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Novel Macrocyclic Inhibitors of Hepatitis C NS3/4A Protease Featuring a 2-Amino-1,3-thiazole as a P4 Carbamate Replacement

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Our laboratories recently reported the discovery of P2−P4 macrocyclic inhibitors of HCV NS3/4A protease, characterized by high levels of potency and liver exposure. Within this novel class of inhibitors, we here describe the identification of a structurally diverse series of compounds featuring a 2-amino-1,3-thiazole as replacement of the carbamate in P4. Optimization studies focused on structural modifications in the P3, P2, and P1 regions of the macrocycle as well as on the linker chain and resulted in the discovery of several analogues characterized by excellent levels of enzyme and cellular activity. Among these, compound **59** displayed an attractive pharmacokinetic profile in preclinical species and showed sustained liver levels following oral administration in rats.

Introduction

Hepatitis C virus (HCV[a]) infection is a major cause of liver disease and represents a serious burden to global health care that is likely to increase over the coming years. It is estimated that approximately 170 million people, ca. 3% of the worldwide population, are currently infected with HCV, and 3−4 million new cases are reported every year.[1] In the majority of cases, HCV infection leads to chronic hepatitis, which in turn can evolve into cirrhosis and ultimately hepatocellular carcinoma (HCC).[2] HCV infection is believed to be responsible for 50−76% of all cases of liver cancer and is the leading cause of liver transplantation.[1,3] The current standard of care for HCV is based on a combination of pegylated interferon alpha (PEG-IFN-α), a protein that stimulates the immune system, and ribavirin, a nucleoside that augments the antiviral action of IFN-α by a not yet completely elucidated mechanism.[4] However, the current therapy suffers major limitations due to significant side effects and limited response rate. Only about 50% of the treated population achieves sustained virological response (SVR) depending on patient characteristics and viral genotype, with particularly low response rates for patients infected with genotype 1 virus, the most prevalent in Europe, North America, and Japan.[5] The discovery of novel, more efficacious, and better tolerated therapies for the treatment of HCV infection is therefore a medical problem that needs urgent attention.

HCV, which replicates mainly in the liver, is a positive single-stranded RNA virus of approximately 9.6 kilobases that encodes a polyprotein precursor of about 3000 amino acids. The latter is processed by cellular and viral proteinases to produce structural and nonstructural (NS) proteins.[6] The virally encoded NS3 protease plays an essential role in the viral cycle being responsible for processing of the nonstructural portion of the polypeptide and has long been recognized as a validated target for antiviral intervention.[7] More recently, NS3 has also been associated with silencing of the host's immune response, further raising its relevance as a therapeutic target.[8] NS3 is a bifunctional protein that harbors a canonical serine protease catalytic triad (Asp-His-Ser) and a helicase/ATP-ase domain.[9] Formation of a heterodimeric complex with the NS4A cofactor is essential for maximum protease efficiency and anchoring to the endoplasmatic reticulum. The unusually shallow substrate binding cleft requiring extensive substrate−protein interactions has posed significant challenges to the development of effective small molecule inhibitors of NS3/4A. The discovery that N-terminal hexapeptide cleavage products are competitive inhibitors of NS3/4A[10] opened new avenues to drug discovery and ultimately resulted in the identification of BILN-2061 (**1**, Figure 1), a non covalent P1−P3 macrocyclic NS3/4A inhibitor that first achieved clinical proof of concept (POC).[11] While the development of **1** was stopped due to significant side effects, several other inhibitors have since then progressed through late stage clinical investigations, including both covalent reversible inhibitors such as telaprevir (VX-950)[12] and boceprevir (SCH503034),[13] and noncovalent ones like TMC435350[14] and ITMN-191.[15]

Among the strategies explored to improve the pharmacologic properties of the initial peptide leads, several macrocyclic analogues have been investigated as β-strand mimetics. Molecular modeling and NMR studies suggested hydrophobic contacts between the P4 and the P2 residues, inspiring the design of bis-arylether macrocycles.[16] Subsequently combination of distinct P4−P2 macrocyclic cores with ketoamide warheads resulted then in several potent NS3/4A inhibitors spanning from the P4 to the P2′ sites.[17]

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[a] Abbreviations: HCV, hepatitis C virus; HCC, hepatocellular carcinoma; PEG-IFN-α, pegylated interferon alpha; SVR, sustained virological response; NS, nonstructural; RCM, ring closing metathesis; FBS, fetal bovine serum; NHS, normal human serum; SAR, structure−activity relationship.

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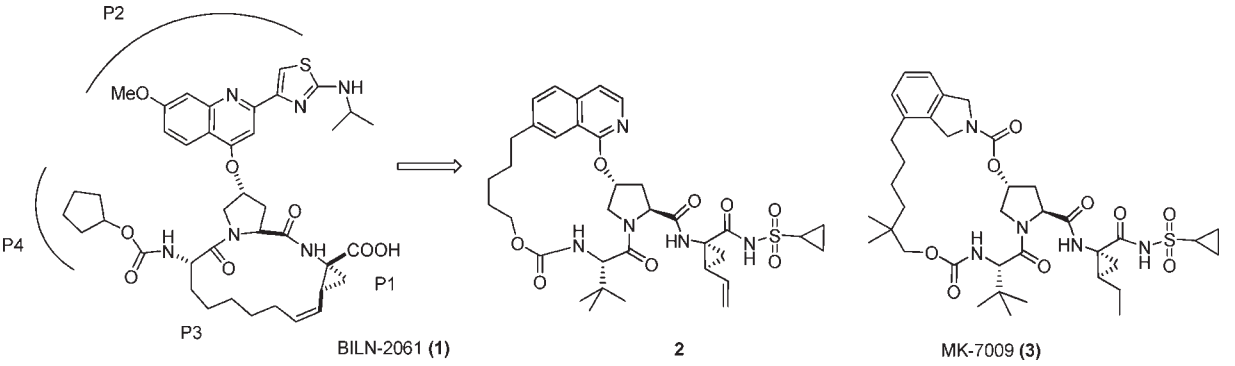


Figure 1. Inhibitors of HCV NS3/4A protease.

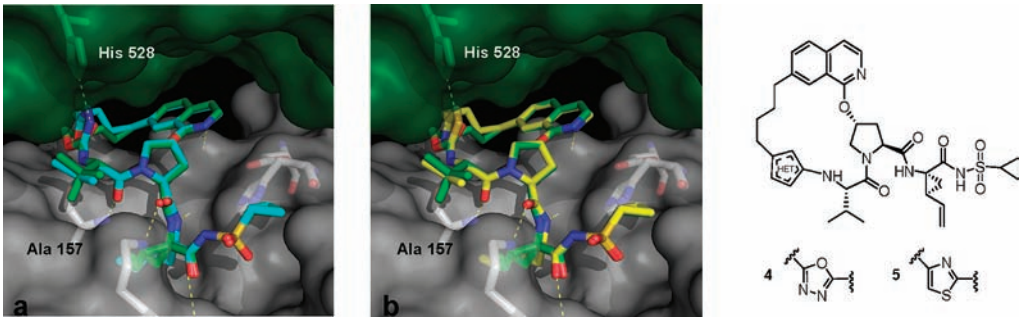


Figure 2. Molecular model of carbamate **2** (green) superimposed with oxadiazole **4** (a, blue) and thiazole **5** (b, yellow) in the context of NS3-4A active site (gray)[22] and helicase domain (green).

Recently, our laboratories reported the discovery of a novel class of potent macrocyclic inhibitors bearing an aliphatic chain linking the P4 carbamate and the P2 moiety, here exemplified by compound **2** (Figure 1).[18] The design of these analogues derived from molecular modeling studies of **1** docked within the full length NS3/4A protease, which highlighted how the space between the helicase domain and the protease might accommodate a connection of the spatially close P2 and P4 residues. Further optimization of the macrocyclic scaffold, including bioisosteric replacement of the P1 carboxylic acid moiety with a cyclopropylacylsulfonylamide,[19] resulted in the identification of numerous potent and selective inhibitors of NS3/4A protease.[18] Noticeably, several of them offered remarkably high liver exposure following oral administration in preclinical species, a key feature in any potential treatment for HCV infection.[18a] Further optimizations led to the discovery of MK-7009 (**3**), which demonstrated excellent preclinical in vivo efficacy in infected chimpanzee and is currently in phase II clinical trials.[20]

This paper describes efforts toward the identification of a structurally diverse series of P2−P4 macrocyclic inhibitors with comparable potency and liver exposure. In particular, we set out to explore modifications of the P4 carbamate moiety and, on the basis of previous examples in which five-membered heterocyclic rings have been used as ester and amide bioisosters,[21] we hypothesized that five-membered amino-heterocycles could be a suitable replacement for the carbamate group. We reasoned that such modification, by restricting the flexibility of the macrocyclic backbone, could further reduce the peptide-like character of these macrocyclic inhibitors and therefore have a favorable impact on their in vivo profile while retaining high inhibitory potency against NS3/4A. To support our initial hypothesis, we modeled carbamate **2** in the context of the full length NS3 structure[22] and superimposed it with analogue **4**, containing a 2-amino-1,3,4-oxadiazole moiety, and with analogue **5**, featuring a 2-amino-1,3-thiazole in P4 (parts a and b of Figure 2, respectively).

We were pleased to observe that in both cases the proposed macrocyclic inhibitors showed a very favorable overlap with the corresponding carbamate analogue, with only minimal effect on the overall conformation. In particular, despite the replacement of the P4-carbamate with a five-membered heterocyclic ring, the network of key intermolecular hydrogen bonds involving the carbonyl and amino groups of the P3 residue with the protein backbone (Ala 157) appeared unperturbed.[23] Additionally, the proposed replacement of the P4-carbamate with an amino-heterocycle did not appear to produce an unfavorable steric clash with His 528, a residue proposed by previous studies to be involved in specific helicase−inhibitor interactions.[18a]

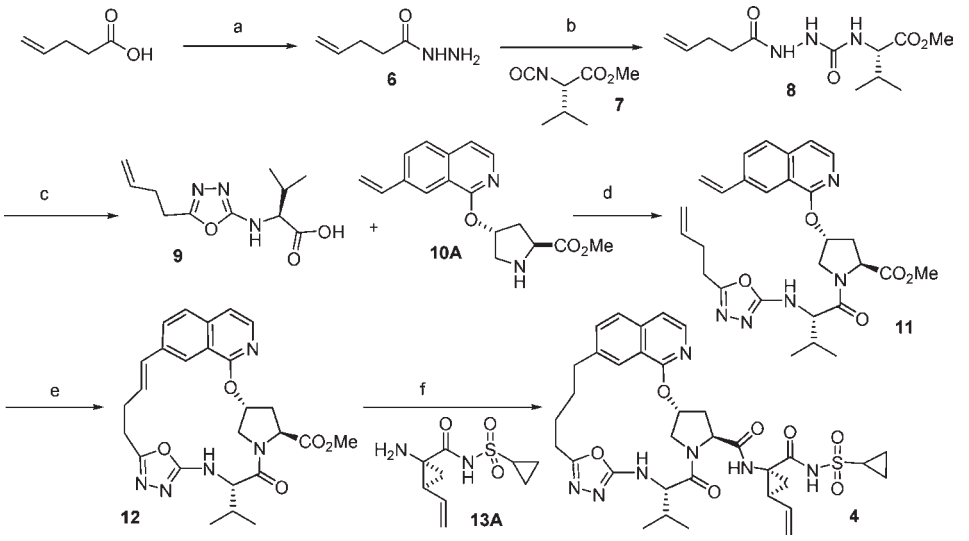
In this paper, we report the studies that led to the identification of the 2-amino-1,3-thiazole moiety as a suitable replacement of the P4 carbamate group in the context of macrocyclic inhibitors of NS3/4A and describe the development of a series of novel inhibitors characterized by nanomolar cellular potency and desirable pharmacokinetic properties in preclinical species.

Chemistry

The synthesis of the initial macrocyclic analogues containing a 2-amino-1,3,4-oxadiazole and a 2-amino-1,3-thiazole in P4 is outlined in Schemes 1 and 2, respectively. In both cases, we started our investigations by introducing the unsubstituted isoquinoline residue in P2 to allow direct comparison with carbamate **2** and we selected a ring closing metathesis (RCM) as the key macrocyclization step.[24]

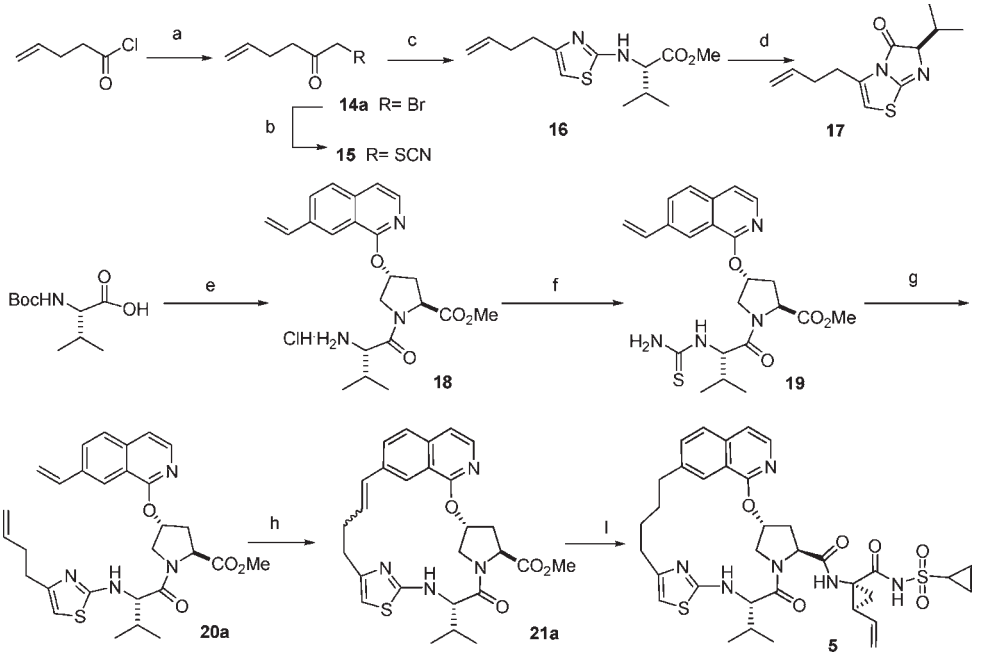
Synthesis of oxadiazole **4** commenced with conversion of 4-pentenoic acid into the corresponding hydrazide **6** by coupling

Scheme 1. Synthesis of Oxadiazole Derivative **4**[a]



[a] Reagents and conditions: (a) (i) EDCI·HCl, Boc-$NHNH_2$, DCM; (ii) TFA, DCM. (b) Dioxane. (c) (i) $POCl_3$, 80 °C; (ii) LiOH, THF/H_2O. (d) TBTU, DCM. (e) Zhan 1 catalyst, 1,2-DCE, 80 °C. (f) (i) Pd/C, H_2, EtOH; (ii) LiOH, THF/H_2O; (iii) **13A**, TBTU, DIPEA, DMF.

