A Novel, Picomolar Inhibitor of Human Immunodeficiency Virus Type 1 Protease†

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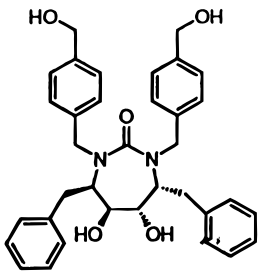
The design, synthesis, and molecular modeling studies of a novel series of azacyclic ureas, which are inhibitors of human immunodeficiency virus type 1 (HIV-1) protease that incorporate different ligands for the S_1', S_2, and S_2' substrate-binding sites of HIV-1 protease are described. The synthesis of this series is highly flexible in the sense that the P_1', P_2, and P_2' residues of the inhibitors can be changed independently. Molecular modeling studies on the phenyl ring of the P_2 and P_2' ligand suggested incorporation of hydrogen-bonding donor/acceptor groups at the 3- and 4-positions of the phenyl ring should increase binding potency. This led to the discovery of compound **7f** (A-98881), which possesses high potency in the HIV-1 protease inhibition assay and the *in vitro* MT-4 cell culture assay (K_i = ∼5 pM and EC_{50} = 0.002 μM). This compares well with the symmetrical cyclic urea **1** pioneered at DuPont Merck.

Introduction

The widespread occurrence and extremely unfavorable prognosis of human immunodeficiency virus (HIV) infection makes the discovery of new and effective therapy very urgent. The World Health Organization has projected that the number of people infected with HIV would grow to over 30 million by the turn of the century.[1] Of the currently licensed therapeutic agents which target the HIV reverse transcriptase, most have undesirable toxicities and select resistant mutant virus strains in a relatively short time.[2] The HIV-1 protease, which is responsible for the maturation of HIV into infectious viral particles,[3] has become an attractive therapeutic target. Most of the potent inhibitors of the HIV−1 protease thus far are peptidomimetic compounds containing transition-state analogs in place of the dipeptide cleavage sites of the protease−substrate sequences.[4−8] The prospect of developing such inhibitors into useful drugs is formidable, due to the poor pharmacokinetic properties of most peptidomimetics.[9] Recent advances, however, have resulted in HIV protease inhibitors that are more orally bioavailable.[10,11] Another formidable challenge is the seemingly limitless capacity of the AIDS retrovirus to evolve drug resistance mutations.[12] At present, the most durable suppression of HIV replication has been achieved with a combination regimen of 3TC and AZT, reverse transcriptase inhibitors which appear to be highly refractory to the selection of cross-resistant mutants.[13] An analogous combination of protease inhibitors depends on the discovery of individually potent, orally bioavailable compounds which lack significant cross-resistance. In advance of relevant clinical data on resistance to ABT-538,[14] it seemed advisable to pursue this goal in a structurally distinct chemical series. Utilizing the C_2 symmetric cyclic urea



1 (DMP 323)

Figure 1. Structure of **1** (DMP323).

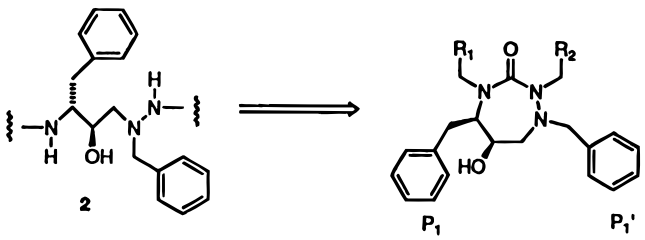


Figure 2. Pseudosymmetric dipeptide isostere-based azacyclic urea.

inhibitors (Figure 1) pioneered at DuPont Merck as a starting point,[15] we have now discovered a new class of highly potent, nonsymmetric inhibitors of the HIV-1 protease.[16] This new series of compounds was based on a linear aza backbone-modified peptidomimetic series reported before (see Figure 2).[17]

Chemistry

The synthesis of this new series of compounds is outlined in Scheme 1. The opening of the 2*S*,3*R*-epoxide **3**,[17] derived from Cbz-D-phenylalaninol, with Boc-protected benzylhydrazine[18] provided the pseudosymmetric dipeptide isostere **4** in 70% yield. Other Boc-protected hydrazines with different side chains can be synthesized by condensation of Boc-carbazate with the corresponding aldehyde followed by hydrogenation. Exchange of the Boc protecting group with Cbz, followed by masking of the secondary alcohol as a SEM ether,

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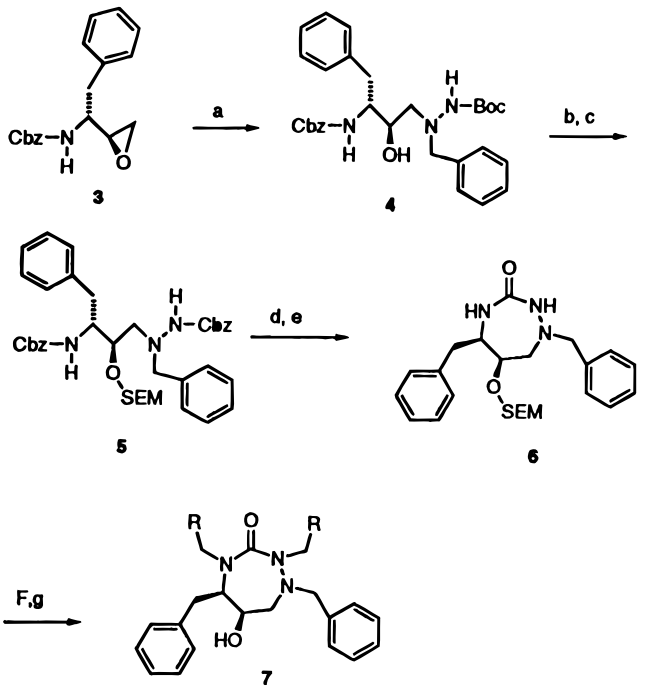
Scheme 1[a]



