Development of a New Type of Protease Inhibitors, Efficacious against FIV and HIV Variants

Taekyu Lee,† Van-Duc Le,† Dongyeol Lim,† Ying-Chuan Lin,‡ Garrett M. Morris,‡ Andrew L. Wong,† Arthur J. Olson,‡ John H. Elder,*,‡ and Chi-Huey Wong*,†

Contribution from the Department of Chemistry and the Skaggs Institute for Chemical Biology and the Department of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Received August 12, 1998

Abstract: Based on the structural analysis of FIV protease and drug-resistant HIV proteases and molecular modeling, a new type of inhibitors with a small P3 residue has been developed. These inhibitors are effective against HIV and its drug-resistant mutants, as well as SIV and FIV. Modification of existing HIV protease inhibitors by reducing the size of the P3 residue has the same effect. This finding provides a new strategy for the development of HIV protease inhibitors effective against the wild-type and drug-resistant mutants. It further supports the use of FIV protease as a useful model for drug-resistant HIV proteases, which often have a more constricted binding region for the P3 group or the combined P3 and P1 groups.

The aspartyl protease (PR) of human immunodeficiency virus (HIV) has been the subject of extensive research for the development of therapeutically useful inhibitors to control the progression of human acquired immunodeficiency syndrome (AIDS). Four competitive inhibitors[1] of this enzyme have been approved, and several others are in clinical trials. Despite the high potency and high selectivity of these inhibitors used in AIDS therapy, many drug-resistant variants of HIV have been identified,[2] including 45 distinct drug-resistant variants found in the past 3 years. The drug-resistant mutants are generated through incomplete suppression of the virus by inhibitors and usually contain multiple substitutions in their proteases.[2] Moreover, these mutant enzymes often exhibit cross-resistance to many structurally distinct protease inhibitors.[2] Therefore, development of new broad-based protease inhibitors efficacious against a wide spectrum of HIV variants may be necessary in order to slow the development of drug resistance.

As a first step toward this goal, we have developed potent inhibitors against feline immunodeficiency virus protease (FIV PR), which has been shown to be a useful animal model for drug-resistant mutant HIV PRs.[3,4] FIV is a retrovirus which causes an immunodeficiency syndrome in cats comparable to AIDS in humans.[5] Both HIV and FIV PRs are C_2-symmetric homodimeric enzymes, and they have almost superimposable active-site structures that facilitate catalysis by an identical mechanism.[3,6] Similar to HIV PR, FIV PR also processes both structural proteins of *gag* and the enzymes encoded by *pol* during FIV replication.[7] Furthermore, six mutated residues in HIV PR causing drug resistance (K20I, V32I, I50V, N88D, L90M, Q92K)[2a,8] are found in the structurally aligned native residues of FIV PR. Kinetic studies have also demonstrated that various potent HIV PR inhibitors which interact with the binding region from S4 to S4′ are less efficient inhibitors of FIV PR by a factor of 100 or more,[3,4] and good inhibitors of FIV PR are often better inhibitors of the wild-type and drug-resistant HIV PRs.[4] In addition to the observation that FIV PR resembles many known drug-resistant HIV PRs, the cat offers a potential animal system to test the effectiveness of anti-lentiviral agents *in vivo* to speed up the drug development process.

† Department of Chemistry and the Skaggs Institute.
‡ Department of Molecular Biology.

(1) (a) Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359−1472. (b) De Lucca, G. V.; Erickson-Viitanen, S.; Lam, P. Y. S. *Drug Discovery Today* **1997**, *2*, 6−18. (c) Vacca, J. P.; Condra, J. H. *Drug Discovery Today* **1997**, *2*, 261−272. (d) Huff, J. R. *J. Med. Chem.* **1991**, *34*, 2305. (e) Wlodawer, A.; Erickson, J. W. *Annu. Rev. Biochem.* **1993**, *62*, 543−585.

(2) (a) Schinazi, R. F.; Larder, B. A.; Mellors, J. W. *Int. Antiviral News* **1997**, *5*, 129−142. (b) Wilson, S. I.; Phylip, L. H.; Mills, J. S.; Gulnik, S. V.; Erickson, J. W.; Dunn, B. M.; Kay, J. *Biochim. Biophys. Acta* **1997**, *1339*, 113−125. (c) Erickson, J. W.; Burt, S. K. *Annu. Rev. Pharmacol. Toxicol.* **1996**, *36*, 545−571. (d) Gulnik, S. V.; Suvorov, L. I.; Liu, B.; Yu, B.; Anderson, B.; Mitsuya, H.; Erickson, J. W. *Biochemistry* **1995**, *34*, 9282−9287. (e) Erickson, J. W. *Nature Struct. Biol.* **1995**, *2*, 523−529. (f) Condra, J. H.; Schleif, W. A.; Blahy, O. M.; Gabryelski, L. J.; Grayyam, D. J.; Quintero, J. C.; Rhodes, A.; Robbins, H. L.; Roth, E.; Shivaprakash, M.; Titus, D.; Yang, T.; Teppler, H.; Squires, K. E.; Deutsch, P. J.; Emini, E. A. *Nature* **1995**, *374*, 569−571. (g) Otto, M. J.; Garber, S.; Winslow, D. L.; Reid, C. D.; Aldrich, P.; Jadhav, P. K.; Patterson, C. E.; Hodge, C. N.; Cheng, Y.-S. E. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7543−7547. (h) Wong, J. K.; Hezareh, M.; Gunthard, H. F.; Halvir, D. V.; Ignacio, C. C.; Spina, C. A.; Richman, D. D. *Science* **1997**, *278*, 1291−1295 (i) Finzi, D.; Hermankova, M.; Pierson, T.; Carruth, L. M.; Buck, C.; Chaisson, R. E.; Quinn, T. C.; Chadwick, K.; Margolick, J.; Brookmeyer, R.; Gallant, J.; Markowitz, M.; Ho, D. D.; Richman, D. D.; Siliciano, R. F. *Science* **1997**, *278*, 1295−1300.

(3) Slee, D. H.; Laslo, K. L.; Elder, J. H.; Ollmann, I. R.; Gustchina, A.; Kervinen, K.; Zdanov, A.; Wlodawer, A.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 11867−11878.

(4) Lee, T.; Laco, G. S.; Torbett, B. E.; Fox, H. S.; Lerner, D. L.; Elder, J. H.; Wong, C.-H. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 939−944.

(5) (a) Talbott, R. L.; Sparger, E. E.; Lovelace, K. M.; Fitch, W. M.; Pedersen, N. C.; Luciw, P. A.; Elder, J. H. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5743−5747. (b) Pedersen, N. C.; Ho, E. W.; Brown, M. L.; Yamamoto, J. K. *Science* **1987**, *235*, 790−793.

(6) Wlodawer, A.; Gustchina, A.; Reshetnikova, L.; Lubkowski, J.; Zdanov, A.; Hui, K. Y.; Angleton, E. L.; Farmerie, W. G.; Goodenow, M. M.; Bhatt, D.; Zhang, L.; Dunn, B. M. *Nat. Struct. Biol.* **1995**, *2*, 480−488.

(7) Kramer, R. A.; Schaber, M. D.; Skalka, A. M.; Ganguly, K.; Wong-Staal, F.; Reddy, E. P. *Science* **1986**, *231*, 1580−1584.

(8) Mellors, J. W.; Larder, B. A.; Schinazi, R. F. *Int. Antiviral News* **1995**, *3*, 8−13.

10.1021/ja982893p CCC: $18.00
Published on Web 02/03/1999

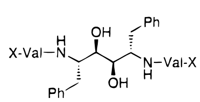
Table 1. Inhibition of FIV and HIV PRs by Small P3 Residue-Containing Inhibitors and Their Parent Compounds[a]

compd	FIV PR,[b] K_i (nM)	HIV PR[c] K_i (nm)	HIV PR[c] IC_{50} (nM)	HIV (G48V),[c] IC_{50} (nm)	HIV (V82F),[c] IC_{50} (nM)
1a	17000 ± 300[d]	1.1 ± 0.2[d]			
1b	41 ± 7[d]	1.5 ± 0.3[d]	3.8	20.5	14.9
1c	nd	nd	5.3	158	60.8
1d	nd	nd	13.3	277	64.4
2a	ni		60000[e]		
2b	7300 ± 300	499 ± 81			
3a	ni		300000[e]		
3b	9400 ± 900	308 ± 70			
4a	ni		2000[e]		
4b	212 ± 23	5.4 ± 0.7	10.3	131	86.1
5a	ni	214[e]			
5b	46 ± 5	2.5 ± 0.4	7.8	68.7	44.4
6b	3700 ± 600	3.0 ± 0.6	5.0	34.5	24.0
7a	2600 ± 300	1.5 ± 0.2	4.0	26.1	13.3
7b	133000 ± 38000	11.3 ± 1.3			
RO31-8959	76000 ± 300	1.6 ± 0.6	4.4	106	26.5

[a] K_i and IC_{50} values were determined in duplicate using fluorescent substrate (see ref 4 for procedures; for HIV PR substrate, see Toth, M. V.; Marshall, G. R. *Int. J. Pept. Res.* **1990**, *36*, 544). For a different assay condition, see ref 2b. nd, not determined. ni, No inhibition at 800 μM of inhibitor. [b] Data were obtained at pH 5.25 at 37 °C in 0.1 M NaH_2PO_4, 0.1 M sodium citrate, 0.2 M NaCl, 0.1 mM DTT, 5% glycerol, and 5% DMSO in volume. [c] Data were obtained at pH 5.25 at 37 °C in 0.1 M MES, 5% glycerol, and 5% DMSO in volume. [d] From ref 4. [e] From ref 3.

Results and Discussion

Redesign of C_2-Symmetric Inhibitors with small P3 Residues. Our initial work on the development of protease inhibitors efficacious against both HIV and FIV was focused on the systematic analysis of the S3 and S3′ subsite specificities of the enzymes using a series of C_2-symmetric inhibitors containing (1*S*,2*R*,3*R*,4*S*)-1,4-diamino-1,4-dibenzyl-2,3-butanediol as a P1 and P1′ core and Val as P2 and P2′ residues.[4] We have demonstrated that FIV PR exhibited a strong preference for small hydrophobic groups at the S3 and S3′ subsites, in contrast to the high flexibility for the P3 and P3′ residues binding to HIV PR.[4] Kinetic studies have also indicated that the binding preference observed in drug-resistant mutant HIV PRs is very similar to that found in FIV PR.[4] In addition, the most potent FIV PR inhibitor, **1b**, strongly inhibits FIV, HIV, and SIV infections in tissue culture with virtually the same degree of effectiveness.[4]



1a X = Cbz
1b X = Cbz-Ala

The X-ray structure of FIV PR complexed with inhibitor **1b** has been determined[9] and has shown that the P1 and P3 side chains are positioned very closely, consistent with previous structural studies for HIV and FIV PRs that show that S1 and S3 subsites are neighboring hydrophobic pockets that accommodate the corresponding P1 and P3 side chains.[1e,4] Models of **1b** bound to HIV and FIV PR (Figure 1) indicate that the S1 and S3 subsites in HIV PR constitute a much larger hydrophobic pocket than the corresponding subsites found in FIV PR. Because of its smaller size, FIV PR can only accommodate inhibitors with a smaller size for P1 and P3 residues together, and several drug-resistant HIV PRs are, indeed, found to have smaller S3 and S1 subsites. Based on the X-ray structures of HIV and FIV PRs complexed with inhibitors,[1e,4] the residues which define the S subsites are shown in Figure 2. In HIV PR, Pro 81 and Val 82 are components for both S1 and S3 subsites since they can affect binding of both P1 and P3 moieties,[11] whereas Ile 98 and Gln 99 are the structurally aligned residues for FIV PR.[6] The X-ray structures also revealed that three residues at S3 and S3′ subsites, Gly 48, Pro 81, and Val 82 of HIV PR, were replaced with Ile 57, Ile 98, and Gln 99 in FIV PR.[6,11] As a result, the S3 and S3′ subsites of FIV PR are sterically more congested than those in HIV PR, and these three different residues may define the S3 and S3′ subsite specificities of the enzymes.[6] The results of these studies point to a new direction for development of inhibitors effective against both HIV PR and its drug-resistant variants.

