Renin Inhibitors. Dipeptide Analogues of Angiotensinogen Utilizing a Structurally Modified Phenylalanine Residue To Impart Proteolytic Stability[1]

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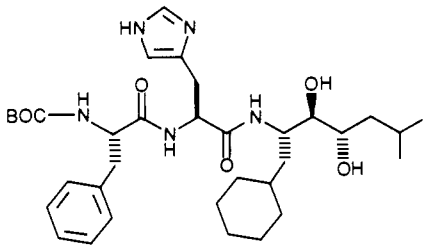
A series of renin inhibitors have been prepared and evaluated for their susceptibility to cleavage by the serine protease chymotrypsin. The compounds were designed by consideration of the structural requirements in the active-site region of renin and chymotrypsin. By systematic alteration of the P_3 phenylalanine residue, compounds with varying degrees of renin inhibitory potency and chymotrypsin susceptibility were obtained. Selected analogues from this group were examined in vivo for both their hypotensive effects and metabolic patterns.

The search for a renin inhibitor possessing therapeutic utility as an antihypertensive agent continues to represent a challenging target for medicinal chemists.[2] Previous publications from our laboratory have described several novel classes of low molecular weight renin inhibitors obtained by structural modification of the Leu-Val portion of the renin substrate.[3] More recently, we have reported a new class of renin inhibitors characterized by the presence of an erythro glycol function replacing the scissile amide bond of angiotensinogen.[4] These dipeptide analogues, of which compound I is prototypical, display as-



I

toundingly high inhibitory potency relative to their low molecular weight. The high affinity of compounds such as I for human renin is also accompanied by a facile susceptibility to the serine protease chymotrypsin (which rapidly cleaves these derivatives between the Phe and His residues in vitro). Since stability to proteolytic enzymes has been implicated as a requirement for developing orally active peptides,[5] we sought to identify analogues of I that are stable to degrading enzymes and that retain a high inhibitory potency for human renin. The results of this study are reported herein.[6]

Chemistry

The compounds prepared for this study are shown in Table I and their syntheses are outlined in Schemes I–VIII. The majority of the dipeptide analogues depicted in Table I were prepared by coupling a modified phenylalanine derivative 1 to the histidyl intermediate **2**[4] by using the standard DCC/HOBT procedure (Scheme I). Small amounts of racemization occurred in the coupling procedure; however, the resulting dipeptide analogues **3** were readily purified by chromatography and/or recrystallization. Preparation of the requisite phenylalanine intermediates containing an N-terminal urea function was carried out as shown in Scheme II. L-Phenylalanine methyl ester hydrochloride was converted to the corresponding α-isocyanato derivative as reported.[7] Reaction of this intermediate with the appropriate secondary amine at 0 °C gave the substituted ureas, which produced the desired carboxylic acids **1a–e** by hydrolysis with LiOH in dioxane/H_2O. Acids **1f**, **1g**, and **1h** were prepared analogously, starting with the methyl esters of homo-Phe, D-Phe, and *p*-OMe-Phe. The synthesis of carbamate intermediate **1i** started with L-phenyllactic acid (Scheme III). Esterification of this acid with MeOH/H_2SO_4 was followed by treatment with phosgene to give the corresponding chloroformate. Acylation of morpholine and ester hydrolysis then completed the synthesis. The preparation of **1j** began with α-ethyl α-benzalsuccinate (*E* stereochemistry), prepared by Stobbe condensation of benzaldehyde with diethyl succinate.[8] Acylation of morpholine with this acid was followed by ester hydrolysis to give **1j** (Scheme IV).

Succinate **1k** was synthesized in enantiomerically pure form by application of the Evans' chiral enolate methodology (Scheme V).[9] Thus, dihydrocinnamoyl chloride was used to acylate the lithio salt of 4-(2-propyl)oxazolidin-2-one to give **4**. Deprotonation of **4** was followed by alkylation with *tert*-butyl bromoacetate to afford the chiral

(1) Portions of this work were presented in December 1987 at the Japanese–United States Congress of Pharmaceutical Sciences in Honolulu, HI.

(2) Greenlee, W. J. *Pharm. Res.* **1987**, *4*, 364.

(3) (a) Rosenberg, S. H.; Plattner, J. J.; Woods, K. W.; Stein, H. H.; Marcotte, P. A.; Cohen, J. *J. Med. Chem.* **1987**, *30*, 1224. (b) Luly, J. R.; Yi, N.; Soderquist, J.; Stein, H.; Cohen, J.; Perun, T. J.; Plattner, J. J. *J. Med. Chem.* **1987**, *30*, 1609. (c) Bolis, G.; Fung, A. K. L.; Greer, J.; Kleinert, H. D.; Marcotte, P. A.; Perun, T. J.; Plattner, J. J.; Stein, H. *J. Med. Chem.* **1987**, *30*, 1729. (d) Kempf, D. J.; de Lara, E.; Stein, H. H.; Cohen, J.; Plattner, J. J. *J. Med. Chem.* **1987**, *30*, 1978.

(4) Luly, J. R.; BaMaung, N.; Soderquist, J.; Fung, A. K. L.; Stein, H. H.; Kleinert, H. D.; Marcotte, P. A.; Egan, D. A.; Bopp, B. A.; Merits, I.; Bolis, G.; Greer, J.; Perun, T. J.; Plattner, J. J. *J. Med. Chem.*, preceding paper in this issue.

(5) Humphrey, M. J.; Ringrose, P. S. *Drug Metab. Rev.* **1986**, *17*, 283.

(6) For an alternative approach to this problem, see: (a) Kempf, D. J.; de Lara, E.; Plattner, J. J.; Stein, H.; Cohen, J.; Marcotte, P. *Abstracts of Papers*, 194th National Meeting of the American Chemical Society, New Orleans, LA, September, 1987; American Chemical Society: Washington, DC, 1987; MEDI 85. (b) Luly, J. R.; Fung, A. K. L.; Plattner, J. J.; Marcotte, P.; BaMaung, N.; Soderquist, J. L.; Stein, H. *Proceedings of the Tenth American Peptide Symposium*; Marshall, G. R., Ed.; ESCOM Science Publishers: Leiden, The Netherlands, 1988; pp 500–502. (c) Sham, H. L.; Bolis, G.; Stein, H. H.; Fesik, S. W.; Marcotte, P. A.; Plattner, J. J.; Rempel, C. A. Greer, J. *J. Med. Chem.* **1988**, *31*, 284.

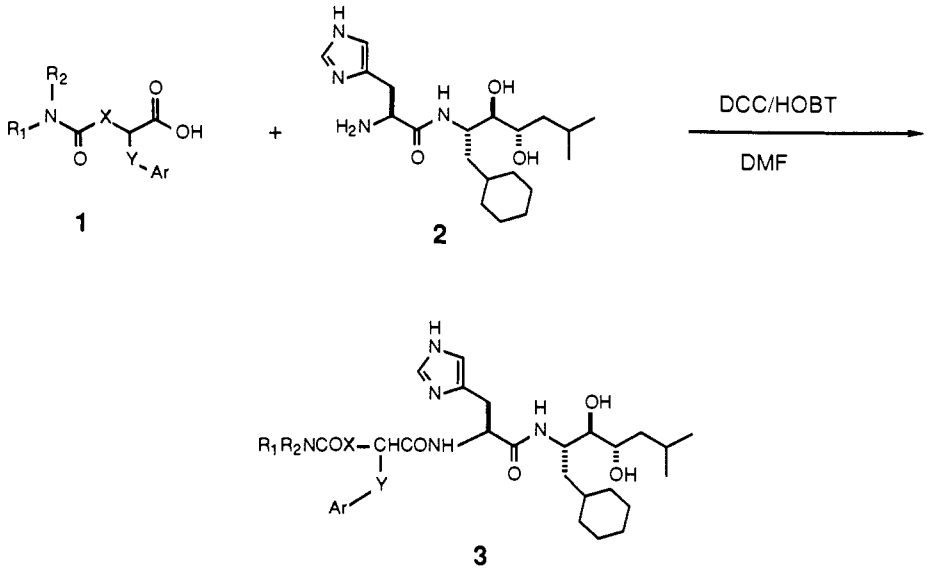
(7) Lombardino, J. G.; Gerber, C. F. *J. Med. Chem.* **1964**, *7*, 97.

(8) Cohen, S. G.; Milovanovic, A. *J. Am. Chem. Soc.* **1968**, *90*, 3495.

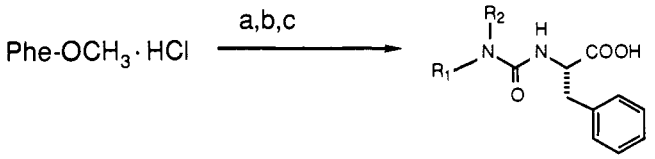
(9) Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 1737.

0022-2623/88/1831-2277$01.50/0

Scheme I

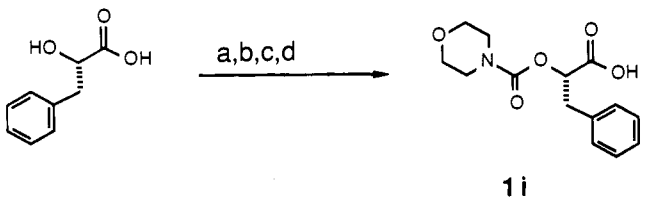


Scheme II[a]



[a] (a) $COCl_2$, toluene, 100 °C. (b) R_1R_2NH, CH_2Cl_2. (c) LiOH, H_2O, dioxane.

Scheme III[a]



[a] (a) H_2SO_4, MeOH. (b) $COCl_2$, CH_2Cl_2, DMF. (c) Morpholine, CH_2Cl_2. (d) LiOH, H_2O, dioxane.

intermediate **5**. Removal of the chiral auxiliary and standard carboxyl modifications of the resulting succinate derivative (**6**) then gave the desired intermediate **1k**.

The synthesis of dehydro-Phe derivative **31** began with β-phenylserine (Scheme VI). Successive esterification and acylation afforded the hydroxy urea **7**. Ester hydrolysis was followed by azlactone formation mediated by acetic anhydride to give **8**.[10] Acylation of dipeptide **2** with **8** then gave **31**. The stereochemistry for the double bond in **31** was assigned as *Z* based upon analogy with literature precedent.[10b]

The β,β-dimethyl Phe intermediate **1m** was synthesized as outlined in Scheme VII. Conjugate addition of phenylmagnesium bromide to diethyl isopropylidenemalonate gave diester **9**.[11] Successive steps of ester hydrolysis, Curtius rearrangement,[12] and ester hydrolysis produced the desired intermediate **1m**.