[a] Reagents: (a) Boc-NHNH-benzyl/2-propanol; (b) i, TFA/CH_2Cl_2; ii, Cbz-NOS; (c) SEM-Cl/diisopropylethylamine; (d) H_2, Pd/C; (e) CDI/CH_2Cl_2; (f) NaH/DMF, then R-halide; (g) TMS-Cl/MeOH.

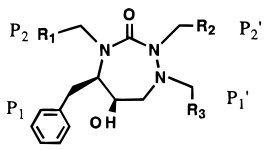
allowed for simultaneous deprotection of the amino termini by hydrogenolysis. Cyclization with 1,1′-carbonyldiimidazole proceeded smoothly to afford the key triazacycloheptanone **6** in 80% yield. Alkylation of the unsubstituted urea nitrogens was accomplished by deprotonation with sodium hydride in DMF followed by the addition of the appropriate halide (e.g., benzyl bromide, 85% yield). Finally, deprotection of the SEM ether by anhydrous HCl in methanol provided the series of azacyclic ureas **7** which are shown in Table 1.

Despite the replacement of two chiral centers by an achiral methylene unit and nitrogen atom, we were gratified to find that compound **7a** has essentially the same inhibitory potency against the HIV-1 protease as compound **1**.[15] As importantly, the synthetic strategy outlined above, unlike the symmetry restricted dimerization route reported for the synthesis of **1** and its congeners, permits the independent selection of all four ring substituents. This versatility is exemplified by the syntheses of compounds **7b,g** (Table 1). In the first instance, variation of the P_1' group was achieved simply by replacing benzaldehyde with 3-furancarboxaldehyde in the synthesis of the corresponding hydrazine. In the second case, regioselective introduction of an alkyl group in the P_2' position was achieved by exploiting the pK_a differential of the urea nitrogens. High-field NMR experiments confirmed our expectation that the first alkylation would occur predominantly at the aza-linked nitrogen.[19] The independent variation of all four ring substituents provides the opportunity to modulate the physicochemical properties of the resulting inhibitors.

Molecular Modeling

Models of inhibitors **7c**−**e** bound to the active site of HIV protease were created (see the Experimental Section for details) prior to their synthesis. Inspection of the model of **7c** indicated that there was unoccupied volume near the Asp 29/30 and Asp 129/130 positions. Furthermore, the unoccupied volume near Asp 29/129 was large enough for us to postulate that water molecules could bind to the backbone NH groups of Asp 29/129 as observed in the uninhibited complex of Rous sarcoma viral protease (waters 287 and 450).[20] The distances from the protein backbone nitrogens to the inhibitor aryl group were between 4 and 4.5 Å. These distances were of sufficient length that placement of hydrogen bond acceptors in the meta and/or para positions of the inhibitor's *N*-benzyl side chain was predicted to yield analogs which could make hydrogen bond interactions with the main chain amide N-H groups of the enzyme. Additionally, displacement of the waters affiliated with Asp 29/129 was also expected to have a favorable effect upon increasing inhibitor affinity for the protease due to the increase in entropy of such a process. Inspection of the structural models of compounds **7d,e** confirmed that hydrogen bonds with the four protein residues Asp 29, Asp 30, Asp 129, and Asp 130 were possible. In summary, consideration of both enthalpic and entropic factors indicated that these analogs of **7c** might possess the desired potency increases. The increased potencies of compounds **7d,e** (with their 4-hydroxy and 3-methoxy substituents, respectively) relative to compound **7c** were in accord with this structural prediction. The models suggested that the potency enhancements were due to separate interactions in the two compounds: an Asp 29/129 interaction in the case of **7e** and an Asp 30/130 interaction in the case of **7d**. Thus, the potency increase was anticipated to be additive when the two substituent

Table 1. HIV-1 Protease Inhibition and *in Vitro* Antiviral Activities of Azacyclic Ureas



compd	R_1	R_2	R_3	K_i[a] (nM)	EC_{50}[b] (μM)
1				0.23	0.03
7a	4-(hydroxymethyl)phenyl	4-(hydroxymethyl)phenyl	phenyl	0.22	0.09
7b	4-(hydroxymethyl)phenyl	4-(hydroxymethyl)phenyl	3-furanyl	0.49	0.10
7c	phenyl	phenyl	phenyl	2.0	2.86
7d	4-hydroxyphenyl	4-hydroxyphenyl	phenyl	0.07	0.01
7e	3-methoxyphenyl	3-methoxyphenyl	phenyl	0.22	0.93
7f	4-hydroxy-3-methoxyphenyl	4-hydroxy-3-methoxyphenyl	phenyl	0.005	0.002
7g	4-hydroxy-3-methoxyphenyl	cyclopropyl	phenyl	0.062	0.17

[a,b] For procedures for the enzyme inhibition assay and MT-4 cell culture assay, see ref 20.