Scheme 2. Synthesis of Thiazole Derivative **5**[a]

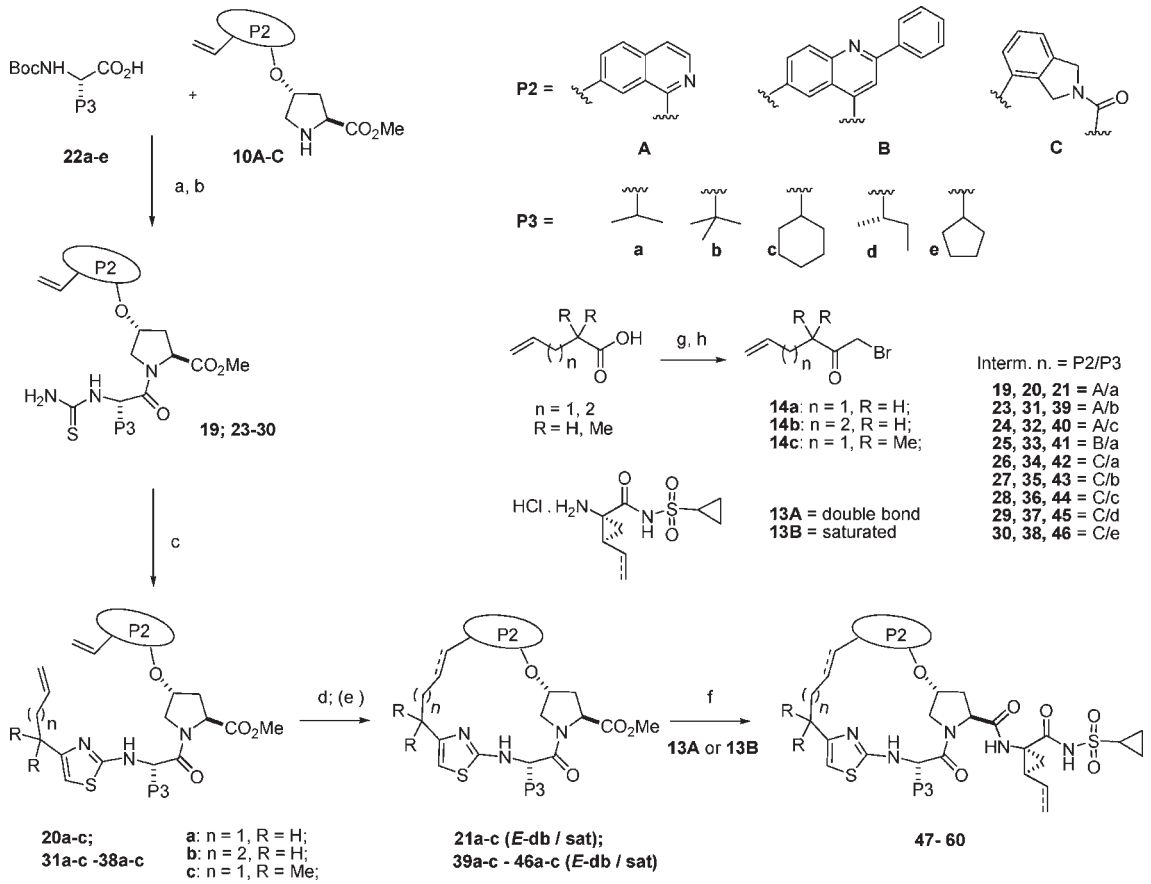


[a] Reagents and conditions: (a) CH_2N_2, Et_2O, then aq HBr, 0 °C. (b) NaSCN, CH_3CN. (c) Methyl valinate, dioxane, 50 °C. (d) (i) LiOH, THF, H_2O; (ii) **10A**, EDCI·HCl, HOBt, $^iPr_2EtN$, DCM. (e) (i) **10A**, TBTU, $^iPr_2EtN$, DMF; (ii) HCl, dioxane. (f) (i) Fmoc-SCN, $^iPr_2EtN$, DCM, 0 °C, then 20% piperidine in MeOH. (g) **14a**, dioxane, 60 °C. (h) (i) Zhan 1 catalyst, 1,2-DCE, 80 °C. (l) (i) Pd/C, H_2, MeOH; (ii) LiOH THF, H_2O; (iii) **13A**, TBTU, $^iPr_2EtN$, DMF.

with *tert*-butyl hydrazine carboxylate and subsequent *N*-Boc deprotection. Reaction of **6** with the valine derived isocyanate **7**[25] afforded the oxadiazole precursor **8**, which upon treatment with $POCl_3$ promoted the desired intramolecular cyclization to give, after hydrolysis, the required functionalized 1,3,4-oxadiazole **9**. Coupling of the latter with the isoquinoline-proline derivative **10A**[26] produced the macrocyclization precursor **11**, which upon treatment with the RCM catalyst Zhan 1[27] in 1,2-dichloroethane gave efficiently and selectively the desired macrolactam ester **12** as the *E*-stereoisomer. Hydrogenation of the double bond, hydrolysis of the methyl ester, and coupling of the resulting carboxylic acid with aminoacylsulfonamide **13A**[28] completed the synthesis of the targeted oxadiazole macrocyclic derivative **4**.

A similar approach was used for the synthesis of the thiazole derivative **5** (Scheme 2). Accordingly, 4-pentenoyl chloride was treated sequentially with diazomethane and aqueous HBr to give the corresponding α-bromo-methyl ketone **14a**. The latter was further progressed to isothiocyanate **15** by reaction with NaSCN. Moderate heating of **15** in the presence of methyl valinate afforded smoothly the required 1,3-thiazole-intermediate **16**. Disappointingly, upon activation with

Scheme 3. Modular Approach to the Synthesis of Macrocyclic Aminothiazole Analogues **47**−**60**[a]



[a] Reagents and conditions: (a) (i) EDCI·HCl, HOBt, $^{i}Pr_2EtN$, DCM; (ii) HCl/dioxane. (b) Fmoc-SCN, $^{i}Pr_2EtN$, DCM, 0 °C, then 20% piperidine in MeOH. (c) **14a**−**c**, dioxane, 60 °C. (d) Zhan 1 catalyst, 1,2-DCE, 80 °C. (e) Pd/C, H_2, MeOH; (f) (i) LiOH, THF, H_2O; (ii) **13A** or **13B**, EDCI·HCl, HOBt, $^{i}Pr_2EtN$, DCM. (g) $(COCl)_2$, cat. DMF, DCM. (h) CH_2N_2, Et_2O; then aq HBr, 0 °C; *E*-db = *E*-double bond; sat = saturated.

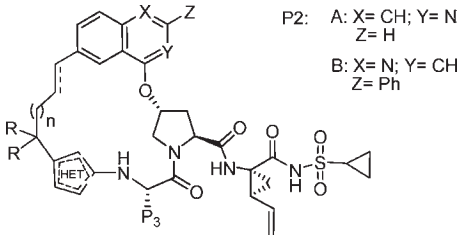
EDCI·HCl, the corresponding carboxylic acid underwent a facile intramolecular cyclization to give the bicyclic derivative **17**, thus preventing further elaboration into the targeted macrocycle. Undeterred by these results, we sought an alternative synthetic route by assembling the P2−P3-dipeptide intermediate **18** first, followed by further elaboration of its terminal P3-amino group to install the required aminothiazole moiety. Also in this case, elaboration of the macrocycle proved to be quite challenging due to facile intramolecular reaction of the P3 amino group with the proline methyl ester of **18**. Ultimately, the issue was overcome by liberating the terminal amino group in carefully controlled conditions and in the presence of an excess of Fmoc-thioisocyanate, thus preventing the undesired lactam formation. Removal of the Fmoc protecting group then afforded thiourea **19**, which was reacted with the previously prepared α-bromo-methyl ketone **14a** to yield the required aminothiazole core. RCM macrocyclization of **20a** proceeded smoothly to give **21a** as a mixture of *E*:*Z* stereoisomers (ca. 4:1 ratio). Further functional group manipulations as previously described afforded then aminothiazole **5**.

Having identified reliable synthetic routes to access efficiently both the aminoxadiazole and the aminothiazole analogues, a modular synthetic approach was developed allowing different regions of the macrocyclic cores to be explored in a systematic manner. The key intermediates were assembled in a convergent manner as depicted in Scheme 3, yielding analogues **47**−**60** efficiently. The appropriate proline residues **10**, carrying the P2 heterocyclic fragments **A**,[26] **B**,[29] and **C**,[30] were coupled with one of the selected P3 amino acid building blocks **22a**−**e** and elaborated to the corresponding thioureas **23**−**30** by means of the previously described transformations. The latter were then converted to the aminothiazole derivatives **31**−**38** by treatment with the appropriate α-bromomethylketones **14a**−**c**, each of different chain lengths and optionally bearing a *gem*-dimethyl substituent. Macrocyclization by RCM reaction and optional reduction of the linker olefin by hydrogenation gave intermediates **39**−**46**. In turn, following methyl ester hydrolysis, the latter could finally be coupled with either unsaturated or saturated P1 fragments **13A**[28] or **13B**[31] to give the targeted analogues **47**−**60**.

Results and Discussion

All the compounds prepared were evaluated in an enzymatic assay using the genotype 1b NS3/4A enzyme, and results are reported as K_i values.[32] The cellular activity was determined using a subgenomic 1b replication assay (replicon),[33] where compounds were tested both in the presence of 10% fetal bovine serum (FBS) and in the more physiological conditions represented by the use of 50% normal human serum (NHS). Initial pharmacokinetic evaluation of the analogues focused on concentration in liver and plasma exposure levels in rats after a single oral dose (5 mg/kg). Selected compounds were further evaluated with a full pharmacokinetic analysis in rat.

Table 1. Aminoheterocyclic Derivatives in the Isoquinoline and Quinoline Series[e]



Entry	Compound (linker)	HET	P3	P2	n	R	1b Ki [a] (nM)	1b replicon EC_{50} (nM)[b] 10%FBS	1b replicon EC_{50} (nM)[b] 50%NHS	$AUC^{c}_{0\text{-}4\ h}$ (μM·h)	liver conc 4h[d] (μM)
1	**2**						0.2	8.7	46	0.27	13.4
2	**4** (sat)			A	1	H	0.9	80	180	0.1	0.03
3	**5** (sat)		"	A	1	H	1.7	60	920	9.9	5.5
4	**47** (sat)	"	"	A	2	H	1.5	120	830	--	--
5	**48** (*E*-db)	"	"	A	1	H	5.0	30	730	0.9	2.4
6	**49** (sat)	"	"	A	1	CH_3	3.0	20	100	0.8	3.6
7	**50** (sat)	"		A	1	H	1.3	30	350	4.7	3.4
8	**51** (sat)	"		A	1	H	0.2	20	130	0.1	1.0
9	**52** (sat)	"		B	1	H	0.5	40	390	<0.01	<0.01

[a] Enzyme potency measured in a NS3/4A time-resolved fluorescence assay; for details, see ref 32; reported K_i values are the mean of ≥3 experiments, standard deviation was within ±20% of the reported value. [b] Cellular activity determined in the HCV bicistronic replicon assay, using HuH-7 cells stably transfected with genotype 1b HCV replicon RNA; for details, see ref 33; EC_{50} are measured in the presence of 10% fetal bovine serum (FBS) and 50% normal human serum (NHS); values are the mean of ≥3 experiments, standard deviation was within ±20% of the reported value. [c] Area under the curve following oral administration to Sprague−Dawley rats at 5 mg/kg (solution, PEG-400), 3 animals per group. [d] Concentration of compound measured in rat liver at 4 h from oral dosing. [e] Compounds 5 and 47−52 are trifluoroacetate salts; sat = saturated; db = double bond.

