Cyclic Urea Amides: HIV-1 Protease Inhibitors with Low Nanomolar Potency against both Wild Type and Protease Inhibitor Resistant Mutants of HIV

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Received August 13, 1996[⊗]

Cyclic urea amides, a novel series of HIV-1 protease (HIV PR) inhibitors, have increased activity against drug-resistant mutants of the HIV PR. The design strategy for these inhibitors is based on the hypotheses that (i) the hydrogen-bonding interactions between the inhibitor and the protease backbone will remain constant for wild-type and mutant enzymes and (ii) inhibitors which are capable of forming many nonbonded interactions, distributed throughout the active site, will experience a lower percent change in binding energy as a result of mutation in the target enzyme than those that form fewer interactions by partial occupation of the active site. The cyclic urea amide, SD146, forms 14 hydrogen bonds and 191 van der Waals contacts to HIV PR. SD146 is a very potent antiviral agent (IC_{90} = 5.1 nM) against wild-type HIV and maintains the same or improved level of high potency against a range of mutant strains of HIV with resistance to a wide variety of HIV protease inhibitors.

Introduction

Inhibition of the virally encoded protease has been demonstrated to be an effective antiviral drug therapy against HIV infection.[1] However, emergence of resistance to protease inhibitors may ultimately limit the usefulness of such drugs. Monitoring of clinical trials of HIV PR inhibitors has documented the emergence of virus variants that are highly resistant to the prescribed protease inhibitors and that, in some cases, are also cross resistant to different structural classes of protease inhibitors.[2] More effective and durable antiviral therapy using HIV PR inhibitors may depend on developing highly potent inhibitors which are also equipotent against mutant strains resistant to major classes of protease inhibitors. In this article, we report the discovery of cyclic urea amides that are equipotent inhibitors of a set of viral strains selected for resistance to the four HIV PR inhibitors that either are approved for marketing (saquinavir, ritonavir, indinavir) or are in advanced clinical development (VX478).

Origin of Resistance

The emergence of HIV resistance to chemotherapeutic agents can be attributed to two major factors. HIV reverse transcriptase is a highly error prone polymerase; virus replication is dependent on this enzyme and may result in as many as 3.4×10^{-5} mutations per base pair per virus replication cycle.[3] In addition, HIV replication occurs at an extraordinarily high rate, and virus can accumulate to a very high total body burden. It has been estimated that in an infected person plasma virion particles are produced at the rate of 10.3×10^9 virions per day.[4] These factors facilitate the emergence of HIV drug resistance. It is very likely that mutations leading to drug resistance arise spontaneously during HIV replication but remain undetectable until the selective pressure of antiviral therapy is initiated.[5] In the presence of an antiviral drug, viable drug resistant mutants would be expected to have a replicative advantage over the wild-type virus and could thus rapidly dominate the HIV population.

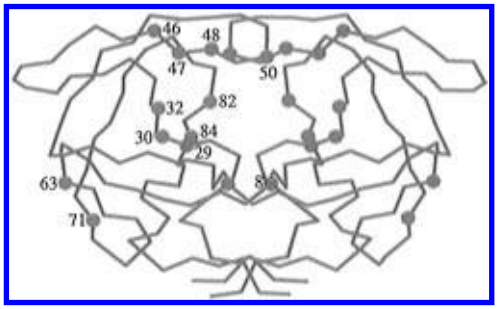

Figure 1. The C-α carbon trace of HIV-1 protease complexed with the cyclic urea inhibitor SD146. The residues associated with resistance are drawn as blue circles and the numbers correspond to the sequence positions of the amino acids. The atoms of each monomer of the homodimer are drawn in red and green, and the inhibitor was omitted for clarity. The list of residues drawn in blue circles is not a complete list of known mutations.

Mechanism of Drug Resistance

At the molecular level, a qualitative understanding of the origin of drug resistance in HIV PR due to amino acid (AA) changes in the active site of the enzyme may be developed by examination of three-dimensional (3D) structures of the enzyme−inhibitor complexes (Figure 1). For example, at position 82, the reduced sensitivity of A, T, or F mutants to C2 symmetric inhibitors, relative to the wild type virus, is due to decreased van der Waals (VDW) interactions between the P1 group of the inhibitor and residue 82 of the protease.[6] However, the precise structural role of mutations remote from the active site, often observed in association with active site mutations, is more difficult to rationalize. Some of these mutations may represent changes which do not directly confer drug resistance but compensate for AA changes in the active site.[7] In general, the origin of resistance can be attributed to the modulation of hydrophobic (e.g.

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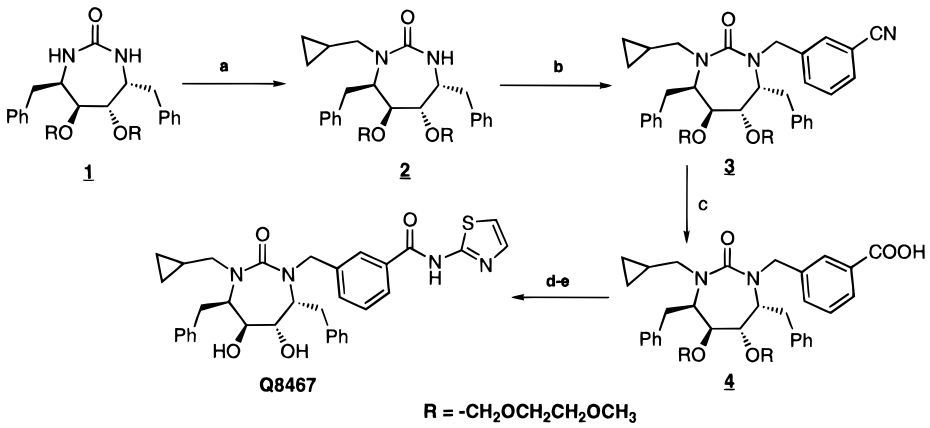
[⊗] Abstract published in *Advance ACS Abstracts,* January 1, 1997.

S0022-2623(96)00586-9 CCC: $14.00

Scheme 1. Synthesis of Nonsymmetric Cyclic Urea Amides[a]



[a] (a) 2.5 equiv of KOH, 2 equiv of (bromomethyl)cyclopropane, toluene, reflux, 86%; (b) 2.2 equiv of NaH, 1.6 equiv of 3-cyanobenzyl bromide, DMF, 83%; (c) 10 equiv of KOH, 140 °C ethylene glycol, 97%; (d) 2 equiv of DDC, 1.5 equiv of HOBT, DMF, 1.5 equiv of 2-aminothiazole; (e) 2 N HCl in 1:1 dioxane/CH_3OH, 36.2% through two steps.

48V; 50V; 82A, F, T; 84V) and electrostatic (e.g. 8Q, K, 30N) interactions between the inhibitor and AA side chains in active site of the enzyme. With the exception of 8Q and 30N mutants,[7] the largest decrease in sensitivity to the protease inhibitors is caused by reduction of the hydrophobic interactions between the inhibitor and AA side chains in the protease. In contrast, mutations which result in the loss of electrostatic interactions between the backbone of the enzyme and the inhibitor have not been described.

The first generation of protease inhibitors selected for clinical evaluation was optimized for activity against the wild-type enzyme. As drug resistant mutants were identified for each major class of HIV PR inhibitor, the need for inhibitors designed to be equally potent against a range of resistant mutants became apparent. Our approach to this problem is based on the assumption that the protease of viable drug resistant viruses would not display major alterations in the protein backbone of the enzyme. Thus electrostatic interactions between the backbone of the enzyme and the inhibitor would remain constant for the wild-type and mutant enzymes, even though interactions with side chain residues could vary as a consequence of mutation. Consequently, inhibitors designed to maximize hydrogen bonds (H bonds) to the backbone of the wild-type enzyme might retain effectiveness against mutant strains. Furthermore, we envisioned that inhibitors which are capable of forming many nonbonded interactions (viz. electrostatic, VDW, ionic, dipole−dipole, $\pi-\pi$, $\pi-$cationic, etc.), distributed throughout the active site, might have superior resistance characteristics as compared to inhibitors which only partially occupy the active site and therefore form fewer interactions with the enzyme. For example in the case of an inhibitor which occupies six subsites (S3, S2, S1, S1′, S2′, and S3′) of the enzyme, the total binding energy (ΔG_{total}) will be a sum of binding energies of six subsites (e.g. ΔG_{S3}, ΔG_{S2}, etc.; eq 1). Similarly, eq 2 can be used to represent the total binding energy of an inhibitor which occupies four subsites of the enzyme.

$$\Delta G_{total} = \Delta G_{S3} + \Delta G_{S2} + \Delta G_{S1} + \Delta G_{S1'} + \Delta G_{S2'} + \Delta G_{S3'} \quad (1)$$

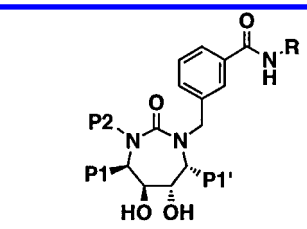
$$\Delta G_{total} = \Delta G_{S2} + \Delta G_{S1} + \Delta G_{S1'} + \Delta G_{S2'} \quad (2)$$

An inhibitor which derives its binding energy by occupation of six subsites (eq 1) is likely to have broader potency against mutant proteases than the inhibitor which occupies four subsites of the enzyme (eq 2) since the percent change in binding energy experienced by the former as a consequence of protease mutation will be lower than the latter. A solution to the drug resistance problem may thus depend on the successful design of inhibitors which are capable of forming the maximum number H bonds to the protein backbone and the maximum number of VDW contacts distributed throughout the active site of HIV PR.

