Design and Synthesis of Potent and Specific Renin Inhibitors Containing Difluorostatine, Difluorostatone, and Related Analogues

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Peptides that contain difluorostatine and difluorostatone residues have been shown to be potent inhibitors of the aspartyl protease renin. The readily hydrated fluoro ketone is proposed to mimic the tetrahedral intermediate that forms during the enzyme-catalyzed hydrolysis of a peptidic bond. It is suggested that the sp^3-hybridized ketal acts as a transition-state analogue renin inhibitor. The fluoro ketone is shown to be a much more effective inhibitor than the corresponding nonfluorinated ketone, which acts as a pseudosubstrate. More lipophilic side chains at the P_1 site can enhance the inhibitory potency of the difluorostatine analogue, but this cannot be demonstrated in the difluorostatone series. Additionally, high renin specificity has been shown for a difluorostatone-containing peptide.

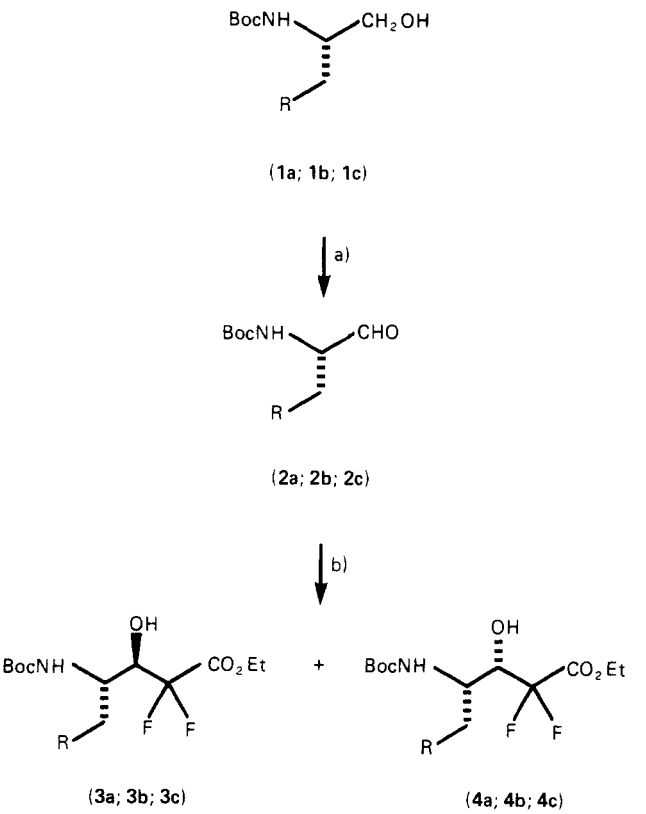
The renin–angiotensin system has been implicated in several forms of hypertension.[1] Renin is an aspartyl protease, which is produced mainly in the juxtaglomerular apparatus of the kidney.[2] It is a highly specific proteolytic enzyme and cleaves the circulating α-globulin angiotensinogen, produced by the liver, to form the decapeptide angiotensin I.[3] The N-terminal sequence of human angiotensinogen is shown in Figure 1, the cleavage site being the peptidic bond between amino acids 10 and 11.[4] Angiotensin I has no known biological activity, but it is converted to the octapeptide angiotensin II by the angiotensin-converting enzyme present in lungs and other organs by removal of the C-terminal dipeptide histidylleucine. Angiotensin II is a very potent vasoconstrictor and also stimulates the release of aldosterone from the adrenal gland. This mineralocorticoid induces sodium and water retention, and this, in conjunction with vasoconstriction, can lead to an increase in blood pressure.[3]

The antihypertensive activity of inhibitors of converting enzyme is not clear mechanistically due to its involvement in the kinin system. Renin, however, is an enzyme of high substrate specificity, and inhibitors of renin should effect the clear involvement of the renin–angiotensin system.[5] Interest in the blockade of renin has led to rapid development of potent inhibitors based on the angiotensinogen sequence. The most successful approach has been based upon the concept of a transition-state analogue[6] of the amide hydrolysis. Modifications at the cleavage site to mimic the tetrahedral species have generated analogues of the minimum substrate with high inhibitory potency in vitro.[7] Successful renin inhibitors could provide agents for control of cases of renin-associated hypertension.

Highly potent inhibitors of renin have been reported in which statine, 4(*S*)-amino-3(*S*)-hydroxy-6-methylheptanoic acid, was incorporated into the N-terminal sequence of angiotensinogen.[8] This novel amino acid was first found in pepstatin,[9] which is a naturally occurring pentapeptide with a general aspartyl protease inhibitory activity. It has been proposed that the statine residue acts as a structural analogue[10] of the tetrahedral species **B** (see Figure 2) formed during enzymatic hydrolysis of the corresponding peptidic bond typified in structure **A**.

A ketone analogue **C** has been prepared by the oxidation of the hydroxyl functionality of statine in a pepstatin analogue.[11] The ketone carbonyl of this pseudosubstrate has been demonstrated by ^{13}C NMR spectroscopy to be converted upon binding to pepsin to a tetrahedral ketal-type structure **D** via enzyme-catalyzed addition of water.[12] However, the ketone is a weaker inhibitor of pepsin than the corresponding statine-containing peptide.[11] The decreased binding might be attributed to the poor tendency of the ketone carbonyl to undergo hydration.

Scheme I. Preparation of 2,2-Difluoro-3-Hydroxy Esters[a]



[a] Compounds: **a**, R = $(CH_3)_2CH$; **b**, R = C_6H_5; **c**, R = c-C_6H_{11}. Reagents: (a) Me_2SO, $(COCl)_2$, CH_2Cl_2, Et_3N; (b) BrF_2CCO_2Et, Zn, THF.

† Physical and Analytical Chemistry.

(1) Davis, J. O. *Circ. Res.* **1977**, *40*, 439. Swales, J. D. *Pharmacol. Ther.* **1979**, *7*, 172.
(2) Peach, M. J. *Physiol. Rev.* **1977**, *57*, 313.
(3) Ondetti, M. A.; Cushman, D. W. *Annu. Rev. Biochem.* **1982**, *51*, 283.
(4) Skeggs, L.; Lentz, K.; Kahn, J.; Hochstrasser, H. *J. Exp. Med.* **1968**, *128*, 13.
(5) Haber, E. *N. Engl. J. Med.* **1984**, *311*, 1631.
(6) Wolfenden, R. *Transition States of Biochemical Processes*; Gandour, R. D., Schowen, R. L., Eds.; Plenum: New York, 1978; p 555.
(7) Szelke, M.; Jones, D. M.; Atrash, B.; Hallett, A.; Leckie, B. J. *Peptides: Structure and Function*, Proceedings of the Eighth American Peptide Symposium; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Co.: Rockford, IL, 1983; p 579; Boger, J. *Ibid.*, p 569.

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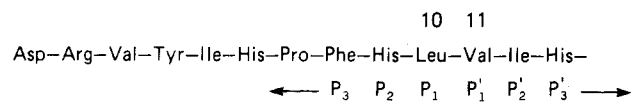


Figure 1. N-terminus of human angiotensinogen.

In our work, we proposed to introduce electron-withdrawing fluorine atoms on the unsubstituted methylene carbon between the carbonyl groups of statine as depicted in **E**. The resulting highly electrophilic nature of the carbonyl should facilitate hydration to form the tetrahedral ketal-type structure **F**.[13]

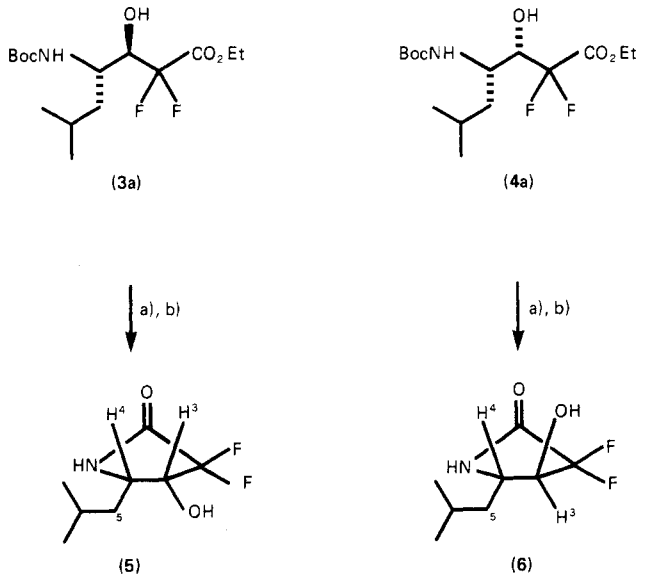
Chemistry

The suitably protected statine analogues **3a** and **4a** were prepared in a concise manner as shown in Scheme I. Boc-L-leucinol (**1a**) was oxidized[14] to the aldehyde **2a**, which was immediately used in the next reaction. A Reformatsky reaction[15] of Boc-L-leucinal (**2a**) with ethyl bromodifluoroacetate in the presence of activated zinc dust afforded the diastereomeric adducts **3a** and **4a**, vide infra, in good yield. Two sets of reaction conditions were examined. When the reaction vessel was placed in a sonicating bath at room temperature, a 70:30 mixture of **3a** and **4a** was isolated in 80% yield. However, under refluxing tetrahydrofuran, **3a** was essentially the only diastereomer isolated. That this latter outcome was a result of thermodynamic equilibration was substantiated by the observation that heating the sonicated reaction mixture to reflux for 30 min returned only the thermodynamically favored **3a** isomer.

