H-2′ and H-4′), 6.04 (d, J = 1.1 Hz, H-1′), 6.83 (br s, 2 H, NH_2), 7.23 (m, trityl), 8.13 (s, 1 H, H-2), 8.27 (s, 1 H, H-8).

9-(2-Fluoro-2,3-dideoxy-β-D-*threo*-pentofuranosyl)adenine (11). A mixture of 493 mg (1 mmol) of 5′-*O*-tritylcordycepin and 0.5 mL of DAST in anhydrous CH_2Cl_2 (10 mL) was refluxed for 3 h, cooled to room temperature, and poured into a $NaHCO_3$ solution (10%). The organic layer was separated, dried, and evaporated. The resulting oil was dissolved in 80% acetic acid, heated for 20 min at 100 °C, and evaporated. The reaction mixture was purified by preparative TLC ($CHCl_3$–MeOH, 85:15, two developments), and trace amounts of an unidentified side compound were removed by chromatography on a XAD column (100–200 μm) with EtOH–H_2O (1:9) as eluent. The UV-absorbing fractions were collected, and the solvent was removed by lyophilization, giving 25 mg (0.1 mmol, 10%) of **11**: UV (MeOH) λ_{max} 258 nm (ε 15 100); MS, m/e (relative intensity) 253 (19.3, M^+), 223 (0.9, M – CH_2O), 222 (4.9, M – CH_2OH), 202 (9.8, M – CH_2OH – HF), 164 (66.4, BCHO + H), 135 (100, B + H), 108 (32.8, B + H – HCN); ^1H NMR (DMSO-d_6) δ 2.00–2.92 (m, H-3′ and H-3″), 3.57 (m, H-5′ and H-5″), 4.23 (m, H-4′), 5.41 (m, $J_{2',F}$ = 54.7 Hz, H-2′), 6.28 (dd, $J_{1',2'}$ = 3.6 Hz, $J_{1',F}$ = 16.7 Hz, H-1′), 7.27 (br s, NH_2), 8.16 (s, H-2), 8.23 (d, J = 2.4 Hz, H-8); ^{13}C NMR (DMSO-d_6) 139.5 (d, J = 4.9 Hz, C-8), 152.7 (C-2) ppm. Anal. ($C_{10}H_{12}N_5O_2F$) C, H, N.

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Registry No. 1, 4097-22-7; **2**, 110143-00-5; **3**, 110142-98-8; **4**, 110143-01-6; **5**, 110143-04-9; **6**, 110142-99-9; **7**, 79872-72-3; **8**, 66323-44-2; **9**, 110143-03-8; **9** (6-*N*-benzoyl), 110143-02-7; **10**, 110143-05-0; **11**, 110143-10-7; **12**, 87418-35-7; **13**, 110143-09-4; **14′**, 51763-58-7; **15**, 6998-75-0; **15′**, 110142-97-7; **17′**, 108895-39-2; **18**, 7057-48-9; **20**, 110142-94-4; **21**, 110142-95-5; **22**, 110142-96-6; **23**, 10992-57-1; **27**, 20535-16-4; **28**, 110143-06-1; **29**, 110143-07-2; **30**, 110143-08-3; adenosine, 58-61-7; cordycepin, 73-03-0; 6-*N*-benzoyl-5′-*O*-(monomethoxytrityl)-2′-deoxyadenosine, 24816-13-5; tritylcordycepin, 90813-62-0.

Optimization and in Vivo Evaluations of a Series of Small, Potent, and Specific Renin Inhibitors Containing a Novel Leu-Val Replacement[1,2]

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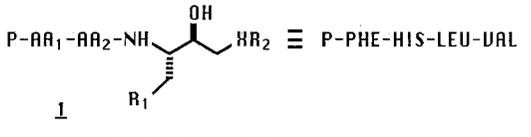
Further structure–activity relationships (SAR) for a novel dipeptide series of renin inhibitors are reported. These inhibitors retain the Phe^8-His^9 portion of angiotensinogen and employ a unique Leu^{10}-Val^{11} replacement [(LVR), ref 2]. SAR at the Leu^{10} side chain revealed that the LVR derived from cyclohexylalanine provided a nearly 10-fold boost in potency for the final inhibitor. In addition SAR work was carried out to delineate the relationships between binding potency and (1) the size, shape, and charge of the side chain at the His^9 position; (2) the size and topology of the side chain at the Phe^8 site; and (3) the size of the Phe^8 N-protecting group. One of the more potent inhibitors, **12**, was shown to provide a substantial antihypertensive effect in a sodium depleted monkey model when administered intravenously. Metabolism work, in Sprague–Dawley rats, provided insights into the susceptibility of **12** to significant hepatic clearance and provided encouraging evidence for intestinal absorption.