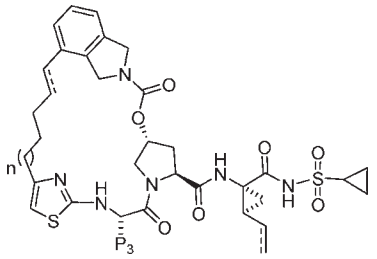
Evaluation of analogues **4** and **5** in vitro confirmed that a five-membered amino-heterocycle could be a very effective replacement of the P4 carbamate. Oxadiazole **4** proved in fact to be a potent enzyme inhibitor, with a K_i = 0.9 nM (entry 2, Table 1), and showed submicromolar activity in the replicon, with EC_{50} = 80 nM in the presence of 10% FBS. In addition, **4** retained interesting levels of cellular activity with 50% NHS (EC_{50} = 180 nM), albeit with a 4-fold loss in comparison with **2** (EC_{50} = 46 nM). Oral administration of **4** in Sprague−Dawley rat at 5 mg/kg resulted in detectable but lower levels of plasma exposure compared to **2** (AUC = 0.1 μM·h and AUC = 0.27 μM·h for **4** and **2**, respectively). The oxadiazole **4** also displayed significantly reduced liver exposure (liver levels at 4 *h* = 0.03 and 13.4 μM for **4** and **2**, respectively).

On the other hand, thiazole **5**, which compared less favorably than both **4** and **2** in the enzyme and in the replicon assay (K_i = 1.7 nM; EC_{50} = 60/920 nM 10% FBS/50% NHS), showed a dramatic increase in systemic exposure following oral administration at 5 mg/kg, with an AUC = 9.9 μM·h (entry 3, Table 1). Moreover, liver levels for **5** were also significantly improved over oxadiazole **4** and only 2-fold lower than **2** (5.5 μM). An excellent oral bioavailability (*F* = 60%) and a low plasma clearance (Clp = 7 mL/min/kg) completed the favorable in vivo profile of **5** and warranted further investigations of this series.[34]

Indeed, these findings prompted us to embark in extensive structural modifications within the thiazole series, addressing systematically the different regions of the macrocyclic core with the primary objective to improve potency while maintaining systemic and liver exposure. As described earlier, the targeted analogues were efficiently prepared by means of modular assembly of key intermediates. The selection of the appropriate building blocks originated from extensive structure−activity investigations previously conducted by us and others on both substrate based peptide inhibitors as well as P2−P4 macrocyclic inhibitors. In particular, it has been shown that a variety of small lipophilic β-branched amino acids are well tolerated in the P3 position, including both natural residues such as valine and isoleucine and unnatural ones such as *tert*-butyl- and cyclohexyl-glycine.[20,35,36] It has also been disclosed that a significant boost in potency could be obtained by introducing large aromatic substituents stemming from the P2 proline and that this region of the inhibitor tolerates substantial structural modification.[23,37] In the context of the P2−P4 macrocycles, these findings led to subnanomolar inhibitors bearing, among others, a 2-phenylquinoline, an isoquinoline, or an indoline substituent in the P2 region.[18,20] On the basis of the aforementioned findings, we selected these structural features as starting points in the optimization of our thiazole series and set out to combine the above P2-substituents with a variety of appropriate P3 amino acids. We also planned to investigate a limited number of structural modifications within the P2−P4 linker chain such as length, saturation, and presence of small alkyl groups, given that in related series these features had positively affected the inhibitory potency.[20]

With **5** as a starting point, we started our investigations by homologating the linker chain to a C5 tether. Despite comparable levels of enzyme potency, analogue **47** (entry 4, Table 1) showed a modest reduction in replicon activity with 10% FBS and did not offer advantage over **5** when tested with 50% NHS (K_i = 1.5 nM; EC_{50} = 120/830 nM 10% FBS/50% NHS). Unlike previous findings reported for the carbamate

Table 2. Aminothiazole Derivatives in the Isoindoline Series[g]



Entry	Compound (linker)	P_1	P_3	n	1b Ki[a] (nM)	1b replicon EC_{50} (nM)[b] 10%FBS	50%NHS	AUC[c] 0-4 h (μM·h)	F[d] (%)	Clp[e] (mL/min/Kg)	liver conc 4h[f] (μM)
1	**53** (*E*-db)	db		1	0.16	5	50	0.4*	8*	21*	0.60*
2	**54** (*E*-db)	db	"	0	0.28	90	635	--	--	--	--
3	**55** (*E*-db)	db		1	0.16	3	23	0.06	6	85	0.13
4	**56** (sat)	db	"	1	0.16	4	19	0.06*	5*	95*	0.06*
5	**57** (*E*-db)	db		1	0.04	3	16	0.40	8	17	0.35
6	**58** (*E*-db)	sat	"	1	0.16	12	50	<0.01	--	56	0.57
7	**59** (*E*-db)	db		1	0.04	2	18	0.70	13	17	0.35
8	**60** (*E*-db)	db		1	0.08	2	16	0.70	36	25	0.18

[a] See footnote details in Table 1. [b] See footnote details in Table 1. [c] Area under the curve following oral administration to Sprague−Dawley rats at 4 mg/kg or 5 mg/kg* (solution, PEG-400; 3 animals per group). [d] Oral bioavailability. [e] Plasma clearance (intravenous dosing vehicle: DMSO/PEG-400/water 20%/60%20%; 3 animals per group). [f] Concentration of compound measured in rat liver at 4 h from oral dosing. [g] All compounds are trifluoroacetate salts; sat = saturated; db = double bond.

series,[18a] the introduction of an *E*-double bond adjacent to the P2-substitutent as in analogue **48** (entry 5, Table 1) resulted in a 3-fold loss of enzyme potency, although the conformational restraint did result in a marginal improvement in replicon potency (K_i = 5 nM; EC_{50} = 30/730 nM 10% FBS/50% NHS). However, following oral administration in rats at 5 mg/kg, **48** showed 2-fold lower liver levels and a significant reduction in plasma exposure in comparison to the saturated analogue **5** (liver levels at 4 *h* = 2.4 μM; AUC = 0.9 μM·h). We continued our investigations by introducing in the position adjacent to the thiazole ring a *gem*-dimethyl group, a structural feature that proved to be beneficial in related series both in terms of potency as well as liver levels.[20] Indeed, the *gem*-dimethyl analogue **49** (entry 6, Table 1) was only slightly less potent than **5** in the enzyme assay and showed a 9-fold boost in cellular potency in high serum conditions (K_i = 3 nM; EC_{50} = 20/100 nM 10% FBS/50% NHS). On the other hand, following single oral dosing at 5 mg/kg, **49** showed lower liver levels and a significant reduction in plasma exposure in respect to its unsubstituted analogue **5** (liver levels at 4 *h* = 3.6 μM; AUC = 0.8 μM·h). These findings prompted us to focus our efforts toward analogues bearing a saturated and unsubstituted C4-linker, and additional opportunities to boost potency within the series came from screening of different residues in P3, a small subset of which is reported in Table 1 (entries 7 and 8). The cyclohexyl-glycine analogue **50** offered a moderate increase in replicon potency over **5** (K_i = 1.3 nM; EC_{50} = 30/350 nM 10% FBS/50% NHS), and the introduction of *tert*-butyl-glycine in P3 as in **51** resulted in a more significant gain of potency. Compound **51** showed in fact a 10-fold boost over **5** with subnanomolar inhibition of NS3/4A protease (K_i = 0.2 nM) and a 6-fold increase in replicon potency in high serum conditions (EC_{50} = 20/130 nM 10% FBS/50% NHS). Evaluation of the pharmacokinetic profile of **50** and **51** in rat at 5 mg/kg showed an inverse correlation between their in vitro potency and plasma and liver exposure after oral administration. The most potent *tert*-butyl glycine derivative **51** was characterized by a drastic decrease in plasma exposure (AUC = 0.1 μM·h) and a 5-fold reduction in liver levels in comparison to the valine analogue **5** (1.0 and 5.5 μM, respectively). On the other hand, the cyclohexyl-glycine derivative **50** showed higher liver and plasma exposure, with liver levels = 3.4 μM and AUC = 4.7 μM·h. These findings proved that it was indeed possible to achieve satisfactory levels of systemic and liver exposure with 2-aminothiazole analogues and prompted us to further explore the series beyond the initial isoquinoline derivatives in the hope of obtaining a significant boost in potency by means of fine-tuning the structure.

Progressing further in our quest for potency, we then turned our attention to the variation of the extended P2 region. On the basis of previous results in related series of macrocyclic inhibitors, we focused our efforts on the introduction of the phenylquinoline and the isoindoline P2 residues,[18,20] exemplified by compounds **52** (entry 9, Table 1) and **53**, respectively (entry 1, Table 2). In both cases, the structural modifications produced subnanomolar enzyme inhibitors (**52**: K_i = 0.5 nM; **53**: K_i = 0.16 nM) and, while the phenylquinoline analogue **52** showed a moderate boost in replicon potency compared to the corresponding quinoline analogue **5** (EC_{50} = 40/390 nM 10% FBS/50% NHS), the isoindoline **53** displayed superior cellular activity, with single digit nanomolar replicon potency in 10% FBS and an almost 20-fold boost in 50% NHS compared to **5** (EC_{50} = 5/50 nM 10% FBS/50% NHS). Evaluation of

Table 3. Pharmacokinetic Parameters and Liver Levels for Amorphous **59**-Potassium Salt Following Oral and Intravenous Administration in Rat and Dog (3 Animals per Group)

compd	species	dose (mg/kg)	Clp[a] (mL/m in/kg)	AUCpo[b] (μM*h)	C_{max}[c] (μM)	F (%)[d]	liver conc[e] 24 h, po (μM)
59	rat	4[f]	17	0.7	0.4	13	
59-K^+	rat	5[g]		1.7	0.5	26[h]	0.01
59-K^+	rat	10[g]		2.5	1.4		
59-K^+	rat	20[g]		13.3	5.9		0.04
59-K^+	rat	30[g]		21.0	8.0		0.06
59-K^+	dog	2[f]	38	0.2	0.06	17	
59-K^+	dog	5[g]		0.5	1.16		
59-K^+	dog	10[g]		10	4.0		

[a] Plasma clearance. [b] Area under the curve following oral administration. [c] Maximum plasma concentration after oral dosing. [d] Oral bioavailability. [e] Concentration of compound measured in rat liver at 24 h from oral dosing. [f] Dosed intravenously; rat: DMSO/PEG-400/water 20%/60%20%; dog: DMSO/saline 30%/70%. [g] Dosed orally; rat: solution, PEG-400; dog: solution, PEG-400/water 70%/30%. [h] Compared to **59**-free base dosed intravenously at 4 mg/kg.

the pharmacokinetic profile of the two analogues in rat gave additional insights toward the potential for further development of the two distinct structural series. In particular, after oral administration at 5 mg/kg, compound **52** was undetectable, while the potent isoindoline derivative **53** displayed plasma exposure with AUC = 0.4 μM·h and liver concentration = 0.60 μM at 4 h, offering therefore a promising starting point for further optimization.

Following the significant boost in enzyme and replicon potency observed for the isoindoline analogue **53**, we focused our efforts on 2-amino-thiazole analogues bearing this particular P2 residue. Within this series, we planned to capitalize on our previous SAR in the linker tether and in P3 to positively impact on the overall in vitro and in vivo profile of these inhibitors.

Unlike previous results within the isoquinoline series, it was found that a C5 linker chain offered a significant advantage over the C4 tether in terms of replicon potency. In fact, although the C4-analogue **54** maintained high levels of enzyme inhibition (K_i = 0.28 nM; entry 2, Table 2), it was affected by a more than 10-fold loss in replicon potency compared to its C5 analogue **53** (EC_{50} = 90/635 nM 10% FBS/50% NHS).

Optimization of the series progressed with the introduction of *tert*-butyl- and cyclohexyl-glycine residues in P3 to give analogues **55** and **57**, respectively (Table 2, entries 3 and 5). In line with our previous findings within the isoquinoline series, these two structural modifications proved to be beneficial in terms of potency, with a further boost in replicon EC_{50} in 50% NHS observed for both the analogues (EC_{50} = 3/23 nM and EC_{50} = 3/16 nM in 10% FBS/50% NHS, respectively). Notably, the introduction of the cyclohexyl-glycine residue in P3 as in compound **57** resulted in a K_i = 0.04 nM, a level of enzyme potency comparable with the most potent protease inhibitors. When profiled in rats (4 mg/kg iv and po), **57** proved to have a significantly higher plasma exposure in comparison with the *tert*-butyl-glycine derivative **55** (oral AUC = 0.40 μM·h and 0.06 μM·h, respectively) and 3-fold higher liver levels (**57**: 0.35 μM; **55**: 0.13 μM). Moreover, **57** showed a considerably reduced plasma clearance in comparison to **55** (Clp = 17 and = 85 mL/min/kg, respectively).

Having secured high levels of potency across the series and identified analogues such **57** with improved liver levels and pharmacokinetic properties, we then turned our attention to explore additional structural variations within the macrocyclic scaffold, with the aim to identify opportunities to optimize further the in vivo profile. Removal of the *E*-double bond within the linker chain did not affect potency, as exemplified by analogue **56** (Table 2, entry 4; K_i = 0.16 nM; EC_{50} = 4/19 nM 10% FBS/50% NHS). However, this structural modification resulted in a 2-fold decrease in liver levels compared to the unsaturated analogue **55** (0.06 μM). Saturation of the P1-double bond to give analogue **58** proved also to be detrimental (Table 2, entry 6), as this compound showed a 4-fold decrease in enzyme potency and a lower activity in replicon compared to the unsaturated analogue **57** (K_i = 0.16 nM; EC_{50} = 12/50 nM 10% FBS/50% NHS). Moreover, despite the good liver exposure (0.57 μM), **58** suffered from undetectable plasma levels, which was considered likely to hinder further development.

As a consequence of these findings, the efforts focused on isoindoline derivatives bearing a double bond both within the linker chain as well as in P1. Further screening of a variety of P3 residues identified the isoleucine and cyclopentyl glycine derivatives **59** and **60** as other promising candidates within the series (Table 2, entry 7 and 8). Both analogues displayed excellent potency against NS3/4A (K_i = 0.04 nM and K_i = 0.08 nM respectively) and maintained the high levels of replicon potency observed for **57**, with EC_{50} = 2/18 nM and 2/16 nM (10% FBS/50% NHS) for **59** and **60**, respectively, values comparable to those of several protease inhibitors currently under clinical investigation. Remarkable also was the replicon EC_{90} measured for **59** and **60**: 3 nM in 10% FBS, 40 nM and 34 nM, respectively, in 50% NHS.