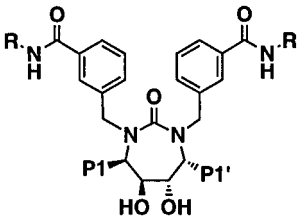
Synthesis of Cyclic Urea Amides

The cyclic urea class of inhibitors was designed to displace the structural water molecule which has been observed in complexes formed between HIV PR and linear inhibitors.[8] The synthesis and structure−activity relationship of cyclic ureas have been recently disclosed.[9,10] The nonsymmetric cyclic urea amides as represented by Q8467 were synthesized as shown in Scheme 1. The cyclic urea[9] **1** was alkylated sequentially with (bromomethyl)cyclopropane followed by 3-cyanobenzyl bromide to provide nonsymmetric bisalkylated cyclic urea **3**. Hydrolysis of the cyano derivative **3** to carboxylic acid **4** proceeded in excellent yield under aqueous alkaline conditions. Coupling of the intermediate **4** with 2-aminothiazole followed by deprotection of the MEM (methoxyethoxymethyl) protecting group provided the target compound Q8467. The peptide coupling reagents[11] such as DCC, EDC, and BOP provide amides in moderate to good yields. Alternatively, the amides can be prepared first by conversion of the acid (e.g. **4**) to the corresponding acyl chloride by treatment with oxalyl chloride followed by reaction with 2-aminothiazole, 2-aminoimidazole, or 2-aminobenzimidazole with the resulting acid chloride. Carboxylic acid derivatives with benzyl or *n*-butyl as P2 substituent were prepared in an analogous manner as described for **4**. Nonsymmetric cyclic urea amides (Figure 2) were prepared by a general procedure described for Q8467.

Symmetric cyclic urea amides as exemplified by SD146 were synthesized as shown in Scheme 2. Cyclic urea **1** was alkylated with 3-cyanobenzyl bromide to provide symmetric bisalkylated cyclic urea **5**. Hydrolysis of the biscyano derivative **5** to biscarboxylic acid **6**



Q8467 : P1 = P1' = benzyl; P2 = cyclopropylmethyl; R = 2-thiazolyl
XV655 : P1 = P1' = P2 = benzyl; R = 2-thiazolyl
XV651 : P1 = P1' = benzyl; P2 = cyclopropylmethyl; R = 2-imidazolyl
SD143 : P1 = P1' = P2' = benzyl; R = 2-imidazolyl
SD152 : P1 = P1' = benzyl; P2 = n-butyl; R = 2-imidazolyl
SD145 : P1 = P1' = benzyl; P2 = cyclopropylmethyl; R = 2-benzimidazolyl



XV638 : P1 = P1' = benzyl; R = 2-thiazolyl
XV652 : P1 = P1' = benzyl; R = 2-imidazolyl
SD146 : P1 = P1' = benzyl; R = 2-benzimidazolyl

Figure 2. Chemical structures of cyclic urea amides.

proceeded in excellent yield under basic conditions. Coupling of the intermediate with 2-aminobenzimidazole followed by deprotection of the MEM protecting group provided the target compound SD146. Symmetric cyclic urea amides (Figure 2) were prepared in an analogous manner from **6**.

Construction of Recombinant Mutant HIV Strains

A panel of isogenic recombinant mutant HIV strains was constructed in the HXB2 viral background in order to test the potency of the inhibitors against drug resistant viruses (Table 1).[12] The drug susceptibility of these recombinant HIV strains was similar to that reported[2] for drug resistant viruses of similar genotype isolated by *in vitro* or *in vivo* selection from a variety of genetic backgrounds (Table 1). An 82A virus showed little resistance to DMP323[8,9] and DMP450[13] but was 6−7-fold resistant to Q8024,[14] a linear diol, and to ABT538.[15] An 82F virus showed moderate resistance to DMP323 and DMP450 as well as to ABT538 and Q8024. An 84V virus showed greater resistance to DMP323 and DMP450 than did the 82F virus and represents the highest degree resistance to cyclic urea protease inhibitors we have observed as a result of a single AA change from the wild-type HXB2 sequence. Markowitz *et al.*[7] reported the selection of a virus with five AA changes in the protease gene which demonstrated significant resistance to ABT538 and other classes of protease inhibitors. A HXB2-based virus with the same five AA changes (46I/63P/71V/82F/84V, designated ABT538 virus) in the protease gene showed substantial resistance (7.3−93-fold) to the cyclic urea protease inhibitors tested, as well as significant resistance to ABT538 itself (30 X), MK639[16] (6.5 X), and VX478[17] (13 X) but no change in IC_{90} for RO31-8959[18] (Table 1). A second combination of five AA changes including changes at positions 82 and 84 has been described in a patient treated with MK639.[2i] The resistance of a reconstructed virus containing the same five AA changes (10R/63P/71V/82T/84V, designated MK639 virus) to each of the cyclic urea protease inhibitors was less than that of viruses containing 82F/84V mutations, although resistance to MK639[19] itself was greater. We constructed viruses resistant to RO31-8959 (48V/90M) and VX478 (46I/47V/50V), as well as viruses with protease mutations reported to confer resistance to A77003 (32I/82I and 8K/46I, Ho *et al.* and Kaplan *et al.*[2]), and these were included in our panel of drug resistant viruses (Table 1). The 32I/82I and 8K/46I viruses showed no resistance to any of the protease inhibitors we tested and are not described further.

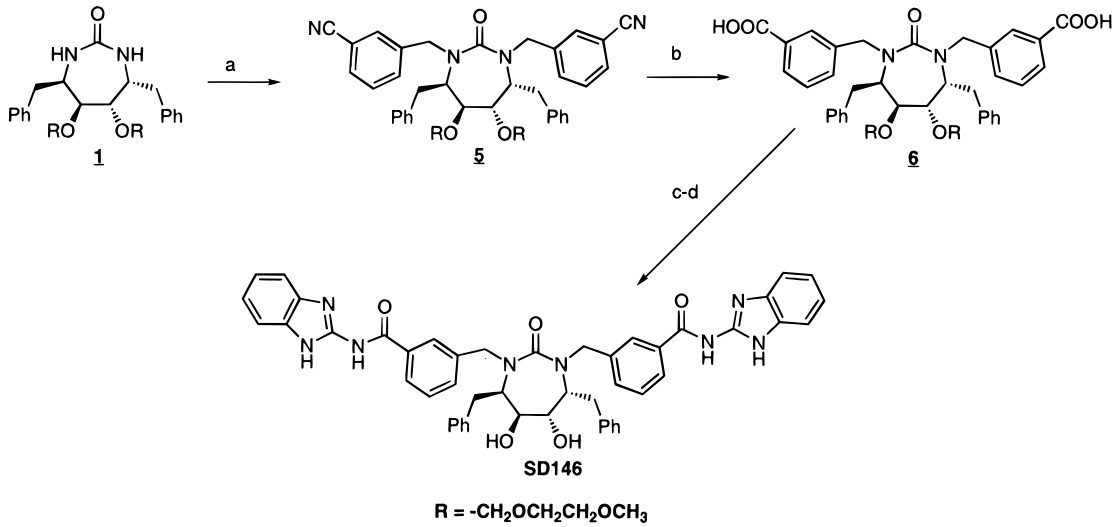
Resistance Profiles of Cyclic Urea Amides

We used constructed viruses to generate a "resistance profile", i.e. potency of a compound against the entire panel of drug resistant mutant viruses. None of the cyclic urea amides showed any significant loss of potency against the 48V/90M (Table 2), the 32I/82I, or the 8Q/46I viruses (data not shown). Indeed, some inhibitors were reproducibly at least 5-fold more potent against the 48V/90M mutant virus than against the HXB2 wild-type virus. Resistance of other viruses with position 82A virus was low, less than 5-fold, for the cyclic urea amides. Resistance of other position 82 and/or 84 mutants in general seemed to vary concordantly between the different cyclic ureas amides, with the 82F virus the least resistant and the ABT538 virus the most resistant. The mutant viruses with single AA changes (82A, F or 84V) exhibited mild (2−8-fold) degrees of resistance to the nonsymmetrical cyclic urea amides (Q8467, XV655, SD143, SD145, SD152). However, the ABT538 virus with five AA changes was more (14−86-fold) resistant to these nonsymmetrical amides. Among symmetrical cyclic urea amides, XV638 was 24-fold less potent against ABT538 virus and 23-fold less potent against the VX478 resistant virus (46I/47V/50V) as compared to the wild-type. The resistance profiles of XV652 and SD146 were exceptional. Both of these compounds maintained full potency against all of the mutant virus strains.

