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P2–P4 Macrocyclic inhibitors of hepatitis C virus NS3-4A serine protease

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Abstract—Synthesis and HCV NS3 serine protease inhibitory activity of 4-hydroxyproline derived macrocyclic inhibitors and SAR around this macrocyclic core is described in this communication. X-ray structure of inhibitor **38** bound to the protease is discussed.

Hepatitis C virus (HCV) infection is a global health crisis leading to liver cirrhosis, hepatocellular carcinoma, and liver failure in humans.[1] It has been estimated that 3% of the human population worldwide is chronically infected with HCV.[2] α-Interferon monotherapy and α-interferon–ribavirin combination therapy are the standard treatment for this infection.[3] While therapeutic effectiveness has been improved with introduction of pegylated version of α-interferon, PEG-INTRON and PEGASYS, it is still far from ideal.[4] Hence there is an unmet medical need to discover and develop new and more effective protocol for the treatment of HCV infection.

Since identification of this virus, the NS3 serine protease contained within the N-terminal region of the NS3 protein has been studied extensively.[5] This chymotrypsin-like serine protease is implicated in viral replication and therefore, an attractive target for HCV antiviral therapeutics.[6] Proof of concept studies in humans have validated this hypothesis.[7]

Our earlier exploration toward developing inhibitors of HCV NS3 serine protease resulted in P_6–P′ hexapeptide derivatives of type **1**, containing α-ketoamide serine trap, with good binding potency.[8] Other groups have also reported their results using a similar strategy.[9] More recently we disclosed that introduction of an appropriate P'_2 residue allowed us to truncate these inhibitors at P_3 without adversely affecting the potency (compd **2**, Fig. 1).[10] X-ray crystal structure of inhibitor bound complex revealed that the P_1-P'_2 moiety formed a C-clamp over Lys 136 of the protease, resulting in exten-

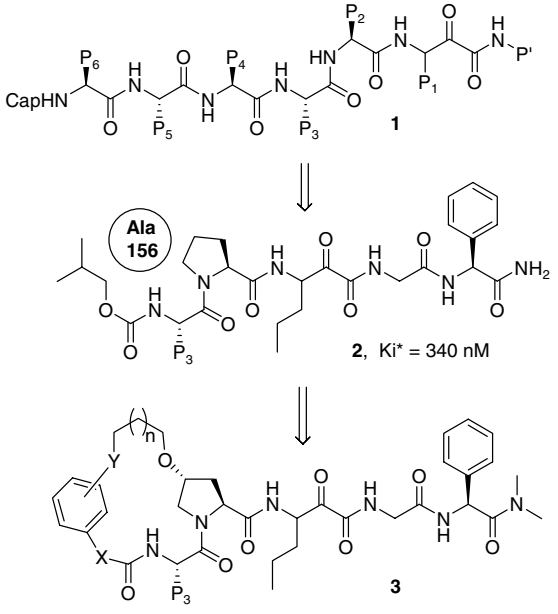

Figure 1. Design of macrocyclic inhibitors.

Keywords: HCV NS3-4A serine protease inhibitor; HCV protease inhibitor; Macrocycle; Ketoamide.
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sive hydrophobic interaction which translated into improved binding potency. Further analysis of the X-ray structure revealed the presence of Ala 156 in close proximity to the P_2/P_3 capping region, and thus could be exploited advantageously to improve the potency. We therefore decided to cyclize the P_2 residue to the P_3 capping moiety, thus forming P_2–P_4 macrocyclic inhibitors.

Since HCV NS3 serine protease inhibitors containing P_2 4-hydroxy proline derivatives have been reported,[11] we envisioned using the hydroxyl group as a handle to introduce linkers. The choice of P_3 capping moiety to be cyclized was more challenging, as most of our acyclic derivatives contained *t*-Boc or *i*-Boc groups as P_3 caps. Our early investigations in the P_3 capping area had identified some substituted benzamido functionality as a good replacement for *i*-Boc residue.[12] Based on these observations we planned on incorporating an aromatic group as the P_3 capping moiety, with suitable substitution pattern that will allow for cyclization onto the P_2 proline via an appropriate linker.