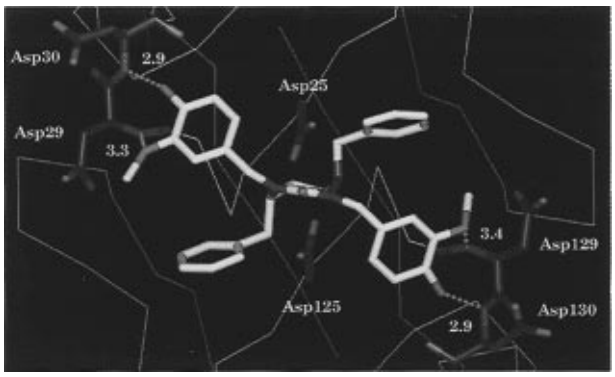


Figure 3. X-ray crystal structure of inhibitor **7f** bound to HIV-1 protease. Distances are in Å.

changes were combined in one molecule. The observed potency for **7f** was indeed higher than either that of **7d** or **7e** and represented a 200-fold decrease in K_i value relative to **7c** (see Table 1). Compound **7f** (A-98881), with a K_i of 5 pM, is one of the most potent HIV-1 protease inhibitors reported to date. The interaction of the 3-OCH_3/4-OH substituents with the main chain N-H groups of Asp 29, Asp 30, Asp 129, and Asp 130 was confirmed by X-ray crystallographic analysis[21] of compound **7f** bound to HIV-1 protease as shown in Figure 3. The central 7-membered azacyclic urea unit of **7f** has an orientation between the active site aspartates, Asp 25/125, and the flap Ile 50/150 residues very similar to that observed for the 7-membered cyclic urea series reported by DuPont Merck (PDB entry 1HVR). It should be noted that the structure 1HVR was released into the public domain and available for inspection after the work described in this paper was completed. As previously observed for **1** (DMP 323), the urea carbonyl of **7f** assumes the position normally occupied by a water molecule (Wat 301) in crystal structures of linear, peptidomimetic inhibitors.[20] Importantly, the inhibitor's phenolic oxygens in the S_2/S_2' subsites are both located at 2.9 Å from the backbone N-H of Asp 30/130, suggesting the existence of strong hydrogen-bonding interactions. The distances between the methoxy oxygens of **7f** and the backbone N-H of Asp 2/129 are slightly longer at 3.3 and 3.4 Å but still suggestive of significant hydrogen-bonding interactions. After completion of this work, an analogous pattern of hydrogen bonding to Asp 29 and Asp 30 by an elegantly designed fused bis-tetrahydrofuran P_2 unit for an acyclic inhibitor series was reported by Merck.[22]

Cross-Resistance

The evolution of resistance to the protease inhibitor ABT-538 in HIV-infected patients has been observed.[23,24] Resistant variants isolated from patients undergoing therapy with the protease inhibitor MK-639 exhibiting cross-resistance to a panel of six structurally diverse protease inhibitors has been reported.[25] Compound **7f** was tested for antiviral activity against two virus clones of resistant variants isolated from patients undergoing monotherapy with ABT-538, and the result are shown in Table 2. The azacyclic urea **7f** retains high potency against the two ABT-538 resistant virus variants (~3-fold decrease in potency as compared to ~16−18-fold for ABT-538.

The pharmacokinetic behavior of **7a**−**e** was examined following a 10 mg/kg oral dose in rats using a previously reported protocol.[26] Unfortunately all of the compounds reported in Table 1 showed poor oral bioavailability, ranging from 0.2% to 4.7%. Comparison of the rate of metabolic transformation of compound **7a** to that of the orally bioavailable peptidomimetic inhibitor ABT-538 in rat hepatic microsomes showed **7a** was metabolized ≥10-fold faster.[27] The major metabolite from **7a** was found to be the dicarboxylic acid resulting from oxidation of the benzylic hydroxy groups. We believe that the poor oral bioavailabilities of the compounds in Table 1 may stem from a combination of poor aqueous solubility and metabolic instability. Current efforts are directed at addressing these problems.

Table 2. Antiviral Activities of **7f** against ABT-538 Resistant Mutant Virus (EC_{50} Values in μM)

compd	wild type HXB2	HXB2 mutant[a] 460.2	HXB2 mutant[a] 460.6
ABT-538	0.078	1.293	1.439
7f	0.006	0.021	0.023

[a] 460.2 and 460.6 are virus clones of patient isolates that have the following point mutations in their protease sequence: 460.2, V82T, I54V, A71V, M36I, and K20R; 460.6, V82T, I54V, A71V, and M36I.

Experimental Section

All melting points were recorded on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton magnetic resonance spectra were recorded on a GE-300 spectrometer using TMS as the internal standard. Mass spectra were obtained by the Structural Chemistry Department, Abbott Laboratories. Elemental analyses were performed by Robertson Microlit Laboratories, Inc. (Madison, NJ). Flash column chromatography was performed on silica gel 60, 0.04−0.063 mm (E. Merck). Thin-layer chromatography was performed on precoated silica gel F-254 plates (0.25 mm; E. Merck) and visualized with phosphomolybdic acid. All reactions are performed under an argon atmosphere except where noted.

