m), 2.20–2.73 (4 H, br s), 2.77–3.10 (2 H, m), 3.70 (2 H, m), 6.30 (1 H, d, J = 5.5 Hz), 6.98–7.50 (4 H, m), 7.68 (1 H, m), 8.65 (1 H, br s); ^{13}C NMR ($CDCl_3$) δ 18.8, 22.9 (×2), 24.7, 34.8, 35.9, 41.2, 52.1 (×2), 70.2, 111.1, 118.2, 118.5, 118.9, 121.1, 121.8, 127.3, 130.5, 136.5; high-resolution mass spectrum (CI) calcd for (M + 1) $C_{20}H_{24}N_2D$ 294.20795, found 294.2119.

Ketanserin Binding Assay. The binding of [^{3}H]ketanserin to $5HT_2$ receptors was conducted predominantly as described by Leysen et al.,[3a] with modification. Cerebral cortical tissue, isolated from adult BALB/cBy mice, was homogenized in 10:1 (v/w) ice-cold 0.25 M sucrose (pH 7.4) with a Brinkman polytron at a setting of 6 for 15 s and centrifuged at 1000g for 10 min. The supernatant (S_1) was removed with a pipet and diluted 1:40 (w/v) in Tris buffer (50 mM Tris-HCl, pH 7.4). This suspension was centrifuged at 35000g for 10 min. The resulting pellet was then washed once by resuspension with a polytron in an identical volume of Tris buffer and centrifuged at 35000g for 10 min. This final pellet was again resuspended, with a polytron, in Tris buffer.

One-milliliter aliquots of the membrane preparation were incubated in triplicate at 37 °C for 15 min with 0.15 nM [^{3}H]-ketanserin in the presence or absence of varying concentrations of indolylethylamine analogues in a final volume of 2 mL (Tris buffer). The incubation was terminated by the addition of 5 mL of ice-cold Tris buffer and filtration was effected with a cell harvester (Brandel) through Whatman GF/B glass-fiber filters presoaked with Tris buffer. Two 5-mL washes were used to rinse through the filters. Radioactivity was measured by scintillation spectroscopy with an efficiency of approximately 50%. Protein concentrations were determined by the method of Lowry et al.[17] Nonspecific binding of [^{3}H]ketanserin was defined with unlabeled methysergide at a final concentration of 1 μM.

8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) Binding Assay. Tissue of the hippocampus, isolated from adult BALB/cBy mice, was homogenized in 40 volumes (v/w) of ice-cold 50 mM Tris-HCl (pH 7.4) using a Brinkman polytron at a setting of 4.5 for 5 s, and was centrifuged at 40000g for 20 min. The pellet was washed twice by resuspension in an identical volume of Tris with a polytron and was centrifuged at 40000g for 20 min. After the second wash and subsequent resuspension the tissue was incubated for 10 min at 37 °C to remove endogenous serotonin. After incubation the tissue was washed twice more before final resuspension in Tris buffer (method of Hall et al.).[18]

Aliquots of 100 μL of the membrane preparation were incubated in triplicate at 23 °C for 30 min (method of Cossery et al.)[19] with 1.2 nM [^{3}H]8-OH-DPAT in the presence or absence of varying concentrations of indolylethylamine analogues in a final volume of 0.5 mL of Tris buffer. The incubation was terminated by the addition of 5 mL of ice-cold Tris buffer and filtration was effected through a Whatman GF/B glass fiber filter, pretreated with 0.05% polyethylenimine. The incubation tube and the filter were each rinsed once with 5 mL of Tris buffer. Radioactivity and protein content were determined as described above. The nonspecific binding of [^{3}H]8-OH-DPAT was defined with unlabeled serotonin at a final concentration of 10 μM.

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Synthesis of Peptidyl Fluoromethyl Ketones and Peptidyl α-Keto Esters as Inhibitors of Porcine Pancreatic Elastase, Human Neutrophil Elastase, and Rat and Human Neutrophil Cathepsin G

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Comparison of MeO-Suc-Val-Pro-Phe-CO_2Me (**29**) and MeO-Suc-Ala-Ala-Pro-Phe-CO_2Me (**25**) with their corresponding trifluoromethyl ketones **9a** and **9b**, respectively, in rat and human neutrophil cathepsin G assays showed the α-keto esters to be more potent inhibitors. Likewise, Ac-Pro-Ala-Pro-Ala-CO_2Me (**21**) was more potent than its corresponding trifluoromethyl ketone (**9c**) in both porcine pancreatic elastase and human neutrophil elastase assays. Within a set of Ala-Ala-Pro-Val-CF_3 elastase inhibitors, the carbobenzyloxy (Cbz) N-protecting group conferred greater potency as a P_5 site recognition unit for elastase than did dansyl, methoxysuccinyl, or *tert*-butyloxycarbonyl. Initial inhibition of elastase was greater when trifluoromethyl ketone **9f** was added from a stock solution of dimethyl sulfoxide than when it had been buffer-equilibrated prior to assay, which suggests that the nonhydrated ketone is the more effective form of the inhibitor. The most potent elastase inhibitor we report is N^{α}-(Ad-SO_2)-N^{ϵ}-(MeO-Suc)Lys-Pro-Val-CF_3 (**16**) which has a K_i of 0.58 nM.

Replacement of the scissile amide unit of proteolytic enzyme substrate analogues by atom assemblies containing electrophilic carbonyl groups is a relatively new approach to proteinase inhibition effected through transition-state mimicry. This approach is effective for cysteine, serine, and aspartyl proteases. We[2] and others[3-13] have employed

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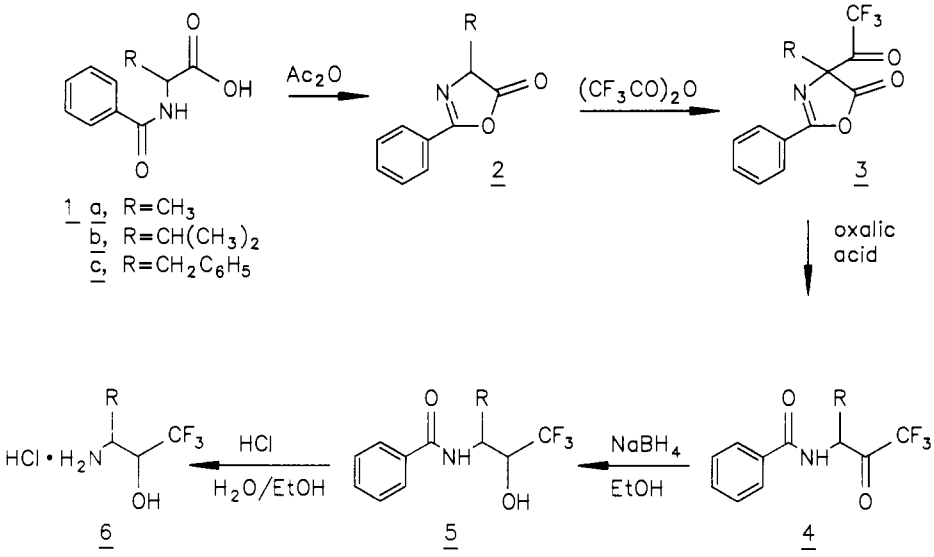
0022-2623/90/1833-0394$02.50/0

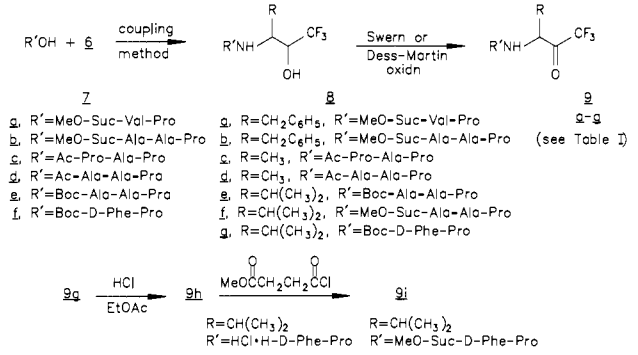
Scheme I



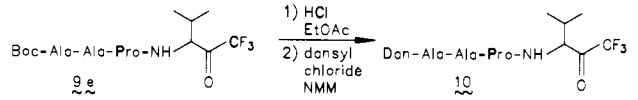
fluorinated ketones at the scissile site in inhibitor design. α-Keto esters have also been employed as scissile-site replacements.[14a,15-17] In this report we describe and compare the chemistry and enzymology of peptidyl fluorinated ketones and peptidyl α-keto esters.

Formation of a tetrahedral intermediate has been postulated as an early event in the hydrolysis of substrates by serine proteases such as elastase even though its demonstration may be uncertain for specific substrates.[18,19] A reasonable corollary is that substratelike carbonyl compounds which could form stable tetrahedral intermediates within the active site of the enzyme should be potent, reversible inhibitors. Ac-Pro-Ala-Pro-alaninal, in which the terminal amino acid is present as an aldehyde, is a potent inhibitor of porcine pancreatic elastase, K_i = 0.8 μM,[20] whereas the corresponding acid,[21] alcohol,[20] and methyl ketone[21] are poorer inhibitors by several orders of magnitude. The hydroxyl function of the active site serine residue of elastase is essential for binding of the aldehyde, consistent with a proposed tetrahedral structure of enzyme-bound inhibitor.[22] Binding of another aldehyde inhibitor as a hemiacetal to a serine protease has been demonstrated by X-ray crystallography.[23]

Scheme II



Scheme III



The concept of inhibition by an electrophilic aldehyde carbonyl group has been extended to other carbonyl-containing peptide analogues in which an electron deficiency is effected by electron-withdrawing groups positioned adjacent to the carbonyl group in a unit approximating the P_1' substrate amino acid. Inhibitors thus derived have great potential for therapeutic applications, since proteolysis by enzymes such as elastase and cathepsin G released from leukocytes can have harmful consequences for the host. Neutrophil elastase, for instance, may play a role in inflammation and emphysema.[24]

The fluorinated ketone peptidyl inhibitors and related α-keto esters prepared for this comparative study were

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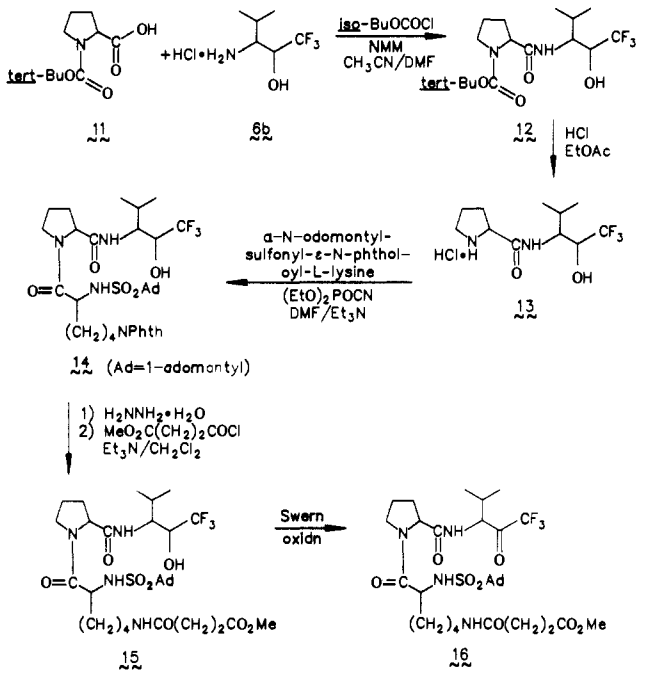
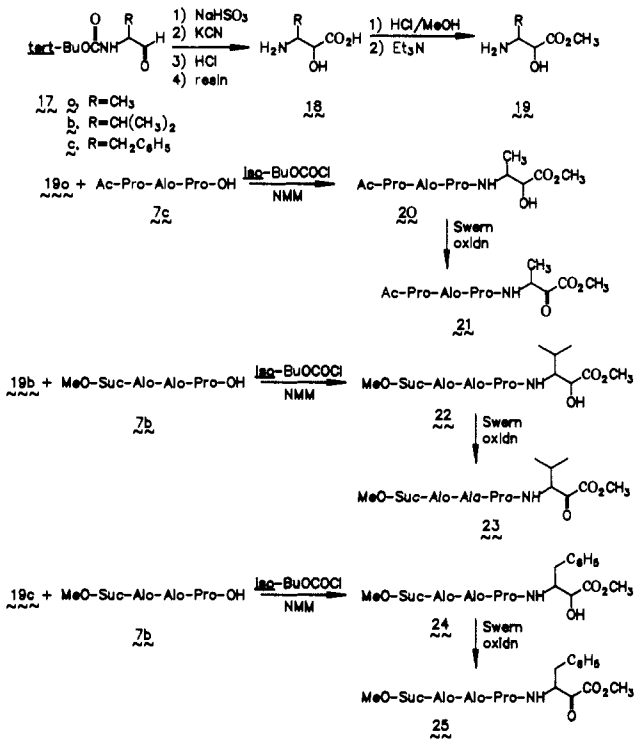
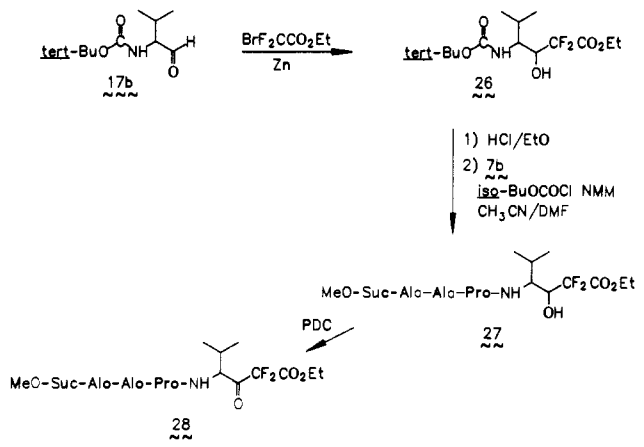
Scheme IV



Scheme V



Scheme VI



evaluated in purified preparations of porcine pancreatic elastase, human neutrophil elastase, and rat and human neutrophil cathepsin G.

Chemistry

N-Benzoylamino acids **1a–c** were converted to N-benzoylated α-amino trifluoromethyl ketones **4** as shown in Scheme I, by using a modified Dakin-West reaction,[25,26] which we recently described.[2] Thus, treatment of **1a–c** with acetic anhydride gave oxazolones **2a–c**, which were trifluoroacetylated to provide **3a–c**. Subsequent oxalic acid-promoted decarboxylation followed by hydrolytic work-up gave trifluoromethyl ketones **4a–c**. Ketones **4a–c** were reduced to the corresponding alcohols **5a–c**, which were subsequently hydrolyzed[27] to provide trifluoromethyl amino alcohol hydrochloride salts **6a–c**.

Amino alcohols **6a–c** were coupled[28] with N-protected di- and tripeptides **7a–f** (Scheme II), where the protecting groups chosen to mimic an additional P-side amino acid were methoxysuccinyl (MeO-Suc), acetyl (Ac), and *tert*-butyloxycarbonyl (Boc). The resulting trifluoromethyl alcohols **8a–g** were then oxidized to afford trifluoromethyl ketones **9a–g**. Several oxidative procedures were investigated for the conversion of **8a–g** to **9a–g**,[31] and the best

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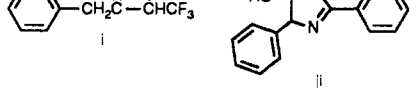
(27) Attempted cleavage of the benzamide function at the level of ketone **4** was unsuccessful: acid hydrolysis (concentrated HCl, reflux) of benzamide trifluoromethyl ketone **4c** resulted in loss of the amine functionality. Isolated was *i*: MS (EI) *m/z* (rel intensity) 218 (M^+, 20) 91 (100); ^{1}H NMR ($CDCl_3$) δ 7.2 (m, 5 H, aryl), 4.55 (q, 1 H, J = 7.5 Hz, CH), 3.95 (s, 2 H, CH_2); ^{19}F NMR ($CDCl_3$) ϕ 84.2 (d, J = 7.5 Hz, CF_3); R_f = 0.4 ($CHCl_3$). Attempted O-alkylation of amide trifluoromethyl ketone **5c** with $(Et_3)O^+BF_4^-$ or $MeOSO_2CF_3$ in order to render it hydrolyzable under mild conditions did not give the expected product. Isolated was ii: IR ($CDCl_3$) 1660 cm^{-1}; ^{19}F NMR ($CDCl_3$) ϕ 82 (s, CF_3).



i

ii

(28) We generally relied on the mixed anhydride coupling method[29] for peptide assembly. However, we also successfully employed 4,6-diphenylthieno[3,4-*d*][1,3]dioxol-2-one 5,5-dioxide,[30] a relatively obscure but useful peptide-activating reagent. A novel feature of this reagent is the stability of the active esters it forms with *N*-acyl amino acids and peptides. These esters can be isolated, recrystallized, and stored.

