Orally Active Trifluoromethyl Ketone Inhibitors of Human Leukocyte Elastase

Chris A. Veale,*,† Peter R. Bernstein,† Claudia M. Bohnert,‡ Frederick J. Brown,† Craig Bryant,† James R. Damewood, Jr.,† Roger Earley,§ Scott W. Feeney,† Philip D. Edwards,† Bruce Gomes,‡ James M. Hulsizer,† Ben J. Kosmider,† Robert D. Krell,‡ Gary Moore,† Theodora W. Salcedo,‡ Andrew Shaw,† David S. Silberstein,‡ Gary B. Steelman,† Mark Stein,† Anne Strimpler,‡ Roy M. Thomas,† Edward P. Vacek,† Joseph C. Williams,‡ Donald J. Wolanin,† and Sheila Woolson†

Departments of Medicinal Chemistry, Drug Disposition and Metabolism, and Pharmacology, ZENECA Pharmaceuticals, A Business Unit of ZENECA Inc., 1800 Concord Pike, Wilmington, Delaware 19897

Received April 14, 1997[⊗]

This paper describes the development a series of peptidyl trifluoromethyl ketone inhibitors of human leukocyte elastase which are found to have excellent pharmacological profiles. Methods have been developed that allow for the synthesis of these inhibitors in stereochemically pure form. Two of these compounds, **1k** and **1l**, have high levels of oral bioavailability in several species. Compound **1l** has entered development as ZD8321 and is presently undergoing clinical evaluation. These compounds demonstrate that peptidyl trifluoromethyl ketone inhibitors can achieve high levels of oral activity and bioavailability, and therefore they may prove useful as therapeutic agents in the treatment of diseases in which elastase is implicated.

Introduction

Human leukocyte elastase (HLE) is a serine protease contained within the azurophilic granules of polymorphonuclear leukocytes.[1] Under normal conditions, when HLE is released from the cell its proteolytic activity is tightly controlled by a number of proteinaceous inhibitors, such as α_1-protease inhibitor and secretory leukocyte protease inhibitor. However, it is possible that in a number of pathophysiological states these proteins ineffectively regulate elastase activity. For example, unrestrained elastolytic activity is associated with the abnormal degradation of connective tissue structural proteins found in pulmonary emphysema,[2] in diseases such as cystic fibrosis and chronic bronchitis in which mucus hypersecretion and impaired host defense are major components, and in acute respiratory distress syndrome (ARDS).[3−5]

ARDS is a severe, life-threatening sequela to sepsis, trauma, and other conditions, for which there is no current effective treatment. The syndrome is characterized by a massive infiltration of the lungs by neutrophils, wherein the neutrophils release a number of toxic substances, including elastase, which damage lung tissue. Two principal mechanisms are proposed to be involved: (1) direct injury to the microvasculature, connective tissue, epithelial, and parenchymal lung cells and (2) the lysis of connective tissues to facilitate neutrophil migration. Thus a potent neutrophil inhibitor could be expected to reduce both the elastase-mediated injury and the recruitment of neutrophils into the lung.[6−10]

Thus, unregulated proteolytic activity of elastase has implicated this enzyme as having a causal or contributory role in a number of acute and chronic disease states, making it potentially an important target for drug development.[11] For treatment of chronic diseases, it would be most advantageous to develop an orally active inhibitor of HLE. Our efforts to obtain orally active inhibitors have focused on two approaches, one of which was the development of nonpeptidic inhibitors of the enzyme. This work gave rise to several different series of nonpeptidic inhibitors, some of which possess excellent levels of oral activity in animal models.[12] A second approach followed the discovery of several peptidic trifluoromethyl ketones which were also found to have excellent oral profiles.[13] These peptidyl trifluoromethyl ketones were identified by screening a large pool of N-substituted Val-Pro-Val-trifluoromethyl ketones (e.g. **1**, Figure 1), whereby it was found that three neutral P_3 nitrogen atom substituents (R_1, Table 1, entries **1a**−**c**) were associated with high levels of oral activity. A shortcoming of these compounds was their existence as noncrystalline mixtures of diastereoisomers due to the lack of stereochemical control at the P_1 α-carbon. The lack of crystallinity would present many obstacles in the development of these compounds. Thus, we focused our attention on the incorporation of these P_3 nitrogen substituents into other peptidyl-inhibitor series in which control of the chirality at P_1 had been achieved (e.g. **2**−**4**, Figure 1). In addition, we sought to develop synthetic methodology which would allow for efficient stereochemical control in the trifluoromethyl ketone series. This paper details our efforts in both of these areas.[14]

Results and Discussion

General Synthetic Methods. The synthesis of compounds in the trifluoromethyl ketone (TFMK) series (Table 1 and Scheme 1) began with the tripeptide amino alcohol **5**, which was obtained as a mixture of two diastereoisomers by our published procedure.[15] The acylation of **5** was accomplished using the appropriate acid chloride, or in cases where the acid chloride was not readily available, via a carbodiimide (EDC)-mediated coupling with the corresponding acid. Oxidation of the trifluoromethyl alcohol to yield the ketone **1** was carried out using a modified Pfitzner−Moffat procedure.[16] TFMK's obtained in this manner were 1:1 mixtures of diastereoismers at the P_1 α-carbon together with variable amounts of the hydrated form of the ketone. Characterization of these compounds was best achieved by prehydrating the TFMK using a D_2O/

† Department of Medicinal Chemistry.
‡ Department of Pharmacology.
§ Department of Drug Disposition and Metabolism.
⊗ Abstract published in *Advance ACS Abstracts,* September 1, 1997.

S0022-2623(97)00250-1 CCC: $14.00

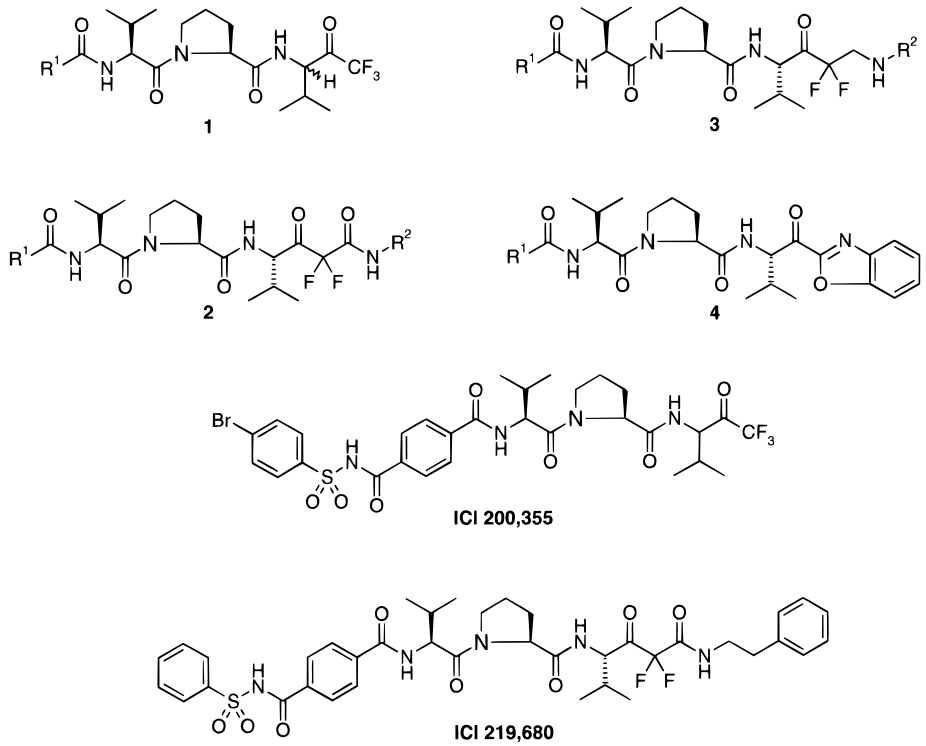
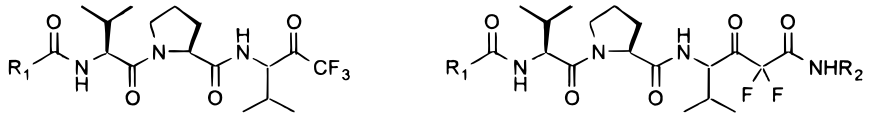


