Unexpected Binding Mode of a Cyclic Sulfamide HIV-1 Protease Inhibitor

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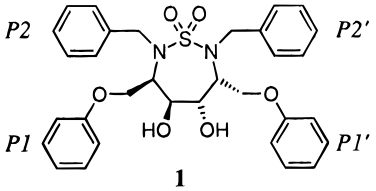
Two cyclic, C_2-symmetric HIV-1 protease inhibitors, one sulfamide and one urea derivative, both comprising phenyl ether groups in the P1/P1′ positions, were cocrystallized with HIV-1 protease, and the crystal structures were determined to 2.0 Å resolution. The structure of the urea **2** showed a conformation similar to that reported for the related urea **3** by Lam et al., while the sulfamide **1** adopted an unanticipated conformation in which the P1′ and P2′ side chains were transposed.

Introduction

The disease acquired immune deficiency syndrome (AIDS) is caused by the retrovirus human immunodeficiency virus (HIV).[1,2] Retroviruses produce polyproteins which are specifically cleaved into enzymes and structural proteins by viral proteases. The HIV-1 protease (HIV PR) is essential for maturation of the virus into infectious viral particles,[3] and this enzyme is considered, therefore, a suitable target for drugs against AIDS.[4−7] HIV PR is an aspartyl protease that is functional as a dimer of two identical subunits with 99 amino acid residues.[8−12] The dimer has one active site region, situated at the interface between the two monomers, with one catalytic triad (Asp-Thr-Gly) from each monomer.

Guidance in the design of inhibitors has come from the crystal structures of HIV PR complexed with different inhibitors.[13−21] There is a significant difference in the conformation of the protease in these inhibitor−complex structures compared to the native structures.[10−12] The largest change is seen in the movement of the two flaps, the β-hairpins (residues 42−58 and 142−158, respectively) which cover the active site.[22] In the inhibitor−protease complexes a water molecule was found in the active site, connecting the inhibitor to the amide hydrogens of Ile50 and Ile150 of the flaps in a tetrahedral arrangement.[14,17,18] This water molecule was postulated to be important for the fit of the flaps over the inhibitor and for the binding of the inhibitor. The functional replacement of this solvent molecule was suggested as a target for inhibitor design for the improvement of the inhibitors with respect to binding and specificity to the retroviral proteases.[17,18] Lam et al. have successfully incorporated the binding features of this structural water molecule into non-peptide cyclic urea and sulfamide inhibitors.[23,24] On the basis of that concept, we have used carbohydrates as chiral precursors for the synthesis of several C_2-symmetric urea and sulfamide inhibitors.[25] Among these the cyclic sulfamide **1** and the cyclic urea **2** inhibited the HIV PR with K_i values of 19.1 and 12.2 nM, respectively.[25] We herein present X-ray crystallographic analyses of these inhibitors in complex with the protease and report an unexpected binding mode of the sulfamide **1** as compared to the urea **2.**

Chart 1



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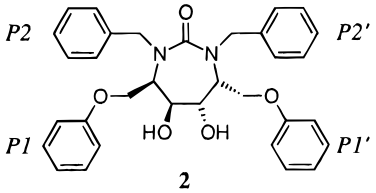

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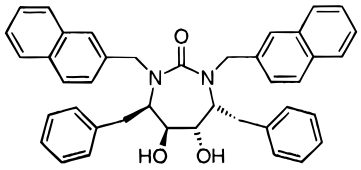

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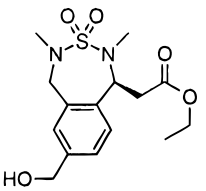

