Novel Potent Hepatitis C Virus NS3 Serine Protease Inhibitors Derived from Proline-Based Macrocycles

Kevin X. Chen,* F. George Njoroge, Ashok Arasappan, Srikanth Venkatraman, Bancha Vibulbhan, Weiying Yang, Tejal N. Parekh, John Pichardo, Andrew Prongay, Kuo-Chi Cheng, Nancy Butkiewicz, Nanhua Yao, Vincent Madison, and Viyyoor Girijavallabhan

Schering-Plough Research Institute, 2015 Galloping Hill Road, K-15-3-3545, Kenilworth, New Jersey 07033

Received August 17, 2005

The hepatitis C virus (HCV) NS3 protease is essential for viral replication. It has been a target of choice for intensive drug discovery research. On the basis of an active pentapeptide inhibitor, **1**, we envisioned that macrocyclization from the P2 proline to P3 capping could enhance binding to the backbone Ala156 residue and the S4 pocket. Thus, a number of P2 proline-based macrocyclic α-ketoamide inhibitors were prepared and investigated in an HCV NS3 serine protease continuous assay (K_i*). The biological activity varied substantially depending on factors such as the ring size, number of amino acid residues, number of methyl substituents, type of heteroatom in the linker, P3 residue, and configuration at the proline C-4 center. The pentapeptide inhibitors were very potent, with the C-terminal acids and amides being the most active ones (**24**, K_i* = 8 nM). The tetrapeptides and tripeptides were less potent. Sixteen- and seventeen-membered macrocyclic compounds were equally potent, while fifteen-membered analogues were slightly less active. *gem*-Dimethyl substituents at the linker improved the potency of all inhibitors (the best compound was **45**, K_i* = 6 nM). The combination of *tert*-leucine at P3 and dimethyl substituents at the linker in compound **47** realized a selectivity of 307 against human neutrophil elastase. Compound **45** had an IC_{50} of 130 nM in a cellular replicon assay, while IC_{50} for **24** was 400 nM. Several compounds had excellent subcutaneous AUC and bioavailability in rats. Although tripeptide compound **40** was 97% orally bioavailable, larger pentapeptides generally had low oral bioavailability. The X-ray crystal structure of compounds **24** and **45** bound to the protease demonstrated the close interaction of the macrocycle with the Ala156 methyl group and S4 pocket. The strategy of macrocyclization has been proved to be successful in improving potency (>20-fold greater than that of **1**) and in structural depeptization.

Introduction

Hepatitis C virus (HCV) is a human pathogen affecting nearly 3% of the world's population. The disease was described as a "silent epidemic" because only about 20% of patients develop acute clinical hepatitis when infected with HCV. Most infections progress to a chronic state that can lead to cirrhosis, liver failure, or liver cancer.[1] Currently, the most effective therapies for HCV infection involve treatment with pegylated α-interferon, either alone or in combination with the antiviral agent ribavirin. These therapeutics have limited efficacy with a sustained response rate of only about 50%.[2] They are also accompanied by considerable side effects in certain patients. Hence, more effective antiviral agents for HCV with fewer side effects are in urgent need.

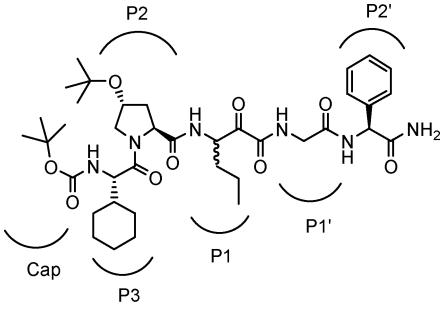
The HCV RNA genome encodes a polyprotein of approximately 3000 amino acids, which must be processed by host and viral proteases into structural and nonstructural (NS) polypeptides. The NS3 protease is located at the N-terminal portion of the NS3 protein. It is responsible for processing four cleavage sites of the nonstructural region and, thus, is essential for viral replication.[3] It has been determined that the HCV NS3 protease belongs to the trypsin or chymotrypsin superfamily of serine proteases.[4] For efficient processing, the protease forms a complex with a small polypeptide cofactor, NS4A.[5] The structure data of the protease have revealed a shallow and solvent-exposed substrate binding region, where the binding energy is mainly derived from weak lipophilic and electrostatic interactions.[6] Despite tremendous difficulty encountered in the process, intensive efforts have been focused on NS3 serine protease, and a number of novel inhibitors have been reported.[7] Among them, two compounds have been progressed into phase I clinical trials in human beings: BILN-2061 from Boehringer-Ingelheim and VX-950 from Vertex Pharmaceuticals.[8]

The cleavage products of the substrates have been frequently used as the starting point for the development of many protease inhibitors. However, these strategies resulted in compounds that are mostly peptidic in nature. It has been difficult to obtain desirable pharmacological profiles because peptides are susceptible to cleavage by peptidases.[9] Various peptidomimetics have been designed to develop drug candidates with less peptide character. Macrocyclization of a substrate has proven to be a promising strategy,[10] with BILN-2061 as a recent example.[8a,b] Many naturally occurring macrocyclic molecules, such as the vancomycin family of antibiotics and chloropeptins,[11] are biologically active. Potentially, macrocycles could offer certain advantages over acyclic peptides as drug candidates. For example, they are conformationally restrained for enhanced binding, and they are less prone to degradation by peptidases.[12]

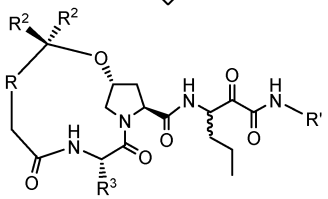
The hexapeptides, *N*-terminal cleavage products from the substrates, have been shown to be competitive inhibitors of the NS3 protease.[13] These peptides have been used as lead compounds and templates in developing more potent and smaller HCV inhibitors. Various inhibitors with diverse structural types have been discovered.[8] One class of compounds employs a reactive electrophile to form a reversible covalent bond with active site serine hydroxyl. These compounds, exemplified by α-ketoacids and ketoamides, have demonstrated their potential as hepatitis C therapeutics.[14] In the course of our search for

* To whom correspondence should be addressed. Phone: (908) 740-7556. Fax: (908) 740-7152. E-mail: kevin.chen@spcorp.com.

10.1021/jm050820s CCC: $33.50
Published on Web 01/07/2006

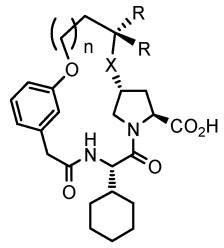


1 Ki* = 220 nM



2

Figure 1. Pentapeptide HCV inhibitor.



3

3a R = H, n = 0, X = O
3b R = H, n = 2, X = O
3c R = H, n = 1, X = S
3d R = H, n = 1, X = SO_2
3e R = H, n = 1, X = NSO_2Ph

Figure 2. Macrocyclic left-hand fragment.

potent HCV inhibitors, we discovered a pentapeptide α-ketoamide, **1** (Figure 1). As a lead, compound **1** shows moderate potency against NS3 protease (K_i* = 220 nM).[15] Modeling studies on the enzyme surface from the X-ray crystal structure[7] revealed the close proximity of the *tert*-butyl group of P2 proline and the Boc capping group. We reasoned that it would be interesting to investigate the potency of P2- and cap-cyclized analogues such as a structure of type **2**. The macrocycle would be expected to provide enhanced binding with the methyl group of Ala156 of the enzyme backbone. We also reckoned that synthesis would be more facile through the use of simple amide capping at P3. To possibly further improve binding in the S4 pocket, the *tert*-butyl group was also replaced with a phenyl group. On the basis of modeling results, we estimated that the appropriate ring size would be between 15 and 17.

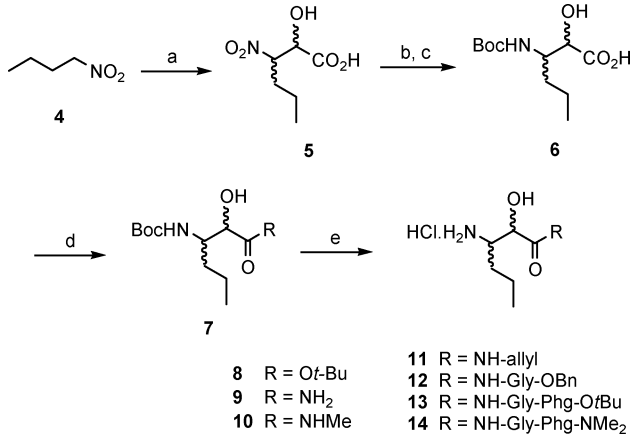
Synthesis of Macrocyclic Inhibitors

The synthesis of the left-hand-side macrocyclic methyl esters starting from 4-hydroxyprolines has been reported in our earlier publications.[16] A number of macrocyclic acid intermediates (**3**, Figure 2) with different ring sizes (15−17), heteroatoms (X = O, N, S), and substituents (R = H, Me) were prepared through hydrolysis of these esters.

The right-hand segments of the inhibitors were prepared from α-hydroxy-β-amino acid **6**, which in turn was synthesized from 1-nitrobutane (**4**) **(**Scheme 1). Thus, when treated with triethylamine, the reaction between **4** and glyoxylic acid afforded compound **5** as a mixture of four diastereomers. The nitro group was reduced to give an amine, which was protected as a Boc-amino acid, **6**. The acid was converted to a *tert*-butyl ester, a primary amide, a methylamide, or an allylamide under ester- or amide-forming conditions. Treatment of intermediates of type **7** with hydrochloric acid removed the Boc protecting group to provide the desired right-hand subunits **8**−**11**. On the other hand, acid **6** was coupled to glycine benzyl ester, glycine−phenylglycine *tert*-butyl ester, or glycine−phenylglycine dimethylamide to give the corresponding di- or tripeptide compounds, which upon removal of the Boc protecting group afforded necessary amine hydrochloride intermediates **12**−**14**.

With both left-hand macrocyclic acids and right-hand mono-, di-, and tripeptide amine intermediates in hand, the stage was set to put together the final target molecules (Scheme 2). Thus, carboxylic acid **15** was coupled to amine hydrochlorides **8** and **10**−**14** under standard conditions (HOOBt, EDC, NMM) to afford a series of α-hydroxyester or α-hydroxyamide intermedi-

Scheme 1[a]

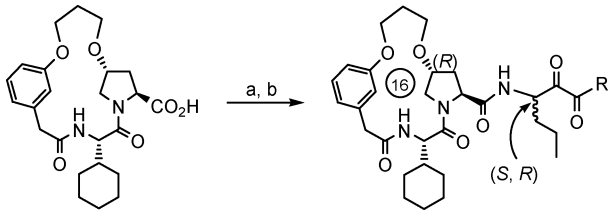


4 **5** **6** **7**

8 R = O*t*-Bu
9 R = NH_2
10 R = NHMe
11 R = NH-allyl
12 R = NH-Gly-OBn
13 R = NH-Gly-Phg-O*t*Bu
14 R = NH-Gly-Phg-NMe_2

[a] Reaction conditions: (a) glyoxylic acid monohydrate, Et_3N, MeOH, 0 °C, then rt, 99%; (b) H_2, 59 psi, Pd−C, AcOH, rt, 66%; (c) NaOH, $(Boc)_2O$, dioxane/H_2O, rt, 89%; (d) (for *tert*-butyl ester) *t*-BuOH, EDC, DMAP, CH_2Cl_2, rt, 34%; (for amides) amide coupling using one of the following amines, NH_4Cl, methylamine hydrochloride, allylamine, glycine benzyl ester hydrochloride, glycine−phenylglycine *tert*-butyl ester hydrochloride, or glycine−phenylglycine dimethylamide hydrochloride, and HOOBt, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 45−95%; (e) 4 M HCl in dioxane for **9**−**12** and **14**, 2 M HCl in EtOAc/dioxane for **8** and **13**, quantitative.

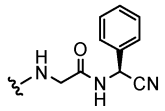
Scheme 2[a]



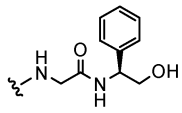
15

16 R = OH
17 R = NHMe
18 R = NH-allyl
19 R = NH-Gly-OBn
20 R = NH-Gly-OH
21 R = NH-Gly-NHBn
22 R = NH-Gly-Phg-O*t*Bu
23 R = NH-Gly-Phg-OH
24 (*S*)-P1, R = NH-Gly-Phg-NMe_2
25 (*R*)-P1, R = NH-Gly-Phg-NMe_2

26 R =

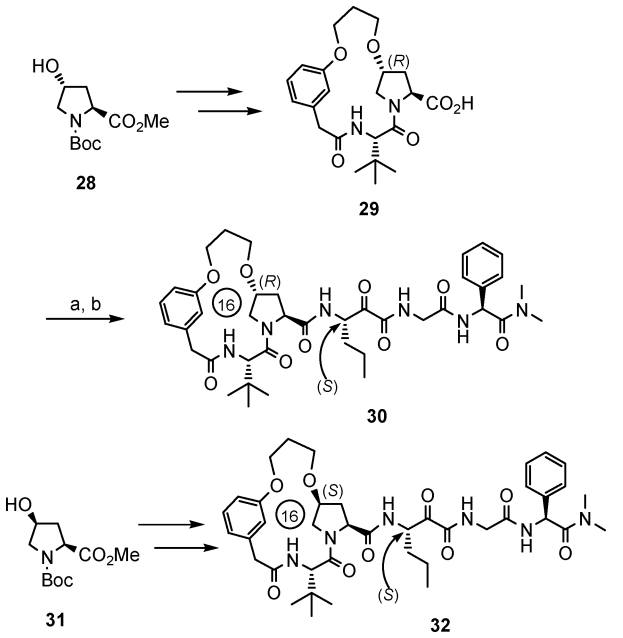


27 R =



[a] Reaction conditions: (a) amine hydrochlorides **8** and **10**−**14**, HOOBt, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 50−95%; (b) Dess−Martin periodinane, CH_2Cl_2, rt, 60−95%; then (for **16**) CF_3CO_2H, CH_2Cl_2, rt, quantitative; (for **20**) **19**, LiOH, THF/MeOH/water, rt; (for **21**, **26**, or **27**) **20**, $BnNH_2$, HCl·$H_2NCH(Ph)CN$, or $H_2NCH(Ph)CH_2OH$, HOOBt, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 85%; (for **23**) **22**, CF_3CO_2H, CH_2Cl_2, rt, quantitative.