Asymmetric Inhibitors with Small P3 Groups. The S3 and S3′ subsite specificities of FIV PR and drug-resistant HIV PRs with mutations affecting the S3 subsite were investigated further to determine if there is a correlation between them. The new inhibitors of HIV PR were synthesized with Ala at P3, Val or Asn at P2, and various Phe-Pro isosteric cores for the P1−P1′ residues (Figure 3). The inhibitory activities of each compound against FIV, HIV, and drug-resistant mutant HIV PRs were determined as described previously,[4] and the results are summarized in Table 1.

Each compound tested in this study is a competitive inhibitor and is significantly more potent against HIV PR than FIV PR, as expected. However, the new inhibitors **1b**−**6b** and **7a** showed a remarkably different pattern of inhibitory activities compared to their parent compounds **1a**−**5a**, ABT−538, and RO31-8959. Compounds **2a**−**4a**, containing no P3 moieties, exhibited marginal activities, with IC_{50} values in the range of 2−300 μM, against HIV PR and did not show any significant inhibition of FIV PR at 800 μM.[3] Only the α-keto amide **5a** showed reasonable potency against HIV PR, with a K_i value of 214 nM.[3] On the other hand, the modified inhibitors **2b**−**5b**, which contain a methyl group as the P3 residue, displayed 120−1000-fold improved inhibitory activities against HIV PR and at least 3 orders of magnitude higher potency for FIV PR compared to

(9) The X-ray structures of **1b** complexed with HIV, FIV (3X), FIV (V59I), and FIV (Q99V) PRs have been determined and will be published separately: Li, M.; Morris, G.; Lee, T.; Laco, G. S.; Wong, C.-H.; Olson, A.; Elder, J. H.; Wlodawer, A.; Gustchina, A. Submitted.

(10) Schnölzer, M.; Rackwitz, H.-R.; Gustchina, A.; Laco, G. S.; Wlodawer, A.; Elder, J. H.; Kent, S. B. H. *Virology* **1996**, *224*, 268−275.

(11) Hosur, M. V.; Bhat, T. N.; Kempf, D. J.; Baldwin, E. T.; Liu, B.; Gulnik, S.; Wideburg, N. E.; Norbeck, D. W.; Appelt, K.; Erickson, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 847−855.

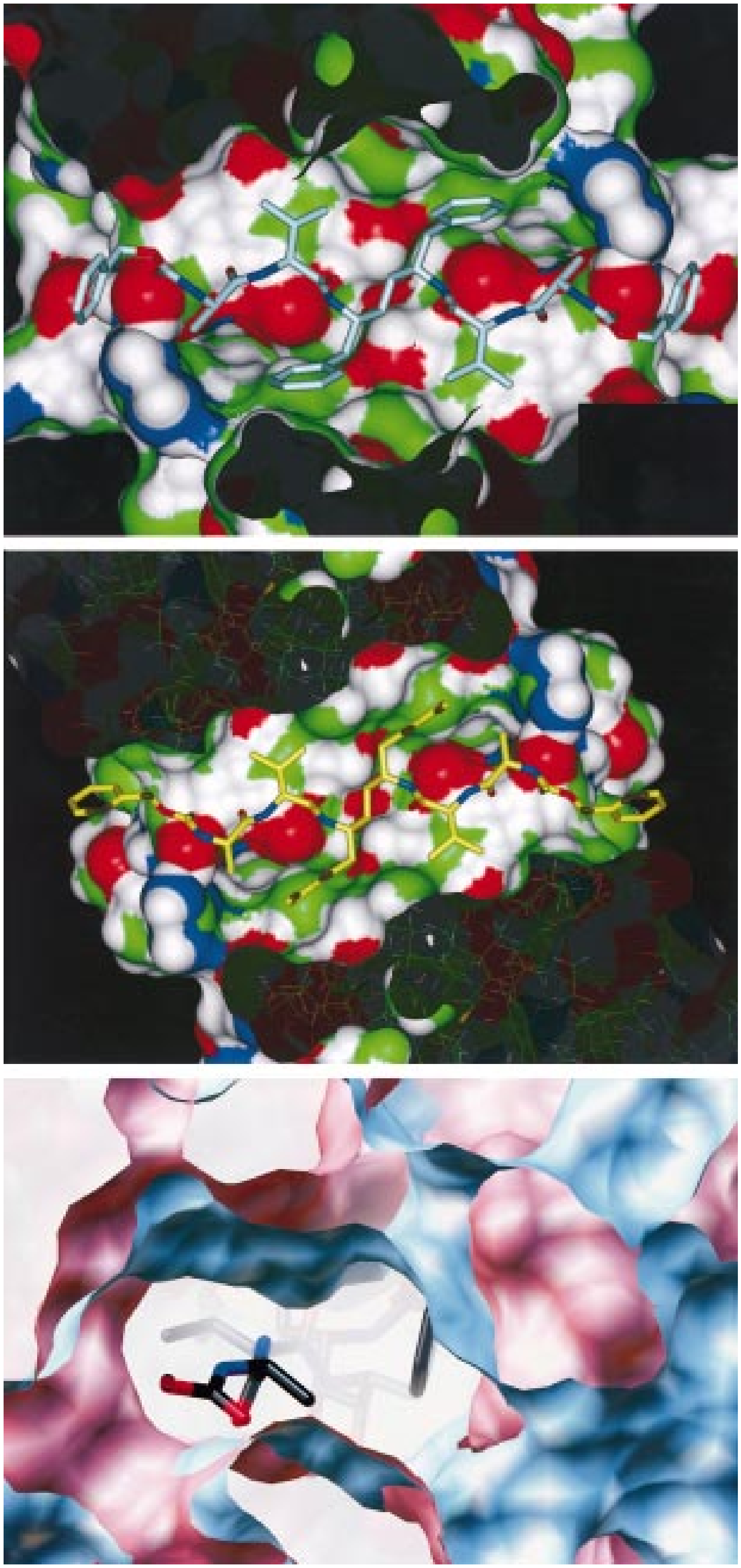


Figure 1. Models of HIV protease (top) and FIV protease (middle) complexed with **1b**, indicating the small S3 site in FIV protease and the close proximity of the P3 (CH_3) and P1 ($PhCH_2$) residues, a structural feature found in many drug-resistant HIV proteases. Bottom: a cut-away view of the molecular surfaces of HIV (pink) and FIV PR (blue), in which the clipping plane is parallel to the plane of the two aspartates and cuts through the P3 site.

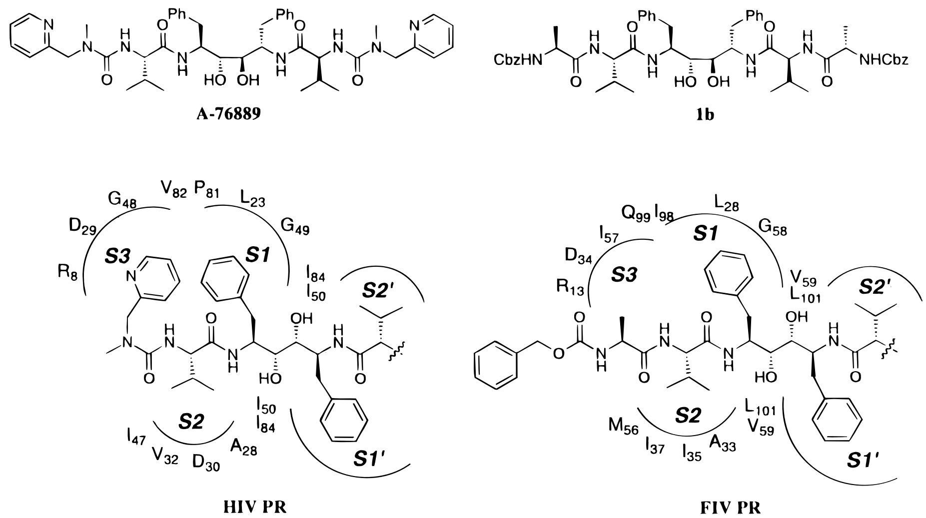


Figure 2. Amino acid side chains in the S subsites interacting with the inhibitors.

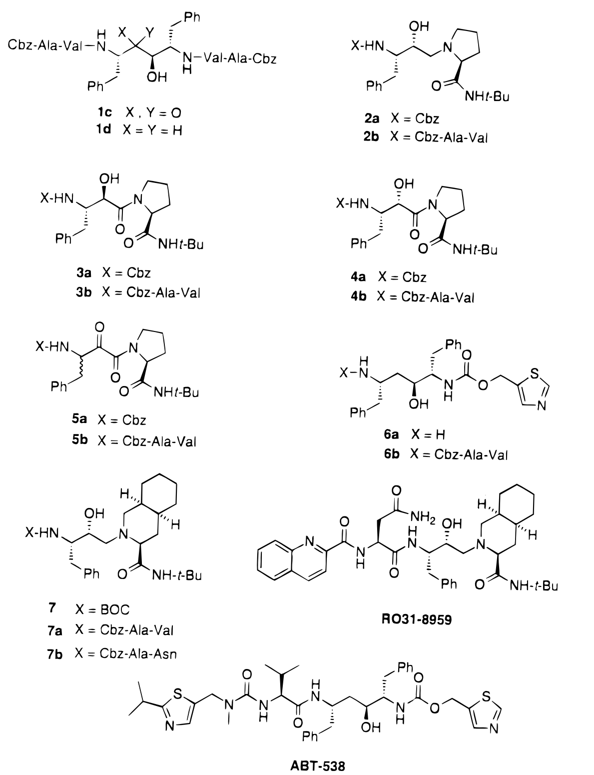


Figure 3. Dissymmetric inhibitors with small P3 residues. Compounds **2b**−**6b** and **7** contain a methyl group as the P3 residue.

their parent compounds. In particular, **5b** was found to be a slow binding inhibitor, with K_i values of 2.5 and 46 nM against HIV and FIV PR, respectively. This level of potency against FIV PR by the inhibitor with a molecular weight of only 649 was truly remarkable, considering that the smallest efficient substrate for the enzyme is an eight-residue peptide, Ac-Pro-Gln-Ala-Tyr∼Pro-Ile-Gln-Thr.[10] Since the ketone moiety is not hydrated in aqueous solution according to ^{13}C NMR studies, the increased inhibitory activity of **5b** may occur via enzyme-assisted hydration of the ketone moiety within the active site to form a *gem*-diol as transition-state mimic, similar to the case of a related α-keto amide inhibitor observed previously by X-ray structure and ^{13}C NMR analyses[3] (Figure 4).

