Cyclic HIV-1 Protease Inhibitors Derived from Mannitol: Synthesis, Inhibitory Potencies, and Computational Predictions of Binding Affinities

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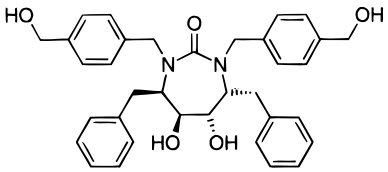
Ten C_2-symmetric cyclic urea and sulfamide derivatives have been synthesized from L-mannonic γ-lactone and D-mannitol. The results of experimental measurement of their inhibitory potencies against HIV-1 protease were compared to calculated free energies of binding derived from molecular dynamics (MD) simulations. The compounds were selected, firstly, to enable elucidation of the role of stereochemistry for binding affinity (**1a**−**d**) and, secondly, to allow evaluation of the effects of variation in the link to the P1 and P1′ phenyl groups on affinity (**1a** and **2**−**5**). Thirdly, compounds with hydrogen bond-accepting or -donating groups attached to the phenyl groups in the P2 and P2′ side chains (**6** and **7**) were selected. Binding free energies were estimated by a linear response method, whose predictive power for estimating binding affinities from MD simulations was demonstrated.

Introduction

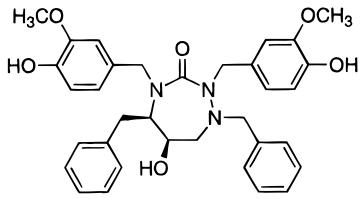
Human immunodeficiency virus (HIV) is the causative agent of acquired immune deficiency syndrome, AIDS.[1] HIV protease processes the viral polyproteins $Pr55_{gag}$ and $Pr160_{gag\text{-}pol}$ into structural proteins and enzymes, including the protease itself. The activity of the protease is vital for proper assembly and maturation of infectious virons.[2] Therefore HIV protease is an important chemotherapeutic target.[3]

Numerous examples of potent inhibitors of HIV-1 protease have been disclosed.[4] A common feature observed in the X-ray crystal structures of linear inhibitor−HIV-1 protease complexes is the presence of a tetracoordinated structural water molecule linking the bound inhibitors to the flexible flaps of the HIV-1 protease dimer.[5] On the basis of this structural information, Lam et al. have performed an elegant design of nonpeptide cyclic ureas, constituting an entirely new class of HIV-1 protease inhibitors.[6] More recently, Sham et al. have reported a series of new azacyclic ureas possessing high potency as inhibitors of HIV-1 protease.[7] The fundamental characteristic of these inhibitors, e.g., DMP 323 and A-98881 (Figure 1), is the cyclic urea carbonyl oxygen mimicking the structural water molecule.[6]

The application of computer modeling and simulation of molecular interactions to drug design is a field currently in rapid growth. Methods allowing reliable predictions of affinity and specificity of ligands would be of paramount value in the discovery process, aiding in the selection of ligands with high potential before synthesis is commenced. In cases where the three-dimensional structure of the drug target is known, methods for scoring putative inhibitors from single conformations of ligand−target complexes have been developed.[8] But even with a 3D-structure of the target available, computational predictions of binding affinity for new ligands is difficult in the absence of accurate 3D-structures of the corresponding complexes. The main reason for this lies in the difficulty of predicting the exact structure of the complex. Force field-based structural optimization and scoring has been applied recently to HIV-1 protease inhibitors with promising results.[9] Åqvist et al. have described another approach, which has yielded good results for several systems,[10] in which absolute binding free energies are estimated by a linear response approximation from thermal averaging of the structure and interaction energies by molec-



DMP 323



A-98881

Figure 1. Structures of DMP 323 and A-98881.

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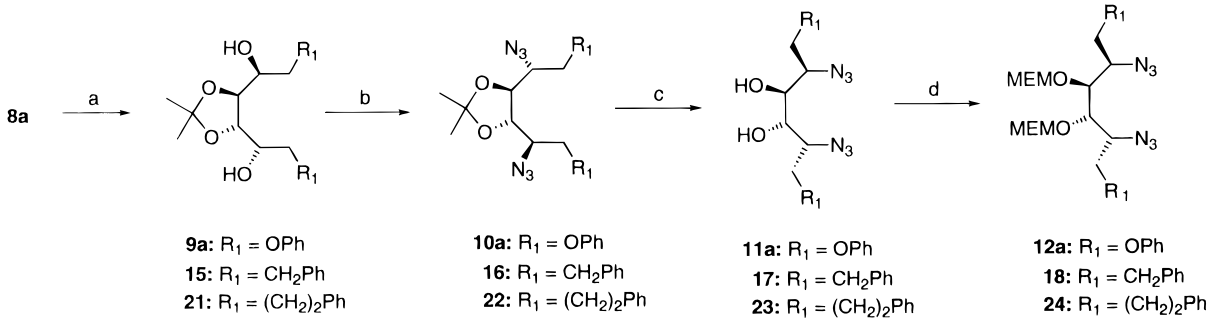
Scheme 1[a]



9a: R_1 = OPh
15: R_1 = CH_2Ph
21: R_1 = $(CH_2)_2Ph$

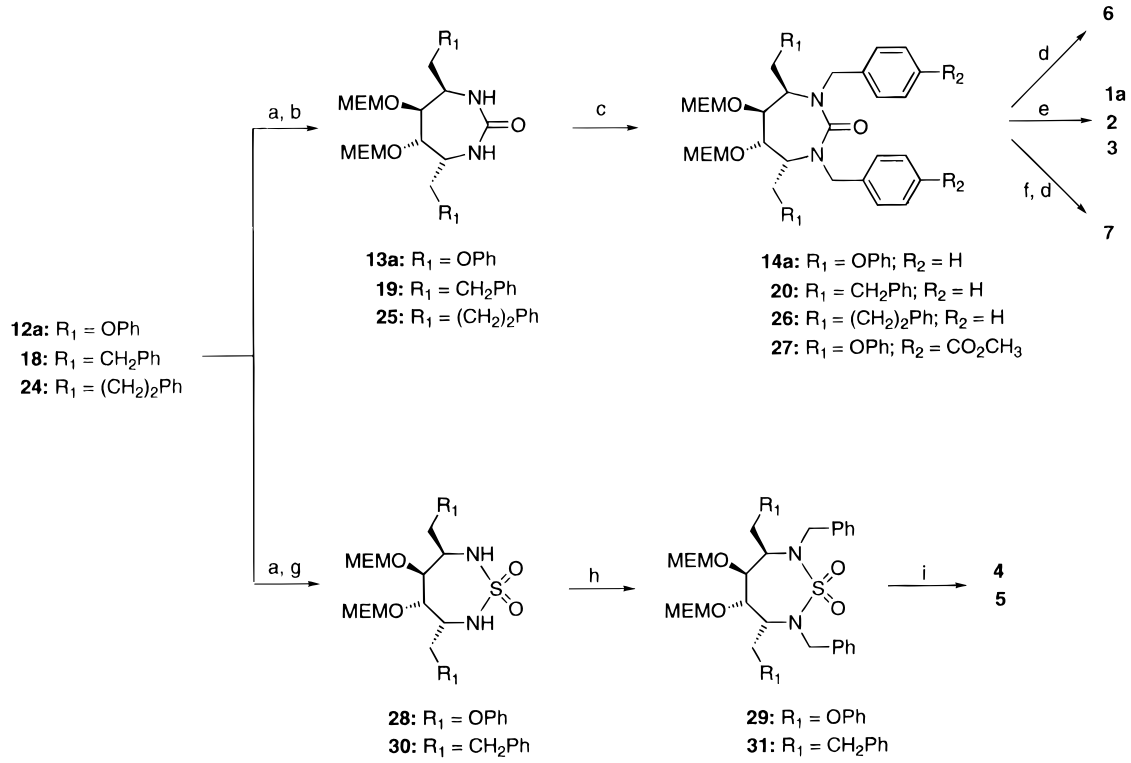
10a: R_1 = OPh
16: R_1 = CH_2Ph
22: R_1 = $(CH_2)_2Ph$

11a: R_1 = OPh
17: R_1 = CH_2Ph
23: R_1 = $(CH_2)_2Ph$

12a: R_1 = OPh
18: R_1 = CH_2Ph
24: R_1 = $(CH_2)_2Ph$

[a] (a) PhOH, NaH, THF or BnMgCl, CuI, THF or $Ph(CH_2)_2Br$, Mg, CuI, THF; (b) DEAD, DPPA, PPh_3; (c) CH_3CN, 3 M HCl; (d) MEMCl, NaH, THF.

Scheme 2[a]



12a: R_1 = OPh
18: R_1 = CH_2Ph
24: R_1 = $(CH_2)_2Ph$

13a: R_1 = OPh
19: R_1 = CH_2Ph
25: R_1 = $(CH_2)_2Ph$

14a: R_1 = OPh; R_2 = H
20: R_1 = CH_2Ph; R_2 = H
26: R_1 = $(CH_2)_2Ph$; R_2 = H
27: R_1 = OPh; R_2 = CO_2CH_3

28: R_1 = OPh
30: R_1 = CH_2Ph

29: R_1 = OPh
31: R_1 = CH_2Ph

[a] (a) H_2, Pd/C (10%), EtOAc; (b) CDI, CH_2Cl_2; (c) BnBr or methyl 4-(bromomethyl)benzoate, NaH, DMF; (d) CH_3OH, concentrated HCl; (e) CH_3CN, 3 M HCl; (f) $LiBH_4$, ether; (g) sulfamide, pyridine; (h) BnBr, NaH, DMF; (i) HCl in ether, methanol.

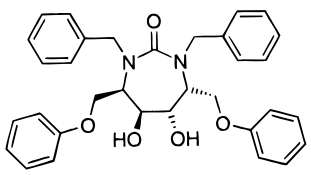
ular dynamics (MD) simulations. This procedure was applied recently to DMP 323 and two linear peptide HIV-1 protease inhibitors.[11]

Several groups have used carbohydrates as chiral precursors for the synthesis of linear HIV-1 protease inhibitors.[12] Here we describe the synthesis of 10 cyclic C_2-symmetric compounds related to DMP 323, which were prepared from the readily available L-mannonic γ-lactone or D-mannitol.[13] For these 10 model compounds, we compare our results from experimental elucidation of their inhibitory potencies against HIV-1 protease to calculated free energies of binding derived from MD simulations. The compounds were selected to enable comparison of (a) the C_2-symmetric diastereomers **1a**−**d**, (b) the ureas **1a**, **2**, and **3** and the sulfamides **4** and **5**, which were modified in the link to the P1 and P1′ phenyl groups, and (c) the compounds **6** and **7**, modified in the P2 and P2′ side chains with groups combining hydrogen bond acceptor and donor capacity, respectively.

Results

Chemistry. The diepoxides **8a,c** were used as key intermediates for the synthesis of **1a, 2**−**7**, and **1c**, respectively, and the diepoxides **8b,d** for the synthesis of **1b,d**, respectively (Figure 3). For the preparation of the diepoxides **8b,d** D-mannitol was used as starting material.[14] We employed this procedure for the preparation of **8a,c** from L-mannonic γ-lactone.[13] The synthetic methods used for the preparation of **1a**−**d** and **2**−**7** are shown in Tables 1−4, and the syntheses of the *RSSR* stereoisomers **1a** and **2**−**7** are outlined in Schemes 1 and 2.

Nucleophilic ring opening of the diepoxides occurs with high regio- and stereoselectivity,[15] and accordingly, ring opening of **8a**, shown in Scheme 1, and **8b** by phenolate smoothly provided **9a,b**[15c] after 7 h. Ring opening of **8c,d** provided the corresponding diastereomers **9c,d** after 24 h reaction times, in yields ranging of 61−72%. Standard Mitsunobu reaction[16] lead to the azides **10a**−**d**, with inverted configuration at carbons



1a

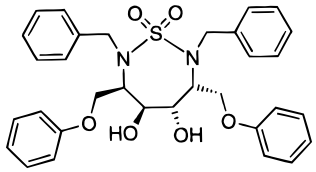


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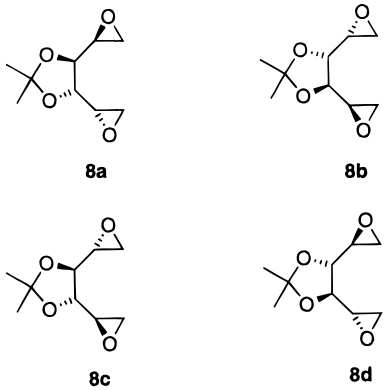
Figure 2. Structures of one urea (**1a**) and one sulfamide (**4**) compound with *RSSR* stereochemistry.



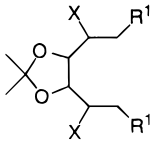
8a **8b** **8c** **8d**

Figure 3. Structures of the bisepoxides **8a**−**d**.

