Discovery and Structure−Activity Relationship of P_1-P_3 Ketoamide Derived Macrocyclic Inhibitors of Hepatitis C Virus NS3 Protease

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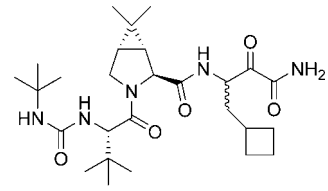
Hepatitis C virus (HCV) infection is the major cause of chronic liver disease, leading to cirrhosis and hepatocellular carcinoma, and affects more than 200 million people worldwide. Although combination therapy of interferon-α and ribavirin is reasonably successful in treating majority of genotypes, its efficacy against the predominant genotype (genotype 1) is moderate at best, with only about 40% of the patients showing sustained virological response. Herein, the SAR leading to the discovery of a series of ketoamide derived P_1-P_3 macrocyclic inhibitors that are more potent than the first generation clinical candidate, boceprevir (**1**, Sch 503034), is discussed. The optimization of these macrocyclic inhibitors identified a P_3 imide capped analogue **52** that was 20 times more potent than **1** and demonstrated good oral pharmacokinetics in rats. X-ray structure of **52** bound to NS3 protease and biological data are also discussed.

Introduction

An estimated 200 million people worldwide are infected with HCV, making it an impending public threat that leads to liver cirrhosis, carcinoma, or liver failure.[1] The slow progression of the disease in combination with mild symptoms has made early detection difficult. Pegylated α-interferon alone or in combination with ribavirin is the preferred treatment for HCV viral infection.[2] Although 80% of genotype-2 infected patients respond initially, only 40% of genotype-1 infected patients show sustained response to interferon treatment. Lack of effective methods to treat genotype-1 HCV infections and patients relapsing from interferon therapy necessitates discovery of new drugs. Significant efforts are now directed toward development of therapies that target key enzymes vital to HCV replication and maturation.[3]

Hepatitis C virus is a positive strand RNA virus with a single open frame of ∼9600 nucleotides. It encodes a single polypeptide of ∼3000 amino acids that is post-translationally modified to produce mature virions.[4] The single polypeptide contains all the structural and nonstructural proteins C-E1-E2-P7-NS2-NS3-NS4A-NS4B-NS5A-NS5B. NS3, a trypsin-like serine protease, catalyzes cis cleavage of the NS3-NS4A junction, followed by trans cleavage of the NS4A-NS4B, NS4B-NS5A, and NS5A-NS5B to produce functional proteins.[5] The central role played by NS3 protease in the development of mature hepatitis C virus makes it an excellent target for drug discovery. Development of small molecule inhibitors for this enzyme would potentially arrest the processing of the aforementioned polyprotein required for viral replication. This has been a field of intense investigation by various groups worldwide.[6] BILN-2061, Sch 503034 (**1**), VX-950, ITMN-191, TMC435350, and MK-7009 are novel protease inhibitors that have been advanced into clinical studies in humans and demonstrated to be efficacious.[6] X-ray crystal structure of the enzyme reveals a featureless, shallow, highly solvent exposed active site located near the surface in a cleft between two β-barrel subdomains.[7] Characteristic residues of the catalytic triad, histidine-57, and aspartic acid-81 are located in the N-terminal, whereas Ser-139 forms part of the C-terminal subdomain. Cysteine is conserved in P_1 position of natural substrate of NS3 protease in all three trans-cleavage sites and is replaced by threonine in the cis-cleavage event. The P_1' is a small amino acid, either a serine or alanine. The P_2, P_3, and P_4 sites are hydrophobic amino acids, and P_5 and P_6 usually contain acidic amino acids such as aspartic acid or glutamic acid. We recently disclosed the synthesis and development of **1**, a selective, potent, orally bioavailable HCV NS3 protease inhibitor that is currently undergoing phase III clinical trials.[8]



1

K_i^* =14 nM
HNE/HCV =2200
Replicon EC_{90} = 0.35 μM

Figure 1

As outlined in Figure 1, **1** had a $K_i^* = 14$ nM in the continuous binding assay[9] and an $EC_{90} = 350$ nM in the replicon based cellular assay[10] with excellent selectivity against the human neutrophil elastase (HNE), an enzyme that structurally resembles HCV NS3 protease. In an effort to develop a potential backup to **1**, we explored various novel scaffolds, most of them directed toward depeptidization. Macrocyclization has been a novel approach that has been widely explored to depeptidize inhibitors. In some cases it has resulted in realizing compounds with improved potency and better physiochemical properties resulting in improved PK and bioavailability.[11] On examination of the X-ray structure of acyclic inhibitors of type **1** bound to NS3 protease, we observed the proximity of the P_2 and P_4 residues and P_1 and P_3 residues (Figure 2). We hypothesized that syntheses of macrocyclic inhibitors that connected these

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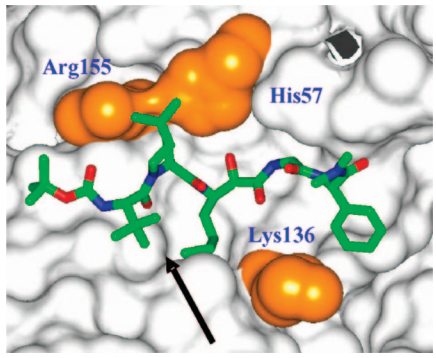
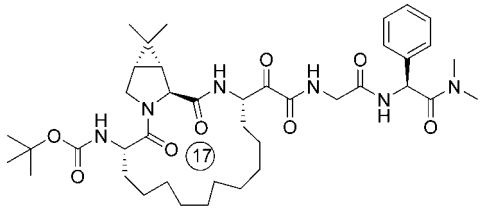


Figure 2. Structure of acyclic inhibitors of type **1** bound to NS3 protease.



2

Figure 3

residues would produce depetidized macrocyclic inhibitors that would bind to the NS3 enzyme mimicking the acyclic conformation. Earlier in this project we reported novel macrocyclic P_2-P_4 inhibitors and optimized their enzyme binding potencies to the low nanomolar range.[12] Herein, we describe the development and SAR of P_1-P_3 series of macrocyclic inhibitors that demonstrate excellent enzyme binding (K_i*), replicon cellular potency (EC_{90}) and PK. Concomitant to our efforts in optimization of these ketoamide derived inhibitors, researchers from Boehringer Ingelheim disclosed the discovery and optimization of BILN-2061, a potent P_1-P_3 macrocyclic inhibitor of HCV NS3 protease that had a carboxylic acid at P_1 and mimicked the natural substrate.[6a]

Examination of X-ray structures of acyclic inhibitors bound to the NS3 protease and modeling initially suggested that a 17-membered macrocycle would possibly be appropriate for binding. We therefore chose this ring size to investigate our first generation of P_1-P_3 macrocyclic series of inhibitor. In addition to this, we had previously established from our acyclic series of inhibitors that a 3,4-dimethylcylopropyl fused proline was an excellent P_2 residue.[13] Even though our previous studies had shown a *tert*-butylglycine or cyclohexylglycine as the preferred P_3, we decided to have an aliphatic straight chain P_3 for ease of synthesis. Therefore, we embarked on the synthesis of our first P_1-P_3 macrocyclic inhibitor, compound **2** (Figure 3).

Chemistry

In general, inhibitors were synthesized following the procedures previously reported.[14] Syntheses of macrocycle were accomplished using olefin metathesis with Grubbs first generation catalyst.[15] The P_1 and P_3 amino acid required for the synthesis of macrocyclic precursor was achieved using the method of Burk with asymmetric hydrogenation as shown in Scheme 1.[16]

Thus, condensation of pentenal **3** with ethyl acetamidomalonate monoester in CH_2Cl_2, in the presence of acetic anhydride and pyridine, resulted in enamide **4**. Enantioselective reduction of enamide **4** was accomplished by treatment with 0.002 mol % of chiral rhodium catalyst and selective hydrogenation of the proximal double bond to yield amino acid **5**. The required amino acid was thus obtained in high stereoselectivity and chemoselectivity. The nitrogen of the acetamide group was Boc protected using Boc_2O and DMAP to form **6**, which on treatment with aqueous LiOH resulted in hydrolysis of the ethyl ester and the acetamide group to form Boc protected amino acid **7**. A similar scheme was used for the synthesis of the homologous amino acid **13**. Thus, oxidation of 5-hexenol with PCC resulted in formation of 5-hexenal **9**, which was converted to the amino acid **11** using similar conditions outlined for the synthesis of **5**. The acetamide compound **11** was once again treated with Boc_2O, and further treatment of the Boc protected derivative with hydrazine resulted in selective deprotection of the acetamide group in the presence of ethyl ester to form compound **12**. Treatment of **12** with 4 M HCl in dioxane yielded amine salt **13** that was used for the syntheses of desired inhibitors as shown in Scheme 2.