The relative inhibitory activities of inhibitors **2b**−**5b** against FIV PR are similar to the relative activities against HIV PR. For example, **5b** is a superior inhibitor of both enzymes, and

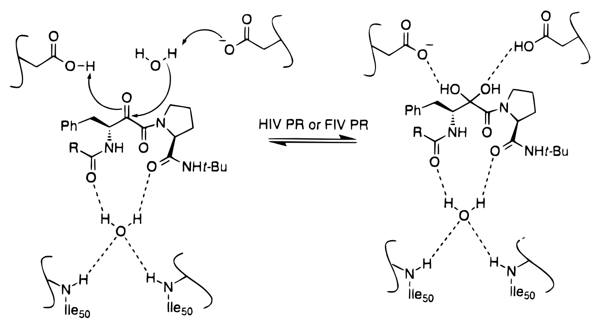
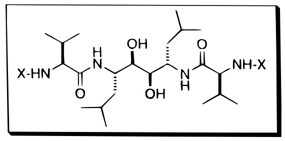


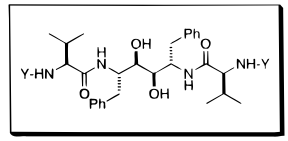
Figure 4. Proposed mechanism of inhibition by **5b**. A water molecule is added, with assistance of the enzyme, to the α-keto group to form a *gem*-diol.

the hydroxyamide **4b** is 57- and 44-fold more effective than its diastereomer **3b** against HIV and FIV PR, respectively. In addition, the relative effectiveness of the P1−P1′ core structures in **2b**−**5b** against HIV PR is also consistent with the binding pattern[3] of their parent compounds **2a**−**5a**. These results indicate that introduction of Val and Ala as P2 and P3 residues can improve the inhibition against both enzymes despite some changes for the P1−P1′ core unit. In addition, extension of the backbone of an inhibitor to contain an appropriate P3 moiety is essential to exhibit high potency against FIV PR, which is also consistent with our previous results[4] with the C_2-symmetric inhibitor **1b**.

The kinetic results of inhibitors **6b** and **7a**, which were modified from existing drugs ABT-538 and RO31-8959 (Saquinavir), respectively, were even more promising. Compound **7a** was found to have K_i values of 1.5 nM and 2.6 μM against HIV and FIV PR, respectively. The inhibitory activities of the original drug RO31-8959, were also evaluated under the same assay conditions, and it was found to have K_i values of 1.6 nM and 76 μM for HIV and FIV PR, respectively. These results clearly indicated that **7a** retained the original inhibitory activity of RO31-8959 against HIV PR, which was already optimized for the wild-type enzyme, but showed 29-fold enhanced potency against FIV PR. Inhibitor **6b** also exhibited activities comparable to those of **7a**, with K_i values of 3.0 nM and 3.7 μM for HIV and FIV PR, respectively. However, **7b** was 7.5- and 51-fold less potent than **7a** against HIV and FIV PR, respectively. Changing the P2 Val residue of **7a** to Asn increased the hydrophilicity of the inhibitor, which could be a major cause of lower activity found in **7b** for both enzymes. In

Table 2. Inhibition of FIV and HIV PRs by C_2-Symmetric Diols[a]





inhibitor (X)	FIV PR[b] K_i (nM)	FIV PR[b] IC50 (nM)	HIV PR,[c] K_i (nM)	inhibitor (Y)	FIV PR[b] K_i (nM)	FIV PR[b] IC50 (nM)	HIV PR,[c] K_i (nM)
8 (Ala-Cbz)	62 ± 9	235	6.5 ± 1.3	**1b** (Ala-Cbz)	41 ± 7[d]	72	1.5 ± 0.3[d]
9 (Leu-Cbz)	230 ± 34	11600	0.87 ± 0.12	**9b** (Leu-Cbz)	159 ± 15[d]	8200	1.4 ± 0.3[d]
10 (Phe-Cbz)	487 ± 20	29300	5.5 ± 0.8	**10b** (Phe-Cbz)	7000 ± 500[d]	92400	2.6 ± 0.4[d]
11 (Val-Cbz)	248 ± 47	18800	nd	**12** (Ser-Cbz)	32 ± 5	66	0.58 ± 0.1
				13 (Thr-Cbz)	142 ± 25	7250	7.7 ± 1.9
				14 (Abu-Cbz)	nd	95	nd
				15 (Nva-Cbz)	nd	6700	nd
				16 (Nle-Cbz)	nd	29400	nd

[a] K_i values were determined in duplicate. nd, not determined. [b] Data were obtained at pH 5.25 at 37 °C in 0.1 M NaH_2PO_4, 0.1 M sodium citrate, 0.2 M NaCl, 0.1 mM DTT, 5% glycerol, and 5% DMSO in volume. [c] Data were obtained at pH 5.25 at 37 °C in 0.1 M MES, 5% glycerol, and 5% DMSO in volume. [d] From ref 4.

fact, **7b** was more soluble in water than **7a** and thus would require higher desolvation energy to bind the hydrophobic active sites of enzymes. It is interesting to note that desymmetrization of **1b** by changing one hydroxy group to a ketone (**1c**) or a methylene (**1d**) reduces the HIV PR potency by 1.4- or 3.5-fold, respectively. The above results suggest that inhibitors with a small P3 residue are effective against both FIV PR and HIV PR. These results are consistent with the results of molecular modeling, which show HIV and FIV PR binding to Saquinavir and its modified derivative with a small P3 residue (Figure 5).

Inhibition of Drug-Resistant Mutant HIV PRs. The modified inhibitors with dual efficacy against FIV and HIV PR (**1b**–**d**, **4b**, **5b**, **6b**, and **7a**) were also tested against drug-resistant mutant HIV PRs G48V and V82F. These mutant enzymes were selected since Gly 48 and Val 82 are within the S3 and S3′ subsites[6,11] and have been identified as some of the most frequently mutated residues associated with development of drug resistance.[2] Against these mutant enzymes, all modified inhibitors retained most of their original potency, and their relative inhibitory activity was also directly proportional to the efficacy against wild-type HIV PR. In particular, modification of the FDA-approved drugs ABT-538 and RO31-8959 containing a bulky P3 group to the ones with a methyl group at P3 (i.e., **6b** and **7a**) showed significantly improved inhibitory activity against the mutants, with only 3.2- and 6.5-fold higher IC_{50} values for the V82F and G48V mutant variants, respectively, compared to 6- and 27-fold higher IC_{50} values for the parent compounds. It is noteworthy that V82F and G48V mutants are known to be less efficient enzymes than the wild-type HIV PR.[2b,d] Therefore, inhibitory activities of compounds tested against these mutant enzymes are expected to be lower compared to wild-type HIV PR. Compound **1b** was also active in cell culture.[12] The IC_{90} values of **1b** against the wild-type HIV PR and the I84V and 82F/84V mutants are 0.1, 0.4, and 0.9 μM, respectively.

***C_2*-Symmetric Inhibitors with an *iso*-Butyl-2,3-diol Core as P1 and P1′.** Since the side chains of P1 and P3 residues of **1b** are positioned very close to each other in the S1 and S3 subsites of the enzyme, we have investigated the activities of new C_2-symmetric inhibitors (**8**–**11**) with an *iso*-butyl group at P1 and P1′. In addition, since water molecules were identified in the S3 and S3′ subsites of both HIV and FIV PR complexed with **1b**,[9] compounds (**12**, **13**) containing a hydroxy group at P3 and P3′ residues were synthesized with the hope that further improvement in inhibition would be obtained, as favorable H-bonding interactions of these polar side chains with the water molecule may occur.

The inhibitory effects of each C_2-symmetric inhibitor on HIV and FIV PRs were determined, and the results are recorded in Table 2. For comparison, the K_i values for inhibition of FIV and HIV PR by the C_2-symmetric inhibitors with a benzyl group at P1 and P1′ are also included (**1b**, **9b**, **10b**).

All the C_2-symmetric diols tested in this study showed competitive inhibition of both the feline and human lentivirus PRs in every case, but with at least an order of magnitude higher potency against HIV PR. Among the inhibitors with *iso*-butyl groups at the P1–P1′ core (**8**–**11**), compound **8,** with Ala at P3 and P3′, is the best inhibitor of FIV. This maximum inhibitory activity observed in **8** was reduced by increasing the size of the side chain of the P3 and P3′ residues. In fact, the measured K_i values of inhibitors **9**, **10**, and **11** were 3.7-, 7.9-, and 4-fold higher, respectively, than that of **8**. This specificity for small hydrophobic groups at P3 and P3′ sites found among the C_2-symmetric inhibitors **8**–**10** with *iso*-butyl groups as the P1–P1′ core against FIV PR was consistent with the observation for the analogous inhibitors **1b**, **9b**, and **10b**. In addition, **8** and **9** exhibited almost the same degree of potency against FIV PR compared to the benzyl analogues **1b** and **9b**, respectively. This result indicates that Leu can bind the S1 and S1′ subsites of the enzyme as effectively as Phe. In the previous report,[4] the explanation for the observed preference for small P3 and P3′ moieties in FIV PR was that bulky groups at the P3 and P3′ positions of the inhibitor could provoke unfavorable interactions at the sterically congested S3 and S3′ subsites of FIV PR. In particular, **10b** (K_i = 7.0 μM) exhibited 170-fold lower potency than **1b**. This proposal is still valid for the lower inhibitory activities of **9**, **10**, and **11** compared to that of **8** against FIV PR. However, it is noteworthy that **10** showed 14-fold higher inhibitory activity than its analogue **10b**. This improved potency against FIV PR observed in **10** cannot be explained by the above proposal, since both inhibitors contain Phe as P3 and P3′ residues. Therefore, current observations also suggest that the steric interaction between neighboring P1 and P3 side chains is another crucial factor to be considered in the design of new inhibitors. Indeed, **10**, containing smaller P1 and P1′ side chains than inhibitor **10b**, can provide more room at S3 and S3′ binding

(12) Bacheler, L. T.; Paul, M.; Jadhar, P. K.; Otto, M.; Stone, B.; Miller, J. *Antiviral Chem. Chemother*. **1994**, *5*, 111.

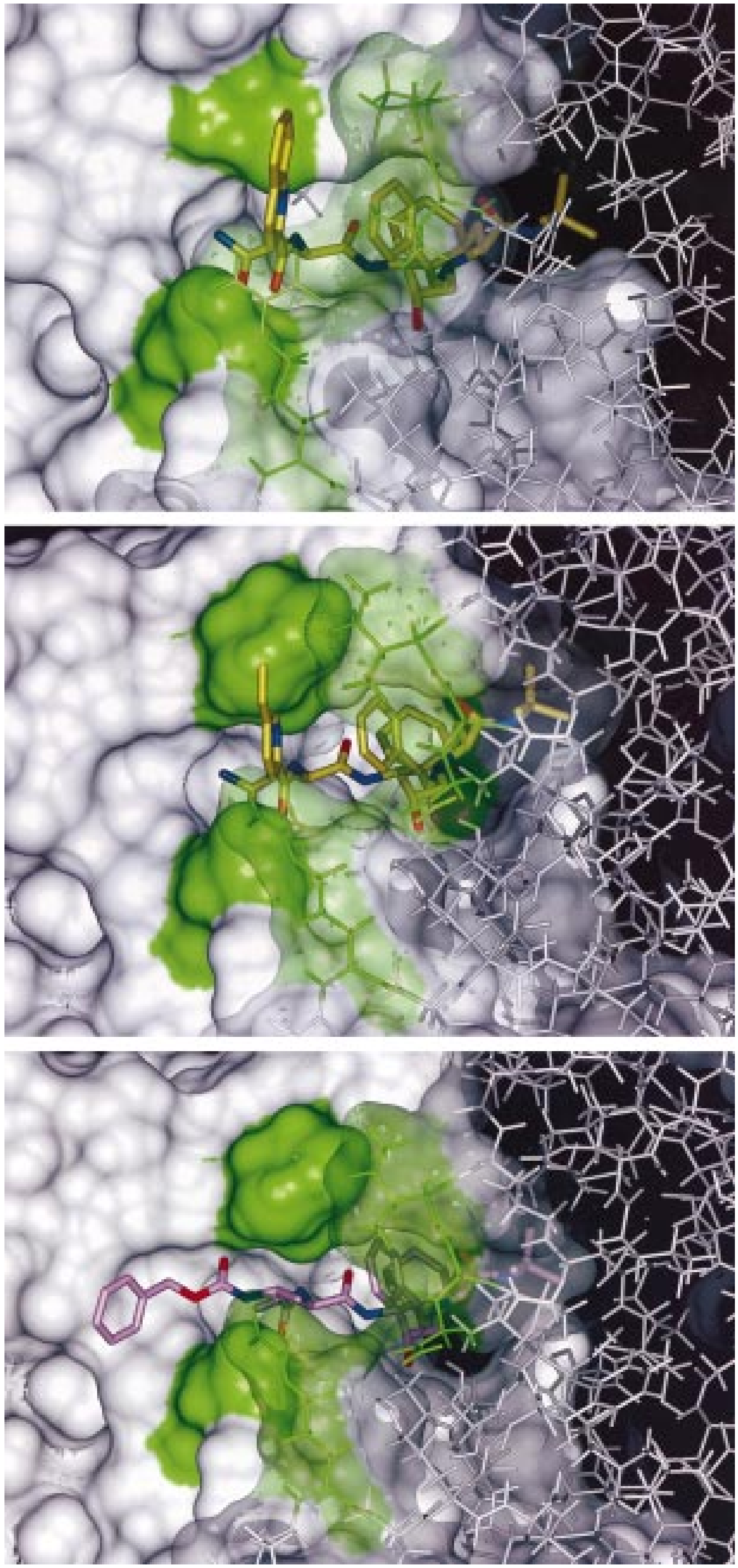


Figure 5. Models of HIV protease (top) and FIV protease (middle) complexed with RO31-8959, and FIV PR complexed with the modified inhibitor **7a** (bottom). The P3 group of RO31-8959 is too big to fit the S3 subsite of FIV PR, whereas **7a**, with methyl group as P3, residue shows a good fit.

