Design and Synthesis of Potent, Selective, and Orally Active Fluorine-Containing Renin Inhibitors[1]

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A series of primate renin inhibitors containing difluorocarbinol and difluoroketone groups at the P_1-P_1' position have been synthesized and studied both in vitro and in vivo. In vitro, the compounds were evaluated as inhibitors of monkey renin and the closely related aspartic proteinase, cathepsin D (bovine), as a measure of enzyme selectivity. Interestingly, the difluoroketone derivatives showed greatly reduced selectivity compared with the corresponding alcohols. However, selectivity could be enhanced by judicious choice of other substituents. Sites influencing selectivity, included not only P_2, which is well-known to strongly affect selectivity, but also the P_4, P_1-P_1', and P_2' sites. These results make possible the design of inhibitors with a greater selectivity for either renin versus cathepsin D. In vivo several of the compounds in the difluoroketone series have shown good oral activity in the salt depleted normotensive cynomolgus monkey model.

Introduction

The renin–angiotensin system (RAS) plays a major role in the control of blood pressure and in maintaining water and electrolyte balance.[2] The octapeptide angiotensin II is a potent vasoconstrictor and also stimulates aldosterone release from the adrenal cortex, leading to sodium and water retention. Several approaches to blockade of the RAS have been studied for the treatment of essential hypertension and heart failure.[3]

Converting-enzyme inhibitors have been successful as antihypertensive agents[4] and more recently there is evidence that these agents improve survival in patients with congestive heart failure.[4,5]

The recent discovery of orally active non-peptide angiotensin II antagonists provides another potential means of intervention, although their therapeutic potential has not been proven.[6] The search for orally active inhibitors of human renin continues to challenge medicinal chemists.[7] Since angiotensinogen is the only known substrate for renin, inhibition at this step in the RAS may provide some clinical advantages over ACE inhibition.

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(1) Abbreviations follow the recommendations of the IUPAC–IUB Joint Commission on Biochemical Nomenclature for amino acids and peptides: *Eur. J. Biochem.* **1984**, *158*, 9–31. Additional abbreviations: ACE, angiotensin converting enzyme; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; BOC, *tert*-butoxycarbonyl; Z, benzyloxycarbonyl; TFA, trifluoroacetic acid; TEA, triethylamine; HOBT, 1-hydroxybenzotriazole; Sta, statine ((3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid); ACHPA, (3*S*,4*S*)-4-amino-3-hydroxy-5-cyclohexylpentanoic acid; DFSta, (3*R*,4*R*)-4-amino-2,2-difluoro-3-hydroxy-6-methylheptanoic acid; FCS, (3*R*,4*S*)-4-amino-2,2-difluorohydroxy-5-cyclohexylpentanoic acid; DFO, (4*S*)-4-amino-2,2-difluoro-3-oxo-6-methylheptanoic acid; FCO, (4*S*)-4-amino-2,2-difluoro-3-oxo-5-cyclohexylpentanoic acid; AEM, 4-(2-aminoethyl)-morpholine; MBA, 2(S)-methylbutylamine.

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Although many potent and selective human renin inhibitors have been reported[8] and some of these have been studied in man,[9] oral activity has been poor. First-pass metabolism appears to be a major problem with many of the renin inhibitors studied to date, due to their size and lipophilicity.[10,11] Low aqueous solubility also compromises absorption.[8,11]

Peptides that contain statine, difluorostatine, and difluorostatone residues at the cleavage site of the substrate have been found to be potent inhibitors of renin.[12-15] The readily hydrated difluoroketone is proposed to mimic the tetrahedral intermediate that forms during the enzyme-catalyzed hydrolysis of a peptidic bond.[13] We have prepared a range of difluoroketone containing inhibitors of renin and their precursor difluorocarbinol analogues in an attempt to design potent, selective, and orally active renin inhibitors. By modification of the substituents particularly at the P_2 and P_2'[16] sites, it has been possible to attain good aqueous solubility, and indeed many of the compounds in the difluoroketone series have shown good oral efficacy.

We and others have studied the factors involved in increasing selectivity for renin over the closely related aspartic proteinase cathepsin D (bovine).[8g,17-21] In this study, the difluoroketone analogues showed greatly reduced selectivity for renin compared with the corresponding alcohols. However judicious choice of substituents at the other sites has enabled the design of potent and selective renin inhibitors.

Chemistry

The renin inhibitors in Table I–V were synthesized as illustrated in Scheme I for compounds **5** and **9**. The Reformatsky reaction of ethyl bromodifluoroacetate[22-25] with Boc-cyclohexylalaninal or Boc-leucinal has previously been reported under both ultrasonic and heating conditions.[12] The aldehyde has been found to be very susceptible to racemization and for this reason the ultrasonic conditions initially appeared to be more desirable. However, although the sonication method is successful on a small scale, in our

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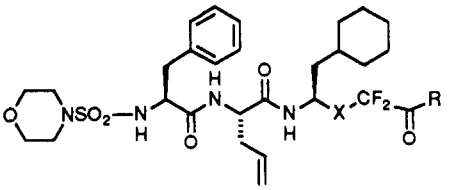
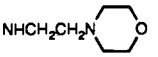
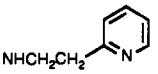
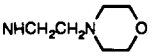
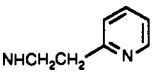
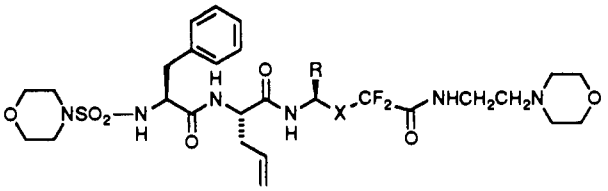
Table I. P_2' Modifications

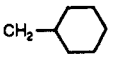
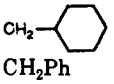


compd	X	R	IC50, nM renin[a]	IC50, nM cathepsin D[b]	ratio C/R	molecular formula[c]
5	CHOH		0.70	2710	3871	$C_{35}H_{54}F_2N_8O_8S\cdot CH_3OH$
6	CHOH	$NHCH_2CH(S)(Me)CH_2CH_3$	0.32	36.5	114	$C_{34}H_{53}F_2N_5O_7S$
7	CHOH	OCH_2CH_3	1.89	767	406	$C_{31}H_{46}F_2N_4O_8S\cdot 0.5(CH_3)_2NCHO$
8	CHOH		0.21	165	788	$C_{36}H_{50}F_2N_8O_7S\cdot 1.6CH_3OH$
9	C=O		1.20	164	137	$C_{35}H_{52}F_2N_6O_8S\cdot CH_3SO_3H\cdot 2H_2O$
10	C=O	$NHCH_2CH(S)(Me)CH_2CH_3$	0.81	18.6	23	$C_{34}H_{51}F_2N_5O_7S\cdot 0.25C_{13}H_{24}N_2O$
11	C=O	OCH_2CH_3	0.40	69.3	173	$C_{31}H_{44}F_2N_4O_8S\cdot 0.2C_{13}H_{24}N_2O$
12	C=O		0.23	24.5	108	$C_{36}H_{48}F_2N_8O_7S\cdot CH_3SO_3H\cdot 1.8H_2O$

[a] IC_{50} values determined using monkey plasma. [b] IC_{50} values determined using bovine cathepsin D. [c] Analyses for C, H, N were within ±0.4%.

Table II. $P_{1\text{-}1}'$ Modifications



compd	X	R	IC50, nM renin[a]	IC50, nM cathepsin D[b]	ratio C/R	molecular formula
5	CHOH		0.70	2710	3871	$C_{35}H_{54}F_2N_8O_8S\cdot CH_3OH$
13	CHOH	CH_2Ph	0.46	1828	3974	$C_{35}H_{48}F_2N_8O_8S\cdot 0.32H_2O\cdot 0.5CHCl_3$
14	CHOH		0.87	$>10^{-4}$ M	≫10000	$C_{34}H_{52}F_2N_8O_8S\cdot 0.12CHCl_3\cdot 0.36H_2O$
9	C=O		1.20	164	137	$C_{35}H_{52}F_2N_8O_8S\cdot CH_3SO_3H\cdot 2H_2O$
15	C=O	CH_2Ph	0.36	90	251	$C_{35}H_{46}F_2N_8O_8S\cdot CH_3SO_3H\cdot 1.90H_2O$
16	C=O		0.31	761	2455	$C_{34}H_{50}F_2N_8O_8S\cdot 1.15MeSO_3H\cdot 1.14H_2O$

[a] IC_{50} values determined using monkey plasma. [b] IC_{50} values determined using bovine cathepsin D. [c] Analyses for C, H, N were within ±0.4%.

hands these conditions have been low yielding and unpredictable upon attempted scale up. Under the thermal conditions, careful preparation of the reagents allowed the scale up of the Reformatsky reaction with reproducible yields (30–40%). Since the organozinc reagent derived from ethyl bromodifluoroacetate is unstable above room temperature,[26] an excess is used in the reaction. Attempts to carry out the transformation using ethyl chlorodifluoroacetate[27] under either ultrasonic or heating conditions in a variety of solvents have been unsuccessful in our hands. Crude alcohol **2** (*R* and *S* mixture) was crystallized from hexane to give the pure *R* isomer **2A** which was used for subsequent transformations. Further coupling with chiral amino acids indicated that the enantiomeric purity of **2A** was high (>95%).

The remainder of the difluorocarbinol synthesis follows standard peptide chemistry to compound **5**.[28] The synthesis of the difluoroketones however, has not been trivial.

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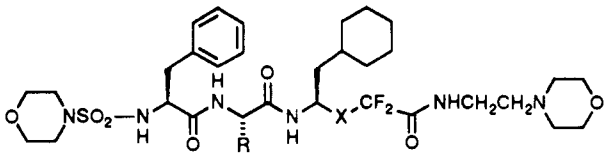
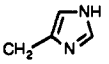
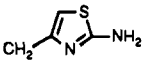
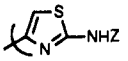
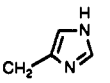
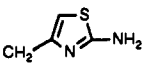
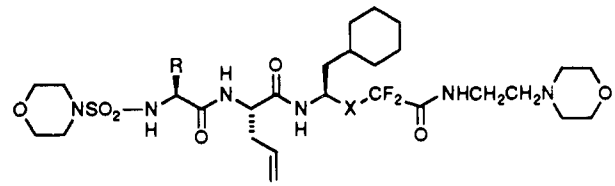
Table III. P_2 Modifications



