Cyclic HIV Protease Inhibitors: Synthesis, Conformational Analysis, P2/P2′ Structure−Activity Relationship, and Molecular Recognition of Cyclic Ureas

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High-resolution X-ray structures of the complexes of HIV-1 protease (HIV-1PR) with peptidomimetic inhibitors reveal the presence of a *structural water molecule* which is hydrogen bonded to both the mobile flaps of the enzyme and the two carbonyls flanking the transition-state mimic of the inhibitors. Using the structure−activity relationships of C_2-symmetric diol inhibitors, computed-aided drug design tools, and first principles, we designed and synthesized a novel class of cyclic ureas that incorporates this structural water and preorganizes the side chain residues into optimum binding conformations. Conformational analysis suggested a preference for pseudodiaxial benzylic and pseudodiequatorial hydroxyl substituents and an enantiomeric preference for the *RSSR* stereochemistry. The X-ray and solution NMR structure of the complex of HIV-1PR and one such cyclic urea, DMP323, confirmed the displacement of the structural water. Additionally, the bound and "unbound" (small-molecule X-ray) ligands have similar conformations. The high degree of preorganization, the complementarity, and the entropic gain of water displacement are proposed to explain the high affinity of these small molecules for the enzyme. The small size probably contributes to the observed good oral bioavailability in animals. Extensive structure-based optimization of the side chains that fill the S2 and S2′ pockets of the enzyme resulted in DMP323, which was studied in phase I clinical trials but found to suffer from variable pharmacokinetics in man. This report details the synthesis, conformational analysis, structure−activity relationships, and molecular recognition of this series of C_2-symmetry HIV-1PR inhibitors. An initial series of cyclic ureas containing nonsymmetric P2/P2′ is also discussed.

Introduction

The RNA genome of the human immunodeficiency virus (HIV), the causative agent of acquired immunedeficiency syndrome (AIDS), encodes an essential aspartic protease (PR)[1] that processes the viral *gag* and *gag-pol* polyproteins into structural and functional proteins. Inhibition of HIVPR *in vitro* results in the production of progeny virions which are immature and noninfectious.[2] Under clinical settings, three HIVPR inhibitors, recently approved by FDA for AIDS therapy in combination with reverse transcriptase inhibitors, have been shown to reduce the viral load and increase the number of $CD4^+$ lymphocytes in HIV-infected patients.[3] In addition, the large abundance of structural information on the HIVPR has made the enzyme an attractive target for computer-aided drug design strategies.[4] Consequently, HIVPR has become a prime focus for the development of HIV therapeutics.[5] However, the daunting ability of the virus to rapidly generate resistant mutants[3e,f,6] suggests that there is an ongoing need for new HIVPR inhibitors with superior pharmacokinetic and efficacy profiles.

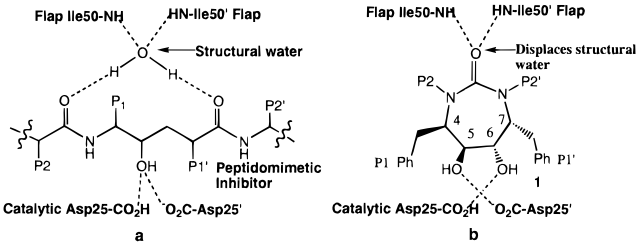

Figure 1. (a) Peptidomimetic inhibitor binding at HIV-1PR active site via a bridging structural water. (b) Preorganized cyclic urea inhibitor **1** at the active site showing the urea oxygen displacing the structural water.

High-resolution X-ray structures of the complexes of HIV-1 protease (HIV-1PR) with peptidomimetic inhibitors reveal the presence of a *structural water molecule* which is hydrogen bonded to both the mobile flaps of the enzyme and the two carbonyls flanking the transition-state mimic of the inhibitors[4] (Figure 1a). The relevance of this structural water to the generation of HIVPR inhibitors had been previously noted.[7] This water molecule is unique to all retroviral aspartic proteases but is absent in mammalian aspartic proteases such as renin.[8] We recently described the rational design of a class of novel and highly potent cyclic urea inhibitors.[9] The urea oxygen in the cyclic urea scaffold was designed to incorporate this structural water molecule (Figure 1b). Specifically, the design of these molecules was accomplished using structure−activity relationship (SAR) data generated from a series of acyclic C_2-symmetric diols,[5a,10] computer-aided drug

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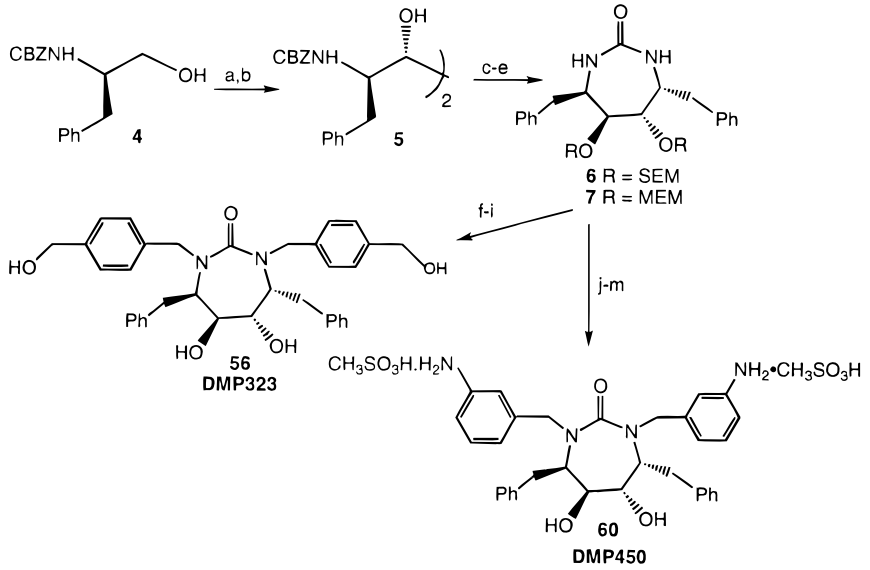
Scheme 1. Synthesis of Symmetric Cyclic Ureas[a]



[a] (a) DMSO, $COCl_2$, TEA, CH_2Cl_2, −78 °C; (b) VCl_3·$(THF)_3$, Zn, rt, THF; (c) SEMCl or MEMCl, Hunig's base, DMF; (d) H_2, Pd/C, EtOAc, MeOH; (e) CDI, CH_2Cl_2; (f) NaH, *p*-$NCC_6H_4CH_2Br$, DMF; (g) DIBAL-H, THF, −78 °C; (h) $NaBH_4$, EtOH; (i) 4 M HCl in dioxane, MeOh; (j) NaH, *m*-$NO_2C_6H_4CH_2Br$, DMF; (k) 4 M HCl in dioxane; (l) H_2, Pd/C; (m) CH_3SO_3H.

design tools, and first principles.[9c,11] The displacement of the unique structural water molecule by incorporation into the inhibitor structure is expected to increase retroviral protease specificity and gain in entropy of binding. The entropic gain of liberating a tightly bound water molecule in proteins to the bulk solvent has been estimated as high as 2 kcal/mol.[12] The cyclic urea scaffold also takes advantage of the principle of preorganization[13] which states that "the more highly hosts and guests are organized for binding and low solvation prior to their complexation, the more stable will be their complexes". In our case, the preorganized inhibitor is anticipated to have higher affinity for HIVPR than its flexible counterpart since no conformational entropic penalty need be paid during binding, the penality having been prepaid during the synthesis of the preorganized structure. As a result of the increased potency of the cyclic ureas due to water displacement and preorganization, fewer subsites of the HIVPR active site need to be occupied to achieve a given level of inhibitory potency. An important consequence of reducing molecular weight is a higher probability of improved oral bioavailability.[14] We describe here the conformational prediction, synthesis, P2/P2′ structure−activity relationship, and molecular recognition of these cyclic urea HIV-1PR inhibitors.

Results and Discussion

Conformational Prediction. One of the critical features of our design strategy is the qualitative prediction of the conformation of seven-membered ring cyclic ureas. It is only with the correct prediction that the preferred stereochemistry complementary to the active site can be successfully evaluated using interactive graphics. The conformational analysis is shown in Figure 2.

Seven-membered ring cyclic ureas can exist in two pseudochair conformations. When the nitrogens are unsubstituted, 1,3-diaxial strain[15] dominates and conformer **2** with pseudodiequatorial benzyl groups and pseudodiaxial hydroxyl groups is preferred. In contrast, when the two nitrogens are substituted with P2/P2′

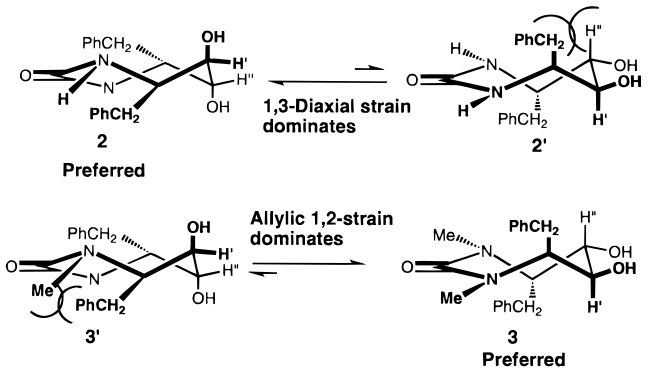


Figure 2. Conformational analysis of designed cyclic ureas predicting that **2** is preferred when the nitrogens are not substituted, whereas conformation **3** is preferred when the nitrogens are substituted due to $A_{1,2}$ strain.

groups, the converse is true even for a substituent as small as a methyl group. The partial double bond character of the urea C−N bond introduces severe allylic 1,2-strain[16] between the benzylic groups and the nitrogen substituents. This allylic 1,2-strain overcomes the 1,3-diaxial strain, and conformer **3** with pseudodiaxial benzyl groups and pseudodiequatorial hydroxyl groups is preferred. This conformational prediction by first principles was subsequently confirmed by small-molecule X-ray crystallography (*vide infra*).

