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Pyrrolidine-5,5-*trans*-lactams. 1. Synthesis and Incorporation into Inhibitors of Hepatitis C Virus NS3/4A Protease

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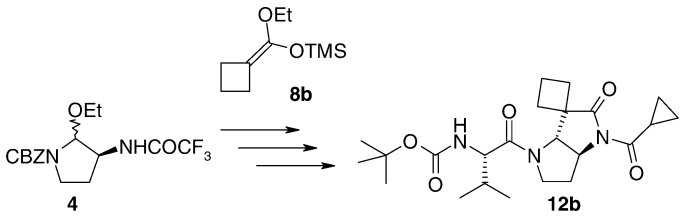
ABSTRACT



In this, the first of two letters, we outline the use of the pyrrolidine-5,5-*trans*-lactam template to design small, neutral, mechanism-based inhibitors of hepatitis C NS3/4A protease. The hitherto unreported reaction of the acyl iminium ion precursor 4 with dialkyl-substituted silyl ketene acetals (e.g., 8b) is described. Compound 12b, with a spirocyclobutyl P1 substituent and a cyclopropylacyl substituent on the lactam nitrogen, has a k_{obs}/I of 400 M^{-1} s^{-1} and demonstrates activity in a replicon cell-based surrogate HCV assay.

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] It has been estimated that of those currently infected, 20% and 4% are likely to develop liver cirrhosis and liver cancer, respectively, in the next decade.[3] 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.[4] The development of new therapies to treat HCV infection effectively is thus of paramount importance, and is currently an intensive area of research.[5]

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. This polyprotein is processed by cellular signal peptidases to produce the structural viral proteins (C, E1, E2, p7), whereas viral proteases (NS2, NS3) are responsible for the production of mature nonstructural replicative proteins. The multifunctional 70 kD NS3 protein is the most extensively studied viral protein.[6] The amino terminal third of the protein is a trypsin-

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like serine protease that cleaves the NS3−4A, NS4A−NS4B, NS4B−NS5A, and NS5A−NS5B junctions. Although isolated NS3 is enzymatically active, it forms a heterodimer with the NS4A cofactor, which is believed to be the physiologically most relevant form of the enzyme.[7] It has been reported that when appropriate mutations were introduced into the NS3 protease region of the HCV genome, the infectivity of these RNAs in chimpanzees was abolished.[8] NS3 protease is thus an essential viral function and should prove to be an excellent target for the development of novel anti-HCV agents.

A number of peptidic and nonpeptidic inhibitors of the NS3/4A serine protease have been reported.[9] The majority of the former have been competitive inhibitors designed from peptide substrates or cleavage products, while nonpeptidic molecules have emerged through random screening and have displayed noncompetitive mechanisms of action. As a starting point for the design of inhibitors we sought to take advantage of the pyrrolidine-5,5-*trans*-lactam template, suitable substitution of which allows access to the S1, S1′, and S3−S4 specificity pockets[10] and traps the active site serine hydroxyl group by acylation (Figure 1).

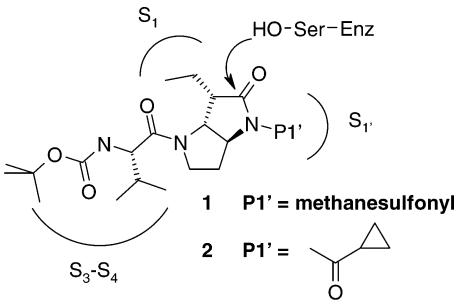

Figure 1. Ethyl pyrrolidine-5,5-*trans*-lactam template: potential interactions with protease subsites.

