# An Orally Bioavailable Pyrrolinone Inhibitor of HIV-1 Protease: Computational Analysis and X-ray Crystal Structure of the Enzyme Complex

Amos B. Smith, III,* Ralph Hirschmann,* Alexander Pasternak, Wenquing Yao, and Paul A. Sprengeler

*Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104*

M. Katharine Holloway,* Lawrence C. Kuo,* Zhongguo Chen, Paul L. Darke, and William A. Schleif

*Departments of Molecular Design and Diversity, Molecular Biology, and Virus and Cell Biology, Merck Research Laboratories, West Point, Pennsylvania 19486*

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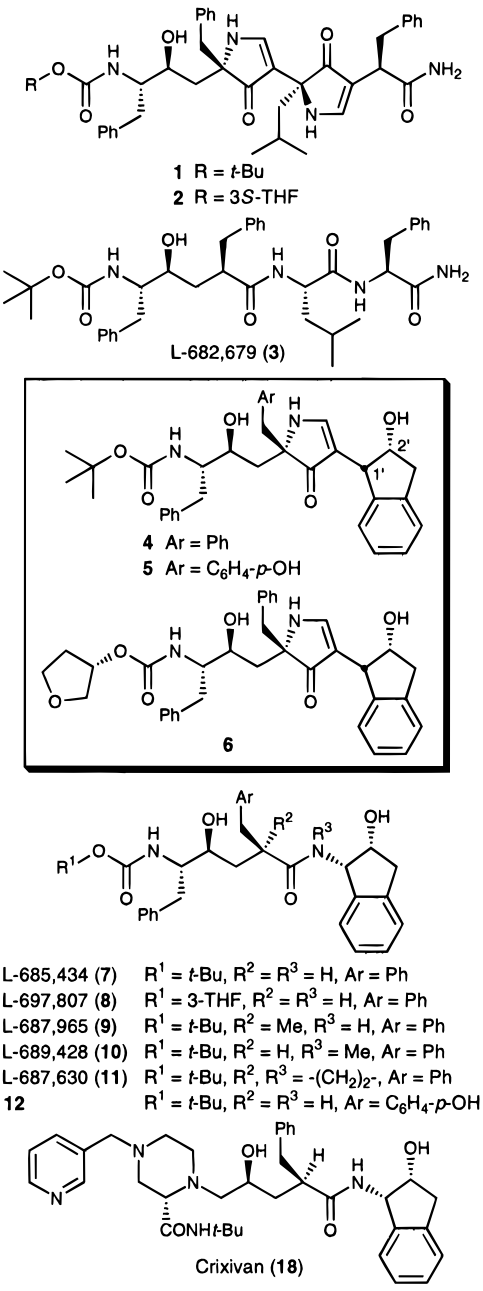
The importance of HIV protease inhibitors in the treatment of AIDS is now well established.[1] A very promising current strategy employs a combination of drugs to inhibit both the protease and the HIV reverse transcriptase.[2] The remarkable mutability of the HIV retrovirus however remains a major obstacle to the successful long-term management and cure of the disease. More recently, the concept of a single-mechanism cocktail (e.g., a mixture of protease inhibitors) has attracted considerable interest.[3,4] Assuming that the single-mechanism approach proves viable, we suggest that the best protection against the wild-type and mutant strains may be obtained by maximizing structural diversity of the protease inhibitors.

We have described several potent inhibitors of the HIV-1 protease (e.g., **1** and **2**), based on the novel 3,5-linked bispyrrolinone scaffold which we developed to mimic the peptidal $\beta$-strand/$\beta$-sheet structural motif.[5] The first-generation inhibitor **1** proved less effective than its exact peptidal counterpart **3** in purified enzyme inhibition assays ($IC_{50}$) but displayed higher cellular antiviral activity ($CIC_{95}$). Importantly, the corresponding $CIC_{95}/IC_{50}$ (C/I) ratios[6] indicated that the bispyrrolinone entered infected lymphocytes more readily than the analogous peptide. We have also confirmed that polypyrrolinones are not cleaved by the proteolytic enzyme α-chymotrypsin.[7] Nonetheless, our most active bispyrrolinone **2** ($IC_{50}$ 1.3 nM, $CIC_{95}$ 800 nM) was not orally bioavailable in dogs,[5c,g] reflecting either nonabsorption from the gastrointestinal tract, first-pass metabolism in the liver, or excretion into the bile. The relatively high molecular weight of 735 may account for the poor oral bioavailability of **2**.[8] We therefore sought to design and synthesize monopyrrolinone inhibitors of HIV-1 protease with molecular weights <600.

