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Discovery of potent sulfonamide P4-capped ketoamide second generation inhibitors of hepatitis C virus NS3 serine protease with favorable pharmacokinetic profiles in preclinical species

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ABSTRACT

Hepatitis is a disease characterized by inflammation of the liver, usually producing swelling and, in many cases, permanent damage to liver tissues. Viral hepatitis C (HCV), a small (+)-RNA virus, infects chronically 3% of the world's population. Boceprevir, SCH 503034, (**1**) our first generation HCV inhibitor, has already established proof-of- concept and is currently in late stage (phase III) clinical trials. 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 by introducing a new sulfonamide moiety and optimization of the $P1/P'_1$ capping led to the discovery of a novel series of inhibitors of the HCV NS3 serine protease. Optimization of the P_1 residue significantly improved potency and selectivity. The combination of optimal moieties led to the discovery of compound **47** which, in addition to being a potent inhibitor of HCV subgenomic RNA replication, was also found to have good PK profile in rat, dog and monkey.

1. Introduction

Hepatitis C is a major cause of acute hepatitis and the most prevalent liver disease. Viral hepatitis C (HCV), a small (+)-RNA virus, infects chronically 3% of the world's population and million are newly infected each year. Because of mild symptoms, HCV has been referred by many as stealth virus. It can hide in the cell, undetected, for a long period of time and can take as long as 20 years to go from infection to chronic liver disease. Untreated HCV infections can progress to cirrhosis, hepatocellular carcinoma and liver failure, a primary cause for liver transplantation.[1] Although the use of alpha 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.[2] With the opportunity to improve response rates, especially for genotype 1 patients, and given the side effects associated with the current therapy, it is necessary to search for novel, potent and drug-like inhibitors of HCV with the intent of improving treatment outcomes and potentially shorten treatment duration.[3]

The NS3 protease has demonstrated a vital role in the replication of the HCV virus. It is a pivotal enzyme required for maturation of hepatitis C virons and assists in the 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]

Several NS3/4A protease inhibitors have been moved to human clinical trials. 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 that now has been discontinued.[6] More recently, new macrocyclic inhibitors were reported with **ITMN-191** (phase I),[7] **TMC435350** (phase II)[8] and the most advanced **MK-7009** currently in phase II with proof-of-concept achieved.[9] As another class, covalent reversible α-ketoamide electrophilic serine trap inhibitors have been reported by our group[10] and others[11] 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 **1**.[12] Clinical candidates Telaprevir (**VX-950**) and **1** belonging to the α-ketoamide series established proof of concept and phase III clinical studies are underway.[13] In this manuscript, we report our efforts aimed at optimizing the overall profile of our first generation inhibitor. Our goal for a second generation inhibitor was to improve the cellular potency and exposure across species.

Lately, we reported that P_4-capped inhibitors could provide enhanced enzyme and cellular potency.[14] Initially, we identified

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a novel series of potent P_4 ester derived inhibitors, as exemplified by compound **2** (Fig. 1), with a spirocyclohexyl group at P_4 that demonstrated 18-fold improvements in cellular potency (EC_{90}) but lacked oral exposure in rat (Table 1, compound **1**). Derivatization of the ester to a ketone moiety followed by modification of the P_4 and P'_1 capping residues drastically improved rat oral exposure but resulted in weak cell potency. P_1 butynylglycine was identified as a moiety that, when used in combination with a cyclopropyl ketone group at P_4, provided compound **3** (Fig. 1) with excellent binding and much improved cellular potencies versus **1**. Unfortunately, P_1 butynylglycine was not the optimal substitution for HNE/HCV selectivity (Table 1, compound **3**) and the electrophilic ketone moiety at P_4 was not a desirable feature for a progression candidate. Due to the excellent cellular potency observed with compound **2**, we decided to further explore the scope of the 1-substituted-cyclohexyl ureas series. We discovered that incorporation of a sulfonamide moiety at P_4 and optimization of the P_1 residue not only significantly improved potency and maintained selectivity but also provided good PK profile in three preclinical species (Table 2, compound **47**). 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.

2. Chemistry

Synthesis of the inhibitors was generally carried out as depicted in Scheme 1. The *gem*-dimethylcyclopropylproline P_2 core **4**, essential for potency, was used to prepare dipeptide **5** according previously described protocols.[12b] Substituted cyclohexyl sulfonamides of type **6** were converted to either an isocyanate or a 4-nitrophenylcarbamate intermediate before being reacted with amine

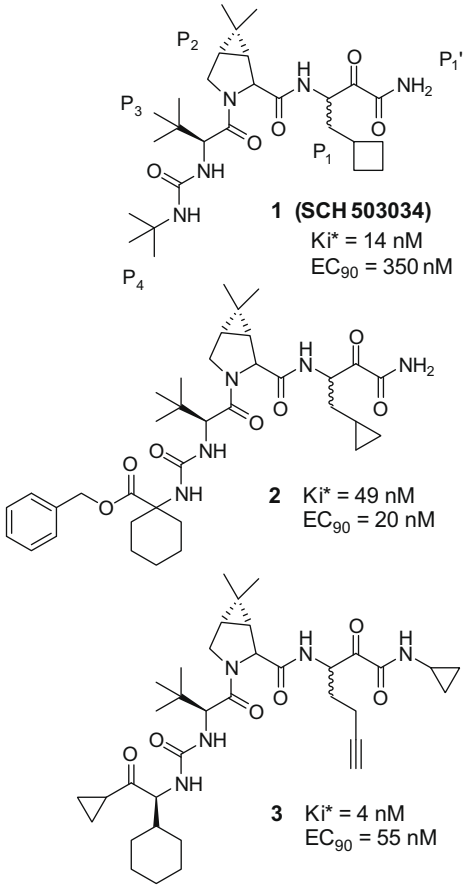
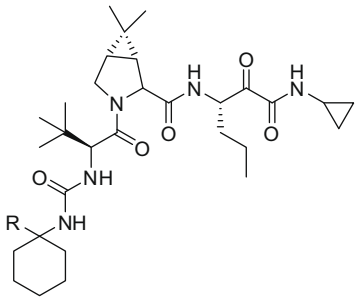
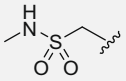
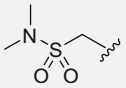
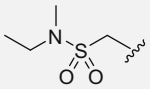
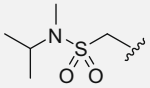
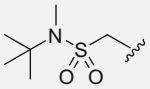
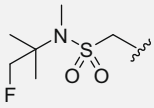
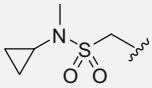
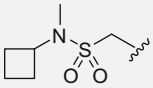
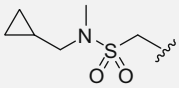
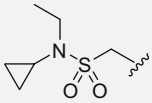
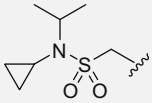
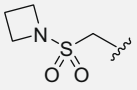
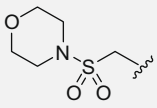


Figure 1. Profile of **1** (SCH 503034), **2** and **3**.

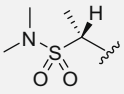
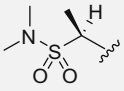
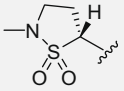
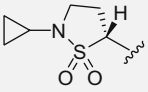
Table 1



Compd	R	K_i^* (nM)[a]	EC_{90} (nM)[b]	HNE/ 2HCV[c]	AUC (μM h)$_{0-6h}$ Rat[d]	Dog[e]	Monkey[f]
1	–	14	350	4400	0.1	3.1[g]	0.3
2	–	49	20	55	0	0	0.05
3	–	4	55	20	11	0.1	–
28		15	300	58	–[h]	–	–
29		6	55	82	0.6	6.1	0.1
30		9	65	64	1.5	0.5	0.1
31		7	70	97	0.9	0.2	–
32		8	55	36	0.6	0.2	–
33		2.8	45	–	0.1	0.2	–
34		8	55	82	1.8	6.9	3.3
35		7	90	–	0.3	0.2	–
36		9	135	–	–	–	–
37		5	65	131	1	7.9	0
38		6	100	–	1.7	0.5	–
39		7	80	–	0.8	0.8	–
40		8	45	135	0.3	0.3	–

(*continued on next page*)

Table 1 (*continued*)

Compd	R	K_i^* (nM)[a]	EC_{90} (nM)[b]	HNE/ 2HCV[c]	AUC (μM h)$_{0-6h}$ Rat[d]	Dog[e]	Monkey[f]
41		2	85	—	0.6	0.1	—
42		22	—	—	—	—	—
43		4	20	340	0.1	—	—
44		4	20	340	0.1	—	—

[a] K_i^* value represents binding for a single diastereomer at P_1 and within twofold for 95% confidence limit.
[b] HNE/HCV is a ratio of selectivity between human neutrophil elastase (HNE), a serine protease and HCV serine proteases.
[c] Replicon (genotype 1) EC_{90} value is within threefold for 95% confidence (single assay) and within twofold for 95% confidence (multiple assays).
[d] Rat PO were dosed at 10 mpk (no IV arm). Number of animals used: 2. Vehicle: PO (0.4%HPMC).
[e] Dog PO were dosed at 2 mpk (no IV arm). Number of animals used: 2. Vehicle: PO (0.4%HPMC).
[f] Monkey PO were dosed at 10 mpk (no IV arm). Number of animals used: 2. Vehicle: PO (0.4%HPMC).
[g] Dog PO were dosed at 3 mpk (no IV arm). Number of animals used: 3. Vehicle: PO (0.4%HPMC).
[h] Not determined.

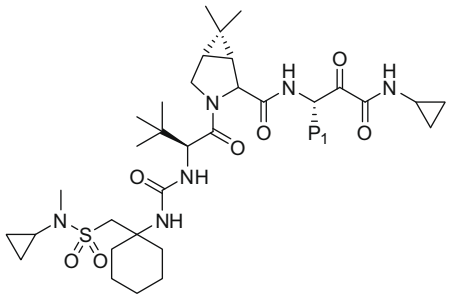
hydrochloride **5**. Hydrolysis of the resulting methyl ester intermediate to carboxylic acid **7** was follwed with HATU mediated coupling to P_1 α-hydroxy-β-aminoamide intermediates of type **8**. The resulting secondary hydroxyamide moieties were oxidized using Dess–Martin conditions to deliver targets **28–47** included in Tables 1 and 2.[15]

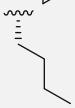
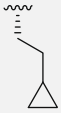
The sulfonamide capping moieties were prepared according to two different procedures outlined in Scheme 2 and 3. With the goal of quickly exploring various sulfonamides, we identified sulfonyl chloride **12** as a key intermediate. The iodomethyl cyclohexanecarboxylic acid methyl ester **10** was efficiently prepared by reacting the enolate of cyclohexanecarboxylic acid methyl **9** with diiodomethane. Displacement of **10** with potassium thioacetate in DMF afforded sulfonyl intermediate **11** in good yield. Preparation of **12** was challenging. Thus, the use of 4 equiv of water and 3 equiv of chlorine gas in AcOH were found optimal to suppress over chlorination and deliver exclusively desired sulfonyl chloride **12** in very high yield. Various sulfonamides of type **13** were accessed upon treatment of **12** with primary or secondary amines. Hydrolysis of the resulting methyl ester to carboxylic acid intermediate followed by a Curtius rearrangement yielded isocyanates **14**.[16]

Chiral acyclic and cyclic sulfonamides were prepared according Scheme 3, thus (*S*)-*tert*-butanesulfinamide was reacted with cyclohexanone **15** in the presence of titanium tetraethoxide to generate sulfinamide **16**. Alkylation of **16** with various lithiated acyclic and cyclic sulfonamides was used to prepare intermediates **17** as a mixture of diastereomers. HCl-mediated removal of the chiral auxiliary of intermediates **17** and subsequent treatment with either phosgene or 4-nitrophenylchloroformate led to the corresponding isocyanates **18** and activated carbamates **19** needed for P_4 capping exploration.

