synthesis of triazine 41 (Table III) by the general procedure.

Physicochemical Constants. The π constants of all of these substituents have been well documented[12] while others have been calculated.[19] MR values are scaled by 0.1 to keep them roughly equiscalar with π values. Indicator variables are defined as follows: I_{OR} = 1 for alkoxy derivatives, e.g., 3-OCH_3, and 0 for all others.

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Novel Renin Inhibitors Containing Analogues of Statine Retro-Inverted at the C-Termini: Specificity at the P_2 Histidine Site[1,2]

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Substituted 1,3- and 1,4-diamines were prepared from epoxides derived from Boc-leucine or Boc-cyclohexylalanine. These diamines were incorporated into renin inhibitors (IC_{50} = 4–1500 nM) replacing the Leu-Val scissile bond in small peptide analogues of angiotensinogen. Replacement of the P_2 histidine imidazole with other heterocycles maintained or enhanced binding while changing the overall basicity of the inhibitor. Finally, substitution of *O*-methyltyrosine for the P_3 phenylalanine suppressed chymotrypsin cleavage of the P_3–P_2 bond.

Renin is a proteolytic enzyme that selectively cleaves its substrate angiotensinogen to begin the cascade that produces the potent pressor octapeptide angiotensin II. The basic strategy behind the design of renin inhibitors has been to modify the Leu-Val scissile bond in an analogue of the natural substrate angiotensinogen in order to maintain binding while preventing proteolytic cleavage. One approach has been to replace this bond with a hydroxyethylene isostere.[3] This modification not only suppresses proteolysis but also enhances binding by mimicking the presumed transition state for cleavage.[4,5] It has been discovered that the unusual γ-amino acid statine is a suitable replacement for the Leu-Val hydroxyethylene isostere even though it is one atom shorter and lacks the valine side chain.[6]

In this paper we describe novel renin inhibitors based upon statine and the one carbon extended analogue homostatine that have been retro-inverted at the C-terminus. We also have examined several amino acids as replacements for the P_2 histidine and have determined some of the stereoelectronic requirements of this site. Finally, a modification that prevents chymotrypsin cleavage between the P_2 and P_3 residues is disclosed.

Results

Synthesis: Diamines and Amino Nitriles Syntheses of the monoprotected diamines **3a** and **3b**, which are precursors to structures related to statine retro-inverted at the C-terminus, are shown in Scheme I. Treatment of epoxide **1a** (96% ee, 15:1 2*R*:2*S*)[7] with sodium azide and isolation of the major product afforded (2*S*,3*S*)-azido alcohol **2a**, which was hydrogenated over palladium on carbon to provide **3a**. Similarly, epoxide **1b** (94% ee, >10:1 2*R*:2*S*)[7] afforded the monoprotected diamine **3b**.

The synthesis of the homologous (3*S*,4*S*)-1-amino-3-hydroxy-4-[(*tert*-butyloxycarbonyl)amino]-5-cyclohexylpentane (**6**) began with Boc-cyclohexylalanal (**4**) as shown in Scheme I.

Condensation of this aldehyde with the lithium enolate of acetonitrile afforded a readily separable mixture of the (3*S*,4*S*)- and (3*R*,4*S*)-hydroxynitriles **5** in a 3:2 ratio. Alternately, epoxide **1b**[7] could be opened with cyanide to provide as the major product the 3*S* isomer **5a**, thereby establishing the stereochemistry of the products derived from the enolate condensation. Hydrogenation of nitrile **5a** over Raney Ni provided the monoprotected diamine **6**.

The optical purity of nitrile **5b** was established by the preparation of diastereomeric derivatives. Deprotection of **5b** with 4 M HCl/dioxane followed by coupling with both (+)- and (−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride[8] provided the diastereomeric amides. Analysis by proton NMR indicated that each diastereomer was contaminated with 3.0% of the other, demonstrating that the material prepared by the acetonitrile enolate condensation had 94% ee.

Synthesis: Extension at N-1. Monoprotected diamines **3a,b** and **6** were acylated with short-chain aliphatic acids designed to mimic the P_2' isoleucine side chain of human angiotensinogen (Scheme II). Amine **6** was also reductively alkylated with isovaleraldehyde and epoxide **1b** was opened with isoamylamine to provide examples of reduced amides in these series. The secondary amines

(1) Presented in part at the 191st National Meeting April 1986.
(2) Abbreviations follow IUPAC–IUB Joint Commission on Biochemical Nomenclature for amino acids and peptides (*Eur. J. Biochem.* **1984**, *158*, 9–31). Additional abbreviations used are as follows: THF, tetrahydrofuran; DMF, dimethylformamide; TFA, trifluoroacetic acid; Boc, *tert*-butyloxycarbonyl; Cbz, benzyloxycarbonyl; Me_2SO, dimethyl sulfoxide; BSA, bovine serum albumin; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethanesulfonyl fluoride.
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Scheme I. Synthesis of Substituted 1,3- and 1,4-Diamines