The in vitro profiling of analogues **57**, **59**, and **60** was further extended to assess their stability in liver microsomes in the presence of NADPH and in hepatocytes. In both systems, the compounds showed an encouraging profile, with stability rank order dog > rat ~ human. Metabolite identification studies in rat, dog, and human hepatocytes showed a similar profile for the three analogues, with the formation of several species derived by oxidation in multiple parts of the macrocyclic structure with the exception of the isoindoline core.

Proceeding in the characterization of these analogues, we were pleased to observe that for the isoindoline derivatives the most promising in vivo profiles were associated with the highest potency, in contrast with our previous findings in the isoquinoline series. Indeed following iv and po dosing in rat at 4 mg/kg, both **59** and **60** showed moderate plasma clearance (17 and 25 mL/min/kg, respectively). However, **59** and **60** displayed improved systemic exposure and bioavailability over **57**, with oral AUC = 0.70 μM·h and F = 13% and 36%, respectively. An additional attribute for the isoleucine derivative **59** was the superior liver concentration seen with this compound, which provided a 2-fold improvement in liver exposure over **60** (liver levels at 4 h = 0.35 and 0.18 μM, respectively). Overall, the profile of **59** prompted us to select

this particular analogue for further investigation. The pharmacokinetic properties of **59** in rats were further evaluated following conversion of the acid *N*-acyl-sulphonamide moiety into the corresponding amorphous potassium salt (Table 3). We were pleased to find that oral dosing of the potassium salt form in rats at 5 mg/kg resulted in higher plasma exposure compared to the free base, with a more than 2-fold increase in the measured AUC (1.7 μM·h). Importantly, the K^+ salt of **59** displayed detectable liver levels at 24 h from dosing, with liver concentration of 0.01 μM. On the basis of these encouraging data, we selected the K^+ salt of **59** for a dose proportionality study in rat, administering the compound orally at 10, 20, and 30 mg/kg, respectively. As reported in Table 3, a more than dose proportional increase was observed for both plasma exposure and C_{max}, which reached the values of 21 μM·h and 8 μM, respectively, at the highest dose. In contrast, the liver levels measured at 24 h increased in a linear manner and, noticeably, the measured concentration of **59** in liver following a single oral dose of 20 mg/kg and higher was comparable or higher than the replicon EC_{90} measured in high serum conditions (EC_{90} = 40 nM). The pharmacokinetic profile of the K^+ salt of **59** in beagle dogs was also encouraging. The compound was dosed at 2 mg/kg intravenously and orally at 2, 5, and 10 mg/kg and, despite a high plasma clearance of 38 mL/min/kg, **59** displayed acceptable oral bioavailability with F = 17% at 2 mg/kg (Table 3). Similarly to what was previously observed in rat, also in dog a more than linear increase was found in oral exposure with increasing dose, reaching the values of AUC = 10 μM·h and C_{max} = 4 μM at 10 mg/kg. These data showed that it is possible for inhibitors belonging to this structural class to reach satisfactory levels of systemic exposure following oral dosing in at least two different preclinical species and, in combination with the sustained liver levels observed in rat, suggest a potential to achieve significant liver exposure also in the clinical setting.

In view of the interesting overall profile shown by **59**, the compound was further evaluated and found to be an inhibitor of wild type NS3 protease of genotypes 2a and 3a with K_i = 0.4 nM and K_i = 45 nM, respectively. Interestingly, **59** also proved to be a low nanomolar inhibitor of the mutant enzyme carrying the R155K single point mutation, a variant associated with decreased sensitivity to protease inhibitors both in vitro and in the clinical setting (K_i = 4 nM).[38] Moreover, **59** did not significantly inhibit other serine proteases (>15000-fold selectivity over trypsin and chymotripsin) and showed more than 100000-fold selectivity in a broad range screen on more than 150 different enzyme and receptor assays.

Conclusions

Within the class of P2−P4 macrocyclic inhibitors of HCV NS3/4A we investigated the possibility of replacing the P4 carbamate with a suitably substituted heterocycle. We discovered that the introduction of a 2-amino-1,3-thiazole within the macrocyclic linker was well tolerated in terms of potency and can offer an acceptable pharmacokinetic profile. Following on from these initial findings, optimization studies within the aminothiazole series were conducted to address structural modifications in the P3, P2, and P1 region of the macrocyclic core as well as in the linker chain and resulted in the identification of a series of compounds characterized by levels of enzyme and cellular potency comparable to those of current clinical candidates. Among these analogues, the isoindoline analogue **59** was evaluated in more detail and proved to be also a potent inhibitor of genotype 2a NS3 protease as well as of the clinically relevant mutant R155K. Moreover, **59** had an acceptable preclinical pharmacokinetic profile and displayed sustained lever levels following oral administration in rat.

Experimental Section

Molecular Modeling. The crystal structure of a peptidomimetic inhibitor bound to the NS3 protease was used as a starting point to build the macrocyclic inhibitor.[22] After minimization, a conformational analysis was performed with MacroModel[39] generating 10000 initial conformers for each compound using the Monte Carlo algorithm. The conformers were subsequently energy-minimized with the MMFF method.[40,41] Finally, the compound was optimized in the active site of the protein.

Chemistry. Reagents and solvents were obtained from commercial suppliers and were used without further purification. Flash chromatography purifications were performed on Merck silica gel (200−400 mesh) as the stationary phase or were conducted using prepacked cartridges on a Biotage system, eluting with PE 40−60 °C/EtOAc or DCM/MeOH mixtures. Analytical high performance and ultraperformance liquid chromatography−mass analysis (HPLC-MS and UPLC-MS) were performed on either a Waters Alliance 2795 apparatus or a Waters Acquity UPLC instrument, equipped with a PDA detector and a Waters ZQ mass spectrometer, using a X-Terra C_{18} column (5 μm, 4.6 mm × 50 mm), an Acquity UPLC BEH C_{18} column (1.7 μm, 2.1 mm × 50 mm), or an Acquity UPLC BEH Shield RP_{18} column (1.7 μm, 2.1 mm × mm 50 mm). Mobile phase comprised a linear gradient of binary mixtures of H_2O and MeCN, both containing 0.1% of HCO_2H. Preparative RP-HPLC was carried out on a Waters Micromass system incorporating a 2525 pump module, a Micromass ZQ detector and a 2767 collection module under Fraction Linx software or on a Shimadzu 10AV-VP coupled with a diode array detector SPD10AV-VP, using either a Waters Symmetry C_{18} (7 μm, 19 mm × 300 mm) or a Waters XBridge C_{18} (5 μm, 19 mm × 100 mm) column. Eluting conditions comprised a linear gradient of binary mixtures of H_2O and MeCN, both containing 0.1% of TFA, flow rate 20 mL/min. All final compounds within the aminothiazole series were isolated as TFA salts.

Nuclear magnetic resonance spectra (^{1}H NMR recorded at frequencies between 300 and 600 MHz) were obtained on Bruker Avance spectrometers and are referenced in ppm relative to TMS. Unless otherwise indicated, spectra were acquired at 300 K.

Low resolution mass spectra (MS) were obtained on a Perkin-Elmer API 100 or on a Waters MicroMass ZQ, operating in negative (ES^-) or positive (ES^+) electrospray ionization mode, and results are reported as the ratio of mass over charge (m/z). High resolving power accurate mass measurement electrospray mass spectral data (HRMS) were acquired by use of a Bruker Daltonics apex-Qe Fourier transform ion cyclotron resonance mass spetrometer (FT-ICR MS). External calibration was accomplished with oligomers of polypropylene glycol.

The purity of final compounds was assessed by two different analytical UPLC methods and found to be ≥95% in all cases. The UPLC analysis was perfomed on a Waters Acquity UPLC instrument, equipped with a PDA detector, using one of the following two methods: (1) Method A: column Acquity UPLC BEH C_{18} (1.7 μm, 2.1 mm × 50 mm); gradient: 10% to 100% MeCN/H_2O (0.1% HCO_2H) in 2.5 min, flow rate 0.5 mL/min. 2). Method B: column Acquity UPLC BEH Shield RP_{18} (1.7 μm, 2.1 mm × 50 mm); gradient: 30% to 100% MeCN/H_2O (0.1% HCO_2H) in 2.6 min, flow rate 0.5 mL/min.

Compounds **7**,[25] **10A**,[26] **10B**,[29] **10C**,[30] **13A**,[28] and **13B**[31] were prepared according to reported literature procedures.

Synthesis of 2-Amino-1,3,4-oxadiazole Analogue 4. Pent-4-enohydrazide (6). To a solution of pent-4-enoic acid (3.96 g, 39.6 mmol) in DCM (400 mL) was added *tert*-butyl carbazate (10.46

g, 79.0 mmol), followed by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (7.74 g, 40.4 mmol), and the mixture was stirred at RT for 16 h. The reaction mixture was then diluted with DCM, washed sequentially with sat. aq $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated under reduced pressure to give *tert*-butyl 2-pent-4-enoylhydrazinecarboxylate as a colorless oil (8.48 g). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.33 (bs, 1H), 6.51 (bs, 1H), 5.82 (m, 1H), 5.10−4.99 (m, 2H), 2.43−2.38 (m, 2H), 2.32 (t, J = 6.8 Hz, 2H), 2.03 (s, 9H). The obtained hydrazide was then dissolved in DCM (70 mL), and trifluoroacetic acid (75 mL, 973 mmol) was added dropwise at 0 °C. The resulting mixture was allowed to warm to RT over 1 h and brought to pH = 7 by addition of sat. aq $NaHCO_3$. The phases were separated and the organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give **6** as a pale-yellow oil (2.1 g, 44% yield from pent-4-enoic acid). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.95 (bs, 1H), 5.79 (m, 1H), 5.04 (dd, J = 17.0, 1.6 Hz, 1H), 4.97 (d, J = 10.0 Hz, 1H), 4.26 (bs, 2H), 2.45−2.23 (m, 2H), 2.12−2.08 (m, 2H).

Methyl *N*-[(2-Pent-4-enoylhydrazino)carbonyl]-L-valinate (8). Isocyanate **7**[25] (3.92 g, 24.9 mmol) was added to a solution of **6** (1.9 g, 16.6 mmol) in dioxane (10 mL), and the resulting mixture was stirred 3 h at RT. The volatiles were then removed under reduced pressure, and the residue was purified by SiO_2 gel chromatography (PE/EtOAc = 1/1) to obtain **8** as a pale-yellow oil (3.0 g, 66% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.55 (d, J = 2.1 Hz, 1H), 7.86 (d, J = 2.2 Hz, 1H), 6.48 (d, J = 8.7 Hz, 1H), 5.81 (m, 1H), 5.04 (dd, J = 17.2, 1.5 Hz, 1H), 4.96 (dd, J = 10.3, 1.5 Hz, 1H), 4.09 (dd, J = 8.7, 5.7 Hz, 1H), 3.64 (s, 3H), 2.32−2.24 (m, 2H), 2.22−2.15 (m, 2H), 1.94 (m, 1H), 0.86 (d, J = 6.9 Hz, 3H), 0.83 (d, J = 6.9 Hz, 3H). MS (ES$^+$) $C_{12}H_{21}N_3O_4$ requires 271; found 272 (M + H$^+$).

***N*-(5-But-3-en-1-yl-1,3,4-oxadiazol-2-yl)-L-valine (9).** A mixture of compound **8** (1.0 g, 3.69 mmol) and $POCl_3$ (4 mL, 42.9 mmol) was heated at 80 °C for 2 h. After cooling to 0 °C, water (5 mL) was carefully added and the mixture was brought to pH = 7 by addition of solid $NaHCO_3$. The mixture was then extracted with EtOAc and the organic layer dried (Na_2SO_4) and concentrated under reduced pressure to give methyl *N*-(5-but-3-en-1-yl-1,3,4-oxadiazol-2-yl)-L-valinate as a pale-yellow oil (0.93 g). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (d, J = 8.8 Hz, 1H), 5.83 (m, 1H), 5.08 (d, J = 17.2 Hz, 1H), 5.01 (d, J = 10.3 Hz, 1H), 3.93 (dd, J = 8.8, 6.1 Hz, 1H), 3.64 (s, 3H), 2.75 (t, J = 7.3 Hz, 2H), 2.43−2.39 (m, 2H), 2.10 (m, 1H), 0.94 (d, J = 6.8 Hz, 3 H), 0.92 (d, J = 6.8 Hz, 3 H). MS (ES$^+$) $C_{12}H_{19}N_3O_3$ requires 253; found 254 (M + H$^+$). A solution of the obtained methyl ester (0.45 g, 1.78 mmol) and LiOH (85 mg, 3.55 mmol) in H_2O/THF (1/1 v/v, 10 mL) was stirred for 1 h at RT. HCl (1N aq) was then added dropwise until pH = 2 was reached and the mixture was extracted with EtOAc. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to obtain **9** as a pale-yellow solid (0.42 g, 98% yield over two steps). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.98 (bs, 1H), 7.63 (d, J = 8.8 Hz, 1H), 5.82 (m, 1H), 5.08 (d, J = 17.1 Hz, 1H), 5.01 (d, J = 10.3 Hz, 1H), 3.86 (dd, J = 9.1, 5.8 Hz, 1H), 2.75 (t, J = 7.5 Hz, 2H), 2.42−2.37 (m, 2H), 2.12 (m, 1H), 0.98−0.92 (m, 6H). MS (ES$^+$) $C_{11}H_{17}N_3O_3$ requires 239; found 240 (M + H$^+$).

