MeI, 74-88-4; C_2H_5I, 75-03-6; $Me(CH_2)_2I$, 107-08-4; Me_2CHI, 75-30-9; $Me(CH_2)_3I$, 542-69-8; $Me(CH_2)_7I$, 629-27-6; $C_6H_5CH_2I$, 620-05-3; C_6H_5NCS, 103-72-0; 4-MeC_6H_4NCS, 622-59-3; 3,4-$Me_2C_6H_3NCS$, 19241-17-9; 4-$F_3CC_6H_4NCS$, 1645-65-4; 4-$MeCOC_6H_4NCS$, 2131-57-9; 3-NCC_6H_4NCS, 3125-78-8; 4-NCC_6H_4NCS, 2719-32-6; 4-$O_2NC_6H_4NCS$, 2131-61-5; 4-$MeOC_6H_4NCS$, 2284-20-0; 4-$MeSC_6H_4NCS$, 15863-41-9; 4-MeS(O)C_6H_4NCS, 109335-70-8; 3-FC_6H_4NCS, 404-72-8; 4-FC_6H_4NCS, 1544-68-9; 2-ClC_6H_4NCS, 2740-81-0; 3-ClC_6H_4NCS, 2392-68-9; 4-ClC_6H_4NCS, 2131-55-7; 3,4-$Cl_2C_6H_3NCS$, 6590-94-9; 3-BrC_6H_4NCS, 2131-59-1; 4-BrC_6H_4NCS, 1985-12-2; C_6H_5NCO, 103-71-9; C_6H_5NHMe, 100-61-8; 4-$MeC_6H_4NH_2$, 106-49-0.

Renin Inhibitors. Statine-Containing Tetrapeptides with Varied Hydrophobic Carboxy Termini

Mark G. Bock,* Robert M. DiPardo, Ben E. Evans, Kenneth E. Rittle, Joshua Boger,† Martin Poe,† Bruce I. LaMont, Robert J. Lynch, Edgar H. Ulm, George P. Vlasuk, William J. Greenlee,† and Daniel F. Veber

Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486, and Rahway, New Jersey 07065.
Received April 10, 1987

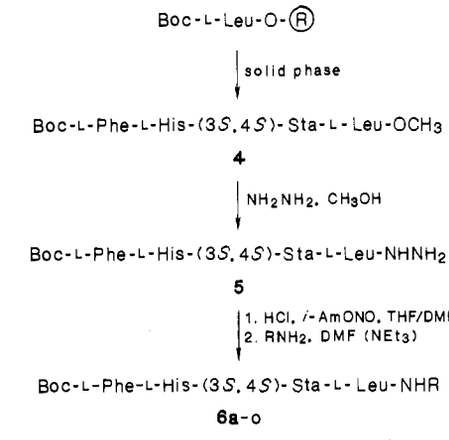
A series of statine-containing tetrapeptides, systematically modified at the carboxy terminus with various hydrophobic aromatic groups, is described. These compounds were tested in vitro for their ability to inhibit porcine, human plasma, and purified human kidney renins. These analogues help to define optimal binding aspects in a region of the enzyme that appears to be specific for spatial arrangement of aromatic groups. Replacement of the metabolically labile Phe amide with nonpeptidal groups proved possible while achieving inhibitory potency in the nanomolar range vs. porcine kidney renin. For the compounds **6i**, **6m**, and **6o**, a large discrepancy in potency between the human plasma and the purified human kidney renin assays was observed. This disparity does not appear to be a consequence of a previously proposed plasma binding component.

In two recent reports[1,2] we described the synthesis and renin-inhibitory activity of modified tetrapeptides. The common features of these potent renin inhibitors are twofold. First, they contain the unusual amino acid statine, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid, which has been postulated to serve as a $Leu^{10}Leu^{11}$ dipeptide surrogate in renin inhibitors designed to resemble angiotensinogen[3,4] (e.g. compound 1, Table II) and, by extrapolation, to mimic the tetrahedral intermediate for the renin–angiotensinogen reaction. Secondly, these renin inhibitors are smaller peptides than most of the previously reported renin inhibitors of equal potency and contain nonpeptidal fragments. Considering the high substrate specificity exhibited by renin, for which the minimum kinetically competent substrate is an octapeptide around the cleavage site,[5] and the demonstrated rate-enhancing effects of peptide substituents distal to the cleavage site,[5,6] decreasing peptide chain length and making fundamental alterations of the substrate peptide might have been expected to result in less potent renin inhibitors. However, our preliminary studies[1,2] showed that a reduction of peptide chain length was possible without suffering unacceptable losses in inhibitor potency and further, that alterations at the carboxy terminus of the known substrate analogue inhibitors Boc-Phe-His-Sta-Leu-Phe-NH_2 (**2**) and Boc-Phe-Phe-Sta-Leu-Phe-NH_2 (**3**) were allowed and in some instances highly favorable.

