Communications

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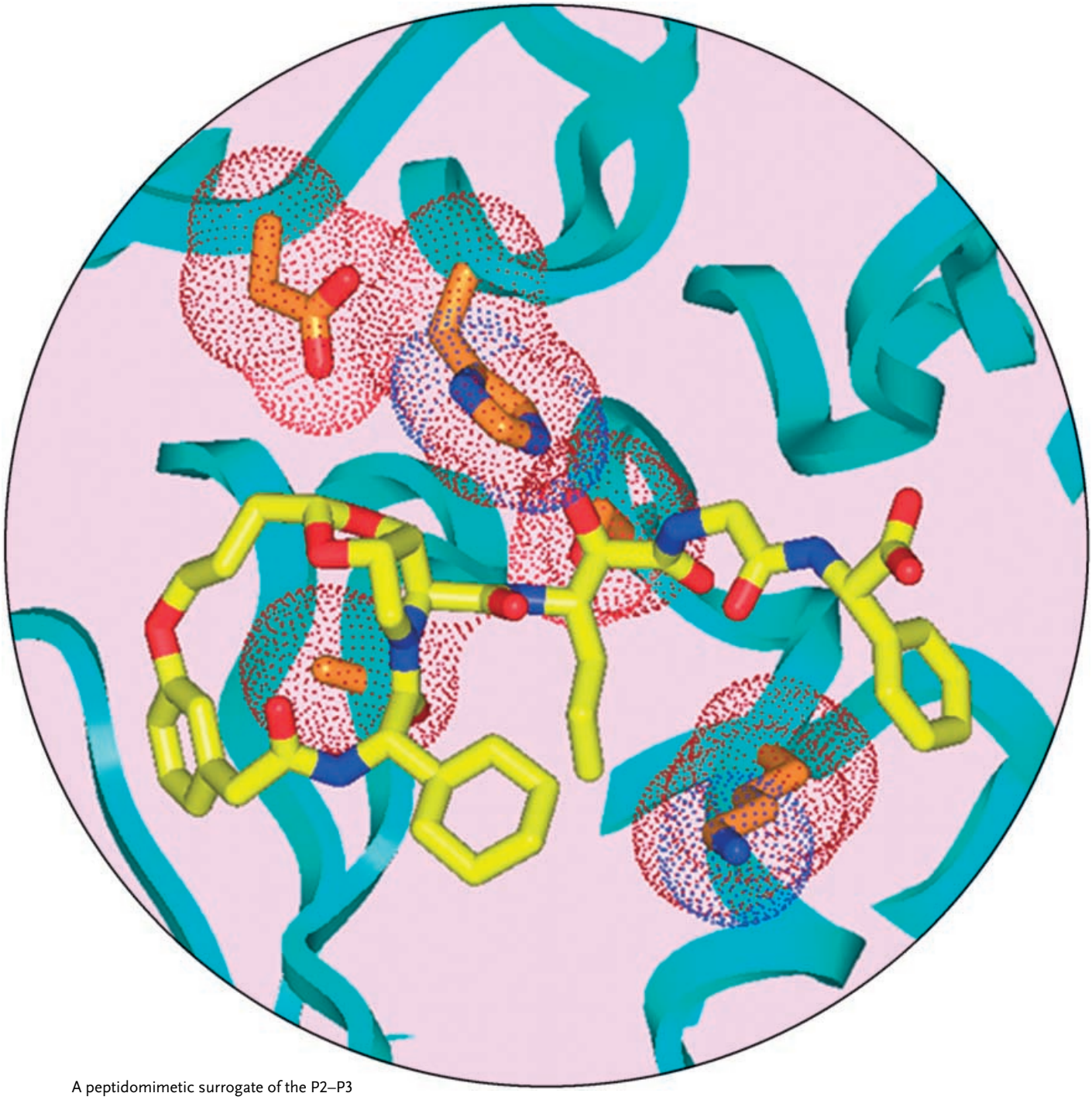

A peptidomimetic surrogate of the P2–P3 dipeptide moiety acts as a potent hepatitis C virus (HCV) inhibitor. This proline-based macrocycle binds tightly with the Ala156 methyl group of HCV NS3 protease while the P2′ phenyl and P1 *n*-propyl groups form a C-shaped clamp around the Lys136 side chain. For more information, see the Communication by K. X. Chen et al. on the following pages.

