Renin Inhibitors. Dipeptide Analogues of Angiotensinogen Utilizing a Dihydroxyethylene Transition-State Mimic at the Scissile Bond To Impart Greater Inhibitory Potency

Jay R. Luly,* Nwe BaMaung, Jeff Soderquist, Anthony K. L. Fung, Herman Stein, Hollis D. Kleinert, Patrick A. Marcotte, David A. Egan, Barbara Bopp, Ilmar Merits, Giorgio Bolis, Jonathan Greer, Thomas J. Perun, and Jacob J. Plattner

Abbott Laboratories, Pharmaceutical Products Division, Abbott Park, Illinois 60064. *Received April 29, 1988*

The synthesis of diol-containing renin inhibitors has revealed that a simple vicinal diol functionality corresponding to the scissile Leu-Val bond in human angiotensinogen is capable of imparting inhibitory activity at a comparable or higher level than either the corresponding aldehyde or hydroxymethyl functionality (compare inhibitors **2a-c** or **3a-c**). This finding has led to the further optimization of a series of small transition-state analogue inhibitors by the inclusion of a second hydroxyl group in the Leu-Val surrogate to give compounds that inhibited human renin in the 200-700-pM range (e.g. **43**, **45**, **63**, **66**). The magnitude of effect of the second hydroxyl group on potency is not only dictated by the absolute stereochemistry of the diol but also by the side chain of the P_1 residue. Molecular modeling of the diol-containing inhibitors suggests that one of the hydroxyl groups hydrogen bonds to Asp 32 and Asp 215, while the second hydrogen bonds to Asp 215. These diol inhibitors are extremely selective for human renin over the related enzymes cathepsin D, pepsin, and gastricsin. At high concentrations, compounds containing a leucine or phenylalanine rather than a histidine at the P_2 position gave only minor amounts of inhibition of the other enzymes. Inhibitor **43** suppressed plasma renin activity completely and lowered mean blood pressure in monkeys after both intravenous and intraduodenal administration, but the blood pressure drop lasted less than 1 h. Monitoring the blood levels of **43** by enzyme inhibition assay after intraduodenal administration to monkeys or oral administration to rats revealed low absorption and rapid clearance. While intratracheal administration to dogs gave approximately 50% bioavailability, rapid clearance was still a problem. After examination of inhibitor **45** in a sensitive primate model in which monkeys were rendered both hypertensive and hyperreninemic, the effects on lowering systolic but not diastolic pressure were apparent even after 22 h postdosing. Details on the synthesis, in vitro structure-activity relationships, molecular modeling, in vivo activity, and metabolism of these inhibitors are described.

The renin angiotensin system (RAS, Figure 1) continues to be explored intently in the search for new agents that will improve cardiovascular therapy.[1] While it is known that agents that inhibit angiotensin converting enzyme (ACE) have proven to be effective in the treatment of hypertension and congestive heart failure, a number of research groups are examining another enzyme in the RAS, renin. Recently, we described a novel class of low molecular weight renin inhibitors that had increased potency due to the introduction of a dihydroxyethylene replacement for the scissile Leu-Val amide bond.[2] In this report, we detail the synthesis and structure proof of this class of compounds, and we delineate the structure-activity relationships at each residue. In addition, drug metabolism and in vivo activity data are provided on selected compounds.

The appearance of compounds **1a-d** in the literature[3] led to several interesting observations. Compound **1a** illustrates that the introduction of a single hydroxyl group in the form of leucinol at the P_1 site and attachment to a protected dipeptide results in a micromolar renin inhibitor. The next observation is that the corresponding aldehyde, **1b**, is nearly 50 times more potent, presumably due to hydration of the aldehyde by a molecule of water in the active site, thus producing a tetrahedral species capable of mimicking the transition state for the P_1-P_1' amide bond hydrolysis.[4] In this series of inhibitors the spatial requirements are such that the two hydroxyl groups require a geminal relationship as in the hydrate of **1b**, rather than a 1,2 or 1,3 relationship found in compounds **1c** and **1d**, which, even though both contain the preferred statine-like stereochemistry,[5,6] are no more potent than the monohydroxy inhibitor **1a**.

Concurrent with this work, we explored series **2** and **3**, revealing some interesting biological differences from series **1**. Series **2** differs from series **1** in the protected dipeptide portion of the molecule. Phenylalanine, the residue that

(1) For recent reviews and discussions, see: (a) Vallotton, M. B. *TIPS* **1987**, *8*, 69. (b) Menard, J.; Corvol, P. *Clin. Invest. Med.* **1986**, *9*, 309. (c) Haber, E. *Hypertension* **1986**, *8*, 1093. (d) Greenlee, W. J. *Pharm. Res.* **1987**, *4*, 364. (e) Boger, J. In *Annual Reports in Medicinal Chemistry*; Bailey, D. M., Egan, R. W., Eds.; Academic: New York, 1985; Vol. 20, p 257. (f) Reid, I. A. *Arch. Intern. Med.* **1985**, *145*, 1475.

(2) (a) Luly, J. R.; Plattner, J. J. European Patent Application 0229667, 1986. (b) Luly, J. R.; Plattner, J. J.; Rosenberg, S. H.; Fung, A. European Patent Application 0189203, 1986. (c) Luly, J. R.; Soderquist, J. L.; Yi, N.; Perun, T. J.; Kleinert, H. D.; Stein, H.; Plattner, J. J. *Abstracts of Papers*, 193rd National Meeting of the American Chemical Society, Division of Medicinal Chemistry, Denver, April 5-10, 1987; American Chemical Society: Washington, DC, 1987; MEDI 81. (d) Luly, J. R.; Fung, A. K. L.; Plattner, J. J.; Marcotte, P.; BaMaung, N.; Soderquist, J. L.; Stein, H. In *Peptides: Chemistry and Biology*; Marshall, G. R., Ed.; ESCOM Science Publishers B. V.: Leiden, The Netherlands, 1988; p 487. (e) Kleinert, H. D.; Martin, D.; Chekal, M. A.; Kadam, J.; Luly, J. R.; Plattner, J. J.; Perun, T. J., Luther, R. R. *Hypertension* **1988**, *11*, 613. (f) Kleinert, H. D.; Luly, J. R.; Marcotte, P. A.; Perun, T. J.; Plattner, J. J.; Stein, H. *FEBS Lett.* **1988**, *230*, 38.

(3) (a) Kokubu, T.; Hiwada, K.; Sato, Y.; Iwata, T.; Imamura, Y.; Matsueda, R.; Yabe, Y.; Kogen, H.; Yamazaki, M.; Iijima, Y.; Baba, Y. *Biochem. Biophys. Res. Commun.* **1984**, *118*, 929. (b) Matsueda, R.; Yabe, Y.; Kogen, H.; Higashida, S.; Koike, H.; Iijima, Y.; Kokubu, T.; Hiwada, K.; Murakami, E.; Imamura, Y. *Chem. Lett.* **1985**, 1041. (c) Kokubu, T.; Hiwada, K.; Murakami, E.; Imamura, Y.; Matsueda, R.; Yabe, Y.; Koike, H.; Iijima, Y. *Hypertension* **1985**, *7*, I-8.

(4) Akahane, K.; Umeyama, H.; Setsuko, N.; Moriguchi, I.; Hirose, S.; Iizuka, K.; Murakami, K. *Hypertension* **1985**, *7*, 3.

(5) "Statine-like" refers 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.[6] For example, the stereochemistry of C2 of inhibitor **2c** is statine-like, but of the *R* configuration.

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

0022-2623/88/1831-2264$01.50/0 © 1988 American Chemical Society

....P4 P3 P2 P1 P1' P2' P3'....
1 2 3 4 5 6 7 8 9 10 11 12 13.....
H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-.....-Protein
Renin
H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-OH *AI*
ACE
H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH *AII*
Aminopeptidase
H-Arg-Val-Tyr-Ile-His-Pro-Phe-OH *AIII*

Figure 1. Renin–angiotensin system.

Table I.[a] In Vitro Activity for Inhibitors

Z-(1)Nal-His-NH–R (1); Boc-Phe-Ala-NH–R (2); Boc-Phe-Ala-NH–R (3, cyclohexylmethyl)

	R	1	2	3
a		61% inhibn @10^{-6} IC_{50} = 3.6 × 10^{-6}	9% inhibn @10^{-5}	73% inhibn @10^{-5} IC_{50} = 4 × 10^{-6}
b		88% inhibn @10^{-6} IC_{50} = 8.0 × 10^{-8}	49% inhibn @10^{-5}	93% inhibn @10^{-5} IC_{50} = 4.5 × 10^{-7}
c		52% inhibn @10^{-6}	65% inhibn @10^{-5} [b] IC_{50} = 4 × 10^{-6}	98% inhibn @10^{-5} [c] IC_{50} = 3.5 × 10^{-7}
d		63% inhibn. @10^{-6} IC_{50} = 6.4 × 10^{-6}	13% inhibn @10^{-5}	
e			0% inhibn @10^{-5}	
f			85% inhibn @10^{-5} IC_{50} = 2 × 10^{-6}	100% inhibn @10^{-5}, 94% inhibn @10^{-6}, IC_{50} = 2 × 10^{-7}

[a] Compound concentrations are given expressed in molarity. Data for compounds **1a–d** were reported in ref 3, and testing was performed at pH 7.4 with sheep angiotensinogen rather than at pH 6.0 with human angiotensinogen. Compounds **2b**, **3b** (ref 8b), and **2f** (ref 8a) were reported by us previously. [b] Compound **2c** as a 5:7 2*R*:2*S* mixture of isomers gave 46% inhibition at 10^{-5} M. [c] Compound **3c** is a 1:1 2*R*:2*S* mixture of isomers.

is present at position P_3 in human renin substrate, angiotensinogen, was used rather than 1-naphthylalanine, an amino acid that, while reducing water solubility, has in some cases led to enhancement in binding over phenylalanine.[3,7,8a] Histidine is the amino acid at position P_2 in

(7) (a) Sham, H. L.; Stein, H.; Rempel, C. A.; Cohen, J.; Plattner, J. J. *FEBS Lett.* **1987**, *220*, 299.

(8) (a) Luly, J. R.; Yi, Y.; Soderquist, J.; Stein, H.; Cohen, J.; Perun, T. J.; Plattner, J. J. *J. Med. Chem.* **1987**, *30*, 1609. (b) Luly, J. R.; Bolis, G.; BaMaung, N.; Soderquist, J.; Dellaria, J. F.; Stein, H.; Cohen, J.; Perun, T. J.; Greer, J.; Plattner, J. J. *J. Med. Chem.* **1988**, *31*, 532. (c) Dellaria, J. F.; Maki, R. G.; Bopp, B. A.; Cohen, J.; Kleinert, H. D.; Luly, J. R.; Merits, I.; Plattner, J. J.; Stein, H. H. *J. Med. Chem.* **1987**, *30*, 2137. (d) Kempf, D. J.; de Lara, E.; Stein, H. H.; Cohen, J.; Plattner, J. J. *J. Med. Chem.* **1987**, *30*, 1978.

Scheme I

Boc-Phe-Ala-NH– 11 → 9-BBN → 2d
1. mixed anhydride 2. H_2N– 10
Boc-Phe-Ala-OH 4 → 1. mixed anhydride 2. HCl H_2N– 5 → 2a,3a
1. mixed anhydride 2. HCl H_2N– 6
Boc-Phe-Ala-NH– 7 → OsO_4 / $NaIO_4$ → 2b, 3b
OsO_4 NMMNO → 2c, 3c
9-BBN → 2e
H_2 / Pd
Boc-Phe-Ala-NH– O-Bn 9 ← 1. NaOBn / DMF 2. $Ba(OH)_2$ 3. mixed anhydride coupling ← Boc-NH– 8

human substrate, but alanine was used for synthetic ease (see Chemistry section) since we have previously shown in other series of inhibitors[8] that later replacement of alanine with histidine routinely gives us an enhancement in activity. Not surprisingly, the simplest inhibitor in the series, **2a**, was effectively devoid of inhibitory activity. Preparation of the corresponding aldehyde and 1,2-diol **2b** and **2c**, respectively, resulted in increased inhibitory activity. That **2c** was considerably more potent than **2a** was in contrast to the results found in series 1 and offered us the first insight that a vicinal glycol at this position might be capable of enhancing the binding potency of members of properly chosen classes of inhibitors. We explored this finding further by synthesizing analogues **2d** and **2e**; both homologation to the 1,3-diol and removal of the secondary alcohol had an adverse effect on potency. To increase the potency of these inhibitors, we modified the P_1 side chain from isobutyl to cyclohexylmethyl.[8b–d,9] We were gratified to see that both aldehyde- and 1,2-diol-containing inhibitors **3b** and **3c**, respectively, were more potent than alcohol **3a**, preserving the relative trend observed in **2a–c**.

With this information in hand, we entertained the notion that this 1,2-diol moiety might be incorporated into a wealth of available monohydroxy-containing inhibitors to augment their binding potency while increasing their molecular weight negligibly. One such monohydroxy series that we have studied extensively[8a–c] is represented by compounds **2f** and **3f**. In this series of inhibitors, an iso-

(9) (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.

pentyl group, which formally mimics the P_1' valine side chain in human substrate, is appended to the leucinol/cyclohexylalaninol fragment. As shown in Table I, this simple aliphatic appendage increases the potency dramatically over compounds **2a** and **3a**. For this reason we set out to explore all four possible configurations of the 1(*S*)-amino-2,3-dihydroxy analogues of compounds like **2f** and **3f**. The synthesis, structure determination, and biological profiles of diol derivatives shown in Tables II and III are described herein.