The purpose of this study was to identify suitable amino acid replacements for the Phe residue and to determine if they could be incorporated at the C-terminus in the renin inhibitor sequence Boc-L-Phe-L-His-(3*S*,4*S*)-Sta-L-Leu-L-Phe-NH_2 (**2**) with positive effect. As the dominant influence in this position appears to be hydrophobic in nature, these substitutions were chosen to probe varying placements of aryl and substituted aryl groups. An additional aim of this work was to further define a previously described phenomenon in which weak inhibition was obtained in the human plasma assay (I_{50}) for a compound(s) determined to be a good inhibitor(s) in the purified human renin assay (K_i).[2,7]

† Merck Sharp & Dohme Research Laboratories, Rahway, NJ.

Scheme I



Results

Chemistry. The tetrapeptide **4** (Scheme I) was synthesized according to standard solid-phase methodology and elaborated to the amides **6a–o** via the intermediacy of acyl hydrazide **5**, followed by azide coupling with the

(1) Bock, M. G.; DiPardo, R. M.; Evans, B. E.; Rittle, K. E.; Boger, J. S.; Freidinger, R. M.; Veber, D. F. *J. Chem. Soc., Chem. Commun.* **1985**, 109.
(2) Evans, B. E.; Rittle, K. E.; Bock, M. G.; Bennett, C. D.; DiPardo, R. M.; Boger, J.; Poe, M.; Ulm, E. H.; LaMont, B. I.; Blaine, E. H.; Fanelli, G. M.; Stabilito, I. I.; Veber, D. F. *J. Med. Chem.* **1985**, *28*, 1755.
(3) 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 (London)* **1983**, *303*, 81.
(4) Boger, J. in *Peptides: Structure and Function*; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Co.: Rockford, IL, 1983; p 569.
(5) His-Pro-Phe-His-Leu-Leu-Val-Tyr: Skeggs, L. T.; Lentz, K. E.; Kahn, J. R.; Hochstrasser, H. J. *J. Exp. Med.* **1968**, *128*, 13.
(6) Fruton, J. S. *Adv. Enzymol.* **1976**, *44*, 1.
(7) 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.

0022-2623/87/1830-1853$01.50/0

corresponding amines. These amines were obtained either commercially or prepared according to the following literature procedures: 1,2-diphenylethylamine (**6c** and **6d**) was resolved[8] with tartaric acid; 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptenamine (**6k**) was synthesized according to the procedure of Evans et al.;[9] *dl-erythro*-1,2-diphenyl-2-hydroxyethylamine,[10] required for the synthesis of **6m**, was derived from benzoin oxime via hydrogenation; and the corresponding *dl-threo*-1,2-diphenyl-2-hydroxyethylamine (**6n**)[10] was available from the *erythro* isomer by inversion of the hydroxyl group via the sequence of N-formylation, oxazoline formation, and hydrolysis. The various compounds synthesized in this manner and their characterization data are collected in Table I.

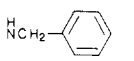
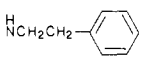
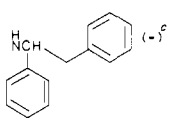
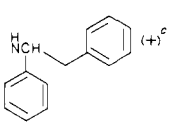
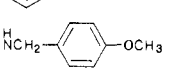
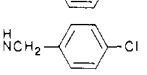
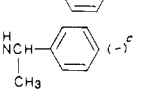
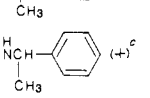
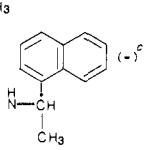
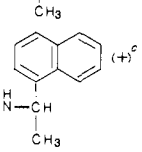
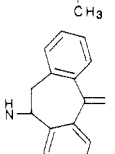
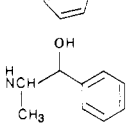
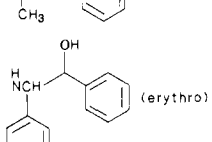
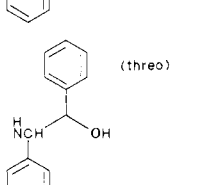
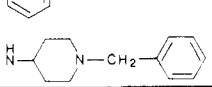
In Vitro Enzyme Inhibition. The inhibitor potencies vs. porcine and human renins of the statine-containing substrate analogues are shown in Table II. The hog kidney inhibitions were measured in an assay using radioactively labeled synthetic decapeptide substrate, Ile-His-Pro-Phe-His-Leu-[^{14}C]Leu-Val-Tyr-Ser, using the protocol described in the Experimental Section.[11] Inhibitions are expressed as I_{50} values and were calculated for several inhibitor concentrations bracketing the I_{50}. The human renin inhibitions were determined in four different assays: human plasma renin activity was measured by radioimmunoassay for angiotensin I production from the endogenous plasma substrate[12] (column 2); inhibition of highly purified human kidney renin[13] was determined by radioimmunoassay using the natural substrate partially pure angiotensinogen (column 3) by radioimmunoassay using a synthetic tetradecapeptide renin substrate (column 4), and by a fluorometric assay using the same synthetic tetradecapeptide renin substrate[14] (column 5). Analyses of the inhibition kinetics were consistent with competitive inhibition, and K_i values were calculated from fits of the experimental data to a standard competitive inhibition equation.[14] For the human plasma renin assay, the concentrations of human renin and renin substrate (angiotensinogen) were not measured.

