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Depeptidization efforts on P_3–P_2' α-ketoamide inhibitors of HCV NS3-4A serine protease: Effect on HCV replicon activity

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Abstract—Depeptidization efforts of the P_3–P_2 region of P_3 capped α-ketoamide inhibitor of HCV NS3 serine protease **1** are reported. We clearly established that N-methylation of the P_2 nitrogen and modification of the P_2' carboxylic acid terminus were essential for activity in the replicon assay.

Hepatitis C virus (HCV), a small (+)-RNA virus belonging to the Flaviviridae family, infects 200 million people worldwide.[1] Untreated HCV infections can progress to liver cirrhosis and hepatocellular carcinoma.[2] Currently, antiviral drug ribavarin in combination with the immune system booster α-interferon is the only available treatment.[3] Although combination therapy is reasonably successful with genotypes 2 and 3, its efficacy against the predominant genotype 1 is moderate at best. Therefore, several research groups have been working toward the development of a more effective, convenient, and tolerable treatment. Because of its vital role in viral replication,[4] HCV NS3 serine protease has been actively pursued as a viral protein target.[5] Oligopeptide derivatives containing α-ketoamide electrophilic trap have been reported by our group[6] and others[7] to be potent inhibitors of HCV NS3 serine protease.

More recently, we demonstrated, through inhibitor **1**, that introduction of a cyclopropyl alanine side chain both at P_2 and P_1 combined with a phenylglycine residue at P_2' improved the binding potency of our earlier P_3 capped inhibitors by about 10-fold (Fig. 1).[8] However, we reasoned that less peptidic inhibitors would be more desirable not only for cellular activity but also to get benefits such as oral bioavailability and improved pharmacokinetics. From the hydrogen bond model depicted

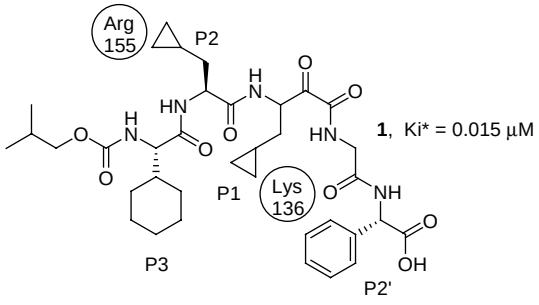

Figure 1.

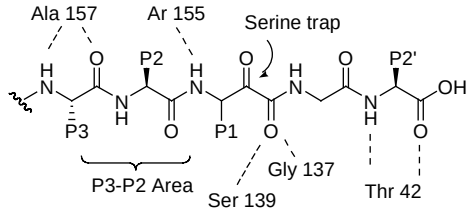

Figure 2.

in Figure 2, it is clear that substrate and inhibitor were bonded through a combination of hydrogen bonds and side-chain hydrophobic interactions. In designing our peptide modification, we had to make sure that parts of the ligand that formed crucial H-bonds with the enzyme backbone were left in place. Therefore, the P_3–P_2 area of inhibitor **1** was targeted for depeptidization (Fig. 2).