Methyl *N*-(5-But-3-en-1-yl-1,3,4-oxadiazol-2-yl)-L-valyl-4-[(7-vinylisoquinolin-1-yl)oxy]-L-prolinate (11). To a solution of **10A** (0.67 g, 1.81 mmol) in DCM (15 mL) was added **9** (0.42 g, 1.78 mmol), followed by ^{i}Pr$_2$NEt (0.93 mL, 5.33 mmol) and TBTU (0.63 g, 1.95 mmol). The resulting reaction mixture was stirred for 12 h at RT and partitioned between EtOAc and brine. The organic layer was dried (Na_2SO_4), concentrated under reduced pressure, and the residue was purified by SiO_2 gel chromatography (PE/EtOAc = 1/1) to give **11** as a colorless oil (0.3 g, 33% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (bs, 1H), 7.95 (d, J = 5.7 Hz, 1H), 7.83 (dd, J = 8.8, 1.3 Hz, 1H), 7.68 (d, J = 8.9 Hz, 1H), 7.22 (d, J = 5.7 Hz, 1H), 6.90 (dd, J = 17.6, 10.9 Hz, 1H), 5.91(m, 1H), 5.87 (d, J = 17.5 Hz, 1H), 5.81 (m, 1H), 5.35 (d, J = 10.9 Hz, 1H), 5.29 (d, J = 8.8 Hz, 1H), 5.06 (d, J = 17.5 Hz, 1H), 5.02 (d, J = 10.0 Hz, 1H), 4.78 (t, J = 8.3 Hz, 1H), 4.39 (dd, J = 8.9, 5.5 Hz, 1H), 4.27 (d, J = 11.5 Hz, 1H), 4.13 (dd, J = 11.5, 4.5 Hz, 1H), 3.78 (s, 3H), 2.76 (m, 1H), 2.67 (t, J = 7.7 Hz, 2H), 2.42−2.34 (m, 3H), 2.19 (m, 1H), 1.11 (d, J = 6.8 Hz, 3H), 1.01 (d, J = 6.8 Hz, 3H). MS (ES$^+$) $C_{28}H_{33}N_5O_5$ requires 519; found 520 (M + H$^+$).

Methyl (3*R*,5*S*,8*S*,16-*E*)-8-Isopropyl-7-oxo-2,27-dioxa-6,9,11,12,24-pentaazapentacyclo[16.6.2.1^{3,6}.1^{10,13}.0^{21,25}]octacosa-1(24),10,12,16,18,20,22,25-octaene-5-carboxylate (12). To a solution of **11** (0.1 g, 0.19 mmol) in DCE (20 mL) at 80 °C was added the Zhan 1^{23} catalyst (25 mg, 0.038 mmol), and the resulting reaction mixture was stirred at 80 °C for 2 h (only *E*-isomer detected by UPLC-MS analysis). After cooling to RT, the volatiles were removed under reduced pressure and the residue was purified by SiO_2 gel chromatography (PE/EtOAc = 3/7) to obtain **12** as a pale-yellow oil (55 mg, 55% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, J = 5.9 Hz, 1H), 7.78 (bs, 1H), 7.60 (d, J = 8.3 Hz, 1H), 7.46 (dd, J = 8.3, 1.7 Hz, 1H), 7.16 (d, J = 5.9 Hz, 1H), 6.47 (d, J = 16.0 Hz, 1H), 5.85 (ddd, J = 16.0, 8.8, 6.6 Hz, 1H), 5.74 (t, J = 3.3 Hz, 1H), 5.38 (d, J = 9.9 Hz, 1H), 4.68 (dd, J = 9.9, 8.1 Hz, 1H), 4.61−4.53 (m, 2H), 3.92 (dd, J = 11.4, 3.3 Hz, 1H), 3.75 (s, 3H), 2.96−2.86 (m, 2H), 2.67−2.60 (m, 2H), 2.26 (m, 1H), 2.14 (m, 1H), 1.16 (d, J = 6.7 Hz, 3H), 1.14 (d, J = 6.7 Hz, 3H). MS (ES$^+$) $C_{26}H_{29}N_5O_5$ requires 491; found 492 (M + H$^+$).

3*R*,5*S*,8*S*)-*N*-((1*R*,2*S*)-1-{[(Cyclopropylsulfonyl)amino]carbonyl}-2-vinylcyclopropyl)-8-isopropyl-7-oxo-2,27-dioxa-6,9,11,12,24-pentazapentacyclo[16.6.2.1^{3,6}.1^{10,13}.0^{21,25}]octacosa-1(24),10,12,18,20,22,25-heptaene-5-carboxamide (4). To a solution of **12** (55 mg, 0.047 mmol) in EtOH (10 mL) was added 10% Pd/C (0.005 mg, 10% w/w), and the resulting reaction mixture was stirred at RT under an atmosphere of H_2 for 2 h. The catalyst was filtered off, and the volatiles were removed under reduced pressure to give methyl (3*R*,5*S*,8*S*)-8-isopropyl-7-oxo-2,27-dioxa-11-thia-6,9,11,12,24-pentaazapentacyclo[16.6.2.1^{3,6}.1^{10,13}.0^{21,25}]octacosa-1(24),10,12,18,20,22,25-heptaene-5-carboxylate as a colorless oil (49 mg, 89% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (d, J = 5.7 Hz, 1H), 7.64 (s, 1H), 7.60 (d, J = 8.3 Hz, 1H), 7.43 (d, J = 8.3 Hz, 1H), 7.18 (d, J = 6.1 Hz, 1H), 5.83 (m, 1H), 5.19 (m, 1H), 4.78 (d, J = 11.4 Hz, 1H), 4.64 (dd, J = 10.2, 8.0 Hz, 1H), 4.43 (d, J = 9.7 Hz, 1H), 3.99 (dd, J = 11.7, 3.2 Hz, 1H), 3.77 (s, 3H), 3.75−3.73 (m, 2H), 3.49 (t, J = 3.5 Hz, 1H), 2.97−2.90 (m, 2H), 2.60 (m, 1H), 2.27 (m, 1H), 2.17 (m, 1H), 1.18−1.77 (m, 2H), 0.93−0.88 (m, 6H). MS (ES$^+$) $C_{26}H_{31}N_5O_5$ requires 493; found 494 (M + H$^+$). A solution of the obtained methyl ester (49 mg, 0.099 mmol) and LiOH (9.5 mg, 0.40 mmol) in H_2O/THF (1/1 v/v, 2 mL) was stirred for 1 h at RT. HCl (1N aq) was then added dropwise until pH = 2 was reached and the mixture was extracted with EtOAc. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to obtain (3*R*,5*S*,8*S*)-8-isopropyl-7-oxo-2,27-dioxa-6,9,11,12,24-pentaazapentacyclo[16.6.2.1^{3,6}.1^{10,13}.0^{21,25}]-octacosa-1(24),10,12,18,20,22,25-heptaene-5-carboxylic acid as a white solid (47 mg, 99% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.91 (d, J = 6.1 Hz, 1H), 7.71 (s, 1H), 7.65 (d, J = 8.3 Hz, 1H), 7.49 (d, J = 8.3 Hz, 1H), 7.24 (d, J = 6.1 Hz, 1H), 5.96 (m, 1H), 4.75 (t, J = 9.0 Hz, 1H), 4.61 (d, J = 11.8 Hz, 1H), 4.40 (d, J = 6.8 Hz, 1H), 3.99 (dd, J = 11.9, 3.2 Hz, 1H), 2.98−2.82 (m, 4H), 2.67−2.53 (m, 2H), 2.25−2.17 (m, 2H), 1.83−1.70 (m, 2H), 1.60 (m, 1H), 1.11−1.06 (m, 6H). MS (ES$^+$) $C_{25}H_{29}N_5O_5$ requires 479; found 480 (M + H$^+$). To a solution the obtained carboxylic acid in DMF (1 mL) was added **13A**[28] (29 mg, 0.108 mmol), followed by ^{i}Pr$_2$NEt (0.019 mL, 0.108 mmol) and TBTU (32 mg, 0.099 mmol). The resulting reaction mixture was stirred for 12 h at RT and purified by reverse phase HPLC to give the title compound as an off-white solid (25 mg, 37% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 8.93 (s, 1H), 7.95 (d, J = 9.2 Hz, 1H), 7.92 (d, J = 5.9 Hz, 1H), 7.78 (d, J = 8.3 Hz, 1H), 7.68 (s, 1H), 7.60 (d, J = 8.3 Hz, 1H), 7.36 (d, J = 5.9 Hz, 1H), 5.76 (m, 1H), 5.60 (m, 1H), 5.21 (d, J = 17.1 Hz, 1H), 5.08 (d, J = 10.7

Hz, 1H), 4.90 (d, J = 11.3 Hz, 1H), 4.25−4.16 (m, 2H), 3.97 (dd, J = 11.3, 3.3 Hz, 1H), 2.96−2.89 (m, 4H), 2.86−2.58 (m, 2H), 2.19−2.07 (m, 3H), 1.75−1.62 (m, 5H), 1.41 (m, 1H), 1.29 (m, 1H), 1.10−1.05 (m, 3H), 1.01 (d, J = 6.6 Hz, 3H), 0.96 (d, J = 6.6 Hz, 3 H). HRMS (ES^+) expected for $C_{34}H_{42}N_7O_7S$ ($M + H^+$) 692.2861; found 692.2886.

Synthesis of 2-Amino-1,3-thiazole Analogue 5. 1-Bromohex-5-en-2-one (14a). A solution of 4-pentenoyl chloride (0.78 g, 6.6 mmol) in Et_2O (5 mL) was added portionwise to a freshly prepared solution of diazomethane (ca. 16.6 mmol, 2.5 equiv) in Et_2O (50 mL) at 0 °C. The resulting reaction mixture was stirred for 30 min at 0 °C and for further 12 h at RT in an open flask. After cooling at 0 °C, HBr (48% aq, 1.3 mL, 8.6 mmol) was added dropwise, and the resulting reaction mixture was stirred at RT for 15 min, after which time the gas evolution had ceased. The ethereal solution was then washed with sat. aq $NaHCO_3$ (2 × 50 mL), brine (50 mL), dried (Na_2SO_4), and concentrated under reduced pressure to give the title compound as a pale-yellow oil (1.09 g, 94% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.80 (m, 1H), 5.06 (dd, J = 17.5, 1.3 Hz, 1H), 5.01 (dd, J = 10.5, 1.2 Hz, 1H), 3.88 (s, 2H), 2.76 (t, J = 7.3 Hz, 2H), 2.37 (m, 2H).

Methyl L-Valyl-(4*R*)-4-[(7-vinylisoquinolin-1-yl)oxy]-L-prolinate Hydrochloride (18). To a solution of **10A** hydrochloride (1.0 g, 2.72 mmol) in DMF (10 mL) was added Boc-(L)-Val (0.61 g, 2.83 mmol), followed by ^{i}Pr$_2$NEt (1.65 mL, 9.45 mmol) and TBTU (9.09 g, 2.83 mmol). The resulting reaction mixture was stirred 12 h at RT and partitioned between EtOAc and 1N aq HCl. The organic layer was further washed with sat. aq $NaHCO_3$, brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by SiO_2 gel chromatography (PE/EtOAc = 7/3) to give methyl *N*-(*tert*-butoxycarbonyl)-L-valyl-(4*R*)-4-[(7-vinylisoquinolin-1-yl)oxy]-L-prolinate as a colorless oil (0.65 g, Y = 54%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.92 (d, J = 5.8 Hz, 1H), 7.80 (dd, J = 8.0, 1.6 Hz, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.21 (d, J = 5.4 Hz, 1H), 6.87 (dd, J = 17.5, 11.0 Hz, 1H), 5.89−5.83 (m, 2H), 5.34 (d, J = 11.0 Hz, 1H), 5.19 (bd, J = 8.8 Hz, 1H), 4.79 (bt, J = 8.3 Hz, 1H), 4.28−4.21 (m, 2H), 4.08 (dd, J = 11.5, 4.4 Hz, 1H), 3.77 (s, 3H), 2.72 (m, 1H), 2.36 (m, 1H), 2.05 (m, 1H), 1.03 (d, J = 6.7 Hz, 3H), 0.93 (d, J = 6.7 Hz, 3H). MS (ES^+) $C_{27}H_{35}N_3O_6$ requires 497; found: 498 ($M + H^+$). A solution of the above compound (0.65 g, 1.31 mmol) in HCl/dioxane (4N, 10 mL) was stirred at RT for 2 h. The volatiles were then removed under reduced pressure to give **18** as an off-white solid (0.56 g). MS (ES^+) $C_{22}H_{27}N_3O_4$ requires 397; found 398 ($M + H^+$).

