New Inhibitors of Human Renin That Contain Novel Leu-Val Replacements. Examination of the P_1 Site

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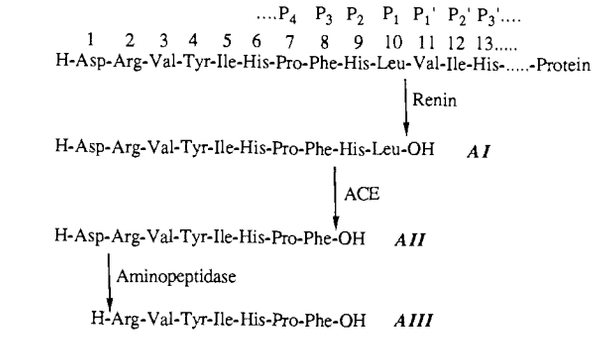
Stereoselective syntheses of several nonpeptide sulfidoethanol fragments that function as Leu^{10}-Val^{11} (P_1-P_1') scissile bond replacements in human angiotensinogen are presented. These fragments are prepared from a variety of amino acids with formal P_1 side chains varying in size and lipophilicity by converting them to their corresponding N-protected aminoalkyl epoxide **5** followed by ring opening with isopropyl mercaptan. The coupling of these fragments to either Boc-Phe-Ala-OH or Boc-Phe-His-OH produces inhibitors of human renin, **6** and **7**, respectively, which are compared to a series of dipeptide-aldehyde inhibitors, **4**, by molecular modeling and biochemical methods. Qualitatively, histidine-containing (P_2) inhibitors **7** possess greater inhibitory potency than their corresponding alanine (P_2) analogues **6**, which are more potent than the corresponding aldehydic inhibitors from series 4. Within a given series, inhibitors with the cyclohexylmethyl P_1 side chain are more potent than the benzyl analogues, which in turn are more potent than cyclohexyl or isobutyl derivatives. Inhibitors with larger P_1 side chains (e.g. adamantylmethyl and benzhydryl) are much less active. The inhibitory potency of these compounds against human renin is discussed in terms of specific interactions with the enzyme.

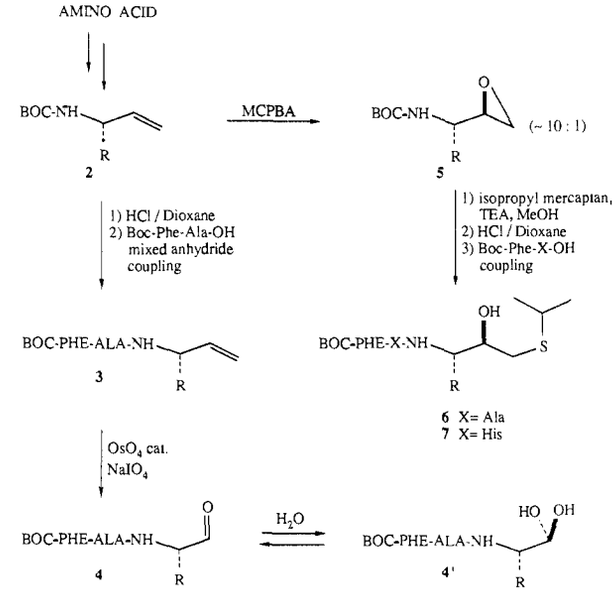
Renin, an aspartic proteinase, is the first enzyme in the renin angiotensin system (RAS, Scheme I). The role it plays in blood pressure regulation and electrolyte homeostasis by way of the potent pressor octapeptide angiotensin II (AII) has been well documented,[1] and it is thought that an inhibitor of renin may have a place in antihypertensive therapy. Recently we reported the synthesis and biological activity of new classes of inhibitors, represented generically by **1**[2] (Figure 1). These compounds are generally easily synthesized, specific for human renin, and, in the case of at least one analogue,[3] efficacious in an animal model. Prompted by these initial results, we have therefore undertaken a study to further our understanding of how these compounds interact with renin. In this paper we describe the synthesis and molecular modeling of a series of these inhibitors with variations at what is formally the P_1 site of angiotensinogen (Scheme I).

After a detailed evaluation of the X–Y group[2] in **1**, we chose *S*-isopropyl for this study as a small group that consistently exhibited good binding potency in both series **6** and **7**. Inhibitors such as **7** can be lined up with the natural angiotensinogen sequence residues 8–11, where the R group and *S*-isopropyl group correspond to the P_1 and P_1' side chains. Additionally, we have synthesized and modeled many of the P_1 modifications in a series of aldehydic inhibitors **4** to try to identify possible series to series variations. The utility of α-acylamino aldehydes in the study of biological systems has been recognized for some time,[4] and the discovery of naturally occurring peptidic proteinase inhibitors containing a C-terminal aldehyde group[5] led to recent syntheses of peptidic aldehyde inhibitors of human renin.[6] It was assumed that these aldehydic peptides act as transition-state analogues, undergoing hydration to give a tetrahedral intermediate[6b,7] such as **4'**.

Scheme I



Scheme II



Chemistry

Inhibitors **6** and **7** with P_1 variations R were prepared by following the procedures used to prepare **6a** and **7a**[2] as shown in Scheme II. Amino acids were *N*-Boc protected, converted to the corresponding amino aldehyde, and transformed to allylic amines **2** by Wittig olefination. Epoxidation with 3-(chloroperoxy)benzoic acid (MCPBA) then provided an appropriately protected aminoalkyl epoxide **5** with a threo:erythro selectivity of about 10:1.[8] Opening of the epoxide with isopropyl mercaptan[2] proceeded smoothly to give the corresponding Boc-protected

† Cardiovascular Research Division.
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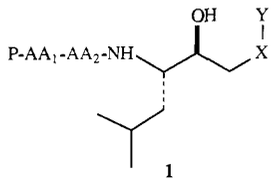

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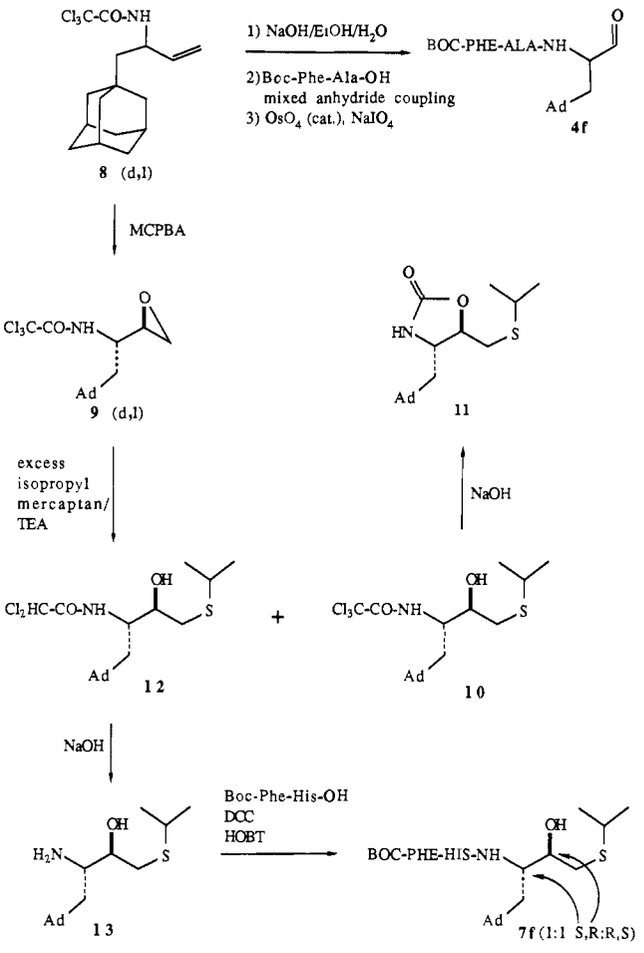
Figure 1. General inhibitor structure.

amino alcohol, which was deprotected with 4 M HCl/dioxane and then coupled either to Boc-Phe-Ala-OH under mixed anhydride conditions to give **6** or to Boc-Phe-His-OH under DCC/HOBT conditions to give **7**.

Synthetic routes to aldehyde-containing inhibitors prepared in the literature have involved the coupling of a suitably protected peptide fragment to an α-amino aldehyde masked as either a semicarbazone[6c] or an *N*-methylhydroxamate.[6b] In another case, an aldehyde was generated by LAH reduction of an arginine lactam derivative and was protected as its diethyl acetal after experiencing difficulties with semicarbazone deprotection.[4g] We have developed a route to peptide aldehydes **4**, which employs a stable allylic amine as a masked α-amino aldehyde. Thus, the aldehydes in this paper were prepared by deprotecting allylic amines **2** with 4 M HCl, coupling the resulting amines to Boc-Phe-Ala-OH, and cleaving olefins **3** oxidatively with $OsO_4/NaIO_4$. To insure that epimerization at the α-center had not occurred, **4a** was reduced to the corresponding alcohol, which was shown to be identical with material derived by coupling Boc-Ala with an authentic sample of L-leucinol.

