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Acyl Sulfonamides as Potent Protease Inhibitors of the Hepatitis C Virus Full-Length NS3 (Protease-Helicase/NTPase): A Comparative Study of Different C-Terminals

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Abstract—Synthesis and inhibitory potencies of three types of protease inhibitors of the hepatitis C virus (HCV) full-length NS3 (protease-helicase/NTPase) are reported: (i) inhibitors comprising electrophilic serine traps (pentafluoroethyl ketones, α-keto acids, and α-ketotetrazoles), (ii) product-based inhibitors comprising a C-terminal carboxylate group, and (iii) previously unexplored inhibitors comprising C-terminal carboxylic acid bioisosteres (tetrazoles and acyl sulfonamides). Bioisosteric replacement with the tetrazole group provided inhibitors equally potent to the corresponding carboxylates, and substitution with the phenyl acyl sulfonamide group yielded more potent inhibitors. The hexapeptide inhibitors Suc-Asp-D-Glu-Leu-Ile-Cha-Nva-$NHSO_2$Ph and Suc-Asp-D-Glu-Leu-Ile-Cha-ACPC-$NHSO_2$Ph with K_i values of 13.6 and 3.8 nM, respectively, were approximately 20 times more potent than the corresponding inhibitors with a C-terminal carboxylate and were comparable to the carboxylate-based inhibitor containing the native cysteine, Suc-Asp-D-Glu-Leu-Ile-Cha-Cys-OH ($K_i = 28$ nM). The acyl sulfonamide group constitutes a very promising C-terminal functionality that allows for prime site optimization.

Introduction

It is estimated that the Hepatitis C Virus (HCV), first identified in 1989, has infected more than 170 million people globally, which is almost five times as many as infected by the HIV virus.[1,2] Acute HCV infection results in chronic hepatitis in approximately 80% of the cases, and can eventually progress into liver cirrhosis, hepatocellular carcinoma or liver failure.[2–4] Thus, HCV is the primary reason for liver transplantation in the western countries.[4,5] Approved therapies are based on interferon-α (IFN-α) and more recently pegylated IFN-α in combination with ribavirin. There is an urgent need for more effective and safe therapies, especially as no vaccine is yet on the market.[6,7]

The HCV genome is a single stranded RNA of positive polarity, which encodes the polyprotein NH_2-C-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-COOH, that is subsequently processed into ten mature viral proteins by proteolytic enzymes.[8–10] The non-structural protein 3 (NS3) is a bi-functional enzyme, of which the first N-terminal 180 amino acids possess protease activity and the remaining 450 amino acids RNA helicase/NTPase activity. The NS3 protease is responsible for auto-catalytic *cis* cleavage at the NS3-NS4A junction and for *trans* cleavages at the NS4A/NS4B, NS4B/NS5A and NS5A/NS5B junctions. The consensus sequence for the trans processing is *Asp/Glu-x-x-xCys* ↓ *Ser/Ala*. The P1 Cys residue has been shown to be of major importance for active site recognition in substrates.[8,11] Enzyme inhibitors that prevent the liberation of NS proteins could ultimately inhibit the replication of the HCV virus. Therefore, the NS3 protease, classified as a chymotrypsin-like serine protease, has been studied

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intensively and represents one of the primary targets in the search for new effective antiviral agents against HCV.[6,7,10,12,13]

To date, several types of inhibitors of the HCV NS3 serine protease have been discovered,[7,13–15] of which the classical serine-trap inhibitors represent one category. The electrophilic groups, forming reversible covalent bonds with the active site serine hydroxyl group, employed in these inhibitors are, for example, aldehydes, α-keto amides, α-keto esters, α-diketones, boronic acids, and α-keto acids.[16–29] The second category of HCV NS3 protease inhibitors is derived from the observation that the HCV NS3 protease is significantly inhibited by its N-terminal hexamer cleavage products comprising a C-terminal carboxylate.[30,31] Such product inhibition, to the extent demonstrated in the NS3 protease case, is rare for serine proteases. The binding exhibited by the carboxylate in the active site seems to be a non-covalent interaction with the oxyanion cavity (NH of Gly137 and Ser139) and with the catalytic histidine as deduced from a 3-D-structure.[32] Several structurally optimized product-based inhibitors have been prepared, which have led successively to inhibitors possessing less peptide-like properties.[7,13,15,22,33–35] The product-based NS3 protease inhibitors display high specificity for the HCV NS3 serine protease over other serine proteases,[27] and the first drug-like product-based HCV NS3 protease inhibitor, currently undergoing extensive clinical evaluation has now been disclosed.[36]

A literature survey reveals that most of the inhibitors of the NS3 protease have been evaluated in an artificial truncated NS3 (protease domain) assay.[16,18–23,25–27,29–31,33,34,37,38] Previously, we have compared results from a truncated NS3 assay to a full-length NS3 (protease-helicase/NTPase) assay that we considered to be more relevant to use for evaluation of inhibitors, since the latter assay should better mimic the in vivo situation.[39,40] Different structure–activity relationships were encountered, in addition to retained inhibitory potencies of shorter product-based peptide inhibitors in the full-length NS3 assay.[39] As indicated by molecular modelling, potential binding interactions with the helicase domain might explain the data obtained.[40] In this context, it should be noted that full-length NS3 assays have been utilized more frequently in recent HCV related patent applications, with a few examples cited herein.[41–44] Hence, we felt prompted to examine protease inhibitors with different characteristic features, with a special focus on the C-terminals, as inhibitors of the full-length NS3 (protease-helicase/NTPase) protein.

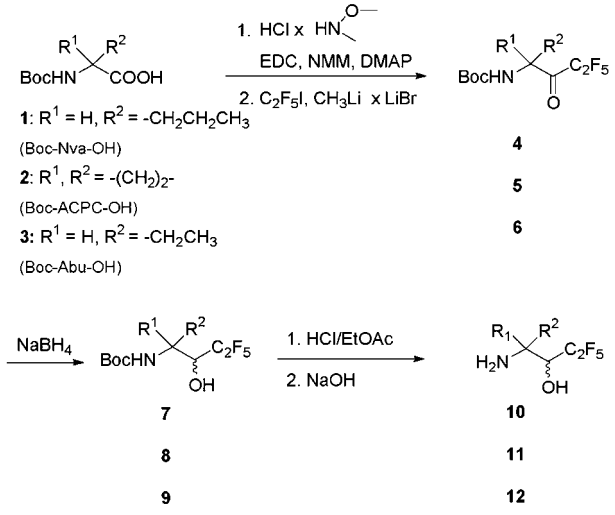
Herein, we report results for three types of HCV NS3 protease inhibitors as assessed in the full-length NS3 assay: (i) inhibitors comprising electrophilic serine traps, (ii) product-based inhibitors with a C-terminal carboxylate group, and (iii) previously unexplored inhibitors comprising C-terminal carboxylic acid bioisosteres. Incorporation of a C-terminal phenyl acyl sulfonamide group provided the most potent inhibitors.

Results

Chemistry

We aimed to synthesize penta- and hexapeptides containing C-terminal pentafluoroethyl ketones, α-keto acids, α-ketotetrazoles, carboxylates, tetrazoles, and acyl sulfonamides. We decided to utilize a direct method, previously reported by us as a means of peptide assembly in the N to C (inverse) direction on solid-phase, which thus allows for C terminal modifications.[45] Classical solid-phase peptide synthesis (SPPS) based on the work of Merrifield is executed in the C to N direction, with the C-terminal consequently attached to the solid support.[46] The strategy used herein for the preparation of the target compounds relied on the preparation of various P1 building blocks for ultimate attachment to resin bound peptides in a combinatorial fashion. The synthesis of the P1 building blocks and the corresponding HCV NS3 protease inhibitors, evaluated in the full-length NS3 assay (Table 1), are outlined in Schemes 1–7.

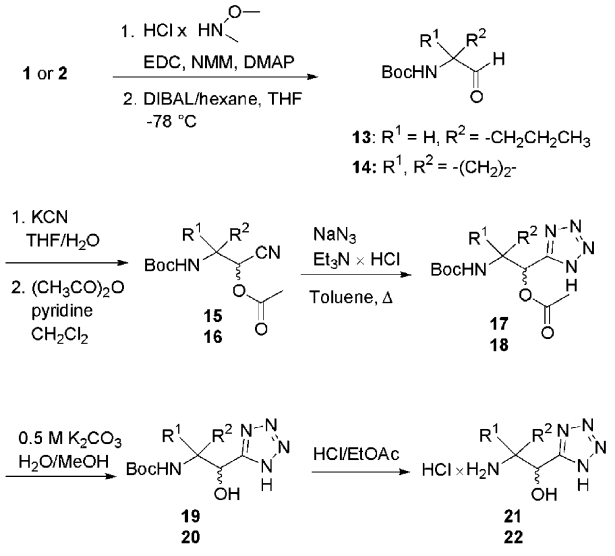
The building blocks for the preparation of inhibitors with a C-terminal pentafluoroethyl ketone function were obtained from the commercially available Boc-protected amino acids, Boc-L-norvaline (Boc-Nva-OH) **1**, Boc-1-aminocyclopropane-1-carboxylic acid (Boc-ACPC-OH) **2**, and Boc-L-2-aminobutyric acid (Boc-Abu-OH) **3**, respectively, as is illustrated in Scheme 1. The acids were converted to the corresponding pentafluoroethyl ketones **4**–**6** using a standard procedure through the synthesis of Weinreb amides, as described by Peet and coworkers.[47–49] Addition of pentafluoroethyl lithium (generated in situ) delivered the pentafluoroethyl ketones **4**–**6**, in acceptable total yields (>63%). Notably, the final yields were affected considerably by the tendency of the compounds **4**–**6** (and **7**–**12**, vide infra) to sublimation, which made handling difficult. Direct coupling of Boc deprotected pentafluoroethyl ketone building blocks to the resin bound peptide **37a** using either *N*-[(1*H*-benzotriazole-1-yl)-(dimethylamino)methylene] - *N* - methylmethanaminium tetrafluoroborate *N*-oxide (TBTU) and *N*,*N*-diiso-



Scheme 1.

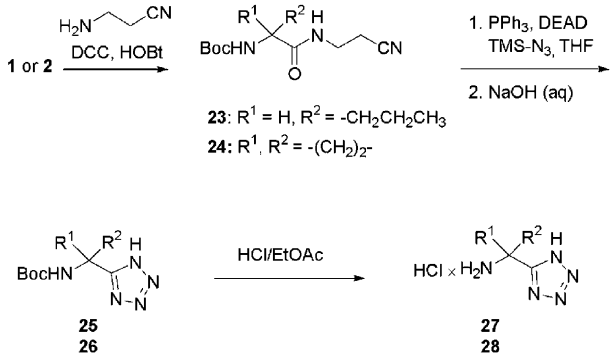
propylethylamine (DIEA), or by the acid fluoride method failed. This forced us to reduce the ketones to the corresponding alcohols prior to the couplings, which were executed subsequently in quantitative yields (vide infra). Thus, reduction of the ketones **4**–**6** using sodium borohydride gave the alcohols **7**[50] to **9** as diastereomeric mixtures in 63–91% yield,[51] which upon Boc deprotection followed by pH adjustment gave the free amines **10**–**12** in good yields.

Synthesis of the building blocks for the preparation of the α-ketotetrazole inhibitors is outlined in Scheme 2, and commenced with the generation of the aldehydes **13**[52,53] and **14**,[54,55] via the corresponding Weinreb amides, and subsequent reduction using diisobutylaluminium hydride (DIBAL).[49,56] Reaction of the aldehydes **13** and **14** with potassium cyanide afforded the cyanohydrins,[57] which were subsequently trapped by acetylation with acetic anhydride in the presence of pyridine to yield the cyano compounds **15** and **16**. The equilibrium between the aldehyde and the corresponding cyanohydrin in the case of Boc-Nva-H was forced almost to completion, in contrast to the case with the more sterically hindered Boc-ACPC-H, where approximately 50% conversion was achieved. The generation of the tetrazoles **17** and **18** was accomplished readily in almost quantitative yields using the protocol adopted from Koguro et al.,[58] by heating sodium azide and **15** or **16** in toluene, in the presence of triethylamine hydrochloride, followed by simple extraction. Subsequent deacetylation using potassium carbonate gave the alcohols **19** and **20**, and Boc deprotection finally yielded the alcohols **21** and **22** as hydrochloride salts in excellent yields.



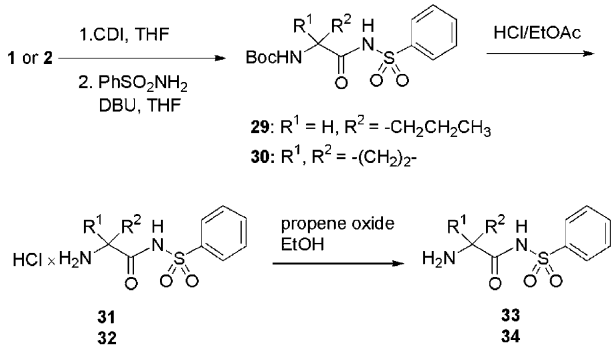
Scheme 2.

The synthesis of the tetrazole analogues, **27** and **28**, of the amino acids Nva and ACPC, where the carboxyl group is replaced by the bioisosteric 5-tetrazolyl group are presented in Scheme 3. The synthesis relies on the method previously reported by Duncia et al., which involves a one-step transformation of an amide into a protected tetrazole.[59] The cyanoethyl amides **23** and **24** were prepared from the corresponding Boc-protected amino acids **1** and **2** using aminopropionitrile, *N*,*N*′-dicyclohexylcarbodiimide (DCC) and *N*-hydroxybenzotriazole (HOBt) in 83 and 85% yields, respectively. The amides were exposed to diethylazodicarboxylate (DEAD), triphenylphosphine and trimethylsilyl azide to generate the corresponding cyanoethyl protected tetrazoles, which were deprotected subsequently to yield the tetrazoles **25** and **26**. Tetrazole formation is highly dependent on the steric hindrance around the neighboring carbon, which was reflected in the yields of the two derivatives.[59] Thus, the total yield of the ACPC-based derivative **26** possessing a quaternary neighboring carbon was 15%, in comparison to 56% of the Nva-based derivative **25**. Boc deprotection finally afforded the hydrochloride salt of the tetrazoles **27** and **28** in high yields.