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Results and Discussion

Asymmetry in Position and Conformation. The inhibitors **1** and **2** are C_2-symmetric (Chart 1) in addition to the HIV-1 protease.[11] The active site, which is the target of the inhibitors, exhibits exact crystallographic, 2-fold rotational (C_2) symmetry in the native structure; thus the inhibitors might be expected to bind in a symmetric fashion to the protease dimer with the sulfur atom of the sulfamide group in **1** and the carbonyl carbon and oxygen atoms in **2** located on the 2-fold axis. This would lead to an exact 2-fold mode of interaction of the hydroxyl groups with the catalytic aspartic acid residues 25 and 125. Structural studies of the protease in complex with symmetric inhibitors have shown that there is a variation from strictly symmetric to asym-

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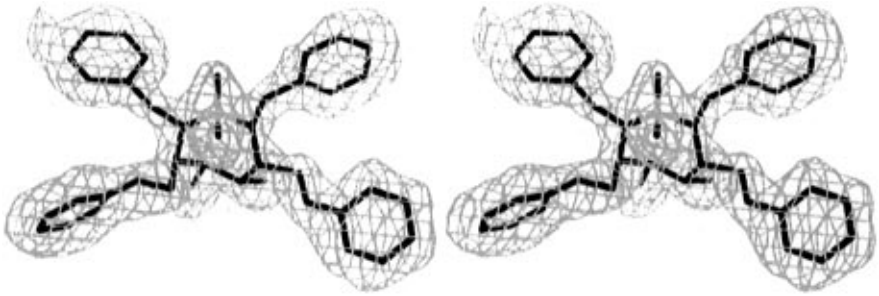

Figure 1. $F_o - F_c$ inhibitor-omit electron density map for **1** showing the inhibitor modeled in the electron density. The electron density map was calculated after removal of the inhibitor from the refined structure and one round of refinement.

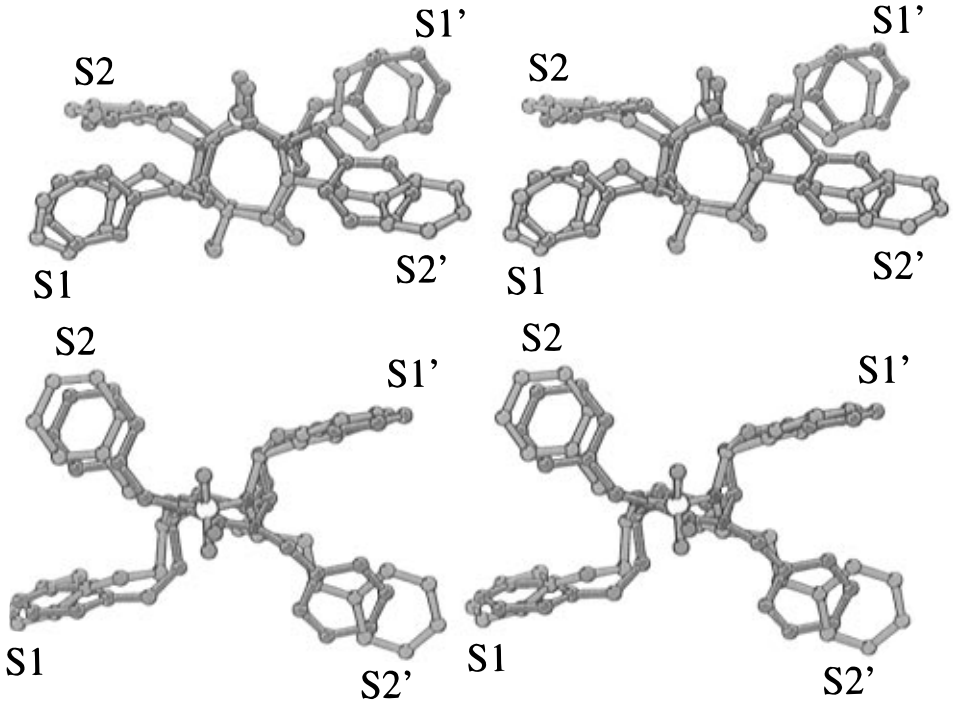

Figure 2. (Top) Stereodrawing of the comparison of the conformations of the inhibitors **1** and **2**. The protein Cα coordinates were used for the superpositioning of **1** on **2**. Compound **1** is displayed in green and **2** in blue. To the left are the P1 and P2 side chains, and to the right are the P1′ and P2′ side chains. (Bottom) Same as above but viewed approximately along the C_2 axis, which is a rotation of about 75° about the vertical axis of the view in the top panel. Figures 2−4 were prepared by MOLSCRIPT.[43]

