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Small-Sized Human Immunodeficiency Virus Type-1 Protease Inhibitors Containing Allophenylnorstatine to Explore the S_2' Pocket[‖,⊥]

Koushi Hidaka,[†] Tooru Kimura,[†] Hamdy M. Abdel-Rahman,[†] Jeffrey-Tri Nguyen,[†] Keith F. McDaniel,[‡] William E. Kohlbrenner,[‡] Akhteruzzaman Molla,[‡] Motoyasu Adachi,[§] Taro Tamada,[§] Ryota Kuroki,[§] Noriko Katsuki,[†] Yoshiaki Tanaka,[†] Hikaru Matsumoto,[†] Jun Wang,[†] Yoshio Hayashi,[†] Dale J. Kempf,[‡] and Yoshiaki Kiso*[,†]

[†]*Department of Medicinal Chemistry, Center for Frontier Research in Medicinal Science, 21st Century COE Program, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8412, Japan,* [‡]*Global Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, Illinois 60064, and* [§]*Molecular Structural Biology Group, Quantum Beam Science Directorate, Japan Atomic Energy Agency, Tokai, Ibaraki, 319-1195, Japan*

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A series of HIV protease inhibitor based on the allophenylnorstatine structure with various P_2' moieties were synthesized. Among these analogues, we discovered that a small allyl group would maintain potent enzyme inhibitory activity compared to the *o*-methylbenzyl moiety in clinical candidate **1** (KNI-764, also known as JE-2147, AG-1776, or SM-319777). Introduction of an anilinic amino group to **2** (KNI-727) improved water-solubility and anti-HIV-1 activity. X-ray crystallographic analysis of **13k** (KNI-1689) with a β-methallyl group at P_2' position revealed hydrophobic interactions with Ala28, Ile84, and Ile50′ similar to that of **1**. The presence of an additional methyl group on the allyl group in compound **13k** significantly increased anti-HIV activity over **1** while providing a rational drug design for structural minimization and improving membrane permeability.

Introduction

Improvements in antiretroviral therapy have changed the fatal human immunodeficiency virus type-1 (HIV-1[a]) infection to a manageable chronic illness.[1] Highly active antiretroviral therapy (HAART) using more than three drugs ranging from HIV reverse transcriptase to protease inhibitors brought about this remarkable breakthrough. Among this effective concoction, HIV protease inhibitors are so essential for HAART that the FDA has approved 10 different protease inhibitors. Recent studies on HIV protease inhibitors including FDA-approved tipranavir and darunavir focused on drug-resistant HIV.[2,3] However, clinically used HIV protease inhibitors have been associated with the emergence of drug resistant viruses,[4] unfavorable side effects, and long-term high dose requirements. These concerns urged us to rekindle our effort to develop a larger variety of HIV protease inhibitors.

A substrate transition-state mimic that possesses one or two hydroxyl groups is introduced in each HIV protease inhibitor to bind with the two catalytic aspartic acids of the enzyme. Fundamentally, this binding inhibits the processing of the gag and gag-pol polyproteins that are essential for propagation of the infectious virion. On the basis of the transition-state mimic concept, we previously reported a series of potent peptidomimetic HIV protease inhibitors containing allophenylnorstatine[5] [Apns: (2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid] with a hydroxymethylcarbonyl (HMC) isostere. Interactions between the HMC isostere and HIV-1 protease have been proven by X-ray crystallography, NMR, and neutron crystallography analyses to reveal hydrogen bonding interactions between the HMC's hydroxyl and carbonyl groups with the corresponding carboxylic/carboxylate groups of the two catalytic aspartic acids.[6−8] This ideal transition-state mimic forms the basis for the development of highly potent small HIV protease inhibitors, such as **1** (KNI-764, also known as JE-2147, AG-1776, or SM-319777)[9,10] and **2** (KNI-727)[5c,11] shown in Figure 1. The potency of these compounds resides in a conformationally constrained P_1-P_1' mimetic moiety, Apns-Dmt [Dmt: (*R*)-5,5-dimethylthiazolidine-4-carboxylic acid]. Compound **1** has been shown to be effective against mutant proteases that are resistant to clinically available HIV protease inhibitors.[12] Mimoto and co-workers reported further modifications from **1** to prevent glucuronidation of the P_2 phenolic hydroxyl group to improve the pharmacokinetic profile.[13] Compound **1** was designed with an *o*-methylbenzyl structure at the P_2' position to accommodate for the symmetric character of the HIV protease.[10] The flexibility of this benzyl moiety is thought to compensate for the binding energy loss found in drug resistant protease mutants.[14] Herein, we focused on the P_2' *o*-methylbenzyl group of **1**, mainly

*To whom correspondence should be addressed. Phone: +81-75-595-4635. Fax: +81-75-591-9900. E-mail: kiso@mb.kyoto-phu.ac.jp.

‖ The atomic coordinates have been deposited in the Protein Data Bank, www.rscb.org (PDB code 3A2O).

⊥ On the 100th Anniversary of the Division of Medicinal Chemistry, we present this work in this commemorative MEDI Centennial issue.

[a] Abbreviations: HIV, human immunodeficiency virus; AIDS, acquired immune deficiency syndrome; HAART, highly active antiretroviral therapy; Apns, allophenylnorstatine; HMC, hydroxymethylcarbonyl; Dmt, (*R*)-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid; SAR, structure−activity relationship; Boc, *tert*-butyloxycarbonyl; BOP, benzotriazole-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; EDC, 1-ethyl-3-(3,3-dimethylaminopropyl)carbodiimide; HOBt, 1-hydroxybenzotriazole; QSAR, quantitative structure−activity relationship.

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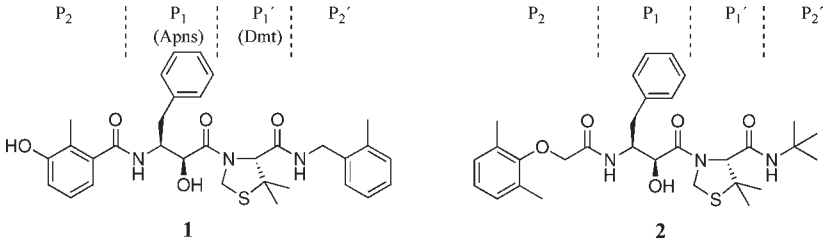


Figure 1. Structures for **1** and **2**.

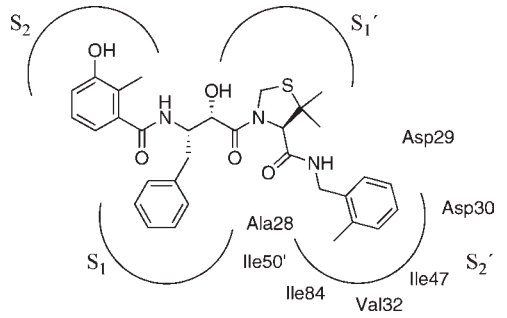


Figure 2. Schematic representation of the residues surrounding the 2-methylbenzyl group of **1**.

because P_2' optimization is limited to a small number of moieties and substrate specificity at the S_2' pocket of the enzyme is relatively low. In the present study, structure−activity relationships at the P_2' position of allophenylnorstatine-containing inhibitors were explored by introducing various moieties to the structure of **1**. To confirm our observations, similar P_2' modifications along with some P_2 improvements were applied to another reference inhibitor **2**.

Strategy behind Structure−Activity Relationship Studies

In recent years, five complexes of **1** with a wild or mutant type HIV-1 protease have been disclosed.[14,15] These complexes revealed hydrophobic interactions between the *o*-methylbenzyl moiety of **1** and S_2' pocket of the enzyme. The PDB data (entries 1MSM and 1KZK) show proximity between the methyl group of the *o*-methylbenzyl moiety and the side chains of Ala28, Ile84, and Ile50′ in a wild type protease, while the phenyl group occupies the S_2' space (Figure 2). In our previous structure−activity relationship (SAR) study at the P_2' position, ortho-methyl substitution on the benzyl structure was favored for inhibitory activity over meta- or para-substitution.[10] Herein, while keeping in mind the importance of *o*-methyl group interactions, we introduced variants of the benzyl structure. We also incorporated various P_2' non-benzyl structures to form van der Waals contacts with the aforementioned key residues of the enzyme.

Although **2** was reported as a potent dipeptide-type HIV-1 protease inhibitor with low anti-HIV activity,[5c] we speculated that the disappointing results in cell-based assays were due to the compound's high hydrophobic nature. In order to improve its hydrophilicity and form hydrogen bonding interactions with the carboxylic acid side chain found in Asp30 of the enzyme, we introduced an additional para-amino group to the aromatic P_2 structure of **2** to generate a 4-amino-2,6-dimethylphenoxyacetyl moiety, which was also used for a malarial plasmepsin inhibitor study.[16] Effective P_2' moieties with potent HIV protease inhibition among the derivatives of **1** were selected and applied to this combination.

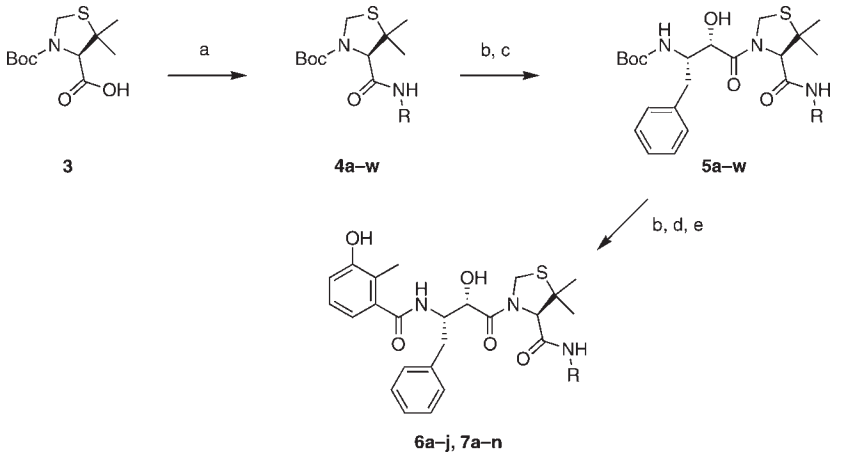
Chemistry

Introduction of various amines to the P_2' position of **1** was performed as shown in Scheme 1. Commercially available amines were reacted with Boc-Dmt-OH (**3**) using BOP as coupling reagent to afford intermediate **4**. 2,6-Dichlorobenzylamine, 2-chloro-6-fluorobenzylamine, 3-hydroxy-2-methylbenzylamine, 3-fluoro-2-methylbenzylamine, and *cis*-4-hydroxy-2-butenylamine were separately synthesized from their corresponding substituted benzoic acids, benzyl bromide, and allyl chloride. After deprotection of the Boc group with HCl, Boc-Apns-OH was coupled by the EDC−HOBt method (compound **5**). Subsequent deprotection of the Boc group and BOP coupling with 3-acetoxy-2-methylbenzoic acid, followed by hydrolysis of the acetyl group, afforded the desired compounds (**6a**−**j**, **7a**−**n**). The synthesis of N-protected 4-amino-2,6-dimethylphenoxyacetic acid and its derivatives is shown in Scheme 2. 4-Nitro-2,6-dimethylphenol was alkylated with ethyl bromoacetate and reduced to an amino group to afford intermediate **8**, followed by protection with $(Boc)_2O$ to give intermediate **9**. 4-(Boc-amino)-2,6-dimethylphenoxyacetic acid **10** was obtained by saponification of intermediate **9**. A portion of intermediate **9** was deprotonated by sodium hydride and methylated with iodomethane and simultaneously hydrolyzed to form a monomethylated analogue **11**. The amino group of intermediate **8** was fully methylated by reductive alkylation using formaldehyde and subsequently hydrolyzed to give dimethylated analogue **12**. Scheme 3 describes the synthesis of **2** derivatives starting from the set of intermediates **5**. For each intermediate found in compound set **5**, the Boc moiety was removed. Then 4-(Boc)amino-2,6-dimethylphenoxyacetic acid **10** was coupled using BOP, followed by a final deprotection to give target compounds **13a**, **13b, and 13e**−**l**. In the case of compounds with a P_2' *o*-methylbenzyl group, a monomethylated amino analogue **11** was introduced using BOP to give compound **13c**, while the dimethylated amino analogue **13d** was synthesized using **12** without a final deprotection.