Figure 1. Elastase inhibitors.

Table 1. Biological Data for Trifluoromethyl Ketones and Difluoro β-Ketoamides



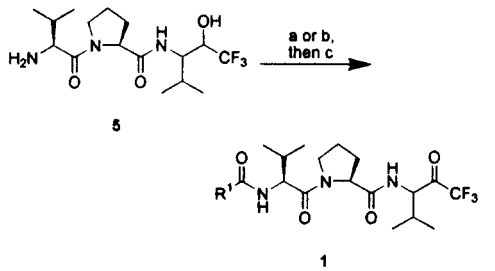
compd	R_1	R_2	series	stereochemistry at P1 position	molecular formula[a]	K_i (nM)[b]	acute hemorrhagic assay % inhibition,[c] 10 mg/kg oral dose
1a	4-$MeOC_6H_5$		TFMK	*S,R*	$C_{24}H_{32}N_3O_5F_3 \cdot 0.7H_2O$	3.3 ± 0.6	70
1b	$C_6H_5CH_2O$		TFMK	*S,R*	$C_{24}H_{32}N_3O_5F_3 \cdot 0.6H_2O$	2.6 ± 0.3	42
1c	C_6H_5O		TFMK	*S,R*	$C_{23}H_{30}N_3O_5F_3 \cdot 0.4H_2O$	1.5 ± 0.3	93
1d	cyclopentyl-O		TFMK	*S,R*	$C_{22}H_{34}N_3O_5F_3 \cdot 0.8H_2O$	1.9 ± 1	43
1e	isobutyl-O		TFMK	*S,R*	$C_{21}H_{34}N_3O_5F_3 \cdot 0.3H_2O$	20 ± 3	3
1f	isopropyl-O		TFMK	*S,R*	$C_{20}H_{32}N_3O_5F_3 \cdot 0.5H_2O$	33 ± 12	69
1g	CH_3CH_2O		TFMK	*S,R*	$C_{19}H_{30}N_3O_5F_3 \cdot 0.4H_2O$	43 ± 14	74
1h	CH_3O		TFMK	*S,R*	$C_{18}H_{28}N_3O_5F_3 \cdot 0.3H_2O$	36 ± 7	92
1j	$C_6H_5OCH_2$		TFMK	*S,R*	$C_{24}H_{32}N_3O_5F_3$	17 ± 7	8
2a	4-$MeOC_6H_5$	*n*-propyl	DFKA	*S*	$C_{28}H_{40}N_4O_6F_2 \cdot 0.6H_2O$	8.9 ± 1.5	17
2b	4-$MeOC_6H_5$	CH_2-(2-pyridyl)	DFKA	*S*	$C_{31}H_{39}N_5O_6F_2 \cdot 1.0H_2O$	3.1 ± 0.9	43
2c	4-$MeOC_6H_5$	CH_2-(2-pyridyl)	DFKA	*R*	$C_{31}H_{39}N_5O_6F_2 \cdot 0.7H_2O$	58 ± 18	31
2d	4-$MeOC_6H_5$	methyl	DFKA	*S*	$C_{26}H_{36}N_4O_6F_2 \cdot 0.5H_2O$	3.8 ± 1.4	25
2e	$C_6H_5CH_2O$	methyl	DFKA	*S*	$C_{26}H_{36}N_4O_6F_2 \cdot 0.5H_2O$	3.0 ± 0.9	7
2f	$C_6H_5CH_2O$	H	DFKA	*S*	$C_{25}H_{34}N_4O_6F_2 \cdot 0.8H_2O$	0.38 ± 0.1	17
2g	C_6H_5O	methyl	DFKA	*S*	$C_{25}H_{34}N_5O_6F_2 \cdot 0.2H_2O$	0.85 ± 0.2	80
2h	C_6H_5O	CH_2-(2-pyridyl)	DFKA	*S*	$C_{30}H_{37}N_5O_6F_2 \cdot 0.5H_2O$	1.7 ± 0.9	69

[a] All compounds were analyzed for C, H, N; the results agreed to within ±0.4% of the theoretical values. [b] Method reported in ref 25. [c] Percent inhibition of elastase-induced lung damage when the compound was dosed orally at 10 mg/kg, 30 min prior to the instillation of a 50 μg challange of elastase.

DMSO-d_6 mixture prior to analysis by ^{1}H NMR. Purity was evaluated by reverse phase HPLC analysis in acetonitrile/water mixtures which showed a single peak (hydrated ketone form) for each of the two diasteroisomers present.

Compounds in the difluoro β-ketoamide (DFKA) series (Scheme 2) were obtained as single diastereoisomers which were nonhydrated. The synthesis of DFKAs begins with the stereochemically pure alcohol **7**, which was obtained via a Reformatsky reaction between Cbz-valinal and ethyl 2-bromo-2,2-difluoroacetate.[17] The major diastereoisomer from this reaction (**7**, 3*R*,4*S*) was then isolated by column chromatography. The ester group in **7** could be converted to the amide **8** by either direct amidation with the appropriate amine or hydrolysis of the ester to the acid followed by EDC-mediated amide formation. Hydrogenolysis of the nitrogen protecting group gave amine **9**. The substituted Val-Pro dipeptide **11** was obtained by acylation of **10** and subsequent TFA cleavage of the *tert*-butyl ester to give

Scheme 1[a]



[a] Generic group R_1 is defined in Tables 1 and 2. Reagents: (a) $R^1C(O)Cl$, $NaHCO_3$, THF; (b) R^1CO_2H, EDC, HOBT, Et_3N, DMF; (c) EDC, Cl_2CHCO_2H, DMSO, toluene.

the acid **11**. Coupling of **9** and **11** using EDC gave the penultimate alcohol **12** which was oxidized to give **2** as a single *S*,*S*,*S* stereoisomer.

