Renin Inhibitors Based on Novel Dipeptide Analogues. Incorporation of the Dehydrohydroxyethylene Isostere at the Scissile Bond[1]

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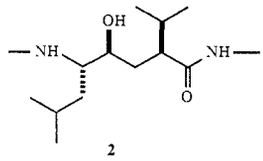
The design and synthesis of renin inhibitors that incorporate the novel dipeptide isostere (4*S*,5*S*)-5-amino-6-cyclohexyl-4-hydroxyhex-1-ene-2-carboxylic acid as a transition-state analogue are described. Titanium-promoted condensation of dilithiated *N*-alkylmethacrylamides with protected amino aldehydes results in efficient preparation of protected dipeptide analogues **7** and **8**. Incorporation of **7** into the partial sequence of angiotensinogen affords potent in vitro inhibitors of human renin. Further chemical manipulation of the unsaturated amide moiety allows the study of structure–activity relationships in both the $P_{1'}$ and $P_{2'}$ sites. Details of the syntheses, stereochemical determinations, and in vitro renin inhibition are presented.

The inhibition of renin, an aspartic proteinase whose action initiates the renin–angiotensin cascade, has been the object of intense investigation in recent years.[2] The potential for treatment of hypertension and related ailments through the inhibition of renin[3] has resulted in the preparation of a variety of potent renin inhibitors based on the peptide sequence of the natural substrate angiotensinogen (**1**). Most notable among these are a series of inhibitors that incorporate the hydroxyethylene dipeptide isostere (**2**) at the scissile site[4] and a series based on the natural hydroxy amino acid statine (**3**).[5] Recently, Boger and co-workers have introduced inhibitors that contain the novel statine analogue **4**, derived from cyclohexylalanine, which show dramatically increased potency.[6a]

Hexapeptide--Pro-Phe-His-Leu-Val-Ile-His--Protein

P_4 P_3 P_2 P_1 $P_{1'}$ $P_{2'}$ $P_{3'}$

1



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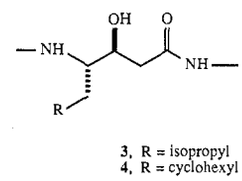
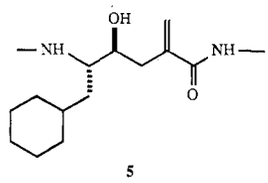


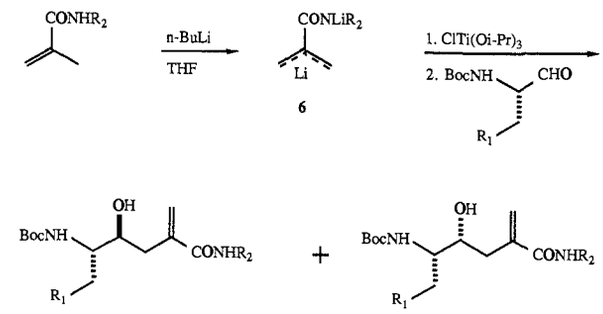
3, R = isopropyl
4, R = cyclohexyl



5

Our interest in this area has been focused on the synthesis and evaluation of renin inhibitors based on novel dipeptide analogues. We envisioned that incorporation of (4*S*,5*S*)-5-amino-6-cyclohexyl-4-hydroxyhex-1-ene-2-carboxylic acid (**5**) into the scissile site of the angiotensinogen sequence might provide potent inhibitors of human renin. Structurally, **5** embodies several characteristics that we deemed beneficial. In addition to the cyclohexylmethyl side chain, the *S* stereochemistry of the hydroxyl group promotes tight binding either as a tetrahedral transition-state mimic[7] or by other mechanism-based inhibition.[8] Moreover, the methylidene side chain rigidifies the system due to conjugation with the adjacent peptide bond. Finally, the presence of an α,β-unsaturated amide in the active site presents the possibility of irreversible inactivation through covalent bond formation. The synthesis and biological evaluation of novel, potent renin inhibitors based on dipeptide analogue **5** are reported herein.

Scheme I



7 a–g, R_1 = cyclohexyl; h–i, R_1 = isopropyl **8**

a, $R_2 = CH_2CH_2CH(CH_3)_2$
b, $R_2 = CH_2CH(CH_3)_2$
c, $R_2 = CH_2C_6H_{11}$
d, $R_2 = CH_2CH_2Ph$
e, $R_2 = CH_3$
f, $R_2 = CH_2CH_2N(CH_3)_2$
g, $R_2 = CH_2C(CH_3)_2CH_2N(CH_3)_2$
h, $R_2 = CH_2CH_2CH(CH_3)_2$
i, $R_2 = CH_2CH(CH_3)_2$

Results and Discussion

The synthetic approach, outlined in Scheme I, follows in part our previously reported synthesis of hydroxyethylene dipeptide isosteres.[9] Thus, β'-lithiation[10] of *N*-alkylmethacrylamides with 2 equiv of *n*-butyllithium in tetrahydrofuran/hexane gave a symmetrical dianion **6**. Subsequent treatment of **6** with chlorotitanium triisopropoxide[9] followed by Boc-cyclohexylalaninal or Boc-leucinal[11] led to a ca. 1:1 mixture of diastereomeric hydroxy

(1) Presented in part at the 191st Meeting of the American Chemical Society, New York, NY, April 1986.
(2) For a recent review, see: Boger, J. *Annu. Rep. Med. Chem.* **1985**, *20*, 257.
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(8) Rich, D. H. *J. Med. Chem.* **1985**, *28*, 263.
(9) Kempf, D. J. *J. Org. Chem.* **1986**, *51*, 3921.
(10) (a) Beak, P.; Kempf, D. J.; Wilson, K. D. *J. Am. Chem. Soc.* **1985**, *107*, 4745. (b) Beak, P.; Kempf, D. J. *J. Am. Chem. Soc.* **1980**, *102*, 4550. (c) Fitt, J. J.; Gschwend, H. W. *J. Org. Chem.* **1980**, *45*, 4257.

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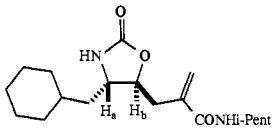
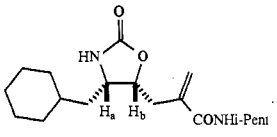
amides **7** and **8**. In most cases, **7** and **8** were separated chromatographically; however, **7f/8f** and **7g/8g** were carried on as inseparable mixtures.