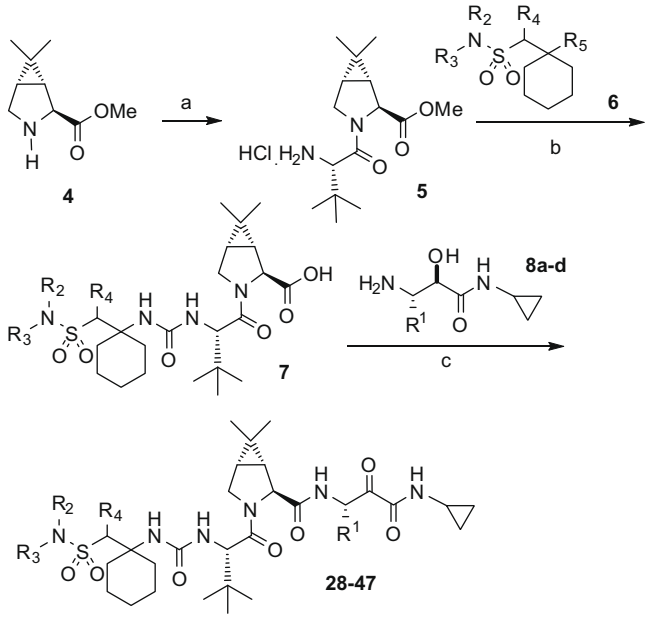
The P_1 α-hydroxy-β-aminocyclopropylamides **8a–d** were prepared according the procedures outlined in Scheme 4. Synthesis of (*S*)-Homo-cyclopropyl alanine hydroxyamide **8d** was initiated

Table 2

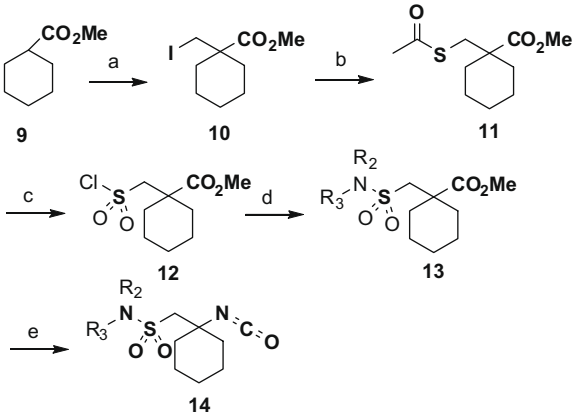


Compd	P1	K_i^* (nM)[a]	EC_{90} (nM)[b]	HNE/ HCV[c]	AUC (μM h)$_{0-6h}$ Rat[d]	Dog[e]	Monkey[f]
45		15	60	113	2.2	1.4	—[g]
46		11	105	959	—	—	—
47		6	75	2485	0.9	4	5.4

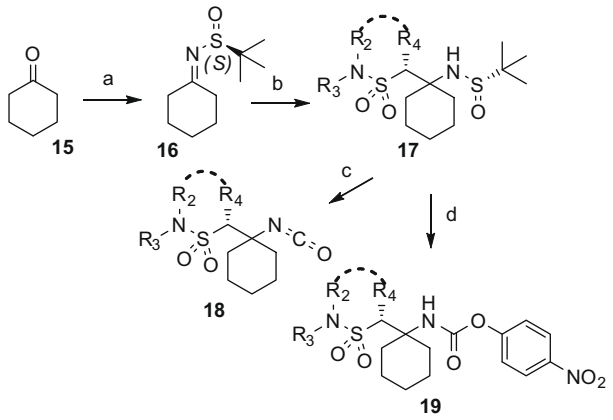
[a] K_i^* value represents binding for a single diastereomer at P_1 and within twofold for 95% confidence limit.
[b] HNE/HCV is a ratio of selectivity between human neutrophil elastase (HNE), a serine protease and HCV serine proteases.
[c] Replicon (genotype 1) EC_{90} value is within threefold for 95% confidence (single assay) and within twofold for 95% confidence (multiple assays).
[d] Rat PO were dosed at 10 mpk (no IV arm). Number of animals used: 2. Vehicle: PO (0.4%HPMC).
[e] Dog PO were dosed at 2 mpk (no IV arm). Number of animals used: 2. Vehicle: PO (0.4%HPMC).
[f] Monkey PO were dosed at 10 mpk (no IV arm). Number of animals used: 2. Vehicle: PO (0.4%HPMC).
[g] Not determined.



Scheme 1. Reaction conditions: (a) (i) Boc-*t*-leucine, EDC, HOOBt; DMF/CH_2Cl_2, *i*Pr_2NEt, 0 °C; (ii) 4 M HCl; *p*-dioxane, rt; (b) (i) (R_5 = isocyanate) **5**, DCM, aq $NaHCO_3$ or (R_5 = 4-nitrophenylcarbamate) **5**, DCM, DIPEA; (ii) **4**, DCM, DIPEA, 0 °C; (iii) LiOH, THF·MeOH/H_2O, 0 °C; (c) (i) HATU; DMF/CH_2Cl_2, *i*Pr_2NEt; (ii) Dess–Martin periodinane.



Scheme 2. Reaction conditions: (a) (i) LDA, THF, −10 °C; (ii) CH_2I_2, 0 °C to rt, 100%; (b) KSCOMe, DMF, 15 °C to rt overnight, 81%; (c) AcOH, H_2O (4 equiv), Cl_2 (3 equiv), 93%; (d) R_2R_3NH, DCM, DIPEA, 0 °C to rt; (e) (i) KOH, MeOH, 60 °C; (ii) DPPA, Et_3N, PhMe, 110 °C.

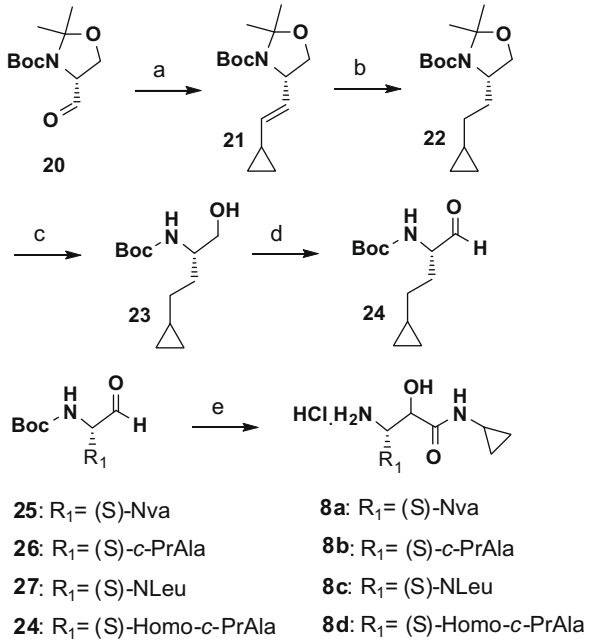


Scheme 3. Reaction conditions: (a) (*S*)-$H_2NS(O)tBu$, $Ti(OEt)_4$, THF, 60 °C, 76%; (b) $R_2R_3NSO_2CH_2R_4$, *n*-BuLi, toluene, −78 °C, 55–60%; (i) 4 M HCl/Dioxane, 0 °C to rt; (ii) phosgene (20% in toluene), DCM, aq $NaHCO_3$, 0 °C to rt, 1 h; (d) (i) 4 M HCl/dioxane, 0 °C to rt, 100%; (ii) 4-nitrophenylchloroformate, DIPEA, DCM, 0 °C to rt.

from D-Garner aldehyde.[17] Cyclopropylmethyl triphenylphosphonium bromide, after treatment with KHMDS, was reacted with **20** to generate **21** in very high yield. Standard hydrogenation reaction for **21** failed to provide the saturated intermediate **22**. Sodium acetate and *p*-toluenesulfono hydrazide were used to chemoselectively reduce the olefin of **21** without reducing the adjacent cyclopropyl ring. *p*-Toluenesulfonic acid mediated removal of the acetonide protection of intermediate **22** and subsequent TEMPO-catalyzed oxidation of **23** with bleach[18] led to aldehyde **24** which was immediately subjected to the Passerini reaction conditions.[19] Thus, use of cyclopropylisonitrile and acetic acid in DCM generated a diastereomeric mixture of acetamides. Hydrolysis of the acetate moiety using potassium carbonate in MeOH followed by HCl-mediated removal of the Boc protecting group yielded desired α-hydroxy-β-aminocyclopropylamides **8d** in 47% overall yield. Norvaline **8a**, Cyclopropylalanine **8b** and Norleucine **8c** were prepared in a similar fashion starting from the corresponding aldehyde **25**, **26** and **27** or by following reported procedures.[12b,20]

3. Discussion

Inhibitors listed in Tables 1 and 2 were tested in continuous assay using the NS4A-tethered single chain NS3 serine protease.[21]



Scheme 4. Reaction conditions: (a) cyclopropylmethyltriphenylphospho-nium bromide, KHMDS, THF, −78 °C, 96%; (b) Tos-NH–NH_2, dimethoxyethane, sodium acetate, H_2O, 90 °C, 4 h, 95%; (c) MeOH, TsOH cat, 95%; (d) NaOCl (5%, 1.1 equiv), LiBr (0.076 equiv), $NaHCO_3$ (1.4 equiv), TEMPO (0.014 equiv), EtOAc, 0 °C; (e) (i) cyclopropylisonitrile, AcOH, DCM, (2–1 ratio); (ii) K_2CO_3, MeOH, H_2O; (iii) 4 M HCl/Dioxane, 0 °C to rt.

HCV replicon inhibitory activity for the targets synthesized was obtained using previously reported assay.[22] To address the issue of selectivity among serine proteases, inhibitors were assayed against human neutrophil elastase (HNE), a serine protease with most structural similarity to HCV NS3 serine protease. The ratio of activity in these two proteases (HNE/HCV) was used as a measure of the selectivity. As a general trend, previous compounds had GAPDH CC_{50} >5 μM when tested in counter screen measuring cellular toxicity. Thus, cytotoxicity was never an issue for this class of inhibitors, and GAPDH was not measured for most recent compounds. We evaluated our initial set of P_4 extended inhibitors **28**–**45** listed in Table 1 by varying the R^2, R^3 and R^5 moieties. As discussed before, the P_4 was maintained as a spirocyclohexyl residue since we had previously observed that the use of 1-methyl-cyclohexyl urea as a P_4 capping group in inhibitor **2** improved the replicon activity by 18-fold compared to **1**. *tert*-Leucine moiety which was previously optimized as a P_3 surrogates and the *gem*-dimethylcyclopropylproline P_2 were used as the substituents of choice in these positions during that entire study.[12b] 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.[12b] For the initial set of compound, we decided to focus on single diastereomer and keep the P_1 constant as norvaline. From our previous experience, we were aware of the fact that the P_1 norvaline was not the optimal substitution for potency and selectivity over HNE but as a readily available fragment we used it for the initial studies.[12b] Finally, based on recently published results from our laboratories,[14] we choose to use the P'_1 cyclopropyl amide capping of compound **3** and maintained it for the entire study.