Protease Inhibitors

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Proline-Based Macrocyclic Inhibitors of the Hepatitis C Virus: Stereoselective Synthesis and Biological Activity

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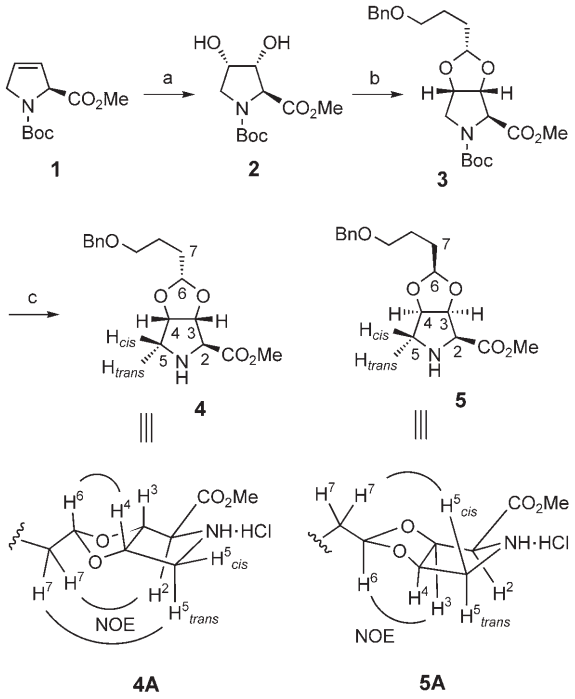
The hepatitis C virus (HCV) is the leading cause of chronic liver disease and has infected more than 170 million people worldwide. It has emerged as a major public health threat.[1] Currently, the standard treatment is α-interferon that is attached to polyethylene glycol, in combination with the antiviral agent ribavirin.[2] This therapy is effective only in approximately 50% of patients and has considerable associated side effects. Given the prevalence of HCV infections, there is an urgent need to develop more-effective, less-toxic, and orally bioavailable small-molecule antiviral agents.

HCV is a single-stranded RNA genome that encodes a polyprotein of about 3000 amino acids. The polyprotein is processed into structural and nonstructural (NS) proteins by host peptidases and virally encoded proteases. The NS3 protease, located towards the N-terminal side of the NS3 protein, is a chymotrypsin-like serine protease.[3] As a result of its essential role in HCV replication, it has been an attractive target for intensive research for new anti-HCV therapy.[4] However, the fact that the NS3 protease has a shallow and solvent-exposed substrate-binding region makes it a formidable task to develop apropriate small-molecule inhibitors.[5]

During the course of our research in potency optimization and structure depeptization of peptide-based substrates of HCV NS3 protease,[6] we were interested in the incorporation of macrocyclic structures into our target molecules. The bicyclic-acetal proline-based macrocycle **9** (Scheme 2) was designed as a peptidomimetic surrogate for the P2–P3 dipeptide moiety on the substrate. We envisioned that the additional contact of the macrocycle with the Ala156 methyl group of the enzyme backbone would enhance binding of the inhibitors. We anticipated two major challenges in the synthesis: first, the stereoselective construction of 3,4-proline cyclic acetal core 3 (Scheme 1), and second, macrocyclization to the 17-membered ring **9**. The former could be achieved through an acid-catalyzed reaction between an aldehyde (or acetal) and 3,4-dihydroxyproline, and the latter through aryl alkyl ether formation under Mitsunobu reaction conditions.[7]

The synthesis started with osmium-catalyzed dihydroxylation of commercially available *N*-Boc-3,4-dehydroproline methyl ester (**1**; Scheme 1), to give an inseparable mixture of



Scheme 1. a) OsO_4, NMO, *t*BuOH/H_2O/acetone/THF, RT, 77%; b) $BnO(CH_2)_3CHO$, *p*-TsOH, $MgSO_4$, CH_2Cl_2, RT, 91%; c) HCl (2 M, dioxane/EtOAc, RT, quant. NMO = *N*-methylmorpholine *N*-oxide, Boc = *tert*-butyloxycarbonyl, *p*-TsOH = *p*-toluenesulfonic acid.

3*S*,4*R*-diol **2** (major) and 3*R*,4*S*-diol (minor, structure not shown).[8] Both diols were then treated with 4-benzyloxybutyraldehyde in the presence of a catalytic amount of *p*-TsOH.[9] The product **3**, and its isomer were isolated as a mixture in excellent yield (91%) when 2 equivalents of the aldehyde were used. The two isomers could be separated in a ratio of 2:1, respectively, by column chromatography. 2D ^{1}H NMR spectroscopy experiments were conducted to determine the absolute configuration of the products and the stereochemical outcome of the acetal formation. To avoid interference from rotamers in **3**, it was necessary to perform the ^{1}H NMR spectroscopic analysis on amine **4** instead. The Boc protecting group was removed selectively in the presence of acid-sensitive acetal functionality when **3** was treated with hydrochloric acid (2 M). NOSEY experiments on compound **4** revealed the presence of NOE interactions between 6-H and 4-H, 7-H and 2-H, and 7-H and 5-H_{trans}. However, there was no observed coupling between protons 2-H and 3-H, which therefore indicates a *trans* relationship. This evidence led to the structural assignment of **4**, with **4A** as its preferred conformation. On the other hand, the amine derived from the minor isomer demonstrated NOE signals between 7-H and 5-H_{cis} as well as 6-H and 3-H, but not between 7-H and 2-H. The coupling constant between 2-H and 3-H ($J = 5.2$ Hz) suggests