Scheme 3[a]



[a] Reaction conditions: (a) **14**, HOOBt, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 69%; (b) Dess−Martin periodinane, CH_2Cl_2, rt, 80%.

ates. These coupling products were then converted to α-ketoesters or α-ketoamides (**17**−**19**, **22**, **24**, **25**) using Dess−Martin periodinane.[17] The α-keto-*tert*-butyl ester was treated with trifluoroacetic acid (TFA) to give the α-ketoacid **16**. The glycine benzyl ester product **19** was hydrolyzed to its corresponding carboxylic acid **20**. Compound **20** was then converted to glycine benzylamide **21**. Treatment of pentapeptide *tert*-butyl ester **22** with TFA provided phenylglycine acid derivative **23**. Finally, coupling of compound **20** with (*S*)-α-aminophenylacetonitrile and phenylglycinol gave α-aminonitrile analogue **26** and *β*-amino alcohol analogue **27**, respectively. The α-ketoester or α-ketoamide products were a mixture of two diastereomers at the P1 α-center, and in most cases, they are inseparable. However, the dimethylamide derivative was separated by flash chromatography to give rise to pure P1 *S*-isomer **24** and *R*-isomer **25**.

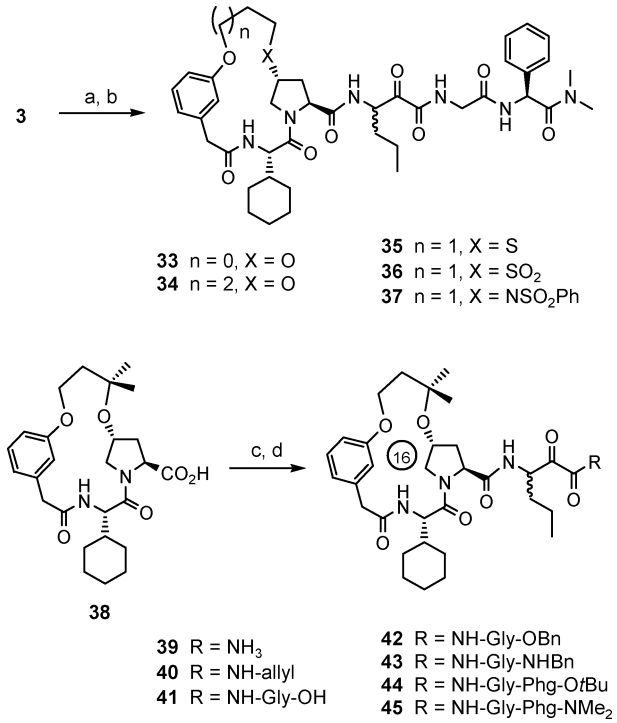
Similarly, when *tert*-leucine was used as the P3 moiety and synthesis carried out as previously described,[16] macrocyclic acid **29** was obtained (Scheme 3). Coupling of acid **29** and amine **14** with subsequent oxidation afforded inhibitor **30**. The same reaction sequence with 4-*cis*-hydroxyproline **31** as the starting material provided the final target **32**. Compounds **30** and **32** were isolated as a single *S*-isomer at the P1 α-center.

To determine the optimal ring size of the macrocycle, 15- and 17-membered rings were also prepared using a previously reported synthetic sequence.[16] The macrocyclic acids were coupled to amine hydrochloride **14** under standard conditions to afford the desired pentapeptide compounds **33** and **34** (Scheme 4).

To explore the potential electronic or substitution effect from the proline C-4 substituent ("X" in Scheme 4), the ether functionality was replaced with a sulfide, sulfone, or sulfonamide moiety. Thus, 16-membered macrocyclic acids with X = S, SO_2, and $NHSO_2Ph$ were coupled to amine **14** to provide, after Dess−Martin periodinane oxidation, α-ketoamide inhibitors **35**−**37** (Scheme 4).

The good potency realized from (*tert*-butyloxy)proline derivative **1** prompted us to investigate the *tert*-alkyl ether linked proline macrocycle. Thus, dimethyl-substituted 16-membered

Scheme 4[a]



[a] Reaction conditions: (a) **14**, HOOBt, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 50−95%; (b) (for **35**) DMSO, Cl_2CHCO_2H, EDC, toluene, rt, 60%; (for all others) Dess−Martin periodinane, CH_2Cl_2, rt, 60−95%; (c) **9**, **11**−**14**, HOOBt, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 48−90%; (d) (for **39**) DMSO, Cl_2CHCO_2H, EDC, toluene, rt, 35%; (for **40**, **42**, and **44**−**45**, Dess−Martin periodinane, CH_2Cl_2, rt, 55−88%; (for **41**) **40**, LiOH, THF/MeOH/water, rt, 98%; (for **43**) **41**, $BnNH_2$, HOOBt, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 80%.

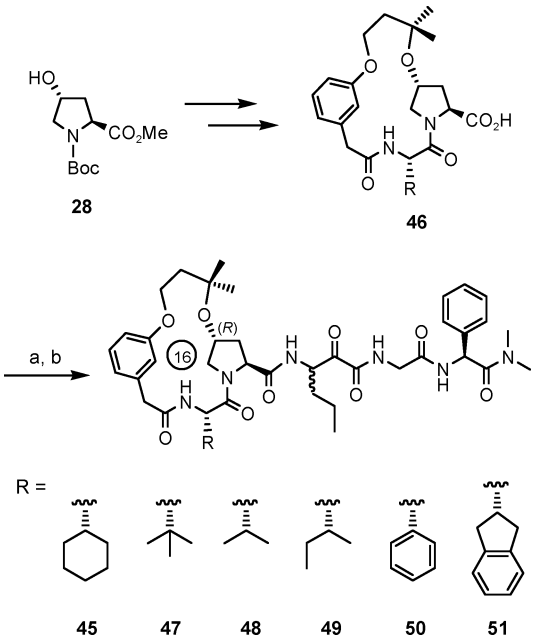
macrocyclic acid **38** was prepared (Scheme 4).[16] The coupling of **38** to amine hydrochlorides **9** and **11**−**14** under standard conditions provided α-hydroxyamide intermediates which were oxidized to the respective ketoamides. Primary ketoamide **39** was obtained through a modified Moffatt oxidation.[21] Secondary ketoamides **40**−**45** were obtained through a Dess−Martin periodinane oxidation.

Variation of the P3 moiety was also of interest. The amino acid residue can be varied readily. A series of macrocyclic acids (**46**, Scheme 5) with a number of P3 hydrocarbon substituents were prepared starting from 4-*trans*-hydroxyproline **28** following previously described procedures.[16] These acids were coupled to amine **14** under standard conditions. The intermediate α-hydroxyamides were oxidized to α-ketoamides **47**−**51** with Dess−Martin periodinane.

Results and Discussion

Sixteen-Membered Macrocyclic HCV Inhibitors: P′-Section SAR. All macrocyclic inhibitors described above were tested in an HCV continuous assay[18] using the NS4A-tethered single-chain NS3 serine protease.[19] The K_i* values in the assay reflected the equilibrium constant determined by the reversible covalent bond formed between the ketone and serine and other interactions between the inhibitors and the enzyme.[20] Most compounds were isolated as an inseparable mixture of two diastereomers at the P1 α-center. The ratio of two isomers varied between 2:1 and 1:2. It should be noted that all our P2′ amides were dimethylamides, instead of a primary amide as in compound **1**. Compound **1** was prepared through a solid-state synthesis. During the course of our SAR study, we found that compounds with dimethylamides were equally potent with

Scheme 5. Inhibitors of HCV NS3 Protease[a]



[a] Reaction conditions: (a) **14**, HOOBt, EDC, NMM, DMF/CH_2Cl_2, −20 °C, 50−95%; (b) Dess−Martin periodinane, CH_2Cl_2, rt, 60−95%.

Table 1. Biological Activity of HCV Inhibitors **16**−**27**, **30**, and **32**

compd	P1−P2 moiety	K_i* (nM)	compd	P1−P2 moiety	K_i* (nM)
16	OH	6100	**23**	NH-Gly-Phg-OH	10
17	NHMe	9900	**24**	NH-Gly-Phg-NMe_2	8[a]
18	NH-allyl	10000	**25**	NH-Gly-Phg-NMe_2	500[b]
19	NH-Gly-OBn	430	**26**	NH-Gly-NHCH(Ph)CN	310
20	NH-Gly-OH	7400	**27**	NH-Gly-NHCH(Ph)-CH_2OH	60
21	NH-Gly-NHBn	1100	**30**	NH-Gly-Phg-NMe_2	15[a]
22	NH-Gly-Phg-O*t*Bu	44	**32**	NH-Gly-Phg-NMe_2	130[a]

[a] Single isomer (*S*) at the P1 α-center. [b] Single isomer (*R*) at the P1 α-center. All others are a mixture of *S*- and *R*-isomers at the P1 α-center.

primary amides.[15] But primary amides were less metabolically stable, which led to poor PK.

Results for compounds **16**−**27** derived from 16-membered macrocycle **15** are presented in Table 1. Two other inhibitors (**30**, **32**) with *tert*-leucine P3 are also included. All but four compounds were tested as P1 diastereomeric mixtures. Compounds **24**, **25**, **30**, and **32** were tested as single isomers. Clearly, the general trend was that larger inhibitors were more potent than the smaller analogues. All tripeptides (**16**−**18**) were modestly active and had high single-digit micromolar K_i*. The results for three tetrapeptides (**19**−**21**) were mixed, with the benzyl ester **19** activity being in the submicromolar range (0.43 μM), that of acid **20** in the high micromolar range, and that of benzylamide **21** in between (1.1 μM). The pentapeptides **22**−**27**, **30**, and **32** were all more potent inhibitors than their truncated analogues. The carboxylic acid **23** and dimethylamide **24** had excellent K_i* (10 and 8 nM, respectively). Compound **24** was a single diastereomer with *S*-configuration at the P1 α-center. The corresponding *R*-diastereomer **25** was much less potent (500 nM), a 63-fold increase in K_i*. Replacement of cyclohexyl with a *tert*-butyl group at P3 slightly increased K_i* of dimethylamide **30** to 15 nM. Other P2′ phenylglycine derivatives such as *tert*-butyl ester **22**, nitrile **26**, and amino alcohol **27** were found to be less potent than their acid and amide counterparts, presumably due to the diminished or loss of hydrogen bond acceptor capability. On the other hand, when the stereocenter at C-4 of the P2 proline was changed from *R* to *S* in **32**, its K_i* increased more than 8-fold to 130 nM. The *R*-substitution was clearly preferred to *S*-substitution.

Table 2. Inhibitors of HCV NS3 Protease

compd	n	ring size	X	K_i* (nM)
33	0	15	O	30[b]
24	1	16	O	8[a]
34	2	17	O	7[a]
35	1	16	S	10[a]
36	1	16	SO_2	3300[b]
37	1	16	NSO_2Ph	36[b]

[a] Single isomer (*S*) at the P1 α-center. [b] Mixture of *S*- and *R*-isomers at the P1 α-center.

Table 3. Inhibitors of HCV NS3 Protease[a]

compd	P1−P2 moiety	K_i* (nM)	compd	P1−P2 moiety	K_i* (nM)
39	NH_2	220	**43**	NH-Gly-NHBn	269
40	NH-allyl	530	**44**	NH-Gly-Phg-O*t*Bu	16
41	NH-Gly-OH	720	**1**	see Figure 1	220
42	NH-Gly-OBn	88	**45**	NH-Gly-Phg-NMe_2	6

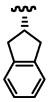
[a] All compounds were a mixture of *S*- and *R*-isomers at the P1 α-center.

Ring Size and P2 Substituent Variations. Compounds with 15- to 17-membered macrocycles were examined to evaluate the effect of ring size on potency (Table 2). As a mixture of two isomers at P1, the 15-membered inhibitor **33**, with a K_i* value of 30 nM, was less potent than 16-membered compound **24** (K_i* = 8 nM), assuming that K_i* of a single isomer is approximately half of that of a mixture. However, 17-membered analogue **34** had an excellent potency of 7 nM, which was similar to that achieved by **24**. These results demonstrated that a 16- or 17-membered ring was the preferred ring size rather than a 15-membered ring.

Table 2 also lists the assay results for compounds **35**−**37**. The linker from proline C-4 was changed from oxygen to sulfur and nitrogen to explore the effects these different heteroatoms would have on potency. The sulfide analogue **35** was nearly as potent (K_i* = 10 nM) as ether analogue **24**. However, introduction of a sulfone moiety as in compound **36** resulted in greatly reduced activity (K_i* = 3300 nM). The phenylsulfonamide derivative **37** was an active inhibitor (36 nM), but not as potent as either the ether or the sulfide analogues. Apparently, more polar functionalities such as sulfone and sulfonamide were less tolerated in this region. The presence of a larger group (phenyl) did not provide any improvement to the potency.