compd	X	R	IC_{50}, nM renin[a]	IC_{50}, nM cathepsin D[b]	ratio C/R	molecular formula[c]
5	CHOH	Alg	0.70	2710	3871	$C_{35}H_{54}F_2N_6O_8S\cdot CH_3OH$
17	CHOH		2.96	$>10^{-4}$ M	>10000	$C_{36}H_{54}F_2N_6O_8S\cdot CH_3OH$
18	CHOH		0.69	95000	>10000	$C_{36}H_{54}F_2N_6O_8S_2\cdot H_2O$
19A	CHOH		164	16940	103	$C_{43}H_{58}F_2N_6O_{10}S_2$
20	CHOH	^{n}Bu	0.24	1111	4629	$C_{36}H_{58}F_2N_6O_8S$
21	CHOH	H	12.3	$>10^{-4}$ M	>1000	$C_{32}H_{50}F_2N_6O_8S\cdot 0.8CH_2Cl_2$
22A	CHOH	O^{n}Pr (fast)	0.44	700	1590	$C_{35}H_{58}F_2N_6O_9S\cdot 0.3CH_3OH$
23	CHOH	CO_2Me	0.62	17730	>10000	$C_{34}H_{52}F_2N_6O_{10}S\cdot 0.5CH_3OH$
24	CHOH	CHOHMe	0.82	7946	9690	$C_{34}H_{54}F_2N_6O_9S\cdot 0.12CH_2Cl_2$
25	CHOH	$(CH_2)_4NHC(=S)NHMe$	2.33	9954	4272	$C_{38}H_{62}F_2N_6O_8S_2\cdot 0.90CHCl_3$
9	C=O	Alg	1.2	164	137	$C_{35}H_{52}F_2N_6O_8S\cdot CH_3SO_3H\cdot 2H_2O$
26	C=O		0.9	$>10^{-4}$ M	>10000	$C_{36}H_{52}F_2N_6O_8S\cdot H_2O$[d]
27	C=O		0.50	3696	7392	$C_{36}H_{52}F_2N_6O_8S\cdot MeSO_3H\cdot 3.2H_2O$
28	C=O	^{n}Bu	0.35	36	102	$C_{36}H_{56}F_2N_6O_8S\cdot 0.9CH_2Cl_2$
29	C=O	H	0.25	67200	>100000	$C_{32}H_{48}F_2N_6O_8S\cdot CH_3SO_3H\cdot EtOH$
30	C=O	O^{n}Pr	2.00	237	119	$C_{35}H_{54}F_2N_6O_9S\cdot C_6H_8O_7\cdot 1.5H_2O$
31	C=O	CO_2Me	0.79	515	652	$C_{34}H_{50}F_2N_6O_{10}S\cdot 2.5CH_3SO_3H\cdot 0.7H_2O$
32	C=O	COMe	1.17	202	173	$C_{34}H_{50}F_2N_6O_9S\cdot CH_3SO_3H\cdot 0.5EtOH\cdot H_2O$

[a] IC_{50} values determined using monkey plasma. [b] IC_{50} values determined using bovine cathepsin D. [c] Analyses for C, H, N were within ±0.4%, except where noted. [d] N: calcd, 13.78; found, 13.05.

Table IV. P_3 Modifications



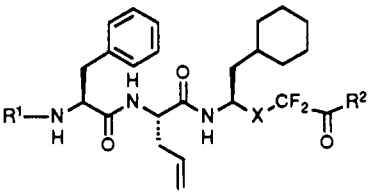
compd	X	R	IC_{50}, nM renin[a]	IC_{50}, nM cathepsin D[b]	ratio C/R	molecular formula[c]
5	CHOH	CH_2Ph	0.70	2710	3871	$C_{35}H_{54}F_2N_6O_8S\cdot CH_3OH$
33	CHOH	CH_2Ph(4-OMe)	1.50	4570	3047	$C_{36}H_{56}F_2N_6O_9S\cdot CH_3SO_3H\cdot 2H_2O$
34	CHOH	CH(OH)Ph	97.8	$>10^{-4}$ M	>1000	$C_{35}H_{54}F_2N_6O_9S\cdot H_2O$
9	C=O	CH_2Ph	1.20	164	137	$C_{35}H_{52}F_2N_6O_8S\cdot CH_3SO_3H\cdot 2H_2O$
35	C=O	CH_2Ph(4-OMe)	0.30	71	236	$C_{36}H_{54}F_2N_6O_9S\cdot CH_3SO_3H\cdot 2.0H_2O$
36	C=O	C(O)Ph	10	1807	181	$C_{35}H_{50}F_2N_6O_9S\cdot CH_3SO_3H\cdot 1.1H_2O$

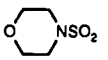
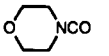
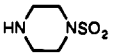
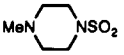
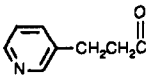
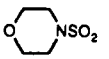
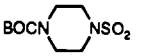
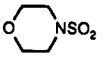
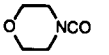
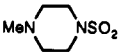
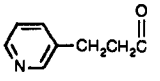
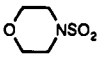
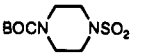
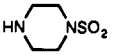
[a] IC_{50} values determined using monkey plasma. [b] IC_{50} values determined using bovine cathepsin D. [c] Analyses for C, H, N were within ±0.4%.

Under a variety of different oxidative reaction conditions including use of Swern,[12,29] Collins,[30] PDC,[31] and Dess–Martin[32,33] reagents, some epimerization α to the difluoroketone group was unavoidable. In addition, the ease with which this epimerization occurred was found to depend on the nature of the other substituents present in the target renin inhibitors. For example, compound **9** with one basic center at P_2' was difficult to isolate as a single isomer from several oxidation reactions, particularly on a

(29) Omura, K.; Swern, D. Oxidation of Alcohols by 'Activated' Dimethylsulfoxide. A Preparative Steric and Mechanistic Study. *Tetrahedron* **1978**, *34*, 1651–1660.
(30) Collins, J. C.; Hess, W. W.; Frank, F. J. Dipyridine-Chromium (VI) Oxide Oxidation of Alcohols in Dichloromethane. *Tetrahedron Lett.* **1968**, 3363–3366.
(31) Tarnus, C.; Jung, M. J.; Rémy, J.-M.; Baltzer, S.; Schirlin, D. G. New Fluoroketones as Human Renin Inhibitors. *FEBS Lett.* **1989**, *249*, 47–50.
(32) Linderman, R. J.; Graves, D. M. Oxidation of Fluoroalkyl-Substituted Carbinols by the Dess–Martin Reagent. *J. Org. Chem.* **1989**, *54*, 661–668.
(33) Takahashi, L. H.; Radhakrishnan, R.; Rosenfield, R. E.; Meyer, E. F.; Trainor, D. A. Crystal Structure of the Covalent Complex Formed by a Peptidyl α,α-Difluoro-β-keto Amide With Porcine Pancreatic Elastase at 1.78 Å Resolution. *J. Am. Chem. Soc.* **1989**, *111*, 3368–3374.

Table V. P_4 Modifications



compd	X	R^1	R^2	IC_{50}, nM renin[a]	IC_{50}, nM cathepsin D[b]	ratio C/R	molecular formula[c]
5	CHOH		AEM	0.70	2710	3871	$C_{35}H_{54}F_2N_6O_8S\cdot CH_3OH$
37	CHOH		AEM	0.28	2377	8489	$C_{36}H_{54}F_2N_6O_7\cdot 0.5H_2O$
38	CHOH		AEM	0.57	6850	12018	$C_{35}H_{55}F_2N_7O_7S\cdot 2CH_3SO_3H\cdot 2H_2O$
39	CHOH		AEM	0.42	25300	>10^5	$C_{36}H_{57}F_2N_7O_7S\cdot 1.5H_2O$
40	CHOH	BOC	AEM	19.1	18820	985	$C_{36}H_{55}F_2N_5O_7$
41	CHOH		AEM	0.22	>10^{-4} M	>10^5	$C_{39}H_{54}F_2N_6O_8\cdot 2CH_3SO_3H$
6	CHOH		MBA	0.32	36.5	114	$C_{34}H_{53}F_2N_5O_7S$
42	CHOH		MBA	1.49	259	174	$C_{39}H_{82}F_2N_6O_8S\cdot 0.7(CH_3)_2NCHO$
9	C=O		AEM	1.20	164	137	$C_{35}H_{52}F_2N_6O_8S\cdot CH_3SO_3H\cdot 2H_2O$
43	C=O		AEM	0.10	32.9	329	$C_{36}H_{52}F_2N_6O_7\cdot 2H_2O\cdot CH_3SO_3H\cdot 0.5C_2H_5OH$
44	C=O		AEM	0.92	728	791	$C_{36}H_{55}F_2N_7O_7S\cdot 2CH_3SO_3H\cdot 3.0H_2O$
45	C=O	BOC	AEM	3.55	1416	399	$C_{36}H_{53}F_2N_5O_7\cdot 0.60H_2O$[d]
46	C=O		AEM	0.23	1655	7196	$C_{39}H_{52}F_2N_6O_8$
10	C=O		MBA	0.81	18.6	23	$C_{34}H_{51}F_2N_5O_7S\cdot 0.25C_{13}H_{24}N_2O$
47	C=O		MBA	2.46	11.4	4.6	$C_{39}H_{60}F_2N_6O_8S$
48	C=O		MBA	0.35	47.4	135	$C_{34}H_{52}F_2N_6O_8S\cdot HCl\cdot 0.8H_2O$

[a] IC_{50} values determined using monkey plasma. [b] IC_{50} values determined using bovine cathepsin D. [c] Analysis for C, H, N were within ±0.4%, except where noted. [d] N: calcd, 9.77; found, 8.66.

large scale. Indeed, stability studies of **9** indicate that it undergoes slow epimerization in phosphate buffer at pH 7.4.

Compounds without a basic group were found to be more stable to this epimerization reaction. We have developed a procedure employing *modified* Pfitzner–Moffatt conditions[34] with 1.5 equiv of dichloroacetic acid (for each basic center, to form the acid addition salt of a basic amine) and using CH_2Cl_2 instead of DMSO as the reaction solvent for ease of workup. At least 10 equiv of DCC is required for reaction completion; smaller quantities always resulted in recovered starting material. DMSO was used in a 10–20-fold excess. Careful acidic workup as the phosphoric acid salt allowed isolation of the pure oxidized product without chromatography in good yield (usually 70–95%). The procedure was found to be fairly general, allowing isolation of most of the compounds in Tables I–V predominantly as single isomers.

However compound **44** (Table V) with two basic centers in the molecule was found to be very susceptible to epimerization; indeed HPLC analysis of aliquots of the oxidation reaction indicated partial epimerization even before attempted isolation.