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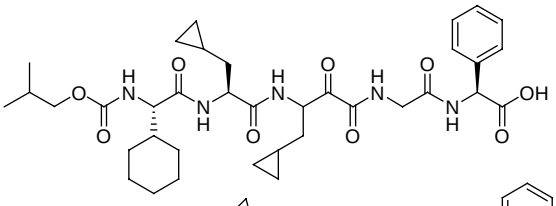
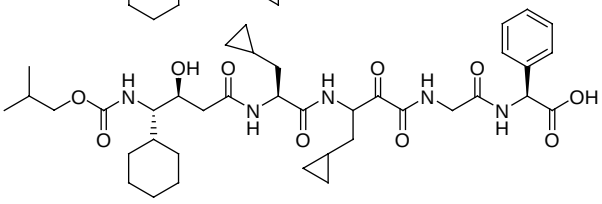
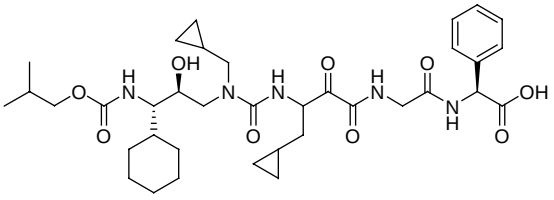
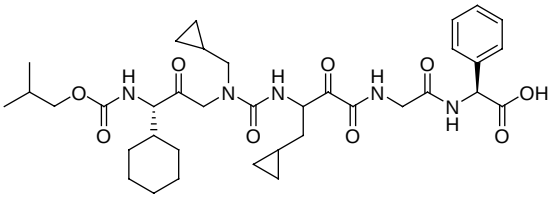
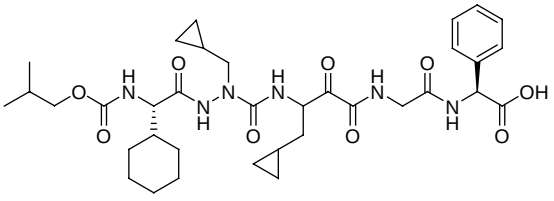
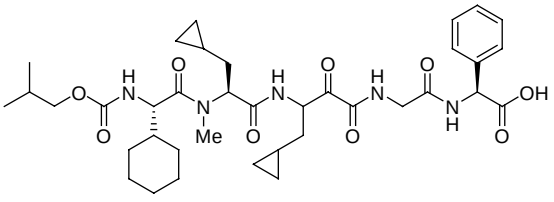
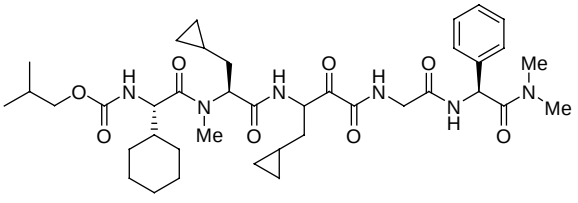
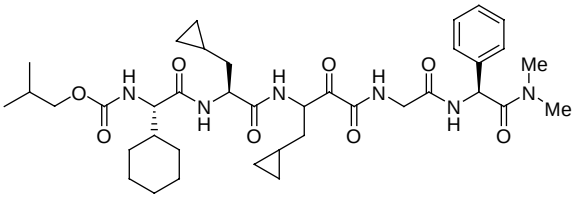
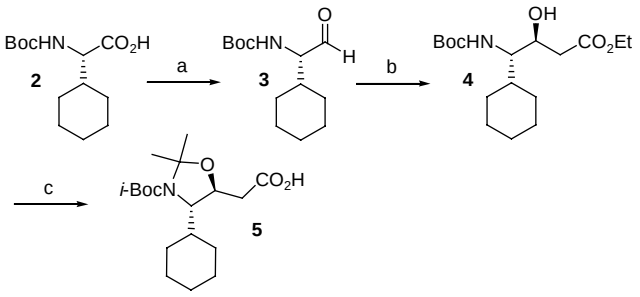
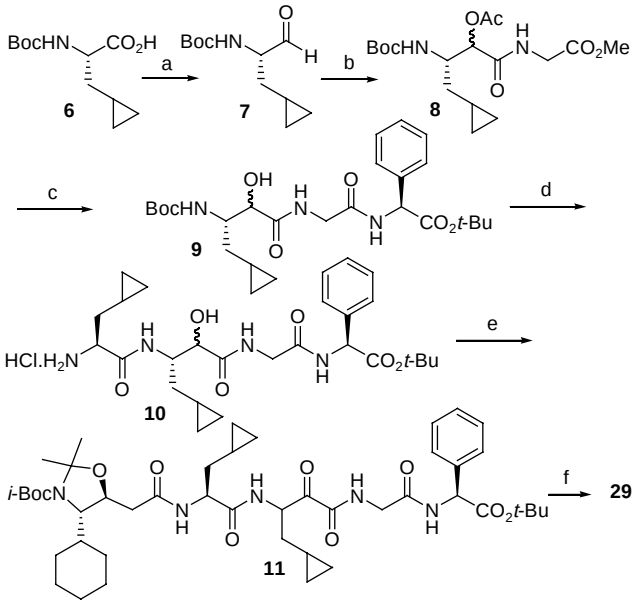
Table 1.