Chemistry

The compounds shown in Table I were prepared as shown in Scheme I. Inhibitors **2a** and **3a** were synthesized by coupling L-leucinol or cyclohexylalaninol to Boc-Phe-Ala-OH by using the mixed anhydride procedure. The coupling of olefins **6**[10] (R = isobutyl or cyclohexylmethyl) to Boc-Phe-Ala-OH provided the versatile intermediate **7**. Oxidative cleavage of the olefin proceeded smoothly to aldehydes **2b** and **3b** with $NaIO_4$/cat. OsO_4. Hydroboration of **7** using 9-borabicyclo[3.3.1]nonane (9-BBN) proceeded to give exclusively primary alcohol **2e**. Oxidation of **7** using catalytic OsO_4 and *N*-methylmorpholine *N*-oxide (NMMNO) gave **2c** and **3c**, each as a near 1:1 mixture of diastereomers at the center bearing the chiral hydroxyl group. To prove which diastereomer was the more active, a stereospecific synthesis of **2c** starting from epoxide **8**[10] was performed. Compound **8** was treated with the sodium salt of benzyl alcohol to perform epoxide opening followed by attack of the formed oxide on to the Boc group to give the corresponding oxazolidin-2-one in analogy to previous work.[8a] Cleavage of the oxazolidin-2-one using barium hydroxide provided the corresponding amino alcohol, which was then coupled to Boc-Phe-Ala-OH by using the mixed anhydride procedure.

The preparation of **2d** proceeded in two steps by first coupling allylic alcohol **10** to dipeptide **4** followed by oxidation of olefin **11** to the primary olefin with 9-BBN.

Both methods used in the preparation of **2c** were attempted in the preparation of inhibitor **30**; however the presence of the unprotected histidine hampered both the olefin osmylation in the first route and the benzyl ether removal (H_2/cat. or Na/NH_3) in the second route. After difficulties in purification or deprotection were encountered with various imidazole protecting groups, inhibitor **30** was finally prepared (Scheme III) from olefin **27**[10] by osmylation to a 3:2 mixture of diastereomeric amino diols **28** followed by *N*-Boc deprotection, O-protection using *tert*-butyldimethylsilyl chloride/imidazole, carbodiimide–HOBT coupling to Boc-Phe-His-OH, and O-deprotection using tetrabutylammonium fluoride. The O-protection/deprotection sequence aided in purification and allowed the isolation of pure **30**, which was the isomer produced in crude form from the above-mentioned O-debenzylation reactions, thus confirming the 2*R* stereochemistry.

Olefins **13–17** were prepared by an extension of our method of preparing chiral allylic amines from amino acids without racemization as shown in Scheme II.[10] Thus, Boc-L-cyclohexylalanine methyl ester **12** was treated sequentially with diisobutylaluminum hydride (DIBAH) followed by the appropriate phosphonium ylide in a one flask operation. That the conversion of **12** to olefins **13–17** proceeds without racemization was proven by conversion to MTPA-amides,[11] a method that we found to be useful in a series of related olefins.[10] Analysis of these amides on HPLC or capillary GC showed >95% enantiomeric excess. ^1H NMR analysis of olefin **13** revealed a predominance of the cis isomer as deduced from the characteristic olefin hydrogen coupling constant of 10.9 Hz (trans isomer, 15.4 Hz). From the cis isomer only two diols can be formed by the subsequent osmylation reaction, **18a** and **18d**, which are necessarily anti at C3–C4. Facial selectivity favored the formation of **18d** over **18a**, but only slightly (1.5:1). Similarly, *trans*-**13** can only give rise to syn isomers **18b** and **18c**. That the stereochemistry of the most active diol, which is derived from the cis olefin, is as shown in **18d**, and not **18a**, could be demonstrated by conversion of each

Scheme II

12 → 1. DIBAH; 2. Ph_3P^+–CH^-–R →
13, R= isobutyl
14, R= methyl
15, R= ethyl
16, R= isopropyl
17, R= isopentyl

OsO_4, NMMNO → 18

18, R= isobutyl
a, (3S,4R)
b, (3S,4S)
c, (3R,4R)
d, (3R,4S)

19a-d, R= methyl
20a-d, R= ethyl
21a-d, R= isopropyl
22a-d, R= isopentyl

18 → 1. Boc-AA_2-OH coupling; 2. deprotection; 3. X-AA_1-OH **25** coupling; or X-AA_1-AA_2-OH **26** coupling → X-AA_1-AA_2-NH… tables 2 &3

18 → NaH, DMF → 23

23, R= isobutyl
a, (5S,1'R)
b, (5S,1'S)
c, (5R,1'R)
d, (5R,1'S)

Scheme III

27 → OsO_4, NMMNO → 28 → 1. HCl; 2. TBDMS-Cl; 3. Boc-Phe-His-OH coupling → Boc-Phe-His-NH…O-X

29, 2RS mixture, X= TBDMS
30, 2R, X= H (F^-)

(10) Luly, J. R.; Dellaria, J. F.; Plattner, J. J.; Soderquist, J. L.; Yi, N. *J. Org. Chem.* **1987**, *52*, 1487.
(11) Dale, J. S.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.

Table II.[a] In Vitro Activity and Characterization Data for Inhibitors

X-AA$_1$-AA$_2$-NH, OH, R$_1$, R$_2$

no.	X	AA$_1$	AA$_2$	R$_1$	R$_2$	IC$_{50}$, nM	% yield[b]	mp,[c] °C	formula[d]	synth[e]	purifn[f] (solvent)[g]
31	Boc	Phe	His	isobutyl	H	1500	*h*	*h*	*h*		
32	Boc	Phe	His	cyclohexylmethyl	H	10	63	108–109 (100)	$C_{34}H_{53}N_5O_5 \cdot {}^1/_2H_2O$	A	A (A)
33	Boc	Phe	His	isobutyl	OH	11	25	186–188	$C_{31}H_{49}N_5O_6 \cdot {}^1/_4H_2O$	A	A (B)
34	Boc	Phe	His	cyclohexylmethyl	OH	1.5	43	119–123 (115)[i]	$C_{34}H_{53}N_5O_6 \cdot H_2O$	A	A (D)
43	Etoc	Phe	His	cyclohexylmethyl	OH	0.5–0.7[j]	*k*	*k*	*k*		
45	Etoc	Phe	Leu	cyclohexylmethyl	OH	0.3[j]	*k*	*k*	*k*		
47	Etoc	Leu	His	cyclohexylmethyl	OH	2.0	*k*	*k*	*k*		
48	Boc	Phe	Ala	cyclohexylmethyl	OH	5	86	150–155 (130)	$C_{31}H_{51}N_3O_6$	A	
49	Boc	Phe	Phe	cyclohexylmethyl	OH	1.5	84	178–181	$C_{37}H_{55}N_3O_6$	A	B
50	Boc	Phe	Leu	cyclohexylmethyl	OH	0.5	90	184–185	$C_{34}H_{57}N_3O_6$	A	C
51	Boc	Phe	(*S*-Me)-Cys	cyclohexylmethyl	OH	0.5	96	189–190	$C_{32}H_{53}N_3O_6S \cdot {}^1/_4H_2O$	A	A (C)
52	Boc	Phe	(pyrazole)-Ala[l]	cyclohexylmethyl	OH	1.0	30	130–135 (95–110)	$C_{34}H_{53}H_5O_6 \cdot {}^1/_2H_2O$	A	A (D)
53	Boc	Leu	His	cyclohexylmethyl	OH	1.0	35	163–164 (130)	$C_{31}H_{55}N_5O_6 \cdot {}^1/_2H_2O$	B	D
54	Boc	Leu	Leu	cyclohexylmethyl	OH	2.5	62	158–160	$C_{31}H_{59}N_3O_6$	C	A (B)
55	Boc	(1)Nal	His	cyclohexylmethyl	OH	1.5	62	155–160 (130)	$C_{38}H_{55}N_5O_6$	B	A (G)
56	Boc	4-(OCH$_3$)-Phe	His	cyclohexylmethyl	OH	1.0	59	183–185	$C_{35}H_{55}N_5O_7$	B	E
57	Boc	Ala	His	cyclohexylmethyl	OH	200	81	138–140	$C_{28}H_{49}N_5O_6 \cdot {}^3/_2H_2O$	B	A (E)
58	Boc	His	His	cyclohexylmethyl	OH	55	50	157–160 (145)	$C_{31}H_{51}N_7O_6 \cdot {}^1/_4H_2O$	B	A (F)
59	*i*-Boc	Phe	His	cyclohexylmethyl	OH	1.0	49	154–158 (115)	$C_{34}H_{53}N_5O_6$	B	A (D)
60	Tba	Phe	His	cyclohexylmethyl	OH	1.0	47	198–200 (125)	$C_{35}H_{55}N_5O_5 \cdot {}^3/_4H_2O$	B	A (H)
61	Tbac	Phe	His	cyclohexylmethyl	OH	2	14	168–171	$C_{34}H_{54}N_6O_5$	B	I
62	Ac	Phe	His	cyclohexylmethyl	OH	0.75	39	185–187	$C_{31}H_{47}N_5O_5 \cdot {}^1/_2H_2O$	B	A (A)
63	iso-butryl	Phe	His	cyclohexylmethyl	OH	0.3	62	200–202	$C_{33}H_{51}N_5O_5 \cdot {}^1/_2H_2O$		
64	Chxc	Phe	His	cyclohexylmethyl	OH	0.7	61	216–218	$C_{36}H_{55}N_5O_5 \cdot {}^1/_2H_2O$	B	A (D)
65	Etoc	(1)Nal	Leu	cyclohexylmethyl	OH	0.25	77	188–189	$C_{36}H_{55}N_3O_6$	D	D
66	Etoc	4-(OCH$_3$)-Phe	Leu	cyclohexylmethyl	OH	0.2[j]	86	176–177	$C_{33}H_{55}N_3O_7$	D	G
67	Etoc	4-(OCH$_3$)-Phe	His	cyclohexylmethyl	OH	1.5[j]	22	195–198 (172)	$C_{33}H_{51}N_5O_7$	B	A (D)
68	Etoc	Phe	Phe	cyclohexylmethyl	OH	1.0	82	204–205	$C_{35}H_{51}N_3O_6$	D	C
69	Etoc	Leu	Leu	cyclohexylmethyl	OH	2.0	54	164–165	$C_{29}H_{55}N_3O_6$	A	C
70	Etoc	4-I-Phe	Leu	cyclohexylmethyl	OH	2.5	73	200–201	$C_{32}H_{52}N_3O_6I$	D	G

[a] Abbreviations: *t*-Boc is *tert*-butyloxycarbonyl, *i*-Boc is isobutyloxycarbonyl, Etoc is ethoxycarbonyl, Tba is *tert*-butylacetyl, Tbac is (*tert*-butylamino)carbonyl, Chxc is cyclohexylcarbonyl, (1)Nal is L-3-(1′-naphthyl)alanine, (pyrazole)-Ala is *dl*-(3-pyrazolyl)alanine. All others are standard amino acid abbreviations. [b] Yield for last coupling step. [c] Provided for nonamorphous solids. Parenthetical values are temperatures where transitions occurred. [d] Analyses for C, H, and N (within ±0.4% of the calculated values) or high-resolution mass spectra (±5 ppm) were obtained. [e] A, carbodiimide coupling to **26**. B, carbodiimide coupling to Boc-AA$_2$-OH, deprotection, and coupling to **25**. C, mixed anhydride coupling to Boc-AA$_2$-OH, deprotection, and mixed anhydride coupling to **25**. D, mixed anhydride coupling to **26**. [f] Flash chromatography (A) or recrystallization from chloroform (B), ether (C), ether/hexane (D), chloroform/ether (E), dichloromethane (F), dichloromethane/methanol (G), dichloromethane/ether/hexane (H), chloroform/ether/hexane (I), or ethyl acetate (J). [g] The following dichloromethane/methanol mixtures were used: A (95:5 to 90:10), B (98:2 to 95:5), C (98:2), D (95:5), E (90:10), F (88:12 to 85:15). The following chloroform/methanol mixtures were used: G (97.5:2.5 to 95:5), H (95:5 to 92.5:7.5), I (95:5 to 90:10), J (97.5:2.5 to 92.5:7.5). [h] See ref 8a. [i] Another crystalline form melted at 180–185 °C. [j] Reference 2d. [k] See Table III. [l] *d,l* mixture.

to the corresponding oxazolidinones **23d** and **23a** followed by ^{1}H NMR analysis. Similarly, this protocol allowed one to assign the relative stereochemistry at C2–C3 in diols **18b** and **18c**. Measurement of the ring hydrogen coupling constants and chemical shifts of oxazolidinones is a reliable diagnostic method that has been used to confirm the relative stereochemistry of amino alcohols, and by using this technique, we were confident of the stereochemistry of **23a–d**, and therefore of **18a–d**. In accord with literature precedent,[12] **23c** and **23d** have trans ring hydrogen coupling constants of 4–5 Hz, while **23a** and **23b** have cis ring hydrogen coupling constants of 7.5–8 Hz. The C5 ring hydrogen chemical shifts of 4.25, 4.38, 4.00, and 4.03 ppm for **23a–d**, respectively, are also consistent with this assignment since the cis ring hydrogens are typically downfield from the trans. The order of elution on silica gel eluting with 1/1 ether/hexane, from fastest running to slowest, was **18d**, **18b**, **18c**, and **18a**. Diols **19–22** were prepared in an analogous manner and exhibited similar chromatographic behavior. Incorporation of the dipeptide was accomplished either by deprotection of the Boc-diol and direct coupling to the dipeptide **26** or by two sequential coupling steps to protected amino acids.

