11)] On the basis of these specifications, we considered the heptapeptide amide Ser–Phe–Asn–Phe–Pro–Ile–Val–NH_2, a chimeric structure of the *TF/PR* and *p17/p24* sequences (Table 1). Then, we incorporated an unnatural amino acid, phenylnorstatine [Pns;(2*R*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid) or allophenylnorstatine [Apns; (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid),[12)] containing the HMC isostere as a transition-state mimic at the P1 site in this peptide amide (compounds **2a, b**). In order to obtain smaller inhibitors, Ser (P4) was deleted, and Phe (P3) was replaced with the isosteric 3-phenylpropionyl (Pp) group (compounds **3a, b**). Moreover, Val (P3′) was deleted, Ile (P2′) was replaced with the isosteric *tert*-butylamine, and the Pp group was replaced with benzyloxycarbonyl (Z) group (compounds **7a, b**, Fig. 3).

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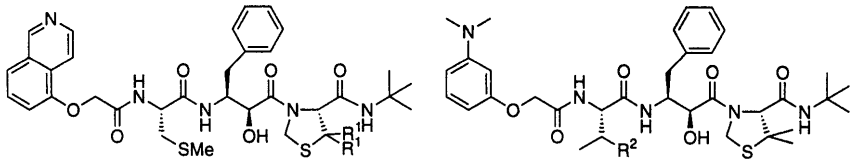


R^1 = Me : KNI-227 (**24c**)
R^1 = H : KNI-272 (**24d**)

R^2 = Me : JE-2178 (**24g**)
R^2 = H : JE-2179 (**24h**)

Fig. 1. Chemical Structures of KNI-227, -272, and JE-2178, -2179

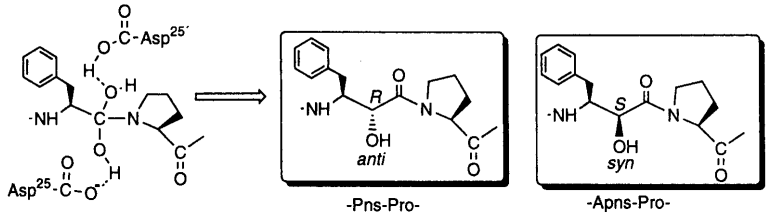


Fig. 2. The Phe–Pro Transition State in HIV-1 Protease, Pns–Pro and Apns–Pro (P1–P1′) with the Hydroxymethylcarbonyl (HMC) Isostere Mimicking the Transition State

Pns=phenylnorstatine=(2*R*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid, Apns=allophenylnorstatine=(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid.

Table 1. Amino Acid Sequences of Cleavage Sites for HIV-1 Protease[9] and Model Substrate

Cleavage site	Amino acid sequence							
	P4	P3	P2	P1	P1′	P2′	P3′	P4′
p17/p24	–Ser	Gln	Asn	Tyr	Pro	Ile	Val	Gln–
p24/p1	–Ala	Arg	Val	Leu	Ala	Glu	Ala	Met–
p1/p9	–Ala	Thr	Ile	Met	Met	Gln	Arg	Glu–
p9/p6	–Pro	Gly	Asn	Phe	Leu	Gln	Ser	Arg–
TF/PR	–Ser	Phe	Asn	Phe	Pro	Gln	Ile	Thr–
PR/RT	–Thr	Leu	Asn	Phe	Pro	Ile	Ser	Pro–
RT/RN	–Ala	Glu	Thr	Phe	Tyr	Val	Asp	Gly–
RN/IN	–Arg	Lys	Ile	Leu	Phe	Leu	Asp	Gly–
Model substrate	Ser	Phe	Asn	Phe	Pro	Ile	Val	NH_2

TF=transframe protein; *PR*=protease; *RT*=reverse transcriptase; *RN*=ribonuclease H; *IN*=integrase.

Chemistry Boc–Apns–OH (**1a**), and Boc–Pns–OH (**1b**) were prepared according to the methods described previously.[12,13] Compounds **2a, b**, and **3a, b** were synthesized by the solid-phase method using Boc strategy on *p*-methylbenzhydrylamine (MBHA) resin.[14] The protected peptide resin thus obtained was treated with anhydrous HF containing *m*-cresol at 0 °C for 60 min, to obtain fully deprotected peptides. The crude peptides were purified by preparative HPLC on an ODS-column. Compounds **7a, b** were synthesized by the solution method in a stepwise manner (Chart 1). The amide bond formation of P1′–P2′ and of P1–P1′ was achieved by use of carbodiimide/1-hydroxybenzotriazol (HOBt) as a condensation reagent. Z-Asn (P3–P2 segment) was incorporated *via* its *p*-nitrophenyl (Np) ester in the presence of HOBt. The inhibitors in Tables 3, 4 (**13a—h**) were obtained as shown in Chart 2. Compounds **13a—e** were prepared by use of the fragment coupling method (P3–P2–P1+P1′–P2′, route A), while compounds **13f, g, h** were obtained by the stepwise elongation method (route B). Boc-protected (*R*)-1,3-thiazolidine-4-carboxylic acid derivatives **8b, c** were prepared from the corresponding L-cysteine analogs by cyclization with formaldehyde, followed by *tert*-butoxycarbonylation with Boc_2O in a one-pot reaction. The amide bonds of P1′–P2′ were formed by use of *N,N′*-dicyclohexylcarbodiimide (DCC)/HOBt or diphenylphosphoryl chloride (DPP-Cl) as a condensation reagent. The mixed anhydride prepared with DPP-Cl was effective for the preparation of Boc-protected 5,5-dimethyl-1,3-thiazolidine-4-carboxamide **9h**. The compounds in Tables 5—8 were synthesized by the following methods (Charts 3—8): Chart 3 shows the preparative method of the aryloxyacetic acids corresponding to the P3 building block. Aryloxyacetic acids **16a—f** were obtained by the reaction of phenols and chloroacetyl esters in the presence of K_2CO_3 or sodium methoxide followed by saponification or hydrogenation. 3-(Dimethylamino)phenoxyacetic acid (**16f**) was isolated as the corresponding crystalline *N*-hydroxy-5-norbornene-2,3-carboxamide (HONB) active ester **17**. Chart 4 illustrates the procedures to synthesize Boc-protected P2–P1–P1′–P2′ intermediates **18a—k**. Compounds **18a, b**, and **h** incorporated asparagine residue at the P2 site were obtained by an active ester method, in order to avoid the contamination of nitrile compounds. Other intermediates **18c—g, i—k** were synthesized under *N*-ethyl-*N′*-[3-(dimethylamino)propyl]carbodiimide (EDC)/HOBt coupling conditions. Removal of the Boc group under acidic conditions and coupling to the corresponding P3 building blocks provided the inhibitors (Charts 5—8). The carbamate compound **21** was synthesized by coupling with the appropriate carbonate **20** derived from *p*-nitrophenyl chloroformate and the corresponding alcohol **19**. The aryloxyacetamide inhibitors in this article except **24g** and **h** were obtained by the coupling to the corresponding aryloxyacetic acids under EDC–HOBt conditions, and compounds **24g** and **h** were prepared by the HONB active ester method (Charts 7—8).

Structure–Activity Relationship against HIV-1 Protease The compounds synthesized in this study were firstly tested for HIV-1 protease inhibitory activity. HIV-1 protease activity was determined by an HPLC method using chemically synthesized [$Ala^{67,95}$] HIV-1 protease (NY-5)[15] and synthetic peptide Ac–Arg–Ala–Ser–Gln–Asn–Tyr–Pro–Val–Val–NH_2[16] as a substrate. Table 2 shows the results of the

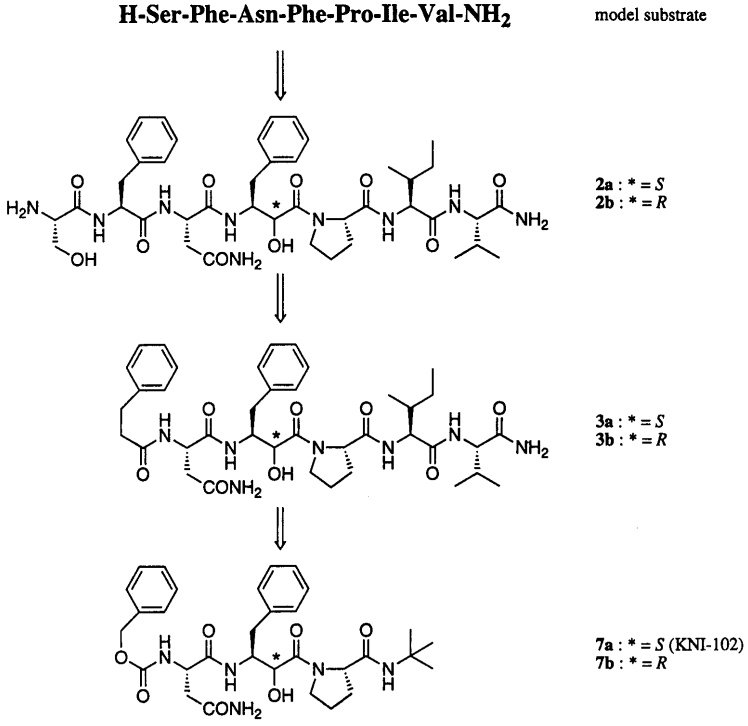
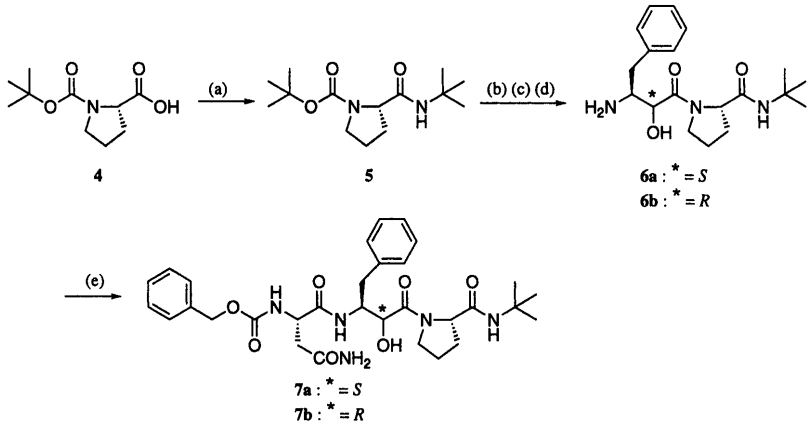
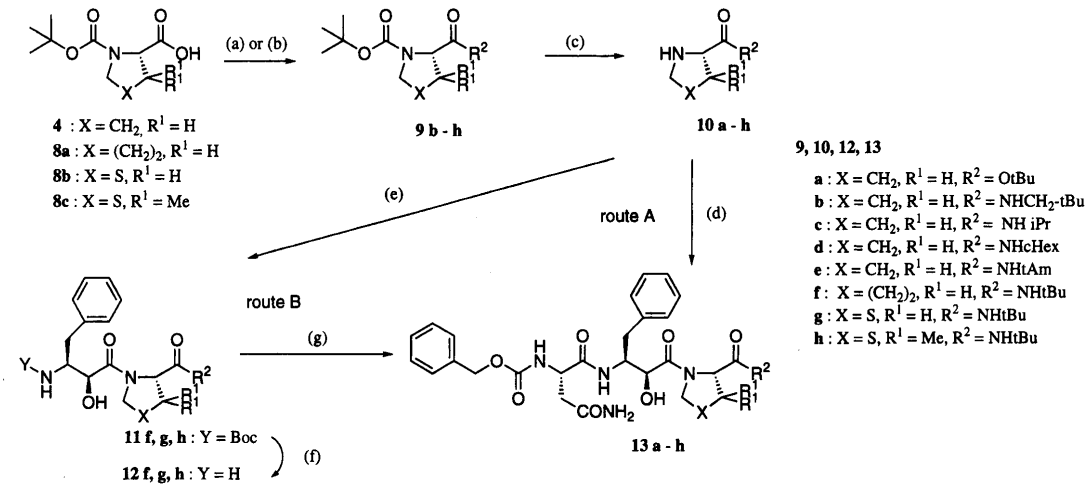


Fig. 3. Design of Substrate-Based HIV Protease Inhibitors



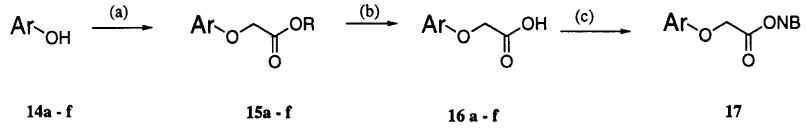
Reagents : (a) EDC-HOBt, *tert*-butylamine, CH_2Cl_2; (b) 4 N HCl/dioxane; (c) **1a** or **1b**, EDC-HOBt, DMF; (d) 4 N HCl/dioxane; (e) Z-Asn-ONp, HOBt, TEA, DMF

Chart 1



Reagents : (a) EDC-HOBt, *tert*-butylamine, CH_2Cl_2; (b) DPP-Cl, TEA, EtOAc, and then *tert*-butylamine; (c) 4 N HCl/dioxane, (d) Z-Asn-Apns-OH[6b], EDC-HOBt, DMF; (e) **1a**, DCC-HOBt, EtOAc; (f) 4 N HCl/dioxane; (g) Z-Asn-ONp, HOBt, TEA, DMF

Chart 2



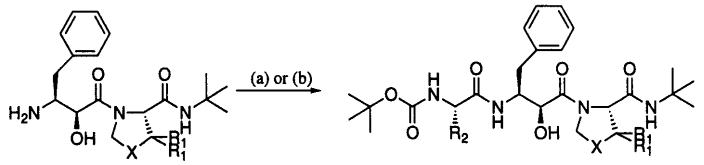
14a - f 15a - f 16 a - f 17

14 - 16 **a**: Ar = 2-biphenyl, R = Me
b: Ar = 3-biphenyl, R = Me
c: Ar = 4-biphenyl, R = Me
d: Ar = 3-(phenylamino)phenyl, R = Me
e: Ar = 5-isoquinolinyl, R = Me
f: Ar = 3-(dimethylamino)phenyl, R = Bn

Ar = 3-(dimethylamino)phenyl

Reagents : (a) methyl chloroacetate or benzyl chloroacetate , K_2CO_3, DMF; (b) NaOH, MeOH aq, or H_2, Pd/C, MeOH; (c) *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB), DCC, CH_2Cl_2

Chart 3

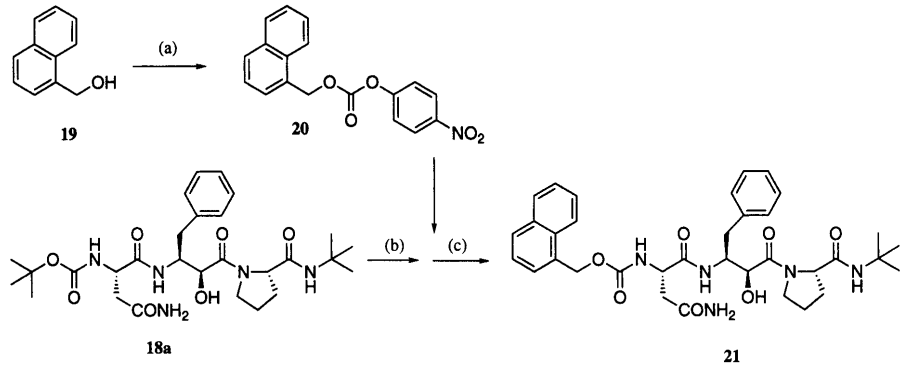


6a: X = CH_2, R^1 = H
12g: X = S, R^1 = H
12i: X = S, R^1 = Me

18a: X = CH_2, R^1 = H, R^2 = CH_2CONH_2
b: X = S, R^1 = H, R^2 = CH_2CONH_2
c: X = S, R^1 = H, R^2 = nPr
d: X = S, R^1 = H, R^2 = iPr
e: X = S, R^1 = H, R^2 = tBu
f: X = S, R^1 = H, R^2 = CH_2SMe
g: X = S, R^1 = H, R^2 = Et
h: X = S, R^1 = Me, R^2 = CH_2CONH_2
i: X = S, R^1 = Me, R^2 = Et
j: X = S, R^1 = Me, R^2 = iPr
k: X = S, R^1 = Me, R^2 = CH_2SMe

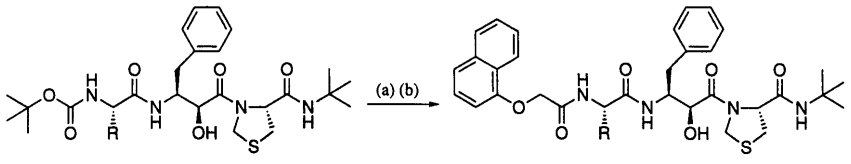
Reagents : (a) Boc-Asn-ONp, HOBt, TEA, DMF; (b)Boc-AA-OH, EDC-HOBt, DMF

Chart 4



Reagents : (a) 4-nitrophenyl chloroformate, pyridine; (b) 4 N HCl/dioxane; (c) HOBt, TEA, DMF

