Improved P1/P1′ Substituents for Cyclic Urea Based HIV-1 Protease Inhibitors: Synthesis, Structure−Activity Relationship, and X-ray Crystal Structure Analysis

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We present several novel P1/P1′ substituents that can replace the characteristic benzyl P1/P1′ moiety of the cyclic urea based HIV protease inhibitor series. These substituents typically provide 5−10-fold improvements in binding affinity compared to the unsubstituted benzyl analogs. The best substituent was the 3,4-(ethylenedioxy)benzyl group. Proper balancing of the molecule's lipophilicity facilitated the transfer of this improved binding affinity into a superior cellular antiviral activity profile. Several analogs were evaluated further for protein binding and resistance liabilities. Compound **18** (IC_{90} = 8.7 nM) was chosen for oral bioavailability studies based on its log *P* and solubility profile. A 10 mg/kg dose in dogs provided modest bioavailability with $C_{max} = 0.22$ μg/mL. X-ray crystallographic analysis of two analogs revealed several interesting features responsible for the 3,4-(ethylenedioxy)benzyl-substituted analog's potency: (1) Comparing the two complexes revealed two distinct binding modes for each P1/P1′ substituent; (2) The ethylenedioxy moieties are within 3.6 Å of Pro 81 providing additional van der Waals contacts missing from the parent structure; (3) The enzyme's Arg 8 side chain moves away from the P1 substituent to accommodate the increased steric volume while maintaining a favorable hydrogen bond distance between the para oxygen substituent and the guanidine NH.

Introduction

Advances in acquired immune deficiency syndrome (AIDS) chemotherapy revealed several promising discoveries used to combat this disease.[1] In particular, recent FDA approvals of Saquinavir, Indinavir, and Ritonavir show that human immunodeficiency virus (HIV) protease inhibitors (HIVPR) are valid approaches to AIDS therapy.[2] Despite the potency and good oral bioavailability of these entities, the emergence of resistant forms of HIV renders their long-term utility in question.[3] Clearly, there is a need for structurally unique compounds that may overcome these limitations. Much of the current literature on HIVPR inhibitors emphasizes structural elements that interact with the enzyme's S2/S2′ and S3/S3′ binding domains. Few reports deal with issues regarding the S1/S1′ pocket.[4]

Several papers have appeared describing the use of a novel 7-membered cyclic urea scaffold for the preparation of inhibitors of HIVPR.[5] This research culminated in the discovery of a promising clinical candidate DMP450.[6] The cyclic urea's structural rigidity allows for well-aligned interactions of its substituents with their corresponding binding elements in the active site of HIVPR. Typically, the preferred substituent at the P1/P1′ position was an unsubstituted phenyl ring.

We recently reported an extensive study of P1/P1′ analogs based on this 7-membered cyclic urea scaffold.[7] A few analogs had modestly improved binding affinity compared to the parent unsubstituted benzyl compound. By altering the P1/P1′ substitution pattern, we were also able to modulate the molecule's physical properties resulting in improved translation (defined as the ratio between the IC_{90} in a cellular assay and the K_i) and better overall antiviral activity. In general, the better antiviral activity resulted mainly from improvement in whole-cell translation rather than increasing enzyme affinity.

We have since expanded the scope of our investigation into this class of compounds and have found other P1 substituents that provide an increase in binding affinity. This culminated in the discovery of a novel P1 substituent that generated compounds with up to a 10-fold increase in binding affinity. To our knowledge, this was the first P1/P1′ substituent that provided such a dramatic increase in potency. In addition, we were able to crystallize two of these analogs in the active site of HIVPR. This helped determine the source of the increased binding affinity. This paper describes the scope and limitations of designing potent P1/P1′ modified cyclic urea based HIVPR inhibitors and their use as bioavailable antiviral agents.

Chemistry

The P1/P1′-modified compounds were prepared as shown in Scheme 1. The sequence begins with the key diepoxide intermediate **1** derived from L-mannonic γ-lactone. Diepoxide **1** was treated with 4 equiv of an organocuprate reagent prepared from 3,4-(ethylenedioxy)bromobenzene to give the diol **2** in 85% yield. This diol was converted to diazide **3** using standard Mitsunobu methodology[8] in 28% yield. This reaction was plagued by the elimination side reaction of one hydroxyl group from diol **2** to give an olefin byproduct. This competing side reaction has been observed previously[9] and was especially prevalent when the displaced alcohol was homobenzylic. The diazide was reduced to the diamine in 90% yield using LAH. The crude diamine

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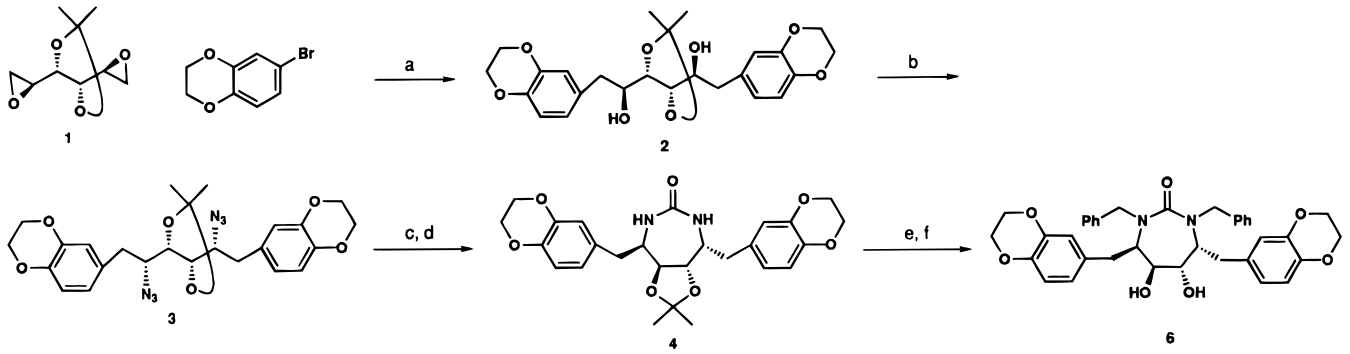
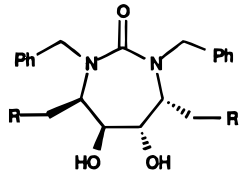
Scheme 1[a]



[a] Reagents and conditions: (a) *n*-BuLi, CuCN, THF, −78 to 0 °C, 85%; (b) Ph_3P, DEAD, $(PhO)_2P(O)N_3$, THF, 0−25 °C, 28%; (c) $LiAlH_4$, THF, room temperature, 90%; (d) CDI, tetrachloroethane, room temperature to reflux, 70%; (e) K^+ *t*-BuO^-, BnBr, THF, room temperature, 68%; (f) 20% concentrated HCl, CH_3CN, room temperature, 95%.

Table 1



compd	R	K_i (nM)[a]	IC_{90} (nM)[a]
parent	phenyl	3.4	790
6	3,4-(ethylenedioxy)phenyl	0.44	112
19	β-naphthyl	9.8	3200
20	3,5-dimethoxyphenyl	8.0	>5000
21	3,4-dimethoxyphenyl	6.4	640
22	3,4-(methylenedioxy)phenyl	1.0	504
23	2,3-dihydro-5-benzofuranyl	1.2	305
24	4-methyl-3,4-dihydro-6-benzoxazinyl	0.7	218

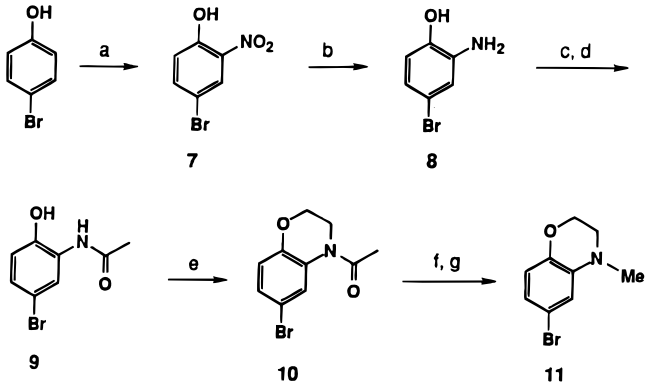
[a] Values determined using methods described in refs 19 and 20 for $n = 2$

was then cyclized using 1,1-carbonyldiimidazole in refluxing 1,1,2,2-tetrachloroethane to give the cyclic urea **4** in 70% yield. Elevated temperatures were required for the cyclization due to the presence of the *trans* fused acetonide protecting group. The desired P2/P2′ substituent was introduced using potassium *tert*-butoxide and the corresponding alkylating agent. Removal of the acetonide protecting group using 20% concentrated HCl in methanol gave the target urea **6** in 95% yield.