The stereochemistry of **7a** and **8a** was determined by conversion to the corresponding oxazolidinones **9** and **10** (sodium hydride in dimethylformamide). Coupling constants of 5.7 and 7.8 Hz for the ring protons of **9** and **10**,



9 J_{ab} = 5.7 Hz



10 J_{ab} = 7.8 Hz

respectively, are consistent with trans and cis stereochemistry as shown.[12] The relative stereochemistry of **7b–i** was assigned by analogy of the NMR spectra to the spectrum of **7a**. The integrity of the absolute stereochemistry was confirmed by conversion of **7b** to the corresponding (+)- and (−)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid (MTPA) amides (HCl, dioxane; (+)- or (−)-MTPA chloride).[13] Examination of the proton NMR spectra of the resulting diastereomers indicated that no racemization had occurred, in agreement with previous results.[9]

The conversion of **7** to intact renin inhibitors was accomplished through standard solution-phase peptide methodology. Thus, removal of the Boc protecting group with HCl in dioxane followed by coupling via either mixed anhydride or carbodiimide methods led to compounds **11–32**. General procedures are detailed in the Experimental Section, and characterization data are shown in Table I. The inhibitory potencies of **11–32** against purified human renal renin[14] were determined by radioimmunoassay for angiotensin I production. Details are given in the Experimental Section. Inhibitions, expressed as IC_{50} values, for **11–32** are shown in Table II. For synthetic ease, initial structure–activity studies employed alanine in the P_2 site in place of histidine. In accordance with previous observations,[15] we discovered that reasonable potencies can be obtained without the attachment of additional peptide units on the carboxyl terminus of the dipeptide analogue. Thus **11**, with only a small alkyl group in the $P_{2'}$ position, has a respectable IC_{50} value of 10^{-8} M. As expected,[6] the inclusion of the cyclohexylmethyl side chain at the P_1 position in **11** and **12** dramatically increases activity (20–40-fold) over **13** and **14**, which contain the native isobutyl side chain. The 30-fold difference in potency between **13** and **15**, which is derived from **8h**, demonstrates that the *S* configuration of the hydroxyl group is also crucial for tight binding.

Structure–activity relationships at the $P_{2'}$ site are demonstrated with compounds **11**, **12**, and **16–20**. Increasing the bulk of the terminal alkyl group to cyclohexylmethyl or β-phenylethyl results in significant (5–15-fold) loss of activity. Decreasing the size to a methyl group also results in lowered potency; thus it seems likely that lipophilic interactions in the $P_{2'}$ position are contributing to overall binding. Further support for this interaction is evident from compound **19**, in which the isopentyl group of **11** is replaced with the polar, isoelectronic 2-(dimethylamino)-ethyl function. The result is a ca. 20-fold loss of activity. Binding can apparently be maintained, however, if the polar group is attached via a longer alkyl chain, as in **20**. The IC_{50} for the 4*S* isomer of **20**, as well as for **19** and **26–28**, can only be estimated since these compounds were tested as ca. 1:1 mixtures. Assuming the 4*R* isomer to be much less active, however (vide supra), the potency of **12** is nearly maintained when the isobutyl group is appended to give **20**.

As expected, replacement of alanine in the above compounds with histidine in the P_2 site results in a further boost in potency toward renin, as evidenced by IC_{50} values of 1.5, 2, and 5 nM for **21**, **24**, and **26**, respectively. Leucine and phenylalanine are also tolerated in the P_2 position without significant loss of activity (cf. **22**, **25**, **27**, and **28**). The replacement of Boc by acid-stable groups such as ethoxycarbonyl, acetyl, or *tert*-butylacetyl does not seriously affect the potency in this series. However, if the protecting group is removed and 3-phenyllactic acid is substituted for phenylalanine to remove the basic nitrogen (compound **32**), a 70-fold loss of activity results. Reduced potency is also generally observed for significant changes in the P_3 site. Replacement of phenylalanine with a hy-

Table I. Chemical Data for Renin-Inhibiting Compounds

no.	synthesis[a]	R_f (solvent)[b]	formula[c]
11	A	0.38 (A)	$C_{35}H_{56}N_4O_6$
12	A	0.32 (A)	$C_{34}H_{54}N_4O_6$
13	A	0.37 (A)	$C_{32}H_{52}N_4O_6 \cdot 0.5H_2O$
14	A	0.32 (A)	$C_{31}H_{50}N_4O_6$
15	A	0.30 (A)	$C_{32}H_{52}N_4O_6$
16	A	0.32 (A)	$C_{37}H_{58}N_4O_6$
17	A	0.39 (A)	$C_{38}H_{54}N_4O_6 \cdot 0.5H_2O$[d]
18	A	0.29 (A)	$C_{31}H_{48}N_4O_6$
19	A	0.17 (C)	$C_{34}H_{55}N_5O_6 \cdot 0.5H_2O$
20	A	0.40 (C)	$C_{37}H_{61}N_5O_6 \cdot 2H_2O$[e]
21	B	0.20 (B)	$C_{38}H_{58}N_6O_6 \cdot 0.5H_2O$
22	A	0.41 (A)	$C_{38}H_{62}N_4O_6 \cdot 0.5H_2O$
23	C	0.12 (A)	$C_{36}H_{54}N_6O_6 \cdot 0.5H_2O$
24	B	0.23 (B)	$C_{37}H_{56}N_6O_6 \cdot H_2O$
25	A	0.40 (A)	$C_{35}H_{56}N_4O_6$
26	B	0.16 (C)	$C_{40}H_{63}N_7O_6 \cdot H_2O$
27	A	0.43 (C)	$C_{43}H_{65}N_5O_6 \cdot 0.5H_2O$
28	A	0.42 (C)	$C_{38}H_{64}ClN_5O_6 \cdot 1.25H_2O$
29	B	0.07 (B)	$C_{35}H_{52}N_6O_5 \cdot H_2O$
30	C	0.10 (B)	$C_{35}H_{52}N_6O_5 \cdot H_2O$
31	C	0.12 (B)	$C_{32}H_{54}N_6O_7 \cdot H_2O$
32	C	0.18 (B)	$C_{33}H_{49}N_5O_5 \cdot 0.5H_2O$
33	*f*	0.37, 0.40 (A)	$C_{35}H_{56}N_4O_7$
34	*f*	0.28, 0.31 (A)	$C_{31}H_{48}N_4O_7 \cdot 0.25H_2O$
41	B	0.16 (A)	$C_{36}H_{60}N_6O_7 \cdot 2H_2O$
42	B	0.17 (A)	$C_{36}H_{60}N_6O_7 \cdot 0.5H_2O$
43	B	0.22 (A)	$C_{37}H_{57}N_9O_7 \cdot 0.5H_2O$[g]
44	B	0.24 (A)	$C_{37}H_{57}N_9O_7 \cdot 0.5H_2O$[h]
45	B	0.19 (A)	$C_{40}H_{64}N_6O_7 \cdot 1.5H_2O$
46	B	0.24 (A)	$C_{37}H_{57}ClN_6O_7 \cdot H_2O$[i]
47	B	0.14 (A)	$C_{37}H_{57}ClN_6O_7 \cdot 0.5H_2O$
48	*f*	0.16 (C)	$C_{37}H_{59}N_7O_7$[j]
49	*f*	0.14 (C)	$C_{37}H_{59}N_7O_7$[j]

[a] General procedures for peptide couplings are given in the Experimental Section. [b] TLC solvent systems: A, 7.5% methanol/chloroform; B, 10% methanol/chloroform; C; 2% isopropylamine/2.5% methanol/chloroform. [c] Analyses for C, H, N were within ±0.4% of calculated values for formulas shown unless otherwise indicated. [d] C, H; N: calcd, 8.34; found, 7.60. [e] C, N; H: calcd, 9.25; found, 8.74. [f] Final synthetic step was not peptide coupling; see Experimental Section. [g] C, H; N: calcd, 16.83; found, 16.00 (see ref 17). [h] C, H; N: calcd, 16.83; found, 15.74 (see ref 17). [i] C, N; H: calcd, 7.91; found, 7.36; exact mass calcd for $C_{37}H_{58}ClN_6O_7$ (M + H) 733.4055, found 733.4038. [j] Exact mass calcd for $C_{37}H_{60}N_7O_7$ (M + H) 714.4554, found 714.4544 (48), 714.4577 (49).

