Discovery of (1*R*,5*S*)-*N*-[3-Amino-1-(cyclobutylmethyl)-2,3-dioxopropyl]-3-[2(*S*)-[[[(1,1-dimethylethyl)amino]carbonyl]amino]-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

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Hepatitis C virus (HCV) infection is the major cause of chronic liver disease, leading to cirrhosis and hepatocellular carcinoma, which affects more than 170 million people worldwide. Currently the only therapeutic regimens are subcutaneous interferon-α or polyethylene glycol (PEG)-interferon-α alone or in combination with oral ribavirin. Although combination therapy is reasonably successful with the majority of genotypes, its efficacy against the predominant genotype (genotype 1) is moderate at best, with only about 40% of the patients showing sustained virological response. Herein, the SAR leading to the discovery of **70** (SCH 503034), a novel, potent, selective, orally bioavailable NS3 protease inhibitor that has been advanced to clinical trials in human beings for the treatment of hepatitis C viral infections is described. X-ray structure of inhibitor **70** complexed with the NS3 protease and biological data are also discussed.

Introduction

An estimated 170 million people worldwide are infected with hepatitis C virus (HCV), making it an impending public threat that leads to liver cirrhosis, carcinoma, or liver failure.[1] The slow progression of the disease in combination with mild symptoms has made early detection difficult. Pegylated α-interferon alone or in combination with ribavirin is the preferred treatment for HCV viral infection.[2] Although 80% of genotype-2-infected patients respond initially, only 40% of genotype-1-infected patients show sustained response to interferon treatment. Lack of effective methods to treat genotype-1 HCV infections and patients relapsing from interferon therapy necessitate discovery of new drugs. Significant efforts are now directed toward development of therapies that target key enzymes vital to HCV replication and maturation.[3]

Hepatitis C virus is a positive strand RNA with a single open frame of ∼9600 nucleotides. It encodes a single polypeptide of 3000 amino acids that is post-translationally modified to produce mature virions.[4] The single polypeptide contains all the structural and nonstructural proteins: C-E1-E2-P7-NS2-NS3-NS4A-NS4B-NS5A-NS5B. NS3, a trypsin-like serine protease, catalyzes cis cleavage of the NS3−NS4A junction, followed by trans cleavage of the NS4A−NS4B, NS4B−NS5A, and NS5A−NS5B to produce functional proteins.[5] The central role played by NS3 protease in the development of mature hepatitis C virus makes it an excellent target for drug discovery. Development of small-molecule inhibitors for this enzyme would potentially arrest the processing of the aforementioned polyprotein required for viral replication. This has been a field of intense investigation by various groups worldwide.[6] BILN-2061 and VX-950 are two novel protease inhibitors that have been advanced into clinical studies in humans and demonstrated to be efficacious.[7] The X-ray crystal structure of the enzyme reveals a featureless, shallow, highly solvent exposed active site located near the surface in a cleft between two β-barrel subdomains.[8] Characteristic residues of the catalytic triad, histidine-57, and aspartic acid-81 are located in the N-terminal, whereas serine-139 forms part of the C-terminal subdomain. Cysteine is conserved in the P_1 position of the natural substrate of NS3 protease in all three trans-cleavage sites and is replaced by threonine in the cis-cleavage event. The P_1' is a small hydrophobic amino acid, either a serine or alanine. The P_2, P_3, and P_4 sites are hydrophobic amino acids, and P_5 and P_6 usually contain acidic amino acids such as aspartic acid or glutamic acid.

Strategy and Initial Leads

We initially screened numerous compound libraries in an effort to identify a potential lead candidate that could be appropriately modified into a "druglike" entity. Our initial efforts failed to generate potential leads; thus, we resorted to structure-based drug design. We envisioned trapping the catalytic serine with conventional electrophiles such as aldehydes, ketones, trifluoromethyl ketones, and ketoamides. Most of these traps did not impart desirable activity with the inhibitors evaluated. A ketoamide containing undecapeptide **1** that spanned from P_6 to P_5' (Figure 1), a mixture of diasteromers at P_1, had an excellent activity of $K_i^* = 1.9$ nM in the inhibition of NS3 protease and thus became our starting point for drug development.

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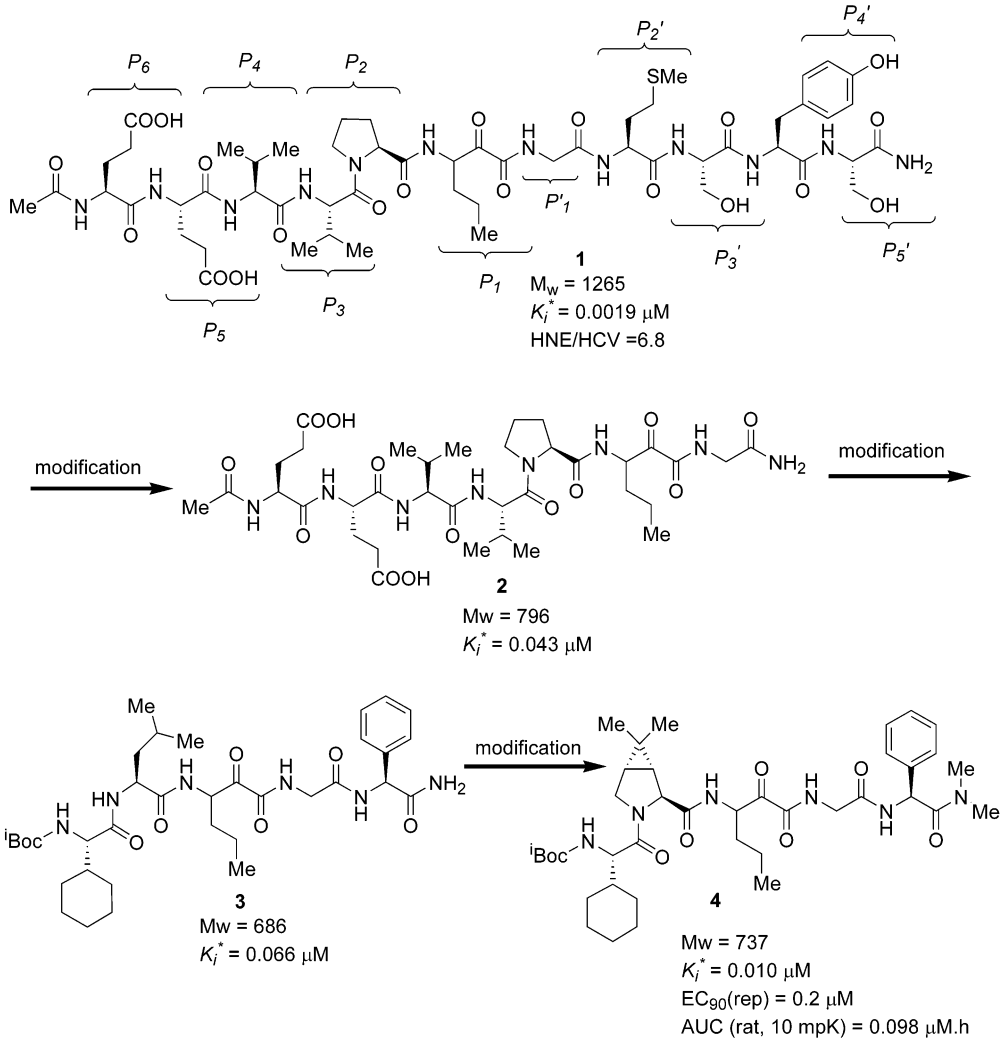


Figure 1. Truncation of lead undecapeptide **1** to P_3-P_2' derived pentapeptide inhibitors.

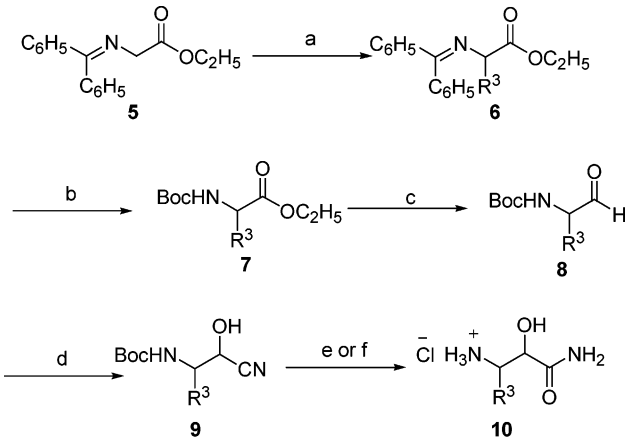
Inhibitor **1**, though an excellent inhibitor of HCV NS3 protease, lacked selectivity against human neutrophil elastase (HNE), a structurally related serine protease. Moreover, with such a large number of peptidic bonds in the molecule, the compound was bound to have poor pharmacokinetics (PK). It was therefore necessary to modify this compound by incorporating appropriate properties that would render it druglike. Replacement of C-terminal tetrapeptide methionine−serine−tyrosine−serine from $P_2'-P_5'$ with $-NH_2$ yielded truncated compound **2** with a $K_i^* = 0.043$ μM. Although this truncation resulted in a 25-fold loss in potency in comparison to **1**, the molecular weight of the resultant inhibitor had been drastically reduced by half. Side chain modification and introduction of various amino acids at P sites of inhibitor **1** pointed to cyclohexylglycine as an excellent P_3 and to leucine as a desirable P_2 residue that provided potent inhibitors. Parallel results from our laboratories also showed that introduction of phenylglycine amide at P_2' yielded P_3 truncated inhibitors of type **3**, which were equipotent to inhibitor **2**.[9] Although P_2 leucine containing inhibitors of type **3** were very potent in an enzyme inhibition assay, they lacked activity in cellular assay.[10] However, P_2 proline containing inhibitors displayed good enzyme binding and moderate cellular potency in a replicon-based assay.[11] Comparing inhibitors **2** and **3**, we envisioned introducing a nonproteinogenic amino acid 3, 4-dimethylcyclopropylproline as a P_2 residue because it had previously been established to mimic constrained leucine.[12] Incorporation of the 3,4-dimethylcyclopropylproline motif at the P_2 position resulted in compound **4** with a K_i^* of 0.010 μM and EC_{90}(rep) of 0.2 μM. Despite their good potencies, compounds of type **4** did not have appreciable oral bioavailability in rats, dogs, and monkeys.[11]

To obtain potent compounds with improved PK, we decided to modify **4** by varying side chain residues at P_1', P_3, P_1, and P_3-capping sites. We now report herein our efforts toward modification of compound **4** through extensive truncations and the discovery of **70** as a selective, potent HCV NS3 protease inhibitor with oral bioavailability, a potential therapeutic agent for the treatment of HCV infections.