**Design and Initial Molecular Modeling.** Replacement of the C-terminal pyrrolinone moiety in **1** and **2** with a $P_{2'}$ indanol, as employed in amide inhibitors L-685,434 (**7**)[9] and L-697,807 (**8**),[10] generated the 3,5,5-trisubstituted pyrrolin-4-one structures **4** (MW 569) and **6** (MW 583). Monopyrrolinones cannot form $\beta$-strand-inducing intramolecular hydrogen bonds between a pyrrolinone NH and the carbonyl of an adjacent heterocycle, as observed in our polypyrrolinones. However, the potential does exist for a weaker H-bond between the pyrrolinone NH and the transition-state-mimetic secondary hydroxyl.




To investigate the influence of this variation in hydrogen bonding on the solution conformation, a Monte Carlo conformational search[11,12] was performed on **6** and the resultant stuctures were minimized with both chloroform and water continuum-solvation models.[13] The backbone conformations in the lowest-energy calculated conformers closely resembled the X-ray stuctures of peptidal inhibitors such as MVT-101 and JG-365 cocrystallized with the HIV-1 protease.[14] The major difference between the chloroform and water models proved to be a hydrogen bond in the former linking the catalytic site and indanol hydroxyls; this interaction caused a slight rotation of the indanol relative to the pyrrolinone. The majority of the other preferred conformers for both solvents displayed the same overall backbone shape, with minor changes in the $P_1$ and $P_1'$ $\chi_1$ angles and the indanol−pyrrolinone torsion angle.

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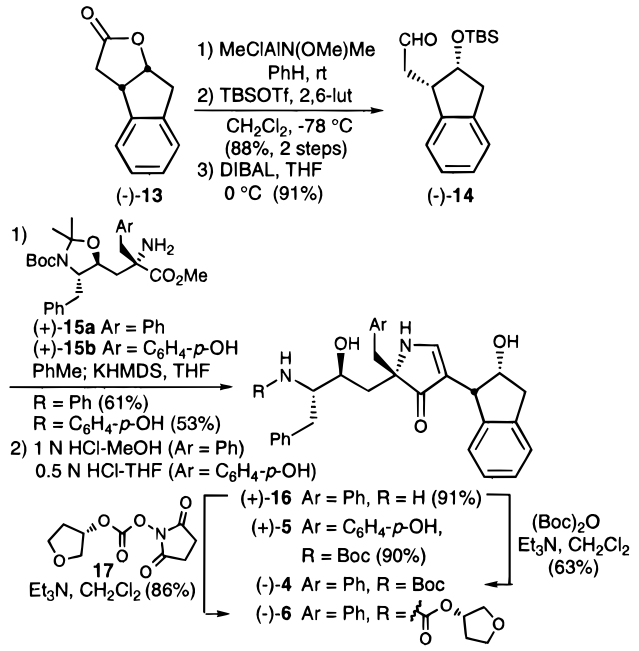
**Synthesis and Biological Activity.** Monopyrrolinones **4** and **6** were synthesized from lactone (−)-**13**[15] via methodology we developed earlier (Scheme 1).[5c−i]

**Scheme 1**




In the purified enzyme inhibition assay,[16,6a] $IC_{50}$s of 11.9 and 2.0 nM for **4** and **6** demonstrated that monopyrrolinones can strongly inhibit the HIV-1 protease (Table 1). Comparison with the analogous amide-based inhibitors **7** and **8** (0.4 and 0.03 nM) suggested a less favorable fit for **4** and **6** in the enzyme active site. As we observed for the related bispyrrolinones,[5c,g] replacement of the N-terminal Boc functionality in **4** with the (3*S*)-hydroxytetrahydrofuranyl carbamate in **6** led to improved potency. Subsequently, three amide-based analogs of **7** were reported:[17] L-687,965 (**9**, α-methyl), L-689,428 (**10**, N-methyl), and L-687,630 (**11**, γ-lactam). These compounds were 18-, 465-, and 215-fold less active than **7**, possibly because an H-bond to the carbonyl of $Gly^{27}$ is important for high affinity. The decrease in activity was much smaller for **4** (30-fold relative to **7**) than for **10** and **11**, suggesting that H-bonding of the pyrrolinone NH group may partially compensate for the loss in affinity caused by steric interactions.