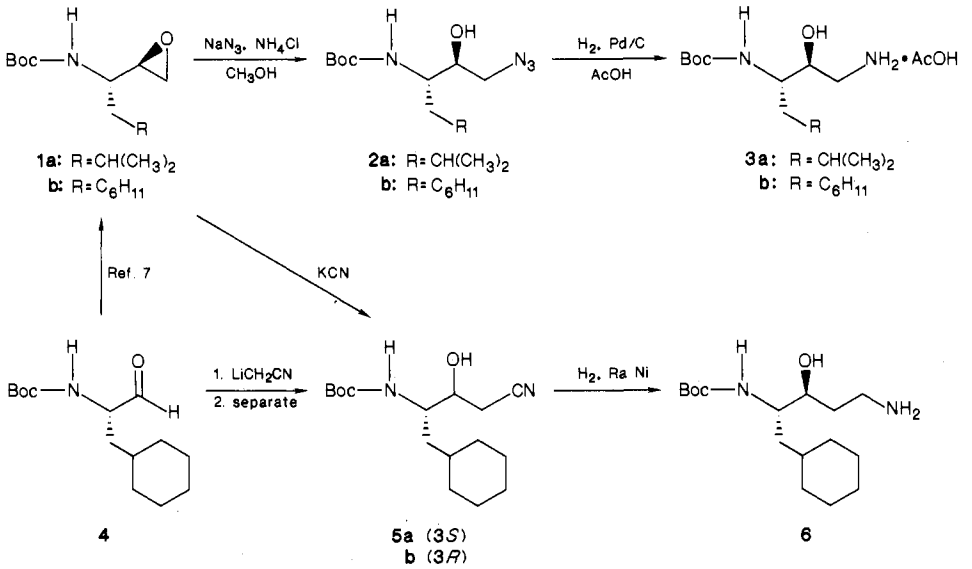
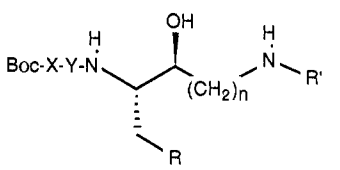


Table I. Renin Inhibition by Compounds Containing Substituted 1,3- and 1,4-Diamines



no.	X	Y	R	*n*	R′	IC_{50},[a] nM
16	Phe	His	$CH(CH_3)_2$	1	$COCH_2CH(CH_3)_2$	600
17	Phe	His	C_6H_{11}[b]	1	$COCH_2CH(CH_3)_2$	50
18	Phe	His	C_6H_{11}	1	$CO(CH_2)_2CH(CH_3)_2$	35
19	Phe	His	C_6H_{11}	2	$COCH_2CH(CH_3)_2$	20
20[c]	Phe	Ala	C_6H_{11}	2	$CO(CH_2)_2CH(CH_3)_2$	30
21	Phe	Phe	C_6H_{11}	2	$CO(CH_2)_2CH(CH_3)_2$	15
22[c]	Phe	His	C_6H_{11}	2	$CO(CH_2)_2CH(CH_3)_2$	15
23	Phe	(τ-*N*-Me)His	C_6H_{11}	2	$CO(CH_2)_2CH(CH_3)_2$	15
24	Phe	(π-*N*-Me)His	C_6H_{11}	2	$CO(CH_2)_2CH(CH_3)_2$	100
25[c]	Phe	*dl*-(4-thiazolyl)Ala	C_6H_{11}	2	$CO(CH_2)_2CH(CH_3)_2$	10
26	Phe	*dl*-(3-pyrazolyl)Ala	C_6H_{11}	2	$CO(CH_2)_2CH(CH_3)_2$	6
27	Phe	*dl*-(4-pyrimidinyl)Ala	C_6H_{11}	2	$CO(CH_2)_2CH(CH_3)_2$	20
28	(*O*-Me)Tyr	His	C_6H_{11}	2	$CO(CH_2)_2CH(CH_3)_2$	4
29	Phe	His	C_6H_{11}	2	$COCH_2N(CH_3)_2$	100
30	Phe	His	C_6H_{11}	1	$(CH_2)_2CH(CH_3)_2$	900
31	Phe	His	C_6H_{11}	2	$(CH_2)_2CH(CH_3)_2$	1500
32	Boc-Phe-His-Sta-$NHCH_2CH(CH_3)_2$[d,e]					500
33	Boc-Phe-His-ACHPA-$NH(CH_2)_2CH(CH_3)_2$[d,f]					5
45	pepstatin					3200

[a] Single determination. [b] Cyclohexyl. [c] 0% inhibition against bovine cathepsin D and porcine pepsin at 10^{-5} M. [d] See ref 9. [e] Sta, statine, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylhexanoic acid. [f] AHCPA, (3*S*,4*S*)-4-amino-3-hydroxy-5-cyclohexylpentanoic acid; see ref 10.

were protected (Cbz) prior to further manipulation.

Synthesis: Extension at N-3(4) (N-Terminus). Final synthesis of the renin inhibitors listed in Table I was accomplished by using standard solution methodology. In most cases a single coupling with a preformed dipeptide provided the final product, but some inhibitors were prepared by the sequential coupling of two amino acid residues. Peptides were purified by silica gel chromatography.

