Conformationally Constrained Renin Inhibitory Peptides: γ-Lactam-Bridged Dipeptide Isostere as Conformational Restriction

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A model of the conformation of the enzyme-bound inhibitor of human renin suggested the possibility of a γ-lactam conformational restriction at the P_2–P_3 site. Synthetic routes to these γ-lactam dipeptide isosteres and their incorporation into potential renin inhibitors are described. Peptide VIa,b with a γ-lactam conformational constraint and a hydroxyethylene isostere at the cleavage site inhibited human plasma renin with an IC_{50} value of 6.5 nM. The flexibility of these syntheses should make available a number of potential enzyme inhibitors with this structural feature for the study of enzyme-bound conformers.

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

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

Many renin inhibitors have been shown to lower blood pressure during intravenous infusion. However, blood pressure usually recovers within minutes after stopping an infusion.[8] Efforts to obtain renin inhibitors with longer duration of action have continued to make progress.[9,10] We have initiated a program with the intention of overcoming the problem of short biological half-life of these peptides, and in our previous papers,[10] we described our work on peptide backbone modification that led to the demonstration of an orally active renin inhibitory peptide. The present study reports a continuation of our work in this area utilizing backbone conformational constraint of peptides.

We chose to examine the amide bond between the amino acids at P_2 and P_3 sites in potential renin inhibitory peptides as a likely point of cleavage by proteolytic enzymes.[10] Figure 2 shows the proposed conformation of the peptidic bond at the P_2–P_3 sites as bound to the computer graphic model of the human renin active site.[11] We have shown earlier[10] that potent renin inhibitory peptides that contain the N-methylated amide at this site could be prepared. It suggested that the NH group of this peptidic bond is not involved in a necessary hydrogen bond to the enzyme. Inspection of the model in Figure 2 also suggested that this N–H bond and the α-carbon C–H bond, as indicated in this drawing, are nearly eclipsed. We propose to replace each of these two hydrogens with a methylene group and to join them into a γ-lactam cyclic constraint as shown in structure A in Figure 3.

Lactams have already been shown to be useful conformational constraints in peptides, and the syntheses of several varieties of protected lactam-bridged dipeptides have been reported.[13] Backbone conformational constraints are of interest as a means of limiting the number of available conformers. Stabilization of a biologically active conformer can lead to improved selectivity and increased potency through restriction on the number of

(1) Davis, J. O. *Circ. Res.* **1977**, *40*, 439. Swales, J. D. *Pharmacol. Ther.* **1979**, *7*, 172.
(2) Peach, M. J. *Physiol. Rev.* **1977**, *57*, 313.
(3) Ondetti, M. A.; Cushman, D. W. *Annu. Rev. Biochem.* **1982**, *51*, 283.
(4) Skeggs, L.; Lentz, K.; Kahn, J.; Hochstrasser, H. *J. Exp. Med.* **1968**, *128*, 13.
(5) Haber, E. *N. Engl. J. Med.* **1984**, *311*, 1631.
(6) Pauling, L. *Am. Sci.* **1948**, *36*, 58. Jencks, W. P. *Current Aspects of Biochemical Energetics*; Kaplan, N. O., Kennedy, E. P., Eds.; Academic: New York, 1966; p 273. Wolfenden, R. *Nature (London)* **1969**, *223*, 704; *Acc. Chem. Res.* **1972**, *5*, 10.
(7) (a) Szelke, M.; Jones, D. M.; Atrash, B.; Hallett, A.; Leckie, B. J. *Peptide Symposium*; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Co.: Rockford, IL, 1983; p 579. (b) Boger, J. *Ibid.*, p 569.
(8) Szelke, M.; Leckie, B. J.; Tree, M.; Brown, A.; Grant, J.; Hallett, A.; Hughes, M.; Jones, D. M.; Lever, A. F. *Hypertension* **1982**, *4* (Suppl. II), II-59. Blaine, E. H.; Schorn, T. W.; Boger, J. *Ibid.* **1984**, *6*, I-111. Wood, J. M.; Forgiarini, P.; Hofbauer, K. G. *Hypertension* **1983**, *1* (Suppl. II), 189. Leckie, B. J.; Grant, J.; Hallett, A.; Hughes, M.; Jones, D. M.; Szelke, M.; Tree, M. *Scott. Med. J.* **1984**, *29*, 125.
(9) Wood, J. M.; Gulati, N.; Forgiarini, P.; Fuhrer, W.; Hofbauer, K. G. *Hypertension* **1985**, *7*, 797. DeClaviere, M.; Cazaubon, C.; Lacour, C.; Nisato, D.; Gagnol, J. P.; Evin, G.; Corvol, P. *J. Cardiovasc. Pharmacol.* **1985**, *7* (Suppl. 4), S58.
(10) (a) Thaisrivongs, S.; Pals, D. T.; Harris, D. W.; Kati, W. M.; Turner, S. R. *J. Med. Chem.* **1986**, *29*, 2088. (b) Pals, D. T.; Thaisrivongs, S.; Lawson, J. A.; Kati, W. M.; Turner, S. R.; DeGraaf, G. L.; Harris, D. W.; Johnson, G. A. *Hypertension* **1986**, *8*, 1105.
(11) Carlson, W.; Karplus, M.; Haber, E. *Hypertension* **1985**, *7*, 13.
(12) HMPA = hexamethylphosphoramide; DCC = dicyclohexylcarbodiimide; 1-HOBT = 1-hydroxybenzotriazole; LDA = lithium diisopropylamide; THF = tetrahydrofuran.
(13) Freidinger, R. M.; Perlow, D. S.; Veber, D. F. *J. Org. Chem.* **1982**, *47*, 104. Freidinger, R. M.; Veber, D. F.; Perlow, D. S.; Brooks, J. R.; Saperstein, R. *Science (Washington, D.C.)* **1980**, *210*, 656. Freidinger, R. M. In *Peptides: Proceedings of the 7th American Peptide Symposium*; Rich, D., Gross, E., Eds.; Pierce Chemical Co.: Rockford, IL, 1981; p 673. Freidinger, R. M.; Veber, D. F.; Hirschmann, R.; Paege, L. M. *Int. J. Pept. Protein Res.* **1980**, *16*, 464. Freidinger, R. M.; Schwenk, D. A.; Veber, D. F. In *Peptides: Proceedings of the 6th American Peptide Symposium*; Gross, E., Meinhofer, J., Eds.; Pierce Chemical Co.: Rockford, IL, 1979; p 703.

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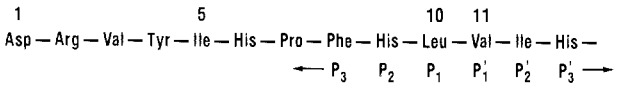


Figure 1. Human angiotensinogen.

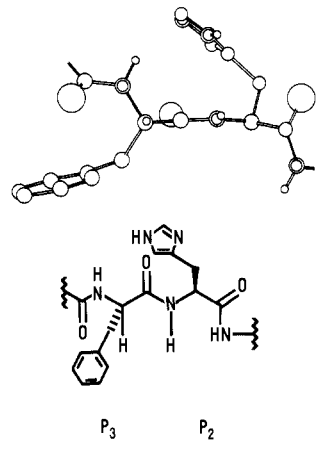


Figure 2. Model of the conformation of the P_2–P_3 site of human angiotensinogen.

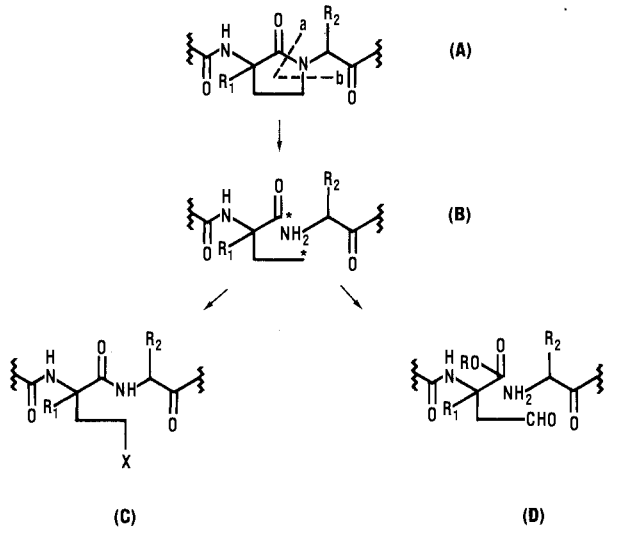


Figure 3. Synthetic analysis of the γ-lactam ring construction.