One might speculate that the formation of the adduct **3a** is favored over the adduct **4a** both kinetically and thermodynamically. As shown in Figure 3, the nucleophile can add to the chelated aldehyde species **G** via a less hindered pathway (a) much more readily than a more hindered pathway (b). This preference would lead to the predominant formation of isomer **3a** over isomer **4a**. Moreover, one might also propose equilibration of the two chelated alcoholate species **H** and **I** via a retroreaction to species **G**. The trans isomer **H** (as drawn) is then thermodynamically favored over the more congested cis isomer **I**. It is significant that, under refluxing tetrahydrofuran conditions, the alcoholate species **H** has essentially complete dominance.

In addition to the statine analogues, we also had some interest in congeners with more lipophilic side chains in the P_1 site since the renin inhibitory activity of peptides has previously been found to be markedly dependent on the hydrophobicity of the amino acid residue at P_1.[16]

Scheme II. Assignment of Relative Stereochemistry[a]



[a] Reagents: (a) Et_2O, HCl(g); (b) THF, *i*-Pr_2NEt.

Starting with Boc-L-phenylalaninol (**1b**) and Boc-L-cyclohexylalaninol (**1c**), the corresponding Reformatsky adducts **3b**, **4b** and **3c**, **4c** were prepared in the same manner as described above.

The relative stereochemistry of the diastereomeric Reformatsky adducts was assigned as follows. Treatment of isomers **3a** and **4a** with dry hydrogen chloride in ether effected the removal of the *tert*-butyloxycarbonyl group. The hydrochloride salts were neutralized with diisopropylethylamine in tetrahydrofuran, and the amino esters spontaneously ring closed to the corresponding γ-lactams **5** and **6** as shown in Scheme II.

The ^1H NMR spectrum of compound **5** in methanol-d_4 showed H-4 as a multiplet. However, this signal simplified to a doublet with a coupling constant of 6 Hz when H-5's were irradiated. This J_{H4H3} was confirmed by the signal from H-3 that appeared as ddd with coupling constants of 6, 8, and 12 Hz. The latter two coupling constants were due to the two fluorine atoms. The ^1H NMR spectrum of compound **6** in methanol-d_4 also showed H-4 as a multiplet. This signal also simplified to a doublet with a coupling constant of 6 Hz when H-5's were irradiated. Again, this J_{H4H3} was confirmed by the signal from H-3 that appeared as ddd with coupling constants of 6, 11, and 12 Hz. The latter two coupling constants were due to the two fluorine atoms.

On the basis of proton coupling constants, the cis/trans stereochemistry of the lactams **5** and **6** could not be assigned. However, their ^{13}C NMR spectra were more informative in this regard. The signal from C-5 of compound **5** appeared at 39.3 ppm whereas the corresponding C-5 of compound **6** appeared at 44.05 ppm. An upfield shift in the isomer **5** with respect to the isomer **6** was indicative of the cis disposition of the two substituents[17] on the lactam ring as that shown in compound **5**.

This assignment of relative stereochemistry received strong support from an NOE experiment. Irradiation of H-4 in lactam **6** caused a 2% enhancement of the signal from H-3. On the other hand, irradiation of H-4 in lactam **5** led to a larger observed NOE enhancement of 9% in the

(8) Boger, J.; Lohr, N. S.; Ulm, E. H.; Poe, M.; Blaine, E. H.; Fanelli, G. M.; Lin, T.-Y.; Payne, L. S.; Schorn, T. W.; LaMont, B. I.; Vassil, T. C.; Stabilito, I. I.; Veber, D. F.; Rich, D. H.; Bopari, A. S. *Nature*, **1983**, *303*, 81.
(9) Umezawa, H.; Aoyagi, T.; Morishima, H.; Matsuzaki, M.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1970**, *23*, 259. Workman, R. J.; Burkitt, D. S. *Arch. Biochem. Biophys.* **1977**, *194*, 157.
(10) Marciniszyn, J.; Hartsuck, J. A.; Tang, J. *J. Biol. Chem.* **1976**, *251*, 7088.
(11) Rich, D. H.; Bopari, A. S.; Bernatowicz, M. S. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 1127.
(12) Rich, D. H.; Bernatowicz, M. S.; Schmidt, P. G. *J. Am. Chem. Soc.* **1982**, *104*, 3535.
(13) For a preliminary communication of this work, see: Thaisrivongs, S.; Pals, D. T.; Kati, W. M.; Turner, S. R.; Thomasco, L. M. *J. Med. Chem.* **1985**, *28*, 1553. For related work, see: Gelb, M. H.; Svaren, J. P.; Abeles, R. H. *Biochemistry* **1985**, *24*, 1813.
(14) Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651.
(15) Hallinan, E. A.; Fried, J. *Tetrahedron Lett.* **1984**, 2301.
(16) See for example: 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.; Veber, D. F. *J. Med. Chem.* **1985**, *28*, 1779.
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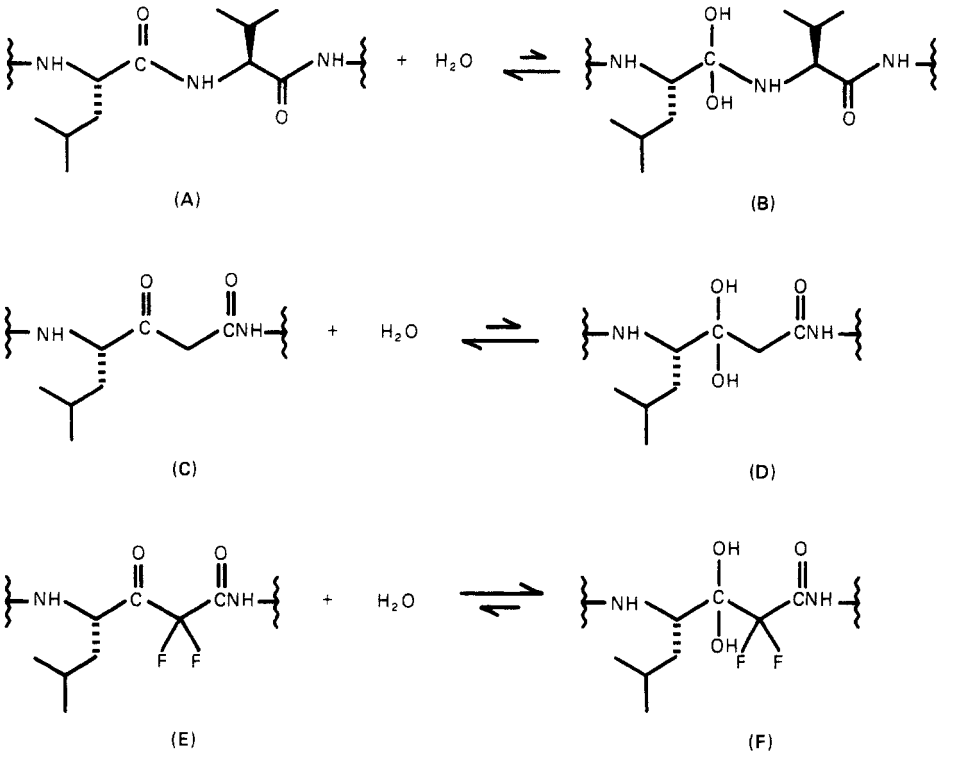


Figure 2. Hydration of a peptidic bond, a ketone, and a difluoro ketone.