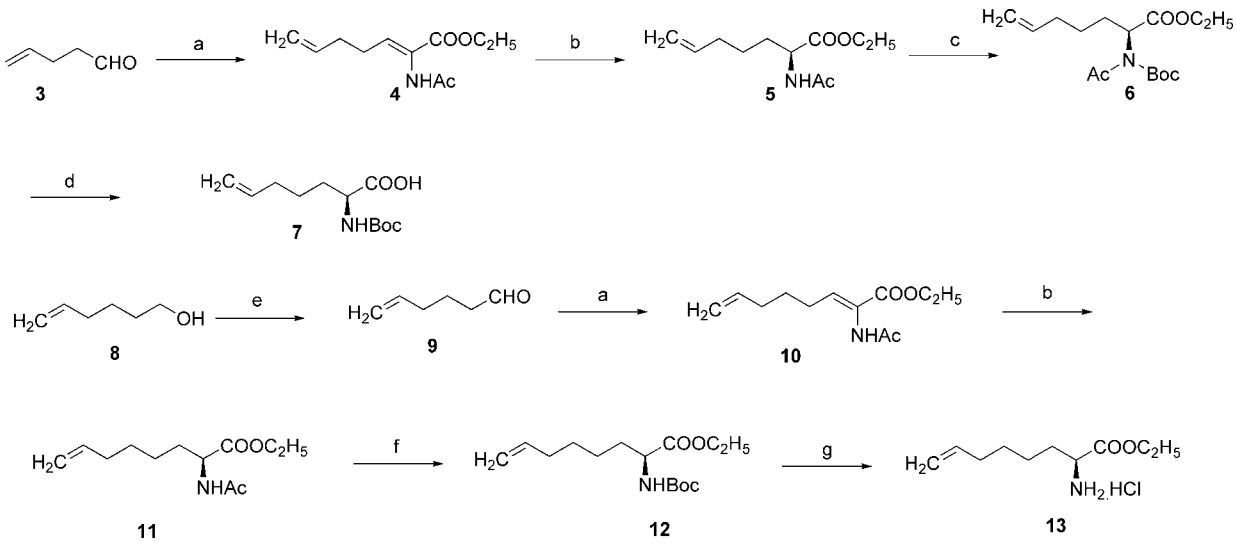
Coupling of Boc protected amino acid **7** with 3,4-dimethylcylopropylproline amino acid **14**[17] using HATU[18] and NMM resulted in dipeptide **15**. Alkaline hydrolysis of dipeptide **15** yielded an intermediate acid, which was coupled with amine salt **13** to yield **16**. Treatment of macrocyclic precursor **16** with Grubbs first generation metathesis catalyst resulted in formation of macrocycle **17** as a mixture of two isomeric olefinic compounds. The first generation catalyst proved to be as good as the more active carbene ligand derived catalysts. The inseparable cis and trans mixture of compound **17** was catalytically hydrogenated using Pd/C and further reduced with $LiBH_4$ to yield alcohol **18**. Oxidation of alcohol **18** with Dess−Martin's reagent[19] yielded aldehyde **19**, which on treatment with alkyl isocyanide and acetic acid yielded hydoxyamide derivative **20**. Analysis of the aldehyde by NMR showed no epimerization of the aldehyde. Basic hydrolysis with aqueous lithium hydroxide followed by oxidation with either Moffat conditions or Dess−Martin's reagent affords ketoamide **22**. The P_3-capped analogues of **22** were obtained by deprotection of Boc group first using 4 M HCl/dioxane and further treatment with various isocyanates to form inhibitors of type **23**. Synthesized inhibitors were assayed for enzyme binding (K_i*) and replicon cellular potency (EC_{90}). In addition, selected interesting compounds were evaluated for their ability to inhibit human neutraphil elastase. The ratio of K_i*(HNE)/K_i*(HCV) was taken as a measure of selectivity. Compounds with good enzyme binding and cellular activity were also evaluated in a rapid rat assay to assess compound plasma levels in rats.

Discussion

As shown in Table 1, the first set of compounds synthesized were those that spanned from P_3-P_2'. As established from our earlier acyclic series, we reasoned that these types of compounds were more likely to be active and provide an excellent model to test the utility of our new scaffold.

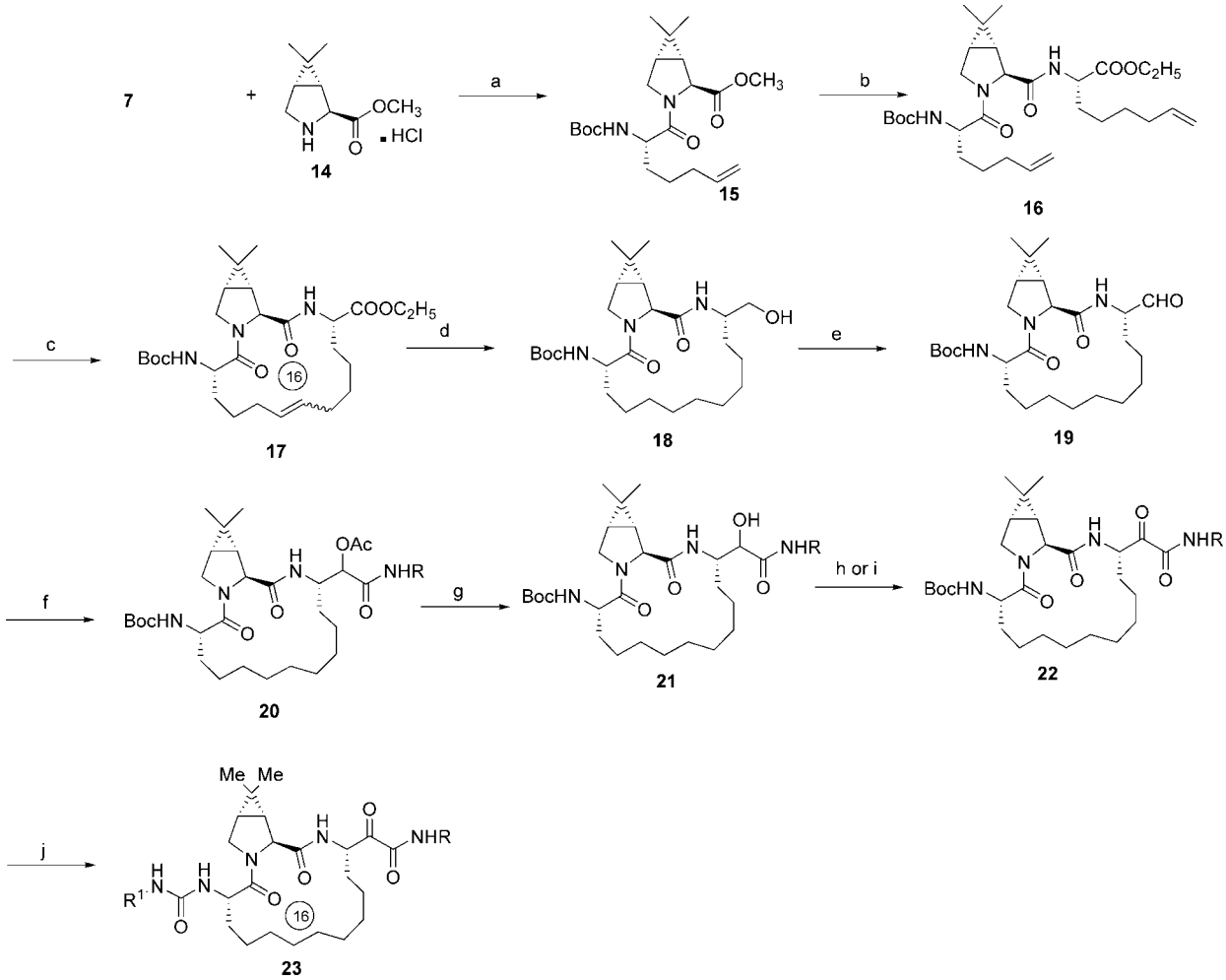
The double bond containing inhibitor **24** as an (*E,Z*)-mixture had excellent enzyme binding (K_i* = 0.005 μM) and desirable HNE/HCV selectivity. It was encouraging to observe that this compound had a good cellular activity in the replicon cellular assay (EC_{90} = 0.3 μM). The saturated macrocyclic analogue **25** was equipotent in the enzyme binding assay (K_i* = 0.006 μM) with cellular potency of EC_{90} = 0.6 μM. Replacement of the Boc group with *tert*-butylurea resulted in compound **26** with K_i* = 0.006 μM and EC_{90} = 1.0 μM. The P_1-P_3 macrocyclic scaffold provided inhibitors that were as potent as those derived

Scheme 1[a]



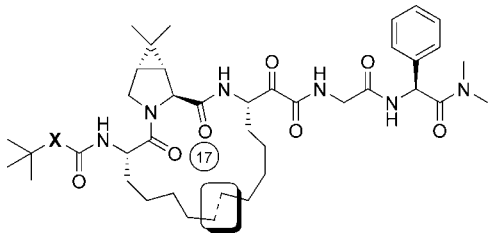
[a] Reagents and conditions: (a) (i) $AcHNCH(COOH)COOC_2H_5$, Ac_2O, pyridine, room temp, 24 h; (b) H_2, Rh(Et-Duphos]OTf, ethanol, room temp, 0.5 h; (c) Boc_2O, DMAP, THF, reflux; (d) aq LiOH, room temp; (e) PCC, CH_2Cl_2, room temp, 12 h; (f) (i) Boc_2O, DMAP, THF reflux; (ii) N_2H_4, ethanol, room temp; (g) 4 M HCl in dioxane, room temp, 1 h.

Scheme 2[a]



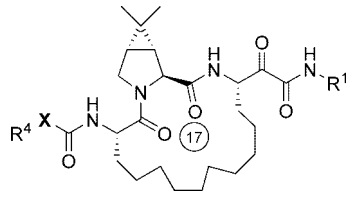
[a] Reagents and conditions: (a) HATU, CH_2Cl_2/DMF, NMM; (b) (i) aq LiOH, THF, room temp; (ii) **13**, HATU, CH_2Cl_2/DMF, NMM; (c) $(Cy_3P)_2RuCl_2CHC_6H_5$, toluene, 60 °C; (d) (i) H_2, Pd/C, EtOAc; (ii) $LiBH_4$, THF, room temp; (e) Dess−Martin periodinane, CH_2Cl_2, room temp; (f) RNC, AcOH, room temp; (g) aq LiOH, THF, room temp; (h) when R = H, DMSO, EDCI·HCl, $Cl_2CHCOOH$, toluene; (i) when R ≠ H, Dess−Martin periodinane, CH_2Cl_2, room temp; (j) (i) 4 M HCl dioxane; (ii) R^1NCO, NMM, CH_2Cl_2.