**Table 1.** Bioassay Data and Calculated Interaction Energies for Pyrrolinones **1** and **4**−**6** and Related Amide-Based Inhibitors

| inhibitor | type | $IC_{50}$ (nM) | $CIC_{95}$ (nM) | C/I | $E_{Inter}$ (kcal/mol) |
|---|---|---|---|---|---|
| **1** | pyrrolinone | 10 | 1500 | 150 | |
| L-682,679 (**3**) | amide | 0.6 | 6000 | 10000 | |
| **4** | pyrrolinone | 11.9 | 800 | 67 | −120.0 |
| L-685,434 (**7**) | amide | 0.3 | 400 | 1333 | −145.0 |
| L-687,965 (**9**) | amide | 7.0 | | | |
| L-689,428 (**10**) | *N*-Me-amide | 186 | | | |
| L-687,630 (**11**) | γ-lactam | 86 | | | |
| **6** | pyrrolinone | 2.0 | 100 | 50 | −134.1 |
| L-697,807 (**8**) | amide | 0.03 | 3 | 100 | −150.9 |
| **5** | pyrrolinone | 6.0 | | | −134.3 |
| **12** | amide | 0.16 | | | |
| Crixivan (**18**)[18] | amide | 0.36 | 25−100 | 69−277 | |

In contrast with the bispyrrolinone inhibitors,[5d,g] the monopyrrolinones are less potent antiviral agents than their amide counterparts in the cellular assay, with $CIC_{95}$ values of 800 and 100 nM for **4** and **6** compared with 400[9] and 3 nM[10] for **7** and **8**. Nevertheless, the C/I ratios[6] for **4** and **6** (67 and 50, respectively) are improved vis-à-vis **7** and **8** (1333 and 100), suggesting that **4** and **6** are more readily transported into HIV-infected lymphocytes.[19] We previously attributed[2c,g] the improvement in $CIC_{95}/IC_{50}$ ratios for the bispyrrolinones to hydrogen bonding between adjacent rings, which would decrease the desolvation energy required for transport from the aqueous extracellular environment into the lipophilic cell membrane.[20,21] In the monopyrrolinones an intramolecular six-membered-ring H-bond can link the pyrrolinone NH with the nearby transition-state-mimetic hydroxyl group, but this interaction is conformationally and energetically less favorable than H-bonding between neighboring pyrrolinone rings, perhaps accounting for the more modest improvements in C/I ratios for the monopyrrolinones (relative to their amide counterparts). The less polar nature of the pyrrolinone enaminone functionality, compared with the amide group in **7** and **8**, may also contribute to the improved C/I indices for **4** and **6**.

Evaluation of the pharmacokinetic properties of **6** in two dogs revealed an oral bioavailability of 13%, with a $t_{1/2}$ of 33−36 min, a clearance rate of 20.6 mL/min/kg, a volume distribution of 0.81 L/kg, and an area under the curve of 1.83 μM/h. Although the corresponding data for amide **8** do not exist, **6** proved to be 5-fold less bioavailable than the amide inhibitor Crixivan (**18**). Our findings demonstrate that useful bioavailability can be attained by replacing the peptide backbone with a pyrrolinone scaffold.[22] In addition, the results suggest that high molecular weight may indeed be responsible for the lack of oral bioavailability of bispyrrolinone **2**.

**Interpretation via Modeling and X-ray Crystallography.** We minimized complexes of pyrrolinones **4** and **6** and their peptidal counterparts (**7** and **8**) in the active site of HIV-1 protease, using the MM2X force field according to published protocols.[23] The key question was whether the pyrrolinone NHs participate in β-sheet-like hydrogen bonding with the enzyme, as suggested by the biological activity (vide supra). The energy-minimized bound conformations of **4** and **6** proved to be nearly identical. In each case, the pyrrolinone NH was displaced relative to the $P_{2'}$ amide NH in **7** or **8**, as expected; the angle between the pyrrolinone NH and the $Gly^{27}$ carbonyl would accommodate a weak hydrogen bond at best. H-Bonding may also be disfavored by the close contact between the $Gly^{27}$ carbonyl and the adjacent carbon in the pyrrolinone ring as well as the distance (3.4 Å) between the indanol hydroxyl and the backbone NH of $Asp^{29}$. These unfavorable conformational changes and steric effects were reflected in the interaction energies calculated for the pyrrolinone inhibitors and amides **7** and **8** (Table 1). A linear correlation ($R = 0.988$) was observed between the calculated values of $E_{Inter}$ and the experimental binding affinities ($IC_{50}$s); importantly, analogous correlations have heretofore been observed[23] for groups of molecules that bind in similar fashion, suggesting that the pyrrolinones and amides have adopted essentially equivalent bound conformations.