Results and Discussion

Structure and in Vitro Activity. The novel series of renin inhibitors composed of dipeptides attached to a nonpeptidic replacement for the scissile Leu–Val amide bond is summarized in Tables II and III. The parent Leu-Val surrogate is composed of a hydroxyethylene isostere to replace the amide bond, and it is truncated at the C-terminus in order to remove all functionality beyond the Val side chain. When compared to Boc-Phe-His-L-leucinol (IC$_{50}$ = 1.3 × 10^{-5} M), it is clear that the additional isopentyl appendage in **31** leads to enhance binding to the enzyme. In an effort to improve the biological properties for this series of small inhibitors, we felt it important to optimize a number of characteristics, the first of which is potency. We intended to do this by improving existing or creating new inhibitor–enzyme interactions rather than by incorporating additional amino acid residues. By taking this approach to potency enhancement, we simplify our

(12) (a) Rich, D. H.; Sun, E. T. O. *J. Med. Chem.* **1980**, *23*, 27. (b) Futagawa, S.; Inui, T.; Shiba, T. *Bull. Chem. Soc.* **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.

Table III. In Vitro Activity and Characterization Data for Inhibitors

no.	X	AA_1	AA_2	R	config C3	config C4	IC_{50}, nM	% yield	mp, °C	formula	synth[a]	purifn[a] (solvent)[a]
30	Boc	Phe	His	H	*R*		50	15	198–200	$C_{30}H_{45}N_5O_6 \cdot {}^1/_2H_2O$	*b*	J
34	Boc	Phe	His	isobutyl	*R*	*S*	1.5	*a*	*a*	*a*	*a*	*a*
35	Boc	Phe	His	isobutyl	*R*	*R*	35	56	130–132 (110)	$C_{34}H_{53}N_5O_6 \cdot H_2O$	A	A (D)
36	Boc	Phe	His	isobutyl	*S*	*S*	70	72	135–136 (120)	$C_{34}H_{53}N_5O_6 \cdot {}^1/_2H_2O$	A	A (D)
37	Boc	Phe	His	isobutyl	*S*	*R*	95	53	197–202	$C_{34}H_{53}N_5O_6 \cdot {}^3/_4H_2O$	A	A (D)
38	Boc	Phe	His	methyl	*R*	*S*	2	19	*c*	$C_{31}H_{47}N_5O_6$	A	A (I)
39	Boc	Phe	His	ethyl	*R*	*S*	0.6	52	192–194	$C_{32}H_{49}N_5O_6 \cdot {}^1/_4H_2O$	A	A (I)
40	Boc	Phe	His	isopropyl	*R*	*S*	1.5	64	177–182	$C_{33}H_{51}N_5O_6$	A	A (J)
41	Boc	Phe	His	isopentyl	*R*	*S*	4	46	115–120	$C_{35}H_{55}N_5O_6 \cdot {}^3/_4H_2O$	A	A (H)
42	Etoc	Phe	His	ethyl	*R*	*S*	0.5	53	200–201	$C_{30}H_{45}N_5O_6$	B	B
43	Etoc	Phe	His	isobutyl	*R*	*S*	0.6	81	174–176 (165)	$C_{32}H_{49}N_5O_6$	B	F
44	Etoc	Phe	Leu	ethyl	*R*	*S*	1.1	94	174–176	$C_{30}H_{49}N_3O_6 \cdot {}^1/_4H_2O$	D	
45	Etoc	Phe	Leu	isobutyl	*R*	*S*	0.3	80	188–189	$C_{32}H_{53}N_3O_6$	D	C
46	Etoc	Leu	His	ethyl	*R*	*S*	10	37	202–203	$C_{27}H_{47}N_5O_6 \cdot {}^1/_2H_2O$	B	H
47	Etoc	Leu	His	isobutyl	*R*	*S*	2	45	172–174 (160)	$C_{29}H_{51}N_5O_6 \cdot H_2O$	B	H

[a] Refer to Table II. [b] See Scheme III. [c] Amorphous solid.

second problem, that of stabilizing the inhibitor to potential degradative enzymes such as chymotrypsin, by having fewer amide bonds to stabilize. We found that the inhibitors were improved by changing the P_1 side chain from isobutyl to cyclohexylmethyl, a side chain that after extensive investigation proved to be optimal in a series of closely related sulfidoethanol analogues.[8b] Thus, compound **32** is more than 100 times more potent than **31**. Introducing a second hydroxyl group into this Leu-Val replacement[2] also increased inhibitory potency. Comparison of compounds **31** and **33** reveals a greater than 100-fold increase in inhibitory potency. The inhibitory potency of **33** could be improved even further by replacement of the isobutyl P_1 side chain with cyclohexylmethyl to give compound **34**. It is interesting to note that this modification does not improve **33** as much as **31**, perhaps due to indirect effects that the P_1 side chain might be exerting on the optimal diol geometry of **33** (see Molecular Modeling Studies).

We reasoned that these dihydroxyethylene isostere-containing inhibitors were profiting by interaction with an additional binding site in renin such as an active site carboxyl group (Asp 32 or Asp 215) or with some other hydrogen bond donating or accepting functionality. One would expect this type of interaction to be stereoselective, and inhibitory potency should be sensitive to variation of the hydroxyl group geometry. For this reason we explored all four possible configurations of the 1-amino-2,3-dihydroxy nucleus shown in Table III, compounds **34–37**. A stepwise decrease in activity can be seen in going from the configuration of **34** to that of **37**. Others have recently also incorporated a dihydroxyethylene isostere into various renin inhibitors[13] since Matsueda et al.[3b] first showed that **1c** possessed inhibitory activity, but until now there has been no demonstration that either the diol is more potent than the alcohol or that the potency could vary with the stereochemistry. It is interesting to note that, like most renin inhibitors, the two most active diastereomeric diols in our series, **34** and **35**, are more potent than their counterparts, **36** and **37**, respectively, which do not have the statine-like absolute configuration at C3 (numbering as shown in Table III).

Like the monohydroxyethylene compounds, removal of the aliphatic appendage, R, from **34** to give **30** results in a significant loss in inhibitory potency, underscoring the importance of each interaction in the Leu-Val surrogate. We therefore felt the need to explore the nature of this R group in greater detail. Compounds **38–41** cover a broad range of R group size ranging from methyl to isopentyl. We were surprised to see, however, how little variation there was among members of this series. That even the methyl analogue, **38**, possesses significantly improved activity relative to **30** and that it also compares well with the **34** suggest that this appendage may serve mainly to anchor the diol into a favorable conformation. Before committing to an R group to be held constant throughout the remaining structure–activity investigations, we explored briefly how two of the most favorable R groups, ethyl and isobutyl, compared when changes were made to the remainder of the molecule. When the N-terminal protecting group of **39** and **34** was changed from Boc to Etoc (ethoxycarbonyl) to give subnanomolar inhibitors **42** and **43**, respectively, relatively little difference in the R group was seen. Changing the P_2 residue from His to Leu (compounds **44** and **45**) or the P_3 residue from Phe to Leu (compounds **46** and **47**) distinguished the R = isobutyl series as the more tolerant to change, and we therefore chose it to examine the protecting group and P_1 and P_2 positions in greater detail.

Compounds **48–52** contain replacements for histidine, the P_2 residue of **34** and of human substrate. The data shown in Table II indicate that a number of different substituents lend subnanomolar potency at this position, including lipophilic groups as well as nonbasic aromatic groups. However, as will be discussed below, this position does seem to play a role in dictating binding specificity.

Compounds **53–58** explore the effect of variation at the P_3 phenylalanine position. Substituting Leu for Phe giving compounds **53** and **54** revealed that the effect of this

(13) (a) Thaisrivongs, S.; Pals, D. T.; Kroll, L. T.; Turner, S. R.; Han, F.-S. *J. Med. Chem.* 1987, *30*, 976. (b) Hanson, G. J.; Baran, J. S.; Lindberg, T.; Walsh, G. M.; Papaiannou, S. E.; Babler, M.; Bittner, S. E.; Yang, P.-C.; Corobbo, M. D. *Biochem. Biophys. Res. Commun.* 1985, *132*, 155. (c) Hanson, G. J.; Baran, J. S.; Lowrie, H. S.; Sarussi, S. J.; Yang, P-C.; Babler, M.; Bittner, S. E.; Papaioannou, S. E.; Walsh, G. M. *Biochem. Biophys. Res. Commun.* 1987, *146*, 959.

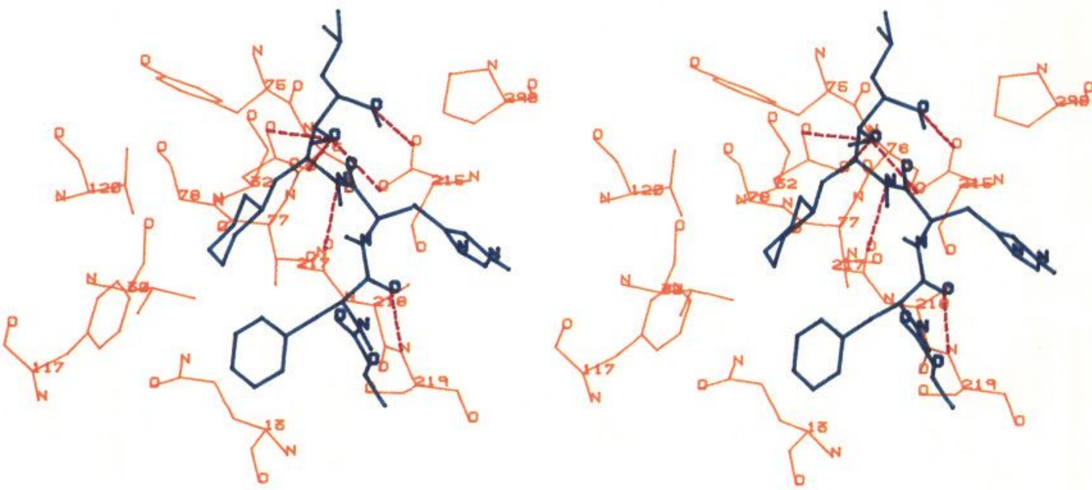

Figure 2. View of the active site of renin (orange lines) with the inhibitor (blue lines), compound **34**, bound. The important hydrogen bond interactions are shown (red dashed lines). The main chain NH of cyclohexylalanine P_1 interacts with carbonyl oxygen of 217. The first hydroxyl is interacting with both Asp 32 and Asp 215, while the second hydroxyl bonds only the other oxygen of Asp 215.

change was somewhat dependent on the nature of the P_2 residue. Having His at P_2 resulted in effectively no change (compare **53** to **34**) while having Leu at P_2 resulted in a 5-fold loss in inhibitory potency (compare **54** to **50**). Compounds **55–58** are other P_3 analogues of **34**. Compounds **55** and **56**, although they possess varied electronic and/or steric properties at this position, had remarkably little effect on binding. It should be noted that the reported potency-enhancing effects of the (1)Nal substitution at the P_3 site[3] did not improve the inhibitory potency of **55** and **65** relative to the corresponding parent Phe compounds, **34** and **45**, respectively. We observed similar behavior in an earlier series of inhibitors[8a] in which the (1)Nal substitution at the P_3 site improved compounds with the isobutyl P_1 side chain nearly 10-fold (as is the case in ref 3); those with the cyclohexylmethyl side chain, as is the case in **55** and **65**, were improved only minimally. Dramatic effects on potency were observed with the Ala replacement (**57**) or the His replacement (**58**). It is interesting to note how the P_3 site accepts the Phe/His interchange much less well than the P_2 position.

The N-terminal Boc group of **34** was next replaced with isomeric and isosteric groups, giving carbamate, amide, and urea compounds **59**, **60**, and **61**, respectively, which were effectively equipotent with each other and with their parent. Examination of amides **62**, **63**, and **64** with alkyl substituents progressing in size from methyl to isopropyl to cyclohexyl revealed surprisingly little variation in activity at this position. Conversion to the ethoxycarbonyl N-terminal group led to a slight improvement in potency (compared **34** and **43**) and also an improvement in acid lability and a reduction in size over the Boc group, so we incorporated this change into a series of inhibitors and reexamined the P_2 and P_3 sites briefly. Comparison of the compound pairs **43**/**34**, **45**/**50**, **67**/**56**, **68**/**49**, and **69**/**54** revealed that the ethoxycarbonyl group in each case led to comparable or improved activity. In particular, compounds with the ethoxycarbonyl group at the N-terminus and Leu at the P_2 site, such as **45**, **65**, and **66**, were in the 200–300 pM range and stand as some of the most potent inhibitors for their size, reported to date. Inhibitor **70**, possessing a 4-iodo substituent, was prepared so that facile radiolabeling by catalytic hydrogenolysis using tritium gas would give rise to a tritiated analogue of **45** for the drug metabolism studies described below.

