Peptidomimetic Inhibitors of the Human Cytomegalovirus Protease

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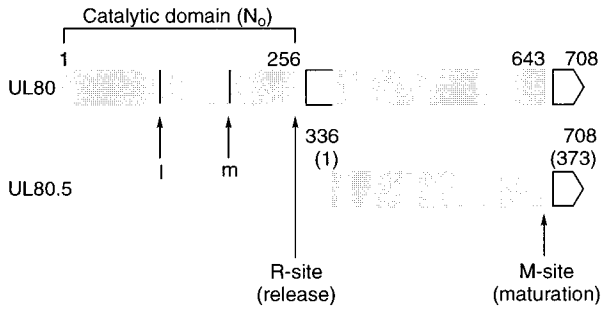
The development of peptidomimetic inhibitors of the human cytomegalovirus (HCMV) protease showing sub-micromolar potency in an enzymatic assay is described. Selective substitution of the amino acid residues of these inhibitors led to the identification of tripeptide inhibitors showing improvements in inhibitor potency of 27-fold relative to inhibitor **39** based upon the natural tetrapeptide sequence. Small side chains at P_1 were well tolerated by this enzyme, a fact consistent with previous observations. The S_2 binding pocket of HCMV protease was very permissive, tolerating lipophilic and basic residues. The substitutions tried at P_3 indicated that a small increase in inhibitor potency could be realized by the substitution of a *tert*-leucine residue for valine. Substitutions of the N-terminal capping group did not significantly affect inhibitor potency. Pentafluoroethyl ketones, α,α-difluoro-β-keto amides, phosphonates and α-keto amides were all effective substitutions for the activated carbonyl component and gave inhibitors which were selective for HCMV protease. A slight increase in potency was observed by lengthening the P_1' residue of the α-keto amide series of inhibitors. This position also tolerated a variety of groups making this a potential site for future modifications which could modulate the physicochemical properties of these molecules.

The human cytomegalovirus (HCMV) is a highly prevalent member of the herpesvirus family infecting up to 80% of the general population.[1] This virus is responsible for opportunistic infections in immunocompromised individuals including organ transplant recipients and AIDS sufferers.[2] Clinical manifestations include disseminated disease, pneumonitis, retinitis, and gastrointestinal infections such as oesophagitis and colitis. Of particular significance are HCMV infections of neonates. This disease is the most common congenitally acquired viral infection in the world.[3] It is estimated that 1% of newborn infants are infected and up to 10% of these are symptomatic and may experience severe complications. Mortality in this latter group approaches 30%.

All members of the herpesvirus family express a protein late in the virus life cycle which appears to function as a self-assembling scaffold during the manufacture of the viral capsid.[4] This assembly protein is present in immature B-capsids and must be processed to remove a short segment of the C-terminus in order to permit the entry of viral DNA and produce an infectious virus particle. Recently it has been shown that this processing is mediated by a protease which is encoded by the virus.[5] The protease itself is expressed as a precursor protein which is autocatalytically cleaved at least twice (Scheme 1). Cleavage occurs near the C-terminal end of the UL80 gene product (M-site) to remove a small fragment, and also at a position located near the center of the precursor (R-site) to excise the catalytic domain (N_o).[6] Both N_o and the full length protease (UL80 gene product) are catalytically active.[5]

HCMV protease N_o shows significant sequence homology with other herpesvirus proteases.[5] Affinity labeling experiments[7] and site-directed mutagenesis[8] indicate that this enzyme is a serine protease. Recent

Scheme 1



crystallographic results[9] have shown that HCMV protease represents a novel structure of serine proteases and in fact possesses a unique catalytic triad.

While it has not been demonstrated that HCMV protease is absolutely required for viral replication, it has been shown that HSV-1 mutants lacking the analogous enzyme or expressing defective variations of it are unable to grow.[10] The high degree of homology between the proteases of HSV and HCMV support the idea that specific inhibitors of HCMV protease would show antiviral activity and thus have therapeutic value. In this paper, we describe the development of inhibitors of HCMV protease based on the amino acid sequences of the two cleavage sites.[11]

Chemistry

The syntheses of the various inhibitors and the required intermediates are described in Schemes 2−7. Inhibitors containing a trifluoromethyl ketone function were obtained in one of three ways (Schemes 2 and 3). Inhibitors which incorporate an asparagine residue at P_2 (Scheme 2) were most conveniently prepared through solid-phase synthesis using the asparagine side chain as an attachment point to the resin.[12] Thus a Henry reaction between hemiacetal **1** and nitroethane gave

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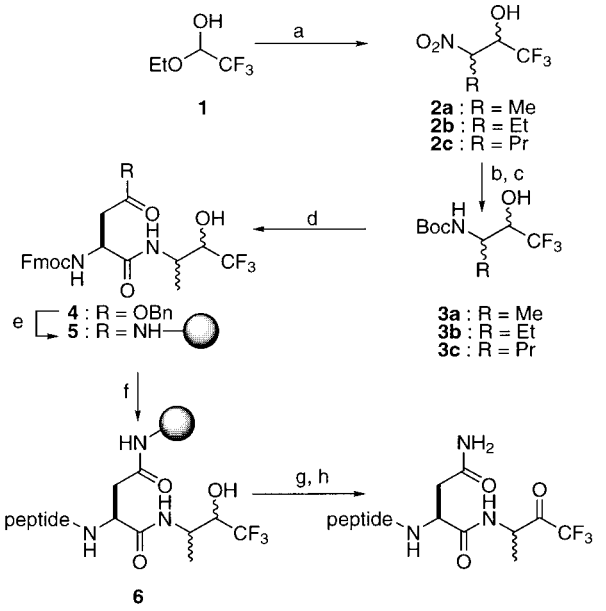
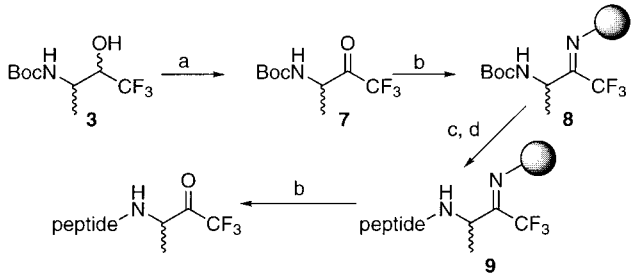
Scheme 2[a]



[a] Reagents and conditions: (a) RCH_2NO_2, K_2CO_3; (b) Raney nickel, EtOH, then HCl; (c) $(Boc)_2O$, K_2CO_3, THF; (d) HCl, dioxane, then Fmoc-Asp(γOBn), DCC, HOBT, CH_2Cl_2, DMF; (e) TFA, CH_2Cl_2, then DCC, HOBT, Knorr resin, NMP; (f) piperidine, NMP, then Fmoc-amino acid, DCC, HOBT, NMP; (g) TFA; (h) DMSO, $CHCl_2CO_2H$.

Scheme 3[a]

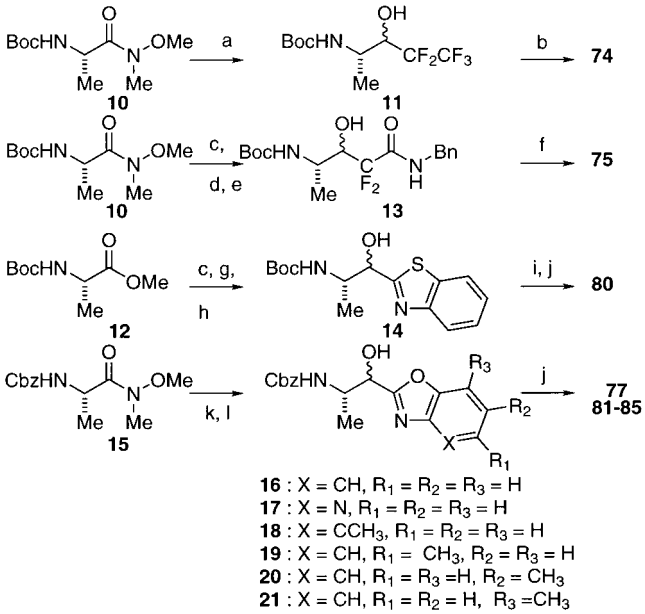


[a] Reagents and conditions: (a) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C; (b) ref 14; (c) TFA, CH_2Cl_2; (d) Fmoc-amino acid, TBTU, HOBT, DMF, *i*-Pr_2NEt.

nitro alcohol **2a** which was immediately reduced and protected to yield alcohol **3a**. After removal of the Boc group, coupling with a suitably protected aspartic acid derivative gave **4**. This compound was then deprotected by hydrolysis and incorporated onto a polymer support to afford the derivatized amide resin **5**. The required amino acids were then introduced by standard methods. Hydrolysis from the resin and oxidation of the resulting alcohol using Moffatt's procedure[13] gave the desired peptides. Trifluoromethyl ketones which contain a P_2 residue other than asparagine were prepared using solution methods from alcohol **3a** or by solid-phase techniques. In these latter cases the trifluoromethyl ketone was used as an attachment point to the resin (Scheme 3).[14] Thus ketone **7**, derived from alcohol **3a**, was attached to a suitable support to give resin **8**. Peptide extension was then accomplished in the usual manner. Subsequent cleavage under acidic conditions gave the required peptides.

Peptides containing an activated carbonyl group other than a trifluoromethyl ketone are shown in Tables 6 and 7. In general, these compounds were prepared by sequentially coupling a suitably protected amino alcohol with the required amino acids or peptide segment. After

Scheme 4[a]

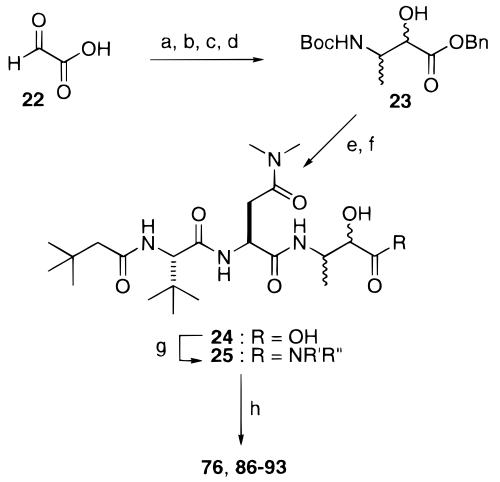


[a] Reagents and conditions: (a) CF_3CF_2I, MeLi·LiBr, Et_2O, −78 to −30 °C, then $NaBH_4$, MeOH:THF:H_2O 3:1:1, 0 °C; (b) method D; (c) $LiAlH_4$, THF; (d) Zn, $BrCF_2CO_2Et$, THF, ultrasound; (e) NH_2CH_2Ph, *i*-Pr_2NEt, EtOH, reflux; (f) method E; (g) SO_3·py, Et_3N, DMSO, CH_2Cl_2; (h) BuLi, benzothiazole, THF, −78 °C; (i) HCl, dioxane; (j) method F; (k) $LiAlH_4$, THF, 0 °C, then $NaHSO_3$, NaCN, CH_2Cl_2, H_2O; (l) EtOH, AcCl, $CHCl_3$, 0 °C, then aminophenol, EtOH, reflux.

the complete backbone was established, oxidation of the resulting alcohols gave the desired compounds. The preparations of the various building blocks are shown in Schemes 4−6. Condensation of Weinreb amide **10** with CF_3CF_2Li[15] followed by reduction with $NaBH_4$ gave pentafluoroethyl-substituted alcohol **11** (Scheme 4). The α,α-difluoro amide **13** was prepared from an ultrasonic Reformatsky reaction[16] between ethyl bromodifluoroacetate and Boc-alaninal followed by treatment with benzylamine. Benzothiazole **14** was obtained in a straightforward manner when 2-lithiobenzothiazole was added to this same aldehyde. The remaining benzoxazole derivatives **16**−**21** were synthesized as shown from amide **15**. Reduction to the aldehyde by the action of $LiAlH_4$ was followed by cyanohydrin formation, partial hydrolysis, and cyclization using procedures previously described.[17] The various α-keto amide derivatives were prepared according to the procedures depicted in Scheme 5. A Henry reaction between glyoxylic acid and nitroethane gave **23** after reduction and suitable protection. Coupling the required amino acids using standard methods gave **24** after removal of the benzyl ester. Incorporation of the appropriate amide function gave a series of alcohols **25** which were readily oxidized to the desired ketones using the Dess−Martin reagent.[18] The β-lactam containing inhibitor **78** was prepared as shown in Scheme 6 from *O*-benzyl hydroxamic acid **26** (synthesized from serine) by employing the procedure described by Miller.[19]

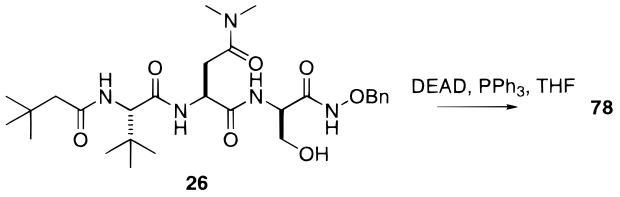
The preparations of the unnatural amino acids adamantylglycine and β,β-dimethylaspartic acid are shown in Scheme 7. Thus oxazolidinone **29** was obtained from acid **27** using procedures described previously.[20] Formation of the enolate followed by treatment with $TrisN_3$[21] gave azide **30**, which upon hydrolysis gave acid **31** in a straightforward manner. To the carboxylate

Scheme 5[a]

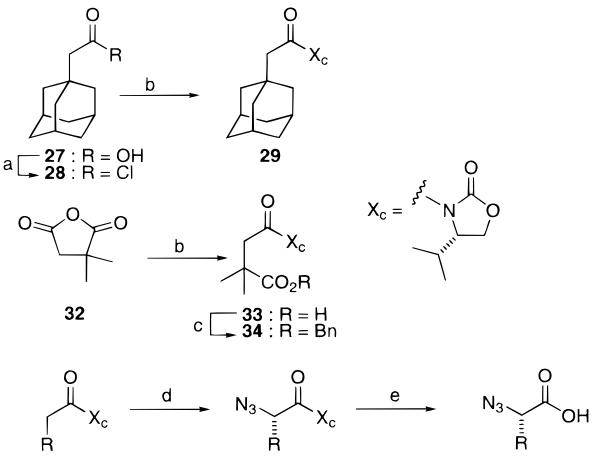


[a] Reagents and conditions: (a) $CH_3CH_2NO_2$, NaOH, EtOH; (b) $(Boc)_2O$, Et_3N, Raney nickel, H_2, EtOH; (c) BnBr, K_2CO_3, DMF; (d) HCl/dioxane; (e) RCO_2H, EDAC, HOBT, DMF; (f) 10% Pd/C, H_2, EtOH; (g) HNR′R″, HOBT, EDAC, *i*-Pr_2NEt, DMF; (h) Dess–Martin periodinane, CH_2Cl_2.

Scheme 6



Scheme 7[a]



29 : R = adamantane **30** : R = adamantane **31** : R = adamantane
34 : R = $C(CH_3)_2CO_2Bn$ **35** : R = $C(CH_3)_2CO_2Bn$ **36** : R = $C(CH_3)_2CO_2Bn$

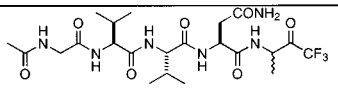
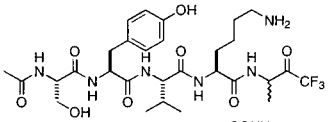
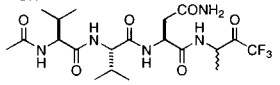
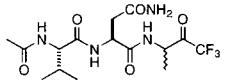
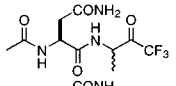
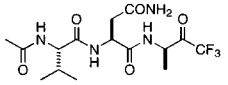
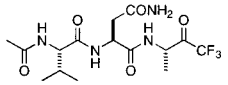
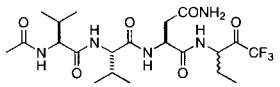
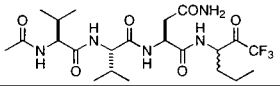
[a] Reagents and conditions: (a) $(COCl)_2$, CH_2Cl_2; (b) BuLi, oxazolidinone, THF, −78 °C; (c) BnBr, DBU, CH_3CN; (d) KHMDS, $TrisN_3$, −78 °C; (e) LiOH, H_2O_2, THF, 0 °C.

group of this azido acid was then introduced the appropriate amino acid residues. Capping the N-terminus was accomplished using standard coupling methods after reduction of the azide moiety. Using a similar approach, anhydride **32** was converted to protected azido acid **36**.

Results and Discussion

The current SAR began with a consideration of the amino acid sequences of the major cleavage sites of HCMV protease.[5] It had previously been shown that proteolysis of oligopeptides corresponding to the M-site sequence of HCMV is somewhat more efficient than cleavage of peptides corresponding to the R-site.[22] This trend was also observed in the case of trifluoromethyl ketone inhibitors. As shown in Table 1, compound **37**, whose amino acid sequence corresponds to that of the M-site, was a slightly more potent inhibitor of HCMV protease[23] than was compound **38** which is based on the R-site sequence. The amino acid sequence of compound **37** was also more amenable to SAR work than was that of **38** since minimal side chain protection was required in the former case. We therefore selected pentapeptide **37** as our initial lead structure.

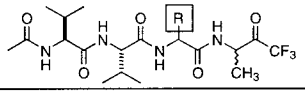
Table 1. Identification of the Minimum Core Required for Optimum Inhibitor Binding

Entry	Compound	Structure	IC_{50} (μM)
1	**37**		1.8 ± 0.3
2	**38**		2.6 ± 0.4
3	**39**		3.0 ± 0.3
4	**40**		80 ± 15
5	**41**		>300
6	**42**		>300
7	**43**		37 ± 4
8	**44**		9 ± 2
9	**45**		>300

The results obtained from an exploration of the minimum core length required for optimal inhibitor potency are also shown in Table 1. Entries 1 and 3–5 illustrate the effect of successive truncations from the N-terminus of compound **37**. The results of entries 3 and 4 suggested that the minimum length required for an inhibitor of HCMV protease with low micromolar activity was a tetramer.

The synthetic methodology used in this study resulted in the formation of diastereomers which were epimeric at the position α to the activated carbonyl function (P_1). This epimerization was unavoidable and the epimers difficult to separate. It has been reported that such activated carbonyl species epimerize readily in vivo.[24] In the case of inhibitor **40**, it was possible to separate the P_1 epimers and to test them individually (entries 6 and 7).[25] Although the configuration at this position had a profound effect on inhibitor potency, during our subsequent SAR we made no attempt to separate these epimers for individual testing.

Table 2. Substitutions of the P_2 Side Chain

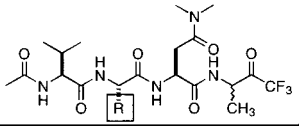


Entry	Compound	R	IC_{50} (μM)
1	**39**		3.0 ± 0.3
2	**46**		9 ± 3
3	**47**		24 ± 5
4	**48**		52 ± 6
5	**49**		19 ± 3
6	**50**		6 ± 1
7	**51**		2.0 ± 0.3
8	**52**		11 ± 2
9	**53**		5 ± 1
10	**54**		61 ± 8
11	**55**		83 ± 15
12	**56**		>300

With the minimum core established, we then proceeded to sequentially examine each amino acid position moving from P_1 to P_4. The optimum side chain found at each position was then adopted in the substitutions tested at subsequent positions. Entries 8 and 9 (Table 1) show the results obtained from changes to the P_1 side chain.[26] Lengthening the P_1 side chain by one carbon atom was tolerated (entry 8); however, side chains larger than an ethyl group gave inactive compounds (entry 9). A glycine-type residue was not tested in this series of inhibitors. Previously it has been reported that such trifluoromethyl ketones enolize readily.[24] This tendency led to problems of stability of trifluoromethyl ketones based on glycine in the present series of inhibitors. Compounds lacking a side chain at P_1 but incorporating other activated carbonyl groups were prepared (data not shown) and were found to have potencies similar to those of the corresponding inhibitors based on alanine. These compounds were also found to be much less stable than the corresponding inhibitors possessing a P_1 side chain. The remaining SAR was performed while maintaining a methyl side chain at P_1.

Table 2 shows some of the modifications to the P_2 residue that were tested. Lengthening the asparagine side chain slightly resulted in a small loss in potency (entries 1 and 2). Entries 3 and 4 show that acidic and small polar residues were less well tolerated at this position. Incorporation of a lysine (found at the R-site) gave a 6-fold drop in inhibitor potency (entry 5). The use of a small basic side chain (entry 6) led to an inhibitor that was slightly less effective than was **39**. The dimethyl-substituted asparagine amide **51** gave a small increase in activity with respect to **39** (entry 7).[27] Entries 8 and 9 show the effect of large, lipophilic residues at this position, which were found to attenuate slightly the activity of these inhibitors relative to compound **39**. Branched side chains such as the one found in inhibitor **54** (entry 10) produced a significant drop in inhibitory activity, as did the incorporation of a small side chain (entry 11). That the configuration of the α-carbon at this position was important is shown by the result of entry 12 in which the use of D-alanine gave a compound which was not an inhibitor of HCMV protease. In general, it appears that the S_2 binding pocket of HCMV protease is tolerant to a variety of inhibitor substitutions, including large lipophilic and basic residues. Examination of the optimum amino acid residue at position P_3 was undertaken using compound **51** as a starting point. Compound **51** was chosen because of the slight potency increase relative to **39** and also because the incorporation of substituted asparagine amides gave compounds which were more stable, more easily purified, and more compatible with solid-phase synthesis[14] than those incorporating asparagine at P_2.

Table 3. Substitutions of the P_3 Side Chain

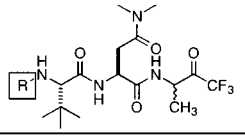


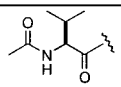
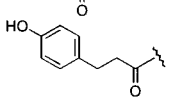
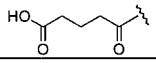
Entry	Compound	R	IC_{50} (μM)
1	**51**		2.0 ± 0.3
2	**57**		4.4 ± 0.5
3	**58**		1.1 ± 0.2
4	**59**		3.6 ± 0.5
5	**60**		6 ± 1
6	**61**		15 ± 4

Decreasing the size of the side chain at the P_3 position by one carbon led to a compound which was 2-fold less active (Table 3, entries 1 and 2). Inhibitor potency was increased slightly when a carbon was added to the isopropyl side chain (entry 3). Extending the side chain resulted in a small loss of inhibitory activity (entry 4). The incorporation of an adamantyl group resulted in only a 3-fold loss in potency relative to **51**. Introduction of a polar function led to a 7-fold potency loss relative to this same compound. The results of these substitutions suggest that the enzyme prefers a bulky lipophilic residue at this position, a result consistent with the observation that valine is highly conserved at this position in the natural cleavage site sequences.

We then considered the optimization of the P_4 portion of the molecule while maintaining the substitution of a *tert*-leucine residue for valine at P_3 (Table 4). As demonstrated previously (Table 1), the minimum peptide core for effective inhibition was found to be a tetramer. To determine if this still held true, we began our investigation of possible changes to this position by removing the P_4 amino acid. This deletion resulted in

Table 4. Substitutions of the P_4 Capping Group



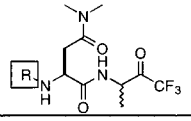
Entry	Compound	R	IC_{50} (μM)
1	**58**		1.1 ± 0.2
2	**62**		2.8 ± 0.4
3	**63**		1.0 ± 0.3
4	**64**		1.4 ± 0.1
5	**65**		1.1 ± 0.1
6	**66**		3.2 ± 0.2
7	**67**		6 ± 1
8	**68**		2.4 ± 0.2
9	**69**		3.4 ± 0.5
10	**70**		1.6 ± 0.4

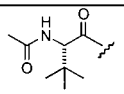
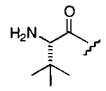
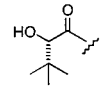
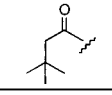
a loss of potency relative to the longer peptide only by a factor of 2−3 (entries 1 and 2). This experiment suggested that the amino acid at P_4 was expendable and that it might be possible to incorporate an optimized capping group at this position. Examination of desamino acids corresponding to those residues found at the R- and M-cleavage sites of HCMV protease indicated that the residues were equally effective capping groups (entries 3 and 4). Further substitution of the 3-methylbutanamide group was also tolerated (entry 5). The importance of the carbonyl functionality was explored by comparing a series of functional group substitutions (entries 5−8). The results obtained from these changes suggested that the nature of the carbonyl function and perhaps the orientation of the N-terminal *tert*-butyl group may play a minor role in inhibitor binding. The effect of the lipophilic *tert*-butyl group was also probed using the charged residues shown in entries 9 and 10. The capping groups screened at this position did not give large improvements in activity although the peptidic nature of the inhibitors was reduced.

The effect of further truncations to the current series of inhibitors was briefly examined (Table 5). Deletion of the capping acetamide to give amine **71** resulted in an inhibitor which was 4-fold less active. The corresponding alcohol showed a further potency loss by a factor of 2. The use of a *tert*-butyl acetate group led to a decrease in activity by a factor of 20 relative to compound **62** (entry 4). The results of this exercise show that while it may be possible to further truncate the inhibitors, there is a price to pay in terms of a loss in potency by approximately an order of magnitude.

Using compound **65** as a template, we then considered the effect of changes to the activated carbonyl group. This functionality is of particular importance for the

Table 5. Truncated Inhibitors of HCMV Protease

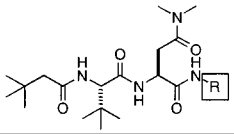


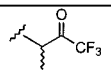
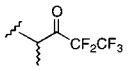
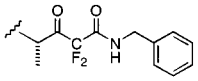
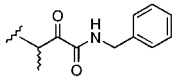
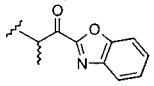
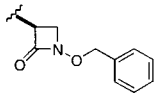
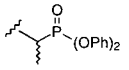
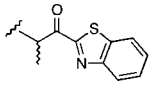
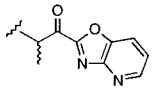
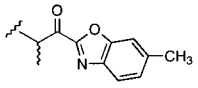
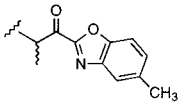
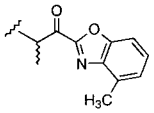
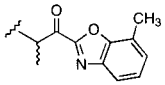
Entry	Compound	R	IC_{50} (μM)
1	**62**		2.8 ± 0.4
2	**71**		12 ± 2
3	**72**		20 ± 2
4	**73**		57 ± 10

inhibition of serine proteases because of the formation of a reversible covalent bond with the active site serine. A number of effective activated carbonyl groups have been described in the literature suitable for use with peptidomimetic inhibitors.[28] We investigated seven major classes of these (Table 6, entries 1 to 7). Compared with trifluoromethyl ketones, the use of pentafluoroethyl ketones, α,α-difluoro-β-keto amides, α-ketobenzoxazoles, α-keto amides, and diphenyl phosphonates gave significant increases in activity (entries 1−5, 7). Inhibitors **74** and **76** showed increases in potency by factors of 10 and 5, respectively. A simple β-lactam group, although able to inhibit the activity of HCMV protease, was much less effective than was **65** (entries 1 and 6).