Analogue **13**, which contains an N-methylated Phe–His amide bond, was prepared as outlined in Scheme VIII. *N*-Boc-*N*-methyl-N^{im}-tosyl-L-histidine (**10**)[13] was coupled to **11**[4] with diethoxyphosphoryl cyanide/triethylamine, and the resulting intermediate detosylated with HOBT/MeOH to give **12**. Cleavage of the Boc group in **12** was followed by coupling with **1e** to afford **13**.

Biological Results and Discussion

Structure–Activity Relationships. In earlier publications from our laboratory, we have shown that renin inhibitors containing Boc-Phe-His or Tba-Phe-His[14] fragments in their structure are susceptible to facile cleavage between the Phe and His residues by chymotrypsin in vitro.[3a,c] Since chymotrypsin is known to be present in the gastrointestinal tract,[15] stability to this enzyme may be a necessary condition for good oral absorption of the intact molecule. Reasoning that the nature of the N-terminal blocking group on a renin inhibitor could alter its susceptibility toward chymotrypsin cleavage, we initially examined several peptide analogues containing N-terminal ureas. In vitro evaluation of these compounds (**3a–e**, Table II) indicated that this structural change led to potent inhibition of both purified and plasma renin. Urea **3b** was also examined in vivo by intravenous administration to salt-depleted, anesthetized cynomolgus monkeys. The data for two separate experiments are shown in Figure 1. A bolus injection of 0.1 mg/kg resulted in an average fall in mean arterial blood pressure of approximately 45 mmHg, in association with complete inhibition of plasma renin activity within 5 min of injection. The duration of both responses persisted for $2^1/_2$ h. Disappointingly, the excellent profile of **3b** as an in vitro and in vivo renin inhibitor was also accompanied by a high susceptibility to chymotrypsin (Table II). This compound is rapidly cleaved by bovine chymotrypsin at 37 °C in vitro with a half-life of 3.3 min, under our screening assay conditions (see the Experimental Section). Thus, N-ter-

(10) (a) Konno, S.; Stammer, C. H. *Int. J. Pept. Protein Res.* **1978**, *12*, 222. (b) Brady, S. F.; Cochran, D. W.; Nutt, R. F.; Holly, F. W.; Bennett, C. D.; Paleveda, W. J.; Curley, P. E.; Arison, B. H.; Saperstein, R.; Veber, D. F. *Int. J. Pept. Protein Res.* **1984**, *23*, 212

(11) Holmberg, C. *Liebigs Ann. Chem.* **1981**, 748.

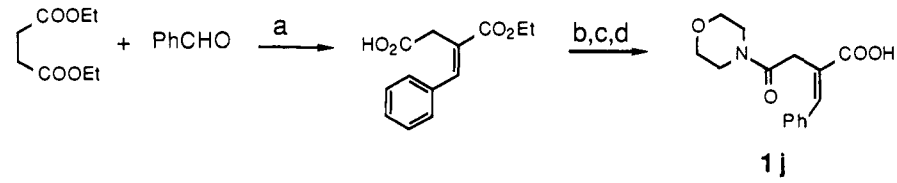
(12) Shioiri, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203.

(13) Thaisrivongs, S.; Pals, D. T.; Harris, D. W.; Kati, W. M.; Turner, S. R. *J. Med. Chem.* **1986**, *29*, 2088.

(14) Tba = *tert*-butylacetyl.

(15) Berne, R. M.; Levy, M. N., Eds. *Physiology*; Mosby: St. Louis, 1983; p 784.

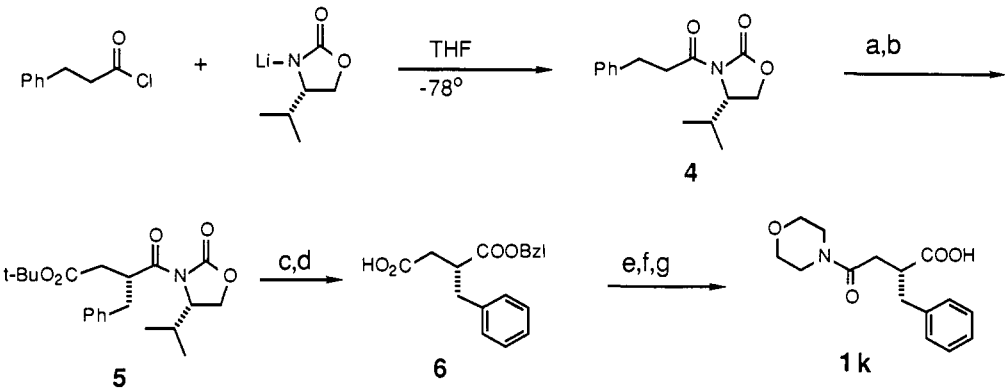
Scheme IV[a]



1j

[a] (a) NaOEt, EtOH. (b) $ClCO_2$-*i*-Bu, *N*-Methylmorpholine. (c) Morpholine. (d) NaOH, H_2O.

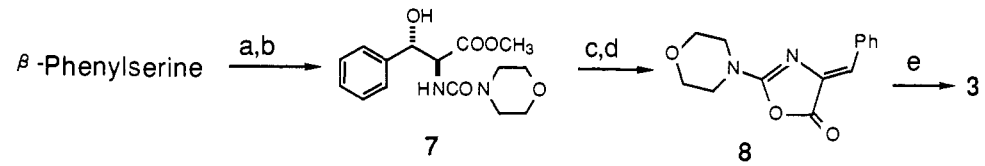
Scheme V[a]



5 **6** **1k**

[a] (a) Sodium hexamethyldisilylamide, THF, –78 °C. (b) $BrCH_2CO_2$-*t*-Bu. (c) LiOBzl, THF, –0 °C. (d) CF_3COOH, CH_2Cl_2. (e) $ClCO_2$-*i*-Bu, *N*-methylmorpholine. (f) Morpholine. (g) H_2/Pd–C.

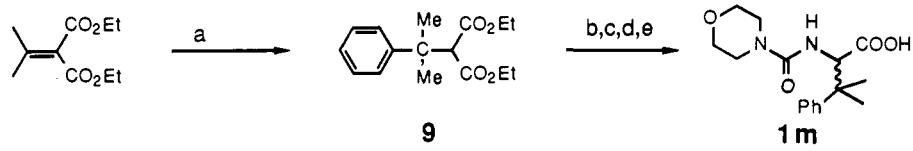
Scheme VI[a]



7 **8**

[a] (a) $SOCl_2$, MeOH. (b) *N*-(Chloroformyl)morpholine. (c) NaOH. (d) Ac_2O. (e) Compound **2**.

Scheme VII[a]



9 **1m**

[a] (a) PhMgBr, CuBr·MeSMe, Et_2O. (b) KOH, EtOH, H_2O. (c) DPPA, Et_3N. (d) Morpholine. (e) NaOH.

minal urea blocking groups are similar to both carbamate and amide functions with respect to their influence on the stability of the adjacent amide bond.

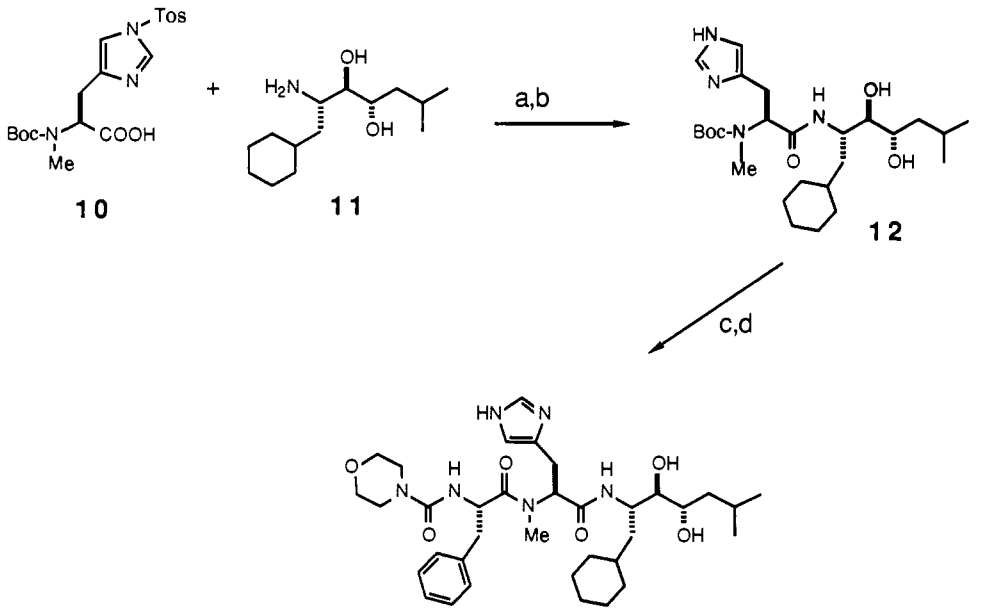
The inability to stabilize the Phe–His amide bond in renin inhibitors by alteration of the N-terminal blocking group prompted us to evaluate other portions of the structure as a means to impart enzymatic stability. Examination of the published specificity requirements for chymotrypsin[16] reveals several critical interactions for efficient binding of a substrate to this enzyme (Figure 2). These include a hydrogen bond of the α-NH of Phe with Ser-214 and a van der Waals interaction of the benzyl side chain with a hydrophobic pocket adjacent to the active site. By blocking or modulating either of these interactions, one could presumably reduce the affinity of the renin inhibitor toward chymotrypsin and thus prevent degradation in the intestinal tract. With use of this strategy, compounds **3f–m** and **13** were prepared and evaluated for both enzymatic stability and renin inhibition (Table II). Compound **3e** was selected as the parent molecule for this study, due to its high potency as a renin inhibitor and rapid cleavage by chymotrypsin. Single structural alterations of **3e** were then systematically carried out in order to identify an analogue exhibiting stability toward chymotrypsin while maintaining potent inhibition of human renin.

As can be seen from the data in Table II, the α-nitrogen of Phe can be replaced with either a carbon[17] (**3k**) or an oxygen (**3i**) with maintenance of good renin inhibitory activity and protection against chymotrypsin. In these compounds, the hydrogen bond from the α-NH of the Phe to the carbonyl oxygen of Ser 214 of chymotrypsin (Figure 2) cannot form, making these molecules poor substrates for chymotrypsin. Compounds **3f**, **3h**, and **3m** represent an attempt to prevent optimal binding of the Phe side chain of the renin inhibitor into the hydrophobic pocket of chymotrypsin. As the data indicate, analogues **3h** with

(16) Steitz, T. A.; Henderson, R.; Blow, D. M. *J. Mol. Biol.* **1969**, *46*, 337.

(17) Replacement of the α-NH of Phe with a methylene group has been reported for norstatine-derived renin inhibitors: Iizuka, K.; Kamijo, T.; Kubota, T.; Akahane, K.; Umeyama, H.; Kiso, Y. *J. Med. Chem.* **1988**, *31*, 701.