Molecular Modeling Studies. The modeling of the human renin active site has been reported elsewhere.[8b,14] Briefly, the model of human renin was built by structural homology from four other aspartic proteinases.[14] The entire renin molecule was derived; but, for routine modeling and calculation of different inhibitors interacting with renin, we restricted our analysis to the active-site region.[14] From the crystal data of the known aspartic proteinases it is apparent that, because of the close distance between the carboxyl groups of aspartates 32 and 215 (numbering is that of the porcine pepsin sequence[15]), one of the two aspartates is protonated. In our model Asp 215 is protonated and Asp 32 is left in a charged state.

With the above described structure of renin, we modeled several compounds into the active site. Then, for each complex, molecular mechanics calculations were performed in the form of molecular dynamics simulations of times varying from 6 to 10 ps. The net charge of the charged residues present in the complex was reduced to approximate the screening effect of solvent which was not included explicitly in the calculation. The molecular mechanics calculations were performed by using the program DISCOVER of Biosym Technologies on a FPS-164 array processor hosted by a VAX 11/785.

The aim of the modeling and simulation work was to observe the behavior of different inhibitors interacting with the active site, and in particular to assess the importance of the second hydroxy group (R_2 in Table II) in different compounds. It can be noted that the presence of this hydroxyl has a remarkable effect when R_1 is an isobutyl: 100-fold improvement over the compound lacking this hydroxyl group (compare **31** and **33** in Table II). On the other hand, the difference is less striking when R_1 is cyclohexylmethyl: only a 7-fold improvement (compare **32** and **34**).

Figure 2 shows the pattern of hydrogen bonds that compound **34** forms with the enzyme. In particular, the first hydroxyl binds to Asp 32 and Asp 215 while the second hydrogen bonds to Asp 215. From molecular dy-

(14) Sham, H. L.; Bolis, G.; Stein, H. H.; Fesik, S. W.; Marcotte, P. A.; Plattner, J. J.; Rempel, C. A.; Greer, J. *J. Med. Chem.* **1988**, *31*, 285.
(15) Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 162.

Table IV. Enzyme Inhibition Selectivity

no.	X	cathepsin D (bovine): % inhibn @ 10^{-5}	pepsin (porcine): % @ 10^{-5}	pepsin (human): % @ 10^{-5}	gastricsin (human): % @ 10^{-5}	renin (human): % @ 10^{-9}
34	Boc-Phe-His	0	0	0	0	33
43	Etoc-Phe-His	0	0	0	0	78
45	Etoc-Phe-Leu	23	32	11	7	71
47	Etoc-Leu-His	0	0	0	0	3
48	Boc-Phe-Ala	0	0	0	0	15–20
49	Boc-Phe-Phe	0	10			37
53	Boc-Leu-His	0	0	0	0	44
54	Boc-Leu-Leu	81	63	25	0	23
55	Boc-(1)Nal-His	0	0			26
69	Etoc-Leu-Leu	87	42			29

namics simulations of these compounds we made the following obsesrvations: compound **31** has a greater mobility within the active site than **33**, the latter being more restricted by the hydrogen bond of the second hydroxyl to Asp 215 (the hydrogen bond network of this inhibitor is similar to the one shown in Figure 2). On the other hand, compounds **32** and **34** are both less mobile than the previous two because of the presence of the larger cyclohexylmethyl group at position P_1. In this case, the presence of the second hydroxyl is not immobilizing the inhibitor within the active site as much as in the isobutyl series.

This hypothesis would maintain that in the series with an isobutyl group at the P_1 position, the second hydroxyl has a 2-fold function: hydrogen bonding with the enzyme and, in so doing, restricting the mobility of the compound, thereby permitting improved dispersion interactions between the inhibitor atoms and the enzyme. In the cyclohexylmethyl series, the binding is tighter and the interaction energy is higher because the larger side chain fills the hydrophobic pocket at position P_1 more optimally, thereby increasing the dispersion energy of interaction between the inhibitor and the enzyme.[8b] The presence of the second hydroxyl in this case therefore simply adds the binding energy of an additional hydrogen bond.

Enzyme Specificity Studies. The compounds in Table IV illustrate the specificity of these diol inhibitors toward inhibition of a variety of related enzymes. The data are presented as percent inhibition at 10^{-9} M for human renin and at 10^{-5} M for the other enzymes. Even at the high concentration, none of the inhibitors with histidine or alanine at the P_2 site show any significant inhibitory activity for other than human renin irrespective of whether the P_3 residue is phenylalanine, leucine, or (1′-naphthyl)alanine. When the P_2 residue was changed to leucine or phenylalanine, low yet measurable amounts of inhibition of the other enzymes were observed.

In Vivo Studies on Inhibitor 43

Intravenous Profile of 43 in Anesthetized Salt-Depleted Monkeys. In preliminary experiments ($n = 2$), the mean blood pressure fell maximally by 14 ± 3.7% at 10 min following a dose of 0.1 mg/kg, iv, of **43** (Figure 3). Low basal pressures and high plasma renin activity (PRA) values reflected lowered blood volumes following salt depletion. Corresponding plasma renin activity values indicate nearly 100% suppression at the 10-min time point and 95% inhibition still present at 40 min postdosing, at

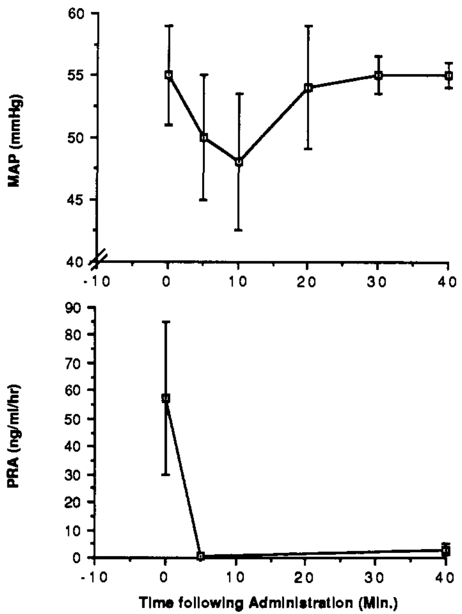

Figure 3. Effect of 0.1 mg/kg, iv of inhibitor **43** on mean arterial pressure (MAP) and plasma renin activity (PRA) in two salt-depleted monkeys. Results are shown as mean ± SE.

which time blood pressure had already returned to control. No heart rate changes were observed (data not shown).

Intraduodenal Administration of 43·HCl in Anesthetized, Salt-Depleted Monkeys. Figure 4 illustrates the blood pressure and plasma renin activity responses of two salt-depleted, anesthetized monkeys to the intraduodenal administration of 6 mg/kg of **43**. Mean arterial pressure (MAP) fell 6 mmHg on the average by 10 min postdosing, but recovered completely by 1 h. Concomitantly, plasma renin activity was maximally suppressed at 5 and 30 min following dosing but, unlike blood pressure, slowly returned toward control. Peak changes in MAP and PRA coincided temporally with peak blood levels of **43**.

Oral Absorption of 43 in Conscious, Fed Rats. Arterial blood levels of **43** were determined by extractive enzyme inhibition assay for a period of 3 h following an oral dose of 10 mg/kg **43**·HCl solution given to conscious, fed rats (Figure 5). The maximum circulating level of approximately 31 ± 11 ng/mL blood was observed at the 5-min time point. Blood levels fell rapidly by 30 min. This early (<15 min postdosing), relative availability of drug has also been a noted pattern in the oral availability of

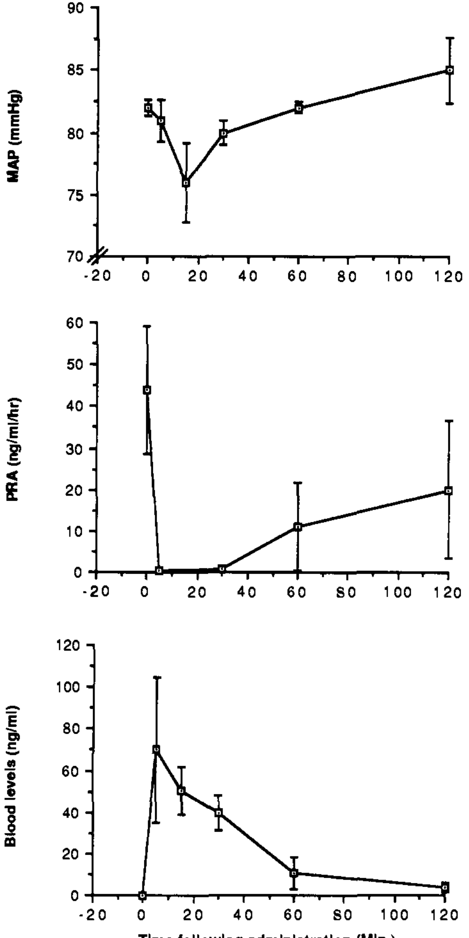

Figure 4. Effect of 6.0 mg/kg of inhibitor 43·HCl on mean arterial pressure (MAP), plasma renin activity (PRA), and drug blood level when given intraduodenally to two anesthetized, salt-depleted monkeys. Mean ± SE values are shown.

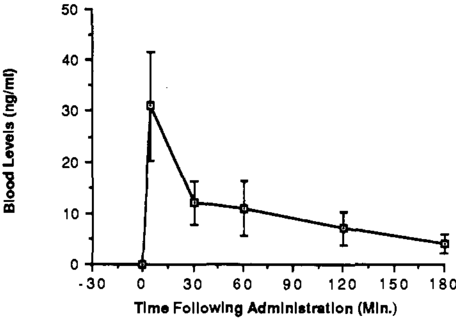

Figure 5. Arterial blood levels of inhibitor 43 in conscious, fed rats following a dose of 10 mg/kg given orally. Data are expressed as mean ± SE (n = 7).

other compounds of this type in rats (data for additional compounds not shown). It is clear that bioavailability of 43 after oral administration to rats is limited.

Pharmacokinetics of Inhibitor 43·HCl after Parenteral Administration to Dogs. In order to determine the bioavailability of 43 in the dog, the compound was dissolved in alcohol/saline and administered to conscious male beagles either intravenously (iv) or directly into the lungs through a permanent tracheostomy stoma (it) surgically prepared as described by Ritter.[16] The compound in blood samples taken from the jugular vein from 1 min to 120 min postdosing was determined by enzyme inhib-

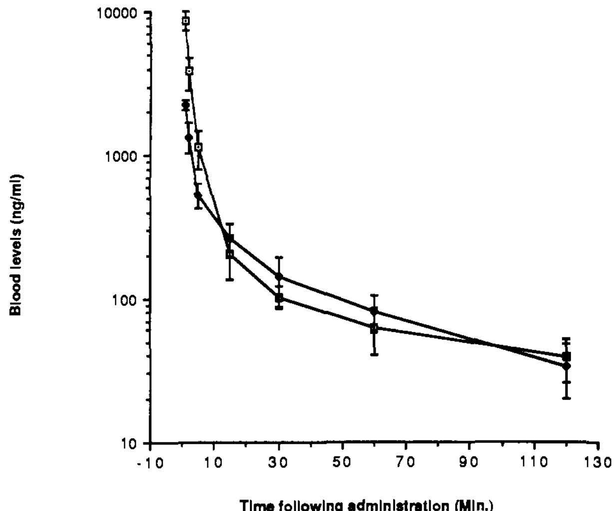

Figure 6. Level of 43·HCl in dog blood following intravenous (□, n = 5) or intratracheal (●, n = 4) administration (1 mg/kg) as determined by enzyme inhibition assay.

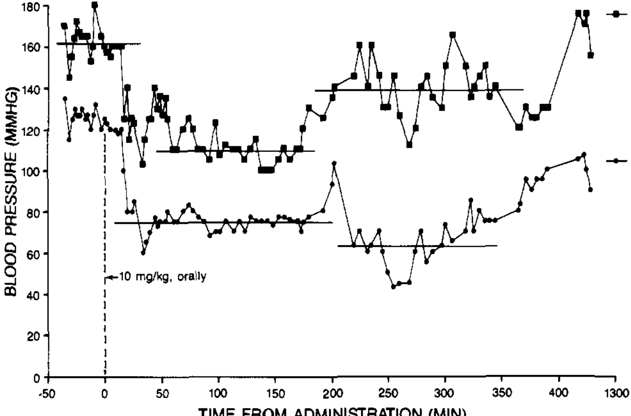

Figure 7. Systolic (■) and diastolic (●) blood pressure responses of a conscious chronically, renin dependent-hypertensive monkey (2 kidney–1 clip Goldblatt) to 10 mg/kg of inhibitor **45** given orally.

ition assay after extraction of the whole blood with acetonitrile. The results of these experiments are shown in Figure 6. The mean absorption of the compound after intratracheal administration was found to be 57%; nearly identical rates of elimination were observed after the two routes ($t_{1/2}$ = 30.1 min, iv; $t_{1/2}$ = 31.5 min, it). Thus, intratracheal administration of this inhibitor is an effective method for delivery and avoids many of the problems commonly encountered with oral administration of peptide drugs such as stability and absorption. However, once delivered, the compound is cleared rapidly in the dog as in the rat and monkey.