Chart 5



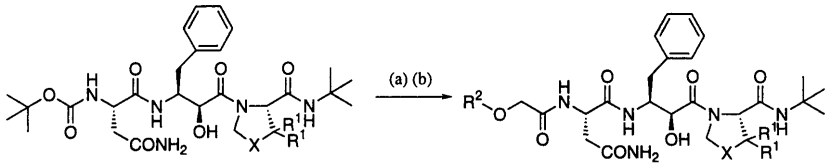
18b - f

22a: R = CH_2CONH_2
b: R = nPr
c: R = iPr
d: R = tBu
e: R = CH_2SMe

Reagents : (a) 4 N HCl/dioxane; (b) 1-naphthoxyacetic acid, EDC-HOBt, DMF

Chart 6

SAR study on the peptide size and stereochemistry of the hydroxyl group in an unnatural amino acid on HIV-1 protease inhibition.[4] Compound **2a** (KNI-93) containing Apns with an HMC isostere exhibited a potent inhibitory activity against HIV-1 protease with an IC_{50} value of 5 nM. Compound **3a**, with the deletion of Ser (P4) and replacement of Phe (P3) by isosteric Pp, also maintained a sufficient inhibitory activity (IC_{50}=468 nM). The Apns-containing tripeptide **7a** (KNI-102), in which Phe (P3) was replaced by its isosteric Z group, Val (P3′) was deleted, and Ile (P2′) was replaced by its isosteric *tert*-butylamine, exhibited a strong activity compared with the pentapeptide **3a**. The stereochemistry of the hydroxyl group is very important for the inhibition of aspartyl proteases. In the case of substrate-based HIV

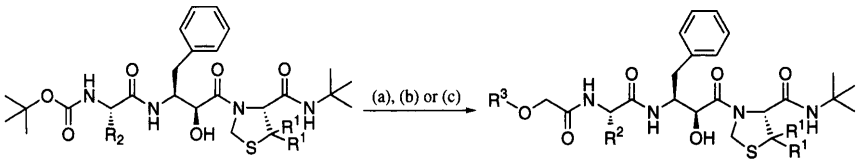


18a: X = CH_2, R^1 = H
18h: X = S, R^1 = Me

23 a: R^2 = 1-naphthyl, X = CH_2, R^1 = H
b: R^2 = 2-naphthyl, X = CH_2, R^1 = H
c: R^2 = 1-naphthyl, X = S, R^1 = Me
d: R^2 = 2-biphenyl, X = S, R^1 = Me
e: R^2 = 3-biphenyl, X = S, R^1 = Me
f: R^2 = 4-biphenyl, X = S, R^1 = Me
g: R^2 = 3-(phenylamino)phenyl, X = S, R^1 = Me
h: R^2 = 5-isoquinolinyl, X = S, R^1 = Me

Reagents : (a) 4 N HCl/dioxane; (b) aryloxyacetic acid in Chart 3, EDC-HOBt, DMF

Chart 7



18d: R^1 = H, R^2 = iPr
f: R^1 = H, R^2 = CH_2SMe
g: R^1 = H, R^2 = Et
i: R^1 = Me, R^2 = Et
j: R^1 = Me, R^2 = iPr
k: R^1 = Me, R^2 = CH_2SMe

24 a: R^1 = Me, R^2 = CH_2SMe, R^3 = 1-naphthyl
b: R^1 = Me, R^2 = CH_2SMe, R^3 = 3-(phenylamino)phenyl
c: R^1 = Me, R^2 = CH_2SMe, R^3 = 5-isoquinolinyl
d: R^1 = H, R^2 = CH_2SMe, R^3 = 5-isoquinolinyl
e: R^1 = H, R^2 = iPr, R^3 = 5-isoquinolinyl
f: R^1 = H, R^2 = Et, R^3 = 5-isoquinolinyl
g: R^1 = Me, R^2 = iPr, R^3 = 3-(dimethylamino)phenyl
h: R^1 = Me, R^2 = Et, R^3 = 3-(dimethylamino)phenyl

Reagents : (a) 4 N HCl/dioxane; (b) aryloxyacetic acid in Chart 3, EDC-HOBt, DMF; (c) **17**, TEA, DMF

Chart 8

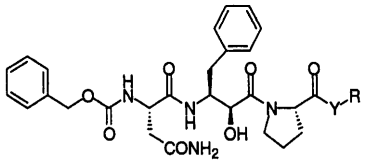
Table 2. Inhibitory Activity against HIV-1 Protease and Anti-HIV-1 IIIB Activity of HIV Protease Inhibitors Containing an Unnatural Amino Acid Such as Apns or Pns

No.		Structure								HIV-1 protease inhibition (IC_{50}, nM)	Anti HIV-1[7] (IC_{50}, μM)
		P4	P3	P2	P1	P1′	P2′	P3′	P4′		
2a	(KNI-93)	Ser	Phe	Asn	Apns	Pro	Ile	Val	NH_2	5.0	>10
2b		Ser	Phe	Asn	Pns	Pro	Ile	Val	NH_2	100	>10
3a			Pp	Asn	Apns	Pro	Ile	Val	NH_2	468	>10
3b			Pp	Asn	Pns	Pro	Ile	Val	NH_2	3000	N.D.
7a	(KNI-102)		Z	Asn	Apns	Pro	NH*t*Bu			89	1.1
7b			Z	Asn	Pns	Pro	NH*t*Bu			>10000	N.D.

Apns=allophenylnorstatine; (2*S*,3*S*)-3-amimo-2-hydroxy-4-phenylbutyryl, Pns=phenylnorstatine; (2*R*,3*S*)-3-amino-2-hydroxy-4-phenylbutyryl, Pp=3-phenylpropionyl, Z=benzyloxycarbonyl, tBu=*tert*-butyl, N.D.=not determined.

protease inhibitors containing HMC–Pro at the scissile peptide bond, the 2*S*-HMC inhibitor (*syn*-diastereomer) containing Apns (**2a**, **3a**, **7a**) was more active than the anti-diastereomer containing Pns (**2b**, **3b**, **7b**) in contrast to the case of the renin inhibitor, which showed a preference of anti-diastereomer over the *syn*-diastereomer.[8] In the case of hydroxyethyl (HE)–Pro type HIV protease inhibitors, the preference shifted from the *syn*-diastereomer in short-chain inhibitors to the anti-diastereomer in long-chain inhibitors.[17,18] The discrepancy between the HMC–Pro inhibitors and the HE–Pro inhibitors seems to be due to the conformational difference between the constrained peptide bond and the relatively flexible methylene-amine bond. On the other hand, HMC–Phe type compounds preferred the anti-hydroxyl group.[4] A preference for the *syn*-hydroxyl group shown in this series of HMC–Pro inhibitors is exceptional among various inhibitors of aspartyl protease such as HIV protease, renin and pepsin, which implies the uniqueness of the HMC–Pro structure. The HIV-1 cytopathic inhibition assay of these HMC compounds was carried out by using $CD4^+$ATH8 cell.[19] The long-chain compounds **2a**, **b** and **3a**, containing natural peptide bonds, showed no inhibitory activity against the HIV cytopathic effect in spite of their potent HIV-1 protease inhibitory activity (Table 2). These compounds seemed to be decomposed by cellular peptidases, or not to be able to penetrate the cell membrane. In contrast, a small-sized compound **7a**, having no natural peptide bonds, showed a potent anti-viral activity with an IC_{50} value of 1.1 μM.[7] In the case

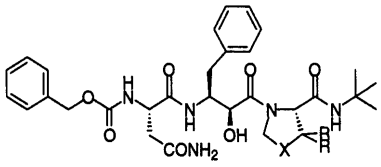
Table 3. HIV-1 Protease Inhibitory Activity (P2′ Site)



Compound	Structure		HIV-1 protease inhibition
	Y	R	IC_{50} (nM)
7a	NH	*t*Bu	89
13a	O	*t*Bu	868
13b	NH	CH_2*t*Bu	520
13c	NH	*i*Pr	320
13d	NH	*c*Hex	572
13e	NH	*t*Am	182

*i*Pr=isopropyl; *c*Hex=cyclohexyl; *t*Am=*tert*-amyl.

Table 4. HIV-1 Protease Inhibitory Activity (P1′ Site)



Compound	Structure			HIV-1 protease inhibition
	X	R		IC_{50} (nM)
7a	CH_2	H	(Pro)	89
13f	$(CH_2)_2$	H		450
13g	S	H	(Thz)	31
13h	S	Me	(Dmt)	3.5

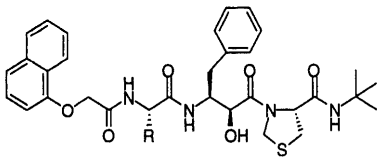
Thz=(*R*)-1,3-thiazolidine-4-carbonyl; Dmt=(*R*)-5,5-dimethyl-1,3-thiazolidine-4-carbonyl.

of our HMC–Pro type compounds, the tripeptide compound was considered to be maximum size required for showing a potent anti-HIV activity.

Table 3 presents the results of the SAR study on the P2′ site. The amide compound **7a** was about 10 fold more preferable than the corresponding ester compound **13a**. According to the result of the analysis of the X-ray crystal structure of KNI-272 complexed to HIV-1 protease,[20] the amide nitrogen of *tert*-butyl (P2′) binds to a water molecule, which forms bridging hydrogen bonds between the amide nitrogen atom of the P2′ group and the backbone nitrogen of Asp29. This causes the 10-fold preference in the HIV protease inhibition of the amide linkage over the ester linkage. The tertiary amides **7a**, **13e**, which might be constrained in their conformation, were more suitable than the primary **13b** or secondary amide **13c**, **d**. Moreover, *tert*-butyl **7a** was more suitable than the bulkier group **13e**.

The SAR study on the P1–P1′ site (P1′ amino acid), which seemed to influence the conformation of the whole compound, is summarized in Table 4. Replacement of the pyrrolidine ring **7a** by the expanded piperidine ring **13f** decreased the HIV-1 protease inhibitory activity; and compound **13g**, with the pyrrolidine ring replaced by the thiazolidine ring (Thz), showed three times greater potency in HIV-1 protease inhibitory activity as compared with compound **7a**. In the case of the hydroxyethylamine (HEA) type inhibitor,

Table 5. HIV-1 Protease Inhibitory Activity (P2 Site)



Compound	Structure		HIV-1protease inhibition
	R		IC_{50} (nM)
22a	$-CH_2CONH_2$	(Asn)	8.8
22b	*n*Pr		6.1
22c	*i*Pr	(Val)	4.1
22d	*t*Bu		12
22e	$-CH_2SMe$	(Mta)	3.2

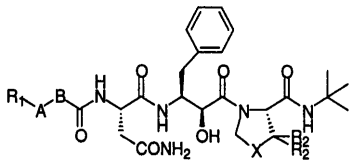
Mta=(*R*)-methylthioalanine.

the replacement of the pyrrolidine ring by the piperidine ring increased the inhibitory activity.[18] As shown in Table 1, HIV-1 protease is known to recognize various sequences as cleavage sites. In addition to Phe–Pro and Tyr–Pro sites, some hydrophobic sites resembling the angiotensinogen cleavage site were recognized.[9] Some compounds, which were designed as renin inhibitors, were also reported to inhibit HIV-1 protease.[21,22] Especially, U-81749, containing ChaΨ[CH(OH)-CH_2]Val at P1–P1′ as a transition-state mimic of the angiotensinogen cleavage site (Leu–Val), exhibited a potent HIV-1 PR inhibitory activity.[22] Accordingly we examined the β-branched amino acid, 5,5-dimethylthiazolidine carboxylic acid (Dmt), a chimeric structure of Thz and Val. The additional dimethyl groups caused an alternate hydrophobic interaction with the S1′ site, and compound **13h** showed a highly potent HIV-1 PR inhibition (IC_{50}=3.5 nM).

Table 5 presents the results of the SAR study on the P2 site. The P2 amino acid of the substrates that have an Xaa–Pro cleavage site, is always Asn, but that of substrates having other cleavage site is a hydrophobic amino acid (Table 1). We investigated some hydrophobic amino acids in addition to Asn at the P2 site of the tripeptide. The structural restriction of this site seemed to be relatively generous in the case of small hydrophobic side chains. The β-branched amino acid **22c** (Val) and a C3 straight chain amino acid **22b** showed good inhibitory activity, but a bulkier group **22d** reduced the inhibitory activity. The introduction of the sulfur atom **22e** (Mta: methylthioalanine) gave more potent interaction, because the bulky sulfur atom helps to fill the subsite volume.[20]

Table 6 presents the results of the SAR study on the P3 site. Replacement of the phenyl group of compound **7a** by the bulkier naphthyl group **21** enhanced the inhibitory activity. Moreover, the 1-naphthoxyacetyl group containing compound **23a** was preferable to the corresponding urethane type compounds **21**. Whereas the 2-naphthoxyacetyl group **23b**, a regioisomer of compound **23a**, reduced the inhibitory activity. The phenyl substituent on the phenoxyacetyl group **23d**—**f** also enhanced the HIV-1 protease inhibitory activity, especially the *m*-substituent **23e** was more suitable than the other regioisomers **23d**, **f**. Although these compounds showed a highly potent inhibitory activity (IC_{50}=2—3 nM), these P3 ligands increased the hydrophobicity, especially in combination with a hydrophobic amino acid at the P2 site

Table 6. HIV-1 Protease Inhibitory Activity (P3 Site)



Compound	Structure						HIV-1 protease inhibition
	P3						
	R^1	A	B		X	R^2	IC_{50} (nM)
7a	Phenyl	CH_2	O	(Z)	CH_2	H	89
21	1-Naphthyl	CH_2	O		CH_2	H	24
23a	1-Naphthyl	O	CH_2	(1-Noa)	CH_2	H	12
23b	2-Naphthyl	O	CH_2		CH_2	H	19
23c	1-Naphthyl	O	CH_2		S	Me	2.8
23d	2-Biphenyl	O	CH_2		S	Me	3.0
23e	3-Biphenyl	O	CH_2	(3-Bpoa)	S	Me	2.2
23f	4-Biphenyl	O	CH_2		S	Me	3.0
23g	3-(Phenylamino)phenyl	O	CH_2	(3-Papoa)	S	Me	2.2
23h	5-Isoquinolinyl	O	CH_2	(5-iQoa)	S	Me	3.6

1-Noa=1-naphthoxyacetyl; 3-Bpoa=3-biphenyloxyacetyl; 3-PaPoa=3-(phenylamino)phenoxyacetyl; 5-iQoa=5-isoquinolinyloxyacetyl.

Table 7. Selectivity against Other Aspartyl Proteases

No.		Structure					HIV-1 PR	Pepsin[a)]	Renin[b)]	Cathepsin D[c)]
		P3	P2	P1	P1′	P2′	IC_{50} (nM)			
7a		Z	Asn	Apns	Pro	NH*t*Bu	87	>80000	>100000	>100000
23a		1-Noa	Asn	Apns	Pro	NH*t*Bu	12.3	>80000	>100000	>100000
22a		1-Noa	Asn	Apns	Thz	NH*t*Bu	8.8	>80000	>100000	18000
23c	(KNI-174)	1-Noa	Asn	Apns	Dmt	NH*t*Bu	2.8	>80000	>100000	6500
23e		3-Bpoa	Asn	Apns	Dmt	NH*t*Bu	2.2	>80000	>100000	2900
23g	(KNI-241)	3-Paoa	Asn	Apns	Dmt	NH*t*Bu	2.2	>80000	>100000	1600
23h		5-iQoa	Asn	Apns	Dmt	NH*t*Bu	3.6	>80000	>100000	>100000
24a		1-Noa	Mta	Apns	Dmt	NH*t*Bu	2.7	18600	>100000	1500
24c	(KNI-227)	5-iQoa	Mta	Apns	Dmt	NH*t*Bu	2.6	12900	>100000	23000
24d	(KNI-272)	5-iQoa	Mta	Apns	Thz	NH*t*Bu	6.6	>80000	>100000	>100000
Pepstatin A							940	2600	1690	62

a) porcine pepsin, *b*) human plasma renin, *c*) bovine cathepsin D; IC_{50} values against HIV-1 PR of this table were determined by an HPLC method using [$Ala^{67,95}$]HIV-1 protease and synthetic peptide Ac–Arg–Ala–Ser–Gln–Asn–Tyr–Pro–Val–Val–NH_2 as a substrate.

(*e.g.* **24a**). This increased hydrophobicity seemed to cause cytotoxicity, and to reduce the oral absorption. Compound **23h**, which incorporated a nitrogen atom at position 6 of the naphthalene ring (corresponding to 5-isoquinoline), reduced hydrophobicity but retained the HIV-1 PR inhibitory activity.

Enzyme Selectivity for Other Aspartyl Proteases Some of the potent inhibitors obtained in this study were examined for enzyme selectivity. The inhibitory activities toward the closely related aspartyl proteases, *i.e.*, pepsin, renin, and cathepsin D, are shown in Table 7. All compounds tested showed more than 500 fold selectivity for HIV-1 PR over other aspartyl proteases, whereas pepstatin A,[23] a typical aspartyl protease inhibitor, showed no selectivity. The high selectivity of these compounds is due to the unique *syn*-configuration of the hydroxyl group of the HMC-Pro like transition state analogue, which is exceptional among various inhibitors of aspartyl proteases. Especially the compounds containing Asn at the P2 site showed higher selectivity over the compounds containing a hydrophobic amino acid (Mta). Increase in HIV-1 protease inhibition by the replacement of P1′ or P3 residues was accompanied by the inhibition of cathepsin D. The relatively hydrophilic 5-isoquinolyloxyacetyl (5-iQoa) group (P3) improved these selectivities, and compounds **23h** and **24d** (KNI-272) showed a high enzyme selectivity without loss of HIV-1 protease inhibitory activity.