***N*-[(Benzyloxy)carbonyl]-(2*R*)-amino-(3*S*)-3,4-epoxybutane (3).** To a dry 250 mL 3-neck flask was added 14.34 g of triphenylmethylphosphonium bromide. To this was added 70 mL of THF, the mixture was cooled to 0 °C, and 4.42 g of 35% potassium hydride dispersion in oil was added. The mixture was stirred at room temperature for 24 h. To this mixture was added 30 mL of toluene, and the mixture was rested for 30 min. The supernatant was cannulated over into a solution of 3.37 g of *N*-[(benzyloxy)carbonyl]-D-phenylalaninal in 50 mL of toluene at −78 °C for 2 h followed by 0.5 h at room temperature.

Saturated ammonium chloride (50 mL) was added. The layers were separated, and the aqueous layer was extracted with ethyl acetate (3 × 100 mL). The combined organic layer was washed with saturated NaCl solution, dried over anhydrous sodium sulfate, filtered, and concentrated. Purification of the crude product by silica gel flash column chromatography (30% ether/70% hexane) provided 3.02 g (89%) of *N*-[(benzyloxy)carbonyl]-(2*R*)-amino-1-phenylbut-3-ene: ^{1}H NMR ($CDCl_3$) δ 2.88 (d, J = 6.0 Hz, 2H), 4.50 (m, 1H), 4.70 (m, 1H), 5.10 (m, 3H), 5.80 (m, 1H), 7.10−7.40 (m, 10H).

To a solution of 2.97 g of the above olefin in 75 mL of dichloromethane at 0 °C was added 9 g of MCPBA. The solution was stirred for 1 h and then at room temperature overnight. It was added to 250 mL of ether and washed successively with cold 10% sodium thiosulfate, 10% sodium carbonate, and then saturated NaCl solution. The organic layer was dried and concentrated to a colorless oil which was purified by silica gel column chromatography (20% EtOAc/80% hexane) to provide 2.7 g of the epoxide **3**: ^{1}H NMR ($CDCl_3$) δ 2.57 (m, 1H), 2.70 (t, J = 4.5 Hz, 1H), 2.90−3.05 (m, 1H), 4.20 (m, 1H), 4.70 (br d, 1H), 7.20−7.38 (m, 10H); MS $(M + H)^+$ = 298. Anal. ($C_{18}H_{19}NO_3$) C, H, N.

2-[[(*tert*-Butyloxy)carbonyl]amino]-(4*R*)-hydroxy-(5*R*)-[[(benzyloxy)carbonyl]amino]-1,6-diphenyl-2-azahexane (4). To a solution of 1.2 g of compound **3** in 36 mL of 2-propanol was added 1.07 g (1.2 equiv) of N^1-[(*tert*-butyloxy)-carbonyl]-N^2-benzylhydrazine. The solution was heated at reflux for 24 h, cooled to room temperature, and concentrated *in vacuo*. Silica gel column chromatography (20% EtOAc/80% hexane) provided 1.5 g (72%) of **4**: ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 9H), 2.43 (m, 1H), 2.78 (m, 1H), 2.96 (d, J = 7.5 Hz, 2H), 3.57 (m, 2H), 3.70−4.00 (m, 3H), 5.48 (s, 2H), 5.19 (br s, 1H), 5.37 (m, 1H), 7.18−7.36 (m, 15H); MS $(M + H)^+ = 520$. Anal. ($C_{30}H_{37}N_3O_5$) C, H, N.

2-[[(Benzyloxy)carbonyl]amino]-(4*R*)-[[(trimethylsilyl)-ethoxy]methoxy]-(5*R*)-[[(benzyloxy)carbonyl]amino]-1,6-diphenyl-2-azahexane (5). The (*tert*-butyloxy)carbonyl protecting group of compound **4** was exchanged to a (benzyloxy)carbonyl protecting group by deprotection with trifluoroacetic acid and methylene chloride, and then reaction of the resulting amino compound with *N*-[[(benzyloxy)carbonyl]oxy]-succinimide provided the di-Cbz compound in 90% yield. To 1.5 g of di-Cbz compound in 12 mL of dimethylformamide were added to 1.8 mL of diisopropylethylamine and then 1.14 mL of [(trimethylsilyl)ethoxy]methyl chloride. The reaction mixture was stirred at room temperature for 19 h. The solvent was removed *in vacuo*. The residue was extracted with EtOAc (3 × 80 mL) and washed with saturated NaCl solution. The organic layer was dried with anhydrous sodium sulfate and filtered and the solvent evaporated *in vacuo*. Purification of the crude product by silica gel column chromatography (20% acetone/80% hexane) provided 1.23 g (83%) of compound **5**: ^{1}H NMR ($CDCl_3$) δ 0.05 (s, 9H), 0.95 (t, J = 9 Hz, 2H), 2.80 (m, 3H), 3.10 (m, 1H), 3.65 (m, 3H), 3.97 (m, 1H), 4.26 (m, 1H), 4.70 (ABq, J = 7.5 Hz, 2H), 5.0 (m, 4H), 5.30 (br d, 1H), 7.25 (m, 20H); MS $(M + H)^+ = 684$. Anal. ($C_{39}H_{49}N_3O_6Si$) C, H, N.

