Design, Synthesis, and Evaluation of Oxygen-Containing Macrocyclic Peptidomimetics as Inhibitors of HCV NS3 Protease

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HCV infection is considered a silent epidemic because most people infected do not develop acute symptoms. Instead, the disease progresses to a chronic state leading to cirrhosis and hepatocarcinoma. Novel therapies are needed to combat this major health threat. The HCV NS3 serine protease has been the target of continuous investigation because of its pivotal role in viral replication. Herein, we present the P1−P3 macrocyclization approach followed for identification of HCV NS3 inhibitors as potential backup candidates to our first generation drug candidate, Sch 503034 (**1**). Different P1−P3 linkers were investigated to identify novel macrocyclic scaffolds. SAR exploration of P3-caps in the macrocyclic cores allowed the identification of L-serine derived macrocycle **32** (K_i* = 3 nM, EC_{90} = 30 nM) and allo-threonine derived macrocycle **36** (K_i* = 3 nM, EC_{90} = 30 nM) as potent HCV NS3 protease inhibitors.

Introduction

Hepatitis C (HCV[a]) virus infection represents a global health threat by infecting nearly 3% of the world population.[1] This disease has been referred to as a "silent epidemic" in numerous occasions because of the fact that only 20% of the people infected develop acute symptoms. The majority of the patients become aware of the infection after chronic symptoms, such as cirrhosis, liver failure, or hepatocarcinoma, have developed.[2] The standard of care for HCV infection is interferon-α in combination with ribavirin.[3] Excellent results following this treatment have been observed for patients infected with genotypes 2 and 3. However, only about 50% of patients infected with genotype 1 achieve a sustained antiviral response.[3,4]

The HCV genome consists of a positive single strand RNA of ∼9600 nucleotides in length that encodes a polyprotein of approximately 3000 amino acids. This polyprotein contains both the structural and nonstructural proteins, and its cleavage is mediated by host and viral proteases.[5,6] The NS3 region is a trypsin-like serine protease that plays a very important role in viral replication. NS3 is responsible for the cis-cleavage of the NS3−NS4A junction, followed by trans-cleavage of the NS4B, NS5A, and NS5B junctions to deliver functional proteins essential for replication.[6,7] Considerable effort from medicinal chemists has been directed toward finding HCV NS3 inhibitors as new therapeutic agents for treatment of this disease.[8] Our group has recently reported the identification of Sch 503034 (boceprevir, compound **1**), a selective, potent, orally bioavailable small molecule for inhibition of HCV NS3 protease.[9] This compound is currently undergoing phase III clinical trials, and its efficacy was established in previous studies through sustained viral load reductions in patients infected with HCV virus.[10] Other HCV NS3 inhibitors such as VX-950 (telaprevir)[11] and BILN 2061 (ciluprevir)[12] have also been identified and progressed into clinic by different research groups. Medicinal chemistry efforts continue in the design, synthesis, and evaluation of new inhibitors with improved potency and pharmacological properties.

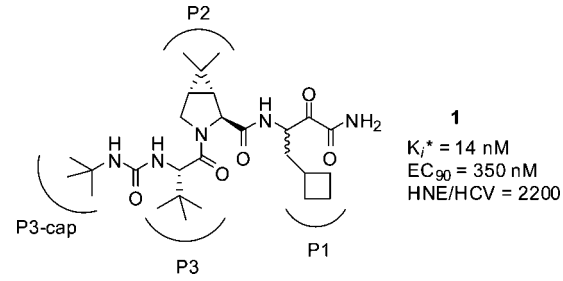

Figure 1. Keto-amide derived **1**, a potent and selective HCV NS3 inhibitor.

Macrocyclization Strategy

The structure and potency data for compound **1** are shown in Figure 1. As indicated, **1** had low nanomolar enzyme affinity (K_i* = 14 nM) in the continuous binding assay and moderate activity in the replicon based cellular assay (EC_{90}= 350 nM). During the development of **1**, we addressed concerns about our compounds acting not only as inhibitors of HCV NS3 but also as indiscriminate inhibitors of other serine proteases by measuring their selectivity for HCV NS3 instead of other serine proteases. The human neutrophil elastase (HNE) was chosen for the selectivity measurement because of its high level of structural homology compared to HCV NS3. Thus, the HNE/HCV selectivity represented the ratio of the inhibitory activity against HNE over the inhibitory activity against HCV NS3. An important characteristic of **1** was its high selectivity (HNE/HCV = 2200), which is a desirable characteristic to maintain in our compounds. Our continued efforts in the HCV area focused in the identification of a potential backup to compound **1** and relied on the exploration of novel scaffolds to depeptidize this molecule and improve its potency. Herein, we describe the design and

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[a] Abbreviations: HCV NS3, hepatitis C virus nonstructural-3 protease; HNE, human neutrophil elastase; SAR, structure−activity relationship; RCM, ring-closing metathesis.

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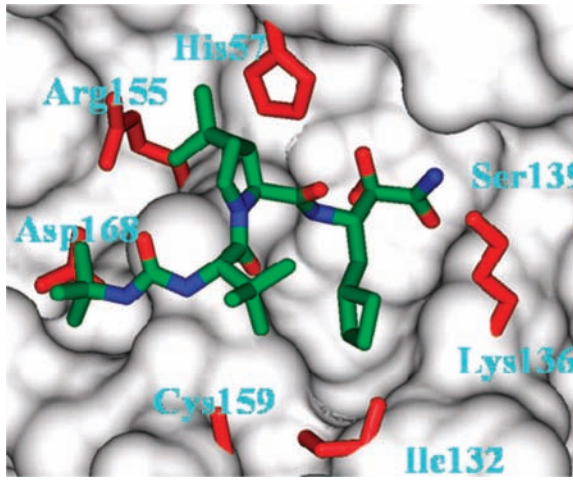


Figure 2. Compound **1** bound to HCV NS3 active site.

SAR development of novel macrocyclic inhibitors of HCV NS3 protease as potential backup compounds for **1**.

Macrocyclization of peptidic molecules is a strategy that has been successfully applied in numerous occasions for depeptidization of bioactive molecules.[13] Compounds with improved potency and pharmacokinetic profiles have been obtained through this method. Upon examination of X-ray structures of acyclic inhibitors bound to the active site of HCV NS3, the proximity of the P1/P3 and P2/P4 residues was evident (see Figure 1 for numbering of the amino acid residues). To illustrate this observation, the structure of **1** bound to the HCV NS3 protease active site is depicted in Figure 2. We hypothesized that macrocyclization via attachment of the appropriate residues would provide depeptidized inhibitors with increased activity. The improvement in potency would arise from more rigid conformations that could achieve a better fit in the active site. Also, improved pharmacokinetic profiles for these compounds could potentially be obtained as a result of modification of their peptidic backbone. Our research group has previously reported the design and synthesis of novel P2−P4 macrocyclic inhibitors.[14] Those reports presented in detail the SAR optimization to achieve low nanomolar inhibition of HCV NS3.

The P1−P3 macrocyclization strategy was also investigated in our group with very encouraging results. On the basis of X-ray structures of acyclic inhibitors bound to HCV NS3 and molecular modeling, we proposed the synthesis of macrocycles with various ring sizes ranging from 15- to 17-membered atoms. Initially, the proposed linkers for the attachment of the P1 and P3 residues were all-carbon linear chains. However, evaluation of the effect of heteroatoms and other functionalities, such as carbamates, was also required and different scaffolds were synthesized. A fused dimethylcyclopropyl proline was chosen as P2 on the basis of data from acyclic inhibitors previously synthesized in our group. It had been established that this unique fragment rendered the resulting compounds more active in both enzymatic and cellular assays.[9,15] We also proposed to use a keto-amide serine trap attached to the macrocyclic core for our inhibitors. Our group and others have previously demonstrated that keto-amide derived inhibitors possessed excellent binding and cellular activities.[16]

Chemistry

The synthetic strategy for the preparation of our P1−P3 macrocyclic inhibitors was based on the utilization of ring-closing metathesis (RCM) for the assembly of the macrocyclic

