Articles

Inhibition of Human Neutrophil Elastase. 4. Design, Synthesis, X-ray Crystallographic Analysis, and Structure−Activity Relationships for a Series of P_2-Modified, Orally Active Peptidyl Pentafluoroethyl Ketones

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A series of P_2-modified, orally active peptidic inhibitors of human neutrophil elastase (HNE) are reported. These pentafluoroethyl ketone-based inhibitors were designed using pentafluoroethyl ketone **1** as a model. Rational structural modifications were made at the P_3, P_2, and activating group (A_G) portions of **1** based on structure−activity relationships (SAR) developed from in vitro (measured K_i) data and information provided by modeling studies that docked inhibitor **1** into the active site of HNE. The modeling-based design was corroborated with X-ray crystallographic analysis of the complex between **1** and porcine pancreatic elastase (PPE) and subsequently the complex between **1** and HNE.

Introduction

Human neutrophil elastase (HNE) is a major granule proteinase of human neutrophils and one of a variety of destructive enzymes involved in phagocytosis. During this process, HNE is released and controlled by endogenous proteinase inhibitors. However, during some pathological conditions, intense neutrophil infiltration results in an imbalance between the amount of HNE and endogenous inhibitors.[1,2] The excess HNE which accumulates can then cause abnormal degradation of healthy tissue.[3,4]

HNE has been implicated in the degradation of a variety of connective tissue proteins, including elastin, collagen, laminin, fibronectin, and proteoglycan.[5] As a result, it is thought to have a major role in the development of diseases such as pulmonary emphysema, rheumatoid arthritis, cystic fibrosis, adult respiratory syndrome (ARDS), and chronic bronchitis.[6,7] Therefore, it would be of therapeutic interest to develop a synthetic inhibitor of HNE which would restore the balance between the free enzyme and the endogenous inhibitors. Since HNE has been implicated in a variety of different clinical ailments, the ideal HNE inhibitor should be available by oral (po), intravenous (iv), or aerosol administration.

There have been several reports describing both peptidic[8−10] and nonpeptidic[11−14] HNE inhibitors. Peptidyl pentafluoroethyl ketone **1** is a potent, orally active inhibitor of HNE[8b] and was selected to enter human clinical trials. With the aid of molecular modeling, inhibitor **1** was rationally modified at the P_2 and P_3 sites in an attempt to improve its affinity for the catalytic site of the enzyme.

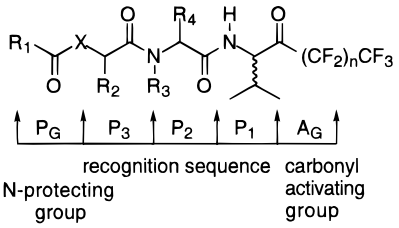
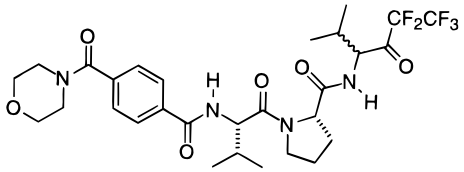


Figure 1. Generalized structure of tripeptidyl elastase inhibitors (P_G-P_3-P_2-P_1-A_G).



1 (MDL 101,146)

In parts 2[8b] and 3[8a] of this series, the effects of varying the N-protecting group (P_G) portion and the P_1 portion of inhibitor **1** (Figure 1) were reported. In an effort to explore the effects of modifying the P_3, P_2, and activating group (A_G) portions[15] of the inhibitor, computer modeling was used to guide the synthetic design.

Chemistry

The synthesis of **1** started with commercially available Boc-Val-OCH_3 **2a**. The HCl salt of Val pentafluoroethyl ketone **3**, prepared by the alkylation of **2a** with

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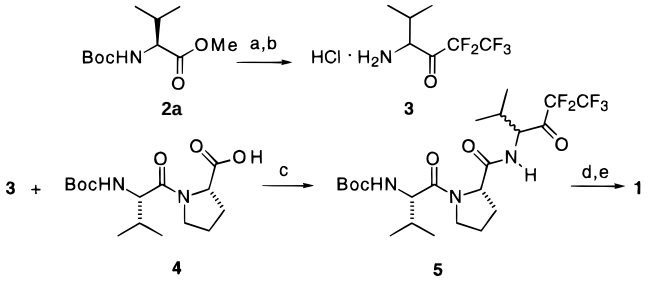
Scheme 1. Synthesis of **1** (MDL 101,146)[a]



[a] Reagents: (a) ICF_2CF_3, MeLi·LiBr, −78 °C; (b) HCl(g), EtOAc, rt; (c) IBCF, NMM, −20 °C; (d) HCl(g), EtOAc, rt; (e) 4-[(4-morpholinyl)carbonyl]benzoyl chloride, NMM, −20 °C.

pentafluoroethyllithium,[16] was coupled to the commercially available protected dipeptide **4** via the mixed anhydride (method I). Treatment of **5** with HCl (gas) provided the corresponding salt which was stored in a refrigerator prior to use. Pentafluoroethyl ketone **5**, following deprotection, was coupled with 4-[(4-morpholinyl)carbonyl]benzoyl chloride to give **1** (Scheme 1). Our strategy in preparing analogues of **1** was to follow this route, when possible, substituting the desired peptide/nonpeptide residue for the Boc-Val-Pro-OH portion of the molecule in the sequence.

The syntheses of constrained analogues **15**−**18** were carried out by coupling the appropriate carboxylic acid to pentafluoroethyl ketone **3** (Scheme 2). *N*-Boc-3-amino-2-oxopiperidineacetic acid **8** was readily synthesized from *N*-α-(*tert*-butoxycarbonyl)-L-ornithine via reductive alkylation with glyoxylic acid followed by intramolecular cyclization.[17] The corresponding γ-lactam was prepared from the sulfonium salt of Boc-L-Met-Gly-OMe followed by intramolecular cyclization to give **6**. Both lactams have the S configuration at the α-position. Coupling of acids **6** and **8** to the pentafluoroethyl ketone **3** gave products **9** and **11**, respectively. Deprotection and acylation with 4-[(4-morpholinyl)-carbonyl]benzoyl chloride gave the respective constrained inhibitors **15** and **18**.

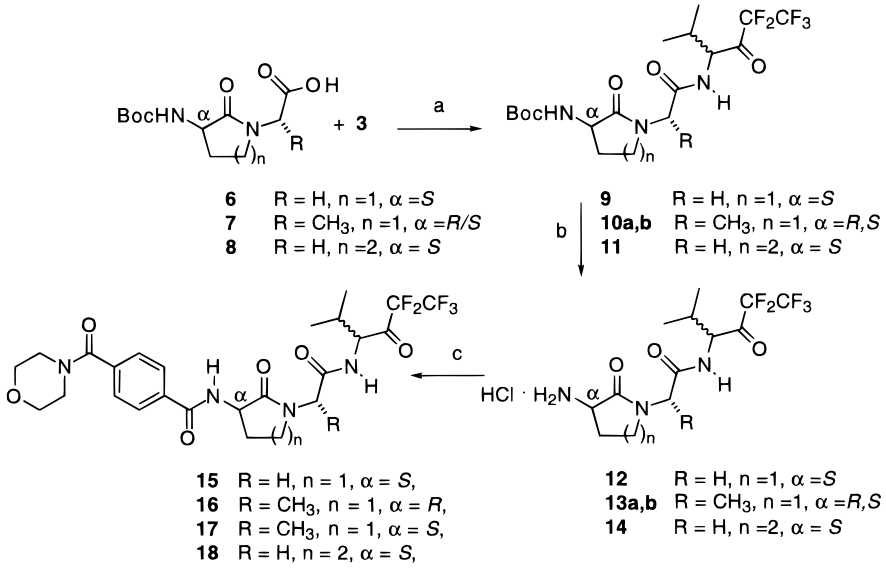
The intramolecular cyclization of Boc-D-Met-Ala-OMe, under similar conditions, gave an inseparable mixture of diastereomeric carboxylic acids **7** as evidenced by the lack of coalescence of the alanyl α-proton quartets in the NMR spectrum. Although the site of racemization is not known, it is speculated to be at the lactam α-carbon, since proton abstraction at this position and cyclization to an intermediate spirocyclopropane (which could proceed by ring opening with iodide followed by intramolecular cyclization to the lactam) would give a mixture of diastereoisomers. Coupling of acid **7** with pentafluoroethyl ketone **3**, using the mixed anhydride method (method I), gave the easily separable diastereomeric pentafluoroethyl ketones **10a**,**b** in 75% yield. When HOBT/EDC was used as the coupling reagent, only a 19% yield of mixture **10a**,**b** was obtained. Finally, deprotection and acylation of **10a**,**b** gave the isomeric products **16** and **17**, respectively.

The phenylketomethylene and morpholinoketomethylene isosteres of the P_G-Val portion of inhibitor **1** were prepared as both *R* and *S* enantiomers. The corresponding keto acids **19a**,**b** and **20a**,**b** were then coupled respectively to Pro-Val pentafluoroethyl ketone **21** to afford isosteres of inhibitor **1** (Scheme 3). To accomplish this as efficiently as possible, pentafluoroethyl ketone **21** was synthesized by coupling Boc-Pro-OH via the mixed anhydride method (method I) to **3** in 90% yield. Deprotection and coupling to the appropriate unit provided the desired isosteric analogues **22** and **23a**,**b**. Attempts to couple the *R* enantiomer **19a** with **21** resulted in internal cyclization of the starting keto acid **19a** to give a β-enollactone, and none of the desired coupled product was isolated.

To complement this strategy, the P_3 D-valine and dehydrovaline analogues, **32** and **33**, were readily synthesized by coupling the corresponding Boc-protected D-Val or dehydrovaline amino acids, **24** and **25**, to the methyl ester of proline hydrochloride using BOP (coupling method IV, Scheme 4). Hydrolysis to the corresponding acid gave a dipeptide intermediate analogous to **4** (in Scheme 1). Coupling to **3**, deprotection, and acylation gave P_3 analogues **32** and **33**.

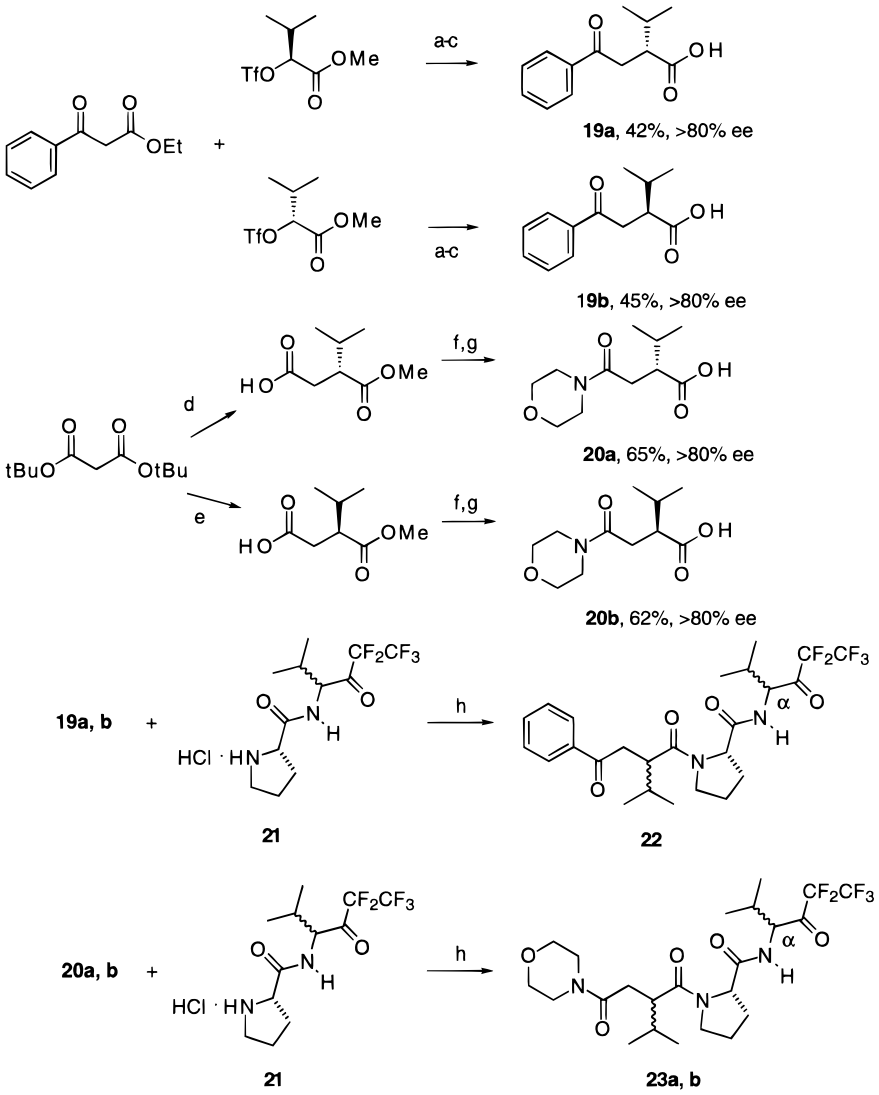
The P_2-modified analogues **54**−**56** (Scheme 5) were synthesized using the same sequence of coupling reac-

Scheme 2. Synthesis of P_2/P_3-Constrained Analogues **15**−**18**[a]



[a] Reagents: (a) IBCF, NMM, −20 °C; (b) HCl(g), EtOAc, rt; (c) 4-[(4-morpholinyl)carbonyl]benzoyl chloride, NMM, −20 °C.

Scheme 3. Synthesis of P_3 Isosteres **22** and **23a**,**b**[a]



[a] Reagents: (a) NaH, THF; (b) LiOH, H_2O; (c) H^+/PhH, Δ; (d) NaH, DMF, (*S*)-TfOCH$(CH_3)_2CO_2$Me, 2,6-lutidine; (e) NaH, DMF, (*R*)-TfOCH$(CH_3)_2CO_2$Me, 2,6-lutidine; (f) EDCI, HOBt, DMF, morpholine; (g) LiOH, H_2O; (h) EDCI, HOBt, NMM, CH_2Cl_2/DMF.

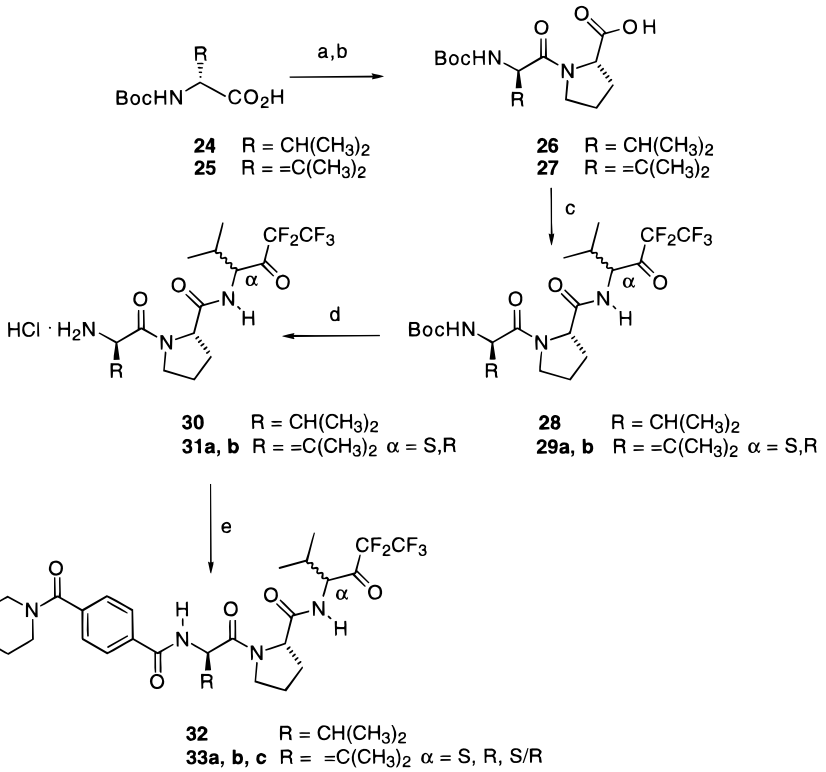
tions shown in Scheme 1. Coupling the P_2 unit with Boc-Val-OSu **2** (method II) gave dipeptides **39**−**42**. Coupling of these dipeptide units to **3** followed by deprotection and acylation with 4-[(4-morpholinyl)-carbonyl]benzoyl chloride gave the corresponding inhibitors **52**−**56**. In some cases, different coupling reagents were used to optimize the yield in the more difficult coupling reactions such as those incorporating the hindered homoproline and tetrahydroisoquinolinecarboxylic acids (vide infra). In the case of the 4-substituted proline analogues, derivatization of the 4-hydroxyl group had to precede incorporation of the hydroxyproline unit into the tripeptide. Attempts to acylate intermediate **45** directly led to complex mixtures and incomplete reactions. During the deprotection of compound **45**, a minor product was isolated and identified as the acetoxy derivative **50**. This intermediate was carried through the same sequence of reactions as hydroxy and *O*-benzyl intermediates **49** and **51**, respectively, to give **55**. Intermediates **49** and **51** gave products **54** and **56**, respectively.

The thiaproline analogue **59** (Scheme 6) and isoquinoline analogues **62a,b** (Scheme 7) were prepared as described for the other P_2 analogues. Coupling of L-thiaproline and tetrahydro-1-isoquinolinecarboxylic acid to Boc-Val-OSu **2** (method II) provided the dipeptides **57** and **60**, respectively. Coupling of dipeptides **57** and **60** to pentafluoroethyl ketone **3** followed by deprotection and acylation with 4-[(4-morpholinyl)-carbonyl]benzoyl chloride gave the corresponding inhibitors **59** and **62a**,**b**.

Positional isomers **69** and **70** were also prepared following the general synthetic scheme described for inhibitor **1**. Formation of dipetides **63** and **64** using coupling method II and subsequent coupling to **3** provided pentafluoroethyl ketones **65** and **67**. Deprotection of these *N*-*tert*-butyloxycarbonyl intermediates and acylation with 4-[(4-morpholinyl)carbonyl]benzoyl chloride provided **69** and **70** (Scheme 8).

In a previous report[8a] conditions are described for the direct nucleophilic perfluoroalkylation of peptide esters to form the corresponding perfluoroalkyl ketones, which is the procedure chosen for the preparation of heptafluoropropyl ketone **73**. Thus, the dipeptide unit **4** was coupled with Val-OMe·HCl to provide **71** in quantitative yield. Nucleophilic perfluoroalkylation with heptafluo-

Scheme 4. Synthesis of P_3 Analogues **32** and **33**[a]



[a] Reagents: (a) ProOMe·HCl, BOP, Et_3N, CH_3CN, rt; (b) LiOH, MeOH/H_2O, rt; (c) HCl·ValCF_2CF_3 (**3**), IBCF, NMM, −20 °C; (d) HCl(g), EtOAc, rt; (e) 4-[(4-morpholinyl)carbonyl]benzoyl chloride, NMM, −20 °C.

ropropyllithium gave heptafluoropropyl ketone **72**, which was deprotected and acylated under the usual conditions to give the heptafluoropropyl ketone **73** (Scheme 9).