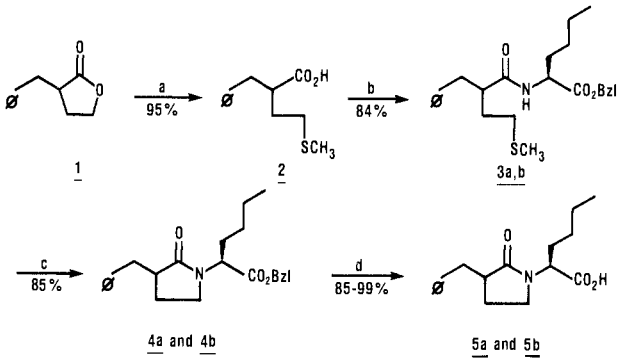
undesired conformers. Potential metabolic stability of the backbone could be realized by eliminating metabolized conformers and by modification of the peptidic bond. We hope to take advantage of some of these properties by incorporating a γ-lactam at the P_2–P_3 sites of renin inhibitory peptides in which the bioactive conformers are not excluded.

Chemistry[12]

We have explored two synthetic routes to the γ-lactam dipeptide isostere A as summarized in Figure 3. As shown in B, the amine component can be attached to the indicated synthon by the formation of bonds a and b. In the first sequence, an intermolecular amide bond formation will lead to structure C. A subsequent intramolecular displacement reaction[13] will then form the γ-lactam A. In a different sequence, an intermolecular reductive amination reaction of the two components as depicted in D is to be followed by an intramolecular amide bond formation to give the γ-lactam A.

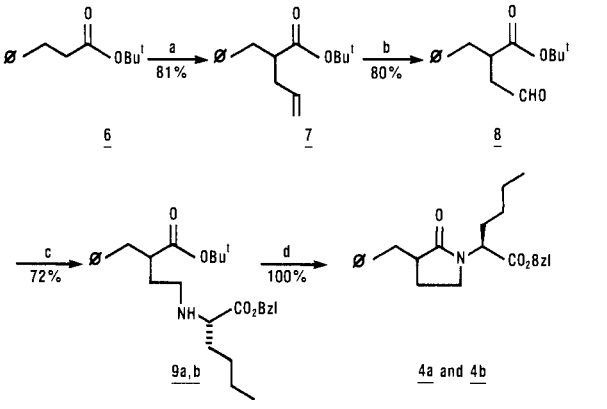
We chose to work with norleucine as the amine component in B to establish the feasibility of the reaction sequences without the potential complication of the imidazole side chain of the normal histidine residue of the

Scheme I. Synthesis of the γ-Lactam 4 via the Intramolecular Displacement Reaction[a]



[a] (a) $LiSCH_3$, HMPA; (b) $NH_2CH(C_4H_9)CO_2Bn$, DCC, 1-HOBT, CH_2Cl_2; (c) Me_3OBF_4, CH_2Cl_2; CH_3CONCH_3Li, THF; (d) Pd/C, H_2; CH_3OH.

Scheme II. Synthesis of the γ-Lactam 4 via the Intermolecular Reductive Amination Reaction[a]



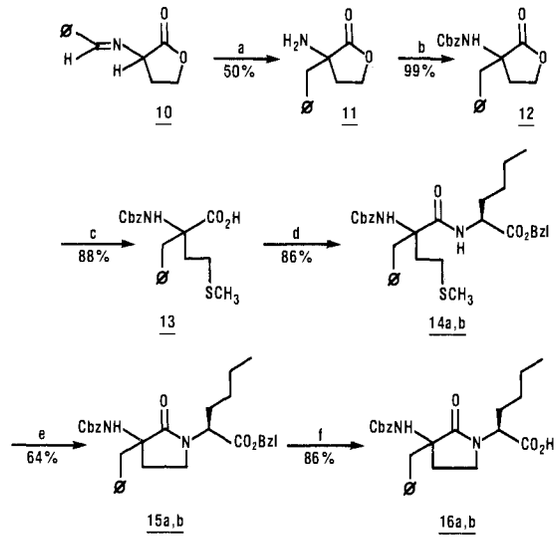
[a] (a) LDA, THF; CH_2=$CHCH_2Br$; (b) OsO_4, $NaIO_4$, THF, H_2O; (c) $NH_2CH(C_4H_9)CO_2Bn$, THF, HOAc; $NaCNBH_3$; (d) HCl(g), Et_2O, CH_2Cl_2; *i*-Pr_2 NEt, $(EtO)_2P(O)CN$, CH_2Cl_2.

P_2 site of the angiotensinogen. In addition to the γ-lactam dipeptide isostere **16**, we also explored the simpler γ-lactam **5** without the acylamino group in anticipation of N-terminal truncated renin inhibitory peptides.

As shown in Scheme I, alkylation of the lithium salt of γ-butyrolactone with benzyl bromide gave the substituted lactone **1**. Treatment of the γ-lactone with lithium thiomethoxide in hexamethylphosphoramide[14] afforded the acid **2**. The thiomethyl group serves as a latent leaving group for the intramolecular displacement reaction.[13] Coupling[15] of this acid to L-norleucine benzyl ester gave the amide **3a,b** as a mixture of diastereomers. Methylation on sulfur with trimethyloxonium tetrafluoroborate was followed by base treatment to induce a ring closure to give the γ-lactams **4a** and **4b**, which were separated by chromatography. Freidinger et al.[13] observed 12–15% epimerization of the ester when sodium hydride was used and when phenylalanine was involved. We have found that the use of a stoichiometric amount or less of the lithium salt of *N*-methylacetamide[16] for the displacement reaction resulted in nonobservable epimerization even in the case of phenylalanine. The benzyl esters **4a** and **4b** were then hydrogenolyzed to give the acids **5a** and **5b**.

(14) Kelly, T. R.; Dali, H. M.; Tsang, W.-G. *Tetrahedron Lett.* **1977**, 3859.
(15) Konig, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788.
(16) Moon, M. W.; Lahti, R. A.; VonVoigtlander, P. F. In *Proceedings of the 7th American Peptide Symposium*; Rich, D., Gross, E., Eds.; Pierce Chemical Co.: Rockford, IL, 1981; p 641.

Scheme III. Synthesis of the γ-Lactam **15** via the Intramolecular Displacement Reaction[a]



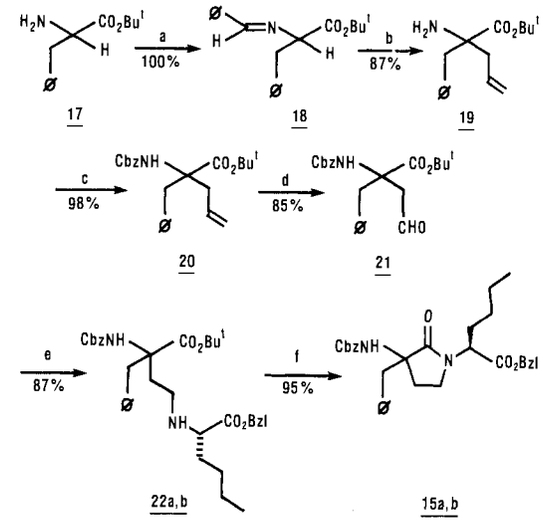
[a] (a) LDA, THF; $C_6H_5CH_2Br$; 10% aqueous HCl; (b) $C_6H_5CH_2OCOCl$, Na_2CO_3, THF; (c) $LiSCH_3$, HMPA; (d) $NH_2CH(C_4H_9)CO_2Bn$, DCC, 1-HOBT, CH_2Cl_2; (e) Me_3OBF_4, CH_2Cl_2; CH_3CONCH_3Li, THF; (f) aqueous NaOH, THF.

The alternative synthetic sequence is shown in Scheme II. Alkylation of the ester enolate of **6** with allyl bromide gave compound **7**. Oxidative cleavage of the terminal olefin with osmium tetraoxide/sodium periodate[17] led to the aldehyde **8**. A reductive amination reaction between this aldehyde and L-norleucine benzyl ester with sodium cyanoborohydride[18] gave the adduct **9a,b** as a mixture of diastereomers. Acidic treatment to remove the *tert*-butyl protecting group was followed by an intramolecular condensation reaction with diethyl phosphorocyanidate[19] to give the γ-lactams **4a** and **4b**, which were identical with those prepared in Scheme I.

The preparation of the γ-lactam **16** is shown in Scheme III. Alkylation[20] of the lithium salt of *N*-benzylidene-α-amino-γ-butyrolactone (**10**) with benzyl bromide was followed by an acidic workup to give the amine **11**. This was protected as the corresponding benzyloxycarbonyl derivative **12**. Treatment of this lactone with lithium thiomethoxide in hexamethylphosphoramide gave the acid **13**. Coupling of this acid with L-norleucine benzyl ester gave a diastereomeric mixture **14a,b**. Methylation on sulfur with trimethyloxonium tetrafluoroborate was followed by base-induced ring closure to the γ-lactam **15a,b** as a mixture of diastereomers. Base hydrolysis of the ester then afforded the corresponding acid **16a,b**.