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Table I. Peptidyl Fluorinated Ketones and Keto Esters as Proteinase Inhibitors

		K_i*,[a] μM			
		elastase		cathepsin G	
no.	structure	porcine	human	rat	human
9a	MeO-Suc-Val-Pro-Phe-CF_3			0.2	3
29	MeO-Suc-Val-Pro-Phe-CO_2CH_3[b]				1.1
9b	MeO-Suc-Ala-Ala-Pro-Phe-CF_3[c]		>1500	1.6	17
25	MeO-Suc-Ala-Ala-Pro-Phe-CO_2CH_3[d]		>1000	0.2	7
9c	Ac-Pro-Ala-Pro-Ala-CF_3	0.24	41		>800
21	Ac-Pro-Ala-Pro-Ala-CO_2Me[e]	0.029	0.85		
9d	Ac-Ala-Ala-Pro-Ala-CF_3	0.15	24		>800
9d	Ac-Ala-Ala-Pro-Ala-CF_3[f]		0.25		
30	Cbz-Ala-Ala-Ala-Ala-CO_2Et[b]	0.14	0.3		
10	Dan-Ala-Ala-Pro-Val-CF_3	0.088	0.010[g]		>600
9e	Boc-Ala-Ala-Pro-Val-CF_3	0.032	0.044		
31	Cbz-Ala-Ala-Pro-Val-CF_3[h]		0.001		
9f	MeO-Suc-Ala-Ala-Pro-Val-CF_3	0.033	0.014[i]		
23	MeO-Suc-Ala-Ala-Pro-Val-CO_2CH_3[j]	0.68	0.20		
28	MeO-Suc-Ala-Ala-Pro-Val-CF_2CO_2Et	9.9	4.3		
9g	Boc-D-Phe-Pro-Val-CF_3	1.8	0.16		
9i	MeO-Suc-D-Phe-Pro-Val-CF_3	28	0.66		
16	N^{α}-(Ad-SO_2)-N^{ϵ}-(MeO-Suc)Lys-Pro-Val-CF_3	0.015	0.00058		

[a] Values are those of steady-state inhibition. [b] Reference 15. [c] K_i = 0.017 μM for α-chymotrypsin; assayed as described in ref 9. [d] K_i = 0.079 μM for α-chymotrypsin; assayed as described in ref 9. [e] For the corresponding acid Ac-Pro-Ala-Pro-Ala-CO_2H, K_i = 0.69 μM for porcine pancreatic elastase and K_i = 66 μM for human neutrophil elastase. [f] Reference 9. [g] The apparent K_i was 0.0063 μM for the more active diastereomer, and 0.11 μM for the less active diastereomer. HPLC ratios of the more active to less active diastereomers in the samples used for these assays were 99.2:0.8 and 1.2:98.8, respectively. [h] Reference 6. [i] The apparent K_i was 0.0070 μM for the more active diastereomer, and 0.092 μM for the less active diastereomer. HPLC ratios of the more active to less active diastereomers in the samples used for these assays were 98.5:1.5 and 0.2:99:8, respectively. [j] Synthesis of 23 proceeded without appreciable racemization to give a single diastereomer; all other compounds are pairs of diastereomers.

procedure in general was the Swern oxidation. Exchange of the Boc group in **9g** for MeO-Suc was accomplished by treatment of **9b** with hydrogen chloride to give amine hydrochloride **9h**, which was acylated with methoxysuccinyl chloride to provide **9i**.

Preparation of fluorescent derivative **10** was accomplished as shown in Scheme III. Thus, removal of the Boc group with hydrogen chloride gave the amino trifluoromethyl ketone hydrochloride salt, which was treated directly with *N*-methylmorpholine (NMM) and dansyl chloride to provide **10**. Again, we were able to produce and utilize an unprotected amino trifluoromethyl ketone without encountering problems with self-condensation.

Preparation of trifluoromethyl ketone **16**, containing a differentially protected lysine unit at the P_3 site, is shown in Scheme IV. Coupling of *N*-(*tert*-butyloxycarbonyl)-L-proline (**11**) with amino alcohol **6b** using the mixed anhydride method[28] gave **12**, which was deprotected to provide amino alcohol **13**. Coupling of **13** with N^{α}-(adamantylsulfonyl)-N^{ϵ}-phthaloyl-L-lysine using diethyl cyanophosphonate[32] gave **14**. The phthaloyl group of **14** was exchanged for the methoxysuccinyl group by treatment with hydrazine hydrate followed by in situ acylation with 3-carbomethoxypropionyl chloride. Finally, Swern oxidation of **15** provided the desired trifluoromethyl ketone **16**.

Preparation of the α-keto esters required for this study is detailed in Scheme V. [(*tert*-Butyloxycarbonyl)amino]acetaldehydes **17a–c**[33a,b] were prepared by reduction of the corresponding amino acid methyl esters with diisobutylaluminum hydride.[33c,d] The β-Amino-α-hydroxy acids **18a–c** were prepared by converting aldehyde bisulfite addition products to cyanohydrins, hydrolyzing the cyanohydrins, and isolating the amino acids via ion-exchange chromatography.[33e] Fischer esterification of the acids provided esters **19a–c**. Peptidyl α-keto ester **21** was prepared from **19a** in a two-step process by mixed-anhydride coupling with N-protected tripeptide **7c**, followed by Swern oxidation. α-Keto esters **23** and **25** were prepared in an analogous fashion. We have subsequently found that the Dess–Martin periodinane is a preferred oxidant for α-hydroxy esters.[14]

One α,α-difluoro ketone was prepared for this study, which is shown in Scheme VI. Aldehyde **17b** was treated with ethyl bromodifluoroacetate under Reformatsky conditions to afford difluoro alcohol **26**. Removal of the *N*-*tert*-butyloxycarbonyl group followed by mixed anhydride coupling with **7b** gave difluoro alcohol **27**, which was converted to difluoro ketone **28** by oxidation with pyridinium dichromate (PDC).

Enzyme Inhibitory Activities and Discussion

Steady-state constants characterizing inhibition of porcine pancreatic elastase, human neutrophil elastase, rat cathepsin G, and human cathepsin G by α-keto esters and peptidyl fluorinated ketones are presented in Table I. Selection of the peptide portion was guided by the considerable amount of information available on binding preferences of substrates and designed inhibitors at the enzyme active sites and the assumption that the inhibitors would initially interact with target enzymes as pseudosubstrates. Extended binding regions are well-established for porcine pancreatic and human neutrophil elastases,[34,35] with significant contributions to binding occurring out to

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the P_5 residue. A binding region to P_3 is suggested for cathepsin G.[15] The preference for a P_2 proline residue for these enzymes is documented.[36] Thus, tri- and tetrapeptide recognition units were incorporated into the inhibitors.

Although only four cathepsin G inhibitors were evaluated, some conclusions can be drawn. In two pairs of compounds having the same amino acid sequence, i.e., the first four compounds in Table I, where direct comparisons can be made between trifluoromethyl ketones and α-keto esters, the α-keto esters were more potent than the trifluoromethyl ketones. All the inhibitors were more potent for rat cathepsin G than for human cathepsin G. Interestingly, the order of potency was reversed with α-chymotrypsin, where trifluoromethyl ketone **9b** was more potent than the analogous α-keto ester **25**. It should be pointed out that the data for **29** are those of Hori, Yasutake, Minematsu, and Powers.[15] Inhibitors **9a**, **9b**, and **25** were more potent against rat cathepsin G than they were against human cathepsin G.

For the pair of analogous elastase inhibitors **9c** and **21**, α-keto ester **21** was similarly significantly more potent than trifluoromethyl ketone **9c**, for both porcine and human elastases. Note that for **21** the corresponding α-keto acid was much less potent. Cbz-Ala-Ala-Ala-Ala-CO_2Et (**30**)[15] was quite potent in spite of the lack of proline at P_2.

Among the tetrapeptides, the Ala-Ala-Pro-Val set of inhibitors (**10**, **9e**, **31**,[6] **9f**, **23**, and **28**) was clearly the most potent for the elastases. In this set of tetrapeptides, trifluoromethyl ketone **9f** was superior to the analogous α-keto ester **23** for both porcine pancreatic and human neutrophil elastases. Replacement of one fluorine for carboethoxy (compound **28**) was detrimental to potency for both enzymes. Among protecting groups, which approximate a P_5 site, dansyl (Dan), Boc, and MeO-Suc were all active, but carbobenzyloxy (Cbz)[6] appeared to be best in conferring potency.

Although all of the inhibitors in Table I (with the exception of **23**) are racemic at the asymmetric center α to the activated carbonyl group, two of the compounds were separated by HPLC to afford pairs of diastereomers. As expected, in both cases one of the diastereomers (presumably the isomer analogous to the L isomer of a tetrapeptide substrate) was more potent. For dansyl compound **10**, K_i was 0.0063 μM for the more active diastereomer and K_i was 0.11 μM for the less active diastereomer. The numbers were similar for methoxysuccinyl compound **9f**, where K_i was 0.0070 μM for the more active diastereomer and K_i was 0.092 μM for the less active diastereomer. Again, it should be noted that the data in Table I for **9d** (second entry) are those of Imperiali and Abeles;[9] for Cbz-Ala-Ala-Ala-Ala-CO_2Et (**30**) are those of Hori, Yasutake, Minematsu, and Powers;[15] and for Cbz-Ala-Ala-Pro-Val-CF_3 (**31**) are those of Dunlap, Stone, and Abeles.[6]

Three additional tripeptides (**9g**, **9i**, and **16**) were prepared as elastase inhibitors, which are shown at the bottom of Table I. All of these compounds were significantly more potent against human neutrophil elastase than for porcine pancreatic elastase. Presumably, the different N-protecting groups account for the differences in inhibitory potencies between **9g** and **9i**.[37] The last entry in Table I, N^{α}-(Ad-SO_2)-N^{ϵ}-(MeO-Suc)Lys-Pro-Val-CF_3 (**16**), was

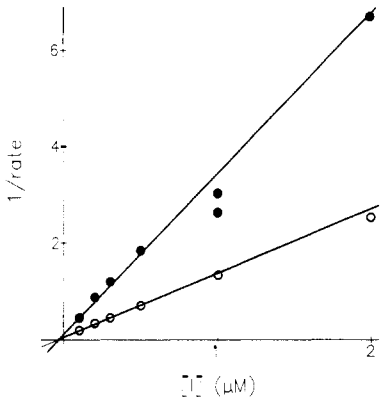

Figure 1. Dixon plot of steady-state inhibition of porcine pancreatic elastase by MeO-Suc-Ala-Ala-Pro-Val-CF_3 (**9f**). The shallower line derives from assays at 0.625 mM concentration of MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide substrate; the steeper line is from assays at 0.25 mM. Each (reciprocal) rate derives from a pair of assays, in one of which elastase was added to a mixture of substrate and inhibitor and in the other substrate was added to a preincubated mixture of elastase plus inhibitor. The molar excess of inhibitor over enzyme was 9-fold or greater. Total elastase concentration ranged from 6 to 220 nM, but rates were normalized to a single enzyme concentration. The two lines intersect at $K_i^* = 0.033$ μM.

the most potent inhibitor of both elastases. This compound appears to be slightly less potent against human leukocyte elastase than the related tetrapeptide N^{α}-Cbz-N^{ϵ}-Cbz-Lys-Val-Pro-Val-CF_3, which was recently reported by Stein et al.[4] to have a K_i value of less than 0.1 nM.

Nearly all of the evaluated inhibitors exhibited slow-binding kinetics with both elastases. Kinetic parameters were obtained for the two simplest models typically used to characterize slow formation of 1:1 enzyme–inhibitor complexes; all of these values are presented in the supplementary material. The steady-state inhibition constants in Table I are adequate for making comparisons between inhibitors. All of the compounds, whether slow-binding or not, were simple competitive inhibitors: at a single inhibitor concentration, the extent of inhibition was reduced in assays carried out at higher substrate concentration. This is illustrated by the Dixon plot in Figure 1. In addition, substrate added to preformed enzyme–inhibitor complex reduced the extent of inhibition; progress curves for the slow-binding inhibitors in this assay showed an increase in the rate of product formation with time, up to the steady-state rate of the equilibrated enzyme–substrate–inhibitor mixture.

Two explanations for the slow development of inhibition of serine proteases with this class of inhibitors have been suggested. First, the similarity of enzyme-bound inhibitor to the tetrahedral intermediate of a substrate undergoing scission creates great stability of the complex, which is reflected in a slow rate of dissociation.[4] The dissociation rates measured for some of our inhibitors (see supplementary material) are in agreement with those reported by Stein et al.[4] and Imperiali and Abeles[9] for similar inhibitors and support this explanation. Second, whereas the association rate constants for selected peptidyl fluorinated ketones and α-keto esters are similar to those of other small ligands and enzymes, the rate of formation of the enzyme–inhibitor complexes is determined by the products of those constants with concentration of inhibitor. Should the concentrations of effective, e.g., nonhydrated, inhibitor be very low, the formation of the enzyme–inhibitor complex could be slow enough to be detectable within the time scale of a typical assay.[4,38] A few assays

(36) Powers, J. C.; Gupton, B. F.; Harley, A. D.; Nishino, N.; Whitley, R. J. *Biochim. Biophys. Acta* **1977**, *485*, 156.

(37) The D-phenylalanine residue was incorporated into **9g** and **9i** to provide compounds with potential for increased proteolytic stability. The D-residue was intentionally added at the most remote recognition site.

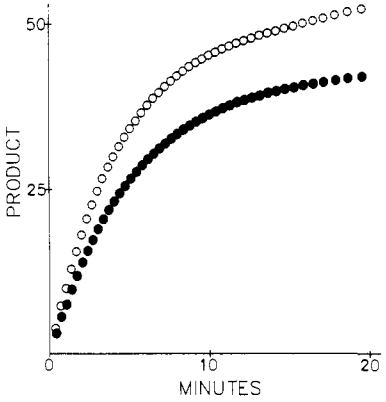

Figure 2. Progress curves of the hydrolysis of MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (0.375 mM) by human neutrophil elastase (8 nM): (●) MeO-Suc-Ala-Ala-Pro-Val-CF_3 (**9f**, 1 μM) was added to the assay mixture from a 10 mM stock solution in dimethyl sulfoxide prior to, but nearly simultaneously with, the addition of enzyme; (○) Inhibitor (1 μM) was allowed to equilibrate in the assay mixture for 20 min before enzyme was added. Product is expressed in nanomoles of *p*-nitroaniline.

we have carried out bear out the second explanation. When trifluoromethyl ketone **9f** dissolved in dimethyl sulfoxide was added to an assay mixture followed as quickly as possible by the addition of human elastase, the time course of substrate hydrolysis was as illustrated in Figure 2. If equilibration of inhibitor with the aqueous assay mixture prior to addition of enzyme was allowed, a reduced inhibition at the outset of the assay period was observed before the rate of product formation following equilibration of the ternary enzyme–inhibitor–substrate system became similar for the two assays (Figure 2). These assays may be interpreted to indicate a higher concentration of effective inhibitor, i.e., ketone, with consequent effect on the association rate, in the assay mixture prepared by nearly simultaneous addition of inhibitor and enzyme components. ^{13}C NMR studies support the concept that trifluoromethyl ketones are hydrated in the presence of water and not hydrated in its absence. The ^{13}C NMR spectrum for **9f** (Experimental Section) recorded in deuteriochloroform shows only the nonhydrated species. However, **9f** in dimethyl-d_6 sulfoxide with water added is completely hydrated. A similar result was obtained with α-keto ester inhibitor **23**.

In summary, we have described the synthesis and enzyme (elastase and cathepsin G) inhibitory activities for sets of peptidyl trifluoromethyl ketones and α-keto esters. In several cases, analogous α-keto esters and trifluoromethyl ketones were prepared and evaluated to permit meaningful comparisons to be made for these two inhibitor types. Studies with both hydrated and nonhydrated versions of a trifluoromethyl ketone and an α-keto ester in the presence of enzyme suggests that nonhydrated species are more effective inhibitors.

We are currently investigating the structures of enzyme–inhibitor complexes using X-ray crystallographic studies of the enzyme–inhibitor complex formed from α-keto ester **25** and α-chymotrypsin. We are also pursuing NMR studies of the enzyme–inhibitor complex derived from trifluoromethyl ketone **9f** individual diastereomers with human sputum elastase and racemization studies with the individual diastereomers of trifluoromethyl ketone **10** in human serum and selected solvents.

(38) Schultz, R. M.; Varma-Nelson, P.; Ortiz, R.; Kozlowski, K. A.; Orawski, A. T.; Pagast, P.; Frankfater, A. *Biochemistry* In press.

Experimental Section

TLC analyses were performed with Merck-DC-F_{254} plates, with detection by irradiation with a Mineralight UVS 11, I_2, ninhydrin, alkaline permanganate, or charring after spraying with vanillin/sulfuric acid;[39] flash chromatography[40] was performed with Merck silica gel 60 (0.040–0.063 mm); melting points were determined with a Thomas-Hoover capillary, a Büchi SMP-20, or a Kofler hot bank melting point apparatus and are uncorrected. IR spectra were recorded with Perkin-Elmer Model 337, 180, and 1800 spectrophotometers; UV spectra were recorded with Beckman DU-7 or Cary 118 spectrophotometers; ^1H, ^{19}F, and ^{13}C NMR spectra were recorded with Varian T-60, Varian FT80A (^{19}F = 74.8 MHz, ^{13}C = 20.0 MHz), Varian XL-300, Varian VXR-300 (^{19}F = 282.2 MHz, ^{13}C = 75.4 MHz), Varian EM-390 (^{19}F = 84.6 MHz), or Bruker AM-360 ^{19}F = 338.8 MHz, ^{13}C = 90.5 MHz) spectrometers; MS data were collected at 70 eV with Finnigan MAT 4600, GC/MS Ribermag R10-10, or Finnigan TSQ GC/MS/MS instruments, and HRMS data were collected at 70 eV with a VG ZABZ-SE spectrometer, using computerized peak matching with perfluorokerosene as the reference and a resolution of 10 000. Chemical shifts for ^1H NMR signals are reported in ppm downfield from TMS (δ); ^{19}F NMR signals are reported in ppm downfield from C_6F_6 (φ). Combustion analyses fell within ±0.4% of the calculated values.