Pharmacological Evaluation

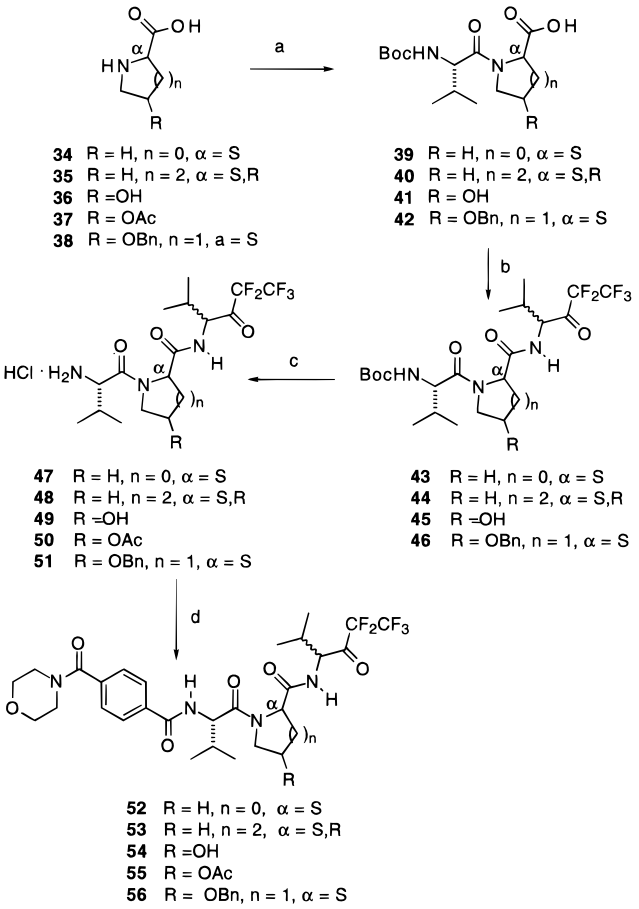
The in vitro HNE inhibitory activity (K_i)[18] and in vivo oral potency of selected compounds are listed in Table 1. The hamster HNE-induced pulmonary hemorrhage model, described previously,[19] was used as the primary screening model to determine if the compounds, when orally administered, were effective inhibitors of HNE-induced pulmonary hemorrhage. Intratracheal (i.t.) instillation of HNE into rodents induced acute pulmonary hemorrhage which was measured by the hemoglobin content in the bronchoalveolar fluid.[20]

In a routine screening protocol, 25 and 50 mg/kg of the test compound were administered, as a suspension in 20% Emulphor (GAF Corp.), orally by gavage 45 min prior to i.t. instillation of 100 μg of HNE/animal. One hour later the animals were sacrificed, the lungs were lavaged with saline, and the amount of hemoglobin in the fluid was measured spectrophotometrically at 536−546 nm. The percent inhibition was calculated as the ratio of pulmonary hemorrhage (BAL Hgb) in the drug-treated animals compared to the vehicle-treated animals. For those compounds with activity at the screening dose, a dose−response curve was obtained. The duration of action of selected compounds was determined by administering compound orally at various times before i.t. In vivo HNE inhibitory activity, at various time points, is presented in Table 2.

Results and Discussion

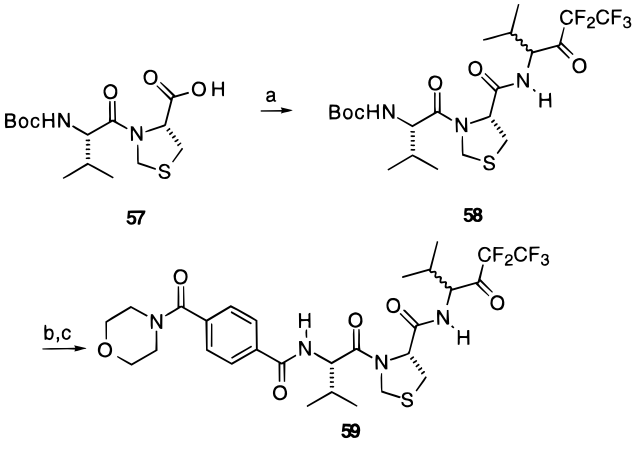
Construction of the Complex between HNE and Inhibitor 1. HNE inhibitor **1** is a reversible, mechanism-based[21] inhibitor of elastase. The pentafluoroethyl functionality activates the carbonyl group of **1**, which corresponds to the scissile amide carbonyl group of substrate, to promote nucleophilic addition at the electrophilic carbonyl group and afford a stable hemiketal with the hydroxyl group of Ser 195 at the active site of the enzyme.[22] Since fluorinated ketone inhibitors have a tendency to epimerize at the chiral center α to the ketone,[23] inhibitor **1** was prepared as approximately a 1:1 mixture of diastereomers. Since the natural substrates of elastase contain only L-peptides and inhibitor **1** is a tripeptide, only the L-stereoisomer was modeled in the enzyme. Coordinates from the Brookhaven Protein Data Bank[24] (code 1HNE) were used for constructing models of the enzyme−inhibitor complex.[25] The structure of HNE inhibited by methoxysuccinyl-Ala-Ala-Pro-Ala chloromethyl ketone as the hemiketal, covalently bonded to Ser 195, was used to model all subsequent inhibitors using the corresponding atoms in the crystal structure to guide placement of analogous recognition units in our inhibitors. The initial model was constructed using inhibitor **1**. The binding site was defined by any protein residue having any atom located within 8 Å from any atom of the inhibitor in its initial conformation. Protein atoms and crystallographic water outside the binding site were held fixed during all calculations. The average structure for the complex was determined and the final structure was minimized and used to rationalize modifications to the lead compound (Figure 2). This model was validated by the X-ray

Scheme 5. Synthesis of P_2 Analogues **54**−**56**[a]



[a] Reagents: (a) Boc-Val-OSuc (**2**), DMF, Et_3N, Δ; (b) HCl·$ValCF_2CF_3$ (**3**), IBCF, NMM, −20 °C; (c) HCl(g), EtOAc, rt; (d) 4-[(4-morpholinyl)carbonyl]benzoyl chloride, NMM, −20 °C.

Scheme 6. Synthesis of Thiazoles **59**[a]

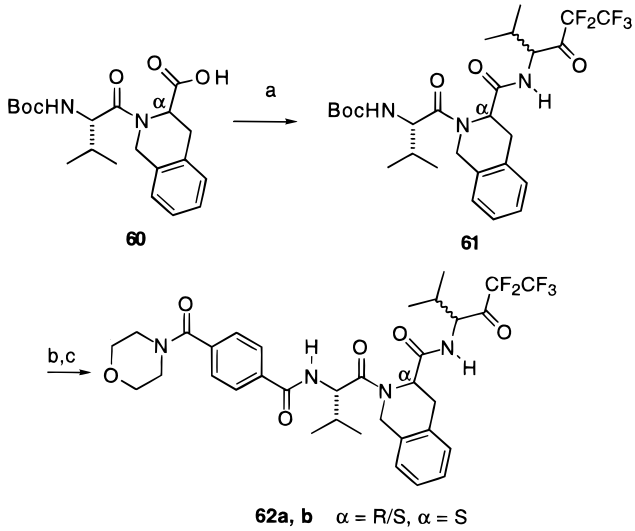


[a] Reagents: (a) HCl·$ValCF_2CF_3$ (**3**), IBCF, NMM, −20 °C; (b) HCl(g), EtOAc, rt; (c) 4-[(4-morpholinyl)carbonyl]benzoyl chloride, NMM, −20 °C.

crystal structure of **1** cocrystallized in porcine pancreatic elastase (PPE) and subsequently complexed with HNE. The high-resolution (1.8 Å) crystal structure of the PPE complex showed that only the LLL-diastereomer binds to the active site, even though cocrystallization was done with a racemic mixture.

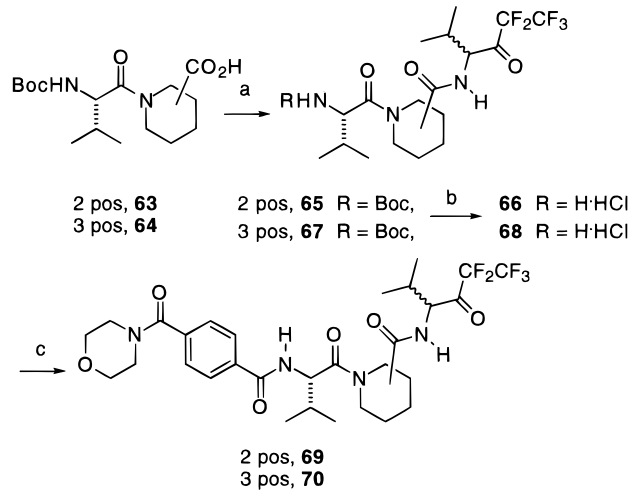
Conformationally Constrained Analogues of Inhibitor 1. The primary specificity site of HNE (S_3) will accommodate small lipophilic residues at P_3 such as

Scheme 7. Synthesis of Isoquinolines **62a**,**b**[a]



[a] Reagents: (a) HCl·$ValCF_2CF_3$ (**3**), IBCF, NMM, −20 °C; (b) HCl(g), EtOAc, rt; (c) 4-[(4-morpholinyl)carbonyl]benzoyl chloride, NMM, −20 °C.

Scheme 8. Synthesis of P_2-Positional Isomers **69** and **70**[a]

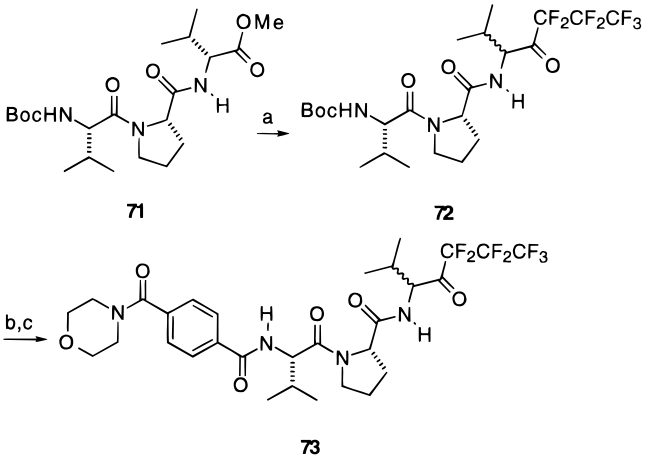


[a] Reagents: (a) HCl·$ValCF_2CF_3$ (**3**), IBCF, NMM, −20 °C; (b) HCl(g), EtOAc, rt; (c) 4-[(4-morpholinyl)carbonyl]benzoyl chloride, NMM, −20 °C.

valine or isoleucine.[26] While the predominant local feature of the S_2 and S_3 subsites of the enzyme is the presence of hydrophobic pockets, there are several oxygen atoms that line the interface of these subsites, namely, HO Tyr 94, O Pro 98, and O Val 99. The side chains of Asn 61 and Asn 99 may also contribute carbonyl oxygens to this array. Substitution in the P_2 and P_3 positions of the inhibitor could exploit interactions with these oxygen atoms as well as with the neighboring hydrophobic areas.

Conformationally constrained analogues were prepared by substituting five- and six-membered cyclic lactams for the P_3 valine and P_2 proline of inhibitor **1**. These were intended to simulate a tethered Val-Pro serving as a P_3-P_2 dipeptide surrogate (Figure 3). For synthetic simplicity the γ-carbon of L-valine was omitted. The use of a lactam in this manner has been shown to improve metabolic stability of substrate-based inhibitors and improved potency.[27]

Scheme 9. Synthesis of A_G Analogue **73**[a]



[a] Reagents: (a) $ICF_2CF_2CF_3$, MeLi·LiBr, −78 °C; (b) HCl(g), EtOAc, rt; (c) 4-[(4-morpholinyl)carbonyl]benzoyl chloride, NMM, −20 °C.

Table 1. In Vitro and In Vivo HNE Inhibitory Activity of Selected Compounds in the Hamster[a]

compd no.	K_i (nM)	% inhibition (n)[b]
1	20	74
33c[d]	340	0
52[c]	34	79*
53	150	54*
54	120	0
55	40	0
56	20	7
59	70	0
62a	100	NT
73	18	47*

[a] See Pharmacological Evaluation section for methodology. The compounds (25 mg/kg) were administered orally to hamsters 30 min before i.t. instillation of HNE (25 μg). [b] Percent inhibition of lung hemorrhage is presented as the mean ± SEM of BAL Hgb from 6−8 hamsters (*p < 0.05 is the criterion for statistical significance). [c] Compound given 15 min prior to administration of HNE. [d] 50 mg/kg of compound p.o. NT, not tested.

Table 2. In Vivo Inhibitory Activity of Selected Compounds at Various Time Points in the Hamster

		% inhibition[a]			
compd no.	dose (mg/kg)	1 h	1.5 h	2 h	4 h
52	25	57*			
	50			0	0
53	25	43*		39	
	50	73*		49*	52*
55	25			0	0
56	50		44		
73	25	30		25	

[a] Compounds were administered orally to hamsters at the times indicated before i.t. instillation of HNE (25 μg). Percent inhibition of lung hemorrhage is presented as the mean ± SEM of BAL Hgb from 6−8 hamsters (*p < 0.05 is the criterion for statistical significance).

The constrained analogues of **1** showed a loss in binding affinity with respect to **1**, e.g., **15** (K_i = 400 μM), **16** (K_i = 14 μM), **17** (K_i = 1100 μM), and **18** (K_i = 95 μM). The restricted conformation of these compounds had shifted the backbone of the inhibitor enough that the 5-amino group and carbonyl function of the lactam and the Val 216 and Val 214 of the enzyme were now not within hydrogen-bonding distances. In contrast to **17**, with the natural *S* configuration at P_3, **16**, with the unnatural *R* configuration, did not show a change in the position of the backbone carbons relative to **1** in the model. There was a good overlap of low-energy conformations between the two compounds in the active site of the enzyme. When **15** was modeled in HNE, a hydrophobic pocket was present at the P_2 glycine site. To provide a better fit with this pocket, the glycine at P_2 was replaced with an alanine residue to give compound **16**. Although **16** showed improved binding affinity, the binding was 1000-fold less than with **1**.

The corresponding γ-lactam, with the *R* configuration at the α-carbon, was prepared. An alanine residue was substituted for glycine at P_2, e.g., compound **18**, to accommodate a hydrophobic pocket observed in the model of this inhibitor docked with the enzyme at this site. These subunits provided constrained analogues of **1** without replacing the peptide backbone of the original tripeptide-based structure.

Isosteric Replacement of the P_3 Val and P_3 Analogues. The carbonyl oxygen of P_3 Val of inhibitor **1** accepts a hydrogen bond from the amide nitrogen of Val 216 of the enzyme, while the amide nitrogen of P_3 Val donates a hydrogen bond to the carbonyl group of Val 216. The strength of this latter hydrogen bond is dependent on the orientation of the amide plane between the P_3 Val and the aromatic ring. The dihedral angle (Ψ_1) is −82° (−32° in the X-ray structure of **1** + PPE, Figure 4). Thus, the internal strain energy of this angle is more than compensated by the strength of the resulting hydrogen bond.

To explore the importance of the hydrogen bonding of the inhibitor Val 216 NH to the carbonyl of Val 216 of the enzyme observed from the model (Figure 5), ketomethylene isosteres of the P_G-Val portion of inhibitor **1** were prepared as both *R* and *S* enantiomers.[28] These analogues, e.g., **22** (K_i = 4.2 μM, (*S*)-ketomethylene), **23a** (K_i = 41 μM, (*R*)-ketomethylene), and **23b** (K_i = 9.8 μM, (*S*)-ketomethylene), while showing better affinity for the enzyme than the constrained analogues, clearly demonstrated the importance of the P_3 valine amide group for enzyme affinity. Replacing the P_3 valine with the D-stereoisomer **32** (K_i = 5.5 μM) and dehydrovaline **33** (K_i = 340 nM) demonstrated the importance of the L-stereocenter at P_3. Compound **32** showed a 100-fold loss in activity versus inhibitor **1**. Dehydro analogue **33c** showed only a 10-fold loss of binding affinity (K_i = 340 nM). With the absence of the stereocenter at P_3, the L- and D-diastereomers of **33c** at P_1 were separated to give the L-isomer **33a** (K_i = 240 nM) and the D-isomer **33b** (K_i = 1100 nM). These differences in affinity clearly demonstrate that the L-stereocenters of the P_1 and P_3 valines are critical for optimal binding activity. The observed in vitro results substantiated the hydrogen-bonding interactions between the P_3 unit of the inhibitor and Val 216 of the enzyme suggested by the model and also the importance of the stereochemistry at the P_3 valine of the inhibitor.

P_2-Positional Isomers, Azetidine, Homoproline, Thiazolidine, Isoquinoline, and 4-Substituted Proline Analogues. The S_2 pocket of the HNE enzyme is hydrophobic. In particular, the side chain of Leu 99 and the hydrophobic face of His 57 help to delineate this region. The upper interface of the pocket is bordered by the carbonyl oxygen of Pro 98 and the side chain of Tyr 94. There appears to be a hydrophobic cavity proximal to the 4-position of the proline residue of the

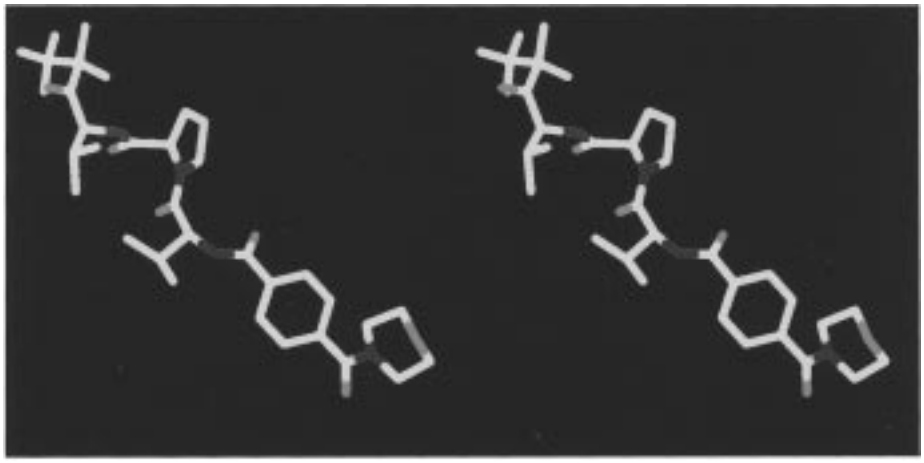


Figure 2. Stereoview of inhibitor **1**. Only the non-hydrogen atoms are shown for clarity. The coordinates of the inhibitor were taken from the crystal structure of the complex of inhibitor **1** with PPE.

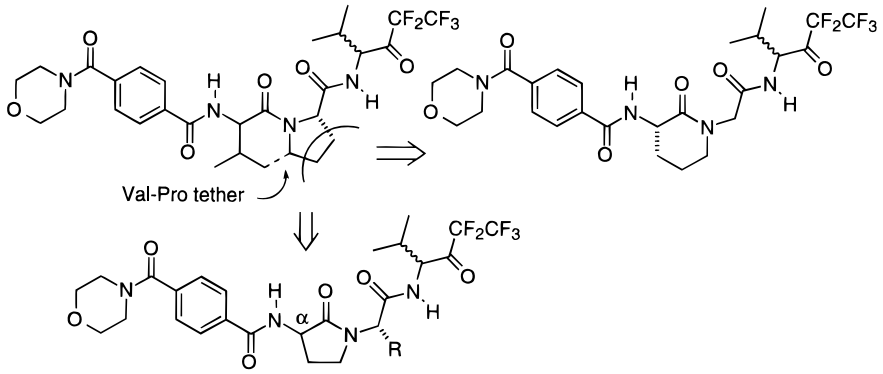


Figure 3.

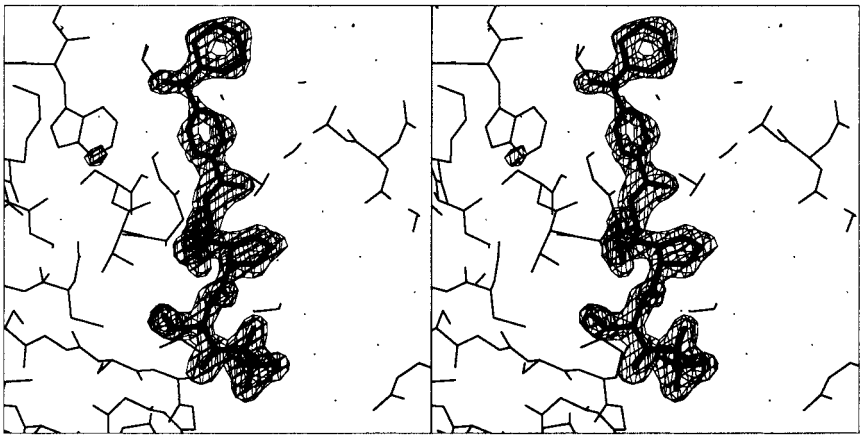


Figure 4. Stereodiagram of the electron density of the bound inhibitor **1** and PPE. The protein molecule is drawn in thin lines, the inhibitor in thick lines. To calculate this map, the inhibitor was omitted from the model and an $F_{obs} - F_{calc}$ difference map was calculated. This means that not all the information of the bound inhibitor is present in the phases and that the electron density visible is determined solely by the experimental (reflection) data. The map is contoured at 4σ.

inhibitor. Placement of the appropriate lipophilic group in this region via substitution or modification of the P_3 and/or P_2 residues could result in better inhibitor–enzyme interaction.