Effect of *gem*-Dimethyl Substitution: *tert*-Alkoxyproline Derivatives. Inspired by *tert*-butoxyproline P2 in compound **1**, a number of *tert*-alkoxy-substituted proline macrocyclic inhibitors were prepared and tested. The enzyme assay results for these compounds are listed in Table 3. The smallest tripeptides, compounds **39** and **40**, were active inhibitors (220 and 530 nM, respectively), realizing an at least 10-fold improvement in activity over analogues of similar size listed in Table 1. The same trend held for tetrapeptide inhibitors **41**−**43**, albeit with a smaller magnitude of improvement (5−10-fold) in activity when compared to compounds **19**−**21**. The larger pentapeptides also demonstrated superiority in K_i*, with that of *tert*-butyl ester **44** being 16 nM and that of dimethylamide **45** being 6 nM. Considering the fact that all these compounds were tested as a mixture of two diastereomers at P1, a 3-fold enhancement in these potencies was achieved in **44** and **45** when compared to the activities of compounds **22** and **24**, respectively. These results have clearly demonstrated that *gem*-dimethyl substituents provided additional binding which contributed to better potency. The magnitude of improvement was more pronounced with

Table 4. SAR at the P3 Position[a]

Compound	P3 Residue	Ki* (nM)
45		6
47		17
48		19
49		70
50		46
51		40

[a] All compounds were a mixture of *S*- and *R*-isomers at the P1 α-center.

Table 5. Results from a Cell-Based Replicon Assay

	24[a]	**30**[a]	**40**[b]	**45**[b]	**47**[b]
K_i* (nM)	8	15	530	6	17
IC_{50} (nM)	400	700	400	130	200

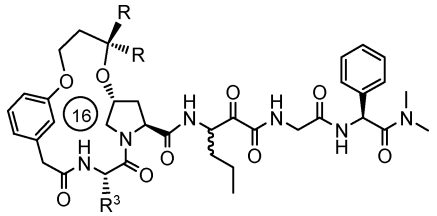
[a] Single isomer (*S*) at the P1 α-center. [b] Mixture of *S*- and *R*-isomers at the P1 α-center.

smaller tri- and tetrapeptide inhibitors (**18**−**21** vs **40**−**43**). A possible explanation is that less potent compounds were more sensitive to any additional binding, thus giving rise to a larger enhancement. Extra binding was not as important to the most potent inhibitors. If compared with acyclic analogue compound **1**, the macrocyclic pentapeptide **45** achieved a 36-fold improvement in potency.

SAR of the P3 Amino Acid Residue. The S3 region of HCV NS3 protease is highly lipophilic. The P3 moiety binds the enzyme mainly through hydrophobic interactions. Six compounds (**45** and **47**−**51**) with P3 side chains of various sizes were examined in an HCV protease continuous assay, and the results are included in Table 4. They were all tested as a mixture of diastereomers at P1. It is evident that cyclohexylglycine remained as the best moiety at the P3 position (**45**, 6 nM). Two other amino acid (*tert*-leucine and valine) derivatives, **47** and **48**, were 2−3-fold less potent (17 and 19 nM, respectively). The analogues derived from isoleucine (**49**), phenylglycine (**50**), and indanylglycine (**51**) were all significantly less active (7−12-fold) than compound **45**. These data suggested that cyclohexylglycine is the amino acid of choice for the P3 position.

Potency of Selected Compounds in a Cell-Based Replicon Assay. To further evaluate the potency and the "in vivo" characteristics of these inhibitors, a few compunds were tested in a replicon-based cellular assay,[22] and the results are included in Table 5. IC_{50}, the concentration required for inhibition of 50% of virus replication, was recorded as a measure of cell-based replicon potency, which is a more accurate reflection of the potency of the inhibitors in a "real" living environment. The IC_{50} values of pentapeptides **24** and **30** were 400 and 700 nM, respectively. However, compounds **45** and **47** were superior with IC_{50} values of 130 and 200 nM, respectively. Apparently, as was observed in the enzyme assay, the *gem*-dimethyl substituents had a positive impact of about 3-fold on replicon potency. Comparing compound **24** with **30**, and **45** with **47**, there was a slight advantage (~2-fold) with a cyclohexyl group at the P3 position rather than a *tert*-butyl group. Finally, the IC_{50} of tripeptide **40** (400 nM) was very intriguing. It was unusual that IC_{50} was similar to its K_i* value. A possible explanation is that smaller molecules such as **40** have better cell permeability than the larger analogues. Overall, the replicon potency of compounds **24** and **40** was in the same range as that of VX-950 (IC_{50} = 350 nM),[8d] and **45** and **47** were more potent than VX-950.

Table 6. HNE Selectivity of Representative HCV NS3 Protease Inhibitors



	24	**45**	**30**	**47**
R	H	Me	H	Me
R^3	cyclohexyl	cyclohexyl	*t*-Bu	*t*-Bu
K_i*$_{HCV}$ (nM)	8[a]	6[b]	15[a]	17[b]
K_i*$_{HNE}$/K_i*$_{HCV}$	46	107	147	306

[a] Single isomer (*S*) at the P1 α-center. [b] Mixture of *S*- and *R*-isomers at the P1 α-center.

Table 7. PK of Selected Compounds

	24	**34**	**45**	**39**	**40**
macrocycle ring size	16	17	16	16	16
AUC(PO) (μM·h)	1.5	0.40	0.46	0.3 0	26.3
AUC(IV) (μM·h)	23.1	5.5	2.2		13.6
F(PO) (%)	3	0.8	2.1		97
AUC(SC) (μM·h)	41.6	34.7	16.5		34.7
F(SC) (%)	90	63	77		

Selectivity of the Inhibitors against Human Neutrophil Elastase. The ubiquitous existence of serine proteases presents a challenge for any protease inhibitor with regard to selectivity. The macrocyclic inhibitors were screened against human neutrophil elastase (HNE), which is a serine protease structurally closely related to HCV NS3 serine protease. Our goal was to achieve a minimum selectivity of 100. Representative data for 16-membered macrocyclic inhibitors are shown in Table 6.

After examination of the HNE inhibition data, an SAR was observed for various combinations of linker dimethyl substituents and P3 residues. When R^3 was cyclohexyl and R was hydrogen, the selectivity (HNE/HCV) of compound **24** was 46 with respect to HNE. The presence of two methyl groups in the linker (**45**, R = Me) improved not only the potency to 6 nM, but also the selectivity to 107. Replacement of cyclohexyl with a *tert*-butyl group at P3 further enhanced the selectivity against HNE. Thus, compounds **30** and **47** had an approximately 3-fold improvement in selectivity over **24** and **45**, respectively. As a mixture of two diastereomers, compound **47** exhibited both good potency and good selectivity.

Pharmacokinetic Properties of Selected Compounds. A number of the macrocyclic HCV protease inhibitors described were evaluated in various PK studies. Results from these studies, on selected compounds, are listed in Table 7. As expected, oral bioavailability was quite low (0.8−3.0%) for the larger pentapeptide compounds **24**, **34**, and **45**. However, rat oral (PO) AUC of compound **24** was reasonable (1.5 μM·h) while IV AUC was excellent (23.1 μM·h). The fact that **24** had very good IV AUC but low PO AUC implied that absorption could be a

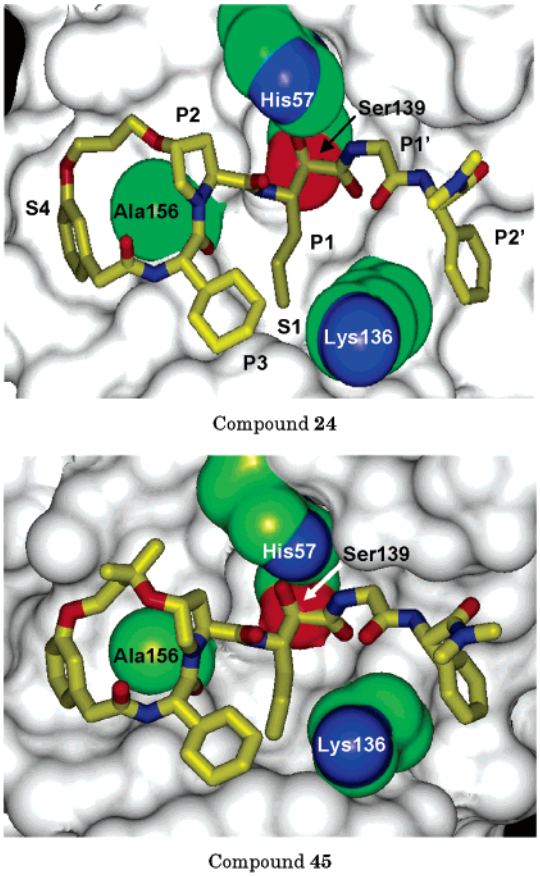


Figure 3. X-ray crystal structures of compounds **24** and **45** bound to HCV NS3 protease.

potential problem due to the size of the molecules. Though smaller in size, the P1 primary ketoamide **39** was among the compounds with very poor PK (rat PO AUC = 0.30 μM·h). Presumably, the stability of the primary amide moiety was an issue in compound **39**. The tripeptide allylamide **40**, however, demonstrated excellent AUC values (PO, 26.3 μM·h; IV, 13.6 μM·h) and an exceptional oral bioavailability of 97%. It was also encouraging to observe that AUCs in rat were all very good (16.5−41.6 μM·h) when the compounds were administered subcutaneously (sc). Excellent subcutaneous bioavailability (63−90%) provides a viable alternative way of administering the compound if it becomes necessary.

X-ray Crystal Structures of Compounds 24 and 45 Bound to Protease. The X-ray crystal structures of two compounds, **24** and **45**, bound to the HCV NS3 protease active site were obtained through soaking the compounds to enzyme crystals (Figure 3). They bound to the enzyme surface with similar conformations. As expected, one of the major interactions was the formation of a reversible covalent bond between the active site serine (Ser139) hydroxyl and the ketone carbonyl of the inhibitor. However, as a result of the nucleophilic attack on the face opposite that usually observed for serine protease, the oxyanion hole was occupied by the P1 carboxylamide carbonyl, rather than by the hemiketal oxygen atom. Instead, the hemiketal oxygen was interacting with and stabilized by a catalytic histidine residue (His57). The macrocycles encircled the methyl group of alanine 156, forming a nice doughnut-shaped crown. The phenyl group in the ring also had good contact within the S4 pocket. Another interesting feature was that the *n*-propyl side chain of the P1 norvaline and the aromatic ring of the P2′ phenylglycine wrapped around the side chain of lysine 136 in such a manner that they formed a C-shaped clamp around it. The P1 *n*-propyl group extended snugly into the S1 pocket. The cyclohexyl moiety fit comfortably in the lipophilic S3 region. In addition, the inhibitor also made multiple hydrogen bonds with the enzyme backbone through its amide chain. The enhancement in potency from the dimethyl substituents seemed to come from the contact between one of the methyl groups and the aromatic ring of the His57 residue.

Both structures demonstrated that the macrocycles had excellent interaction with the protease, especially with the Ala156 methyl group and S4 pocket. The concept of macrocyclization was demonstrated to be successful in achieving compounds with better potency and that were more depeptized. A 36-fold improvement in potency was accomplished from compound **1** to compound **45**. Although the oral bioavailability was still not satisfactory for the most potent and selective compounds, some exciting PK data in the subcutaneous study and from a smaller compound such as **40** indicated that the future is promising for some of these macrocyclic inhibitors.

Conclusion

In summary, a number of P2 proline-based macrocyclic α-ketoamide inhibitors were prepared and investigated in an HCV NS3 serine protease continuous assay. The biological activity varied substantially depending on several factors such as the ring size, number of amino acid residues, number of methyl substituents, type of heteroatom in the linker, P3 residue, and configuration at the proline C-4 center. The pentapeptide inhibitors were very potent, with the C-terminal acid and amide analogues being the most active ones. Single-digit nanomolar potency (K_i*) was achieved with some of the macrocyclic compounds (**45**, 6 nM). The tetrapeptides were less potent, while the tripeptides were only moderately active. The 16- and 17-membered macrocyclic compounds were equally potent, while 15-membered analogues were less active. A sulfide linker from proline C-4 was as effective as an ether linkage. However, the corresponding sulfone and nitrogen analogues were less effective. The dimethyl substituents at the carbon adjacent to the oxygen improved the potency of all inhibitors across the board. Compound **45** had very good potency in a cell-based replicon assay (IC_{50} = 130 nM). The *tert*-leucine at P3 and dimethyl substituents at the linker enhanced the selectivity of the inhibitors against HNE. The combination of these two factors in compound **47** realized a good selectivity of 307. Several compounds had excellent subcutaneous AUC and bioavailability in rats. A less potent smaller inhibitor, **40**, had a remarkable oral bioavailability of 97%. The X-ray crystal structures of compounds **24** and **45** bound to the protease confirmed the excellent potency of these compounds resulted from good binding of the macrocyclic ring with the Ala156 methyl group and S4 pocket. They also revealed the formation of a covalent bond between the inhibitor ketone carbonyl and the Ser139 hydroxyl of the protease. A C-shaped clamping around the Lys136 side chain by *n*-propyl at P1 and phenyl at P2′ was also observed. All these and other interactions contributed to the enhanced activity of these inhibitors. The strategy of macrocyclization has been proved to be successful in both improving potency and structural depeptization.

Experimental Section

General Methods. Reagents and solvents, including anhydrous THF, dichloromethane, and DMF, were purchased from Aldrich or other commercial sources and were used without further purification. Reactions that were moisture sensitive or used anhydrous solvents were performed under either a nitrogen or an argon atmosphere. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel plates obtained from Analtech.

Visualization was accomplished with UV light or by staining with basic $KMnO_4$ solution, ethanolic H_2SO_4, or Vaughn's reagent. Compounds were purified by flash chromatography either on a glass column using Merck silica gel 60 (230−400 mesh) or on an ISCO RediSep disposable silica gel column. NMR spectra were recorded at 300, 400, or 500 MHz for ^{1}H and at 75, 100, or 125 MHz for ^{13}C on a Bruker or Varian spectrometer with $CDCl_3$ or DMSO-d_6 as solvent. The chemical shifts are given in parts per million, referenced to the internal TMS or deuterated solvent signal.