2 and 5. Reduction to the primary amines occurred smoothly, but cyclization was not facile, probably due to ring strain imposed by the *trans*-fused five-membered acetonide ring.[17] Deketalization of the azides furnished **11a**−**d**. Treatment with MEM-chloride[18] afforded the bis-MEM ethers **12a**−**d** in 83−88% isolated yields. Hydrogenation on Pd/C provided the crude amines, which were reacted with carbonyldiimidazole[19] to give the cyclic ureas **13a**−**d** in 55−68% yields from the appropriate azide. Benzylation[20] to give **14a** and the enantiomer **14b** was complete in 12 h, while formation of **14c,d** required 24 h reaction time for full conversion.[21] Deprotection gave the target compounds **1a**−**d** in 6−17% overall yield from **8a**−**d**.

For the preparation of **2**[13] and **3** a similar reaction sequence was adopted. Ring opening of **8a** with benzylmagnesium chloride or phenylethylmagnesium bromide in the presence of CuI[12a] delivered **15** and **21**, respectively. The Mitsunobu reaction followed by deketalization and reprotection with MEM-chloride provided **18** and **24**. After hydrogenation, cyclization to give the ureas **19** and **25** occurred smoothly. Benzylation and subsequent deprotection furnished **2** and **3** in 14−18% overall yield from the epoxide. Alkylation with methyl 4-(bromomethyl)benzoate and deprotection provided the ester **6**, while reduction[22] before the deprotection furnished the hydroxymethyl derivative **7**. Sulfamides **4** and **5**[6b] were prepared by heating the crude amine with sulfamide in refluxing pyridine. This cyclization[23] followed by benzylation using sodium hydride as base in DMF and subsequent deprotection with dry HCl in ether−methanol afforded compounds **4** and **5** in 18% and 16% overall yield from the diepoxide as depicted in Scheme 2.

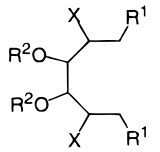
Table 1. Structures and Preparation Methods for Mannitol Derivatives



compd[a]	config	R^1	X	prepn method[b]
9a	*SSSS*	OPh	OH	I
9b	*RRRR*	OPh	OH	I
9c	*RSSR*	OPh	OH	I
9d	*SRRS*	OPh	OH	I
10a	*RSSR*	OPh	N_3	II
10b	*SRRS*	OPh	N_3	II
10c	*SSSS*	OPh	N_3	II
10d	*RRRR*	OPh	N_3	II
15	*SSSS*	CH_2Ph	OH	VIII
16	*RSSR*	CH_2Ph	N_3	II
21	*SSSS*	$(CH_2)_2Ph$	OH	IX
22	*RSSR*	$(CH_2)_2Ph$	N_3	II

[a] For experimental data of compounds **9b**−**d** and **10b**−**d**, see Supporting Information. [b] See the Experimental Section.

Table 2. Structures and Preparation Methods for Mannitol Derivatives

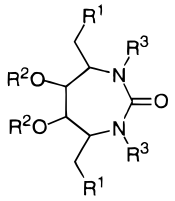


compd[a]	config	R^1	R^2	X	prepn method[b]
11a	*RSSR*	OPh	H	N_3	III
11b	*SRRS*	OPh	H	N_3	III
11c	*SSSS*	OPh	H	N_3	III
11d	*RRRR*	OPh	H	N_3	III
12a	*RSSR*	OPh	MEM	N_3	IV
12b	*SRRS*	OPh	MEM	N_3	IV
12c	*SSSS*	OPh	MEM	N_3	IV
12d	*RRRR*	OPh	MEM	N_3	IV
17	*RSSR*	CH_2Ph	H	N_3	III
18	*RSSR*	CH_2Ph	MEM	N_3	IV
23	*RSSR*	$(CH_2)_2Ph$	H	N_3	III
24	*RSSR*	$(CH_2)_2Ph$	MEM	N_3	IV

[a] For experimental data of compounds **11b**−**d** and **12b**−**d**, see Supporting Information. [b] See the Experimental Section.

Inhibition of HIV-1 Protease. The inhibitory effect of the synthesized compounds was determined with purified HIV-1 protease in a standardized assay.[24] The results are presented as IC_{50}-values, i.e., the concentration of inhibitor resulting in 50% inhibition in this assay (Table 5). Since some compounds were not sufficiently potent to allow determination of IC_{50}-values, results are also given as percent inhibition at 10 μM (Table 5). Compounds that showed significant inhibition were further characterized, and K_i-values were determined (Table 5). The model for tight-binding inhibitors used for analysis[25] was adequate except for the most potent inhibitors, as judged by the correlation between k_{cat}- and K_m-values, obtained when fitting data for different inhibitors and by statistical criteria (not shown). When the K_i-values are lower than the enzyme concentration used, the portion of free inhibitor becomes insignificant, since the enzyme is essentially titrated with inhibitor under these conditions; thus, in such cases the method

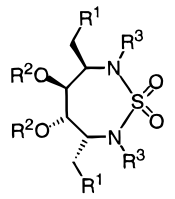
Table 3. Structures and Preparation Methods for Cyclic Urea Compounds



compd[a]	config	R^1	R^2	R^3	prepn method[b]
1a	*RSSR*	OPh	H	CH_2Ph	VII
1b	*SRRS*	OPh	H	CH_2Ph	VII
1c	*SSSS*	OPh	H	CH_2Ph	VII
1d	*RRRR*	OPh	H	CH_2Ph	VII
2	*RSSR*	CH_2Ph	H	CH_2Ph	VII
3	*RSSR*	$(CH_2)_2Ph$	H	CH_2Ph	VII
6	*RSSR*	OPh	H	CH_2-4-$(CO_2CH_3)Ph$	X
7	*RSSR*	OPh	H	CH_2-4-$(CH_2OH)Ph$	XI
13a	*RSSR*	OPh	MEM	H	V
13b	*SRRS*	OPh	MEM	H	V
13c	*SSSS*	OPh	MEM	H	V
13d	*RRRR*	OPh	MEM	H	V
14a	*RSSR*	OPh	H	CH_2Ph	VI
14b	*SRRS*	OPh	MEM	CH_2Ph	VI
14c	*SSSS*	OPh	MEM	CH_2Ph	VI
14d	*RRRR*	OPh	MEM	CH_2Ph	VI
19[c]	*RSSR*	CH_2Ph	MEM	H	V
20	*RSSR*	CH_2Ph	MEM	CH_2Ph	VI
25[c]	*RSSR*	$(CH_2)_2Ph$	MEM	H	V
26	*RSSR*	$(CH_2)_2Ph$	MEM	CH_2Ph	VI
27[d]	*RSSR*	OPh	MEM	CH_2-4-$(CO_2CH_3)Ph$	VI

[a] For experimental data of compounds **13b**−**d** and **14b**−**d**, see Supporting Information. [b] See the Experimental Section. [c] 2 days reaction time. [d] Methyl 4-(bromomethyl)benzoate was used instead of BnBr.

Table 4. Structures and Preparation Methods for Cyclic Sulfamide Compounds



compd	config	R^1	R^2	R^3	prepn method[a]
4	*RSSR*	OPh	H	CH_2Ph	XIV
5	*RSSR*	CH_2Ph	H	CH_2Ph	XIV
28	*RSSR*	OPh	MEM	H	XII
29	*RSSR*	OPh	MEM	CH_2Ph	XIII
30	*RSSR*	CH_2Ph	MEM	H	XII
31	*RSSR*	CH_2Ph	MEM	CH_2Ph	XIII

[a] See the Experimental Section.

cannot be used. The standard errors for determination of the parameters were ≤10%.

The stereochemistry of the compounds was found to be critical for their inhibitory effect. Only the *RSSR* isomer **1a** inhibits the enzyme significantly, K_i = 12.2 nM. The other isomers (**1b**−**d**) showed little (<15%) or no inhibitory effect even at micromolar concentrations. Small structural modifications of compound **1a** gave significant changes in the inhibitory potency. Changing the P1/P1′ side chains from phenoxymethyl (**1a**) to phenylethyl (**2**) resulted in a 20-fold reduction of the K_i-value. A 50-fold loss of inhibitory effect relative to **1a** was found when the P1/P1′ side chain was further extended to give the phenylpropyl derivative **3**. The cyclic sulfamides **4** and **5** both showed inhibitory potencies of the same order of magnitude as **1a**. Interestingly, exchanging the P1/P1′ ether oxygens for methylene groups in the sulfamides gave essentially equipotent derivatives (**4** vs **5**), contrary to the observation with the ureas (**1a** vs **2**). Finally, both of the *p*-hydroxymethyl compounds (**7** and DMP 323) exhibited high potencies, while compound **6** exhibited lower binding affinity than **1a**.

Dynamics Simulations and Free Energy Calculations. Binding free energies were estimated by an approximation described elsewhere,[10] which we will refer to here as the linear interaction energy (LIE) method. Two MD simulations were performed for each inhibitor, one for the inhibitor free in water and the other for the inhibitor bound inside the solvated protein. The simulation results are summarized in Table 6 in terms of the average electrostatic (polar) and van der Waals (nonpolar) ligand interaction energies for the bound and unbound states. Hydrogen bonds are included implicitly in these two quantities and are mainly reflected in the electrostatic energy. Table 6 also gives the resulting free energies of binding obtained with the LIE approximation. The predicted absolute binding free energies are in reasonable agreement with the experimental values (Table 5) but are generally ca. 2 kcal/mol too negative. The simulations identify **1a** as a good inhibitor and give the correct ranking of the compounds **1a**−**d**. In particular, the two compounds **1c,d** are found to bind poorly, while the binding energy of **1b** is somewhat overestimated. The water simulations of the enantiomeric pairs converged as expected to give very similar values within each pair. The average of the two simulations is given in Table 6 for each pair. For the compounds **2** and **3**, with the ether oxygens replaced by methylene and ethylene groups, **2** is predicted to be

Table 5. Inhibitory Activity of Cyclic C_2-Symmetric Urea and Sulfamide Compounds against HIV-1 Protease

				K_i (nM)		ΔG_{bind} (kcal/mol)	
compd	config	inhibition (%) (at 10 μM)	IC_{50} (μM)	expt[a]	calcd	expt	calcd
DMP 323	*RSSR*	nd	0.015	<1[b]	0.083	<−12.8	−14.3 ± 1.0
1a	*RSSR*	nd	0.08	12.2	1.1	−11.2	−12.7 ± 0.5
1b	*SRRS*	15	nd	nd	15		−11.1 ± 0.7
1c	*SSSS*	10	nd	nd	3200		−7.8 ± 0.9
1d	*RRRR*	0	nd	nd	36000		−6.3 ± 0.5
2	*RSSR*	nd	4.0	214	7.8	−9.5	−11.5 ± 1.4
3	*RSSR*	nd	10.0	570	0.80	−8.9	−12.9 ± 0.7
4	*RSSR*	nd	0.200	19.1	0.80	−10.9	−12.9 ± 1.7
5	*RSSR*	nd	0.200	14.7	0.94	−11.1	−12.8 ± 0.5
6	*RSSR*	nd	8.0	nd	9.2		−11.4 ± 0.9
7	*RSSR*	nd	0.010	<1[b]	0.051	<−12.8	−14.6 ± 1.6

[a] The standard error was less than 10%. [b] K_i(DMP 323) = 0.27 nM and K_i(**7**) = 0.23 nM have been determined by a different method (Nillroth et al., to be published). nd = not determined.