The P_2 proline of inhibitor **1** was replaced with nipecotic and isonipecotic acids which provided positional isomers of inhibitor **1**. This introduced an additional carbon into the backbone of the molecule which affected the hydrogen bonding between the inhibitor and the enzyme. Azetidinone and homoproline analogues were synthesized to observe the effect of ring size on in vitro and in vivo activity. A thiazolidamide analogue was synthesized to observe the effect of a heteroatom near the S_2 enzyme subsite, while L-1,2,3,4-tetrahydroisoquinoline and 4-substituted proline analogues were prepared based on spacial volume and hydrophobicity of the S_2 subsite of the enzyme.

When proline was replaced by azetidinone and homoproline, to provide inhibitors **52** (K_i = 34 nM) and **53** (K_i = 150 nM), respectively, both compounds showed good binding affinity for the enzyme and oral activity was similar to that of inhibitor **1**. The homoproline analogue **53** showed a longer duration of activity in vivo than **1**. On the basis of these observations, the thiazo-

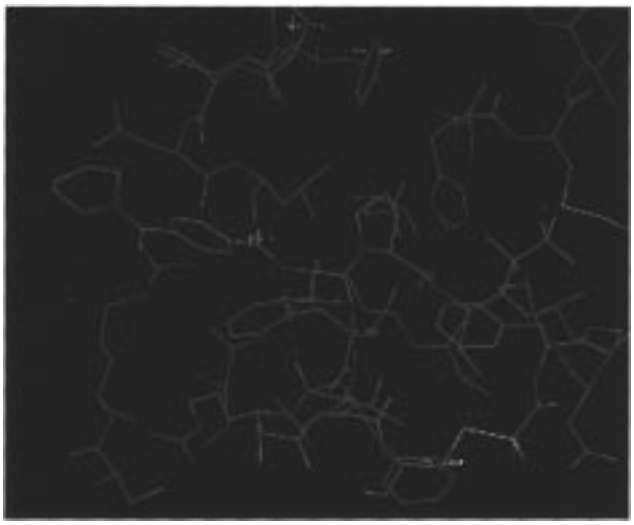


Figure 5. Model of inhibitor **1** covalently bonded at Ser 195 of HNE. H-Bonding is apparent between N of Val (P_3) and Val 216, the carbonyl of Pro (P_2) and Val 216, and N of Val (P_1) and Ser 214 of HNE.

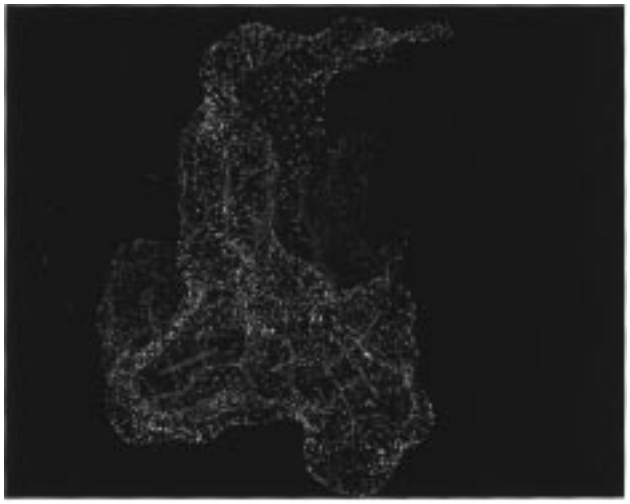


Figure 6. Connolly surface model of inhibitor **56** and HNE. The P_2 extension of inhibitor **56** fits nicely in the S_2 pocket of HNE. The hydrophobic region is shown in green and white. The region of positive charge is blue, and the negative charge is red.

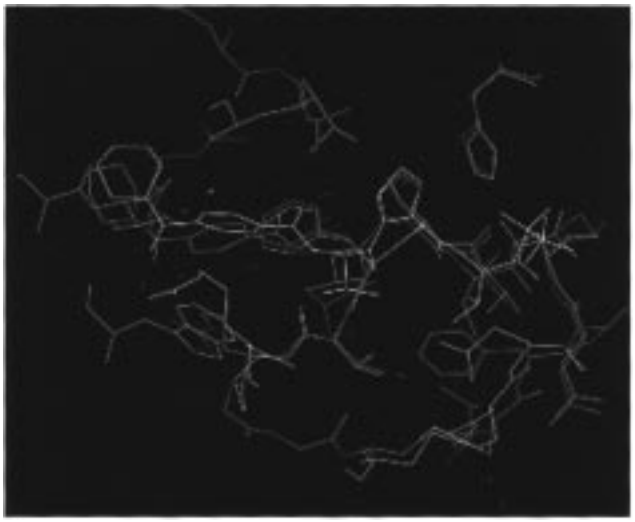


Figure 7. Overlay of the X-ray structure of HNE (green; PDB code 1HNE) with the modeled inhibitor **1** (blue) and the X-ray structure of the complex of PPE (orange) with inhibitor **1** (white).

lidine analogue **59** and tetrahydroisoquinoline analogues **62a**,**b** were prepared. Although these compounds showed improved binding affinity over **53**, there was loss of oral activity when compound **59** was tested in vivo (Table 1).

The spacial volume and hydrophobicity of the S_2 subsite of the enzyme, observed in the model, suggested that the synthesis of the 4-subtituted proline analogues **54**−**56** may provide better binding affinity. Substitution of 4-hydroxyproline for proline afforded compound **54** which had a K_i value of 120 nM. Long aliphatic side chains terminating with basic groups (such as lysine) have been shown to bind in this region.[29] The acetoxy derivative of 4-hydroxyproline, **55**, had a K_i value of 40 nM, reflecting the lesser hydrophilic and greater lipophilic nature of this group. The *O*-benzyl ether compound **56** bound with a K_i value of 20 nM, showing that fairly large groups are accommodated at this position (Figure 6), as has been shown by others.[30]

Positional isomers **69** ($K_i = 8.5\ \mu$M) and **70** ($K_i = 200\ \mu$M) of inhibitor **1** showed a decrease in binding activity as the distance between the ring nitrogen and the amide carbonyl increased, demonstrating that changes in the backbone carbonyl and nitrogen positions are not tolerated by the enzyme.

Variation of the Carbonyl Activating Group (A_G). The oxyanion produced by addition of the Ser 195 hydroxyl group to the pentafluoroethyl ketone of the inhibitor **1** forms hydrogen bonds with the amide protons of Gly 193 and Ser 195. The amide NH of the P_1 Val hydrogen-bonds with the carbonyl oxygen of Ser 214. The X-ray crystal structure was consistent with only the LLL-diastereomer of the inhibitor **1** binding to the enzyme, although it is known that the inhibitor quickly racemizes at blood serum pH. The P_1 dehydrovaline analogue was previously synthesized[8a] to explore the effect on binding activity.

To observe the effect of increasing the electrophilicity of the activating group of the inhibitor **1** on the formation of the hemiketal in the active site of the enzyme, the heptafluoro-*n*-propyl ketone analogue **73** was synthesized. Although binding and oral activity for **73** ($K_i = 18$ nM) were comparable to those of **1** ($K_i = 20$ nM), the lack of both longer duration of in vivo activity and significant increase in binding did not warrant further investigation.

Conclusions

A number of P_3- and P_2-modified peptidyl pentafluoroethyl ketones were synthesized based on rational design. The model of inhibitor **1** in the enzyme, using the Brookhaven Protein Data Bank coordinates for HNE (from file entry 1HNE), proved to be very useful in guiding the synthetic effort. When the X-ray crystal structures were solved for complex PPE−**1** and HNE−**1**, there was good correlation between the model and crystal structure (Figure 7). Through structure modification, the tolerance of the enzyme to structural changes to inhibitor **1** could be monitored. Varying the P_2 and P_3 regions of inhibitor **1** had a dramatic effect on the in vitro potency. In general, analogues which

Table 3. Statistics on the PPE−**1** Complex

space group	$P3_1$
cell dimensions	$a = b = 69.1$ Å, $c = 51.1$ Å, $\alpha = \beta = 90°$, $\gamma = 120°$
resolution	1.8 Å
no. of observations	101240
no. of unique reflections	24302
R-sym	5.0%
completeness	93.4%
R-factor	0.180 for 24038 reflections between 8.0 and 1.8 Å

Table 4. Statistics on the HNE−**1** Complex

space group	$P4_322$
cell dimensions	$a = b = 75.8$ Å, $c = 108.5$ Å, $\alpha = \beta = \gamma = 90°$
resolution	3.0 Å
no. of observations	48509
no. of unique reflections	6729
R-sym	9.9%
completeness	99.1%
R-factor	0.160 for 5799 reflections between 8.0 and 3.0 Å

varied the position of the nitrogens and carbonyls of inhibitor showed a loss in binding activity. The constrained analogues (**15**, $K_i = 400$ μM; **16**, $K_i = 140$ μM; **17**, $K_i = 1100$ μM; and **18**, $K_i = 95$ μM) and the isosteres (**22**, $K_i = 4.2$ μM; **23a**, $K_i = 41$ μM; and **23b**, $K_i = 9.8$ μM), which did not contain the P_3 Val nitrogen, showed a loss of binding affinity. The latter suggests that the hydrogen bonds formed between the P_3 valine of the inhibitor and Val 216 of the enzyme, observed in the X-ray crystal structure, were critical for activity.

Although the homoproline analogue **53** showed acceptable binding ($K_i = 150$ nM) compared with inhibitor **1**, and a longer duration of action, the difficulty in preparing this analogue and reaction yield made this an unlikely candidate for replacing inhibitor **1** as the lead compound. This was also the case with the heptafluoro-*n*-propyl ketone analogue **73** ($K_i = 18$ nM). Although compound **73** was orally active, there was not a significant enough increase in the duration of activity to warrant development of reaction conditions which would provide adequate amounts of the material for additional evaluation.

The 4-substituted prolines (analogues **54**−**56**) proved to be successful modifications of inhibitor **1** which were based on the model. Substitution with various groups defined a cavity in the S_2 subsite of HNE which is very hydrophobic. This finding may allow placement of a lipophilic residue (e.g., aryl) in this region which could lead to enhanced binding and an increased duration of action. Further exploration of this pocket may lead to a more potent, orally active HNE inhibitor.

Experimental Section

X-ray Crystal Structure. 1. Crystal Structure of Porcine Pancreatic Elastase (PPE) with 1. Crystals of the complex formed with PPE and **1** were prepared in buffer using the sitting drop method.[31] Part of a big needle (dimensions $1.0\times0.5\times0.2$ mm^3) was mounted. Data were collected using a Siemens multiwire area detector mounted on a Siemens rotating anode generator. The crystal diffracted to approximately 1.8 Å to give a completely different space group ($P3_1$) from what has been previously reported ($P2_12_12_1$).[32] Data were processed with the XDS package,[33] and the statistics of the data collection are given in Table 3. The crystal form of the complex differed considerably from the form of native PPE crystals. Whereas native PPE crystals were cube-shaped, crystals of this complex had a needle shape.

The crystal structure was solved by molecular replacement with X-plor[34] using the structure of native elastase (PDB code 3EST) as a model and refined also using X-plor. The procedure was straightforward, yielding clear peaks for the rotation and translation functions. The starting *R*-factor for data between 8.0 and 3.0 Å was 0.309 after rigid body refinement. A map calculated at 3.0 Å clearly showed the bound inhibitor which was built into the density. Five cycles of map inspection, rebuilding, and refinement yielded a final model with an *R*-factor of 0.180 for all 24 038 measured unique reflections between 8.0 and 1.8 Å. A mean coordinate error of 0.2 Å can be estimated from a Luzzati plot. With the exception of a few surface loops, which are presumably quite mobile, the electron density maps were of sufficient quality (1.8 Å) to unambiguously determine the chirality of the bound inhibitor.

Superposition of the crystal structure of the PPE−**1** complex with the crystal structure of HNE taken from the Brookhaven Protein Data Bank (code 1HNE) revealed that the folding of the porcine and the human enzyme is very similar. The rms difference is 1.8 Å for 210 equivalent C^α atoms, and the rms difference is 0.5 Å for 141 equivalent C^α atoms that differ less than 3σ. The inhibitor forms a covalent tetrahedral adduct with the enzyme, mimicking the tetrahedral transition state that occurs during hydrolysis of peptide substrates. The carbonyl carbon of the perfluoroethyl ketone group is linked to the active site Ser 195, and the carbonyl oxygen occupies the oxyanion hole. As expected, no buried hydrogen bond donors or acceptors are present which are unable to make at least one hydrogen bond.

2. Crystal Structure of Human Neutrophile Elastase (HNE) with 1. Crystals of the HNE−**1** complex were obtained by the hanging drop method.[35] Data were collected to 3.0 Å resolution using a Siemens multiwire area detector, mounted on a Siemens rotating anode generator. As with the complex formed with the porcine enzyme, the complex crystallized in a crystal form which has not previously been published. The space group is $P4_322$. Data were processed with XDS,[33] and statistics of the data collection are given in Table 4.

The crystal structure was solved by molecular replacement using the AMoRe[36] package, and refinement was done with X-plor.[34] The structure of HNE inhibited by methoxysuccinyl-Ala-Ala-Pro-Ala chloromethyl ketone (PDB code 1HNE) was used as a model. The procedure was performed using data between 15.0 and 3.5 Å for the two possible enantiomorphic space groups $P4_122$ and $P4_322$. The correct solution was very clear with a correlation of 0.346 and an *R*-factor of 0.471 in space group $P4_322$. A map calculated at 3.0 Å clearly showed the bound inhibitor and carbohydrate attachment sites at Asn 109 and 159. Four cycles of map inspection, rebuilding, and refinement yielded a final model with an *R*-factor of 0.160 for 5799 reflections between 8.0 and 3.0 Å. A mean coordinate error of 0.2−0.3 Å can be estimated from a Luzzati plot. The electron density is well-defined considering the relatively low resolution of 3.0 Å.

Superposition of the HNE−**1** complex with the crystal structure of HNE taken from the Protein Data Bank (code 1HNE) revealed that the folding of HNE does not change very much upon binding of the different inhibitors. The rms difference is 0.6 Å for 217 equivalent C^α atoms and 0.2 Å for 175 equivalent C^α atoms that differ less than 3σ. The largest difference in C^α position is 4.2 Å for Asn 148 in loop 147−151, a flexible surface loop. The rms differences between the HNE−**1** complex and the PPE−**1** complex are 1.7 Å for all 214 equivalent C^α atoms and 0.5 Å for 138 C^α atoms deviating less than 3σ, which are virtually identical to the differences between PPE−**1** and the 1HNE complex. Figure 8 shows that the binding mode of **1** is identical for the C_2F_5 unit through the proline moiety, but the positions of the phenyl and especially the morpholine ring differ considerably. This is due to steric hindrance with loop 173−175 in the PPE structure. It should be noted, however, that the inhibitor bound to HNE is involved in crystal contacts which may lead to subtle alterations of the binding mode.

3. Chirality of the Bound Inhibitor. At 3.0 Å resolution, it is not possible to establish unambiguously the chirality of

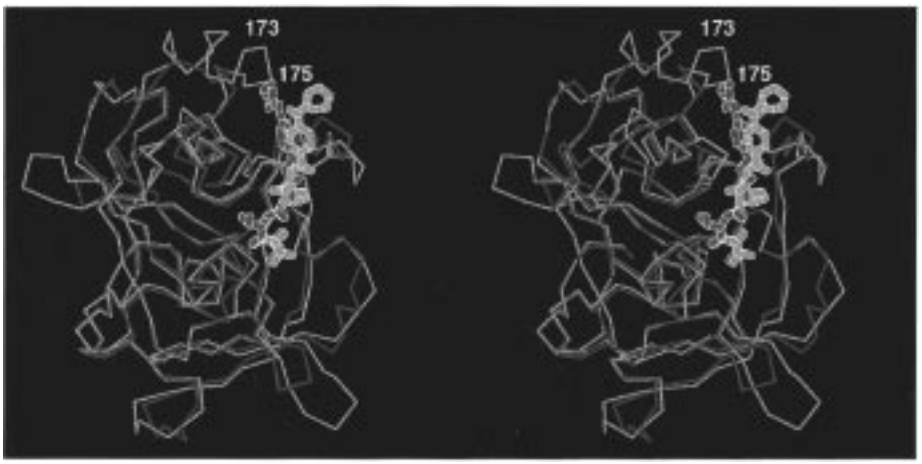


Figure 8. Superposition of the X-ray crystal structure of HNE−**1** and PPE−**1** complexes. Shown are the backbones of HNE (thin green lines) and PPE (thin orange lines) and inhibitor **1** as bound to HNE (thick green lines) and PPE (thick orange lines). Also indicated is loop 173−175 of PPE, which hinders entrance of the morpholine ring of **1** into the active site cleft, resulting in a different binding mode for this part of the inhibitor.

the bound inhibitor in HNE. However, inhibitor **1** was used (the LLL-diastereomer) for cocrystallation, and this diastereomer fits well to the electron density maps. Since the binding mode of this part of the molecule is identical in the PPE complex and the stereochemistry was determined unambiguously as being LLL in PPE, it is almost certain that this is the diastereomer which binds to HNE as well.

The inhibitor forms a covalent tetrahedral adduct with the enzyme, mimicking the tetrahedral transition state that occurs during hydrolysis of peptide substrates. The carbonyl carbon atom of the pentafluoroethyl ketone is linked to the active site Ser 195 and the carbonyl oxygen occupies the oxyanion hole. As was observed for the PPE−**1** complex, the fluorine atoms F75 and F76 hydrogen-bond with the active site His 57.

4. Modeling Parameter Estimation. After placement of the inhibitor in the binding site, all water molecules were removed and the inhibitor geometry was optimized for 100 steps of steepest descent, holding all enzyme atoms fixed. A distance-dependent dielectric constant was used in conjunction with a 12 Å cutoff in all calculations. The inhibitor was then subjected to 20 ps of molecular dynamics (MD) at 300 K and reoptimized using the conjugate gradient algorithm until the gradient norm fell below 0.01 kcal/mol/Å. Two solvent layers of thickness 8 and 14 Å were added to the binding region, keeping crystallographic positions where possible. Hydrogen positions were established by placing harmonic positional restraints on oxygen atoms in the second solvent cap and all crystallographic waters, fixing the position of protein heavy atoms during a 30-ps MD simulation. This was followed by relaxation of all protein and solvent positions for 30-ps and a 50 ps production run during which coordinates were saved every picosecond.

The conformation of the terminal *N*-[4-[(4-morpholinyl)-carbonyl]benzoyl] group is largely governed by the values of the dihedral angles between the aromatic ring and the adjacent amide groups. *N*-Methylbenzamide is an appropriate template by which to judge the quality of the molecular mechanics parameters used for this torsion potential. The default CVFF force field[37] of DISCOVER 2.9[38] yields a 2-fold rotational barrier in excess of 14 kcal/mol for *N*-methylbenzamide. Similiar results were obtained with the CFF91 force field.[39] This steep barrier tends to keep the amide group coplanar with the aromatic ring and will affect the capability of this group to form proper hydrogen-bonding geometries with atoms at the receptor. The energy profile for this dihedral as measured by the semiempirical AM1[40] Hamiltonian in MOPAC 6.0[41] is 4-fold, with the minimum at about 45° and barriers of 0.6 and 1.1 kcal/mol to bring the amide group in-plane and perpendicular to the aromatic ring, respectively.[42] Similiar results have recently been noted by others[43] and are in accordance with NMR data.[44] Adjustments to the parameters (V_2 = 7.0 kcal/mol and V_4 = 0.1 kcal/mol) for the C−C−C−N torsion were made in order to reproduce the semiempirical curve.