All characterization data for macrocyclic acids, other intermediates, and some inhibitors and general reaction conditions are included in the Supporting Information.

(2-{3-[(12-Cyclohexyl-11,14-dioxo-2,6-dioxa-10,13-diazatricyclo-[14.3.1.1^{7,10}]henicosa-1(19),16(20),17-triene-9-carbonyl)amino]-2-oxohexanoylamino}acetylamino)phenylacetic Acid *tert*-Butyl Ester (22). For detailed reaction conditions for the preparation of the inhibitor, see the Supporting Information. The product **22** (78% yield, two steps) was isolated as a mixture of two diastereomers: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.75 (t, J = 6.0 Hz) and 8.64−8.63 (m, 1H), 8.71 (d, J = 7.0 Hz, 1H), 8.42 (dd, J = 2.4, 9.6 Hz, 1H), 8.26 and 8.08 (d, J = 7.0 and 7.9 Hz, 1H), 7.41−7.33 (m, 5H), 7.15 (app t, J = 7.8 Hz, 1H), 6.80 (s, 1H), 6.72 (app d, J = 7.6 Hz, 1H), 5.27 (dd, J = 4.0, 7.1 Hz, 1H), 5.07−4.97 (m, 1H), 4.45 (dt, J = 3.0, 9.7 Hz, 1H), 4.35 and 4.28 (t, J = 8.5 and 8.6 Hz, 1H), 4.19−3.80 (m, 7H), 3.68 (d, J = 16.9 Hz, 1H), 3.53−3.50 (m, 2H), 2.39−2.32 (m, 1H), 1.84−1.59 (m, 10H), 1.46−1.23 (m, 12H), 1.19−1.06 (m, 3H), 0.91−0.74 (m, 5H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 196.7, 196.6, 171.7, 171.30, 171.25, 170.0, 169.7, 169.41, 169.38, 168.5, 168.4, 167.60, 167.58, 160.9, 160.7, 158.5, 158.4, 138.2, 138.15, 138.12, 136.7, 136.5, 129.7, 128.71, 128.69, 128.6, 128.1, 127.42, 127.35, 127.3, 121.7, 115.4, 112.2, 112.1, 81.32, 81.29, 76.9, 76.8, 64.94, 64.89, 64.87, 63.4, 63.3, 58.1, 57.4, 56.92, 56.87, 56.81, 56.76, 56.7, 54.6, 54.52, 54.45, 53.8, 53.70, 53.67, 53.4, 53.2, 49.6, 41.8, 41.44, 41.39, 33.9, 33.7, 31.7, 29.2, 29.1, 29.0, 28.5, 28.3, 27.5, 26.0, 25.3, 18.7, 18.63, 18.60, 13.82, 13.75, 13.5; HRMS *m*/*z* calcd for $C_{44}H_{61}N_5O_{10}$ (M + H)$^+$ 820.4497, found 820.4493.

(2-{3-[(12-Cyclohexyl-11,14-dioxo-2,6-dioxa-10,13-diazatricyclo-[14.3.1.1^{7,10}]henicosa-1(19),16(20),17-triene-9-carbonyl)amino]-2-oxohexanoylamino}acetylamino)phenylacetic Acid (23). A solution of the *tert*-butyl ester **22** (26 mg, 0.032 mmol) in trifluoroacetic acid (2 mL) and CH_2Cl_2 (2 mL) was stirred at room temperature for 3 h. After the volatiles were removed in vacuo, the residue was dissolved in 50% MeOH−CH_2Cl_2 and concentrated to dryness in vacuo to afford an off-white solid (24 mg, quantitative): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73−8.65 (m, 2H), 8.40 (dd, J = 9.5, 2.6 Hz, 1H), 8.24−8.05 (1 H), 7.64−7.55 (m, 1H), 7.41−7.32 (m, 5H), 7.15 (t, J = 7.8 Hz, 1H), 6.80−6.71 (m, 3H), 5.35 (dd, J = 7.5, 1.9 Hz, 1H), 5.04−4.96 (m, 1H), 4.48−4.43 (m, 1H), 4.37−4.22 (m, 1H), 4.16−3.27 (m, 11H), 2.35−2.31 (m, 1H), 1.84−0.70 (m, 21 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.7, 171.7, 171.4, 171.3, 170.0, 169.7, 167.5, 161.0, 160.7, 158.5, 158.5, 158.4, 138.2, 138.2, 137.1, 137.0, 132.1, 132.1, 131.6, 131.5, 131.5, 129.2, 128.8, 128.7, 128.7, 128.6, 128.0, 127.7, 127.5, 127.5, 121.8, 115.4, 112.2, 76.9, 76.8, 65.0, 64.9, 63.4, 63.3, 58.2, 5 7.4, 56.3, 56.2, 56.2, 54.6, 54.5, 53.8, 53.4, 53.2, 41.5, 41.5, 41.4, 40.2, 33.9, 33.7, 31.9, 31.7, 29.2, 29.0, 28.6, 28.3, 26.1, 25.3, 18.7, 18.6, 13.5; HRMS *m*/*z* calcd for $C_{40}H_{51}N_5O_{10}$ (M + H)$^+$ 762.3714, found 762.3705.

12-Cyclohexyl-11,14-dioxo-2,6-dioxa-10,13-diazatricyclo[14.3.1.1^{7,10}]henicosa-1(19),16(20),17-triene-9-carboxylic Acid ((([1-(Dimethylcarbamoylphenylmethyl)carbamoyl]methyl)aminooxalyl)butyl)amide (24 and 25). The *S*-isomer **24** and *R*-isomer **25** (82% combined yield, 2 steps) were separated by flash chromatography (2−4% MeOH/CH_2Cl_2). Data for **24**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.70 (t, J = 5.2 Hz, 1H), 8.56 (d, J = 7.7 Hz, 1H), 8.42 (d, J = 6.6 Hz, 1H), 8.25 (d, J = 6.6 Hz, 1H), 7.36−7.29 (m, 5H), 7.16−7.13 (m, 1H), 6.79−6.78 (m, 2H), 6.71 (app dd, J = 2.0, 7.9 Hz, 1H), 5.81 (d, J = 7.8 Hz, 1H), 5.00−4.96 (m, 1H), 4.46−4.42 (m, 1H), 4.35 (dd, J = 7.6, 9.8 Hz, 1H), 4.18−3.99 (m, 5H), 3.86−3.74 (m, 2H), 3.67 (app d, J = 15.1 Hz, 1H), 3.53−3.48 (m, 2H), 3.36 (d, J = 15.6 Hz, 1H), 2.92 (s, 3H), 2.84 (s, 3H), 2.33 (dd, J = 7.6, 13.6 Hz, 1H), 1.84−1.59 (m, 10H), 1.47−1.13 (m, 6H), 0.90−0.76 (m, 5H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 196.7, 171.2, 169.69, 169.67, 169.2, 166.9, 160.7, 158.3, 138.2, 137.5, 129.1, 128.6, 127.84, 127.76, 121.7, 115.4, 112.1, 76.9, 64.9, 63.4, 57.4, 54.4, 53.7, 53.3, 53.0, 41.6, 41.3, 36.6, 35.3, 33.7, 31.7, 28.9, 28.5, 28.3, 26.0, 25.3, 18.7, 13.5. HRMS calcd for $C_{42}H_{58}N_6O_9$(M + H)$^+$: 789.4187. Found: 789.4184. Data for **25**: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.75 (t, J = 6.2 Hz, 1H), 8.56 (d, J = 7.5 Hz, 1H), 8.43 (d, J = 9.8 Hz, 1H), 8.07 (d, J = 7.8 Hz, 1H), 7.38−7.29 (m, 5H), 7.15 (t, J = 7.8 Hz, 1H), 6.80 (s, 1H), 6.72 (app d, J = 8.1 Hz, 2H), 5.82 (d, J = 7.7 Hz, 1H), 5.04 (ddd, J = 3.9, 7.9, 9.4 Hz, 1H), 4.78−4.43 (m, 1H), 4.28 (dd, J = 7.9, 9.5 Hz, 1H), 4.20−4.00 (m, 4H), 3.96 (d, J = 10.8 Hz, 1H), 3.81 (t, J = 5.8 Hz, 1H), 3.76 (d, J = 6.3 Hz, 1H), 3.70−3.64 (m, 2H), 3.52−3.48 (m, 1H), 3.39−3.35 (m, 1H), 2.92 (s, 3H), 2.84 (s, 3H), 2.38−2.34 (m, 1H), 1.84−1.59 (m, 10H), 1.43−1.11 (m, 6H), 0.91−0.77 (m, 5H). ^{13}C NMR (125 MHz, DMSO-d_6), δ, 196.6, 171.3, 169.9, 169.6, 169.2, 166.9, 160.9, 158.4, 138.1, 137.4, 129.1, 128.6, 128.3, 127.8, 127.75, 121.7, 115.3, 112.1, 76.7, 64.9, 63.3, 58.1, 54.5, 53.7, 53.1, 53.0, 41.6, 41.4, 36.5, 35.3, 33.8, 31.9, 29.1, 28.5, 28.3, 26.0, 25.3, 18.6, 13.4. HRMS calcd for $C_{42}H_{58}N_6O_9$(M + H)$^+$: 789.4187. Found: 789.4192.

12-Cyclohexyl-11,14-dioxo-2,6-dioxa-10,13-diazatricyclo[14.3.1.1^{7,10}]henicosa-1(19),16(20),17-triene-9-carboxylic Acid ((([1-(Cyanophenylmethyl)carbamoyl]methyl)aminooxalyl)butyl)-amide (26). Compound **26** was prepared through standard coupling of **20** and (*S*)-α-aminophenylacetonitrile hydrochloride according to standard coupling procedures. It was isolated as a mixture of two diastereomers: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.33−9.29 (m, 1H), 8.9 and 8.84 (t, J = 6.0 Hz, 1H), 8.43 (d, J = 10.0 Hz, 1H), 8.27 (d, J = 7.0 Hz) and 8.11 (dd, J = 5.5, 3.0 Hz, 1H), 7.50−7.40 (m, 5H), 7.15 (t, J = 8.0 Hz, 1H), 6.80 (m, 1H), 6.73 (m, 2H), 6.21−6.18(m, 1H), 5.11−5.07 and 5.03−4.99 (m, 1H), 4.46 (dt, J = 3.5, 10 Hz, 1H), 4.36 and 4.30 (t, J = 9.5 and 8.5 Hz, 1H), 4.17−4.15 (m, 4H), 3.88−3.85 (m, 2H), 3.71−3.64 (m, 2H), 3.55−3.49 (m, 2H), 2.34−2.33 (m, 1H), 2.00−1.40 (m, 10H), 1.38−0.98 (m, 7H), 0.91−0.84 (m, 5H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 197.3, 197.2, 172.2, 170.8, 170.6, 168.7, 161.8, 161.7, 159.3, 159.2, 139.1, 139.0, 134.9, 130.0, 129.8, 127.9, 122.6, 119.3, 116.3, 113.0, 77.8, 77.7, 65.8, 64.2, 59.0, 58.3, 55.4, 55.3, 54.6, 54.3, 54.0, 44.3, 44.2, 42.6, 42.5, 42.3, 42.2, 34.7, 34.6, 33.0, 32.6, 30.0, 29.8, 29.4, 29.2, 26.9, 26.2, 19.6, 19.5, 14.4; HRMS *m*/*z* calcd for $C_{40}H_{51}N_6O_8$ (M + H)$^+$ 743.3768, found 743.3773.

12-Cyclohexyl-11,14-dioxo-2,6-dioxa-10,13-diazatricyclo[14.3.1.17,10]henicosa-1(19),16(20),17-triene-9-carboxylic Acid ((([1-(2-Hydroxy-1-phenylethylcarbamoyl)methyl]aminooxalyl)butyl)-amide (27). Compound **27** was prepared through standard coupling of **20** and (*S*)-2-amino-2-phenylethanol: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.71 and 8.65 (t, J = 6.0 and 6.2 Hz, 1H), 8.42 (dd, J = 3.3, 9.3 Hz, 1H), 8.34 (dd, J = 8.4, 11.1 Hz, 1H), 8.27 and 8.08 (d, J = 6.8, 8.2 Hz, 1H), 7.31−7.21 (m, 5H), 7.15 (t, J = 7.7 Hz, 1H), 6.79 (br s, 1H), 6.72 (dd, J = 2.1, 8.1 Hz, 2H), 5.09−4.98 (m, 1H), 4.84 (q, J = 6.8 Hz, 1H), 4.44 (dt, J = 5.5, 9.7 Hz, 1H), 4.35 and 4.28 (dd, J = 7.7, 9.6 and 8.2, 9.4 Hz, 1H), 4.18−4.02 (m, 2H), 3.97−3.83 (m, 2H), 3.81−3.63 (m, 2H), 3.59−3.47 (m, 4H), 3.38−3.35 (m, 1H), 2.35 (ddd, J = 7.5, 13.2, 21.2 Hz, 1H), 1.84−1.59 (m, 10H), 1.48−1.23 (m, 3H), 1.17−1.09 (m, 3H), 0.90−0.78 (m, 5H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 197.53, 197.50, 172.19, 172.16, 170.8, 170.57, 170.55, 168.2, 161.7, 161.5, 159.3, 159.2, 141.79, 141.76, 139.1, 139.0, 130.0, 128.92, 128.90, 127.76, 127.75, 127.66, 122.6, 116.3, 113.0, 77.8, 77.6, 65.81, 65.76, 65.48, 65.46, 64.22, 64.17, 59.0, 58.2, 55.98, 55.95, 55.4, 55.3, 54.62, 54.57, 54.2, 54.0, 42.7, 42.6, 42.3, 42.2, 34.7, 34.6, 32.9, 32.6, 30.5, 30.0, 29.8, 29.4, 29.2, 26.9, 26.2, 19.6, 19.5, 14.40, 14.36; HRMS *m*/*z* calcd for $C_{40}H_{54}N_5O_9$ (M + H)$^+$ 748.3922, found 748.3903.