Several compounds were investigated further in order to better characterize their interactions with HCMV protease in terms of mode of inhibition. Figure 1 shows a Dixon plot obtained for compound **76** which clearly demonstrates that this compound was a competitive inhibitor of HCMV protease.[29] Compounds **63**, **74**, and **77** gave similar results indicating that these were all inhibiting in a competitive fashion (data not shown). It is well-known that the interaction of trifluoromethyl ketone-based inhibitors with serine proteases is characterized by a slow onset of inhibition. This phenomenon has been explained by the observation that trifluoromethyl ketones exist in solution almost exclusively in the hydrated form.[30] This produces a very low concentration of the inhibitory ketone form and results in time-dependent inhibition. As shown in Figure 2, trifluoromethyl ketone **65** exhibits slow onset of inhibition with an apparent rate constant of 5.4×10^{-3} s^{-1}. Other carbonyl activating groups were found to be less susceptible to this slow binding behavior. Shown in Figure 3 is the progress curve obtained for compound **76**, in which equilibrium is reached more rapidly. Compound **74** showed slow binding behavior intermediate between that of **76** and **65**, while **77** gave a progress curve comparable to **76**. The very slow turnover rate shown by HMCV protease,[7,22] coupled with slow binding kinetics for the present series of inhibitors, has implications for the reliability of the enzymatic data. To ensure that the IC_{50} values obtained were a true reflection of inhibitory power, we utilized assay conditions in which

Table 6. Various Activated Carbonyl Groups



Entry	Compound	R	IC_{50} (μM)
1	**65**		1.1 ± 0.1
2	**74**		0.11 ± 0.01
3	**75**		0.46 ± 0.06
4	**76**		0.20 ± 0.05
5	**77**		0.6 ± 0.1
6	**78**		33 ± 8
7	**79**		0.66 ± 0.06
8	**80**		1.1 ± 0.3
9	**81**		11 ± 2
10	**82**		0.6 ± 0.1
11	**83**		0.6 ± 0.1
12	**84**		0.9 ± 0.2
13	**85**		2.8 ± 0.3

the inhibitors were preincubated with the enzyme before introduction of the substrate (see the Experimental Section). Some consequences of slow binding kinetics for trifluoromethyl ketones and other peptidomimetic inhibitors of HCMV protease will be described elsewhere.[31]

Because serine proteases are common, we investigated the inhibitory activity of our compounds toward a variety of these enzymes. Compounds **65** and **74**−**85** were tested for inhibitory activity against porcine pancreatic elastase (PPE), human leukocyte elastase (HLE), bovine pancreatic α-chymotrypsin (BPC), and the cysteine protease human liver cathepsin B (cat-B) (Table 7). Compounds **65** and **74**−**79** all showed good selectivity profiles against HLE, BPC, and cat-B. Some of these compounds were weak inhibitors of PPE (which like HCMV protease shows a preference for alanine at P_1) but with selectivity windows of 19−300-fold. One important exception to this last trend is α-ketobenzoxazole **77** which was actually 7-fold *more* potent against PPE than against HCMV protease. We carried out a limited SAR of benzoxazole substitutions to try to improve the selectivity profile of these compounds. Benzothiazole **80** proved to be a potent inhibitor of HCMV (IC_{50} 1.1 μM) and also interacted with PPE (IC_{50}

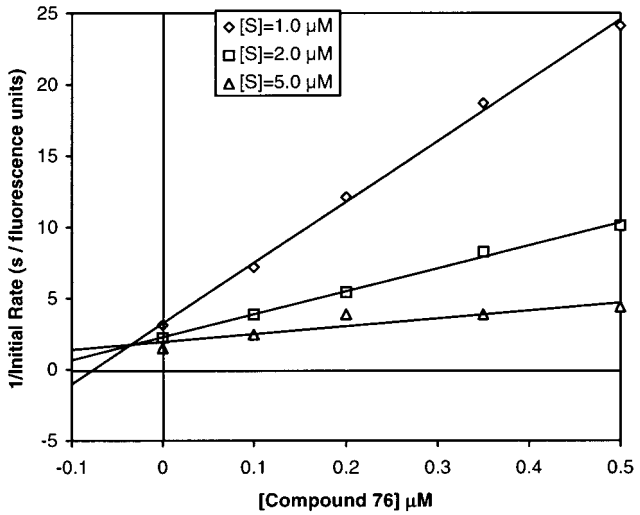


Figure 1. Dixon plot for competitive inhibition of compound **76** against HCMV protease.

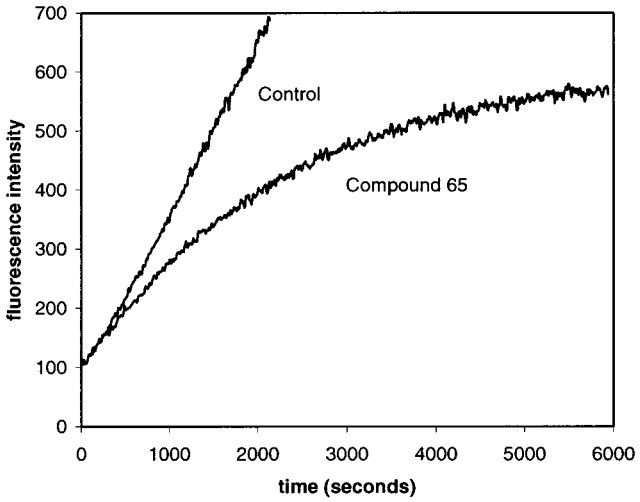


Figure 2. Progress curve for the inhibition of HCMV protease by compound **65**.

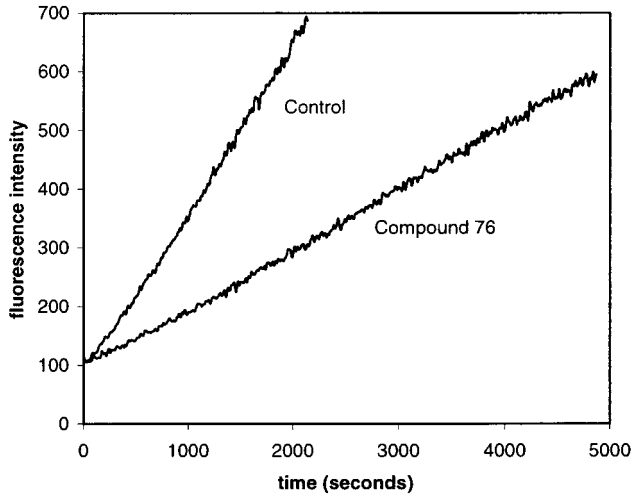


Figure 3. Progress curve for the inhibition of HCMV protease by compound **76**.

Table 7. Activity of Various Inhibitors of HCMV Protease against Other Serine Proteases

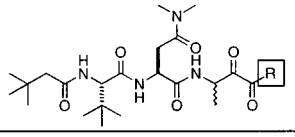
		IC_{50} (μM)			
entry	compd	PPE	BPC	cat-B	HLE
1	**65**	21	>300	>300	>300
2	**74**	33	14	>300	>300
3	**75**	39	>300	>300	>300
4	**76**	5	>300	>300	>300
5	**77**	0.08	>300	>300	183
6	**78**	>300	>75	>300	>300
7	**79**	>300	>300	>300	>300
8	**80**	9	>300	19	>300
9	**81**	>300	>300	59	>300
10	**82**	0.2	>300	225	>300
11	**83**	0.2	>300	>300	>300
12	**84**	0.07	>300	>300	>300
13	**85**	0.3	>300	>300	>300

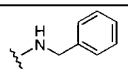
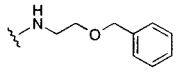
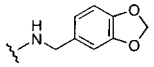
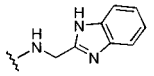
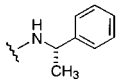
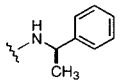
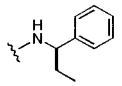
9 μM). Compound **81** was not an inhibitor of PPE, but this improvement was accompanied by an 18-fold loss in activity toward HCMV protease (Table 6, entry 9). The various methylated benzoxazoles **82**−**85** were all more potent inhibitors of PPE than of HCMV protease (Table 7, entries 10−13). The use of the α-ketobenzoxazole group gave very effective inhibitors of HCMV protease; however, we were unable to improve the selectivity profile of these compounds relative to PPE.

Compound **76** represented one of the most potent inhibitors of HCMV protease described so far. This structure also suggested the possibility of further increasing potency by extending the C-terminal amide moiety of this inhibitor into the S_1' binding pocket of the enzyme. The observation that the P_1' amino acids are fairly conserved (alanine or serine) prompted us to extend the C-terminus of the α-keto amide class of inhibitors in order to try to take advantage of interactions in the S′ pocket.

Decreasing the bulk of the C-terminal amide function resulted in a decrease in activity by a factor of 6 (Table 8, entries 1 and 2), suggesting that some binding could be realized by changes to this moiety. Only monosubstituted amides were tolerated at this position as evidenced by the results of entry 3. Further modifications to this substituent did not result in large changes in inhibitor activity (entries 4−6). One interesting effect is illustrated in entries 7 and 8. Incorporation of an (*R*)-methylbenzyl amide gave an inhibitor which was similar in potency to the corresponding *N*-benzyl-substituted compound (entries 1 and 8). The use of an (*S*)-methylbenzyl amide group however resulted in an 18-fold loss in activity relative to compound **76** (entry 7). A slight increase in potency was observed by lengthening the side chain of benzyl amide **92** (entries 8 and 9). These experiments show that it is possible to improve inhibitor potency by taking advantage of binding opportunities in the S_1' pocket of HCMV protease.

Table 8. Extensions into the S′ Binding Pocket



Entry	Compound	R	IC_{50} (μM)
1	**76**		0.20 ± 0.05
2	**86**		1.1 ± 0.3
3	**87**		>300
4	**88**		0.14 ± 0.03
5	**89**		0.10 ± 0.01
6	**90**		0.21 ± 0.05
7	**91**		3.7 ± 0.8
8	**92**		0.28 ± 0.04
9	**93**		0.11 ± 0.03

Summary

The development of peptidomimetic inhibitors of HCMV protease showing sub-micromolar activity in vitro has been described. Selective substitution of the amino acid residues of these inhibitors has led to the identification of tripeptide inhibitors showing up to 27-fold increases in inhibitor potency relative to inhibitor **39** based upon the natural tetrapeptide sequence. This increase in activity is more dramatic when the natural tripeptides are considered (compounds **74** and **89** are 730-fold more potent than **40**). Only methyl or ethyl groups were well-tolerated by this enzyme at the P_1 position. It appears that the S_2 binding pocket of HCMV protease was very permissive, tolerating lipophilic and basic residues. This suggested that the P_2 side chain could be an important position to utilize in terms of modulating future inhibitor specificity or physicochemical properties. The substitutions examined at position 3 of the present series of inhibitors indicated that a small increase in inhibitor potency could be realized by the substitution of a *tert*-leucine residue for valine. The effect of the N-terminal capping group was also probed. These substitutions indicated that this position was also somewhat permissive. Pentafluoroethyl ketones, α,α-difluoro-β-keto amides, α-ketobenzoxazoles, α-keto amides, and diphenyl phosphonates were all effective substitutions for the activated carbonyl component, although inhibitors incorporating an α-ketobenzoxazole group were not specific for HCMV protease. A slight increase in potency of the α-keto amide inhibitors was observed by lengthening the P_1' residue. This position also tolerated a variety of groups, making this a potential site for future modifications which could modify the physicochemical properties of these molecules.

Many of these compounds have also been tested in cell culture. Some members of the present series of compounds show EC_{50} values in the 100 μM range,[32] despite having potencies near 50 nM in the enzymatic assay. The reasons for this are not clear at this time and may involve problems of cell penetration and/or degradation in the assay conditions required for measuring antiviral activity. We are currently working to improve this aspect by investigating the cell permeability of our compounds and by assessing their stability in the cell culture medium.

Table 9. Synthetic and Physical Data

compd	method[a]	amount synthesized (mg)	overall yield (%)	HPLC 1[b] (%)	HPLC 2[b] (%)	formula	anal.	FAB (*m*/*z*) (MH^+)
37	C	25	30	97 (A)	98 (B)	$C_{22}H_{36}F_3N_6O_7$	HRMS	553
38	C	53	27	98 (A)	99 (B)	$C_{29}H_{44}F_3N_6O_8$	HRMS	661
39	A	22	2	100 (A)	100 (B)	$C_{20}H_{33}F_3N_5O_6$	HRMS	496
40	B	46	7	100 (A)	98 (D)	$C_{15}H_{24}F_3N_4O_5$	HRMS	397
41	B	17	32	99 (A)	99 (D)	$C_{10}H_{15}F_3N_3O_4$	HRMS	298
42	A	27.6	10	99 (A)	97 (D)	$C_{15}H_{23}F_3N_4O_3$	HRMS	397
43	A	27.6	10	97 (A)	100 (D)	$C_{15}H_{23}F_3N_4O_3$	HRMS	397
44	C	15	10	100 (A)	100 (D)	$C_{21}H_{35}F_3N_5O_6$	HRMS	510
45	B	10	14	89 (A)	99 (D)	$C_{22}H_{37}F_3N_5O_6$	HRMS	524
46	C	67	44	86 (A)	82 (D)	$C_{21}H_{35}F_3N_5O_6$	HRMS	510
47	C	23	15	96 (A)	97 (B)	$C_{20}H_{32}F_3N_4O_7$	HRMS	497
48	C	17	12	94 (A)	99 (B)	$C_{19}H_{32}F_3N_4O_6$	HRMS	469
49	C	35	23	98 (A)	96 (B)	$C_{22}H_{39}F_3N_5O_5$	HRMS	510
50	C	107	67	98 (A)	98 (D)	$C_{22}H_{33}F_3N_5O_5S$	HRMS	536
51	C	73	46	100 (A)	99 (B)	$C_{22}H_{37}F_3N_5O_6$	HRMS	524
52	C	40	27	97 (A)	97 (D)	$C_{22}H_{38}F_3N_4O_5$	HRMS	495
53	C	30	19	98 (A)	100 (B)	$C_{25}H_{36}F_3N_4O_5$	HRMS	529
54	C	42	29	84 (A)	83 (B)	$C_{21}H_{36}F_3N_4O_5$	HRMS	481
55	C	59	43	99 (A)	99 (B)	$C_{19}H_{32}F_3N_4O_5$	HRMS	453
56	C	61	45	99 (A)	99 (B)	$C_{19}H_{32}F_3N_4O_5$	HRMS	453
57	C	72	52	100 (A)	100 (D)	$C_{21}H_{35}F_3N_5O_6$	HRMS	510
58	C	82	51	100 (A)	99 (B)	$C_{23}H_{39}F_3N_5O_6$	HRMS	538
59	C	71	43	100 (A)	100 (D)	$C_{24}H_{41}F_3N_5O_6$	HRMS	552
60	B	107	16	100 (A)	99 (D)	$C_{29}H_{45}F_3N_5O_6$	HRMS	616
61	B	85	16	96 (A)	98 (D)	$C_{23}H_{37}F_3N_5O_8$	HRMS	568
62	B	134	24	99 (A)	100 (E)	$C_{18}H_{30}F_3N_4O_5$	HRMS	439
63	C	65	40	97 (A)	95 (B)	$C_{25}H_{36}F_3N_4O_6$	HRMS	545
64	C	55	38	95 (A)	99 (B)	$C_{21}H_{36}F_3N_4O_5$	HRMS	481
65	C	28	63	99 (A)	99 (B)	$C_{22}H_{38}F_3N_4O_5$	HRMS	495
66	C	20	14	99 (A)	97 (D)	$C_{22}H_{40}F_3N_4O_4$	HRMS	481
67	C	36	24	99 (A)	98 (B)	$C_{21}H_{36}F_3N_4O_6$	HRMS	497
68	B	136	26	99 (A)	100 (D)	$C_{21}H_{37}F_3N_5O_5$	HRMS	496
69	B	12	4	100 (A)	98 (D)	$C_{20}H_{35}F_3N_5O_5$	HRMS	482
70	B	70	3	100 (A)	100 (D)	$C_{21}H_{34}F_3N_4O_7$	C,H,N	511
71	C	190	48	99 (D)	99 (E)	$C_{16}H_{28}F_3N_4O_4$	HRMS	397
72	B	26	16	92 (A)	99 (D)	$C_{16}H_{27}F_3N_3O_5$	HRMS	398
73	B	105	18	97 (D)	99 (E)	$C_{16}H_{27}F_3N_3O_4$	HRMS	382
74	D	217	19	98 (A)	99 (D)	$C_{23}H_{38}F_5N_4O_5$	HRMS	545
75	E	115	37	100 (A)	100 (C)	$C_{30}H_{46}F_2N_5O_6$	C,H,N	610
76	G	51	6	100 (C)	96 (D)	$C_{29}H_{46}N_5O_6$	HRMS	560
77	F	99	22	97 (A)	95 (B)	$C_{28}H_{42}N_5O_6$	C,H,N	544
78	H	11	20	100 (A)	100 (D)	$C_{28}H_{43}N_5O_6$	HRMS	546
79	I	155	16	100 (A)	99 (D)	$C_{32}H_{48}N_4O_7P$	C,H,N	631
80	F[c]	28	25	99 (A)	100 (D)	$C_{28}H_{42}N_5O_5S$	HRMS	560
81	F	35.6	15	97 (A)	98 (D)	$C_{27}H_{43}N_6O_7$	HRMS	563
82	F	45	37	97 (A)	100 (C)	$C_{29}H_{44}N_5O_6$	C,H,N	558
83	F	53	39	96 (A)	99 (C)	$C_{29}H_{44}N_5O_6$	C,H,N	558
84	F	47.9	49	97 (A)	99 (C)	$C_{29}H_{44}N_5O_6$	C,H,N	558
85	F	57.3	48	97 (A)	97 (C)	$C_{29}H_{44}N_5O_6$	C,H,N	558
86	G	6	1	100 (C)	98 (D)	$C_{23}H_{42}N_5O_6$	HRMS	484
87	G	8	1	99 (C)	99 (D)	$C_{24}H_{44}N_5O_6$	HRMS	498
88	G	37	28	97 (C)	95 (D)	$C_{31}H_{50}N_5O_7$	HRMS	604
89	G	35	8	99 (C)	100 (D)	$C_{30}H_{46}N_5O_8$	HRMS	604
90	G	6	2	93 (C)	87 (D)	$C_{30}H_{46}N_7O_6$	HRMS	600
91	G	40	4	99 (C)	98 (D)	$C_{30}H_{48}N_5O_6$	HRMS	574
92	G	12	1	99 (C)	97 (D)	$C_{30}H_{48}N_5O_6$	HRMS	574
93	G	33	5	100 (C)	96 (D)	$C_{31}H_{50}N_5O_6$	HRMS	588

[a] See Experimental Section. [b] HPLC solvent system given in parentheses. [c] Initial deprotection was done with HCl/dioxane.

Experimental Section

Chemistry. Unless otherwise noted, materials were obtained from commercial sources and used without further purification. The purity of each inhibitor was determined by HPLC using two systems, and/or by elemental analysis. ^{1}H NMR spectra were obtained on a Bruker AMX 400 spectrometer. FAB mass spectra were recorded on an Autospec, VG spectrometer. Column chromatography was performed either on silica gel (10−40 μm or 230−400 mesh ASTM, E. Merck) or by preparative HPLC using a Partisil 10 ODS-3, C18 preparative column (50 cm × 22 mm). Analytical HPLC were carried out on the following systems. System A: Vydac C18, 10 μm analytical column (24 cm × 4.6 mm); mobile phase, acetonitrile/0.06% trifluoroacetic acid (TFA) in water/0.06% TFA. System B: Vydac C18, 5 μm analytical column (15 cm × 4.6 mm); mobile phase, acetonitrile in 50 mM NaH_2PO_4 at pH 4.4. System C: Vydac C8, 10 μm analytical column (24 cm × 4.6 mm); mobile phase, acetonitrile in 20 mM Na_2HPO_4 at pH 8.0. System D: symmetry shield C8, 10 μm analytical column (15 cm × 3.9 mm); mobile phase, acetonitrile in 20 mM Na_2HPO_4 at pH 9.0. System E: Supelcosil C8, 5 μm analytical column (15 cm × 4.6 mm); mobile phase, acetonitrile/0.1% TFA in water/0.1% TFA at pH 2.0. Methods of preparation and analytical data for target compounds are summarized in Table 9.

4-[(*tert*-Butoxycarbonyl)amino]-1,1,1-trifluorobutan-2-ol (3a). This compound was prepared according to the procedure described for the preparation of the valine analogue.[33] Thus a solution of nitroethane (0.2 mol, 14.4 mL), trifluoroacetaldehyde ethyl hemiacetal 90% (0.2 mol, 25.7 mL),

and anhydrous K_2CO_3 (4 mol %, 0.008 mol, 1.10 g) was heated at 50 °C behind a safety shield for 6 h and then stirred at room temperature for 40 h. The mixture was diluted with Et_2O (200 mL) and was washed with 5% citric acid, saturated aqueous $NaHCO_3$, and saturated brine. The organic layer was dried (Na_2SO_4) and most of the ether evaporated under reduced pressure to give a yellow oil. This oil was dissolved in absolute ethanol (150 mL) and was hydrogenated at 40 psi of hydrogen over 1.5 g of ethanol-washed Raney nickel (from 3 g of a 50% aqueous suspension) until no more hydrogen was consumed. The solution was degassed, the catalyst was filtered, and most of the ethanol was evaporated under reduced pressure. A pure sample was obtained by recrystalization from ether−hexane (1:1) (200 mL) at −20 °C. The white precipitate which formed was collected by filtration and washed with hexane (6.59 g, 23% overall): mp 67−69 °C; IR (KBr) ν 3070 (br), 2980 (br), 1154 (s) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.78 (m, 1H), 2.98 (br t, J = 5.2 Hz, 1H), 1.02 (d, J = 6.4 Hz, 3H); HRMS (CI-NH_3) calcd for $C_4H_9F_3NO$ (MH^+) 144.0636, found 144.0632. Anal. ($C_4H_9F_3NO$) C, H, N. The mother liquors were concentrated under reduced pressure, and the residue was used as such in the next step (19.4 g). To a solution of the crude amino alcohol (19.4 g, 0.13 mol) and di-*tert*-butyl dicarbonate (29.6 g, 0.13 mol) in a mixture of THF (300 mL) and water (150 mL) was added a solution of K_2CO_3 (18.7 g, 0.13 mol) in water (60 mL). The mixture was vigorously stirred for 48 h, diluted with EtOAc (250 mL), and washed with H_2O (3 × 100 mL). The organic layer was dried (Na_2SO_4), and the solvent was evaporated under reduced pressure to give a yellow oil (33.2 g). This material was purified by flash chromatography (200 g of TLC grade silica gel) using 20% EtOAc/hexane to afford the desired protected trifluoromethyl alcohol **3** as a crystalline, off-white solid (20.8 g, 66%): mp 62−65 °C; IR (KBr) ν 3360 (br), 2983, 1680 cm^{-1}; ^1H NMR (DMSO-d_6) 1:1 mixture of diastereomers, δ 6.84 (d, J = 8.3 Hz, 0.5H), 6.44 (d, J = 8.9 Hz, 0.5H), 6.32 (d, J = 6.9 Hz, 0.5H), 6.23 (d, J = 7.4 Hz, 0.5H), 3.93−3.81 (m, 1.5H), 3.70 (m, 0.5H), 1.37 (s, 9H), 1.10 (d, J = 7.4 Hz, 1.5H), 1.07 (d, J = 6.4 Hz, 1.5 Hz, 0.5H); ^{13}C NMR (100.6 MHz, DMSO-d_6) 1:1 mixture of diastereomers δ 155.5 and 155.4 (s), 126.0 (dq, J = 28.5, 10.9 Hz), 78.6 (s), 71.0 (q, J = 28.2 Hz), 46.3 (s), 28.9 (s) 18.1 (s), 16.3 (s); IR (KBr) ν 3360 (br), 2983, 1680 cm^{-1}; MS (CI-NH_3) m/z 244 (MH^+), 261 ($MH^+ + H_2O$). Anal. ($C_9H_{16}F_3NO_3$) C, H, N.

4-[(*tert*-Butoxycarbonyl)amino]-1,1,1-trifluoropentan-2-ol (3b). This compound was prepared in 64% yield from nitropropane (1.71 g, 19.2 mmol) using the procedure described above for compound **3a**: mp 78−79 °C; IR (KBr) 3600−3100 broad, 1677 (s), 1530 (s); ^1H NMR (DMSO-d_6) 3:2 mixture of diastereomers, δ 6.72 (d, J = 9.2 Hz, 0.6H), 6.28 (d, J = 7.3 Hz, 0.6H), 6.25 (d, J = 9.9 Hz, 0.4H), 6.18 (d, J = 7.6 Hz, 0.4H), 3.95−3.91 (m, 0.4H), 3.90−3.75 (m, 0.6H), 3.69−3.62 (m, 0.4H), 3.55−3.48 (m, 0.6H), 1.69−1.64 (m, 0.6H), 1.56−1.37 (m, 1.4H), 1.37 (s, 9H), 0.86−0.80 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 155.5, 125.8 (q, J = 285 Hz), 125.5 (q, J = 285 Hz), 77.9, 77.8, 71.09−69.5 (m), 51.4, 51.2, 28.3, 28.1, 24.5, 22.7, 10.6, 10.0; HRMS calcd for $C_{10}H_{19}F_3NO_3$ (MH^+) 258.1317, found 258.1310. Anal. ($C_{11}H_{20}F_3NO_3$) C, H, N.

4-[(*tert*-Butoxycarbonyl)amino]-1,1,1-trifluorohexan-2-ol (3c). This compound was prepared in 72% yield from nitrobutane (2.50 g, 24.2 mmol) using the procedure described above for compound **3a**: IR (KBr) ν 3360 (br), 2965, 1669 cm^{-1}; ^1H NMR (DMSO-d_6) 1:1 mixture of diastereomers, δ 6.75 (d, J = 9.2 Hz, 0.5H), 6.19 (d, J = 7.6 Hz, 0.5H), 6.31 (d, J = 9.5 Hz, 0.5H), 6.29 (d, J = 7.0 Hz, 0.5H), 3.90 (m, 0.5H), 3.60 (m, 0.5H), 3.79−3.76 (m, 1H), 1.37 (s, 9H), 1.55, 1.45 and 1.24 (3 m, 4H), 1.36 (s, 9H), 0.85 (t, J = 7 Hz, 3H); MS (CI-NH_3) m/z 272 (MH^+). Anal. ($C_{11}H_{20}F_3NO_3$) C, H, N.