Antiviral Activity and Pharmacokinetics *Ki* values, antiviral activity, and pharmacokinetics for selected compounds are shown in Table 8. *Ki* values against HIV-1 protease were determined by an HPLC method using recombinant HIV-1 protease (NY-5) and synthetic peptide H–Ser–Gln–Asn–Tyr–Pro–Ile–Val–OH as a substrate. Antiviral activity was determined in cell culture against HIV-1 strain IIIB in CEM-SS cells.[24]

The compound containing 3-(phenylamino)phenoxyacetyl (3-Papoa) as the P3 ligand preferred Asn (**23g**) to Mta (**24b**) for the P2 residue, whereas 5-iQoa preferred Mta (**24c**) to Asn (**23h**), which was caused by the different binding mode between Asn and Mta for the S2 subsite (hydrogen bond formation and hydrophobic interaction, respectively). Tanaka *et al.*[25] reported that KNI-272-resistant HIV-1 was nearly com-

Table 8. HIV-1 Protease Inhibitory Activity, Anti-HIV Activity, and Pharmacokinetic Profile in Rats

No		Structure					*Ki* (nM)	HIV-1 IIIB IC_{50} (nM)	C_{max} (μM)	T_{max} (min)	$t_{1/2\beta}$ (min)	*AUC* (μM·min)	*F* (%)
		P3	P2	P1	P1′	P2′							
23g	(KNI-241)	3-Papoa	Asn	Apns	Dmt	NH*t*Bu	0.058	50	N.D.	N.D.	70	N.D.	N.D.
23h		5-iQoa	Asn	Apns	Dmt	NH*t*Bu	0.349	225	0.04	10	17	1.6	0.7
24b		3-Papoa	Mta	Apns	Dmt	NH*t*Bu	0.184	6.8	N.D.	N.D.	42	N.D.	N.D.
24c	(KNI-227)	5-iQoa	Mta	Apns	Dmt	NH*t*Bu	0.088	5.7	0.37	30	48	79	29
24d	(KNI-272)	5-iQoa	Mta	Apns	Thz	NH*t*Bu	0.744	25	1.29	60	22	117	28
24e		5-iQoa	Val	Apns	Thz	NH*t*Bu	1.498	31	2.46	30	29	127	32
24f		5-iQoa	Abu	Apns	Thz	NH*t*Bu	2.144	102	1.58	30	28	74	23
24g	(JE-2178)	3-Dapoa	Val	Apns	Dmt	NH*t*Bu	0.318	15	0.70	90	63	157	89
24h	(JE-2179)	3-Dapoa	Abu	Apns	Dmt	NH*t*Bu	0.399	24	1.16	60	110	228	82

Ki values of this table were determined by an HPLC method using recombinant HIV-1 protease (NY-5) and synthetic peptide H–Ser–Gln–Asn–Tyr–Pro–Ile–Val–OH as a substrate. Antiviral activity was determined based on the HIV-1 IIIB-induced cytopathic effects evaluated by the use of the tetrazolium reagent as described in the Experimental Section. *F* (%) which is percent bioavailability *via* i.d. route, was determined by comparing the mean areas under the curves (*AUC*) after i.v. and i.d. doses., 3-Dapoa=3-(dimethylamino)phenoxyacetyl; Abu=(*S*)-2-aminobutyryl; *CL*, plasma clearance rate; V_{dss}, volume of distribution; $t_{1/2\beta}$, plasma half-life; C_{max}, maximum plasma concentration; T_{max}, time of maximum plasma concentration; N.D.=not detected.

pletely sensitive to compound KNI-241 (**23g**). The low resistance profile among these compounds seems to be due to this different binding mode of each compound. Although compound **23g** showed an extremely low *Ki* value, its anti-HIV activity was limited to 50 nM. The compounds bearing Asn at P2 site **23g, h** did not translate their HIV-1 protease inhibitory action into anti-HIV activity in CEM-SS cells. The hydrophilic carboxamide group of Asn, which would be expected to hydrate easily, would interrupt penetration of the drug through the cell membrane. On the other hand, the compounds containing hydrophobic Mta at the P2 site **24b, c** showed a potent anti-HIV activity in CEM-SS cells with IC_{50} values below 10 nM.

These selected compounds were examined for pharmacokinetics when administered by the intraduodenal (i.d.) route to rats as a 50% polyethylene glycol (PEG) solution (10 mg/kg). The plasma levels of the compounds, bearing Asn residue at P2 or the 3-Papoa structure at P3, could not be detected as significant (**23g, h, 24b**). The hydrophilicity of asparagine, or the oxidation sensitive nature of the diphenylamine structure (3-Papoa) would explain these undesirable results. Compound **24c** (KNI-227, Fig. 1), bearing an hydrophobic Mta residue at P2, showed the plasma concentration over 0.3 μM, and had 29% bioavailability after i.d. administration in rats. The replacement of Dmt with Thz at P1′ gave compound **24d** (KNI-272, Fig. 1), having a higher plasma concentration (C_{max}=1.29 μM, Fig. 5) and potent anti-HIV activity. Moreover, the maximum plasma concentration achieved was 4.18 μM when KNI-272 was orally administered at a dose of 15 mg/kg to dogs, and the bioavailability was estimated to be 29%.[26] When we found KNI-272 to be a promising HIV protease inhibitor, there were no known orally potent HIV protease inhibitors available (saquinavir was only several percentage points orally bioavailable in animals[27]); therefore we chose this compound as our first clinical candidate.

Improvement on KNI-272 Clinical trials of KNI-272 were undertaken and the result showed a decrease in the viral load in the blood of patients.[28] Unfortunately, as the relatively short plasma half-life of KNI-272 limits our ability to maintain plasma levels, KNI-272 requires frequent dosing for clinical use. Some HIV protease inhibitors, especially ritonavir,[29] enhanced the plasma concentration of KNI-272 by

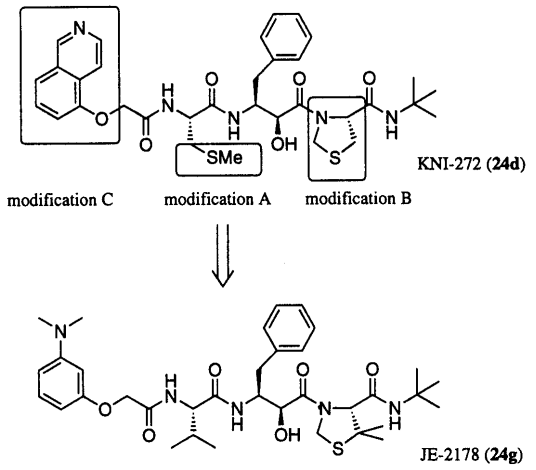


Fig. 4. Chemical Modification of KNI-272 to Improve Pharmacokinetic Profile

Modifications A and B, preventing the oxidation of sulfur atom, modification C, reducing the hydrophobicity.

inhibiting cytochrome P-450 CYP3A4, a major metabolic enzyme of KNI-272. Therefore we tried to co-administer KNI-272 with other HIV protease inhibitors to improve its pharmacokinetic profile.[30] On the other hand, we further tried to improve its pharmacokinetic profile by the structural modifications. We studied the metabolic fate of KNI-272 *in vivo* by means of the distribution of ^{14}C-labeled KNI-272 after i.v. administration to bile-exteriorized dogs and rats. In each case, three major metabolites were produced: the sulfoxide resulting from oxidation at either methylthioalanine or thiazolidine, and the sulfone of thiazolidine. Although minor hydroxyl compounds of the isoquinoline ring were also detected, the structural features responsible for the rapid clearance of KNI-272 were mainly the two oxidized sulfur atoms existing at P2 and P1′ sites.[31] With the above information in hand, we sought structural modifications of KNI-272 that would improve its pharmacokinetic profile without sacrificing its antiviral potency (another approach to the structural modification, *i.e.* reducing the molecular size, was reported in another article.[32]) As shown in Fig. 4, to reduce the rate of oxidation, we carried out the following two structural modifications: 1) replacement of the oxidation-sensitive Mta residue with an aliphatic amino acid residue (modification

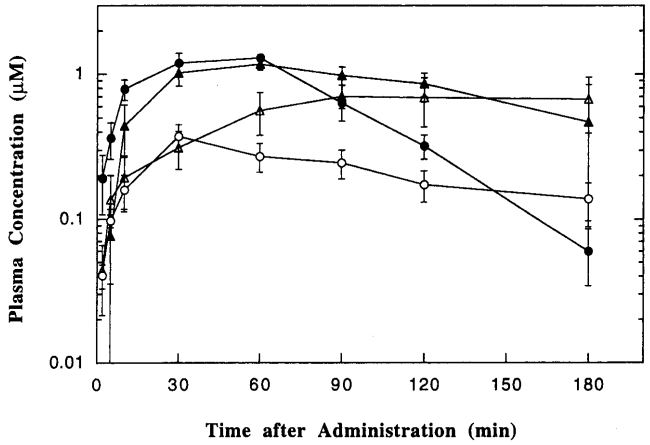


Fig. 5. Plasma Concentration of Selected Compounds after i.d. Administration (10 mg/kg) in Rats

○, compound **24c**; ●, compound **24d**; △, compound **24g**; ▲, compound **24h**.

A), 2) substitution of the sterically hindered dimethyl group on the thiazolidine ring (replacement with Dmt) to avoid the oxidation of the sulfur atom (modification B). The first structural modification (P2) was accomplished in consideration of the following matters: The mutation of Ile84 of HIV-1 protease to Val was widely observed in protease inhibitor-resistant HIV proteases,[33] and the mutation of this site often causes cross-resistance between protease inhibitors. The terminal methyl group of Mta contacts I84 of HIV protease tightly,[20] so KNI-272 is strongly decreased in its inhibitory activity by mutation of this site.[33] Therefore, norvaline, the isosteric analogue of Mta, was avoided, and Val or 2-aminobutyric acid (Abu) was selected as the aliphatic P2 residue. Although the compound **24e** with replacement by Val at the P2 site showed a higher level of maximum plasma concentration (C_{max}=2.46 μM) after i.d. administration than KNI-272, the maintenance of its plasma concentration was not improved; the elimination half-life and *AUC* did not change compared with those for KNI-272. These data suggested that preventing the oxidation of Mta was not sufficient to improve the pharmacokinetic profile of KNI-272. Therefore, the next structural modification (modification B), preventing the oxidation of thiazolidine ring, was expected to improve the plasma half-life (*e.g.* **24c**: $t_{1/2\beta}$=48 min *vs.* **24d**: $t_{1/2\beta}$=22 min). Substitution of dimethyl groups on the thiazolidine ring was also expected to improve the HIV-1 protease inhibitory activity, but this modification seemed to be accompanied by decreased solubility. Although the isoquinoline structure of the P3 site contributed to HIV protease inhibitory activity, the hydrophobicity of this structure limited its absorption especially in basic medium. Therefore, other structural modification at the P3 site was required to compensate for the lack of solubility (modification C). As a result of our SAR study of another series of inhibitors, the 3-(dimethylamino)phenoxyacetyl (3-Dapoa) group was found to be a P3 sunstituent with favorable pharmacokinetics, so we introduced the 3-Dapoa group into these Dmt containing compounds **24g** and **24h** (Fig. 1). These compounds had higher solubility than **24d** in basic medium at pH 7.4 (the solubility of **24g**, **24h** and **24d** were 28.2, 37.2 and 2.7 μg/ml, respectively), and showed a good pharmacokinetic profile in rats. After i.v. administration (10 mg/kg), the elimination half-life of these compounds was 63 min and 110 min, respectively, which was more than 3—5 times longer than that of KNI-272. The bioavailabilty of these compounds after i.d. administration was estimated to be over 80%. Although their maximum plasma concentration after i.d. administration did not reach the level of KNI-272, the duration of their plasma concentration was superior to that of KNI-272 (Fig. 5). Moreover compounds **24g** and **24h** presented a potent antiviral activity in CEM-SS cells compared with KNI-272. Study to obtain additional pharmacokinetic profiles and additional antiviral profile (*e.g.*; antiviral activity in various cell lines, or resistance profiles against HIV) of these compounds are now in progress.

Conclusions

In summary, we designed and synthesized a series of a novel class of substrate-based peptidomimetic HIV protease inhibitors containing Apns based on the transition-state isostere concept. From the SAR study of HIV-1 protease inhibition, enzyme selectivity for other aspartyl proteases, the antiviral activity, and pharmacokinetic study in rats, **24c** (KNI-227) and **24d** (KNI-272, our first clinical candidate) were found to be selective and orally potent HIV protease inhibitors. Although KNI-272 decreased of the viral load in the blood of patients in a clinical trial, the relatively short plasma half-life of this compound did not permit its plasma level to be adequately maintained without an inhibitor of its metabolic enzyme. Therefore, we sought to improve the pharmacokinetic feature of KNI-272, and in doing so found the long lasting ($t_{1/2\beta}$>60 min) and highly bioavailable (F>80%) compounds **24g** (JE-2178) and **24h** (JE-2179). The pharmacokinetic profiles of these compounds indicated that **24g, h** are promising as orally available HIV protease inhibitors.

Experimental

HIV-1 Protease Inhibition 1) HIV-1 protease inhibitory activity of the compounds in Tables 2—7 was determined by the following method using chemically synthesized [Ala^{67,95}]HIV-1 protease (NY5-type sequence): The [Ala^{67,95}]HIV-1 protease was synthesized by the general solid-phase method described below using hydroxymethyl-phenylacetoamidemethyl (PAM) resin (0.3 meq/g). The protease was removed from the resin by treatment with HF/10% *m*-cresol at 0 °C for 1 h, and the product was purified by the gel-filtration (Sephadex G-50, G-75) in 50% AcOH. The crude HIV-1 protease was folded to an active protease by dialysis in 50 mM 2-[*N*-morpholino]ethanesulfonic acid (MES)-NaOH buffer, pH 6.0 containing 1 mM ethylenediaminetetraacetic acid (EDTA)-2Na and 2.5 mM dithiothreitol (DTT). In the inhibition assay, the reaction mixture contained 100 mM MES–NaOH buffer (pH 5.5), 40 mM substrate (Ac–Arg–Ala–Ser–Gln–Asn–Tyr–Pro–Val–Val–NH_2 trifluoroacetate), inhibitors at various concentrations dissolved in dimethylsulfoxide (DMSO), and 9.2 μg of the HIV-1 protease in a total volume of 15 μl. After incubation for 60 min at 37 °C, the reaction was terminated by the addition of 15 μl of acetonitrile; and the C-terminal cleavage fragment (Pro–Val–Val–NH_2) was separated by reversed phase HPLC on a C_{18} column with linear gradient of water to acetonitrile (both solutions containing 0.1% trifluoroacetic acid [TFA]), detected by absorbance at 215 nm, and quantified by comparison with a synthetic product standard. 2) HIV-1 protease inhibitory activity of the compounds in Table 8 was determined by the following method using recombinant HIV-1 protease (NY5-type sequence): Recombinant HIV-1 protease was expressed in *Escherichia coli* and purified to a single band by sodium dodecylsulfate–polyacrylamide gel electrophoresis. In the inhibition assay, 25 μl of 200 mM MES–NaOH buffer, pH 6.0, containing 2 mM DTT and 2 mM EDTA-2Na was mixed with 5 μl of various concentrations of the inhibitor dissolved in DMSO and 10 μl of titrated HIV-1 protease (10.5 nM) in 50 mM MES–NaOH, pH 6.0, containing 2.5 mM DTT, 1 mM EDTA-2Na, 0.2% Nonidet P-40 and 15% glycerol. The mixture of protease and inhibitor was preincubated for 5 min at 37 °C, and the enzymatic reaction was initiated by addition of 10 μl of a 75-mM

substrate solution in the above-described assay buffer. After incubation for 60 min at 37 °C, the reaction was terminated by addition of 75 μl of TFA (4%); and the C-terminal cleavage fragment (Pro–Ile–Val–OH) was separated by reverse phase HPLC on a C_{18} column with a linear gradient of water to acetonitrile (both solutions containing 0.1% TFA), detected by absorbance at 215 nm, and quantified by comparison with a synthetic product standard. The *Ki* values of the inhibitors were analyzed by a mathematical model for tight-binding inhibitors,[34] in which the concentration of inhibitor is less than or approximately equal to the enzyme concentration. The initial velocity data of HIV protease in the presence of various inhibitor concentrations were fitted by nonlinear regression analysis to equation 1, showed below, with KaleidaGraph (Version 3.08 d) for Macintosh, where V is the initial velocity with an inhibitor; V_0 is the measured initial velocity in the absence of the inhibitor; the substrate *Km* is estimated to be 21.4 mM; and *S*, *Et*, and *It* are the concentrations of substrate, active enzyme, and inhibitor, respectively.

$$V=\frac{V_0}{2Et}\left(\left\{\left[Ki\left(1+\frac{S}{Km}\right)+It-Et\right]^2+4Ki\left(1+\frac{S}{Km}\right)Et\right\}^{1/2}-\left[Ki\left(1+\frac{S}{Km}\right)+It-Et\right]\right) \quad (1)$$

Enzyme Selectivity Assay Inhibition of renin (human plasma renin) was measured by a radioimmunoassay using RENIN RIABEAD (Dainabbott).[35] Activities of pepsin (porcine pepsin, Nakalai) and cathepsin D (bovine cathepsin D, Sigma) were measured by a spectorphotomeric assay using albumin-BPB[36] and Phe–Ala–Phe(4-NO_2)-Phe–Val–Leu–OM4P,[37] respectively, as substrate.

Antiviral Activity Antiviral activity of test compounds was determined based on inhibition of HIV-1 IIIB-induced cytopathic effects in CEM-SS cells *in vitro*. The CEM-SS cells (2.5×10^4 cells/ml) were incubated in a total volume of 200 μl of tissue culture medium (RPMI-1640 medium plus 10% fetal calf serum with 50 μg of gentamicin/ml) containing test compound and HIV-1 IIIB for 6 days at 37 °C in a 5% CO_2 incubator. The virus was added to each well as a titer sufficient to give complete cell killing at 6 days post-infection. After incubation, HIV-1 III-induced cytopathic effects were analyzed by staining with the tetrazolium dye XTT.[24] The antiviral activity of a given compound was expressed as the 50% inhibitory concentration (IC_{50}).