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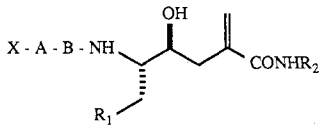
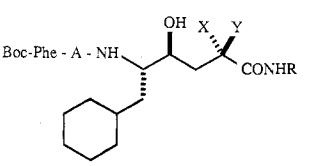
Table II. Inhibition of Human Renin by Compounds Containing (4*S*,5*S*)-5-Amino-6-cyclohexyl-4-hydroxyhex-1-ene-2-carboxylic Acid



no.	X	A	B	R_1	R_2	IC_{50}, nM
11	Boc	Phe	Ala	Cyc[a]	*i*-Pent	10
12	Boc	Phe	Ala	Cyc	*i*-Bu	10
13	Boc	Phe	Ala	*i*-Pr	*i*-Pent	200
14	Boc	Phe	Ala	*i*-Pr	*i*-Bu	400
15	Boc	Phe	Ala	*i*-Pr	*i*-Pent (α-OH)[b]	6000
16	Boc	Phe	Ala	Cyc	CH_2Cyc	50
17	Boc	Phe	Ala	Cyc	CH_2CH_2Ph	150
18	Boc	Phe	Ala	Cyc	CH_3	50
19	Boc	Phe	Ala	Cyc	$CH_2CH_2N(CH_3)_2$	400 (200)[c]
20	Boc	Phe	Ala	Cyc	DADP[d]	25 (13)[c]
21	Boc	Phe	His	Cyc	*i*-Pent	1.5
22	Boc	Phe	Leu	Cyc	*i*-Pent	4
23	Etoc[e]	Phe	His	Cyc	*i*-Pent	3
24	Boc	Phe	His	Cyc	*i*-Bu	2
25	Etoc	Phe	Leu	Cyc	*i*-Bu	5
26	Boc	Phe	His	Cyc	DADP	5 (2.5)[c]
27	Boc	Phe	Phe	Cyc	DADP	8.5 (4)[c]
28	Etoc	Phe	Leu	Cyc	DADP[f]	10 (5)[c]
29	TBA[g]	Phe	His	Cyc	CH_3	2
30	Ac	Phe	His	Cyc	*i*-Pent	4
31	Boc	Ser	His	Cyc	*i*-Pent	2000
32		phenyllactic	His	Cyc	*i*-Pent	100

[a] Cyc = cyclohexyl. [b] The hydroxyl group in **15** has the 4*R* stereochemistry. [c] Estimated IC_{50} value of active 4*S*,5*S* diastereomer based on 1:1 mixture. [d] DADP = 3-(*N*,*N*-dimethylamino)-2,2-dimethylpropyl. [e] Etoc = ethoxycarbonyl. [f] Tested as the hydrochloride salt. [g] TBA = *tert*-butylacetyl.

Table III. Inhibition of Renin by α,α-Disubstituted Analogues

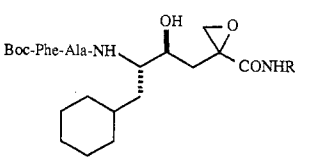


no.	A	X	Y	R	IC_{50}, nM
33	Ala	CH_2O[a]		*i*-Pent	25
34	Ala	CH_2O[a]		CH_3	40
41	His	OH	CH_3	*i*-Pent	5.5
42	His	CH_3	OH	*i*-Pent	50
43	His	OH	CH_2N_3	*i*-Bu	1
44	His	CH_2N_3	OH	*i*-Bu	20
45	His	OH	*i*-Bu	*i*-Bu	30
46	His	OH	CH_2Cl	*i*-Bu	0.8
47	His	CH_2Cl	OH	*i*-Bu	20
48	His	OH	CH_2NH_2	*i*-Bu	15
49	His	CH_2NH_2	OH	*i*-Bu	35

[a] 1:1 mixture of diastereomers.

drophilic amino acid (e.g., **31**) causes a drastic (>1000-fold) loss in inhibition.

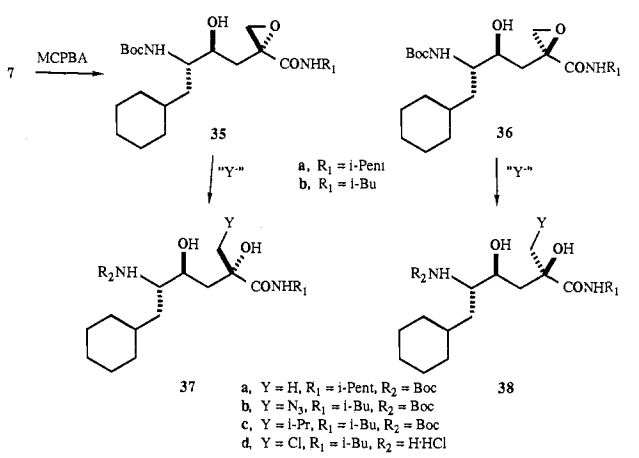
Structure–activity relationships in the $P_{1'}$ site were investigated through chemical manipulation of the α,β-unsaturated amide. Epoxidation of inhibitors **11** and **18** (*m*-chloroperoxybenzoic acid, CH_2Cl_2) led in good yield to **33** and **34**, respectively, as 1:1 mixtures of diastereomers.



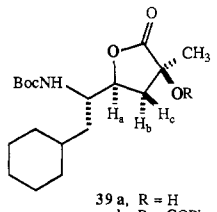
33, R = i-Pent
34, R = CH_3

In the former case, epoxidation results in loss of activity (Table III) while a small increase is observed in the latter case. Epoxidation of intermediates **7** provided **35** and **36**, which could be separated chromatographically (Scheme II). Further elaboration was accomplished by the addition of various nucleophiles to the less hindered end of the resulting α,β-epoxy amides. Thus, hydrogenation (Pd/C) of **35a** and **36a** gave 1,3-diols **37a** and **38a**, respectively, while addition of sodium azide (NH_4Cl, MeOH) to **35b** and **36b** led to azido diols **37b** and **38b**, respectively, in quantitative yield. Reaction of **35b** with isopropylmagnesium chloride was sluggish to give **37c** in moderate yield. Finally, removal of the Boc protecting group of **35b** and **36b** (HCl, dioxane) resulted in concomitant ring opening to give chlorohydrins **37d** and **38d**.