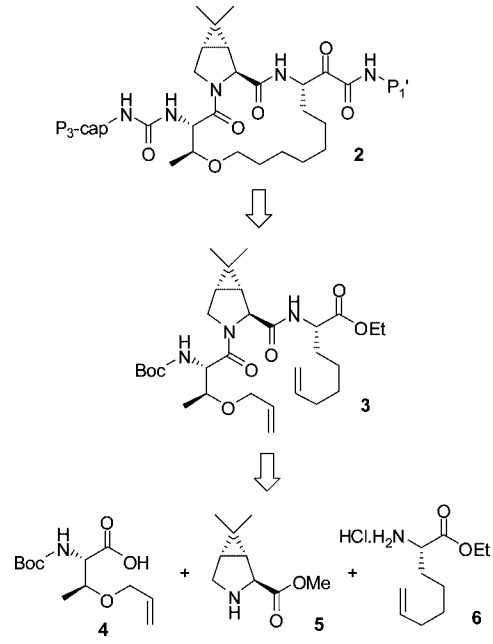


Figure 3. Retrosynthetic analysis for preparation of allo-threonine derived P1−P3 macrocyclic inhibitors of HCV NS3.

core. Our group reported the details for the preparation of all-carbon and L-serine derived macrocycles in a previous communication.[19] The retrosynthetic analysis for the allo-threonine macrocycles is depicted in Figure 3. Macrocycle **2** could be synthesized from tripeptide **3** via RCM followed by hydrogenation. The ethyl ester could be converted to the required ketoamide moiety via Passerini reaction, and the P3-cap group could be installed after *N*-Boc deprotection followed by formation of urea.

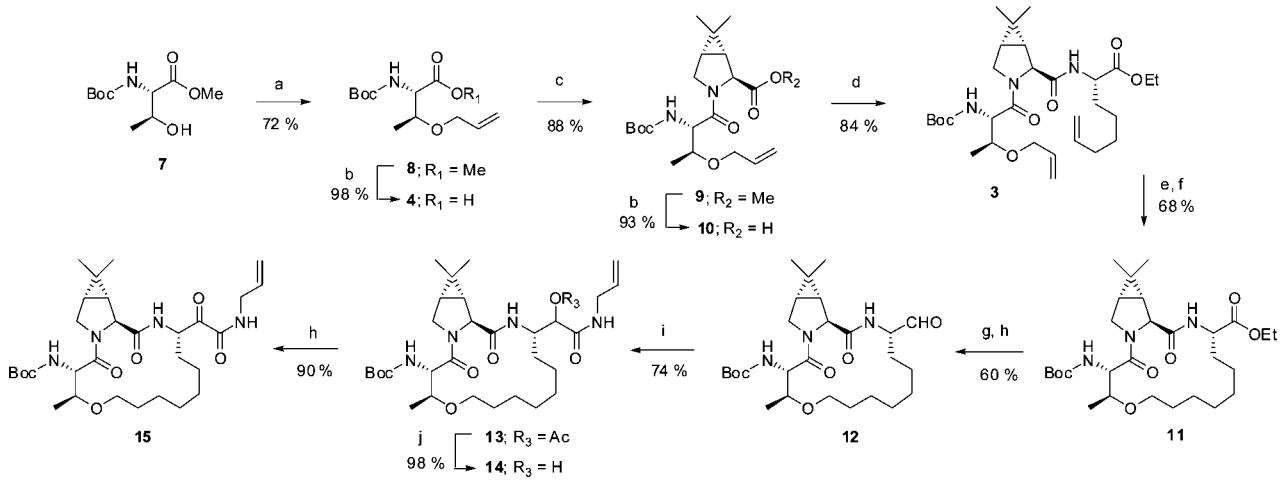
Tripeptide **3** could be obtained from sequential coupling reactions between *O*-allylated allo-threonine **4**, fused dimethylcyclopropyl proline **5**, and the *ω*-unsaturated amino acid **6**. The synthesis of allo-threonine macrocycle **15** is shown in Scheme 1. Thus, allo-threonine **7** underwent *O*-allylation under neutral conditions followed by hydrolysis of the ester functionality to give **4**. Acid **4** was coupled with dimethylcyclopropylproline **5** followed by hydrolysis of the ester to give **10**. Dipeptide **10** was coupled with *ω*-unsaturated amino acid derivative **6** to give the required substrate for RCM **3**. Utilization of Grubb's first generation catalyst for the RCM delivered the corresponding unsaturated macrocycle, which was hydrogenated to give the fully assembled macrocycle **11**. Then installation of the keto-amide moiety was achieved from the ethyl ester functionality of **11**. A two-step transformation involving reduction−oxidation of **11** gave aldehyde **12**, which underwent Passerini reaction with allyl isocyanide to obtain α-acetoxyamide **13**. Methanolysis of the acetate in **13** followed by Dess−Martin oxidation gave the allo-threonine macrocycle **15** which contains the required ketoamide moiety.

Introduction of the P3-cap in **15** was achieved in a one-pot reaction involving cleavage of the *N*-Boc protecting group with 4 M HCl followed by formation of the urea using the corresponding P3-isocyanate **16** under mild basic conditions to avoid epimerization of the P1 residue (Scheme 2).

Discussion

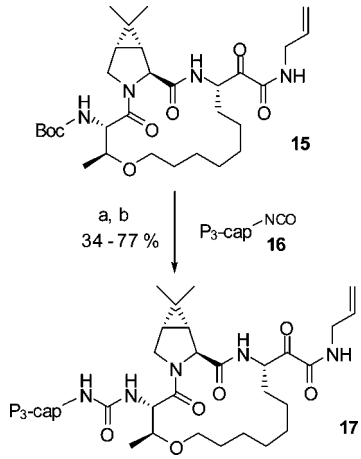
HCV NS3 Inhibitors **18**−**36** (Tables 1−3) were tested in an HCV continuous assay using the NS4A-tethered single chain NS3 serine protease. Inhibition of the NS3 serine protease was

Scheme 1. Synthesis of the P1−P3 Allo-Threonine Derived Macrocyclic Core[a]



[a] Reagents and conditions: (a) allyl-OC(O)OMe, $Pd(PPh_3)_4$, THF, 60 °C; (b) LiOH, THF/H_2O; (c) **5**, HATU, NMM, DMF/CH_2Cl_2; (d) **6**, HATU, NMM, DMF/CH_2Cl_2; (e) Grubb's catalyst, PhMe, 60 °C; (f) H_2 (35 psi), $Pd(OH)_2/C$, MeOH; (g) $LiBH_4$, THF, 0 °C; (h) Dess−Martin periodinane, CH_2Cl_2, 25 °C; (i) allyl-NC, AcOH, CH_2Cl_2; (j) LiOH, $THF/MeOH/H_2O$.

Scheme 2. General Procedure for Introduction of the P3-Cap in Allo-Threonine Derived Macrocycles[a]

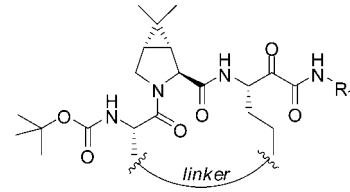


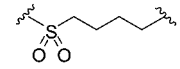
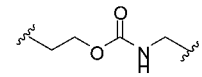
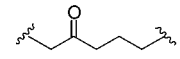
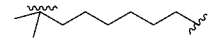
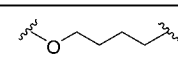
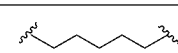
[a] Reagents and conditions: (a) 4 M HCl in dioxane, room temp; (b) **16**, CH_2Cl_2, aqueous $NaHCO_3$.

measured using a chromogenic assay previously reported.[17] The replicon activity (cellular potency) for inhibitors **26**−**36** (Tables 2 and 3) was determined using previously reported HCV replicon cell lines and assays.[18] In order to explore the SAR of the P1−P3 linker, different compounds having a diverse array of linkers were synthesized (Table 1). As demonstrated in Table 1, the data clearly indicated that the nature of the linker had a profound effect on the potency of the compounds.