Pharmacokinetics Pharmacokinetic parameters of the protease inhibitors were studied in rats and dogs. In the rat i.v. or i.d. administration studies, three male Sprague–Dawley rats (300—400 g) received the compound at 10 mg/kg in 50% PEG (1 ml/kg) under anesthesia in combination with KETARAL (Sankyo Co. Ltd, Tokyo)/SELACTAL (Bayer AG, Germany). The i.v. administration was made *via* a femoral vein. In the i.d. dosing study, rats were incised subphrenically for *ca.* 3 cm along the abdominal median line, a polyethylene tube (INTRAMEDIC, PE10) was inserted into duodenum, and then the test solution was injected into the duodenum through the tube. Heparinized blood samples (0.5 ml) were obtained after dosing at appropriate times, and plasma (0.2 ml) was obtained by immediate centrifugation and kept frozen (−80 °C) until analyzed. A plasma aliquot (0.2 ml) was combined with 4 ml of *tert*-butylmethyl ether containing an appropriate internal standard. Samples were vortexed vigorously for 10 s, shaken for 1 h at room temperature., and then centrifuged at 2500×***g*** for 15 min at 4 °C. The organic layer (3.6 ml) was evaporated to dryness at 40 °C, and then the samples were reconstituted in 0.3 ml of 50% methanol with vortexing. The parent inhibitors and the respective internal standard were separated from plasma contaminants on a CAPCELLPAK C_{18} column (4.6×150 mm; Shiseido Ltd., Tokyo). The elution condition was a linear gradient of 45% to 60% acetonitrile in 0.1% TFA for 12 min at a flow rate of 1.0 ml/min with UV detection at 210 nm. The drug concentration in each plasma sample was calculated by the internal standard method. Standard plasma samples spiked with specified amounts of each compound were analyzed, and the calibration curve was prepared by plotting the concentration of test compound and its ratio to the internal standard. The assays for each inhibitor were linear (correlation coefficients, >0.999) over the concentration range of 0 to 10 μg/ml, and the detection limit of quantification was 0.01 μg/ml. Pharmacokinetic parameters for inhibitors were estimated by a non-compartmental method. Maximum plasma concentration (C_{max}), and time of maximum plasma concentration (T_{max}) were determined by inspection of individual subject concentration–time curves, and the mean area under the plasma concentration–time curve (*AUC*) was determined by the linear trapezoidal rule. The apparent plasma half-life ($t_{1/2}$) was estimated from the slope of the terminal phase fitted to the log plasma concentration–time data by the method of least squares. The apparent distribution volume (V_{dss}) of the inhibitor was determined by the following equation:

$$V_{dss}=\text{Dose i.v.}\times AUMC(0\text{—}\infty)/AUC\ \text{i.v.}(0\text{—}\infty), \quad (2)$$

where *AUMC* (0—∞) is the total area under the first moment of the drug concentration curve from zero to infinity. The plasma clearance (*CL*) was calculated as the dose divided by the *AUC* from zero to infinity $[AUC]_0^\infty$.

Chemistry In general, reagents and solvents were used as purchased without further purification. All compounds except free peptides and resins were routinely checked by TLC with Merck Silica gel $60F_{254}$ precoated plates. Column chromatography was performed on Wakogel C-200 (Wako, 70—150 μm) or Wakogel C-300 (Wako, 45—75 μm). Preparative HPLC were conducted with a Shimadzu LC-4A. Melting points were measured with a Yanagimoto melting point apparatus and left uncorrected. Proton and carbon NMR spectra were recorded on a JEOL GSX270 FT NMR spectrometer. Chemical shifts were expressed in δ ppm from the internal standard tetramethylsilane, and following abbreviations were used: s=singlet, d=doublet, t=triplet, m=multiplet, br=broad, bs=broad singlet, and dd=double doublet. Time-of-flight mass spectrometry (TOF-MS) was recorded on a KOMPACT MALDI III spectrometer. FAB-MS was obtained on a JEOL JMS-SX102A spectrometer equipped with a JMA-DA7000 data system.

General Procedure for Solid-Phase Peptide Coupling *p*-Methylbenzhydrylamine (MBHA) resin or hydroxymethyl-PAM resin was used as a solid support, and standard solid-phase techniques were used for Boc–amino acid coupling, that is, 1) selective deprotection of Boc group using 0.5 M methanesulfonic acid (MSA)/CH_2Cl_2 : 1,4-dioxane (9 : 1), 2% anisole (1 and 20 min), 2) weak-basewash with 2% pyridine/DMF; and 3) coupling using Boc-amino acid (2 eq), benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium-hexafluoro-phosphate (BOP, 2 eq), and triethylamine (TEA, 4 eq) in *N*,*N*-dimethylformamide (DMF, 60 min), and capping with 0.3 M decanoic anhydride in DMF (30 min).

H–Ser–Phe–Asn–Apns–Pro–Ile–Val–NH_2 Trifluoroacetate (2a) Compound **2a** was synthesized by the general solid-phase method on MBHA resin, using **1a**, and removed from the resin by treatment with HF containing 10% *m*-cresol at 0 °C for 1 h. The crude peptide was purified by reverse phase chromatography followed by lyophilization from H_2O. Yield, 38%; purity, >98% by analytical HPLC; HRFAB-MS for $C_{42}H_{61}N_9O_{10}+H_1$: Calcd, 852.4619. Found, 852.4612.

H–Ser–Phe–Asn–Pns–Pro–Ile–Val–NH_2 Trifluoroacetate (2b) Compound **2b** was synthesized in a manner similar to that described for compound **2a**, using **1b**. Yield, 44%; purity, >98% by analytical HPLC; HRFAB-MS for $C_{42}H_{61}N_9O_{10}+H_1$: Calcd, 852.4619. Found, 852.4614.

3-Phenylpropionyl-Asn–Apns–Pro–Ile–Val–NH_2 (3a) Compound **3a** was synthesized in a manner similar to that described for compound **2a**, using **1a**. Yield, 45%; purity, >98% by analytical HPLC; HRFAB-MS for $C_{39}H_{55}N_7O_8+H_1$: Calcd, 750.4190. Found, 750.4183.

3-Phenylpropionyl-Asn–Pns–Pro–Ile–Val–NH_2 (3b) Compound **3b** was synthesized in a manner similar to that described for compound **2a**, using **1b**. Yield, 37%; purity, >98% by analytical HPLC; HRFAB-MS for $C_{39}H_{55}N_7O_8+H_1$: Calcd, 750.4190. Found, 750.4200.

(*S*)-1-*tert*-Butoxycarbonyl-2-*N*-*tert*-butylcarbamoylpyrrolidine (5) To a solution of Boc–Pro–OH (10.0 g, 46.5 mmol) and HOBt (6.30 g, 46.5 mmol) in CH_2Cl_2 (30 ml), EDC·HCl (9.80 g, 51.2 mmol) was added in an ice-bath. After 30 min, *tert*-butylamine (5.86 ml, 55.8 mmol) in CH_2Cl_2 (30 ml) was added dropwise to the reaction mixture and stirred overnight. The reaction mixture was sequentially washed with 3% K_2CO_3, 1 N HCl, and brine, dried over $MgSO_4$, and then evaporated. The obtained solid was recrystallized from *n*-hexane to give 11.7 g of the title compound. Yield, 93%, mp, 118—119 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.25 (s, 9H), 1.35 (s, 9H), 1.6—1.9 (m, 3H), 1.9—2.1 (m, 1H), 3.2—3.4 (m, 2H), 3.9—4.1 (m, 1H), 7.38 (s, 1H); HRFAB-MS *m/z*: 271.2028 for $(M+H)^+$ (Calcd 271.2021 for $C_{14}H_{27}N_2O_3$).

(*S*)-*N*-*tert*-Butyl-1-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoyl]-pyrrolidine-2-carboxamide (6a) To a solution of **5** (2.71 g, 10.0 mmol) in CH_2Cl_2 (10 ml), 4 N HCl in dioxane (10 ml) was added and stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in DMF followed by neutralization with TEA (1.39 ml, 10.0 mmol). To this solution, Boc–Apns–OH (**1a**, 2.95 g, 10.0 mmol), HOBt (1.35 g, 10.0 mmol)

and EDC·HCl (2.10 g, 11.0 mmol) were added, and the mixture was stirred overnight. To the reaction mixture, CH_2Cl_2 and 1 N HCl were added; and then the organic layer was washed with 3% K_2CO_3 and brine, dried over $MgSO_4$ and evaporated to give the residue. To the solution of this residue in CH_2Cl_2 (10 ml), 4 N HCl in dioxane (10 ml) was added and stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in H_2O, washed with CH_2Cl_2, adjusted to pH 10 with 3 N NaOH, and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was recrystallized from *n*-hexane/EtOAc to give 2.62 g of the title compound. Yield, 75%; mp 152—155 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.19 (s, 9H), 1.3—1.4 (br, 2H), 1.7—1.9 (m, 3H), 1.9—2.1 (m, 1H), 2.35 (dd, 1H, J=13.2 Hz, 10.0 Hz), 2.8—3.0 (m, 1H), 3.01 (d, 1H, J=13.2 Hz), 3.5—3.7 (m, 2H), 3.9—4.1 (m, 1H), 4.31 (t, 1H, J=4.2 Hz), 4.96 (d, 1H, J=7.8 Hz), 7.1—7.4 (m, 5H), 7.47 (s, 1H); TOF-MS *m/z*: 348 $(M+H)^+$; *Anal.* Calcd for $C_{19}H_{29}N_3O_3$: C, 65.68; H, 8.41; N, 12.09. Found: C, 65.39; H, 8.51; N, 12.31.

(*S*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-2-hydroxy-3-[(*S*)-2-benzyloxycarbonylaminosuccinamyl]amino-4-phenylbutanoyl]pyrrolidine-2-carboxamide (7a) To the solution of **6a** (1.00 g, 2.88 mmol) and HOBt (0.39 g, 2.88 mmol) in DMF (10 ml), Z–Asn–ONp (1.34 g, 3.46 mmol) and TEA (0.40 ml, 3.46 mmol) were added and stirred overnight. To the reaction mixture, CH_2Cl_2 and 3% K_2CO_3 were added, and then the organic layer was washed sequentially with 3% K_2CO_3, 1 N HCl, and brine, and then dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH), and recrystallized from *n*-hexane/EtOAc to give 1.47 g of the title compound. Yield, 86%; mp 102—104 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.25 (s, 9H), 1.7—1.9 (m, 2H), 1.9—2.1 (m, 2H), 2.2—2.4 (m, 2H), 2.5—2.8 (m, 2H), 3.5—3.7 (m, 2H), 4.0—4.2 (m, 1H), 4.2—4.4 (m, 3H), 4.91 (d, 1H, J=7.3 Hz), 5.01 (s, 2H), 6.89 (bs, 1H), 7.1—7.4 (m, 12H), 7.54 (s, 1H), 7.89 (d, 1H, J=8.4 Hz); TOF-MS *m/z*: 596 $(M+H)^+$; *Anal.* Calcd for $C_{31}H_{41}N_5O_7$: C, 62.50; H, 6.94; N, 11.76. Found: C, 62.22; H, 7.20; N, 11.77.

(*S*)-*N*-*tert*-Butyl-3-[(2*R*,3*S*)-2-hydroxy-3-[(*S*)-2-benzyloxycarbonylaminosuccinamyl]amino-4-phenylbutanoyl]pyrrolidine-2-carboxamide (7b) To a solution of **5** (271 mg, 1.0 mmol) in CH_2Cl_2 (2 ml), 4 N HCl in dioxane (2 ml) was added and stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in DMF followed by neutralization with TEA (139 μl, 1.0 mmol). To this solution, Boc–Pns–OH (**1b**, 295 mg, 1.0 mmol), HOBt (135 mg, 1.0 mmol) and EDC·HCl (210 mg, 1.1 mmol) were added, and the mixture was then stirred overnight. To the reaction mixture, CH_2Cl_2 and 1 N HCl were added, and then the organic layer was washed with 3% K_2CO_3 and brine, dried over $MgSO_4$ and evaporated. The oily residue thus obtained was dissolved in CH_2Cl_2 (2 ml), to which 4 N HCl in dioxane (2 ml) was added, and the mixture was stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in H_2O, washed with CH_2Cl_2, and adjusted to pH 10 with 3 N NaOH, and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to give 200 mg of crude **6b**. This oily residue was dissolved in DMF (3 ml), and Z–Asn–ONp (241 mg, 0.68 mmol), HOBt (77 mg, 0.57 mmol), and TEA (95 μl, 0.68 mmol) were added; and the mixture was stirred overnight. To the reaction mixture, EtOAc and 3% K_2CO_3 were added, and then the organic layer was washed sequentially with 3% K_2CO_3, 1 N HCl and brine, and then dried over $MgSO_4$. After removal of the solvent, the residue was recrystallized from *n*-hexane/EtOAc to give 239 mg of the title compound. Yield, 40%; mp, 189—191 °C; (DMSO-d_6) δ (ppm): 1.20 (s, 9H), 1.6—2.0 (m, 4H), 2.2—2.4 (m, 2H), 2.6—2.9 (m, 2H), 3.3—3.4 (m, 2H, overlapped with H_2O), 4.1 (br, 2H), 4.2—4.4 (br, 2H), 4.73 (d, 1H, J=6.8 Hz), 5.02 (s, 2H), 6.91 (bs, 1H), 7.2—7.4 (m, 13H), 7.78 (d, 1H, J=9.2 Hz); TOF-MS *m/z*: 596 $(M+H)^+$; *Anal.* Calcd for $C_{31}H_{41}N_5O_7$: C, 62.50; H, 6.94; N, 11.76. Found: C, 62.26; H, 7.13; N, 11.61.

(*S*)-3-[(2*S*,3*S*)-2-Hydroxy-3-[(*S*)-2-benzyloxycarbonylaminosuccinamyl]amino-4-phenylbutanoyl]pyrrolidine-2-carboxylic Acid *tert*-Butylester (13a) To a solution of H–Pro–OtBu (**10a**, 51 mg, 0.3 mmol), Z–Asn–Apns–OH[6b] (133 mg, 0.3 mmol), HOBt (41 mg, 0.3 mmol) in DMF (2 ml), and EDC·HCl (63 mg, 0.33 mmol) were added, and the mixture was stirred overnight. To the reaction mixture, CH_2Cl_2 and 1 N HCl were added, and then the organic layer was washed with 3% K_2CO_3, then with brine, dried over $MgSO_4$, and evaporated. The residue was recrystallized from *n*-hexane/EtOAc to give 136 mg of the title compound. Yield, 76%; mp, 149—150 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.40 (s, 9H), 1.8—2.0 (m, 3H), 2.1—2.4 (m, 3H), 2.6—2.8 (m, 2H), 3.6—3.8 (m, 2H), 4.1—4.2 (br, 1H), 4.2—4.4 (m, 3H), 5.01 (s, 2H), 5.10 (d, 1H, J=7.3 Hz), 6.89 (br, 1H), 7.1—7.4 (m, 12H), 7.96 (d, 1H, J=8.4 Hz); TOF-MS *m/z*: 597 $(M+H)^+$; *Anal.* Calcd for $C_{31}H_{40}N_4O_8$: C, 62.40; H, 6.76; N, 9.39. Found: C, 62.23; H, 6.84; N, 9.32.

(*S*)-1-*tert*-Butoxycarbonyl-2-(*N*-2,2-dimethylethyl)carbamoylpyrrolidine (9b) Compound **9b** was prepared from Boc–Pro–OH and neopentylamine in a similar manner as described for compound **5**. Yield, 46%; mp, 114—116 °C; HRFAB-MS *m/z*: 285.2184 for $(M+H)^+$ (Calcd 285.2178 for $C_{15}H_{29}N_2O_3$).

(*S*)-*N*-2,2-Dimethylethyl-3-[(2*S*,3*S*)-2-hydroxy-3-[(*S*)-2-benzyloxycarbonylaminosuccinamyl]amino-4-phenylbutanoyl]pyrrolidine-2-carboxamide (13b) To a solution of **9b** (94 mg, 0.33 mmol) in CH_2Cl_2 (2 ml), 4 N HCl in dioxane (2 ml) was added and stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in DMF followed by neutralization with TEA (46 μl, 0.33 mmol). To this solution, Z–Asn–Apns–OH (133 mg, 0.3 mmol), HOBt (41 mg, 0.3 mmol), and EDC·HCl (63 mg, 0.33 mmol) were added; and the mixture was stirred overnight. To the reaction mixture, CH_2Cl_2 and 1 N HCl were added, and then the organic layer was washed with 3% K_2CO_3 and brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH), and then reprecipitated from *n*-hexane/EtOAc to give 117 mg of the title compound. Yield, 64%; mp, 108—110 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.83 (s, 9H), 1.7—1.9 (m, 2H), 1.9—2.0 (m, 1H), 2.0—2.2 (m, 1H), 2.2—2.4 (m, 2H), 2.6—2.8 (m, 2H), 2.90 (d, 1H, J=6.5 Hz), 3.6—3.7 (br, 2H), 4.0—4.2 (br, 1H), 4.2—4.4 (m, 2H), 4.4—4.5 (m, 1H), 4.96 (d, 1H J=7.0 Hz), 5.01 (s, 2H), 6.88 (bs, 1H), 7.1—7.4 (m, 12H), 7.96 (d, 1H, J=7.0 Hz); TOF-MS *m/z*: 610 $(M+H)^+$; *Anal.* Calcd for $C_{32}H_{43}N_5O_7 \cdot 0.25$EtOAc: C, 62.74; H, 7.18; N, 11.09. Found: C, 62.43; H, 7.33; N, 11.31.