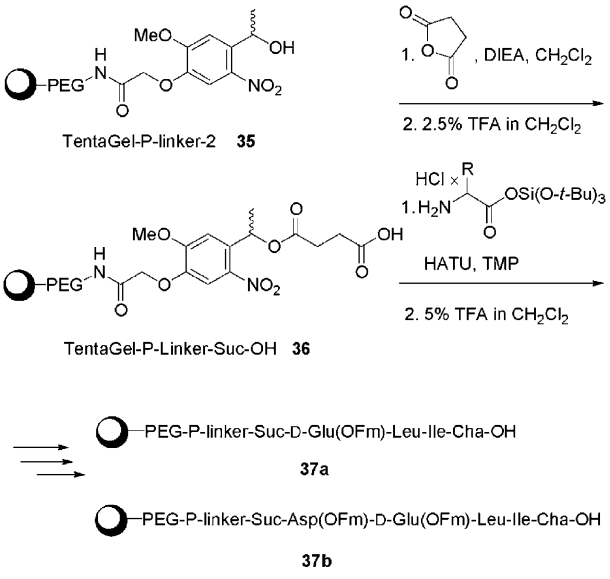
Scheme 3.

As an alternative to the tetrazoles, we replaced the C-terminal carboxyl group with the bioisosteric acyl sulfonamide group. The synthesis of the unnatural amino acids comprising a phenyl acyl sulfonamide group is shown in Scheme 4. The commercially available Boc-protected amino acids **1** and **2** were coupled to benzenesulfonamide using 1,1-carbonyldiimidazole (CDI) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to afford the acyl sulfonamides **29** and **30** in 64 and 90% yield, respectively.[60] Boc deprotection gave the hydrochlorides **31** and **32** in high yields (95 and 90%), which were transformed subsequently into the corresponding zwitterions **33** and **34** by propene oxide.[45,61]



Scheme 4.

The general method for the synthesis of the resin bound tetra- and pentapeptides **37a**,**b** is described in Scheme 5. A TentaGel based solid support with a photolabile linker, that is, TentaGel-P-Linker-2 **35**, was used as starting resin.[62,63] The capping group, succinic acid, was introduced efficiently through coupling of succinic anhydride in presence of DIEA to yield **36**. A full conversion was achieved as confirmed by ^{13}C NMR. The peptides were assembled by coupling of the amino acid tri-*tert*-butoxysilyl (Sil) esters using *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) and 2,4,6-trimethylpyridine (TMP), without any preactivation.[45] The Sil esters were cleaved by using 5% trifluoroacetic acid (TFA) in dichloromethane. The base labile fluorenylmethyl (Fm) ester side chain was selected to protect Asp and Glu. Peptide assembly in the N to C direction is normally associated with a higher risk of epimerization, since the amino acid residue being activated is *N*-acylated. As shown by us previously, epimerization can be suppressed when fast (HATU promoted) coupling methods are used and preactivation avoided.[45] Accordingly, HPLC analysis after cleavage of analytical samples of **37a**,**b** shows the desired product as the dominant peak.

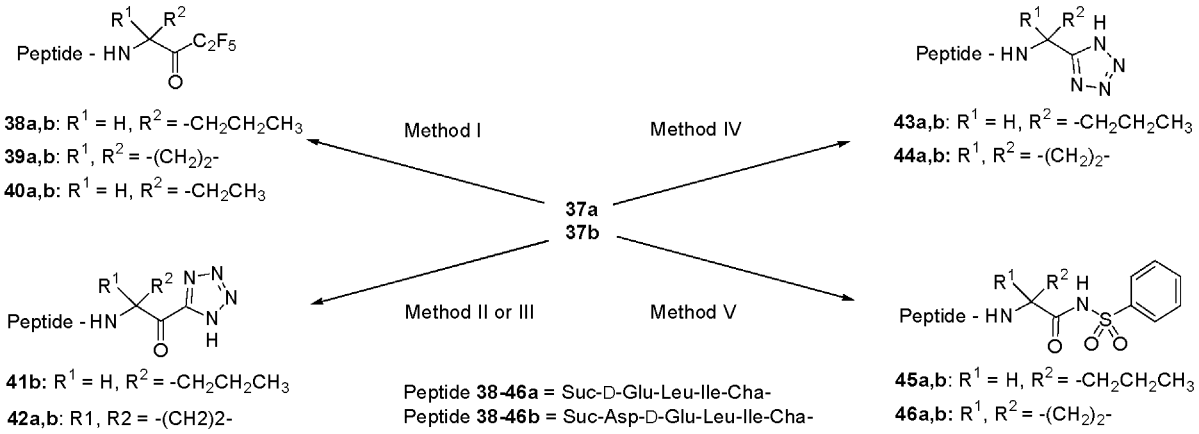


Scheme 5.

For generation of the inhibitors **38**–**46a**,**b** the P1 building blocks, described in Schemes 1–4, were coupled in a parallel fashion to aliquots of **37a** and **37b** using HATU as coupling reagent, as outlined in Scheme 6. TMP was used as base for the coupling of the free amines **10**–**12**, **33** and **34**. To effect the coupling of the hydrochlorides **21**, **22**, **27**, and **28** the stronger base *N*,*N*-diisopropylamine (DIEA) was employed. The resins were treated subsequently with 25% piperidine in DMF and 2.5% TFA in CH_2Cl_2 to remove the Fm groups and the formed piperidine salt, respectively. The peptide target compounds comprising the C-terminal tetrazole **43a**,**b** and **44a**,**b** and acyl sulfonamide group **45a**,**b** and **46a**,**b** were liberated from the resin as major products by photolysis at 350 nm, and were finally purified by RP-HPLC. The resin bound pentafluoroethyl alcohols were oxidized readily using Dess–Martin periodinane[64,65] and were cleaved thereafter from the resin to smoothly provide the desired peptide ketones **38**–**40a**,**b** as the predominant products, which were purified subsequently by RP-HPLC. The same oxidation and cleavage procedure was used initially for the preparation of the α-ketotetrazoles **41** and **42**. As deduced by HPLC-MS analysis of the crude reaction mixtures, complex mixes of compounds had been formed. Only the α-ketotetrazoles **42a**,**b**, based on ACPC, could be isolated after purification by RP-HPLC. No products of the Nva derivatives **41** could be traced after oxidation and photolysis. As an alternative method, the alcohol product (after coupling of the tetrazole containing alcohol **21** to **37b**) was released from the solid support prior to oxidation. According to the HPLC-MS analysis only partial coupling of **21** had been achieved. The mixture was mainly composed of the desired alcohol product, the starting material and the dimethylamide of **37b** (the dimethylamine group most probably originating from HATU and not from DMF, since the dimethylamide side-product was formed to a greater extent when dichlormethane was used as solvent). The hydroxylpeptide was purified by RP-HPLC and was oxidized subsequently in solution using the Dess–Martin reagent. Thus, the α-ketotetrazole inhibitor **41b** could be isolated after RP-HPLC purification.

In some cases, the crude products after coupling of the P1 building blocks (Scheme 6) contained isomers which had the same molecular weight as the predominantly-formed target compound. A partial epimerization of the Cha residue presumably accounts for the side-product formation. The major products were evaluated in the bioassay only after separation from the epimer, unless otherwise stated. Apart from the epimerization of the Cha residue it is well recognized that epimerisation α to the activated carbonyl function of electrophilic inhibitors, such as in the pentafluoroethyl ketones **38a**,**b** and **40a**,**b**, is difficult to avoid.[48,51] Furthermore, in aqueous solution, hydration of the electrophilic ketones **38**–**40a**,**b** occurs.[48] Hence, a mixture of the ketone/hydrate form and possibly epimers were used in the biological evaluation.

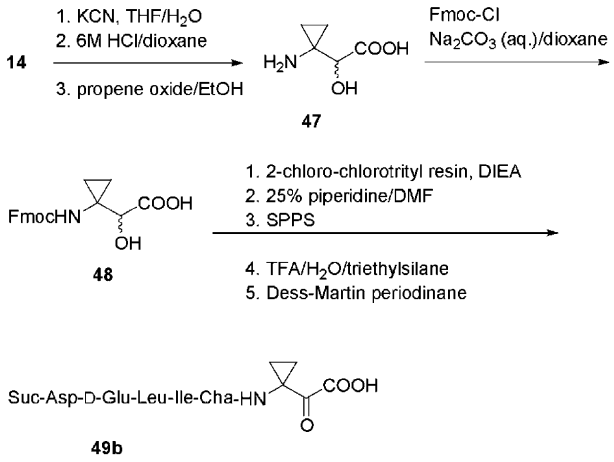
The preparation of the α-keto acid inhibitor **49b** is outlined in Scheme 7. The α-hydroxy-β-amino acid compound **48** was prepared according to established methods using a modified isolation procedure.[52,66,67] Thus, the aldehyde **14** was transformed into the corresponding cyanohydrin (see the discussion above concerning cyanohydrins from Boc-ACPC-H in the synthesis of **16**) by potassium cyanide. The crude cyanohydrin was hydrolyzed by concentrated hydrochloride acid in dioxane under reflux conditions. Isolation of the zwitterionic product **47**, in a total yield of 41%, was achieved smoothly by removal of the hydrochloride salt by propene oxide in an ethanolic solution,[45,61] whereupon the product precipitated. The Fmoc protected α-hydroxy-β-amino acid **48** was obtained by reaction



Scheme 6. Method I: (a) **10**, **11** or **12**, HATU, TMP; (b) 25% piperidine/DMF; (c) 2.5% TFA/CH_2Cl_2; (d) Dess–Martin periodinane, (e) hv (350 nm), MeOH. Method II: (a) **21**, HATU, DIEA; (b) 25% piperidine/DMF; (c) 2.5% TFA/CH_2Cl_2; (d) hv (350 nm), MeOH; (e) Dess–Martin periodinane. Method III: (a) **22**, HATU, DIEA; (b) 25% piperidine/DMF; (c) 2.5% TFA/CH_2Cl_2; (d) Dess–Martin periodinane; (e) hv (350 nm), MeOH. Method IV: (a) **27**, **28**, HATU, DIEA; (b) 25% piperidine/DMF; (c) 2.5% TFA/CH_2Cl_2; (d) hv (350 nm), MeOH. Method V: (a) **33**, **34**, HATU, TMP; (b) 25% piperidine/DMF, (c) 2.5% TFA/CH_2Cl_2, (d) hv (350 nm), MeOH.

with Fmoc-Cl and aqueous sodium carbonate in dioxane in a rather poor yield (34%) and was attached subsequently to a polystyrene-based 2-chlorotrityl chloride resin in presence of DIEA, using a slightly modified literature procedure.[68] The loading of the resin could be monitored readily by IR analysis of the resin through the appearance of a carbonyl stretch vibration band at 1732 cm^{-1}. The resin bound derivative was then subjected to classical SPPS according to the standard Fmoc/*t*-Bu protocol using TBTU as coupling reagent and DIEA as base to deliver desired peptide sequence. Treatment with TFA released the alcohol-containing peptide from the resin. Oxidation using the Dess–Martin reagent in solution finally gave the α-keto acid **49b** in reasonable 43% yield, calculated from the weight of the crude peptide used in the oxidation.

The remaining peptide inhibitors which possess a C-terminal carboxylic acid, that is **50–54a**,**b** were synthesized using standard SPPS conditions.



Scheme 7.

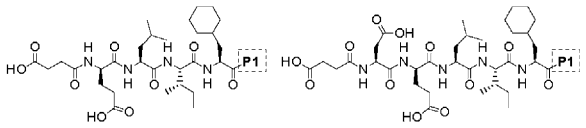
Protease inhibition of full-length HCV NS3 (protease-helicase/NTPase)

The inhibitors **38–40a**,**b**, **41b**, **42–46a**,**b**, and **50–54a**,**b** (Table 1) were evaluated in an in vitro assay comprising the full-length NS3 protein (amino acids 1027–1657 of the HCV polyprotein) and the central part of NS4A (2K-NS4A) as cofactor.[39,40,69] The enzyme, cofactor and inhibitor were pre-incubated for 10 min, whereafter the reaction was initiated by addition of the substrate. As a consequence, potentially slow kinetics of binding and dissociation of the electrophilic inhibitors was not studied herein. The reference compound Ac-Asp-D-Gla-Leu-Ile-Cha-Cys was examined in parallel, yielding an average inhibition of 70±11% at 0.104 μM. The inhibitors are divided into three categories: A, electrophilic; B, product-based; and C, carboxylic acid bioisosteres, and their K_i values are presented in Table 1.