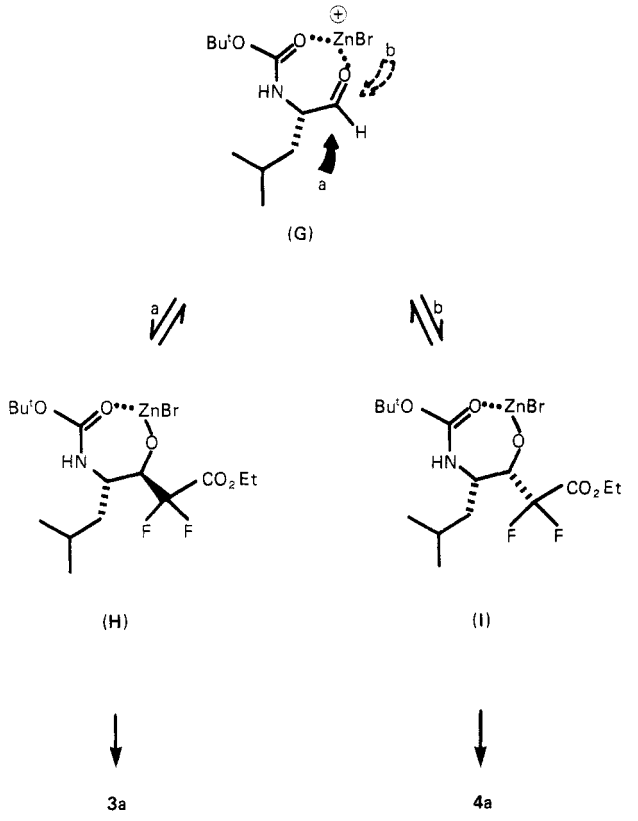
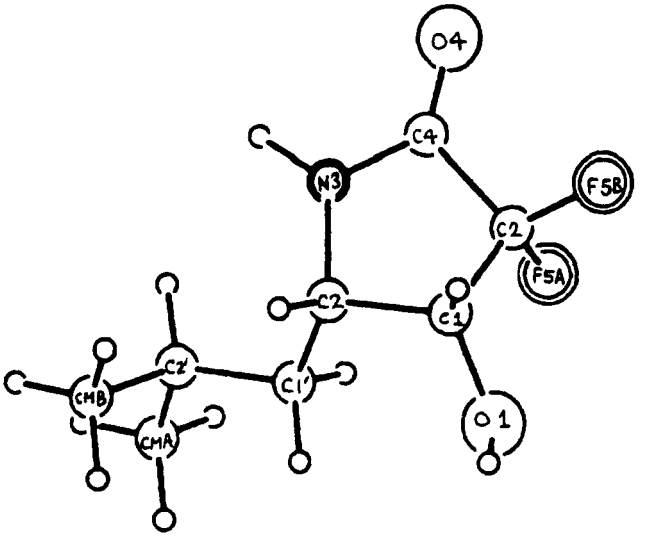


Figure 3. Reformatsky reaction.

signal from H-3. This is again indicative of the cis disposition of H-3 and H-4 on the lactam ring of compound **5**.

Finally, the crystalline compound **5** was submitted to X-ray analysis, which conclusively demonstrated its stereochemistry.

We were concerned about the extent of possible racemization of the aldehyde during the Reformatsky reaction. Therefore, the enantiomeric purity of adducts such as **3a** was determined via its Mosher ester.[18] A racemic sample of the Reformatsky adduct (**3a** and **3a'**) was prepared from

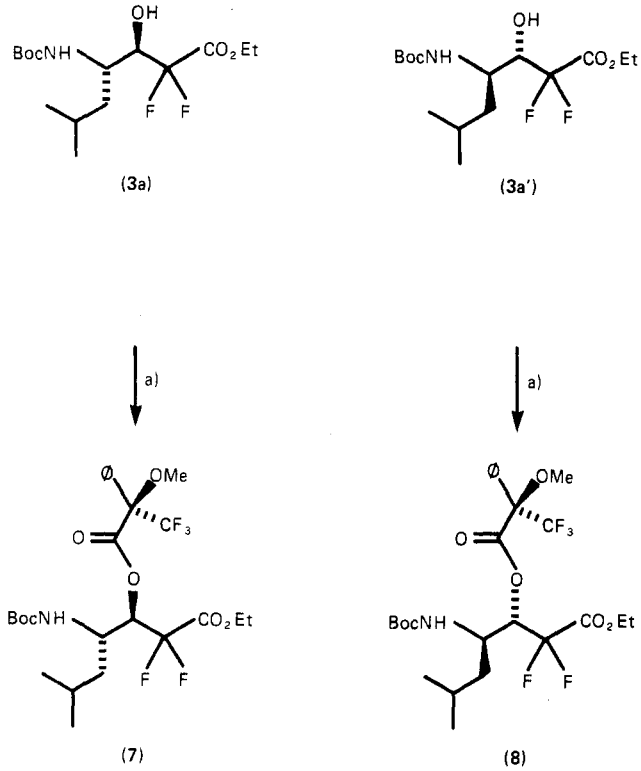


	X^a	Y	Z	B_{eq}
C(1)	−4758 (5)	−3104 (3)	−4255 (4)	1.4 (1)
O(1)	−6703 (3)	−3439 (0)	−3475 (3)	1.7 (1)
C(2)	−2508 (5)	−3393 (3)	−3110 (4)	1.3 (1)
N(3)	−1065 (4)	−2381 (3)	−3682 (3)	1.2 (1)
C(4)	−2123 (5)	−1416 (3)	−4500 (4)	1.4 (1)
O(4)	−1321 (4)	−464 (2)	−5092 (3)	1.7 (1)
C(5)	−4644 (5)	−1706 (3)	−4588 (5)	1.6 (1)
F(5A)	−5570 (3)	−1020 (2)	−3317 (3)	2.2 (1)
F(5B)	−5730 (3)	−1337 (2)	−6167 (3)	2.5 (1)
C(1')	−2689 (5)	−3361 (3)	−1126 (4)	1.6 (1)
C(2')	−453 (5)	−3568 (4)	29 (5)	1.8 (1)
C(MA)	−747 (7)	−3284 (4)	1964 (5)	2.9 (2)
C(MB)	419 (7)	−4905 (4)	−139 (6)	2.8 (2)

[a] Fractional coordinates ($\times 10^4$) and B_{eq}(Å^2). Estimated standard deviations are in parentheses. $B_{eq}a = {}^4/_3(a^2B_{11} + b^2B_{22} + c^2B_{33} + (ab\cos\gamma)B_{12} + (ac\cos\beta)B_{13} + (bc\cos\alpha)B_{23})$.

Figure 4.

Scheme III. Determination of Enantiomeric Purity[a]



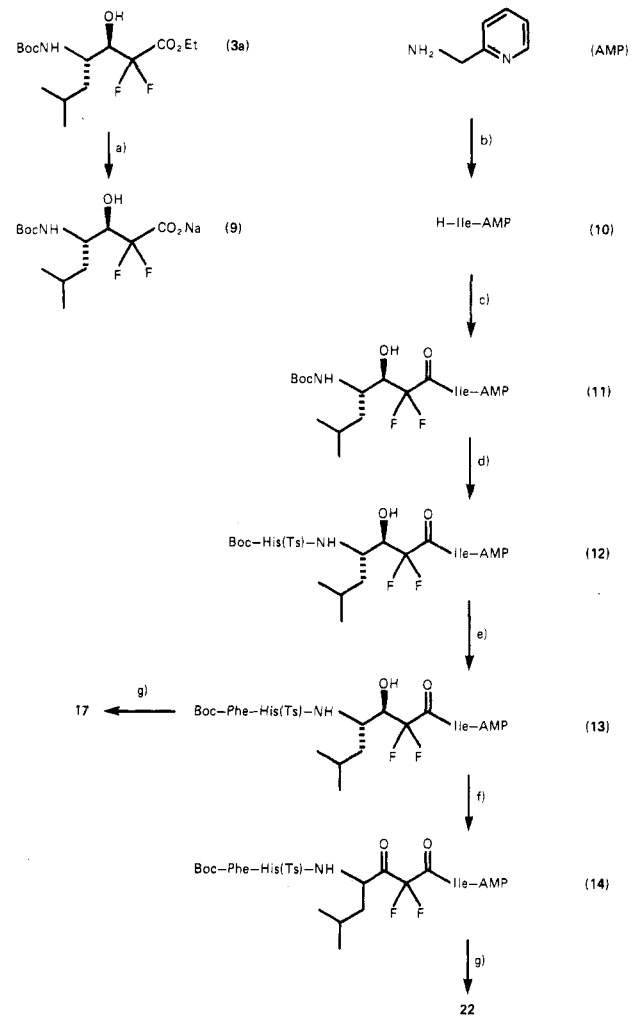
[a] Reagents: (a) (*S*)-$C_6H_5C(OCH_3)(CF_3)COCl$, pyridine, CCl_4.

