0.1 M Tris, 5 mM $MgCl_2$ solution, pH ~8, and was activated by following the procedure of Andersson et al.[16] Leucine *p*-nitroanilide hydrochloride (LPNA) was purchased from Sigma and was used without further purification. The buffer used for all assays was 50 mM Tris, pH 8.6, containing 5.0 mM $MgCl_2$ and 1 mM LPNA, unless otherwise stated. The initial hydrolysis rates of LPNA were determined by placing a solution of substrate and inhibitor in a cuvette and initiating the reaction by addition of ~15 μg of LAP/1.00 mL final volume. Substrate hydrolysis at 25 °C was followed by observing the increase in absorbance at λ = 405 nm ($\Delta\epsilon$ = 9620 M^{-1} cm^{-1}).

Slow-Binding of (*R*)-(1-Amino-3-methylbutyl)phosphonic Acid ((*R*)-3e). A series of eight assays were performed, with the concentration of (*R*)-**3e** varied as 0.5, 1, 2, 5, 10, and 15 μM; each reaction was followed until steady state was reached. The initial exponential phase of the reaction was analyzed by subtracting the steady-state rate for the reaction progress curve (Figure 1); a Newtonian algorithm involving a nonlinear least-squares fit to the resulting curve was used to determine the exponential constant, k_{app}. A representative, fitted curve is shown in Figure 2. A plot of k_{app} vs. inhibitor concentration was linear, indicating a one-step slow-binding mechanism (E + I ⇌ EI). The on rate, k_{on}, calculated from slope = $k_{on}/(1 + S/K_m)$, is 403 ± 55 M^{-1} s^{-1}; the off rate, k_{off}, calculated from $k_{off} = K_i \times k_{on}$, is 9.26 × 10^{-4} s^{-1}.

Slow Binding of (*R*)-(1-Amino-2-phenylethyl)phosphonic Acid ((*R*)-3h). The slow-binding behavior of the phenylalanine analogue **9a** was determined similarly, by using inhibitor concentrations of 3, 6, 9, 12, 15, 60, 80, and 100 μM: k_{on} = 445 ± 50 M^{-1} s^{-1}; k_{off} = 1.87 × 10^{-4} s^{-1}.

Inhibition of LAP by *N*-[[Amino(1-amino-3-methylbutyl)phosphinyl]carbonyl]-L-leucine (17-NH_2). The K_i of compound 17-NH_2 was determined by standard methods, using inhibitor concentrations of 30, 60, 90, 120, 150, and 180 μM. This compound is stable and does not show slow-binding behavior under the assay conditions. A Dixon plot was used to determine the K_i of compound 17-NH_2 as 40 μM;[48] a similar procedure was used to determine the K_i values of (*S*)-**3e**, **9e**, and **9h**.

Inhibition by (*R*)-L-(1-Amino-3-methylbutyl)phosphonate ((*R*)-3e). The final K_i of compound (*R*)-**3e** was determined by using inhibitor concentrations of 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 μM; each reaction was followed until an equilibrium rate was reached (16 min). A K_i value of 0.23 μM was determined from a Dixon plot.[48] A similar procedure was used to determine the K_i values of compounds **3c**, **3d**, **3f**, **3g**, and (*R*)-**3h**.

Inhibition of LAP by (2*S*)-2-[[Hydroxy(2-phenyl-1-aminoethyl)phosphinyl]oxy]-4-methylpentanoate (8h). The K_i of compound **8h** was also determined by standard methods. A series of 16 assays were performed, with substrate concentrations of 0.67, 0.1, 0.25, and 1.0 mM and inhibitor concentration of 0, 0.01, 0.1, and 1.0 mM. The reaction velocity, determined during the first 4.0 min of reaction, was plotted in Lineweaver–Burk form, and the slopes were replotted vs. [I]; the K_i value for compound **8h** was found to be 340 μM. A similar procedure was used to determine the K_i values for compounds **8e**, **5e**, **5h**, **3a**, **3b**, and (*S*)-**3h**.

Acknowledgment. We express our appreciation to John Hanson for programming the least-squares-fit algorithm and to Lynette Young for providing us with the resolved isomers of **3h**. This work was supported by a grant from the National Institutes of Health (Grant No. CA-22747).

Supplementary Material Available: Characterization of the inhibitors and synthetic intermediates not described above (3 pages). Ordering information is given on any current masthead page.

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New Inhibitors of Human Renin That Contain Novel Leu-Val Replacements

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Stereoselective syntheses of several nonpeptide fragments that function as Leu^{10}-Val^{11} scissile bond replacements in human angiotensinogen are presented. The opening of N-protected aminoalkyl epoxide **3** with a variety of sulfur, oxygen, nitrogen, and carbon nucleophiles is a key reaction in the preparation of these novel fragments **4**–**8**. The coupling of these fragments to protected dipeptides that mimic positions 8 and 9 in angiotensinogen produces inhibitors of human renin even though the molecules contain no functionality beyond what is formally the Val^{11} side chain of angiotensinogen. R groups that closely resemble that of the Val side chain are preferable; thus, isopropyl ≥ higher alkyl > phenyl > substituted phenyl. Sulfur is the best X group; oxidation leads to slight (X = SO_2) and significant (X = SO) decreases in inhibitory potency. One such inhibitor, **60**, has an IC_{50} of 13 nM when tested with purified human renin at pH 6.0. The significant activity of these small inhibitors is thought to be due in part to the hydroxyl group of the fragment functioning as a transition-state analogue. Of these, the inhibitors that contain histidine show marked selectivity toward renin over a related aspartic proteinase, pepsin.

Renin is an aspartic proteinase that is released from the kidney and catalyzes a specific hydrolysis of the glycoprotein angiotensinogen to give the decapeptide angiotensin I (AI). A dipeptidylcarboxypeptidase, angiotensin converting enzyme (ACE), then converts it to the octapeptide AII, which, in addition to being an extremely potent vasoconstrictor, is also a promoter of aldosterone release and thereby sodium retention. A variety of other effects on the kidneys, brain, and pituitary are also due to the actions of AII. Aminopeptidases further act on AII to give AIII, which produces effects similar to those produced by AII, but to a lesser extent. This cascade, known as the renin–angiotensin system (RAS),[1] is therefore an important area for study in the regulation of blood pressure and electrolyte homeostasis[2] (Scheme I).

The design of ACE inhibitors as useful drugs in the treatment of hypertension and cardiac failure has been reviewed.[3] However, since renin has remarkable specificity

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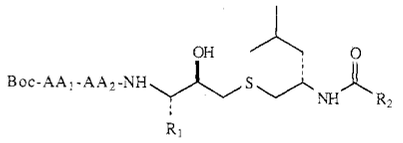
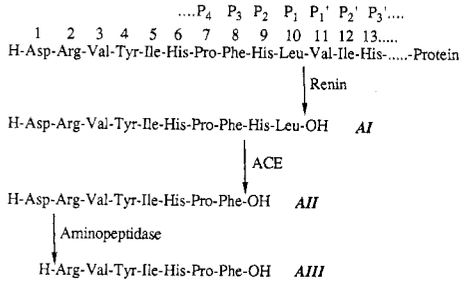


Figure 1.

Scheme I

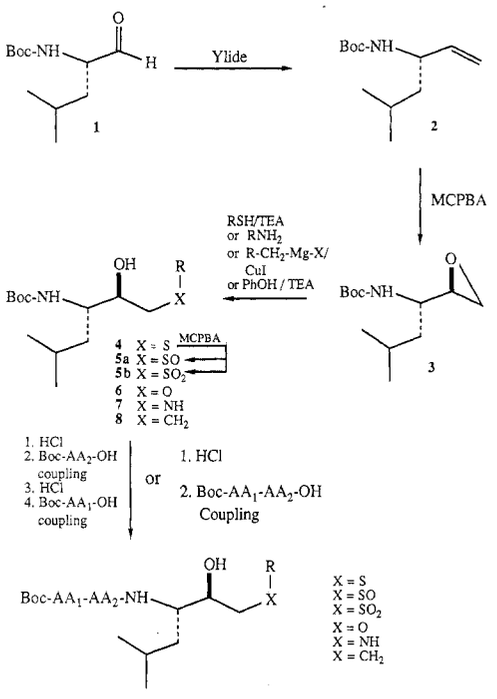


toward its substrate, inhibitors of this enzyme should provide a more direct probe of the RAS itself compared to the inhibitors of the relatively nonspecific ACE, whose effects may also be due in part to the action of kinins[4] and vasoactive prostaglandins[5] in addition to suppression of AII levels.