Chemistry

Syntheses of P_1 fragments were accomplished following the general methods shown in Schemes 1−3. As outlined in Scheme 1, method A using cyanohydrin chemistry was employed for the syntheses of P_1 segments where the corresponding amino acids were readily available or easily synthesized. Thus, alkylation of diphenylimine protected glycine derivative **5** with appropriate alkyl halide followed by hydrolysis and Boc protection yielded amino esters of type **7**. The corresponding *N*-Boc protected amino acids were converted to the aldehyde **8** by reduction of the intermediate Wienreb amide using $LiAlH_4$.[13] These aldehydes were converted to cyanohydrins of type **9** by treatment with acetone cyanohydrin and triethylamine.[14] Intermediates **9** were hydrolyzed using aqueous or methanolic HCl to the corresponding hydroxy acids or hydroxy esters, which

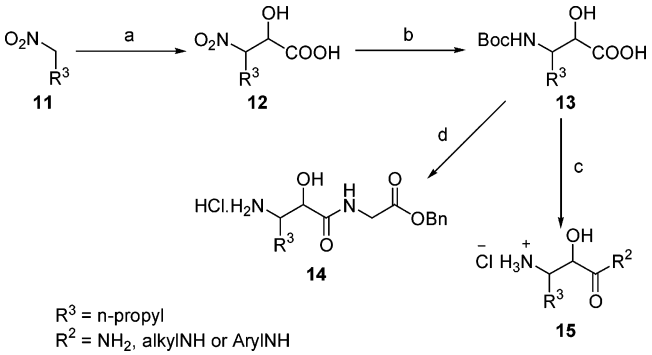
Scheme 1. Cyanohydrin Route: Method A[a]



R^3 = cyclopropylmethyl, cyclobutylmethyl, cyclopentylmethyl, cyclohexylmethyl, isobutyl, isobutenyl, n-butenyl

[a] Reagents and conditions: (a) KO^{t}Bu, THF, −78 °C → room temp, R^{3}X, room temp, 12 h; (b) (i) 1 M aqueous HCl, THF; (ii) Boc_2O; (c) (i) aqueous LiOH, THF/H_2O/MeOH; (ii) BOP or EDCI, CH_3NHOCH_3·HCl, CH_2Cl_2, DMF; (iii) $LiAlH_4$, THF, 0 °C → room temp, 0.5 h; (d) $(CH_3)_2C(OH)CN$, Et_3N, CH_2Cl_2, room temp; (e) (i) K_2CO_3, H_2O_2, DMSO; (ii) 4 M HCl/dioxane; (f) (i) 6 M methanolic HCl, reflux, 12 h; (ii) Boc_2O, (^{i}Pr)$_2$EtN; (iii) aqueous LiOH, THF/H_2O/MeOH; (iv) NH_4Cl, EDCI, NMM, DMF; (iv) 4 M HCl/dioxane.

Scheme 2. Henry Reaction: Method B[a]



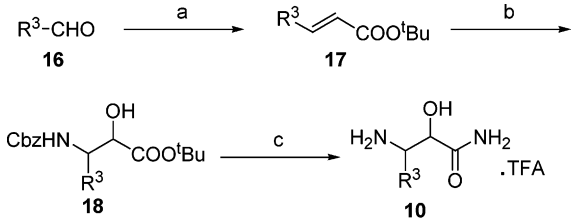
R^3 = n-propyl
R^2 = NH_2, alkylNH or ArylNH

[a] Reagents and conditions: (a) OHC−COOH·H_2O, MeOH, Et_3N; (b) H_2/Pd/C, CH_3COOH; (ii) Boc_2O, $NaHCO_3$, aqueous dioxane; (c) (i) NH_4Cl or R^{2}H, EDCI, NMM, DMF/CH_2Cl_2; (ii) 4 M HCl/dioxane; (d) (i) EDCI, NMM, Gly-OBn·HCl; (ii) 4 M HCl/dioxane.

were protected with the Boc group and subsequently coupled with ammonium chloride. Deprotection of the Boc group using HCl/dioxane resulted in a P_1 fragment of type **10**. In some cases, intermediates **9** were directly hydrolyzed to the corresponding primary amides using aqueous basic hydrogen peroxide, which were then followed by deprotection using 4 M HCl to yield α-hydroxyamide of type **10**.[15]

The Henry reaction (method B) was employed in the synthesis of the P_1 fragment where the corresponding nitroalkanes were commercially available (Scheme 2).[16] Nitropropane or nitrobutane was condensed with glyoxalic acid to form 3-substituted 2-hydroxy-3-nitropropionic acid derivatives **12**. The nitro group of **12** was catalytically hydrogenated using Pd/C, and the resulting amines were protected as the *tert*-butylcarbamate to form hydroxyacid of type **13**. Coupling of **13** with ammonium chloride followed by Boc deprotection resulted in P_1 segments used for syntheses of inhibitors outlined in Table 3. Compounds summarized in Table 2 were obtained via coupling acid **13** with various aliphatic and aromatic amines. P_1 segments required for the preparation of compounds in Table 1 were synthesized by coupling acid **13** with glycine benzyl ester to form dipeptide

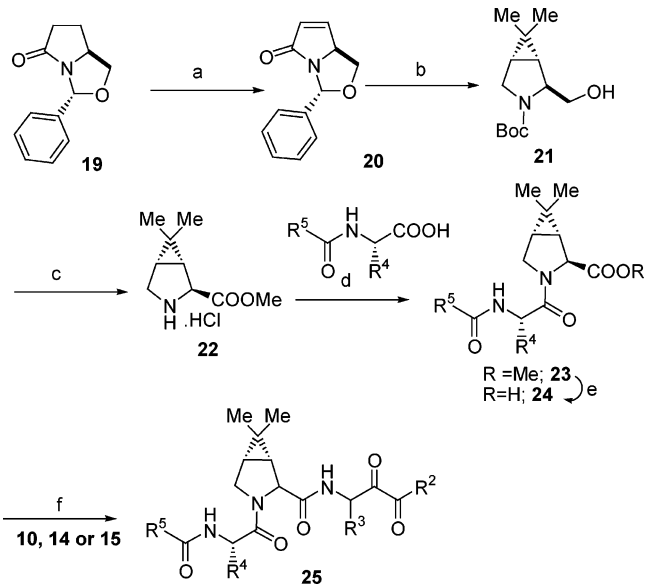
Scheme 3. Aminohydroxylation Reaction: Method C[a]



R^3 = trifluoroethyl, 3-methyloxetane, 2-methyloxetane

[a] Reagents and conditions: (a) $(EtO)_2P(O)CH_2COO^tBu$, Et_3N; (b) $(DHQ)_2Phal$, Cbz-NH_2, ^{t}BuOCl, aqueous NaOH; (c) (i) TFA/CH_2Cl_2; (ii) NH_4Cl, EDCI, NMM; (iii) H_2/Pd/C, MeOH or Me_2S, TFA.

Scheme 4[a]



[a] Reagents and conditions: (a) (i) KHMDS, PhSeCl, −78 °C → room temp; (ii) H_2O_2, pyridine; (b) (i) BuLi, $[(CH_3)_2CHPPh_3]Br$, −78 °C → room temp, THF; (ii) $LiAlH_4$, THF, reflux; (iii) H_2/Pd/C, Boc_2O; (c) (i) Jones oxidation, acetone, −5 °C, 3 h; (ii) TMS-diazomethane, toluene/MeOH; (iii) 4 M HCl/dioxane; (d) (i) EDCI, HOOBt, NMM, CH_2Cl_2/DMF, 12 h or HATU, NMM, CH_2Cl_2/DMF; (e) aqueous LiOH, THF/H_2O/MeOH; (f) (i) **10, 14,** or **15,** EDCI, HOOBt, NMM, CH_2Cl_2/DMF, room temp, 12 h; (ii) Cl_2COOH, EDCI·HCl, DMSO.

14. The benzyl ester was subsequently deprotected and coupled with various amines.

As shown in Scheme 3, Sharpless amino hydroxylation (method C) was the third method employed for syntheses of P_1 fragments of other compounds shown in Table 3.[17] Thus, aldehydes of type **16** were converted to the α,β-unsaturated *tert*-butyl esters **17** via the Horner Emmons reaction,[18] and the resultant olefins were subjected to aminohydroxylation to yield hydroxy esters of type **18**. Deprotection of *tert*-butyl esters of intermediates **18** generated hydroxy acids, which were used as described previously to prepare **10**.

Scheme 4 outlines representative syntheses of inhibitors **26**−**72**. The synthesis of the P_2 piece was carried out via phenylselenation of pyroglutamic acid derivative **19**, followed by oxidation of intermediate selenide with H_2O_2 and subsequent elimination to provide α,β-unsaturated lactam **20**.[19] Treatment of lactam **20** with isopropylphosphonium ylide followed by reduction of the lactam with $LiAlH_4$ resulted in the 3,4-dimethylcyclopropaned *N*-benzylprolinol.[20] The *N*-benzyl group deprotection using catalytic hydrogenation followed by Boc protection yielded prolinol derivative **21**. Jones oxidation of **21**, followed by esterification of the resultant acid with TMS-

diazomethane and Boc deprotection, yielded P_2 amine salt **22.** Coupling of **22** with the appropriately capped P_3 amino acid using the EDCI protocol gave dipeptide **23** that was hydrolyzed to acid **24** using aqueous LiOH. Subsequent couplings of acids **24** with appropriate P_1 segments resulted in intermediate hydroxyamides, which were oxidized to the corresponding ketoamides of type **25** using modified Moffat[21] conditions with EDCI, DMSO, and $Cl_2CHCOOH$ to yield compounds summarized in Tables 3−7. Alternatively, compounds in Tables 1 and 2 were readily obtained by coupling **24** with either intermediate **15** or **14** followed by oxidation with Dess−Martin periodinane reagent.[22]

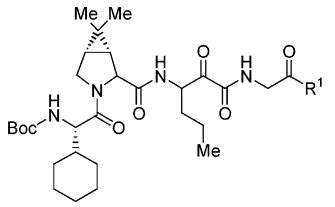
Discussion

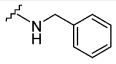
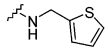
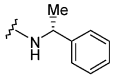
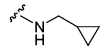
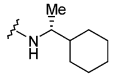
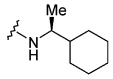
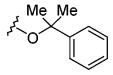
Synthesized inhibitors shown in Tables 1−7 were evaluated for their ability to inhibit the hydrolysis of chromogenic 4-phenylazophenyl (PAP) ester from the peptide fragment Ac-DTEDVVP(Nva)-O-4-PAP in a HCV protease continuous assay.[23] Inhibitors exhibited reversible slow tight binding kinetics, and K_i* data generated reflected equilibrium binding efficiency.[24] Potent compounds were then evaluated in a replicon-based cellular assay.[25] EC_{90}, the concentration required for inhibition of 90% of virus replication in hepatocytes, was recorded as a measure of replicon cellular potency. A multiplex Taqman RT reaction was done measuring both the HCV and endogenous GAPDH RNA levels. GAPDH RNA levels did not change when measured up to a maximum concentration of 5 μM. MTS assay as a mesure of toxicity was conducted on compounds of interest. Compounds of interest were evaluated for binding to human neutrophil elastase and the ratio of activity, HNE/HCV, reflected a measure of selectivity. Neutrophil elastase was chosen as a measure of selectivity because of its close structural similarity to HCV NS3 protease. Selected compounds were evaluated in a rapid rat assay[26] to obtain plasma levels. Compounds with good enzyme binding, selectivity, cellular potency, and rapid rat plasma levels were evaluated for full PK in rats, dogs, and monkeys.