Scheme VIII[a]



[a](a) $(EtO)_2POCN$, TEA. (b) MeOH, HOBT. (c) HCl/dioxane. (d) BOP-Cl, TEA, compound **1e**.

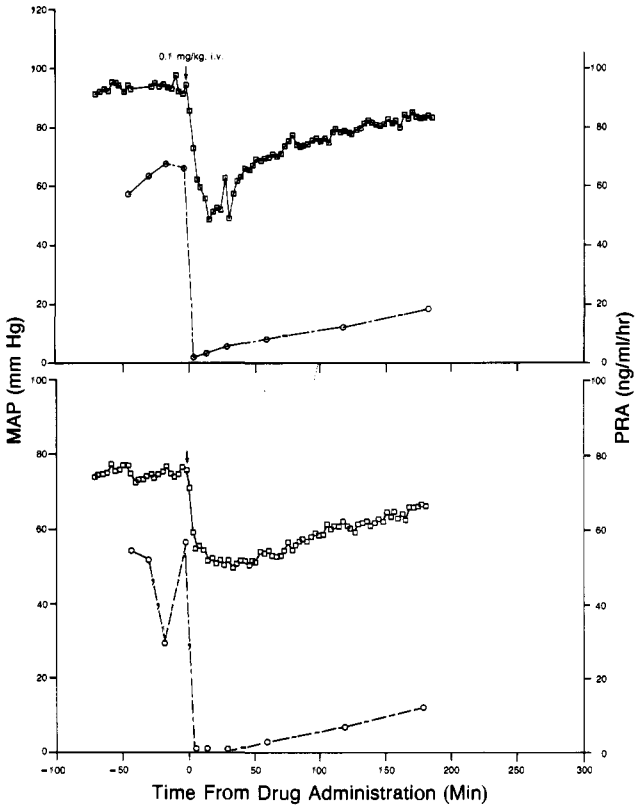


Figure 1. Effect of a single bolus dose of **3b** on mean arterial pressure and plasma renin activity in two anesthetized, salt-depleted monkeys. MAP = mean arterial pressure (□) PRA = plasma renin activity (○).

the *p*-methoxybenzyl side chain and **3m** with the β,β-dimethyl substituents proved to be substantially stabilized against chymotrypsin degradation whereas **3f** with the longer phenethyl substituent was readily cleaved by this enzyme.

These results can be explained by examining the binding mode of these side chains to their respective binding

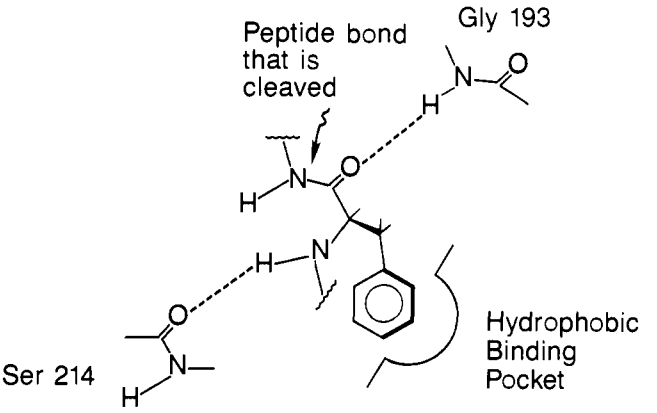
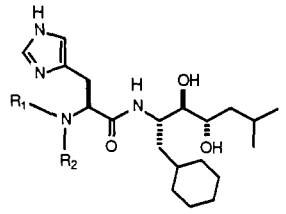
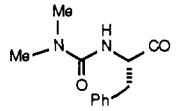
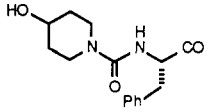
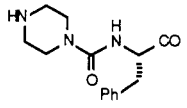
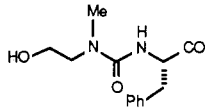
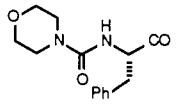
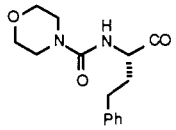
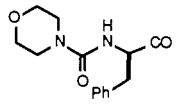
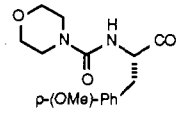
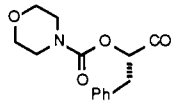
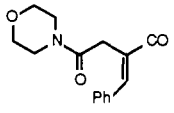
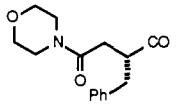
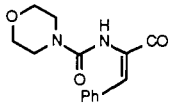
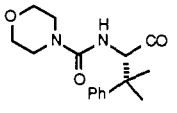
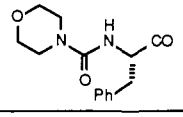


Figure 2. Schematic representation of a peptide bond that is cleaved by chymotrypsin, showing several important interactions of the substrate with the enzyme.

pockets in chymotrypsin and renin (Figure 3). The chymotrypsin pocket is narrower than in renin and normally binds the aromatic side chains of Phe, Tyr, and Trp. The phenyl ring of Phe fits snugly into this site. The phenolic oxygen of **3h** is accommodated as it is in Tyr itself; however, the *O*-methyl collides severely with the wall of the pocket, primarily with the main chain of Ser 217 (Figure 3a). In the same way, the β,β-dimethyls of **3m** also overlap with the residues lining the pocket, with the main-chain portions of residues Cys 191 and Ser 195 as well as with the side chain of Ser 195. The more flexible phenethyl side chain of **3f**, on the other hand, fits very nicely into the chymotrypsin pocket with the phenyl group occupying almost the same volume as would the six-membered ring of the indole in Trp. When these side chains are modeled into the appropriate subsite of our renin model (Figure 3b), all of these side chains fit quite well. Of course, the conformation of these side chains when binding to this pocket may be quite different from that in chymotrypsin (Figure 3a). The closest contacts in renin are a minor overlap of one of the β-methyls in **3m** with the side-chain hydroxyl of Ser 219 of renin that usually forms a hydrogen bond

Table I



compd	R_1	R_2	mp, °C	% yield[a]	formula
3a		H	108–110	61.5	$C_{32}H_{50}N_6O_5 \cdot 2.25H_2O$
3b		H	130–132	42	$C_{35}H_{54}N_6O_6 \cdot 1.5H_2O$
3c		H	170–175	45	$C_{38}H_{61}N_7O_9$
3d		H	78–85	16	$C_{33}H_{52}N_2O_6 \cdot 1.5H_2O$
3e		H	147–150	62.5	$C_{34}H_{52}N_6O_6$
3f		H	137–139	63	$C_{35}H_{54}N_6O_6 \cdot 0.5H_2O$
3g		H	204–205	59	$C_{34}H_{52}N_6O_6 \cdot 0.5H_2O$
3h		H	140–142	66	$C_{35}H_{54}N_6O_7$
3i		H	100–102	35.4	$C_{34}H_{51}N_5O_7 \cdot 0.5H_2O$
3j		H	110–112	40	$C_{35}H_{51}N_5O_6 \cdot 0.5H_2O$
3k		H	181–182	43	$C_{35}H_{53}N_5O_6$
3l		H	138–141	60	$C_{34}H_{50}N_6O_6$
3m		H	120–122	80	$C_{36}H_{56}N_6O_6 \cdot 1.25H_2O$
13		Me	130–135	38.5	$C_{35}H_{54}N_6O_6 \cdot 0.5H_2O$

[a] Represents yield for last coupling step.

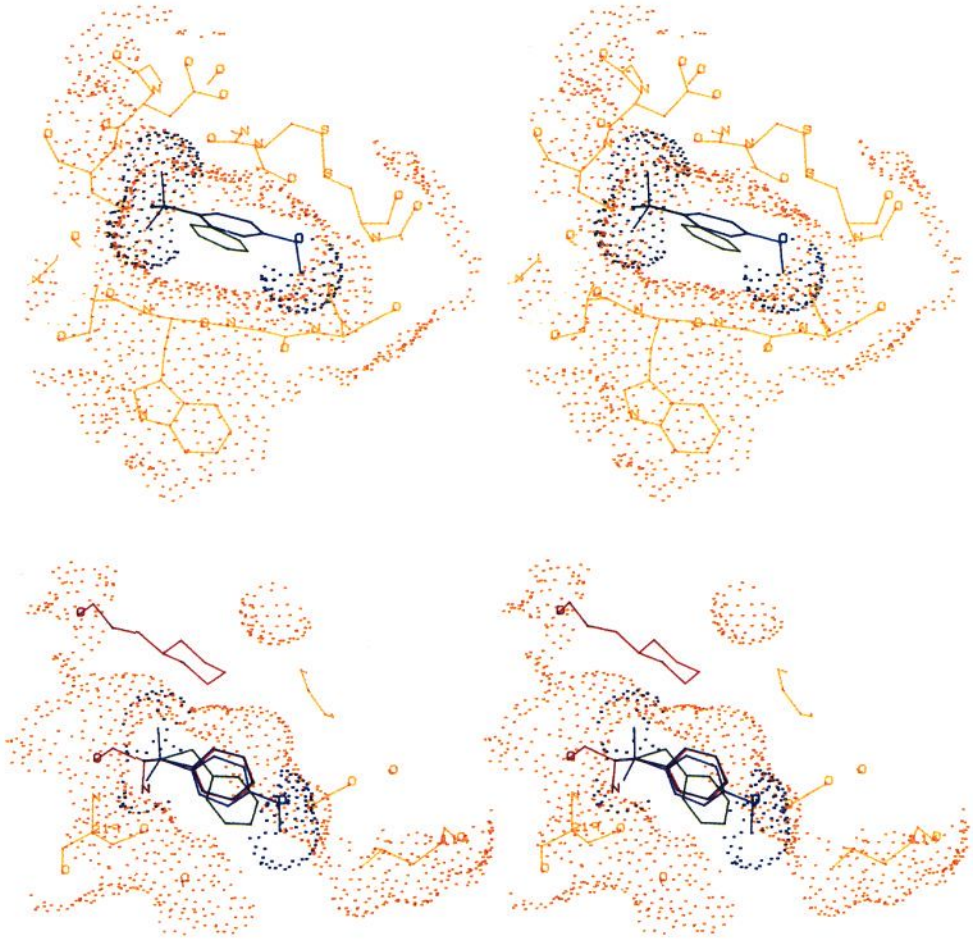


Figure 3. Stereo presentation of the binding pockets for the phenylalanine side chain in chymotrypsin (a, top) and in renin (b, bottom). The view into the respective pocket has been corresponded by aligning the C_α, C_β, and C_γ of the Phe. The enzyme is shown in yellow. The yellow dots represent the solvent exclusion, molecular surface of the enzyme as calculated by Connolly's program.[27] The *p*-methoxybenzyl side chain **3h** (blue), phenethyl **3f** (green), and β,β-dimethyl-Phe **3m** (purple) are shown bound in these two pockets. The van der Waals surfaces for the possible overlapping atoms of **3f** and **3h** are shown in the respective colors. In the renin pocket (b), the cyclohexylalanine residue of the inhibitor is shown in red, as it is an important contributor to the surface of the Phe binding site. Clear overlap is observed for the *O*-methyl of **3h** and the β-dimethyls of **3m** in chymotrypsin. Both of these side chains are accommodated in the renin pocket; the latter with only minor overlap at the hydroxy of Ser 219 (see text).

with the main-chain NH of the dimethyl-Phe of the inhibitor.[6c,18] This close contact can be relieved by small movements of the two groups. However, this effect may explain the somewhat weaker inhibition of renin by **3m** compared to the other modified side chains in this series (Table II).