General Methods. All reactions were performed in oven-dried, magnetically stirred 50-, 100-, or 250-mL, round-bottomed flasks under an atmosphere of nitrogen. Except where noted otherwise, reagents and starting materials were obtained from common commercial sources and used as received. Anhydrous solvents were purchased from Aldrich Chemical Co., Inc. in Sure/Seal bottles. Other reaction solvents and all chromatographic, recrystallization, and work-up solvents were spectroscopic grade and used as received. The organic extracts were dried over anhydrous $MgSO_4$ or Na_2SO_4 prior to solvent evaporation. Thin-layer chromatograms were visualized first with UV light and then by I_2, unless specified otherwise.[45] Flash chromatography[46] was performed on Merck silica gel 60 (230−400 mesh ASTM, EM Science).

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian VXR-300, Unity 300, Unity 400, or Gemini-300 spectrometer in $CDCl_3$, unless otherwise stated. ^{1}H NMR chemical shifts (δ) are reported in ppm relative to TMS (0.00 ppm) or chloroform (7.26 ppm) as a reference, ^{19}F NMR (282 MHz unless otherwise indicated) signals are reported in ppm from $CDCl_3$ with an external reference standard of $CFCl_3$, and coupling constants (J) are reported in hertz (Hz). Signals were designated as s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet), br (broad), etc. First-order analysis of spectra were attempted when possible; consequently, chemical shifts and coupling constants for multiplets may only be approximate. ^{13}C NMR spectra were recorded on the Varian Gemini instrument (75 MHz) with chemical shifts (δ) reported in ppm relative to $CDCl_3$ (77.0 ppm), unless stated otherwise. IR spectra were recorded on a Perkin-Elmer model 1800 or a Mattson Galaxy 5020 FT-IR spectrophotometer. Mass spectra (MS) data were collected at 70 eV on a Finnigan MAT 4600, MAT TSQ-700, or VG Analytical Limited ZAB2-SE mass spectrophotometer using electron impact (EI) or chemical ionization (CI) with the molecular ion designated as M^+ and the relative peak height in percent given in parentheses. Computerized peak matching with perfluorokerosene as the reference was used for HRMS. Combustion analysis was performed using a Perkin-Elmer model 2400 elemental analyzer, and analysis fell within ±0.4% of the calculated values.

Starting materials were purchased from Aldrich with the exception of some specified amino acids which were purchased from Bachem Bioscience Inc. and Advanced Chem Tech. Hydrogen chloride gas was purchased from AGA Burdox, and

the pentafluoroethyl iodide (CF_3CF_2I) used was purchased from Aldrich Chemical Co. (lot #04512HZ, purity 97%).

The coupling reactions between the amino acids were performed by one of four methods: method I, using a mixed carbonic−carboxylic acid anhydride (isobutyl chloroformate, IBCF); method II, generating the *N*-hydroxysuccinic imido ester of valine **2**; method III, using a carbodiimide (dicyclohexylcarbodiimide, diisopropylcarbodiimide, or water-soluble carbodiimide, EDCI); or method IV, using phosphorus reagents such as (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP). Each method is initially described in detail where appropriate and then referred to as general method I, II, III, or IV for subsequent experimental procedures. Also, repetitious experimental procedures are described initially in detail and subsequently referred to as general procedures.

Abbreviations: Boc, *tert*-butyloxycarbonyl; NMM, *N*-methylmorpholine; IBCF, isobutyl chloroformate; DMF, *N*,*N*-dimethylformamide; HOBT, 1-hydroxybenzotriazole hydrate; HMPA, hexamethylphosphoramide; DCC, 1,3-dicyclohexylcarbodiimide; EDCI, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide·HCl; HOSu, *N*-hydroxysuccinamide; BOP, (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; DME, ethylene glycol dimethyl ether; TMS, tetramethylsilane; PhMe, toluene; RB, round-bottom; rt, room temperature; TFA, trifluoroacetic acid; conc, concentrated.

(*tert*-Butyloxycarbonyl)-L-valylsuccinimide (2). To a cooled (ice bath) stirred solution of *N*-Boc-L-valine (4.56 g, 21 mmol) and *N*-hydroxysuccinimide (2.41 g, 21 mmol) in DME (50 mL) was added DCC (4.75 g, 23 mmol). The reaction mixture was stirred for 6 h at 5 °C and then left to stand in the refrigerator overnight. The reaction mixture was then cold-filtered, the solid washed with Et_2O, and the combined filtrate conc to yield a solid, which was crystallized from EtOAc/hexane to afford **2** (4.59 g, 69.5%) as a white crystalline solid: mp 123−124 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.00−4.95 (d, 1H, J = 9.3 Hz), 4.62 (dd, 1H, J = 4.97 Hz), 2.85 (s, 4H), 2.75−2.44 (m, 1H), 1.45 (s, 9H), 1.25−0.90 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 168.6, 167.9, 155.1, 155.0, 80.4, 77.4, 77.2, 77.0, 76.6, 76.5, 57.0, 31.6, 31.1, 28.2, 28.1, 28.0, 27.99, 27.93, 25.5, 18.6, 17.3; MS (CI/CH_4) m/z 315 (MH^+), 299, 287, 259, 241, 215 (base peak), 173, 172, 145, 144, 116, 100, 72. Anal. ($C_{14}H_{22}N_2O_6$) C, H, N.

4-Amino-1,1,1,2,2-pentafluoro-5-methyl-3-hexanone Hydrochloride (3). A solution of *N*-Boc-L-valine methyl ester **2a** (2.27 g, 9.81 mmol) in Et_2O (14 mL)/toluene (11.3 mL) was cooled to −50 °C and treated with CF_3CF_2I (3.7 mL, 31.1 mmol, 3.2 equiv) and then further cooled to −60 °C and treated dropwise with MeLi·LiBr (1.5 M in Et_2O, 20 mL, 30.0 mmol, 3.1 equiv) in 55 min, −60 to −50 °C. The resulting reaction mixture was stirred for 1 h and then treated dropwise with 2-propanol (10 mL, 20 min; < −50 °C). After stirring for 30 min, the reaction mixture was allowed to warm to 0 °C and then poured into 1 M $KHSO_4$ (60 mL). Phases were separated, and the aqueous phase was extracted with Et_2O (50 mL). The organic phases were combined, dried ($MgSO_4$), and filtered, and the filtrate was conc in vacuo (room temperature, 15 mmHg) to provide a white solid. This solid was chromatographed on SiO_2 [40 g, eluting with hexane (400 mL) and then 10% EtOAc/hexane (400 mL)] to provide 4-[(*tert*-butyloxycarbonyl)amino]-1,1,1,2,2-pentafluoro-5-methylhexan-3-one (2.22 g) as a white solid. This solid was crystallized from hexane (40 mL, reflux and then cooled to 0 °C) to provide an analytically pure sample (1.62 g, 57%) (first crop; remaining material in the mother liquor): R_f = 0.77 in 20% EtOAc/hexane; mp 69−70 °C; IR ($CHCl_3$) ν_{max} 3443, 2976, 1753, 1716, 1500, 1369, 1234, 1197, 1163 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.00 (m, 1H), 4.80 (m, 1H), 2.30 (m, 1H), 1.44 (s, 9H), 1.10 (d, 3H, J = 6.8 Hz), 0.84 (d, 3H, J = 6.9 Hz); ^{19}F NMR (282 MHz, $CDCl_3$) δ −82.1 (s), −121.4 (d, J = 297 Hz), −122.8 (d, J = 297 Hz); UV (MeOH) λ_{max} 225 nm (ϵ = 754); MS (CI/CH_4) m/z (rel intensity) 320 (MH^+, 100). Anal. ($C_{12}H_{18}F_5NO_3$) C, H, N. To a stirred, ice-cooled solution of 4-[(*tert*-butyloxycarbonyl)amino]-1,1,1,2,2-pentafluoro-5-methylhexan-3-one (1.6 g, 5.0 mmol) in EtOAc (30 mL) was bubbled HCl gas for 3 min. The reaction was then capped with a drying tube and stirred for 1 h at room temperature. The reaction was conc and then triturated with Et_2O to afford **3** as white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.10 (br s, 3H, NH_3), 4.75 (br s, 1H, CH), 2.70−2.51 (m, 1H, CH), 1.35 (d, 3H, CH_3), 1.09 (d, 3H, CH_3).

(*tert*-Butyloxycarbonyl)-3-amino-2-oxo-1-pyrrolidineacetic Acid (6). A solution of Boc-α-Met-Gly methyl ester[17] (5.0 g, 15.6 mmol) and methyl iodide (30 mL, 0.48 mol) was stirred at room temperature for 16 h. The supernatant was drawn off, and the residue was dried under high vacuum to give the methyl sulfonium salt as an amorphous solid. To this, dissolved in DMF/CH_2Cl_2 (1:1, 312 mL) under N_2, was added NaH (1.25 g, 31.5 mmol, as a 60% oil dispersion), and the reaction was stirred at 0 °C for 2.5 h. EtOAc (104 mL) was then added followed by H_2O (24 mL), and the resulting solution was left at room temperature overnight, then conc in vacuo, and partitioned beween H_2O (50 mL) and CH_2Cl_2 (50 mL). The aqueous phase was then acidified to pH 4 with 0.5 M citric acid and continuously extracted with CH_2Cl_2. Conc in vacuo yielded a solid which was crystallized from CH_2Cl_2 to give **6** (1.0 g, 3.8 mmol) as a white solid: mp 168−169 °C; ^{1}H NMR (DMSO-d_6) δ 12.80 (br s, 1H), 7.13 (d, 1H, J = 8.8 Hz), 4.10 (br q, 1H, J = 9.6 Hz), 4.02 (d, 1H, J = 17.6 Hz), 3.82 (d, 1H, J = 17.6 Hz), 3.40−3.23 (m, 2H), 2.30−2.19 (m, 1H), 1.90−1.75 (m, 1H); MS (CI/CH_4) m/z 259 (MH^+), 243, 231, 217, 203 (base peak), 185, 173, 159, 141, 114, 88, 74.

(*tert*-Butyloxycarbonyl)-3-amino-α-methyl-2-oxo-1-pyrrolidineacetic Acid (7). Boc-D-Met-L-Ala-methyl ester[17] (6.08 g, 18.2 mmol) was dissolved with stirring in CH_3I (25 mL, 0.40 mol) in a stoppered flask wrapped in aluminum foil. After 6 days, the solution was concentrated in vacuo; CH_2Cl_2 was added, and the solution was concentrated in vacuo three times to give 8.10 g of an amorphous solid, which was dissolved in CH_2Cl_2 (125 mL). This solution (and a CH_2Cl_2 (25 mL) rinse) was added rapidly to a vigorously stirred suspension of pentane-washed NaH (1.82 g of 60% dispersion, 45.5 mmol, 2.5 equiv) in DMF (150 mL) at 0 °C under nitrogen. The reaction mixture was allowed to stir at 0 °C for 3 h, then poured into cold dilute HCl, and extracted twice with ether. The combined extracts were washed twice with water and brine and dried ($MgSO_4$). Concentration in vacuo gave 2.94 g of pale-yellow oil. Cystallization from cyclohexane/EtOAc gave 819 mg (17%) of fine white crystals. The analytical sample was obtained after a second recystallization from cyclohexane/EtOAc to give acid **7** as a pale-yellow cystalline powder. Variable temperature NMR showed a coalescence of the methyl doublets, but not of the coupled α-proton quartets, indicating that the sample was a mixture of diastereomers: mp 143−151 °C dec; IR (KBr) 3387, 2982, 1744, 1701, 1669, 1518, 1246, 1167 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.10 (m, 1H), 4.54 and 4.48 (two overlapping q, 1H, J = 7.4 Hz), 4.24−4.01 (m, 1H), 3.37−3.15 (m, 4H − H_2O peak + 3H), 2.25 (m, 1H), 1.79 (m, 1H), 1.39 (s, 9H), 1.31 and 1.30 (2d in 3:2 ratio, 3H, J = 7.4 Hz); MS (CI/CH_4) m/z (rel intensity) 310 (M^+ + 29), 273 (MH^+), 245, 218, 217 (100), 216, 199, 173. Anal. ($C_{12}H_{20}N_2O_5$) C, H, N.

(*tert*-Butyloxycarbonyl)-3-L-amino-2-oxo-1-piperidineacetic Acid (8). A solution of *N*-α-(*tert*-butyloxycarbonyl)-*N*-β-(carboxymethyl)ornithine[17] (1.0 g, 3.45 mmol) in DMF (30 mL) was heated at 55 °C for 16 h and then conc in vacuo to provide a yellow oil which crystallized from EtOAc−hexane affording **8** (800 mg, 85%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 7.38 (br m, 1H), 5.4 (m, 1H), 4.34−3.15 (series of m, 5H), 2.35 (m, 1H), 2.0−1.54 (m, 3H), 1.44 (s, 9H, tBu).

(*tert*-Butyloxycarbonyl)-3-amino-2-oxo-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-1-pyrrolidineacetamide (9). Coupling Method I. To a stirred solution of **6** (225 mg, 0.872 mmol) and NMM (0.10 mL, 0.872 mmol) in CH_2Cl_2 (10 mL) at −20 °C was added IBCF (0.11 mL, 0.872 mmol). The reaction was stirred at −20 °C for 20 min when additional NMM (0.10 mL, 0.872 mmol) was added followed by addition of acid **3** (223 mg, 0.87 mmol) as a solid in one portion. After stirring at −15 °C for 1 h the reaction was allowed to warm to room temperature, diluted with additional CH_2Cl_2, extracted

with 10% HCl (3 × 20 mL), saturated $NaHCO_3$ (2 × 20 mL), and brine (1 × 20 mL), dried (Na_2SO_4), and conc to afford **9** (400 mg, 100%) as an amorphous solid: ^{1}H NMR ($CDCl_3$) δ 7.45 (d, 1H, J = 4.5 Hz), 5.15−4.90 (m, 1H), 4.65−4.30 (br d, 1H), 4.10−3.40 (m, 4H), 2.60−2.10 (m, 3H), 1.45 (s, 9H), 1.16−0.85 (dd, 6H, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 192.4, 172.4, 168.7, 168.36, 168.32, 155.2, 115.6, 78.0, 59.2, 51.0, 50.9, 50.8, 44.9, 44.8, 44.79, 44.77, 43.8, 43.7, 43.6; ^{19}F NMR ($CDCl_3$) δ −194.3 (s, 3F), −234 (s, 2F).

(*tert*-Butyloxycarbonyl)-3-amino-α-methyl-2-oxo-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-1-pyrrolidineacetamide (10a,b). A stirred solution of acid **7** (274 mg, 1.01 mmol) was coupled to **3** (269 mg, 1.05 mmol) by method I to give, after flash chromatography, a less polar diastereomer of pentafluoroethyl ketone **10a** (248 mg, 41%) as a colorless oil and the more polar diastereomer of pentafluoroethyl ketone **10b** (163 mg, 34%) as white crystals. For the less polar diastereomer **10a**: ^{1}H NMR ($CDCl_3$) δ 7.1−6.98 (m, 1H), 5.23 (m, 1H), 5.02−4.65 (m, 2H), 4.23 (m, 1H), 3.52−3.1 (m, 2H), 2.61 (m, 1H), 2.45 (m, 1H), 1.94 (m, 1H), 1.45 (2s) and 1.44 (m) and 1.39 (d, J = 7.2 Hz) [12H total], 1.07 (d, J = 6.9 Hz) and 0.99 (d, J = 6.8 Hz) and 0.85 (d, J = 6.9 Hz) [6H total]; ^{19}F NMR ($CDCl_3$) δ −79.44 (s) and −82.11 (s) in 1:12 ratio, −121.25 (d, J = 296 Hz), −121.86 and −122.17 (inner peaks of an AB pattern), −122.80 (d, J = 296 Hz). Recrystallization from cyclohexane gave the analytical sample with mp 152−154 °C, for the more polar diastereomer **10b**: IR (KBr) 3395, 3295, 2976, 1684, 1532, 1505, 1285, 1235, 1200, 1167 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.3 (m, 1H), 5.25 (m, 1H), 4.97−4.8 (m, 2H), 4.09−3.94 (m, 1H), 3.4−3.24 (m, 2H), 2.6−2.33 (m, 2H), 2.3−2.04 (m, 1H), 1.43 (s, 9H), 1.40 and 1.38 (2d in 1:2 ratio, 3H, J = 7.2 Hz), 1.05, 1.00, and 0.93 (3d, in a 2:1:3 ratio, 6H, J = 6.8, 6.7, 6.8 Hz, respectively); ^{19}F NMR ($CDCl_3$) δ −82.05 (s, 3F), −121.73, −121.76, and −121.81 (3s in 5:4:18 ratio, 2F); MS (CI/CH_4) m/z (rel intensity) 514 (M^+ + 41), 502 (M^+ + 29), 474 (MH^+), 446, 419, 418 (100), 400; $[\alpha]_D$ +90.4° (c 0.52, $CHCl_3$). Anal. ($C_{19}H_{28}F_5N_3O_5$) C, H, N.

(*tert*-Butyloxycarbonyl)-3-L-amino-2-oxo-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-1-piperidineacetamide (11). A stirred solution of acid **8** (750 mg, 2.75 mmol) was coupled to **3** (702 mg, 2.75 mmol) via method I to give **11** (1.10 g, 85%) as an amorphous solid: ^{1}H NMR ($CDCl_3$) δ 7.37 (m, 1H), 4.97 (m, 1H), 4.1−3.3 (series of m, 6H), 2.35 (m, 2H), 2.1−1.33 (series of m, 3H), 1.44 (s, 9H, tBu), 0.97 (m, 6H, 2 × CH_3).

General Procedure I: Representing Removal of an *N*-*t*-Boc Protecting Group. 3-Amino-2-oxo-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-1-pyrrolidineacetamide Hydrochloride Salt (12). A stirred solution of **9** (400 mg, 0.87 mmol) in EtOAc (10 mL) at ice bath temperature was treated with HCl gas for 4 min; then the reaction was stoppered with a drying tube and stirred at room temperature for 1 h. Evaporation of the solvent in vacuo yielded **12** (300 mg, 87.3%) as an amorphous solid: ^{19}F NMR ($CDCl_3$) δ −52.5 (s, 3F), −92.1 (s, 2F).

L-3-Amino-2-oxo-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-1-piperidineacetamide Hydrochloride Salt (14). A stirred solution of **11** (900 mg, 1.90 mmol), treated as in general procedure I, afforded **14** (770 mg, 99%) as an amorphous solid: ^{1}H NMR ($CDCl_3$) δ 7.98 (m, 0.5H, NH), 7.35 (m, 1H), 4.92 (m, 1H), 4.28−3.2 (series of m, 6H), 2.41 (m, 2H), 2.29−1.54 (series of m, 3H), 1.01 (m, 6H, 2 × CH_3).

