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Inhibitors of the Hepatitis C Virus NS3 Protease with Basic Amine Functionality at the P3-Amino Acid N-Terminus: Discovery and Optimization of a New Series of P2−P4 Macrocycles

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In a follow-up to our recent disclosure of P2−P4 macrocyclic inhibitors of the hepatitis C virus (HCV) NS3 protease (e.g., **1**, Chart 1), we report a new but related compound series featuring a basic amine at the N-terminus of the P3-amino acid residue. Replacement of the electroneutral P3-amino acid capping group (which is a feature of almost all tripeptide-like inhibitors of NS3 reported to date) with a basic group is not only tolerated but can result in advantageous cell based potency. Optimization of this new class of P3-amine based inhibitors gave compounds such as **25** and **26** that combine excellent cell based activity with pharmacokinetic properties that are attractive for an antiviral targeting HCV.

Introduction

Hepatitis C virus (HCV[a]) is a human pathogen from the flaviviridae family of positively stranded RNA viruses and infects around 200 million individuals worldwide.[1] Chronic infections of HCV are initially asymptomatic but can ultimately lead to serious liver disorders such as hepatocellular carcinoma and cirrhosis. HCV is consequently a leading cause of liver transplantation.[2] The current standard of care for HCV infections is based on subcutaneous injection of a modified interferon-α usually combined with ribavarin. Such treatment regimens are inconvenient, poorly tolerated, and only successful at curing HCV infection in around 50% of patients. New therapies targeting virally encoded enzymes are under investigation,[3,4] but to date, no direct-acting antiviral agent has reached the market.

Replication of HCV occurs predominantly in hepatocytes where the genome is translated into a polyprotein that is subsequently cleaved to produce the structural components of HCV, as well as the nonstructural proteins that constitute the viral replication machinery. Four out of the five polyprotein cleavages that occur in the nonstructural region are mediated by the virally encoded heterodimeric NS3/NS4A (NS3) enzyme.[5] This enzyme is a serine protease that has been shown to be essential for viral replication and that has been studied intensively as a target for direct antivirals. BILN-2061, a reversible P1−P3 macrocyclic "product-like" inhibitor[6] of NS3 was the first compound for which clinical proof of concept was achieved, validating the NS3 protease as an excellent target for drug intervention. Subsequently related noncovalent inhibitors[7] (e.g., ITMN-191 and TMC435350) and serine-trap based covalent compounds[8] (e.g., VX-950 and Sch503034) have elicited strong clinical reductions in viral titers, further illustrating the potential benefit to human health from development of drugs against NS3. Toward this goal, we have recently described the design and initial evaluation of a novel series of P2−P4 macrocyclic inhibitors of the HCV NS3/NS4A protease.[9] These compounds (e.g., **1**, Chart 1) demonstrated strong inhibition of the NS3 enzyme, had robust cell-based potency, and showed promising preliminary pharmacokinetic profiles. The evolution of this compound class to bismacrocyclic analogues (e.g., **2**) and to a clinical candidate MK-7009 has also been disclosed.[10] As part of our continuing effort in this area, we report here an exploration of structure−activity relationships in the linker group that tethers the nitrogen atom of the P3-amino acid to the heterocyclic group attached to the hydroxyproline residue in P2.[11] This work led to the finding that basic alkylamine functionality at the N-terminus of active-site P1−P3 peptidomimetic inhibitors of NS3 is not only tolerated but can be beneficial in terms of enhanced cell based potency or improved pharmacokinetic properties.

Biology

Compounds were assessed for activity (K_i) against recombinant NS3 protease using a time-resolved fluorescence assay.[12] Inhibition of subgenomic HCV RNA replication was measured in HUH-7 cells using modifications[13] of the procedure described by Bartenschlager;[14] reported data were generated using cellular HCV RNA as the assay readout and were confirmed (results not shown) in an independent assay using a monoclonal antibody against the viral NS3 protease as the readout. Cell-based activity (EC_{50}) in the replicon assay was measured routinely in the presence of 10% fetal bovine serum; high serum conditions refer to data collected in the presence

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[a] Abbreviations: HCV, hepatitis C virus; NS, nonstructural; HATU, azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; TBTU *O*-benzotriazole-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; NOESY, nuclear Overhauser enhanced spectroscopy; RCM, ring closing metathesis; RP-HPLC, reversed phase high performance liquid chromatography; po, per os; iv, intravenous.

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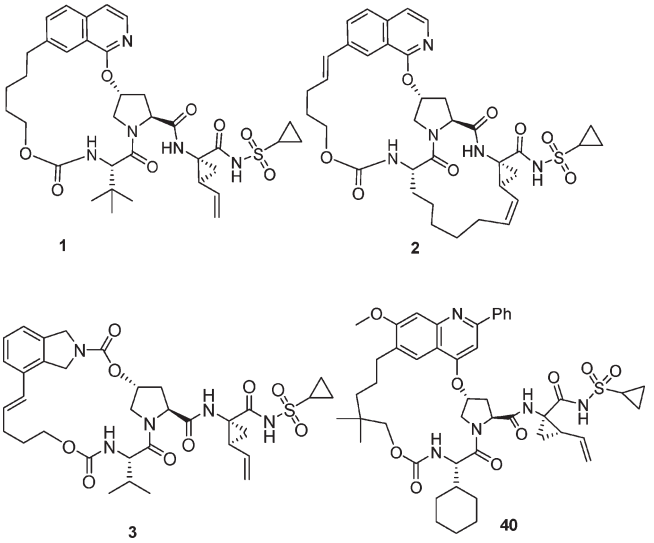
Chart 1. P2−P4 Macrocyclic Inhibitors of the HCV NS3 Protease Enzyme



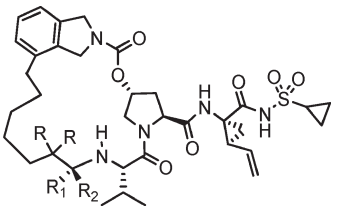
of 50% normal human serum. Enzyme inhibition data and replicon potencies were generated against genotype 1b HCV. Preliminary pharmacokinetic studies were conducted through oral administration of compounds to male Sprague−Dawley rats at 5 mg/kg using PEG_{400} as vehicle. In these studies plasma exposure was determined from the area under the plasma concentration/time curve between 0 and 4 h, and liver tissue concentrations were determined at the 4 h time point. Follow-up pharmacokinetic studies were conducted for compound **25** in both male Sprague−Dawley rat and male beagle dog. The dosing parameters used were the following: dog, intravenous (iv) 1 mg/kg ($DMSO/PEG_{400}/H_2O$, 20%:60%:20% as vehicle); dog, per os (po) 5 mg/kg (PEG_{400}/H_2O, 80%:20% as vehicle); rat, iv 5 mg/kg ($DMSO/PEG_{400}/H_2O$ 20%:60%:20% as vehicle); rat, po 5 mg/kg (PEG_{400} as vehicle). Pharmacokinetic parameters were determined from the area under the plasma concentration/time curve between 0 and 24 h (or to the last point at which compound levels were quantifiable). Liver tissue concentrations were determined (in rat) at the 24 h time point.

Chemistry

The P2−P4 macrocyclic amine compounds described (Tables 1, 3, and 4) were assembled using the modular approach outlined in Figure 1. This route relied on ring closing metathesis (RCM) methodology to construct the central P2−P4 macrocyclic ring (**10**) followed by installation of the P1−P1′ functionality by amide coupling with the known amino acid acylsulfonamide fragment **11**.[15] The RCM precursors **9** were available through amide coupling between a *trans*-4-hydroxyproline methyl ester derivative (**4**−**7**) functionalized with the required P2-heterocyclic group, and the N-alkylated P3-amino acid derivatives **8**. A vinyl group on the P2-heterocycle in compounds **4**−**7** served as a partner for the RCM step with the terminal olefin in the functionalized amino acids **8**.

Scheme 1 illustrates the synthesis of the P3-amino acid derivatives **8** that were employed herein. Treatment of the known oxazolidine **27**[16] with 6-bromohex-1-ene afforded a mixture of the amino alcohols **28a**,**b**. The diastereomeric ratio was 7:1, and the individual isomers were separated by reversed phase high performance liquid chromatography (RP-HPLC).

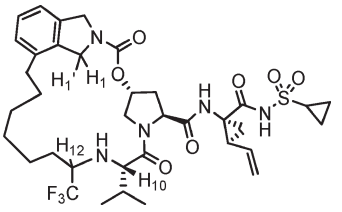
Table 1. P2−P4 Macrocyclic Amine Inhibitors Containing a Trifluoroethylamine Amide Isostere (**12a**,**b**) and Early Structural Analogues (**13**−**15**)



compd	R	R_1	R_2	K_i (nM)[a]	EC_{50} (nM)[b]
3	See Chart 1.			0.04 ± 0.01	12
12a	H	CF_3	H	1.1 ± 0.1	18
12b	H	H	CF_3	5.7 ± 0.3	470
13a[c]	H	Me	H	0.27 ± 0.07	1
13b[c]	H	H	Me	1.7 ± 0.5	40
14	H	H	H	0.51 ± 0.2	7
15	F	H	H	0.35 ± 0.01	14

[a] K_i values in this and subsequent tables are quoted as the arithmetic mean ± standard error (or half-range where $n = 2$) for two to nine independent determinations. [b] EC_{50} values are measured in the presence of 10% fetal bovine serum. [c] The absolute stereochemistry of compounds **13a** and **13b** is assigned by analogy to **12a**,**b** (see ref 21 for further details).

Table 2. Most Significant NOE[23] Intensities for Compounds **12a** and **12b** in Comparison with Predicted Interatomic Distances from Molecular Modeling

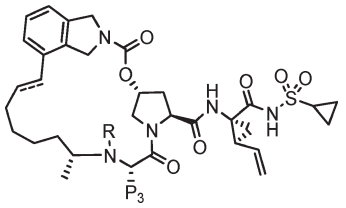


	12a (12-*S*)		**12b** (12-*R*)	
proton	calculated (Å)	measured[a]	calculated (Å)	measured[a]
$H_{12}-H_{10}$	2.9	s	3.6	m
$H_{12}-H_{1(centroid)}$	3.5	m	6.2	nd

[a] NOE intensities determined from NOESY experiments: w = weak; m = medium; s = strong; nd = not detected.

Oxidation of these compounds with Jones reagent furnished the corresponding carboxylic acids **8a**,**b**. The remaining P2−P4 linking fragments **8c**−**j** were prepared by reductive amination using the appropriate amino ester derivative. Thus, reaction of 7-octen-2-one **29** with the methyl esters of valine, cyclopentylglycine, or cyclohexylglycine afforded (after methyl ester hydrolysis) the functionalized amino acids **8c**−**e**. The reductive amination step proceeded with a diastereomeric ratio of around 1:1, and compounds **8c**−**e** were used as mixtures in the subsequent coupling reactions (see Scheme 3). A reductive amination/ester hydrolysis sequence also proceeded in efficient yield with the aldehydes **30**, **31**, and **32**[17] giving **8f**−**j**. 2,2-Difluoro-6-heptenal **31** (which was used in the preparation of **8g**) was obtained by fluorination of **30** with *N*-fluoro-*N*-(phenylsulfonyl)benzenesulfonamide using racemic proline as the organocatalyst.[18]

Table 3. Structural Changes to the P3-Amino Acid Residue and Their Effect on Rat Pharmacokinetic Properties



compd	bond	P_3	R	K_i (nM)	EC_{50} (nM)[a]	AUC_{po} (μM·h)[b]	Liver (μM)[c]
13a	single	Val	H	0.27 ± 0.07	1	< 0.1	< 0.1
17a	double[d]	C_5H_9	H	0.29 ± 0.07	0.5	< 0.1	0.26
17c	single	C_5H_9	H	0.1 ± 0.03	0.7	< 0.1[e]	< 0.1[e]
18a	single	C_6H_{11}	H	0.06 ± 0.01	2	< 0.1	1.04
19	single	C_6H_{11}	Me	0.35 ± 0.01	3	< 0.1[e]	0.56[e]

[a] EC_{50} values were measured in the presence of 10% fetal bovine serum. [b] Area under the plasma concentration/time curve between 0 and 4 h following oral administration of the compounds (5 mg/kg) to Sprague−Dawley rats. [c] Rat liver concentrations are measured 4 h after oral administration (5 mg/kg) to Sprague−Dawley rats. [d] *E*-Olefin geometry. [e] 4 mg/kg oral dose.

Scheme 2 illustrates the construction of compound **12a**. Thus, TBTU mediated amide coupling between **4**[19] and **8a** furnished the RCM precursor **9a** as a single diastereoisomer, and this material was smoothly cyclized to **10a** using Zhan I[20] as catalyst. Direct hydrogenation of the crude product from the RCM reaction gave the saturated macrocyclic compound *sat*-**10a** (62% yield from **9a**). The synthesis was completed by $LiOH \cdot H_2O$ mediated hydrolysis of the proline methyl ester followed by TBTU mediated amide coupling with **11**. Compound **12a** was isolated following purification by RP-HPLC. In identical fashion as outlined in Scheme 2, the inhibitor **12b** (which is a diastereoisomer of **12a**, Table 1) was accessed from **4** and **8b**. The intermediates (not shown) were **9b** (RCM precursor) and **10b** (P2−P4 macrocycle).

The construction of compounds **13a**,**b**, **17a**−**c**, and **18a**,**b** is shown in Scheme 3. Although the P3 fragments **8c**−**e** used contain both a free amine and a carboxylic acid group (which have the potential to undergo a homocoupling reaction), their reaction with **4** was successfully accomplished using HATU as the peptide reagent. The RCM precursors **9c**−**e** were isolated in 43−76% yield. The amine functionality in **9c**−**e** was deactivated as a TFA salt and in this form did not impede the Zhan I mediated RCM reaction (45−73% yield). Macrocycles **10c**−**e** (each a mixture of diastereoisomers at the chiral center adjacent to the P3-N atom) were purified by RP-HPLC to give the individual 12-*R* and 12-*S* isomers; only *E*-olefins were isolated from the ring closing metathesis reaction. The individual diastereoisomers[21] (*R*)-**10c**−**e** and (*S*)-**10c**−**e** were elaborated separately through the final steps of the synthesis. Thus, hydrolysis of the proline methyl ester and a hydrogenation step set up the final TBTU-mediated coupling with **11**. Compounds **13a**, **17c**, and **18a** (from (*R*)-**10c**, (*R*)-**10d**, and (*R*)-**10e**, respectively) and compounds **13b** and **18b** (from (*S*)-**10c** and (*S*)-**10e**, respectively) were isolated following RP-HPLC purification. In a similar vein **17a** and **17b** were obtained from (*R*)-**10d** and (*S*)-**10d** by omitting the catalytic hydrogenation step. Compound **19** was prepared by direct reductive-formylation of **18a**, which was smoothly achieved (55% yield following RP-HPLC purification) in the presence of a Lewis acid ($ZnCl_2$) using $NaBH_3CN$ as the reducing agent.

An analogous reaction sequence as has already been described was used to access compounds **14** and **15** (Scheme 4). Thus, coupling of **4** with the P3 fragments **8f** or **8g** gave the RCM precursors **9f**,**g** and Zhan I mediated RCM furnished the olefins **10f**,**g**. Hydrogenation, proline ester deprotection, and amide coupling with **11** again completed the synthesis.

The macrocyclic amine compounds **20**−**26** were prepared (Scheme 5) from the *trans*-4-hydroxyproline heterocyclic derivatives **5**,[9] **6**,[10] and **7**[22] together with the P3-amino acid fragments **8h**−**j**. HATU mediated coupling of **8h** or **8i** with **6** gave the ring closing metathesis precursors **9h**,**i** from which Zhan I catalyzed RCM generated the *trans*-olefins **10h**,**i**. Following removal of the proline methyl ester, the corresponding carboxylic acids were elaborated either to **22**/**24** by direct coupling with **11** or to **23**/**25** by a hydrogenation/coupling sequence. Likewise, reaction of **8j** with **5** or of **8h** with **7** afforded the macrocycles **20**−**21** or **26**, respectively.

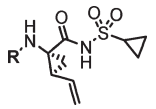
Having been unable to obtain functionalized amino acids **8** through reductive amination of aldehydes such as **30** with sterically hindered amino acids, compound **16** was accessed as outlined in Scheme 6. Coupling of **4** with (D,L)-*N*-Boc-trifluoromethylglycine followed by *N*-Boc deprotection furnished **33**. Reductive amination between this compound and **30** was possible but gave **9l** as a single diastereoisomer in modest yield (17%). Following elaboration of **9l** to **16** as has previously been described, the P3-amino acid residue was confirmed to have the desired (*R*)-configuration by ^1H,^1H NOESY experiments.[23]

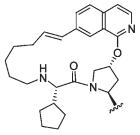
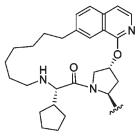
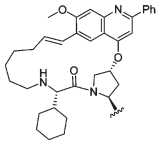
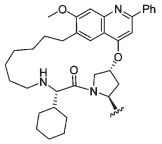
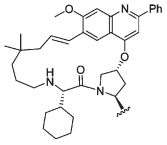
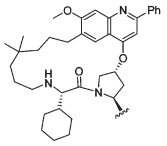
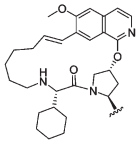
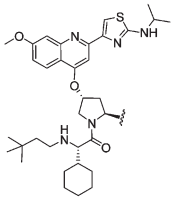
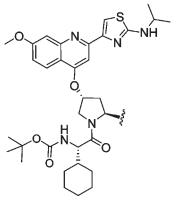
The acyclic tripeptide analogues **38** and **39** were prepared as outlined in Scheme 7. The intermediates **35a**,**b**[24] containing the P2-proline residue from BILN-2061 were elaborated either at the N-terminus to furnish the P2−P3 fragment **37** or at the carboxylic acid to give the P1−P2 dipeptide analogue **36**. Coupling of **36** with **34** (which was available following reductive amination of cyclohexylglycine methyl ester and 3,3-dimethylbutanal followed by routine protecting group manipulations) led after *N*-Boc-deprotection to the amine **38**. TBTU mediated coupling of **37** with **11** provided the urethane capped tripeptide analogue **39**.