Results and Discussion

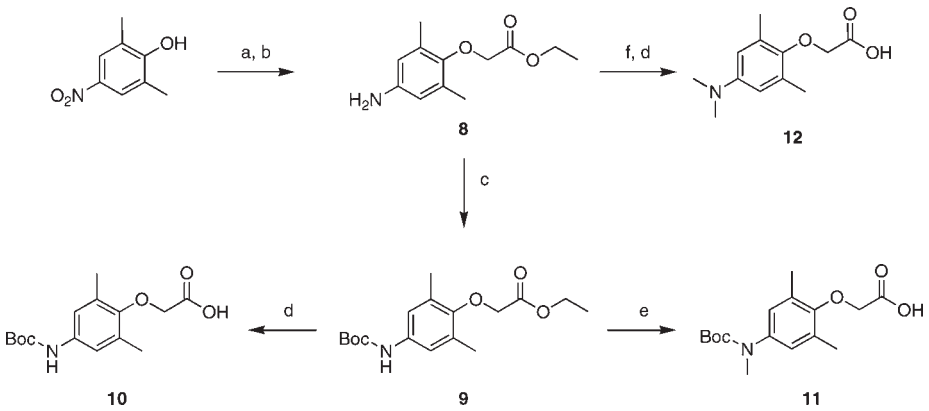
HIV-1 protease inhibitory activity of the synthesized compounds was evaluated. Table 1 summarizes the enzymatic assay results from compounds possessing a P_2' benzyl structure as determined at 50 and 1 nM of the inhibitor. A log linear correlation was observed between the results obtained at 50 and 1 nM ($r^2 = 0.93$). This well-fitted log linear correlation indicates that inhibitory data determined at 50 nM is almost as reliable as those obtained at 1 nM. Substitution at the ortho-position on the phenyl ring afforded compounds **6a**−**g** that effectively exhibited high inhibitory activity (>90% at 50 nM). Compounds with a double substitution on both ortho positions (**6a**−**e**) exhibited potency similar to that of **1** at 50 nM. The most potent activity was exhibited by double

Scheme 1[a]



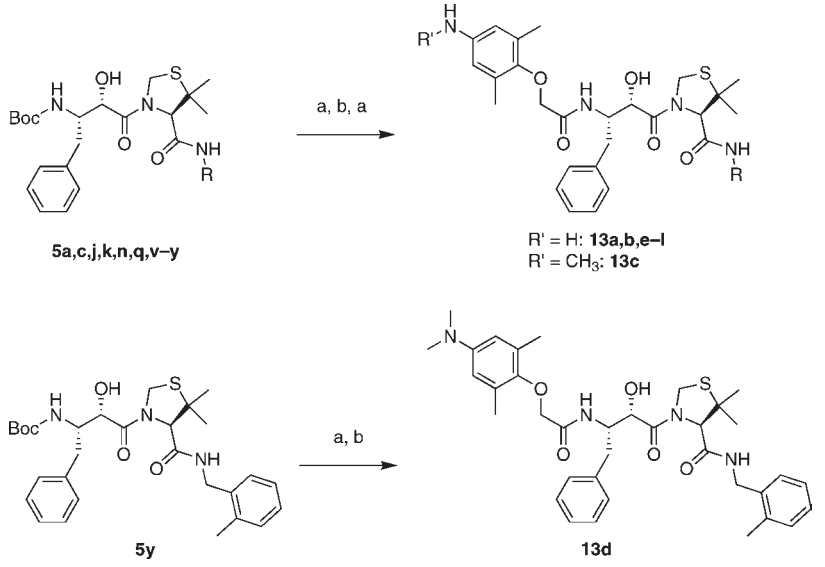
[a] Reagents: (a) amine (R-NH_2), BOP, Et_3N DMF; (b) 4 N HCl/dioxane, anisole; (c) Boc-Apns-OH, EDC, HOBt, Et_3N, DMF; (d) 3-acetoxy-2-methylbenzoic acid, BOP, Et_3N, DMF; (e) 1 N aqueous NaOH, MeOH.

Scheme 2[a]



[a] Reagents: (a) $BrCH_2CO_2Et$, K_2CO_3 DMF; (b) 10% Pd−C, MeOH, H_2; (c) $(Boc)_2O$, THF−H_2O; (d) 1 N aqueous NaOH, MeOH; (e) NaH, CH_3I, THF; (f) HCHO, 10% Pd−C, THF, H_2.

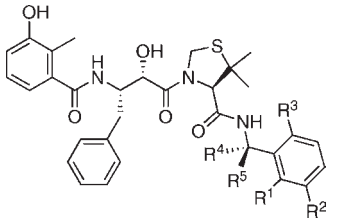
Scheme 3[a]



[a] Reagents: (a) 4 N HCl/dioxane, anisole; (b) **10**, **11**, or **12**, BOP, Et_3N, DMF.

methyl-substituted **6a** (82% at 1 nM, K_i = 2.4 pM). The presence of aromatic halogen atoms reduced inhibitory activity (**6b**−**e**) in the general order of methyl > chlorine > fluorine, down to 24% inhibition at 1 nM for the case of

Table 1. HIV Protease Inhibitory and Anti-HIV Activity of P_2'-Benzyl Derivatives[a]



compd	R^1	R^2	R^3	R^4	R^5	HIV-1 PR % inhibition at 50 nM	HIV-1 PR % inhibition at 1 nM	anti-HIV-1_{IIIB} EC_{50} (μM)
1	CH_3	H	H	H	H	99[b]	72	0.033
6a (KNI-814)	CH_3	H	CH_3	H	H	99[c]	82	nd
6b (KNI-1526)	CH_3	H	Cl	H	H	99	77	0.032
6c (KNI-1365)	Cl	H	Cl	H	H	98	66	0.039
6d (KNI-1405)	Cl	H	F	H	H	97	50	0.032
6e (KNI-1367)	F	H	F	H	H	91	24	0.051
6f (KNI-1098)	CH_3	OH	H	H	H	95	41	0.120
6g (KNI-1366)	CH_3	F	H	H	H	97	53	0.056
6h (KNI-1352)	H	H	H	CH_3	H	95	31	0.079
6i (KNI-1174)	H	H	H	CH_3	CH_3	82[d]	nd	0.123
6j (KNI-1359)	H	H	H	H	CH_3	52	nd	0.617

[a] nd: not determined. [b] K_i = 0.031 nM. [c] K_i = 0.0024 nM. [d] Reference 17.

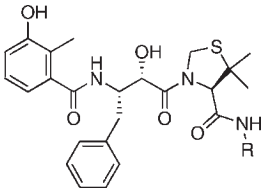
difluoro-substituted **6d**. A hydroxyl moiety was introduced at the meta-position (R^2 group) in consideration of the symmetric nature of the protease (cf. the P_2 and P_2' residues) to afford inhibitor **6f**. However, compound **6f** exhibited lower inhibitory activity than **1**, which does not possess a P_2' *m*-hydroxyl moiety. This result suggests a disruption of interactions between the *o*-methyl group and the side chains of Ala28, Ile84, and Ile50′. Compound **6g** possessing a *m*-fluorine showed a lessened disruption of interactions. From the crystal structure of **1** and HIV-1 protease, the R^4 group is believed to be near the R^1 *o*-methyl group. As expected, compound **6h** maintained inhibitory activity because the R^4 group could alternate for the R^1 group. On the other hand, the R^5 group is located in proximity to the amide group of Gly49. The presence of an R^5 group would essentially interfere with the enzyme's flap region. To no big surprise, compounds **6i** and **6j** possessing a methyl R^5 exhibited relatively reduced potencies (cf. **6h**−**j**). These results brought to light the importance of side chain interactions with Ala28, Ile84, and Ile50′. Inhibitory activity against wild-type HIV-1 of these compounds was examined. Among them, only three compounds, **6b**−**d**, possessed potencies similar to that of **1**.

Table 2 depicts the inhibitory activity of compounds possessing a non-benzyl structure at the P_2' position. The presence of a chiral indane (**7a** and **7b**) instead of *o*-methylbenzyl structure (**1**) maintained potent activity against the enzyme. This result indicates tolerable conformational constraints of the *o*-methylbenzyl structure. Chiral compound **7a** exhibited slightly higher activity than the compound with opposite chirality (**7b**). An additional hydroxyl group on the indane structure attenuated potency (**7c** and **7d**). The stereochemistry on the indanol compound possessing higher potency (**7d**) is consistent with that of indinavir.[18] The crystal structure of a complex of indinavir and HIV protease revealed direct interactions between the indanolphenyl ring and side chains of Ala28, Ile84, and Ile50′, thereby pushing the hydroxyl group toward Asp30. In the case of compound **7d**, we assume that the interactions of the P_2' moiety are similar to that of indinavir. Cycloalkyl groups (**7e**−**h**) were introduced to fill the S_2' space while maintaining interactions with the enzyme's hydrophobic residues. Gradually decreasing the ring size from a seven- to four-membered cyloalkyl ring, we observed that the five-membered ring compound (**7g**) is most likely to have the better fit in the S_2' space. The relatively small size of the five-membered ring compared to that of the benzyl structure motivated us to focus on downsizing the P_2' group. The S_2' pocket seems to prefer an *n*-propyl moiety (**7j**) over an ethyl group (**7i**). Compound **7k** possessing an allyl group instead of an *n*-propyl group (**7j**) exhibited increased inhibitory activity (93% vs 88% at 50 nM, respectively), while a triple bond slightly decreased activity (**7l**, 90%). We presume that a more constrained conformation with a double bond would promote hydrophobic contacts with the side chains of Ala28, Ile84, and Ile50′. An additional methyl group on the allyl moiety intensified potency to 98% (**7m**). Interestingly, compound **7m** with a β-methallyl moiety also exhibited strong antiviral potency against HIV-1 (EC_{50} = 17 nM, using wild-type pNL4-3) over that of **1** (EC_{50} = 60 nM, using wild-type pNL4-3). These results confirmed the effectiveness of small ligands on inhibitory activities against HIV protease and the virus. Compound **7n** with an additional hydroxymethyl instead of an allyl group (**7k**) possessed equipotent enzymatic inhibitory activity. Anti-HIV activity of inhibitor **7n** was 10-fold less effective than inhibitor **7k**. This difference suggests that the hydroxymethyl moiety is detrimental to the delivery of the inhibitor to the HIV protease target.

After completing our work on the **1** derivatives,[19] we were made aware that some compounds (**6g**−**j** and **7a**,**b**,**e**−**h**,**k**−**m**) have been synthesized via different routes, assayed, and declared as patented inventions by Agouron Pharmaceuticals, Inc. using **1** as reference compound.[20] Consequently, although reached by convergent research, we are not claiming the aforementioned compounds to be novel. However, we believe that the results from our SAR studies to be of interest.