Methyl *N*-(Aminocarbonothioyl)-L-valyl-(4*R*)-4-[(7-vinylisoquinolin-1-yl)oxy]-L-prolinate (19). ^{i}Pr$_2$NEt (0.44 mL, 2.5 mmol) was added to a suspension of **18** (0.54 g, 1.25 mmol) in DCM (5 mL) at 0 °C, and the resulting solution was immediately added to a solution of FMOC isothiocyanate (0.39 g, 1.37 mmol) in DCM (5 mL) at 0 °C. The resulting mixture was stirred at RT for 30 min and then treated with a 20% solution of piperidine in MeOH (5 mL). After 3 h, the volatiles were removed under reduced pressure and the residue was purified by SiO_2 gel chromatography (gradient elution, PE/EtOAc = 8/2 to DCM/MeOH = 95/5) to give the title compound as a pale-yellow foam (0.53 g, 93% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (s,1H), 7.90 (d, J = 5.8 Hz, 1H), 7.78 (dd, J = 8.5, 1.7 Hz, 1H), 7.65 (d, J = 8.5 Hz, 1H), 7.19 (d, J = 5.7 Hz, 1H), 6.91 (dd, J = 17.7, 10.9 Hz, 1H), 6.26 (bs, 2H), 5.91−5.86 (m, 2H), 5.34 (d, J = 11.1 Hz, 1H), 4.94 (m, 1H), 4.85 (bd, J = 11.1 Hz, 1H), 4.64 (bt, J = 8.6 Hz, 1H), 4.14−4.07 (m, 2H), 3.72 (s, 3H), 2.77 (m, 1H), 2.36 (m, 1H), 2.07 (m, 1H), 1.11 (d, J = 6.8 Hz, 3H), 1.06 (d, J = 6.7 Hz, 3H). MS (ES^+) $C_{23}H_{28}N_4O_4S$ requires 456; found 457 ($M + H^+$).

Methyl *N*-(4-But-3-en-1-yl-1,3-thiazol-2-yl)-L-valyl-(4*R*)-4-[(7-vinylisoquinolin-1-yl)oxy]-L-prolinate (20a). A solution of thiourea **19** (0.19 g, 0.42 mmol) and **14a** (0.11 g, 0.63 mmol) in dioxane (3 mL) was stirred at 65 °C for 1 h. The volatiles were then removed under reduced pressure and the residue was purified by SiO_2 gel chromatography (DCM/MeOH = 98/2) to give the title compound as a pale-brown oil (0.21 g, 93% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, J = 5.7 Hz, 1H), 7.87 (bs, 1H), 7.79 (dd, J = 8.5, 1.7 Hz, 1H), 7.67 (d, J = 8.5 Hz, 1H), 7.20 (d, J = 6.0 Hz, 1H), 6.77 (dd, J = 17.1, 11.0 Hz, 1H), 5.92 (m, 1H), 5.81 (d, J = 17.6 Hz, 1H), 5.68 (s, 1H), 5.59 (m, 1H), 5.39 (bd, J = 10.5 Hz, 1H), 5.33 (d, J = 10.9 Hz, 1H), 4.85−4.76 (m, 2H), 4.44 (d, J = 11.7 Hz, 1H), 4.25 (bt, J = 8.2 Hz, 1H), 4.11 (dd, J = 11.4, 4.4 Hz, 1H), 3.76 (s, 3H), 2.67 (m, 1H), 2.40−2.23 (m, 3H), 2.13−2.05 (m, 3H), 1.10 (d, J = 6.7 Hz, 3H), 1.04 (d, J = 6.7 Hz, 3H). MS (ES^+) $C_{29}H_{34}N_4O_4S$ requires 534; found 535 ($M + H^+$).

Methyl (3*R*, 5*S*, 8*S*)-8-Isopropyl-7-oxo-2oxa-11-thia-6,9,24,27-tetraazapentacyclo[16.6.2.1^{3,5}.1^{10,13}.0^{21,25}]octacosa-1(24),10(27),12,18,20,22,25-heptaene-5-carboxylate (21a). To a solution of **20a** (0.2 g, 0.37 mmol) in DCE (7 mL) was added Zhan 1^{27} catalyst (36 mg, 0.055 mmol), and the resulting reaction mixture was stirred at 85 °C for 2 h. The volatiles were then removed under reduced pressure, and the residue was purified by SiO_2 gel chromatography (PE/EtOAc = 1/1) to give methyl (3*R*,5*S*,-8*S*,16*E* or *Z*)-8-isopropyl-7-oxo-2oxa-11-thia-6,9,24,27-tetraazapentacyclo[16.6.2.1^{3,5}.1^{10,13}.0^{21,25}]octacosa-1(24),10(27),12,18,20,22,25-heptaene-5-carboxylate as a ca. 4:1 mixture of *E*- and *Z*-olefins as determined by UPLC-MS analysis (pale-brown glass; 0.10 g, 55% yield). MS (ES^+) $C_{27}H_{30}N_4O_4S$ requires 506; found 507 ($M + H^+$). To a solution of the above compound (100 mg, 0.19 mmol) in MeOH/EtOAc (1/1 v/v, 4 mL) was added 10% Pd/C (10 mg, 10% w/w), and the resulting reaction mixture was stirred at RT under an atmosphere of H_2 for 2 h. The catalyst was filtered off, replaced with a fresh aliquot (10 mg), and the reaction mixture was stirred in the above conditions for a further period of time. The process was repeated as previously described until complete conversion to product was observed (typically 36 h, 3 fresh aliquots of catalyst). After filtering off the catalyst, the volatiles were removed under reduced pressure to give **21a** as a pale-yellow oil (64 mg, 62% yield over two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, J = 5.8 Hz, 1H), 7.86 (bs, 1H), 7.63 (d, J = 8.4 Hz, 1H), 7.45 (d, J = 8.6 Hz, 1H), 7.21 (d, J = 5.5 Hz, 1H), 6.01 (s, 1H), 5.97 (m, 1H), 4.76 (bt, J = 8.8 Hz, 1H), 4.67 (m, 1H), 4.23 (d, J = 11.9 Hz, 1H), 3.96 (dd, J = 11.5, 3.7 Hz, 1H), 3.76 (s, 3H), 2.89−2.74 (m, 3H), 2.55−2.50 (m, 2H), 2.37−2.17 (m, 3H), 1.75−1.67 (m, 4H), 1.13 (d, J = 6.7 Hz, 3H), 1.01 (d, J = 6.8 Hz, 3H). MS (ES^+) $C_{27}H_{32}N_4O_4S$ requires 508; found 509 ($M + H^+$).

(3*R*,5*S*,8*S*)-*N*-((1*R*,2*S*)-1-{[(Cyclopropylsulfonyl)amino]-carbonyl}-2-vinylcyclopropyl)-8-isopropyl-7-oxo-2oxa-11-thia-6,9,24,27-tetraazapentacyclo[16.6.2.1^{3,5}.1^{10,13}.0^{21,25}]octacosa-1-(24),10(27),12,18,20,22,25-heptaene-5-carboxylate (5). To a solution of methyl ester **21a** (64 mg, 0.126 mmol) in THF/H_2O (1/1 v/v, 1 mL) was added LiOH (30 mg, 1.26 mmol). The resulting reaction mixture was stirred at RT for 2 h and then treated with 1N aq HCl until pH = 2 was reached. The volatiles were removed under reduced pressure, and the residue was taken up in toluene (3 mL) and concentrated under reduced pressure three times to give the corresponding carboxylic acid. MS(ES^+) $C_{26}H_{30}N_4O_4S$ requires 494; found 495 ($M + H^+$). To a solution of the latter in DMF (1 mL was added hydrochloride **13A** (37 mg, 0.138 mmol) followed by ^{i}Pr$_2$NEt (0.80 mL, 0.44 mmol) and TBTU (44 mg, 0.138 mmol). The resulting reaction mixture was stirred 12 h at RT and purified by preparative RP HPLC to give **5** as a trifluoroacetate salt (off-white solid, 47 mg, 62% yield). ^{1}H NMR (600 MHz, DMSO-d_6 + TFA) δ 10.70 (s, 1H), 9.60 (d, J = 8.9 Hz, 1H), 8.74 (s, 1H), 7.97 (d, J = 5.8 Hz, 1H), 7.91 (bs, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.61 (dd, J = 8.4, 1.5 Hz, 1H), 7.39 (d, J = 5.8 Hz, 1H), 6.67 (s, 1H), 5.95 (bt, J = 3.5 Hz, 1H), 5.56 (ddd, J = 17.2, 10.4, 9.1 Hz, 1H), 5.21 (dd, J = 17.2, 1.3 Hz, 1H), 5.09 (dd, J = 10.4, 1.3 Hz, 1H), 5.02 (bs, 1H), 4.44 (dd, J = 9.9, 7.6 Hz, 1H), 4.35 (d, J = 11.4 Hz, 1H), 3.93 (dd, J = 11.4, 3.3 Hz, 1H), 2.94−2.86 (m, 2H), 2.72−2.64 (m, 3H), 2.52 (m,

1H), 2.40 (m, 1H), 2.20 (ddd, J = 14.0, 10.4, 4.5 Hz, 1H), 2.13 (app q, J = 8.6 Hz, 1H), 1.78−1.63 (m, 3H), 1.62−1.50 (m, 2H), 1.28 (dd, J = 9.4, 5.3 Hz, 1H), 1.13−0.98 (m, 4H), 1.06 (d, J = 6.8 Hz, 3H), 0.96 (d, J = 6.8 Hz, 3H). HRMS (ES^+) expected for $C_{35}H_{43}N_6O_6S_2$ ($M + H^+$) 707.2680; found 707.2679.

General Procedure for the Synthesis of the α-Bromo-methylketones 14b and 14c. To a solution of the appropriate carboxylic acid (15 mmol) in DCM (10 mL) at 0 °C was added dropwise oxalylchloride (2 M in DCM, 22.5 mmol) and a catalytic amount of DMF (0.2 mL). The resulting mixture was stirred at RT for 2 h and then concentrated under reduced pressure. The residue was dissolved in Et_2O (10 mL) and added portionwise to a freshly prepared solution of diazomethane (ca. 33 mmol, 2.2 equiv) in Et_2O (110 mL) at 0 °C. The resulting mixture was stirred for 30 min at 0 °C and a further 12 h at RT in an open flask. After cooling to 0 °C, HBr (48% aq, 3 mL) was added dropwise and the solution stirred at RT for 30 min until the gas evolution had ceased. The ethereal solution was washed with aq sat. $NaHCO_3$, brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was typically used as such in the following step. Alternatively, it could be purified by SiO_2 gel chromatography, eluting with PE/Et_2O = 98/2.

General Procedure for the Synthesis of the Thioureas 23−30. Step 1. To a 0.3 M solution of one of the P2-building blocks **10A−C** in DCM was added the appropriate *N*-Boc-protected amino acid **22a−e** (1.1 equiv), followed by $^iPr_2NEt$ (2.0 equiv) and TBTU (1.5 equiv), and the reaction mixture was stirred for 1 h at RT. The volatiles were removed under reduced pressure, and the residue was dissolved in EtOAc. The organic layer was washed with 1N aq HCl, sat. aq $NaHCO_3$, brine, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by SiO_2 gel chromatography (typically with gradient elution: PE/EtOAc = 8/2 to 7/3) to give the *N*-Boc-protected P2−P3 coupling product.

Step 2. The product from step 1 was dissolved in 4N HCl in dioxane, and the resulting solution (0.4 M) was stirred at RT for 2 h. The volatiles were removed under reduced pressure to give the P2−P3 coupling product as hydrochloride salt.

Step 3. To a 0.25 M solution of the compound from step 2 in DCM at 0 °C was added $^iPr_2NEt$ (2.0 equiv), and the resulting mixture was added immediately to a 0.25 M solution of Fmoc-isothiocyanate (1.1 equiv) in DCM at 0 °C. The resulting mixture was stirred at RT for 30 min and then treated with a 20% solution of piperidine in MeOH (2.5−5.0 mL) and stirred for further 3 h at RT. The volatiles were removed under reduced pressure, and the residue was purified by SiO_2 gel chromatography (typically with gradient elution PE/EtOAc = 8/2 to DCM/MeOH = 95/5).

General Procedure for the Synthesis of the Aminothiazoles 20b−c and 31a−c to 38a−c. A 0.14 M solution of the selected thiourea **19** or **23−30** and the chosen α-bromomethylketone **14a−c** (1.2 equiv) in dioxane was stirred at 65 °C for 1 h. The solution was then cooled to RT, the volatiles were removed under reduced pressure, and the residue was usually employed in the following step without further purification; when required, the compound was purified by SiO_2 gel chromatography (typically with gradient elution PE/EtOAc = 8/2 to DCM/MeOH = 98/2).

General Procedure for the Synthesis of the Methyl Esters 21b−c and 39a−c to 46a−c. Step 1. To a 0.015 M solution of the selected aminothiazole **20b−c** or **31a−c** to **38a−c** in DCE at 85 °C was added the Zhan 1^{27} catalyst (0.15 equiv), and the resulting reaction mixture was stirred at 85 °C for 2 h. After cooling to RT, the volatiles were removed under reduced pressure and the residue was either used as such in the following step or purified by SiO_2 gel chromatography (typically with gradient elution PE/EtOAc = 8/2 to DCM/MeOH = 98/2).

Step 2. (Performed only en route to analogues bearing a saturated linker chain). To a 0.05 M solution of the methyl ester from step 1 in MeOH/EtOAc (1/1 v/v) was added 10% Pd/C (10% w/w), and the resulting reaction mixture was stirred under an atmosphere of H_2 at RT for 2 h. The catalyst was filtered off, replaced with a fresh aliquot, and the reaction mixture was stirred in the above conditions for a further period of time. The process was repeated as previously described until complete conversion to product was observed (typically 36 h, 3 fresh aliquots of catalyst). After filtering off the catalyst, the volatiles were removed under reduced pressure to give the saturated macrocyclic methyl esters, which were used directly in the following step.

General Procedure for the Synthesis of Aminothiazole Macrocyclic Analogues 47−60. Step 1. To a 0.13 M solution of the selected methyl ester **21** or **39−46** in THF/H_2O (1/1 v/v, 1 mL) was added LiOH (10 equiv). The resulting reaction mixture was stirred at RT for 2 h and then treated with 1N aq HCl until pH = 2 was reached. The volatiles were removed under reduced pressure, the residue was taken up in toluene, and concentrated three times to give the corresponding carboxylic acid.