Method A (Scheme 2). Procedure for the Preparation of Trifluoromethyl Ketones. *N*1-(3,3,3-Trifluoro-1(*R*)-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-2-methyl-1-(methylcarboxamido)propyl]carboxamido]butanediamide (42) and *N*1-(3,3,3-Trifluoro-1(*S*)-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-2-methyl-1-(methylcarboxamido)propyl]carboxamido]-butanediamide (43). To a solution of *N*-(9-fluorenylmethoxycarbonyl)-L-aspartic acid β-*tert*-butyl ester (1.85 g, 4.5 mmol) in CH_2Cl_2 (40 mL) and DMF (5 mL) were added HOBT (0.64 g, 4.72 mmol) and DCC (4.72 mL of a 1 M solution in CH_2Cl_2, 4.72 mmol). After 10 min of stirring, the hydrochloride salt of trifluoromethyl alcohol **3a** (0.8 g, 4.45 mmol) [prepared by prior treatment of **3a** (1.08 g, 4.45 mmol) with 4 N HCl in dioxane at room temperature] was added together with *i*-Pr_2-NEt (1.64 mL, 9.44 mmol). The reaction was stirred for 1 h before the mixture was washed with water (2 × 15 mL) and brine (15 mL), dried ($MgSO_4$), and concentrated in vacuo to give an oil. This material was purified by flash chromatography (TLC grade silica gel) to give 2.39 g of the desired dipeptide **4** (100%): HPLC (system A) 98%, (system B) 99%; IR (KBr) ν 3300, 1721, 1697, 1661, 1540 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.99−7.93 (m, 1H), 7.89 (d, J = 7.3 Hz, 2H), 7.70 (m, 2H), 7.55 (dd, J_1 = 15.9 Hz, J_2 = 8.6 Hz, 1H), 7.45−7.39 (m, 2H), 7.35−7.29 (m, 2H), 6.41 (d, J = 7.0 Hz, 1H), 4.40−4.19 (m, 4H), 4.06−3.88 (m, 2H), 2.66−2.58 (m, 1H), 2.44−2.40 (m, 1H), 1.37 (s, 9H), 1.08 (d, J = 6.7 Hz, 3H); FAB MS m/z 537 (MH^+); HRMS calcd for $C_{27}H_{32}F_3N_2O_6$ (MH^+) 537.2212, found 537.2229. Compound **4** (2.39 g, 4.45 mmol) was treated with 40% TFA/CH_2Cl_2 (25 mL) to give the free acid of the aspartic acid residue. This material was dissolved in *N*-methylpyrrolidone (40 mL) to which HOBT (0.63 g, 4.67 mmol) in CH_2Cl_2 (5 mL) and DCC (4.7 mL of a 1 M solution in CH_2Cl_2, 4.7 mmol) were added. The reaction mixture was stirred for 30 min at room temperature before being filtered. The filtrate containing the activated ester was added to Knorr resin (4.38 g, 3.41 mmol) which had been deprotected with 20% piperidine/NMP (treatments of 4 and 20 min) in a peptide synthesizer. The reaction was left overnight, washed with CH_2Cl_2 (3×), and dried to give 5.12 g of the resin **5** (titer of resin, 0.43 mmol/g). Resin **5** (0.766 g, 0.33 mmol) was treated with 20% piperidine/NMP (two treatments) as before followed by a wash cycle with NMP (5×). This deprotected resin was then treated with a solution of the activated ester of Fmoc-Val-OH which was prepared in the following manner: To the Fmoc-Val-OH (0.34 g, 1 mmol) in NMP (10 mL) were added HOBT (0.135 g, 1 mmol) and DCC (1.0 mL of a 1 M solution in CH_2Cl_2, 1 mmol). After 30 min of stirring, the mixture was filtered to give the activated ester which was shaken with the resin for 5 h. The resulting resin peptide was washed with CH_2Cl_2 (3×) and deprotected with NMP as before. The peptide was then capped with acetic anhydride (0.47 mL, 5 mmol). The resin was washed with CH_2Cl_2 and dried to give 0.67 g of **6**. The peptide was cleaved from the resin with TFA (9.5 mL) and water (0.5 mL) at 0 °C and then at room temperature for 1.5 h. The mixture was filtered and rinsed with TFA (2 mL) and CH_2Cl_2 before being concentrated and co-evaporated with CH_2Cl_2 (2×) and toluene. The crude peptide was precipitated with Et_2O, filtered, and dried to give 0.13 g of the trifluoromethyl alcohol. This alcohol (0.08 g, 0.19 mmol) was dissolved in anhydrous DMSO (1.5 mL) and anhydrous toluene (1.5 mL). To this solution was added solid DCC (0.237 g, 1.16 mmol) followed by dichloroacetic acid (9.6 μL, 0.12 mmol).[13] The reaction was stirred for 30 min at room temperature before being quenched with oxalic acid (0.105 g, 1.16 mmol) in MeOH (3 mL) for 10 min. The solid was filtered, and the filtrate was diluted with EtOAc and washed with saturated aqueous $NaHCO_3$. The aqueous phase was lyophilized, and the solid was taken up in water (5 mL) and neutralized with AcOH before being purified by preparative HPLC [gradient 0−18% CH_3CN/H_2O] to give compounds **42** (27.6 mg) and **43** (27.6 mg). **42**: IR (KBr) ν 1685, 1671, 1638 cm^{-1}; ^1H NMR (DMSO-d_6) 20:1 mixture of hydrate/non-hydrate, δ 8.11 (d, J = 7.5 Hz, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.35 (br s, 1H), 7.32 (d, J = 9.3 Hz, 1H), 6.92 (br s, 1H), 4.52 (q, J = 7.2 Hz, 1H), 4.11 (m, 2H), 2.45 (m, 2H), 1.95 (m, 1H), 1.26 (d, J = 6.9 Hz, 0.05H), 1.07 (d, J = 6.9 Hz, 2.95H), 0.84 (t, J = 6.6 Hz, 6H); HRMS calcd for $C_{15}H_{23}F_3N_4O_3$ (MH^+) 397.1699, found 397.1707. **43**: IR (KBr) ν 1685, 1663, 1626 cm^{-1}; ^1H NMR (DMSO-d_6) hydrated form only, δ 8.19 (d, J = 7.5 Hz, 1H), 7.93 (d, J = 8.4 Hz, 1H), 7.43 (d, J = 9.6 Hz, 1H), 7.34 (br s, 1H), 6.90 (br s, 1H), 4.51 (q, J = 6.9 Hz, 1H), 4.11 (m, 2H), 2.45 (m, 2H), 1.93 (m, 1H), 1.90 (s, 3H), 1.06 (d, J = 6.9 Hz, 3H), 0.84 (m, 6H); HRMS calcd for $C_{15}H_{23}F_3N_4O_3$ (MH^+) 397.1699, found 397.1712.

Method B. General Procedure for the Preparation of

Trifluoromethyl Ketone Analogues by the Solution Method. *N*1-(3,3,3-Trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-2-methyl-1-(methylcarboxamido)propyl]carboxamido]butanediamide (40). The trifluoromethyl alcohol **3a** (2.43 g, 10.0 mmol) was treated with 4 N HCl/dioxane (10 mL) for 1.5 h before being concentrated in vacuo. The hydrochloride salt (1.80 g, 10 mmol) was combined with Boc-Asn-OH (2.56 g, 11 mmol), EDAC (2.11 g, 11 mmol), HOBT (2.72 g, 20 mmol), and *i*-Pr_2NEt (3.8 mL, 22 mmol) in CH_2Cl_2 (15 mL). The reaction mixture was stirred for 2.5 h before being diluted with EtOAc. The organic phase was washed with 10% citric acid, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), filtered, and concentrated. The solid was recrystallized from EtOAc:Et_2O:hexane (1:5:1) to give a white solid (1.81 g, 51%): ^{1}H NMR (DMSO-d_6) δ 7.72 and 7.70 (2 d, J = 8.3 and 8.3 Hz, 1H), 7.46−7.42 (m, 0.2H), 7.25 (d, J = 14.9 Hz, 1H), 6.93−6.80 (m, 1.8H), 6.55 (d, J = 7.0 Hz, 0.2H), 6.38 (d, J = 6.7 Hz, 0.8H), 4.25−4.07 (m, 1H), 4.06−3.86 (m, 2H), 2.44−2.25 (m, 2H), 1.37 (s, 9H), 1.13−1.03 (m, 3H). To this material (0.60 g, 1.68 mmol) was added 4 N HCl/dioxane (5 mL) for 1.5 h before being concentrated in vacuo. The hydrochloride salt was combined with Boc-Val-OH (0.40 g, 1.85 mmol), EDAC (0.36 g, 1.85 mmol), HOBT (0.45 g, 3.36 mmol), and *i*-Pr_2NEt (0.65 mL, 3.7 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred 18 h before being diluted with EtOAc. The organic phase was washed with 10% citric acid, saturated $NaHCO_3$, and brine and dried ($MgSO_4$), filtered, and concentrated. The crude material was trituated with Et_2O to give the tripeptide as a white solid (0.60 g, 79%). To a portion of this peptide (0.30 g, 0.66 mmol) was added 4 N HCl (5 mL) for 1.5 h before being concentrated in vacuo. The hydrochloride salt was dissolved in MeCN (7 mL) and treated with *i*-Pr_2NEt (0.27 mL, 1.52 mmol) and acetyl chloride (50 μL, 0.69 mmol) and stirred for 2 h. The reaction mixture was concentrated and trituated with Et_2O to give a white solid (0.23 g, 80%): ^{1}H NMR (DMSO-d_6) δ 9.0 (bs, 1H), 8.23−7.90 (m, 2H), 7.60 (2 d, J = 8.3 and 8.3 Hz, 1H), 7.33 (bs, 1H), 6.88 (bs, 1H), 6.48 (2 d, J = 7.0 and 7.0 Hz, 0.2H), 6.40 and 6.35 (2 d, 0.8H), 4.54−4.43 (m, 1H), 4.15−3.87 (m, 3H), 3.18−3.08 (m, 2H), 1.89 and 1.88 (2 s, 3H), 1.12−1.03 (m, 3H), 0.89−0.80 (m, 6H). To this alcohol (0.23 g, 0.53 mmol) dissolved in DMSO (2.0 mL) was added dichloroacetic acid (70 μL, 0.85 mmol) and a 1.0 M solution of DCC in CH_2Cl_2 (5.3 mL, 5.3 mmol). The reaction was stirred for 18 h before oxalic acid (0.75 g, 5.83 mmol) was added slowly and stirred 30 min. The mixture was filtered and the residue purified by preparative HPLC to give **40** (46 mg, 22%) as a white solid: IR (KBr) ν 1685, 1655, 1627 cm^{-1}; ^{1}H NMR (DMSO-d_6) 7:1 mixture of hydrate/non-hydrate, 1:1 mixture of diastereomers at P_1, δ 8.19 and 8.11 (2 d, J = 7.6 and 7.6 Hz, 1H), 7.94 (m, 1H), 7.38 (m, 2H), 6.91 (m, 3H), 4.51 (m, 1H), 4.10 (m, 2H), 2.40 (m, 1H), 1.94 (m, 1H), 1.89 and 1.87 (2 s, 3H), 1.26 (m, 0.4H), 1.07 (d, J = 5.4 Hz, 2.6H), 0.83 (t, J = 6.3 Hz, 6H); HRMS calcd for $C_{15}H_{24}F_3N_4O_5$ (MH^+) 397.1699, found 397.1712.

Method C (Scheme 3). General Procedure for the Synthesis of Trifluoromethyl Ketones.[14] ***N*1-(3,3,3-Trifluoro-1-methyl-2-oxopropyl)-(2*S*)-6-amino-2-[[(1*S*)-1-[[(1*S*)-1-[[(1*S*)-2-hydroxy-1-(methylcarboxamido)ethyl]carboxamido]-2-phenylethyl]carboxamido]-2-methylpropyl]carboxamido]hexanamide (38).** To a solution of oxalyl chloride (34.1 mL, 0.39 mol) in anhydrous CH_2Cl_2 (700 mL) was added dropwise at −60 °C a solution of DMSO (64 mL, 0.9 mol) dissolved in CH_2Cl_2 (50 mL). After 30 min a solution of **3a** (72.9 g, 0.3 mol) in CH_2Cl_2 (200 mL) was added over 2 h. Triethylamine (125 mL, 0.9 mol) was added and the reaction allowed to warm slowly to 0 °C before water (300 mL) was slowly added. The organic phase was separated and washed successively with 5% citric acid, saturated $NaHCO_3$, and brine. The organic phase was dried (Na_2SO_4), filtered, and concentrated to give a yellow oil which was purified by flash chromatography (20−30% EtOAc/hexane) to give the trifluoromethyl ketone **7** (36 g, 50%): mp 79−87 °C; IR (KBr) ν 3390, 3280 (br), 1660, 1530 cm^{-1}; ^{1}H NMR (DMSO-d_6) 80% hydrated form, δ 7.71 (d, J = 6.7 Hz, 0.2H), 6.85 (s, 0.75H), 6.77 (s, 0.75H), 6.35 (d, J = 9.6 Hz, 0.8H), 4.51(q, J = 6.7 Hz, 0.2H), 3.82 (q, J = 7.0 Hz, 0.8H), 1.37 (s, 9H), 1.25 (d, J = 7.0 Hz, 0.6H), 1.08 (d, J = 6.7 Hz, 2.4H); ^{13}C NMR (DMSO-d_6) 181.5 (d, J = 20.1 Hz), 154.6 (s), 123.5 (q, J = 29.08 Hz), 92.8 (q, J = 28.8 Hz), 77.7 (s), 48.6 (s), 27.7 (s), 14.5 (s); HRMS (CI-NH_3) calcd for $C_9H_{15}F_3NO_3$ (MH^+) 242.1004, found 242.1000. Anal. ($C_9H_{14}F_3NO_3$) C, H, N. Ketone **7** (27.4 g, 0.114 mol) was refluxed with *trans*-4-(semicarbazidomethyl)cyclohexanecarboxylic acid benzyl ester[34] (34.7 g, 0.114 mol) in toluene (300 mL) with a catalytic amount of TsOH (1.08 g, 5.7 mmol) for 3 h. The mixture was cooled, diluted with EtOAc (300 mL), and washed successively with 1 M aqueous HCl (2 × 100 mL), a saturated aqueous solution of $NaHCO_3$ (2 × 100 mL), a 0.6% aqueous solution of bleach (2 × 100 mL), a 5% aqueous solution of sodium thiosulfate (2 × 100 mL), and a saturated aqueous solution of brine (2 × 100 mL). The organic solution was dried (Na_2SO_4) and was concentrated under reduced pressure to half its initial volume. The compound precipitated from the mixture. It was collected by filtration, washed with several portions of EtOAc/hexane (6:4), and dried under vacuum to give the desired semicarbazone as a white solid. A second crop was obtained by concentrating the mother liquor: total yield 31 g, 52%; mp 162.5−163 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.24 (s, 1 H), 7.50 (broad s, 1 H), 7.39−7.29 (complex m, 5 H), 6.80 (t, J = 6.8 Hz, 1 H), 5.07 (s, 2 H), 4.74 (broad quintet, J = 6.7 Hz, 1 H), 2.99 (t, J = 6.4 Hz, 2 H), 2.30 (triple t, J_1 = 12.0 Hz, J_2 = 3.3 Hz, 1 H), 1.92 (d, J = 10.8 Hz, 2 H), 1.71 (d, J = 11.2, 2 H), 1.43−1.27 (complex m, 12 H), 1.19 (d, J = 7.6 Hz, 3 H), 0.93 (dq, J_1 = 13.1 Hz, J_2 = 2.6 Hz, 2 H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 175.6, 155.5, 137.2, 136.7 (q, J = 29.5 Hz), 129.3, 128.7, 128.5, 121.9 (q, J = 277 Hz), 79.3, 66.0, 45.8, 43.5, 43.3, 38.1, 30.0, 29.0, 28.9; IR (KBr) 1726 (s), 1690 (s), 1550 (s), 1520 (s) cm^{-1}; MS (CI-NBA) 529 (MH^+). Anal. ($C_{25}H_{35}F_3N_4O_5$) C, H, N. The ester so obtained (21.9 g, 41.4 mmol) was dissolved in EtOAc (300 mL) and ethanol (300 mL) and stirred in the presence of 5% Pd/C (2.2 g) under 45 psi of hydrogen for 15 h. The acid was not soluble in this solvent system, and so the mixture was thoroughly degassed with nitrogen and was diluted with CH_2Cl_2 (200 mL) and MeOH (500 mL). The medium was gently refluxed for 30 min on a water bath, and the catalyst was removed by filtration through Celite and was washed with several 20 mL portions of CH_2Cl_2 followed by 20 mL portions of MeOH. The solvent was removed under reduced pressure, and the resulting white solid was triturated with 250 mL of Et_2O and filtered. The acid was isolated as a white solid (18.0 g, 99% yield): mp 210−212 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.96 (s, 1 H), 10.20 (s, 1 H), 7.47 (br, 1 H), 6.77 (t, J = 5.9 Hz, 1 H), 4.74 (quintet, J = 6.9 Hz, 1 H), 3.31 (br, 1 H), 2.99 (t, J = 6.4 Hz, 2 H), 2.11 (tt, J_1 = 12.0 Hz, J_2 = 0.8 Hz, 1 H), 1.89 (d, J = 10.3 Hz, 2 H), 1.70 (d, J = 10.8 Hz, 2 H), 1.36 (s, 9 H), 1.30−1.19 (m, 7 H), 0.91 (dq, J_1 = 12.5 Hz, J_2 = 2.6 Hz, 2 H); ^{13}C NMR (400 MHz, DMSO-d_6) δ 175.7, 153.9, 153.6, 134.9 (q, J = 29 Hz), 120.1 (q, J = 276.1 Hz), 77.5, 44.0, 41.7, 41.5, 36.3, 28.3, 27.2, 27.0, 15.0; FT-IR (KBr) 1700 (s), 1538 (s); MS (CI-NBA) 439 (MH^+). Anal. ($C_{18}H_{29}F_3N_4O_5$) C, H, N. The acid so obtained was dissolved in DMSO (100 mL) and NMP (600 mL) and treated with BHA·HCl resin (49.1 g, 30 mmol), TBTU (12.5 g, 39 mmol), HOBT (5.26 g, 39 mmol), and *i*-Pr_2NEt (20.9 g, 120 mmol). The slurry was stirred with a mechanical stirrer for 4 h. The resin was washed with 500 mL portions of NMP, CH_2Cl_2, MeOH, CH_2Cl_2, and MeOH. The resin was dried and titrated (picric acid method, titer of resin = 0.42 mmol/g). The resin (0.25 mmol) was deprotected in an automated peptide synthesizer by treatment with 45% TFA/CH_2Cl_2 over 25 min. Standard FMOC coupling procedures were used to prepare the fully elaborated peptide. Thus to the deprotected resin (0.25 mmol) was added sequentially the amino acid (0.9 mmol), HOBT (121.5 mg, 0.9 mmol), TBTU (289 mg, 0.9 mmol) all as 0.5 M solutions in DMF, and *i*-Pr_2NEt (314 μL, 1.8 mmol) as a 1.0 M solution in NMP. Double couplings were performed for each residue. Deprotection of the FMOC group was carried out by sequential treatments with 25% piperidine/NMP (5 and 20 min). Subsequent couplings were performed following the same protocol. Side chain deprotections were then carried out by treatment with 75% TFA/CH_2Cl_2 with 5% anisole (3 h), followed by washes with CH_2Cl_2, MeOH, 5% *i*-Pr_2NEt/CH_2Cl_2, and CH_2Cl_2. Cleavage was accomplished by refluxing the resin

peptide in THF (9 mL) with a cocktail of AcOH (500 μL), H_2O (250 μL), and 1 M HCl (100 μL) for 4 h. The resin was filtered and treated to the same conditions once more. The procedure was repeated once (for a total of three cleavages). All of the mother liquors were combined and were concentrated in vacuo. The crude peptide was purified by preparative HPLC to give compound **38** as a white solid (53 mg, 27% overall yield from resin **8**): IR (KBr) ν 3500−2800 (br), 1643, 1516 cm^{-1}; ^{1}H NMR (DMSO-d_6) 3:1 mixture of hydrate/non-hydrate, 1:1 mixture of diastereomer at P_1, δ 9.14 (s, 1H), 8.71 and 8.68 (d, J = 5.7 and 5.7 Hz, 0.25H), 8.08 and 8.03 (d, J = 8.0 and 8.0 Hz, 1H), 7.91 (t, J = 7.1 Hz, 2H), 7.75 (quartet, J = 7.9 Hz, 1H), 7.63 (br s, 3H), 7.57 and 7.56 (d, J = 10.9 and 8.9 Hz, 1H), 7.02−6.95 (m, 2.75H), 6.61 (d, J = 8.3 Hz, 2H), 4.94 (m, 1 H), 4.71−4.62 (m, 0.25H), 4.43 (m, 1H), 4.26 (m, 2H), 4.13 (m, 1.75H), 3.48 (t, J = 5.7 Hz, 2H), 2.92 (m, 1H), 2.74 (br m, 3H), 1.95 (q, J = 6.8 Hz, 1H), 1.83 (s, 3H), 1.62 (m, 1H), 1.51 (m, 2H), 1.08 (m, 3H), 1.08 and 1.07 (d, J = 6.7 and 6.6 Hz, 3H), 0.86−0.81 (m, 6H); HRMS calcd for $C_{29}H_{44}F_3N_6O_8$ (MH^+) 661.3173, found 661.3195.

4-[(*tert*-Butoxycarbonyl]amino]-1,1,1,2,2-pentafluoropentan-3-ol (11). To a solution of *N*-(*tert*-butoxycarbonyl)-(*S*)-alanine (3.00 g, 15.9 mmol) in CH_2Cl_2 (50 mL) at 0 °C was added 1,1′-carbonyldiimidazole (3.34 g, 20.6 mmol). After 30 min of stirring at 0 °C, Et_3N (2.08 g, 20.6 mmol) was added followed by the addition of *O*,*N*-dimethylhydroxylamine hydrochloride (2.01 g, 20.6 mmol). The mixture was stirred 1 h at 0 °C and then at room temperature for 4 h. Ether (200 mL) was added and the organic phase was washed twice with 10% aqueous HCl, twice with saturated $NaHCO_3$, and once with brine. The organic phase was dried ($MgSO_4$), filtered, and concentration in vacuo to give **10** as a white solid (3.35 g, 91% yield) which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 5.24 (br, 1H), 4.69 (br, 1H), 3.77 (s, 3H), 3.21 (s, 3H) 1.44 (s, 9H), 1.31 (d, J = 7.0 Hz, 3H). To a dry 500 mL round-bottom flask was added anhydrous Et_2O (100 mL) and a 1.5 M solution of MeLi·LiBr in Et_2O (100 mL, 150 mmol). This solution was cooled to −78 °C. A second flask was cooled to −78 °C and charged with Et_2O (100 mL) and CF_3CF_2I (44.7 g, 182 mmol). The contents of the second flask were then added via cannula over 15 min to the MeLi·LiBr slurry. The resulting solution was stirred for 30 min at −78 °C before the Weinreb amide **10** (10.6, 45.5 mmol) was added in one portion. The reaction mixture was stirred at −78 °C for 90 min and then allowed to warm to −30 °C for 2 h. The reaction was quenched by the addition of saturated NH_4Cl (125 mL). The organic phase was washed with H_2O, dried (Na_2SO_4), filtered, and concentrated to provide an orange oil. The oil was redissolved in 20% MeOH/THF (100 mL) and transferred to a 500 mL round-bottom flask. The solution was cooled to 0 °C before $NaBH_4$ (1.9 g, 50.1 mmol) was added portionwise over 5 min (**Caution**! foaming occurs). The reaction mixture was subsequently stirred for 1 h at 0 °C. Ether (200 mL) was added followed by 10% citric acid (100 mL). The aqueous layer was extracted with Et_2O (3 × 50 mL). The combined organic extracts were washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue was purified by flash chromatography (20% ethyl acetate in hexanes) to provide as a colorless oil **11** (12.3 g, 92%): HPLC (system D) 100%; IR (KBr) ν 3368, 2987, 1687 cm^{-1}; ^{1}H NMR (DMSO-d_6) 2:1 mixture of diastereomers, δ 6.87 (d, J = 8.0, 0.6H), 6.42 (d, J = 8.9 Hz, 0.3H), 6.32 and 6.25 (2 d, J = 8.0 and 8.3 Hz, 1H), 4.08−3.78 (m, 2H), 1.37 (s, 9H), 1.13 and 1.08 (2 d, J = 7.0 and 6.7 Hz, 3H); FAB MS *m*/*z* 294 (MH^+); HRMS calcd for $C_{10}H_{17}F_5NO_3$ (MH^+) 294.1129, found 294.1138. Anal. ($C_{10}H_{16}F_5NO_3$) C, H, N.

Method D: *N*4,*N*4-Dimethyl-*N*1-(3,3,4,4,4-pentafluoro-1-methyl-2-oxobutyl)-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]butanediamide (74). Compound **11** (0.92 g, 3.14 mmol) was treated with 4 N HCl/dioxane (1.5 h) before being concentrated in vacuo. The resulting hydrochloride salt (3.14 mmol) was combined with Boc-Asn(γ-NMe_2)-OH (0.93 g, 3.45 mmol), TBTU (1.21 g, 3.77 mmol), HOBT (0.51 g, 1.2 mmol), and *i*-Pr_2NEt (1.64 mL, 9.42 mmol) in CH_2Cl_2 (10 mL). After 3 h at room temperature, the mixture was extracted into EtOAc and washed with 1 N HCl, saturated aqueous $NaHCO_3$, and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography (4:1 EtOAc/hexane) to give the coupled product (0.594 g, 43%): HPLC (system A) 93%; ^{1}H NMR ($CDCl_3$) 1.3:1 mixture of diastereomers, δ 6.92 (bs, 0.6H), 6.66 (d, J = 7.95 Hz, 0.4H), 5.80 (m, 1H), 4.71 (bs, 0.4H), 4.50 (m, 1.6H), 4.45−4.20 (m, 2H), 3.34 (dd, J = 16.85 and 16.85 Hz, 0.4H), 3.06−2.97 (m, 0.6H), 3.045 and 3.04 (2 s, 3H), 3.02 and 3.00 (2 s, 3H), 2.67 (dd, J = 7.63 and 7.63 Hz, 0.6H), 2.58 (dd, J = 16.85 and 16.85 Hz, 0.4H), 1.48 and 1.46 (2 s, 9H), 1.32 (t, J = 7.95 Hz, 3H). The dipeptide (0.43 g, 0.99 mmol) was treated with 4 N HCl/dioxane (10 mL) for 2 h before being concentrated in vacuo. The resulting hydrochloride salt (0.99 mmol) was combined with Boc-Tbg-OH (0.254 g, 1.1 mmol), BOP (0.487 g, 1.1 mmol), and *i*-Pr_2NEt (0.52 mL, 3.0 mmol) in CH_2Cl_2 (10 mL). After 2.5 h at room temperature, the mixture was extracted into EtOAc and washed with 1 N HCl, saturated aqueous $NaHCO_3$, and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography (1:1 EtOAc/hexane) to give the coupled product as a white solid (0.33 g, 60%): HPLC (system A) 99%; ^{1}H NMR ($CDCl_3$) 1.3:1 mixture of diastereomers, δ 8.15 (m, 0.4H), 7.73 (m, 0.6H), 7.37 (m, 0.6H), 7.10 (m, 0.4H), 5.12 (m, 1H), 4.77 (m, 0.7H), 4.69 (m, 1.3H), 4.25 (m, 2H), 3.76 (m, 1H), 3.26 (dd, J = 15.9 and 15.9 Hz, 0.4H), 3.16 (dd, J = 12.4 and 12.4 Hz, 0.6H), 3.06 and 3.03 (2 s, 3H), 2.94 and 2.92 (2 s, 3H), 2.55 (dd, J = 7.0 and 7.0 Hz, 0.6H), 2.39 (dd, J = 11.4 and 11.4 Hz, 0.4H), 1.46 and 1.45 (2 s, 9H), 1.37−1.25 (m, 3H), 1.06 and 1.03 (2 s, 9H). This peptide (0.30 g, 0.67 mmol) was then treated with 4 N HCl/dioxane (10 mL) and concentrated in vacuo. The hydrochloride salt was combined with *tert*-butylacetic acid (0.094 μL, 0.74 mmol), BOP (0.33 g, 0.74 mmol), and *i*-Pr_2NEt (0.23 mL, 1.34 mmol) in CH_2Cl_2 (10 mL) and stirred 3.5 h at room temperature. The mixture was diluted with EtOAc and washed with 1 N HCl, saturated aqueous $NaHCO_3$, and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was purified by flash chromatography to give the desired peptide as a white solid (0.297 g, 88%): HPLC (system A) 99%); ^{1}H NMR ($CDCl_3$) δ 7.79 (d, J = 7.6 Hz, 1H), 7.32 (d, 8.3 Hz, 1H), 6.18 (d, J = 6.7 Hz, 1H), 5.21 (d, J = 6.7 Hz, 1H), 4.82−4.78 (m, 1H), 4.39−4.31 (m, 1H), 4.23−4.10 (m, 2H), 3.11−3.06 (dd, J = 16.2 and 15.7 Hz, 1H), 2.99 (s, 3H), 2.91 (s, 3H), 2.52 (dd, J = 16.2 and 15.9 Hz, 1H), 2.18 (s, 2H), 1.27 (d, J = 6.7 Hz, 3H), 1.06 (s, 9H), 1.05 (s, 9H). The alcohol (0.26 g, 0.48 mmol) so obtained was combined with Dess−Martin periodinane (0.51 g, 1.19 mmol) in CH_2Cl_2 (5 mL) and stirred for 4 h. The reaction mixture was diluted with EtOAc and treated with a 1:1 mixture of 10% $Na_2S_2O_3$:saturated $NaHCO_3$ (10 mL) for 15 min. After extraction with EtOAc the organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. The pentafluoroethyl ketone was obtained by trituration with 3:1 hexane/EtOAc to give **74** as a white solid (0.217 g, 84%): IR (KBr) ν 1685, 1618 cm^{-1}; ^{1}H NMR (DMSO-d_6) 3:1 mixture of diastereomers at P_1, δ 8.20 and 8.14 (2 d, J = 7.5 and 7.5 Hz, 1H), 7.66 (d, J = 9.0 Hz, 1H), 7.51 (d, J = 8.8 Hz, 1H), 6.91 (m, 2H), 4.58 (m, 1H), 4.16 (m, 2H), 2.95 and 2.80 (2 s, 6H), 2.70 (m, 2H), 2.11 (m, 2H), 1.08 (d, J = 6.6 Hz, 3H), 0.95 (s, 9H), 0.91 (s, 9H); HRMS calcd for $C_{23}H_{38}F_5N_4O_5$ (MH^+) 545.2762, found 545.2775.