12-*tert*-Butyl-11,14-dioxo-2,6-dioxa-10,13-diazatricyclo[14.3.1.1^{7,10}]henicosa-1(19),16(20),17-triene-9-carboxylic Acid ((([1-(Dimethylcarbamoylphenylmethyl)carbamoyl]methyl)amino-oxalyl)butyl)amide (30). The products were separated into the

S-isomer and *R*-isomer (57% combined yield, two steps). Data for the *S*-isomer: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.72 (t, J = 6.3 Hz, 1H), 8.57 (d, J = 7.5 Hz, 1H), 8.37 (d, J = 9.8 Hz, 1H), 8.26 (d, J = 6.9 Hz, 1H), 7.38−7.35 (m, 4H), 7.34−7.30 (m, 1H), 7.16 (dd, J = 7.2 and 8.9 Hz, 1H), 6.74−6.71 (m, 3H), 5.83 (d, J = 7.7 Hz, 1H), 5.07−5.03 (m, 1H), 4.69 (d, J = 9.7 Hz, 1H), 4.39 (dd, J = 7.6 and 10.2 Hz, 1H), 4.14−4.04 (m, 3H), 3.97 (d, J = 10.4 Hz, 1H), 3.88−3.75 (m, 3H), 3.54−3.48 (m, 2H), 3.40−3.34 (m, 2H), 2.93 (s, 3H), 2.85 (s, 3H), 2.34−2.30 (m, 1H), 1.87−1.79 (m, 1H), 1.77−1.67 (m, 3H), 1.47−1.35 (m, 3H), 0.95 (s, 9H), 0.86 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 196.6, 171.2, 170.1, 169.6, 169.2, 166.9, 160.6, 158.2, 138.4, 137.4, 129.0, 128.5, 127.8, 127.7, 121.6, 115.1, 111.7, 76.7, 65.0, 63.1, 57.3, 56.2, 54.8, 54.1, 53.1, 53.0, 41.5, 41.2, 36.5, 35.3, 34.9, 33.7, 31.6, 28.5, 26.2, 18.5, 13.4; HRMS *m*/*z* calcd for $C_{40}H_{55}N_6O_9$ $(M + H)^+$ 763.4031, found 763.4047.

12-*tert*-Butyl-11,14-dioxo-2,6-dioxa-10,13-diazatricyclo[14.3.1.1^{7,10}]henicosa-1(19),16(20),17-triene-9-carboxylic Acid ((([1-(Dimethylcarbamoylphenylmethyl)carbamoyl]methyl)aminooxalyl)butyl)amide (32). The macrocyclic methyl ester was prepared according to the published procedures.[16] It was hydrolyzed to its corresponding carboxylic acid. On the basis of ^{1}H NMR analysis, the proline α-center was partially racemized (ca. 20%) during hydrolysis: ^{13}C NMR (DMSO-d_6) δ 172.6, 172.1, 169.6, 159.9, 139.4, 129.3, 122.0, 115.7, 112.8, 74.6, 65.9, 63.7, 59.1, 58.1, 51.6, 43.6, 38.9, 36.5, 30.3, 27.4; HRMS *m*/*z* calcd for $C_{22}H_{31}N_2O_6$ $(M + H)^+$ 419.2182, found 419.2176. The coupling and oxidation were carried out according to general procedures. The *S*-isomer and *R*-isomer were isolated (35% combined yield, two steps). Data for the *S*-isomer: ^{13}C NMR (DMSO-d_6) δ 197.3, 171.7, 170.1, 169.5, 167.8, 161.6, 159.4, 138.7, 138.4, 130.3, 129.5, 128.72, 128.66, 123.3, 116.3, 114.4, 110.0, 76.8, 65.9, 65.1, 58.4, 57.8, 55.8, 53.9, 51.3, 42.6, 42.4, 37.5, 36.2, 35.5, 34.8, 32.43 29.4, 27.5, 19.4, 14.4; HRMS calcd for $C_{40}H_{55}N_6O_9$ $(M + H)^+$ 763.4031, found 763.4040.

11-Cyclohexyl-10,13-dioxo-2,5-dioxa-9,12-diazatricyclo[13.3.1.1^{6,9}]icosa-1(18),15(19),16-triene-8-carboxylic Acid ((([1-(Dimethylcarbamoylphenylmethyl)carbamoyl]methyl)aminooxalyl)butyl)amide (33). Compound **33** was isolated as an inseparable mixture of two diastereomers: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.73 and 8.68 (t, J = 6.1 and 6.0 Hz, 1H), 8.55 (d, J = 7.6 Hz, 1H), 8.46 (t, J = 10.0 Hz, 1H), 8.23 and 7.98 (d, J = 6.6 and 7.9 Hz, 1H), 7.47−7.29 (m, 5H), 7.11 (t, J = 7.8 Hz, 1H), 7.04 and 7.01 (s, 1H), 6.75 (d, J = 7.2 Hz, 1H), 6.72−6.70 (m, 1H), 5.84 and 5.82 (d, J = 7.8 and 7.7 Hz, 1H), 5.04−4.94 (m, 1H), 4.38−4.10 (m, 3H), 4.03 (br s, 1H), 3.88−3.85 (m, 1H), 3.83 (d, J = 6.3 Hz, 1H), 3.79 (d, J = 5.9 Hz, 1H), 3.76−3.72 (m, 1H), 3.65 (d, J = 14.2 Hz, 1H), 3.47−3.40 (m, 2H), 3.24 (d, J = 13.9 Hz, 1H), 2.92 (s, 3H), 2.84 (s, 3H), 2.45 (dd, J = 7.4, 13.4 Hz, 1H), 1.75−1.53 (m, 8H), 1.50−1.24 (m, 3H), 1.22−1.14 (m, 3H), 0.89−0.76 (m, 5H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 196.5, 173.2, 171.2, 170.2, 169.4, 169.2, 169.1, 166.9, 160.6, 159.3, 138.3, 137.4, 128.7, 128.5, 127.8, 127.7, 122.5, 116.0, 113.6, 77.5, 68.6, 67.7, 57.3, 54.4, 53.31, 53.27, 53.2, 53.0, 52.9, 41.8, 41.5, 38.7, 38.5, 36.5, 35.3, 32.9, 31.6, 29.3, 28.0, 26.0, 25.3, 25.2, 18.6, 13.5, 13.4; HRMS *m*/*z* calcd for $C_{41}H_{54}N_6O_9$ $(M + H)^+$ 775.4031, found 775.4048.

5-Cyclohexyl-3,6-dioxo-11,16-dioxa-4,7-diazatricyclo[15.3.1.1^{7,10}]docosa-1(21),17,19-triene-8-carboxylic Acid ((([1-(Dimethylcarbamoylphenylmethyl)carbamoyl]methyl)aminooxalyl)butyl)amide (34). Compound **34** was isolated as the *S*-isomer: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.61 (d, J = 7.2 Hz, 1H), 7.57 (app t, J = 5.2 Hz, 1H), 7.39−7.33 (m, 7H), 7.18 (app t, J = 7.9 Hz, 1H), 6.79−6.70 (m, 3H), 5.85 (d, J = 7.6 Hz, 1H), 5.29−5.25 (m, 1H), 4.70 (app t, J = 8.8 Hz, 1H), 4.48 (dd, J = 7.9 and 9.6 Hz, 1H), 4.10−3.94 (m, 4H), 3.83−3.80 (m, 1H), 3.62−3.54 (m, 3H), 3.50−3.46 (m, 1H), 3.43−3.39 (m, 1H), 3.02 (s, 3H), 2.92 (s, 3H), 2.34−2.29 (m, 1H), 2.23−2.17 (m, 1H), 1.84−1.56 (m, 13H), 1.32−1.00 (m, 7H), 0.84 (t, J = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 195.5, 172.6, 171.5, 170.7, 166.7, 159.5, 159.2, 137.1, 136.9, 130.1, 129.5, 129.0, 128.3, 121.7, 116.9, 111.7, 68.8, 68.6, 58.7, 55.9, 55.1, 54.8, 54.5, 43.9, 42.6, 42.1, 37.3, 36.5, 34.0, 32.7, 29.6, 29.3, 27.1, 26.6, 26.3, 19.2, 14.0; HRMS *m*/*z* calcd for $C_{43}H_{59}N_6O_9$ $(M + H)^+$ 803.4344, found 803.4347.

12-Cyclohexyl-11,14-dioxo-2-oxa-6-thia-10,13-diazatricyclo-[14.3.1.1^{7,10}]henicosa-1(19),16(20),17-triene-9-carboxylic Acid ((([1-(Dimethylcarbamoylphenylmethyl)carbamoyl]methyl)aminooxalyl)butyl)amide (35). Standard coupling of **3c** and **14** provided an α-hydroxyamide intermediate: HRMS *m*/*z* calcd for $C_{42}H_{59}N_6O_8$ $(M + H)^+$ 807.4115, found 807.4103. To a solution of this intermediate (180 mg, 0.220 mmol) in dichloromethane at room temperature were added sequentially DMSO (0.313 mL, 4.40 mmol), DCC (908 mg, 4.40 mmol), and dichloroacetic acid (0.0364 mL, 0.440 mmol). The reaction mixture was stirred overnight (16 h) and was then quenched by addition of a 5% aqueous citric acid solution (5 mL) and MeOH (1 mL), and the resulting mixture was stirred for 30 min. The solid material was filtered off, and the filtrate was washed with saturated aqueous sodium bicarbonate solution and brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash column chromatography using 0−2% methanol in dichloromethane to give the product (105 mg, 60%) as a mixture of diastereomers. One part of the mixture (36 mg) was purified again by column chromatography to provide more polar pure P1 *S*-isomer (8 mg) and its less polar *R*-isomer (6 mg). Data for the *S*-isomer: ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.56 (app t, J = 5.3 Hz, 1H), 7.51−7.49 (m, 1H), 7.41−7.32 (m, 7H), 7.21 (app t,, J = 7.9 Hz, 1H), 7.03 (s, 1H), 6.82−6.77 (m, 2H), 5.85 (d, J = 6.9 Hz, 1H), 5.23−5.19 (m, 1H), 4.61−4.55 (m, 2H), 4.32−4.28 (m, 1H), 4.18−4.13 (m, 1H), 4.06−3.91 (m, 2H), 3.81−3.78 (m, 1H), 3.64−3.61 (m, 2H), 3.47−3.42 (m, 2H), 3.01 (s, 3H), 2.91 (s, 3H), 2.61 (app t, J = 7.1 Hz, 2H), 2.52−2.47 (m, 1H), 2.13−2.04 (m, 1H), 1.92−1.54 (m, 11H), 1.37−1.31 (m, 1H), 1.24−1.15 (m, 1H), 0.90 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 172.4 171.4, 170.4, 169.6, 166.6, 159.1, 137.1, 137.0, 130.2, 129.6, 129.5, 129.0, 128.3, 122.3, 116.3, 114.9, 67.8, 59.2, 56.3, 54.8, 54.6, 54.0, 43.9, 42.9, 42.7, 41.1, 37.3, 36.5, 33.8, 30.0, 29.9, 29.2, 27.2, 26.6, 26.10, 26.06, 19.3, 14.1; HRMS *m*/*z* calcd for $C_{42}H_{57}N_6O_8S$ $(M + H)^+$ 805.3959, found 805.3950.

12-Cyclohexyl-6,6,11,14-tetraoxo-2-oxa-6λ^6-thia-10,13-diazatricyclo[14.3.1 .1^{7,10}]henicosa-1(19),16(20),17-triene-9-carboxylic Acid ((([1-(Dimethylcarbamoylphenylmethyl)carbamoyl]methyl)aminooxalyl)butyl)amide (36). Compound **36** was obtained as a mixture of P1 diastereomers: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.74 (dd, J = 5.8, 10.6 Hz, 1H), 8.54 (d, J = 7.6 Hz, 1H), 8.49−8.45 (m, 1H), 8.33 and 8.10 (d, J = 7.1 and 8.0 Hz, 1H), 7.38−7.29 (m, 5H), 7.22−7.19 (m, 1H), 6.91−6.88 (m, 2H), 6.82−6.80 (m, 1H), 5.82 and 5.81 (d, J = 7.6 and 8.0 Hz, 1H), 4.99 (ddd, J = 3.6, 7.8, 9.3 Hz, 1H), 4.52−4.45 (m, 1H), 4.42−4.32 (m, 2H), 4.27−4.22 (m, 1H), 4.08 (q, J = 5.3 Hz, 1H), 3.98−3.79 (m, 5H), 3.64 (d, J = 14.4 Hz, 1H), 3.17−3.12 (m, 1H), 3.01−2.95 (m, 1H), 2.92 (s, 3H), 2.84 (s, 3H), 2.61−2.56 (m, 1H), 2.19−2.13 (m, 1H), 1.99−1.93 (m, 2H), 1.75−1.60 (m, 7H), 1.49−1.24 (m, 3H), 1.18−1.12 (m, 3H), 0.97−0.84 (m, 5H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 196.4, 196.2, 170.4, 170.3, 170.2, 170.1, 169.28, 169.25, 169.1, 166.8, 160.7, 160.5, 156.95, 156.88, 138.0, 137.9, 137.4, 129.3, 129.2, 128.4, 127.65, 127.60, 127.59, 122.7, 122.6, 115.8, 115.7, 115.4, 65.7, 65.6, 61.0, 60.5, 59.5, 59.0, 58.4, 55.1, 54.7, 53.4, 52.9, 48.4, 47.5, 47.3, 45.44, 45.41, 41.5, 41.4, 41.1, 40.9, 36.4, 35.2, 31.5, 28.9, 28.7, 28.4, 28.3, 28.21, 28.16, 26.9, 25.8, 25.2, 20.6, 19.8, 19.7, 18.5, 18.4, 13.9, 13.30, 13.26; HRMS *m*/*z* calcd for $C_{42}H_{57}N_6O_{10}S$ $(M + H)^+$ 837.3857, found 837.3865.