A major regulatory mechanism for the maintenance of blood pressure in mammals is the renin–angiotensin system (RAS).[3,4,5a] The RAS is a multiregulated cascade of enzyme mediated proteolytic events that converts angiotensinogen to angiotensin II (AII) and angiotensin III (AIII), the principle pressor agents of the system (Scheme I). The major pharmacological effects of AII and AIII are vasoconstriction and stimulation of the adrenal cortex to release aldosterone, which in turn induces sodium retention. Inhibition of angiotensin converting enzyme (ACE),[6] which catalyzes the second step in the RAS, has established that obstruction of this system prior to formation of AII or AIII can effectively reduce blood pressure in hypertensive patients.

The success of ACE inhibitors as antihypertensive agents has encouraged many to seek an inhibitor of the first step of the RAS, namely, the renin catalyzed cleavage of angiotensinogen to angiotensin I (AI). In previous work from our laboratories,[2] a novel series of small, potent, and selective renin inhibitors of the general structure **1** was



1

disclosed (in which P = an N-protecting group; AA_1 and AA_2 = amino acid residues; X = NH, O, CH_2, or SO_n (n = 0 or 2); R_1 = isopropyl; and R_2 = a lipophilic group). These inhibitors were designed as "transition-state

(1) Abbreviations follow IUPAC-IUB Commission on Biochemical Nomenclature for amino acids and peptides: *Eur. J. Biochem.* **1984**, *158*, 9–31. Additional abbreviations used are as follows: Ac, acetyl; Bn, benzyl; BSA, bovine serum albumin; Cbz, benzyloxycarbonyl; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; Etoc, (ethyloxy)carbonyl; Ibu, isobutyryl; LVR, Leu-Val replacement; MCPBA, *m*-chloroperoxybenzoic acid; PMSF, phenylmethanesulfonyl fluoride; Poa, phenoxyacetyl; sg, silica gel; Tba, *tert*-butylacetyl.

(2) For the previous paper in this series, see: Luly, J. R.; Yi, N.; Soderquist, J.; Stein, H.; Cohen, J.; Perun, T. J.; Plattner, J. J. *J. Med. Chem.* **1987**, *30*, 1609.

(3) Peach, M. *Physiol. Rev.* **1977**, *57*, 313.

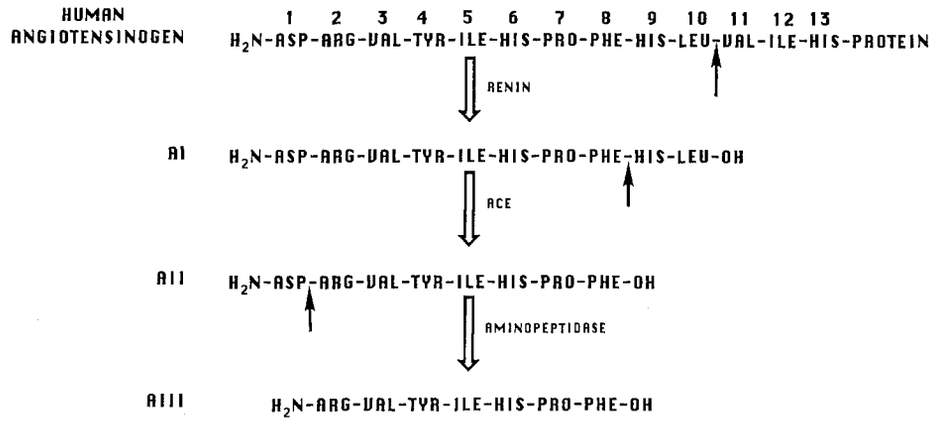
(4) Ondetti, M. A.; Cushman, D. W. *Annu. Rev. Biochem.* **1982**, *51*, 283.

(5) For other relevant literature, refer to the preceding paper in this series, ref 2 this paper: (a) ref 1–3; (b) ref 13–15 provided historical background concerning the previous use of transition-state analogues in renin inhibitors.

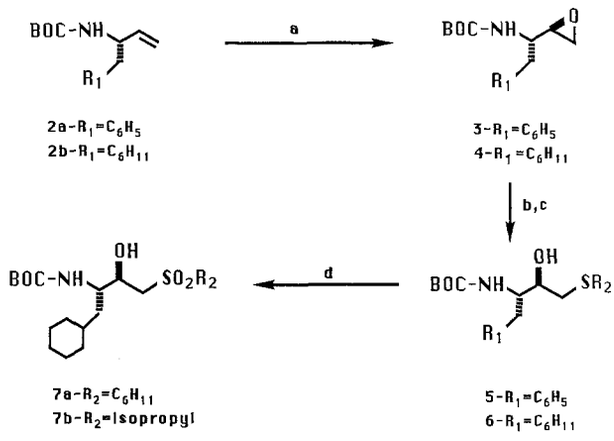
(6) For a recent review, refer to: Wyvratt, M. J.; Patchett, A. A. *Med. Res. Rev* **1985**, *5*, 483.