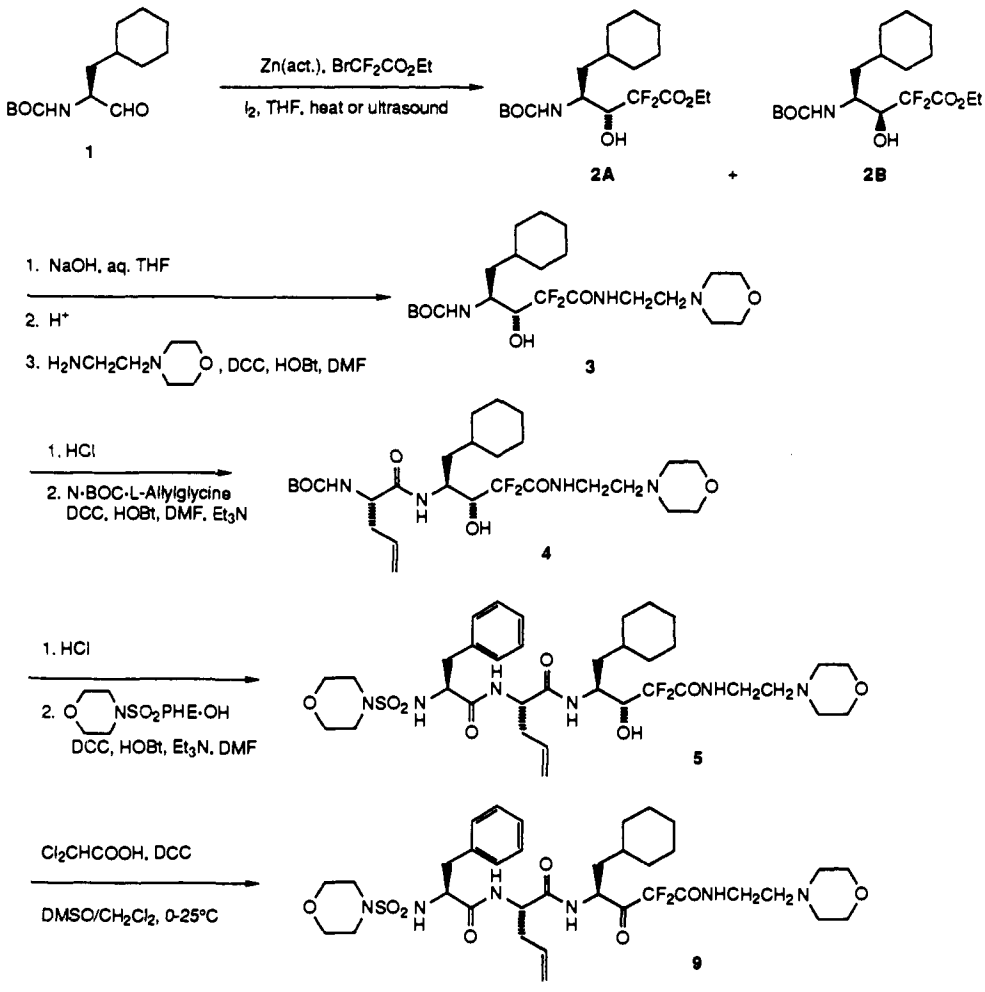
Discussion

Structure–Activity Relationships. The compounds **5–48** listed in Tables I–V have been prepared in an attempt to study structure–activity relationships systematically within the difluorocarbinol and difluoroketone series. Most of these compounds are highly potent primate renin inhibitors. From previous observations we have found that inhibition of monkey plasma renin activity (PRA) is predictive of inhibition of human PRA.[8g] However since baseline PRA values are considerably higher in the monkey

(34) Pfitzner, K. A.; Moffatt, J. G. Sulfoxide-Carbodiimide Reactions. II. Scope of the Oxidation Reaction. *J. Am. Chem. Soc.* **1965**, *87*, 5670–5678.

(35) We thank Professor H. Rapoport for helpful advice regarding workup and isolation of the difluorostatone products.

Scheme I



than in the human, IC_{50} values for the former can be determined more precisely. In most cases both the alcohols and corresponding ketones are of comparable potency toward inhibition of renin.

In Table I variation of the P_2' position allowed control of the log *P* values of compounds and those with basic groups at this position can form water-soluble addition salts. Changes at this position have little effect on the in vitro renin inhibitory potency among the compounds studied.

Variation at the P_1-P_1' site shown in Table II indicates that the benzyl, cyclohexyl, and cyclohexylmethyl analogues are of similar potency, contrary to previous results with other series of renin inhibitors.[36] In Table III, variation at the P_2 site is shown and it is obvious that a wide range of groups are tolerated at this position. Within the difluorocarbinol series the glycine derivative **21** and CBZ-protected heterocyclic analogue **19** were the least potent.

In Table IV, P_3 variation illustrates that Phe- and Tyr(OMe)-containing inhibitors are at least 1 order of magnitude more potent than derivatives **34** and **36**.

P_4 variation in Table V provided some very interesting compounds with a wide range of physicochemical properties. The oral activity of compound **44** will be highlighted.

Selectivity: Renin vs Cathepsin D. A number of interesting structure–activity trends related to the selectivity of the target inhibitors for renin versus the closely related aspartic proteinase cathepsin D have been observed. These results are of particular interest because cathepsin D has been implicated in the pathophysiology of a number of diseases,[37,38] including breast cancer and rheumatoid arthritis. The difluorocarbinol and difluoroketone series show different structure–activity relationships and are discussed separately.

Variation of the P_2' in the difluorocarbinol series indicates that for higher renin selectivity it is preferable for a basic group such as 4-(2-aminoethyl)morpholine (AEM) to be present. Small, neutral groups tend to decrease the selectivity for renin versus cathepsin D. This is seen by comparison of compounds **5** and **8** with **6** and **7**. Changes at the P_2' site have less effect on the selectivity within the difluoro ketone series (compounds **9–12**).

Since it appears that hydration at the active site markedly increases the binding to the cathepsin D enzyme relative to the alcohol, variation at the P-P_1' site was studied in an attempt to increase the selectivity for renin. In Table II all of the difluorocarbinols are moderately selective with compound **14** showing a large increase in

(36) Luly, J. R.; Bolis, G.; BaMaung, N.; Soderquist, J.; Dellaria, J. F.; Stein, H.; Cohen, J.; Perun, T. J.; Greer, J.; Plattner, J. J. New Inhibitors of Human Renin that Contain Novel Leu-Val Replacements. Examination of the P_1 Site. *J. Med. Chem.* **1988**, *31*, 532–539.

(37) Lenarcic, B.; Kos, J.; Dolenc, I.; Lucovnik, P.; Krizaj, I.; Turk, V. Cathepsin D Inactivates Cysteine Proteinase Inhibitors Cystatins. *Biochem. Biophys. Res. Commun.* **1988**, *154*, 765–772.

(38) Greenbaum, L. M.; Sutherland, J. H. Host Cathepsin D Response to Tumor in the Normal and Pepstatin-Treated Mouse. *Cancer Res.* **1983**, *43*, 2584–2587.

selectivity for renin. In the difluoroketone series this same trend is observed, compound **16** being considerably more selective than **9** or **15**. Thus it seems possible to control renin selectivity by judicious variation at the P_1-P_1' site.

Table III shows the results of modification at the P_2 site upon selectivity. It has previously been reported that the P_2 substitution is important for renin selectivity.[17-21] The data in Table III verify this; indeed it is clearly the most important position for control of selectivity toward renin. Within the difluorocarbinol series, substitution with heterocyclic groups vs alkyl or heteroalkyl groups causes a large decrease in the cathepsin D inhibition (cf. compounds **17** and **18** with **5** and **20**, **22A**, **24**, **25**). Thus compounds **17** and **18** are highly potent and selective primate renin inhibitors.

Surprisingly the ester derivative **23** is a selective renin inhibitor, but it should be noted that this compound is in fact a mixture of diastereomers at P_2. Within the difluoroketone series, compounds with heterocyclic groups at P_2 still possess superior selectivity (cf. compounds **26** and **27** with **9**, **28**, **30–32**) to those with alkyl or ester functionality. The unexpectedly high selectivity of glycine analogues **21** and **29** could not be explained.

In general the corresponding difluoroketone analogues are less selective than their difluorocarbinol precursors.

Table IV illustrates the effect of P_3 variation upon selectivity for renin. Again the difluorocarbinols (**5**, **33**, and **34**) are more selective than the corresponding difluoro ketones (**9**, **35**, and **36**).

In Table V we investigated variation at the P_4 site. Among the difluorocarbinols substitution with basic groups tends to increase renin selectivity (cf. compounds **38**, **39**, and **41** with **5**, **37**, and **40**). Conversely, inhibition of cathepsin D can be increased by substitution with neutral groups. Within the difluoroketone series this same trend is observed (cf. compounds **44** and **46** with **9**, **43**, and **45**) although to a lesser extent. It is interesting to note than when there is a neutral group at the P_2' position, which is known to decrease the cathepsin D/renin ratio (earlier discussion), the beneficial effect of the basic P_4 group upon selectivity is reduced (compound **48**).

Oral Efficacy

We have evaluated selected compounds in Tables I–V for oral efficacy. Most of the difluorocarbinol derivatives showed only modest blood pressure lowering effects (10–17 mmHg) upon oral administration (30 mg/kg) to salt-depleted cynomolgus monkeys. The best profile was observed for compound **5** and is shown in Figure 1. The ratio of the maximum blood pressure lowering effect of the drug and of the receptor antagonist saralasin (see Biological Methods) provided a method of normalizing the blood pressure responses for different monkeys. For compound **5** this ratio was found to be 0.50.

By contrast the difluoroketone series showed good oral efficacy, particularly those compounds with a basic group at P_2 and/or P_2'. The hypotensive effects of several members of this series upon oral administration (30 mg/kg) to the salt-depleted normotensive cynomolgus monkey are shown in Figure 2.

Compounds **9** and **44** showed the greatest oral efficacy with maximum blood pressure drops of 34 and 29 mmHg, respectively. Compounds **10**, **35**, and **46** showed moderate oral activity (only compounds **9**, **10**, and **44** shown in Figure 2). Oral efficacy does not appear to correlate with log *P* (Table VI). The saralasin ratio for these compounds was close to unity. The superior antihypertensive activity of the difluoroketones compared with the difluorocarbinols is not completely understood. The difluoroketones are

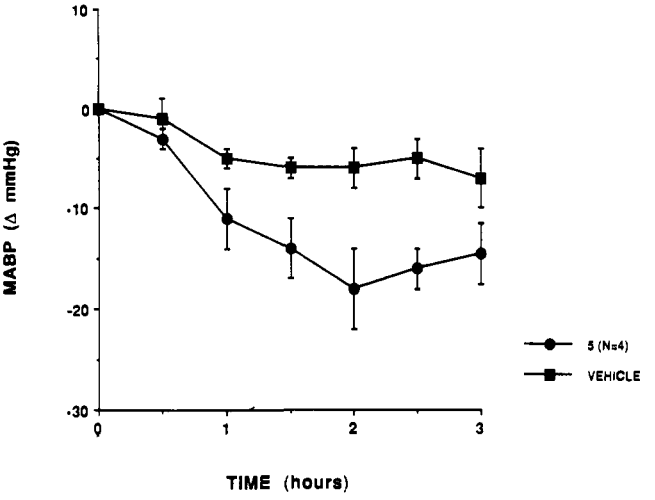


Figure 1. Activity of compound **5** in salt-depleted normotensive monkeys after an oral dose of 30 mg/kg.

