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Design, synthesis, and biological evaluation of monopyrrolinone-based HIV-1 protease inhibitors possessing augmented P2′ side chains

Amos B. Smith, III,[a,*] Adam K. Charnley,[a] Hironori Harada,[a] Jason J. Beiger,[a] Louis-David Cantin,[a] Craig S. Kenesky,[a] Ralph Hirschmann,[a] Sanjeev Munshi,[b] David B. Olsen,[b] Mark W. Stahlhut,[b] William A. Schleif[b] and Lawrence C. Kuo[b]

[a]*Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104, USA*
[b]*Merck Research Laboratories, West Point, PA 19486, USA*

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Abstract—A series of monopyrrolinone-based HIV-1 protease inhibitors possessing rationally designed P2′ side chains have been synthesized and evaluated for activity against wild-type HIV-1 protease. The most potent inhibitor displays subnanomolar potency in vitro for the wild-type HIV-1 protease. Additionally, the monopyrrolinone inhibitors retain potency in cellular assays against clinically significant mutant forms of the virus. X-ray structures of these inhibitors bound in the wild-type enzyme reveal important insights into the observed biological activity.

Despite significant advances in the treatment of AIDS, in particular the advent of HAART (highly active anti-retroviral therapy), the development of new HIV therapeutics remains essential to combat this epidemic.[1] The remarkable ability of the virus to circumvent current clinical agents via mutation clearly highlights the need for improved therapeutics. In fact, development of a cocktail of highly potent, structurally diverse inhibitors of HIV-1 encoded enzymes may provide the best opportunity to thwart viral resistance.[2]

A principal goal of the Smith/Hirschmann collaboration at Penn has been the development of the pyrrolinone scaffold as a privileged peptidomimetic. The homochiral 3,5-linked pyrrolinone scaffold (Fig. 1) was first introduced in 1992 and has been demonstrated to be a highly successful β-strand/β-sheet mimic,[3] while the heterochiral 3,5-linked pyrrolinone (Fig. 1) scaffold has recently been shown to hold considerable potential as a β-turn mimic.[4]

Aspartic acid proteases including the HIV-1 protease most often bind their substrate in an extended β-strand conformation, and thus, we initiated a program to develop potent HIV-1 protease inhibitors based on our pyrrolinone β-strand peptidomimetic scaffold. Our early work directed at pyrrolinone-based HIV-1 protease inhibitors resulted in a series of bispyrrolinones which displayed both potent (nM) activity, and improved cell permeability relative to peptidal inhibitors, yet lacked oral bioavailability in dogs.[5] Reasoning that the lack of oral bioavailability was due, at least in part, to their high molecular weight, we subsequently designed and synthesized a monopyrrolinone-based HIV-1 protease inhibitor (−)-**1** (Fig. 2), which proved to be both potent (nM) and to possess 13% oral bioavailability in dogs.[6] X-ray structure determination of (−)-**1** cocrystallized

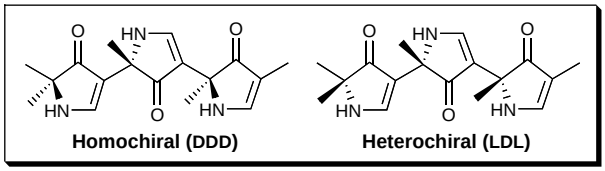

Figure 1. Homochiral (DDD) and heterochiral (LDL) polypyrrolinones.

Keywords: Pyrrolinone; HIV-1; Protease inhibitors.
* Corresponding author. Tel.: +1 215 898 4860; fax: +1 215 898 5129; e-mail: smithab@sas.upenn.edu