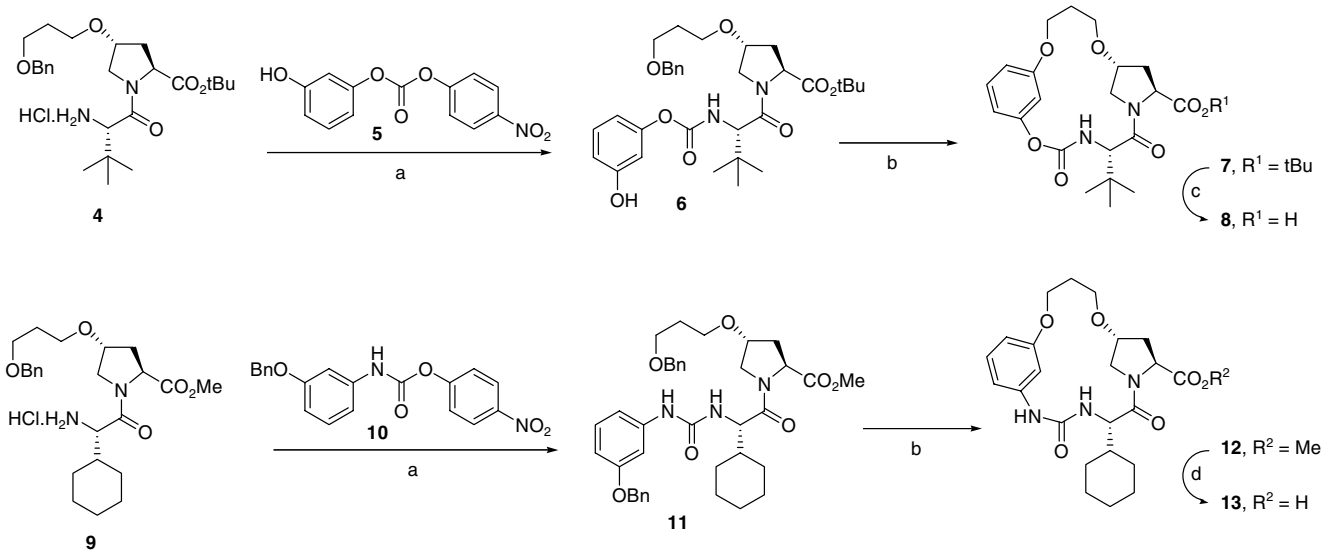
Furthermore, we opted to cap the P_2' carboxyl terminus with *N*,*N*-dimethylamide residue, since earlier SAR studies in the P_2' area indicated this functionality provided superior C-terminus cap compared to parent $-NH_2$.[12] Modeling results suggested that 16- to 18-membered ring size would provide maximum contact with the enzyme surface. Recently, we disclosed our efforts directed toward effective implementation of this macrocyclization strategy.[13] Herein, we report further modifications around the macrocyclic core leading to potent HCV NS3 serine protease inhibitors.

Synthetic approach for preparation of the designed macrocycles started from previously described dipeptide derivative **4**[14] (Scheme 1). Treatment of **4** with activated carbonate **5**[15] resulted in formation of carbamate **6**. Removal of benzyl protecting group on the linker set the stage for macrocyclization, which was realized via Mitsunobu protocol using ADDP and triphenylphosphine.[14] Cleavage of the *tert*-butyl ester of **7** under acidic conditions provided the 16-membered carbamate macrocycle **8**. The choice of *tert*-butyl ester protecting group was essential so that the deprotection could be performed under acidic conditions. Attempted removal of the corresponding methyl ester (structure not shown) using LiOH conditions afforded the acid with concomitant ring opening of the macrocyclic phenyl carbamate. Urea macrocycle **13**, shown in Scheme 1, was prepared in an analogous manner employing the activated carbamate **10**.