Table 1 shows several examples (**19**, **21**, and **22**) prepared in similar fashion from commercially available substituted aryl bromides. Compound **23** was prepared from the known 5-bromodihydrobenzofuran[10] while urea **20** was prepared using an amino acid-based approach starting with 3,5-dimethoxy-D-phenylalanine as described previously.[11]

The synthesis of 4-methyl-3,4-dihydro-2*H*-benzo[*b*]-[1,4]oxazin-6-yl bromide, used to prepare **24**, is shown in Scheme 2. Selective mononitration of *p*-bromophenol proceeded smoothly using nitric acid impregnated silica gel[12] to give the desired 4-bromo-2-nitrophenol **7** in 97% yield. Reduction of the nitro group using $SnCl_2$ gave the desired aniline **8** in 74% yield. Bisacylation of the amino phenol **8** with acetic anhydride followed by selective removal of the phenolic acetate gave the amide **9** in 97% yield for the two steps. The *N*-acyl group was critical for efficient introduction of the ethylene bridging group in the next step.[13] This was accomplished with

Scheme 2[a]



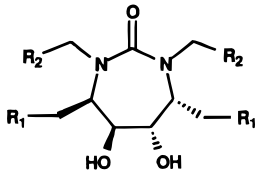
[a] Reagents and conditions: (a) HNO_3-SiO_2, CH_2Cl_2, room temperature, 97%; (b) $SnCl_2$, HCl, 74%; (c) Ac_2O, pyridine, quant; (d) NaOH, MeOH, room temperature, 97%; (e) NaOH, 1,2-dibromomethane, Aliquat 336, CH_2Cl_2/CH_3CN, room temperature, 90%; (f) KOH, $MeOH/H_2O$, 55 °C, quant; (g) H_2CO, $NaCNBH_3$, CH_3CN, 95%.

1,2-dibromoethane using Aliquat 336 as the base and a CH_2Cl_2/CH_3CN solvent mixture at room temperature to give the desired product **10** in 90% yield. A simple two-step protocol transformed the *N*-acyl group to the desired *N*-methyl group to give the bromide **11** in 95% overall yield.

Several of the P2 variants shown in Table 2 (**25**, **26**, and **28**) were prepared according to Scheme 1 using commercially available alkylating agents. The methyl ketone analogs (**13**, **30**, **35** and **36**) were prepared according to Scheme 3 using **13** as an example. The urea **4** was alkylated with *m*-cyanobenzyl bromide under standard conditions to give compound **12** in 94% yield. Conversion of the nitrile to the methyl ketone was accomplished using excess methylmagnesium bromide in THF followed by an acetic acid workup to give a quantitative yield of the desired ketone. The acetonide protecting group was removed as previously described to give **13** in 90% overall yield for the two steps. The *p*-fluoro methyl ketone analogs **29** and **34** were prepared in a similar fashion from 4-fluoro-3-cyanobenzyl bromide.[14]

The *m*-amino-containing analogs (**15**, **17**, **18**, **31**, and **32**) were prepared according to Scheme 4 using **18** as an example. Alkylation of urea **4** with *m*-nitrobenzyl bromide provided the desired bis-alkylated urea **14** in 84% yield. Reduction to the amine and subsequent deprotection of the acetonide protecting group was done in a one-pot procedure and provided **15** in 83% yield for

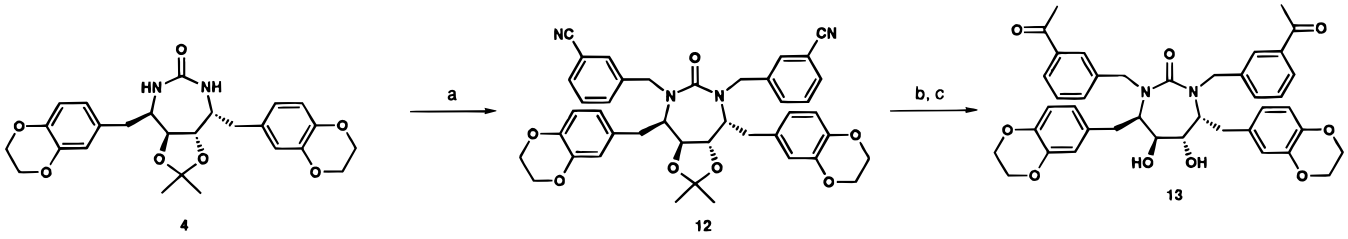
Table 2



compd	R_1	R_2	K_i (nM)[a]	IC_{90} (nM)[a]
13	3,4-(ethylenedioxy)phenyl	*m*-$CH_3C(O)C_6H_4$	0.04 (0.07)	4.2 (40)
15		*m*-$NH_2C_6H_4$	0.04 (0.23)[b]	40 (150)[b]
17		R_2 = *m*-$NH_2C_6H_4$ $R_{2'}$ = *m*-$MeNHC_6H_4$	0.11	27
18		*m*-$MeNHC_6H_4$	0.14 (0.15)	8.7 (88)
25		cyclopropyl	0.27 (2.1)	928 (1800)
26		β-naphthyl	0.04 (0.31)	98 (3900)
27		*p*-$HOCH_2C_6H_4$	0.05 (0.27)	83 (136)
28		*p*-HOC_6H_4	0.03 (0.12)	107 (32)
29		*m*-$CH_3C(O)$-*p*-FC_6H_4	0.64 (4.4)	84 (860)
30	2,3-dihydro-5-benzofuranyl	*m*-$CH_3C(O)C_6H_4$	0.048	10
31		*m*-$NH_2C_6H_4$	0.11	30.6
32		*m*-$MeNHC_6H_4$	0.20	61.6
33	3,4-(methylenedioxy)phenyl	*m*-$CH_3C(O)C_6H_4$	0.043	14.7
34		*m*-$CH_3C(O)$-*p*-FC_6H_4	1.5	265
35	3,5-dimethoxyphenyl	*m*-$CH_3C(O)C_6H_4$	0.4	46.4
36	4-methyl-3,4-dihydro-6-benzoxazinyl	*m*-$CH_3C(O)C_6H_4$	0.08	16.4

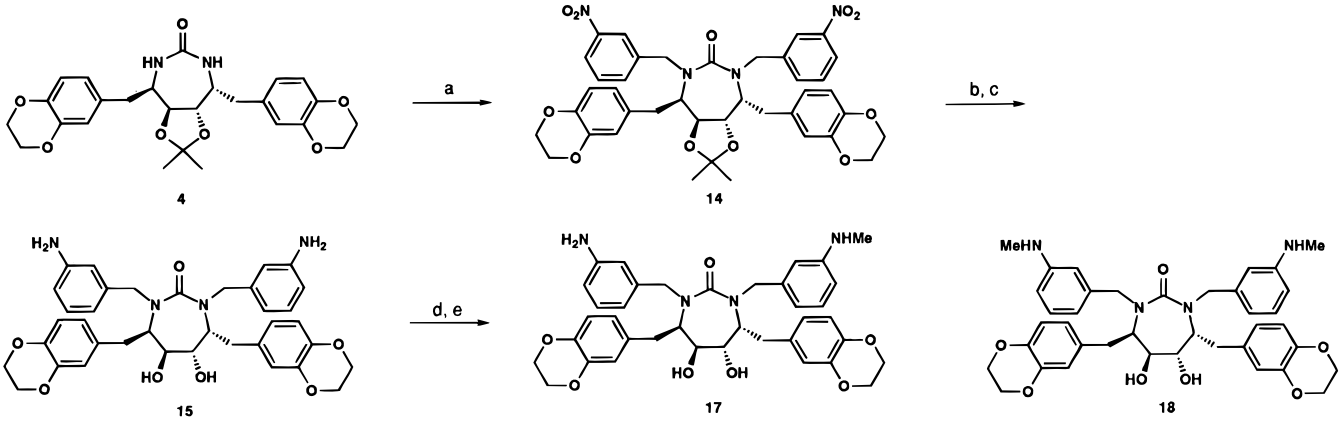
[a] Values determined using methods described in refs 19 and 20 for $n = 2$. Values in parentheses correspond to the parent compounds having an unsubstituted phenyl ring at the P1 position. [b] Value for DMP450 taken from ref 6.

Scheme 3[a]



[a] Reagents and conditions: (a) *m*-cyanobenzyl bromide, K^+t-BuO^-, THF, room temperature, 94%; (b) methylmagnesium bromide, THF, reflux, 95%; (c) HCl, THF:MeOH, room temperature, 88%.

Scheme 4[a]



[a] Reagents and conditions: (a) *m*-nitrobenzyl bromide, K^+t-BuO^-, THF, room temperature, 84%; (b) H_2, 5% Pd/C, THF, 94%; (c) 20% concentrated HCl in MeOH, room temperature, 91%; (d) neat butyl formate, reflux, 94%; (e) BH_3−THF, THF, reflux, 88%.

the two steps. Selective monomethyl introduction was accomplished using a two-step protocol. Formylation of the aniline nitrogen using butyl formate gave compound **16** in 80% yield. Initial reduction conditions using borane−THF complex gave a mixture of two products corresponding to **17** and **18** in a 1:1 ratio. The desired product could be prepared cleanly by reduction with LAH in refluxing THF for 15 min to give **18** as the sole product in 95% yield.