The 16-membered macrocycle **18**, which contained a sulfone moiety, had weak binding (K_i* = 670 nM). The carbamate containing 17-membered macrocycle **19** (K_i* = 660 nM) was equipotent to compound **18**. It is noteworthy that the difference in ring size for **18** and **19** had little effect on activity. Introduction of a carbonyl in the linker resulted in compound **20** (K_i* = 320 nM); this modification proved beneficial, giving a 2-fold improvement in potency compared to **18** and **19**. SAR for acyclic inhibitors (such as compound **1**) had previously demonstrated that a bulky amino acid such as *tert*-butylglycine or cyclohexylglycine was preferred as P3 residue. Therefore, compound **21**, which had a *gem*-dimethyl substitution resembling *tert*-butylglycine, was synthesized. However, the binding data of **21** (K_i* = 250 nM) did not confirm our hypothesis,

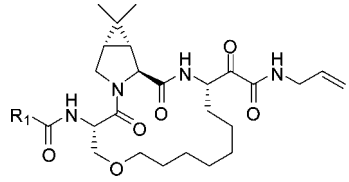
Table 1. Novel Macrocyclic Scaffolds for HCV NS3 Inhibitors

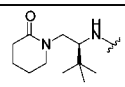
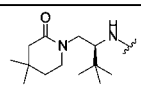
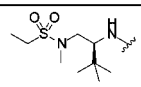
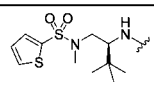
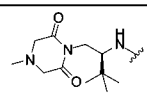
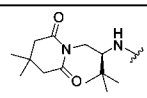
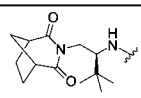


cmpd	*linker*	R_1	Ki* (nM)
18			670
19			660
20			320
21		H	250
22			150
23			36

resulting in a weaker inhibitor compared to the acyclic analogues. X-ray structure of **21** bound to HCV NS3 (data not shown) showed that one of the methyl groups of the P3-*gem*-dimethyl had a counterproductive interaction with the enzyme backbone. More potent inhibitors were subsequently obtained using linear nonbranched linkers. Thus, the oxygen containing macrocycle **22** with K_i* = 150 nM represented a new class of compounds that were as potent as those derived from acyclic series. The all-carbon linker gave macrocycle **23** with excellent binding (K_i* = 36 nM). The gain in potency represented a 5-fold improvement over the oxygen containing analogues. This gain

Table 2. Data for Oxygen Containing Macrocyclic Inhibitors **24** − **32** (L-Serine Derived)



cpd	R_1	K_i* (nM)	HNE/HCV	EC_{90} (μM)
24		400	na[a]	na[a]
25		160	9	na[a]
26		21	160	0.250
27		21	140	0.100
28		18	350	na[a]
29		14	100	0.150
30		15	300	0.080
31		7	300	0.050
32		3	1500	0.030

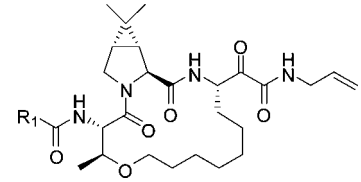
[a] na: not available.

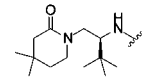
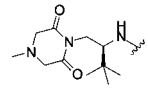
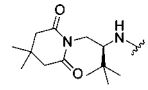
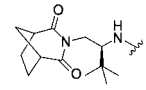
in potency could be attributed to the hydrophobic nature of the P1 and P3 regions in the HCV NS3 active site which favored nonpolar groups.

We have previously reported the synthetic approach developed for preparation of all-carbon and oxygen containing macrocyclic cores.[19] The synthetic approach is based on the utilization of ring closing metathesis for construction of the macrocyclic core and the late installation of the required keto-amide moiety using Passerini reaction. Herein, we present SAR data and optimization for oxygen containing macrocyclic analogues.

Oxygen Containing Macrocycles: L-Serine Derived Inhibitors. Binding data obtained from compound **22** established that an oxygen substitution in the macrocyclic core was well tolerated and provided a suitable scaffold that could be more metabolically stable than the all-carbon macrocyclic analogue. Therefore, we focused our efforts in further improving the potency of compounds in these series. Previous SAR

Table 3. Data for Oxygen Containing Macrocyclic Inhibitors **33**−**36** Derived from Allo-Threonine



cpd	R_1	K_i* (nM)	HNE/HCV	EC_{90} (μM)
33		8	1300	0.060
34		6	1100	0.060
35		7	1400	0.040
36		3	2800	0.030

developed for acyclic inhibitors has shown that introducing an appropriate P3-cap moiety led to improvement in both enzymatic and cellular potency. Thus, a set of compounds were prepared with the premise of exploring the P3-cap region using the L-serine derived macrocyclic core. Table 2 shows data for compounds prepared in this series.

Replacement of P3-Boc group in **22** with hydrogen gave compound **24**. Binding data showed that **24** had poor enzymatic activity with K_i* = 400 nM. Introduction of a P3-cap moiety derived from cyclohexylglycine resulted in compound **25**, which showed a 2-fold improvement in potency (K_i* = 160) compared to **24**. However, compound **25** had almost no selectivity against HNE (HNE/HCV = 9). To further improve the binding and cellular activity of our compounds, we investigated P3-caps derived from *tert*-butylglycine. These P3-caps included different groups such as lactams, sulfonamides, and imides. The results obtained are shown in Table 2.

Macrocycle **26**, which contains a lactam moiety in the P3-cap, was prepared and resulted in an 8-fold improvement in binding activity (K_i* = 21 nM) compared to cyclohexylglycine derived **25**. Compound **26** also gained selectivity against elastase (HNE/HCV = 160), and its replicon activity (EC_{90} = 160 nM) was better than that of compound **1**. Introduction of a *gem*-dimethyl group at the 4-position of the lactam ring in **26** resulted in compound **27**. This small modification did not affect the binding activity or the elastase selectivity of **27**; it remained equipotent to **26**. However, the replicon activity of **27** (EC_{90} = 100 nM) was improved by 2-fold compared to **26** and 3-fold compared to **1**. Thus, it became evident that the oxygen containing macrocycle was an excellent core capable of delivering compounds with better potency than **1**. In order to further improve the potency of our macrocyclic inhibitors, we investigated other P3-caps based on the SAR of acyclic analogues. Two different sulfonamide type P3-caps were attached to the

macrocyclic core to give compounds **28** and **29**. A slight improvement was observed for these compounds in the binding assay compared to lactam analogues. Compound **28** had $K_i^* =$ 18 nM, whereas **29** had $K_i^* = 14$ nM. However, the improvement in binding did not translate into better replicon potency compared to lactam **27**. A significant improvement in binding and cellular potency was achieved when imide type P3-caps were attached to the macrocyclic core leading to compounds with replicon EC_{90} values below 100 nM. Macrocycle **30** ($K_i^* = 15$ nM), which contained the *N*-methylimide derived P3-cap, showed slightly better replicon potency ($EC_{90} = 80$ nM). Concomitant to the improvement in cellular potency, a gain in HNE selectivity was also observed. Compound **30** had a 2-fold improvement in elastase selectivity (HNE/HCV = 300) compared to **27**. The *gem*-dimethylimide P3-cap was appended to the macrocyclic core to give compound **31** ($K_i^* = 7$ nM), which showed single digit nanomolar activity. This represented a 2-fold improvement in binding compared to the *N*-methylimide analogue **30**. Finally, an excellent compound was obtained when the bicyclic imide P3-cap was attached to our macrocyclic core to give **32**. Binding potency for **32** was in the low single digit nanomolar range ($K_i^* = 3$ nM), and the cellular potency for **32** was $EC_{90} = 30$ nM, which was 10-fold more active than **1**. The HNE selectivity for **32** (HNE/HCV = 1500) was higher than any other compound in this series and very close to that of **1**. Thus, the L-serine derived macrocyclic core proved to be an excellent scaffold for the synthesis of potent and selective inhibitors. The potency and selectivity of these macrocycles could be modulated by modification of the P3-cap region. Our goal to identify a macrocyclic core that could provide inhibitors with better potency than **1** was fulfilled. Furthermore, we established that the SAR of the P3-cap region for acyclic inhibitors translated very well into the macrocyclic series. Consequently, we directed efforts to further improve the overall profile of macrocyclic inhibitors by direct modification of the macrocyclic core.