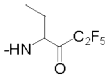
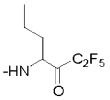
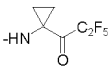
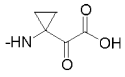
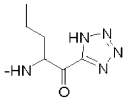
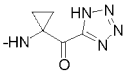
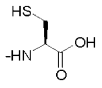
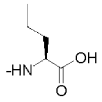
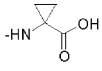
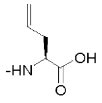
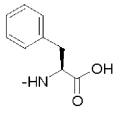
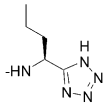
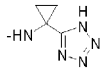
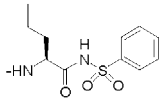
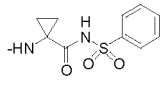
Discussion

The penta- and hexapeptide inhibitors employed for the assessment of the impact of different C-terminal residues (P1 building blocks) contain two or three carboxyl groups in the N-terminal region, while the central part of the peptides encompass identical lipophilic amino acid residues. As is shown in Table 1, all P1-building blocks provided penta- and hexapeptidic protease inhibitors of the full-length NS3 (protease-helicase/NTPase) protein with K_i values in the micro- to nanomolar range. Peptides with the native Cys in the P1 position were included in the series as controls. The thiol side-chain was superior to all lipophilic side chain substitutes as apparent from a comparison of the product-based inhibitors **50–54a**,**b** (category B, Table 1). This observation is in reasonable agreement with previous results using the protease domain assay.[27,33] Thus, the cysteine peptides **50a**,**b** were approximately ten times more active than the Nva-, ACPC- and allylglycine-based inhibitors **51–53a**,**b**,

Table 1. Inhibition of the NS3 protease activity of the full-length NS3 (protease-helicase/NTPase)



a b

Inhibitor type	Compd	P1	K_i (μM)±SD
A: Electrophilic	**40a**		2.0
	40b		0.081±0.007
	38a		2.9±0.3
	38b		0.12±0.02
	39a		10% (83 μM)[a]
	39b		1.8±0.1
	49b		0.098±0.006
	41b[b]		0.30±0.03
	42a		13±2
	42b		1.9±0.4
B: Product-based	**50a**		0.12±0.01
	50b		0.028±0.004
	51a		1.9±0.1
	51b		0.22±0.03
	52a		1.8±0.2
	52b		0.091±0.005
	53a		3.0±0.2
	53b		0.12±0.01
	54a		30±3.5
	54b		1.5±0.2
C: Carboxylic acid bioisosteres	**43a**		5.3±0.6
	43b		0.156±0.005
	44a[c]		10.2±1.1
	44b		0.19±0.01
	45a		0.21±0.04
	45b		0.0136±0.0005
	46a		0.56±0.1
	46b		0.0038±0.0004

[a]Percent inhibition at 83 μM.
[b]Purity = 91%.
[c]5:3 mixture of L-/D-Cha diastereomers.

which in turn were more than ten times more active than the inhibitors with Phe in P1 (**54a**,**b**).

Evaluation of the electrophilic inhibitors (category A, Table 1) shows that the pentafluoroethyl ketones of Nva **38a**,**b** (and Abu **40a**,**b**) as inhibitors were equally or slightly more active than the corresponding acids (**51a**,**b**), However the combination of the pentafluoroethyl ketone and the cyclopropane ring of **39a**,**b** was deleterious, possibly due to unfavorable steric interactions. The postulated tetrahedral hemiketal formed with the active site serine hydroxyl might not be combined successfully with a neighboring disubstituted α-carbon as present in **39a**,**b**. The α-ketotetrazole inhibitors **41b** and **42a**,**b** were primarily designed as bioisosteres to the very potent α-keto acid inhibitors discovered by Narjes et al.[21] Therefore, the α-keto acid **49b** was made as a reference substance. In the full-length NS3 assay **49b** exhibited a 20-fold higher potency compared to the corresponding α-ketotetrazole **42b**. This result correlates with a recent observation by Narjes' group who studied tripeptide inhibitors with difluoroAbu in the P1 postion.[20] However, the Nva-based inhibitor **41b** was fairly active, again demonstrating that serine-trap carbonyl groups are not compatible with the disubstituted α-carbon found in the ACPC based inhibitors in the present series (**39a**,**b**, **42a**,**b** compared to **38a**,**b** and **41b**).

Bioisosteric replacements in lead substances have frequently been utilized in order to retain or enhance potencies and to simultaneously improve pharmacokinetic properties.[70–72] The inhibitors **43**–**46a**,**b** (category C, Table 1) were prepared as bioisosteres to the corresponding product-based inhibitors with a C-terminal carboxylate. Two common carboxylic acid bioisosteres, the tetrazole and the acyl sulfonamide, with calculated pK_a-values similar to that of the carboxyl group were explored.[72] It was found that both the tetrazole and the acyl sulfonamide were suitable substitutes for the C-terminal carboxylate (see compounds **43**–**46a**,**b**, **51a**,**b** and **52a**,**b**, Table 1). It is noteworthy that the acyl sulfonamide group had a pronounced positive impact on the inhibitory potencies. Thus, both the Nva and ACPC based inhibitors **45a**,**b** and **46a**,**b** exhibited approximately 10-fold lower K_i values than the carboxyl analogues **51a**,**b** and **52a**,**b**. The acyl sulfonamide comprising hexapeptide inhibitors **45b** and **46b** (K_i = 13.6 and 3.8 nM, respectively) were indeed as potent as the inhibitor that encompasses the native Cys (**50b**, K_i = 28 nM). Bearing in mind that these inhibitors have a non-optimal P1 side chain, it is tempting to postulate that novel interactions of the acyl sulfonamide group at the active site might account for the improved inhibitory potency. This hypothesis was supported by computer modeling, where the 3-D-structure of full-length NS3 (as a scNS3-NS4A construct) disclosed by Yao et al.,[32] was used as a starting point for docking of the inhibitor **45a** by the procedure recently reported.[40] Different binding modes seem possible, of which two typical examples are explored in Figure 1a and b. Thus, one of the oxygen of the sulfone group might be engaged in hydrogen bonding with the catalytic His57 (1a) or with the oxyanion

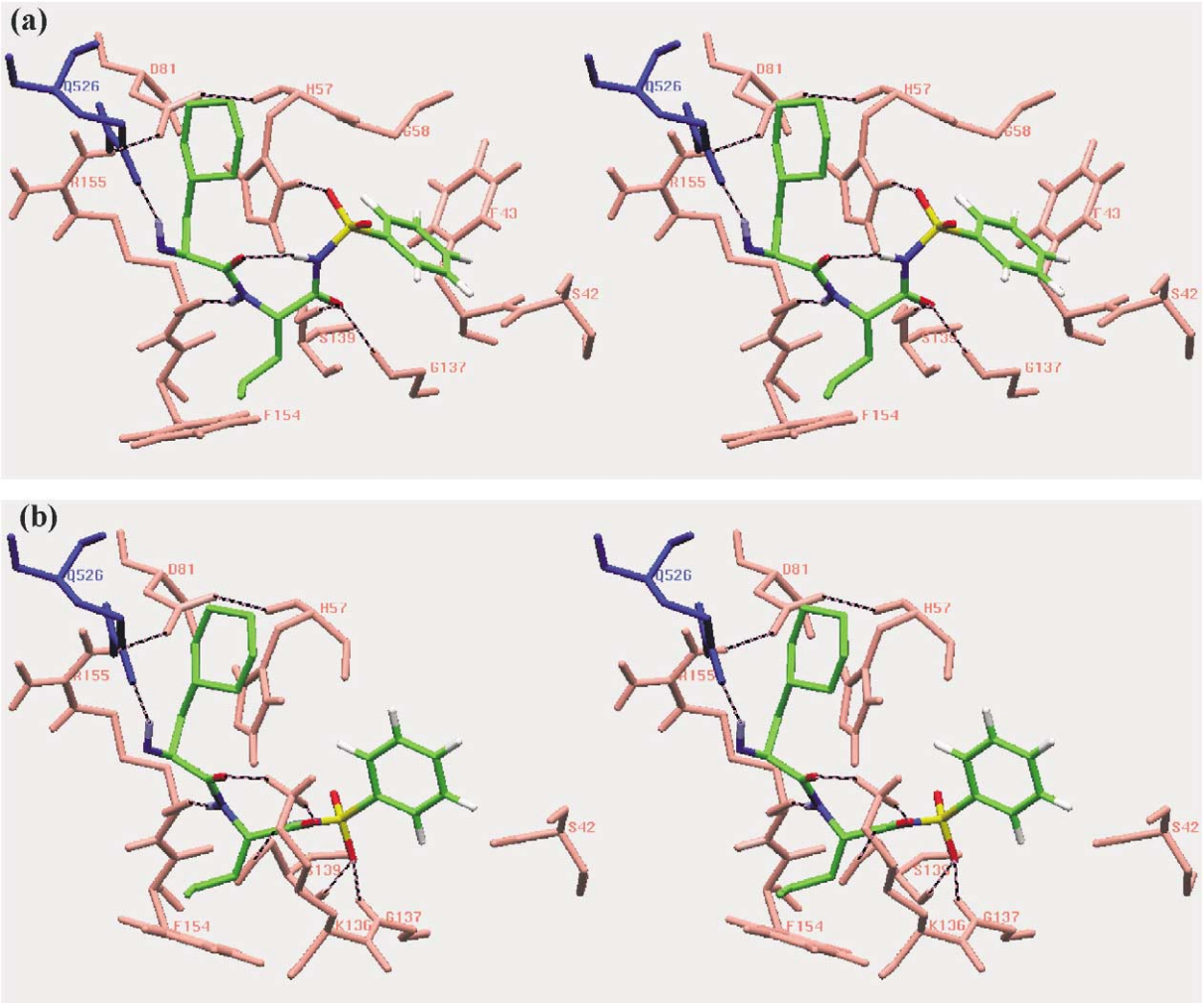


Figure 1. Stereoview of two possible binding modes (**a** and **b**) of Suc-D-Glu-Leu-Ile-Cha-Nva-$NHSO_2Ph$ **45a** docked into the protease active site of the full-length scNS3–NS4A protein. Protease (3–181) residues depicted in pink, helicase (182–631) residues in blue, and the inhibitor (only the P2–P1 part of the inhibitor shown) color-coded with all carbons in green, oxygens in red, nitrogens in blue, and hydrogens in gray. Only residues close to the inhibitor (within 3.0 Å) are shown. Hydrogen bonds are indicated.

cavity (NH of Gly137 and Ser139) (1b). Furthermore the carbonyl of the acyl sulfonamide group could act as a hydrogen bond acceptor of the NH hydrogen of Gly137 and of the OH hydrogen of the catalytic Ser139 (1a), or with the flexible Lys136 (1b). Additionally, there is sufficient space to accommodate the phenyl moiety of the acyl sulfonamide (1a and b) allowing for favorable hydrophobic interactions with the closely situated Phe43 in one case (1a). Two hydrogen bonds from the helicase residues Gln526 (1a and b) and His 528 to the NH of the P2 residue and the carbonyl of the P4 residue, respectively, were established in the model (the latter not shown). Very recently, while this paper was in preparation, Campbell et al. disclosed a patent application covering potent HCV NS3 protease inhibitors with P1 acyl sulfonamides.[44] We are convinced that the acyl sulfonamide group constitutes a very promising C-terminal residue that allows for prime site optimization with the phenyl group serving as a handle. Peptides, in particular those containing multiple carboxylates, as preferred by the HCV NS3 protease, are unlikely to be orally bioavailable. However, the bioisostere approach presented here could serve as a starting point for refinements which might ultimately yield more drug-like inhibitors.

Conclusions

The inhibitory potencies of three types of HCV NS3 protease inhibitors assessed in the relevant full-length NS3 (protease-helicase/NTPase) assay have been compared, and consist of: (i) inhibitors comprising electrophilic serine traps, (ii) product-based inhibitors with a C-terminal carboxylate, and (iii) previously unexplored, inhibitors with C-terminal carboxylic acid bioisosteres. To summarize, bioisosteric replacement of the C-terminal carboxylic acid of the product-based inhibitors of the HCV NS3 protease with tetrazoles and, especially, acyl sulfonamide groups was successful. In fact, the phenyl acyl sulfonamide residue produced product-based inhibitors up to 20-fold more potent than the corresponding carboxylates. In addition to possessing better pharmacokinetic properties than a carboxylate, the aryl acyl sulfonamide group also allows for structural elongations and modifications. Thus, the bioisosteric acyl

sulfonamide group has great potential in such inhibitors and will be explored further in our on-going drug discovery efforts.

Experimental

Chemistry. General methods

^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-EX270 spectrometer at 270 (68) MHz or a JEOL JNM-EX400 at 400 (100) MHz at ambient temperature. Chemical shifts are reported as δ values (ppm) indirectly referenced to TMS by the solvent signal ($CHCl_3$) δ 7.26, ($CDCl_3$) δ 77.23, (methanol-d_3) δ 3.31, (methanol-d_4) δ 49.15, (DMSO-d_5) δ 2.50 and (DMSO-d_6) δ 39.51. IR spectra were recorded on a Perkin–Elmer Model 1605 FT-IR and are reported as ν_{max} (cm^{-1}), and for measurement of neat solids the IR spectrometer was equipped with a Microfocus Beam Condenser with ZnSe lenses in a Diasqueeze Plus Diamond Compressor Cell (Graseby Specac Inc., Smyrna, USA). Column chromatography was executed on Merck silica gel 60 (40–63 μm). Elemental analyses were performed by Mikro Kemi AB, Uppsala, Sweden or Analytische Laboratorien, Lindlar, Germany. Optical rotations were recorded on a Perkin–Elmer Model 241 polarimeter at ambient temperature. Specific rotations ($[\alpha]_D$) are reported in deg/dm, and the concentration (*c*) is given in g/100 mL in the specified solvent. Cleavage of the peptides from the photolabile resin was performed in a RPR-200 Rayonet Chamber Reactor equipped with the Rayonet Merry-Go-round Model-RMA-500 (22 samples) working as a photoreactor. All samples were rotated past the light source at a speed of 5 RPM. The stirring in each vial was conducted using a rebuilt magnetic stirrer placed underneath the reactor. The photoreactor was equipped with 16 25W lamps emitting 350 nm UV-light. The distance to the sample was 1.7 cm. The Quest 210™ organic synthesizer (Argonaut Technologies) was utilized in the peptide syntheses. RP-HPLC-MS was performed on a Gilson-Finnigan ThermoQuest AQA system in ESI mode, on a Zorbax Stable Bond C8 column (20×50 mm or 4.6×50 mm), using an acetonitrile/H_2O gradient with 50 mM NH_4OAc (pH 6.3) (method A) or an acetonitrile/H_2O gradient with 0.05% HCOOH (method B), using UV (214, 255 nm) and MS detection. RP-HPLC was performed on an analytical LiChrosphere C18 column (0.4×25 cm, 5 μm particles, 100 Å pores) using an acetonitrile/H_2O gradient with 0.1% TFA (method C), or an analytical Vydac C18 column (0.46×15 cm, 10 μm particles, 300 Å pores). Amino acid analyses were performed at the Department of Biochemistry, Uppsala University, Sweden, on 24 h hydrolyzates (on oxidized samples of Cys containing peptides) with an LKB 4151 alpha plus analyser using ninhydrin detection.