Boc-DL-leucinol. Reaction with (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride gave the diastereomeric pair **7** and **8**. The ^{19}F NMR signals from the trifluoromethyl groups were well-resolved singlets at 89.9 and 90.6 ppm (downfield from hexafluorobenzene). The Mosher ester derived from the sample of **3a** prepared under refluxing condition with Boc-L-leucinal (**2a**) indicated an optical purity of approximately 90% for the adduct **3a**. On the other hand, the Mosher ester from the sample **3a** prepared under sonicating condition showed no detectable peak at 89.9 ppm in its ^{19}F NMR spectrum. In this latter case, a high optical purity of the adduct **3a** could be assured.

These presumed transition-state analogue inserts were incorporated into potential renin inhibitory peptides in a straightforward manner. Synthesis of the peptides **17** and **22** as shown in Scheme IV will serve as illustrative examples.

The ester **3a** was hydrolyzed with 1 equiv of sodium hydroxide, and the resulting salt **9** was then lyophilized. Coupling to L-isoleucyl-2-pyridylmethylamine with *N*,*N*′-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole gave the adduct **11**. Two more amino acids were sequentially added by using diethylphosphoryl cyanide with triethylamine as coupling reagent[19] to afford the intermediate **13**. Removal of the tosyl group on histidine with 1-hydroxybenzotriazole gave the desired peptide **17**. On the other hand, oxidation of the alcohol **13** with dimethyl sulfoxide and oxalyl chloride[14] led to the corresponding ketone **14**. After removal of the tosyl group on histidine, the resulting peptide **22** could be isolated as an epimeric mixture at C-4 (refer to carbon numbering in Scheme II) due to the ready epimerization of the difluoro ketone under the reaction condition. Analytical HPLC showed two components that could be separated by preparative HPLC to give peptide **19** and its C-4 epimer, which exhibited identical FAB mass spectra.

Scheme IV. Synthesis of Compounds **17** and **22**[a]



[a] Reagents: (a) 1 equiv NaOH, H_2O, THF; (b) Boc-Ile-OH, DCC, HOBT, CH_2Cl_2; TFA, CH_2Cl_2; (c) salt **9**, DCC, HOBT, CH_2Cl_2; (d) TFA, CH_2Cl_2; Boc-His(Ts)-OH, DEPC, Et_3N; (e) TFA, CH_2Cl_2; Boc-Phe-OH, DEPC, Et_3N; (f) Me_2SO, $(COCl)_2$, CH_2Cl_2; Et_3N; (g) HOBT, CH_3OH.

Biology

Table I displays the comparative inhibition of human plasma renin by congeneric peptides that contain different structural analogues of statine. The difluorostatine-containing peptide **17** is an effective renin inhibitor whereas the epimeric isomer **18** shows about 60-fold reduction in inhibitory activity. This finding is in accord with the earlier finding that the 3(*S*)-hydroxyl group of statine in a pepstatin analogue results in a much better inhibitor of pepsin than the corresponding 3(*R*)-hydroxyl analogue.[20] It is to be noted that the difluorostatine-containing peptide **17** is, however, less potent than the corresponding statine analogue **15**. This might suggest the importance of the hydroxyl group as a hydrogen-bond acceptor. The two fluorine atoms in the isomer **17** reduce the electron density on oxygen relative to that in compound **15**.

The statone-containing peptide **16** shows 20-fold reduction in inhibitory potency when compared to the sta-

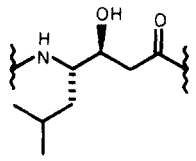
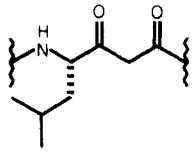
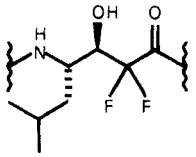
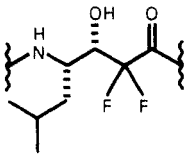
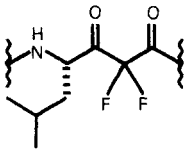
(18) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512.

(19) Yamada, S.; Kasai, Y.; Shioiri, T. *Tetrahedron Lett.* **1973**, 1595.

(20) Rich, D. H.; Sun, E.; Singh, J. *Biochem. Biophys. Res. Commun.* **1977**, *74*, 762. Rich, D. H.; Sun, E. T. O.; Ulm, E. *J. Med. Chem.* **1980**, *23*, 27.

Table I. Inhibition of Human Plasma Renin

Boc-Phe-His-X-Ile-AMP

X	no.	IC_{50}, nM
	**15**	1.7
	**16**	34
	**17**	12
	**18**	730
	**19**	0.52

tine analogue **15**. The decreased binding might be attributed to the poor tendency of the C-3 ketone carbonyl to undergo hydration toward sp^3 hybridization. The corresponding fluorinated ketone **19**, however, is about 65 times more active than the nonfluorinated congener **16**. The increased propensity toward sp^3 hybridization is reflected in the tighter binding of the fluorinated analogue.

We have also studied some analogues with more lipophilic side chains in the P_1 site.[16] As shown in Table II, the hydroxyl congener **20** with a benzyl side chain shows renin inhibitory activity essentially identical with that of the statine analogue **17**. A dramatic outcome, however, can be found in the cyclohexyl methyl analogue **21**, which is 20 times more active than the previous two congeners.

With this encouraging finding, we proceeded to examine the fluorinated ketone series in the hope of discovering more potent renin inhibitors. Compounds **22–24** were tested as epimeric mixtures at C-4. Again, the phenyl analogue **23** shows renin inhibitory potency essentially identical with that of the statone analogue **22**. Contrary to expectation, the cyclohexyl analogue **24** shows no improvement in inhibitory activity.

A high degree of enzyme specificity is desirable for a potentially useful therapeutic agent. We chose to examine the comparative inhibition of a few selected proteases by compound **22**. As illustrated in Table III, pepstatin[9] is a general aspartyl protease inhibitor and exhibits poor selectivity for renin. The substrate analogue RIP (Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys)[21] is a weak inhibitor and does not discriminate between renin and converting enzyme. The difluoro ketone **22**, however, exhibits high renin specificity. It has negligible inhibitory activity against converting enzyme and is 3–4 orders of magnitude less effective against pepsin and cathepsin D when compared to renin.

(21) Burton, J.; Cody, R. J., Jr.; Herd, J. A.; Haber, E. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5476.