Solvents and reagents were dried prior to use when deemed necessary. Anhydrous oxalic acid refers to reagent obtained from commercially available oxalic acid after drying overnight at 110 °C followed by sublimation at 60–70 °C at 0.05 Torr.

General Procedure (GP) for the Preparation of Trifluoromethyl α-Benzamidoalkyl Ketone (4). GP 1. A mixture of freshly prepared 5(4*H*)-oxazolone **2** and TFAA (1.2–2.9 equiv) was stirred at 20–40 °C (oil-bath temperature) under N_2 for a given time interval (^1H and ^{19}F NMR monitoring; see detailed description of compounds) to afford acylated oxazolone **3**. Excess TFAA and the formed TFA were then removed thoroughly under vacuum (50–80 °C, oil-bath temperature, 0.01 Torr), and the residue was mixed with rigorously dried oxalic acid (1.5–1.7 equiv). The stirred mixture was heated to 110–120 °C (oil-bath temperature). After cessation of gas evolution (about 10–15 min), the mixture was allowed to cool to room temperature and EtOAc/H_2O (4:1, 25 mL/mmol) was added. The aqueous layer was separated and extracted with EtOAc (2 × 5 mL/mmol) and dried ($MgSO_4$). Flash evaporation (20 Torr, 30 °C) gave crude trifluoromethyl α-benzamidoalkyl ketone (4), which was taken up in CCl_4 and flash evaporated again (20 Torr, 30 °C); residual solvents were removed under vacuum (0.01 Torr, room temperature). Further purification was achieved by recrystallization or flash chromatography (see detailed description of compounds).

General Procedure for the Preparation of Trifluoromethyl Benzamido Alcohol 5. GP 2. $NaBH_4$ was added to a rigorously stirred, cold solution (ice bath) of trifluoromethyl benzamido ketone 4 in EtOH (2 mL/mmol). After completion of the reaction (for details, see individual compounds), the mixture was acidified carefully with 6 N HCl and extracted with EtOAc (2 × 10 mL/mmol). The organic layers were combined, washed with brine (10 mL/mmol), and dried ($MgSO_4$) to give, after removal of the solvents by flash evaporation (rotatory evaporator, 20 Torr, 40 °C), trifluoromethyl benzamido alcohol **5** in good yield.

General Procedure for the Preparation of Trifluoromethyl Amino Alcohol 6. GP 3a,b. A solution of the trifluoromethyl benzamido alcohol **5**, obtained by GP 2, in concentrated HCl/H_2O/EtOH (2:1:1, 70 mL/mmol) was refluxed for several hours (for details, see individual compounds). The reaction mixture was then flash evaporated (rotary evaporator, 30–40 °C, 20 Torr) to give crude, solid amino alcohol hydrochloride **6**. Further purification was achieved with either of the following methods.

(a) Dissolving the residue in H_2O (10 mL/mmol), extraction with Et_2O (4–5 × 5 mL/mmol), flash evaporation of the aqueous layer (rotary evaporator, 30–40 °C, 20 Torr), and recrystallization of the solid residue to afford pure trifluoromethyl amino alcohol hydrochloride **6**.

(39) Matthews, J. S. *Biochim. Biophys. Acta* **1963**, *69*, 163.
(40) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(b) Dissolving the residue in H_2O (10 mL/mmol), basification (pH 14) by addition of solid NaOH, and extraction with EtOAc (5 × 5 mL/mmol). The organic layers were combined, dried (Na_2SO_4), and flash evaporated (40 °C, 20 Torr) to afford, after recrystallization, pure amino alcohol as the free base.

General Procedure for Acylation of Amino Alcohol 6 or 19 To Give the Trifluoromethyl Acylamino Alcohol 8 or Acylamino Hydroxy Ester 20, 22, or 24. GP 4. A solution of NMM (1.1 equiv) and the N-protected peptide in CH_3CN (5–11 mL/mmol) was cooled to −20 °C and *i*-BuOCOCl (1 equiv) was added. To this solution was added a precooled (−20 °C) mixture of the amino alcohol **6** or **19**, or the respective hydrochloride (1.1 equiv) and NMM (1.1 equiv) in DMF (1.2–1.5 mL/mmol) or $CHCl_3$ (1–7 mL/mmol). The reaction mixture was stirred at −20 °C for 4 h, allowed to warm to room temperature, and stirred overnight. The reaction mixture was poured into H_2O and extracted with EtOAc (2 × 15 mL/mmol), and the combined organic extracts were dried (Na_2SO_4). Solvents were evaporated, and the residue was flash chromatographed to yield trifluoromethyl acylamino alcohol **8** or acylamino hydroxy ester **20**, **22**, or **24** (for details, see individual description of compounds below).

General Procedure for Oxidation of Alcohol 8 or 20, 22, or 24 To Give Trifluoromethyl Acylamino Ketone 9 or Acylamino Keto Ester 21, 23, or 25. GP 5a,b,c. To a stirred solution of oxalyl chloride (1.1–1.5 equiv) in CH_2Cl_2 (2 mL/mmol), cooled to −60 °C, was added a solution of DMSO (2–3 equiv) in CH_2Cl_2 (0.4 mL/mmol). After 2–5 min a solution of the alcohol **8** or **21**, **24**, or **26** in CH_2Cl_2 (2 mL/mmol), and in some examples more DMSO, was added (for details, see individual description of compounds). Stirring was continued for 15 min at −60 °C, Et_3N (3–6 equiv) was added, and the mixture was allowed to warm to room temperature. Acylamino ketone **9** or **21**, **23**, or **25** was isolated after use of one of the following workups.

(a) With CH_2Cl_2/H_2O: 1 N HCl (4 mL/mmol) and CH_2Cl_2 (20 mL/mmol) were added. The layers were separated, the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL/mmol), and the organic layers were combined, washed (concentrated $NaHCO_3$, H_2O, and brine, each 40 mL/mmol), and dried ($MgSO_4$). Flash evaporation (rotary evaporator, 20 Torr, 40 °C) gave the ketone. (For further purification, see individual compounds.)

(b) With EtOac/H_2O: H_2O (20 mL/mmol) was added and the mixture was extracted with EtOAc (2 × 30 mL/mmol). The organic layers were combined, dried (Na_2SO_4), and flash evaporated to give the ketone. (For purification, see individual compounds.)

(c) The mixture was concentrated in vacuo to about one-third of its volume, applied to silical gel, and flash chromatographed. The product-containing fractions were combined and the solvents were removed to afford the ketone. (For details, see description of individual compounds.)

General Procedure for the Preparation of α-Hydroxy Acids 18a–c. GP 6. To a stirred suspension of aldehyde **17a**, **17b**, or **17c** in H_2O (2–3 mL/mmol) cooled in an ice bath was added a solution of $NaHSO_3$ (1 equiv) in H_2O (0.6–0.9 mL/mmol). After stirring at 0 °C for several hours, and for two examples at room temperature for several hours, the reaction was stored at 4 °C overnight. The stirred reaction mixture was allowed to warm to room temperature and EtOAc (15 mL/mmol) was added followed by a solution of KCN (1 equiv) in H_2O (1 mL/mmol). After 4–4.5 h, the layers were separated, and the organic layer was washed with H_2O (2 × 7–8 mL/mmol) followed by brine. Drying ($MgSO_4$) and concentration gave a quantitative yield of cyanohydrin as a viscous oil. The cyanohydrin was dissolved in dioxane (3.5–4.4 mL/mmol) and concentrated aqueous HCl (3–4 mL/mmol) was added. After gas evolution ceased, the solution was heated at reflux overnight. The cooled reaction mixture was washed with Et_2O (2 × 10 mL/mmol) and the aqueous layer was concentrated. The residue was dissolved in H_2O/2 N NH_4OH (pH 7–10), loaded onto an ion-exchange resin (H^+ form) column and eluted with H_2O followed by 2 N NH_4OH. Product-containing fractions were combined and concentrated. Crystallization of the residue from H_2O/CH_3COCH_3 gave the α-hydroxy acid.

General Procedure for Preparation of α-Hydroxy Esters 19a–c. GP 7. Into a stirred suspension of β-amino-α-hydroxy acid **18a**, **18b**, or **18c** in CH_3OH was bubbled HCl. After solution resulted, the reaction was cooled in an ice bath and the bubbling of the HCl gas was continued for several minutes. The reaction was capped with a drying tube and allowed to warm slowly to room temperature. The solution was concentrated, the residue was redissolved in CH_3OH, and the solution was saturated with HCl at ice bath temperature. The suspension was concentrated and the crude hydrochloride salt was dried under vacuum in a desiccator over KOH pellets. The free amine was generated by dissolving the crude hydrochloride in Et_3N (2.5 mL/mmol) and CH_3OH (3 mL/mmol), cooling the stirred solution in an ice bath, and adding Et_2O in portions to precipitate Et_3N·HCl. The suspension was stirred for several minutes and filtered, and the filtrate was concentrated. The free amine was used the same day without further purification.

***N*-(3,3,3-Trifluoro-1-methyl-2-oxopropyl)benzamide (4a).** A 13.2-g (0.075 mol) quantity of oxazolone **2a** in 29.3 mL (0.21 mol) of TFAA was heated at reflux for 1.75 h and then stirred at room temperature overnight. Treatment with oxalic acid (11.5 g, 0.13 mol) followed by workup and flash chromatography on silica gel (EtOAc/hexane, 3:7; recrystallized from CCl_4) gave 3.0 g (16%) of **4a** as an off-white solid, as a 3:1 mixture of ketone **4a** and its hydrate: ^1H NMR (CD_3COCD_3) δ 8.30 (br s, 1 H, NH), 8.0–7.7 (m, 3 H, aryl, NH) 7.6–7.3 (m, 3 H, aryl), 6.80 and 6.53 (2 br s, OH), 4.97 and 4.34 (2 quint, 3:1, 1 H, J = 7 Hz, CH), 1.55 and 1.47 (2 d, 3:1, 3 H, J = 7 Hz, CH_3); IR (KBr) 3440, 3300, 1770 ($COCF_3$), 1635 (CONH), 1575, 1530 cm^{-1}. Sublimation of a portion of this mixture gave ketone **4a** as a hygroscopic white solid: mp 115–117 °C; ^1H NMR (CD_3COCD_3) δ 8.26 (br s, 1 H, NH), 7.9–7.7 (m, 2 H, aryl), 7.6–7.3 (m, 3 H, aryl), 4.97 (quint, 1 H, J = 7 Hz, CH), 1.55 (d, 3 H, J = 7 Hz, CH_3); ^{19}F NMR (CD_3COCD_3) φ 87.9 (s, CF_3CO), 82.5 (s, $CF_3C(OH)_2$), ratio 13:1; IR (Nujol) 3290 (NH), 2910, 2840, 1765 ($COCF_3$), 1635 (CONH), 1525 cm^{-1}. Anal. ($C_{11}H_{10}F_3NO_2$) C, H, N.

Addition of 3 drops of H_2O to the NMR tube containing the sublimed material gave spectra, listed below, consistent with the hydrated form of **4a**: ^1H NMR (CD_3COCD_3/H_2O) δ 7.98 (br d, 1 H, NH), 7.95–7.9 (m, 2 H, aryl), 7.6–7.4 (m, 3 H, aryl), 4.50 (quint, 1 H, J = 7 Hz, CH), 1.41 (d, 3 H, J = 7 Hz, CH_3); ^{19}F NMR (CD_3COCD_3/H_2O) φ 82.5 (s, $CF_3C(OH)_2$); ^{13}C NMR (CD_3COCD_3/H_2O) δ 168.8 (CONH), 134.6, 132.3, 129.1 and 128.0 (aryl), 124.7 (q, J = 289 Hz, CF), 94.8 (q, J = 29 Hz, $C(OH)_2$), 50.6, 14.8.

***N*-[3,3,3-Trifluoro-2-oxo-1-(2-methylethyl)propyl]benzamide (4b) (GP 1).** A 70.9-g (0.35 mol) quantity of freshly distilled oxazolone **2b** and 141 mL (1.00 mol) of TFAA was heated for 15 h at reflux. Treatment with oxalic acid (54.0 g, 0.60 mol) and workup gave 42.8 g of ketone **4b** as a yellow solid, which crystallized from CCl_4 to give 31.5 g (33%) of analytically pure **4b** as white crystals, mp 94 °C: R_f = 0.70 (EtOAc); ^1H NMR ($CDCl_3$) δ 7.9 (m, 2 H, aroyl), 7.5 (m, 3 H, aroyl), 6.7 (m, 1 H, NH), 5.30 (dd, 1 H, J = 4, 7 Hz, NCH), 2.6–2.3 (m, 1 H, $CHMe_2$), 1.13 and 0.97 (2 d, 6 H, J = 6 Hz, 2 CH_3); ^{19}F NMR ($CDCl_3$) φ 85.3 (s, $CF_3C{=}O$), 79.0 (s, $CF_3C(OH)_2$), ratio 25:1; IR (KBr) 3340, 1765 (CF_3CO), 1640 (CONH), 1625 cm^{-1}; UV λ_{max} (ε) 223 (11 344); c = 5.24 × 10^{-5} M (CH_3CN). Anal. ($C_{13}H_{14}F_3NO_2 \cdot {}^1/_2H_2O$) C, H, N.

***N*-[3,3,3-Trifluoro-2-oxo-1-(phenylmethyl)propyl]benzamide (4c) (GP 1).** A 2.51-g (0.01 mol) quantity of freshly crystallized oxazolone **2c** was treated with TFAA (2.52 g, 12.0 mmol) for 24 h and then with oxalic acid (1.35 g, 15.0 mmol) to afford 2.02 g (63%) of a ketone/hydrate mixture of **4c** as a white powder: mp 162–163 °C (EtOAc/hexane); R_f = 0.78 (EtOH, NH_4OH 17%); ^1H NMR (CD_3COCD_3) δ 7.8 (m, 2 H, aroyl), 7.3 (m, 9 H, aroyl, aryl, NH), 5.1 and 4.3 (2 m, 1:4, 1 H, CHCO and $CHC(OH)_2$), 3.42 and 3.07 (ABX, 2 H, J = 15, 5, 7 Hz, CH_2-aryl); ^{19}F NMR (CD_3COCD_3, CF_3CO_2H) φ 86.4 (s, CF_3CO), 80.5 (s, $CF_3C(OH)_2$), ratio 1:4; IR (KBr) 3340, 1780 ($COCF_3$), 1680 (CONH) cm^{-1}; UV λ_{max} (ε) 226 nm (13 702), c = 4.16 × 10^{-5} M (CH_3CN). Anal. ($C_{17}H_{14}F_3NO_2 \cdot H_2O$) C, H, N.

***N*-(3,3,3-Trifluoro-2-hydroxy-1-methylpropyl)benzamide (5a) (GP 2).** A 1.00-g (0.0263 mol) quantity of $NaBH_4$ and 6.45 g (0.026 mol) of the ketone/hydrate mixture **4a** gave 6.19 g (95%) of alcohol **5a**, a yellow solid, used in the preparation of amino alcohol **6a** without further purification; ^1H NMR (CD_3COCD_3) δ 7.9–7.2 (m, 6 H, aryl, NH), 5.97 and 5.64 (2 br s, 1 H, OH), 4.6–4.1 (m, 2 H, CHO, CHN), 1.36 (br d, 3 H, J = 7 Hz, CH_3); ^{19}F NMR (CD_3COCD_3) φ 88.4 and 87.6 (2 d, J = 7.3 Hz), ratio 3.8:1; IR

(Nujol) 3380 (NH), 3290 (OH), 2920, 2840, 1640 (CONH), 1535 cm^{-1}.

***N*-[3,3,3-Trifluoro-2-hydroxy-1-(1-methylethyl)propyl]-benzamide (5b) (GP 2).** A 27.4-g (0.100 mol) quantity of ketone **4b** was reduced with 1.9 g (0.05 mol) of $NaBH_4$. Workup gave 25.1 g (91%) of crystalline (EtOAc/hexane) alcohol **5b**: mp 155–156 °C; R_f = 0.3 (EtOAc/hexane, 2:1); ^1H NMR ($CDCl_3$, CD_3OD) δ 7.8 (m, 2 H, aroyl), 7.5 (m, 3 H, aroyl), 6.9 (m, 1 H, NH), 4.4 (m, 1 H, CHN), 4.13 (q, 1 H, J = 7 Hz, CHO), 2.3 (m, 1 H, CH), 1.05 (d, 6 H, J = 6 Hz, CH_3); ^{19}F NMR ($CDCl_3$, CD_3OD) φ 87.2 (d, J = 7 Hz); IR (KBr) 3400–3300 (NH, OH), 1640 (CONH) cm^{-1}; MS (CI, NH_3) m/z (rel intensity) 276 (MH^+, 100), 176 ($M^+ - CF_3CH_2OH$, 20), 105 (C_6H_5CO, 10). Anal. ($C_{13}H_{16}F_3NO_2$) C, H, N.