metric binding.[26] Hosur et al. have designated three different categories of binding to distinguish different combinations of positional and conformational symmetry.[27] According to this classification **2** belongs to type B, that is, the inhibitor adopts symmetric conformation but binds asymmetrically, with the C_2 axes of the inhibitor and enzyme noncolinear. The electron density map of **1**, however, revealed a different conformation of the seven-membered ring compared to that of **2,** leading to reversed orientations of the P1′ and P2′ side chains. This was verified by modeling of the inhibitor **1** in the $F_o - F_c$ inhibitor-omit electron density map (Figure 1). Despite being C_2-symmetric, **1** not only binds asymmetrically to the protein but also adopts an asymmetric conformation and thus should be classified as a type C inhibitor.

In order to determine the deviation from 2-fold symmetry, the enzyme dyad was calculated by superposition of the 99 Cα atoms of subunit 2 on the corresponding Cα atoms of subunit 1. The entire enzyme−inhibitor complex was rotated using this enzyme dyad. The inhibitor **2** and enzyme C_2 axis are displaced by 0.15 Å through translation. The differences between equivalent carbon atoms from the two halves of the inhibitor are 0.1−1.2 Å. The deviation from symmetry is not merely the result of translation of the entire inhibitor but is also due to differences in the positions of the P1 and P2 side chains, with the largest differences in the P1/P1′ side chains. The analysis of **1** showed that the equivalent side chains did not superimpose. The asymmetric conformation of **1** is shown in Figure 2, where **1** is superimposed on **2**. The Cα atoms of the two enzyme complexes were used for the positioning. The P1/P2 sides of the inhibitors overlap well. One interesting and important feature is demonstrated as follows: on the P1′/P2′ side, the side chains are shifted such that the P1′ of **1** overlaps with the P2′ of **2** and vice versa. The overlap of P1′ of **1** and P2′ of **2** is almost as good as that of the P1 and P2 side chains, while there are significant differences in the positions of the P2′ side chain of **1** and the P1′ side chain of **2**. Related unexpected shifts of binding sites of linear inhibitors have been reported recently.[28,29]

Binding Interactions of the Inhibitors in the Active Site of HIV PR. Compound **2** is positioned in a fashion similar to the related inhibitor **3**[23] (entry 1HVR in the Protein Data Bank[30]). The P1/P1′ side chains are in close contact, i.e., within van der Waals