Table II. Inhibition of Human Plasma Renin

Boc-Phe-His-X-Ile-AMP

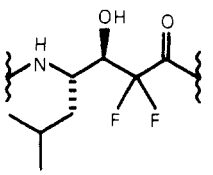
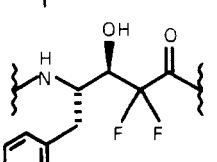
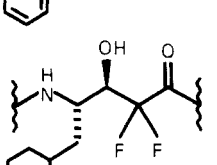
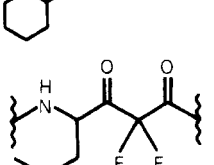
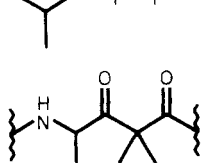
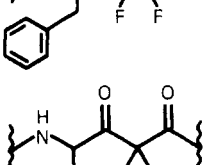
X	no.	IC_{50}, nM
	**17**	12
	**20**	13
	**21**	0.57
	**22**	1.4
	**23**	1.3
	**24**	2.5

Table III. Comparative Inhibition of Selected Proteases

	IC_{50}, M			
	human plasma renin	porcine pepsin	bovine cathepsin D	rabbit ACE
22	1.4×10^{-9}	4.2×10^{-5}	1.7×10^{-6}	24% at 10^{-3}
pepstatin[9]	6.0×10^{-6}	1.0×10^{-8}	2.8×10^{-8}	
RIP[21]	9.4×10^{-6}	0% at 10^{-4}	0% at 10^{-3}	1.2×10^{-5}

Summary

The use of the transition-state analogue concept is an effective approach in the design of potent enzyme inhibitors. In the case of aspartyl proteases, introduction of units that are proposed to mimic the hydrolysis intermediate has been invaluable in the design of effective inhibitors.[22] Previous work on the mechanistic aspect of the statone-containing peptide as a pseudosubstrate[12] presented an opportunity to create more effective inhibitors. Interest in the blockade of renin, a therapeutically important enzyme, has led us to develop potent inhibitors that contain difluorostatine and difluorostatone as transition-state analogues. Based on the premise that an electron-deficient carbonyl analogue would show much

(22) Rich, D. H. *J. Med. Chem.* **1985**, *28*, 263.

greater propensity to form a tetrahedral ketal, the fluorinated ketone has been shown to be a more effective inhibitor than the corresponding nonfluorinated analogue and is proposed to mimic the tetrahedral intermediate formed during the hydrolysis of a peptidic bond by renin. These potent renin inhibitors could provide a basis for a therapeutic agent in the control of renin-associated hypertension.

Experimental Section

Chemistry. Mass spectra, infrared spectra, optical rotations, melting points, and combustion analyses were obtained by the Physical and Analytical Chemistry Department of The Upjohn Co. ^{1}H NMR spectra were determined on a Varian CFT-20 at 80 MHz, and chemical shifts were reported as δ units relative to tetramethylsilane. ^{19}F NMR spectra were determined on a Varian T-60 at 84.67 MHz, and chemical shifts were reported as δ units relative to hexafluorobenzene as internal standard.

Thin-layer chromatography was conducted with Analtech 0.25-mm glass plates precoated with silica gel GF. For column chromatography, E. Merck silica gel 60, 230–400 mesh, was used. All solvents for chromatography were Burdick and Jackson reagent grade distilled in glass.

Tetrahydrofuran was distilled under argon from sodium metal in the presence of benzophenone. Dichloromethane was distilled from phosphorus pentoxide. Triethylamine was distilled from calcium hydride. Diethylphosphoryl cyanide was freshly distilled before use.

Peptides **15–24** were analyzed on a Perkin-Elmer Series 4 liquid chromatograph with a Kratos Spectroflow 773 detector (254 nm) and a Perkin-Elmer LCI-100 integrator using a Brownlee RP-18, 10 μm, 25 cm × 4.6 mm analytical column at a flow rate of 1.5 mL/min. The mobile phase was an isocratic mixture of 90% methanol and 10% aqueous phosphate pH 3 buffer. Preparative HPLC for the difluoro ketone peptides **22** used an isocratic mixture of 70% acetonitrile and 30% aqueous phosphate pH 3 buffer, with 1% tetrabutylammonium dihydrogen phosphate.

(*tert*-Butyloxycarbonyl)-L-leucinal (2a). To a stirred solution of 0.65 mL (7.45 mmol) of oxalyl chloride in 15 mL of dichloromethane at −78 °C under argon was added 1.1 mL (15.5 mmol) of dimethyl sulfoxide. After 15 min, a solution of 1.3 g (6.0 mmol) of (*tert*-butyloxycarbonyl)-L-leucinol (**1a**) in 15 mL of dichloromethane was added. After 15 min, 2.2 mL (15.8 mmol) of triethylamine was added and the resulting mixture allowed to warm to room temperature. Water was added to dissolve the salt, and the mixture was then partitioned between dichloromethane and saturated aqueous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The resulting residue was passed rapidly through a pad of silica gel with 1:1 ether/pentane. The filtrate was concentrated to give 1.23 g (5.71 mmol, 95%) of **2a** as an oil that was used immediately in the next reaction: ^{1}H NMR ($CDCl_3$) δ 9.58 (br s, 1 H), 1.45 (s, 9 H), 0.96 (2 d, 2 × 3 H, J = 6 Hz).

Ethyl 4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-2,2-difluoro-3(*R* and *S*)-hydroxy-6-methylheptanoates (3a and 4a): Sonicating Condition. To a suspension of 1.138 g (17.5 mmol) of activated zinc in 2 mL of tetrahydrofuran under argon in a sonicating bath was added dropwise a solution of 1.23 g (5.71 mmol) of (*tert*-butyloxycarbonyl)-L-leucinal (**2a**) and 3.37 g (16.6 mmol) of ethyl bromodifluoroacetate in 9 mL of tetrahydrofuran. After complete addition, the resulting suspension was allowed to mix for an additional 15 min. The mixture was then partitioned between dichloromethane and aqueous 1 M $KHSO_4$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 20% ethyl acetate in hexane to give two diastereomers.

3*R* Isomer (3a): 1.05 g (3.09 mmol, 54%); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.96 (d, 3 H, J = 6 Hz), 0.97 (d, 3 H, J = 6 Hz), 1.39 (t, 3 H, J = 7 Hz), 1.46 (s, 9 H), 4.37 (q, 2 H, J = 7 Hz), 4.83 (d, 1 H, J = 10 Hz); IR (neat) 3390, 1760, 1690 cm^{-1}; $[\alpha]_D$ −11° (c 0.615, $CHCl_3$). Anal. ($C_{15}H_{27}NO_5F_2$) C, H, N.

3*S* Isomer (4a): 0.477 g (1.40 mmol, 25%); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.95 (d, 3 H, J = 6 Hz), 0.98 (d, 3 H, J = 7 Hz), 1.39 (t, 3 H, J = 7 Hz), 1.47 (s, 9 H), 4.38 (q, 2 H, J = 7 Hz), 4.71 (d, 1 H, J = 7 Hz); IR (neat) 3390, 1760, 1695 cm^{-1}; $[\alpha]_D$ −31° (c 0.646, $CHCl_3$). Anal. ($C_{15}H_{27}NO_5F_2$) C, H, N.

Ethyl 4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-2,2-difluoro-3(*R*)-hydroxy-6-methylheptanoate (3a): Refluxing Condition. To a stirred suspension of 2.21 g (33.88 mmol) of activated zinc in 17 mL of tetrahydrofuran under argon in an oil bath at 75 °C was added 203 mg (1.0 mmol) of ethyl bromodifluoroacetate. After 1 min, a solution of 2.914 g (13.55 mmol) of (*tert*-butyloxycarbonyl)-L-leucinal (**2a**) and 6.397 g (31.52 mmol) of ethyl bromodifluoroacetate in 13 mL of tetrahydrofuran was slowly added. After complete addition, the resulting mixture was allowed to reflux for an additional 30 min. The cooled reaction mixture was partitioned between dichloromethane and aqueous 1 M $KHSO_4$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel to give 2.704 g (8.0 mmol, 60%) of the (3*R*) isomer (**3a**).

Ethyl 4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-2,2-difluoro-3(*R* and *S*)-hydroxy-5-phenylpentanoates (3b and 4b). By the same procedure as in the preparation of compounds **3a** and **4a** under sonicating condition, 118 mg (1.8 mmol) of activated zinc, 180 mg (0.72 mmol) of (*tert*-butyloxycarbonyl)-L-phenylalaninal (**2b**) and 0.23 mL (1.8 mmol) of ethyl bromodifluoroacetate in 2 mL of tetrahydrofuran afforded 234 mg (0.63 mmol, 87%), after chromatography on silica gel with 40% ethyl acetate in hexane, of two diastereomers.

3*R* Isomer (3b): ^{1}H NMR ($CDCl_3$) δ 1.34 (t, 3 H, J = 7 Hz), 1.41 (s, 9 H), 4.31 (q, 2 H, J = 7 Hz), 7.28 (br s, 5 H); IR (mull) 3445, 3230, 1760, 1680 cm^{-1}; $[\alpha]_D$ −30° (c 0.984, $CHCl_3$); mp 137.0–138.6 °C (EtOAc/hexane). Anal. ($C_{18}H_{25}NO_5F_2$) C, H, N.