With an idea of the preferred conformation in hand, we proceeded to determine the stereochemistry necessary for active site complementarity by interactive graphics. The preferred stereochemistry was predicted to be 4*R*,5*S*,6*S*,7*R*. It is with this stereochemistry that the P1/P1′/P2/P2′ side chains can project optimally into the S1/S1′/S2/S2′ pockets, respectively. The preferred *RSSR* stereochemistry is derived from unnatural D-phenylalanine. This contrasted with the SAR of acyclic C_2-symmetric diols where the unnatural D-phenylalanine at P1/P1′ is highly disfavored.[17] The stereochemical predictions were subsequently confirmed by biological activity and X-ray structures (*vide infra*).

Synthesis. The synthesis of cyclic ureas is shown in Scheme 1 as exemplified by **56**, DMP323. Oxidation

Table 1. Physical Data for HIVPR Inhibitors

compd	mp (°C)	formula	anal.
3	171−173	$C_{21}H_{26}N_2O_3 \cdot 0.5H_2O$	C,H,N
8	212−214	$C_{23}H_{30}N_2O_3 \cdot 0.75H_2O$	C,H,N
9	180−182	$C_{25}H_{34}N_2O_3$	C,H,N
10	140−142	$C_{27}H_{38}N_2O_3$	C,H,N
11	125−127	$C_{29}H_{42}N_2O_3$	C,H,N
12	110−112	$C_{31}H_{46}N_2O_3 \cdot 0.25H_2O$	C,H,N
13	100−101	$C_{33}H_{50}N_2O_3$	C,H,N
14	183−185	$C_{25}H_{34}N_2O_5 \cdot 0.25H_2O$	C,H,N
15	148−150	$C_{27}H_{38}N_2O_5 \cdot 0.5H_2O$	C,H,N
16	108−110	$C_{29}H_{42}N_2O_7 \cdot 0.1CHCl_3$	C,H,N
17	207	$C_{27}H_{38}N_2O_3$	HRMS
18	198−199	$C_{29}H_{42}N_2O_3 \cdot 0.5H_2O$	C,H,N
19	120−122	$C_{31}H_{46}N_2O_3$	HRMS
20	105−107	$C_{33}H_{50}N_2O_3$	HRMS
21	144−145	$C_{35}H_{54}N_2O_3 \cdot 0.15CHCl_3$	C,H,N
22	244−245	$C_{31}H_{46}N_2O_3$	HRMS
23	164−166	$C_{25}H_{30}N_2O_3$	C,H,N
24	205−207	$C_{27}H_{34}N_2O_3$	HRMS
25	167−168	$C_{29}H_{38}N_2O_3 \cdot 0.25H_2O$	C,H,N
26	nd	$C_{27}H_{34}N_2O_5$	HRMS
27	196−197	$C_{25}H_{26}N_2O_3$	HRMS
28	210−212	$C_{27}H_{34}N_2O_3$	C,H,N
29	215−216	$C_{29}H_{38}N_2O_3 \cdot 0.25H_2O$	C,H,N
30	227−228	$C_{31}H_{42}N_2O_3 \cdot 0.5H_2O$	C,H,N
31	242−243	$C_{33}H_{46}N_2O_3$	C,H,N
32	120−122	$C_{31}H_{44}N_2O_3 \cdot 2H_2O$	C,H,N
33	171	$C_{33}H_{36}N_2O_3$	C,H,N
34	151−153	$C_{31}H_{32}N_4O_3$	HRMS
35	104	$C_{31}H_{32}N_4O_3$	HRMS
36	nd	$C_{31}H_{32}N_4O_3$	HRMS
37	231−233	$C_{41}H_{38}N_2O_3 \cdot 0.5H_2O$	C,H,N
38	202−204	$C_{41}H_{38}N_2O_3$	C,H,N
39	203	$C_{3}33H_{32}N_2O_3F_2 \cdot 1.5H_2O$	C,H,N
40	185	$C_{33}H_{32}N_2O_3F_2 \cdot 0.67H_2O$	C,H,N
41	133	$C_{33}H_{32}N_2O_3F_2$	C,H,N
42	211	$C_{33}H_{32}N_2O_3Cl_2 \cdot 0.75H_2O$	C,H,N
43	210	$C_{33}H_{32}N_2O_3Cl_2 \cdot 0.67H_2O$	C,H,N
44	73	$C_{33}H_{32}N_2O_3Cl_2 \cdot H_2O$	C,H,N
45	211	$C_{33}H_{32}N_2O_3Br \cdot H_2O$	C,H,N
46	91	$C_{33}H_{32}N_2O_3Br \cdot 0.5H_2O$	C,H,N
47	205	$C_{35}H_{38}N_2O_3 \cdot -.5H_2O$	C,H,N
48	72	$C_{35}H_{38}N_2O_3 \cdot H_2O$	C,H,N
49	246	$C_{35}H_{32}N_2O_3F_6 \cdot 0.1CHCl_3$	C,H,N
50	71	$C_{35}H_{32}N_2O_3F_6 \cdot 1.25H_2O$	C,H,N
51	228	$C_{35}H_{38}N_2O_5 \cdot 0.75H_2O$	C,H,N
52	179	$C_{35}H_{38}N_2O_5 \cdot H_2O$	C,H,N
53	162	$C_{35}H_{38}N_2O_5$	C,H,N
54	248	$C_{33}H_{32}N_4O_7 \cdot 0.33H_2O$	C,H,N
55	184	$C_{33}H_{32}N_2O_3I$	C,H,N
56	194−195	$C_{35}H_{38}N_2O_5$	C,H,N
57	197	$C_{35}H_{38}N_2O_5 \cdot 0.25H_2O$	C,H,N
58	115−117	$C_{33}H_{34}N_2O_3 \cdot H_2O$	C,H,N
59	101−103	$C_{33}H_{34}N_2O_3 \cdot 0.5H_2O$	C,H,N
60	208 dec	$C_{33}H_{36}N_4O_3 \cdot 2CH_4O_3S \cdot 3H_2O$	C,H,N
61	198	$C_{35}H_{38}N_2O_5 \cdot H_2O$	C,H,N
62	150−152	$C_{33}H_{36}N_2O_3 \cdot 1.5H_2O$	C,H,N
63	78−80	$C_{34}H_{38}N_2O_3 \cdot 1.25H_2O$	C,H,N
64	172−174	$C_{33}H_{34}N_2O_3 \cdot 1.5H_2O$	C,H,N
65	175−178	$C_{34}H_{36}N_2O_3 \cdot 1.25H_2O$	C,H,N
66	80−92	$C_{36}H_{40}N_2O_3 \cdot 0.25CHCl_3$	C,H,N
67	90−92	$C_{37}H_{36}N_2O_3$	HRMS
68	94−96	$C_{36}H_{35}N_3O_3 \cdot H_2O$	C,H,N
69	118−120	$C_{36}H_{35}N_3O_3 \cdot H_2O$	C,H,N
70	105−107	$C_{37}H_{37}N_3O_3 \cdot 0.1CH_2Cl_2$	C,H,N
71	199−201	$C_{38}H_{31}N_2O_4 \cdot 0.5H_2O$	C,H,N
72	nd	$C_{37}H_{37}N_3O_3$	HRMS
73	137−138	$C_{37}H_{36}N_2O_4 \cdot 0.25H_2O$	C,H,N
74	194−195	$C_{21}H_{24}N_2O_4 \cdot 0.25H_2O$	C,H,N
75	116	$C_{33}H_{36}N_2O_3 \cdot 0.25H_2O$	C,H,N

of *N*-(benzyloxycarbonyl)-(*R*)-phenylalaninol under Swern[18] conditions gave 84% yield of the corresponding aldehyde. The aldehyde was then treated with $VCl_3 \cdot (THF)_3$ and zinc in methylene chloride at room temperature[19] to give diol **5** in 55% yield with a diastereomeric purity of 98:2 (*RSSR*:*RRRR*) after purification. The diol was protected by treatment with [2-(trimethylsilyl)-ethoxy]methyl chloride (SEMCl) and *N*,*N*-diisopropylethylamine in dry DMF at 50 °C in 91% yield, and the benzyloxycarbonyl (Cbz) protecting groups were removed by hydrogenolysis with 10% Pd/C in ethyl acetate/methanol. The crude diamine was cyclized with carbonyl diimidazole (CDI) and pyridine in methylene chloride to give cyclic urea **6** in 52% yield (two steps). Alternatively, (2-methoxyethoxy)methyl chloride (MEM-Cl) could be used as the protecting group to give **7** in 62% overall yield from **5**.

Alkylation of **7** with *p*-cyanobenzyl bromide and NaH in anhydrous DMF gave the corresponding nitrile in 40% yield. Diisobutylaluminum hydride (DIBAL) reduction of the nitrile gave the corresponding aldehyde in 43% yield. The aldehyde was further reduced to benzyl alcohol with sodium borohydride in ethanol followed by deprotection with HCl in methanol to give the desired cyclic urea **56** in 64% yield (two steps).