Step 2. To a 0.13 M solution of the carboxylic acid from step 1 in DMF was added hydrochloride **13A** or **13B** (1.1 equiv), followed by $^iPr_2NEt$ (3.5 equiv) and TBTU (1.1 equiv). The resulting reaction mixture was stirred 12 h at RT and purified by preparative RP HPLC to give compounds **47−60** as a trifluoroacetate salts. The reported yields for the final compounds are all given after preparative RP HPLC purification and calculated over two steps from the macrocyclic methyl ester unless otherwise specified.

(3*R*,5*S*,8*S*)-*N*-((1*R*,2*S*)-1-{[(Cyclopropylsulfonyl)amino]carbonyl}-2-ethenylcyclopropyl)-8-(1-methylethyl)-7-oxo-2-oxa-11-thia-6,9,25,28-tetraazapentacyclo[17.6.2.1^{3,6}.1^{10,13}.0^{22,26}]nonacosa-1(25),10(28),12,19,21,23,26-heptaene-5-carboxamide (47). The title compound was prepared using ester **21b**(sat) and **13A**; off-white solid (7 mg, 10% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.57 (s, 1H), 8.98 (s, 1H), 8.01 (d, J = 5.8 Hz, 1H), 7.90−7−77 (m, 2H), 7.67 (d, J = 8.4 Hz, 1H), 7.43 (d, J = 5.8 Hz, 1H), 6.20 (bs, 1H), 6.08 (m, 1H), 5.64 (m, 1H), 5.24 (d, J = 17.0 Hz, 1H), 5.12 (d, J = 10.2 Hz, 1H), 4.48−4.38 (m, 2H), 4.19−3.95 (m, 2H), 2.97 (m, 1H), 2.88−2.74 (m, 2H), 2.48−2.41 (m, 3H), 2.31−2.13 (m, 3H), 1.80−1.69 (m, 3H), 1.54 (m, 1H), 1.36−1.26 (m, 4H), 1.13−0.98 (m, 10H). MS (ES^+) $C_{36}H_{44}N_6O_6S_2$ requires 720; found 721 ($M + H^+$).

(3*R*,5*S*,8*S*,16*E*)-*N*-((1*R*,2*S*)-1-{[(Cyclopropylsulfonyl)amino]carbonyl}-2-vinylcyclopropyl)-8-isopropyl-7-oxo-2-oxa-11-thia-6,9,24,27-tetraazapentacyclo[16.6.2.13,5.110,13.021,25]octacosa-1(24),10(27),12,16,18,20,21,23,25-nonaene-5-carboxamide (48). The title compound was prepared using ester **21b**(*E*-**db**) and **13A**; off-white solid (24 mg, 36% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 8.69 (s, 1H), 8.33 (s, 1H), 7.98 (d, J = 5.7 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.64 (d, J = 8.4 Hz, 1H), 7.40 (d, J = 5.7 Hz, 1H), 6.68−6.53 (m, 2H), 6.36 (m, 1H), 5.78−5.54 (m, 2H), 5.24 (d, J = 17.0 Hz, 1H), 5.15−5.04 (m, 2H), 4.46−4.41 (m, 2H), 3.95 (d, J = 11.4 Hz, 1H), 3.00−2.65 (m, 5H), 2.32−2.11 (m, 4H), 1.73 (m, 1H), 1.28 (m, 1H), 1.17−0.96 (m, 10H). HRMS (ES^+) expected for $C_{35}H_{41}N_6O_6S_2$ ($M + H^+$) 705.2524; found 705.2557.

(3*R*,5*S*,8*S*)-*N*-((1*R*,2*S*)-1-{[(Cyclopropylsulfonyl)amino]carbonyl}-2-vinylcyclopropyl)-8-isopropyl-14,14-dimethyl-7-oxo-2-oxa-11-thia-6,9,24,27-tetraazapentacyclo[16.6.2.1^{3,6}.1^{10,13}.0^{21,25}]octacosa-1(24),10(27),12,18,20,22,25-heptaene-5-carboxamide (49). The title compound was prepared using ester **21c**(sat) and **13A**; off-white solid (33 mg, 28% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 8.88 (s, 1H), 7.96 (d, J = 5.8 Hz, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.75 (s, 1H), 7.58 (bd, J = 8.4 Hz, 1H), 7.38 (d, J = 5.8 Hz, 1H), 6.21 (bs, 1H), 5.89 (bs, 1H), 5.59 (dt, J_1 = 17.1 Hz, J_2 = J_3 = 10.1 Hz, 1H), 5.22 (d, J = 17.1 Hz, 1H), 5.10 (d, J = 10.1 Hz, 1H), 4.40 (m, 1H), 4.17 (d, J = 11.4 Hz, 1H), 3.98 (bd, J = 11.4 Hz, 1H), 2.94 (m, 1H), 2.75−2.69 (m, 2H), 2.59 (dd, J = 13.8, 7.6 Hz, 1H), 2.27−2.07 (m, 4H), 1.79−1.66 (m, 2H), 1.51 (m, 1H), 1.42−1.28 (m, 3H), 1.23 (s, 3H), 1.14−1.02 (m, 12H), 0.97 (d, J = 6.6 Hz, 3H).

HRMS (ES^+) expected for $C_{37}H_{47}N_6O_6S_2$ ($M + H^+$) 735.2993; found 735.3029.

(3*R*,5*S*,8*S*)-8-Cyclohexyl-*N*-((1*R*,2*S*)-1-{[(cyclopropylsulfonyl)-amino] carbonyl}-2-ethenylcyclopropyl)-7-oxo-2-oxa-11-thia-6,9,24,27-tetraazapentacyclo[16.6.2.1^{3,6}.1^{10,13}.0^{21,25}]octacosa-1(24),10-(27),12,18,20,22,25-heptaene-5-carboxamide (50). The title compound was prepared using ester **40a**(sat) and **13A**; off-white solid (33 mg, 13% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H), 8.81 (s, 1H), 7.98 (d, J = 5.8 Hz, 1H), 7.91 (s, 1H), 7.83 (d, J = 8.3 Hz, 1H), 7.61 (d, J = 8.3 Hz, 1H), 7.40 (d, J = 5.8 Hz, 1H) 6.47 (bs, 1H), 5.98 (m, 1H), 5.56 (m, 1H), 5.22 (d, J = 17.2 Hz, 1H), 5.10 (d, J = 10.1 Hz, 1H), 4.79 (m, 1H), 4.41 (t, J = 8.3 Hz, 1H), 4.28 (m, 1H), 3.98 (bd, J = 12.1 Hz, 1H), 2.98−1.85 (m, 9H), 1.83−1.46 (m, 9H), 1.45−0.84 (m, 11H). HRMS (ES^+) expected for $C_{38}H_{47}N_6O_6S_2$ ($M + H^+$) 746.2993; found 747.2947.

(3*R*,5*S*,8*S*)-*N*-((1*R*,2*S*)-1-{[(Cyclopropylsulfonyl)amino]carbonyl}-2-ethenylcyclopropyl)-8-(1,1-dimethylethyl)-7-oxo-2-oxa-11-thia-6,9,24,27-tetraazapentacyclo[16.6.2.1^{3,6}.1^{10,13}.0^{21,25}]octacosa-1(24),10(27),12,16,18,20,22,25-octaene-5-carboxamide (51). The title compound was prepared using ester **39a**(sat) and **13A**; off-white solid (30 mg, 22% yield). ^{1}H NMR (600 MHz, DMSO-d_6 + TFA) δ 10.74 (s, 1H), 10.08 (bs, 1H), 8.61 (s, 1H), 7.96 (d, J = 5.7 Hz, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.78 (bs, 1H), 7.58 (d, J = 7.8 Hz, 1H), 7.38 (d, J = 5.7 Hz, 1H), 6.70 (s, 1H), 5.64 (bt, J = 3.6 Hz, 1H), 5.57 (ddd, J = 17.2, 10.1, 9.0 Hz, 1H), 5.22 (d, J = 17.2 Hz, 1H), 5.10 (d, J = 10.1 Hz, 1H), 4.98 (d, J = 9.1 Hz, 1H), 4.77 (d, J = 12.3 Hz, 1H), 4.51 (t, J = 8.4 Hz, 1H), 3.86 (dd, J = 12.3 Hz, 3.8 Hz, 1H), 2.93 (m, 1H), 2.87−2.79 (m, 2H), 2.80−2.63 (m, 3H), 2.30 (m, 1H), 2.19 (app q, J = 9.0 Hz, 1H), 1.84 (m, 1H), 1.73 (dd, J = 7.8, 5.6 Hz, 1H), 1.72−1.61 (m, 2H), 1.52 (m, 1H), 1.32 (dd, J = 9.4, 5.6 Hz, 1H), 1.08 (s, 9H), 1.10−1.02 (m, 4H). HRMS (ES^+) expected for $C_{36}H_{45}N_6O_6S_2$ ($M + H^+$) 721.2837; found 721.2799.

(3*R*,5*S*,8*S*)-*N*-((1*R*,2*S*)-1-{[(Cyclopropylsulfonyl)amino]carbonyl}-2-ethenylcyclopropyl)-8-(1-methylethyl)-7-oxo-23-phenyl-2-oxa-11-thia-6,9,22,27-tetraazapentacyclo[16.6.2.1^{3,6}.1^{10,13}.0^{21,25}]-octacosa-1(24),10(27),12,18,20,22,25-heptaene-5-carboxamide (52). The title compound was prepared using ester **41a** (sat) and **13A**; off-white solid (30 mg, 26% yield). ^{1}H NMR (600 MHz, C_5D_5N) δ 10.58 (s, 1H), 8.46 (d, J = 7.3 Hz, 2H), 8.29 (d, J = 8.5 Hz, 1H), 8.11 (s, 1H), 7.97 (d, J = 9.5 Hz, 1H), 7.55−7.49 (m, 4H), 7.30 (s, 1H), 6.33 (s, 1H), 6.10 (dt, J_1 = 16.9, J_2 = J_3 = 10.0 Hz, 1H), 5.27−5.18 (m, 2H), 5.07 (d, J = 10.0 Hz, 1H), 4.89 (t, J = 8.1 Hz, 1H), 4.59 (d, J = 12.1 Hz, 1H), 4.35 (bd, J = 12.1 Hz, 1H), 3.30 (m, 1H), 2.80−2.47 (m, 11H), 2.24 (t, J = 6.9 Hz, 1H), 1.87−1.79 (m, 2H), 1.77−1.70 (m, 3H), 1.44 (m, 1H), 1.35 (m, 1H), 1.25 (d, J = 6.7 Hz, 3H), 1.21 (d, J = 6.7 Hz, 3H), 0.97−0.84 (m, 2H). MS (ES^+) $C_{41}H_{46}N_6O_6S_2$ requires 782; found 783 ($M + H^+$).

(1*R*,12*E*,22*S*,25*S*)-*N*-((1*R*,2*S*)-1-{[(Cyclopropylsulfonyl)amino]-carbonyl}-2-ethenylcyclopropyl)-22-(1-methylethyl)-3,23-dioxo-2-oxa-19-thia-4,21,24,28-tetraazapentacyclo [22.2.1.1^{4,7}.1^{17,20}.0^{6,11}] octacosa-6,8,10,12,17,20(28)-hexaene-25-carboxamide (53). The title compound was prepared using ester **42b**(*E*-**db**) and **13A**; off-white solid (36 mg, 24% yield from thiazole **34b**). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.62 (s, 1H), 8.84 (s, 1H), 7.31−7.21 (m, 3H), 6.38 (d, J = 16.2 Hz, 1H), 6.36 (bs, 1H), 6.05 (dt, J_1 = 16.2 Hz, J_2 = J_3 = 6.9 Hz, 1H), 5.60 (ddd, J = 17.2, 10.3, 9.8 Hz, 1H), 5.29 (bt, 1H), 5.23 (d, J = 17.2 Hz, 1H), 5.10 (d, J = 10.3 Hz, 1H), 4.71−4.59 (m, 6H), 4.30 (bt, J = 8.4 Hz, 1H), 4.16 (d, J = 11.8 Hz, 1H), 3.97 (m, 1H), 2.93 (m, 1H), 2.59 (m, 2H), 2.34−2.13 (m, 6H), 1.89−1.76 (m, 2H), 1.71 (dd, J = 7.7, 5.2 Hz, 1H), 1.32 (dd, J = 9.2, 5.2 Hz, 1H), 1.10−1.03 (m, 6H), 0.93 (d, J = 6.7 Hz, 3H). HRMS (ES^+) expected for $C_{36}H_{45}N_6O_6S_2$ ($M + H^+$) 737.2786; found 737.2788.

(1*R*,12*E*,21*S*,24*S*)-*N*-((1*R*,2*S*)-1-{[(Cyclopropylsulfonyl)amino]-carbonyl}-2-ethenylcyclopropyl)-21-(1-methylethyl)-3,22-dioxo-2-oxa-18-thia-4,20,23,27-tetraazapentacyclo[21.2.1.1^{4,7}.1^{16,19}.0^{6,11}]-octacosa-6,8,10,12,16,19(27)-hexaene-24-carboxamide (54). The title compound was prepared using ester **42a**(*E*-**db**) and **13A**; off-white solid (19 mg, 24% yield from thiazole **34a**). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.55 (bs, 1H), 9.95 (bs, 1H), 7.30−7.20 (m, 2H), 7.15 (s, 1H), 6.00−5.88 (m, 3H), 5.72 (m, 1H), 5.52 (m, 1H), 5.29 (d, J = 12.0 Hz, 1H), 5.16 (d, J = 6.5 Hz, 1H), 4.76−4.69 (m, 2H), 4.48−4.40 (m, 2H), 4.24 (m, 1H), 4.08 (m, 1H), 3.94 (m, 1H), 3.64 (m, 1H), 3.05 (m, 1H), 2.95 (m, 1H), 2.59 (m, 2H), 2.68−2.49 (m, 7H), 2.14 (m, 1H), 2.03 (m, 1H), 1.48−1.33 (m, 3H), 1.15 (d, J = 6.0 Hz, 3H), 1.05 (d, J = 6.0 Hz, 3H). HRMS (ES^+) expected for $C_{35}H_{43}N_6O_6S_2$ ($M + H^+$) 723.2629; found 723.2639.