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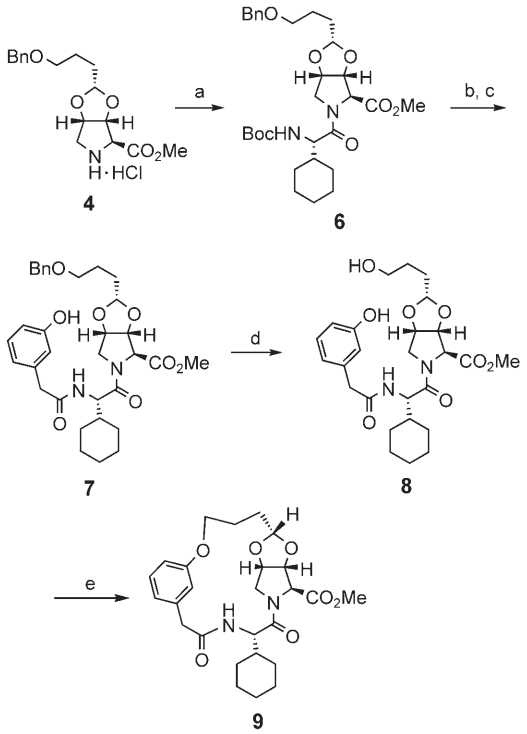
a *cis* relationship. As a result, the structure **5** and its preferred conformation **5A** was assigned. Significantly, the formation of the acetal with both 3*S*,4*R*- and 3*R*,4*S*-dihydroxyprolines led exclusively to the *endo* products.[10]

Once the stereochemistry was established, amine **4** was then coupled to *N*-Boc-cyclohexylglycine under standard conditions to give dipeptide **6** (Scheme 2). Selective removal

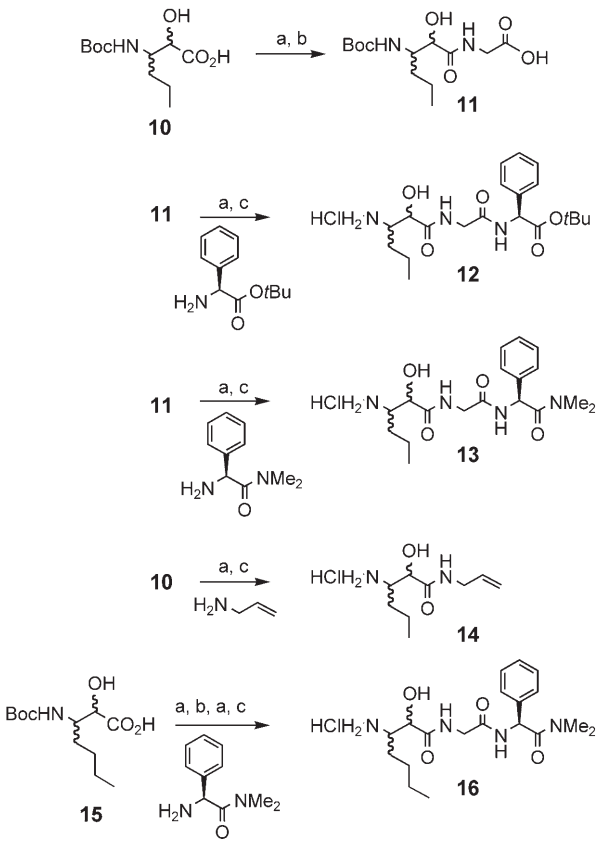


Scheme 2. a) *N*-Boc-Chg-OH, HATU, *i*Pr$_2$NEt, CH_2Cl_2, 0 °C→RT, 76%; b) HCl (2 M), dioxane/EtOAc, RT, quant.; c) 3-OH-PhCO$_2$H, HATU, *i*Pr$_2$NEt, CH_2Cl_2, 0 °C to RT, 98%; d) H_2, Pd C 10%, EtOH/EtOAc, RT, quant.; e) PPh_3, ADDP, CH_2Cl_2, RT, 72%. Chg-OH = cyclohexylglycine, HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′*N*′-tetramethyluronium hexafluorophosphate, ADDP = 1,1′-(azodicarbonyl)dipiperidine.