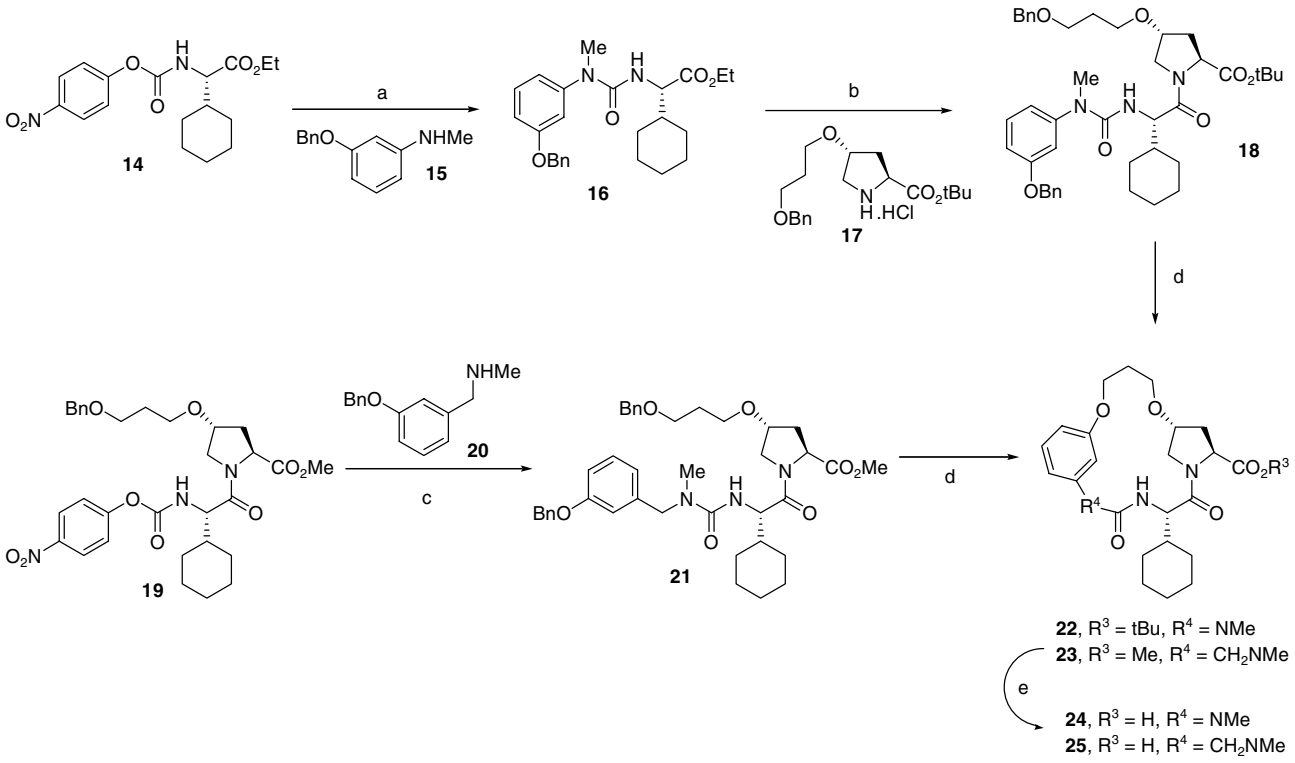
For the synthesis of *N*-methyl urea macrocycles, **24** and **25**, the isocyanate surrogate (4-nitrophenyl carbamoyl moiety) was introduced at the P_3 nitrogen terminus. Reaction of the *N*-methylaniline derivative **15** with the activated carbamoyl derivative **14**, resulted in the formation of *N*-methyl urea **16**. Hydrolysis of the ethyl ester of **16**, and subsequent coupling with 4-hydroxyproline derived amino ester **17** resulted in the macrocyclic precursor **18**. Mitsunobu protocol described above was then utilized to synthesize the *N*-methyl urea macrocycle **22**. Deprotection of the *tert*-butyl ester using TFA provided acid **24**. Similar strategy employing the dipeptide derived 4-nitrophenyl carbamate **19** and *N*-methylbenzylamine derivative **20** afforded the 17-membered *N*-methylurea macrocycle **25** (Scheme 2).

Having constructed the macrocyclic acids, installation of the P_1–P' residue **26**[13] was done under coupling condition using EDCI, HOOBt, and NMM. The resulting mixture of hydroxyl amide diastereomers were oxidized using Dess–Martin's periodinane[16] to provide the P_2–P_4 macrocyclic α-ketoamide targets **27**–**30** (Scheme 3).