Cyclic urea **60**, DMP450, was synthesized by alkylation of **7** with *m*-nitrobenzyl bromide and NaH in DMF to give a 58% yield of the bisalkylated cyclic urea.[34] Deprotection (82% yield) with HCl in methanol followed by hydrogenolysis (91% yield) with 10% Pd/C gave the free base of **60**. Treatment of the free base with methanesulfonic acid provided **60**. Cyclic ureas **3**−**55**, **57**−**59**, and **61** were synthesized using similar procedures. A summary of the physical data is given in Table 1.

The nonsymmetrical cyclic urea **69** was synthesized as shown in Scheme 2. Alkylation of cyclic urea **7** with 4.0 equiv of NaH and 1.1 equiv of β-naphthylmethyl bromide in dry DMF under nitrogen gave a 23% chromatographed yield of monoalkylated product (monoalkylation:bisalkylation is 1.4:1.0). Second alkylation (31% yield) of the monoalkylated product with 4 equiv of 4-picolyl chloride and 12 equiv of NaH in dry DMF followed by HCl deprotection (100% yield) gave nonsymmetric cyclic urea **69**. Nonsymmetric cyclic ureas **62**−**73** were synthesized by a similar procedure.

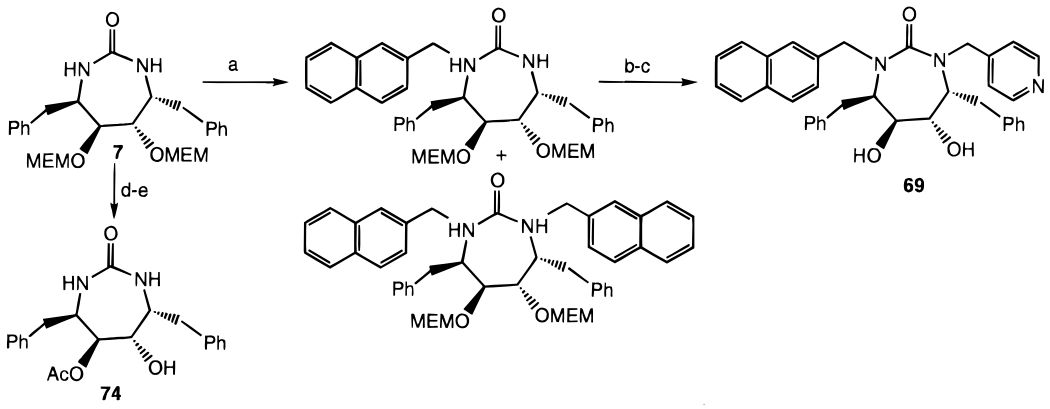
Deprotection of **7** with HCl in methanol followed by treatment with triethyl orthoacetate and catalytic *p*-toluenesulfonic acid in acetonitrile provided crystalline unsubstituted cyclic urea **74** in 57% yield for the purpose of X-ray structural determination of conformation.

Urea **75** was synthesized as shown in Scheme 3. (*R*)-Phenylalaninol **4** was protected as MEM ether. Removal of the *N*-Cbz protecting group with hydrogen and 5% Pd/C in ethanol gave the amine which was coupled with CDI to form the urea. Benzylation of the urea with benzyl bromide and sodium hydride in DMF followed by deprotection with concentrated HCl and MeOH gave compound **75** in 21% overall yield.

P2/P2′ Structure−Activity Relationship and Molecular Recognition. HIVPR is an attractive protein to study the molecular recognition[20] between macromolecules and small molecules, due to the abundance of high-resolution crystal structures of the HIVPR−inhibitor complexes.[4] The study is further simplified due to the C_2-symmetric nature and small size (99 amino acids/monomer) of the HIVPR.[4] We would like to describe the P2/P2′ structure−activity relationship and molecular recognition study in terms of enantiomeric recognition, conformation, water displacement, and preorganization.

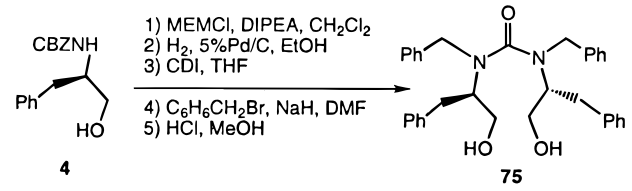
Cyclic urea **3**, with methyl substituents, inhibits HIV-1PR with a K_i[21] of 5700 nM as shown in Table 2. When

Scheme 2. Synthesis of Nonsymmetric Cyclic Ureas[a]



[a] (a) 4 equiv of NaH, 1.1 equiv of β-naphthylmethyl bromide, DMF, separation; (b) 12 equiv of NaH, 4 equiv of 4-picolyl chloride, DMF; (c) HCl, MeOH; (d) $CH_3C(OEt)_3$, *p*-TSA, CH_3CN; (e) H_2O.

Scheme 3. Synthesis of Seco Compound



the size of the alkyl group was increased incrementally from methyl to *n*-heptyl as in **3** and **8**−**13**, the optimal size was found to be *n*-butyl (**10**) with a K_i of 1.4 nM. Published X-ray structures[4] revealed that the S2/S2′ pockets are essentially lipophilic except toward the edge of the pockets near the entrance to the active site. The hydrophobic residues lining the S2/S2′ pockets are Val 32/32′, Ile 47/47′, Gly 48/48′, Ile 50/50′, and Ile 84/84′. The *n*-butyl group has the optimal length to form hydrophobic interactions with most of these lipophilic residues of the S2/S2′ pockets. The antiviral activity in terms of IC_{90}, the concentration of inhibitor resulting in 90% inhibition of viral RNA production in HIV-1-infected MT-2 cells, was determined by quantifying the viral RNA with an oligonucleotide-based sandwich hybridization assay.[21] For this series of *n*-alkyl compounds, **10** shows the lowest IC_{90}. The translation of K_i to IC_{90} also seems to be optimal for **10**, relative to the shorter or longer *n*-alkyls. The hydrophobic nature of the S2/S2′ pockets was further demonstrated by the 2−3 orders of magnitude decrease in binding when hydrophilic oxygens were inserted into the *n*-alkyl side chains as in **14** (570×), **15** (690×), and **16** (30×) compared with the corresponding *n*-alkyls **10**, **11**, and **13**, respectively. Methyl branching along the *n*-alkyl chains also decreases binding by 4.4−8.6-fold for **17**−**20** relative to **9**−**12**, respectively. Additional methyl branching as in **22** decreases binding 3.0-fold further, compared with **18**. On the other hand, insertion of double bonds in order to rigidify the alkyl groups improves binding of **23** (1.5×), **24** (6.7×), **25** (6.7×), and **26** (18×) compared with **9**, **17**, **18**, and **15**, respectively. Conversion of the allyl group to the alkynyl group (**27**) loses 4.2-fold in binding.

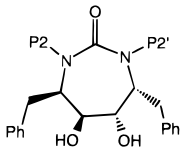
In the cycloalkyl series, **28**−**31**, cyclobutylmethyl cyclic urea **29** was found to have the best K_i among the cycloalkylmethyl cyclic ureas. The oral bioavailability (*F*%) of cyclopropylmethyl cyclic urea **28** in rat was determined to be 100% with a C_{max} of 4.3 μM at 10 mg/kg dose. In dog, the oral bioavailability is 48% with a C_{max} of 9.2 μM at similar dose. The low molecular weight of this compound (434 g/mol) probably contributes to its excellent bioavailability. *N*-Morpholino-2-ethyl cyclic urea **32**, made to increase water solubility, was found to be a poor binder. This is probably due to the deleterious effect of the hydrophilic nitrogen in the lipophilic region of the S2/S2′ pockets, as seen with oxygen above.

Benzyl cyclic urea **33** showed a K_i of 3.0 nM, making it an attractive side chain for analoging. Picolyl substituents were introduced as in **34**−**36**, and 3-picolyl cyclic urea **35** was found to be the best, with a K_i of only 3.2-fold weaker than that of the corresponding **33**. According to the X-ray structures,[4] the S2/S2′ pockets are very large. α-Naphthylmethyl was introduced at P2/P2′ as in **37** and found to be a poor binder. On the other hand, β-naphthylmethyl cyclic urea **38** was found to be a subnanomolar inhibitor with a K_i of 0.31 nM. However, the translation of K_i to IC_{90} for **38** is rather poor, probably due to the extremely high lipophilicity of the molecule (clog*P*[23] 9.2).

A series of regioisomeric halo (**39**−**46**), methyl (**47, 48**), trifluoromethyl (**49,50**), and methoxy (**51**−**53**) substituents were introduced on P2/P2′ benzyl side chains. In general, the regioisomeric preference in decreasing order is meta, para, and ortho. Several of the para-isomers are within 2−3-fold of the meta-isomers in terms of K_i. Nitro substitution at the meta-position as in **54** gave a K_i of 2.8 nM. On the other hand, iodo substitution at the meta-position gave a K_i of 0.42 nM, making **55** the best binder in the *m*-halo series.