Results and Discussion

Peptidomimetic inhibitors that span the P1−P3 region (or P1′−P3 region) of the NS3 protease enzyme are ubiquitous in the HCV literature. Compound series based upon both acyclic structures[25] and several classes of P1−P3 macrocycles[7,8,26]

Table 4. Activity and Preliminary Rat Pharmacokinetic Profile for P2−P4 Macrocyclic Amine Inhibitors: SAR at the P2 Heterocyclic Group and Evaluation of an Acyclic Analogue **38**



No.	R	Ki (nM)	[a]EC_{50} (nM)[a]	[b]AUC (μM.h)	[c]Liver (μM)
20		1.5±0.3	120	[d]nd	nd
21		1.3±0.2	410	nd	nd
22		0.16±0.04	19	<0.1	0.64
23		0.11±0.02	23	<0.1	0.35
24		0.12±0.03	33	0.5	5.6
25		0.24±0.06	48	0.45	13.7
26		0.32±0.01	25	7	2.8
38		0.24±0.04	74	nd	nd
39		0.07±0.03	65	nd	nd

[a] EC_{50} values were measured in the presence of 50% normal human serum. [b] Area under the plasma concentration/time curve between 0 and 4 h following oral administration of the compounds (5 mg/kg) to Sprague−Dawley rats. [c] Rat liver concentrations are measured 4 h after oral administration (5 mg/kg). [d] nd = not determined.

have been reported. More recently, P2−P4 macrocycles[9,10,27] have been shown to be a viable class of tripeptide mimetics that strongly inhibit HCV replication. A structural feature of almost all HCV inhibitors from these classes[28] is an electroneutral capping group at their P3 N-terminus, most usually an alkyl (or cycloalkyl) amide, urea, or urethane. The role of the capping group may be seen as twofold; the lipophilic alkyl group generates binding energy through interaction toward the S4 subsite of NS3, while the P3-amidic NH group can engage in a hydrogen bonding interaction with the backbone carbonyl of Ala157.[29] In light of these considerations we were attracted to a recently reported[30] trifluoroethylamine isostere for an amide bond and questioned whether in the context of our P2−P4 macrocyclic inhibitors (e.g., **3**, Chart 1) this might provide a viable alternative to the urethane functionality found in this compound class to date. We envisioned that the alkyl chain of the tether between the P2 and P3 residues might still engage S4, while the nonbasic amine group of the P3-amino acid would retain the potential to act as a hydrogen bond donor. The suitability of this hypothesis was borne out by molecular modeling studies[31] on **3** and the trifluoroethylamine analogues **12a**,**b** (Table 1) which have opposite configuration at the CF_3 substituted carbon α to the P3-N atom. Superposition of **3** with **12b** (which has *R*-configuration at the α-CF_3 group) suggested that a steric clash between the enzyme and the CF_3 group would result in significant distortion of the P2−P4 region of the ligand and ultimately diminish interaction with the NS3 enzyme. In contrast, the isomer based on (*S*)-configuration at the α-CF_3 group (**12a**) overlaid well with urethane **3**. For this isomer the CF_3 group is oriented away from the enzyme surface, allowing the ligand to interact with NS3 in a manner similar to that of the (predicted) bioactive conformation of **3**.

Compounds **12a**,**b** were prepared as is outlined in Scheme 2 and then tested in our NS3 enzyme and cell based replicon assays. Results showed that although both compounds caused somewhat lower inhibition of the NS3 enzyme than the urethane **3**,[32] they nonetheless retained potency in the low nanomolar range (Table 1). Furthermore, in the cell based replicon assay a comparable response for the more active trifluoroethylamine **12a** (EC_{50} = 18 nM) as for urethane **3** (EC_{50} = 12 nM) was measured, highlighting the suitability of a trifluoroethylamine based linker in the context of a P2−P4 macrocycle.

The absolute stereochemistry at C_{12} for **12a** and **12b** was assessed by NMR analysis. Significantly different interatomic distances between H_{12} and both H_{10} and the H_1 centroids were expected for the two diastereoisomers on the basis of molecular modeling, and these predictions were confirmed by $^1H,^1H$-NOESY experiments[23] performed on both compounds. As reported in Table 2, a strong match was observed between the calculated distances and the measured NOE intensities for both epimers, indicating (*S*)-configuration at C_{12} for the more active isomer **12a** and (*R*)-configuration for **12b**. Both compounds had similar *Z*-conformations at their proline amide bonds. Thus, the stronger biochemical potency that is predicted (and observed) for compound **12a** with respect to **12b** together with measured NMR data and molecular modeling make a strong case for the assignment of (12*S*)-configuration at the α-CF_3 group in **12a**.

A feature that distinguishes **12a** from previously reported cathepsin K inhibitors that incorporate a trifluoroethylamine isostere of an amide is the pK_a of the amine functionality β to the CF_3 group. The predicted[33] pK_a of this N-atom in **12a** is

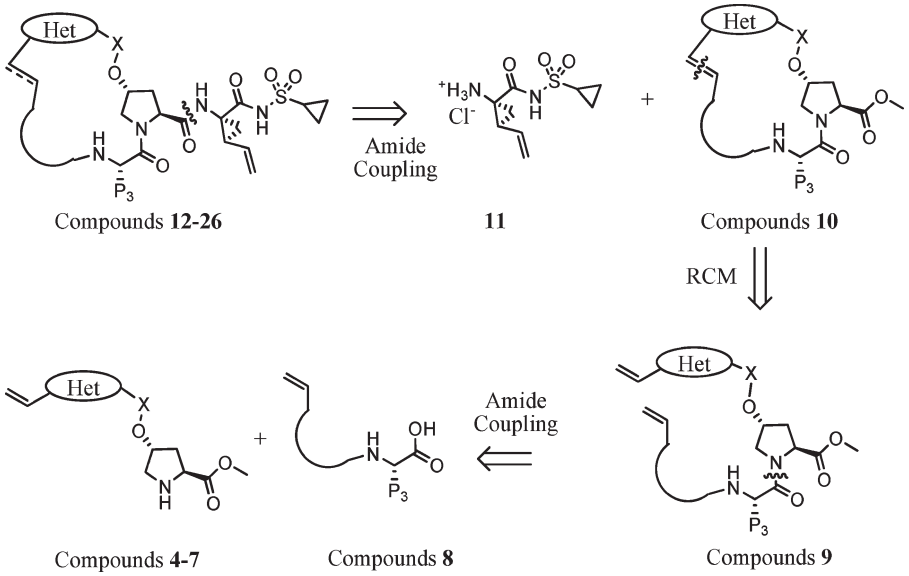


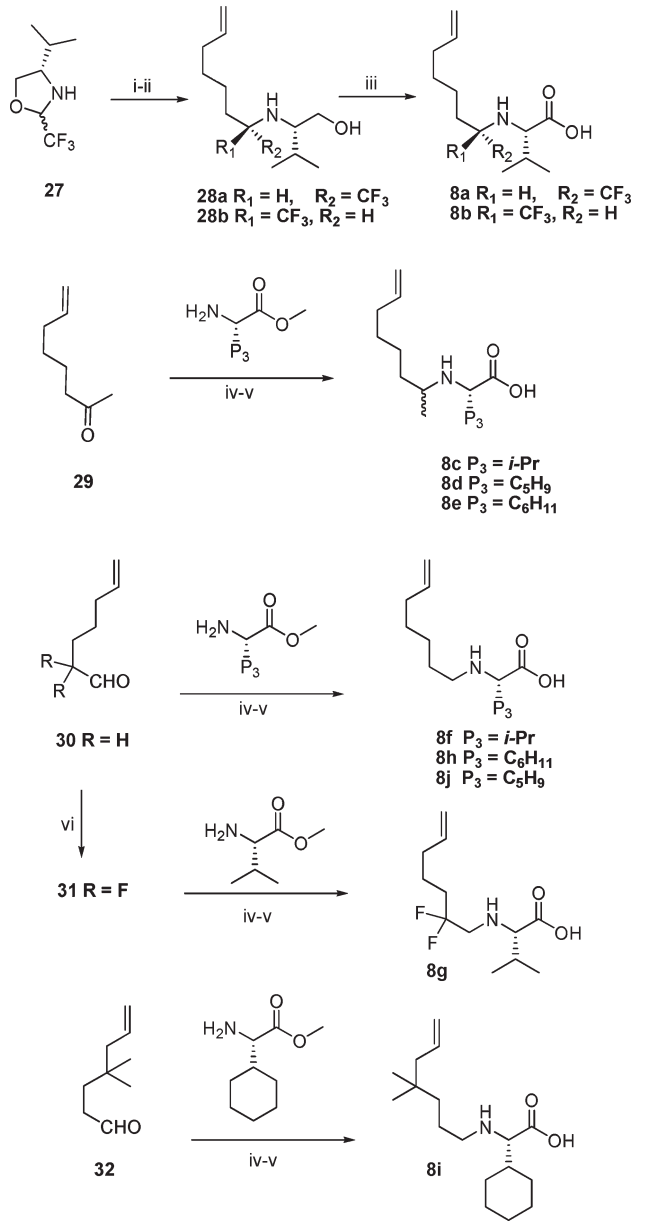
Figure 1. General retrosynthetic analysis of P2−P4 macrocyclic amine inhibitors **12**−**26**.

6.3, around 3 log units higher than that reported for trifluoroethylamine amide isosteres in the cathepsin K area. The strong enzyme inhibition shown by **12a** was notable given that a precipitous drop in activity (of around 3 orders of magnitude) was observed in the cathepsin area when an amine with pK_a above 6 was employed as the amide isostere.[30b] Although **12a** would be expected to be around 90% unprotonated at the physiological pH of the biological assays, a significant amount of the protonated form would still be present in the assay media. To assess the contribution to enzyme inhibition from this protonated species and to further probe the role of amine pK_a, the α-methyl branched compounds **13a**,**b** (which aside from amine pK_a have strong structural similarity to **12a**,**b**) were evaluated. Remarkably, the more active of these α-methyl diastereoisomers, **13a**,[21] had enzyme inhibition that was *enhanced* over the CF_3 compound **12a**. While it cannot be excluded that modification of the CF_3 group to the sterically less demanding CH_3 may be favorable, the presence of a basic amine likely improves potency through electrostatic interaction with the acidic residue D168 which lies close to the P3 amino acid (Figure 2C). A further attraction of amine **13a** was that it elicited a much stronger cell based response (EC_{50} = 1 nM) than either **12a** or the original urethane **3**. The cell based potency of **13a** was especially attractive when the replicon assay was conducted in the presence of human plasma. Under these conditions, which may be considered to be more physiologically relevant,[34] **12a** and **13a** had EC_{50} values of 320 and 3 nM, respectively. The improved cell-based potency of **13a** is in line with reduced affinity for serum albumin when a basic amine (which renders the inhibitor formally zwitterionic) is present.[35] In vitro binding experiments with **12a** and **13a** showed that their free fractions in human plasma are 1.9% and 10.6%, respectively.

On the basis of these initial findings, a research campaign to optimize P2−P4 linked macrocyclic amine inhibitors was initiated. The first target was evaluation of structurally simplified inhibitors devoid of the chiral center resulting from the branching functionality α to the P3-amine group. Compound **14** fulfills this goal and demonstrates that removal of the branch can be achieved without significant loss of biological activity; **14** showed similar intrinsic potency and cell based efficacy to the α-branched analogue **13a**. Two simplified analogues of **12a**, both of which retained a P3 N-terminal nonbasic amine, were also evaluated. The P3-trifluoromethylglycine compound **16** (Scheme 6) retained enzyme inhibition comparable to **12a** (K_i values for **16** and **12a** are 1.8 and 1.1 nM, respectively) but showed much reduced efficacy in the cell based assay (EC_{50} of 160 nM vs 18 nM). In contrast, the *gem*-difluoro compound **15** had somewhat improved biochemical potency over the prototype trifluoroethylamine inhibitor **12a** and demonstrated comparable cell based efficacy as either **12a** or **14**. However, the absence of a basic amine group in **15** again resulted in a less favorable serum shift than for **13a** or **14**; when assayed in the presence of human serum, **15** was around a 100 nM inhibitor of HCV replication.

Rat pharmacokinetic studies were performed on all compounds (**12a**−**15**) in Table 1. After oral administration consistently low exposure of these compounds in rat plasma was measured. AUC values ranged from zero (compound **14** was undetectable in rat plasma following administration at 4 mg/kg) to 0.05 μM·h (compound **12a**). Low compound levels were also detected in rat liver tissue 4 h post-administration,[36] the highest concentration being measured for compound **13b** (0.22 μM). The combination of low plasma and liver levels was viewed as a limitation for these macrocyclic amines given that their main target is the suppression of HCV replication in hepatocytes. The low observed plasma exposure and liver tissue concentration likely reflects modest absorption and uptake of the inhibitors from the gastrointestinal tract. It has been reported[9] that in the carbamate series a change of side chain in the P3 α-amino acid residue had a profound effect on uptake, suggesting that an approach to improving pharmacokinetics of the current inhibitors might be to evaluate changes at P3. The results in Table 3 reflect our efforts in this direction and further emphasize that for P2−P4 macrocycles very subtle changes in the structure of the P3-amino acid side chain can strongly influence the pharmacokinetic profile of the inhibitor. Thus, little benefit came from the presence of a cyclopentylglycine residue in P3, with compound **17c** showing a profile similar to that of its direct P3-valine analogue **13a**. Somewhat improved liver tissue levels were recorded when a benzylic double bond was present in the

Scheme 1[a]

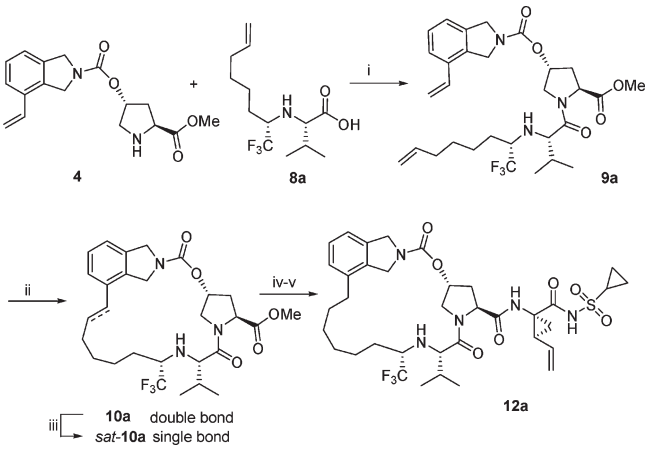


[a] Reagents: (i) 6-bromohex-1-ene, Mg, Et_2O; (ii) RP-HPLC; (iii) Jones reagent, Me_2CO; (iv) $NaBH_3CN$, $ZnCl_2$, MeOH; (v) $LiOH \cdot H_2O$, THF/H_2O; (vi) $(PhSO_2)_2NF$, D,L-proline, THF.

P2−P4 linker group (compound **17a**), but a stronger improvement was noted for ring expanded cyclohexyl analogue **18a**. The liver levels of this compound were at least 100 times higher than for initial macrocyclic amine inhibitors such as **13a**. The N-methylated compound **19**, which interestingly demonstrated that tertiary amine functionality at the P3 N-terminus of these inhibitors is tolerated, showed a similarly improved concentration in rat liver. Ultimately, however, although liver concentrations were improved for the cyclohexyl analogues **18a** and **19**, the lack of plasma exposure remained an issue that could not be addressed via changes to the P3 residue alone.