The construction of amide derived macrocycles is shown in Scheme 4. The dipeptide derivative **31** was coupled with commercially available acids **32** and further elabo-

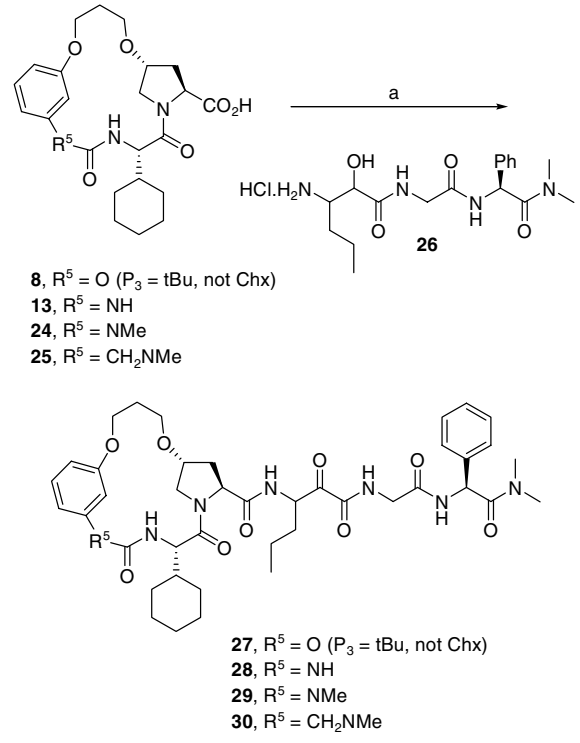


Scheme 1. Reagents and conditions: (a) Et_3N, imidazole (cat.), CH_2Cl_2/DMF (20–38%); (b) see Ref. 14; (c) TFA/CH_2Cl_2 (10%, three steps); (d) aq 1 M LiOH/THF/MeOH (15%, three steps).



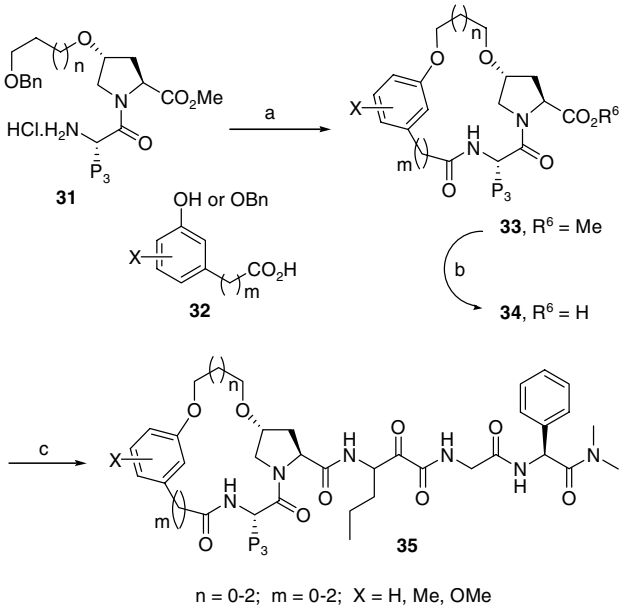
Scheme 2. Reagents and conditions: (a) Et_3N, imidazole (cat.), $CHCl_3$, 50 °C (56%); (b) i—aq LiOH, EtOH; ii—**17**, EDCI, HOOBt, NMM, CH_2Cl_2/DMF (34%, two steps); (c) Et_3N, imidazole (cat.), CH_2Cl_2 (63%); (d) see Ref. 14; (e) for **24**, TFA/CH_2Cl_2 (21%, three steps); for **25**, aq 1 M LiOH/MeOH (35%, three steps).

rated to provide the macrocyclic acids **34**.[14] Conversion of these acids **34** to the target compounds of type **35** essentially followed procedures described in Scheme 3.



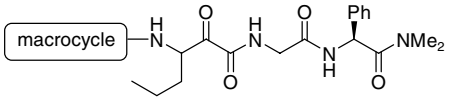
Scheme 3. Reagents and conditions: (a) i—**26**, EDCI, HOOBt, NMM, CH_2Cl_2/DMF; ii—Dess–Martin's periodinane, CH_2Cl_2 (50–60%, two steps).

HCV NS3 serine protease inhibitory data[17] for the macrocyclic targets were obtained using the continuous spectrophotometric assay described earlier[18] and the results are summarized in Table 1. Cyclization of the P_2 moiety to the P_3 capping group with an appropriate



Scheme 4. Reagents and conditions: (a) i—**32**, EDCI, HOOBt, NMM, CH_2Cl_2/DMF; ii—see Ref. 14; (b) aq 1 M LiOH/THF/MeOH; (c) i—**26**, EDCI, HOOBt, NMM, CH_2Cl_2/DMF; ii—Dess–Martin's periodinane, CH_2Cl_2 (50–60%, two steps).