Attempts to prevent access of the nucleophilic serine residue of chymotrypsin[16] to the susceptible Phe–His amide bond was approached by preparation of the *N*-Me (**13**) and also by the β,β-di-Me (**3m**) analogues (Figure 3a).[19] In both cases, complete stability of the resultant peptide was achieved, although renin inhibitory activity fell off somewhat for **3m** as described above.

The consequences of rigidifying the Phe side chain in the renin inhibitors were explored with analogues **3j** and **3l**. Both of these compounds displayed considerable stability toward chymotrypsin as well as good renin inhibitory activity. Previously, workers have shown that dehydro amino acids are effective in producing enzymatic stability.[10a] Modeling the dehydrophenylalanine compounds into the chymotrypsin active site shows that they cannot bind to chymotrypsin in a productive conformation without causing a major collision with this enzyme. This collision is mainly with residues lining the primary specificity pocket, including the main-chain atoms of Trp 215 and Gly 216. It is due to the trigonal geometry at the α-carbon and the double bond of the dehydrophenylalanine forcing the side chain to lie cis to its main-chain amino group (compound **3l**) or carbon isostere (compound **3j**). The last modification that was evaluated for this series of compounds involved introduction of a D-Phe residue (**3g**). As expected, this change led to complete enzymatic stability but also resulted in a considerable loss of renin inhibitory potency. In this case, the side chain in the D configuration points out into solvent and does not collide with the enzyme. Yet, because the phenyl group cannot reach into the Phe binding pocket at all, binding to the active site is greatly decreased in both cases.

Although the inhibitory potencies of the compounds were always lower when measured against plasma renin at pH 7.4 as compared to renin in the purified assay system at pH 6.0, there was no obvious consistent effect as a function of structure; there were, however, some trends which were noted. The greatest losses in inhibitor po-

(18) Plattner, J. J.; Greer, J.; Fung, A. K. L.; Stein, H.; Kleinert, H. D.; Sham, H. L.; Smital, J. R.; Perun, T. J. *Biochem. Biophys. Res. Commun.* **1986**, *139*, 982.

(19) N-Methylation of the P_3–P_2 amide bond in renin inhibitors has previously been reported (ref 6c and 13).

Table II. Enzyme Inhibition and Chymotrypsin Stability of Renin Inhibitors

compd	IC_{50}, nM: purified human renin	IC_{50}, nM: human plasma renin	ratio[a]	% inhibition[b]: pepsin	% inhibition[b]: cathepsin D	chymotrypsin susceptibility[c]
3a	0.50	3.3	6.6	11	3	17.5
3b	0.42	0.68	1.6	4	2	3.3
3c	0.65	4.5	6.9	0	0	6.9
3d	0.55	0.98	1.8	3	0	34.3
3e	0.35	0.89	2.5	0	0	2.2
3f	0.30	2.0	6.7	6	0	9.6
3g	14	140	10	2	1	stable[d]
3h	0.76	2.4	3.2	4	3	727
3i	0.82	3.0	3.7	0	0	233
3j	0.79	2.1	2.7	8	0	1260
3k	0.29	1.3	4.5	0	0	227
3l	0.58	5.3	9.1	0	0	stable[d]
3m	2.6	65	25	3	3	stable[d]
13	0.94	2.5	2.7	0	0	stable[d]

[a] Ratio of IC_{50} value for human plasma renin (pH 7.4) and purified human renin (pH 6.0). [b] Percent inhibition at 1×10^{-5} M. [c] $t_{1/2}$ (min) for degradation by chymotrypsin. [d] No detectable degradation at 6 h.

tencies in plasma occurred when the Phe residue was perturbed: dimethyl substitution (**3m**, 25-fold), D configuration (**3g**, 10-fold), or introduction of a dehydro bond (**3l**, 9.1-fold). A dehydro bond alone was not sufficient for a large potency loss since **3j**, which was a dehydro Phe compound without the α-NH as in **3l**, demonstrated only a 2.7-fold loss. Those compounds with an OH function as part of the N-terminal blocking group (**3b** and **3d**) suffered the smallest losses in plasma, factors of 1.6 and 1.8, respectively. The remainder of the compounds with unsubstituted L-Phe demonstrated losses in the range of 2.5–6.9-fold; p-OCH_3 substitution (**3h**) and the next higher homologue (**3f**) also fell into this range.

The marked specificity of this class of inhibitors for renin, as opposed to other aspartic proteinases, can be seen from the data in Table II; the IC_{50} for inhibition of renin was in the nanomolar range while very little or zero inhibition of either pepsin or cathepsin D was observed at 1×10^{-5} M.

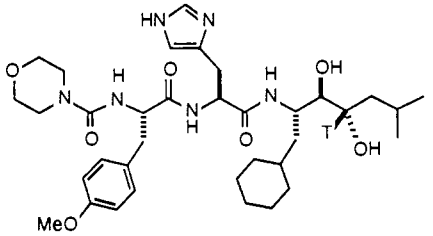
In Vivo Studies. Our ultimate goal in designing a metabolically stable peptide analogue is to identify a renin inhibitor with good oral bioavailability and which shows a long-lasting hypotensive effect. We expected that the characteristic of enzymatic stability would not only promote enhanced gastrointestinal transportability but would also lead to a prolonged half-life in the blood. In order to explore these objectives we have examined the biological effects of compounds **3e** and **3h** in cynomolgus monkeys. These two analogues were selected for detailed evaluation since they represent "nonstabilized" and "stabilized" versions of a peptide analogue and they possess similar structures with reasonably close IC_{50} values against human plasma renin.

We first examined the effects of intravenous administration of **3e** and **3h** to anesthetized, salt-depleted cynomolgus monkeys. A 0.01 mg/kg bolus injection of **3e** produced a striking hypotensive response accompanied by a precipitous drop in PRA. A typical trace for this experiment is shown in Figure 4a. Note the rapid rebound in PRA even before the blood pressure has returned to normal. Unexpectedly, bolus injection of **3h** at the same dose led to a much weaker hypotensive effect (data not shown). Since this analogue is approximately 2.7 times less potent in inhibiting human plasma renin, we also examined **3h** at a higher dose of 1 mg/kg. A representative trace for this experiment is depicted in Figure 4b. The response for the "stabilized" analogue (**3h**) clearly differs from the "nonstabilized" congener (**3e**). The former is characterized by a relatively moderate drop in blood pressure but with a complete and prolonged inhibition of PRA. Reasons for the substantially different intravenous profiles for these two analogues are not known at this time.

Intraduodenal administration (id) of **3e** (Figure 5) caused mild, dose-related effects on mean arterial blood pressure and PRA. Treatment with 1 mg/kg, id, transiently lowered PRA from 96.3 ± 23.8 to 20.9 ± 7.1 ($P < 0.05$ at 15 min) and provoked a reactive rise in PRA ($P < 0.05$ at 180 min) without altering blood pressure. Administration of 3 mg/kg id, induced a maximal fall in mean arterial pressure of 6 mmHg accompanied by an 85% suppression of PRA, with recovery of both parameters occurring within 1–2 h postdosing. The highest dose of 10 mg/kg id, caused an 8 mmHg reduction in MAP which lasted for 2–3 h in association with a maximal suppression of PRA of 85% as noted in the 3 mg/kg group; however, PRA remained 47% below base line at 4 h following drug treatment.

Results assessing intraduodenal activity of **3h** at doses of 3, 10, and 30 mg/kg are shown in Figure 6. The 3 mg/kg, id, dose caused a fall of 10 mmHg ($P < 0.05$, 60 min) which recovered to base line within 2–3 h, but did not cause a statistically significant inhibition of PRA. The blood pressure response to 10 mg/kg, id, was similar in magnitude and duration of action to the lower 3 mg/kg, except that PRA was progressively suppressed during the course of the experiment ($P < 0.05$, 60–180 min). Disappointingly, blood pressure did not significantly change from base line (7 mmHg, maximal response) following a dose of 30 mg/kg id, and although PRA was significantly suppressed during the first 30 min following dosing (95% at 5 min, 90% at 30 min, and 72% at 60 min), recovery toward base line was relatively rapid.

Drug Metabolism Studies. To gain additional insight into the in vivo behavior of these compounds, we have examined the disposition and metabolism of analogue **3h** in rats. This compound, which we demonstrated to be stable in vitro to chymotrypsin, was labeled with tritium on one of the glycol carbons as indicated below:[20]