X-ray Structures of Cyclic Urea Amides

In order to gain an understanding of the nature of the interactions between the protease and the cyclic urea amides, the structures of HIV PR complexed with Q8467, XV638, and SD146 were determined. The overall structures of these complexes were very similar to those previously described for DMP323[8] and DMP450[13] complexed with HIV PR except that Q8467, XV638, and SD146 contain groups that bind in a third subsite (S3). The conformations of the three inhibitor molecules have been clearly defined on the basis of the residual electron density within the active sites of the proteases (Figure 3). A superposition of the bound inhibitor structures revealed that the seven-membered cyclic urea ring and the P1 and P1′ groups were not significantly altered by changes in the size or chemical nature of the P2 and P2′ groups (Figure 3). In each complex, the urea oxygen formed two H bonds to the amide nitrogens of residues Ile50/50′ of the flaps and the two diol oxygens formed a total of four H bonds to the catalytic aspartates (Asp25/25′) at the base of the pocket.

Scheme 2. Synthesis of Symmetric Cyclic Urea Amides[a]



[a] (a) 6 equiv of NaH, 4 equiv of 3-cyanobenzyl bromide, DMF, 89%; (b) 11 equiv of KOH, 140 °C ethylene glycol, 95%; (c) 8 equiv of EDC, 2.0 equiv of HOBT, Et_3N, 8 equiv of 2-aminobenzimidazole, DMF; (d) 2 N HCl in 1:1 dioxane/CH_3OH, 15.3% through two steps.

Table 1. Resistance of Reconstructed Mutant Viruses to HIV-1 Protease Inhibitors

HIV protease inhibitor	K_i (nM)[a]	IC_{90}wt (nM)[a]	resistance of constructed mutant viruses (IC_{90} mutant/IC_{90} wt)						
			82A	82F	84V	ABT538 virus[b]	48V/90M	MK639 virus[c]	46I/47V/50V
Cyclic Ureas									
XK234[d]	1.9	2100	1.4	4	6.6	7.2	1.3	11	1.7
DMP323	0.33	95	2.8	7.1	22	93	0.3	18	11
DMP450	0.34	120	2.5	5.3	9.9	49	1.5	27	7.5
Other									
ABT 538	0.37	150	7.1	8.9	2.4	30	5	5.5	6.0
MK-639	0.37	28	2.5	2.8	0.5	6.5	3.6	18	1.8
Ro31−8959	0.25	15	0.9	0.4	0.7	0.6	20	5.3	ND
VX478	0.16	81	2.2	1.9	1.5	13	1.1	3.8	39
Q8024	0.25	195	6.0	1.8	2.1	13	1.3	>150	6.5

[a] K_i's for protease inhibitors against a purified wild-type HIV-1 protease were determined as previously described.[9] IC_{90}'s were measured as described in biological methods. [b] ABT538 virus carries five mutations, 46I/63P/71V/82F/84V, and is named for its significant resistance to ABT538, while MK639 virus (*c*) carries five mutations, L10/46I/63P/82T/84V, and shows resistance to MK-639. [d] XK234 is a cyclic urea with cyclopropylmethyl at P2/P2′; also see ref 8, Table 1, compound **2** for the structure of XK234.

Table 2. Resistance of Reconstructed Mutant Viruses to Cyclic Urea Amides

inhibitor	no. of H-bond interactions[a]	K_i (nM)[b]	IC_{90} (nM)[b]	resistance of constructed mutant viruses[b] (IC_{90} mutant/IC_{90} wt)						
				82A	82F	84V	ABT 538 virus	48V/90M	MK639 virus	46I/47V/50V
Q8467	10	0.058	40	1.8	4.1	6.1	41	0.9	18.5	11
XV655	10	0.074	74	4.9	3.0	7.2	31	1.9	10	10.2
XV651	11	0.016	28	ND[c]	ND	ND	83	ND	12	ND
SD143	11	0.033	21	1	0.5	2.9	29	0.6	15	ND
SD152	11	0.025	18	1.4	14.5	4.2	27	1	14.5	ND
SD145	11	0.037	17	1.9	2.1	8.4	86	1.9	26	2.1
XV638	12	0.027	4.2	2.2	0.9	1.2	24	0.2	8.7	23
XV652	14	0.014	19	2.7	1.6	0.7	0.2	0.1	0.4	0.4
SD146	14	0.024	5.1	0.8	0.3	0.6	1.2	0.3	0.7	1.0

[a] Except for Q8467, XV638, and SD146, the number of H bonds were estimated from their models. [b] K_i's, IC_{90}'s for wild-type virus, and resistance of mutant viruses were measured as described in Biological Methods. [c] ND = not determined.

Examination of the structures of these complexes revealed differences between the individual inhibitor−protease complexes in the number of electrostatic and hydrophobic interactions formed. Q8467 is a nonsymmetric molecule that binds in two different orientations related by a pseudo-2-fold axis that is coincident with the carbonyl double bond of the cyclic urea moiety. The two nitrogen atoms near the amide junction point toward the flaps, and the carbonyl oxygen and the sulfur atom point toward the base of the pocket. The nitrogen atoms form two H bonds to Gly48′ (C═O; NH) while the carbonyl oxygen forms two hydrogen bonds to Asp29′ (NH) and Asp30′ (NH) (Figure 4). The sulfur atom of the thiazole ring is located near the side chain of Asp30′. However, no hydrogen bond could be formed between the sulfur atom and carboxy group of Asp30′ (Figure 4). The cyclopropyl group of P2 is much smaller than that of P2′ and is only involved in five VDW contacts with the protease. A total of 10 possible H bonds and 111 VDW interactions were formed between Q8467 and the enzyme (Figure 4 and Table 3). The symmetrical bisthiazole amide XV638 binds symmetrically to HIV PR with a pseudo-2-fold axis nearly coincident with the pseudo-2-fold axis relating the homodimer of HIV PR. The minor deviations from C2 symmetry are due to small differences in the torsion angles of the substituents. The electrophilic atoms of the P2 are arranged in a linear fashion just below the flaps analogous to an

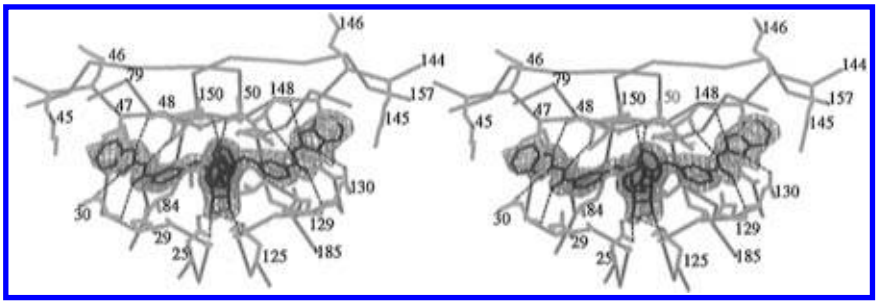


Figure 3. SD146 bound in the active site of HIV PR. The residues that interact with SD146 are drawn in blue (Table 3) and the dashed lines indicate hydrogen bonds. The C-α carbons of the enzyme are drawn in red, the numbers correspond to the sequence positions of the amino acids, and a $|2F_o - F_c|$ simulated annealed omit map of SD146 at 1.0σ is shown in pink.