Benzyl 4-[(*tert*-Butoxycarbonyl)amino]-2,2-difluoro-3-hydroxy-(4*S*)-pentanoate (13). This material was prepared using a modification of the procedure previously described.[16] Thus amide **10** (14.9 g, 64.3 mmol) was dissolved in THF (230 mL) at 0 °C. $LiAlH_4$ (4.90 g, 129 mmol) was added in several portions over a period of 20 min, and the suspension was then stirred for 2 h at 0 °C. This suspension was transferred via cannula into 500 mL of 10% aqueous citric acid and stirred for 1 h. The mixture was extracted with Et_2O (3×), and the combined organic phases were washed with water and brine, dried ($MgSO_4$), filtered, and concentrated to give the corresponding aldehyde as a white solid (10.7 g, 97%): ^{1}H NMR ($CDCl_3$) δ 9.57 (s, 1H), 5.09 (br, 1H), 4.23 (br, 1H), 1.46 (s, 9H), 1.34 (d, J = 7.3 Hz, 3H). Zinc dust (16.2 g, 24.8 mmol) was placed in THF (40 mL) and sonicated for 30 min. A

solution of the aldehyde (10.7 g, 62 mmol) and ethyl bromodifluoroacetate (20 g, 99 mmol) was added over 30 min using a syringe pump while sonicating. Sonication was continued for 1.5 h before the suspension was poured into 500 mL of 10% aqueous citric acid and extracted with EtOAc (3×). The combined organic extracts were washed with water, brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The oil obtained contained 20% of the starting aldehyde but was used without further purification. The hydroxy ester (2.20 g, 7.40 mmol), benzylamine (3.96 g, 37.0 mmol), and *i*-Pr_2NEt (4.76 g, 37.0 mmol) were heated in refluxing ethanol for 18 h. The solution was concentrated to dryness, taken up into EtOAc, washed with 1 N HCl and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo to give a yellow oil. This material was purified by flash chromatography using TLC grade silica gel to give **13** as a white solid (1.17 g, 44% over two steps): HPLC (system B) 100%, (system C) 99%; IR (KBr) ν 3344, 2979, 1684 cm^{-1}; ^1H NMR ($CDCl_3$) 4:1 mixture of isomers, δ 7.20 (m, 5H), 6.90 (br s, 1H), 4.75 (m, 1H), 4.45 (m, 2H), 4.08 (br s, 1H), 3.88 (m, 2H), 1.32 (s, 9H), 1.16 (d, 3H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 164.58, 164.30, 164.02, 155.61, 139.31, 129.11, 127.97, 127.72, 119.99, 117.47, 114.88, 78.59, 71.91, 71.65, 71.44, 46.28, 43.04, 29.10, 19.35; FAB MS *m*/*z* 359 (MH^+); HRMS calcd for $C_{17}H_{25}F_2N_2O_4$ (MH^+) 359.1782, found 359.1768. Anal. ($C_{17}H_{24}F_2N_2O_4$) C, H, N.

Method E: *N*1-[3-(Benzylcarbamoyl)-3,3-difluoro-1-methyl-2-oxopropyl]-*N*4,*N*4-dimethyl-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]-butanediamide (75). Compound **13** (0.263 g, 0.73 mmol) was treated with 4 N HCl/dioxane (6 mL) for 30 min before being concentrated in vacuo. The resulting hydrochloride salt was combined with BOP (0.39 g, 0.88 mmol), Boc-Asn(γ-NMe_2)-OH (0.191 g, 0.73 mmol), and *i*-Pr_2NEt (0.32 mL, 1.83 mmol) in CH_2Cl_2 (5 mL). After 4 h, the reaction mixture was poured into EtOAc and washed sequentially with 1 N HCl, saturated aqueous $NaHCO_3$, and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. The material was trituated with 3:7 hexane:EtOAc to give a white solid (0.30 g, 82%): ^1H NMR ($CDCl_3$) δ 7.41−7.28 (m, 6H), 6.98−6.94 (m br, 1H), 5.56−5.51 (m br, 1H), 4.59−4.42 (m, 4H), 4.11−4.03 (m, 2H), 3.11−3.05 (m, 1H), 2.99 (s, 3H), 2.88 (s, 3H), 2.61−2.54 (m, 1H), 1.45 (s, 9H), 1.32 (d, J = 6.6 Hz, 3H). This material (0.27 g, 0.54 mmol) was treated with 4 N HCl/dioxane (6 mL) for 30 min before being concentrated in vacuo. The hydrochloride salt (0.54 mmol) was combined with Boc-Tbg-OH (0.125 g, 0.54 mmol), BOP (0.286 g, 0.65 mmol), and *i*-Pr_2-NEt (0.23 mL, 1.35 mmol) in CH_2Cl_2 (3 mL) and stirred for 4 h. The mixture was diluted with EtOAc and washed sequentially with 1 N HCl, saturated aqueous $NaHCO_3$, and brine before being dried ($MgSO_4$), filtered, and concentrated in vacuo. The product was purified by flash chromatography using TLC grade silica gel to afford a white solid (0.25 g, 76%): ^1H NMR ($CDCl_3$) δ 8.06 (s br, 1H), 7.68 (s br, 1H), 7.33−7.26 (m, 6H), 5.11 (d, J = 4.4 Hz, 1H), 4.67 (dd, J = 14.6, 7.0 Hz, 1H), 4.50 (s br, 1H), 4.32−4.22 (m, 3H), 4.13−4.07 (m, 1H), 3.71 (d, J = 5.7 Hz, 1H), 3.23−3.20 (m, 1H), 3.00 (s, 3H), 2.87 (s, 3H), 2.43−2.38 (dd, J = 15.9, 5.7 Hz, 1H), 1.49 (s, 9H), 1.22 (d, J = 6.7 Hz, 3H), 1.02 (s, 9H). This peptide (0.25 g, 0.41 mmol) was treated with 4 N HCl/dioxane (3 mL) and stirred 1 h before being concentrated in vacuo. The hydrochloride salt (0.41 mmol) was combined with *tert*-butylacetic acid (52 μL, 0.41 mmol), BOP (0.216 g, 0.49 mmol), and *i*-Pr_2NEt (0.18 mL, 1.02 mmol) in CH_2Cl_2 (3 mL) and stirred 4 h. The mixture was diluted with EtOAc and washed sequentially with 1 N HCl, saturated aqueous $NaHCO_3$, and brine before being dried ($MgSO_4$), filtered, and concentrated in vacuo. The product was purified by flash chromatography using TLC grade silica gel (5% MeOH/EtOAc) to give the fully elaborated peptide as a white solid (0.191 g, 77%): ^1H NMR ($CDCl_3$) δ 7.65 (d, J = 7.6 Hz, 1H), 7.37−7.27 (m, 7H), 5.97 (d, J = 6.7 Hz, 1H), 4.65−4.59 (m, 2H), 4.46 (d, J = 9.2 Hz, 1H), 4.35 (dd, J = 15.0, 5.4 Hz, 1H), 4.28−4.24 (m, 1H), 4.15−4.06 (m, 1H), 4.02 (d, J = 6.7 Hz, 1H), 3.16 (dd, J = 15.9, 3.5 Hz, 1H), 2.99 (s, 3H), 2.87 (s, 3H), 2.45 (dd, J = 15.6, 9.2 Hz, 1H), 2.19 (dd, J = 18.1, 13.0 Hz, 2H), 1.24 (d, J = 7.0 Hz, 3H), 1.05 (s, 9H), 1.03 (s, 9H). The peptide (0.15 g, 0.245 mmol) was dissolved in CH_2Cl_2 (15 mL) and treated with Dess−Martin periodinane (0.10 g, 0.245 mmol) and stirred at room temperature for 5 h. The mixture was diluted with EtOAc and treated with a 1:1 mixture of 10% $Na_2S_2O_3$:saturated $NaHCO_3$ (15 min). The organic phase was washed sequentially with saturated $NaHCO_3$, 10% citric acid, and brine before being dried ($MgSO_4$), filtered, and concentrated in vacuo. The final product was purified by preparative HPLC to give, after lyophilization, compound **75** as a white solid (0.115 g, 77%): IR (KBr) ν 3293, 1680, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) 1:3 mixture of hydrate/nonhydrate, δ 9.65−9.55 (m, 0.25H), 8.99−8.47 (m, 0.75H), 8.20−8.15 (m, 1H), 7.65−7.55 (m, 1H), 7.40−7.20 (m, 6H), 6.55 (br s, 0.25H), 6.34 (br s, 0.75H), 4.80−4.72 (m, 0.25H), 4.59−4.53 (m, 1H), 4.42−4.26 (m, 2H), 4.26−4.16 (m, 1.5H), 4.11 (d, J = 8.3 Hz, 0.25), 2.96 (s, 2.25H), 2.93 (s, 0.75H), 2.79 (s, 0.75H), 2.77 (s, 2.25H), 2.68 (m, 2H), 2.25−2.18 (m, 1H), 2.05−1.95 (m, 1H), 1.24 (d, J = 7.0 Hz, 0.75H), 1.06 (d, J = 6.6 Hz, 2.25H), 0.95 (s, 9H), 0.90 (s, 9H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 197.32, 197.06, 196.79, 171.46, 171.08, 170.98, 170.49, 170.33, 169.86, 169.34, 161.15, 160.88, 160.62, 138.54, 138.04, 128.53, 128.40, 127.43, 127.27, 127.21, 126.98, 112.32, 109.68, 107.04, 60.45, 59.80, 50.04, 49.87, 49.58, 48.44, 48.26, 42.60, 42.36, 36.81, 35.03, 34.28, 33.89, 30.74, 29.84, 26.83, 15.54; HRMS calcd for $C_{30}H_{46}F_2N_5O_6$ (MH^+) 610.3416, found 610.3395.

(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-1-benzo[d][1,3]-thiazol-2-yl-1-propanol (14). This compound was prepared from methyl ester **12** using the procedure previously described.[17d] Thus a solution of **12** (28.1 g, 132 mmol) in THF (50 mL) was added dropwise to a suspension of $LiAlH_4$ (3.76 g, 396 mmol) in THF (200 mL) at 0 °C. After complete addition the mixture was stirred at room temperature for 1 h. Celite (34 g) was then added followed by the careful addition of water (34 mL), 2 N NaOH (34 mL), and water (100 mL), and stirring was continued for 1 h. The resulting white suspension was filtered, and the filter cake was washed with EtOAc. The desired alcohol was obtained as a colorless oil (21.07 g, 91%): ^1H NMR ($CDCl_3$) δ 4.66 (br s, 1H), 3.77 (br s, 1H), 3.68−3.61 (m, 1H), 3.53−3.47 (m, 1H), 2.65 (br s, 1H), 1.45 (s, 9H), 1.14 (d, J = 6.7 Hz, 3H). To a solution of this alcohol (3.25 g, 18.5 mmol) and Et_3N (7.75 mL, 55.6 mmol) in anhydrous CH_2Cl_2 (60 mL) and DMSO (28 mL) at 0 °C was added SO_3·py (8.85 g, 55.6 mmol) in small portions. The solution was then stirred at room temperature for 1.5 h before being poured into ice water and extracted three times with CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated to give an oil which was purified by flash chromatography to give the desired aldehyde (2.34 g, 73%) which was used immediately: ^1H NMR ($CDCl_3$) δ 9.57 (s, 1H), 5.09 (br, 1H), 4.23 (br, 1H), 1.46 (s, 9H), 1.34 (d, J = 7.3 Hz, 3H). To a solution of benzothiazole (4.43 mL, 40.5 mmol) in THF (100 mL) at −78 °C was added *n*-BuLi (26.5 mL of a 1.4 M solution in hexanes, 37.15 mmol). After 30 min of stirring, a solution of the above aldehyde (2.34 g, 13.51 mmol) in THF was added. The solution was stirred for 72 min before being quenched by the addition of saturated NH_4Cl. Extraction with EtOAc was followed by a wash with brine and drying over $MgSO_4$. Flash chromatography afforded the desired product as an orange oil: HPLC (system A) 100%, (system D, pH 7.4) 100%; IR (KBr) ν 3272, 1713 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) 6:1 mixture of diastereomers at P_1, δ 8.07−8.05 (m, 1H), 7.96−7.92 (m, 1H), 7.50−7.45 (m, 1H), 7.41−7.37 (m, 1H), 6.81 and 6.44 (2 d, J = 8.6 Hz, 1H), 6.47 (d, J = 5.4 Hz, 1H), 4.92−4.90 (m, 1H), 4.03−3.96 (m, 1H), 1.32 and 1.29 (2 s, 9H), 1.08 and 0.98 (2 d, J = 6.7 and 6.7 Hz, 3H); FAB MS *m*/*z* 309 (MH^+); HRMS calcd for $C_{15}H_{21}N_2O_3S$ (MH^+) 309.1273, found 309.1283.

(2*S*)-2-[(Benzyloxycarbonyl)amino]-1-benzo[*d*][1,3]-oxazol-2-yl-1-propanol (16). This compound was prepared from **15** and 2-aminophenol using the procedure previously described.[17] To a solution of *N*-(benzyloxycarbonyl)-(*S*)-alanine (20.0 g, 89.7 mmol) in CH_2Cl_2 (200 mL) at 0 °C, was added 1,1′-carbonyldiimidazole (18.2 g, 115.7 mmol). After 30 min of stirring at 0 °C, Et_3N (16.1 mL, 115.7 mmol) was added followed by the addition of *O*,*N*-dimethylhydroxylamine hydrochloride (11.3 g, 115.7 mmol). The mixture was stirred 1 h at 0 °C and then at room temperature for 4 h. CH_2Cl_2 was added, and the organic phase was washed twice with 10%

aqueous HCl, saturated $NaHCO_3$, and brine and dried over $MgSO_4$. Removal of the solvent in vacuo gave amide **15** (24.2 g) which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 7.4−7.3 (m, 5H), 5.65−5.55 (m, 1H), 5.15−5.05 (m, 2H), 4.82−4.74 (m, 1H), 3.77 (s, 3H), 3.21 (s, 3H), 1.34 (d, J = 6.9 Hz, 3H). This compound was dissolved in THF (350 mL) at 0 °C. A 1.0 M solution of $LiAlH_4$ in THF (110 mL, 110 mmol) was added dropwise over 30 min. Stirring was then continued at room temperature for 2 h. The mixture was then cooled to 0 °C, and a solution of $KHSO_4$ (22.4 g) in water (250 mL) was added carefully. After 1 h of stirring at 0 °C, the solution was extracted with ether and washed twice with 10% aqueous HCl, twice with saturated $NaHCO_3$, and once with brine. The organic phase was dried ($MgSO_4$), filtered, and concentration in vacuo to give the desired aldehyde (19.4 g) which was immediately dissolved in CH_2Cl_2 (350 mL) and cooled to 0 °C. A solution of $NaHSO_3$ (55.9 g, 540 mmol) in water (150 mL) was introduced, and the resulting mixture was stirred for 1 h. NaCN (25.0 g, 511 mmol) was then added, and stirring was continued overnight. The suspension was diluted with EtOAc (250 mL) and hexanes (250 mL), and the layers were separated. Washing with water and brine was followed by drying over $MgSO_4$ to afford the desired cyanohydrin (18.26 g, 83%) which was dissolved in benzene (350 mL) and stored at − 20 °C. To a mixture of ethanol (47.1 mL, 803 mmol) and $CHCl_3$ (50 mL) at 0 °C was added AcCl (53.5 mL, 752 mmol). After 30 min of stirring at 0 °C, a solution of the above cyanohydrin (5.87 g, 25.1 mmol) in $CHCl_3$ (50 mL) was added dropwise and stirring was continued for an additional 2 h. The mixture was then concentrated in vacuo and taken up in ethanol (60 mL). The solution was refluxed in the presence of 2-aminophenol (3.01 g) overnight. The ethanol was removed and the residue taken up in EtOAc, washed twice with 15% NaOH, 10% HCl, $NaHCO_3$, and brine, and dried ($MgSO_4$). Flash chromatography afforded the desired compound **16** as an orange syrup (4.81 g, 59%) which was used without further purification. An analytical sample was obtained by recrystallization from 30% EtOAc in hexanes: HPLC (system A) 99%, (system D) 99%; IR (KBr) ν 1692 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) 1:1 mixture of diastereomers at P_1, δ 7.75−7.68 (m, 2H), 7.42−7.19 (m, 8H), 6.22 and 6.10 (2 d, J = 6.0 and 5.4 Hz, 1H), 5.03−4.71 (m, 3H), 4.14−4.01 (m, 1H), 1.20 and 1.11 (2 d, J = 6.7 and 7.0 Hz, 3H); FAB MS m/z 327 (MH^+); HRMS calcd for $C_{18}H_{19}N_2O_4$ (MH^+) 327.1345, found 327.1355. Anal. ($C_{18}H_{18}N_2O_4$) C, H, N.

(2*S*)-2-[(Benzyloxycarbonyl)amino]-1-(oxazolo[4,5-*b*]-pyridin-2-yl)-1-propanol (17). This material was prepared as a 1:1 mixture of isomers in 12% yield from the above cyanohydrin (978 mg, 4.18 mmol) and 2-amino-3-hydroxypyridine (505 mg, 4.60 mmol) using the procedure described above for compound **16**. An analytical sample was obtained by recrystallization from EtOAc in hexanes (one isomer): mp 159−161 °C; IR (KBr) ν 1719, 1699 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (dd, J = 4.8, 1.2 Hz, 1H), 8.18 (d, J = 7.8 Hz, 1H), 7.44 (dd, J = 8.1, 5.1 Hz, 1H), 7.39−7.08 (m, 6H), 6.33 (d, J = 6.0 Hz, 1H), 5.00−4.82 (m, 2H), 4.74 (m, 1H), 4.10−4.00 (m, 1H), 1.21 (d, J = 6.7 Hz, 3H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 169.34, 155.32, 154.73, 146.08, 142.32, 136.99, 128.21, 127.59, 127.37, 120.57, 119.04, 69.99, 64.99, 49.91, 16.31; FAB MS m/z 328 (MH^+); HRMS calcd for $C_{17}H_{18}N_3O_4$ (MH^+) 328.1297, found 328.1286. Anal. ($C_{17}H_{17}N_3O_4$) C, H, N.

(2*S*)-2-[(Benzyloxycarbonyl)amino]-1-(4-methylbenzo-[*d*][1,3]oxazol-2-yl)-1-propanol (18). This material was prepared as a 1:1 mixture of isomers in 35% yield from the above cyanohydrin (707 mg, 3.02 mmol) and 2-amino-*m*-cresol (409 mg, 3.32 mmol) using the procedure described above for compound **16**. An analytical sample was obtained by recrystallization from EtOAc in hexanes (1.3:1 mixture of isomers): mp 98 °C; IR (KBr) ν 1701, 1690 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38−7.20 (m, 7H), 7.15−7.10 (m, 1H), 5.39 and 5.29 (2 d, J = 7.9 and 5.4 Hz, 1H), 5.15−4.90 (m, 3H), 4.40 and 4.23 (2 br s, 2H), 2.58 (s, 3H), 1.34 and 1.14 (2 d, J = 6.7 and 7.0 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 164.82, 164.22, 156.28, 156.12, 150.72, 150.65, 139.40, 136.27, 130.61, 130.43, 128.51, 128.40, 128.14, 128.01, 127.91, 125.20, 125.14, 125.04, 124.98, 108.20, 108.13, 71.02, 70.62, 66.96, 66.77, 50.45, 50.27, 17.35, 16.39, 12.24; FAB MS m/z 341 (MH^+); HRMS calcd for $C_{19}H_{21}N_2O_4$ (MH^+) 341.1501, found 341.1490. Anal. ($C_{19}H_{20}N_2O_4$) C, H, N.

(2*S*)-2-[(Benzyloxycarbonyl)amino]-1-(5-methylbenzo-[*d*][1,3]oxazol-2-yl)-1-propanol (19). This material was prepared as a 1:1 mixture of isomers in 53% yield from the above cyanohydrin (1.10 g, 4.70 mmol) and 2-amino-*p*-cresol (636 mg, 5.17 mmol) using the procedure described above for compound **16**. An analytical sample was obtained by recrystallization from EtOAc in hexanes (7:1 mixture of isomers): mp 134−135 °C; IR (KBr) ν 1718, 1691 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47−7.09 (m, 8H), 5.47 (d, J = 8.6 Hz, 1H), 5.12−4.87 (m, 4H), 4.54−4.30 (m, 1H), 2.44 (s, 3H), 1.32 and 1.13 (2 d, J = 6.7 and 6.7 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 165.90, 165.19, 156.09, 149.04, 140.24, 136.28, 134.38, 128.34, 128.09, 127.91, 126.32, 119.87, 119.69, 110.23, 70.84, 70.57, 66.92, 66.67, 50.48, 50.29, 21.34, 17.24, 15.32; FAB MS m/z 341 (MH^+); HRMS calcd for $C_{19}H_{21}N_2O_4$ (MH^+) 341.1501, found 341.1490. Anal. ($C_{19}H_{20}N_2O_4$) C, H, N.

(2*S*)-2-[(Benzyloxycarbonyl)amino]-1-(6-methylbenzo-[*d*][1,3]oxazol-2-yl)-1-propanol (20). This material was prepared as a 1:1 mixture of isomers in 71% yield from the above cyanohydrin (1.44 g, 6.15 mmol) and 6-amino-*m*-cresol (832 mg, 6.77 mmol) using the procedure described above for compound **16**. An analytical sample was obtained by recrystallization from EtOAc in hexanes (8:1 mixture of isomers): mp 108−109 °C; IR (KBr) ν 1692 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, J = 8.3 Hz, 1H), 7.36−7.10 (m, 7H), 5.40 and 5.32 (d, J = 8.9 and 8.9 Hz, 1H), 5.14−4.85 (m, 3H), 4.42−4.28 (m, 2H), 2.47 (s, 3H), 1.33 and 1.14 (2 d, J = 6.7 and 6.7 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 165.08, 156.11, 151.24, 137.95, 136.28, 135.76, 128.52, 128.16, 127.99, 127.90, 125.89, 125.79, 119.38, 119.20, 111.09, 70.99, 70.69, 66.99, 66.76, 50.55, 50.26, 21.70, 17.29, 15.38; FAB MS m/z 341 (MH^+); HRMS calcd for $C_{19}H_{21}N_2O_4$ (MH^+) 341.1501, found 341.1490. Anal. ($C_{19}H_{20}N_2O_4$) C, H, N.

(2*S*)-2-[(Benzyloxycarbonyl)amino]-1-(7-methylbenzo-[*d*][1,3]oxazol-2-yl)-1-propanol (21). This material was prepared as a 1:1 mixture of isomers in 57% yield from the above cyanohydrin (609 mg, 2.60 mmol) and 6-amino-*o*-cresol[35] (330 mg, 2.60 mmol) using the procedure described above for compound **16**. An analytical sample (1.2:1 mixture of isomers) was obtained by flash chromatography (30% EtOAc in hexanes): oil; IR (neat) ν 1705 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (m, 1H), 7.35−7.10 (m, 7H), 5.50 and 5.38 (2 d, J = 8.9 and 8.3 Hz, 1H), 5.15−4.90 (m, 3H), 4.41 (s, 1H), 2.36 (s, 3H), 1.35 and 1.14 (2 d, J = 6.7 and 7.0 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 165.41, 164.85, 156.29, 156.12, 150.14, 139.67, 136.28, 128.50, 128.39, 128.13, 127.97, 127.84, 126.36, 126.31, 124.59, 124.51, 121.54, 117.28, 117.12, 70.99, 70.56, 66.96, 66.71, 50.42, 50.25, 17.41, 15.26, 15.08; FAB MS m/z 341 (MH^+); HRMS calcd for $C_{19}H_{21}N_2O_4$ (MH^+) 341.1501, found 341.1490. Anal. ($C_{19}H_{20}N_2O_4$) C, H, N.