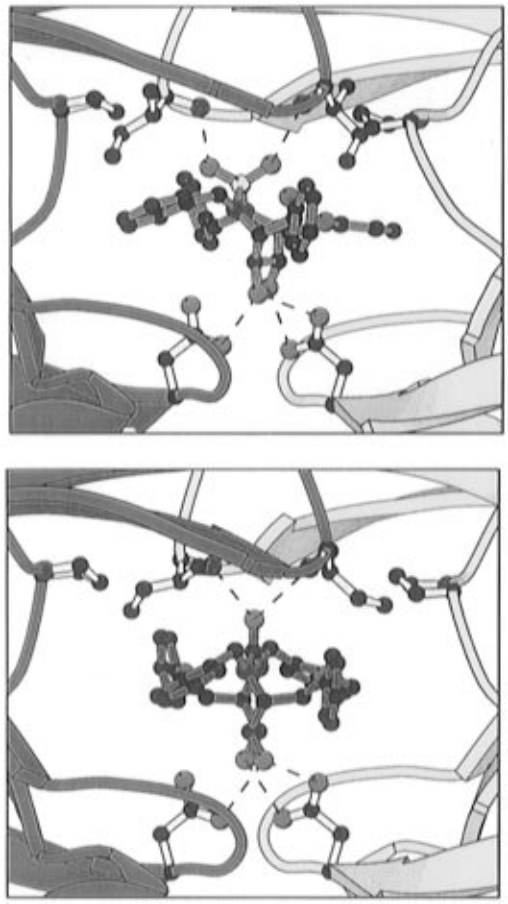

Figure 3. Positions of the inhibitors (top) **1** (in green) and (bottom) **2** (in blue) in the protease active site. The protease subunit 1 is shown in green and subunit 2 in yellow. Asymmetry in the inhibitor−protease interaction is apparent in the hydrogen-bonding interactions to the catalytic amino acid residues Asp25/125 and in the close packing interactions with the hydrophobic residues Ile50/150 and Pro81/181. The hydrogen bond distances between the sulfamide oxygens of compound **1** and the Ile50/150 nitrogens are significantly shorter (both 2.9 Å) than the corresponding distances to the carbonyl oxygen of compound **2** (2.9 and 3.3 Å).

distance, with the S1/S1′ subsite residues Ile50/150, Ile123/23, Asp125/25, Pro181/81, and Ile184/84 and the carbonyl oxygens of Gly27/127. The P2/P2′ side chains are in contact with the S2/S2′ subsite residues Ala28/128, Asp30/130, Val32/132, and Ile150/50 and, in addition, the S1′/S1 residues Ile84/184. In the complex of HIV PR with **1** the P1 and P2 side chains are in contact with the S1 and S2 residues as are the corresponding side chains of **2**. As a consequence of the shift, the P1′ side chain interacts with the residues of the S2′ site, the P2′ side chain interacts with the S1′ subsite, and only small differences in the interactions with the protein residues are observed compared to P1 and P2. However, the P1′ and P2′ side chains penetrate deeper into the pockets and are also in close contact with the residues Ile47/147.

The inhibitors form hydrogen bonds with the enzyme through the hydroxyl groups and the oxygens of the sulfamide and carbonyl groups. The hydroxyl groups of **1** and **2** show a similar, but not symmetric, hydrogen-bonding pattern (Figure 3). One of the hydroxyl groups is hydrogen bonded to both catalytic aspartates (2.6 and 2.6 Å for **1**, 2.7 and 3.1 Å for **2**), while the other hydroxyl group is hydrogen bonded to only Asp125 (2.7 Å for **1**, 2.8 Å for **2**). This asymmetry is in agreement with that observed with other dihydroxyethylene−HIV PR complexes[26] and furthermore concurs with what has been suggested for the asymmetric binding of the catalytic substrate intermediate to the aspartates in the proposed mechanism.[31]

As predicted,[23] the carbonyl oxygen in **2**, mimicking the structural water, forms hydrogen bonds to each of the backbone amide hydrogens of the protease residues Ile50 (3.3 Å) and Ile150 (2.9 Å) (Figure 3, bottom). In the compound **1**, both of the sulfamide oxygens are engaged in hydrogen bonding to the flap. One of the oxygens is hydrogen bonded to the amide nitrogen of Ile50 with the distance 2.9 Å, and with the same distance the other oxygen binds to Ile150 (Figure 3, top).

The phenyl ether oxygens in the P1 and P1′ side chains of **2** and the P1 side chain of **1** are at distances of 4.4 Å to the amide nitrogens of Ile50 and Ile150. These distances are longer than a hydrogen bond distance by about 1.4 Å but shorter than a van der Waals interaction by 0.6−1.9 Å. It is unclear whether there are tangible interactions between these dipoles.