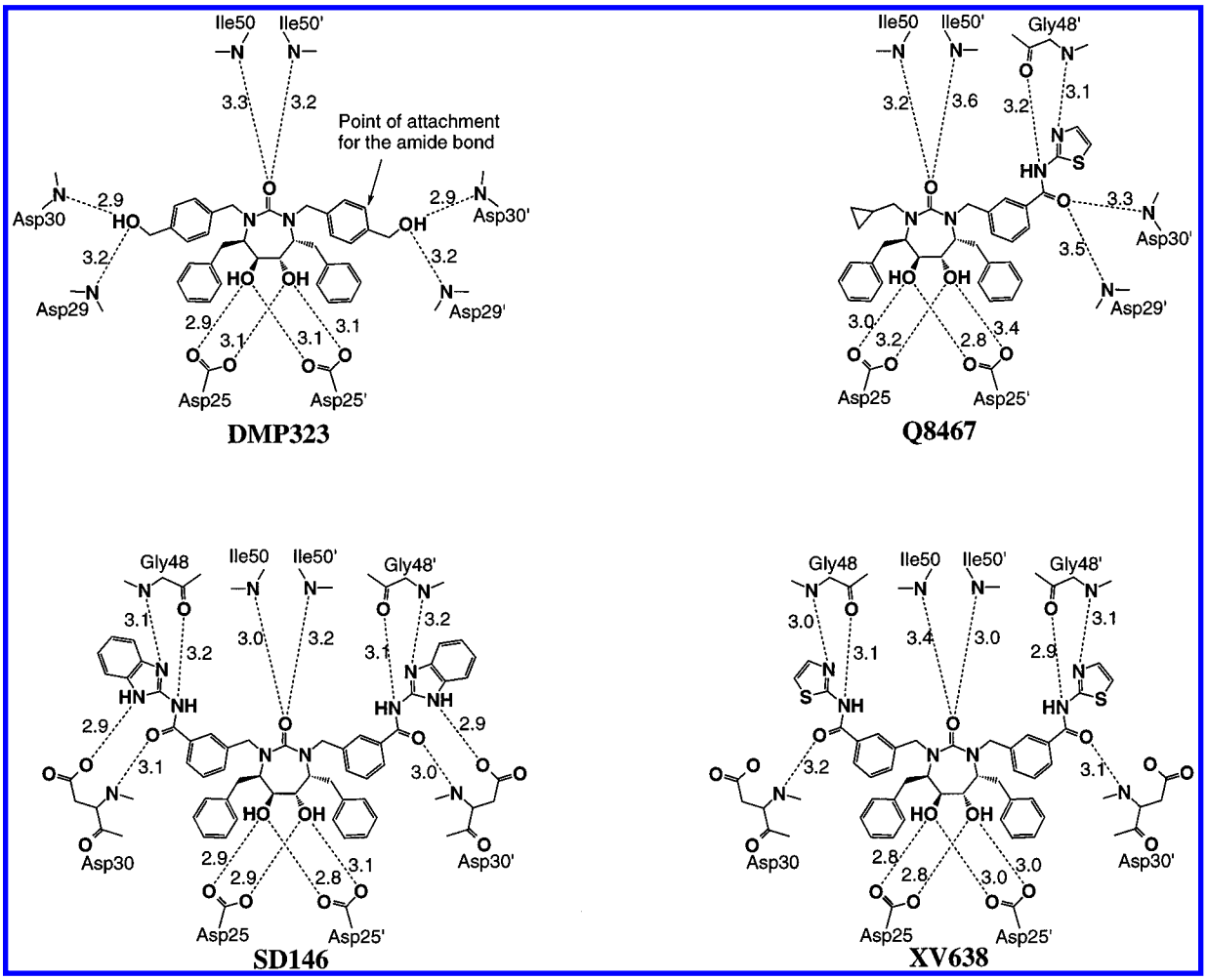


Figure 4. Schematic diagram showing the hydrogen bond interactions between the HIV PR and the inhibitors, DMP323, Q8467 (one of two orientations), XV638, and SD146.

additional strand of a β-sheet. This arrangement generates six H bonds to the flap residues (Gly48/48′, Ile50/50′), and six H bonds to the residues at the base of the pocket (Asp25/25′, Asp30/30′) (Figure 4 and Table 3). The bound conformation of SD146 is very similar to that of XV638 with the addition of solvent-exposed phenyl moieties at P3/P3′. SD146 forms two additional hydrogen bonds to Asp30/30′ for a total of 14 H bonds, which is the largest number described for a HIV PR inhibitor complex. A detailed tabulation of the electrostatic and hydrophobic interactions formed between the protease and inhibitors is given in Table 3.

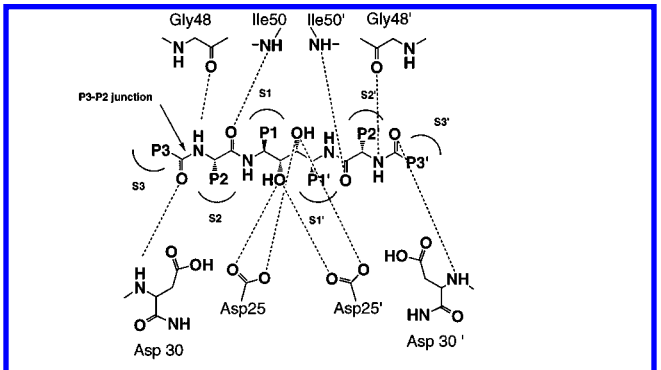
Discussion

X-ray crystallographic analysis of HIV PR complexed with DMP323 revealed that the inhibitor occupied only four of six possible subsites of the enzyme (S1/S1′ and S2/S2′) and formed 10 H bonds to Asp25/25′, Ile50/50′, Asp29/29′, and Asp30/30′. We thought that it might be possible to design more potent inhibitors that would occupy all six subsites of the enzyme and would have complementary groups for additional H bonds to the backbone of the enzyme. The structure−activity relationship in a C2 symmetric diol (Figure 5) series of protease inhibitors we studied previously suggested that the amide bond between the P2−P3 junction was critical for maintaining nanomolar binding affinity.[14] The significance of this amide bond between the P2−P3 junction became evident after examination of HIV PR complexes with linear inhibitors.[21] There is an array of H bonds donors and acceptors at the S2−S3 junction of the enzyme active site comprised of Asp29 (NH;

Table 3. Interactions between HIV-1 and Inhibitors[a]

	8 25 30 45 50 81 84																		Total number of Contacts (<4.1 Å)	Total number of H–bonds (<3.6 Å)	Buried surface area (Å^2)♦
	R	P.....	D	T	G	A	D	D	T	V	L.....	K	M	I	G	G	I	G.....T	P	V	N	I			
XM323			10		5	4	3	10		2				3	3	5	7		2	7		3			
			11		5	5	4	11		2				2	2	5	5		2	4		3	125	10	837
Q8467		2	14		5	4	0	0		0				0	2	7	9		0	2		2			
		0	12		5	6	6	5		1				6	12	1	2		0	6		2	111	10	810
XV638		2	12		5	6	3	7		1				7	13	7	10		4	2		6			
		0	12		5	3	5	9		2				7	13	6	7		3	3		6	166	12	941
SD146		2	12		5	6	2	10		2		1	1	16	11	8	8		3	2		6			
		2	12		5	5	4	12		2		4	0	8	12	6	11		3	4		6	191	14	993

[a] The boxed letters identify the wild-type residues that contact (<4.1 Å) the inhibitors. The numbers correspond to the actual number of contacts between one residue and the inhibitor. The first and second rows of numbers represent the distribution of contacts for each monomer. (♦) Amount of accessible surface area, around the inhibitor, excluded on complex formation.



P9941 : P1 = P1' = benzyl; P2 = P2'= 2-butyl; P3 = P3' = 2-pyridylmethyl
Q8024 : P1 = P1' = benzyl; P2 = P2'= 2-propyl; P3 = P3' = benzyloxy

Figure 5. Schematic diagram showing the proposed hydrogen bond interactions between the HIV PR and the *C2* symmetric inhibitors, P9941 and Q8024.

COOH), Asp30 (NH; COOH), and Gly48(C═O; NH) pointing toward the inhibitor binding cleft. Indeed, the amide linkage between P2 and P3 of the linear inhibitors made critical H bonds with Gly48 (C═O) and Asp29 (NH).[11] We thought that it might be possible to capture these hydrogen bonding interactions in the cyclic urea series by attaching a P3 group *via* an amide linkage. Attachment of P3 might also enable the formation of additional VDW interactions.

Molecular modeling suggested that the 3-position of the benzyl group of DMP323 on the P2 substituent in the cyclic urea series was the most appropriate place for attaching the P3 group. This led to the synthesis of a cyclic urea amide Q8467 containing cyclopropylmethyl at P2 and 3-(2-thiazolylaminocarbonyl) benzyl at P2′. It is a potent inhibitor of HIV PR (K_i = 0.058 nM) and a potent antiviral agent against the wild-type virus (IC_{90} = 40 nM; Table 2). Symmetric cyclic urea carboxamide XV638 containing 3-(2-thiazolylaminocarbonyl)benzyl groups at both P2 and P2′ is an even more potent inhibitor of HIV PR (K_i = 0.027 nM) and the wild-type virus (IC_{90} = 4.2 nM). Structural studies of HIV PR·Q8467 and HIV PR·XV638 complexes indicated that the heteroatoms of the amide linkage as well as the heteroatoms of the thiazole ring (CO, NH, S, and N) are within the hydrogen-bonding distances to Asp30 (NH), Gly48 (CO), Asp30 (COOH), and Gly48 (NH) respectively (Figure 4). The proximity of the sulfur atom of the thiazole moiety in XV638 to the carboxyl group of Asp30 suggested that replacing the sulfur atom with an NH would result in the formation of two additional H bonds one on each side of the C2 symmetric inhibitor.