Compounds in the difluoromethyleneamine (**3**), and α-ketobenzoxazole (**4**) series were made using previously reported procedures.[18,19]

Pharmacological Evaluation. Having identified several substituted tripeptide TFMK's with oral activity ($ED_{50} \leq 10$ mg/kg, **1a**−**c**, Table 1), we broadened the scope of our work to investigate whether additional improvements in their oral profile could be obtained by altering the electrophilic ketone portion of the molecule. In this regard we focused our attention on the three P_3 nitrogen substituents which conveyed the best oral activity in the TFMK series, these being the 4-methoxybenzamide, phenylurethane, and Cbz groups.[13] We then incorporated these substituents into a variety of other electrophilic ketone series focusing primarily on series in which chemical precedent existed for the preparation of single diastereoisomers. The results of this work are shown in Tables 1 and 2. *In vivo* testing utilized a hamster-based acute hemorrhagic assay (AHA).[20] This assay measures the ability of an orally dosed inhibitor to protect the lung from hemorrhage induced by a subsequent intratracheal challenge of a 50 μg/animal dose of HLE. Variation of the time interval between oral administration of inhibitor and instillation of HLE was used as a measure of the compounds' duration of action.

A number of difluoro β-ketoamides were prepared which contained 4-methoxybenzamide, phenylurethane, or Cbz groups. Previous work had found that placement of large lipophilic groups in the $P_{1'}$ position produced very potent inhibitors *in vitro* (e.g. ICI 219,680, Figure 1, $K_i = 0.06$ nM). However, we chose to keep the size of the $P_{1'}$ group small in order to maintain good aqueous solubility. As is shown in Table 1, small $P_{1'}$ substituents gave *in vitro* potencies comparable to those found in the TFMK series. In fact the DFKA with a primary amide substituent (**2f**) was the most potent compound. Comparison of compounds **2b** and **2c** demonstrates that the *S* stereochemistry at the P_1 position is required for good potency as would be expected based upon the preference of HLE for amino acids of the L configuration. Compounds containing the phenylurethane substituent showed good activity *in vivo*, with **2g** being the most potent of the DFKA series. However, stability problems with the phenylurethane moiety prevented the further development of **2g** or **2h** (*vide infra*).

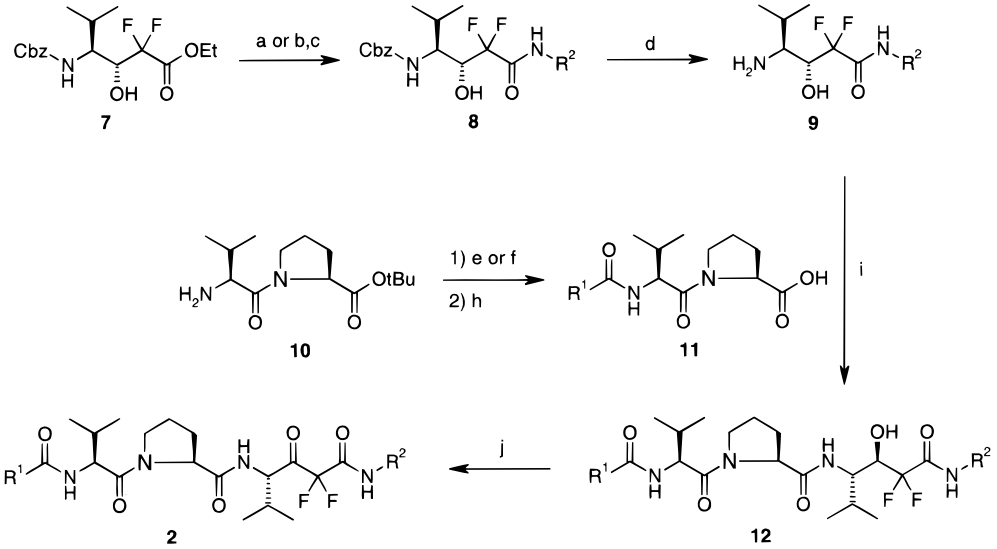
A recent publication from our laboratories demonstrated that difluoromethyleneamines (DFMA) (e.g. **3**, Figure 1) were effective inhibitors of HLE *in vitro*.[19] In the current context, we found that while these compounds were potent inhibitors of the enzyme *in vitro*, they were generally inactive upon oral dosing (Table 2).

α-Ketobenzoxazoles (AKBOs) have been shown to be effective inhibitors of HLE, and good precedent exists for stereochemical control in this series.[21] A number of compounds in this series were prepared and tested with the results shown in Table 2. Several of these compounds were found to have excellent affinity for the enzyme, with **4b**, **4c**, **4d**, and **4f** showing subnanomolar inhibition. Compounds from this series were previously shown to have good activity as aerosolized agents.[22] However, when administered by the oral route, these compounds gave only low levels of activity.

The results with the AKBO, DFKA, DFMA, and TFMK series demonstrate that the type of electrophilic carbonyl employed in the inhibitor can have a very dramatic effect on *in vivo* activity. Our work with these series has not revealed any metabolic weakness of one series over another, and thus the differences in oral activity are most likely related to subtle effects that each of these electrophilic ketone moieties has on the overall physical properties of the molecule. Of the various combinations of P_3 nitrogen substituents and electrophilic ketones evaluated, the trifluoromethyl ketones had a superior overall profile, and as is shown in Table 1 these compounds were found to be very potent inhibitors of the enzyme both *in vitro* and *in vivo*. Phenylurethane **1c** had a particularly strong oral profile, with >90% inhibition in the acute hemorrhagic assay at a 10 mg/kg oral dose. However, the stability of the phenylurethane moiety was poor. When compound **1c** was placed in plasma from a number of species including human, the compound was found to undergo significant decomposition ($t_{1/2} < 15$ min). Related TFMK's such as **1a** were found to be completely stable under similar conditions. Chemical stability studies also found compound **1c** to be unstable, resulting in a pH dependent hydrolysis of the phenylurethane moiety. These concerns prompted us to prepare other analogs which would be expected to have greater stability such as compounds **1d**−**h**. We were pleased to find that several of these compounds had excellent oral activity. In fact the simple methylurethane **1h** gave 92% inhibition in the oral assay. Measurement of the chemical stability of compound **1h** also found a large increase in its stability relative to **1c**, as would be expected.[23] Incubation of **1h** in rat and human plasma under identical conditions as used to test compound **1c** found no instability over a 24 h period.

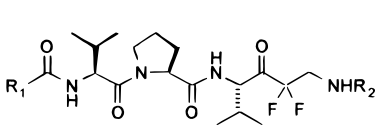
While the level of oral activity of several compounds in the TFMK series was excellent, their lack of crystallinity necessitated that they be purified by extensive column chromatography. While this is an acceptable situation in a research environment, it would be a major disadvantage in producing the large quantities of material needed to support further development of an elastase inhibitor as a possible therapeutic agent. We were unsure as to whether the lack of crystallinity in this series was a consequence of the compounds being prepared as diastereomeric mixtures or whether the compounds were intrinsically noncrystalline. We knew that the α-stereocenter in trifluoromethyl ketones un-

Scheme 2[a]

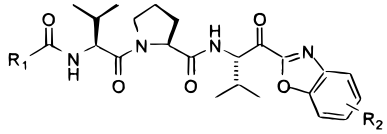


[a] Generic groups R^1 and R^2 are defined in Tables 1 and 2. Reagents: (a) R^2NH_2, EtOH; (b) LiOH, THF, MeOH, H_2O; (c) R^2NH_2, EDC, HOBT, *N*-methylmorpholine, THF; (d) H_2, 10% Pd/C, EtOH; (e) $R^1C(O)Cl$, $NaHCO_3$, THF; (f) R^1CO_2H, EDC, HOBT, Et_3N, DMF; (h) TFA; (i) HOBT, **9**, *N*-methylmorpholine, EDC, THF; (j) EDC, Cl_2CHCO_2H, DMSO, toluene.