Table 1



Cpd.	X		*K_i* (μM)*	*HNE/ HCV*	*EC_{90} (μM)*
24	O		0.005	1400	0.30
25	O		0.006	780	0.60
26	NH		0.006	980	1.0

Table 2



Cpd.	X	R^4	R^1	*K_i* (μM)*	*EC_{90} (μM)*
27	O		NHBn	0.089	2.00
28	O			6.0	NA
29	O		H	0.059	0.90
30	NH		H	0.059	1.0
31	NH		H	0.036	0.40
32	NH	^{t}BuOOC	H	0.037	0.40

from acyclic series. From our previous experience in the discovery and development of boceprevir (**1**), we hypothesized that truncation would provide inhibitors with improved PK and cellular potencies. The effects of these modifications are outlined in Table 2.

Results from Table 2 demonstrate that truncations had a profound effect on the enzyme binding of these inhibitors. Replacement of $P_1'-P_2'$ glycine−phenylglycine fragment of compound **25** with Gly-NHBn yielded compound **27** with $K_i^* = 0.089\ \mu M$ and $EC_{90} = 2.0\ \mu M$ resulting in a 20-fold loss in activity compared to **25**. Further truncation by replacement of $P_1'-P_2'$ glycine−phenylglycine with *tert*-butylamide resulted in compound **28** with $K_i^* = 6.0\ \mu M$. However, replacement of $P_1'-P_2'$ glycine−phenylglycine with primary amide resulted in compound **29** with $K_i^* = 0.059\ \mu M$ and $EC_{90} = 0.9\ \mu M$. This modification resulted in a 10-fold loss in enzyme activity compared to inhibitor **25** and in less than 2-fold loss in cellular potency. Similarly, replacement of *tert*-butyl carbamate P_3 capping of compound **29** with *tert*-butylurea resulted in compound **30**, which had a similar activity as the Boc derivative **29**. To further improve binding and cellular activity, we explored the replacement of P_3-*tert*-butyl carbamate of **29** with α-methylcyclohexylurea, resulting in compound **31** with $K_i^* = 0.036\ \mu M$ and $EC_{90} = 0.40\ \mu M$. This was encouraging because the cellular activity of compound **31** was similar to our first generation clinical compound **1**. Similarly, the introduction of *tert*-butylglycine derivative as P_3 capping resulted in compound **32** ($K_i^* = 0.037\ \mu M$ and $EC_{90} = 0.40\ \mu M$), which had a binding activity similar to **39**, clearly indicating that modification of P_3 capping in the primary amide series of inhibitors would allow improvement in enzyme and cellular activity.

Effect of Macrocyclization. In order to evaluate the effect of macrocyclization, the binding efficiency of the acyclic compound compared to cyclic derivative (Scheme 3) was evaluated. The acyclic derivative **33** had $K_i^* = 0.25\ \mu M$, whereas the 17-membered cyclic compound **27** had $K_i^* = 0.059\ \mu M$. Thus, macrocyclization of **33** resulted in ∼5-fold improvements in binding activity. Macrocyclization of the P_1 and P_3 residues resulted in preorganizing the binding conformation of the inhibitor, thus contributing to improved potency.

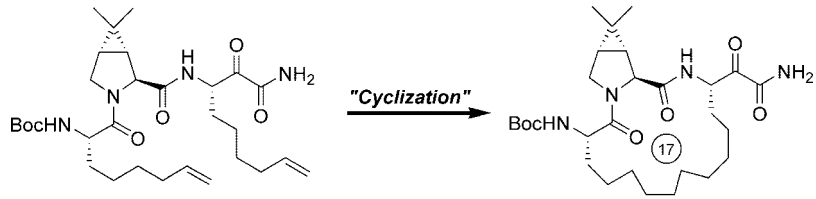
Effect of Ring Size. In an effort to identify the optimal ring size, macrocyclic inhibitors ranging from 14 to 17 membered rings were synthesized and evaluated for their enzyme activity. Inhibitors containing 14, 15, and 17 membered ring were synthesized using similar methods outlined in Scheme 2 by appropriately changing the combination of P_1 and P_3 amino acids. Results from these compounds are tabulated in Table 3.

From Table 3 it was evident that the 15- and 16-membered rings were the most preferred. Thus, the 15-membered compound **35** had a $K_i^* = 0.036\ \mu M$ and $EC_{90} = 1.0\ \mu M$. Similarly the 16-membered compound **36** had $K_i^* = 0.03\ \mu M$ and $EC_{90} = 0.6\ \mu M$. The activities of these compounds were marginally better than the 17-membered compound **29**. However, the 14-membered compound **34** ($K_i^* = 0.210\ \mu M$) demonstrated much diminished binding activity in comparison to the larger ring sized systems. It was therefore concluded that a minimum of a 15-membered ring was required for the compound to exhibit desirable potency. We therefore decided to evaluate SAR with a 16-membered macrocycle and subsequently to extend important findings to 15- and 17-membered rings as well.

Since we had observed from previous SARs (Table 2, compounds **31** and **32**) that modification of P_3 capping improved both enzyme binding potency and replicon cellular activity, we decided to extend the studies further. We embarked on incorporating these residues to evaluate their effect on the activity of **36**. A summary of these modifications is shown in Table 4.

The introduction of P_3 caps had a profound effect in improving enzyme binding and replicon cellular activity. Thus, incorporation of cyclopropyl derived ketone moiety resulted in compounds **37** ($K_i^* = 0.011\ \mu M$ and $EC_{90} = 0.1\ \mu M$) and **38** ($K_i^* = 0.011\ \mu M$ and $EC_{90} = 0.18\ \mu M$), a 5-fold improvement in enzyme binding and 5- to 10-fold enhancement in cellular activity compared to *tert*-butylurea compound **29**. Similarly, introduction of a *tert*-butylglycineurea P_3 cap that was further modified with methylsulfonamide and thiophenesulfonamide

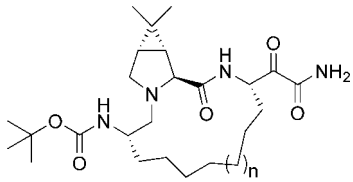
Scheme 3



33
K_i^* =0.25 μM

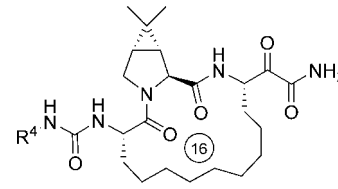
27
K_i^* =0.059 μM

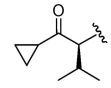
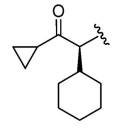
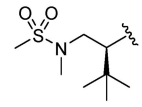
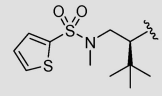
Table 3



Cpd.	n	Ring Size	K_i* (μM)	EC_{90} (μM)
34	0	14	0.210	NA
35	1	15	0.036	1.0
36	2	16	0.030	0.6
29	3	17	0.059	1.0

Table 4



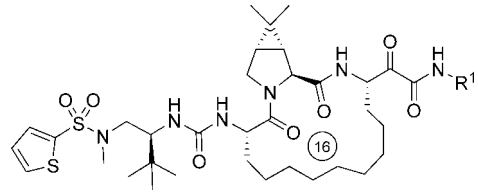
Cpd.	R^4	K_i* (μM)	EC_{90} (μM)
30		0.059	1.0
37		0.011	0.10
38		0.011	0.18
39		0.003	0.25
40		0.002	0.07

resulted in compounds **39** (K_i* = 0.003 μM and EC_{90} = 0.25 μM) and **40** (K_i* = 0.002 μM and EC_{90} = 0.07 μM), respectively. It is noted that the thiophenesulfonamide derived compound **40** had K_i* = 0.002 μM, a 25-fold improvement in potency compared to the Boc compound **29**, and a cellular potency of EC_{90} = 0.07 μM, a 15-fold improvement in activity over **29**. This was the first time in this series that we achieved a compound with $EC_{90} \leq 0.1$ μM. Having established that compounds with excellent cellular activities could be achieved, we evaluated the PK properties of compound **40**. Analysis of **40** in rapid rat assay indicated plasma levels of **40** were low (AUC = 0.07 μM·h), presumably because of poor absorption. Our previous experience in the acyclic series had clearly demonstrated that the replacement of P_1' primary amide with secondary amides greatly improved PK profiles of the inhibitors. However, in most cases this change was accompanied with loss in activity. In an effort to improve the potency and PK of these molecules, we studied the structure−activity relationship at the P_1' region. Small aliphatic isocyanides were synthesized and incorporated using methods outlined in Scheme 3. Compounds resulting from these modifications are outlined in Table 5.

Introduction of small aliphatic groups at P_1' was well tolerated. Thus, incorporation of ethyl- and propylamide moieties at P_1' resulted in compounds **41** (K_i* = 0.010 μM and EC_{50} = 0.030 μM) and **42** (K_i* = 0.014 μM and EC_{50} = 0.080 μM), respectively. These compounds were slightly less potent than the corresponding primary amide derivative **40** (K_i* = 0.002 μM and EC_{50} = 0.020 μM). Similarly, introduction of cyclopropylmethylamide as the P_1' group resulted in compound **43** (K_i* = 0.021 μM and EC_{50} = 0.050 μM), which was less active than the corresponding primary amide analogue **40**. However, the introduction of allyl amide at P_1' was well tolerated resulting in compound **44** (K_i* = 0.005 μM and EC_{50} = 0.020 μM). The allylamide derivative **44** was equipotent to the corresponding primary amide compound **40** and demonstrated good oral plasma levels in rats (AUC = 1.26 μM·h). Since the P_1' allylamide group provided the combination with the best activity and acceptable PK, we further investigated the modification of P_3 capping residues in this series. The improved activity of compound **44** compared to **42** may result from the π-character of the double bond in the allyl group.