3*S* Isomer (4b): ^{1}H NMR ($CDCl_3$) δ 1.34 (t, 3 H, J = 7 Hz), 1.37 (s, 9 H), 4.38 (q, 2 H, J = 7 Hz), 7.28 (br s, 5 H); IR (mull) 3425, 3360, 1755, 1700 cm^{-1}; $[\alpha]_D$ −43° (c 0.84, $CHCl_3$); mp 117.2–119.0 °C (EtOAc/hexane). Anal. ($C_{18}H_{25}NO_5F_2$) C, H, N.

Ethyl 4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-5-cyclohexyl-2,2-difluoro-3(*R* and *S*)-hydroxypentanoates (3c and 4c). By the same procedure as in the preparation of compounds **3a** and **4a** under sonicating condition, 200 mg (3.06 mmol) of activated zinc, 292 mg (1.14 mmol) of (*tert*-butyloxycarbonyl)-L-cyclohexylalaninal (**2c**), and 0.39 mL (3.07 mmol) of ethyl bromodifluoroacetate in 4 mL of tetrahydrofuran afforded 423 mg (1.11 mmol, 97%), after chromatography on silica gel with 20% ethyl acetate in hexane, of two diastereomers.

3*R* Isomer (3c): ^{1}H NMR ($CDCl_3$) δ 1.37 (t, 3 H, J = 7.5 Hz), 1.45 (s, 9 H), 4.32 (q, 2 H, J = 7 Hz); IR (mull) 3400, 1770, 1690 cm^{-1}; $[\alpha]_D$ −19° (c 0.504, $CHCl_3$); mp 113.0–116.1 °C (EtOAc/hexane). Anal. ($C_{18}H_{31}NO_5F_2$) C, H, N.

3*S* Isomer (4c): ^{1}H NMR ($CDCl_3$) δ 1.37 (t, 3 H, J = 7 Hz), 1.45 (s, 9 H), 4.34 (q, 2 H, J = 7 Hz); IR (mull) 3425, 1760, 1695 cm^{-1}; $[\alpha]_D$ −40° (c 0.481, $CHCl_3$); mp 72.8–74.5 °C (EtOAc/hexane). Anal. ($C_{18}H_{31}NO_5F_2$) C, H, N.

4(*S*)-Amino-2,2-difluoro-3(*R*)-hydroxy-6-methylheptanoic Acid, γ-Lactam (5). A solution of 144.5 mg (0.426 mmol) of the ester **3a** in 15 mL of ether saturated with dry HCl was allowed to stir at room temperature for 75 min. The concentrated residue was suspended in 10 mL of tetrahydrofuran, and then 0.11 mL (0.63 mmol) of diisopropylethylamine was added. The resulting solution was heated to reflux for 3 h and then cooled. The concentrated residue was chromatographed on silica gel with ethyl acetate to give 75 mg (0.39 mmol, 91%) of the lactam **5**: ^{1}H NMR (CD_3OD) δ 0.94 (d, 3 H, J = 6 Hz), 0.97 (d, 3 H, J = 6 Hz), 3.75 (m, 1 H), 4.26 (ddd, 1 H, J = 6, 8, 12 Hz); IR (mull) 2370, 3175, 1746, 1713 cm^{-1}; $[\alpha]_D$ −40° (c 0.67, CH_3OH). Anal. ($C_8H_{17}NO_2F_2$) C, H, N.

4(*S*)-Amino-2,2-difluoro-3(*S*)-hydroxy-6-methylheptanoic Acid, γ-Lactam (6). By the same procedure used for the preparation of lactam **5**, 139.2 mg (0.41 mmol) of the ester **4a** afforded 73 mg (0.38 mmol, 92%) of the lactam **6**: ^{1}H NMR (CD_3OD) δ 0.97 (d, 3 H, J = 6 Hz), 0.98 (d, 3 H, J = 6 Hz), 3.4 (m, 1 H), 3.9 (ddd, 1 H, J = 6, 11, 12 Hz); IR (neat) 3265, 1740 cm^{-1}; $[\alpha]_D$ −55° (c 0.534, $CHCl_3$). Anal. ($C_8H_{17}NO_2F_2$) C, H, N.

(3*R*,4*S* and 3*S*,4*R*)-Ethyl 4-[(*tert*-Butyloxycarbonyl)amino]-2,2-difluoro-3-*O*-[(*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl]-6-methylheptanoates (7 and 8). A solution of 474 mg (1.4 mmol) of racemic (3*R*,4*S* and 3*S*,4*R*)-ethyl-4-[(*tert*-butyloxycarbonyl)amino]-2,2-difluoro-3-hydroxy-6-methylheptanoates (**3a** and **3a′**), 0.59 mL (3.1 mmol) of (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, and 0.45 mL of carbon tetrachloride was heated at 50 °C for 1 day. The reaction mixture was then chromatographed on silica gel with 5% ethyl acetate in hexane to give 645 mg (1.16 mmol, 83%) of the esters **7** and **8**: ^{1}H NMR ($CDCl_3$) δ 0.83 and 0.93 (2 d, 2 × 6 H,

Table IV. Characterization of Renin-Inhibitory Peptides

peptides[a]	TLC[b] R_f	HPLC[c] k'	amino acid anal. Phe	His	Ile	formula	FAB-MS calcd	found
15	0.14	8.45	1.00	0.87	0.91	$C_{40}H_{58}N_8O_7$	763.4506	763.4522
16	0.18	4.23	1.00	1.10	1.06	$C_{40}H_{56}N_8O_7$	761.4350	761.4357
17	0.34	5.82	1.00	1.01	0.97	$C_{40}H_{56}N_8O_7F_2$	799.4318	799.4305
18	0.36	6.96	1.00	1.01	0.93	$C_{40}H_{56}N_8O_7F_2$	799.4318	799.4305
19	0.45	5.74	1.00	1.01	1.16	$C_{40}H_{54}N_8O_7F_2$	797.4161	797.4169
20	0.34	6.68	1.00	1.02	0.97	$C_{43}H_{54}N_8O_7F_2$	833.4161	833.4174
21	0.42	8.55	1.00	1.03	0.97	$C_{43}H_{60}N_8O_7F_2$	839.4631	839.4607
22	0.37, 0.45	5.01, 5.74	1.00	1.02	1.01	$C_{40}H_{54}N_8O_7F_2$	797.4161	797.4193
23	0.38, 0.48	6.36, 7.03	1.00	0.97	0.95	$C_{43}H_{52}N_8O_7F_2$	831.4005	831.4016
24	0.37, 0.47	7.41, 9.31	1.00	1.09	0.96	$C_{43}H_{58}N_8O_7F_2$	837.4474	837.4474

[a] ^{1}H NMR in $CDCl_3$ at 80 MHz found consistent with structures. [b] Solvent system of 9:1 EtOAc/CH_3OH. [c] See the Experimental Section for conditions; k' is the partition ratio.

J = 6 Hz), 1.29 and 1.31 (2 t, 2 × 3 H, J = 7 Hz), 1.42 (2 s, 2 × 9 H), 3.47 and 3.59 (2 br q, 2 × 3 H), 4.25 and 4.33 (2 q, 2 × 2 H, J = 7 Hz); ^{19}F NMR ($CDCl_3$, 84.67 MHz) δ 4.65 (d, 1 F, J = 28 Hz), 4.73 (d, 1 F, J = 32.5 Hz), 5.28 (d, 1 F, J = 32.5 Hz), 5.43 (d, 1 F, J = 28 Hz), 89.9 (s, 3 F), 90.6 (s, 3 F); IR (neat): 1768, 1720 cm^{-1}; MS (FAB) [M + H]$^+$ at m/z 556.2337 (calcd 556.2333).

(3*R*,4*S*)-Ethyl 4-[(*tert*-Butyloxycarbonyl)amino]-2,2-difluoro-3-*O*-[(*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl]-6-methylheptanoates (7): (a) From Sonicating Condition. Reaction of 147 mg (0.433 mmol) of ester **3a**, 0.18 mL (0.93 mmol) of (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride, 0.14 mL (1.7 mmol) of pyridine, and a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine in 0.75 mL of carbon tetrachloride afforded 201.4 mg (0.36 mmol, 84%) of the ester 7: ^{1}H NMR ($CDCl_3$) δ 0.83 (d, 6 H, J = 6 Hz), 1.31 (t, 3 H, J = 7 Hz), 1.42 (s, 9 H), 3.59 (br q, 3 H), 4.33 (q, 2 H, J = 7 Hz); ^{19}F NMR ($CDCl_3$, 84.67 MHz) δ 4.65 (d, 1 F, J = 28 Hz), 5.43 (d, 1 F, J = 28 Hz), 90.6 (s, 3 F); IR (neat) 1768, 1720 cm^{-1}; MS (FAB) [M + H]$^+$ at m/z 556.2320 (calcd 556.2333).