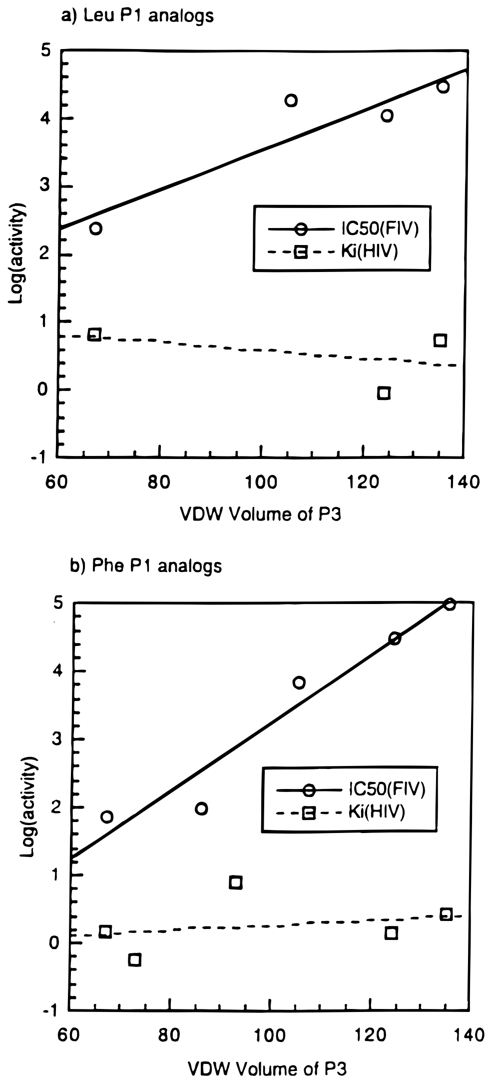


Figure 6. Effect of the size of the P3 side chain (based on the van der Waals volume in Å^3) on the inhibition of FIV and HIV proteases.

sites to accommodate bulky benzyl groups. This proposal was further confirmed by the modeling of FIV PR complexed with **8**. The structure reveals that the side chains of P1 and P3 residues are positioned closely, and the combined S1 and S3 pocket is not wide enough to accommodate two benzyl groups. It is also expected that binding of P1 and P3 moieties can be affected by each other, and an appropriate combination of P1 and P3 residues is essential for good binding. Figure 6 shows the effects of the size of P3 side chain on the inhibition of FIV and HIV proteases.

It has been determined that HIV PR exhibits a high degree of flexibility in binding at the S3 and S3′ subsites.[4] The results from the inhibition studies of the diols **8**−**10** against HIV PR showed very different patterns compared to their analogues **1b**, **9b**, and **10b**. The K_i values of **8** and **10** were 4.3- and 2.0-fold higher than those of **1b** and **10b**, respectively. This indicates that Phe at P1 and P1′ is better than Leu for binding to HIV PR. However, **9** is a more effective inhibitor than **9b** and also showed 7.5- and 6.3-fold higher potency against HIV PR compared to **8** and **10**, respectively. This significant improvement was not observed from **1b**, **9b**, and **10b**. These kinetic results indicate that introduction of *iso*-butyl groups at S3 and S3′ subsites can improve the potency of an inhibitor containing a medium-size P1−P1′ core and suggest that the overall size of the combined P1 and P3 residues will significantly affect the binding specificity of the enzymes. We have also investigated the effect of P2 residue in **1b** on the inhibition of FIV and HIV PR and found that Val is better than L-α-aminobutyric acid (IC50 = 41 and 1400 for HIV and FIV PR), norvaline (IC50 = 137 and >10 000 for HIV and FIV PR), and norleucine (IC50 = 9800 for HIV PR).

Finally, compounds containing a hydroxy group at P3 and P3′ side chains (**12** and **13**) were also effective inhibitors of both HIV and FIV PR. In particular, **12** displayed the highest potency, with K_i values of 0.58 and 32 nM against HIV and FIV PRs, respectively. It is noteworthy that **12** is more hydrophilic than **1b** by two additional hydroxy groups and would require more desolvation energy in order to bind to the hydrophobic active sites of enzymes. However, **12** exhibited a 3-fold higher potency against HIV PR and similar inhibition against FIV PR, compared to **1b**. These results indicate that binding of **12** is significantly enhanced by the two hydroxyl groups of the P3 and P3′ side chains, presumably by promoting favorable electrostatic interactions with the crystallographic water molecules at the S3 and S3′ subsites. The ability of **12** to prevent infection of FIV in tissue culture was also examined. It was, however, not as effective as **1b**. It has been known that introduction of hydrophilic functional groups, such as a hydroxyl or carboxyl group, to the P2 and P2′ positions of C_2-symmetric inhibitors would cause a dramatic loss of potency against HIV PR in tissue culture, though the inhibitory activity in vitro has little change.[15] Our *ex vivo* assay results have confirmed a similar activity loss in tissue culture by the presence of a hydrophilic group at P3 and P3′. These results suggest that increasing the overall polarity and hydrophilicity of compounds may result in the reduction of efficacy *in vivo*.

The above results indicate that the interaction of P1 and P3 residues in the active sites of HIV and FIV PR is a crucial factor to be considered for maximizing the binding affinity of inhibitors. Although it has been considered that Phe can provide ideal binding at the S1 subsite of HIV PR,[1] our observations suggest that, with appropriate P3 and P3′ moieties, one can also develop potent inhibitors with P1 and P1′ side chains smaller than the benzyl group.

Synthesis

The key intermediates (1*S*,2*R*,3*R*,4*S*)-1,4-bis[(*N*-Cbz)amino]-1,4-dibenzyl-2,3-diol and (1*S*,2*R*,3*R*,4*S*)-1,4-bis[(*N*-Cbz)amino]-1,4-diisobutyl-2,3-diol (**17**) (Scheme 1) were prepared by the stereoselective pinacol coupling of L-Cbz-phenylalanal and L-Cbz-leucinal, respectively, using Pedersen's procedure.[16] The minor diastereomeric impurities of the coupling reaction were removed by flash column chromatography after protection of the diol as an isopropylidene derivative (**18**). The Cbz groups of **18** were removed to yield the diamine **19** by hydrogenation.

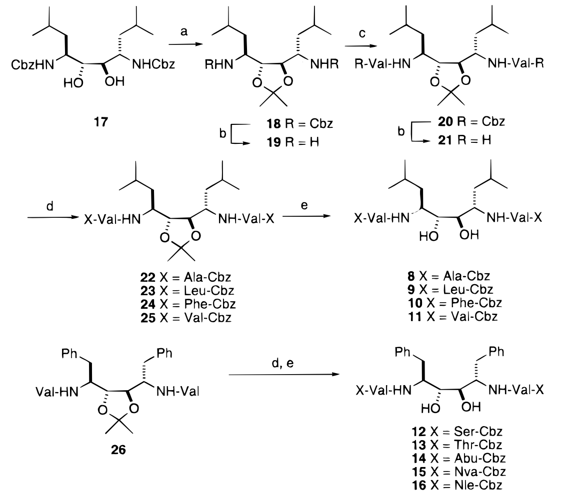
(13) (a) Kempf, D. J.; Sham, H. L.; Marsh, K. C.; Flentge, C. A.; Betebenner, D.; Green, B. E.; McDonald, E.; Vasavanonda, S.; Saldivar, A.; Wideburg, N, E.; Kati, W. M.; Ruiz, L.; Zhao, C.; Fino, L.; Patterson, J.; Molla, A.; Plattner, J. J.; Norbeck, D. W. *J. Med. Chem.* **1998**, *41*, 602−617. (b) Kempf, D. J.; Marsh, K. C.; Fino, L. C.; Bryant, P.; Craig-Kennard, A.; Sham. H. L.; Zhao, C.; Vasavanond, S.; Kohlbrenner, W. E.; Wideburg, N. E.; Saldivar, A.; Green, B. E.; Herrin, T.; Norbeck, D. W. *Bioorg. Med. Chem.* **1994**, *2*, 847−858.

(14) Thompson, W. J.; Ghosh, A. K.; Holloway, M. K.; Lee, H. Y.; Munson, P. M.; Schwering, J. E.; Wai, J.; Darke, P. L.; Zugay, J.; Emini, E. A.; Schleif, W. A.; Huff, J. R.; Anderson, P. S. *J. Am. Chem. Soc.* **1993**, *115*, 801−803.

(15) Budt, K.-H.; Peyman, A.; Hansen, J.; Knolle, J.; Meichsner, C.; Paessens A.; Ruppert, D.; Stowasser, B. *Bioorg. Med. Chem.* **1995**, *3*, 559−571.

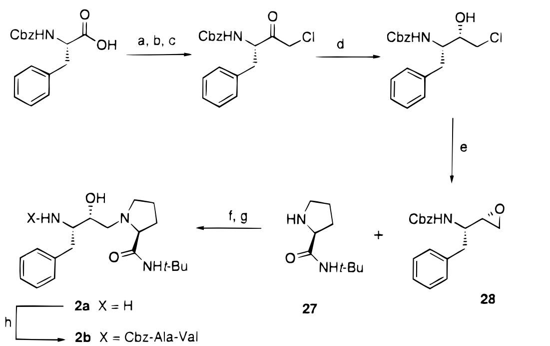
(16) Konradi, A. W.; Pedersen, S. F. *J. Org. Chem.* **1992**, *57*, 28−32.

Scheme 1[a]



[a] Conditions: (a) 2,2-dimethoxypropane, *p*-TsOH (80%); (b) Pd/C, H_2, MeOH (99%); (c) HBTU, Cbz-Val, Et_3N, CH_3CN (89%); (d) HBTU, Cbz-amino acids, Et_3N, CH_3CN; (e) *p*-TsOH, MeOH.

Scheme 2. Synthesis of **2b**[a]

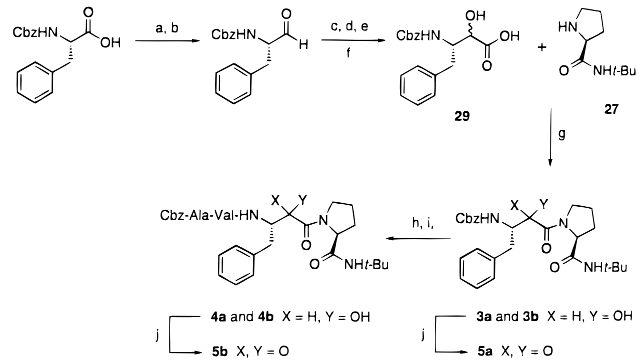


[a] Conditions: (a) NMM, THF, *i*-BuOCOCl; (b)CH_2N_2, Et_2O; (c) HCl (85%, 3 steps); (d) $NaBH_4$, EtOH (90% de, 81%); (e)NaOMe, MeOH (96%); (f) MeOH, Et_3N (90%); (g) Pd/C, H_2, EtOAc; (h) CBz-Ala-Val-OH, HBTU, Et_3N,CH_3CN (49%, 2 steps).