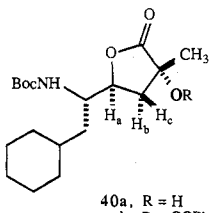
Scheme II



The stereochemistry of **37a** and **38a** (and thus **35** and **36**) was established by conversion first to lactones **39a** and **40a**, respectively (xylenes, reflux),[9] followed by benzoylation (PhCOCl, Et_3N, DMAP) in order to facilitate spectral interpretation. Analysis of **39b** and **40b** by NOE methods revealed that irradiation of either the methyl resonance (1.74 ppm) or H_a (4.50 ppm) in **39b** results in significant



39a, R = H
b, R = COPh



40a, R = H
b, R = COPh

enhancement of H_b (2.32 ppm). In contrast, irradiation of the methyl resonance (1.73 ppm) of **40b** results in enhancement of H_c (2.35 ppm) while irradiation of H_a (4.88 ppm) gives significant enhancement of H_b (2.63 ppm). These results are consistent with structures of **39** and **40** as shown.

Removal of the Boc protecting group from **37** and **38** followed by coupling to Boc-Phe-His-OH led to inhibitors **41–47**. Inhibitory potencies are shown in Table III. With each set of diastereomers, greater potency is observed with the compounds (e.g., **41**, **43**, **46**) in which the absolute configuration of the lipophilic side chain corresponds to the L-valine side chain of angiotensinogen. Interestingly, azido and chloro diols **43** and **46** show greatest potency against renin whereas either small (**41**) or large alkyl groups (**45**) in the $P_{1'}$ position result in loss of activity. The amino diols **48** and **49**, produced by reduction (H_2, Pd/C, CH_3OH) of **43** and **44**, respectively, show a further loss, indicating that polar groups are not well-tolerated in the $P_{1'}$ site. The loss of potency observed when **21** is compared to **41** suggests that the presence of the second hydroxyl group in compounds **41–49** may be deleterious. Only when lipophilic binding at the $P_{1'}$ position is increased as in **43** and **46** is activity maintained.

The unique structure of dipeptide analogue **5** allows for the possibility of irreversible inhibition of renin via conjugate addition to the α,β-unsaturated system. This possibility was explored in the in vitro assay by increasing the preincubation time prior to addition of angiotensinogen. The results of 5- and 60-min preincubation times at the IC_{50} concentration of compounds **11**, **14**, **16**, **18**, **33**, and **34** are given in Table IV. Within experimental error, no change in inhibitory activity was observed, supporting the idea that inhibitors derived from **5** act as transition-state mimics rather than through irreversible binding. Since these inhibitors lack most of the bulk of the valine side chain of angiotensinogen, it seems likely that the olefinic moiety stabilizes the enzyme–inhibitor complex by providing a favorable conformation for binding.

In conclusion, a new series of renin inhibitors based on the dipeptide analogue **5** has been developed. The synthesis of **5** via dilithiated *N*-alkylmethacrylamides provides a direct and efficient route to these novel inhibitors. Structure–activity studies have provided a number of compounds that display potent (IC_{50} = 0.8–2 × 10^{-9} M) in vitro inhibition of human renin. Further modifications of this series and in vivo studies will be the subject of future reports.

Experimental Section

Solvents and other reagents were of reagent grade or higher. Tetrahydrofuran was freshly distilled from sodium benzophenone ketyl before use. All reactions involving organometallics were performed in flame-dried glassware under an inert atmosphere. Proton magnetic resonance spectra were measured on a Nicolet QE-300 (300 MHz) instrument using tetramethylsilane as an internal standard. Elemental analyses were performed by the Analytical Research Department, Abbott Laboratories. Flash column chromatography[16] was performed on silica gel 60, 0.04–0.063 mm (E. Merck). Thin-layer chromatography was performed on precoated silica gel F-254 plates (0.25 mm; E. Merck) and was visualized with phosphomolybdic acid.

(16) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

Table IV. Inhibition of Renin following Variable Preincubation Times

no.	concn,[a] M	% inhibn, 5 min[b]	% inhibn, 60 min[b]
11	10^{-8}	38	31
14	4×10^{-7}	48	43
16	5×10^{-8}	49	53
18	5×10^{-8}	44	37
33	2.5×10^{-8}	66	61
34	4×10^{-8}	49	38

[a] Concentration of inhibitor: assay was performed at IC_{50} concentration for individual inhibitors determined at 5-min preincubation in another experiment. [b] Preincubation time prior to addition of angiotensinogen.

General Procedure for the Condensation of Dilithiated Amides with Boc-amino Aldehydes. (4*S*,5*S*)- and (4*R*,5*S*)-*N*-Isopentyl-5-[[(*tert*-butyloxy)carbonyl]amino]-6-cyclohexyl-4-hydroxyhex-1-ene-2-carboxamide (7a and 8a). A solution of *N*-isopentylmethacrylamide (643 mg, 4.15 mmol) in 25 mL of dry tetrahydrofuran was cooled under an N_2 atmosphere to −78 °C and treated dropwise with 3.28 mL (8.5 mmol) of *n*-butyllithium in hexane. The resulting solution was warmed to 0 °C for 20 min, recooled to −78 °C, and treated with 6.2 mL (6.2 mmol) of chlorotitanium triisopropoxide in hexane. After again warming to 0 °C for 5 min, the dark solution was recooled to −78 °C, treated with a solution of Boc-cyclohexylalaninal[11] (670 mg, 2.3 mmol) in 5 mL of tetrahydrofuran, stirred for 5 min at −78 °C, warmed to 0 °C for 20 min, and quenched with saturated aqueous ammonium chloride. The resulting suspension was treated with ca. 50 mL of ether, stirred until the salts became white, extracted with two 100-mL portions of ether, dried over $MgSO_4$, and concentrated in vacuo. The crude mixture was separated by flash column chromatography using 4:1 chloroform/ethyl acetate to give 249 mg (26%) of **7a** (R_f 0.44), 292 mg (31%) of **8a** (R_f 0.36, 3:2 chloroform/ethyl acetate), and 184 mg (20%) of a ca. 1:1 mixture of the two products.

7a: ^{1}H NMR δ 0.8–1.9 (m, 16 H), 0.94 (d, J = 6 Hz, 6 H), 1.43 (s, 9 H), 2.42 (m, 2 H), 3.32 (br q, J = 7 Hz, 2 H), 3.62 (m, 1 H), 3.68 (m, 1 H), 4.79 (br d, J = 9 Hz, 1 H), 5.08 (br s, 1 H), 5.43 (s, 1 H), 5.56 (s, 1 H), 6.03 (br t, 1 H); MS, m/z 410. Anal. ($C_{23}H_{42}N_2O_4$) C, H, N.

(4*S*,5*S*)- and (4*R*,5*S*)-*N*-isobutyl-5-[[(*tert*-butyloxy)carbonyl]amino]-6-cyclohexyl-4-hydroxyhex-1-ene-2-carboxamide (7b and 8b): R_f (3:2 chloroform/ethyl acetate 0.39 (**7b**), 0.31 (**8b**). **7b**: ^{1}H NMR δ 0.8–1.55 (m, 8 H), 0.94 (d, J = 7 Hz, 6 H), 1.44 (s, 9 H), 1.6–1.7 (m, 4 H), 1.8–1.9 (m, 2 H), 2.45 (m, 2 H), 3.11 (dt, J = 13, 7 Hz, 1 H, 3.18 (dt, J = 13, 7 Hz, 1 H), 3.62 (m, 1 H), 3.70 (m, 1 H), 4.55 (br, 1 H), 4.79 (br d, J = 10 Hz, 1 H), 5.44 (s, 1 H), 5.59 (s, 1 H), 6.17 (br t, J = 7 Hz, 1 H); MS, m/z 396. Anal. ($C_{22}H_{40}N_2O_4$) C, H, N.