Entry	Compound	Structure	K_i^* (μM)	Replicon IC_{90} (μM)
1	**1**		0.015	>5.0
2	**29**		42.0	>5.0
3	**30**		10.0	>5.0
4	**31**		2.1	>5.0
5	**32**		0.23	>5.0
6	**33**		0.12	>5.0
7	**34**		0.06	0.95
8	**35**		0.05	> 5.0

The preparation of compound **29** (Table 1) is depicted in Schemes 1 and 2. The protected γ-amino acid **5** used in the synthesis of **29** was prepared as shown in Scheme 1. Thus, Boc-L-cyclohexylglycine **2** was converted to the corresponding Weinreb amide that was reduced with $LiAlH_4$ to give carbinol **3** in high yield. The protocol



Scheme 1. Reagents and conditions: (a) i—HCl·HN(OMe)Me, EDCI, NMM, CH_2Cl_2, −10 °C (100%); ii—LAH, THF, −30 °C (100%); (b) LDA, $MeCO_2Et$, $ZnBr_2$, THF, −78 °C, (49% yield, 80% de); (c) i—4 M HCl, dioxane, (100%); ii—*i*-BuOCOCl, Na_2CO_3, dioxane, H_2O, (74%); iii—DMP, $Et_2O{\cdot}BF_3$, (78%); iv—aq 1 N LiOH, THF/H_2O (95%).

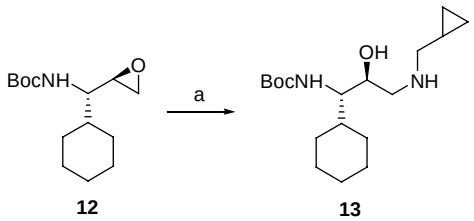


Scheme 2. Reagents and conditions: (a) i—HCl·HN(OMe)Me, EDCI, NMM, CH_2Cl_2, −10 °C (100%); ii—LAH, THF, −30 °C (95%); (b) AcOH, methyl-isocyanoacetate, CH_2Cl_2, (70% yield, 2 to 1 ratio); (c) i—aq 1 N LiOH, THF/H_2O; (100%); ii—H-PhG-*O*-*t*-Bu, EDCI, HOOBt, NMM, CH_2Cl_2, −20 °C, (95%); (d) i—4 M HCl in dioxane, (100%); ii—**6**, EDCI, HOOBt, NMM, CH_2Cl_2, −20 °C, (52%); iii—4 M HCl in dioxane, (100%); (e) i—**5**, EDCI, HOOBt, NMM, CH_2Cl_2, (41%); ii—Dess–Martin's periodinane, CH_2Cl_2, (100%); (f) 50% TFA in CH_2Cl_2, 1 h, then *n*-heptane, (95%).

