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Pyrrolidine-5,5-*trans*-lactams. 2. The Use of X-ray Crystal Structure Data in the Optimization of P3 and P4 Substituents

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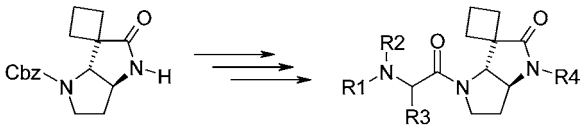
ABSTRACT



In this, the second of two letters, we describe the elaboration of the pyrrolidine-5,5-*trans*-lactam template to delineate the requirements for optimal substitution of the pyrrolidine and lactam nitrogen atoms. Central to the strategy is the use of rapid iterative synthesis in conjunction with X-ray crystal structure determination of ligand–protein complexes.

Hepatitis C virus (HCV) infects chronically an estimated 3% of the global human population,[1] often leading to cirrhosis, hepatocellular carcinoma, and liver failure in later life.[2] Current therapies are based upon interferon-α, alone or in combination with ribavirin. Although sustained response rates are markedly improved using combination therapies, at least 50% of patients fail to show a sustained response. Additionally, current therapies have the disadvantage of frequent and severe side-effects.[3] The development of new therapies for treating HCV infection effectively is thus of paramount importance and is currently an intensive area of research.[4]

HCV is a small, enveloped virus, the genome of which is a 9.5 kb single-stranded RNA that encodes for a single polyprotein of 3010−3030 amino acids. Mature nonstructural replicative proteins are released from this polyprotein by the action of the viral proteases NS2 and NS3. It has been established that introducing mutations into the NS3 protease region of the HCV genome abolishes infectivity,[5] demonstrating that NS3 protease is thus an essential viral function and should prove to be an excellent target for the development of novel anti-HCV agents.

In the preceding Letter,[6] we outlined the synthesis and incorporation of the α,α-disubstituted *trans*-lactam core into nonpeptidic inhibitors of the NS3 protease; the optimization of the P1 substituent was described. Herein, we describe how initial X-ray structures and molecular models obtained with **1** and **2** (Figure 1) were used to guide the selection of potency enhancing substituents on the pyrrolidine and lactam nitrogen atoms.

Crystallographic Evaluation. The potency of spirocylobutyl inhibitor **2** can be rationalized by modeling the inhibitor into previously reported crystal structures;[7] the spirocylobutyl fills the S1 pocket more optimally than the α-ethyl side chain of **1**. Modeling the interaction of the Boc-

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(2) (a) Gerber, M. A. *J. Hepatol*. **1993**, *17* (suppl. 3), 108. (b) Alter, M. J. *Hepatology* **1997**, *26*, 62. (c) Hoofnagle, J. H. *Hepatology* **1997**, *26*, 15S. (d) Kwong, A. D. *Antiviral Res.* **1998**, *40*, 1. (e) Hoofnagle, J. H. *Digestion* **1998**, *59*, 563.

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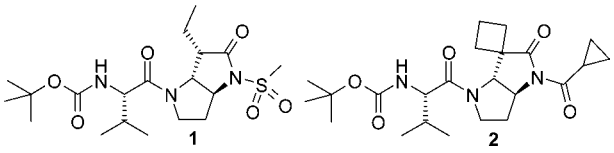


Figure 1. trans-Lactams used in X-ray crystallographic evaluation and modeling.

valine substituent offers considerably greater challenges, however, and only a direct determination of an X-ray crystal structure can unequivocally establish how these inhibitors bind.

