Toward the Back-Up of Boceprevir (SCH 503034): Discovery of New Extended P_4-Capped Ketoamide Inhibitors of Hepatitis C Virus NS3 Serine Protease with Improved Potency and Pharmacokinetic Profiles

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Hepatitis C is the most prevalent liver disease. Viral hepatitis C (HCV), a small (+)-RNA virus, infects chronically an estimated 300 million people worldwide. Results of Phase I clinical studies with our first generation HCV inhibitor Boceprevir, SCH 503034 (**1**), presented at the 56th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD) were encouraging, and thus, additional human clinical studies are underway. In view of the positive data from our first generation compound, further work aimed at optimizing its overall profile was undertaken. Herein, we report that extension of our earlier inhibitor to the P_4 pocket and optimization of the P_1' capping led to the discovery of new ketoamide inhibitors of the HCV NS3 serine protease with improved in vitro potency. In addition to being potent inhibitors of HCV subgenomic RNA replication, some of the new P_4-capped inhibitors were also found to have improved PK profile.

Introduction

Hepatitis C is the most prevalent liver disease. Viral hepatitis C (HCV[a]), a small (+)-RNA virus, infects chronically an estimated 300 million people worldwide. HCV displays genetic heterogeneity with the genotype 1 being most common in the U.S., Europe, and Japan, and the most challenging to eradicate. Untreated HCV infections can progress to cirrhosis, hepatocellular carcinoma, and liver failure, a primary cause for liver transplantation.[1] Subcutaneous and intramuscular injections of α interferons (immune system booster) have been used since the late 1980s in the treatment of chronic hepatitis C. Currently, PEGylated-interferon in combination with antiviral drug ribavirin has brought the rate of sustained virological response to approximately 50% in patients infected with the genotype 1.[2] Although the use of α interferons is an integral component in the management of chronic hepatitis C infection, major adverse events can occur and subsequently result in dose reduction or discontinuation of treatment. Thus, several research groups have been working toward development of a more effective, convenient, and tolerable treatment.[3]

Upon entering a liver cell, the hepatitis C virus dissociates, liberating the viral RNA genome. The HCV genome serves as a template for cap-independent translation through an internal ribosome entry (IRES) located in the 5′ untranslated region. The resulting polyprotein undergoes both co- and post-translational proteolytic maturation by host and virally encoded proteases. The NS3 serine protease, a pivotal enzyme required for maturation of hepatitis C virons, assists in processing of the HCV polyprotein by cleaving four downstream sites. Because of its central role in viral replication,[4] inhibition of HCV NS3 serine protease has been actively pursued as target for antiviral therapy.[5]

The Boehringer Ingelheim group was the first one to report phase Ib clinical antiviral efficacy with BILN 2061, a competitive inhibitor of the HCV NS3 serine protease.[6] Derivatives containing an α-ketoamide electrophilic trap have been reported by our group[7] and others[8] to be potent inhibitors of HCV NS3 serine protease. Recently, we published our work that led to the identification of our first generation clinical candidate Boceprevir, (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]hexane-2(*S*)-carboxamide (SCH 503034), **1** (Figure 1).[9] Clinical candidates (1*S*,3a*R*,6a*S*)-2-((*S*)-2-((*S*)-2-cyclohexyl-2-(pyrazine-2-carboxamido)acetamido)-3,3-dimethylbutanoyl)-*N*-((*S*)-1-(cyclopropylamino)-1,2-dioxohexan-3-yl)octahydrocyclopenta[*c*]pyrrole-1-carboxamide (VX950) and **1** belonging to the α-ketoamide series established proof-of-concept in the aforementioned studies.[10] Recently, several other NS3 protease inhibitors have been advanced to clinical trial.[11] Results of phase I clinical studies with **1**, presented at the 56th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD) in November 2005, were encouraging.[12] The drug was rapidly absorbed, well tolerated, and demonstrated potent antiviral activity, both as monotherapy and in combination with peginterferon α-2b. Phase II clinical studies of **1** are still underway, and phase III trials have been initiated. Consequently, further work aimed at optimizing the overall profile of our first generation inhibitor was undertaken.

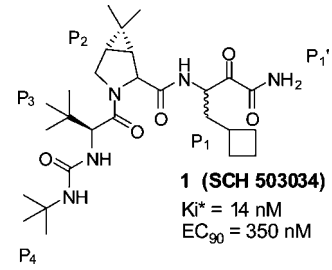

Figure 1. Profile of **1** (SCH 503034).

While investigating the P_3 capping SAR that led to the discovery of **1**, we noticed that incorporation of the larger

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[a] Abbreviations: HCV, hepatitis C virus; DM periodinane, Dess−Martin's periodinane; Cys159, cysteine159; C_{6h}, liver concentration at 6 h.

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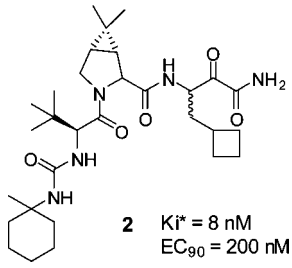


Figure 2. Profile of **2**.

hydrophobic moiety in **2** (Figure 2) achieved improvement both in enzyme and in cellular activity compared to **1**.

Exploring the scope of the 1-substituted-cyclohexyl ureas series, we discovered that incorporation of a benzyl ester moiety provided a dramatic improvement of the cell potency (Table 1, compound **20**, $EC_{90} = 20$ nM) compared to **1**. Herein, we report our finding that led to the discovery of new P_4 extended ketoamide inhibitors of the HCV NS3 serine protease with improved potency and DMPK properties.

Chemistry. Synthesis of the inhibitors was generally carried out as depicted in Scheme 1. The *gem*-dimethylcyclopropylproline P_2 core **3** was known to be essential for potency and was used to prepare the Boc-protected P_3 amino acid, dipeptide **4** according previously described protocols.[9b] With the goal of exploring the P_4 area, we identified **6** as a key intermediate. Thus, acid **4** was coupled with the appropriate P_1 hydroxyamides **5** following standard HATU coupling methodology. Oxidation of primary hydroxyamide and secondary hydroxyamide moieties was accomplished using Moffatt and Dess−Martin conditions, respectively.[13] After Boc removal, intermediate **6** was reacted with isocyanates of type **7** to efficiently deliver targets of type **8**.

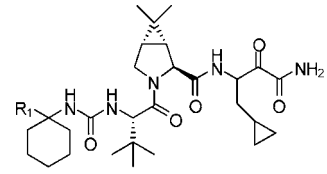
The various capping moieties were prepared according to the general procedures outlined in Scheme 2. Esterification of protected amino acid **9** bearing the desired functionality R^4 proceeded smoothly using EDCI and 4-DMAP to yield structures of type **10**. Standard HATU coupling delivered corresponding amides of type **11**. Ketones of type **12** were prepared by reacting various Grignard or organolithium reagents with the appropriate Weinreb amide. Finally, Boc removal from intermediates **10**−**12** and subsequent treatment of the amine salts with phosgene in aqueous sodium bicarbonate led to the corresponding isocyanates of type **7** that were needed for P_4 capping exploration.

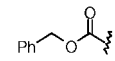
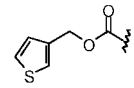
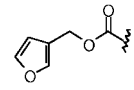
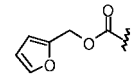
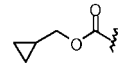
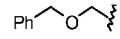
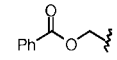
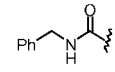
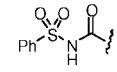
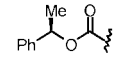
Derivatization of ketone **13** was accomplished according to Scheme 3. The alkene analogue **14** was prepared from **13** using a Peterson olefination.[14] Sodium borohydride reduction in MeOH generated the benzylic alcohol **15** that was further hydrogenated to **16** using Pd/C. Phenyl ketone **13** was reacted with *O*-methylhydroxylamine in pyridine to provide oximes **17** as a mixture of isomers. Isocyanates **18** were prepared from their corresponding HCl or TFA salt following the aforementioned conditions.

Discussion

Inhibitors listed in Tables 1,3−5 were tested in continuous assay using the NS4A-tethered single chain NS3 serine protease.[15] HCV replicon inhibitory activity for the targets synthesized was obtained using previously reported assay.[16] As a general trend, compounds listed in Tables 1,3−5 had GAPDH $CC_{50} > 5\ \mu$M when tested in counter screen measuring cellular toxicity and maintained good selectivity vs HNE similar to previously reported inhibitors.[9b] We evaluated our initial set of

Table 1[a]



Cpd	R_1	Ki*(nM)	EC_{90}(nM)
19	CH_3	9	200
20		49	20
21		31	20
22		39	20
23		28	30
24		39	60
25		30	200
26[b]		60	600
27		120	1000
28		100	2000
29[b]		44	5000
30		9	5000
31		110	5000
32		80	600

[a] K_i* value represents binding for a mixture of diastereomers at P_1 and within 2-fold for 95% confidence limit; Replicon (genotype 1) EC_{90} value is within 3-fold for 95% confidence (single assay) and within 2-fold for 95% confidence (multiple assays). [b] P_1 cyclobutylalanine.