of the Boc protecting group and subsequent coupling with 3-hydroxyphenylacetic acid provided compound **7**. Cleavage of the benzyl ether gave the phenol alcohol **8**, which is the substrate for the key Mitsunobu macrocyclization reaction.[11] Treatment of **8** with triphenylphosphane and 1,1′-(azodicarbonyl)dipiperidine furnished the desired macrocycle **9** in excellent yield (72%).

The "right-hand" segment (P1–P2′) of the molecule was prepared through a series of peptide couplings and deprotections, starting with α-hydroxy-β-amino acid norvaline (Nva) and norleucine (Nle) derivatives[12] (**10** and **15**, respectively; Scheme 3). The hydrochloride intermediates of the tripeptide amines **12**, **13**, **16**, and allyl amide amine **14** were obtained as mixtures of four diastereomers.

Finally, the macrocyclic "left-hand" segment and the α-hydroxyamide "right-hand" moiety were combined (Scheme 4). The methyl ester **9** was hydrolyzed to the carboxylic acid **17**, which was then coupled with amines **12**–**14** and **16**. The resulting hydroxyamides were oxidized to the

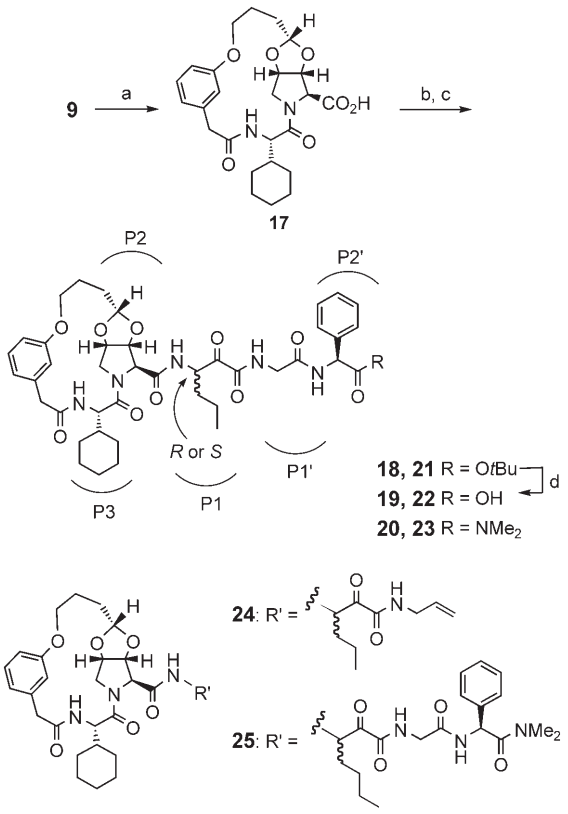


Scheme 3. a) respective amine, DhBtOH, $ClCH_2CH_2Cl$, NMM, DMF/CH_2Cl_2, −20 °C, 65–95%; b) H_2, Pd-C, EtOH, RT, 98%; c) 2 M HCl, dioxane/EtOAc, RT, quant. DhBtOH = 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine, NMM = *N*-methylmorpholine, DMF = *N*,*N*-dimethylformamide.

corresponding α-ketoamides by using Dess–Martin periodinane.[13] The products were mixtures of two diastereomers at the P1–Nva/Nle α center. The *tert*-butyl ester isomers **18** and **21** and the dimethyl amides **20** and **23**, were separated. Treatment of **18** and **21** with TFA gave carboxylic acids **19** and **22**, respectively. The allyl amide **24** and the P1–Nle analogue **25** were an inseparable mixture of diastereomers.

All macrocyclic inhibitors **18**–**25** were tested in an HCV continuous assay[14] (Table 1). The inhibition constants (K_i^*) were determined by using NS4A-tethered single-strain NS3 serine protease.[15] The K_i^* values for compounds **18**, **19**, and **20** were 0.81, 0.36, and 0.21 μM, respectively. However, the opposite diastereomers **21**, **22**, and **23** were much more potent (K_i^* = 0.038, 0.038, and 0.044 μM, respectively). Based on the structure–activity relationship results from our acyclic peptide HCV protease inhibitors,[16] the more potent compounds **21**–**23** were assigned the *S* configuration at P1 and the less potent compounds **18**–**20** the *R* configuration. The P1–Nle analogue **25** was equally potent (0.076 μM) considering it was a mixture of two isomers, whereas the smaller tripeptide inhibitor **24** was only moderately active (3.1 μM).