A 2.6 Å resolution X-ray structure of **1** was obtained by soaking preformed crystals of the truncated NS3 protease domain: NS4A cofactor (tNS3:NS4A)[7] (pH 6.5, compound concentration 2mM, 4% DMSO, 48 h).[8] Although the crystals were found to be only modestly diffracting, a number of key features could be established (Figure 2). The core of the

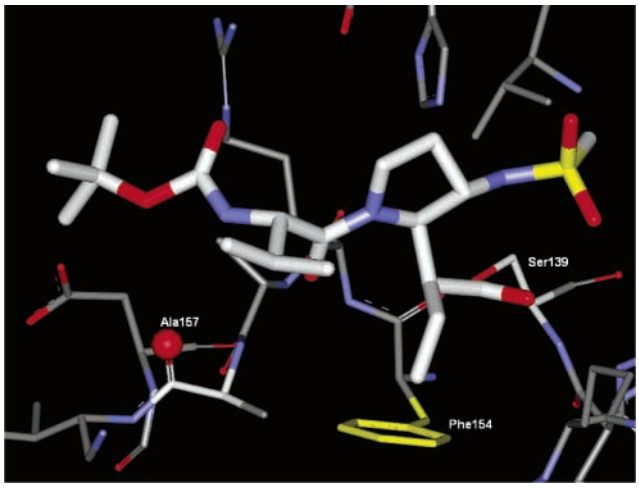


Figure 2. Crystal structure of **1** complexed with HCV NS3 protease. Bold structure is the ring-opened form of **1** covalently bound to Ser139. Key protein residues (referred to in the text) are drawn with thick bonds, and other NS3 residues are drawn with thin bonds.

molecule corresponds to the areas of electron density that are best defined, and the inhibitor is clearly seen as being covalently linked to the protein through Ser139. The α-ethyl side chain is seen in S1, which has Phe154 at the base of the pocket, accounting for the substrate preference for cysteine at this position. The isopropyl side chain of valine overlays a shallow, solvent-exposed indentation on the protein surface, suggesting that it should be possible to substitute this position with a variety of groups; however, it is unlikely that potency will be significantly impacted, either positively or negatively. The urethane nitrogen is within 3.4 Å of the carbonyl of Ala157, which is slightly too far to be considered a viable hydrogen bond, although we considered that this distance would be more likely in the region of 2.9 Å in the modeled preacylation complex. The carbonyl oxygen of the urethane is solvent exposed and unlikely to contribute much to binding; the bridging oxygen may also be within hydrogen-bonding distance to the oxygen of Ala157. This oxygen−oxygen interaction is clearly not a hydrogen-bonding combination, but it suggests that in future analogues, the urethane should be replaced with a urea moiety. The *tert*-butyl group is just above and to the edge of S4, suggesting that a methylene spacer linking to a small hydrophobic group may improve binding. At the other end of the molecule, it is very difficult to rationalize the role of the lactam substituent because the structure only presents a picture of the postacylation complex. More useful from the perspective of molecule design is a model of the preacylation complex, and thus an attempt was made to construct a model of the preacylation complex computationally. Figure 3 illustrates

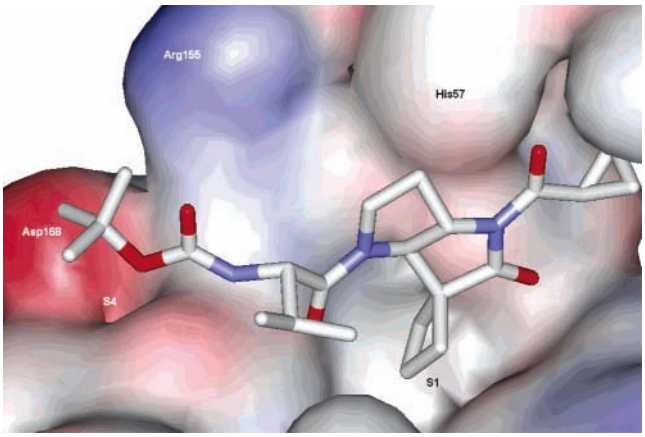


Figure 3. Computational model of preacylation complex of **2** with HCV NS3 protease. Solvent-accessible protein surface is colored by electrostatic potential, and residues and subsites referred to in the text are marked.

a model built on the basis of compound **2**, utilizing the crystallographic data on the acylation complex of **1**. The rigidity of the *trans*-lactam core made the reconstitution of the *trans*-lactam system a trivial exercise, and this fragment was then docked into the enzyme active site. Constraining the spirocyclobutyl to fit into S1 and maintaining the distance between the side chain oxygen of Serine-139 and the lactam carbonyl to ca. 2.6 Å ensures correct overall orientation.