Modeling studies suggested that there was ample room for the P_4 cap to be extended beyond the spirocyclohexyl group. The Cys159 amino acid residue of the protein backbone in the S4 region was identified as an opportunity for additional hydrogen bonding

interaction. We envisioned that extended inhibitors having sulfonyl or carbonyl groups at the P_4 position could engage in a hydrogen bonding with Cys159. Recent results from our laboratories confirmed our hypothesis.[23] In the course of our extensive search for the optimal functionality to establish the hydrogen bond, we discovered that secondary methylsulfonamide **28** (Table 1, K_i^* = 15 nM, EC_{90} = 300 nM), had enzyme and cellular potency similar to that of **1**. The activity demonstrated by compound **28** prompted us to further investigate the SAR of various P_4 residues. Compounds prepared to evaluate this effect are outlined in Table 1. Dimethylsulfonamide **29** (EC_{90} = 55 nM) established that tertiary sulfonamides provided enhanced cellular potency (sixfold improvement vs **28**). The rat, dog and monkey pharmacokinetic properties of inhibitor **29** were investigated and the data is summarized in Table 1. Although we were glad to see excellent exposure in dog (6.1 μM h), oral area-under-curve (PO AUC) level in rat and monkey were similar to **1** and needed improvement for a second generation compound. Modifications of the dimethyl sufonamide moiety with larger substituents were explored. Thus, incorporation of an ethyl, isopropyl and *tert*-butyl group in sulfonamides **30**, **31** and **32** resulted in similar enzyme and cellular activity but lower exposure in dog compared to **29**. Fluorinated sulfonamide **33** was prepared with the aim of improving the pharmacokinetic profile of compound **32**. Unfortunately, while improving the ezyme potency (K_i^* = 2.8 nM) and retaining good cellular potency (EC_{90} = 45 nM), rat and dog exposure of fluorinated sulfonamide **33** was lower compared to **32**. Cyclic substituents (Table 1, entry **34** and **35**) were investigated and results obtained with cyclopropyl sulfonamide **34** were encouraging. Although the potency profile of compound **34** (Table 1, K_i^* = 8 nM, EC_{90} = 55 nM) was similar to **29**, this cyclopropyl *N*-methyl sulfonamide capped inhibitor exhibited for the first time good oral exposure in all three species with tenfold exposure enhancement in monkey compared to **1**. A compound with larger ring size was prepared but the analog **35**, with a cyclobutyl substituent (Table 1, EC_{90} = 90 nM) was less potent in the cellular assay and displayed poorer PK in rat and dog compared to the cyclopropyl counterpart **34**. The improved overall profile demonstrated by compound **34** in comparison to **1** prompted us to further investigate the SAR of this cyclopropyl *N*-methyl sulfonamide P_4 residue. The bulkier cyclopropylmethyl group in inhibitor **36** (K_i^* = 9 nM, EC_{90} = 135 nM) was detrimental to the cell activity. We further probed the P_4 cyclopropyl *N*-methyl sulfonamide series and we discovered that incorporation of small groups was tolerated. Thus, cyclopropyl *N*-ethyl sulfonamide **37** was prepared and showed similar potency profile to the cyclopropyl *N*-methyl sulfonamide analog **34**. Moreover, the HNE selectivity seemed improved for that series with HNE/HCV = 131. Unfortunately, while maintaining good exposure in rat and dog, **37** completely lacked oral exposure in monkey. Incorporation of the larger isopropyl group led to inhibitor **38** (K_i^* = 6 nM, EC_{90} = 100 nM) that resulted in loss of cellular activity compared to **34** (EC_{90} = 55 nM). Cyclic amines (Table 1, inhibitors **39**, **40**) were also studied. Cyclobutylsulfonamide **39** (Table 1, K_i^* = 7 nM, EC_{90} = 80 nM) had a similar potency profile when compared to the simple dimethylsulfonamide **29** but did not show exposure in dog after oral administration. Inhibitor **40** bearing a morpholine sulfonamide moiety with potentially better solubility properties was also prepared. Unfortunately, although the cellular potency of compound **40** (Table 1, K_i^* = 8 nM, EC_{90} = 45 nM) was improved, no enhancement of oral exposure in rat or dog was observed.

To further probe the P_4 region we also envisioned substitution onto the methylene spacer between the sulfonamide and the spirocyclohexyl groups. Thus, the diastereoselective synthesis of this class of chiral sulfonamide was initiated. As a direct comparison with our earlier dimethylsulfonamide lead **29**, we prepared both *R*- and *S*- diastereomer **41** and **42**. The *R* stereochemistry seemed to be essential for activity as inhibitor **41** (K_i^* = 2 nM) was tenfold more potent than inhibitors **42** (K_i^* = 22 nM), the correspondent (*S*) isomer. However, when compared to dimethylsulfonamide **29** (EC_{90} = 55 nM), substitution onto the methylene spacer between the sulfonamide and the spirocyclohexyl groups resulted in lower cellular potency (Table 1, entry **41**, EC_{90} = 85 nM) and much lower exposure in dog. Tethering of the methyl group was envisioned to access a more rigid cyclic core. The (*R*) diastereomers were prepared since we had established earlier that this is the desired stereochemistry requirement. We discovered that the incorporation of the five-membered cyclic sulfonamide provided inhibitor **43** with more than fourfold improvement in cell potency (EC_{90} = 20 nM, HNE/HCV = 340) compared to **41** and good elastase selectivity. Inhibitor **43** was the first of its class to show such enhanced activity in the replicon assay. Based on our earlier SAR finding, we incorporated the cyclopropyl moiety of our best inhibitor **34** into the cyclic sulfonamide series. Thus, compound **44** was prepared and showed excellent cellular potency as well (Table 1, EC_{90} = 20 nM). The pharmacokinetic properties of inhibitor **43** and **44** were investigated and the data is listed in Table 1. Unfortunately, both compounds showed poor exposure in rat after oral administration and efforts in this series were discontinued.

Having established that the cyclopropyl *N*-methyl sulfonamide moiety as the optimal substitution for the acyclic sulfonamide series, we turned our effort toward improving the elastase selectivity of inhibitor **34**. During our earlier exploratory studies that led to the discovery of **1**, we observed that the P_1 norvaline was not the optimal substitution for HNE/HCV selectivity.[12b] Cyclopropyl alanine analog **45** and nor-leucine analogs **46** were prepared and provided improvements in elastase selectivity. However, it was clear from the data presented in Table 2 that introduction of homocyclopropylalanine at P_1 had a profound effect on the HNE/HCV selectivity while maintaining cellular potency of compound **47** (K_i^* = 6 nM, EC_{90} = 75 nM) when compared to inhibitor **34**. Furthermore, **47** exhibited not only good oral exposure in rat (10 mpk, AUC $(\mu M\ h)_{0-6h}$ = 0.9) and dog (2 mpk, AUC $(\mu M\ h)_{0-6h}$ = 4) but in monkey as well (10 mpk, AUC $(\mu M\ h)_{0-6h}$ = 5.4).

To illustrate the interactions of the P_4 cap with the protein, Figure 2 shows compound **47** modeled in the active site of the NS3/NS4A protease domain. The overall binding mode of the P1–P4 residues would be as expected predefined by the backbone hydrogen bonding network between the inhibitor and the protein. The P_4 spirocyclohexyl of compound **47** was found to bind to the

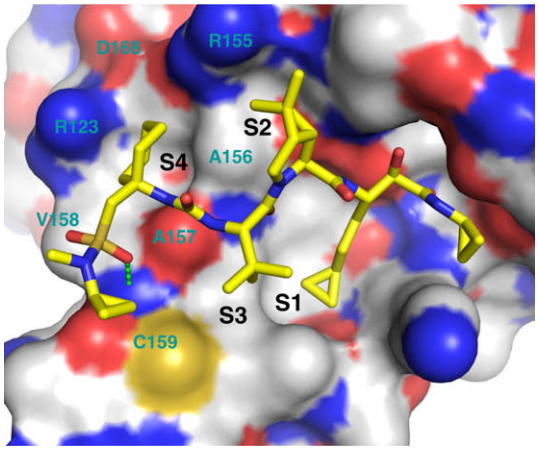


Figure 2. Model of **47** bound to the active site of HCVNS3/NS4A protease domain. The inhibitor is shown as stick model (yellow carbon) and the protein as surface (gray carbon). The S1–S4 subsites and the residues that occupy the S4 pocket as well as Cys 159 are labeled. The hydrogen bond between one of the sulfone oxygens and the NH of Cys159 is indicated (green dashed line).

S4 subsite, a shallow hydrophobic pocket consisting of residues Arg 123, Asp 168, Arg 155, Ala 156 and Val 158. For the P_4 cap, one of the sulfone oxygens was found to make a hydrogen bond with the backbone NH of Cys159. The cyclopropyl group off the sulfonamide moiety also makes hydrophobic contacts with the sidechain of Cys159. The favorable hydrophobic contacts of the P_4 cyclopropyl cap with the side chain of Cys159 and the additional hydrogen bond with the backbone NH of Cys159 may attribute to the enhancement in enzyme potency of compound **47** compared with **1**.

4. Conclusion

As we moved towards the discovery of a second generation HCV protease inhibitor, we discovered that P_4-capped inhibitors could provide enhanced enzyme and cellular potency. Initially, we observed that the use of 1-methyl-cyclohexyl urea as a P_4 capping group in inhibitor **2** was well tolerated and could lead to improvement in cellular potency. Based on modeling studies, we envisioned that extended inhibitors having sulfonyl or carbonyl groups at the P_4 position could engage in a hydrogen bonding with Cys159. We discovered that the use of sulfonamides moieties at P_4 provided potent inhibitors with good pharmacokinetics properties. Derivatization of the sulfonamide via systematic optimization of the substituents led to the identification of the cyclopropyl methyl sulfonamide that generated compound **34** with excellent enzyme potency, improved replicon activity and the best overall exposure in rat, dog and monkey. Optimization of the P_1 norvaline residue resulted in the identification of homocyclopropylalanine P_1 fragment that, when used in combination with cyclopropyl amide P_1' capping, provided compound **47** with excellent HNE/HCV selectivity, much improved cellular potencies over **1** and good oral exposure in three preclinical species.

5. Experimental

5.1. Molecular modeling

To model compound **47** 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 sidechains and terminal caps corresponding to **47** were built. From the crystal structure, the backbone atoms form a network of hydrogen bonds with the protein, therefore predefine the backbone orientation of the compounds. The sidechain 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.

5.2. General

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. The chemical shifts are given in ppm, referenced to the internal TMS or deuterated solvent signal.

General experimental procedures were described in earlier publications[12b] for the synthesis of P2 core, peptide coupling, Boc group deprotection, hydrolysis of ester to carboxylic acid and Dess–Martin periodinane oxidation.

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 micron, 50 mm × 4.6 mm ID, 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.

5.2.1. (1*R*,2*S*,5*S*)-3-((*S*)-2-(3-(1-((*N*-Cyclopropyl-*N*-methylsulfamoyl)methyl)cyclohexyl)ureido)-3,3-dimethylbutanoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxylic acid (6; R_3 = c-Pr, R_2 = Me, R_4 = H)

To a 0 °C DMF (50 mL) solution of (1*R*,2*S*,5*S*)-methyl 3-((*S*)-2-amino-3,3-dimethylbutanoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxylate hydrochloride **4**[12b] (10 mmol, 3.18 g) was added isocyanate **14** (0.26 M in DCM, 12 mmol, 47 mL) followed by DIPEA (60 mmol, 10.2 mL). The reaction was warmed-up to rt and left stirring overnight. The reaction mixture was diluted with DCM and washed twice with HCl (1.0 M) followed by brine. The organic layer was dried over $MgSO_4$, filtered and concentrated down to yield 5.96 g of (1*R*,2*S*,5*S*)-methyl 3-((*S*)-2-(3-(1-((*N*-cyclopropyl-*N*-methylsulfamoyl)methyl)cyclohexyl)-ureido)-3,3-dimethylbutanoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxylate as a brown foam that was immediately dissolved in THF (50 mL). To this solution was added at 0 °C aqueous LiOH (11.5 equiv, 16.2 mL, 1.0 M). The reaction was warmed-up to rt and left stirring overnight. The product was then evaporated to dryness and NaOH (1.0 N, 50 mL) and Et_2O (50 mL) were added. The phases were separated and the aqueous phase was acidified with 6 N HCl to pH of 1.5. The product was extracted with EtOAc and washed with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated down to yield 4.8 g (88% over 2 steps) of (1*R*,2*S*,5*S*)-3-((*S*)-2-(3-(1-((*N*-cyclopropyl-*N*-methylsulfamoyl)methyl)cyclohexyl)ureido)-3,3-dimethylbutanoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxylic acid (**6**; R_3 = c-Pr, R_2 = Me, R_4 = H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.24 (d, J = 9.6 Hz, 1H), 6.00 (br s, 1H), 4.18 (d, J = 9.6 Hz, 1H), 4.07 (s, 1H), 3.91 (d, J = 9.6 Hz, 1H), 3.76–3.68 (m, 2H), 3.59–3.54 (m, 1H), 2.68 (s, 3H), 2.32–2.82 (m, 1H), 2.19–2.06 (m, 2H), 1.55–1.35 (m, 8H), 1.36 (d, J = 7.6 Hz, 1H), 1.20 (d, J = 6.4 Hz, 1H), 0.98 (s, 3H), 0.91 (s, 9H), 0.78 (s, 3H), 0.65–0.59 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.1, 171.2, 157.2, 66.8, 59.2, 56.7, 53.5, 53.3, 47.4, 37.0, 34.9, 34.4, 31.5, 30.3, 27.2, 26.7, 26.2, 25.4, 21.1, 19.1, 12.7, 7.5, 7.4. HRMS calcd for $C_{26}H_{45}N_4O_6S$: 541.3059 $(M + H)^+$. Found: 541.3045.