Table 6. Dynamics Averages of Nonbonded Terms in the Simulations[a]

compd	$\langle V_{vdw}\rangle_{prot}$	$\langle V_{vdw}\rangle_{wat}$	$\langle V_{el}\rangle_{prot}$	$\langle V_{el}\rangle_{wat}$	ΔG_{bind} vdw	ΔG_{bind} el	ΔG_{bind} calcd
DMP 323	−86.57 ± 1.17	−42.07 ± 0.19	−82.22 ± 1.08	−67.89 ± 0.48	−7.16 ± 0.2	−7.16 ± 0.8	−14.3 ± 1.0
1a	−85.76 ± 0.17	−46.66 ± 0.88	−50.99 ± 0.10	−38.19 ± 0.44	−6.30 ± 0.2	−6.40 ± 0.3	−12.7 ± 0.5
1b	−90.61 ± 1.08	−46.66 ± 0.88	−46.31 ± 0.20	−38.19 ± 0.44	−7.08 ± 0.3	−4.06 ± 0.4	−11.1 ± 0.7
1c	−88.30 ± 0.70	−46.63 ± 0.12	−41.42 ± 1.30	−39.32 ± 0.12	−6.71 ± 0.2	−1.05 ± 0.7	−7.8 ± 0.9
1d	−86.79 ± 0.52	−46.63 ± 0.12	−38.99 ± 0.65	−39.32 ± 0.12	−6.47 ± 0.1	+0.16 ± 0.4	−6.3 ± 0.5
2	−86.32 ± 0.21	−48.42 ± 0.25	−45.17 ± 1.66	−34.29 ± 0.97	−6.10 ± 0.1	−5.44 ± 1.3	−11.5 ± 1.4
3	−90.37 ± 0.23	−52.59 ± 0.44	−50.57 ± 0.21	−37.00 ± 0.96	−6.08 ± 0.1	−6.78 ± 0.6	−12.9 ± 0.7
4	−88.85 ± 0.03	−50.91 ± 0.09	−49.70 ± 1.91	−36.09 ± 1.31	−6.11 ± 0.1	−6.80 ± 1.6	−12.9 ± 1.7
5	−88.42 ± 0.04	−49.11 ± 0.04	−48.97 ± 0.22	−36.01 ± 0.57	−6.33 ± 0.1	−6.48 ± 0.4	−12.8 ± 0.5
6	−103.61 ± 0.10	−53.65 ± 1.39	−57.38 ± 0.63	−50.68 ± 0.51	−8.04 ± 0.3	−3.35 ± 0.6	−11.4 ± 0.9
7	−90.86 ± 0.43	−44.13 ± 0.53	−84.98 ± 1.33	−70.78 ± 1.34	−7.52 ± 0.2	−7.10 ± 1.4	−14.6 ± 1.6

[a] Values are averages over 125 ps (250 ps for DMP 323 and **7**). The error estimates are one-half the differences between the first and last 62.5 ps (or 125 ps) and thus measure precision rather than accuracy. All values are given in kcal/mol. The contributions from the two terms in the binding free energy approximation are also shown.

less active than **1a**, in agreement with the binding experiments. However, the calculated binding energy for **3** is about the same as for **1a**, which is a significant overestimation compared to experimental values. Calculations on the sulfamides **4** and **5** yielded binding free energies close to those obtained for **1a**, in agreement with experimental findings. The ester **6** was predicted to be similarly potent to **2**, which is indeed also indicated by the experimentally observed IC_{50}-value. The calculations predict essentially equal, large negative, binding energies for DMP 323 and **7**, pointing to a negligible effect of the added oxygen. There are, however, significant uncertainties in this prediction, since we find that a major part of the interaction energy of these two inhibitors arises from interactions of the hydroxymethyl groups with aspartates 30/230, which are mobile and in contact with bulk water, leading to large variations during simulation time. For these two inhibitors, data collection times for the protein simulations were therefore doubled. The free energy of binding obtained for DMP 323 is more negative than has been reported earlier[11] due to the interaction with Asp 30/230, since the aspartate side chains need to move (χ_1 rotation) from their crystallographic starting positions for this interaction to occur, a conformational change that was neither anticipated nor observed in the calculations on DMP 323.[11] As noted above, exact experimental values of the inhibition constants could not be obtained for the potent inhibitors **7** and DMP 323 (K_i of 0.27 nM has been reported for DMP 323).[6]

The MD average structures from the calculations give a picture of binding arrangements over some tens of picoseconds. On this time scale, all inhibitors were found to bind somewhat asymmetrically in the binding pocket, especially **1a,b**. The reason for this asymmetry of binding was the protonation state chosen for the catalytic aspartates. Asp 25 was considered to be negatively charged, Asp 225 was protonated,[26] and both hydroxyls of the inhibitor were found to point toward Asp 25, tilting the seven-membered ring slightly. Strong hydrogen bonds can form between diequatorial hydroxyl groups and the charged aspartate. Only one of the diaxial hydroxyl groups found in relaxed conformations of the free compounds **1c,d** would be able to hydrogen bond to Asp 25. A strong charge−dipole interaction would then be replaced by a weaker dipole−dipole interaction with Asp 225, leading to weaker binding. For **1c**, the calculations indeed predict a conformational change in the seven-membered cyclic urea ring, yielding diequatorial hydroxyls, but this seems to be at the expense of other favorable interactions and still results in poor binding. The average structures for the *RSSR* inhibitors are summarized in Figure 4, which summarizes the average hydrogen-bonding arrangement in the vicinity of the inhibitor on the time scale of the simulations.

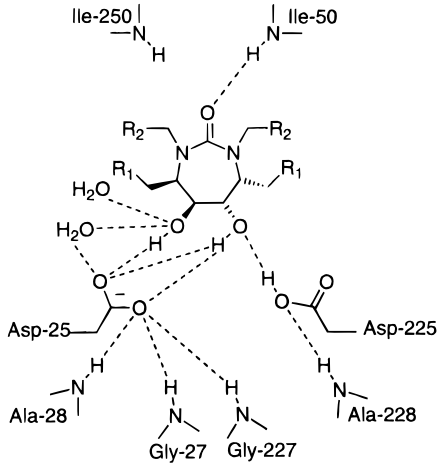


Figure 4. Schematic picture showing the general hydrogen-bonding arrangements in the vicinity of the inhibitors with *RSSR* stereochemistry, on the tens-of-picoseconds time scale of the simulations. Distances in the figure are not to scale, and all water molecules depicted are in contact with bulk water. Dashed lines indicate that atom distances were on average favorable for hydrogen bond formation.

Discussion

High binding affinity is attained by both steric and electrostatic complementarity between the ligand and its target. Imperfect steric fit generally can be relaxed by small adjustments in the protein. Electrostatic complementarity, which involves both polar interactions (e.g., hydrogen bonds) and nonpolar interactions (e.g., hydrophobic effects), usually cannot be adjusted easily due to the long-range nature of electrostatic fields and is therefore a major determinant of binding specificity. These effects are apparent in the results for the stereoisomers **1a**−**d**. For all four compounds, shape complementarity alone provides a basic level of affinity, as demonstrated by the van der Waals term of the MD simulation results, but the exact polar−hydrophobic matching within this shape results in the observed (Table 5) large differences, leading to high affinity in

some cases (in the nanomolar range for **1a**). The present results indicate that the stereochemistry of the compounds is critical for their inhibitory potency. It is especially important for the hydroxyl groups to be positioned optimally, and the *RSSR* stereoconfiguration of **1a** enables favorable hydrogen bonds to the charged aspartate.

The MD calculations suggest the following explanation for the 20-fold enhancement of binding affinity of **1a** as compared **2**: A dipole is formed by each partially negative ether oxygen and its two partially positive neighboring carbons, since the angle between these three atoms is less than 180°. Analysis of the average structure from the MD simulations shows that for **1a**, these dipoles are aligned to an electrostatic field arising from the protein environment, with the major contribution from the charged Asp 25 but also with significant contributions from the flap peptide bonds from residues 49−50 and 249−250.

The cyclic sulfamides **4** and **5** were synthesized with *RSSR* stereochemistry since this configuration was found to be optimal for the urea compounds. The binding energies measured for the sulfamide compounds **4** and **5** are both approximately equal to that found for **1a**, in agreement with the result derived from the MD simulations. One might expect the same electrostatic effects in **4** and **5** as in **1a** and **2**, but the average structures of **4** and **5** predict that there is not enough space available for the ether oxygens to be aligned to the field, due to the extra volume from the sulfonyl group compared to the carbonyl group. Thus, no decrease in affinity results from the substitution of the ether oxygen in compound **4** with the methylene group in compound **5**.

Compounds **6** and **7** were synthesized in order to study hydrogen-bonding capacity in the S2/S2′ cavity. As expected, **7** exhibited high binding affinity in analogy to DMP 323. This is probably due to the ability of **7** to interact with the negatively charged aspartates 30/230, as discussed above. The simulations point to a large net loss of electrostatic binding energy of **6** compared to **1a**. This illustrates a general principle of ligand binding energetics, namely, that a comparison always must be made to the unbound reference state, where the ligand is free in water. Therefore, not only must the addition of a group to the ligand lead to favorable interactions in the bound state, but the interactions with the receptor must be stronger than the interactions of the same added group with surrounding water molecules of the unbound state, or no net increase in binding energy will be achieved (Table 6).

One interesting structural feature emerging from the simulations is that the hydrogen bonds between the inhibitor and the flaps seem not to be as strong as possible, as reflected by the fact that only one strong hydrogen bond to the NH groups 50/250 is observed in the tens-of-picoseconds average structure (Figure 4). This could indicate that the cyclic urea carbonyl group might not be the optimal mimic of the crystallographically found water molecule, but this could also be due to imperfections in the representation of the groups involved in the force field used in this work. Moreover it is interesting to note that water molecules (in contact with bulk water) can interact with the cylic urea ring hydroxyls even when the inhibitor is bound to the protein.

The asymmetric protonation state[26] of the catalytic Asp 25/225 used in the present simulations seems to explain the asymmetric binding mode of the inhibitors on the short time scales of the simulations, depicted in Figure 4. However, even if this asymmetry is correct on the short time scale of the simulations, it need not be observable necessarily in crystallographic studies, since these involve implicit averaging over many protein dimers that need not be isomorphous with respect to the aspartate protonation throughout the crystal. Instead, such experiments may reflect the average of Figure 4 and its symmetry-related counterpart, in addition to any contributions from other protonation states, depending on the pH of the crystal.

Conclusion

Synthesis of these inhibitors from readily available L-mannonic γ-lactone or D-mannitol is relatively straightforward, and the ether oxygens, easily incorporated by the synthetic methodology employed, in fact seem to enhance binding 20-fold as compared to the corresponding methylene analog (**2**) in the cyclic urea series. Furthermore, the methylene analog in the sulfamide series (**5**) is 15-fold more potent than the corresponding cyclic urea derivative, but steric effects seem to preclude addition of these two enhancement factors.

The predictive power of the LIE method for estimating binding affinities for the ligands from MD simulations has been demonstrated. The enzymatic measurements give the following ranking of inhibitors (in decreasing potency) DMP 323, **7** > **1a, 4, 5** > **2, 3, 6** > **1b**−**d**, whereas the theoretical predictions are DMP 323, **7** > **1a, 3**−**5** > **1b, 2, 6** > **1c,d**, showing that the relative ordering of the inhibitors is well reproduced by the calculations, with the exception of the large overestimation of the potency of compound **3** and the slight overestimation for compound **1b**.

The reason for the apparently systematic overestimation of the binding strength of the compounds studied here by ca. 2 kcal/mol is not clear. It is, of course, conceivable that our linear response formula with its current parameterization is not accurate enough (although earlier calculations suggested so). Another possibility is that differences between the experimental conditions and the simulation model (e.g., ionic strength) affect the result in a systematic way. A third possibility is that some force field deficiency is responsible for the error. One may, for example, question the non-polar-extended atom representation of phenyl groups in the GROMOS force field[27] in this context. This somewhat oversimplified model could lead to an exaggeration of the hydrophobic effect which may be especially significant here since the compounds studied each have four phenyl groups, in contrast to those considered in earlier applications of the method. Nevertheless, the results obtained here suggest that the LIE method is useful as a tool in design projects where the structure of the molecular target is known.

Experimental Section

Chemistry. General Information. Melting points were recorded on an Electrothermal melting point apparatus and are uncorrected. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. Specific rotations ($[\alpha]_D$) are reported in deg/dm, and the concentration (c) is given in g/100 mL in the specific solvent. ^{1}H and ^{13}C NMR spectra were recorded

on a JEOL JNM-EX 270 spectrometer at 270.2 and 67.8 MHz, respectively, or on a JEOL JNM-EX 400 spectrometer at 399.8 and 100.5 MHz, respectively; the chemical shifts are given in ppm relative to tetramethylsilane. Infrared spectra were recorded on a Perkin-Elmer 1600 series FTIR instrument. Elemental analyses were performed by Mikro Kemi AB, Sweden, or Analytische Laboratorien, Germany, and were within ±0.4% of calculated values. Mass spectroscopy was carried out on a JEOL SX 102 instrument. Flash column chromatography was performed on silica gel 60, 0.04−0.063 mm (E. Merck), with gradient elution unless otherwise noted. Thin-layer chromatography was performed on precoated silica gel F-254 plates (0.25 mm; E. Merck) and visualized with UV light and H_2SO_4 in ethanol, phosphomolybdic acid, or ninhydrin. Standard workup: organic layers were dried with $MgSO_4$ and concentrated *in vacuo*.

3,4-*O*-Isopropylidene-1,2:5,6-dianhydro-D-iditol (8c): mp 69−70 °C; IR (KBr) ν 2994, 1379, 1249, 1180 cm^{-1}; $[\alpha]_D$ = +17.5° (c = 1.20, $CHCl_3$, 24 °C); ^{1}H NMR (270.2 MHz, $CDCl_3$) δ 3.85 (dd, J = 3.1, 1.6 Hz, 2H, C*H*OC), 3.07 (m, 2H, C*H*O), 2.84 (dd, J = 5.1, 4.1 Hz, 2H, CH_2O), 2.74 (dd, J = 5.1, 2.1 Hz, 2H, CH_2O), 1.40 (s, 6H, CH_3); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 110.8, 78.1, 51.2, 43.9, 26.8. Anal. ($C_9H_{14}O_4$) C, H.