A wide range of P_3 caps were selected and incorporated in these inhibitors to evaluate their activities. These results are summarized in Table 6. Many of the P_1' allyl amide derivatives demonstrated excellent enzyme binding (K_i*) and replicon cellular potency combined with good PK in rats. The allylamide derivatives **45** and **46** demonstrated K_i* = 0.020 and 0.006 μM and EC_{90} = 0.48 and 0.28 μM, respectively. The cellular activities of these compounds were slightly worse than the corresponding primary amide analogues **37** and **38**. However, the introduction of allylamide in inhibitors that contained other modified P_3 caps demonstrated improved K_i* as well as EC_{90}. Thus, the methylsulfonamide compound **47** had a binding K_i* = 0.003 μM and EC_{90} = 0.055 μM and the 2-pyridylsulfona-

Table 5[a]



Cpd.	R^1	K_i* (μM)	EC_{50} (μM*)	*Rat PK (PO) AUC (μM.h.)*[20]
41		0.010	0.030	-
42		0.014	0.080	-
43		0.021	0.050	0.4
44		0.005	0.020	1.26

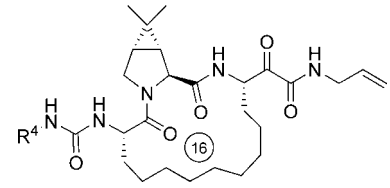
[a] (∗) EC_{50} was used for comparison, since EC_{90} could not be determined for all compounds.

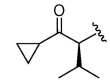
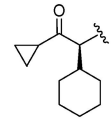
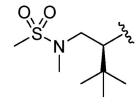
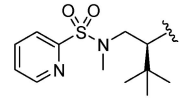
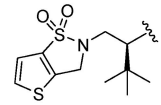
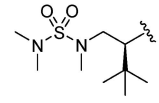
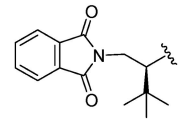
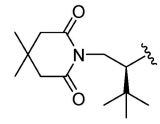
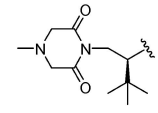
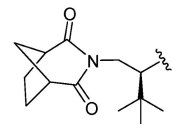
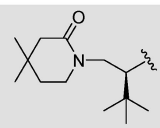
mide derived inhibitor **48** had an enzyme activity of K_i* = 0.007 μM and EC_{90} = 0.090 μM. Synthesis of the cyclic sulfonamide derivative of inhibitor **40** resulted in compound **49** (K_i* = 0.007 μM and EC_{90} = 0.15 μM), which was less potent than acyclic analogue **40**. Introduction of dimethylsulfonylurea derived P_3 capping resulted in compound **50** with K_i* = 0.014 μM and EC_{90} = 0.06 μM. It was encouraging to note that the dimethylsulfonylurea compound **50** demonstrate reasonable PK with AUC = 1.04 μM·h in rats when dosed orally at 5 mg/kg.

The introduction of imide derived P_3 caps had a profound effect on the cellular activity of the P_1−P_3 macrocyclic inhibitors. Thus, incorporation of pthalimide derived cap resulted in compound **51** with K_i* = 0.016 μM and EC_{90} = 0.1 μM, which was less potent than **50**. However introduction of dimethylglutarimide derived P_3 capping resulted in compound **52** (K_i* = 0.001 μM and EC_{90} = 0.020 μM). This resulted in a marked improvement in replicon cellular potency compared to the primary amide analogue **40**. It also demonstrated good plasma levels in rats with AUC = 1.1 μM·h. Similarly, introduction of a bicyclic derived imide cap resulted in compound **54**, which once again demonstrated excellent binding (K_i* = 0.003 μM) and improved replicon cellular activity (EC_{90} = 0.015 μM). The modification of the dimethylglutarimide moiety to the corresponding dimethyl lactam derivative resulted in inhibitor **55** (K_i* = 0.002 μM and EC_{90} = 0.020 μM), which had similar activity to **52** and also demonstrated good plasma levels in rats with AUC = 1.5 μM·h.

The X-ray structure of inhibitor **52** bound to HCV NS3 protease was solved and is shown in Figure 4. From the structure, it was clear that the aliphatic chain linking the P_1 and P_3 made excellent contact in the lypophilic region connecting the S_1 and S_3 pockets. The P_2 (1*R*,5*S*)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane ring adopted a bent conformation that allowed maximum overlap of the methylenes of proline and cyclopropyl ring to Ala-156. The conformation adopted by cyclopropyl group allowed the methyl group proximal to the carbonyl to interact with His-57 and the methyl group distal to carbonyl to interact with Ala-156 and Arg-155. The *tert*-butyl group of P_3 imide capping occupied the S_4 region of space,

Table 6[a]



Cpd.	R^4	K_i* (μM)	EC_{90} (μM)	*Rat PK (PO) AUC (μM.h.)*[20]†
45		0.020	0.48	NA
46		0.006	0.28	NA
47		0.003	0.055	0.36
48		0.007	0.090	0.84
49		0.007	0.150	0.84
50		0.013	0.060	1.04*
51		0.016	0.100	1.2
52		0.002	0.020	1.1
53		0.004	0.030	0.28
54		0.003	0.015	0.31
55		0.002	0.020	1.5

[a] (†) The po dosing was at 10 mg/kg in 0.4% hpmc, and AUC was measured from 0 to 6 h. (∗) The po dosing was 5 mg/kg in 0.4% hpmc.

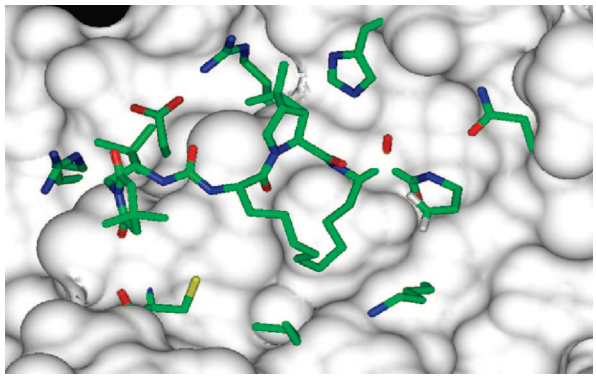


Figure 4. X-ray structure of inhibitor **52** bound to NS3 protease.

making excellent van der Walls contact with the protein, and the carbonyl of the imides group formed a hydrogen bond with Csy-159. The electrophilic ketoamide group reversibly trapped Ser-139 to form a covalent bond with the enzyme, and the hydrogen of the P_1' allylamide donated a hydrogen bond to the peptidic backbone of the protein, locking the inhibitor to the surface.

In addition to van der Waals contacts, inhibitor **52** formed a series of specific hydrogen bonds with the protein surface. When the various hydrogen bonding interactions that existed between inhibitor **52** and NS3 protease were mapped out, it was evident that the P_1' allylamide donated a hydrogen bond to the protein, and the carbonyl oxygen in turn made two hydrogen bonds with the nitrogens of Ser-139 and Gly-137. The urea nitrogens donated two hydrogen bonds to Ala-157, thus improving potency and HCV specificity. Additionally, the nitrogen of the P_1 residue donated a hydrogen bond to Arg-155 and the oxygen of P_3 carbonyl group accepted a hydrogen bond from Ala-157. In combination with hydrophobic interaction the array of hydrogen bonds also contributed greatly to the binding potency and selectivity of compound **52**.

Conclusions

Analysis of the X-ray structure of the acyclic series of inhibitors bound to NS3 protease clearly revealed the proximity of S_1 and S_3 sites. This prompted the syntheses of P_1-P_3 macrocyclic series of inhibitors that could potentially bind to the enzyme in a preorganized binding conformation. Guided by modeling, a 17-membered ring was initially determined to be the optimal size. Macrocyclic inhibitors spanning from P_3-P_2' were synthesized using Grubbs olefin metathesis reaction. These inhibitors bound to protein with excellent enzyme binding and displayed moderate cellular activity in the replicon based cellular assay ($EC_{90} \approx 0.5-1.0$ μM). In an attempt to improve cellular activity, P_1' primary amide compounds similar to **1** were synthesized. These inhibitors were active and had further improved cellular activity to our first generation clinical candidate (i.e., $EC_{90} \approx 0.35$ μM). SAR studies in the primary amide series of inhibitors clearly demonstrated a 16-membered ring as the optimal ring size.

SAR in the 16-membered macrocycle demonstrated that the introduction of novel P_3 caps further improved cellular activity. Introduction of these P_3 caps allowed identification of inhibitors that had greatly improved cellular activity compared to our first generation clinical candidate, boceprevir. However, these compounds had poor PK in rats. In an attempt to obtain compounds with better plasma exposure, secondary amides were explored. Structure−activity relationship at the P_1' site identified allylamide as an optimal P_1' residue that was well tolerated. Reinvestigation of P_3 capping in the allylamide series identified compounds with enzyme binding and cellular activity ($EC_{90} \approx 0.05-0.06$ μM) improved over **1**. The introduction of imide derived P_3 caps provided compounds with improved cellular activity by an additional 3-fold, leading to compound **52** with $EC_{90} = 0.02$ μM, ~20 times more potent than our first generation compound **1**. These compounds not only had greatly improved cellular activity but also had good plasma level when evaluated for PK in rats and are being further evaluated for PK in higher species. We thus clearly demonstrated that P_1-P_3 macrocyclic inhibitors that incorporated a ketoamide warhead demonstrated excellent binding and cellular activity, which was optimized to generate compounds that were more potent than **1**. X-ray structure of inhibitor **52** bound to enzyme also revealed a crucial hydrogen bonding of the imide oxygens to Cys-159 that contributed to improved potency and improved cellular activity.