Modeling revealed that there are a few hydrogen bond donors/acceptors, namely, the side chains and backbone amides of Asp 29/29′ and Asp 30/30′, close to the meta- and para-positions of the *N*-benzyl groups. Hydroxy and hydroxymethyl groups were incorporated as in **56**−**59**. These compounds indeed have K_i values in the subnanomolar range. Moreover, because of the reduced lipophilicity, the translation from K_i to IC_{90} is greatly improved. For example, cyclic urea **56** (clog*P* and HPLC log *P* are 4.8 and 3.6, respectively[23]) translates 2 orders of magnitude better than other subnanomolar inhibitors **38** (clog*P* 9.2), **43** (clog*P* 8.3), and **55** (clog*P* 9.1). The IC_{90}'s of these cyclic ureas, **56**−**59**, are in the range of 0.032−0.057 μM. These cyclic ureas are orally bioavailable with C_{max} of 0.39−0.83 μM at 10 mg/kg in rats and *F*% of 18−30.[24] Among the hydroxy compounds, **56** has the best oral bioavailability in dogs, with 37% oral bioavailability and a C_{max} of 2.8 μM at 10 mg/kg dose. In addition to hydroxy groups, amino groups were also

Table 2. Symmetric Cyclic Urea Inhibitors of HIVPR



				bioavailability[c]	
compd	P2/P2′	K_i[a] (nM)	IC_{90}[b] (μM)	C_{max} (μM)	*F*%
3	methyl	5700	>141		
8	*n*-ethyl	100	>132		
9	*n*-propyl	8 ($n = 1$)	54		
10	*n*-butyl	1.4	0.68 ($n = 2$)		
11	*n*-pentyl	1.6 ($n = 3$)	1.5 ($n = 2$)		
12	*n*-hexyl	4.6	>102		
13	*n*-heptyl	260	>96		
14	$CH_2CH_2OCH_3$	800	>114		
15	$CH_2CH_2OCH_2CH_3$	1100	>106		
16	$CH_2CH_2OCH_2CH_2OCH_3$	7700	>94		
17	*i*-butyl	49	>100		
18	*i*-pentyl	12	3.2		
19	*i*-hexyl	7	8.1		
20	*i*-heptyl	30	>95		
21	*i*-octyl	110	>18		
22	neohexyl	36			
23	allyl	5.2 ($n = 12$)	4.7 ($n = 14$)	2.7	49
24	2-methylpropen-3-yl	7.3	7.6		
25	isoprenyl	1.8	0.87	<0.4	
26	$CH_2CH_2OCHCH_2$	60	>107		
27	3-propynyl	22	42		
28	cyclopropylmethyl	2.1	1.8 ($n = 49$)	4.3 9.2 (dog)	100 48 (dog)
29	cyclobutylmethyl	1.3	1.0		
30	cyclopentylmethyl	4.3	1.7 ($n = 4$)	0.2	
31	cyclohexylmethyl	37	>96 ($n = 2$)		
32	*N*-morpholino-2-ethyl	4000	>91		
33	benzyl	3.0	0.83	1.3	
34	2-picolyl	145	49		
35	3-picolyl	9.7	8.8		
36	4-picolyl	90	98		
37	α-naphthylmethyl	86	16		
38	*β*-naphthylmethyl	0.31 ($n = 3$)	3.9 ($n = 5$)	0.38	
39	*o*-fluorobenzyl	34	5.5		
40	*m*-fluorobenzyl	3.0	0.71		
41	*p*-fluorobenzyl	1.4	0.60	1.6	
42	*o*-chlorobenzyl	240	11.3		
43	*m*-chlorobenzyl	0.89	1.3	0.3	
44	*p*-chlorobenzyl	5.2	4.5		
45	*m*-bromobenzyl	1.4	1.2		
46	*p*-bromobenzyl	27	8.1		
47	*m*-methylbenzyl	7.0	2.4		
48	*p*-methylbenzyl	5.7	4.3		
49	*m*-(trifluoromethyl)benzyl	22	7.8		
50	*p*-(trifluoromethyl)benzyl	51	7.2		
51	*o*-methoxybenzyl	1,870	22.9		
52	*m*-methoxybenzyl	1.6	1.3		
53	*p*-methoxybenzyl	157	7.6		
54	*m*-nitrobenzyl	2.8	0.97		
55	*m*-iodobenzyl	0.42	3.0		
56	*p*-(hydroxymethyl)benzyl (DMP323)	0.34 ($n = 91$)	0.057 ($n = 30$)	0.78 2.8 (dog)	27 37 (dog)
57	*m*-(hydroxymethyl)benzyl	0.14	0.038 ($n = 4$)	0.83	18
58	*p*-hydroxybenzyl	0.12	0.032 ($n = 2$)	0.39	22
59	*m*-hydroxybenzyl	0.12	0.054 ($n = 14$)	0.81 2.0 (dog)	30 16 (dog)
60	*m*-(aminobenzyl)·$2CH_3SO_3H$ (DMP450)	0.28	0.13	2.25 11.2 (dog)	71 79 (dog)
61	*p*-($HOCH_2$)benzyl (enantiomer of DMP323)	1650	51 ($n = 2$)		

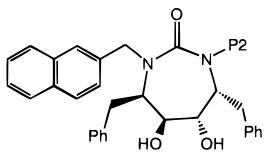
[a] K_i values were measured as described,[21] with 62.5−250 pM HIV-1PR dimer and four concentrations of inhibitor (0.1−10 000 nM, depending on inhibitor); $n = 2$ except as indicated. **56** has $K_i = 0.34 \pm 0.16$. [b] Inhibition of viral replication was quantified in HIV-1 (RF)-infected MT-2 cells by measurement of viral RNA with an oligonucleotide-based sandwich hybridization assay;[22] $n = 1$ except as indicated. **56** has IC_{90} of 0.057 ± 0.028 μM. [c] Bioavailability was determined in groups of rats, unless otherwise indicated ($n = 4$/group), dosed with compound in formulations containing propylene glycol, poly(ethylene glycol) 400, water at 10 mg/kg.[24] The maximum plasma concentration (C_{max}) is the observed peak plasma concentration after an oral dose. Oral bioavailability (*F*%) was determined by the ratio AUC(po)/AUC, where AUC is the area under the plasma concentration−time curve from time zero to infinity and is normalized for dose.

introduced as in **60** to provide another potent compound (IC_{90} 130 nM) with greatly improved oral bioavailability in both rats and dogs. The oral bioavailability of **60** in dogs was as high as 79%, with a C_{max} of 11.2 μM at a dose of 10 mg/kg.[34]

Cyclic urea **56** has a K_i of 0.34 nM, while its enantiomer **61** has a K_i of 1650 nM. This 4900-fold difference in K_i confirmed our modeling prediction during the design stage that the *RSSR* stereochemistry as in **56** is the preferred one. Based on modeling, cyclic urea **61** cannot project P2/P2′ into the corresponding S2/S2′ pockets.

The cyclic urea scaffold is symmetric, designed to be complementary to the C_2-symmetric HIVPR.[4] This

Table 3. Nonsymmetrical Cyclic Urea Inhibitors of HIVPR



compd	P2	K_i[a] (nM)	IC_{90}[b] (μM)	bioavail[c] C_{max} (μM)
38	β-naphthylmethyl	0.31 ($n = 3$)	3.9 ($n = 5$)	0.38
62	*n*-propyl	1.1	3.3	
63	*n*-butyl	0.6	0.75	
64	allyl	1.4	0.99	
65	cyclopropylmethyl	1.5	1.3	
66	cyclopentyl	0.28	0.66	0.32
67	benzyl	2.3	7.5	
68	3-picolyl	5.2	0.72	
69	4-picolyl	6.9	2.7	
70	*p*-fluorobenzyl	3.6	1.1	
71	*p*-(hydroxymethyl)benzyl	0.93	0.21	
72	*m*-aminobenzyl	1.0	0.084	
73	*m*-hydroxybenzyl	0.33	0.145	0.96 2.7 (dog)

[a–c] See footnotes a–c in Table 2.

provides the synthetic advantage in structure–activity relationship studies as cyclic ureas with symmetric P2/P2′ side chains can be prepared easily to find the best P2/P2′ side chains. Subsequently, the best P2/P2′ side chains can be combined to make cyclic ureas with nonsymmetric P2/P2′ side chains. Nonsymmetric cyclic ureas have the advantage of better solubility than symmetric cyclic ureas. For example, the symmetric cyclic ureas described above are in general poorly soluble in chloroform, whereas nonsymmetric cyclic ureas (*vide infra*) are more soluble in chloroform. We next describe the study of an initial series of nonsymmetric cyclic ureas containing a tight binding β-naphthylmethyl group as one of the P2/P2′ side chains as shown in Table 3.

Nonsymmetric cyclic ureas **62**–**73** were prepared to examine the effect of nonsymmetric P2/P2′ side chains on K_i. In general, the K_i of the nonsymmetric cyclic ureas falls within the range of the K_i constituted by the two corresponding parent symmetric cyclic ureas. Only **70**–**72** are outside the range; however, even these compounds are only 3–4-fold weaker. Cyclic urea **66** is an interesting compound. It is a combination of **11** (cyclopentylmethyl, K_i 4.3 nM) and **38** (β-naphthylmethyl, K_i 0.31 nM). It had a K_i of 0.28 nM, which is identical with that of the tighter binder **38**, even though it contains a weaker cyclopentylmethyl group as one of the P2 substituents.

The translation of K_i to IC_{90} of all these nonsymmetric cyclic ureas is 1–2 orders of magnitude better than the parent symmetric **38**. This is due to the fact that **38** is too lipophilic (*vide supra*). We are currently actively exploring nonsymmetric cyclic ureas.