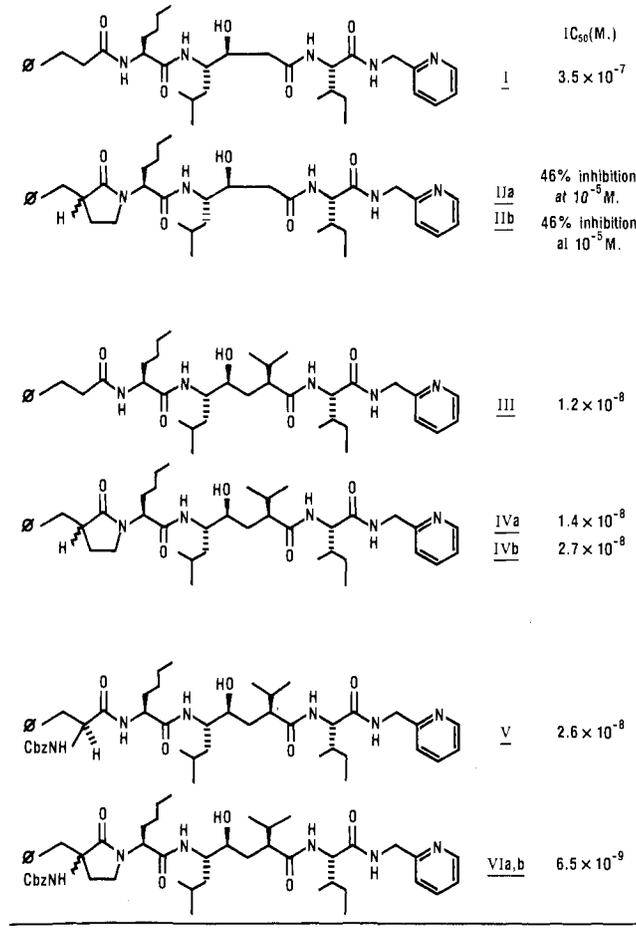
The alternative synthesis of the γ-lactam **15** is shown in Scheme IV. The benzylidene of phenylalanine *tert*-butyl ester (**18**) could be alkylated with allyl bromide. Mild acidic treatment removed the benzylidene group to give the amine **19**, which was protected as the benzyloxycarbonyl protecting group in **20**. Oxidative cleavage of the olefin gave the aldehyde **21**. Reductive amination of this aldehyde with L-norleucine benzyl ester gave the diastereomeric mixture **22a,b**. Removal of the *tert*-butyl group with acid was followed by an intramolecular condensation reaction to give the γ-lactam mixture **15a,b** as already prepared in Scheme III.

Scheme IV. Synthesis of the γ-Lactam **15** via the Intermolecular Reductive Amination Reaction[a]



[a] (a) C_6H_5CHO, Et_3N; (b) LDA, THF; $CH_2{=}CHCH_2Br$; aqueous citric acid; (c) $C_6H_5CH_2OCOCl$, Na_2CO_3, THF; (d) OsO_4, $NaIO_4$, THF, H_2O; (e) $NH_2CH(C_4H_9)CO_2Bn$, THF, HOAC; $NaCNBH_3$; (f) HCl(g), Et_2O, CH_2Cl_2; *i*-Pr_2NEt, $(EtO)_2P(O)CN$, CH_2Cl_2.

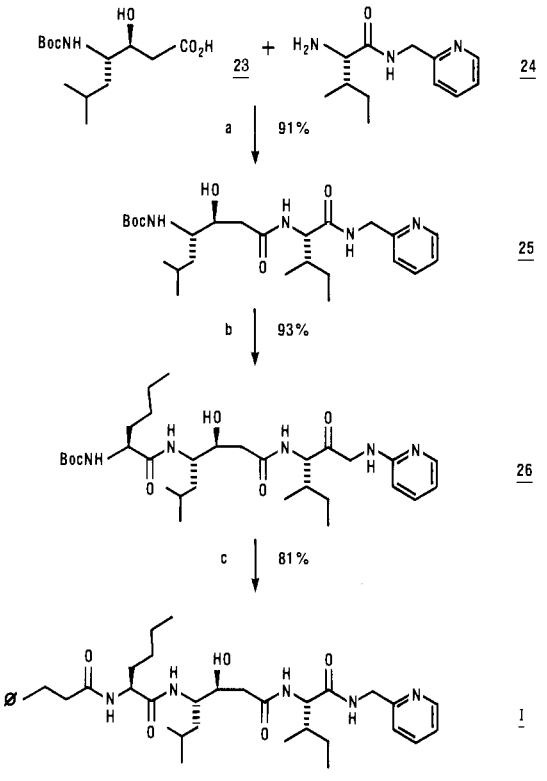
Table I. Inhibition of Human Plasma Renin



We chose to examine renin inhibitory peptides that contain two different transition-state-analogue inserts:[21] statine and the hydroxyethylene isostere of Leu(10)–Val(11) bond. These two building blocks for the dipeptide isosteres of the scissile site, 4(*S*)-[(*tert*-butyloxycarbonyl)amino]-3(*S*)-hydroxy-6-methylheptanoic acid

(17) Pappo, R.; Allen, D. S., Jr.; Lemieux, R. U.; Johnson, W. S. *J. Org. Chem.* **1956**, *21*, 478.
(18) Borch, R. F. *Org. Synth.* **1972**, *52*, 124.
(19) Yamada, S.; Kasai, Y.; Shiori, T. *Tetrahedron Lett.* **1973**, 1595.
(20) Stork, G.; Leong, A. Y. W.; Touzin, A. M. *J. Org. Chem.* **1976**, *41*, 3491.
(21) Boger, J. *Annu. Rep. Med. Chem.* **1985**, *20*, 257.

Scheme V. Synthesis of Peptide I[a]



[a] (a) $(EtO)_2P(O)CN$, *i*-Pr_2NEt, CH_2Cl_2; (b) HCl(g), Et_2O; $BocNHCH(C_4H_9)CO_2H$, $(EtO)_2P(O)CN$, *i*-Pr_2NEt, CH_2Cl_2; (c) TFA, CH_2Cl_2; $C_6H_5CH_2CH_2CO_2H$, $(EtO)_2P(O)CN$, *i*-Pr_2NEt, CH_2Cl_2.

Table II. Physical Characteristics of Compounds in Table I

				FAB-HRMS	
compd[a]	yield,[b] %	HPLC[c] k'	formula	calcd	found
I		6.75	$C_{35}H_{54}N_5O_5$	624.4125	624.4161
IIa	77	4.82	$C_{37}H_{56}N_5O_5$	650.4281	650.4270
IIb	85	4.95	$C_{37}H_{56}N_5O_5$	650.4281	650.4315
III		4.10	$C_{39}H_{62}N_5O_5$	680.4751	680.4742
IVa	80	3.71	$C_{41}H_{64}N_5O_5$	706.4907	706.4882
IVb	92	2.92	$C_{41}H_{64}N_5O_5$	706.4907	706.4917
V		6.46	$C_{47}H_{69}N_6O_7$	829.5224	829.5227
VIa,b	100	4.23, 5.80	$C_{49}H_{71}N_6O_7$	855.5384	855.5422

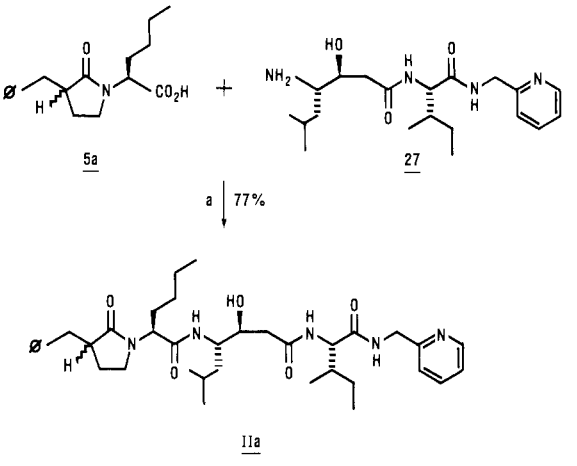
[a] ^1H NMR found consistent with structures. [b] Coupling step of the γ-lactam dipeptide isostere as in the preparation of compound IIa. [c] See the Experimental Section for conditions; k' is the partition ratio.