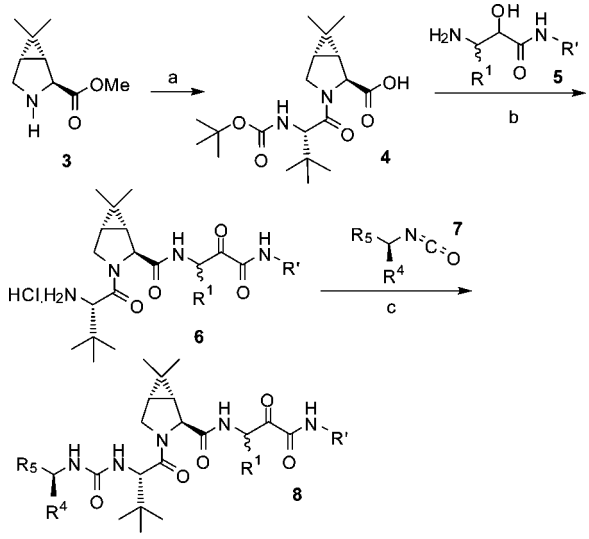
P_4 extended inhibitors **19**−**32** listed in Table 1 by varying the R^1 moieties. The P_1 was kept constant as a cyclopropyl or cyclobutyl alanine residue. as these two motifs previously provided similar binding potencies. The *tert*-Leucine moiety, which was previously optimized as a P_3 surrogates, was used during that entire study.[9b] As reported earlier, the nature of P_3 capping could greatly influence the enzyme activity. While investigating newer substitutions, we observed that the use of

Table 2. Full Rat and Monkey Pharmacokinetic Properties of Inhibitor **20**[a]

	rat	monkey
AUC (PO)	0	0.05
AUC (IV)	9.3	3.4
$t_{1/2}$ (IV)	2.9	3.1
Cl	28	43
$V_{d(ss)}$	3.5	1.7
F (PO)	0	3

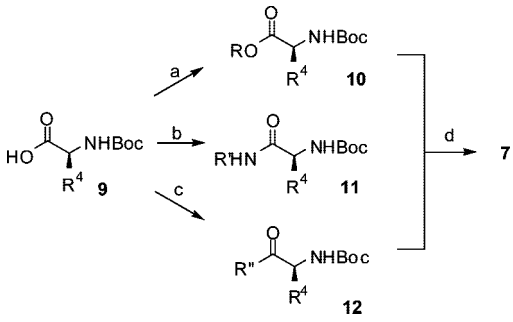
[a] AUC(μM·h); $t_{1/2}$ (h); Cl(mL/min/kg); $V_{d(ss)}$ (L/kg); F (%); rat PO and IV were dosed at 10 mpk, 3 animals; monkey PO and IV were dosed at 3 mpk, 3 animals; vehicle: rat IV (40%HPBCD); monkey IV (20%HPBCD); rat and monkey PO (0.4%MC).

Scheme 1[a]



[a] Reaction conditions: (a) (i) Boc-*t*-leucine, EDC, HOOBt; DMF/CH_2Cl_2, iPr_2NEt, 0 °C; (ii) LiOH, THF·MeOH/H_2O, 0 °C; (b) (i) HATU, DMF, DIPEA, −20 °C; (ii) Cl_2CO_2H, EDCI·HCl, DMSO, 0 °C (R′ = H); Dess−Martin periodinane, RT (R′ = alkyls); (iii) 4 M HCl; *p*-dioxane, RT; (c) CH_2Cl_2, iPr_2NEt, 0 °C.

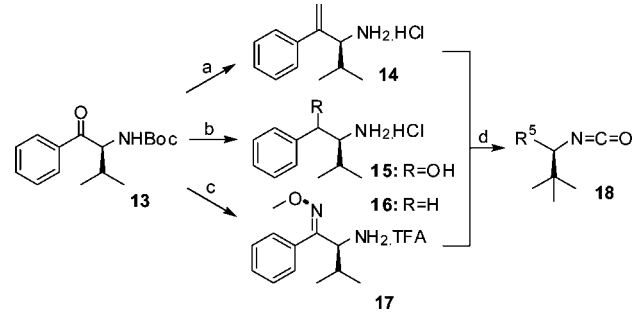
Scheme 2[a]



[a] Reaction conditions: (a) EDCI, 4-DMAP, ROH, 0 °C; (b) HATU, DMF, DIPEA, R'NH_2, −20 °C. (c) (i) EDCI, NMM, HCl·HN(OMe)Me, CH_2Cl_2, 0 °C; (ii) R″MgBr or R″MgCl or R″Li, Et_2O or THF, −78 °C. (d) (i) 4 M HCl *p*-dioxane, RT; (ii) phosgene, $NaHCO_3$, H_2O /CH_2Cl_2, 0 °C.

1-methyl-cyclohexyl urea as a P_3 capping group in inhibitor **19** boosted further the enzyme and replicon activity by 2-fold compared to **1**. While exploring the scope of the 1-substituted-cyclohexyl ureas, we discovered that the incorporation of a benzyl ester moiety provided inhibitor **20** with a dramatic 10-fold improvement in cell potency (EC_{90} = 20 nM) compared to **19**. Inhibitor **20** was the first of its class to show such enhanced activity in the replicon assay. PAMPA permeability of similar analogues was recently disclosed in a communication and seemed to correlate well with cellular activity.[17] Because

Scheme 3[a]

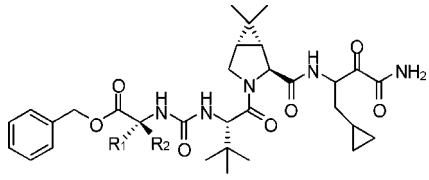


[a] Reaction conditions: (a) (i) $TMSCH_2MgCl$, THF, 0 °C; (ii) KHMDS, THF, 0 °C; (iii) 4 M HCl *p*-dioxane, RT. (b) (i) $NaBH_4$, MeOH, 0 °C (R = OH); (ii) H_2, MeOH, 10%Pd/C, RT (R = H); (iii) 4 M HCl *p*-dioxane, RT. (c) (i) $MeONH_2$.HCl, pyridine, 50 °C; (ii) 50% TFA in CH_2Cl_2, RT. (d) Phosgene, $NaHCO_3$, H_2O /CH_2Cl_2, 0 °C.

of the improved cell potency, we quickly explored the SAR of this new series and we identified additional esters that showed low EC_{90} in the replicon assay (Table 1, compound **21**−**23**). Aromatic moieties seemed essential for improvement in potency, whereas alkyl esters were less potent (Table 1, compound **24**, **25**). Modification of the benzyl ester moiety to a benzyl ether **26** and benzoyl ester **27** resulted in a significant loss in cellular activity. Although the carbonyl group seemed to be required for potency, phenyl ketone **28** (K_i* = 100 nM) showed weak enzyme activity and cell potency. Benzyl amide **29** was also found to be less active. The rat and monkey pharmacokinetic properties of inhibitor **20** were investigated, and the data is summarized in Table 2. Clearance values reported for compound **20** indicated moderate metabolic stability in rat but low in monkey (rat Cl = 28 mL/min/kg, monkey Cl = 43 mL/min/kg). Cynomolgus monkeys (n = 2) were dosed at 2 mg/kg PO with the ^{14}C ketoamide of **20**. There was no observed radioactivity for the plasma sample. The (0−24 h) recovery of radioactivity (% of oral dose) was 4.3% in bile and 0.8% in urine. Furthermore, the major response in bile was generated by the ester hydrolysis metabolite **30**. Unfortunately, while retaining good enzyme potency (K_i* = 9 nM), the carboxylic acid **30** completely lacked activity in cell assay (EC_{90} = 5 μM), conceivably due to poor cell penetration. Carboxylic acid isostere **31** was prepared, but the acid sulfonamide showed weak enzyme potency and poor activity in cell (K_i* = 110 nM, EC_{90} = 5 μM). Hindered ester **32** (K_i* = 80 nM, EC_{90} = 600 nM) was synthesized in an attempt to improve pharmacokinetics properties. Unfortunately, introduction of steric bulk at the benzylic position, primarily architectured to prevent hydrolysis, led to a decrease in both enzyme and cell potency.

The improved cellular activity demonstrated by compound **20** in comparison to **1** prompted us to further investigate the SAR of various P_4 residues. Compounds prepared to evaluate this effect are outlined in Table 3. Regarding spirocycloalkyl series, compounds with the larger ring sizes such as compounds **20** and **33** had optimal potency, whereas those with smaller ring sizes such as spirocyclopropyl analogue **34** (K_i* = 170 nM and EC_{90} = 1000 nM) lost potency. Incorporation of 2-aminoisobutyric acid as the P_4 residue resulted in compound **35** with K_i* = 210 nM, a similar activity to derivative **34**. Inhibitors **36** (K_i* = 290 nM) and **37** (K_i* = 44 nM) having D and L α methyl valine were used to establish which stereochemistry at P_4 was required for activity. Further evaluation of the P_4 residues was done by using various L amino acids; incorporation of L-valine provided **38** (K_i* = 13 nM and EC_{90} = 35 nM), a potent inhibitor in both enzyme and replicon assay. The

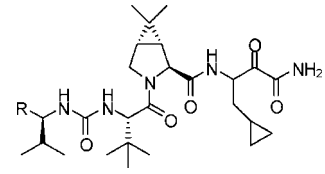
Table 3[a]

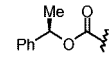
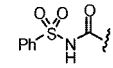


Cpd	R_1	R_2	Ki*(nM)	EC_{90}(nM)
33			58	30
34			170	1000
35			210	1480
36			290	nt[b]
37			44	500
38	H		13	35
39	H		67	50
40	H		10	20
41	H		9	25
42	H		10	20

[a] K_i* value represents binding for a mixture of diastereomers at P_1 and within 2-fold for 95% confidence limit; Replicon (genotype 1) EC_{90} value is within 3-fold for 95% confidence (single assay) and within 2-fold for 95% confidence (multiple assays). [b] Not tested (nt).