Table 2. Biological Data for α-Ketobenzoxazoles and Difluoromethyleneamines



Difluoromethyleneamines Series **3** (DFMA)



α-Ketobenzoxazoles Series **4** (AKBO)

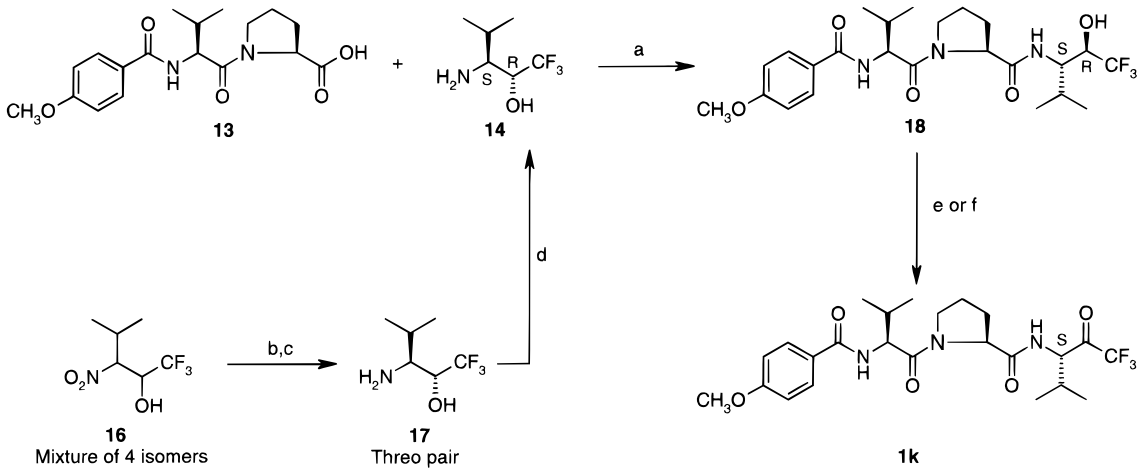
compd	R_1	R_2	series	stereochemistry at P1 position	molecular formula[a]	K_i (nM)[b]	acute hemorrhagic assay % inhibition,[c] 10 mg/kg oral dose
3a	4-$MeOC_6H_5$	$(O)COC_4H_9$	DFMA	*S*	$C_{30}H_{44}N_4O_7F_2 \cdot 1.0H_2O$	3.4 ± 0.9	24
3b	4-$MeOC_6H_5$	H	DFMA	*S*	$C_{25}H_{36}N_4O_5F_2 \cdot 1.0TFA$[d]	15 ± 2.5	10
3c	4-$MeOC_6H_5$	$CH_2C_6H_5$	DFMA	*S*	$C_{32}H_{42}N_4O_5F_2 \cdot 1.0TFA$	0.35 ± 0.1	17
3d	4-$MeOC_6H_5$	$(O)CC_6H_5$	DFMA	*S*	$C_{32}H_{40}N_4O_6F_2$	6.4 ± 2.2	18
3e	$C_6H_5CH_2O$	$(O)COC_4H_9$	DFMA	*S*	$C_{30}H_{44}N_4O_7F_2 \cdot 0.5H_2O$	1.1 ± 0.3	0
3f	$C_6H_5CH_2O$	H	DFMA	*S*	$C_{25}H_{36}N_4O_5F_2 \cdot 1.0TFA$	130 ± 7	
3g	$C_6H_5CH_2O$	$CH_2C_6H_5$	DFMA	*S*	$C_{32}H_{42}N_4O_5F_2 \cdot 1.0TFA$	1.6 ± 0.3	0
3h	C_6H_5O	$(O)COC_4H_9$	DFMA	*S*	$C_{29}H_{42}N_4O_7F_2$	0.35 ± 0.04	0
3i	C_6H_5O	H	DFMA	*S*	$C_{24}H_{34}N_4O_5F_2 \cdot 1.4TFA$	0.81 ± 0.1	45
3j	C_6H_5O	$CH_2C_6H_5$	DFMA	*S*	$C_{31}H_{40}N_4O_5F_2 \cdot 1.2TFA$	4.1 ± 0.4	18
3k	C_6H_5O	$(O)CCH_3$	DFMA	*S*	$C_{26}H_{36}N_4O_6F_2$	2.3 ± 0.4	42
3l	C_6H_5O	(O)C(3-pyridyl)	DFMA	*S*	$C_{30}H_{37}N_5O_6F_2$	0.35 ± 0.05	46
4a	$C_6H_5CH_2O$	H	AKBO	*S*	$C_{30}H_{36}N_4O_6$	4.7 ± 0.9	0
4b	C_6H_5O	3-CO_2CH_3	AKBO	*S*	$C_{31}H_{36}N_4O_8 \cdot 0.25H_2O$	0.64 ± 0.03	27
4c	$C_6H_5CH_2O$	2-CO_2CH_3	AKBO	*S*	$C_{31}H_{36}N_4O_8$	0.58 ± 0.2	34
4d	4-$MeOC_6H_5$	2-CO_2CH_3	AKBO	*S*	$C_{32}H_{38}N_4O_8$	0.65 ± 0.1	0
4e	4-$MeOC_6H_5$	2-$NHC(O)CH_3$	AKBO	*S*	$C_{32}H_{39}N_5O_7$	8.0 ± 0.8	11
4f	C_6H_5O	2-CO_2CH_3	AKBO	*S*	$C_{31}H_{36}N_4O_8$	0.64 ± 0.05	12

[a] All compounds were analyzed for C, H, N; the results agreed to within ±0.4% of the theoretical values. [b] Method reported in ref 25. [c] Percent inhibition of elastase-induced lung damage when the compound was dosed orally at 10 mg/kg, 30 min prior to the instillation of a 50 μg challange of elastase. [d] TFA = trifluoroacetic acid.

dergo a rapid epimerization *in vivo*. Thus obtaining TFMKs as single isomers would not be expected to dramatically improve the biological profile of the molecule, but could confer important advantages in the isolation and purification of the compound. Thus we focused our attention on obtaining single isomers of several of the most promising compounds in Table 1, first to see if these compounds would be crystalline, and second, if crystallization would be an effective method for their purification.