As a result of the unpredictable effect of minor changes in chemical structure on pharmacokinetic parameters, the early optimization of all our P2−P4-macrocyclic compound series relied heavily on in vivo studies run on closely related analogues. Analysis of the data generated from these studies (which

Scheme 2[a]

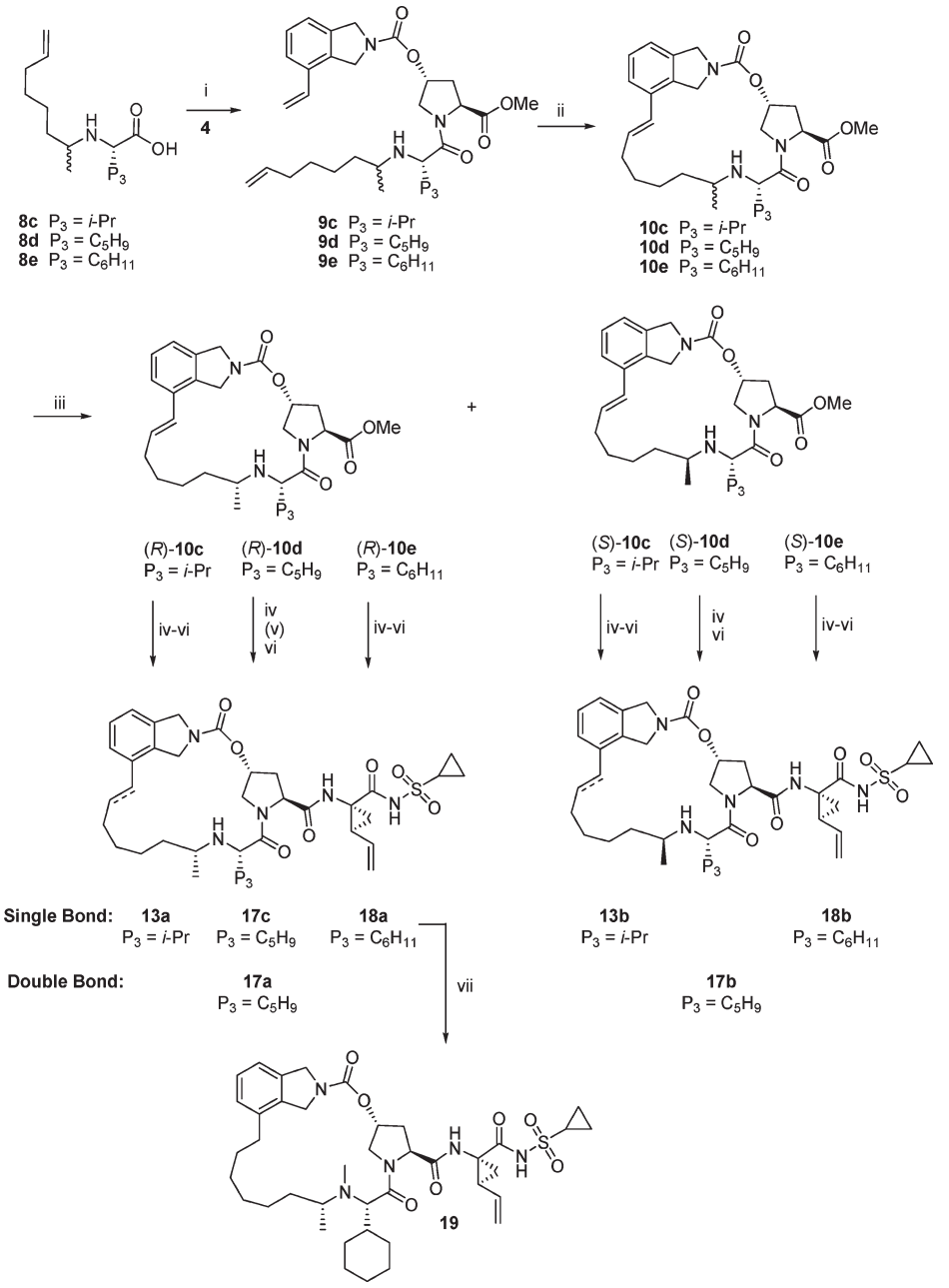


[a] Reagents: (i) TBTU, CH_2Cl_2, DIEA; (ii) Zhan I, DCE, TFA then RP-HPLC; (iii) H_2(g), Pd/C; (iv) $LiOH.H_2O$, THF/H_2O; (v) TBTU, **11**, DIEA, DMAP, then RP-HPLC.

were conducted on compounds from across a range of subseries differing in structure at the P3 amino acid residue, the P2−P3 linker group, and the P2-heterocycle) was subsequently performed with a view to establishing trends. Figure 3 illustrates an interrogation of this early rat pharmacokinetic data set aimed at establishing relationships between oral plasma exposure and liver levels as a function of structural changes to the P2 heterocyclic group. This plot of dose normalized plasma vs dose normalized liver levels makes a clear illustration that the choice of P2-heterocycle alone is insufficient to ensure either strong plasma exposure or liver levels in the micromolar range. However, a trend suggesting that ether linked 2-isoquinoline P2-heterocyclic compounds (indicated in yellow) tend to be more profitable emerges, and the presence of additional functionality on the isoquinoline ring, such as a methoxy group at the 6-position (blue), can apparently bring a further improvement in plasma exposure. In contrast, while compounds based on the isoindoline carbamate P2-heterocycle (which was a feature of our early macrocyclic amine compounds and which are indicated in grey) do on occasion achieve high liver concentrations ($>10\,\mu$M), they consistently fail to provide plasma exposure above $0.5\,\mu\text{M}\cdot\text{h}$.

Changes to the P2-heterocyclic group were consequently explored as an approach toward remedying the poor rat pharmacokinetics of early P2−P4 macrocyclic amine inhibitors. Disappointingly, and for reasons not clearly understood, inhibitors based on an unsubstituted 1,7-linked 2-isoquinoline P2-heterocycle (Table 4, **20**, **21**) tended to lack potency against the NS3 enzyme. Macrocyclic amine inhibitors based on this P2-heterocycle are also modest inhibitors in the cell based assay (e.g., **21** $EC_{50} = 410$ nM), and compounds of this type were not further pursued. In contrast 1,7-linked 3-phenyl-4-quinoline based inhibitors showed excellent potency. Compounds **22** and **23** highlight this, both showing subnanomolar enzyme blockade and strong efficacy in the replicon assay when it was conducted under high serum conditions (EC_{50} values were in the 20 nM range). The presence of a 6-OMe substituent on the P2 heterocycle of **22** and **23** (a structural feature that had appeared to favor plasma exposure in a related series; see Figure 3) did not strongly benefit PK properties; no improvement in plasma exposure or rat liver levels was achieved with respect to previous

Scheme 3[a]



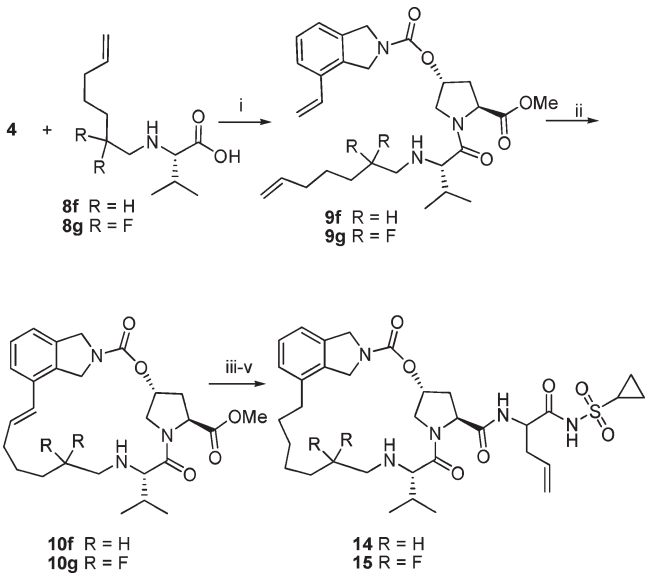
[a] Reagents: (i) TBTU, CH_2Cl_2, DIEA; (ii) Zhan I, DCE, TFA, then RP-HPLC; (iii) RP-HPLC; (iv) $LiOH \cdot H_2O$, THF/H_2O; (v) H_2(g), Pd/C; (vi) TBTU, **11**, DIEA, DMAP, then RP-HPLC; (vii) HCHO, $NaBH_3CN$, $ZnCl_2$, MeOH, then RP-HPLC.

P3-cyclohexylglycine compounds such as **18a**. However, a strong improvement was achieved by installation of *gem*-dimethyl functionality into the linker group that joins the P2 and P3 amino acids. This change again highlights how subtle structural features can dramatically impact pharmacokinetics; compounds **24** and **25** were among our first macrocyclic amine inhibitors that following oral administration to rat achieved plasma exposure significantly higher ($\sim$0.5 μM·h) than detection limits together with high compound concentrations in liver (5.6 and 13.7 μM for **24** and **25**, respectively). As projected on the basis of the analysis in Figure 3, further augmented plasma levels came from inhibitors based on a 6-methoxy-2-isoquinoline heterocycle at P2. Gratifyingly this change also restored biochemical and cell based potency to a more attractive, low nanomolar level (compare **21** and **26**). Compound **26** had excellent plasma exposure in rat (7 μM·h following a 5 mg/kg dose), and although the liver concentration of this inhibitor was not as high as had been achieved with compounds **24** and **25**, it was nonetheless detected at a level more than 2 orders of magnitude above its replicon EC_{50}.

Although there have been occasional reports in the patent literature relating to tripeptide based inhibitors of NS3 that feature an amine group at their N-terminus,[28] the inhibitors described here are to the best of our knowledge the first potent compound series to be based on this feature. The rareness of protease inhibitors featuring an amine at P3 is probably a reflection of early work in the NS3 area, where enzyme bound crystal structures were used toward the design of smaller and more druglike inhibitors.

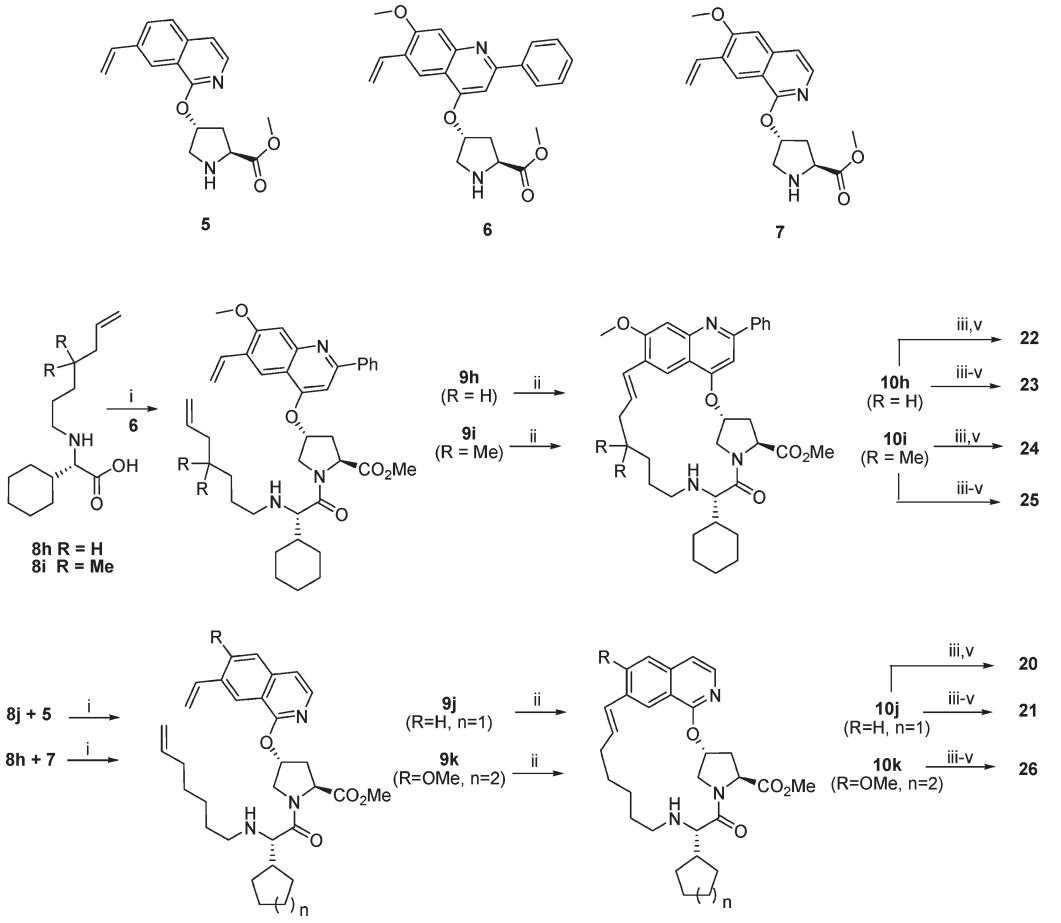
The presence of a hydrogen bonding interaction between Ala157 and the (neutral) P3 NH group of the ligand is a feature in these X-ray structures, and retention of this structural element during subsequent phases of optimization is understandable.[37] However, the compounds described show that neutral functionality is not a strong requirement at the N-terminus, and in fact, benefits in terms of cell based potency or pharmacokinetic properties can come from its replacement with basic amine functionality. Thus, compound **13a** showed around a 100-fold stronger cell based response (when assayed under high serum conditions) than the closely related P3-urethane compound **3**. During the optimization of **13a** a degree of cell based potency could be sacrificed to favor inhibitors with pharmacokinetic properties that are attractive in an antiviral targeting HCV. Compound **25** (Table 4) highlights this approach. Although the cell-based potency of this inhibitor is not superior to the carbamate analogue **40** (Chart 1; the compounds are equipotent in our cell based assays), it does reach somewhat higher levels in rat liver (13.7 μM vs 9.9 μM) following oral administration of a 5 mg/kg dose and also has improved plasma exposure (0.45 μM·h vs 0.15 μM·h). Compound **25** was 44% bioavailable in rat, with moderate plasma clearance (27 mL/min/kg); the half-life was 3.3 h. Plasma levels were not quantifiable 24 h after oral administration, but the compound was still detected in liver at a concentration (0.3 μM) well above its replicon EC_{50}, suggesting at least the potential for prolonged suppression of HCV replication in the target organ. Significantly higher exposure was also measured in dog for the amine based inhibitor **25** vs **40** (68.8 μM·h vs 5.7 μM·h). The overall

Scheme 4[a]



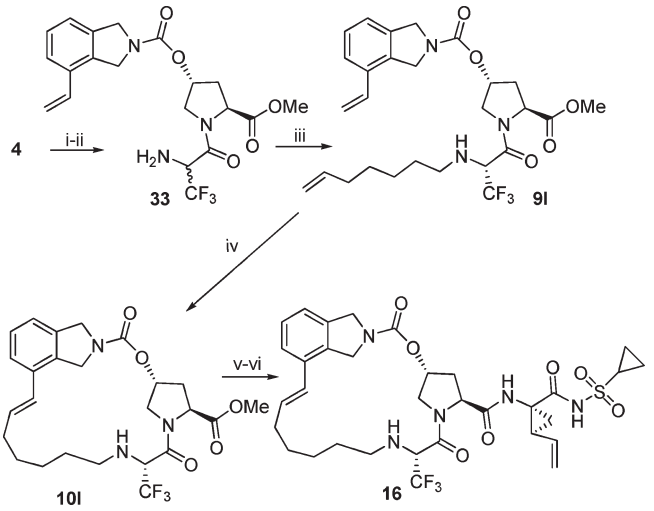
[a] Reagents: (i) TBTU, CH_2Cl_2, DIEA; (ii) Zhan I, DCE, TFA, then RP-HPLC; (iii) H_2(g), Pd/C; (iv) LiOH·H_2O, THF/H_2O; (v) TBTU, **11**, DIEA, DMAP, then RP-HPLC.

Scheme 5[a]



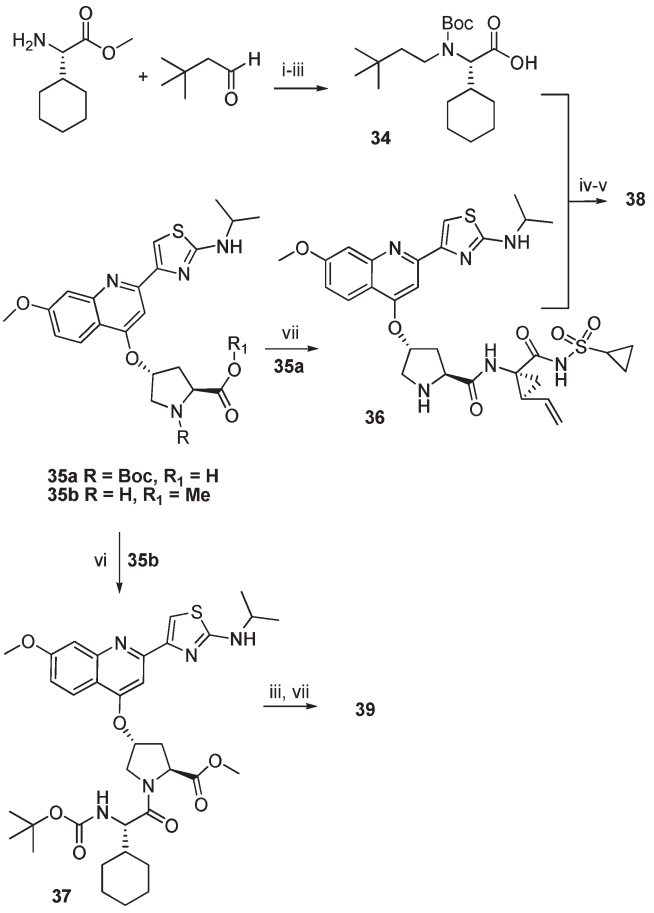
[a] Reagents: (i) HATU, CH_2Cl_2, DIEA; (ii) Zhan I, DCE, TFA; (iii) LiOH·H_2O, THF/H_2O; (iv) H_2(g), Pd/C; (v) TBTU, **11**, DIEA, DMAP, then RP-HPLC.

Scheme 6[a]



[a] Reagents: (i) (D,L)-*N*-Boc-trifluoromethylglycine, HATU, NMM; (ii) 4 N HCl in 1,4-dioxane; (iii) **30**, $NaBH_3CN$, then RP-HPLC; (iv) Zhan I, DCE, TFA; (v) $LiOH \cdot H_2O$, THF/H_2O; (vi) TBTU, **11**, DIEA, DMAP, then RP-HPLC.

Scheme 7[a]



[a] Reagents: (i) $NaBH_3CN$, $ZnCl_2$, MeOH; (ii) Boc_2O, Et_3N, MeOH; (iii) $LiOH \cdot H_2O$, $THF/dioxane/H_2O$; (iv) TBTU, DIEA, DMAP; (v) TFA/CH_2Cl_2, then RP-HPLC; (vi) Boc-Chg-OH, HATU, Et_3N; (vii) **11**, TBTU, DIEA, DMAP.

pharmacokinetic profile of **25** in dog was attractive, with the compound showing low clearance (1 mL/min/kg), a reasonable plasma half-life (3 h), and 92% bioavailability.