(1*R*,12*E*,22*S*,25*S*)-*N*-((1*R*,2*S*)-1-{[(Cyclopropylsulfonyl)amino]-carbonyl}-2-ethenylcyclopropyl)-22-(1,1-dimethylethyl)-3,23-dioxo-2-oxa-19-thia-4,21,24,28-tetraazapentacyclo[22.2.1.1^{4,7}.1^{17,20}.0^{6,11}]-nonaacosa-6,8,10,12,17,20(28)-hexaene-25-carboxamide (55). The title compound was prepared using ester **43b**(*E*-**db**) and **13A**; off-white solid (50 mg, 45% yield from thiazole **35b**). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.48 (s, 1H), 9.73 (s, 1H), 8.72 (bs, 1H), 7.28 (t, J = 7.5 Hz, 1H), 7.21 (d, J = 7.5 Hz, 1H), 7.19 (d, J = 7.5 Hz, 1H), 6.39 (d, J = 16.1 Hz, 1H), 6.29 (s, 1H), 6.00 (dt, J_1 = 16.1 Hz, J_2 = J_3 = 6.9 Hz, 1H), 5.62 (dt, J_1 = 17.2, J_2 = J_3 = 10.0 Hz, 1H), 5.25 (bs, 1H), 5.21 (d, J = 17.2 Hz, 1H), 5.09 (d, J = 10.0 Hz, 1H), 4.77 (d, J = 14.8 Hz, 1H), 4.72−4.64 (m, 3H), 4.61−4.52 (m, 2H), 4.34 (t, J = 8.2 Hz, 1H), 4.24 (d, J = 11.9 Hz, 1H), 4.07 (dd, J = 11.9, 5.2 Hz, 1H), 2.93 (m, 1H), 2.67 (m, 1H), 2.54 (m, 1H), 2.42 (dd, J = 13.4, 7.2 Hz, 1H), 2.34−2.28 (m, 2H), 2.24−2.14 (m, 2H), 1.88−1.76 (m, 2H), 1.69 (dd, J = 7.9, 5.5 Hz, 1H), 1.35 (dd, J = 9.4, 5.5 Hz, 1H), 1.11−1.02 (m, 4H), 1.06 (s, 9H). HRMS (ES^+) expected for $C_{37}H_{47}N_6O_7S_2$ ($M + H^+$) 751.2942; found 751.2956.

(1*R*,22*S*,25*S*)-*N*-((1*R*,2*S*)-1-{[(Cyclopropylsulfonyl)amino]-carbonyl}-2-ethenylcyclopropyl)-22-(1,1-dimethylethyl)-3,23-dioxo-2-oxa-19-thia-4,21,24,28-tetraazapentacyclo[22.2.1.1^{4,7}.1^{17,20}.0^{6,11}]-nonaacosa-6,8,10,17,20(28)-pentaene-25-carboxamide (56). The title compound was prepared using ester **43b**(sat) and **13A**; off-white solid (50 mg, 33% yield from thiazole **35b**). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.54 (s, 1H), 8.78 (s, 1H), 7.23 (t, J = 7.4 Hz, 1H), 7.15 (d, J = 7.4 Hz, 1H), 7.09 (d, J = 7.4 Hz, 1H), 6.27 (bs, 1H), 5.64 (m, 1H), 5.26 (m, 1H), 5.22 (d, J = 17.2 Hz, 1H), 5.09 (d, J = 11.1 Hz, 1H), 4.77−4.46 (m, 5H), 4.28 (t, J = 8.1 Hz, 1H), 4.22 (d, J = 11.4 Hz, 1H), 4.04 (m, 1H), 2.93 (m, 1H), 2.68−2.29 (m, 6H), 2.24 (app q, J = 8.6 Hz, 1H), 2.13 (m, 1H), 1.78−1.62 (m, 3H), 1.58−1.41 (m, 3H), 1.37 (dd, J = 9.6, 5.5 Hz, 1H), 1.15−0.93 (m, 13H). HRMS (ES^+) expected for $C_{37}H_{49}N_6O_7S_2$ ($M + H^+$) 753.3099; found 753.3110.

(1*R*,12*E*,22*S*,25*S*)-22-Cyclohexyl-*N*-((1*R*,2*S*)-1-{[(cyclopropyl-sulfonyl)amino]carbonyl}-2-vinylcyclopropyl)-3,23-dioxo-2-oxa-19-thia-4,21,24,28-tetraazapentacyclo[22.2.1.1^{4,7}.1^{17,20}.0^{6,11}]-nonacosa-6,8,10,12,17,20(28)-hexaene-25-carboxamide (57). The title compound was prepared using ester **44b**(*E*-**db**) and **13A**; off-white solid (58 mg, 24% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.64 (s, 1H), 8.80 (s, 1H), 7.32−7.20 (m, 3H), 6.40 (bs, 1H), 6.36 (d, J = 16.2 Hz, 1H), 6.05 (dt, J_1 = 16.2 Hz, J_2 = J_3 = 6.7 Hz, 1H), 5.58 (m, 1H), 5.27 (m, 1H), 5.23 (d, J = 17.1 Hz, 1H), 5.11 (d, J = 11.1 Hz, 1H), 4.79 (d, J = 14.6 Hz, 1H), 4.72−4.56 (m, 4H), 4.29 (t, J = 8.3 Hz, 1H), 4.15 (d, J = 11.9 Hz, 1H), 3.97 (m, 1H), 2.93 (m, 1H), 2.66−2.36 (m, 4H), 2.35−2.09 (m, 9H), 1.90−1.59 (m, 7H) 1.34−0.99 (m, 7H). HRMS (ES^+) expected for $C_{39}H_{49}N_6O_7S_2$ ($M + H^+$) 777.3099; found 777.3068.

(1*R*,12*E*,22*S*,25*S*)-22-Cyclohexyl-*N*-((1*R*,2*S*)-1-{[(cyclopropyl-sulfonyl) Amino]carbonyl}-2-ethylcyclopropyl)-3,23-dioxo-2-oxa-19-thia-4,21,24,28-tetraazapentacyclo[22.2.1.1^{4,7}.1^{17,20}.0^{6,11}] nonacosa-6,8,10,12,17,20(28)-hexaene-25-carboxamide (58). The title compound was prepared using ester **44b**(*E*-**db**) and **13B**; off-white solid (58 mg, 29% yield). ^{1}H NMR (600 MHz, DMSO-d_6 + TFA) δ 10.62 (s, 1H), 8.59 (s, 1H), 7.29 (d, J = 7.6 Hz, 1H), 7.27 (t, J = 7.6 Hz, 1H), 7.21 (d, J = 7.6 Hz, 1H), 6.63 (s, 1H), 6.28 (d, J = 16.2 Hz, 1H), 6.09 (dt, J_1 = 16.2 Hz, J_2 = J_3 = 7.0 Hz, 1H), 5.32 (bt, J = 3.8 Hz, 1H), 4.87 (bs, 1H), 4.77 (d, J = 14.8 Hz, 1H), 4.70 (d, J = 14.8 Hz, 1H), 4.64−4.57 (m, 2H), 4.33 (t, J = 8.8 Hz, 1H). 4.22 (d, J = 11.9 Hz, 1H), 3.87 (dd, J = 11.9, 3.8 Hz, 1H), 2.94 (m, 1H), 2.63−2.57 (m, 2H), 2.40 (dd, J = 14.0, 7.6 Hz, 1H),

2.29 (m, 1H), 2.16−2.09 (m, 2H), 2.00−1.90 (m, 2H), 1.80−1.70 (m, 4H), 1.65 (bd, J = 12.0 Hz, 1H), 1.58 (bd, J = 11.7 Hz, 1H) 1.52−1.36 (m, 4H), 1.35−0.96 (m, 10H), 0.90 (t, J = 7.2 Hz, 3H). MS (ES^+) $C_{39}H_{50}N_6O_7S_2$ requires 778; found 779 ($M + H^+$).

(1*R*,12*E*,22*S*,25*S*)-*N*-((1*R*,2*S*)-1-{[(Cyclopropylsulfonyl)amino]carbonyl}-2-ethenylcyclopropyl)-22-[(1*S*)-1-methylpropyl]-3,23-dioxo-2-oxa-19-thia-4,21,24,28-tetraazapentacyclo[22.2.1.1^{4,7}.1^{17,20}.0^{6,11}]nonaacosa-6,8,10,12,17,20(28)-hexaene-25-carboxamide (59). The title compound was prepared using ester **45b**(*E*-**db**) and **13A**; off-white solid (52 mg, 27% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.70 (s, 1H), 9.72 (bs, 1H), 8.69 (s, 1H), 7.31 (d, J = 7.5 Hz, 1H), 7.27 (t, J = 7.5 Hz, 1H), 7.21 (d, J = 7.5 Hz, 1H), 6.64 (s, 1H), 6.28 (d, J = 16.2 Hz, 1H), 6.10 (dt, J_1 = 16.2 Hz, J_2 = J_3 = 6.8 Hz, 1H), 5.56 (dt, J_1 = 17.3 Hz, J_2 = J_3 = 10.1 Hz, 1H), 5.32 (bt, 1H), 5.23 (d, J = 17.3 Hz, 1H), 5.10 (d, J = 10.1 Hz, 1H), 4.91 (bs, 1H), 4.78 (d, J = 14.7 Hz, 1H), 4.70−4.58 (m, 3H), 4.34 (dd, J = 9.7, 7.4 Hz, 1H), 4.23 (d, J = 11.7 Hz, 1H), 3.85 (dd, J = 11.7, 3.8 Hz, 1H), 2.93 (m, 1H), 2.62−2.58 (m, 2H), 2.42 (dd, J = 14.2, 7.4 Hz, 1H), 2.29 (m, 1H), 2.17−2.09 (m, 3H), 2.05 (m, 1H), 1.94 (m, 1H), 1.77−1.69 (m, 2H), 1.45 (m, 1H), 1.25 (dd, J = 9.3, 5.3 Hz, 1H), 1.18 (m, 1H), 1.13−1.03 (m, 4H), 1.01 (d, J = 6.7 Hz, 3H), 0.90 (t, J = 7.2 Hz, 3H). HRMS (ES^+) expected for $C_{37}H_{47}N_6O_7S_2$ ($M + H^+$) 751.2942; found 751.2921.

(1*R*,12*E*,22*S*,25*S*)-22-Cyclopentyl-*N*-((1*R*,2*S*)-1-{[(cyclopropylsulfonyl)amino]carbonyl}-2-vinylcyclopropyl)-3,23-dioxo-2-oxa-19-thia-4,21,24,28-tetraazapentacyclo[22.2.1.1^{4,7}.1^{17,20}.0^{6,11}]nonacosa-6,8,10,12,17,20(28)-hexaene-25-carboxamide (60). The title compound was prepared using ester **46b**(*E*-**db**) and **13A**; off-white solid (60 mg, 30% yield). ^{1}H NMR (600 MHz, DMSO-d_6, T = 330 K) δ 10.43 (s, 1H), 8.79 (s, 1H), 7.70 (bs, 1H), 7.28 (t, J = 7.6 Hz, 1H), 7.21 (d, J = 7.6 Hz, 1H), 7.20 (d, J = 7.6 Hz, 1H), 6.40 (d, J = 16.2 Hz, 1H), 6.28 (bs, 1H), 6.02 (dt, J_1 = 16.2 Hz, J_2 = J_3 = 7.0 Hz, 1H), 5.62 (ddd, J = 17.2, 10.4, 10.0 Hz, 1H), 5.29 (bt, J = 3.9 Hz, 1H), 5.23 (dd, J = 17.2, 1.4 Hz, 1H), 5.10 (dd, J = 10.4, 1.4 Hz, 1H), 4.80 (d, J = 14.4 Hz, 1H), 4.71−4.59 (m, 4H), 4.31 (dd, J = 9.0, 7.7 Hz, 1H), 4.17 (d, J = 11.7 Hz, 1H), 4.04 (dd, J = 11.7, 5.1 Hz, 1H), 2.93 (m, 1H), 2.62 (m, 1H), 2.57 (m, 1H), 2.39 (dd, J = 12.1, 7.8 Hz, 1H), 2.34−2.24 (m, 3H), 2.21−2.14 (m, 2H), 1.87−1.76 (m, 3H), 1.74−1.68 (m, 2H), 1.65−1.60 (m, 2H), 1.55−1.46 (m, 2H), 1.43−1.35 (m, 2H), 1.33 (dd, J = 9.4, 5.2 Hz, 1H), 1.13−1.10 (m, 2H), 1.07−1.03 (m, 2H). HRMS (ES^+) expected for $C_{38}H_{47}N_6O_6S_2$ ($M + H^+$) 763.2942; found 763.2939.

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Supporting Information Available: Characterization data for intermediates **14**, **20**, **21**, **23**−**30**, **32**−**38**, and **39**−**46**; additional characterization data for compounds **4**, **5**, **51**, **55**, **57**, **59**, and **60**. This material is available free of charge via the Internet at http://pubs.acs.org.

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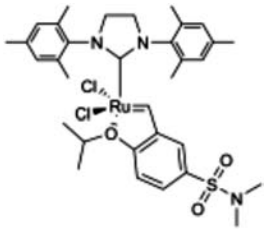

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