Discussion

The design of renin inhibitors containing nonpeptidal carboxy termini was initially guided by the structure–activity data derived from the porcine renin assay. The results of the present study corroborate our earlier findings[1,2] that, for good inhibition, the analogy between renin substrate and statine-containing renin inhibitors need not be exact with respect to substrate positions 12 and 13. As indicated in Table II, column 1, the peptide sequence of our inhibitors could be shortened to at least four amino acids without subtantial loss of inhibitor potency (cf. 1 with **6a–o**). Further, it appears that the site that interacts with the C-terminal Phe side chain in 1 and 2 adequately accommodates the phenyl ring of the benzyl amide **6a**. The phenethylamide **6b** represents an attempt to optimize the placement of the aromatic moiety relative to the Leu–

Table I. Characterization of Statine-Containing Tetrapeptide Substrate Analogues

Boc-L-Phe[8]-L-His[9]-(3*S*,4*S*)-Sta-L-Leu[12]-R

compd	R	TLC, R_f[a]	HPLC, purity	formula[b]
6a		0.40	97.8	$C_{41}H_{59}N_7O_7$
6b		0.30	97.0	$C_{42}H_{61}N_7O_7$
6c		0.43	99.9	$C_{48}H_{65}N_7O_7$
6d		0.43	93.9	$C_{48}H_{65}N_7O_7$
6e		0.37	98.9	$C_{42}H_{61}N_7O_8$
6f		0.32	95.5	$C_{41}H_{58}ClN_7O_7$
6g		0.43	99.9	$C_{42}H_{61}N_7O_7$
6h		0.46	99.5	$C_{42}H_{61}N_7O_7$
6i		0.51	85[d]	$C_{46}H_{63}N_7O_7$
6j		0.51	93[e]	$C_{46}H_{63}N_7O_7$
6k		0.54	96.7	$C_{50}H_{65}N_7O_7$
6l		0.37	98.2[f]	$C_{43}H_{63}N_7O_8$
6m		0.35	96.5	$C_{48}H_{65}N_7O_8$
6n		0.32	90.8	$C_{48}H_{65}N_7O_8$
6o		0.33	90.1	$C_{46}H_{68}N_8O_7$

[a] Chloroform–ethanol–concentrated ammonia, 80:10:1. [b] Amino acid analyses are within 3% of the theoretical value; the value for Sta was uniformly low (approximately 5–15%) for all compounds including the reference samples Sta and Boc-Sta. [c] Refers to the optical rotation of the amine. [d] Sample contaminated with approximately 15% of the (+)-α-methylnaphthyl diastereomer (HPLC). [e] Contaminated with approximately 7% of the (−)-α-methylnaphthyl diastereomer (HPLC). [f] 3:1 mixture of *dl*-norpseudoephedrine diastereomer (HPLC).

(8) Nakazaki, M.; Mita, I.; Toshioka, N. *J. Chem. Soc. Jpn.* **1963**, *36*, 161.
(9) Evans, B. E.; Anderson, P. S.; Christy, M. E.; Colton, C. D.; Remy, D. C.; Rittle, K. E.; Engelhardt, E. J. *J. Org. Chem.* **1979**, *44*, 3127.
(10) Weijlard, J.; Pfister, K., III; Swanezy, E. F.; Robinson, C. A.; Tishler, M. *J. Am. Chem. Soc.* **1951**, *73*, 1216.
(11) Rich, D. H.; Sun, E. T. O.; Ulm, E. *J. Med. Chem.* **1980**, *23*, 27.
(12) Haber, E.; Koerner, T.; Page, L. B.; Kliman, B.; Purnode, A.-J. *J. Clin. Endocrinol.* **1969**, *29*, 1349.
(13) Slater, E. E.; Strout, H. V. *J. Biol. Chem.* **1981**, *256*, 8164.
(14) Poe, M.; Bergstrom, A. R.; Wu, J. K.; Bennett, C. D.; Rodkey, J. A.; Hoogsteen, K. *J. Biol. Chem.* **1984**, *259*, 8358.