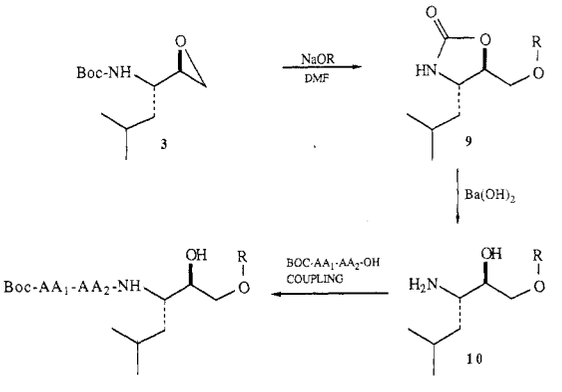
In a limited number of cases, the manipulation of the RAS by renin inhibitors (among them substrate analogue inhibitors,[6] peptides derived from the profragment segment,[7] pepstatin/statine-based inhibitors,[8] and other transition-state or intermediate analogues of substrate[9]) has led to blood-pressure lowering in experimental animals as well as in humans; the literature describing design, synthesis, and biological activity of renin inhibitors has been the subject of a succinct review.[10] Most of the inhibitors used in the above studies were peptidic in nature and/or high in molecular weight. We set as our goal to minimize these two features in hopes of ultimately identifying a new, easily synthesized class of compounds that would be absorbed well after oral administration. Our preliminary effects toward this end are reported here.

Previously, we synthesized a series of inhibitors,[11] depicted generally in Figure 1, which contain a novel mercaptoethanol amide bond replacement at the scissile bond site (residues P_1-$P_{1'}$). That a stable isostere containing a hydroxyl group of the *S* configuration[12] (statine-like)[13] at this position in angiotensinogen fragments can lead to increased potency has been demonstrated by the incorporation of the hydroxyethylene isostere[14] and statine[15] into inhibitors. In a related series of inhibitors, we have now discovered that when a (phenylthio)ethanol replace-

Scheme II



Scheme III



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(12) The "*S*" and "*R*" configurations are as defined by the IUPAC 1974 Recommendations for Section E, Fundamental Stereochemistry: *Pure Appl. Chem.* **1976**, *45*, 13.

(13) "Statine-like stereochemistry" is a descriptor that will be used in this report to refer to compounds that have an absolute stereochemistry of the carbon bearing the hydroxyl group like that of statine (4(*S*)-amino-3(*S*)-hydroxy-6-methylheptanoic acid). This will avoid possible confusion brought on by the *S*/*R* stereochemical convention.[12] For example, the stereochemistry of the carbon bearing the hydroxyl group of inhibitor **15** is statine-like, but of the *R* configuration.

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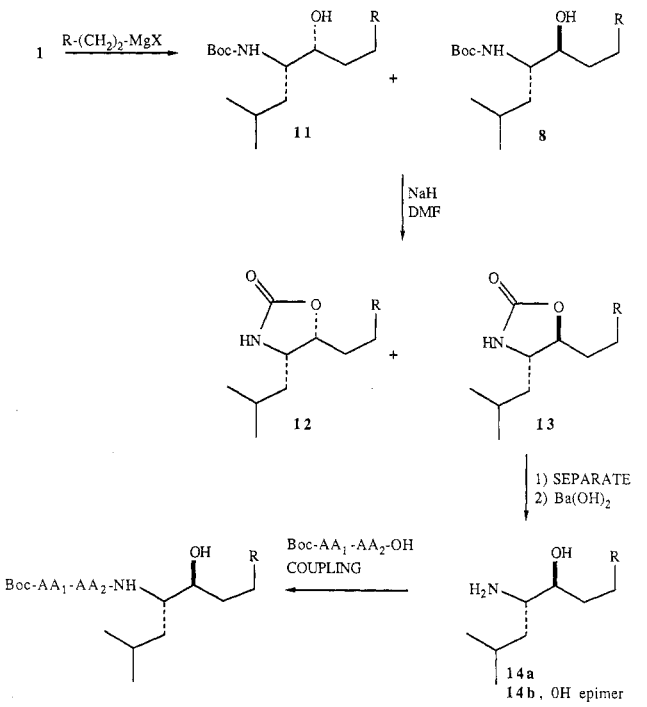
Table I. Chemical Data

no.	synthesis[a]	purifn[b] (solvent)[c]	yield,[d] %	formula[e]
15	A	A (A)	55	$C_{33}H_{44}N_5O_5S$
16	B	A (A)	84	$C_{30}H_{45}N_3O_5S \cdot H_2O$
17	B	A (B)	88	$C_{36}H_{47}N_3O_5S$
18	B	A (B)	71	$C_{36}H_{47}N_3O_6S$
19	B	A (A)	86	$C_{30}H_{42}N_3O_5SBr \cdot {}^1/_4H_2O$
20	B	A (B)	50	$C_{34}H_{51}N_3O_5S$
21	B	A (A)	81	$C_{34}H_{45}N_3O_5S \cdot {}^1/_2H_2O$
22	B	A (A)	57	$C_{34}H_{45}N_3O_5S \cdot {}^1/_2H_2O$
23	B	A (B)	57	$C_{35}H_{47}N_3O_5S \cdot {}^1/_4H_2O$
24	B	A (A)	51	$C_{31}H_{45}N_3O_5S \cdot {}^1/_2H_2O$
25	B	A (A)	35	$C_{32}H_{47}N_3O_5S$
26	B	A (A)	84	$C_{33}H_{49}N_3O_5S$
27	B	A (B)	39	$C_{27}H_{45}N_3O_5S$
28	B	A (B)	51	$C_{28}H_{47}N_3O_5S \cdot {}^1/_4H_2O$
29	B	A (B)	62	$C_{29}H_{49}N_3O_5S$
30	B	A (B)	52	$C_{28}H_{47}N_3O_5S$
31	B	A (A)	67	$C_{30}H_{48}N_3O_5S$
32	B	A (B)	42	$C_{29}H_{47}N_3O_5S$
33	B	A (A)	72	$C_{30}H_{43}N_3O_6 \cdot {}^1/_2H_2O$
34	B	A (B)	22	$C_{30}H_{44}N_4O_5 \cdot {}^1/_2H_2O$
35	B	A (B)	73	$C_{31}H_{45}N_3O_5$
36	B	A (B)	86	$C_{27}H_{45}N_3O_6$
37	B	B (C)	37	$C_{27}H_{46}N_4O_5 \cdot {}^1/_2H_2O$
38	B	A (D)	94	$C_{28}H_{47}N_3O_5$
39	B	C	88	$C_{28}H_{47}N_3O_5 \cdot {}^1/_2H_2O$
40	B	A (D)	74	$C_{28}H_{47}N_3O_6 \cdot {}^1/_2H_2O$
41	B	A (B)	59	$C_{29}H_{49}N_3O_5 \cdot {}^1/_2H_2O$
42	B	A (A)	32	$C_{30}H_{43}N_3O_6S \cdot {}^1/_4H_2O$
43	B	A (B)	56	$C_{30}H_{43}N_3O_7S$
44	D	A (D)	27	$C_{30}H_{49}N_3O_6S$
45	E	A (A)	70	$C_{30}H_{49}N_3O_7S$
46	A	A (C)	45	$C_{30}H_{47}N_5O_6 \cdot {}^1/_3H_2O$
47	A	D	61	$C_{30}H_{48}N_6O_5 \cdot 1^1/_2H_2O$
48	A	A (C)	57	$C_{31}H_{49}N_5O_5$
49	A	A (C)	52	$C_{31}H_{49}N_5O_6 \cdot {}^1/_2H_2O$
50	A	A(A)	50	$C_{32}H_{51}N_5O_5 \cdot H_2O$
51	A	A (E)	41	$C_{30}H_{47}N_5O_5S \cdot {}^1/_2H_2O$
52	A	A (F)	58	$C_{30}H_{47}N_5O_7S$
53	A	A (F)	48	$C_{31}H_{49}N_5O_7S \cdot H_2O$
54	A	A (F)	58	$C_{32}H_{51}N_5O_7 \cdot {}^2/_3H_2O$
55	A	A (E)	24	$C_{33}H_{52}N_5O_5S$
56	A	A (E)	43	$C_{33}H_{52}N_5O_5S$
57	A	A (A)	49	$C_{33}H_{51}N_5O_7S$
58	C	A (B)	36	$C_{35}H_{51}N_5O_5 \cdot H_2O$
59	C	A (D)	41	$C_{35}H_{51}N_5O_5 \cdot {}^1/_2H_2O$
60	C	A (A)	28	$C_{37}H_{53}N_5O_5S$
61	A	A (E)	36	$C_{37}H_{53}N_5O_5S$