Structures of HIV PR·DMP323,[8,20] HIV PR·Q8467, HIV PR·XV638, and HIV PR·SD146 complexes were useful in developing the correlation between the non-bonded interactions between inhibitor and enzyme and the resistance profiles of cyclic urea amides. The increase in potency against WT HIV and the retention of potency against protease inhibitor resistant viruses parallels the increasing number of H bonds formed in the inhibitor enzyme complex. There are 10 H bonds between HIV PR and DMP323, 10 between HIV PR and Q8467, 12 between HIV PR and XV638, and 14 between HIV PR and SD146. The carboxamide junction present in the 3-(2-benzimidazolylaminocarbonyl)benzyl moiety of number of these cyclic urea amides is unique. Every heteroatom of the carboxamide junction forms a hydrogen bond with a nearby residue in HIV PR. SD146 forms a total of 14 H bonds with HIV PR of which 8 H bonds are between the inhibitor and the backbone of the enzyme. These H bonds to the backbone are likely be maintained even in drug resistant mutant proteases. There are 4 H bonds between the diol moiety and the active-site aspartates. The loss of these H bonds in viable drug resistant mutant viruses is unlikely because the active-site aspartates are required for enzyme activity and the formation of fully infectious virus particles.[22] The remaining two H bonds between the NH of the benzimidazole ring and Asp30 side chains could be lost as a result of mutation; enzymes with N or Y substitutions have been reported to retain some enzymatic activity.[23] A viable virus containing a 30N mutation has recently been reported following *in vitro* selection against nelfinavir and observed in patients experiencing a viral RNA load rebound on nelfinavir therapy.[24] Thus, at least 12 if not all 14 H bonds could be maintained between SD146 and mutant proteases. A comparision of the resistance profile of XV652 (14 H bonds) and SD146 (14 H bonds) with XV638 (12 H bonds) against ABT538 and MK639 viruses suggests that the two additional H bonds in XV652 and SD146 contribute to broader potency against mutant viruses. The relative potency of XV652 in an antiviral assay is about 4-fold less than SD146; however, the resistance profile of XV652 is as good as that of SD146. It should

be pointed out that while the improved potency of SD146 and XV652 is maintained against resistant mutants and strongly correlates to our design efforts, contributions from biological components aside from intrinsic enzyme binding cannot be ruled out.

Compounds with an improved resistance profile have increased number of VDW contacts between the HIV PR and the inhibitors. The number of VDW contacts increases from 111 for Q8467 to 166 for XV638 and to 191 for SD146 (Table 3). Similarly, the buried surface of the inhibitor upon complex formation increases from 810 Å^2 for Q8467 to 941 Å^2 for XV638 and to 993 Å^2 for SD146 (Table 3). In comparision to XV638 and SD146, DMP323 and DMP450 exhibit a relatively poor resistance profile which correlated with a decreased number of H bonds and VDW interactions. The nonsymmetric cyclic urea amides (e.g. Q8467) have a reduced number of H bonds and VDW interactions as compared to the symmetric cyclic urea amides. Indeed, the resistance profile of symmetric cyclic urea amides is superior to that of the nonsymmetric cyclic urea amides. SD146 is very potent antiviral agent ($IC_{90} = 5.1$ nM) and has an exceptional resistance profile. Unfortunately, because of its extreme insolubility in water and oils, to date no formulation of SD146 could be developed for oral or intravenous administration to animals.

Conclusions

These results demonstrate that the ability of an HIV PR inhibitor to form the largest number of H bonds to the backbone and the largest number of VDW interactions in the active site correlates strongly with the retention of high potency against drug resistant mutant viruses. We believe that the remarkable resistance profile of SD146 stems from its ability to occupy all six enzyme subsites and form a large number of H bonds and extensive VDW contacts. Structure-based approaches can be successfully applied to the design of HIV-1 protease inhibitors to combat drug resistance. The structure-based design strategies developed during the course of this study may have broader implications in therapeutic areas where the potency of the drug decreases due to the mutation of the drug target.

Experimental Section

Chemical Methods. All procedures were carried out under inert gas in oven-dried glassware unless otherwise indicated. Proton NMR spectra were obtained on VXR or Unity 300 or 400 MHz instruments (Varian Instruments, Palo Alto) with chemical shifts δ in ppm downfield from TMS as an internal reference standard. Melting points were determined on a Electrothermal digital melting point apparatus and are uncorrected. Elemental analyses were performed by Quantitative Technologies, Inc., Bound Brook, NJ 08805. Mass spectra were measured with a HP 5988A mass spectrometer with particle beam interface using NH_3 for chemical ionization or a Finnigan MAT 8230 mass spectrometer with NH_3−DCI or VG TRIO 2000 for ESI. High-resolution mass spectra were measured on a VG 70-VSE instrument with NH_3 chemical ionization. Optical rotations were obtained on Perkin-Elmer 241 polarimeter at 25 °C. Solvents and reagents were obtained from commercial vendors in the appropriate grade and used without further purification unless otherwise indicated.

[4*R*-(4α,5α,6*β*,7*β*)]-1-(Cyclopropylmethyl)hexahydro-5,6-bis[(2-methoxyethoxy)methoxy]-4,7-bis(phenylmethyl)-2*H*-1,3-diazepin-2-one (2). A solution of [4*R*-(4α,5α,6*β*,7*β*)]-hexahydro-5,6-bis[(2-methoxyethoxy)methoxy]-4,7-bis(phenylmethyl)-2*H*-1,3-diazepin-2-one (**1**) (21.0 g, 42 mmol), poly(ethylene glycol) (MW 1000, 3.0 g), and potassium hydroxide (85%) (6.9 g, 105 mmol) in dry toluene (200 mL) was heated at reflux using a Dean−Stark apparatus until ∼21 mmol of water was collected. A suspension resulted. The mixture was cooled to room temperature and the treated with (bromomethyl)cyclopropane (25.5 g, 84 mmol) dropwise and heated at 60 °C for 18 h. The mixture was poured into water, and the organic layer was separated and washed with water (2 × 100 mL). The organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by flash chromatography (1 kg of silica gel) using 2:3 EtOAc:hexane to afford 20.0 g of **2** as a colorless oil (86% yield): ^{1}H NMR ($CDCl_3$) δ 0.09−0.10 (m, 2H), 0.36−0.44 (m, 2H), 0.90−0.97 (m, 1H), 1.93−1.98 (m, 1H), 2.88-2.97 (m, 1H), 2.98−3.04 (m, 1H), 3.09−3.13 (m, 1H), 3.22−3.55 (m, 3H), 3.37 (s, 6H), 3.57−3.63 (m, 4H), 3.69−3.78 (m, 3H), 3.85−4.09 (m, 4H), 4.60 (d, $J = 7.3$ Hz, 1H), 4.86 (d, $J = 9.9$ Hz, 1H), 4.88 (d, $J = 9.9$ Hz, 1H), 4.96 (d, $J = 7.7$ Hz, 1H), 4.98 (d, $J = 6.9$ Hz, 1H), 7.16−7.35 (m, 10H).

[4*R*-(4α,5α,6*β*,7*β*)]-3-[[3-(Cyclopropylmethyl)hexahydro-5,6-bis[(2-methoxyethoxy)methoxy]-2-oxo-4,7-bis(phenylmethyl)-1*H*-1,3-diazepin-1-yl]methyl]benzonitrile (3). A solution of **2** (20.0 g, 36 mmol) in dry DMF (150 mL) was cooled in a 0 °C ice bath, treated with 60% sodium hydride in mineral oil (3.2 g, 80 mmol), and stirred in the ice bath for 15 min. The mixture was then treated with α-bromo-*m*-tolunitrile (11.5 g, 58 mmol) and stirred at room temperature for 18 h. The mixture was poured into ice water and extracted with EtOAc (2 × 100 mL). The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (500 g silica gel) using 1:1 EtOAc:hexane to afford 20.0 g of **3** (83% yield): ^{1}H NMR ($CDCl_3$) δ 0.08−0.11 (m, 2H), 0.42−0.45 (m, 2H), 0.94 (m, 1H), 1.96−2.02 (m, 1H), 2.77−3.21 (m, 4H), 3.27 (d, $J = 14.3$ Hz, 1H), 3.36 (d, $J = 6.2$ Hz, 6H), 3.47−3.65 (m, 7H), 3.68−3.78 (m, 4H), 3.88−3.95 (m, 1H), 3.98−4.03 (m, 1H), 4.61 (d, $J = 14.3$ Hz, 1H), 4.72 (d, $J = 7.0$ Hz, 1H), 4.81 (d, $J = 6.9$ Hz, 1H), 4.82 (d, $J = 6.9$ Hz, 1H), 4.94 (d, $J = 7.0$ Hz, 1H), 6.92−6.94 (m, 2H), 7.16−7.52 (m, 12H).

[4*R*-(4α,5α,6*β*,7*β*)]-3-[[3-(Cyclopropylmethyl)hexahydro-5,6-bis[(2-methoxyethoxy)methoxy]-2-oxo-4,7-bis(phenylmethyl)-1*H*-1,3-diazepin-1-yl]methyl]benzoic Acid (4). A solution of **3** (14.0 g, 20.9 mmol) in dry ethylene glycol (200 mL) was treated with potassium hydroxide (14.0 g, 212 mmol) and heated at 140 °C for 18 h. The mixture was diluted with water and neutralized with concentrated HCl and extracted with EtOAc (2 × 100 mL). The organic extracts were combined and washed with water, dried over $MgSO_4$, filtered, and concentrated to afford 14.0 g of **4** as a tan oil (97% yield): ^{1}H NMR ($CDCl_3$) δ 0.10−0.13 (m, 2H), 0.43−0.45 (m, 2H), 0.95−0.97 (m, 1H), 2.00−2.07 (m, 1H), 2.98−3.23 (m, 5H), 3.35 (s, 6H), 3.41−3.48 (m, 2H), 3.51−3.62 (m, 6H), 3.68−3.76 (m, 3H), 3.88−3.94 (m, 1H), 3.99−4.03 (m, 1H), 4.64 (d, $J = 7.0$ Hz, 1H), 4.73 (d, $J = 6.6$ Hz, 1H), 4.77 (d, $J = 13.9$ Hz, 1H), 4.84 (d, $J = 6.9$ Hz, 1H), 4.93 (d, $J = 7.0$ Hz, 1H), 7.01−7.03 (m, 2H), 7.13−7.48 (m, 10H), 7.89 (s, 1H), 7.97 (d, $J = 7.7$ Hz, 1H).