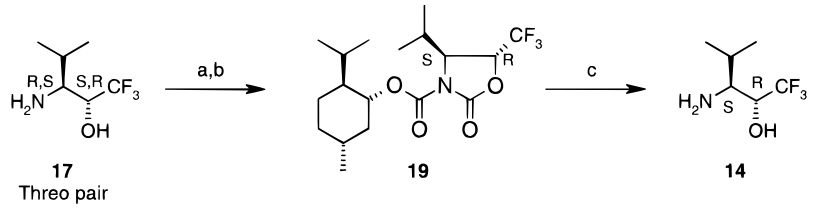
Synthesis of Diastereomerically Pure TFMKS. We have previously reported methodology to prepare stereochemically enriched TFMK's via the addition of (trifluoromethyl)zinc reagents to aldehydes such as valinal derivatives.[24] Oxidation of the resulting alcohol to the ketone can be effected by procedures, such as the Dess−Martin periodinane reagent, which do not lead to epimerization. For the present work, the strategy we used to prepare stereochemically pure TFMKs is shown in Scheme 3. This approach avoids the use of valinal derivatives, which can be easily epimerized, and also removes the safety concerns which would be faced with the large-scale use of the Dess−Martin periodinane reagent. Thus, the coupling of acid **13** and pure 2*R*,3*S* amino alcohol **14** gave the precursor alcohol **18** as a crystalline single isomer. The amino alcohol **14** was

Scheme 3[a]



[a] Reagents: (a) HOBT, *N*-methylmorpholine, EDC, THF; (b) fractional crystallization of threo isomers from pentane; (c) Raney nickel, H_2, 2-propanol; (d) *d*-tartaric acid, ethanol; (e) EDC, Cl_2CHCO_2H, DMSO, toluene; (f) $KMnO_4$, NaOH, ^tBuOH, H_2O.

Scheme 4[a]



[a] Reagents: (a) triphosgene, NaOH, toluene, H_2O; (b) (i) *n*-BuLi, THF, then (−)-menthyl chloroformate; (ii) fractional crystallization; (c) KOH, dioxane, H_2O.

obtained by resolution of **17** as the *d*-tartaric acid salt. The racemic *threo*-amino alcohols used in the resolution were obtained by fractional crystallization of a mixture of nitro alcohols **16**, followed by reduction of the nitro group using Raney nickel.[15] The mother liquors from this fractional crystallization, which were enriched in the erythro isomers, could be reequilibrated to an erythro/threo mixture by treatment with K_2CO_3 to allow for additional fractional crystallizations. Thus efficient use could be made of all the nitro alcohol product. While the tartaric acid resolution of **17** was initially used to obtain pure amino alcohol **14**, this process was cumbersome and low yielding, especially upon scale-up. Seven recrystallizations of the tartrate salt of **17** were required to obtain stereochemically pure **14**; thus a more efficient process was required. After examining a number of alternative resolving agents without success, we developed a process (Scheme 4) in which a covalent diastereomeric compound is formed between **17** and (−)-menthyl chloroformate. In this procedure conversion of **17** into the oxazolidone derivative dramatically enhances the crystallinity of the desired adduct **19** and avoids the poor chemical yields obtained by directly coupling (−)-menthyl chloroformate to **17**. This resolution procedure is remarkably efficient, as crystallization of the desired stereoisomer **19** from the crude reaction mixture proceeds to give material of 99% de in 67% of the theoretical yield. The chiral auxiliary and oxazolidone ring in **19** are then easily cleaved by treatment with base to furnish stereochemically pure **14**.

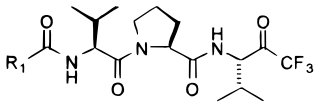
Oxidation of **18** (Scheme 3) using a modified Pfitzner−Moffat procedure proceeded with some epimerization to give the ketone as a 80:20 mixture of *SSS* to *SSR* isomers. Without further purification this mixture was crystallized to furnish **1k** in 55% yield and in 94% diastereomeric purity. We were greatly encouraged by the fact that crystallization had enriched the stereochemical purity of the compound and found that a single recrystallization from ether gave exclusively the desired *SSS* isomer. Subsequent work has found that $KMnO_4$ under basic conditions is a more efficient oxidant and proceeds without epimerization of the α-center. Using this procedure, high yields (89%) of stereochemically pure material are obtained.

Using the methodology described above, the TFMKs with the best oral activity (**1a**,**f**,**h**) were prepared as single isomers (**1k**−**m**, Table 3). Interestingly, compound **1k** could be isolated in crystalline form, as either the hydrate or ketone form depending upon isolation conditions, while **1l** and **1m** were obtained in crystalline form as the hydrated species.

Biological Evaluation. Biological data for compounds **1k**−**m** is shown in Table 3. All the compounds are very potent inhibitors of the enzyme *in vitro* and have high levels of inhibition of the elastase-induced hemorrhage when administered to hamsters orally at 10 mg/kg. Four-point dose−response curves (0.1−10 mg/kg) were generated to determine the ED_{50} value for each compound. This work found compound **1l** to be the most potent with an ED_{50} of 2.0 mg/kg. Compounds **1k** and **1l** were also evaluated in the AHA model after intravenous administration, which found their ED_{50}'s to be 0.59 and 0.51 mg/kg, respectively.

Compounds **1k** and **1l** were found to possess advantages in their physical property profiles and were selected for further evaluation.[26] Enzyme selectivity studies were carried out for **1k** and **1l** vs a variety of proteolytic enzymes. The data is shown in Table 4. As

Table 3. Biological Data for Single-Ddiastereomer Trifluoromethyl Ketones



compd	R_1	stereochemistry at P1 position	molecular formula[a]	K_i (nM)[b]	acute hemorrhagic assay: % inhibition,[c] 10 mg/kg oral dose	acute hemorrhagic assay: intravenous ED_{50} (mg/kg)	acute hemorrhagic assay: oral ED_{50} (mg/kg)
1k	4-$MeOC_6H_5$	*S*	$C_{24}H_{32}N_3O_5F_3$	1.3 ± 0.3	84	0.59	4.9
1l	CH_3O	*S*	$C_{18}H_{28}N_3O_5F_3 \cdot 1.0H_2O$	13 ± 1.7	92	0.51	2.0
1m	isopropyl-O	*S*	$C_{20}H_{32}N_3O_5F_3 \cdot 1.5H_2O$	5.8 ± 1.3	80		3.0

[a] All compounds were analyzed for C, H, N; the results agreed to within ±0.4% of the theoretical values. [b] Method reported in ref 25. [c] Percent inhibition of elastase-induced lung damage when the compound was dosed orally at 10 mg/kg, 30 min prior to the instillation of a 50 μg challange of elastase.

Table 4. *In Vitro* Enzyme Selectivity Ratios of Compounds **1k** and **1l**

enzyme	compound **1k**: K_i (nM)	compound **1k**: selectivity ratio[a]	compound **1l**: K_i (nM)	compound **1l**: selectivity ratio
serine proteases				
human leukocyte elastase	6.7		13	
porcine pancreatic elastase	200	153	149	11
human pancreatic chymotrypsin	200 000	153 846	66 600	5123
human pancreatic trypsin	NI[b]		NI	
human plasma thrombin	NI		NI	
human plasma kallikrein	NI		NI	
human urine kallikrein	NI		NI	
human leukocyte cathepsin G	250 000	192 307	NI	
cysteine proteases				
papaya papain	NI		NI	
bovine spleen cathepsin L	NI		NI	
metalloproteases				
rabbit lung angiotensin converting enzyme	NI		NI	
carboxypeptidase A	170 000	130 769	NI	
others				
human erythrocyte acetyl cholinesterase	NI		NT[c]	
human plasma monoamine oxidase	NI		NI	

[a] Selectivity ratio refers to the ratio K_i(enzyme)/K_i(HLE). [b] NI, no inhibition at highest concentration tested [I] = 188 μM. [c] NT = not tested.