(4*S*,5*S*)- and (4*R*,5*S*)-(cyclohexylmethyl)-5-[[(*tert*-butyloxy)carbonyl]amino]-6-cyclohexyl-4-hydroxyhex-1-ene-2-carboxamide (7c and 8c): R_f (3:2 chloroform/ethyl acetate) 0.46 (**7c**), 0.38 (**8c**). **7c**: ^{1}H NMR δ 0.8–1.0 (m, 4 H), 1.1–1.6 (m, 10 H), 1.45 (s, 9 H), 1.6–1.8 (m, 9 H), 1.87 (br d, 1 H), 2.45 (m, 2 H), 3.15 (q, J = 7 Hz, 2 H), 3.62 (m, 1 H), 3.69 (m, 1 H), 4.79 (br d, J = 10 Hz, 1 H), 5.11 (br s, 1 H), 5.43 (s, 1 H), 5.58 (s, 1 H), 6.14 (br t, 1 H); MS, m/z 436.

(4*S*,5*S*)- and (4*R*,5*S*)-*N*-(2-phenylethyl)-5-[[(*tert*-butyloxy)carbonyl]amino]-6-cyclohexyl-4-hydroxyhex-1-ene-2-carboxamide (7d and 8d): R_f (7:3 chloroform/ethyl acetate) 0.15 (**7d**), 0.10 (**8d**). **7d**: ^{1}H NMR δ 0.8–1.5 (m, 8 H), 1.44 (s, 9 H), 1.7 (m, 4 H), 1.86 (br d, J = 13 Hz, 1 H), 2.42 (m, 2 H), 2.87 (t, J = 7 Hz, 2 H), 3.5–3.7 (m, 4 H), 4.76 (br d, J = 10 Hz), 4.92 (br s, 1 H), 5.39 (s, 1 H), 5.45 (s, 1 H), 6.09 (br t, 1 H), 7.2–7.4 (m, 5 H); MS, m/z 444.

(4*S*,5*S*)- and (4*R*,5*S*)-*N*-methyl-5-[[(*tert*-butyloxy)carbonyl]amino]-6-cyclohexyl-4-hydroxyhex-1-ene-2-carboxamide (7e and 8e): R_f (3:2 chloroform/ethyl acetate) 0.13 (**7e**), 0.08 (**8e**). **7e**: ^{1}H NMR δ 0.8–1.5 (m, 8 H), 1.44 (s, 9 H), 1.7 (m, 4 H), 1.87 (br d, 1 H), 2.45 (m, 2 H), 2.89 (d, J = 5 Hz, 3 H),

3.62 (m, 1 H), 3.70 (m, 1 H), 4.80 (br d, J = 10 Hz, 1 H), 5.09 (br s, 1 H), 5.43 (s, 1 H), 5.58 (s, 1 H), 6.20 (br, 1 H); MS, m/z 354.

N-[2-(N,N-Dimethylamino)ethyl]-5-[[(*tert*-butyloxy)carbonyl]amino]-6-cyclohexyl-4-hydroxyhex-1-ene-2-carboxamide (7f and 8f): R_f 0.17 (1:1 methanol/acetonitrile); chromatographic separation of **7f** and **8f** was not possible; ^{1}H NMR δ 0.8–1.0 (m, 4 H), 1.1–1.5 (m, 12 H), 1.45 (s, 18 H), 1.6–1.7 (m, 8 H), 1.90 (br d, 2 H), 2.24 (s, 12 H), 2.45 (m, 8 H), 3.38 (m, 4 H), 3.66 (m, 4 H), 4.80 (br d, J = 10 Hz, 1 H), 4.90 (br d, J = 10 Hz, 1 H), 5.43 (s, 2 H), 5.61 (s, 1 H), 5.65 (s, 1 H), 6.75 (m, 2 H); MS, m/z 412 (M + H).

N-[2,2-Dimethyl-3-(N,N-dimethylamino)propyl]-5-[[(*tert*-butyloxy)carbonyl]amino]-6-cyclohexyl-4-hydroxyhex-1-ene-2-carboxamide (7g and 8g): R_f 0.39 (1:1 methanol/chloroform); chromatographic separation of **7g** and **8g** was not possible; ^{1}H NMR δ 0.8–1.0 (m, 4 H), 0.95 (s, 6 H), 0.97 (s, 6 H), 1.1–1.5 (m, 12 H), 1.44 (s, 18 H), 1.6–1.7 (m, 8 H), 1.88 (br d, 2 H), 2.33 (s, 12 H), 2.4–2.5 (m, 8 H), 3.22 (m, 4 H), 3.6–3.7 (m, 4 H), 4.72 (br d, J = 10 Hz, 1 H), 4.83 (br d, J = 10 Hz, 1 H), 5.43 (s, 2 H), 5.55 (s, 1 H), 5.58 (s, 1 H), 9.0 (br, 1 H), 9.1 (br, 1 H); MS, m/z 453 (M + H).

(4*S*,5*S*)- and (4*R*,5*S*)-*N*-isopentyl-5-[[(*tert*-butyloxy)carbonyl]amino]-4-hydroxy-7-methyloct-1-ene-2-carboxamide (7h and 8h): R_f (3:2 chloroform/ethyl acetate) 0.49 (**7h**), 0.42 (**8h**). **7h**: ^{1}H NMR δ 0.92 (d, J = 7 Hz, 3 H), 0.93 (d, J = 7 Hz, 9 H), 1.1–1.7 (m, 6 H), 1.43 (s, 9 H), 2.43 (m, 2 H), 3.32 (br q, 2 H), 3.65 (m, 2 H), 4.82 (br d, J = 9 Hz, 1 H), 5.17 (br s, 1 H), 5.43 (s, 1 H), 5.56 (s, 1 H), 6.07 (br t, 1 H); MS, m/z 370.

(4*S*,5*S*)- and (4*R*,5*S*)-isobutyl-5-[[(*tert*-butyloxy)carbonyl]amino]-4-hydroxy-7-methyloct-1-ene-2-carboxamide (7i and 8i): R_f (3:2 chloroform/ethyl acetate) 0.48 (**7i**), 0.40 (**8i**). **7i**: ^{1}H NMR δ 0.92 (d, J = 7 Hz, 3 H), 0.93 (d, J = 7 Hz, 3 H), 0.94 (d, J = 7 Hz, 6 H), 1.1–1.6 (m, 3 H), 1.45 (s, 9 H), 1.83 (heptet, J = 7 Hz, 1 H), 2.44 (m, 2 H), 3.14 (m, 2 H), 3.63 (m, 2 H), 4.82 (br d, J = 10 Hz, 1 H), 5.13 (br s 1 H), 5.44 (s, 1 H), 5.59 (s, 1 H), 6.16 (br t, 1 H); MS, m/z 356.