P_2' Modified Analogues. Initially, phenylglycine dimethylamide at P_2' was replaced with benzyl and alkyl derivatives that mimicked the aryl portion of **4**. These modifications were intended to remove the terminal amide bond from **4**, thus making the resulting inhibitors less peptidic. Results of these modifications are summarized in Table 1.

Thus, replacement of phenylglycine of **4** with benzylamide resulted in **26** with K_i* = 0.056 μM, 5-fold less active than **4**. Introduction of thiophenemethylamide at P_2' gave compound **27** with activity similar to that of inhibitor **26** (K_i* = 0.060 μM). Both these compounds were evaluated for oral PK in rats and found to have AUC values of 1.27 and 0.73 μM·h, respectively. It was encouraging to observe that truncation of a single amide resulted in compounds with appreciable plasma levels in rats. Incorporation of a methyl group at the benzylic position of **26** resulted in compound **28** with similar potency, K_i* = 0.055 μM, but improved rat PK (AUC = 2.66 μM·h). Replacement of phenylglycine dimethylamide at P_2' with cyclopropylmethylamide resulted in compound **29** with K_i* = 0.130 μM, a 2-fold loss in activity compared to **26**. This could have been due to the fact that the cyclopropylmethyl group was too small to effect the desired interaction at P_2'. Compound **30** lacking the benzyl group or aliphatic group lost potency, indicating the preference of an aryl or alkyl moiety at P_2'. Introduction of (*R*)-methylcyclohexylamide at P_2' gave compound **31** (a saturated analogue of **28**) that resulted in a 2-fold loss in activity. Moreover, the rapid rat AUC of inhibitor **31** was poor with AUC = 0.09 μM·h. The role of phenylglycine NH at P_2' was probed by replacing it with oxygen to give compound **33** (K_i* = 0.050 μM). This result was similar to the activity of NH compound **26**, demonstrating that the phenylglycine NH was not critical for maintaining activity. This suggested that suitably modified P_1' analogues could also provide potent inhibitors.

Table 1. [a]



Cpd. *	*R^1*	*K_i^* (μM)*[§]
26		0.056
27		0.060
28		0.055
29		0.130
30		0.790
31		0.100
32		0.092
33		0.050

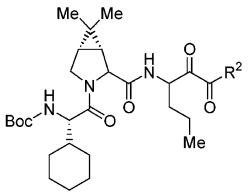
[a] Table notes are as follows. *P_1 fragment synthesized using method B. §K_i* value represents binding for a mixture of diasteromers at P_1 and within 2-fold for 95% confidence limit.

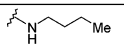
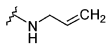
P_1' Modified Analogues. Further exploration of P_1' truncated analogues were carried out retaining norvaline as P_1 and cyclohexylglycine as P_3 residues. Initially, we decided to explore the P_1' site using smaller aliphatic amines. Results of these modifications are summarized in Table 2.

Replacement of the glycine−phenylglycine−dimethylamide segment of inhibitor **4** with butylamide resulted in compound **34** with K_i* = 1.9 μM, 50-fold less potent than **33**. However, introduction of allylamide at the P_1' site resulted in compound **35** with K_i* = 0.28 μM. Although the potency of **35** was 7-fold less than that of **33**, it was encouraging to see this kind of activity with a P_1' truncated compound. More interestingly, **35** had an excellent oral PK in rats with AUC = 26 μM·h. Incorporation of a smaller group such as methylamide at P_1' provided compound **36** with K_i* = 1.5 μM and excellent PK in rats with AUC = 13.4 μM·h. Further truncation to a primary amide functionality resulted in **37** (K_i* = 0.100 μM), a 3-fold improvement in potency over allylamide derivative **35**. Compound **37** also had a good PK in rats with AUC = 2.52 μM·h.

Enhanced potency of primary amide **37** was hypothesized to result from multiple hydrogen bondings to the enzyme backbone. This was confirmed by replacement of the primary amide with

Table 2. [a]



Cpd.[◊]	R^2	K_i*(μM)[§]	*Rat PK (PO) AUC (μM.h.)*[29]
34		1.90	NA
35		0.28	26
36[†]		1.50	13.4
37		0.10	2.52
38[†]		>13.0	NA
39[30]		0.11	NA

[a] Table notes are as follows. [†] *tert*-Butylglycine was used as P_3 residue. [◊] Method B was used for syntheses of P_1 fragment. [§] K_i* value represents binding for mixture of diasteromers at P_1 and within 2-fold for 95% confidence limit. NA = data not available.

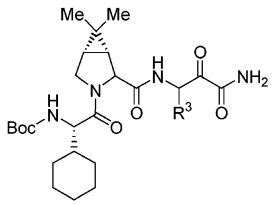
dimethylamide, resulting in compound **38**, which was essentially inactive (K_i* > 13.0 μM). Isosteric replacement of NH_2 with OH resulted in ketoacid **39** with K_i* = 0.110 μM, which once again demonstrated the importance of the P_1' hydrogen bond in maintaining activity. X-ray crystal structures of compounds of type **37** bound to protease established the participation of the primary amide functionality in hydrogen-bonding to glutamine-41 of the enzyme (Figure 3).[27]

Modifications at P_1. Analysis of the X-ray structure of NS3 enzyme revealed the S_1 pocket to be uniquely shallow and hydrophobic in nature. We sought to explore this pocket by introducing a variety of motifs. Enzyme binding and cellular data of P_1 modified compounds are tabulated in Table 3.

The carbon bearing P_1 residue is adjacent to the electrophilic ketoamide moiety where serine-139 from the enzyme attacks to form a covalent bond. The α-proton is acidic, making this center readily epimerizable. Thus, it was our initial intention to quarternize this center to alleviate potential epimerization. Unfortunately, incorporation of 1-aminocyclopropyl P_1 resulted in compound **40** that did not have appreciable activity. This loss in potency was ascribed to the inability of serine-139 to attack ketoamide functionality because of steric hindrance. Consequently, follow-up analogues were mostly prepared with monosubstituted amino acids at P_1.

Since a propyl group was readily accommodated at the P_1 site, we decided to further study the effect of changing the length of the carbon chain by incorporating a 2-aminobutyric acid moiety at this position. This resulted in the formation of compound **41** with K_i* = 0.74 μM, a 7-fold loss in activity in comparison to norvaline derivative **37**. Incorporation of the 2-aminohex-5-enoic acid derivative resulted in a less potent inhibitor **42** with K_i* = 0.150 μM. This demonstrated that extension of the carbon chain at P_1 was undesirable. We therefore explored the effect of branching substitution at the S_1 site. Replacement of norvaline with 2-amino-4-methylpent-4-enoic acid and isoleucine derivatives resulted in compounds **43** and **44** with K_i* = 0.3 μM and K_i* = 0.4 μM, respectively.

Table 3. [a]



Cpd.[◊]	R^3	K_i* (μM)[§]	*HNE/ HCV*	EC_{90} (μM)	*GAPDH toxicity(μM)*[▼]
37[b]		0.100	3	NA	NA
40[a]		>14.0	NA	NA	NA
41[b]		0.740	NA	NA	NA
42[a]		0.150	2	NA	NA
43[a]		0.300	2	NA	NA
44[a]		0.400	9	NA	NA
45[a]		0.025	23	0.400	>5
46[a]		0.008	138	0.700	>5
47[a]		0.150	370	NA	NA
48[a]		>12.0	NA	NA	NA
49[c]		0.400	NA	>1.00	>5
50[c]		0.090	140	NA	NA
51[c]		0.050	2	0.700	>5

[a] Table notes are as follows. [◊]Methods used for syntheses of P_1 fragments (Scheme 1): a = cyanohydrin route; b = Henry reaction; c = Sharpless method. [§]K_i* value represents binding for a mixture of diasteromers at P_1 and within 2-fold for 95% confidence limit. NA = data not available. [▼]Highest tested concentration is 5 μM.

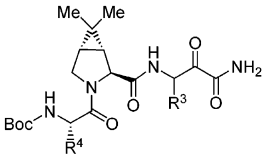
The loss in activity indicated that branching at P_1 was detrimental. Incorporation of cyclopropylalanine derived P_1 resulted in compound **45** with K_i* = 0.025 μM, a 4-fold improvement in activity over **37**. This result was encouraging because, for the first time, we achieved an activity of K_i* less than 0.1 μM in a truncated compound. The profound effect of constraining the two methyl groups of leucine into a ring to form cyclopropylalanine (compare compounds **45** and **44**) resulted in a 16-fold improvement in potency. Introduction of cyclobutylalanine at the P_1 position resulted in compound **46** with further enhancement in activity (K_i* = 0.008 μM). Further expansion of the ring size to the cylcopentylalanine derivative

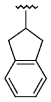
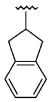
yielded compound **47** that resulted in a loss in activity with $K_i^* = 0.150\ \mu M$. Even worse, introduction of cyclohexylalanine at P_1 yielded compound **48** that was not active even at 12 μM. These studies clearly established that small cyclic groups were well tolerated at the S_1 pocket with the optimum ring being cyclobutylalanine. Potent inhibitors **45** and **46** were further profiled for their selectivity against human neutrophil elastase, HCV replicon cellular activity, and PK in rats and higher species. As summarized in Table 3, the HNE/HCV selectivities of these compounds were dependent on the size of the P_1 substituents. The greater the size of the P_1 residue, the better was the HNE/HCV selectivity. Comparison of compounds **45**−**47** demonstrated that the cyclopentylalanine, analogue **47**, was more selective (HNE/HCV = 370) than cyclobutylalanine compound **46** (HNE/HCV = 138), which was in turn more selective than cyclopropylalanine derivative **45** (HNE/HCV = 23). The replicon cellular activity of compounds **45** and **46** were 0.4 and 0.7 μM, respectively. In full rat PK studies compound **45** had an AUC of 1.3 μM·h and bioavailability of 4% whereas compound **46** had an AUC of 1.5 μM·h and bioavailability of 28%. However, compound **46** had poor PK in monkeys (Table 7). Introduction of small polar groups, such as oxygen, in the ring resulted in compounds **49** and **50** with $K_i^* = 0.40$ and 0.090 μM, respectively, a 50-fold loss in activity compared to **46**. This result clearly reinforced the fact that the S_1 position preferred lypophilic groups and introduction of polar functionality at P_1 resulted in loss of potency.