Oxygen Containing Macrocycles: Allo-Threonine Derived Inhibitors. In order to modify the oxygen containing macrocyclic core with the goal to identify a scaffold that gave more potent compounds, we aimed to combine information gained from the L-serine derived macrocyclic inhibitors and the SAR for acyclic inhibitors in our program. Thus, we established that the 16-membered ring might be optimum for activity and the position of the oxygen within the macrocycle seemed to be favorable for binding. Since the macrocyclic system spanned from −P1−P2−P3−linker−, there were several possibilities for further modifications. Our fused dimethylcyclopropylproline P2 had proven superior to other P2 groups, and we decided to leave it unchanged. SAR of acyclic series for the P1 regions had shown that small hydrophobic groups gave analogues with better profiles. It seemed reasonable to leave the P1 region of our macrocyclic core unaffected as well. Therefore, the P3 region and the linker seemed to be more suitable for modification to gain access to a new scaffold. Modification of the linker length would lead to a macrocycle with different ring size, and we had established that a 16-membered macrocycle was excellent for activity. Thus, modification of the linker length was not pursued. Instead, we focused our efforts in modifying the P3 region to access new macrocyclic cores. Previous SAR for acyclic inhibitors had shown that bulky hydrophobic amino acids, such as *tert*-butylglycine, cyclohexylglycine, or β-methyl cyclohexylglycine, were excellent groups for the P3 region.[9,20] However, when we introduced a *gem*-dimethyl moiety in the macrocyclic core to mimic *tert*-butylglycine, a severe loss in activity was observed (Table 1, compound **21**, $K_i^* = 250$ nM). As indicated above, X-ray later demonstrated that one of the methyl groups had an adverse steric interaction with the enzyme backbone. We realized that introduction of a similar *gem*-dimethyl substitution in the oxygen containing macrocyclic core would have the same detrimental effect for activity. Therefore, we proposed to investigate a P3 group which did not possess the methyl group that gave the undesired interaction. Instead, only one methyl group was introduced in the oxygen containing macrocyclic core. This new scaffold was synthesized from allo-threonine following the synthetic approach based on RCM.

The results for allo-threonine derived macrocycles are shown in Table 3. It was evident that the proposed modification resulted in a new oxygen containing macrocyclic core that yielded more potent compounds than the L-serine macrocyclic analogues. Compound **33** ($K_i^* = 8$ nM), an allo-threonine derived macrocycle, was a much better compound compared to its L-serine derived analogue **27** (Table 2, $K_i^* = 21$ nM). The gain in binding activity for **33** was almost 3-fold compared to **27**. More importantly, the replicon activity of **33** ($EC_{90} = 60$ nM) underwent a 2-fold improvement. Compound **33** also showed a tremendous improvement in elastase selectivity (HNE/HCV = 1300). This number represents a 10-fold improvement compared to **27**. The data obtained for **33** clearly validated our hypothesis for modification of the macrocyclic core at the P3 position. The imide derived P3-caps used for L-serine derived macrocycles were also introduced in the allo-threonine macrocyclic series. Compound **34**, which had the *N*-methylimide derived P3-cap, was equipotent ($K_i^* = 6$ nM; $EC_{90} = 60$ nM) to **33**. The allo-threonine macrocycle **35**, which had the dimethylimide derived P3-cap, gave an improvement in cellular potency ($EC_{90} = 40$ nM) compared to its L-serine derived analogue **31** (Table 2, $EC_{90} = 50$ nM). Although both compounds showed similar binding activity ($K_i^* = 7$ nM), the elastase selectivity of **35** was significantly improved for the allo-threonine analogue (HNE/HCV = 1400, 5-fold increase compared to **31**). The allo-threonine macrocycle containing the bicyclic imide derived P3-cap, compound **36**, also showed excellent potency ($K_i^* = 3$ nM, $EC_{90} = 30$ nM). Its potency was similar to that of L-serine analogue **32**. Once more, an increased elastase selectivity was observed for **36** (HNE/HCV = 2800), providing an almost 2-fold improvement compared to **32**.

The replicon activity of the allo-threonine macrocycle **36** ($EC_{90} = 30$ nM) represented an improvement of more than 10-fold compared to **1** ($EC_{90} = 350$ nM). Furthermore, allo-threonine derived macrocycle **36** was much superior to **1** in terms of elastase selectivity (HNE/HCV = 2800). Compound **36** had excellent potency and selectivity profiles that make it a strong candidate for backup of **1**. Further investigations for macrocycles **32**, **36**, and related analogues are underway in our laboratories.

Conclusion

A new class of HCV NS3 protease inhibitors was discovered on the basis of the macrocyclization of acyclic inhibitors via their P1−P3 residues. X-ray analysis and molecular modeling played an important role in the design of the new macrocyclic scaffolds. It was demonstrated that the nature of the P1−P3 linker had a profound effect on activity and the most active compounds derived from linear all-carbon and oxygen-containing linkers. These linkers provided novel scaffolds for compounds with excellent enzymatic and cellular potencies. Other linker-substitutions, such as sulfones and carbamates, resulted in compounds with poorer binding activity. Further investigation

of oxygen-containing macrocyclic core in combination with various P3-caps resulted in the identification of **31** (K_i* = 7 nM; EC_{90} = 50 nM) and **32** (K_i* = 3 nM; EC_{90} = 30 nM). These inhibitors showed a remarkable potency profile that surpassed by 10-fold the potency of **1**. Further modifications of the L-serine derived macrocyclic core led to the identification of a new scaffold based on allo-threonine. The novel macrocyclic core provided more potent compounds that displayed better elastase selectivity as well. Thus, the idea of improving potency and selectivity by direct modification of the macrocyclic core was fulfilled by introduction of a methyl group in a strategic position identified on the basis of SAR and X-ray analysis of acyclic inhibitors. The new allo-threonine derived core in combination with different P3-caps resulted in the identification of **35** (K_i* = 7 nM; EC_{90} = 40 nM) and **36** (K_i* = 3 nM; EC_{90} = 30 nM). Macrocycle **36** also showed a 10-fold improvement in cellular potency compared to **1**, and it also showed enhanced elastase selectivity. In conclusion, a new class of HCV NS3 keto-amide containing macrocyclic inhibitors was identified. Heteroatoms were introduced into the macrocyclic system, leading to the identification of the oxygen-containing macrocyclic core as one of the best for potency and selectivity. Further improvements in potency and selectivity were achieved by investigation of various P3-caps attached to the macrocyclic core. Investigation of the DMPK profile for these oxygen-containing macrocyclic inhibitors is in progress.

Experimental Section

Dry solvents were purchased from Aldrich or Acros and used without further purification. Other solvents or reagents were used as obtained except when otherwise noted. Analytical thin layer chromatography (TLC) was performed on precoated silica gel plates available from Analtech. Visualization was accomplished with UV light or by staining with basic $KMnO_4$ solution, methanolic H_2SO_4, or Vaughn's reagent. Column chromatography was performed using Merck silica gel 60 (particle size 0.040−0.055 mm, 230−400 mesh) or using Biotage or Isco chromatographic systems. NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 in 400 or 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR). Low and high resolution mass spectra were obtained using electron spray or FAB ionization methods.

(2*S*,3*S*)-Methyl 3-(Allyloxy)-2-(*tert*-butoxycarbonylamino)-butanoate (8). A solution of *N*-Boc-L-allo-threonine methyl ester (**7**) (6.8 g, 29.15 mmol) in 250 mL of dry THF was degassed (vacuum/N_2-flush) and treated with allylmethyl carbonate (1.3 equiv, 4.3 mL, d 1.022). A catalytic amount of tetrakis(triphenylphosphine)palladium (0.02 mol%, 673 mg) was added. The slightly yellow mixture was degassed again and heated at 60 °C for 3 h. The mixture was concentrated under reduced pressure and the residue was chromatographed on silica gel (ethyl acetate/hexanes, 1:9) to afford the product **8** (5.72 g, 72%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.86 (1H, ddt, J = 5.1, 10.2, 17.5 Hz), 5.26 (1H, dd, J = 2.1, 17.5 Hz), 5.25 (1H, m), 5.16 (1H, dd, J = 1.4, 10.2 Hz), 4.44 (1H, dd, J = 3.6, 8.7 Hz), 4.02 (2H, m), 3.78 (1H, m), 3.74 (3H, s), 1.43 (9H, s), 1.20 (3H, d, J = 6.6 Hz). LRMS (ESI): calcd for $C_{13}H_{24}NO_6$ [M + H]$^+$ 274.17, found 274.28.