(b) From Refluxing Condition. Reaction of 198.7 mg (0.585 mmol) of ester **3a**, 0.25 mL (1.3 mmol) of (*S*)-(+)-α-methoxy-α-trifluoromethyl)phenylacetyl chloride, 0.19 mL (2.3 mmol) of pyridine, and a catalytic amount of 4-(*N*,*N*-dimethylamino)-pyridine in 1 mL of carbon tetrachloride afforded 288.3 mg (0.52 mmol, 89%) of the ester 7: ^{19}F NMR spectrum indicated ca. 95:5 ratio for the signals at δ 90.6 and at 89.9; MS (FAB) [M + H$^+$] at m/z 556.2350 (calcd 556.2333).

L-Isoleucyl-2-pyridylmethylamine (10). To a stirred solution of 9.25 g (40 mmol) of (*tert*-butyloxycarbonyl)-L-isoleucine, 4.2 mL (40.7 mmol) of 2-(aminomethyl)pyridine, and 5.95 g (44 mmol) of 1-hydroxybenzotriazole in 40 mL of dichloromethane was added 8.66 g (42 mmol) of dicylohexylcarbodiimide. After stirring at room temperature for 14 h, the resulting mixture was filtered and the filtrate washed with saturated aqueous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with ethyl acetate to give 11.34 g (35.3 mmol, 88%) of *N*-(*tert*-butyloxycarbonyl)-L-isoleucyl-2-pyridylmethylamine: ^{1}H NMR ($CDCl_3$) δ 0.92 (2 d, 2 × 3 H, J = 7 Hz), 1.41 (s, 9 H), 4.0 (dd, 1 H, J = 6, 9 Hz), 4.39 (d, 2 H, J = 5 Hz), 5.11 (br d, 1 H, J = 9 Hz), 7.17 (m, 2 H), 7.63 (m, 1 H), 8.50 (br d, 1 H, J = 5 Hz).

A solution of 11.3 g (35.2 mmol) of Boc-Ile-AMP above in 25 mL of dichloromethane and 25 mL of trifluoroacetic acid was allowed to stir at room temperature for 1 h. The reaction mixture was then concentrated and the residue treated with excess aqueous $NaHCO_3$. The aqueous phase was then continuously extracted with dichloromethane for 1 day. The concentrated residue was chromatographed on silica gel with 3% methanol and 3% methanol (saturated with gaseous ammonia) in dichloromethane to give 7.76 g (35.1 mmol, 100%) of the amine **10**: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3 H, J = 7 Hz), 0.93 (d, 3 H, J = 7 Hz), 1.42 (s, 9 H), 4.05 (dd, 1 H, J = 6, 9 Hz), 4.57 (d, 2 H, J = 5 Hz), 5.12 (br d, 1 H, J = 9 Hz), 7.21 (m, 2 H), 7.65 (m, 1 H), 8.54 (br d, 1 H, J = 5 Hz).

[[4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-2,2-difluoro-3-(*R*)-hydroxy-6-methylheptanoyl]-L-isoleucyl]-2-pyridylmethylamine (11). To a stirred solution of 394.4 mg (1.16 mmol) of the ester **3a** in 2.4 mL of tetrahydrofuran was added 1.2 mL (1.2 mmol) of a 1 M aqueous NaOH. After 2 h, tetrahydrofuran was removed by evaporation and the remaining aqueous phase was diluted with 2 mL of water. The resulting solution was lyophilized to give the salt **9** as a white solid.

To a stirred solution of this salt, 385 mg (1.74 mmol) of L-isoleucyl-2-pyridylmethylamine, and 361 mg (2.67 mmol) of 1-hydroxybenzotriazole in 6 mL of dichloromethane was added 311 mg (1.51 mmol) of dicyclohexylcarbodiimide. After 2 days, the reaction mixture was filtered and the filtrate partitioned between dichloromethane and saturated aqueous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 60% ethyl acetate in hexane to give 444 mg (0.86 mmol, 74%) of compound **11**: ^{1}H NMR ($CDCl_3$) δ 0.91 (m, 12 H), 1.4 (s, 9 H), 8.44 (br d, 1 H, J = 5 Hz). MS (FAB) [M + H]$^+$ at m/z 514.

[4(*S*)-[[*N*-(*tert*-Butyloxycarbonyl)-*N*^{im}-tosyl-L-histidyl]amino]-2,2-difluoro-3(*R*)-hydroxy-6-methylheptanoyl]-L-isoleucyl-2-pyridylmethylamine (12). A solution of 157.5 mg (0.305 mmol) of compound **11** in 2 mL of dichloromethane and 2 mL of trifluoroacetic acid was allowed to stir at room temperature for 1 h. The reaction mixture was then concentrated and the residue treated with excess aqueous $NaHCO_3$. The aqueous phase was extracted with several portions of dichloromethane. The combined organic phase was dried ($MgSO_4$) and then concentrated.

To a stirred solution of the above residue, 156 mg (0.38 mmol) of (*tert*-butyloxycarbonyl)-*N*^{im}-tosyl-L-histidine, and 60 μL (0.43 mmol) of triethylamine in 2 mL of dichloromethane was added 60 μL (0.39 mmol) of diethylphosphoryl cyanide. After 15 h, the reaction mixture was partitioned between dichloromethane and saturated aqueous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 70% ethyl acetate in hexane to give 220 mg (0.273 mmol, 90%) of compound **12**: ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9 H), 2.41 (s, 3 H), 8.50 (br d, 1 H, J = 5 Hz).

[4(*S*)-[[[*N*-(*tert*-Butyloxycarbonyl)-L-phenylalanyl]-*N*^{im}-tosyl-L-histidyl]amino]-2,2-difluoro-3(*R*)-hydroxy-6-methylheptanoyl]-L-isoleucyl-2-pyridylmethylamine (13). By the same procedure as in the preparation of compound **12**, 220 mg (0.273 mmol) of compound **12** was treated with 2 mL of dichloromethane and 2 mL of trifluoroacetic acid to give the free amine. This material with 90 mg (0.34 mmol) of (*tert*-butyloxycarbonyl)-L-phenylalanine, 60 μL (0.43 mmol) of triethylamine, and 60 μL (0.39 mmol) of diethylphosphoryl cyanide in 2 mL of dichloromethane afforded 162 mg (0.17 mmol, 62%) of compound **13** after chromatography on silica gel with ethyl acetate: ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 9 H), 2.41 (s, 3 H), 8.45 (br d, 1 H, J = 5 Hz).

[4(*S*)-[[[*N*-(*tert*-Butyloxycarbonyl)-L-phenylalanyl]-L-histidyl]amino]-2,2-difluoro-3(*R*)-hydroxy-6-methylheptanoyl]-L-isoleucyl-2-pyridylmethylamine (17). A solution of 15 mg (0.016 mmol) of compound **13** and 30 mg (0.22 mmol) of 1-hydroxybenzotriazole in 0.5 mL of methanol was allowed to stir at room temperature for 14 h. The concentrated reaction mixture was then chromatographed on silica gel with 5% methanol (saturated with gaseous ammonia) in ethyl acetate to give 9 mg (0.011 mmol, 70%) of peptide **17**: ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 9 H), 8.47 (br d, 1 H, J = 5 Hz).

[4(*R* and *S*)-[[[*N*-(*tert*-Butyloxycarbonyl)-L-phenylalanyl]-*N*^{im}-tosyl-L-histidyl]amino]-2,2-difluoro-6-methyl-3-oxoheptanoyl]-L-isoleucyl-2-pyridylmethylamines (14). To a stirred solution of 30 μL (0.34 mmol) of oxalyl chloride in 0.5 mL of dichloromethane at −78 °C under argon was added 55 μL

(0.78 mmol) of dimethyl sulfoxide. After 10 min, a solution of 240 mg (0.252 mmol) of [4(*S*)-[[[(*tert*-butyloxycarbonyl)-L-phenylalanyl]-N^{im}-tosyl-L-histidyl]amino]-2,2-difluoro-3(*R*)-hydroxy-6-methylheptanoyl]-L-isoleucyl-2-pyridylmethylamine (**13**) in 2 mL of dichloromethane was added. The resulting mixture was allowed to stir at −20 °C for 60 min. It was then treated with 110 μL (0.79 mmol) of triethylamine. After 30 min, the resulting mixture was partitioned between dichloromethane and saturated aqueous $NaHCO_3$. The aqueous phase was extracted with several portions of dichloromethane. The combined organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 5% methanol in dichloromethane to give 234 mg (0.246 mmol, 97%) of compound **14** as a mixture of epimers: ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 9 H), 2.42 (s, 3 H), 8.50 (br d, 1 H, J = 5 Hz).