(*S*)-1-*tert*-Butoxycarbonyl-2-*N*-isopropylcarbamoylpyrrolidine (9c) Compound **9c** was prepared from Boc–Pro–OH and isopropylamine in a similar manner as described for compound **5**. Yield, 55%; mp, 132—134 °C; HRFAB-MS *m/z*: 257.1870 for $(M+H)^+$ (Calcd 257.1865 for $C_{13}H_{25}N_2O_3$).

(*S*)-*N*-Isopropyl-3-[(2*S*,3*S*)-2-hydroxy-3-[(*S*)-2-benzyloxycarbonylaminosuccinamyl]amino-4-phenylbutanoyl]pyrrolidine-2-carboxamide (13c) Compound **13c** was prepared from Z–Asn–Apns–OH and **9c** in a similarly as described for compound **13b**. Yield, 40%; mp, 160—162 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.03 (d, 3H, J= 6.5 Hz), 1.05 (d, 3H, J=6.2 Hz), 1.7—1.9 (m, 2H), 1.9—2.1 (m, 2H), 2.2—2.4 (m, 2H), 2.6—2.8 (m, 2H), 3.6—3.7 (br, 2H), 3.7—3.9 (m, 1H), 4.0—4.2 (br, 1H), 4.2—4.4 (m, 3H), 4.95 (d, 1H, J=7.6 Hz), 5.01 (s, 2H), 6.89 (bs, 1H), 7.1—7.4 (m, 12H), 7.74 (d, 1H, J=7.8 Hz), 7.87 (d, 1H, J=8.9 Hz); TOF-MS *m/z*: 582 $(M+H)^+$; *Anal.* Calcd for $C_{30}H_{39}N_5O_7$: C, 61.95; H, 6.76; N, 12.04. Found: C, 61.99; H, 6.91; N, 11.78.

(*S*)-1-*tert*-Butoxycarbonyl-2-*N*-cyclohexylcarbamoylpyrrolidine (9d) Compound **9d** was prepared from Boc–Pro–OH and cyclohexylamine in a manner similar to that described for compound **5**. Yield, 65%; mp, 141—143 °C; HRFAB-MS *m/z*: 297.2181 for $(M+H)^+$ (Calcd 297.2178 for $C_{16}H_{29}N_2O_3$).

(*S*)-*N*-Cyclohexyl-3-[(2*S*,3*S*)-2-hydroxy-3-[(*S*)-2-benzyloxycarbonylaminosuccinamyl]amino-4-phenylbutanoyl]pyrrolidine-2-carboxamide (13d) Compound **13d** was prepared from Z–Asn–Apns–OH and **9d** similarly as described for compound **13b**. Yield, 42%; mp, 103—105 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.0—1.4 (m, 5H), 1.4—1.6 (m, 1H), 1.6—1.9 (m, 6H), 1.9—2.0 (m, 1H), 2.0—2.1 (m, 1H), 2.2—2.4 (m, 2H), 2.6—2.8 (m, 2H), 3.4—3.5 (br, 1H), 3.5—3.6 (m, 2H), 4.0—4.2 (m, 1H), 4.2—4.4 (m, 3H), 4.95 (d, 1H, J=7.6 Hz), 5.01 (s, 2H), 6.88 (br, 1H), 7.1—7.4 (m, 12H), 7.72 (d, 1H, J=7.8 Hz), 7.88 (d, 1H, J=8.4 Hz); TOF-MS *m/z*: 622 $(M+H)^+$; *Anal.* Calcd for $C_{33}H_{43}N_5O_7 \cdot 0.8$EtOAc: C, 62.81; H, 7.19; N, 10.12. Found: C, 62.49; H, 7.13; N, 10.54.

(*S*)-1-*tert*-Butoxycarbonyl-2-*N*-*tert*-amylcarbamoylpyrrolidine (9e) Compound **9e** was prepared from Boc–Pro–OH and *tert*-amylamine similarly as described for compound **5**. Yield, 68%; mp, 110—112 °C; HRFAB-MS *m/z*: 285.2184 for $(M+H)^+$ (Calcd 285.2178 for $C_{15}H_{29}N_2O_3$).

(*S*)-*N*-*tert*-Amyl-3-[(2*S*,3*S*)-2-hydroxy-3-[(*S*)-2-benzyloxycarbonylaminosuccinamyl]amino-4-phenylbutanoyl]pyrrolidine-2-carboxamide (13e) Compound **13e** was prepared from Z–Asn–Apns–OH and **9e** similarly as described for compound **13b**. Yield, 34%; mp, 96—98 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.77 (t, 1H, J=7.3 Hz), 1.19 (s, 6H), 1.6—1.7 (m, 2H), 1.7—1.9 (m, 2H), 1.9—2.2 (m, 2H), 2.2—2.4 (m, 2H), 2.5—2.8 (m, 2H), 3.5—3.7 (m, 2H), 4.0—4.2 (m, 1H), 4.2—4.4 (m, 3H), 4.89 (d, 1H, J=7.3 Hz), 5.01 (s, 2H), 6.89 (s, 1H), 7.1—7.4 (m, 13H), 7.88 (d, 1H, J= 8.4 Hz); TOF-MS *m/z*: 610 $(M+H)^+$; *Anal.* Calcd for $C_{32}H_{43}N_5O_7$ 0.25EtOAc: C, 62.74; H, 7.18; N, 11.09. Found: C, 62.28; H, 7.28; N, 11.06.

(*S*)-1-*tert*-Butoxycarbonyl-2-*N*-*tert*-butylcarbamoylpiperidine (9f) To a solution of Boc-protected L-pipecolinic acid (530 mg, 2.31 mmol) and HOBt (328 mg, 2.43 mmol) in CH_2Cl_2 (10 ml), EDC·HCl (485 mg,

2.54 mmol) was added in an ice-bath. After 30 min, *tert*-butylamine (0.73 ml, 6.93 mmol) was added dropwise to the reaction mixture, which was then stirred overnight. The reaction mixture was washed with 3% K_2CO_3, 1 N HCl, and brine, and dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH) to give 500 mg of the title compound. Yield, 76%; mp, 126—128 °C; HRFAB-MS *m/z*: 285.2174 for $(M+H)^+$ (Calcd 285.2178 for $C_{15}H_{29}N_2O_3$).

(*S*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-2-hydroxy-3-[(*S*)-2-benzyloxycarbonylaminosuccinamyl]amino-4-phenylbutanoyl]piperidine-2-carboxamide (13f) To a solution of **9f** (114 mg, 0.40 mmol) in CH_2Cl_2 (2 ml), 4 N HCl in dioxane (2 ml) was added and stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in DMF followed by neutralization with TEA (56 μl, 0.40 mmol). To this solution, Boc–Apns–OH (**1a**, 118 mg, 0.40 mmol), HOBt (54 mg, 0.40 mmol) and EDC·HCl (84 mg, 0.44 mmol) were added, and the mixture was stirred overnight. To the reaction mixture, CH_2Cl_2 and 1 N HCl were added, and then the organic layer was washed with 3% K_2CO_3, brine, and dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH) to give 87 mg (0.19 mmol) of the crude compound (**11f**). The crude compound thus obtained was dissolved in CH_2Cl_2 (2 ml), and then 4 N HCl in dioxane (2 ml) was added, and the mixture was stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in DMF followed by neutralization with TEA (26 μl, 0.19 mmol). To the solution, Z–Asn–ONp (81 mg, 0.23 mmol), HOBt (26 mg, 0.19 mmol) and TEA (32 μl, 0.23 mmol) were added and stirred overnight. To the reaction mixture, CH_2Cl_2 and 3% K_2CO_3 were added, and then the organic layer was washed sequentially with 3% K_2CO_3, 1 N HCl, and brine, and then dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH), and recrystallized from *n*-hexane/EtOAc to give 63 mg of the title compound. Yield, 26%; mp, 96—98 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.27 (s, 9H), 1.3—1.5 (m, 2H), 1.5—1.7 (br, 3H), 2.0—2.2 (br, 1H), 2.2—2.4 (m, 2H), 2.56 (d, 1H, *J*=6.8 Hz), 2.6—2.8 (m, 1H), 3.5—3.7 (m, 1H), 3.9—4.0 (m, 2H), 4.1—4.2 (m, 1H), 4.2—4.4 (m, 1H), 4.47 (d, 1H, *J*=4.9 Hz), 4.85 (d, 1H, *J*=7.3 Hz), 5.01 (s, 2H), 5.0—5.4 (br, 1H), 6.88 (br, 1H), 7.1—7.4 (m, 13H), 7.87 (d, 1H, *J*=8.4 Hz); TOF-MS *m/z*: 610 $(M+H)^+$; *Anal.* Calcd for $C_{32}H_{43}N_5O_7\cdot$0.5EtOAc: C, 62.46; H, 7.25; N, 10.71. Found: C, 62.04; H, 7.11; N, 11.00.

(*R*)-*N*-*tert*-Butyl-1,3-thiazolidine-4-carboxamide (10g) To a solution of Boc–Thz–OH (11.65 g, 50 mmol) and HOBt (6.75 g, 50 mmol) in CH_2Cl_2 (80 ml), DCC (11.33 g, 55 mmol) was added in an ice-bath. After 30 min, *tert*-butylamine (13.75 ml, 150 mmol) in CH_2Cl_2 (60 ml) was added dropwise to the reaction mixture, which was then stirred overnight. The reaction mixture was washed sequentially with 3% K_2CO_3, 1 N HCl, and brine, dried over $MgSO_4$, and then evaporated. The residue was redissolved in CH_2Cl_2 (125 ml), 4 N HCl in dioxane (125 ml) was added, and the mixture was stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in H_2O and filtered. The filtrate was washed with CH_2Cl_2, adjusted to pH 8 with K_2CO_3, and extracted with CH_2Cl_2. After drying and concentrating, the obtained solid was recrystallized from *n*-hexane/toluene to give 7.87 g of the title compound. Yield, 84%; mp, 63—65 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.26 (s, 9H), 2.75—2.82 (m, 1H), 2.91—2.98 (m, 1H), 3.10—3.21 (m, 1H), 3.62—3.71 (m, 1H), 4.01 (t, 1H, *J*=9.7 Hz), 4.14 (t, 1H, *J*=8.8 Hz), 7.56 (s, 1H); TOF-MS *m/z*: 189 $(M+H)^+$; *Anal.* Calcd for $C_8H_{16}N_2OS$: C, 51.03; H, 8.56; N, 14.88. Found: C, 51.13; H, 8.41; N, 15.02.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (12g) To a solution of **10g** (1.05 g, 5.59 mmol) in DMF (10 ml), Boc–Apns–OH (1.50 g, 5.09 mmol), HOBt (0.69 g, 5.09 mmol), and EDC·HCl (1.07 g, 5.60 mmol) were added and the mixture was stirred overnight. To the reaction mixture, CH_2Cl_2 and 1 N HCl were added, and then the organic layer was washed with 3% K_2CO_3 and then with brine, dried over $MgSO_4$, and evaporated to give **11g**. To a solution of **11g** in CH_2Cl_2 (13 ml), 4 N HCl in dioxane (13 ml) was added, and the mixture was stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in H_2O, washed with CH_2Cl_2 and adjusted to pH 8 with K_2CO_3 to give a solid. The obtained solid was washed with hot methanol to give 1.57 g of the title compound. Yield, 85%; mp, 208—210 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.19 (s, 9H), 1.40 (br, 2H), 2.31—2.40 (m, 1H), 2.90 (t, 1H, *J*=8.1 Hz), 3.01—3.07 (m, 2H), 3.16—3.26 (m, 1H), 4.11 (t, 1H, *J*=7.6 Hz), 4.60 (d, 1H, *J*=8.9 Hz), 4.77—4.82 (m, 1H), 4.89 (d, 1H, *J*=8.6 Hz), 5.20 (d, 1H, *J*=7.8 Hz), 7.16—7.31 (m, 5H), 7.57 (s, 1H); TOF-MS *m/z*: 366 $(M+H)^+$; *Anal.* Calcd for $C_{18}H_{27}N_3O_3S$: C, 59.15; H, 7.45; N, 11.50. Found: C, 58.93; H, 7.56; N, 11.12.

(*R*)-*N*-*tert*-Butyl-3-[(2S,3*S*)-2-hydroxy-3-[(S)-2-benzyloxycarbonylaminosuccinamyl]amino-4-phenylbutanoyl]thiazolidine-4-carboxamide (13g) To a solution of **12g** (500 mg, 1.37 mmol) and HOBt (185 mg, 1.37 mmol) in DMF (5 ml), Z–Asn–ONp (636 mg, 1.64 mmol) and TEA (190 μl, 1.37 mmol) were added; and the mixture was stirred overnight. To the reaction mixture, CH_2Cl_2 and 3% K_2CO_3 were added, and then the organic layer was washed sequentially with 3% K_2CO_3, 1 N HCl, and brine, and then dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH), and recrystallized from toluene to give 690 mg of the title compound. Yield, 82%; mp, 102—104 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.26 (s, 9H), 2.2—2.4 (m, 2H), 2.6—2.7 (br, 2H), 2.98 (dd, 1H, *J*=6.2 Hz, 11.1 Hz), 3.3—3.4 (m, 1H, overlapped with H_2O), 4.0—4.2 (br, 1H), 4.2—4.5 (br, 2H), 4.60(d, 1H, *J*=9.5 Hz), 4.76 (t, 1H, *J*=7.0 Hz), 4.94 (d, 1H, *J*=9.7 Hz), 5.01 (s, 2H), 5.19 (d, 1H, *J*=7.0 Hz), 6.88 (bs, 1H), 7.1—7.4 (m, 12H), 7.67 (s, 1H), 7.94 (d, 1H, *J*=8.4 Hz); TOF-MS *m/z*: 614 $(M+H)^+$; *Anal.* Calcd for $C_{30}H_{39}N_5O_7S\cdot$0.4EtOAc: C, 58.48; H, 6.55; N, 10.79. Found: C, 57.98; H, 6.67; N, 10.58.

(*R*)-*N*-*tert*-Butyl-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (10h) To a solution of Boc–Dmt–OH (5.22 g, 20.0 mmol) and TEA (3.34 ml, 24.0 mmol) in EtOAc (100 ml), DPP-Cl (4.55 ml, 22.0 mmol) was added in an ice-bath, and the mixture was stirred for 1 h. Then to the reaction mixture, *tert*-butylamine (6.30 ml, 60.0 mmol) was added in an ice-bath. After overnight stirring, the reaction mixture was washed sequentially with 1 N HCl, 3% K_2CO_3, and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was redissolved in CH_2Cl_2 (30 ml), to which was added 4 N HCl in dioxane (30 ml), and then stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in H_2O and filtered. The filtrate was washed with CH_2Cl_2, adjusted to pH 8 with K_2CO_3, and extracted with CH_2Cl_2. After drying and concentrating, the obtained solid was recrystallized from *n*-heptane to give 3.01 g of the title compound. Yield, 70%; mp, 75—77 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.16 (s, 3H), 1.27 (s, 9H), 1.52 (s, 3H), 3.16 (d, 1H, *J*=13.2 Hz), 3.46—3.58 (m, 1H), 3.99 (dd, 1H, *J*=11.8 Hz, 9.2 Hz), 4.26 (dd, 1H, *J*=7.3 Hz, 9.2 Hz), 7.47 (s, 1H); TOF-MS *m/z*: 217 $(M+H)^+$; *Anal.* Calcd for $C_{10}H_{20}N_2OS$: C, 55.52; H, 9.32; N, 12.95. Found: C, 55.52; H, 9.16; N, 13.08.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (12h) To a solution of **10h** (2.90 g, 13.43 mmol) in EtOAc (30 ml), Boc–Apns–OH (3.77 g, 12.79 mmol), HOBt (1.73 g, 12.79 mmol) and DCC (3.03 g, 14.71 mmol) were added; and the mixture was stirred overnight. The reaction mixture was then washed with 3% K_2CO_3, 1 N HCl, and brine in this order, dried over $MgSO_4$ and evaporated. The residue was redissolved in CH_2Cl_2 (20 ml), and then 4 N HCl in dioxane (20 ml) was added, and the mixture was stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in H_2O and filtered. The filtrate was washed with CH_2Cl_2, adjusted to pH 8 with K_2CO_3, and extracted with CH_2Cl_2. After drying with $MgSO_4$ and concentrating, the obtained solid was recrystallized from *n*-hexane/EtOAc to give 3.73 g of the title compound. Yield, 74%; mp, 177—180 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.23 (s, 9H), 1.35 (s, 3H), 1.3—1.5 (m, 2H), 1.49 (s, 3H), 2.30—2.38 (m, 1H), 2.88—3.04 (m, 2H), 4.10 (t, 1H, *J*=7.3 Hz), 4.36 (s, 1H), 4.90 (s, 1H), 5.19 (d, 1H, *J*=7.3 Hz), 7.16—7.31 (m, 5H), 7.52 (s, 1H); TOF-MS *m/z*: 394 $(M+H)^+$; *Anal.* Calcd for $C_{20}H_{31}N_3O_3S$: C, 61.04; H, 7.94; N, 10.68. Found: C, 61.03; H, 8.06; N, 10.69.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-2-hydroxy-3-[(*S*)-2-benzyloxycarbonylaminosuccinamyl]amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (13h) To a solution of **12h** (500 mg, 1.27 mmol) and HOBt (172 mg, 1.27 mmol) in DMF (5 ml), Z–Asn–ONp (591 mg, 1.52 mmol) and TEA (177 μl, 1.27 mmol) were added; and the mixture was stirred overnight. To the reaction mixture, CH_2Cl_2 and 3% K_2CO_3 were added; and then the organic layer was washed sequentially with 3% K_2CO_3, 1 N HCl, and brine, and dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH), and recrystallized from *n*-hexane/EtOAc to give 710 mg of the title compound. Yield, 87%; mp, 113—115 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.27 (s, 9H), 1.39 (s, 3H), 1.48 (s, 3H), 2.2—2.4 (m, 2H), 2.6—2.7 (m, 2H), 4.0—4.2 (br, 1H), 4.2—4.4 (m, 2H), 4.50 (s, 1H), 4.8—5.0 (m, 2H), 5.01 (s, 2H), 5.20 (d, 1H, *J*=7.3 Hz), 6.89 (bs, 1H), 7.1—7.4 (m, 12H), 7.65 (s, 1H), 7.95 (d, 1H, *J*=8.1 Hz); TOF-MS *m/z*: 628 $(M+H)^+$; *Anal.* Calcd for $C_{32}H_{43}N_5O_7S$: C, 59.89; H, 6.75; N, 10.91. Found: C, 59.62; H, 7.03; N, 10.90.