(2*R*,4*S*,5*S*)- and (2*S*,4*S*,5*S*)-5-[[(*tert*-Butyloxy)carbonyl]amino]-6-cyclohexyl-4-hydroxy-2-(*N*-isopentylcarbamoyl)hex-1-ene 1,2-Oxide (35a and 36a). A solution of **7a** (206 mg, 0.50 mmol) in 8 mL of dichloromethane was treated with 217 mg (1.0 mmol) of 3-chloroperoxybenzoic acid and allowed to stand at ambient temperature. After 18 h, the solution was diluted with 5 mL of ether, treated with 10% aqueous $Na_2S_2O_3$, stirred vigorously for 1.5 h, extracted with 25 mL of ether, washed sequentially with 3 N NaOH and saturated brine, dried over $MgSO_4$, and concentrated in vacuo to give a 1.1:1 mixture of **35a** (R_f 0.49) and **36a** (R_f 0.40, 3:2 chloroform/ethyl acetate), respectively, in 100% yield. The diastereomeric products were separated by flash column chromatography using 5.5:1 chloroform/ethyl acetate. **35a**: ^{1}H NMR δ 0.8–1.7 (m, 16 H), 0.91 (d, J = 6 Hz, 6 H), 1.43 (s, 9 H), 1.86 (br d, 1 H), 2.28 (dd, J = 15, 10 Hz, 1 H), 2.85 (br d, J = 5 Hz, 1 H), 2.92 (d, J = 5 Hz, 1 H), 3.25 (q, J = 7 Hz, 2 H), 3.62 (m, 1 H), 3.75 (br d, J = 10 Hz, 1 H), 4.76 (br d, J= 10 Hz, 1 H), 5.60 (s, 1 H), 6.42 (br t, 1 H); MS, m/z 427 (M + H).

36a: ^{1}H NMR δ 0.8–1.7 (m, 16 H), 0.91 (d, J = 6 Hz, 6 H), 1.45 (s, 9 H), 1.83 (br d, 1 H), 2.50 (dd, J = 15, 10 Hz, 1 H), 2.82 (d, J = 5 Hz, 1 H), 2.93 (d, J = 5 Hz, 1 H), 3.12 (d, J = 5 Hz, 1 H), 3.24 (q, J = 7 Hz, 2 H), 3.60 (m, 1 H), 3.85 (m, 1 H), 4.66 (br d, J = 10 Hz, 1 H), 6.37 (br t, 1 H); MS, m/z 427 (M + H).

(2*R*,4*S*,5*S*)- and (2*S*,4*S*,5*S*)-5-[[(*tert*-Butyloxy)carbonyl]amino]-6-cyclohexyl-4-hydroxy-2-(*N*-isobutylcarbamoyl)hex-1-ene 1,2-Oxide (35b and 36b). In a manner analogous to the manner of preparation of **35a** and **36a**, **7b** was converted in 100% yield to a 1.1:1 mixture of **35b** (R_f 0.44) and **36b** (R_f 0.37, 3:2 chloroform/ethyl acetate), which were separated by flash column chromatography using 5:1 chloroform/ethyl acetate. **35b**: ^{1}H NMR δ 0.8–1.8 (m, 14 H), 0.90 (d, J = 6 Hz, 6 H), 1.43 (s, 9 H), 1.86 (br d, 1 H), 2.28 (dd, J = 14, 10 Hz, 1 H), 2.86 (d, J = 5 Hz, 1 H), 2.93 (d, J = 5 Hz, 1 H), 3.06 (t, J = 7 Hz, 2 H), 3.62 (m, 1 H), 3.76 (br d, J = 10 Hz, 1 H), 4.76 (br d, J = 9 Hz, 1 H), 5.56 (s, 1 H), 6.53 (br t, 1 H); MS, m/z 412.

36b: ^{1}H NMR δ 0.8–1.8 (m, 14 H), 0.90 (d, J = 6 Hz, 6 H), 1.42 (s, 9 H), 1.83 (br d, 1 H), 2.51 (dd, J = 15, 10 Hz, 1 H), 2.84 (d, J = 5 Hz, 1 H), 2.95 (d, J = 5 Hz, 1 H), 3.05 (t, J = 7 Hz, 2 H), 3.13 (d, J = 5 Hz, 1 H), 3.60 (m, 1 H), 3.84 (m, 1 H), 4.68 (br d, J = 10 Hz, 1 H), 6.48 (br t, 1 H); MS, m/z 412.

(2*S*,4*S*,5*S*)-*N*-Isopentyl-5-[[(*tert*-butyloxy)carbonyl]amino]-6-cyclohexyl-2,4-dihydroxyhexane-2-carboxamide (37a). A suspension of 159 mg (0.38 mmol) of **35a** and 160 mg of 20% palladium on charcoal in 20 mL of methanol was shaken under a H_2 atmosphere for 12 h. After filtration and concentration in vacuo, purification by flash column chromatography using 3:1 chloroform/ethyl acetate gave 99 mg (62%, 81% based on recovered **35a**) of **37a** (R_f 0.45, 3:2 chloroform/ethyl acetate): ^{1}H NMR δ 0.8–1.8 (m, 17 H), 0.92 (d, J = 6 Hz, 6 H), 1.38 (s, 3 H), 1.45 (s, 9 H), 2.16 (dd, J = 15, 2 Hz, 1 H), 3.2–3.4 (m, 3 H), 3.66 (m, 1 H), 4.43 (br s, 1 H), 4.68 (br d, J = 7 Hz, 1 H), 5.34 (br s, 1 H), 7.08 (br t, 1 H); MS, m/z 429 (M + H).

(2*R*,4*S*,5*S*)-*N*-Isopentyl-5-[[(*tert*-butyloxy)carbonyl]amino]-6-cyclohexyl-2,4-dihydroxyhexane-2-carboxamide (38a). In a manner analogous to the manner of preparation of **37a**, 145 mg of **36b** was converted to 99 mg (68%) of **38a** (R_f 0.32, 3:2 chloroform/ethyl acetate) after purification by flash column chromatography using 3:1 chloroform/ethyl acetate: ^{1}H NMR δ 0.8–1.8 (m, 16 H), 0.92 (d, J = 6 H), 1.42 (s, 3 H), 1.45 (s, 9 H), 1.89 (br d, 2 H), 3.27 (q, J = 7 Hz, 2 H), 3.44 (br d, J = 4 Hz, 1 H), 3.63 (m, 1 H), 3.84 (m, 1 H), 4.68 (br d, J = 9 Hz, 1 H), 5.09 (br s, 1 H), 6.95 (br t, 1 H); MS, m/z 429 (M + H).