Results and Discussion

Initial SAR. Additional oxygen-bearing P1 benzyl analogs related to compound **6** were prepared to examine the source of the observed increased binding affinity. The binding affinity (K_i) and cellular antiviral activity (IC_{90}) of these analogs are presented in Table 1. Clearly, the 3,4-(ethylenedioxy)benzyl substituent was unique. Breaking the ethylene bridge carbon−carbon bond,

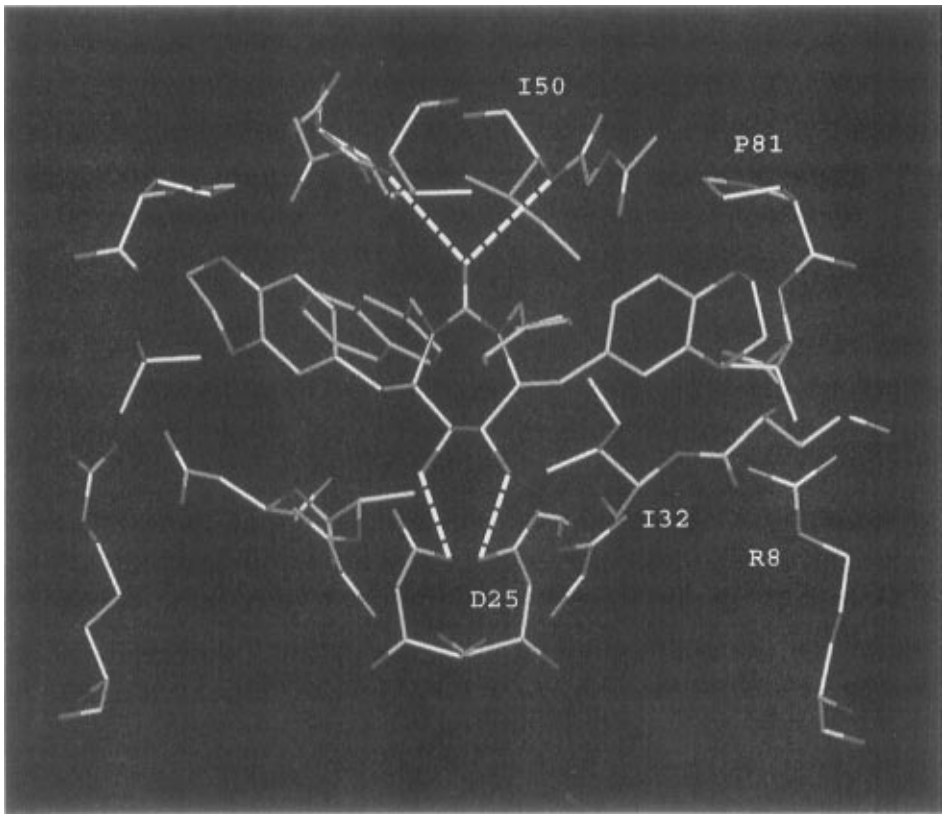


Figure 1. Inhibitor **26** bound within the active site of HIVPR. Protein residues located within 4.1 Å from the inhibitor are shown. Hydrogen bonds between the inhibitor and key enzyme residues are shown as yellow dotted lines.

exemplified by compound **21**, eliminates any advantageous binding phenomenon. The binding affinity of **21** was comparable to the parent unsubstituted P1 phenyl analog, and only a slight improvement was seen in the whole-cell antiviral activity. The increased binding affinity does not appear to be related to the P1 phenyl ring's electronic character. MOPAC calculations showed similar electron density coefficients for the P1 phenyl ring carbons, resulting in similar phenyl ring π distributions for compounds **6** and **21**. The order of magnitude loss in antiviral activity observed with compound **20** results from poor translation. The source of this loss in translation is unclear. Both compound **20** and the parent have identical clogP values.

The piperonyl analogs **22** and **23** also show an improved binding affinity profile when compared to the parent. Shortening the bridge between the oxygen substituents from two carbons to a methylene group affects the binding compared to **6**, but both are still more potent than the parent. Removing one oxygen atom from the piperonyl group (**23**) has little effect on binding affinity. In addition, the translation improves as indicated by the lower IC_{90} for **23**. It seems that both oxygen substituents may not be critical for improved binding affinity.

This is further exemplified by compound **24**. Replacing the meta oxygen with an *N*-methyl nitrogen shows a slight decrease in K_i compared with **23**. This suggests the meta oxygen lacks critical interactions with S1 pocket residues. In addition, the *N*-methyl group's increased lipophilicity relative to the oxygen atom further improves the translation of compound **24** with antiviral activity now 3.5-fold greater than the parent. Replacing the oxygen atom with an *N*-methyl group has an added advantage. The additional basic nitrogen site allows for the formation of water soluble salts, thereby increasing the chances for good bioavailability.

Pure steric or hydrophobic interactions alone cannot account for the improved binding affinity. Compound **19** shows that simply filling the hydrophobic S1 pocket with a large hydrophobic substituent is not sufficient for increased binding affinity. A combination of both van der Waals interactions and hydrogen bond interactions seems to be responsible for the improved binding affinity. These interactions can be seen in the crystal structures of two analogs **15** and **26** bound in the active site of HIVPR (Figures 1 and 2).

X-ray Crystal Structure Analysis. Figure 1 shows all the structural elements of inibitor **26** interacting with their corresponding binding domains. The urea carbonyl oxygen creates a hydrogen bond with both flap residues Ile 50 and 150. The diol oxygens create two hydrogen bonds with both catalytic aspartic acid residues Asp 25 and 125. The P2 naphthyl substituent fills the S2 pocket and has several van der Waals contacts with residues Ile 28, Asp 30, Val 32, and Gly 48. The P2′ substituent has the same contacts mentioned above in addition to Ile 47′ and Ile 84′. The 3,4-(ethylenedioxy)benzyl P1 substituent fills the S1 pocket. The ethylenedioxy moiety's para oxygen atom is within 3.6 Å of Pro 81. This interaction contributes additional van der Waals contacts missing from the parent structure. In addition, the meta-substituted oxygen lies within 3.7 Å of the Arg 8 guanidine NH creating a weak hydrogen bond interaction also not present in the parent. An efficient network of hydrogen bond and van der Waals interactions contribute to the excellent binding affinity observed for this analog (K_i = 0.04 nM).

Figure 2 shows an overlap of the crystal structures of DMP450 and its close analog **15**. The crystal struc-

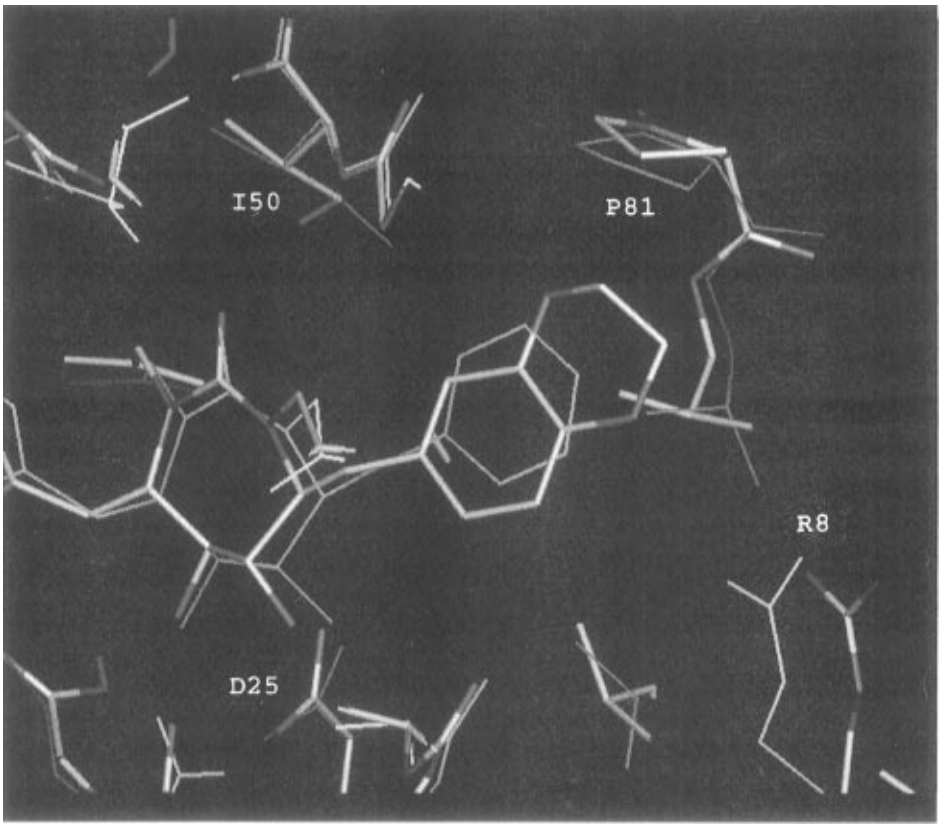


Figure 2. Overlap of inhibitor **15** and DMP450 in the active site of HIVPR. For **15**, carbon atoms are colored gray, oxygen atoms are red, and nitrogen atoms are blue. All atoms of DMP450 are colored yellow.

ture of DMP450 is shown in yellow, and the overlapped structure of **15** is colored by element type. This figure allows us to analyze how the enzyme accommodates the structurally related inhibitors in different ways. Little movement is seen in the enzyme's C_α trace when comparing the two structures.[15] Since the P2/P2′ *m*-aminobenzyl substituents of DMP450 and **15** show little variance, they were Z-clipped out of the image for clarity. Both substituents have similar spatial orientations of their perspective phenyl rings. In addition, the hydrogen bond trajectory between the meta amino group and the side chain carboxylate of Asp 29 is very similar. The hydrogen bond distance differs by only 0.5 Å.