2-Biphenyloxyacetic Acid (16a) To a solution of *o*-phenylphenol (**14a**, 0.85 g, 5.0 mmol) and K_2CO_3 (1.04 g, 7.5 mmol) in DMF (10 ml), methyl chloroacetate (0.52 ml, 6.0 mmol) was added in an ice-bath, and the reaction mixture was stirred overnight. To the reaction mixture, H_2O (30 ml) was

added, followed by extraction with EtOAc. The organic layer was washed with 1 N HCl, and then with brine, and evaporated. The obtained oily residue was dissolved in MeOH (20 ml) and after the addition of 1 N NaOH (7.5 ml, 7.5 mmol), was stirred for 2 h. The reaction mixture was acidified to pH 3 with conc.HCl, and then extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and then evaporated. The obtained solid was recrystallized from *n*-hexane/EtOAc to give 1.02 g of the title compound. Yield, 84%; mp, 92—93 °C; *Anal.* Calcd for $C_{14}H_{12}O_3$: C, 73.67; H, 5.30. Found: C, 74.00; H, 5.38.

3-Biphenyloxyacetic Acid (16b) Compound **16b** was prepared from *m*-phenylphenol (**14b**) in a similar manner as described for compound **16a**. Yield, 82%; mp, 98—100 °C; *Anal.* Calcd for $C_{14}H_{12}O_3$: C, 73.67; H, 5.30. Found: C, 74.00; H, 5.35.

4-Biphenyloxyacetic Acid (16c) Compound **16c** was prepared from *p*-phenylphenol (**14c**) similarly as described for compound **16a**. Yield, 61%; mp, 192—194 °C; *Anal.* Calcd for $C_{14}H_{12}O_3$: C, 73.67; H, 5.30. Found: C, 74.01; H, 5.34.

3-(Phenylamino)phenoxyacetic Acid (16d) To a solution of 3-(phenylamino)phenol (**14a**, 0.93 g, 5.0 mmol) and K_2CO_3 (1.04 g, 7.5 mmol) in DMF (10 ml), methyl chloroacetate (0.52 ml, 6.0 mmol) was added; and the mixture was then stirred 3 h at 80 °C. To the reaction mixture, H_2O (30 ml) was added, followed by extraction with EtOAc. The organic layer was washed with 1 N HCl, then with brine, and evaporated. The obtained oily residue was dissolved in MeOH (20 ml); and 3 N NaOH (7.5 ml, 7.5 mmol) was added to it, followed by stirring for 1 h. To the reaction mixture, H_2O and EtOAc were added, and the aqueous layer was acidified to pH 3 with conc.HCl, and then extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and then evaporated. The obtained solid was recrystallized twice from *n*-hexane/EtOAc to give 374 mg of the title compound. Yield, 31%; mp, 134—135 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.59 (s, 2H), 6.35 (dd, 1H, J=1.6 Hz, 6.4 Hz), 6.56 (s, 1H), 6.64—6.67 (m 1H), 6.82 (t, 1H, J=5.8 Hz), 7.02—7.13 (m, 3H), 7.22 (t, 2H, J=6.3 Hz), 8.17 (s, 1H), 12.95 (br, 1H); *Anal.* Calcd for $C_{14}H_{13}NO_3$: C, 69.12; H, 5.39; N, 5.76. Found: C, 69.45; H, 5.47; N, 5.56.

5-Isoquinolinyloxyacetic Acid (16e) To a solution of 5-hydroxyisoquinoline (2.90 g, 20 mmol) in DMF (40 ml), sodium methoxide (1.10 g, 20.4 mmol) was added in ice bath and stirred 1 h, and then methyl chloroacetate (1.79 ml, 20.4 mmol) was added and stirred overnight. After removal of the solvent, H_2O (30 ml) was added, followed by extraction with EtOAc. The organic layer was washed with H_2O, and then evaporated. The obtained oily residue was dissolved in MeOH (10 ml), to which 3 N NaOH (8.0 ml, 24 mmol) was added; and then the mixture was stirred for 1 h. The reaction mixture was concentrated (MeOH was removed), H_2O (10 ml) and acetone (20 ml) were added, and then the mixture was acidified to pH 3 with conc.HCl to afford the precipitate. This precipitate was filtered and then washed with H_2O and acetone to give 2.96 g of the title compound. Yield, 73%, mp, 218—220 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 4.99 (s, 2H), 7.20 (d, 1H, J=8.1 Hz), 7.60 (t, 1H, J=8.0 Hz), 7.72 (d, 1H, J=7.8 Hz), 8.59 (d, 1H, J=8.1 Hz), 9.32 (s, 1H), 11.36 (br, 1H); *Anal.* Calcd for $C_{11}H_9NO_3$: C, 65.02; H, 4.46; N, 6.89. Found: C, 65.39; H, 4.53; N, 6.67.

3-(Dimethylamino)phenoxyacetic Acid *N*-Hydroxy-5-norbornene-2,3-dicarboxamide ester (17) To a solution of 3-(dimethylamino)phenol (**14a**, 15.1 g, 110 mmol) and K_2CO_3 (20.7 g, 150 mmol) in DMF (150 ml), benzyl chloroacetate (15.2 ml, 100 mmol) was added and stirred 5 h at 80 °C. To the reaction mixture, H_2O was added; and the product was then extracted with CH_2Cl_2, washed with 1 N HCl, followed with brine, dried over $MgSO_4$, and then evaporated. The oily residue was dissolved in MeOH (150 ml), and 5% Pd/C (2.0 g) was added to the solution, which was then stirred for 2 h at H_2 atmosphere. The reaction mixture was filtered, and the filtrate was evaporated to afford the crude **16f** (19.3 g, yield: 99%). To a solution of **16f** (1.95 g, 100 mmol) and HONB (1.79 g, 100 mmol) in CH_2Cl_2 (20 ml), DCC (2.06 g, 10.0 mmol) was added; and the mixture was stirred overnight. The reaction mixture was filtered, and the filtrate was washed with H_2O and brine, dried over $MgSO_4$, and then evaporated. The obtained residue was recrystallized from methanol to give 1.63 g of the title compound. Yield, 46%; mp, 133—135 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.53 (d, 1H, J=8.9 Hz), 1.61 (d, 1H, J=8.9 Hz), 2.88 (s, 6H), 3.33 (bs, 2H, overlapped with H_2O), 3.53 (bs, 2H), 5.17 (s, 2H), 6.1—6.2 (br, 2H), 6.23—6.26 (m, 2H), 6.36 (d, 1H, J=9,7 Hz), 7.07 (t, 1H, J=8.6 Hz): *Anal.* Calcd for $C_{19}H_{20}N_2O_5$: C, 64.04; H, 5.66; N, 7.86. Found: C, 64.33; H, 5.66; N, 7.70.

(*S*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(*tert*-butoxycarbonyl)aminosuccinamyl]amino-2-hydroxy-4-phenylbutanoyl]pyrrolidine-2-carboxamide (18a) To a solution of **6a** (1.04 g, 3.00 mmol) and HOBt (0.41 g, 3.00 mmol) in DMF (10 ml), Boc–Asn–ONp (1.27 g, 3.60 mmol) and TEA (0.50 ml, 3.60 mmol) were added; and the mixture was then stirred overnight. To the reaction mixture, EtOAc and 3% K_2CO_3 were added, and then the organic layer was washed sequentially with 3% K_2CO_3, 1 N HCl, and brine, and then dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH), and recrystallized from *n*-hexane/EtOAc to give 0.79 g of the title compound. Yield, 47%; mp, 120—123 °C; *Anal.* Calcd for $C_{28}H_{43}N_5O_7$: C, 59.87; H, 7.72; N, 12.47. Found: C, 59.59; H, 7.84; N, 12.27.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(*tert*-butoxycarbonyl)aminosuccinamyl]amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (18b) To a solution of **12g** (0.73 g, 2.00 mmol) and HOBt (0.27 g, 2.00 mmol) in DMF (10 ml), Boc–Asn–ONp (0.85 g, 2.40 mmol) and TEA (0.33 ml, 2.40 mmol) were added; and the mixture was then stirred overnight. To the reaction mixture, EtOAc and 3% K_2CO_3 were added; and then the organic layer was washed sequentially with 3% K_2CO_3, 1 N HCl, and brine, and then dried over $MgSO_4$. After removal of the solvent, the residue was recrystallized from *n*-hexane/EtOAc to give 0.81 g of the title compound. Yield, 70%; mp, 125—128 °C; *Anal.* Calcd for $C_{27}H_{41}N_5O_7S$: C, 55.94; H, 7.13; N, 12.08. Found: C, 55.98; H, 7.30; N, 12.08.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(*tert*-butoxycarbonyl)aminopentanoyl]amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (18c) To a solution of **12g** (0.53 g, 1.47 mmol), Boc-protected L-norvaline (0.35 g, 1.61 mmol), and HOBt (0.22 g, 1.61 mmol) in DMF (5 ml), EDC·HCl (0.34 g, 1.76 mmol) was added; and the mixture was stirred overnight. To the reaction mixture, 3% K_2CO_3 was added, and the obtained precipitate was washed sequentially with 3% K_2CO_3, 1 N HCl and H_2O. After drying, the crude product was stirred in EtOAc under refluxed conditions, and filtered to give 0.47 g of the title compound. Yield, 57%; mp, 232—234 °C; *Anal.* Calcd for $C_{28}H_{44}N_4O_6S$: C, 59.55; H, 7.85; N, 9.92. Found: C, 59.66; H, 7.78; N, 10.23.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(*tert*-butoxycarbonyl)amino-3-methylbutanoyl]amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (18d) Compound **18d** was prepared from Boc–Val–OH and compound **12g** in a similar manner as described for compound **18c**. Yield, 78%; mp, 235—237 °C; *Anal.* Calcd for $C_{28}H_{44}N_4O_6S$: C, 59.55; H, 7.85; N, 9.92. Found: C, 59.55; H, 7.76; N, 10.22.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(*tert*-butoxycarbonyl)amino-3,3-dimethylbutanoyl]amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (18e) Compound **18e** was prepared from Boc-protected L-*tert*-leucine and compound **12g** in a manner similar to that described for compound **18c**. Yield, 79%; mp, 238—240 °C; *Anal.* Calcd for $C_{29}H_{46}N_4O_6S$: C, 60.18; H, 8.01; N, 9.68. Found: C, 59.80; H, 7.90; N, 9.96.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(*tert*-butoxycarbonyl)amino-3-methylthiopropionyl]amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (18f) Compound **18f** was prepared from Boc–Mta–OH and compound **12g** similarly as described for compound **18c**, except for washing in refluxing MeOH. Yield, 84%; mp, 218—220 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.26 (s, 9H), 1.38 (s, 9H), 1.99 (s, 3H), 2.4—2.7 (m, 4H, overlapped with DMSO), 2.98 (dd, 1H, J=6.5 Hz, 11.6 Hz), 3.29—3.36 (m, 1H, overlapped with H_2O), 4.0—4.2 (m, 2H), 4.4—4.5 (m, 1H), 4.61 (d, 1H, J=9.5 Hz), 4.76 (t, 1H, J=6.8 Hz), 4.94 (d, 1H, J=9.5 Hz), 5.20 (d, 1H, J=6.8 Hz), 6.84 (d, 1H, J=9.5 Hz), 7.13—7.22 (m, 3H), 7.34 (d, 2H, J=6.8 Hz), 7.68 (s, 1H), 7.99 (d, 1H, J=8.4 Hz); *Anal.* Calcd for $C_{27}H_{42}N_4O_6S_2$: C, 55.65; H, 7.26; N, 9.61. Found: C, 55.65; H, 7.17; N, 10.05.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(*tert*-butoxycarbonyl)aminosuccinamyl]amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (18h) Compound **18h** was prepared from Boc–Asn–ONp and compound **12h** in a manner similar to that described for compound **18b**. Yield, 71%; mp, 126—128 °C; *Anal.* Calcd for $C_{29}H_{45}N_5O_7S$ 0.5H2O: C, 56.47; H, 7.52; N, 11.35. Found: C, 56,63; H, 7.56; N, 11.62.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(*tert*-butoxycarbonyl)aminobutanoyl]amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (18i) To a solution of **12h** (0.39 g, 1.0 mmol), Boc-protected (*S*)-2-aminobutyric acid (0.20 g, 1.0 mmol) and HOBt (0.14 g, 1.0 mmol) in DMF (6 ml), EDC·HCl (0.21 g, 1.1 mmol) was added; and the mixture was stirred overnight. To the reaction mixture, EtOAc and 3% K_2CO_3 were added, and then the organic layer was washed sequentially with 3% K_2CO_3, 1 N HCl, and brine, and then dried over $MgSO_4$. After removal of the solvent, the residue was recrystallized from *n*-hexane/EtOAc to give 0.56 g of the title compound. Yield, 96%; mp, 196—198 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.66 (t, 3H, J=7.3 Hz), 1.27 (s, 9H), 1.37 (s, 9H), 1.40 (s, 3H), 1.49 (s, 3H), 2.6—2.8 (m, 2H), 3.7—3.9 (br, 1H), 4.1—4.2 (br, 1H), 4.36—4.38 (m, 1H), 4.50 (s, 1H), 4.87 (d, 1H, J=9.2 Hz), 5.01 (d, 1H,

J=8.6 Hz), 5.12 (d, 1H, J=7.3 Hz), 6.73 (d, 1H, J=8.9 Hz), 7.1—7.3 (m, 3H), 7.35 (d, 2H, J=7.0 Hz), 7.65 (s, 1H), 7.87 (d, 1H, J=8.1 Hz); *Anal.* Calcd for $C_{29}H_{46}N_4O_6S$: C, 60.18; H, 8.01; N, 9.68. Found: C, 60.08; H, 7.96; N, 9.99.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(*tert*-butoxycarbonyl)amino-3-methylbutanoyl]amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (18j) Compound **18j** was prepared from Boc–Val–OH and compound **12h** similarly as described for compound **18i**. Yield, 94%; mp, 190—192 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.55 (d, 3H, J=6.5 Hz), 0.65 (d, 3H, J=6.5 Hz), 1.27 (s, 9H), 1.38 (s, 9H), 1.40 (s, 3H), 1.49 (s, 3H), 2.6—2.8 (m, 2H), 3.6—3.8 (m, 1H), 4.1—4.3 (m, 1H), 4.38 (d, 1H, J=5.7 Hz), 4.52 (s, 1H), 4.87 (d, 1H, J=8.6 Hz), 5.06 (d, 2H, J=7.8 Hz), 6.65 (d, 1H, J=9.2 Hz), 7.1—7.3 (m, 3H), 7.36 (d, 2H, J=7.3 Hz), 7.69 (s, 1H), 7,88 (d, 1H, J=8.1 Hz); *Anal.* Calcd for $C_{30}H_{486}N_4O_6S$: C, 60.78; H, 8.16; N, 9.45. Found: C, 60.96; H, 8.37; N, 9.38.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(*tert*-butoxycarbonyl)amino-3-methylthiopropionyl]amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (18k) To a solution of **12h** (0.79 g, 2.0 mmol), Boc–Mta–OH (0.52 g, 2.2 mmol), and HOBt (0.30 g, 2.2 mmol) in DMF (5 ml), EDC·HCl (0.46 g, 2.4 mmol) was added; and the mixture was stirred overnight. To the reaction mixture, EtOAc and 3% K_2CO_3 were added, and then the organic layer was washed with 3% K_2CO_3, 1 N HCl, and brine in this order, and then dried over $MgSO_4$. After removal of the solvent, the residue was recrystallized from *n*-hexane to give 1.09 g of the title compound. Yield, 90%; mp, 175—177 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.27 (s, 9H), 1.38 (s, 12H), 1.48 (s, 3H), 1.99 (s, 3H), 2.4—2.8 (m, 4H, overlapped with DMSO), 4.0—4.2 (m, 2H), 4.3—4.4 (m, 1H), 4.50 (s, 1H), 4.87 (d, 1H, J=8.6 Hz), 4.98 (d, 1H, J=8.6 Hz), 5.20 (d, 1H, J=7.3 Hz), 6.84 (d, 1H, J=8.9 Hz), 7.1—7.3 (m, 3H), 7.33 (d, 2H, J=6.8 Hz), 7.65 (s, 1H), 7.99 (d, 1H, J=8.4 Hz); *Anal.* Calcd for $C_{29}H_{46}N_4O_6S_2$: C, 57.02; H, 7.59; N, 9.17. Found: C, 57.02; H, 7.57; N, 9.40.