The chemistry used to prepare inhibitors **4f** and **7f** differed only slightly and is shown in Scheme III. Racemic olefin **8**, which was prepared by rearrangement of a trichloroacetimidate,[8] was deprotected with hydroxide in aqueous ethanol, coupled to Boc-Phe-Ala-OH in the usual manner, and then oxidatively cleaved with $OsO_4/NaIO_4$ to give **4f** as a 1:1 mixture of *R*:*S* isomers. Epoxidation of **8** provided **9**,[8] and treatment with isopropyl mercaptan/TEA as before gave the expected protected amino alcohol **10** as well as the reduction product, dichloroacetyl

Scheme III



analogue **12**. Difficulty was encountered at this point in the removal of the trichloroacetyl protecting group of **10**. Following the conditions that were successful in the deprotection of **8**, we discovered that base only caused expulsion of trichloromethyl anion and cyclization to oxazolidin-2-one **11**. Attempted reductive removal of the trichloroacetyl group with $NaBH_4$[9] also failed to give significant amounts of **13**. Fortunately dichloromethyl analogue **12**, with diminished leaving group capability, underwent facile deprotection in hydroxide to provide desired amine **13** in 93% yield; no cyclization product **11** was observed. Standard carbodiimide coupling then provided **7f** as an inseparable 1:1 mixture of *S,R*:*R,S* isomers. Intermediate **12** can be made to predominate in the epoxide opening reaction by treatment of **9** with a larger excess of the mercaptan at higher temperature. In addition to effecting the epoxide ring opening, these conditions more completely effect reduction of the trichloroacetyl to dichloroacetyl group.

Molecular Modeling

A number of groups[7,10,11] have modeled the structure of the human renin molecule using comparative modeling techniques.[12-14] Our model structure for renin has been previously described.[15,16]

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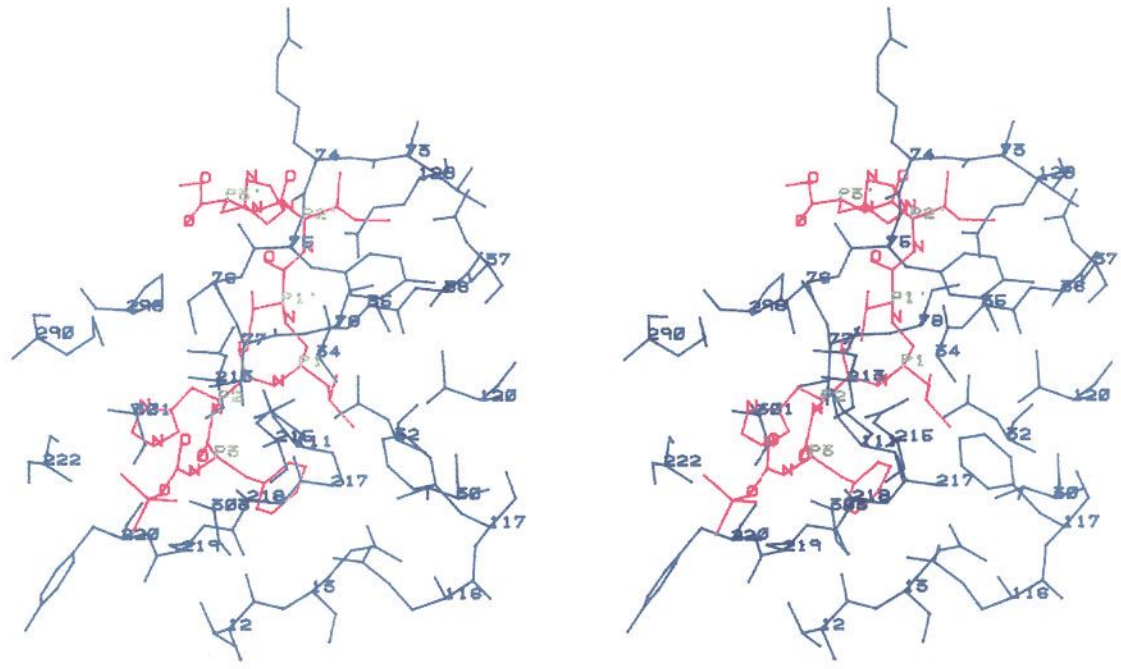

Figure 2. Parent hexapeptide and selected residues of the model renin active site.

The peptide Boc-Phe-His-Leu-ψ(CH_2-NH)-Val-Ile-His-OCH_3 was chosen as the parent inhibitor mimicking the renin substrate and was modeled in the active site of renin[16] with the aid of the published crystallographic structure of the complex of pepstatin in rhizopus pepsin[17] and of a pepstatin fragment in penicillopepsin[18,19] as guides. The model of human renin was obtained by comparing the structural homology with four other aspartic proteinases.[16] Since the latter are very similar in the active-site region and tend to diverge in other regions, it was decided to restrict our analysis to the active-site region. The active site, for modeling purposes, was defined as all of the residues of the enzyme within 7 Å of any atom of the parent compound; nine fragments of the renin molecule consisting of 95 residues were defined in this way. The N- and C-terminal residues of each fragment that lay outside the 7 Å volume were kept fixed during the energy calculations in order to avoid significant departures from the model resulting from exclusion of the rest of the protein. The size reduction of the molecular complex also reduced considerably the computational time involved in performing the energy calculations. Using the above described structure of the renin–inhibitor complex as a guide, we modeled the compounds listed in Table I by superimposing the main chain of residues AA_1 and AA_2 (see Figure 1) onto the main chain of the Phe-His residues of the parent compound (positions P_3 and P_2, respectively). The compounds in series **4** were modeled in the form of hydrated aldehydes. For eight of the complexes thus obtained, energy calculations were performed in the form of energy minimizations and molecular dynamics simulations in order to better understand the interactions of these inhibitors with renin. The compounds selected were from series **4** and **6** having as R: **a**, isobutyl; **b**, cyclohexylmethyl, **c**, cyclohexyl, and **d**, cyclohexylethyl (see Table I). The net charge of the charged residues present in the complex was reduced to approximate the screening effect of solvent that was not included explicitly in the calculations. The molecular mechanics calculations were performed with the program DISCOVER of Biosym Technologies on a FPS-164 array processor hosted by a VAX 11/785.

Table I

R	IC_{50}, nM or (inhibn @ 10^- M)		
	4	**6**	**7**
(**a**) isobutyl	20000	700	81
(**b**) cyclohexylmethyl	460	9	4
(**c**) cyclohexyl	1000	1500	150
(**d**) cyclohexylethyl	55000	(45%)	1500
(**e**) dicyclohexylmethyl	40000[a]		
(**f**) adamantylmethyl	35000[a]		2500[b]
(**g**) benzhydryl		(11%)[b]	(3%)[b]
(**h**) 2,4,6-trimethylbenzyl		(5%)[b]	
(**i**) benzyl		170	19

[a] Prepared starting from *d,l* olefin **2** and was determined by ^1H NMR spectroscopy to be a 1:1 *R*:*S* mixture of diastereomers at the chiral center bearing the R group. [b] Prepared starting from *d,l* olefin **2** and was determined by ^1H NMR spectroscopy to be a 1:1 mixture of *S,R*:*R,S* diastereomers at the chiral centers bearing the R and OH groups, respectively.

Results and Discussion

Table I shows the IC_{50} values for the inhibitors determined in a renin inhibition assay at pH 6. Compounds less potent that 10 μM are expressed only in terms of percent inhibition at 10^{-5} M. Qualitatively one can see that histidine-containing inhibitors series **7** are more potent than their corresponding Ala analogues series **6** and that these inhibitors are generally more potent than the aldehydic inhibitors series **4**. Within each series, the qualitative preference for the P_1 side chain, R, is cyclohexylmethyl over isobutyl or cyclohexyl, but judging qualitative preferences across series is more complex. For example, while isobutyl and cyclohexyl side chains are comparably potent in series **6** and **7**, they differ by a factor of 20 in series **4**. To better understand the trends in biological activities, we examined the molecular interactions between renin and these inhibitors by using molecular modeling techniques.