5.2.2. (*S*)-3-Amino-*N*,5-dicyclopropyl-2-hydroxypentanamide hydrochloride (7d)

To a 0 °C solution of alcohol **23** (30 g, 131 mmol) in EtOAc (250 mL) was added LiBr (0.076 equiv, 10 mmol, 870 mg) followed by $NaHCO_3$ (14 g, 184 mmol) in water (140 mL) and TEMPO (0.014 equiv, 1.84 mmol, 300 mg). Bleach (5%, 1.1 equiv, 150 mol, 200 mL) was added at a rate of about 10 mL/min .At the end of addition, TLC analysis indicated complete conversion. 250 mL of a ~1 M $Na_2S_2O_3$ solution was added and stirring was maintained for 10 min. Layers were separated and EtOAc layer was washed with $NaHCO_3$ then brine. Organic layer (~500 mL of EtOAc) was dried over MgSO4, filtered and processed directly to the next step without further isolation. To a 0 °C solution of crude aldehyde **24** (131 mmol) in EtOAc (~500 mL) was added acetic acid (1.2 equiv, 157 mmol, 9 mL) followed by cyclopropylisonitrile (1.05 equiv, 138 mmol, 11.5 mL). The mixture was stirred at to rt for 18 h then concentrated to dryness to provide (*S*)-3-(*tert*-butoxycarbonylamino)-5-cyclopropyl-1-(cyclopropylamino)-1-oxopentan-2-yl acetate as a (1/0.8) mixture of two diastereomers. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 and 7.95 (d, J = 3.6 Hz, 1H), 6.74 and 6.54 (d, J = 9.2 Hz, 1H), 4.97 and 4.74 (d, J = 4.8 Hz, 1H), 3.95–3.85 (m, 1H), 2.73–2.59 (m, 1H), 2.13 and 2.10 (s, 3H), 1.52–1.35 (m, 11H), 1.27–1.13 (m, 2H), 0.69–0.61 (m, 3H), 0.47–0.37 (m, 4H), 0.03–(−)0.02 (m, 2H). MS (ESI) 377[$(M+Na)^+$, 10], 355 (20), 299 (30), 237 (100). HRMS calcd for $C_{18}H_{31}N_2O_5$: 355.2233 $(M+H)^+$. Found: 355.2223. To a 1L flask containing (*S*)-3-(*tert*-butoxycarbonylamino)-5-cyclopropyl-1-(cyclopropylamino)-1-oxopentan-2-yl acetate (131 mmol) was added MeOH/H_2O (300 mL each) and K_2CO_3 (327 mol, 45 g). Reaction was stirred at rt for 2 h. TLC

analysis (60/40/DCM/EtOAc) indicated complete conversion. The reaction mixture was concentrated under vacuum to remove methanol then the aqueous solution was extracted with EtOAc (200 mL). EtOAc layer was washed with HCl (1 N), then brine and was dried over $MgSO_4$, filtered and concentrated down to yield (*S*)-*tert*-butyl 5-cyclopropyl-1-(cyclopropylamino)-2-hydroxy-1-oxopentan-3-ylcarbamate as a (1/0.8) mixture of two diastereomers (35 g, 77% over three steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 and 7.69 (br s, 1H), 6.37 and 6.01 (d, J = 8.8 Hz, 1H), 5.49 and 5.32 (d, J = 6.0 Hz, 1H), 3.82–3.75 (m, 1H), 2.70–2.54 (m, 1H), 1.55–1.35 (m, 2H), 1.33 (br s, 9H), 1.28–1.08 (m, 2H), 0.65–0.50 (m, 3H), 0.44–0.42 (m, 2H), 0.35–0.29 (m, 2H), 0.04–(−)0.07 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.9, 173.5, 155.6, 155.5, 77.9, 74.0, 72.9, 53.2, 53.1, 31.7, 31.2, 31.1, 28.7, 28.6, 22.5, 22.4, 11.0, 6.1, 6.0, 5.0, 4.9, 4.6, 4.4 MS (ESI) 335[(M+Na)$^+$, 10], 313 (20), 213 (100). HRMS calcd for $C_{18}H_{29}N_2O_4$: 313.2127 (M+H)$^+$. Found: 313.2123. To (*S*)-*tert*-butyl 5-cyclopropyl-1-(cyclopropylamino)-2-hydroxy-1-oxopentan-3-ylcarbamate (30 g) was added HCl dioxane (4.0 N, 10 mL per g, 300 mL) and the reaction was stirred at high speed. After 15 min, TLC analysis indicated complete conversion. Et_2O (1 L) was added and stirring was maintained for 10 min. The resulting homogenous slurry was filtered off and dried under vacuum to provide (*S*)-3-amino-*N*,5-dicyclopropyl-2-hydroxypentanamide hydrochloride **7d** as a (1/0.8) mixture of two diastereomers (24.5 g, 100%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 and 8.09 (d, J = 4.4 Hz, 1H), 8.03–8.01 and 7.89–7.80 (m, 2H), 6.39 and 6.27 (br s, 1H), 4.16 and 3.97 (br s, 1H), 2.73–2.58 (m, 1H), 1.75–1.43 (m, 2H), 1.28–1.12 (m, 2H), 0.64–0.57 (m, 3H), 0.53–0.46 (m, 2H), 0.39–0.35 (m, 2H), 0.02–(−)0.05 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 172.3, 71.1, 70.0, 66.8, 53.3, 53.1, 30.2, 30.1, 29.4, 27.8, 22.7, 22.6, 11.0, 10.9, 6.0, 5.9, 5.8, 5.7, 4.8, 4.7, 4.6, 4.5. MS (ESI) 335[(M+Na)$^+$, 10], 313 (20), 213 (100). HRMS calcd for $C_{11}H_{21}N_2O_2$: 213.16030 (M+H)$^+$. Found: 213.1606.

5.2.3. 1-Iodomethyl-cyclohexanecarboxylic acid methyl ester (10)

To a −78 °C of freshly prepared LDA solution (120 mmol, 75 mL) was added a THF solution (50 mL) of cyclohexane carboxylic acid methyl ester **1** (15.57 g, 109 mmol). After 1 h, diiodomethane (109 mmol, 8.78 mL) was added while keeping the internal temperature below 10 °C. After addition, the reaction was warmed to rt and stirred overnight. After a total of 18 h, the reaction was quenched with a saturated NH_4Cl solution and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated down to provide 1-Iodomethyl-cyclohexanecarboxylic acid methyl ester **10** (35 g, 100%) that was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.72 (s, 3H), 3.31 (s, 2H), 2.14–2.10 (m, 2H), 1.59–1.32 (m, 8H).

5.2.4. 1-Acetylsulfanylmethyl-cyclohexanecarboxylic acid methyl ester (11)

To 0 °C solution of crude **10** (109 mmol) in DMF (90 ml) was added Potassium Thioacetate (1.2 equiv, 131 mmol, 15 g) while maintaining the temperature below 30 °C. The reaction was stirred for 18 h at rt then cooled to 10 °C. Water (160 mL) was added and the reaction mixture was extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ then brine. The organic layer was dried over MgSO4, filtered and concentrated down to a dark oil. SiO_2 column purification (Biotage, 75+M) using 1% to 5% EtOAc in Hexanes provided 1-acetylsulfanylmethyl-cyclohexanecarboxylic acid methyl ester **11** (43 g, 81% yield over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.67 (s, 3H), 3.14 (s, 2H), 2.32 (s, 3H), 2.05–2.01 (m, 2H), 1.60–1.29 (m, 8H). MS (ESI) 304[(M+Na)$^+$, 5], 210 (100).

5.2.5. 1-Chlorosulfonylmethyl-cyclohexanecarboxylic acid methyl ester (12)

To a stirred solution of thioester **11** (53 mmol, 12.23 g) in acetic acid (55 mL) was added water (212 mmol, 3.8 g). Then, Chlorine gas (11.3 g, 159 mmol) was bubbled lightly through for about 15–20 min. The internal temp rose to 55 °C and the reaction was cooled down. TLC analysis indicated complete conversion to a more polar material. Reaction was diluted in DCM, washed with water (twice), then NaOH (0.5 N) and brine. DCM layer was dried over $MgSO_4$, filtered and concentrated down to yield 1-chlorosulfonylmethyl-cyclohexanecarboxylic acid methyl ester **12** obtained as a light yellow oil (12 g, 90% yield) that was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.16 (s, 2H), 3.76 (s, 3H), 2.16–2.13 (m, 2H), 1.67–1.10 (m, 8H); MS (ESI) 253[(M+Na)$^+$, 100].

5.2.6. 1-[(Cyclopropyl-methyl-sulfamoyl)-methyl]-cyclohexanecarboxylic acid methyl ester (13; R_3 = c-Pr, R_2 = Me)

To a rt solution of DCM (10 mL) containing sulfonyl chloride **12** (4 mmol, 1 g) was added cyclopropyl amine (1.5 equiv, 6 mmol, 0.36 g) followed by Et_3N (2 mL). After 5 h, reaction was poured into EtOAc and washed with $NaHCO_3$, HCl 1.0 N and brine. Organic layer was dried over $MgSO_4$, filtered and concentrated down to yield methyl 1-(*N*-cyclopropylsulfamoylmethyl)cyclohexanecarboxylate intermediate (1.28 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.68–4.65 (m, 1H), 3.76 (s, 3H), 3.41 (s, 2H), 2.56–2.54 (m, 1H), 2.16–2.13 (m, 2H), 1.67–1.10 (m, 8H), 0.73–0.71 (m, 4H). MS (ESI) 298[(M+Na)$^+$, 100]. To a 0 °C solution of methyl 1-(*N*-cyclopropylsulfamoylmethyl)cyclohexanecarboxylate (4 mmol) in DMF (10 mL) was added Cs_2CO_3 (1.5 equiv, 6 mmol, 2 g) followed by MeI (1.8 equiv, 7.2 mmol, 0.45 mL). Reaction was stirred overnight then diluted with EtOAc, washed with water (2 × 50 mL), NH_4Cl and brine. Organic layer was dried over $MgSO_4$, filtered and concentrated down to 1-[(cyclopropyl-methyl-sulfamoyl)-methyl]-cyclohexanecarboxylic acid methyl ester **13** obtained as a light yellow oil (1.15 g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.60 (s, 3H), 3.37 (s, 2H), 2.40–2.38 (m, 1H), 1.97–1.92 (m, 2H), 1.58–1.30 (m, 8H), 0.66–0.64 (m, 4H). MS (ESI) 312[(M+Na)$^+$, 100].