The selectivity of these inhibitors against human neutrophil elastase (HNE), a structurally closely related serine protease, was also measured (Table 1). Compounds **18**–**23** were moderately selective against elastase (HNE/HCV =



Scheme 4. a) LiOH, MeOH/THF/H_2O, RT, 93%; b) **12–14** or **16**, DhBtOH, EDC, NMM, DMF/CH_2Cl_2, −20°C, 50–85%; c) Dess–Martin periodinane, CH_2Cl_2, RT, 51–89%; d) TFA, CH_2Cl_2, RT, quant. TFA = trifluoroacetic acid

Table 1: Potency and selectivity of compounds **18–25** against HCV protease.

Compound	R	P1	K_i^*[μM]	HNE/HCV
18	O*t*Bu	(*R*)-Nva	0.81 ± 0.04	6
19	OH	(*R*)-Nva	0.36 ± 0.03	11
20	NMe_2	(*R*)-NVa	0.21 ± 0.04	32
21	O*t*Bu	(*S*)-Nva	0.038 ± 0.001	3
22	OH	(*S*)-Nva	0.038 ± 0.001	4
23	NMe_2	(*S*)-Nva	0.044 ± 0.009	5
24	–	(*R*,*S*)-Nva	3.1 ± 0.3	–
25	NMe_2	(*R*,*S*)-Nle	0.076 ± 0.006	383

3:32), whereas the P1–Nle derivative **25** was highly selective (383-fold). This indicates that the P1 residue plays an important role in selectivity. Pharmacokinetic (PK) studies in rats showed that compound **23** had a low oral AUC (area under the curve) value (0.1 μM h@3 mpk) and bioavailability (0.3%), whereas compound **24** gave a respectable AUC value (2.6 μM h). Presumably, the smaller size and fewer amide bonds of **24** contributed to the improved PK profile. The solubility of **18–23** in pH 7.4 buffer was in the range of 5–80 μM, with **19** and **20** at the higher end and **18**, **21, 22**, and **23** at the lower end.

The X-ray crystallography structure of compound **22** bound to the active site of HCV protease was obtained (Figure 1). It confirmed the absolute configurations at both

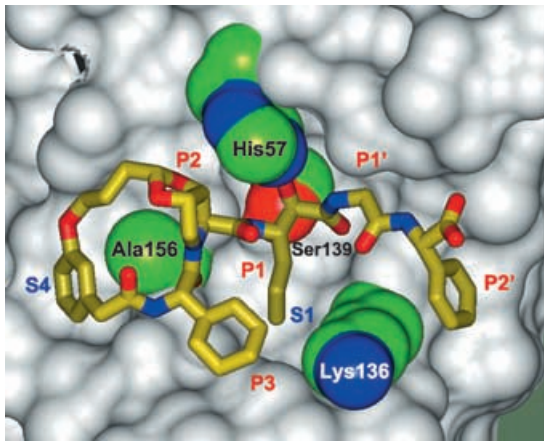


Figure 1. The X-ray crystal structure of compound **22** bound to HCV protease.

the acetal center and the P1 α center. As expected a reversible covalent-bond formation occurred between the carbonyl group of the ketone and the hydroxyl group of Ser139. Most importantly, the 17-membered macrocycle formed a perfect "donut" that surrounded the Ala156 methyl group. The P1 *n*-propyl side chain was buried in the S1 pocket and a "C"-shaped clamp was formed around the side chain of Lys136 along with the P2′ phenyl group. The P2 bicyclic praline derivitive, the P3 cyclohexyl ring and the phenyl group in the macrocycle all had hydrophobic interactions with the protein surface. Furthermore, there were several hydrogen bonds between the inhibitor peptide chain and that of the enzyme backbone. All these interactions contributed to the excellent potency of **22**.

In summary, a concise and highly stereoselective synthesis of the 17-membered macrocycle **9** was completed and the *endo* product was shown to form exclusively at the acetal center of the bicyclic proline core. The key macrocyclization provided excellent yields through a Mitsunobu protocol. The resultant macrocyclic inhibitors were active against HCV NS3 serine protease with the (*S*)-P1 isomers being the more potent. The X-ray crystal structure of compound **22** bound to the enzyme revealed that the macrocycle formed good contact with the enzyme by adopting a "donut"-shaped conformation above the methyl group of Ala156. Further evaluation of these compounds and the design of inhibitors with better potency and PK profiles are currently in progress.

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