Discussion

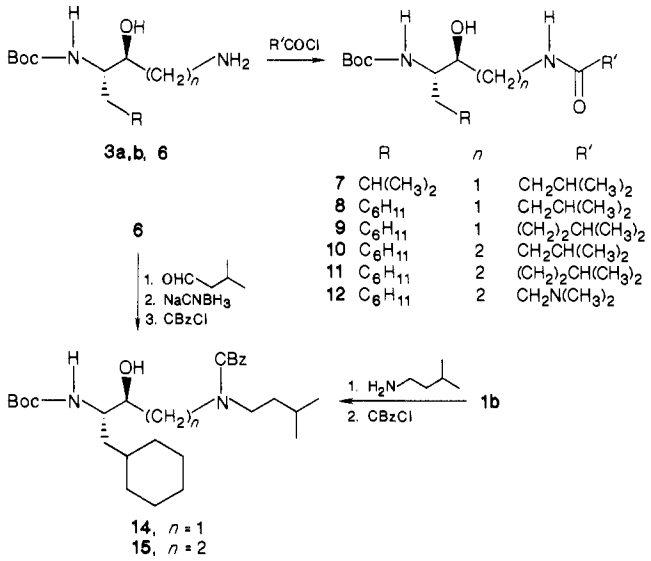
The inhibitory potencies for the compounds in the retro-inverted statine series are shown in Table I. Comparison with the parent statine containing compounds (**16** vs. **32**[9]) indicates that interchanging the amide nitrogen and carbonyl does not affect binding. It has been demonstrated independently by our group and others[10,11] that replacement of the isopropyl portion of the statine side chain with a cyclohexyl group significantly enhances activity. The corresponding change in the retro-inverted statine compounds increased binding 10-fold (**17**, **18**); however, those compounds are now less active than the non-retro-inverted structures (**18** vs. **33**[9]).

The compounds that are homologues of the above series are related to homostatine retro-inverted at the C-terminus. This modification places the "C-terminal" nitrogen into the same position as in statine amide-containing renin

(9) Sham, H., unpublished results.

(10) 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. *J. Med. Chem.* **1985**, *28*, 1779.
(11) Dellaria, J. D., unpublished results.

Scheme II. Conversion of Diamines **3** and **6** into Renin Inhibitor C-Terminal Fragments



inhibitors and results in a doubling of potency (**19** vs. **17**, **22** vs. **18**).

As a potential means of controlling the pharmacokinetic properties of these inhibitors, we sought to make changes that would vary the overall basicity of the molecules without affecting in vitro activity. We first attempted to increase the inhibitor pK_a by incorporating a basic nitrogen into the inhibitor either as part of the C-terminal end group (**29**) or as part of the backbone of the molecule (**30**, **31**). In each case, however, we observed a significant loss in activity.

A more successful approach was to vary the amino acid occupying the P_2 histidine site. First, we briefly examined the steric requirements of this position. We found that methylation of the histidine τ-nitrogen preserved activity while the corresponding (π-*N*-methyl)histidine derivative was 7-fold less potent. We attribute this to steric interactions between the π-*N*-methyl and the peptide backbone that force the imidazole ring into a conformation unfavorable for binding. Incorporating the larger phenylalanine residue into the P_2 site provided an inhibitor that was equipotent with the parent histidine-containing compound. Thus not only are the steric requirements of this site lenient but neither the basicity nor the hydrogen-bonding capabilities of the imidazole ring are important for binding. We were confident, therefore, that the imidazole ring could be replaced by other less basic heterocycles without a loss in activity and this indeed proved to be true (**25**–**27**). In each case potency was preserved or actually increased to provide nanomolar inhibitors as exemplified by the pyrazole- and thiazole-containing compounds. The pK_a's of the parent heterocycles range from 1.3 to 2.5[12] (pK_a of imidazole = 7.2[12]) and thus these inhibitors will be neutral and uncharged in all parts of the body except the stomach, yet they are sufficiently basic that they can be formulated as salts of strong acids.

In order to determine whether the basicity of the amino acid occupying the P_2 histidine site affected the specificity of these renin inhibitors, **20**, **22**, and **25** were tested against bovine cathepsin D and porcine pepsin. No inhibition was observed at 10^{-5} M.

One of the main reasons for poor oral absorption of many drug molecules is extensive degradation in the intestine. The Phe-His amide bond in **22** was demonstrated to be cleaved in vitro by chymotrypsin ($t_{1/2}$ = 4 min). Since this enzyme is present in the intestinal tract, stability toward chymotrypsin may be a necessary condition for good oral absorption. Examination of the published specificity requirements for chymotrypsin (esterase activity) revealed that a critical interaction for efficient binding of a substrate to this enzyme involves a van der Waals interaction of the phenylalanine benzyl side chain with a hydrophobic pocket in the active site.[13] While this site will accept a phenylalanine or tyrosine side chain, *O*-alkylated tyrosine derivatives do not bind to chymotrypsin presumably because the side chains are too bulky to fit the active site.

Reasoning that the smallest of the alkylated tyrosines might still fit the renin active site, we replaced phenylalanine with *O*-methyltyrosine (**28**). Gratifyingly, this compound was a 4 nM renin inhibitor, 4 times more active than the parent phenylalanine-containing material (**22**). Furthermore, **28** was completely stable in the in vitro chymotrypsin assay (4-h incubation).