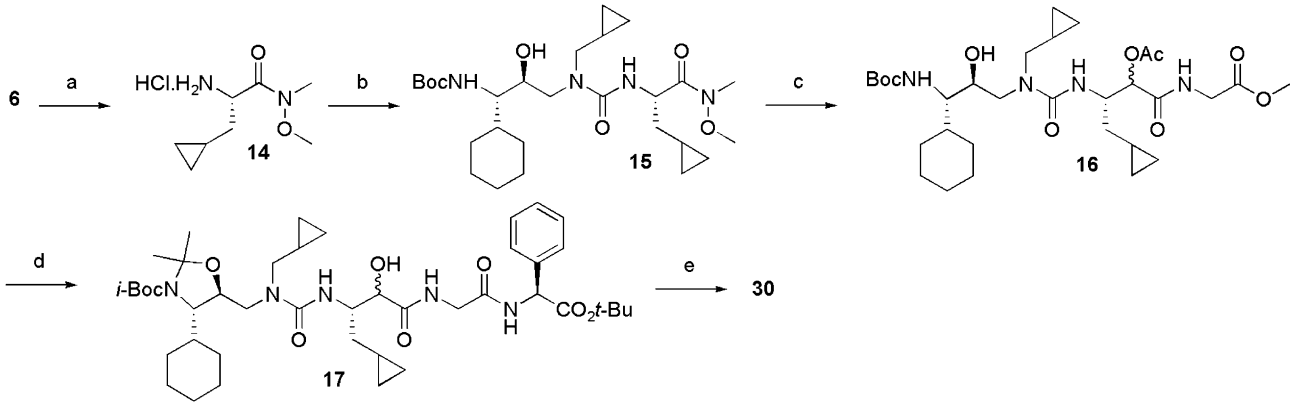
developed by Roberts et al.[9] was applied to **3** to introduce the hydroxyethylene scaffold. Thus, condensation of aldehyde **3** with the zinc enolate of ethyl acetate at −78 °C furnished the 3*S*,4*S* β-hydroxy-γ-amino ester **5**. Protecting group manipulation on the amino end was performed to introduce the *i*-Boc functionality of the P_3 capped inhibitors. The hydroxyl and the carbamate amino groups were tied up as acetonide using DMP and $Et_2O{\cdot}BF_3$ to provide, after base hydrolysis of the methyl ester group, the desired protected γ-amino acid **5** in 27% overall yield (Scheme 1).

Preparation of the α-hydroxyl amide core of our inhibitors is depicted in Scheme 2. Carbinol **7**, prepared following the same methodology used for the preparation of **3**, was subjected to the Passerini reaction conditions.[10] Thus, use of methyl-isocyanoacetate and acetic acid in CH_2Cl_2 generated the acetamides **8** as a mixture of diastereomers in good yield. The acetate protecting group and the methyl ester moiety of **8** were removed in one pot with LiOH. The resulting carboxylic acid was then subjected to standard coupling conditions (EDCI, HOOBt, and NMM) with protected phenylglycine amino acid to deliver the P_1–P_2' intermediate **9**. After chemoselective removal of the *t*-Boc-protecting group of **9**, the cyclopropylalanine moiety **6** was incorporated at P_2 using the coupling and deprotection conditions described above to deliver the HCl salt **10** for final assembly. Thus, intermediate **10** and protected γ-amino acid **5** prepared earlier were subjected to aforementioned coupling conditions to provide, after Dess–Martin's periodinane oxidation,[11] the desired α-ketoamide **11** in excellent yield. One-pot deprotection of the acetonide and the terminal *tert*-butyl ester moieties was performed with a 50% TFA in CH_2Cl_2 solution and provided the desired target **29** in high yield (Scheme 2).[12]

Preparation of compound **30** (Table 1) is depicted in Schemes 3 and 4. A different approach for the synthesis of this compound was adopted to avoid extensive protecting group manipulation. Thus, the *threo* carbamate **12** (Scheme 3) was constructed from Boc-L-cyclohexylglycine **2** in four steps following the methodology developed by Luly et al.[13] Addition of aminomethylcyclopropane to epoxide **12** was achieved by heating both components together in MeOH at 60 °C providing the amine **13** in 85% yield. The preparation of the α-hydroxyl amide core of inhibitor **30** was anticipated to proceed again via a Passerini reaction, albeit on a more elaborate intermediate **15** (Scheme 4). Thus, conversion of the Boc-L-cyclopropylalanine **6** to the corresponding Weinreb amide followed by removal of the *t*-Boc-protecting group under acidic condition provided the HCl salt **14**. Amino alcohol **13** (Scheme 3), treated with carbonyl-diimidazole and Et_3N in CH_2Cl_2, readily reacted with amine **14** to give urea peptoid **15**. The corresponding aldehyde of **15**, obtained by LAH reduction, was successfully subjected to the Passerini conditions as described earlier to provide acetates **16** in 72% yield. Protecting group manipulation on the amino end of **16** was performed as shown in Scheme 4 to introduce the *i*-Boc and the acetonide functionalities. Acetate and methyl ester hydrolysis was per-



Scheme 3. Reagents and conditions: (a) aminomethylcyclopropane, MeOH, 60 °C, 16 h, (85%).