5.2.7. *N*-Cyclopropyl-C-(1-isocyanato-cyclohexyl)-*N*-methyl-methanesulfonamide (14; R_3 = c-Pr, R_2 = Me)

To a rt solution of MeOH (30 mL) containing ester **13** (R_3 = c-Pr, R_2 = Me; 4 mmol, 1.1 g) was added aquous KOH (17.6 mL, 61.7 mmol) and the reaction was brought to 55 °C for 18 h. Volatile were removed under vacuum and residue was acidified to Ph 4 with HCl 1.0 N. Reaction was diluted with EtOAc and washed with water (2 × 20 mL) and brine. Organic layer was dried over $MgSO_4$, filtered and concentrated down to give 1-[(cyclopropyl-methyl-sulfamoyl)-methyl]-cyclohexanecarboxylic acid (1.09 g, 96%). To the crude acid (3.96 mmol, 1.09 g) in toluene (15 mL) was added DPPA (0.85 mL, 3.96 mmol) followed by Et_3N (0.56 mL, 3.96 mmol). The reaction was stirred at rt for 10 min then refluxed for 2 h. Reaction was cooled down to rt and diluted with DCM (50 mL). The organic layer was washed with $NaHCO_3$, brine and dried over $MgSO_4$. The volatile were removed and the residue was diluted with DCM (15 mL) to give *N*-cyclopropyl-C-(1-isocyanato-cyclohexyl)-*N*-methyl-methanesulfonamide **14** that was used as a 0.26 M solution in DCM.

5.2.8. 2-Methyl-*N*-[1-(2-methyl-1,1-dioxido-5(*R*)-isothiazolidinyl]cyclohexyl)-2-propane-(*S*)-sulfinamide (16, R_3 = Me, R_2 = R_1 = -CH_2)

n-Butyllithium (1.3 equiv, 6.6 mL of a 2.5 M soln in hexanes) was added dropwise to a cooled (−78 °C) solution of *N*-methyl sultam **16** (1.35 equiv, 2.32 g) in 100 mL of dry THF under anhydrous atomosphere. The mixture was stirred for 30 min at that

temperature and then transferred via cannula to a solution of sulfinyl imine (2.56 g) in 100 mL of dry THF at −78 °C. After addition was completed, the reaction mixture was stirred for 30 min. The reaction was quenched at −78 °C by addition of 20 mL of aqueous saturated ammonium chloride solution. The mixture was allowed to reach room temperature and then partitioned between ethyl acetate (200 mL) and aqueous saturated sodium bicarbonate solution (50 mL). The aqueous layer was back extracted with ethyl acetate (2 × 150 mL). The combined organic layers were washed with brine (80 mL), dried over magnesium sulfate, filtered and concentrated. The residue was chromatographed on silica gel (gradient: dichloromethane/hexanes; 1:1 to 30% acetone in dicloromethane/hexanes; 1:1) to afford the *R,S*-isomer **17** (660 mg; 15.4%). ^{1}H NMR ($CDCl_3$; 500 MHz) δ 4.87 (1H, s), 3.75 (1H, *J* = 9.4 Hz, t), 3.12 (1H, ddd, *J* = 3.5 & 8.2 & 9.5 Hz), 3.04 (1H, m), 2.65 (3H, s), 2.27 (2H, m), 2.19 (1H, *J* = 3.5 & 7.3 & 9.1 & 12.6 Hz, dddd), 2.09 (1H, *J* = 3.8 & 10.1 & 13.6 Hz, ddd), 2.02 (1H, *J* = 3.8 & 10.1 & 13.9 Hz, ddd), 1.73 (2H, m), 1.61 (1H, m), 1.54 (2H, m), 1.37 (2H, m), 1.23 (9H, s). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 61.5, 58.4, 56.2, 46.6, 36.0, 32.6, 30.4, 24.8, 22.9, 22.0, 21.5, 20.3. LRMS (ESI) calcd for $C_{14}H_{29}N_2O_3S_2$ $[M+H]^+$: 337.16, found 336.97.

5.2.9. 5(*R*)-(1-Isocyanatocyclohexyl)-2-methylisothiazolidine 1,1-dioxide (18, R_3 = Me, R_2 = R_1 = -CH_2)

The *R,S*-sulfinamide **16** (R_3 = Me, R_2 = R_1 = –CH_2; 640 mg, 1.901 mmol) was dissolved in 40 mL of methanol and treated with 10 mL of 4 M HCl solution in dioxane. The mixture was stirred for about 30 min until all the starting material had been consumed as determined by TLC (40% acetone in hexanes). The mixture was evaporated to dryness. Dichloromethane was added (10 mL) to make a cloudy solution. Upon addition of 80 mL of ether a white precipitated formed. The mixture was placed in a cooling bath (0 °C) for 15 min and then the solids were recovered by filtration to afford 1-(2-methyl-1,1-dioxido-5(*R*)-isothiazolidinyl)cyclohexanamine hydrochloride (510 mg, 99%) as a white powder. LRMS (ESI) calcd for $C_{10}H_{21}N_2O_2S$ $[M+H]^+$: 233.13, found 232.95. A solution of amine hydrochloride (510 mg, 1.897 mmol) in 40 mL of dichloromethane was treated with 20 mL of aqueous saturated sodium bicarbonate solution and stirred vigorously for 10 min at 0 °C. Stirring was stopped and layers were allowed to separate. Phosgene (10 mL of 20% soln in toluene) was added through a needle to the organic layer (lower layer) in one portion. The mixture was vigorously stirred immediately after addition for 20 min at 0 °C and further stirred at room temp for 3 h. The mixture was diluted with 100 mL of dichloromethane and layers were separated. The organic layer was washed with 50 mL of cold aqueous saturated sodium bicarbonate solution and dried over magnesium sulfate. The organic layer was filtered and diluted with 15 mL of toluene. The resulting solution was concentrated under reduced pressure and the product was used as a 0.126 M solution in toluene.

5.2.10. (*S*)-*tert*-Butyl 4-(2-cyclopropylvinyl)-2,2-dimethyloxazolidine-3-carboxylate (21)

To a −78 °C solution of Cyclopropylmethyl triphenylphosphonium bromide (1.5 equiv, 40 mmol, 15.9 g) in THF (180 mL) was added KHMDS (1.4 equiv, 36.7 mmol, 73.4 mL). The resulting orange solution was warmed-up to rt for 1 h then cooled down to −78 °C. To the above solution was added a solution of D-Gardner aldehyde **20** (1 equiv, 26.2 mmol, 6 g) in THF (20 mL). After reaction was completed by TLC, methanol (200 mL) was added at −78 °C and the reaction was gradually warmed-up to rt over 1 h. Reaction was diluted with EtOAc then washed with water and brine. Organic layer was dried over $MgSO_4$, filtered and concentrated down. Ph_3PO was filtered trough a pad of celite and the filtrate was concentrated to dryness to provide 7.2 g of **21**.^{1}H NMR (400 MHz, $CDCl_3$) δ 5.51–5.30 (m, 1H), 5.15–4.70 (m, 2H), 4.10 and 4.00 (dd, *J* = 8.9 & 6.0 Hz, 1H), 3.73–3.70 (m, 1H), 1.59–1.45 (m, 16 H), 0.80–0.68 (m, 2H), 0.42–0.28 (m, 2H). MS (ESI) 290[$(M+Na)^+$, 5], 212 (70), 168 (30).

5.2.11. (*S*)-*tert*-Butyl 4-(2-cyclopropylethyl)-2,2-dimethyloxazolidine-3-carboxylate (22)

To a solution of **21** (22.5 mmol, 6 g) and *p*-toluenesulfono hydrazide (225 mmol, 42 g) in 200 mL of dimethoxyethane warmed to reflux was added dropwise a solution of sodium acetate (375 mmol, 52.5 g) in water (300 mL) over 4 h. After the addition, reaction was cooled down to rt and poured into 200 mL of water and extracted twice with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated down to afford (*S*)-*tert*-butyl 4-(2-cyclopropylethyl)-2,2-dimethyloxazolidine-3-carboxylate **22** obtained as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92–3.80 (m, 2H), 3.72 (d, *J* = 7.60 Hz, 1H), 1.91–1.60 (m, 2H), 1.59 (s, 3H), 1.54 (s, 3H), 1.48 (s, 9H), 1.25–1.15 (m, 2 H), 0.67–0.65 (m, 1H), 0.42–0.40 (m, 2H), 0.01–0.00 (m, 2H). MS (ESI) 292[$(M+Na)^+$, 20], 214 (40), 170 (100).

5.2.12. (*S*)-*tert*-Butyl 4-cyclopropyl-1-hydroxybutan-2-ylcarbamate (23)

To a stirred solution of acetonide **22** (6 g, 22 mmol) in MeOH (120 mL) was added 1 mol% of TsOH (2.2 mmol, 418 mg). After 30 min, TLC analysis indicated completion. Reaction was diluted with EtOAc and washed with water, $NaHCO_3$ and brine. Organic layer was dried over $MgSO_4$, filtered and concentrated down to a viscous oil. The crude product was purified on Biotage (40+M, 10 to 40% EtOAc in DCM) to provide (*S*)-*tert*-butyl 4-cyclopropyl-1-hydroxybutan-2-ylcarbamate **23** (4.84 g, 96%).^{1}H NMR (400 MHz, $CDCl_3$) δ 4.58 (br s, 1H), 3.69–3.67 (m, 2H), 3.54–3.52 (m, 1H), 1.65–1.49 (m, 2H), 1.45 (s, 9H), 1.30–1.15 (m, 2 H), 0.68–0.65 (m, 1H), 0.45–0.42 (m, 2H), 0.07–0.00 (m, 2H). MS (ESI) 252[$(M+Na)^+$, 70], 174 (30), 130 (100).

5.2.13. (1*R*,5*S*)-*N*-[1(*S*)-[2-(Cyclopropylamino)-1,2-dioxoethyl]-butyl]-3-[3,3-dimethyl-2(*S*)-[[[[1-[[(methylamino)sulfonyl]-methyl]cyclohexyl]amino]carbonyl]amino]-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (28)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.72 (d, *J* = 4.7 Hz, 1H), 8.38 (d, *J* = 7.0 Hz, 1H), 6.68 (d, *J* = 4.7 Hz, 1H), 6.22 (d, *J* = 9.4 Hz, 1H), 5.96(s, 1H), 5.00–4.91 (m, 1H), 4.29 (s, 1H), 4.16 (d, *J* = 10.9 Hz, 2H), 3.91 (d, *J* = 10.9 Hz, 1H), 3.79–3.70 (m, 1H), 3.56 (d, *J* = 14.1 Hz, 1H), 3.30 (d, *J* = 14.8 Hz, 1H), 2.79–2.69 (m, 1H), 2.51(s, 3H), 2.17 (d, *J* = 11.7 Hz, 1H), 2.07 (d, *J* = 12.5 Hz, 1H), 1.75–1.13 (m, 13H), 0.99 (s, 3H), 0.98–0.96 (m,1H), 0.89–0.75 (m, 14H), 0.66–0.62 (m, 2H), 0.59–0.55 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.1, 171.1, 170.4, 161.9, 156.8, 67.3, 58.9, 56.5, 53.4, 52.7, 38.0, 34.3, 34.2, 33.9, 33.5, 31.6, 28.6, 28.3, 26.2, 26.1, 22.4, 22.3, 20.5, 18.7, 18.4, 13.8, 13.4, 12.4, 10.7, 5.3 ppm. HRMS (ESI) calcd for $C_{37}H_{60}N_6O_7S$ $[M+H]^+$: 667.3866, found 667.3865.

5.2.14. (1*R*,5*S*)-*N*-[1(*S*)-[2-(Cyclopropylamino)-1,2-dioxoethyll]-butyl]-3-[2(*S*)-[[[[1-[[(dimethylamino)sulfonyl]methyl]cyclo-hexyl]amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (29)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (d, *J* = 5.5 Hz, 1H), 8.36 (d, *J* = 7.0 Hz, 1H), 6.62 (d, *J* = 9.4 Hz, 1H), 5.99 (s, 1H), 4.94–4.91 (m, 1H), 4.27 (s, 1H), 4.16 (d, *J* = 9.4 Hz, 1H), 3.81 (d, *J* = 10.2 Hz, 1H), 3.73–3.69 (m, 1H), 3.56 (d, *J* = 14.1 Hz, 1H), 3.27 (d, *J* = 14.1 Hz, 1H), 2.75–2.69 (m, 1H), 2.65 (s, 6H), 2.16 (d, *J* = 10.2 Hz, 1H), 2.07 (d, *J* = 10.2 Hz, 1H), 1.70–1.65 (m, 1H), 1.49–1.27 (m, 14H), 0.98 (s, 3H), 0.87 (br s, 9H), 0.86–0.83 (m, 1H), 0.803 (s, 3H), 0.80–0.76 (m, 1H), 0.64–0.62 (m, 2H), 0.56–0.53 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.6, 171.6, 170.9, 162.4, 157.3, 61.5, 59.0, 56.4, 53.4, 52.6, 47.4, 36.8, 34.3, 33.8, 31.6, 30.7, 26.9, 26.2,

m26.0, 24.9, 22.6, 22.4, 20.5, 19.1, 18.7, 18.4, 13.4, 12.5, 5.4, 5.3. HRMS (ESI) calcd for $C_{33}H_{56}N_6O_7S$ $[M+H]^+$: 681.4010, found 681.4012.