The *trans*-lactam template has been found to be widely applicable to a number of serine proteases. These templates, developed as thrombin,[11] human neutrophil elastase (HNE),[12] and human cytomegalovirus (HCMV) inhibitors,[13] are active intracellularly, stable in plasma, and orally active in vivo. The first report concerning the application of this template to HCV NS3/4A protease described α- and *β*-ethyl-substituted *trans*-lactams.[14] The first generation inhibitors (e.g., **1**) suffered from modest hydrolytic stability in plasma (mediated by nonspecific esterases/hydrolases, analogous to experience in the elastase area[15]) and limited synthetic accessibility. We hypothesised that the stability issue could be overcome by preparing the α,α-disubstituted *trans*-lactam templates **3a**−**c** (Figure 2), which would also lead to a

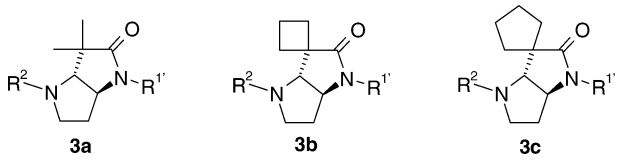

Figure 2. α,α-Disubstituted pyrrolidine-5,5-*trans*-lactams.

considerable simplification of the synthetic challenge posed by the template, since it would remove a difficult to introduce chiral center. Our earlier results had demonstrated that the S1 pocket was capable of accommodating an ethyl side chain in either the α- or *β*-configuration. Modeling studies (based upon crystal structures of ethyl *trans*-lactams soaked into NS3 protease) suggested that the S1 pocket should be capable of accommodating an α,α-dimethyl or spirocyclobutyl-substituted *trans*-lactam. Concise syntheses of **3a**−**c** and their elaboration into potent, low molecular weight, nonpeptidic NS3/4A protease inhibitors are reported below.

Dimethyl compound **3a** can be accessed by the direct alkylation methodology of Borthwick et al.,[16] but we chose to utilize the more flexible acyliminium ion methodology developed by Macdonald et al.[12,17] (Scheme 1).

Key intermediate **4** was prepared in a chirally pure form from L-methionine by reported methods.[12] Silyl ketene acetals **8b** and **8c** were obtained by modification of the method of Ainsworth et al.[18] and subjected to Lewis acid-mediated coupling with acyliminium ion precursor **4** to yield fully protected amino esters **5a**−**c**. Trifluoroacetamide hydrolysis was performed on unpurified **5a**−**c** since a facile acid/base extraction was found to be sufficient to permit isolation of the amino esters **6a**−**c** in excellent yield and purity. In all cases, ring closure with tBuMgCl to form the *trans*-lactam proceeded in excellent yield.

Our previous studies had shown that a simple Boc-Valine substituent at R^2 could furnish compounds displaying excellent activity in combination with small, electron-withdrawing substituents on the lactam nitrogen (see Figure 2). Our earlier studies had demonstrated that substituting the lactam with a methanesulfonyl group generated a highly electrophilic series of compounds susceptible to rapid plasma hydrolysis. Although α,α-disubstitution on the lactam ring improved stability, hydrolysis still proceeded at an unacceptable rate

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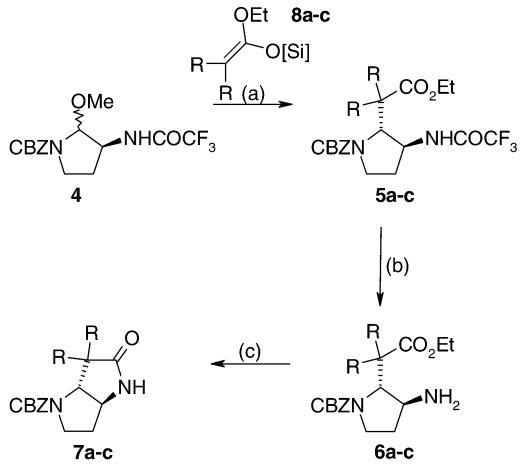
Scheme 1. Synthesis of α,α-Disubstituted Pyrrolidine-5,5-*trans*-lactam Core Template



Reagents and Conditions :
a) $BF_3.OEt_2$, DCM
b) K_2CO_3, EtOH
c) tBuMgCl, THF

			Yield (%)		
			5	**6**	**7**
R = R =	methyl	**a**	Quant.	65	90
R =	Cyclobutyl	**b**	Quant.	Quant.	78
R =	Cyclopentyl	**c**	Quant.	92	71

(data not shown). In parallel with our structure-based design efforts, we also sought to capitalize on experience gained by our co-workers working on the HNE and HCMV protease targets, and to this end, representative compound collections from both programs were screened in a medium-throughput manner. One compound highlighted as a result of this exercise was an α-methyl-substituted *trans*-lactam **9**,[19] bearing a cyclopropylacyl lactam substituent (Figure 3). We