(2*R*,4*S*,5*S*)-*N*-Isobutyl-1-azido-5-[[(*tert*-butyloxy)carbonyl]amino]-6-cyclohexyl-2,4-dihydroxyhexane-2-carboxamide (37b). A solution of 51.0 mg (0.124 mmol) of **35b**, 24 mg (0.37 mmol) of sodium azide, and 15 mg (0.28 mmol) of ammonium chloride in 7 mL of methanol was heated at reflux for 18 h. The resulting mixture was partitioned between chloroform and water, dried over Na_2SO_4, and concentrated to give 55 mg (98%) of **37b**, which was homogeneous by TLC (R_f 0.54, 3:2 chloroform/ethyl acetate): ^{1}H NMR δ 0.8–1.9 (m, 15 H), 0.95 (d, J = 7 Hz, 6 H), 1.46 (s, 9 H), 2.06 (br d, J = 14 Hz, 1 H), 3.12 (m, 2 H), 3.31 (d, J = 12 Hz, 1 H), 3.35 (m, 1 H), 3.46 (d, J = 12 Hz, 1 H), 3.70 (m, 1 H), 4.72 (m, 2 H), 5.83 (br s, 1 H), 7.30 (br t, 1 H); IR (KBr) 2103 cm^{-1}; MS, m/z 456 (M + H).

(2*S*,4*S*,5*S*)-*N*-Isobutyl-1-azido-5-[[(*tert*-butyloxy)carbonyl]amino]-6-cyclohexyl-2,4-dihydroxyhexane-2-carboxamide (38b). In a manner analogous to the manner of preparation of **37b**, 50 mg (0.12 mmol) of **36b** was converted to 54 mg (99%) of **38b** (R_f 0.46, 3:2 chloroform/ethyl acetate): ^{1}H NMR δ 0.8–1.9 (m, 14 H), 0.94 (d, J = 6 Hz, 6 H), 1.46 (s, 9 H), 1.95 (t, J = 6 Hz, 2 H), 3.11 (m, 3 H), 3.37 (d, J = 12 Hz, 1 H), 3.47 (d, J = 12 Hz, 1 H), 3.7–3.8 (m, 2 H), 4.73 (br d, J = 9 Hz, 1 H), 5.98 (br s, 1 H), 7.22 (br t, 1 H); MS, m/z 456 (M + H).

(2*S*,3*S*,5*S*)-*N*-Isobutyl-2-[[(*tert*-butyloxy)carbonyl]amino]-1-cyclohexyl-3,5-dihydroxy-7-methyloctane-5-carboxamide (37c). A solution of 21.4 mg (0.052 mmol) of **35b** in 1 mL of tetrahydrofuran was cooled under a N_2 atmosphere to 0 °C and treated with 0.13 mL (0.26 mmol) of isopropylmagnesium chloride in ether. After 45 min, the mixture was treated with saturated aqueous NH_4Cl, extracted with ether, and dried over $MgSO_4$. Separation by flash column chromatography using 3:1 hexane/ethyl acetate gave 6 mg (25%, 57% based on recovered **35b**) of **37c** (R_f 0.30, 6:1 chloroform/ethyl acetate): ^{1}H NMR δ 0.8–1.8 (m, 18 H), 0.88 (d, J = 6 Hz, 3 H), 0.93 (d, J = 6 Hz, 6 H), 0.96 (d, J = 6 Hz, 3 H), 1.45 (s, 9 H), 2.06 (dd, J = 14, 2 Hz, 1 H), 3.11 (m, 2 H), 3.35 (m, 1 H), 3.66 (m, 1 H), 4.51 (br d, J = 5 Hz, 1 H), 4.72 (br d, J = 7 Hz, 1 H), 5.32 (br s, 1 H), 7.25 (br t, 1 H); MS, m/z 457 (M + H).

(3*S*,5*S*,1′*S*)-5-[1-[[(*tert*-Butyloxy)carbonyl]amino]-2-cyclohexylethyl]-3-hydroxy-3-methyldihydrofuran-2(3*H*)-one (39a). A solution of 16 mg (0.037 mmol) of **37a** in 5 mL of xylenes was heated at reflux for 8 h. After removal of the solvent, purification by flash column chromatography using 3:1 chloroform/ethyl acetate afforded 7.7 mg (60%) of **39a** (R_f 0.16, 4:1 chloroform/ethyl acetate): ^{1}H NMR δ 0.8–1.7 (m, 12 H), 1.45 (s, 9 H), 1.50 (s, 3 H), 1.84 (br d, 1 H), 2.26 (d, J = 8 Hz, 2 H), 2.77 (m, 1 H), 3.90 (m, 1 H), 4.36 (br t, 1 H), 4.51 (br d, 1 H); MS, m/z 341.

(3*R*,5*S*,1′*S*)-5-[1-[[(*tert*-Butyloxy)carbonyl]amino]-2-cyclohexylethyl]-3-hydroxy-3-methyldihydrofuran-2(3*H*)-one (40a). In a manner analogous to the manner of preparation of **39a**, 17 mg (0.040 mmol) of **38a** was converted to 5.9 mg (44%) of **40a** (R_f 0.12, 3:1 hexane/ethyl acetate) after purification by flash column chromatography using 3:1 hexane/

ethyl acetate: ^{1}H NMR δ 0.8–1.7 (m, 11 H), 1.44 (s, 9 H), 1.50 (s, 3 H), 1.82 (br d, 1 H), 2.00 (dd, J = 14, 9 Hz, 1 H), 2.35 (m, 2 H), 3.86 (m, 1 H), 4.38 (br d, J = 9 Hz, 1 H), 4.64 (br t, 1 H); MS, m/z 341.

(3*S*,5*S*,1′*S*)-3-(Benzoyloxy)-5-[1-[[(*tert*-butyloxy)carbonyl]amino]-2-cyclohexylethyl]-3-methyldihydrofuran-2(3*H*)-one (39b). A solution of 6.6 mg (0.019 mmol) of **39a**, 9 μL (0.08 mmol) of benzoyl chloride, 11 μL (0.08 mmol) of triethylamine, and 1 mg of 4-(dimethylamino)pyridine in 0.3 mL of dichloromethane was allowed to stand at ambient temperature for 26 h. Extractive workup followed by flash column chromatography using 4:1 hexane/ethyl acetate afforded 6.6 mg (77%) of **39b** (R_f 0.25, 3:1 hexane/ethyl acetate): ^{1}H NMR δ 0.8–1.8 (m, 12 H), 1.40 (s, 9 H), 1.74 (s, 3 H), 1.85 (br d, 1 H), 2.32 (dd, J = 13, 7 Hz, 1 H), 2.81 (dd, J = 13, 11 Hz, 1 H), 3.92 (m, 1 H), 4.50 (m, 1 H), 4.95 (br d, J = 10 Hz, 1 H), 7.46 (t, J = 7 Hz, 2 H), 7.60 (t, J = 7 Hz, 1 H), 8.04 (d, J = 7 Hz, 2 H); irradiation at either 1.74 ppm or 4.50 ppm resulted in NOE enhancement at 2.32 ppm; MS, m/z 446 (M + H).