(1*R*,2*S*,5*S*)-Methyl 3-((2*S*,3*S*)-3-(Allyloxy)-2-(*tert*-butoxycarbonylamino)butanoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]-hexane-2-carboxylate (9). A solution of **8** (5.72 g, 20.92 mmol) in 250 mL of a 4:2:1 mixture of THF/water/MeOH was cooled to 0 °C and treated with lithium hydroxide monohydrate (2.5 equiv, 2.19 g). The cooling bath was removed after 30 min, and the mixture was stirred at room temp for a further 4 h until all the starting material had been consumed. The reaction mixture was treated with 200 mL of aqueous 1 M HCl, and the product was taken into dichloromethane (4 × 100 mL). The combined organic extracts were dried over magnesium sulfate, filtered, and concentrated under reduced pressure to afford acid **4** (5.42 g, 98%) which was used without further purification. LRMS (ESI): calcd for $C_{12}H_{22}NO_5$ [M + H]$^+$ 260.15, found 260.34.

A solution of **4** (5.42 g, 20.92 mmol) in 200 mL of dry dichloromethane and 100 mL of dry DMF was stirred at 0 °C and treated with HATU (1.4 equiv, 11.16 g). The amine hydrochloride **5** (1.2 equiv, 5.16 g) was added followed by *N*-methylmorpholine (4.0 equiv, 9.19 mL, d 0.920). The reaction mixture was stirred overnight. All the volatiles were removed under vacuum, and the residue was dissolved in 500 mL of ethyl acetate. The organic layer was washed with water (200 mL), aqueous 1 M HCl (100 mL), aqueous saturated sodium bicarbonate solution (100 mL), and brine (100 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was chromatographed on silica gel (ethyl acetate/hexanes, 2:8) to give the product **9** (7.6 g, 88%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.92 (1H, ddt, J = 5.1, 10.9, 16.8 Hz), 5.26 (1H, dd, J = 1.4, 16.8 Hz), 5.13 (3H, m), 4.47 (1H, dd, J = 7.3, 10.2 Hz), 4.39 (1H, s), 3.93 − 4.07 (3H, m), 3.84 (1H, d, J = 10.2 Hz), 3.72 (3H, s), 3.66 (2H, m), 1.39 (9H, s), 1.18 (3H, d, J = 6.6 Hz), 1.03 (3H, s), 0.92 (3H, s). LRMS (ESI): calcd for $C_{21}H_{35}N_2O_6$ [M + H]$^+$ 411.25, found 411.59.

(*S*)-Methyl 2-((1*R*,2*S*,5*S*)-3-((2*S*,3*S*)-3-(Allyloxy)-2-(*tert*-butoxycarbonylamino)butanoyl)-6,6-dimethyl-3-azabicyclo-[3.1.0]hexane-2-carboxamido)oct-7-enoate (3). A solution of **9** (7.6 g, 18.51 mmol) in 300 mL of a 2:1 mixture of THF/water was cooled to 0 °C and treated with lithium hydroxide monohydrate (2.5 equiv, 1.93 g). The cooling bath was removed after 30 min, and the mixture was stirred at room temp for a further 4 h. The reaction mixture was treated with 200 mL of aqueous 1 M HCl, and the product was taken into dichloromethane (4 × 100 mL). The combined organic extracts were dried over magnesium sulfate, filtered, and concentrated under reduced pressure to afford the acid **10** (6.86 g, 93%) as a colorless solid which was used without further purification. LRMS (ESI): calcd for $C_{20}H_{33}N_2O_6$ [M + H]$^+$ 397.23, found 397.60.

A solution of **10** (6.86 g, 17.30 mmol) in 100 mL of dry dichloromethane and 100 mL of dry DMF was stirred at 0 °C and treated with HATU (1.4 equiv, 9.23 g). The amine hydrochloride **6** (1.1 equiv, 4.21 g) was added to 100 mL of dichloromethane followed by addition of *N*-methylmorpholine (4 equiv, 7.6 mL, d 0.920). The reaction mixture was stirred at 0 °C overnight. All the volatiles were removed under vacuum, and the residue was dissolved in 500 mL of ethyl acetate. The organic layer was washed with water (2 × 100 mL), aqueous 1 M HCl (100 mL), aqueous saturated sodium bicarbonate solution (100 mL), and brine (100 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was chromatographed on silica gel (ethyl acetate/hexanes, 3:7) to afford the product **3** (8.17 g, 84%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.85 (1H, d, J = 7.3 Hz), 5.80 (2H, m), 5.21 (1H, dd, 1.4, 19.0 Hz), 5.16 (1H, s), 5.13 (1H, dd, J = 1.4, 10.2 Hz), 4.97 (1H, dd, J = 1.4, 16.8 Hz), 4.92 (1H, dd, J = 2.1, 10.2 Hz), 4.47 (2H, m), 4.36 (1H, s), 4.17 (2H, dq, J = 1.4, 7.3 Hz), 3.99 (2H, d, J = 5.1 Hz), 3.87 (2H, s), 3.65 (1H, m), 2.03 (1H, s), 2.02 (2H, q, J = 6.6 Hz), 1.80 (2H, m), 1.65 (1H, d, J = 7.3 Hz), 1.63 (1H, m), 1.47 (1H, m), 1.39 (9H, s), 1.26 (3H, t, J = 7.3 Hz), 1.25 (2H, m), 1.17 (3H, d, J = 5.9 Hz), 1.02 (3H, s), 0.88 (3H, s). LRMS (ESI): calcd for $C_{30}H_{50}N_3O_7$ [M + H]$^+$ 564.36, found 564.81.

3(*S*)-*tert*-Butoxycarbonylamino-4(*S*),18,18-trimethyl-2,15-dioxo-5-oxa-1,14-diazatricyclo[14.4.0.0^{17,19}]icosane-13(*S*)-carboxylic Acid Ethyl Ester (11). A solution of **3** (8.17 g, 14.49 mmol) in 1.5 L of toluene was degassed for 30 min (argon bubbling) and treated with Grubb's first generation catalyst (0.2 equiv, 2.38 g). The pink solution was heated to 60 °C for 18 h. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel (ethyl acetate/hexanes, 3:7) to give the unsaturated macrocyclic product (7.0 g, 90%) as a mixture of *E*- and *Z*-isomers (approximately 4:1). LRMS (ESI): calcd for $C_{28}H_{46}N_3O_7$ [M + H]$^+$ 536.33, found 536.79

A solution of the unsaturated macrocyclic product (7.0 g, 13.06 mmol) in 300 mL of methanol was treated with palladium on carbon (0.1 mol%, 1.37 g of 10% Pd/C). The mixture was hydrogenated at 35 psi for 3 h. The reaction mixture was diluted with 300 mL of dichloromethane and filtered through a short path of Celite. The filtrate was concentrated and the residue was chromatographed on silica gel (ethyl acetate/hexanes, 3:7) to afford the product **11** (5.33 g, 76%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.14 (1H, d, J = 8.8 Hz), 5.26 (1H, d, J = 10.2 Hz), 4.66 (1H, ddd, J = 2.9, 8.7, 9.5), 4.32 (1H, s), 4.29 (1H, d, J = 9.5 Hz), 4.18 (2H, q, J = 7.3 Hz), 3.93 (1H, d, J = 10.2 Hz), 3.89 (1H, m), 3.63 (1H, dd, J = 6.6, 8.7 Hz), 3.57 (1H, m), 3.21 (1H, ddd, J = 5.1, 6.6, 9.5 Hz), 1.84 (1H, m), 1.60 (1H, m), 1.36−1.53 (5H, m), 1.22−1.35 (6H, m), 1.39 (9H, s), 1.26 (3H, t, J = 7.3 Hz), 1.17 (3H, d, J = 5.8 Hz), 1.01 (3H, s), 0.85 (3H, s). LRMS (ESI): calcd for $C_{28}H_{48}N_3O_7$ $[M + H]^+$ 538.35, found 538.69.