The X-ray structure of the complex of **56** and HIV-1PR was determined.[25] As shown in Figure 3, the complex displays reasonably symmetric binding between the inhibitor and protease. The benzyl alcohols accept hydrogen bonds from the backbone NH of Asp 30/30′ and Asp 29/29′ as shown in Figure 4. The diols form multiple hydrogen bonds with catalytic Asp 25/25.[26] The urea oxygen accepts two hydrogen bonds from the backbone NH of Ile 50/50′. Thus, the inhibitor links the protease catalytic aspartates to the flexible flaps via a hydrogen bond network that does not include an intervening water molecule. The displacement of the structural water was further confirmed by NMR ROESY experiments of the complex of isotopically enriched HIVPR-1/**56**.[26] This water displacement is entropically favorable,[12] contributing to the high potency of the cyclic ureas.

The displacement of the structural water by inhibitors has been an area of active research.[27,28] Recently the Parke Davis[28a–c] and Upjohn[28d–f] groups had independently discovered pyrone inhibitors based on random screening of in-house chemical collections. These pyrones have been confirmed to displace the structural water based on X-ray studies of the complexes.[28]

Attempts at preparing high-quality crystals of N-unsubstituted cyclic urea for X-ray crystallographic studies were unsuccessful. However, we were able to obtain crystals of **74** where one of the hydroxyl groups had been acetylated. The X-ray structure of **74**[29] shows that the conformation of N-unsubstituted cyclic urea has pseudodiequatorial benzyl and pseudodiaxial acetoxy and hydroxyl groups (Figure 5). For the methyl-substituted cyclic urea **3**, the X-ray structure[29] shows that **3** has adopted the alternative ring conformation with pseudodiaxial benzyl and pseudodiequatorial hydroxyl groups.

X-ray structures of other N-substituted cyclic ureas have also been solved.[29,30] They all share the same ring conformation as **3**. Extensive NMR studies of the ring conformation of **60** in water had been performed. The result indicates that the ring conformation of **60** in water is similar to its solid-state X-ray conformation. These NMR experiments are being described in detail elsewhere.[29]

Figure 6 shows an overlap of bound and "unbound" (small-molecule crystal structure[2]) **56**. The similarity of the two structures suggests that **56** is indeed highly preorganized[13] for binding. To further evaluate the contribution of preorganization in the cyclic urea system, the corresponding seco compound **75** was used for comparison as shown in Figure 7. Cyclic urea **33** has a K_i of 2.5 nM, whereas urea **75** has a K_i of 6700 nM. This corresponds to a 4.8 kcal/mol gain in binding energy for **33** versus **75**. This gain in binding energy reflects the preorganization of the side chains and diols of the cyclic urea structure relative to **75**. In general, preorganization includes, but is not limited to, conformational entropic penality,[13] hydrophobic collapse penalty,[31] and desolvation cost.[13] In our case, it is not possible to dissect these contributions. The above analysis was performed based on the assumption that **75** binds in a loose, relatively unstrained "pseudocyclic" conformation, close to that of **33** but with the methylene carbons bearing the hydroxy groups not in van der Waals contacts. This binding mode is not unreasonable since the diols would want to bind to the catalytic Asp 25/25′.[4] Nevertheless, this may or may not be the case. The extended conformation of urea **75** may be the low-energy binding conformation, whereas the loose "pseudocyclic" conformation needed for comparison in Figure 7 is a higher energy conformation. In this case, the value of 4.8 kcal/mol as determined is not valid. Obtaining the X-ray structure of a complex of **75** and HIVPR can provide the answer.

In consideration of the overall pharmacokinetic and safety parameters in animals, cyclic urea **56** was chosen as the first clinical candidate and designated DMP323.[32]

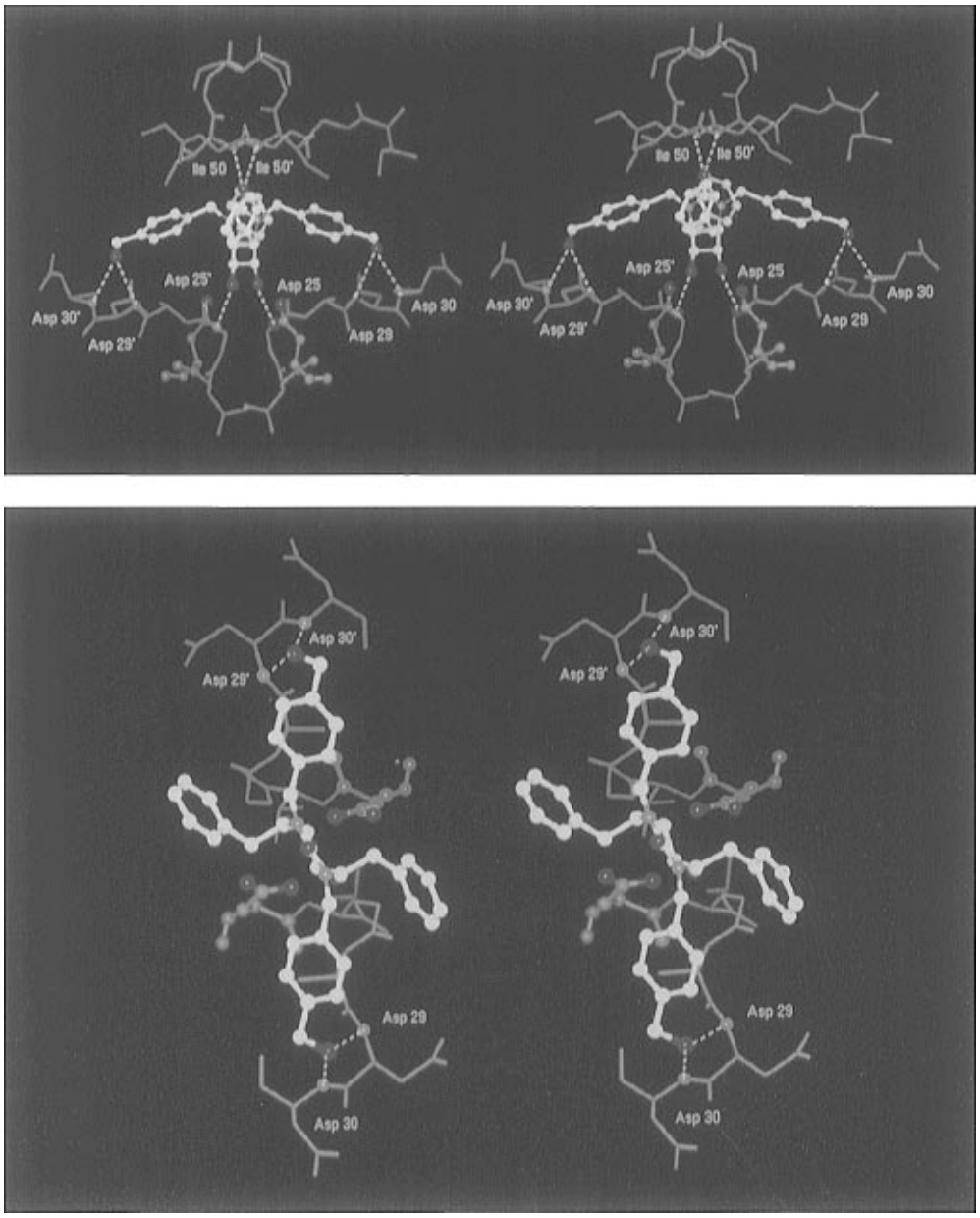


Figure 3. X-ray structure of HIV-1PR dimer (brown/green) complexed with cyclic urea **56** (DMP323, white). Oxygen is colored red and nitrogen colored blue. (Top) Front view showing the hydrogen-bonding network from the floor of the active site to the flaps without the intervention of the structural water. (Bottom) C_2-Axis view from the top showing the benzyl alcohol of **56** accepts two hydrogen bonds from Asp 29/29′ and Asp 30/30′.

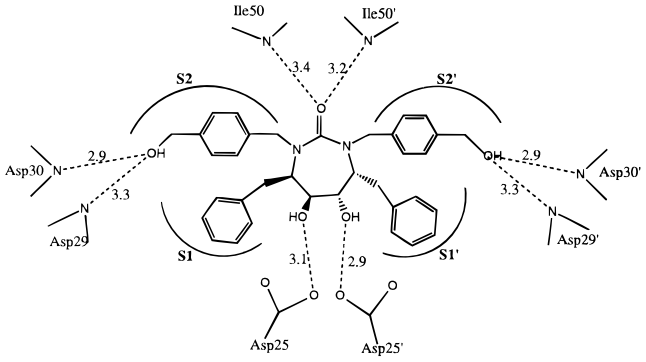


Figure 4. Interatomic distances of cyclic urea **56** binding to HIV-1PR as derived from the X-ray crystal structure. Distances are measured between heavy atoms in angstroms.

However due to the low aqueous solubility of **56** (6 μg/mL), highly variable human oral bioavailability was observed ranging from 0 to 4.6 μM at 1.5 g dose, with a mean of 1.4 μM.[33] This clinical trial was subsequently

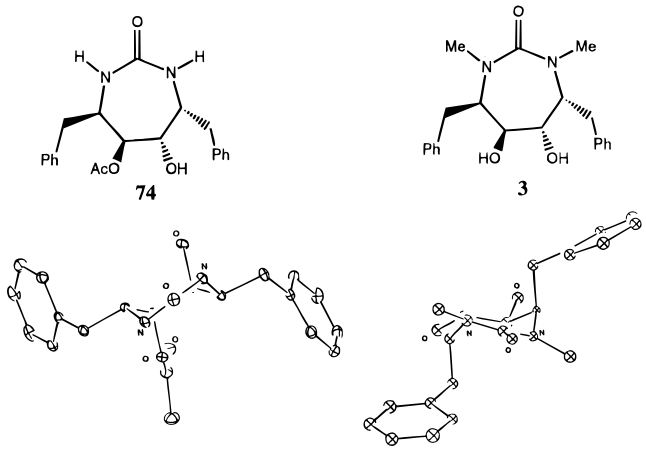


Figure 5. Small-molecule X-ray structures of unsubstituted cyclic urea **74** showing pseudodiequatorial benzyl and pseudodiaxial diol substituents and of methyl-substituted cyclic urea **3** showing the alternate conformation, consistent with the design hypothesis.