Materials and abbreviations

The resin used in the inverse SPPS was the photolabile TentaGel-P-linker-2 **35** (loading capacity: 0.167 mmol/g, determined from N-analysis), prepared by coupling of 4-acetyl-2-methoxy-5-nitrophenoxy acetic acid to TentaGel-S-NH_2 (RAPP Polymer), followed by reduction with $NaBH_4$.[62,63] The amino acid tri-*tert*-butoxysilyl (Sil) esters used in the solid-phase syntheses in the inverse direction were prepared as described by us previously.[45] 2-chloro-tritylchloride, H-Cys(Trt)-2-chlorotrityl and Fmoc-Phe-Wang resins were obtained from Alexis Corporation, Senn Chemicals and Nova Biochem, respectively. The *N*-Fmoc protected amino acids, Boc-Nva-OH **1**, Boc-ACPC-OH **2** and Boc-Abu-OH **3** were purchased from Senn Chemicals, Alexis Corporation, Nova Biochem or Bachem. *N*-[(1*H*-benzotriazole-1-yl)-(dimethylamino)methylene]-*N*-methylmethanaminium tetrafluoroborate *N*-oxide (TBTU) and *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) were obtained from Richelieu Biotech and Millipore or Applied Biosystems, respectively. The bases 2,4,6-trimethylpyridine (TMP, collidine) (Merck, p.a) or *N,N*-diisopropylethylamine (DIEA), redistilled, 99.5% from Aldrich were used in the peptide synthesis. DMF (Aldrich, 99.9+%, HPLC grade) was stored over molecular sieves (4 Å); dichloromethane (Riedel-deHaën) was freshly distilled from calcium hydride, and THF from sodium and benzophenone. Pentafluoroethyl iodide, min 97%, was obtained from Stream Chemicals and Dess–Martin periodinane [1,1,1-tris(acetyloxy)-1,1-dihydro-1,2-benziodoxol-3-[1*H*]-one], 97% from Aldrich or Lancaster Synthesis. Other materials were obtained from commercial sources and used without further purification.

***tert*-Butyl (1*S*)-3,3,4,4,4-pentafluoro-2-oxo-1-propylbutylcarbamate (4).** Boc-Nva-OH **1** was transformed into the corresponding Weinreb amide[52] by standard procedures[48,49] (data for the Weinreb amide, *tert*-butyl (1S)-1-{[methoxy(methyl)amino]carbonyl}butylcarbamate: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.13 (br d, 1H, NH), 4.68 (m, 1H), 3.77 (s, 3H), 3.20 (s, 3H), 1.73–1.58 (m, 2H), 1.57–1.30 (m, 2H), 1.44 (s, 9H), 0.92 (dd, $J=7.3$, 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 155.7, 79.6, 61.7, 50.3, 35.3, 32.2, 28.5, 18.8, 13.9. IR (neat) 3326, 1716, 1663 cm^{-1}. Anal. calcd for $C_{12}H_{24}N_2O_4$: C, 55.4; H, 9.3; N, 10.8. Found: C, 55.1; H, 9.4; N, 10.7). Condensed pentafluoroethyl iodide (9.0 g, 37 mmol) was added to freshly distilled ether (150 mL) in a dried three-necked flask under a stream of N_2, at −78 °C. A solution of the Weinreb amide (3.30 g, 12.7 mmol) in freshly distilled ether (50 mL) was added, followed by dropwise addition of 1.5 M methyl lithium–lithium bromide complex in ether (24 mL, 36 mmol), at a rate that maintained the temperature below −62 °C. The reaction mixture was stirred for 1.5 h under N_2 atmosphere at −65 to −73 °C. Cooling was halted and the reaction mixture allowed to warm to 0 °C. Quenching was performed by addition of water (200 mL), followed by 1 M aqueous $NaHSO_4$. The organic layer was separated and the aqueous layer extracted with ether (100 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel

(5% EtOAc/pentane) to yield **4** as a white solid (2.55 g, 63%), with a tendency to sublime, which affected the yield. An analytical sample was further purified by sublimation. ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.08–4.90 (br d, 1H, NH), 4.88–4.72 (m, 1H), 1.92–1.73 (m, 1H), 1.61–1.36 (m, 3H), 1.43 (s, 9H), 0.95 (dd, J = 7.2, 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.8 (dd, J = 26, 26 Hz), 155.2, 118.0 (qdd, J = 288, 34, 34 Hz), 107.4 (ddq, J = 268, 268, 36 Hz), 80.8, 56.1, 32.9, 28.4, 18.9, 13.1. IR (neat) 3274, 1757, 1712 cm^{-1}. Anal. calcd for $C_{12}H_{18}F_5NO_3$: C, 45.1; H, 5.7; N, 4.4. Found: C, 45.0; H, 6.1; N, 4.4.

***tert*-Butyl 1-(2,2,3,3,3-pentafluoropropanoyl)cyclopropylcarbamate (5).** Compound **5** was prepared according to the procedure described for **4** using the Weinreb amide[73] (1.60 g, 6.55 mmol) prepared from Boc-ACPC-OH **2**, pentafluoroethyl iodide (16 g, 65 mmol) and 1.5 M methyl lithium–lithium bromide complex in ether (37 mL, 56 mmol), with the exception that no column chromatography was needed. Concentration under reduced pressure was carried out carefully and not to complete dryness, to minimize sublimation, to yield **5** as white solid (2.24 g, 113%, including ether). An analytical sample was further purified by sublimation. ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.28 (br s, 1H, NH), 1.82–1.74 (m, 2H), 1.64–1.40 (m, 2H), 1.44 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.6 (t, J = 24.5 Hz), 155.5, 118.0 (qt, J = 285, 34 Hz), 108.0 (tq, J = 269, 38 Hz), 81.1, 40.6, 28.3, 22.8. IR (neat) 3354, 1734, 1690 cm^{-1}. Anal. calcd for $C_{11}H_{14}F_5NO_3$: C, 43.6; H, 4.7; N, 4.6. Found C, 43.8; H, 4.8; N, 4.6.

***tert*-Butyl (1S)-1-ethyl-3,3,4,4,4-pentafluoro-2-oxobutylcarbamate (6).** Compound **6** was prepared according to the procedure described for **4** using the Weinreb amide[52] (2.00 g, 8.13 mmol) prepared from Boc-Abu-OH **3** (data for the Weinreb amide, *tert*-butyl (1*S*)-1-{[methoxy(methyl)amino]carbonyl}propylcarbamate: ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.16 (br d, 1H, NH), 4.63 (m, 1H), 3.77 (s, 3H), 3.21 (s, 3H), 1.77 (m, 1H), 1.67–1.53 (m, 1H), 1.43 (s, 9H), 0.94 (dd, J = 7.3, 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 155.7, 79.6, 61.7, 51.7, 32.2, 28.5, 26.2, 10.0. IR (neat) 3350, 1702, 1662 cm^{-1}. Anal. calcd for $C_{11}H_{22}N_2O_4$: C, 53.6; H, 9.0; N, 11.4. Found: C, 53.9; H, 9.1; N, 11.4), pentafluoroethyl iodide (19.5 g, 79.3 mmol) and 1.5 M methyl lithium–lithium bromide complex in ether (20.5 mL, 30.7 mmol). The crude product was purified by column chromatography on silica gel (10% EtOAc/isohexane) to yield **6** (1.63 g, 66%) as a white solid. In this case the product also sublimed, which affected the yield. An analytical sample was further purified by sublimation. ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.10–4.90 (br d, 1H, NH), 4.87–4.72 (m, 1H), 1.97–1.88 (m, 1H), 1.72–1.54 (m, 1H), 1.44 (s, 9H), 0.99 (dd, J = 7.4, 7.4 Hz, 3H). ^{13}C NMR (68 MHz, $CDCl_3$) δ 194.6 (dd, J = 27, 27 Hz), 155.2, 118.0 (qdd, J = 286, 33, 33 Hz), 107.4 (ddq, J = 267, 267, 38 Hz), 80.9, 57.4, 28.4, 24.3, 9.7. IR (neat) 3364, 1756, 1706 cm^{-1}. Anal. calcd for $C_{11}H_{16}F_5NO_3$: C, 43.3; H, 5.3; N, 4.6. Found C, 43.1; H, 5.4; N, 4.6.

***tert*-Butyl (1*S*)-3,3,4,4,4-pentafluoro-2-hydroxy-1-propylbutylcarbamate (7).** Boc-Nva-C_2F_5 **4** (1.10 g, 3.44 mmol) was dissolved in a mixture of THF (27 mL) and MeOH (7 mL). The solution was cooled to 0 °C, sodium borohydride (0.360 g, 9.52 mmol) was added and the resulting reaction mixture was stirred for 1.5 h. The mixture was diluted with ether (60 mL) and washed with 2% aqueous citric acid solution (2×15 mL). The combined aqueous layers were extracted with ether (3×15 mL). The combined ether layers were successively washed with saturated aqueous $NaHCO_3$ (15 mL), water (20 mL), brine (20 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure to yield crude **7**[50] as a 1:4 mixture of diastereomers (A/B) (1.03 g, 93%). The crude product was further purified by column chromatography on silica gel (15% EtOAc/isohexane). Separation of the two diastereomers (A, B) was achieved. Total yield 0.699 g (63%) In this case, the product also sublimed, which affected the yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ (A) 5.03 (br d, 1H), 4.81 (br d, 1H), 4.21–3.81 (m, 1H), 3.69 (m, 1H), 1.89–1.24 (m, 4H), 1.44 (s, 9H), 0.94 (dd, J = 7.2, 7.2 Hz, 3H). ^{13}C NMR (68 MHz, $CDCl_3$); (B) 4.74 (br d, 1H), 4.62 (br d, 1H), 4.17 (m, 1H), 3.92 (m, 1H), 1.70–1.21 (m, 4H), 1.45 (s, 9H), 0.95 (dd, J = 7.2, 7.2 Hz, 3H). ^{13}C NMR (68 MHz, $CDCl_3$) δ (A) 157.4, 119.2 (qdd, J = 287, 36, 36 Hz), 114.0 (ddq, 263, 257, 36 Hz), 80.9, 70.7 (dd, J = 26, 20.5 Hz), 50.8, 33.4, 28.4, 19.5, 13.8; (B) 157.6, 119.2 (qdd, J = 287, 35, 35 Hz), 114.5 (ddq, 263, 257, 35.5 Hz), 81.0, 71.7 (dd, J = 26, 20 Hz), 53.5, 32.4, 28.4,19.9, 13.8. IR (neat) 3550-3150, 1684 cm^{-1}. Anal. calcd for $C_{12}H_{20}F_5NO_3$: C, 44.9; H, 6.3; N, 4.4. Found C, 44.9; H, 6.5; N, 4.3.

***tert*-Butyl 1-(2,2,3,3,3-pentafluoro-1-hydroxypropyl)cyclopropylcarbamate (8).** Compound **8** was prepared according to the procedure described for **7** using Boc-ACPC-C_2F_5 **5** (1.00 g, 3.30 mmol). The crude product was purified by column chromatography on silica gel (5% EtOAc/pentane) to yield **8** (0.820 g, 81%) as a white solid. In this case the product also sublimed, which affected the yield. ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.08 (d, J = 7.1 Hz, 1H), 5.09 (br s, 1H), 3.31 (m, 1H), 1.44 (s, 9H), 1.22–1.11 (m, 1H), 1.10–0.91 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.5, 119.1 (qdd, J = 287, 35, 35 Hz), 114.1 (ddq, J = 261, 259, 36 Hz), 81.9, 75.7 (dd, J = 24, 20 Hz) 33.5, 28.3, 14.8, 14.1. IR (neat) 3500–3300, 1661 cm^{-1}. Anal. calcd for $C_{11}H_{16}F_5NO_3$: C, 43.3; H, 5.3; N, 4.6. Found C, 43.3; H, 5.4; N, 4.6.

***tert*-Butyl (1*S*)-1-ethyl-3,3,4,4,4-pentafluoro-2-hydroxybutylcarbamate (9).** Compound **9** was prepared according to the procedure described for **7** using Boc-Abu-C_2F_5 **6** (0.670 g, 2.20 mmol). The crude product [1:4 mixture of diastereomers (A/B)] was purified by column chromatography on silica gel (7% EtOAc/pentane) to give **9** (0.62 g, 91%) as a white solid. Separation of the two diastereomers (A, B) was achieved. In this case, the product also sublimed. ^{1}H NMR (400 MHz, $CDCl_3$) δ (A) 5.03 (br d, 1H), 4.21–3.77 (br m, 1H), 4.03 (m, 1H), 3.60 (m, 1H), 1.93–1.66 (m, 2H), 1.43 (s, 9H), 0.98 (dd, J = 7.3, 7.3 Hz, 3H); (B) 4.79 (br d, 1H), 4.79–4.39 (br m, 1H) 4.17 (m, 1H), 3.88–3.70 (m, 1H), 1.79–1.54 (m, 2H), 1.44 (s, 9H) 1.00 (dd, J = 7.3 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (A) 157.4, 119.2 (qdd,

J=287, 36, 36 Hz), 114.5 (ddq, J=262, 256, 35 Hz), 80.9, 70.4 (dd, J=24, 21 Hz), 52.5, 28.4, 24.5, 10.7; (B) 157.6, 119.0 (qdd, J=288, 36, 36 Hz), 114.5 (ddq, J=258, 258, 35 Hz), 81.0, 71.5 (dd, J=26, 20 Hz), 55.3, 28.4, 23.5, 11.2. IR (neat) 3500–3250, 1660 cm^{-1}. Anal. calcd for $C_{11}H_{18}F_5NO_3$: C, 43.0; H, 5.9; N, 4.6. Found C, 42.9; H, 6.0; N, 4.5.