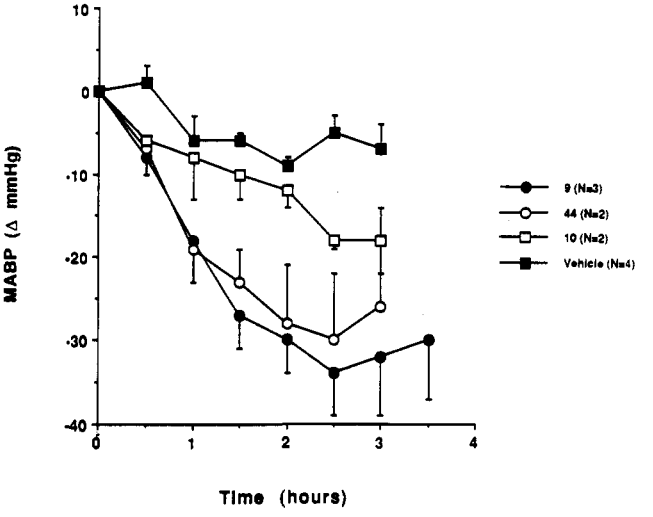


Figure 2. Activity of several difluoroketone renin inhibitors in salt-depleted normotensive monkeys after an oral dose of 30 mg/kg.

hydrated in aqueous solution and this may contribute to their oral efficacy. Molecular weight and log *P* values are comparable for the two series.[39]

Figure 3 illustrates the oral efficacy of compound **9** compared with PD 132002 and PD 134672 (Figures 4 and 5), two orally active renin inhibitors from different series that we have recently reported.[40a,b]

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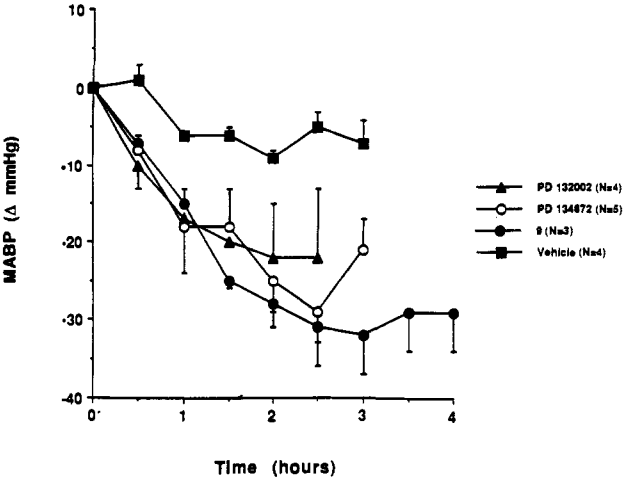
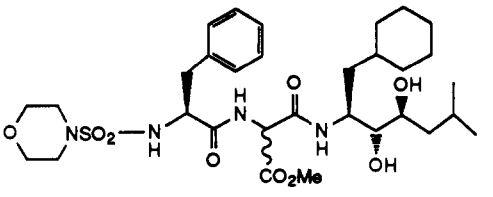


Figure 3. Comparative activity of PD 132002, PD 134672, and compound **9** in salt-depleted normotensive monkeys after an oral dose of 30 mg/kg.

Table VI

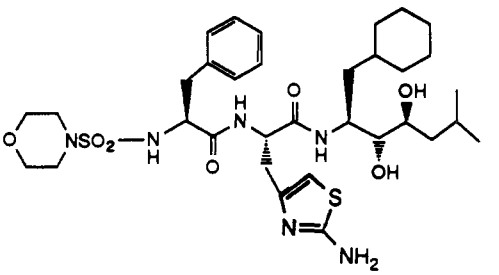
compd	log P^a	compd	log P^a
5	3.17	**35**	2.73
9	3.39	**44**	2.73
10	4.63	**46**	2.90

[a] log P values measured at pH 7.4.



PD 132002

Figure 4. Structure of PD 132002.



PD 134672

Figure 5. Structure of PD 134672.

Summary

Novel renin inhibitors containing difluorocarbinol and difluoroketone groups at the P_1-P_1' site were prepared and tested in vitro and in vivo. Most of these compounds were highly potent primate renin inhibitors.

Selectivity for renin over cathepsin D could be controlled by judicious choice of the substituents at the P_4-P_3, P_1-P_1', and P_2' sites, in addition to the P_2 site, which is well-known to strongly affect selectivity. Small neutral groups at the P_2' and P_4 sites decrease renin selectivity. Heterocyclic groups at P_2 are preferred for high renin selectivity. Oxidation of the difluorocarbinol P_1-P_1' group markedly decreased renin vs cathepsin D selectivity.

Many of the difluoroketones exhibited good oral activity in the salt-depleted normotensive cynomolgus monkey model.

Experimental Section

The NMR spectra were recorded on a Varian XL-200 instrument. The FAB-MS was determined on a VG analytical 7070E/HF mass spectrometer in a thioglycerol matrix using xenon as the target gas. Rotations were recorded on a Perkin-Elmer Model 142 polarimeter. TLC was done on precoated sheets (silica gel 60F 254, Merck). Silica gel chromatography was done with Kieselgel 60 (70–230 mesh or 230–400 mesh for flash).

Most compounds were purified by chromatography on silica gel (5% MeOH/CH_2Cl_2 unless otherwise stated) and were usually obtained as foams that often retained solvent, even on prolonged drying under vacuum. Intermediates and the compounds in Tables I–V all showed the correct molecular ion in the FAB mass spectrum. The NMR was consistent with the assigned structures. Most compounds were analyzed by HPLC for purity employing an Alltech RP C18 column (4.6 × 250 mm); 5-μm particle size, 1.5 mL/min flow rate (unless otherwise noted), 214-nm detection, and various mobile phases including 65:30:5 0.05 M Et_3N (pH 7.0 with H_2PO_4–CH_3CN–THF or various ratios of MeOH–0.05 M $NH_4H_2PO_4$ (aqueous) (pH 3.0 with H_3PO_4)). The log P values of the compounds in Table VI were determined using a standard HPLC correlation method.[41]

Ethyl 4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-5-(cyclohexylmethyl)-2,2-difluoro-3(*R*)-hydroxypentanoate (2). Activated zinc dust (9.2 g, 0.14 mol) was suspended in anhydrous THF (300 mL) under N_2. One crystal of I_2 was added and the reaction brought to reflux. Ethyl bromodifluoroacetate (freshly distilled, 0.1 mL) was added to initiate the reaction, and after about 1 min, a mixture of the *N*-BOC-L-cyclohexylalaninal (1)[42,43] (18.0 g, 0.071 mol) and bromo difluoro ester (13.6 mL, 0.107 mol) in THF (100 mL) was added at such a rate as to control the reflux (as rapidly as possible). After refluxing for a further 30 min, the mixture was allowed to cool and partitioned between ethyl acetate (~400 mL) and 1 M $KHSO_4$ (~100 mL). The organic phase was washed with water and brine and dried (Na_2SO_4). After evaporation, the residue was chromatographed on silica gel eluting with 10 → 20% ethyl acetate–petroleum ether to afford the product as a yellowish oil (10.7 g, 40%). Addition of hexane to this oil allowed precipitation of a white solid which was removed by filtration to give **2A** (4.59 g, 17%) as a single diastereomer (HPLC, mobile phase 80:20 CH_3CN–H_2O, t_R = 3.9 min, 100%, single isomer). The stereochemistry at the hydroxyl center was assigned as previously reported.[25] The remaining residue was recolumned to afford further product (3.39 g, 12.6%, 5:1 ratio of diastereomers and 0.74 g, 2.7%, ca. 3:2 ratio of diastereomers). For **2A**: MS (CI + CH_4) m/z 380 (MH^+), 324 (MH^+ – ^{t}Bu); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.67 (1 H, br d), 4.38 (2 H, dd, J = 10.5, 5.8 Hz), 3.98 (3 H, m), 1.68–0.90 (12 H, m), 1.40 (9 H, s), 1.38 (3 H, t, J = 5.8 Hz).

1,1-Dimethylethyl [1(*S*)-(Cyclohexylmethyl)-3,3-difluoro-2(*R*)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]carbamate (3). To a solution of ester **2A** (1.50 g, 4.0 mmol) in THF (30 mL) was added NaOH (0.17 g in 5 mL of H_2O) and the resulting solution was stirred for 4 h. The pH was brought to 2–3 with dilute HCl and the solution extracted three times with EtOAc. The combined organic extracts were washed with H_2O and brine. Drying (Na_2SO_4) and removal of the solvent under reduced pressure gave the acid (1.10 g, 79%) as a white foam which was used directly in subsequent reactions. To the acid (1.25 g,

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(43) Boger, J.; Payne, L. S.; Perlow, D. S.; Lohr, N. S.; Poe, M.; Blaine, E. H.; Ulm, E. H.; Schorn, T. W.; LaMont, B. I.; Lin, T.-Y.; Kawai, M.; Rich, D. H.; Veber, D. F. Renin Inhibitors. Syntheses of Subnanomolar, Competitive, Transition-State Analogue Inhibitors Containing a Novel Analogue of Statine. *J. Med. Chem.* **1985**, *28*, 1779–1790.

3.50 mmol) and HOBT (0.47 g, 3.50 mmol) in anhydrous DMF at 0 °C was added DCC (0.72 g, 3.50 mmol) in DMF (20 mL). 4-(2-Aminoethyl)morpholine (0.46 g, 3.50 mmol) in DMF (30 mL) was then added and the resulting mixture stirred at 0 °C for 1 h before warming to room temperature. After ca. 16 h the dicyclohexylurea was removed by filtration and the solvent evaporated under reduced pressure. The residue was taken up in ethyl acetate, refiltered, and washed sequentially with saturated $NaHCO_3$ solution and brine. After drying (Na_2SO_4), filtration, and evaporation, the crude product was purified by column chromatography on silica gel eluting with MeOH in $CHCl_3$ (5 → 7% gradient). The appropriate fractions were combined to afford product **3** as a white foam (0.92 g, 56%): MS (CI + CH_4) m/z 464 (MH^+); HPLC, 80:20 CH_3CH–H_2O, t_R = 4.07 min, 96.6%; ^1H NMR (200 MHz, $CDCl_3$) δ 8.02 (1 H, s), 7.10 (1 H, br s), 5.02 (1 H, br d, J = 7.2 Hz), 4.68 (1 H, br s), 3.96 (2 H, m), 3.74 (4 H, br t), 3.49 (2 H, m), 2.56 (5 H, m), 1.80–0.90 (13 H, m), 1.45 (9 H, s).

1,1-Dimethylethyl [1-[[[1(*S*)-(Cyclohexylmethyl)-3,3-difluoro-2(*R*)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]amino]carbonyl]-3(*S*)-butenyl]carbamate (4). To a solution of **3** (7.34 g, 0.016 mol) in $CHCl_3$ (200 mL) and MeOH (20 mL) at room temperature was bubbled HCl gas for 10 min. After stirring for 1 h, the reaction mixture was concentrated and the dihydrochloride partitioned between chloroform and saturated $NaHCO_3$ solution. The aqueous layer was washed with $CHCl_3$, and combined organic extracts were dried ($MgSO_4$), filtered, and concentrated to a white foam (5.21 g, 93%). After drying for several hours under high vacuum this material (FCS-AEM) was used directly in the coupling reaction.

To a solution of *N*-α-BOC-L-allylglycine[44] (3.23 g, 0.015 mol) and HOBT (4.06 g, 0.03 mol) in CH_2Cl_2 (100 mL) at 0 °C was added DCC (3.67 g, 0.017 mol) followed by FCS-AEM (5.21 g, 0.015 mol) in CH_2Cl_2 (20 mL). After 2 h the reaction was allowed to warm to room temperature and stirred for a further 16 h. The reaction was then filtered and washed with saturated $NaHCO_3$ solution, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel eluting with 4% MeOH in $CHCl_3$ to afford **4** (6.53 g, 80%) as a white foam: MS (FAB, thioglycerol) m/z 561 (M^+); ^1H NMR (200 MHz, $CDCl_3$) δ 7.93 (1 H, br s), 5.70 (1 H, m), 5.20 (2 H, m), 4.17 (1 H, m), 3.96–3.50 (7 H, m), 3.22 (1 H, m), 2.80 (1 H, br m), 2.60–2.34 (10 H, m), 1.82–0.80 (13 H, m), 1.44 (9 H, s).