Table II. Inhibition of Porcine and Human Kidney Renin by Statine-Containing Substrate Analogues

		I_{50},[a] 10^{-9} M		K_i, 10^{-9} M: purified human kidney renin[b]		
no.	compound	hog kidney renin (1)	human plasma renin (2)	Angio(RIA)[c] (3)	Syn(RIA)[d] (4)	Syn(Fluor)[e] (5)
1	Iva-His-Pro-Phe-His-Sta-Leu-Phe-NH_2	31	3.0	1.5	17	19[f]
2	Boc-Phe-His-Sta-Leu-Phe-NH_2	63	140	70	1000	190
6a	Boc-Phe-His-Sta-Leu-benzylamide	25	26	70	55	18
6b	Boc-Phe-His-Sta-Leu-phenethylamide	170	164	120	350	36
6c	Boc-Phe-His-Sta-Leu-(−)-1,2-diphenylethylamide	23	1326	700	68	28
6d	Boc-Phe-His-Sta-Leu-(+)-1,2-diphenylethylamide	6.9	151	280	38	29
6e	Boc-Phe-His-Sta-Leu-*p*-methoxybenzylamide	6.7	33	12	100	ND[g]
6f	Boc-Phe-His-Sta-Leu-*p*-chlorobenzylamide	5.0	81	280	50	240
6g	Boc-Phe-His-Sta-Leu-(−)-α-phenylethylamide	22	21	36	20	27
6h	Boc-Phe-His-Sta-Leu-(+)-α-phenylethylamide	14	49	100	6	19
6i	Boc-Phe-His-Sta-Leu-(−)-α-naphthylethylamide	14	51	140	13	0.98
6j	Boc-Phe-His-Sta-Leu-(+)-α-naphthylethylamide	11	484	600	230	130
6k	Boc-Phe-His-Sta-Leu-10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cycloheptenamide	5.8	569	320	520	120
6l	Boc-Phe-His-Sta-Leu-1-hydroxy-1-phenyl-2-propylamide	480	178	220	36	67
6m	Boc-Phe-His-Sta-Leu-*erythro*-1,2-diphenyl-2-hydroxy-ethylamide	90	134	110	0.20	0.12
6n	Boc-Phe-His-Sta-Leu-*threo*-1,2-diphenyl-2-hydroxy-ethylamide	24	841	350	47	28
6o	Boc-Phe-His-Sta-Leu-4-amido-1-benzylpiperidide	280	127	97	0.04	0.064

[a] I_{50} values were reproducible within ±20%. [b] K_i values had an estimated error of ±20%. [c] Radioimmunoassay using angiotensinogen. [d] Radioimmunoassay using synthetic tetradecapeptide renin substrate. [e] Fluorimetric assay for peptidal amino termini (using synthetic tetradecapeptide renin substrate). [f] Reference 1. [g] Not determined.

amide bond since the position in space of the phenyl ring in **6a** must be quite different from that of the Phe side chain in **2**. A priori, the phenethyl group in **6b** would have appeared a better choice than the benzyl group in **6a**, since it more closely resembles the C-terminal phenylalanine side chain in **2**. Nevertheless, **6b** was approximately 7 times less potent than the simple benzylamide **6a** and suggests the possibility of independent binding sites for two different locations of the phenyl ring. The expedient of combining the features of **6a** and **6b** afforded the 1,2-diphenylethylamides **6c** and **6d**. The 1,2-diphenylethylamide **6d**, whose absolute configuration corresponds to that of L-Phe, was even more potent than **6a** and approximately 10 times more potent than the standard pentapeptide **2** while the diastereomer **6c** is only as potent as the benzylamide **6a**. These small but significant differences are consistent with the phenyl group of the benzyl amide interacting with a binding site for the amide group of **2** and this group contributing more than the phenyl of the side chain. Thus, **6d**, which corresponds to the L-amino acid, allows interaction of both phenyl rings while **6c**, wherein the phenethyl group corresponds to the D-amino acid, allows only the "best" interaction (the benzyl group) and places the second best (the phenethyl group) in an area of no interaction. A conformationally restricted analogue of the diphenylethyl amides **6c** and **6d**, the racemic cycloheptenamide **6k**, was as potent as the most active isomer **6d** and may thus indicate a possible conformation in the bound state.

When a hydroxy group was added to the diphenethyl amide in an unsuccessful attempt to improve water solubility, potency was reduced but is optimal in the racemic threo isomer **6n**. There was little stereoselectivity in those derivatives that contained enantiomeric C-termini (**6g** and **6h**, **6i** and **6j**) when only one aromatic moiety was present while the diastereomeric C-termini in **6m** and **6n** resulted in differences similar to those seen between **6c** and **6d**. Additional modifications of the benzyl group revealed further increased potency by para substitution with a methoxy or a chloro group (**6e**, **6f**).