Although **2** was also reported to exhibit potent inhibition against HIV protease, it exhibited low activity against HIV (Table 3).[11] We assume that its excessive hydrophobic character reduces its membrane permeability. Not only to improve its hydrophilic profile but also to induce additional

Table 2. HIV Protease Inhibitory and Anti-HIV Activity of P_2' Derivatives



Compound	R	HIV-1 PR % inhibition at 50 nM	Anti-HIV-1_{IIIB} EC_{50} (μM)	Compound	R	HIV-1 PR % inhibition at 50 nM	Anti-HIV-1_{IIIB} EC_{50} (μM)
7a (KNI-1267)		97	0.073	**7h** (KNI-1370)		65	0.119
7b (KNI-1271)		91	0.353	**7i** (KNI-1465)		64	0.182
7c (KNI-1243)		66	0.550	**7j** (KNI-1459)		88	0.091
7d (KNI-1241)		92	0.100	**7k** (KNI-1317)		93	0.123
7e (KNI-1291)		76	0.332	**7l** (KNI-1357)		90	0.110
7f (KNI-1128)		86	0.207	**7m** (KNI-1614)		98[a]	0.017[b]
7g (KNI-1249)		94	0.125	**7n** (KNI-1403)		94	1.20

[a] K_i = 0.19 nM. [b] pNL4-3 strain.

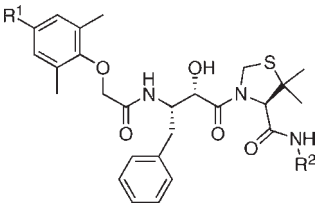
hydrogen bonding interactions with Asp30′, a *p*-amino group was inserted on the P_2 ligand. The resultant compound **13a** exhibited equipotent enzymatic inhibitory activity as **2**, 89-fold increase in water solubility, and 10-fold improvement in anti-HIV activity. This improvement in anti-HIV activity using a *p*-amino group was more effective than our previously reported examples using pseudosymmetric compounds.[21] Compound **13b** possessing an *o*-methylbenzyl group at the P_2' position, not unlike **1**, exhibited enhanced activity against both the enzyme and virus. Additional methylation of the R^1 amino group (**13c** and **13d**) failed to improve potency. Consequently, in order to improve inhibitory profiles of the compound, we only focused on the free R^1 amino group for further modifications. Eight other P_2' ligands were selected for combinatorial study. As expected, the HIV-1 protease inhibitory activity profiles for compounds **13e**−**l** were greater than 90% at 50 nM. Although most derivatives exhibited equal or slightly lower potencies, only the indanol derivative (**13h**) exhibited higher enzyme inhibition than the corresponding **1** analogue **7d**. We observed a drastic decrease in anti-HIV activity as a result of an additional hydroxymethyl group to the allyl structure (cf. **13j** and **13l**). This result is consistent in derivatives of **1** (cf. **7k** and **7n**). However, an additional methyl group, i.e., *β*-methallyl, was significantly effective against anti-HIV activity (**13k**, EC_{50} = 10 nM), similar to that of derivative **7m**.

We succeeded in obtaining an X-ray crystallographic structure of a complex of compound **13k** (Figure 3) and HIV-1 protease. The data disclosed that the HMC of the inhibitor interacts with the two catalytic Asp residues in a similar fashion as previously reported complexes (Figure 4a,b).[7,14,15] The additional P_2 *p*-amino group was aligned near the side chain of Asp30′ with possible hydrogen bonding interactions. The side chain of Asp30′ was more distant than to that of **1** observed in PDB 1MSM (Figure 4c). The equipotency of **2** and compound **13a** suggests that additional hydrogen bonding interactions from the amino group could compensate for the undesired steric hindrance from this same group. The *β*-methallyl moiety in inhibitor **13k** was within hydrophobic contacts with the side chains of Ala28, Ile84, and Ile50′ in the S_2' pocket (Figure 4d). These hydrophobic interactions are believed to play a similar role as the *o*-methyl group in **1**. On the other hand, another part of *β*-methallyl in compound **13k**, the allyl moiety, compensated for the absence of the phenyl group found in compound **1**, interacting with Val32 and Ile47. These interactions would explain the relatively sustained activity against HIV protease from **13k** (K_i = 0.83 nM) and **7m** (K_i = 0.19 nM) compared to that of inhibitor **1** (K_i = 0. 031 nM).

In regard to water solubility, these analogues dissolved moderately with a range of 1−13 mg/mL compared with previously reported water-soluble prodrug forms (Table 3).[22] However, the moderate solubility of these compounds would be an asset for future pharmaceutical development as anti-HIV therapeutical medicine. The anti-HIV activity of the compounds seems to correlate to the potency against the enzyme better than water solubility (r^2 = 0.41 vs 0.16, from each respective log linear equation).

At first sight there does not seem to be any significant relationship between HIV-1 PR inhibition and antiviral activity. Only a coefficient of determination (r^2) of 0.41 was observed

Table 3. Introduction of *p*-Amino Group into **2** and Its Derivatives[c]



Compound	R^1	R^2	HIV-1 PR % inhibition at 50 nM	Anti-HIV-1_{IIIB}, EC_{50} (μM)			Water solubility of TFA salt (mg/mL)
				0%HS	50% HS	SBE	
2	H		91	1.29	4.5	3.5	0.068
13a (KNI-1030)	NH_2		92	0.132	0.601	4.5	6.02
13b (KNI-1369)	NH_2		98	0.083	0.249	3.0	2.64
13c (KNI-1431)	$NHCH_3$		85	0.191	0.560	2.9	2.99
13d (KNI-1433)	$N(CH_3)_2$		85	0.758	3.9	5.1	3.83
13e (KNI-1303)	NH_2		99	0.041	0.150	3.6	1.25
13f (KNI-1436)	NH_2		95	0.158	0.648	4.1	1.18
13g (KNI-1364)	NH_2		97	0.032	0.241	7.5	1.71
13h (KNI-1293)	NH_2		96	0.357	1.5	4.2	3.74
13i (KNI-1292)	NH_2		90	0.373	1.9	5.0	7.48
13j (KNI-1350)	NH_2		91	0.066	0.284	4.3	12.8
13k (KNI-1689)	NH_2		98[a]	0.010[b]	0.082[b]	8.2	8.48
13l (KNI-1454)	NH_2		91	0.184	0.433	2.3	9.32

[a] 43% inhibition at 1 nM inhibitor, K_i = 0.83 nM. [b] pNL4-3 strain. [c] HS: human serum. SBE: serum binding effect.

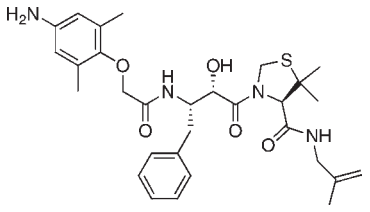


Figure 3. Structure for **13k**.

in the **2** derived series, as previously mentioned. Indeed, when a drug is tested for antiviral activity, several factors are involved including solvation, internalization into the cell, localization to the enzyme, and intrinsic affinity for the enzyme. Whereas X-ray crystal structures may be used to explain HIV-1 PR inhibition, various factors influence the overall antiviral activity, thus rendering antiviral activity predictions somewhat more challenging.

We performed a quantitative structure−activity relationships (QSAR) study to correlate the biophysicochemical properties of the inhibitors with antiviral activity. A reliable equation, using only four descriptors was derived (eq 1): All possible permutations of 214 descriptors calculated with the Molecular Operating Environment (MOE 2005.06, Chemical Computing Group, Inc., Montreal, Canada) software were evaluated and correlated with antiviral activity for compounds

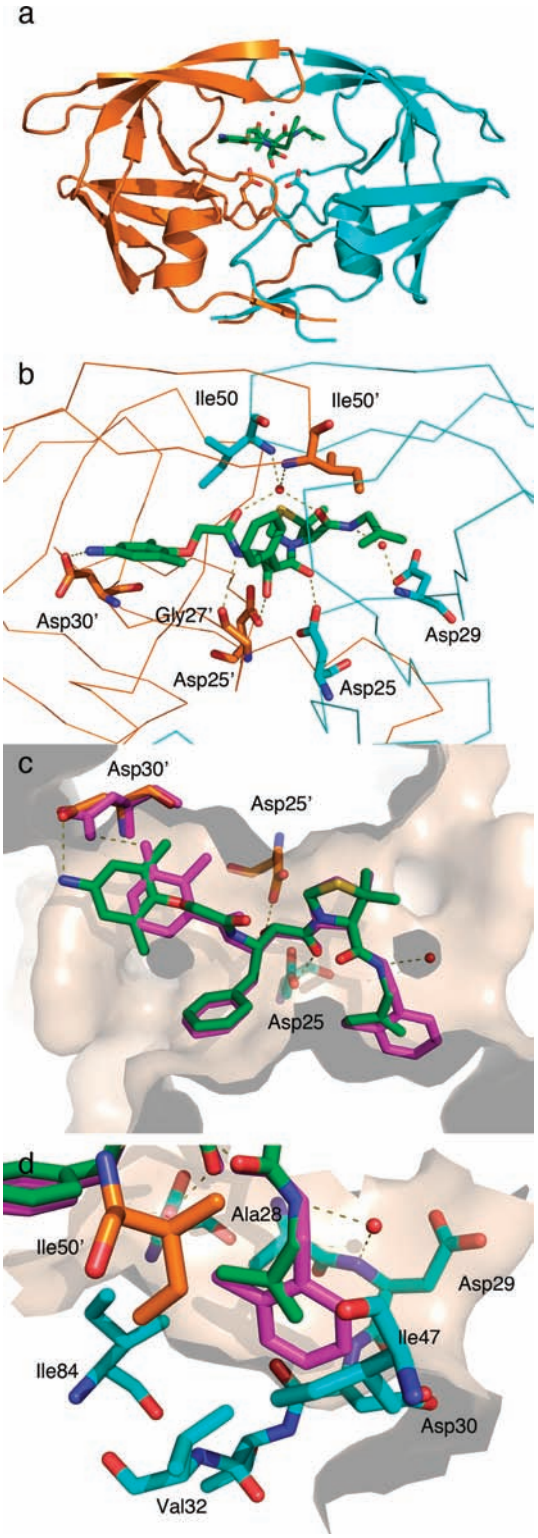


Figure 4. X-ray crystal structure of a complex of compound **13k** (green sticks) and dimeric HIV-1 protease (orange and light-blue ribbons): (a) full structure; (b) hydrogen bonding interactions (dotted lines); (c) superimposition of **1**. Compound **1** and Asp30′ from PDB 1MSM is represented in magenta sticks. (d) Residues surrounding the β-methallyl group in S_2' pocket. Figures were generated using MacPyMOL (DeLano Scientific, LLC, CA).

in which HIV-1 PR inhibition at 50 nM and EC_{50} have been determined, namely, compounds **1**, **2**, **6b**−**j**, **7a**−**n**, and **13a**−**l**. Although the EC_{50} values for inhibitors **7m** and **13k** were

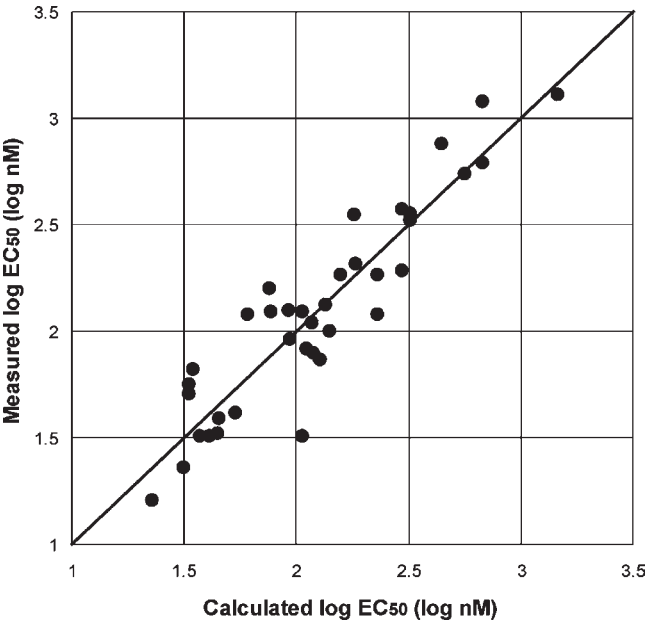


Figure 5. Plot of calculated versus measured anti-HIV activity for eq 1 for **1**, **2**, **6b**−**j**, **7a**−**n**, and **13a**−**l**.

determined using the pNL4-3 wild-type strain while the other compounds' EC_{50} values were determined with the IIIB wild-type strain, we observed a relationship between both wild types (IIIB vs pNL4-3, $r^2 = 0.76$, log linear equation), and the EC_{50} values used in deriving eq 1 for compounds **7m** and **13k** were extrapolated from the correlation. The scoring criteria for choosing the appropriate descriptor combination in eq 1 were that the final equation must have an r^2 value greater than 0.80 and the lowest deviation error (Figures 5). Deviation error was calculated as the sum of squares of the floor values of the absolute differences between calculated and measured −log(EC_{50}) divided by 0.1. The final equation is well fitted ($r^2 = 0.85$) and statistically significant ($p < 0.01$). A form of *K*-fold cross-validation, namely, leave-one-out cross-validation, was performed using a single observation from the original sample as the validation data and the remaining observations as the training data, and the process was repeated such that each observation in the sample was used once as the validation data. The equation is valid because the root-mean-square deviations (rmsd) of the coefficients and intercept are relatively small, and most importantly, the coefficient of determination did not greatly vary during cross-validation. The narrow r^2 range ($r^2 = 0.82$−0.88) indicates the absence of outlier data in the training set.