Modifications at the P_3 Site. The S_3 site of HCV NS3 protease is a shallow hydrophobic pocket occupied by isoleucine in the natural substrate. We explored the tolerance of this site by introducing a wide variety of substituents. Data for the resulting compounds are summarized in Table 4. Compounds described in this table were synthesized using procedures outlined in Schemes 1−4. In this series, cyclopropylalanine and cyclobutylalanine were retained as P_1 residues.

Truncation of cyclohexylglycine to valine at the S_3 site resulted in inhibitor **52** with $K_i^* = 0.210\ \mu M$, an 8-fold loss in potency compared to analogous cyclohexylglycine compound **45**. Similarly, introduction of cyclopentylglycine at P_3 gave **53**, a slightly less active compound with $K_i^* = 0.100\ \mu M$. The decrease in potencies for compounds **52** and **53** in comparison to P_3 cyclohexylglycine was attributed to insufficient van der Waals interaction of side chains at the S_3 site. To achieve better overlap, indanylglycine was introduced resulting in compound **54** with $K_i^* = 0.082\ \mu M$. Introduction of indanylglycine at P_3 with P_1 cyclobutylalanine resulted in compound **55** ($K_i^* = 0.27$ μM), a 30-fold loss in activity. Further exploration of the P_3 site with lypophilic *tert*-butylglycine resulted in compound **56** ($K_i^* = 0.057\ \mu M$) containing P_1 cyclopropylalanine and compound **57** ($K_i^* = 0.076\ \mu M$) containing P_1 cyclobutylalanine. It is worth mentioning that these compounds had a 3- to 4-fold improvement in binding activity relative to P_3 valine compound **52**. P_3 *tert*-butylglycine derived inhibitors **56** and **57** demonstrated improved HNE/HCV selectivity compared to P_3 cyclohexylglycine containing compounds. Once again the P_1 cyclobutylalanine analogue **57** had a better HNE/HCV selectivity than the P_1 cyclopropylalanine analogue **56**. Cellular activity of **56** and **57** in the replicon-based cellular assay was similar to that of cyclohexylglycine derived compounds **45** and **46** with $EC_{90} = 0.6$ and 0.8 μM, respectively. Introduction of amino(1-methylcyclopropyl)acetic acid, a constrained *tert*-butylglycine analogue at P_3, resulted in inhibitors **58** and **59** with diminished activity: $K_i^* = 0.300$ and 0.700 μM, respectively. Incorporation of 2-amino-3-hydroxy-3-methylbutyric acid at P_3 gave **60** with $K_i^* = 0.220\ \mu M$. This demonstrated that the S_3 site was less tolerant to polar substituents.

Table 4. [a]



Cpd	R^4	R^3	Ki* (μM)[§]	HNE/HCV	EC_{90} (μM)	GAPDH toxicity(μM)[▼]
45			0.025	23	0.400	>5
46			0.008	138	0.700	>5
52			0.210	19	NA	NA
53			0.100	11	NA	NA
54			0.082	49	>1.00	>5
55			0.270	NA	2.00	>5
56			0.057	112	0.600	>5
57			0.076	684	0.800	>5
58			0.300	NA	NA	NA
59			0.700	NA	NA	NA
60			0.220	55	NA	NA

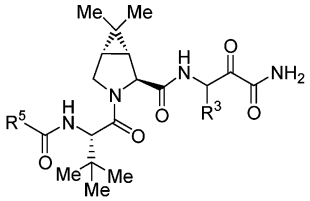
[a] Table notes are as follows. [§]K_i^* value represents binding for a mixture of diasteromers at P_1 and within 2-fold for 95% confidence limit. NA = data not available. [▼]Highest tested concentration is 5 μM.

Modifications at the P_3-Capping Site. From X-ray crystal structure studies of inhibitors bound to NS3 protease, it was clear that the *tert*-butyl group of Boc carbamate occupied the S_4 pocket. To explore this site further, a wide variety of carbamates and ureas were synthesized (Table 5). *tert*-Butylglycine was retained as the P_3 moiety because it demonstrated improved elastase selectivity in comparison to other P_3 residues. Cyclobutylalanine and cyclopropylalanine were similarly retained as the P_1 residues.

Incorporation of isopropyl carbamate capping with the P_1 cyclopropylalanine residue resulted in compound **61** with $K_i^* = 0.150\ \mu M$, 3-fold less active than the base compound **56** containing P_3 Boc capping. On the other hand, the cyclopropyl carbamate P_3-capped compound **62** had a slightly lower activity with $K_i^* = 0.300\ \mu M$.

Replacement of the Boc group in **56** with isobutyl carbamate resulted in compound **63** with similar potency ($K_i^* = 0.07\ \mu M$)

Table 5. [a]



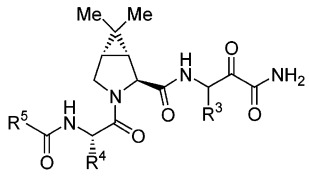
Cpd.	R^5	R^3	K_i* (μM)[§]	*HNE/HCV*	EC_{90} (μM)	*GAPDH toxicity*(μM)[▼]
56			0.057	112	0.600	>5
57			0.076	684	0.800	>5
61			0.150	100	NA	NA
62			0.300	NA	NA	NA
63			0.070	160	NA	NA
64			0.036	3800	0.700	>5
65			0.140	160	NA	NA
66			0.016	4200	0.730	>5
67			0.035	380	0.400	>5
68			0.013	369	0.400	>5
69			0.080	100	0.900	>5
70			0.014	2200	0.350	>5
71			0.060	45	>1.00	>5
72			0.100	270	NA	NA

[a] Table notes are as follows. [§]K_i* value represents binding for a mixture of diasteromers at P_1 and within 2-fold for 95% confidence limit. NA = data not available. [▼]Highest tested concentration is 5 μM.

and HNE/HCV selectivity (HNE/HCV = 160). Introduction of carbamate derived from (*R*)-3-methylbutan-2-ol resulted in compound **64** with K_i* = 0.036 μM, a 2-fold improvement in activity over the base compound. It was encouraging to see that the elastase selectivity of **64** (HNE/HCV = 3800) was greatly improved while retaining replicon cellular potency (EC_{90} = 0.7 μM). Overall, introduction of different carbamate P_3 caps did not appreciably improve enzyme binding or cellular activity; therefore, urea surrogates were pursued.

The isopropylurea capped compound **65** (K_i* = 0.140 μM) did not enhance activity in the P_1 cyclopropylalanine series. However, introduction of isopropylurea in the P_1 cyclobutylalanine series resulted in compound **66** with a 3-fold improvement in potency (K_i* = 0.016 μM) and 420-fold improvement in selectivity against elastase. Incorporation of *tert*-butylurea as a replacement of the Boc group resulted in compounds **68** and **70** with improved binding (K_i* = 0.013 and 0.014 μM, respectively), elastase selectivities, and cellular potencies. Inhibitor **70** with the *tert*-butylurea cap demonstrated HNE/HCV selectivity of 2200, a 15-fold improvement relative to the corresponding carbamate derivative **57**. *tert*-Butylurea capped compound **70** also demonstrated an improved cellular potency in the replicon assay with EC_{90} = 0.350 μM. Introduction of *N*-methyl-*tert*-butylurea capping resulted in compounds **71** and **72** with K_i* = 0.060 and 0.100 μM, respectively, a 4- to 7-fold loss in potency suggesting the involvement of urea NH in hydrogen-bonding to the peptide backbone.

Table 6. [a]



Cpd.	R^5	R^4	R^3	K_i* (μM)[§]	*HNE/HCV*
45				0.025	23
56				0.057	112
73				0.050	38
68				0.013	369
46				0.008	140
57				0.076	684
74				0.050	90
70				0.014	2200

[a] Table notes are as follows. [§]K_i* value represents binding for a mixture of diasteromers at P_1 and within 2-fold for 95% confidence limit.

Synergistic Effects of Residues on Binding and Selectivity. As the SAR of various substituents at P_1, P_3, and P_3-cappings were investigated, synergistic effects that influenced potency and selectivity were observed. These synergistic effects are discussed below.

(a) Effect of P_3 and P_3-Capping on Binding Activity. As shown in Table 6, the potency of inhibitors discussed greatly depended on the substituents at P_3 and P_3-capping. Thus, in a given series with P_1 cyclopropylalanine, Boc capped compound **45** (K_i* = 0.025 μM) with P_3 cyclohexylglycine had a better enzyme activity than compound **56** (K_i* = 0.057 μM) containing P_3 *tert*-butylglycine. However, *tert*-butylurea capped compound **68** (K_i* = 0.013 μM) with P_3 *tert*-butylglycine was found to be more potent than compound **73** (K_i* = 0.050 μM) that contained P_3 cyclohexylglycine. A similar observation was also seen in the P_1 cyclobutylalanine containing series of compounds; the Boc capped P_3 cyclohexylglycine containing compound **46** (K_i* = 0.008 μM) was more potent than Boc capped P_3 *tert*-butylglycine compound **57** (K_i* = 0.076 μM) and vice versa,

Table 7. PK Data of Selected Potent Compounds

compd	po AUC (μM·h)	% bioavailability
	Rat	
45[a]	1.3	3.4
46[a]	1.5	28
66[a]	0.83	13
68[a]	1.00	16
70[a]	1.52	26
	Monkey	
46[c]	0.11	1
68[b]	0.05	2
70[c]	0.12	4−11
	Dog	
68[b]	1.6	36
70[c]	3.08	30

[a] 10 mg/kg. [b] 2 mg/kg. [c] 3 mg/kg.

and the urea capped P_3 *tert*-butylglycine derivative **70** (K_i* = 0.014 μM) was more potent than P_3 cyclohexylglycine derivative **74** (K_i* = 0.050 μM). This synergy could not be explained by assessment of the X-ray structures of these compounds bound to the protease.