[4(*R* and *S*)-[[[*N*-(*tert*-Butyloxycarbonyl)-L-phenylalanyl]-L-histidyl]amino]-2,2-difluoro-6-methyl-3-oxoheptanoyl]-L-isoleucyl-2-pyridylmethylamines (22). A solution of 234 mg (0.246 mmol) of compound **14** and 100 mg (0.74 mmol) of 1-hydroxybenzotriazole in 2 mL of methanol was allowed to stir at room temperature for 15 h. The concentrated reaction mixture was chromatographed on silica gel with 5% methanol (saturated with gaseous ammonia) in dichloromethane to give 180 mg (0.23 mmol, 92%) of peptide **22** as a mixture of C-4 epimers: ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 9 H), 8.45 (br d, 1 H, J = 5 Hz).

Other peptides reported here were synthesized by the same sequence of reactions utilizing different statine analogues as required. Physical characteristics for the final products **15–24** are listed in Table IV.

Crystallography for the γ-Lactam 5: $C_8H_{13}NO_2F_2$; M_r 193.19; monoclinic; $P2_1$; a = 5.995 (1), b = 10.676 (1), c = 7.590 (1) Å; β = 96.30 (1)°; V = 482.8 (2) Å^3; Z = 2; D_{calcd} = 1.33 g/cm^3; Cu Kα,λ = 1.5418 Å, μ(Cu Kα) = 0.9 cm^{-1}; T = 123 K; R = 0.031 for 886 unique reflections.

A clear, chunky prism of dimensions 0.23 × 0.26 × 0.31 mm was used for intensity measurements on a Syntex P1 diffractometer controlled by a Harris computer. Cu Kα radiation and a graphite monochromator were used for intensity measurement. The step-scan technique was used with a scan speed of 4°/min, a scan width of 3.4°, and $2\theta_{max}$ = 136°. Ten reflections periodically monitored showed no loss of intensity during the data collection. Of the 886 unique reflections measured, 864 had intensities greater than 3σ. Standard deviations in the intensities were approximated by the equation $\sigma^2(I) = \sigma^2(I)_{count\ stat} + (0.007I)^2$, where the coefficient of I was calculated from the variations in intensities of the monitored reflections. Unit cell parameters were determined accurately by least-squares fit of Cu $K\alpha_1$ 2θ values (λ(Cu $K\alpha_1$) = 1.5402 Å) for 25 high-2θ reflections.[23] *Lp* corrections appropriate for a monochromator with 50% perfect character were applied with no absorption correction. The structure was solved by direct methods, using MULTAN80.[24] Hydrogen atoms found in difference maps were very close to positions generated from planar or tetrahedral geometry, so generated positions were used. The structure was refined by least squares with the coordinates and anisotropic thermal parameters for non-hydrogen atoms included in the refinement. Hydrogen parameters were included in the calculations but not refined. Isotropic thermal parameters for hydrogen atoms were set $^1/_2$ unit higher than the isotropic equivalent of the thermal parameters of the attached heavier atom. The function minimized in the refinement was $\sum w(F_o^2 - F_c^2)^2$, where weights w were $1/\sigma^2(F_o^2)$. Atomic form factors were from Doyle and Turner,[25] except for hydrogen which was from Stewart, Davidson, and Simpson.[26] In the final refinement cycle, all shifts were <0.35σ. The final R was 0.031, and the standard deviation of fit was 4.13. A final difference map showed no peaks >0.3 e Å^{-3}. The CRYM system of computer programs[27] was used.

Biology. Inhibition of Human Plasma Renin. Compounds **15–24** were assayed for plasma renin inhibitory activity as follows: Lyophilized human plasma with 0.1% EDTA was obtained commercially (New England Nuclear). The angiotensin I generation step utilized 250 μL of plasma, 2.5 μL of phenylmethanesulfonyl fluoride, 25 μL of maleate buffer (pH 6.0), and 10 μL of an appropriate concentration of inhibitor in a 1% Tween 80 in water vehicle. Incubation was for 90 min at 37 °C. Radioimmunoassay for angiotensin I was carried out with a commercial kit (Clinical Assays). Plasma renin activity values for inhibitor tubes were compared to control tubes to estimate percent inhibition. The inhibition results were expressed as IC_{50} values that were obtained by plotting three to four inhibitor concentrations on semilog graph paper and estimating the concentration producing 50% inhibition.

Protease Specificity. Inhibition of pepsin was determined as described[28] with porcine pepsin (Sigma), porcine hemoglobin (Sigma), and 0.02 M KCl–HCl buffer (pH 2). The compounds were dissolved in the buffer, and after incubation for 25 min at 37 °C with the enzyme and substrate, perchloric acid was added and the absorbance of the acid-soluble fractions measured at 280 nm. The percent inhibition was estimated from the net absorbance of inhibited assays in relation to uninhibited control assays. A plot of percent inhibition vs. log inhibitor concentration was constructed with the IC_{50} defined as the inhibitor concentration causing 50% inhibition.

Bovine cathepsin D (Sigma) inhibition was measured via a slightly modified literature procedure.[29] The compounds were dissolved in 0.2 M acetate buffer (pH 3.2) as was the porcine hemoglobin (Sigma) substrate. Incubations for 30 min at 37 °C were terminated in ice, and 1.7 M perchloric acid was added to all mixtures. Extinction at 280 nm was measured and the IC_{50} determined as above.

Inhibition of angiotensin II converting enzyme activity was estimated as described in the literature.[30] The enzyme was extracted from rabbit lung. The substrate was hippuryl-L-histidyl-L-leucine (Aldrich), and the buffer was K_2HPO_4 (pH 8.3). The compounds were dissolved in 50% dimethylformamide. Incubations were for 60 min at 37 °C and were terminated via HCl addition. Absorbance of the ethyl acetate extracted hippuric acid was measured at 228 nm. The IC_{50} was calculated as above.

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Registry No. **1a**, 82010-31-9; **1b**, 66605-57-0; **1c**, 103322-56-1; **2a**, 58521-45-2; **2b**, 72155-45-4; **2c**, 98105-42-1; **3a**, 96056-65-4; (±)-**3a**, 103420-33-3; **3b**, 103322-57-2; **3c**, 103322-58-3; **4a**, 97920-08-6; **4b**, 103322-59-4; **4c**, 103420-29-7; **5**, 103322-60-7; **6**, 103322-61-8; **7**, 103322-62-9; **8**, 103420-30-0; **9**, 103322-63-0; **10**, 97920-16-6; **11**, 97920-09-7; **12**, 103346-52-7; **13**, 97920-10-0; (4*R*)-**14**, 97995-54-5; (4*S*)-**14**, 97920-15-5; **15**, 97920-11-1; **16**, 97920-13-3; **17**, 97920-12-2; **18**, 97995-52-3; **19**, 97920-14-4; **20**, 103322-64-1; **21**, 103322-65-2; (4*R*)-**22**, 97995-53-4; (4*R*)-**23**, 103322-66-3; (4*S*)-**23**, 103420-31-1; (4*R*)-**24**, 103322-67-4; (4*S*)-**24**, 103420-32-2; ACE, 9015-82-1; ethyl bromodifluoroacetate, 667-27-6; (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, 20445-33-4; (*tert*-butyloxycarbonyl)-L-isoleucine, 13139-16-7; 2-(aminomethyl)-pyridine, 3731-51-9; *N*-(*tert*-butyloxycarbonyl)-L-isoleucyl-2-pyridylmethylamine, 103322-68-5; (*tert*-butyloxycarbonyl)-N^{im}-tosyl-L-histidine, 35899-43-5; (*tert*-butyloxycarbonyl)-L-phenylalanine, 13734-34-4; renin, 9015-94-5; pepsin, 9001-75-6; cathepsin D, 9025-26-7.

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