In conclusion, we have developed a series of small nanomolar renin inhibitors based upon novel diamines related to statine and homostatine retro-inverted at the C-terminus. By changing the residue occupying the P_2 histidine site, we have been able to modulate the basicity of these inhibitors. Finally, replacing the P_3 phenylalanine with *O*-methyltyrosine stabilized the P_2–P_3 bond to chymotrypsin cleavage in vitro.

Experimental Section

Solvents and other reagents were reagent grade and used without further purification. Final product solutions were dried over anhydrous Na_2SO_4 prior to evaporation on a rotary evaporator. Tetrahydrofuran was distilled from sodium/benzophenone ketal and methylene chloride was distilled from P_2O_5. L-Boc-(π-*N*-methyl)histidine, L-Boc-(τ-*N*-methyl)histidine, and Boc-*O*-methyltyrosine were purchased from Bachem. NMR spectra were recorded at 300 MHz and are expressed in ppm downfield from tetramethylsilane as an internal standard. Column chromatography was performed on silica gel 60, 0.04–0.063 mm (E. Merck), eluting with 5–10 psi air pressure. Thin-layer chromatography was done on silica gel plates (E. Merck), and components were visualized with ninhydrin or phosphomolybdic acid reagents. The following systems were used: 20% ethyl acetate/80% hexane (I); 50% ethyl acetate/50% hexane (II); 25% ethyl acetate/25% water/25% 1-butanol/25% acetic acid (III); and 10% methanol/90% chloroform (IV).

(2*S*,3*S*)-1-Azido-2-hydroxy-3-[(*tert*-butyloxycarbonyl)amino]-4-cyclohexylbutane (2b). Epoxide **1b**[7] (600.0 mg, 2.23 mmol), sodium azide (350 mg, 5.40 mmol), and ammonium chloride (210 mg, 3.90 mmol) in methanol (10 mL) were heated at reflux for 16 h and then cooled and concentrated in vacuo. The residue was diluted with ethyl acetate, washed with water and brine, and then dried and evaporated. Chromatography on silica gel (70 g) with 15% ethyl acetate in hexane afforded 399 mg (57%) of the desired 2*S*,3*S* isomer followed by traces of the 2*R*,3*S* isomer.

2*S*,3*S* isomer: TLC, R_f 0.26 (I); ^{1}H NMR ($CDCl_3$) δ 4.63 (1 H, NH, br d), 3.68 (2 H, m), 3.38 (2 H, CH_2N_3, m), 1.45 (9 H, s), 1.9–0.8 (13 H, m). Anal. ($C_{15}H_{28}N_4O_3$) C, H, N.

2*R*,3*S* isomer: TLC, R_f 0.22 (I); ^{1}H NMR ($CDCl_3$) δ 4.54 (1 H, NH, br d), 3.75 (2 H, m), 3.33 (2 H, CH_2N_3, m).

(2*S*,3*S*)-1-Azido-2-hydroxy-3-[(*tert*-butyloxycarbonyl)amino]-5-methylhexane (2a): prepared (4.90 g, 82%) from epoxide **1a** according to the procedure for compound **2**, mp 50–52 °C. Anal. ($C_{12}H_{24}N_4O_3$) C, H; N: calcd, 20.57; found, 20.07.

(2*S*,3*S*)-1-Amino-2-hydroxy-3-[(*tert*-butyloxycarbonyl)amino]-4-cyclohexylbutane Acetic Acid Salt (3b). Azide **2b** (345 mg, 1.10 mmol) and 10% palladium on carbon (300 mg) in acetic acid (10 mL) were stirred under a hydrogen atmosphere

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(13) Kundu, N.; Roy, S.; Maenza, F. *Eur. J. Biochem.* **1972**, *28*, 311.

for 9 h. The reaction was filtered and evaporated to yield 334 mg (87%). This material was used without further purification: ^{1}H NMR ($CDCl_3$) δ 2.85 (2 H, CH_2N, m).

(2*S*,3*S*)-1-Amino-2-hydroxy-3-[(*tert*-butyloxycarbonyl)amino]-5-methylhexane Acetic Acid Salt (3a): prepared (1.10 g, 97%) from azide **2a** according to the procedure for compound 3b; EI-MS, M^+ at 240.

(3*S*,4*S*)-3-Hydroxy-4-[(*tert*-butyloxycarbonyl)amino]-5-cyclohexylpentanenitrile (5a). To lithium diisopropylamide (4.4 mmol) in THF (4 mL) at −78 °C was added acetonitrile (0.25 mL, 4.4 mmol). After 10 min Boc-cyclohexylalanal[7,10] (0.76 g, 3.0 mmol) in THF (5 mL) was added, and the reaction mixture was stirred for 15 min and quenched with 0.5 M H_3PO_4 (25 mL). The mixture was extracted with ethyl acetate, which was dried and evaporated. Chromatography of the resisue on silica gel with 20% ethyl acetate in hexane afforded 0.362 g (41%) of 3*S*,4*S* isomer **5a** followed by 0.150 g (17%) of 3*R*,4*S* isomer **5b** plus 0.201 g of mixed fractions.