QSAR Equation for **1**, **2**, **6b**−**j**, **7a**−**n**, and **13a**−**l**:

$$\begin{aligned} -\log(EC_{50}) &= 3.147\log(Inh_{enz}) \\ &+ (5.775 \times 10^{-2} \cdot VSAp - 0.060 \times 10^{-2} \cdot VSAp^2) \\ &+ (3.070 \cdot ASAp - 0.290 \cdot ASAp^2) \\ &- (4.886 \times 10^{-2} \cdot SMR - 0.051 \times 10^{-2} \cdot SMR^2) \\ &- 9.886 \end{aligned} \quad (1)$$

$$n = 37, \quad r^2 = 0.85, \quad F = 23, \quad p < 0.01$$

EC_{50} is the anti-HIV inhibitory activity (μM). Inh_{enz} is the percent HIV-1 protease inhibition at 50 nM of the test compound, expressed as a number from 0 to 100. VSAp uses the partial equalization of orbital electronegativity method[23] for calculating the van der Waals surface area of atoms with partial charges in the range of +0.05 to +0.10, i.e., slight positively charged compounds. ASAp is the fractional, positive partial charge, weighted, water accessible surface area[24] using a probe of 1.4 Å. SMR uses the subdivided surface area method[25] for calculating the van der Waals surface area of atoms with contributions to molar refractivity in the range of 0.485−0.560 and represents the bulkiness of the compound.

Optimal values for the appropriate descriptor (VSAp, ASAp, and SMR) can be determined because each descriptor is described as a parabolic quadratic. Thus, an inhibitor with potent enzyme inhibitory activity, a VSAp value of ~48.41 (extrapolated), ASAp of ~5.287 (extrapolated), and SMR of ~48.36 (intrapolated) is expected to exhibit "optimally" potent antiviral activity. The contribution of each descriptor to the equation can be estimated when each descriptor's quadratic is normalized: VSAp (40%), ASAp (35%), SMR (13%), and Inh_{enz} (12%). The low contribution of Inh_{enz} confirms our previous observation that enzyme affinity plays a small yet important role in antiviral activity to a similar extent as SMR. However, because Inh_{enz} is the lowest contributor in the equation, it may be possible to very roughly predict the antiviral activity of a compound that is yet synthesized, from the compound's calculated VSAp, ASAp, and SMR values while keeping in mind the aforementioned calculated "optimal" values. The major contributors, VSAp and ASAp, both suggest that compounds with proportionally large areas of slightly positively charged surfaces in the range of +0.05 to +0.10 are expected to exhibit potent antiviral activity. Of interest, there is a correlation between the VSAp and ASAp descriptors ($r^2 = 0.76$) because both descriptors only differ in their consideration of either van der Waals or water accessible surface area. However, exclusion of either one of VSAp or ASAp descriptor from the overall QSAR equation used to predict anti-HIV_{IIIB} would form equations with low correlations ($r^2 < 0.43$). Nonetheless, the contribution of the VSAp and ASAp descriptors is consistent with the observed reduced anti-HIV activity in compound **6f** possessing a partially negatively charged P_2' *m*-hydroxy moiety compared with compound **6g** which possessed a *m*-fluorine moiety. The same line of reasoning could explain that the addition of the partially negatively charged hydroxymethyl to a P_2' allyl moiety is detrimental to anti-HIV activity (cf. **7k** vs **7n**, and **13j** vs **13l**). Indeed, compound **13k** was accurately predicted by the equation to exhibit the most potent antiviral activity and **2** as the least potent. It should, however, be noted that most of our compounds belong to a predefined subset of potent enzyme inhibitors with similar chemical structures, and the equation may not accurately predict the antiviral activity of compounds that are vastly structurally different. In fact, other research groups[26,27] have observed a correlation between the octanol−water partition coefficient (log *P*) to predict lipophilicity and antiviral activity, which we did not observe as one of the top-three calculated contributors to the equation in our series of compounds. Although log *P* is in fact found in the fifth top QSAR equation, it was omitted from eq 1 to keep the equation more statistically reliable. It should, however, be noted that most descriptors are somewhat related to each other because an equation formed with log *P* instead of SMR would only be slightly less reliable ($r^2 = 0.83$ vs 0.85). Some reports suggested a correlation with molar refractivity (i.e., SMR), thus re-enforcing our current equation.[27]

Table 4. Inhibitory Activity against Wild-Type and Resistant HIV Strain[a]

	EC_{50} (μM)		
compd	wt (pNL4-3)	IND-R[b]	FR
lopinavir	0.016[c]	0.385[c]	24
1	0.060	0.100	1.7
6d	0.025	0.157	6.3
6f	0.162	0.155	1.0
7f	0.240	1.73	7.2
7g	0.290	2.20	7.6
7k	0.141	0.173	1.2
7l	0.558	1.93	3.5
13a	0.280	2.03	7.3
13b	0.103	0.568	5.5
13e	0.122	0.409	3.4
13g	0.048	0.416	8.7
13h	0.450	1.42	3.2
13i	0.620	3.01	4.9
13j	0.054	0.470	8.7

[a] wt: wild-type. FR: fold resistance. [b] IND-R: L10R/M46I/L63P/V82T/I84V. [c] From ref 31.

Attenuation of inhibitors' potential activity in vivo is another hurdle for developing clinical candidates. Evaluation of activity in the presence of 50% human serum has been suggested as an alternative method to provide deeper insights into the in vivo potency of the inhibitors.[28] Serum binding effect of compounds **13a**−**l** ranged from 2 to 8 (Table 3). Among the series, compound **13k** exhibited the most potent activity with human serum (EC_{50} = 82 nM, 8.2-fold attenuation). These moderate reductions in activity in the presence of human serum suggest promising potentials for future clinical studies.

A few compounds were preliminary tested against indinavir-resistant strain[29] IND-R (Table 4). Among these compounds, **1** exhibited the most potent inhibitory activity against IND-R. Moreover, we identified three compounds (**6d**, **6f**, and **7k**) possessing relatively higher potency than lopinavir. Relative difference in activity against the IND-R and wild-type (pNL4-3) strains ranged from 1- to 9-fold. Apparent preferences for specific P_2 or P_2' moieties against IND-R were not observed. The reason behind the comparably high activities of **6d** and **6f** might be derived from hydrogen bonding interaction at the P_2' position. The activity of the tested compounds was lower against lopinavir-resistant strain[30] A17 (data not shown). The ineffectiveness against A17 suggests dependencies on the interacting protease residues. Further extensions on the inhibitor, such as a tripeptide-type compounds exhibiting specific interactions with the S3 pocket of the protease, are in progress to develop compounds with high activity against drug-resistant viral strains. Statistically, we observed a correlation between both wild-type strains (IIIB vs pNL4-3, $r^2 = 0.76$, log linear equation). Low correlation was observed between wild-type pNL4-3 and indinavir-resistant strain IND-R ($r^2 = 0.55$). These low correlations suggest that predicting anti-HIV activity against resistant viral strains based on wild-type anti-HIV activity remains quite a challenge.

Conclusion

We investigated structure−activity relationships at the P_2' position of allophenylnorstatine-containing HIV protease

inhibitors. Modifications based on the benzyl structure optimized the substitution of the P_2' *o*-methyl group in compound **1**. Introduction of non-benzyl moieties to the P_2' position led us to uncover the effectiveness of relatively small allyl moieties for enzymatic inhibition. An additional methyl group, more specifically a β-methallyl moiety, marked potent inhibitory activity against both the HIV protease and virus. Compound **13k**, possessing a β-methallyl group, exhibited potent anti-HIV activity and moderate water solubility because of a hydrophilic P_2 moiety. These results provide a rational drug design for size-minimizing a lead compound and providing new insights on membrane permeability for drug candidates against other enzymes or receptors. Further evaluations against resistant HIV strains of synthetic analogues are underway in order to develop clinical candidates for HIV therapy.

Experimental Section

Reagents and solvents used were purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan), Nacalai Tesque (Kyoto, Japan), Aldrich Chemical Co. Inc. (Milwaukee, WI), and Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan) and were used without further purification. TLC was performed using Merck silica gel 60 F_{254} precoated plates. Column chromatography was performed on Merck 107734 silica gel 60 (70−230 mesh). Melting points were measured on a Yanagimoto micromelting apparatus without corrections. The purities of the desired compounds were confirmed by HPLC or elemental analyses as greater than 95%. Analytical HPLC was performed using C18 reversed-phase column (4.6 mm × 150 mm, YMC Pack ODS AM302) with binary solvent systems: (A) linear gradient of CH_3CN 40−100% in 0.1% aqueous TFA in 15 min, (B) linear gradient of CH_3CN 20−80% in 0.1% aqueous TFA in 30 min at a flow rate of 0.9 mL/min, detected at 230 nm. Preparative HPLC was carried out on a C18 reversed-phase column (20 mm × 250 mm, YMC Pack ODS SH343-5) with a binary solvent system: a linear gradient of CH_3CN in 0.1% aqueous TFA with a flow rate of 5.0 mL/min and detection at 230 nm. ^{1}H NMR spectra were obtained on a JEOL AL300 (300 MHz) spectrometer with TMS as an internal standard. Mass spectra (electrospray ionization, methanol as the mobile phase) were obtained from a Finnigan SSQ 7000 spectrometer. FAB-MS was performed on a JEOL JMS-SX102A spectrometer equipped with the JMA-DA7000 data system. MALDI-TOF MS was performed on a Voyager-DE RP spectrometer (PerSeptive Biosystems, Inc.).

(*R*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (6a). Compound **6a** was prepared from compound **5j** in a manner similar to that described for compound **6c**. Yield 91%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.35 (s, 1H), 8.22 (d, J = 8.4 Hz, 1H), 8.08 (br, 1H), 7.38 (d, J = 7.0 Hz, 1H), 7.29−7.14 (m, 3H), 7.11−6.92 (m, 4H), 6.78 (d, J = 7.5 Hz, 1H), 6.56 (d, J = 6.8 Hz, 1H), 5.18 (d, J = 9.0 Hz, 1H), 4.99 (d, J = 9.0 Hz, 1H), 4.54 (s, 1H), 4.52 (d, J = 3.7 Hz, 1H), 4.46 (dd, J = 13.9 Hz, 6.1 Hz, 1H), 4.41−4.32 (m, 1H), 4.18 (dd, J = 13.9 Hz, 3.3 Hz, 1H), 2.85−2.65 (m, 2H), 2.31 (s, 6H), 1.81 (s, 3H), 1.46 (s, 3H), 1.35 (s, 3H). HRMS (FAB) *m*/*z*: calcd for $C_{33}H_{39}N_3O_5SNa$ [M + Na]$^+$ 612.2508; found 612.2502.