[4*R*-(4α,5α,6*β*,7*β*)]-3,3′-[[Tetrahydro-5,6-bis[(2-methoxyethoxy)methoxy]-2-oxo-4,7-bis(phenylmethyl)-1*H*-1,3-diazepine-1,3(2*H*)-diyl]bis(methylene)]bis[benzonitrile] (5). A solution of **1** (61.6 g, 120 mmol) in dry DMF (300 mL) was cooled in a 0 °C ice bath. The mixture was treated with 60% sodium hydride in mineral oil (29.4 g, 735 mmol) which was added in portions followed by the dropwise addition of bromo-*m*-tolunitrile (94.1 g, 480 mmol). The mixture was stirred at room temperature for 18 h. The mixture was then cooled in a 0 °C ice bath and treated with saturated sodium bicarbonate solution. The mixture was extracted with EtOAc (3 × 150 mL). The organic layers were combined and washed with saturated sodium bicarbonate solution, water, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated and the residue purified by flash chromatography (1 kg silica gel) using 1:1 EtOAc:hexane to afford 78.1 g of **5** (88.8% yield): ^{1}H NMR ($CDCl_3$) δ 2.70−2.78 (m, 2H), 2.99−3.03 (m, 2H), 3.26 (d, $J = 14.3$ Hz, 2H), 3.35 (s, 6H), 3.48−3.81 (m, 12H), 4.61 (d, $J = 14.3$ Hz, 2H), 4.71 (d, $J = 6.6$ Hz, 2H), 4.79 (d, $J = 6.9$ Hz, 2H), 6.88−6.91 (m, 4H), 7.22−7.55 (m, 14H).

[4*R*-(4α,5α,6*β*,7*β*)]-3,3′-[[Tetrahydro-5,6-bis[(2-methoxyethoxy)methoxy]-2-oxo-4,7-bis(phenylmethyl)-1*H*-1,3-diazepine-1,3(2*H*)-diyl]bis(methylene)]bis[benzoic acid] (6). A solution of **5** (70 g, 96 mmol) in dry ethylene glycol (600 mL) was treated with potassium hydroxide (70 g, 1.06 mol) and the mixture heated at 140 °C for 18 h. The mixture was diluted with water, acidified with concentrated HCl, and extracted with EtOAc (3 × 100 mL). The organic layers were combined and washed with water. The organic layer was dried over $MgSO_4$, filtered, and concentrated to afford 70.31 g of **6** (95.0% yield): ^{1}H NMR ($CDCl_3$) δ 2.88−3.06 (m, 4H), 3.20 (d, J = 14.3 Hz, 2H), 3.33 (s, 6H), 3.36−3.70 (m, 12H), 4.61 (d, J = 6.6 Hz, 2H), 4.69 (d, J = 6.6 Hz, 2H), 4.81 (d, J = 14.3 Hz, 2H), 7.01−7.03 (m, 4H), 7.19−7.30 (m, 6H), 7.38−7.51 (m, 4H), 7.93 (s, 2H), 7.99 (d, J = 7.3 Hz, 2H).

[4*R*-(4α,5α,5*β*,7*β*)]-3-[[3-(Cyclopropylmethyl)hexahydro-5,6-dihydroxy-2-oxo-4,7-bis(phenylmethyl)-1*H*-1,3-diazepin-1-yl]methyl]-*N*-2-thiazolylbenzamide (Q8467). A solution of **4** (1.4 g, 2.0 mmol) and 1-hydroxybenzotriazole (405 mg, 3 mmol) in dry DMF (10 mL) was cooled in a 0 °C ice bath. The mixture was treated with 2-aminothiazole (300 mg, 3.0 mmol) followed by dicyclohexylcarbodiimide (825 mg, 4.0 mmol) using dry DMF (5 mL) to aid in the addition. A precipitate forms within 15 min after the addition, and the mixture was stirred at room temperature for 18 h. The mixture was diluted with ethyl acetate and filtered. The mother liquor was washed with water (75 mL) followed by dilute sodium bicarbonate (2 × 50 mL) followed by water (50 mL). The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated. The residue was treated with 2.0 M HCl in 1:1 MeOH:dioxane (10 mL) and the mixture stirred at room temperature for 18 h. The mixture was neutralized by pouring into saturated sodium bicarbonate (evolution!) and extracting with ethyl acetate (2 × 75 mL). The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash chromatography (75 g silica gel) using 1% MeOH:$CHCl_3$ followed by 2% and 3% to afford 432 mg of Q8467 as a white solid (36.2% yield); mp 135.9 °C; $[\alpha]_D$ +87.61° (*c* 0.226, DMSO); ^{1}H NMR ($CDCl_3$) δ 0.01−0.03 (m, 2H), 0.31−0.43 (m, 2H), 0.89 (m, 1H), 2.77−2.85 (m, 1H), 3.03−3.19 (m, 3H), 3.34 (d, J = 14.3 Hz, 1H), 3.50−3.57 (m, 1H), 3.7 (m, 2H), 3.88 (bs, 1H), 4.00 (bs, 1H), 4.10 (bs, 1H), 4.7 (d, J = 14.3 Hz, 1H), 6.82 (m, 2H), 6.92 (d, J = 3.6 Hz, 1H), 7.10−7.25 (m, 9H), 7.33−7.39 (m, 1H), 7.46 (d, J = 7.7 Hz, 1H), 7.75 (s, 1H), 7.84 (d, J = 7.7 Hz, 1H), 11.7 (s, 1H); ^{13}C NMR (DMSO) δ 4.00, 4.13, 10.96, 32.53, 55.83, 56.78, 66.96, 70.81, 71.03, 114.22, 126.27, 126.55, 127.32, 128.49, 128.74, 129.01, 129.50, 129.59, 129.75, 132.61, 133.55, 137.96, 139.60, 140.63, 140.89, 159.17, 161.41, 165.29; HRMS calcd for $C_{34}H_{37}N_4O_4S$ $[M + H]^+$ 597.2535, found 597.2523. Anal. ($C_{34}H_{36}N_4O_4S$): C, H, N, S.

[4*R*-(4α,5α,6*β*,7*β*)]-3-[[Hexahydro-5,6-dihydroxy-2-oxo-3,4,7-tris(phenylmethyl)-1*H*-1,3-diazepin-1-yl]methyl]-*N*-2-thiazolylbenzamide (XV655). Compound XV655 was prepared in an analagous manner as described in the synthesis for Q8467: 411 mg as a white solid (65.0% yield); mp 246.6 °C; $[\alpha]_D$ +93.93° (*c* 0.214, DMSO); ^{1}H NMR (DMSO) δ 2.70−2.76 (m, 1H), 2.82−2.90 (m, 2H), 2.95−3.02 (m, 2H), 3.09 (d, J = 13.9 Hz, 1H), 3.42−3.59 (m, 4H), 4.67 (d, J = 13.9 Hz, 1H), 4.70 (d, J = 13.9 Hz, 1H), 5.14 (d, J = 12.1 Hz, 1H), 5.15 (d, J = 12.1 Hz, 1H), 6.92−6.95 (m, 2H), 7.01 (d, J = 6.6 Hz, 2H), 7.14 (d, J = 6.6 Hz, 2H), 7.23−7.37 (m, 10H), 7.42−7.53 (m, 2H), 7.57 (d, J = 3.6 Hz, 1H), 7.96 (s, 1H), 8.02 (d, J = 7.6 Hz, 1H), 12.7 (s, 1H); ^{13}C NMR (DMSO) δ 32.50, 55.64, 55.98, 65.76, 66.89, 70.66, 114.19, 126.43, 126.48, 127.32, 127.68, 128.55, 128.64, 128.82, 128.98, 129.34, 129.47, 129.58, 129.62, 132.53, 133.53, 138.05, 138.68, 139.42, 140.45, 140.53, 159.20, 161.55, 165.22. HRMS calcd for $C_{37}H_{37}N_4O_4S$ $[M + H]^+$ 633.2535, found 633.2517. Anal. ($C_{37}H_{36}N_4O_4S$) C, H, N, S.