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-*N*-[1(*S*)-(cyclohexylmethyl)-3,3-difluoro-2(*R*)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]-4,5-didehydro-L-norvalinamide (5).** To a solution of **4** (3.65 g, 6.52 mmol) in CH_2Cl_2 (150 mL) and MeOH (30 mL) at room temperature was passed a stream of HCl gas for about 15 min. The solution was stirred for a further 15 min and then evaporated under reduced pressure. The residue was azeotroped several times with portions of $CHCl_3$ and then dried in vacuo for several hours. The dihydrochloride was utilized directly in the coupling reaction (3.50 g).

To the amine dihydrochloride prepared above (1.83 g, 3.43 mmol), HOBT (0.46 g, 3.43 mmol) and *N*-(4-morpholinosulfonyl)-L-phenylalanine[8g,45] (1.07 g, 3.43 mmol) in anhydrous DMF (50 mL) at 0 °C was added DCC (0.71 g, 3.43 mmol) in DMF (10 mL), followed by Et_3N (0.96 mL, 6.86 mmol). After 1 h the reaction was allowed to warm to room temperature and stirred for a further 16 h. The reaction was filtered and the DMF distilled under reduced pressure. The resulting residue was taken up in ethyl acetate and washed with saturated $NaHCO_3$ solution followed by brine. The organic layer was dried (Na_2SO_4), filtered, and evaporated.

Crude product **5** was purified by column chromatography on silica gel eluting with 5 → 8% MeOH in $CHCl_3$ to afford pure product (1.82 g, 70%) as a white foam. MS (FAB) m/z 757.3 (MH^+); HPLC, 65:30:5 MeOH–0.05 M Et_3N (pH 7.0 with H_3PO_4)–THF, 1.2 mL/min, t_R = 6.83 min, 95%, single isomer; ^1H NMR (200 mHz, $CDCl_3$) δ 7.46 (1 H, br s), 7.36 (4 H, m), 7.24 (3 H, m), 6.84 (1 H, d, J = 6.3 Hz), 5.68 (1 H, m), 5.19 (1 H, s), 5.12 (1 H, d, J = 5.6 Hz), 4.92 (1 H, d, J = 6.0 Hz), 4.36 (1 H, dd, J = 6, 10 Hz), 4.10–3.88 (2 H, m), 3.72 (4 H, t, J = 3.5 Hz), 3.52 (6 H, m), 3.20 (1 H, dd, J = 3.7, 11.3 Hz), 2.95 (3 H, m), 2.72 (3 H, m), 2.53 (6 H, m), 1.90–1.50 (9 H, m), 1.40–1.08 (4 H, m), 0.92 (2 H, m).

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-*N*-[1(*S*)-(cyclohexylmethyl)-3,3-difluoro-2(*R*)-hydroxy-4-[(2-methylbutyl)amino]-4-oxobutyl]-4,5-didehydro-L-norvalinamide (6).** Title compound **6** was prepared as described above for **5** using 2(*S*)-methylbutylamine instead of 4-(2-aminoethyl)morpholine to yield a white foam (3.30 g, 84.2%): MS (FAB) m/z 714 ($M + H)^+$; HPLC, 45:55:5 Et_3N (0.025 M, pH 3)–CH_3CN–THF; t_R = 29.4 min; 94.4:5.6 ratio of isomers.

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-*N*-[1(*S*)-(cyclohexylmethyl)-4-ethoxy-3,3-difluoro-2(*R*)-hydroxy-4-oxobutyl]-4,5-didehydro-L-norvalinamide (7).** Title compound **7** was prepared as described above for **5** from **2**. Product **7** (5.0 g, 89%) was obtained as a pale yellow foam: MS (FAB) m/z 673 ($M + H)^+$.

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-*N*-[1(*S*)-(cyclohexylmethyl)-3,3-difluoro-2(*R*)-hydroxy-4-oxo-4-[[2-(2-pyridinyl)ethyl]amino]butyl]-4,5-didehydro-L-norvalinamide (8).** Title compound **8** was prepared as described previously for **5** using 2-(aminoethyl)pyridine instead of 4-(2-aminoethyl)-morpholine to yield a white foam (1.50 g, 98%): MS (FAB) m/z 749.2 ($M + H)^+$.

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-*N*-[1(*S*)-(cyclohexylmethyl)-3,3-difluoro-4-[[2-(4-morpholinyl)ethyl]-amino]-2,4-dioxobutyl]-4,5-didehydro-L-norvalinamide, Methanesulfonate (9).** To alcohol **5** (1.70 g, 2.0 mmol) in dry CH_2Cl_2 (40 mL) at 0 °C was added dichloroacetic acid (0.24 mL, 2.9 mmol) followed by anhydrous DMSO (4.0 mL) and then DCC (4.10 g, 20 mmol). The mixture was stirred at 0 °C for 1–2 h and then at room temperature overnight (18 h). Oxalic acid (2.6 g, 20 mmol) was dissolved in methanol and added slowly to the reaction. After about 20 min, the solid was filtered and the filtrate evaporated and diluted with EtOAc. The precipitate present was removed by filtration once again. The ethyl acetate layer (300 mL) was washed with aqueous H_3PO_4 (4:1) (4 × 25 mL). The acidic aqueous layer was washed with EtOAc (2 × 25 mL) and the pH of the solution brought to pH 4 (pH meter) with cold aqueous NH_4OH solution. The precipitated product was extracted into EtOAc (4 × 100 mL), and the combined organic layers were washed with brine. After drying (Na_2SO_4) the solution was filtered and evaporated to an off-white foam (1.70 g). The foam was dissolved in EtOH (15 mL) and methanesulfonic acid (147 μL, freshly distilled) was added. After stirring for 10 min the solution was evaporated to dryness under reduced pressure. The residue was dissolved in H_2O (50 mL) and lyophilized to yield a fluffy foam (1.70 g, 85%): MS (FAB) m/z 755.2 ($M + H)^+$; HPLC, 65:30:5 MeOH–0.05 M Et_3N (pH 7.0 with H_3PO_4)–THF; t_R = 8.83, 9.82 min, 98:2 ratio of diastereoisomers; ^1H NMR (200 mHz, $CDCl_3$) δ 8.78 (1 H, m), 8.05 (1 H, d, J = 9.3 Hz), 7.36 (4 H, m), 7.25 (2 H, m), 6.72 (1 H, m), 5.78 (1 H, m), 5.20 (1 H, d, J = 14.1 Hz), 5.15 (1 H, d, J = 8.5 Hz), 4.65 (1 H, m), 4.48 (1 H, m), 4.20–3.20 (16 H, m), 3.05 (2 H, m), 2.80 (3 H, m), 2.60 (2 H, m), 1.90–1.50 (8 H, m), 1.40–1.05 (5 H, m), 0.90 (2 H, m).

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-*N*-[1(*S*)-(cyclohexylmethyl)-3,3-difluoro-4-[(2-methylbutyl)amino]-2,4-dioxobutyl]-4,5-didehydro-L-norvalinamide (10).** Title compound **10** was prepared as described above for **9** from **6**. The product was obtained as a white foam (1.30 g, 68.4%): MS (FAB) m/z 712.3 ($M + H)^+$.

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-*N*-[1(*S*)-(cyclohexylmethyl)-4-ethoxy-3,3-difluoro-2,4-dioxobutyl]-4,5-didehydro-L-norvalinamide (11).** Title compound **11** was prepared as described above **9** from **7**. The product was obtained as a white foam (0.80 g, 88.9%): MS (FAB) m/z 671 ($M + H)^+$; HPLC, 33:67 Et_3N (0.05 M, pH 3 with H_3PO_4)–MeOH, t_R = 5.48, 9.32 min, 90.9:9.1 ratio of isomers.

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-*N*-[1(*S*)-(cyclohexylmethyl)-3,3-difluoro-2,4-dioxo-4-[[2-(2-pyridinyl)-ethyl]amino]butyl]-4,5-didehydro-L-norvalinamide, Methanesulfonate (12).** Title compound **12** was prepared as described

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(45) Wegler, R.; Bodenbenner, K. *N,N*-Dialkylamidosulfonyl Chlorides. *Ann. Chem.* **1959**, *624*, 25–29.

above for **9** from compound **8**. The product (0.80 g, 80%) was converted to the methanesulfonic acid salt by treatment of an ethanol solution of **12** with 74 μL of methanesulfonic acid and stirring for 10 min. After evaporation the residue was taken up in ethanol once again and evaporated under high vacuum to a white foam (0.90 g): MS (FAB) m/z 747.3 $(M + H)^+$; HPLC, 40:60 CH_3CN–0.05 M Et_3N (pH 3 with H_3PO_4), t_R = 9.87, 12.73 min, 96.6:3.4 ratio of diastereoisomers.

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-4,5-didehydro-*N*-[3,3-difluoro-2(*R*)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxo-1(*S*)-(phenylmethyl)butyl]-L-norvalinamide (13).** Title compound **13** was prepared as described previously for **5** using α-*N*-BOC-L-phenylalanine as the starting material for the Reformatsky reaction. Alcohol **13** was obtained as a white foam (2.06 g, 92%): MS (FAB) m/z 751.3 $(M + H)^+$; HPLC, 60:35:5 CH_3CN–0.05 M $NH_4H_2PO_4$ (pH 3 with H_3PO_4)–THF, t_R = 9.82 min, 99.9%, single diastereoisomer.

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-*N*-[1(*S*)-cyclohexyl-3,3-difluoro-2(*R*)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]-4,5-didehydro-L-norvalinamide (14).** Title compound **14** was prepared as described previously for **5** using α-*N*-BOC-L-cyclohexylglycine as the starting material for the Reformatsky reaction. Alcohol **14** was obtained as a white form (7.43 g, 91%): MS (FAB) m/z 743.3 $(M + H)^+$.

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-4,5-didehydro-*N*-[3,3-difluoro-4-[[2-(4-morpholinyl)ethyl]amino]-2,4-dioxo-1(*S*)-(phenylmethyl)butyl]-L-norvalinamide, Methanesulfonate (15).** Title compound **15** was prepared by oxidation of **13** according to the procedure outlined for compound **9**. Difluoroketone **15** was obtained as a white foam (1.03 g) which was dissolved in MeOH (20 mL) and treated with freshly distilled methanesulfonic acid (84 μL). After stirring for 15 min, the solvent was removed under reduced pressure and the residue dissolved in H_2O and lyophilized to obtain a white foam (2.03 g, 93%). MS (FAB) m/z 749.3 $(M + H)^+$; HPLC, 60:35:5 CH_3CN–0.05 M $NH_4H_2PO_4$ (pH 3 with H_3PO_4)–THF, t_R = 9.56 min, 96%, single diastereoisomer.