General Procedure II: Representative Coupling with 4-[(4-Morpholinyl)carbonyl]benzoyl Chloride. *N*-[4-[(4-Morpholinyl)carbonyl]benzoyl]-3-amino-2-oxo-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-1-pyrrolidinamide (15). To a stirred suspension of 4-[(4-morpholinyl)-carbonyl]benzoic acid (0.717 g, 3.1 mmol) and benzyltriethylammonium chloride (5 mg, 0.02 mmol) in 1,2-dichloroethane (20 mL) was added thionyl chloride (0.225 mL, 3.1 mmol), and the reaction was heated at reflux. After 2.5 h, the reaction was allowed to cool to room temperature and conc in vacuo. The residue was then azeotroped with CCl_4 and placed under vacuum to give the acid chloride as a light-orange oil (quantitative) which was used without further purification. In a separate RB flask, a stirred solution of **12** (1.0 g, 2.50 mmol) in CH_2Cl_2 (15 mL) was cooled to −20 °C. NMM (0.33 mL, 3.0 mmol) was added and immediately followed by the dropwise addition of the acid chloride in CH_2Cl_2 (5 mL) at such a rate as to maintain the internal reaction temperature at −10 °C or less. After the addition was complete, the reaction mixture was allowed to warm to room temperature. After 2 h at room temperature, the reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with 1 N HCl (2 × 20 mL), saturated $NaHCO_3$ (2 × 20 mL), and brine (1 × 20 mL). Drying ($MgSO_4$) and conc in vacuo afforded a crude yellow oil which was immediately flash chromatographed (2- × 15-cm column eluted with 1:27 MeOH−CH_2Cl_2) to give **15** (876 mg, 60%) as an amorphous solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.05−8.90 (t, 1H, J = 8.8 Hz), 8.62−7.60 (t, 1H, J = 8.8 Hz), 7.93 (d, 2H, J = 8.5 Hz), 7.50 (d, 2H, J = 7.9 Hz), 4.79−4.69 (m, 2H), 4.16−3.92 (m, 2H), 3.80−3.10 (m, 10H), 2.26−2.20 (m, 2H), 2.00 (m, 1H), 0.98 (m, 3H), 0.91 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 172.08, 172.0, 168.3, 165.4, 138.4, 138.3, 134.6, 134.5, 127.3, 126.9, 65.9, 59.3, 59.2, 50.3, 50.2, 44.9, 44.2, 44.1, 40.3, 40.0, 39.9, 39.7, 39.5, 39.2, 38.9, 38.6, 27.9, 25.2, 19.2, 19.1, 17.1, 17.0; MS (CI/CH_4) m/z 577 (MH^+), 533, 490, 429, 404, 358, 330, 272, 228, 220, 200 (base peak), 180, 152, 91, 70. Anal. ($C_{25}H_{29}F_5N_4O_6$·1H_2O) C, H, N.

[3*R*-[1(α*S),3*R**]]-α-Methyl-3-[[4-[(4-morpholinyl)carbonyl]benzoyl]amino]-2-oxo-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-1-pyrrolidineacetamide (16).** A flask containing the less polar diastereomer of pentafluoroethyl ketone **10a** (241 mg, 0.509 mmol) was treated as described in general procedure I to give **13a**. **13a** was then treated according to general procedure II to afford **16** (174 mg, 58%) as an amorphous solid: IR (KBr) 3422, 3376, 3322, 1682, 1645, 1539, 1281, 1223, 1202 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.85 (d, 2H, J = 8.2 Hz), 7.47 and 7.46 (2d, 2H, J = 8.3, 8.2 Hz), 6.90 (m, 1.3H), 6.78 (d, 0.7H, J = 8.1 Hz), 5.03 (ddd, 0.3H, J = 8.5, 4.6, 0.9 Hz), 4.93 (ddd, 0.7H, J = 8.1, 3.9, 1.0 Hz), 4.79 (q, 0.7H, J = 7.1 Hz), 4.64−4.44 (m, 1.3H), 3.88−3.17 (m, 11H), 2.83 (m, 1H), 2.38 (m, 1H), 2.12−1.94 (m, 1H), 1.50 and 1.44 (2d in a 1:2 ratio, 3H, J = 7.2 Hz), 1.09 and 1.01 (2d in a 1:2 ratio, 3H, J = 6.8 Hz), 0.085 (d, 3H, J = 6.8 Hz); ^{19}F NMR ($CDCl_3$) δ major −82.11 (s), −121.23 (d, J = 295 Hz), −122.91 (d, J = 295 Hz); minor −82.09 (s), −121.25 (d, J = 295 Hz), −122.71 (d, J = 295 Hz); MS (CI/CH_4) m/z (rel intensity) 631 (M^+ + 41), 619 (M^+ + 29), 592, 591 (MH^+, 100), 305, 139, 99. Anal. ($C_{26}H_{31}F_5N_4O_6$) C, H, N.

[3*S*-[1(α*R),3*R**]]-α-Methyl-3-[[4-[(4-morpholinyl)carbonyl]benzoyl]amino]-2-oxo-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-1-pyrrolidineacetamide (17).** The more polar diastereomer of pentafluoroethyl ketone **10b** (147 mg, 0.310 mmol) was deprotected as described in general procedure I to give **13b**. This was then treated as in general procedure II to provide **17** (99 mg, 54%) as an amorphous solid: IR (KBr) 3310, 1684, 1645, 1541, 1281, 1223, 1200 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.99 (d, <0.5H, J = 6.1 Hz), 7.76 and 7.72 (2d in 2:1 ratio, 2H, J = 8.3 Hz), 7.63 (d, <1H, J = 8.2 Hz), 7.57 (d, <1H, J = 6.5 Hz), 7.40 and 7.35 (2d in 2:1 ratio, 2H, J = 8.3 Hz), 5.00 (m, 1H), 4.92 (q, 1H, J = 7.3 Hz), 4.44−4.35 and 4.28−4.17 (2m in 2:1 ratio), 3.85−3.25 (m, 10H), 2.63 (m, 1H), 2.47 (m, 1H), 2.27 (m, 1H), 1.48 and 1.44 (2d in a 1:2 ratio, 3H, J = 7.3 Hz), 1.10 and 1.06 (2d in a 2:1 ratio, 3H, J = 6.8 Hz), 1.01 and 0.096 (2d in 1:2 ratio, 3H, J = 6.9 Hz); ^{19}F NMR ($CDCl_3$) δ −81.96 and −82.03 (2s in 1:2 ratio, 3F), −121.70 (apparent d, inner peaks of AB pattern), −121.81 (s in 2:1 ratio, 2F); MS (CI/CH_4) m/z (rel intensity) 631 (M^+ + 41), 619 (M^+ + 29), 592, 591 (MH^+, 100), 99. Anal. ($C_{26}H_{31}F_5N_4O_6$) C, H, N.

***N*-[4-[(4-Morpholinyl)carbonyl]benzoyl]-L-3-amino-2-oxo-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-1-piperidineacetamide (18).** A stirred solution of **14** (690 mg, 1.68 mmol) was treated as described in general procedure II to give **18** (160 mg, 16%) as an amorphous solid: IR (KBr) 3059, 3423, 3381, 3373, 3362, 3310, 2966, 2931, 2862, 1753, 1639, 1541, 1496, 1464, 1438 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$)

δ 7.84 (d, 2H, J = 8.5 Hz, aryl), 7.46 (d, 2H, J = 7.9 Hz), 7.32 (m, 1H, NH), 7.05 (d, 1H, J = 8.5 Hz, NH), 5.02 (m, 1H), 4.75 and 4.44 (2d, 1H, J = 18 Hz), 4.29 (m, 1H), 3.9−3.25 (series of m, 11H), 2.60−2.32 (m, 2H), 1.98 (m, 3H), 1.04 (d, 3H, J = 9 Hz, CH_3), 0.91 (dd, 3H, J = 12, 9 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 168.6, 138.56, 134.9, 127.47, 127.41, 127.3, 127.2, 77.5, 77.4, 77.3, 77.2, 77.1, 77.0, 76.8, 76.79, 76.72, 76.5, 66.8, 59.6, 59.3, 52.1, 51.9, 51.8, 49.37, 49.33, 29.6, 28.9, 28.7, 27.4, 27.2, 21.1, 21.0, 19.86, 19.81, 16.8, 16.7; ^{19}F NMR ($CDCl_3$) δ −82.04 (d, J = 5.6 Hz, CF_3), −121.76 and −122.01 (d, J = 11.3 Hz, CF_2); MS (CI/CH_4) m/z 631, 619, 591 (MH^+, 100), 571, 504, 443, 416, 372, 357, 301, 218, 153, 104, 71. Anal. ($C_{26}H_{31}F_5N_4O_6$) C, H, N.

L-2-Isopropyl-4-oxo-5-phenylbutanoic Acid (19a).[47] To a stirred, cold (0 °C) solution of l-methylhydroxyisovaleric acid (1.32 g, 10 mmol) in CH_2Cl_2 (15 mL) was added triflic anhydride (1.84 mL, 11 mmol) followed by 2,6-lutidine (1.28 mL, 1 mmol) under N_2. The resulting solution was stirred for 10 min and used for the next reaction without further purification. Meanwhile, to a stirred cold (0 °C) suspension of NaH (1.0 g of 60% in oil, 22 mmol) in dry THF (50 mL) was added, dropwise, a solution of ethyl benzoylacetate (3.84 g, 20 mmol) in THF (50 mL) under N_2. After 10 min of stirring, the above triflate was diluted with CH_2Cl_2 (10 mL) and added dropwise to the gray suspension. The resulting solution was stirred at reflux for 13 h. Upon cooling, 1 N HCl solution (60 mL) was added and the reaction mixture was extracted with EtOAc (3 × 80 mL). The organic extracts were combined, washed with brine (100 mL), dried ($MgSO_4$), passed through a short pad of silica gel, and conc by rotary evaporator (bath temperature 30 °C) to provide a yellow oil. This oil was dissolved in H_2O (70 mL) and THF (70 mL), treated with $LiOH{\cdot}H_2O$ (2.5 g), and stirred at room temperature for 5 h. The resulting solution was acidified by 6 N HCl (pH 5) and heated at reflux for 12 h. After the reaction cooled to room temperature, the solution was basified by solid KOH (pH 10) and washed with Et_2O (70 mL). The aqueous phase was acidified by 6 N HCl (pH 3) and extracted with EtOAc (3 × 80 mL). The combined EtOAc extracts were dried ($MgSO_4$), passed through a short pad of silica gel, and conc to give **19a** as an oil (920 mg, 42%) after purification by radial chromatography (hexane/EtOAc, 95:5, 90:10, 80:20). The oily product solidified upon addition of hexane/EtOAc (1:1) to give a white solid: $[\alpha]^{25}_D$ −24.06 (*c* 0.64, MeOH); mp 103−105 °C; IR (neat) 3600−2500 (br), 2996, 1802, 1708, 1598, 1582, 1450, 1214 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.96 and 7.46 (m, 5H, phenyl), 3.48 (dd, 1H, B of ABX, CH_2CH), 3.30 (m, 2H, A and X for ABX, CH_2CH), 2.10 (m, 1H, $CH(CH_3)_2$), 1.02 (d, 6H, J = 8 Hz, $CH(CH_3)_2$). Anal. ($C_{13}H_{16}O_3$) C, H, N.

D-2-Isopropyl-4-oxo-5-phenylbutanoic Acid (19b).[47] The corresponding D-isomer was prepared in the same manner from D-methylhydroisovaleric acid, as described above, in 45% yield: $[\alpha]^{25}_D$ +32.36 (*c* 0.72, MeOH).

L-2-Isopropyl-4-oxo-5-morpholinylbutanoic Acid (20a).[47] To a stirred suspension of NaH (2.4 g, 60% oil dispersion, 60 mmol) in DMF (50 mL) was added di-*tert*-butylmalonate (4.32 g, 20 mmol). The reaction mixture was stirred at 0 °C for 3 h. Meanwhile to a stirred solution of L-2-methylhydroxyisovaleric acid ester (2.64 g, 20 mmol) in CH_2Cl_2 (20 mL) was added triflic anhydride (4 mL, 22 mmol), followed by 2,6-lutidine (2.8 mL, 24 mmol) at 0 °C, and the solution was stirred for 10 min. This triflate was diluted with CH_2Cl_2 (10 mL) and added to the above enolate. The resulting mixture was then stirred at 0 °C for 3 h. The reaction was then quenched with H_2O (100 mL) and extracted with EtOAc (3 × 80 mL), and the combined extracts were washed successively with 1 N HCl (100 mL) and brine (100 mL), dried ($MgSO_4$), passed through a short pad of silica gel, and conc to a yellow oil. This oil was dissolved in toluene (50 mL), treated with TFA (5 mL) at room temperature for 1 h, and heated at reflux for an additional 5 h. After cooling to room temperature the reaction was concentrated to a residue which was dissolved in EtOAc (150 mL), washed with H_2O (100 mL) and brine (100 mL), passed through a short pad of $MgSO_4$ and silica gel, and conc to provide 2-isopropyl methyl succinate as a sticky oil (2.35 g, 71%) after purification by flash chromatography (hexane/EtOAc, 90:10, 80:20, 60:40): $[\alpha]^{25}_D$ −14.0 (*c* 0.4, MeOH); IR (neat) 3500−2500 (br), 2977, 1745, 1716, 1442, 1377, 1242, 1183 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.70 (s, 3H, OCH_3), 2.76 (m, 2H, B and X for ABX, CH_2CH), 2.46 (dd, 1H, J = 16, 2.8 Hz, A of ABX, CH_2CH), 2.00 (septet, 1H, J = 6.8 Hz, $CH(CH_3)_2$), 0.94 (dd, 6H, J = 6.8, 7.2 Hz, $CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.5, 175.0, 51.8, 47.2, 32.9, 30.2, 20.0, 19.6.

To a stirred solution of the above oil (2.02 g, 13.8 mmol) in DMF (50 mL) were added HOBt (2.16 g, 15.5 mmol) and morpholine (1.4 mL, 16 mmol), followed by EDCI (3.0 g, 16 mmol) at 0 °C. The resulting solution was stirred at 0 °C for 3 h and then allowed to warm to room temperature and stir overnight; 1 N HCl (100 mL) and H_2O (100 mL) were added, and the reaction was extracted with EtOAc (3 × 100 mL). The combined extracts were washed with saturated $NaHCO_3$ (100 mL) and brine (100 mL), passed through a short pad of $MgSO_4$/silica gel, and conc to provide a colorless oil (2.15 g, 64%). Without further purification this oil (2.15 g, 8.8 mmol) was dissolved in THF and H_2O (50 mL/50 mL) and treated with $LiOH{\cdot}H_2O$ (420 mg, 10 mmol) at room temperature overnight. After 16 h the reaction was washed with Et_2O (100 mL) and the remaining aqueous phase acidified with 6 N HCl (pH 3). The aqueous phase was then extracted with EtOAc (3 × 100 mL), and the combined extracts were dried ($MgSO_4$), conc, and flash chromatographed (hexane/EtOAc, 60:40, 20:80) to provide **20a** as a colorless oil (1.3 g, 65%). Crystallization from hexane and EtOAc gave a white solid: $[\alpha]^{25}_D$ −24.9 (*c* 0.45, MeOH); mp 79−28 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.47 (br, 1H, C00H), 3.66 (set of m, 8H, CH_2CH_2), 2.82 (m, 2H, B and X for ABX, CH_2CH), 2.32 (dd, 1H, J = 15.6, 2.8 Hz, A of ABX, CH_2-CH), 2.04 (m, 1H, $CH(CH_3)_2$), 0.98 (dd, 6H, J = 6.8, 7.2 Hz, $CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.0, 170.6, 66.8, 66.5, 47.5, 46.0, 42.2, 31.4, 30.0, 20.2, 19.8.

D-2-Isopropyl-4-oxo-5-morpholinylbutanoic Acid (20b).[47] The corresponding D-isomer was prepared from D-2-methylhydroxyisovaleric acid ester in the same manner as described above in 62% yield: $[\alpha]^{25}_D$ +26.36 (*c* 0.33, MeOH).

***N*-(1,1,1,2,2-Pentafluoro-5-methyl-3-oxo-4-hexanyl)-L-prolinamide (21).** Boc-proline (2.15 g, 0.01 mol) and **3** (2.26 g, 0.01 mol) were coupled by method I to yield Boc-Pro-Val-C_2F_5 (3.30 g, 79%) as an amorphous solid: ^{1}H NMR ($CDCl_3$) δ 7.85 (m, 1H, NH), 4.98 (m, 1H), 4.38 (m, 1H, α-CH), 3.43 (m, 2H), 2.36 (m, 1H, β-CH Val), 2.3−1.71 (m, 4H), 1.44 (s, 9H, tBu), 1.06, (d, 3H, J = 6.6 Hz, CH_3), 0.87 (d, 3H, J = 6.6 Hz, CH_3); MS (CI/CH_4) m/z (rel intensity) 457, 445 (M^+ + 29), 417 (MH^+), 389, 361, 341, 317 (100), 269, 213, 186, 170, 114, 84, 70.

Treating this Boc-Pro-Val-C_2F_5 (624 mg, 1.50 mmol) as described in general procedure I gave HCl salt **21** (544 mg) as a very hygroscopic white foam.

[2*S*-[1(*R),2*R**]]-1-[2-(1-Methylethyl)-4-phenyl-1,4-dioxobutyl]-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-prolinamide (22).** Amine hydrochloride **21** (620 mg, 1.77 mmol) was coupled to **19b** (380 mg, 1.77 mmol) using coupling method III affording **22** (140 mg, 15%) as a colorless glass: ^{1}H NMR ($CDCl_3$) δ 8.19 (d, 1H, J = 7.5 Hz), 7.98−7.89 (m, 3H), 7.6−7.3 (m, 5H), 4.91 (m, 1H), 4.65 and 4.60 (2dd, 1H, J = 8.0, 1.4 Hz and J = 8.1, 2.0 Hz), 4.14 and 3.98 (q and m, 1H, J = 7.0 Hz), 3.75−3.60 (m, 2H), 3.13−3.07 (m, 2H), 2.97 (m, 1H), 2.55−2.23 (m, 2H), 2.13−1.78 (m, 4H), 1.30 (d, 1H, J = 7.0 Hz), 1.17−1.00 (m, 2H), 0.89−0.85 (m, 12H, d at 0.88, J = 6.9 Hz, and d at 0.86, J = 6.9 Hz, discernible); ^{19}F NMR ($CDCl_3$) mixture of isomers (6:4) A:B δ A −82.20 (s, CF_3), −121.45 and −122.86 (AB quartet, J = 296 Hz, CF_2); B −82.17 (s, CF_3), −121.37 and −122.71 (AB quartet, J = 296 Hz, CF_2); MS (CI/CH_4) m/z (rel intensity) 519 (MH^+), 499, 399, 317, 300, 272, 203 (100), 105, 84, 70. Anal. ($C_{25}H_{31}F_5N_2O_4$) C, H, N.

[2*S*-[1(*S),2*R**]]-1-[2-(1-Methylethyl)-4-(4-morpholinyl)-1,4-dioxobutyl]-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-2-pyrrolidinecarboxamide (23a). Coupling Method III.** Coupling of the amine hydrochloride **21** (370 mg, 1.05 mmol) with acid **20a** (232 mg, 1.01 mmol) using HOBT (159 mg, 1.04 mmol), EDCI (256 mg, 1.34 mmol), and NMM

(115 mL, 1.05 mmol) in 2:1 CH_2Cl_2/DMF (4.5 mL) gave 479 mg of yellow oil which was combined with 89 mg of crude material from a similar experiment and purified by flash chromatography (10% acetone/EtOAc) to give 333 mg (47%) of **23a** as a very pale-yellow tacky glass: IR ($CHCl_3$) 2967, 1674, 1634, 1466, 1439, 1227, 1198, 1117 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.60 (d, 0.5H, J = 8.7 Hz), 7.57 (d, 0.5H, J = 9.1 Hz), 4.67 (m, 1H), 4.06−3.89 (m, 1H), 3.76−3.37 (m, 10H), 2.96−2.65 (m, 2H), 2.51 and 2.50 (2dd, 1H, J = 16.3, 6.8 Hz and J = 16.3, 6.6 Hz), 2.38−2.23 (m, 1H), 2.14−1.79 (m, 4H), 1.01−0.85 (m, 12H, d at 0.99, J = 6.6 Hz), 0.92 (d, J = 6.9 Hz), 0.87 (J = 6.9 Hz, discernible); ^{19}F NMR ($CDCl_3$) δ −79.29 and −79.45 (2s, trace), −82.01 and −82.13 (2s, 3F), −120.97 (d, 0.5F, J = 293 Hz), −121.25 (d, 0.5F, J = 297 Hz), −123.09 (d, 0.5F, J = 297 Hz), −123.32 (d, 0.5F, J = 293 Hz); MS (CI/CH_4) m/z (rel intensity) 568 (M^+ + 41), 556 (M^+ + 29), 528 (MH^+), 441, 213, 212 (100). Anal. ($C_{23}H_{34}F_5N_3O_5$) C, H, N.