Figure 2 depicts selected residues of the renin model around the parent hexapeptide inhibitor. The P_1 side chain is close to the P_3 side chain and both lie in a single hydrophobic pocket formed by residues Val^{30}, Phe^{117}, Val^{120}, and Tyr^{75} of the enzyme (porcine pepsin sequence numbering). Due to the highly hydrophobic nature of this

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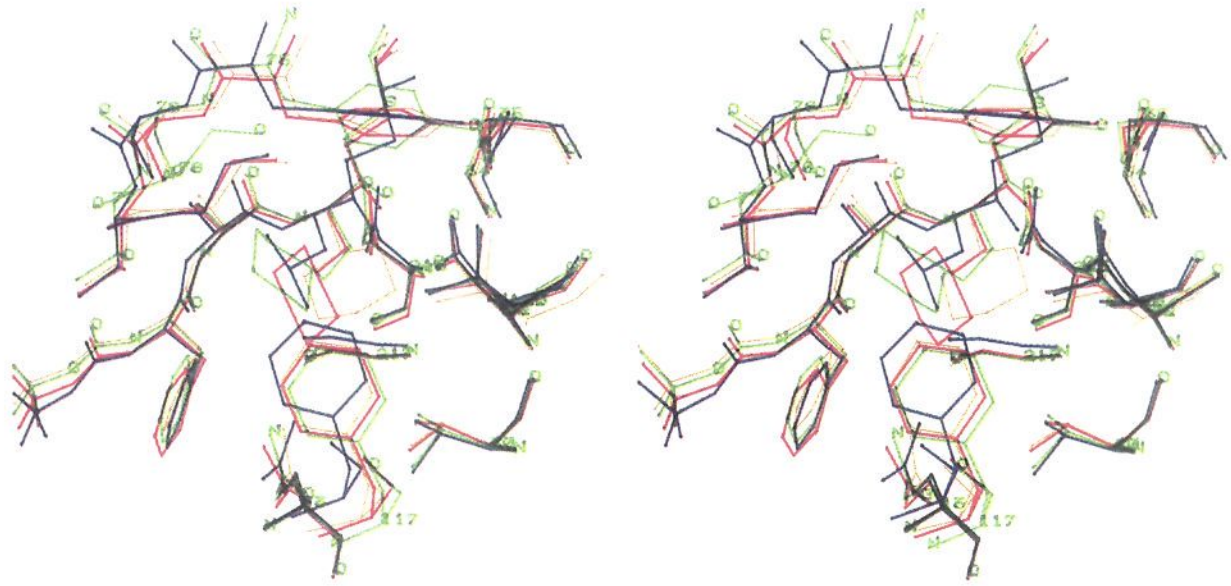

Figure 3. Minimized structures of renin complexed with inhibitors of series **6** with the residues of the active site around the P_1 position highlighted: blue, P_1 = isobutyl; orange, P_1 = cyclohexyl; red, P_1 = cyclohexylmethyl; green, P_1 = cyclohexylethyl. The average Ψ, Φ angles for the backbone of the compound **6b** as found in the active site are Phe (225°, 136°), Ala (221°, 102°), cyclohexylalanine (245°, 64°). The following two angles in the backbone are C_α–C–C–S = 172° and C–C–S–C = 219°.

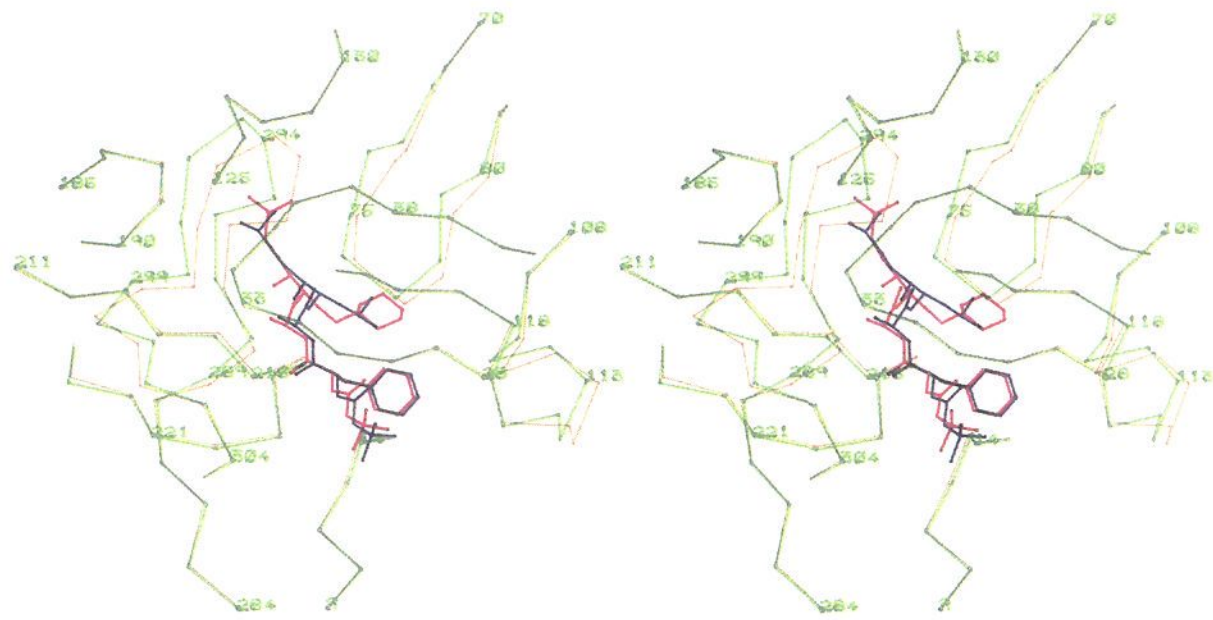

Figure 4. Carbon α trace of the active site of renin with two inhibitors from series **6**. P_1 = isobutyl (blue and orange); P_1 = cyclohexylethyl (red and green).

pocket then, a strategy for designing novel inhibitors is to modify the hydrophobic P_1 side chain and to fill the pocket as much as possible in order to maximize the dispersion energy between the enzyme and this side chain. Therefore P_1 side chains were varied from the relatively small isobutyl to the rather bulky dicyclohexyl and adamantyl derivatives.[20]

The series of compounds reported here differ significantly from the parent compound in that they lack three residues P_1', P_2', and P_3'; it was observed,[15] in fact, that the binding of inhibitors did not depend critically on the last three residues of the parent compound. The loop 290–300 of the active site is very flexible, and it is found near residues P_1' and P_3' of the parent inhibitor. The major change observed in the structure of the active site when the shorter new inhibitors replaced the parent hexapeptide was the movement of loop 290–300 (2–4 Å depending on the compound) to adjust to the shorter size of the inhibitors.

(20) Two side chain variations at the P_1 position have been explored recently in statine analogues: (a) Boger, J.; Payne, L. S.; Perlow, D. S.; Lohr, N. S.; Poe, M.; Blaine, E. H.; Ulm, E. H.; Schorn, T. W.; LaMont, B. I.; Lin, T.-Y.; Kawai, M.; Rich, D. H.; Veber, D. F. *J. Med. Chem.* **1985**, *28*, 1779. (b) Thaisrivongs, S.; Pals, D. T.; Kati, W. M.; Turner, S. R.; Thomasco, L. M.; Watt, W. *J. Med. Chem.* **1986**, *29*, 2080.

The common features of all the structures subjected to energy calculations were as follows: all the inhibitors maintained the hydrogen bond between the carbonyl of Phe (P_3) and the NH of Ser^{219}. Also Asp^{32} and Asp^{215} hydrogen bond with the hydroxyl of compounds of series **6** or with the two hydroxyls in the case of series **4**. These two strong interactions maintain the inhibitor aligned to the 217–219 segment of the enzyme. Finally the side chains of residues P_1 (the variable R) and P_3 remained close to one another in the same hydrophobic pocket. The largest changes in the active site occurred in the regions near the C-terminus of the inhibitors including loop 290–300 and the segment of residues 72–80. Other parts of the enzyme moved to a lesser degree.