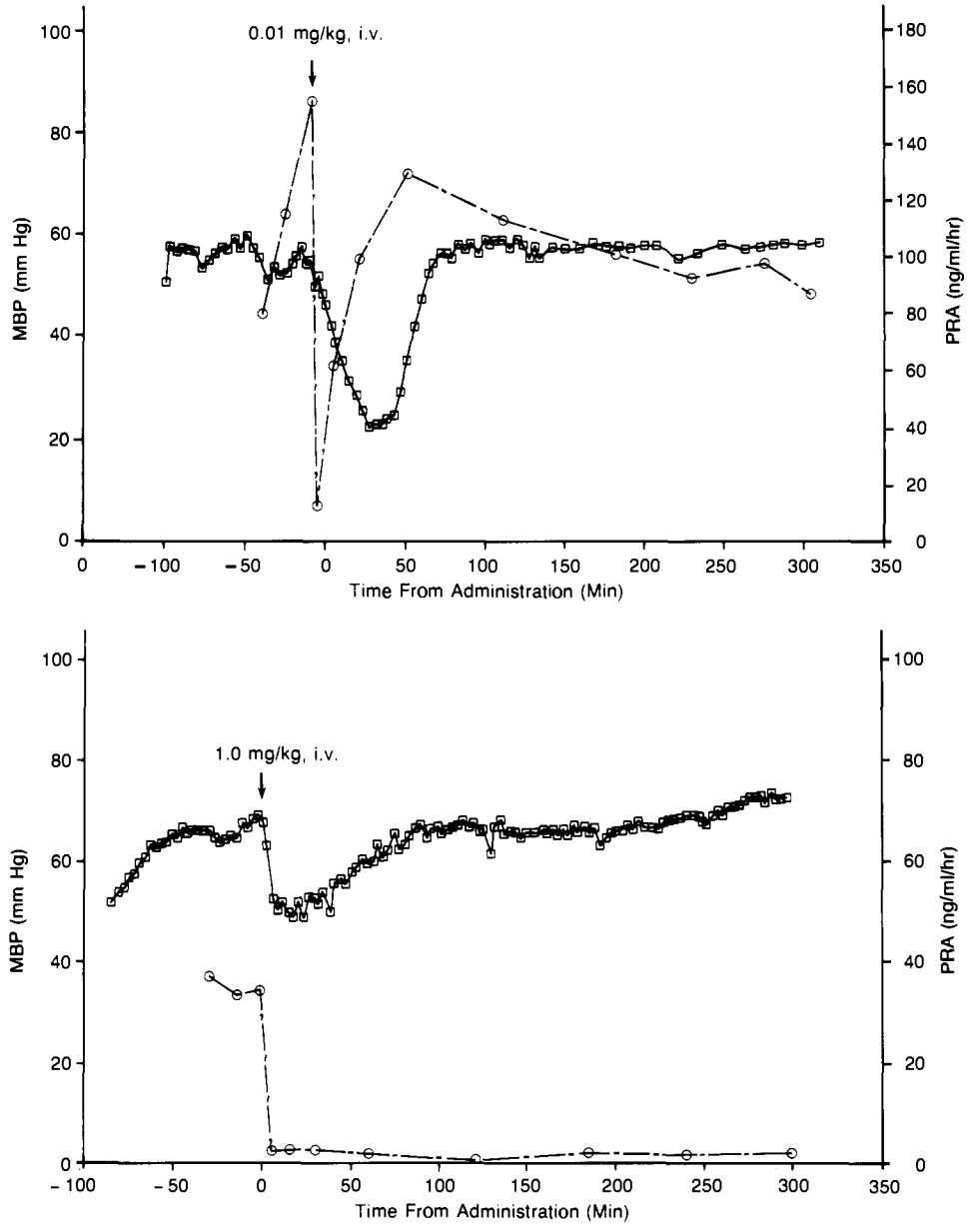


Figure 4. The original tracings of the effect of a single bolus dose of **3e** (a) and **3h** (b) on mean arterial pressure and plasma renin activity are shown in representative anesthetized, salt-depleted monkey. MBP = mean blood pressure (□), PRA = plasma renin activity (○). Each compound was tested at the given dose in duplicate. Duplicate results were similar for **3e** and **3h**, respectively.

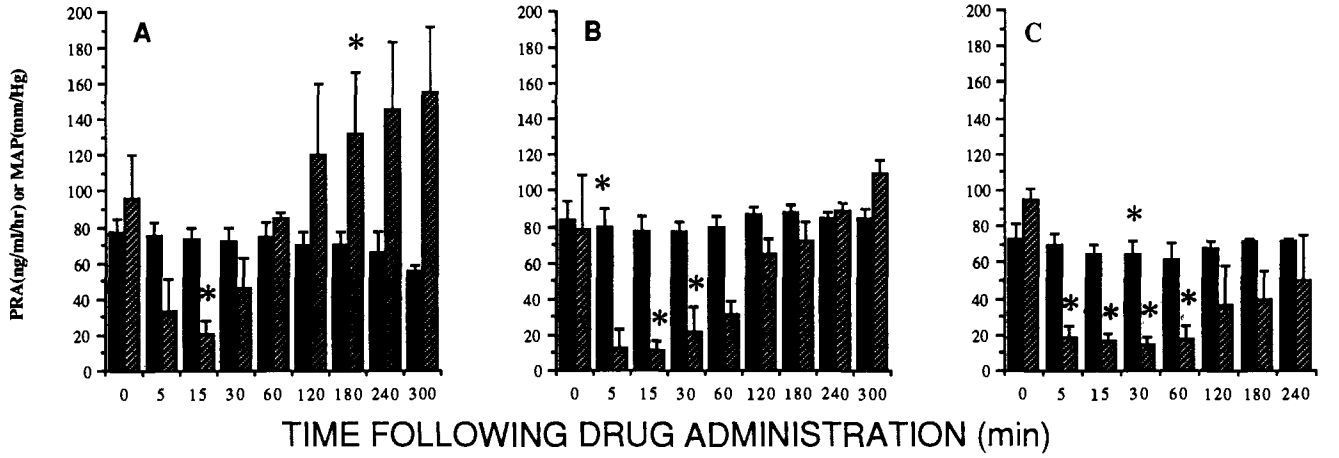


Figure 5. Effects of intraduodenal administration of **3e** at doses of 1 mg/kg (panel A), 3 mg/kg (panel B), and 10 mg/kg (panel C) on MAP and PRA in anesthetized, salt-depleted monkeys (n = 3, each group). Results are shown as mean ± SE and were considered significantly different from time = 0, if $P \leq 0.05$. MAP = mean arterial pressure (■), PRA = plasma renin activity (▨).

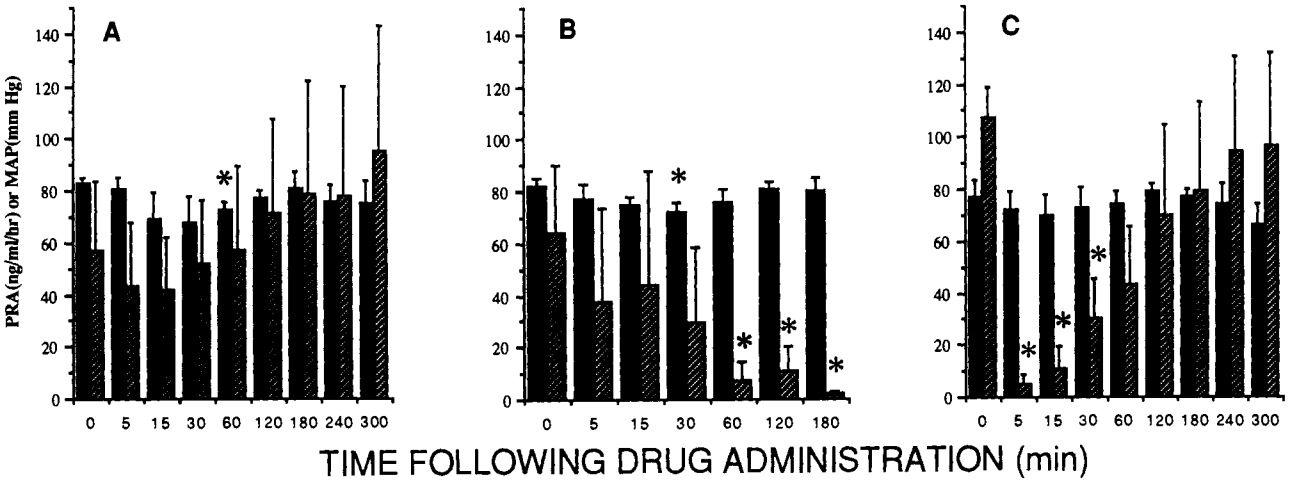


Figure 6. Effects of intraduodenal administration of **3h** at doses of 3 mg/kg (panel A), 10 mg/kg (panel B) and 30 mg/kg (panel C) on MAP and PRA in anesthetized, salt-depleted monkeys (n = 3, each group). Results are shown as mean ± SE and were considered significantly different from time = 0, if $P \leq 0.05$. MAP = mean arterial pressure (■), PRA = plasma renin activity (▨).

Table III. Excretion of Radioactivity and Parent Drug in Urine and Feces of Rats after Intravenous Administration of [^{3}H]-**3h**

	percent of tritium dose		
	urine	feces	total
total ^{3}H	29.3	56.8	86.1
[^{3}H]-**3h**	6.5	26.4	32.9

After intravenous administration of [^{3}H]-**3h** (0.3 mg/kg), the levels of radioactivity in the plasma averaged 24 ng equiv/mL at 15 min and declined to 5 ng equiv/mL at 1 h. Thus, this compound and/or its metabolites are rapidly cleared from the plasma. At 15 min after dosing, unchanged **3h** accounted for over 90% of the radioactivity in the plasma, corresponding to a concentration of 22 ng/mL. Very low levels of **3h** (1–3 ng/mL) could still be detected in the plasma at 6 h.

During the 48-h study, 29.3% of the iv ^{3}H dose was excreted in the urine and 56.8% was eliminated in the feces (Table III). The parent drug accounted for 26% of the urinary radioactivity and 46% of the fecal radioactivity. Thus, about one-third of the ^{3}H dose was excreted as unchanged **3h**, illustrating the reasonably good metabolic stability of the molecule. Hepatobiliary clearance of **3h** was confirmed in an additional rat, in which the parent drug accounted for 64% of the radioactivity excreted in the bile.[21] Metabolites of **3h** have not been identified, although small amounts (~6% of the ^{3}H dose) of the amino diol portion of the molecule were detected in the feces, suggesting that cleavage after the histidyl residue may have occurred.

Summary. In this study we have examined the in vitro stability of renin inhibitors to the serine protease chymotrypsin. Compounds containing an amino diol fragment appended to an N-protected Phe-His dipeptide were found to be readily cleaved by this enzyme. On the basis of the known specificity requirements for chymotrypsin, a series of modified phenylalanine residues was designed so as to minimize the affinity toward chymotrypsin while maintaining tight binding to renin. Incorporation of these altered Phe derivatives into transition-state analogues of angiotensinogen led to compounds with varying degrees of renin inhibitory potency and a range of susceptibilities toward chymotrypsin. Hypotensive responses were observed for both a "stabilized" (**3h**) and "nonstabilized" (**3e**) analogue, following intraduodenal administration in cynomolgus monkeys. However, analogue **3h** showed no clear advantage over **3e** in terms of the magnitude of response and the duration of action, even though **3h** is not cleaved by chymotrypsin. The limiting factor for a useful pharmacologic effect for these compounds may be their short half-life in the plasma. This notion is supported by the study with radiolabeled **3h**, where this derivative was found to be rapidly cleared from the plasma with significant excretion into the bile.[22] Thus, the search for a therapeutically useful renin inhibitor must overcome a number of obstacles, including poor absorption/transportability, proteolytic degradation, and rapid biliary excretion. Our efforts to address these issues will be reported in future publications.

Experimental Section

Solvents and other reagents were reagent grade and used without further purification. Anhydrous tetrahydrofuran was distilled from sodium/benzophenone ketyl. Proton magnetic resonance spectra were measured on a Nicolet QE-300 (300 MHz). Chemical shifts are reported as δ values (parts per million) relative to Me_4Si as an internal standard. Elemental analyses and the above determinations were performed by the Analytical Research Department, Abbott Laboratories. Column chromatography was performed on silica gel 60, 0.04–0.063 mm (E. Merck), eluting with 5–10 psi air pressure. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected.