[2*S*-[1(*R),2*R**]]-1-[2-(1-Methylethyl)-4-(4-morpholinyl)-1,4-dioxobutyl]-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-2-pyrrolidinecarboxamide (23b).** Coupling of the amine hydrochloride **21** (402 mg, 1.14 mmol) with acid **20b** (229 mg, 1.00 mmol) using coupling method III gave pentafluoroethyl ketone **23b** (303 mg, 57%) as a colorless glass. A second chromatography (3.5−6.5% *i*-PrOH/ CH_2Cl_2) taking only the center fractions failed to remove an impurity (presumably a third diastereomer), giving **23b** (225 mg) as a colorless glass: IR (film) 2969, 1686, 1643, 1437, 1225, 1200, 1117 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.25, 8.16, and 7.93 (3d in 1:7:5 ratio, 1H, J = 8.8, 7.5, 7.3 Hz), 4.97−4.88 (m, 1H), 4.79−4.43 (m, 1H), 3.96−3.84 (m, 1H), 3.74−3.43 (m, 10H), 3.00−2.84 (m, 2H), 2.70−2.23 (m, 3H), 2.11−1.75 (m, 4H), 1.09−0.85 (m, 12H, d at 1.07, J = 6.8 Hz, d at 0.88, J = 6.9 Hz, and d at 0.87, J = 6.9 Hz, discernible); ^{19}F NMR ($CDCl_3$) 53:34:8 mixture of isomers A:B:C δ A −82.18 (s), 121.45 (d, J = 295 Hz), −122.84 (d, J = 295 Hz); B −82.14 (s), −121.33 (d, J = 295 Hz), −122.69 (d, J = 295 Hz); C −82.22 (s), −120.60 (d, J = 292 Hz), −123.57 (d, J = 292 Hz); MS (CI/CH_4) m/z (rel intensity) 556 (M^+ + 29), 529, 528 (MH^+, 100), 212. Anal. ($C_{23}H_{34}F_5N_3O_5$) C, H, N.

2-[(*tert*-Butyloxycarbonyl)amino]-3-methyl-2-butenoic Acid (25). *N*-Boc-dehyrovaline methyl ester (730 mg, 3.20 mmol), prepared from the dehydrovaline methyl ester hydrochloride,[48] was hydrolyzed in MeOH/H_2O (4:1, 20 mL) with LiOH·H_2O (2.13 mg, 51 mmol). The reaction mixture was heated at 50 °C for 24 h, then allowed to cool to room temperature, and conc in vacuo to a residue which was dissolved in H_2O and washed with EtOAc. The aqueous solution was then acidified with 3 M HCl, with ice bath cooling, to pH 2 and extracted with EtOAc. The organic extract was then washed with brine, dried (Na_2SO_4), and conc in vacuo to afford **25** (637 mg, 92%) as a white glass. This was used in the next step without further purification.

Boc-D-valyl-L-proline (26). Coupling Method IV. To a stirred solution of Boc-D-valine (**24**) (from Novabiochem; 2.4 g, 0.011 mol), L-proline methyl ester HCl (1.8 g, 0.011 mol), and BOP (Novabiochem; 4.9 g, 0.011 mol) in CH_3CN (100 mL) at room temperature was added Et_3N (3.1 mL, 0.022 mmol). After stirring at room temperature for 2 h, the reaction was treated with brine (100 mL) and extracted with EtOAc (3 × 100 mL). The combined extracts were washed with 1 N HCl (100 mL) and then saturated $NaHCO_3$ (100 mL) and dried ($MgSO_4$). The solvent was removed in vacuo, and the crude oil was immediately flashed chromatographed (3- × 21-cm column eluted with 2:3 Et_2O−hexane) to give the dipeptide methyl ester (1.97 g, 55%) as a colorless oil: ^1H NMR (300 MHz, $CDCl_3$) δ 5.27 (d, 1H, J = 8 Hz, NH), 4.44 (m, 1H, α-CH of Val), 4.35 (m, 1H), 3.9 (m, 1H), 3.73 (s, 3H, OMe), 3.6 (m, 1H), 2.27−1.9 (pr m, 5H), 1.44 (s, 9H, t-Bu), 1.00 (d, 3H, J = 6.8 Hz, CH_3), 0.92 (d, 3H, J = 6.6 Hz, CH_3). Saponification of the ester (1.63 g, 4.96 mmol) in MeOH (30 mL) at room temperature with 1 N NaOH (4.0 mL) at room temperature for 3 h gave **26** (1.0 g, 64%) as an amorphous solid: ^1H NMR (300 MHz, $CDCl_3$) δ 5.38 (d, 1H, J = 8 Hz, NH), 4.51 (m, 1H, a-CH of Val), 4.3 (m, 1H), 3.92 (m, 1H), 3.57 (m, 1H), 2.3−1.9 (series of m, 5H), 1.44 (s, 9H, tBu), 0.95 (m, 6H, 2 × CH_3).

Boc-dehydrovalyl-L-prolinamide (27). 25 (592 mg, 2.75 mmol) and L-proline methyl ester hydrochloride (455 mg, 2.75 mmol) were coupled using method IV to give the dipeptide methyl ester as an amorphous solid (406 mg, 45%): ^1H NMR ($CDCl_3$) δ 6.07−5.80 (br s, 1H), 4.57 (br t, 1H, J = 6.1 Hz), 3.94−3.60 (m, 4H), 3.60−3.42 (m, 1H), 2.39−2.19 (m, 1H), 2.12−1.84 (m, 6H), 1.72 (s, 3H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 172.6, 166.8, 153.1, 127.9, 124.4, 79.9, 58.3, 52.0, 47.9, 29.6, 28.2, 24.9, 19.5, 18.9. Hydrolysis in CH_3OH/H_2O (4:1, 20 mL) and LiOH·H_2O (78.3 mg, 1.8 mmol) and heating at 50 °C for 2.5 h provided **27** (281 mg, 79%) as an amorphous solid: ^1H NMR ($CDCl_3$) δ 6.17 (br s, 1H), 4.70−4.56 (br m, 1H), 3.79−3.48 (m, 2H), 2.41−2.07 (br m, 2H), 2.04−1.90 (m, 2H), 1.79 (s, 3H), 1.74 (s, 3H), 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 172.7, 168.3, 153.4, 125.9, 124.0, 80.9, 59.6, 48.5, 28.8, 28.2, 24.6, 19.8, 18.6.

(*tert*-Butyloxycarbonyl)-D-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-L-prolinamide (28). Acid **26** (1.0 g, 3.18 mmol) was coupled to **3** (816 mg, 3.18 mmol) via method I to yield **28** (1.4 g, 85%) as a white solid: ^1H NMR (300 MHz, $CDCl_3$) δ 8.02 (d, 1H, J = 8 Hz, NH), 7.8 (br d, 0.5H, NH), 5.3 (br d, 0.5H, NH), 5.18 (dd, 0.5H, J = 8.6, 4.6 Hz), 4.91 (dd, 1 H, J = 7.6, 4.3 Hz), 4.68 (dd, 0.5H, J = 7.8, 6.2 Hz), 4.26 (m, 1H), 3.85 and 3.65 (pr m, 2H, CH_2N), 2.55−1.7 (series of m, 6H, β-CH of Val, CH_2CH_2), 1.44 (s, 9H, tBu), 1.1−0.82 (m, 12H, 4 × CH_3); ^{19}F NMR ($CDCl_3$) δ −82.12 (s, CF_3), −121.31 and −122.75 (AB quartet, J = 296 Hz, CF_2).

(*tert*-Butyloxycarbonyl)dehydrovalyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-L-prolinamide (29a,b). A stirred solution of **27** (280 mg, 0.90 mmol) and **3** (229 mg, 0.90 mmol) were coupled using method I to provide, after flash chromatography, diastereomers **29b** (higher R_f, 84.7 mg, 18%) and **29a** (lower R_f, 67.7 mg, 15%) as amorphous solids. **29a**: ^1H NMR ($CDCl_3$) δ 7.99 (d, 1H, J = 6.9 Hz), 6.12 (s, 1H), 4.97 (t, 1H, J = 7.6 Hz), 4.67 (dd, 1H, J = 8.2, 4.4 Hz), 3.58−3.38 (m, 2H), 2.46−2.05 (m, 4H), 1.97−1.83 (m, 2H), 1.74 (s, 3H), 1.72 (s, 3H), 1.44 (s, 9H), 1.00 (d, 3H, J = 6.8 Hz), 0.97 (d, 3H, J = 6.9 Hz); ^{19}F NMR ($CDCl_3$) δ −79.25, −82.13, −121.90, −122.11. **29b**: ^1H NMR ($CDCl_3$) δ 8.00 (d, 1H, J = 6.9 Hz), 6.05 (s, 1H), 4.82 (t, 1H, J = 7.0 Hz), 4.68 (dd, 1H, J = 11.9, 3.8 Hz), 3.68−3.55 (m, 1H), 3.55−3.42 (m, 1H), 2.48−2.25 (m, 2H), 2.14−1.86 (m, 3H), 1.72 (s, 3H), 1.71 (s, 3H), 1.44 (s, 9H), 1.01 (d, 3H, J = 6.7 Hz), 0.93 (d, 3H, J = 6.9 Hz); ^{19}F NMR ($CDCl_3$) δ −79.35, −82.09, −121.99, −122.03.

D-Valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-L-prolinamide Hydrochloride Salt (30). Treatment of **28** (1.3 g, 2.5 mmol) as described in general procedure I afforded **30** (964 mg, 85%) as a white foam.

Dehydrovalyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-L-prolinamide Hydrochloride Salt (31a,b). A solution of **29b** (67.7 mg, 0.132 mmol) was treated as described in general procedure I to afford **31b**. The synthesis of **31a** was carried out in the same fashion starting with **29a**.

***N*-[4-[(4-Morpholinyl)carbonyl]benzoyl]-D-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-L-prolinamide (32).** A solution of **30** (960 mg, 2.12 mmol) in CH_2Cl_2 (20 mL) was treated as described in general procedure II to afford **32** (900 mg, 67%) as an amorphous solid: IR (KBr) 3427, 3313, 3053, 2968, 2935, 2897, 2877, 1751, 1635, 1533, 1435, 1394, 1327, 1302, 1280, 1261, 1222, 1197, 1161, 1114, 1068, 1026, 1014, 918, 896, 862, 842, 785, 738, 655, 597 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.9−7.7 and 7.52−7.40 (pr m, 5H, aryl and NH), 6.83 (m, 1H, NH), 4.93 (m, 1H, α-CH of Val), 4.7 (m, 2H, α-CH of Val and CH of Pro), 4.08−3.25 (series of m, 10 H), 2.55−1.82 (series of m, 6H), 1.16−0.70 (m, 12H, 4 × CH_3); ^{13}C NMR ($CDCl_3$) δ 172.1, 170.8, 170.6, 169.3, 169.2, 166.6, 166.4, 138.6, 138.5, 134.7, 127.4, 127.36, 127.34, 127.2, 66.83, 66.80, 59.9, 59.7, 59.5, 59.3, 58.9, 57.1, 56.8, 55.93, 55.90, 52.2, 48.1, 48.0, 47.6, 47.2, 42.5, 31.7, 31.1, 31.0, 29.1, 28.8, 28.7, 27.5, 27.3, 24.67, 24.61, 24.4, 19.7, 19.5, 19.4, 18.5, 18.3, 17.6, 17.5, 16.4, 16.3; ^{19}F NMR ($CDCl_3$) δ −82.10, −82.15 (2s, CF_3), −121.39, −122.69 (AB quartet, J = 298 Hz, CF_2) also

−121.96 (s); MS (CI/CH_4) *m/z* (rel intensity) 661 ($M^+ + 29$), 634, 633 (MH^+), 446, 333, 317 (100), 305, 237, 218, 193, 144, 130. Anal. ($C_{29}H_{37}F_5N_4O_6$) C, H, N.

***N*-[4-[(4-Morpholinyl)carbonyl]benzoyl]dehydrovalyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-L-prolinamide (33a,b).** A solution of **31b** (0.132 mmol) was treated as described in general procedure II to give **33b** (38.1 mg, 47%) as a colorless glass: IR (KBr) 3435, 3298, 3048, 2972, 1753, 1638, 1528, 1497, 1437, 1319, 1302, 1281, 1260, 1223, 1198, 1117 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.90 (s, 1H), 8.05−7.75 (m, 3H), 7.54−7.33 (m, 2H), 4.97 (t, 1H, J = 7.5 Hz), 4.66 (dd, 1H, J = 8.1, 4.0 Hz), 4.03−3.18 (br m, 4H), 2.46−2.09 (m, 3H), 2.09−1.87 (m, 2H), 1.77 (s, 3H), 1.66 (s, 3H), 0.96 (d, 3H, J = 6.6 Hz), 0.84 (d, 3H, J = 6.7 Hz); ^{19}F NMR ($CDCl_3$) δ −82.02, −121.91, −121.95; MS (CI/CH_4) *m/z* 631 (MH^+), 317 (base peak). Anal. ($C_{29}H_{35}F_5N_4O_6 \cdot 0.2\ H_2O$) C, H, N.

The same procedure as above, using **31a** as the starting material, provided **33a** (53.6 mg, 52%) as a colorless glass: IR (KBr) 3435, 3295, 2972, 1753, 1640, 1528, 1497, 1437, 1302, 1280, 1223, 1198, 1117 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.12 (s, 1H), 7.87 (d, 2H, J = 8.4 Hz), 7.60 (d, 1H, J = 9.0 Hz), 7.44 (d, 2H, J = 8.4 Hz), 5.00 (dd, 1H, J = 8.3, 5.6 Hz), 4.61 (dd, 1H, J = 8.1, 4.5 Hz), 4.02−3.18 (br m, 4H), 2.51−2.08 (m, 3H), 2.85−2.08 (m, 2H), 1.74 (s, 3H), 1.58 (s, 3H), 1.00 (d, 3H, J = 6.8 Hz), 0.94 (d, 3H, J = 6.9 Hz); ^{19}F NMR ($CDCl_3$) δ −81.97, −121.89; MS (CI/CH_4) *m/z* 631 (MH^+), 317 (base peak). Anal. ($C_{29}H_{35}F_5N_4O_6 \cdot 0.4\ H_2O$) C, H, N.

***N*-[4-[(4-Morpholinyl)carbonyl]benzoyl]dehydrovalyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-L-prolinamide (33c).** The D,L-mixture at P_1, **33**, was carried through the same sequence of reactions to provide **33c** (600 mg, 54%) as an amorphous solid after flash chromatography (eluted with 1:27 MeOH/CH_2Cl_2): ^1H NMR ($CDCl_3$) δ 8.19 (s, 0.5H, NH), 8.02−7.93 (m, 1H, NH), 7.87 (m, 2H, aryl), 7.68 (d, 0.5H, J = 8 Hz, NH), 4.97 (dd, 1H, J = 9, 7 Hz), 4.68 (2dd, 1H, ratio 3:2, J = 8, 4 Hz), 3.9−3.3 (series of m, 10H), 2.4−1.9 (series of m, 5H),1.77 (m, 3H, CH_3), 1.66 (d, 3H, J = 9 Hz, CH_3), 0.97 (m, 3H, CH_3), 0.85 (d, 3H, J = 7 Hz); ^{19}F NMR ($CDCl_3$) δ −82.10 (s, CF_3), −121.99 (d, J = 8.5 Hz), −122.03 (overlapping s); MS (CI/CH_4) *m/z* 659, 631 (MH^+), 357, 345, 317 (base peak), 297, 84, 70. Anal. ($C_{29}H_{35}F_5N_4O_6 \cdot 0.6H_2O$) C, H, N.

***trans*-4-Benzyloxyproline Hydrochloride Salt (38).** HCl (g) was bubbled for 2 min into an ice-cooled solution of (*tert*-butyloxycarbonyl)-*O*-benzyl-L-hydroxyproline (5.0 g, 15.6 mmol) in EtOAc (30 mL). The reaction was then stoppered, stirred at room temperature for 1 h, and conc in vacuo to provide a white solid which was triturated with Et_2O and dried under vacuum to yield **38** (3.92, 98%): mp 188−190 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 9.1 (br s, 1H), 7.3 (m, 5H), 4.56 (s, 2H), 4.4−4.2 (m, 2H), 3.6−3.38 (m, 3H), 2.6−2.58 (m, 1H), 2.26−2.1 (m, 1H); MS *m/z* (rel intensity) 262 ($M^+ + C_3H_5$),250 ($M^+ + C_2H_5$), 222 (MH^+, 100), 176, 130, 107, 91, 85, 69.

(*tert*-Butyloxycarbonyl)-L-valyl-L-azetidinecarboxylic Acid (39). Coupling Method II. To a stirred solution of (*S*)-(−)-2-azetidinecarboxylic acid (**34**) (1.0 g, 10 mmol) and Et_3N (1.5 mL, 11 mmol) in DMF (30 mL) was added **2** (2.8 g, 9.0 mmol), and the reaction mixture was heated to 120 °C for 2.5 h. Upon cooling the reaction mixture was conc in vacuo, and the oily residue was dissolved in EtOAc, washed with 1 N HCl (2 × 30 mL), dried ($MgSO_4$), and conc to afford **39** (1.88 g, 65%) as an amorphous solid: ^1H NMR (300 MHz, $CDCl_3$) δ 9.15 (br s, 1H), 5.09 (d, 1H, J = 8.8 Hz, NH), 5.00 (dd, 1H, J = 9.2, 7.2 Hz, α-CH of Val), 4.43 (dd, 1H, J = 7.0, 1.49 Hz), 4.17 (m, 1H), 3.94 (app t, 1H, J = 8 Hz), 2.62 (m, 3H), 1.44 (s, 9H, tBu), 0.97 (d, 6H, J = 6.7 Hz, 2 × CH_3).

(*tert*-Butyloxycarbonyl)-L-valyl-D,L-pipecolinic Acid (40). Prepared by method II using d,L-pipecolinic acid (**35**) (1.30 g, 10 mmol) to afford **40** (579 mg, 29%), a mixture of two enantiomers, as an amorphous solid: ^1H NMR (300 MHz, $CDCl_3$) δ 8.94 (br s, 1H), 5.62 (d, 1H, J = 9.8 Hz, NH), 5.45 (d, 0.5H, J = 3.98 Hz), 5.07 (d, 0.5H, J = 9.25, D-Pec), 4.60 (dd, 0.5H, J = 8.83, 5.06 Hz, α-CH of Val), 4.25 (dd, 0.5H, J = 8.69, 4.66 Hz, Val of D-compd), 3.93 (app d, 1H, J = 12.47 Hz, Pec), 3.25 (m, 1H, Pec), 2.31 (m, 1H, Pec), 2.20 (m, 0.5H, Val of D-compd), 2.01 (m, 0.5H, Val), 1.78−1.33 (m, 5H), 1.44 (s, 9H, tBu), 1.01−0.86 (m, 6H, 2 × CH_3).

(*tert*-Butyloxycarbonyl)-L-valyl-*trans*-4-hydroxyproline (41). *trans*-4-Hydroxy-L-proline (1.31 g, 10 mmol) was treated as described in method II to give **41** (1.85 g, 56%) as an amorphous solid: ^1H NMR (300 MHz, $CDCl_3$) δ 5.70−5.25 (m, 1H, NH), 5.05 (m, 1H), 4.80−3.95 (m, 4H), 3.85−2.80 (series of m, 3H), 2.35−1.80 (m, 2H), 1.44 (s, 9H, tBu), 1.01−0.95 (m, 6H, 2 × CH_3); MS (CI/CH_4) *m/z* (rel intensity) 331 (MH^+), 303, 275 (100), 259, 231, 217, 172, 162, 144, 132, 116, 86, 72.