Comparing the complexes containing compounds within series **6** (Figure 3), we observed that the backbone at position P_1 in compounds with R = cyclohexyl, cyclohexylmethyl, and cyclohexylethyl retained a similar position relative to the active site while the compounds with R = isobutyl showed a displacement that caused a readjustment of the active site. A reason for such a displacement is that the isobutyl group, the smallest of the side chains, moves deeper into the large hydrophobic pocket to fill it more completely. The C_α connected to the isobutyl group is 0.7 Å away from the one connected to the cyclohexylmethyl. When the corresponding distances of the compounds with R = cyclohexyl and cyclohexylethyl are

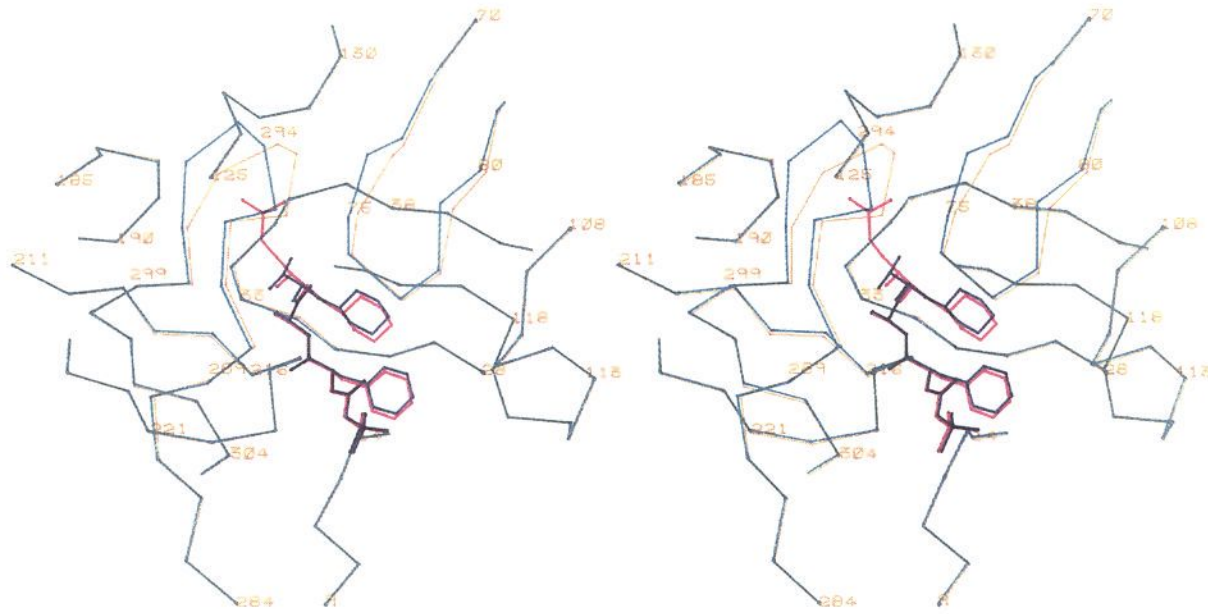

Figure 5. Carbon α trace of the active site of renin with two inhibitors having P_1 = cyclohexylmethyl. Series **4** (blue and orange); series **6** (red and cyan).

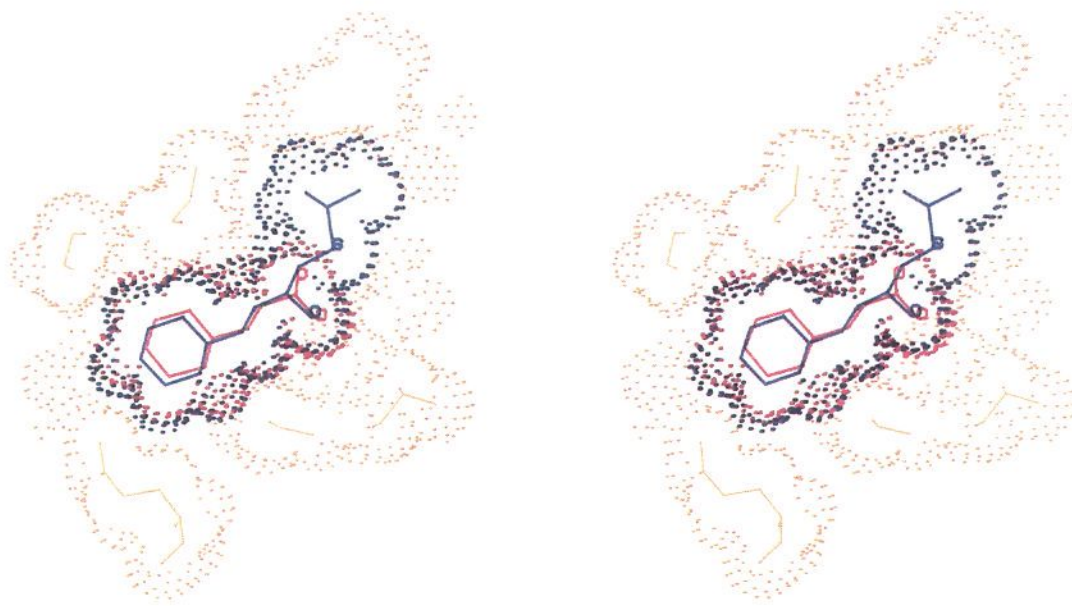

Figure 6. van der Waals surface of renin around the cyclohexylmethyl P_1 side chain. Series **4** (red); series **6** (blue).

compared to that with R = cyclohexylmethyl, they are found to be less than 0.3 Å. It can be observed, also in Figure 3, that there is a concerted movement of the residues of the active site and of the inhibitor to maximize the dispersion energy. The extent to which the enzyme can accommodate different inhibitors is shown in Figure 4 where the C_α trace of the active site residues is displayed when bound to inhibitors with a small (R = isobutyl) and a large (R = cyclohexylethyl) P_1 side chain. The root mean square difference of the enzyme structures containing the two compounds is 0.9 Å; the largest deviation from one structure to the other is of 2.3 Å at residue Pro^{294}.

In complexes of inhibitors from series **4**, we observed similarities with those of series **6**, but the relative displacement of the P_1 residues was more pronounced; namely the movement of the isobutyl group into the hydrophobic pocket caused the connected C_α to be shifted 0.9 Å relative to the position of the C_α connected to the cyclohexylmethyl analogue. The C_α connected to cyclohexyl and cyclohexylethyl side chains are found to be 0.5 Å away from the C_α connected to cyclohexylmethyl.

When we compared compounds with the same R group from two different series, **4** and **6**, we observed that the largest changes in the enzyme structure also occur in the loop segment of residues 290–300 and in residues 74–79. This appears to be primarily due to the difference in the size of the C-terminal group in the two series. Figure 5 shows the C_α trace of the active site and illustrates the extent of the changes when the compound with R = cyclohexylmethyl of series **4** is compared with the corresponding compound of series **6**.

A significant difference in the contact surfaces (which can be taken as approximately representing the dispersion energy) between the inhibitors and the enzyme can be observed in Figure 6 where the R group is cyclohexylmethyl and the C-terminus of the inhibitors of the two different series **4** and **6** are displayed with their van der Waals surfaces in contact with the surface of the neighboring residues of the active site. It is apparent that the extended C-terminus of series **6** compounds restricts the mobility of the inhibitor in the active site and improves binding relative to series **4**.

Using the above modeled and energy-determined structures, we can explain qualitatively the very different potencies reported in Table I for the different R substituents at position P_1. Compounds with cyclohexylmethyl are seen to fill the hydrophobic pocket optimally, maximizing the dispersion energy. Compounds with R = benzyl were analyzed by model-building techniques and it was observed that the benzyl structure was very similar to and was occupying the same space as the cyclohexylmethyl, but the complementarity of the phenyl ring to the hydrophobic pocket was not as adequate as the cyclohexyl ring. The smaller isobutyl side chain, despite conformational changes, still does not fill the pocket and therefore binds more weakly. Cyclohexyl, by virtue of the ring being sit-