3,4-*O*-Isopropylidene-1,6-di-*O*-phenyl-L-mannitol (9a). Method I. To phenol (8.6 g, 91.5 mmol) in THF/toluene (1:3, 500 mL) was added NaH (1.8 g, 60.8 mmol) under N_2 atmosphere; this was stirred for 20 min at 25 °C. The diepoxide **8a** (2.83 g, 15.2 mmol) was added, and the temperature was raised to 95 °C for 7 h. The solution was allowed to cool and washed with 1 M NaOH (2 × 150 mL). The combined aqueous extracts were extracted with ether (100 mL). The combined organic extracts were dried and concentrated. Purification by flash chromatography (CH_2Cl_2 to CH_2Cl_2/CH_3OH, 100:1) gave the product as a white solid (4.09 g, 72%): mp 108−111 °C; IR (CH_2Cl_2) ν 3600−3300, 3050, 2932, 1594, 1492 cm^{-1}; $[\alpha]_D$ = −38.0° (c = 1.03, $CHCl_3$, 25 °C); ^{1}H NMR (270.2 MHz, $CDCl_3$) δ 7.3 (dd, J = 7.5, 6.9 Hz, 4H, OAr*H*[m]), 6.95 (m, 6H, OAr*H*[o + p]), 4.27 (d, J = 7.6 Hz, 2H, C*H*OC), 4.05 (m, 6H, C*H*OH, CH_2OPh), 3.6 (s, 2H, O*H*), 1.39 (s, 6H, CH_3); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 158.5, 129.4, 121.1, 114.7, 109.8, 79.7, 71.7, 69.4, 26.9. Anal. ($C_{21}H_{26}O_6$) C, H.

2,5-Diazido-3,4-*O*-isopropylidene-1,6-di-*O*-phenyl-2,5-dideoxy-D-iditol (10a). Method II. To a solution of compound **9a** (0.98 g, 2.62 mmol) and triphenylphosphine PPh_3 (1.44 g, 5.5 mmol) in THF (10 mL) at −15 °C was added diethyl azodicarboxylate (DEAD) (1.11 g, 5.5 mmol), and this was stirred for 5 min. Diphenyl phosphorazidate (DPPA) (1.51 g, 5.5 mmol) was added, and the reaction mixture was stirred at 25 °C overnight. The solvent was removed, and the crude residue was purified by dry flash chromatography (pentane/CH_2Cl_2, 6:1) followed by flash chromatography (pentane/CH_2Cl_2, 10:1−1:1) to give the product as a white solid (0.72 g, 65%): mp 75−77 °C; IR (CH_2Cl_2) ν 3042, 2936, 2113, 1595, 1492 cm^{-1}; $[\alpha]_D$ = −39.0° (c = 1.09, $CHCl_3$, 25 °C); ^{1}H NMR (270.2 MHz, $CDCl_3$) δ 7.29 (dd, J = 8.6, 7.6 Hz, 4H, OAr*H*[m]), 6.98 (t, J = 7.4 Hz, 2H, OAr*H*[p]), 6.92 (d, J = 8.6 Hz, 4H, OAr*H*[o]), 4.33 (m, 2H, C*H*OC), 4.25 (d, J = 6.3 Hz, 4H, CH_2OPh), 3.75 (m, 2H, C*H*N$_3$), 1.46 (s, 6H, CH_3); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 157.9, 129.6, 121.6, 114.6, 110.9, 76.9, 67.8, 59.5, 26.9. Anal. ($C_{21}H_{24}N_6O_4$) C, H, N.

2,5-Diazido-1,6-di-*O*-phenyl-2,5-dideoxy-D-iditol (11a). Method III. Compound **10a** (2.0 g, 4.71 mmol) was added to a mixture of acetonitrile (60 mL) and 3 M HCl (15 mL). The temperature was raised to 50 °C and maintained for 4 h. The solvent was removed, and the crude product was purified by flash chromatography (CH_2Cl_2 to CH_2Cl_2/CH_3OH, 25:1) to give the product as a white solid (1.45 g, 80%); IR (film) ν 3427, 3064, 2932, 2110, 1595, 1493, 1239 cm^{-1}; $[\alpha]_D$ = −25.1° (c = 1.0, $CHCl_3$, 25 °C); ^{1}H NMR (270.2 MHz, $CDCl_3$) δ 7.29 (dd, J = 7.9, 6.6 Hz, 4H, OAr*H*[m]), 7.0 (t, J = 7.3 Hz, 2H, OAr*H*[p]), 6.92 (d, J = 7.9 Hz, 4H, OAr*H*[o]), 4.29 (m, 4H, CH_2OPh), 3.96 (m, 4H, C*H*OH, C*H*N$_3$), 2.91 (s, 2H, O*H*); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 157.9, 129.6, 121.7, 114.6, 70.9, 68, 62.4; HRMS calcd for $C_{18}H_{20}N_6O_4$ 384.1546, found 384.1544.

2,5-Diazido-3,4-bis-*O*-[(2-methoxyethoxy)methyl]-1,6-di-*O*-phenyl-2,5-dideoxy-d-iditol (12a). Method IV. Compound **11a** (1.39 g, 3.63 mmol) in THF (75 mL) was stirred with NaH (0.43 g, 14.5 mmol) for 15 min. (2-Methoxyethoxy)-methyl chloride (MEMCl) (1.81 g, 14.5 mmol) was added, and the reaction mixture was stirred overnight. The reaction was quenched with aqueous saturated NH_4Cl; the mixture was diluted with water and extracted with ether (2 × 100 mL). The combined ether extracts were dried and concentrated. The crude product was purified by flash chromatography (pentane/CH_2Cl_2, 1:1, to CH_2Cl_2) to give the product as a colorless oil (1.73 g, 85%): IR (film) ν 3041, 2927, 2104, 1599, 1498 cm^{-1}; $[\alpha]_D$ = −29.6° (c = 1.0, $CHCl_3$, 25 °C); ^{1}H NMR (270.2 MHz, $CDCl_3$) δ 7.29 (dd, J = 8.2, 7.6 Hz, 4H, OAr*H*[m]), 6.98 (t, J = 7.2 Hz, 2H, OAr*H*[p]), 6.94 (d, J = 8.2 Hz, 4H, OAr*H*[o]), 4.9 (d, J = 6.9 Hz, 2H, OCH_2O), 4.81 (d, J = 6.9 Hz, 2H, OCH_2O), 4.31 (m, 4H, CH_2OPh), 4.08 (m, 4H, C*H*OCH$_2$, C*H*N$_3$), 3.78 (dt, J = 11.2, 4.3 Hz, 2H, OCH_2CH$_2$O), 3.69 (dt, J = 11.6, 4.6 Hz, 2H, OCH_2CH$_2$O), 3.51 (t, J = 4.5 Hz, 4H, OCH$_2$CH_2O), 3.35 (s, 6H, CH_3); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 158.1, 129.5, 121.4, 114.6, 97.3, 77.6, 71.6, 68.3, 68, 60.6, 59. Anal. ($C_{26}H_{36}N_6O_8$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-5,6-Bis[(2-methoxyethoxy)methoxy]-4,7-bis(phenoxymethyl)-1,3-diazepan-2-one (13a). Method V. To compound **12a** (716 mg, 1.28 mmol) in ethyl acetate (25 mL) was added a catalytic amount of 10% Pd/C. Hydrogen was added to the system at atmospheric pressure, and the reaction mixture was stirred overnight. The suspension was filtered through Celite and concentrated to give a colorless oil. In order to ensure high dilution conditions, the crude amine (595 mg) was dissolved in CH_2Cl_2 (50 mL) and the carbonyldiimidazole (CDI) (230 mg, 1.41 mmol) was dissolved in CH_2Cl_2 (50 mL), respectively; this was slowly added with a syringe pump to a reservoir of CH_2Cl_2 (300 mL), and the reaction mixture was stirred overnight. The solvent was removed, and purification by flash chromatography (CH_2Cl_2 to CH_2Cl_2/CH_3OH, 100:1) gave the product as a white solid (426 mg, 67%): mp 218−220 °C; IR (KBr) ν 3228, 3106, 2936, 1687, 1600, 1498, 1466, 1243 cm^{-1}; $[\alpha]_D$ = +50.1° (c = 1.14, $CHCl_3$, 25 °C); ^{1}H NMR (399.8 MHz, $CDCl_3$) δ 7.28 (dd, J = 8.8, 7.5 Hz, 4H, OAr*H*[m]), 6.99 (t, J = 7.4 Hz, 2H, OAr*H*[p]), 6.95 (d, J = 8.8 Hz, 4H, OAr*H*[o]), 4.86 (d, J = 7.1 Hz, 2H, OCH_2O), 4.79 (d, J = 7.3 Hz, 2H, OCH_2O), 4.73 (br s, 2H, N*H*), 4.11 (app s, 6H, C*H*OCH$_2$, CH_2OPh), 3.97 (br s, 2H, C*H*N), 3.75 (dt, J = 11.3, 4.2 Hz, 2H, OCH_2CH$_2$O), 3.71 (dt, J = 11.5, 4.2 Hz, 2H, OCH_2-CH$_2$O), 3.52 (t, J = 4.5 Hz, 4H, OCH$_2$CH_2O), 3.35 (s, 6H, CH_3); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 164.0, 157.9, 129.5, 121.4, 114.6, 96.3, 74.6, 72.0, 67.8, 67.3, 58.9, 50.5. Anal. ($C_{27}H_{38}N_2O_9$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-1,3-Dibenzyl-5,6-bis[(2-methoxyethoxy)-methoxy]-4,7-bis(phenoxymethyl)-1,3-diazepan-2-one (14a). Method VI. To compound **13a** (150 mg, 0.28 mmol) in DMF (10 mL) were added NaH (80% suspension) (50 mg, 1.68 mmol) and benzyl bromide (287 mg, 1.68 mmol). The reaction mixture was stirred under a N_2 atmosphere overnight. Excess NaH was quenched carefully with ethanol, and the reaction mixture was diluted with water and extracted with ether (2 × 50 mL). The combined ether extracts were washed with brine, dried, and concentrated. Purification by flash chromatography (CH_2Cl_2/CH_3OH, 100:1) gave a colorless oil (180 mg, 89%): IR (film) ν 3062, 2888, 1653, 1599, 1496, 1471 cm^{-1}; $[\alpha]_D$ = −56.2° (c = 1.26, $CHCl_3$, 25 °C); ^{1}H NMR (270.2 MHz, $CDCl_3$) δ 7.25 (m, 14H, Ar*H*), 6.95 (t, J = 7.2 Hz, 2H, OAr*H*[p]), 6.82 (d, J = 7.9 Hz, 4H, OAr*H*[o]), 5.16 (d, J = 14.2 Hz, 2H, CH_2Ph), 4.58 (d, J = 7.0 Hz, 2H, OCH_2O), 4.49 (d, J = 6.9 Hz, 2H, OCH_2O), 4.32 (app t, J = 9.8 Hz, 2H, CH_2OPh), 4.22 (dd, J = 9.9, 2.6 Hz, 2H, CH_2OPh), 4.09 (d, J = 14.1 Hz, 2H, CH_2Ph), 3.89 (br d, J = 9.6, 2H, C*H*N), 3.45−3.3 (m, 10H, C*H*OCH$_2$, OCH_2CH_2O), 3.28 (s, 6H, CH_3); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 161.8, 158.6, 138.5, 129.6 (2 C), 128.9, 127.7, 121.2, 114.9, 96.5, 77.0, 71.7, 67.8, 65.7, 59.9, 59.1, 55.9. Anal. ($C_{41}H_{50}N_2O_9$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-1,3-Dibenzyl-4,7-bis(phenoxymethyl)-5,6-dihydroxy-1,3-diazepan-2-one (1a). Method VII. To a solution of acetonitrile (4 mL) and 3 M HCl (1 mL) was added **14a** (100 mg, 0.14 mmol). The reaction mixture was heated

to 50 °C for 5 h. The solvent was removed, and purification by flash chromatography (CH_2Cl_2 to CH_2Cl_2/CH_3OH, 100:1) gave a white solid (67.2 mg, 89%): mp 218−221 °C; IR (KBr) ν 3600−3300, 3026, 2892, 1586, 1496 cm^{-1}; $[\alpha]_D = -29.5°$ (c = 0.43, DMSO, 25 °C); ^1H NMR (270.2 MHz, DMSO-d_6) δ 7.31 (m, 14H, Ar*H*), 6.96 (t, J = 7.3 Hz, 2H, OAr*H*[p]), 6.86 (d, J = 8.1 Hz, 4H, OAr*H*[o]), 5.36 (br s, 2H, O*H*), 4.96 (d, J = 14.3 Hz, 2H, CH_2Ph), 4.29 (m, 4H, CH_2OPh), 4.00 (d, J = 14.4 Hz, 2H, CH_2Ph), 3.70 (br m, 2H, C*H*N), 3.42 (m, 2H, C*H*OH); ^{13}C NMR (67.8 MHz, DMSO-d_6) δ 161.8, 158.9, 139.4, 130.2, 129.5, 129.2, 127.9, 121.5, 115.3, 70.8, 66.0, 62.2, 56.2. Anal. ($C_{33}H_{34}N_2O_5$) C, H, N.