1,1,1-Trifluoro-3-amino-2-butanol Hydrochloride (6a) (GP 3a). A 6.0-g (24.3 mmol) quantity of benzamide **5a** was cleaved upon 23 h of reflux. Trituration and digestion with EtOAc gave **6a** (2.11 g, one pair of enantiomers) as a white solid. Cooling the filtrate yielded additional **6a** (1.46 g, mixture of both pairs of enantiomers). The combined yield of the crops was 3.57 g (83%). Compound **6a** (one pair of enantiomers): mp 179–187 °C; ^1H NMR (D_2O) δ 4.43 (dq, 1 H, J = 3.4, 7.4 Hz, CHO), 3.82 (dq, 1 H, J = 3.4, 7.0 Hz, CHN), 1.37 (d, 3 H, J = 7.0 Hz, CH_3); ^{19}F NMR (D_2O) φ 88.0 (d, J = 7.1 Hz); MS (EI) m/z (rel intensity) 144 (MH^+, 1), 142 [$(M - H)^+$, 0.7], 128 (14), 80 (91), 74 (83), 69 (100); MS (DCI, CH_4) m/z (rel intensity) 144 (MH^+, 100), 126 (6). Anal. ($C_4H_8F_3NO \cdot HCl$) C, H, N.

Compound **6a** (mixture of both pairs of enantiomers, ratio 1.1:1): mp 123–157 °C; ^1H NMR (D_2O) δ 4.43 and 4.25 (dq and m, 1 H, CHO), 3.82 and 3.74 (dq and m, 1 H, CHN), 1.43 and 1.37 (2 d, 3 H, CH_3); ^{19}F NMR (D_2O) φ 88.0 and 87.0 (2 d, J = 7.1 Hz), ratio 1.1:1; MS (EI) m/z (rel intensity) 144 (MH^+, 78), 142 [$(M - H)^+$, 9], 128 (92), 124 (13), 90 (17), 80 (67), 74 (100), 69 (61); MS (DCI, CH_4) m/z (rel intensity) 144 (MH^+, 100), 126 (5). Anal. ($C_4H_8F_3NO \cdot HCl$) C, H, N.

1,1,1-Trifluoro-3-amino-4-methyl-2-pentanol Hydrochloride (6b) (GP 3a). A 25.1-g (0.091 mol) quantity of benzamide **5b** was cleaved (20 h reflux) to give 12.2 g (64%) of recrystallized (EtOAc/hexane) amino alcohol hydrochloride **6b**: mp 152–153 °C; R_f = 0.54 ($C_4H_9OH/AcOH/H_2O$, 3:1:1); ^1H NMR (D_2O) δ 4.60 (dq, 1 H, J = 7, 6 Hz, $CHCF_3$), 3.43 (t, 1 H, J = 6 Hz, CHN), 2.30 (m, 1 H, CH), 1.08 (dd, 6 H, J = 9, 2.2 Hz, CH_3); ^{19}F NMR (D_2O) φ 87.20 and 83.37 (2 d, J = 7 Hz), ratio 95:5; IR (KBr) 3180 (NH, OH), 1630, 1520 cm^{-1}; MS (DCI, NH_3) m/z (rel intensity) 170 (MH^+, 100). Anal. ($C_6H_{12}F_3NO \cdot HCl$) C, H, N.

1-[*N*-[(1,1-Dimethylethoxy)carbonyl]-L-valyl]-L-proline Phenylmethyl Ester. To a stirred soln of Boc-L-Val-OH (2.17 g, 10.0 mmol) in DMF (20 mL) cooled in an ice-CH_3OH bath was added 1-hydroxybenzotriazole hydrate (3.37 g). The resultant suspension was diluted with DMF (14 mL) to give a solution and L-proline phenylmethyl ester hydrochloride (2.42 g, 10.0 mmol) was added followed by NMM (1.10 mL, 10.0 mmol). DCC (2.3 g, 11.0 mmol) was added and the suspension was stirred at less than 5 °C for 3 h and then at room temperature overnight. Water (0.5 mL) was added, the reaction suspension was filtered, and the filtrate was concentrated [rotary evaporator and then under vacuum (0.4 mmHg) at 80 °C]. To the residue was added EtOAc (50 mL), and the suspension was stirred for several minutes and then filtered. The filtrate was diluted with EtOAc (75 mL) and the organic layer was washed with 0.5 N HCl (3 × 25 mL), H_2O (25 mL), saturated $NaHCO_3$ (2 × 25 mL), and brine (25 mL). Drying ($MgSO_4$) and concentration followed by flash chromatography using EtOAc/hexane (3:7) as the eluant gave Boc-Val-Pro-OBz (3.70 g, 91%) as a colorless liquid. An analytical sample was obtained by heating a portion of the sample under vacuum in a drying pistol containing refluxing acetone: ^1H NMR ($CDCl_3$) δ 7.33 (s, 5 H, Ar), 5.23 (br d, 1 H, NH), 5.18 and 5.13 (2 d, 2 H, CH_2O), 4.6–4.55 (m, 1 H, CHN), 4.27 (dd, 1 H, CHN), 3.8–3.7 and 3.7–3.6 (2 m, 2 H, CH_2N), 2.3–2.1 and 2.1–1.9 (2 m, 1 H and 4 H, CH_2CH_2, CH), 1.40 (s, 9 H, $(CH_3)_3C$), 1.00 (d, 3 H, CH_3), 0.89 (d, 3 H, CH_3); MS (DCI/CH_4) m/z (rel intensity) 405 (MH^+, 30), 349 (100), 331 (12), 305 (28), 206 (5); HRMS (MH^+) calcd for $C_{22}H_{32}N_2O_5$ 405.2389, found 405.2393.

L-Valyl-L-proline Phenylmethyl Ester Hydrochloride. Into a stirred solution of Boc-Val-Pro-OBz (3.49 g, 8.63 mmol) in EtOAc (65 mL) at 0 °C was rapidly bubbled HCl for 15 min. The reaction flask was capped with a drying tube and the solution was allowed to warm to room temperature. After 2 h, the solution was concentrated and the residue was dried under vacuum in a desiccator over KOH pellets to give 2.81 g of Val-Pro-OBz, which was used without further purification: ^1H NMR ($CDCl_3$) δ 8.32 (br s, 3 H, NH_3), 7.4–7.3 (m, 5 H, aryl), 5.14 and 5.05 (2 d, 2 H, CH_2O), 4.67 (dd, 1 H, CH), 4.20 (d, 1 H, CH), 4.0–3.9 and 3.6–3.4 (2 m, 2 H, CH_2N), 2.4–2.2 and 2.1–1.8 (2 m, 5 H, CH_2CH_2, CH), 1.08 and 1.06 (2 d, 6 H, CH_3); MS (EI) m/z (rel intensity) 305 (MH^+, 3), 304 (M^+, 4), 261 (4), 204 (2), 91 (36), 72 (100); MS (DCI/CH_4) m/z (rel intensity) 305 (MH^+, 100), 206 (14).

1-[*N*-(4-Methoxy-1,4-dioxobutyl)-L-valyl]-L-proline Phenylmethyl Ester. To a stirred solution of succinic acid monomethyl ester (1.07 g, 8.10 mmol) in CH_3CN (100 mL) at −15 °C was added NMM (0.89 mL, 8.10 mmol) followed after 6 min by *i*-BuOCOCl (1.05 mL, 8.10 mmol). After 11 min, a solution of Val-Pro-OBz (2.76 g, 8.10 mmol) and NMM (0.89 mL, 8.10 mmol) in CH_3CN (20 mL) was slowly added. The reaction was stirred at −10 to −20 °C for 45 min, at −5 to −20 °C for 45 min, and then allowed to warm to room temperature. After 2 h, the reaction mixture was concentrated, EtOAc (75 mL) was added, and the resulting suspension was filtered. The filtered solid was washed with EtOAc (3 × 10 mL) and the combined filtrate and washes were concentrated. The residue was flash chromatographed with EtOAc/hexane (9:1) as the eluant to give MeO-Suc-Val-Pro-OBz (3.14 g, 93%) as a colorless, viscous oil. An analytical sample was obtained by heating a portion of the sample under high vacuum in a drying pistol containing refluxing toluene: ^1H NMR ($CDCl_3$) δ 7.4–7.3 (m, 5 H, aryl), 6.39 (d, 1 H, NH), 5.18 and 5.14 (2 d, 2 H, CH_2O), 4.7–4.5 (m, 2 H, CHN), 3.9–3.7 and 3.7–3.6 and 3.67 (2 m and s, 5 H, CH_2N, CH_3O), 2.8–2.4 (m, 4 H, $COCH_2CH_2CO$), 2.3–2.1 and 2.1–1.9 (2 m, 5 H, CH_2CH_2, CH), 0.97 (d, 3 H, CH_3), 0.90 (d, 3 H, CH_3); MS (EI) m/z (rel intensity) 419 (MH^+, 21), 418 (M^+, 29), 387 (17), 214 (15), 204 (11), 186 (100), 115 (21), 91 (23), 72 (41), 70 (28). MS (DCI/CH_4) m/z (rel intensity) 419 (MH^+, 41), 206 (100). Anal. ($C_{22}H_{30}N_2O_6$) C, H, N.

1-[*N*-(4-Methoxy-1,4-dioxobutyl)-L-valyl]-L-proline (7a). To a solution of MeO-Suc-Val-Pro-OBz (2.21 g, 5.28 mmol) in EtOAc (110 mL) under an argon atmosphere was added 10% palladium on charcoal (0.44 g). The stirred mixture was hydrogenated under 1 atm of hydrogen at room temperature for 4 h. The catalyst was removed by filtration through Celite and the filtrate was concentrated to give a quantitative yield of **7a** as an oily, white foam: ^1H NMR ($CDCl_3$) δ 7.12 (d, 1 H, NH), 6.70 (br s, 1 H, OH), 4.63 (t, 1 H, CHN), 4.51 (dd, 1 H, CHN), 3.9–3.8 and 3.73–3.6 and 3.73 (2 m and s, 5 H, CH_2N, CH_3O), 2.8–2.4 (m, 4 H, $COCH_2CH_2CO$), 2.3–1.9 (m, 5 H, CH, CH_2CH_2), 1.01 (d, 3 H, CH_3), 0.94 (d, 3H, CH_3); MS (DCI/CH_4) m/z (rel intensity) 329 (MH^+, 100), 311 (2), 297 (3), 214 (35), 186 (6), 144 (6), 116 (87); HRMS (MH^+) calcd for $C_{15}H_{24}N_2O_6$ 329.1713, found 329.1716.

***N*-(4-Methoxy-1,4-dioxobutyl)-L-valyl-*N*-[3,3,3-trifluoro-2-hydroxy-1-(phenylmethyl)propyl]-L-prolinamide (8a) (GP 4).** A solution of CH_3O-Suc-Val-Pro-OH (**7a**; 1.22 g, 3.72 mmol) and NMM (0.41 mL, 3.72 mmol) in CH_3CN (80 mL) was treated with *i*-BuOCOCl (0.48 mL, 3.72 mmol) and amino alcohol **6c** (0.81 g, 3.72 mmol, essentially one pair of enantiomers) in CH_3CN (8 mL) to afford, after flash chromatography (EtOAc), 1.83 g (93%) of amide alcohol **8a** as an oily, white foam: ^{19}F NMR ($CDCl_3$) φ 88.1 (d, J = 7.6 Hz), 88.0 (d, J = 7.3 Hz), 87.9 (d, J = 7.9 Hz), 87.5 (d, J = 7.6 Hz), ratio 40:13:13:34; MS (EI) m/z (rel intensity) 529 (M^+, 6), 316 (5), 214 (11), 186 (18), 115 (14), 70 (100); MS (DCI/CH_4) m/z (rel intensity) 530 (MH^+, 100), 510 (8), 317 (72), 115 (14); HRMS (MH^+) calcd for $C_{25}H_{34}N_3F_3O_6$ 529.2400, found 529.2391.

***N*-(4-Methoxy-1,4-dioxobutyl)-L-alanyl-L-alanyl-*N*-[3,3,3-trifluoro-2-hydroxy-1-(phenylmethyl)propyl]-L-prolinamide (8b) (GP 4).** A solution of MeO-Suc-Ala-Ala-Pro-OH (**7b**;[41,42] 1.00 g, 2.68 mmol) and NMM (0.30 mL, 2.74 mmol) in CH_3CN (25 mL) was treated with *i*-BuOCOCl (0.35 mL, 2.68 mmol), and the free base was generated from amino alcohol hydrochloride **6c** (0.60 g, 2.74 mmol, essentially one pair of enantiomers) in CH_3CN (4

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mL). The reaction mixture was concentrated and the residue was flash chromatographed (gradient: 0–40% CH_3COCH_3 in EtOAc). Concentration of the combined product-containing fractions gave a white foam, which was dissolved in a minimum amount of hot CH_2Cl_2. Ether was added to precipitate prolinamide **8b** [0.36 g, 94% of two diastereoisomers (12:1) plus 6% of three other components by reverse-phase HPLC] as a white solid. Concentration of the filtrate gave more of the title compound [0.60 g, 87% of same two isomers (1:21) plus 13% of four other components by reverse-phase HPLC] as an oily foam. Both mixtures were used separately without further purification in the subsequent oxidation step to afford **9b**. The white, solid mixture: ^{1}H NMR ($CDCl_3/CD_3OD$, 1:1) δ 7.15 (s, 5 H, aryl), 3.65 (s, 3 H, OCH_3), 1.58 (d, 6 H, CH_3); ^{19}F NMR ($CDCl_3/CD_3OD$, 1:1) ϕ 88.0 (d, J = 8 Hz) and several minor signals; MS (EI) m/z (rel intensity) 572 (M^+, 13), 541 (9), 473 (2), 414 (3), 371 (4), 354 (10), 326 (14), 257 (89), 186 (70), 158 (23), 115 (30), 70 (100); MS (DCI/CH_4) m/z (rel intensity) 573 (MH^+, 100), 317 (16). Oily, foamy mixture: ^{1}H NMR ($CDCl_3$) δ 7.20 (s, 5 H, aryl), 3.65 (s, 3 H, OCH_3), 1.5–1.1 (m, 6 H, CH_3); ^{19}F NMR ($CDCl_3$) ϕ 87.6 (d, J = 7 Hz), 87.2 (t, J = 7 Hz) and several minor signals; MS (EI) m/z (rel intensity) 573 (MH^+, 3), 572 (M^+, 2), 541 (12), 473 (3), 414 (6), 354 (13), 257 (100), 186 (78), 158 (28), 115 (37), 70 (45); MS (DCI, *iso*-butane) m/z (rel intensity) 573 (MH^+, 100).

***N*-Acetyl-L-prolyl-L-alanyl-*N*-(3,3,3-trifluoro-2-hydroxy-1-methylpropyl)-L-prolinamide (8c).** A suspension of NMM (0.40 mL, 3.61 mmol) and **7c** (1.17 g, 3.61 mmol) in CH_3CN (55 mL) was cooled to −20 °C and *i*-BuOCOCl (0.47 mL, 3.61 mmol) was added. After 10 min, a suspension of amino alcohol **6a** (648 mg, 3.61 mmol) and NMM (0.40 mL, 3.61 mmol) in $CHCl_3$ (25 mL) was added and the reaction mixture was allowed to warm slowly to room temperature. After 4 h the reaction mixture was concentrated, and the residue was dissolved in H_2O (65 mL) and treated with a mixed-bed resin (J.T. Baker: ANG MI-615, 17 g). The resin was removed by filtration, the filtrate was concentrated and the residue was flash chromatographed (CH_3OH/CH_2Cl_2, 1:9) to give a foamy, white solid. Trituration with Et_2O gave **8c** (365 mg, 23%) as a hygroscopic, white solid: ^{1}H NMR (D_2O) δ 4.6–3.8 (m, 5 H, CH), 3.8–3.3 (m, 4 H, CH_2N), 2.4–1.6 and 2.01 (m and s, 11 H, CH_2CH_2, CH_3CO), 1.5–1.05 (m, 6 H, CH_3); ^{1}H NMR ($CDCl_3$) δ 8.4–7.1 (m, 2 H, NH), 6.25–5.3 (m, 1 H, OH), 4.9–3.2 (m, 9 H, CH, CH_2N), 2.4–1.7 and 2.11 (m and s, 11 H, CH_2CH_2, CH_3CO), 1.32 (br t, 6 H, CH_3); IR (KBr) 3300, 2985, 1645, 1530, 1475 cm^{-1}.