(13(*S*)-Formyl-4(*S*),18,18-trimethyl-2,15-dioxo-5-oxa-1,14-diazatricyclo[14.4.0.0^{17,19}]icos-3(*S*)-yl)carbamic Acid *tert*-Butyl Ester (12). A solution of **11** (5.33 g, 9.91 mmol) in 100 mL of dry THF was treated with lithium borohydride (2.1 equiv, 10.4 mL of a 2 M solution in THF). The reaction mixture was stirred at room temperature. After 2 h, more lithium borohydride solution was added (1 equiv) and stirring was continued for 1 h. The excess lithium borohydride was quenched by addition of aqueous saturated ammonium chloride solution. The mixture was partitioned between ethyl acetate (300 mL) and aqueous saturated sodium bicarbonate solution (100 mL). The aqueous layer was back-extracted with ethyl acetate (2 × 100 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was chromatographed on silica gel (acetone/hexanes, 3:7) to afford the alcohol intermediate (3.93 g, 80%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.92 (1H, d, J = 8.7 Hz), 5.45 (1H, d, J = 10.2 Hz), 4.32 (1H, d, J = 9.5 Hz), 4.29 (1H, s), 4.09 (2H, m), 3.98 (1H, m), 3.92 (2H, m), 3.60−3.73 (3H, m), 3.56 (1H, m), 3.50 (2H, m), 3.23 (1H, m), 1.22−1.63 (10H, m), 1.38 (9H, s), 1.17 (3H, d, J = 5.8 Hz), 1.01 (3H, s), 0.85 (3H, s). LRMS (ESI): calcd for $C_{26}H_{46}N_3O_6$ $[M + H]^+$ 496.34, found 496.74.

A solution of the alcohol intermediate (1.0 g, 2.01 mmol) in 40 mL of dry dichloromethane was treated with Dess−Martin periodinane (1.5 equiv, 1.28 g). The reaction mixture was stirred at room temperature for 3 h. The mixture was treated with aqueous 1 M sodium thiosulfate solution (10 mL) and stirred for 5 min. Aqueous saturated sodium bicarbonate solution (30 mL) was also added, and stirring was continued for further 10 min. The mixture was extracted with dichloromethane (3 × 80 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated. The residue was chromatographed on silica gel (gradient, ethyl acetate/hexanes, 4:6 to 8:2) to afford the product **12** (750 mg, 75%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.57 (1H, d, J = 8.7 Hz), 7.27 (1H, broad s), 5.39 (1H, d, J = 10.2 Hz), 4.65 (1H, m), 4.34 (1H, s), 4.31 (1H, t, J = 9.5 Hz), 3.95 (1H, d, J = 10.9 Hz), 3.89 (1H, dd, J = 5.1, 10.9 Hz), 3.64 (1H, dd, J = 6.6, 8.7 Hz), 3.57 (1H, m), 3.20 (1H, m), 1.92 (1H, m), 1.19−1.63 (13H, m), 1.39 (9H, s), 1.17 (3H, d, J = 5.8 Hz), 1.02 (3H, s), 0.86 (3H, s). LRMS (ESI): calcd for $C_{26}H_{44}N_3O_6$ $[M + H]^+$ 494.32, found 494.71.

Acetic Acid Allylcarbamoyl-(3(*S*)-*tert*-butoxycarbonylamino-4(*S*),18,18-trimethyl-2,15-dioxo-5-oxa-1,14-diazatricyclo[14.4.0.0^{17,19}]icos-13(*S*)-yl)methyl Ester (13). A solution of **12** (750 mg, 1.51 mmol) in 20 mL of dry dichloromethane was treated with allylisocyanide (2 equiv, 210 mg) and acetic acid (2 equiv, 0.17 mL, d 1.049). The mixture was stirred at room temperature for 5 h. All the volatiles were removed under vacuum and the residue was chromatographed on silica gel (gradient, acetone/hexanes, 1:9 to 5:5) to afford the product **13** (700 mg, 74%) as a white solid. ^{1}H NMR reported as diastereomeric mixture (400 MHz, $CDCl_3$): δ 6.87 (0.3H, d, J = 9.5 Hz), 6.68 (0.7H, d, J = 9.5 Hz), 6.34 (0.3H, dd, J = 5.1, 5.8 Hz), 6.26 (0.7H, dd, J = 5.1, 5.8 Hz), 5.81 (1H, m), 5.15 (4H, m), 4.49 (0.7H, m), 4.41 (0.3H, m), 4.30 (1H, m), 4.21 (0.3H, s), 4.16 (0.7H, s), 3.90 (4H, m), 3.59 (2H, m), 3.23 (1H, m), 2.19 (2.1H, s), 2.17 (0.9H, s), 1.22 − 1.62 (14H, m), 1.39 (9H, s), 1.18 (3H, d, J = 5.8 Hz), 1.03 (0.9H, s), 1.01 (2.1H, s), 0.85 (3H, s). LRMS (ESI): calcd for $C_{32}H_{53}N_4O_8$ $[M + H]^+$ 621.39, found 621.85.

(13(*S*)-Allylaminooxalyl-4(*S*),18,18-trimethyl-2,15-dioxo-5-oxa-1,14-diaza-tricyclo[14.4.0.0^{17,19}]icos-3(*S*)-yl)carbamic Acid *tert*-Butyl Ester (15). A solution of **13** (700 mg, 1.12 mmol) in 20 mL of a 2:1:1 mixture of THF/water/methanol was treated with lithium hydroxide monohydrate (2.5 equiv, 118 mg) and stirred for 30 min. The reaction mixture was diluted with 50 mL of aqueous saturated sodium bicarbonate solution and extracted with dichloromethane (3 × 80 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated to afford the product **14** (651 mg, 98%) as a colorless solid which was used without further purification. LRMS (ESI): calcd for $C_{30}H_{51}N_4O_7$ $[M + H]^+$ 579.38, found 579.75.

A solution of **14** (652 mg, 1.127 mmol) in 25 mL of dry dichloromethane was treated with Dess−Martin periodinane (2.0 equiv, 956 mg). The reaction mixture was stirred at room temperature for 30 min. The mixture was treated with aqueous 1 M sodium thiosulfate solution (20 mL) and stirred for 5 min. Aqueous saturated sodium bicarbonate solution (30 mL) was also added, and stirring was continued for a further 10 min. The mixture was extracted with dichloromethane (3 × 80 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated. The residue was chromatographed on silica gel (gradient, acetone/hexanes, 1:9 to 5:5) to afford the product **15** (585 mg, 90%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 (1H, d, J = 8.0 Hz), 7.05 (1H, t, J = 5.8 Hz), 5.83 (1H, ddt, J = 5.8, 10.2, 17.5 Hz), 5.46 (1H, ddd, J = 2.9, 8.0, 9.5 Hz), 5.30 (1H, d, J = 10.2 Hz), 5.21 (1H, d, J = 16.8 Hz), 5.18 (1H, m), 4.36 (1H, s), 4.32 (1H, dd, J = 8.0, 10.2 Hz), 3.93 (3H, m), 3.85 (1H, dd, J = 5.1, 10.9 Hz), 3.64 (1H, dd, J = 6.6, 8.0 Hz), 3.57 (1H, ddd, J = 5.1, 5.8, 9.5 Hz), 3.22 (1H, ddd, J = 5.1, 5.8, 9.5 Hz), 1.92 (2H, m), 1.62 (1H, d, J = 8.0 Hz), 1.22−1.55 (11H, m), 1.39 (9H, s), 1.17 (3H, d, J = 6.6 Hz), 1.01 (3H, s), 0.85 (3H, s). LRMS (ESI): calcd for $C_{30}H_{49}N_4O_7$ $[M + H]^+$ 577.36, found 577.74.