5a: mp 71–74 °C; TLC, R_f 0.57 (II); ^{1}H NMR ($CDCl_3$) δ 2.61 (1 H, CH_2CN, dd), 2.52 (1 H, CH_2CN, dd). Anal. ($C_{16}H_{28}N_2O_3 \cdot 0.4H_2O$) C, H; N: calcd, 9.23; found, 8.81.

5b: TLC, R_f 0.53 (II); ^{1}H NMR ($CDCl_3$) δ 2.51 (2 H, CH_2CN, d).

B. From Epoxide 1b. Epoxide 1b[7] (2.9503 g, 10.95 mmol) in methanol (60 mL) was treated with a solution of KCN (2.00 g, 30.7 mmol) in water (20 mL). The mixture was stirred at room temperature for 18 h, concentrated in vacuo to 20 mL, partitioned between ethyl acetate and saturated $NaHCO_3$ solution, and then washed with saturated $NaHCO_3$ solution. The organic phase was dried, evaporated, and chromatographed on silica gel with 20% ethyl acetate in hexane to afford 2.1124 g (65%) of **5a** followed by 0.1136 g (4%) of **5b**.

(3*S*,4*S*)-1-Amino-3-hydroxy-4-[(*tert*-butyloxycarbonyl)amino]-5-cyclohexylpentane (6). Nitrile **5a** (2.11 g, 7.12 mmol) and Raney Ni (0.50 g) were hydrogenated at 3 atm in methanol (70 mL) and ammonia (10 mL). The mixture was filtered, evaporated, dissolved in ethyl acetate, and extracted into 0.5 M H_3PO_4. The aqueous phase was made basic with solid K_2CO_3 and extracted with 25% 2-propanol in chloroform, which was dried and evaporated to afford 2.03 g (95%) of a foam: TLC, R_f 0.79 (III); $[\alpha]^{23}_D$ −35.4° (c 1, EtOH); ^{1}H NMR ($CDCl_3$) δ 4.81 (1 H, d), 3.83 (1 H, m), 3.60 (1 H, m), 3.23 (1 H, m), 2.88 (1 H, m), 2.35 (2 H, br m), 1.95–0.75 (16 H, m), 1.45 (9 H, s); exact mass ($C_{16}H_{32}N_2O_3$) calcd 300.2411, found 300.2439.

(3*S*,4*S*)-1-[[(3-Methylbutyl)carbonyl]amino]-3-hydroxy-4-[(*tert*-butyloxycarbonyl)amino]-5-cyclohexylpentane (11). Amine **6** (496.2 mg, 1.65 mmol) in CH_2Cl_2 (10 mL) at 0 °C was treated with triethylamine (0.40 mL, 2.8 mmol) and isocaproyl chloride (312.1 mg, 2.32 mmol). The mixture was stirred at 0 °C for 1 h, then evaporated, dissolved in methanol (5 mL), and treated with 1 M NaOH (5 mL). After 1 h at room temperature the mixture was diluted with ethyl acetate, washed with 0.5 M H_3PO_4, saturated $NaHCO_3$ solution, and brine, then dried, and evaporated to afford 602.2 mg (91%) of an oil: TLC, R_f 0.50 (IV). Anal. ($C_{22}H_{42}N_2O_4 \cdot 0.25H_2O$) C, H, N.

(2*S*,3*S*)-1-[[(2-Methylpropyl)carbonyl]amino]-2-hydroxy-3-[(*tert*-butyloxycarbonyl)amino]-5-methylhexane (7): prepared from amine **3a** (109.8 mg, 90%) according to the procedure for compound 11. Anal. ($C_{17}H_{34}N_2O_4 \cdot 0.5H_2O$) H, N; C: calcd, 60.15; found, 60.58.

(2*S*,3*S*)-1-[[(2-Methylpropyl)carbonyl]amino]-2-hydroxy-3-[(*tert*-butyloxycarbonyl)amino]-4-cyclohexylbutane (8): prepared from amine **3b** (104.6 mg, 96%) according to the procedure for compound 11; TLC, R_f 0.52 (IV); ^{1}H NMR ($CDCl_3$) δ 6.42 (1 H, RCONH, br t), 4.67 (1 H, Boc-NH, d), 2.08 (2 H, CH_2CO, m), 1.45 (9 H, s), 0.96 (6 H, d); EI-MS, M^+ at 370.

(2*S*,3*S*)-1-[[(3-Methylbutyl)carbonyl]amino]-2-hydroxy-3-[(*tert*-butyloxycarbonyl)amino]-4-cyclohexylbutane (9): prepared from amine **3b** (107.4 mg, 96%) according to the procedure for compound 11; TLC, R_f 0.52 (IV); ^{1}H NMR ($CDCl_3$) δ 6.42 (1 H, RCONH, br t), 4.67 (1 H, Boc-NH, d), 2.22 (2 H, CH_2CO, m), 1.45 (9 H, s), 0.90 (6 H, d); EI-MS, M^+ at 384.

(3*S*,4*S*)-1-[[(2-Methylpropyl)carbonyl]amino]-3-hydroxy-4-[(*tert*-butyloxycarbonyl)amino]-5-cyclohexylpentane (10): prepared from amine **6** (36.1 mg, 91%) according to the procedure for compound 11; TLC, R_f 0.46 (IV). Anal. ($C_{21}H_{40}N_2O_4 \cdot 1.75H_2O$) C, H, N.