6-Benzenesulfonyl-12-cyclohexyl-11,14-dioxo-2-oxa-6,10,13-triazatricyclo[14.3.1.1^{7,10}]henicosa-1(19),16(20),17-triene-9-carboxylic Acid ((([1-(Dimethylcarbamoylphenylmethyl)carbamoyl]-methyl)aminooxalyl)butyl)amide (37). Compound **37** was obtained as a mixture of P1 diastereomers: ^{1}H NMR (500 MHz, DMSO-d_6) δ .86 and 8.73 (t, J = 6.3 and 6.2 Hz, 1H), 8.62 and 8.60 (d, J = 7.7 and 7.4 Hz, 1H), 8.30 and 8.25 (d, J = 9.7 and 10.0 Hz, 1H), 8.17 and 8.03 (d, J = 6.8 and 7.8 Hz, 1H), 7.95 (d, J = 8.5 Hz, 1H), 7.82 (d, J = 9.6 Hz, 1H), 7.71−7.60 (m, 3H), 7.38−7.30 (m, 5H), 7.20−7.16 (m, 1H), 6.81 (d, J = 7.0 Hz, 1H), 6.76−6.74 (m, 2H), 5.85 (d, J = 8.8 Hz, 1H), 5.04 and 4.99 (m, 1H), 4.57−

4.48 (m, 2H), 4.40 and 4.30 (t and d, J = 9.9 and 10.9 Hz, 1H), 4.18−4.10 (m, 1H), 3.95−3.74 (m, 3H), 3.63−3.59 (d, 2H), 3.36−3.33 (m, 1H), 3.12−3.01 (m, 3H), 2.94 and 2.93 (s, 3H), 2.853 and 2.850 (s, 3H), 1.88−1.60 (m, 11H), 1.45−1.31 (m, 2H), 1.17−1.08 (m, 3H), 0.89−0.83 (m, 5H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 197.3, 197.1, 172.1, 171.8, 171.51, 171.50, 170.1, 169.9, 169.7, 167.89, 167.87, 161.4, 161.3, 158.0, 157.9, 139.9, 139.8, 139.2, 139.1, 138.4, 133.97, 133.96, 130.6, 130.5, 130.28, 130.27, 129.5, 128.74, 128.69, 128.67, 127.8, 127.7, 123.2, 123.1, 116.7, 113.6, 113.5, 110.0, 65.1, 59.2, 58.2, 55.2, 54.3, 54.1, 53.93, 53.91, 42.69, 42.67, 42.6, 42.5, 37.5, 36.2, 32.4, 32.0, 31.2, 30.9, 30.3, 29.7, 28.8, 27.1, 26.9, 26.5, 26.31, 26.25, 20.0, 19.6, 18.8, 18.7, 14.4, 14.3; HRMS *m*/*z* calcd for $C_{48}H_{62}N_7O_{10}S$ $(M + H)^+$ 928.4279, found 928.4290.

12-Cyclohexyl-5,5-dimethyl-11,14-dioxo-2,6-dioxa-10,13-diazatricyclo[14.3.1.1^{7,10}]henicosa-1(19),16(20),17-triene-9-carboxylic Acid (1-Aminooxalylbutyl)amide (39). Oxidation of intermediate coupling product α-hydroxyamide to **39** through a modified Moffatt oxidation was described above in the preparation of compound **35** (35% yield, two steps): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (br s, 1H), 7.27 (br s, 1H), 7.14 (app t, J = 12.7 Hz, 1H), 6.72−6.65 (m, 4H), 6.51 (t, J = 8.6 Hz, 1H), 4.79 (dd, J = 4.5, 8.5 Hz, 1H), 4.69 (dd, J = 9.8, 17.5 Hz, 1H), 4.27−4.07 (m, 3H), 3.97 (d, J = 11.2 Hz, 1H), 3.70−3.50 (M, 4H), 2.48−2.41 (m, 1H), 2.06−1.96 (m, 2H), 1.84−1.49 (m, 11H), 1.43−1.11 (m, 11H), 0.95−0.90 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.6, 195.5, 172.8, 170.9, 170.5, 170.2, 160.9, 158.9, 156.4, 151.8, 145.8, 136.5, 132.5, 129.5, 121.2, 116.2, 110.5, 76.3, 74.9, 69.3, 63.9, 58.2, 58.1, 56.8, 56.7, 55.4, 55.3, 54.1, 54.0, 53.4, 43.1, 41.8, 41.0, 40.9, 35.8, 33.4, 29.2, 29.1, 29.0, 26.1, 25.8, 25.73, 25.68, 19.0, 18.9, 13.7; HRMS *m*/*z* calcd for $C_{32}H_{47}N_4O_7$ $(M + H)^+$ 599.3445, found 599.3456.

12-Cyclohexyl-5,5-dimethyl-11,14-dioxo-2,6-dioxa-10,13-diazatricyclo[14.3.1.1^{7,10}]henicosa-1(19),16(20),17-triene-9-carboxylic Acid (1-Allylaminooxalylbutyl)amide (40). Product **40** was obtained as a mixture of two P1 diastereomers: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.86 and 8.82 (t, J = 6.0 and 6.0 Hz, 1H), 8.43 (dd, J = 1.9, 9.5 Hz, 1H), 8.29 and 8.19 (d, J = 6.6 and 7.9 Hz, 1H), 7.13 (t, J = 7.9 Hz, 1H), 6.70−6.69 (m, 2H), 6.64 (d, J = 7.6 Hz, 1H), 5.83−5.75 (m, 1H), 5.13−5.05 (m, 3H), 5.02 and 5.4.94 (ddd, J = 3.8, 8.0, 9.6 and 2.7, 5.7, 9.2 Hz, 1H), 4.48 (t, J = 9.6 Hz, 1H), 4.43−4.35 (m, 2H), 4.30−4.24 (m, 1H), 4.10−4.04 (m, 1H), 3.86 (dd, J = 10.7, 19.5 Hz, 1H), 3.77−3.72 (m, 3H), 3.66 and 3.56 (dd, J = 3.6, 10.6 and 3.3, 10.5 Hz, 1H), 3.36 (d, J = 15.5 Hz, 1H), 2.12−2.06 (m, 1H), 1.89−1.80 (m, 2H), 1.78−1.55 (m, 7H), 1.49−1.23 (m, 4H), 1.21−1.12 (m, 8H), 0.95−0.79 (m, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 197.1, 197.0, 171.30, 171.26, 169.72, 169.58, 169.1, 160.9, 160.8, 158.1, 138.25, 138.20, 134.1, 129.1, 121.2, 121.1, 115.49, 115.46, 115.29, 115.27, 110.7, 110.6, 73.99, 73.96, 69.50, 69.47, 63.1, 58.3, 57.7, 54.3, 53.4, 53.1, 41.3, 41.2, 40.8, 40.7, 40.66, 40.59, 40.58, 37.7, 37.6, 36.6, 31.9, 31.5, 28.8, 28.4, 26.2, 26.1, 25.9, 25.3, 25.2, 25.04, 24.98, 24.89, 22.5, 22.3, 22.0, 18.6, 18.5, 13.4; HRMS calcd for $C_{35}H_{51}N_4O_7S$ $(M + H)^+$ 639.3758, found 639.3765.

(2-{3-[(12-Cyclohexyl-5,5-dimethyl-11,14-dioxo-2,6-dioxa-10,13-diazatricyclo[14.3.1.1^{7,10}]henicosa-1(19),16(20),17-triene-9-carbonyl)amino]-2-oxohexanoylamino}acetylamino)phenylacetic Acid *tert*-Butyl Ester (44). Compound **44** was obtained as a mixture of two inseparable P1 diastereomers: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.74−8.67 (m, 2H), 8.42 (d, J = 9.5 Hz, 1H), 8.27 and 8.13 (d, J = 7.0 and 7.8 Hz, 1H), 7.41−7.33 (m, 5H), 7.14−7.11 (m, 1H), 6.70−6.69 (m, 2H), 6.65 (d, J = 7.5 Hz, 1H), 5.27 (dd, J = 3.4, 7.3 Hz, 1H), 5.08−4.98 (m, 1H), 4.51−4.25 (m, 4H), 4.10−4.04 (m, 1H), 3.92−3.78 (m, 3H), 3.74 (d, J = 3.4 Hz, 1H), 3.70−3.64 and 3.58−3.56 (m, 1H), 3.36 (d, J = 15.8 Hz, 1H), 2.14−2.08 (m, 1H), 1.91−1.52 (m, 10H), 1.51−1.38 (m, 2H), 1.341 and 1.336 (s, 9H), 1.27−1.03 (m, 10H), 0.95−0.76 (m, 5H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 196.64, 196.55, 171.30, 171.26, 169.9, 169.7, 169.61, 169.58, 169.4, 169.3, 167.5, 160.8, 160.6, 158.1, 138.3, 138.2, 136.5, 136.4, 133.0, 132.2, 132.0, 129.1, 128.7, 128.6, 128.1, 127.37, 127.35, 121.2, 121.1, 115.30, 115.27, 110.7, 110.6, 81.21, 81.19, 74.6, 74.0, 69.54, 69.48, 63.2, 63.1, 58.4, 57.8, 56.83, 56.79, 55.8, 55.6, 54.4, 54.3, 53.3, 53.0, 41.4, 41.31, 41.28, 41.25, 40.7, 40.6, 37.7, 37.6, 31.9, 31.5, 28.8, 28.6, 28.4, 27.4, 26.2, 26.1, 25.9, 25.25, 25.16, 25.0, 24.9, 18.6, 18.5, 13.4; HRMS *m*/*z* calcd for $C_{46}H_{64}N_5O_{10}$ $(M + H)^+$ 846.4653, found 846.4644.

12-Cyclohexyl-5,5-dimethyl-11,14-dioxo-2,6-dioxa-10,13-diazatricyclo[14.3.1.1^{7,10}]henicosa-1(19),16(20),17-triene-9-carboxylic Acid ((([1-(Dimethylcarbamoylphenylmethyl)carbamoyl]-methyl)aminooxalyl)butyl)amide (45). Compound **45** was obtained as a mixture of two inseparable P1 diastereomers: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (app t, J = 8.5 Hz, 1H), 7.68 and 7.60 (t, J = 4.9 and 4.9 Hz, 1H), 7.45−7.22 (m, 6H), 7.20−6.74 (m, 2H), 6.67−6.64 (m, 3H), 5.86 (d, J = 7.4 Hz, 1H), 5.29 (dt, J = 4.7, 7.6 Hz, 1H), 4.82−4.63 (m, 2H), 4.26−3.93 (m, 6H), 3.64 (s, 2H), 3.56 (dd, J = 3.3, 11.0 Hz, 1H), 2.97 (s, 3H), 2.90 (s, 3H), 2.37−1.97 (m, 3H), 1.86−1.47 (m, 9H), 1.36−0.97 (m, 13H), 0.88 and 0.84 (t, J = 7.1 and 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 195.1, 195.0, 172.6, 172.5, 170.4, 169.4, 166.4, 165.0, 162.6, 159.2, 158.7, 142.5, 136.7, 136.6,129.4, 129.0, 128.8, 128.4, 128.2, 127.7, 127.6, 127.5, 125.4, 121.1, 116.0, 110.5, 103.4, 82.0, 80.2, 74.74, 74.68, 69.4, 63.8, 58.4, 58.3, 56.7, 55.3, 55.1, 54.1, 53.9, 42.8, 42.3, 41.4, 41.3, 41.0, 36.9, 36.4, 36.0, 33.4, 32.5, 30.3, 29.1, 28.9, 28.6, 26.3, 26.1, 26.0, 25.7, 18.8, 13.7; HRMS *m*/*z* calcd for $C_{44}H_{61}N_6O_9$ $(M + H)^+$ 817.4500, found 817.4528.

12-*tert*-Butyl-5,5-dimethyl-11,14-dioxo-2,6-dioxa-10,13-diazatricyclo[14.3.1.1^{7,10}]henicosa-1(19),16(20),17-triene-9-carboxylic Acid [1-(Dimethylcarbamoylphenylmethyl)carbamoyl]methyl)-aminooxalyl)butyl)amide (47). Compound **47** was prepared through the same general procedures as described above, except that Boc-*tert*-butylglycine was substituted for Boc-cyclohexylglycine at P3. It was isolated as a mixture of two diastereomers: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54−7.23 (m, 8H), 7.13 (t, J = 7.7 Hz, 1H), 6.70−6.64 (m, 3H), 6.58 (br s, 1H), 5.80 (d, J = 7.0 Hz, 1H), 5.34−5.20 (m, 1H), 4.92 (t, J = 8.9 Hz, 1H), 4.72 and 4.65 (t, J = 8.9 and 8.9 Hz, 1H), 4.24−3.92 (m, 6H), 3.68 and 3.59 (ABq, J = 15.3 Hz, 1H), 3.47 (dt, J = 2.6, 12.0 Hz, 1H), 2.98 and 2.95 (s, 3H), 2.88 and 2.87 (s, 3H), 2.49−2.33 (m, 1H), 2.09−1.80 (m, 3H), 1.65−1.29 (m, 4H), 1.23 (s, 3H), 1.21 (s, 3H), 1.03 and 1.00 (s, 9H), 0.88 (q, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.9, 197.4, 171.0, 170.5, 170.3, 170.1, 169.1, 167.9, 166.1, 165.9, 159.0, 158.8, 158.7, 145.4, 136.6, 129.4, 129.0, 128.9, 128.5, 127.8, 127.4, 121.10, 121.07, 116.1, 116.0, 110.15, 110.09, 74.8, 69.3, 69.1, 67.9, 63.9, 63.7, 58.4, 57.9, 57.5, 57.4, 57.2, 54.24, 54.17, 43.1, 42.2, 41.0, 40.9, 36.8, 36.3, 36.2, 36.0, 35.6, 33.6, 33.1, 32.5, 26.4, 26.3, 25.9, 25.7, 25.4, 25.1, 18.9, 18.8, 13.63, 13.57; HRMS *m*/*z* calcd for $C_{42}H_{59}N_6O_9$ $(M + H)^+$ 791.4344, found 791.4342.