Amine **19** was directly coupled with Cbz-Val using HBTU[17] to give adduct **20**. Four different P3 and P3′ residues were then introduced to adduct **20** by applying the same deprotection and coupling procedures described above to give **22**−**25**. Finally, the target inhibitors **8**−**11** were prepared by deprotection of the isopropylidene group from the corresponding precursors (Scheme 1). Compounds **12**−**16** were obtained by applying the same procedure described previously using (1*S*,2*R*,3*R*,4*S*)-1,4-bis[(*N*-Cbz)amino]-1,4-dibenzyl-2,3-diol as a starting material.

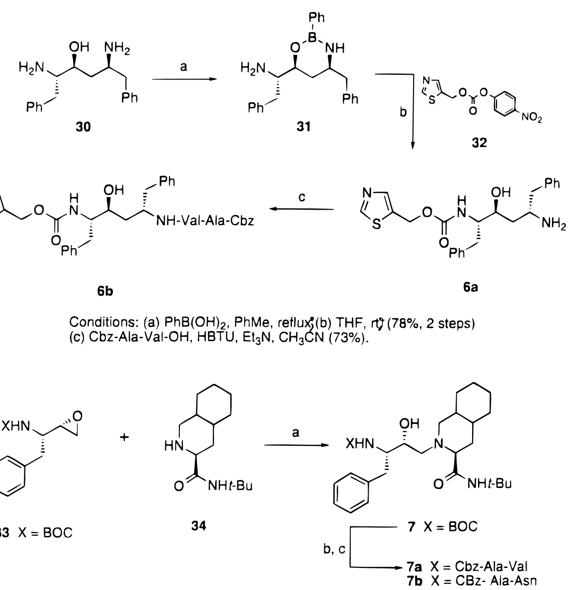
The hydroxyethylamine inhibitor **2b** was prepared by coupling the proline derivative **27** to the epoxide **28**[3,19] via reflux in methanol, using triethylamine as shown in Scheme 2. The synthesis of the core isostere **5a** has been modified from the method previously employed by our group. The α-hydroxy acid **29**, prepared from known procedures,[3,20] was coupled to the proline derivative **27** to give the α-hydroxy amides **3a** and **3b**. Hydrogenation of the diasteromers **3a** and **3b** followed by HBTU-mediated coupling with Cbz-Ala-Val-OH gave **4a** and **4b** in 65% yield. Dess−Martin oxidation[21] of the α-hydroxy amides **3a**−**4b** gave the corresponding α-keto amides **5a** and **5b** in moderate yield (Scheme 3).

Scheme 3. Synthesis of the α-Ketoamides **5a** and **5b**[a]



[a] Conditions: (a) BH_3, THF; (b) Swern oxidation (90%); (c) $NaHSO_3$, H_2O; (d) KCN; (e) HCl (6 N in dioxane); (f) Cbz-Cl, NaOH, H_2O (52%, 4 steps); (g) HBTU, Et_3N, CH_3CN (73%); (h) Pd/C, H_2, EtOAc; (i) Cbz-Ala-Val-OH, HBTU, Et_3N, CH_3CN (65%, 2 steps); (j) Dess−Martin (63%).

Scheme 4. Synthesis of the Modified Existing Drugs **6a**[a] and **7a**,**b**[b]



[a] Conditions: (a) $PhB(OH)_2$, PhMe, reflux; (b) THF, rt (78%, 2 steps); (c) Cbz-Ala-Val-OH, HBTU, Et_3N, CH_3CN (73%). [b] Conditions: (a) MeOH, Et_3N (80%); (b) TFA, CH_2Cl_2; (c) HBTU, Et_3N, CH_3CN, Cbz-Ala-Val-OH (76%) or Cbz-Ala-Asn-OH (78%).

The synthesis of **6b** began with the cyclic boronate **31** prepared by refluxing the diamine **30** with phenylboric acid.[13] Condensation of the phenyl boronate **31** with carbonate **32** followed by HBTU-mediated coupling with Cbz-Ala-Val-OH gave the desired product **6b** (Scheme 4). The synthesis of the modified Saquinavir derivatives **7a** and **7b** began with condensation of the isoquinoline derivative **34** with epoxide **33** to give the adduct **7** in 80% yield.[14] Removal of the BOC group in **7** with TFA followed by HBTU coupling with Cbz-Ala-Val-OH or Cbz-Ala-Asn-OH gave the modified inhibitors **7a** and **7b**, respectively (Scheme 4).

Conclusion

In summary, manipulation of the backbone of HIV PR inhibitors to create appropriate P3 residues can improve potency against FIV, HIV, and mutant HIV PRs, regardless of the structure of the P1−P1′ core units. The inhibitors with small

(17) Dourtoglou, V., Gross, B. *Synthesis* **1984**, 572−574.
(18) Biosym Technologies, San Diego, CA, 1998.
(19) Hung, R. R.; Straub, J. A.; Whitesides, G. M. *J. Org. Chem*. **1991**, *56*, 3849−3855.
(20) Munoz, B.; Giam, C.-Z.; Wong. C.-H. *Bioorg. Med. Chem*. **1994**, *2*, 1085−1090.
(21) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc*. **1991**, *113*, 7277−7287.

P3 and bulky P1 described in this study are effective against FIV and HIV PR and exhibit high potency against drug-resistant mutant HIV PRs. In addition, modification of some existing HIV PR inhibitors to have a small P3 group also enhances their inhibition of FIV PR and mutant HIV proteases without affecting the activity against wild-type HIV PR. The development of broad-based inhibitors described in this study contributes significantly to our understanding of the specificity and resistance development of the aspartyl protease and provides a new strategy for the development of new antiviral pharmaceuticals. Although the current results represent only an initial step toward development of therapeutic agents efficacious against both native and mutant HIV proteases, using FIV PR as a general model for the drug-resistant mutant HIV PRs is clearly an effective strategy. One may thus use cats as a drug-resistant animal model to accelerate the drug development process.

Experimental Section.

Chemical Synthesis. General Procedures. Analytical TLC was performed on precoated plates (Merck, silica gel 60F-254). Silica gel used for flash column chromatography was Mallinckrodt Type 60 (230−400 mesh). NMR (^{1}H, ^{13}C) spectra were recorded on either a Bruker AMX-400 or an Am-500 MHz Fourier transform spectrometer. Coupling constants (*J*) are reported in hertz (Hz), and chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (TMS, 0.0 ppm) with $CDCl_3$, DMSO, or CD_3OD as solvent.

All other reagents were commercial compounds of the highest purity available and purchased from the Aldrich, Sigma, Nova Biochem, or Bachem.

General Procedure for Coupling Reaction. To a solution of a free amine (1.0 mol equiv) and carboxylic acid (1.0 mol equiv) in dry CH_3CN (0.1−0.15 M) was added HBTU (1.0 mol equiv) followed by Et_3N (1.0 mol equiv) at 20 °C under Ar atmosphere. The reaction mixture was stirred for 15 min and then quenched by addition of brine and extracted with EtOAc. The organic layer was then washed sequentially with 1 N HCl, saturated aqueous $NaHCO_3$, and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography to give the desired product.

Compound 1c. Compound **1b**[4] (5.3 mg, 0.0058 mmol) was treated with a mixture of freshly prepared dimethyldioxirane in acetone (0.047 M, 0.372 mL, 3 equiv) and $CDCl_3$ (0.3 mL) at 25 °C. The reaction was monitored with analytical HPLC. After 3 days of stirring, the reaction mixture was concentrated and purified on a semipreparative C18 column (HPLC yield 63%): ^{1}H NMR (500 MHz, DMSO-d_6, 20 °C) δ 0.65−0.72 (12H, m), 1.11 (3H, d, *J* = 5.9 Hz), 1.14 (3H, d, *J* = 5.9 Hz), 1.75−1.85 (2H, m), 2.67−2.77 (2H, m), 2.82 (1H, dd, *J* = 4.1, 11.6 Hz), 3.02 (1H, bd, *J* = 10.2 Hz), 4.05−4.13 (4H, m), 4.26 (1H, bd, *J* = 5.9 Hz), 4.57−4.62 (1H, m), 4.95−5.03 (4H, m), 5.11 (1H, d, *J* = 5.2 Hz), 7.10−7.15 (4H, m), 7.16−7.23 (6H, m), 7.26−7.37 (9H, m), 7.43−7.47 (2H, m), 7.52 (1H, d, *J* = 7.5 Hz), 7.54 (1H, d, *J* = 7.4 Hz), 7.72 (1H, d, *J* = 7.4 Hz), 8.42 (1H, d, *J* = 6.8 Hz); HRMS (FAB+) calcd for MCs^{+} $C_{50}H_{62}N_6O_{10}Cs$ *m*/*e* 1139.3582, found *m*/*e* 1139.3538.

Compound 1d. (2*S*,3*S*,5*S*)-3-Hydroxy-2,5-bisamino-1,6-diphenylhexane[13b] (15.7 mg, 0.055 mmol) was coupled to *N*-Cbz-Ala-Val-OH (39 mg, 0.12 mmol) to give the product (39 mg, 79%) as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6, 20 °C) δ 0.56−0.61 (2H, m), 0.64−0.68 (2H, m), 0.72−0.76 (6H, m), 0.84−0.88 (2H, m), 1.17−1.25 (12H, m), 1.49−1.53 (2H, m), 1.79−1.91 (2H, m), 2.55−2.60 (1H, m), 2.69−2.76 (2H, m), 3.60 (1H, m), 4.03−4.14 (6H, m), 4.47 (1H, m), 5.00 (4H, m), 7.08−7.43 (20H, m); ^{13}C NMR (100 MHz, DMSO-d_6, 20 °C, major peaks) δ 17.9, 18.1, 18.2, 18.2, 19.2, 19.3, 30.7, 30.9, 37.4, 38.8, 46.8, 50.0, 53.2, 57.6, 57.7, 65.3, 68.2, 78.7, 79.2, 125.7, 125.8, 127.7, 127.74, 127.8, 127.83, 127.9, 128.0, 128.3, 129.0, 129.2, 137.0, 138.5, 139.2, 155.6, 169.9, 170.5, 172.0, 172.2, 172.4; HRMS (FAB+) calcd for MCs^{+} $C_{50}H_{64}N_6O_9Cs$ *m*/*e* 1025.3789, found *m*/*e* 1025.3748.

Compound 2b. Compound **2a**[3] (18 mg, 0.039 mmol) was hydrogenated with 10% Pd/C (15 mg) in EtOAc (2 mL) to give an amine as a colorless oil. The crude amine, Cbz-Ala-Val-OH (12 mg, 0.039 mmol), HBTU (14 mg, 0.038 mmol), and Et_3N (4.7 mg, 0.046 mmol) in CH_3CN (1 mL) gave **2b** (12 mg, 49%) as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6, 60 °C) δ 0.76 (6H, d, *J* = 6.7 Hz), 1.20 (3H, d, *J* = 7.1 Hz), 1.26 (9H, s), 1.64−1.70 (3H, br), 1.86−1.90 (1H, m), 1.98−2.00 (1H, m), 2.67 (1H, m), 2.69 (1H, m), 2.93 (1H, b), 2.96 (1H, br), 3.03 (3H, m), 3.50 (1H, br), 4.00−4.13 (3H, m), 5.04 (2H, s), 7.10−7.50 (14H, m); ^{13}C NMR (100 MHz, DMSO-d_6, 60 °C) δ 18.0, 19.2, 28.3, 30.0, 30.8, 34.7, 38.2, 49.5, 50.1, 52.8, 55.5, 57.7, 59.4, 65.3, 67.8, 71.0, 125.7, 127.6, 127.7, 127.8, 128.3, 129.2, 137.0, 138.8, 155.6, 170.2, 170.3, 172.0; HRMS (FAB+) calcd for MCs^{+} $C_{35}H_{51}N_5O_6Cs$ *m*/*e* 770.2894, found *m*/*e* 770.2922.