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-*N*-[1(*S*)-cyclohexyl-3,3-difluoro-4-[[2-(4-morpholinyl)ethyl]amino]-2,4-dioxobutyl]-4,5-didehydro-L-norvalinamide, Methanesulfonate (16).** Title compound **16** was prepared by oxidation of **14** according to the procedure outlined for compound **9**. Difluoroketone **16** was obtained as a white foam (1.93 g, 96%) which was dissolved in MeOH (40 mL) and treated with freshly distilled methanesulfonic acid (0.25 mL, 2.60 mmol). After stirring for 15 min, the solvent was evaporated under reduced pressure and the residue dissolved in H_2O and lyophilized to obtain a white foam (2.06 g, 94%): MS (FAB) m/z 741.2 $(M + H)^+$; HPLC, 65:35:5 MeOH–0.05 M Et_3N–THF (pH 7 with H_3PO_4), 1.0 mL/min, t_R = 6.72, 7.2 min, 95%, 90:10 ratio of diastereoisomers.

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-*N*-[1(*S*)-(cyclohexylmethyl)-3,3-difluoro-2(*R*)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]-L-histidinamide (17).** Title compound **17** was prepared as described above for compound **5**, using an *N*-imidazole trityl-protected histidine. The final deprotection was performed by dissolving *N*-(4-morpholinylsulfonyl)-L-phenylalanyl-His(trit)-FCS-AEM (0.70 g, 0.67 mmol) in 80% AcOH (10 mL) and heating the reaction for 10 min at 80 °C. After cooling to room temperature and evaporation under reduced pressure, the residue was taken in ethyl acetate (20 mL) and washed twice with saturated $NaHCO_3$ solution before drying (Na_2SO_4). Column chromatography (5 → 15% MeOH in $CHCl_3$) on silica gel gave a white foam product (0.23 g, 42.8%) as an 87:10 mixture of diastereoisomers (HPLC) and a minor less polar isomer (80 mg, 14.9%). Major product (87:10 mixture): MS (FAB) m/z 797.4 $(M + H)^+$; HPLC, 60:35:5 MeOH–0.05 M Et_3N (pH 7 with H_3PO_4)–THF, 1.2 mL/min, t_R = 9.10, 8.70 min.

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-3-(2-amino-4-thiazolyl)-*N*-[1(*S*)-(cyclohexylmethyl)-3,3-difluoro-2(*R*)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]-L-alaninamide (18).** Title compound **18** was prepared as described above for compound **5** from the novel aminothiazole amino acid.[46] The product was obtained as a white solid (0.56 g, 41%): mp = 103–115 °C; MS (FAB) m/z 829.3 $(M + H)^+$.

[4-[2-[[1(*S*)-(Cyclohexylmethyl)-3,3-difluoro-2(*R*)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]amino]-1-[[2(*S*)-[(4-morpholinylsulfonyl)amino]-1-oxo-3-phenylpropyl]amino]-2-oxoethyl]-2-thiazolyl]carbamic Acid, 1,1-Dimethyl Ether Ester (19A and 19B). Title compound **19A** was prepared as described above for compound **5**. The product was finally purified by chromatography on silica gel eluting with 3 → 10% MeOH in a 1:1 mix of EtOAc and CH_2Cl_2, to afford two diastereoisomers: fast isomer (**19A**) [1.26 g, 58%; MS (FAB) m/z 949.3 $(M + H)^+$. Anal. C, H, N] and a slow isomer **19B** (0.66 g, 30.3%) and additionally a mixture of the two isomers (0.20 g, 10%).

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-*N*-[1(*S*)-(cyclohexylmethyl)-3,3-difluoro-2(*R*)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]-4,5-didehydro-L-norleucinamide (20).** Title compound **20** was prepared as described above for compound **5**. The product was obtained as a white foam (2.84 g, 70.1%): MS (FAB) m/z 773 $(M + H)^+$.

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-*N*-[1(*S*)-(cyclohexylmethyl)-3,3-difluoro-2(*R*)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]-4,5-didehydro-L-glycinamide (21).** Title compound **21** was prepared as described above for compound **5**. The product was purified by chromatography on silica gel eluting with 0 → 4% MeOH in CH_2Cl_2 to afford the product as a white solid: mp = 85–92 °C; MS (FAB) m/z 717 $(M + H)^+$.

***N*-[2-[[1(*S*)-(Cyclohexylmethyl)-3,3-difluoro-2(*R*)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]amino]-2-oxo-1-propoxyethyl]-α-[(4-morpholinylsulfonyl)amino]benzenepropanamide (22).** Title compound **22** was prepared as described above for compound **5** from the novel α-heteroatom amino acid.[47] The products were obtained as (two diastereoisomers) white foams: fast isomer **22A** [0.99 g, 19.6%; MS (FAB) m/z 775.5 $(M + H)^+$; HPLC, mobile phase A, t_R = 4.54 min, 98.6% single isomer], slow isomer **22B** [0.77 g, 15.3%, MS (FAB) m/z 775.5 $(M + H)^+$], and a mixture of the two isomers (1.40 g, 27.8%) to give a total yield of 62.7%.

3-[[1(*S*)-(Cyclohexylmethyl)-3,3-difluoro-2(*R*)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]amino]-*N*-[*N*-(4-morpholinylsulfonyl)-L-phenylalanyl]-3-oxo-DL-alanine Methyl Ester (23). Compound **23** was prepared as described previously for compound **5**. The product was purified by flash chromatography on silica gel eluting with 5% MeOH in $CHCl_3$ to afford **23** as a white foam (3.90 g, 41.9%). Repurification was necessary by flash chromatography (eluting with 5% MeOH in EtOAc) to afford **23** as a mixture of diastereoisomers (3.17 g, 34%): MS (FAB) m/z 775.4 $(M + H)^+$; HPLC, 70:30 Et_3N (0.05 M, pH 3)–CH_3CN; 1.5 mL/min, t_R = 19.52, 21.44 min, 97.2%, 72:28 ratio of isomers.

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-*N*-[1(*S*)-(cyclohexylmethyl)-3,3-difluoro-2(*R*)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]-L-threoninamide (24).** Compound **24** was prepared as described previously for compound **5**. The product was finally purified by chromatography on silica gel eluting with 0 → 4% MeOH in CH_2Cl_2 to afford the product as a white solid: mp = 107–112 °C, MS (FAB) m/z 761.4 $(M + H)^+$.

***N*-(4-Morpholinylsulfonyl)-L-phenylalanyl-*N*-[1(*S*)-(cyclohexylmethyl)-3,3-difluoro-2(*R*)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]-*N*-6-[(methylamino)thioxomethyl]-L-lysinamide (25).** Title compound **25** was prepared as described previously for **5** by DCC/HOBT coupling of *N*-(4-morpholinylsulfonyl)-L-phenylalanine with δ-*N*-Z-Lys-FCS-AEM to afford the product as a white foam (1.81 g, 88.7%). The benzyl protecting group was removed by hydrogenation (20% Pd/C, H_2, 100 mL of MeOH) to afford the free amine (1.57 g, 100%). The crude amine (1.57 g, 2.0 mmol) was

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(47) Repine, J. T.; Kaltenbronn, J. S.; Bonadies, L. A.; Doherty, A. M.; Hamby, J. M.; Himmelsbach, R. J.; Kornberg, B. E.; Taylor, M. D.; Rapundalo, S. T.; Batley, B. L. Renin Inhibitors Containing α-Heteroatom Amino Acids as P2 Replacements. 199th National Meeting of the American Chemical Society, Boston, MA, April 22–27, 1990.

dissolved in CH_2Cl_2 (80 mL) at 0 °C, and methyl isothiocyanate[89] (145 mg, 2.0 mmol) in CH_2Cl_2 (5 mL) was added. After 24 h of stirring at room temperature, the solvent was removed under reduced pressure and the residue chromatographed on silica gel eluting with 5 → 20% MeOH in $CHCl_3$ to afford the product as a white foam (0.89 g, 52%): MS (FAB) m/z 861.6 $(M + H)^+$; HPLC, 60:35:5 MeOH–Et_3N (0.05 M, pH 7.0 with H_3PO_4)–THF, 1.5 mL/min, t_R = 10.80, 11.70 min, 95.2%, 95.2:1 ratio of isomers.

N-(4-Morpholinylsulfonyl)-L-phenylalanyl-N-[1(S)-(cyclohexylmethyl)-3,3-difluoro-4-[[2-(4-morpholinyl)ethyl]amino]-2,4-dioxobutyl]-L-histidinamide (26). Title compound 26 was obtained by oxidation of N-(4-morpholinylsulfonyl)-L-phenylalanyl-His(trit)-FCS-AEM followed by deprotection according to the procedure described for compounds 17 and 9. The crude product was obtained as a mixture of diastereoisomers (44:56 by HPLC) and was chromatographed on silica gel eluting with 5 → 15% MeOH in $CHCl_3$ to afford the product as a white foam (0.40 g, 40%) dried under high vacuum: MS (FAB) m/z 795.2 $(M + H)^+$.

N-(4-Morpholinylsulfonyl)-L-phenylalanyl-3-(2-amino-4-thiazolyl)-N-[1(S)-(cyclohexylmethyl)-3,3-difluoro-4-[[2-(4-morpholinyl)ethyl]amino]-2,4-dioxobutyl]-L-alaninamide, Methanesulfonate (27). Title compound 27 was obtained by oxidation of the TROC [[(trichloromethyl)oxy]carbonyl] protected analogue of 18 by the procedure described previously for 9. Removal of the TROC group was performed by treatment of N-(4-morpholinylsulfonyl)-L-Phe-ATM(TROC)-FCO-AEM (crude material, 0.86 g) with Zn (0.27 g) and solid NH_4Cl (2 g) in MeOH–THF (1:4, 30 mL) for 24 h. Further portions of zinc were added periodically (2 × 0.27 g) until TLC indicated reaction completion. The suspension was then filtered through Celite and evaporated. The yellowish residue was purified by preparative HPLC on an Econosil column eluting with pH 7 buffer system to afford the required product 27 (0.49 g, 75%) as a 98:2 mixture of diastereoisomers (white foam). The material was converted to the monomethanesulfonic acid salt in the usual way. MS (FAB) m/z 827.0 $(M + H)^+$; HPLC 65:30:5 MeOH–0.05 M Et_3N (pH 7 with H_3PO_4)–THF, 1 mL/min, t_R = 7.93 min, 95%, single isomer.

N-(4-Morpholinylsulfonyl)-L-phenylalanyl-N-[1(S)-(cyclohexylmethyl)-3,3-difluoro-4-[[2-(4-morpholinyl)ethyl]amino]-2,4-dioxobutyl]-4,5-didehydro-L-norleucinamide (28). Title compound 28 was obtained by oxidation of 20 by the procedure described previously for the preparation of 9. The product was obtained as a white foam (1.70 g, 78.3%): MS (FAB) m/z 771.4 $(M + H)^+$.

N-(4-Morpholinylsulfonyl)-L-phenylalanyl-N-[1(S)-(cyclohexylmethyl)-3,3-difluoro-4-[[2-(4-morpholinyl)ethyl]amino]-2,4-dioxobutyl]glycinamide (29). Title compound 29 was obtained by oxidation of compound 21 by the procedure described for the preparation of 9. The compound (0.80 g, 84.2%) was finally converted to the methanesulfonate salt by dissolving in EtOH (15 mL) and CH_3SO_3H (80 μL). Evaporation gave a white foam (0.90 g): MS (FAB) m/z 715.3 $(M + H)^+$.