Table 4[a]



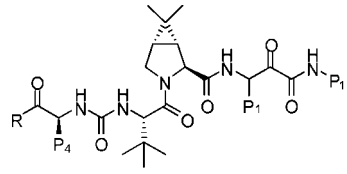
Cpd	R	Ki*(nM)	EC_{90}(nM)
43		80	600
44		6	5000
45		10	1000
46		5	80
47		50	nt[b]
48		59	nt
49		77	nt
50		60	nt
51		50	nt

[a] K_i* value represents binding for a mixture of diastereomers at P_1 and within 2-fold for 95% confidence limit; replicon (genotype 1) EC_{90} value is within 3-fold for 95% confidence (single assay) and within 2-fold for 95% confidence (multiple assays). [b] Not tested (nt).

bulkier *tert*-butyl group in inhibitor **39** (K_i* = 67 nM) was detrimental to the enzyme activity. We further probed the P_4 region, and we discovered that small L-cycloakyls amino acids were well tolerated. Thus, incorporation of cyclopropyl, cyclopentyl, and cyclohexyl glycine resulted in inhibitors **40**, **41**, and **42**, respectively, with improved enzyme and cell potency. In summary, SAR studies of P_4 groups identified L-valine and L-cycloakyls amino acids as optimal substituent that provided improved enzyme binding as well as cellular activity. Commercially available L-valine and L-cyclohexyl glycine were used to further investigate SAR, and the results are outlined in Tables 4 and 5.

As observed in the spirocyclohexyl P_4 series, introduction of steric bulk at the benzylic position in compound **43** led to a 17-fold decrease in cell potency compare with **38**. The acid sulfonamide **44** (K_i* = 6 nM) was also prepared with L-valine at P_4 and provided 18-fold improvement in enzyme potency compared to **31** (K_i* = 110 nM), but cell potency remained poor (EC_{90} = 5 μM). Tetrazole analogue **45** was made but like the other carboxylic acid isostere previously evaluated, it exhibited excellent enzyme potency but weak replicon activity (K_i* = 10 nM, EC_{90} = 1 μM). In contrast with the spirocyclohexyl P_4 series, incorporation of a phenyl ketone in the L-valine series yielded inhibitor **46** with more than 20-fold improvement in both enzyme and replicon activity when compared to compound **28**. In addition to good potency, inhibitor **46** also showed some exposure after oral administration in rats (AUC = 0.26 μM.hr). Simple modifications of the ketone moiety demonstrated that this functional group was essential for activity with compounds **47**−**51** exhibiting more than 10-fold loss in enzyme potency. With this information in hand, we further explored the ketone series by introducing various aromatic and alkyl modifications. Incorporation of a pyridine group improved the PK profile of analogue **52** but resulted in some decrease in cell potency (EC_{90} = 200 nM) as compared to **46** (EC_{90} = 80 nM). The thiophene and thiazole analogues **53** and **54** were almost equipotent to the phenyl ketone **46** but did not show better oral PK in rat. We then looked at small cycloakyl and alkyl substituents. Thus, cyclopropyl ketone **55**, cyclobutyl ketone **56**, and cyclohexyl ketones **57** were prepared. The smaller cyclopropyl group seemed optimal for both enzyme and replicon potency with similar overall profile compared to the original ketone **46**. Small alkyls groups were also probed (compounds **58**−**60**) but did not provide any improvements over previous compounds. As established earlier in the ester series, incorporation of cyclohexyl glycine at P_4 provided inhibitor **42** with better overall potency profile. With that information in

Table 5[a]



Cpd	R	P4	P1	P1'	Ki*(nM)	EC_{90}(nM)
52				H	8	200
53				H	8	100
54				H	9	90
55				H	7	100
56				H	20	350
57				H	13	200
58				H	14	400
59				H	12	150
60				II	12	200
61				II	7	90

Cpd	R	P4	P1	P1'	Ki*(nM)	EC_{90}(nM)
62				H	8	100
63					25	600
64					10	500
65					27	450
66					38	900
67					9	250
68					4	55
69					8	70

[a] K_i* value represents binding for a mixture of diastereomers at P_1 and within 2-fold for 95% confidence limit; replicon (genotype 1) EC_{90} value is within 3-fold for 95% confidence (single assay) and within 2-fold for 95% confidence (multiple assays).

hand, we prepared the 2-pirydine ketone analogue **61** with the aim of enhancing the replicon potency of this series. Interestingly, through this manipulation, we were able to improve the cell potency of **61** to EC_{90} = 90 nM and also obtain an inhibitor with good oral exposure and liver concentration in rats (Table 6). Similar improvement of pharmacokinetics profile for the cyclopropyl ketone **62** was observed when combined with the cyclohexylglycine at P_4.

During our earlier exploratory studies that led to the discovery of **1**, we observed that P_1' secondary amides, especially allyl amides, when used in combination with norvaline at P_1 provided inhibitors with very good rat PK profile but weak enzyme and replicon potency.[9b] With the improved potency of our P_4-capped inhibitors, we decided to introduce the allyl residue at the P_1' position with the goal of further improving the PK profile. Thus, the nor-valine P_1 and allyl amide P_1' combinations were used to generate inhibitors **63** and **64** bearing the cyclopropyl ketone moiety. Introduction of the allyl moiety at P_1' resulted in compounds with poorer cellular activity compared to primary amide derivatives **55** and **61**, however, they exhibited excellent rat PK with oral exposure improved by 40- to 200-fold, respectively. In addition, concentration in the liver for inhibitor **64** was also very high (5.8 μM at 6 h). To further improve the cellular activity of these inhibitors, we decided to explore the effect of variation of P_1 residue. From our previous experience with the discovery of **1**, we were aware of the fact that the P_1 norvaline was not the optimal substitution.[9b] Cyclopropyl alanine analogue **65** and norleucine analogues **66** were prepared but provided similar weak replicon potency. However, from Table 5, it was clear that introduction of butynylglycine at P_1 had a profound effect on the cellular potency of compound **67** (K_i* = 9 nM, EC_{90} = 250 nM) compared to earlier inhibitors. Furthermore, **67** exhibited very good rat oral exposure (AUC(μM·h)0−6 h = 24) and high concentration in the liver (8.5 μM at 6 h) (Table 6). Because of the potential in vivo liability of the allyl moiety, we turned our effort toward the identification of a more desirable surrogates at P_1'. Replacement of the allyl group with cyclopropyl resulted in compound **68.** The cyclopropyl at P_1' was well tolerated and resulted in 5-fold improvement in cell potency (EC_{90} = 55 nM) compared to the allyl amide analogue **67** (EC_{90} = 250 nM). Similar potency profile was achieved when the P_1 butynylglycine and P_1' cyclopropyl amide combinations were used with L-valine at P_4 to generate inhibitor **69** (EC_{90} = 70 nM). However, as observed earlier with **59** and **62**, the cyclohexylglycine at P_4 in inibitor **68** ($AUC(\mu M\cdot h)_{0-6\ h}$ = 11) provided better oral exposure compared to the L-valine residue as in inibitor **69** (AUC(μM·h)0−6 h = 4.8).

Table 6. Rapid Rat PK Data for Selected Compounds[a]

compd	AUC (μM·h)$_{0-6\ h}$	liver conc (μM)$_{6h}$
1	0.14	0.3[b]
45	0.26	nd[c]
52	0.42	nd
53	0.08	nd
54	0.15	nd
55	0.12	nd
61	1.4	0.8
62	1.4	2.7
63	26	nd
64	55	5.8
67	24	8.5
68	11	2.9
69	4.8	0.9

[a] Rat PO were dosed at 10 mpk (no IV arm). Number of animals used: 2. Vehicle: PO (40%HPBCD). [b] Rat liver concentration at 8 h. [c] Rat liver concentration in was not determined (nd).