The inhibition of human plasma renin by the compounds **1**, **2**, and **6a–o** is shown in Table II, column 2 and points out differences between the hog and human enzymes. For example, no corresponding potency increase was obtained with the addition of a phenyl group to the benzyl amide **6a** (cf. **6c** and **6d**). This result, perhaps a consequence of the differences in the substrate sequences required for hog and human renin (e.g. His-13 replaces Tyr-13 in human), contradicts the analysis based on porcine kidney renin inhibition that invokes the presence of a separate binding site for the additional phenyl group. Inspection of the data obtained for **6c**, **6d**, **6i**, and **6j** speaks against the existence of a selective aromatic binding site on human renin. The absolute configuration of the (−)-α-naphthylethylamide moiety in **6i** is *S*; consequently, this places the naphthyl group in an area occupied by the Phe–amide bond in **2**. The naphthyl group in **6i** is, therefore, in the same relationship to the peptide chain as the one phenyl group of the (+)-1,2-diphenylethylamide moiety **6d**, which was also optimum for hog.

Slightly enhanced stereoselectivity was observed in derivatives with enantiomeric C-termini (**6c** and **6d**, **6i** and **6j**) in the human plasma renin assay. On the other hand, with the diastereomeric C-termini in **6m** and **6n**, the threo isomer **6n** was optimum for the hog, whereas the erythro isomer **6m** was more potent in the human. In the latter compounds, the addition of the hydroxy group adds little in terms of potency, in view of the similar potency of **6d**.

In addition to the human plasma renin assay, compounds **1**, **2**, and **6a–o** were also measured for their ability to inhibit purified human kidney renin, Table II, columns 3–5. While the system using endogenous renin and substrate is likely to provide a more direct answer to the question of in vivo activity of these compounds in humans, it was judged that the purified human kidney renin assay could be used to gauge the intrinsic potency of these compounds and, therefore, possibly offer important information to guide synthetic activities. Within this frame of reference, the recently reported studies from this laboratory[2] were extended to include compounds **6a–o**. Analysis of these data brings several discrepancies to the fore. For a number of compounds, the most prominent being **6i**, **6m**, and **6o**, we note a sizeable disparity in potency between human plasma and the human kidney renin

assays employing synthetic substrate (column 2 vs. 4 and 5). In previous reports[2,7] it has been suggested that the disparity in potencies between the human kidney (column 5) and the human plasma (column 2) assays, for certain lipophilic compounds (e.g. **6o**), could be attributed to some extremely tight specific or nonspecific absorption to some plasma components, thereby preventing the intrinsic renin inhibition from being manifested. Since not all hydrophobic compounds show this discrepancy between the human plasma assay and the purified human renin fluorescence assay results, (cf. **6a**, **6g**, **6h**) the variable(s) responsible for this phenomenon was not readily defined. Others have noted differences in activity when human renin inhibition is determined in both plasma and human kidney renin assays.[15-17] In some cases, degradation of the inhibitor in plasma may be responsible; in others, lower pH in the human kidney renin assay or other differences may contribute. Although we have obtained direct physical evidence for binding of some inhibitors by plasma components,[2] it was not clear that such an effect could markedly reduce their inhibitor potency. We have now found, however, that when measurement of purified human kidney renin inhibition is carried out using human angiotensinogen as substrate, activity for inhibitors **6i**, **6m**, and **6o** matches closely that determined in the human plasma assay. The potency determined for **6o** in the angio (RIA) assay is also in agreement with that reported by Arrowsmith and co-workers[18] (IC_{50} = 190 nM) in a RIA using human kidney renin and synthetic substrate (hog sequence). Thus it is not clear that the synthetic (tetradecapeptide) substrate is alone responsible for the occasional overestimaton of potency obtained by the fluorometric assay. Additional factors, such as the absence of other proteinase inhibitors, or difficulties involving the detection method (fluorescence vs RIA) may be responsible.[19] A definitive explanation for this effect and other aspects of the fluorescence assay remain to be determined.

Conclusions

A systematic attempt to replace the carboxy terminal portion of the peptidal renin inhibitor **2** with nonpeptidal equivalents has been partially successful. Differences in binding selectivity for this region of the molecule have been recognized between human and porcine renins. The phenylalanine amide group, known to be a site of hepatic metabolism,[20] can be replaced by several classes of aromatic groupings in both unconstrained and conformationally constrained form. Our present findings have uncovered additional discrepancies between human plasma renin and purified human kidney renin assays. Indications are that the subnanomolar potencies of shortened peptide renin inhibitors observed in the purified human kidney renin assays employing synthetic tetradecapeptide substrate are in certain cases artifactually high.