Compounds 3b and 4b. A mixture of diastereomer **3a** and **4a**[3] (85 mg, 0.170 mmol) was hydrogenated with 10% Pd/C (20 mg) in EtOAc (3 mL) to give an amine as a colorless oil. The crude amine, Cbz-Ala-Val-OH (55 mg, 0.170 mmol), HBTU (65 mg, 0.170 mmol), and Et_3N (20 mg, 0.20 mmol) in CH_3CN (4 mL) gave separable mixture of **3b** and **4b** (72 mg, 65%) as a white solid.

Compound 3b: ^{1}H NMR (400 MHz, CD_3OD, 20 °C) δ 0.82 (6H, br s), 1.28−1.33 (12H, m), 1.80−2.05 (5H, m), 2.80−2.97 (4H, m), 3.36 (1H, br), 4.10−4.20 (2H, m), 4.25 (1H, br s), 4.44 (1H, br s), 5.09 (2H, s), 7.20 (1H, br), 7.24−7.55 (13H, m); ^{13}C NMR (100 MHz, CD_3OD, 20 °C) δ 18.0, 18.3, 19.9, 25.5, 28.8, 30.7, 32.1, 38.8, 47.8, 52.0, 52.9, 60.1, 62.3, 67.7, 70.4, 127.7, 128.9, 129.0, 129.5, 129.6, 130.5, 139.4, 158.1, 172.0, 172.7, 173.8; HRMS (FAB+) calcd for MCs^{+} $C_{35}H_{51}N_5O_6Cs$ *m*/*e* 770.2894, found *m*/*e* 770.2922.

Compound 4b: ^{1}H NMR (400 MHz, DMSO-d_6, 60 °C) δ 0.74−0.77 (6H, m), 1.21−1.23 (3H, overlapping), 1.23 (9H, s), 1.81−1.91 (5H, br), 2.70 (1H, m), 2.71 (1H, m), 2.78 (1H, br), 2.89 (1H, br), 3.00 (1H, br), 3.36−3.41 (4H, m), 5.04 (2H, s), 7.16−7.44 (14H, m); ^{13}C NMR (100 MHz, DMSO-d_6, 60 °C) δ 17.1, 17.6, 18.7, 23.3, 28.1, 30.1, 36.8, 37.8, 45.7, 49.6, 50.0, 57.3, 60.1, 65.1, 69.4, 125.6, 127.1, 127.2, 127.6, 127.8, 128.6, 136.6, 138.2, 155.1, 169.9, 171.6; HRMS (FAB+) calcd for MCs^{+} $C_{35}H_{51}N_5O_6Cs$ *m*/*e* 770.2894, found *m*/*e* 770.2947.

Compound 5b. Dess−Martin reagent (22 mg, 0.074 mmol) was added to a solution of diasteromers **3b** and **4b**[3] (16 mg, 0.025 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was stirred for 12 h and then quenched by addition of brine and extracted with EtOAc. The organic layer was then washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography to give **5b** (10 mg, 63%) as a mixture of isomers (1:1): ^{1}H NMR (500 MHz, DMSO-d_4, 60 °C) δ 0.76−0.85 (6H, m), 1.16, 1.18 (3H, d, *J* = 7.0 Hz), 1.24, 1.26 (9H, s), 1.70−1.80 (3H, m), 1.82−2.08 (3H, m), 2.15−2.22 (1H, m), 2.60 (1H, dd, *J* = 10.5, 14.6 Hz), 2.96 (1H, dd, *J* = 9.1, 14.2 Hz), 3.17 (1H, dd, *J* = 4.9, 14.2 Hz), 3.24−3.26 (1H, m), 3.38−3.65 (5H, m), 4.07−4.12 (3H, m), 4.19 (1H, t, *J* = 6.6 Hz), 4.24 (2H, t, *J* = 5.7 Hz), 4.62 (1H, q, *J* = 4.3 Hz), 4.95−4.99 (1H, m), 5.01 (2H, s), 5.26 (1H, m), 7.15−7.45 (12H, m); ^{13}C NMR (100 MHz, DMSO-d_4, 60 °C) δ 17.6, 18.0, 19.1, 21.7, 24.4, 28.4, 29.1, 31.0, 32.4, 33.7, 34.2, 35.6, 47.4, 50.0, 50.2, 55.9, 56.7, 56.8, 57.1, 57.5, 59.8, 60.4, 65.3, 126.2, 126.5, 127.7, 127.7, 128.1, 128.2, 128.3, 128.4, 128.6, 128.9, 137.1, 137.2, 138.0, 140.5, 155.6, 162.0, 162.2, 169.8, 170.7, 171.0, 171.4, 172.1, 172.2, 196.0, 198.1; HRMS (FAB+) calcd for MCs^{+} $C_{35}H_{47}N_5O_7Cs$ *m*/*e* 782.2530, found *m*/*e* 782.2558.

Compound 6b. The amine **6a**[13] (20 mg, 0.047 mmol), Cbz-Ala-Val-OH (15 mg, 0.047 mmol), HBTU (18 mg, 0.047 mmol), and Et_3N (5.7 mg, 0.056 mmol) in CH_3CN (1 mL) gave the title compound **6b** (25 mg, 73%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6, 80 °C) δ 0.75−0.78 (6H, m), 1.21 (3H, d, *J* = 7.1 Hz), 1.48−1.56 (2H, m), 1.85−1.94 (1H, m), 2.62−2.79 (3H, m), 3.58 (1H, m), 3.79 (1H, m), 4.05−4.08 (1H, m), 4.10−4.13 (2H, m), 5.04 (2H, d, *J* = 2.5 Hz), 5.14 (2H, s), 7.08−7.34 (15H, m), 7.80 (1H, s), 8.98 (1H, s); ^{13}C NMR (100 MHz, DMSO-d_6, 80 °C) δ 17.4, 18.7, 30.2, 37.0, 38.0, 46.9, 50.0, 52.9, 55.5, 57.0, 57.5, 65.1, 68.6, 125.3, 127.1, 127.2, 127.4, 127.4, 127.8, 128.5, 128.6, 128.7, 133.5, 136.9, 138.2, 138.8, 142.3, 154.4, 155.0, 169.2, 170.0, 171.5; HRMS (FAB+) calcd for MCs^{+} $C_{39}H_{47}N_5O_7SCs$ *m*/*e* 862.2277, found *m*/*e* 862.2251.

Compound 7a. The amine[14] (30 mg, 0.075 mmol) prepared from the BOC derivative **7**, Cbz-Ala-Val-OH (24 mg, 0.075 mmol), HBTU

(28 mg, 0.075 mmol), and Et_3N (9.1 mg, 0.09 mmol) in CH_3CN (1 mL) gave the title compound **7a** (40 mg, 76%) as a white solid: ^1H NMR (500 MHz, DMSO-d_6, 80 °C) δ 0.73 (3H, d, *J* = 6.8 Hz), 0.75 (3H, d, *J* = 6.8 Hz), 1.19 (3H, d, *J* = 7.08 Hz), 1.26 (9H, s), 1.31−1.43 (4H, br), 1.48 (2H, br), 1.59 (1H, br), 1.63 (1H, br), 1.73 (1H, br), 1.82−1.98 (3H, m), 2.05−2.13 (2H, m), 2.57−2.66 (3H, m), 2.93−2.98 (2H, m), 4.06−4.20 (3H, m), 5.03 (2H, s), 7.09−7.39 (14H, m); ^{13}C NMR (100 MHz, DMSO-d_6, 80 °C) δ 16.9, 17.8, 18.2, 18.6, 19.3, 20.4, 24.8, 25.9, 28.4, 29.8, 30.6, 31.2, 32.4, 33.3, 35.7, 49.9, 52.1, 57.3, 58.0, 65.3, 69.8, 125.4, 127.6, 127.7, 127.8, 128.3, 129.3, 137.0, 140.2, 155.6, 170.1, 172.0, 172.7; HRMS (FAB+) calcd for MCs^+ $C_{40}H_{59}N_5O_6Cs$ *m*/*e* 838.3520, found *m*/*e* 838.3546.

Compound 7b. The amine[14] (49 mg, 0.122 mmol) prepared from the BOC derivative **7**, Cbz-Ala-Asn-OH (41 mg, 0.122 mmol), HBTU (46 mg, 0.122 mmol), and Et_3N (12.4 mg, 0.09 mmol) in DMF (1 mL) gave the title compound **7a** (42 mg, 78%) as a white solid: ^1H NMR (500 MHz, DMSO-d_6, 60 °C) δ 1.22 (3H, d, *J* = 7.1 Hz), 1.27 (9H, s), 1.31−1.85 (10H, m), 1.90 (2H, m), 2.22 (1H, br), 2.33 (1H, br), 2.49−2.58 (3H, m), 2.71 (1H, dd, *J* = 9.4, 14.3 Hz), 2.81 (1H, m), 2.91−2.94 (1H, m), 3.18−3.25 (2H, m), 3.74 (1H, m), 4.08 (1H, q, *J* = 7.3 Hz), 4.52 (1H, q, *J* = 6.3 Hz), 5.03 (2H, s), 7.13−7.35 (10H, m); ^{13}C NMR (100 MHz, DMSO-d_6, 20 °C) δ 18.3, 20.2, 21.3, 25.2, 26.0, 28.3, 29.5, 30.5, 32.8, 34.7, 35.6, 37.5, 49.5, 50.1, 50.3, 58.3, 58.5, 61.2, 65.5, 68.0, 126.7, 127.8, 127.9, 128.4, 128.7, 129.6, 137.1, 137.4, 155.7, 158.0, 158.3, 172.1, 173.2; HRMS (FAB+) calcd for MH^+ $C_{39}H_{57}N_6O_7$ *m*/*e* 721.4262, found *m*/*e* 721.4289.

Compound 18. To a solution of 1,4-bis[(*N*-Cbz)amino]-1,4-diisobutyl-2,3-diol (**17**) (450 mg, 0.90 mmol) in 2,2-dimethoxypropane (24 mL) was added a catalytic amount of *p*-TsOH. The reaction mixture was heated at 60 °C for 5 h and cooled to 20 °C. The reaction mixture was diluted with EtOAc (200 mL), and the resulting solution was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was then purified by flash chromatography to give 2,3-protected (1*S*,2*R*,3*R*,4*S*)-diastereomer **18** (467 g, 96%) as a white solid: ^1H NMR (400 MHz, DMSO-d_6, 80 °C) δ 0.80−0.87 (6H, m), 1.11−1.20 (1H, m), 1.27 (3H, s), 1.42−1.49 (1H, m), 1.53−1.61 (1H, m), 3.58 (1H, s), 3.72−3.83 (1H, m), 5.00 (1H, d, *J* = 12.8 Hz), 5.08 (1H, d, *J* = 12.8 Hz), 6.42 (1H, br s), 7.26−7.32 (5H, m); ^{13}C NMR (100 MHz, DMSO-d_6, 80 °C) δ 21.1, 22.3, 23.8, 26.4, 41.0, 48.1, 64.8, 78.9, 126.8, 127.0, 127.6, 136.7, 153.4; HRMS (FAB+) calcd for MCs^+ $C_{31}H_{44}N_2O_6Cs$ *m*/*e* 673.2254, found *m*/*e* 673.2228.