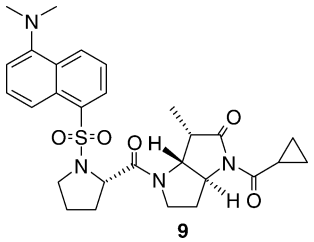


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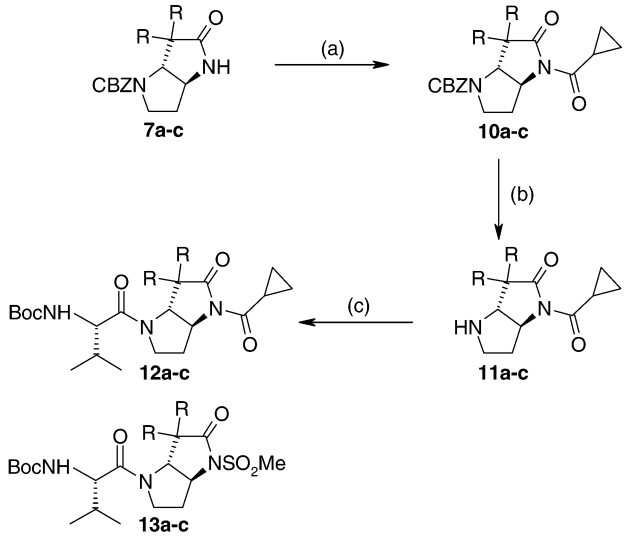
Figure 3. Cyclopropyl substituted pyrrolidine-5,5-*trans*-lactam.

considered that this compound was probably a genuine "hit" since, although the single α-methyl substituent fills S1 suboptimally, the small lactam substituent should be well accommodated in the S1′ pocket of NS3 protease. Thus, compounds **12** and **13** were selected as final targets. Deprotonation of **7a**−**c** with lithium hexamethyldisilazide followed by addition of cyclopropane carbonyl chloride yielded **10a**−**c**. Removal of the benzyl carbamate with

Scheme 2. Elaboration of Core Template



Reagents and Conditions :
a) LiHMDS, cyclopropane carbonyl chloride (or MsCl), THF
b) H_2, Pd/C, ethyl acetate/ethanol, HCl
c) HATU, DIPEA, RCO_2H, MeCN or DMF

			Yield (%)			
			10	**11**	**12**	**13**
R = R =	R = methyl	**a**	83	82	Quant.	93
R =	Cyclobutyl	**b**	73	99	76	89
R =	Cyclopentyl	**c**	66	87	76	84

hydrogen over 10% Pd/C in the presence of 1 equiv of HCl proceeded smoothly to provide amines **11a**−**c** as hydrochloride salts. HATU-mediated coupling was employed to introduce the Boc-Valine substituent to provide **12a**−**c** in excellent yields. Compounds **13a**−**c** were analogously prepared using methanesulfonyl chloride in place of cyclopropane carbonyl chloride.

Compounds were initially assayed in a chromogenic assay, determining an IC_{50} following 4 h of enzyme/inhibitor preincubation, and this enabled the compounds to be crudely ranked (Table 1). It is apparent that α,α-dimethyl and spirocyclopentyl are roughly equivalent in potency, being approximately 10-fold less potent than the spirocyclobutyl series. The enhanced potency of the latter could be a consequence of ring strain (reactivity) or a more optimal fit in S1. In the case of the spirocyclopentyl substituent (and for dimethyl), the side-chain internal bond angle at the spiro junction is tetrahedral, whereas in the case of the spirocyclobutyl, the angle is near 90°.[20] This may contribute to increased lactam ring strain in the latter, which may result in enhanced reactivity. Future work (based upon the methodology of Sykes et al.[21]) will investigate this point. Alternatively, the improved potency of spirocyclobutyl is a consequence of a better fit in S1.