Experimental Section

Chemical. Melting points were determined in open capillary tubes and are uncorrected. ^1H NMR spectra were recorded on a Varian EM-390, a Varian XL-300, or a Nicolet NT-360 spectrometer. Chemical shifts are reported as δ values relative to Me_4Si as internal standard. IR spectra were obtained on a Perkin-Elmer 295 spectrophotometer. Amino acid analyses were carried out on a 121 MB Beckman amino acid analyzer using Beckman Type AA-10 spherical resin. HPLC was carried out on a Hewlett-Packard 1084B instrument. Flash chromatography was performed on silica gel (E. Merck, 0.04–0.063 mm), and thin-layer chromatography (TLC) and preparative thick-layer chromatography (PLC) were carried out on E. Merck 60F-254 precoated silica gel plates (0.25, 0.5 and 2 mm). Visualization was done with UV light, iodine vapor, ninhydrin, phosphomolybdic acid, and/or *tert*-butyl hydroperoxide–starch–iodine. When elemental analyses are indicated only by symbols of the elements, the analytical results obtained for these elements are within 0.4% of the theoretical values or 3% in the case of amino acid analyses.

(*tert*-Butyloxycarbonyl)-L-phenylalanyl-L-histidyl-(3*S*,4*S*)-statyl-L-leucine Methyl Ester (4). This material was prepared by standard solid-phase methodology[21] using Boc-L-Leu esterified to 2% cross-linked polystyrene–divinylbenzene copolymer (15.32 g, 15 mmol). The N^{α}-Boc derivatives of Sta, His-DNP, and Phe were coupled with dicyclohexylcarbodiimide (DCC) with 1 equiv of the additive 1-hydroxybenzotriazole hydrate. The Sta was prepared in accordance with Evans et al.[22] The Boc groups were removed with 40% trifluoroacetic acid. A coupling of 16 h (2.5 equiv of each Boc-amino acid) was used except for Sta. Complete coupling were judged by the method of Kaiser.[23] In order to conserve the amounts of Sta employed, an initial coupling using 1.10 equiv of N^{α}-Boc-Sta for 72 h gave approximately 90% complete reaction. The addition of 0.25 equiv more of N^{α}-Boc-Sta plus an equal amount of DCC gave complete coupling after an additional 16 h. The DNP protecting group on His was removed by using 50% mercaptoethanol in DMF. The finished resin peptide was dried and suspended in 150 mL of dry methanol containing 30 mL of triethylamine. The resulting suspension was stirred under nitrogen for 1 h and filtered. This cycle was repeated twice at 6 and 14 h. The combined filtrates were rotoevaporated to give 12 g of the crude methyl ester 4. Flash chromatography (methylene chloride–methanol, 9:1) gave 8.5 g of a yellow oil which was rechromatographed (chloroform–ethanol, 95:5) to afford 6.85 g of the analytical product as a yellow solid: mp 90–94 °C; HPLC >99%; ^1H NMR (Me_2SO-d_6) δ 0.97 (12H, m, CH_3), 1.3 (9 H, s, Boc), 2.1 (2 H, br d, α-CH_2-Sta), 3.6 (3 H, s, OCH_3), 7.2 (5 H, arom). AA Anal. ($C_{35}H_{54}N_6O_8$).

(*tert*-Butyloxycarbonyl)-L-phenylalanyl-L-histidyl-(3*S*,4*S*)-statyl-L-leucine Hydrazide (5). The methyl ester 4 (0.92 g, 1.34 mmol) was dissolved at room temperature in 10 mL of a 1:1 mixture of dry methanol and hydrazine (95%). After 15 min the burgundy-colored reaction mixture was rotoevaporated to dryness at room temperature and the resulting residue was partitioned between ethyl acetate (75 mL) and H_2O (50 mL). The organic layer was washed with H_2O (4 × 50 mL), dried ($MgSO_4$), and concentrated to afford 0.81 g of 5 as a yellow solid: R_f 0.23 (chloroform–ethanol–concentrated ammonia, 80:10:1); ^1H NMR (Me_2SO-d_6) δ 0.95 (12 H, m, CH_3), 1.3 (9 H, s, Boc), 2.05 (2 H, br d, α-CH_2-Sta), no methyl ester. Anal. ($C_{34}H_{54}N_8O_7$) C; H; N: calcd, 16.32; found, 15.69.