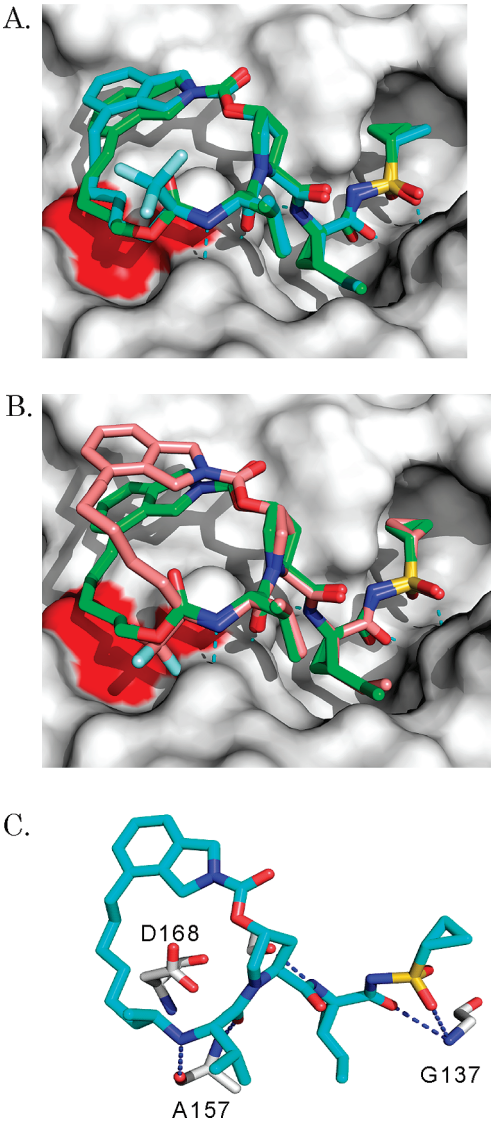


Figure 2. Superposition of urethane inhibitor **3** (green) with trifluoroethylamine analogues **12a** (cyan, A) and **12b** (pink, B) and the proposed binding orientation for compound **12a** (cyan, C). Inhibitors were minimized and docked into the active site of the genotype 1b NS3/peptide4A crystal structure. The enzyme surface and individual amino acid residues are shown in white (with the exception of the side chain of residue D168 which is shown in red in parts A and B). Potential hydrogen bonding interactions between the ligand and enzyme are shown as broken lines.

To demonstrate that the tolerance for amine functionality at the P3 N-terminus is not limited to P2−P4 macrocyclic protease inhibitors (where constraint of the P3-capping group into a ring may render it more permissive toward modification), compounds **38** and **39** that are based on the P2 heterocycle found in BILN-2061 were prepared. Amine analogue **38** showed marginally weaker inhibition (4-fold) than the corresponding P3-urethane analogue **39** but nonetheless retained subnanomolar inhibition of the NS3 enzyme. In line with the results described for the P2−P4 macrocyclic compounds, amine **38** showed a similar response as urethane **39** in the cell-based assay. These results confirm that the tolerance for simple amine functionality at the P3-amino acid N-terminus is not confined to P2−P4 macrocyclic inhibitors of the NS3 protease.

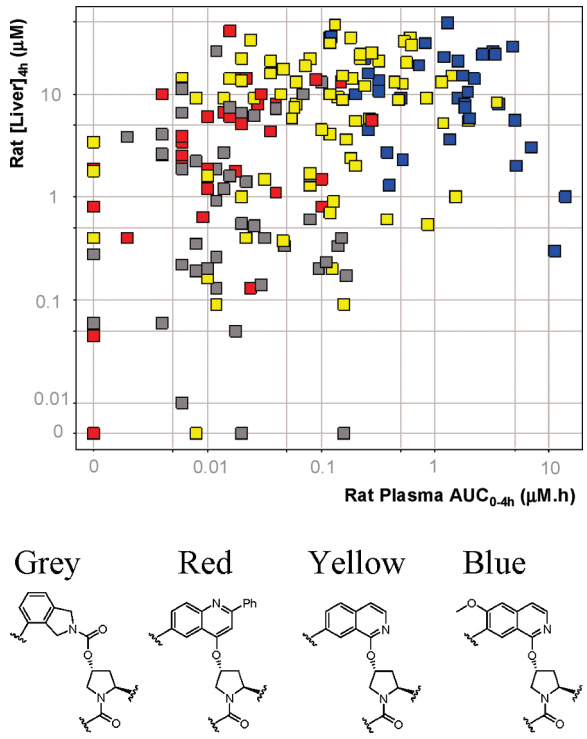


Figure 3. Pharmacokinetic data for P2−P4 macrocyclic compounds measured following oral administration to Sprague−Dawley rats at doses between 3 and 5 mg/kg. Plasma exposure (x-axis, μM·h) was measured as the area under the plasma concentration/time curve between 0 and 4 h; liver concentrations (y-axis, μM) were measured at 4 h. Data are colored according to the heterocycle attached to the P2-hydroxyproline residue, as indicated below the graph. Exposure values and liver concentrations for compounds that were administered at doses below 5 mg/kg are normalized by linear adjustment to 5 mg/kg.

Conclusion

In summary we have described the discovery and optimization of a series of P2−P4 macrocyclic amine inhibitors of the HCV NS3 protease. The starting point for this work was **12a/13a**, which showed promising cell based potency but had poor pharmacokinetic properties in rat. Through optimization of the P2 heterocycle, the P3 amino acid, and the P2−P3 linking group, these issues were addressed to generate inhibitors with attractive properties for anti-HCV agents. Basic amine functionality at the P3 N-terminus of HCV NS3 inhibitors has been shown to be generally tolerated, and optimized inhibitors that incorporate this structural feature have cell-based potency and pharmacokinetic profiles that compare favorably with their related P3-N-capped (urethane) analogues.

Experimental Section

Solvents were obtained from commercial suppliers (Fluka, puriss. grade) and were used without further purification. Organic extracts were dried over anhydrous Na_2SO_4 (Merck). Flash chromatography purifications were performed on Biotage prepacked silica gel columns using Biotage Horizon and SP2 instruments. NMR spectra were recorded on Bruker Avance spectrometers and, unless otherwise stated, were acquired at 300 K. Proton chemical shifts are reported in parts per million (δ) and are referenced to the residual proton signal of the deuterated solvent (DMSO-d_6 at 2.50 ppm, $CDCl_3$ at 7.26 ppm, CD_3OD at 3.31 ppm). Carbon chemical shifts are referenced to the solvent signal of DMSO-d_6 at 39.5 ppm. Compounds were assessed for purity under two independent RP-HPLC conditions and were judged >95% pure by peak area (UV detection at 254 nm); for the purity of specific compounds, see below. RP-HPLC was performed on Waters Alliance instruments (analytical scale) or Waters preparative-scale instrument equipped for automated mass or UV-triggered collection. In all cases mixtures of MeCN and H_2O (each buffered with 0.1% TFA) were used as the mobile phase. The following conditions were employed. Conditions A: stationary phase, Waters Symmetry C_{18}, 7 μm, 19 mm × 300 mm; flow rate of 20 mL/min; gradient of 40% MeCN for 4 min and then from 40% to 90% MeCN over 16 min. Conditions B: same as for conditions A but using the following gradient, 20% MeCN for 4 min and then from 20% to 70% MeCN over 16 min. Conditions C: stationary phase, Atlantis C_{18}, 5 μm, 3 mm × 150 mm; flow rate of 1 mL/min; gradient of 20% MeCN for 1 min and then from 20% to 80% MeCN over 7 min. Conditions D: stationary phase, X-Bridge 5 μm, 4.6 mm × 50 mm; flow rate of 1 mL/min; gradient of 20% MeCN for 1 min and then from 20% to 100% over 4 min. Conditions E: stationary phase, X-Bridge C_{18}, 5 μm, 19 mm × 100 mm; flow rate of 20 mL/min; gradient of 20% MeCN for 1 min and then from 20% to 80% MeCN over 16 min. Low resolution mass spectrometry data were generated on Waters ZQ micromass systems. High resolution data were collected on Waters Synapt Q-tof instruments.

Methyl *N*-[(1*S*)-1-(Trifluoromethyl)hept-6-en-1-yl]-L-valyl-(4*R*)-4-{[(4-vinyl-1,3-dihydro-2*H*-isoindol-2-yl)carbonyl]oxy}-L-prolinate (9a). A solution of **8a** (82 mg, 0.29 mmol) in CH_2Cl_2 (7 mL) was treated with DIEA (112 μL, 0.64 mmol) and TBTU (112 mg, 0.35 mmol). The mixture was stirred for 5 min and then treated with **4** (113 mg, 0.32 mmol). The mixture was stirred for 14 h and then diluted with aqueous HCl (1 N). The organic layer was separated and washed with saturated aqueous $NaHCO_3$ and brine and then dried. Removal of the volatiles gave a residue that was purified by flash chromatography on silica gel (EtOAc/petroleum ether gradient from 5:95 to 15:85) to furnish the title compound (50 mg, 30%) as a white foam. ^{1}H NMR (DMSO-d_6, 600 MHz; 9:1* mixture of rotamers about the proline amide bond and a 1$^{\#}$:1$^{\#\#}$ mixture of rotamers about the isoindoline urethane bond) δ 7.48 (dd, J = 7.8, 2.4 Hz, 1H), 7.30 (t, J = 7.8 Hz, 1H), 7.26$^{\#}$ and 7.14$^{\#\#}$ (d, J = 7.8 Hz, 1H), 6.73$^{\#\#}$ and 6.62$^{\#}$ (dd, J = 17.5, 11.1 Hz, 1H), 5.82−5.68 (m, 1H), 5.76 (d, J = 17.5 Hz, 1H), 5.38$^{\#}$ and 5.33$^{\#\#}$ (d, J = 11.1 Hz, 1H), 5.30−5.26 and 5.22−5.17* (m, 1H), 5.04−4.86 (m, 2H), 4.83−4.77* and 4.55−4.48 (m, 1H), 4.74−4.56 (m, 4H), 3.91 and 3.86* (d, J = 11.9 Hz, J* = 12.1 Hz, 1H), 3.79−3.75 and 3.58−3.50* (m, 1H), 3.69* and 3.64 (s, 3H), 3.26 and 2.97* (d, J = 7.8 Hz, 1H), 2.90−2.82* and 2.81−2.74 (m, 1H), 2.61−2.54* and 2.49−2.42 (m, 1H), 2.41−2.36* and 2.19−2.12 (m, 1H), 2.07−1.90 (m, 3H), 1.73−1.67 (m, 1H), 1.44−1.35 (m, 3H), 1.29−1.19 (m, 3H), 0.91 (d, J = 6.8 Hz, 3H), 0.90 (d, J = 6.8 Hz, 3H); ^{19}F NMR (376 MHz, DMSO-d_6, two rotamers with relative intensity 9:1* are observed) δ −77.0, −77.5*; MS (ES$^+$) m/z 580 (M + H)$^+$.

Methyl *N*-[(1*R*)-1-(Trifluoromethyl)hept-6-en-1-yl]-L-valyl-(4*R*)-4-{[(4-vinyl-1,3-dihydro-2*H*-isoindol-2-yl)carbonyl]oxy}-L-prolinate (9b). Following the procedure described for **9a**, treatment of **8b** (250 mg, 0.89 mmol) with **4** furnished the title compound (89 mg, 17%) as a solid. ^{1}H NMR (600 MHz, DMSO-d_6; a 1:1* mixture of rotamers about the isoindoline urethane bond and a >95:5 mixture of rotamers about the proline amide bond were observed; only the major isomer is assigned) δ 7.47 (d, J = 7.8 Hz, 1H), 7.30 (t, J = 7.8 Hz, 1H), 7.25 and 7.17* (d, J = 7.8 Hz, 1H), 6.73 and 6.63* (dd, J = 17.5, 11.1 Hz, 1H), 5.80−5.71 (m, 2H), 5.37 and 5.35−5.25* (d and m*, J = 11.1 Hz, 1H), 5.32−5.25 (m, 1H), 4.97 (d, J = 17.2 Hz, 1H), 4.92 (d, J = 10.1 Hz, 1H), 4.74−4.41 (m, 5H), 3.92 and 3.90* (d, J = 12.0 Hz, 1H), 3.75−3.68 (m, 1H), 3.63 and 3.62* (s, 3H), 3.51 and 3.46* (dd, J=8.0, 5.3 Hz, 1H), 3.19−3.09 (m, 1H), 2.47−2.38 (m, 1H), 2.14−2.05 (m, 2H), 2.04−1.91 (m, 2H), 1.78−1.68 (m, 1H), 1.51−1.28 (m, 6H), 0.95 and 0.94* (d, J = 6.8 Hz, 3H), 0.80 and 0.78* (d, J = 6.8 Hz, 3H); ^{19}F NMR (376 MHz, DMSO-d_6; two rotamers with relative ratio 1:1 are observed) δ −74.3, −74.4; MS (ES$^+$) m/z 580 (M + H)$^+$.

Methyl *N*-[(2*R* and 2*S*)-Oct-7-en-2-yl]-L-valyl-(4*R*)-4-{[(4-ethenyl-1,3-dihydro-2*H*-isoindol-2-yl)carbonyl]oxy}-L-prolinate Trifluoroacetate (9c). Following the general procedure described for **9a**, treatment of **8c** (467 mg, 2.06 mmol) with **4** furnished a solid that was further purified by RP-HPLC (conditions B) to give a 3:2 mixture of diastereoisomers of the title compounds (999 mg, 76%) as a white powder. MS (ES^+) m/z 526 $(M+H)^+$.

(3*R*,5*S*)-1-{(2*S* and 2*R*)-2-Cyclopentyl-2-[oct-7-en-2-ylamino]-acetyl}-5-(methoxycarbonyl)pyrrolidin-3-yl 4-ethenyl-1,3-dihydro-2*H*-isoindole-2-carboxylate (9d). Following the general procedure described for **9a**, treatment of **8d** (324 mg, 1.28 mmol) with **4** furnished the title compounds (301 mg, 43%) as an oil. ^1H NMR (400 MHz, DMSO-d_6, 1:1* mixture of diastereoisomers) δ 7.54−7.43 (m, 1H), 7.36−7.24 (m, 2H), 7.36−7.24 and 7.20* (m and t*, J^* = 9.7 Hz, 1H), 6.80−6.61 (m, 1H), 5.84−5.63 (m, 1H), 5.42−5.19 (m, 2H), 5.02−4.82 (m, 2H), 4.77−4.36 (m, 6H), 4.01−3.87 (m, 1H), 3.83−3.67 (m, 1H), 3.65 (s, 3H), 3.31−3.17 (m, 1H), 2.33−0.96 (m, 19H), 0.79 (br d, J = 6.9 Hz, 3H); MS (ES^+) m/z 552 $(M+H)^+$.

(3*R*,5*S*)-1-[(2*S* and 2*R*)-2-Cyclohexyl-2-(oct-7-en-2-ylamino)-acetyl]-5-(methoxycarbonyl)pyrrolidin-3-yl 4-ethenyl-1,3-dihydro-2*H*-isoindole-2-carboxylate (9e). Following the general procedure described for **9a**, treatment of **8e** (1.51 g, 5.0 mmol) with **4** furnished the title compound (1.28 g, 45%) as an oil. MS (ES^+) m/z 566 $(M+H)^+$.

Methyl *N*-(Hept-6-en-1-yl)-L-valyl-(4*R*)-4-{[(4-ethenyl-1,3-dihydro-2*H*-isoindol-2-yl)carbonyl]oxy}-L-prolinate (9f). Following the general procedure described for **9a**, treatment of **8f** (990 mg, 3.96 mmol) with **4** furnished the title compound (1.82 g, 90%) as a white solid. MS (ES^+) m/z 512 $(M+H)^+$.

Methyl *N*-(2,2-Difluorohept-6-en-1-yl)-L-valyl-(4*R*)-4-{[(4-ethenyl-1,3-dihydro-2*H*-isoindol-2-yl)carbonyl]oxy}-L-prolinate (9g). Following the general procedure described for **9a** (HATU used in place of TBTU), treatment of **8g** (286 mg, 1.15 mmol) with **4** furnished the title compound (247 mg, 39%) as a foam. MS (ES^+) m/z 548 $(M+H)^+$.

Methyl (4*R*)-1-[(2*S*)-2-Cyclohexyl-2-(hept-6-en-1-ylamino)-acetyl]-4-[(7-methoxy-2-phenyl-6-vinylquinolin-4-yl)oxy]-L-prolinate (9h). Following the general procedure described for **9a** (HATU used in place of TBTU), treatment of **8h** (269 mg, 1.06 mmol) with **6** (375 mg, 0.925 mmol) furnished the title compound (512 mg, 35%) as an oil. MS (ES^+) m/z 640 $(M+H)^+$.

Methyl (4*R*)-1-{(2*S*)-2-Cyclohexyl-2-[(4,4-dimethylhept-6-en-1-yl)amino]acetyl}-4-[(6-ethenyl-7-methoxy-2-phenylquinolin-4-yl)oxy]-L-prolinate (9i). Following the general procedure described for **9a** (HATU used in place of TBTU), treatment of **8i** (293 mg, 1.04 mmol) with **6** (402 mg, 0.99 mmol) furnished the title compound (308 mg, 47%) as an oil. MS (ES^+) m/z 668 $(M+H)^+$.

Methyl (4*R*)-1-[(2*S*)-2-Cyclopentyl-2-(hept-6-en-1-ylamino)-acetyl]-4-[(7-ethenylisoquinolin-1-yl)oxy]-L-prolinate (9j). Following the general procedure described for **9a** (HATU used in place of TBTU), treatment of **8j** (263 mg, 1.10 mmol) with **5** (345 mg, 1.15 mmol) furnished the title compound (466 mg, 82%) as an oil. ^1H NMR (400 MHz, $CDCl_3$) δ 7.97 (br s, 1H), 7.94 (d, J = 5.9 Hz, 1H), 7.81 (br d, J = 8.5 Hz, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.22 (d, J = 5.9 Hz, 1H), 6.83 (dd, J = 17.4, 10.9 Hz, 1H), 5.89−5.85 (m, 1H), 5.89 (d, J = 17.4 Hz, 1H), 5.74−5.63 (m, 1H), 5.38 (dd, J = 10.9, 1.2 Hz, 1H), 4.94−4.85 (m, 3H), 4.17 (d, J = 11.8 Hz, 1H), 4.00 (br d, J = 11.8 Hz, 1H), 3.79 (s, 3H), 3.12 (d, J = 8.5 Hz, 1H), 2.79−2.71 (m, 1H), 2.39−2.30 (m, 2H), 2.09−1.98 (m, 3H), 1.88−1.71 (m, 4H), 1.59−1.24 (m, 5H), 1.19−1.07 (m, 4H), 1.01−0.81 (m, 2H); MS (ES^+) m/z 520 $(M+H)^+$.