In Vivo Studies on Inhibitor 45

Oral Activity in the 2 Kidney–1 Clip Goldblatt Monkey. Figure 7 depicts the original tracing showing the effect of oral administration of **45**, 10 mg/kg, to a 2 kidney–1 clip, conscious monkey which was rendered hypertensive and hyperreninemic. This experiment, which was reproduced in two additional monkeys and reproducible in the monkey shown, was selected for demonstration purposes because the response was striking and was monitored for 24 h. The base-line systolic/diastolic blood pressures of this monkey were 160/128 mmHg. Within 20 min of drug administration, both systolic and diastolic blood pressures fell to normotensive levels and plateaued thereafter at 110/73 mmHg. At 200 min postdosing, the

(16) Ritter, J. W. *Lab. Anim. Sci.* **1984**, *34*, 79.

Table V. Excretion of Radioactivity by Rats Given a 0.3 or 5 mg/kg Oral Dose of [^{3}H]-**45**

	% ^{3}H dose	
	0.3 mg/kg	5 mg/kg
urine	9.3	2.5
feces	74.0	90.0

Table VI.[a] Metabolic Profiles in 0–24-h Urine and Fecal Samples from Rats Given a 0.3 mg/kg Oral Dose of [^{3}H]-45

		% urinary or fecal ^{3}H	
zone	ref compd	urine	feces
1	Phe, ^3H_2O	6–7	1–2
2		20–24	nd
3	Etoc-Phe-OH	70	7–14
4	Etoc-Phe-Leu-OH	nd	8–15
5	H-Phe-Leu-amino diol	nd	7–12
6	parent drug	nd	48–75

[a] Values represent determinations in two rats; nd = not detected; urine and fecal samples contained 6–8% and 66–70% of administered ^{3}H dose, respectively.

monkey received supplemental saline, iv, to provide adequate hydration and maintenance of blood volume, which as expected increased systolic blood pressure but did not affect diastolic pressure presumably due to the vasodilatory action of **45** on arterioles. Remarkably, 22 h (1300 min) after dosing, systolic but not diastolic pressure had returned to control values. The blood pressure response shown here was impressive in light of the fact that peak circulating titers of **45** were below 50 ng/mL. Heart rate remained unchanged from base line throughout the treatment period.

Drug Metabolism Studies on Inhibitor 45. During the first hour after intraarterial administration (see the Experimental Section) of [^{3}H]-**45** to rats, the parent drug levels in plasma were in the low nanogram/milliliter range and decreased rapidly, with a half-life of about 10 min. At 60 min, unchanged [^{3}H]-**45** still accounted for over 80% of the circulating radioactivity. In contrast, the total ^{3}H levels in the plasma of the rats given a 5 mg/kg oral dose increased slowly between 1 and 6 h after dosing. At best, trace quantities of the parent drug were detected in the plasma, and (ethoxycarbonyl)phenylalanine, formed by cleavage of the Phe–Leu bond, was tentatively identified as a major circulating metabolite.

After oral administration of [^{3}H]-**45** (5 or 0.3 mg/kg), 2–9% of the ^{3}H dose was excreted in the urine and 90–74% was eliminated in the feces (Table V). No parent drug was detected in the urine, and about 70% of the urinary radioactivity was due to (ethoxycarbonyl)phenylalanine (Table VI). However, the parent drug accounted for about half to three-quarters of the radioactivity in the feces. This probably represented unabsorbed drug since **45** was not detected in the bile of another rat given the drug intragastrically. Fecal metabolites were tentatively identified as (ethoxycarbonyl)phenylalanine, (ethoxycarbonyl)-phenylalanylleucine, and phenylalanylleucyl-amino diol, suggesting that, in addition to the Phe–Leu bond, the Leu–amino diol and ethoxycarbonyl–Phe bonds were also cleaved.

Summary

The synthesis of this class of diol-containing renin inhibitors has revealed that a simple vicinal diol functionality corresponding to the scissile Leu–Val bond in human angiotensinogen is capable of imparting inhibitory activity at a comparable or higher level than either the corresponding aldehyde or hydroxymethyl functionality. Inhibitory potency was increased both by finding new, as well as by improving existing, binding groups through introduction of a second hydroxyl group of specific stereoconfiguration and through modification of the P_1 and P_1' lipophilic side chains, respectively, giving compounds that inhibited human renin in the 200–300 pM range. The magnitude of effect of the second hydroxyl group on potency is not only dictated by the absolute stereochemistry of the diol but also by the side chain of the P_1 residue. While molecular modeling could not readily distinguish among the four diol diastereomers in a quantitative manner, the model does suggest that, in the most potent stereoconfiguration of the diol-containing inhibitors, one of the hydroxyl groups hydrogen bonds to Asp 32 and Asp 215, while the second hydrogen bonds to Asp 215. A variety of N-terminal protecting groups were found to be tolerated; among them, the ethoxycarbonyl group seemed to offer a good combination of size, acid stability, and potency.

In vitro, these diol inhibitors were extremely selective for renin over the related enzymes cathepsin D, pepsin, and gastricsin. At high concentrations, compounds containing a leucine or phenylalanine rather than a histidine at the P_2 position, while givng high levels of renin inhibition, gave only minor amounts of inhibition of the other enzymes. Other positions of the molecules studied seemed to have little effect on enzyme selectivity.

While a member of this series, **43**, is capable of lowering mean arterial blood pressure and plasma renin activity in salt-depleted monkeys after both intravenous and intraduodenal administration, the effects are not long lived. The blood levels of inhibitor **43** were monitored by quantitative enzyme inhibition assay in the monkey, rat, and dog. Intraduodenal administration to monkeys and oral administration to rats gave low blood levels while intratracheal administration to dogs gave approximately 60% bioavailability; however rapid clearance is a significant problem in all models. Inhibitor **45**, when evaluated orally in a sensitive primate model in which monkeys were rendered both hypertensive and hyperreninemic (2K–1C), gave a long-lived effect on diastolic pressure even though peak blood levels were low. Blood levels of this compound after oral administration to rats were also low, and elimination of the bulk of the radiolabeled material as unabsorbed drug would suggest either that the 2K–1C model was, as expected, extremely sensitive to even low levels of a potent renin inhibitor that possibly acts on an active pool of renin other than the plasma pool or that the distribution profile of **45** in monkeys differs markedly from that of rats. Further work to modify the properties of this potent class of inhibitors in an effort to improve plasma half-lives and hypotensive efficacy is under way.

Experimental Section

All amino acids and protected amino acids were obtained from Sigma Chemical Co. (St. Louis, MO), Chemical Dynamics (South Plainfield, NJ), or Bachem (Torrance, CA) 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.

Catalytic tritiation of compound **70** was carried out by New England Nuclear. Proton magnetic resonance spectra were measured on a Nicolet QE-300 (300 MHz). Chemical shifts are reported as δ values (parts per million) relative to Me_4Si as an internal standard. 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 with E. Merck precoated silica gel F-254

plates (thickness, 0.25 mm). Flash column chromatography was carried out with Baker silica gel (40 μm).

General Procedures. The following experimental procedures provide representative conditions for the preparation of all compounds shown in the tables.

A. Deprotection 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. Mixed Anhydride Coupling. To a stirred −12 °C solution of the Boc-protected amino acid or dipeptide (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 the amine hydrochloride (prepared by deprotecting 0.25 mmol of the corresponding Boc-amine 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 to homogeneity on silica gel (dichloromethane/methanol mixtures) to give the desired compound.

C. Carbodiimide–HOBT Coupling. The corresponding amine hydrochloride (0.2 mmol) was dissolved in dimethylformamide (5 mL) along with the N-protected amino acid or dipeptide (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 to homogeneity on silica gel (dichloromethane/methanol mixtures) to give the desired material.

D. Optical Purity Determination. Olefins **13–17** were converted to their MTPA amides[11] according to our previous procedure.[10] The crude product mixture was then analyzed on a Varian Aerograph 3700 gas chromatograph equipped with a flame-ionization detector and RSL150 column (0.25 mm × 30 m) at a helium flow rate of 24 psi. The following temperature settings were used: oven, 215 °C; injector, 220 °C; and detector, 280 °C. Under these conditions, the (+)-MTPA-*S*-olefin and (−)-MTPA-*S*-olefin derivatives of cis olefin **13** had retention times of 16.40 and 15.99 min, respectively. The (+)-MTPA derivatives were typically prepared so that the minor product, (+)-MTPA-*R*-olefin, would elute first, thus removing it from the tail of the second peak.

(2*S*)-2-[(Boc-L-phenylalanyl-L-alanyl)amino]-1-hydroxy-4-methylpentane (2a). L-Leucinol was coupled to Boc-Phe-Ala-OH by using the general mixed anhydride coupling procedure to give **2a** in 98% yield: mp 111–113 °C; mass spectrum, $(M + H)^+$ = 436; ^1H NMR ($CDCl_3$) δ 0.92 (d, 6 H, *J* = 7 Hz), 1.34 (d, 3 H, *J* = 7 H), 1.42 (s, 9 H), 1.54–1.68 (m, 3 H), 2.8 (br, 1 H), 3.00 (dd, 1 H, *J* = 8, 14 Hz), 3.13 (dd, 1 H, *J* = 6, 14 Hz), 3.50 (br dd, 1 H, *J* = 6, 11 Hz), 3.67 (br dd, 1 H, *J* = 3, 11 Hz), 4.01 (m, 1 H), 4.25–4.4 (m, 2 H) 4.93 (d, 1 H, *J* = 5 Hz), 6.42 (d, 1 H, *J* = 6 Hz), 6.51 (d, 1 H, *J* = 8 Hz), 7.15–7.38 (m, 5 H). Anal. ($C_{23}H_{37}N_3O_5$) C, H, N.

(2*RS*,3*S*)-3-[(Boc-L-phenylalanyl-L-alanyl)amino]-1,2-dihydroxy-5-methylhexane (2c). Method A. Inhibitor **2c** was prepared in 84% yield by using an analogous procedure to that used to prepare **3c**, below: mp 100–103 °C; mass spectrum, M^+ = 475; comparison of NMR spectrum of the 2*R*,*S* mixture with that of the pure 2*R* isomer below indicates a 5:7 mixture of *R* and *S* isomers. Useful diagnostic resonances are the 4.11 ppm multiplet for the 2*R* isomer and the 3.90 ppm multiplet for the 2*S* isomer. Anal. ($C_{24}H_{39}N_3O_6 \cdot {}^1/_4H_2O$) C, H, N.

Method B. Alternatively, **2c** could be prepared as a pure 2*R*,3*S* diastereomer by hydrogenolysis of benzyl ether **9**, which was prepared in analogy to the corresponding isobutyl ether.[8a] Compound **9** (50 mg, 0.090 mmol) in methanol (20 mL) was hydrogenated with 20% Pd/C (25 mg) at 4 atm of hydrogen for 18 h. Filtration and evaporation provided 30 mg (72%) of the desired product: mp 117–121 °C; ^1H NMR ($CDCl_3$) δ 0.91 (m, 6 H), 1.37 (d, 3 H, *J* = 7 Hz), 1.42 (s, 9 H), 1.3–1.8 (several br m), 2.98 (dd, 1 H, *J* = 8, 14 Hz), 3.13 (dd, 1 H, *J* = 6, 14 Hz), 3.4–3.75 (br m, 3 H), 4.12 (br m, 1 H), 4.23–4.43 (br m, 1 H), 5.00 (br, 1 H), 6.5 (br, 1 H), 6.72 (br d, 1 H, *J* = 9 Hz), 7.18–7.4 (m, 5 H).

(3*S*,4*S*)-4-[(Boc-L-phenylalanyl-L-alanyl)amino]-1,3-dihydroxy-6-methylheptane (2d). Alcohol **10** was prepared from the corresponding oxazolidinone[17] by hydrolysis under basic conditions[8a] and coupled to Boc-Phe-Ala-OH by the mixed anhydride procedure described above to give **11** in 92% yield; mass spectrum, M^+ = 461. To a stirred solution of **11** (200 mg, 0.433 mmol) in dry THF (10 mL) was added 9-BBN (5.2 mL of a 0.5 M solution in THF). After 18 h the sequential addition of water (3 mL), 3 M NaOH (0.87 mL), and, 2 min later, 30% H_2O_2 (1.0 mL) was performed. The mixture was heated to 50 °C for 1 h, cooled to room temperature, and partitioned between ether (20 mL) and water (5 mL). The organic phasic was washed (5 mL of brine), dried (Na_2SO_4), concentrated, and chromatographed (40 g of 40-μm SiO_2; 95:5 CH_2Cl_2/CH_3OH) to give 126 mg (61%) of the desired product: mass spectrum, M^+ = 479. Anal. ($C_{25}H_{41}N_3O_6$) C, H, N.

(3*S*)-3-[(Boc-L-phenylalanyl-L-alanyl)amino]-1-hydroxy-5-methylhexane (2e). Subjecting olefin **7** to the conditions used in the oxidation of **11** to **2d** above gave the desired product in 76% yield: mass spectrum, M^+ = 449; ^1H NMR ($CDCl_3$) δ 0.87 (d, 3 H, *J* = 7 Hz), 0.89 (d, 3 H, *J* = 7 Hz), 1.25–1.9 (m, several H), 1.34 (d, 3 H, *J* = 7 Hz), 1.42 (s, 9 H), 3.03 (dd, 1 H, *J* = 8, 14 Hz), 3.13 (dd, 1 H, *J* = 6, 14 Hz), 3.45–3.65 (m, 3 H), 4.12 (br m, 1 H), 4.29 (m, 1 H), 4.39 (m, 1 H), 4.88 (d, 1 H, *J* = 6 Hz), 6.33 (br, 1 H), 6.43 (br d, 1 H, *J* = 7 Hz), 7.17–7.37 (m, 5 H). Anal. ($C_{24}H_{39}N_3O_5$) C, H, N.