Compound 20 (General Procedure for the Coupling Reaction). Compound **18** (480 mg, 0.89 mmol) in EtOAc (30 mL) containing 10% Pd/C (170 mg) was stirred under H_2 (1atm) at 20 °C for 20 h. The reaction mixture was filtered through Celite and then concentrated in vacuo to give diamine **19** (226 mg, 93%) as a colorless oil, which was used for the coupling reaction without purification.

To a solution of diamine **19** (194 mg, 0.71 mmol) and *N*-Cbz-L-valine (377 mg, 1.50 mmol) in CH_3CN (8 mL) was added HBTU (569 mg, 1.50 mmol) followed by Et_3N (166 mg, 1.64 mmol). The reaction mixture was stirred for 15 min at 20 °C under Ar and then quenched by addition of brine (20 mL) and extracted with EtOAc (4 × 20 mL). The organic layer was washed sequentially with 1 M HCl (5 mL), saturated aqueous $NaHCO_3$ (5 mL), and saturated aqueous NaCl (5 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography to give **20** (430 mg, 82%) as a white solid: ^1H NMR (400 MHz, DMSO-d_6, 25 °C) δ 0.78−0.88 (12H, m), 1.05−1.15 (1H, m), 1.22 (3H, s), 1.47−1.63 (2H, m), 2.01 (1H, q, *J* = 6.4 Hz), 3.42 (1H, s), 3.97 (1H, dd, *J* = 9.0, 6.2), 4.02−4.10 (1H, m), 5.01 (2H, dd, *J* = 17.3, 12.6 Hz), 7.27−7.41 (7H, m); ^{13}C NMR (100 MHz, DMSO-d_6, 20 °C) δ 17.6, 19.3, 21.4, 23.9, 27.0, 30.1, 41.7, 44.5, 60.2, 65.4, 79.2, 107.3, 127.6, 127.7, 128.3, 136.8, 156.0, 171.1; HRMS (FAB+) calcd for MCs^+ $C_{41}H_{62}N_4O_8Cs$ *m*/*e* 871.3622, found *m*/*e* 871.3648.

Compound 8. Compound **20** (170 mg, 0.23 mmol) was hydrogenated with 10% Pd/C (50 mg) in EtOAc (8 mL) to give **21** (106 mg, 99%) as a colorless viscous oil. It was used for the coupling reaction without purification.

Compound **21** (20 mg, 0.043 mmol) was coupled with *N*-Cbz-Ala (20 mg, 0.089 mmol) to give **22** (28 mg, 75%) as a white solid: ^1H NMR (500 MHz, DMSO-d_6, 80 °C) δ 0.80−0.88 (12H, m), 1.15−1.20 (1H, m), 1.22 (3H, d, *J* = 7.0 Hz), 1.45−1.51 (1H, m), 1.53−1.60 (1H, m), 2.04 (1H, q, *J* = 6.2 Hz), 3.48 (1H, s), 4.06−4.15 (2H, m), 4.24 (1H, dd, *J* = 7.5, 2.5 Hz), 5.03 (2H, dd, *J* = 16.5, 11.5 Hz), 7.23 (1H, d, *J* = 9.0 Hz), 7.26−7.31 (1H, m), 7.33−7.40 (5H, m), 7.45 (1H, d, *J* = 8.9 Hz); HRMS (FAB+) calcd for MCs^+ $C_{47}H_{72}N_6O_{10}Cs$ *m*/*e* 1013.4364, found *m*/*e* 1013.4324.

General Procedure for Deprotection. To a solution of **22** (25 mg, 0.030 mmol) in MeOH (1.5 mL) was added a catalytic amount of *p*-TsOH. The reaction mixture was heated at 60 °C for 24 h and then diluted with EtOAc (10 mL). The organic solution was washed with saturated aqueous $NaHCO_3$ (2 mL) and saturated aqueous NaCl (2 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo to give free diol **21** (14 mg, 57%) as a white solid: ^1H NMR (500 MHz, DMSO-d_6, 80 °C) δ 0.82−0.88 (12H, m), 1.14−1.20 (1H, m), 1.24 (3H, d, *J* = 7.1 Hz), 1.38−1.58 (2H, m), 1.53−1.60 (1H, m), 2.01 (1H, q, *J* = 6.8 Hz), 2.86 (1H, s), 4.06−4.16 (3H, m), 5.03 (2H, s), 6.84−6.89 (2H, m), 7.25−7.70 (1H, m), 7.31−7.74 (4H, m), 7.40 (1H, d, *J* = 8.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6, 80 °C) δ 17.24, 17.5, 18.6, 21.5, 22.6, 23.6, 29.5, 41.5, 47.2, 49.8, 57.7, 65.0, 72.5, 126.9, 127.0, 127.6, 137.0, 154.54, 169.6, 171.6; HRMS (FAB+) calcd for MCs^+ $C_{44}H_{68}N_6O_{10}Cs$ *m*/*e* 973.4051, found *m*/*e* 973.4028.

The preparations of **9**−**16** were carried out using the general procedures for coupling and deprotection.

Compound 9. In a similar manner, **21** (19 mg, 0.041 mmol) was coupled to *N*-Cbz-Leu (23 mg, 0.086 mmol) to give **23** (23 mg, 58%) as a white solid: ^1H NMR (500 MHz, DMSO-d_6, 80 °C) δ 0.75−0.88 (18H, m), 1.15−1.19 (1H, m), 1.28 (3H, s), 1.40−1.70 (5H, m), 1.95−2.02 (1H, m), 3.49 (1H, s), 4.06−4.12 (2H, m), 4.20−4.28 (1H, m), 5.03 (2H, dd, *J* = 17.1, 12.0 Hz), 7.12−7.20 (1H, br), 7.22−7.41 (6H, m), 7.45 (1H, d, *J* = 9.0 Hz); HRMS (FAB+) calcd for MCs^+ $C_{53}H_{84}N_6O_{10}Cs$ *m*/*e* 1097.5303, found *m*/*e* 1097.5351.

Compound **23** (20 mg, 0.021) was deprotected to give **9** (9.0 mg, 46%) as a white solid: ^1H NMR (500 MHz, DMSO-d_6, 80 °C) δ 0.80−0.95 (18H, m), 1.12−1.19 (1H, m), 1.40−1.55 (4H, m), 1.60−1.68 (1H, m), 1.95−2.02 (1H, m), 3.17 (1H, s), 4.03−4.13 (3H, m), 5.03 (2H s), 7.00 (1H, d, *J* = 9.5 Hz), 7.02−7.10 (1H, br), 7.25−7.35 (5H, m), 7.49 (1H, d, *J* = 9.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C) δ 18.1, 19.4, 21.5, 21.9, 23.1, 23.6, 23.9, 24.2, 30.2, 40.7, 42.1, 46.9, 53.2, 57.7, 65.3, 73.2, 127.6, 127.8, 128.3, 137.1, 155.8, 170.2, 172.1; HRMS (FAB+) calcd for MCs^+ $C_{50}H_{80}N_6O_{10}Cs$ *m*/*e* 1057.4990, found *m*/*e* 1057.4954.

Compound 10. Compound **21** (22 mg, 0.047 mmol) was coupled to *N*-Cbz-Phe (30 mg, 0.098 mmol) to give **24** (30 mg, 63%) as a white solid: ^1H NMR (500 MHz, DMSO-d_6, 80 °C) δ 0.76−0.92 (12H, m), 1.15−1.20 (1H, m), 1.29 (3H, s), 1.45−1.60 (2H, m), 1.98−2.10 (1H, m), 2.78−2.85 (1H, m) 3.51 (1H, s), 4.08−4.15 (1H, br), 4.25−4.35 (2H, m), 4.95 (2H s), 7.12−7.35 (12H, m), 7.56 (1H, d, *J* = 8.5 Hz); HRMS (FAB+) calcd for MCs^+ $C_{59}H_{80}N_6O_{10}Cs$ *m*/*e* 1165.4990, found *m*/*e* 1165.4936.

Compound **24** (20 mg, 0.019) was deprotected to give **10** (11 mg, 58%) as a white solid: ^1H NMR (400 MHz, DMSO-d_6, 80 °C) δ 0.83−0.89 (12H, m), 1.18−1.25 (1H, m), 1.43−1.58 (2H, m), 2.02 (1H, q, *J* = 6.7 Hz), 2.82 (1H, dd, *J* = 14.1, 9.8 Hz), 3.04 (1H, dd, *J* = 14.2, 4.4 Hz), 3.23 (1H, s), 4.10−4.20 (2H, m), 4.32−4.38 (1H, m), 4.95 (2H, s), 6.95−7.00 (2H, m), 7.15−7.32 (10H, m), 7.54 (1H, d, *J* = 8.5 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C) δ 17.3, 18.7, 21.5, 22.6, 23.6, 29.7, 37.0, 41.5, 47.3, 55.7, 57.7, 64.9, 72.5, 125.5, 126.7, 127.0, 127.3, 127.6, 128.5, 137.4, 139.1, 156.0, 169.6, 170.6; HRMS (FAB+) calcd for MCs^+ $C_{56}H_{76}N_6O_{10}Cs$ *m*/*e* 1125.4677, found *m*/*e* 1125.4709.

Compound 11. Compound **21** (22 mg, 0.047 mmol) was coupled to *N*-Cbz-Val (25 mg, 0.098 mmol) to give **25** (31 mg, 70%) as a white solid: ^1H NMR (500 MHz, DMSO-d_6, 80 °C) δ 0.75−0.88 (18H, m), 1.12−1.19 (1H, m), 1.28 (3H, s), 1.45−1.60 (2H, m), 2.00 (2H, br s), 3.50 (1H, s), 3.92−3.99 (1H, m), 4.03−4.12 (1H, br s), 4.25−4.32 (1H, m), 4.98−5.08 (2H m), 6.89−6.95 (1H, br), 7.25−7.38 (6H, m), 7.48 (1H, d, *J* = 8.9 Hz); HRMS (FAB+) calcd for MCs^+ $C_{51}H_{80}N_6O_{10}Cs$ *m*/*e* 1069.4990, found *m*/*e* 1069.4943.

Compound **25** (24 mg, 0.025) was deprotected to give **11** (11 mg, 50%) as a white solid: ^1H NMR (400 MHz, DMSO-d_6, 80 °C) δ 0.80−0.88 (18H, m), 1.13−1.22 (1H, m), 1.41−1.56 (2H, m), 1.95−2.04

(2H, m), 3.20 (1H, s), 3.94 (1H, dd, $J = 8.9$, 6.6 Hz), 4.11 (1H, se, $J = 4.4$ Hz), 4.17 (1H, dd, $J = 8.8$, 6.6 Hz), 5.04 (2H, s), 6.74 (1H, d, $J = 8.2$ Hz), 6.94 (1H, d, $J = 9.3$ Hz), 7.25−7.35 (5H, m), 7.43 (1H, d, $J = 8.8$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6, 80 °C) δ 17.3, 18.5, 18.7, 21.5, 22.6, 23.6, 29.6, 29.7, 41.5, 47.3, 57.6, 60.0, 65.0, 72.5, 126.9, 127.0, 127.6, 137.4, 156.0, 169.7, 170.3; HRMS (FAB+) calcd for MCs$^+$ $C_{48}H_{76}N_6O_{10}Cs$ *m*/*e* 1029.4677, found *m*/*e* 1029.4701.