The S1 pocket shows a bit more diversity. The P1 substituent's orientation of **15** has rotated 180° compared to **26**. This demonstrates the enzyme's flexibility at accommodating the same substituent in different conformations. The same interaction between the ethylenedioxy group and Pro 81 was observed as shown previously in Figure 1 except here both carbon and oxygen atoms contribute to the interaction. In addition, the Arg 8 side chain has now moved away from the P1 substituent compared to DMP450. This movement accommodates the increased steric bulk of the substituted P1 phenyl ring while maintaining a weak hydrogen bond (4.1 Å) between the para oxygen substituent and the guanidine moiety. These additional interactions contribute to the improved binding affinity observed for compound **15** ($K_i = 0.04$) compared to that for DMP450 ($K_i = 0.23$).

Antiviral Potency Optimization and *in Vivo* Studies. To further improve this series' binding affinity and overall antiviral activity, we introduced P2 substituents having additional hydrogen bond generating capabilities. These examples are shown in Table 2. For comparison, the parent structure's values for both K_i and IC_{90} are presented in parentheses. In almost every case, a superior binding affinity profile was observed compared to the parent structures having an unsubstituted P1 phenyl ring. It was essential to have the correct polarity balance between the P1 and P2 substituents for good antiviral activity. Compounds **15**, **27**, and **28** have P2 substituents that are relatively polar. Despite their excellent binding affinities, they do not translate well and have mediocre antiviral properties. Lipophilic substituents at P2 complement the slightly polar nature of the 3,4-(ethylenedioxy)benzyl substituent. Judicious choice of the P2 substituent's characteristics leads to good translation of the increased binding affinity. The substituents that provided the best overall profile are exemplified by compounds **13**, **18**, **30**, **33**, and **36**. Meta-substituted *N*-methylamines and methyl ketones gave the most potent compounds in this series. An attempt at increasing the translation of compounds **13** and **33** by introducing a para fluoro substituent (see **29** and **34**) failed. The fluorine atom disrupts the hydrogen-bonding capability of the carbonyl group. This resulted in a substantial increase in these analogs' K_i values. Nonetheless, they were still 3−7-fold better in binding affinity compared to the corresponding parent.

The five best compounds in this series were further evaluated. This entailed determining the compounds' resistance profiles and protein binding characteristics. The information is presented in Table 3. The selected I84V mutant strain is used in our primary resistance screen due to the severe susceptibility of some members of the cyclic urea class of HIVPR inhibitors to this mutant. In addition, this mutation plays a critical role in the development of cross-resistance to a structurally diverse set of HIVPR inhibitors.[16] The effects of resis-

Table 3. Adjusted IC_{90} Values for Selected Compounds

compd	IC_{90} (nM)[a]	PB potency shift[b]	I84V resistance shift[c]	adj IC_{90} (nM)[d]
13	4.2	30	10	1260
18	8.7	30	10	2610
30	10	17	31	5270
33	14.7	9	10	1323
36	16.4	>50	10	>8200

[a] Values determined using methods described in ref 20 for $n = 2$. [b] Corresponds to the fold shift in IC_{90} due to protein binding. [c] Corresponds to the fold shift in IC_{90} due to an I84V mutation. [d] Adj IC_{90} = IC_{90} × PB potency shift × I84V resistance shift.

tance and protein binding are expressed as the fold shift in the original IC_{90} value determined from Table 2. A final value is expressed as the adjusted IC_{90} which is the product of the original IC_{90}, the protein binding shift, and the I84V resistance shift. This value allows us to evaluate three key issues simultaneously, presenting a more accurate antiviral potency profile. The results show that these compounds are also susceptible to the I84V mutant of HIVPR and are substantially protein bound, in effect limiting the compound's antiviral activity. In particular, compound **36** was highly protein bound with its initial promising antiviral activity (IC_{90} = 16 nM) completely negated. By designing into the molecule a basic site for salt formation, with the hope of increased water solubility, we inadvertently increased its protein binding.

On the basis of a superior aqueous solubility and log *P* profile, compound **18** was chosen for further *in vivo* evaluation. The compound was dosed in both rats and dog to determine its bioavailability. At 10 mg/kg the compound failed to attain any measurable blood levels in rats. At the same dose in dogs, a measure of bioavailability was seen with a C_{max} = 0.22 μg/mL and a $F\%$ = 4.6. The iv data indicated the compound was cleared quite rapidly from circulation with a half-life of 0.8 h. No additional studies were conducted to determine the source of the high clearance values observed.

Conclusion

We presented several novel P1/P1′ substituents that provide increased binding affinity for HIVPR. The most potent substituent found was the 3,4-ethylenedioxy group that consistently provided a 5−10-fold improvement in binding affinity. This improvement in binding affinity frequently translated into an improved antiviral profile provided the P2/P2′ substituents are carefully chosen. Several potent analogs were further evaluated for their resistance and protein binding profiles. These compounds were found to be highly protein bound, limiting their antiviral efficacy. The most promising analog **18** was further evaluated *in vivo*. The compound did not attain any detectable blood levels in rats. A 10 mg/kg dose resulted in low blood levels in dogs with a C_{max} = 0.22 μg/mL. X-ray crystallographic analysis of two analogs bound in the active site of HIVPR revealed several interesting features. Both hydrophobic interactions and the presence of a weak hydrogen bond were responsible for this substituent's improved potency profile. Further evaluation of these P1/P1′ substituents is ongoing and will be reported in due course.

Experimental Section

All reactions were carried out with continuous stirring under an atmosphere of dry nitrogen. Commercial reagents were used as received without additional purification. THF was distilled from sodium benzophenone ketyl. ^{1}H NMR (300 MHz) spectra were recorded using tetramethylsilane as an internal standard. Melting points are uncorrected. TLC was performed on E. Merck 15719 silica gel plates. Flash chromatography was carried out using EM Science silica gel 60 (230−400 mesh). All final targets were obtained as noncrystalline amorphous solids unless specified otherwise. Elemental analysis was performed by Quantitative Technologies, Inc., Bound Brook, NJ. For compounds where analysis was not obtained, HPLC analysis was used, and purity was determined to be >98%. Analytical data for compounds **19** and **22** were disclosed previously.[7]

1,6-Dideoxy-1,6-bis(3,4-(ethylenedioxy)phenyl)-3,4-*O*-isopropylidene-L-mannitol (2). To a solution of 3,4-(ethylenedioxy)bromobenzene (27.75 g, 129 mmol) in THF (150 mL) at −78 °C was added *n*-butyllithium (51.5 mL, 129 mmol, 2.5 M in hexane). After 10 min the reaction was allowed to reach −20 °C to which copper(I) cyanide (5.77 g, 64.4 mmol) was added in one portion. After complete dissolution of the solid, the reaction was warmed to 0 °C and diepoxide **1** (3.0 g, 16.1 mmol) in THF (10 mL) was added via cannula. The reaction was allowed to reach room temperature, and stirring was continued for 15 min. The reaction was quenched with saturated NH_4Cl solution (25 mL), diluted with ether (600 mL), and washed with saturated NH_4Cl solution (2 × 200 mL). The organic layer was separated and dried ($MgSO_4$) and the solvent removed at reduced pressure. Chromatography (silica gel, 30−70% EtOAc/hexane) gave diol **2** as a foam (6.30 g, 85%). **2**: NMR ($CDCl_3$) δ 6.8 (m, 4 H), 6.7 (m, 2 H), 4.25 (s, 8 H), 3.75 (bs, 4 H), 3.05 (m, 2 H), 2.95 (bs, 2 H), 2.6 (m, 2 H), 1.4 (s, 6 H); CIMS (NH_3) *m*/*z* 459 (M + H^+, 34).

(2*R*,3*S*,4*S*,5*R*)-1,6-Bis(3,4-(ethylenedioxy)phenyl)-2,5-diazido-3,4-*O*-isopropylidenehexanediol (3). To a solution of diol **2** (6.3 g, 13.7 mmol) and triphenylphosphine (9.0 g, 34.3 mmol) in THF (30 mL) at 0 °C was added slowly diethyl azodicarboxylate (5.40 mL, 34.3 mmol). After 10 min diphenyl phosphorazidate (7.40 mL, 34.3 mmol) was added to the reaction slowly. The reaction was allowed to reach room temperature and stirred for 2.5 h. The reaction was quenched with methanol (10 mL) at 0 °C. The mixture was then stirred at room temperature for 10 min and the solvent removed at reduced pressure. Chromatography (silica gel, benzene to 1% EtOAc/benzene) gave the diazide **3** as an oil (1.95 g, 28%). **3**: NMR ($CDCl_3$) δ 6.8 (d, J = 8.0 Hz, 2 H), 6.75 (m, 2 H), 6.7 (m, 2 H), 4.25 (s, 8 H), 4.1 (bs, 2 H), 3.25 (m, 2 H), 2.9 (m, 4 H), 1.5 (s, 6 H).