(*R*)-*N*-(2-Chloro-6-methylbenzyl)-3-[(2*S*,3*S*)-3-(3-hdroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (6b). Compound **6b** was prepared from compound **5b** in a manner similar to that described for compound **6c**. Yield 47%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.36 (br, 1H), 8.20−8.23 (m, 2H), 7.37 (d, J = 7.3 Hz, 1H), 7.31−7.14 (m, 6H), 6.95 (t, J = 7.8 Hz, 1H), 6.78 (d, J = 8.0 Hz, 1H), 6.55 (d, J = 7.3 Hz, 1H), 5.15 (d, J = 9.0 Hz, 1H), 5.49 (d, J = 9.0 Hz, 1H), 4.55 (s, 1H), 4.51 (d, J = 3.3 Hz, 1H), 4.46 (d, J = 5.0 Hz, 1H), 4.41 (d, J = 4.1 Hz, 1H), 4.37 (d, J = 3.5 Hz, 1H), 2.83−2.64 (m, 2H), 2.38 (s, 3H), 1.81 (s, 3H), 1.46 (s, 3H), 1.33 (s, 3H). HRMS (FAB) *m*/*z*: calcd for $C_{32}H_{36}ClN_3O_5SNa$ [M+ Na]$^+$ 632.1962; found 632.1968.

(*R*)-*N*-(2,6-Dichlorobenzyl)-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (6c). A mixture of **5c** (95.5 mg, 0.16 mmol), anisole (34.6 μL, 0.32 mmol), and 4 M HCl in dioxane (1.0 mL) was stirred for 30 min at room temperature. After removal of the solvent in vacuo, the residue was precipitated from ether to give the hydrochloride salt. To a solution of the salt in DMF (2 mL) were added triethylamine (55.9 μL, 0.4 mmol), 3-acetoxy-2-methylbenzoic acid (34.2 mg, 0.18 mmol), and BOP (77.8 mg, 0.18 mmol) in an ice bath, and the mixture was stirred overnight at room temperature. After removal of the solvent in vacuo, the residue was dissolved in EtOAc, washed sequentially with 10% citric acid, 5% $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated in vacuo. To a solution of the residue in methanol (2 mL) was added 1 N aqueous NaOH (0.32 mL), and the mixture was stirred 1 h at room temperature. After removal of the methanol in vacuo, the residue was acidified with citric acid, extracted with EtOAc, washed with 5% $NaHCO_3$ and then brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the product by preparative TLC gave 59.8 mg of the titled compound as a white solid (yield 59%). Additional purification of the product by preparative HPLC gave compound **6c** as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.34 (s, 1H), 8.30 (t, J = 4.6 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 7.51−7.14 (m, 8H), 6.95 (t, J = 7.8 Hz, 1H), 6.77 (d, J = 7.2 Hz, 1H), 6.55 (d, J = 7.2 Hz, 1H), 5.31 (d, J = 6.9 Hz, 1H), 5.13 (d, J = 8.7 Hz, 1H), 5.01 (d, J = 8.7 Hz, 1H), 4.64−4.64 (m, 4H), 4.43−4.32 (m, 1H), 2.84−2.64 (m, 2H), 1.81 (s, 3H), 1.46 (s, 3H), 1.34 (s, 3H). HRMS (FAB) *m*/*z*: calcd for $C_{31}H_{33}Cl_2N_3O_5SNa$ [M + Na]$^+$ 652.1416; found 652.1412.

(*R*)-*N*-(2-Chloro-6-fluorobenzyl)-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (6d). Compound **6d** was prepared from compound **5d** in a manner similar to that described for compound **6c**. Yield 92%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.35 (br, 1H), 8.38 (t, J = 4.7 Hz, 1H), 8.19 (d, J = 8.4 Hz, 1H), 7.42−7.14 (m, 8H), 6.95 (t, J = 7.8 Hz, 1H), 6.77 (d, J = 8.1 Hz, 1H), 6.55 (d, J = 7.3 Hz, 1H), 5.12 (d, J = 9.2 Hz, 1H), 5.00 (d, J = 9.2 Hz, 1H), 4.54−4.46 (s, 3H), 4.42−4.29 (m, 2H), 2.83−2.64 (m, 2H), 1.81 (s, 3H), 1.45 (s, 3H), 1.28 (s, 3H). HRMS (FAB) *m*/*z*: calcd for $C_{31}H_{33}ClFN_3O_5SNa$ [M + Na]$^+$ 636.1711; found 636.1716.

(*R*)-*N*-(2,6-Difluorobenzyl)-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (6e). Compound **6e** was prepared from compound **5e** in a manner similar to that described for compound **6c**. Yield 92%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.36 (br, 1H), 8.44 (t, J = 5.1 Hz, 1H), 8.16 (d, J = 8.6 Hz, 1H), 7.38−7.16 (m, 6H), 7.07 (t, J = 7.8 Hz, 2H), 6.95 (t, J = 7.7 Hz, 1H), 6.77 (d, J = 8.1 Hz, 1H), 6.54 (d, J = 7.4 Hz, 1H), 5.11 (d, J = 9.0 Hz, 1H), 4.99 (d, J = 9.2 Hz, 1H), 4.47 (d, J = 4.4 Hz, 1H), 4.45 (s, 1H), 4.43−4.32 (m, 2H), 4.25 (dd, J = 13.9 Hz, 4.6 Hz, 1H), 2.84−2.64 (m, 2H), 1.81 (s, 3H), 1.44 (s, 3H), 1.24 (s, 3H). HRMS (FAB) *m*/*z*: calcd for $C_{31}H_{33}F_2N_3O_5SNa$ [M + Na]$^+$ 620.2007; found 620.2001.

(*R*)-*N*-(3-Hydroxy-2-methylbenzyl)-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (6f). Compound **6f** was prepared from compound **5f** in a manner similar to that described for compound **6c**. Yield 71%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.37 (s, 1H), 9.19 (s, 1H), 8.24 (t, J = 5.2 Hz, 1H), 8.14 (d, J = 8.4 Hz, 1H), 7.34 (d, J = 7.1 Hz, 12H), 7.23−7.13 (m, 3H), 6.97−6.87 (m, 2H), 6.77 (dd, J = 7.2 Hz, 4.7 Hz, 1H), 6.69 (d, J = 7.7 Hz, 1H), 6.55 (d, J = 7.3 Hz, 1H), 5.13 (d, J = 9.0 Hz, 1H), 5.01 (d, J = 9.0 Hz, 1H), 4.50 (s, 1H), 4.48 (d, J = 4.6 Hz, 1H), 4.44−4.31 (m, 2H), 4.07 (dd, J = 14.9 Hz, 4.8 Hz, 1H), 2.89−2.67 (m, 2H),

2.06 (s, 3H), 1.83 (s, 3H), 1.49 (s, 3H), 1.24 (s, 3H). HRMS (FAB) *m*/*z*: calcd for $C_{32}H_{37}N_3O_6SNa$ [M + Na]$^+$ 614.2301; found 614.2297.

(*R*)-*N*-(3-Fluoro-2-methylbenzyl)-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (6g). Compound **6g** was prepared from compound **5g** in a manner similar to that described for compound **6c**. Yield 69%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.37 (s, 1H), 8.39 (t, J = 5.6 Hz, 1H), 8.14 (d, J = 8.3 Hz, 1H), 7.34−6.91 (m, 9H), 6.78 (d, J = 7.3 Hz, 1H), 6.55 (d, J = 6.6 Hz, 1H), 5.46 (br, 1H), 5.14 (d, J = 9.2 Hz, 1H), 5.01 (d, J = 9.2 Hz, 1H), 4.51−4.36 (m, 4H), 4.14 (dd, J = 15.1 Hz, 4.8 Hz, 1H), 2.88−2.66 (m, 2H), 2.17 (s, 3H), 1.82 (s, 3H), 1.50 (s, 3H), 1.34 (s, 3H). HRMS (FAB) *m*/*z*: calcd for $C_{32}H_{36}FN_3O_5SNa$ [M + Na]$^+$ 616.2257; found 616.2252.

(*R*)-*N*-[(*S*)-1-Phenylethane-1-yl]-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (6h). Compound **6h** was prepared from compound **5h** in a manner similar to that described for compound **6c**. Yield 54%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.36 (s, 1H), 8.45 (d, J = 7.9 Hz, 1H), 8.19 (d, J = 8.4 Hz, 1H), 7.37−7.16 (m, 10H), 6.95 (t, J = 7.8 Hz, 1H), 6.78 (d, J = 7.7 Hz, 1H), 6.55 (d, J = 7.2 Hz, 1H), 5.36 (d, J = 6.8 Hz, 1H), 5.10 (d, J = 9.2 Hz, 1H), 5.01 (d, J = 9.2 Hz, 1H), 5.00−4.94 (m, 1H), 4.55−4.52 (m, 1H), 4.50 (s, 1H), 4.46−4.37 (m, 1H), 2.87−2.66 (m, 2H), 1.81 (s, 3H), 1.49 (s, 3H), 1.39 (d, J = 7.0 Hz, 1H), 1.22 (s, 3H). HRMS (FAB) *m*/*z*: calcd for $C_{32}H_{37}N_3O_5SNa$ [M + Na]$^+$ 598.2352; found 598.2359.

(*R*)-*N*-[(*R*)-1-Phenylethane-1-yl]-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (6j). Compound **6j** was prepared from compound **5i** in a manner similar to that described for compound **6c**. Yield 83%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.35 (s, 1H), 8.37 (d, J = 7.9 Hz, 1H), 8.15 (d, J = 8.4 Hz, 1H), 7.38 (d, J = 7.3 Hz, 2H), 7.29−7.12 (m, 8H), 6.94 (t, J = 7.8 Hz, 1H), 6.77 (d, J = 7.9 Hz, 1H), 6.54 (d, J = 7.3 Hz, 1H), 5.39 (d, J = 7.0 Hz, 1H), 5.17 (d, J = 8.8 Hz, 1H), 5.04−4.94 (m, 2H), 4.54 (s, 1H), 4.49 (dd, J = 6.6 Hz, 4.2 Hz, 1H), 4.43−4.33 (m, 1H), 2.81−2.63 (m, 2H), 1.81 (s, 3H), 1.52 (s, 3H), 1.40 (s, 3H), 1.36 (d, J = 7.0 Hz, 1H). HRMS (FAB) *m*/*z*: calcd for $C_{32}H_{37}N_3O_5SNa$ [M + Na]$^+$ 598.2352; found 598.2358.

(*R*)-*N*-[(*S*)-Indan-1-yl]-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (7a). Compound **7a** was prepared from compound **5k** in a manner similar to that described for compound **6c**. Yield 52%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.38 (s, 1H), 8.35 (d, J = 7.8 Hz, 1H), 8.22 (d, J = 8.4 Hz, 1H), 7.35−7.12 (m, 9H), 6.95 (t, J = 7.8 Hz, 1H), 6.78 (d, J = 7.8 Hz, 1H), 6.57 (d, J = 7.2 Hz, 1H), 5.36−5.26 (m, 2H), 5.15 (d, J = 9.0 Hz, 1H), 5.04 (d, J = 9.3 Hz, 1H), 4.52 (d, J = 3.9 Hz, 1H), 4.48 (s, 1H), 4.45−4.35 (m, 1H), 3.02−2.68 (m, 4H), 2.44−2.32 (m, 1H, overlapped with DMSO), 1.81 (s, 3H), 1.92−1.77 (m, 1H), 1.50 (s, 3H), 1.45 (s, 3H). HRMS (FAB) *m*/*z*: calcd for $C_{33}H_{37}N_3O_5SNa$ [M + Na]$^+$ 610.2352; found 610.2357.