It is also clear that although substituting the lactam nitrogen with a methanesulfonyl moiety should render the compounds much more electrophilic than the more sterically

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Table 1. HCV NS3/4A Protease Inhibitory Activity and Plasma Stability of Pyrrolidine-5,5-*trans*-lactams

R =	R1′ = methanesulfonyl	HCV protease IC_{50} (uM)[22]	R1′ = cyclopropylacyl	HCV protease IC_{50} (uM)	HCV protease K_{obs}/I (M^{-1} s^{-1})[23]	human plasma stability (% turnover after 4 h)[24]
α-ethyl	**1**	30	**2**	8.9		64
α-methyl			**9**	97		42
dimethyl	**13a**	34	**12a**	3	31	21
cyclobutyl	**13b**	4.4	**12b**	0.51	400	28
cyclopentyl	**13c**	31	**12c**	2.5	36	39

hindered cyclopropylacyl compounds, it is the latter that are better recognized by the protease. The 10-fold enhancement in potency demonstrated by this modification arises from the fact that the planar bond angle of the acyl group secures better binding than the tetrahedral methanesulfonyl. In addition, it is suggested that the cyclopropyl moiety fills the S1′ pocket more completely than the methyl group of the methanesulfonyl.

Using similar methods to those reported previously, **2** was synthesized and used as the reference compound to facilitate comparison of the plasma stability of **12a**−**c**. Compounds were incubated for 4 h in the presence of human plasma, and the percentage remaining was analyzed. It was particularly gratifying to note that our hypothesis that it should be possible to increase potency with concomitant improvement in stability had been realized (cf. **12b** with **2**, Table 1).

Once a range of spirocyclobutyl *trans*-lactam analogues had been prepared, it was very evident that recording IC_{50}s following 4 h of preincubation was not capable of delivering a robust SAR. In the chromogenic assay, the enzyme concentration was approximately 0.2 uM, so the more potent analogues were acting as active site titrants. For this reason, the format of the assay was changed to employ a fluorogenic substrate and the assay run in continuous readout mode to generate kobs/I data − a more robust, kinetic measure of potency. Under these modified conditions, SAR trends were much more apparent and this assay forms the basis upon which all future results will be described.

In conclusion, we have demonstrated that placing a spirocyclobutyl substituent α to the lactam carbonyl, in combination with cyclopropylacyl substitution on the lactam itself, increases the potency of pyrrolidine-5,5-*trans*-lactams by approximately 2 orders of magnitude compared to the previously reported compounds. In addition, the disubstituted compounds show markedly enhanced synthetic accessibility and stability to human plasma hydrolysis. Compounds such as **12b**, with k_{obs}/I ca. 400 M^{-1} s^{-1} are sufficiently potent to show replicon[25] potency in the single figure micromolar range (see the following letter in this issue).

In the following paper, we describe our efforts to capitalize on the superior binding of the spirocyclobutyl *trans*-lactam template by further investigation of the requirements of the lactam substituent as well as the optimization of the amino acid substituent on the pyrrolidine nitrogen.

Acknowledgment. We thank Drs. Berwyn Clarke and George Hardy for support and encouragement; Drs. Graham Baker, Sue Bethell, and Malcolm Ellis for provision of NS3 protease protein and initial assay systems; Dr. Martin Johnson for synthesis of chiral reference materials; Derek Evans for provision of intermediates; and Drs. Tony Pateman and Angela Patikis for human plasma stability data.

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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(22) Chromogenic assay: enzyme NS3 protease domain only, 0.2 μM final concentration; 4A concentration, 10 μM; substrate (Ac-EDVVPC-pNA) final concentration, 1.5mM; substrate Km = 1.85 mM; absorbance read at 405 nm. Test compounds were preincubated with enzyme for 4 h before the addition of substrate.

(23) Fluorogenic assay: enzyme NS3−4A full-length, 20 nM final concentration; substrate (aminobenzoyl-E-D-V-V-P-C-S-M-S-Y(3-NO_2)-NH_2) 25 μM final concentration (signal increase at Em_{420} nm (Ex_{320} nm)); K_{obs}/I values were obtained at three concentrations (approximately 5-fold apart); the K_{obs}/I value was calculated by dividing the K_{obs} value by the molar concentration used. Units of measurement are M^{-1} s^{-1}.

(24) Each compound was incubated in fresh human plasma at a concentration of 25 uM and a temperature of 37 °C; an aliquot was withdrawn and deproteinated with acetonitrile for 4 h. Samples were assayed by LC-MS on an API-300 using APCI source and single-ion monitoring. Results were expressed as percentage turnover.

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