(4*S*,5*R*,6*R*,7*S*)-1,3-Dibenzyl-4,7-bis(phenoxymethyl)-5,6-dihydroxy-1,3-diazepan-2-one (1b). Compound **1b** was synthesized from **14b** according to method VII in 72% yield: mp 219−223 °C; IR ($CHCl_3$) ν 3585, 3056, 2937, 1645, 1596, 1463, 1449, 1269, 1237 cm^{-1}; $[\alpha]_D = +23.3°$ (c = 0.30, DMSO, 25 °C); ^1H NMR (270.2 MHz, DMSO-d_6) δ 7.3 (m, 14H, Ar*H*), 6.95 (t, J = 7.3 Hz, 2H, OAr*H*[p]), 6.85 (d, J = 8.1 Hz, 4H, OAr*H*[o]), 5.39 (br s, 2H, O*H*), 4.97 (d, J = 14.2 Hz, 2H, CH_2-Ph), 4.3 (m, 4H, CH_2OPh), 4.0 (d, J = 15.0 Hz, 2H, CH_2Ph), 3.75 (br m, 2H, C*H*N), 3.37 (m, 2H, C*H*OH); ^{13}C NMR (67.5 MHz, DMSO-d_6) δ 160.9, 158.0, 138.5, 129.4, 128.7, 128.4, 127.1, 120.7, 114.4, 69.8, 65.0, 61.3, 55.3. Anal. ($C_{33}H_{34}N_2O_5$) C, H, N.

(4*S*,5*S*,6*S*,7*S*)-1,3-Dibenzyl-4,7-bis(phenoxymethyl)-5,6-dihydroxy-1,3-diazepan-2-one (1c). Compound **1c** was synthesized from **14c** according to method VII in 76% yield: IR (KBr) ν 3600−3200, 3030, 2927, 1621, 1598, 1495, 1470, 1241 cm^{-1}; $[\alpha]_D = +47.3°$ (c = 0.69, $CHCl_3$, 25 °C); ^1H NMR (399.8 MHz, $CDCl_3$) δ 7.35 (m, 4H, Ar*H*), 7.28 (m, 10H, Ar*H*), 7.00 (t, J = 7.3 Hz, 2H, OAr*H*[p]), 6.85 (d, J = 8.8 Hz, 4H, OAr*H*[o]), 4.98 (d, J = 14.4 Hz, 2H, CH_2Ph), 4.13 (dd, J = 9.7, 5.3 Hz, 2H, CH_2OPh), 4.08 (dd, J = 9.7, 5.3 Hz, 2H, CH_2OPh), 4.02 (d, J = 14.4 Hz, 2H, CH_2Ph), 3.93 (m, 2H, C*H*OH), 3.42 (m, 2H, C*H*N), 2.62 (br s, 2H, O*H*); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 163.3, 158.2, 137.8, 129.5, 129.2, 128.7, 127.7, 121.4, 114.6, 71.0, 66.9, 63.2, 52.9. Anal. ($C_{33}H_{34}N_2O_5$) C, H, N.

(4*R*,5*R*,6*R*,7*R*)-1,3-Dibenzyl-4,7-bis(phenoxymethyl)-5,6-dihydroxy-1,3-diazepan-2-one (1d). Compound **1d** was synthesized from **14d** according to method VII in 70% yield: IR (KBr) ν 3600−3200, 3030, 2926, 1621, 1598, 1494, 1469 cm^{-1}; $[\alpha]_D = -43.3°$ (c = 0.51, $CHCl_3$, 25 °C); ^1H NMR (399.8 MHz, $CDCl_3$) δ 7.37−7.25 (m, 14H, Ar*H*), 7.00 (t, J = 7.3 Hz, 2H, OAr*H*[p]), 6.85 (d, J = 7.8 Hz, 4H, OAr*H*[o]), 4.99 (d, J = 14.4 Hz, 2H, CH_2Ph), 4.12 (dd, J = 9.6, 5.1 Hz, 2H, CH_2OPh), 4.09 (dd, J = 9.6, 5.1 Hz, 2H, CH_2OPh), 4.03 (d, J = 14.2 Hz, 2H, CH_2Ph), 3.95 (m, 2H, C*H*OH), 3.42 (m, 2H, C*H*N), 2.55 (br s, 2H, O*H*); ^{13}C NMR (67.5 MHz, $CDCl_3$) δ 163.2, 158.2, 137.8, 129.5, 129.3, 128.7, 127.7, 121.4, 114.6, 71.0, 66.9, 63.2, 52.9. Anal. ($C_{33}H_{34}N_2O_5$) C, H, N.

1,6-Dibenzyl-3,4-*O*-isopropylidene-1,6-dideoxy-L-mannitol (15). Method VIII. To a stirred suspension of CuI (6.1 g, 32 mmol) in THF (50 mL) at −20 °C was added benzylmagnesium chloride (32 mL of a 2.0 M solution, 64 mmol). After 1 h at 0 °C, the diepoxide **8a** (2.0 g, 10.75 mmol) was added and the reaction mixture was stirred for 30 min at 0 °C. The reaction was quenched with saturated aqueous NH_4Cl; the mixture was diluted with water (50 mL) and extracted with ether (3 × 150 mL). The combined ether extracts were washed with brine (50 mL), dried, and concentrated. Purification by flash chromatography (CH_2Cl_2 to CH_2Cl_2/CH_3OH, 100:1) gave a white solid (3.47 g, 87%): mp 80−83 °C; IR (KBr) ν 3600−3100, 2922, 1600, 1494, 1453, 1374, 1069 cm^{-1}; $[\alpha]_D = -34.6°$ (c = 0.786, $CHCl_3$, 25 °C); ^1H NMR (270.2 MHz, $CDCl_3$) δ 7.2 (m, 10H, Ar*H*), 3.67 (m, 4H, C*H*OH, C*H*OC), 3.24 (s, 2H, O*H*), 2.87 (ddd, J = 13.5, 10.2, 5.6 Hz, 2H, CH_2Ph), 2.74 (ddd, J = 13.9, 9.6, 6.6 Hz, 2H, CH_2Ph), 2.11 (dddd, J = 13.9, 10.2, 5.9, 4.0 Hz, 2H, CH_2CH$_2$Ph), 1.78 (dddd, J = 13.9, 9.3, 5.3, 5.3 Hz, 2H, CH_2CH$_2$Ph), 1.35 (s, 6H, CH_3); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 141.9, 128.5, 128.4, 125.8, 108.9, 82.9, 72.5, 35.7, 31.3, 26.8. Anal. ($C_{23}H_{30}O_4$) C, H.

2,5-Diazido-1,6-dibenzyl-3,4-*O*-isopropylidene-1,2,5,6-tetradeoxy-D-iditol (16). Compound **16** was synthesized from **15** according to method II in 70% yield: IR (CH_2Cl_2) ν 3073, 2937, 2107, 1603, 1496, 1234 cm^{-1}; $[\alpha]_D = -47.7°$ (c = 1.42, $CHCl_3$, 25 °C); ^1H NMR (399.8 MHz, $CDCl_3$) δ 7.34−7.18 (m, 10H, Ar*H*), 4.08 (app s, 2H, C*H*OC), 2.93 (dd, J = 9.8, 4.4 Hz, 2H, C*H*N$_3$), 2.85 (ddd, J = 13.9, 9.1, 5.4 Hz, 2H, CH_2Ph), 2.73 (ddd, J = 13.9, 8.8, 7.3 Hz, 2H, CH_2Ph), 2.14 (dddd, J = 14.4, 9.0, 9.0, 5.4 Hz, 2H, CH_2CH$_2$Ph), 1.93 (dddd, J = 14.2, 9.3, 7.3, 4.6 Hz, 2H, CH_2CH$_2$Ph), 1.48 (s, 6H, CH_3); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 140.4, 128.6, 128.4, 126.2, 110.4, 79.7, 59.3, 32.4, 26.8. Anal. ($C_{23}H_{28}N_6O_2$) C, H, N.

2,5-Diazido-1,6-dibenzyl-1,2,5,6-tetradeoxy-D-iditol(17). Compound **17** was synthesized from **16** according to method III in 86% yield: mp 72−73 °C; IR (KBr) ν 3537, 3500−3200, 3030, 2941, 2103, 1602, 1495, 1454, 1280 cm^{-1}; $[\alpha]_D = +6.3°$ (c = 1.09, $CHCl_3$, 25 °C); ^1H NMR (270.2 MHz, $CDCl_3$) δ 7.33−7.25 (m, 4H, Ar*H*), 7.23−7.15 (m, 6H, Ar*H*), 3.61 (dt, J = 7.2, 4.0 Hz, 2H, C*H*OH), 3.29 (ddd, J = 9.5, 5.6, 3.9 Hz, 2H, C*H*N$_3$), 2.79 (ddd, J = 13.9, 11.2, 6.6 Hz, 2H, CH_2Ph), 2.72 (ddd, J = 13.9, 11.9, 7.6 Hz, 2H, CH_2Ph), 2.56 (d, J = 5.6 Hz, 2H, O*H*), 1.95 (m, 4H, CH_2CH$_2$Ph); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 140.5, 128.6, 128.3, 126.2, 73.4, 62.9, 32.1, 32.0. Anal. ($C_{20}H_{24}N_6O_2$) C, H, N.

2,5-Diazido-1,6-dibenzyl-3,4-bis-*O*-[(2-methoxyethoxy)methyl]-1,2,5,6-tetradeoxy-D-iditol (18). Compound **18** was synthesized from **17** according to method IV in 70% yield: IR (film) ν 3026, 2926, 2103, 1603, 1496, 1453 cm^{-1}; $[\alpha]_D = -47.8°$ (c = 1.32, $CHCl_3$, 25 °C); ^1H NMR (399.8 MHz, $CDCl_3$) δ 7.3 (m, 4H, Ar*H*), 7.21 (m, 6H, Ar*H*), 4.90 (d, J = 7.1 Hz, 2H, OCH_2O), 4.81 (d, J = 6.8 Hz, 2H, OCH_2O), 3.82 (m, 2H, C*H*OCH$_2$), 3.76 (ddd, J = 11.0, 5.1, 3.6 Hz, 2H, OCH_2CH$_2$O), 3.64 (ddd, J = 10.7, 5.9, 3.7 Hz, 2H, OCH_2CH$_2$O), 3.46 (m, 4H, OCH$_2$CH_2O), 3.33 (s, 6H, CH_3), 3.18 (m, 2H, C*H*N$_3$), 2.80 (ddd, J = 13.9, 9.3, 5.6 Hz, 2H, CH_2Ph), 2.71 (ddd, J = 13.7, 9.3, 6.8 Hz, 2H, CH_2Ph), 2.1−1.93 (m, 4H, CH_2CH$_2$Ph); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 140.7, 128.5, 128.4, 126.1, 97.3, 81.2, 71.5, 68.0, 60.7, 58.9, 32.4, 32.3. Anal. ($C_{28}H_{40}N_6O_6$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-5,6-Bis[(2-methoxyethoxy)methyl]-4,7-bis-(2-phenylethyl)-1,3-diazepan-2-one (19). Compound **19** was synthesized from **18** according to method V in 66% yield: mp 147−150 °C; IR (KBr) ν 3264, 3025, 2926, 1677, 1454, 1361 cm^{-1}; $[\alpha]_D = +30.9°$ (c = 1.25, $CHCl_3$, 25 °C); ^1H NMR (270.2 MHz, $CDCl_3$) δ 7.26 (m, 4H, Ar*H*), 7.16 (m, 6H, Ar*H*), 4.76 (d, J = 6.9 Hz, 2H, OCH_2O) 4.67 (d, J = 7.3 Hz, 2H, OCH_2O), 4.17 (br s, 2H, N*H*), 3.66 (br s, 2H, C*H*OCH$_2$), 3.53 (m, 6H, C*H*N, OCH_2CH$_2$O), 3.42 (m, 4H, OCH$_2$CH_2O), 3.31 (s, 6H, CH_3), 2.72 (m, 4H, CH_2Ph), 1.87 (m, 4H, CH_2CH$_2$Ph); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 163.7, 140.6, 128.6, 128.4, 126.2, 96.3, 77.2, 71.5, 67.6, 59.0, 51.1, 33.9, 32.4. Anal. ($C_{29}H_{42}N_2O_7$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-1,3-Dibenzyl-5,6-bis[(2-methoxyethoxy)-methoxy]-4,7-bis(2-phenylethyl)-1,3-diazepan-2-one (20). Compound **20** was synthesized from **19** according to method VI in 86% yield: IR (film) ν 3061, 2926, 1641, 1495, 1453, 1357 cm^{-1}; $[\alpha]_D = -60.9°$ (c = 1.17, $CHCl_3$, 25 °C); ^1H NMR (270.2 MHz, $CDCl_3$) δ 7.22 (m, 16H, Ar*H*), 7.07 (m, 4H, Ar*H*), 5.09 (d, J = 14.2 Hz, 2H, NCH_2Ph), 4.50 (d, J = 6.9 Hz, 2H, OCH_2O), 4.41 (d, J = 6.6 Hz, 2H, OCH_2O), 3.92 (d, J = 13.9 Hz, 2H, NCH_2Ph), 3.39 (m, 12H, C*H*OCH$_2$, C*H*N, OCH_2CH_2O), 3.32 (s, 6H, CH_3), 2.68 (ddd, J = 14.2, 9.2, 5.9 Hz, 2H, CH$_2$CH_2-Ph), 2.46 (dt, J = 14.2, 8.2 Hz, 2H, CH$_2$CH_2Ph), 1.96 (m, 4H, CH_2CH$_2$Ph); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 126.3, 141.5, 138.1, 129.5, 128.6, 128.4, 128.3, 127.5, 125.9, 96.2, 77.1, 71.6, 67.1, 60.3, 59.0, 56.0, 33.0, 28.6. Anal. ($C_{43}H_{54}N_2O_7$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-1,3-Dibenzyl-4,7-bis(2-phenylethyl)-5,6-dihydroxy-1,3-diazepan-2-one (2).[13] Compound **2** was synthesized from **20** according to method VII in 91% yield: mp 210−212 °C; IR (KBr) ν 3600−3100, 3026, 2910, 1579, 1483, 1451, 1233 cm^{-1}; $[\alpha]_D = -22.6°$ (c = 1.17, $CHCl_3$, 23 °C); ^1H NMR (399.8 MHz, $CDCl_3$) δ 7.33−7.26 (m, 4H, Ar*H*), 7.25−7.17 (m, 12H, Ar*H*), 7.13 (m, 4H, Ar*H*), 5.13 (d, J = 14.1 Hz, 2H, NCH_2Ph), 3.91 (d, J = 14.2 Hz, 2H, NCH_2Ph), 3.48 (s, 2H, C*H*OH), 3.35 (m, 2H, C*H*N), 2.78 (ddd, J = 13.2, 9.3, 6.1 Hz, 2H, CH$_2$CH_2Ph), 2.56 (ddd, J = 14.0, 9.8, 7.1 Hz, 2H, CH$_2$CH_2-Ph), 2.18 (s, 2H, O*H*), 2.10−1.96 (m, 4H, CH_2CH$_2$Ph); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 162.4, 141.3, 137.9, 129.3, 128.5, 128.3, 128.2, 127.5, 125.9, 71.1, 60.7, 55.7, 33.1, 28.1. Anal. ($C_{35}H_{38}N_2O_3$) C, H, N.