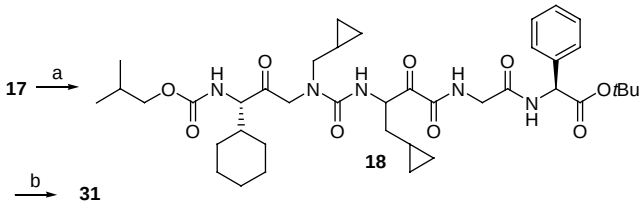


Scheme 4. Reagents and conditions: (a) 4 M HCl in dioxane, (100%); (b) **13**, CDI, Et_3N, CH_2Cl_2, (47%); (c) i—LAH, THF, −30 °C; ii—AcOH, methyl-isocyanoacetate, CH_2Cl_2, (72% over two steps); (d) i—4 M HCl in dioxane, (100%); ii—*i*-BuOCOCl, CH_2Cl_2, DIPEA, (83%); iii—DMP, $Et_2O{\cdot}BF_3$, (70%); iv—aq 1 N LiOH, THF/H_2O (100%); v—H-PhG-*O*-*t*-Bu, HATU, DMF, DIPEA, −20 °C, (95%); (e) i—Dess–Martin's periodinane, CH_2Cl_2, (91%); ii—50% TFA in CH_2Cl_2, 1 h, then *n*-heptane (100%).

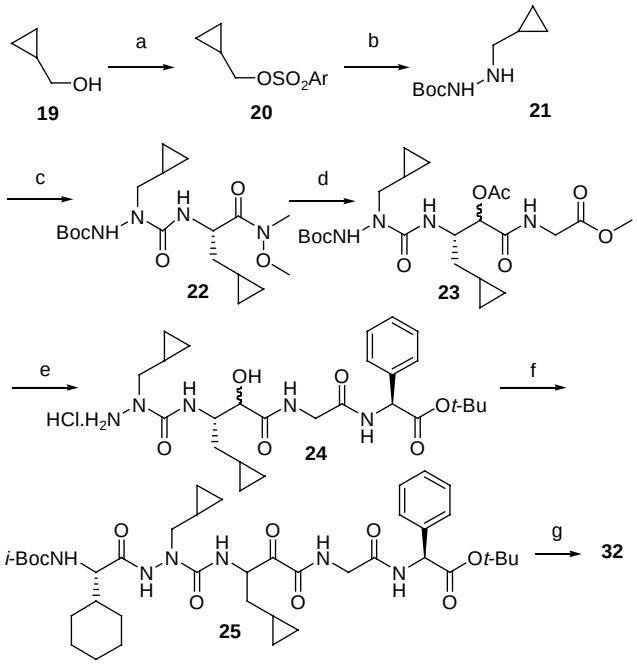
formed as described earlier and the resulting carboxylic acid was subjected to coupling conditions with protected phenylglycine amino acid to deliver the P_3–P'_2 intermediate **17** in high yield. Dess–Martin's periodinane oxidation of α-ketoamide **17** followed by a one-pot deprotection of the acetonide and the terminal *tert*-butyl ester moieties, with a 50% TFA in CH_2Cl_2 solution, provided the desired target **30**.

Preparation of compound **31** (Table 1) is outlined in Scheme 5. Thus, the acetonide moiety of α-ketoamide **17** was removed chemoselectively with a 0.5 M solution of TFA in CH_2Cl_2. Dess–Martin's periodinane oxidation of the resulting diol provided α-ketoamide **18** in good yield. Deprotection of the terminal *tert*-butyl ester group with a 50% TFA in CH_2Cl_2 solution yielded the desired target **31**.

Preparation of compound **32** (Table 1) is depicted in Scheme 6. First attempts to prepare hydrazine **21** via reduction of the corresponding hydrazone were unsuccessful and led only to the dicyclopropyl adduct.[14] Synthesis of the desired hydrazine **21** was achieved via the displacement of tosylate **20** in 60% yield. About 20 equiv of *tert*-butyl carbazate were required to observe good chemoselectivity. Using conditions similar to those described earlier, carbonyl-diimidazole was used to couple amine **14** and the hydrazine **21**. The resulting Weinreb amide **22** was reduced to the corresponding aldehyde that was immediately subjected to the Passerini condi-



Scheme 5. Reagents and conditions: (a) i—0.5 M TFA in CH_2Cl_2; ii—Dess–Martin's periodinane, CH_2Cl_2, (73%); (b) 50% TFA in CH_2Cl_2, 1 h, then *n*-heptane (95%).