***N*-Acetyl-L-alanyl-L-alanyl-*N*-(3,3,3-trifluoro-2-hydroxy-1-methylpropyl)-L-prolinamide (8d) (GP 4).** A solution of Ac-Ala-Ala-Pro-OH[35] (**7d**; 0.30 g, 0.001 mol) and NMM (0.11 mL, 0.001 mol) in CH_3CN (20 mL) at −15 °C was treated with *i*-BuOCOCl (0.13 mL, 0.001 mol) and amino alcohol hydrochloride **6a** (0.18 g, 0.001 mol, one pair of enantiomers) in NMM (0.11 mL, 0.001 mol) and $CHCl_3$ (6 mL). The reaction mixture was concentrated and the residue was flash chromatographed (CH_3CO-CH_3) to give fluorinated prolinamide analogue **8d** (0.36 g, 85%) as a white solid. A portion of the white solid was dissolved in H_2O and lyophilized to obtain an analytically pure sample: ^{1}H NMR (CD_3COCD_3) δ 7.9–7.3 (m, 3 H, NH), 5.8–5.65 (m, 1 H, OH), 4.7–4.1 (m, 5 H, CH), 3.8–3.4 (m, 2 H, CH_2N), 2.3–1.8, 1.94 and 1.93 (m and 2 s, 7 H, CH_2CH_2 and CH_3CO), 1.3–1.1 (m, 9 H, CH_3); ^{19}F NMR (CD_3COCD_3) ϕ 88.4 (d, J = 7.6 Hz), 88.3 (d, J = 7.6 Hz), 88.2 (d, J = 8.2 Hz), ratio 51:34:15; MS (EI) m/z (rel intensity) 424 (M^+, 1), 338 (1), 325 (1), 282 (1), 254 (4), 185 (3), 114 (4), 70 (100), 44 (82); MS (DCI/CH_4) m/z (rel intensity) 425 (MH^+, 70), 325 (13), 312 (15), 282 (8), 254 (8), 241 (100); HRMS calcd for $C_{17}H_{27}F_3N_4O_5$ 424.1934, found 424.1923.

***N*-[(1,1-Dimethylethoxy)carbonyl]-L-alanyl-L-alanyl-*N*-[3,3,3-trifluoro-2-hydroxy-1-(1-methylethyl)propyl]-L-prolinamide (8e) (GP 4).** A solution of Boc-Ala-Ala-Pro-OH (**7e**; 1.0 g, 2.80 mmol) and NMM (0.34 mL, 3.06 mmol) in CH_3CN (25 mL) was treated with *i*-BuOCOCl (0.37 mL, 2.88 mmol) and amino alcohol hydrochloride **6b** (0.61 g, 2.91 mmol) in DMF (4 mL) and NMM (0.34, 3.06 mmol) to give, after flash chromatography (EtOAc), the desired product **8e** (1.09 g, 76%): ^{1}H NMR ($CDCl_3$) δ 7.8–7.1 (m, 3 H, 3 × NH), 5.5–4.6 (m, 4 H, 3 × CH), 1.80 (s, 9 H, CH_3), 1.8–0.8 (m, 12 H, CH_3); IR (KBr) 3440, 3320, 2980, 2940, 2880, 1770, 1740, 1665 cm^{-1}; MS (DCI/CH_4) m/z (rel intensity) 511 (MH^+, 100), 455 (90), 411 (12), 269 (22); HRMS (MH^+) calcd for $C_{25}H_{37}F_3N_4O_6$ 511.2743, found 511.2794.

***N*-(4-Methoxy-1,4-dioxobutyl)-L-alanyl-L-alanyl-*N*-[3,3,3-trifluoro-2-hydroxy-1-(1-methylethyl)propyl]-L-prolinamide (8f) (GP 4).** A solution of MeO-Suc-Ala-Ala-Pro-OH (**7b**;[41,42] 1.07 g, 2.88 mmol) and NMM (0.34 mL, 3.06 mmol) in CH_3CN (25 mL) was treated with *i*-BuOCOCl (0.37 mL, 2.88 mmol) and amino alcohol hydrochloride **6b** (0.61 g, 2.91 mmol, essentially one pair of enantiomers) in NMM (0.34 mL, 3.06 mmol) and DMF (4 mL). The reaction mixture was concentrated and the residue was flash chromatographed (gradient: 0–25% CH_3COCH_3 in EtOAc) to give 1.22 g (81%) of amide alcohol **8f** as a white solid; ^{1}H NMR ($CDCl_3$) δ 8.4 and 8.2 (2 br d, 1 H, NH), 7.53 (m, 1 H, NH), 6.9 (m, 1 H, NH), 5.3–4.5 (m, 4 H, NCHCO, OH), 4.3–3.5 and 3.70 (m and s, 7 H, CHO, CHN, CH_2N and CH_3O), 2.8–2.4 (m, 4 H, $COCH_2CH_2CO$), 2.4–1.8 (m, 5 H, CH_2CH_2, CH), 1.5–0.8 (m, 12 H, CH_3); ^{19}F NMR ($CDCl_3$) ϕ 88.0, 87.5, 86.9, 85.6, and 85.2 (5 d, J = 7 Hz), ratio 46:41:9:2:2; IR ($CDCl_3$) 3300, 3080, 2970, 2940, 2880, 1740, 1630, 1540 cm^{-1}. Anal. ($C_{22}H_{35}F_3N_4O_7$) C, H, N.

***N*-[(1,1-Dimethylethoxy)carbonyl]-D-phenylalanyl-*N*-[3,3,3-trifluoro-2-hydroxy-1-(1-methylethyl)propyl]-L-prolinamide (8g) (GP 4).** A solution of Boc-D-Phe-Pro-OH (**7f**; 3.6 g, 10.0 mmol) and NMM (1.0 g, 10 mmol) in CH_3CN (50 mL) was treated at −10 °C with *i*-BuOCOCl and 3-amino-1,1,1-trifluoro-4-methyl-2-pentanol hydrochloride (**6b**) to afford after flash chromatography (EtOAc/hexane, 3:1), prolinamide **8g** (4.11 g, 7.9 mmol) in 80% yield: ^{1}H NMR ($CDCl_3$) δ 7.23 (s, 5 H, aryl), 5.4 (m, 1 H, CH), 5.2 (m, 1 H, CH), 2.96 (d, 2 H, J = 6 Hz, CH_2), 1.42 (s, 9 H, CH_3), 1.1–0.8 (m, 6 H, CH_3); IR (KBr) 3360, 2970, 1695, 1665, 1640 cm^{-1}; MS (DCI/CH_4) m/z (rel intensity) 516 (MH^+, 28), 198 (100); HRMS (MH^+) calcd for $C_{25}H_{32}F_3N_3O_6$ 516.2685, found 516.2683.

***N*-(4-Methoxy-1,4-dioxobutyl)-L-valyl-*N*-[3,3,3-trifluoro-2,2-dihydroxy-1-(phenylmethyl)propyl]-L-prolinamide (9a).** To a stirred suspension of the Dess–Martin periodinane[14b] (2.64 g, 6.22 mmol) in CH_2Cl_2 (100 mL) under an argon atmosphere was added CF_3CO_2H (0.48 mL, 6.22 mmol) followed after 8 min by alcohol **8a** (0.89 g, 1.68 mmol) in CH_2Cl_2 (25 mL). After 6 days at room temperature, the reaction mixture was diluted with Et_2O (60 mL) and a solution of $NaHCO_3$ (3.33 g) and $Na_2S_2O_3 \cdot 5H_2O$ (9.23 g) in H_2O (90 mL) was added. The biphasic system was stirred until both layers were clear. The layers were separated, the aqueous layer was extracted with additional CH_2Cl_2 (30 mL), and the combined extracts were washed with saturated aqueous $NaHCO_3$ (2 × 45 mL) followed by 90 mL of a mixture of brine/H_2O (2:1). After drying ($MgSO_4$) and concentration, the residue was flash chromatographed with EtOAc/hexane (9:1) as the eluant to give an oily, off-white foam. Addition of Et_2O followed by hexane and concentration gave **9a** (0.83 g, 90%) as a white solid: ^{1}H (Me_2SO-d_6) δ 8.08 (br t, 1 H, NH), 7.90 and 7.77 (2 br d, 1 H, NH), 7.3–7.1 and 7.12 and 7.10 (m and 2 s, 6 H, aryl, OH), 7.06 and 6.83 (2 s, 1 H, OH), 4.4–4.1 (7, 3H, CHN), 3.7–3.4 and 3.553 and 3.549 (m and 2 s, 5 H, CH_2N, CH_3O), 3.2–3.0 and 2.8–2.55 (2 m, 2 H, aryl CH_2), 2.55–2.2 (m, $COCH_2CH_2CO$), 2.0–1.5 (m, 5 H, CH_2CH_2, CH), 1.0–0.75 (m, 6 H, CH_3); ^{19}F NMR (Me_2SO-d_6) ϕ 88.8, 88.5, 83.6, 83.2, 83.1, 83.0 (6 s), ratio 1.4: 1.1:4.0:54.4:36.1:2.9; MS (DCI/CH_4) m/z (rel intensity) 528 ($MH^+ - H_2O$, 33), 315 (100), 214 (11), 186 (5), 115 (8), 70 (8). Anal. ($C_{25}H_{32}F_3N_3O_6 \cdot H_2O$) C, H, N.

***N*-(4-Methoxy-1,4-dioxobutyl)-L-alanyl-L-alanyl-*N*-[3,3,3-trifluoro-2,2-dihydroxy-1-(phenylmethyl)propyl]-L-prolinamide (9b) (GP 5c).** A 0.6-g quantity (1 mmol) of the oily foamy mixture from the preparation of **8b** was oxidized with oxalyl chloride (0.13 mL, 1.57 mmol) and DMSO (0.22 mL, 3.14 mmol) in CH_2Cl_2 (5.5 mL). Flash chromatography (EtOAc/CH_3COCH_3, 3:1) gave 0.35 g of **9b** as a white foam [90% of two diastereomers (43:47), plus 5% of starting material plus 5% of three other components by reverse-phase HPLC]: ^{1}H NMR (Me_2SO-d_6) δ 7.3–7.1 (m, 5 H, aryl), 3.57 (s, 3 H, OCH_3), 1.2–1.1 (m, 6 H, CH_3); ^{13}C NMR (Me_2SO-d_6) δ 92.97 (q, J = 29.3 Hz), 92.88 (q, J = 29.3 Hz); ^{19}F NMR (Me_2SO-d_6) ϕ 83.7, 83.53 and 83.45 (3 s) and several minor signals; IR ($CDCl_3$) 3280, 3080, 2980, 1735, 1650, 1540, 1450 cm^{-1}; MS m/z (rel intensity) 571 ($MH^+ - H_2O$, 100), 539 (80), 315 (82), 257 (27), 183 (25), 142 (54), 118 (40); HRMS ($MH^+ - H_2O$) calcd for $C_{26}H_{35}F_3N_4O_8$ 571.2380, found 571.2394.

***N*-Acetyl-L-prolyl-L-alanyl-*N*-(3,3,3-trifluoro-2,2-dihydroxy-1-methylpropyl)-L-prolinamide (9c) (GP 5c).** Oxalyl

chloride (0.082 mL, 0.94 mmol) in CH_2Cl_2 (1 mL), DMSO (0.14 mL, 1.89 mmol) in CH_2Cl_2 (0.5 mL), and alcohol prolinamide **8c** (340 mg, 0.75 mmol) in CH_2Cl_2 (1.5 mL) gave crude **9c**. Repetitive flash chromatography with CH_3OH/CH_2Cl_2 (8:92) and trituration with Et_2O/hexane (1:9) gave **9c** (105 mg, 30%) as a white solid: ^1H NMR ($CDCl_3$) δ 8.0–5.9 (m, 3 H, NH), 4.9–4.0 (m, 4 H, CH), 4.0–3.2 and 3.40 (m and br s, 6 H, CH_2N, OH), 2.5–1.6 and 2.11 (m and s, 11 H, CH_2CH_2, CH_3CO), 1.5–1.1 (m, 6H, CH_3); MS (DCI/CH_4) *m/z* (rel intensity) 449 ($MH^+ - H_2O$, 55), 239 (100), 211 (28), 140 (23).

***N*-Acetyl-L-alanyl-L-alanyl-*N*-(3,3,3-trifluoro-2,2-dihydroxy-1-methylpropyl)-L-prolinamide (9d) (GP 5c).** Oxalyl chloride (83 μL, 0.95 mmol) in CH_2Cl_2 (1.5 mL), DMSO (0.14 mL, 1.91 mmol), and alcohol **8d** (0.27 g, 0.64 mmol) in 1.5 mL of CH_2Cl_2 gave 0.12 g (43%) of trifluoro ketone hydrate **9d**, as an off-white solid after lyophilization: R_f = 0.28 (CH_3COCH_3); ^1H NMR (CD_3COCD_3) δ 8.1–7.8 and 7.7–7.5 and 7.4–7.3 (3 m, 3 H, NH), 6.5–6.2 and 6.2–6.1 (2 m, 2 H, OH), 4.7–4.1 (m, 4 H, CH), 3.8–3.4 (m, 2 H, CH_2N), 2.3–1.8 and 1.93 and 1.92 (m and 2 s, 7 H, CH_2CH_2, CH_3CO), 1.3–1.2 (m, 9 H, CH_3); ^{13}C NMR (CD_3COCD_3) δ 124.63 (q, *J* = 288.0 Hz, CF_3), 124.58 (q, *J* = 287.4 Hz, CF_3), 94.90 (q, *J* = 30.2 Hz, $C(OH)_2$), 94.83 (q, *J* = 30.1 Hz, $C(OH)_2$); ^{19}F NMR (D_2O) φ 82.0 and 81.9 (2 s), ratio 1.3:1; ^{19}F NMR (CD_3COCD_3) φ 82.5, 82.4, 82.1, and 82.0 (4 s), ratio 36:42:13:9; MS (EI) *m/z* (rel intensity) 422 ($M^+ - H_2O$, 1), 336 (1), 254 (4), 185 (3), 167 (4), 157 (3), 114 (9), 86 (7), 70 (98), 44 (100); MS (CDI/*i*-butane) *m/z* (rel intensity) 423 ($MH^+ - H_2O$, 100), 254 (5), 239 (9), 185 (3); HRMS calcd for $C_{17}H_{25}F_3N_4O_5$ 422.1777, found 422.1777.

***N*-[(1,1-Dimethylethoxy)carbonyl]-L-alanyl-L-alanyl-*N*-[3,3,3-trifluoro-1-(1-methylethyl)-2-oxopropyl]-L-prolinamide (9e) (GP 5c).** Oxalyl chloride (0.078 mL, 0.9 mmol) in CH_2Cl_2 (2 mL), DMSO (0.125 mL, 1.8 mmol), and alcohol prolinamide **8e** (260 mg, 0.53 mmol) gave 180 mg (70%) of ketone **9e** (elution: EtOAc): ^1H NMR ($CDCl_3$) δ 7.8 (m, 1 H, NH), 5.1–4.6 (m, 4 H, CH), 1.45 (s, 9 H, CH_3), 1.4–0.8 (m, 12 H, CH_3); ^{19}F NMR ($CDCl_3$) φ 85.90 and 85.87 (2 s); ratio 1:1; IR (KBr) 3430, 3320, 2980, 2940, 2880, 1765, 1690, 1635 cm^{-1}.

***N*-(4-Methoxy-1,4-dioxobutyl)-L-alanyl-L-alanyl-*N*-[3,3,3-trifluoro-1-(1-methylethyl)-2-oxopropyl]-L-prolinamide (9f) (GP 5c).** Oxalyl chloride (0.13 mL, 1.50 mmol), DMSO (0.21 mL, 3.00 mmol), and prolinamide **8f** (0.53 g, 1.00 mmol) in CH_2Cl_2 (2.5 mL) and DMSO (0.4 mL) gave, after flash chromatography, (gradient: 25–35% CH_3COCH_3 in EtOAc) 0.39 g (74%) of a mixture of ketone **9f** and its hydrate (9:1), as white foam: ^1H NMR ($CDCl_3$) δ 8.3–7.7 (m, 2 H, NH) 6.9–6.7 (m, 1 H, NH), 5.0–4.0 (br m, 4 H, CH), 3.8–3.4 and 3.63 (m and s, 5 H, CH_2N, CH_3O), 2.7–2.4 (m, 4 H, $COCH_2CH_2CO$), 2.4–1.8 (m, 5 H, CH_2CH_2, CH), 1.4–0.7 (m, 12 H, CH_3); ^{19}F NMR ($CDCl_3$) φ 85.8, 85.7, 80.1, and 79.7 (4 s), ratio 9:9:1:1; IR ($CDCl_3$) 3420, 3300, 3030, 3005, 2970, 2930, 2880, 1770, 1735, 1675, 1640, 1520 cm^{-1}; MS (DCI, NH_4) *m/z* (rel intensity) 523 (MH^+, 100), 267 (46); HRMS (MH^+) calcd for $C_{22}H_{33}F_3N_4O_7$ 523.2380, found 523.2443.