General Procedure for the Synthesis of Compounds 6a–o. (*tert*-Butyloxcarbonyl)-L-phenylalanyl-L-histidyl-(3*S*,4*S*)-statyl-L-leucine Benzylamide (6a). Hydrazide 5 (285 mg, 0.415 mmol) was dissolved in 2 mL of dry, degassed DMF,

(15) Plattner, J.; Fung, A. K. L.; Stein, H.; Kleinert, H. D.; Marcotte, P.; Smital, J. R.; Dellaria, J. G.; Sham, H. L.; Luly, J. R.; Rosenberg, S. H.; Kempf, D. J.; Grear, J.; Perun, T. J. *Abstracts of Papers*, 191st National Meeting of the American Chemical Society, New York, NY, April 8-13, 1986; American Chemical Society: Washington, DC, 1986; MEDI 28.
(16) Miyazakki, M.; Toda, N.; Etoh, Y.; Kubota, T.; Kinji, I. *Jpn. J. Pharmcol.* **1986**, *40*, 70P.
(17) Cumin, F.; Evin, G.; Fehrentz, J.-A.; Seyer, R.; Castro, B.; Menard, J.; Corvol, J. *J. Biol. Chem.* **1985**, *260*, 9154.
(18) Arrowsmith, R. J.; Carter, K.; Dann, J. G.; Davies, D. E.; Harris, C. J.; Morton, J. A.; Lister, P.; Robinson, J. A.; Williams, D. J. *J. Chem. Soc., Chem. Commun.* **1986**, 755.
(19) It is interesting to note that the anomalously high potency obtained for **6o** using the fluorescence assay is eliminated by the addition of 1 mM EDTA to the assay mixture. M. Poe: et al., unpublished results.
(20) Boger, J.; Bennett, C. D.; Payne, L. S.; Ulm, E. H.; Blaine, E. H.; Homnick, C. F.; Schorn, T. W.; LaMont, B. I.; Veber, D. F. *Regulatory Peptides (Suppl. 4)*, **1985**, 8.
(21) Erickson, B.; Merrifield, R. B. *Proteins* **1976**, *2*, 257.
(22) Rittle, K. E.; Homnick, C. F.; Ponticello, G. S.; Evans, B. E. *J. Org. Chem.* **1982**, *47*, 3016.
(23) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.

and cooled to −25 °C under nitrogen. To this stirred solution was added 1.1 mmol of freshly prepared 7.1 N hydrochloric acid in dry THF (pH of reaction mixture approximately 1, moist pH paper). To this acidic solution was then added 55 μL (0.42 mmol) of isoamyl nitrite over a period of 10 min. After approximately 1 h, there were added 2 equiv (89 mg) of benzylamine and the reaction mixture was held at −20 °C for 12–14 h. The reaction solution was concentrated under reduced pressure and the residue was partitioned between ethyl acetate (75 mL) and 10% citric acid solution. The phases were separated whereupon the organic layer was washed in succession with 10% citric acid solution (2 × 25 mL), saturated $NaHCO_3$ solution (2 × 25 mL), and brine. The dried organic extracts ($MgSO_4$) were concentrated to give a yellow solid which was purified via PLC (chloroform–ethanol–concentrated ammonia, 80:10:1) to afford the analytical sample as an off-white powder.

(*tert*-Butyloxycarbonyl)-L-phenylalanyl-L-histidyl-(3*S*,4*S*)-statyl-L-leucine-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptenamide (6k). This reaction was carried out by using identical reaction conditions to those described for **6a** except that after the addition of the dibenzo[*a*,*d*]cycloheptenamine (1.1 equival) the pH of the reaction mixture was adjusted to 8–8.5 (moist pH paper) with triethylamine.

Biological Methods. Porcine kidney renin was assayed as described by Rich et al.,[11] using the ^{14}C-Leu-labeled decapeptide substrate Ile-His-Pro-Phe-His-Leu-[^{14}C]Leu-Val-Tyr-Ser, except that the pH was raised to pH 7.3 by the use of 0.05 M citrate/phosphate buffer, 30 °C. Enzyme concentration was determined to be 9×10^{-9} M, substrate concentration was 8.0×10^{-5} M, and substrate $K_M = 5 \times 10^{-5}$ M. IC_{50} values (concentration for 50% inhibition) were determined by linear regression of logit vs. log concentration over a 15–90% inhibition range, using three to six concentrations. IC_{50} values were found to be reproducible within ±20%.

Human plasma renin inhibition was determined by radioimmunoassay for angiotensin I, as described by Haber et al.,[12] using a commercial kit (Clinical Assays, Cambridge, MA) at pH 7.4 (phosphate), 37 °C. Plasma inhibition values (I_{50}) were obtained as described[24] and generally were reproducible within ±30%. 8-Hydroxyquinoline or phenylmethanesulfonyl fluoride were used as angiotensinase inhibitors and both were found to give similar results at pH 7.4.