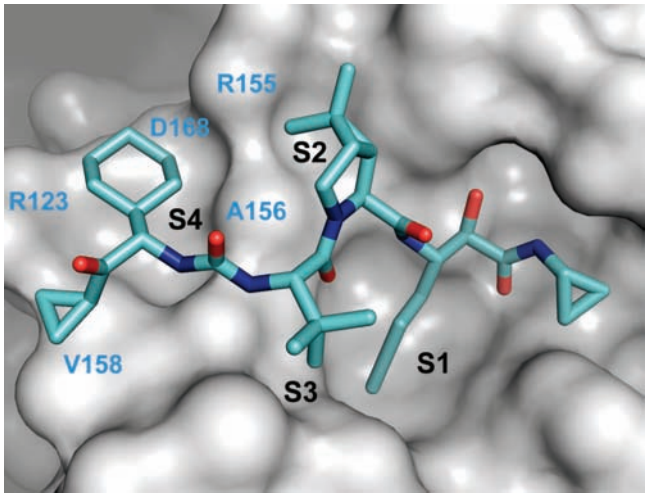


Figure 3. Model of compound **68** bound to the active site of HCV NS3/NS4A protease domain. The inhibitor is shown as stick and the protein as surface. The S1−S4 subsites and the residues that occupy the S4 pocket are indicated.

To understand the interactions, especially the P_4 and P_4 cap, with the protein, compound **68** was modeled in the active site of the NS3/NS4A protease domain (Figure 3). Similar to the *t*-butyl glycine P_3 cap of compound **1**, the P_4 cyclohexyl glycine binds to the S_4 subsite, which is a shallow hydrophobic pocket consisting of residues Arg 123, Asp 168, Arg 155, Ala 156, and Val 158. The cyclopropyl P_4 cap sits on top of Val 158 side chain. The ketone oxygen points toward the solvent. The favorable hydrophobic contacts of the P_4 cyclopropyl cap with the side chain of Val 158 may attribute to the enhancement in enzyme potency of compound **68** compared with **1**.

Conclusion

As we moved toward the back-up for **1**, we discovered that P_4-capped inhibitors could provide enhanced enzyme and cellular potency. Initially, we identified novel series of P_4 ester derived inhibitors that showed 18-fold improvements in cellular potency (EC_{90}) but lacked oral exposure in rat. Derivatization of the ester and optimization of the P_4 residue led to the identification of the ketone series that provided compounds with excellent enzyme potency, improved replicon activity, and modest oral exposure in rat. Modification of the primary amide P_1' capping group to a secondary allyl amide drastically improved rat oral exposure but resulted in weak cell potency. By systematic optimization of the P_1 and the P_1' residues, P_1 butynylglycine and P_1' cyclopropyl amide were identified as moieties that, when used in combination, provided compounds with excellent binding and much improved cellular potencies over **1**. Evaluation of these potent compounds in rapid rats allowed us to identify inhibitor **68** with excellent enzyme and replicon potency ($K_i^* = 4$ nM and $EC_{90} = 55$ nM) and with an improved oral exposure in rats (AUC = 11 μM·h) compared to **1**.

Experimental Section

NMR spectra were recorded at 300, 400, or 500 MHz for ^{1}H and at 75, 100, or 125 MHz for ^{13}C on a Bruker or Varian spectrometer with $CDCl_3$ or DMSO-d_6 as solvent, respectively. The chemical shifts are given in ppm, referenced to the internal TMS or deuterated solvent signal.

General experimental procedures were described in earlier publications[9] for the synthesis of P2 core, peptide coupling, Boc group deprotection, hydrolysis of ester to carboxylic acid, and Dess−Martin periodinane oxidation. The syntheses of ketones and analogues were performed from various starting material according to literature procedures with some minor modifications.

Purity of target compounds were determined using LC-MS analysis. LC-MS analyses were performed using an Applied Biosystems API-150 mass spectrometer and Shimadzu SCL-10A LC system. Column: Phenomenex Gemini C18, 5 μm, 50 mm × 4.6 mm i.d., gradient: from 90% water, 10% CH_3CN, 0.05%TFA, 5 min to 5% water, 95% CH_3CN, 0.05% TFA in 5 min, UV detection: 254 nm. All targets compounds were >95% pure.

Molecular Modeling. To model compound **68** in the NS_3/NS_{4A} protease active site, the crystal structure of compound **1** bound to NS_3/NS_{4A} protease (PDB ID 2OBO) was used as a template upon which the P_1 and P_4 side chains and terminal caps corresponding to **68** were built. From the crystal structure, the backbone atoms form a network of hydrogen bonds with the protein and therefore predefine the backbone orientation of the compounds. The side chain atoms was energy minimized for 500 steps, followed by another 500 steps of minimization of the whole inhibitor molecule. SYBYL (Tripos, Inc.) molecular modeling package was used in model building and optimization.

Phenylmethyl 1-[[[[1(*S*)-[[(1*R*,5*S*)-2(*S*)-[[[3-Amino-1-(cyclopropylmethyl)-2,3-dioxopropyl]amino]carbonyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hex-3-yl]carbonyl]-2,2-dimethylpropyl]amino]carbonyl]amino]cyclohexan Carboxylate (20). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.41 and 8.34 (d, J = 6.93 and 7.56 Hz, 1H), 8.02 (d, J = 7.88 Hz, 1H), 7.75 (bs, 1H), 7.35−7.30 (m, 5H), 6.47 (bs, 1H), 6.20 (dd, J = 10.08 and 16.07 Hz, 1H), 5.12−5.08 (m, 1H), 5.06 (d, J = 12.92 Hz, 1H), 4.94 (dd, J = 4.72 and 12.92 Hz, 1H), 4.33 (s, 1H), 4.18 (t, J = 8.19 Hz, 1H), 3.88 (t, J = 9.77 Hz, 1H), 3.78−3.73 (m, 1H),1.96 (d, J = 11.03 Hz, 1H), 1.82 (d, J = 12.29 Hz, 1H), 1.71−1.17 (m, 12H), 0.99 (d, J = 11.35 Hz, 3H), 0.93−0.84 (m, 10H), 0.77 (d, J = 12.92 Hz, 3H), 0.43−0.35 (m, 2H), 0.12−0.009 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 198.47, 197.92, 175.47, 171.82, 171.55, 171.52, 171.32, 163.78, 163.49, 157.63, 157.57, 137.30, 129.11, 128.56, 128.31, 128.30, 128.28, 66.32, 60.20, 59.96, 58.09, 57.76, 57.68, 55.02, 48.34, 35.85, 35.16, 35.14, 33.94, 32.55, 31.56, 31.45, 27.67, 27.58, 27.13, 27.11, 26.97, 26.93, 25.69, 21.62, 21.52, 19.33, 19.30, 13.32, 13.24, 8.75, 8.55, 5.90, 5.21, 4.90. HRMS calcd for $C_{36}H_{52}N_5O_7$, 666.3867 $(M + H)^+$; found, 666.3844.

3-Thienylmethyl 1-[[[[1(*S*)-[[(1*R*,5*S*)-2(*S*)-[[[3-Amino-1-(cyclopropylmethyl)-2,3-dioxopropyl]amino]carbonyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hex-3-yl]carbonyl]-2,2-dimethylpropyl]amino]carbonyl]amino]cyclohexanecarboxylate (21). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.40 and 8.35 (d, J = 6.62 and 7.25 Hz, 1H), 8.00 (d, J = 8.51 Hz, 1H), 7.75 (bs, 1H), 7.49−7.44 (m, 2H), 7.05−7.04 (m, 1H), 6.46 (bs, 1H), 6.20 (dd, J = 10.09 and 16.39 Hz, 1H), 5.15−5.02 (m, 2H), 4.96−4.95 and 4.93−4.92 (m, 1H), 4.33 (bs, 1H), 4.17 (t, J = 9.45 Hz, 1H), 3.89−3.85 (m, 1H), 3.78−3.72 (m, 1H), 1.98−1.91 (m, 1H), 1.82−1.79 (m, 1H), 1.72−1.13 (m, 13H), 0.99−0.97 (m, 3H), 0.89 (bs, 9H), 0.77−0.75 (m, 3H), 0.39−0.38 (m, 2H), 0.10−0.01

(m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 198.47, 197.92, 175.41, 171.81, 171.51, 171.31, 163.78, 163.48, 157.65, 157.60, 138.20, 128.09, 127.26, 124.18, 62.08, 60.21, 59.97, 58.11, 57.77, 57.70, 55.02, 48.35, 35.87, 35.17, 33.88, 32.59, 31.57, 31.45, 27.69, 27.60, 27.12, 26.98, 26.93, 25.69, 21.63, 21.54, 19.34, 13.31, 13.23, 8.75, 8.54, 5.90, 5.23, 4.90. HRMS calcd for $C_{34}H_{50}N_5O_7S$, 672.3431 $(M + H)^+$; found, 672.3437.