1,6-Bis(2-phenylethyl)-3,4-*O*-isopropylidene-1,6-dideoxy-L-mannitol (21). Method IX. To Mg (763 mg, 31.8 mmol), ether (10 mL), and a small amount of iodine was added phenylethyl bromide (5.88 g, 31.8) under mild reflux. The phenylethylmagnesium bromide was added to a suspension of CuI (3.07 g, 16.1 mmol) in dry THF (35 mL) at −40 °C. The temperature was slowly raised to −20 °C over 60 min and then lowered to −40 °C followed by addition of the diepoxide **8a** (1.0 g, 5.3 mmol). The temperature was once again slowly raised to −20 °C. The reaction was quenched with saturated aqueous NH_4Cl; the mixture was diluted with water (40 mL) and extracted with ether (3 × 150 mL). The combined ether extracts were washed with brine (40 mL), dried, and concentrated. Purification by flash chromatography (isohexane/ethyl acetate, 10:1−6:1) gave a white solid (1.37 g, 64%): IR ($CHCl_3$) ν 3600−3200, 3026, 2860, 1603, 1496, 1453 cm^{-1}; $[\alpha]_D = -26.9°$ ($c = 0.81$, $CHCl_3$, 25 °C); ^1H NMR (270.2 MHz, $CDCl_3$) δ 7.3−7.1 (m, 10H, Ar*H*), 3.7−3.5 (m, 4H, C*H*OC, C*H*OH), 3.28 (br s, 2H, O*H*), 2.7−2.5 (m, 4H, CH_2CH_2Ph), 1.9−1.4 (m, 8H, $CH_2CH_2CH_2Ph$), 1.34 (s, 6H, CH_3); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 142.3, 128.3, 128.2, 125.7, 108.7, 83.0, 72.9, 35.8, 33.8, 26.8. Anal. ($C_{25}H_{34}O_4$) C, H.

2,5-Diazido-1,6-bis(2-phenylethyl)-3,4-*O*-isopropylidene-1,2,5,6-tetradeoxy-D-iditol (22). Compound **22** was synthesized from **21** according to method II in 67% yield: IR ($CHCl_3$) ν 3050, 2974, 2111, 1602, 1496, 1453 cm^{-1}; $[\alpha]_D = -46.2°$ (c = 1.05, $CHCl_3$, 25 °C); ^1H NMR (270.2 MHz, $CDCl_3$) δ 7.28 (m, 4H, Ar*H*), 7.18 (m, 6H, Ar*H*), 3.99 (m, 2H, C*H*OC), 2.93 (m, 2H, C*H*N_3), 2.66 (t, J = 7.2 Hz, 4H, CH_2CH_2Ph), 1.93−1.55 (m, 8H, $CH_2CH_2CH_2Ph$), 1.44 (s, 6H, CH_3); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 141.5, 128.4, 128.3, 126.0, 110.3, 79.8, 60.5, 35.4, 30.2, 28.0, 26.8. Anal. ($C_{25}H_{32}N_6O_2$) C, H, N.

2,5-Diazido-1,6-bis(2-phenylethyl)-1,2,5,6-tetradeoxy-D-iditol (23). Compound **23** was synthesized from **22** according to method III in 91% yield: mp 130−131 °C; $[\alpha]_D = +4.7°$ (c = 0.51, DMSO, 25 °C); IR (KBr) ν 3600−3200, 3026, 2936, 2110, 1495 cm^{-1}; ^1H NMR (399.8 MHz, DMSO-d_6) δ 7.28 (m, 4H, Ar*H*), 7.17 (m, 6H, Ar*H*), 5.29 (d, J = 7.8 Hz, 2H, O*H*), 3.45 (m, 2H, C*H*OH), 3.32 (m, 2H, C*H*N_3), 2.58 (m, 4H, CH_2CH_2Ph), 1.74−1.42 (m, 8H, $CH_2CH_2CH_2Ph$); ^{13}C NMR (67.8 MHz, DMSO-d_6) δ 141.8, 128.2, 128.1, 125.6, 73.3, 62.5, 34.8, 29.2, 27.6. Anal. ($C_{22}H_{28}N_6O_2$) C, H, N.

2,5-Diazido-3,4-*O*-bis[(2-methoxyethoxy)methyl]-1,6-bis(2-phenylethyl)-1,2,5,6-tetradeoxy-D-iditol (24). Compound **24** was synthesized from **23** according to method IV in 78% yield: IR (film) ν 3026, 2929, 2107, 1603, 1496, 1454 cm^{-1}; $[\alpha]_D = -39.2°$ (c = 0.63, $CHCl_3$, 25 °C); ^1H NMR (270.2 MHz, $CDCl_3$) δ 7.3−7.1 (m, 10H, Ar*H*), 4.85 (d, J = 6.9 Hz, 2H, OCH_2O), 4.75 (d, J = 6.9 Hz, 2H, OCH_2O), 3.72 (m, 4H, OCH_2CH_2O), 3.61 (m, 2H, C*H*OCH_2), 3.48 (m, 4H, OCH_2CH_2O), 3.35 (s, 6H, CH_3), 3.17 (m, 2H, C*H*N_3), 2.65 (m, 4H, CH_2CH_2Ph), 1.72 (m, 8H, $CH_2CH_2CH_2Ph$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 141.5, 128.3, 128.2, 125.8, 97.3, 81.1, 71.5, 68.0, 61.3, 58.9, 35.4, 30.0, 27.9. Anal. ($C_{30}H_{44}N_6O_6$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-5,6-Bis[(2-methoxyethoxy)methoxy]-4,7-bis(3-phenylpropyl)-1,3-diazepan-2-one (25). Compound **25** was synthesized from **24** according to method V in 78% yield: IR ($CHCl_3$) ν 3421, 3042, 2934, 1672, 1453 cm^{-1}; $[\alpha]_D = +18.6°$ (c = 1.09, $CHCl_3$, 25 °C); ^1H NMR (270.2 MHz, $CDCl_3$) δ 7.25 (m, 4H, Ar*H*), 7.15 (m, 6H, Ar*H*), 4.77 (d, J = 7.6 Hz, 2H, OCH_2O), 4.68 (d, J = 7.6 Hz, 2H, OCH_2O), 4.03 (br s, 2H, N*H*), 3.62 (m, 6H, C*H*OCH_2, OCH_2CH_2O), 3.5 (br t, J = 6.5 Hz, 2H, C*H*NH), 3.43 (m, 4H, OCH_2CH_2O), 3.31 (s, 6H, CH_3), 2.63 (t, J = 6.9 Hz, 4H, CH_2CH_2Ph), 1.82−1.50 (m, 8H, $CH_2CH_2CH_2Ph$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 164.0, 141.4, 128.4, 128.3, 126.0, 96.1, 76.6, 71.5, 67.7, 59.0, 51.5, 35.4, 32.0, 28.0. Anal. ($C_{31}H_{46}N_2O_7$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-1,3-Dibenzyl-5,6-bis[(2-methoxyethoxy)-methoxy]-4,7-bis(3-phenylpropyl)-1,3-diazepan-2-one (26). Compound **26** was synthesized from **25** according to method VI in 78% yield: IR (film) ν 2927, 1641, 1495, 1453, 1110, 1035 cm^{-1}; $[\alpha]_D = -55.7°$ (c = 0.67, $CHCl_3$, 25 °C); ^1H NMR (270.2 MHz, $CDCl_3$) δ 7.25 (m, 14H, Ar*H*), 7.12 (m, 6H, Ar*H*), 4.96 (d, J = 13.9 Hz, 2H, NCH_2Ph), 4.50 (d, J = 6.9 Hz, 2H, OCH_2O), 4.42 (d, J = 6.9 Hz, 2H, OCH_2O), 3.83 (d, J = 14.2 Hz, 2H, NCH_2Ph), 3.37 (m, 12H, C*H*OCH_2, C*H*N, OCH_2CH_2O), 2.42 (m, 4H, CH_2CH_2Ph), 1.80−1.33 (m, 8H, $CH_2CH_2CH_2Ph$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 162.0, 141.9, 138.3, 129.6, 128.5, 128.3, 128.2, 127.4, 125.8, 96.3, 76.5, 71.6, 67.0, 61.3, 58.9, 56.0, 35.8, 28.9, 26.9. Anal. ($C_{45}H_{58}N_2O_7$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-1,3-Dibenzyl-4,7-bis(3-phenylpropyl)-5,6-dihydroxy-1,3-diazepan-2-one (3). Compound **3** was synthesized from **26** according to method VII in 74% yield: IR (film) ν 3600−3200, 3026, 2923, 1602, 1495, 1475, 1453 cm^{-1}; $[\alpha]_D = -3.8°$ (c = 0.476, $CHCl_3$, 25 °C); ^1H NMR (270.2 MHz, $CDCl_3$) δ 7.25 (m, 10H, Ar*H*), 7.15 (m, 10H, Ar*H*), 5.01 (d, J = 14.2 Hz, 2H, NCH_2Ph), 3.82 (d, J = 14.2 Hz, 2H, NCH_2Ph), 3.39 (br s, 2H, C*H*OH), 3.24 (br d, J = 9.9 Hz, 2H, C*H*N), 2.49 (m, 4H, CH_2CH_2Ph), 1.95 (br s, 2H, O*H*), 1.85−1.42 (m, 8H, $CH_2CH_2CH_2Ph$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 162.2, 141.8, 138.1, 129.5, 128.6, 128.4, 128.3, 127.6, 125.9, 71.2, 61.8, 56.1, 35.9, 29.1, 26.5. Anal. ($C_{37}H_{42}N_2O_3$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-1,3-Bis{[4-methoxycarbonyl)phenyl]-methyl}-5,6-bis[(2-methoxyethoxy)methoxy]-4,7-bis(phenoxymethyl)-1,3-diazepan-2-one (27). Compound **27** was synthesized from **13a** according to method VI in 72% yield, with methyl 4-(bromomethyl)benzoate as alkylating agent: IR (film) ν 3040, 2890, 1720, 1655, 1598, 1467 cm^{-1}; $[\alpha]_D = -46.4°$ (c = 1.21, $CHCl_3$, 25 °C); ^1H NMR (399.8 MHz, $CDCl_3$) δ 7.93 (d, J = 8.3 Hz, 4H, CH_2ArH[o]), 7.29 (m, 8H, Ar*H*), 6.99 (t, J = 7.3 Hz, 2H, OAr*H*[p]), 6.79 (d, J = 8.0 Hz, 4H, OAr*H*[o]), 5.25 (d, J = 14.2 Hz, 2H, NCH_2Ar), 4.66 (d, J = 6.9 Hz, 2H, OCH_2O), 4.59 (d, J = 7.1 Hz, 2H, OCH_2O) 4.23 (d, J = 6.3 Hz, 4H, CH_2Ph), 4.18 (d, J = 14.2 Hz, 2H, NCH_2Ar), 3.93 (app s, 8H, C*H*N, CH_3OOC), 3.51−3.34 (m, 10H, C*H*OCH_2, OCH_2-CH_2O), 3.33 (s, 6H, CH_3); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 166.9, 161.4, 158.4, 143.7, 130.1, 129.6, 129.5, 129.4, 121.3, 114.8, 96.7, 71.7, 67.7, 65.5, 61.0, 59.1, 56.1, 52.2. Anal. ($C_{45}H_{54}N_2O_{13}$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-1,3-Bis{[4-(methoxycarbonyl)phenyl]-methyl}-4,7-bis(phenoxymethyl)-5,6-dihydroxy-1,3-diazepan-2-one (6). Method X. To compound **27** (37 mg, 0.044 mmol) in methanol (4 mL) was added concentrated HCl (0.16 mL). The reaction mixture was heated to 40 °C for 5 h. The solvent was removed. Purification by flash chromatography (CH_2Cl_2 to CH_2Cl_2/CH_3OH, 100:1) gave the product as a white solid (26.4 mg, 92%): mp 187−189 °C; IR (KBr) ν 3600−3300, 3040, 2950, 1720, 1604, 1471 cm^{-1}; $[\alpha]_D = +3.2°$ (c = 1.43, $CHCl_3$, 25 °C); ^1H NMR (399.8 MHz, $CDCl_3$) δ 7.95 (d, J = 8.3 Hz, 4H, CH_2ArH[o]), 7.31 (m, 8H, Ar*H*), 7.02 (t, J = 7.3 Hz, 2H, OAr*H*[p]), 6.8 (d, J = 7.8 Hz, 4H, OAr*H*[o]), 5.15 (br d, J = 11.9 Hz, 2H, NCH_2Ar), 4.39 (m, 2H, CH_2OPh), 4.20 (m, 4H, NCH_2Ar, CH_2OPh), 3.92 (s, 6H, CH_3OOC), 3.80 (m, 2H, C*H*N), 3.59 (br s, 2H, C*H*OH), 2.8 (br s, 2H, O*H*); ^{13}C NMR (100.2 MHz, $CDCl_3$) δ 166.8, 161.5, 157.8, 143.3, 130.1, 129.7, 129.6, 129.2, 121.7, 114.6, 71.5, 65.1, 60.0, 55.8, 52.2. Anal. ($C_{37}H_{38}N_2O_9$) C, H, N.