(b) Effect of P_3 and P_1 Substituents on Selectivity. Another interesting synergy was observed on selectivity against elastase by the interaction of P_1 and P_3 residues. Thus, a close examination of inhibitors demonstrated that P_1 cyclobutylalanine derived inhibitors had better selectivity against HNE than P_1 cyclopropylalanine compounds. Moreover, in the series containing P_1 cyclobutylalanine, inhibitors containing P_3 *tert*-butylglycine (compounds **57** and **70**) were more selective than P_3 cyclohexylglycine counterparts (compounds **46** and **74**).

Pharmacokinetic Studies. On the basis of potency and selectivity, key compounds were selected and evaluated for their PK profiles in rats, monkeys, and dogs.

As shown in Table 7, the Boc capped compound **46** containing P_1 cyclobutylalanine had a good bioavailability of 28% in rats with AUC = 1.5 μM·h, whereas the corresponding P_1 cyclopropylalanine containing compound **45** had a bioavailability of 4% with albeit similar AUC = 1.3 μM·h. However, PK in monkeys for compound **46** was poor with AUC = 0.11 μM·h and a short half-life of 1 h. The isopropylurea capped compound **66** demonstrated moderate PK in rats with AUC = 0.83 μM·h and bioavailability of 13%. Thus, it was not further evaluated in monkeys and dogs. Evaluation of *tert*-butylurea capped compound **68** and **70** in rats gave AUC = 1.0 and 1.5 μM·h and bioavailabilities of 16% and 26%, respectively. The PK data of these inhibitors in dogs were similar to those in rats; P_1 cyclopropylalanine derived compound **68** had bioavailability of 36% with AUC = 1.6 μM·h at 2 mg/kg, and P_1 cyclobutylalanine analogue compound **70** had bioavailability of 30% with AUC = 3.1 μM·h at 3 mg/kg. Both compounds showed variable monkey PK with bioavailability ranging between 2% and 11%. Inhibitor **70** was moderately absorbed with an absorption of 36% in rats and absorption of 23% in dogs. It was well distributed in the body especially in the target organ liver, which demonstrated a liver/plasma ratio of ~30.

Since compound **70** had good enzyme binding, selectivity against HNE, desirable PK properties, excellent target organ exposure, and no hERG or CYP inhibition, it was progressed into clinical studies in humans (Figure 2).

The X-ray structure of inhibitor **70** bound to HCV NS3 protease was solved and is shown in Figure 3.

From the structure, it was clear that cyclobutylalanine moiety occupied most of the space available at the S_1 pocket. The P_2 dimethylcyclopropylproline residue adopted a bent conformation that allowed maximum overlap of the methylenes of proline and cyclopropyl ring to Ala-156. The conformation adopted by cyclopropanated proline allowed the methyl group proximal to the carbonyl to interact with His-57 and the methyl group distal to carbonyl to interact with Ala-156 and Arg-155. The side chain of P_3 *tert*-butylglycine occupied the S_3 pocket with the two methyl groups of the *tert*-butyl group interacting with the protease effectively and the third methyl group being solvent-exposed. The tertiary butyl group of P_3-urea capping occupied the S_4 pocket, enhancing binding of the inhibitor to protease. The electrophilic ketoamide reversibly trapped serine-139 to form a covalent bond with the enzyme and the hydrogen of the primary amide donated a hydrogen bond to the peptidic backbone of the protein, locking the inhibitor to the surface.

In addition to van der Waals contacts, inhibitor **70** formed a series of specific hydrogen bonds with the protein surface. A schematic representation of these interactions to the protease is shown in Figure 4. When the various hydrogen-bonding interactions that existed between inhibitor **70** and NS3 protease were mapped out, it was evident that the primary ketoamide donated a hydrogen bond to glutamine-41, and the carbonyl oxygen in turn made two hydrogen bonds with the nitrogens of serine-139 and glycine-137. The urea nitrogens donated two hydrogen bonds to alanine-157, thus improving potency and HCV specificity. Additionally, the nitrogen of the P_1 cyclobu-

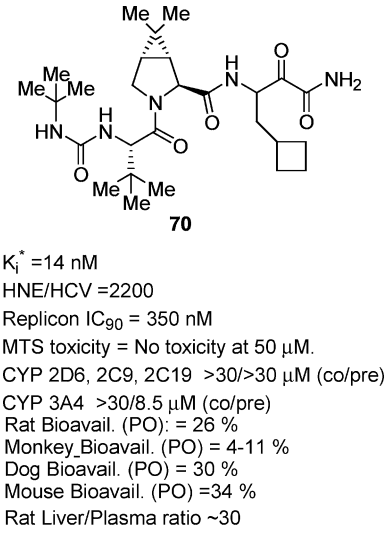


Figure 2. Compound **70**.

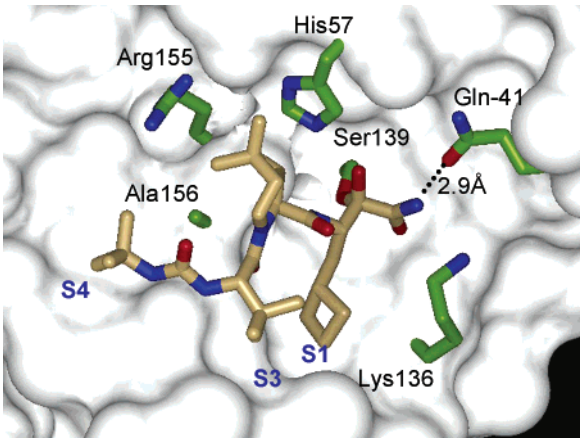


Figure 3. X-ray structure of inhibitor **70** bound to NS3 protease.

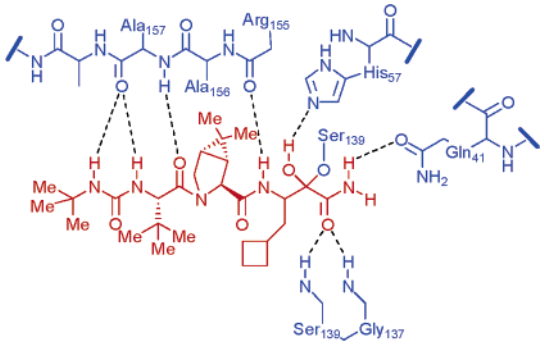


Figure 4. Hydrogen bond interactions of inhibitor **70** with HCV protease.

tylalanine donated a hydrogen bond to Arg-155, and the oxygen of the P_3 carbonyl group accepted a hydrogen bond from the Ala-157. In combination with hydrophobic interaction, the array of hydrogen bonds also contributed greatly to the binding potency and selectivity of compound **70**.

Conclusions

A series of systematic truncations and modifications of amino acid residues of the lead undecapeptide **1** led to the discovery of **70**, a potent, low molecular weight HCV NS3 protease inhibitor. Initial truncation efforts of inhibitor **1** led to the discovery of compounds that spanned from P_3 to P_2', which had good enzyme binding as exemplified by compound **4**. Lack of PK and cellular potency in earlier series necessitated further truncation and depeptidization of initial molecules. The relationship between lower molecular weight inhibitors and rat PK was established early with the identification of P_2' truncated analogues. P_1' SAR studies allowed identification of primary ketoamide moiety as an excellent residue that improved binding potency of the P_3-P_1 truncated compounds. The identification of primary ketoamides series with improved enzyme activity and PK allowed further optimization to identify **37**. Systematic SAR studies in the P_1 site identified cyclopropylalanine and cyclobutylalanine as excellent P_1 groups that provided potent inhibitors. Cyclopropylalanine and cyclobutylalanine at P_1 in combination with cyclohexylglycine at P_3 yielded inhibitors **45** and **46** that were active in enzyme and replicon cellular assays. Even though cyclohexyglycine was an acceptable P_3 residue, further SAR at this site showed *tert*-butylglycine as an alternative group that provided inhibitors with improved HNE selectivity. In the course of these investigations, we discovered a synergy between P_1, P_3, and P_3-capping residues that governed enzyme binding and HNE selectivity. Finally, P_3-capping SAR led to the identification of *tert*-butylurea as an excellent cap, which in turn improved potency of the P_3 *tert*-butylglycine series leading to the identification of compound **70** as a candidate with excellent binding, selectivity, and cellular potency.

The PK profile of inhibitor **70** demonstrated good oral bioavailabilties of 26% and 30% in rats and dogs with excellent distribution in animals. Target organ analysis in rats revealed a desirable 30-fold liver/plasma concentration of **70** with a concentration of ~14 times the replicon EC_{90}.

Finally, the X-ray of **70** was solved and illustrated the major interactions between the inhibitor and the enzyme. Compound **70** has been advanced to phase 1 clinical trials for the treatment of hepatitis C viral disease.

Experimental Section

General. 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) were performed on precoated silica gel plates available from Analtech. Column chromatography was performed using Merck silica gel 60 (particle size 0.040−0.055 μm, 230−400 mesh) or using Biotage or Isco chromatographic systems. Many compounds were further purified using Varian normal-phase HPLC with YMC-diol column with solvent A (hexanes) and solvent B (2-propanol, CH_2Cl_2, and acetonitrile). Visualization was accomplished with UV light or by staining with basic $KMnO_4$ solution, methanolic H_2SO_4, or Vaughn's reagent. NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 unless otherwise noted at 300, 400, or 500 MHz (^{1}H NMR) or at 75, 100, or 125 MHz (^{13}C NMR). Mass spectra were obtained using electron spray FAB ionization methods. Where applicable ^{1}H NMR and ^{13}C NMR of compounds are reported as two sets of signals (integration adding up to two molecules) representing two diasteromers.

Representative Procedure for the Synthesis of 3-Amino-4-cyclobutyl-2-hydroxybutyramide Hydrochloride Salt (R^3 = Cyclobutylmethyl, 10) Using Cyanohydrin Route. Similar methods were employed for the syntheses of intermediates **10** where R^3 was cyclopropylmethyl, cyclopentylmethyl, cyclohexylmethyl, isobutyl, isobutylene, or *n*-butenyl.