(*R*)-*N*-[(*R*)-Indan-1-yl]-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (7b). Compound **7b** was prepared from compound **5l** in a manner similar to that described for compound **6c**. Yield 54%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.37 (s, 1H), 8.38 (d, J = 8.7 Hz, 1H), 8.18 (d, J = 8.7 Hz, 1H), 7.41−7.07 (m, 9H), 6.94 (t, J = 7.7 Hz, 1H), 6.78 (d, J = 7.5 Hz, 1H), 6.53 (d, J = 7.5 Hz, 1H), 5.39 (d, J = 8.1 Hz, 1H), 5.33 (d, J = 8.4 Hz, 1H), 4.47 (s, 1H), 4.44−3.38 (m, 1H), 2.94−2.70 (m, 4H), 2.41−2.26 (m, 1H, overlapped with DMSO), 1.82 (s, 3H), 1.87−1.68 (m, 1H), 1.52 (s, 3H), 1.44 (s, 3H). HRMS (FAB) *m*/*z*: calcd for $C_{33}H_{37}N_3O_5SNa$ [M + Na]$^+$ 610.2352; found 610.2358.

(*R*)-*N*-[(1*R*,2*S*)-2-Hydroxyindan-1-yl]-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (7c). Compound **7c** was prepared from compound **5m** in a manner similar to that described for compound **6c**. Yield 62%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.35 (s, 1H), 8.21 (d, J = 8.2 Hz, 1H), 8.16 (d, J = 7.9 Hz, 1H), 7.34−7.12 (m, 9H), 6.95 (t, J = 7.7 Hz, 1H), 6.67 (d, J = 8.0 Hz, 1H), 6.56 (d, J = 7.5 Hz, 1H), 5.52 (d, J = 6.2 Hz, 1H), 5.26−5.16 (m, 2H), 5.06 (d, J = 9.4 Hz, 1H), 4.64 (s, 1H), 4.60 (d, J = 3.1 Hz, 1H), 4.53−4.32 (m, 3H), 3.05 (dd, J = 16.6 Hz, 5.0 Hz, 1H), 2.88−2.64 (m, 3H), 1.80 (s, 3H), 1.54 (s, 3H), 1.49 (s, 3H). HRMS (FAB) *m*/*z*: calcd for $C_{33}H_{37}N_3O_6SNa$ [M + Na]$^+$ 626.2301; found 626.2294.

(*R*)-*N*-[(1*S*,2*R*)-2-Hydroxyindan-1-yl]-3-[(2*S*,3*S*)-3-(3-hdroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (7d). Compound **7d** was prepared from compound **5n** in a manner similar to that described for compound **6c**. Yield 70%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.38 (s, 1H), 8.28 (d, J = 8.4 Hz, 1H), 8.10 (d, J = 8.7 Hz, 1H), 7.42−7.05 (m, 9H), 6.95 (t, J = 7.7 Hz, 1H), 6.78 (d, J = 7.8 Hz, 1H), 6.57 (d, J = 7.2 Hz, 1H), 5.30 (d, J = 4.8 Hz, 1H), 5.27 (d, J = 4.8 Hz, 1H), 5.17 (d, J = 9.6 Hz, 1H), 5.03 (d, J = 9.0 Hz, 1H), 4.75 (s, 1H), 4.57 (d, J = 3.3 Hz, 1H), 4.45−4.32 (m, 2H), 3.05 (dd, J = 16.1 Hz, 5.3 Hz, 1H), 2.91−2.68 (m, 3H), 1.82 (s, 3H), 1.56 (s, 3H), 1.49 (s, 3H). HRMS (FAB) *m*/*z*: calcd for $C_{33}H_{37}N_3O_6SNa$ [M + Na]$^+$ 626.2301; found 626.2305.

(*R*)-*N*-Cycloheptyl-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)-amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (7e). Compound **7e** was prepared from compound **5o** in a manner similar to that described for compound **6c**. Yield 59%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.35 (s, 1H), 8.16 (d, J = 8.3 Hz, 1H), 7.85 (d, J = 7.7 Hz, 1H), 7.35 (d, J = 7.0 Hz, 2H), 7.24 (t, J = 7.2 Hz, 2H), 7.17 (d, J = 7.0 Hz, 1H), 6.94 (t, J = 7.7 Hz, 1H), 6.77 (d, J = 7.9 Hz, 1H), 6.54 (d, J = 7.5 Hz, 1H), 5.32 (br, 1H), 5.11 (d, J = 9.0 Hz, 1H), 5.00 (d, J = 9.0 Hz, 1H), 4.50 (d, J = 3.8 Hz, 1H), 4.44 (s, 1H), 4.42−4.35 (m, 1H), 3.81−3.69 (m, 1H), 2.83−2.65 (m, 2H), 1.81 (s, 3H), 1.79−1.70 (m, 2H), 1.61−1.39 (m, 13H), 1.37 (s, 3H). HRMS (FAB) *m*/*z*: calcd for $C_{31}H_{41}N_3O_5SNa$ [M + Na]$^+$ 590.2665; found 590.2671.

(*R*)-*N*-Cyclohexyl-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)-amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (7f). Compound **7f** was prepared from compound **5p** in a manner similar to that described for compound **6c**. Yield 32%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.36 (s, 1H), 7.80 (d, J = 7.9 Hz, 1H), 7.34 (d, J = 7.1 Hz, 1H), 7.37−7.13 (m, 5H), 6.94 (t, J = 7.8 Hz, 1H), 6.73 (d, J = 7.3 Hz, 1H), 6.54 (d, J = 7.1 Hz, 1H), 5.33 (d, J = 7.1 Hz, 1H), 5.10 (d, J = 9.0 Hz, 1H), 5.01 (d, J = 9.2 Hz, 1H), 4.51 (dd, J = 7.0 Hz, 4.2 Hz, 1H), 4.43 (s, 1H), 4.42−4.35 (m, 1H), 3.63−3.44 (m, 1H), 2.84−2.65 (m, 2H), 1.81 (s, 3H), 1.77−1.59 (m, 4H), 1.50 (s, 3H), 1.37 (s, 3H), 1.29−1.06 (m, 6H). HRMS (FAB) *m*/*z*: calcd for $C_{30}H_{39}N_3O_5SNa$ [M + Na]$^+$ 576.2508; found 576.2503.

(*R*)-*N*-Cyclopentyl-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)-amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (7g). Compound **7g** was prepared from compound **5q** in a manner similar to that described for compound **6c**. Yield 83%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.34 (s, 1H), 8.15 (d, J = 8.7 Hz, 1H), 7.88 (d, J = 7.2 Hz, 1H), 7.37−7.15 (m, 5H), 6.93 (t, J = 7.5 Hz, 1H), 6.77 (d, J = 7.8 Hz, 1H), 6.54 (d, J = 7.5 Hz, 1H), 5.32 (d, J = 6.6 Hz, 1H), 5.11 (d, J = 9.3 Hz, 1H), 5.01 (d, J = 9.3 Hz, 1H), 4.50 (dd, J = 6.8 Hz, 4.4 Hz, 1H), 4.46−4.34 (m, 2H), 4.06−3.96 (m, 1H), 2.84−2.65 (m, 2H), 1.87−1.32 (m, 17H). HRMS (FAB) *m*/*z*: calcd for $C_{29}H_{37}N_3O_5SNa$ [M + Na]$^+$ 562.2352; found 562.2357.

(*R*)-*N*-Cyclobutyl-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)-amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (7h). Compound **7h** was prepared from compound **5r** in a manner similar to that described for compound **6c**. Yield 22%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.36 (s, 1H), 8.18−8.14 (m, 2H), 7.34 (d, J = 7.1 Hz, 2H),

7.25 (t, J = 7.1 Hz, 2H), 7.18 (d, J = 7.1 Hz, 1H), 7.34 (d, J = 7.1 Hz, 2H), 6.94 (t, J = 7.8 Hz, 1H), 6.77 (d, J = 7.9 Hz, 1H), 6.54 (d, J = 7.5 Hz, 1H), 5.38 (d, J = 6.8 Hz, 1H), 5.11 (d, J = 9.0 Hz, 1H), 5.01 (d, J = 9.2 Hz, 1H), 4.51−4.34 (m, 3H), 4.26−4.11 (m, 1H), 2.84−2.65 (m, 2H), 1.99−1.89 (m, 2H), 1.81 (s, 3H), 1.68−1.57 (m, 2H), 1.50 (s, 3H), 1.35 (s, 3H). HRMS (FAB) m/z: calcd for $C_{28}H_{35}N_3O_5SNa$ $[M + Na]^+$ 548.2195; found 548.2191.

(*R*)-*N*-Ethyl-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (7i). Compound **7i** was prepared from compound **5s** in a manner similar to that described for compound **6c**. Yield 37%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.36 (s, 1H), 8.11 (d, J = 8.2 Hz, 1H), 7.91 (t, J = 5.5 Hz, 1H), 7.35−7.14 (m, 5H), 6.94 (t, J = 7.8 Hz, 1H), 6.77 (d, J = 7.5 Hz, 1H), 6.53 (d, J = 7.3 Hz, 1H), 5.42 (d, J = 6.4 Hz, 1H), 5.10 (d, J = 9.2 Hz, 1H), 5.01 (d, J = 9.2 Hz, 1H), 4.49−4.38 (m, 2H), 4.36 (s, 1H), 3.13−3.04 (m, 2H), 2.87−2.67 (m, 2H), 1.81 (s, 3H), 1.50 (s, 3H), 1.36 (s, 3H), 1.00 (t, J = 7.1 Hz, 3H). HRMS (FAB) m/z: calcd for $C_{26}H_{33}N_3O_5SNa$ $[M + Na]^+$ 522.2039; found 522.2043.

(*R*)-*N*-Propyl-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (7j). Compound **7j** was prepared from compound **5t** in a manner similar to that described for compound **6c**. Yield 48%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.36 (s, 1H), 8.12 (d, J = 8.2 Hz, 1H), 7.90 (t, J = 5.7 Hz, 1H), 7.35−7.14 (m, 5H), 6.94 (t, J = 7.7 Hz, 1H), 6.77 (d, J = 8.1 Hz, 1H), 6.54 (d, J = 7.4 Hz, 1H), 5.41 (d, J = 6.8 Hz, 1H), 5.10 (d, J = 9.2 Hz, 1H), 5.01 (d, J = 9.2 Hz, 1H), 4.50−4.39 (m, 2H), 4.38 (s, 1H), 3.10−2.95 (m, 2H), 2.87−2.66 (m, 2H), 1.82 (s, 3H), 1.50 (s, 3H), 1.47−1.31 (s, 5H), 0.84 (t, J = 7.3 Hz, 3H). HRMS (FAB) m/z: calcd for $C_{27}H_{35}N_3O_5SNa$ $[M + Na]^+$ 536.2195; found 536.2202.

(*R*)-*N*-Allyl-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (7k). Compound **7k** was prepared from compound **5a** in a manner similar to that described for compound **6c**. Yield 51%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.39 (s, 1H), 8.16−8.02 (m, 2H), 7.36−7.13 (m, 8H), 6.95 (t, J = 7.8 Hz, 1H), 6.77 (d, J = 8.1 Hz, 1H), 6.54 (d, J = 7.5 Hz, 1H), 5.84−5.70 (m, 1H), 5.21 (dd, J = 17.1 Hz, 1.5 Hz, 1H), 5.12 (d, J = 9.0 Hz, 1H), 5.02 (dd, J = 10.2 Hz, 1.5 Hz, 1H), 5.01 (d, J = 9.0 Hz, 1H), 4.48−4.35 (m, 3H), 3.84−3.56 (m, 2H), 2.88−2.66 (m, 2H), 1.81 (s, 3H), 1.50 (s, 3H), 1.36 (s, 3H). HRMS (FAB) m/z: calcd for $C_{27}H_{33}N_3O_5SN$ $[M + Na]^+$ 534.2039; found 534.2043.