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-*O*-isopropylidene-4,7-bis-((3,4-(ethylenedioxy)phenyl)methyl)-2*H*-1,3-diazapin-2-one (4). A solution of **3** (1.95 g, 3.83 mmol) in THF (30 mL) was cooled to 0 °C and was treated with a solution of LAH (11.5 mL, 11.5 mmol, 1.0 M in THF). The cooling bath was removed and the reaction reached room temperature. After 20 min the reaction was recooled to 0 °C and quenched with 1 N NaOH, diluted with EtOAc (50 mL), dried (Na_2SO_4), and filtered (Celite). The solvent was removed at reduced pressure, and the crude diamine was used without further purification. A solution of the diamine in tetrachloroethane (130 mL) was treated with carbonyldiimidazole (0.62 g, 3.80 mmol) in one portion. After 15 min the reaction was added via cannula to tetrachloroethane (10 mL) at reflux. After 15 min the reaction was cooled and chromatographed directly (silica gel, 2:1 to 1:1 EtOAc/hexane to 2.5% MeOH/CH_2Cl_2 to 5% MeOH/CH_2Cl_2) to give urea **4** as a white solid (1.18 g, 70%). **4**: mp 242−243 °C; NMR ($CDCl_3$) δ 6.8 (d, J = 8.0 Hz, 2 H), 6.75 (m, 2 H), 6.7 (m, 2 H), 4.9 (m, 2 H), 4.25 (s, 8 H), 3.5 (m, 4 H), 2.95 (m, 2 H), 2.7 (m, 2 H) 1.5 (s, 6 H); CIMS (NH_3) *m*/*z* 483 (M + H^+, 100). Anal. ($C_{26}H_{30}N_2O_7$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-*O*-isopropylidene-1,3-bis-(phenylmethyl)-4,7-bis((3,4-(ethylenedioxy)phenyl)methyl)-2*H*-1,3-diazapin-2-one (5). To a solution of **4** (0.1 g, 0.21 mmol) in dry THF (2 mL) at room temperature was added potassium *tert*-butoxide (0.62 mL, 0.62 mmol, 1 M in THF). After 5 min benzyl bromide (0.08 mL, 0.62 mmol) was added and the reaction stirred at room temperature for 1 h. The reaction was quenched with H_2O, diluted with ether (30 mL), washed with water (10 mL), dried ($MgSO_4$), and concentrated.

Chromatography (silica gel, 20% EtOAc/hexane) gave urea **5** as a foam (93 mg, 68%). **5**: NMR ($CDCl_3$) δ 7.3 (m, 6 H), 7.2 (m, 4 H), 6.8 (d, J = 8.0 Hz, 2 H), 6.65 (m, 2 H), 6.55 (m, 2 H), 5.0 (d, J = 14.0 Hz, 2 H), 4.3 (s, 8 H), 3.8 (bs, 2 H), 3.7 (m, 2 H), 3.2 (d, J = 14.0 Hz, 2 H), 2.8 (m, 4 H), 1.3 (s, 6 H); CIMS (NH_3) m/z 663 ($M + H^+$, 100).

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis(phenylmethyl)-4,7-bis((3,4-(ethylenedioxy)phenyl)methyl)-2*H*-1,3-diazapin-2-one (6). To a solution of urea **5** (93 mg, 0.14 mmol) in THF (2 mL) at room temperature was added 20% concentrated HCl in methanol (1.0 mL). After 15 min the reaction was poured into saturated $NaHCO_3$ (4 mL) and extracted with ethyl acetate (20 mL). The organic layer was separated, dried ($MgSO_4$), and concentrated. The residue was crystallized from ether to give **6** as a white solid (83 mg, 95%). **6**: mp 195−197 °C; NMR ($CDCl_3$) δ 7.3 (m, 4 H), 7.25 (m, 2 H), 7.2 (m, 4 H), 6.8 (d, J = 8.1 Hz, 2 H), 6.65 (m, 2 H), 6.6 (dd, J = 8.1, 2.0 Hz, 2 H), 4.95 (d, J = 14.2 Hz, 2 H), 4.3 (s, 8 H), 3.6 (m, 4 H), 3.25 (d, J = 14.2 Hz, 2 H), 2.9 (m, 4 H), 2.1 (bs, 2 H); CIMS (NH_3) m/z 623 ($M + H^+$, 100); HRMS calcd for $C_{37}H_{39}N_2O_7$ ($M + H^+$) 623.2757; found 623.2758. Anal. ($C_{37}H_{38}N_2O_7$) C, H, N.

2-Nitro-4-bromophenol (7). Silica gel 60 (EM Science) was added to nitric acid (69−71%, 150 mL) in one portion and the mixture stirred at room temperature for 2 h. The mixture was then filtered and air dried on a Buchner funnel. The solid was left to air dry in a crystallizing dish for 3 days. The loading level was determined by titration with 0.1 M NaOH to be 3.03 mmol g^{-1} nitric acid. This nitric acid impregnated silica gel (57.8 mmol, 19.1 g) was suspended in dichloromethane (450 mL) and cooled to 0 °C. The mixture was treated with 4-bromophenol (5.0 g, 23.0 mmol) in several portions. After 5 min the mixture was filtered, and the silica gel cake was washed with dichloromethane. The concentrated filtrate gave the desired product of sufficient purity for use in the next step (12.3 g, 97%). **7**: NMR ($CDCl_3$) δ 10.5 (s, 1 H), 8.26 (d, J = 2.6 Hz, 1 H), 7.67 (dd, J = 6.6, 2.6 Hz, 1 H), 7.09 (d, J = 8.8 Hz, 1 H); GC/MS (CH_4) m/z 218 ($M + H^+$, 20), 200 (100).

2-Amino-4-bromophenol (8). A mixture of stannous chloride dihydrate (24.8 g, 0.11 mmol) and concentrated hydrochloric acid (55 mL) in methanol (100 mL) was cooled to 15 °C and treated with 4-bromo-2-nitrophenol (5.0 g, 23.0 mmol) in one portion. After the addition was complete, the cooling bath was removed and the reaction stirred at room temperature for 16 h. The reaction mixture was diluted with ethyl acetate (200 mL) and the pH adjusted to 7 with saturated sodium bicarbonate solution. The mixture was filtered (Celite) and the filter cake washed with ethyl acetate. The organic phase was separated, the aqueous phase was extracted with ethyl acetate (100 mL), and the combined extracts were dried ($MgSO_4$) and concentrated to give the desired product as a white solid (3.5 g, 74%). **8**: NMR (DMSO-d_6) δ 9.28 (s, 1 H), 6.70 (s, 1 H), 6.57−6.47 (m, 2 H), 4.80 (s, 2 H); CIMS (CH_4) m/z 188 ($M + H^+$, 100).

***N*-Acetyl-2-amino-4-bromophenol (9).** 2-Amino-4-bromophenol (3.5 g, 18.6 mmol) in acetic anhydride (100 mL) containing several drops of pyridine was heated on a steam bath for 5 min and then at room temperature for another 5 min before the mixture was cooled in ice. The solid was collected on a Buchner funnel, washed with *n*-hexane, and air-dried to give the bisacetylated product. This solid was added to 1 M sodium hydroxide (50 mL) and stirred at room temperature until the mixture became homogeneous. The reaction mixture was then poured into a mixture of crushed ice (25 g) and 6 M hydrochloric acid (10 mL). The solid was collected on a Buchner funnel and air-dried to give the desired product as a tan solid (4.1 g, 97%). **9**: NMR (CD_3OD) δ 7.96 (d, J = 2.2 Hz, 1 H), 7.05 (dd, J = 8.8, 2.2 Hz, 1 H), 6.74 (d, J = 2.2 Hz, 1 H), 2.14 (s, 3 H). CIMS (NH_3) m/z 247/249 ($M + NH_4^+$, 97/98), 230/232 ($M + H^+$, 100/99).

1-(6-Bromo-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-4-yl)-1-ethanone (10). A solution of **9** (2.8 g, 12.2 mmol), 1.2-dibromoethane (4.2 mL, 49.0 mmol), sodium hydroxide (2.0 g, 49.0 mmol), and Aliquat 336 (0.6 g) in dichloromethane (25 mL) and acetonitrile (15 mL) was stirred at room temperature for 18 h. The reaction mixture was filtered, and the solids were washed with ether. The combined filtrates were concentrated at reduced pressure. Chromatography (silica gel, 50% ethyl acetate/hexane) gave the desired product as an oil (2.8 g, 90%). **10**: NMR ($CDCl_3$) δ 7.17 (d, J = 8.8 Hz, 1 H), 6.80 (d, J = 8.8 Hz, 1 H), 4.30−4.26 (m, 2 H), 3.91 (m, 2 H), 2.33 (s, 3 H); CIMS (NH_3) m/z 273/274 ($M + NH_4^+$, 100/98), 256/258 ($M + H^+$, 96/90).