(4*S*) - 4 - Amino - 1,1,1,2,2 - pentafluoro - 3 - heptanol (10). Compound **7** (0.420 g, 1.31 mmol, only the major diastereomer) was dissolved in 3.6 M HCl/EtOAc (3.70 mL, 13.3 mmol) and stirred for 3 h. The solvent was evaporated to give a solid. The residue was dissolved in water (15 mL) for removal of the hydrochloride salt. pH was adjusted to 12.5 by addition of 1M aqueous NaOH. The aqueous layer was extracted with ether (4×20 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give the free amine **10** (0.200 g, 69%) as a white solid. In this case, the product also sublimed, which affected the yield. ^1H NMR (270 MHz, DMSO-d_6) 3.90 (ddd, J=23.0, 5.3, 5.3 Hz, 1H), 2.94–2.81(m, 1H), 1.62–1.39 (m, 2H), 1.38–1.16 (m, 2H), 0.91–0.79 (m, 3H). ^{13}C NMR (68 MHz, DMSO-d_6) 121.2 (qdd, J=287.3, 37.4, 35.4 Hz), 115.2 (ddq, J=261.2, 255.9, 34.6 Hz), 71.9 (dd, J=24, 20 Hz), 50.5, 33.8, 18.6, 14.0. IR (neat) 3378, 3330, 1613 cm^{-1}. Anal. calcd for $C_7H_{12}F_5NO$: C, 38.0; H, 5.5; N, 6.3. Found C, 38.1; H, 5.5; N, 6.2.

1-(1-Aminocyclopropyl)-2,2,3,3,3-pentafluoro-1-propanol (11). Compound **11** was prepared according to the procedure described for **10**, using **8** (0.770 g, 2.52 mmol), to give the product as an oil (0.490 g, 95%), which crystallized upon storage. In this case the product also sublimed. ^1H NMR (400 MHz, $CDCl_3$) δ 3.25 (dd, J=18.7, 6.7 Hz, 1H), 2.96 (br s, 2H), 0.97–0.81 (m, 2H), 0.71–0.62 (m, 1H), 0.61–0.53 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 119.1 (qdd, J=286, 35, 35 Hz), 114.8 (ddq, J=260, 258, 36 Hz), 73.4 (dd, J=24, 21 Hz) 34.2, 14.3, 13.0. IR (neat) 3386, 3324, 1611 cm^{-1}. Anal. calcd for $C_6H_8F_5NO$: C, 35.1; H, 3.9; N, 6.8. Found C, 35.5; H, 4.0; N, 6.5.

(4*S*) - 4 - Amino - 1,1,1,2,2 - pentafluoro - 3 - hexanol (12). Compound **12** was prepared according to the procedure described for **10**, using **9** (0.540 g, 1.76 mmol, 1:4 mixture of diastereomers). Concentration under reduced pressure was performed carefully and not to complete dryness, to minimize sublimation, giving **12** as a white solid (0.370 g, 101%) in a 1:4 mixture of diastereomers (A:B). ^1H NMR (400 MHz, $CDCl_3$) δ (A + B) 3.97 (ddd, J=24.2, 4.4, 4.4 Hz, 0.8H), 3.78–3.67 (ddd, J=23.6, 5.1, 1.1 Hz, 0.2H), 3.24 (m, 0.2H), 2.94 (m, 0.8H), 3.23–2.26 (br m, 2H), 1.88–1.40 (m, 2H), 1.03–0.95 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (A + B) 119 (m), 115 (m), 69.9 (dd, J=27, 21 Hz, B), 68.7 (dd, J=28, 21 Hz, A), 54.3 (B), 49.7 (A), 27.5 (A), 25.4 (B), 10.9(B), 10.4 (A). IR (neat) 3375, 3298, 1594 cm^{-1}. Anal. calcd for $C_6H_{10}F_5NO$: C, 34.8; H, 4.9; N, 6.8. Found C, 35.0; H, 5.3; N, 6.4.

(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-1-cyanopentyl acetate (15). Boc-Nva-H[52,53] **13** (2.63 g, 13.1 mmol) was dissolved in a mixture of THF (70 mL) and water (80 mL). KCN (3.40 g, 52.2 mmol) was added and the reaction mixture was stirred at room temperature for 1 h. The mixture was partitioned between water (100 mL) and EtOAc (200 mL). The water phase was further extracted with EtOAc (2×200 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (2×300 mL) and brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to yield crude cyanohydrin, which was directly protected: The crude cyanohydrin was dissolved in CH_2Cl_2 (100 mL). Acetic anhydride (2.46 mL, 26.1 mmol) and pyridine (2.11 mL, 26.1 mmol) were added and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 and washed with aqueous 1 M HCl (2×200 mL), H_2O (100 mL), saturated aqueous $NaHCO_3$ (2×200 mL), and brine (1×200 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (gradient system: 5% to 20% EtOAc/pentane) to afford **15** (3.28 g, 93%) as an oil in a 2:3 mixture of diastereomers. ^1H NMR (400 MHz, $CDCl_3$) δ 5.43 (br d, NH) and 5.38 (d, J=4.6 Hz, NH)(1H), 4.72–4.55 (m, 1H), 4.14–4.03 (m) and 4.02–3.90 (m)(1H), 2.14 (s) and 2.11 (s) (3H), 1.75–1.30 (m, 4H), 1.43 (s, 9H), 0.94 (dd, J=7.3, 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1,168.9, 155.5, 155.2, 115.6, 115.4, 80.7, 80.4, 64.7, 63.6, 51.3, 51.1, 32.5, 32.2, 20.5, 20.4, 19.1, 19.0, 13.8, 13.7. IR (neat) 3351, 1760, 1714, 1694 cm^{-1}. Anal. calcd for $C_{13}H_{22}N_2O_4\times1/5H_2O$: C, 57.0; H, 8.2; N, 10.2. Found C, 57.1; H, 8.0; N, 10.0.

{1-[(*tert*-Butoxycarbonyl)amino]cyclopropyl}(cyano)methyl acetate (16). Compound **16** was prepared according to the procedure described for **15** using Boc-ACPC-H[54,55] **14** (2.32 g, 12.5 mmol) and KCN (4.23 g, 64.9 mmol), to yield **16** (1.59 g, 50%). ^1H NMR (400 MHz, $CDCl_3$) δ 5.47 (s, 1H), 5.10 (br s, 1H), 2.15 (s, 3H), 1.44 (s, 9H), 1.30–0.79 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.1, 155.2, 115.7, 80.6, 64.8, 34.0, 20.5, 14.0, 11.9. IR (neat) 3345, 1752, 1686 cm^{-1}. Anal. calcd for $C_{12}H_{18}N_2O_4$: C, 56.7; H, 7.1; N, 11.0. Found C, 56.8; H, 7.3; N, 10.7.

(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-1-(1*H*-tetrazol-5-yl)-pentyl acetate (17). A mixture of **15** (2.68 g, 9.91 mmol), triethylamine hydrochloride (2.73 g, 19.8 mmol) (prepared from triethylamine and HCl/ether), and sodium azide (1.29 g, 19.8 mmol) in toluene (75 mL) was heated at reflux overnight. The reaction mixture was diluted with toluene and extracted with water (3×200 mL). The combined water layers were acidified to pH 1 with concentrated HCl, and extracted with EtOAc (3×400 mL). The combined organic phases were washed with brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure to give pure **17** (2.86 g, 92%). The product is a mixture of diastereomers and a pair of rotamers as deduced by NMR. ^1H NMR (400 MHz, $CDCl_3$) δ 6.33–6.05 (m, 1H), 5.7 (br d), 5.46 (br d), 5.41 (bd) and 5.24 (m) (1H), 4.30–4.06 (m, 1H), 2.11 (s) and 2.10 (s) (3H), 1.65–1.20 (m, 4H), 1.36 (s) and 1.35 (s)(9H), 0.95–0.85 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 170.2, 169.9, 156.7, 156.6, 82.0, 81.1, 80.8, 69.2, 68.5, 68.1,

54.8, 53.0, 52.5, 33.5, 33.0, 28.4, 28.1, 20.9, 20.7, 19.3, 19.2, 13.9, 13.7. IR (KBr) 3364, 1760, 1694 cm^{-1}. Anal. calcd for $C_{13}H_{23}N_5O_4$: C, 49.8; H, 7.4; N, 22.4. Found C, 49.6; H, 7.4; N, 22.3.

{1-[(*tert*-Butoxycarbonyl)amino]cyclopropyl}(1*H*-tetrazol-5-yl)methyl acetate (18). Compound **18** was prepared according to the procedure described for **17**, using **16** (12.5 mmol), to give **18** (1.98 g, 53% over three steps from compound **14**). NMR recorded at ambient temperature showed traces of a rotamer. However, at 55 °C free rotation was achieved. ^1H NMR (400 MHz, $CDCl_3$, 55 °C) δ 5.90 (s, 1H), 5.61 (br s, 1H), 2.13 (s, 3H), 1.38 (s, 9H), 1.22–1.2 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$, 55 °C) δ 169.9, 157.2, 155.1, 81.7, 70.1, 34.6, 28.4, 20.8, 15.4, 13.2. IR (KBr) 3366, 1732, 1698 cm^{-1}. Anal. calcd for $C_{12}H_{19}N_5O_4$: C, 48.5; H, 6.4; N 23.6. Found: C, 48.7; H, 6.6; N 23.3.

***tert*-Butyl (1*S*)-1-[hydroxy(1*H*-tetrazol-5-yl)methyl]butylcarbamate (19).** Aqueous 1 M K_2CO_3 (100 mL) was added to a solution of **17** (2.61 g, 8.34 mmol) in MeOH (100 mL), and the resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with water and acidified to pH 1 with concentrated HCl. The water phase was extracted with EtOAc (3×300 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure to give pure **19** (2.99 g, 99%) as a white solid. ^1H NMR (400 MHz, CD_3OD) 2:3 mixture of diastereomers δ 5.12 (d, $J=3.1$ Hz) and 4.93 (d, $J=6.3$ Hz)(1H), 3.91–3.78 (m, 1H), 1.70–1.20 (m, 4H), 1.35 (s) and 1.34 (s) (9H), 1.00–0.87 (m, 3H). ^{13}C NMR (100 MHz, CD_3OD) 2:3 mixture of diastereomers δ 159.7,159.5, 158.1, 157.9, 80.3, 80.2, 69.5, 68.5, 55.9, 34.0, 33.0, 28.80, 28.76, 20.5, 20.3, 14.29, 14.25, including traces of rotamers. (KBr) 3330, 1671 cm^{-1}. Anal. calcd for $C_{11}H_{21}N_5O_3$: C, 48.7; H, 7.8; N, 25.8. Found C, 48.8; H, 8.0; N, 25.6.

***tert*-Butyl 1-[hydroxy(1*H*-tetrazol-5-yl)methyl]cyclopropylcarbamate (20).** Compound **20** was prepared according to the procedure described for **19** using **18** (1.87 g, 6.29 mmol), to yield **20** (1.48 g, 92%) as a white solid. NMR recorded at ambient temperature showed traces of a rotamer. However, at 55 °C free rotation was achieved: ^1H NMR (400 MHz, CD_3OD, 55 °C) δ 4.89 (s, 1H), 1.35 (s, 9H), 1.21–1.05 (m, 2H), 0.93–0.75 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD, 55 °C) δ 159.5, 159.1, 80.9, 70.2, 37.5, 28.7, 12.7, 12.3. IR (KBr) 3284, 1665 cm^{-1}. Anal. calcd for $C_{10}H_{17}N_5O_3$: C, 47.1; H, 6.7; N, 27.4. Found C, 47.4; H, 6.8; N, 27.5.

(2*S*)-2-Amino-1-(1*H*-tetrazol-5-yl)-1-pentanol hydrochloride (21). Compound **19** (1.89 g, 6.97 mmol) were dissolved in 3.6 M HCl/EtOAc (20.0 mL, 72.0 mmol) and stirred for 3 h. The solvent was evaporated and the precipitated crude product was suspended in CH_2Cl_2, filtered, washed with more CH_2Cl_2, and dried in vacuo (50 °C) to yield pure **21** (1.36 g, 94%) as a white solid. ^1H NMR (270 MHz, CD_3OD) 2:3 mixture of diastereomers δ 5.45 (d, $J=3.6$ Hz) and 5.25 (d, $J=5.0$ Hz)(1H), 3.78–3.64 (m, 1H), 1.85–1.20 (m, 4H), 0.98 (dd, $J=7.3$, 7.3 Hz) and 0.91 (dd, $J=7.2$, 7.2 Hz) (3H). ^{13}C NMR (68 MHz, CD_3OD) 2:3 mixture of diastereomers δ 158.8, 158.4, 66.2, 65.4, 56.3, 56.2, 32.6, 30.8, 19.8, 19.6, 14.2. IR (neat) 3600–2400, 1594, 1500, 1065 cm^{-1}. Anal. calcd for $C_6H_{14}ClN_5O$: C, 34.7; H, 6.8; N, 33.7. Found C, 34.9; H, 6.9; N, 33.9.

(1-Aminocyclopropyl)(1*H*-tetrazol-5-yl)methanol hydrochloride (22). Compound **22** was prepared according to the procedure described for **21**, using **20** (1.28 g, 5.03 mmol), to yield **22** in quantitative yield (0.970 g). ^1H NMR (400 MHz, CD_3OD) δ 5.04 (s, 1H), 1.25–1.08 (m, 4H). ^{13}C NMR (100 MHz, CD_3OD) δ 158.2, 68.2, 39.0, 10.1, 9.0. IR (neat) 3600–2400, 1599, 1523, 1074 cm^{-1}. Anal. calcd for $C_5H_{10}ClN_5O$: C, 31.3; H, 5.3; N 36.6. Found: C, 31.2; H, 5.4; N 36.3.