Ethyl 2-*tert*-Butoxycarbonylamino-3-cyclobutylpropionate (7). A stirred solution of ketimine **5** (50 g, Aldrich, 187.1 mmol) in dry THF (400 mL) under N_2 was cooled to −78 °C and treated with a solution of K^{t}BuO (220 mL, 1.15 equiv, 1 M in THF). The reaction mixture was stirred at 0 °C for 1 h and treated with bromomethylcyclobutane (28 mL, 249 mmol) and further stirred at room temperature for 48 h. The reaction mixture was concentrated in vacuo, and the residue was dissolved in Et_2O (300 mL) and treated with aqueous HCl (2 M, 300 mL). The resulting solution was stirred at room temperature for 5 h and extracted with Et_2O (1 L). The aqueous layer was basified (pH ~12−14) with aqueous NaOH (50%) and extracted with CH_2Cl_2 (3 × 300 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to give pure cyclobutylalanine ethyl ester (18 g) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.13 (q, 2 H, J = 7.2 Hz), 3.33 (dd, 1 H, J = 5.5 and 2.2 Hz), 2.42 (h, 1 H, J = 7.7 Hz), 2.08−1.98 (m, 2 H), 1.89−1.55 (m, 6 H), 1.25 (t, 3 H, J = 7.2 Hz).

A solution of cyclobutylalanine ethyl ester (18 g, 105.2 mmol) in CH_2Cl_2 (350 mL) was treated with di-*tert*-butyl dicarbonate (23 g, 105.4 mmol) at 0 °C and stirred at room temperature for 12 h. After the completion consumption of starting material (TLC), the reaction mixture was concentrated in vacuo and purified by chromatography (SiO_2, EtOAc/hexanes, 0−20% gradient). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.00 (d, 1 H, J = 7.3 Hz), 4.22 (dq, 3 H, J = 6.9 and 1.0 Hz), 2.42 (p, 1 H, J = 8.2 Hz), 2.15−2.10 (m, 2 H), 1.94−1.68 (m, 6 H), 1.49 (s, 9 H), 1.33 (t, 3 H, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.5, 155.7, 80.1, 61.6, 52.9, 40.2, 33.0, 28.9, 28.8, 19.1, 14.6; MS (ESI), 294 [(M + Na)$^+$, 80], 216 (50), 172 (100).

(2-Cyclobutyl-1-formylethyl)carbamic Acid *tert*-Butyl Ester (8). Ester **7** was dissolved in THF/H_2O (200 mL, 1:1) and treated with LiOH·H_2O (6.5 g, 158.5 mmol) and stirred at room temperature for 3 h. The reaction mixture was acidified with concentrated HCl (pH ~1−0) and extracted with CH_2Cl_2. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo to yield Boc protected cyclobutylalanine as a colorless viscous oil, which was used for next step without further purification.

A solution of acid (15.0 g, 61.7 mmol) in CH_2Cl_2 (250 mL) was treated with BOP reagent (41.1 g, 93 mmol), *N*-methylmorpholine (27 mL), and *N*,*O*-dimethylhydroxylamine hydrochloride (9.07 g, 93 mmol) and stirred at room temperature for 16 h. The reaction mixture was diluted with 1 N aqueous HCl (250 mL), and the organic layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 300 mL). The combined organic layers were dried ($MgSO_4$), filtered, concentrated in vacuo, and purified by chromatography (SiO_2, EtOAc/hexanes, 2:3) to yield the Wienreb amide (15.0 g) as a colorless solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.11 (d, 1 H, J = 9.3 Hz), 4.62−4.55 (m, 1 H), 3.77 (s, 3 H), 3.20

(s, 3 H), 2.40 (p, 1 H, J = 7.7 Hz), 2.14−2.00 (m, 2 H), 1.91−1.59 (m, 6 H), 1.43 (s, 9 H).

A solution of the amide (15 g, 52.1 mmol) in dry THF (200 mL) was treated with a solution of $LiAlH_4$ (1 M, 93 mL, 93.0 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 1 h and carefully quenched at 0 °C with a solution of $KHSO_4$ (10% aqueous) The resultant slurry was filtered, and the organic layer was diluted with aqueous HCl (1 M, 150 mL) and extracted with CH_2Cl_2 (3 × 200 mL). The combined organic layers were washed with aqueous HCl (1 M), saturated $NaHCO_3$, and brine and dried ($MgSO_4$). The mixture was filtered and concentrated in vacuo to yield aldehyde **8** as viscous colorless oil (14 g). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.62 (s, 1 H), 5.06 (bs, 1 H), 4.22 (q, 2 H, J = 6.3 Hz), 2.46 (s, 1 H, J = 7.9 Hz), 2.17−2.08 (m, 2 H), 2.03−1.83 (m, 3 H), 1.72 (s, 3 H, J = 10.4 Hz), 1.50 (s, 9 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 200.3, 155.8, 80.4, 59.5, 36.8, 32.9, 29.2, 28.7, 19.0; MS (ESI) 250 [$(M + Na)^+$, 5], 172 (20), 128 (100).

(2-Cyano-1-cyclobutylmethyl-2-hydroxyethyl)carbamic Acid *tert*-Butyl Ester (9). A solution of the aldehyde **8** (14 g, 61.6 mmol) in CH_2Cl_2 (50 mL) was treated with Et_3N (10.73 mL, 74.4 mmol) and acetone cyanohydrin (10.86 g, 127.57 mmol) and stirred at room temperature for 12 h. The reaction mixture was concentrated in vacuo and diluted with aqueous HCl (1 M, 200 mL) and extracted into CH_2Cl_2 (3 × 200 mL). The combined organic layers were washed with H_2O and brine, dried ($MgSO_4$), filtered, concentrated in vacuo, and purified by chromatography (SiO_2, EtOAc/hexanes, 1:4) to yield **9** (10.3 g) as a colorless oil as a mixture of diastereomers.

3-Amino-4-cyclobutyl-2-hydroxybutyramide Hydrochloride Salt (10). (a) Basic Hydrogen Peroxide Hydrolysis. A solution of cyanohydrin **9** (4.5 g, 17.7 mmol) in CH_3OH (50 mL) was treated with H_2O_2 (10 mL) and LiOH (820 mg, 20.8 mmol) and stirred at 0 °C for 3 h. The reaction mixture was diluted with aqueous $Na_2S_2O_3$ (5%, 50 mL) and extracted with CH_2Cl_2 (3 × 150 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo to yield Boc proteced hydroxyamide (3.25 g, 67%). The residue was dissolved in 4 N HCl in dioxane and stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo to give **10**, which was used without further purification. ^{1}HNMR (500 MHz, DMSO-d_6, mixture of diastereomers) δ 8.10 (s, 3 H), 7.85 (s, 3 H), 7.56−7.49 (m, 4 H), 6.45 (d, 1 H, J = 6.0 Hz), 6.28 (d, 1 H, J = 5.0 Hz), 4.19 (q, 1 H, J = 2.5 Hz), 3.98 (dd, 1 H, J = 2.0 and 4.0 Hz), 3.26 (b, 1H), 3.13 (b, 1H), 2.46−2.37 (m, 2 H), 2.1−2.00 (m, 4 H), 1.85−1.45 (m, 8 H); ^{13}C NMR δ 174.4, 174.0, 71.7, 70.2, 52.1, 51.9, 36.5, 35.1, 32.0, 31.9, 28.4, 28.3, 28.1, 18.7, 18.5; MS (ESI) 173 [$(M + Na)^+$, 100].

(b) Acidic Hydrolysis of Cyanohydrin (Alternative Procedure). Saturated methanolic HCl, prepared by bubbling HCl gas to CH_3OH (700 mL) at 0 °C, was treated with cyanohydrin **9** and heated at reflux for 24 h. The mixture was concentrated in vacuo to yield methyl[3-amino-4-cyclobutyl-2-hydroxybutyrate]·HCl, which was used in the next step without purification. ^{1}H NMR (CH_3OH-d_4, 400 MHz) δ 4.48 (d, 1 H, J = 3.3 Hz), 4.26 (d, 1 H, J = 3.3 Hz), 3.79 (s, 3 H), 3.51−3.38 (m, 2 H), 3.33 (s, 3 H), 2.5−2.35 (m, 2 H), 2.34−2.00 (m, 4 H), 1.93−1.58 (m, 12 H).

A suspension of the amine salt in CH_2Cl_2 (200 mL) was treated with Et_3N (45.0 mL, 315 mmol) and Boc_2O (45.7 g, 209 mmol) at −78 °C. The reaction mixture was then stirred at room temperature overnight and diluted with aqueous HCl (2 M, 200 mL) and extracted into CH_2Cl_2. The organic layer was dried ($MgSO_4$), filtered, concentrated in vacuo, and purified by chromatography (EtOAc/hexanes, 1:4) to yield methyl 3-*tert*-butoxycarbonylamino-4-cyclobutyl-2-hydroxybutyrate.

A solution of methyl ester obtained in the previous step (3 g, 10.5 mmol) in THF/H_2O (1:1) was treated with LiOH·H_2O (645 mg, 15.75 mmol) and stirred at room temperature for 2 h. The reaction mixture was acidified with aqueous HCl (1 M, 15 mL) and concentrated in vacuo. The residue was dried in a vacuum.

A solution of the acid in CH_2Cl_2 (50 mL) and DMF (25 mL) was treated with NH_4Cl (2.94 g, 5.5 mmol), EDCI (3.15 g, 16.5 mmol), HOOBt (2.69 g, 16.5 mmol), and NMM (4.4 g, 43.6 mmol). The reaction mixture was stirred at room temperature for 3 days. The solvents were removed in vacuo, and the residue was diluted with aqueous HCl (250 mL) and extracted with CH_2Cl_2. The combined organic layers were washed with aqueous saturated $NaHCO_3$, dried ($MgSO_4$), filtered, and concentrated in vacuo to obtain (2-carbamoyl-1-cyclobutylmethyl-2-hydroxyethyl)carbamic acid *tert*-butyl ester. ^{1}H NMR (CH_3OH-d_4, 400 MHz) δ 4.13 (d, 1 H, J = 3.9 Hz), 4.06 (d, 1 H, J = 3.0 Hz), 3.89−3.70 (m, 2 H), 2.42−2.30 (m, 2 H), 2.26−1.98 (m, 4 H), 1.98−1.55 (m, 1 2 H), 1.42 (s, 9 H), 1.40 (s, 9 H). It was deprotected as described before to yield **10** as beige solid.