Table 1.



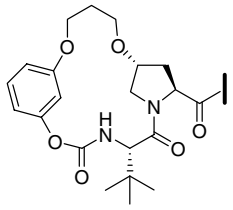
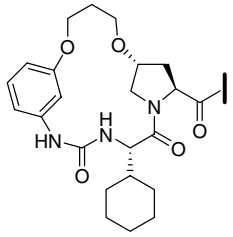
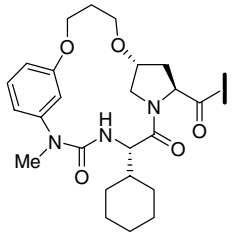
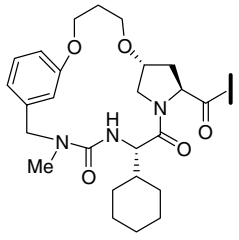
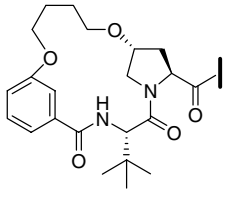
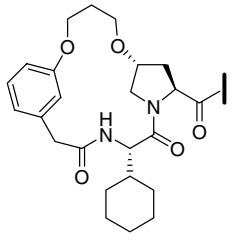
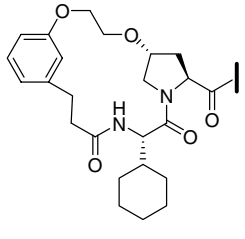
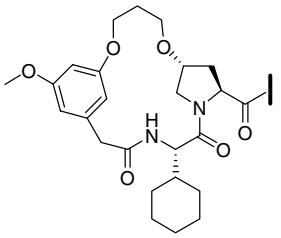
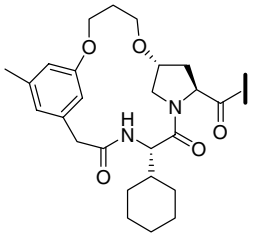
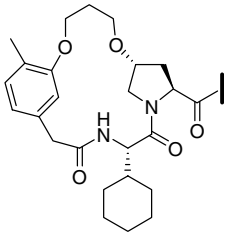
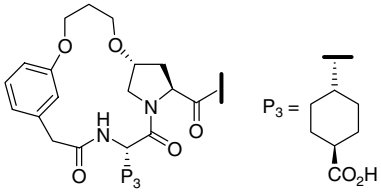
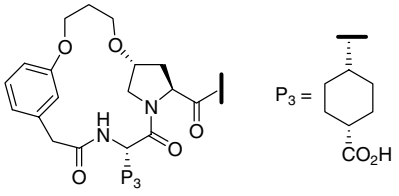
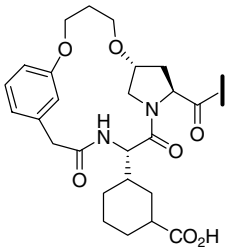
Compound	Macrocycle	K_i^* (nM)
27		200
28		1600
29		90
30[a]		26
36[a]		210
37[a]		*8*
38[a]		25

Table 1 (*continued*)

Compound	Macrocycle	K_i^* (nM)
39[a]		32
40[a]		30
41[a]		12
42		19
43		30
44		6

[a] (*S*)-Isomer at P_1 center.

linker resulted in the carbamate macrocycle, **27**, with a binding potency of 200 nM. While this result was very encouraging in that the potency was retained, further improvement was needed.

We first decided to concentrate on modifying the P_3 capping linkage in order to improve the potency and also