Active Site of the Protein. The changes in the active site area between different inhibitor−HIV PR complexes are generally small (within 0.3−0.8 Å rms deviation).[26] This is also true for the complexes of HIV PR with **1** and **2**. In these complexes there are only small shifts of less than 0.3 Å in the side chains in the active site area. There are two notable exceptions, namely, the amino acids Ile50/150 and Pro81/181, which are asymmetrically positioned, have different conformations and deviate from symmetry by 1−2 Å (Figure 3). These residues are involved in close packing contacts with the inhibitors and with each other. Consequently, these residues and a possible asymmetric electronic environment surrounding the active-site aspartic acids[26] may be of importance for the asymmetric positioning of the inhibitors in the active site. Whether the conformational change of the seven-membered ring and the accompanying shift of the P1′ and P2′ side chains of compound **1** are induced through interactions with the protease could not be determined from the crystallographic structure analysis.

Conformation of the Seven-Membered Sulfamide Ring. In order to study the origin of the difference in binding conformation, a conformational analysis of the two inhibitors was attempted. The lack of high-quality force field parameters available to use around the sulfamide moiety precluded a good analysis of **1**. However, a Monte Carlo conformational search of this compound *in vacuo* was performed using the parameters present in the Amber* force field. The ring conformation of **1** as identified in the complex of HIV PR can be reproduced approximately (rms of 0.07 Å of atoms in the central ring), but it is nearly 17 kJ/mol higher in energy than the lowest energy conformation found. Work is currently in progress to develop new force field parameters.

The Cambridge Structure Database (CSD)[32] was searched for sulfamides in order to ascertain the novelty of the conformation of **1**. A seven-membered cyclic sulfamide, compound **4**, was found (CSD code SEKFUN).[33] It is, however, uncertain what effect the fused benzene ring in **4** has on the ring geometry of the cyclic sulfamide. The overlap of the central rings of **1** and **4**

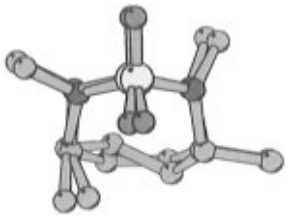

Figure 4. Superposition of the seven-membered ring of compounds **1** and **4**. Compound **1** is displayed in green and **4** in magenta. For clarity only the methylene groups of **1** are shown. Compound **4** was modeled with methyl groups.

is good if one accepts the differences at the site of the ring fusion (rms of 0.23 Å of the central rings). To simulate the presentation of the P2, P1, P1′, and P2′ side chains, methyl groups were modeled to **4** at standard bond lengths, bond angles, and appropriate chirality. The methyl groups of **4,** representing P1, P1′, and P2′, overlap well with the appropriate methylenes of **1**. A flip of the nitrogen carrying P2 of **4** also brings this methyl into alignment (Figure 4; rms of 0.29 Å of the central rings and the methyls of P2, P1, P1′, and P2′).

The good fit of the central ring (except where the benzene is fused) and the modeled P2′, P1′, P1, and P2 side chains supports the hypothesis that the unexpected switch of the roles of P1′ and P2′ is a result of the ring geometry adopted by the cyclic sulfamide.

Summary and Conclusions

The unanticipated binding mode of the cyclic sulfamide **1** as compared to the structurally very similar cyclic urea **2** illustrates the difficulties in predicting the conformation of an inhibitor in complex with an enzyme. The structural information reported herein might have implications for future design of inhibitors comprising seven-membered cyclic sulfamides as a core structure.

Experimental Section

Purification. HIV-1 protease was expressed in *Escherichia coli* as described by Bäckbro et al.[34] The enzyme was isolated from inclusion bodies through a slightly modified version of the method by Taylor et al.[35] The procedure involved denaturation in 8 M urea, separation on Poros Q (PerSeptive Biosystems), refolding at 4 °C, and ion exchange chromatography using a Poros S column. The material was concentrated by ammonium sulfate precipitation and desalted using a PD-10 column (Pharmacia), and final concentration was carried out through ultra filtration on Centricon concentrators (Amicon).