Optimization of P3 Substituent. Inspection of the crystal structures and models (Figures 2 and 3) suggested amino acid scanning as a means of enhancing binding in this region. Boc- or Cbz-protected α-amino acids bearing the normal range of functionality were synthesized as described for **2**, and a selection of the results obtained are summarized (Figure 4, information on rapid array synthesis, compound characterization, and further analogues are described in Supporting Information).

(8) Atomic coordinates have been deposited in the Protein Data Bank as 1N1L.

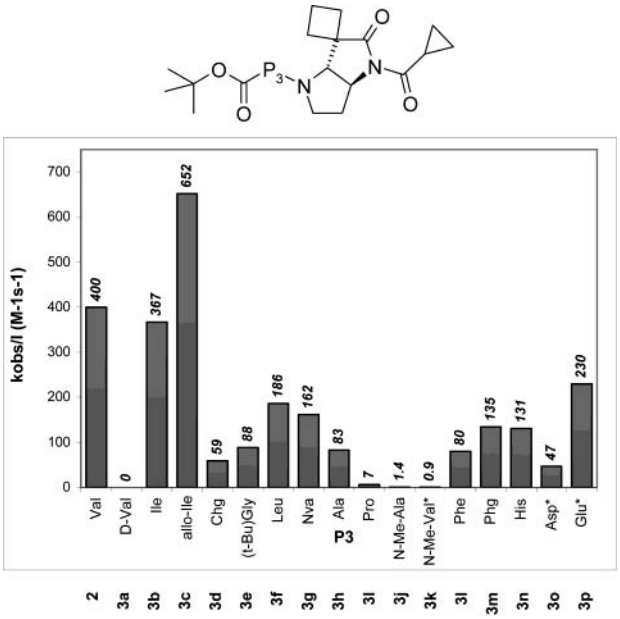


Figure 4. Optimization of P3 substituent.

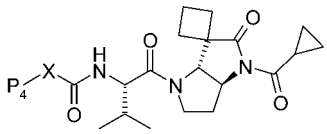
Data are consistent with the X-ray structural and modeling data. Only the L-α-amino acids are accommodated, with a strong preference for the more bulky *β*-branched residues (cf. Val, Ile, and notably *allo*-Ile (**3c**) with Nva; cf. Phg with Phe). Charged residues are less potent than Valine despite the known ability of the enzyme to accommodate acidic residues in the 4A/4B substrate.[9] N-Methylated amino acids are very poorly active, confirming the likely critical role of the hydrogen bond to the carbonyl of Ala157.

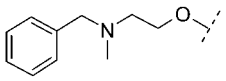
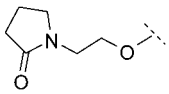
Optimization of the P4 Substituent. Having investigated the requirements for the P3 substituent, we next turned our attention to P4. Figures 2 and 3 show the *tert*-butyl group to lie just at the lip of the S4 pocket. An array of compounds was synthesized by rapid array methodology with the objective of improving both the potency and overall molecule properties. (Information on rapid array synthesis and compound characterization is described in Supporting Information). Several groups containing a basic nitrogen were synthesized with the objective of enhancing the solubility (**4a**−**d**, Table 1) and interacting with Asp168.

Linear, flexible capping groups were investigated (**4a** and **4b**) along with more compact, cyclic groups connected by a linker of varying length (**4c** and **4d**). The poor potency of the groups investigated illustrates that it is difficult to obtain increases in potency by making interactions with highly solvent-exposed charged residues. Other substituents bearing hydrogen-bonding functionality also failed to show any improvement (**4e**).