(**23**)[22] and 4(*S*)-[(*tert*-butyldimethylsilyl)oxy]-5(*S*)-[(*tert*-butyloxycarbonyl)amino]-2(*S*)-isopropyl-7-methyloctanoic acid[10,23] were prepared by known procedures.

The linear peptide I in Table I was prepared in a straightforward manner from the acid **23** and the amine **24**[10] as shown in Scheme V. The other two peptides III and V were also prepared in a similar manner but with a different acid[10] as the starting material. The γ-lactam-containing peptides IIa, IIb, IVa, IVb, and VIa,b were prepared in the same manner and an example is shown in Scheme VI. The yields of these condensation reactions are given in Table II.

Peptide VIa,b was obtained as an epimeric mixture. Peptides IIa and IIb are two distinct epimers. Peptides IVa and IVb are also two separate epimers.

Scheme VI. Synthesis of Peptide IIa[a]



[a] (a) $(EtO)_2P(O)CN$, Et_3N, CH_2Cl_2.

Results and Discussion

Compounds in Table I were evaluated as inhibitors of human plasma renin with IC_{50} values as shown. The statine-containing linear peptide I is an effective inhibitor of renin with an IC_{50} value of 3.5×10^{-7} M. The corresponding conformationally constrained γ-lactam congeners IIa and IIb, however, show drastically reduced renin inhibitory potency. This result would tend to suggest that the conformational restriction introduced into peptide I has seriously affected the proper binding conformers. The result is very different with the hydroxyethylene-isostere-containing series. The linear peptide III and the two epimeric γ-lactam congeners IVa and IVb all show nearly the same IC_{50} values. In this instance, the γ-lactam restriction on peptide III did not preclude the bioactive conformers. If one were to assume that the phenylmethyl groups in both epimers IVa and IVb bind approximately to the same S_3 site of the enzyme, the stereochemical orientation of these two epimers relative to the enzyme would then differ only in the interchange of the ethylene unit and the carbonyl group. This result seems to suggest that the carbonyl is not involved in an essential hydrogen-bonding interaction with the enzyme since the two epimers IVa and IVb show similar binding affinity.

Compounds II and IV differ in only one methylene unit at the transition-state-analogue inserts. Powers et al.[24] has suggested on the basis of pepsin substrates to pepstatin that statine might better be an isosteric replacement of a dipeptide even though it is one atom too short to match a dipeptide. On the basis of the X-ray data for pepstatin bound to *Rhizopus chinensis* aspartyl protease, Boger[7b] proposed a model in which statine resembles a dipeptide in its tetrahedral intermediate form. The use of statine as a dipeptide replacement has generated numerous potent renin inhibitory peptides.[25] Szelke et al.[7a] has also described the hydroxyethylene isostere for the preparation of very active renin inhibitors. The close agreement in the resulting potencies of the peptides with statine and hydroxyethylene isostere is further evidence for statine

(22) Rich, D. H.; Sun, E. T.; Bopari, A. S. *J. Org. Chem.* **1978**, *43*, 3624.
(23) Hester, J. B.; Pals, D. T.; Saneii, H. H.; Sawyer, T. K.; Schostarez, H. J.; TenBrink, R. E.; Thaisrivongs, S. *Eur. Pat. Appl.* 0173481.
(24) Powers, J. C.; Harley, A. D.; Myers, D. V. In *Acid Proteases—Structure, Function and Biology*; Tang, J.; Ed.; Plenum: New York, 1977; p 141.
(25) 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.; Sabilito, I. I.; Veber, D. F.; Rich, D. H.; Bopari, A. S. *Nature (London)* **1983**, *303*, 81.

functioning as a dipeptide replacement. In the present investigation, however, introduction of a γ-lactam restriction at the P_2–P_3 site has rendered this concept of statine as a dipeptide replacement invalid in this template. There is a difference of 3 orders of magnitude in inhibitory potencies between compounds II and IV. We have made similar observation earlier for renin inhibitory peptides that contain N^{α}-methylation of the amino acid at P_2 site.[10a] These results suggest that the mode of binding of statine-containing inhibitors differs from that of inhibitors with a hydroxyethylene dipeptide isostere. This difference significantly effects the resulting binding affinity in certain templates.

With the more appropriately substituted γ-lactam **16a,b** we have prepared the peptide VIa,b as a mixture of epimers. As compared to the linear congener V, the restricted analogue showed some enhancement in inhibitory potency. The γ-lactam dipeptide isostere replacement can also be expected to render the normal peptidic bond at P_2–P_3 site no longer a potential site of cleavage by proteolytic enzymes.

It is noted here that these γ-lactam-containing peptides can potentially be prepared as single isomers with known absolute configuration. Individual isomers of γ-lactam building blocks **4** and **15** are required. In Scheme II, an asymmetric alkylation[26] of a chiral ester of hydrocinnamic acid would yield an optically active 2-allyl derivative **7**. Continuing the same sequence of reactions would then afford **4a** or **4b** as single isomers. In Scheme IV, an asymmetric alkylation[27] of a phenylalanine derivative would give the optically active 2-allyl derivative **19**. Following the same synthetic route, the preparation of the γ-lactam **15a** or **15b** as single isomers should also be feasible.

Summary

In this paper, a model of the conformation of enzyme-bound inhibitor of human renin has led to the proposal of a γ-lactam cyclic constraint at the P_2–P_3 site. The intent is to minimize metabolized conformers of the resulting peptides in addition to the replacement of the peptidic bond by a nonmetabolized isostere. It is also hoped that through such restriction the biologically active conformers can be optimized and that selectivity of the resulting congeners can also be improved.

We have outlined two different synthetic routes to each of the two differently substituted γ-lactams. One reaction scheme is based on an intramolecular displacement reaction for the ring closure step, and the other is based on an intermolecular reductive amination reaction. We have demonstrated their feasibility and efficiency in the present paper. These sequences should prove useful in the preparation of a wide variety of other dipeptide isosteres with flexibility in the two sidechains. We have also eluded to the feasibility in securing optically active isomers with known absolute configuration.

We have demonstrated by way of examples that this type of conformational constraint as suggested by the model can lead to active renin inhibitors. It is noted that the successful application of such a restriction unit is substrate-dependent as demonstrated in the difference between the statine-containing and the hydroxyethylene-isostere-containing congeneric peptides. Further structure–activity analysis on these series of compounds and additional testings of these conformationally restricted congeners will offer additional useful information for the design of therapeutically useful inhibitors of renin.

Experimental Section

Chemistry. Mass spectra, infrared spectra, optical rotations, and combustion analyses were obtained by the Physical and Analytical Chemistry Department of The Upjohn Co. ^{1}H NMR spectra were recorded at 80 MHz with a Varian Model CFT-20 spectrometer and at 300 MHz with a Bruker Model AM-300 spectrometer. Chemical shifts were reported as δ units relative to tetramethylsilane as internal standard.

Thin-layer chromatography was conducted with Analtech 0.25-mm glass plates precoated with silica gel GF. For column chromatography, E. Merck silica gel 60, 70–230 and 230–400 mesh, were used. All solvents for chromatography were Burdick and Jackson reagent grade distilled in glass.

Tetrahydrofuran was distilled under argon from sodium metal in the presence of benzophenone. Dichloromethane was dried over 4-Å molecular sieves. Hexamethylphosphoramide, diisopropylamine, diisopropylethylamine and triethylamine were distilled from calcium hydride. Diethyl phosphorocyanidate was freshly distilled before use.

4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3(*S*)-hydroxy-6-methylheptanoic acid was prepared according to Rich et al.[22] 4(*S*)-[*tert*-Butyldimethylsilyl)oxy]-5(*S*)-[(*tert*-butyloxycarbonyl)amino]-2(*S*)-isopropyl-7-methyloctanoic acid was prepared according to Hester et al.[23] Other materials are commercially available reagent-grade chemicals.

Compounds in Table I were analyzed on a Perkin-Elmer Series 4 liquid chromatograph with a Kratos Spectroflow 773 detector (215 or 254 nm) and a Perkin-Elmer LCI-100 integrator with a Brownlee RP-18, 10 μm, 25 cm × 4.6 mm analytical column at a flow rate of 1.5 mL/min. The mobile phase for compounds IIa, IIb, III, and IV was an isocratic mixture of 75% methanol and 25% aqueous phosphate pH 3 buffer; for compound I, a 70:30 mixture; and for compounds IVa, IVb, and VIa,b, an 80:20 mixture.