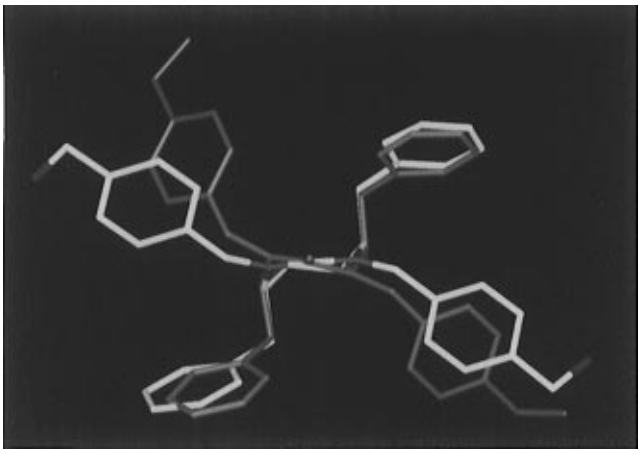
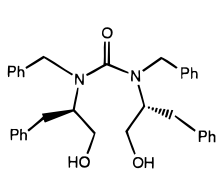
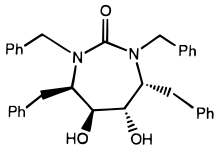


Figure 6. Comparison of bound (white) and "unbound" (brown) conformations of cyclic urea **56** showing the high degree of preorganization.



75 Ki: 6,700 nM; ΔΔG: 0 Kcal/mol



33 Ki: 2.5; ΔΔG: -4.8

Figure 7. Comparison of cyclic urea **33** and the seco compound **74** showing a gain of 4.8 kcal/mol of binding energy upon preorganizing the side chains and the diols.

terminated. DMP450, with excellent water solubility (>170 mg/mL), was chosen as the second clinical candidate, and the human pharmacokinetics were found to be greatly improved, analogous to the animal models. The results of the clinical study of DMP450 are being described in detail elsewhere.[34]

Conclusion

Cyclic ureas are in general potent inhibitors of HIVPR for molecules of their size.[5] The molecular recognition features discussed above reveal that this is a result of preorganization and high complementarity in terms of conformation, stereochemistry, hydrophobic interactions, hydrogen bondings, and water displacement. The high potency results in relatively smaller size which probably contributes to good oral bioavailability in rats and dogs.[14] We are continuing to explore nonsymmetric cyclic HIVPR inhibitors in order to find candidates with superior pharmacokinetic and efficacy profiles.

Experimental Section

Biological Methods. Inhibition of HIVPR was measured by assaying the cleavage of a fluorescent peptide substrate using HPLC.[21] The antiviral potency of compounds was assessed by measuring their effect on the accumulation of viral RNA transcripts 3 days after infection of MT-2 cells with HIV-1 RF.[22] Uninfected cells were incubated in microtiter plate wells with serial dilutions of test compound in cell culture medium for 30 min; then virus was added. After 3 days of culture at 37 °C and 5% CO_2, infected cell cultures were lysed and the levels of HIV RNA determined using a microtiter plate-based hybridization assay. The concentration of test compound which reduced the concentration of HIV viral RNA by 90% from the level measured in an untreated infected culture was designated the IC_{90}. The cellular toxicity of compounds was assayed by measuring the extent of MTT dye reduction in uninfected MT-2 cell cultures grown for 3 days in the presence of various concentrations of test compound. The compound concentration which decreased the level of MTT dye reduction by 50% was designated the TC_{50}. Only compounds which displayed an antiviral IC_{90} concentration which was at least 3-fold less than the TC_{50} concentration were considered to have a specific antiviral effect. Oral bioavailability was measured as previously described.[24,32c]

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, CA) with TMS as an internal reference standard. Melting points were determined on a Mettler SP61 apparatus and are uncorrected. Elemental analyses were performed by Quantitative Technologies, Inc., Bound Brook, NJ. Mass spectra were measured with a HP5988A 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. Separation of isomers was performed using supercritical fluid chromatography with a Chiracel OD column (Daicel Chemical Ind. Ltd.) and a 20% methanol-modified CO_2 mobile phase. Optical rotations were obtained on a Perkin Elmer 241 polarimeter. Solvents and reagents were obtained from commercial vendors in the appropriate grade and used without further purification unless otherwise indicated. All compounds were determined to be homogeneous by TLC, elemental analysis, and/or HPLC. Phenylalaninol I, (*R*)-phenylmethyl [2-hydroxy-1-(phenylmethyl)ethyl]carbamate, was obtained from Synthetech, Inc. The optical rotation of the lot employed in the following sequence was $[\alpha]^{25}_D$ +41° (*c* 1.0, EtOH).

(1*R*,2*S*,3*S*,4*R*)-Bis(phenylmethyl)[2,3-Dihydroxy-1,4-bis(phenylmethyl)-1,4-butanediyl]bis[carbamate] (5). A solution of 52 mL of oxalyl chloride (0.59 mol) in 500 mL of dichloromethane (CH_2Cl_2) was cooled to −78 °C, and 57 mL of anhydrous dimethyl sulfoxide (0.81 mol) was added in 500 mL of CH_2Cl_2 over 1 h while the temperature was maintained below −70 °C (caution: exothermic). Stirring was continued at −78 °C for 0.5 h, and 125 g of (*R*)-*N*-Cbz-phenylalaninol (**4**) (0.44 mol) was added in 800 mL of CH_2Cl_2 over 1 h, again maintaining the temperature below −70 °C, followed by stirring for 0.5 h at −78 °C. Triethylamine (244 mL) was added in 300 mL of CH_2Cl_2 over 0.5 h followed by stirring for 2 h at −70 °C. Addition of 800 mL of 20% aqueous $KHSO_4$ was followed by allowing the mixture to warm to room temperature and addition of 300 mL of water. The aqueous phase was separated and washed with 400 mL of CH_2Cl_2. The combined organic layers from two such runs were washed with 1 L of saturated aqueous $NaHCO_3$, 1 L of water, and 1 L of saturated aqueous NaCl, dried over $MgSO_4$, and concentrated to 700 mL. Hexane (2 L) was added, the mixture was cooled in an ice bath for 1 h, and the solids were filtered and washed with cold hexane. After drying to constant weight at 40−50 °C, 209 g (84%) of a white solid was obtained: mp 82−83 °C; $[\alpha]^{25}_D$ +61.58° (*c* 0.406, MeOH); ^{1}H NMR ($CDCl_3$) δ 3.15 (d, J = 7.0 Hz, 2H), 4.52 (q, J = 7.0 Hz, 1H), 5.14 (s, 2H), 5.35 (br d, J = 6.0 Hz, 1H), 7.10−7.42 (m, 10H), 9.65 (br s, 1H). The aldehyde, when pure, could be stored under inert atmosphere without racemization or trimerization. Runs were pooled as necessary for the following step.

A 4-necked reaction flask was charged with 467 g of VCl_3·$(THF)_3$ (1.25 mol) and 1 L of CH_2Cl_2 in a drybox, removed from the drybox, and fitted with a reflux condenser, a nitrogen bubbler, and a thermocouple. Zinc dust (54 g, 0.83 mol, also weighed in the drybox) was added, and the temperature rose to 40 °C. The prepared aldehyde (350 g, 1.2 mol) was added rapidly in 700 mL of CH_2Cl_2, and the reaction mixture was stirred overnight. A flask containing 6 L of water and 500 mL of concentrated HCl was warmed until the solution reached about 65 °C. The reaction mixture was added by addition funnel and CH_2Cl_2 collected in a cooled flask as it