[4*R*-(4α,5α,6*β*,7*β*)]-3-[[3-(Cyclopropylmethyl)hexahydro-5,6-dihydroxy-2-oxo-4,7-bis(phenylmethyl)-1*H*-1,3-diazepin-1-yl]methyl]-*N*-1*H*-imidazol-2-ylbenzamide (XV651). A solution of **4** (3.0 g, 4.3 mmol) and pyridine (1.29 g, 16.2 mmol) in dry benzene (30 mL) was cooled in a 0 °C ice bath. The mixture was treated dropwise with 2.0 M oxalyl chloride in dichloromethane (16.2 mL, 32.4 mmol). A precipitate formed immediately, and after the addition was complete, the mixture was stirred at room temperature for 18 h. The solvents and excess oxalyl chloride were pumped off. The residue in dry pyridine (15 mL) was treated with 2-aminoimidazole sulfate (1.704 g, 12.9 mmol) and the mixture stirred at room temperature for 18 h. The mixture was diluted with ethyl acetate and washed with water (2 × 50 mL). The residue after removal of solvent was dissolved in dry methanol (10 mL) and 4 M HCl in dioxane (10 mL) and stirred at room temperature for 18 h. The mixture was diluted with ethyl acetate and washed with saturated sodium bicarbonate. The organic layer was dried over $MgSO_4$, filtered, and concentrated and the residue purified by flash chromatography (100 g of silica gel) using 1:1 EtOAc:hexane followed by 10:1:10 EtOAc:EtOH:hexane. The solvent was switched to 1% MeOH:$CHCl_3$ followed by 2% and finally 5% to afford 711 mg of white solid compound XV651 (28.5% yield). mp 156.7 °C; $[\alpha]_D$ +91.41° (*c* 0.256, DMSO); ^{1}H NMR ($CDCl_3$) δ 0.01−0.03 (m, 2H), 0.33−0.37 (m, 2H), 0.87 (m, 1H); 1.89−1.96 (m, 1H), 2.86−2.94 (m, 1H), 3.04−3.13 (m, 5H), 3.50−3.71 (m, 3H), 3.85−3.88 (m, 1H), 4.04−4.08 (m, 1H), 4.71 (d, J = 14.2 Hz, 1H), 6.61 (s, 2H), 6.91 (d, J = 6.6 Hz, 2H), 7.10−7.32 (m, 13H), 7.75 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 3.45, 3.91, 10.43, 32.36, 32.61, 55.64, 56.95, 65.84, 66.24, 70.95, 71.05, 117.52, 126.12, 126.35, 126.73, 128.28, 128.47, 128.77, 129.22, 129.38, 133.23, 133.35, 139.13, 139.46, 139.82, 142.43, 161.74, 167.20. HRMS calcd for $C_{34}H_{38}N_5O_4$ $[M + H]^+$ 580.2923, found: 580.2912. Anal. ($C_{34}H_{37}N_5O_4 \cdot 0.6H_2O$) C, H, N.

[4*R*-(4α,5α,6*β*,7*β*)]-3-[[Hexahydro-5,6-dihydroxy-2-oxo-3,4,7-tris(phenylmethyl)-1*H*-1,3-diazepin-1-yl]methyl]-*N*-1*H*-imidazol-2-ylbenzamide (SD143). Compound SD143 was prepared in a manner analagous to that described in the synthesis for XV651: 246 mg as a white solid (23.6% yield); mp 155.0 °C; ^{1}H NMR (DMSO) δ 2.81−2.89 (m, 3H), 2.98−3.63 (m, 3H), 3.43−3.55 (m, 4H), 4.67 (d, J = 13.9 Hz, 1H), 4.71 (d, J = 14.2 Hz, 1H), 5.11−5.14 (m, 2H), 6.80 (s, 2H), 6.96−7.47 (m, 17H), 7.93 (s, 1H), 7.98 (d, J = 7.7 Hz, 1H), 11.8 (s, 2H); ^{13}C NMR (DMSO) δ 32.48, 55.65, 55.82, 65.75, 66.42, 70.71, 126.50, 127.14, 127.68, 128.65, 128.83, 129.23, 129.35, 129.62, 132.66, 134.67, 138.70, 139.05, 140.49, 140.55, 143.18, 161.63, 166.61; HRMS calcd for $C_{37}H_{38}N_5O_4$ $[M + H]^+$ 616.2923, found 616.2918. Anal. ($C_{37}H_{37}N_5O_4$) C, H, N.

[4*R*-(4α,5α,6*β*,7*β*)]-3-[[3-Butylhexahydro-5,6-dihydroxy-2-oxo-4,7-bis(phenylmethyl)-1*H*-1,3-diazepin-1-yl]methyl]-*N*-1*H*-imidazol-2-ylbenzamide (SD152). Compound SD152 was prepared in a manner analagous to that described in the synthesis for XV651: 254 mg as a white solid (22.6% yield); mp 150.0 °C; $[\alpha]_D$ +90.65° (*c* 0.246, DMSO); ^{1}H NMR (DMSO) δ 0.75 (t, J = 7.3 Hz, 3H), 1.11−1.28 (m, 4H), 1.93−1.97 (m, 1H), 2.78−3.08 (m, 5H), 3.40−3.50 (m, 4H), 3.65−3.69 (m, 1H), 4.63 (d, J = 14.3 Hz, 1H), 5.12−5.23 (m, 2H), 6.78 (s, 2H), 6.89 (d, J = 6.6 Hz, 2H), 7.10−7.43 (m, 10H), 7.87 (s, 1H), 7.94 (d, J = 7.7 Hz, 1H), 11.8 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 13.76, 20.27, 31.19, 32.66, 32.78, 49.21, 49.49, 49.78, 52.59, 55.79, 66.18, 66.44, 71.04, 71.26, 117.72, 126.24, 126.47, 126.87, 128.39, 128.48, 128.57, 128.81, 129.34, 129.46, 133.38, 133.43, 139.24, 139.52, 139.77, 142.69, 161.71, 167.34. HRMS calcd for $C_{34}H_{40}N_5O_4$ $[M + H]^+$ 582.3080, found 582.3078. Anal. ($C_{34}H_{39}N_5O_4$) C, H, N.

[4*R*-(4α,5α,6*β*,7*β*)]-*N*-1*H*-Benzimidazol-2-yl-3-[[3-(cyclopropylmethyl)hexahydro-5,6-dihydroxy-2-oxo-4,7-bis(phenylmethyl)-1*H*-1,3-diazepin-1-yl]methyl]benzamide (SD145). Compound SD145 was prepared in a manner analagous to that described in the synthesis for **9**: 617 mg as a white solid (67.6% yield); mp 170.4 °C; $[\alpha]_D$ +69.16° (*c* 0.214, DMSO); ^{1}H NMR (DMSO) δ 0.00 (m, 2H), 0.32−0.34 (m, 2H), 0.84−0.89 (m, 1H), 1.76−1.83 (m, 1H), 2.80−3.07 (m, 6H), 3.45−3.52 (m, 3H), 3.73−3.76 (m, 1H), 4.64 (d, J = 14.3 Hz, 1H), 5.11−5.21 (m, 2H), 6.90 (d, J = 6.6 Hz, 2H), 7.05−7.43 (m, 14H), 7.93 (s, 1H), 8.02 (d, J = 7.7 Hz, 1H), 12.2 (s, 2H); ^{13}C NMR (DMSO) δ 3.91, 4.02, 10.89, 32.40, 55.54, 56.70, 66.25, 66.85, 70.75, 70.98, 121.91, 126.24, 126.51, 127.51, 128.48, 128.70, 129.41, 129.59, 129.71, 132.89, 135.07, 139.19, 140.63, 140.90, 161.47. HRMS calcd for $C_{38}H_{40}N_5O_4$ $[M + H]^+$ 630.3080, found 630.3060. Anal. ($C_{38}H_{39}N_5O_4$) C, H, N.

[4*R*-(4α,5α,6*β*,7*β*)]-3,3′-[[Tetrahydro-5,6-dihydroxy-2-oxo-4,7-bis(phenylmethyl)-1*H*-1,3-diazepine-1,3(2*H*)-diyl]-bis(methylene)]bis[*N*-2-thiazolylbenzamide] (XV638). A solution of **6** (1.08 g, 1.4 mmol) and 1-hydroxybenzotriazole (570 mg, 4.2 mmol) in dry DMF (10 mL) was cooled in a 0 °C ice bath and treated with 2-aminothiazole (421 mg, 4.2 mmol). To this was added a solution of 1,3-dicyclohexylcarbodiimide (1.15 g, 5.6 mmol) in dry DMF (6 mL). The reaction mixture was stirred at room temperature for 16 h, diluted with ethyl acetate, and filtered over Celite. The solution was washed with water (2 × 50 mL), saturated sodium bicarbonate, and water. The solution was the dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (75 g silica gel) using 2:3 EtOAc:hexane, followed by 1:8 MeOH:$CHCl_3$ to elute 1.2 g of a white solid. The solid 1.2 g in dry methanol (6 mL) was treated with 4 N HCl in dioxane (6 mL). The mixture was stirred at room temperature for 18 h and then poured into chloroform (75 mL). The organic layer was washed with saturated sodium bicarbonate. The aqueous layer was extracted with chloroform (2 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (75 g silica gel) using chloroform, followed by 1% MeOH:$CHCl_3$, 2%, and finally 4% to elute 880 mg of XV638 as a white solid (82.0% yield); mp 179.8 °C; $[\alpha]_D$ +76.76° (*c* 0.284, DMSO); ^{1}H NMR (DMSO) δ 2.67−2.75 (m, 2H), 2.93−2.97 (m, 2H), 3.05 (d, J = 13.9 Hz, 2H), 3.54−3.57 (m, 4H), 4.63 (d, J = 13.9 Hz, 2H), 5.16 (s, 2H), 6.88−6.91 (m, 4H), 7.16−7.23 (m, 6H), 7.24 (d, J = 3.7 Hz, 2H), 7.39−7.50 (m, 4H), 7.53 (d, J = 3.7 Hz, 2H), 7.93 (s, 2H), 7.98 (d, J = 7.3 Hz, 2H), 12.4 (s, 2H); ^{13}C NMR (DMSO) δ 32.44, 55.89, 66.81, 70.54, 114.03, 126.28, 127.19, 128.42, 128.84, 129.34, 129.41, 132.43, 133.38, 137.76, 139.25, 159.0, 161.23, 165.11; HRMS calcd for $C_{41}H_{39}N_6O_5S_2$ [M + H]$^+$ 759.2423, found 759.2406. Anal. ($C_{41}H_{38}N_6O_5S_2$) C, H, N, S.