Scheme 6. Reagents and conditions: (a) TosCl, pyridine, 0 °C, (66%); (b) *tert*-butylcarbazate, EtOH, 0–40 °C, (60%); (c) **14**, CDI, Et_3N, CH_2Cl_2, (30%); (d) i—LAH, THF, −30 °C; ii—AcOH, methyl-isocyanoacetate, CH_2Cl_2, (70% over two steps); (e) i—aq 1 N LiOH, THF/H_2O (77%); ii—H-PhG-*O*-*t*-Bu, HATU, DMF, DIPEA, −20 °C, (90%); iii—4 M HCl in dioxane, (100%); (f) i—*i*-Boc-cyclohexylglycine, EtOCOCl, THF, Et_3N, −5 °C, (50%); ii—Dess–Martin's periodinane, CH_2Cl_2, (95%); (g) 50% TFA in CH_2Cl_2, 1 h, then *n*-heptane (100%).

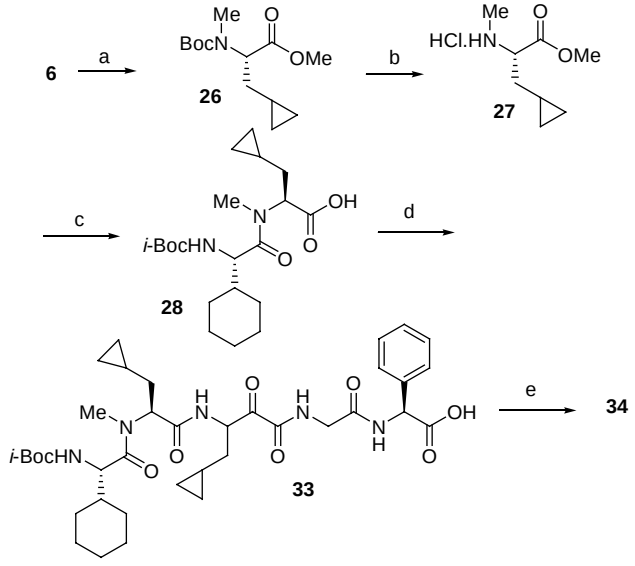
tions to yield **23**. As described earlier, after acetate and methyl ester hydrolysis, the resulting carboxylic acid was subjected to coupling conditions with protected phenylglycine amino acid which delivered, after chemoselective removal of the Boc-protecting group, the P_2–P'_2 hydrazine intermediate **24** in high yield. However, incorporation of the last residue at P_3 was problematic. Thus, standard coupling condition (HATU and DIPEA) with *i*-Boc-cyclohexylglycine did proceed, albeit in very poor yield. Fortunately, preparation of the mixed anhydride of the cyclohexylglycine moiety followed by displace-

ment with the hydrazine **24** led, after Dess–Martin oxidation, to the desired azapeptide **25** in 47% isolated yield. Deprotection of the terminal *tert*-butyl ester group with a 50% TFA in CH_2Cl_2 solution yielded the desired target **32**.

Preparation of compounds **33** and **34** (Table 1) is outlined in Scheme 7. Thus, Boc-L-cyclopropylalanine **6** was converted to the *N*-methyl carbamate **26** following a two-step, high-yielding, sequence. Removal of the Boc-protecting group under acidic condition provided the HCl salt **27**. Coupling with *i*-Boc cyclohexylglycine followed by hydrolysis of the methyl ester moiety provided intermediate **28**. Following a similar methodology as shown in Scheme 2 for the preparation of target **29**, compound **9** was used to deliver inhibitor **33**. Standard coupling conditions with dimethylamine hydrochloride provided the dimethyl amide P_2' capped inhibitor **34**.