(5*R*,6*R*)-1,5-Dibenzyl-3-oxo-6-[[(trimethylsilyl)ethoxy]-methoxy]-1,2,4-triazacycloheptane (6). To a suspension of 100 mg of 10% Pd/C in 20 mL of methanol was added 1.2 g of compound **5**. The mixture was stirred vigorously under a hydrogen atmosphere (balloon filled with hydrogen) for 1 h. The catalyst was filtered off, and the filtrate was concentrated *in vacuo* to give a colorless oil which was dissolved in 120 mL of dichloromethane. To this solution was added 300 mg of 1,1′-carbonyldiimidazole. The solution was kept at room temperature for 72 h and then concentrated *in vacuo* and the crude product purified by silica gel column chromatography (20% EtOAc/80% CH_2Cl_2) to provide 617 mg of compound **6** (80%, two steps): ^{1}H NMR ($CDCl_3$) δ 0.02 (s, 9H), 0.90 (t, J = 9.0 Hz, 2H), 2.96 (m, 2H), 3.17 (m, 2H), 3.70 (m, 3H), 3.97 (m, 1H), 4.05 (ABq, J = 13.5 Hz, 2H), 4.45 (br s, 1H), 4.73 (ABq, J = 7.5 Hz, 2H), 5.62 (br s, 1H), 7.30 (m, 10H); MS $(M + H)^+ = 442$. Anal. ($C_{24}H_{35}N_3O_3Si$) C, H, N.

1-(Hydroxymethyl)-4-[[[(trimethylsilyl)ethoxy]methoxy]-methyl]benzene. To 3.08 g (21.7 mmol) of 1,4-benzenedimethanol in 5 mL of DMF/200 mL of dichloromethane were added 4.6 mL (1.2 equiv) of diisopropylethylamine and 4.0 mL of SEM-Cl. After 3 h at room temperature, solvent was removed *in vacuo* and the residue taken up in 150 mL of EtOAc and washed with saturated $NaHCO_3$ (50 mL). The organic layer was dried and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (10% EtOAc/90% CH_2Cl_2) to give 2.98 g (51%) of product: ^{1}H NMR ($CDCl_3$) δ 0.01 (s, 9H), 0.95 (t, J = 10 Hz, 2H), 1.60 (br s, 1H), 3.65 (t, J = 10 Hz, 2H), 4.58 (s, 2H), 4.67 (s, 2H), 4.72 (s, 2H), 7.32 (s, 4H); MS $(M + NH_4)^+ = 286$.

1-[[(Methylsulfonyl)oxy]methyl]-4-[[[(trimethylsilyl)-ethoxy]methoxy]methyl]benzene. To 622 mg (2.32 mmol) of the hydroxymethyl compound described above in 25 mL of dichloromethane were added at 0 °C 0.404 mL of triethylamine (1.25 equiv) and 0.183 mL of methanesulfonyl chloride (1.0 equiv). After 0.5 h at 0 °C, the solvent was removed *in vacuo* and the crude product purified directly by silica gel column chromatography (5% EtOAc/95% CH_2Cl_2) to give 638 mg (79%) of product: ^{1}H NMR ($CDCl_3$) δ 0.02 (s, 9H), 0.95 (t, J = 9.0 Hz, 2H), 2.88 (s, 3H), 3.65 (t, J = 9.0 Hz, 2H), 4.60 (s, 2H), 4.72 (s, 2H), 5.20 (s, 2H), 7.38 (s, 2H); MS $(M + NH_4)^+ = 364$.

(5*R*,6*R*)-2,4-Bis[(hydroxymethyl)benzyl]-1,5-dibenzyl-6-hydroxy-3-oxo-1,2,4-triazacycloheptane (7a)—General Procedure for Alkylation of Urea 6 and Deprotection of the SEM Groups. To a solution of 180 mg of urea **6** (0.41 mmol) in 3 mL of DMF was added 165 mg of NaH (60% oil dispersion, 10 equiv). After 15 min at room temperature and the evolution of hydrogen stopped, a solution of 680 mg (5 equiv) of the mesylate described above was added. After 3 h at room temperature, the reaction was quenched carefully with saturated NH_4Cl at 0 °C and the mixture extracted with ether (3 × 50 mL). The combined organic layer was washed with saturated NaCl solution and dried with anhydrous Na_2SO_4. Concentration and purification by silica gel column chromatography (10% EtOAc/90% hexane) provided 285 mg (73%) of the SEM-protected product. To a solution of 280 mg of this product in 2.8 mL of methanol was added 0.28 mL of chlorotrimethylsilane. The solution was stirred at room temperature for 1.25 h. The sovlent was removed *in vacuo*, and the crude product was purified by silica gel column chromatography (5% MeOH/95% CH_2Cl_2) to give 150 mg (88%) of compound **7a**: ^{1}H NMR ($CDCl_3$) δ 1.55 (br s, 3H), 2.70 (d, J = 9.0 Hz, 2H), 2.92 (m, 2H), 3.08 (m, 1H), 3.40 (m, 1H), 3.80 (m, 1H), 4.06 (ABq, J = 9 Hz, 4H), 4.70 (s, 4H), 4.90 (m, 2H), 7.06−7.50 (m, 18H); MS $(M + H)^+ = 552$. Anal. ($C_{34}H_{37}N_3O_4$) C, H, N.