(4*R*,5*S*,6*S*,7*R*)-1,3-Bis{[4-(hydroxymethyl)phenyl]methyl}-4,7-bis(phenoxymethyl)-5,6-dihydroxy-1,3-diazepan-2-one (7). Method XI. To compound **27** (60 mg, 0.072 mmol) in ether (2 mL) was added $LiBH_4$ (4.70 mg, 0.216 mmol). The reaction mixture was heated to reflux for 12 h. Excess $LiBH_4$ was quenched carefully with 1 M HCl (20 mL), and ether (20 mL) was added. The layers were separated, and the water phase was extracted with ether (2 × 20 mL). The combined ether extracts was washed with saturated $NaHCO_3$ (20 mL), dried, and concentrated to yield 45 mg of the crude product, which was used without further purification. To a solution of the crude diol in methanol (3 mL) was added concentrated HCl (1 mL); this was stirred overnight at 25 °C. The solvent was removed, and the crude product was purified by flash chromatography (CH_2Cl_2/CH_3OH, 50:1−20:1) to give a white solid (27.1 mg, 63%): mp 161−163 °C; IR (KBr) ν 3600−3200, 3040, 2923, 1600, 1473, 1301 cm^{-1}; $[\alpha]_D = -10.6°$ (c = 0.491, CH_3OH, 25 °C); ^1H NMR (399.8 MHz, CD_3OD) δ 7.34−7.18 (m, 12H, Ar*H*), 6.98 (t, J = 7.4 Hz, 2H, OAr*H*[p]), 6.90 (d, J = 8.0 Hz, 4H, OAr*H*[o]), 5.12 (d, J = 13.9 Hz, 2H, NCH_2Ar), 4.58 (s, 4H, CH_2OH), 4.34 (m, 4H, CH_2OPh), 4.06 (d, J = 14.2 Hz, 2H, NCH_2Ar), 3.88 (m, 2H, C*H*N), 3.36 (br s, 2H, C*H*OH); ^{13}C NMR (100.2 MHz, CD_3OD) δ 163.4, 159.5, 141.8, 138.0, 130.1, 130.0, 128.0, 121.7, 115.4, 71.1, 65.8, 64.5, 62.3, 56.3. Anal. ($C_{35}H_{38}N_2O_7$) C, H, N.

(3*R*,4*S*,5*S*,6*R*)-4,5-Bis[(2-methoxyethyl)methoxy]-3,6-bis(phenoxymethyl)-1,2,7-thiadiazepane 1,1-Dioxide (28). Method XII. To compound **12a** (225 mg, 0.4 mmol) in ethyl acetate (5 mL) was added a catalytic amount of 10% Pd/C. Hydrogen was added to the system at atmospheric pressure, and the reaction mixture was stirred overnight. The suspension was filtered through Celite and concentrated to give a colorless oil. The crude amine (180 mg) was dissolved in dry pyridine (2.5 mL) under a N_2 atmosphere, and sulfamide (36 mg, 0.37 mmol) was added.[23] The reaction mixture was heated to reflux for 16 h. After cooling, the solution was diluted with ether (20 mL). The solution was washed with water (2 × 20 mL), then with 1 M aqueous HCl (2 × 10 mL), and finally with 10% aqueous Na_2CO_3 (10 mL). The ether solution was dried and concentrated. The residue was purified by flash chromatography (CH_2Cl_2 to CH_2Cl_2/CH_3OH, 19:1). This gave **29** as a pure white solid (138 mg, 60%): mp 72−74 °C; IR (mineral oil) ν 3356, 3278, 1587, 1490, 1350, 1251, 1226, 1162, 1125 cm^{-1}; $[\alpha]_D = -15.3°$ ($c = 1.0$, $CHCl_3$, 25 °C); ^1H NMR (270.2 MHz, $CDCl_3$) δ 7.29 (dd, $J = 8.2$, 7.9 Hz, 4H, OAr*H*[m]), 6.98 (t, $J = 7.3$ Hz, 2H, OAr*H*[p]), 6.88 (d, $J = 8.3$ Hz, 4H, OAr*H*[o]), 5.35 (d, $J = 10.9$ Hz, 2H, N*H*), 4.83 (d, $J = 7.3$ Hz, 2H, OCH_2O), 4.78 (d, $J = 7.3$ Hz, 2H, OCH_2O), 4.3 (s, 2H, C*H*OCH$_2$), 4.13 (m, 2H, CH_2OPh), 4.04 (m, 2H, CH_2OPh), 3.90 (ddd, $J = 8.6$, 8.6, 5.5 Hz, 2H, C*H*N), 3.77 (m, 2H, OCH_2CH$_2$O), 3.61 (m, 2H, OCH_2CH$_2$O), 3.46 (m, 4H, OCH$_2$CH_2O), 3.32 (s, 6H, CH_3); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 157.8, 129.6, 121.5, 114.5, 96.4, 74.1, 71.6, 68, 66.1, 59, 49.6. Anal. ($C_{26}H_{38}N_2O_{10}S$) C, H, N.

(3*R*,4*S*,5*S*,6*R*)-2,7-Dibenzyl-4,5-bis[(2-methoxyethoxy)-methoxy]-3,6-bis(phenoxymethyl)-1,2,7-thiadiazepane 1,1-Dioxide (29). Method XIII. To compound **28** (30 mg, 0.053 mmol) in DMF (0.75 mL) was added NaH (80% suspension) (6.3 mg, 0.21 mmol). The reaction mixture was stirred at room temperature under a N_2 atmosphere for 30 min. Benzyl bromide (36 mg, 0.21 mmol) was added, and the reaction was allowed to proceed overnight. Excess NaH was quenched by dilution with water (5 mL), and the reaction mixture was extracted with ether (2 × 10 mL). The combined ether extracts were dried and concentrated. Purification by flash chromatography (CH_2Cl_2 to CH_2Cl_2/CH_3OH, 99:1) gave a colorless oil (39 mg, 99%): IR (film) ν 3052, 2927, 2886, 1600, 1497, 1329, 1243, 1157, 1112, 1036 cm^{-1}; $[\alpha]_D = -27.6°$ ($c = 1.0$, $CHCl_3$, 25 °C); ^1H NMR (270.2 MHz, $CDCl_3$) δ 7.48 (d, $J = 6.9$ Hz, 4H, Ar*H*), 7.33 (dd, $J = 7.6$, 6.9 Hz, 4H, Ar*H*), 7.22 (m, 6H, OAr*H*[m], Ar*H*), 6.93 (t, $J = 7.3$ Hz, 2H, OAr*H*[p]), 6.68 (d, $J = 8.6$ Hz, 4H, OAr*H*[o]), 5.03 (d, $J = 17.2$ Hz, 2H, NCH_2Ph), 4.84 (m, 6H, NCH_2Ph, OCH_2O), 4.47 (dd, $J = 7.3$, 6.6 Hz, 2H, C*H*N), 4.27 (s, 2H, C*H*OCH$_2$), 4.01 (m, 4H, CH_2OPh), 3.74 (m, 2H, OCH_2CH$_2$O), 3.67 (m, 2H, OCH_2CH$_2$O), 3.4 (m, 4H, OCH$_2$CH_2O), 3.28 (s, 6H, CH$_3$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 157.9, 139.6, 129.5, 128.6, 127.1, 127, 121.3, 114.4, 96, 76.8, 71.5, 67.9, 65.9, 59, 53.5, 52. Anal. ($C_{40}H_{50}N_2O_{10}S$) C, H, N.

(3*R*,4*S*,5*S*,6*R*)-2,7-Dibenzyl-3,6-bis(phenoxymethyl)-4,5-dihydroxy-1,2,7-thiadiazepane 1,1-Dioxide (4). Method XIV. To a solution of compound **29** (14 mg, 0.019 mmol) in methanol (1 mL) was added saturated HCl in ether (2 mL). The reaction mixture was stirred overnight at room temperature. The solvent was removed, and purification by flash chromatography (CH_2Cl_2 to CH_2Cl_2/CH_3OH, 49:1) gave a white solid (10 mg, 93%): mp 194−195 °C; IR (mineral oil) ν 3546, 3436, 1600, 1497, 1325, 1248, 1146 cm^{-1}; $[\alpha]_D = +7.4°$ ($c = 1.0$, $CHCl_3$, 25 °C); ^1H NMR (270.2 MHz, acetone-d_6) δ 7.54 (d, $J = 7.2$ Hz, 4H, Ar*H*), 7.34−7.18 (m, 10H, Ar*H*, OAr*H*[m]), 6.91 (t, $J = 7.3$ Hz, 2H, OAr*H*[p]), 6.79 (d, $J = 7.9$ Hz, 4H, OAr*H*[o]), 5.05 (d, $J = 17.5$ Hz, 2H, NCH_2Ph), 4.96 (m, 4H, NCH_2Ph, O*H*), 4.45 (t, $J = 6.6$ Hz, 2H, C*H*N), 4.27 (m, 4H, C*H*OH, CH_2OPh), 4.16 (dd, $J = 9.4$, 6.3 Hz, 2H, CH_2OPh); ^{13}C NMR (67.8 MHz, acetone-d_6) δ 159.8, 141.6, 130.8, 129.6, 128.5, 128.1, 122.3, 115.9, 75.3, 67.6, 56.4, 53.4. Anal. ($C_{32}H_{34}N_2O_6S$) C, H, N.