(1*R*,5*S*)-*N*-[3-Amino-1-(cyclobutylmethyl)-2,3-dioxopropyl]-3-[3,3-dimethyl-1-*oxo*-2(*S*)-[[[[1-[(phenylmethoxy)methyl]cyclohexyl]-amino]carbonyl]amino]butyl]-6,6-dimethyl-3-azabicyclo[3.1.0]-hexan-2(*S*)-Carboxamide (26). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.29 and 8.17 (d, J = 7.25 and 7.25 Hz, 1H), 8.02 (d, J = 17.34 Hz, 1H), 7.76(bs, 1H), 7.35−7.27 (m, 5H), 6.15 (t, J = 8.82 Hz, 1H), 5.79(bs, 1H), 4.99−4.95 and 4.88−4.84 (m, 1H), 4.43 (bs, 1H), 4.28 (d, J = 5.04 Hz, 1H), 4.17 (t, J = 6.62 Hz, 1H), 4.02−3.89 (m, 2H), 3.75 (d, J = 12.29 Hz, 1H), 3.53 (d, J = 8.82 Hz, 1H), 3.46 (d, J = 9.14 Hz, 1H), 2.05−1.07 (m, 21H), 1.00 (d, J = 5.99 Hz, 3H), 0.91(s, 9H), 0.87(d, J = 14.81 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 198.65, 197.98, 173.72, 171.86, 171.80, 171.61, 171.60, 163.89, 163.69, 158.03, 157.94, 139.76, 132.43, 130.03, 129.55, 129.03, 129.09, 100.16, 75.93, 73.34, 72.01, 60.34, 60.26, 59.98, 57.62, 57.54, 55.17, 54.33, 52.97, 52.74, 48.28, 37.61, 37.55, 37..39, 35.05, 33.07, 33.00, 32.78, 32.08, 31.50, 31.41, 29.87, 28.80, 28.66, 28.49, 28.34, 28.27, 27.22, 26.98, 26.80, 26.26, 25.37, 22.86, 21.60, 21.52, 19.74, 19.35, 18.73, 18.60, 13.40, 13.34.

2-Furanylmethyl 1-[[[[1(*S*)-[[(1*R*,5*S*)-2(*S*)-[[[3-Amino-1-(cyclopropylmethyl)-2,3-dioxopropyl]amino]carbonyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hex-3-yl]carbonyl]-2,2-dimethylpropyl]amino]carbonyl]amino]cyclohexanecarboxylate (28). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.40 and 8.32 (d, J = 6.62 and 7.25 Hz, 1H), 8.01 (d, J = 7.88 Hz, 1H), 7.74(bs, 1H), 7.62 (bs, 1H), 6.44 (d, J = 6.93 Hz, 2H), 6.16 (dd, J = 10.08, 16.39 Hz, 1H), 5.11−5.07 and 5.06−5.02 (m, 1H), 4.96−4.87(m, 2H), 4.32 (s, 1H), 4.15 (t, J = 8.82 Hz, 1H), 3.86 (t, J = 10.71 Hz, 1H), 3.77−3.71 (m, 2H), 1.90−1.74 (m, 1H), 1.71−1.15 (m, 14H), 1.00 (d, J = 9.77 Hz, 3H), 0.88 (bs, 9H), 0.80 (d, J = 13.24 Hz, 3H), 0.41−0.37 (m, 2H), 0.12−0.01 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 198.47, 197.90, 175.18, 171.82, 171.53, 171.49, 171.27, 163.77, 163.48, 157.53, 157.46, 150.31, 144.29, 111.48, 111.14, 60.20, 59.95, 58.65, 58.11, 57.64, 57.56, 55.01, 54.98, 48.32, 35.85, 35.11, 35.16, 33.88, 32.57, 32.53, 31.55, 31.44, 27.65, 27.55, 27.08, 27.07, 26.98, 26.82, 25.64, 21.59, 21.49, 19.33, 19.30, 13.30, 13.22, 8.73, 8.53, 5.89, 5.21, 4.90.

(1*R*,5*S*)-*N*-[3-Amino-1-(cyclopropylmethyll)-2,3-dioxopropyl]-3-[3,3-dimethyl-1-*oxo*-2(*S*)-[[[[1-[[(phenylmethyl)amino]carbonyl]-cyclohexyl]amino]carbonyl]amino]butyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-2(*S*)-carboxamide (29). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.40 and 8.33 (d, J = 6.62 and 10.71 Hz, 1H), 8.02 (d, J = 9.14 Hz, 1H), 7.93 (bs, 1H), 7.74 (s, 1H), 7.24 (m, 5H), 6.54-.6.45 (m, 1H), 6.20 (s, 1H), 5.11−5.06 and 5.05−5.01 (m, 1H), 4.31 (s, 1H), 4.29 (d, J = 6.62 Hz, 1H), 4.24 (t, J = 9.14 Hz, 1H), 4.20−4.13 (m, 1H), 3.91−3.74 (m, 2H), 2.05 (d, J = 14.50 Hz, 1H), 1.86 (d, J = 9.45 Hz, 1H), 1.71−1.14 (m, 12H), 0.97−0.88 (m, 13H), 0.74 (d, J = 12.61 Hz, 3H), 0.43−0.36 (m, 2H), 0.17−0.02 (m, 2H).

1-[[[[1(*S*)-[[(1*R*,5*S*)-2(*S*)-[[[3-Amino-1-(cyclopropylmethyl)-2,3-dioxopropyl]amino]carbonyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hex-3-yl]carbonyl]-2,2-dimethylpropyl]amino]carbonyl]amino]cyclohexanecarboxylate (30). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.39 and 8.32 (d, J = 6.62 and 7.25 Hz, 1H), 8.01 (d, J = 7.25 Hz, 1H), 7.75(bs, 1H), 6.30 (d, J = 4.09 Hz, 2H), 6.20 (d, J = 10.08 Hz, 1H), 5.11−5.08 and 5.07−5.03 (m, 1H), 4.33 (s, 1H), 4.24−4.15 (m, 1H), 3.91−3.84 (m, 1H), 3.80−3.71 (m, 1H), 1.96−1.93 (m, 1H), 1.81 (d, J = 12.61 Hz, 1H), 1.70−1.11 (m, 12H), 1.01−0.99 (m, 3H), 0.95−0.85 (m, 10H), 0.81−0.77 (m, 3H), 0.47−0.35 (m, 2H), 0.14−0.02 (m, 2H). HRMS calcd for $C_{29}H_{46}N_5O_7$, 576.3397 $(M + H)^+$; found, 576.3403.

(1*R*,5*S*)-*N*-[3-Amino-1-(cyclopropylmethyl)-2,3-dioxopropyl]-3-[3,3-dimethyl-1-*oxo*-2(*S*)-[[[[1-[[(phenylsulfonyl)amino]carbonyl]-cyclohexyl]amino]carbonyl]amino]butyl]-6,6-dimethyl-3-azabicyclo-[3.1.0]hexan-2(*S*)-carboxamide (31). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.41 and 8.34 (d, J = 7.25 and 7.56 Hz, 1H), 8.02 (d, J = 11.35 Hz, 3H), 7.88 (bs, 1H), 7.79−7.74 (m, 2H), 7.59 (t, J = 10.40 Hz, 1H), 6.63−6.58 (m, 1H), 6.54 (s, 1H), 6.16 (s, 1H), 5.12−5.08 and 5.06−5.02 (m, 1H), 4.34 (bs, 1H), 4.25 (dd, J = 6.62, 9.77 Hz, 1H), 3.87−3.74 (m, 2H), 2.12−2.08 (m, 1H), 2.02−1.98 (m, 1H), 1.85−1.13 (m, 13H), 1.01 (d, J = 10.71 Hz, 3H), 0.91 (s, 9H), 0.80 (d, J = 11.66 Hz, 3H), 0.47−0.36 (m, 2H), 0.12−0.01 (m, 2H). HRMS calcd for $C_{35}H_{51}N_6O_8S$, 714.8880 $(M + H)^+$; found, 714.8892.

Benzyl 2-(3-((2*S*)-1-((1*R*,2*S*,5*S*)-2-(4-Amino-1-cyclopropyl-3,4-dioxobutan-2-yl)carbamoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-3-yl)-3,3-dimethyl-1-oxobutan-2-yl)ureido)-2-methylpropanoate (33). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 −7.60 (m, 1H), 7.05 (s, 1H), 6.95 (s, 1H), 5.85 (d, J = 9.85 Hz, 1H), 5.62−5.56 (m, 1H), 5.17−5.02 (m, 3H), 4.46−4.37 (m, 2H), 4.13−4.00 (m, 2H), 3.84−3.74 (m, 1H), 2.48 (s, 1H), 1.89−1.64 (m, 2H), 1.58−1.37 (m, 5H), 1.27−1.20 (m, 1H), 1.01−0.78 (m, 20H), 0.75−0.64 (m, 1H), 0.40 (t, J = 7.07 Hz, 2H), 0.13−0.05 (m, 2H). LCMS for $C_{33}H_{47}N_5O_7$: 626.10 $(M + H)^+$, purity 98.7%.

(2*R*)-Benzyl 2-(3-((2*S*)-1-((1*R*,2*S*,5*S*)-2-(4-Amino-1-cyclopropyl-3,4-dioxobutan-2-yl)carbamoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]-hexan-3-yl)-3,3-dimethyl-1-oxobutan-2-yl)ureido)-2,3-dimethylbutanoate (36). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (s, 5H), 6.96 (s, 1H), 6.89 (s, 1H), 6.81 (s, 1H), 5.51−5.33 (m, 1H), 5.23 (s, 2H), 5.15 (d, J = 12.64 Hz, 1H), 5.03 (d, J = 12.64 Hz, 1H), 4.44−4.34 (m, 2H), 4.05−3.97 (m, 1H), 3.83−3.73 (m, 1H), 3.82−3.67 (m, 2H), 3.66−3.60 (m, 1H), 2.19−2.07 (m, 1H), 1.97−1.78 (m, 1H), 1.70−1.54 (m, 2H), 1.52−1.43 (m, 2H), 1.05−0.85 (m, 17H), 0.84−0.63 (m, 3H), 0.49−0.37 (m, 2H), 0.17−0.07 (m, 2H). LCMS for $C_{35}H_{52}N_5O_7$: 654.50 $(M + H)^+$, purity 98.9%.