(5*R*,6*R*)-1-(3-Furanylmethyl)-5-benzyl-3-oxo-6-[[(trimethylsilyl)ethoxy]methoxy]-1,2,4-triazacycloheptane. The synthesis of this urea followed the same sequence of reactions leading to **6**, except the N^1-[(*tert*-butyloxy)carbonyl]-N^2-benzylhydrazine was replaced by N^1-[(*tert*-butyloxy)carbonyl]-N^2-(3-furanylmethyl)hydrazine: ^{1}H NMR ($CDCl_3$) δ 0.02 (s, 9H), 0.92 (ABq, J = 7 Hz, 2H), 2.95 (d, J = 7.0 Hz, 2H), 3.10 (dd, J = 4, 7 Hz, 1H), 3.20 (dd, J = 4, 7 Hz, 1H), 3.65 (m, 3H), 3.95 (m, 3H), 4.45 (br s, 1H), 4.72 (ABq, J = 7.5 Hz, 2H), 5.65 (d, J = 1.5 Hz, 1H), 6.48 (t, J = 1.5 Hz, 1H), 7.20 (m, 5H), 7.38 (d, J = 1.5 Hz, 1H); MS $(M + H)^+ = 431$. Anal. ($C_{22}H_{33}N_3O_4Si$) C, H, N.

(5*R*,6*R*)-2,4-Bis[4-(hydroxymethyl)benzyl]-1-(3-furanylmethyl)-5-benzyl-6-hydroxy-3-oxo-1,2,4-triazacycloheptane (7b). The above-described urea was alkylated and the SEM group deprotected using the identical procedures described for **7a** to give **7b** in 89% yield: ^{1}H NMR ($CDCl_3$) δ 1.55 (br s, 3H), 2.70−3.00 (m, 5H), 3.40 (m, 1H), 3.80 (m, 1H), 3.90 (ABq, J = 13.5 Hz, 2H), 4.10 (m, 1H), 4.66 (s, 2H), 4.68 (s, 2H), 4.85 (m, 2H), 6.83 (d, J = 1.5 Hz, 1H), 7.05−7.48 (m, 15H); MS $(M + H)^+ = 542$. Anal. ($C_{32}H_{35}N_3O_5 \cdot 0.25H_2O$) C, H, N.

(5*R*,6*R*)-1,2,4,5-Tetrabenzyl-6-hydroxy-3-oxo-1,2,4-triazacycloheptane (7c). Alkylation of the urea **6** with benzyl bromide and deprotection of the SEM group using identical procedures described for **7a** provided **7c** in 91% yield: ^{1}H NMR ($CDCl_3$) δ 1.18 (d, J = 6 Hz, 1H), 2.71 (d, J = 10 Hz, 1H), 2.91 (dd, J = 3, 10 Hz, 1H), 2.96 (d, J = 3 Hz, 1H), 3.10 (dd, J = 10, 15 Hz, 1H), 3.46 (m, 1H), 3.82 (m, 1H), 4.05 (m, 3H), 4.92 (d, J = 15 Hz, 1H), 7.10−7.50 (m, 20H); MS $(M + H)^+ = 504$. Anal. ($C_{32}H_{33}N_3O_2 \cdot 0.5H_2O$) C, H, N.

(5*R*,6*R*)-2,4-Bis(4-hydroxybenzyl)-1,5-dibenzyl-6-hydroxy-3-oxo-1,2,4-triazacycloheptane (7d). Alkylation of the urea **6** with 4-[[(trimethylsilyl)ethoxy]methoxy]benzyl chloride and deprotection of the SEM groups using identical procedures described for **7a** provided **7d** in 85% yield: ^{1}H NMR (DMSO-d_6) δ 2.64 (d, J = 13.5 Hz, 1H), 2.87 (m, 2H), 3.48 (m, 3H), 3.60 (m, 1H), 3.77 (d, J = 13.5 Hz, 1H), 3.90 (ABq, J = 10 Hz, 2H), 4.57 (dd, J = 3, 12, Hz, 2H), 4.90 (d, J = 4.5 Hz, 1H), 6.72 (d, J = 9 Hz, 4H), 6.90 (d, J = 9 Hz, 2H), 7.04 (d, J = 9 Hz, 2H), 7.25 (m, 10H), 9.27 (s, 1H), 9.38 (s, 1H); MS $(M + H)^+ = 524$. Anal. ($C_{32}H_{33}N_3O_4 \cdot 0.25H_2O$) C, H, N.

(5*R*,6*R*)-2,4-Bis(3-methoxybenzyl)-1,5-dibenzyl-6-hydroxy-3-oxo-1,2,4-triazacycloheptane (7e). Alkylation of urea **6** with 3-methoxybenzyl bromide and deprotection of the SEM group using identical procedures described for **7a** provided **7e** in 75% yield: ^{1}H NMR ($CDCl_3$) δ 1.20 (d, J = 6 Hz, 1H), 2.72 (m, 2H), 2.93 (m, 2H), 3.10 (dd, J = 12, 13.5 Hz, 1H), 3.47 (dt, J = 4, 13.5 Hz, 1H), 3.78 (s, 3H), 3.80 (s, 3H), 3.86 (m, 2H), 4.0 (m, 1H), 4.08 (s, 2H), 4.90 (d, J = 13.5 Hz, 2H), 6.75 (m, 4H), 7.10 (m, 4H), 7.30 (m, 10H); MS $(M + H)^+ = 552$. Anal. ($C_{34}H_{37}N_3O_4$) C, H, N.