Method F: General Procedure for the Preparation of α-Keto Heterocycles. *N*1-[2-(1,3-Benzoxazol-2-yl)-1-methyl-2-oxoethyl]-*N*4,*N*4-dimethyl-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]-butanediamide (77). A mixture of **16** (265 mg, 0.81 mmol) and 10% Pd on carbon (79 mg) in ethanol (20 mL) was stirred under an atmosphere of hydrogen for 1 h. The solution was then filtered through a glass microfiber and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (6 mL), and Boc-Asn(γ-NMe_2)-OH (222 mg, 0.85 mmol), HOBT (220 mg, 1.63 mmol), *i*-Pr_2NEt (0.56 mL, 3.25 mmol), and EDAC (169 mg, 0.88 mmol) were added. Additional *i*-Pr_2NEt was introduced to bring the pH above 8, and stirring was continued overnight. The resulting mixture was diluted with EtOAc, washed sequentially with 10% citric acid, 10% Na_2CO_3, and water, and dried by passing through a plug of glass wool. Flash chromatography (EtOAc) gave the desired compound (314 mg, 95%): ^{1}H NMR (DMSO-d_6) δ 7.82−7.66 (m, 3H), 7.42−7.33 (m, 3H), 6.78 and 6.73 (2 d, J = 7.8 and 7.5 Hz, 1H), 6.23−6.18 and 6.16−6.11 (2 m, 1H), 4.84−4.82 and 4.66−4.50 (2 m, 1H), 4.33−4.17 (m, 2H), 2.88, 2.77, 2.74 (3 s, 6H), 2.58−2.20 (m, 2H), 1.34 (s, 9H), 1.18−1.15 (m, 3H). This

product was stirred in a mixture of CH_2Cl_2 (8 mL) and TFA (2 mL) for 2 h. After removal of the solvent, residual TFA was removed by azeotropic distillation with benzene using a rotary evaporator. The residue was dissolved in CH_2Cl_2 (6 mL), and Boc-Tbg-OH (188 mg, 0.81 mmol), HOBT (209 mg, 1.55 mmol), *i*-Pr_2NEt (0.54 mL, 3.10 mmol), and EDAC (161 mg, 0.84 mmol) were added. Additional *i*-Pr_2NEt was introduced to bring the pH above 8, and stirring was continued overnight. The resulting mixture was diluted with EtOAc, washed sequentially with 10% citric acid, 10% Na_2CO_3, and water, and dried by passing through a plug of glass wool. Flash chromatography (EtOAc) gave the desired compound (264 mg, 62%): ^{1}H NMR (DMSO-d_6) δ 7.89−7.82 and 7.73−7.68 (m, 4H), 7.42−7.33 (m, 2H), 6.45 (d, J = 6.9 Hz, 1H), 6.19 and 6.06 (2 d, J = 6.0 and 5.4 Hz, 1H), 4.84 and 4.68 (2 t, J = 5.1 and 6.3 Hz, 1H), 4.61−4.51 (m, 1H), 4.33−4.23 (m, 1H), 3.84−3.77 (m, 1H), 2.89, 2.78, 2.72 (3 s, 6H), 2.58−2.27 (m, 2H), 1.38 (s, 9H), 1.12 (m, 3H), 0.86 and 0.84 (2 s, 9H). This product was stirred in a mixture of CH_2Cl_2 (8 mL) and TFA (2 mL) for 2 h. After removal of the solvent, residual TFA was removed by azeotropic distillation with benzene using a rotary evaporator. The residue was dissolved in CH_2Cl_2 (6 mL), and *tert*-butylacetic acid (64 mg, 0.50 mmol), HOBT (129 mg, 0.96 mmol), *i*-Pr_2NEt (0.33 mL, 1.91 mmol), and EDAC (99 mg, 0.52 mmol) were added. Additional *i*-Pr_2NEt was introduced to bring the pH above 8, and stirring was continued overnight. The resulting mixture was diluted with EtOAc, washed sequentially with 10% citric acid, 10% Na_2CO_3, and water, and dried by passing through a plug of glass wool. Flash chromatography (EtOAc) gave the desired compound (162 mg, 62%) which was immediately dissolved in CH_2Cl_2 (8 mL). Dess−Martin periodinane (252 mg, 0.59 mmol) was added and the resulting mixture stirred for 1 h. A 1:1 mixture of 10% $Na_2S_2O_3$:saturated $NaHCO_3$ was introduced, and stirring was continued until both layers were clear (10 min). The residue was extracted with CH_2Cl_2, washed with aqueous $NaHCO_3$, dried ($MgSO_4$), filtered, and concentrated in vacuo. Flash chromatography using TLC grade silica gel (3% ethanol in EtOAc) afforded the compound as a colorless oil. This material was dissolved in a minimum amount of CH_3CN, diluted with water, and lyophilized to afford the desired compound **77** as a white solid (99.3 mg, 61%): IR (KBr) ν 3311, 1713, 1657 cm^{-1}; ^{1}H NMR (DMSO-d_6) 2.7:1 mixture of diastereomers, δ 8.28 (d, J = 5.7 Hz, 1H), 8.16 and 8.08 (2 d, J = 7.5 and 7.5 Hz, 1H), 8.00 (d, J = 8.1 Hz, 1H), 8.28 (d, J = 8.1 Hz, 1H), 7.75−7.53 (m, 2H), 7.45−7.34 (m, 1H), 6.99 and 6.75 (2 s, 1H, hydrate), 5.30−5.22 and 4.41−4.35 (2 m, 1H), 4.62 and 4.52 (2 q, J = 6.0 and 7.2 Hz, 1H), 4.18 and 4.13 (2 d, J = 9.0 and 8.4 Hz, 1H), 2.91 and 2.82 (2 s, 3H), 2.77 and 2.71 (2 s, 3H), 2.73−2.59 (m, 2H), 2.20 and 2.16 (2 d, J_{AB} = 12.6 and 12.9 Hz, 1H), 2.03 and 2.02 (2 d, J_{AB} = 12.6 and 12.9 Hz, 1H), 1.42 and 1.06 (d, J = 7.2 and 6.9 Hz, 3H), 0.95−0.87 (m, 18H); HRMS calcd for $C_{28}H_{42}N_5O_6$ (MH^+) 544.3135, found 544.3154. Anal. ($C_{28}H_{41}N_5O_6$) C, H, N.

3-[(*tert*-Butoxycarbonyl)amino]-2-hydroxybutyric Acid Benzyl Ester (23). To a solution of nitroethane (4.0 g, 53 mmol) in ethanol (15 mL) was added aqueous NaOH (68 mL of 2 N solution, 136 mmol). To this rapidly stirred solution was added glyoxylic acid (5.9 g, 64 mmol). The solution was stirred 15 h and then acidified with 10% aqueous HCl (pH 2), and the aqueous phase was saturated with NaCl before extraction with EtOAc (3 × 150 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated to give a viscous yellow oil (8.1 g). This crude material was dissolved in ethanol (50 mL) containing Et_3N (18 mL, 119 mmol) and treated with di-*tert*-butyl dicarbonate (12.2 g, 56 mmol) and Raney nickel (3 g) which had been washed with water and ethanol immediately before use. Hydrogenation at 45 psi for 20 h afforded after filtration through Celite and concentration the crude acid (11.1 g). A portion of the crude acid (3.07 g, 14 mmol) was dissolved in DMF (30 mL) and treated with anhydrous K_2CO_3 (4.3 g, 30.8 mmol) and benzyl bromide (2.5 mL, 21 mmol). After 3 h of stirring at room temperature, the DMF was removed under reduced pressure and the residue dissolved in EtOAc (150 mL) and washed sequentially with water and brine before being dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude yellow oil (4.3 g) was purified by flash chromatography on silica gel (230−400 mesh), eluting with 33% EtOAc in hexane to provide pure benzyl ester **23** (1.8 g, 42% from nitroethane): HPLC (system C) 99%, (system D) 97%; IR (KBr) ν 3422, 3361, 1740, 1684 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.36 (s, 5H), 5.27 (d, J = 12.1 Hz, 1H), 5.19 (d, J = 12.1 Hz, 1H), 4.82 (m, 1H), 4.36 and 4.35 (2 d, J = 5.7 and 5.4 Hz, 1H), 4.11 (m, 1H), 3.10 (m, 1H), 1.43 (s, 9H), 0.97 (d, J = 7.0 Hz, 3H); FAB MS m/z 310 (MH^+), 210 ($MH^+ - 100$); HRMS calcd for $C_{16}H_{24}NO_5$ (MH^+) 310.1654, found 310.1644. Anal. ($C_{16}H_{23}NO_5$) C, H, N.

Method G (Scheme 5): General Procedure for the Synthesis of α-Keto Amides. 3-[[2-[[2-[(3,3-Dimethylbutyryl)amino]-3,3-dimethylbutyryl]amino]-3-(dimethylcarbamoyl)propionyl]amino]-2-oxobutyric Acid Benzyl Amide (76). Ester **23** (4.0 g, 12.9 mmol) was treated with 4 N HCl/dioxane (30 mL) for 45 min at 0 °C before being concentrated in vacuo. The hydrochloride salt (12.9 mmol) was combined with EDAC (2.6 g, 13.6 mmol), HOBT (1.8 g, 13.6 mmol), and Boc-Asn(γ-NMe_2)-OH (3.4 g, 12.9 mmol) in DMF (50 mL) under a nitrogen atmosphere. The solution was cooled to 0 °C (ice bath) before *i*-Pr_2NEt (7.9 mL, 45.3 mmol) was added. The solution was then stirred at room temperature for 16 h. The reaction mixture was diluted with EtOAc (250 mL) and washed sequentially with saturated aqueous $NaHCO_3$, 5% aqueous HCl, and brine before being dried ($MgSO_4$), filtered, and concentrated in vacuo to give the crude material (6.0 g). The crude material was suitable for subsequent coupling without purification. This compound was coupled to *tert*-butylacetic acid and Boc-Tbg-OH using identical procedures. After the final coupling, the α-hydroxy benzyl ester peptide was purified by flash chromatography. The acid **24** was then obtained from the benzyl ester (1.10 g, 2.0 mmol) by hydrogenation over 10% Pd/C (55 mg) in ethanol (30 mL) at atmospheric pressure for 3 h to afford after filtration through a pad of Celite a white solid (0.95 g, 100% yield): HPLC (system A) 100%, (system C) 100%; IR (KBr) ν 3316, 1727, 1642 cm^{-1}; ^{1}H NMR ($CDCl_3$) mixture of four diastereomers, δ 8.06 and 8.01 (2 d, J = 7.3 and 8.6 Hz, 1H), 7.87, 7.79, 7.70, and 7.54 (4 d, J = 8.6, 8.6, 8.9, and 8.6 Hz, 1H), 7.09 and 7.03 (2 d, J = 7.9 and 8.6 Hz, 0.5H), 6.72 (m, 0.5H), 6.52 (m, 0.25), 6.34 and 6.29 (2 d, J = 7.6 and 7.3 Hz, 0.75H), 6.10−5.4 (br s, 1H), 4.99−4.88 (m, 0.5H), 4.87−4.78 (m, 0.5H), 4.66−4.37 (m, 2H), 4.33−4.09 (m, 1H), 3.30−3.15 (m, 0.3H), 3.05−2.85 (m, 6.7 H), 2.75−2.65 and 2.60−2.50 (m, 1H), 2.25−2.10 (m, 2H), 1.28−1.19 (m, 3H), 1.10−0.97 (m, 18H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 174.9, 173.5, 173.1, 173.0, 171.5, 171.0, 170.9, 170.8, 170.7, 170.5, 170.2, 73.03, 72.74, 60.9, 60.67, 50.3, 50.1, 49.5, 49.4, 48.1, 47.9, 47.7, 37.56, 35.9, 35.8, 35.7, 34.7, 34.4, 34.3, 33.8, 31.1, 29.9, 29.8, 26.9, 26.8, 26.7, 17.4, 17.2; FAB MS m/z 473 (MH^+), 495 (M + 23); HRMS calcd for $C_{22}H_{41}N_4O_7$ (MH^+) 473.2975, found 473.2990. The acid **24** (0.15 g, 0.32 mmol) was combined with benzylamine (41.5 μL, 0.38 mmol), EDAC (0.121 g, 0.63 mmol), HOBT (86 mg, 0.63 mmol), and *i*-Pr_2NEt (0.39 mL, 2.22 mmol) in DMF (8 mL) and stirred 16 h. The reaction mixture was diluted with EtOAc (40 mL), washed sequentially with 1 N HCl, saturated $NaHCO_3$, and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The final oxidation step was performed by treatment of α-hydroxy amide **25** (R = $NHCH_2Ph$) (62 mg, 0.11 mmol) with the Dess−Martin periodinane (94 mg, 0.22 mmol) in DMF (1 mL) for 4 h. The reaction mixture was diluted with EtOAc and treated with a 1:1 mixture of 10% $Na_2S_2O_3$ and saturated $NaHCO_3$ (15 min). The organic phase was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. Final purification was performed using preparative HPLC to afford after lyophilization **76**, (51 mg, 82% yield) as a white solid: IR (KBr) ν 3316, 1641, 1529 cm^{-1}; ^{1}H NMR (DMSO-d_6) 1:1 mixture of diastereoisomers at P_1, δ 9.21−9.15 (m, 1H), 8.14 and 8.09 (2 d, 7.3 and 7.6 Hz, 1H), 8.03 and 7.97 (2 d, J = 6.4 and 5.7 Hz, 1H), 7.60 (d, J = 8.3, 1H), 7.35−7.17 (m, 5H), 5.02−4.88 (m, 1H), 4.64−4.49 (m, 1H), 4.39−4.23 (m, 2H), 4.13 and 4.12 (2 d, J = 8.6 and 8.6 Hz, 1H), 2.92 and 2.91 (2 s, 3H), 2.79 and 2.78 (2 s, 3H), 2.74−2.54 (m, 2H), 2.19 (br d, J = 12.4 Hz, 1H), 2.03 and 2.02 (2 d, J = 12.4 and 12.7 Hz, 1H), 1.25 and 1.23 (2 d, J = 7.3 and 7.0 Hz, 3H), 0.94 and

0.91 (2 s, 18H); HRMS calcd for $C_{29}H_{46}N_5O_6$ (MH^+) 560.3448, found 560.3426.

Method H (Scheme 6): *N*1-[1-(Benzyloxy)-2-oxo-(3*S*)-azetanyl]-*N*4,*N*4-dimethyl-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]-butanediamide (78). A solution of Boc-D-Ser(OBn)-OH (1.10 g, 3.72 mmol), benzyl bromide (490 μL, 4.10 mmol), and DBU (610 μL, 4.10 mmol) in CH_3CN (20 mL) was stirred at room temperature for 2 h. The resulting mixture was poured into 0.1 M HCl and extracted with EtOAc (2 × 30 mL), dried ($MgSO_4$), and concentrated in vacuo. The pale yellow oil (1.45 g) obtained was used as such without any purification. This material was then treated with a 4 N HCl/dioxane solution (10 mL) and the resulting mixture stirred 2.5 h at room temperature. Concentration under vacuum afforded a colorless oil (1.30 g) that solidified upon standing: ^{1}H NMR (DMSO-d_6) δ 8.70 (s, 3H), 7.40−7.25 (m, 10H), 5.25 (dd, 2H), 4.55 (d, 1H), 4.52 (dd, 2H), 3.95−3.88 (m, 2H). To a solution of Boc-Asp(γ-NMe_2)-OH (131 mg, 0.50 mmol) and the above hydrochloride salt (162 mg, 0.50 mmol) was added TBTU (170 mg, 0.53 mmol) in CH_3CN (5 mL). This mixture was stirred at −10 °C before adding NMM (116 μL, 1.06 mmol). The resulting mixture was slowly allowed to warm to room temperature and stirred 23 h. The mixture was then concentrated in vacuo, poured into 0.1 M HCl, extracted with EtOAc (2 × 10 mL), dried ($MgSO_4$), and concentrated to dryness. The residue was purified by flash chromatography (EtOAc), giving a pale yellow oil (213 mg, 80%): ^{1}H NMR ($CDCl_3$) δ 7.60 (d, 1H), 7.35−7.20 (m, 10H), 6.20 (br s, 1H), 5.25 (d, 1H), 5.15 (d, 1H), 4.75 (q, 1H), 4.60−4.55 (m, 1H), 4.50 (d, 1H), 4.42 (d, 1H), 3.88 (dd, 1H), 3.70 (dd, 1H), 3.50 (d, 1H), 3.18 (d, 1H), 2.97 (s, 3H), 2.90 (s, 3H), 2.55 (dd, 1H). Boc-Asp(γ-NMe_2)-D-Ser(OBn)-OBn (213 mg, 0.40 mmol) was dissolved in a 4 N HCl/dioxane solution (5 mL) and stirred for 2 h. The mixture was then concentrated in vacuo. The resulting hydrochloride salt (0.40 mmol) was combined with Boc-Tbg-OH (93 mg, 0.40 mmol) and TBTU (136 mg, 0.42 mmol) in CH_3CN (5 mL) at 0 °C before NMM (93 μL, 0.84 mmol) was added. The reaction was stirred at room temperature for 48 h. The residue was extracted into EtOAc, washed sequentially with saturated $NaHCO_3$, 1 N HCl, and brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. This material was purified by flash chromatography on silica gel (80% EtOAc/hexanes), giving a pale colorless oil (208 mg, 81%): ^{1}H NMR ($CDCl_3$) δ 7.80 (br s, 1H), 7.50 (d, 1H), 7.35−7.20 (m, 10H), 5.27 (d, 1H), 5.20 (d, 1H), 5.16 (d, 1H), 4.92−4.86 (m, 1H), 4.77−4.71 (m, 1H), 4.48 (d, 1H), 4.42 (d, 1H), 3.87 (dd, 1H), 3.69 (dd, 1H), 3.15 (d, 1H), 2.95 (s, 3H), 2.91 (s, 3H), 2.50 (dd, 1H), 1.49 (s, 9H), 0.98 (s, 9H). A solution of Boc-Tbg-Asp(γ-NMe_2)-D-Ser(OBn)-OBn (206 mg, 0.32 mmol) was treated with 4 N HCl/dioxane (5 mL) for 1 h before being concentrated in vacuo. The resulting hydrochloride salt was combined with *tert*-butylacetyl chloride (49 μL, 0.35 mmol) and Et_3N (94 μL, 0.68 mmol) in CH_2Cl_2 (5 mL). The resulting mixture was stirred 30 min before being poured into 0.1 M HCl and extracted with EtOAc (2 × 10 mL). The combined organic phases were washed with saturated $NaHCO_3$, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (EtOAc), affording a white solid (176 mg, 86%): ^{1}H NMR ($CDCl_3$) δ 7.79 (d, 1H), 7.46 (d, 1H), 7.35−7.19 (m, 10H), 6.08 (d, 1H), 5.20 (d, 1H), 5.15 (d, 1H), 4.92−4.86 (m, 1H), 4.77−4.72 (m, 1H), 4.48 (d, 1H), 4.42 (d, 1H), 4.23 (d, 1H), 3.87 (dd, 1H), 3.69 (dd, 1H), 3.11 (d, 1H), 2.94 (s, 3H), 2.91 (s, 3H), 2.48 (dd, 1H), 2.11 (d, 2H), 1.05 (s, 9H), 0.98 (s, 9H). A solution of (*t*-$BuCH_2CO$)-Tbg-Asp(γ-NMe_2)-D-Ser(OBn)-OBn (172 mg, 0.72 mmol) and $Pd(OH)_2$/C (10 mg) in methanol (10 mL) was stirred at room temperature under a hydrogen atmosphere (1 atm) for 2.5 h. The catalyst was filtered through Celite and concentrated in vacuo. The white solid (124 mg, 100%) obtained was used as such without any purification. A solution of (*t*-$BuCH_2CO$)-Tbg-Asp(γ-NMe_2)-D-Ser-OH (100 mg, 0.21 mmol), *O*-benzylhydroxylamine hydrochloride (100 mg, 0.66 mmol), NMM (80 μL, 0.66 mmol), and HOAt (30 mg, 0.23 mmol) in DMF (6 mL) was stirred at 0 °C before a 2.3 M solution of DCC in CH_2Cl_2 (100 μL, 0.23 mmol) was added. The resulting mixture allowed to warm slowly to room temperature and stirred 18 h. The mixture was poured into 0.1 M HCl and extracted with EtOAc (2 × 10 mL). The combined organic extracts were washed with 0.1 M HCl, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by preparative HPLC using a reverse phase column (0−70% acetonitrile/water gradient over 80 min) to give **26** as a white solid (90 mg, 75%): ^{1}H NMR ($CDCl_3$) δ 10.05 (s, 1H), 7.50−7.30 (m, 5H), 6.00 (d, 1H), 4.94−4.81 (m, 3H), 4.50−4.43 (m, 1H), 4.22 (dd, 1H), 4.00 (d, 1H), 3.78 (dd, 1H), 3.39 (dd, 1H), 3.00 (s, 3H), 2.89 (s, 3H), 2.52 (dd, 1H), 2.13 (d, 2H), 1.04 (s, 9H), 1.01 (s, 9H). To a solution of the alcohol **26** (77 mg, 0.14 mmol) and PPh_3 (54 mg, 0.20 mmol) in THF (5 mL) at room temperature was added diethyl azodicarboxylate (32 μL, 0.20 mmol). The resulting mixture was stirred 17 h, followed by concentration in vacuo. The residue was purified by flash chromatography on silica gel (5% MeOH/EtOAc). A white solid (40 mg, 53%) was obtained. For analytical purposes, trituration in EtOAc afforded 11 mg of compound **78**: mp 198−200 °C; IR (KBr) ν 1764 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.88 (d, J = 7.3 Hz, 1H), 7.57 (d, J = 7.3 Hz, 1H), 7.45−7.35 (m, 5H), 5.95 (d, J = 7.0 Hz, 1H), 4.98 (d, J = 1.0 Hz, 2H), 4.75 (ddd, J = 7.3, 7.3, 3.5 Hz, 1H), 4.65 (m, 1H), 4.07 (d, J = 7.3 Hz, 1H); 3.48 (t, J = 4.9 Hz, 1H), 3.34 (dd, J = 4.8, 2.5 Hz, 1H), 3.15 (dd, J = 16.2, 3.2 Hz, 1H), 2.99 (s, 3H), 2.93 (s, 3H), 2.44 (dd, J = 10.2, 7.0 Hz, 1H), 2.21 (d, J = 12.9 Hz, 1H), 2.13 (d, J = 12.9 Hz, 1H), 1.04 (s, 9H), 1.03 (s, 9H); HRMS calcd for $C_{28}H_{43}N_5O_6$ (MH^+) 546.3292, found 546.3273.

(4*S*)-3-[2-(1-Adamantyl)acetyl]-4-isopropyl-1,3-oxazolan-2-one (29). 1-Adamantaneacetic acid (1.0 g, 5.14 mmol) was dissolved in CH_2Cl_2 (15 mL) containing 1 drop of DMF. The mixture was stirred magnetically at 5 °C under an atmosphere of nitrogen and oxalyl chloride (1.1 equiv, 0.72 g, 496 μL, 5.66 mmol) was added dropwise over 20 min. After 2 h, dichloromethane was evaporated under vacuum. The residual oil was dissolved in benzene (10 mL) and concentrated to afford compound **28** (1.09 g, 100%) as a pale yellow oil which was used as such in the next reaction. To a solution of (4*S*)-(−)-4-isopropyl-2-oxazolidinone (0.66 g, 5.14 mmol) in anhydrous THF (10 mL) at −40 °C was added dropwise *n*-BuLi (3.22 mL, 1.6 M in hexanes, 5.14 mmol). After 30 min at −40 °C, the reaction mixture was cooled to −78 °C. The crude acid chloride **28** (1.09 g, 5.14 mmol) dissolved in THF (1 mL) was added dropwise. The mixture was then stirred magnetically at 0 °C for 1 h. Ethyl acetate was added, and the organic phase was washed with 20% aqueous citric acid and saturated aqueous $NaHCO_3$, dried ($MgSO_4$), filtered, and concentrated. The residual solid was purified by flash chromatography on silica gel, eluting with hexane:ethyl acetate (5:1) to provide pure **29** (1.26 g, 80%) as a white solid: mp 105−107 °C; [α] +62° (*c* 1.54, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.48−4.44 (m, 1H), 4.24−4.16 (m, 2H), 2.91 (d, J = 14 Hz, 1H), 2.71 (d, J = 14 Hz, 1H), 2.38−2.33 (m, 1H), 2.00−1.94 (m, 3H), 1.75−1.61 (m, 12H), 0.92 (d, J = 7.5 Hz, 3H), 0.89 (d, J = 7.5 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 171.21, 154.08, 62.84, 58.54, 46.71, 42.23, 36.73, 33.81, 28.63, 28.53, 18.04, 14.68; FAB MS *m*/*z* 306 (MH^+); HRMS calcd for $C_{18}H_{28}NO_3$ (MH^+) 306.2069, found 306.2058. Anal. ($C_{18}H_{27}NO_3$) C, H, N.

(2*S*)-2-(1-Adamantyl)-2-azidoethanoic Acid (31). The oxazolidinone **29** (4.2 g, 13.7 mmol) was dissolved in THF (15 mL) and was added dropwise over 15 min to a solution of potassium bis(trimethylsilyl)amide (20.1 mL, 0.69 M in THF, 13.9 mmol) at −78 °C. After 45 min at −78 °C, 2,4,6-triisopropylbenzenesulfonyl azide (4.9 g, 15.8 mmol) in THF (10 mL) at −78 °C was added in one portion to the enolate. After 5 min, glacial acetic acid (4.6 equiv, 3.8 g, 3.61 mL, 63.2 mmol) was added and the mixture was stirred at 40 °C for 1 h. Tetrahydrofuran was evaporated under reduced pressure and the residue was dissolved in a mixture of EtOAc and water. The organic phase was washed with saturated aqueous $NaHCO_3$, followed by brine, dried ($MgSO_4$), filtered, and concentrated. The residual oil was dissolved in 75 mL of hexane/ethyl acetate (2/1). After 16 h at 25 °C, the white precipitate was removed by filtration, and the filtrate was concentrated to give an oily residue which was filtered through a pad of silica, washed with hexane/ethyl acetate (8/1). This material (crude **30**, 1.3 g, 27%) was dissolved in THF/water

(70 mL, 3/1) and H_2O_2 (4 equiv, 30%, 1.69 mL, 15 mmol) was added at 0 °C followed by $LiOH·H_2O$ (2.1 equiv, 0.33 g, 7.88 mmol). After 45 min, 10% aqueous $Na_2S_2O_3$ (48 mL) and solid $NaHCO_3$ (0.22 g) were added. The reaction mixture was concentrated and ca. 50 mL of water was added. The aqueous phase was washed with chloroform (four times), acidified at 0 °C with 15% HCl, and extracted with EtOAc (three times). The combined EtOAc extracts were dried ($MgSO_4$), filtered, and concentrated to provide compound **31** (0.79 g, 90% from crude **30**) as a white solid: mp 110−112 °C; [α] −36° (*c* 1.60, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.60 (s, 1H), 2.16−2.00 (m, 3H), 1.76−1.62 (m, 12H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 174.19, 72.51, 38.68, 37.50, 36.51, 28.19; FAB MS *m*/*z* 236 (MH^+); HRMS calcd for $C_{12}H_{18}N_3O_2$ (MH^+) 236.1399, found 236.1389. Anal. ($C_{12}H_{17}N_3O_2$) C, H, N.