Experimental Section

General. Dry solvents were purchased from Aldrich or Acros and used without further purification. Other solvents or reagents were used as obtained except when otherwise noted. Analytical thin layer chromatography (TLC) was performed on precoated silica gel plates available from Analtech. Column chromatography were performed using Merck silica gel 60 (particle size 0.040−0.055 mm, 230−400 mesh) or using Biotage or Isco chromatographic systems. All compounds were synthesized as a single diastereomer at P_1, and most compounds were further purified using Varian normal phase HPLC with YMC-diol column with solvent system solvent A (hexanes) and solvent B (a mixture of isopropanol, CH_2Cl_2, and acetonitrile). Visualization was accomplished with UV light or by staining with basic $KMnO_4$ solution, methanolic H_2SO_4, or Vaughn's reagent. NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 unless otherwise noted at 300, 400, or 500 MHz (^{1}H NMR), or 75, 100, or 125 MHz (^{13}C NMR). Mass spectra were obtained using electron spray or FAB ionization methods.

1,1-Dimethylethyl-[(17a*S*,18a*R*,18b*S*)-3(*S*)-[2-[[2-[[2-(dimethylamino)-2-oxo-1(*S*)-phenylethyl]amino]-2-oxoethyl]amino]-1,2-dioxoethyl]-1,2,3,4,5,6,7,10,11,12,13,14,15,17,17a,18,18a,18b-octadecahydro-18,18-dimethyl-1,15-dioxocyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacycloheptadecin-14(*S*)-yl]carbamate [24]. ^{1}H NMR (500 MHz, DMSO-d_6), δ, 8.78−8.76 (m, 1 H), 8.60−8.29 (m, 2 H), 7.36−7.31 (m, 5 H), 5.83 (d, 1 H, J =7.9 Hz), 5.26−5.13 (m, 2 H), 4.40−3.66 (m, 8 H), 2.93 (s, 3 H), 2.85 (s, 3 H), 2.02−0.85 (bm, 18 H), 1.35 (s, 9 H), 0.99 (s, 3 H), 0.88 (s, 3 H). MS (ESI, *m*/*z*, relative intensity), 773 [(M + 1)$^+$, 70], 751 [(M + 1)$^+$, 60], 651 (100).

1,1-Dimethylethyl-[(17a*S*,18a*R*,18b*S*)-3(*S*)-[2-[[2-[[2-(dimethylamino)-2-oxo-1(*S*)-phenylethyl]amino]-2-oxoethyl]amino]-1,2-dioxoethyl]eicosahydro-18,18-dimethyl-1,15-dioxocyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacycloheptadecin-14(*S*)-yl]carbamate [25]. ^{1}H NMR (400 MHz, DMSO-d_6), δ, 8.77 (t, 1H, J = 5.9 Hz), 8.55 (d, 1 H, J = 7.3 Hz), 8.32 (d, 1 H, J = 8.8 Hz), 7.35−7.28 (m, 5 H), 7.05 (d, 1 H, J = 6.1 Hz), 5.80 (d, 1 H, J = 8.1 Hz), 5.27 (bt, 1 H, J = 8.8 Hz), 4.32 (s, 1 H), 4.10−3.96 (m, 2 H), 3.81−3.70 (m, 3 H), 2.91 (s, 3 H), 2.83 (s, 3 H), 1.84−0.84 (m, 22 H), 1.33 (s, 9 H), 0.99 (s, 3 H), 0.89 (s, 3 H). MS (ESI, *m*/*z*, relative intensity), 775 [(M + 1)$^+$, 70], 753 [(M + 1)$^+$, 60], 653 (100), 277 (80), 232 (60), 148 (80), 117 (95).

1,1-Dimethylethyl-[(17a*S*,18a*R*,18b*S*)-3(*S*)-[2-[(1,1-dimethylethyl)amino]-1,2-dioxoethyl]eicosahydro-18,18-dimethyl-1,15-dioxocyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacycloheptadecin-14(*S*)-yl]carbamate [28]. ^{1}H NMR (500 MHz, DMSO-d_6), δ, 8.30 (d, 1 H, J = 9.1 Hz), 7.97 (s, 1 H), 7.05 (d, 1 H, J = 7.9 Hz), 5.23 (t, 1 H, J =9.5 Hz), 4.34 (s, 1 H), 4.10 (bs, 1 H), 4.01 (d, 1 H, J = 10.1 Hz), 3.75 (dd, 1 H, J = 5.7 and 4.8 Hz), 1.79−1.15 (m, 22 H), 1.35 (s, 9 H), 1.31 (s, 9 H), 0.99 (s, 3 H), 0.91 (s, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6), δ, 171.9, 171.0, 162.3, 155.9, 78.7, 60.4, 52.4, 52.3, 51.7, 47.7, 32.1, 31.9, 31.2, 27.9, 27.6, 27.4, 26.9,

26.9, 26.3, 24.8, 23.2, 19.6, 13.5, MS (ESI, *m/z*, relative intensity), 591 [(M + 1)$^+$, 70], 491 (100).

1,1-Dimethylethyl-[(17a*S*,18a*R*,18b*S*)-3(*S*)-(2-amino-1,2-dioxoethyl)-eicosahydro-18,18-dimethyl-1,15-dioxocyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacycloheptadecin-14(*S*)-yl]carbamate [29]. ^{1}H NMR (400 MHz, DMSO-d_6), δ, 8.29 (d, 1 H, J = 8.8 Hz), 8.03 (s, 1 H), 7.77 (s, 1 H), 7.04 (d, 1 H, J = 7.7 Hz), 5.24 (t, 1 H, J = 10.4 Hz), 4.32 (s, 1 H), 4.08 (bt, 1 H), 3.98 (d, 1 H, J = 9.3 Hz), 3.72 (dd, 1 H, J = 4.4 and 5.5 Hz), 1.77−1.11 (m, 22 H), 1.32 (s, 9 H), 0.98 (s, 3 H), 0.89 (s, 3 H). MS (ESI, *m/z*, relative intensity), 557 [(M + Na)$^+$, 10]$^+$, 535 (10), 435 (80), 390 (100).

(17a*S*,18a*R*,18b*S*)-14(*S*)-[[[(1,1-Dimethylethyl)amino]carbonyl]amino]eicosahydro-18,18-dimethyl-α,1,15-trioxocyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacycloheptadecine-3(*S*)-acetamide [30]. ^{1}H NMR (500 MHz, DMSO-d_6), δ, 8.32 (d, 1 H, J = 9.0 Hz), 8.06 (s, 1 H), 7.80 (s, 1 H), 5.90 (d, 1 H, J = 9.5 Hz), 5.76 (s, 1 H), 5.27−5.23 (m, 1 H), 5.35 (s, 1 H), 4.30−4.23 (m, 1 H), 3.99 (bd, 1 H, J = 10.0 Hz), 3.75 (dd, 1 H, J = 3.0 and 4.0 Hz), 1.18 (s, 9 H), 1.48−0.89 (m, 22 H), 1.00 (s, 3 H), 0.89 (s, 3 H). MS (ESI, *m/z*, relative intensity), 556 [(M + Na)$^+$, 15]$^+$, 534 (35), 450 (85), 435 (100), 126 (70)

(17a*S*,18a*R*,18b*S*)-Eicosahydro-18,18-dimethyl-14(*S*)-[[[(1-methylcyclohexyl)amino]carbonyl]amino]-α,1,15-trioxocyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacycloheptadecine-3(*S*)-acetamide [31]. ^{1}H NMR (500 MHz, DMSO-d_6), δ, 8.32 (d, 1 H, J = 9.1 Hz), 8.05 (s, 1 H), 7.80 (s, 1 H), 6.09−6.01 (m, 1 H), 5.57 (s, 1 H), 5.24 (bt, 1 H, J = 10.4), 4.35 (s, 1 H), 4.30−4.25 (m, 1 H), 3.99 (bd, 1 H, J = 11.0 Hz), 3.75−3.72 (m, 1 H), 1.89−1.15 (m, 35 H), 1.00 (s, 3 H), 0.88 (s, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6), δ, 199.0, 171.9, 157.3, 60.2, 52.4, 51.7, 50.9, 47.6, 37.7, 37.2, 32.8, 31.8, 31.1, 30.4, 29.0, 28.3, 27.9, 27.6, 27.0, 26.9, 26.4, 26.1, 24.9, 23.4, 22.2, 19.5, 13.6. MS (ESI, *m/z*, relative intensity), 574 [(M + 1)$^+$, 40], 435 (100).

1,1-Dimethylethyl-[(15a*S*,16a*R*,16b*S*)-3(*S*)-(2-amino-1,2-dioxoethyl)-octadecahydro-16,16-dimethyl-1,13-dioxocyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacyclopentadecin-12(*S*)-yl]carbamate [35]. ^{1}H NMR (500 MHz, DMSO-d_6), δ, 8.34 (d, 1 H, J = 9.1 Hz), 8.05 (s, 1 H), 7.79 (s, 1 H), 6.93 (d, 1 H, J =7.6 Hz), 5.28 (dt, 1 H, J = 2.8 and 11.4 Hz), 4.40 (s, 1 H), 4.11 (bt, 1 H, J = 8.1 Hz), 3.98 (d, 1 H, J = 9.8 Hz), 3.79 (dd, 1 H, J = 5.4 and 4.7 Hz), 1.88−1.72 (m, 2 H), 1.48−1.06 (m, 16 Hz), 1.35 (s, 9 H), 1.01 (s, 3 H), 0.90 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$), δ, 198.9, 171.7, 171.2, 164.3, 155.8, 78.8, 60.3, 52.6, 52.4, 47.7, 31.2, 30.7, 30.5, 29.0, 28.9, 28.3, 28.1, 27.0, 26.7, 26.1, 25.7, 24.5, 24.0, 19.5, 13.5. MS (ESI, *m/z* relative intensity), 507 [(M + 1)$^+$, 65], 450 (25), 407 (100).