[a] A. Carbodiimide coupling to Boc-AA_1-AA_2-OH. B. Mixed anhydride coupling to Boc-AA_1-AA_2-OH. Carbodiimide coupling to Boc-AA_2-OH, deprotection, and active ester coupling to Boc-AA_1-OSu. D. Oxidation of **31** with 1 equiv of MCPBA. E. Oxidation of **31** with 2 equiv of MCPBA. [b] A. Flash chromatography on silica gel. B. Preparative TLC on silica gel. C. Recrystallization from ether/hexane. D. Trituration with ethyl acetate/hexane. [c] The following dichloromethane/methanol mixtures were used: A (95:5), B (98:2), C (90:10), D (gradient with 2–5% methanol in dichloromethane), E (gradient with 5–7% methanol in dichloromethane); F, chloroform/methanol (95:5). [d] Percent yield from **4**–**8** or **10**. [e] Analyses for C, H, and N (within ±0.4% of the calculated values) or high-resolution mass spectra (±5 ppm) were obtained for the above compounds.

ment is attached to an N-protected dipeptide, a reasonably potent inhibitor results even though all functionality beyond what is formally the carbon bearing the $P_{1'}$ side chain is absent (**15**). The effect of oxidation state of the X group was studied by changing sulfur to sulfoxide and sulfone. Other variations of the X group include the following: X = NH, in order to provide a series of inhibitors with enhanced water solubility; X = SO and X = SO_2, in order to study possible metabolites of the X = S series; X = O and X = CH_2, in order to provide two series that, unlike that with X = S, would not be prone to oxidation/reduction processes in vivo.

Scheme IV



Chemistry

The synthesis of peptides containing X = S, SO, SO_2, NH, and CH_2 is summarized in Scheme II and Table I. Previously, we detailed a highly diastereoselective two-step synthesis of protected aminoalkyl epoxides.[16] Thus, we synthesized epoxide **3** (92–94% threo, 99% ee) from (*tert*-butyloxycarbonyl)leucinal by Wittig olefination followed by oxidation with 3-chloroperoxybenzoic acid (MCPBA). Epoxide **3** underwent facile ring opening upon treatment with mercaptans in triethylamine/methanol. Alcohol **4** was then deprotected with acid and coupled to the dipeptide Boc-AA_1-AA_2-OH to give the inhibitor with X = S. Alternatively these inhibitors could be prepared from alcohol **4** by deprotection, coupling to Boc-AA_2-OH, deprotection, and coupling to Boc-AA_1-OH.

Oxidation of **4** with 200 mol% or 100 mol% MCPBA provided sulfone **5b** or a diastereomeric mixture of sulfoxides **5a**, respectively. Deprotection and coupling of each in a similar manner provided inhibitors with X = SO and X = SO_2. In an analogous manner, epoxide **3** could be opened with amines to provide **7**. After deprotection, coupling to the protected dipeptide occurred only with the less hindered, primary amine to afford inhibitors with X = NH.

A copper-assisted Grignard addition to epoxide **3** was used to prepare **8**, R = isobutyl. As will be discussed below, this method is an alternative to the direct addition of a Grignard to aldehyde **1**. Deprotection of **8** and coupling to a protected dipeptide also proceeded in a straightforward manner.

Peptides containing X = O could be prepared by the above route only when R = phenyl. When oxygen anions more basic than phenoxide were used, cyclization of the epoxide-opened product **6** onto the Boc group afforded 2-oxazolidinone **9** (Scheme III). Barium hydroxide hydrolysis in dioxane/water and coupling under standard conditions then provided the corresponding inhibitors.

Addition of a Grignard reagent to aldehyde **1** provided diastereomeric alcohols **8** and **11** (Scheme IV). The hy-

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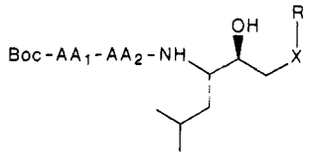
Table II. Inhibitors and in Vitro Activity



no.	AA_1	AA_2	X	R	renin IC_{50}, μM (pH 6.0)	renin % inhibn (10^{-5} M)	pepsin: % inhibn (10^{-5} M)
15	Phe	His	S	phenyl	0.96	96	0
16	Phe	Ala	S	phenyl	8	56	
17	Phe	Phe	S	phenyl		48	11
18	Phe	Tyr	S	phenyl		21	11
19	Phe	Ala	S	4-bromophenyl		21	
20	Phe	Ala	S	4-*tert*-butylphenyl		15	
21	Phe	Ala	S	2-naphthyl		23	
22	Phe	Ala	S	1-naphthyl		7	
23	Phe	Ala	S	1-naphthylmethyl		48	
24	Phe	Ala	S	benzyl	10	57	96
25	Phe	Ala	S	phenethyl	4.5	73	
26	Phe	Ala	S	phenylpropyl	1	88	74
27	Phe	Ala	S	isopropyl	0.7	90	73
28	Phe	Ala	S	isobutyl	1.5	89	
29	Phe	Ala	S	isopentyl	1.5	93	
30	Phe	Ala	S	*tert*-butyl	3	88	
31	Phe	Ala	S	cyclohexyl	0.8	94	100
32	Phe	Ala	S	cyclopentyl	1	94	
33	Phe	Ala	O	phenyl		48	27
34	Phe	Ala	NH	phenyl		30	
35	Phe	Ala	CH_2	phenyl		35[a]	
36	Phe	Ala	O	isopropyl	7	54	
37	Phe	Ala	NH	isopropyl		5	
38	Phe	Ala	CH_2	isopropyl	2	85	
39	Phe	Ala	CH_2	isopropyl		16(OH)epimer	
40	Phe	Ala	O	isobutyl	7	59	68
41	Phe	Ala	CH_2	isobutyl	3.5	71	
42	Phe	Ala	SO	phenyl		12	
43	Phe	Ala	SO_2	phenyl		25	
44	Phe	Ala	SO	cyclohexyl		46	
45	Phe	Ala	SO_2	cyclohexyl	2	76	
46	Phe	His	O	isopropyl	1.5	85	0
47	Phe	His	NH	isopropyl		35	
48	Phe	His	CH_2	isopropyl	1.5	89	
49	Phe	His	O	isobutyl	0.65	94	0
50	Phe	His	CH_2	isobutyl	0.60	97	
51	Phe	His	S	isopropyl	0.081	92 (10^{-6} M), 54 (10^{-7} M)	
52	Phe	His	SO_2	isopropyl	0.20	99	3
53	Phe	His	SO_2	isobutyl	0.35[b]	98	
54	Phe	His	SO_2	isopentyl	0.50[b]	94	
55	Phe	His	S	cyclohexyl	0.035	78 (10^{-7} M)	
56	Phe	His	S	cyclohexyl	0.52	95 (OH epimer)	
57	Phe	His	SO_2	cyclohexyl	0.090	92 (10^{-6} M), 51 (10^{-7} M)	
58	1-Nal	His	CH_2	isopropyl	0.15[b]	93 (10^{-6} M)	6
59	2-Nal	His	CH_2	isopropyl	0.60	87 (10^{-6} M)	
60	1-Nal	His	S	cyclohexyl	0.013	85 (10^{-7} M)	
61	2-Nal	His	S	cyclohexyl	0.040[b]	85 (10^{-7} M)	0

[a] 2:1 mixture of *S*:*R* diastereomers at the carbon bearing the hydroxyl group.[12] [b] IC_{50} measurements at pH 7.4 for inhibitors **53**, **54**, **58**, and **61** were found to be 0.87, 1.5, 0.16, and 0.10 μM, respectively.

droxyl stereochemistry was established by cyclizing to the corresponding 2-oxazolidinones with NaH/DMF, separating the mixture, and examining the coupling constants and chemical shifts of the ring hydrogens in the ^{1}H NMR spectra. A coupling constant of 7–8 Hz suggests **12** while 4–6 Hz suggests **13**.[17] Also, the chemical shifts of ring hydrogens of **12** are generally downfield from those of **13**, presumably due to shielding of the hydrogens in **13** by substituents on adjacent ring carbons. Oxazolidin-2-one **13** with the desired 5*S* configuration was then hydrolyzed to the amine, which was then coupled to the protected dipeptide to give the inhibitors with X = CH_2. This route allowed the synthesis of the analogous inhibitor derived from the diastereomeric *R* alcohol **11** to confirm that the *S* (statine-like)[13] configuration is indeed the more potent.