(*R*)-*N*-Propargyl-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)-amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (7l). Compound **7l** was prepared from compound **5u** in a manner similar to that described for compound **6c**. Yield 38%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.37 (s, 1H), 8.40 (t, J = 5.4 Hz, 1H), 8.11 (d, J = 8.0 Hz, 1H), 7.33 (d, J = 7.2 Hz, 2H), 7.26 (t, J = 7.2 Hz, 2H), 7.21−7.14 (m, 1H), 6.95 (t, J = 7.8 Hz, 1H), 6.77 (d, J = 8.1 Hz, 1H), 6.54 (d, J = 7.1 Hz, 1H), 5.49 (d, J = 6.4 Hz, 1H), 5.11 (d, J = 9.2 Hz, 1H), 5.01 (d, J = 9.2 Hz, 1H), 4.47−4.40 (m, 2H), 4.39 (s, 1H), 3.87−3.82 (m, 2H), 3.09 (t, J = 2.2 Hz, 1H), 2.90−2.66 (m, 2H), 1.81 (s, 3H), 1.50 (s, 3H), 1.36 (s, 3H). HRMS (FAB) m/z: calcd for $C_{27}H_{31}N_3O_5SNa$ $[M + Na]^+$ 532.1882; found 532.1878.

(*R*)-*N*-(2-Methylallyl)-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (7m). Compound **7m** was prepared from compound **5v** in a manner similar to that described for compound **6c**. Yield 70%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.36 (s, H), 8.11−8.03 (m, 2H), 7.36−7.13 (m, 5H), 6.95 (t, J = 7.8 Hz, 1H), 6.78 (d, J = 7.1 Hz, 1H), 6.54 (d, J = 7.5 Hz, 1H), 5.11 (d, J = 9.2 Hz, 1H), 5.00 (d, J = 9.2 Hz, 1H), 4.88 (s, 1H), 4.73 (s, 1H), 4.49−4.36 (m, 4H), 3.74 (dd, J = 15.7 Hz, 6.1 Hz, 1H), 3.52−3.47 (m, 1H, overlapped with H_2O), 2.88−2.67 (m, 2H), 1.82 (s, 3H), 1.65 (s, 3H), 1.51 (s, 3H), 1.36 (s, 3H). HRMS (FAB) m/z: calcd for $C_{28}H_{35}N_3O_5SNa$ $[M + Na]^+$ 548.2195; found 548.2189.

(*R*)-*N*-(*cis*-4-Hydroxy-2-buten-1-yl)-3-[(2*S*,3*S*)-3-(3-hydroxy-2-methylbenzoyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (7n). Compound **7n** was prepared from compound **5w** in a manner similar to that described for compound **6c**. Yield 25%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.37 (s, 1H), 8.15−8.05 (m, 2H), 7.34 (d, J = 7.1 Hz, 2H), 7.25 (t, J = 7.3 Hz, 2H), 7.20−7.13 (m, 1H), 6.95 (t, J = 7.3 Hz, 1H), 6.78 (d, J = 8.0 Hz, 1H), 6.54 (d, J = 7.3 Hz, 1H), 5.58−5.48 (m, 1H), 5.45 (d, J = 6.1 Hz, 1H), 5.36−5.26 (m, 1H), 5.11 (d, J = 9.0 Hz, 1H), 5.01 (d, J = 8.8 Hz, 1H), 4.69−4.67 (m, 1H), 4.49−4.40 (m, 2H), 4.38 (s, 1H), 4.06−3.99 (m, 2H), 3.75−3.68 (m, 2H), 2.90−2.66 (m, 2H), 1.82 (s, 3H), 1.49 (s, 3H), 1.35 (s, 3H). HRMS (FAB) m/z: calcd for $C_{28}H_{35}N_3O_6SNa$ $[M + Na]^+$ 564.2144; found 564.2151.

(*R*)-*N*-(*tert*-Butyl)-3-[(2*S*,3*S*)-3-(4-amino-2,6-dimethyphenoxyacetyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (13a). Compound **13a** was prepared from Boc-Apns-Dmt-NHtBu[10] in a manner similar to that described for compound **13b**. Yield 68%. Mp 122−124 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.13 (d, J = 8.8 Hz, 1H), 7.67 (s, 1H), 7.35 (d, J = 6.8 Hz, 2H), 7.27−7.13 (m, 5H), 6.82 (s, 2H), 4.98 (d, J = 8.4 Hz, 1H), 4.92 (d, J = 8.8 Hz, 1H), 4.53 (s, 2H), 4.48 (d, J = 3.3 Hz, 1H), 4.37−4.27 (m, 1H), 4.19 (d, J = 14.1 Hz, 1H), 3.99 (d, J = 13.9 Hz, 1H), 2.81−2.71 (m, 2H), 2.14 (s, 6H), 1.49 (s, 3H), 1.40 (s, 3H), 1.26 (s, 9H). HRMS (FAB) m/z: calcd for $C_{30}H_{43}N_4O_5S$ $[M + H]^+$ 571.2944; found 571.2960.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-(4-amino-2,6-dimethyphenoxyacetyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (13b). A mixture of Boc-Apns-Dmt-NH(*o*-methylbenzyl)[10] (54.2 mg, 0.1 mmol), anisole (21.6 μL, 0.2 mmol), and 4 N HCl in dioxane (1.0 mL) was stirred for 30 min at room temperature. After removal of the solvent in vacuo, the residue was precipitated from ether to give the hydrochloride salt. To a solution of the HCl salt in DMF (2 mL) were added triethylamine (34.9 μL, 0.25 mmol), 4-(Boc-amino)-2,6-dimethylphenoxyacetic acid (**10**) (32.5 mg, 0.11 mmol), and BOP (48.7 mg, 0.11 mmol) in an ice bath, and the mixture was stirred overnight at room temperature. After removal of the solvent in vacuo, the residue was added to EtOAc, washed sequentially with 10% citric acid, 5% $NaHCO_3$, and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was mixed with anisole (21.6 μL) and 4 N HCl in dioxane (1.0 mL), and then the mixture was stirred for 1 h at room temperature. After removal of the solvent in vacuo, the residue was precipitated from ether to give a product as hydrochloride salt, 46 mg. Purification of the product by preparative HPLC gave compound **13b** as a white powder. Yield 70%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.38 (t, J = 5.8 Hz, 1H), 8.14 (d, J = 8.8 Hz, 1H), 7.33−7.06 (m, 11H), 6.81 (s, 2H), 4.96 (s, 2H), 4.51 (s, 1H), 4.47 (d, J = 3.7 Hz, 1H), 4.43−4.34 (m, 2H), 4.22−4.13 (m, 2H), 4.00 (d, J = 14.1 Hz, 1H), 2.82−2.71 (m, 2H), 2.26 (s, 3H), 2.14 (s, 6H), 1.51 (s, 3H), 1.36 (s, 3H). HRMS (FAB) m/z: calcd for $C_{34}H_{42}N_4O_5SNa$ $[M + Na]^+$ 641.2774; found 641.2777.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-(4-methylamino-2,6-dimethyphenoxyacetyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (13c). Compound **13c** was prepared from Boc-Apns-Dmt-NH(*o*-methylbenzyl) and **11** in a manner similar to that described for compound **13b**. Yield 68%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.37 (br, 1H), 8.10 (d, J = 9.3 Hz, 1H), 7.34−7.05 (m, 11H), 6.58 (s, 1H), 4.96 (d, J = 9.0 Hz, 2H), 4.51 (s, 1H), 4.47 (d, J = 3.7 Hz, 1H), 4.44−4.31 (m, 2H), 4.24−4.03 (m, 2H), 3.95 (d, J = 14.1 Hz, 1H), 2.82−2.67 (m, 5H), 2.26 (s, 3H), 2.11 (s, 6H), 1.50 (s, 3H), 1.36 (s, 3H). HRMS (FAB) m/z: calcd for $C_{35}H_{44}N_4O_5SNa$ $[M + Na]^+$ 655.2930; found 655.2927.

(*R*)-*N*-(2-Methylbenzyl)-3-[(2*S*,3*S*)-3-(4-dimethylamino-2,6-dimethyphenoxyacetyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (13d). To a solution of

HCl salt of H-Apns-Dmt-NH(*o*-methylbenzyl)[10] (74 mg, 0.14 mmol) in DMF were added triethylamine (19 μL, 0.14 mmol), HOBt·H_2O (23 mg, 0.15 mmol), 4-(dimethylamino)-2,6-dimethylphenoxyacetic acid (**12**) (89 mg, 0.14 mmol), and EDC·HCl (29 mg, 0.15 mmol) in an ice bath, and the mixture was stirred overnight at room temperature. After removal of the solvent in vacuo, the residue was added into EtOAc, washed sequentially with 5% $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. Purification of the product by silica gel column chromatography gave 56 mg of compound **13d** as a white solid. Yield 63%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.37 (t, J = 5.4 Hz, 1H), 8.09 (d, J = 8.3 Hz, 1H), 7.33−7.07 (m, 11H), 6.53 (br, 1H), 4.98 (d, J = 9.3 Hz, 1H), 4.94 (d, J = 9.0 Hz, 1H), 4.50 (s, 1H), 4.47 (d, J = 3.5 Hz, 1H), 4.45−4.35 (m, 2H), 4.24−4.01 (m, 2H), 3.95−3.87 (m, 1H), 2.90−2.72 (m, 10H), 2.26 (s, 3H), 2.11 (s, 6H), 1.51 (s, 3H), 1.36 (s, 3H). HRMS (FAB) m/z: calcd for $C_{36}H_{46}N_4O_5SNa$ $[M + Na]^+$ 669.3087; found 669.3081.

(*R*)-*N*-(2,6-Dimethylbenzyl)-3-[(2*S*,3*S*)-3-(4-amino-2,6-dimethyphenoxyacetyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (13e). Compound **13e** was prepared from compound **5j** in a manner similar to that described for compound **13a**. Yield 94%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.18 (d, J = 9.0 Hz, 1H), 8.10 (br, 1H), 7.39−6.96 (m, 10H), 6.77 (s, 2H), 4.98 (d, J = 8.7 Hz, 1H), 4.93 (d, J = 8.7 Hz, 1H), 4.54 (s, 1H), 4.51−4.12 (m, 2H), 4.38−4.26 (m, 1H), 4.22−4.11 (m, 2H), 4.00 (d, J = 14.4 Hz, 1H), 2.82−2.70 (m, 2H), 2.30 (s, 6H), 2.14 (s, 6H), 1.46 (s, 3H), 1.37 (s, 3H). HRMS (FAB) m/z: calcd for $C_{35}H_{44}N_4O_5SNa$ $[M + Na]^+$ 655.2930; found 655.2934.

(*R*)-*N*-(2,6-Dichlorobenzyl)-3-[(2*S*,3*S*)-3-(4-amino-2,6-dimethyphenoxyacetyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (13e). Compound **13e** was prepared from compound **5c** in a manner similar to that described for compound **13b**. Yield 31%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.30 (t, J = 5.1 Hz, 1H), 8.12 (d, J = 9.2 Hz, 1H), 7.50−7.14 (m, 10H), 6.65 (s, 2H), 4.94 (d, J = 2.2 Hz, 1H), 4.63−4.57 (m, 1H), 4.55 (s, 1H), 4.52−4.43 (m, 1H), 4.38−4.28 (m, 1H), 4.15 (d, J = 14.1 Hz, 1H), 3.96 (d, J = 14.1 Hz, 1H), 2.80−2.71 (m, 2H), 2.11 (s, 6H), 1.46 (s, 3H), 1.35 (s, 3H). HRMS (FAB) m/z: calcd for $C_{33}H_{38}Cl_2N_4O_5SNa$ $[M + Na]^+$ 695.1838; found 695.1845.