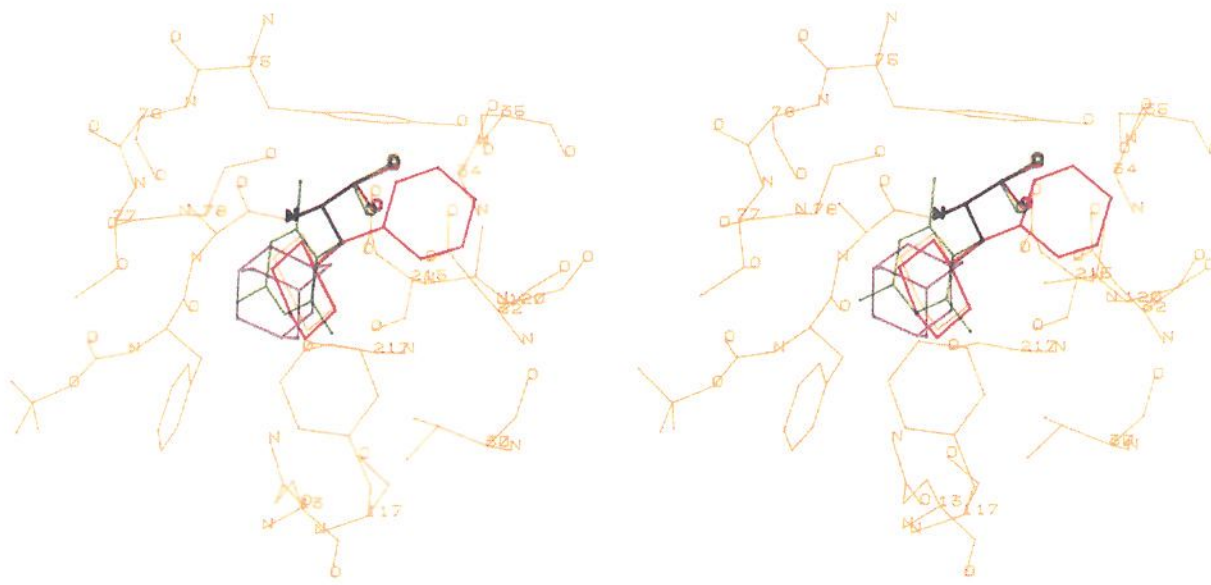

Figure 7. Selected residues of the renin active site around the P_1 site: orange, P_1 = cyclohexylmethyl; green, P_1 = 2,4,6-trimethylbenzyl; red, P_1 = dicyclohexylmethyl; magneta, P_1 = adamantylmethyl.

uated closer to the main chain, not only fails to fill the pocket but also causes quite different conformational changes in the P_1 binding pocket. Both of these effects can be expected to diminish binding. Compounds containing cyclohexylethyl fit the hydrophobic pocket with difficulty. In series **4** the ring of this side chain is accommodated by displacing the phenyl ring of Phe P_3 which is lying in the same pocket; in series **6** the ring has bad steric interactions with residues Tyr^{75}, Gly^{78} and Phe^{117} of the active site. Complexes with inhibitors having larger P_1 side chains, such as **e**, **f**, **g**, **h** (Table I and Figure 7), showed such large overlaps and bad steric interactions when modeled that molecular mechanics calculations were not feasible. Clearly, these compounds fit very poorly in the P_1 binding site (Figure 7). The generally better binding showed by compounds of series **6** compared to series **4** can be accounted for by the presence of the longer C-terminal group in series **6**, which provides these compounds with more dispersion energy interaction with the enzyme. The higher potencies of compounds of series **7** compared to the other two series can be rationalized in a similar fashion: the His side chain fills the pocket at P_2 site better than the smaller Ala side chain, therefore "anchoring" in a more favorable conformation the inhibitors to the active site.

Experimental Section

All amino acids and protected amino acids were obtained from Sigma Chemical Co. unless otherwise noted. Inhibitors **6a**,[2] **7a**,[2] and **7b**[3] were prepared as previously described. Anhydrous solvents used were dried and freshly distilled. All reactions unless otherwise noted were run in oven-dried glassware under an atmosphere of dry nitrogen or argon.

Proton magnetic resonance spectra were measured on a Nicolet QE-300 instrument (300 MHz). Chemical shifts are reported as δ values (parts per million) relative to Me_4Si as an internal standard. Mass spectra were obtained with Hewlett-Packard HP5985 (CI, EI), Varian CH7 (EI), and Kratos MS50 (FAB, HRMS) spectrometers. Elemental analyses and the above determinations were performed by the Analytical Research Department, Abbott Laboratories. Thin-layer chromatography (TLC) was carried out by using E. Merck precoated silica gel F-254 plates (thickness, 0.25 mm). Flash column[21] chromatography was carried out with Baker silica gel (40 μm).

The following experimental procedures provide representative conditions for the preparation of the compounds shown in Table I.

Deprotections. A. Boc-amines. The Boc-amine (1 mmol) was treated with anhydrous 4 M HCl/dioxane (10–20 mmol) for 1 h. Evaporation and chasing several times with toluene provided the corresponding amine hydrochloride, which was used in the coupling reaction without further purification.

B. Trichloroacetyl- and Dichloroacetylamines. To a stirred solution of the protected amine (0.05 mmol) in ethanol (2 mL) was added 3 M NaOH (2 mL). After 16 h, the mixture was diluted with water (8 mL) and extracted with ether (2 × 8 mL). The combined extracts were dried filtered and evaporated to give the corresponding amine, which was coupled without further purification.

Mixed-Anhydride Coupling. Synthesis of Olefins 3. (3*S*)-3-[(Boc-L-phenylalanylalanyl)amino]-5-methylhex-1-ene (3a). To a stirred −12 °C solution of Boc-Phe-Ala-OH (84.1 mg, 0.25 mmol) in anhydrous tetrahydrofuran (3 mL) was added sequentially *N*-methylmorpholine (28 μL, 0.25 mmol) and isobutyl chloroformate (32 μL, 0.25 mmol). After 3 min, a −12 °C solution of 3-amino-5-methylhex-1-ene hydrochloride (prepared by deprotecting 0.25 mmol of **4a** according to the above procedure) in anhydrous tetrahydrofuran (3 mL) containing *N*-methylmorpholine (0.25 mmol) was added. Ten minutes later, the mixture was allowed to warm to room temperature for 2 h, at which time the solvent was evaporated, and the resulting residue was partitioned between ethyl acetate (20 mL) and saturated $NaHCO_3$ (5 mL). The organic phase was washed sequentially with 0.1 M H_3PO_4 (5 mL) and brine (5 mL). Drying (Na_2SO_4) and evaporating provided crude material, which was chromatographed on silica gel (dichloromethane/methanol, 98/2) to give 100 mg (93%) of the desired compound: mass spectrum, M^+ = 431; mp 143–145 °C; ^{1}H NMR ($CDCl_3$) δ 0.91 (d, 6 H, J = 7 Hz), 1.33 (d, 3 H, J = 7 Hz), 1.35 (m, 2 H), 1.41 (s, 9 Hz), 1.58 (m, 1 H), 3.18 (m, 2 H), 4.3–4.5 (m, 3 H), 4.90 (br d, 1 H, J = 6 Hz), 5.05–5.2 (m, 2 H), 5.73 (m, 1 H), 6.18 (br, 1 H), 6.51 (d, 1 H, J = 7 Hz), 7.15–7.35 (m, 5 H). Anal. ($C_{24}H_{37}N_3O_4 \cdot {}^1/_4H_2O$) C, H, N.

(2*S*)-2-[(Boc-L-phenylalanylalanyl)amino]-1-cyclohexylbut-3-ene (3b): 81% yield of an amorphous solid; mass spectrum, $(M + H)^+$ = 472; ^{1}H NMR ($CDCl_3$) δ 0.8–1.8 (m, 13 H), 1.33 (d, 3 H, J = 7 Hz), 1.40 (s, 9 H), 3.08 (m, 2 H), 4.33 (m, 1 H), 4.40 (m, 1 H), 4.48 (m, 1 H), 4.88 (m, 1 H), 5.05–5.18 (br, 1 H), 5.73 (m, 1 H), 6.12 (br, 1 H), 6.47 (d, 1 H, J = 8 Hz), 7.15–7.35 (m, 5 H).

(1*S*)-1-[(Boc-L-phenylalanylalanyl)amino]-1-cyclohexylprop-2-ene (3c): 76% yield; mass spectrum, M^+ = 457; mp 176–177 °C; ^{1}H NMR ($CDCl_3$) δ 0.85–1.3 (m, 6 H), 1.33 (d, 3 H, J = 7 Hz), 1.42 (s, 9 H), 1.6–1.8 (m, 5 H, 3.08 (m, 2 H), 4.26 (m, 1 H), 4.35 (m, 1 H), 4.43 (m, 1 H), 4.88 (m, 1 H), 5.08–5.17 (m, 2 H), 5.73 (m, 1 H), 6.21 (m, 1 H), 6.52 (br d, 1 H, J = 7 Hz), 7.17–7.35 (m, 5 H). Anal. ($C_{26}H_{39}N_3O_4$) C, H, N.

(3*S*)-3-[(Boc-L-phenylalanylalanyl)amino]-1-cyclohexylpent-4-ene (3d): 93% yield; mass spectrum, $(M + H)^+$ = 486; mp 142–144 °C; ^{1}H NMR ($CDCl_3$) δ 0.8–1.75 (m, 15 H), 1.33 (d, 3 H, J = 7 Hz), 1.40 (s, 9 H), 3.04 (dd, 1 H, J = 8, 14 Hz), 3.10 (dd, 1 H, J = 6, 14 Hz), 4.28–4.47 (m, 3 H), 4.92 (d, 1 H, J = 7 Hz), 5.07–5.17 (m, 2 H), 5.74 (m, 1 H), 6.22 (d, 1 H, J = 8 Hz), 6.54 (d, 1 H, J = 7 Hz), 7.16–7.34 (m, 5 H).