***N*-[(1,1-Dimethylethoxy)carbonyl]-D-phenylalanyl-*N*-[3,3,3-trifluoro-1-(1-methylethyl)-2-oxopropyl]-L-prolinamide (9g) (GP 5b).** Oxalyl chloride (0.65 mL, 7.5 mmol) in CH_2Cl_2 (15 mL), DMSO (1.05 mL, 15 mmol) in CH_2Cl_2 (2.5 mL), and alcohol prolinamide **8g** (2.5 g, 4.85 mmol) in CH_2Cl_2 (5 mL) gave, after purification by flash chromatography with EtOAc/hexane (4:1), 1.8 g (72%) of ketone **9g**: ^1H NMR ($CDCl_3$) δ 7.3–7.2 (m, 5 H, aryl), 4.8 (m, 1 H, CH), 4.6 (m, 1 H, CH), 4.49 (t, 1 H, *J* = 6.0 Hz, CH), 1.4 (m, 9 H, CH_3), 1.03 (d, 3 H, *J* = 6.9 Hz, CH_3), 0.96 (d, 3 H, *J* = 6.6 Hz, CH_3), 0.89 (m, 6 H, CH_3); ^{19}F NMR ($CDCl_3$), φ 85.9 and 85.8 (2 s), ratio 1:1; IR (KBr) 3380, 2970, 2930, 2875, 1760, 1625 cm^{-1}; MS (DCI/CH_4) *m/z* (rel intensity) 514 (MH^+, 28), 458 ($M - C_4H_9$, 41), 414 ($M - C_5H_9O_2$, 46); HRMS (MH^+) calcd for $C_{25}H_{34}F_3N_3O_5$ 514.2528, found 514.2526.

D-Phenylalanyl-*N*-[3,3,3-trifluoro-1-(1-methylethyl)-2-oxopropyl]-L-prolinamide Hydrochloride (9h). A solution of 1.10 g (2.14 mmol) of protected trifluoromethyl ketone **9g** in EtOAc (100 mL) was cooled to 0 °C and treated with HCl gas for 5 min. The reaction mixture was stirred at 0 °C for 1.5 h and removal of solvent in vacuo gave amino ketone hydrochloride **9h** (0.95 g, 2.14 mol) in 99% yield. The product was used in subsequent reactions without purification: ^1H NMR ($CDCl_3$) δ 7.28 (s, 5 H, aryl), 2.4–1.7 (br m, 6 H, CH_2), 1.1–0.7 (br m, 6 H, CH_3); IR (KBr) 3420, 2970, 2880, 1765, 1650 cm^{-1}; MS (CDI/CH_4) *m/z* (rel intensity) 414 (MH^+, 100), 396 (82); HRMS (MH^+) calcd for $C_{20}H_{26}F_3N_3O_3$ 414.2004, found 414.1991.

***N*-(4-Methoxy-1,4-dioxobutyl)-D-phenylalanyl-*N*-[3,3,3-trifluoro-1-(1-methylethyl)-2-oxopropyl]-L-prolinamide (9i).** To a solution of amino ketone hydrochloride **9h** (0.40 g, 0.9 mmol) in pyridine (2 mL) was added 3-carbomethoxypropionyl chloride (0.27 g, 1.8 mmol). The reaction mixture was stirred for 2 h, poured into H_2O, and extracted with EtOAc. The combined organic fractions were dried (Na_2SO_4), solvents were removed in vacuo, and the residue was purified via flash chromatography (EtOAc/hexane, 3:1) to yield 0.31 g (59%) of **9i**: ^1H NMR ($CDCl_3$) δ 7.65 (m, 1 H, NH), 7.3–7.2 (m, 5 H, aryl), 6.5 (m, 1 H, NH), 4.9 (m, 1 H, CH), 4.8 (m, 1 H, CH), 4.5 (m, 1 H, CH), 3.69 (s, 3 H, CH_3), 3.02 (d, 2 H, *J* = 6 Hz, CH_2), 1.0 (m, 6 H, CH_3); ^{19}F NMR ($CDCl_3$) φ 85.9 and 85.7 (2 s); ratio, 1:1; IR (neat) 3300, 2960, 1760, 1740, 1650 cm^{-1}; MS (DCI/NH_4) *m/z* (rel intensity) 528 (MH^+, 100), 267 (23); HRMS (MH^+) calcd for $C_{25}H_{32}F_3N_3O_6$ 528.2321, found 528.2355.

***N*-L-Alanyl-L-alanyl-*N*-[3,3,3-trifluoro-1-(1-methylethyl)-2-oxopropyl]-L-prolinamide Hydrochloride.** HCl gas was bubbled through a cold (0 °C) solution of protected trifluoromethyl ketone **9e** (0.180 g, 0.35 mmol) in EtOAc (50 mL) for 5 min. The reaction mixture was stirred at 0 °C for 1.5 h, and solvents were removed in vacuo to afford 0.151 g (96%) of the amino ketone hydrochloride salt, which was used as such in the preparation of compound **10**: IR (KBr) 3300, 2980, 1680, 1640, 1545, 1470, 1180 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.7 (m, 1 H, NH), 8.25 (m, 3 H, $HCl{\cdot}H_2N$), 4.65–4.40 (m, 3 H, CH), 2.23 (m, 1 H), 2.0–1.8 (m, 4 H, CH_2), 1.32 (d, 3 H, *J* = 7.5 Hz, alanine CH_3), 1.25 (d, 3 H, *J* = 7.5 Hz, alanine CH_3), 1.90 (d, 3 H, *J* = 6.0 Hz, valine CH_3), 1.80 (d, 3 H, *J* = 6.0 Hz, valine CH_3); MS (DCI/CH_4) *m/z* (rel intensity) 409 (MH^+, 100), 389 (5), 267 (85); HRMS (MH^+) calcd for $C_{17}H_{27}F_3N_4O_4$ 409.2062, found 409.2030.

***N*-[[5-(Dimethylamino)-1-naphthalenyl]sulfonyl]-L-alanyl-L-alanyl-*N*-[3,3,3-trifluoro-1-(1-methylethyl)-2-oxopropyl]-L-prolinamide (10).** To a suspension of 50 mg of the above ketone hydrochloride (0.11 mmol) in CH_2Cl_2 (1 mL) was added NMM (50 mg, 0.5 mmol). The solution was stirred for 5 min and dansyl chloride (50 mg) was added. The reaction mixture was stirred for 2 h at room temperature with the exclusion of light and then purified by flash chromatography. Elution with EtOAc gave 48 mg (68%) of dansyl peptide **10**: ^1H NMR ($CDCl_3$) δ 8.52 (d, 1 H, *J* = 6.0 Hz, aryl), 8.2 (m, 2 H, aryl), 7.67 (d, 1 H, *J* = 8.7 Hz, NH), 7.5 (m, 3 H, aryl), 7.17 (d, 1 H, *J* = 6.0 Hz, aryl), 7.05 (d, 1 H, *J* = 6.0 Hz, NH), 6.95 (d, 1 H, *J* = 6.0 Hz, NH), 4.8 (m, 1 H, CH), 4.69 (dd, 1 H, CH), 4.55 (q, 1 H, *J* = 6.8 Hz, CH), 2.89 (s, 6 H, NCH_3), 1.2–0.9 (m, 12 H, CH_3); ^{19}F NMR ($CDCl_3$) φ 85.9 and 85.8 (2 s), ratio 1:1; IR (KBr) 3350, 2980, 2940, 2880, 2840, 2790, 1760, 1680, 1635 cm^{-1}; MS (DCI/NH_4) *m/z* (rel intensity) 642 (MH^+, 49), 251 (100); HRMS (MH^+) calcd for $C_{29}H_{38}F_3N_5O_6S$ 642.2572, found 642.2571. Anal. ($C_{29}H_{38}F_3N_5O_6S$) C, H, N.

***N*^{1}-[(1,1-Dimethylethoxy)carbonyl]-*N*^{α}-[3,3,3-trifluoro-2-hydroxy-1-(1-methylethyl)propyl]-L-prolinamide (12).** A solution of 1.14 g (5.80 mmol) of *N*-(*tert*-butyloxycarbonyl)-L-proline (**11**) and NMM (0.68 mL, 6.12 mmol) in CH_3CN (25 mL) was cooled to −17 °C and *i*-BuOCOCl (0.74 mL, 5.80 mmol) was added. The solution was stirred for 10 min and a 0 °C mixture of **6b** (1.22 g, 6.12 mmol), NMM (0.68 g, 6.12 mmol), and DMF (4 mL) was added dropwise. The reaction mixture was stirred for 4 h at −17 °C, allowed to warm to room temperature and stirred overnight. The mixture was concentrated and partitioned between EtOAc and water. The organic layer was dried (Na_2SO_4) and concentrated and the crude product was purified by flash chromatography (EtOAc) to give 1.12 g (52%) of **12**: IR (KBr) 3420, 3300, 2980, 1680, 1630, 1170 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.5 (d, 1 H, *J* = 9.0 Hz, NH), 6.3 (m, 1 H, NH), 4.2–3.8 (m, 3 H, CH), 3.25 (m, 2 H, CH_2), 2.3–1.6 (m, 5 H), 1.45 (s, 9 H, $C(CH_3)_3$), 0.85 (d, *J* = 6.0 Hz, 6 H, valine CH_3 groups); MS (DCI/CH_4) *m/z* (rel intensity) 369 (MH^+, 30), 313 (57), 269 (100), 170 (14); HRMS (MH^+) calcd for $C_{16}H_{27}F_3N_2O_4$ 369.2001, found 369.1999.

***N*-[3,3,3-Trifluoro-2-hydroxy-1-(1-methylethyl)propyl]-L-prolinamide Hydrochloride (13).** A solution of **12** (330 mg, 0.89 mmol) in EtOAc (50 mL) was cooled to 0 °C and saturated with HCl gas (5 min). After 1.5 h of stirring, the solvent was removed to leave 271 mg (quantitative yield) of **13**: ^1H NMR

(Me_2SO-d_6/D_2O) δ 0.84 and 0.82 (2 d, J = 6 Hz, 6 H, valine CH_3 groups); HRMS (MH^+) calcd for $C_{11}H_{20}N_2O_2F_3$ 269.1477, found 269.1473.

N^1-[N^α-(Adamantylsulfonyl)-N^ϵ-phthaloyl-L-lysyl]-N^α-[3,3,3-trifluoro-2-hydroxy-1-(1-methylethyl)propyl]-L-prolinamide (14). To a solution of N^α-(adamantylsulfonyl)-N^ϵ-phthaloyl-L-lysine (3.10 g, 0.65 mmol), amine hydrochloride **13** (2.00 g, 0.65 mmol), and diethyl cyanophosphonate (200 μL, 1.14 mmol) in freshly distilled DMF (5 mL) at 0 °C was added Et_3N (200 μL, 2.73 mmol). The reaction mixture was stirred at 0 °C for 1 h, allowed to warm to room temperature, and stirred for 1 h. The solution was poured into H_2O (100 mL) and extracted with EtOAc (2 × 75 mL). The combined extracts were washed with dilute HCl, 5% $NaHCO_3$, saturated NaCl and then dried (Na_2SO_4). Removal of solvent in vacuo and purification by flash chromatography with EtOAc gave 1.80 g (38%) of **14**: ^1H NMR ($CDCl_3$) δ 7.9–7.6 (m, 4 H, aryl), 5.25–4.95 (br s, 1 H, CH), 4.8–4.6 (m, 1 H, CH), 1.83–1.56 (br s, 12 H, adamantyl), 1.1–0.9 (m, 6 H, CH_3); IR (KBr) 3430, 2910, 2850, 1710, 1640 cm^{-1}; MS (DCI/CH_4) m/z (rel intensity) 725 (MH^+, 56), 661 (6), 269 (18), 135 (100); HRMS (MH^+) calcd for $C_{35}H_{47}F_3N_4O_7S$ 725.3196, found 725.3175.

N^1-[N^α-(Adamantylsulfonyl)-N^ϵ-(4-methoxy-1,4-dioxobutyl)-L-lysyl]-N^α-[3,3,3-trifluoro-2-hydroxy-1-(1-methylethyl)propyl]-L-prolinamide (15). To a solution of lysyl prolinamide **14** (170 mg, 0.23 mmol) in EtOH (2 mL) was added hydrazine monohydrate (0.27 mL, 5.2 mmol). The solution was stirred for 4 h at room temperature, during which time a precipitate formed. The reaction mixture was filtered and the solvent was removed in vacuo. The crude free amine was diluted with EtOH (2 × 10 mL) and the mixture was concentrated in vacuo to aid the evaporation of residual hydrazine. The crude product was suspended in CH_2Cl_2 (5 mL) and Et_3N (300 μL) and 3-carbomethoxypropionyl chloride (28 μL, 0.23 mmol) were added. The resulting solution was stirred overnight, followed by reduction of the solvent volume in vacuo to 1 mL and purification by flash chromatography. Elution with EtOAc yielded 60 mg (37%) of trifluoromethyl alcohol **15**: ^1H NMR ($CDCl_3$) δ 7.2–6.5 (m, 2 H, NH), 5.6–5.2 (m, 2 H, NH, CH) 5.5 (m, 1 H, CH), 3.63 (s, 3 H, OCH_3), 3.2 (m, 2 H), 2.58 (m, 3 H), 2.25–1.90 (m, 10 H), 1.80–1.40 (m, 10 H), 1.96 (m, 6 H, valine CH_3 groups); MS (DCI/CH_4) m/z (rel intensity) 709 (MH^+, 79), 645 (8), 496 (25), 135 (100).

N^1-[N^α-(Adamantylsulfonyl)-N^ϵ-(4-methoxy-1,4-dioxobutyl)-L-lysyl]-N^α-[3,3,3-trifluoro-1-(1-methylethyl)-2-oxopropyl]-L-prolinamide (16) **(GP 5b)**. Oxalyl chloride (70 μL, 0.8 mmol) in CH_2Cl_2 (2 mL), DMSO (110 μL, 1.5 mmol), and alcohol **15** (40 mg, 0.06 mmol) afforded 25 mg (63%) of crude ketone **16**. The product was purified by flash chromatography (EtOAc); LC/MS [Vestec thermospray/Finnigan TSQ-46 mass spectrometer, 5-μm Spherisorb column (25 cm × 4.6 mm i.d.), mobile phase 0.01 M ammonium acetate (pH 4.5)/acetonitrile 65/35] analysis showed two diastereomers in a ratio of 1.5:1: ^1H NMR ($CDCl_3$) δ 4.80 (br m, 3 H, CH) 3.66 (s, 3 H, OCH_3), 2.00 (br m, OH), 1.73 (br m, 9 H), 1.02 and 0.90 (2 d, 3 H, J = 6.0 Hz, valine CH_3 groups); MS (DCI/CH_4) m/z (rel intensity) 707 (MH^+, 20), 495 (2.5) 413 (5.8), 383 (15), 269 (100); HRMS (MH^+) calcd for $C_{32}H_{49}F_3N_4O_8S$ 707.3308, found 707.3313.

3-Amino-2-hydroxybutanoic Acid (18a) **(GP 6)**. Aldehyde **17a** (2.5 g, 14.4 mmol) in H_2O (30 mL) was treated with $NaHSO_3$ (1.50 g, 14.4 mmol) in 10 mL of H_2O. EtOAc (200 mL) and KCN (935 mg, 14.4 mmol) in H_2O (10 mL) were used to form the cyanohydrin. The cyanohydrin was heated at reflux in dioxane (50 mL) and concentrated aqueous HCl (50 mL). Ion-exchange chromatography and crystallization gave **18a** (1.05 g, 61%) as a white solid: ^1H NMR (D_2O) δ 4.09 and 3.92 (2 d, 1 H, CHO), 3.8–3.3 (m, 1 H, CHN), 1.27 and 1.11 (2 d, 3 H, CH_3).

3-Amino-2-hydroxy-4-methylpentanoic Acid (18b) **(GP 6)**. Aldehyde **17b** (2.3 g, 11.4 mmol) in H_2O (30 mL) was treated with $NaHSO_3$ (1.2 g, 11.4 mmol) in H_2O (10 mL). EtOAc (200 mL) and KCN (0.74 g, 11.4 mmol) in H_2O (15 mL) were used to form the cyanohydrin. The cyanohydrin was heated at reflux in dioxane (50 mL) and concentrated aqueous HCl (50 mL). Ion-exchange chromatography and crystallization gave **18b** (0.94 g, 56%) as a white solid: ^1H NMR (D_2O) δ 4.3–4.0 (m, 1 H, CHO), 3.3–3.0 (m, 1 H, CHN), 2.3–1.8 (m, 1 H, CH), 1.00 and 0.96 (2 d, 6 H, CH_3). Anal. ($C_6H_{13}NO_3$) C, H, N.

3-Amino-2-hydroxy-4-phenylbutanoic Acid (18c) **(GP 6)**. Aldehyde **17c** (3.92 g, 15.7 mmol) in H_2O (40 mL) was treated with $NaHSO_3$ (1.64 g, 15.7 mmol) in H_2O (10 mL). EtOAc (200 mL) and KCN (1.02 g, 15.7 mmol) in H_2O (10 mL) were used to form the cyanohydrin. The cyanohydrin was heated at reflux in dioxane (65 mL) and concentrated HCl (50 mL). Ion-exchange chromatography and crystallization gave **18c** (1.94 g, 63%) as a white solid: mp 240–244 °C dec; ^1H NMR ($CDCl_3$/CF_3CO_2D) δ 7.4–6.9 (m, aryl, exchangeable protons), 4.84 and 4.54 (2 d, 1 H, J = 3.9 Hz, CHO), 4.25–4.0 (m, 1 H, CHN), 3.3–3.0 (m, 2 H, CH_2). Anal. ($C_{10}H_{13}NO_3$) C, H, N.