(3*S*,4*S*)-1-[[[(Dimethylamino)methyl]carbonyl]amino]-3-hydroxy-4-[(*tert*-butyloxycarbonyl)amino]-5-cyclohexylpentane (12): prepared from amine **6** (59.6 mg, 46%) according to the procedure for compound 11; TLC, R_f 0.16 (IV). Anal. ($C_{20}H_{39}N_3O_4 \cdot 0.25H_2O$) C, H, N.

(2*S*,3*S*)-1-[(3-Methylbutyl)(benzyloxycarbonyl)amino]-2-hydroxy-3-[(*tert*-butyloxycarbonyl)amino]-4-cyclohexylbutane (14). Epoxide 1b[7] (189.0 mg, 0.702 mmol) was stirred for 24 h in isoamylamine (0.8 mL). The mixture was evaporated and the residue was dissolved in methylene chloride (5 mL) and cooled to 0 °C. After addition of benzyl chloroformate (0.100 mL, 0.70 mmol) and triethylamine (0.12 mL, 0.16 mmol), the reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 30 min, diluted with ethyl acetate, washed with 0.5 M H_3PO_4 saturated $NaHCO_3$ solution and brine, then dried, and evaporated. Chromatography of the residue on silica gel afforded 134.6 mg (40%) as an oil: TLC, R_f 0.26 (0.20 for 2*S*,3*R* isomer, I). Anal. ($C_{28}H_{46}N_2O_5$) C, H. N.

(3*S*,4*S*)-1-[(3-Methylbutyl)(benzyloxycarbonyl)amino]-3-hydroxy-4-[(*tert*-butyloxycarbonyl)amino]-5-cyclohexylpentane (15). Amine **6** (245.6 mg, 0.847 mmol) in THF (4 mL) was treated with 5A molecular sieves (0.85 g, freshly activated) and isovaleraldehyde (91 μL, 0.85 mmol). After being allowed to stand for 24 h, the mixture was cooled to 0 °C, treated with $NaCNBH_3$ (64.0 mg, 1.02 mmol), stirred for 30 min, diluted with CH_2Cl_2 (30 mL), filtered, and evaporated. Chromatography of the residue on silica gel with 3% methanol in chloroform afforded 148.1 mg (47%, 0.400 mmol) of product as an oil: ^{1}H NMR ($CDCl_3$) δ 4.81 (1 H, Boc-NH, br d), 3.82 (1 H, m), 3.56 (1 H, m), 3.23 (2 H, m), 3.00 (2 H, m), 1.46 (9 H, s), 0.96 (6 H, d). This amine was dissolved in CH_2Cl_2 (5 mL), cooled to 0 °C, and then treated with benzyl chloroformate (63 μL, 0.44 mmol) and triethylamine (70 μL, 0.50 mmol). After 30 min at 0 °C and 45 min at room temperature, the mixture was diluted with ethyl acetate, washed with 0.5 M H_3PO_4, saturated $NaHCO_3$ solution and brine, then dried, and evaporated. Chromatography of the residue on silica gel with 20% ethyl acetate in hexane afforded 145.3 mg (72%) of an oil: TLC, R_f 0.20 (I). Anal. ($C_{29}H_{48}N_2O_5$) C, H, N.

General Procedure for Peptide Coupling. The Boc-protected C-terminal fragment (1 equiv) was stirred for 1 h at ambient temperature in 4 M HCl/dioxane. The mixture was evaporated with ether chasers and the residue was dissolved in DMF (final concentration 50–100 mM). To this solution was added *N*-methylmorpholine (2 equiv), 1-hydroxybenzotriazole monohydrate (3 equiv), and the appropriate N-protected amino acid or dipeptide (1 equiv). The solution was cooled to −23 °C and treated with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (1 equiv). After 2 h the mixture was warmed to room temperature, stirred for 12 h, then poured into saturated $NaHCO_3$ solution, and extracted into ethyl acetate. The organic phase was washed with water and brine and then dried and evaporated. Chromatography on silica gel with 2–5% methanol in chloroform afforded the pure products (Table II, vida infra).

Intermediates Prepared by the Above Protocol. Boc-(π-*N*-methyl)His Amide of (3*S*,4*S*)-1-[[(3-Methylbutyl)carbonyl]amino]-3-hydroxy-4-amino-5-cyclohexylpentane (34). Anal. ($C_{29}H_{51}N_5O_5 \cdot 0.5H_2O$) C, H, N.

Boc-(τ-*N*-methyl)His Amide of (3*S*,4*S*)-1-[[(3-Methylbutyl)carbonyl]amino]-3-hydroxy-4-amino-5-cyclohexylpentane (35). Exact mass calcd for $C_{29}H_{51}N_5O_5$ 549.3887, found 549.3917.

Boc-*dl*-(4-pyrimidinyl)Ala Amide of (3*S*,4*S*)-1-[[(3-Methylbutyl)carbonyl]amino]-3-hydroxy-4-amino-5-cyclohexylpentane (36). Anal. ($C_{29}H_{49}N_5O_5 \cdot 0.75H_2O$) H, N; C: calcd, 62.09; found, 62.52.