Benzyl (3-*tert*-Butoxycarbonylamino-2-hydroxyhexanoylamino)acetate Hydrochloride Salt (14). To a stirred solution of **13**[28] (3.00 g, 12.0 mmol) in DMF (15 mL) and CH_2Cl_2 (15 mL) at −20 °C was added HOOBt (1.97 g, 12.0 mmol), *N*-methylmorpholine (4.0 mL, 36.0 mmol), and EDCI (2.79 g, 14.5 mmol). The mixture was stirred for 10 min, followed by addition of HCl·H_2N-Gly-OBn (2.56 g, 13.0 mmol). The resulting solution was stirred at −20 °C for 12 h, then kept in the refrigerator overnight and concentrated to dryness, followed by dilution with EtOAc (150 mL). The EtOAc solution was then washed twice with saturated $NaHCO_3$, H_2O, 5% H_3PO_4, and brine, dried over Na_2SO_4, filtered, concentrated to dryness, and purified by chromatography (SiO_2, acetone/hexanes, 1:4 → 1:1) (4.5 g, 94%) to yield Boc-protected **14**. ^{1}H NMR (DMSO-d_6, 500 MHz, mixture of diastereomers) δ 8.21 and 8.18 (t, 2 H, J = 6.5 Hz), 7.40−7.33 (m, 10 H), 6.39 and 5.94 (d, 2 H, J = 9.0 and 9.5 Hz), 5.89 and 5.76 (d, 2 H, J = 6 and 5.5 Hz), 5.14 (bs, 4 H), 3.99−3.70 (m, 8 H), 1.39 and1.36 (s, 18 H), 1.48−1.07 (m, 8 H), 0.85 and 0.77 (t, 6 H, J = 7.0 and 6.5 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 173.9, 173.2, 170.5, 170.4, 156.1, 136.8, 129.3, 128.9, 128.8, 128.7, 78.5, 78.4, 74.5, 73.1, 66.6, 53.5, 41.4, 30.5, 29.1, 29.0, 19.8, 19.7, 14.7, 14.6. MS (ESI) 395 [$(M + 1)^+$, 100].

A solution of Boc protected compound was dissolved in 4 M HCl in dioxane and stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo and dried to yield **14** as a pale-brown solid.

Representative Procedure for the Synthesis of 3-Amino-2-hydroxyhexanoic Acid Butylamide Hydrochloride Salt (R^3 = Propyl, R^2 = C_4H_9NH; 15). Similar methods were employed for the syntheses of intermediates of type **15** where R^3 was propyl and R^2 was NH_2, C_4H_9NH, allylNH, CH_3NH, and $(CH_3)_2N$.

A solution of the hydroxy acid (300 mg, 1.21 mmol) **13** in CH_2Cl_2 (5 mL) and DMF (5 mL) was treated with *n*-butylamine (106 mg, 1.46 mmol), EDCI (347 mg, 1.82 mmol), HOOBt (296 mg, 1.81 mmol), and NMM (305 mg, 3.02 mmol). The reaction mixture was stirred at 0 °C for 16 h. The reaction mixture was concentrated in vacuo, and the residue was diluted with aqueous HCl (100 mL) and extracted with EtOAc. The combined organic layers were washed with aqueous saturated $NaHCO_3$, dried ($MgSO_4$), filtered, concentrated in vacuo, and purified by chromatography (SiO_2, acetone/hexanes, 2:3) to obtain Boc protected 3-amino-2-hydroxyhexanoic acid butylamide (140 mg). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.72 (t, 1 H, J = 6.6 Hz), 7.67 (t, 1 H, J = 7.7 Hz), 6.34 (d, 1 H, J = 9.5 Hz), 5.95 (d, 1 H, J = 9.5 Hz), 5.52 (d, 1 H, J = 5.1 Hz), 5.47 (d, 1 H, J = 6.7 Hz), 3.86−3.83 (m, 2 H), 3.79−3.60 (m, 2 H), 3.12−2.97 (m, 4 H), 1.36 (s, 18 H), 1.36−1.25 (m, 4 H), 1.28−1.13 (m, 8 H), 1.13−1.06 (m, 4 H), 0.84 (t, 6 H, J = 7.3 Hz), 0.77 (t, 6 H, J = 7.00 Hz); MS (ESI) 325 [$(M + Na)^+$, 40], 303 [$(M + 1)^+$, 30], 247 (30), 203 (70), 130 (100).

The Boc group was deprotected as described before using 4 M HCl in dioxane to yield **15** as a beige solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.07 (t, 1 H, J = 5.9 Hz), 8.04 (t, 1 H, J = 6.7 Hz), 8.00 (b, 3 H), 7.78 (b, 3 H), 6.40 (d, 1 H, J = 5.8 Hz), 6.31 (d, 1 H, J = 5.9 Hz), 4.15 (dd, 1 H, J = 2.9 and 2.2 Hz), 4.00 (t, 1 H, J = 4.4 Hz), 3.68−3.65 (m, 1 H), 3.49−3.44 (m, 1 H), 3.15−3.00 (m, 4 H), 1.59−1.19 (m, 16 H), 0.87−0.79 (2t, 12 H); MS (ESI) 203 [$(M + 1)^+$, 100], 132 (80).

Representative Procedures for the Synthesis of Target Compounds Described in Tables 2−7. Preparation of {2-[2-(2-Carbamoyl-1-cyclobutylmethyl-2-oxoethylcarbamoyl)-6,6-di-

methyl-3-azabicyclo[3.1.0]hex-3-yl]-1-cyclohexyl-2-oxoethyl}-carbamic Acid *tert*-Butyl Ester (46; R^4 = Cyclohexyl, R^5 = O^{t}Bu, R^3 = Methylcyclobutyl). Similar methods with minor modifications were employed for the syntheses of target compounds described in Tables 2−7. The amino ester **22**[10] was prepared following the method of Zhang and Madalengoitia with some modifications. The introduction of the cyclopropyl ring was achieved by the reaction of **20** with the corresponding isopropyl-lphosphonium ylide, and the Boc group was cleaved using methanolic HCl.

A solution of Boc-Chg-OH (Senn Chemicals, 6.64 g, 24.1 mmol) and amine salt **22** (4.5 g, 22 mmol) in CH_2Cl_2 (100 mL) at 0 °C was treated with BOP reagent and stirred at room temperature for 15 h. The reaction mixture was concentrated in vacuo and diluted with aqueous 1 M HCl. The aqueous layer was extracted with EtOAc (3 × 200 mL). The combined organic layers were washed with saturated $NaHCO_3$ (200 mL), dried ($MgSO_4$), filtered, concentrated in vacuo, and purified by chromatography (SiO_2, EtOAc/hexanes, 3:7) to obtain **23** (R^4 = cyclohexyl, R^5 = O^{t}Bu, 6.0 g) as a colorless solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.05 (d, 1 H, J = 8.5 Hz), 4.18 (s, 1 H), 4.01 (d, 1 H, J = 10 Hz), 3.88 (t, 1 H, J = 9.0 Hz), 3.76 (dd, 1 H, J = 5.0 and 5.5 Hz), 3.65 (s, 3 H), 1.79−1.53 (m, 7 H), 1.41 (d, 2 H), 1.35 (s, 9 H), 1.13 (s, 3 H), 0.88 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.4, 171.6, 156.5, 78.8, 59.7, 57.4, 52.8, 47.4, 39.3, 30.5, 29.6, 29.3, 29.0, 27.9, 26.8, 26.7, 26.4, 26.2; MS (ESI) 431 [(M + Na)$^+$, 60], 409 (90), 353 (80), 309 (100).

A solution of methyl ester **23** (R^4 = cyclohexyl, R^5 = O^{t}Bu, 4.0 g, 9.79 mmol) in THF/H_2O (1:1) was treated with LiOH·H_2O (401 mg, 9.79 mmol) and stirred at room temperature for 3 h. The reaction mixture was acidified with aqueous HCl and concentrated in vacuo to obtain the free acid that was used in subsequent coupling without further purification. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 5.23 (d, 2 H, J = 9.8 Hz), 4.49 (s, 1 H), 4.22 (t, 1 H, J = 8.8 Hz), 4.06 (d, 1 H, J = 10.5 Hz), 3.38 (dd, 1 H, J = 4.7 and 5.8 Hz), 1.88−1.69 (m, 8 H), 1.56−1.53 (m, 1 H), 1.45 (m, 9 H), 1.33−1.18 (m, 4 H), 1.11 (s, 3 H), 0.98 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.3, 156.2, 80.1, 77.7, 60.4, 57.3, 48.0, 40.7, 30.1, 29.8, 29.0, 28.7, 27.7, 26.6, 26.3, 26.2, 19.8, 13.0; MS (ESI) 417 [(M + Na)$^+$, 44], 395 [(M + 1)$^+$, 30] 339 (40), 295 (100).

A solution of acid **23** (R^4 = cyclohexyl, R^5 = O^{t}Bu, 1.5 g, 3.74 mmol) in DMF/CH_2Cl_2 (1:1 50 mL) was treated with amine salt **10** (R^3 = cyclobutylmethyl) (772 mg, 3.74 mmol), EDCI (1.07 g, 5.61 mmol), HOOBt (959 mg, 5.61 mmol), and NMM (2.15 mL, 14.96 mmol) at −10 °C. The reaction mixture was stirred at 0 °C for 48 h and concentrated in vacuo. The residue was diluted with aqueous 1 M HCl and extracted with CH_2Cl_2. The combined organic layers were extracted with aqueous $NaHCO_3$, aqueous HCl, and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to obtain the hydroxyamide (2.08 g) as a tan solid.