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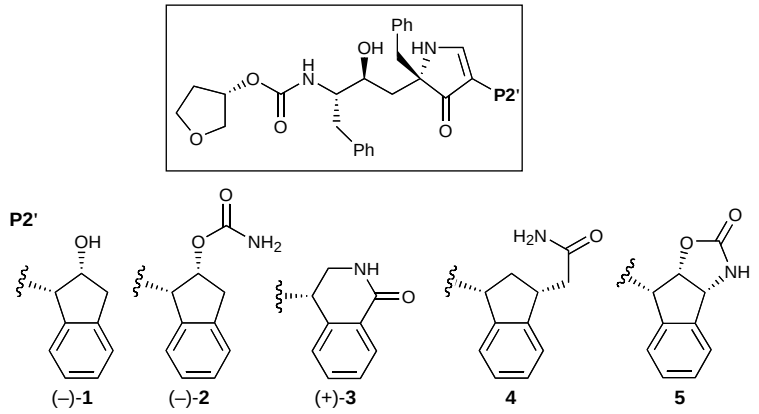


Figure 2. Monopyrrolinone-based HIV-1 protease inhibitors.

with the wild-type HIV-1 protease revealed the presence of a water molecule bridging the indanol hydroxyl on the P2′ side chain and the Asp29 NH in the S2 pocket.[6] Based on X-ray crystallographic information, molecular modeling led to the design of inhibitors with modified P2′ side chains [cf. (−)-**2** and (+)-**3**] holding the promise of improved potency via displacement of the adventitious water molecule. Unfortunately, these early attempts proved unsuccessful.[6b]

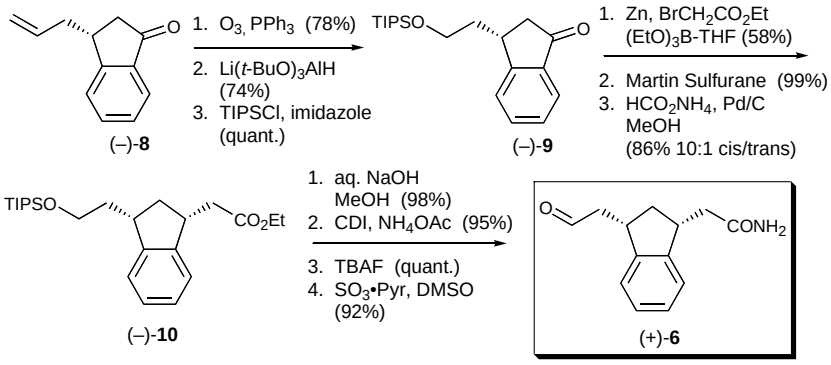
In continuation of our efforts to prepare highly potent pyrrolinone-based HIV-1 protease inhibitors with improved P2′ side chains, we report here the design, synthesis, and biological evaluation of a third-generation series of monopyrrolinone-based inhibitors (**4** and **5**), including the X-ray analysis of the inhibitors cocrystallized with wild-type HIV-1 protease.

Molecular modeling suggested that P2′ side chains incorporating the benzylic acetamide and cyclic carbamate substituted indane skeletons (**4** and **5**, respectively) would improve the hydrogen-bonding interactions in the S2′ pocket, particularly with Asp29. In addition, the cyclic carbamate could be anticipated to gain additional potency via preorganization of the side chain, thereby reducing the entropy penalty associated with binding.[7]

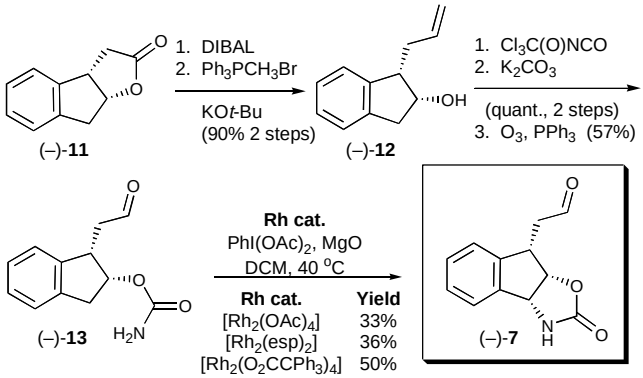
From the synthetic perspective, incorporation of the P2′ side chains into our pyrrolinone synthetic protocol called for preparation of aldehydes (+)-**6** and (−)-**7** (Schemes 1 and 2, respectively). The synthesis of (+)-**6** began with allyl indanone (−)-**8**, which was readily available in enantiopure form via the Johnson sulfoxide resolution method.[8] Ozonolysis of the olefin followed in turn by chemoselective reduction of the aldehyde and protection as the triisopropylsilyl ether (TIPS) provided ketone (−)-**9**. Attachment of the side chain onto ketone (−)-**9** was achieved via Reformatsky addition, followed by elimination of the resulting alcohol[9] and hydrogenation to afford ester (−)-**10** as a mixture (10:1) of diastereomers favoring the desired *cis*-product. Ester (−)-**10** was then converted to the primary amide in two steps, followed by removal of the silyl-protecting group and oxidation of the resulting alcohol to furnish aldehyde (+)-**6**.