To explore this relationship further we modeled **5**, a *p*-hydroxy analog of **4.** The calculated interaction

energy ($E_{Inter}$ −134.3 kcal/mol) led to a predicted $IC_{50}$ of 2.7 nM. The latter value was expected to be slightly too low; an H-bond in the model, between the *p*-OH in $P_{1'}$ and a nitrogen of $Arg^8$, is not likely to be significant in solution because $Arg^8$ lies exposed to solvent at the end of the active-site cleft. The observed $IC_{50}$ of 6.0 nM agreed well with the calculation.

Ultimately the binding of **6** to the HIV-1 protease was elucidated via X-ray crystallographic analysis of the complex (Figure 1a). Single crystals, obtained at room temperature via vapor diffusion in hanging drops,[24] diffracted to 2.0 Å resolution. The space group was $P2_12_12$ (cell constants: $a = 58.26$ Å, $b = 87.48$ Å, and $c = 46.41$ Å) with one molecule of the complex per asymmetric unit. Following data collection and processing, as described previously, the structure was determined via the difference Fourier method, using the Crixivan complex as the starting model.[24] Data collected at 2−6 Å resolution were refined with XPLOR,[25] yielding an *R* factor of 0.183 with rms bond-length and bond-angle deviations of 0.016 Å and 2.048°, respectively. A single orientation was observed for **6** bound to the protease.

Comparison of the crystal structures of enzyme-bound Crixivan (**18**) and **6** (Figure 1c) revealed shifts of the protease backbone in both the flap and $Thr^{26}$−$Asp^{30}$ regions to accommodate the pyrrolinone ring. As predicted, the pyrrolinone NH does not hydrogen bond to $Gly^{27}$. Surprisingly, this NH does H-bond to the carboxylate of $Asp^{25}$, a catalytic residue of the protease (N−O distance 3.209 Å), resulting in a 90° rotation of the carboxylate relative to the Crixivan complex. In addition, the distance between the indanol hydroxyl and $Asp^{29}$ (5.40 Å) not only is larger than the corresponding values for similar amide-based inhibitors but also exceeds the modeling prediction for **6.** Not unexpectedly, we found that this space is occupied by a water molecule which H-bonds to both the NH of $Asp^{29}$ and the indanol hydroxyl, compensating for the loss of the $Gly^{27}$ hydrogen bond. Recent work has revealed similar binding of water to crystalline HIV-1 protease even in the absence of an inhibitor.[26] The entropically unfavorable inclusion of a water molecule in the active site may contribute significantly (up to ca. 2 kcal/mol) to the lower affinity of **6**.[27] The bound conformation of **6** differed only slightly from the calculated structures in water and chloroform (Figure 1b), and a small rotation (ca. 30°) of the scissile-mimetic bond yields identical backbones (rms 0.164 Å). This preorganization probably accounts for the relatively high affinity observed for the monopyrrolinones despite the disadvantages noted above.

**Summary.** Our search for novel scaffolds with improved pharmacokinetic properties[5c,g] has led to several potent pyrrolinone-based inhibitors of HIV-1 protease. These monopyrrolinones are completely stable to proteases, and **6** proved to be orally bioavailable in dogs. Cocrystallization of **6** with the enzyme and X-ray analysis revealed an unexpected hydrogen bond between $Asp^{25}$ and the pyrrolinone NH as well as the incorporation of a water molecule linking the indane hydroxyl and the NH of $Asp^{29}$. The protease complexes of pyrrolinone **6** and Crixivan both crystallize in a single orientation. Notwithstanding the overall similarity of the latter structures, significant differences in the conformation and H-bonding of the enzyme are discernible both in the active site and in other regions.