3-Methoxy-4-[[(trimethylsilyl)ethoxy]methoxy]-benzaldehyde. To a solution of 2.5 g of vanillin in 100 mL of dichloromethane were added 3.43 mL (1.2 equiv) of diisopropylethylamine and 3.1 mL (1.0 equiv) of SEM-Cl. After 15 min at room temperature, the solvent was removed *in vacuo*. The residue was washed with saturated $NaHCO_3$ and extracted with EtOAc (3 × 100 mL), and the combined organic layer was dried and concentrated. The crude product was purified by silica gel column chromatography (20% EtOAc/80% hexane) to give 4.54 g (99%) of product: ^{1}H NMR ($CDCl_3$) δ 0.01 (s, 9H), 0.97 (t, J = 7.5 Hz, 2H), 3.80 (t, J = 7.5 Hz, 2H), 3.96 (s, 3H), 7.30 (d, J = 9 Hz, 1H), 7.42 (m, 2H); MS $(M + H)^+ = 283$.

3-Methoxy-4-[[(trimethylsilyl)ethoxy]methoxy]-benzyl Chloride. The benzaldehyde described above was reduced with $NaBH_4$ quantitatively to the corresponding alcohol. To 2.30 g of this alcohol in 80 mL of dichloromethane were added 1.4 mL (1.25 equiv) of triethylamine and 0.64 mL (1.0 equiv) of methanesulfonyl chloride at 0 °C. After 30 min at 0 °C, the solution was stirred at room temperature for 1.25 h. The solvent was removed *in vacuo*, and the light yellow oil was purified directly by silica gel column chromatography (CH_2Cl_2) to give 1.56 g (64%) of product: ^{1}H NMR ($CDCl_3$) δ 0.01 (s, 9H), 0.96 (t, J = 7.5 Hz, 2H), 3.80 (t, J = 7.5 Hz, 2H), 3.90 (s, 3h), 4.58 (s, 2H), 5.29 (s, 2H), 6.90 (m, 2H), 7.15 (d, J = 7.0 Hz, 1H); MS $((M + H)_4)^+ = 320$.

(5*R*,6*R*)-2,4-Bis(4-hydroxy-3-methoxybenzyl)-1,5-dibenzyl-6-hydroxy-3-oxo-1,2,4-triazacycloheptane (7f). Alkylation of urea **6** with the substituted benzyl chloride described above and deprotection of the SEM groups using the identical procedures described for **7a** provided **7f** in 80% yield: ^{1}H NMR (DMSO-d_6) δ 2.55 (m, 2H), 2.65 (d, J = 13.5 Hz, 1H), 2.88 (m, 2H), 3.42 (m, 1H), 3.68 (m, 1H), 3.73 (s, 3H), 3.74 (s, 3H), 3.77 (d, J = 13.5 Hz, 1H), 3.96 (m, 2H), 4.60 (d, J = 13.5 Hz, 1H), 4.62 (d, J = 13.5 Hz, 1H), 4.95 (d, J = 4.5 Hz, 1H), 6.47 (dd, J = 2, 7.5 Hz, 1H), 6.64 (d, J = 2 Hz, 1H), 6.74 (m, 2H), 6.83 (dd, J = 2, 7.5 Hz, 1H), 7.0 (d, J = 2 Hz, 1H), 7.08 (d, J = 7.5 Hz, 2H), 7.25 (m, 9H), 8.84 (s, 1H), 8.97 (s, 1H); MS $(M + H)^+ = 584$. Anal. ($C_{34}H_{37}N_3O_6$) C, H, N.

(5*R*,6*R*)-2-(Cyclopropylmethyl)-4-(4-hydroxy-3-methoxybenzyl)-1,5-dibenzyl-6-hydroxy-3-oxo-1,2,4-triazacycloheptane (7g). Stepwise alkylation of urea **6**, first with (bromomethyl)cyclopropane and then with 3-methoxy-4-[[(trimethylsilyl)ethoxy]methoxy]benzyl chloride, and deprotection of the SEM groups using identical procedures described for **7a** provided **7g** in 60% yield: ^{1}H NMR ($CDCl_3$) δ 0.35 (m, 2H), 0.60 (m, 2H), 1.28 (m, 1H), 1.30 (d, J = 6 Hz, 1H), 3.0 (m, 5H), 3.25 (t, J = 12 Hz, 1H), 3.37 (dd, J = 9, 13.5 Hz, 1H), 3.48 (dt, J = 3, 10.5 Hz, 1H), 3.83 (s, 3H), 3.90 (m, 1H), 4.10 (s, 2H), 4.77 (d, J = 13.5 Hz, 1H), 5.57 (s, 1H), 6.62 (d, J = 9 Hz, 1H), 6.63 (s, 1H), 6.76 (d, J = 9 Hz, 1H), 7.25 (m, 10H); MS $(M + H)^+ = 502$. Anal. ($C_{30}H_{35}N_3O_4$) C, H, N.