12-Isopropyl-5,5-dimethyl-11,14-dioxo-2,6-dioxa-10,13-diazatricyclo[14.3.1.17,10]henicosa-1(19),16(20),17-triene-9-carboxylic Acid ((([1-(Dimethylcarbamoylphenylmethyl)carbamoyl]-methyl)aminooxalyl)butyl)amide (48). Compound **48** was prepared by the same general procedures using Boc-valine at P3. It was isolated as a mixture of two diastereomers: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.74 and 8.68 (t, J = 6.2 and 6.2 Hz, 1H), 8.56 (dd, J = 6.1, 7.6 Hz, 1H), 8.50 (d, J = 9.4 Hz, 1H), 8.28 and 8.19 (d, J = 6.9 and 8.2 Hz, 1H), 7.39−7.27 (m, 5H), 7.15−7.12 (m, 1H), 6.71−6.70 (m, 2H), 6.66−6.64 (m, 1H), 5.825 and 5.818 (d, J = 7.5 and 7.6 Hz, 1H), 5.06−5.02 (m, 1H), 4.44−4.25 (m, 4H), 4.11−4.04 (m, 1H), 3.89−3.57 (m, 5H), 3.38 and 3.37 (d, J = 15.4 and 15.1 Hz, 1H), 3.31 (d, J = 3.6 Hz, 1H), 2.92 and 2.90 (s, 3H), 2.84 and 2.83 (s, 3H), 2.12−1.80 (m, 4H), 1.70−1.58 (m, 2H), 1.45−1.14 (m, 9H), 0.89−0.77 (m, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 196.6, 196.5, 171.29, 171.25, 170.0, 169.8, 169.64, 169.61, 169.2, 166.9, 160.8, 158.2, 138.25, 138.18, 137.4, 129.1, 128.5, 127.8, 127.72, 127.70, 121.2, 115.3, 110.8, 73.98, 73.96, 69.5, 63.2, 58.2, 57.7, 55.8, 55.7, 54.8, 53.2, 53.0, 52.9, 41.53, 41.46, 41.35, 41.28, 40.7, 40.6, 37.7, 36.5, 35.3, 31.8, 30.6, 30.5, 28.3, 26.3, 26.2, 26.1, 24.9, 24.8, 19.0, 18.9, 18.8, 18.74, 18.68, 18.6, 18.5, 13.41, 13.37; HRMS *m*/*z* calcd for $C_{41}H_{57}N_6O_9$ $(M + H)^+$ 777.4187, found 777.4171.

12-*sec*-Butyl-5,5-dimethyl-11,14-dioxo-2,6-dioxa-10,13-diazatricyclo[14.3.1.1^{7,10}]henicosa-1(19),16(20),17-triene-9-carboxylic Acid ((([1-(Dimethylcarbamoylphenylmethyl)carbamoyl]-methyl)aminooxalyl)butyl)amide (49). Compound **49** was prepared by the same general procedures using Boc-isoleucine at P3. It was isolated as a mixture of two diastereomers: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.74 and 8.69 (t, J = 6.2 and 6.1 Hz), 8.56 (t, J = 7.1 Hz, 1H), 8.50 (dd, J = 1.5, 9.4 Hz, 1H), 8.28 and 8.20 (d, J = 6.9 and 8.2 Hz, 1H), 7.37−7.28 (m, 5H), 7.13 (dt, J = 1.6, 7.9 Hz, 1H), 6.70−6.69 (m, 2 H), 6.65 (d, J = 7.6 Hz, 1H), 5.82 (d, J = 7.6 Hz, 1H), 5.06−4.99 (m, 1H), 4.48−4.25 (m, 4H), 4.11−4.05 (m, 1H), 3.90 (dd, J = 10.8, 20.6 Hz, 1H), 3.83−3.72 (m, 3H), 3.66 and 3.57 (dd, J = 3.4, 10.8 and 3.2, 10.6 Hz, 1H), 3.37 (d, J = 15.4 Hz, 1H), 2.92 (s, 3H), 2.842 and 2.838 (s, 3H), 2.14−2.08 (m, 1H), 1.90−1.58 (m, 4H), 1.52−1.33 (m, 3H), 1.29−1.01 (m, 9H), 0.89−0.76 (m, 9H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 196.6, 196.5, 171.29, 171.26, 170.1, 169.9, 169.52, 169.51, 169.2, 166.9, 160.8, 160.6, 158.1, 138.23, 138.17, 137.4, 129.1, 128.5, 127.8, 127.71, 127.70, 121.2, 121.1, 115.3, 110.7, 110.6, 74.0, 73.9, 69.6, 69.5, 63.1, 58.3, 57.7, 55.8, 55.6, 54.8, 54.2, 54.1, 53.2, 52.95, 52.93, 41.54, 41.47, 41.3, 41.2, 40.7, 40.6, 37.7, 37.6, 36.5, 36.4, 35.3, 31.8, 31.5, 31.2, 28.3, 26.1, 26.0, 25.0, 24.9, 24.3, 22.0, 18.6, 18.5, 15.0, 14.9, 13.9, 13.41, 13.38, 10.65, 10.63; HRMS m/z calcd for $C_{42}H_{59}N_6O_9$ $(M + H)^+$ 791.4340, found 791.4340.

5,5-Dimethyl-11,14-dioxo-12-phenyl-2,6-dioxa-10,13-diazatricyclo[14.3.1.1^{7,10}]henicosa-1(19),16(20),17-triene-9-carboxylic Acid ((([1-(Dimethylcarbamoylphenylmethyl)carbamoyl]-methyl)aminooxalyl)butyl)amide (50). Compound **50** was prepared by the same general procedures using Boc-phenylglycine at P3. It was isolated as a mixture of two diastereomers: ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.26 (dd, J = 6.4, 9.1 Hz) and 9.20 (t, J = 8.2 Hz, 1H), 8.74−8.70 (m, 1H), 8.68 (t, J = 7.0 Hz, 1H), 8.58−8.54 (m, 1H), 7.37−7.13 (m, 11H), 6.86−6.81 (m, 1H), 6.77−6.67 (m, 2H), 5.92 (dd, J = 5.5, 8.7 Hz, 1H), 8.22 (dd, J = 6.3, 8.0 Hz, 1H), 5.03−4.88 (m, 2H), 4.49−4.06 (m, 4H), 3.95−3.58 (m, 5H), 3.33−3.30 (m) and 3.22 (dt, J = 2.6, 11.9 Hz, 1H), 2.93 and 2.92 (s, 3H), 2.841 and 2.836 (s, 3H), 2.37−2.34 (m, 1H), 1.95−1.62 (m, 4H), 1.44−1.13 (m, 9H), 0.87−0.75 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 196.5, 196.2, 172.0, 171.1, 170.1, 170.0, 169.24, 169.17, 166.87, 166.85, 160.6, 158.53, 158.46, 138.62, 138.59, 138.2, 138.0, 137.42, 137.39, 129.2, 129.1, 128.5, 127.84, 127.79, 127.75, 127.73, 127.70, 127.64, 127.61, 127.4, 127.1, 127.0, 121.6, 121.4, 115.7, 115.3, 111.6, 111.1, 110.8, 74.3, 73.9, 73.8, 69.5, 68.0, 63.9, 63.6, 58.1, 54.8, 53.4, 53.3, 52.9, 41.55, 41.50, 41.47, 41.45, 41.1, 40.9, 40.5, 37.7, 37.6, 36.5, 35.3, 31.4, 31.2, 28.3, 27.2, 27.0, 25.2, 24.7, 24.5, 24.4, 22.0, 18.7, 18.6, 13.9, 13.4; HRMS calcd for $C_{44}H_{55}N_6O_9$ $(M + H)^+$ 811.4031, found 811.4026.

12-Indan-2-yl-11,14-dioxo-2,6-dioxa-10,13-diazatricyclo[14.3.1.1^{7,10}]henicosa-1(19),16(20),17-triene-9-carboxylic Acid ((([1-(Dimethylcarbamoylphenylmethyl)carbamoyl]methyl)aminooxalyl)butyl)amide (51). Compound **51** was prepared by the same general procedures using Boc-2-indanylglycine at P3. It was isolated as a mixture of two diastereomers: ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.78 and 8.73 (t and m, 2H), 8.59 and 8.58 (d, J = 6.0 and 5.6 Hz, 1H), 8.31 and 8.13 (d, J = 7.2 and 8.0 Hz, 1H), 7.65−7.61 and 7.58−7.54 (m, 1H), 7.38−7.31 (m, 5H), 7.20−7.10 (m, 4H), 6.75−6.72 (m, 2H), 6.68 (t, J = 7.9 Hz, 1H), 5.84 and 5.82 (d, J = 3.8 and 3.4 Hz, 1H), 5.11−5.07 and 5.04−5.00 (m, 1H), 4.71 and 4.66 (t, J = 9.6 and 9.6 Hz, 1H), 4.44 and 4.32 (t and m, J = 8.4 Hz, 1H), 4.12−4.05 (m, 1H), 3.85−3.68 (m, 5H), 3.58−3.53 (m, 1H), 3.44−3.41 (m, 1H), 2.98−2.90 (m, 1H), 2.93 and 2.92 (s, 3H), 2.88−2.79 (m, 1H), 2.86 and 2.85 (s, 3H), 2.76−2.69 (m, 2H), 2.12−2.06 (m, 1H), 1.89−1.84 (m, 2H), 1.76−1.68 (m, 1H), 1.66−1.62 (m, 1H), 1.50−1.38 and 1.32−1.28 (m, 3H), 1.19 and 1.18 (s, 3H), 1.17 and1.15 (s, 3H), 0.91−0.87 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 197.7, 172.3, 170.9, 170.8, 170.4, 170.3, 170.1, 167.8, 161.8, 161.6, 159.3, 159.2, 143.4, 143.2, 139.0, 138.9, 138.4, 138.4, 132.4, 132.3, 130.1, 129.7, 129.6, 129.5, 128.7, 128.7, 127.0, 127.0, 127.0, 125.3, 125.3, 125.2, 125.1, 122.3, 122.2, 116.3, 112.4, 75.0, 73.2, 70.5, 70.3, 64.6, 64.5, 64.2, 58.8, 54.4, 54.22, 54.17, 54.1, 53.9, 53.9, 42.4, 41.7, 37.5, 36.2, 36.2, 32.6, 32.4, 27.8, 27.4, 25.8, 25.7, 19.7, 19.6, 14.4, 14.4; HRMS (FAB) m/z calcd for $C_{47}H_{59}N_6O_9$ 851.4344 $(M + H)^+$, found 851.4149.

Acknowledgment. We thank the Structural Chemistry Department at the Schering-Plough Research Institute for NMR and MS analysis for all new compounds. We also thank Ms. Wanli Wu and Regina Huelgas for their assistance in the preparation and characterization of some compounds.

Supporting Information Available: Compound characterization data for intermediates and general procedures for hydrolysis and amide coupling. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Cohen, J. The Scientific Challenge of Hepatitis C. *Science* **1999**, *285*, 26−30. (b) Alter, M. J.; Kruszon-Moran, D.; Nainan, O. V.; McQuillan, G. M.; Gao, F.; Moyer, L. A.; Kaslow, R. A.; Margolis, H. S. The Prevalence of Hepatitis C Virus Infection in The United States, 1988 Through 1994. *N. Engl. J. Med.* **1999**, *341*, 556−562. (c) Cuthbert, J. A. Hepatitis C: Progress and Problems. *Clin. Microbiol. Rev.* **1994**, *7*, 505−532.

(2) (a) Neumann, A. U.; Lam, N. P.; Dahari, H.; Gretch, D. R.; Wiley: T. E.; Layden, T. J.; Perelson, A. S. Hepatitis C Virus Dynamics *in vivo* and the Antiviral Efficacy of Interferon-α Therapy. *Science* **1998**, *282*, 103−107. (b) Rosen, H. R.; Gretch, D. R. Hepatitis C Virus: Current Understanding and Prospects for Future Therapies. *Mol. Med. Today* **1999**, *5*, 393−399. (c) Di Bisceglie, A. M.; McHutchison, J.; Rice, C. M. New therapeutic strategies for hepatitis C. *Hepatology* **2002**, *35*, 224−231.

(3) (a) Lindenbach, B. D.; Rice, C. M. Unravelling hepatitis C virus replication from genome to function. *Nature* **2005**, *436*, 933−938. (b) Kolykhalov, A. A.; Mihalik, K.; Feinstone, S. M.; Rice, C. M. Hepatitis C Virus-Encoded Enzymatic Activities and Conserved RNA Elements in the 3′ Nontranslated Region Are Essential for Virus Replication In Vivo. *J. Virol.* **2000**, *74*, 2046−2051.

(4) Lesk, A. M.; Fordham, W. D. Conservation and Variability in the Structure of Serine Proteinase of the Chymotrypsin Family. *J. Mol. Biol.* **1996**, *258*, 501−537.

(5) De Francesco, R.; Steinkuhler, C. Structure and Function of the Hepatitis C Virus NS3−NS4A Protease. *Curr. Top. Microbiol. Immunol.* **2000**, *242*, 149−169.