the metabolic stability of these inhibitors. Hence the urea macrocycle derived target **28** was next synthesized. Unfortunately inhibitor **28** had lost most of the potency which maybe attributed to high degree of conformational restriction around the macrocyclic region. Interestingly, alkylation (i.e., methylation) of the distal urea nitrogen resulted in restoring, if not improving, the potency (compd **29**, $K_i^* = 90$nM). Increasing the ring size provided macrocyclic inhibitor **30**, with further improvement in potency. Since our early work in the P_3 capping region with acyclic inhibitors[12] indicated that substituted benzamido functionality was well tolerated, we then investigated P_3 amide linked macrocyclic cores. In order to maintain the ring size as 16-membered, we prepared the benzamido P_3 capped inhibitor **36**, containing four-carbon linker. Encouragingly, this inhibitor **36** was equipotent to the parent carbamate macrocyclic inhibitor, **27**. Moving the aromatic group one-carbon away from the P_3 amide linkage, while retaining the ring size, resulted in target **37** with excellent binding potency, $K_i^* = 8$ nM.[13] Further adjustment of the aromatic group resulted in **38**, another potent inhibitor with $K_i^* = 25$ nM. Having established the requirements for the macrocyclic core, which resulted in an excellent HCV NS3 serine protease inhibitor (**37**), we then set out to study the effect of substitution on the aromatic ring. While introduction of 3-methoxy (compd **39**) or 3-methyl (compd **40**) functionality seemed to affect the binding in a negative way, the 2-methyl group (compd **41**) essentially retained the potency. The effect of substitution on the P_3 cyclohexyl ring was investigated next. Preparation of the 4-carboxyl substituted cyclohexylglycine derivatives has been reported previously.[19] Introduction of *trans* or *cis*-carboxyl group at the 4-position of cyclohexyl P_3 residue resulted in minimal loss in potency (compounds **42** and **43**) compared to the parent amide linked inhibitor, **37**. However, 3-carboxyl cyclohexylglycine at P_3 afforded inhibitor **44** which was equipotent to **37** ($K_i^* = 6$ nM). Thus, the hypothesis of appropriately positioned carboxyl groups for interaction with the amino sidechain of Lys 136 did not prove to be the case.

X-ray crystal structure of the inhibitor **38** bound to the protease is shown in Figure 2.[20] The peptidic core is bound to the protease through a series of hydrogen bonding interactions. The catalytic Ser 139 attacked the electrophilic keto functionality in a covalent and reversible manner. Interestingly, the amide carbonyl group adjacent to the keto moiety occupied the oxyanion hole, instead of the tetrahedral oxygen anion resulting from the attack of Ser 139, as expected. The propyl residue at P_1 is buried into the shallow hydrophobic S_1 pocket. The cyclohexyl moiety at P_3 occupied the S_3 pocket, providing hydrophobic contact with the enzyme. As observed in our previous inhibitors, the P′ residue wrapped over the sidechain of lysine 136, resulting in P_1-P_2' C-clamp, thus locking Lys 136 in place. Most interestingly, the macrocyclic core formed a ring around Ala 156, while the aromatic group at the P_3 cap region buried deep into the S_4 pocket. These resultant hydrophobic interactions with the enzyme translated into improved potency.

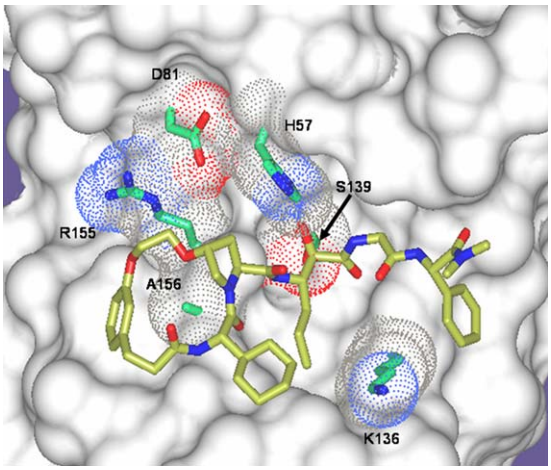


Figure 2. X-ray structure of **38** bound to the protease.

In summary we have established that cyclization of P_2 moiety with P_3 capping residue via a suitable linker provided additional binding contact with the protease surface. Targets derived from these designed macrocycles exhibited potent HCV NS3 serine protease inhibitory activity. While the carbamate and N-methylurea linked macrocycles provided a few fold improvement in binding, the 16-membered amide linked macrocyclic targets resulted in inhibitors with single-digit nanomolar potency. X-ray crystal structure of one of the inhibitors (**38**) bound to the protease revealed that the P region macrocyclic core formed a ring around Ala 156, and the P_3 capped aromatic group was positioned well in the S_4 cavity. Together, these hydrophobic interactions resulted in tremendous improvement in potency.

Acknowledgment

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20. Crystallographic data for the inhibitor–protease complex included in this paper has been deposited with the RCSB Protein Data Bank as PDB ID 2GVF. The structural details can be viewed at www.rscb.org using the ID number above.