Table 5. Pharmacokinetic Parameters for Compounds **1k** and **1l**[a,b]

compd	species	$t_{1/2}$ (h)	C_{max}[c] (ng/mL)	T_{max}[d] (h)	intravenous AUC[e] (ng·h/mL)	oral AUC (ng·h/mL)	bioavailability[f] (%)
1k	hamster	1.2	373	0.50	1365	355	26
	rat	0.82	972	0.25	1702	1400	82
	dog	2.0	382	0.50	3179	1254	39
1l	hamster	2.0	1460	0.25	2890	2160	75
	rat	1.3	3690	0.50	6540	5480	84
	dog	0.81	5440	0.33	9366	6540	70

[a] Compounds were administered as a solution in 10% PEG400/H_2O. [b] All data has been normalized to a 10 mg/kg dose and refers to the concentration of *SSS* diastereoisomer present. [c] Maximum concentration of unchanged drug in plasma recorded in the period 0−6 h postdose. [d] Time of maximum concentration. [e] Integrated area under the concentration vs time curve. [f] Bioavailability as measured by the ratio of intravenous to oral AUC.

can be observed from the data in the table, both compounds are very selective for HLE over other enzymes tested.

Pharmacokinetics. Pharmacokinetic parameters for **1k** and **1l** were measured in three different species: hamster, rat, and dog (Table 5). The data presented in the table only represent the amount of *SSS* isomer present in plasma and therefore underrepresent the amount of drug available. Studies in plasma have shown that these compounds undergo epimerization to a mixture of *SSS* and *SSR* isomers, whose composition is approximately 60:40 *SSS* to *SSR*. The epimerization is a reversible process as dosing either the *SSS* or *SSR* isomer independently produces the same ratio of isomers. In general, these compounds have excellent oral bioavailability as the data in the table indicate. The bioavailability for **1k** did show some dependence upon species, with rat giving the highest result. Compound **1l**, however, showed uniformly high bioavailability in all three species measured. Metabolic studies on **1k** and **1l** have found the trifluoromethyl alcohols to be the major metabolites of these compounds. This metabolite has also been observed for a number of other trifluoromethyl ketones, both peptidic and nonpeptidic, suggesting that this mode of metabolism is independent of the rest of the molecule.

Summary

We have developed a series of peptidyl trifluoromethyl ketone inhibitors of HLE which have excellent pharmacological profiles. Methods have been developed that allow for the synthesis of these compounds in stereo-

chemically pure form, which should greatly facilitate their large-scale manufacture and purification. Two of these compounds, **1k** and **1l**, have high levels of oral bioavailability in several species. Compound **1l** has entered development as ZD8321 and is presently undergoing clinical evaluation.[27] These compounds demonstrate that peptidyl trifluoromethyl ketone inhibitors can achieve high levels of oral activity and bioavailability, and therefore they may prove useful as therapeutic agents in the treatment of diseases in which elastase is implicated.

Experimental Section

General Methods. Analytical samples were homogeneous by TLC and afforded spectroscopic results consistant with the assigned structures. Proton NMR spectra were obtained using either a Bruker WM-250 or AM-300 spectrometer. Chemical shifts are reported in parts per million relative to Me_4Si as internal standard. Mass spectra (MS) were recorded on a Kratos MS-80 instrument operating in the chemical ionization (CI) mode. Elemental analyses for carbon, hydrogen, and nitrogen were determined by the ZENECA Pharmaceuticals Analytical Department, on a Perkin-Elmer 241 elemental analyzer, and are within ±0.4% of theory for the formulas given. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel plates (60F-254, 0.2 mm thick, E. Merck). Visualization of the plates was accomplished by using UV light or phosphomolybdic acid/ethanol charring procedures. Chromatography refers to flash chromatography conducted on Kieselgel 60 230−400 mesh (E. Merck, Darmstadt) using the indicated solvents. Solvents used for reactions or chromatography were either reagent grade or HPLC grade. Reactions were run under an argon atmosphere at ambient temperature unless otherwise noted. Solutions were evaporated under reduced pressure on a rotary evaporator. The following abbreviations are used: THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; EDC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; TFA, trifluoroacetic acid; HOBT, *N*-hydroxybenzotriazole.

***N*-(4-Methoxybenzoyl)-L-valyl-L-proline (13).** To a 0 °C solution of **10** (12.5 g, 46 mmol) in THF (100 mL) was added a solution of anisoyl chloride (8.37 g, 49 mmol) in THF (200 mL) followed by triethylamine (18 mL, 129 mmol). After 1 h the reaction was judged complete by TLC, and the solvent was then removed by evaporation. The resulting material was dissolved in ethyl acetate and was washed with water, aqueous saturated sodium bicarbonate, aqueous saturated ammonium chloride, and brine. The organic solution was dried ($MgSO_4$) and the solvent removed. The resulting material was purified by silica gel chromatography (methanol:methylene chloride, 3:97) to provide the *tert*-butyl ester (14.9 g, 80%) as a white foam. A portion of this ester (7.13 g, 17.6 mmol) was dissolved in trifluoroacetic acid (85 mL) and the resulting solution allowed to stir for 0.5 h. The solvent was then removed under vacuum and the residue dissolved in ethyl acetate and washed with water. The organic solution was dried and the solvent removed. The resulting material was purified by chromatography (methanol:methylene chloride:acetic acid, 97:2:1) to provide **13** (6.5 g): ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.37 (d, 1H), 7.90 (d, 2H), 6.96 (d, 2H), 4.44 (t, 1H), 4.24 (dd, 1H), 3.97 (m, 1H), 3.80 (s, 3H), 3.64 (m, 1H), 2.15 (m, 2H), 1.88 (m, 3H), 0.99 (d, 3H), 0.87 (d, 3H).

***threo*-(2*R*,*S*,3*S*,*R*)-3-Amino-4-methyl-1,1,1-trifluoro-2-pentanol (17).** To a solution of nitro alcohol **16** (11.1 kg of a 44:56 mixture of threo to erythro isomers plus other impurities) in CH_2Cl_2 (5 L) was added potassium carbonate (0.766 kg, 5.54 mol) and the mixture stirred for 2 days at room temperature. The reaction mixture was cooled to 0 °C and then quenched with 3 N HCl (3.6 L) to a final pH of 1. The organic layer was separated, and the aqueous layer was washed with CH_2Cl_2. The combined organic fractions were dried ($MgSO_4$), and the solvent was concentrated to give a thick oil which was 66:34 (threo:erythro) by GC. This oil was diluted with pentane (3 L) and placed in a freezer overnight. The resulting solid was collected and washed with pentane to afford the nitro alcohol (2.14 kg, 10.6 mol, >90% threo isomer). The filtrate (~1:1 threo/erythro) was distilled under vacuum (at 1 mmHg) using a Vigreux column at a pot temperature of 85 °C. (**Caution!** This compound has been shown to be thermally unstable at high temperatures and heating must be monitored so that the temperature does not exceed 100 °C!) A light yellow distillate (bp 65−70 °C) was collected. Crystallization of this distillate from pentane gave 1.3 kg of additional threo isomer: MS (CI) 202 (M + H). A portion of the threo nitro alcohol (800 g, 3.98 mol) in isopropyl alcohol (2 L) was added to Raney Ni (48 g) in isopropyl alcohol (400 mL) under a N_2 atmosphere in 4 L stainless steel Parr bottle. The reaction mixture was placed on a Parr shaker under 50 psi of hydrogen atmosphere. An exothermic reaction resulted with the temperature reaching 50 °C. The reaction mixture was cooled in an ice bath to bring it back to 20 °C. The reaction mixture was run for 36 h until H_2 uptake had ceased. An aliquot was removed and analyzed by capillary GC which indicated that the reaction was complete. The reaction mixture was filtered through Celite and the filtercake washed with methanol. The reaction mixture was concentrated to 1.5 L, diluted with hexane (1.5 L), and stored in a freezer for 48 h. The resulting solid was collected and washed with hexane. The filtrate was concentrated with hexane to provide additional material. The solids were combined to give 496 g (73%) of pure amino alcohol **17**: ^{1}H NMR (300 MHz, acetone-d_6) δ 8.45 (s, 1H), 5.11 (m, 1H), 3.61 (m, 1H), 1.72 (m, 1H), 0.86 (d, 6H); MS (CI) 172 (M + H).