***tert*-Butyl 1-{[(2-cyanoethyl)amino] carbonyl}butylcarbamate (23).** Boc-Nva-OH **1** (3.00 g, 13.8 mmol), 3-aminopropionitrile (1.02 mL, 13.8 mmol), dicyclohexylcarbodiimide (DCC) (2.85 g, 13.8 mmol), *N*-hydroxybenzotriazole (HOBT) (1.86 g, 13.8 mmol) were dissolved in DMF (50 mL) and stirred at 0 °C for 48 h. The reaction mixture was filtered and the solvent evaporated. The residue was chromatographed on silica gel (3:2 ethyl acetate/pentane) to yield **23** (3.10 g, 83%) as a white solid. $[\alpha]_D = -31.9°$ (*c* 1.0, MeOH). ^1H NMR (270 MHz, $CDCl_3$) δ 6.74 (br t, 1H, NH), 4.91 (br d, 1H, NH), 4.05 (m, 1H), 3.59–3.44 (m, 2H), 2.62 (dd, $J=6.4$, 6.4 Hz, 2H), 1.86–1.25 (m, 4H), 1.45 (s, 9H), 0.94 (dd, $J=7.3$, 7.3 Hz, 3H). ^{13}C NMR (68 MHz, $CDCl_3$) δ 173.4, 156.0, 118.2, 80.4, 54.5, 35.7, 34.7, 28.5, 19.0, 18.4, 13.9. IR (KBr) 2246, 1685, 1662 cm^{-1}. Anal. calcd for $C_{13}H_{23}N_3O_3$: C, 58.0; H, 8.6; N, 15.6. Found C, 58.1; H, 8.6; N, 15.4.

***tert*-Butyl 1-{[(2-cyanoethyl)amino] carbonyl}cyclopropylcarbamate (24).** Compound **24** was prepared according to the procedure described for **23**, using Boc-ACPC-OH **2** (1.02 g, 5.07 mmol), 3-aminopropionitrile (0.373 mL, 5.07 mmol), DCC (1.10 g, 5.32 mmol), HOBT (0.86 g, 5.32 mmol) and DMF (20 mL). The residue was chromatographed on silica gel (5% MeOH/dichloromethane) to yield **24** (1.09 g, 85%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 7.10–6.90 (br, 1H , NH), 5.18 (br s, 1H, NH), 3.53 (dt, $J=6.3$, 6.3 Hz, 2H), 2.61 (t, $J=6.3$ Hz, 2H), 1.56 (m, 2H), 1.46 (s, 9H), 1.04 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 156.0, 118.4, 81.4, 36.2 (C, NCH$_2$), 28.5, 18.7, 18.2. IR (neat) 2249, 1694, 1660 cm^{-1}. Anal. calcd for $C_{12}H_{19}N_3O_3$: C, 56.9; H, 7.6; N, 16.7. Found C, 57.2; H, 7.7; N, 16.6.

***tert*-Butyl 1-(1*H*-tetrazol-5-yl)butylcarbamate (25).** Diethylazodicarboxylate (DEAD) (2.6 mL, 16.6 mmol) was added dropwise to a mixture of **23** (1.77 g, 6.59 mmol), triphenylphosphine (4.33 g, 16.4 mmol), and trimethylsilyl azide (2.16 mL, 16.5 mmol) in THF (40 mL) at 0 °C under N_2 atmosphere. The mixture was slowly warmed to room temperature and stirred for 48 h. The reaction mixture was diluted with water and extracted with EtOAc (2×100 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (2×100 mL) and brine (100 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The residue was

chromatographed on silica gel (35% EtOAc/pentane) to give the cyanoethyl protected tetrazole (not completely pure), which was subsequently deprotected: 1 M Aqueous NaOH (9.60 mL, 9.60 mmol) was added to the tetrazole derivative dissolved in THF (60 mL) and stirred at room temperature for 5 h. The THF was evaporated and the residue diluted with saturated aqueous $NaHCO_3$ (100 mL) and washed with ether (3×100 mL). The water phase was acidified with 1M aqueous HCl and extracted with EtOAc (3×300 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), filtrered, and concentrated under reduced pressure to yield pure **25** (0.883 g, 56%) as a white solid. $[\alpha]_D = -46.2°$ (*c* 1.0, MeOH). ^1H NMR (270 MHz, CD_3OD) δ 4.95 (m, 1H, overlap with H_2O signal), 2.01–1.78 (m, 2H), 1.52–1.20 (m, 2H), 1.44 (s, 9H), 0.97 (dd, $J = 7.3$, 7.3 Hz, 3H). ^{13}C NMR (100 MHz, relaxation delay = 6 s, CD_3OD) δ 160.2, 157.8, 80.9, 47.2, 36.8, 28.8, 20.0, 13.9. IR (KBr) 3330, 1672, 1520 cm^{-1}. Anal. calcd for $C_{10}H_{19}N_5O_2$: C, 49.8; H, 7.9; N, 29.0. Found C, 49.8; H, 7.8; N, 28.6.

***tert*-Butyl 1-(1*H*-tetrazol-5-yl)cyclopropylcarbamate (26).** Compound **26** was prepared according to the procedure described for **25**, using **24** (2.83 g, 11.2 mmol), DEAD (4.39 mL, 27.9 mmol), triphenylphosphine (7.33 g, 27.9 mmol), trimethylsilyl azide (3.65 mL, 27.9 mmol) and THF (60 mL), with following exceptions: the reaction was stirred for 3 days, the cyanoethyl protected tetrazole was chromatographed on silica gel (gradient system: 35–75% EtOAc/pentane) and then treated with 1M aqueous NaOH (10.0 mL, 10.0 mmol) in THF (70 mL) and stirred at room temperature overnight, to yield **26** as a slightly yellow solid (0.365 g, 15%). ^1H NMR (400 MHz, CD_3OD) δ 1.57–1.51 (m, 2H), 1.46 (s, 9H), 1.44–1.38 (m, 2H). ^{13}C NMR (100 MHz, relaxation delay = 4 s, CD_3OD) δ 162.0, 158.4, 81.4, 28.8, 20.0. IR (neat) 3248, 1681, 1537 cm^{-1}. Anal. calcd for $C_9H_{15}N_5O_2 \times 1/10H_2O$: C, 47.6; H, 6.8; N, 30.8. Found C, 47.6; H, 6.6; N, 30.6.

1-(1*H*-Tetrazol-5-yl)-1-butanamine hydrochloride (27). Compound **25** (0.766 g, 3.17 mmol) was dissolved in 2.7 M HCl/EtOAc (12.0 mL, 32.4 mmol) and stirred for 3 h. Precipitated product was isolated by filtration, washed with EtOAc, and dried in vacuo to afford pure **25** (0.479 g, 85%) as a white solid. $[\alpha]_D = -7.0°$ (*c* 1.0, MeOH). ^1H NMR (400 MHz, CD_3OD) δ 4.81 (dd, $J = 7.1$, 7.1 Hz, 1H), 2.18–2.05 (m, 2H), 1.49–1.28 (m, 2H), 0.99 (dd, $J = 7.3$, 7.3 Hz, 3H). ^{13}C NMR (100 MHz, relaxation delay = 6 s, CD_3OD) δ 159.5, 47.8, 35.9, 19.4, 13.9. IR (KBr) 1548, 1073 cm^{-1}. Anal. calcd for $C_5H_{12}ClN_5$: C, 33.8; H, 6.8; N, 39.4. Found C, 33.7; H, 6.8; N, 39.3.

1-(1*H*-tetrazol-5-yl)cyclopropanamine hydrochloride (28). Compound **28** was prepared according to the procedure described for **27**, using **26** (0.336 g, 1.49 mmol) with the following exceptions: the crystals were washed with CH_2Cl_2 and dried in vacuo (60 °C) to afford **28** in quantitative yield. ^1H NMR (400 MHz, CD_3OD) δ 1.75–1.57 (m, 4H). ^{13}C NMR (100 MHz, CD_3OD) δ 162.4, 31.2, 15.0. IR (neat) 1578, 1042 cm^{-1}. Anal. calcd for $C_4H_8ClN_5$: C, 29.7; H, 5.0; N, 43.3. Found C, 29.5; H, 5.0; N, 43.0.

***tert*-Butyl (1*S*)-1-{[(phenylsulfonyl)amino]carbonyl}butylcarbamate (29).** 1,1-carbonyldiimidazole (CDI) (5.78 g, 35.7 mmol) was added to a solution of Boc-Nva-OH **1** (2.50 g, 11.5 mmol) in dry THF (200 mL) and stirred for 30 min. A mixture of DBU (2.58 mL, 17.3 mmol) and benzenesulfonamide (3.62 g, 23.0 mmol) in dry THF (20 mL) was added and the reaction mixture was stirred for a further 6 h. The solvent was reduced to half the initial volume by evaporation and thereafter was diluted in CH_2Cl_2 (300 mL) and washed with 5% aqueous citric acid (2×250 mL), water (250 mL) and brine (250 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (5% MeOH/$CHCl_3$) to yield **29** (2.64 g, 64%) as a white solid. $[\alpha]_D = -24.1°$ (*c* 1.0, MeOH). ^1H NMR (270 MHz, $CDCl_3$) δ 9.70 (br s, 1H), 8.20–7.85 (m, 2H), 7.67–7.59 (m, 1H), 7.58–7.47 (m, 2H), 5.08–4.85 (m, 1H), 4.07 (m, 1H), 1.83–1.62 (m, 1H), 1.59–1.17 (m, 3H), 1.43 (s, 9H), 0.86 (dd, $J = 7.3$, 7.3 Hz, 3H). ^{13}C NMR (68 MHz, $CDCl_3$) δ 170.4, 156.4, 138.7, 134.1, 129.1, 128.5, 81.3, 54.7, 33.1, 28.4, 18.8, 13.8. IR(neat) 3349, 1717, 1665 cm^{-1}. Anal. calcd for $C_{16}H_{24}N_2O_5S \times 2/3H_2O$: C, 52.2; H, 6.9; N, 7.6. Found: C, 52.4; H, 6.9; N, 7.2.

***tert*-Butyl 1-{[(phenylsulfonyl)amino]carbonyl}cyclopropylcarbamate (30).** Compound **30** was prepared according to the procedure described for **29** using Boc-ACPC-OH **2** (2.41 g, 12.0 mmol), CDI (6.42 g, 39.6 mmol), DBU (4.00 mL, 26.8 mmol) and benzenesulfonamide (4.99 g, 31.8 mmol). The reaction was heated to reflux for 24 h and then an additional 76 h. Purification by column chromatography on silica gel (5% MeOH/$CHCl_3$) gave **30** (3.68 g, 90%) as a white solid. ^1H NMR (270 MHz, $CDCl_3$) δ 9.69 (br s, 1H), 8.12–7.98 (m, 2H), 7.69–7.58 (m, 1H), 7.57–7.47 (m, 2H), 5.81–5.16 (br m, 1H), 1.57–1.46 (m, 2H), 1.42 (s, 9H), 1.12–1.02 (m, 2H). ^{13}C NMR (68 MHz, $CDCl_3$) δ 170.7, 156.1, 138.5, 133.8, 128.9, 128.3, 81.8, 36.5, 28.1, 19.1. IR (neat) 3331, 3262, 1710, 1686 cm^{-1}. Anal. calcd for $C_{15}H_{20}N_2O_5S$: C, 52.9; H, 5.9; N, 8.2. Found: C, 52.6; H, 6.1; N, 7.9.

***N*-[(2*S*)-2-Aminopentanoyl]benzenesulfonamide hydrochloride (31).** Compound **29** (2.48 g, 6.96 mmol) was dissolved in 3.6 M HCl/EtOAc (19.3 mL, 69.6 mmol) and stirred at room temperature for 3 h. The reaction was concentrated under reduced pressure to yield pure **31** (1.93 g, 95%) as a beige solid. $[\alpha]_D = +58.5°$ (*c* 1.0, MeOH). ^1H NMR (270 MHz, CD_3OD) δ 8.10–7.95 (m, 2H), 7.77–7.66 (m, 1H), 7.65–7.53 (m, 2H), 3.89 (dd, $J = 5.6$, 6.8 Hz, 1H), 1.86–1.66 (m, 2H), 1.43–1.11 (m, 2H), 0.81 (dd, $J = 7.3$, 7.3 Hz, 3H). ^{13}C NMR (68 MHz, CD_3OD) δ 169.6, 140.5, 135.3, 130.3, 129.4, 54.9, 34.1, 18.8, 14.0. IR (neat) 3500–2400, 1724 cm^{-1}. Anal. calcd for $C_{11}H_{17}ClN_2O_3S$: C, 45.1; H, 5.9; N, 9.6. Found: C, 45.4; H, 6.0; N, 9.3.

***N*-[(1-Aminocyclopropyl)carbonyl]benzenesulfonamide hydrochloride (32).** Compound **32** was prepared accord-

ing to the procedure described for **31**, using **30** (3.68 g, 10.8 mmol), to yield the **32** (2.69 g, 90%) as a beige solid. ^{1}H NMR (270 MHz, CD_3OD) δ 8.10–8.01 (m, 2H), 7.77–7.67 (m, 1H), 7.66–7.55 (m, 2H), 1.73–1.38 (m, 4H). ^{13}C NMR (CD_3OD, 68 MHz) δ 170.3, 140.4, 135.3, 130.2, 129.6, 37.4, 14.0. IR (neat) 3500–2400, 1686. Anal. calcd for $C_{10}H_{13}ClN_2O_3S$: C, H, N. C, 43.40; H, 4.73; N, 10.12. Found: C, 43.42; H, 4.84; N, 9.98.