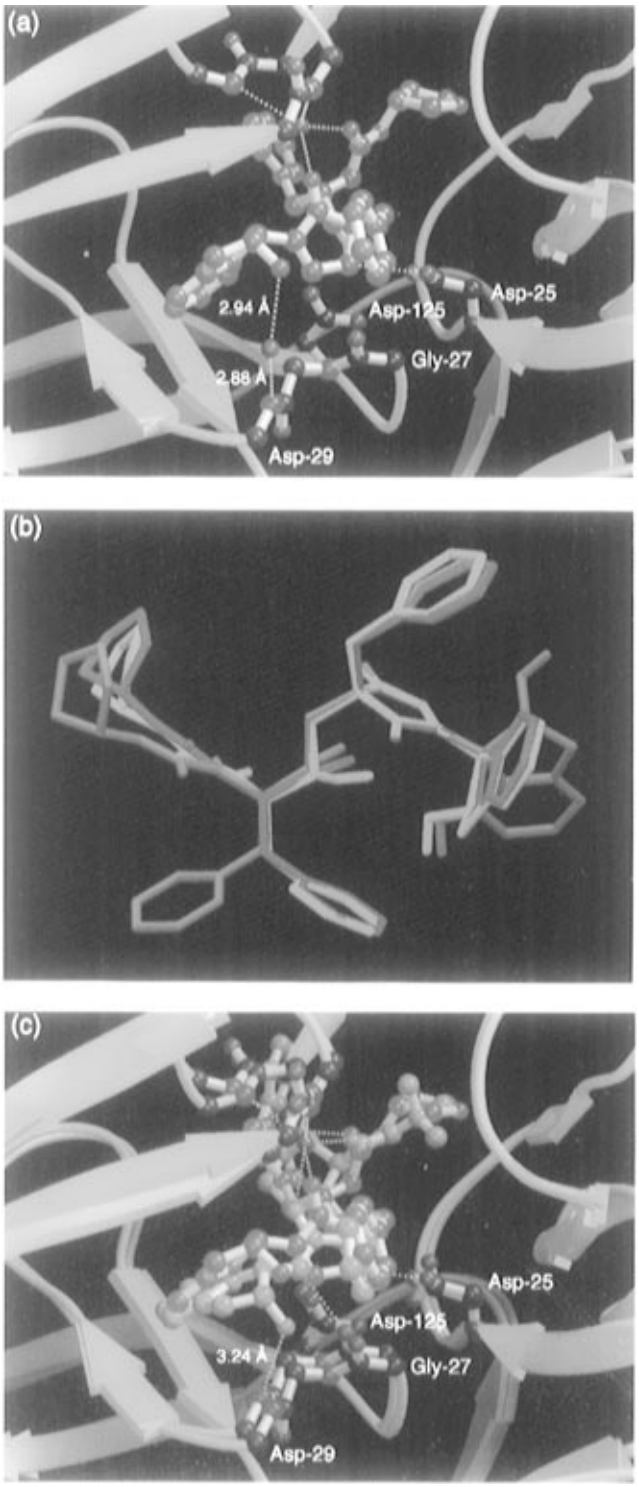



**Figure 1.** (a) X-ray crystal structure of monopyrrolinone **6** bound to HIV-1 protease.[28] (b) Overlay of the lowest-energy linear structures of **6** minimized in chloroform (green) and water (blue) with the enzyme-cocrystallized conformation (purple). (c) Overlay of the HIV-1 protease cocrystal structures of **6** (purple; enzyme, blue) and Crixivan (**18**) (orange; enzyme, yellow).[28]

We believe that if the concept of single-mechanism cocktails[3] of HIV-1 protease inhibitors is eventually validated in the clinic, its benefits can be optimized by maximizing the structural diversity of the protease inhibitors comprising the cocktail. We are currently synthesizing several pyrrolinones designed to provide

higher affinity by preventing the entropically unfavorable binding of water in the active site while maintaining the H-bond to Asp[25].

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**Supporting Information Available:** Plots of the plasma levels of **6** in dogs (po and iv) and of binding affinity vs calculated interaction energy for the pyrrolinone- and amide-based inhibitors; characterization data for **4**−**6**, **13**, **14**, and **16** (5 pages). See any current masthead page for ordering information.

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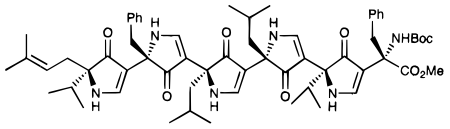


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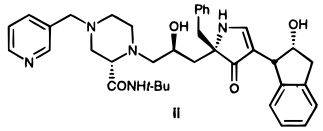


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