N-[2-[[1(S)-(Cyclohexylmethyl)-3,3-difluoro-4-[[2-(4-morpholinyl)ethyl]amino]-2,4-dioxobutyl]amino]-2-oxo-1-propoxyethyl]-α-[(4-morpholinylsulfonyl)amino]benzenepropanamide, 2-Hydroxy-1,2,3-propanetricarboxylate (30). Title compound 30 was obtained by oxidation of 22AB according to the procedure outlined previously for 9. The product was obtained as a white foam (1.36 g, 76%). The compound was converted to its citrate salt (1.19 g) to afford a white foam: MS (FAB) m/z 773.7 $(M + H)^+$.

3-[[1-(Cyclohexylmethyl)-3,3-difluoro-4-[[2-(4-morpholinyl)ethyl]amino]-2,4-dioxobutyl]amino]-N-[N-(4-morpholinylsulfonyl)-L-phenylalanyl]-3-oxo-(S)-DL-alanine, Methyl Ester, Methanesulfonate (31). Title compound 31 was prepared by oxidation of 23 according to the procedure described previously for 9. The product (1.04 g, 99%) was dissolved in 20 mL of MeOH and 0.12 mL of CH_3SO_3H. After stirring for 15 min, the solution was evaporated, redissolved in 15 mL of H_2O and lyophilized to afford a white foam (1.15 g, 100%): MS (FAB) m/z 773.4 $(M + H)^+$.

N-(4-Morpholinylsulfonyl)-L-phenylalanyl-α-amino-N-[1(S)-(cyclohexylmethyl)-3,3-difluoro-4-[[2-(4-morpholinyl)ethyl]amino]-2,4-dioxobutyl]-3-oxo-L-butanamide, Methanesulfonate (32). Title compound 32 was prepared by oxidation of 24 according to the procedure described previously for 9. The product (0.80 g, 47%) obtained from the H_3PO_4 extraction (0.80 g, 47%) was converted to the methanesulfonate by addition of CH_3SO_3H (37 μL) in EtOH (5 mL) followed by evaporation, redissolving in H_2O, and lyophilization to a white foam: MS (FAB) m/z 756.6 $(M + H)^+$. From the ethyl acetate extract was obtained a further 0.41 g of product 32.

O-Methyl-N-(4-morpholinylsulfonyl)-L-tyrosyl-N-[1(S)-(cyclohexylmethyl)-3,3-difluoro-2(R)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]-4,5-didehydro-L-norvalinamide, Methanesulfonate (33). Title compound 33 was obtained by the methods described previously for 5. The product was finally purified by chromatography on silica gel eluting with 4% MeOH in CH_2Cl_2 to afford a white foam (3.38 g, 77.5%): MS (FAB) m/z 787.4 $(M + H)^+$. The product (0.70 g, 0.89 mmol) was dissolved in EtOH (10 mL) followed by addition of methanesulfonic acid (56 μL). After stirring for 30 min, the solution was evaporated and the residue redissolved in H_2O (100 mL) and lyophilized to afford a white foam (774 mg, 99%): MS (FAB) m/z 786.9 $(M + H)^+$; HPLC, 85:15 MeOH–0.05 M $NH_4H_2PO_4$ (pH 3 with H_3PO_4), 2.5 mL/min, t_R = 13.1, 10.2 min, 92:6 ratio of isomers.

β-Hydroxy-N-(4-morpholinylsulfonyl)-L-phenylalanyl-N-[1(S)-(cyclohexylmethyl)-3,3-difluoro-2(R)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]-4,5-didehydro-L-norvalinamide (34). Title compound 34 was prepared by the methods described previously for 5. The product was finally purified by chromatography on silica gel eluting with 5% MeOH in EtOAc to obtain a white foam (2.00 g, 51.8%): MS (FAB) m/z 773.3 $(M + H)^+$.

O-Methyl-N-(4-morpholinylsulfonyl)-L-tyrosyl-N-[1(S)-(cyclohexylmethyl)-3,3-difluoro-4-[[2-(4-morpholinyl)ethyl]amino]-2,4-dioxobutyl]-4,5-didehydro-L-norvalinamide, Methanesulfonate (35). Title compound 35 was obtained by oxidation of 33 according to the procedure described for 9 to obtain a white foam (0.76 g, 84%) as a 98:2 mixture of diastereoisomers (HPLC). The product was dissolved in EtOH (15 mL) and methanesulfonic acid (63 μL) was added. After stirring for 15 min, the solution was evaporated and the residue dissolved in H_2O and lyophilized to a white foam (1.00 g): MS (FAB) m/z 785.4 $(M + H)^+$; HPLC, 55:45:5 0.05 M Et_3N (pH 7.0)–CH_3CN–THF, 1.5 mL/min, t_R = 6.05, 6.68, 90:10 ratio of isomers.

N-(4-Morpholinylsulfonyl)-β-oxo-L-phenylalanyl-N-[1(S)-(cyclohexylmethyl)-3,3-difluoro-4-[[2-(4-morpholinyl)ethyl]amino]-2,4-dioxobutyl]-4,5-didehydro-L-norvalinamide, Methanesulfonate (36). Title compound 36 was obtained from 34 according to the procedure outlined for compound 9. The product was obtained as a pale yellow foam (1.40 g, 70%) that was converted to the CH_3SO_3H salt by dissolving in EtOH (15 mL) and CH_3SO_3H (125 μL). After evaporation, 1.25 g (87%) was obtained: MS (FAB) m/z 769.3 $(M + H)^+$.

N-(4-Morpholinylcarbonyl)-L-phenylalanyl-N-[1(S)-(cyclohexylmethyl)-3,3-difluoro-2(R)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]-4,5-didehydro-L-norvalinamide (37). Title compound 37 was prepared as described previously for compound 5 from BOC-N-(4-morpholinylcarbonyl)-L-phenylalanine (prepared in a similar manner to (4-morpholinylsulfonyl)-L-phenylalanine. The compound was finally purified by column chromatography on 0 → 4% MeOH in CH_2Cl_2 to afford a white foam (2.20 g, 54.4%): MS (FAB) m/z 721 $(M + H)^+$.

N-(1-Piperazinylsulfonyl)-L-phenylalanyl-N-[1(S)-(cyclohexylmethyl)-3,3-difluoro-2(R)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]-4,5-didehydro-L-norvalinamide, Methanesulfonate (38). Title compound 38 was prepared from BOC-N-(1-piperazinylsulfonyl)-L-phenylalanine by the methods described previously for 5. The BOC-protected derivative of 38 (3.33 g, 85%) was finally deprotected with excess HCl in $CHCl_3$ (150 mL) followed by neutralization with solid $NaHCO_3$. The organic layer was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), filtered, and concentrated. The white foam obtained was washed with Et_2O and dried under high vacuum (2.56 g, 89%): MS (FAB) m/z 756.6 $(M + H)^+$. The product was converted to its methanesulfonate salt by addition of methanesulfonic acid (2 equiv) in EtOH (10 mL). After evaporation the residue was dissolved in H_2O and lyophilized.

N-[(4-Methyl-1-piperazinyl)sulfonyl]-L-phenylalanyl-N-[1(S)-(cyclohexylmethyl)-3,3-difluoro-2(R)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]-4,5-didehydro-L-norvalinamide (39). Title compound **39** was prepared from **38** (1.96 g, 2.60 mmol) by treatment of a mixture of 37% formalin (2.0 mL, 0.024 mol), formic acid (10 mL), and EtOH (20 mL). The above mixture was refluxed for 3 h and then concentrated. The residue was partitioned between EtOAc and 10% K_2CO_3 solution and the organic layer dried (Na_2SO_4). After filtration and evaporation the product was purified by flash chromatography on silica gel eluting with 15% MeOH in $CHCl_3$ to afford a white foam (1.18 g, 59%): MS (FAB) m/z 770.3 (M + H)$^+$; HPLC, 60:35:5 MeOH–0.05 M Et_3N (pH 7.0 with H_3PO_4)–THF; t_R = 8.31 min, 97.1%.

N-[(1,1-Dimethylethoxy)carbonyl]-L-phenylalanyl-N-[1-(S)-(cyclohexylmethyl)-3,3-difluoro-2(R)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]-4,5-didehydro-L-norvalinamide (40). Title compound **40** was prepared by methods described previously for **5**. The product was finally purified by chromatography on silica gel eluting with 4% MeOH in CH_2Cl_2 to afford a white foam (3.89 g, 82%): MS (FAB) m/z 708.2 (M + H)$^+$; HPLC, CH_3CN–H_2O (50:50), t_R = 12.1 min, 97.6%, single isomer.

N-[1-Oxo-3-(3-pyridinyl)propyl]-L-phenylalanyl-N-[1-(S)-(cyclohexylmethyl)-3,3-difluoro-2(R)-hydroxy-4-[[2-(4-morpholinyl)ethyl]amino]-4-oxobutyl]-4,5-didehydro-L-norvalinamide, Methanesulfonate (41). Title compound **41** was prepared by methods described previously for **5**. The product was finally purified by filtration followed by washing several times with EtOAc–Et_2O. The product was dried at 65 °C in vacuo for 2 h to afford **41** as a white solid, mp = 195–201 °C (4.02 g, 90.9%). The product (1.0 g, 1.35 mmol) was converted to the bis(methanesulfonic acid) salt by treatment with methanesulfonic acid (0.18 mL) and 10 mL of MeOH followed by evaporation to a white foam: MS (FAB) m/z 741.4 (M + H)$^+$; HPLC, 48:52 0.05 M Et_3N (pH 3 with H_3PO_4)–CH_3CN, t_R = 10.4 min, 98.2%, single isomer.

N-[[4-[(1,1-Dimethylethoxy)carbonyl]-1-piperazinyl]sulfonyl]-L-phenylalanyl-N-[1-(cyclohexylmethyl)-3,3-difluoro-2(R)-hydroxy-4-[(2-methylbutyl)amino]-4-oxobutyl]-4,5-didehydro-L-norvalinamide (42). Title compound **42** was prepared by methods described previously for **5** from (piperazinylsulfonyl)-L-phenylalanine and substituting 2(S)-methylbutylamine (MBA) for 4-(2-aminoethyl)morpholine (AEM). The product was finally purified by column chromatography on silica gel eluting with 2.5% MeOH in CH_2Cl_2 to afford the product as a white foam (3.4 g, 76%): MS (FAB) m/z 813.3 (M + H)$^+$ (NMR confirmed presence of DMF).

N-(4-Morpholinylcarbonyl)-L-phenylalanyl-N-[1(S)-(cyclohexylmethyl)-3,3-difluoro-4-[[2-(4-morpholinyl)ethyl]amino]-2,4-dioxobutyl]-4,5-didehydro-L-norvalinamide, Methanesulfonate (43). The title compound **43** was prepared by oxidation of **37** by the method described previously for **9**. The methanesulfonate salt was obtained as a white foam (1.60 g, 95%) from **43**: MS (FAB) m/z 718.85 (M + H)$^+$.