Assays were carried out as follows. (a) Radioimmunoassay with natural angiotensinogen substrate: 0.02 ng human kidney renin, purified accorindg to Slater and Strout,[13] was incubated with 500 ng of partially purified human angiotensinogen (0.08 μM substrate concentration; about 1% pure; purified from outdated human plasma up through the DE-52 step according to Kokubu et al.[25]), for 60 min at 37 °C in 0.10 ml of 0.1 M citrate phosphate (pH 7.20) with 0.001 mL of inhibitor in 10% methanol or *N*,*N*-dimethylformamide. The reaction was quenched by boiling the mixture for 1 min. The angiotensin I produced was quantitated by a radioimmunoassay by competition with ^{125}I-Ang I (Travenol Labs Ang I radioimmunoassay kit). The assay was linear with time, linear with concentration, and linear with angiotensinogen concentration. (b) Radioimmunoassay with synthetic tetradecapeptide substrate: 0.02 ng purified human kidney renin[13] was incubated at 37 °C for 30 min in 0.10 mL of 0.1 M citrate phosphate buffer (pH 7.20) with 3.8 μM of H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Ser-OH and 0.01 mL of inhibitor, previously dissolved in *N*,*N*-dimethylformamide. The reaction was quenched by heating at 100 °C for 2 min. The angiotensin I generated by renin was assayed with a Gamma Coat [^{125}I] Plasma Renin Activity Radioimmunoassay Kit (Travenol-Genentech Diagnostics) by the procedure described in the kit. Data analysis to derive inhibitor potencies was carried out as previously described.[14] Inhibitor titrations were consistent with rapidly equilibrating, competitive inhibitors. (c) Fluorometric method with synthetic tetradecapeptide substrate: the assay was carried out with purified human kidney renin[13] as previously described[14] at 37 °C, pH 7.20 (0.10 M citrate phosphate). K_i values were determined from linear I/V vs. [I] plots,[26] with an estimated error of ±20%.

Acknowledgment. We thank C. F. Homnick, S. L. Fitzpatrick, J. S. Murphy, J.-P. Moreau, and Dr. D. W. Cochran for analytical support. We thank Dr. R. M. Freidinger for useful discussions and acknowledge the support and encouragement of Dr. P. S. Anderson.

Registry No. **2**, 87063-27-2; **4**, 87700-33-2; **5**, 100901-97-1; **6a**, 109585-11-7; **6b**, 109585-12-8; **6c**, 87727-77-3; **6d**, 87691-40-5; **6e**, 87691-45-0; **6f**, 109585-13-9; **6g**, 87691-42-7; **6h**, 87758-54-1; **6i**, 109585-14-0; **6j**, 109667-72-3; **6k**, 87691-46-1; **6l** (diastereomer 1), 109667-73-4; **6l** (diastereomer 2), 109667-74-5; **6m**, 87691-48-3; **6o**, 87691-51-8; BOC-Sta-OH, 58521-49-6; BOC-His(DNP)-OH, 25024-53-7; BOC-Phe-OH, 13734-34-4; H_2NCH_2Ph, 100-46-9; $H_2NCH_2CH_2Ph$, 64-04-0; (*R*)-(−)-$H_2NCHPhCH_2Ph$, 34645-25-5; (*S*)-(+)-$H_2NCHPhCH_2Ph$, 3082-58-4; $H_NCH_2C_6H_4$-*p*-OMe, 2393-23-9; $H_2NCH_2C_6H_4$-*p*-Cl, 104-86-9; (*S*)-(−)-$H_2NCHMePh$, 2627-86-3; (*R*)-(+)-$H_2NCHMePh$, 3886-69-9; (*S*)-(−)-1-(2-naphthyl)ethylamine, 3082-62-0; (*R*)-(+)-1-(2-naphthyl)ethylamine, 3906-16-9; (±)-10,11-dihydro-5*H*-5-methylenedibenzo[a,d]cyclohepten-10-amine, 87747-58-8; *dl*-norpseudoephedrine, 54680-46-5; *dl*-*erythro*-1,2-diphenyl-2-hydroxyethylamine, 23412-95-5; *dl*-*threo*-1,2-diphenyl-2-hydroxyethylamine, 39664-87-4; *N*-benzyl-4-piperidinamine, 50541-93-0; renin, 9015-94-5.

(24) Poe, M.; Wu, J. K.; Florance, J. R.; Rodkey, J. A.; Bennett, C. D.; Hoogsteen, K. J. *J. Biol. Chem.* **1983**, *258*, 2209.
(25) Kokubu, T.; Hiwada, K.; Sogo, Y. *Jpn. Circ. J.* **1980**, *44*, 274.
(26) Dixon, M. *J. Biochem.* **1953**, *55*, 170.