(2*R*/*S*)-2-[(Boc-L-phenylalanylalanyl)amino]-1,1-dicyclohexylbut-3-ene (3e): 89% yield; mass spectrum, M^+ = 553; ^{1}H NMR ($CDCl_3$) δ 1.0–1.8 (m, 24 H), 1.32, 1.35 (2 d, 1:1, 3 H total,

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

J = 7, 7 Hz), 1.39 (s, 9 H), 3.0–3.15 (m, 2 H), 3.25–4.05 (m, 2 H), 4.64 (m, 1 H), 4.88 (m, 1 H), 5.03–5.13 (m, 2 H), 5.65–5.83 (m, 1 H), 6.08, 6.18 (2 d, 1:1, 1 H total, J = 9, 9 Hz), 6.56, 6.67 (2 d, 1:1, 1 H total, J = 7, 7 Hz), 7.15–7.35 (m, 5 H). Anal. ($C_{33}H_{51}N_3O_4$) C, H, N.

(2*R*/*S*)-2-[(Boc-L-phenylalanylalanyl)amino]-1-(1-adamantyl)but-3-ene (3f): 89% yield; mass spectrum, M^+ = 523; ^{1}H NMR ($CDCl_3$) δ 1.32, 1.34 (2 d, 1:1, 3 H total, J = 7, 7 Hz), 1.40, 1.42 (2 s, 1:1, 9 H total), 1.15–1.75 (m, 14 H), 1.94 (m, 3 H), 3.08 (m, 2 H), 4.27–4.44 (m, 2 H), 4.54 (m, 1 H), 4.86 (m, 1 H), 4.95–5.15 (m, 2 H), 5.7 (m, 1 H), 6.0, 6.1 (2 m, 1:1, 1 H total), 6.39, 6.47 (2 m, 1:1, 1 H total), 7.15–7.35 (m, 5 H). Anal. ($C_{31}H_{45}N_3O_4 \cdot {}^1/_2H_2O$) C, H, N.

Oxidative Cleavage. Synthesis of Aldehydes 4. To a rapidly stirred solution of olefin **3** (0.212 mmol) in tetrahydrofuran (5 mL) containing OsO_4 (65 μL of a 2.5 w/v % solution in *tert*-butyl alcohol) was added $NaIO_4$ (113 mg, 0.53 mmol) in water (3 mL). After the reaction was shown to be complete by TLC analysis (ca. 5 h), the mixture was diluted with water (10 mL) and extracted with ether (4 × 8 mL). The combined organic phase was washed with 10% aqueous Na_2SO_3 (3 × 6 mL) and brine (6 mL). Drying (Na_2SO_4), filtering, and evaporating provided the corresponding aldehyde **4**. When necessary, minor impurities were removed by chromatography on silica gel.

(2*S*)-2-[(Boc-L-phenylalanylalanyl)amino]-4-methylpentan-1-al (4a): 95% yield of an amorphous solid; mass spectrum, M^+ = 433, $(M + 1)^+$ = 434; ^{1}H NMR ($CDCl_3$) δ 0.93 (d, 3 H, J = 6 Hz), 0.96 (d, 3 H, J = 6 Hz), 1.3–1.75 (m, 3 H), 1.37 (d, 3 H, J = 7 Hz), 1.41 (s, 9 H), 3.18 (m, 2 H), 4.25–4.45 (m, 2 H), 4.48 (m, 1 H), 4.98 (br, 1 H), 6.45 (d, 1 H, J = 7 Hz), 6.70 (br, 1 H), 7.15–7.35 (m, 5 H), 9.53 (s, 1 H). Anal. ($C_{23}H_{35}N_3O_5 \cdot {}^1/_4H_2O$) C, H, N.

(2*S*)-2-[(Boc-L-phenylalanylalanyl)amino]-3-cyclohexylpropan-1-al (4b): 99% yield of an amorphous solid; mass spectrum, M^+ = 473; ^{1}H NMR ($CDCl_3$) δ 0.8–1.8 (m, 13 H), 1.36 (d, 3 H, J = 7 Hz), 1.39 (s, 9 H), 3.18 (m, 2 H), 4.3–4.55 (m, 3 H), 4.96 (d, 1 H, J = 7 Hz), 6.58 (d, 1 H, J = 8 Hz), 6.79 (br d, 1 H, J = 6 Hz), 7.15–7.35 (m, 5 Hz), 9.53 (s, 1 H). Anal. ($C_{26}H_{39}N_3O_5 \cdot {}^1/_2H_2O$), C, H, N.

(2*S*)-2-[(Boc-L-phenylalanylalanyl)amino]-2-cyclohexylethan-1-al (4c): 82% yield; mass spectrum, M^+ = 459; mp 143–145 °C; ^{1}H NMR ($CDCl_3$) δ 1.0–1.45 (m, 6 H), 1.34 (d, 3 H, J = 7 Hz), 1.41 (s, 9 H), 1.65–1.85 (m, 5 H), 3.08 (m, 2 H), 4.3–4.45 (m, 2 H), 4.48 (m, 1 H), 4.9 (br, 1 H), 6.49 (d, 1 H, J = 7 Hz), 6.68 (m, 1 H), 7.15–7.35 (m, 5 H), 9.62 (s, 1 H). Anal. ($C_{25}H_{37}N_3O_5 \cdot {}^1/_4H_2O$) C, H. N.

(2*S*)-2-[(Boc-L-phenylalanylalanyl)amino]-4-cyclohexylbutan-1-al (4d): 88% yield; mass spectrum, M^+ = 487; ^{1}H NMR ($CDCl_3$) δ 0.8–1.75 (m, 14 H), 1.35 (d, 3 H, J = 7 Hz), 1.42 (s, 9 H), 1.87 (br m, 1 H), 3.08 (m, 2 H), 4.33 (m, 2 H), 4.47 (m, 1 H), 4.89 (m, 1 H), 6.46 (d, 1 H, J = 7 Hz), 6.72 (m, 1 H), 7.15–7.35 (m, 5 H), 9.53 (s, 1 H). Anal. ($C_{27}H_{41}N_3O_5 \cdot {}^1/_2H_2O$) C, H, N.

(2*R*/*S*)-2-[(Boc-L-phenylalanylalanyl)amino]-3,3-dicyclohexylpropan-1-al (4e): 21% yield; mass spectrum, M^+ = 555. Anal. ($C_{32}H_{49}N_3O_5 \cdot H_2O$) C, H, N.

(2*R*/*S*)-2-[(Boc-L-phenylalanylalanyl)amino]-3-(1-adamantyl)propan-1-al (4f): 86% yield; mass spectrum, M^+ = 525; ^{1}H NMR ($CDCl_3$) δ 1.15–1.75 (m, 14 H), 1.35, 1.36 (2 d, 1:1, 3 H total, J = 7, 7 Hz), 1.38, 1.40 (2 s, 1:1, 9 H total), 1.97 (m, 3 H), 3.0–3.2 (m, 2 H), 4.30 (m, 1 H), 4.35–4.55 (m, 2 H), 4.86 (m, 1 H), 6.33, 6.44 (2 d, 1:1, 1 H total, J = 7, 8 Hz), 6.58, 6.73 (2 m, 1:1, 1 H total), 7.15–7.35 (m, 5 H), 9.41, 9.48 (2 s, 1:1, 1 H total). Anal. ($C_{30}H_{43}N_3O_5 \cdot {}^1/_4H_2O$) C, H, N.

Synthesis of Inhibitors 6. Epoxides **5** were prepared[8] and opened with isopropyl mercaptan[2] as described previously. The protected amino alcohols thus obtained were deprotected with HCl and coupled by the mixed-anhydride method using the procedures described above for the preparation of peptides **3**.

(2*R*,3*S*)-3-[(Boc-L-phenylalanylalanyl)amino]-2-hydroxy-4-cyclohexyl-1-(isopropylthio)butane (6b): 62% yield; mass spectrum, $(M + 1)^+$ = 564; mp 149–151 °C; ^{1}H NMR ($CDCl_3$) δ 1.26 (d, 3 H, J = 7 Hz), 1.28 (d, 3 H, J = 7 Hz), 1.33 (d, 3 H, J = 7 Hz), 1.41 (s, 9 H), 0.75–1.85 (sev m, 19 H), 2.46 (dd, 1 H, J = 10, 14 Hz), 2.71 (dd, 1 H, J = 3, 10 Hz), 2.85–3.1 (m, 3 H), 3.61 (m, 1 H), 4.05 (m, 1 H), 4.34 (m, 1 H), 4.92 (br m, 1 H), 6.23 (br, 1 H), 6.40 (br d, 1 H, J = 7 Hz, 7.15–7.35 (m, 5 H). Anal. ($C_{30}H_{49}N_3O_5 \cdot {}^1/_4H_2O$) C, H, N.