N-[(4-Methyl-1-piperazinyl)sulfonyl]-L-phenylalanyl-N-[1-(cyclohexylmethyl)-3,3-difluoro-4-[[2-(4-morpholinyl)ethyl]amino]-2,4-dioxobutyl]-4,5-didehydro-L-norvalinamide, Methanesulfonate (44). Title compound **44** was prepared by oxidation of **39** by the method described previously for **9**. HPLC of the crude material indicated a 36:64 mixture of diastereoisomers (92% purity). The product was finally purified by column chromatography on silica gel eluting with 5 → 10% MeOH in CH_2Cl_2 to afford a white foam dried under high vacuum (0.45 g, 62%). The product was converted to the bis(methanesulfonate) by treatment with methanesulfonic acid (76 μL) in EtOH (10 mL) followed by evaporation under reduced pressure and lyophilization from H_2O: MS (FAB) m/z 768.4 (M + H)$^+$; HPLC, 60:35:5 MeOH–0.05 M Et_3N (pH 7.0 with H_3PO_4)–THF; t_R = 9.79, 11.15, 94%, 42:52 ratio of isomers.

N-[(1,1-Dimethylethoxy)carbonyl]-L-phenylalanyl-N-[1-(S)-(cyclohexylmethyl)-3,3-difluoro-4-[[2-(4-morpholinyl)-ethyl]amino]-2,4-dioxobutyl]-4,5-didehydro-L-norvalinamide (45). Title compound **45** was prepared by oxidation of **40** by the method described previously for **9**. The product was purified by chromatography on silica gel eluting with 0 → 5% MeOH in CH_2Cl_2 to afford a foam which was rechromatographed eluting with 30% EtOAc in CH_2Cl_2 to 2:49:49 MeOH–EtOAc–CH_2Cl_2 to afford the product as a white foam: MS (FAB) m/z 706 (M + H)$^+$.

N-[3-(3-Pyridinyl)-1-oxopropyl]-L-phenylalanyl-N-[1-(S)-(cyclohexylmethyl)-3,3-difluoro-4-[[2-(4-morpholinyl)-ethyl]amino]-2,4-dioxobutyl]-4,5-didehydro-L-norvalinamide, Methanesulfonate (46). Title compound **46** was prepared by oxidation of **41** by the method described previously for **9**. The methanesulfonic acid salt was prepared by addition of methanesulfonic acid (0.32 g, 3.98 mmol) and ethanol (20 mL) to the off-white foam **46** (2.48 g). The solution was evaporated and dried under reduced pressure to afford the salt as an off-white foam (2.40 g, 81.5%): MS (FAB) m/z 739.3 (M + H)$^+$; HPLC, 30:70 0.05 M Et_3N (aqueous)–CH_3CN (pH 3 with H_3PO_4), t_R = 1.56 min, 95.3:4.7 ratio of isomers.

N-[[4-[(1,1-Dimethylethoxy)carbonyl]-1-piperazinyl]-sulfonyl]-L-phenylalanyl-N-[1(S)-(cyclohexylmethyl)-3,3-difluoro-4-[(2-methylbutyl)amino]-2,4-dioxobutyl]-4,5-didehydro-L-norvalinamide (47). Title compound **47** was prepared by oxidation of **42** according to the method described previously for **9**. The product was finally purified by chromatography on silica gel eluting with 20% EtOAc in hexane to afford the product as a white foam (1.60 g, 61.5%): MS (FAB) m/z 811.4 (M + H)$^+$.

N-(1-Piperazinylsulfonyl)-L-phenylalanyl-N-[1(S)-(cyclohexylmethyl)-3,3-difluoro-4-[(2-methylbutyl)amino]-2,4-dioxobutyl]-4,5-didehydro-L-norvalinamide, Monohydrochloride (48). Title compound **48** was prepared from **47** (1.0 g, 1.23 mmol) by deprotection with HCl(g) in CH_2Cl_2 (100 mL) and MeOH (1 mL). After bubbling through HCl for 15 min the solution was evaporated. Additional CH_2Cl_2 was added to the residue and then evaporated, this procedure being repeated twice more. Finally Et_2O was added and the solution evaporated to dryness. The product was dried under vacuum to afford the hydrochloride salt as a white foam: FAB (MS) m/z 711.3 (M + H)$^+$; HPLC, mobile phase 60:35:5 CH_3CN–Et_3N (0.025 M) pH 7 with H_3PO_4–CH_3CN–THF, t_R = 5.16 min, 96.3%.

Biological Methods. Inhibition of renin activity by novel inhibitor drugs was determined by a radioimmunoassay for angiotensin I, based on the method of Haber et al.[48,8g] The in vitro angiotensin I generation step utilized 500 μL of monkey plasma (containing native renin and angiotensinogen), 50 μL of maleate buffer (pH 6.0), 5 μL of phenylmethanesulfonyl fluoride (PMSF) and 2 μL of an appropriate concentration of inhibitor in a dimethyl sulfoxide (DMSO) vehicle. Incubation was for 60 min at 37 °C. Following incubation, each mixture was analyzed (in duplicate) for angiotensin I via radioimmunoassay using ^{125}I-labeled angiotensin I and carried out in tubes coated with rabbit anti-angiotensin I antibody (Gamma Coat RIA Kit, Dade Clinical Assays). Monkey plasma renin activity ranged from 3–8 AI/mL per h. Values for inhibitor tubes were compared to those for vehicle (DMSO) control tubes to estimate percent inhibition. At the concentration used, DMSO inhibits the generation of angiotensin I by <10%. The inhibition results were expressed as IC_{50} values, which were obtained by plotting six inhibitor concentrations and estimating the concentration producing 50% inhibition using nonlinear regression analysis.

Inhibition of bovine cathepsin D (Sigma) activity was assessed in duplicate by the hydrolysis of bovine hemoglobin (2X crystallized, Sigma) at pH 3.2 and 37 °C (modified from Aoyagi et al. and Kokubu et al.).[49,50] Net absorbance (at 280 nm) was measured in acid-precipitated supernatant fractions of inhibited

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vs uninhibited control assays. The IC_{50} values were determined as described above.

Conscious High-Renin Normotensive Monkey Model. Male cynomolgus monkeys weighing between 4.9 and 7.7 kg were placed on a low-sodium diet (Bio-Serv Inc., Frenchtown, NJ) 7–10 days prior to testing. Each monkey was then treated with furosemide (Lasix, INJ 5%, Hoechst-Roussel) at 2 mg/kg per day im for 4 consecutive days prior to testing.

Solutions were prepared using a vehicle of DMA 7.5:30:62.5–Tween 80–H_2O. Concentrations were adjusted to allow the total dose to be administered in a volume of 2 mL/kg. The solution was administered by oral gavage using a 16-French rectal–colon tube (Davol, Cranston, RI). The monkeys were instrumented with vascular access parts (Norfolk Medical Products, Skokie, IL) for intraarterial blood pressure monitoring. Blood pressure was measured using a computer data acquisition system. Monkeys selected for these studies had been trained to rest quietly in a basic macaque restrainer (Primate Products, Woodside, CA). At the end of each experiment monkeys were challenged with an intravenous infusion (20 μg/mg per min for 30 min) of the angiotensin II receptor antagonist saralasin (Sar^1-Val^5-Ala^8-AII; Bachem Inc., Torrance, CA). Maximum blood pressure lowering effect of the drug compared to that with saralasin infusion, expressed as a ratio, provided a measure of the response.

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Registry No. 1, 98105-42-1; **2A**, 103322-58-3; **3**, 137429-58-4; **4**, 137302-30-8; **5**, 137302-31-9; **6**, 137302-32-0; **7**, 137302-33-1; **8**, 137302-34-2; **9**·$MeSO_3H$, 137302-36-4; **9** (free base), 137302-35-3; **10**, 137302-37-5; **11**, 137302-38-6; **12**·$MeSO_3H$, 137302-40-0; **12** (free base), 137302-39-7; **13**, 137302-41-1; **14**, 137302-42-2; **15**·$MeSO_3H$, 137302-44-4; **15** (free base), 137302-43-3; **16**·$MeSO_3H$, 137302-46-6; **16** (free base), 137302-45-5; **17**, 137302-47-7; **17** (N^{His}-trityl), 137302-84-2; **18**, 137429-59-5; **18** (*N*-TROC), 137302-86-4; **19A**, 137429-60-8; **19B**, 137429-64-2; **20**, 137302-48-8; **21**, 137302-49-9; **22A**, 137302-50-2; **22B**, 137429-65-3; **23** (epimer 1), 134334-93-3; **23** (epimer 2), 134452-00-9; **24**, 137302-51-3; **25**, 132101-82-7; **26** (epimer 1), 137302-52-4; **26** (epimer 2), 137429-66-4; **27**·$MeSO_3H$, 137429-61-9; **27** (free base), 135760-09-7; **27** (*N*-TROC), 137302-87-5; **28**, 137302-53-5; **29**·$MeSO_3H$, 137302-55-7; **29** (free base), 137302-54-6; **30**·citrate, 137302-57-9; **30** (free base), 137302-56-8; **31**·$^5/_2MeSO_3H$ (epimer 1), 134334-96-6; **31**·$^5/_2MeSO_3H$ (epimer 2), 134334-98-8; **31** (free base, epimer 1), 134453-04-6; **31** (free base, epimer 2), 134334-94-4; **32**·$MeSO_3H$, 137302-59-1; **32** (free base), 137302-58-0; **33**·$MeSO_3H$, 137302-61-5; **33** (free base), 137302-60-4; **34**, 137302-62-6; **35**·$MeSO_3H$, 137302-64-8; **35** (free base), 137302-63-7; **36**·$MeSO_3H$, 137302-66-0; **36** (free base), 137302-65-9; **37**, 137302-67-1; **38**·$2MeSO_3H$, 137432-19-0; **38** (free base), 137302-82-0; **39**, 137302-68-2; **40**, 137302-69-3; **41**·$2MeSO_3H$, 137302-71-7; **41** (free base), 137302-70-6; **42**, 137302-72-8; **43**·$MeSO_3H$, 137302-74-0; **43** (free base), 137302-73-9; **44**·$2MeSO_3H$ (epimer 1), 137302-76-2; **44**·$2MeSO_3H$ (epimer 2), 137490-10-9; **44** (free base, epimer 1), 137302-83-1; **44** (free base, epimer 2), 137429-62-0; **45**, 137302-77-3; **46**·$MeSO_3H$, 137302-79-5; **46** (free base), 137302-78-4; **47**, 137302-80-8; **48**·HCl, 137302-81-9; **48** (free base), 137429-63-1; AEM, 2038-03-1; MBA, 34985-37-0; $BrCF_2COOEt$, 667-27-6; (*S*)-$BocNHCH(CH_2CH{=}CH_2)COOH$, 90600-20-7; Boc-Phe-OH, 13734-34-4; (*S*)-$BocNHCH(c\text{-}C_6H_{11})$-COOH, 109183-71-3; H-Lys(Z)-FCS-AEM, 137302-85-3; MeNCS, 556-61-6; *N*-(4-morpholinosulfonyl)-L-phenylalanine, 124278-39-3; 2-(aminoethyl)pyridine, 2706-56-1; Boc-*N*-(4-morpholinylcarbonyl)-L-phenylalanine, 137302-88-6; Boc-*N*-(1-piperazinylsulfonyl)-L-phenylalanine, 137302-89-7; renin, 9015-94-5; cathepsin D, 9025-26-7.