***N*-[(2*S*)-2-Aminopentanoyl]benzenesulfonamide (33).** The hydrochloride **31** (1.88 g, 6.43 mmol) was dissolved in EtOH (50 mL). Propene oxide (4.95 mL, 70.7 mmol) was added. The resulting mixture was stirred and heated at 50 °C for 3 h. The precipitated product was collected by filtration and washed with diethyl ether and dried in vacuo (50 °C) to yield the zwitterion **33** (1.10 g, 67%) as a white solid. $[\alpha]_D = +14.0°$ (*c* 1.0, DMSO). ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.82–7.73 (m, 2H), 7.75–7.51 (br s, 1H); 7.44–7.33 (m, 3H), 3.55–3.20 (m, 1H, overlap with H_2O signal), 1.74–1.46 (m, 2H), 1.33–1.08 (m, 2H), 0.81 (dd, $J = 7.3$, 7.3 Hz, 3H). ^{13}C NMR (68 MHz, DMSO-d_6) 172.4, 145.4, 130.2, 127.7, 126.9, 54.7, 31.8, 17.7, 13.8. IR(neat) 3300–2450, 1620 cm^{-1}. Anal. calcd for $C_{11}H_{16}N_2O_3S$: C, 51.5; H, 6.3; N, 10.9. Found: C, 51.7; H, 6.3; N, 11.0.

***N* - [(1 - Aminocyclopropyl)carbonyl]benzenesulfonamide (34).** Compound **34** was prepared according to the procedure described for **33**, using the hydrochloride **32** (2.41 g, 8.71 mmol), to yield **34** (1.87 g, 89%) as a beige solid. ^{1}H NMR (270 MHz, DMSO-d_6) δ 8.09 (br s, 1H), 7.86–7.75 (m, 2H), 7.47–7.33 (m, 3H), 1.27–1.02 (m, 2H) 1.01–0.80 (m, 2H). ^{13}C NMR (68 MHz, DMSO-d_6) δ 172.5, 145.5, 130.2, 127.8, 127.1, 36.5, 12.2. IR (neat) 3300–2800, 1627 cm^{-1}. Anal. calcd for $C_{10}H_{12}N_2O_3S$: C, 50.0; H, 5.0; N, 11.7 Found: C, 50.3; H, 5.3; N, 11.6.

TentaGel-P-linker-Suc-OH (36). TentaGel-P-linker-2 **35** (2.50 g, 0.418 mmol) was swollen in CH_2Cl_2 (25 mL) for 10–20 min in a column equipped with a polypropylene frit. Succinic anhydride (0.418 g, 4.18 mmol) and DIEA (0.714 mL, 4.18 mmol) were added. The column was placed in an overhead mixer for 18 h. The resin was washed with CH_2Cl_2 (5×20 mL), treated with 5% TFA/CH_2Cl_2 (2×10 mL) and successively washed with CH_2Cl_2 (5×20 mL) and MeOH (5×20 mL). The resin **36** was dried in vacuo and analyzed by ^{13}C NMR. ^{13}C NMR (270 MHz, $CDCl_3$) δ 21.8 (CH_3), 28.3 (CH_2), 29.9 (CH_2), 38.7 (CH_2NH), 40.9 (resin), 56.3 (OCH_3), 67.0–75.5 (CH and PEG), 108.5, 111.2 (CH Ar), 130.0 (broad signal, resin), 135.0, 139.2, 145.5, 154.2 (ipso Ar), 167.3 (NHC=O), 171.0 (OC=O), 174.0 (HOC=O).

Preparation of TentaGel-P-linker-Suc-D-Glu(OFm)-Leu-Ile-Cha-OH (37a) and TentaGel-P-linker-Suc-Asp(OFm)-D-Glu(OFm)-Leu-Ile-Cha-OH (37b). Aliquots of the resin **36** (75 μmol) were swollen in DMF (2.3 mL) for 20–30 min in columns equipped with polypropylene frits or in Quest 210 columns. A freshly prepared solution of the amino acid Sil ester hydrochloride (H-AA-OSil×HCl, where AA = Asp(OFm), D-Glu(OFm), Leu, Ile or Cha)[45] (5 equiv) in CH_2Cl_2 (2 mL), a solution of HATU (4 equiv) in DMF (2 mL) and TMP (9 equiv) were added, in the written order. The columns were placed in an overhead mixer or magnetically stirred in the Quest 210 apparatus for 2 h. The resin was washed with DMF (8×5 mL) and CH_2Cl_2 (7×5 mL). Removal of the Sil group was accomplished by treatment with 5% TFA/CH_2Cl_2 (6 mL) for 20 min. Finally, the resin was washed with CH_2Cl_2 (7×5 mL) and MeOH (7×5 mL) and dried. The procedure was repeated to yield **37a** and **37b**, respectively. The resins were dried in vacuo and analyzed by amino acid analysis, ^{13}C NMR (DMSO-d_6, not shown), and LC–MS (after cleavage of a analytical sample). **37a**: MS (calcd 804.4) 805.5 ($M+H^+$). Amino acids analysis (on-resin) Glu, 1.11; Leu, 1.03; Ile, 0.98; Cha, 0.88 (corrupted). **37b**: MS (calc. 1097.6) 1098.6 ($M+H^+$). Amino acids analysis (on-resin) Asp, 1.08, Glu, 1.05; Leu, 0.98; Ile, 0.93; Cha, 0.95.

General procedure for the preparation of the inhibitors Suc-D-Glu-Leu-Ile-Cha-P1 (38–46a) and Suc-Asp-D-Glu-Leu-Ile-Cha-P1 (38–46b). Aliquots of the resins **37a** or **37b** (42 μmol) were swollen in DMF (and CH_2Cl_2) (1.7 mL) for 20–30 min in columns equipped with polypropylene frits or in Quest 210 columns. The P1 building block (**10**–**12**, **21**, **22**, **27**, **28**, **33** and **34**) (5 equiv) dissolved in DMF or CH_2Cl_2 (0.5 mL), a solution of HATU (4 equiv) in DMF (2 mL), and the base (DIEA or TMP, 4–14 equiv) were added to the resin in the written order. The final solvent composition was 4:1 DMF/CH_2Cl_2 (3.5 mL). The columns were placed in an overhead mixer or magnetically stirred in the Quest 210 apparatus for 2–5 h and then washed with DMF (8×5 mL). Removal of the fluorenylmethyl ester (Fm group) was accomplished by stirring the resin for 20 min in 25% piperidine/DMF. Successive washings were done with DMF (8×5 mL), CH_2Cl_2 (7×5 mL), 5% TFA/CH_2Cl_2 (3×5 mL), CH_2Cl_2 (7×5 mL), and finally MeOH (7×5 mL). The resins were dried in vacuo. Cleavage of the peptides from the resins was performed with UV irradiation (350 nm). Thus, aliquots of resin (50 mg) in MeOH (1.5 mL) were irradiated for 2 h under N_2-atmosphere in quartz tubes equipped with septa. The solid support was removed by filtration and the solvent evaporated to yield the crude residues as oils or solids, which were purified by preparative RP-HPLC (according to method A, B, or C). The fractions corresponding to the major peak were combined, lyophilized and subsequently redissolved in a small volume of H_2O/MeCN (occasionally supplemented with aqueous NH_4OAc to assist solubilization) for analysis by MS, analytical RP-HPLC and amino acid analysis. The concentration of the solutions was calculated from amino acid analysis data and aliquots containing 0.5 or 1 μmol were transferred to 2 mL vials and lyophilized. The purity of the target compounds was >95% according to the HPLC analyses (UV, 220 nm), unless otherwise stated. The average total yield based on the loading of TentaGel-P-linker-2 **35** (i.e., over 6–8 steps) was 10–25%. It should be noted that the photolysis releases only ca. 75% of the products, as determined

from amino acid analysis of the resin after cleavage. Some of the target peptides were oxidized before or after cleavage as described under the specific heading below.

Suc-D-Glu-Leu-Ile-Cha-Nva-C_2F_5 (38a). The compound was prepared according to the general method using **10** and TMP (4 equiv), with the exception that oxidation was performed prior to cleavage from the resin. Thus, the peptide resin comprising the alcohol **10** was swollen in CH_2Cl_2 (3.5 mL) for 20 min. Dess–Martin periodinane (5 equiv) was added and the resulting mixture rotated for 18 h. The resin was washed with CH_2Cl_2 (7×5 mL) and MeOH (7×5 mL). Final purification of **38a** was done according to method B. MS (calcd 827.4) 828.2 ($M+H^+$), 946.2 ($M+H^++H_2O$). Amino acids analysis Glu, 0.99; Leu, 1.01; Ile, 0.99; Cha, 1.00.

Suc-Asp-D-Glu-Leu-Ile-Cha-Nva-C_2F_5 (38b). The compound was prepared according to the general method using **10** and TMP (4 equiv), and oxidized and purified as described for compound **38a**. MS (calcd 942.4) 943.4 ($M+H^+$) 961.4 ($M+H^++H_2O$). Amino acids analysis Asp, 1.01; Glu, 1.03; Leu, 1.00; Ile, 0.97; Cha, 1.00.

Suc-D-Glu-Leu-Ile-Cha-ACPC-C_2F_5 (39a). The compound was prepared according to the general method using **11** and TMP (4 equiv), and oxidized and purified as described for compound **38a**. MS (calcd 811.4) 812.2 ($M+H^+$) 930.2 ($M+H^++H_2O$). Amino acids analysis Glu, 1.02; Leu, 0.99; Ile, 1.00; Cha, 0.99.

Suc-Asp-D-Glu-Leu-Ile-Cha-ACPC-C_2F_5 (39b). The compound was prepared according to the general method using **11** and TMP (4 equiv), and oxidized and purified as described for compound **38a**. MS (calcd 926.4) 927.4 ($M+H^+$) 945.4 ($M+H^++H_2O$). Amino acids analysis Asp, 1.02; Glu, 1.02; Leu, 1.00; Ile, 0.97; Cha, 0.99.

Suc-D-Glu-Leu-Ile-Cha-Abu-C_2F_5 (40a). The compound was prepared according to the general method using **12** and TMP (4 equiv), and oxidized as described for compound **38a**. Final purification of **40a** was done using method C. MS (calcd 813.4) 814.2 ($M+H^+$) 832.2 ($M+H^++H_2O$). Amino acids analysis Glu, 0.91; Leu, 0.93; Ile, 1.07; Cha, 1.08.

Suc-Asp-D-Glu-Leu-Ile-Cha-Abu-C_2F_5 (40b). The compound was prepared according to the general method using **12** and TMP (4 equiv), and oxidized and purified as described for compound **38a**. MS (calcd 928.4) 929.4 ($M+H^+$) 947.4 ($M+H^++H_2O$). Amino acids analysis Asp, 1.00; Glu, 1.02; Leu, 1.01; Ile, 0.97; Cha, 1.01.

Suc-Asp-D-Glu-Leu-Ile-Cha-Nva-CN_4H (41b). The compound was prepared according to the general method using **21** and DIEA (14 equiv), with the exception that oxidation was performed in solution since the on-resin procedure produced a complex mixture of compounds. Thus, the peptidic alcohol was cleaved from the resin (mixture of compounds, see the chemistry section) and purified by preparative HPLC according to method B and lyophilised. The resulting alcohol was dissolved in DMF/CH_2Cl_2 (4:1, 2.5 mL) and cooled to 0 °C. Dess–Martin periodinane (approximately 5 equiv) was added and the resulting reaction mixture was stirred for 7 h. The reaction was quenched with 10% aqueous $Na_2S_2O_3$ and the solvent evaporated. The product was purified according to method B to yield **41b** with a final purity of 91%. MS (calcd 892.5) 893.4 ($M+H^+$). Amino acids analysis Asp, 1.10; Glu, 1.05; Leu, 1.05; Ile, 0.95; Cha, 0.85.

Suc-D-Glu-Leu-Ile-Cha-ACPC-CN_4H (42a). The compound was prepared according to the general method using **22** and DIEA (14 equiv), and oxidized and purified as described for compound **38a**. The desired product was not the major component in the crude mixture after cleavage (before purification) as indicated by in the HPLC chromatogram. MS (calcd 761.4) 762.5 ($M+H^+$). Amino acids analysis Glu, 1.09; Leu, 0.98; Ile, 1.00; Cha, 0.94.

Suc-Asp-D-Glu-Leu-Ile-Cha-ACPC-CN_4H (42b). The compound was prepared according to the general method using **22** and DIEA (14 equiv), and oxidized as described for compound **38a**. Final purification of **42b** was carried out according to method C. The desired product was not the major component in the crude mixture after cleavage (before purification) as indicated by in the HPLC chromatogram. MS (calcd 876.4) 877.1 ($M+H^+$). Amino acids analysis Asp, 1.04; Glu, 1.03; Leu, 1.00; Ile, 0.96; Cha, 0.96.

Suc-D-Glu-Leu-Ile-Cha-1-(1*H*-tetrazol-5-yl)-1-butanamide (43a). The compound was prepared according to the general method using **27** and DIEA (14 equiv). Final purification of **43a** was done according to method B. MS (calcd 749.4) 750.6 ($M+H^+$). Amino acids analysis Glu, 1.02; Leu, 1.00; Ile, 0.97; Cha, 1.02.

Suc-Asp-D-Glu-Leu-Ile-Cha-1-(1*H*-tetrazol-5-yl)-1-butanamide (43b). The compound was prepared according to the general method using **27** and DIEA (14 equiv). Final purification of **43b** was performed according to method B. MS (calcd 864.5) 865.7 ($M+H^+$). Amino acids analysis Asp, 1.00; Glu, 1.05; Leu, 1.02; Ile, 0.97; Cha, 0.96.

Suc-D-Glu-Leu-Ile-Cha-1-(1*H*-tetrazol-5-yl)cyclopropanamide (44a). The compound was prepared according to the general method using **28** and DIEA (14 equiv). Final purification of **44b** was performed according to method B to yield **44a** as a (3:5) mixture of D-/L-Cha diastereomers. MS (calcd 733.4) 734.2 ($M+H^+$). Amino acids analysis Glu, 1.01; Leu, 1.04; Ile, 0.99; Cha, 0.95.