***threo*-(2*R*,3*S*)-3-Amino-4-methyl-1,1,1-trifluoro-2-pentanol (14).** To a solution of amino alcohol **17** (1.9 kg, 11.1 mol) in toluene (7 L) was added a solution of sodium hydroxide (888 g, 22.2 mol) in water (8 L). Triphosgene (1098 g, 3.7 mol) was added in 100 g portions with cooling to maintain the temperature between 20 and 30 °C. The reaction was stirred for 1 h after the addition of triphosgene was complete. The reaction was judged complete after analysis by capillary GC. The organic portion was separated and the aqueous phase extracted with a mixture of THF and toluene (1:1). The organic phases were combined and washed with 0.5 N HCl, saturated aqueous bicarbonate, and brine. The solution was dried ($MgSO_4$), filtered, and concentrated to a thick slurry in toluene. The slurry was diluted with hexane and the resulting solid collected and washed with hexane. Concentration of the filtrate provided additional material. The solids were combined to give 1939 g (89%) of pure oxazolidinone: white solid; mp 71 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45 (s, 1H), 5.11 (m, 1H), 3.61 (m, 1H), 1.72 (m, 1H), 0.86 (d, 6H).

A solution of the oxazolidinone prepared above (1434 g, 7.27 mol) in THF (16 L) was cooled to −70 °C, and to this was added *n*-butyllithium (800 mL, 8.0 mol, from a 10 M solution in hexane) while the temperature was maintained between −60 and −40 °C. When the addition was complete, the solution was stirred for 0.5 h and then (−)-menthyl chloroformate (1670 g, 7.64 mol) was added (temperature maintained at −60 °C). The reaction was allowed to warm to room temperature overnight. The next day the reaction was cooled to −50 °C and quenched by addition of saturated aqueous sodium bicarbonate (200 mL) and then diluted with water. The layers were separated, and the organic portion was washed with brine. The aqueous layers were combined, diluted with 1 N NaOH to pH 10, and then extracted with ethyl acetate (2×). The organic layers were combined, dried ($MgSO_4$), filtered, and concentrated. The residue was crystallized from ether/hexane to give 1295 g, 47% of crystalline material which was recrystallized from ether/hexane to provide 929 g (33%, 66% of theoretical) of product which was found by ^{19}F NMR to be pure *S*,*R* isomer (>99% de). 2*R*,3*S* isomer: white solid; mp 138−140 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.51 (dd, 1H), 4.68 (m, 1H), 4.26 (m, 1H), 2.27 (m, 1H), 1.94 (d, 1H), 1.78 (m, 1H), 1.62 (d, 2H), 1.42 (m, 2H), 1.01 (dd, 2H), 0.95−0.84 (m, 24H), 0.71 (d, 3H); ^{19}F NMR (376.5 MHz, DMSO) δ −76.9910. 2*S*,3*R* isomer: mp 80−81.5 °C; ^{19}F NMR (376.5 MHz, DMSO-d_6) δ −77.0019.

To a solution of the urethane adduct prepared above (311 g, 820 mmol) in dioxane (800 mL) was added a solution of potassium hydroxide (460 g, 8.2 mol) in water (460 mL) at room temperature, followed by heating to reflux (95 °C) for 40 h. The mixture was cooled to 0 °C and made acidic to pH 1 with 6 N HCl (1.4 L). The layers were separated, and the aqueous layer was washed with CH_2Cl_2. The combined organic portions were back-extracted with 1 N HCl, and all of the aqueous layers were combined and made basic with 20% NaOH to pH 13. The aqueous base was extracted with CH_2Cl_2 (3 × 1 L), dried ($MgSO_4$), filtered, and concentrated to give 122.5 g (87%) of pure amino alcohol **14**: characterized as the hemioxalate salt; white solid; mp 184−186 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.71 (bs, 3H), 4.08 (ddd, 1H), 2.88 (m, 1H), 1.81 (m, 1H), 0.92 (m, 6H). Anal. Calcd $C_6H_{12}F_3NO\cdot 0.5C_2H_2O_4$: C, 38.89; H, 6.06; N, 6.48. Found: C, 38.75; H, 5.95; N, 6.47.

(*S*)-1-[(*S*)-2-(4-Methoxybenzamido)-3-methylbutyryl]-*N*-[(*S*)-2-methyl-1-((*R*)-2,2,2-trifluoro-1-hydroxyethyl)-propyl]pyrrolidine-2-carboxamide (18). To a solution of **13** (7.1 g, 20 mmol), **14** (3.8 g, 22 mmol, free base), and *N*-hydroxybenzotriazole hydrate (5.5 g, 41 mmol) in DMF (40 mL) was added triethylamine (7.1 mL, 51 mmol) followed by EDC (5.9 g, 31 mmol). The resulting mixture was allowed to stir for 12 h. The reaction mixture was diluted with ethyl acetate and washed with 1 N HCl (2×), water, 1 N NaOH, and brine. The solution was dried ($MgSO_4$) and the solvent removed by evaporation. The resulting material was purified by silica gel chromatography (gradient elution, ethyl acetate: methylene chloride (30:70) to methanol:methylene chloride (5: 95)) to give pure **18** (6.6 g, 65%) as a white foam: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.33 (t, 1H), 7.87 (d, 2H), 7.61 (d, 1H), 7.24 (d, 1H), 6.98 (d, 2H), 6.57 (d, 1H), 6.41 (d, 1H), 4.42 (m, 2H), 4.10 (m, 1H), 3.76 (m, 3H), 3.62 (m, 1H), 1.73−2.15 (m, 6H), 0.93 (m, 12H); MS (CI) 502 (M + H).