4-Nitrophenyl 1-Naphthylmethylcarbonate (20) To a solution of 1-naphthalenemethanol (**19**, 1.00 g, 6.3 mmol) in pyridine (5 ml), 4-nitrophenyl chloroformate (1.27 g, 6.3 mmol) was added in an ice-bath and stirred for 3 h. After removal of the solvent *in vacuo*, the oily residue was extracted with CH_2Cl_2, washed with 1 N HCl and brine, and dried over $MgSO_4$. The solvent was removed *in vacuo*, and the obtained residue was recrystallized from ethanol to give 1.08 g of the title compound. Yield, 53%; mp, 140—141 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 5.79 (s, 2H), 7.5—7.7 (m, 6H), 8.01 (d, 2H, J=8.4 Hz), 8.18 (d, 1H, J=7.8 Hz), 8.29—8.35 (m, 2H); *Anal.* Calcd for $C_{18}H_{13}NO_5$: C, 66.87; H, 4.05; N, 4.33. Found: C, 67.32; H, 4.22; N, 4.27.

(*S*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-2-hydroxy-3-[(*S*)-2-(1-naphthylmethyloxycarbonyl)aminosuccinamyl]amino-4-phenylbutanoyl]pyrrolidine-2-carboxamide (21) To a solution of **18a** (169 mg, 0.3 mmol) in CH_2Cl_2 (5 ml), 4 N HCl in dioxane (5 ml) was added and stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in DMF followed by neutralization with TEA (42 μl, 0.3 mmol). To this solution, compound **20** (116 mg, 0.36 mmol), HOBt (41 mg, 0.3 mmol) and TEA (50 μl, 0.36 mmol) were added; and the mixture was stirred overnight. To the reaction mixture, EtOAc and 3% K_2CO_3 were added, and then the organic layer was washed sequentially with 3% K_2CO_3, 1 N HCl, and brine, and then dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH), and recrystallized from *n*-hexane/EtOAc to give 31 mg of the title compound. Yield, 16%; mp, 110—112 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.25 (s, 9H), 1.7—1.8 (m, 2H), 1.9—2.1 (m, 2H), 2.2—2.4 (m, 2H), 2.5—2.8 (m, 2H), 3.6—3.8 (br, 2H), 4.0—4.2 (br, 1H), 4.2—4.4 (m, 3H), 4.90 (d, 1H, J=7.3 Hz), 5.48 (s, 1H), 6.8—6.9 (br, 1H), 7.1—7.3 (m, 4H), 7.31—7.34 (m, 3H), 7.4—7.7 (m, 5H), 7.87—8.05 (m, 4H); TOF-MS *m/z*: 647 $(M+H)^+$; *Anal.* Calcd for $C_{35}H_{43}N_5O_7 \cdot 0.5H_2O$: C, 64.20; H, 6.77; N, 10.70. Found: C, 64.08; H, 6.71; N, 10.53.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(1-naphthoxyacetyl)aminosuccinamyl]amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (22a) To a solution of **18b** (116 mg, 0.2 mmol) in CH_2Cl_2 (3 ml), 4 N HCl in dioxane (3 ml) was added; and the mixture was stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in DMF followed by neutralization with TEA (28 μl, 0.2 mmol). To this solution, *l*-naphthoxyacetic acid (40 mg, 0.2 mmol), HOBt (27 mg, 0.2 mmol), and EDC·HCl (42 mg, 0.22 mmol) were added; and the mixture was then stirred overnight. To the reaction mixture, CH_2Cl_2 and 3% K_2CO_3 were added, and then the organic layer was washed with 1 N HCl, then with brine, and dried over $MgSO_4$. After removal of the solvent, the residue was reprecipitated from *n*-hexane/CH_2Cl_2 to give 61 mg of the title compound. Yield, 46%; mp, 112—115 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.25 (s, 9H), 2.50—2.53 (m, 1H, overlapped with DMSO), 2.6—2.7 (br, 2H), 2.98—3.02 (m, 1H), 3.30—3.37 (m, 1H, overlapped with H_2O), 4.1—4.2 (br, 1H), 4.47—4.50 (m, 1H), 4.62—4.67 (m, 4H), 4.76 (t, 1H, J=6.9 Hz), 4.96 (d, 1H, J=9.5 Hz), 5.20 (d, 1H, J=7.0 Hz), 6.9—7.2 (m, 5H), 7.3—7.6 (m, 7H), 7.68 (s, 1H), 7.90 (d, 1H, J=6.8 Hz), 8.04 (d, 1H, J=8.1 Hz), 8.32 (m, 2H); TOF-MS *m/z*: 665 $(M+H)^+$; *Anal.* Calcd for $C_{34}H_{41}N_5O_7S$: C, 61.52; H, 6.23; N, 10.55. Found: C, 61.16; H, 6.33; N, 10.17.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(1-naphthoxyacetyl)aminopentanoyl]amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (22b) Compound **22b** was prepared from 1-naphthoxyacetic acid and compound **18c** in a similar manner as described for compound **22a** (recrystallization from *n*-hexane/EtOAc). Yield, 45%; mp, 162—164 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.81 (t, 3H, J=7.3 Hz), 1.26 (s, 9H), 1.4—1.6 (br, 2H), 2.6—2.7 (br, 2H), 3.00 (dd, 1H, J=11.6 Hz, 6.8 Hz), 3.15—3.37 (m, 1H, overlapped with H_2O), 4.1—4.2 (br, 1H), 4.3—4.4 (m, 1H), 4.46 (d, 1H, J=5.1 Hz), 4.65—4.71 (m, 3H), 4.77 (t, 1H, J=6.9 Hz), 4.99 (d, 1H, J=9.5 Hz), 5.17 (d, 1H, J=6.8 Hz), 6.88 (d, 1H, J=7.3 Hz), 7.10—7.21 (m, 3H), 7.34—7.42 (m, 3H), 7.50—7.55 (m, 3H), 7.69 (s, 1H), 7.81—7.91 (m, 1H), 7.99 (d, 1H, J=8.1 Hz), 8.18—8.24 (m, 2H); TOF-MS *m/z*: 649 $(M+H)^+$; *Anal.* Calcd for $C_{35}H_{44}N_4O_6S$: C, 64.79; H, 6.84; N, 8.64. Found: C, 64.87; H, 6.88; N, 8.55.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(1-naphthoxyacetyl)amino-3-methylbutanoyl]amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (22c) Compound **22c** was prepared from 1-naphthoxyacetic acid and compound **18d** in a manner similar to that described for compound **22a** (recrystallization from n-hexane/EtOAc). Yield, 27%; mp, 151—153 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.75 (t, 6H, J=6.9 Hz), 1.26 (s, 9H), 1.9—2.1 (m, 1H), 2.6—2.7 (br, 2H), 2.95—3.05 (m, 1H), 3.30—3.37 (m, 1H, overlapped with H_2O), 4.1—4.3 (m, 1H), 4.3—4.5 (m, 1H), 4.67—4.81 (m, 4H), 4.99 (d, 1H, J=9.5 Hz), 5.13 (d, 1H, J=6.8 Hz), 6.89 (d, 1H, J=7.8 Hz), 7.11—7.21 (m, 3H), 7.35—7.42 (m, 3H), 7.50—7.56 (m, 3H), 7.70 (s, 1H), 7.82—7.91 (m, 2H), 8.19—8.24 (m, 2H); TOF-MS *m/z*: 649 $(M+H)^+$; *Anal.* Calcd for $C_{35}H_{44}N_4O_6S$: C, 64.79; H, 6.84; N, 8.64. Found: C, 64.57; H, 6.80; N, 8.85.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(1-naphthoxyacetyl)amino-3,3-dimethylbutanoyl]amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (22d) Compound **22d** was prepared from 1-naphthoxyacetic acid and compound **18e** similarly as described for compound **22a** (recrystallization from *n*-hexane/EtOAc). Yield, 26%; mp, 222—225 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.86 (s, 9H), 1.25 (s, 9H), 2.6—2.7 (br, 2H), 2.99 (dd, 1H, J=11.6 Hz, 6.2 Hz), 3.3—3.4 (m, 1H, overlapped with H_2O), 4.1—4.3 (br, 1H), 4.34 (d, 1H, J=9.2 Hz), 4.48—4.50 (m, 1H), 4.66—4.82 (m, 4H), 5.00—5.08 (m, 2H), 6.87 (d, 1H, J=7.8 Hz), 7.07—7.21 (m, 3H), 7.35—7.41 (m, 3H), 7.49—7.56 (m, 3H), 7.71 (d, 2H, J=11.3 Hz), 7.88—7.91 (m, 1H), 8.15—8.22 (m, 2H); TOF-MS *m/z*: 663 $(M+H)^+$; *Anal.* Calcd for $C_{36}H_{46}N_4O_6S$: C, 65.23; H, 6.99; N, 8.45. Found: C, 65.35; H, 7.12; N, 8.22.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(1-naphthoxyacetyl)amino-3-methylthiopropionyl]amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (22e) Compound **22e** was prepared from 1-naphthoxyacetic acid and compound **18f** in a manner similar to that described for compound **22a** (recrystallization from *n*-hexane/EtOAc). Yield, 30%; mp, 176—178 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.26 (s, 9H), 2.04 (s, 3H), 2.6—2.8 (m, 3H), 2.9—3.0 (m, 1H), 3.3—3.4 (m, 1H, overlapped with H_2O), 4.1—4.2 (br, 1H), 4.46—4.50 (m, 1H), 4.5—4.8 (m, 5H), 4.97 (d, 1H, J=9.5 Hz), 5.24 (d, 1H, J=7.3 Hz), 6.93 (d, 1H, J=7.8 Hz), 7.10 (d, 1H, J=6.8 Hz), 7.18 (t, 2H, J=7.2 Hz), 7.32—7.45 (m, 3H), 7.50—7.55 (m, 3H), 7.69 (s, 1H), 7.88—7.91 (m, 1H), 8.14 (d, 1H, J=8.9 Hz), 8.25—8.33 (m, 2H); TOF-MS *m/z*: 667 $(M+H)^+$; *Anal.* Calcd for $C_{34}H_{42}N_4O_6S_2$: C, 61.24; H, 6.35; N, 8.40. Found: C, 61.37; H, 6.39; N, 8.27.

(*S*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(1-naphthoxyacetyl)aminosuccinamyl]amino-2-hydroxy-4-phenylbutanoyl]pyrrolidine-2-carboxamide (23a) To a solution of **18a** (169 mg, 0.3 mmol) in CH_2Cl_2 (3 ml), 4 N HCl in dioxane (3 ml) was added; and the mixture was stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in DMF followed by neutralization with TEA (42 μl, 0.3 mmol). To this solution, 1-naphthoxyacetic acid (61 mg, 0.3 mmol), HOBt (41 mg, 0.3 mmol), and EDC·HCl (63 mg, 0.33 mmol) were added; and the mixture was stirred overnight. To the reaction mixture, CH_2Cl_2 and 3% K_2CO_3 were added, and then the organic layer was washed sequentially with 1 N HCl and brine, and then dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH), and reprecipitated from *n*-hexane/EtOAc to give 80 mg of the title compound. Yield, 41%;

mp, 103—106 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.23 (s, 9H), 1.7—1.9 (br, 2H), 1.9—2.1 (br, 2H), 2.5—2.8 (br, 4H, overlapped with DMSO), 3.5—3.7 (br, 2H), 4.1—4.2 (br, 1H), 4.3—4.4 (br, 2H), 4.6—4.7 (br, 3H), 4.90 (d, 1H, J=7.3 Hz), 6.9—7.2 (m, 5H), 7.30—7.53 (m, 8H), 7.89 (d, 1H, J=7.3 Hz), 7.97 (d, 1H, J=8.6 Hz), 8.34 (d, 1H, J=9.5 Hz), 8.43 (d, 1H, J=7.8 Hz); TOF-MS m/z: 647 $(M+H)^+$; *Anal.* Calcd for $C_{35}H_{43}N_5O_7 \cdot 0.5$EtOAc: C, 64.42; H, 6.87; N, 10.15. Found: C, 64.51; H, 6.95; N, 10.20.