Methyl (4*R*)-1-[(2*S*)-2-Cyclohexyl-2-(hept-6-en-1-ylamino)-acetyl]-4-[(6-methoxy-7-vinylisoquinolin-1-yl)oxy]-L-prolinate (9k). Following the general procedure described for **9a** (HATU used in place of TBTU), treatment of **8h** (222 mg, 0.88 mmol) with **7** (320 mg, 0.88 mmol) furnished the title compound (280 mg, 57%) as an oil. MS (ES^+) m/z 564 $(M+H)^+$.

Methyl 3,3,3-Trifluoro-*N*-(hept-6-en-1-yl)-L-alanyl-(4*R*)-4-{[(4-ethenyl-1,3-dihydro-2*H*-isoindol-2-yl)carbonyl]oxy}-L-prolinate (9l). A solution of **34** (730 mg, 1.65 mmol) in THF (3 mL) was treated with **30** (186 mg, 1.65 mmol) and $NaCNBH_3$ (312 mg, 4.96 mmol). The mixture was stirred for 1 h, and then the volatiles were evaporated in vacuo. The resulting residue was purified by RP-HPLC (conditions B) to furnish the title compound (155 mg, 17%) as a white solid. MS (ES^+) m/z 538 $(M+H)^+$.

Methyl (5*R*,7*S*,10*S*,12*S*,17*E*)-10-Isopropyl-3,9-dioxo-12-(trifluoromethyl)-6,7,9,10,11,12,13,14,15,16-decahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7-carboxylate (10a). General Procedure for Ring Closing Metathesis. A solution of **9a** (100 mg, 0.17 mmol) in DCE (10 mL) was treated with Zhan catalyst I (17 mg, 0.026 mmol), and the mixture was heated under reflux for 1 h. The solution was cooled and filtered, and then the volatiles were evaporated under reduced pressure. The residue obtained contained the title compound and was used without further purification in the following reaction step. ^1H NMR (600 MHz, DMSO-d_6) δ 7.27 (t, J = 7.5 Hz, 1H), 7.21 (d, J = 7.5 Hz, 1H), 7.14 (d, J = 7.5 Hz, 1H), 6.40 (d, J = 16.1 Hz, 1H), 5.88 (ddd, J = 16.1, 7.4, 6.7 Hz, 1H), 5.42 (t, J = 3.2 Hz, 1H), 4.77 (d, J = 15 Hz, 1H), 4.70−4.60 (m, 2H), 4.55 (d, J = 15 Hz, 1H), 4.49 (dd, J = 10.6, 7.3 Hz, 1H), 3.97 (dd, J = 11.7, 1.4 Hz, 1H), 3.73 (dd, J = 11.7, 3.1 Hz, 1H), 3.62 (s, 3H), 3.26 (t, J = 8 Hz, 1H), 2.91−2.82 (m, 1H), 2.41−2.35 (m, 1H), 2.28−2.16 (m, 2H), 2.11 (br s, 1H), 2.05 (ddd, J = 13.4, 10.8, 3.9 Hz, 1H), 1.73−1.66 (m, 1H), 1.64−1.59 (m, 2H), 1.57−1.50 (m, 1H), 1.47−1.39 (m, 1H), 1.38−1.30 (m, 1H), 1.26−1.18 (m, 1H), 0.91 (d, J = 6.6 Hz, 6H); ^{19}F NMR (376 MHz, DMSO-d_6) δ −78.2; MS (ES^+) m/z 552 $(M+H)^+$.

Methyl (5*R*,7*S*,10*S*,12*R* or *S*,17*E*)-10-Isopropyl-3,9-dioxo-12-(trifluoromethyl)-6,7,9,10,11,12,13,14,15,16-decahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7-carboxylate (10b). Treatment of **9b** (89 mg, 0.154 mmol) as described in the general procedure for **10a** furnished the title compound that was used directly in the subsequent step. MS (ES^+) m/z 552 $(M+H)^+$.

(5*R*,7*S*,10*S*,12*R*,17*E*)-10-Isopropyl-7-(methoxycarbonyl)-12-methyl-3,9-dioxo-6,7,9,10,11,12,13,14,15,16-decahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosin-11-ium Trifluoroacetate ((*R*)-10c). A sealed vial was charged with a solution of **9c** (448 mg, 0.70 mmol) and Zhan catalyst I (93 mg, 0.14 mmol) in CH_2Cl_2 (45 mL). The mixture was heated under microwave irradiation at 100 °C for 40 min and then cooled and filtered. The volatiles were removed under reduced pressure and the residue was purified by RP-HPLC (conditions B) to furnish in the first fractions (t_R = 14.58 min) the title compound[17] (160 mg, 37%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 7.30−7.23 (m, 1H), 7.16−7.09 (m, 2H), 6.36 (d, J = 16.2 Hz, 1H), 5.82 (dt, J = 16.2, 5.7 Hz, 1H), 5.32−5.27 (br s, 1H), 4.83−4.65 (m, 4H), 4.59 (d, J = 14.6 Hz, 1H), 4.11−4.06 (br s, 1H), 3.96−3.81 (m, 2H), 3.78 (s, 3H), 2.93 (dd, J = 15.1, 8.3 Hz, 1H), 2.87−2.78 (m, 1H), 2.58−2.09 (m, 4H), 1.86−0.97 (m, 12H), 1.47 (d, J = 6.3 Hz, 3H); MS (ES^+) m/z 498 $(M+H)^+$. The later fractions (t_R = 15.12 min) were freeze-dried to give (5*R*,7*S*,10*S*,12*S*,17*E*)-10-isopropyl-7-(methoxycarbonyl)-12-methyl-3,9-dioxo-6,7,9,10,11,12,13,14,15,16-decahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosin-11-ium trifluoroacetate ((*S*)-10c) (155 mg, 36%) as a white powder. ^1H NMR (400 MHz, $CDCl_3$) δ 7.30−7.22 (m, 2H), 7.15 (d, J = 7.1 Hz, 1H), 6.22 (d, J = 16.4 Hz, 1H), 5.95 (dt, J = 16.4, 6.3 Hz, 1H), 5.62−5.57 (br s, 1H), 4.82−4.70 (m, 3H), 4.64 (d, J = 14.7 Hz, 1H), 4.49 (d, J = 14.7 Hz, 1H), 4.11−4.07 (br m, 1H), 3.92−3.79 (m, 2H), 3.79 (s, 3H), 2.88−2.80 (m, 1H), 2.66 (dd, J = 14.2, 7.8 Hz, 1H), 2.60−2.51 (m, 1H), 2.38−2.13 (m, 3H), 1.88−1.73 (m, 2H), 160−0.97 (m, 4H), 1.40 (d, J = 6.6 Hz, 3H), 1.21 (d, J = 7.0 Hz, 3H), 1.10 (d, J = 6.6 Hz, 3H); MS (ES^+) m/z 498 $(M+H)^+$.

Methyl (5*R*,7*S*,10*S*,12*R*,17*E*)-10-Cyclopentyl-12-methyl-3,9-dioxo-6,7,9,10,11,12,13,14,15,16-decahydro-1*H*,5*H*-2,22:5,8-di-

methano-4,2,8,11-benzoxatriazacycloicosine-7(3*H*)-carboxylate trifluoroacetate ((*R*)-10d). Following the procedure described for **10c**, treatment of **9d** (300 mg, 0.54 mmol) and TFA (54 μL, 0.71 mmol) gave a residue that was purified by RP-HPLC (conditions B) to afford in the first fractions (t_R = 14.81 min) the title compound[17] (93.2 mg, 27%) as a white powder. MS (ES^+) *m*/*z* 524 $(M + H)^+$. The later fractions (t_R = 15.37 min) were freeze-dried to give methyl (5*R*,7*S*,10*S*,12*S*,17*E*)-10-cyclopentyl-12-methyl-3,9-dioxo-6,7,9,10,11,12,13,14,15,16-decahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7(3*H*)-carboxylate trifluoroacetate ((*S*)-**10d**) as a white powder (101.5 mg, 29%). MS (ES^+) *m*/*z* 524 $(M+H)^+$.

(5*R*,7*S*,10*S*,12*R*)-10-Cyclohexyl-7-(methoxycarbonyl)-12-methyl-3,9-dioxo-6,7,9,10,11,12,13,14,15,16-decahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosin-11-ium trifluoroacetate ((*R*)-10e). Following the procedure described for **10c**, treatment of **9e** (1.39 g, 2.05 mmol) gave a residue that was purified by RP- HPLC (conditions B) to afford in the first fractions the title compound[17] (410 mg, 31%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 8.60 (br s, 2H), 7.35−7.15 (m, 3H), 6.27 (d, *J* = 16.4 Hz, 1H), 6.07 (dt, *J* = 16.4, 5.7 Hz, 1H), 5.42 (br s, 1H), 4.89−4.54 (m, 4H), 4.46 (d, *J* = 14.8 Hz, 1H), 4.40−4.31 (m, 1H), 4.26 (d, *J* = 12.2 Hz, 1H), 3.76−3.60 (m, 4H), 2.95−2.77 (m, 1H), 2.47−2.40 (m, 1H, partially obscured by residual DMSO signal), 2.30−2.03 (m, 3H), 2.01−1.71 (m, 6H), 1.70−0.97 (m, 14H); MS (ES^+) *m*/*z* 538 $(M + H)^+$. The later fractions were freeze-dried to afford (5*R*,7*S*,10*S*,12*S*)-10-cyclohexyl-7-(methoxycarbonyl)-12-methyl-3,9-dioxo-6,7,9,10,11,12,13,14,15,16-decahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosin-11-ium trifluoroacetate (*S*)-**10e** (190 mg, 14%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 8.46 (br s, 2H), 7.33−7.15 (m, 3H), 6.35 (d, *J* = 16.2 Hz, 1H,), 6.04 (dt, *J* = 16.2, 5.8 Hz, 1H), 5.19 (br s, 1H), 4.80 (d, *J* = 15.5 Hz, 1H), 4.73−4.50 (m, 3H, partially obscured by residual water), 4.49−4.39 (m, 1H), 4.34 (d, *J* = 11.7 Hz, 1H), 3.74 (dd, *J* = 12.4, 4.0 Hz, 1H), 3.69−3.60 (m, 3H), 2.87 (bs, 1H), 2.74−2.58 (m, 1H), 2.42−2.02 (m, 3H), 2.01−1.46 (m, 7H), 1.45−0.94 (m, 14H). MS (ES^+) *m*/*z* 538 $(M + H)^+$.

Methyl (5*R*,7*S*,10*S*,17*E*)-3,9-Dioxo-10-(propan-2-yl)-6,7,9,10,11,12,13,14,15,16-decahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7(3*H*)-carboxylate trifluoroacetate (10f). Treatment of **9f** (2.24 g, 3.58 mmol) as described in the general procedure for **10a** furnished a residue that was crystallized (petroleum ether/EtOAc, 8:2) to furnish the title compound (1.3 g, 78%) as a pale solid. MS (ES^+) *m*/*z* 484 $(M + H)^+$.

Methyl (5*R*,7*S*,10*S*,17*Z*)-13,13-Difluoro-10-isopropyl-3,9-dioxo-6,7,9,10,11,12,13,14,15,16-decahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7-carboxylate (10g). Following the procedure described for **10c**, treatment of **9g** (247 mg, 0.45 mmol) gave a residue that was purified by flash chromatography on silica gel (EtOAc/petroleum ether, 2:8) to furnish the title compound (154 mg, 66%) as a foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (t, *J* = 7.5 Hz, 1H), 7.19 (d, *J* = 7.5 Hz, 1H), 7.13 (d, *J* = 7.5 Hz, 1H), 6.30 (d, *J* = 16.3 Hz, 1H), 5.95 (dt, *J* = 16.3, 6.1 Hz, 1H), 5.39 (t, *J* = 3.3 Hz, 1H), 4.83−4.55 (m, 5H), 3.94 (d, *J* = 11.1 Hz, 1H), 3.78−3.71 (m, 1H), 3.76 (s, 3H), 3.34 (d, *J* = 6.6 Hz, 1H), 3.03 (ddd, *J* = 26.2, 13.9, 5.5 Hz, 1H), 2.87−2.66 (m, 2H), 2.51−1.72 (m, 8 H), 1.05 (d, *J* = 6.4 Hz, 3H), 1.03 (d, *J* = 6.4 Hz, 3H); MS (ES^+) *m*/*z* 520 $(M + H)^+$.

(2*R*,4*S*,7*S*,14*E*)-7-Cyclohexyl-23-methoxy-4-(methoxycarbonyl)-6-oxo-20-phenyl-3,4,6,7,8,9,10,11,12,13-decahydro-2H-16,18-etheno-2,5-methanopyrido[3,4-*r*][1,5,8]oxadiazacyclononadecin-8-ium Trifluoroacetate (10h). Treatment of **9h** (350 mg, 0.55 mmol) and TFA (57 μL, 0.74 mmol) as described in the general procedure for **10c** afforded a residue containing the title compound. MS (ES^+) *m*/*z* 612 $(M + H)^+$. Treatment of this material according to step 2 of the general procedure described for **12a** (vide infra) followed by RP-HPLC purification (conditions B) gave the corresponding carboxylic acid (2*R*,4*S*,7*S*,14*E*)-4-carboxy-7-cyclohexyl-23-methoxy-6-oxo-20-phenyl-3,4,6,7,8,9,10,11,12,13-decahydro-2*H*-16,18-etheno-2,5-methanopyrido[3,4-*r*][1,5,8]oxadiazacyclononadecin-8-ium trifluoroacetate (251 mg, 65% from **9h**) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.30−12.60 (br s, 1H), 9.00−8.73 (br m, 2H), 8.28−8.18 (m, 2H), 7.99 (s, 1H), 7.70−7.48 (m, 5H), 6.72 (d, *J* = 15.7 Hz, 1H), 6.14−6.03 (m, 1H), 5.75 (br s, 1H), 4.74−4.64 (m, 1H), 4.60−4.50 (m, 2H), 3.99 (s, 3H), 3.95−3.87 (m, 1H), 3.29−3.16 (m, 1H), 2.89−2.77 (m, 1H), 2.74−2.60 (m, 1H), 2.46−2.29 (m, 2H), 2.22−2.11 (m, 1H), 2.05−1.05 (m, 17H); MS (ES^+) *m*/*z* 598 $(M + H)^+$.

(2*R*,4*S*,7*S*,14*E*)-7-Cyclohexyl-23-methoxy-4-(methoxycarbonyl)-12,12-dimethyl-6-oxo-20-phenyl-3,4,6,7,8,9,10,11,12,13-decahydro-2*H*-16,18-etheno-2,5-methanopyrido[3,4-*r*][1,5,8]oxadiazacyclononadecin-8-ium Trifluoroacetate (10i). Treatment of **9i** (307 mg, 0.46 mmol) and TFA (48 μL, 0.62 mmol) as described in the general procedure for **10c** afforded a residue containing the title compound. MS (ES^+) *m*/*z* 640 $(M + H)^+$. Treatment of this material with $LiOH \cdot H_2O$ according to step 2 of the general procedure described for **12a** (vide infra) followed by evaporation of the volatiles and trituration of the resulting residue with MeCN afforded lithium (2*R*,4*S*,7*S*,14*E*)-7-cyclohexyl-23-methoxy-12,12-dimethyl-6-oxo-20-phenyl-3,4,6,7,8,9,10,11,12,13-decahydro-2*H*-16,18-etheno-2,5-methanopyrido-[3,4-*r*][1,5,8]oxadiazacyclononadecine-4-carboxylate (233 mg, 80% from 9i) as a white solid. ^{1}H NMR (400 MHz, CD_3OD + TFA) δ 8.26 (s, 1H), 8.10−8.04 (m, 2H), 7.79−7.70 (m, 3H), 7.64 (s, 1H), 7.54 (s, 1H), 6.82 (d, *J* = 15.8 Hz, 1H), 6.43 (dt, *J* = 15.8, 7.9 Hz, 1H), 5.88 (br s, 1H), 4.75 (t, *J* = 8.8, 1H), 4.66 (d, *J* = 12.4 Hz, 1H), 4.48 (d, *J* = 4.8 Hz, 1H), 4.11 (s, 3H), 4.11−4.05 (m, 1H), 3.20 (td, *J* = 11.7, 5.5 Hz, 1H), 2.99−2.90 (m, 2H), 2.57−2.48 (m, 1H), 2.28−2.22 (m, 2H), 2.11−1.70 (m, 8H), 1.47−1.21 (m, 7H), 1.00 (s, 3H), 0.99 (s, 3H); MS (ES^+) *m*/*z* 626 $(M+H)^+$.

(2*R*,4*S*,7*S*,14*E*)-7-Cyclopentyl-4-(methoxycarbonyl)-6-oxo-3,4,6,7,8,9,10,11,12,13-decahydro-2*H*-16,18-etheno-2,5-methanopyrido[3,2-*r*][1,5,8]oxadiazacyclononadecin-8-ium Trifluoroacetate (10j). Treatment of **9j** (350 mg, 0.673 mmol) and TFA (70 μL, 0.91 mmol) as described in the general procedure for **10c** afforded a residue containing the title compound that was used in the following step without further purification. MS (ES^+) *m*/*z* 492 $(M + H)^+$.

(2*R*,4*S*,7*S*,14*E*)-7-Cyclohexyl-23-methoxy-4-(methoxycarbonyl)-6-oxo-3,4,6,7,8,9,10,11,12,13-decahydro-2*H*-16,18-etheno-2,5-methanopyrido[3,2-*r*][1,5,8]oxadiazacyclononadecin-8-ium Trifluoroacetate (10k). Treatment of **9k** (280 mg, 050 mmol) and TFA (35 μL, 0.50 mmol) as described in the general procedure for **10a** afforded a residue containing the title compound that was purified by RP-HPLC (conditions E) to give the title compound (35 mg, 10%) as a white solid. MS (ES^+) *m*/*z* 536 $(M+H)^+$.