α-Benzyl-γ-butyrolactone (1). To a stirred solution of 31.5 mL (31.5 mmol) of 1 M lithium hexamethyldisilazide in tetrahydrofuran at −78 °C under argon was slowly added to a solution of 2.3 mL (29.9 mmol) of γ-butyrolactone in 10 mL of tetrahydrofuran. After complete addition, the reaction mixture was allowed to stir for 10 min, and then 4.3 mL (36.2 mmol) of benzyl bromide was slowly added. After 30 min, the cooling bath was replaced with an ice water bath. After 1 h, the mixture was allowed to warm to room temperature. After stirring overnight, the concentrated reaction mixture was partitioned between dichloromethane and half-saturated aqueous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 5–10% ethyl acetate in hexane. The dialkylated γ-butyrolactone eluded off first, followed by 3.3 g (18.7 mmol, 63%) of the desired compound 1: ^{1}H NMR ($CDCl_3$) δ 1.6–3.2 (m, 5 H), 4.1–4.3 (m, 2 H), 7.3 (br s, 5 H); IR (neat) 1768 cm^{-1}; HRMS, *m/z* 176.0829 (calcd for $C_{11}H_{12}O_2$ 176.0837). Anal. ($C_{11}H_{12}O_2$) C, H.

2-[(Methylthio)ethyl]hydrocinnamic Acid (2). A mixture of 1.0326 g (5.87 mmol) of the lactone 1 and 0.64 g (11.8 mmol) of lithium thiomethoxide in 5 mL of hexamethylphosphoramide was allowed to stir at room temperature. After 2 days, the reaction mixture was taken up in 50 mL of water and then extracted with three 30-mL portions of dichloromethane. The aqueous phase was acidified (methyl orange as indicator) with concentrated HCl. The resulting mixture was extracted with three 30-mL portions of ether. The combined ethereal phases were washed with three 20-mL portions of water. The organic phase was then dried ($MgSO_4$) and concentrated to give 1.25 g (5.57 mmol, 95%) of the acid **2**: ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 3 H) 7.24 (br s, 5 H).

***N*-[2-[(Methylthio)ethyl]hydrocinnamoyl]-L-norleucine Benzyl Esters (3a,b).** To a stirred solution of 1.24 g (5.54 mmol) of the acid **2**, 6.1 mmol of L-norleucine benzyl ester (2.4 g of the tosyl salt in dichloromethane was washed with saturated aqueous $NaHCO_3$ and dried over $MgSO_4$), and 0.75 g (5.6 mmol) of 1-hydroxybenzotriazole in 50 mL of dichloromethane was added 1.25 g (6.1 mmol) of dicyclohexylcarbodiimide. After 14 h, the reaction mixture was filtered, and the filtrate was washed with aqueous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and

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

(27) Seebach, D.; Aebi, J. D.; Naef, R.; Weber, T. *Helv. Chim. Acta* **1985**, *68*, 144.

then concentrated. The residue was chromatographed on silica gel to give 2 g (4.68 mmol, 84%) of the amides **3a,b** as a mixture of diastereomers: ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 3 H), 5.05 (d, 1 H, J = 12 Hz), 5.22 (d, 1 H, J = 12 Hz), 5.9 (d, 1 H, J = 9 Hz), 7.3 (m, 10 H); IR (mull) 1724, 1639 cm^{-1}; HRMS, m/z 427.2176 (calcd for $C_{25}H_{33}NO_3S$ 427.2181). Anal. ($C_{25}H_{33}NO_3S$) C, H, N.

3(*R*)- and 3(*S*)-Benzyl-2-oxo-1-pyrrolidine-2(*S*)-hexanoic Acid Benzyl Esters (4a and 4b). A mixture of 747 mg (1.75 mmol) of compound **3a,b** and 272 mg (1.84 mmol) of trimethyloxonium tetrafluoroborate in 7 mL of dichloromethane was allowed to stir at room temperature for 2 h. The solution was then concentrated and dried.

To a solution of 190 mg (2.6 mmol) of *N*-methylacetamide in 15 mL of tetrahydrofuran at 0 °C under argon was added 1.75 mL (1.75 mmol) of lithium hexamethyldisilazide. After 30 min, a solution of the above residue in 15 mL of tetrahydrofuran was added. After 90 min, the reaction mixture was partitioned between ethyl acetate and aqueous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 10–15% ethyl acetate in hexane to give 221 mg (0.58 mmol, 33%) of isomer **4a** and 342 mg (0.90 mmol, 52%) of isomer **4b**.

Compound **4a**: ^{1}H NMR ($CDCl_3$) δ 5.2 (s, 2 H), 7.2 (s, 5 H), 7.3 (s, 5 H); IR (neat) 1740, 1692 cm^{-1}; $[\alpha]_D$ +22° (c 0.525, $CHCl_3$); HRMS, m/z 379.2146 (calcd for $C_{24}H_{29}NO_3$ 379.2147). Anal. ($C_{24}H_{29}NO_3$) C, H, N.

Compound **4b**: ^{1}H NMR ($CDCl_3$) δ 5.2 (s, 2 H), 7.2 (s, 5 H), 7.3 (s, 5 H); IR (neat) 1740, 1691 cm^{-1}; [α] 60° (c 0.658, $CHCl_3$); HRMS, m/z 379.2146 (calcd for $C_{24}H_{29}NO_3$ 379.2147). Anal. ($C_{24}H_{29}NO_3$) C, H, N.

3(*R*)- and 3(*S*)-Benzyl-2-oxo-1-pyrrolidine-2(*S*)-hexanoic Acids (5a and 5b). A suspension of 152 mg (0.40 mmol) of the benzyl ester **4a** and 15 mg of 10% palladium on activated charcoal or 272 mg (0.717 mmol) of the benzyl ester **4b** and 25 mg of 10% palladium on activated charcoal in 3 mL of methanol was stirred under hydrogen atmosphere for 2 h. The suspension was then filtered, and the filtrate was concentrated to give 107 mg (0.34 mmol, 85%) of the acid **5a** or 206 mg (0.71 mmol, 99%) of the acid **5b**.

Acid **5a**: ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3 H, J = 7 Hz), 4.7 (dd, 1 H, J = 5, 10 Hz), 7.3 (br s, 5 H); IR (mull) 1729, 1642 cm^{-1}; $[\alpha]_D$ +37° (c 0.481, $CHCl_3$); HRMS, m/z 289.1674 (calcd for $C_{17}H_{23}NO_3$ 289.1678). Anal. ($C_{17}H_{23}NO_3$) C, H, N.

Acid **5b**: ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3 H, J = 7 Hz), 4.7 (dd, 1 H, J = 5, 11 Hz), 7.3 (br s, 5 H); IR (neat) 1734, 1689, 1643; $[\alpha]_D$ −34° (c 1.037, $CHCl_3$); HRMS, m/z 289.1677 (calcd for $C_{17}H_{23}NO_3$ 289.1678).

2-Allylhydrocinnamic Acid *tert*-Butyl Ester (7). To a stirred solution of 5.4 mL (39 mmol) of diisopropylamine in 32 mL of tetrahydrofuran at −78 °C under argon was added 20.6 mL (35 mmol) of a 1.70 M solution of *n*-butyllithium in hexane. After 15 min, a solution of 6.55 g (31.7 mmol) of *tert*-butyl hydrocinnamate in 16 mL of tetrahydrofuran was slowly added. After 15 min, 5.5 mL (64 mmol) of allyl bromide was added, and the resulting solution was allowed to warm to room temperature. After 30 min, 100 mL of 0.5 M aqueous $KHSO_4$ was added, and the resulting mixture extracted with several portions of ether. The organic phase was washed with diluted aqueous HCl and saturated aqueous NaCl and then dried ($MgSO_4$). The organic layer was concentrated, and the residue was chromatographed on silica gel with 5% ether in hexane to give 6.30 g (25.6 mmol, 81%) of compound **7**: ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 9 H), 2.2–3.0 (m, 5 H), 5.1 (m, 2 H), 5.8 (m, 1 H), 7.2 (m, 5 H); IR (neat) 1728 cm^{-1}; HRMS, m/z 246.1631 (calcd for $C_{16}H_{22}O_2$ 246.1620). Anal. ($C_{16}H_{22}O_2$) C, H.

2-(Formylmethyl)hydrocinnamic Acid *tert*-Butyl Ester (8). To a stirred solution of 739 mg (3.0 mmol) of compound **7** in 10 mL of tetrahydrofuran was added 3.9 mL of water and small amount of osmium tetraoxide. To this vigorously stirred mixture was added 1.93 g (9.0 mmol) of sodium periodate in portions. After 20 min, the mixture was partitioned between ether and water. The organic phase was washed with aqueous $Na_2S_2O_3$ and aqueous NaCl and then dried ($MgSO_4$). The organic layer was concentrated, and the residue was chromatographed on silica gel with 15% ethyl acetate in hexane to give 598 mg (2.41 mmol, 80%) of the aldehyde **8**: ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9 H), 2.5 (m, 1 H), 2.8 (m, 2 H), 3.1 (m, 2 H), 7.2 (m, 5 H), 9.7 (s, 1 H); IR (neat) 1725 cm^{-1}; HRMS, m/z 248.1417 (calcd for $C_{15}H_{20}O_3$ 248.1412). Anal. ($C_{15}H_{20}O_3$) C, H.