(*tert*-Butyloxycarbonyl)-L-valyl-*trans*-4-benzyloxyproline (42). *trans*-4-Benzyloxy-L-proline, hydrochloride salt (**38**) (2.57 g, 10 mmol) was coupled to **2** (3.14 g, 10 mmol) as described in method II to give **42** (1.8 g, 42%). Crystallization (EtOAc/hexane) afforded a white solid: mp 125−128 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 8.0 (br s, 1H), 7.4−7.2 (m, 5H), 5.35 (d, 1H, J = 9.2 Hz, NH), 4.65 (m, 1H), 4.52 (m, 2H), 3.70 (m, 1H), 2.5−1.8 (series of m, 3H), 1.44 (s, 9H, tBu), 0.98 (d, 3H, J = 6.8 Hz, CH_3), 0.92 (d,, 3H, J = 6.8 Hz, CH_3).

(*tert*-Butyloxycarbonyl)-L-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-L-azetidinamide (43). Acid **39** (1.80 g, 6.0 mmol) was couple to **3** (1.54 g, 6.0 mmol) via method I to give **44** (2.20 g, 73%) as a colorless oil: ^1H NMR (300 MHz, $CDCl_3$) δ 8.4 (m, 1H, NH), 5.12−4.90 (m, 2H), 4.38 (m, 1H), 4.11 (m, 1H), 4.00 (m, 1H), 2.73 (m, 1H), 2.42 (2m, 2H), 1.92 (m, 2H), 1.45 (s, 9H, tBu), 1.11−0.85 (m, 12H, 4 × CH_3); ^{19}F NMR ($CDCl_3$) δ −82.14 (s, CF_3), −120.99 and −123.09, −121.23 and −122.84 (2AB quartets, J = 296 Hz, CF_2).

(*tert*-Butyloxycarbonyl)-L-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-D,L-pipecolinamide (44). Prepared from **40** (450 mg, 1.37 mmol) by method II to afford **44** (580 mg, 80%) as an amorphous solid: ^1H NMR (300 MHz, $CDCl_3$) δ 6.98 (m, 1H, NH), 5.36−4.88 (m, 3H), 4.60 (m, 1H), 4.43 (m, 1H), 3.92 (m, 1H), 3.10 (m, 1H, CH of Pec), 2.36−1.92 (series of m, 3H, Pec and CH of Val), 1.79−1.24 (m, 4H), 1.45 (s, 9H, tBu), 1.05−0.83 (m, 12H, 4 × CH_3); ^{19}F NMR ($CDCl_3$) δ −82.10 (s, CF_3), −120.74, −123.40 (m, CF_2).

(*tert*-Butyloxycarbonyl)-L-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-*trans*-4-hydroxyprolinamide (45). Acid **41** (1.80 g, 5.60 mmol) was coupled by method II to yield **45** (1.44 g, 48%) as an amorphous solid: ^1H NMR (300 MHz, $CDCl_3$) δ 7.98 (br d, 0.5H, J = 7.6 Hz, NH), 7.73 (br d, 0.5H, J = 7.6 Hz, NH), 5.47 (br d, 1H, J = 8.6 Hz, NH), 5.04−4.95 (m, 1H), 4.76−4.67 (m 1H), 4.49 (br s, 1H), 4.23 (m, 1H), 3.94 (br d, 1H, J = 10.8 Hz), 3.66−3.59 (m, 1H), 2.50−1.98 (series of m, 4H), 1.42 (s, 9H, tBu), 1.09−0.88 (m, 12H, 4 × CH_3); ^{13}C NMR ($CDCl_3$) δ 173.9, 173.2, 170.6, 170.4, 156.3, 156.2, 80.5, 80.4, 77.4, 77.2, 77.0, 76.5, 70.0, 69.8, 59.7, 59.5, 58.2, 57.7, 57.6, 55.7, 55.5, 35.7, 34.7, 31.0, 30.9, 29.0, 28.6, 28.3, 28.2, 20.2, 19.9, 19.3, 19.7, 18.3, 18.2, 16.4, 16.1; ^{19}F NMR ($CDCl_3$) δ −82.17 (s, CF_3), −82.18 (s, CF3), −121.37 and −122.92 (AB quartet, J = 296 Hz, CF_2), −121.6 and −122.8 (AB quartet, J = 296 Hz, CF_2); MS (CI/CH_4) *m/z* (rel intensity) 549 (MNH_4^+, 12), 532 (MH^+, 54), 482 (11), 330 (15), 245 (100), 189 (15).

(*tert*-Butyloxycarbonyl)-L-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-*trans*-4-benzyloxyprolinamide (46). Acid **22** (1.40 g, 3.30 mmol) was coupled to **3** (840 mg, 3.30 mmol) via method I to yield **46** (2.0 g, 95%) as a white solid: mp 91−93 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.96 (0.5H, NH), 7.33 (m, 5H), 5.28 (m, 1H, NH), 4.95 (m, 1H), 4.83 (m, 1H), 4.70 (t, 0.5H), 4.54 (q, 2H, CH_2Ph), 4.30 (m, 2H), 4.07−3.9 (m, 1H), 3.60 (m, 1 H), 2.68 (dt, 0.5H), 2.49 (dt, 0.5H), 2.33 (m, 1H), 2.2−1.88 (m, 3H), 1.44 (s, 9H, tBu), 1.09−0.86 (m, 12H, 4 × CH_3).

L-Valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-L-azetamide Hydrochloride Salt (47). Pentafluoroethyl ketone **43** (2.0 g, 3.98 mmol) was treated as described in general procedure I to afford **47** (1.63 g, 96%) as an amorphous solid: IR (film) 3196, 2972, 2937, 2883, 2636, 1755, 1655; ^1H NMR (300 MHz, $CDCl_3$) δ 8.09 (d, 1H, J = 8.2 Hz, NH), 8.02

(d, 1H, J = 8.5 Hz, NH), 5.15 (m, 1H), 5.00 (m, 1H), 4.55 (m, 1H), 4.13 (m, 1H), 3.90 (m, 1H), 2.56 (m, 2H), 2.30 (m, 3H), 1.12−0.84 (m, 12H, 4 × CH_3); ^{13}C NMR ($CDCl_3$) δ 193.2, 170.7, 170.4, 170.3, 169.9, 62.2, 61.5, 59.7, 59.5, 55.1, 55.0, 50.1, 30.0, 29.9, 28.9, 28.7, 19.8, 19.7, 18.8, 18.5, 18.2, 18.1, 17.8, 16.6, 16.3; ^{19}F NMR ($CDCl_3$) δ −82.06 and −82.14 (2s, CF_3), −121.16 and −122.76, −121.33 and −122.88 (2AB quartets, J = 296 Hz, CF_2); MS (CI/CH_4) m/z (rel intensity) 442 (6), 430 (18), 402 (MH^+, 100), 303 (9), 72 (42). Anal. ($C_{16}H_{24}F_5N_3O_3$·HCl) C, H, N.

L-Valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-D,L-pipecolinamide Hydrochloride Salt (48). Prepared from **44** (530 mg, 1.0 mmol) as described in general procedure I to afford **48** (460 mg, 99%) as an amorphous solid.

***N*-L-Valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-*trans*-4-hydroxyprolinamide Hydrochloride Salt (49) and *N*-L-Valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-*trans*-4-acetoxyprolinamide Hydrochloride Salt (50).** Pentafluoroethyl ketone **45** (1.44 g, 2.70 mmol) was treated as described in general procedure I to afford **49** and the acetoxy derivative **50** as a minor product (1.27 g, 100%) as an amorphous solid.

***N*-L-Valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-*trans*-4-benzyloxyprolinamide Hydrochloride Salt (51).** Pentafluoroethyl ketone **46** (330 mg, 0.53 mmol) was treated as described in general procedure I to afford **51** (240 mg, 81%) as an amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (m, 2H, NH_2), 7.33 (m, 5H), 5.04 (app q, 1H), 4.88 (dq, 1H), 4.52 (q, 2H, CH_2Ph), 4.33 (m, 1H), 4.0−3.8 (series of m, 4H), 2.42−2.1 (m, 4 H), 1.09−0.86 (m, 12H, 4 × CH_3).

***N*-[4-[(4-Morpholinyl)carbonyl]benzoyl]-L-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-L-azetamide (52).** Pentafluoroethyl ketone **47** (1.5 g, 3.43 mmol) was treated as described in general procedure II to afford **52** (335 mg, 16%) as an amorphous solid: IR (KBr) 3690, 3678, 3429, 3271, 3011, 2972, 2931, 2899, 2876, 2862, 1755, 1680, 1631; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32 (m, 1H, NH), 7.86 (d, 2H, J = 8.5 Hz, aryl), 7.49 (d, 2H, J = 7.9 Hz, aryl), 6.70 (m, 1H, NH), 5.00 (m, 2H), 4.50 (m, 2H), 4.19 (m, 1H), 4.86−3.30 (series of m, 8H), 2.82−1.95 (series of m, 4H), 1.05 (m, 9H, 3 × CH_3), 0.88 (d, 3H, J = 6.9 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 174.07, 174.05, 170.6, 169.2, 166.4, 138.66, 138.62, 135.0, 134.9, 127.4, 127.3, 66.8, 66.7, 62.0, 61.7, 59.7, 59.6, 54.1, 54.0, 49.3, 31.5, 31.4, 28.7, 28.5, 20.0, 19.0, 18.8, 18.2, 18.1, 18.09, 18.05, 16.1, 16.0; ^{19}F NMR ($CDCl_3$) δ −82.12 (s, CF_3), −120.98 and −123.12, −121.20 and −122.86 (2AB quartets, J = 296 Hz, CF_2); MS (CI/CH_4) m/z (rel intensity) 647, 619 (MH^+), 303 (100), 289, 218. Anal. ($C_{28}H_{35}F_5N_4O_6$·0.3 H_2O) C, H, N.

***N*-[4-[(4-Morpholinyl)carbonyl]benzoyl]-L-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-D,L-pipecolinamide (53).** Prepared from **48** (450 mg, 10.0 mmol) as described in general procedure II to afford **53** (270 mg, 42%) as an amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95−7.76 (m, 2H, aryl), 7.58−7.39 (m, 2H), 7.20−6.86 (m, 2H, aryl), 5.40−4.30 (m, 4H), 4.20−3.20 (m, 10H, 2 × NCH_2CH_2O and NCH_2 of Pro), 2.60−1.95 (m, 3H), 1.90−1.82 (m, 4H), 1.25−0.75 (m, 12H); ^{19}F NMR ($CDCl_3$) δ −81.97 (m, CF_3), −121.82 and −119.87 (m, CF_2); MS (CI/CH_4) m/z (rel intensity) 647 (MH^+), 564, 536, 474, 428, 363, 331 (100), 317, 289, 246, 218, 186, 158, 104, 84, 72.

***N*-[4-[(4-Morpholinyl)carbonyl]benzoyl]-L-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-*trans*-4-hydroxyprolinamide (54) and *N*-[4-[(4-Morpholinyl)-carbonyl]benzoyl]-L-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-*trans*-4-acetoxyprolinamide (55).** Prepared from **49** and **50** (800 mg, 1.71 mmol) as described in general procedure II to afford **55** (higher R_f fractions, 90 mg, 7.6%) as an amorphous solid and **54** (lower R_f fractions, 160 mg, 14.5%) also as an amorphous solid. **54**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (m, 2H, aryl), 7.45 (d, 2H, J = 8.3 Hz, aryl), 7.35 (d, 0.5H, J = 8.4 Hz, NH), 6.91 (d, 1H, J = 8.7 Hz, NH), 4.99 (m, 1H), 4.84 (t, 1H), 4.72 (t, 1H), 4.68 (m, 1H), 4.55 (br s, 1H), 4.18 (m, 1H), 3.8−3.4 (br s overlapping m, 9H), 2.86−2.03 (series of m, 4H), 1.26 (m, 9H, 3 × CH_3), 0.96 (m, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 173.2, 172.5, 170.7, 170.4, 169.2, 167.1, 138.8, 134.9, 127.52, 127.50, 127.4, 127.3, 77.55, 77.52, 77.51, 77.46, 77.44, 77.3, 77.2, 77.1, 77.0, 76.88, 76.85, 76.6, 76.56, 76.54, 70.1, 69.9, 66.8, 59.6, 59.5, 58.5, 57.9, 57.0, 56.0, 56.0, 55.7, 36.0, 34.9, 31.42, 31.40, 31.3, 29.2, 28.8, 20.0, 19.85, 19.83, 19.4, 19.2, 18.4, 16.5, 16.4, 16.3, 16.2; ^{19}F NMR ($CDCl_3$) δ −82.1, −82.15 (s, CF_3), −121.31, −123.02 (AB quartet, J = 293 Hz, CF_2), −121.35, −122.82 (AB quartet, J = 298 Hz, CF_2); MS (CI/CH_4) m/z (rel intensity) 649 (MH^+), 361, 334, 333 (100), 317, 289, 218, 200, 111, 86. Anal. ($C_{29}H_{37}F_5N_4O_7$) C, H, N. **55**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, 2H, J = 8.4 Hz, aryl), 7.72 (0.5H, J = 8.4 Hz, NH), 7.47 (d, 2H, J = 8.3 Hz, aryl), 6.74 (d, 1H, J = 8.6 Hz, NH), 5.36 (m, 1H), 5.00 (m, 1H), 4.83 (dd, 0.5H, J = 8.6, 7.2 Hz), 4.74 (dd, 1H, J = 8.0, 7.2 Hz), 4.69 (t, 0.5H), 4.08 (br d, 1H), 3.9−3.3 (br s overlapping m, 9H), 2.81 (m, 0.5H), 2.64 (m, 0.5H), 2.41−2.06 (series of m, 3H), 2.04 (s, 3H, OCH_3), 1.02 (m, 9H, 3 × CH_3), 0.94 (m, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 173.0, 172.3, 170.4, 170.1, 169.9, 169.2, 166.3, 138.7, 127.4, 127.3, 72.5, 72.4, 66.8, 59.6, 59.5, 58.5, 57.8, 56.3, 56.2, 53.2, 52.9, 34.0, 32.8, 31.8, 31.7, 31.6, 29.3, 29.2, 28.8, 24.9, 20.9, 20.0, 19.8, 19.5, 19.3, 17.95, 17.92, 17.7, 16.4, 16.2; ^{19}F NMR ($CDCl_3$) δ −82.1, −82.13 (s, CF_3), −121.22, −123.06 (AB quartet, J = 296 Hz, CF_2), −121.28, −122.86 (AB quartet, J = 301 Hz, CF_2); MS (CI/CH_4) m/z (rel intensity) 691 (MH^+), 631, 472, 444, 389, 375 (100), 349, 318, 317, 289, 264, 225, 218, 128, 100. Anal. ($C_{31}H_{39}F_5N_4O_8$) C, H, N.

***N*-[4-[(4-Morpholinyl)carbonyl]benzoyl]-L-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-*trans*-4-benzyloxyprolinamide (56).** Prepared from **51** (558 mg, 1.0 mmol) as described in general procedure II to afford **56** (520 mg, 70%) as an amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (dd, 2H, aryl), 7.78 (d, 0.5H, NH), 7.45 (dd, 2H, aryl), 7.29 (m, 5H), 7.21 (d, 0.5H, NH), 6.87 (d, 1H, NH), 4.98 (m, 1H), 4.82 (m, 1H), 4.69 (t, 0.5H), 4.54 (dq, 2H, CH_2Ph), 4.31 (br s, 1H), 4.08 (dq, 1H), 3.9−3.25 (series of m, 9H), 2.69 (dt, 0.5H), 2.46 (dt, 0.5H), 2.34 (m, 1H), 2.17 (m, 1H), 1.02 (m, 9H, 3 × CH_3), 0.89 (m, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 193.1, 172.9, 172.2, 170.7, 170.4, 169.3, 166.2, 138.4, 137.5, 137.4, 135.2, 128.5, 128.4, 128.2, 127.9, 127.8, 127.78, 127.73, 127.71, 127.5, 127.4, 127.3, 119.5, 115.76, 107.1, 106.6, 71.4, 71.2, 66.7, 59.6, 59.4, 58.7, 58.0, 56.0, 52.6, 52.3, 48.14, 48.11, 48.10, 48.0, 42.6, 42.57, 42.52, 42.4, 33.5, 32.4, 31.8, 29.1, 28.6, 20.0, 19.8, 19.4, 19.3, 17.8, 17.6, 16.3, 16.1; ^{19}F NMR ($CDCl_3$) δ −82.10, −82.13 (s, CF_3), −121.3, −122.9 and −121.4, −122.8 (AB quartet, J = 296 Hz, CF_2); MS (CI/CH_4) m/z (rel intensity) 767 (M^+ + 29), 740 (10), 739 (MH^+, 27), 632 (11), 520 (7), 492 (5), 424 (18), 423 (100), 403 (3), 345 (5), 317 (30), 289 (4), 218 (3), 176 (11), 91 (2). Anal. ($C_{36}H_{43}F_5N_4O_7$·0.4 H_2O) C, H, N.

(*tert*-Butyloxycarbonyl)-L-valyl-L-thiazolidine-4-carboxylic Acid (57). Prepared from l-thiazolidine-4-carboxylic acid (1.3 g, 10 mmol) using coupling method II to afford **57** (2.27 g, 68%) as an amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (br s, 1H), 5.57 (app t, 0.5H), 5.39 (d, 0.5H, J = 9.5 Hz), 5.11 (t, 1H, J = 5.6 Hz), 4.94 (d, 1H, J = 8.6 Hz), 4.55 (d, 1H, J = 8.3 Hz), 4.35 (m, 1H), 3.29 (d, 1H, J = 5.6 Hz), 2.30−1.54 (series of m, 2H), 1.43 (s, 9H, tBu), 1.04−0.92 (m, 6H, 2 × CH_3).

(*tert*-Butyloxycarbonyl)-L-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-L-thiazolidamide (58). Prepared from **57** (2.27 g, 6.8 mmol) using coupling method I to give **58** (3.28 g, 92%) as an amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (br d, 1H, J = 8 Hz, NH), 5.27 (m, 1H, NH), 5.00 (m, 2H), 4.51 (m, 1H), 4.38 (m, 1H), 3.64−3.38 (pr m, 1H), 3.12 (m, 1H), 2.40−1.84 (series of m, 3H), 1.44 (s, 9H, tBu), 1.15−0.84 (m, 12H, 4 × CH_3).

***N*-[4-[(4-Morpholinyl)carbonyl]benzoyl]-L-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-L-thiazolidamide (59).** The HCl salt was prepared from **58** (3.2 g, 6.0 mmol) using general procedure I to afford an amorphous solid (2.68 g, 96%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (m, 2H, NH_2), 5.36 (m, 1H), 5.12 (m, 1H), 4.95 (m, 1H), 4.58 (app t, 1H), 4.40 (m, 1H), 3.42 (m, 1H), 3.20 (m, 1H), 2.39 (m, 3H), 1.30−0.84 (m, 12H, 4 × CH_3); ^{19}F NMR ($CDCl_3$) δ −82.0 (2s,

ratio 1:3, CF_3), −120.0 and −123.8 (overlapping AB quartets, ratio 1:3, CF_2).