Compound 12. (1*S*,2*R*,3*R*,4*S*)-1,4-Bis[(*N*-Cbz)amino]-1,4-dibenzyl-2,3-diol derivative **26** was prepared by applying the procedure described.[4] Compound **26** (40 mg, 0.074 mmol) was coupled to *N*-Cbz-Ser (38 mg, 0.16 mmol) to give the adduct (60 mg, 83%) as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6, 80 °C) δ 0.66 (3H, d, $J = 6.5$), 0.72 (3H, d, $J = 6.0$), 1.31 (3H, s), 1.90 (1H, d, $J = 6.2$ Hz), 2.65−2.80 (2H, m), 3.13−3.17 (1H, m), 3.58 (2H, s), 4.07−4.16 (2H, m), 4.23−4.31 (1H, m), 4.60−4.70 (1H, m), 5.05 (2H, s), 6.90−6.99 (1H, br), 7.07−7.19 (6H, m), 7.25−7.35 (5H, m), 7.45−7.50 (1H, br); HRMS (FAB+) calcd for MCs$^+$ $C_{53}H_{68}N_6O_{12}Cs$ *m*/*e* 1113.3990, found *m*/*e* 1113.3897.

The adduct (30 mg, 0.031 mmol) was then deprotected to give **12** (15 mg, 51%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6, 80 °C) δ 0.70 (3H, d, $J = 6.5$ Hz), 0.72 (3H, d, $J = 7.0$ Hz), 1.91 (1H, se, $J = 6.5$), 2.68−2.82 (2H, m), 3.33 (1H, s), 3.60−3.65 (2H, m), 4.07 (1H, dd, $J = 9.0$, 6.5 Hz), 4.16 (1H, q, $J = 8.0$), 4.29 (1H, s), 4.37−4.43 (1H, m), 4.63−4.70 (1H, m), 5.07 (2H, s), 6.82−6.92 (1H, br), 7.05−7.40 (12H, m); ^{13}C NMR (100 MHz, DMSO-d_6, 80 °C) δ 17.6, 19.3, 30.3, 38.5, 50.5, 57.0, 57.8, 61.8, 65.5, 73.2, 125.7, 127.8, 128.4, 129.1, 136.9, 139.0, 155.9, 169.9, 170.2; HRMS (FAB+) calcd for MCs$^+$ $C_{50}H_{64}N_6O_{12}Cs$ *m*/*e* 1073.3637, found *m*/*e* 1073.3685.

Compound 13. Compound **26** (33 mg, 0.061 mmol) was coupled to *N*-Cbz-Thr (33 mg, 0.13 mmol) to give the adduct (52 mg, 85%) as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6, 80 °C) δ 0.69 (3H, d, $J = 7.0$ Hz), 0.74 (3H, d, $J = 6.5$ Hz), 1.03 (3H, d, $J = 6.5$ Hz), 1.32 (3H, s), 1.93 (1H, se, $J = 6.0$ Hz), 2.68−2.80 (2H, m), 3.60 (1H, s), 3.90−3.98 (1H, m), 4.02 (1H, dd, $J = 8.5$, 5.0 Hz), 4.20 (1H, dd, $J = 8.5$, 6.0 Hz), 4.27−4.32 (1H, m), 4.56 (1H, d, $J = 5.5$ Hz), 5.06 (2H, s), 6.65−6.75 (1H, br), 7.10−7.19 (6H, m), 7.28−7.35 (5H, m), 7.44 (1H, d, $J = 9.0$ Hz); HRMS (FAB+) calcd for MCs$^+$ $C_{55}H_{72}N_6O_{12}Cs$ *m*/*e* 1141.4263, found *m*/*e* 1141.4316.

The adduct (35 mg, 0.037 mmol) was then deprotected to give **13** (20 mg, 56%) as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6, 80 °C) δ 0.69 (3H, d, $J = 6.5$ Hz), 0.72 (3H, d, $J = 7.0$ Hz), 1.04 (3H, d, $J = 6.5$ Hz), 1.87−1.93 (1H, m), 2.68−2.80 (2H, m), 3.33 (1H, s), 3.90−4.15 (3H, m), 4.22−4.38 (2H, m), 5.06 (2H, s), 6.60−6.70 (1H, br), 7.05−7.40 (11H, m), 7.50 (1H, d, $J = 8.0$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6, 20 °C) δ 17.6, 19.3, 19.7, 30.6, 38.5, 50.6, 57.5, 60.3, 65.5, 66.8, 73.1, 125.5, 127.7, 127.8, 128.0, 128.4, 129.0, 136.9, 138.9, 156.0, 169.7, 170.2; HRMS (FAB+) calcd for MCs$^+$ $C_{52}H_{68}N_6O_{12}Cs$ *m*/*e* 1101.3950, found *m*/*e* 1101.3900.

Compound 14. Compound **26** (22 mg, 0.041 mmol) was coupled to *N*-α-Cbz-2-aminobutyric acid (19.6 mg, 0.082 mmol). The adduct was then deprotected to give **14** (16.4 mg, 43%) as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6, 20 °C) δ 0.66 (3H, d, $J = 5.5$ Hz), 0.70 (3H, d, $J = 5.6$ Hz), 0.79 (3H, t, $J = 6.2$ Hz), 1.42−1.50 (1H, m), 1.52−1.58 (1H, m), 1.78−1.84 (1H, m), 2.57−2.65 (1H, m), 2.73−2.82 (1H, m), 3.24 (1H, s), 3.90−3.95 (1H, m), 4.10 (1H, dd, $J = 7.4$, 5.4 Hz), 4.42−4.49 (1H, m), 4.65 (1H, s), 5.01 (2H, s), 7.07 (1H, t, $J = 5.8$ Hz), 7.11−7.18 (4H, m), 7.28−7.33 (1H, m), 7.32−7.37 (4H, m), 7.41 (1H, d, $J = 6.8$ Hz), 7.46 (1H, d, $J = 7.7$ Hz), 7.52 (1H, d, $J = 7.4$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6, 20 °C) δ 10.4, 17.8, 19.3, 25.2, 30.7, 38.5, 50.3, 56.1, 57.4, 65.3, 73.2, 125.6, 127.8, 128.3, 129.0, 137.0, 138.9, 155.9, 170.3, 171.4; HRMS (FAB+) calcd for MCs$^+$ $C_{52}H_{68}N_6O_{10}Cs$ *m*/*e* 1069.4051, found *m*/*e* 1069.4004.

Compound 15. Compound **26** (22 mg, 0.041 mmol) was coupled to *N*-α-Cbz-L-norvaline (20.6 mg, 0.082 mmol). The adduct was then deprotected to give **15** (14 mg, 35%) as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6, 20 °C) δ 0.67 (3H, d, $J = 6.7$ Hz), 0.70 (3H, d, $J = 6.7$ Hz), 0.83 (3H, t, $J = 7.3$ Hz), 1.15−1.34 (2H, m), 1.38−1.53 (2H, m), 1.77−1.85 (1H, m), 2.57−2.64 (1H, m), 2.73−2.80 (1H, m), 3.25 (1H, s), 3.96−4.02 (1H, m), 4.10 (1H, dd, $J = 8.7$, 6.6 Hz), 4.41−4.47 (1H, m), 4.64 (1H, s), 5.01 (2H, s), 7.05−7.10 (1H, m), 7.11−7.17 (4H, m), 7.26−7.31 (1H, m), 7.32−7.37 (4H, m), 7.44 (1H, d, $J = 8.3$ Hz), 7.49 (1H, d, $J = 9.7$ Hz), 7.51 (1H, d, $J = 9.3$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6, 20 °C) δ 13.7, 17.8, 18.7, 19.3, 22.5, 30.7, 34.0, 38.5, 50.3, 54.5, 57.3, 65.3, 73.2, 125.6, 127.7, 127.8, 128.3, 129.0, 137.0, 138.9, 155.9, 170.3, 171.6; HRMS (FAB+) calcd for MCs$^+$ $C_{54}H_{72}N_6O_{10}Cs$ *m*/*e* 1097.4364, found *m*/*e* 1097.4317.

Compound 16. Compound **26** (22 mg, 0.041 mmol) was coupled to *N*-α-Cbz-L-norleucine (21.8 mg, 0.082 mmol). The adduct was then deprotected to give **16** (20.5 mg, 50%) as a white solid: ^{1}H NMR (500 MHz, DMSO-d_6, 20 °C) δ 0.66 (3H, d, $J = 6.7$ Hz), 0.70 (3H, d, $J = 6.7$ Hz), 0.83 (3H, t, $J = 6.8$ Hz), 1.15−1.28 (4H, m), 1.40−1.48 (1H, m), 1.49−1.57 (1H, m), 1.75−1.85 (1H, m), 2.61 (1H, dd, $J = 3.9$, 13.8 Hz), 2.76 (1H, dd, $J = 9.9$, 13.8 Hz), 3.25 (1H, s), 3.94−4.01 (1H, m), 4.10 (1H, dd, $J = 8.8$, 6.6 Hz), 4.38−4.45 (1H, m), 4.63 (1H, s), 5.01 (2H, s), 7.04−7.09 (1H, m), 7.10−7.17 (4H, m), 7.27−7.31 (1H, m), 7.32−7.37 (4H, m), 7.45 (1H, d, $J = 8.4$ Hz), 7.48 (1H, d, $J = 9.6$ Hz), 7.51 (1H, d, $J = 9.6$ Hz); ^{13}C NMR (100 MHz, DMSO-d_6, 20 °C) δ 13.9, 17.8, 19.3, 21.9, 27.7, 30.7, 31.6, 38.4, 50.4, 54.8, 57.4, 65.3, 73.0, 125.6, 127.7, 127.8, 128.3, 129.0, 137.1, 138.9, 155.9, 170.3, 171.6; HRMS (FAB+) calcd for MCs$^+$ $C_{56}H_{76}N_6O_{10}Cs$ *m*/*e* 1125.4677, found *m*/*e* 1125.4729.

Molecular Modeling

Models of the protease inhibitor **1b**, complexed with HIV-1 protease and FIV protease, were built using the Brookhaven Protein Data Bank entries 1HVI[11] and 1FIV[6] as starting points. The entry 1HVI has a resolution of 1.8 Å and contains the C_2-symmetric Abbott inhibitor A77003 (*R*,*S*) co-crystallized with the HIV-1 protease of strain HIVLAI expressed in *E. coli*. The structure of A77003 is quite similar to that of **1b**. The stereochemistry of one of the “core” hydroxyls had to be inverted, and the terminal pyrimidine groups were replaced by the Cbz groups. The 1FIV structure has a resolution of 2.0 Å and contains the inhibitor LP-149 co-crystallized with the feline immunodeficiency virus protease from *Felis catus* and expressed in *E. coli*.

The model of **1b** bound to HIV-1 protease was built first, using InsightII 97.0’s Biopolymer[18] and Discover modules and the AMBER force field. Molecular mechanics minimization was performed using the conjugate gradients minimizer until the minimization converged, i.e., the derivative of the energy was less than 0.001. Distance constraints were used in the early stages of the modeling to ensure that the hydrogen bonds were preserved between the inhibitor, water, and protease. Initially, only the inhibitor model, five active-site water molecules, and the residues of the active site that were in contact with the inhibitor were allowed to move. These five waters consisted of the water bound between the tips of the flaps, and four waters, two in each subunit of the dimer, that bind inside a pocket adjacent to the Arg-8 residue in HIV (Arg-13 in FIV). The resulting model was minimized while allowing all atoms to move. A similar procedure was applied to the modeling shown in Figure 5, using HIV protease complexed with RO31-8959.

Acknowledgment. We thank Dr. M. C. Fitzgerald at Scripps for providing HIV and FIV PR substrates, Dr. George Trainor at Du Pont Pharmaceuticals for the inhibition analysis of **1b** in cell culture, and Dr. A. Wlodawer at NCI for providing the coordinate of FIV protease complexed with **1b**. Support from Grants P01GM48870 (C.H.W. and A.J.O.) and R01AI40882 (J.H.E.) from the National Institutes of Health is gratefully acknowledged. The contribution of reagents supplied by the AIDS Reagents and Reference Program of the National Institutes of Health is also gratefully acknowledged.

JA982893P