(16a*S*,17a*R*,17b*S*)-13(*S*)-[[[[1(*S*)-[(2,4-Dioxo-3-azabicyclo-[3.2.1]oct-3-yl)methyl]-2,2-dimethylpropyl]amino]carbonyl]-amino]hexadecahydro-12(*S*),17,17-trimethyl-α,1,14-trioxo-*n*-(2-propenyl)-2*H*,12*H*-cyclopropa[3,4]pyrrolo[1,2-*e*][1,5,8]-oxadiazacyclohexadecine-3(*S*)-acetamide (36). The *N*-Boc allothreonine macrocycle **15** (60 mg, 0.104 mmol) was dissolved in 10 mL of 4 M HCl solution in dioxane. The resulting solution was stirred at room temperature for 30 min. All the volatiles were removed under reduced pressure, and the residue was placed under high vacuum for 3 h. The resulting hydrochloride salt was dissolved in 5 mL of dichloromethane and cooled to 0 °C. Then 20 drops of aqueous saturated sodium bicarbonate solution were added. After 10 min, a solution of the isocyanate **16** was added dropwise (1.2 equiv, 0.8 mL of a 0.155 M solution in toluene) and stirring was continued for 10 min. The cooling bath was removed and the mixture was stirred at room temp for 3 h. The reaction mixture was diluted with dichloromethane (50 mL) and dried over magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified on silica gel (gradient, acetone/hexanes, 2:8 to 6:4) to afford the product **36** (54 mg, 70%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.12 (1H, broad s), 7.39−7.79 (1H, broad s), 6.29 (1H, broad s), 5.91 (1H, ddt, J = 5.9, 10.4, 17.0 Hz), 5.71 (1H, broad s), 5.40 (1H, broad s), 5.27 (1H, dd, J = 1.2, 17.0 Hz), 5.23 (1H, dd, J = 1.2, 10.4 Hz), 4.67 (1H, dd, J = 7.8, 8.1 Hz), 4.50 (1H, broad s), 4.24 (1H, d, J = 10.7 Hz), 4.07 (1H, dd, J = 5.3, 10.4 Hz), 4.03 (1H, m), 3.97 (1H, ddd, J = 5.6, 5.9, 15.7 Hz), 3.81 (2H, m), 3.73 (1H, m), 3.67 (1H, d, J = 12.2 Hz), 3.62 (1H, m), 3.20 (2H, s), 3.07 (1H, s), 2.29 (1H, d, J = 11.0 Hz), 2.07 (3H, broad s), 1.93 (2H, broad s), 1.83 (3H, broad s), 1.28−1.68 (10H, m), 1.17 (3H, d, J = 5.9 Hz), 1.11 (1H, m), 1.01 (3H, s), 0.99 (9H, s), 0.83 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 198.3, 176.7, 172.3, 171.0, 158.8, 157.5, 132.9, 117.2, 75.0, 67.6, 60.3, 56.9, 55.3, 48.2, 44.9, 41.7, 39.9, 34.3, 32.3, 31.2, 28.1, 27.3,

27.1, 26.9, 26.4, 26.3, 24.2, 23.8, 22.6, 18.9, 15.7, 13.0 ppm. HRMS (FAB) calcd for $C_{39}H_{61}N_6O_8$ $[M + H]^+$: 741.4551, found 741.4543.

Supporting Information Available: Spectroscopical data for macrocyclic compounds **18**−**23**, L-serine derived macrocycles **24**−**32**, and allo-threonine derived macrocycles **33**−**35**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Hepatitis C. Fact Sheet No. 164; World Health Organization (WHO): Geneva, Switzerland, Revised October 2000. WHO Web site: http://www.who.int/mediacentre/factsheets/fs164/en/. (b) Wasley, A.; Alter, M. J. Epidemiology of hepatitis C: geographic differences and temporal trends. *Semin. Liver Dis.* **2000**, *20*, 1–16. (c) Brown, R. S. Jr; Gaglio, P. J. Scope of worldwide hepatitis C problem. *Liver Transplant.* **2003**, *9*, S10–S13.

(2) Lauer, G. M.; Walker, B. D. Hepatitis C virus infection. *N. Engl. J. Med.* **2001**, *345*, 41–52.

(3) (a) McHutchison, J. G.; Gordon, S. C.; Schiff, E. R.; Shiffman, M. L.; Lee, W. M.; Rustgi, V. K.; Goodman, Z. D.; Ling, M-H.; Cort, S.; Albrecht, J. K. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. *N. Engl. J. Med.* **1998**, *339*, 1485–1492. (b) Fried, M. W.; Shiffman, M. L.; Reddy, K. R.; Smith, C.; Marinos, G.; Gonçales, F. L., Jr.; Häussinger, D.; Diago, M.; Carosi, G.; Dhumeaux, D.; Craxi, A.; Lin, A.; Hoffman, J.; Yu, J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N. Engl. J. Med.* **2002**, *347*, 975–982.

(4) Poynard, T.; Marcellin, P.; Lee, S. S.; Niederau, C.; Minuk, G. S.; Ideo, G.; Bain, V.; Heathcote, J.; Zeuzem, S.; Trepo, C.; Albrecht, J. Randomised trial of interferon α2b plus ribavirin for 48 weeks or for 24 weeks versus interferon α2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. *Lancet* **1998**, *352*, 1426–1432.

(5) Choo, Q. L.; Kuo, G.; Weiner, A. G.; Overby, L. R.; Bradley, D. W.; Houghton, M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* **1989**, *244*, 359–362.

(6) Lindenbach, B. D.; Rice, C. M. Unraveling hepatitis C virus replication from genome to function. *Nature* **2005**, *436*, 933–938.

(7) Bartenschlager, R.; Ahlborn-Laake, L.; Mous, J.; Jacobsen, H. Nonstructural protein 3 of the hepatitis C virus encodes a serine-type proteinase required for cleavage at the NS3/4 and NS4/5 junctions. *J. Virol.* **1993**, *67*, 3835–3844.

(8) (a) Goudreau, N.; Llinàs-Brunet, M. The therapeutic potential of NS3 protease inhibitors in HCV infection. *Expert Opin. Invest. Drugs* **2005**, *14*, 1129–1144. (b) Manns, M. P.; Foster, G. R.; Rockstroh, J. K.; Zeuzem, S.; Zoulim, F.; Houghton, M. The way forward in HCV treatment—finding the right path. *Nat. Rev. Drug Discovery* **2007**, *6*, 991–1000. (c) Sheldon, J.; Barreiro, P.; Soriano, V. Novel protease and polymerase inhibitors for the treatment of hepatitis C virus infection. *Expert Opin. Invest. Drugs* **2007**, *16*, 1171–1181.

(9) Venkatraman, S.; Bogen, S. L.; Arasappan, A.; Bennett, F.; Chen, K.; Jao, E.; Liu, Y-T.; Lovey, R.; Hendrata, S.; Huang, Y.; Pan, W.; Parekh, T.; Pinto, P.; Popov, V.; Pike, R.; Ruan, S.; Santhanam, B.; Vibulbhan, B.; Wu, W.; Yang, W.; Kong, J.; Liang, X.; Wong, J.; Liu, R.; Butkiewicz, N.; Chase, R.; Hart, A.; Agrawal, S.; Ingravallo, P.; Pichardo, J.; Kong, R.; Baroudy, B.; Malcolm, B.; Guo, Z.; Prongay, A.; Madision, Broske, L.; Cui, X.; Cheng, K-C.; Hsieh, T. Y.; Brisson, J-M.; Prelusky, D.; Korfmacher, W.; White, R.; Bogonowich-Knipp, S.; Pavlovsky, A.; Prudence, B.; Saksena, A. K.; Ganguly, A.; Piwinski, J.; Girijavallabhan, V.; Njoroge, F. G. Discovery of (1*R*,5*S*)-*N*-[3-amino-1-(cyclobutylmethyl)-2−3-dioxopropyl]-3-[2(*S*)-[[[(1,1-dimethylethyl)-amino]carbonyl]amio]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-2(*S*)-carboxamide (SCH 503034), a selective, potent, orally bioavailable, hepatitis C virus NS3 protease inhibitor: a potential therapeutic agent for the treatment of hepatitis C infection. *J. Med. Chem.* **2006**, *49*, 6074–6086.

(10) Schering-Plough Press Release Aug 4th, 2008: Top-Line Results of Boceprevir Phase II Study Showed High Rate of Sustained Response (SVR) in Genotype 1 Treatment-Naive Hepatitis C Patients; Schering-Plough: Kenilworth, NJ, 2008. Schering-Plough Web site: http://www.schering-plough.com/news/news_article.aspx?reqid=1182855.

(11) (a) Yip, Y.; Victor, F.; Lamar, J.; Johnson, R.; Wang, Q. M.; Glass, J. I.; Yumibe, N.; Wakulchik, M.; Munroe, J.; Chen, S.-H. P4 and P1′ optimization of bicycloproline P2 bearing tetrapeptidyl α-keto-amides as HCV protease inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5007–5011. (b) Chen, S.-H.; Lamar, J.; Yip, Y.; Victor, F.; Johnson, R. B.; Wang, Q. M.; Glass, J. I.; Heinz, B.; Colacino, J.; Guo, D.; Tebbe, M.; Munroe, J. E. P1 and P1′ optimization of [3,4]-bicycloproline P2 incorporated tetrapeptidyl α-ketoamide based HCV protease inhibitors. *Lett. Drug Des. Discovery* **2005**, *2*, 118–123. (c) Perni, R. B.; Almquist, S. J.; Byrn, R. A.; Chandorkar, G.; Chaturvedi, P. R.; Courtney, L. F.; Decker, C. J.; Dinehart, K.; Gates, C. A.; Harbeson, S. L.; Heiser, A.; Kalkeri, G.; Kolaczkowski, E.; Lin, K.; Luong, Y.-P.; Rao, B. G.; Taylor, W. P.; Thomson, J. A.; Tung, R. D.; Wei, Y.; Kwong, A. D.; Lin, C. Preclinical profile of VX-950, a potent, selective, and orally, bioavailable inhibitor of hepatitis C virus NS3-4A serine protease. *Antimicrob. Agents Chemother.* **2006**, *50*, 899–909.