Methyl 3-Amino-2-hydroxybutanoate (19a) **(GP 7)**. Acid **18a** (1.0 g, 8.4 mmol) in CH_3OH (25 mL and then 20 mL) was treated with HCl. A portion of the crude hydrochloride (1.1 g, 6.5 mmol) in Et_3N (15 mL) and CH_3OH (15 mL) was treated with Et_2O (75 mL). Concentration of the filtrate gave the free amine, which was used the same day without further purification: ^1H NMR ($CDCl_3$) δ 4.22 and 3.97 (2 d, 1 H, CHO), 3.75 (s, 3 H, CH_3O), 3.7–3.1 and 3.49 (m and br s, 4 H, CHN, OH, NH_2), 1.21 and 1.10 (2 d, 3 H, CH_3).

Methyl 3-Amino-2-hydroxy-4-methylpentanoate (19b) **(GP 7)**. Acid **18b** (0.89 g, 6.05 mmol) in CH_3OH (25 mL and then 20 mL) was treated with HCl. The crude hydrochloride salt in Et_3N (15 mL) and CH_3OH (20 mL) was treated with Et_2O (100 mL). Concentration of the filtrate gave the free amine, which was used the same day without further purification.

Methyl 3-Amino-2-hydroxy-4-phenylbutanoate (19c) **(GP 7)**. Acid **18c** (0.43 g, 2.20 mmol) in CH_3OH (30 mL and then 20 mL) was treated with HCl. The crude hydrochloride salt in Et_3N (6 mL) and CH_3OH (8 mL) was treated with Et_2O (70 mL). Concentration of the filtrate gave the free amine, which was used the same day without further purification.

Methyl 3-[(*N*-Acetyl-L-prolyl-L-alanyl-L-prolyl)amino]-2-hydroxybutanoate (20). A suspension of NMM (0.22 mL, 2.00 mmol) and **7c**[35] (651 mg, 2.00 mmol) in CH_3CN (20 mL) was cooled to −20 °C and *i*-BuOCOCl (0.26 mL, 2.00 mmol) was added. After 10 min a solution of amino alcohol **19a** (532 mg, 4.00 mmol) in $CHCl_3$ (3 mL) was added and the reaction mixture was allowed to warm slowly to room temperature. After 2.5 h the reaction mixture was concentrated and the residue was dissolved in H_2O (20 mL) and treated with a mixed-bed resin (J.T. Baker: ANG MI-615, 11 g). The resin was removed by filtration, the filtrate was concentrated and the residue was flash chromatographed [CH_3OH/CH_2Cl_2 (1:9)] to give **20** (315 mg, 36%) as a white foam: ^1H NMR ($CDCl_3$) δ 8.1–7.1 (m, 2 H, NH), 4.9–3.9 (m, 6 H, CHN, CHO, OH), 3.9–3.3 (m, 7 H, CH_2N, CH_3O), 2.5–1.7 and 2.11 (m and s, 11 H, CH_2CH_2, CH_3CO), 1.6–1.1 (m, 6 H, CH_3).

***N*-Acetyl-L-prolyl-L-alanyl-*N*-(3-methoxy-1-methyl-2,3-dioxopropyl)-L-prolinamide** (21). To a stirred solution of oxalyl chloride (0.12 mL, 1.43 mmol) in CH_2Cl_2 (1.5 mL), cooled to −60 °C, was added DMSO (0.20 mL, 2.86 mmol) in CH_2Cl_2 (0.5 mL). After 5 min alcohol **20** (315 mg, 0.72 mmol) in CH_2Cl_2 (1.5 mL) was added. Stirring was continued for 25 min at −60 °C. After the addition of Et_3N (0.50 mL, 3.58 mmol) the mixture was allowed to warm to room temperature and applied directly to a column and flash chromatographed [CH_3OH/CH_2Cl_2 (1:9)]. Product-containing fractions were combined and concentrated. D_2O was added to the residue, the solution was concentrated, and the process was repeated to give hydrated **21** (34%) as an off-white solid: ^1H NMR (CD_3COCD_3) δ 7.9–7.5 (m, 2 H, NH), 4.9–4.1 (m, 4 H, CH), 3.9–3.3 and 3.80 (m and s, 7 H, CH_2N, CH_3O), 2.3–1.7 and 2.03 (m and s, 11 H, CH_2CH_2, CH_3CO), 1.5–1.1 (m, 6 H, CH_3).

***N*-(4-Methoxy-1,4-dioxobutyl)-L-alanyl-L-alanyl-*N*-[2-hydroxy-3-methoxy-1-(1-methylethyl)-3-oxopropyl]-L-prolinamide** (22) **(GP 4)**. A solution of MeO-Suc-Ala-Ala-Pro-OH (**7b**;[41,42] 2.23 g, 6.00 mmol) and NMM (0.70 mL, 6.30 mmol) in CH_3CN (60 mL) was treated with *i*-BuOCOCl (0.77 mL, 6.00 mmol) and amino alcohol **19b** (0.97 g, 6.00 mmol) in $CHCl_3$ (5 mL). The reaction mixture was concentrated and the residue was flash chromatographed (gradient: 40–50% CH_3COCH_3 in EtOAc) to give **22** (2.63 g, 85%) as a white foam: ^1H NMR ($CDCl_3$) δ 8.37 and 8.18 (2 d, 1 H, NH), 7.30 (d, 1 H, NH), 6.89 (d, 1 H, NH), 5.0–4.4 (m, 3 H, CH), 4.4–3.4 and 3.67 and 3.60 (m and 2 s, 10 H, CHO, CHN, CH_2N, CH_3O), 2.8–2.3 (m, 4 H, $COCH_2CH_2CO$), 2.3–1.6 (m, 5 H, CH_2CH_2, CH), 1.4–1.0 (m, 6 H, CH_3), 1.0–0.6 (m, 6 H, CH_3); MS (EI) m/z (rel intensity) 514 (M^+, 2), 483 (3), 354

(16), 257 (67), 186 (39), 158 (10), 115 (18), 70 (100); MS (DCI/CH_4) m/z (rel intensity) 515 (MH^+, 100), 259 (91); HRMS (MH^+) calcd ($C_{23}H_{38}N_4O_9$) 515.2717, found 515.2789.

***N*-(4-Methoxy-1,4-dioxobutyl)-L-alanyl-L-alanyl-*N*-[3-methoxy-1-(1-methylethyl)-2,3-dioxopropyl]-L-prolinamide (23) (GP 5c).** Oxalyl chloride (0.26 mL, 3.00 mmol) in CH_2Cl_2 (7 mL), DMSO (0.43 mL, 6.00 mmol) in CH_2Cl_2 (1 mL), and alcohol prolinamide **22** (1.03 g, 2.00 mmol) in CH_2Cl_2 (4 mL) gave crude **23**. Flash chromatography with CH_3COCH_3/EtOAc (2:3) gave a white, foamy solid (0.80 g), which was crystallized (CH_2Cl_2/Et_2O) to give keto ester **23** (0.44 g, 43%) as a white solid: ^1H NMR ($CDCl_3$) δ 8.15 (br d, 1 H, NH), 7.68 (br d, 1 H, NH), 6.75 (br d, 1 H, NH), 5.2–4.5 (m, 4 H, CHN), 4.0–3.4 and 3.82 and 3.65 (m and s and s, 8 H, CH_2N, CH_3O, CH_3O), 3.0–1.8 (m, 9 H, $COCH_2CH_2CO$, CH_2CH_2, CH), 1.34 and 1.18 and 0.95 and 0.76 (4 d, 12 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 192.0 (ketone), 173.0, 171.8, 171.5, 171.3, 170.9, 161.0, 59.8, 59.1, 52.7, 51.5, 48.1, 47.3, 45.9, 30.6, 29.1, 28.8, 28.5, 24.6, 19.6, 19.3, 18.1, 17.0; MS (EI) m/z (rel intensity) 512 (M^+, 4), 481 (7), 354 (30), 257 (100), 186 (64), 158 (19), 115 (24), 70 (77); MS (DCI/CH_4) m/z (rel intensity) 513 (MH^+, 97), 257 (100). Anal. ($C_{23}H_{36}N_4O_9$) C, H, N.

***N*-(4-Methoxy-1,4-dioxobutyl)-L-alanyl-L-alanyl-*N*-[2-hydroxy-3-methoxy-3-oxo-1-(phenylmethyl)propyl]-L-prolinamide (24) (GP 4).** A soln of MeO-Suc-Ala-Ala-Pro-OH (**7b**;[41,42] 0.82 g, 2.20 mmol) and NMM (0.24 mL, 2.20 mmol) in CH_3CN (25 mL) was treated with *i*-BuOCOCl (0.29 mL, 2.20 mmol) and amino alcohol **19c** (2.20 mmol) in $CHCl_3$ (5 mL). The reaction mixture was concentrated and the residue was flash chromatographed (gradient: 0–50% CH_3COCH_3 in EtOAc) to give **24** (0.78 g, 63%) as an oily, white foam: ^1H NMR ($CDCl_3$) δ 8.3–7.9 (m, 1 H, NH), 7.7–6.9 (m, 6 H, aryl, NH), 6.80 and 6.54 (2 br m, 1 H, NH), 5.0–3.1 and 3.63 and 3.61 (m and 2 s, 13 H, CHN, CHO, CH_2N, CH_3O), 3.1–2.3 (m, 6 H, $COCH_2CH_2CO$, CH_2-aryl), 2.3–1.8 (m, 4 H, CH_2CH_2), 1.5–1.1 (m, 6 H, CH_3); MS (DCI/CH_4) m/z (rel intensity) 563 (MH^+, 16), 378 (10), 354 (7), 307 (100); HRMS (MH^+) calcd for $C_{27}H_{38}N_4O_9$ 563.2717, found 563.2702.

***N*-(4-Methoxy-1,4-dioxobutyl)-L-alanyl-L-alanyl-*N*-[3-methoxy-2,3-dioxo-1-(phenylmethyl)propyl]-L-prolinamide (25) (GP 5c).** Oxalyl chloride (0.17 mL, 1.97 mmol) in CH_2Cl_2 (6 mL), DMSO (0.28 mL, 3.95 mmol), and alcohol prolinamide **24** (0.74 g, 1.32 mmol) in chilled (−78 °C) CH_2Cl_2 (5 mL) gave crude **25**. Flash chromatography with CH_3COCH_3/EtOAc (2:3) gave **25** (0.20 g, 27%) as an oily, white foam: R_f = 0.21 (CH_3COCH_3/EtOAc, 1:1); ^1H NMR ($CDCl_3$) δ 8.3–7.6 (m, 2 H, NH), 7.4–7.0 (m, 5 H, aryl), 6.74 (br d, 1 H, NH), 5.4–4.4 (m, 4 H, CHN), 3.9–3.3 and 3.73 and 3.70 and 3.63 (m and 3 s, 8 H, CH_2N, CH_3O), 3.3–2.3 (m, 6 H, CH_2-aryl, $COCH_2CH_2CO$), 2.3–1.5 (m, 4 H, CH_2CH_2), 1.5–1.0 (m, 6 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 191.3 (ketone), 160.7 (ester α to ketone), plus some component of hydrate at 94.4 ($C(OH)_2$); MS (EI) m/z (rel intensity) 560 (M^+, 0.5), 529 (1), 501 (1), 473 (14), 402 (25), 354 (94), 305 (25), 257 (100), 186 (41), 158 (12), 91 (36); MS (DCI/CH_4) m/z (rel intensity) 561 (MH^+, 100), 305 (48); HRMS (MH^+) calcd for $C_{27}H_{36}N_4O_9$ 561.2561, found 561.2539.

Ethyl 4-[[(1,1-Dimethylethoxy)carbonyl]amino]-2,2-difluoro-3-hydroxy-5-methylhexanoate (26). A mixture of *N*-(*tert*-butyloxycarbonyl)valinal[33] (**17b**; 5.40 g, 27 mmol) and ethyl bromodifluoroacetate (7.10 g, 35 mmol) in THF (50 mL) was added dropwise under nitrogen over a period of 30 min to a refluxing suspension of zinc wool (2.28 g, 35 mg-atom) in THF (10 mL). After the addition was complete, the mixture was allowed to cool to room temperature and was then diluted by the addition of EtOAc (50 mL), 1 M $KHSO_4$ (60 mL), and brine (60 mL). The organic layer was separated and the aqueous layer was extracted twice with EtOAc (2 × 50 mL). Filtration and removal of the solvent in vacuo left an oil, which was purified by column chromatography (SiO_2, EtOAc/hexane, 1:9) to yield 3.13 g (36%) of alcohol **26**: ^1H NMR ($CDCl_3$) δ 5.17 (br d, 1 H, J = 9 Hz, NH), 4.35 (q, 2 H, J = 7 Hz, OCH_2), 4.3–3.8 (m, 2 H, CHOH), 3.6 (m, 1 H, CHN), 2.0 (m, 1 H, Me_2CH), 1.47 (s, 9 H, CH_3), 1.37 (t, 3 H, J = 7 Hz, OCH_2CH_3), 1.00 and 1.02 (2 d, 6 H, J = 6 Hz, valine CH_3 groups). Anal. ($C_{14}H_{25}F_2NO_5$) C, H, N.

Ethyl 4-Amino-2,2-difluoro-3-hydroxy-5-methylhexanoate Hydrochloride. Protected amino ester **26** (0.220 g, 0.68 mmol) was dissolved in a 4 N HCl/Et_2O solution (10 mL) at 0 °C. The mixture was stirred 1 h at 0 °C and 14 h at room temperature. The white precipitate was filtered off and rinsed thoroughly with Et_2O. The solid was dried over KOH (0.01 Torr) overnight to yield 0.170 g (96%) of the hydrochloride salt: mp 181–182 °C, ^1H NMR (D_2O) δ 4.8–4.25 (m, 1 H, CHO), 4.45 (q, 2 H, J = 7.5 Hz, OCH_2), 3.5 (m, 1 H, $CHMe_2$), 2.15 (m, 1 H, $CHMe_2$), 1.33 (t, 3 H, J = 7.5 Hz, OCH_2CH_3), 1.05 (d, 6 H, J = 7.5 Hz, $CHMe_2$).

Ethyl 2,2-Difluoro-3-hydroxy-4-[[*N*-(4-methoxy-1,4-dioxobutyl)-L-alanyl-L-alanyl-L-prolyl]amino]-5-methylhexanoate (27) (GP 4). MeO-Suc-Ala-Ala-Pro-OH (**7b**; 0.371 g, 1 mmol) in CH_3CN (10 mL) was treated with NMM (0.106 g, 1.05 mmol), *i*-BuOCOCl (0.136 g, 1 mmol), and difluoro amino alcohol hydrochloride (0.275 g, 1.05 mmol) from above. After stirring for 15 h at room temperature, the mixture was concentrated (0.01 Torr, 40°C) and the residue was chromatographed (SiO_2, EtOAc/acetone, 7:3) to yield 0.490 g (85%) of **27** as a 4:1 mixture of diastereoisomers: ^1H NMR ($CDCl_3$) δ 8.6, 7.6, 7.0 (br d, 3 H, J = 9 Hz, 3 NH), 5.1–4.7 (m, 3 H, NCHCO), 4.33 (q, 2 H, J = 7.5 Hz, OCH_2), 4.6–4.05 (m, 2 H, CHOH), 4.0–3.6 (m, 3 H, NCH_2, CH), 3.70 (s, 3 H, OCH_3), 2.8–2.4 (m, 4 H, $OCCH_2CH_2CO$), 2.4–1.7 (m, 5 H, CH, 2 CH_2), 1.5–1.1 (m, 9 H, CH_3), 0.97–0.90 (2 d, 6 H, J = 6 Hz, CH_3); ^{19}F NMR ($CDCl_3$) (minor isomer) φ 37.2 (dd, J = 260, 20 Hz), 48.6 (dd, J = 260, 3 Hz); ^{19}F NMR ($CDCl_3$) (major isomer) φ 40.7 (dd, J = 260, 16 Hz), 46.9 (dd, J = 260, 6 Hz); MS (DCI, NH_3) m/z 579 (MH^+).

Ethyl 2,2-Difluoro-4-[[*N*-(4-methoxy-1,4-dioxobutyl)-L-alanyl-L-alanyl-L-prolyl]amino]-5-methyl-3-oxohexanoate (28). A solution of hydroxy ester **27** (0.230 g, 0.4 mmol) in CH_2Cl_2 (4 mL) was added to a suspension of pyridinium dichromate (0.228 g) and 3A molecular sieves (0.336 g) in CH_2Cl_2 (4 mL) containing glacial AcOH (20 μL). Stirring was continued for 15 h at room temperature. Florisil (0.200 g) was added and after 0.25 h of stirring the mixture was filtered. Removal of the solvent and chromatography (SiO_2, EtOAc/acetone, 7:3) afforded 0.150 g (65%) of ketone **28** as a white solid: mp 102–103 °C, ^1H NMR ($CDCl_3$) δ 7.70, 7.60 (2 br d, 2 H, J = 9 Hz, 2 NH), 6.45 (br d, 1 H, J = 8 Hz, NH), 4.95 (m, 1 H, NCH), 4.9–4.7 (m, 3 H, NCHCO), 4.37, 4.35 (2 q, 2 H, J = 7.5 Hz, OCH_2), 3.70 (s, 3 H, OCH_3), 3.75–3.6 (m, 2 H, NCH_2), 2.75–2.5 (m, 4 H, $OCCH_2CH_2CO$), 2.4–2.0 (m, 5 H, 2 CH_2, CH), 1.35 (t, 3 H, J = 7.5 Hz, CH_3), 1.32 (d, 3 H, J = 7 Hz, CH_3), 1.25 (d, 3 H, J = 7 Hz, CH_3), 0.92 (d, 3 H, J = 7 Hz, CH_3), 0.80 (d, 3 H, J = 7 Hz, CH_3); ^{19}F NMR ($CDCl_3$) φ 48.4 and 49.35 (2 d, AB, J = 280 Hz); MS (DCI, NH_3) m/z 577 (MH^+). Anal. ($C_{25}H_{38}F_2N_4O_9$) C, H, N.