(2*S*)-2-[(Boc-L-phenylalanyl-L-alanyl)amino]-3-cyclohexyl-1-hydroxypropane (3a). Inhibitor **3a** was prepared in 92% yield by using an analogous procedure to that used to prepare **2a**: mp 100–103 °C; mass spectrum, M^+ = 475; ^1H NMR ($CDCl_3$) δ 0.8–1.8 (several m), 1.34 (d, 3 H, *J* = 7 Hz), 1.42 (s, 9 H), 3.01 (dd, 1 H, *J* = 8, 14 Hz), 3.13 (dd, 1 H, *J* = 6, 14 Hz), 3.48 (dd, 1 H, *J* = 6, 11 Hz), 3.68 (dd, 1 H, *J* = 3, 11 Hz), 4.03 (m, 1 H), 4.24–4.38 (m, 1 H), 4.90 (d, 1 H, *J* = 5 Hz), 6.37 (d, 1 H, *J* = 6 Hz), 6.47 (br, 1 H), 7.15–7.38 (m, 5 H). Anal. ($C_{26}H_{41}N_3O_5 \cdot {}^1/_4H_2O$) C, H, N.

(2*RS*,3*S*)-3-[(Boc-L-phenylalanyl-L-alanyl)amino]-4-cyclohexyl-1,2-dihydroxybutane (3c). To a stirred solution of **7**[8b] (R = cyclohexylmethyl, 100 mg, 0.212 mmol) in THF (95 mL) were added OsO_4 solution (0.065 mL of a 2.5 w/v % solution in *tert*-butyl alcohol) and *N*-methylmorpholine *N*-oxide (57 mg, 0.424 mmol) sequentially. After 4.5 h, brine (10 mL) was added, and the mixture was extracted with ether (4 × 8 mL). The combined organic phase was washed with 10% Na_2SO_3 (3 × 6 mL), 0.1 M H_3PO_4 (5 mL), and brine (5 mL). Drying, filtering, and evaporating provided the desired product (97 mg, 91%) as a 1:1 mixture of diastereomers at the carbon bearing the secondary alcohol: mp 125–128 (transition at 95–100) °C; mass spectrum, M^+ = 505; ^1H NMR ($CDCl_3$) δ 0.75–1.8 (several m), 1.34, 1.36 (2 d, 3 H total, *J* = 7, 7 Hz, 1:1 ratio), 1.42 (s, 9 H), 2.93–3.2 (m, 2 H), 3.3–3.73 (m, 3 H), 3.94, 4.16 (2 br m, 1 H total, 1:1 ratio), 4.2–4.4 (m, 2 H), 4.89, 4.95 (2 br d, 1 H total, 1:1 ratio), 6.41 (br, 1 H), 6.58, 6.65 (2 br d, 1 H total, *J* = 8, 9 Hz, 1:1 ratio), 7.15–7.4 (m, 5 H). Anal. ($C_{27}H_{43}N_3O_6$) C, H, N.

(2*S*,3*S*)-2-[(Boc-L-phenylalanyl-L-alanyl)amino]-1-cyclohexyl-3-hydroxy-6-methylheptane (3f). Following the procedure used to prepare **2f**,[8a] but using cyclohexylalanine rather than leucine, gave **3f** in 92% yield: mass spectrum, $(M + H)^+$ = 546; ^1H NMR ($CDCl_3$) δ 0.8–1.87 (m, several H), 0.86 (d, 3 H, *J* = 7 Hz), 0.87 (d, 3 H, *J* = 7 Hz), 1.34 (d, 3 H, *J* = 7 Hz), 1.42 (s, 9 Hz), 2.98–3.17 (m, 2 H), 3.51 (br, 1 H), 3.97 (br m, 1 H), 4.25–4.45 (m, 2 H), 4.94 (d, 1 H, *J* = 6 Hz), 6.34 (br d, 1 H, *J* = 9 Hz), 6.4

(17) Bolis, G.; Fung, A. K. L.; Greer, J.; Kleinert, H. D.; Marcotte, P. A.; Perun, T. J.; Plattner, J. J.; Stein, H. H. *J. Med. Chem.* **1987**, *30*, 1729.

(br, 1 H), 7.17–7.37 (m, 5 H). Anal. ($C_{31}H_{51}N_3O_5$) C, H, N.

Olefins 13–17. The procedure for the preparation of olefin 13 is representative.

(2*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-1-cyclohexyl-6-methylhept-3-ene (13). To a stirred −78 °C solution of Boc-cyclohexylalanine methyl ester[9a] (40 g, 140 mmol) in anhydrous toluene (250 mL) was added diisobutylaluminum hydride (130 M %, 1.5 M solution in toluene, 121.4 mL) at a rate to keep the internal temperature below −60 °C. After stirring for an additional 20 min at −78 °C, the aldehyde solution was used immediately as described below.

To a poassium hydride (35% dispersion in oil, 32.09 g) suspension in a 0 °C mixture of anhydrous THF/DMSO (1000 mL/200 mL) under dry N_2 was added 1,1,1,3,3,3-hexamethyldisilazane (209 M %, 49.07 g) dropwise. After stirring at 0 °C for 1 h, the resulting solution was added via cannula to a 0 °C flask containing isopentyltriphenylphosphonium bromide (209 M %, 125.66 g). The mixture was stirred vigorously for 1 h at which time it was cooled to −78 °C. The −78 °C aldehyde solution prepared above was then added via cannula. After stirring at −78 °C for 15 min, the mixture was allowed to slowly warm to room temperature and then heated to 40 °C for 12 h. The mixture was then cooled to room temperature and quenched with methanol (7.65 mL) followed by aqueous Rochelle salts (100 mL of saturated solution and 500 mL of H_2O). The mixture was then extracted with ethyl acetate (2×). The combined extracts were washed with water and brine. Drying ($MgSO_4$) and evaporating provided crude alkene which was chromatographed on silica gel (ether/hexane) to give 16.5 g (38%) of the desired compound as an 85:15 mixture of cis/trans isomers based on the NMR spectrum. Quantities (10–15%) of slightly impure but useable olefin were also typically carried on and purified after the next step. **13**: mp 53–55 °C; mass spectrum, M^+ = 309; ^{1}H NMR ($CDCl_3$) δ 0.90 (d, 3 H, *J* = 7 Hz), 0.92 (d, 3 H, *J* = 7 Hz), 1.43 (s, 9 H), 0.8–1.85 (m, 15 H), 2.03 (m, 2 H), 4.33 (br m, 1 H), 4.44 (br m, 1 H), 5.18 (m, 1 H), 5.43 (ddd, 1 H, *J* = 7.5, 7.5, 10.8 Hz). Irradiation of the allylic methylene simplified the 5.18 ppm resonance to a dd, *J* = 9.0, 10.8 Hz and the 5.43 resonance to a d, *J* = 10.8 Hz. The olefinic protons for the minor, trans isomer could be seen also: 5.33 (dd, *J* = 6.7, 15.4 Hz), 5.55 (m). Anal. ($C_{19}H_{35}NO_2$) C, H, N.

(2*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-1-cyclohexyl-3-pentene (14): 48% yield; mass spectrum, M^+ = 267; ^{1}H NMR ($CDCl_3$) δ 0.8–1.8 (m, 1 H), 1.44 (s, 9 H), 4.0–4.5 (m, 2 H), 5.18, 5.33 (1 H total, 3:2 ratio of cis and trans isomers, respectively; cis, ddq, *J* = 10, 10, 2 Hz; trans, br dd, *J* = 7, 16 Hz), 5.45–5.65 (m, 1 H). Anal. ($C_{16}H_{29}NO_2$) C, H, N.

(2*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-1-cyclohexyl-3-hexene (15): 47% yield; mass spectrum, $(M + H)^+$ = 282; ^{1}H NMR ($CDCl_3$) δ 0.8–1.8 (m, 13 H), 0.97, 0.99 (2 t, 3 H total, *J* = 7.5, 7.5 Hz), 1.43 (s, 9 H), 2.03, 2.14 (2 m, 2 H total), 4.05–4.5 (br m, 2 H), 5.12, 5.29 (1 H total, 3:2 ratio of cis and trans isomers, respectively; cis, br dd, *J* = 10, 10 Hz; trans, br dd, *J* = 7, 16 Hz), 5.42, 5.60 (1 H total, 3:2 ratio of cis and trans isomers, respectively; cis, br ddd, *J* = 7, 7, 10 Hz; trans, br ddd, *J* = 7, 7, 16 Hz). Anal. ($C_{17}H_{31}NO_2\cdot{}^1/_4H_2O$) C, H, N.

(2*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-1-cyclohexyl-5-methylhex-3-ene (16): 39% yield; mass spectrum, M^+ = 295; ^{1}H NMR ($CDCl_3$) δ 0.8–1.8 (m, 13 H), 0.95 (d, 3 H, *J* = 7 Hz), 0.98 (d, 3 H, *J* = 7 Hz), 1.44 (s, 9 H), 2.73 (br, 1 H), 4.33 (br, 1 H) 4.52 (br m, 1 H), 5.01 (dd, 1 H, *J* = 10, 10 Hz), 5.23 (dd, 1 H, *J* = 10, 10 Hz), a trans olefin proton representing 5% of the mixture could be seen at 5.53 (dd, *J* = 6, 15 Hz). Anal. ($C_{18}H_{33}NO_2\cdot{}^1/_4H_2O$) C, H, N.

(2*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-1-cyclohexyl-7-methyloct-3-ene (17): 37% yield; mass spectrum, M^+ = 323; ^{1}H NMR ($CDCl_3$) δ 0.8–1.8 (m, 16 H), 0.88 (d, 6 H, *J* = 7 Hz), 1.42 (s, 9 H), 2.12 (m, 2 H), 4.25–4.40 (m, 2 H), 5.12 (br dd, 1 H, *J* = 10, 10 Hz), 5.40 (br ddd, 1 H, *J* = 7, 7, 10 Hz), an olefin proton centered at approximately 5.15 ppm could be partially resolved from the 5.12 m, indicating approximately 10% of the trans isomer. Anal. ($C_{20}H_{37}NO_2$) C, H, N.

Diols 18–22. The procedure for the preparation of 18a–d is representative.

(2*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (18a–d): The 3*R*,4*S*, 3*S*,4*S*, 3*R*,4*R*, and 3*S*,4*R* Diastereomers. To a solution of 13 (8.50 g, 27.5 mmol) in dry THF (150 mL) were added OsO_4 (2.8 mL of a 2.5% solution in *tert*-butyl alcohol) and *N*-methylmorpholine *N*-oxide (9.28 g, 68.7 mmol). After 4 days the mixture was partitioned between ether (200 mL) and brine (100 mL). The aqueous layer was back-extracted with ether (2 × 100 mL), and the combined organic phase was washed with 10% Na_2SO_3, 0.1 M H_3PO_4, and brine. Drying ($MgSO_4$) and evaporating provided a residue (10.81 g) which was chromatographed on silica gel to elute a 60–80% yield of the four diols in the order **18d**, **18b**, **18c**, and **18a**. The exact composition of the diol mixture varied with the ratio of cis/trans olefins 13–17. Olefin mixtures enriched in the cis isomer led to proportionately greater yields of erythro diols **18–22a** and **18–22d**, with the facial selectivity of osmylation giving preferentially (1.5:1) the **18–22d** isomer over the **18–22a** isomer. Stereochemistry was proven by conversion to oxazolidinones **23a–d** as described below.

18d (3*R*,4*S*): mp 127.5–130 °C; mass spectrum, $(M + H)^+$ = 344; ^{1}H NMR ($CDCl_3$) δ 0.8–1.85 (m, 15 H), 0.88 (d, 3 H, *J* = 7 Hz), 0.94 (d, 3 H, *J* = 7 Hz), 1.46 (s, 9 H), 1.93 (m, 1 H), 3.2 (br d, 1 H, *J* = 8 Hz), 3.33 (m, 1 H), 4.04 (m, 1 H), 4.25 (br 1 H), 4.58 (br d, 1 H, *J* = 9 Hz). The below data were obtained in CD_3CN: 0.8–1.95 (m, 16 H), 0.84 (d, 3 H, *J* = 7 Hz), 0.92 (d, 3 H, *J* = 7 Hz), 1.42 (s, 9 H), 2.84 (d, 1 H, *J* = 7 Hz), 3.02 (m, 1 H), 3.22 (m, 1 H), 3.89 (m, 1 H), 4.04 (d, 1 H, *J* = 4 Hz), 5.23 (d, 1 H, *J* = 10 Hz), when D_2O was added, the 2.84, 4.04, and 5.23 ppm resonances exchanged out. The 3.02 resonance shifted to 3.06 and simplified to a dd, *J* = 1.5, 9 Hz. The 3.22 resonance shifted to 3.28 and simplified to a ddd, *J* = 2.5, 9, 9 Hz. The 3.89 resonance shifted to 3.88 and simplified to a ddd, *J* = 1.5, 5, 10 Hz). Anal. ($C_{19}H_{37}NO_4$) C, H, N.