[(Dimethylamino)carbonyl]-L-phenylalanine (1a). A suspension of L-phenylalanine methyl ester hydrochloride (7 g, 0.032 mol) in toluene (125 mL) was heated to 100 °C while phosgene gas was bubbled into the reaction mixture. After approximately 1.5–2 h, the mixture became homogeneous. The passage of phosgene was continued for an additional 15 min, while the temperature was maintained at 90–100 °C. The toluene was

(20) The synthesis of [^{3}H]-**3h** was carried out by reduction of the corresponding ketone precursor with $NaBT_4$. A description of the ketone synthesis and reduction conditions is described elsewhere: Luly, J. R.; Hsiao, C-N.; BaMaung, N.; Plattner, J. J. *J. Org. Chem.*, in press.

(21) Extensive biliary clearance of a proteolytically stable renin inhibitor has previously been reported: Boger, J.; Bennett, C. D.; Payne, L. S.; Ulm, E. H.; Homnick, C. F.; Schorn, T. W.; Lamont, B. I.; Veber, D. F. *Regul. Pept.* **1985**, *54* (Suppl. 4), 8.

(22) Since the in vivo efficacy experiments and drug metabolism studies were conducted in different species, it is not certain that rapid biliary clearance is the cause of the poor hypotensive efficacy for **3e** and **3h**. However, the rapid biliary uptake for peptidic renin inhibitors observed in the rat (described herein) and in the dog[21] suggests that this elimination pathway may also occur in the monkey.

then evaporated and the residue chased several times with benzene. A 6.5-g (0.031 mol) sample of the crude α-isocyanato-L-phenylalanine methyl ester was dissolved in 50 mL of CH_2Cl_2 and cooled to 0 °C. Dimethylamine (1.4 g, 0.031 mol) dissolved in 5 mL of CH_2Cl_2 was added dropwise. After 10 min at 0–5 °C, the reaction mixture was distributed between 0.5 N HCl and CH_2Cl_2. The organic layer was washed with aqueous $NaHCO_3$ and dried over $MgSO_4$. Evaporation of the solvent gave 6.4 g (81%) of product after trituration with hexane, mp 79–80 °C. Anal. ($C_{13}H_{18}N_2O_3$) C, H, N.

To a 0 °C solution of the above ester (0.91 g, 3.63 mmol) in dioxane (15 mL) was added a solution of lithium hydroxide (0.174 g, 4.15 mmol) in H_2O (7.5 mL). After stirring for 1 h at 0–5 °C, the reaction mixture was diluted with cold H_2O and extracted two times with Et_2O. The aqueous portion was acidified with 6 N HCl and extracted with Et_2O. The organic extract was washed with brine and evaporated to give an 87% yield of product as a viscous liquid. NMR ($CDCl_3$): δ 2.82 (s, 6 H), 3.2 (m, 2 H), 4.6 (m, 1 H), 7.25 (m, 5 H).

[(4-Hydroxypiperidinyl)carbonyl]-L-phenylalanine (1b) was prepared from α-isocyanato-L-phenylalanine methyl ester in a fashion analogous to that for **1a** in 72% yield. NMR ($CDCl_3$): δ 1.45 (m, 2 H), 1.80 (m, 2 H), 3.0 (m, 2 H), 3.2 (m, 4 H), 4.58 (m, 1 H), 4.95 (m, 1 H), 7.25 (m, 5 H).

[(4-Cbz-1-piperazinyl)carbonyl]-L-phenylalanine (1c) was prepared from (carbobenzyloxycarbonyl)piperazine and α-isocyanato-L-phenylalanine methyl ester as described for **1a**. NMR ($CDCl_3$): δ 3.12 (dd, 2 H), 3.28 (m, 4 H), 3.44 (m, 4 H), 4.65 (dd, 1 H), 4.96 (d, 1 H), 5.13 (s, 2 H), 7.16–7.38 (m, 10 H).

[[*N*-Methyl-*N*-(2-hydroxyethyl)amino]carbonyl]-L-phenylalanine (1d) was prepared in the same manner as **1a** in 73% yield. NMR ($CDCl_3$): δ 2.85 (s, 3 H), 3.03 (dd, 1 H), 3.20 (dd, 1 H), 3.27 (m, 2 H), 3.68 (m, 2 H), 4.5 (m, 1 H), 5.72 (m, 1 H), 7.3 (m, 5 H).

(4-Morpholinylcarbonyl)-L-phenylalanine (1e) was prepared as described for **1a** in 74% yield. NMR ($CDCl_3$): δ 3.13 (dd, 2 H), 3.27 (m, 4 H), 3.62 (m, 4 H), 4.66 (dd, 1 H), 4.9 (d, 1 H), 7.17–7.35 (m, 5 H).

(4-Morpholinylcarbonyl)-L-homophenylalanine (1f). (+)-α-Amino-4-phenylbutyric acid (homo-Phe) methyl ester hydrochloride was converted to the corresponding α-isocyanato derivative as described for L-Phe-OCH_3·HCl. Reaction with morpholine and hydrolysis in the same manner as described for **1a** gave **1f** in 68% yield. NMR (DMSO-d_6): δ 1.92 (m, 2 H), 2.62 (m, 2 H), 3.28 (m, 4 H), 3.53 (m, 4 H), 4.00 (m, 1 H), 6.68 (d, J = 8 Hz, 1 H), 7.18 (m, 3 H), 7.25 (d, J = 7 Hz, 2 H).

(4-Morpholinylcarbonyl)-D-phenylalanine (1g) was prepared from D-Phe-OCH_3·HCl by using the procedure described for **1a**. NMR ($CDCl_3$): δ 3.13 (dd, 2 H), 3.26 (m, 4 H), 3.63 (m, 4 H), 4.66 (dd, 1 H), 4.9 (d, 1 H), 7.16–7.34 (m, 5 H).

(4-Morpholinylcarbonyl)-L-*p*-methoxyphenylalanine (1h) was obtained from L-*p*-MeO-Phe-OCH_3·HCl by using the procedure described for **1a**. NMR ($CDCl_3$): δ 3.11 (dd, 2 H), 3.27 (m, 4 H), 3.62 (m, 4 H), 4.62 (dd, 1 H), 4.98 (d, 1 H), 6.82 (d, 2 H), 7.10 (d, 2 H).

2(*S*)-[(4-Morpholinylcarbonyl)oxy]-3-phenylpropionic Acid (1i). To L-phenyllactic acid methyl ester (3.2 g, 0.018 mol) was added 150 mL of 12.5% phosgene in toluene and 25 drops of DMF. After the mixture was stirred for 16 h at room temperature, the solvent was evaporated and the residue chased several times with benzene. The resulting product was dissolved in CH_2Cl_2 (50 mL), cooled to 0 °C, and treated by dropwise addition with 3.86 g (0.044 mol) of morpholine. The reaction mixture was stirred for 2 h at 0–5 °C and then distributed between 0.5 N HCl and CH_2Cl_2. The organic phase was washed with aqueous $NaHCO_3$ and brine and evaporated to a residue. Flash chromatography on silica gel eluting with 2/1 Et_2O–hexane gave 3.38 g (65%) of methyl 2(*S*)-[(4-morpholinylcarbonyl)oxy]-3-phenylpropionate as a viscous liquid. Hydrolysis of this ester as described for **1a** then gave the desired product in 86% yield. NMR ($CDCl_3$): δ 3.08 (dd, 1 H), 3.20 (dd, 1 H), 5.19 (dd, 1 H), 7.17–7.38 (m, 5 H).

(*E*)-2-[(4-Morpholinylcarbonyl)methyl]cinnamic Acid (1j). To a stirred −12 °C solution of α-ethyl α-benzalsuccinate[8] (1.6 g, 6.83 mmol) in anhydrous THF (50 mL) were added *N*-methylmorpholine (1.0 mL, 9.09 mmol) and isobutyl chloroformate (1.2 mL, 9.25 mmol) sequentially. After 5 min, a −12 °C solution of morpholine (0.8 mL, 9.17 mmol) was added. After 1 h, the solution was filtered and the filtrate concentrated. The residual oil was dissolved in 200 mL of EtOAc and was washed successively with 10% sodium bisulfate, aqueous $NaHCO_3$, and brine. The organic layer was dried (Na_2SO_4), filtered, and evaporated. The residue was chromatographed on silica gel with 2% MeOH in CH_2Cl_2 as eluent to give 985 mg (48%) of ethyl 2-[(4-morpholinylcarbonyl)methyl]-*trans*-cinnamate. NMR ($CDCl_3$): δ 1.35 (t, 3 H, J = 7 Hz), 3.50 (s, 2 H), 3.51–3.72 (m, 8 H), 4.27 (q, 2 H, J = 7 Hz), 7.35–7.40 (m, 5 H), 7.92 (s, 1 H). Hydrolysis of the above ethyl ester using LiOH in dioxane/H_2O as described for **1a** gave **1j** in 92% yield. NMR ($CDCl_3$): δ 3.80 (s, 2 H), 3.55–3.70 (m, 8 H), 3.32–3.40 (m, 5 H), 7.90 (s, 1 H).

(4*S*)-3-(3-Phenylpropionyl)-4-(2-propyl)oxazolidin-2-one (4). To a stirred solution of 4-(3-propyl)oxazolidin-2-one in anhydrous THF (250 mL) under a nitrogen atmosphere at −78 °C was added in a dropwise fashion a solution of *n*-butyllithium in hexane (50 mL, 77.4 mmol) over 5–10 min. After the mixture was stirred an additional 20 min at −78 °C, 3-phenylpropionyl chloride (12.7 mL, 85.2 mmol) was added neat. The reaction was warmed to room temperature and stirred 2 h at room temperature. The reaction was quenched by adding 100 mL of saturated aqueous NH_4Cl, and the volatiles were removed by rotary evaporation. The resulting aqueous residue was extracted three times with Et_2O, and the combined organic phases were washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. Recrystallization from hexanes/EtOAc provided the title compound (16.6 g, 82%), mp 86.5–87.5 °C. Anal. ($C_{15}H_{19}NO_3$) C, H, N.

(4*S*)-3-[(2*R*)-2-[(*tert*-Butyloxycarbonyl)methyl]-3-phenylpropionyl]-4-(2-propyl)-oxazolidin-2-one (5). To a stirred solution of **4** (2.28 g, 8.72 mmol) in anhydrous tetrahydrofuran (30 mL) under a nitrogen atmosphere at −78 °C was added a solution of sodium hexamethyldisilylamide (9.6 mL, 9.59 mmol) in THF. After the mixture was stirred for 30 min at −78 °C, *tert*-butyl bromoacetate (2.21 g, 11.34 mmol) was added in anhydrous THF and the resulting solution stirred 1 h at −78 °C. The reaction was quenched by adding 20 mL of saturated aqueous NH_4Cl and partitioned between Et_2O and H_2O. The aqueous layer was drawn off and extracted with Et_2O. The combined organic phases were washed with 10% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, dried (Na_2SO_4), filtered, and concentrated to give the product (2.59 g, 79%), mp 167–168 °C. Anal. ($C_{21}H_{29}NO_3$) C, H, N.