0022-2623/87/1830-2137$01.50/0

Scheme I. Renin-Angiotensin System



Scheme II[a]



[a] (a) MCPBA (2.5 equiv); (b) R_2SH, Et_3N, MeOH; (c) separation; (d) MCPBA (2.2 equiv).

analogues"[5b] of angiotensinogen, where the P-AA_1-AA_2 portion mimics the Phe[8]-His[9] positions of angiotensinogen with the remainder being a novel Leu[10]-Val[11] replacement (LVR). We now wish to reveal the results of continued investigations of this exciting series of inhibitors. The structure–activity relationships (SAR) revealed herein are complementary to those previously reported[2] and have led to further increases in potency. In addition, whole animal studies, for one of the most potent inhibitors, will address the antihypertensive efficacy in an anesthetized monkey model and metabolic patterns in rats.

Chemistry

Synthesis: Leu[10]-Val[11] Replacement (LVR). The preparation of the LVR parallels the previously described work[2] and is summarized in Scheme II. The epoxides **3** and **4** were obtained in a highly enantio- and diastereoselective synthesis starting from the corresponding *N*-*tert*-butyloxycarbonyl L-amino alcohols via olefins **2a** and **2b**, as recently described;[7] this provided the threo epoxides (threo/erythro ≥ 11:1) having ≥99% ee. The epoxides were readily opened with the mercaptans in the presence of triethylamine in refluxing methanol to afford a diastereomeric mixture at the hydroxyl center (introduced in the epoxidation), which was separated at this stage via chromatography to provide the desired 2(*R*)-hydroxy-LVR, **5** or **6**, as single diastereomers. Oxidation to the sulfone **7** was achieved by treatment with 2.2 molar equiv of *m*-chloroperoxybenzoic acid (MCPBA). The Boc protecting group of the desired LVR (**5–7**) was removed (EtOH/HCl, room temperature, 1–2 h), and the resulting salts were utilized without further purification.

Synthesis: Final Inhibitors. The final renin inhibitors were prepared by standard solution phase peptide chemistry. Sequential coupling of the two amino acids to the desired LVR minimized racemization often encountered when the corresponding dipeptide (P_1-AA_1-AA_2-OH) was coupled to the LVR. Purification by normal phase chromatography and/or recrystallization provided the final inhibitors for biological evaluation. The diastereomeric purity of the final inhibitors was accurately reflected by doubling of various signals in the 300-MHz ^1H NMR. The detection limits were estimated to be ±3% by comparison of analytical HPLC and ^1H NMR results on selected compounds. The imidazole methine signals (singlets at ≃7.4 and 6.8 ppm), the *tert*-butyl signals of *N*-*t*-Boc-protected inibitiors, and amide and urethane NH signals (≃5–7 ppm in $CDCl_3$) all proved to be sensitive indicators of the diastereomeric purity of the final inhibitors. In all cases the 300-MHz ^1H NMR spectrum of the final inhibitors for testing indicated these compounds to be single homogeneous diastereomers.

Biological Results and Discussion

In Vitro Enzyme Inhibition. The inhibitory potencies, obtained at pH 6, and chemical data for the new compounds are summarized in Tables I and II. The first two compounds **7** and **8** were designed to investigate the leucine pocket in the LVR. As others have found,[8] this site provides a sequential boost in potency from 35 nM to 15 nM and 4 nM by exchanging the isopropyl side chain[2] for a phenyl or a cyclohexyl moiety, respectively. Further investigations at this site will be the topic of a future disclosure.[9] Subsequent SAR work was carried out on the cyclohexyl replacement at the leucine site of the LVR to take advantage of the attendant potency boost.

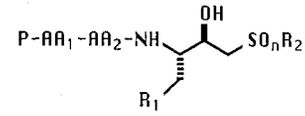
Compounds **8–14** were prepared to probe the requirements for lipophilicity, size, and oxidation state of the SO_nR_2 portion of the LVR. These results indicate that branching α to the sulfur (S or SO_2) is preferable and that when R_2 is cyclohexyl or isopropyl, the inhibitors are virtually equipotent (compare **8** versus **10** and **12** versus **13**). The importance of the 2(*R*)-hydroxyl group was es-

(7) Luly, J. R.; Dellaria, J. F.; Plattner, J. J.; Soderquist, J. L.; Yi, N. *J. Org. Chem.* **1987**, *52*, 1487.