Boc-Phe-His Amide of (2*S*,3*S*)-1-[(3-Methylbutyl)(benzyloxycarbonyl)amino]-2-hydroxy-3-amino-4-cyclohexylbutane (37). Anal. ($C_{43}H_{62}N_6O_7 \cdot 0.5H_2O$) C, H, N.

Boc-Phe-His Amide of (3*S*,4*S*)-1-[(3-Methylbutyl)(benzyloxycarbonyl)amino]-3-hydroxy-4-amino-5-cyclohexylpentane (38). Anal. ($C_{44}H_{64}N_6O_7 \cdot 0.5H_2O$) C, H, N.

Boc-His Amide of (3*S*,4*S*)-1-[[(3-Methylbutyl)carbonyl]amino]-3-hydroxy-4-amino-5-cyclohexylpentane (39). ^{1}H NMR ($CDCl_3$) δ 7.58 (1 H, im-CH, s), 6.84 (1 H, im-CH,

Table II. Physical Data for Renin-Inhibiting Compounds

no.[a]	formula[b]	TLC, R_f[c]
16	$C_{32}H_{50}N_6O_6 \cdot 0.5H_2O$	0.34
17	$C_{35}H_{54}N_6O_6 \cdot 0.5H_2O$	0.37
18	$C_{36}H_{56}N_6O_6 \cdot 0.75H_2O$	0.41
19	$C_{36}H_{56}N_6O_6 \cdot 0.25H_2O$	0.38
20	$C_{34}H_{56}N_4O_6$	0.62
21	$C_{40}H_{60}N_4O_6 \cdot H_2O$	0.73
22	$C_{37}H_{58}N_6O_6$	0.45
23	$C_{38}H_{60}N_6O_6$	0.52
24	$C_{38}H_{60}N_6O_6 \cdot 0.75H_2O$	0.43
25	$C_{37}H_{57}N_5O_6S \cdot H_2O$	0.62
26	$C_{37}H_{58}N_6O_6 \cdot 0.75H_2O$	0.53
27	$C_{38}H_{58}N_6O_6 \cdot 0.5H_2O$	0.55, 0.57[d]
28	$C_{38}H_{60}N_6O_7 \cdot 1.5H_2O$[e]	0.50
29	$C_{35}H_{55}N_7O_6$[f]	0.08
30	$C_{35}H_{56}N_6O_5 \cdot 2H_2O$[g]	0.69[h]
31	$C_{36}H_{58}N_6O_5 \cdot H_2O$	0.67[h]

[a] See Table I for structure. [b] Analyses for C, H, N were +0.4% of expected values (for formulae shown) unless otherwise noted. [c] 15% methanol in chloroform unless otherwise noted (see Experimental Section). [d] Diastereomers separable. [e] H: calcd, 8.58; found, 8.15. [f] Exact mass calcd for $C_{35}H_{56}N_7O_6$ (M + H) 670.4292, found 670.4302. [g] N: calcd, 12.41; found, 11.74. [h] 1:1:1:1 H_2O/1-BuOH/AcOH/EtOAc (system III, see Experimental Section).

s), 6.46 (1 H, NH, br d), 6.32 (1 H, NH, br t), 6.07 (1 H, NH, br s), 2.20 (1 H, CH_2CO, dd), 1.46 (9 H, $(CH_3)_3$, s), 0.90 (6 H, d); DCI-MS, M + H at 536.

Removal of Benzyloxycarbonyl (CBz) Protecting Group. The appropriate CBz-protected inhibitor (**37** or **38**) and an equal weight of 10% palladium on carbon in acetic acid were stirred under a hydrogen atmosphere for 12 h. The mixture was filtered and evaporated. The residue was dissolved in water, basified with Na_2CO_3, and extracted into ethyl acetate, which was dried and evaporated to afford the final product (Table II).

Boc-*dl*-(4-pyrimidinyl)alanine (40). To *dl*-(4-pyrimidinyl)alanine[14] (359.2 mg, 2.31 mmol) in a solution of sodium hydroxide (105 mg, 2.62 mmol) in water (2.5 mL) and *tert*-butyl alcohol (1.7 mL) was added di-*tert*-butyl dicarbonate (520 mg, 2.39 mmol) in THF (0.5 mL). The mixture was stirred for 16 h, poured into saturated $NaHCO_3$ solution, and washed with ether. The aqueous phase was acidified with 2 M HCl to pH 5–6 and the resulting solid was filtered and dried to afford 417.2 mg (71%): TLC, R_f 0.56 (III); ^1H NMR (CD_3OD) δ 9.06 (1 H, br s), 8.66 (1 H, br s), 7.43 (1 H, d), 4.63 (1 H, dd). Anal. ($C_{12}H_{17}N_3O_4$) C, H, N.