1,1-Dimethylethyl[(13*S*,16a*S*,17a*R*,17b*S*)-3-(2-amino-1,2-dioxoethyl)-octadecahydro-17,17-dimethyl-1,14-dioxo-2*H*-cyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacyclohexadecin-13-yl]carbamate [36]. ^{1}H NMR (500 MHz, DMSO-d_6), δ, 8.34 (d, 1 H, J = 9.5 Hz), 8.06 (s, 1 H), 7.80 (s, 1 H), 7.09 (d, 1 H, J = 7.6 Hz), 5.31 (t, 1 H, J = 11.0 Hz), 4.36 (s, 1 H), 4.11−4.05 (m, 1 H)), 3.99 (d, 1 H, J = 10.4 Hz), 3.76 (dd, 1 H, J = 5.4 and 4.7 Hz), 1.88−1.19 (m, 20 H), 1.34 (s, 9 H), 1.01 (s, 3 H), 0.92 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$), δ, 198.8, 172.0, 171.2, 164.2, 155.9, 78.7, 60.7, 52.5, 52.4, 47.7, 42.9, 36.5, 31.8, 31.6, 31.2, 31.3, 28.9, 28.6, 28.1, 27.9, 27.4, 26.9, 26.5, 26.4, 24.2, 22.9, 22.2, 19.6, 14.8, 13.5. MS (ESI, *m/z* relative intensity), 521 [(M + 1)$^+$, 100], 421 (40).

(16a*S*,17a*R*,17b*S*)-13(*S*)-[[[[1(*S*)-(cyclopropylcarbonyl)-2-methylpropyl]amino]carbonyl]amino]octadecahydro-17,17-dimethyl-α,1,14-trioxo-2*H*-cyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacyclohexadecine-3(*S*)-acetamide [37]. ^{1}H NMR (500 MHz, DMSO-d_6), δ, 8.32 (d, 1 H, J = 9.4 Hz), 8.04 (s, 1 H), 7.78 (s, 1 H), 6.38 (d, 1 H, J = 8.2 Hz), 6.23 (d, 1 H, J = 8.5 Hz), 5.30 (t, 1 H, J = 9.5 Hz, 4.36 (s, 1 H), 4.33 (dd, 1 H, J = 4.7 and 4.1 Hz), 4.29−4.24 (m, 1 H), 3.89 (d, 1 H, J = 10.4 Hz), 3.77 (dd, 1 H, J = 5.4 and 4.7 Hz), 2.24−2.13 (m, 2 H), 1.72−0.71(m, 30 H), 1.00 (s, 3 H), 0.86 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$), δ, 210.8, 198.7, 172.0, 171.4, 164.2, 157.9, 64.5, 60.5, 52.6, 51.4, 47.6, 32.2, 31.5, 31.3, 30.1, 28.3, 27.8, 28.0, 27.3, 26.9, 26.6, 26.2, 24.4, 22.4, 20.4, 19.4, 18.8, 17.8, 13.6, 11.6, 11.2. MS (ESI, *m/z* relative intensity), 588 [(M + 1)$^+$, 100], 421(40).

(16a*S*,17a*R*,17b*S*)-13(*S*)-[[[[1(*S*)-Cyclohexyl-2-cyclopropyl-2-oxoethyl]amino]carbonyl]amino]octadecahydro-17,17-dimethyl-α,1,14-trioxo-2*H*-cyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacyclohexadecine-3(*S*)-acetamide [38]. ^{1}H NMR (500 MHz, DMSO-d_6), δ, 8.34 (d, 1 H, J = 9.1 Hz), 8.05 (s, 1 H), 7.79 (s, 1 H), 6.36 (d, 1 H, J = 8.8 Hz), 6.26 (d, 1 H, J = 8.8 Hz), 5.30 (t, 1 H, J = 9.0 Hz), 4.36 (s, 1 H), 4.36 (dd, 1 H, J = 5.0 and 4.7 Hz), 4.29−4.25 (m, 1 H), 3.89 (dd, 1 H, J = 10.4 Hz), 3.77 (q, 1 H, J = 5.4 Hz), 2.18−2.13 (m, 2 H), 1.80−0.76 (m, 34 H), 1.00 (s, 3 H), 0.88 (s, 3 H). MS (ESI, *m/z* relative intensity) 628 [(M + 1)$^+$, 20], 421 (10), 130 (100).

(16a*S*,17a*R*,17b*S*)-13(*S*)-[[[[2,2-Dimethyl-1(*S*)-[[methyl(methylsulfonyl)amino]methyl]propyl]amino]carbonyl]amino]octadecahydro-17,17-dimethyl-α,1,14-trioxo-2*H*-cyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacyclohexadecine-3(*S*)-acetamide [39]. ^{1}H NMR (DMSO-d_6, 500 MHz), δ, 8.32 (d, 1 H, J = 9.0 Hz), 8.03 (s, 1 H), 7.77 (s, 1 H), 6.21 (d, 1 H, J = 8.8 Hz), 5.87 (s, 1 H), 5.28 (t, 1 H, J =10.1 Hz), 4.34 (s, 1 H), 4.26−4.22 (m, 1 H), 3.85 (d, 1 H, J = 10.1 Hz), 3.75 (dd, 1 H, J =10.1 and 5.4 Hz), 3.59 (d, 1 H, J = 13.6 Hz), 3.41 (d, 1 H, J = 13.9 Hz), 3.31 (s, 6 H), 2.21−2.11 (m, 2 H), 1.67−1.06 (m, 18 H), 1.24 (s, 9 H), 0.99 (s, 3 H), 0.87 (s, 3 H). ^{13}C NMR (DMSO-d_6, 125 MHz), δ 209.2, 182.5, 181.8, 174.7, 167.7, 70.9, 70.6, 64.8, 63.1, 61.7, 61.5, 58.0, 45.8, 45.6, 42.8, 42.0, 41.8, 38.7, 38.5, 38.4, 37.7, 37.5, 37.5, 37.4, 37.2, 37.0, 36.6, 36.3, 35.0, 33.8, 32.9, 32.0, 32.0, 29.9, 24.1. MS (ESI, *m/z* relative intensity) 693 [(M + K)$^+$, 15], 677 [(M + Na)$^+$, 25], 655 [(M + 1)$^+$, 100]. HRMS calcd for $C_{31}H_{54}N_6NaO_7S$ [M + 1]$^+$: 677.3672. Found: 677.3685.

(16a*S*,17a*R*,17b*S*)-13(*S*)-[[[[2,2-Dimethyl-1(*S*)-[[methyl(2-thienylsulfonyl)amino]methyl]propyl]amino]carbonyl]amino]-*N*-ethyloctadecahydro-17,17-dimethyl-α,1,14-trioxo-2*H*-cyclopropa[3,4]pyrrolo[1,2-*a*]-[1,4]diazacyclohexadecine-3(*S*)-acetamide [41]. ^{1}H NMR (500 MHz, DMSO-d_6), δ, 8.71 (t, 1 H, J = 5.7 Hz), 8.35 (d, 1 H, J = 8.8 Hz), 8.00 (dd, 1 H, J = 1.3 and 5.0 Hz), 7.65 (d, 1 H, J = 3.8 Hz), 7.26 (dd, 1 H, J = 3.8 and 1.0 Hz), 6.15 (d, 1 H, J = 8.8 Hz), 5.89 (d, 1 H, J = 9.7 Hz), 5.31 (t, 1 H, J = 10.7 Hz), 4.35 (s, 1 H), 4.29 (bt, 1 H, J = 9.1 Hz), 3.93 (d, 1 H, J = 9.8 Hz), 3.77 (dd, 1 H, J = 5.4 and 4.7 Hz), 3.64 (dt, 1 H, J = 3.2 and 8.8 Hz), 3.20−3.11 (m, 2 H), 2.96−2.87 (m, 2 H), 2.68 (s, 3 H), 1.75−1.59 (m, 2 H), 1.47−0.83 (m, 21 H), 0.99 (s, 3 H), 0.85 (s, 3 H), 0.81 (s, 9 H). ^{13}C NMR (125 MHz, $CDCl_3$), δ, 198.4, 172.0, 171.7, 161.8, 158.2, 137.8, 133.8, 133.0, 128.9, 60.4, 55.7, 52.7, 51.6, 51.4, 47.6, 36.0, 35.0, 34.3, 32.2, 31.6, 31.3, 28.4, 28.0, 27.8, 27.4, 27.0, 26.9, 26.6, 26.3, 24.4, 22.3, 19.4, 15.1, 13.6. MS (ESI, *m/z* relative intensity), 773 [(M + Na)$^+$, 100], 751 [(M + 1)$^+$, 60].

(16a*S*,17a*R*,17b*S*)-13(*S*)-[[[[2,2-Dimethyl-1(*S*)-[[methyl(2-thienylsulfonyl)amino]methyl]propyl]amino]carbonyl]amino]octadecahydro-17,17-dimethyl-α,1,14-trioxo-*N*-propyl-2*H*-cyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacyclohexadecine-3(*S*)-acetamide [42]. ^{1}H NMR (500 MHz, DMSO-d_6), δ, 8.71 (t, 1 H, J = 6.0 Hz), 8.36 (d, 1 H, J = 9.1 Hz), 8.00 (dd, 1 H, J = 1.3 and 3.8 Hz), 7.65 (dd, 1 H, J = 1.3 and 2.5 Hz), 7.26 (dd, 1 H, J = 3.8 and 1.3 Hz), 6.15 (d, 1H, J = 9.1 Hz), 5.88 (d, 1 H, J = 10.1 Hz), 5.31 (t, 1H, J = 9.1 Hz), 4.34 (s, 1 H), 4.30 (t, 1 H, J = 8.2 Hz), 3.93 (d, 1 H, J = 10.4 Hz), 3.77 (dd, 1 H, J = 5.4 and 4.7 Hz), 3.64 (dt, 1 H, J = 4.4 and 9.8 Hz), 3.13−3.04 (m, 2 H), 2.95−2.87 (m, 2 H), 2.67 (s, 3 H), 1.73−1.63 (m, 2 H), 1.50−1.07 (m, 20 H), 0.99 (s, 3 H), 0.84 (s, 3 H), 0.81 (s, 9 H), 0.85−0.81 (m, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6), δ,198.5, 172.0, 171.7, 162.1, 158.2, 137.7, 133.9, 133.0, 129.0, 60.4, 55.7, 55.7, 52.7, 51.6, 51.4, 47.6, 41.1, 36.0, 35.0, 32.2, 31.6, 31.2, 28.4, 28.0, 27.8, 27.4, 27.0, 26.9, 26.6, 26.3, 24.4, 22.8, 22.3, 19.4, 13.6, 12.1. MS (ES, *m/z* relative intensity) 788 [(M + Na)$^+$, 40], 766 [(M + 1)$^+$, 100].