(3*R*,4*S*,5*S*,6*R*)-4,5-Bis[(2-methoxyethoxy)methoxy]-3,6-bis(2-phenylethyl)-1,2,7-thiadiazepane 1,1-Dioxide (30). Compound **30** was synthesized from **18** according to method XII in 77% yield: mp 98−99 °C; IR (KBr) ν 3208, 3029, 2917, 1604, 1495, 1456, 1418 cm^{-1}; $[\alpha]_D = +55.3°$ ($c = 0.64$, $CHCl_3$, 23 °C); ^1H NMR (399.8 MHz, $CDCl_3$) δ 7.30−7.16 (m, 10H, Ar*H*), 5.08 (d, $J = 11.2$ Hz, 2H, N*H*), 4.75 (d, $J = 7.4$ Hz, 2H, OCH_2O), 4.70 (d, $J = 7.0$ Hz, 2H, OCH_2O), 3.72 (app s, 2H, C*H*OCH$_2$), 3.6 (ddd, $J = 11.0$, 11.0, 3.9 Hz, 2H, C*H*N), 3.48 (m, 4H, OCH_2CH$_2$O), 3.41 (m, 4H, OCH$_2$CH_2O), 3.33 (s, 6H, CH_3), 2.88 (ddd, $J = 10.2$, 8.6, 5.2 Hz, 2H, CH$_2$CH_2Ph), 2.72 (ddd, $J = 14.0$, 8.0, 8.0 Hz, 2H, CH$_2$CH_2Ph), 1.89 (dddd, $J = 13.9$, 11.0, 8.8, 5.2 Hz, 2H, CH_2CH$_2$Ph), 1.66 (dddd, $J = 12.2$, 8.3, 8.3, 3.9 Hz, 2H, CH_2CH$_2$Ph); ^{13}C NMR (100.2 MHz, $CDCl_3$) δ 141.6, 128.8, 128.5, 126.0, 96.8, 80.4, 71.6, 67.8, 59.0, 50.2, 35.4, 32.0. Anal. ($C_{28}H_{42}N_2O_8S$) C, H, N.

(3*R*,4*S*,5*S*,6*R*)-2,7-Dibenzyl-4,5-bis[(2-methoxyethoxy)-methoxy]-3,6-bis(2-phenylethyl)-1,2,7-thiadiazepane 1,1-Dioxide (31). Compound **31** was synthesized from **30** according to method XIII in 88% yield: IR (KBr) ν 3062, 2924, 1603, 1496, 1452, 1308 cm^{-1}; $[\alpha]_D = -3.5°$ ($c = 1.27$, $CHCl_3$, 23 °C); ^1H NMR (399.8 MHz, DMSO-d_6) δ 7.45 (d, $J = 7.1$ Hz, 4H, Ar*H*), 7.36 (app t, $J = 7.8$, 7.1 Hz, 4H, Ar*H*), 7.30 (t, $J = 7.3$ Hz, 2H, Ar*H*), 7.18 (app t, $J = 7.5$, 6.9 Hz, 4H, Ar*H*), 7.11 (t, $J = 7.3$ Hz, 2H, Ar*H*), 6.90 (d, $J = 7.0$ Hz, 4H, Ar*H*), 4.82 (d, $J = 16.6$ Hz, 2H, NCH_2Ph), 4.73 (d, $J = 6.9$ Hz, 2H, OCH_2O), 4.69 (d, $J = 6.6$ Hz, 2H, OCH_2O), 5.53 (br m, 2H, NCH_2Ph), 3.81 (br s, 2H, C*H*OCH$_2$), 3.70 (br s, 2H, C*H*N), 3.52 (br m, 4H, OCH_2CH$_2$O), 3.39 (t, $J = 4.6$ Hz, 4H, OCH$_2$CH_2O), 3.19 (s, 6H, CH_3), 2.55 (br m, 2H, CH$_2$CH_2Ph), 2.33 (br m, 2H, CH$_2$CH_2Ph), 1.96 (br m, 2H, CH_2CH$_2$Ph), 1.85 (br m, 2H, CH_2-CH$_2$Ph); ^{13}C NMR (100.2 MHz, DMSO-d_6) δ 143.0, 140.8, 129.9, 129.8, 129.7, 129.4, 128.8, 127.4, 97.6, 81.1, 72.8, 68.8, 59.7, 58.2, 53.6, 34.2, 32.7. Anal. ($C_{42}H_{54}N_2O_8S$) C, H, N.

(3*R*,4*S*,5*S*,6*R*)-2,7-Dibenzyl-3,6-bis(2-phenylethyl)-4,5-dihydroxy-1,2,7-thiadiazepane 1,1-Dioxide (5). Compound **5** was synthesized from **31** according to method XIV in 77% yield: IR (KBr) ν 3600−3200, 3027, 2924, 1603, 1495, 1453, 1295 cm^{-1}; $[\alpha]_D = +47.9°$ ($c = 1.21$, $CHCl_3$, 23 °C); ^1H NMR (399.8 MHz, $CDCl_3$) δ 7.33 (d, $J = 7.8$ Hz, 4H, Ar*H*), 7.23 (m, 6H, Ar*H*), 7.11 (app t, $J = 7.3$ Hz, 4H, Ar*H*), 7.06 (t, $J = 7.3$ Hz, 2H, Ar*H*), 6.88 (d, $J = 6.9$ Hz, 4H, Ar*H*), 4.68 (d, $J = 15.6$ Hz, 2H, NCH_2Ph), 4.28 (d, $J = 15.6$ Hz, 2H, NCH_2-Ph), 3.70 (s, 2H, C*H*OH), 3.47 (d, $J = 8.8$ Hz, 2H, C*H*N), 2.68 (ddd, $J = 13.2$, 10.8, 4.9 Hz, 2H, CH$_2$CH_2Ph), 2.28 (br s, 2H, O*H*), 2.15 (ddd, $J = 13.1$, 9.8, 6.4 Hz, 2H, CH$_2$CH_2Ph), 1.98 (dddd, $J = 14.6$, 10.2, 10.2, 4.9 Hz, 2H, CH_2CH$_2$Ph), 1.85 (m, 2H, CH_2CH$_2$Ph); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 141.8, 137.6, 128.85, 128.81, 128.7, 128.5, 128.1, 126.0, 74.1, 59.9, 53.9, 33.1, 29.4. Anal. ($C_{34}H_{38}N_2O_4S$) C, H, N.

Expression and Purification of HIV-1 Protease. HIV-1 protease was cloned and heterologously expressed in *Escherichia coli* and purified as described elsewhere.[28]

Inhibition Studies. The degree of inhibition of HIV-1 protease was determined essentially as described previously[24] in a spectrophotometric assay using a chromophoric peptide, HIV substrate III (Bachem Feinchemicalien AG, Bubendorf, Switzerland). The measurements were performed in a total volume of 120 μL by preincubating 60.8 μM substrate and inhibitor in 50 mM glycine, 50 mM sodium acetate, 50 mM MES, 50 mM Tris-HCl, 1.0 M NaCl, 1 mM EDTA, and 1 mM DTT at pH 5.5 and 37 °C for 5 min. The reaction was started by addition of enzyme (30 nM), and the rate of cleavage was followed by continuously registering the change in absorbance at 300 nm. Inhibitors were dissolved in DMSO; the final concentration of DMSO was kept below 2.5%. Solubility was limited: high concentrations resulted in cloudiness in the assay cuvette and nonlinear traces of absorbance versus time. Inhibition was determined at 10 μM for compounds not potent enough for IC_{50}-value determination. The IC_{50}-value is taken as the inhibitor concentration that results in 50% activity. It is estimated by plotting activity versus the logarithmic inhibitor concentration.

Determination of K_i-values was carried out by measuring the activity of 20 nM HIV-1 protease at different substrate and inhibitor concentrations. The mathematical analysis required an extensive data set comprising a wide range of substrate and inhibitor concentrations. The lowest substrate concentration that gave reproducible results and a detectable absorbance change was used (10 μM). Due to limited solubility the highest concentration of substrate that could be used was 100 μM. Two intermediate concentrations were also used (20

and 50 μM). The inhibitor concentration was chosen to be of the order of an initially estimated K_i-value and 2−4 times this concentration. At least three concentrations were used. Nanomolar concentrations of enzyme were used in order to obtain sufficiently rapid and accurate estimates of reaction velocities. Autoproteolysis of the enzyme and the sensitivity, which is limited by absorbance change upon cleavage of substrate, limit the time that can be used for the experiment, precluding the possibility of compensating lower rates achieved by decreasing the enzyme concentration with longer reactions. The total enzyme concentration is therefore of the same order as the concentration of inhibitor and requires the use of a rate equation that accounts for the presence of an enzyme−inhibitor complex for determination of K_i.[25] A rate equation for tight-binding inhibitors was fitted to the data by nonlinear regression analysis using QNFIT in SIMFIT.[29]

Molecular Dynamics Simulations. MD simulations were performed with a modified version (Åqvist, unpublished) of the ENZYMIX program,[30] using the GROMOS force field[27] with modifications reported elsewhere.[31,10] Two spherical simulation systems of radius 16 Å were set up for each inhibitor. In one, the inhibitor was placed in the center of the sphere, and SPC water molecules[32] were added to fill the sphere. For the other simulation, the inhibitor was docked into the HIV proteinase, the simulation sphere was centered on the inhibitor, and water molecules were added to fill the nonprotein parts of the sphere. Protein atoms outside the simulation sphere were strongly restrained to their crystallographic positions and only interacted through bonds over the sphere boundary. The 16 Å radius sphere used in this work in fact contains most of the proteinase. MD simulations were then performed, first to allow the system to attain equilibrium and thereafter to collect data for the free energy calculations. For inhibitor−water simulations, the inhibitor was kept in the center of the sphere by restraining the carbonyl carbon. The simulation temperature was 300 K, the MD time step was 1 fs, and SCAAS surface restraints[33] were employed to hold the water surface at the desired radius. A nonbonded interaction cutoff radius of 10 Å was used for interactions between neutral noninhibitor groups, but the inhibitor and all charged groups interacted with everything inside the simulation sphere. Initial protein coordinates were from Brookhaven Protein Data bank entry 5HVP,[5g] conformation 1. Net charges were left unmodified for the residues Asp 25, Arg 8, Arg 208, Asp 29, Asp 229, Asp 30, Asp 230, Arg 87, and Arg 287, while all other charged residues (far from the inhibitor) were replaced from the alternate set of GROMOS[27] residues with neutral dipolar groups. Asp 225 was considered to be protonated. Tetramethyl-substituted *RSSR* and *RRRR* cyclic urea rings were energy minimized by repeated simulated annealing using InsightII and Discover (Biosym/MSI, San Diego, CA), and the resulting minimum energy rings and their mirror images were used for modeling the ligands. The ligands were graphically docked into the protein, without manipulation of protein coordinates, to yield the starting models. We used Insight II (Biosym/MSI) for docking except for **3**, where the ligand was docked into the protein by a conformational search with the Macro Model program.[34]

The systems consisting of the inhibitor in water were equilibrated for 190 ps at 300 K and then run for 125 ps, collecting data every 5 fs. The protein−inhibitor−water systems were heated under decreasing restraints in a 15 ps stepwise heating scheme, equilibrated for 175 ps at 300 K, and then run for 125 ps for data collection (250 ps for DMP 323 and **7**). Additional harmonic distance restraints of 4 kcal/mol Å^2 were applied during the heating and the first 25 ps of the equilibration to ensure correct initial geometries of the system. These were applied between the inhibitor carbonyl oxygen and the nitrogens of the peptide NH groups of Ile 50 and Ile 250 and between the inhibitor carbonyl carbon and the side chain carboxylate carbon of Asp 25 and Asp 225. For DMP 323 and **7**, similar additional restraints of 5 kcal/mol were applied between one carboxylate oxygen of aspartates 30 and 230 and the appropriate inhibitor hydroxyl group oxygen and hydrogen atoms, to ensure that the aspartates would find the possibility of hydrogen bonding to these hydroxyl groups. Otherwise, the time needed for the aspartates to swing around to this equilibrium position from the crystallographic starting coordinates was very long. The data collected in inhibitor−water and inhibitor−protein−water simulations were the nonbonded force field interaction energies for inhibitor-surrounding interactions (where 'surrounding' refers to water or water + protein, respectively), as required by the LIE procedure[10] used to evaluate the free energy (see below). Other details were as in the recently reported similar calculations on other HIV-1 proteinase inhibitors.[11]

Free Energy Calculations. The LIE method is based on a linear response model of binding energetics. The simulation averages of the interaction energies between the inhibitor and its surroundings (no interactions within the inhibitor or within the surroundings are included in the average) are assumed to be linearly related to the free energy of solvation in that type of surrounding. The interaction energies are divided into electrostatic and van der Waals parts, with different proportionality constants for the different terms in the final expression for the binding energy below. As described elsewhere,[10,35] these considerations result in the following semiempirical formula, where < > denotes MD time averages:

$$\Delta G_{bind} = \Delta G_{el} + \Delta G_{vdw} \approx \alpha(<V_{vdw}>_{prot} - <V_{vdw}>_{wat}) + \beta(<V_{el}>_{prot} - <V_{el}>_{wat}) \quad (1)$$

Here, α is an empirically determined constant, and β is predicted to be $^1/_2$ by the electrostatic linear response approximation.[10,35] As in earlier work, we here use $\alpha = 0.161$ and $\beta = ^1/_2$ to estimate the binding free energies.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectral data, IR, optical rotations, and elemental analyses for compounds **9b**−**14b**, **9c**−**14c**, and **9d**−**14d** (6 pages). Ordering information is given on any current masthead page.

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