***N*-[4-Phenyl-3(*R*)- and -3(*S*)-(*tert*-butyloxycarbonyl)-butyl]-L-norleucine Benzyl Esters (9a,b).** To a stirred solution of 295 mg (1.3 mmol) of L-norleucine benzyl ester and 330 mg (1.3 mmol) of the aldehyde **8** in 5 mL of tetrahydrofuran was added 4-Å molecular sieves. After 30 min, 76 μL (1.3 mmol) of acetic acid and 84 mg (1.3 mmol) of sodium cyanoborohydride were successively added. After 2 h, the reaction mixture was partitioned between dichloromethane and aqueous NaHCO3. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 20% ethyl acetate in hexane to give 424 mg (0.93 mmol, 72%) of a diastereomeric mixture **9a,b**: ^{1}H NMR ($CDCl_3$) mixture of *tert*-butyl and benzyl esters; IR (mull) 1742, 1726 cm^{-1}; HRMS, m/z 454.2948 (calcd for $C_{28}H_{40}NO_4$ 454.2957).

3(*R*)- and 3(*S*)-Benzyl-2-oxo-1-pyrrolidine-2(*S*)-hexanoic Acid Benzyl Esters (4a,b). To a stirred solution of 170 mg (0.37 mmol) of compounds **9a,b** in 2 mL of dichloromethane and 13 mL of ether was passed a stream of HCl gas. After 15 min, the resulting mixture was concentrated. To a stirred mixture of this residue in 4 mL of dichloromethane was added 0.14 mL (0.8 mmol) of diisopropylethylamine and 0.17 mL (0.45 mmol) of diethyl phosphorocyanidate. After being stirred overnight, the concentrated reaction mixture was chromatographed on silica gel with 20% ethyl acetate in hexane to give 167 mg as a mixture of compounds **4a** and **4b** as prepared earlier.

***N*-Benzylidene-α-amino-γ-butyrolactone (10).** To a suspension of 3.84 g (21.1 mmol) of α-amino-γ-butyrolactone hydrogen bromide in 40 mL of dichloromethane was added 2.15 mL (21.15 mmol) of benzaldehyde, followed by 5.9 mL (42.3 mmol) of triethylamine and excess $MgSO_4$. After being stirred at room temperature for 20 h, the mixture was filtered, and the filtrate was concentrated. a 200-mL portion of ether was added, and the resulting suspension was filtered. The filtrate was washed with 50 mL of saturated aqueous NaCl. The aqueous phase was extracted with two 100-mL portions of ether. The combined organic phase was dried ($MgSO_4$) and then concentrated. The residue was evaporatively distilled at 0.05 mmHg (Kugelrohr oven 200–250 °C) to give 3.5 g of an oil, which solidified on storage in a freezer (18.5 mmol, 88%): ^{1}H NMR ($CDCl_3$) δ 2.9 (m, 2 H), 4.3 (m, 3 H), 7.35 (m, 3 H), 7.7 (m, 2 H), 8.3 (s, 1 H).

α-Benzyl-α-amino-γ-butyrolactone (11). To a stirred solution of 2.8 mL (20.0 mmol) of diisopropylamine in 12 mL of tetrahydrofuran at −78 °C under argon was added 11.7 mL (18.2 mmol) of *n*-butyllithium in hexane. After 15 min, a solution of 3.16 g (16.7 mmol) of compound **10** in 10 mL of tetrahydrofuran at −78 °C under argon was cannulated into the stirred reaction mixture. After 15 min, 2.14 mL (18.0 mmol) of benzyl bromide was added. After 5 min, the reaction mixture was allowed to stir at room temperature for 24 h. It was then cooled in an ice bath, and 20 mL of 10% aqueous HCl was added. After the mixture was stirred at room temperature for 1 h, it was recooled in an ice bath, and an excess of saturated aqueous $NaHCO_3$ was slowly added. The resulting mixture was added to 200 mL of dichloromethane and washed with 50 mL of saturated aqueous $NaHCO_3$. The aqueous phase was extracted with three 100-mL portions of dichloromethane. The combined organic phase was dried ($MgSO_4$) and then concentrated. The resulting residue was chromatographed on silica gel with ethyl acetate to 5% methanol in ethyl acetate to give 1.6 g (8.37 mmol, 50%) of a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.56 (br s, 2 H), 2.81 (d, 1 H, J = 15 Hz), 3.03 (d, 1 H, J = 15 Hz), 7.2 (m, 5 H).

α-Benzyl-α-[(benzyloxycarbonyl)amino]-γ-butyrolactone (12). To a stirred solution of 1.55 g (8.1 mmol) of the amine **11** in 16 mL of tetrahydrofuran was added 2.0 g (19 mmol) of powdered sodium carbonate, followed by 1.27 mL (8.9 mmol) of benzyl chloroformate. After the mixture was stirred at room temperature for 18 h, water was added to dissolve the salt, and the resulting mixture was diluted with 100 mL of ethyl acetate. It was washed with 50 mL of saturated aqueous NaCl. The aqueous phase was extracted with two 50-mL portions of ethyl acetate. The combined organic phase was dried ($MgSO_4$) and then concentrated. The residue was passed through 20 g of silica gel with ethyl acetate, and the filtrate was concentrated to a white solid, 2.6 g (8.0 mmol,

99%): ^{1}H NMR ($CDCl_3$) δ 2.67 (m, 2 H), 2.97 (d, 1 H, J = 14 Hz), 3.2 (d, 1 H, J = 14 Hz), 3.44 (dd, 1 H, J = 9, 16 Hz), 4.15 (m, 1 H), 5.10 (s, 2 H), 5.34 (br s, 1 H), 7.26 (s, 5 H), 7.34 (s, 5 H); IR (mull) 1754, 1777 cm^{-1}; MS, m/z 325. Anal. ($C_{19}H_{19}NO_4$) C, H, N.

2-[(Benzyloxycarbonyl)amino]-2-[(methylthio)ethyl]-hydrocinnamic Acid (13). A mixture of 2.475 g (7.6 mmol) of the γ-lactone **12** and 540 mg (10 mmol) of lithium thiomethoxide in 8 mL of hexamethylphosphoramide was allowed to stand at room temperature for 4 days. It was then added to 150 mL of dichloromethane and 50 mL of saturated aqueous NaCl. To this vigorously stirred mixture was added 10% aqueous HCl (methyl orange indicator) until acidic. The organic phase was further washed with 50 mL of saturated aqueous NaCl. The aqueous phases were extracted with the same two 60-mL portions of dichloromethane. The combined organic phase was dried ($MgSO_4$) and then concentrated. The residue was taken up in 200 mL of water, and the aqueous phase was extracted with three 200-mL portions of ether. The combined organic phase was dried ($MgSO_4$) and then concentrated to give 2.5 g of the acid **13** (6.69 mmol, 88%): ^{1}H NMR ($CDCl_3$) δ 2.1 (s, 3 H), 3.1 (d, 1 H, J = 14 Hz), 3.6 (d, 1 H, J = 14 Hz), 5.1 (d, 1 H, J = 12 Hz), 5.5 (d, 1 H, J = 12 Hz), 6.8–7.4 (m, 10 H).

***N*-[2(*R*)- and 2(*S*)-[(Benzyloxycarbonyl)amino]-2-[(methylthio)ethyl]hydrocinnamoyl]-L-norleucine Benzyl Esters (14a,b).** To a stirred solution of 1.43 mmol of L-norleucine benzyl ester (from 562 mg of the tosyl salt CH_2Cl_2/aqueous $NaHCO_3$), 590 mg (1.58 mmol) of the acid **13**, and 210 mg (1.55 mmol) of 1-hydroxybenzotriazole in 20 mL of dichloromethane was added 320 mg (1.55 mmol) of dicylohexylcarbodiimide. After 6 h, the mixture was filtered, and the filtrate was partitioned between dichloromethane and aqueous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was triturated with EtOAc and then filtered. The concentrated filtrate was chromatographed on silica gel with 15% ethyl acetate in hexane to give a white solid, 713 mg (1.24 mmol, 86%): ^{1}H NMR ($CDCl_3$) shows approximately equal mixture of two diastereomers; IR (mull) 1744, 1721, 1651 cm^{-1}. Anal. ($C_{33}H_{40}N_2O_5S$) C, H, N, S.