Benzyl (2*R*)-2-(Carboxymethyl)-3-phenylpropionate (6). To a stirred solution of dry benzyl alcohol (0.55 mL, 5.33 mmol) in anhydrous THF (18 mL) under a nitrogen atmosphere at 0 °C was added a hexane solution of *n*-butyllithium (2.58 mL, 4.00 mmol). To this solution was added **5** (2.59 g) in anhydrous THF (10 mL). After the mixture was stirred 1 h at 0 °C, the reaction was quenched by adding excess saturated aqueous NH_4Cl. The volatiles were removed by rotary evaporation, and the resulting aqueous residue was extracted two times with Et_2O. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The residue oil was purified by chromatography on SiO_2 (15% EtOAc–hexanes) to provide the desired product (0.89 g, 94%) as a colorless oil. Mass spectrum: $(M)^+$ = 354. The above product (0.52 g, 1.47 mmol) was dissolved in a 1:1 (v/v) solution (6 mL) of trifluoroacetic acid and CH_2Cl_2 and stirred at room temperature for 1 h. The volatiles were removed in vacuo to provide the title compound (0.437 g, 100%) as an oil, which crystallized on standing. the unpurified material was of sufficient purity to employ in subsequent steps. NMR ($CDCl_3$): δ 2.46 (dd, J = 5.4, 17.7 Hz, 1 H), 2.74 (dd, J = 9.3, 17.7 Hz, 1 H), 2.8 (dd, J = 8.4, 12.3 Hz, 1 H), 3.07 (dd, J = 6.3, 12.3 Hz, 1 H), 3.17 (m, 1 H), 5.12 (s, 2 H), 7.1–7.37 (m, 10 H).

(2*R*)-2-[(4-Morpholinylcarbonyl)methyl]-3-phenylpropionic Acid (1k). Acid **6** was coupled to morpholine in 80% yield by using the mixed-anhydride procedure described for **1j**. The resulting product (1.76 g, 4.79 mmol) was dissolved in EtOAc (5 mL) and syringed into a flask charged with 10% Pd/C (0.3 g). The resulting suspensions was exposed to 1 atm of gaseous hydrogen for 4 h. The catalyst was removed by filtration through a Celite pad. The filtrate was concentrated in vacuo to provide the desired compound (1.33 g, 100%) as a cream-colored foam, which was employed without further purification. NMR ($CDCl_3$):

δ 2.39 (dd, J = 3.6, 17.7 Hz, 1 H), 2.62 (dd, J = 8.7, 17.7 Hz, 1 H), 2.77 (dd, J = 11.7, 15.3 Hz, 1 H), 3.17–3.35 (m, 4 H), 3.57–3.68 (m, 6 H), 7.07–7.45 (m, 5 H).

2(*R*,*S*)-[(4-Morpholinylcarbonyl)amino]-3,3-dimethyl-3-phenylpropionic Acid (1m). A solution of diethyl (α,α-dimethylbenzyl)malonate[11] (42.1 g, 0.15 mol) in EtOH (100 mL) was treated by dropwise addition with a solution of KOH (8.48 g, 0.13 mol) in 100 mL of EtOH. After heating at 90 °C for 1 h and at 50 °C for 20 h, the reaction mixture was evaporated on the rotary evaporator to a residue. The residue was diluted with H_2O and extracted with Et_2O to remove unreacted starting material. The aqueous phase was cooled to 5 °C, acidified to pH 3 with 6 N HCl, and extracted with CH_2Cl_2. The organic layer was washed with brine solution and dried over magnesium $MgSO_4$. Evaporation of the solvent gave 27.3 g (84%) of liquid product. NMR ($CDCl_3$): δ 1.05 (t, 3 H), 1.6 (s, 6 H), 3.78 (s, 1 H), 3.96 (m, 2 H), 7.2–7.4 (m, 5 H).

To a solution of the above product (4 g, 0.016 mol) in toluene was added triethylamine (2.23 mL, 0.016 mol) and diphenyl phosphorazidate (4.4 g, 0.016 mol). The reaction mixture was heated at 100 °C for 2.5 h, cooled to 5 °C, and treated with 1.4 mL (0.016 mol) of morpholine. After stirring overnight at room temperature, the toluene solution was washed successively with 1 N HCl and aqueous $NaHCO_3$ solution. The dried organic solution was evaporated to a residue, which was purified by column chromatography on silica gel. There was obtained 3.7 g (69%) of product after trituration with hexane, mp 93–94 °C. Anal. ($C_{18}H_{26}N_2O_4$) C, H, N.

A solution of the above ester (2 g, 5.99 mmol) in dioxane (10 mL) was treated with 0.26 g (6.5 mmol) of NaOH in 5 mL of H_2O. After the mixture was stirred for 16 h at 35 °C, the reaction was worked up as described for **1a** to give 1.5 g (82%) of **1m**. NMR ($CDCl_3$): δ 1.5 (d, 6 H), 3.2 (m, 4 H), 3.6 (m, 4 H), 4.66 (s, 2 H), 7.35 (m, 5 H).

(4-Morpholinylcarbonyl)-β-phenylserine Methyl Ester (7). A suspension of 10.0 g (46.3 mmol) of β-phenylserine hydrochloride in 50 mL of MeOH was treated cautiously with 3.7 mL (51 mmol) of thionyl chloride. The resulting solution was heated overnight at 40 °C. After removal of the solvent, recrystallization from MeOH–EtOAc acetate gave 9.62 g (90%) of the corresponding methyl ester. Toluene (40 mL) was cooled to 0 °C and treated with gaseous phosgene for 20 min. During the second 10-min period, a solution of 1.1 mL (12.6 mmol) of morpholine in 20 mL of toluene was added dropwise. After addition was complete, the mixture was warmed in a water bath and nitrogen was bubbled through the solution for 30 min to remove excess phosgene. After filtration, removal of the solvent gave a colorless liquid, which was taken up in 10 mL of CH_2Cl_2 and added dropwise to a precooled (0 °C) solution of 1.45 g (6.3 mmol) of the above ester and 2.64 mL (20 mmol) of triethylamine in 50 mL of CH_2Cl_2. After addition was complete, the resulting solution was stirred at ambient temperature overnight, diluted with CH_2Cl_2, washed sequentially with aqueous HCl, H_2O, and aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated. Purification by flash column chromatography using 4% methanol in chloroform gave 1.60 g (83%) of pure **7** as a white foam. ^1H NMR ($CDCl_3$): δ 3.30 (m, 4 H), 3.62 (t, J = 5 Hz, 4 H), 3.72 (s, 3 H), 4.72 (dd, J = 4, 9 Hz, 1 H), 5.21 (br t, J = 4 Hz, 1 H), 5.28 (br d, J = 9 Hz, 1 H), 7.3–7.4 (m, 5 H).

(*Z*)-4-Benzylidene-2-(4-morpholinylcarbonyl)oxazolin-5-one (8). A solution of 0.50 g (1.6 mmol) of **7** in 5 mL of THF was treated with 1.1 mL (3.3 mmol) of aqueous NaOH. After being allowed to stir at ambient temperature for 1 h, the solvent was removed in vacuo to give a white solid. The residue was sitrred with 1.5 mL of Ac_2O for 3 days. After removal of the solvent, purification by flash column chromatography using 7:3 hexane–EtOAc (R_f 0.24) afforded 0.22 g (53%) of pure **8**. ^1H NMR ($CDCl_3$): δ 3.71 (m, 4 H), 3.80 (t, J = 5 Hz, 4 H), 6.72 (s, 1 H), 7.25–7.45 (m, 3 H), 8.00 (d, J = 7 Hz, 2 H).

(2*S*,3*R*,4*S*)-2-[[(4-Morpholinylcarbonyl)-*Z*-dehydrophenylalanyl-L-histidyl]amino]-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (3l). A solution of 0.24 mmol of **2** (dihydrochloride salt) and 62 mg (0.24 mmol) of **8** in 2 mL of DMF was treated with 0.083 mL of triethylamine and stirred at ambient temperature overnight. After removal of the solvent in vacuo, the residue was partitioned between EtOAc and aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated. Purification by flash column chromatography using 7.5% MeOH in $CHCl_3$ (R_f 0.08) gave 91.8 mg (60%) of pure **3l** as a white solid. FABMS: 639 $(M + H)^+$.

(2*S*,3*R*,4*S*)-2-[[[(Dimethylamino)carbonyl]-L-phenylalanyl-L-histidyl]amino]-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (3a). To a stirred 0 °C solution of **1a** (0.078 g, 0.33 mmol) in anhydrous DMF (2 mL) was added a solution of **2** (dihydrochloride, 0.136 g, 0.3 mmol)[4] in DMF (5 mL). Hydroxybenzotriazole (0.054 g, 0.4 mmol) and DCC (0.068 g, 0.33 mmol) were then added sequentially. After 2.5 h the mixture was warmed to 25 °C, stirred 12 h, filtered, and evaporated in vacuo. The residue was distributed between $CHCl_3$ and saturated $NaHCO_3$. The organic phase was then washed separately with saturated $NaHCO_3$ and brine. Drying (Na_2SO_4) and evaporating provided a residue, which was chromatographed on silica gel with 5% MeOH in $CHCl_3$ as eluent to give 0.11 g (61.5%) of **3a**, mp 108–110 °C. Analogues **3b** and **3d–k** were prepared by the same procedure (see Table I).

(2*S*,3*R*,4*S*)-2-[[(1-Piperazinylcarbonyl)-L-phenylalanyl-L-histidyl]amino]-1-cyclohexyl-3,4-dihydroxy-6-methylheptane Bis(acetic acid) Salt (3c). With intermediate **1c** and **2** and the procedure described for **3a**, the corresponding Cbz-protected product was obtained in 52% yield. A solution of this material (0.22 g, 28.7 mmol) in acetic acid (100 mL) was hydrogenated at 1 atm with 20% Pd/C (0.22 g) for 3 h. Filtration, extration of the catalyst with acetic acid, and evaporation of the combined acetic acid solutions gave a residue which was dissolved in H_2O (5 mL) and lyophilized to provide 190 mg (87%) of the desired product, mp 170–175 °C.