X-ray Crystallography. Recombinant HIV-1 protease expressed in *Escherichia coli* (Rittenhouse, J.; Turon, M. C.; Helfrich, R. J.; Albrecht, K. S.; Weigl, D.; Simmer, R. L.; Mordini, F.; Erickson, J.; Kohlbrenner, W. E. *Biochem. Biophys. Res. Commun.* **1990**, *171*, 60−66) was purified to >95% purity. Approximately 5-fold molar excess of **7f** was dissolved in DMSO to make a 2% DMSO in 2−2.5 mg/mL protease solution buffered with 0.1 M *N*-ethylmorpholine at pH 5.4. After overnight incubation of the protease and inhibitor mixture, a 20% ammonium sulfate in sodium acetate buffer of pH 5.5 was used as precipitants in the hanging drop method for crystallization at room temperature. Crystals of *P*61 space group with $a = b = 63.08$ Å and $c = 83.68$ Å were obtained in 7−10 days.

Diffraction data were collected with a Rigaku R-AXIS II imaging plate detector system (Molecular Structure Corp., Woodlands, TX) and processed to a final R merge of 5.0% with a program by T. Higashi, Rigaku Corp. The total number of reflections was 16 426 from a resolution range of 33−1.8 Å with an average redundancy of 4.7 and 93% completion. For the range of 2.0−1.8 Å, data had 85% completion and the average FSQ/SIG was 3.1.

The starting model of protease dimer was from the in-house coordinates of the refined structure of ABT-538 and protease complex.[11] The parameters of **7f** were generated using QUANTA (version 4.0; Molecular Simulations Inc., Burlington, MA). A quantum mechanical calculation for the core of the 7-membered ring of **7f** was carried out with Gaussian 92 (Gaussian, Inc., Pittsburgh, PA, 1992) to clarify the bond characteristics of nitrogen atoms of the ring. An initial $F_o - F_c$ difference Fourier map calculated with protease coordinates using PROTEIN (W. Steigeman, Max Planck Institut, Muenchen, FRG) clearly revealed the density for **7f**. The inhibitor model was built into the difference density with manual adjustment using PRODO (Jones, T. A. *Methods Enzymol.* **1985**, *115*, 157). The model of the complex was then refined using X-PLOR (Brunger, A.T. *X-PLOR, Version 3.1*; Yale University Press: New Haven, CT, 1992). Following the protocol of X-PLOR, rounds of model building and refinement with simulated annealing (temperature from 1000 to 300 K) were performed. Due to the near C_2 symmetry of **7f**, two orientations of the inhibitor were tried, but only one orientation was preferred judging from the density for the hydroxyl group of **7f**. The R value for the final model was 20.1% from 10.0−1.8 Å resolution. The rms deviations from ideal bond distances and angles are 0.014 Å and 3.1°, respectively. Full details of the crystallographic analysis will be published elsewhere with other inhibitor complexes.

Determination of Apparent K_i. HIV-1 protease activity was measured by a continuous fluorometric assay[28] using the internally quenched fluorogenic substrate DABCYL-GABA-Ser-Gln-Tyr-Pro-Ile-Val-Gln-EDANS (BACHEM; M-1865) and a SPEX FluoroMax spectrofluorometer (excitation 340 nm, emission 490 nm). The reactions were run at 30 °C and pH 4.7 in a final volume of 300 μL. The reaction mixture contained 1 M sodium chloride, 125 mM sodium acetate, 1 mg/mL bovine serum albumin, 3 μM substrate, the appropriate concentration of inhibitor, about 0.5 nM recombinant protease, and DMSO at a final concentration of 3%. The substrate and inhibitor were dissolved in DMSO and diluted with DMSO to 100 times the final concentration in the reaction. The protease was preincubated with the inhibitor at 30 °C for 8 min prior to initiation of the reaction with substrate. The reaction rate was determined from the linear portion of the progress curve between 1 and 6 min. At least five concentrations of inhibitor were used to give bewteen 15% and 85% inhibition. The apparent K_i was estimated by nonlinear regression (GraFit, Erithacus Software) to the equation for tight-binding inhibitor:[29,30]

$$v = C\{((K + I - fE)^2 + 4KfE)^{1/2} - (K + I - fE)\}/2$$

where v = reaction rate, K = apparent K_i, I = total inhibitor concentration, E = total enzyme concentration, f = fraction of active enzyme, and $C = k_{cat}S/(K_m + S)$. The regression program provides estimates for the parameters K, f, and C.

Molecular Modeling. Molecular modeling was carried out using the BIOSYM software package using the DISCOVER CVFF force field. Structures of **7c**−**e** were created by modifying the P_1/P_1' residues of the *S*,*S*-diol C_2 symmetric inhibitor A76928 (entry 1HVK in the Protein Data Bank)[31] to include an additional urea substituent to occupy the position of Wat 415 of 1HVK (corresponds to Wat 301 in ref 18). Note that the *S*-hydroxy configuration in A-76928 corresponds to the desired *R*-hydroxy configuration in the **7a**−**f** series. Benzyl groups were added to the urea nitrogens and oriented within the S_2/S_2' subsites. The aza nitrogen of **7c** was assumed to be sp^3-hybridized in accord with literature precedent.[32] Energy minimization of the inhibitor structure was carried out while keeping the protein atoms fixed in position and terminated when the rms energy gradient decreased to below 0.4 kcal/mol/Å.

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Supporting Information Available: 300 MHz ^{1}H NMR data for compounds **7a**−**g** (7 pages). Ordering information can be found on any current masthead page.

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