18b (3*S*,4*S*): mass spectrum, $(M + H)^+$ = 344; ^{1}H NMR ($CDCl_3$) δ 0.8–1.85 (several m), 0.89 (d, 3 H, *J* = 7 Hz), 0.93 (d, 3 H, *J* = 7 Hz), 1.46 (s, 9 H), 2.23 (br d, 1 H, *J* = 9 Hz), 2.93 (m, 1 H), 3.52 (br m, 2 H), 3.73 (br m, 1 H), 4.42 (br d, 1 H, *J* = 9 Hz). Anal. ($C_{19}H_{37}NO_4$) C, H, N.

18c (3*R*,4*R*): mass spectrum, $(M + H)^+$ = 344; ^{1}H NMR ($CDCl_3$) δ 0.8–1.9 (several m), 0.92 (d, 3 H, *J* = 7 Hz), 0.95 (d, 3 H, *J* = 7 Hz), 1.44 (s, 9 H), 1.98 (br d, 1 H, *J* = 4.5 Hz), 2.91 (br d, 1 H, *J* = 4.5 Hz), 3.25 (m, 1 H) 3.63 (m, 1 H), 3.78 (m, 1 H), 4.66 (br d, 1 H, *J* = 9 Hz).

18a (3*S*,4*R*): mass spectrum, $(M + H)^+$ = 344; ^{1}H NMR ($CDCl_3$) δ 0.75–2.0 (several m), 0.92 (d, 3 H, *J* = 7 Hz), 0.96 (d, 3 H, *J* = 7 Hz), 1.44 (s, 9 H), 3.43 (m, 1 H), 3.72 (m, 1 H), 3.82 (m, 1 H), 4.54 (br d, 1 H, *J* = 9 Hz). Anal. ($C_{19}H_{37}NO_4$) C, H, N.

In a similar way, **19d**, **20d**, **21d**, and **22d** could be prepared.

(2*S*,3*R*,4*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-1-cyclohexyl-3,4-dihydroxypentane (19d): 21% yield; mass spectrum, $(M + H)^+$ = 302; ^{1}H NMR ($CDCl_3$) δ 0.75–1.85 (several m), 1.29 (d, 3 H, *J* = 6.5 Hz), 1.46 (s, 9 H), 3.18 (m, 1 H), 3.47 (m, 1 H), 4.04 (m, 1 H), 4.35 (br d, 1 H, *J* = 3 Hz), 4.48 (br d, 1 H, *J* = 9 Hz).

(2*S*,3*R*,4*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-1-cyclohexyl-3,4-dihydroxyhexane (20d). 19% yield; mass spectrum, $(M + H)^+$ = 316; ^{1}H NMR ($CDCl_3$) δ 0.8–1.85 (several m), 1.03 (t, 3 H, *J* = 7 Hz), 1.46 (s, 9 H), 3.15–3.30 (m, 2 H), 4.04 (br m, 1 H), 4.20 (m, 1 H), 4.53 (br d, 1 H, *J* = 9 Hz).

(2*S*,3*R*,4*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-1-cyclohexyl-3,4-dihydroxy-5-methylhexane (21d): 26% yield; mass spectrum, $(M + H)^+$ = 330; ^{1}H NMR ($CDCl_3$) δ 0.8–1.85 (several m), 0.93 (d, 3 H, *J* = 7 Hz), 1.03 (d, 3 H, *J* = 7 Hz), 1.46 (s, 9 H), 1.99 (m, 1 H), 3.15 (m, 1 H), 3.40 (m, 1 H), 3.97 (m, 1 H), 4.03 (m, 1 H), 4.56 (br d, 1 H, *J* = 9 Hz). Anal. ($C_{18}H_{35}NO_4$) C, H, N.

(2*S*,3*R*,4*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-1-cyclohexyl-3,4-dihydroxy-7-methyloctane (22d). 30% yield; mass spectrum, $(M + H)^+$ = 358; ^{1}H NMR ($CDCl_3$) δ 0.8–1.8 (several m), 0.94 (d, 3 H, *J* = 7 Hz), 0.96 (d, 3 H, *J* = 7 Hz), 1.44 (s, 9 H), 3.23 (m, 2 H), 4.04 (m, 1 H), 4.22 (br s, 1 H), 4.53 (br d, 1 H, *J* = 9 Hz). Anal. ($C_{20}H_{39}NO_4$) C, H, N.

Oxazolidinones 23a–d. The corresponding diol, 18 (253 mg, 0.736 mmol), in dry dimethylformamide (1.0 mL) was added to a stirred suspension of NaH (44 mg of a 60% dispersion in oil, 1.10 mmol, hexane-washed) in dry dimethylformamide (7 mL). After 3 h, the mixture was quenched with 0.1 M H_3PO_4 and extracted into chloroform. The combined organic phase was

washed with brine, dried ($MgSO_4$), filtered, and evaporated to give 156 mg of a white solid (79%).

(4*S*,5*S*)-4-(Cyclohexylmethyl)-5-[(1*R*)-1-hydroxy-3-methylbutyl]oxazolidin-2-one (23a): 79% yield; mass spectrum, M^+ = 269; ^1H NMR ($CDCl_3$) δ 0.7–2.0 (several m, 16 H), 0.88 (d, 3 H, J = 7 Hz), 0.92 (d, 3 H, J = 7 Hz), 3.79–3.93 (m, 2 H), 4.25 (dd, 1 H, J = 7.6, 8.2 Hz), 6.02 (br s, 1 H). Anal. ($C_{15}H_{27}NO_3$) C, H, N.

(4*S*,5*S*)-4-(Cyclohexylmethyl)-5-[(1*S*)-1-hydroxy-3-methylbutyl]oxazolidin-2-one (23b): 74% yield; mass spectrum, $(M - CHOHCH_2CH(CH_3)_2)^+$ = 183; ^1H NMR ($CDCl_3$) δ 0.8–1.95 (several m, 22 H), 3.88 (m, 1 H), 3.97 (m, 1 H), 4.38 (dd, 1 H, J = 4.3, 7.9 Hz), 5.43 (br s, 1 H).

(4*S*,5*R*)-4-(Cyclohexylmethyl)-5-[(1*R*)-1-hydroxy-3-methylbutyl]oxazolidin-2-one (23c): 68% yield; mass spectrum, $(M - H_2O)^+$ = 251; ^1H NMR ($CDCl_3$) δ 0.8–1.95 (several m, 16 H), 0.93 (d, 3 H, J = 7 Hz), 0.96 (d, 3 H, J = 7 Hz), 3.63 (m, 1 H), 3.85 (m, 1 H), 4.00 (dd, 1 H, J = 4.0, 5.4 Hz), 5.10 (br s, 1 H).

(4*S*,5*R*)-4-(Cyclohexylmethyl)-5-[(1*S*)-1-hydroxy-3-methylbutyl]oxazolidin-2-one (23d): 83% yield; mass spectrum, M^+ = 269; ^1H NMR ($CDCl_3$) δ 0.85–1.9 (several m, 16 H), 0.92 (d, 3 H, J = 7 Hz), 0.98 (d, 3 H, J = 7 Hz), 1.93 (br d, 1 H, J = 5 Hz), 3.88–3.98 (m, 2 H), 4.03 (dd, 1 H, J = 3.8, 5.2 Hz), 5.07 (br s, 1 H). Anal. ($C_{15}H_{27}NO_3$) C, H, N.

Etoc-(1)Nal-OH (25). The procedure used to prepare Etoc-(1)Nal-OH is representative of the syntheses of the other Etoc derivatives. To a stirred solution of H-(1)Nal-OH (2.00 g, 9.29 mmol) in dioxane (50 mL) and water (25 mL) was added 3 M NaOH (3.3 mL), and the resulting solution was cooled to 0 °C. Ethyl chloroformate (1.11 g, 10.22 mmol) in dioxane (10 mL) and 3 M NaOH (3.3 mL) in water (6.7 mL) were then added alternately in a portionwise manner. After stirring for 10 min at 0 °C, the reaction mixture was allowed to warm to room temperature for 1 h. The reaction mixture was then evaporated, diluted with water (75 mL), and washed with ether. The aqueous layer was acidified with 1 M H_3PO_4 to pH 2–4 and was then extracted with EtOAc (3 × 30 mL). The combined extracts were washed (brine), dried (Na_2SO_4), and concentrated in vacuo to provide 2.55 g (96%) of Etoc-(1)Nal-OH. **25**: mass spectrum, M^+ = 287. Anal. ($C_{16}H_{17}NO_4 \cdot {}^1/_4H_2O$) C, H, N.

Dipeptides 26. Boc-Phe-His-OH (**26a**),[18] Boc-Phe-(pyrazole)Ala-OH (**26b**),[19] Boc-Phe-Ala-OH (**26c**),[20] Boc-Phe-Phe-OH (**26d**),[21] and Boc-Phe-Leu-OH (**26e**)[22] were prepared as described. Etoc-Phe-Leu-OH (**26f**), Etoc-Phe-Phe-OH (**26g**), Etoc-(4-I)-Phe-Leu-OH (**26h**), Etoc-(1)Nal-Leu-OH (**26i**), and Etoc-Leu-Leu-OH (**26j**) were all synthesized by preparing the appropriate Etoc-amino acid, **25**, as described above and coupling to either Phe or Leu methyl ester by using the general mixed anhydride procedure described above. The methyl ester was then hydrolyzed according to the representative procedure used for Etoc-Phe-Phe-OCH_3. To a stirred solution of Etoc-Phe-Phe-OCH_3 (1.5 g, 3.8 mmol) in dioxane (30 mL) and water (15 mL) at 0 °C was added slowly lithium hydroxide monohydrate (0.17 g, 4.1 mmol) in water (15 mL) over 20 min. The reaction was allowed to warm to room temperature for 2 h. The mixture was then diluted with water (30 mL) and washed with ether (2 × 20 mL), and the aqueous layer was acidified with 1 M H_3PO_4 to pH 2–4. The aqueous phase was then extracted with EtOAc (2 × 50 mL), and the combined extracts were washed with brine, dried (Na_2SO_4), and evaporated to give 1.35 g (93%) of the desired compound. The product was recrystallized to homogeneity when necessary. The yield below reflects the coupling, hydrolysis, and purification steps.

Etoc-Phe-Leu-OH (26f): 75% yield; mass spectrum, M^+ = 350; mp 139–141 °C; ^1H NMR ($CDCl_3$) δ 0.92 (d, 6 H, J = 7 Hz), 1.22 (t, 3 H, J = 7 Hz), 1.45–1.7 (m, 3 H), 3.07 (m, 2 H), 4.09 (q, 2 H, J = 7 Hz), 4.45 (m, 1 H), 4.55 (m, 1 H), 5.32 (br d, 1 H, J = 8 Hz), 6.40 (br d, 1 H, J = 8 Hz), 7.15–7.33 (m, 5 H). Anal. ($C_{18}N_{26}N_2O_5$) C, H, N.

Etoc-Phe-Phe-OH (26g): 87% yield; mass spectrum, M^+ = 384; mp 154–156 °C; ^1H NMR ($CDCl_3$) δ 1.21 (br t, 3 H, J = 7 Hz), 3.02 (m, 3 H), 3.15 (dd, 1 H, J = 6, 14 Hz), 4.07 (q, 2 H, J = 7 Hz), 4.40 (m, 1 H), 4.76 (m, 1 H), 5.16 (br, 1 H), 6.45 (br d, 1 H, J = 6 Hz), 7.04 (m, 2 H), 7.13–7.33 (m, 8 H). Anal. ($C_{21}H_{24}N_2O_5 \cdot {}^1/_4H_2O$) C, H, N.

Etoc-(4-I)Phe-Leu-OH (26h): 76% yield; mass spectrum, M^+ = 476; ^1H NMR ($CDCl_3$) δ 0.92 (d, 3 H, J = 7 Hz), 0.93 (d, 3 H, J = 7 Hz), 1.23 (t, 3 H, J = 7 Hz), 1.47–1.74 (m, 3 H), 3.03 (m, 2 H), 4.10 (q, 2 H, J = 7 Hz), 4.43 (m, 1 H), 4.55 (m, 1 H), 5.25 (br, 1 H), 6.39 (br d, 1 H, J = 8 Hz), 6.96 (d, 2 H, J = 9 Hz), 7.62 (d, 2 H, J = 9 Hz). Anal. ($C_{18}H_{25}N_2O_5I$) C, H, N.

Etoc-(1)Nal-Leu-OH (26i): 78% yield; mass spectrum, M^+ = 400; mp 153–154 °C; ^1H NMR ($CDCl_3$) δ 0.87 (d, 3 H, J = 7 Hz), 0.88 (d, 3 H, J = 7 Hz), 1.22 (m, 3 H), 1.35–1.65 (m, 3 H), 3.43–3.65 (m, 2 H), 4.08 (m, 2 H), 4.46 (m, 1 H), 4.57 (m, 1 H), 5.44 (d, 1 H, J = 8 Hz), 6.0 (m, 1 H), 7.3–7.4 (m, 2 H), 7.47–7.6 (m, 2 H), 7.75 (br d, 1 H, J = 9 Hz), 7.85 (br d, 1 H, J = 9 Hz), 8.18 (br d, 1 H, J = 9 Hz). Anal. ($C_{22}H_{28}N_2O_5$) C, H, N. Another crystalline form gave mp 165–166 °C. Anal. ($C_{22}H_{28}N_2O_5 \cdot H_2O$) C, H, N.