(*S*)-1-[(*S*)-2-(4-Methoxybenzamido)-3-methylbutyryl]-*N*-[(*S*)-2-methyl-1-(trifluoroacetyl)propyl]pyrrolidine-2-carboxamide (1k). A three-neck, 3 L flask fitted with an addition funnel and nitrogen inlet, charged with a solution of **18** (25.7 g, 0.051 mol) in a mixture of *tert*-butyl alcohol (205 mL) and water (255 mL) was cooled to 0 °C. To this solution was added 0.6 N sodium hydroxide (260 mL, 0.153 mol) followed by the dropwise addition of an aqueous solution of potassium permanganate (24.2 g, 0.153 mol in 385 mL water), over 1 h. After 1 h of stirring at 0 °C, the reaction appeared complete by TLC. Methanol (100 mL) was added to the reaction mixture, and after stirring at 0 °C for an additional 2 h, the mixture was filtered through Celite. The filtrate was cooled to 0 °C and acidified with 1 N hydrochloric acid (200 mL), which resulted in formation of a voluminous white precipitate. Sodium chloride was added to the mixture to the point of saturation, followed by addition of ethyl ether (200 mL) and ethyl acetate (200 mL), and the biphasic mixture was stirred until all solid had dissolved. The layers were separated, and the aqueous layer was extracted with additional ethyl ether:ethyl acetate (1:1), the combined organic layers were washed with brine and dried ($MgSO_4$), and the solvent was removed. The resulting foam was dissolved in warm toluene (150 mL) and allowed to stand overnight at 25 °C. The resulting crystals were collected, washed with ether, and vacuum dried at 40 °C for 3 h to yield **1k** (16.5 g, 65%) as a fine white solid (mp 149−151 °C). Trituration of the filtrate with ether resulted in the recovery of an additional crop of **1k** (6.24 g) to give a combined yield of 89%. Reversed phase HPLC analysis of both product crops indicated diasteresomeric excess of 99.5% (column, SUPELCO LC-18 reversed-phase 25 cm × 4.6 mm, 5 μm; eluent, 60:40 water:acetonitrile; flow rate, 1.0 mL/min, 210 nm, *SSS* isomer t_R = 6.5 min, *SSR* isomer t_R = 8.6 min): white solid; mp 149−151 °C; ^{1}H NMR (300 MHz, DMSO-d_6/D_2O) δ 7.76 (d, 2H), 6.94 (d, 2H), 4.37 (m, 2H), 3.98 (d, 1H), 3.84 (m, 1H), 3.75 (s, 3H), 3.56 (m, 1H), 2.19 (m, 6H), 1.70−0.75 (d, 3H), 0.89 (m, 9H); MS (CI): M + H = 500 (100). Anal. ($C_{24}H_{32}F_3N_3O_5$) C, H, N.

4(*S*)-[(Benzyloxycarbonyl)amino]-2,2-difluoro-3(*R*)-hydroxy-*N*,5-dimethylhexanamide (8, R_2 = CH$_3$). To a solution of ethyl 4(*S*)-[(benzyloxycarbonyl)amino]-2,2-difluoro-3(*R*)-hydroxy-5-methylhexanoate (1.0 g, 2.78 mmol) in ethanol (28 mL) was added aqueous methylamine (4.8 mL, 139 mmol) and the resulting solution allowed to stir for 5 h. The reaction mixture was diluted with ethyl acetate and washed with 0.1 N HCl and brine. The solution was dried ($MgSO_4$) and the solvent removed. The resulting material was chromatographed (gradient elution, ethyl acetate:methylene chloride, 20:80 to 50:50) to provide pure **8** (796 mg, 83%): ^{1}H NMR (300 MHz, DMSO-d_6/TFA-d_4) δ 7.37 (m, 5H), 5.07 (dd, 2H), 4.16 (dd, 1H), 3.58 (d, 1H), 2.68 (s, 3H), 1.82 (m, 1H), 0.90 (dd, 6H).

4(*S*)-Amino-2,2-difluoro-3(*R*)-hydroxy-*N*,5-dimethylhexanamide (9, R_2 = CH$_3$). To a solution of **8** (0.78 g, 2.27 mmol) in ethanol (25 mL) was added 10% Pd/C (50 mg) and the mixture placed under a hydrogen atmosphere (50 psi) and shaken for 4 h. The reaction mixture was filtered through Celite and the solvent removed to yield pure **9** (470 mg, 100%) as an oil sufficiently pure for further use: ^{1}H NMR (300 MHz DMSO-d_6/TFA-d_4) δ 4.22 (t, 1H), 3.19 (m, 1H), 2.74 (s, 3H), 2.04 (m, 1H), 0.96 (dd, 6H); MS (CI) 211 (M + H).

***N*-[(Phenoxycarbonyl)-L-valyl-L-prolyl]-4(*S*)-amino-2,2-difluoro-3(*R*)-hydroxy-*N*,5-dimethylhexanamide (12, R_1 = PhO, R_2 = CH$_3$).** To a solution of the acid **11** (0.66 g, 2 mmol), amine **9** (0.46 g, 2.2 mmol), HOBT (0.454 g, 4 mmol), and *N*-methylmorpholine (0.55 mL, 5 mmol) in THF (20 mL) was added EDC (0.42 g, 2.2 mmol) and the resulting solution allowed to stir for 24 h. The reaction mixture was then diluted with ethyl acetate and washed with water, and brine. The organic solution was dried ($MgSO_4$) and the solvent removed. The crude material was chromatographed (methanol:methylene chloride, 5:95) to give pure **12** (0.87 g, 83%) as a white foam: ^{1}H NMR (300 MHz, DMSO-d_6/TFA-d_4) δ 7.41 (t, 2H), 7.21 (t, 1H), 7.10 (d, 2H), 4.50 (m, 1H), 4.20 (dd, 1H), 4.10 (d, 1H), 3.78 (d, 1H), 3.74 (m, 1H), 3.59 (m, 1H), 2.69 (s, 3H), 1.95 (m, 6H), 1.00 (m, 6H), 0.87 (t, 6H); MS (CI) 433 (M + H − phenol).

***N*-[(Phenoxycarbonyl)-L-valyl-L-prolyl]-4(*S*)-amino-2,2-difluoro-3-oxo-*N*,5-dimethylhexanamide (2, R_1 = PhO, R_2 = CH$_3$).** To a 0 °C solution of **12** (0.78 g, 1.48 mmol) in DMSO (7.5 mL) and toluene (7.5 mL) was added sequentially EDC (2.84 g, 14.8 mmol) and dichloroacetic acid (0.49 mL, 5.92 mmol). The resulting solution was allowed to stir for 2 h and then diluted with ethyl acetate and washed with H_2O and brine. The organic solution was dried ($MgSO_4$) and the solvent removed. The crude product was purified by chromatography (methanol:methylene chloride, 5:95) to provide **2** (400 mg, 52%) as a foam: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.05 (d, 1H), 8.32 (d, 1H), 7.99 (d, 1H), 7.38 (t, 2H), 7.20 (m, 1H), 7.05 (d, 2H), 4.76 (m, 1H), 4.47 (m, 1H), 4.05 (t, 1H), 3.70 (m, 1H), 3.56 (m, 1H), 2.67 (d, 3H), 2.23 (m, 1H), 1.89 (m, 5H), 0.94 (d, 3H), 0.92 (d, 3H), 0.88 (d, 3H), 0.75 (d, 3H). Anal. ($C_{25}H_{34}F_2N_4O_6\cdot 0.2H_2O$) C, H, N.

Acknowledgment. We would like to thank Dr. Steven. J. Pegg, Mr. George J. Sependa, and Mr. Elwin P. Davies of the Zeneca Process Development Department for their help and contributions to this work.

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JM970250Z