Benzyl 4-[(4*S*)-4-Isopropyl-2-oxo-1,3-oxazolan-3-yl]-2,2-dimethyl-4-oxobutanoate (34). *n*-Butyllithium (2.4 mL, 1.6 M in hexanes, 3.9 mmol) was added dropwise to a solution of (4*S*)-(−)-4-isopropyl-2-oxazolidinone (0.5 g, 3.9 mmol) in THF (5 mL) at −40 °C under an atmosphere of nitrogen. After 30 min at −40 °C, the reaction mixture was cooled to −78 °C and 2,2-dimethylsuccinic anhydride (0.5 g, 3.9 mmol) dissolved in THF (2 mL) was added dropwise. The mixture was then stirred magnetically at 0 °C for 1 h. Ethyl acetate was added, and the organic phase was washed with 20% aqueous citric acid and brine, dried ($MgSO_4$), and concentrated to afford crude **33** as a pale yellow solid (1.0 g) which was dissolved in acetonitrile (5 mL) at 0 °C. 1,8-Diazabicyclo[5.4.0]undec-7-ene (0.59 g, 583 μL, 3.9 mmol) and benzyl bromide (0.67 g, 463 μL, 3.9 mmol) were added, and the reaction mixture was then stirred at 25 °C for 16 h. Acetonitrile was evaporated in vacuo. The residue was partitioned between EtOAc and 20% aqueous citric acid. The organic phase was washed with water and brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography on silica gel eluting with hexane:ethyl acetate (4:1) to give pure **34** (0.88 g, 61% from **32**) as a colorless oil: [α] +56° (*c* 1.30, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31−7.28 (m, 5H), 5.11 (m, 2H), 4.36−4.32 (m, 1H), 4.23−4.15 (m, 2H), 3.25 (s, 2H), 2.30−2.22 (m, 1H), 1.33 (s, 3H), 1.31 (s, 3H), 0.86 (d, *J* = 7 Hz, 3H), 0.81 (d, *J* = 7 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 176.73, 170.75, 154.03, 136.25, 128.35, 127.88, 66.24, 63.44, 58.27, 45.31, 40.24, 28.31, 25.62, 25.58, 17.84, 14.56; FAB MS *m*/*z* 348 (MH^+); HRMS calcd for $C_{19}H_{26}NO_5$ (MH^+) 348.1811, found 348.1822. Anal. ($C_{19}H_{25}NO_5$) C, H, N.

Benzyl 3-Azido-2,2-dimethylsuccinic Acid (36). The oxazolidinone **34** (8.67 g, 24.9 mmol) was dissolved in THF (27 mL) and was added dropwise over 15 min to a solution of potassium bis(trimethylsilyl)amide (36.5 mL, 0.69 M in THF, 25.2 mmol) at −78 °C. After 45 min at −78 °C, 2,4,6-triisopropylbenzenesulfonyl azide (8.89 g, 28.7 mmol) in THF (15 mL) at −78 °C was added in one portion to the enolate. After 5 min, glacial acetic acid (4.6 equiv, 6.90 g, 6.56 mL, 0.12 mol) was added and the mixture was stirred at 35−40 °C for 90 min. Tetrahydrofuran was evaporated under reduced pressure, and the residue was dissolved in a mixture of EtOAc and water. The organic phase was washed with saturated aqueous $NaHCO_3$, brine, dried ($MgSO_4$), filtered, and concentrated. The residue was dissolved in 150 mL of hexane/ethyl acetate (2/1). After 16 h at 25 °C, the white precipitate was removed by filtration, and the filtrate was concentrated to give an oil which was filtered through a pad of silica, rinsing with hexane/ethyl acetate (5/1). This pale yellow oil (crude **35**, 5.37 g, 55%) was dissolved in THF/water (260 mL, 3/1) and H_2O_2 (4 equiv, 30%, 6.24 mL, 55 mmol) was added at 0 °C, followed by $LiOH·H_2O$ (2.1 equiv, 1.22 g, 29 mmol). After 45 min, 10% aqueous $Na_2S_2O_3$ (175 mL) and solid $NaHCO_3$ (0.81 g) were added. Tetrahydrofuran was evaporated, and the aqueous phase was extracted with chloroform (continuous liquid−liquid extraction, 24 h). The aqueous phase was then acidified with concentrated HCl at 0 °C, and extracted with EtOAc (three times). The combined EtOAc extracts were dried ($MgSO_4$), filtered, and concentrated. The residual oil was purified by flash chromatography on Merck silica gel eluting with ethyl acetate/acetic acid (400/1) to give compound **36** (0.82 g, 21%) as a colorless oil: [α] −74° (*c* 1.43, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39−7.32 (m, 5H), 5.20−5.12 (m, 2H), 4.48 (s, 1H), 1.34 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 174.74, 173.86, 135.42, 128.56, 128.35, 128.13, 67.86, 67.18, 45.98, 23.01, 20.29; FAB MS *m*/*z* 278 (MH^+); HRMS calcd for $C_{13}H_{16}N_3O_4$ (MH^+) 278.1141, found 278.1130. Anal. ($C_{13}H_{15}N_3O_4$) H, N; C: calcd, 56.31; found, 55.90.

Method I: *N*4,*N*4-Dimethyl-*N*1-[1-(diphenylphosphonyl)ethyl]-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]butanediamide (79). To a warm solution of 1-[*N*-(benzyloxycarbonyl)amino]ethyl phosphonate[36] (8.50 g, 21.0 mmol) in ethanol (75 mL) was added a solution of 4 N HCl/dioxane (5.25 mL, 21.0 mmol) and 10% Pd/C (850 mg, 10% w/w). The mixture was stirred vigorously, flushed three times with hydrogen, and stirred 16 h under a hydrogen atmosphere (balloon). The catalyst was filtered through Celite and the filtrate concentrated in vacuo. The residual oil was trituated in Et_2O (150 mL) until a white solid was obtained. This was filtered and dried to give 6.10 g (93%) of the corresponding hydrochloride salt: ^{1}H NMR (DMSO-d_6) δ 9.18 (s, 3H), 7.40−7.44 (m, 4H), 7.24−7.27 (m, 6H), 4.18 (dt, *J* = 7.2, 20.3 Hz, 1H), 1.61 (dd, J_1 = 7.2 Hz, J_2 = 18.0 Hz, 3H). A stirred solution containing Boc-Asn(γ-NMe_2)-OH (500 mg, 1.92 mmol), the hydrochloride salt from above (663 mg, 2.11 mmol), *i*-Pr_2NEt (836 μL, 4.80 mmol), and TBTU (677 mg, 2.11 mmol) in DMF (8 mL) was stirred initially at 0 °C for 15 min, and then at room temperature for 3 h under an atmosphere of nitrogen. The solution was poured into brine and the product extracted with EtOAc (2 × 25 mL). The combined organic extracts were washed sequentially with 5% aqueous $NaHCO_3$, 1 M citric acid, and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo to give 0.975 g of an amorphous solid. The product was purified by flash chromatography (gradient 15−30% *i*-PrOH/hexane) to yield the coupled phosphonate derivative as an amorphous solid (0.81 g, 81%): HPLC (system A) 99.5%; ^{1}H NMR ($CDCl_3$) 1:1 mixture of diastereomers at P_1, δ 7.65−7.35 (m, 1H), 7.30−7.05 (m, 10H), 6.20−5.95 (m, 1H), 4.86−4.64 (m, 1H), 4.54−4.42 (m, 1H), 3.16−3.05 (m, 1H), 2.97−2.74 (m, 6H), 2.56−2.41 (m, 1H), 1.55−1.45 (m, 3H), 1.38 (s, 9H); FAB MS *m*/*z* 520 (MH^+), 420 (MH^+ − 100). This material (0.75 g, 1.44 mmol) was treated with 4 N HCl/dioxane (30 min) before being concentrated in vacuo. The hydrochloride salt (1.44 mmol) was combined with Boc-Tbg-OH (0.40 g, 1.73 mmol), TBTU (0.555 g, 1.73 mmol), and *i*-Pr_2NEt (1.05 mL, 6.05 mmol) in DMF (8 mL) initially at 0 °C (15 min) and then at room temperature 16 h. The reaction mixture was diluted with EtOAc and washed sequentially with 5% aqueous $NaHCO_3$, 1 M citric acid, and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by flash chromatography using TLC grade silica gel (20% *i*-PrOH/hexane) gave the desired dipeptide fragment as a white solid (0.773 g, 85%): ^{1}H NMR ($CDCl_3$) δ 8.06−7.80 (m, 1H), 7.50−7.40 (m, 1H), 7.38−7.10 (m, 10H), 5.24−5.12 (m, 1H), 4.86−4.68 (m, 2H), 3.84−3.76 (m, 1H), 3.21−3.07 (m, 1H), 2.95−2.78 (m, 6H), 2.58−2.35 (m, 1H), 1.61−1.49 (m, 3H), 1.43 (s, 9H), 0.96 (s, 9H); FAB MS *m*/*z* 633 (MH^+), 533 (MH^+ − 100). This compound (0.70 g, 1.0 mmol) was treated with 4 N HCl/dioxane (30 min) before being concentrated in vacuo. The hydrochloride salt (1.0 mmol) was combined with *tert*-butylacetic acid (191 μL, 1.50 mmol), TBTU (0.385 g, 1.20 mmol), and *i*-Pr_2NEt (0.52 mL, 3.0 mmol) in DMF (10 mL) for 16 h. The reaction mixture was diluted with EtOAc and washed sequentially with 5% aqueous $NaHCO_3$, 1 M citric acid, and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification was performed by preparative HPLC to give compound **79** (155 mg, 25%): IR (KBr) ν 3289, 1642 cm^{-1}; ^{1}H NMR (DMSO-d_6) 2:1 mixture of diastereomers at P_1, δ 8.35 (d, *J* = 8.9 Hz, 0.34H), 8.24 (d, *J* = 7.3 Hz, 0.66H), 8.20 (d, *J* = 9.2 Hz, 0.66H), 8.15 (d, *J* = 7.6 Hz, 0.34H), 7.63 (d, *J* = 8.6 Hz, 0.66H), 7.58 (d, *J* = 8.6 Hz, 0.34H), 7.33−7.40 (m, 4H), 7.14−7.23 (m, 6H), 4.57−4.72 (m, 2H), 4.18 (d, *J* = 8.6 Hz, 0.66H), 4.17 (d, *J* = 8.6 Hz, 0.34H), 3.62 (s, broad, 1H), 2.94 (s, 1H), 2.88 (s, 2H), 2.79 (s, 1H), 2.77 (s, 2H), 2.59−2.74 (m, 1H), 2.20 (d, *J* = 12.7 Hz, 0.66H), 2.17 (d, *J* = 12.7 Hz, 0.34H), 2.02 (d, *J* = 12.7 Hz, 0.66H), 1.98 (d, *J* = 12.7 Hz, 0.34H), 1.44 (d, *J* = 7.3 Hz, 1.5H), 1.39 (d, *J* = 7.3 Hz, 1.5H), 0.95 (s,

5.9H), 0.92 (s, 5.9H), 0.91(s, 3.1H), 0.88 (s, 3.1H); HRMS calcd for $C_{32}H_{48}N_4O_7P$ (MH^+) 631.3260, found 631.3279. Anal. ($C_{32}H_{47}N_4O_7P$) C, H, N.

***N*1-(3,3,3-Trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-2-methyl-1-[[(1*S*)-2-methyl-1-[[(methylcarboxamido)-methyl]carboxamido]propyl]carboxamido]propyl]-carboxamido]butanediamide (37).** Final purification was performed by preparative HPLC: IR (KBr) ν 3400−3000 (br), 1637, 1548 cm^{-1}; ^1H NMR (DMSO-d_6) 1:1 mixture of hydrate/non-hydrate, 1:1 mixture of diastereomers at P_1, δ 8.51 and 8.49 (d, J = 6.0 and 6.0 Hz, 0.5H), 8.08−8.02 (m, 2H), 7.83−7.76 (m, 2H), 7.47−7.30 (m, 1.5H), 6.95−6.88 (m, 2H), 4.61−4.52 (m, 1.5H), 4.26−4.06 (m, 2.5H), 3.79−3.67 (m, 2H), 2.67−2.32 (m, 2H), 1.99−1.93 (m, 2H), 1.85 (s, 3H), 1.26 and 1.25 (d, J = 4.1 and 3.8 Hz, 1.5H), 1.06 (d, 6.7 Hz, 1.5H), 0.84−0.79 (m, 12H); HRMS calcd for $C_{22}H_{36}F_3N_6O_7$ (MH^+) 553.2597, found 553.2617.

***N*1-(3,3,3-Trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-2-methyl-1-[[(1*S*)-2-methyl-1-(methylcarboxamido)propyl]-carboxamido]propyl]carboxamido]butanediamide (39).** Final purification was performed by preparative HPLC: IR (KBr) ν 3600−2800, 1636, 1546 cm^{-1}; ^1H NMR (DMSO-d_6) 3:1 mixture of hydrate/non-hydrate, 1:1 mixture of diastereomers at P_1, δ 8.53 (m, 0.25H), 8.09 and 8.06 (d, J = 7.9 and 7.4 Hz, 1H); 7.89 and 7.87 (d, J = 4.9 and 5.4 Hz, 1H), 7.72 and 7.70 (d, J = 4.5 and 4.0 Hz, 1H), 7.46 (d, J = 9.8 Hz, 0.4H), 7.37−7.30 (m, 1.5H), 6.96−6.89 (m, 2.6H), 4,54 (m, 1H), 4.21−4.06 (m, 3H), 2.48−2.34 (m, 2H), 2.00−1.91 (m, 2H), 1.871 and 1.868 (s, 3H), 1.27 and 1.25 (d, J = 4.5 and 3.9 Hz, 0.75H), 1.06 (d, 6.9 Hz, 2.25H), 0.84−0.80 (m, 12H); HRMS calcd for $C_{20}H_{33}F_3N_5O_6$ (MH^+) 496.2382, found 496.2387.

***N*1-(3,3,3-Trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-(methylcarboxamido)butanediamide (41).** Final purification was performed by preparative HPLC: IR (KBr) ν 3387, 1696, 1653 cm^{-1}; ^1H NMR (DMSO-d_6) 1:1 mixture of diastereoisomers at P_1, δ 8.65 and 8.59 (2 d, J = 6.1 and 5.7 Hz, 0.1H), 8.09 and 8.04 (2 d, J = 7.9 and 7.9 Hz, 1H), 7.53 and 7.36 (2 d, J = 9.2 and 9.2 Hz, 1H), 7.31 (br s, 1H), 6.96−6.87 (m, 3H), 4.61−4.47 (m, 1H), 4.05−4.15 (m, 1H), 2.49 and 2.31 (m, 2H), 1.25 (dd, J = 7.0 and 4.4 Hz, 0.3H), 1.07 (dd, J = 7.0 and 3.5 Hz, 2.7H); HRMS calcd for $C_{10}H_{15}F_3N_3O_4$ (MH^+) 298.1015, found 298.1026.

***N*1-(1-Ethyl-3,3,3-trifluoro-2-oxopropyl)-(2*S*)-2-[[(1*S*)-2-methyl-1-[[(1*S*)-2-methyl-1-(methylcarboxamido)propyl]-carboxamido]propyl] carboxamido]butanediamide (44).** Final purification was performed by preparative HPLC: IR (KBr) ν 1640 cm^{-1}; ^1H NMR (DMSO-d_6) 7:1 mixture of hydrate/non-hydrate, 1:1 mixture of diastereomers at P_1, δ 8.55−8.47 (m, 0.14H), 8.09 and 8.05 (2 d, J = 7.3 and 7.3 Hz, 1H), 7.90−7.87 (m, 1H), 7.71−7.68 (m, 1H), 7.40−7.24 (m, 2H), 6.92−6.77 (m, 3H), 4.59−4.52 (m, 1H), 4.23−4.15 (m, 2H), 3.96−3.87 (m, 1H), 2.67−2.32 (m, 2H), 2.01−1.90 (m, 2H), 1.87 and 1.86 (2 s, 3H), 1.79−1.71 (m, 1H), 1.40−1.28 (m, 1H), 0.89−0.74 (m, 15H); HRMS calcd for $C_{21}H_{35}F_3N_5O_6$ (MH^+) 510.2539, found 510.2558.

***N*1-[1-(2,2,2-Trifluoroacetyl)butyl]-(2*S*)-2-[[(1*S*)-2-methyl-1-[[(1*S*)-2-methyl-1-(methylcarboxamido)propyl]carboxamido]propyl]carboxamido]butanediamide (45).** This compound was prepared by the same procedure as for **3**, except 1-nitroethane was replaced by 1-nitrobutane. Purification was performed by preparative HPLC: IR (KBr) ν 3280, 1663, 1637, 1546 cm^{-1}; ^1H NMR (DMSO-d_6) 7:1 mixture of hydrate/non-hydrate, 1:1 mixture of diastereomers at P_1, δ 8.50 (m, 0.25H), 8.18 (d, J = 7.3 Hz, 0.12H), 8.07 (m, 1H), 8.02 (d, J = 7.95 Hz, 0.25H), 7.89 and 7.88 (2 d, J = 8.6 and 8.9 Hz, 1H), 7.77 (d, J = 8.3 Hz, 0.12H), 7.70 (d, J = 8.6 Hz, 1H), 7.46−7.24 (m, 2H), 6.97−6.72 (m, 2.7H), 4.63−4.51 (m, 1H), 4.30 and 4.28 (2 d, J = 6.7 and 6.7 Hz, 0.12H), 4.24−4.13 (m, 2H), 4.09−3.96 (m, 1H), 2.50−2.32 (m, 2H), 2.02−1.91 (m, 2H), 1.87 (s, 3H), 1.72−1.57 (m, 1H), 1.43−1.21 (m, 2H), 1.18−1.04 (m, 1H), 0.89−0.75 (m, 15H); HRMS calcd for $C_{22}H_{37}F_3N_5O_6$ (MH^+) 524.2696, found 524.2705.

***N*1-(3,3,3-Trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-2-methyl-1-[[(1*S*)-2-methyl-1-(methylcarboxamido)propyl]-carboxamido]propyl] carboxamido]pentanediamide (46).** This compound was prepared by the same procedure as for **3**, except 1-nitroethane was replaced by 1-nitropentane. Final purification was performed by preparative HPLC: IR (KBr) ν 3281, 3079, 1647 cm^{-1}; ^1H NMR (DMSO-d_6) 2:3 mixture of hydrate/non-hydrate, 1.2:1 mixture of diastereoisomers at P_1, δ 8.72 (d, J = 5.7 Hz, 0.16H), 8.70 (d, J = 5.7 Hz, 0.18H), 7.90−8.02 (m, 0.8H), 7.90 (d, J = 8.9 Hz, 1H), 7.68−7.74 (m, 1H), 7.56−7.61 (m, 0.3H), 7.10−7.21 (m, 1H), 6.90−7.00 (m, 1H), 6.70−6.75 (m, 1H), 4.60−4.69 (m, 0.4H), 4.07−4.29 (m, 3.7H), 2.00−2.09 (m, 2H), 1.90−1.98 (m, 2H), 1.86 (s, 3H), 1.83−1.61 (m, 2H), 1.28 (d, J = 7.0 Hz, 0.5H), 1.27 (d, J = 7.0 Hz, 0.6H), 1.08 (d, J = 6.5 Hz, 1.4H), 1.07 (d, J = 6.4 Hz, 1.2H), 0.81−0.84 (m, 12H); HRMS calcd for $C_{21}H_{35}F_3N_5O_6$ (MH^+) 510.2539, found 510.2521.

(3*S*)-3-[[(1*S*)-2-Methyl-1-[[(1*S*)-2-methyl-1-(methylcarboxamido)propyl]carboxamido]propyl]carboxamido]-3-[(3,3,3-trifluoro-1-methyl-2-oxopropyl)carbamoyl]propanoic Acid (47). This compound was prepared on solid phase using the trifluoromethyl ketone resin (Scheme 3). Final purification was performed by preparative HPLC: IR (KBr) ν 3500−2800 (br), 1639, 1546 cm^{-1}; ^1H NMR (DMSO-d_6) hydrated form only, 1:1 mixture of diastereomers at P_1, δ 12.29 (br s, 1H), 8.22 and 8.14 (d, J = 7.8 and 7.9 Hz, 1H), 7.89 (m, 1H), 7.70 (m, 1H), 7.40 and 7.35 (d, J = 8.8 and 9.4 Hz, 1H), 6.93 (t, J = 7.8 Hz, 2H), 4.53 (m, 1H), 4.17 (m, 2H), 4.11 (q, J = 6.9 Hz, 1H), 2.67−2.58 (m, 1H), 2.47 (m, 1H), 1.94 (m, 2H), 1.87 (s, 3H), 1.06 (m, 3H), 0.83 (m, 12H); HRMS calcd for $C_{20}H_{32}F_3N_4O_7$ (MH^+) 497.2223, found 497.2237.

***N*1-[(1*S*)-1-[(1*S*)-2-Hydroxy-1-[(3,3,3-trifluoro-1-methyl-2-oxopropyl)carbamoyl]ethylcarbamoyl]-2-methylpropyl]-(2*S*)-3-methyl-2-(methylcarboxamido)butanamide (48).** This compound was prepared on solid phase using the trifluoromethyl ketone resin (Scheme 3). Final purification was performed by preparative HPLC: IR (KBr) ν 3500−2800, 1637, 1543 cm^{-1}; ^1H NMR (DMSO-d_6) hydrated form only, 1:1 mixture of diastereomers at P_1, δ 7.94 (d, J = 7.9 Hz, 1H), 7.90 (d, J = 8.9 Hz, 1H), 7.73 and 7.71 (d, J = 5.4 and 4.9 Hz, 1H), 7.55 and 7.50 (8.9 and 9.4 Hz, 1H), 6.93 (br m, 2H), 4.31−4.10 (m, 4H), 3.57−3.47 (m, 3H), 1.97 (m, 2H), 1.87 (s, 3H), 1.09 and 1.08 (d, J = 6.9 and 6.9 Hz, 3H), 0.86−0.81 (m, 12H); HRMS calcd for $C_{19}H_{32}F_3N_4O_6$ (MH^+) 469.2274, found 469.2261.

***N*1-(3,3,3-Trifluoro-1-methyl-2-oxopropyl)-(2*S*)-6-amino-2-[[(1*S*)-2-methyl-1-[[(1*S*)-2-methyl-1-(methylcarboxamido)propyl]carboxamido]propyl]carboxamido]-hexanamide (49).** This compound was prepared on solid phase using the trifluoromethyl ketone resin (Scheme 3). Final purification was performed by preparative HPLC: IR (KBr) ν 3227, 1638, 1545, 1189 cm^{-1}; ^1H NMR (DMSO-d_6) hydrated form only, 1:1 mixture of diastereomers at P_1, δ 7.99 (2 d, J = 7.9 and 7.4 Hz, 1H), 7.88 (d, J = 8.8 Hz, 1H), 7.71 and 7.70 (2 d, J = 8.7 and 8.4 Hz, 1H), 7.58 (t, J = 8.9 Hz, 1H), 6.99 (m, 2H), 4.29−4.09 (m, 4H), 2.73 (m, 2H), 2.00−1.92 (m, 2H), 1.93 (s, 3H), 1.63−1.46 (m, 4H), 1.28−1.25 (m, 2H), 1.09 and 1.07 (2 d, J = 6.9 and 6.9 Hz, 3H), 0.85−0.82 (m, 12H); HRMS calcd for $C_{22}H_{39}F_3N_5O_5$ (MH^+) 510.2903, found 510.2888.

***N*1-[(1*S*)-2-Methyl-1-[[(1*S*)-2-(1,3-thiazol-4-yl)-1-[(3,3,3-trifluoro-1-methyl-2-oxopropyl)carbamoyl]ethyl]carbamoyl]propyl]-(2*S*)-3-methyl-2-(methylcarboxamido)butanamide (50).** This compound was prepared on solid phase using the trifluoromethyl ketone resin (Scheme 3). Final purification was performed by preparative HPLC: IR (KBr) ν 3276, 3084, 1638 cm^{-1}; ^1H NMR (DMSO-d_6) 1:1.2 hydrate/non-hydrate, 1.2:1 mixture of diastereomers at P_1, δ 8.99 (d, J = 5.4 Hz, 0.55H), 8.98 (d, J = 5.4 Hz, 0.45H), 8.77 (d, J = 5.7 Hz, 0.19H), 8.71 (d, J = 5.7 Hz, 0.23H), 8.09−8.17 (m, 1H), 7.84−7.88 (m, 1H), 7.67−7.90 (m, 1.43H), 7.55 (d, J = 8.9 Hz, 0.25H), 7.29−7.34 (m, 1H), 6.94 (br s, 0.75H), 4.58−4.73 (m, 1.5H), 4.06−4.18 (m, 2.5H), 2.97−3.17 (m, 2H), 1.86−1.93 (m, 2H), 1.86 (s, 1.65H), 1.85 (s, 1.35H), 1.22 (d, J = 6.7 Hz, 0.61H), 1.21 (d, J = 6.7 Hz, 0.74H), 1.07 (d, J = 7.0 Hz, 0.93H), 0.98 (d, J = 6.7 Hz, 0.72H), 0.76−0.80 (m, 12 H); HRMS calcd for $C_{22}H_{33}F_3N_5O_5S$ (MH^+) 536.2154, found 536.2170.

***N*4,*N*4-Dimethyl-*N*1-(3,3,3-trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-2-methyl-1-[[(1*S*)-2-methyl-1-(methylcarboxamido)propyl]carboxamido]propyl]carboxamido]-butanediamide (51).** This compound was prepared on solid

phase using the trifluoromethyl ketone resin (Scheme 3). Final purification was performed by preparative HPLC: IR (KBr) 1638 cm^{-1}; ^1H NMR (DMSO-d_6) 1:1 mixture of diastereomers at P_1, δ 8.56−8.49 (m, 0.1H), 8.10−8.03 (m, 0.8H), 7.89−7.86 (m, 0.8H), 7.73−7.70 (m, 0.8H), 7.44−7.41 (m, 1H), 6.95−6.80 (m, 1.5H), 4.63−4.54 (m, 1H), 4.20−4.06 (m, 3H), 2.94−2.93 (m, 3H), 2.80−2.78 (m, 3H), 2.67−2.62 (m, 2H), 1.99−1.93 (m, 2H), 1.87−1.86 (m, 3H), 1.30−1.25 (m, 0.5H), 1.07−1.06 (m, 2.5H), 0.84−0.83 (m, 12H); HRMS calcd for $C_{22}H_{37}F_3N_5O_6$ (MH^+) 524.2696, found 524.2710.

***N*1-(3,3,3-Trifluoro-1-methyl-2-oxopropyl)-(2*S*)-4-methyl-2-[[(1*S*)-2-methyl-1-[[(1*S*)-2-methyl-1-(methylcarboxamido)propyl]carboxamido]propyl]carboxamido]pentanamide (52).** This compound was prepared on solid phase using the trifluoromethyl ketone resin (Scheme 3). Final purification was performed by preparative HPLC: IR (KBr) ν 3268, 3080, 1632 cm^{-1}; ^1H NMR (DMSO-d_6) 1:1.2 hydrate/non-hydrate, 1.2:1 mixture of diastereomers at P_1, δ 8.72 (d, J = 6.5 Hz, 0.25H), 8.70 (d, J = 6.5 Hz, 0.3H), 7.91−8.10 (m, 1H), 7.84−7.88 (m, 1H), 7.74−7.80 (m, 1H), 7.55 (d, J = 8.6 Hz, 0.2H), 7.53 (d, J = 8.9 Hz, 0.25H), 6.90−6.96 (m, 1H), 4.57−4.68 (m, 0.5H), 4.26−4.39 (m, 1H), 4.15−4.21 (m, 1H), 4.05−4.14 (m, 1.4H), 1.87−1.95 (m, 2H), 1.85 (s, 3H), 1.53−1.63 (m, 1H), 1.30−1.50 (m, 2H), 1.26 (d, J = 7.0 Hz, 0.8H), 1.25 (d, J = 7.0 Hz, 1H), 1.07 (d, J = 6.7 Hz, 0.8H), 1.06 (d, J = 6.7 Hz, 0.6H), 0.78−0.88 (m, 18H); HRMS calcd for $C_{22}H_{38}F_3N_4O_5$ (MH^+) 495.2794, found 495.2803.