Recently, we reported the discovery of inhibitor **1** as a potent inhibitor of the hepatitis C virus NS3-4A serine protease.[8] While **1** showed very good enzyme inhibitory activity ($K_i^* = 0.015$ μM), it obviously lacked drug-like properties. Alteration of peptides to peptidomimetics could afford compounds with improved biological potencies and increased resistance to enzymatic degradation. It was therefore decided to modify inhibitor **1** to bring in these desirable attributes. After analyzing the H-bond network outlined in Figure 2, we targeted the P_3–P_2 area of inhibitor **1** for depeptidization.

HCV NS3 serine protease inhibitory activity and HCV replicon inhibitory activity for the targets synthesized were obtained using previously reported assays.[15,16]



Scheme 7. Reagents and conditions: (a) i—NaH (3 equiv), MeI (8 equiv), THF; ii—CH_2N_2, Et_2O (90%); (b) 4 M HCl in dioxane, (100%); (c) i—*i*-Boc-cyclohexylglycine, DEPBT, DIPEA, THF (50%); ii—aq 1 N LiOH, THF/H_2O (83%); (d) i—**9,** 4 M HCl in dioxane, (100%); ii—EDCI, HOOBt, NMM, CH_2Cl_2, −20 °C, (92%); iii—Dess–Martin's periodinane, CH_2Cl_2, (100%); iv—50% TFA in CH_2Cl_2, 1 h, then *n*-heptane, (95%); (e) HCl·$HNMe_2$, EDCI, HOOBt, NMM, CH_2Cl_2, −20 °C, (45%).

Introduction of a hydroxyethylene spacer between P_3 and P_2 was detrimental for the activity of **29** with a K_i^* of 42 μM. The non-peptidic linkage at the bond between P_2 and P_3 building blocks, longer than the corresponding peptide bond, displaced the P_3 side chain outwardly relative to a native peptide substrate. The loss of interaction with Ala 157 certainly contributed to the loss in activity. Thus, we decided to keep the length of the P_3 side chain identical to that of the native peptide substrate for later compounds. We then turned our efforts toward the incorporation of a urea peptoid moiety in the P_3–P_2 region. Replacement of the α-carbon by a trivalent nitrogen was realized and led to the urea peptoid **30**. We envisioned that the hydroxyl group of **30** was well positioned to interact with Ala 157 but unfortunately, compound **30**, with a $K_i^* = 10$ μM, lacked good affinity with the enzyme backbone. Importance of the interaction with Ala 157 was further demonstrated with the oxidation of hydroxyl group of compound **30**. Thus, incorporation of a carbonyl function at P_3 had a positive effect on the potency: inhibitor **31**, with a $K_i^* = 2.1$ μM, show a 5-fold improvement in potency compared to **30**. Since lack of inhibitory activity of peptidomimetics **30** and **31** could also be attributed to the loss of asymmetry associated with this transformation, we decided to introduce an aza-amino acid moiety in that position. While some researchers have considered that the loss of asymmetry associated with this transformation could lead to a conformation that can be considered intermediate between the D- or L-amino acid, others have shown that the adjacent nitrogen could confer a sp^3 character to the α-trivalent nitrogen that could largely preserve the original peptide conformation in the resulting peptidomimetic.[17] Thus, incorporation of cyclopropyl aza-amino acyl fragment at P_2 led to inhibitor **32**. While azapeptide **32** ($K_i^* = 0.23$ μM) was substantially less potent than inhibitor **1** ($K_i^* = 0.015$ μM), incorporation of the cyclopropyl aza-amino acid motif at P_2 resulted in 10-fold improvement in activity compared to urea peptoid **31** ($K_i^* = 2.1$ μM). X-ray crystal structure of the inhibitor **32** bound to the protease is shown in Figure 3.[18] It can be seen that the peptidic core binds to the protease through a series of hydrogen bonding interac-