For the preparation of aldehyde (−)-**7**, we turned to the rhodium-mediated nitrene C–H insertion chemistry developed by Du Bois et al.[10] Here we envisioned **7** to arise from carbamate **13** (Scheme 2) via a late-stage C–H insertion. In the event, (−)-**13** was prepared from known lactone (−)-**11**, the latter readily available from the synthesis of (−)-**1**. Initially, the C–H insertion proceeded only in modest yield with $[Rh_2(OAc)_4]$ and $[Rh_2(esp)_2]$; better results (ca. 50%) were obtained with $[Rh_2(O_2CPh_3)_4]$.

With aldehydes (+)-**6** and (−)-**7** in hand, application of our pyrrolinone cyclization protocol involving imine formation followed by metalloenamine-mediated



Scheme 1. Preparation of aldehyde (+)-**6**.

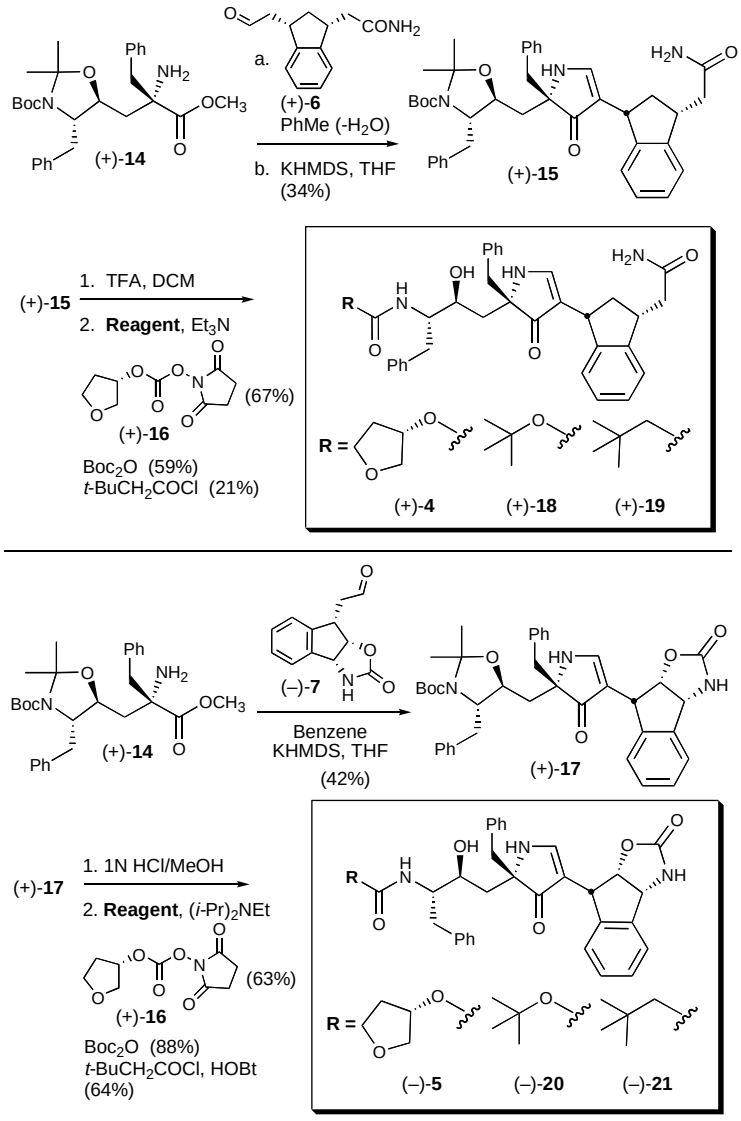


Scheme 2. Preparation of aldehyde (−)-**7**.