Biological Activities and Discussion

The analogues related to **15** were evaluated as inhibitors of human renal renin at the pH optimum 6.0. The results of these studies are summarized in Table II. Inhibition at pH 7.4 was determined for selected compounds and was found to be the same or slightly lower (Table II, note *b*). In general, IC_{50} determinations were not made on compounds with less than 50% inhibition at 10^{-5} M. Com-

(17) (a) Rich, D. H.; Sun, E. T. O. *J. Med. Chem.* **1980**, *23*, 27. (b) Futagawa, S.; Inui, T.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 3308. (c) Foglia, T. A.; Swern, D. *J. Org. Chem.* **1969**, *34*, 1680. (d) Cardillo, G.; Orena, M.; Sandri, S.; Tomasini, C. *Tetrahedron* **1985**, *41*, 163.

pounds **15–18** explore variations of AA_2. Although His, the amino acid found at position 9 of angiotensinogen, is clearly the optimal substituent of those examined, Ala represents a convenient alternative, especially in terms of the ease of purification and higher yields of inhibitors containing it. We therefore proceeded with several AA_2 = Ala inhibitors because, as will be seen, resubstitution of Ala with His gives a nearly 10-fold boost in activity.

The enzyme is less tolerant of phenyl rings bearing bromo and *tert*-butyl substituents in the para position (inhibitors **19** and **20**) or of the bulkier naphthyl rings, regardless of the regioisomer (**21** and **22**). Extending a naphthyl group by one methylene spacer to give R = 1-naphthylmethyl leads to a tighter binding inhibitor, **23**. In the more potent RX = phenylthio series, the inhibitor with a one-methylene spacer was of about the same activity (**24** vs. **16**), but increasing the aliphatic spacer from one to two to three methylenes led to a stepwise boost in potency (**24**, **25**, **26**).

Since the R group corresponds to what is formally the $P_{1'}$ side chain of valine, residue 11, we examined changes with R = alkyl (**27–30**). Again, the side chain of natural substrate (isopropyl) emerged as the best of those examined, although R = isobutyl, isopentyl, and *tert*-butyl still displayed reasonable potency. Inhibitors with R = cyclohexyl (**31**) and cyclopentyl (**32**) preserve the isopropyl backbone, but the extra lipophilicity lent by the ring offered no improvement.

The variation in activity with X = O, NH, and CH_2 was studied with both aromatic and aliphatic R groups. When R = phenyl, analogues **33** (X = O), **34** (X = NH), and **35** (X = CH_2) were less potent than **16** (X = S), although it should be noted that **35** existed as a 2:1 mixture of *S*/*R* isomers about the carbon bearing the hydroxyl group, and it is known in this series (compound **39**) as well as in others[15] that the *R* isomer is relatively inactive compared to the *S* (statine-like)[13] isomer. This being the case, **35**, if in isomerically pure form, would probably compare well with **33** and **16**. When R = isopropyl, analogues **36** (X = O), **37** (X = NH), and **38** (X = CH_2) were again less active than their sulfur counterpart, **27**. In this comparison, only carbon analogue **38** approached the potency of **27**. A similar trend was observed when R = isobutyl (**40**, X = O and **41**, X = CH_2).

The sulfur analogues were explored further to see if their oxidation products, possible metabolites in vivo, possessed inhibitory activity. Sulfoxides and sulfones representing both aromatic (**42** and **43**) and aliphatic (**44** and **45**) R groups were prepared. Without exception, activities fell relative to the respective unoxidized parents (**16** and **31**).

Reintroducing His in place of Ala gave the expected boost in potency in several examples. Compare: **46/36**, **47/37**, **48/38**, **49/40**, **50/41**, **51/27**, **55/31**, **57/45**. The most exceptional in this regard were inhibitors **55** and **57**, which became over 20 times more potent than their Ala analogues. As in the pair **39/38**, a loss in potency of **56** relative to **55** was observed, further underscoring the importance of the statine-like stereochemistry. Diastereomer **56** was prepared by starting with a sample of **3** that was enriched in the erythro isomer.

The AA_1 position was also examined briefly. Renin inhibitors have been previously synthesized with naphthylalanine (Nal) for Phe at what is formally position 8.[9b,11,18] This change was tried in selected inhibitors in our series with two regioisomers, 1- and 2-Nal. When X = CH_2, the 1-Nal-containing inhibitor (**58**) was 4 times more potent than the 2-Nal isomer (**59**), and they were respectively 10 and 2.5 times more potent than the Phe analogue (**48**). The literature provides examples from different series of inhibitors where the 1-Nal isomer imparted more[9b] and less[18] activity and where the 2-Nal imparted less activity.[11] These examples illustrate how minor differences in inhibitor structure among series can lead to altered enzyme conformations and consequently to different tolerances in site variation. In the X = S series, again, the 1-Nal isomer (**60**) was slightly more potent (3 times) than the 2-Nal isomer (**61**), but they were respectively 3 times more potent and equipotent compared to the corresponding Phe analogue (**55**).

The selectivity of representative inhibitors toward renin over a related aspartic proteinase, pepsin, was studied. As shown in Table I, inhibitors that contain AA_2 = His show almost no inhibition of pepsin at 10^{-5} M. Inhibitors that instead contain Phe, Tyr, or Ala at this position are much less specific; in fact, compounds **24** and **31** are actually better pepsin inhibitors than renin inhibitors.

Summary

Several classes of nonpeptide replacements corresponding to the Leu[10]-Val[11] dipeptide of human angiotensinogen were prepared directly (**8**) from aldehyde **1** or indirectly (**4–8**) via epoxide **3**. Renin inhibitors were then prepared by coupling these fragments to a protected dipeptide. R groups that closely resemble that of the Val side chain of angiotensinogen were found to be preferable; thus isopropyl ≥ higher alkyl > phenyl > substituted phenyl. Sulfur was found to be the best X group; oxidized forms led to slight (SO_2) and significant (SO) decreases in activity. The statine-like stereochemistry of the carbon bearing the hydroxyl group in these fragments was shown to be preferable. The selectivity and potency of His-containing compounds were found to be excellent while those of inhibitors with the other amino acids tried in position AA_2 were lower. AA_1 = Phe, 1-Nal, and 2-Nal showed a series to series variation. The inhibitors described here compare well to other low-molecular-weight inhibitors that possess minimal functionality beyond the Leu-10 position,[9b-d] and further chemical, biological, and molecular-modeling studies on these compounds are currently under way and will be reported in due course.

Experimental Section

All amino acids and protected amino acids were obtained from Sigma Chemical Co. unless otherwise noted. 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 (300 MHz) instrument. 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[19] chromatography was carried out by using Baker silica gel (40 μm).

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

Epoxide Openings. A. With Mercaptans. (2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(cyclohexylthio)-2-

(18) Hanson, G. J.; Baran, J. S.; Lindberg, T.; Walsh, G. M.; Papaioannou, S. E.; Babler, M.; Bittner, S. E.; Yang, P.-O.; Corobbo, M. D. *Biochem. Biophys. Res. Commun.* **1985**, *132*, 155.

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

hydroxy-5-methylhexane (4a, R = Cyclohexyl). To a stirred solution of epoxide **3**[16] (200 mg, 0.87 mmol) in methanol (8.7 mL) were added cyclohexyl mercaptan (102 mg, 0.87 mmol) and triethylamine (88 mg, 0.87 mmol). The resultant solution was refluxed for 2 h and then evaporated to give a residue, which was chromatographed on 15 g of 40-μm SiO_2 (7:3 hexane/ether) to give 281 (94%) of the desired compound: mass spectrum M^+ = 345; ^1H NMR ($CDCl_3$) δ 0.92 (d, 3 H, J = 6 Hz), 0.94 (d, 3 H, J = 6 Hz, 1.2–2.05 (several m, 13 H), 1.46 (s, 9 H), 2.52 (dd, 1 H, J = 9, 13 Hz), 2.65 (m, 1 H), 2.79 (dd, 2 H, J = 3, 13 Hz), 2.93 (br s, 1 H), 3.57 (m, 1 H), 3.67 (m, 1 H), 4.70 (br d, 1 H, J = 10 Hz). Anal. Calcd for $C_{18}H_{34}NO_3S$: C, 62.6; H, 10.2; N, 4.0. Found: C, 62.9; H, 10.4; N, 3.9.

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(phenylthio)-2-hydroxy-5-methylhexane (4b, R = phenyl): 82% yield; mass spectrum M^+ = 339; ^1H NMR ($CDCl_3$) δ 0.90 (d, 3 H, J = 6 Hz), 0.92 (d, 3 H, J = 6 Hz), 1.44 (s, 9 H), 1.2–1.7 (m, 3 H), 2.82 (br, 1 H), 2.9 (dd, 1 H, J = 11, 14 Hz), 3.16 (dd, 1 H, J = 3, 14 Hz), 3.57 (m, 1 H), 3.79 (m, 1 H), 4.69 (br d, 1 H, J = 9 Hz), 7.2–7.45 (m, 5 H).