***N*1-[(1*S*)-2-Methyl-1-[[(1*S*)-2-phenyl-1-[(3,3,3-trifluoro-1-methyl-2-oxopropyl)carbamoyl]ethyl]carbamoyl]-propyl]-(2*S*)-3-methyl-2-(methylcarboxamido)butanamide (53).** This compound was prepared by solid phase using the trifluoromethyl ketone resin (Scheme 3). Final purification was performed by preparative HPLC: IR (KBr) ν 3280, 1636, 1546 cm^{-1}; ^1H NMR (DMSO-d_6) 3:1 hydrate/non-hydrate, 1:1 mixture of diastereomers at P_1, δ 8.79 (d, J = 5.7 Hz, 0.12H), 8.71 (d, J = 6.4 Hz, 0.12H), 8.03 (m, 1H), 7.86 (m, 1H), 7.64 (m, 1.76H), 7.22 (m, 5H), 6.93 and 6.90 (2 d, J = 14.3 and 13.7 Hz, 1.6H), 4.63−4.54 (m, 1.25H), 4.16−4.05 (m, 2.75H), 3.00−2.66 (m, 2H), 1.86 (s, 3H), 1.85 (m, 2H), 1.25, 1.21, 1.09 and 0.96 (4 d, J = 7.4, 6.8, 6.9 and 7.9 Hz, 3H), 0.79 (m, 12H); HRMS calcd for $C_{25}H_{36}F_3N_4O_5$ (MH^+) 529.2638, found 529.2619.

***N*1-[(1*S*)-2-Methyl-1-[[(1*S*)-2-methyl-1-[(3,3,3-trifluoro-1-methyl-2-oxopropyl)carbamoyl]propyl]carbamoyl]-propyl]-(2*S*)-3-methyl-2-(methylcarboxamido)butanamide (54).** Final purification was performed by preparative HPLC: IR (KBr) 1633 cm^{-1}; ^1H NMR (DMSO-d_6) 1:1 mixture of diastereoisomers at P_1, δ 8.78 (d, J = 5.5 Hz, 0.25H), 8.71 (d, J = 5.5 Hz, 0.25H), 7.88−7.60 (m, 3.5H), 6.98−6.87 (m, 1H), 4.70−4.64 (m, 0.5H), 4.22−4.09 (m, 3.5H), 1.97−1.91 (m, 3H), 1.86 (s, 3H), 1.28−1.26 (m, 1.7H), 1.09−1.07 (m, 1.3H), 0.88−0.78 (m, 18H); FAB MS *m*/*z* 481 (MH^+); HRMS calcd for $C_{21}H_{36}F_3N_4O_5$ (MH^+) 481.2638, found 481.2627.

***N*1-[(1*S*)-2-Methyl-1-[[(1*S*)-1-[(3,3,3-trifluoro-1-methyl-2-oxopropyl)carbamoyl]ethyl]carbamoyl]propyl]-(2*S*)-3-methyl-2-(methylcarboxamido)butanamide (55).** Final purification was performed by preparative HPLC: IR (KBr) ν 3264, 1627, 1552 cm^{-1}; ^1H NMR (DMSO-d_6) 1:1 mixture of diastereomers at P_1, δ 7.98 and 7.92 (2 d, J = 7.3 and 7.3 Hz, 1H), 7.88−7.83 (m, 1H), 7.72 (d, J = 8.8 Hz, 1H), 7.58 (t, J = 9.4 Hz, 1H), 6.95 and 6.91 (2 br s, 2H), 4.33−4.06 (m, 4H), 1.95 (m, 2H), 1.87 (s, 3H), 1.17−1.13 (m, 3H), 1.08 (d, J = 6.9 Hz, 3H), 0.85−0.81 (m, 12H); HRMS calcd for $C_{19}H_{32}F_3N_4O_5$ (MH^+) 453.2325, found 453.2338.

***N*1-[(1*S*)-2-Methyl-1-[[(1*R*)-1-[(3,3,3-trifluoro-1-methyl-2-oxopropyl)carbamoyl]ethyl]carbamoyl]propyl]-(2*S*)-3-methyl-2-(methylcarboxamido)butanamide (56).** Final purification was performed by preparative HPLC: IR (KBr) 1634 cm^{-1}; ^1H NMR (DMSO-d_6) 1:1 mixture of diastereomers at P_1, δ 8.63 (d, J = 5.5 Hz, 0.1H), 8.56 (d, J = 4.5 Hz, 0.1H), 8.18−8.12 (m, 0.2H), 8.01 (d, J = 7 Hz, 0.3H), 7.96 (d, J = 7.5 Hz, 0.3H), 7.89−7.74 (m, 1.8H), 7.65 (d, J = 9 Hz, 0.3H), 7.60 (d, J = 9.5 Hz, 0.3H), 6.96−6.92 (m, 1.3H), 4.68−4.59 (m, 0.3H), 4.34−4.25 (m, 1H), 4.19−4.02 (m, 2.7H), 1.98−1.89 (m, 2H), 1.86 (s, 3H), 1.31−1.29 (m, 0.8H), 1.19−1.07 (m, 5.2H), 0.86−0.82 (m, 12H); HRMS calcd for $C_{19}H_{32}F_3N_4O_5$ (MH^+) 453.2325, found 453.2338.

***N*4,*N*4-Dimethyl-*N*1-(3,3,3-trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-1-[[(1*S*)-2-methyl-1-(methylcarboxamido)propyl]carboxamido]propyl]carboxamido]butanediamide (57).** Final purification was performed by preparative HPLC: IR (KBr) ν 3283, 1642 cm^{-1}; ^1H NMR (DMSO-d_6) 2:1 mixture of hydrate/non-hydrate, 1:1 mixture of diastereomers at P_1, δ 8.05 and 8.01 (2 d, J = 7.6 and 7.6 Hz, 1H), 7.91−7.82 (m, 2H), 7.43 and 7.39 (2 d, J = 9.2 and 9.2 Hz, 1H), 7.01−6.80 (m, 2H), 4.61−4.52 (m, 1H), 4.22−4.00 (m, 3H), 2.94 and 2.93 (2 s, 3H), 2.80 (s, 3H), 2.71−2.59 (m, 2H), 2.00−1.90 (m, 1H), 1.87 and 1.86 (2 s, 3H), 1.73−1.61 (m, 1H), 1.57−1.45 (m, 1H), 1.06 (d, J = 6.0 Hz, 3H), 0.87−0.78 (m, 9H); HRMS calcd for $C_{21}H_{35}F_3N_5O_6$ (MH^+) 510.2539, found 510.2526.

***N*4,*N*4-Dimethyl-*N*1-(3,3,3-trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-[[(1*S*)-2-methyl-1-(methylcarboxamido)propyl]carboxamido]propyl]-carboxamido]butanediamide (58).** Final purification was performed by preparative HPLC: IR (KBr) ν 3500−2900, 1640, 1538 cm^{-1}; ^1H NMR (DMSO-d_6) hydrated form only, 1:1 mixture of diastereomers at P_1, δ 8.20 and 8.13 (d, J = 7.4 and 7.2 Hz, 1H), 7.91 and 7.91 (d, J = 9.0 and 8.7 Hz, 1H), 7.56 and 7.55 (d, J = 9.3 and 9.3 Hz, 1H), 7.50 and 7.43 (d, J = 9.3 and 9.3 Hz, 1H), 6.93 (br s, 1H), 6.81 (br s, 1H), 4.57 (m, 1H), 4.23 (m, 2H), 4.11 (m, 1H), 2.95 and 2.94 (s, 3H), 2.80 and 2.80 (s, 3H), 2.72−2.57 (m, 2 H), 1.95 (m, J = 6.9 Hz, 1H), 1.87 (s, 3H), 1.07 and 1.06 (d, J = 8.1 and 6.6 Hz, 3H), 0.89 (s, 9H), 0.83 (d, J = 6.8 Hz, 6H); HRMS calcd for $C_{23}H_{39}F_3N_5O_6$ (MH^+) 538.2852, found 538.2843.

***N*4,*N*4-Dimethyl-*N*1-(3,3,3-trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-3,3-dimethyl-1-[[(1*S*)-2-methyl-1-(methylcarboxamido)propyl]carboxamido]butyl]carboxamido]-butanediamide (59).** Final purification was performed by preparative HPLC: IR (KBr) ν 3285, 1644 cm^{-1}; ^1H NMR (DMSO-d_6) 16:1 mixture of hydrate/non-hydrate, 3:2 mixture of diastereomers at P_1, δ 8.05 (d, J = 8.3 Hz, 1H), 7.91−7.79 (m, 2H), 7.43 and 7.39 (2 d, J = 9.2 and 9.2, 1H), 7.11−6.66 (br s, 2H hydrate), 4.62−4.49 (m, 1H), 4.36−4.25 (m, 1H), 4.16−4.02 (m, 2H), 2.94 and 2.93 (2 s, 3H), 2.80 (s, 3H), 2.71−2.55 (m, 2H), 1.99−1.88 (m, 1H), 1.85 and 1.84 (2 s, 3H), 1.67−1.58 (m, 1H), 1.46 (dd, J = 14.2 and 8.9 Hz, 1H), 1.28−1.25 (m, 0.2H), 1.06 (d, J = 6.7 Hz, 2.8H), 0.86 (s, 9H), 0.83 (d, J = 6.7 Hz, 3H), 0.81 (d, J = 6.7 Hz, 3H); HRMS calcd for $C_{24}H_{41}F_3N_5O_6$ (MH^+) 552.3009, found 552.3031.

***N*4,*N*4-Dimethyl-*N*1-(3,3,3-trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-[[(*S*)-1-(1-adamantyl)-1-[[(1*S*)-2-methyl-1-(methylcarboxamido)propyl]carboxamido]methyl]-carboxamido]butanediamide (60).** Final purification was performed by preparative HPLC: IR (KBr) ν 3293, 1641, 1533 cm^{-1}; ^1H NMR (DMSO-d_6) 4:1 mixture of hydrate/non-hydrate, 1:1 mixture of diastereomers at P_1, δ 8.58 (d, J = 5.5 Hz, 0.1H), 8.47 (d, J = 6.5 Hz, 0.1H), 8.22−8.12 (m, 1H), 7.94−7.91 (m, 1H), 7.49−7.38 (m, 1.9H), 6.94 (br s, 1H), 6.86 (br s, 1H), 4.60−4.52 (m, 1H), 4.24−4.20 (m, 1H), 4.13−3.98 (m, 2H), 2.94 (s, 1.5H), 2.93 (s, 1.5H), 2.79 (s, 3H), 2.74−2.57 (m, 2H), 2.00−1.94 (m, 1H), 1.94−1.86 (m, 3H), 1.87 (s, 3H), 1.64−1.48 (m, 12H), 1.26−1.25 (m, 0.6H), 1.07 (d, J = 6.5 Hz, 2.4H), 0.82 (d, J = 6.5 Hz, 6H); HRMS calcd for $C_{29}H_{45}F_3N_5O_6$ (MH^+) 616.3322, found 616.3335.

(3*S*)-3-[[(1*S*)-2-(Dimethylcarbamoyl)-1-[(3,3,3-trifluoro-1-methyl-2-oxopropyl)carbamoyl]ethyl]carbamoyl]-2,2-dimethyl-3-[[(1*S*)-2-methyl-1-(methylcarboxamido)propyl]-carboxamido]propanoic Acid (61). This compound was prepared in solution using standard coupling methods. The β,β-dimethylaspartic acid residue was incorporated as the γ-benzyl ester derivative. Oxidation of the trifluoromethyl alcohol was accomplished with the Dess−Martin periodinane. Final purification was performed by preparative HPLC: IR (KBr) ν 1654, 1532 cm^{-1}; ^1H NMR (DMSO-d_6) 1:1 mixture of diastereomers at P_1, δ 7.98−7.89 (m, 2 H), 7.76 (d, J = 7.5 Hz, 0.5H), 7.67 (d, J = 7.5 Hz, 0.5H), 7.53 (d, J = 9 Hz, 0.5H), 7.48 (d, J = 9 Hz, 0.5H), 6.92 (br s, 1H), 6.83 (br s, 1H), 4.75 (d, J = 9.5 Hz, 1H), 4.57−4.51 (m, 1H), 4.27−4.19 (m, 1H), 4.18−4.03 (m, 1H), 2.94 (s, 1.5H), 2.93 (s, 1.5H), 2.80 (s, 3H), 2.71−2.57 (m, 2H), 2.04−1.95 (m, 1H), 1.87 (s, 3H), 1.08−1.05

(m, 9H), 0.85 (d, J = 6.5 Hz, 3H), 0.83 (d, J = 6.5 Hz, 3H); HRMS calcd for $C_{23}H_{37}F_3N_5O_8$ (MH^+) 568.2594, found 568.2505.

***N*4,*N*4-Dimethyl-*N*1-(3,3,3-trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(methylcarboxamido)-propyl]carboxamido]butanediamide (62).** Final purification was performed by preparative HPLC: IR (KBr) ν 1640 cm^{-1}; ^1H NMR (DMSO-d_6) 1:1 mixture of diastereomers at P_1, δ 8.06 and 7.94 (2 d, J = 7.6 and 7.3 Hz, 1H), 7.69 (d, J = 8.9 Hz, 1H), 7.22 and 7.21 (2 d, J = 8.9 and 9.2 Hz, 1H), 4.42−4.34 (m, 1H), 4.01 and 3.96 (2 d, J = 8.9 and 8.9 Hz, 1H), 3.95−3.89 (m, 1H), 2.77 (d, J = 3.2 Hz, 3H), 2.62 (s, 3H), 2.59−2.42 (m, 2H), 1.72 (d, J = 3.8 Hz, 3H), 1.09 and 0.89 (2 d, J = 7.0 and 6.7 Hz, 3H), 0.73 and 0.72 (2 s, 9H); HRMS calcd for $C_{18}H_{30}F_3N_4O_5$ (MH^+) 439.2168, found 439.2154.

***N*4,*N*4-Dimethyl-*N*1-(3,3,3-trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-1-[(4-hydroxyphenethyl)carboxamido]-2,2-dimethylpropylcarboxamido]butanediamide (63).** Final purification was performed on a preparative HPLC: IR (KBr) ν 1636 cm^{-1}; ^1H NMR (DMSO-d_6) 16:1 mixture of hydrate/non-hydrate, 1.4:1 mixture of diastereomers at P_1, δ 9.20−9.00 (m, 1H), 8.23 and 8.13 (2 d, J = 7.4 and 7.4 Hz, 1H), 7.79 (2 d, J = 9.0 and 9.0 Hz, 1H), 7.41 (2 d, J = 9.0 and 9.0 Hz, 1H), 6.99 (d, J = 8.6 Hz, 2H), 6.99−6.75 (m, 1.5H hydrate), 6.65−6.62 (m, 2H), 4.62−4.52 (m, 1H), 4.20 and 4.17 (2 d, J = 9.0 and 9.0 Hz, 1H), 4.15−4.05 (m, 1H), 2.96 and 2.95 (2 s, 3H), 2.80 (s, 3H), 2.72−2.60 (m, 4H), 2.50−2.45 (m, 1H), 2.45−2.35 (m, 1H), 1.27 (d, J = 7.0 Hz, 0.1H), 1.75 (m, 2.8H), 0.86 (s, 9H); HRMS calcd for $C_{25}H_{36}F_3N_4O_6$ (MH^+) 545.2587, found 545.2602.

***N*4,*N*4-Dimethyl-*N*1-(3,3,3-trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-1-(isobutylcarboxamido)-2,2-dimethylpropyl]carboxamido]butanediamide (64).** Final purification was performed by preparative HPLC: IR (KBr) ν 1636 cm^{-1}; ^1H NMR (DMSO-d_6) 19:1 mixture hydrate/non-hydrate, 1:1 mixture of diastereomers at P_1, δ 8.21 and 8.11 (2 d, J = 7.2 and 7.2 Hz, 1H), 7.73 and 7.72 (2 d, J = 9.0 and 9.0 Hz, 1H), 7.44 and 7.40 (2 d, J = 9.6 and 9.3 Hz, 1H), 7.1−6.7 (br, 1.7H hydrate), 4.60−4.52 (m, 1H), 4.21 and 4.19 (2 d, J = 9.0 and 8.7 Hz, 1H), 4.15−4.04 (m, 1H), 2.96 and 2.95 (2 s, 3H), 2.80 (s, 3H), 2.74−2.58 (m, 2H), 2.14 and 2.12 (2 d, J = 13.2 and 12.9 Hz, 1H), 2.08 (m, 1H), 1.96 (m, 1H), 1.07−1.04 (m, 3H) 0.90 (s, 9H), 0.87 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H); HRMS calcd for $C_{21}H_{36}F_3N_4O_5$ (MH^+) 481.2638, found 481.2627.

***N*4,*N*4-Dimethyl-*N*1-(3,3,3-trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)-propyl]carboxamido]butanediamide (65).** Final purification was performed by preparative HPLC: IR (KBr) ν 1635 cm^{-1}; ^1H NMR (DMSO-d_6) 29:1 mixture of hydrate/non-hydrate, 1:1 mixture of diastereomers at P_1, δ 8.20 and 8.10 (2 d, J = 7.3 and 7.0 Hz, 1H), 7.62 (d, J = 8.9 Hz, 1H), 7.44 and 7.43 (2 d, J = 9.2 and 9.2 Hz, 1H), 6.95−6.75 (m, 1.7H), 4.56 (quintet, J = 6.7 Hz, 1H), 4.19 and 4.16 (2 d, J = 8.9 and 8.9 Hz, 1H), 4.12−4.05 (m, 1H), 2.95 and 2.94 (2 s, 3H), 2.79 (s, 3H), 2.75−2.60 (m, 2H), 2.20−2.15 (m, 1H), 2.04 and 2.01 (2 d, J = 12.7 and 12.4 Hz, 1H), 1.07−1.04 (m, 3H), 0.95 (s, 9H), 0.90 (s, 9H); HRMS calcd for $C_{22}H_{38}F_3N_4O_5$ (MH^+) 495.2794, found 495.2777.

***N*4,*N*4-Dimethyl-*N*1-(3,3,3-trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-1-[(3,3-dimethylbutyl)amino]-2,2-dimethylpropyl]carboxamido]butanediamide (66).** This compound was prepared by solid phase using the trifluoromethyl ketone resin (Scheme 3). The final reductive amination on the terminal *tert*-butylglycine amine (0.3 mmol) was performed on solid phase by addition of 3,3-dimethylbutyraldehyde (376 μL, 3.0 mmol) in DMF (15 mL with acetic acid (150 μL) and $NaBH_3CN$ (63 mg, 1 mmol) for 20 h. After removal of the solvent, the resin was cleaved as described in method C. After purification by preparative HPLC the compound was obtained as a white solid (20.6 mg) after lyophilization: IR (KBr) ν 2960, 1667 cm^{-1}; ^1H NMR (DMSO-d_6) 1:1 mixture of diastereomers at P_1, δ 8.79−8.72 (m, 2H), 8.04 (br s, 1H), 7.78 (d, J = 9.0 Hz, 0.5H), 7.67 (d, J = 9.0 Hz, 0.5H), 6.97−6.96 (m, 1.3H), 6.91 (s, 0.6H), 4.82−4.75 (m, 1H), 4.15−4.11 (m, 1H), 3.61 (d, J = 9.5 Hz, 1H), 2.94 (m, 3H), 2.87 (m, 1H), 2.79 (m, 3H), 2.70−2.65 (m, 3H), 1.66−1.57 (m, 1H), 1.51−1.44 (m, 1H), 1.29−1.24 (m, 1H), 1.08 (d, J = 7.0 Hz, 3H), 1.02 (s, 5.4H), 0.99 (s, 3.6H), 0.89 (s, 3.6H), 0.89 (s, 5.4H); HRMS calcd for $C_{22}H_{40}F_3N_4O_4$ (MH^+) 481.3002, found 481.2991.

***N*4,*N*4-Dimethyl-*N*1-(3,3,3-trifluoro-1-methyl-2-oxopropyl)-2-[[1-[(*tert*-butoxycarbonyl)amino]-2,2-dimethyl-(1*S*)-propyl]carboxamido]-(2*S*)-butanediamide (67).** The final purification was performed by preparative HPLC: IR (KBr) ν 3500−2900, 1641, 1510 cm^{-1}; ^1H NMR (DMSO-d_6) 5:1 mixture of hydrate/non-hydrate, 1:1 mixture of diastereomers at P_1, δ 8.56 (br d, J = 5.1 Hz, 0.15H), 8.10−8.00 (m, 1H), 7.50−7.46 (m, 1H), 6.93−6.82 (m, 1.7H), 6.50−6.49 (m, 1H), 4.62 (m, 1H), 4.12 (m, 1H), 3.84 (m, 1H), 2.95 (m, 3H), 2.80 (s, 3H), 2.65 (m, 2H), 1.38 (s, 9H), 1.26 (d, J = 6.6 Hz, 0.45H), 1.06 (d, J = 6.6 Hz, 2.55H), 0.88 (s, 9H); HRMS calcd for $C_{21}H_{36}F_3N_4O_6$ (MH^+) 497.2587, found 497.2601.

***N*4,*N*4-Dimethyl-*N*1-(3,3,3-trifluoro-1-methyl-2-oxopropyl)-2-[[1-[(*tert*-butylaminocarbonyl)amino]-2,2-dimethyl-(1*S*)-propyl]carboxamido]-(2*S*)-butanediamide (68).** Final purification was performed by preparative HPLC: IR (KBr) ν 1641 cm^{-1}; ^1H NMR (DMSO-d_6) 1:1 mixture of diastereomers at P_1, δ 8.15 and 8.09 (2 d, J = 7.3 and 7.0 Hz, 1H), 7.51 and 7.43 (2 d, J = 8.9 and 9.2 Hz, 1H), 6.00 (s, 1H), 5.97−5.93 (m, 1H), 4.58−4.53 (m, 1H), 4.11−4.03 (m, 1H), 3.94−3.91 (m, 1H), 2.95 (d, J = 5 Hz, 3H), 2.79 (s, 3H), 2.67−2.60 (m, 2H), 1.20 (s, 9H), 1.07−1.05 (m, 3H), 0.86 (s, 9H); HRMS calcd for $C_{21}H_{37}F_3N_5O_5$ (MH^+) 496.2747, found 496.2765.

***N*4,*N*4-Dimethyl-*N*1-(3,3,3-trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-1-[[(dimethylamino)methyl]carboxamido]-2,2-dimethylpropyl]carboxamido]butanediamide (69).** Final purification was performed by preparative HPLC: IR (KBr) ν 1654, 1540, 1186 cm^{-1}; ^1H NMR (DMSO-d_6) 9:1 mixture of hydrate/non-hydrate, 1:1 mixture of diastereomers at P_1, δ 9.61 (br s, 1H), 8.68 and 8.66 (2 d, J = 9.0 and 8.5 Hz, 1 H), 8.37 and 8.30 (2 d, J = 7.5 and 7.0 Hz, 1H), 7.48 and 7.45 (2 d, J = 9.0 and 9.0 Hz, 1H), 6.95−6.87 (m, 2H), 4.63−4.54 (m, 1H), 4.35−4.32 (m, 1H), 4.12−3.94 (m, 3H), 2.95−2.94 (m, 3H), 2.80−2.78 (m, 9H), 2.72−2.56 (m, 2H), 1.26−1.24 (m, 0.3H), 1.07−1.06 (m, 2.7H), 0.92 (s, 9H); HRMS calcd for $C_{20}H_{35}F_3N_5O_5$ (MH^+) 482.2590, found 482.2599.

4-[[(1*S*)-1-[[(1*S*)-2-(Dimethylcarbamoyl)-1-[(3,3,3-trifluoro-1-methyl-2-oxopropyl)carbamoyl]ethyl]carbamoyl]-2,2-dimethylpropyl]carbamoyl]butanoic Acid (70). This compound was prepared using method B. The final residue was introduced by treatment with glutaric anhydride in Et_3N. Final purification was performed by preparative HPLC: IR (KBr) ν 1638, 1537, 1176 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) hydrate form only, 1:1 mixture of diastereomers at P_1, δ 8.22 and 8.12 (2 d, J = 7.5 and 7.0 Hz, 1H), 7.81−7.72 (m, 1H), 7.43 and 7.39 (2 d, J = 9.0 and 9.0 Hz, 1H), 6.93 (br s, 0.8H), 6.81 (br s, 0.8H), 4.60−4.52 (m, 1H), 4.21−4.18 (m, 1H), 4.12−4.05 (m, 1H), 2.95−2.94 (m, 3H), 2.79 (s, 3H), 2.68−2.63 (m, 2H), 2.28−2.14 (m, 4H), 1.73−1.66 (m, 2H), 1.11−1.05 (m, 3H), 0.89 (s, 9H); HRMS calcd for $C_{21}H_{34}F_3N_4O_7$ (MH^+) 511.2379, found 511.2363.

***N*4,*N*4-Dimethyl-*N*1-(3,3,3-trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-1-amino-2,2-dimethylpropyl]carboxamido]butanediamide (71).** Final purification was performed by preparative HPLC: IR (KBr) ν 1670 cm^{-1}; ^1H NMR (DMSO-d_6) 2:1 hydrate/non-hydrate, 1:1 mixture of diastereomers at P_1, δ 8.87 and 8.80 (2 d, J = 5.7 and 6.4 Hz, 0.4H), 8.56−8.49 (m, 1H), 8.02 (br s, 3H), 7.69 and 7.64 (2 d, J = 9.0 and 9.2 Hz, 0.6H), 6.96, 6.95, 6.88 and 6.83 (4 s, 1.3H), 4.73−4.63 (m, 1.4H), 4.14−4.08 (m, 0.6H), 3.55−3.48 (m, 1H), 2.96 (s, 3H), 2.82 (s, 3H), 2.74−2.58 (m, 2H), 1.25 (d, J = 6.7 Hz, 1H), 1.08 and 1.07 (2 d, J = 6.7 and 6.7 Hz, 2H), 1.01−0.96 (m, 9H); HRMS calcd for $C_{16}H_{28}F_3N_4O_4$ (MH^+) 397.2063, found 397.2077.

***N*4,*N*4-Dimethyl-*N*1-(3,3,3-trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-[[(1*S*)-1-hydroxy-2,2-dimethylpropyl]carboxamido]butanediamide (72).** This compound was prepared using method B. The 2-hydroxyisobutyric acid moiety was introduced as the *O*-acetyl derivative. Oxidation of the trifluoromethyl alcohol with the Dess−Martin periodinane was followed by cleavage of the acetate group with aqueous NaOH. Final purification was performed by preparative HPLC: IR (KBr) ν 1641 cm^{-1}; ^1H NMR (DMSO-d_6) 2:1 hydrate:non-hydrate, 1:1 mixture of diastereomers at P_1, δ 7.83 and 7.79

(2 d, J = 7.9 and 7.6 Hz, 1H), 7.73−7.67 (m, 0.3H), 7.57 and 7.52 (2 d, J = 9.2 and 9.2 Hz, 1H), 7.04−6.82 (m, 2H, hydrate), 5.69−5.47 (m, 1H), 4.65−4.55 (m, 1H), 4.19−4.05 (m, 1H), 2.95 and 2.94 (2 s, 3H) 2.80 and 2.79 (2 s, 3H), 2.79−2.53 (m, 3H), 1.39−1.02 (m, 3H), 0.87 (s, 9H); HRMS calcd for $C_{16}H_{27}F_3N_3O_5$ (MH^+) 398.1903, found 398.1892.