distilled off. When addition was completed, the mixture was allowed to cool to room temperature and the precipitate collected and washed with water until colorless. The solid was further washed with 1.2 L of ethanol followed by 1.2 L of hexane. Two of the above runs were combined and recrystallized by dissolving in hot THF and filtering away insoluble material. Hexane was added and the mixture allowed to cool. A total of 374 g (55%) of **5** as a white crystalline solid was recovered: mp 211−213 °C; SCF chromatographic analysis showed 98% 1*R*,2*S*,3*S*,4*R* isomer and 2% 1*R*,2*R*,3*R*,4*R* isomer; $[\alpha]^{25}_D$ +12.5° (*c* 0.042, MeOH; ^{1}H NMR (DMSO) δ 2.65−2.80 (m, 4H), 3.25 (br s, 2H), 4.12−4.26 (m, 2H), 4.55 (br s, 2H), 4.90 (ABd, J = 15.0 Hz), 4.93 (ABd, J = 15.0 Hz), 6.84 (d, J = 8.0 Hz, 2H), 7.00−7.35 (m, 20H); MS *m*/*z* 569 (M + 1, 100); HRMS (M + 1) calcd 569.2651, found 569.2644. Anal. ($C_{34}H_{36}N_2O_6$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-bis[[2-(trimethylsilyl)-ethoxy]methoxy]-4,7-bis(phenylmethyl)-2*H*-1,3-diazepin-2-one (6). To a solution of 40 g (70 mmol) of diol **5** in 150 mL of dry DMF under nitrogen was added 37 mL (211 mmol) of [2-(trimethylsilyl)ethoxy]methoxy chloride (SEMCl) followed by 38 mL (230 mmol) of diisopropylethylamine. The mixture was heated at 50 °C for 48 h. After cooling, the mixture was partitioned between CH_2Cl_2 and 5% HCl. The organic phase was washed with saturated $NaHCO_3$, water, and brine and dried with $MgSO_4$. The solvent was removed and the residue purified by chromatography (30% ethyl acetate/hexane) to give 53 g (91%) of a white solid: mp 53−54 °C; ^{1}H NMR ($CDCl_3$) δ 0.05 (s, 18H), 0.95−1.00 (m, 4H), 2.70−2.80 (m, 4H), 3.40−3.60 (m, 4H), 3.75−4.05 (m, 3H), 4.15 (q, J = 7.5 Hz, 1H), 4.60−5.05 (m, 10H), 7.0−7.4 (m, 20H); MS (DCI) *m*/*z* 847 (M + NH_4, 100); HRMS (M + 1) calcd 829.4280, found 829.4281. Anal. ($C_{46}H_{64}N_2O_8Si_2$) C, H, N.

The white solid (8.2 g, 9.9 mmol) and 1 g of 10% Pd/C in 100 mL of ethyl acetate/methanol (1:1) was stirred under hydrogen overnight. TLC showed complete hydrogenolysis. The catalyst was filtered off through a Celite pad, and the solvent was removed to give 5.3 g of the diamine as a colorless gum. The diamine was dissolved in 50 mL of dry CH_2Cl_2 under nitrogen. Pyridine (1.5 mL, 19 mmol) and 1.9 g (11 mmol) of 1,1′-carbonyldiimidazole in 50 mL of CH_2Cl_2 were added, and the mixture was stirred overnight and then washed with 5% HCl, saturated $NaHCO_3$, and brine and dried with $MgSO_4$. The solvent was removed and the residue chromatographed to give 3.1 g (52% in two steps) of cyclic urea **6** as a white solid: mp 74−75 °C; ^{1}H NMR ($CDCl_3$) δ 0.05 (s, 18H), 0.90 (t, J = 7.5 Hz, 4H), 2.85−2.95 (m, 4H), 3.50−3.70 (m, 4H), 3.65 (s, 2H), 3.90 (t, J = 7.5, 2H), 4.06 (s, 2H), 4.66 (d, J = 7.5 Hz, 2H), 4.75 (d, J = 7.5 Hz, 2H), 7.15−7.30 (m, 10H); MS (DCI) *m*/*z* 587 (M + 1, 100); HRMS (M + 1) calcd 587.3337, found 587.3353. Anal. ($C_{31}H_{50}N_2O_5Si_2$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-bis[(2-methoxyethoxy)-methoxy]-4,7-bis(phenylmethyl)-2*H*-1,3-diazepin-2-one (7). Following the same procedure for **6**, one can obtain (methoxyethoxy)methyl (MEM)-protected cyclic urea **7** in 62% yield from **5** and MEM chloride as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.80−3.00 (m, 4H), 3.34 (s, 6H), 3.38−3.50 (m, 6H), 3.64 (s, 2H), 3.90 (t, J = 8.0 Hz, 2H), 4.26 (s, 2H, NH), 4.75 (ABd, J = 7.5 Hz, 2H), 4.80 (ABd, J = 7.5 Hz, 2H), 7.18−7.35 (m, 10H); MS (ESI) *m*/*z* 503 (M + 1, 100); HRMS (M + 1) calcd 503.2757, found 503.2772.

Representative Procedures for N-Substituted Cyclic Ureas: Method A. (4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis[[4-(hydroxymethyl)phenyl]methyl]-4,7-bis-(phenylmethyl)-2*H*-1,3-diazepin-2-one (56, DMP323). To a solution of 5.50 g (10.9 mmol) of MEM-protected cyclic urea **7** in 50 mL of dry dimethylformamide was added 2.60 g (65.6 mmol) of 60% sodium hydride in mineral oil. The mixture was stirred for 15 min and cooled to 0 °C, and 8.58 g (43.8 mmol) of 4-cyanobenzyl bromide in DMF was added. The mixture was stirred at 0 °C for 15 min and at room temperature for 2 h and then hydrolyzed with water and extracted with ether to give an oil that was purified by flash chromatography using 15% ethyl acetate in methylene chloride. This yielded 3.2 g (40%) of the nitrile as a semisolid: MS *m*/*z* 733 (M + 1, 100).

The nitrile (2.74 g, 3.74 mmol) was dissolved in 15 mL of dry toluene under nitrogen and cooled to −78 °C. To this solution was added 5.48 mL (8.22 mmol) of DIBAL-H (1.5 M in toluene) dropwise, and the mixture was allowed to warm to room temperature and stir overnight. The mixture was then washed with cold 5% HCl, water, saturated sodium bicarbonate, and brine and dried with $MgSO_4$. Evaporation of solvent followed by flash chromatography with 20% ethyl acetate in methylene chloride gave 1.18 g (43%) of aldehyde as a gum: MS *m*/*z* 739 (M + 1, 100).

The aldehyde (1.00 g, 1.35 mmol) was dissolved in 10 mL of ethanol and treated with 65 mg of sodium borohydride. The mixture was refluxed for 3 h. After cooling, the mixture was partitioned between ether and water. The ether phase was washed twice with water and brine and dried with magnesium sulfate. The resulting benzyl alcohol was used with no purification.

The benzyl alcohol (0.87 g, 1.17 mmol) was dissolved in 10 mL of methanol and 10 mL of 4 M HCl in dioxane. After stirring overnight, the solvent was evaporated and the residue purified by chromatography (3% methanol in ethyl acetate) to give 0.49 g (64% in two steps) of **56** as a white solid: mp 197 °C; ^{1}H NMR (CD_3OD) δ 2.93 (d, J = 15 Hz, 2H), 2.90−3.05 (m, 4H), 3.53 (br s, 2H), 3.68 (br d, 2H), 4.57 (s, 4H), 4.75 (d, J = 15 Hz, 2H), 7.05−7.35 (m, 18H); MS (CI) *m*/*z* 567 (100, M + 1); $[\alpha]^{25}_D$ +103° (*c* 0.598, EtOH). Anal. ($C_{35}H_{38}N_2O_5$) C, H, N.

Method A was used to make cyclic ureas **57**−**61**.

Method B. (4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-dimethyl-4,7-bis(phenylmethyl)-2*H*-1,3-diazepin-2-one (3). Following the same procedure for **56** with SEM-protected cyclic urea **6** and methyl iodide as the alkylating agent, one can obtain **3** as a white solid: mp 170−174 °C; ^{1}H NMR ($CDCl_3$) δ 2.58 (s, 6H), 2.80−3.10 (m, 4H), 2.90 (s, 2H), 3.52 (d, J = 9.0 Hz, 2H), 4.02 (br s, 2H); MS (CI) *m*/*z* 355 (100, M + 1). Anal. ($C_{21}H_{26}N_2O_3 \cdot 0.5H_2O$) C, H, N.

Method B was also used to make cyclic ureas **4**−**55**.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3,4,7-tetrakis(phenylmethyl)-2*H*-1,3-diazepin-2-one (33). Following method B for **3** with benzyl chloride as alkylating agent, one can obtain benzyl cyclic urea **33** as a white solid: mp 171 °C; ^{1}H NMR ($CDCl_3$) d 1.60 (br s, 1H), 2.35 (br s, 1H), 2.98−3.10 (m, 6H), 3.57 (br d, J = 15 Hz, 2H), 3.60 (br s, 2H), 7.10−7.40 (m, 20H); MS (CI) *m*/*z* 507 (100, M + 1); $[\alpha]^{25}_D$ +141 (*c* 0.922, MeOH). Anal. ($C_{33}H_{34}N_2O_3$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis[[3-(hydroxymethyl)phenyl]methyl]-4,7-bis(phenylmethyl)-2*H*-1,3-diazepin-2-one (57). Following method A for **56** with 3-cyanobenzyl chloride as alkylating agent, one can obtain benzyl alcohol **57** as a white solid: mp 245−247 °C; ^{1}H NMR (CD_3OD) δ 2.80−3.15 (m, 4H), 3.10 (d, J = 15 Hz, 2H), 3.60 (br s, 2H), 3.62 (br s, 2H), 4.85 (d, J = 15 Hz, 2H), 7.00−7.90 (m, 18H); MS (CI) *m*/*z* 567 (100, M + 1). Anal. ($C_{35}H_{38}N_2O_5 \cdot 0.25H_2O$) C, H, N.