4-Methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-6-yl Bromide (11). A solution of **10** (2.0 g, 7.81 mmol) and potassium hydroxide (2.8 g, 50 mmol) in methanol (10 mL) and water (5 mL) was heated at 55 °C for 30 min before the reaction mixture was poured onto crushed ice and extracted with ether (2 × 75 mL). The combined extracts were dried ($MgSO_4$) and concentrated to give the deacylated product which was carried on to the next step without further purification (1.6 g, quantitative). This product (0.20, 0.93 mmol) was added to a mixture of 37% aqueous formaldehyde (0.15 g, 1.86 mmol) and acetonitrile (15 mL) at room temperature. The mixture was treated with sodium cyanoborohydride (0.10 g, 1.5 mmol) in one portion and stirred for 15 min before acetic acid (3 drops) was added. The reaction mixture was stirred for 30 min, poured into ether (30 mL), and washed with 1 M sodium hydroxide (5 mL) and saturated sodium chloride (5 mL). The organic layer was separated, dried (Na_2SO_4), and concentrated at reduced pressure. Chromatography (silica gel, 30% ether/hexane) gave the product as a yellow oil (0.20 g, 95%): NMR ($CDCl_3$) δ 6.73−6.70 (m, 2 H), 6.61 (d, J = 7.2 Hz, 1 H), 4.27−4.24 (m, 2 H), 3.28−3.25 (m, 2 H), 2.87 (s, 3 H); CIMS (NH_3) m/z 228/230 ($M + H^+$, 95/100).

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis(3-acetylbenzyl)-4,7-bis((3,4-(ethylenedioxy)phenyl)methyl)-2*H*-1,3-diazapin-2-one (13): mp 200−201 °C; NMR ($CDCl_3$) δ 7.85 (d, J = 7.7 Hz, 2 H), 7.8 (s, 2 H), 7.4 (m, 4 H), 6.8 (d, J = 8.8 Hz, 2 H), 6.5 (m, 4 H), 4.9 (d, J = 14.3 Hz, 2 H), 4.25 (s, 8 H), 3.7 (bs, 2 H), 3.55 (m, 2 H), 3.4 (d, J = 14.3 Hz, 2 H), 2.95 (m, 2 H), 2.8 (m, 2 H), 2.6 (s, 6 H); CIMS (NH_3) m/z 724 ($M + NH_4^+$, 100); HRMS calcd for $C_{41}H_{43}N_2O_9$ ($M + H^+$) 707.2969, found 707.2969. Anal. ($C_{41}H_{42}N_2O_9$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-*O*-isopropylidene-1,3-bis((3-nitrophenyl)methyl)-4,7-bis((3,4-(ethylenedioxy)-phenyl)methyl)-2*H*-1,3-diazapin-2-one (14). To a solution of **4** (0.1 g, 0.21 mmol) in dry THF (2 mL) at room temperature was added potassium *tert*-butoxide (0.62 mL, 0.62 mmol, 1.0 M in THF). After 5 min 3-nitrobenzyl bromide (0.08 mL, 0.62 mmol) was added and the reaction stirred at room temperature for 1 h. The reaction was quenched with H_2O diluted with ether (30 mL), washed with water (10 mL), dried ($MgSO_4$), and concentrated. Chromatography (silica gel, 20% EtOAc/hexane) gave urea **14** as a foam (93 mg, 68%). **14**: NMR ($CDCl_3$) δ 7.3 (m, 6 H), 7.2 (m, 4 H), 6.8 (d, J = 8.0 Hz, 2 H), 6.65 (m, 2 H), 6.55 (m, 2 H), 5.0 (d, J = 14.0 Hz, 2 H), 4.3 (s, 8 H), 3.8 (bs, 2 H), 3.7 (m, 2 H), 3.2 (d, J = 14.0 Hz, 2 H), 2.8 (m, 4 H), 1.3 (s, 6 H); CIMS (NH_3) m/z 663 ($M + H^+$, 100).

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis((3-aminophenyl)methyl)-4,7-bis((3,4-(ethylenedioxy)phenyl)-methyl)-2*H*-1,3-diazapin-2-one (15). A solution of urea **14** (0.1 g, 0.13 mmol) in THF (2 mL) was treated with 10% palladium on carbon (20 mg) in one portion and stirred under a atmosphere of hydrogen. After 16 h a solution of 20% concentrated HCl in methanol (2 mL) was added in one portion and the reaction stirred at room temperature for 1 h. The reaction mixture was filtered (Celite) and the filtrate poured into saturated $NaHCO_3$ solution (5 mL). The mixture was extracted with EtOAc (10 mL), the organic layer was separated and dried (Na_2SO_4), and the solvent was evaporated. Chromatography (silica gel, 5% MeOH/CH_2Cl_2) gave the desired product as a white solid (74 mg, 88%). **15**: mp 140−142 °C; NMR (CD_3OD) δ 7.05 (m, 2 H), 6.8 (d, J = 8.3 Hz, 2 H), 6.65 (m, 2 H), 6.6 (m, 4 H), 6.55 (m, 2 H), 6.5 (d, J = 8.9 Hz, 4 H), 4.7 (d, J = 14.2 Hz, 2 H), 4.2 (s, 8 H), 3.5 (m, 4 H), 2.95 (d, J = 14.2 Hz, 2 H), 2.9 (m, 4 H); ESMS m/z 653 ($M + H^+$, 14), 327 ($M + 2H^+$, 100); HRMS calcd for $C_{37}H_{41}N_4O_7$ ($M + H^+$) 653.2975, found 653.2979. Anal. ($C_{37}H_{40}N_4O_7$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis((3-(carboxyamino)phenyl)methyl)-4,7-bis((3,4-(ethylenedioxy)-

phenyl)methyl)-2*H*-1,3-diazapin-2-one (16). Urea **15** (0.13 g, 0.2 mmol) was suspended in butyl formate (2 mL) and heated to reflux for 1 h. All solids dissolve after 5 min. The mixture was cooled and the butyl formate removed at reduced pressure. The residue was chromatographed (silica gel, 5−7% MeOH/CH_2Cl_2) to give the desired product (0.12 g, 85%). The NMR spectrum in MeOH-d_4 shows a multiplicity of signals due to the presence of amide bond rotomers: ESMS *m/z* 709 ($M + H^+$, 100).