[4*R*-(4α,5α,6*β*,7*β*)]-3,3′-[[Tetrahydro-5,6-dihydroxy-2-oxo-4,7-bis(phenylmethyl)-1*H*-1,3-diazepine-1,3(2*H*)-diyl]-bis(methylene)]bis[*N*-1*H*-imidazol-2-ylbenzamide] (XV652). Compound XV652 was prepared in a manner analagous to that described in the synthesis for XV651: 75 mg as a white solid (6.1% yield); mp 136.0 °C; $[\alpha]_D$ +63.55° (*c* 0.214, DMSO); ^{1}H NMR (DMSO) δ 2.83−2.92 (m, 2H), 3.08−3.17 (m, 4H), 3.61−3.71 (m, 4H), 4.77 (d, J = 14.3 Hz, 2H), 6.76 (s, 4H), 6.99 (d, J = 7.7 Hz, 4H), 7.20−7.28 (m, 6H), 7.41−7.43 (m, 4H), 7.77 (s, 2H), 7.83−7.86 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 34.37, 57.91, 68.22, 72.46, 119.45, 128.22, 128.69, 130.31, 130.63, 131.12, 134.94, 135.70, 140.56, 141.35, 144.44, 163.95, 169.31; HRMS calcd for $C_{41}H_{41}N_8O_5$ [M + H]$^+$ 725.3199, found 725.3200. Anal. ($C_{41}H_{40}N_8O_5 \cdot 0.5H_2O$) C, H, N.

[4*R*-(4q,5α,6*β*,7*β*)]-3,3′-[[Tetrahydro-5,6-dihydroxy-2-oxo-4,7-bis(phenylmethyl)-1*H*-1,3-diazepine-1,3(2*H*)-diyl]-bis(methylene)]bis[*N*-1*H*-benzimidazol-2-ylbenzamide] (SD146). Compound SD146 was prepared in a manner analagous to that described in the synthesis for XV638 and isolated as a bishydrochloride: 200 mg as a white solid (15.3% yield); mp 266.4 °C; ^{1}H NMR (DMSO) δ 2.82−2.91 (m,2H), 3.01 (d, J = 14.0 Hz, 4H), 3.51−3.57 (m, 4H), 4.71 (d, J = 14.2 Hz, 2H), 5.16 (s, 2H), 6.99 (d, J = 7.0 Hz, 4H), 7.12−7.16 (m, 4H), 7.20−7.51 (m, 14H), 8.02 (s, 2H), 8.08 (d, J = 7.7 Hz, 2H), 12.3 (s, 4H); ^{13}C NMR (DMSO) δ 32.51, 55.81, 66.31, 70.71, 121.94, 126.49, 127.58, 128.63, 128.76, 129.48, 129.64, 132.93, 139.03, 140.48, 161.59; HRMS calcd for $C_{49}H_{45}N_8O_5$ [M + H]$^+$ 825.3512, found 825.3520. Anal. ($C_{49}H_{46}Cl_2N_8O_5$) C, H, Cl, N.

Biological Methods. Inhibition of HIV protease was measured by assaying the cleavage of a fluorescent peptide substrate using HPLC.[25] The lower limit of the detection for the reported[25] assay has been improved by lowering the enzyme concentration from 62 to 50 pM. The antiviral potency of compounds was assessed by measuring their effect on the accumulation of viral RNA transcripts three days after infection of MT-2 cells with HIV-1 RF.[26] Oral bioavailability was measured as previously described.[27] In order to introduce defined sequence alterations into the protease gene, recombinant DNA plasmids containing the 5′ half of the HIV HXB2 genome were subjected to site directed mutagenesis as previously described.[12] Cloned 5′ half HIV plasmids were linearized with NcoI, ligated with a complementary NcoI linearized 3′ half HIV plasmid, and used to transfect MT4 cells by lipofection. The culture was frozen in aliquots 7−10 days after lipofection, when virally induced cytopathic effect had spread throughout the culture. Virus stocks were not passaged, in order to reduce the opportunity for accumulation of additional sequence changes beyond those encoded in the transfected plasmid.

The drug susceptibility of wild type and protease variant HIV's was tested by measuring the effects of each compound on accumulation of viral p24 antigen following infection of MT4 cells. MT4 cells (2.5 × 10^5 cells/mL) were infected with appropriate dilutions of each virus stock and cultured for 24 h at 37 °C and 5% CO_2 in RPMI 1640 with 10% fetal calf serum, 2 mM glutamine, and 50 μg/mL gentamycin (Gibco/BRL) without protease inhibitors. Infected cultures were then washed three times in medium and plated into microtiter plate wells with various concentrations of each inhibitor at a final cell number of 2.5 × 10^5 infected cells/mL. Following addition of drug, infected cell cultures were incubated for an additional 72 h. Appropriate dilutions of each virus stock were determined in preliminary experiments and were selected to result in the accumulation of between 1000 and 4000 ng/mL of p24 antigen at the end of the 4 day culture period. P24 antigen accumulation was quantitated in all cultures using the DuPont p24 antigen Elisa kit. The potency of each compound is reported as an IC_{90}, that concentration of compound that inhibited the accumulation of viral p24 antigen by 90% from the level accumulated in an infected, untreated culture. Resistance of mutant viruses was calculated as (IC_{90} for a mutant virus/IC_{90} of wt HXB2 virus). Since differences in viral growth rates or initial viral innoculum could affect the IC_{90} values measured, susceptibility to ddC(Sigma), a reverse transcriptase inhibitor, was monitored for all viruses in each experiment. IC_{90} values for mutant viruses were normalized by the variation in the IC_{90} value for the ddC internal control. This normalization was usually less than 2 and always less than 2.5-fold. Despite this normalization, resistance values less than 3-fold are not considered significant.

X-ray Methods. A modified form of HIV-1 PR, Cys to Ala at position 95, was expressed in *E. coli* and purified from inclusion bodies using a solid phase refolding procedure.[20] Frozen aliquots of active protein were thawed in the presence of inhibitor. Crystals of the protease·inhibitor complexes were grown at 18 °C in hanging drops by vapor diffusion as reported.[28] Hexagonal rods (0.07 mm × 0.07 mm × 1.4 mm) appeared within 1 week. Diffraction data were collected at room temperature on a RAXIS IIc imaging plate mounted on a RU-H2R Rigaku rotating anode (Cu Kα) generator using a 0.3 mm cathode and operating at 50 kV and 100 mA. The unit cell parameters were determined from three still frames taken at 15° intervals using the RAXIS processing software. All protease·inhibitor complexes crystallized in the space group *P*61 with a dimer in the asymmetric unit and cell dimensions $a = b = 62.8$ Å and $c = 83.5$ Å, with a dimer in the asymmetric unit. Full data sets were obtained by collecting between 25 and 30 2° oscillation images with an exposure time of 60 min at a detector distance of 70 mm. A total of 58 704, 39 076, and 53 827 raw reflections were measured and reduced to 16 523, 16 197, and 15 429 unique reflections (all the data) for Q8467, XV638, and SD146, respectively. The merging R factors for symmetry-related reflection were 11%, on intensities, for the three complexes. The atomic coordinates of HIV PR complexed with DMP323 complex were used as the starting model in refinement. The complexes with Q8467, XV638, and SD146 were refined to 2.1, 1.8, and 1.8 Å, respectively, by performing several cycles of simulated annealing, positional, and restrained B-refinement.[29] The conformations of the inhibitors were determined by calculating $|F_o - F_c|$ and $|2F_o - F_c|$ maps, and the protein structure was adjusted as necessary using SA-omit maps. The starting R values were approximately 44% and were reduced to 19% with an overall deviations from ideal geometry of 3.07, 2.92, and 2.95 Å for bond distances, and 0.012, 0.012, and 0.012 Å, respectively.

Acknowledgment. We thank Edward R. Holler Jr. for his technical assistance during the synthesis of cyclic urea amides, Ronald M. Klabe for K_i measurements, Dean Winslow and Renay Buckery for participation in the early phases of recombinant virus construction, and Karen B. Gallagher and Renay Buckery for viral resistance data. We also thank Paul S. Anderson, David A. Jackson, and Charles A. Kettner for helpful comments on the manuscript. We are indebted to the HIV Working Group of Dupont Merck.

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JM960586T