(16a*S*,17a*R*,17b*S*)-*N*-(Cyclopropylmethyl)-13(*S*)-[[[[2,2-dimethyl-1(*S*)-[[methyl(2-thienylsulfonyl)amino]methyl]propyl]amino]carbonyl]amino]octadecahydro-17,17-dimethyl-α,1,14-trioxo-2*H*-cyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacyclohexadecine-3(*S*)-acetamide [43]. ^{1}H NMR (500 MHz, DMSO-d_6), δ, 8.80 (d, 1 H, J = 9.1 Hz), 8.00 (dd, 1 H, J = 1.3 and 3.8 Hz), 7.65 (dd, 1 H, J = 9.1 Hz), 8.00 (dd, 1 H, J = 1.3 and 3.8 Hz), 7.65 (dd, 1 H, J = 1.3 and 3.8 Hz), 7.26 (dd, 1 H, J = 3.8 and 1.3 Hz), 6.15 (d, 1 H, J = 8.5 Hz), 5.89 (d, 1H, J = 10.1 Hz), 5.32 (t, 1 H, J = 9.5 Hz), 4.35 (s, 1H), 4.29 (t, 1 H, J = 7.6 Hz), 3.93 (d, 1 H, J = 10.4 Hz), 3.77

(dd, 1 H, J = 5.4 and 4.7 Hz), 3.64 (dt, 1 H, J = 4.4 and 9.7 Hz), 3.07−2.87 (m, 4 H), 2.67 (s, 3 H), 1.70−1.62 (m, 2 H), 1.45−0.87 (m, 18 H), 0.99 (s, 3 H), 0.85 (s, 3 H), 0.81 (s, 9 H), 0.42−0.39 (m, 2 H), 0.21−0.18 (m, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$), δ, 198.5, 172.0, 171.7, 162.0, 158.2, 133.8, 133.0, 128.9, 137.7, 60.4, 55.7, 52.7, 51.4, 47.6, 43.7, 36.0, 35.0, 32.2, 31.6, 31.3, 28.4, 27.8, 28.0, 27.4, 27.1, 27.0, 26.9, 26.6, 26.3, 24.4, 22.3, 19.4, 13.6, 11.4, 4.1, 4.0. MS (ESI, *m*/*z* relative intensity), 799 [(M + Na)$^+$, 60], 777 [(M + 1)$^+$, 100].

(16a*S*,17a*R*,17b*S*)-13(*S*)-[[[[2,2-Dimethyl-1(*S*)-[[methyl(2-thienylsulfonyl)amino]methyl]propyl]amino]carbonyl]amino]octadecahydro-17,17-dimethyl-α,1,14-trioxo-*N*-(2-propenyl)-2*H*-cyclopropa[3,4]pyrrolo-[1,2-*a*][1,4]diazacyclohexadecine-3(*S*)-acetamide [44]. ^{1}H NMR (500 MHz, DMSO-d_6), δ, 8.06 (bs, 1 H), 7.62 (dd, 1 H, J = 1.3 and 3.8 Hz), 7.57 (dd, 1 H, J = 1.3 and 2.5 Hz), 7.49 (b, 1H), 7.16 (dd, 1 H, J = 3.8 and 1.3 Hz), 5.93−5.67 (m, 2 H), 5.69 (bt, 1 H, J = 8.2 Hz), 5.27−5.19 (m, 2 H), 4.72 (m, 1 H), 4.63 (s, 1 H), 4.31 (d, 1 H, J = 10.4 Hz), 4.06−3.90 (m, 5 H), 2.93−2.77 (m, 2 H), 2.85 (s, 3 H), 1.92−1.31 (m, 20 H), 1.04 (s, 3 H), 0.93 (s, 3 H), 0.91 (s, 9 H). ^{13}C NMR (125 MHz, $CDCl_3$), δ, 198.5, 173.7, 171.7, 159.3, 158.2, 138.7, 133.3, 132.2, 131.9, 127.9, 117.6, 60.7, 54.9, 53.7, 51.7, 51.0, 48.4, 42.2, 35.2, 34.5, 33.4, 32.5, 31.0, 27.9, 27.3, 27.1, 27.3, 26.9, 26.4, 25.8, 25.0, 23.3, 26.7, 19.3, 13.5. MS (ESI, *m*/*z* relative intensity) 817 [(M + Na + CH_3OH)$^+$, 60], 795 [(M + Na)$^+$, 65], 763 [(M + 1)$^+$, 100]. HRMS calcd for $C_{37}H_{58}NaN_6O_7S_2$ [M + Na]$^+$: 785.3706. Found: 785.3706.

(16a*S*,17a*R*,17b*S*)-13(*S*)-[[[[1(*S*)-(Cyclopropylcarbonyl)-2-methylpropyl]amino]carbonyl]amino]octadecahydro-17,17-dimethyl-α,1,14-trioxo-*N*-(2-propenyl)-2*H*-cyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacyclohexadecine-3(*S*)-acetamide [45]. ^{1}H NMR (500 MHz, DMSO-d_6), δ, 8.89 (t, 1 H, J = 5.7 Hz), 8.37 (d, 1 H, J = 9.1 Hz), 6.39 (d, 1 H, J = 8.8 Hz), 6.23 (d, 1 H, J = 8.8 Hz), 5.85−5.36 (m, 1H), 5.30 (t, 1 H, J = 9.7 Hz), 5.14−5.06 (m, 2 H), 4.35 (s, 1 H), 4.26 (dt, 1 H, J = 1.9 and 10.1 Hz), 3.91−3.71 (m, 4 H), 2.21−2.15 (m, 2 H), 1.73−1.64 (m, 2 H), 1.52−0.72 (m, 23 H), 1.00 (s, 3 H), 0.87 (s, 3 H), 0.70 (d, 6 H, J = 6.6 Hz). ^{13}C NMR (125 MHz, $CDCl_3$), δ, 210.8, 198.2, 172.0, 171.4, 162.1, 157.9, 135.0, 116.4, 64.5, 60.5, 52.8, 51.4, 47.6, 41.7, 32.1, 31.5, 31.2, 30.1, 28.3, 28.0, 27.8, 27.3, 26.9, 26.6, 26.2, 24.4, 22.4, 20.4, 19.4, 18.8, 17.8, 13.6, 11.7, 11.2, 8.5. MS (ESI, *m*/*z* relative intensity), 628 [(M + 1)$^+$, 100], 461 (20), 450 (10).

(16a*S*,17a*R*,17b*S*)-13(*S*)-[[[[2,2-Dimethyl-1(*S*)-[[methyl(2-pyridinylsulfonyl)amino]methyl]propyl]amino]carbonyl]amino]octadecahydro-17,17-dimethyl-α,1,14-trioxo-*N*-(2-propenyl)-2*H*-cyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacyclohexadecine-3(*S*)-acetamide [48]. ^{1}H NMR (500 MHz, DMSO-d_6), δ, 8.89 (t, 1 H, J = 5.9 Hz), 8.72 (d, 1H, J = 5.1 Hz), 8.37 (d, 1 H, J = 8.8 Hz), 8.07 (t, 1 H, J = 7.3 Hz), 7.88 (d, 1 H, J = 7.3 Hz), 7.66 (dd, 1 H, J = 5.1 and 2.9 Hz), 6.13 (d, 1 H, J = 8.8 Hz), 5.84−5.73 (m, 2 H), 5.28 (t, 1 H, J = 9.5 Hz), 5.06 (dd, 2 H, J = 18.0 and 3.0 Hz), 4.30 (s, 1 H), 4.25 (t, 1 H, J = 8.8 Hz), 3.92 (d, 1 H, J = 10.3 Hz), 3.77−3.60 (m, 4 H), 3.20 (d, 1 H, J = 10.2 Hz), 3.05 (t, 1 H, J = 10.2 Hz), 2.75 (s, 3 H), 1.63 (m, 1 H), 1.45−0.83 (m, 19 H), 0.97 (s, 3 H), 0.83 (s, 3 H), 0.77 (s, 9 H). ^{13}C NMR (125 MHz, DMSO-d_6), δ, 198.3, 172.1, 171.7, 162.1, 162.0, 158.3, 157.1, 151.1, 139.6, 135.0, 128.0, 123.3, 116.5, 60.5, 55.8, 52.8, 52.3, 51.5, 47.6, 41.7, 36.4, 35.0, 32.2, 31.8, 31.6, 28.5, 28.0, 27.9, 27.4, 27.2, 27.1, 26.9, 26.6, 26.3, 22.3, 19.5, 15.0, 13.7. MS (ESI, *m*/*z* relative intensity) 780 [(M + Na)$^+$, 50], 758 [(M + H)$^+$, 100].