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1-((3-aminophenyl)methyl)-3-((3-(methylamino)phenyl)methyl)-4,7-bis((3,4-(ethylenedioxy)phenyl)methyl)-2*H*-1,3-diazapin-2-one (17). A solution of compound **16** (0.12 g, 0.17 mmol) in THF (2 mL) was cooled to 0 °C and treated with BH_3·THF (0.51 mL, 0.51 mmol, 1.0 M in THF) dropwise over 10 min. The reaction mixture was allowed to reach room temperature over 1 h and then heated at reflux for 16 h. The mixture was cooled and quenched by the addition of MeOH (1 mL). Concentrated HCL (1 mL) was added, and the mixture was stirred at room temperature for 30 min. The solvent was removed at reduced pressure, and the residue was taken up in EtOAc (10 mL) and washed with saturated $NaHCO_3$ (5 mL), the organic layer was separated and dried (Na_2SO_4), and the solvent was evaporated. Chromatography (silica gel, 5% MeOH/CH_2Cl_2) gave a higher running R_f product which corresponded to the monomethylated product **17** (60 mg, 53%). Further elution gave the symmetrical di-*N*-methylurea **18** (40 mg, 35%). **17**: NMR (CD_3OD) δ 7.2 (m, 2 H), 6.9−6.5 (m, 12 H), 4.8 (dd, J = 14.0, 3.7 Hz, 2 H), 4.3 (s, 8 H), 3.6 (m, 6 H), 3.4 (s, 3 H), 3.0 (m, 4 H), 2.8 (s, 3 H); ESMS *m/z* 667 ($M + H^+$, 100); HRMS calcd for $C_{38}H_{43}N_4O_7$ ($M + H^+$) 653.2975, found 653.2979.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis((3-(methylamino)phenyl)methyl)-4,7-bis((3,4-(ethylenedioxy)phenyl)methyl)-2*H*-1,3-diazapin-2-one (18). A solution of compound **16** (0.12 g, 0.17 mmol) in THF (3 mL) was cooled to 0 °C and treated with a solution of LAH (0.5 mL, 0.5 mmol, 1.0 M in THF) dropwise over 10 min. The cooling bath was removed and the reaction mixture heated at reflux for 30 min. The reaction mixture was cooled to 0 °C and quenched with 1 N NaOH (4 drops). After quenching, the reaction was allowed to reach room temperature and diluted with EtOAc. Anhydrous sodium sulfate was added directly to the mixture with continued stirring for 20 min. The reaction was then filtered (Celite), and the remaining salts were washed with additional EtOAc. The filtrate was evaporated and the residue chromatographed (silica gel, 2.5−5% MeOH/CH_2Cl_2) to give the desired product (0.11 g, 95%). **18**: NMR (CD_3OD) δ 7.2 (t, J = 6.5 Hz, 2 H), 6.9 (d, J = 7.5 Hz, 2 H), 6.8 (m, 2 H), 6.75 (m, 2 H), 6.6 (m, 2 H), 6.55 (m, 2 H), 6.5 (bs, 2 H), 5.0 (d, J = 14 Hz, 2 H), 4.4 (s, 8 H), 3.8 (bs, 2 H), 3.65 (m, 4 H), 3.2 (d, J = 14 Hz, 2 H), 3.0 (m, 4 H), 2.8 (s, 6 H), 2.5 (bs, 2 H); ESMS *m/z* 681 ($M + H^+$, 100); HRMS calcd for $C_{39}H_{45}N_4O_7$ ($M + H^+$) 681.3288, found 681.3289.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis(phenylmethyl)-4,7-bis((3,5-dimethoxyphenyl)methyl)-2*H*-1,3-diazapin-2-one (20): mp 210−212 °C; NMR (CD_3OD) δ 7.3 (m, 4 H), 7.25 (m, 2 H), 7.2 (d, J = 6 Hz, 4 H), 6.4 (m, 2 H), 6.25 (s, 4 H), 4.75 (d, J = 14 Hz, 2 H), 3.8 (s, 12 H), 3.6 (m, 4 H), 3.1 (d, J = 14 Hz, 2 H), 2.95 (d, J = 8.5 Hz, 2 H), 2.85 (m, 2 H); CIMS *m/z* 627 ($M + H^+$, 100); HRMS calcd for $C_{37}H_{43}N_2O_7$ ($M + H^+$) 627.3070, found 627.3078. Anal. ($C_{37}H_{42}N_2O_7$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis(phenylmethyl)-4,7-bis((3,4-dimethoxyphenyl)methyl)-2*H*-1,3-diazapin-2-one (21): mp 204−206 °C; NMR (CD_3OD) δ 7.3 (m, 6 H), 7.2 (d, J = 5.5 Hz, 4 H), 6.9 (d, J = 5.5 Hz, 2 H), 6.65 (m, 4 H), 4.7 (d, J = 14 Hz, 2 H), 3.85 (s, 6 H), 3.8 (s, 6 H), 3.6 (m, 4 H), 3.1 (d, J = 14 Hz, 2 H), 2.95 (m, 2 H), 2.85 (m, 2 H); CIMS *m/z* 627 ($M + H^+$, 100); HRMS calcd for $C_{37}H_{43}N_2O_7$ ($M + H^+$) 627.3070, found 627.3080. Anal. ($C_{37}H_{42}N_2O_7$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis(phenylmethyl)-4,7-bis(2,3-dihydrobenzo[*b*]furan-5ylmethyl)-2*H*-1,3-diazapin-2-one (23): mp 194−196 °C; NMR ($CDCl_3$) δ 7.4 (m, 6 H), 7.25 (m, 4 H), 6.9 (m, 4 H), 6.8 (d, J = 7 Hz, 2 H), 5.0 (d, J = 14 Hz, 2 H), 4.7 (t, J = 7.5 Hz, 4 H), 3.7 (bs, 2 H), 3.6 (m, 2 H), 3.3 (m, 6 H), 3.0 (m, 4 H); CIMS *m/z* 591 ($M + H^+$, 100); HRMS calcd for $C_{37}H_{39}N_2O_5$ ($M + H^+$) 591.2859, found 591.2866. Anal. ($C_{37}H_{38}N_2O_5$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis(phenylmethyl)-4,7-bis((4-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]-oxazin-6-yl)methyl-2*H*-1,3-diazapin-2-one (24): NMR ($CDCl_3$) δ 7.33 (m, J = 7.7 Hz, 1H), 7.17 (d, J = 4.1), 7.10 (dd, J = 7.7, 10.6 Hz, 3 H), 6.80 (d, J = 3.7 Hz, 1H), 6.80 (d, 8.42, 1H), 6.38 (m, 2H), 4.93 (d, J = 14.7 Hz, 1H), 4.28 (m, 2H), 3.60 (bs, 1H), 3.49 (m, 1 H), 3.25 (m, 2H), 3.17 (d, J = 14.2 Hz, 1H), 2.84 (m, 1H), 2.83 (s, 3H), 2.67 (bs, 1H); ESI-MS *m/z* 874.3 ($(M + H)^+$, 12.1), 424.3 ($(M + 2H)^{2+}$, 100); HRMS calcd for $C_{45}H_{46}N_6O_7S_2$ ($M + H^+$) 847.2939, found 847.2948.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis(cyclopropylmethyl)-4,7-bis((3,4-(ethylenedioxy)phenyl)methyl)-2*H*-1,3-diazapin-2-one (25): mp 125−130 °C; NMR ($CDCl_3$) δ 6.7 (d, J = 9.5 Hz, 2 H), 6.65 (m, 2 H), 6.6 (m, 2 H), 4.25 (s, 8 H), 3.95 (bs, 2 H), 3.55 (m, 2 H), 2.9 (m, 4 H), 2.5 (bs, 2 H), 2.1 (m, 2 H), 0.85 (m, 2 H), 0.35 (m, 4 H), 0.05 (m, 4 H); CIMS (NH_3) *m/z* 551 ($M + H^+$, 100); HRMS calcd for $C_{31}H_{39}N_2O_7$ ($M + H^+$) 551.2757, found 551.2749. Anal. ($C_{31}H_{38}N_2O_7$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis(2-naphthylmethyl)-4,7-bis((3,4-(ethylenedioxy)phenyl)methyl)-2*H*-1,3-diazapin-2-one (26): mp 232−234 °C; NMR ($CDCl_3$) δ 7.85 (m, 6 H), 7.55 (s, 2 H), 7.5 (m, 6 H), 6.8 (d, J = 8.1 Hz, 2 H), 6.7 (m, 2 H), 6.6 (dd, J = 8.1, 1.8 Hz, 2 H), 5.15 (d, J = 14.3 Hz, 2 H), 4.3 (s, 8 H), 3.6 (m, 4 H), 3.4 (d, J = 14.3 Hz, 2 H), 3.0 (m, 4 H), 2.2 (bs, 2 H); CIMS (NH_3) *m/z* 740 ($M + NH_4^+$, 100); HRMS calcd for $C_{45}H_{43}N_2O_7$ ($M + H^+$) 723.3070, found 723.3051. Anal. ($C_{45}H_{42}N_2O_7$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis((4-(hydroxymethyl)phenyl)methyl)-4,7-bis((3,4-(ethylenedioxy)phenyl)methyl)-2*H*-1,3-diazapin-2-one (27): mp 127−130 °C; NMR ($CDCl_3$) δ 7.3 (d, J = 8.1 Hz, 4 H), 7.2 (d, J = 8.1 Hz, 4 H), 6.8 (d, J = 8.3 Hz, 2 H), 6.55 (dd, J = 8.3, 2.0 Hz, 2 H), 6.4 (m, 2 H), 4.8 (d, J = 14.1 Hz, 2 H), 4.6 (s, 4 H), 4.25 (s, 8 H), 3.6 (bs, 2 H), 3.5 (m, 2 H), 3.3 (d, J = 14.1 Hz, 2 H), 2.9 (m, 2 H), 2.8 (m, 2 H); CIMS (NH_3) *m/z* 700 ($M + NH_4^+$, 100); HRMS calcd for $C_{39}H_{43}N_2O_9$ ($M + H^+$) 683.2969, found 683.2970. Anal. ($C_{39}H_{42}N_2O_9$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis((4-hydroxyphenyl)methyl)-4,7-bis((3,4-(ethylenedioxy)phenyl)-methyl)-2*H*-1,3-diazapin-2-one (28): mp 228−230 °C; NMR (CD_3OD) δ 7.1 (d, J = 8.1 Hz, 4 H), 6.8 (m, 6 H), 6.7 (s, 2 H), 6.65 (m, 2 H), 4.75, (d, J = 14.0 Hz, 2 H), 4.35 (s, 8 H), 3.6 (m, 2 H), 3.55 (bs, 2 H), 3.1 (d, J = 14.0 Hz, 2 H), 2.95 (m, 2 H), 2.9 (m, 2 H); CIMS (NH_3) *m/z* 672 ($M + NH_4^+$, 100); HRMS calcd for $C_{37}H_{39}N_2O_9$ ($M + H^+$) 655.2656, found 655.2662. Anal. ($C_{37}H_{38}N_2O_9$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis(3-acetyl-4-fluorobenzyl)-4,7-bis((3,4-(ethylenedioxy)phenyl)methyl)-2*H*-1,3-diazapin-2-one (29): mp 197−199 °C; NMR ($CDCl_3$) δ 7.7 (m, 2 H), 7.45 (m, 2 H), 7.05 (m, 2 H), 6.8 (d, J = 8.1 Hz, 2 H), 6.45 (m, 4 H), 4.7 (d, J = 14.2 Hz, 2 H), 4.25 (s, 8 H), 3.75 (bs, 2 H), 3.5 (m, 4 H), 2.95 (m, 2 H), 2.7 (m, 2 H), 2.6 (d, J = 4.6 Hz, 6 H), 2.3 (bs, 2 H); CIMS (NH_3) *m/z* 743 ($M + H^+$, 100); HRMS calcd for $C_{41}H_{41}F_2N_2O_9$ ($M + H^+$) 743.2780, found 743.2800. Anal. ($C_{41}H_{40}N_2O_9F_2$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis(3-acetylbenzyl)-4,7-bis(2,3-dihydrobenzo[*b*]furan-5-ylmethyl)-2*H*-1,3-diazapin-2-one (30): mp 237−240 °C; NMR (DMSO-d_6) δ 7.9 (d, J = 5 Hz, 2 H), 7.7 (s, 2 H), 7.5 (m, 4 H), 6.6 (m, 6 H), 4.6 (d, J = 14 Hz, 2 H), 4.5 (t, J = 7.5 Hz, 4 H), 3.45 (m, 4 H), 3.2 (d, J = 14 Hz, 2 H), 3.1 (t, J = 7.5 Hz, 4 H), 2.9 (m, 2 H), 2.7 (m, 2 H), 2.55 (s, 6 H); CIMS *m/z* 675 ($M + H^+$, 100); HRMS calcd for $C_{41}H_{43}N_2O_7$ ($M + H^+$) 675.3070, found 675.3088. Anal. ($C_{41}H_{42}N_2O_7$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis((3-aminophenyl)methyl)-4,7-bis(2,3-dihydrobenzo[*b*]furan-5-ylmethyl)-2*H*-1,3-diazapin-2-one (31): mp 232−235 °C; NMR (CD_3OD) δ 7.0 (t, J = 6 Hz, 2 H), 6.9 (s, 2 H), 6.85 (m, 2 H), 6.7 (d, J = 6 Hz, 2 H), 6.6 (m, 2 H), 6.5 (m, 4 H), 4.7 (d, J = 14 Hz, 2 H), 4.5 (t, J = 6.5 Hz, 4 H), 3.6 (m, 4 H), 3.3 (d, J = 14 Hz, 2 H), 3.2 (t, J = 6.5 Hz, 4 H), 2.9 (m, 4 H); CIMS *m/z* 621