Methyl (5*R*,7*S*,10*R*,17*E*)-3,9-Dioxo-10-(trifluoromethyl)-6,7,9,10,11,12,13,14,15,16-decahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7(3*H*)-carboxylate (10l). Following the procedure described for **10c**, treatment of **9l** (150 mg, 0.28 mmol) gave a residue that was purified by RP-HPLC (conditions B). The fraction with t_R = 1.72 min was freeze-dried to furnish the title compound (17 mg, 12%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.30 (d, *J* = 7.6 Hz, 1H), 7.24 (t, *J* = 7.6 Hz, 1H), 7.17 (d, *J* = 7.6 Hz, 1H), 6.24 (d, *J* = 16.4 Hz, 1H), 6.14 (ddd, *J* = 16.4, 5.6, 5.6 Hz, 1H), 5.80−5.70 (m, 1H), 5.36 (br t, *J* = 3.4 Hz, 1H), 4.85−4.72 (m 2H), 4.63 (s, 2H), 4.61 (d, *J* = 16.0 Hz, 1H), 4.38 (d, *J* = 12.4 Hz, 1H), 3.68 (dd, *J* = 12.4, 3.4 Hz, 1H), 3.65 (s, 3H), 3.10 (ddd, *J* = 11.9, 11.9, 5.2 Hz, 1H), 2.87 (ddd, *J* = 11.9, 11.9, 5.0 Hz, 1H), 2.56 (dd, *J* = 13.9, 8.5 Hz, 1H), 2.27−2.17 (m, 3H), 1.85−1.65 (m, 2H), 1.60−1.45 (m, 2H), 1.40−1.25 (m, 2H). MS (ES^+) *m*/*z* 510 $(M+H)^+$.

(5*R*,7*S*,10*R*,12*R*)-*N*-{(1*R*,2*S*)-1-[(Cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-3,9-dioxo-10-(propan-2-yl)-12-(trifluoromethyl)-6,7,9,10,11,12,13,14,15,16,17,18-dodecahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7(3*H*)-carboxamide (12a). Step 1: General Procedure for Catalytic Hydrogenation. Methyl (5*R*,7*S*,10*S*,12*S*)-10-Isopropyl-3,9-dioxo-

12-(trifluoromethyl)-6,7,9,10,11,12,13,14,15,16,17,18-dodecahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7-carboxylate (*sat*-10a). A solution of **10a** (120 mg, 0.22 mmol) in MeOH (20 mL) was treated with Pd/C (10%, 20 mg). The mixture was evacuated and then stirred under an atmosphere of hydrogen for 6 h. The solution was filtered and the filtrate was concentrated in vacuo to give a residue that was purified by flash chromatography on silica gel (EtOAc/petroleum ether, gradient from 1:9 to 3:7) to afford the title compound (106 mg, 62%) as a white foam. MS (ES^+) m/z 554 $(M + H)^+$.

Step 2: General Procedure for Proline Ester Hydrolysis. (5*R*,7*S*,10*S*,12*S*)-10-Isopropyl-3,9-dioxo-12-(trifluoromethyl)-6,7,9,10,11,12,13,14,15,16,17,18-dodecahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7-carboxylic Acid. A solution of the preceding compound (105 mg, 0.19 mmol) in a 2:1 (v/v) mixture of THF and H_2O (6 mL) was treated with $LiOH \cdot H_2O$ (24 mg, 0.57 mmol). The mixture was stirred at 20 °C for 14 h and then cooled and acidified to pH 6 by addition of aqueous HCl (1 N). The mixture was diluted with EtOAc, and the organic layer was separated and dried. Removal of the solvent gave a residue that was used without further purification in the subsequent step. MS (ES^+) m/z 540 $(M + H)^+$.

Step 3: General Procedure for Amide Couplings with 11. (5*R*,7*S*,10*R*,12*R*)-*N*-{(1*R*,2*S*)-1-[(Cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-3,9-dioxo-10-(propan-2-yl)-12-(trifluoromethyl)-6,7,9,10,11,12,13,14,15,16,17,18-dodecahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7(3*H*)-carboxamide (12a). To a stirred mixture of the preceding compound (89 mg, 0.165 mmol) in CH_2Cl_2 (10 mL) were added DIEA (69 μL, 0.396 mmol), DMAP (10 mg, 0.082 mmol), and TBTU (64 mg, 0.198 mmol). The solution was stirred for 5 min and then treated with **11**[18] (53 mg, 0.198 mmol). The resulting mixture was stirred at 20 °C for 14 h and then diluted with saturated aqueous $NaHCO_3$ and EtOAc. The organic layer was separated, washed with brine, and dried. Removal of the volatiles gave a residue that was purified by RP-HPLC (conditions B) to furnish the title compound (42 mg, 34%) as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6, 294 K) δ 10.58 (br s, 1H), 9.22 (br s, 1H), 7.24 (t, J = 7.4 Hz, 1H), 7.17 (d, J = 7.4 Hz, 1H), 7.11 (d, J = 7.4 Hz, 1H), 5.67 (ddd, J = 17.1, 10.2, 9.2 Hz, 1H), 5.42 (br t, J = 3.4 Hz, 1H), 5.28 (dd, J = 17.1, 1.7 Hz, 1H), 5.12 (dd, J = 10.2, 1.7 Hz, 1H), 4.65 (s, 2H), 4.64−4.58 (m, 2H), 4.40 (dd, J = 11.0, 6.7 Hz, 1H), 3.96 (d, J = 11.9 Hz, 1H), 3.76 (dd, J = 11.9, 3.4 Hz, 1H), 3.30 (d, J = 6.5 Hz, 1H), 3.00−2.95 (m, 1H), 2.90−2.85 (m, 1H), 2.51−2.44 (m, 2H), 2.22 (dd, J = 13.4, 6.7 Hz, 1H), 2.16 (q, J = 8.8 Hz, 1H), 2.05 (ddd, J = 13.4, 11.0, 3.4 Hz, 1H), 1.97 (br s, 1H), 1.79−1.71 (m, 2H), 1.56−1.48 (m, 3H), 1.44−1.18 (m, 7H), 1.09−0.99 (m, 4H), 0.91 (d, J = 6.7 Hz, 3H), 0.82 (d, J = 6.7 Hz, 3H). ^{19}F NMR (376 MHz, DMSO-d_6) δ −75.8. ^{13}C NMR (150 MHz, DMSO-d_6) δ 174.1, 172.2, 169.2, 152.9, 137.1, 136.2, 135.0, 133.4, 127.9, 127.6, 126.8 (q, J = 228 Hz), 120.3, 117.9, 73.5, 64.8, 59.0, 58.9 (q, J = 26 Hz), 53.2, 52.1, 50.7, 40.8, 34.6, 33.8, 31.4, 31.2, 30.7, 28.9, 28.5, 26.4, 26.0, 23.5, 22.8, 19.1, 17.9, 5.7, 5.4. Analytical RP-HPLC t_R (conditions C) 5.84 min (peak area 98%), t_R (conditions D) 4.42 min (peak area 98%); HRMS calculated for $C_{36}H_{49}F_3N_5O_7S$ $(M + H)^+$ 752.3305, found 752.3325.

(5*R*,7*S*,10*S*,12*R*)-*N*-((1*R*,2*S*)-1-{[(Cyclopropylsulfonyl)mino]-carbonyl}-2-vinylcyclopropyl)-10-isopropyl-3,9-dioxo-12-(trifluoromethyl)-6,7,9,10,11,12,13,14,15,16,17,18-dodecahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7-carboxamide (12b). Treatment of **10b** (88 mg, 0.160 mmol) as described in the general procedures for **12a** furnished the title compound (12 mg, 10%) as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6, 294 K) δ 10.73 (br s, 1H), 9.32 (br s, 1H), 7.25 (t, J = 7.4 Hz, 1H), 7.18 (d, J = 7.4 Hz, 1H), 7.12 (d, J = 7.4 Hz, 1H), 5.71 (ddd, J = 17.1, 10.2, 9.2 Hz, 1H), 5.40 (br t, J = 3.6 Hz, 1H), 5.30 (dd, J = 17.1, 1.7 Hz, 1H), 5.13 (dd, J = 10.2, 1.7 Hz, 1H), 4.73 (d, J = 14.2 Hz, 1H), 4.67−4.55 (m, 3H), 4.31 (dd, J = 10.9, 6.7 Hz, 1H), 4.05 (d, J = 11.9 Hz, 1H), 3.75 (dd, J = 11.9, 3.6 Hz, 1H), 3.70−3.65 (m, 1H), 3.12 (d, J = 8.1 Hz, 1H), 2.95−2.85 (m, 1H), 2.80 (br s, 1H), 2.59 (dd, J = 12.9, 6.4 Hz, 1H), 2.40−2.35 (m, 1H), 2.26 (dd, J = 13.4, 6.7 Hz, 1H), 2.19 (q, J = 8.8 Hz, 1H), 2.09 (ddd, J = 13.4, 10.9, 3.6 Hz, 1H), 1.89−1.80 (m, 1H), 1.73 (dd, J = 7.9, 5.1 Hz, 1H), 1.63−1.50 (m, 2H), 1.49−1.33 (m, 6H), 1.31−1.22 (m, 2H), 1.15−0.97 (m, 5H), 0.85 (d, J = 6.7 Hz, 3H), 0.83 (d, J = 6.7 Hz, 3H); ^{19}F NMR (376 MHz, DMSO-d_6) δ −75.2. Analytical RP-HPLC t_R (conditions C) 5.81 min (peak area 98%), t_R (conditions D) 4.45 min (peak area 99%); HRMS calculated for $C_{36}H_{49}F_3N_5O_7S$ $(M+H)^+$ 752.3305, found 752.3306.

(5*R*,7*S*,10*R*,12*R*)-*N*-{(1*R*,2*S*)-1-[(Cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-12-methyl-3,9-dioxo-10-(propan-2-yl)-6,7,9,10,11,12,13,14,15,16,17,18-dodecahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7(3*H*)-carboxamide Trifluoroacetate (13a). Treatment of (*R*)-**10c** (192 mg, 0.31 mmol) as described in the general procedure reported for **12a** gave the title compound (22%) as a white powder. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.90 (br s, 1H), 8.73 (br s, 1H), 8.50−8.25 (m, 2H), 7.26 (t, J = 7.4 Hz, 1H), 7.18 (d, J = 7.4 Hz, 1H), 7.14 (d, J = 7.4 Hz, 1H), 5.56 (ddd, J = 17.1, 10.2, 9.2 Hz, 1H), 5.41 (br s, 1H), 5.22 (d, J = 17.1 Hz, 1H), 5.11 (d, J = 10.2 Hz, 1H), 4.67 (m, 2H), 4.62−4.49 (m, 3H), 4.40−4.34 (m, 1H), 4.18 (d, J = 12.2, 1H), 3.74 (dd, J = 12.2, 2.5 Hz, 1H), 2.94−2.82 (m, 2H), 2.52−2.43 (m, 2H, obscured by residual DMSO signal), 2.42−2.34 (m, 1H), 2.23 (m, 1H), 2.15 (q, J = 8.7 Hz, 1H), 2.06 (m, 1H), 1.74 (dd, J = 7.6, 5.5 Hz, 1H), 1.71 (m, 1H), 1.63−1.44 (m, 3H), 1.41−0.97 (m, 20H). Analytical RP-HPLC t_R (conditions C) 5.96 min (peak area 97%), t_R (conditions D) 4.31 min (peak area 98%); HRMS calculated for $C_{36}H_{52}N_5O_7S$ $(M + H)^+$ 698.3587, found 698.3579.

(5*R*,7*S*,10*R*,12*S*)-*N*-{(1*R*,2*S*)-1-[(Cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-12-methyl-3,9-dioxo-10-(propan-2-yl)-6,7,9,10,11,12,13,14,15,16,17,18-dodecahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7(3*H*)-carboxamide Trifluoroacetate (13b). Treatment of (*S*)-**10c** (281 mg, 0.46 mmol) as described in the general procedure reported for **12a** gave the title compound (7%) as a white powder. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.89 (br s, 1H), 8.77 (br s, 1H), 8.39−8.23 (m, 2H), 7.24 (t, J = 7.4 Hz, 1H), 7.18 (d, J = 7.4 Hz, 1H), 7.12 (d, J = 7.4 Hz, 1H), 5.58 (ddd, J = 17.2, 10.3, 9.2 Hz, 1H), 5.34 (br s, 1H), 5.24 (d, J = 17.2 Hz, 1H), 5.12 (d, J = 10.3 Hz, 1H), 4.71 (d, J = 14.6 Hz, 1H), 4.64−4.58 (m, 3H), 4.48 (t, J = 8.8 Hz, 1H), 4.38−4.36 (m, 1H), 4.28 (d, J = 10.6 Hz, 1H), 3.76 (d, J = 10.6 Hz, 1H), 3.07−2.99 (br m, 1H), 2.95−2.89 (m, 1H), 2.58−2.45 (obscured by residual DMSO, 2H), 2.42−2.36 (m, 1H), 2.33−2.25 (m, 1H), 2.17 (q, J = 8.7 Hz, 1H), 2.13−2.05 (m, 1H), 1.78−1.71 (m, 2H), 1.73−1.56 (m, 1H), 1.49−1.19 (m, 9H), 1.28 (d, J = 6.3 Hz, 3H), 1.12 (d, J = 6.7 Hz, 3H), 1.15−0.96 (m, 4H), 0.97 (d, J = 6.8 Hz, 3H). Analytical RP-HPLC t_R (conditions C) 6.01 min (peak area 97%), t_R (conditions D) 4.33 min (peak area 96%); HRMS calculated for $C_{36}H_{52}N_5O_7S$ $(M + H)^+$ 698.3587, found 698.3580.

(5*R*,7*S*,10*S*,12*R*)-*N*-{(1*R*,2*S*)-1-[(Cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-12-methyl-3,9-dioxo-10-(propan-2-yl)-6,7,9,10,11,12,13,14,15,16,17,18-dodecahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7(3*H*)-carboxamide Trifluoroacetate (14). Treatment of **10f** (180 mg, 0.35 mmol) as described in the general procedure reported for **12a** gave the title compound (23%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.74 (s, 1H), 8.74 (s, 1H), 8.70−8.56 (m, 2H), 7.25 (t, J = 7.4 Hz, 1H), 7.17 (d, J = 7.4 Hz, 1H), 7.13 (d, J = 7.4 Hz, 1H), 5.56 (ddd, J = 17.1, 10.3, 9.3 Hz, 1H), 5.39 (br s, 1H), 5.22 (d, J = 17.1 Hz, 1H), 5.11 (d, J = 10.3 Hz, 1H), 4.78−4.48 (m, 5H), 4.27 (br s, 1H), 3.74 (dd, J = 11.6, 2.5 Hz, 1H), 2.95−2.89 (m, 2H), 2.69−2.66 (m, 1H), 2.38−2.32 (m, 1H, partially obscured by residual DMSO signal), 2.21−2.06 (m, 3H), 1.73 (dd, J = 9.7, 6.9 Hz, 2H), 1.69−1.55 (m, 4H), 1.32−1.26 (m, 1H), 4.49−4.62 (m, 7H), 1.15−1.09 (m, 1H), 1.04 (dd, J = 9.7, 6.9 Hz, 9H). Analytical RP-HPLC t_R (conditions C) 5.86 min (peak area 99%), t_R

(conditions D) 4.24 min (peak area 99%); HRMS calculated for $C_{35}H_{50}N_5O_7S$ $(M + H)^+$ 684.3431, found 698.3431.

(5*R*,7*S*,10*S*)-*N*-((1*R*,2*S*)-1-{[(Cyclopropylsulfonyl)amino]carbonyl}-2-vinylcyclopropyl)-13,13-difluoro-10-isopropyl-3,9-dioxo-6,7,9,10,11,12,13,14,15,16,17,18-dodecahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7-carboxamide (15). Treatment of **10g** (22 mg, 0.158 mmol) according to the general procedure described for **12a** gave the title compound (15%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.66 (s, 1H), 8.97 (br s, 1H), 7.25 (t, J = 7.5 Hz, 1H), 7.17 (d, J = 7.5 Hz, 1H), 7.13 (d, J = 7.5 Hz, 1H), 5.61 (ddd, J = 17.2, 10.4, 9.3 Hz, 1H), 5.38 (br s, 1H), 5.25 (d, J = 17.2 Hz, 1H), 5.11 (d, J = 10.4 Hz, 1H), 4.73−4.54 (m, 4H), 4.45 (dd, J = 10.1, 7.3 Hz, 1H), 4.04 (d, J = 12.1 Hz, 1H), 3.75 (dd, J = 12.0, 3.2 Hz, 1H), 3.50−3.02 (m, 3H), 2.96−2.85 (m, 1H), 2.45−2.34 (m, 3H, partially obscured by residual DMSO), 2.24−1.67 (m, 6H), 1.66−1.48 (m, 2H), 1.49−1.20 (m, 5H), 1.14−0.91 (m, 10H). Analytical RP-HPLC t_R (conditions C) 8.03 min (peak area >99%), t_R (conditions D) 5.60 min (peak area 98%); HRMS calculated for $C_{35}H_{48}F_2N_5O_7S$ $(M+H)^+$ 720.3243, found 720.3237.