cyclization,[3b] proceeded with both aldehydes, albeit the yields were modest (Scheme 3). That pyrrolinone formation proved viable in the presence of amide and carbamate hydrogens was however gratifying, and in the case of amide (+)-**6**, represents the first demonstration of such functional compatibility. Global deprotection of the resultant pyrrolinones, followed by treatment with succinimidyl carbonate (+)-**16**,[11] provided the prospective inhibitors (+)-**15** and (+)-**17** in good yield for the two steps. In addition to the targeted compounds, analogues incorporating *t*-butyl carbamate and *t*-butyl acetamide P2 substituents were prepared in a similar fashion [e.g. (+)-**18** to (−)-**21**].

The monopyrrolinone inhibitors were evaluated for activity against wild-type HIV-1 protease in isolated enzyme[12] and cellular[13] assays (Table 1). Pleasingly, the prospective inhibitors, with the exception of those incorporating a *t*-butyl acetamide at P2 [e.g. (+)-**19** and (−)-**21**], displayed potent activity in the HIV-1 protease enzyme assay. Analogue (−)-**5** exhibits subnanomolar activity, and as such represents an order of magnitude improvement in potency relative to our first- and second-generation pyrrolinone-based inhibitors.

Activity in the cellular assays, however, did not reflect the improved in vitro potency, resulting in significant but somewhat diminished CIC_{95} values, with higher



Scheme 3. Preparation of monopyrrolinone-based protease inhibitors.

Table 1. Bioassay data for monopyrrolinone-based HIV-1 protease inhibitors

Inhibitor	IC_{50} (nM)	CIC_{95} (nM)	C/I ratio
(−)-**1**	2	100–250	50–125
(−)-**2**	2	250	125
(+)-**3**	5.7	781	137
(+)-**4**	2.4	781	325
(−)-**5**	0.44	400	909
(+)-**18**	20.5	>400	
(+)-**19**	>250	12,500	
(−)-**20**	2.5	200	80
(−)-**21**	96	3125	32.5
Indinavir	0.36	25–100	69–277

Experimental error for IC_{50} is ±5%, based on standard errors of the mean, and for CIC_{95}, one-half to 2-fold of the value shown, based on serial dilutions of compound concentration.

CIC_{95}/IC_{50} (C/I) ratios relative to our previous inhibitors [ca. (−)-**1**] as well as indinavir.[6b]

Having demonstrated both potent in vitro and significant in vivo activity against wild-type HIV-1, we next tested the most potent third- generation inhibitors against three clinically significant mutant virus strains.[14] Pleasingly, the third- generation pyrrolinone-based inhibitors maintained good inhibitory activity across the panel of clinically significant mutants. Additionally, by comparing wild-type CIC_{95} values, the pyrrolinone-based inhibitors displayed minimal effects from protein binding [in the presence of either 10% fetal bovine serum (Table 2) or 50% normal human serum (Table 3)]. These results are quite encouraging and support our belief that the binding conformation of the pyrrolinone scaffold may be sufficiently unique, so as to overcome established mutations.

Table 2. Bioassay data against different viral isolates in the presence of 10% normal human serum (NHS) or 10% fetal bovine serum (FBS)

Inhibitor	CIC_{95} (nM)				
	Wild type		Mutants		
	H9IIIB (FBS)	NL4-3 (FBS)	1002-60C	4X	1026-60C
(−)-**1**	156	78	1250	625	156
(+)-**4**	1250	312	1250	625	312
(−)-**5**	625	625	1250	156	312
(−)-**20**	156	156	2500	2500	625
Indinavir	50	50	400	100	400

Table 3. Bioassay data against different viral isolates in the presence of 50% normal human serum (NHS)

Inhibitor	CIC_{95} (nM)				
	Wild type		Mutant		
	H9IIIB	NL4-3	1002-60C	4X	1026-60C
(−)-**1**	156	156	1250	1250	312
(+)-**4**	1,250	625	2,500	1250	625
(−)-**5**	625	625	1250	312	312
(−)-**20**	312	625	10,000	5000	1250
Indinavir	25	50	400	100	400

Experimental error for CIC_{95}, one-half to 2-fold of the value shown, based on serial dilutions of compound concentration.