The HCl salt was treated as described in general procedure II to afford **59** (275 mg, 10%) as an amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (m, 2H, aryl), 7.49 (d, 2H, J = 7.9 Hz, aryl), 7.04 (m, 1H, NH), 5.08 (m, 2H), 4.92 (m, 1H), 4.60 (pr d, 1H, J = 9 Hz), 4.25 (br d, 0.5H, J = 11 Hz), 3.88−3.30 (series of m, 7H), 3.14 (m, 1H), 2.34 (m, 1H), 2.22 (m, 1H), 1.92 (pr m, 1H), 1.64 (m, 1H), 1.32 (m, 1H), 1.05 (m, 9H, 3 × CH_3), 0.87 (m, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 172.1, 171.7, 169.3, 169.25, 169.20, 166.5, 156.6, 138.6, 134.97, 134.94, 127.4, 127.3, 127.2, 66.8, 62.5, 61.7, 59.5, 59.4, 58.7, 56.3, 56.2, 50.2, 49.9, 49.1, 48.17, 48.15, 48.12, 48.08, 48.05, 48.02, 47.9, 33.9, 33.8, 31.86, 31.81, 31.1, 30.9, 30.5, 29.1, 29.0, 25.6, 24.9, 20.0, 19.9, 19.7, 19.6, 19.5, 19.4, 19.0, 18.1, 17.8, 17.7, 16.3, 16.2, 16.1; ^{19}F NMR ($CDCl_3$) δ −82.10 (s, CF_3), −121.22 and −122.88 (AB quartets, J = 296 Hz, CF_2); MS (CI/CH_4) m/z (rel intensity) 679, 651 (MH^+), 536, 416, 335, 317 (100), 225, 214.

(*tert*-Butyloxycarbonyl)-L-valyl-D,L-tetrahydroisoquinoline-1-carboxylic Acid (60). A stirred solution of D,L-tetrahydro-1-isoquinolinecarboxylic acid hydrochloride salt (2.1 g, 10 mmol) was coupled to **2** (2.0 g, 6 mmol) via method II to give **60** (484 mg, 13%) as an isomeric mixture amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.2 (m, 4H), 5.6 (d, 0.25H, J = 8 Hz, NH), 5.4 (d, 0.75H, J = 8 Hz, NH), 5.38 (m, 1H), 5.1−4.4 (series of m, 3H), 3.5−3.05 (series of m, 2H), 2.08 and 1.93 (pr of m, 1H, ratio 2:1, l/d), 1.4 (s, 9H, tBu), 1.1−0.8 (m, 6H, 2 × CH_3).

(*tert*-Butyloxycarbonyl)-L-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)-D,L-tetrahydroisoquinolinecarboxamide (61). Prepared from **60** (450 mg, 1.2 mmol) by method I to give **61** (641 mg, 93%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23 (m, 4H), 5.5 (m, 0.25H, NH), 5.45−4.15 (series of m, 6H), 4.1−3.75 (series of m, 1H), 3.5−2.9 (series of m, 2H), 2.4−1.7 (m, 2H), 1.43 (s, 9H, tBu), 1.12−0.63 (m, 12H, 4 × CH_3); ^{19}F NMR ($CDCl_3$) δ −82.01 (s, CF_3), −120.5 to −123.6 (overlapping AB quartets, CF_2).

***N*-[4-[(4-Morpholinyl)carbonyl]benzoyl]-L-valyl-*N*-(1,1,1,2,2,-pentafluoro-5-methyl-3-oxo-4-hexanyl)-D,L-1,2,3,4-tetrahydroisoquinolinecarboxamide (62a) and -L-1,2,3,4-tetrahydroisoquinolinecarboxamide (62b).** The hydrochloride salt of the deprotected amine was prepared from **61** (641 mg, 1.11 mmol) using general procedure I to give an amorphous solid (385 mg, 68%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (br m, 2H), 7.18 (m, 4H), 5.5−4.5 (series of m, 6H), 4.1−3.7 (series of m, 1H), 3.5−2.8 (series of m, 2H), 2.3 (m, 1H), 1.9 (m, 1H), 1.2−0.7 (m, 12H, 4 × CH_3); ^{19}F NMR ($CDCl_3$) δ −82.0 (overlapping s, CF_3), −120.5 to −123.7 (overlapping AB quartets, CF_2).

Using general procedure II the hydrochloride salt of the deprotected amine from **61** (385 mg, 0.749 mmol) gave **62a** (95 mg, 18%) as an amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (m, 2H, aryl), 7.46 (m, 2H, aryl), 7.2 (m, 4H), 7.1 (m, 1H, NH), 6.83 (d, 0.5H, J = 9 Hz, NH), 6.54 (d, 0.5H, J = 9 Hz, NH), 5.45−4.6 (series of m, 5H), 3.9−2.9 (series of m, 10H), 2.4−2.1 (pr of overlapping m, 2H), 1.2−0.65 (m, 12H, 4 × CH_3); ^{19}F NMR ($CDCl_3$) δ −82.13 (app t, CF_3), −121.3 and −123.1 (AB quartet, J = 296 Hz, overlapping m, CF_2); MS (CI/CH_4) m/z 723, 695 (MH^+), 578, 550, 549, 476, 379, 317, 289, 259, 234 (100), 220, 200.

The L-analogue was prepared using the same sequence to yield **62b** (77 mg, 40%) as an amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (m, 2H, aryl), 7.47 (m, 2H, aryl), 7.22 (m, 4H), 7.06 (m, 1H, NH), 6.82 (d, 0.5H, J = 9 Hz, NH), 6.54 (d, 0.5H, J = 9 Hz, NH), 5.5−4.54 (series of m, 5H), 3.9−2.98 (series of m, 10H), 2.4−2.1 (pr of overlapping m, 2H), 1.2−0.65 (m, 12H, 4 × CH_3); ^{19}F NMR ($CDCl_3$) δ −82.13 (app t, CF_3), −121.3 and −123.1, −121.34 and −122.99 (overlapping AB quartets, J = 296 Hz, CF_2); MS (CI/CH_4) m/z 723, 695 (MH^+), 562, 550, 536, 522, 476, 393, 379, 363, 331, 317, 289, 262, 246, 234, 218, 206 (100), 186, 149, 139, 132. Anal. ($C_{34}H_{39}F_5N_4O_6$) C, H, N.

(*tert*-Butyloxycarbonyl)-L-valylnipecotic Acid (63). Nipecotic acid (1.29 g, 10 mmol) was coupled to **2** (3.14 g, 10 mmol) via method II to give **63** (2.96 g, 90%), a mixture of diastereomers, as an amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.10 (br s, 1H), 5.61 (m, 1H, NH), 5.48 (m, 1H), 4.65 (m, 1H), 4.48 (m, 1H), 4.30−2.80 (m, 1H), 3.42−2.35 (series of m, 2H), 2.16 (m, 1H), 2.00−1.30 (m, 4H), 1.44 (s, 9H, tBu), 1.01−0.84 (m, 6H, 2 × CH_3).

(*tert*-Butyloxycarbonyl)-L-valylisonipecotic Acid (64). Isonipecotic acid (1.29 g, 10 mmol) was coupled to **2** (3.14 g, 10 mmol) via method II to give **64** (1.2 g, 36%), a mixture of diastereomers, as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.54 (d, 0.75H, J = 9 Hz, NH), 5.04 (d, 0.25H, J = 9 Hz, NH), 4.55−4.20 (series of m, 2H), 3.95 (m, 1H), 3.22 (m, 1H), 2.93 (m, 1H), 2.61 (m, 1H), 2.3−1.57 (series of m, 5H), 1.44 (s, 9H, tBu), 1.02−0.87 (m, 6H, 2 × CH_3).

(*tert*-Butyloxycarbonyl)-L-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)nipecotamide (65). Acid **63** (820 mg, 2.50 mmol) was coupled to **3** (640 mg, 2.50 mmol) via method I to give **65** (920 mg, 71%) as an amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (m, 1H, NH), 5.45−4.85 (m, 2H), 4.75−4.15 (m, 4H), 3.80−3.05 (m, 4H), 2.60−2.20 (series of m, 4H), 2.10−1.65 (m, 3H), 1.45 (s, 9H, tBu), 1.12−0.75 (m, 12H, 4 × CH_3).

***N*-L-Valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)nipecotamide Hydrochloride Salt (66).** Pentafluoroethyl ketone **65** (920 mg, 1.74 mmol) was treated as described in general procedure I to afford **66** (800 mg, 99%) as an amorphous solid.

(*tert*-Butyloxycarbonyl)-L-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)isonipecotamide (67). Acid **64** (950 mg, 2.9 mmol) and NMM (0.32 mL, 2.9 mmol) were coupled via method I to hydrochloride **3** (737 mg, 2.9 mmol) to give **67** (1.32 g, 86%) as an amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.98 (m, 1H), 5.34 (m, 1H), 5.13 (m, 1H), 4.75−4.25 (m, 2H), 4.12−3.82 (m, 2H), 3.55−2.25 (series of m, 2H), 2.1−1.55 (series of m, 6H), 1.44 (s, 9H, tBu), 1.12−0.75 (m, 12H, 4 × CH_3); ^{19}F NMR ($CDCl_3$) δ −82.02 (s, CF_3), −121.1 and −122.8 (AB quartet, J = 310 Hz, CF_2).

***N*-L-Valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)isonipecotamide Hydrochloride Salt (68).** A stirred solution of **67** (1.3 g, 2.45 mmol) was treated as in general procedure I to afford **68** (1.11 g, 97%) as an amorphous solid.

***N*-[4-[(4-Morpholinyl)carbonyl]benzoyl]-L-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)nipecotamide (69).** A solution of **66** (800 mg, 1.77 mmol) was treated as described in general procedure II to afford **69** (300 mg, 26%) as an amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92−7.70 (m, 2H, aryl), 7.55−7.39 (m, 2H), 5.18−4.82 (m, 2H), 4.76−4.20 (m, 1H), 4.00−2.90 (m, 11H), 2.60−1.50 (m, 9H), 1.18−0.80 (m, 12H); ^{19}F NMR ($CDCl_3$) δ −82.07, −82.11 (m, CF_3), −120.46 and −123.92 (m, CF_2); MS (CI/CH_4) m/z 647 (MH^+), 576, 526, 465, 447, 430, 412, 331 (100), 305, 301, 289, 273, 262, 218, 200.

***N*-[4-[(4-Morpholinyl)carbonyl]benzoyl]-L-valyl-*N*-(1,1,1,2,2-pentafluoro-5-methyl-3-oxo-4-hexanyl)isonipecotamide (70).** A stirred solution of **68** (800 mg, 1.77 mmol) was treated as in general procedure II to afford **70** (300 mg, 26%) as an amorphous solid: IR (KBr) 3427, 3323, 3049, 3034, 2968, 2933, 2874, 1753, 1631, 1527, 1454 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 (d, 2H, J = 7.8 Hz, aryl), 7.47 (d, 2H, J = 8.1 Hz, aryl), 7.12 (dd, 1H, J = 8.5, 3.9 Hz, NH), 6.09 (d overlapping t, 1H, J = 8.6, 5.3 Hz), 5.13 (dd, 1H, J = 8.3, 4.1 Hz), 5.05 (dd, 1H, J = 8.6, 5.3 Hz), 4.53 (m, 1H), 4.12 (br t, 1H, J = 16.4 Hz), 3.86−3.10 (series of m, 10H), 2.78 (m, 1H), 2.55−2.27 (pr of overlapping m, 2H), 2.16−1.55 (series of m, 4H), 1.12−0.8 (m, 12H, 4 × CH_3); ^{13}C NMR ($CDCl_3$) δ 173.9, 173.6, 170.1, 170.0, 169.96, 169.91, 169.3, 166.4, 166.3, 138.3, 135.6, 135.5, 127.4, 127.2, 127.1, 66.8, 58.8, 58.7, 48.16, 48.12, 48.09, 48.05, 48.02, 45.3, 44.8, 42.8, 42.7, 42.6, 42.5, 42.4, 42.3, 41.6, 41.2, 31.9, 29.3, 29.2, 29.1, 28.9, 28.7, 28.6, 28.4, 28.3, 28.1, 19.9, 19.8, 19.7, 17.4, 17.2, 17.2, 16.3; ^{19}F NMR ($CDCl_3$) δ −82.08 (s, CF_3), −121.14 and −122.9, −121.17 and −122.85 (overlapping AB quartet, J = 293, 296 Hz, CF_2); MS (CI/CH_4) m/z 687, 675, 647 (MH^+), 412, 332, 331 (100), 289, 231, 220, 218, 200, 178. Anal. ($C_{30}H_{39}F_5N_4O_6$·0.3H_2O) C, H, N.

Methyl (*tert*-Butyloxycarbonyl)-L-valyl-L-prolyl-L-valinate (71). *N*-Boc-L-valyl-L-proline (from Advanced ChemTech; 3.1 g, 0.01 mol) was coupled to L-valine methyl ester hydrochloride (1.67 g, 0.01 mol; Aldrich) via method I to afford **71** (4.27 g, 100%) as an amorphous solid: $R_f = 0.33$ in 3:1 Et_2O/hexane; IR (KBr) ν_{max} 3553, 3537, 3520, 3510, 3310, 2968, 2935, 2876, 1741, 1687, 1631, 1527, 1440, 1390, 1367, 1338, 1309, 1244, 1203, 1172, 1114 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.22 (br d, 1H, $J = 8.4$ Hz, NH), 5.24 (br d, 1H, $J = 11.0$ Hz, NH), 4.62 (dd, 1H, $J = 8.2$, 2.9 Hz, CH of Pro), 4.43 (app dd, 1H, $J = 8.6$, 5.1 Hz, α-CH of Val), 4.30 (dd, 1H, $J = 9.5$, 6.4 Hz, CH of Val) 3.75−3.70 and 3.63−3.59 (pr m, 2H, CH_2N), 3.70 (s, 3H, OMe), 2.40 (m, 1H, β-CH of Val), 2.17−1.91 (m, 5H, CH_2CH_2 and β-CH of Val), 1.43 (s, 9H, tBu), 1.00 (d, 3H, $J = 6.7$ Hz, CH_3), 0.95−0.90 (m, 9H, 3 × CH_3); ^{13}C NMR ($CDCl_3$) δ 172.5, 172.1, 170.9, 155.8, 79.5, 77.4, 77.1, 76.9, 76.8, 76.5, 59.9, 57.5, 56.7, 52.0, 47.6, 31.4, 31.0, 28.3, 28.2, 27.1, 25.1, 19.5, 18.9, 17.8, 17.3; MS (CI/CH_4) *m*/*z* (rel intensity) 428 (MH^+, 22), 372 (68), 328 (100). Anal. ($C_{21}H_{37}N_3O_6$) C, H, N.

(*tert*-Butyloxycarbonyl)-L-valyl-*N*-(1,1,1,2,2,3,3-heptafluoro-6-methyl-4-oxo-5-heptanyl)prolinamide (72). To a solution of methyl ester **71** (3.8 g, 9.0 mmol) in anhydrous Et_2O (100 mL) at −78 °C was added, dropwise, under N_2, $CF_3CF_2CF_2I$ (6.6 mL, 48.0 mmol; from Aldrich; stabilized with Cu). To this mixture was added dropwise MeLi·LiBr (1.5 M in Et_2O, 28.5 mL, 42.0 mmol) at a rate which maintained an internal reaction temperature below −70 °C. The reaction mixture was stirred at −78 °C for 1 h, the cold bath removed, and stirring continued for 5 min. The mixture was then poured into H_2O (100 mL), the layers were separated, and the aqueous phase was acidified with 1 N HCl. The aqueous phase was then extracted with additional Et_2O (100 mL), and the combined ethereal extracts were dried ($MgSO_4$). The solvent was removed in vacuo to yield a crude yellow foam which was flash chromatographed (3:1 Et_2O−hexane) to give **72** (654 mg, 13%) as an amorphous solid: IR (KBr) 3423, 3292, 2972, 2937, 2879, 2823, 2771, 2739, 2253, 1755, 1687, 1635, 1525, 1444, 1392, 1367, 1348, 1313, 1232, 1178, 1126 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, 1H, $J = 8.2$ Hz, NH), 5.44 (d, 1H, $J = 9.2$ Hz NH), 5.02 (dd, 1 H, $J = 7.8$, 4.5 Hz, CH of Val), 4.64 (dd, 1H, $J = 8.0$, 3.0 Hz, CH of Pro), 4.30 (dd, 1H, $J = 9.2$, 6.8 Hz, α-CH of Val), 3.80−3.74 and 3.66−3.60 (pr of m, 2H, CH_2N), 2.31−1.92 (series of m, 6H, β-CH of Val, CH_2CH_2), 1.44 (s, 9H, tBu), 1.02 (d, 3H, $J = 7.0$ Hz, CH_3), 0.98 (d, 3H, $J = 6.9$ Hz, CH_3), 0.94 (d, 3H, $J = 6.7$ Hz, CH_3), 0.88 (d, 3H, $J = 6.9$ Hz, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.3, 193.0, 192.7, 172.9, 171.1, 155.7, 118.7, 115.8, 111.3, 108.9, 108.6, 108.2, 105.9, 79.6, 77.3, 77.2, 76.9, 76.6, 59.7, 59.3, 56.8, 47.8, 31.4, 29.0, 28.3, 26.9, 25.1, 19.9, 19.8, 19.7, 19.5, 19.4, 17.5, 17.4, 16.3, 16.1; ^{19}F NMR (376.3 MHz, $CDCl_3$) δ −80.91 (t, CF_3), −119.03 and −120.43 (AB quartet, $J = 297$ Hz, CF_2), −126.62 (s, CF_2); MS (CI/CH_4) *m*/*z* (rel intensity) 566 (MH^+, 100); HRMS ($C_{23}H_{34}F_7N_3O_5$) (M^+) calcd 566.2492, obsd 566.2475.

***N*-[4-[(4-Morpholinyl)carbonyl]benzoyl]-L-valyl-*N*-(1,1,1,2,2,3,3-heptafluoro-6-methyl-4-oxo-5-heptanyl)prolinamide (73).** The Boc protection was removed from **72** using general procedure I to give the HCl salt (185 mg, 0.37 mmol), and 4-[(4-morpholinyl)carbonyl]benzoyl chloride was added as described in general procedure II to afford crude **73** (260 mg) as a white foam. This was flash chromatographed (2- × 15-cm column eluted with 1:27 MeOH−CH_2Cl_2) to give **73** (162 mg, 64%) as an amorphous solid: IR (KBr) 3431, 3323, 3049, 2970, 2935, 2877, 1755, 1693, 1631, 1529, 1437, 1394, 1346, 1300, 1278, 1259, 1232, 1161, 1118, 1068 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, 2H, $J = 8.4$ Hz, aryl), 7.52 (d, 1H, $J = 8.4$ Hz, NH), 7.46 (d, 2H, $J = 8.3$ Hz, aryl), 7.12 (d, 1H, $J = 8.7$ Hz, NH), 5.04 (dd, 1H, $J = 8.2$, 4.2 Hz, α-CH of Val), 4.84 (dd, 1H, $J = 8.6$, 7.3 Hz, α-CH of Val), 4.62 (dd, 1H, $J = 7.9$, 2.9 Hz, CH of Pro), 3.94−3.37 (m, 10H, 2 × NCH_2CH_2O and NCH_2 of Pro), 2.29−1.97 (series of m, 6H, 2 × β-CH of Val and CH_2CH_2), 1.06 (d, 3H, $J = 6.8$ Hz, CH_3), 1.01 (d, 6H, $J = 6.7$ Hz, 2 × CH_3), 0.86 (d, 3H, $J = 6.9$ Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 172.2, 170.9, 169.2, 166.3, 138.5, 135.1, 127.4, 127.3, 77.4, 77.1, 76.9, 76.5, 66.7, 59.9, 59.3, 55.9, 47.9, 31.8, 29.1, 27.0, 25.1, 19.8, 19.5, 17.8, 16.2; ^{19}F NMR (470.2 MHz, $CDCl_3$) δ −80.24 (t, $J = 9$ Hz, CF_3), −118.39 and −119.87 (dq, $J = 295$, 9 Hz, $COCF_2$), −125.99 (AB m, CF_2); MS (CI/CH_4) *m*/*z* (rel intensity) 683 (MH^+, 59), 367 (100). Anal. ($C_{30}H_{37}F_7N4O_6 \cdot 1.3H_2O$) C, H, N.

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Supporting Information Available: Nomenclature (Figure 9) and modeling coordinates (Tables 5 and 6) for HNE inhibitor **1** (4 pages). Ordering information is given on any current masthead page.

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