(*S*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(2-naphthoxyacetyl)aminosuccinamyl]amino-2-hydroxy-4-phenylbutanoyl]pyrrolidine-2-carboxamide (23b) Compound **23b** was prepared from 2-naphthoxyacetic acid and compound **18a** in a manner similar to that described for compound **23a**. Yield, 45%; mp, 106—109 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.24 (s, 9H), 1.7—1.9 (m, 2H), 1.9—2.1 (m, 2H), 2.5—2.8 (m, 4H, overlapped with DMSO), 3.5—3.7 (br, 2H), 4.1—4.2 (br, 1H), 4.3—4.4 (br, 2H), 4.59—4.64 (m, 3H), 4.89 (d, 1H, J=7.3 Hz), 6.92 (bs, 1H), 7.08—7.54 (m, 11H), 7.80—7.88 (m, 3H), 7.98 (d, 1H, J=8.1 Hz), 8.26 (d, 1H, J=8.4 Hz); TOF-MS m/z: 647 $(M+H)^+$; *Anal.* Calcd for $C_{35}H_{43}N_5O_7 \cdot H_2O$: C, 63.33; H, 6.83; N, 10.55. Found: C, 63.77; H, 6.73; N, 10.48.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(1-naphthoxyacetyl)aminosuccinamyl]amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (23c) Compound **23c** was prepared from 1-naphthoxyacetic acid and compound **18h** similarly as that described for compound **23a**. Yield, 64%; mp, 115—118 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.26 (s, 9H), 1.40 (s, 3H), 1.49 (s, 3H), 2.5—2.7 (m, 4H, overlapped with DMSO), 4.1—4.2 (br, 1H), 4.46 (dd, 1H, J=7.3 Hz, 2.4 Hz), 4.51 (s, 1H), 4.61—4.68 (m, 3H), 4.88 (d, 1H, J=8.6 Hz), 5.01 (d, 1H, J=8.6 Hz), 5.20 (d, 1H, J=7.3 Hz), 6.92—7.19 (m, 6H), 7.29—7.56 (m, 6H), 7.66 (s, 1H), 7.89 (d, 1H, J=8.6 Hz), 8.03 (d, 1H, J=8.4 Hz), 8.34 (d, 1H, J=7.3 Hz), 8.41 (d, 1H, J=7.8 Hz); TOF-MS m/z: 692 $(M+H)^+$; *Anal.* Calcd for $C_{36}H_{45}N_5O_7S \cdot 0.5H_2O$: C, 61.69; H, 6.62; N, 9.99. Found: C, 61.52; H, 6.60; N, 9.73.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(2-biphenyloxyacetyl)aminosuccinamyl]amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (23d) Compound **23d** was prepared from 2-biphenyloxyacetic acid (**16a**) and compound **18h** similarly as described for compound **23a**. Yield, 33%; mp, 107—109 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.27 (s, 9H), 1.40 (s, 3H), 1.50 (s, 3H), 2.3—2.5 (m, 2H, overlapped with DMSO), 2.5—2.7 (m, 2H), 4.1—4.2 (br, 1H), 4.43—4.59 (m, 5H), 4.87 (d, 1H, J=8.9 Hz), 5.01 (d, 1H, J=8.9 Hz), 5.16 (d, 1H, J=7.6 Hz), 6.92—7.19 (m, 7H), 7.31 (t, 4H, J=7.3 Hz), 7.40 (d, 1H, J=7.3 Hz), 7.55 (d, 1H, J=6.8 Hz), 7.67 (s, 1H), 7.83 (d, 1H, J=7.8 Hz), 8.03 (d, 1H, J=8.4 Hz); TOF-MS m/z: 734 $(M+H)^+$; *Anal.* Calcd for $C_{38}H_{47}N_5O_7S \cdot 0.5H_2O$: C, 62.79; H, 6.66; N, 9.63. Found: C, 62.45; H, 6.59; N, 9.55.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(3-biphenyloxyacetyl)aminosuccinamyl]amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (23e) Compound **23e** was prepared from 3-biphenyloxyacetic acid (**16b**) and compound **18h** similarly as described for compound **23a**. Yield, 62%; mp, 110—112 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.26 (s, 9H), 1.39 (s, 3H), 1.49 (s, 3H), 2.4—2.5 (m, 2H, overlapped with DMSO), 2.6—2.8 (m, 2H), 4.0—4.2 (br, 1H), 4.42—4.68 (m, 5H), 4.87 (d, 1H, J=8.9 Hz), 4.99 (d, 1H, J=8.9 Hz), 5.18 (d, 1H, J=7.3 Hz), 6.90—6.98 (m, 2H), 7.06—7.49 (m, 12H), 7.59—7.69 (m, 3H), 8.02 (d, 1H, J=8.9 Hz), 8.18 (d, 1H, J=8.4 Hz); TOF-MS m/z: 734 $(M+H)^+$; *Anal.* Calcd for $C_{38}H_{47}N_5O_7S \cdot 0.5H_2O$: C, 62.79; H, 6.66; N, 9.63. Found: C, 62.82; H, 6.64; N, 9.46.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(4-biphenyloxyacetyl)aminosuccinamyl]amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (23f) Compound **23f** was prepared from 4-biphenyloxyacetic acid (**16c**) and compound **18h** similarly as described for compound **23a**. Yield, 66%; mp, 114—116 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.27 (s, 9H), 1.40 (s, 3H), 1.49 (s, 3H), 2.4—2.5 (m, 2H, overlapped with DMSO), 2.5—2.7 (m, 2H), 4.0—4.2 (br, 1H), 4.4—4.7 (m, 5H), 4.87 (d, 1H, J=8.9 Hz), 5.00 (d, 1H, J=8.9 Hz), 5.20 (d, 1H, J=7.3 Hz), 6.92 (bs, 1H), 7.04 (d, 2H, J=8.9 Hz), 7.11—7.22 (m, 3H), 7.30—7.32 (m, 4H), 7.43 (t, 2H, J=7.6 Hz), 7.59—7.66 (m, 5H), 8.01 (d, 1H, J=8.4 Hz), 8.19 (d, 1H, J=8.1 Hz); TOF-MS m/z: 734 $(M+H)^+$; *Anal.* Calcd for $C_{38}H_{47}N_5O_7S \cdot 0.5$EtOAc: C, 63.05; H, 6.75; N, 9.19. Found: C, 63.10; H, 6.68; N, 9.25.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(3-phenylaminophenyloxyacetyl)-aminosuccinamyl]amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (23g) Compound **23g** was prepared from 3-(phenylamino)phenoxyacetic acid (**16d**) and compound **18h** in a manner similar to that described for compound **23a**. Yield, 29%; mp, 109—111 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.27 (s, 9H), 1.39 (s, 3H), 1.48 (s, 3H), 2.4—2.5 (m, 2H, overlapped with DMSO), 2.6—2.8 (m, 2H), 4.0—4.2 (br, 1H), 4.40—4.45 (m, 3H), 4.50 (s, 1H), 4.58—4.64 (m, 1H), 4.86 (d, 1H, J=8.9 Hz), 4.99 (d, 1H, J=8.9 Hz), 5.18 (d, 1H, J=6.8 Hz), 6.41 (d, 1H, J=8.1 Hz), 6.68 (d, 2H, J=7.8 Hz), 6.81—6.90 (m, 2H), 7.07—7.31 (m, 11H), 7.67 (s, 1H), 7.98 (d, 1H, J=8.1 Hz), 8.13 (d, 1H, J=8.4 Hz), 8.19 (s, 1H); TOF-MS m/z: 733 $(M+H)^+$; *Anal.* Calcd for $C_{38}H_{48}N_6O_7S \cdot 0.5$EtOAc: C, 61.84; H, 6.75; N, 10.82. Found: C, 61.66; H, 6.70; N, 10.90.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(5-isoquinolinyloxyacetyl)-aminosuccinamyl] amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (23h) To a solution of **18h** (121 mg, 0.2 mmol) in CH_2Cl_2 (3 ml), 4 N HCl in dioxane (3 ml) was added; and the mixture was stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in DMF followed by neutralization with TEA (28 μl, 0.2 mmol). To this solution, 5-isoquinolinyloxyacetic acid (40 mg, 0.2 mmol), HOBt (27 mg, 0.2 mmol), and EDC·HCl (42 mg, 0.22 mmol) were added; and the mixture was stirred overnight. To the reaction mixture, CH_2Cl_2 and 3% K_2CO_3 were added, and then the organic layer was washed with brine, and then dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH), and reprecipitated from *n*-hexane/EtOAc to give 78 mg of the title compound. Yield, 57%; mp, 131—133 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.25 (s, 9H), 1.39 (s, 3H), 1.49 (s, 3H), 2.4—2.5 (m, 2H, overlapped with DMSO), 2.4—2.6 (m, 2H), 4.0—4.2 (br, 1H), 4.46 (dd, 1H, J=7.3 Hz, 2.4 Hz), 4.50 (s, 1H), 4.60—4.71 (m, 3H), 4.87 (d, 1H, J=8.9 Hz), 5.01 (d, 1H, J=8.9 Hz), 5.20 (d, 1H, J=6.8 Hz), 6.99—7.22 (m, 5H), 7.31 (d, 2H, J=7.3 Hz), 7.38 (br, 1H), 7.57—7.74 (m, 3H), 8.02 (d, 1H, J=8.1 Hz), 8.15 (d, 1H, J=5.7 Hz), 8.47 (d, 1H, J=7.8 Hz), 8.52 (d, 1H, J=5.9 Hz), 9.30 (s, 1H); TOF-MS m/z: 693 $(M+H)^+$; *Anal.* Calcd for $C_{35}H_{44}N_6O_7S \cdot H_2O$: C, 59.14; H, 6.52; N, 11.82. Found: C, 59.52; H, 6.45; N, 11.32.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(1-naphthoxyacetyl)amino-3-methylthiopropionyl]amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (24a) Compound **24a** was prepared from 1-naphthoxyacetic acid and compound **18k** similarly as described for compound **22a** (recrystallization from *n*-hexane/EtOAc). Yield, 71%; mp, 113—115 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm); 1.27 (s, 9H), 1.40 (s, 3H), 1.49 (s, 3H), 2.04 (s, 3H), 2.6—2.8 (m, 4H), 4.1—4.3 (br, 1H), 4.45 (dd, 1H, J=7.3 Hz, 2.4 Hz), 4.52 (s, 1H), 4.5—4.7 (m, 1H), 4.89 (d, 1H, J=8.9 Hz), 5.05 (d, 1H, J=8.9 Hz), 5.24 (d, 1H, J=7.3 Hz), 6.93 (d, 1H, J=7.3 Hz), 7.10—7.21 (m, 3H), 7.32—7.42 (m, 3H), 7.50—7.56 (m, 3H), 7.67 (s, 1H), 7.87—7.91 (m, 1H), 8.13 (d, 1H, J=8.1 Hz), 8.24—8.32 (m, 2H); TOF-MS m/z: 695 $(M+H)^+$; *Anal.* Calcd for $C_{36}H_{46}N_4O_6S_2$: C, 62.22; H, 6.67; N, 8.06. Found: C, 61.97; H, 6.64; N, 8.03.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(3-phenylaminophenoxyacetyl)-amino-3-methylthiopropionyl]amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (24b) Compound **24a** was prepared from **16d** and compound **18k** in a manner similar to that described for compound **22a** (reprecipitation from *n*-hexane/CH_2Cl_2). Yield, 82%; mp, 103—105 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.27 (s, 9H), 1.40 (s, 3H), 1.49 (s, 3H), 2.01 (s, 3H), 2.59—2.80 (m, 4H), 4.0—4.2 (br, 1H), 4.41—4.53 (m, 5H), 4.87 (d, 1H, J=8.9 Hz), 5.02 (d, 1H, J=8.9 Hz), 5.21 (d, 1H, J=7.3 Hz), 6.41 (d, 1H, J=9.2 Hz), 6.67 (d, 2H, J=7.6 Hz), 6.83 (t, 1H, J=7.4 Hz), 7.07—7.26 (m, 6H), 7.32 (d, 2H, J=6.8 Hz), 7.67 (s, 1H), 7.94 (d, 1H, J=8.6 Hz), 8.18 (s, 1H), 8.26 (d, 1H, J=8.9 Hz); TOF-MS m/z: 736 $(M+H)^+$; *Anal.* Calcd for $C_{38}H_{49}N_5O_6S_2$: C, 62.02; H, 6.71; N, 9.52. Found: C, 62.32; H, 7.07; N, 9.19.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(5-isoquinolinyloxyacetyl)amino-3-methylthiopropionyl]amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (24c) Compound **24c** was prepared from **16e** and compound **18k** similarly as described for compound **22a** (recrystallization from *n*-hexane/ethanol). Yield, 77%; mp, 107—109 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.27 (s, 9H), 1.40 (s, 3H), 1.49 (s, 3H), 2.04 (s, 3H), 2.6—2.9 (m, 4H), 4.1—4.3 (br, 1H), 4.45 (dd, 1H, J=7.3 Hz, 3.0 Hz), 4.52 (s, 1H), 4.5—4.6 (m, 1H), 4.76 (s, 2H), 4.89 (d, 1H, J=8.9 Hz), 5.04 (d, 1H, J=8.9 Hz), 5.24 (d, 1H, J=7.6 Hz), 7.09—7.21 (m, 4H), 7.33 (d, 2H, J=7.3 Hz), 7.57 (t, 1H, J=8.1 Hz), 7.69 (t, 1H, J=7.8 Hz), 8.06 (d, 1H, J=5.9 Hz), 8.21 (d, 1H, J=8.4 Hz), 8.30 (d, 1H, J=8.4 Hz), 8.54 (d, 1H, J=5.9 Hz), 9.29 (s, 1H); TOF-MS m/z: 696 $(M+H)^+$; *Anal.* Calcd for $C_{35}H_{45}N_5O_6S_2$: C, 60.41; H, 6.52; N, 10.06. Found: C, 59.93; H, 6.53; N, 10.31.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-3-[(*S*)-2-(5-isoquinolinyloxyacetyl)amino-3-methylthiopropionyl]amino-2-hydroxy-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (24d) To a solution of **18f** (600 mg, 1.03 mmol) in CH_2Cl_2 (5 ml), 4 N HCl in dioxane (5 ml) was added; and the mixture was then stirred for 2 h. The reaction mixture was concentrated, and then

the residue was dissolved in DMF followed by neutralization with TEA (143 μl, 1.03 mmol). To this solution, 5-isoquinolinyloxyacetic acid (**16e**, 210 mg, 1.03 mmol), HOBt (139 mg, 1.03 mmol), and EDC·HCl (236 mg, 1.24 mmol) were added; and the mixture was stirred overnight. To the reaction mixture, CH_2Cl_2 and 3% K_2CO_3 were added, and then the organic layer was washed with 3% K_2CO_3 followed by brine, and dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH), and recrystallization from ethanol gave 510 mg of the title compound. Yield, 74%; mp 175 °C,[38] ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.27 (s, 9H), 2.06 (s, 3H), 2.68—2.76 (m, 3H), 2.87 (dd, 1H, J=13.5 Hz, 4.9 Hz), 3.02 (dd, 1H, J=11.9 Hz, 6.5 Hz), 3.32—3.40 (m, 1H, overlapped with H_2O), 4.1—4.3 (br, 1H), 4.52 (dd, 1H, J=7.0 Hz, 2.4 Hz), 4.58—4.84 (m, 4H), 5.00 (d, 1H, J=9.5 Hz), 5.27 (d, 1H, J=7.3 Hz), 7.11—7.23 (m, 3H), 7.37 (d, 1H, J=7.6 Hz), 7.58 (t, 1H, J=8.0 Hz), 7.71—7.73 (m, 2H), 8.09 (d, 1H, J=5.9 Hz), 8.24 (d, 1H, J=8.4 Hz), 8.34 (d, 1H, J=7.8 Hz), 8.56 (d, 1H, J=5.4 Hz), 9.31 (s, 1H); TOF-MS *m/z*: 668 $(M+H)^+$; *Anal.* Calcd for $C_{33}H_{41}N_5O_6S_2\cdot 0.5H_2O$: C, 58.56; H, 6.25; N, 10.35. Found: C, 58.55; H, 6.23; N, 10.06.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-2-hydroxy-3-[(*S*)-2-(5-isoquinolinyloxyacetyl)amino-3-methylbutanoyl]amino-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (24e) Compound **24e** was prepared from **16e** and compound **18d** in a manner similar to that described for compound **22a** (recrystallization from *n*-hexane/EtOAc). Yield, 72%; mp, 172—173 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.73—0.78 (m, 6H), 1.26 (s, 9H), 1.9—2.1 (m, 1H), 2.6—2.7 (m, 2H), 2.95—3.05 (m, 1H), 3.30—3.34 (m, 1H, overlapped with H_2O), 4.1—4.3 (m, 2H), 4.4—4.5 (m, 1H), 4.67—4.81 (m, 4H), 4.99 (d, 1H, J=9.2 Hz), 5.14 (d, 1H, J=7.3 Hz), 7.11—7.22 (m, 4H), 7.36 (d, 2H, J=9.0 Hz), 7.57 (t, 1H, J=7.6 Hz), 7.69—7.72 (m, 2H), 7.93 (d, 1H, J=8.6 Hz), 8.00 (d, 1H, J=5.7 Hz), 8.22 (d, 1H, J=8.4 Hz), 8.54 (d, 1H, J=5.9 Hz), 9.29 (s, 1H); TOF-MS *m/z*: 650 $(M+H)^+$; *Anal.* Calcd for $C_{34}H_{43}N_5O_6S$: C, 62.84; H, 6.67; N, 10.78. Found: C, 63.05; H, 6.76; N, 10.76.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-2-hydroxy-3-[(*S*)-2-(5-isoquinolinyloxyacetyl)aminobutanoyl]amino-4-phenylbutanoyl]-1,3-thiazolidine-4-carboxamide (24f) To a solution of **12g** (730 mg, 2.0 mmol), Boc-protected (*S*)-2-aminobutyric acid (406 mg, 2.0 mmol), and HOBt (270 mg, 2.0 mmol) in DMF (5 ml), EDC·HCl (420 mg, 2.2 mmol) was added; and the mixture was stirred overnight. To the reaction mixture, 3% K_2CO_3 was added; and the obtained precipitate was washed sequentially with 3% K_2CO_3, 1 N HCl and H_2O. After drying, the crude product was stirred in 50% aqueous MeOH under refluxing conditions, and filtered to give 760 mg of **18g** (69%). To the solution of **18g** (165 mg, 0.30 mmol) in CH_2Cl_2 (3 ml), 4 N HCl in dioxane (3 ml) was added; and the mixture was stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in DMF followed by neutralization with TEA (50 μl, 0.30 mmol). To this solution, **16e** (61 mg, 0.30 mmol), HOBt (41 mg, 0.30 mmol), and EDC·HCl (63 mg, 0.33 mmol) were added; and the mixture was stirred overnight. To the reaction mixture, CH_2Cl_2 and 3% K_2CO_3 were added, and then the organic layer was washed with 3% K_2CO_3 and then with brine, and dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH), and reprecipitated from *n*-hexane/CH_2Cl_2 to give 110 mg of the title compound. Yield (total), 40%; mp, 152—154 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.77 (t, 3H, J=7.4 Hz), 1.26 (s, 9H), 1.4—1.7 (m, 2H), 2.6—2.7 (m, 2H), 3.00 (dd, 1H, J=6.5 Hz, 11.9 Hz), 3.30—3.37 (m, 1H, overlapped with H_2O), 4.1—4.2 (m, 1H), 4.2—4.3 (m, 1H), 4.4—4.5 (m, 1H), 4.6—4.8 (m, 4H), 4.99 (d, 1H, J=9.2 Hz), 5.19 (d, 1H, J=7.6 Hz), 7.11—7.22 (m, 4H), 7.35 (d, 2H, J=7.0 Hz), 7.57 (t, 1H, J=8.1 Hz), 7.71 (d, 2H, J=7.3 Hz), 8.03 (t, 2H, J=8.1 Hz), 8.21 (d, 1H, J=8.1 Hz), 8.54 (d, 1H, J=5.9 Hz), 9.29 (s, 1H); TOF-MS *m/z*: 636 $(M+H)^+$; *Anal.* Calcd for $C_{33}H_{41}N_5O_6S$: C, 62.34; H, 6.50; N, 11.02. Found: C, 62.09; H, 6.51; N, 10.82.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-2-hydroxy-3-[(*S*)-2-(3-dimethylaminophenoxyacetyl)amino-3-methylbutanoyl]amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (24g) To a solution of **18j** (1.18 g, 2.0 mmol) in CH_2Cl_2 (10 ml), 4 N HCl in dioxane (10 ml) was added; and the mixture was stirred for 2 h. The reaction mixture was concentrated, and then the residue was dissolved in DMF (8.0 ml) followed by neutralization with TEA (278 μl, 2.0 mmol). To this solution, **17** (748 mg, 2.1 mmol) and TEA (278 μl, 2.0 mmol) were added; and the mixture was stirred overnight. To the reaction mixture, EtOAc and 3% K_2CO_3 were added, and then the organic layer was washed with 3% K_2CO_3 followed with brine, and then dried over $MgSO_4$. After removal of the solvent, the residue was purified by silica gel column chromatography (CH_2Cl_2–MeOH) to give 1.13 g of the title compound. Yield, 84%; mp, 98—100 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.70 (d, 6H, J=6.8 Hz), 1.27 (s, 9H), 1.40 (s, 3H), 1.49 (s, 3H), 1.88—1.95 (m, 1H), 2.5—2.8 (m, 2H), 2.85 (s, 6H), 4.13—4.24 (m, 2H), 4.4—4.7 (m, 4H), 4.89 (d, 1H, J=8.9 Hz), 5.06—5.11 (m, 2H), 6.20—6.23 (m, 2H), 6.31—6.35 (m, 1H), 7.0—7.2 (m, 4H), 7.35 (d, 2H, J=6.8 Hz), 7.58—7.67 (m, 2H), 8.21 (d, 1H, J=8.4 Hz); TOF-MS *m/z*: 670 $(M+H)^+$; *Anal.* Calcd for $C_{35}H_{51}N_5O_6S$: C, 62.75; H, 7.67; N, 10.45. Found: C, 62.66; H, 7.73; N, 10.35.

(*R*)-*N*-*tert*-Butyl-3-[(2*S*,3*S*)-2-hydroxy-3-[(*S*)-2-(3-dimethylaminophenoxyacetyl)aminobutanoyl]amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (24h) Compound **24h** was prepared from **18i** in a manner similar to that described for compound **24g**. Yield, 50%; mp, 93—96 °C; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.73 (t, 3H, J=7.3 Hz), 1.27 (s, 9H), 1.40 (s, 3H), 1.49 (s, 3H), 1.3—1.7 (m, 2H), 2.5—2.8 (m, 2H), 2.85 (s, 6H), 4.1—4.3 (m, 2H), 4.4—4.7 (m, 4H), 4.89 (d, 1H, J=8.9 Hz), 5.04 (d, 1H, J=8.9 Hz), 5.16 (d, 1H, J=7.6 Hz), 6.20—6.23 (m, 2H), 6.32—6.35 (m, 1H), 7.0—7.2 (m, 4H), 7.34 (d, 2H, J=7.3 Hz), 7.65—7.75 (m, 2H), 8.19 (d, 1H, J=8.1 Hz); TOF-MS *m/z*: 656 $(M+H)^+$; *Anal.* Calcd for $C_{34}H_{49}N_5O_6S\cdot 0.5H_2O$: C, 61.42; H, 7.58; N, 10.53. Found: C, 61.49; H, 7.46; N, 10.56.

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