(*R*)-*N*-[(*S*)-Indan-1-yl]-3-[(2*S*,3*S*)-3-(4-amino-2,6-dimethyphenoxyacetyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (13g). Compound **13g** was prepared from compound **5k** in a manner similar to that described for compound **13b**. Yield 92%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.34 (d, J = 7.9 Hz, 1H), 8.10 (d, J = 9.2 Hz, 1H), 7.32−7.12 (m, 11H), 6.62 (s, 2H), 5.30 (dd, J = 15.3 Hz, 8.1 Hz, 1H), 5.04 (d, J = 9.2 Hz, 1H), 4.95 (d, J = 8.8 Hz, 1H), 4.48 (d, J = 3.7 Hz, 1H), 4.46 (s, 1H), 4.42−4.31 (m, 1H), 4.14 (d, J = 14.1 Hz, 1H), 3.94 (d, J = 14.1 Hz, 1H), 2.99−2.70 (m, 4H), 2.44−2.30 (m, 1H, overlapped with H_2O), 2.11 (s, 6H), 1.91−1.77 (m, 1H), 1.51 (s, 3H), 1.45 (s, 3H). HRMS (FAB) m/z: calcd for $C_{35}H_{42}N_4O_5SNa$ $[M + Na]^+$ 653.2774; found 653.2780.

(*R*)-*N*-[(1*S*,2*R*)-2-Hydroxyindan-1-yl]-3-[(2*S*,3*S*)-3-(4-amino-2,6-dimethyphenoxyacetyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (13h). Compound **13h** was prepared from compound **5n** in a manner similar to that described for compound **13b**. Yield 46%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.21 (d, J = 8.8 Hz, 1H), 8.10 (d, J = 8.8 Hz, 1H), 7.37 (d, J = 7.3 Hz, 2H), 7.31−7.10 (m, 8H), 7.03 (t, J = 7.2 Hz, 1H), 6.78 (s, 2H), 5.28 (dd, J = 8.1 Hz, 4.7 Hz, 1H), 4.96 (s, 2H), 4.76 (s, 1H), 4.50 (d, J = 2.9 Hz, 1H), 4.45−4.27 (m, 2H), 4.19 (d, J = 14.3 Hz, 1H), 4.07−3.96 (m, 1H), 3.05 (dd, J = 16.0 Hz, 5.1 Hz, 1H), 2.92−2.71 (m, 3H), 2.14 (s, 6H), 1.56 (s, 3H), 1.48 (s, 3H). HRMS (FAB) m/z: calcd for $C_{35}H_{42}N_4O_6SNa$ $[M + Na]^+$ 669.2723; found 669.2728.

(*R*)-*N*-Cyclopentyl-3-[(2*S*,3*S*)-3-(4-amino-2,6-dimethyphenoxyacetyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (13i). Compound **13i** was prepared from compound **5q** in a manner similar to that described for compound **13b**. Yield 88%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.13 (d, J = 8.7 Hz, 1H), 7.94 (d, J = 7.2 Hz, 1H), 7.37−7.14 (m, 7H), 6.76 (s, 2H), 4.94 (dd, J = 13.2 Hz, 9.0 Hz, 2H), 4.48 (d, J = 3.6 Hz, 1H), 4.43 (s, 1H), 4.40−4.29 (m, 1H), 4.16 (d, J = 14.1 Hz, 1H), 4.04−3.94 (m, 2H), 2.81−2.71 (m, 2H), 2.13 (s, 6H), 1.83−1.71 (m, 2H), 1.40−1.68 (m, 9H), 1.37 (s, 3H). HRMS (FAB): HRMS (FAB) m/z: calcd for $C_{31}H_{42}N_4O_5SNa$) $[M + Na]^+$ 605.2774; found 605.2769.

(*R*)-*N*-Allyl-3-[(2*S*,3*S*)-3-(4-amino-2,6,-dimethyphenoxyacetyl)-amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (13j). Compound **13j** was prepared from compound **5a** in a manner similar to that described for compound **13b**. Yield 92%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.16 (t, J = 5.9 Hz, 1H), 8.11 (d, J = 8.6 Hz, 1H), 7.34−7.14 (m, 7H), 6.86 (s, 2H), 5.47 (br, 1H), 5.84−5.71 (m, 1H), 5.21 (dd, J = 17.1 Hz, 1.7 Hz, 1H), 5.02 (dd, J = 10.4 Hz, 1.7 Hz, 1H), 4.96 (d, J = 4.0 Hz, 2H), 4.47 (d, J = 3.8 Hz, 1H), 4.44 (s, 1H), 4.41−4.37 (m, 1H), 4.17 (d, J = 14.1 Hz, 1H), 4.01 (d, J = 14.3 Hz, 1H), 3.72 (br, 2H), 2.85−2.71 (m, 2H), 2.15 (s, 6H), 1.51 (s, 3H), 1.37 (s, 3H). HRMS (FAB) m/z: calcd for $C_{29}H_{38}N_4O_5SNa$ $[M + Na]^+$ 577.2461; found 577.2456.

(*R*)-*N*-(2-Methylallyl)-3-[(2*S*,3*S*)-3-(4-amino-2,6-dimethyphenoxyacetyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (13k). Compound **13k** was prepared from compound **5v** in a manner similar to that described for compound **13b**. Yield 45%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.15 (t, J = 5.9 Hz, 1H), 8.06 (d, J = 9.2 Hz, 1H), 7.34−7.15 (m, 7H), 6.58 (s, 2H), 5.47 (br, 1H), 4.96 (d, J = 4.1 Hz, 2H), 4.90 (s, 1H), 4.73 (s, 1H), 4.52−4.29 (m, 3H), 4.12 (d, J = 15.0 Hz, 1H), 3.94 (d, J = 14.5 Hz, 1H), 3.78 −3.54 (m, 2H, overlapped with H_2O), 2.81−2.69 (m, 2H), 2.09 (s, 6H), 1.66 (s, 3H), 1.52 (s, 3H), 1.38 (s, 3H). HRMS (FAB) m/z: calcd for $C_{30}H_{40}N_4O_5SNa$ $[M + Na]^+$ 591.2617; found 591.2623.

(*R*)-*N*-(*cis*-4-Hydroxy-2-buten-1-yl)-3-[(2*S*,3*S*)-3-(4-amino-2,6-dimethyphenoxyacetyl)amino-2-hydroxy-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (13l). Compound **13l** was prepared from compound **5w** in a manner similar to that described for compound **13b**. Yield 76%. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 8.18−8.06 (m, 2H), 7.35−7.16 (m, 7H), 6.75 (s, 2H), 5.58−5.47 (m, 1H), 5.39−5.28 (m, 1H), 4.95 (dd, J = 14.7 Hz, 9.1 Hz, 1H), 4.47 (d, J = 3.8 Hz, 1H), 4.39 (s, 1H), 4.38−4.31 (m, 1H), 4.15 (d, J = 14.1 Hz, 1H), 4.03−3.96 (m, 2H), 3.80−3.69 (m, 2H, overlapped with H_2O), 2.81−2.70 (m, 2H overlapped with DMSO), 2.13 (s, 6H), 1.50 (s, 3H), 1.36 (s, 3H). HRMS (FAB) m/z: calcd for $C_{30}H_{40}N_4O_6SNa$ $[M + Na]^+$ 607.2566; found 607.2573.

Water Solubility Test. Compounds were suspended in pure water, left under sonication for 30 min, then cooled to room temperature. The saturated solutions were passed through a centrifugal filter (0.45 nm filter unit, Ultrafree-MC, Millipore). The filtrate was analyzed using RP-HPLC.

HIV Protease Inhibition. HIV protease inhibitory activity of the test compounds was determined on the basis of the inhibition of the HIV protease substrate (H-Lys-Ala-Arg-Val-Tyr-Phe(*p*-NO_2)-Glu-Ala-Nle-NH_2) cleavage using recombinant HIV-1 protease. HIV protease substrate was synthesized by solid phase methods. Recombinant HIV-1 protease was purchased from Bachem AG, Bubendorf, Switzerland. In the inhibition assay, 25 μL of 200 mM 2-(*N*-morpholino)ethanesulfonic acid (MES)−NaOH buffer (pH 5.5), containing 2 mM dithiothreitol, 2 mM EDTA−2Na, and 1 M NaCl was mixed with 5 μL of the inhibitor (500 nM) dissolved in DMSO and 10 μL of HIV-1 protease (2 μg/mL) in 50 mM AcOH (pH 5.0) containing 1 mM EDTA−2Na, 25 mM NaCl, 0.2% 2-mercaptoethanol, 0.2% Nonidet P-40, and 10% glycerol. The reaction was initiated by adding of 10 μL of a 1.0 mM substrate solution. After incubation for 15 min at 37 °C, the reaction was terminated by the addition of 1 N HCl, and the N-terminal cleavage fragment (H-Lys-Ala-Arg-Val-Tyr−OH) was separated by reversed-phase HPLC on a C18

column (3.0 mm × 75 mm. YMC Pack ODS AS-3E7) with a linear gradient of CH_3CN in 0.1% aqueous TFA at a flow rate of 1.0 mL/min, and its quantity was determined by monitoring fluorescence intensity (excitation, 275 nm; emission, 305 nm). In the case of 1 nM assay, 10 nM inhibitor dissolved in DMSO and HIV-1 protease (0.04 μg/mL) were used, and the sample was incubated for 3 h at 37 °C. K_i values were estimated by fitting the data from several substrate and inhibitor concentrations to standard equations for tight binding competitive inhibitors with a similar procedure described in ref 13a.

Anti-HIV Activity. Anti-HIV activity of test compounds was determined on the basis of inhibition of HIV-induced cytopathic effect in MT-4 cells in vitro as previously reported.[28] HIV-1 wild-type (IIIB, pNL4-3) or IND-R was inoculated to MT-4 cells in a 96-well plate. The resulting culture was treated with an equal volume of a 1% DMSO solution of each test compound with several concentrations and 10% fetal bovine serum and was incubated for 5 days in a CO_2 incubator at 37 °C, in triplicate. After treatment with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), the optical density of the plate was measured and the percent cytopathic effect reduction was calculated. Then EC_{50} values were estimated by fitting the data to a median-effect equation. Cytotoxicity (TD_{50}) was determined by incubation in the absence of the virus.

X-ray Crystallographic Analysis. Preparation of HIV protease for crystallization was performed as reported.[8] The hanging drop vapor diffusion method was used for crystallization. Crystals were set up with a 2-fold molar excess of inhibitors to protease of 2.0 mg/mL concentration. The reservoir solution consisted of 126 mM phosphate buffer at pH 5.0, 63 mM sodium citrate, and 0.2 M ammonium sulfate. The obtained crystal was soaked into the precipitant solution containing 45% (w/v) glycerol, then flash-frozen under a N_2 gas cryostream (100 K). Data were collected at the BL41XU beamline in SPring-8 and processed using HKL2000.[32] The crystals of HIV protease/**13k** complex belong to the monoclinic space group $P2(1)2(1)2$, with unit cell dimensions $a = 58.2$ Å, $b = 85.8$ Å, $c = 46.5$ Å, $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$. The structure was refined to a crystallographic R-factor of 10.1% (free R-factor = 11.8%) at 0.88Å resolution using the program SHELX-97.[33]

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Supporting Information Available: Results of HPLC and elemental analysis of target compounds, QSAR equations, and synthetic details for preparation of **4a**−**w** and **5a**−**w**. This material is available free of charge via the Internet at http://pubs.acs.org.

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