Boc-Phe-*dl*-(3-pyrazolyl)alanine Methyl Ester (41). To *dl*-(3-pyrazolyl)alanine methyl ester dihydrochloride[15] (2.05 g, 8.5 mmol) in dimethylformamide (10 mL) at −10 °C were added Boc-Phe succinimido ester (2.50 g, 6.90 mmol) and *N*-methylmorpholine (2.8 mL, 25 mmol). The mixture was stirred at −10 °C for 1 h and then at 25 °C for 12 h. The mixture was partitioned between ethyl acetate and saturated $NaHCO_3$ solution and extracted into ethyl acetate, which was washed with water, dried, and evaporated to afford 2.75 g (95%): TLC, R_f 0.45, 0.41 (IV); ^1H NMR ($CDCl_3$) δ 7.45, 7.41 (total 1 H, d), 6.00 (1 H, s). Anal. ($C_{21}H_{28}N_4O_5 \cdot 0.25H_2O$) C, H, N.

Boc-Phe-*dl*-(4-thiazolyl)alanine Methyl Ester (42). *dl*-(4-Thiazolyl)alanine[16] (440 mg, 1.80 mmol) in methanol (8 mL) at 0 °C was treated with $SOCl_2$ (0.18 mL, 2.5 mmol). The mixture was heated at 58 °C for 15 h and then evaporated with methanol chasers to yield the ester dihydrochloride as a white solid: TLC, R_f 0.59 (III). The solid was suspended in THF (15 mL) and treated with *N*-methylmorpholine (0.60 mL, 3.3 mmol) and Boc-Phe succinimido ester (520 mg, 1.43 mmol). After 18 h the mixture was poured into ethyl acetate, washed with saturated $NaHCO_3$ solution and brine, then dried, and evaporated. Chromatography of the residue on silica gel with 2% methanol in chloroform afforded 574 mg (92%): TLC, R_f 0.47 (IV); ^1H NMR ($CDCl_3$) δ 8.68 (1 H, d), 7.00, 6.92 (total 1 H, d). Anal. ($C_{21}H_{27}N_3O_5S \cdot 0.25H_2O$) C, H, N.

Boc-Phe-*dl*-(3-pyrazolyl)alanine (43). Boc-Phe-*dl*-(3-pyrazolyl)alanine methyl ester (210.3 mg, 0.505 mmol) in dioxane (1.5 mL) and water (1.0 mL) was treated with lithium hydroxide monohydrate (27.2 mg, 0.648 mmol), and the mixture was stirred at 25 °C for 30 min and quenched with 2 M HCl (0.32 mL, 0.64 mmol). The mixture was diluted with chloroform, washed with water, then dried, and evaporated to afford 184.1 mg (91%) of a foam. Anal. ($C_{20}H_{26}N_4O_5 \cdot 0.25H_2O$) C, H, N.

Boc-Phe-*dl*-(4-thiazolyl)alanine (44): prepared from the methyl ester (317.2 mg, 100%) according to the above procedure. Anal. ($C_{20}H_{25}N_3O_5S \cdot 0.5H_2O$) C, H, N.

Biological Methods. Purified human renal renin[17] was assayed with pure human angiotensinogen[18] at pH 6.0 in maleate buffer. Test compounds were dissolved in Me_2SO and diluted so that prior to addition to the assay system the solutions were 10% in Me_2SO and 0.5% in BSA. The final incubation mixture (100 μL) contained the following: maleate buffer, pH 6.0, 0.135 M; EDTA, 3 mM; PMSF, 1.4 mM; angiotensinogen, 0.21 μM; renin, 0.24 mGU;[19] BSA, 0.44%; Me_2SO, 1%. At least three different concentrations of inhibitor that bracketed the IC_{50} were preincubated with renin for 5.0 min at 37 °C, substrate was added, and the incubation was allowed to proceed for 10.0 min. The reaction was stopped by freezing the solution in a methanol/dry ice bath, and after thawing at 4 °C, an aliquot was analyzed for angiotensin I by radioimmunoassay utilizing a commercial kit (NEN Research). The percent inhibition of the reaction was determined and the IC_{50} was calculated by regression analysis. The reaction time of 10 min was on the linear portion of the incubation time–angiotensin I generation curve, and at the highest concentrations tested, none of the compounds cross reacted with the antibody to angiotensin I. The presence of 1% Me_2SO in the final incubation mixture caused no statistically significant effect on the renin activity.

Bovine cathepsin D (Sigma) and porcine pepsin (Sigma) activities were assessed by the hydrolysis of hemoglobin at pH 3.1 and 1.9, respectively, at 37 °C, and measurements of the absorbance at 280 nm of the supernatant after precipitation with trichloroacetic acid.[20]

Stability Studies. Chymotrypsin (porcine pancreas Sigma C4129) was dissolved at 0.1 mg/mL in 30 mM sodium phosphate/100 mM sodium chloride, pH 6.9. Test compounds (**22** and **28**) were introduced at 0.1 mg/mL by dilution of a 5 mg/mL methanol stock solution (20 μL/mL) into the enzyme solution. Aliquots were removed at intervals and quenched by addition of acetonitrile and then centrifuged and filtered. Analyses of the incubations were carried out by HPLC on a Waters μBondpack analytical C-18 column (30 cm) eluted with a gradient from 20% CH_3CN/80% H_2O/0.1% TFA to 80% CH_3CN/20% H_2O/0.1% TFA over 20 min. Peak detection was by UV absorbance at 214 nm with quantitation using a Perkin-Elmer LCI-100 computing integrator.

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