(5*R*,7*S*,10*S*,17*E*)-*N*-{(1*R*,2*S*)-1-[(Cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-3,9-dioxo-10-(trifluoromethyl)-6,7,9,10,11,12,13,14,15,16-decahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7(3*H*)-carboxamide (16). Treatment of **10l** (17 mg, 0.034 mmol) as described in the general procedure reported for **12a** gave the title compound (35%) as a white powder. ^{1}H NMR (600 MHz, DMSO-d_6 + TFA, 294 K) δ 10.98 (bs, 1H), 8.55 (bs, 1H), 7.33 (d, J = 7.6 Hz, 1H), 7.27 (t, J = 7.6 Hz, 1H), 7.21 (d, J = 7.6 Hz, 1H), 6.24 (d, J = 16.4 Hz, 1H), 6.14 (ddd, J = 16.4, 5.5, 5.5 Hz, 1H), 5.61 (m, 1H), 5.51 (ddd, J = 17.1, 10.2, 9.0 Hz, 1H), 5.33 (br t, J = 3.3 Hz, 1H), 5.22 (dd, J = 17.1, 1.5 Hz, 1H), 5.11 (dd, J = 10.2, 1.5 Hz, 1H), 4.71−4.60 (m, 4H), 4.57 (dd, J = 9.7, 8.2 Hz, 1H), 4.36 (d, J = 12.2 Hz, 1H), 3.70 (dd, J = 12.2, 3.3 Hz, 1H), 3.07 (ddd, J = 12.0, 12.0, 4.7 Hz, 1H), 2.96−2.85 (m, 1H), 2.77 (ddd, J = 12.0, 12.0, 5.4 Hz, 1H), 2.56 (dd, J = 14.3, 8.2 Hz, 1H), 2.30−2.15 (m, 2H), 2.14−2.07 (m, 2H), 1.80−1.65 (m, 3H), 1.60−1.45 (m, 2H), 1.42−1.27 (m, 2H), 1.24 (dd, J = 9.3, 5.2 Hz, 1H), 1.10−0.95 (m, 4H); ^{19}F NMR (376 MHz, DMSO-d_6) δ −73.0. Analytical RP-HPLC t_R (conditions C) 6.28 min (peak area 99%), t_R (conditions D) 4.53 min (peak area 99%); HRMS calculated for $C_3H_{41}F_3N_5O_7S$ $(M + H)^+$ 708.2679, found 708.2702.

(5*R*,7*S*,10*S*,12*R*,17*E*)-10-Cyclopentyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-12-methyl-3,9-dioxo-6,7,9,10,11,12,13,14,15,16-decahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7(3*H*)-carboxamide Trifluoroacetate (17a). Treatment of (*R*)-**10d** (42 mg, 0.068 mmol) as described in the general procedure reported for **12a** (steps 2 and 3) gave the title compound (37.5 mg, 59%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.72 (br s, 1H), 8.78−8.68 (m, 2H), 8.64 (br s, 1H), 8.57−8.47 (m, 2H), 7.32−7.22 (m, 3H), 6.24 (d, J = 16.3 Hz, 1H), 6.07 (dt, J = 16.3, 5.7 Hz, 1H), 5.56 (ddd, J = 17.1, 10.3, 9.5 Hz, 1H), 5.42−5.38 (m, 1H), 5.21 (d, J = 17.1 Hz, 1H), 5.11 (d, J = 10.3 Hz, 1H), 4.72−4.54 (m, 4H), 4.51 (dd, J = 10.0, 7.7 Hz, 1H), 4.41 (br t, J = 8.3 Hz, 1H), 4.23 (br d, J = 10.7 Hz, 1H), 3.73 (br d, J = 10.7 Hz, 1H), 2.95−2.82 (m, 2H), 2.54−2.01 (m, 6H, partially obscured by residual DMSO signal), 1.96−1.28 (m, 16H), 1.27 (d, J = 6.6 Hz, 3H), 1.13−0.99 (m, 4H). Analytical RP-HPLC t_R (conditions C) 6.03 min (peak area 97%), t_R (conditions D) 4.31 min (peak area 98%); HRMS calculated for $C_{38}H_{52}N_5O_7S$ $(M + H)^+$ 722.3587, found 722.3599.

(5*R*,7*S*,10*S*,12*R*,17*E*)-10-Cyclopentyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-12-methyl-3,9-dioxo-6,7,9,10,11,12,13,14,15,16-decahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7(3*H*)-carboxamide Trifluoroacetate (17b). Treatment of (*S*)-**22d** (102 mg, 0.160 mmol) as described in the general procedure reported for **12a** (steps 2 and 3) gave the title compound as a white powder (29.5 mg, 22%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.86 (br s, 1H), 8.66 (br s, 1H), 8.51−8.33 (m, 2H), 7.31−7.18 (m, 3H), 6.38 (d, J = 16.2 Hz, 1H), 6.01 (dt, J = 16.2, 6.1 Hz, 1H), 5.58 (ddd, J = 17.2, 10.4, 9.1 Hz, 1H), 5.22 (d, J = 17.2 Hz, 1H), 5.22−5.16 (m, 1H), 5.12 (d, J = 10.4 Hz, 1H), 4.83 (d, 14.7 Hz, 1H), 4.71 (d, 14.7, 1H), 4.65−4.53 (m, 3H), 4.41 (br t, J = 9.0 Hz, 1H), 4.29 (br d, J = 12.3 Hz, 1H), 3.76 (dd, J = 12.3, 3.4 Hz, 1H), 2.96−2.83 (m, 2H), 2.72−2.64 (m, 1H), 2.46−0.98 (m, 25H), 1.28 (d, J = 6.3 Hz, 3H). Analytical RP-HPLC t_R (conditions C) 5.97 min (peak area 99%), t_R (conditions D) 4.41 min (peak area 99%); HRMS calculated for $C_{38}H_{52}N_5O_7S$ $(M+H)^+$ 722.3587, found 722.3593.

(5*R*,7*S*,10*S*,12*R*)-10-Cyclopentyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-12-methyl-3,9-dioxo-6,7,9,10,11,12,13,14,15,16,17,18-dodecahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7(3*H*)-carboxamide Trifluoroacetate (17c). Treatment of (*R*)-**10d** (93.2 mg, 0.146 mmol) as described in the general procedure reported for **12a** gave the title compound (26 mg, 46%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.76 (br s, 1H), 8.72 (br s, 1H), 8.63−8.34 (m, 2H), 7.26 (t, J = 7.5 Hz, 1H), 7.19 (d, J = 7.5 Hz, 1H), 7.14 (d, J = 7.5 Hz, 1H), 5.57 (ddd, J = 17.2, 10.3, 9.1 Hz, 1H), 5.42−5.38 (br s, 1H), 5.22 (d, J = 17.2 Hz, 1H), 5.11 (d, J = 10.3 Hz, 1H), 4.72−4.41 (m, 6H), 4.18 (br d, J = 11.0 Hz, 1H), 3.75 (br d, J = 11.0 Hz, 1H), 2.96−2.79 (m, 2H), 2.54−2.02 (m, 6H, partially obscured by residual DMSO signal), 1.93−0.99 (m, 24H), 1.23 (d, J = 6.4 Hz, 3H). Analytical RP-HPLC t_R (conditions C) 6.13 min (peak area 92%), t_R (conditions D) 4.34 min (peak area 93%); HRMS calculated for $C_{38}H_{54}N_5O_7S$ $(M+H)^+$ 724.3744, found 724.3748.

(5*R*,7*S*, 10*S*, 12*R*)-10-Cyclohexyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-12-methyl-3,9-dioxo-6,7,9,10,11,12,13,14,15,16,17,18-dodecahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7(3*H*)-carboxamide Trifluoroacetate (18a). Treatment of (*R*)-**10e** (170 mg, 0.26 mmol) as described in the general procedure reported for **12a** gave the title compound (20%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (br s, 1H), 8.73 (br s, 1H), 8.43 (br s, 1H), 8.34−8.20 (m, 1H), 7.26 (t, J = 7.5 Hz, 1H), 7.18 (d, J = 7.5 Hz, 1H), 7.14 (d, J = 7.5 Hz, 1H), 5.58 (ddd, J = 17.3, 10.4, 9.1 Hz, 1H), 5.41 (br s, 1H), 5.21 (d, J = 17.3 Hz, 1H), 5.11 (d, J = 10.4 Hz, 1H), 4.72−4.45 (m, 5H), 4.48−4.39 (m, 1H), 4.17 (d, J = 12.1 Hz, 1H), 3.76 (dd, J = 12.9, 2.3 Hz, 1H), 2.97−2.78 (m, 2H), 2.47−2.34 (partially obscured by residual DMSO, 2H), 2.19−2.03 (m, 2H), 1.99−1.42 (m, 11H), 1.41−0.93 (m, 20H). Analytical RP-HPLC t_R (conditions C) 6.39 min (peak area 96%), t_R (conditions D) 4.51 min (peak area 97%); HR-MS calculated for $C_{39}H_{56}N_5O_7S$ $(M + H)^+$ 738.3900, found 738.3925.

(5*R*,7*S*,10*S*,12*S*)-10-Cyclohexyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-12-methyl-3,9-dioxo-6,7,9,10,11,12,13,14,15,16,17,18-dodecahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7(3*H*)-carboxamide Trifluoroacetate (18b). Treatment of (*S*)-**10e** (180 mg, 0.29 mmol) as described in the general procedure reported for **12a** gave the title compound (37%) as a white powder. ^{1}H NMR (DMSO-d_6) δ 10.70 (s, 1H), 8.85−8.73 (m, 1H), 8.41 (br s, 1H), 8.15 (br s, 1H), 7.24 (t, J = 7.4 Hz, 1H), 7.17 (d, J = 7.4 Hz, 1H), 7.12 (d, J = 7.4 Hz, 1H), 5.65−5.55 (m, 1H), 5.36 (br s, 1H), 5.23 (d, J = 17.1 Hz, 1H), 5.12 (d, J = 10.3 Hz, 1H), 4.76−4.53 (m, 4H), 4.48 (t, J = 9.3 Hz, 1H), 4.36−4.21 (m, 2H), 3.81 (d, J = 9.5 Hz, 1H), 3.05 (br s, 1H), 2.91 (m, 1H), 2.60−2.53 (m, 1H, partially obscured by residual DMSO), 2.46−2.38 (m, 1H), 2.22−2.10 (m, 2H), 2.00−1.87 (m, 1H) 1.86−1.71 (m, 5H), 1.70−1.54 (m, 3H), 1.53−1.20 (m, 15H) 1.19−0.98 (m, 6H). Analytical RP-HPLC t_R (conditions C) 6.39 min (peak area 94%), t_R (conditions D) 4.52 min (peak area 96%); HRMS calculated for $C_{39}H_{56}N_5O_7S$ $(M+H)^+$ 738.3900, found 738.3915.

(5*R*,7*S*,10*S*,12*R*)-10-Cyclohexyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-11,12-dimethyl-3,9-dioxo-6,7,9,10,11,12,13,14,15,16,17,18-dodecahydro-1*H*,5*H*-2,22:5,8-dimethano-4,2,8,11-benzoxatriazacycloicosine-7(3*H*)-carboxamide Trifluoroacetate (19). A solution of $NaCNBH_3$ (5.2 mg, 0.083 mmol) and $ZnCl_2$ (5.7 mg, 0.042 mmol) in MeOH (4 mL)

was added dropwise to a solution of **18a** (59 mg, 0.069 mmol) and formaldehyde (37% in H_2O, 0.18 mL, 2.42 mmol) in MeOH (2 mL). The mixture was stirred for 14 h, and then the volatiles were evaporated under reduced pressure. The residue was purified by RP-HPLC (conditions B) to furnish the title compound as a white powder (33 mg, 55%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H), 8.73 (br s, 1H), 8.65 (br s, 1H), 7.26 (t, J = 7.5 Hz, 1H), 7.19 (d, J = 7.5 Hz, 1H), 7.13 (d, J = 7.5 Hz, 1H), 5.60 (ddd, J = 17.2, 10.3, 9.5 Hz, 1H), 5.37 (br s, 1H), 5.20 (d, J = 17.2 Hz, 1H), 5.11 (d, J = 10.3 Hz, 1H), 4.77−5.55 (m, 4H), 4.56−4.39 (m, 2H), 3.92 (d, J = 12.1 Hz, 1H), 3.80 (dd, J = 12.1, 2.5 Hz, 1H), 2.97−2.80 (m, 2H), 2.62 (d, J = 4.0 Hz, 3H), 2.47−2.36 (m, 3H, partially obscured by residual DMSO signal), 2.15 (q, J = 8.9 Hz, 1H), 2.10−1.93 (m, 2H), 1.92−1.41 (m, 12H), 1.40−1.81 (m, 11H), 1.17−0.94 (m, 6H). Analytical RP-HPLC t_R (conditions C) 6.24 min (peak area 97%), t_R (conditions D) 4.46 min (peak area 98%); HRMS calculated for $C_{40}H_{58}N_5O_7S$ $(M+H)^+$ 752.4057, found 752.4050.

(2*R*,4*S*,14*E*)-7-Cyclopentyl-4-({(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}carbamoyl)-6-oxo-3,4,6,7,8,9,10,11,12,13-decahydro-2*H*-16,18-etheno-2,5-methanopyrido[3,2-*r*][1,5,8]oxadiazacyclononadecin-8-ium Trifluoroacetate (20). Treatment of **10j** (408 mg, 0.673 mmol) as described in the general procedure for **12a** (steps 2 and 3) gave the title compound (92 mg, 17%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.72 (br s, 1H), 8.96−8.69 (m, 2H), 8.59 (br s, 1H), 8.09 (m, 1H), 7.99 (d, J = 5.8 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.64 (br d, J = 8.4, 1.5 Hz, 1H), 7.41 (d, J = 5.8 Hz, 1H), 6.69 (d, J = 15.7 Hz, 1H), 6.02 (dt, J = 15.7, 7.3 Hz, 1H), 5.78 (br s, 1H), 5.56 (ddd, J = 17.2, 10.2, 9.6 Hz, 1H), 5.18 (d, J = 17.2 Hz, 1H), 5.09 (d, J = 10.2 Hz, 1H), 4.60−4.48 (m, 2H), 4.43 (dd, J = 10.3, 7.5 Hz, 1H), 3.85 (dd, J = 12.1, 2.3 Hz, 1H), 3.35−3.23 (m, 1H), 2.95−2.87 (m, 1H), 2.79−2.58 (m, 2H), 2.38−2.06 (m, 5H), 1.96−1.83 (m, 2H), 1.79−1.36 (m, 12H), 1.33−1.20 (m, 2H), 1.15−0.95 (m, 4H). Analytical RP-HPLC t_R (conditions C) 6.20 min (peak area 98%), t_R (conditions D) 4.42 min (peak area 98%); HRMS calculated for $C_{37}H_{50}N_5O_6S$ $(M+H)^+$ 690.3325, found 690.3322.

(2*R*,4*S*,7*S*)-7-Cyclopentyl-4-({(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}carbamoyl)-6-oxo-3,4,6,7,8,9,10,11,12,13,14,15-dodecahydro-2*H*-16,18-etheno-2,5-methanopyrido[3,2-*r*][1,5,8]oxadiazacyclononadecin-8-ium Trifluoroacetate (21). Treatment of **10j** (408 mg, 0.67 mmol) as described in the general procedure reported for **12a** gave the title compound (140 mg, 26%) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.66 (br s, 1H), 8.85 (br s, 1H), 8.85−8.61 (m, 2H), 7.98 (d, J = 5.8 Hz, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.85−7.82 (m, 1H), 7.67 (dd, J = 8.4, 1.5 Hz, 1H), 7.41 (d, J = 5.8 Hz, 1H), 6.05 (br s, 1H), 5.57 (dt, J = 17.1, 9.9 Hz, 1H), 5.23 (dd, J = 17.1, 1.5 Hz, 1H), 5.12 (dd, J = 9.9, 1.5 Hz, 1H), 4.66 (dd, J = 10.2, 7.6 Hz, 1H), 4.40−4.30 (m, 2H), 3.88 (dd, J = 11.9, 2.9 Hz, 1H), 2.98−2.83 (m, 3H), 2.77−2.58 (m, 3H), 2.28−2.12 (m, 3H), 1.89−0.95 (m, 24H). Analytical RP-HPLC t_R (conditions C) 6.40 min (peak area 99%), t_R (conditions D) 4.55 min (peak area 99%); HRMS calculated for $C_{37}H_{50}N_5O_6S$ $(M+H)^+$ 692.3482, found 692.3475.

(2*R*,4*S*,7*S*,14*E*)-7-Cyclohexyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-23-methoxy-6-oxo-20-phenyl-3,4,6,7,8,9,10,11,12,13-decahydro-2*H*-16,18-etheno-2,5-methanopyrido[3,4-*r*][1,5,8]oxadiazacyclononadecine-4-carboxamide Trifluoroacetate (22). Treatment of **10h** (350 mg, 0.55 mmol) as described in the general procedure reported for **12a** (steps 2 and 3) gave the title compound (43 mg, 35% from **9h**) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.86 (br s, 1H), 8.83−8.73 (br m, 2H), 8.60 (s, 1H), 8.29−8.22 (m, 2H), 7.92 (s, 1H), 7.65−7.54 (m, 4H), 7.47−7.41 (m, 1H), 6.73 (d, J = 15.5 Hz, 1H), 5.99 (dt, J = 15.5, 7.2 Hz, 1H), 5.76 (br s, 1H), 5.57 (ddd, J = 17.2, 10.3, 9.2 Hz, 1H), 5.16 (d, J = 17.2 Hz, 1H), 5.08 (d, J = 10.3 Hz, 1H), 4.63 (br d, J = 10.9 Hz, 1H), 4.50−4.42 (m, 1H), 4.41 (dd, J = 10.9, 7.9 Hz, 1H), 3.98 (s, 3H), 3.97−3.88 (m, 1H), 3.33−3.19 (m, 1H), 2.94−2.77 (m, 2H), 2.64−0.95 (m, 28H). Analytical RP-HPLC t_R (conditions C) 5.70 min (peak area 99%), t_R (conditions D) 3.91 min (peak area 99%); HRMS calculated for $C_{45}H_{56}N_5O_7S$ $(M+H)^+$ 810.3900, found 810.3911.