5.2.15. (1*R*,5*S*)-*N*-[1(*S*)-[2-(Cyclopropylamino)-1,2-dioxoethyl,]-butyl]-3-[2(*S*)-[[[[1-[[[ethyl(methyl)amino]sulfonyl]methyl]-cyclohexyl]amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (30)

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.73 (d, J = 5.0 Hz, 1H), 8.36 (d, J = 6.6 Hz, 1H), 6.21 (d, J = 9.8 Hz, 1H), 5.99 (s, 1H), 4.97–4.93 (m, 1H), 4.28 (s, 1H), 4.17 (d, J = 10.0 Hz, 1H), 3.89 (d, J = 10.4 Hz, 1H), 3.73 (dd, J = 5.4 Hz, 1H), 3.58 (d, J = 14.1 Hz, 1H), 3.27 (d, J = 14.1 Hz, 1H), 3.04 (dd, J = 6.9 & 14.1 Hz, 2H), 2.77–2.71 (m, 1H), 2.67 (s, 3H), 2.16 (d, J = 11.9 Hz, 1H), 2.07 (d, J = 11.9 Hz, 1H), 1.71–1.66 (m, 1H), 1.51–1.29 (m, 12H), 1.06 (t, J = 6.9 Hz, 3H), 0.99 (s, 3H), 0.89 (s, 9H), 0.89–0.84 (m, 4H), 0.81 (s, 3H), 0.67–0.63 (m, 2H), 0.58–0.55 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 197.1, 170.3, 170.0, 161.9, 156.7, 58.9, 56.3, 54.3, 53.4, 52.6, 47.3, 43.8, 34.3, 33.9, 33.2, 31.6, 30.6, 26.8, 26.1, 26.0, 24.9, 22.4, 20.5, 18.6, 16.4, 13.4, 13.3, 12.4, 5.3, 5.2. HRMS (ESI) calcd for $C_{33}H_{56}N_6O_7S$ $[M+H]^+$: 695.4179, found 695.4178.

5.2.16. (1*R*,5*S*)-*N*-[1(*S*)-[2-(Cyclopropylamino)-1,2-dioxoethyl]-butyl]-3-[3,3-dimethyl-2(*S*)-[[[[1-[[[methyl(1-me5thylethyl)-amino]sulfonyl]methyl]cyclohexyl]amino]carbonyl]amino]-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (31)

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.73 (d, J = 5.0 Hz, 1H), 8.36 (d, J = 6.9 Hz, 1H), 6.19 (d, J = 9.8 Hz, 1H), 5.97 (s, 1H), 4.97–4.93 (m, 1H), 4.28 (s, 1H), 4.18 (d, J = 10.0 Hz, 1H), 3.95–3.86 (m, 2H), 3.73–3.71 (m, 1H), 3.58 (d, J = 13.9 Hz, 1H), 3.25 (d, J = 14.2 Hz, 1H), 2.77–2.71 (m, 1H), 2.58 (s, 3H), 2.16 (d, J = 12.3 Hz, 1H), 2.06 (d, J = 11.3 Hz, 1H), 1.71–1.66 (m, 1H), 1.52–1.29 (m, 11H), 1.07 (d, J = 6.6 Hz, 6H), 0.99 (s, 3H), 0.89 (s, 9H), 0.89–0.84 (m, 5H), 0.81 (s, 3H), 0.66–0.64 (m, 2H), 0.58–0.56 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 197.1, 170.3, 170.0, 161.9, 156.7, 58.9, 56.3, 53.4, 52.8, 47.5, 47.3, 34.3, 34.2, 33.8, 31.6, 30.6, 26.8, 26.6, 26.1, 26.0, 24.9, 22.4, 20.5, 20.4, 19.9, 19.8,18.7, 18.3, 13.4, 12.4, 5.3, 5.2. HRMS (ESI) calcd for $C_{35}H_{60}N_6O_7S$ $[M+H]^+$: 709.4282, found 709.4294.

5.2.17. (1*R*,5*S*)-*N*-[1(*S*)-[2-(Cyclopropylamino)-1,2-dioxoethyl-butyl]-3-[2(*S*)-[[[[1-[[[(1,1-dimethylethyll)methylamino]sulfo-nyl]methyl]cyclohexyl]amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (32)

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.73 (d, J = 5.0 Hz, 1H), 8.36 (d, J = 6.9 Hz, 1H), 6.17 (d, J = 10.0 Hz, 1H), 5.98 (s, 1H), 4.96–4.93 (m, 1H), 4.28 (s, 1H), 4.17 (d, J = 9.7 Hz, 1H), 3.89 (d, J = 10.0 Hz, 1H), 3.77–3.71 (m, 1H), 3.68 (d, J = 14.1 Hz, 1H), 3.26 (d, J = 14.1 Hz, 1H), 2.76–2.72 (m, 1H), 2.71 (s, 3H), 2.18 (d, J = 11.0 Hz, 1H), 2.08 (d, J = 11.3 Hz, 1H), 1.71–1.67 (m, 1H), 1.52–1.30 (m, 11H), 1.31 (s, 9H), 1.14–1.04 (m, 1H), 0.99 (s, 3H), 0.89–0.85 (m, 13H), 0.81 (s, 3H), 0.68–0.63 (m,2H), 0.58–0.53 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 197.1, 171.0, 170.4, 161.9, 156.7, 58.9, 57.5, 56.4, 53.4, 53.0, 47.3, 34.3, 33.9, 31.7, 31.6, 30.6, 28.9, 26.8, 26.1, 26.0, 24.9, 22.3, 20.6, 20.5, 20.4, 18.6, 18.3, 13.4, 12.4, 5.3, 5.2. HRMS (ESI) calcd for $C_{33}H_{56}N_6O_7S$ $[M+H]^+$: 723.4492, found 723.4504.

5.2.18. (1*R*,5*S*)-*N*-[1(*S*)-[2-(Cyclopropylamino)-1,2-dioxoethyl]-butyl]-3-[2(*S*)-[[[[1-[[[(2-fluoro-1,1-dimethylethyl)methyl-amino]sulfonyl]methyl]cyclohexyl]amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]-hexane-2(*S*)-carboxamide (33)

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.73 (d, J = 5.0 Hz, 1H), 8.37 (d, J = 6.6 Hz, 1H), 6.18 (d, J = 9.8 Hz, 1H), 6.00 (s, 1H), 4.98–4.94 (m, 1H), 4.50 (s, 1H), 4.40 (s, 1H), 4.30 (s, 1H), 4.19 (d, J = 9.8 Hz, 1H), 3.89 (d, J = 10.4 Hz, 1H), 3.73–3.68 (m, 2H), 2.77 (s, 3H), 2.16–2.14 (m, 1H), 2.06–2.04 (m, 1H), 1.72–1.67 (m, 1H), 1.51–1.36 (m, 10H), 1.31 (s, 9H), 1.15–1.10 (m, 1H), 0.99 (s, 3H), 0.89–0.83 (m, 13H), 0.81 (s, 3H), 0.66–0.64 (m, 2H), 0.58–0.56 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6, C–F coupled spectrum) δ 197.1, 171.0, 170.3, 161.9, 156.7, 88.6, 87.2, 59.6, 59.5, 58.9, 58.6, 56.3, 53.4, 52.9, 47.3, 34.4, 34.3, 34.2, 33.9, 32.1, 31.5, 30.6, 26.8, 26.1, 26.0, 24.9, 23.3, 23.2, 23.1, 22.3, 20.5, 20.5, 18.6, 18.3, 13.3, 12.4, 5.3, 5.2. HRMS (ESI) calcd for $C_{33}H_{56}N_6O_7S$ $[M+H]^+$: 741.4398, found 723.4393.

5.2.19. (1*R*,5*S*)-*N*-[1(*S*)-[2-(Cyclopropylamino)-1,2-dioxoethyl]-butyl]-3-[2(*S*)-[[[[1-[[[cyclopropyl(methyl)amino]sulfonyl]-methyl]cyclohexyl]amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (34)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (d, J = 5.4 Hz, 1H), 8.36 (d, J = 7.0 Hz, 1H), 6.20 (d, J = 10.2 Hz, 1H), 6.01 (s, 1H), 4.96–4.92 (m, 1H), 4.26 (s, 1H), 4.16 (d, J = 10.9 Hz, 1H), 3.89 (d, J = 10.9 Hz, 1H), 3.73–3.67 (m, 2H), 3.36–3.30 (m, 1H), 2.75–2.70 (m, 1H), 2.68 (s, 3H), 2.32–2.28 (m, 1H), 2.21 (d, J = 11.7 Hz, 1H), 2.08 (d, J = 11.7 Hz, 1H), 1.73–1.63 (m, 1H), 1.53–1.23 (m, 12H), 1.15–1.10 (m, 1H), 0.98 (s, 3H), 0.87–0.81 (m, 12H), 0.80 (s, 3H), 0.65–0.59 (m, 6H), 0.58–0.53 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.6, 171.6, 170.8, 162.4, 157.3, 61.4, 58.5, 56.9, 53.9, 53.3, 46.7, 37.0, 35.0, 34.9, 34.8, 32.1, 31.5, 29.7, 27.9, 27.3, 26.7, 26.6, 25.4, 22.9, 21.0, 19.2, 18.9, 13.9, 12.9, 7.5, 7.4, 5.9, 5.8. HRMS (ESI) calcd for $C_{33}H_{56}N_6O_7S$ $[M+H]^+$: 707.4166, found 707.4168.

5.2.20. (1*R*,5*S*)-3-[2(*S*)-[[[[1-[[[Cyclobutyl(methyl)amino]sulfo-nyl]methyl]cyclohexyl]amino] carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-*N*-[1(*S*)-[2-(cyclopropylamino)-1,2-dioxoethyl]-butyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (35)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (d, J = 5.0 Hz, 1H), 8.37 (d, J = 6.9 Hz, 1H), 6.20 (d, J = 9.8 Hz, 1H), 5.99 (s, 1H), 4.98–4.94 (m, 1H), 4.30 (s, 1H), 4.20 (d, J = 9.8 Hz, 1H), 4.11–4.07 (m, 1H), 3.89 (d, J = 10.0 Hz, 1H), 3.76–3.73 (m, 1H), 3.52 (d, J = 13.6 Hz, 1H), 3.19 (d, J = 13.9 Hz, 1H), 2.78–2.73 (m, 1H), 2.67 (s, 3H), 2.17–2.13 (m, 3H), 2.04–1.95 (m, 3H), 1.72–1.69 (m, 1H), 1.60–1.29 (m, 13H), 1.15–1.09 (m, 1H), 1.00 (s, 3H), 0.90 (s, 9H), 0.89–0.85 (m, 4H), 0.83 (s, 3H), 0.66–0.65 (m, 2H), 0.58–0.57 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.6, 171.6, 170.8, 162.4, 157.2, 59.5, 56.8, 55.0, 53.9, 53.3, 51.8, 47.9, 34.9, 34.8, 34.3, 32.1, 31.2, 29.9, 27.5, 27.4, 27.3, 26.7, 26.6, 25.4, 22.9, 21.1, 21.0, 19.2, 18.9, 14.6, 13.9, 12.9, 5.9. HRMS (ESI) calcd for $C_{36}H_{60}N_6O_7S$ $[M+H]^+$: 721.4335, found 721.4330.