Etoc-Leu-Leu-OH (26j): 68% yield; mass spectrum, M^+ = 316; mp 150–152 °C; ^1H NMR ($CDCl_3$) δ 0.94 (m, 12 H), 1.25 (t, 3 H, J = 7 Hz), 1.45–1.8 (m, 9 H), 4.13 (q, 2 H, J = 7 Hz), 4.22 (m, 1 H), 4.59 (m, 1 H), 5.23 (br d, 1 H, J = 8 Hz), 6.68 (br d, 1 H, J = 8 Hz). Anal. ($C_{15}H_{28}N_2O_5$) C, H, N.

(2*RS*,3*S*)-3-[(*tert*-Butyloxycarbonyl)amino]-4-cyclohexyl-1,2-dihydroxybutane (28). To a stirred solution of 2-(*S*)-[(*tert*-butyloxycarbonyl)amino]-1-cyclohexylbut-3-ene (1.00 g, 3.95 mmol) in THF (20 mL) were added OsO_4 solution (1.2 mL of a 2.5 w/v % solution in *tert*-butyl alcohol) and *N*-methylmorpholine *N*-oxide (1.07 g, 7.90 mmol). After 24 h, the mixture was partitioned between ether (50 mL) and brine (25 mL). The layers were separated, and the organic phase was extracted with ether (3 × 25 mL). The combined organic phase was washed with 10% Na_2SO_3 (4 × 10 mL), 1.0 M H_3PO_4 (2 × 8 mL), and brine 15 mL). Drying and evaporating provided the desired product as an oil (1.14 g, 100%). ^1H NMR shows a 3:2 mixture of diastereomers (NH 4.43 and 4.56 ppm, respectively). **28**: ^1H NMR ($CDCl_3$) δ 0.8–1.85 (several m, 13 H), 1.46 (s, 9 H), 3.25 (br m, 1 H), 3.43–3.9 (m, 5 H), 4.43, 4.56 (2 d, 1 H total, J = 9 and 9 Hz, 3:2 ratio, respectively).

(2*RS*,3*S*)-3-[(Boc-L-phenylalanyl-L-histidyl)amino]-4-cyclohexyl-2-hydroxy-1-[(*tert*-butyldimethylsilyl)oxy]butane (29). Compound **28** (1.10 g, 3.82 mmol) was treated with anhydrous 1 M HCl/CH_3OH (80 mL) for 16 h at which time evaporation and drying provided the corresponding amine hydrochloride (0.85 g, 100%). To a suspension of the hydrochloride salt (344 mg, 1.54 mmol) and imidazole (105 mg) in dichloromethane (15 mL) were added triethylamine (156 mg) and *tert*-butyldimethylsilyl chloride (232 mg). The solvent was evaporated after 31 h, and the residue was then redissolved in anhydrous dimethylformamide (15 mL). Boc-Phe-His-OH (619 mg) and 1-hydroxybenzotriazole (HOBT, 312 mg) were then added. After cooling of the stirred solution to −23 °C, 1,3-dicyclohexylcarbodiimide (DCC, 318 mg) was added. The mixture was warmed to room temperature 3 h later. After 13 h the solvent was evaporated in vacuo, and the residue was dissolved in ethyl acetate (40 mL), filtered, washed with saturated $NaHCO_3$ (2 × 10 mL) and brine (10 mL), and dried (Na_2SO_4). Filtration and evaporation provided a residue which was chromatographed on silica gel with dichloromethane/methanol mixtures as eluant to give 441 mg (42%) of the desired product: mass spectrum, $(M + H)^+$ = 686. Anal. ($C_{36}H_{59}N_5O_6Si$) C, H, N.

(2*R*,3*S*)-3-[(Boc-L-phenylalanyl-L-histidyl)amino]-4-cyclohexyl-1,2-dihydroxybutane (30). To a solution of **29** (200 mg, 0.291 mmol) in anhydrous THF (5 mL) at 0 °C was added tetrabutylammonium fluoride (0.58 mL of a 1 M solution in THF). The solution was warmed to room temperature for 4 h and then evaporated. The residue was dissolved in chloroform and washed

(18) Thomas, A. M. U.S. Patent 4,025,499.
(19) Rosenberg, S. H.; Plattner, J. J.; Woods, K. W.; Stein, H. H.; Marcotte, P. A.; Cohen, J.; Perun, T. J. *J. Med. Chem.* **1987**, *30*, 1224.
(20) Chorev, M.; Goodman, M. *Int. J. Pept. Protein Res.* **1983**, *21*, 258.
(21) Chang, J. K.; Shimizu, M.; Wang, S.-S. *J. Org. Chem.* **1976**, *41*, 3255.
(22) Bower, J. D.; Guest, K. P.; Morgan, B. A. *J. Chem. Soc., Perkin Trans. 1* **1976**, 2488.

with water (3×) and brine (1×). Drying and evaporating provided a gum which was treated with hot ethyl acetate (8 mL). Cooling and filtration provided a 25-mg first crop (15%) of the desired material: mass spectrum, $(M + H)^+ = 572$; ^1H NMR ($CD_3OD/CDCl_3$, 1:1) δ 0.73–1.8 (several m), 1.37 (s, 9 H), 2.86 (m, 1 H), 3.04 (m, 2 H), 3.14 (dd, 1 H, J = 5, 14 Hz), 3.3–3.6 (m, 3 H), 4.03 (m, 1 H), 4.27 (dd, 1 H, J = 5, 9 Hz), 4.54 (m, 1 H), 6.03 (br s, 1 H), 7.2–7.35 (m, 5 H), 7.53 (br s, 1 H). Anal. ($C_{30}H_{45}N_5O_6\cdot{}^1/_2H_2O$) C, H, N.

Biological Methods. A. In Vitro Assays. 1. Enzyme Inhibition. The inhibitory activity of the compounds against purified human renal renin, porcine pepsin, and bovine cathepsin D was determined as described previously.[8a,23] Human gastricsin and pepsin were obtained from gastric juice by IRC-50 ion exchange chromatography and pH elution as described by Tang;[24] the specific activities were 0.055 absorbance unit/min per μg of protein (pH 3.1) and 0.022 absorbance unit/min per μg of protein (pH 2.0), respectively, when hemoglobin served as substrate and the absorbance of the supernatant was measured at 280 nm after precipitation with trichloroacetic acid. The effect of the compounds on these enzymes was assessed by the same procedure as that utilized for the determination of the specific activities.

2. Quantitative Assay of Renin Inhibitor 43 in Blood Samples by Extraction/Enzyme Inhibition Assay. The cells and proteins in whole blood samples (100 μL) were precipitated by addition of 300 μL of acetonitrile, followed by vigorous mixing. The supernatant from the precipitated, centrifuged samples was diluted sequentially in buffer (typically 1:4, 1:16, 1:64, 1:256) and then evaporated. To the dry residue were added one stock solution (50 μL) containing purified human angiotensiongen[25] (ca. 500 ng) and a second solution (50 μL) containing either purified human renin[26] (ca. 0.2 ng) or renin-absent buffer controls. The buffer used for both stock solutions was 0.135 M sodium maleate, pH 6.0, containing 0.0015 M EDTA, 0.046 M NaCl, and 1% bovine serum albumin. A 10-μL aliquot of the incubation, after 30 min at 37 °C, was subjected to radioimmunoassay for angiotensin I using the NEN kit. The control assay, which excludes renin, determined the angiotensin I level of the sample and any other immunoreactive substance in the extract or reagents. The extent of the inhibition of the assay was used to quantitate the concentration of renin inhibitor present in the extract, based on the following equation (derived from the kinetic equation of competitive enzyme inhibition): $[\text{inhibitor}] = (V_0/V_{inh} - 1) \times IC_{50}$. The concentration of compound in the blood sample was determined by multiplication of the calculated value in the assay by the overall dilution factor. Compound 43 was found to extract quantitatively from whole blood with acetonitrile and to inhibit the reconstituted renin assay according to the predicted kinetics (IC_{50} = 0.50 ng/mL after extraction from whole blood).

B. In Vivo Assays. 1. Intravenous and Intraduodenal Activity in Salt-Depleted Monkeys. Intravenous activity was determined in male cynomolgus monkeys (*Macaca fascicularis*), with average weights of 4 kg. The monkeys were salt-depleted by feeding them a low-salt chow and fresh fruit diet, supplemented by furosemide treatment (5 mg/kg, po) 1 week and 1 day before the experiment. The monkeys, which were fasted overnight, were studied under sodium pentobarbital anesthesia (15 mg/kg bolus + 0.1 mg/kg per min maintenance iv infusion). A femoral artery was cannulated in order to measure continually blood pressure and heart rate (Grass Pressure Transducer Model P23dB and Grass Polygraph Model 7, Grass Instruments, Quincy, MA) and to withdraw blood samples for the determination of plasma renin activity.[27] Compounds were introduced into the venous circulation via a leg vein injection or administered intraduodenally by direct injection. Compound 43 was given in a 5% dextrose solution as the hydrochloride salt.

2. Oral Absorption in Rats. Male Sprague–Dawley rats weighing at least 150 g were anesthetized with Brevital (50 mg/kg, ip), and a PE 50 catheter was placed in the carotid artery and exited through the back of the neck. The rats were allowed to recover from anesthesia (minimum of 2 h), and a time zero control blood sample (0.5 mL) was obtained. Each animal was then immediately dosed with 10 mg/kg of the experimental compound by oral gavage, using a blunt-tip needle and syringe. Blood samples (0.5 mL) were taken at 5, 30, 60, 120, and 180 min following dosing and replaced with equal volumes of heparinized (20 units/mL) Normosol. Drug blood levels were determined by enzyme inhibition assay (as described above). Compound 43 was prepared as the hydrochloride salt in 5% dextrose.

3. Oral Activity in 2 Kidney–1 Clip Goldblatt Monkeys (2K–1C). Several monkeys were rendered renin dependent and hypertensive. Under sodium pentobarbital anesthesia, one renal artery was constricted with a silver clip. The opposite renal artery was left untouched. Blood pressure was monitored noninvasively with a pediatric cuff containing a microphone and an electro sphygmomanometer (Narco Systems, Houston, TX). A blood pressure elevation of approximately 30 mmHg was attempted, and the degree of constriction was determined by the acute blood pressure response. Monkeys that remained hypertensive 2 weeks postsurgery were studied in a conscious chaired state, and blood pressure was monitored on the tail. Following a control period, 10 mg/kg of **45** was administered in a piece of banana and the response was followed for a minimum of 6 h.

C. Drug Metabolism Studies. Compound [^{3}H]-**45**, labeled with tritiated phenylalanine, had a specific activity of 1 mCi/mg and was diluted with unlabeled **45** prior to use. Male Sprague–Dawley derived rats, weighing 0.20–0.27 kg, were given a 0.3 mg/kg intraarterial dose or a 0.3 or 5 mg/kg oral dose of [^{3}H]-**45** (~30–60 μCi/rat), dissolved in ethanol. Plasma samples were obtained by cardiac puncture at designated times after dosing. Urine and feces excreted by other rats were collected for 1 or 2 days after drug administration. Bile was obtained from other rats given [^{3}H]-**45** (0.3 mg/kg) intragastrically after surgical implantation of a bile duct cannula under diethyl ether anesthesia. The feces were homogenized in 70% aqueous ethanol, and aliquots were burned in a sample oxidizer. All samples were assayed for total radioactivity by liquid scintillation spectrometry and corrected for quenching with an external standard. Most samples contained negligible quantities of tritiated water, as determined by lyophilization and radioassay of the distillates.

Metabolic patterns in plasma, bile, urine, and fecal samples were determined by high-pressure liquid chromatography on a C18 column. The mobile phase used for the plasma samples was a concave gradient of acetonitrile, tetrahydrofuran, and 0.05 M ammonium acetate from 15:1:84 to 70:1:29 (volume) over a 40-min period. The mobile phase used for the urine, bile, and fecal samples was a linear 25–75% aqueous acetonitrile gradient containing 1% trifluoroacetic acid over a period of 40 min. In both cases, the gradients were followed by isocratic elution with the final solutions for 10 min. The column effluent was collected in 1-mL (1 min) fractions, which were radioassayed by liquid scintillation spectrometry. Radioactive peaks were tentatively identified by comparison of the retention times with those of authentic reference compounds, based on their absorbance at 215 nm.

Acknowledgment. The assistance of Jerome Cohen (in vitro renin inhibition), Edwin Johnson (intratracheal administration to dogs), Gary Rotert (purification of tritiated **45**), Marianne Johnson (metabolism studies), Donald Martin, Gary Young, Dean Gibbons, and Marcia Checkal (pharmacology) is gratefully acknowledged.

(23) Luly, J. R.; Plattner, J. J.; Stein, H.; Yi, N.; Soderquist, J.; Marcotte, P. A.; Kleinert, H. D.; Perun, T. J. *Biochem. Biophys. Res. Commun.* **1987**, *143*, 44.
(24) Tang, J. In *Methods in Enzymology*; Perlmann, G.; Lorand, L., Eds.; Academic: New York, 1970; Vol. XIX, pp 406–421.
(25) Dorer, F. E.; Lentz, K. E.; Kahn, J. R.; Levine, M.; Skeggs, L. T. *Anal. Biochem.* **1978**, *87*, 11.
(26) Stein, H. H.; Fung, A. K. L.; Cohen, J. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1985**, *44*, 1363.
(27) Preibisz, J.; Sealey, J. E.; Aceto, R. M.; Laragh, J. H. *Cardiovasc. Rev. Rep.* **1982**, *3*, 787.