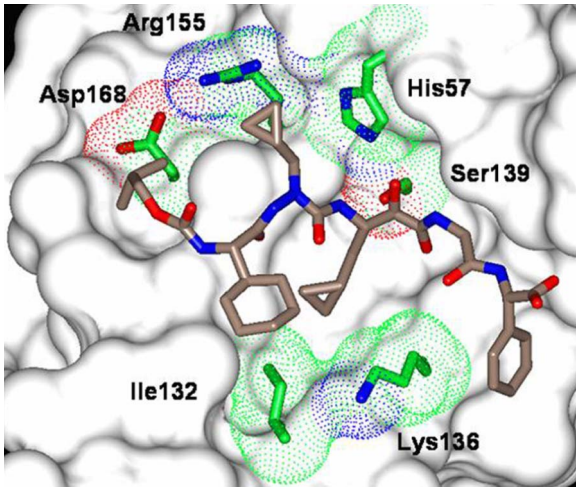


Figure 3. X-ray structure of **32** bound to the protease.

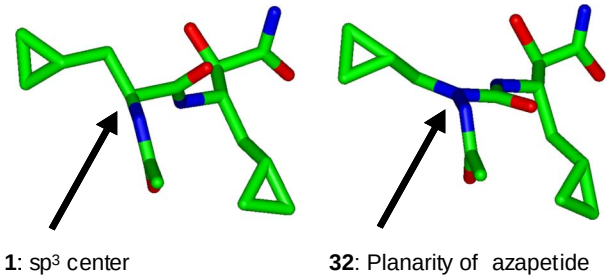


Figure 4. P_2–P_1 region geometry of compounds **1** and **32** bound to the protease.

tions. In the P_2–P_1 region, analysis of the X-ray structure revealed a planar geometry for the azapeptide motif compared to the sp^3 geometry of the P_2 amino acid residue of **1**[8] (Fig. 4). Consequently, we decided to keep the stereochemistry of the P_2 side chain identical to that of the native peptide substrate and turned our efforts toward the replacement of the α-hydrogen of the P_2 amino acid. Replacements of the α-hydrogen of the common amino acids by a methyl group had been reported as another example of depeptidization usually referred to as α-alkyl modification.[19] N-Methylation of the amide linkage at P_2 was found to be not as detrimental as some of the earlier modifications. Compound **33**, with a $K_i^* = 0.12$ μM, was the most active peptidomimetic synthesized. Targets **1** and **29**–**33** included in Table 1 were prepared with a carboxylic acid residue at P_2'. In spite of the fact that these compounds had good enzyme inhibitory activity, none of the derivatives showed cellular activity with IC_{90} values less than 5 μM against HCV replicon.[16] This large difference was certainly related to the physicochemical features of these compounds such as solubility and cell penetration. According to the H-bond model depicted in Figure 2, the hydroxyl group of the carboxylic acid residue at P_2' did not form any crucial interactions with the enzyme backbone. We carried out modifications aimed at removing the charged residue.

We discovered that incorporation of a dimethyl amide cap at the terminal carbonyl provided, for the first time, activity in the replicon assay. Thus, inhibitor **34** (Table 1) with a K_i^* of 0.06 μM exhibited the best cellular potency in that series with $IC_{90} = 0.95$ μM. To further establish the importance of masking the NH at P_2, we also prepared compound **35**. Inhibitor **35**, with a K_i^* of 0.05 μM and a replicon $IC_{90} > 5$ μM, clearly established that removal of the charged residue at P_2' alone was not enough to observe inhibition of the HCV replicon.

In summary, depeptidization of our earlier P_3 capped inhibitor led to the identification of a potent inhibitor of the HCV NS3 serine protease and with good activity against HCV replicon. N-Methylation at P_2 and replacement of the charged residue at P_2' with a dimethyl amide cap did not produce any substantial increase in enzyme inhibitory activity but were essential for inhibition of the HCV replicon system. N-Methylation at P_2 seemed to overcome an apparent 'defect at P_2' conferred by the absence of a proline residue. Consequently, further work aimed at the incorporation and optimization of proline moieties at P_2 is under progress and will be reported shortly.

References and notes

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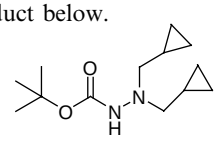

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