(2*S*)-Benzyl 2-(3-((2*S*)-1-((1*R*,2*S*,5*S*)-2-(4-Amino-1-cyclopropyl-3,4-dioxobutan-2-yl)carbamoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]-hexan-3-yl)-3,3-dimethyl-1-oxobutan-2-yl)ureido)-2,3-dimethylbutanoate (37). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (d, J = 5.09 Hz, 1H), 7.32 (s, 5H), 6.93 (s, 1H), 6.79 (s, 1H), 6.23 (s, 1H), 5.33 (d, J = 6.72 Hz, 1H), 5.13 (s, 2H), 4.44 (d, J = 12.11 Hz, 2H), 4.11 (d, J = 10.35 Hz, 1H), 3.83−3.73 (m, 1H), 3.69 (s, 1H), 2.21−2.03 (m, 1H), 1.89−1.66 (m, 2H), 1.61−1.40 (m, 5H), 1.25 (s, 1H), 1.04−0.68 (m, 20H), 0.43 (d, J = 8.22 Hz, 2H), 0.14−0.02 (m, 2H). LCMS for $C_{35}H_{51}N_5O_7$: 654.20 $(M + H)^+$, purity 96.5%.

(1*R*,5*S*)-*N*-[3-Amino-1-(cyclopropylmethyl)-2,3-dioxopropyl]-3-[3,3-dimethyl-2(*S*)-[[[[2-methyl-1(*S*)-[[(phenylsulfonyl)amino]carbonyl]propyl]amino]carbonyl]amino]butyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-2(*S*)-carboxamide (44). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.40 and 8.34 (d, J = 6.62 and 7.56 Hz, 1H), 8.01(d, J = 10.40 Hz, 1H), 7.86 (d, J = 7.56 Hz, 3H), 7.74 (bs, 1H), 7.61 (bs, 1H),7.55 (d, J = 6.93 Hz, 2H), 6.30 (dd, J = 10.40, 13.87 Hz, 1H), 6.23 (d, J = 8.52 Hz, 1H), 5.10−5.06 and 5.04−5.00 (m, 1H), 4.29 (s, 1H), 4.12 (t, J = 9.14 Hz, 1H), 3.99 (bs, 1H), 3.86 (t, J = 10.40 Hz, 1H), 3.76−3.71 (m, 1H), 1.88−1.82 (m, 1H), 1.70−1.52 (m, 1H), 1.44−1.23 (m, 4H), 1.00 (d, J = 10.40 Hz, 3H), 0.83 (s, 9H), 0.79 (d, J = 13.24 Hz, 3H), 0.66 (d, J = 5.35 Hz, 3H), 0.54 (d, J = 6.62 Hz, 3H), 0.39−0.36 (m, 2H), 0.12−0.005 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 198.47, 197.89, 171.87, 171.59, 171.47, 171.27, 163.81, 163.50, 158.41, 158.39, 129.43, 128.09, 60.23, 59.99, 59.23, 58.20, 58.09, 54.98, 54.96, 48.28, 35.83, 35.07, 35.05, 31.86, 31.56, 31.45, 27.78, 27.69, 27.22, 27.19, 26.99, 26.95, 19.86, 19.36, 19.34, 17.75, 13.46, 13.41, 8.74, 8.53, 5.90, 5.21, 4.90. HRMS calcd for $C_{33}H_{49}N_6O_8S$, 689.8500 $(M + H)^+$; found, 689.8591.

(1*R*,5*S*)-*N*-[3-Amino-1-(cyclopropylmethyl)-2,3-dioxopropyl]-3-[2(*S*)-[[[(1(*S*)-benzoyl-2-methylpropyl)amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-2(*S*)-carboxamide (46). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.42 and 8.36 (d, J = 6.93 and 7.25 Hz, 1H), 8.02 (d, J = 9.77 Hz, 1H), 7.97 (d, J = 8.19 Hz, 2H), 7.75(bs, 1H), 7.64 (t, J = 7.56 Hz, 1H), 7.52 (t, J = 7.88 Hz, 2H), 6.58 (d, J = 8.82 Hz, 1H), 6.39 (J = 9.77 and 13.55 Hz, 1H), 5.17 (dd, J = 5.04 and 8.82 Hz, 1H), 5.13−5.09 and 5.07−5.03 (m, 1H), 4.35 (s, 1H), 4.21 (d, J = 8.19 Hz, 1H), 3.90 (t, J = 10.40 Hz, 1H), 3.80−3.74 (m, 1H), 2.04−1.99 (m, 1H), 1.72−1.56 (m, 1H), 1.47−1.31 (m, 3H), 1.01 (d, J = 9.77 Hz, 3H), 0.90−0.83 (m, 16H), 0.70 (d, J = 9.14 Hz, 3H), 0.42−0.36 (m, 2H), 0.11−0.006 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 201.00, 198.48, 197.90, 171.83, 171.55, 171.44, 171.24, 163.79, 163.50, 158.55, 158.53, 136.57, 134.23, 129.27, 129.05, 60.25, 60.02, 59.28, 58.17, 58.07, 55.01, 54.98, 48.32, 35.89, 35.17, 35.16, 31.58, 31.47, 31.25, 31.22, 27.77, 27.67, 27.16, 27.13, 26.99, 26.95, 20.49, 19.35, 19.33, 17.66, 13.48, 13.41, 8.74, 8.54, 5.90, 5.21, 4.90. HRMS calcd for $C_{33}H_{48}N_5O_6$, 611.3672 (M + H)$^+$; found, 611.3674.

(1*R*,5*S*)-*N*-[3-Amino-1-(cyclopropylmethyl)-2,3-dioxopropyl]-3-[3,3-dimethyl-2(*S*)-[[[[2-methyl-1(*R*)-([phenylmethyl)propyl]amino]carbonyl]amino]-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]-hexan-2(*S*)-carboxamide (48). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.39 and 8.31 (d, J = 6.62 and 7.25 Hz, 1H), 8.01 (d, J = 9.14 Hz, 1H), 7.47 (s, 1H), 7.23−7.12 (m, 5H), 5.99−5.94 (m, 2H), 5.11−5.07 and 5.05−5.01 (m, 1H), 4.31 (s, 1H), 4.13 (t, J = 6.93 Hz, 1H), 3.87 (t, J = 10.08 Hz, 1H), 3.76−3.70 (m, 1H), 3.68−3.63 (m, 1H), 2.67 (dd, J = 4.72 and 13.87 Hz, 2H), 1.71−1.54 (m, 2H), 1.46−1.23 (m, 3H), 1.00 (d, J = 10.40 Hz, 3H), 0.83 (s, 9H), 0.81−0.76 (m, 10H), 0.43−0.36 (m, 2H), 0.12−0.01 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 198.47, 197.92, 171.85, 171.72, 171.56, 171.52, 163.78, 163.48, 158.54, 158.49, 140.52, 129.83, 128.82, 126.55, 60.17, 59.91, 57.78, 57.70, 55.87, 55.86, 54.99, 54.96, 48.26, 48.23, 39.45, 39.43, 35.85, 35.13, 35.11, 31.92, 31.55, 31.45, 27.68, 27.58, 27.14, 27.12, 26.99, 26.94, 20.39, 19.32, 19.29, 17.66, 13.38, 13.32, 8.74, 8.54, 5.90, 5.21, 4.90. HRMS calcd for $C_{33}H_{49}NaN_5O_5$, 618.3631 (M + Na)$^+$; found, 618.3655.

(1*R*,5*S*)-*N*-[3-Amino1-(cyclopropylmethyl)-2,3-dioxopropyl]-3-[3,3-dimethyl-1-*oxo*-2(*S*)-[[[[1(*S*)-(2-pyridinylcarbonyl)-2-methylpropyl]amino]carbonyl]amino]butyl]-6,6-dimethyl-3-azabicyclo-[3.1.0]hexan-2(*S*)-carboxamide (52). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.76 (t, J = 4.72 Hz, 1H), 8.03 (d, J = 7.56 Hz, 2H), 7.98 (t, J = 9.14 Hz, 1H), 7.75−7.68 (m, 2H), 6.55−6.52 (m, 2H), 5.67−5.64 (m, 1H), 5.11−5.08 and 5.06−5.03 (m, 1H), 4.33 (d, J = 3.15 Hz, 1H), 4.16 (t, J = 9.14 Hz, 1H), 3.88 (d, J = 9.45 Hz, 1H), 3.78−3.72 (m, 2H), 2.21−2.14 (m, 1H), 1.73−1.15 (m, 5H), 1.02 (d, J = 9.77 Hz, 3H), 0.92−0.71 (m, 15H), 0.66 (d, J = 6.62 Hz, 3H), 0.41−0.35 (m, 2H), 0.11−0.02 (m, 2H). HRMS calcd for $C_{32}H_{47}N_6O_6$, 611.3479 (M + H)$^+$; found, 611.3522.