Compact, hydrophobic groups were also evaluated. The most important observation was that introducing a spacer methylene modestly improved potency, presumably by providing additional conformational flexibility (cf. **4f**−**i** with **2**). However, optimal potency is demonstrated by groups of similar size to a compact C4-methyl unit (**4g** and **4i**) or C5-methyl unit (**4f**), since smaller (**4j**) or larger (**4k**) groups are less potent. In general, replacing the urethane (**4l**) with the more flexible carboxamide linker (**4m**) reduced potency. This trend was reproduced in the cyclohexyl series: interestingly, although the cyclohexyl carbamoyl (**4n**) is suboptimal, changing the linking oxygen to methylene (**4o**) is deleterious, although synthesis of the urea analogue **4p** gives a dramatic enhancement in potency, possibly as a consequence of formation of the putative hydrogen bond. Future communications will elaborate the extent and limitations of this interesting observation.

Table 1. Optimization of P4 Substituent



Cpd #	P4	X	Kobs/I ($M^{-1}s^{-1}$)
2	*tert*-Butyl	O	400
4a	2-Dimethylaminoethyl	O	48
4b		O	54
4c	2-Morpholinoethyl	O	73
4d	3-Piperidinopropyl	O	81
4e		O	140
4f	Cyclopentylmethyl	O	461
4g	Isobutyl	O	516
4h	1-Methylcyclopropylmethyl	O	610
4i	Cyclobutylmethyl	O	658
4j	Cyclopropylmethyl	O	268
4k	Cyclohexylmethyl	O	155
4l	Benzyl	O	328
4m	Phenoxy	CH_2	150
4n	Cyclohexyl	O	128
4o	Cyclohexyl	CH_2	20
4p	Cyclohexyl	NH	901

Replicon Cell-Based Assay Results. Representative compounds were examined in a replicon surrogate cellular assay system.[10] Overall, a reasonable correlation between enzyme and cellular assay data was observed. A number of factors

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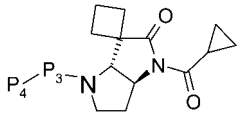
Table 2. Replicon Cell-Based Assay IC_{50} Values for a Range of P3- and P4-Modified Pyrrolidine-5,5-*trans*-lactams



compd	P4	P3	IC_{50} (uM)
2	Boc	Val	5.7
3a	Boc	D-Val	>100
3b	Boc	Ile	10.3
3c	Boc	*allo*-isoloeucine	15.6
3d	Boc	cyclohexylglycine	3.7[1]
3f	Boc	Leu	14.8
3h	Boc	Ala	38
3k	Cbz	*N*-Me-Val	>100
3p	Cbz	Glu	9.3[11]
4c	2-morpholino-ethyloxycarbonyl	Val	24[11]
4d	3-piperidino-propyloxycarbonyl	Val	8.3
4g	isobutyloxycarbonyl	Val	8.6
4l	Cbz	Val	22

impact cellular potency, but it is clear that prerequisites for activity are good cellular penetration in conjunction with k_{obs}/I greater than 150 M^{-1} s^{-1}. Compounds **2**, **3b**−**f**, and **4g**, all rather close structural analogues, demonstrate biochemical potency in a very similar range and, reassuringly, yield replicon IC_{50}s falling in a narrow range. Compounds bearing highly polar groups fail to show activity, e.g., **3p**, **4c**, **4d**, most likely as a result of poor membrane permeability or insufficient inherent biochemical potency. Compound **3d** appears anomalously active (in the light of its biochemical potency); however, in-assay cellular toxicity was evident at concentrations 10 times the apparent IC_{50} and is the probable explanation.

In conclusion, we have shown that rapid array synthesis can be used in conjunction with crystallographic and modeling data to demonstrate that one of the optimal substituents on the *trans*-lactam pyrrolidine nitrogen is valine. We have also shown that the valine nitrogen may be further substituted with small, hydrophobic carbamates to yield drug-like molecules in which potency is maintained and in some cases enhanced.

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Supporting Information Available: Detailed experimental procedures for synthesis of representative compounds and characterization data for test compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) In-assay toxicity observed at concentrations <10-fold greater than the quoted IC_{50}.