Suc-Asp-D-Glu-Leu-Ile-Cha-1-(1*H*-tetrazol-5-yl)cyclopropanamide (44b). The compound was prepared according to the general method using **28** and DIEA (14 equiv). Final purification of **44b** was carried out according to method B. MS (calcd 848.4) 849.6 ($M+H^+$). Amino acids analysis Asp, 1.00; Glu, 1.01; Leu, 1.02; Ile, 0.97; Cha, 1.02.

Suc-D-Glu-Leu-Ile-Cha-Nva-$NHSO_2Ph$ (45a). The compound was prepared according to the general method

using **33** and TMP (9 equiv). Final purification of **45b** was carried out according to method B. MS (calcd 864.4) 865.6 ($M+H^+$). Amino acids analysis Glu, 0.99; Leu, 1.02; Ile, 0.96; Cha, 1.02, Nva, 1.02.

Suc-Asp-D-Glu-Leu-Ile-Cha-Nva-$NHSO_2Ph$ (45b). The compound was prepared according to the general method using **33** and TMP (9 equiv). Final purification of **45b** was performed according to method B. MS (calcd 979.5) 980.4 ($M + H^+$). Amino acids analysis Asp, 1.01; Glu, 1.00; Leu, 1.03; Ile, 0.98; Cha, 0.99, Nva, 0.99.

Suc-D-Glu-Leu-Ile-Cha-ACPC-$NHSO_2Ph$ (46a). The compound was prepared according to the general method described above using **34** and TMP (9 equiv). Final purification of **46a** was performed according to method C. MS (calcd 848.4) 849.7 ($M+H^+$). Amino acids analysis Glu, 0.99; Leu, 0.98; Ile, 1.04; Cha, 0.99.

Suc-Asp-D-Glu-Leu-Ile-Cha-ACPC-$NHSO_2Ph$ (46b). The compound was prepared according to the general method described above using **34** and TMP (9 equiv). Final purification of **46b** was performed according to method C. MS (calcd 963.4) 964.3 ($M+H^+$). Amino acids analysis Asp, 1.02; Glu, 1.00; Leu, 0.96; Ile, 1.01; Cha, 1.00.

(1-Aminocyclopropyl)(hydroxy)acetic acid (47). The Boc-ACPC-H **14** (1.06 g, 5.73 mmol) was dissolved in a mixture of THF (60 mL) and water (80 mL). KCN (1.95 g, 29.9 mmol) was added and the reaction mixture was stirred at room temperature for 3 h. The mixture was extracted with EtOAc (2×150 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (2×150 mL) and brine (150 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure to yield crude cyanohydrin as an oil, which was directly hydrolyzed without further purification. The crude cyanohydrin was dissolved in a mixture of 1,4-dioxane (60 mL) and concentrated HCl (60 mL) and thereafter gently heated to reflux for 20 h. The reaction mixture was concentrated under reduced pressure to afford the crude hydrochloride salt of the α-hydroxy carboxylic acid as a brown paste. Propene oxide (6.0 mL, 85.6 mmol) was added to the hydrochloride salt dissolved in EtOH (70 mL). The reaction mixture was stirred and heated at 50 °C for 3 h, during which time the zwitterionic product precipitated. The reaction mixture was cooled to room temperature and filtered. The isolated product was washed with cold diethyl ether and dried in vacuo (38 °C) to yield the zwitterion **47** (0.310 g, 41%) as a white solid. ^{1}H NMR (270 MHz, DMSO-d_6) δ 3.60 (s, 1H), 0.83–0.75 (m, 1H), 0.73–0.56 (m, 3H). ^{13}C NMR (68 MHz, DMSO-d_6) δ 174.2, 68.1, 36.0, 6.8, 5.5. IR (neat) 3379, 3500–2250, 1636, 1581, 1542 cm^{-1}. Anal. calcd for $C_5H_9NO_3$: C, 45.8; H, 6.9; N, 10.7 Found: C, 46.0; H, 7.1; N, 10.3.

(1 - {[(9*H* - Fluoren - 9 - ylmethoxy)carbonyl]amino}cyclopropyl)(hydroxy)-acetic acid (48). The zwitterion **47** (0.246 g, 1.88 mmol) was dissolved in a mixture of 10% aqueous Na_2CO_3 (35 mL) and 1,4-dioxane (17 mL) and cooled to 0 °C. Fmoc-Cl (2.00 g, 7.74 mmol) dissolved in 1,4-dioxane (17 mL) was added dropwise to the stirred mixture. Stirring was continued at room temperature for 45 h and the pH was kept at around 10–11. 1 M aqueous HCl was added for adjustment to pH 8. The reaction mixture was washed with diethyl ether (4×90 mL). Ether (100 mL) was added to the aqueous layer, which was subsequently acidified to pH 2 with 1M aqueous HCl under vigorous stirring. The two phases were separated and the aqueous layer was extracted with additional ether (2×100 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$) and concentrated under reduced pressure to give the product **48** (0.222 g, 33%) as a white solid. ^{1}H NMR (270 MHz, DMSO-d_6) δ 7.88 (dd, $J=1.3$, 7.4 Hz, 2H), 7.77 (s, 1H), 7.70 (broad d, $J=7.4$), 7.41 (ddd, $J=1.3$, 7.4, 7.4 Hz, 2H), 7.31 (ddd, $J=1.2$, 7.4, 7.4 Hz, 2H), 4.32–4.11 (m, 3H), 3.96 (s, 1H), 1.01–0.75 (m, 2H), 0.70–0.50 (m, 2H). ^{13}C NMR (68 MHz, DMSO-d_6) δ 174.0, 156.4, 143.8, 140.7, 127.6, 127.1, 125.4, 120.1, 71.4, 65.5, 46.6, 35.2, 10.9, 10.1. IR (neat) 3446, 3269, 3500–2400, 1721, 1663 cm^{-1}. Anal. calcd for $C_{20}H_{19}NO_5$: C, 68.0; H, 5.4; N, 4.0. Found: C, 68.1; H, 5.7; N, 3.8.

General procedure for the SPPS of the inhibitors Suc-D-Glu-Leu-Ile-Cha-P1-OH (50–54a) Suc-Asp-D-Glu-Leu-Ile-Cha-P1-OH (49b, 50–54b). The peptide inhibitors **49b** and **50**–**54** were synthesized by standard Fmoc/*t*-Bu solid-phase peptide synthesis techniques using a parallel approach. H-Cys(Trt)-2-chlorotrityl resin and Fmoc-Phe-Wang resin were commercial products. The other starting resins; H-Nva-2-chlorotrityl resin, H-ACPC-2-chlorotrityl resin, H-allylglycine-2-chlorotrityl resin, and (1-aminocyclopropyl)(hydroxy)acetic acid-2-chlorotrityl resin (from **48**) were synthesized by loading of the corresponding Fmoc-protected amino acids using a slightly modified literature procedure.[68] In short, 2-chloro-tritylchloride resin (1.47 mmol/g, 500 mol), Fmoc-AA-OH (300 mol) and DIEA (1.2 mmol) were agitated for 60 min inCH_2Cl_2 (5 mL). End-capping was performed by washing with CH_2Cl_2/MeOH/DIEA (17:2:1) followed by CH_2Cl_2, DMF, and CH_2Cl_2. Peptide assembly was performed on a 33 mol scale using Fmoc-AA-OH (0.13 mmol), TBTU (0.13 mmol) and DIEA (0.26 mmol) in DMF (0.5 mL) with a coupling time of 60 min. The Fmoc group was removed by 25% piperidine in DMF for 1 + 10 min. Terminal acylation was accomplished by succinic anhydride (0.33 mmol) and DIEA (0.33 mmol) in DMF (0.5 mL) for 60 min. The peptides were cleaved from the resin in 0.5 mL TFA/H_2O/triethylsilane (18:1:1) for 60 min, and the resin was subsequently filtered off. The TFA was partially evaporated followed by precipitation of the crude peptide by addition of diethyl ether. The products were collected by centrifugation and washed several times with fresh diethyl ether, air-dried and purified by preparative RP-HPLC (method A, unless otherwise stated). The target compounds were lyophilized and subsequently redissolved in a small volume of H_2O/MeCN (occasionally supplemented with aqueous NH_4OAc to assist solubilization) for analysis by MS, analytical RP-HPLC and amino acid analysis. The concentration of

the solutions was calculated from amino acid analysis data and aliquots containing 1 μmol were transferred to 2-mL vials and lyophilized. The purity of the target compounds was >95% according to the HPLC analyses (UV, 220 nm). The average total yields were 35–45%. Analysis data are described under the specific heading below.

Suc-Asp-D-Glu-Leu-Ile-Cha-ACPC-COOH (49b). The compound was prepared according to the general SPPS method up to the HPLC purification, starting with **48** to yield the corresponding peptidic alcohol, which was oxidized in solution to yield **49b**. Thus, the crude peptide alcohol (38 mg, 44 μmol) was dissolved in DMF (2 mL) and cooled to 0 °C. Dess–Martin periodinane (171 mg, 404 μmol) was added and the resulting reaction mixture was stirred for 4 h. The reaction was quenched with 10% aqueous $Na_2S_2O_3$ and the solvent evaporated. The product was purified by RP-HLPC according to method C to yield the product as a white solid (15.7 mg, 43%). MS (calcd 852.4) 853.2 ($M + H^+$). Amino acids analysis Asp, 1.00; Glu, 1.00; Leu, 1.01; Ile, 0.99; Cha, 0.99.

Suc-D-Glu-Leu-Ile-Cha-Cys-OH (50a). MS (calcd 729.4) 730.1 ($M + H^+$). Amino acids analysis Glu, 1.00; Leu, 1.01; Ile, 1.01; Cha, 1.00; Cys, 0.98.

Suc-Asp-D-Glu-Leu-Ile-Cha-Cys-OH (50b). MS (calcd 844.4) 845.1 ($M + H^+$). Amino acids analysis Asp, 1.01; Glu, 0.99; Leu, 1.01; Ile, 1.00; Cha, 1.00; Cys, 0.99.

Suc-D-Glu-Leu-Ile-Cha-Nva-OH (51a). MS (calcd 725.4) 726.3 ($M+H^+$). Amino acids analysis Glu, 1.00; Leu, 1.00; Ile, 1.00; Cha, 1.01; Nva, 1.00.

Suc-Asp-D-Glu-Leu-Ile-Cha-Nva-OH (51b). MS (calcd 840.5) 841.2 ($M+H^+$). Amino acids analysis Asp, 1.01; Glu, 0.99; Leu, 1.00; Ile, 1.00; Cha, 1.00; Nva, 1.01.

Suc-D-Glu-Leu-Ile-Cha-ACPC-OH (52a). MS (calcd 709.4) 710.2 ($M+H^+$). Amino acids analysis Glu, 1.00; Leu, 1.00; Ile, 1.00; Cha, 1.00.

Suc-Asp-D-Glu-Leu-Ile-Cha-ACPC-OH (52b). MS (calcd 824.4) 825.2 ($M+H^+$). Amino acids analysis Asp, 1.00; Glu, 0.98; Leu, 0.99; Ile, 1.04; Cha, 0.99.

Suc-D-Glu-Leu-Ile-Cha-AllylGly-OH (53a). MS (calcd 723.4) 724.2 ($M+H^+$). Amino acids analysis Glu, 1.02; Leu, 0.99; Ile, 0.99; Cha, 1.00.

Suc-Asp-D-Glu-Leu-Ile-Cha-AllylGly-OH (53b). MS (calcd 838.4) 839.2 ($M+H^+$). Amino acids analysis Asp, 1.00; Glu, 1.02; Leu, 1.00; Ile, 0.98; Cha, 1.01.

Suc-D-Glu-Leu-Ile-Cha-Phe-OH (54a). MS (calcd 773.4) 774.2 ($M+H^+$). Amino acids analysis Glu, 1.00; Leu, 1.00; Ile, 0.99; Cha, 1.01; Phe 0.99.

Suc-Asp-D-Glu-Leu-Ile-Cha-Phe-OH (54b). MS (calcd 888.5) 889.2 ($M+H^+$). Amino acids analysis Asp, 0.99; Glu, 1.02; Leu, 1.01; Ile, 1.00; Cha, 1.00; Phe, 0.98.

Enzyme inhibition

The inhibition of the protease activity of the full-length HCV NS3 (protease-helicase/NTPase) was measured by a continuous fluorescent assay, as described previously.[39,40,69] Briefly, 1 nM enzyme was incubated in 50 mM HEPES pH 7.5, 10 mM DTT, 40% glycerol, 0.1% *n*-octyl-β-D-glucoside, 3.3% DMSO with 25 μM cofactor (KKGSVVIVGRIVLSGK, peptide 2K-NS4A) and inhibitor at 30 °C for 10 min, the reaction was initiated by addition of 0.5 μM substrate (Ac-DED(Edans)EEA-buψ[COO]ASK(Dabcyl)–NH_2).

Molecular modeling

Molecular modeling studies were performed with MacroModel (version 7.1) and the new Maestro interface (version 3).[74] All molecular calculations used the Amber* force field with the united atom charge set.[75,76] The effect of water as solvent was modeled by the GB/SA solvation method.[77] The crystal structure 1CU1 (chain A) was used as starting structure.[32] This structure was fitted to the force field and modified to be suitable for docking studies as described previously.[40] His 528 was assumed to have the proton in position 4. The docking studies were performed as reported. Since the acyl sulfonamides exceed the template residues of the crystal in the P1′ region this part was investigated thoroughly. Various conformations of the acyl sulfonamides were considered by rotating (i) the single bond between the Cα of residue P1 and the carbonyl group and (ii) the single bond between the amide-nitrogen and the sulfur in order to find possible interaction patterns in the P1′ region during docking.

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