(12) (a) Lamarre, D.; Anderson, P. C.; Bailey, M.; Beaulieu, P.; Bolger, G.; Bonneau, P.; Bös, M.; Cameron, D. R.; Cartier, M.; Cordingley, M. G.; Faucher, A.-M.; Goudreau, N.; Kawai, S. H.; Kukolj, G.; Lagacé, L.; LaPlante, S. R.; Narjes, H.; Poupart, M-A.; Rancourt, J.; Sentjens, R. E.; George, T. S.; Simoneau, B.; Steinmann, G.; Thibeault, D.; Tsantrizos, Y. S.; Weldon, S. M.; Yong, C.-L.; Llinàs-Brunet, M. An NS3 protease inhibitor with antiviral effects in humans infected with hepatitis C virus. *Nature* **2003**, *426*, 186–189. (b) Llinas-Brunet, M.; Bailey, M. D.; Bolger, G.; Brochu, C.; Faucher, A.-M.; Ferland, J. M.; Garneau, M.; Ghiro, E.; Gorys, V.; Grand-Maitre, C.; Halmos, T.; Lapeyre-Paquette, N.; Liard, F.; Poirier, M.; Rheaume, M.; Tsantrizos, Y. S.; Lamarre, D. Structure−activity study on a novel series of macrocyclic inhibitors of the hepatitis C virus NS3 protease leading to the discovery of BILN 2061. *J. Med. Chem.* **2004**, *47*, 1605–1608.

(13) For recent examples, see the following: (a) Grieco, P.; Cai, M.; Liu, L.; Mayorov, A.; Chandler, K.; Trivedi, D.; Lin, G.; Campiglia, P.; Novellino, E.; Hruby, V. J. Design and microwave-assisted synthesis of novel macrocyclic peptides active at melanocortin receptors: discovery of potent and selective hMC5R receptor antagonists. *J. Med. Chem.* **2008**, *51*, 2701–2707. (b) Marsault, E.; Hoveyda, H. R.; Peterson, M. L.; Saint-Louis, C.; Landry, A.; Vézina, M.; Ouellet, L.; Wang, Z.; Ramaseshan, M.; Beaubien, S.; Benakli, K.; Beauchemin, S.; Déziel, R.; Peeters, T.; Fraser, G. L. Discovery of a new class of macrocyclic antagonists to the human motilin receptor. *J. Med. Chem.* **2006**, *49*, 7190–7197. For reviews, see the following: (c) Li, P.; Roller, P. P. Cyclization strategies in peptide derived drug design. *Curr. Top. Med. Chem.* **2002**, *2*, 325–341. (d) Li, P.; Roller, P. P.; Xu, J. Current synthetic approaches to peptide and peptidomimetic cyclization. *Curr. Org. Chem.* **2002**, *6*, 411–440.

(14) (a) Venkatraman, S.; Njoroge, F. G.; Girijavallabhan, V. M.; Madison, V.; Yao, N. H.; Prongay, A. J.; Butkiewicz, N.; Pichardo, J. Design and synthesis of depeptidized macrocyclic inhibitors of hepatitis C NS3-4A protease using structure-based drug design. *J. Med. Chem.* **2005**, *48*, 5088–5091. (b) Chen, K. X.; Njoroge, F. G.; Vibulbhan, B.; Prongay, A.; Pichardo, J.; Madison, V.; Buevich, A.; Chan, T.-M. Proline-based macrocyclic inhibitors of the hepatitis C virus: stereoselective synthesis and biological activity. *Angew. Chem., Int. Ed.* **2005**, *44*, 7024–7028. (c) Chen, K. X.; Njoroge, F. G.; Arasappan, A.; Venkatraman, S.; Vibulbhan, B.; Yang, W.; Parekh, T. N.; Pichardo, J.; Prongay, A.; Cheng, K.-C.; Butkiewicz, N.; Yao, N.; Madison, V.; Girijavallabhan, V. Novel potent hepatitis C virus NS3 serine protease inhibitors derived from proline-based macrocycles. *J. Med. Chem.* **2006**, *49*, 995–1005. (d) Arasappan, A.; Njoroge, F. G.; Chen, K. X.; Venkatraman, S.; Parekh, T. N.; Gu, H.; Pichardo, J.; Butkiewicz, N.; Prongay, A.; Madison, V.; Girijavallabhan, V. P2−P4 macrocyclic inhibitors of hepatitis C virus NS3-4A serine protease. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3960–3965.

(15) Bogen, S. L.; Arasappan, A.; Bennett, F.; Chen, K.; Jao, E.; Liu, Y.-T.; Lovey, R. G.; Venkatraman, S.; Pan, W.; Parekh, T.; Pike, R. E.; Ruan, S.; Liu, R.; Baroudy, B.; Agrawal, S.; Chase, R.; Ingravallo, P.; Pichardo, J.; Prongay, A.; Brisson, J.-M.; Hsieh, T. Y.; Cheng, K.-C.; Kemp, S. J.; Levy, O. E.; Lim-Wilby, M.; Tamura, S. Y.; Saksena, A. K.; Girijavallabhan, V.; Njoroge, F. G. Discovery of SCH 446211 (SCH 6): a new ketoamide inhibitor of the HCV NS3 serine protease and HCV subgenomic RNA replication. *J. Med. Chem.* **2006**, *49*, 2750–2757.

(16) (a) Venkatraman, S.; Wu, W.; Prongay, A.; Girijavallabhan, V.; Njoroge, F. G. Potent inhibitors of HCV-NS3 protease derived from boronic acids. *Bioorg. Med. Chem. Lett.* **2008**, doi: 10.1016/j.bmcl.2008.10.124. (b) Bennett, J. M.; Campbell, A. D.; Campbell, A. J.; Carr, M. G.; Dunsdon, R. M.; Greening, J. R.; Hurst, D. N.; Jennings, N. S.; Jones, P. S.; Jordan, S.; Kay, P. B.; O'Brien, M. A.; King-Underwood, J.; Raynham, T. M.; Wilkinson, C. S.; Wilkinson, T. C. I.; Wilson, F. X. The identification of α-ketoamides as potent inhibitors of hepatitis C virus NS3-4A proteinase. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 355–357. (c) Han, W.; Hu, Z.; Jiang, X.; Decicco, C. P. α-Ketoamides, α-ketoesters and α-diketones as HCV NS3 protease inhibitors. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 711–713.

(17) Zhang, R.; Beyer, B. M.; Durkin, J.; Ingram, R.; Njoroge, F. G.; Windsor, W. T.; Malcolm, B. A. A continuous spectrophotometric assay for the hepatitis C virus serine protease. *Anal. Biochem.* **1999**, *270*, 268–275.

(18) Lohmann, V.; Korner, F.; Koch, J.-O.; Herian, U.; Theilmann, L.; Bartenschlager, R. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science* **1999**, *285*, 110–113.

(19) Velázquez, F.; Venkatraman, S.; Wu, W.; Blackman, M.; Prongay, A.; Girijavallabhan, V.; Shih, N.-Y.; Njoroge, F. G. Application of ring-closing metathesis for the synthesis of macrocyclic peptidomimetics as inhibitors of HCV NS3 protease. *Org. Lett.* **2007**, *9*, 3061–3064.
(20) Arasappan, A.; Venkatraman, S.; Padilla, A. I.; Wu, W.; Meng, T.; Jin, Y.; Wong, J.; Prongay, A.; Girijavallabhan, V.; Njoroge, F. G. Practical and efficient method for amino acid derivatives containing β-quaternary center: application toward synthesis of hepatitis C virus NS3 serine protease inhibitors. *Tetrahedron Lett.* **2007**, *48*, 6343–6347.

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