3(*R*)- and 3(*S*)-Benzyl-3-[(benzyloxycarbonyl)amino]-2-oxo-1-pyrrolidine-2(*S*)-hexanoic Acid Benzyl Esters (15a,b). A mixture of 656 mg (1.14 mmol) of compounds **14a,b** and 180 mg (1.22 mmol) of trimethyloxonium tetrafluoroborate in 4.5 mL of dichloromethane was allowed to stir at room temperature for 90 min. It was then concentrated.

To a stirred solution of 125 mg (1.71 mmol) of *N*-methylacetamide in 10 mL of tetrahydrofuran at 0 °C was added 1.1 mL (1.1 mmol) of lithium hexamethyldisilazide in tetrahydrofuran. After 15 min, a solution of the above residue in 5 mL of tetrahydrofuran was added. After 1 h, the reaction mixture was partitioned between dichloromethane and aqueous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 25% ethyl acetate in hexane to give 384 mg (0.73 mmol, 64%): ^{1}H NMR ($CDCl_3$) shows approximately equal mixture of two diastereomers; IR (neat) 1738, 1697 cm^{-1}; HRMS, m/z 529.2695 (calcd for $C_{32}H_{37}N_2O_5$ 529.2702). Anal. ($C_{32}H_{36}N_2O_5$) C, H, N.

3(*R*)- and 3(*S*)-Benzyl-3-[(benzyloxycarbonyl)amino]-2-oxo-1-pyrrolidine-2(*S*)-hexanoic Acids (16a,b). To a stirred solution of 326 mg (0.617 mmol) of the esters **15a,b** in 2 mL of tetrahydrofuran was added 1 mL of 1 M aqueous NaOH and a small amount of methanol to obtain a clear homogeneous solution. After 4 h, tetrahydrofuran was removed on a rotary evaporator. The aqueous phase was extracted with ether and then acidified with concentrated HCl. Extractions with dichloromethane gave the acids **16a,b** (233 mg, 0.53 mmol, 86%): ^{1}H NMR ($CDCl_3$) shows approximately equal mixture of two diastereomers; IR (mull) 1720, 1643 cm^{-1}; HRMS, m/z 439.2211 (calcd for $C_{25}H_{31}N_2O_5$ 439.2233). Anal. ($C_{25}H_{30}N_2O_5$) C, H, N.

***N*-Benzylidenephenylalanine *tert*-Butyl Ester (18).** A mixture of 1.033 g (9.73 mmol) of benzaldehyde, 2.15 g (9.72 mmol) of phenylalanine *tert*-butyl ester, 1.35 mL (9.70 mmol) of triethylamine, and excess $MgSO_4$ in 30 mL of dichloromethane was allowed to stir at room temperature overnight. It was then filtered, and the filtrate was concentrated. The residue was partitioned between ether and aqueous NaCl. The organic phase was dried ($MgSO_4$) and the concentrated to give 3 g (9.7 mmol, 100%) of compound **18**: ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 9 H), 3.1 (dd, 1 H), 3.3 (dd, 1 H), 4.1 (dd, 1 H), 7.1–7.6 (m, 10 H), 8.0 (s, 1 H); IR (neat) 1734, 1642 cm^{-1}; HRMS, m/z 309.1720 (calcd for $C_{20}H_{23}NO_2$ 309.1729).

α-Allylphenylalanine *tert*-Butyl Ester (19). To a stirred solution of 1.85 mL (13.2 mmol) of diisopropylamine in 6.4 mL of tetrahydrofuran at −78 °C under argon was added 6.4 mL (10.6 mmol) of *n*-butyllithium in hexane. After 15 min, a solution of 2.72 g (8.8 mmol) of compound **18** in 4 mL of tetrahydrofuran was added. After 10 min, 1.2 mL (13.9 mmol) of allyl bromide was added, and the resulting mixture allowed to warm to room temperature. After the mixture was stirred overnight, a solution of 4.5 g of citric acid in 30 mL of water was added. After a few hours, the reaction mixture was partitioned between aqueous $NaHCO_3$ and dichloromethane. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 30% ethyl acetate in hexane to give 2.0 g (7.65 mmol, 87%) of compound **19**: ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9 H), 2.27 (dd, 1 H, J = 8, 13 Hz), 2.68 (dd, 1 H, J = 6, 13 Hz), 2.75 (d, 1 H, J = 13 Hz), 3.16 (d, 1 H, J = 13 Hz), 5.16 (m, 2 H), 5.71 (m, 1 H), 7.25 (m, 5 H); IR (neat) 1727 cm^{-1}; HRMS, m/z 262.1803 (calcd for $C_{16}H_{24}NO_2$ 262.1807).

α-Allyl-*N*-(benzyloxycarbonyl)phenylalanine *tert*-Butyl Ester (20). To a stirred solution of 1.69 g (6.466 mmol) of compound **19** in 12 mL of tetrahydrofuran was added 1.7 g (16 mmol) of powdered Na_2CO_3 and 1.2 mL (8.0 mmol) of benzyl chloroformate. After being stirred for 14 h, the reaction mixture was partitioned between water and dichloromethane. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 5% ethyl acetate in hexane to give 2.5 g (6.32 mmol, 98%) of compound **20**: ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9 H), 2.57 (dd, 1 H, J = 7, 14 Hz), 3.05 (d, 1 H, J = 14 Hz), 3.25 (dd, 1 H, J = 7, 14 Hz), 4.12 (d, 1 H, J = 14 Hz), 5.1 (m, 4 H), 5.65 (m, 2 H), 7–7.5 (m, 10 H); IR (neat) 1720 cm^{-1}; HRMS, m/z 396.2176 (calcd for $C_{24}H_{30}NO_4$ 396.2175).

***N*-(Benzyloxycarbonyl)-α-(formylmethyl)phenylalanine *tert*-Butyl Ester (21).** To a stirred solution of 653 mg (1.65 mmol) of the alkene **20** in 5.6 mL of tetrahydrofuran and 2.1 mL of water was added a small amount of osmium tetraoxide and 1.06 g (5.0 mmol) of sodium periodate in portions. After 40 min, the mixture was partitioned between ether and water. The ethereal phase was washed with aqueous $Na_2S_2O_3$ and aqueous NaCl and then dried ($MgSO_4$). The organic phase was concentrated, and the residue was chromatographed on silica gel with 25% ethyl acetate in hexane to give 555 mg (1.40 mmol, 85%) of the aldehyde **21**: ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9 H), 2.97 (d, 1 H, J = 13.5 Hz), 3.04 (d, 1 H, J = 13.5 Hz), 3.59 (d, 1 H, J = 13.5 Hz), 3.85 (d, 1 H, J = 13.5 Hz), 5.02 (d, 1 H, J = 12 Hz), 5.22 (d, 1 H, J = 12 Hz), 5.87 (s, 1 H), 7–7.4 (m, 10 H), 9.68 (s, 1 H); IR (neat) 1727 cm^{-1}.

***N*-[3(*R*)- and 3(*S*)-[(Benzyloxycarbonyl)amino]-4-phenyl-3-(*tert*-butyloxycarbonyl)butyl]-L-norleucine Benzyl Esters (22a,b).** To a stirred solution of 283 mg (1.28 mmol) of L-norleucine benzyl ester and 508 mg (1.28 mmol) of the aldehyde **21** in 6 mL of tetrahydrofuran was added 4-Å molecular sieves. After 30 min, 73 μL (1.28 mmol) of acetic acid and 80 mg (1.28 mmol) of sodium cyanoborohydride were successively added. After being stirred overnight, the reaction mixture was partitioned between dichloromethane and aqueous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 30% ethyl acetate in hexane to give 672 mg (1.11 mmol, 87%) of a diastereomeric mixture, **22a,b**: ^{1}H NMR ($CDCl_3$) mixture of *tert*-butyl and benzyl esters; IR (neat) 1731, 1720 cm^{-1}; HRMS, m/z 603.3423 (calcd for $C_{32}H_{47}N_2O_6$ 603.3434).

3(*R*)- and 3(*S*)-Benzyl-3-[(benzyloxycarbonyl)amino]-2-oxo-1-pyrrolidine-2(*S*)-hexanoic Acid Benzyl Esters (15a,b). To a stirred solution of 587 mg (0.97 mmol) of compounds **22a,b** in 20 mL of ether was passed a stream of gaseous HCl. After 90 min, the resulting mixture was concentrated, and the residue was dissolved in 10 mL of dichloromethane. To this stirred solution was added 0.37 mL (2.1 mmol) of diisopropylethylamine, followed by 0.18 mL (1.2 mmol) of diethyl phosphorocyanidate. After being stirred overnight, the concentrated reaction mixture was chromatographed on silica gel with 30% ethyl acetate in hexane to

give 490 mg (0.93 mmol, 95%) of compounds **15a,b** as prepared earlier.