(1*R*,5*S*)-*N*-[3-Amino-1-(cyclopropylmethyl)-2,3-dioxopropyl]-3-[3,3-dimethyl-2(*S*)-[[[[2-methyl-1(*S*)-(2-thienylcarbonyl)propyl]amino]carbonyl]amino]-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]-hexan-2(*S*)-carboxamide (53). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.42 and 8.37 (d, J = 6.62 and 7.56 Hz, 1H), 8.03 (t, J = 4.72 Hz, 3H), 7.75 (bs, 1H),7.26 (t, J = 4.72 Hz, 1H), 6.60 (d, J = 8.51 Hz, 1H), 6.36 (dd, J = 10.08 and 13.87 Hz, 1H), 5.12−5.08 and 5.06−5.02 (m, 1H), 4.93−4.90 (m, 1H), 4.34 (d, J = 3.15 Hz, 1H), 4.18 (t, J = 9.14 Hz, 1H), 3.87 (t, J = 11.35 Hz, 1H), 3.81−3.73 (m, 1H), 2.08−2.03 (m, 1H), 1.72−1.52 (m, 1H), 1.47−1.23 (m, 4H), 1.02 (d, J = 10.40 Hz, 3H), 0.89−0.81 (m, 15H), 0.77 (d, J = 6.93 Hz, 3H), 0.42−0.37 (m, 2H), 0.12−0.01 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 198.51, 197.89, 193.82, 173.66, 171.82, 171.54, 171.36, 171.19, 163.79, 163.49, 158.42, 158.40, 143.31, 136.23, 134.45, 129.73, 60.65, 60.40, 60.22, 60.01, 58.12, 58.03, 54.98, 48.31, 35.86, 35.22, 35.16, 31.79, 31.75, 31.58, 31.48, 27.73, 27.64, 27.11, 27.05, 27.00, 26.96, 26.88, 20.47, 19.36, 19.33, 18.10, 13.47, 13.43, 8.76, 8.55, 5.91, 5.21, 4.90. HRMS calcd for $C_{33}H_{48}N_5O_6S$, 616.3169 (M + H)$^+$; found, 616.3170.

(1*R*,5*S*)-*N*-[3-Amino-1-(cyclopropylmethyl)-2,3-dioxopropyl]-3-[3,3-dimethyl-2(*S*)-[[[[2-methyl-1(*S*)-(2-thiazolylcarbonyl)propyl]-amino]carbonyl]amino]-1-oxobutyl]-6,6-dimethyl-3-azabicyclo-[3.1.0]hexan-2(*S*)-carboxamide (54). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.42 and 8.36 (d, J = 6.62 and 7.25 Hz, 1H), 8.27 (d, J = 2.87 Hz, 1H), 8.20 (d, J = 2.83 Hz, 1H), 8.02 (d, J = 9.45 Hz, 1H), 7.75 (bs, 1H), 6.62 (d, J = 8.82 Hz, 1H), 6.42 (dd, J = 9.77 and 13.55 Hz, 1H), 5.32−5.30 (m, 1H), 5.12−5.08 and 5.06−5.02 (m, 1H), 4.34 (d, J = 3.15 Hz, 1H), 4.15 (t, J = 9.14 Hz, 1H), 3.85 (t, J = 10.71 Hz, 1H), 3.77−3.72 (m, 1H), 2.29−2.25 (m, 1H), 1.72−1.53 (m, 1H), 1.47−1.30 (m, 3H), 1.01 (d, J = 9.77 Hz, 3H), 0.94−0.79 (m, 16H), 0.74 (d, J = 6.62 Hz, 3H), 0.43−0.35 (m, 2H), 0.12−0.008 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 198.50, 197.94, 193.98, 171.83, 171.55, 171.38, 171.18, 165.91, 163.81, 163.49, 158.52, 158.51, 146.22, 129.31, 60.51, 60.21, 60.02, 58.18, 58.10, 55.00, 54.98, 48.30, 35.86, 35.15, 31.58, 31.49, 31.20, 31.18, 27.75, 27.66, 27.18, 27.16, 27.00, 26.96, 20.60, 19.38, 19.35, 17.58, 13.45, 13.38, 8.76, 8.55, 5.91, 5.21, 4.90. HRMS calcd for $C_{30}H_{45}N_6O_6S$, 617.3121 (M + H)$^+$; found, 617.3134.

(1*R*,5*S*)-*N*-[3-Amino-1-(cyclopropylmethyl)-2,3-dioxopropyl]-3-[2(*S*)-[[[[1(*S*)-(cyclopropylcarbonyl)-2-methylpropyl]amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]-hexan-2(*S*)-carboxamide (55). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.41 and 8.35 (d, J = 6.62 and 7.25 Hz, 1H), 8.00 (d, J = 9.77 Hz, 1H), 7.75 (bs, 1H), 6.44 (d, J = 8.83 Hz, 1H), 6.34 (dd, J = 10.08 and 14.18 Hz, 1H), 5.12−5.08 and 5.06−5.02 (m, 1H), 4.36−4.32 (m, 2H), 4.19 (t, J = 9.45 Hz, 1H), 3.90−3.83 (m, 1H), 3.79−3.73 (m, 1H), 2.24−2.11 (m, 2H), 1.73−1.65 (m, 1H), 1.63−1.52 (m, 1H), 1.49−1.39 (m, 2H), 1.36−1.29 (m, 1H), 1.00 (d, J = 10.4 Hz, 3H), 0.90−0.72 (m, 18H), 0.70−0.69 (m, 4H), 0.45−0.29 (m, 2H), 0.15−0.01 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 210.83, 198.46, 197.88, 171.85, 171.56, 171.48, 171.27, 163.8, 163.48, 158.56, 64.61, 60.24, 60.02, 58.13, 58.04, 54.97, 48.30, 35.86, 35.17, 31.56, 31.45, 30.23, 30.21, 27.76, 27.67, 27.17, 26.98, 20.35, 19.36, 18.81, 17.79, 13.44, 11.78, 11.20, 8.73, 8.53, 5.89, 5.20, 4.89. HRMS calcd for $C_{30}H_{48}N_5O_6$, 574.3604 (M + H)$^+$; found, 574.3616.

(1*R*,5*S*)-*N*-[3-Amino-1-(cyclopropylmethyl)-2,3-dioxopropyl]-3-[3,3-dimethyl-2(*S*)-[[[[3-methyl-1(*S*)-(1-methylethyl)-2-oxobutyl]amino]carbonyl]amino]-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-2(*S*)-carboxamide (59). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (500 MHz, DMSO-d_6) 8.40 and 8.34 (d, J = 6.62 and 7.25 Hz, 1H), 8.01 (d, J = 9.14 Hz, 1H), 7.74 (bs, 1H), 6.41 (d, J = 9.14 Hz, 1H), 6.30 (dd, J = 9.77 and 14.18 Hz, 1H), 5.12−5.08 and 5.06−5.02 (m, 1H), 4.33 (d, J = 2.83 Hz, 1H), 4.27 (q, J = 5.35 and 9.14 Hz, 1H), 4.19 (t, J = 9.45 Hz, 1H), 3.88 (d, J = 10.71 Hz, 1H), 3.79−3.73 (m, 1H), 2.82 (p, J = 6.90 Hz, 1H), 2.07−2.00 (m, 1H), 1.71−1.52 (m, 1H), 1.47−1.40 (m, 2H), 1.35−1.30 (m, 1H), 1.01−0.98 (m, 6H), 0.95−0.87 (m, 13H), 0.82−0.79 (m, 6H), 0.69 (d, J = 6.93 Hz, 3H), 0.42−0.36 (m, 2H), 0.14−0.01 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 214.96, 198.47, 197.92, 171.84, 171.55, 171.44, 171.24, 163.80, 163.49, 158.54, 158.52, 62.34, 60.22, 59.99, 58.10, 58.02, 55.00, 54.98, 48.31, 37.84, 35.85, 35.18, 35.17, 29.86, 29.84, 28.53, 27.73, 27.63, 27.16, 27.13, 26.99, 26.95, 20.49, 19.44, 19.35, 19.33, 18.55, 17.90, 13.41, 13.35, 8.76, 8.55, 5.90, 5.21, 4.90. HRMS calcd for $C_{33}H_{59}N_5O_5$, 576.3761 (M + H)$^+$; found, 576.3738.