(4*R*,5*S*,6*s*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis[(4-hydroxyphenyl)methyl]-4,7-bis(phenylmethyl)-2*H*-1,3-diazepin-2-one (58). Following method A for **56** with 3-(benzyloxy)benzyl chloride as alkylating agent, one can obtain bisalkylated cyclic urea. The benzyl protecting groups were removed by hydrogenolysis in the presence of 10% Pd/C, and the MEM protecting groups were removed by the standard procedure to give phenol **58** as a white solid: mp 115−117 °C; ^{1}H NMR (CD_3OD) δ 2.83 (d, J = 11 Hz, 2H), 2.95−3.05 (m, 4H), 3.60 (br s, 2H), 3.62 (br s, 2H), 4.65 (d, J = 11 Hz, 2H), 6.75 (d, J = 8 Hz, 4H), 6.96 (d, J = 8 Hz, 4H), 7.10−7.40 (m, 8H); MS (CI) *m*/*z* 539 (5, M + 1), 182 (100). Anal. ($C_{33}H_{34}N_2O_5 \cdot 0.5H_2O$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis[(3-hydroxyphenyl)methyl]-4,7-bis(phenylmethyl)-2*H*-1,3-diazepin-2-one (59). Following the procedure for the synthesis of **58** with 4-(benzyloxy)benzyl chloride as alkylating agent, one can obtain phenol **59** as a white solid: mp 101−103 °C; ^{1}H NMR (CD_3OD) δ 2.83 (d, J = 11 Hz, 2H), 2.95−3.20 (m, 4H), 3.60 (br s, 2H), 3.62 (br s, 2H), 4.70 (d, J = 11 Hz, 2H), 6.60 (d, J = 8 Hz, 2H), 6.64 (s, 2H), 6.67 (d, J = 8 Hz, 2H), 7.10−7.40 (m, 12H); MS (CI) *m*/*z* 539 (2, M + 1), 142 (100). Anal. ($C_{33}H_{34}N_2O_5 \cdot 0.5H_2O$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1,3-bis[(3-aminophenyl)methyl]-2*H*-1,3-diazepin-2-one, Bismethane-

sulfonic Acid Salt (60, DMP450). The synthesis of **60** is described in detail in ref 34.

(4*S*,5*R*,6*R*,7*S*)-Hexahydro-5,6-dihydroxy-1,3-bis[[4-(hydroxymethyl)phenyl]methyl]-4,7-bis(phenylmethyl)-2*H*-1,3-diazepin-2-one (61). Following method A for **56**, one can prepare the corresponding enantiomer **61** as a white solid: mp 198 °C; ^{1}H NMR (CD_3OD) δ 2.93 (d, J = 15 Hz, 2H), 2.90−3.05 (m, 4H), 3.53 (br s, 2H), 3.68 (br d, 2H), 4.75 (d, J = 15 Hz, 2H), 7.05−7.35 (m, 18H); MS (CI) m/z 567 (100, M + 1); $[\alpha]^{25}_D$ −101° (c 0.466, EtOH). Anal. ($C_{35}H_{38}N_2O_5 \cdot H_2O$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5,6-dihydroxy-1-(4-pyridylmethyl)-3-(*β*-naphthylmethyl)-4,7-bis(phenylmethyl)-2*H*-1,3-diazepin-2-one (69). To a mixture of 2.03 g (50.9 mmol) of 60% NaH in mineral oil (washed with hexane) in 20 mL of dry DMF under nitrogen was added a solution of 6.40 g (12.7 mmol) of di-MEM-protected cyclic urea **7**. The mixture was stirred at room temperature for 10 min. A solution of 3.16 g (14.0 mmol) of 2-(bromomethyl)naphthylene was added and the mixture stirred at room temperature for 8 h. The reaction was quenched with methanol and partitioned between ethyl acetate and water. The organic phase was washed with water and brine and dried with $MgSO_4$. After rotovaping, 7.5 g of a residue was obtained and chromatographed with a gradient of EtOAc and hexane to give 1.77 g (23%) of mononaphthylmethyl cyclic urea (MS m/z 643 (M + 1), 100), 1.51 g (16%) of bis(naphthylmethyl) cyclic urea, and 2.31 g (36%) of starting material.

The mononaphthylmethyl cyclic urea was alkylated with 4 equiv of 4-picolyl chloride and 12 equiv of NaH at room temperature for 2 weeks following method A to give 31% yield of MEM-protected 4-picolyl cyclic urea. Similar acid deprotection (100% yield) followed by preparative TLC (70% EtOAc/hexane) gave **69** as a white solid: mp 118−120 °C; ^{1}H NMR ($CDCl_3$) δ 2.90−3.40 (m, 4H), 2.98 (d, J = 15 Hz, 1H), 3.25 (d, J = 15 Hz, 1H), 3.65 (br d, 2 H), 4.95 (d, J = 15 Hz, 1H), 5.07 (d, J = 15 Hz, 1H), 7.05−7.35 (m, 17H), 7.67 (d, J = 7.5 Hz, 2H), 8.15 (d, J = 7.5 Hz, 2H); MS (CI) m/z 558 (100, M + 1). Anal. ($C_{35}H_{38}N_2O_5 \cdot H_2O$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-Hexahydro-5-acetoxy-6-hydroxy-4,7-bis(phenylmethyl)-2*H*-1,3-diazepin-2-one (74). Di-MEM-protected cyclic urea **7** (50.2 g, 0.1 mol) was dissolved in MeOH (250 mL) and cooled in an ice bath to 0 °C. HCl (g) was bubbled into the solution for 10 min, and the mixture was stirred at 0 °C for 1 h. The mixture was concentrated at room temperature on a rotary evaporator to give the diol as a white solid. The resulting diol was suspended in acetonitrile (300 mL) and treated with 34.5 g (0.2 mol) of triethyl orthoacetate and 0.5 g of *p*-toluenesulfonic acid. The suspension was stirred until TLC analysis showed all the diol was consumed and formation of the ortho ester was complete. Then 50 mL of water was added and the mixture stirred for 30 min until all the ortho ester was converted to the monoacetate. The mixture was concentrated on a rotary evaporator to give a solid residue. The resulting residue was recrystallized from ethyl acetate to give 21 g (57%) of **74** as a white solid: mp 194−195 °C; ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3H), 2.64−2.83 (m, 3H), 2.86 (d, J = 8.0 Hz, 2H), 3.56 (q, J = 4.4 Hz, 1H), 3.79 (t, J = 7.7 Hz, 1H), 3.92 (br t, J = 7.7 Hz, 1H), 4.80 (d, J = 4.4 Hz, 1H), 7.19−7.12 (m, 4H), 7.33−7.21 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 21.27, 38.45, 38.88, 51.35, 53.75, 57.56, 72.57, 120.44, 127.69, 127.82, 129.39, 129.50, 136.40, 136.80, 164.41, 170.31. Anal. ($C_{21}H_{24}N_2O_4 \cdot 0.25H_2O$) C, H, N.

[*R*-(*R,*R**)]-*N*,*N*′-Bis[2-hydroxy-1-(phenylmethyl)ethyl]-*N*,*N*′-bis(phenylmethyl)urea (75).** A solution of 28.5 g (0.1 mol) of (*R*)-*N*-Cbz-phenylalaninol (**4**), 18.7 g (0.15 mol) of (2-methoxyethoxy)methyl chloride (MEMCI), and 26.1 mL (0.15 mol) of diisopropylethylamine in 200 mL of dry methylene chloride was stirred at room temperature overnight. TLC showed no starting material. The reaction mixture was washed with water, dried with $MgSO_4$, and evaporated to dryness to give 37.9 g of crude MEM ether as a brown oil.

To a solution of 18.7 g (50 mmol) of the crude MEM ether in 200 mL of ethanol was added 3.7 g of 5% Pd/C. The mixture was treated with 1 atm of hydrogen for 2 h until TLC showed no starting material. The mixture was filtered and evaporated to dryness to give 11.9 g of crude diamine. The crude diamine (11.5 g, 48.1 mmol) and carbonyldiimidazole (3.9 g, 24.1 mmol) were dissolved in 200 mL of dry THF under nitrogen and stirred at room temperature overnight until the reaction was completed. The mixture was evaporated and partitioned between CH_2Cl_2 and water. The organic phase was washed with water twice, dried with $MgSO_4$, and evaporated to give 11.6 g of crude urea. The crude urea (2.52 g, 5.0 mmol) was benzylated with 5.13 g (30 mmol) of benzyl bromide and 1.2 g (83 mmol) of 60% NaH in mineral oil in anhydrous DMF under nitrogen. The reaction was completed overnight as monitored by TLC.

The final reaction mixture was poured into ice/water and extracted with EtOAc. The organic phase was washed with water six times, dried with $MgSO_4$, and evaporated to dryness. The crude product was chromatographed (hexane/EtOAc, 1:1) to give 2.03 g of a light colored oil. The oil (1.1 g, 1.6 mmol) was dissolved in 200 mL of methanol and 40 mL of concentrated HCl added slowly. The mixture was allowed to stir overnight and poured into ice/saturated $NaHCO_3$. The crude product was chromatographed (CH_2Cl_2/MeOH, 100:1) to give 300 mg (21% overall yield) of urea diol **75** as a solid: mp 116 °C; ^{1}H NMR ($CDCl_3$) δ 2.88 (d, J = 6.0 Hz, 4H), 3.48−3.70 (m, 6H), 3.75 (d, J = 15 Hz, 2H), 4.18 (d, J = 15 Hz, 2H), 4.82 (br s, 2H), 7.00−7.35 (m, 10H); MS (CI) m/z 509 (M + 1, 100); HRMS calcd 509.2804, found 509.2799; $[\alpha]^{25}_D$ +80.0° (c 0.015, MeOH). Anal. ($C_{33}H_{36}O_3N_2 \cdot 0.25H_2O$) C, H, N.

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Supporting Information Available: Elementary analysis, high-resolution mass spectra, and X-ray crystallographic data (12 pages). Ordering information is given on any current masthead page.

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