X-ray structures of the third-generation inhibitors (+)-**4** and (−)-**5** complexed with wild-type HIV-1 protease were obtained. Analysis of the cocrystal structure of (+)-**4** indicated that, as predicted, the P2′ substituent had displaced the undesired[6b] water molecule (Fig. 3), despite the fact that we did not obtain an increase in potency.[15] Gratifyingly, the cocrystal structure of (−)-**5**, our most potent inhibitor (IC_{50} 0.44 nM), revealed that we had again succeeded in displacing the water molecule, reestablishing important interactions in the S2′ pocket (Fig. 4).[16]

Comparison of the P2′ side chains of (−)-**5** and (+)-**4** indicates that the indane ring and the cyclic carbamate of (−)-**5** are superimposable onto the indane ring and benzylic acetamide, respectively, of (+)-**4**. In the two X-ray structures, the *B*-factors associated with these moieties are indistinguishable within experimental error. For these reasons, the increase in binding affinity (∼1 kcal/mol) of (−)-**5** over that of (+)-**4** can be attributed to a difference in ground-state energetics between the two compounds in the bulk solvent, likely a result of reducing the rotational entropy arising from the benzylic acetamide of (+)-**4** (i.e., preorganization).[7]

The design, synthesis, biological evaluation, and X-ray structural analyses of a series of third-generation monopyrrolinone HIV-1 protease inhibitors have been achieved. These studies culminated in the preparation

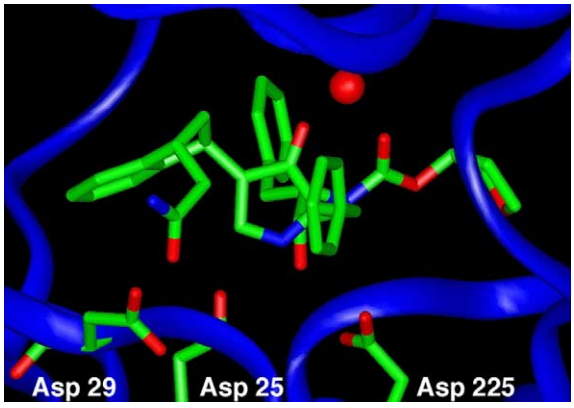


Figure 3. X-ray cocrystal structure of (+)-**4** with HIV-1 protease.

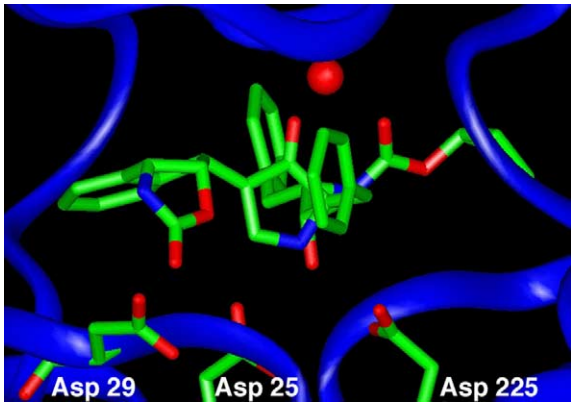


Figure 4. X-ray cocrystal structure of (−)-**5** with HIV-1 protease.

of (−)-**5** displaying subnanomolar activity in vitro. The combination of potent biological activities and cocrystal structure analysis validated our hypothesis that rational modification of the P2′ side chain of these monopyrrolinone-based HIV-1 protease inhibitors would lead to an improvement in in vitro potency. Importantly, the third-generation monopyrrolinone-based inhibitors maintained their in vivo activity against several clinically significant HIV-1 mutants, further illustrating the potential value of the pyrrolinone inhibitor scaffold.

Acknowledgments

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15. This structure has been deposited with the Protein Data Bank with Accession No. 2BBB.
16. This structure has been deposited with the Protein Data Bank with Accession No. 2BB9.