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-[(4-bromophenyl)thio]-2-hydroxy-5-methylhexane (4c, *R* = 4-bromophenyl): 71% yield; mass sppectrum M^+ = 418; ^1H NMR ($CDCl_3$) δ 0.91 (d, 3 H, J = 6 Hz), 0.93 (d, 3 H, J = 6 Hz), 1.45 (s, 9 H), 1.2 – 1.7 (m, 3 H), 2.73 (d, 1 H, J = 3 Hz), 2.90 (dd, 1 H, J = 10, 14 Hz), 3.13 (dd, 1 H, J = 3, 14 Hz), 3.56 (m, 1 H), 3.68 (m, 1 H,), 4.66 (br d, 1 H, J = 10 Hz), 7.25 (d, 2 H, J = 9 Hz), 7.42 (d, 2 H, J = 9 Hz).

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-[(4-*tert*-butylphenyl)thio]-2-hydroxy-5-methylhexane (4d, R = 4-*tert*-butylphenyl): 70% yield; Mass spectrum M^+ = 395; ^1H NMR ($CDCl_3$) δ 0.90 (d, 3 H, J = 7 Hz), 0.92 (d, 3 H, J = 7 Hz), 1.23 (s, 9 H), 1.46 (s, 9 H), 1.2–1.7 (m, 3 H), 2.86 (dd, 1 H, J = 10, 14 Hz), 3.14 (dd, 1 H, J = 4, 14 Hz), 3.55 (m, 1 H), 3.68 (m, 1 H), 4.68 (d, 1 H, J = 10 Hz), 7.33 (s, 4 H). Anal. Calcd for $C_{22}H_{37}NO_3S$: C, 66.8; H, 9.4; N, 3.5. Found: C, 66.5; H, 9.6; N, 3.3.

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(2-naphthylthio)2-hydroxy-5-methylhexane (4e, R = 2-naphthyl): 65% yield; mass spectrum M^+ = 389; ^1H NMR ($CDCl_3$) δ 0.88 (d, 6 H, J = 6 Hz), 1.46 (s, 9 H), 1.2–1.7 (m, 3 H), 2.91 (br d, 1 H, J = 2 Hz), 2.98 (dd, 1 H, J = 10, 14 Hz), 3.27 (dd, 1 H, J = 3, 14 Hz), 3.61 (m, 1 H), 3.73 (m, 1 H), 4.73 (d, 1 H, J = 10 Hz), 7.46 (m, 3 H), 7.97 (m, 4 H).

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(1-naphthylthio)-2-hydroxy-5-methylhexane (4f, R = 1-naphthyl): 45% yield; mass spectrum M^+ = 389; ^1H NMR ($CDCl_3$) δ 0.87 (d, 6 H, J = 6 Hz), 1.15–1.7 (m, 3 H), 1.43 (s, 9 H), 2.9 (br, 1 H), 2.95 (dd, 1 H, J = 10, 14 Hz), 3.18 (dd, 1 H, J = 3, 14 Hz) 3.5 (m, 1 H), 3.65 (m, 1 H), 4.67 (d, 1 H, J = 10 Hz), 7.42 (m, 1 H), 7.54 (m, 2 H), 7.65 (m, 1 H), 7.78 (m, 1 H), 7.86 (m, 1 H), 8.43 (m, 1 H). Anal. Calcd for $C_{22}H_{31}NO_3S$: C, 67.8; H, 8.0; N, 3.6. Found: C, 67.8; H, 8.2; N, 3.4.

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-[(naphthylmethyl)thio]-2-hydroxy-5-methylhexane (4g, R = 1-naphthylmethyl): 88% yield; mass spectrum M^+ = 403; ^1H NMR ($CDCl_3$) δ 0.92 (d, 3 H, J = 6 Hz), 0.94 (d, 3 H, J = 6 Hz), 1.44 (s, 9 H), 1.2–1.7 (m, 3 H), 2.57 (m, 1 H), 2.72 (m, 1 H), 3.53 (m, 1 H), 3.62 (m, 1 H), 4.18 (d, 1 H, J = 14 Hz), 4.25 (d, 1 H, J = 14 Hz), 4.63 (d, 1 H, J = 10 Hz), 7.4 (m, 2 H), 7.56 (m, 2 H), 7.81 (m, 1 H), 7.89 (m, 1 H), 8.16 (d, 1 H, J = 8 Hz). Anal. Calcd for $C_{23}H_{33}NO_3S$: C, 68.4; H, 8.2; N, 3.5. Found: C, 68.6; H, 8.3; N, 3.3.

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(benzylthio)-2-hydroxy-5-methylhexane (4h, R = benzyl): 57% yield; mass spectrum M^+ = 353; ^1H NMR ($CDCl_3$) δ 0.92 (d, 3 H, J = 6 Hz), 0.94 (d, 3 H, J = 6 Hz), 1.43 (s, 9 H), 1.2–1.7 (m, 3 H), 2.48 (dd, 1 H, J = 11, 15 Hz), 2.63 (dd, 1 H, J = 4, 15 Hz), 2.74 (br, 1 H), 3.5 (m, 1 H), 3.59 (m, 1 H), 3.72 (s, 2 H), 4.63 (d, 1 H, J = 10 Hz), 7.3 (m, 5H). Anal. Calcd for $C_{19}H_{31}NO_3S$: C, 64.6; H, 8.8; N, 4.0. Found: C, 64.8; H, 8.7; N, 4.0.

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(phenethylthio)-2-hydroxy-5-methylhexane (4i, R = phenethyl): 90% yield; mass spectrum $(M + 1)^+$ = 368; ^1H NMR ($CDCl_3$) δ 0.93 (d, 3 H, J = 7 Hz), 0.94 (d, 3 H, J = 7 Hz), 1.43 (s, 9 H), 1.2–1.7 (m, 3 H), 2.53 (m, 1 H), 2.7–2.85 (m, 6 H), 3.58 (m, 1 H), 3.66 (m, 1 H), 4.67 (br d, 1 H, J = 10 Hz), 7.17–7.35 (m, 5 H). Anal. Calcd for $C_{20}H_{33}NO_3S$: C, 65.4; H, 9.0; N, 3.8. Found; C, 65.2; H, 9.1, N, 3.8.

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-[(3-phenylpropyl)thio]-2-hydroxy-5-methylhexane (4j, R = 3-phenylpropyl): 48% yield; mass spectrum M^+ = 381; ^1H NMR ($CDCl_3$) δ 0.92 (d, 3 H, J = 6 Hz), 0.94 (d, 3 H, J = 6 Hz), 1.45 (s, 9 H), 1.2–1.75 (m, 3 H), 1.92 (m, 2 H), 2.52 (m, 3 H), 2.72 (m, 3 H), 2.86 (br, 1 H), 3.44 (m, 1 H), 3.66 (m, 1 H), 4.68 (br d, 1 H, J = 10 Hz), 7.15–7.35 (m, 5 H).

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(isopropylthio)-2-hydroxy-5-methylhexane (4k, R = isopropyl): 79% yield; mass spectrum $(M + 1)^+$ = 306; ^1H NMR ($CDCl_3$) δ 0.92 (d, 3 H, J = 7 Hz), 0.94 (d, 3 H, J = 7 Hz), 1.27 (d, 3 H, J = 7 Hz), 1.29 (d, 3 H, J = 7 Hz), 1.44 (s, 9 Hz), 1.2–1.8 (m, 3 H), 2.53 (dd, 1 H, J = 10, 14 Hz), 2.78 (dd, 1 H, J = 3, 14 Hz), 2.86 (br, 1 H), 2.93 (m, 1 H), 3.58 (m, 1 H), 3.67 (m, 1 H), 4.68 (br d, 1 H, J = 10 Hz). Anal. Calcd for $C_{15}H_{31}NO_3S$: C, 60.0; H, 10.2; N, 4.6. Found: C, 58.8; H, 10.2; N, 4.5.

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(isobutylthio)-2-hydroxy-5-methylhexane (4l, R = isobutyl): 76% yield; mass spectrum M^+ = 319; ^1H NMR ($CDCl_3$) δ 0.93 (d, 3 H, J = 7 Hz), 0.94 (d, 3 H, J = 7 Hz), 0.98 (d, 6 H, J = 7 Hz), 1.43 (s, 9 H), 1.3–1.7 (m, 3 H), 1.78 (m, 1 H), 2.40 (m, 2 H), 2.52 (dd, 1 H, J = 10, 14 Hz), 2.72 (dd, 1 H, J = 3, 14 Hz), 2.91 (br, 1 H), 3.58 (m, 1 H), 3.63 (m, 1 H), 4.71 (br d, 1 H, J = 10 Hz). Anal. Calcd for $C_{16}H_{33}NO_3S$: C, 60.2; H, 10.4; N, 4.4. Found: C, 60.4; H, 10.3; N, 4.3.