(2*R*,3*S*)-3-[(Boc-L-phenylalanylalanyl)amino]-2-hydroxy-3-cyclohexyl-1-(isopropylthio)propane (6c): 85% yield of an amorphous solid; mass spectrum, $(M + H)^+$ = 550; ^{1}H NMR ($CDCl_3$) δ 0.9–1.9 (m, 11 H), 1.27 (d, 3 H, J = 7 Hz), 1.28 (d, 3 H, J = 7 Hz), 1.33 (d, 3 H, J = 7 Hz), 1.41 (s, 9 H), 2.46 (dd, 1 H, J = 10, 14 Hz), 2.66 (dd, 1 H, J = 4, 14 Hz), 2.93 (m, 1 H), 3.02 (m, 1 H), 3.07 (m, 2 H), 3.63 (m, 1 H), 3.86 (m, 1 H), 4.33 (m, 1 H), 4.43 (m, 1 H), 5.03 (br d, 1 H, J = 7 Hz), 6.42 (d, 1 H, J = 10 Hz), 6.48 (d, 1 H, J = 7 Hz), 7.17–7.35 (m, 5 H). Anal. ($C_{29}H_{47}N_3O_5$) C, H, N.

(2*R*,3*S*)-3-[(Boc-L-phenylalanylalanyl)amino]-2-hydroxy-5-cyclohexyl-1-(isopropylthio)pentane (6d): 82% yield; mass spectrum, $(M + H)^+$ = 578; ^{1}H NMR ($CDCl_3$) δ 0.87 (m, 2 H), 1.1–1.7 (m, 13 H), 1.27 (d, 3 H, J = 7 Hz), 1.28 (d, 3 H, J = 7 Hz), 1.33 (d, 3 H, J = 7 Hz), 1.41 (s, 9 H), 2.46 (dd, 1 H, J = 10, 13 Hz), 2.68 (dd, 1 H, J = 4, 13 Hz), 2.85–3.15 (m, 4 H), 3.66 (m, 1 H), 3.86 (m, 1 H), 4.25–4.45 (m, 2 H), 4.96 (m, 1 H), 6.32 (d, 1 H, J = 9 Hz), 6.42 (d, 1 H, J = 7 Hz), 7.15–7.35 (m, 5 H). Anal. ($C_{31}H_{51}N_3O_5$) C, H, N.

(2*R*,3*S*)-3-[(Boc-L-phenylalanylalanyl)amino]-2-hydroxy-4,4-diphenyl-1-(isopropylthio)butane and (2*R*,3*S*)-3-[(Boc-L-phenylalanylalanyl)amino]-2-hydroxy-4,4-diphenyl-1-(isopropylthio)butane (6g): 75% combined yield; ^{1}H NMR of the crude mixture showed a 1:1 mixture of *S,R*:*R,S* isomers at the centers bearing the R and OH groups, respectively. Chromatography separated the 2*R*,3*S* and 2*S*,3*R* diastereomers.

Isomer A: mass spectrum, $(M + 1)^+$ = 634; ^{1}H NMR ($CDCl_3$) δ 0.9 (br d, 3 H), 1.18 (d, 6 H, J = 7 Hz), 1.43 (s, 9 H), 2.45–3.08 (m, 5 H), 3.49 (m, 1 H), 4.15 (m, 2 H), 4.33 (d, 1 H, J = 12 Hz), 4.84 (m, 2 H), 5.99 (d, 1 H, J = 7 Hz), 6.27 (br d, 1 H, J = 9 Hz), 7.08–7.38 (m, 15 H); exact mass 634.3302, calcd mass for $C_{36}H_{48}H_3O_5S$ 634.3314 (M + 1).

Isomer B: mass spectrum, $(M + 1)^+$ = 634; ^{1}H NMR ($CDCl_3$) δ 0.67 (d, 3 H, J = 7 Hz), 1.16 (d, 3 H, J = 7 Hz), 1.17 (d, 3 H, J = 7 Hz), 1.40 (s, 9 H), 2.43 (dd, 1 H, J = 10, 14 Hz), 2.65 (dd, 1 H, J = 4, 14 Hz), 2.72 (m, 1 H), 3.0 (m, 3 H), 3.53 (m, 1 H), 4.09 (m, 1 H), 4.1 (br, 1 H), 4.13 (d, 1 H, J = 12 Hz), 4.81 (m, 1 H), 4.98 (br, 1 H), 6.04 (d, 1 H, J = 10 Hz), 6.42 (d, 1 H, J = 7 Hz), 7.05–7.42 (m, 15 H).

(2*R*,3*S*)-3-[(Boc-L-phenylalanylalanyl)amino]-2-hydroxy-4-(2,4,6-trimethylphenyl)-1-(isopropylthio)butane and (2*S*,3*R*)-3-[(Boc-L-phenylalanylalanyl)amino]-2-hydroxy-4-(2,4,6-trimethylphenyl)-1-(isopropylthio)butane (6h): 59% yield; mass spectrum, M^+ = 599, (M + 1) = 600; ^{1}H NMR ($CDCl_3$) δ 1.15–1.33 (m, 15 H), 1.38, 1.43 (2 s, 1:1, 9 H total), 2.23 (s, 3 H), 2.36 (s, 3 H), 2.38 (s, 3 H), 2.43 (m, 1 H), 2.58 (m, 1 H), 2.73–3.25 (m, 6 H), 3.46, 3.53 (2 dd, 1:1, 1 H total), J = 3, 9 Hz, 3, 9 Hz), 4.05–4.2 (m, 1 H), 4.28–4.48 (m, 2 H), 4.97, 5.18 (2 m, 1 H total), 6.43 (m, 1 H), 6.54, 6.65 (2 d, 1 H total, J = 7, 10 Hz), 6.82 (d, 2 H, J = 3 Hz), 7.15–7.35 (m, 5 H). Anal. ($C_{33}H_{49}N_3O_5$) C, H, N.

(2*R*,3*S*)-3-[(Boc-L-phenylalanylalanyl)amino]-2-hydroxy-4-phenyl-1-(isopropylthio)butane (6i): 76% yield; mass spectrum, $(M + 1)^+$ = 624; mp 199–200 °C; ^{1}H NMR ($CDCl_3$) δ 1.22 (d, 6 H, J = 6 Hz), 1.27 (d, 3 H, J = 7 Hz), 1.45 (s, 9 H), 2.50 (*AB*X, 1 H, J = 9, 13 Hz), 2.63 (*AB*X, 1 H, J = 4, 13 Hz), 2.83 (m, 1 H), 2.87–3.27 (m, 5 H), 3.62 (m, 1 H), 4.17 (q, 1 H, 6.6 Hz), 5.12 (d, 1 H, J = 7 Hz), 6.4 (d, 1 H, J = Hz), 6.67 (d, 1 H, J = 9 Hz), 7.15–7.35 (m, 10 H). Anal. ($C_{30}H_{43}N_3O_5S \cdot {}^1/_4H_2O$) C, H, N.

Carbodiimide Coupling. Synthesis of Inhibitors 7. Epoxide **5** (0.2 mmol) was opened and the product was deprotected as described above and previously[2] to give the corresponding amine hydrochloride, which was dissolved in dimethylformamide (5 mL) along with Boc-Phe-His-OH (82 mg, 0.2 mmol), *N*-methylmorpholine (20 mg, 0.2 mmol), and 1-hydroxybenzotriazole hydrate (HOBT; 41.5 mg, 0.31 mmol). The mixture was cooled to −23 °C, and 1,3-dicyclohexylcarbodiimide (DCC; 42 mg, 0.2 mmol) was added. The mixture was allowed to warm to room temperature over 3 h, and stirring was continued for an additional 18 h. Filtration and evaporation of the filtrate provided a solid, which was partitioned between ethyl acetate and saturated aqueous $NaHCO_3$. The organic phase was washed (saturated aqueous $NaHCO_3$ 1×, brine 1×), dried (Na_2SO_4), filtered, and evaporated

to give a residue, which was chromatographed on silica gel (dichloromethane/methanol, 9/1) to give the desired material.