5.2.21. (1*R*,5*S*)-*N*-[1(*S*)-[2-(Cyclopropylamino)-1,2-dioxoethyl]-butyl]-3-[2(*S*)-[[[[1-[[[(cyclopropylmethyl)methylamino]sulfo-nyl]methyl]cyclohexyl]amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (36)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (d, J = 4.7 Hz, 1H), 8.22 (d, J = 7.0 Hz, 1H), 6.04 (d, J = 9.4 Hz, 1H), 5.83 (s, 1H), 4.81–4.77 (m, 1H), 4.13 (s, 1H), 4.01 (d, J = 9.4 Hz, 1H), 3.73 (d, J = 10.2 Hz, 1H), 3.58–3.55 (m, 1H), 3.44 (d, J = 14.1 Hz, 1H), 3.10 (d, J = 14.1 Hz, 1H), 2.73 (d, J = 7.0 Hz, 3H), 2.34 (br s, 1H), 2.00 (d, J = 10.9 Hz, 1H), 1.90 (d, J = 10.2 Hz, 1H), 1.59–1.50 (m, 1H), 1.35–1.12 (m, 12H), 1.04–0.96 (s, 1H), 0.83 (br s, 3H), 0.86–0.82 (m, 1H), 0.73 (br s, 9H), 0.71–0.69 (m, 5H), 0.65 (br s, 3H), 0.65–0.60 (m,1H), 0.49–0.48 (m, 2H), 0.42–0.39 (m, 2H), 0.33–0.31 (m, 2H), 0.02–0.00 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.1, 171.1, 170.4, 161.9, 156.8, 58.9, 58.4, 53.4, 52.9, 52.6, 49.5, 47.4, 34.3, 34.3, 33.8, 33.3, 31.6, 30.6, 26.9, 26.2, 26.1, 25.9, 24.9, 24.7, 22.4, 20.5,

18.7, 18.4, 18.2, 14.4, 13.4, 12.4, 5.4, 5.3. HRMS (ESI) calcd for $C_{36}H_{60}N_6O_7S$ $[M+H]^+$: 721.4322, found 721.4328.

5.2.22. (1*R*,5*S*)-*N*-[1(*S*)-[2-(Cyclopropylamino)-1,2-dioxoethyl]-butyl]-3-[2(*S*)-[[[[1-[[(cyclopropylethylamino)sulfonyl]methyl]-cyclohexyl]aminocarbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (37)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.72 (d, J = 5.4 Hz, 1H), 8.35 (d, J = 7.0 Hz, 1H), 6.17 (d, J = 10.2 Hz, 1H), 5.99 (s, 1H), 4.93–4.91 (m, 1H), 4.68 (s, 1H), 4.16 (d, J = 10.9 Hz, 1H), 3.88 (d, J = 10.9 Hz, 1H), 3.72–3.67 (m, 2H), 3.29 (d, J = 14.0 Hz, 1H), 3.16–3.10 (m, 2H), 2.75–2.70 (m, 1H), 2.39–2.30 (m, 1H), 2.18 (d, J = 10.9 Hz, 1H), 2.06 (d, J = 10.9 Hz, 1H), 1.73–1.62 (m, 1H), 1.52–1.27 (m, 12H), 1.09 (t, J = 7.0 Hz, 3H), 0.97 (s, 3H), 0.87 (s, 9H), 0.86–0.81 (m, 4H), 0.79 (s, 3H), 0.70–0.69 (m, 2H), 0.63–0.60 (m, 4H), 0.55–0.54 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.6, 171.6, 170.8, 162.4, 157.3, 59.5, 56.9, 53.9, 53.4, 47.9, 44.9, 34.9, 34.8, 34.8, 34.4, 32.1, 31.2, 29.5, 29.2, 27.4, 26.7, 26.6, 26.4, 25.2, 22.9, 21.0, 19.2, 18.9, 14.5, 13.9, 12.9, 7.3, 7.2, 5.9. HRMS (ESI) calcd for $C_{37}H_{60}N_6O_7S$ $[M+H]^+$: 721.4322, found 721.4329.

5.2.23. (1*R*,5*S*)-*N*-[1(*S*)-[2-(Cyclopropylamino)-1,2-dioxoethyl]-butyl]-3-[2(*S*)-[[[[1-[[[cyclopropyl(1-methylethyl)amino]-sulfonyl]methyl]cyclohexyl]amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (38)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (d, J = 4.8 Hz, 1H), 8.36 (d, J = 7.0 Hz, 1H), 6.16 (d, J = 9.4 Hz, 1H), 5.99 (s, 1H), 4.95–4.91 (m, 1H), 4.27 (s, 1H), 4.19–4.15 (m, 1H), 3.96–3.82 (m, 2H), 3.73 (d, J = 14.1 Hz, 1H), 3.27 (d, J = 14.1 Hz, 1H), 2.76–2.70 (m, 1H), 2.24–2.15 (m, 2H), 2.05 (d, J = 12.5 Hz, 1H), 1.73–1.24 (m, 13H), 1.18–1.16 (m, 6H), 0.97 (s, 3H), 0.87 (br s, 10H), 0.86–0.82 (m, 4H), 0.79 (s, 3H), 0.78–0.74 (m, 4H), 0.65–0.61 (m, 2H), 0.57–0.51 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.6, 171.6, 170.9, 162.4, 157.2, 59.5, 57.2, 56.8, 53.9, 53.8, 53.5, 51.2, 47.9, 34.9, 34.5, 32.1, 31.2, 27.3, 26.7, 26.6, 25.4, 25,1, 22.9, 21.8, 21.6, 21.1, 19.2, 18.9, 14.5, 13.9, 12.9, 7.1, 7.0, 5.9, 5.8. HRMS (ESI) calcd for $C_{37}H_{62}N_6O_7S$ $[M+H]^+$: 735.4478, found 735.4485.

5.2.24. (1*R*,5*S*)-3-[2(*S*)-[[[[1-[(1-Azetidinylsulfonyl)methyl]-cyclohexyl]amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-*N*-[1(*S*)-[2-(cyclopropylamino)-1,2-dioxoethyl]butyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (39)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.75 (d, J = 5.4 Hz, 1H), 8.41 (d, J = 6.3 Hz, 1H), 6.20 (d, J = 10.2 Hz, 1H), 5.99 (s, 1H), 4.98–4.93 (m, 1H), 4.28 (s, 1H), 4.17 (d, J = 10.2 Hz, 1H), 3.89 (d, J = 10.2 Hz, 1H), 3.74–3.69 (m, 6H), 3.68 (d, J = 14.8 Hz, 1H), 3.39 (d, J = 14.1 Hz, 1H), 2.76–2.67 (m, 1H), 1.73–1.65 (m, 1H), 1.53–1.27 (m, 12H), 1.13–1.03 (m, 1H), 0.98 (s, 3H), 0.87–0.83 (m, 15H), 0.80 (s, 3H), 0.65–0.61 (m, 2H), 0.57–0.53 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.1, 171.1, 170.3, 161.9, 156.7, 59.0, 56.4, 53.6, 53.4, 53.7, 47.4, 34.4, 34.3, 34.1, 33.9, 33.6, 33.4, 31.6, 26.8, 26.2, 26.0, 24.9, 22.4, 20.5, 18.7, 13.4, 12.5, 9.3, 5.3, 5.2, 3.4, 3.3. HRMS (ESI) calcd for $C_{34}H_{56}N_6O_7S$ $[M+H]^+$: 693.4009, found 693.4015.

5.2.25. (1*R*,5*S*)-*N*-[1(*S*)-[2-(Cyclopropylamino)-1,2-dioxoethyl]-butyl]-3-[3,3-dimethyl-2(*S*)-[[[[1-[(4-morpholinylsulfonyl)-methyl]cyclohexyl]amino]carbonyl]amino]-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (40)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (d, J = 5.3 Hz, 1H), 8.36 (d, J = 6.9 Hz, 1H), 6.21 (d, J = 10.0 Hz, 1H), 6.20 (s, 1H), 4.97–4.93 (m, 1H), 4.28 (s, 1H), 4.17 (d, J = 10.0 Hz, 1H), 3.91 (d, J = 10.4 Hz, 1H), 3.74–3.71 (m, 1H), 3.60 (br s, 4H), 3.29 (d, J = 14.1 Hz, 1H), 3.04 (br s, 4H), 2.76–2.72 (m, 1H), 2.16 (d, J = 12.0 Hz, 1H), 2.08 (d, J = 12.0 Hz, 1H), 1.73–1.66 (m, 1H), 1.52–1.26 (m, 12H), 1.18–1.08 (m, 1H), 0.99 (s, 3H), 0.89–0.84 (m, 13H), 0.82 (s, 3H), 0.66–0.64 (m, 2H), 0.58–0.56 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 197.1, 171.0, 170.4, 161.9, 156.7, 65.7, 58.9, 56.4, 53.4, 53.2, 52.7, 47.3, 45.1, 34.4, 34.2, 33.8, 31.6, 30.6, 26.8, 26.6, 26.2, 26.0, 25.1, 24.8, 22.4, 20.9, 20.5, 18. 6, 18.4, 13.4, 12.4, 5.4, 5.3. HRMS (ESI) calcd for $C_{35}H_{58}N_6O_8S$ $[M+H]^+$: 723.4128, found 723.4124.

5.2.26. (1*R*,5*S*)-*N*-[1(*S*)-[2-(Cyclopropylamino)-1,2-dioxoethyl]-butyl]-3-[(2*S*)-2-[[[[1-[1(*R*)-[(dimethylamino)sulfonyl]ethyl]-cyclohexyl]amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyll-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (41)

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.73 (d, J = 5.0 Hz, 1H), 8.36 (d, J = 6.9 Hz, 1H), 6.26 (d, J = 10.1 Hz, 1H), 6.02 (s, 1H), 4.98–4.94 (m, 1H), 4.29 (s, 1H), 4.11 (t, J = 9.5 & 6.9 Hz, 1H), 3.94 (d, J = 10.4 Hz, 1H), 3.72 (dd, J = 5.4 & 10.1 Hz, 1H), 2.75 (s, 6H), 2.18 (d, J = 12.9 Hz, 1H), 2.10 (d, J = 13.6 Hz, 1H), 1.74–1.66 (m, 2H), 1.55–1.25 (m, 12H), 1.09 (d, J = 7.3 Hz, 3H), 1.00 (s, 3H), 0.90 (s, 9H), 0.89–0.85 (m, 5H), 0.82 (s, 3H), 0.66–0.64 (m, 2H), 0.58–0.56 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 197.1, 171.0, 170.6, 161.9, 156.9, 60.1, 58.9, 56.7, 56.1, 53.4, 47.4, 36.9, 33.9, 31.5, 30.6, 30.3, 26.9, 26.3, 26.1, 24.9, 22.4, 20.9, 20.4, 18.7, 18.4, 13.4, 12.4, 10.9, 5.3, 5.2. HRMS (ESI) calcd for $C_{34}H_{58}N_6O_7S$ $[M+H]^+$: 695.4166, found 695.4168.