Table 4. Crystallographic Refinement Statistics

	15	**26**
R factor[a]	0.196	0.173
resolution range (Å)	10.0−2.1	10.0−2.25
no. of reflections used	6197	7032
no. of protein atoms	1514	1514
no. of inhibitor atoms	48	54
rms deviations from ideal values		
bond lengths (Å)	0.019	0.019
bond angles (deg)	3.88	3.71

[a] R factor $= \sum||F_o| - |F_c||/\sum|F_o|$, where $|F_o|$ and $|F_c|$ are the observed and calculated structure factor amplitudes, respectively.

(M + H$^+$, 100); HRMS calcd for $C_{37}H_{41}N_4O_5$ (M + H$^+$) 621.3077, found 621.3070. Anal. ($C_{37}H_{40}N_4O_5$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis((3-(methylamino)phenyl)methyl)-4,7-bis(2,3-dihydrobenzo-[*b*]furan-5-ylmethyl)-2*H*-1,3-diazapin-2-one (32): mp 222−226 °C; NMR (CD_3OD) δ 7.0 (t, J = 6 Hz, 2 H), 6.9 (s, 2 H), 6.85 (m, 2 H), 6.7 (d, J = 6 Hz, 2 H), 6.6 (m, 2 H), 6.5 (m, 4 H), 4.7 (d, J = 14 Hz, 2 H), 4.5 (t, J = 6.5 Hz, 4 H), 3.6 (m, 4 H), 3.3 (d, J = 14 Hz, 2 H), 3.2 (t, J = 6.5 Hz, 4 H), 2.9 (m, 4 H), 2.8 (s, 6 H); CIMS m/z 649 (M + H$^+$, 100); HRMS calcd for $C_{39}H_{45}N_4O_5$ (M + H$^+$) 649.3390, found 649.3389.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis(3-acetylbenzyl)-4,7-bis((3,4-(methylenedioxy)phenyl)methyl)-2*H*-1,3-diazapin-2-one (33): NMR ($CDCl_3$) δ 7.8 (m, 4 H), 7.5−7.3 (m, 4 H), 6.7 (d, J = 7 Hz, 2 H), 6.5 (m, 4 H), 5.9 (m, 4 H), 4.8 (d, J = 14 Hz, 2 H), 3.7 (bs, 2 H), 3.55 (bd, 2 H), 3.4 (d, J = 14 Hz, 2 H), 3.0 (m, 2 H), 2.8 (t, J = 6 Hz, 4 H), 2.5 (s, 6 H); CIMS m/z 679 (M + H$^+$, 100); HRMS calcd for $C_{39}H_{39}N_2O_9$ (M + H$^+$) 679.2656, found 679.2649.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis(4-fluoro-3-acetylbenzyl)-4,7-bis((3,4-(methylenedioxy)phenyl)-methyl)-2*H*-1,3-diazapin-2-one (34): NMR (CD_3OD) δ 7.7 (m, 2 H), 7.4 (m, 2 H), 7.0 (t, J = 8.4 Hz, 2 H), 6.7 (d, J = 7 Hz, 2 H), 6.4 (m, 4 H), 6.0 (d, J = 6.5 Hz, 4 H), 4.6 (d, J = 14 Hz, 2 H), 3.8 (bs, 2 H), 3.5 (m, 4 H), 3.0 (m, 2 H), 2.75 (m, 2 H), 2.6 (d, J = 4 Hz, 6 H), 2.5 (m, 2 H); CIMS m/z 715 (M + H$^+$, 100); HRMS calcd for $C_{39}H_{37}N_2O_9F_2$ (M + H$^+$) 715.2467, found 715.2462.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis(3-acetylbenzyl)-4,7-bis((3,5-dimethoxyphenyl)methyl)-2*H*-1,3-diazapin-2-one (35): NMR (CD_3OD) δ 7.9 (m, 2 H), 7.8 (bs, 2 H), 7.45 (m, 4 H), 6.3 (m, 2 H), 6.2 (m, 4 H), 4.7 (d, J = 14 Hz, 2 H), 3.7 (s, 12 H), 3.65 (m, 4 H), 3.4 (d, J = 14 Hz, 2 H), 3.0 (m, 2 H), 2.8 (m, 2 H), 2.6 (s, 6 H); CIMS m/z 711 (M + H$^+$, 100); HRMS calcd for $C_{41}H_{47}N_2O_9$ (M + H$^+$) 711.3282, found 711.3296.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis(3-acetylbenzyl)-4,7-bis((4-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]-oxazin-6-yl)methyl)-2*H*-1,3-diazapin-2-one (36): NMR (CD_3-OD) δ 7.8 (d, J = 7.7 Hz, 2 H), 7.7 (s, 2 H), 7.5−7.4 (m, 2 H), 6.7 (d, J = 8.4 Hz, 2 H), 6.4−6.3 (m, 4 H), 4.9 (d, J = 14.2 Hz, 2 H), 4.3 (m, 4 H), 3.6 (bs, 2 H), 3.6−3.5 (m, 2 H), 3.3 (bs, 2 H), 3.3−3.2 (m, 6 H), 2.9 (d, J = 6 Hz, 2 H), 2.9−2.75 (m, 2 H), 2.8 (s, 6 H), 2.58 (s, 6 H); CIMS m/z 733 (M + H$^+$, 100); HRMS calculated for $C_{43}H_{49}N_4O_7$ (M + H$^+$) 733.3605, found 733.3601.

X-ray Crystallography. The complexes of **15** and **26** with HIV-1 protease were crystallized as described previously.[17] The unit cell dimensions of both complexes are $a = b = 63.3$ Å and $c = 84.2$ Å. The diffraction data were collected with a R-AXIS II imaging plate mounted on a RU200 Rigaku rotating anode generator operating at 50 kV and 100 mA. For **15**, a total of 25 974 reflections were collected resulting in 14 838 unique reflection and $R_{sym} = 7.2\%$. Although the crystal diffracts up to 1.8 Å, the data can be considered at 2.1 Å resolution with the criteria of $F^2 >= 1.0\sigma F^2$, and 80% complete data. For **26**, 15 938 unique reflections from 36 874 observations were obtained with $R_{sym} = 9.4\%$. Using the same criteria, the data can be considered having 2.25 Å resolution and contains 88% complete data. The difference maps calculated with the protein coordinates of a related cyclic urea structure revealed the corresponding inhibitor position.[5a] The structures were refined using the simulated annealing method, XPLOR.[18] No constraints were applied to maintain identity between the two protease monomers. Standard geometry of the inhibitor was based on the single crystal structure of a cyclic urea. Statistics of the crystallographic refinement are detailed in Table 4.

Binding Affinity Assay. Inhibition of HIV protease was measured by assay of the cleavage of a fluorescent peptide substrate using HPLC.[19]

Whole Cell Antiviral Assay. 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.[20]

I84V Mutant Antiviral Assay. The I84V mutant virus was selected using continuous passage of HIV-RF in PBMC's in the presence of 1 μg/mL of DMP323. The yield reduction plaque assay was determined as described previously.[21]

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