Enzyme Assays for Porcine Pancreatic Elastase and Human Neutrophil Elastase. Porcine pancreatic elastase was purified by using the procedure described by Shotton,[43] slightly modified by the addition of chromatography on (carboxymethyl)cellulose prior to crystallization of the enzyme. Trypsin 1-300 (U.S. Biochemical Corp.) was used as starting material. Human elastase was purified from purulent sputum. (We thank Dr. F. Kellogg, Children's Hospital, Cincinnati, for provision of starting material.) Enzyme extraction and DNase treatment followed the procedure of Twumasi and Liener,[44] and chromatographic steps on aprotinin-Sepharose and (carboxymethyl)-cellulose were similar to those described by Barrett.[45]

Both elastases were assayed spectrophotometrically, with nitroanilide substrates. A buffer of 0.1 M NaCl, 0.01 M *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES), 0.01 M Tris, 0.1% polyethylene glycol 6000,[46] adjusted to pH 8.0, was used. Suc-Ala-Pro-Ala-*p*-nitroanilide, obtained from Peninsula Laboratories, and MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide, obtained from Sigma Chemical Co., are good substrates for the pig enzyme,[18] and for the human enzyme,[34] respectively. Most often, however, the nitroanilide with valine at P_1 was used for inhibition studies of porcine elastase and the substrate with alanine at P_1 was used for inhibition studies of human elastase. Use of a less

(43) Shotton, D. M. *Methods in Enzymology*; Perlman, G. E., Lorand, L., Eds.; Academic Press: New York, 1970; Volume 19, p 113.
(44) Twumasi, D. Y.; Liener, I. E. *J. Biol. Chem.* **1977**, *252*, 1917.
(45) Barrett, A. J. *Methods in Enzymology*; Lorand, L., Ed.; Academic Press: New York, 1980; Volume 80, p 581.
(46) Lottenberg, R.; Hall, J. A.; Blinder, M.; Binder, E. P.; Jackson, C. M. *Biochim. Biophys. Acta* **1983**, *742*, 539.

sensitive assay with a poorer substrate in these experiments facilitated following slow-onset inhibition in continuous assays. Trials using valine-containing substrate contained 1.25% DMSO. Assays were run at 37 °C in a Cary Model 210 spectrophotometer. Generation of 4-nitroaniline was monitored at 410 nm; a molar extinction coefficient of 8800[47] was used to calculate the amount of product formed. Data were transferred via the Cary digital interface port under the control of a VIC 20 microcomputer, which logged up to 100 pairs of time and absorbance values per assay; these data were transferred to other computers for numeric analysis.

Determination of initial rates of product formation for inhibitor-free assays and for inhibitor assays, in which attainment of a steady state was rapid, was performed by the numerical equivalent of the direct-plot method.[48] Michaelis–Menten parameters for hydrolysis of Suc-Ala-Pro-Ala-*p*-nitroanilide by porcine pancreatic elastase were K_m = 1.65 mM and $k_{cat.}/K_m$ = 80.8 s^{-1} mM^{-1}, and for human elastase they were K_m = 1.78 mM and $k_{cat.}/K_m$ = 3.75 s^{-1} mM^{-1}. For the hydrolysis of MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide by porcine pancreatic elastase, the values were K_m = 2.49 mM and $k_{cat.}/K_m$ = 10.9 s^{-1} mM^{-1}, and for human elastase they were K_m = 0.0830 mM and $k_{cat.}/K_m$, 225 $s^{-1}mM^{-1}$.

Most of the inhibitors exhibited slow-binding kinetics; these progress curves were analyzed according to the models concisely described by Morrison.[49] One model envisions rapid formation of one enzyme–inhibitor complex prior to slow formation of another enzyme–inhibitor complex

$$E + I \underset{}{\overset{K_i}{\rightleftharpoons}} EI \underset{k_4}{\overset{k_3}{\rightleftharpoons}} EI^*$$

while the other posits only a single, slowly-formed complex:

$$E + I \underset{k_2}{\overset{k_1}{\rightleftharpoons}} EI^*$$

For both models, the amount of product, P, formed by remaining active enzyme at time t is described by the equation

$$P = v_s t + (v_0 - v_s)[1 - e(-kt)]/k + d$$

where v_s is the steady-state asymptotic rate of product formation at equilibrium (assuming unchanging substrate concn), v_0 is the initial velocity, k is an apparent first order rate constant, and d is the displacement of P from zero at $t = 0$. (This blank value was removed from data sets before further numeric analysis.)

For each trial of a given set of enzyme, substrate, and inhibitor concentration, two assays were run. In the first, enzyme was added to a mixture of substrate and inhibitor. In the second, enzyme and inhibitor were preincubated before the addition of substrate. The progress curves for two such assays are characterized by the same steady-state rate, v_s, and the same rate constant, k, but the tangents to the curves at time zero differ, since one reflects a rate before formation of the slowly-formed complex, whereas the other reflects a rate prior to partial relief of inhibition by substrate. These two rates were termed v_{01} and v_{02}. Parameters were determined by simultaneous analysis of the paired assay data; four values were obtained, two of which, v_s and k, were the best least-squares approximations common to both curves and two of which, v_{01} and v_{02}, each were determined from a single set of data. The only nonlinear parameter of the four is k; thus, an interactive process was employed, at each stage of which exact estimates for linear parameters were calculated.[50]

Only data up to a limit of 10% consumption of substrate were analyzed, in order to justify the assumption of constant substrate concentration. In addition, the ratio of total inhibitor to total enzyme was maximized, insofar as practical, so that the concentration of non-enzyme-bound inhibitor could be approximated by the total inhibitor concentration. However, enough enzyme had to be employed to get useful changes in absorbance due to substrate hydrolysis over the course of the assay. For some of the most potent inhibitors, it was calculated that as much as 25% of total inhibitor could be bound to enzyme in some, but not all, of the assays used to determine inhibition parameters. Enzyme molarity was based on protein content; inhibitor/enzyme ratios would have been increased had active site molarity been determined by titration. Inappropriate equation of unbound inhibitor to total inhibitor would lead to underestimating the potency of the compound. Selwyn plots[51] were made to demonstrate the stability of enzyme activity over the duration of lengthy assays.

The overall inhibition constant, designated K_i^*, was determined by using steady-state rates and rearrangement of the expression for competitive inhibition:

$$v_s = v_{max}\,[S]/([S] + K_m\,(1 + [I]/K_i^*))$$

From a collection of initial rates v_{01}, obtained when enzyme was added last to a mixture of substrate and inhibitor, the likelihood of the two enzyme–inhibitor complex model was assessed. If there was a consistent reduction in v_{01} below the rate of inhibitor-free control assays, the constant for formation of the rapid complex was determined from the competitive inhibition relationship, but with v_{01} values replacing v_s values. The slow reversion rate constant for this model was obtained from the collection of k values, using K_i^* and K_i values obtained from the same data collection:

$$k_4 = k([S] + K_m(1 + [I]/K_i))/([S] + K_m(1 + [I]/K_i^*))$$

If v_{01} was not appreciably sensitive to inhibitor concentration, the single enzyme–inhibitor complex model was assumed, nonetheless realizing that, when $K_i \gg K_i^*$ and if inhibitor concentrations appropriate only to establishing K_i^* were tested, fitting of data to a two complex model was not possible. For this model, the slow formation rate constant was obtained from the slope of the linear relationship

$$k = k_2 + k_1[I]/(1 + [S]/K_m)$$

Enzyme Assays for Rat and Neutrophil Human Cathepsin G. Purulent sputum was used as the source of human neutrophil cathepsin G[44,52] and casein-induced rat peritoneal leukocytes (ca. 90% neutrophils) were used as the source of rat neutrophil cathepsin G. Sputum or peritoneal lavage was homogenized with a tight-fitting Teflon–glass homogenizer in 0.34 M sucrose. The homogenate was treated with DNase and centrifuged at 1000*g* for 10 min. The supernatant was centrifuged at 30000*g* for 30 min to pellet a granule-rich fraction. A granule extract was prepared by suspending the granule pellet in sodium acetate buffer (0.05 M, pH 5.5) containing 1 M NaCl and 0.1% Brij 35 and sonicating the suspension in an ultrasonic bath for 30 min at 4 °C. The suspension was centrifuged at 30000*g* for 30 min, and the supernatant was used directly for assay of cathepsin G activity. Cathepsin G could be further purified by cation-exchange chromatography using the Pharmacia FPLC system and a Mono S column; inhibition constants measured with crude granule lysate agreed with inhibition constants measured using purified enzyme.

Cathepsin G activity was measured at 37 °C and pH 7.5 using Suc-Ala-Ala-Pro-Phe-*p*-nitroanilide (Sigma) as the substrate[53,34] in a buffer consisting of 0.1 M HEPES, 0.5 M NaCl, 0.1% Brij 35, and 10% v/v DMSO. Substrate and inhibitor were added to the required concentration from stock solutions in DMSO. The substrate concentration was at least 10-fold lower than the K_m, and K_i values were calculated by the equation $K_i = [I]/(v_0/v_1 - 1)$, where v_0 is the initial rate in the absence of an inhibitor and v_1 is the initial rate in the presence of an inhibitor at the concentration [I]. When slow binding was observed (rat enzyme with **9a** and **9b**, and human enzyme with **9a**), the final equilibrium velocity was used as v_I. In one instance (rat enzyme and keto ester inhibitor **25**), competitive inhibition was demonstrated by a Dixon plot.

Registry No. **1a**, 1205-02-3; **1b**, 2901-80-6; **1c**, 2901-76-0; **2a**, 51127-13-0; **2b**, 51127-17-4; **2c**, 51127-19-6; **3a**, 123206-04-2; **3b**, 123206-05-3; **3c**, 123206-06-4; **4a**, 123206-07-5; **4b**, 123206-08-6;

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4c, 123206-09-7; (±)-(R*,R*)-5a, 123206-10-0; (±)-(R*,S*)-5a, 123206-11-1; 5b, 106746-07-0; 5c, 123206-12-2; (±)-(R*,R*)-6a, 102614-00-6; (±)-(R*,S*)-6a, 102614-01-7; 6b, 106746-09-2; 6c, 123206-13-3; 7a, 105108-49-4; 7b, 123206-14-4; 7c, 41036-61-7; 7d, 63769-88-0; 7e, 63769-98-2; 7f, 38675-10-4; 8a, 123206-15-5; 8b, 123206-16-6; 8c, 106771-14-6; 8d, 102614-06-2; 8e, 106771-19-1; 8f, 123206-17-7; 8g, 123239-02-1; (R)-9a, 123206-18-8; (S)-9a, 123285-38-1; (R)-9b, 123239-03-2; (S)-9b, 123286-93-1; (R)-9c, 123285-39-2; (S)-9c, 123285-40-5; (R)-9d, 123285-41-6; (S)-9d, 123285-42-7; (R)-9e, 123285-43-8; BOC-deblocked (R)-9e·HCl, 123285-58-5; (S)-9e, 123285-44-9; BOC-deblocked (S)-9e·HCl, 123285-59-6; (R)-9f, 123206-19-9; (S)-9f, 123285-45-0; (R)-9g, 123206-20-2; (S)-9g, 123285-46-1; (R)-9h, 123206-21-3; (S)-9h, 123285-47-2; (R)-9i, 123206-22-4; (S)-9i, 123285-48-3; (R)-10, 123285-49-4; (S)-10, 123285-50-7; 11, 15761-39-4; 12, 123239-04-3; 13, 123239-05-4; 14, 123206-23-5; 15, 123206-24-6; (R)-16, 123206-25-7; (S)-16, 123285-51-8; 17a, 105499-11-4; 17b, 123285-52-9; 17c, 98818-36-1; 18a, 565-81-1; 18b, 117213-88-4; 18c, 62084-21-3; 19a, 106746-17-2; 19a·HCl, 123206-26-8; 19b, 123206-27-9; 19b·HCl, 123206-28-0; 19c, 123206-29-1; 19c·HCl, 123206-30-4; 20, 106771-16-8; (R)-21, 123285-53-0; (S)-21, 123285-54-1; 22, 123206-31-5; 23, 123206-32-6; 24, 123206-33-7; (R)-25, 123206-34-8; (S)-25, 123285-55-2; 26, 106771-28-2; 27, 106785-91-5; (R)-28, 123285-56-3; (S)-28, 123285-57-4; BOC-Val-OH, 13734-41-3; H-Pro-OBzl·HCl, 16652-71-4; BOC-Val-Pro-OBzl, 58872-03-0; H-Val-Pro-OBzl·HCl, 95501-60-3; MeO-Suc-OH, 3878-55-5; MeO-Suc-Val-Pro-OBzl, 123206-35-9; $MeOCOCH_2CH_2COCl$, 1490-25-1; Dan-Cl, 605-65-2; N^{α}-$AdSO_2$-N^{ϵ}-Pht-L-Lys-OH, 98385-08-1; $AdSO_2$-Lys-Pro-NHCH(*i*-Pr)$CH(OH)CF_3$, 123206-36-0; $BrF_2CCOOEt$, 667-27-6; BOC-NHCH(*i*-Pr)$CH(OH)CF_2COOEt$·HCl, 123206-37-1; elastase, 9004-06-2; cathepsin G, 56645-49-9.

Supplementary Material Available: Tables listing kinetic constants for some of the elastase inhibitors synthesized (**9c-g,i**, **10**, **16**, **21**, **23**, and **28**) and analysis data for **4a-c**, **5b**, **6a,b**, **8b,f**, **9a**, **10**, **18b,c**, **23**, **26**, **28**, and MeO-Suc-Val-Pro-OBz (3 pages). Ordering information is given on any current masthead page.

Thiazolo[4,5-*d*]pyrimidine Nucleosides. The Synthesis of Certain 3-β-D-Ribofuranosylthiazolo[4,5-*d*]pyrimidines as Potential Immunotherapeutic Agents

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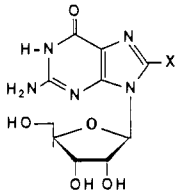
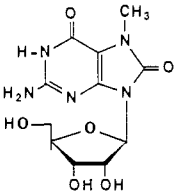
Novel analogues of the naturally occurring purine nucleosides were synthesized in the thiazolo[4,5-*d*]pyrimidine ring system to determine the immunomodulatory effects of insertion of a sulfur atom in place of nitrogen at position 7 of the purine ring. In particular, 5-amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**7**, guanosine analogue), 3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,5,7(3*H*,4*H*,6*H*)-trione (**8**, xanthosine analogue), 3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione (**10**, inosine analogue), and 7-amino-3-β-D-ribofuranosylthiazolo[4,5-*d*]pyrimidin-2(3*H*)-one (**32**, adenosine analogue) were prepared, as well as the 8-mercaptoguanosine (**14**) and 6-mercaptoguanosine (**17**) analogues. Single-crystal X-ray studies confirmed the structural assignment of **17** and **32** as having the β-configuration with the site of glycosylation at N3. The nucleosides were evaluated for their ability to potentiate various murine immune functions in direct comparison to the known active agents 8-bromoguanosine (**1**), 8-mercaptoguanosine (**2**), and 7-methyl-8-oxoguanosine (**3**). Two of the guanosine analogues, **7** and **14**, were found to exhibit significant immunoactivity relative to the positive control compounds (**1-3**), while the adenosine, inosine, xanthosine, and 6-mercaptoguanosine analogues were devoid of activity. Compound **7** exhibited greater immunoactivity than any of the other guanosine analogues and derivatives in all test systems. Specifically, **7** was shown to be about twice as potent as **3** in the murine spleen cell mitogenicity assay. In addition, treatment with **7** produced about a 4-fold increase in natural killer cell cytotoxicity, while treatment with **3** afforded a 3-fold increase over controls. Finally, **7** provided excellent protection (92% survivors compared to 0% for placebo controls) against Semliki Forest virus in mice. Induction of interferon may account for the major mode of action of these guanosine analogues.

The development of clinically useful agents for enhancing host resistance to disease and restoring impaired immune functions has become a major objective of current pharmaceutical research efforts. Indeed, the phenomenal growth in basic understanding of the immune system over the past 15 years and the demonstration of the prevalence of cellular immune defects in cancer, aging, autoimmunity, and infectious diseases have led to the development of a variety of specific and nonspecific immunotherapeutic agents.[1,2]

While many of the naturally occurring cytokines which potentiate the immune response are large glycoproteins, several synthetic small molecules have been shown to modulate immune functions as well.[3] Certain ribonucleosides of guanine substituted at C8 have been extensively studied primarily as modulators of B-cell activation.[4] The most active and most studied derivatives include 8-bromoguanosine (**1**, first prepared in our labo-



1, X = Br
2, X = SH



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