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(isopentylthio)-2-hydroxy-5-methylhexane (4m, R = isopentyl): 87% yield; mass spectrum M^+ = 333; ^1H NMR ($CDCl_3$) δ 0.91 (d, 6 H, J = 7 Hz), 0.94 (d, 3 H, J = 7 Hz), 0.95 (d, 3 H, J = 7 Hz), 1.44 (s, 9 H), 1.3–1.8 (m, 6 H), 2.52 (m, 3 H), 2.74 (dd, 1 H, J = 4, 11 Hz), 2.94 (br, 1 H), 3.55–3.75 (m, 2 H), 4.73 (br d, 1 H, J = 10 Hz). Anal. Calcd for $C_{17}H_{35}NO_3S$: C, 61.2; H, 10.6; N, 4.2. Found: C, 61.6; H, 10.6; N, 4.1.

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(*tert*-butylthio)-2-hydroxy-5-methylhexane (4n, R = *tert*-butyl): 67% yield; mass spectrum M^+ = 319; ^1H NMR ($CDCl_3$) δ 0.93 (d, 3 H, J = 7 Hz), 0.94 (d, 3 H, J = 7 Hz), 1.33 (s, 9 H), 1.44 (s, 9 H), 1.2–1.75 (m, 3 H), 2.58 (dd, 1 H, J = 10, 13 Hz), 2.77 (dd, 1 H, J = 4, 13 Hz), 2.85 (br, 1 H), 3.60 (m, 1 H), 3.68 (m, 1 H), 4.73 (br d, 1 H, J = 10 Hz). Anal. Calcd for $C_{16}H_{33}NO_3S$: C, 60.2; H, 10.4; N, 4.4. Found: C, 60.6; H, 10.6; N, 4.2.

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(cyclopentylthio)-2-hydroxy-5-methylhexane (4o, R = cyclopentyl): 43% yield; mass spectrum $(M + 1)^+$ = 331; ^1H NMR ($CDCl_3$) δ 0.93 (m, 6 H), 1.42 (s, 9 H), 1.2–1.8 (m, 9 H), 1.97 (m, 2 H), 2.52 (m, 1 H), 2.75 (m, 1 H), 2.85, (br, 1 H), 3.07 (m, 1 H), 3.55–3.7 (m, 2 H), 4.67 (d, 1 H, J = 10 Hz).

B. With Amines. (2*S*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-2-hydroxy-5-methyl-1-(phenylamino)hexane (7, R = Phenyl). To a stirred solution of epoxide **3** (200 mg, 0.87 mmol) in methanol (10 mL) was added aniline (79 μL, 0.87 mmol). The solution was refluxed for 20 h and was then evaporated to give a residue, which was chromatographed on SiO_2 (3:2 ether/hexane) to give 140 mg (50%) of the desired product: mass spectrum M^+ = 322; ^1H NMR ($CDCl_3$) δ 0.93 (2 d, 6 H), 1.2–1.85 (m, 3 H), 1.46 (s, 9 H), 2.35 (br, 1 H), 3.2 (m, 2 H), 3.8 (br m, 2 H), 4.6 (br d, 1 H), 6.65–6.75 (m, 3 H), 7.17 (m, 2 H). Anal. Calcd for $C_{18}H_{30}N_2O_3$: C, 67.0; H, 9.4; N, 8.7. Found: C, 67.0; H, 9.5; N, 8.3.

(2*S*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-2-hydroxy-1-(isopropylamino)-5-methylhexane (7, R = Isopropyl). To a stirred solution of epoxide **3** (420 mg, 1.84 mmol) in methanol (18 mL) was added isopropylamine (1.09 g, 18.4 mmol). After 18 h the solution was evaporated and dried under high vacuum to give the title compound as an oil: mass spectrum M^+ = 288; ^1H NMR ($CDCl_3$) δ 0.92 (d, 6 H, J = 6 Hz), 1.09 (d, 3 H, J = 4 Hz), 1.12 (d, 3 H, J = 4 Hz), 1.45 (s, 9 H), 1.1–1.8 (several m, 3 H), 2.58 (dd, 1 H, J = 10, 12 Hz), 2.77 (dd, 1 H, J = 4, 12 Hz), 2.83 (m, 1H), 3.6 (br m, 2 H), 4.76 (br d, 1 H).

C. With Alcohols. (4*S*,5*R*)-4-(2-Methylpropyl)-5-[[(2-methylpropyl)oxy]methyl]oxazolidin-2-one (9a, R = Isobutyl). To a stirred suspension of NaH (209 mg of 60% dispersion in oil, 5.23 mmol, washed with hexane 3×) in dimethylformamide

(4 mL) was added isobutylalcohol (1.0 mL, 10.8 mmol). After H_2 evolution ceased (ca. 20 min), epoxide **3** in dimethylformamide (2 mL) was added. The mixture was stired for 2 h and then poured into saturated aqueous NH_4Cl and extracted with ether. The combined extracted were washed (brine), dried (Na_2SO_4), filtered, and evaporated to give 594 mg (59%) of the desired compound as an oil, which was carried on to the hydrolysis reaction without further purification: mass spectrum $(M + H)^+ = 230$; ^1H NMR ($CDCl_3$) δ 0.90 (d, 6 H, J = 6 Hz), 0.93 (d, 3 H, J = 5.5 Hz), 0.97 (d, 3 H, J = 5.5 Hz), 1.4–1.7 (mm, 3 H), 1.86 (m, 1 H), 3.27 (m, 2 H), 3.58 (dd, 2 H, J = 2.5, 4.5 Hz), 3.77 (m, 1 H), 4.27 (dd, 1 H, J = 5, 11 Hz), 5.23 (br s, 1 H).

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-phenoxy-2-hydroxy-5-methylhexane (6, R = Phenyl). The reaction was run as in part A above except that phenol was used in place of a mercaptan and the solution was refluxed for 24 h: 25% yield; mass spectrum $M^+ = 323$; ^1H NMR ($CDCl_3$) δ 0.95 (d, 3 H, J = 7 Hz), 0.96 (d, 3 H, J = 7 Hz), 1.45 (s, 9 H), 1.3–1.8 (m, 3 H), 2.74 (br, 1 H), 3.78 (m, 1 H), 3.85–4.1 (m, 3 H), 4.77 (br d, 1 H, J = 10 Hz), 6.85–7.0 (m, 3 H), 7.28 (m, 2 H).

D. With Grignard Reagents. (4*S*,5*S*)-4-[(*tert*-Butyloxycarbonyl)amino]-2,9-dimethyl-5-hydroxydecane (8, R = Isobutyl). To a stirred suspension of CuI (2.3 g, 12 mmol) at 0 °C in anhydrous THF (10 mL) was added isopentylmagnesium bromide (30 mL of a 0.84 M solution in THF, 25.2 mmol) dropwise. After 20 min, a solution of epoxide **3** (459 mg, 2.0 mmol) in THF (20 mL) was added. The mixture was stirred at 0–5 °C for 18 h and then filtered through Celite. The filtrate was washed with saturated aqueous NH_4Cl, and the washes were back extracted with ether. The combined organic layers were washed (brine), dried (Na_2SO_4), filtered, and evaporated. The residue was chromatographed on 170 g of silica gel eluting with ethyl acetate/hexane (1:5). Combination of selected fractions provided 420 mg (70%) of the desired product: mass spectrum $M^+ = 301$; ^1H NMR ($CDCl_3$) δ 0.88 (d, 6 H, J = 6 Hz), 0.94 (d, 3 H, J = 7 Hz), 0.95 (d, 3 H, J = 7 Hz), 1.15–1.7 and 1.47 (m and s, 19 H), 3.45–3.65 (2 m, 2 H), 4.58 (br d, 1 H, J = 9 Hz).

(3*S*,4*S*)-4-[(*tert*-Butyloxycarbonyl)amino]-3-hydroxy-6-methyl-1-phenylheptane (8, R = phenyl): 57% yield; mass spectrum $M^+ = 321$; ^1H NMR ($CDCl_3$) δ 0.93 (m, 6 H), 1.2–1.85 (m, 5 H), 1.45 (s, 9 H), 1.95 (br, 1 H), 2.6–2.95 (m, 2 H), 3.5–3.75 (m, 2 H), 4.53, 4.58 (2 br d, 1 H, 1:2 ratio, J = 10 Hz, 10 Hz), 7.15–7.35 (m, 5 H).