(1*R*,5*S*)-*N*-[3-Amino-1-(cyclopropylmethyl)-2,3-dioxopropyl]-3-[2(*S*)-[[[[1(*S*)-cyclohexyl-2-*oxo*-2-(2-pyridinyl)ethyl]amino]carbonyl]-amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]-hexane-2(*S*)-carboxamide (61). The product was isolated as a mixture of two diastereomers (~1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.77 (d, J = 5.04 Hz, 1H), 8.41 and 8.35 (d, J = 6.93 and 7.56 Hz, 1H), 8.04−7.96 (m, 3H), 7.75 (bs, 1H), 7.70−7.67 (m, 1H), 6.56 (d, J = 9.14 Hz, 1H), 6.44−6.39 (m, 1H), 5.68−5.65 (m, 1H), 5.12−5.08 and 5.07−5.03 (m, 1H), 4.34 (bs, 1H), 4.16 (t, J = 9.45 Hz, 1H), 3.89(t, J = 10.71 Hz, 1H), 3.78−3.72 (m, 1H), 1.80−1.75 (m, 1H), 1.66−1.30 (m, 8H), 1.13−0.83 (m, 22H),

0.41−0.36 (m, 2H), 0.11−0.01 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 201.69, 198.49, 197.92, 171.85, 171.57, 171.54, 171.32, 163.82, 163.52, 158.51, 158.49, 152.81, 150.12, 138.67, 128.82, 123.00, 60.23, 60.00, 58.40, 58.39, 58.15, 58.05, 55.01, 54.98, 48.28, 35.88, 35.13, 35.11, 31.59, 31.50, 30.69, 27.82, 27.76, 27.69, 27.20, 27.17, 27.02, 26.98, 26.64, 26.56, 26.44, 19.35, 19.33, 13.38, 13.32, 8.74, 8.54, 5.90, 5.21, 4.91. HRMS calcd for $C_{35}H_{51}N_6O_6$, 651.3870 $(M + H)^+$; found, 651.3872.

(1*R*,5*S*)-*N*-[3-Amino-1-(cyclopropylmethyl)-2,3-dioxopropyl]-3-[2(*S*)-[[[(1(*S*)-cyclohexyl-2-cyclopropyl-2-oxoethyl)amino]carbonyl]-amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]-hexane-2(*S*)-carboxamide (62). The product was isolated as a mixture of two diastereomers (∼1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.41 and 8.36 (d, J = 6.62 and 7.25 Hz, 1H), 8.02 (t, J = 8.82 Hz, 1H), 7.75 (bs, 1H), 6.46 (d, J = 8.82 Hz, 1H), 6.32 (dd, J = 10.08 and 14.50 Hz, 1H), 5.11−5.08 and 5.06−5.02 (m, 1H), 4.33−4.30 (m, 2H), 4.23−4.15 (m, 1H), 3.88 (t, J = 10.71 Hz, 1H), 3.80−3.73 (m, 1H), 2.17−2.10 (m, 1H), 1.83−1.75 (m, 1H), 1.71−1.11 (m, 11H), 1.02−0.97 (m, 5H), 0.94−0.85 (m, 13H), 0.83−0.81 (m, 3H), 0.80−0.75 (m, 2H), 0.47−0.31 (m, 2H), 0.14−0.03 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 210.78, 198.51, 197.90, 171.82, 171.54, 171.46, 171.26, 163.77, 163.50, 158.45, 158.43, 130.23, 115.66, 110.47, 94.63, 64.37, 60.12, 59.93, 58.06, 57.98, 55.01, 48.31, 35.85, 35.24, 35.17, 31.60, 30.41, 28.07, 27.77, 27.63, 27.17, 27.15, 27.09, 27.00, 26.97, 26.62, 26.53, 19.36, 19.32, 18.95, 13.37, 13.32, 13.00, 11.74, 11.34, 8.75, 8.55, 5.91, 5.21, 4.90. HRMS calcd for $C_{33}H_{52}N_5O_6$, 614.3839 $(M + H)^+$; found, 614.3845.

(1*R*,5*S*)-3-[2(*S*)-[[[[1(*S*)-(Cyclopropylcarbonyl)-2-methylpropyl]-amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-*N*-[1-[1,2-dioxo-2-(propenylamino)ethyl]butyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexan-2(*S*)-carboxamide (63). The product was isolated as a mixture of two diastereomers (∼1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.87 and 8.81 (t, J = 6.0 Hz, 1H), 8.39 and 8.34 (d, J = 6.93 and 7.25 Hz, 1H), 6.44 (d, J = 8.82 Hz, 1H), 6.33 (t, J = 10.08 Hz, 1H), 5.83−5.75 (m, 1H), 5.12 (d, J = 1.57 Hz, 1H), 5.09−5.04 (m, 1H), 5.00−4.96 and 4.89−4.85 (m, 1H), 4.35 (q, J = 4.72 and 8.82 Hz, 1H), 4.30 (d, J = 6.93 Hz, 1H), 4.22−4.16 (m, 1H), 3.87 (d, J = 10.08 Hz, 1H), 3.79−3.68 (m, 3H), 2.18−2.15 (m, 2H), 1.74−1.65 (m, 1H), 1.55−1.23 (m, 5H), 1.01 (d, J = 9.45 Hz, 3H), 0.93−0.75 (m, 22H), 0.71 (d, J = 6.93 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 210.82, 198.22, 197.60, 171.88, 171.79, 171.40, 171.24, 162.02, 161.64, 158.59, 158.56, 135.05, 135.00, 116.46, 116.36, 64.61, 60.23, 59.98, 58.07, 54.37, 48.29, 41.70, 35.16, 32.51, 32.47, 31.58, 31.55, 30.23, 30.21, 27.83, 27.75, 27.18, 26.99, 26.96, 20.59, 20.37, 19.57, 19.43, 19.36, 18.81, 17.81, 17.58, 14.45, 14.33, 13.44, 13.38, 11.76, 11.19. HRMS calcd for $C_{32}H_{52}N_5O_6$, 602.3918 $(M + H)^+$; found, 602.3940.

(1*R*,5*S*)-3-[2(*S*)-[[[(1(*S*)-Cyclohexyl-2-cyclopropyl-2-oxoethyl)amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-*N*-[1-[1,2-dioxo-2-(2-propenylamino)ethyl]butyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (64). The product was isolated as a mixture of two diastereomers (∼1:1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.88 and 8.82 (t, J = 5.99 Hz, 1H), 8.40 and 8.35 (d, J = 6.62 and 7.25 Hz, 1H), 6.46 (d, J = 8.50 Hz, 1H), 6.31 (t, J = 10.40 Hz, 1H), 5.83−5.76 (m, 1H), 5.12−5.04 (m, 2H), 5.01−4.96 and 4.89−4.85 (m, 1H), 4.33−4.29 (m, 2H), 4.19−4.16 (m, 1H), 3.87 (t, J = 10.08 Hz, 1H), 3.79−3.71 (m, 3H), 2.17−2.12 (m, 1H), 1.84−1.76 (m, 1H), 1.73−1.03 (m, 16H), 1.07−0.97 (m, 5H), 0.94−0.75 (m, 17H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 210.77, 198.22, 197.61, 171.96, 171.79, 171.41, 171.25, 162.02, 161.64, 158.46, 158.43, 135.05, 135.00, 116.46, 116.36, 64.37, 60.22, 59.96, 58.00, 54.37, 48.28, 41.70, 35.17, 32.52, 32.47, 31.58, 31.55, 30.41, 28.08, 27.83, 27.74, 27.16, 27.00, 26.97, 26.63, 26.54, 19.57, 19.44, 19.35, 18.95, 14.45, 13.35, 13.29, 11.71, 11.32. HRMS calcd for $C_{35}H_{55}N_5NaO_6$, 664.4050 $(M + Na)^+$; found, 664.4059.

(1*R*,2*S*,5*S*)-3-((*S*)-2-(3-((*S*)-1-Cyclohexyl-2-cyclopropyl-2-oxoethyl)ureido)-3,3-dimethylbutanoyl)-*N*-(1-(cyclopropylamino)-1,2-dioxohept-6-yn-3-yl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxamide (68). The product was isolated as a mixture of two diastereomers (∼1:1). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.8.72 (d, J = 5.13 Hz, 1H), 8.44(d, J = 7.32 Hz, 1H), 6.46−6.41 (m, 2H), 6.31−6.24 (m, 2H), 5.00−4.92 (m, 1H), 4.33−4.26 (m, 2H), 4.25 (s, 1H), 4.23−4.18 (m, 1H), 4.14 (d, J = 9.52 Hz, 2H), 3.84 (d, J = 10.25 Hz, 2H), 3.81−3.69 (m, 2H), 2.78 (s, 1H), 2.76−2.68 (m, 1H), 2.34−2.18 (m, 2H), 2.17−2.08 (m, 2H), 2.00−1.90 (m, 1H), 1.83−1.73 (m, 2H), 1.71−1.48 (m, 4H), 1.44−1.38 (m, 1H), 1.36−1.27 (m, 1H), 1.25−1.05 (m, 2H), 1.03−0.92 (m, 4H), 0.88 (s, 9H), 0.82−078 (m, 4H), 0.66−0.60 (m, 2H), 0.58−0.51 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 210.77, 197.40, 172.07, 171,27, 162.92, 158.45, 84.15, 72.73, 60.06, 57.99, 54.18, 48.27, 40.99, 40.82, 40.65, 35.16, 31.50, 30.41, 29.96, 28.08, 27.88, 27.20, 26.98, 26.63, 26.54, 23.33, 20.42, 19.39, 18.95, 15.58, 13.34, 11.73, 11.34, 6.26. LCMS for $C_{36}H_{54}N_5O_3$: 652.3 $(M + H)^+$, purity 96.5%.

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