(2*R*,3*S*)-3-[(Boc-L-phenylalanylhistidyl)amino]-2-hydroxy-3-cyclohexyl-1-(isopropylthio)propane (7c): 40% yield of an amorphous solid; mass spectrum, $(M + H)^+ = 616$; ^1H NMR ($CDCl_3$) δ 0.7–1.85 (m, 11 H), 1.26 (d, 3 H, J = 7 Hz), 1.39 (s, 9 H), 2.5 (m, 2 H), 2.8–3.3 (m, 5 H), 3.62 (m, 1 H), 3.81 (m, 1 H), 4.30 (m, 1 H), 4.67 (m, 1 H), 5.03 (m, 1 H), 6.62 (d, 1 H, J = 10 Hz), 6.83 (s, 1 H), 7.18–7.37 (m, 5 H), 7.52 (s, 1 H). Anal. ($C_{32}H_{49}N_5O_5S \cdot {}^1/_4H_2O$) C, H, N.

(2*R*,3*S*)-3-[(Boc-L-phenylalanylhistidyl)amino]-2-hydroxy-5-cyclohexyl-1-(isopropylthio)pentane (7d): 42% yield; mass spectrum, $(M + H)^+ = 644$; ^1H NMR ($CDCl_3$) δ 0.7–1.7 (m, 15 H), 1.25 (m, 6 H), 1.38 (s, 9 H), 2.4–2.6 (m, 2 H), 2.85–3.05 (m, 3 H), 3.2 (m, 2 H), 3.62 (m, 1 H), 3.85 (m, 1 H), 4.28 (m, 1 H), 4.63 (m, 1 H), 5.03 (d, 1 H, J = 5 Hz), 5.99 (d, 1 H, J = 9 Hz), 6.84 (s, 1 H), 7.17–7.37 (m, 5 H), 7.57 (s, 1 H). Anal. ($C_{34}H_{53}N_5O_5S \cdot {}^1/_2H_2O$) C, H, N.

(2*R*,3*S*)-3-[(Boc-L-phenylalanylhistidyl)amino]-2-hydroxy-4-(1-adamantyl)-1-(isopropylthio)butane and (2*S*,3*R*)-3-[(Boc-L-phenylalanylhistidyl)amino]-2-hydroxy-4-(1-adamantyl)-1-(isopropylthio)butane (7f): 38% yield; exact mass 682.4002, calcd mass for $C_{37}H_{56}N_5O_5S$ 682.4002 (M + 1).

(2*R*,3*S*)-3-[(Boc-L-phenylalanylhistidyl)amino]-2-hydroxy-4,4-diphenyl-1-(isopropylthio)butane and (2*S*,3*R*)-3-[(Boc-L-phenylalanylhistidyl)amino]-2-hydroxy-4,4-diphenyl-1-(isopropylthio)butane (7g): 56% yield; mass spectrum, $(M + H)^+ = 700$; ^1H NMR ($CDCl_3$) δ 1.23 (m, 6 H), 1.38, 1.41 (2 d, 1:1, 9 H), 2.1–3.2 (m, 8 H), 3.5 (m, 1 H), 4.1–4.4 (m, 3 H), 4.85–5.05 (m, 2 H), 6.45 (d, 1 H, 6.55 (m, 1 H), 7.1–7.4 (m, 15 H), 7.48, 7.52 (2 s, 1:1, 1 H total). Anal. ($C_{39}H_{49}N_5O_5S \cdot {}^1/_2H_2O$) C, H, N.

(2*R*,3*S*)-3-[(Boc-L-phenylalanylhistidyl)amino]-2-hydroxy-4-phenyl-1-(isopropylthio)butane (7i): 41% yield; mass spectrum, $(M + H)^+ = 558$; mp 210.5–211.5 °C; ^1H NMR ($CDCl_3$) δ 1.18 (d, 3 H, J = 4 Hz), 1.22 (d, 3 H, J = 4 Hz), 1.40 (s, 9 H), 2.5 (d, 1 H, J = 9.6 Hz), 2.6–3.35 (m, J = 9 Hz), 3.54 (br t, 1 H, J = 6 Hz), 4.1–4.35 (m, 2 H), 4.61 (br q, 1 H, J = 5.5 Hz), 5.07 (d, 1 H, J = 5.5 Hz), 6.71 (s, 1 H), 6.73 (m, 1 H), 7.17–7.38 (m, 11 H), 7.48 (s, 1 H). Anal. ($C_{33}H_{45}N_5O_5S$) C, H, N.

(2*R*,3*S*/2*S*,3*R*)-3-[(Trichloroacetyl)amino]-2-hydroxy-4-(1-adamantyl)-1-(isopropylthio)butane (10) and (2*R*,3*S*/2*S*,3*R*)-3-[(Dichloroacetyl)amino]-2-hydroxy-4-(1-adamantyl)-1-(isopropylthio)butane (12). Epoxide **9** (39.0 mg, 0.107 mmol), isopropyl mercaptan (50 μL, 0.53 mmol), and triethylamine (15 μL, 0.11 mmol) were dissolved in methanol (5 mL) and heated in a sealed tube at 65 °C for 15 h. Evaporation and chromatography on silica (dichloromethane) provide **10** (15 mg, 32%) and **12** (20 mg, 47%). One can favor the formation of **10** by reducing the mercaptan stoichiometry 50% and heating at 40–50 °C while monitoring the reaction for disappearance of starting epoxide by TLC (double elution using hexane/ether, 4/1).

10: mass spectrum, $(M + 1)^+ = 442$; ^1H NMR ($CDCl_3$) δ 1.26 (d, 3 H, J = 7 Hz), 1.28 (d, 3 H, J = 7 Hz), 1.45–1.75 (m, 14 H), 1.96 (m, 3 H), 2.38 (dd, 1 H, J = 11, 14 Hz), 2.82 (dd, 1 H, J = 3, 14 Hz), 2.9 (m, 2 H), 3.62 (m, 1 H), 4.18 (m, 1 H), 6.88 (d, 1 H, J = 10 Hz). Anal. ($C_{19}H_{30}NO_2SCl_3$) C, H, N.

12: mass spectrum, $M^+ = 389$; ^1H NMR ($CDCl_3$) δ 1.26 (d, 3 H, J = 6 Hz), 1.28 (d, 3 H, J = 6 Hz), 1.4–1.75 (m, 14 H), 1.95 (m, 3 H), 2.38 (dd, 1 H, J = 11, 14 Hz), 2.79 (dd, 1 H, J = 3, 14 Hz), 2.90 (m, 1 H), 3.60 (m, 1 H), 4.08 (m, 1 H), 5.92 (s, 1 H), 6.64 (d, 1 H, J = 10 Hz).

(4*S*,5*R*/4*R*,5*S*)-4-(1-Adamantylmethyl)-5-[(2-propylthio)methyl]oxazolidin-2-one (11). The general procedure for deprotection of chloroacetamides (vide supra) was used. **11**: 61% yield; mass spectrum, $(M + 1)^+ = 324$; ^1H NMR ($CDCl_3$) δ 1.28 (d, 3 H, J = 6 Hz), 1.29 (d, 3 H, J = 6 Hz), 1.4–1.8 (m, 14 H), 2.00 (m, 3 H), 2.79 (dd, 1 H, J = 7, 14 Hz), 2.86 (dd, 1 H, J = 5, 14 Hz), 3.04 (m, 1 H), 3.78 (m, 1 H), 4.26 (m, 1 H), 5.03 (m, 1 H).

Biological Methods. Purified human renal renin[22] was assayed with pure human angiotensinogen[23] at pH 6.0 in maleate buffer. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted so that prior to addition to the assay system the solutions were 10% in DMSO and 0.5% in bovine serum albumin (BSA). The final incubation mixture (100 μL) contained the following: maleate buffer, pH 6.0, 0.135 M; ethylenediaminetetraacetic acid, 3 mM; phenylmethane sulfonyl fluoride, 1.4 mM; angiotensinogen, 0.21 μM; renin, 0.24 mGU[24]; BSA, 0.44%; DMSO, 1%. At least three different concentrations of inhibitor that bracketed the IC_{50} (concentration that inhibited 50%) were preincubated with renin for 5.0 min at 37 °C, the substrate was added, and the incubation was allowed to proceed for 10.0 min. The reaction was stopped by freezing the solution in a methanol/dry ice bath, and after thawing at 4 °C, an aliquot was analyzed for angiotensin I by radioimmunoassay utilizing a commercial kit (NEN Research). The percent inhibition of the reaction was determined and the IC_{50} was calculated by regression analysis. The reaction time of 10 min was on the linear portion of the incubation time–angiotensin I generation curve, and at the highest concentrations tested none of the compounds cross reacted with the antibody to angiotensin I. The presence of 1% DMSO in the final incubation mixture caused no statistically significant effect on the renin activity.

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