Oxidations of Sulfides 4 to Sulfoxides 5a and Sulfones 5b. (2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(cyclohexylsulfonyl)-2-hydroxy-5-methylhexane (5b, R = Cyclohexyl). To a stirred solution of **4a** (152 mg, 0.440 mmol) in dichloromethane (8 mL) was added 3-chloroperoxybenzoic acid (MCPBA, 211 mg of 80% reagent, 225 mol%) portionwise. After being stirred overnight, the mixture was cooled to 0 °C, and cold 10% Na_2SO_3 (3 mL) was added. After 15 min, the mixture was washed with saturated $NaHCO_3$ (2×) and brine (1×). Drying ($MgSO_4$), filtering, and evaporating provided a glassy solid, which was recrystallized from hexane to give 110 mg (67%) of the desired product as small cubes: mp 110–111 °C; ^1H NMR ($CDCl_3$) δ 0.92 (d, 3 H, J = 6 Hz), 0.94 (d, 3 H, J = 6 Hz), 1.15–1.8 (several m, 9 H), 1.46 (s, 9 H), 1.84 (m, 2 H), 2.17 (m, 2 H), 2.93 (m, 1 H), 3.1 (m, 2 H), 3.6 (m, 2 H), 4.32 (m, 1 H), 4.74 (d, 1 H, J = 10 Hz). Anal. Calcd for $C_{18}H_{35}NO_5S$: C, 57.3; H, 9.3; N, 3.7. Found: C, 57.3; H, 9.3; N, 3.4.

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(phenylsulfonyl)-2-hydroxy-5-methylhexane (5b, R = phenyl): 68% yield; mass spectrum $M^+ = 371$; ^1H NMR ($CDCl_3$) δ 0.88 (d, 6 H, J = 7 Hz), 1.41 (s, 9 H), 1.25–1.7 (m, 2 H), 3.28 (m, 3 H), 3.5–3.65 (m, 2 H), 4.10 (m, 1 H), 4.71 (br d, 1 H, J = 10 Hz), 7.62(m, 2 H), 7.68 (m, 1 H), 7.83 (d, 2 H, J = 7 Hz).

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(isopropylsulfonyl)-2-hydroxy-5-methylhexane (5b, R = isopropyl): 46% yield; mass spectrum $(M + H)^+ = 338$; ^1H NMR ($CDCl_3$) δ 0.92 (d, 3 H, J = 7 Hz), 0.93 (d, 3 H, J = 7 Hz), 1.4 (d, 6 H, J = 7 Hz), 1.44 (s, 9 H), 1.3–1.75 (m, 3 H), 3.1–3.25 (m, 3 H), 3.55 (br, 1 H), 3.63 (br m, 1 H), 4.33 (m, 1 H), 4.74 (br d, 1 H, J = 10 Hz).

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(isobutylsulfonyl)-2-hydroxy-5-methylhexane (5b, R = isobutyl): 66% yield; mass spectrum $(M + H)^+ = 352$; ^1H NMR ($CDCl_3$) δ 0.92 (d, 3 H, J = 7 Hz), 0.94 (d, 3 H, J = 7 Hz), 1.13 (d, 6 H, J = 7 Hz), 1.3–1.7 (m, 3 H), 2.38 (m, 1 H), 2.97 (d, 2 H, J = 7 Hz), 3.14 (m, 2 H), 3.53 (d, 1 H, J = 3 Hz), 3.63 (br m, 1 H), 4.27 (m, 1 H), 4.72 (d, 1 H, J = 10 Hz).

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(isopentylsulfonyl)-2-hydroxy-5-methylhexane (5b, R = isopentyl): 51% yield; mass spectrum $(M + H)^+ = 366$; ^1H NMR ($CDCl_3$) δ 0.94 (m, 12 H), 1.45 (s, 9 H), 1.2–1.8 (m, 6 H), 3.0–3.2 (m, 4 H), 3.55 (br, 1 H), 3.63 (m, 1 H), 4.27 (m, 1 H), 4.73 (d, 1 H, J = 10 Hz).

Sulfoxides 5a were prepared in an analogous manner with the exception that only 110 mol % of MCPBA was added.

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(phenylsulfinyl)-2-hydroxy-5-methylhexane (5a, R = phenyl): 78% yield; mass spectrum $M^+ = 355$; ^1H NMR ($CDCl_3$) δ 0.83, 0.94 (2 m, 6 H), 1.37, 1.43 (2 s, 9 H), 1.2–1.8 (m, 3 H), 2.63, 2.73 (2 m, 1 H), 3.3, 3.44 (2 m, 1 H), 3.63, 4.04 (2 m, 1 H), 4.25–4.4 (m, 1 H), 4.7–4.8 (m, 1 H), 7.5–7.7 (m, 5 H).

(2*R*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-1-(cyclohexylsulfinyl)-2-hydroxy-5-methylhexane (5a, R = cyclohexyl): 58% yield; mass spectrum $M^+ = 361$; ^1H NMR ($CDCl_3$) δ 0.93 (d, 6 H, J = 7 Hz), 1.45 (s, 9 H), 1.2–2.2 (m, 10 H), 2.6–3.0 (m, 4 H), 3.6–3.75 (m, 1 H), 4.23–4.35 (m, 1 H), 4.82 (m, 1 H).

Hydrolysis of Oxazolidin-2-ones 9. (2*R*,3*S*)-3-Amino-2-hydroxy-1-(isobutyloxy)-5-methylhexane (10, R = Isobutyl). To a stirred solution of oxazolidin-2-one **9** (R = isobutyl, 150 mg, 0.654 mmol) in dioxane/water (4 mL/4 mL) was added $Ba(OH)_2 \cdot 8H_2O$ (180 mg, 0.571 mmol). The mixture was refluxed for 4 h, cooled, filtered, adjusted to pH 5–6 with 1 M H_3PO_4, and washed with ether. After the pH was raised to 8 with 1 M NaOH, the mixture was extracted with $CHCl_3$ (2×) and isopropyl alcohol/$CHCl_3$, 1:3 (2×). The combined $CHCl_3$ extracts were dried (Na_2SO_4), filtered, and evaporated to give 66 mg (50%) of the desired product as an oil, which was coupled to the appropriate peptide without further purification: mass spectrum $(M + H)^+ = 204$; ^1H NMR ($CDCl_3$) δ 0.87–0.95 (several close d, 12 H), 1.27 (m, 2 H), 1.72 (m, 1 H), 1.88 (m, 1 H), 2.79 (m, 1 H), 3.22 (m, 2 H), 3.4–3.55 (m, 3 H).

Addition of Grignard Reagents to Amino Aldehydes 1. (4*S*,5*RS*)-4-[(*tert*-Butyloxycarbonyl)amino]-2,8-dimethyl-5-hydroxynonane (8 and 11, R = Isopropyl). To a −78 °C stirred solution of L-(*tert*-butyloxycarbonyl)leucinal (14.04 g, 65.21 mmol, crude material prepared by DIBAH reduction of Boc-Leu-OCH_3[16,20]) in anhydrous THF (250 mL) was added isopentylmagnesium bromide (163 mL of a 0.8 M solution in THF, 130 mmol). After 2 h, the cold bath was removed, and the reaction was quenched by slow addition of 1 M H_3PO_4 (50 mL). The mixture was diluted with water (300 mL) and then extracted with ether (3 × 75 mL). The combined extracts were washed (brine), dried (Na_2SO_4), filtered, and evaporated to provide an oil, which was chromatographed on silica gel (ethyl acetate/hexane, 15:85). Combination of selected fractions provided 8.7 g (46%) of the desired product as a mixture of diastereomers. Separation of this mixture was easier to carry out after converting the mixture to **12** and **13** as described below: mass spectrum $(M + H)^+ = 288$; ^1H NMR ($CDCl_3$) δ 0.8–1.0 (m, 12 H), 1.1–1.8 (several br m, 8 H), 1.44 (s, 9 H), 3.45–3.75 (br m, 2 H), 4.45–4.65 (br m, 2 H).

Oxazolidin-2-one Synthesis and Hydrolysis. (4*S*,5*R*)-4-(2-Methylpropyl)-5-(3-methylbutyl)oxazolidin-2-one (12, R = Isopropyl) and (4*S*,5*S*)-4-(2-Methylpropyl)-5-(3-methylbutyl)oxazolidin-2-one (13, R = Isopropyl). To a stirred 0 °C suspension of NaH (390 mg of a 60% dispersion in oil, 9.74 mmol, washed 3× with hexane prior to use) in dimethylformamide (20 mL) was added a 0 °C solution of **8**/**11** (1.40 g, 4.87 mmol) in dimethylformamide (5 mL). After 2 h, the reaction mixture was quenched (1 M HCl) and evaporated. The residue was dissolved in 0.5 M HCl (10 mL) and extracted with ether (3 × 20 mL). The combined organic phase was washed (brine), dried (Na_2SO_4), filtered, and evaporated to provide an oil, which was chromatographed on silica gel (ethyl acetate/hexane, 1:3) to give **13** (390 mg, 38%, faster eluting) and **12** (206 mg, 29%).