(3*R*,5*S*,1′*S*)-3-(Benzoyloxy)-5-[1-[[(*tert*-butyloxy)carbonyl]amino]-2-cyclohexylethyl]-3-methyldihydrofuran-2(3*H*)-one (40b). In a manner analogous to the manner of prepraration of **39b**, 5.5 mg (0.016 mmol) of **40a** was converted to 4.0 mg (56%) of **40b** (R_f 0.35, 3:1 hexane/ethyl acetate) following purification by flash column chromatography using 5:1 hexane/ethyl acetate: ^{1}H NMR δ 0.8–1.9 (m, 13 H), 1.46 (s, 9 H), 1.73 (s, 3 H), 2.35 (dd, J = 15, 6 Hz, 1 H), 2.63 (dd, J = 15, 9 Hz, 1 H), 3.89 (m, 1 H), 4.44 (br d, J = 10 Hz, 1 H), 4.88 (br t, 1 H), 7.45 (t, J = 7 Hz, 2 H), 7.60 (t, J = 7 Hz, 1 H), 8.02 (t, J = 7 Hz, 2 H); irradiation at 1.73 ppm resulted in NOE enhancement at 2.35 ppm; irradiation at 4.88 ppm resulted in NOE enhancement at 2.63 ppm; MS, m/z 446 (M + H).

General Procedure for Peptide Couplings. Procedure A: *N*-[*N*-[(*tert*-Butyloxy)carbonyl]-L-phenylalanyl-L-alanyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2-(*N*-isopentylcarbamoyl)hex-1-ene (11). Compound **7a** (31.5 mg, 0.077 mmol) was treated with 0.5 mL of HCl in dioxane (4 M) and allowed to stand at ambient temperature for 1 h. After removal of the solvent in vacuo, the residue was treated twice with 0.5 mL of anhydrous ether followed each time by concentration in vacuo. The crude amine hydrochloride, obtained as a white solid, was dissolved in 0.6 mL of 2:1 dimethylformamide/dichloromethane and treated with 8.4 μL (0.077 mmol) of 4-methylmorpholine. A solution of 39 mg (0.115 mmol) of Boc-Phe-Ala-OH and 13 μL (0.12 mmol) of 4-methylmorpholine in 0.5 mL of dichloromethane and 0.1 mL of dimethylformamide was cooled to −15 °C and treated with 15 μL (0.12 mmol) of isobutyl chloroformate. After being stirred for 5 min, the solution was treated with the solution of the neutralized amine hydrochloride and stirred at −15 °C for 0.5 h and at ambient temperature for 2 h. After dilution with ca. 10 mL of ethyl acetate, the solution was washed sequentially with 1 mL of 1 M HCl, 1 mL of saturated aqueous $NaHCO_3$, and 1 mL of saturated brine, dried over $MgSO_4$, and concentrated in vacuo. Separation by flash column chromatography using 2.5% methanol in chloroform gave 27 mg (56%) of 11, which was recrystallized from chloroform/hexane.

Procedure B: *N*-[*N*-[(*tert*-Butyloxy)carbonyl]-L-phenylalanyl-L-histidyl]-5(*S*)-amino-1-azido-6-cyclohexyl-2(*R*),4(*S*)-dihydroxy-2-(*N*-isobutylcarbamoyl)hexane (43). A solution of the crude amine hydrochloride (prepared from 20.7 mg (0.0455 mmol) of **37b** in accordance with procedure A), 20.1 mg (0.050 mmol) of Boc-Phe-His-OH, 18 mg (0.14 mmol) of 1-hydroxybenzotriazole monohydrate, and 15 μL (0.14 mmol) of 4-methylmorpholine in 0.8 mL of dimethylformamide was cooled to −23 °C and treated with 9.6 mg (0.050 mmol) of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride. The resulting solution was stirred at −23 °C for 2 h and slowly allowed to warm to ambient temperature overnight. After removal of the solvent in vacuo, the residue was partitioned between ethyl acetate and aqueous $NaHCO_3$, washed with H_2O and saturated brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by flash column chromatography using 5% methanol/chloroform gave 24.3 mg (72%) of **43** as a white solid.

Procedure C: *N*-[*N*-(Ethoxycarbonyl)-L-phenylalanyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2-(*N*-isopentylcarbamoyl)hex-1-ene (23). In accordance with procedure B, 295 mg (0.72 mmol) of **7a** was deprotected and coupled to Boc-His-OH to give, after purification by flash column chromatography using 7.5% methanol/chloroform, 298 mg (76%) of *N*-[*N*-[(*tert*-butyloxy)carbonyl]-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2-(*N*-isopentylcarbamoyl)hex-1-ene. A portion (42.5 mg, 0.078 mmol) was coupled in similar fashion to (ethoxycarbonyl)-L-phenylalanine to give, after flash column chromatography using 7.5% methanol/chloroform, 29.4 mg (57%) of **23**.

***N*-[*N*-[(*tert*-Butyloxy)carbonyl]-L-phenylalanyl-L-alanyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2-(*N*-isopentylcarbamoyl)hex-1-ene 1,2-Oxide (33).** In a manner analogous to the manner of preparation of **35b** and **36b**, 11 mg (0.018 mmol) of 11 was converted to 7.6 mg (67%) of **33** following purification by flash column chromatography using 2% methanol/chloroform.

***N*-[*N*-[(*tert*-Butyloxy)carbonyl]-L-phenylalanyl-L-alanyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2-(*N*-methylcarbamoyl)hex-1-ene 1,2-Oxide (34).** In a manner analogous to the manner of preparation of **35b** and **36b**, 30 mg (0.052 mmol) of 18 was converted to 29.8 mg (97%) of **34**, which was recrystallized from dichloromethane/ether/hexane.

1-Amino-5(*S*)-[[*N*-[(*tert*-butyloxy)carbonyl]-L-phenylalanyl-L-histidyl]amino]-6-cyclohexyl-2(*R*),4(*S*)-dihydroxy-2-(*N*-isobutylcarbamoyl)hexane (48). A mixture of 8.2 mg (0.011 mmol) of **43**, 2 μL (0.035 mmol) of acetic acid, and ca. 5 mg of 10% palladium on carbon in 0.5 mL of methanol was stirred under a H_2 atmosphere for 16 h. After filtration and removal of the solvent, the residue was passed through a column of basic alumina using 1:1 methanol/ethyl acetate, concentrated, diluted with chloroform, filtered, and concentrated to give 6.9 mg (88%) of **48** as a white solid.

1-Amino-5(*S*)-[[*N*-[(*tert*-butyloxy)carbonyl]-L-phenylalanyl-L-histidyl]amino]-6-cyclohexyl-2(*S*),4(*S*)-dihydroxy-2-(*N*-isobutylcarbamoyl)hexane (49). In a manner analogous to the manner of preparation of **48**, 9.8 mg (0.013 mmol) of **44** was converted to 7.2 mg (76%) of **49** after purification on basic alumina using 1:1 methanol/ethyl acetate.

Biological Methods. Purified human renal renin[14] was assayed by utilizing pure human angiotensinogen[18] at pH 6.0 in maleate buffer. Tests compounds were dissolved in DMSO and diluted so that prior to addition to the assay system the solutions were 10% in DMSO 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%; DMSO, 1%. At least three different concentrations of inhibitor which bracketed the IC_{50} were preincubated with renin for 5 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} (the concentration causing 50% inhibition) 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% DMSO in the final incubation mixture caused no statistically significant effect on the renin activity.

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