***N*4,*N*4-Dimethyl-*N*1-(3,3,3-trifluoro-1-methyl-2-oxopropyl)-(2*S*)-2-(neopentylcarboxamido)butanediamide (73).** Final purification was performed by preparative HPLC: IR (KBr) ν 1638 cm^{-1}; ^1H NMR (DMSO-d_6) 2:1 mixture of diastereomers at P_1, δ 7.80−7.77 (m, 1H), 7.27 and 7.22 (2 d, J = 9.2 and 9.2 Hz, 1H), 4.49−4.42 (m, 1H), 3.98−3.92 (m, 1H), 2.80 (s, 3H), 2.64 (s, 3H), 2.55−2.40 (m, 2H), 1.84 (s, 2H), 1.10 and 0.92 (2 d, J = 6.9 and 6.6 Hz, 3H), 0.79 (s, 9H); HRMS calcd for $C_{16}H_{27}F_3N_3O_4$ (MH^+) 382.1954, found 382.1968.

***N*1-[2-(1,3-Benzothiazol-2-yl)-1-methyl-2-oxoethyl]-*N*4,*N*4-dimethyl-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]butanediamide (80).** Final purification was performed by preparative HPLC: IR (KBr) ν 1642 cm^{-1}; ^1H NMR (DMSO-d_6) 1.5:1 mixture of diastereomers, δ 8.30−8.13 (m, 4H), 7.73−7.58 (m, 3H), 5.47−5.38 (m, 1H), 4.68−4.60 (m, 1H), 4.18−4.10 (m, 1H), 2.92 (s, 3H), 2.79 (s, 3H), 2.76−2.63 (m, 2H), 2.20 (d, J = 12.5 Hz, 1H), 2.03 (d, J = 12.5 Hz, 1H), 1.43 and 1.37−1.28 (d, J = 7.3 Hz; m, 3H), 0.95 (s, 9H), 0.90 (s, 9H); HRMS calcd for $C_{28}H_{42}N_5O_5S$ (MH^+) 560.2906, found 560.2896.

***N*4,*N*4-Dimethyl-*N*1-(1-methyl-2-[1,3]oxazolo[4,5-*b*]-pyridin-2-yl-2-oxoethyl)-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]butanediamide (81).** Final purification was performed by preparative HPLC: 1:1 mixture of isomers; IR (KBr) ν 1703, 1682, 1643 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.39 and 11.30 (2 s, 1H), 8.19 (d, J = 7.7 Hz, 1H), 7.96−7.83 (m, 1H), 7.63−7.56 (m, 2H), 7.39−7.32 (m, 2H), 7.06−6.95 (m, 1H), 4.65−4.46 (m, 2H), 4.25 and 4.22 (2 d, J = 9.1 and 8.3 Hz, 1H), 2.95 and 2.91 (2 s, 3H), 2.77 and 2.66 (2 s, 3H), 2.72−2.45 (m, 2H), 2.18−2.00 (m, 2H), 1.17 and 1.10 (2 d, J = 6.8 and 6.8 Hz, 3H), 0.98−0.85 (m, 18H); HRMS calcd for $C_{27}H_{43}N_6O_7$ (M+19) 563.3193, found 563.3207.

***N*4,*N*4-Dimethyl-*N*1-[1-methyl-2-(6-methyl-1,3-benzoxazol-2-yl)-2-oxoethyl]-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]butanediamide (82).** Final purification was performed by radial chromatography: IR (KBr) ν 1713, 1650, 1642 cm^{-1}; ^1H NMR (DMSO-d_6) 5:1 mixture of diastereomers, δ 8.36 and 8.15 (2 d, J = 8.4 and 7.2 Hz, 1H), 8.31 and 8.25 (2 d, J = 5.1 and 5.7 Hz, 1H), 7.89−7.86 (m, 1H), 7.73−7.69 (m, 1H), 7.63−7.51 (m, 1H), 7.41−7.36 (m, 1H), 5.29−5.21 and 4.74−7.68 (2 m, 1H), 4.67−4.50 (m, 1H), 4.14 and 3.91 (2 d, J = 8.7 and 6.0 Hz, 1H), 2.93−2.58 (m, 8H), 2.49 (s, 3H), 2.23−2.18 (m, 1H), 2.05−2.01 (m, 1H), 1.45 and 1.41 (2 d, J = 7.2 and 7.2 Hz, 3H), 0.97−0.87 (m, 18H); HRMS calcd for $C_{29}H_{44}N_5O_6$ (MH^+) 558.3292, found 558.3307. Anal. ($C_{28}H_{43}N_5O_6$) C, H, N.

***N*4,*N*4-Dimethyl-*N*1-[1-methyl-2-(5-methyl-1,3-benzoxazol-2-yl)-2-oxoethyl]-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]butanediamide (83).** Final purification was performed by flash chromatography: IR (KBr) ν 1713, 1642 cm^{-1}; ^1H NMR (DMSO-d_6) 1.7:1 mixture of diastereomers, δ 8.38−8.06 (m, 2H), 7.81−7.67 (m, 2H), 7.63−7.37 (m, 2H), 5.28−5.20 and 4.73−4.67 (2 m, 1H), 4.62 and 4.55−4.48 (q, J = 7.2 Hz; m, 1H), 4.13 and 3.91 (2 d, J = 8.4 and 6.3 Hz, 1H), 2.93−2.46 (m, 8H), 2.49 (s, 3H), 2.21 and 2.20 (2 d, J_{AB} = 12.3 and 12.4 Hz, 1H), 2.03 (2 d, J_{AB} = 12.3 Hz, 1H), 1.45 and 1.41 (2 d, J = 7.2 and 7.2 Hz, 3H), 0.97−0.87 (m, 18H); HRMS calcd for $C_{29}H_{44}N_5O_6$ (MH^+) 558.3292, found 558.3307. Anal. ($C_{29}H_{43}N_5O_6$) C, H, N.

***N*4,*N*4-Dimethyl-*N*1-[1-methyl-2-(4-methyl-1,3-benzoxazol-2-yl)-2-oxoethyl]-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]butanediamide (84).** Final purification was performed by flash chromatography: IR (KBr) ν 1713, 1658, 1642 cm^{-1}; ^1H NMR (DMSO-d_6) 4:1 mixture of diastereomers, δ 8.38−8.08 (m, 2H), 7.71−7.15 (m, 4H), 5.32−5.24 and 4.74−4.68 (2 m, 1H), 4.63 and 4.55 (2 q, J = 6.3 and 6.6 Hz, 1H), 4.14 and 3.91 (2 d, J = 8.4 and 6.6 Hz, 1H), 2.93−2.48 (m, 11H), 2.20 (d, J_{AB} = 12.6 Hz, 1H), 2.03 (d, J_{AB} = 12.6 Hz, 1H), 1.45 and 1.42 (2 d, J = 7.2 and 7.2 Hz, 3H), 0.97−0.87 (m, 18H); HRMS calcd for $C_{29}H_{44}N_5O_6$ (MH^+) 558.3292, found 558.3307. Anal. ($C_{29}H_{43}N_5O_6$) C, H, N.

***N*4,*N*4-Dimethyl-*N*1-[1-methyl-2-(7-methyl-1,3-benzoxazol-2-yl)-2-oxoethyl]-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]butanediamide (85).** Final purification was performed by flash chromatography: IR (KBr) ν 1715, 1642 cm^{-1}; ^1H NMR (DMSO-d_6) 4:1 mixture of diastereomers, δ 8.37 and 8.15 (2 d, J = 8.1 and 7.5 Hz, 1H), 8.34 and 8.28 (2 d, J = 5.9 and 5.7 Hz, 1H), 7.83−7.20 (m, 4H), 5.26 and 4.73−4.68 (quint, J = 6.3 Hz; m, 1H), 4.62 and 4.57 (2 q, J = 6.0 and 6.4 Hz, 1H), 4.14 and 3.90 (2 d, J = 8.4 and 6.3 Hz, 1H), 2.93−2.58 (m, 8H), 2.54 (s, 3H), 2.21 and 2.20 (2 d, J_{AB} = 12.3 and 12.6 Hz, 1H), 2.03 (d, J_{AB} = 12.6 Hz, 1H), 1.45 and 1.42 (2 d, J = 7.5 and 7.2 Hz, 3H), 0.97−0.87 (m, 18H); HRMS calcd for $C_{29}H_{44}N_5O_6$ (MH^+) 558.3292, found 558.3307. Anal. ($C_{29}H_{43}N_5O_6$) C, H, N.

***N*4,*N*4-Dimethyl-*N*1-[1-methyl-2-(methylcarbamoyl)-2-oxoethyl]-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]butanediamide (86).** Final purification was performed by preparative HPLC: IR (KBr) ν 3320, 1645 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.61 and 8.56 (2 m, 1H), 8.18−8.05 (m, 1H), 7.97 and 7.93 (2 d, J = 6.4 and 6.3 Hz, 1H), 7.60 (br d, J = 7.3 Hz, 1H), 5.03−4.90 (m, 1H), 4.65−4.50 (m, 1H), 4.13 and 4.12 (2 d, J = 8.6 and 8.6 Hz, 1H), 2.94 (br s, 3H), 2.80 and 2.79 (2 s, 3H), 2.71−2.55 (m, 5H), 2.20 (d, J = 12.4 Hz, 1H), 2.04 and 2.01 (2 d, J = 12.7 and 12.7 Hz, 1H), 1.23 and 1.22 (2 d, J = 7.3 and 7.0 Hz, 3H), 0.95 (s, 9H), 0.91 (br s, 9H); HRMS calcd for $C_{23}H_{42}N_5O_6$ (MH^+) 484.3135, found 484.3148.

***N*1-[2-(Dimethylcarbamoyl)-1-methyl-2-oxoethyl]-*N*4,*N*4-dimethyl-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]butanediamide (87).** Final purification was performed by preparative HPLC: IR (KBr) 3302, 1719, 1644 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.27 and 8.16 (2 d, J = 6.4 and 6.7 Hz, 1H), 8.11 and 8.06 (2 d, J = 7.6 and 7.9 Hz, 1H), 7.59 and 7.58 (2 d, J = 8.6 and 8.0 Hz, 1H), 4.62−4.50 (m, 2H), 4.13 and 4.12 (2 d, J = 8.0 and 8.6 Hz, 1H), 2.94 (br s, 3H), 2.86 and 2.85 (2 s, 6H), 2.80 (s, 3H), 2.72−2.56 (m, 2H), 2.23−2.16 (2 d, J = 12.7 and 12.7 Hz, 1H), 2.10−1.90 (m, 1H), 1.30 and 1.29 (2 d, J = 7.3 and 7.3 Hz, 3H), 0.95 (s, 9H), 0.90 (s, 9H); HRMS calcd for $C_{24}H_{44}N_5O_6$ (MH^+) 498.3292, found 498.3309.

***N*1-[2-[[2-(Benzyloxy)ethyl]carbamoyl]-1-methyl-2-oxoethyl]-*N*4,*N*4-dimethyl-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]butanediamide (88).** Final purification was performed by preparative HPLC: IR (KBr) ν 3299, 1645, 1527 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.67 and 8.62 (2 t, J = 5.7 Hz, 1H), 8.15 and 8.10 (2 d, J = 7.5 and 6.3 Hz, 1H), 7.98 and 7.93 (2 d, J = 6.3 and 6.3 Hz, 1H), 7.61 (d, J = 8.4 Hz, 1H), 7.40−7.20 (m, 5H), 5.0−4.90 (m, 1H), 4.65−4.55 (m, 1H), 4.47 (s, 2H), 4.13 and 4.12 (2 d, J = 8.7 and 8.4 Hz, 1H), 3.51 (t, J = 6 Hz, 2H), 3.37−3.30 (m, 2H), 2.94 and 2.93 (2 s, 3H), 2.80 and 2.79 (2 s, 3H), 2.75−2.60 (m, 2H), 2.20 (d, J = 12.6 Hz, 1H), 2.04 and 2.02 (2 d, J = 12.6 and 12.6 Hz, 1H), 1.23 and 1.22 (2 d, J = 7.2 and 7.2 Hz, 3H), 0.95 (s, 9H), 0.91 (s, 9H); HRMS calcd for $C_{31}H_{50}N_5O_7$ (MH^+) 604.3710, found 604.3690.

***N*1-[2-[(1,3-Benzodioxol-5-ylmethyl)carbamoyl]-1-methyl-2-oxoethyl]-*N*4,*N*4-dimethyl-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]-butanediamide (89).** Final purification was performed by preparative HPLC: IR (KBr) ν 3302, 1644 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.14 and 9.09 (2 t, J = 6.4 and 6.1 Hz, 1H), 8.13 and 8.08 (2 d, J = 7.5 and 7.5 Hz, 1H), 8.01 and 7.96 (2 d, J = 6.6 and 6.0 Hz, 1H), 7.60 (d, J = 8.1 Hz, 1H), 6.85−6.80 (m, 2H), 6.73 (d, J = 7.8 Hz, 1H), 5.97 (s, 2H), 5.02−4.86 (m, 1H), 4.65−4.51 (m, 1H), 4.31−4.08 (m, 3H), 2.92 (br s, 3H), 2.80 and 2.79 (2 s, 3H), 2.74−2.58 (m, 2H), 2.19 (br d, J = 12.6 Hz, 1H), 2.03 and 2.02 (2 d, J = 12.9 and 12.6 Hz, 1H), 1.23 (m, 3H), 0.94 and 0.90 (2 s, 18H); HRMS calcd for $C_{30}H_{46}N_5O_8$ (MH^+) 604.3347, found 604.3333.

***N*1-[2-[(1*H*-Benzo[*d*]imidazol-2-ylmethyl)carbamoyl]-1-methyl-2-oxoethyl]-*N*4,*N*4-dimethyl-(2*S*)-2-[[(1*S*)-2,2-**

dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]-butanediamide (90). Final purification was performed by preparative HPLC: IR (KBr) ν 3294, 1663, 1522 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.41 and 9.35 (2 t, J = 10.8 and 10.5 Hz, 1H), 8.64 (m, 0.5H), 8.18 and 8.09 (2 t, J = 14.3 and 15.2 Hz, 2H), 7.99 (d, J = 6.4 Hz, 0.5H), 7.78−7.52 (m, 3H), 7.48−7.34 (m, 2H), 6.48−6.20 (br d, 1H), 5.08−4.93 (m, 1H), 4.78−4.51 (m, 3H), 4.20−4.05 (m, 1H), 2.96, 2.93, 2.92 and 2.90 (4 s, 3H), 2.80, 2.79, 2.76, and 2.72 (4 s, 3H), 2.71−2.55 (m, 2H), 2.20 and 2.17 (2 d, J = 12.4 and 9.8 Hz, 1H), 2.03 and 2.02 (2 d, J = 12.7 and 12.7 Hz, 1H), 1.28 and 1.275 (2 d, J = 7.3 and 7.3 Hz, 2H), 0.95 (s, 9H), 0.94−0.84 (m, 9H); HRMS calcd for $C_{30}H_{46}N_7O_6$ (MH^+) 600.3509, found 600.3488.

***N*4,*N*4-Dimethyl-*N*1-[1-methyl-2-oxo-2-[[(1*S*)-1-phenylethyl]carbamoyl]ethyl]-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]butanediamide (91).** Final purification was performed by preparative HPLC: IR (KBr) ν 3300, 1647 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.11 and 9.07 (2 d, J = 8.6 and 8.6 Hz, 1H), 8.12 and 8.09 (2 d, J = 7.9 and 7.3 Hz, 1H), 8.01 and 7.93 (2 d, J = 6.4 and 6.3 Hz, 1H), 7.60 (m, 1H), 7.37−7.17 (m, 5H), 5.01−4.86 (m, 2H), 4.67−4.51 (m, 1H), 4.14 and 4.13 (2 d, J = 8.6 and 8.6 Hz, 1H), 2.93 and 2.92 (2 s, 3H), 2.79 (br s, 3H), 2.73−2.54 (m, 2H), 2.19 (d, J = 12.7 Hz, 1H), 2.06 and 2.01 (2 d, J = 8.3 and 8.3 Hz, 1H), 1.44−1.37 (m, 3H), 1.24 and 1.17 (2 d, J = 7.6 and 7.3 Hz, 3H), 0.94 (s, 9H), 0.91 and 0.90 (2 s, 9H); HRMS calcd for $C_{30}H_{48}N_5O_6$ (MH^+) 574.3605, found 574.3586.

***N*4,*N*4-Dimethyl-*N*1-[1-methyl-2-oxo-2-[[(1*R*)-1-phenylethyl]carbamoyl]ethyl]-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]butanediamide (92).** Final purification was performed by preparative HPLC: IR (KBr) ν 3288, 1645, 1525 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.11 and 9.05 (2 d, J = 8.3 and 8.6 Hz, 1H), 8.13 and 8.07 (2 d, J = 7.3 and 7.6 Hz, 1H), 8.00 and 7.95 (2 d, J = 6.4 and 6.4 Hz, 1H), 7.59 (d, J = 8.6 Hz, 1H), 7.37−7.18 (m, 5H), 5.02−4.84 (m, 2H), 4.63−4.51 (m, 1H), 4.14 and 4.12 (2 d, J = 8.6 and 8.6 Hz, 1H), 2.91 and 2.87 (2 s, 3H), 2.80 and 2.77 (2 s, 3H), 2.73 and 2.53 (m, 2H), 2.19 and 2.18 (2 d, J = 12.7 and 12.4 Hz, 1H), 2.09−1.98 (m, 1H), 1.42 (d, J = 7.0 Hz, 3H), 1.23 and 1.19 (2 d, J = 7.3 and 7.0 Hz, 3H), 0.95 (br s, 9H), 0.91 and 0.90 (2 s, 9H); HRMS calcd for $C_{30}H_{48}N_5O_6$ (MH^+) 574.3605, found 574.3591.

***N*4,*N*4-Dimethyl-*N*1-[1-methyl-2-oxo-2-[[(1*R*)-1-phenylpropyl]carbamoyl]ethyl]-(2*S*)-2-[[(1*S*)-2,2-dimethyl-1-(neopentylcarboxamido)propyl]carboxamido]butanediamide (93).** Final purification was performed by preparative HPLC: IR (KBr) ν 3297, 1647 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.07 and 9.02 (2 d, J = 8.6 and 8.9 Hz, 1H), 8.13 and 8.06 (2 d, J = 7.7 and 7.2 Hz, 1H), 7.99 and 7.94 (2 d, J = 5.9 and 5.9 Hz, 1H), 7.59 (d, J = 8.3 Hz, 1H), 7.40−7.18 (m, 5H), 4.98−4.84 (m, 1H), 4.75−4.65 (m, 1H), 4.63−4.55 (m, 1H), 4.13 (2 d, J = 8.4 and 8.4 Hz, 1H), 2.92 and 2.88 (2 s, 3H), 2.80 and 2.77 (2 s, 3H), 2.75−2.58 (m, 2H), 2.19 and 2.18 (2 d, J = 12.6 and 12.6 Hz, 1H), 2.03 and 2.02 (2 d, J = 12.6 and 12.3 Hz, 1H), 1.87−1.69 (m, 2H), 1.23 and 1.17 (2 d, J = 7.2 and 7.2 Hz, 3H), 0.95 and 0.94 (2 s, 9H), 0.91 and 0.90 (2 s, 9H), 0.87−0.78 (m, 3H); HRMS calcd for $C_{31}H_{50}N_5O_6$ (MH^+) 588.3761, found 588.3744.

Biochemistry. Material and Methods. Fluorescence measurements were recorded on a Perkin-Elmer LS-50B spectrofluorimeter equipped with a plate reader accessory. UV measurements were recorded on a Thermomax microplate reader from Molecular Devices. All specificity enzymes and their respective substrates were commercially available from the following suppliers: Boehringer Mannheim (bovine pancreas α-chymotrypsin no. 103314 lot 13724423-58, porcine pancreas elastase no. 1027891 lot 83260521-23), Calbiochem (human neutophil elastase no. 324681 lot B12778, human liver cathepsin B no. 219364 lot B14649, Succ-AAA-pNA no. 573459 lot 510008), Sigma Chemical Co. (Succ-AAPF-pNA no. S7388 lot 31H5805), Bachem (Z-FR-pNA no. L-1242 lot 502774, Succ-AAV-pNA no. L-1405, lot 116699).

HCMV N_o Protease Assay. The concentration of recombinant HCMV N_o protease was determined by the Bradford method[37] and expressed in terms of total monomer concentration. The enzyme was assayed with an internally quenched fluorogenic substrate based on the maturation cleavage site (Abz-VVNASSRLY(3-NO_2)R-OH, k_{cat}/K_M = 260 M^{-1} s^{-1}). The fluorescence increase upon cleavage of the Ala-Ser amide bond was monitored using excitation λ = 312 nm (slit 2.5 Å) and emission λ = 415 nm (slit 5 Å). A protocol adaptable to a 96-well plate format was designed for the determination of IC_{50} values of inhibitors. Thus HCMV N_o protease (125 nM) was preincubated for 5 h at 30 °C with a range of sequentially diluted inhibitor concentrations (300−0.06 μM depending on the potency of each compound). After this period, enzymatic hydrolysis was initiated by addition of the fluorogenic substrate and the reaction was performed for 2 h at 30 °C (∼30% conversion). No quenching was required before fluorescence measurement since the total scanning time by the plate reader accessory was brief relative to the duration of the reaction. The final concentrations of HCMV N_o protease (expressed in terms of total monomer concentration) and substrate were 100 nM and 5 μM, respectively. IC_{50} values were obtained through fitting of the inhibition curve to a competitive inhibition model using SAS NLIN procedure. A description of the assay is summarized below. In a white polystyrene round-bottom 96-well plate (MicroFLUOR W. Dynatech) were added using a Biomek liquid handler (Beckman) 30 μL of enzyme solution (50 mM Tris/HCl pH 8, 0.67 M Na_2SO_4, 50 mM NaCl, 0.1 mM EDTA, 1 mM TCEP, 0.05% w/v casein, 167 nM HCMV protease) and 10 μL of inhibitor solution (50 mM Tris/HCl pH 8, 50 mM NaCl, 0.1 mM EDTA, 1 mM TCEP, 0.05% w/v casein, 1.5 mM to 0.3 μM inhibitor, 15% v/v DMSO). After a 5 h preincubation at room temperature, 10 μL of substrate solution (50 mM Tris/HCl pH 8, 0.5 M Na_2SO_4, 50 mM NaCl, 0.1 mM EDTA, 1 mM TCEP, 0.05% w/v casein, 25 μM Abz-VVNASSRLY(3-NO_2)R-OH) were added to each well and the reaction was further incubated at 30 °C for 2 h after which time the fluorescence was read on the spectrofluorimeter plate reader. Rows of wells were allocated for controls (no inhibitor) and for blanks (no inhibitor and no enzyme). The sequential 2-fold dilutions of the inhibitor solution were performed on a separate plate by the liquid handler using 50 mM Tris/HCl pH 8, 50 mM NaCl, 0.1 mM EDTA, 0.05% w/v casein, 1 mM TCEP, 15% v/v DMSO). The mode of inhibition was determined by measurements of the initial rates (in cuvettes) at various substrate and inhibitor concentrations using the same conditions as above. Data were plotted according to the Cornish-Bowden method[38] ([S]/v *versus* [I]) and Dixon method (1/v *versus* [I]) to visually assess the type of inhibition.

Selectivity Assays. The selectivity of the compounds was determined against a variety of serine proteases (human leukocyte and porcine pancreatic elastases (HLE and PPE), bovine pancreas α-chymotrypsin) and one cysteine protease (human liver cathepsin B). In all cases a 96-well plate format protocol using a colorometric *p*-nitroanilide (pNA) substrate specific for each enzyme was used. Each assay included a 1 h preincubation enzyme−inhibitor at 30 °C followed by addition of substrate and hydrolysis to ∼30% conversion as measured by scanning on a UV Thermomax microplate reader. Substrate concentrations were kept as low as possible compared to K_M to reduce substrate competition. Compound concentrations varied from 300 to 0.06 μM depending on their potency. The final conditions for each assay were as followed: 50 mM Tris/HCl pH 8, 0.5 M Na_2SO_4, 50 mM NaCl, 0.1 mM EDTA, 3% DMSO, 0.01% Tween-20 with [100 μM Succ-AAPF-pNA and 250 pM α-chymotrypsin], [133 μM Succ-AAA-pNA and 8 nM porcine elastase], or [133 μM Succ-AAV-pNA and 8 nM leukocyte elastase], 100 mM NaH_2PO_4 pH 6, 0.1 mM EDTA, 3% DMSO, 1 mM TCEP, 0.01% Tween-20, 30 μM Z-FR-pNA, and 5 nM cathepsin B (the stock enzyme was activated in buffer containing 20 mM TCEP before use). A representative example is summarized below for porcine pancreatic elastase. In a polystyrene flat-bottom 96-well plate (CellWells, Corning) were added using a Biomek liquid handler (Beckman) 40 μL of assay buffer (50 mM Tris/HCl pH 8, 1 M Na_2SO_4, 50 mM NaCl, 0.1 mM EDTA), 20 μL of enzyme solution (50 mM Tris/HCl pH 8, 50 mM NaCl, 0.1 mM EDTA, 0.02% Tween-20, 40 nM porcine pancreatic elastase), and 20 μL of inhibitor solution (50 mM Tris/HCl pH 8, 50 mM NaCl, 0.1 mM EDTA, 0.02% Tween-20, 1.5 mM to 0.3 μM, 15% v/v DMSO). After 60 min

preincubation at 30 °C, 20 μL of substrate solution (50 mM Tris/HCl pH 8, 0.5 M Na_2SO_4, 50 mM NaCl, 0.1 mM EDTA, 665 μM Succ-AAA-pNA) was added to each well, and the reaction was further incubated at 30 °C for 60 min after which time the absorbance was read on the UV Thermomax plate reader. Rows of wells were allocated for controls (no inhibitor) and for blanks (no inhibitor and no enzyme). The sequential 2-fold dilutions of the inhibitor solution were performed on a separate plate by the liquid handler using 50 mM Tris/HCl pH 8, 50 mM NaCl, 0.1 mM EDTA, 0.02% Tween-20, 15% v/v DMSO. The other specificity assays were performed in a similar fashion.

Plaque Reduction Assay. The plaque reduction assay was performed according to Turk[39] with minor modifications. Briefly, Hs-68 cells (ATCC no. CRL 1635) were seeded in 12-well plates at 80 000 cells/well in 1 mL of DMEM medium (Gibco BRL) supplemented with 10% fetal bovine serum (FBS, Gibco BRL). The plates were incubated for 3 days at 37 °C to allow the cells to reach 80−90% confluency prior to the assay. The medium was removed from the cells by aspiration. The cells were then infected with approximately 50 pfu of HCMV (strain AD169, ATCC VR-538) in DMEM medium supplemented with 5% inactivated FBS (assay medium). The virus was allowed to adsorb to the cells for 2 h at 37 °C. Following viral adsorption, the medium was removed from the wells by aspiration. The cells were then incubated with or without 1 mL of appropriate concentrations of test reagent in assay medium. After 4 days of incubation at 37 °C, the medium was exchanged with fresh medium containing test compound, and 4 days later (8 days postinfection) the cells were fixed with 1% aqueous formaldehyde and stained with a 2% crystal violet solution in 20% ethanol. Microscopic plaques were counted using a stereo-microscope. Drug effects were calculated as a percent reduction in the number of plaques in the presence of each drug concentration compared to the number observed in the absence of drug. Ganciclovir was used as a positive control in all experiments. The cytotoxicity of drugs was determined with the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma)[40] under the same cell culture assay conditions used in the plaque reduction assay.

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