Crystallization. Crystallization was performed by vapor diffusion. Protease (2 mg/mL) and inhibitor (40 mM in DMSO) were mixed in a ratio of 1:1 and subjected to cocrystallization by the vapor diffusion method. Drops consisting of 5 μL of the protease−inhibitor mixture plus 5 μL of the crystallization buffer (50 mM MES, pH 5.5, 0.4 M NaCl, and 0.02% (w/v) NaN_3) were equilibrated against the same buffer at 4 °C. Crystals usually appeared within 4 days and grew to a size of 0.3 mm × 0.2 mm × 0.05 mm within 4 weeks.

X-ray Data. X-ray data were recorded at 4 °C on MAR imaging plates on the synchrotron beam lines DW32 at Lure, Orsay (compound **1**), and 9.5 at DRAL, Daresbury (compound **2**). The programs DENZO and SCALEPACK were used for processing[36] and scaling.[37] A summary of data collection statistics is given in Table 1.

Structure Determination. As a starting model for the protease dimer, coordinates were obtained from the data set 4PHV in the Brookhaven Protein Data Bank.[20] This structure is a HIV-1 protease in complex with the psuedosymmetric inhibitor L-700,417. This crystal form, space group $P2_12_12$, is isomorphous with the protease−**1** and protease−**2** crystals and contains one complete protease dimer in the asymmetric unit. The structure of the protease−**1** complex was determined first. These protein coordinates were thereafter used in the structure determination of the protease−**2** complex. The structure of **3** was used for modeling of the inhibitors using the Macromodel (version 4.5) software package with the AMBER* force field.[38] The models were modified to fit the electron densities and refined together with the protein.

Table 1. Data Processing Statistics

	protease−**1**	protease−**2**
space group	$P2_12_12$	$P2_12_12$
wavelength (Å)	0.90	0.92
no. of crystals	1	2
cell dimensions (a, b, c) (Å)	58.0, 85.8, 46.5	59.0, 86.8, 46.8
d_{min} (Å)	2.0	2.0
no. of observations	73 708	62 019
no. of unique reflections	14 846	14 634
completeness (%)	91.7	84.3
R_{merge}[a]	4.1	5.0
reflections $I > 3\sigma$ (%)	86	64
reflections $I > 3\sigma$ (%) in highest resolution shell	75	39
B in resolution (Å)	2.07−2.00	2.07−2.00

[a] $R_{merge} = \Sigma|I_{obs} - \langle I\rangle|/\Sigma\langle I\rangle$.

Table 2. Refinement Statistics of Final Models

	protease−**1**	protease−**2**
resolution of data (Å)	8.0−2.0	8.0−2.0
R_{cryst}[a] (%)	18.7	16.1
R_{free} (%)	24.7	23.7
no. of atoms	1636	1673
no. of water molecules	75	112
mean B-factor, protein (Å^2)	17	21
mean B-factor, inhibitor (Å^2)	14	21
deviations from ideality[b]		
bond lengths (Å)	0.010	0.025
bond angles (deg)	1.90	2.43
dihedrals (deg)	25.5	26.9
impropers (deg)	1.32	1.90

[a] $R_{cryst} = \sum_{hkl} ||F_{obs,hkl}| - |F_{calc,hkl}||/\sum_{hkl}|F_{obs,hkl}|$. [b] Ideal parameters are those defined by Engh and Huber.[42]

Model Building and Refinement. Refinement was performed using the program package X-PLOR.[39] The starting model was refined with rigid-body and slow-cool refinement using only the protein coordinates. The difference Fourier map ($F_o - F_c$) clearly showed the position and orientation of the inhibitor together with the positions of a large number of water molecules. The inhibitor was built manually into the electron density using the program O.[40] Water molecules were added in $F_o - F_c$ peaks at chemically acceptable sites using WATERSORT, a program of the CCP4 package. Only solvent molecules with B-values less than 60 Å^2 were accepted. A total of six cycles of refinement were performed. Data between 8.0 and 2.0 Å were used in all the refinement steps. The free R-factor (R_{free}),[41] based on 10% of the data, was used to monitor the refinement. The refinement statistics are shown in Table 2.

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