(6) (a) Yan, Y.; Li, Y.; Munshi, S.; Sardana, V.; Cole, J. L.; Sardana, M.; Steinkuehler, C.; Tomei, L.; De Francesco, R.; Kuo, L. C.; Chen, Z. Complex of NS3 protease and NS4A Peptide of BK strain Hepatitis C Virus: a 2.2 A resolution structure in a hexagonal crystal form. *Protein Sci.* **1998**, *7*, 837−847. (b) Di Marco, S.; Rizzi, M.; Volpari, C.; Walsh, M. A.; Narjes, F.; Colarusso, S.; De Francesco, R.; Matassa, V. G.; Sollazzo, M. Inhibition of Hepatitis C Virus NS3/NS4A Protease: The Crystal Structures of Two Protease-Inhibitor Complexes. *J. Biol. Chem.* **2000**, *275*, 7152−7157.

(7) (a) De Francesco, R.; Migliaccio, G. Challenges and Successes in Developing New Therapies for Hepatitis C. *Nature* **2005**, *436*, 953−960. (b) Tan, S.-L.; He, Y.; Huang, Y.; Gale, M., Jr. Strategies for Hepatitis C Therapeutic Intervention: Now and Next. *Curr. Opin. Pharmacol.* **2004**, *4*, 465−470. (c) Malancona, S.; Colarrusso, S.; Ontoria, J. M.; Marchetti, A.; Poma, M.; Stansfield, I.; Laufer, R.; Marco, A. D.; Taliani, M.; Verdirame, M.; Gonzalez-paz, O.; Matassa, V. G.; Narjes, F. SAR and Pharmacokinetics Studies on Phenethylamide Inhibitors of the Hepatitis C Virus. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4575−4579. (d) Llinas-Brunet, M.; Bailey, M. D.; Ghiro, E.; Gorys, V.; Halmos, T.; Poirier, M.; Rancourt, J.; Goudreau, N. A Systematic Approach to the Optimization of Substrate-Based Inhibitors of the Hepatitis C Virus NS3 Protease: Discovery of Potent and Specific Tripeptide Inhibitors *J. Med. Chem.* **2004**, *47*, 6584−6594. (e) Andrews, D. M.; Barnes, M. C.; Dowle, M. D.; Hind, S. L.; Johnson, M. R.; Jones, P. S.; Mills, G.; Patikis, A.; Pateman, T. J.; Redfern, T. J.; Ed Robinson, J.; Slater, M. J.; Trivedi, N. Pyrrolidine-5,5-*trans*-Lactams. 5. Pharmacokinetic Optimization of Inhibitors of Hepatitis C Virus NS3/4A Protease. *Org. Lett.* **2003**, *5*, 4631−4634. (f) Zhang, X. Inhibitors of Hepatitis C−A Review of the Current Patent Literature. *Idrugs* **2002**, *5*, 154−158. (g) Dymock, B. W. Emerging Therapies for Hepatitis C Virus Infection. *Expert Opin. Emerging Drugs* **2001**, *6*, 13−42. (h) Dymock, B. W.; Jones, P. S.; Wilson, F. X. Novel Approaches to the Treatment of Hepatitis C Virus. *Antiviral Chem. Chemother.* **2000**, *11*, 79−96. (i) Zhang, R.; Durkin, J. P.; Windsor, W. T. Azapeptides as Inhibitors of the Hepatitis C Virus NS3 Serine Protease. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1005−1008. (j) Ingallinella, P.; Fattori, D.; Altamura, S.;

Steinkuhler, C.; Koch, U.; Cicero, D.; Bazzo, R.; Cortese, R.; Bianchi, E.; Pessi, A. Prime Site Binding Inhibitors of a Serine Protease: NS3/4A of Hepatitis C Virus. *Biochemistry* **2002**, *41*, 5483−5492. (k) Beevers, R.; Carr, M. G.; Jones, P. S.; Jordan, S.; Kay, P. B.; Lazell, R. C.; Raynham, T. M. Solution and Solid-Phase Synthesis of Potent Inhibitors of Hepatitis C Virus NS3 Protease. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 641−643.

(8) For BILN-2061, see: (a) Lamarre, D.; Anderson, P. C.; Bailey, M.; Beaulieu, P.; Bolger, G.; Bonneau, P.; Bös, M.; Cameron, D. R.; Cartier, M.; Cordingley, M. G.; Faucher, A.-M.; Goudreau, N.; Kawai, S. H. Kukolj, G.; Lagacé, L.; LaPlante, S. R. Narjes,H.; Poupart, M.-A Rancourt, J.; Sentjens, R. E.; George, T, S. Simoneau, B.; Steinmann, G.; Thibeault, D.; Tsantrizos, Y. S. Weldon, S. M.; Yong, C-L.; and Llinàs-Brunet M. An NS3 protease inhibitor with antiviral effects in humans infected with hepatitis C virus *Nature* **2003**, *426*, 186−189. (b) Tsantrizos, Y. S.; Bolger, G.; Bonneau, P.; Cameron, D. R.; Gordreau, N.; Kukolj, G.; LaPlante, S. R.; Llinas-Brunet, M.; Nar, H.; Lamarre, D. Macrocyclic Inhibitors of the NS3 Protease as Potential Therapeutic Agents of Hepatitis C Virus Infection. *Angew. Chem., Int. Ed*. **2003**, *42*, 1355−1360. For VX-950, see: (c) Yip, Y.; Victor, F.; Lamar, J.; Johnson, R.; Wang, Q.; May; G.; John I.; Yumibe, N.; Wakulchik, M.; Munroe, J.; Chen, S.-H. P4 and P1′ optimization of bicycloproline P2 bearing tetrapeptidyl α-ketoamides as HCV protease inhibitors. *Bioorg. Med. Chem. Lett*. **2004**, *14*, 5007−5011. (d) Perni, R. The Discovery of VX-950, an Inhibitor of the HCV NS3-4A Protease in Clinical Development. Gordon Research Conference, New London, NH, Aug 7−12, 2005.

(9) West, M. L.; Fairlie, D. P. Targeting HIV-1 protease: A Test of Drug-Design Methodologies. *Trends Pharm. Sci*. **1995**, *16*, 67−75.

(10) Fairlie, D. P.; West, M. L.; Wong, A. K. Towards Protein Surface Mimetics. *Curr. Med. Chem*. **1998**, *5*, 29−62.

(11) Boger, D. L.; Kim, S. H.; Miyazaki, S.; Strittmatter, H.; Weng, J.-H.; Mori, Y.; Rogel, O.; Castle, S. L.; McAtee, J. J. Total Synthesis of the Teicoplanin Aglycon. *J. Am. Chem.* Soc. **2000**, *122*, 7416−7417. (b) Evans, D. A.; Wood, M. R.; Trotter, B. W.; Richardson, T. I.; Barrow, J. C.; Katz, J. L. Total Syntheses of Vancomycin and Eremomycin Aglycons. *Angew. Chem., Int. Ed.* **1998**, *37*, 2700−2704. (c) Evans, D. A.; Dinsmore, C. J.; Watson, P. S.; Wood, M. R.; Richardson, T. I.; Trotter, B. W.; Katz, J. L. Nonconventional Stereochemical Issues in the Design of the Synthesis of the Vancomycin Antibiotics: Challenges Imposed by Axial and Nonplanar Chiral Elements in the Heptapeptide Aglycons *Angew. Chem., Int. Ed*. **1998**, *37*, 2704−2708. (e) Rama Rao, A.; Garjar, M.; Reddy, K.; Rao, A. Studies directed toward the synthesis of vancomycin and related cyclic peptides. *Chem. Rev.* **1995**, *95*, 2135−2167. (f) Mtsuzaki, K.; Ikeda, H.; Ogino, T.; Matsumoto, A.; Woodruff, H. B.; Tanaka, H.; Omura, S. Chloropeptins I and II, Novel Inhibitors Against gp120-CD4 Binding from *Streptomyces* sp. *J. Antibiot.* **1994**, *47*, 1173−1174. (g) Matsuzaki, K.; Ogino, T.; Sunazuka, T.; Tanaka, H.; Omura, S. Chloropeptins, New Anti-HIV Antibiotics Inhibiting gp120-CD4 Binding from *Streptomyces* sp. II. Structure Elucidation of Chloropeptin I. *J. Antibiot.* **1997**, *50*, 66−69.

(12) McGeary, R. P.; Fairlie, D. P. Macrocyclic Peptidomimetics: Potential for Drug Development. *Curr. Opin. Drug Discovery Dev.* **1998**, *1*, 208−217.

(13) (a) Llinas-Brunet, M.; Bailey, M. D.; Fazal, G.; Goulet, S.; Halmos, T.; LePlante, S.; Maurice, R.; Poirier, M.; Poupart, M.-A.; Thibeault, D.; Wernic, D.; Lamarre, D. Peptide-Based Inhibitors of the Hepatitis C Virus Serine Protease. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 1713−1718. (b) Steinkuhler, C.; Biasiol, G.; Brunetti, M.; Urbani, A.; Koch, U.; Cortese, R.; Pessi, A.; De Francesco, R. Product Inhibition of the Hepatitis C Virus NS3 Protease. *Biochemistry* **1998**, *37*, 8899−8905.

(14) (a) Lin, C.; Lin, K.; Luong, Y.-P.; Rao, B. G.; Wei, Y.; Brennan, D. L.; Fulghum, J. R.; Hsiao, H.-M.; Ma, S.; Maxwell, J. P.; Cottrell, K. M.; Perni, R. B.; Gates, C. A.; Kwong, A. D. *In Vitro* Resistance Studies of Hepatitis C Virus Serine Protease Inhibitors, VX-950 and BILN 2061. *J. Biol. Chem.* **2004**, *279*, 17508−17514. (b) Sun, D. X.; Liu, L.; Heinz, B.; Kolykhalov, A.; Lamar, J.; Johnson, R. B.; Wang, Q. M.; Yip, Y.; Chen, S.-H. P4 Cap Modified Tetrapeptidyl α-Ketoamides as Potent HCV NS3 Protease Inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4333−4338. (c) Yip, Y.; Victor, F.; Lamar, J.; Johnson, R.; Wang, Q. M.; Glass, J. I.; Yumibe, N.; Wakulchik, M.; Munroe, J.; Chen, S.-H. P4 and P1′ Optimization of Bicycloproline P2 Bearing Tetrapeptidyl α-Ketoamides as HCV Protease Inhibitors. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5007−5011. (d) Perni, R. B.; Farmer, L. J.; Cottrell, K. M.; Court, J. J.; Courtney, L. F.; Deininger, D. D.; Gates, C. A.; Harbeson, S. L.; Kim, J. L.; Lin, C.; Lin, K.; Luong, Y.-P.; Maxwell, J. P.; Murcko, M. A.; Pitlik, J.; Rao, B. G.; Schairer, W. C.; Tung, R. D.; Van Drie, J. H.; Wilson, K.; Thomson, J. A. Inhibitors of Hepatitis C Virus NS3/4A Protease. Part 3: P2 Proline Variants. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1939−1942.

(15) Schering-Plough Research Institute. Unpublished results.

(16) (a) Chen, K. X.; Arasappan, A.; Njoroge, F. G.; Vibulbhan, B.; Buevich, A.; Chan, T.-M.; Girijavallabhan, V. Syntheses of Novel 4-*tert*-Alkyl Ether Proline-Based 16- and 17-Membered Macrocyclic Compounds. *J. Org. Chem.* **2002**, *67*, 2730−2733. (b) Arasappan, A.; Chen, K. X.; Njoroge, F. G.; Parekh, T. N.; Girijavallabhan, V. Novel Dipeptide Macrocycles from 4-Oxo, -Thio, and -Amino-Substituted Proline Derivatives. *J. Org. Chem.* **2002**, *67*, 3923−3926.

(17) Dess, D. B.; Martin, J. C. A Useful 12-I-5 Triacetoxyperiodinane (the Dess−Martin Periodinane) for the Selective Oxidation of Primary or Secondary Alcohols and a Variety of Related 12-I-5 Species. *J. Am. Chem. Soc.* **1991**, *113*, 7277−7287.

(18) Zhang, R.; Beyer, B. M.; Durkin, J.; Ingram, R.; Njoroge, F. G.; Windsor, W. T.; Malcolm, B. A. A Continuous Spectrophotometric Assay for the Hepatitis C Virus Serine Protease. *Anal. Biochem.* **1999**, *270*, 268−275. The substrate Ac-DTEDVVP(Nva)-O-PAP was used in the present study.

(19) Taremi, S. S.; Beyer, B.; Maher, M.; Yao, N.; Prosise, W.; Weber, P. C.; Malcolm, B. A. Construction, Expression, and Characterization of a Novel Fully Activated Recombinant Single-Chain Hepatitis C Virus Protease. *Protein Sci.* **1998**, *7*, 2143−1249.

(20) For a definition of K_i* and discussions, see: Morrison, J. F.; Walsh, C. T. In *Advances in Enzymology*; Meister, A., Ed.; 1988; Vol. 61, pp 201−301.

(21) (a) Tidwell, T. T. Oxidation of Alcohols to Carbonyl Compounds via Alkoxysulfonium Ylides: The Moffatt, Swern and Related Oxidations. *Org. React.* **1990**, *39*, 297. (b) Epstein, W. W.; Sweat, F. W. Dimethyl Sulfoxide Oxidations. *Chem. Rev.* **1967**, *67*, 247−260.

(22) (a) Chung, v.; Carroll, A. R.; Gray, N. M.; Parry, N. R.; Thommes, P. A.; Viner, K. C.; D′Souza, E. A. Development of Cell-Based Assays for In Vitro Characterization of Hepatitis C Virus NS3/4A Protease Inhibitors. *Antimicrob. Agents Chemother*. **2005**, *49*, 1381−1390. (b) Blight, K. J.; Kolykhalov, A. A.; Rice, C. M. *Science* **2000**, *290*, 1972−1974. (c) Lohmann, V.; Körner, F.; Koch, J.-O.; Herian, U.; Theilmann, L. Bartenschlager, R. Replication of subgenomic Hepatitis C virus RNAs in a Hepatoma cell line. *Science* **1999**, *285*, 110−113.

JM050820S