5.2.27. (1*R*,5*S*)-*N*-[1(*S*)-[2-(Cyclopropylamino)-1,2-dioxoethyl]-butyl]-3-[(2*S*)-[[[[1-[1(*S*)-[(dimethylamino)sulfonyl]ethyl]-cyclohexyl]amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyll-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (42)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.75 (d, J = 5.0 Hz, 1H), 8.38 (d, J = 6.9 Hz, 1H), 6.30 (d, J = 9.8 Hz, 1H), 5.98 (s, 1H), 4.96–4.92 (m, 1H), 4.29 (s, 1H), 4.18 (d, J = 6.9 Hz, 1H), 4.06 (d, J = 10.4 Hz, 1H), 3.87 (d, J = 10.1 Hz, 1H), 3.74–3.71 (m, 1H), 2.74 (s, 7H), 2.26 (d, J = 12.9 Hz, 1H), 1.81–1.71 (m, 2H), 1.56–1.23 (m, 14H), 1.14 (d, J = 6.9 Hz, 3H), 0.99 (s, 3H), 0.89 (s, 9H), 0.87–0.84 (m, 2H), 0.81 (s, 3H), 0.65–0.63 (m, 2H), 0.58–0.54 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.1, 171.0, 170.3, 161.9, 156.5, 60.7, 58.9, 56.4, 56.2, 53.4, 47.4, 36.9, 34.4, 31.6, 30.8, 30.6, 30.3, 26.8, 26.2, 26.0, 25.0, 22.6, 22.4, 20.9, 20.5, 18.7, 18.4, 13.4, 12.4, 10.9, 5.4. LC–MS for $C_{34}H_{58}N_6O_7S$ $[M+H]^+$: 695.4.

5.2.28. (1*R*,5*S*)-*N*-[3-(Cyclopropylamino)-1(*S*)-(2-cyclopropyl-ethyl)-2,3-dioxopropyl]-3-[2(*S*)-[[[[1-[[[cyclopppropyl(methyl)-amino]sulfonyl]methyl]cyclohexyl]amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]-hexane-2(*S*)-carboxamide (43)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, J = 7.8 Hz, 1H), 7.25 (d, J = 6.6 Hz, 1H), 5.49–5.44 (m, 1H), 5.15 (s, 1H), 4.57 (br s, 2H), 4.20 (t, J = 10.7 Hz, 1H), 4.04 (s, 1H), 3.87 (dd, J = 10.4 & 5.4 Hz, 1H), 3.13 (d, J = 6.9 Hz, 1H), 2.96–2.90 (m, 1H), 2.82–2.78 (m, 1H), 2.64 (s, 3H), 2.61 (d, J = 11.9 Hz, 1H), 2.37 (d, J = 9.2 Hz, 1H), 2.23–2.14 (m, 1H), 1.93–1.85 (m, 1H), 1.79–1.69 (m, 1H), 1.60–1.14 (m, 12H), 0.99 (s, 3H), 0.96 (br s, 9H), 0.92–0.86 (m, 5H), 0.84 (s, 3H), 0.82–0.81 (m, 2H), 0.65–0.62 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.9, 172.5, 170.8, 160.3, 156.7, 63.7, 59.6, 57.3, 56.2, 54.1, 53.4, 48.6, 46.9, 35.2, 33.2, 32.1, 31.6, 30.9, 30.2, 29.9, 27.4, 26.4, 25.5, 22.6, 21.7, 20.9, 18.8, 18.7, 14.1, 13.2, 12.8, 6.3, 6.2. HRMS (FAB) calcd for $C_{34}H_{56}N_6O_7S$ $[M+H]^+$: 692.3978, found 693.3981.

5.2.29. (1*R*,5*S*)-*N*-[1(*S*)-[2-(Cyclopropylamino)-1,2-dioxoethyl]-butyl]-3-[2(*S*)-[[[[1-(2-cyclopropyl-1,1-dioxido-5(*R*)-isothiazolidinyl)cyclohexyl]amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (44)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (d, J = 4.7 Hz, 1H), 8.36 (d, J = 7.0 Hz, 1H), 6.24 (d, J = 10.2 Hz, 1H), 6.09 (s, 1H), 4.96–4.91 (m, 1H), 4.27 (s, 1H), 4.20–4.03 (m, 2H), 3.88 (t, J = 10.2 Hz, 1H),

3.75–3.68 (m, 1H), 3.29 (d, J = 10.2 Hz, 1H), 3.16 (m, 1H), 3.01–2.88 (m, 1H), 2.75–2.68 (m, 1H), 2.45 (d, J = 11.8 Hz, 1H), 2.18–2.11 (m, 1H), 2.08–1.99 (m, 1H), 1.95–1.85 (m, 2H), 1.69–1.04 (m, 11H), 0.99 (s, 3H), 0.88–0.83 (m, 12H), 0.81–0.76 (m, 5H), 0.66–0.60 (m, 4H), 0.57–0.48 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 197.6, 171.6, 171.0, 162.4, 157.4, 64.7, 59.5, 58.2, 57.2, 55.4, 53.9, 50.8, 50.5, 47.8, 46.3, 34.5, 32.1, 30.5, 26.8, 26.6, 26.4, 25.5, 22.9, 21.0, 20.8, 19.2, 19.1, 18.9, 13.9, 12.8, 5.9, 5.6, 4.6, 4.5. HRMS (ESI) calcd for $C_{36}H_{58}N_6O_7S$ [M+H]$^+$: 719.4166, found 719.4172.

5.2.30. (1*R*,5*S*)-*N*-[3-(Cyclopropylamino)-1(*S*)-(cyclopropylmethyl)-2,3-dioxopropyl]-3-[2(*S*)-[[[[1-[[[cyclopropyl(methyl)amino]sulfonyl]methyl]cyclohexyl]amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (45)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.74 (d, J = 4.7 Hz, 1H), 8.44 (d, J = 6.2 Hz, 1H), 6.22 (d, J = 10.2 Hz, 1H), 6.02 (s, 1H), 5.08–5.03 (m, 1H), 4.31 (s, 1H), 4.19 (d, J = 10.2 Hz, 1H), 3.92 (d, J = 10.9 Hz, 1H), 3.72 (d, J = 14.1 Hz, 2H), 3.34 (t, J = 6.2 Hz, 1H), 2.69 (s, 3H), 2.33–2.29 (m, 1H), 2.22 (d, J = 11.7 Hz, 1H), 2.10 (d, J = 10.9 Hz, 1H), 1.74–1.66 (m, 1H), 1.51–1.15 (m, 12H), 0.99 (s, 3H), 0.88 (br s, 10H), 0.81 (s, 3H), 0.67–0.56 (m, 8H), 0.41–0.36 (m, 2H), 0.10–0.02 (m, 2H).;^{13}C NMR (100 MHz, DMSO-d_6) δ 197.3, 171.5, 170.9, 162.2, 157.3, 59.5, 58.5, 56.9, 54.9, 53.3, 47.9, 38.7, 37.0, 35.5, 34.9, 34.8, 34.4, 31.5, 31.2, 27.3, 26.7, 26.6, 25.4, 22.9, 21.1, 18.9, 12.9, 8.4, 7.5, 7.4, 5.8, 5.5, 4.8, 4.6. HRMS (ESI) calcd for $C_{36}H_{58}N_6O_7S$ [M+H]$^+$: 719.4166, found 719.4169.

5.2.31. (1*R*,5*S*)-*N*-[1(*S*)-[2-(Cyclopropylamino)-1,2-dioxoethyl]-pemtyl]-3-[2(*S*)-[[[[1-[[[cyclopropyl(methyl)amino]sulfonyl]-methyl]cyclohexyl]amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2(*S*)-carboxamide (46)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (d, J = 5.5 Hz, 1H), 8.35 (d, J = 6.2 Hz, 1H), 6.20 (d, J = 10.2 Hz, 1H), 6.02 (s, 1H), 4.96–4.90 (m, 1H), 4.27 (s, 1H), 4.17 (d, J = 10.2 Hz, 1H), 3.89 (d, J = 10.9 Hz, 1H), 3.71 (d, J = 14.1 Hz, 2H), 3.31 (t, J = 6.2 Hz, 1H), 2.75–2.70 (m, 1H), 2.68 (s, 3H), 2.33–2.28 (m, 1H), 2.21 (d, J = 11.7 Hz, 1H), 2.09 (d, J = 11.7 Hz, 1H), 1.74–1.69 (m, 1H), 1.53–1.04 (m, 15H), 0.98 (s, 3H), 0.88 (br s, 9H), 0.87–0.81 (m, 3H), 0.80 (s, 3H), 0.67–0.59 (m, 6H), 0.57–0.54 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ 197.6, 171.6, 170.9, 162.4, 157.3, 59.5, 58.5, 56.9, 54.0, 52.1, 47.9, 37.0, 34.8, 34.7, 34.3, 33.2, 33.0, 31.5, 31.0, 29.8, 28.0, 27.3, 26.7, 26.6, 25.4, 22.9, 22.2, 21.1, 19.3, 18.9, 14.2, 7.5, 7.4, 5.9. HRMS (ESI) calcd for $C_{36}H_{60}N_6O_7S$ [M+H]$^+$: 721.4322, found 721.4328.

5.2.32. (1*S*,5*R*)-*N*-((*S*)-5-Cyclopropyl-1-(cyclopropylamino)-1,2-dioxopentan-3-yl)-3-((*S*)-2-(3-(1-((*N*-cyclopropyl-*N*-methylsulfamoyl)methyl)cyclohexyl)ureido)-3,3-dimethylbutanoyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2-carboxamide (47)

To the acid 6 (R_3 = c-Pr, R_2 = Me, R_4 = H), (1.2 equiv, 907 mg, 1.68 mmol) in DCM (60 mL) was added amine hydrochloride 7 d (1 equiv, 1.4 mmol, 349 mg) with stirring on ice. To this solution was added HATU (1.2 equiv, 1.68 mmol, 638 mg) followed by DIPEA (60 mmol, 10.2 mL). The reaction was warmed-up to rt and left stirring overnight. The reaction mixture was diluted with EtOAc and washed with 1 N HCl, $NaHCO_3$ then brine. The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuuo. The intermediate product, α-hydroxyamide, was obtained as a mixture of diastereomers (1.35 g) which was used in subsequent reaction without further purification. To the solution of this product (1.35 g) in anhydrous CH_2Cl_2 (30 mL) at rt was added Dess–Martin reagent (1.66 g, 3.92 mmol). The mixture was stirred for 3 h. Saturated $NaHCO_3$ and $Na_2S_2O_3$ solutions (20 mL each) were added. After stirring for 10 min, the layers were separated. The aqueous solution was extracted with EtOAc (2 × 50 mL). The organic solutions were combined, dried with $MgSO_4$, filtered and concentrated in vacuuo. Flash chromatography purification (20–80% EtOAc/Hexane) afforded 47 (1 g, 83% yield over two steps). Analytical HPLC (YMC-Pack Diol NP column, 150 × 3 mm; 7% (CH_3CN (0.3), *i*-PrOH (1.7), DCM (2)) in Hexanes; 1 mL min; 254 nm), rt = 11.0 min, purity 97.0%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.75 (d, J = 4.7 Hz, 1H), 8.35 (d, J = 7.0 Hz, 1H), 6.23 (d, J = 10.2 Hz, 1H), 6.03 (s, 1H), 5.04–4.97 (m, 1H), 4.28 (s, 1H), 4.19 (d, J = 10.2 Hz, 1H), 3.91 (d, J = 10.2 Hz, 1H), 3.73 (d, J = 14.1 Hz, 2H), 3.31 (d, J = 6.2 Hz, 1H), 2.78–2.72 (m, 1H), 2.70 (s, 3H), 2.40–2.30 (m, 1H), 2.23 (d, J = 10.2 Hz, 1H), 2.11 (d, J = 10.9 Hz, 1H), 1.92–1.80 (m, 1H), 1.59–1.07 (m, 14H), 1.00 (s, 3H), 0.90 (br s, 9H), 0.86 (br s, 3H), 0.67–0.58 (m, 8H), 0.37–0.36 (m, 2H), 0.12–0.0 (m, 2H).^{13}C NMR (100 MHz, DMSO-d_6) δ 197.7, 171.6, 170.8, 162.5, 157.3, 59.5, 56.9, 54.0, 53.3, 52.3, 47.9, 37.0, 34.8, 34.3, 34.2, 31.5, 31.2, 31.0, 30.2, 27.4, 26.8, 26.6, 25.4, 22.9, 21.1, 20.6, 18.9, 13.0, 10.6, 7.5, 7.4, 5.9, 5.8, 4.9, 4.3. HRMS (ESI) calcd for $C_{37}H_{60}N_6O_7S$ [M+H]$^+$: 733.4335, found 733.4331.

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