13: mass spectrum $(M + H)^+ = 214$; ^1H NMR ($CDCl_3$) δ

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0.85–1.0 (4 d, 3 H each), 1.1–1.8 (several m, 8 H), 3.51 (ddd, 1 H), 4.11 (ddd, 1 H, J = 4.8, 6.0, 7.6), 6.23 (br s, 1 H). Irradiation of the 3.51-ppm ddd resulted in the collapse of the 4.11-ppm resonance to a dd, J = 4.8, 7.6 Hz, indicating a ring H coupling of 6.0 Hz.

12: mass spectrum $(M + H)^+ = 214$; ^1H NMR ($CDCl_3$) δ 0.91 (d, 9 H, J = 7 Hz), 0.98 (d, 3 H, J = 6.5 Hz), 1.15–1.3 (m, 2 H), 1.4–1.8 (m, 6 H), 3.83 (ddd, 1 H, J = 3.1, 7.5, 10.7 Hz), 4.56 (ddd, 1 H), 6.12 (br s, 1 H). Irradiation of the 4.56-ppm ddd resulted in the collapse of the 3.83-ppm resonance to a dd, J = 3.1, 10.7 Hz, indicating a ring H coupling of 7.5 Hz.

(4*S*,5*S*)-4-Amino-2,8-dimethyl-5-hydroxynonane (14a, R = Isopropyl) and (4*S*,5*R*)-4-Amino-2,8-dimethyl-5-hydroxynonane (14b, R = Isopropyl). Hydrolyses of oxazolidin-2-ones **12** and **13** were carried out as above for **9**.

Deprotection of Boc-Amines 4–8. 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.

Coupling Methods. A. Carbodiimide Coupling. (2*R*,3*S*)-3-[(Boc-L-Phenylalanyl-L-histidyl)amino]-2-hydroxy-5-methyl-1-(phenylthio)hexane (15). Compound **4** (R = phenyl, 129 mg, 0.38 mmol) was deprotected as described above to give the corresponding amine hydrochloride, which was dissolved in dimethylformamide (5 mL) along with Boc-Phe-His-OH (153 mg, 0.38 mmol), *N*-methylmorpholine (39 mg, 0.38 mmol), and 1-hydroxybenzotriazole hydrate (HOBT, 77 mg, 0.57 mmol). The mixture was cooled to −23 °C, and 1,3-dicyclohexylcarbodiimide (DCC, 78 mg, 0.38 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 131 mg (55%) of the desired material: ^1H NMR ($CHCl_3$) δ 0.7–0.9 (m, 6 H), 1.1–1.6 (br m, 3 H), 1.38 (s, 9 H), 2.5–3.3 (several br m, 6 H), 3.58 (m, 1 H), 4.0 (m, 1 H), 4.27 (m, 1 H), 4.63 (m, 1 H), 5.05 (br m, 1 H), 6.65 (br d, 1 H), 6.78 (br s, 1 H), 7.13–7.45 (m, 10 H), 7.48 (d, 1 H, J = 1Hz); mass spectrum (FAB) $(M + H)^+ = 624$. Anal. Calcd for $C_{33}H_{44}N_5O_5S$: C, 63.6; H, 7.1; N, 11.2. Found: C, 63.3; H, 7.4; N, 11.3.

B. Mixed Anhydride Coupling. (2*R*,3*S*)-3-[(Boc-L-Phenylalanyl-L-alanyl)amino]-2-hydroxy-5-methyl-1-(isopropylthio)hexane (27). To a stirred −12 °C solution of Boc-Phe-Ala-OH (84.1 mg, 0.25 mmol) in anhydrous tetrahydrofuran (3 mL) were added *N*-methylmorpholine (28 μL, 0.25 mmol) and isobutyl chloroformate (32 μL, 0.25 mmol) sequentially. After 3 min, a −12 °C solution of 4-amino-3-hydroxy-6-methyl-1-phenylheptane hydrochloride (prepared by deprotecting 0.25 mmol of **4**, R = isopropyl, 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 51 mg (39%) of the desired compound: mass spectrum $(M + H)^+ = 524$; ^1H NMR ($CDCl_3$) δ 0.92 (d, 6 H, J = 6 Hz), 1.26 (d, 3 H, J = 6 Hz), 1.28 (d, 3 H, J = 6 Hz), 1.33 (d, 3 H, J = 7 Hz), 1.41 (s, 9 H), 2.46 (dd, 1 H, J = 10, 15 Hz), 2.73 (dd, 1 H, J = 4, 15 Hz), 2.87–3.13 (br m, 4 H), 3.62 (m, 1 H), 4.02 (m, 1 H), 4.25–4.43 (m, 2 H), 4.94 (br, 1 H), 6.26 (br, 1 H), 6.41 (br, 1 H), 7.17–7.37 (m, 5 H). Anal. Calcd for $C_{27}H_{45}N_3O_5S$: C, 61.9; H, 8.7; N, 8.0. Found: C, 61.6; H, 8.4; N, 8.0.

C. Carbodiimide Coupling Followed by Active Ester Coupling. (4*S*,5*S*)-4-[[Boc-L-(1-Naphthylalanyl)-L-histidyl]-amino]-2,8-dimethyl-5-hydroxynonane (58). Coupling procedure A was used to couple Boc-His-OH to **14a** (R = isopropyl) in 61% yield after chromatography on silica gel (dichloromethane/methanol, 98:2–95:5). Deprotection of this material (54.0 mg, 0.127 mmol) as above gave the amine dihydrochloride, which was used in the below active ester coupling without further purification. Boc-L-(1-naphthyl)alanine and *N*-hydroxysuccinimide were coupled according to procedure A to give the corresponding succinimido ester (62%) after recrystallization from dichloromethane/hexane (mp 168–170). This material was used directly in the following coupling.

To a stirred solution of the above dihydrochloride salt (0.127 mmol) in chloroform (5 mL) containing triethylamine (25.7 mg, 0.254 mmol) was added the succinimido ester (52.4 mg, 0.127 mmol). After 8 h, the mixture was diluted with chloroform (15 mL) and washed sequentially with water, saturated aqueous $NaHCO_3$, and brine. The organic phase was dried (Na_2SO_4), filtered, and evaporated to give a residue, which was chromatographed (dichloromethane/methanol, 98:2). Combination of selected fractions provided 47 mg (59%) of the desired product: ^1H NMR ($CDCl_3$) δ 0.77 (d, 3 H, J = 6 Hz), 0.83 (d, 3 H, J = 6 Hz), 1.05–1.6 (br m and s at 1.36, 27 H), 2.83 (dd, 1 H, J = 5, 15 Hz), 3.15–3.45 (m, 4 H), 3.75–3.95 (m, 3 H), 4.38 (m, 1 H), 4.58 (m, 1 H), 5.07 (m, 1 H), 6.53 (d, 1 H, J = 10 Hz), 6.82 (s, 1 H), 7.3–7.65 (m, 6 H), 7.81 (d, 1 H, J = 8 Hz), 7.88 (d, 1 H, J = 8 Hz), 8.19 (d, 1 H, J = 8 Hz). Anal. Calcd for $C_{35}H_{51}N_5O_5 \cdot H_2O$: C, 65.7; H, 8.4; N, 10.9. Found: C, 65.7; H, 8.1; N, 10.6.

Biological Methods. Purified human renal renin[21] was assayed by utilizing pure human angiotensinogen[22] at pH 6.0 in maleate buffer (or at pH 7.4 in HEPES buffer). Test compounds were dissolved in dimethyl sulfoxide (Me_2SO) and diluted so that prior to addition to the assay system the solutions were 10% in Me_2SO 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; phenylmethanesulfonyl fluoride, 1.4 mM; angiotensinogen, 0.21 μM; renin, 0.24 mGU;[23] BSA, 0.44%; Me_2SO, 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, 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 curve, and at the highest concentrations tested, none of the compounds cross reacted with the antibody to angiotensin I. The presence of 1% Me_2SO in the final incubation mixture caused no statistically significant effect on the renin activity.

Porcine pepsin (Sigma) activities were assessed by the hydrolysis of hemoglobin at pH 1.9 at 37 °C and measurement of the absorbance at 280 nm of the supernatant after precipitation with trichloroacetic acid.[24]

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