(2*S*,3*R*,4*S*)-2-[[(4-Morpholinylcarbonyl)-L-β,β-dimethylphenylalanyl-L-histidyl]amino]-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (3m). Intermediates **1m** and **2** were coupled by using the procedure described for **3a** with the following modifications. The reaction was conducted at room temperature and without the addition of hydroxybenzotriazole. Purification of the crude product by chromatography on silica gel eluting with 5% MeOH in $CHCl_3$ gave an 80% yield of **3m**, mp 120–122 °C. Anal. ($C_{36}H_{56}N_6O_6 \cdot 1.25H_2O$) C, H, N. The above product containing an *R*,*S* mixture of diastereomers at the modified Phe residue was purified by HPLC for enzyme inhibition. The HPLC purification was carried out on an analytical scale with a Waters μBondapak C-18 column (30 cm) with a 1.5 mL/min flow rate and UV detection at 214 nM. A gradient elution of CH_3CN (26%)/H_2O (74%)/TFA (0.1%) adjusted to CH_3CN (74%)/H_2O (26%)/TFA (0.1%) over 20 min was used. The active isomer (IC_{50} = 2.1×10^{-9} M) eluted at 16.17 min and is presumed to be the *S* isomer at the α-carbon of phenylalanine. The other diastereomer, eluting at 15.49 min, was found to be inactive. The mixture was used for stability testing.

(2*S*,3*R*,4*S*)-2-[(Boc-L-α-*N*-methylhistidyl)amino]-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (12). To a stirred solution of *N*-Boc-*N*-methyl-N^{im}-tosyl-L-histidine[13] (3 g, 7.08 mmol) and 1.97 g (7.05 mmol) of **11** (hydrochloride)[4] in DMF (20 mL) was added 2.09 mL (15 mmol) of triethylamine, followed by the slow addition of diethoxyphosphoryl cyanide (1.09 mL, 7.19 mmol). After being stirred at room temperature for 16 h, the reaction mixture was worked up and purified as described for **3a** to give a 75% yield of product, mp 160 °C. NMR ($CDCl_3$): δ 0.9 (m, 6 H), 1.4 (s, 9 H), 1.2–1.6 (m, 16 H), 2.43 (s, 3 H), 2.78 (s, 3 H), 7.07 (s, 1 H), 7.6 (dd, 4 H), 7.9 (s, 1 H).

The above product was stirred in CH_3OH with 5 equiv of HOBT for 16 h. The reaction mixture was filtered. The filtrate was evaporated to a solid, which was taken up with $CHCl_3$, washed with dilute $NaHCO_3$ and brine, and then dried and filtered. The resultant residue after evaporation was chromatographed on silica gel with 5% $CH_3OH/CHCl_3$ as eluent to give the desired product in 60% yield. NMR ($CDCl_3$): δ 0.9 (2 d, 6 H), 1.2–1.7 (m, 16 H), 1.45 (s, 9 H), 2.85 (s, 3 H), 6.85 (s, 1 H), 7.5 (s, 1 H).

(2*S*,3*R*,4*S*)-2-[[(4-Morpholinylcarbonyl)-L-phenylalanyl-L-*N*-methylhistidyl]amino]-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (13). A 650-mg (1.32-mmol) sample of **12** was dissolved in 20 mL of 4 M HCl/dioxane containing 5 drops of MeOH and kept for 1 h at room temperature. The solvents were evaporated, and the residue was distributed between aqueous $NaHCO_3$ and EtOAc. Drying and evaporation of the EtOAc gave the corresponding free base. Using the procedure

described for **3a** but replacing the DCC with bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) and **2** with the free base of **12** gave **13** in 39% yield, mp 130–135 °C. Anal. ($C_{35}H_{54}N_6O_6 \cdot 0.5H_2O$) C, H, N.

Inhibition Studies. Assays of purified human renin in maleate buffer at pH 6.0 and cathepsin D and pepsin were performed as described previously.[3c] Plasma renin assays at pH 7.4 were a modification of the previous method[3c] in that 0.10 M HEPES was substituted for maleate, the incubation time was increased to 2.5 h, and the radioimmunoassay for angiotensin I was performed on the entire incubation sample.

Drug Metabolism Studies. Compound **3h** was labeled with tritium in the 4-position of the amino diol fragment[20] and had a specific activity of 176 μCi/mg. Male Sprague–Dawley derived rats, weighing 0.20–0.25 kg, were given a 0.3 mg/kg intravenous dose of [^{3}H]-**3h** (~10–20 μCi/rat), dissolved in an ethanol/water mixture (1:1, v/volume). Plasma samples were obtained from a tail vein in three rats at designated times after dosing. Urine and feces excreted by three other rats were collected for 2 days after drug administration. Bile was obtained from another rat after surgical implantation of a bile duct cannula under diethyl ether anesthesia. The feces were homogenized in 70% aqueous ethanol, and aliquots were burned in a sample oxidizer. All samples were assayed for total radioactivity by liquid scintillation spectrometry and corrected for quenching with an external standard. The samples contained negligible quantities of tritiated water, as determined by lyophilization and radioassay of the distillates.

Metabolic patterns in plasma, bile, urine, and fecal samples were determined by high-pressure liquid chromatography on a C-18 column with a linear 1–75% aqueous acetonitrile gradient containing 0.01 M tetramethylammonium perchlorate and 0.01 M sodium dodecylsulfate. The column effluent was collected in 1-mL (1 min) fractions, which were radioassayed by liquid scintillation spectrometry. Some of the radioactive peaks in the samples were tentatively identified by comparison of their retention times with those of authentic reference standards, based on absorbance at 215 nm or radioactivity.

Chymotrypsin Stability Experiments. Compounds were incubated at 0.05 mg/mL with 0.1 mg/mL bovine pancreatic chymotrypsin (Sigma #C-4129) in 30 mM sodium phosphate and 100 mM sodium chloride, pH 6.9. Methanol at 2% was included as cosolvent. Aliquots were withdrawn at intervals from 0.5 to 360 min, the reaction was stopped by addition of CH_3CN/TFA, and the composition analyzed by high-pressure liquid chromatography using a Waters μBondapak C-18 column and gradient elution of CH_3CN (26%)/H_2O (26%)/TFA (0.1%) to CH_3CN (74%)/H_2O (26%)/TFA (0.1%) over 20 min. Peaks were detected by UV absorbance at 214 nm and quantified with a Perkin-Elmer LCI-100 integrator. Susceptible compounds exhibited first-order kinetics of reaction. The half-times for compounds **3h** and **3j** in Table II are extrapolated from the extent of hydrolysis at 360 min.

Molecular Modeling. The side chains of the amino acids that were substituted for the phenylalanine were placed in the active site of chymotrypsin[23] with atomic coordinates obtained from the Brookhaven Data Bank.[24] The renin active site was modeled from the structures of the other known aspartic proteinases as previously described.[6c,18] In each case, the main chain of the renin inhibitor, in the conformation that it binds either to chymotrypsin[25] or to renin, was left unchanged. The side-chain dihedral angles were varied in order to remove or minimize bad contacts of the side chain with the respective enzyme binding pocket. The importance of any remaining overlap was assessed.

In Vivo Activity. Intravenous and intraduodenal activity were determined in male cynomolgus monkeys (*M. fascicularis*), weighing between 3 and 5 kg. The monkeys were fed a low-salt chow and fresh fruit diet and treated twice with furosemide (5 mg/kg, po), 1 week and 1 day before the experiment. Following this regimen renders the monkeys in a salt-depleted state, characterized by normal base-line blood pressures but elevated plasma renin activities. The monkeys were fasted overnight and studied under anesthesia on the day of the experiment (sodium pentobarbital, 15 mg/kg bolus + 0.10 mg/kg per min maintenance iv infusion). A femoral artery was catheterized for the direct and continuous measurement of blood pressure and heart rate (Grass Pressure Transducer Model P23dB and Grass Polygraph Model 7, Grass Instruments, Quincy, MA), as well as for the withdrawal of blood samples for the measurement of plasma renin activity.[26] Compounds were tested for intravenous activity by injection via a leg vein or for intraduodenal activity via a catheter placed directly into the proximal segment of the duodenum following a laparotomy. Each monkey received only one dose of compound.

Registry No. **1a**, 108514-97-2; **1b**, 114457-68-0; **1c**, 114457-64-6; **1d**, 116129-72-7; **1e**, 114457-62-4; **1f**, 116129-73-8; **1g**, 116129-74-9; **1h**, 114359-50-1; **1i**, 114343-31-6; **1i** (methyl ester), 114359-57-8; **1j**, 116129-75-0; **1j** (methyl ester), 116129-87-4; **1k**, 112804-14-5; **1k** (benzyl ester), 116129-89-6; **1m**, 114359-59-0; 1m (ethyl ester), 114359-58-9; **2**, 116183-33-6; **2**·2HCl, 104882-47-5; **3a**, 114457-18-0; **3b**, 114457-24-8; **3c** (free base), 114457-20-4; **3c** (diacetate salt), 114457-21-5; **3c** (Cbz-protected), 114457-65-7; **3d**, 114457-19-1; **3e**, 114457-17-9; **3f**, 116129-76-1; **3g**, 114529-31-6; **3h**, 114457-22-6; **3i**, 114457-15-7; **3j**, 116129-77-2; **3k**, 114457-27-1; **3l**, 116129-78-3; **3m**, 116183-34-7; **3m** (diastereomer), 116183-35-8; **4**, 95798-31-5; **5**, 116129-79-4; **6**, 116129-80-7; **6** (*tert*-butyl ester), 116129-88-5; **7**, 116129-81-8; **7** (free acid), 116129-93-2; **8**, 116129-82-9; **9**, 78775-63-0; **10**, 103372-35-6; **11**·HCl, 104882-45-3; **12**, 116129-83-0; **12** (N^{im}-tosyl), 116148-99-3; **13**, 116129-84-1; H-Phe-OMe·HCl, 7524-50-7; (*S*)-(OCN)CH(CH_2Ph)COOMe, 40203-94-9; Me_2NH, 124-40-3; Me_2NCO-Phe-OMe, 116129-85-2; (*S*)-(+)-PhCH_2CH(NH_2)COOMe, 60425-49-2; H-D-Phe-OMe·HCl, 13033-84-6; H-Phe(*p*-OMe)-OMe·HCl, 7479-01-8; (*E*)-PhCH=C(COOEt)-CH_2COOH, 116129-86-3; PhCH_2CH_2COCl, 645-45-4; BrCH_2COOBu-*t*, 5292-43-3; (±)-PhC(CH_3)$_2$CH(COOH)COOEt, 116129-90-9; (*S*)-PhCH_2CH(OH)COOMe, 13673-95-5; *N*-(benzyloxycarbonyl)piperazine, 31166-44-6; morpholine, 110-91-8; (4*S*)-4-isopropyloxazolidin-2-one, 17016-83-0; L-*erythro*-β-phenylserine hydrochloride, 116129-91-0; L-*erythro*-β-phenylserine methyl ester, 116129-92-1; *N*-(chlorocarbonyl)morpholine, 15159-40-7; renin, 9015-94-5; chymotrypsin, 9004-07-3; protease, 9001-92-7.

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