(2*R*,4*S*,7*S*)-7-Cyclohexyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-23-methoxy-6-oxo-20-phenyl-3,4,6,7,8,9,10,11,12,13,14,15-dodecahydro-2*H*-16,18-etheno-2,5-methanopyrido[3,4-*r*][1,5,8]oxadiazacyclononadecine-4-carboxamide Trifluoroacetate (23). Treatment of **10h** (120 mg, 0.166 mmol) as described in the general procedure reported for **12a** gave the title compound (58 mg, 38% from **9h**) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.84 (br s, 1H), 8.81−8.58 (m, 3H), 8.27−8.17 (m, 2H), 7.82 (s, 1H), 7.70−7.54 (m, 4H), 7.52−7.44 (m, 1H), 5.86 (br s, 1H), 5.57 (ddd, J = 17.1, 10.2, 9.1 Hz, 1H), 5.21 (d, J = 17.1 Hz, 1H), 5.12 (d, J = 10.2 Hz, 1H), 4.61 (t, J = 8.8 Hz, 1H), 4.49 (br d, J = 12.1 Hz, 1H), 4.40−4.32 (m, 1H), 3.99 (s, 3H), 3.97−3.89 (m, 1H), 3.07−2.82 (m, 3H), 2.80−2.59 (m, 3H), 2.35−2.24 (m, 1H), 2.14 (q, J = 8.7 Hz, 1H), 1.98−1.49 (m, 11H), 1.39−0.97 (m, 16H). Analytical RP-HPLC t_R (conditions C) 5.49 min (peak area 97%), t_R (conditions D) 3.92 min (peak area 99%); HRMS calculated for $C_{45}H_{58}N_5O_7S$ $(M+H)^+$ 812.4057, found 812.4067.

(2*R*,4*S*,7*S*,14*E*)-7-Cyclohexyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-23-methoxy-12,12-dimethyl-6-oxo-20-phenyl-3,4,6,7,8,9,10,11,12,13-decahydro-2*H*-16,18-etheno-2,5-methanopyrido[3,4-*r*][1,5,8]oxadiazacyclononadecine-4-carboxamide Trifluoroacetate (24). Treatment of **10i** (224 mg, 0.297 mmol) as described in the general procedure reported for **12a** (steps 2 and 3) gave the title compound (104 mg, 37% from **9i**) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (br s, 1H), 8.90−8.63 (m, 3H), 8.29−8.22 (m, 2H), 8.11 (s, 1H), 7.66−7.53 (m, 4H), 7.49−7.44 (m, 1H), 6.73 (d, J = 15.8 Hz, 1H), 6.28 (dt, J = 15.8, 7.8 Hz, 1H), 5.76 (br s, 1H), 5.56 (ddd, J = 17.1, 10.5, 9.1 Hz, 1H), 5.18 (d, J = 17.1 Hz, 1H), 5.08 (d, J = 10.5 Hz, 1H), 4.63 (br d, J = 11.9 Hz, 1H), 4.50−4.39 (m, 2H), 4.00 (s, 3H), 3.97−3.90 (m, 1H), 3.22−3.11 (m, 1H), 2.94−2.86 (m, 1H), 2.80−2.67 (m, 2H), 2.27−2.06 (m, 4H), 2.00−1.61 (m, 9H), 1.32−0.96 (m, 12H), 0.92 (s, 3H), 0.91 (s, 3H). Analytical RP-HPLC t_R (conditions C) 5.70 min (peak area >99%), t_R (conditions D) 3.92 min (peak area >99%); HRMS calculated for $C_{47}H_{60}N_5O_7S$ $(M + H)^+$ 838.4213, found 838.4221.

(2*R*,4*S*,7*S*)-7-Cyclohexyl-*N*-{(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-23-methoxy-12,12-dimethyl-6-oxo-20-phenyl-3,4,6,7,8,9,10,11,12,13,14,15-dodecahydro-2*H*-16,18-etheno-2,5-methanopyrido[3,4-*r*][1,5,8]oxadiazacyclononadecine-4-carboxamide Trifluoroacetate (25). Treatment of **10i** (699 mg, 0.93 mmol) as described in the general procedure reported for **12a** gave the title compound (301 mg, 34% from **9i**) as a white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (br s, 1H), 8.79 (s, 1H), 8.65−8.55 (br s, 1H), 8.51−8.41 (br s, 1H), 8.28−8.20 (m, 2H), 7.83 (s, 1H), 7.67−7.54 (m, 5H), 7.47 (s, 1H), 5.85 (br s, 1H), 5.58 (ddd, J = 17.0, 10.3, 9.5 Hz, 1H), 5.22 (d, J = 17.0 Hz, 1H), 5.12 (d, J = 10.3 Hz, 1H), 4.63 (dd, J = 9.2, 8.2 Hz, 1H), 4.41 (d, J = 12.1 Hz, 1H), 4.38−4.31 (br m, 1H), 3.98 (s, 3H), 4.01−3.89 (m, 1H), 2.97−2.84 (m, 3H), 2.80−2.73 (m, 1H), 2.68−2.57 (m, 2H), 2.35−2.25 (m, 1H), 2.14 (q, J = 8.7 Hz, 1H), 2.01−1.90 (m, 1H), 1.81−0.99 (m, 22H), 0.85 (s, 3H), 0.77 (s, 3H). Analytical RP-HPLC t_R (conditions C) 5.81 min (peak area >99%), t_R (conditions D) 4.14 min (peak area >99%); HRMS calculated for $C_{47}H_{60}N_5O_7S$ $(M + H)^+$ 840.4370, found 840.4381.

(2*R*,4*S*,7*S*,14*E*)-7-Cyclohexyl-4-({(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}carbamoyl)-23-methoxy-6-oxo-3,4,6,7,8,9,10,11,12,13-decahydro-2*H*-16,18-etheno-2,5-methanopyrido[3,2-*r*][1,5,8]oxadiazacyclononadecin-8-ium Trifluoroacetate (26). Treatment of **10k** (90 mg, 0.14 mmol) as described in the general procedure reported for **12a** (steps 2 and 3) gave the title compound (25%) as a white powder. ^{1}H NMR (DMSO-d_6) δ 11.12 (s, 1H), 8.90−8.71 (m, 2H), 8.58 (s, 1H), 7.96−7.90 (m, 2H), 7.33 (d, J = 5.9 Hz, 1H), 7.30 (s, 1H), 6.70 (d, J = 15.6 Hz,

1H), 5.92 (dt, J = 15.6, 7.6 Hz, 1H), 5.74 (br s, 1H), 5.58 (ddd, J = 17.0, 10.2, 9.1 Hz, 1H), 5.18 (d, J = 17.0 Hz, 1H), 5.09 (d, J = 10.2 Hz, 1H), 4.52 (d, J = 11.6 Hz, 1H), 4.48−4.38 (m, 2H), 3.91 (s, 3H), 3.70 (dd, J = 12.4, 3.5 Hz, 1H), 3.21 (br s, 1H), 2.91 (m, 1H), 2.74 (dd, J = 14.1, 7.6 Hz, 1H), 2.60 (br s, 1H), 2.36−2.24 (m, 1H), 2.23−2.07 (m, 3H), 1.99−1.60 (m, 9H), 1.57−1.34 (m, 3H), 1.33−0.93 (m, 11H). Analytical RP-HPLC t_R (conditions C) 6.29 min (peak area 98%), t_R (conditions D) 4.45 min (peak area 98%); HRMS calculated for $C_{39}H_{52}N_5O_7S$ $(M+H)^+$ 734.3587, found 734.3578.

Methyl 3,3,3-Trifluoroalanyl-(4*R*)-4-{[(4-ethenyl-1,3-dihydro-2*H*-isoindol-2-yl)carbonyl]oxy}-L-prolinate (33). A solution of **4** (1.00 g, 2.83 mmol) in CH_2Cl_2 (20 mL) was treated with (D,L)-*N*-Boc-trifluoromethylglycine (827 mg, 3.40 mmol) and NMM (686 μL, 6.24 mmol). HATU (1.51 g, 3.40 mmol) was added, and the mixture was stirred for 14 h. The solution was diluted with EtOAc and aqueous HCl (1 N), and then the layers were separated. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine and then dried. Removal of the volatiles gave a residue (MS (ES^+) m/z 542 $(M+H)^+$) that was taken up in a solution of HCl in dioxane (4 N, 5 mL). The mixture was stirred for 4 h, and then the volatiles were removed in vacuo to afford a residue that was triturated with Et_2O to furnish the title compound (1.3 g, 59%) as a solid that was used directly in the subsequent step. MS (ES^+) m/z 442 $(M+H)^+$.

(2*S*)-[(*tert*-Butoxycarbonyl)(3,3-dimethylbutyl)amino](cyclohexyl)acetic Acid (34). Following the general procedure for reductive amination described for **10c**, treatment of methyl (2*S*)-amino(cyclohexyl)acetate (400 mg, 1.92 mmol) and 3,3-dimethylbutanal (424 mg, 4.24 mmol) furnished (2*S*)-cyclohexyl[(3,3-dimethylbutyl)amino]acetate (490 mg, 100%). MS (ES^+) m/z 256 $(M+H)^+$. This material was taken up in CH_2Cl_2 (35 mL) and treated with Et_3N (0.54 mL, 3.84 mmol) and di-*tert*-butyl dicarbonate (544 mg, 2.49 mmol). The mixture was stirred for 14 h and then diluted with EtOAc and washed with aqueous HCl (1 N) and brine. The dried organic layer was concentrated to afford a residue that was purified by flash chromatography on silica gel (EtOAc/petroleum ether, gradient from 0:100 to 5:95) to give (2S)-[(*tert*-butoxycarbonyl)(3,3-dimethylbutyl)amino](cyclohexyl)-acetate (678 mg, 100%) as a white solid. MS (ES^+) m/z 356 $(M+H)^+$. This material was dissolved in a 2:1 (v/v) mixture of THF/H_2O (15 mL) and treated with $LiOH \cdot H_2O$ (240 mg, 5.74 mmol). The mixture was heated at 50 °C for 3 h and then cooled and diluted with EtOAc. The organic layer was washed with aqueous HCl (1 N) and brine and then dried. Removal of the volatiles in vacuo afforded the title compound (500 mg, 77%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, a mixture of two conformers in a ratio 55:45*) δ 12.51 (br s, 1H), 4.13 and 3.72* (d, J = 9.9 Hz and J* = 8.9 Hz, 1H), 3.24−2.99 (m, 2H), 1.91−1.08 (m, 11H), 1.40 and 1.36* (s, 9H), 0.98−0.83 (m, 2H), 0.87 (s, 9H); MS (ES^+) m/z 342 $(M+H)^+$.

(4*R*)-*N*-{(1*R*,2*S*)-1-[(Cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}-4-({7-methoxy-2-[2-(propan-2-ylamino)-1,3-thiazol-4-yl]quinolin-4-yl}oxy)-L-prolinamide (36). Treatment of **35a** (123 mg, 0.16 mmol) according to the general procedure described for **12a** (step 3, no RP-HPLC purification) gave a residue that was taken up in an 8:2 (v/v) mixture of CH_2Cl_2/TFA (10 mL). The mixture was stirred for 2 h, and then the volatiles were removed in vacuo. Trituration of the residue with Et_2O afforded the title compound (122 mg, 97%) as a solid. MS (ES^+) m/z 641 $(M+H)^+$.

Methyl (4*R*)-1-{(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-2-cyclohexylacetyl}-4-({7-methoxy-2-[2-(propan-2-ylamino)-1,3-thiazol-4-yl]quinolin-4-yl}oxy)-L-prolinate (37). Treatment of *N*-Boc-cyclohexylglycine (520 mg, 2.0 mmol) according to the general procedure described for **12a** (step 3, using **35b** in place of **11**, no RP-HPLC purification) gave a residue that was triturated with a 1:2 (v/v) mixture of CH_2Cl_2/Et_2O to furnish the title compound (1.1 g, 84%) as an oily solid that was used without further purification. MS (ES^+) m/z 682 $(M+H)^+$.

(4*R*)-1-{(2*S*)-2-Cyclohexyl-2-[(3,3-dimethylbutyl)amino]acetyl}-*N*-((1*R*,2*S*)-1-{[(cyclopropylsulfonyl)amino]carbonyl}-2-vinylcyclopropyl)-4-({2-[2-(isopropylamino)-1,3-thiazol-4-yl]-7-methoxyquinolin-4-yl}oxy)-L-prolinamide (38). Treatment of **36** (27 mg, 0.08 mmol) according to the general procedure described for **12a** (step 3 using **34** in place of **11**, no RP-HPLC purification) gave a residue that was taken up in a 4:1 (v/v) mixture of CH_2Cl_2/TFA (5 mL). The mixture was stirred for 2 h, and then the volatiles were removed. The residue was purified by RP-HPLC to furnish the title compound (7.5 mg, 12%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6, mixture of two conformers in a ratio 3:1; only signals for the major conformer are reported) δ 10.86 (s, 1H), 8.90 (s, 1H), 8.64 (br s, 2H), 8.33 (s, 1H), 8.12 (br s, 1H), 8.04 (d, J = 9.2 Hz, 1H), 7.91 (d, J = 2.1 Hz, 1H), 7.82 (s, 1H), 7.31 (dd, J = 9.2, 2.1 Hz, 1H), 5.87 (br s, 1H), 5.61 (ddd, J = 17.1, 11.0, 9.2 Hz, 1H), 5.22 (d, J = 17.1 Hz, 1H), 5.12 (d, J = 11.0 Hz, 1H), 4.64 (dd, J = 9.7, 7.6 Hz, 1H), 4.42 (d, J = 12.0 Hz, 1H), 4.32−4.25 (br s, 1H), 4.22−4.13 (m, 2H), 3.96 (s, 3H), 2.98−2.88 (m, 1H), 2.86−2.70 (m, 2H), 2.63−2.35 (m, 2H, partially obscured by residual DMSO signal), 2.43−2.30 (m, 1H), 2.16 (q, J = 8.8 Hz, 1H), 1.97−1.69 (m, 7H), 1.69−1.60 (m, 1H), 1.50−1.40 (m, 2H), 1.38−1.30 (m, 1H), 1.26 (d, J = 6.3 Hz, 3H), 1.25 (d, J = 6.2 Hz, 3H), 1.21−0.99 (m, 7H), 0.64 (s, 9H). Analytical RP-HPLC t_R (conditions C) 5.05 min (peak area 95%), t_R (conditions D) 4.14 min (peak area 91%); HRMS calculated for $C_{44}H_{62}N_7O_7S_2$ $(M+H)^+$ 864.4152, found 864.4182.

***tert*-Butyl {(1*S*)-1-Cyclohexyl-2-[(2*S*,4*R*)-2-({(1*R*,2*S*)-1-[(cyclopropylsulfonyl)carbamoyl]-2-ethenylcyclopropyl}carbamoyl)-4-({7-methoxy-2-[2-(propan-2-ylamino)-1,3-thiazol-4-yl]quinolin-4-yl}oxy)pyrrolidin-1-yl]-2-oxoethyl}carbamate Trifluoroacetate (39).** Treatment of **37** (100 mg, 0.15 mmol) as outlined in the general procedure for **12a** (steps 2 and 3) gave the title compound (100 mg, 80%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.42 (s, 1H), 9.05 (s, 1H), 8.21 (br s, 1H), 8.18 (d, J = 9.2 Hz, 1H), 8.04 (br s, 1H), 7.84 (br s, 1H), 7.75 (s, 1H), 7.28 (d, J = 9.2 Hz, 1H), 7.11 (d, J = 6.9 Hz, 1H), 5.77 (br s, 1H), 5.62 (ddd, J = 17.1, 10.9, 9.2 Hz, 1H), 5.25 (d, J = 17.1 Hz, 1H), 5.12 (d, J = 10.9 Hz, 1H), 4.67 (d, J = 12.1 Hz, 1H), 4.41 (t, J = 7.0 Hz, 1H), 4.22−4.08 (m, 1H), 3.98 (s, 3H), 3.97−3.93 (m, 1H), 3.88−3.80 (m, 2H), 2.99−2.89 (m, 1H),2.70−2.56 (m, 1H), 2.40−2.24 (m, 1H), 2.14 (q, J = 8.8 Hz, 1H),1.91−1.48 (m, 7H), 1.34−1.28 (m, 1H), 1.26 (d, J = 5.8 Hz, 6H), 1.16 (s, 9H), 1.13−0.8 (m, 8H). Analytical RP-HPLC t_R (conditions C) 7.19 min (peak area 97%), t_R (conditions D) 5.75 min (peak area 97%); HRMS calculated for $C_{43}H_{58}N_7O_9S_2$ $(M+H)^+$ 880.3737, found 880.3731.

Supporting Information Available: Synthetic procedures and characterization of compounds **8a**−**j**, **28a**,**b**, and **31**; rat and dog PK curves and parameters for compound **25**. This material is available free of charge via the Internet at http://pubs.acs.org.

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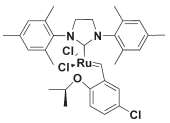

(21) No effort was made to assign the absolute stereochemistry of the diastereoisomers of the ring closing products **10c**−**e**. In all cases the first diastereoisomers of **10c**−**e** eluted during RP-HPLC purification gave rise to the more active inhibitors. Compounds **13a**, **17a**, and **18a** showed 7- to 89-fold improved enzyme inhibition over **13b**, **17b**, and **18b**, respectively. On the basis of our expectation that (12*R*)-stereochemistry is favorable for interaction with NS3 (see Results and Discussion in main text for compounds **12a** and **12b**; note the change of Cahn−Ingold−Prelog priorities for the nonfluorinated analogues **13**, **17**, and **18**), the first eluted isomers of **10c**−**e** (as well as compounds derived from these intermediates) are depicted and named as the (12*R*)-diastereoisomers.

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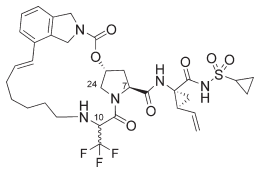

(24) Compounds **35a**,**b** were available through routine protecting group changes from intermediates described in the following published patent application: Rosenquist, A.; Thorstensson, F.; Johansson, P.-O.; Kvarnstrom, I.; Ayesa, S.; Classon, B.; Rakos, L.; Samuelsson, B. HCV NS-3 Serine Protease Inhibitors. WO 2005/073216, 2005.

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