(16a*S*,17a*R*,17b*S*)-13(*S*)-[[[[1(*S*)-[[[(Dimethylamino)sulfonyl]methylamino]methyl]-2,2-dimethylpropyl]amino]carbonyl]amino]octadecahydro-17,17-dimethyl-α,1,14-trioxo-*N*-(2-propenyl)-2*H*-cyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacyclohexadecine-3(*S*)-acetamide [50]. ^{1}H NMR (500 MHz, DMSO-d_6), δ, 8.89 (t, 1 H, J = 6.0 Hz), 8.37 (d, 1 H, J = 9.1 Hz), 6.15 (d, 1 H, J = 9.1 Hz), 5.83−5.76 (m, 2 H), 5.29 (t, 1 H, J = 10.1 Hz), 5.14−5.06 (m, 2 H), 4.33 (s, 1 H), 4.30 (t, 1 H, J = 9.1 Hz), 3.92 (d, 1 H, J = 10.4 Hz), 3.81−3.71 (m, 3 H), 3.61 (dt, 1 H, J = 2.5 and 10.4 Hz), 3.17 (dd, 1 H, J = 2.8 and 10.7 Hz), 2.94 (dd, 1 H, J = 11.0 and 2.5 Hz), 2.71 (s, 3 H), 2.69 (s, 6 H), 1.71−1.63 (m, 2 H), 1.46−1.17 (m, 18 H), 0.99 (s, 3 H), 0.84 (s, 3 H), 0.81 (s, 9 H). ^{13}C NMR (125 MHz, DMSO-d_6), δ, 198.2, 172.0, 171.6, 162.0, 158.1, 135.0, 116.4, 60.4, 55.8, 52.8, 51.6, 51.3, 47.6, 41.7, 38.5, 36.0, 34.9, 32.3, 31.6, 31.2, 28.4, 27.9, 27.3, 27.1, 27.0, 26.9, 26.6, 26.2, 24.4, 22.3, 19.4, 13.6. MS (ESI, *m*/*z* relative intensity) 724 [(M + 1)$^+$, 100], 461 (10).

(16a*S*,17a*R*,17b*S*)-13(*S*)-[[[[1(*S*)-[(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)methyl]-2,2-dimethylpropyl]amino]carbonyl]amino]octadecahydro-17,17-dimethyl-α,1,14-trioxo-*N*-(2-propenyl)-2*H*-cyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacyclohexadecine-3(*S*)-acetamide [51]. ^{1}H NMR (500 MHz, DMSO-d_6), δ, 8.87 (t, 1 H, J = 6.0 Hz), 8.32 (d, 1 H, J = 9.1 Hz), 7.83 (bs, 4 H), 5.99 (d, 1 H, J = 8.8 Hz), 5.84 (d, 1 H, J = 10.4 Hz), 5.82−5.75 (m, 1 H), 5.24 (bt, 1 H, J = 10.4 Hz), 5.13−5.05 (m, 2 H), 4.27 (s, 1 H), 3.88 (dt, 1 H, J = 4.1 and 9.4 Hz), 3.79−3.49 (m, 7 H), 1.66−1.63 (m, 1 H), 1.39−0.92 (m, 19 H), 0.96 (s, 3 H), 0.93 (s, 9 H), 0.77 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$), δ, 198.1, 171.9, 171.0, 168.6, 162.1, 158.0, 135.0, 135.0, 132.5, 123.6, 116.4, 60.4, 56.2, 52.9, 51.3, 47.4, 41.6, 39.5, 34.3, 31.9, 31.4, 31.2, 28.2, 27.8, 27.8, 27.1, 27.0, 26.8, 26.5, 26.0, 24.5, 22.2, 19.3, 13.5. MS (ESI, *m*/*z* relative intensity) 755 [(M + Na)$^+$, 40], 733 [(M + 1)$^+$, 100].

(16a*S*,17a*R*,17b*S*)-13(*S*)-[[[[1(*S*)-[(4,4-Dimethyl-2,6-dioxo-1-piperidinyl)methyl]-2,2-dimethylpropyl]amino]carbonyl]amino]-octadecahydro-17,17-dimethyl-α,1,14-trioxo-*N*-(2-propenyl)-2*H*-cyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacyclohexadecine-3(*S*)-acetamide [52]. ^{1}H NMR (500 MHz, DMSO-d_6), δ, 8.10 (t, 1 H, J = 5.7 Hz), 8.37 (d, 1 H, J = 8.8 Hz), 6.19 (d, 1H, J = 8.5 Hz), 5.83−5.75 (m, 1 H), 5.58 (d, 1 H, J = 10.4 Hz), 5.27 (bt, 1 H, J = 9.1 Hz), 5.13−5.05 (m, 2 H), 4.31 (s, 1 H), 4.18 (t, 1 H, J = 8.8 Hz), 3.83− 3.65 (m, 6 H), 3.58 (d, 1 H, J = 12.6 Hz), 2.43 (dd, 4 H, J = 17.0 and 11.0 Hz), 1.66−1.62 (m, 2 H), 1.44−1.11 (m, 18 H), 0.98 (s, 3 H), 0.96 (s, 6 H), 0.84 (s, 9 H), 0.82 (s, 3 H). ^{13}C NMR (125 MHz, DMSO-d_6), δ, 198.2, 172.8, 172.0, 171.3, 162.1, 158.1, 135.0, 116.4, 60.4, 55.4, 52.8, 51.3, 47.5, 46.3, 41.7, 39.5, 35.0, 32.4, 31.5, 31.2, 29.2, 28.3, 27.9, 27.9, 27.3, 27.0, 26.9, 26.5, 26.1, 24.5, 22.4, 19.4, 13.6. MS (ES, *m*/*z* relative intensity) 749 [(M + Na)$^+$, 20], 727 [(M + 1)$^+$, 100].

(16a*S*,17a*R*,17b*S*)-13(*S*)-[[[[2,2-Dimethyl-1(*S*)-[(4-methyl-2,6-dioxo-1-piperazinyl)methyl]propyl]amino]carbonyl]amino]-octadecahydro-17,17-dimethyl-α,1,14-trioxo-*N*-(2-propenyl)-2*H*-cyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacyclohexadecine-3(*S*)-acetamide [53]. ^{1}H NMR (500 MHz, $CDCl_3$): δ, 8.00 (1H, d, J = 7.88 Hz), 7.61 (1H, broad s), 5.95 (1H, broad s), 5.85 (1H, ddt, J = 5.67, 10.40, 17.02 Hz), 5.63 (1H, t, J = 8.51 Hz), 5.21 (1H, ddt, J = 1.26, 1.57, 17.02 Hz), 5.19 (1H, broad s), 5.18 (1H, dd, J = 1.26, 10.40 Hz), 4.60 (1H, ddd, J = 3.46, 9.45, 10.40 Hz), 4.54 (1H, s), 4.11 (1H, d, J = 10.08 Hz), 3.79−4.02 (6H, m), 3.42 (2H, d, J = 16.07 Hz), 3.33 (2H, d, J = 16.07 Hz), 2.37 (3H, s), 1.84 (4H, m), 1.68 (1H, m), 1.43−1.52 (4H, m), 1.15−1.42 (10H, m), 1.05 (1H, m), 0.96 (3H, s), 0.91 (9H, s), 0.81 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$), δ, 198.2, 172.9, 171.3, 170.1, 158.8, 157.5, 132.9, 117.2, 60.2, 58.0, 55.9, 53.1, 51.1, 48.1, 43.7, 41.8, 39.4, 34.2, 33.5, 32.3, 30.8, 29.2, 27.3, 26.9, 26.87, 26.84, 26.4, 26.3, 26.0, 25.4, 24.5, 22.8, 18.9, 13.0 ppm. HRMS (ESI) calcd for $C_{37}H_{60}N_7O_7$ [M + H]$^+$: 714.4554. Found: 714.4556.

(16a*S*,17a*R*,17b*S*)-13(*S*)-[[[[1(*S*)-[(2,4-Dioxo-3-azabicyclo[3.2.1]oct-3-yl)methyl]-2,2-dimethylpropyl]amino]carbonyl]amino]octadecahydro-17,17-dimethyl-α,1,14-trioxo-*N*-(2-propenyl)-2*H*-cyclopropa[3,4]pyrrolo[1,2-*a*][1,4]diazacyclohexadecine-3(*S*)-acetamide [54]. ^{1}H NMR (500 MHz, $CDCl_3$): δ, 8.03 (1H, d, J = 6.6 Hz), 7.69 (1H, broad s), 6.04 (1H, broad s), 5.85 (1H, ddt, J = 5.67, 10.08, 17.0 Hz), 5.64 (1H, broad s), 5.22 (1H, ddt, J = 1.2, 1.5, 17.0 Hz), 5.21 (1H, broad s), 5.17 (1H, ddt, J = 1.2, 1.2, 10.0 Hz), 4.64 (1H, d, J = 8.8 Hz), 4.56 (1H, broad s), 4.16 (1H, d, J = 10.0 Hz), 3.89−4.02 (3H, m), 3.84 (1H, dt, J = 4.72, 10.0 Hz), 3.73 (1H, s), 3.71 (1H, d, J = 6.3 Hz), 3.13 (1H, broad s), 3.08 (1H, broad s), 2.16 (1H, d, J = 11. Hz), 2.02 (3H, m), 1.86 (2H, m), 1.70 (5H, m), 1.58 (1H, dt, J = 4.0, 12.0 Hz), 1.48 (4H, m), 1.14−1.43 (9H, m), 1.05 (1H, m), 0.96 (3H, s), 0.89 (9H, s), 0.81 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$): δ 198.4, 173.0, 171.3, 158.8, 157.6, 132.9, 117.2, 60.1, 56.0, 53.1, 51.0, 48.1, 44.9, 44.7, 41.8, 38.8, 34.2, 33.6, 32.5, 32.3, 31.5, 30.8, 29.2, 27.3, 27.2, 26.9, 26.87, 26.83, 26.46, 26.42,

26.0, 25.5, 24.6, 22.9, 22.6, 18.9, 13.0 ppm. HRMS (ESI) calcd for $C_{39}H_{61}N_6O_7$ $[M + H]^+$: 725.4602. Found: 725.4581.

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