A solution of hydroxyamide (2.08 g, 3.79 mmol) in toluene and DMSO (1:1, 20 mL) at 0 °C was treated with EDCI (7.24 g, 37.9 mmol) and dichloroacetic acid (2.42 g, 19.9 mmol) and stirred at room temperature. for 4 h. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$), filtered, concentrated, in vacuo, and purified by chromatography (SiO_2, acetone/hexanes, 3:7) to yield **46** as a colorless solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.30 (d, 1 H, J = 6.5 Hz), 8.12 (d, 1 H, J = 7.5 Hz), 8.02 (s, 1 H), 8.00 (d, 1 H, J = 12.0 Hz), 7.77 (brd, 2 H), 6.96 (d, 1 H, J = 6.0 Hz), 6.94 (d, 1 H, J = 6.0 Hz), 4.89−4.96 (m. 2 H), 4.27 (s, 1 H), 4.25 (s, 1 H), 3.94 (d, 2 H, J = 9.5 Hz), 3.83−3.89 (m, 2 H), 3.76−3.79 (m, 1 H), 3.70−3.73 (m, 1 H), 2.46−2.48 (m, 1 H), 2.31−2.37 (m, 1 H), 1.93−2.01 (m, 4 H), 1.74−1.82 (m, 8 H), 1.56−1.64 (m, 16 H), 1.42−1.47 (m, 2 H), 1.37 (s, 9 H), 1.35 (s, 9 H), 1.37−1.35 (m, 2 H), 1.10−1.11 (brd, 6 H), 1.02 (s, 3 H), 1.01 (s, 3 H), 0.9 (s, 3 H), 0.86 (s, 3 H), 1.03−0.87 (bm, 4 H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 198.7, 198.1, 171.8, 171.6, 171.3, 171.2, 163.8, 163.7, 156.5, 78.8, 78.7, 60.7, 60.2, 57.7, 57.6, 52.8, 47.8, 47.7, 39.5, 39.2, 37.7, 37.6, 33.1, 31.6, 29.6, 29.5, 29.0, 28.8, 28.7, 28.3, 28.2, 27.9, 27.5, 27.1, 27.0, 26.84, 26.8, 26.5, 26.4, 26.3, 19.6, 18.7, 18.6. HRMS calcd for $C_{29}H_{47}N_4O_6$: 547.3496 (M + H)$^+$. Found: 547.3453

Synthesis of (1*R*,5*S*)-*N*-[3-Amino-1-(cyclobutylmethyl)-2,3-dioxopropyl]-3-[2(*S*)-[[[(1,1-dimethylethyl)amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-2(*S*)-carboxamide (70; R^4 = ^{t}Bu, R = ^{t}BuNH). A solution of Boc-*tert*-Lue (5.0 g 21.6 mmol) in dry CH_2Cl_2/DMF (50 mL, 1:1) was cooled to 0 °C and treated with the amine salt **22** (5.3 g, 25.7 mmol), NMM (6.5 g, 64.8 mmol), and BOP reagent (11.6 g, 25.7 mmol). The mixture was stirred at room temperature for 24 h, diluted with aqueous HCl (1 M), and extracted with CH_2Cl_2. The combined organic layers were washed with HCl (aqueous, 1 M), saturated $NaHCO_3$, and brine, dried ($MgSO_4$), filtered, concentrated in vacuo, and purified by chromatography (SiO_2, acetone/hexane, 1:5) to yield 3-[2-(3-*tert*-butylureido)-3,3-dimethylbutyryl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxylic acid methyl ester as a colorless solid. ^{1}H NMR (CD_3OD, 400 MHz) δ 6.48 (d, 1 H, J = 9.3 Hz), 4.35 (s, 1 H), 4.21(d, 1 H, J = 9.9 Hz), 4.04−3.88 (m, 2 H), 3.73 (s, 3 H), 1.58−1.54 (m, 1 H), 1.41 (s, 9 H), 1.49−1.41 (m, 1 H), 1.07 (s, 3 H), 1.02 (s, 9 H), 0.93 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.4, 171.5, 156.4, 80.0, 77.7, 59.6, 59.0, 52.7, 48.1, 35.4, 30.8, 28.7, 27.8, 26.7, 19.9, 12.8; MS (ESI) 405 [(M + Na)$^+$, 100], 383 [(M + 1)$^+$, 40], 327 (70), 283 (100).

A solution of methyl ester (4.0 g, 10.46 mmol) was dissolved in HCl (4 M solution of dioxane) and stirred at room temperature for 3 h. The reaction mixture was concentrated in vacuo to obtain the amine hydrochloride salt used in the next step without further purification. A solution of the amine salt (397 mg, 1.24 mmol) in CH_2Cl_2 (10 mL) was cooled to −78 °C and treated with *tert*-butyl isocyanate (250 mg, 2.5 mmol) and stirred at room temperature overnight. The reaction mixture was concentrated in vacuo, and the residue was diluted with aqueous HCl (1 M) and extracted with CH_2Cl_2. The combined organic layers were washed with aqueous HCl (1 M), saturated $NaHCO_3$, and brine. The organic layers were dried, filtered, and concentrated in vacuo, and the residue was purified by chromatography (SiO_2, acetone/hexanes, 1:4) to yield **23** as a colorless solid. ^{1}H NMR (CD_3OD, 400 MHz) δ 6.05 (s, 1 H), 5.88 (d, 1 H, J = 9.3 Hz), 4.32 (s, 1 H), 4.27 (d, 1 H, J = 9.9 Hz), 4.06 (d, 1 H, J = 10.4 Hz), 3.87 (dd, 1 H, J = 4.9 and 5.5 Hz), 1.54 (dd, 1 H, J = 4.9 and 2.2 Hz), 1.44 (d, 1 H, J = 7.2 Hz), 1.25 (s, 9 H), 1.04 (s, 3 H), 0.99 (s, 9 H), 0.92 (s, 3 H); MS (ESI) 404 [(M + Na)$^+$, 90], 382 [(M + 1)$^+$, 50], 319 (40), 283 (100), 213 (70), 170 (30).

A solution of methyl ester (381 mg, 1.0 mmol) in THF/H_2O (1:1, 5 mL) was treated with LiOH·H_2O (62 mg, 1.5 mmol) and stirred at room temperature for 3 h. The reaction mixture was acidified with aqueous HCl and concentrated in vacuo to obtain the free acid that was used in a subsequent step without purification. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 5.94 (s, 1 H), 5.87 (d, 1 H, J = 10.3 Hz), 4.13 (d, 1 H, J = 10.3 Hz), 4.08 (s, 1 H), 3.97 (d, 1 H, J = 10.3 Hz), 3.73 (dd, 1 H, J = 5.9 and 4.4 Hz), 1.89 (s, 1 H), 1.46 (dd, 1 H, J = 5.1 and 2.2 Hz), 1.36 (bd, 1 H, J = 7.3 Hz), 1.15 (s, 9 H), 0.98 (s, 3 H), 0.89 (s, 9 H), 0.80 (s, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 174.9, 173.8, 158.1, 77.7, 60.1, 58.3, 51.0, 48.5, 35.4, 30.5, 29.8, 27.9, 26.8, 26.7, 19.9, 12.9; MS (ESI) 390 [(M + Na)$^+$, 20], 368 [(M + 1)$^+$, 20], 269 (100), 312 (20).

A solution of acid (254.9 mg, 0.69 mmol) in DMF/CH_2Cl_2 (1:1, 5.0 mL) was treated with amine **10** (159 mg, 0.763 mmol), EDCI (199 mg, 1.04 mmol), HOOBt (169.5 mg, 1.04 mmol), and NMM (280 mg, 2.77 mmol) at −20 °C. The reaction mixture was stirred at −20 °C for 48 h and concentrated in vacuo. The residue was diluted with aqueous 1 M HCl and extracted with EtOAc. The combined organic layers were extracted with aqueous $NaHCO_3$, aqueous HCl, and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to obtain a tan solid (470 mg) that was used in the next reaction without further purification.

A solution of hydroxyamide (470 mg, 0.9 mmol) in toluene and DMSO (1:1 20 mL) at 0 °C was treated with EDCI (1.72 g, 9.0 mmol) and dichloroacetic acid (0.37 mL, 4.5 mmol) and stirred at room temperature for 4 h. The reaction mixture was diluted with

CH_2Cl_2 and washed with saturated $NaHCO_3$, and brine. The organic layer was dried ($MgSO_4$), filtered, concentrated in vacuo, and purified by chromatography (SiO_2, acetone/hexanes, 3:7) to yield **70** as a colorless solid as a mixture of diasteromers. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.26 (d, 1 H, J = 7.0 Hz), 8.00 (s, 1 H), 7.75 (s, 1 H), 5.96 (s, 1 H), 5.84 (d, 1 H, J = 10 Hz), 4.96 (m, 1 H), 4.28 (s, 1H), 4.11 (d, 1 H, J = 11 Hz), 3.94 (d, 1H, J = 10 Hz), 3.73 (dd, 1 H, J = 10 and 5 Hz), 2.48 (m, 1 H), 1.95 (m, 2 H), 1.61 (m, 1 H), 1.59 (m, 1 H), 1.77 (m, 1 H), 1.57 (m, 1 H), 1.74 (m, 2 H), 1.42 (dd, 1 H, J = 7.5 and 5 Hz), 1.28 (d, 1 H, J = 7.5 Hz), 1.17 (s, 9 H), 1.01 (s, 3 H), 0.90 (s, 9 H), 0.85 (s, 3 H), 8.15 (d, 1 H, J = 7.0 Hz), 7.96 (s, 1 H), 7.74 (s, 1 H), 5.96 (s, 1 H), 5.86 (d, 1 H, J = 10 Hz), 4.85 (m, 1 H), 4.27 (s, 1H), 4.13 (d, 1 H, J = 11.0 Hz), 3.97 (d, 1H, J = 10 Hz), 3.76 (dd, 1 H, J = 10 and 5 Hz), 2.36 (m, 1 H), 1.97 (m, 2 H), 1.60 (m, 2 H), 1.78 (m, 1 H), 1.64 (m, 1 H), 1.75 (m, 2 H), 1.44 (dd, 1 H, J = 7.5 and 5 Hz), 1.27 (d, 1 H, J = 7.5 Hz), 1.17 (s, 9 H), 1.00 (s, 3 H), 0.89 (s, 9 H), 0.82 (s, 3 H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 197.8, 170.9, 170.8, 162.8, 157.4, 59.1, 56.8, 51.8, 48.9, 47.4, 36.7, 34.0, 32.0, 30.6, 29.1, 27.8, 27.3, 27.1, 26.4, 26.1, 18.5, 17.7, 12.5. 197.1, 171.1, 170.7, 163.0, 157.3, 59.4, 56.9, 52.1, 48.9, 47.4, 36.6, 34.0, 32.1, 30.5, 29.1, 27.9, 27.4, 26.8, 26.4, 26.1, 18.5, 17.8, 12.4. HRMS calcd for $C_{27}H_{46}O_5N_5$ $[M + 1]^+$ 520.3499. Observed: 520.3514. Anal Calcd for $C_{27}H_{45}N_5O_5$: C, 62.40%; H, 8.73%; N, 13.48%. Found: C, 62.48%; H, 8.94%; N, 13.19%.

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Supporting Information Available: NMR and MS data for compounds **26**−**74**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(29) Rats were orally administered with 10 mg of compound in 20% HPBCD. Blood was withdrawn periodically and pooled. AUC was calculated over a 0−6 h period.

(30) Synthesis of hydroxy acid **39**: Acid **13** ($R^3 = C_3H_7$) was converted to the methyl ester and oxidized using Dess−Martin reagent to yield intermediate methyl 3-*tert*-butoxycarbonylamino-2-oxohexanoate. Deprotection of the Boc group of methyl 3-*tert*-butoxycarbonylamino-2-oxohexanoate and coupling with **24** yielded the ketoester, precursor of **39**. Hydrolysis of methyl ester using aqueous $NaHCO_3$ in 2-propanol yielded ketoacid **39**.

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