***N*-[*N*$^{\alpha}$-[4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3(*S*)-hydroxy-6-methylheptanoyl]-L-isoleucyl]-2-pyridylmethylamine (25).** To a stirred solution of 1.05 g (3.81 mmol) of 4-(*S*)-[(*tert*-butyloxycarbonyl)amino]-3(*S*)-hydroxy-6-methylheptanoic acid (**23**) and 0.928 g (4.19 mmol) of *N*-L-isoleucyl-2-pyridylmethylamine (**24**) in 15 mL of dichloromethane was added 1 mL (5.7 mmol) of diisopropylethylamine, followed by 0.75 mL (4.95 mmol) of diethyl phosphorocyanidate. After 6 h, the reaction mixture was partitioned between dichloromethane and saturated aqueous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 3% methanol in ethyl acetate to give 1.66 g (3.46 mmol, 91%) of compound **25**: ^{1}H NMR ($CDCl_3$) δ 0.9 (m, 12 H), 1.43 (s, 9 H), 4.42 (dd, 1 H, J = 6, 9 Hz), 4.56 (d, 2 H, J = 5 Hz), 4.79 (d, 1 H, J = 10 Hz), 6.60 (d, 1 H, J = 8 Hz), 7.19 (m, 1 H), 7.41 (br t, 1 H), 7.65 (m, 1 H), 8.51 (d, 1 H, J = 4 Hz); HRMS, m/z 479.3205 (calcd for $C_{25}H_{43}N_4O_5$ 479.3233).

***N*-[*N*-[*N*$^{\alpha}$-(*tert*-Butyloxycarbonyl)-L-norleucyl]-4-(*S*)-amino-3(*S*)-hydroxy-6-methylheptanoyl]-L-isoleucyl]-2-pyridylmethylamine (26).** To a stirred suspension of 140 mg (0.29 mmol) of compound **25** in 5 mL of ether was passed gaseous HCl. After 15 min, the mixture was concentrated, and the residue suspended in 2 mL of dichloromethane. To this stirred mixture was added 180 μL (1.03 mmol) of diisopropylethylamine, 79 mg (0.34 mmol) of N^{α}-(*tert*-butyloxycarbonyl)-L-norleucine, and 55 μL (0.36 mmol) of diethyl phosphorocyanidate. After being stirred overnight, the concentrated residue was chromatographed on silica gel with 5% methanol in dichloromethane to give 160 mg (0.27 mmol, 93%) of compound **26**: ^{1}H NMR ($CDCl_3$) δ 0.7–1.0 (m, 15 H), 1.44 (s, 9 H), 1.5–5.3 (m), 7.15–7.85 (m, 5 H), 8.5 (d, 1 H, J = 5 Hz); HRMS, m/z 592.4116 (calcd for $C_{31}H_{54}N_5O_6$ 592.4074).

***N*-[*N*-(*N*$^{\alpha}$-Hydrocinnamoyl-L-norleucyl)-4(*S*)-amino-3(*S*)-hydroxy-6-methylheptanoyl]-L-isoleucyl]-2-pyridylmethylamine (I).** A solution of 160 mg (0.27 mmol) of compound **26** in 1 mL of dichloromethane and 1 mL of trifluoroacetic acid was allowed to stir at room temperature for 40 min. The reaction mixture was slowly added to a stirred mixture of 2 g of $NaHCO_3$ in 20 mL of water. The resulting mixture was extracted with several portions of dichloromethane. The combined organic phase was dried ($MgSO_4$) and then concentrated. To a stirred solution of this residue in 2 mL of dichloromethane was added 50 mg (0.33 mmol) of hydrocinnamic acid, 100 μL (0.57 mmol) of diisopropylethylamine, and 55 μL (0.36 mmol) of diethyl phosphorocyanidate. After being stirred overnight, the concentrated reaction mixture was chromatographed on silica gel with 5% methanol in dichloromethane to give 136 mg (0.22 mmol, 81%) of compound I: ^{1}H NMR ($CDCl_3$) δ 0.7–1.0 (m, 15 H), 1.2–4.6 (m), 7.15–7.85 (m, 13 H), 8.5 (d, 1 H, J = 5 Hz); HRMS, m/z 624.4161 (calcd for $C_{35}H_{54}N_5O_5$ 624.4125).

Compounds III and V were prepared in a similar manner to that described for the preparation of compound I. Physical characteristics of these compounds are listed in Table II.

***N*-[*N*$^{\alpha}$-[*N*-[2-(3-Benzyl-2-oxo-1-pyrrolidinyl)hexanoyl]-4(*S*)-amino-3(*S*)-hydroxy-6-methylheptanoyl]-L-isoleucyl]-2-pyridylmethylamine (IIa).** To a stirred solution of 43.6 mg (0.15 mmol) of the acid **5a**, 57 mg (0.15 mmol) of the amine **27**, and 25 μL (0.18 mmol) of triethylamine in 1 mL of CH_2Cl_2 was added 25 μL (0.16 mmol) of diethyl phosphorocyanidate. After 14 h, the reaction mixture was concentrated, and the residue was chromatographed on silica gel with 5% methanol in dichloromethane to give 75 mg (0.115 mmol, 77%) of compound IIa.

Compounds IIb, IVa, IVb, and VIa,b were prepared in a similar manner to that described for the preparation of compound IIa. Physical characteristics of these compounds are listed in Table II.

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

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Registry No. 1, 68975-13-3; **2**, 114155-61-2; **3** (*R* diastereomer), 114155-62-3; **3** (*S* diastereomer), 114155-63-4; **4** (3*R* diastereomer), 114155-76-9; **4** (3*S* diastereomer), 114155-77-0; **5** (3*R* diastereomer), 114155-78-1; **5** (3*S* diastereomster), 114155-79-2; **6**, 16537-10-3; **7**, 114155-64-5; **8**, 114155-65-6; **9** (3*R* diamstereomer), 114155-80-5; **9** (3*S* diastereomer), 114155-81-6; **10**, 99842-74-7; **411**, 114155-66-7; **12**, 114155-67-8; **13**, 114155-68-9; **14** (2*R* diastereomer), 114155-69-0; **14** (2*S* diastereomer), 114155-70-3; **15** (3*R* diastereomer), 114155-82-7; **15** (3*S* diastereomer), 114155-83-8; **16** (3*R* diastereomer), 114155-84-9; **16** (3*S* diastereomer), 114155-85-0; **17**, 16874-17-2; **18**, 114155-71-4; **19**, 114155-72-5; **20**, 114155-73-6; **21**, 114155-74-7; **22** (3*R* diastereomer), 114155-86-1; **22** (3*S* diastereomer), 114155-87-2; **23**, 58521-49-6; **24**, 97920-16-6; **25**, 103372-24-3; **26**, 114155-75-8; **27**, 110695-87-9; I, 114155-88-3; II (*R* diastereomer), 114155-91-8; II (*S* diastereomer), 114247-17-5; III, 114155-89-4; IV (*R* diastereomer), 114155-92-9; IV (*S* diastereomer), 114247-18-6; V, 114155-90-7; VI (*R* diastereomer), 114155-93-0; VI (*S* diastereomer), 114247-19-7; 1-HOBT, 2592-95-2; H-Nle-OCH$_2$Ph, 63219-54-5; BOC-Nle-OH, 6404-28-0; Cbz-Phe-OH, 1161-13-3; BOC-NH-(*S*)-CH(*i*-Bu)-(*S*)-CH(OSiMe$_2$Bu-*t*)CH$_2$-(*S*)-CH(*i*-Pr)COOH, 103335-80-4; BOC-NH-(*S*)-CH(*i*-Bu)-(*S*)-CH(OH)CH$_2$-(*S*)-CH(*i*-Pr)CO-L-Ile-NHCH$_2$(2-pyridyl), 114247-20-0; BOC-L-Nle-NH-(*S*)-CH(*i*-Bu)-(*S*)-CH(OH)CH$_2$-(*S*)-CH(*i*-Pr)CO-L-Ile-NHCH$_2$(2-pyridyl), 114155-94-1; N$_2$N-(*S*)-CH(*i*-Bu)-(*S*)-CH(OH)CH$_2$-(*S*)-CH(*i*-Pr)CO-L-Ile-NHCH$_2$(2-pyridyl), 102562-62-9; γ-butyrolactone, 96-48-0; α-amino-γ-butyrolactone, 6305-38-0; hydrocinnamic acid, 501-52-0; renin, 9015-94-5.