(8) (a) Boger, J.; Payne, L. S.; Perlow, L. 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.

(9) Unpublished results: Luly, J. R.; Dellaria, J. F.

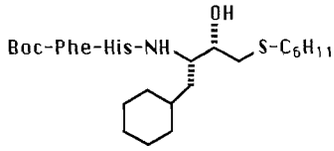
Table I. Inhibitors and in Vitro Activities Obtained at pH 6



no.	P	AA_1	AA_2	R_1	n	R_2	renin IC_{50}, nM	% inhibn at 10^{-5} M: bovine cathepsin D	% inhibn at 10^{-5} M: porcine pepsin
7	Boc	Phe	His	C_6H_6	0	C_6H_{11}	15	0	4
8	Boc	Phe	His	C_6H_{11}	0	C_6H_{11}	4.0	0	9
9	Boc	Phe	His	C_6H_{11}	0	$CH_2CH(CH_3)_2$	6.5	–	–
10	Boc	Phe	His	C_6H_{11}	0	$CH(CH_3)_2$	4.0	0	0
11	Boc	Phe	His	C_6H_{11}	0	CH_3	10	–	–
12	Boc	Phe	His	C_6H_{11}	2	C_6H_{11}	2.5	0	0
13	Boc	Phe	His	C_6H_{11}	2	$CH(CH_3)_2$	2.0	0	0
14	Boc	Phe	His	C_6H_{11}	2	CH_3	40	–	–
15[b]	Boc	Phe	His	C_6H_{11}	0	C_6H_{11}	100	–	–
16	Boc	Phe	Ala	C_6H_{11}	0	$CH(CH_3)_2$	9.9	–	–
17	Boc	Phe	Ala	C_6H_{11}	2	$CH(CH_3)_2$	70	55	0
18	Boc	Phe	Leu	C_6H_{11}	2	$CH(CH_3)_2$	4.0	–	–
19	Boc	Phe	Phe	C_6H_{11}	2	$CH(CH_3)_2$	30	0	4
20	Boc	Phe	Thr	C_6H_{11}	2	$CH(CH_3)_2$	8.0	–	–
21	Boc	Phe	Ser	C_6H_{11}	2	$CH(CH_3)_2$	40	47	0
22	Boc	Phe	Hse	C_6H_{11}	2	$CH(CH_3)_2$	20	–	–
23	Boc	Phe	(Bn)Thr	C_6H_{11}	2	$CH(CH_3)_2$	6.0	95	18
24	Boc	Phe	(Cbz)Orn	C_6H_{11}	2	$CH(CH_3)_2$	60	–	–
25	Boc	Phe	Orn	C_6H_{11}	2	$CH(CH_3)_2$	(43% at 10^{-6} M)[a]	–	–
26	Boc	Phe	(Cbz)Lys	C_6H_{11}	2	$CH(CH_3)_2$	100	–	–
27	Boc	Phe	Lys	C_6H_{11}	2	$CH(CH_3)_2$	(34% at 10^{-6} M)[a]	–	–
28	Boc	Phe	(Ac)Lys	C_6H_{11}	2	$CH(CH_3)_2$	300	–	–
29	Boc	D-Phe	His	C_6H_{11}	2	$CH(CH_3)_2$	150	0	0
30	Boc	(Bn)Ser	His	C_6H_{11}	2	$CH(CH_3)_2$	75	–	–
31	Boc	(Bn)Thr	His	C_6H_{11}	2	$CH(CH_3)_2$	5.5	0	0
32	Etoc	(Bn)Thr	His	C_6H_{11}	2	$CH(CH_3)_2$	20	–	–
33	Boc	(Me)Tyr	His	C_6H_{11}	2	$CH(CH_3)_2$	3.0	0	0
34		Poa	His	C_6H_{11}	0	C_6H_{11}	430	–	–
35		Boc	His	C_6H_{11}	2	C_6H_{11}	(5% at 10^{-5} M)[a]	–	–
36		Dba	His	C_6H_{11}	0	C_6H_{11}	20	0	0
37		Dba	His	C_6H_{11}	2	C_6H_{11}	40	–	–
38		Dba	Leu	C_6H_{11}	2	$CH(CH_3)_2$	25	–	–
39		Dba	His	C_6H_{11}	2	$CH(CH_3)_2$	70	0	0
40		H-(Bn)Thr	His	C_6H_{11}	2	$CH(CH_3)_2$	300	–	–
41	Tba	Phe	His	C_6H_{11}	2	$CH(CH_3)_2$	3.0	0	0
42	HCl·Tba	Phe	His	C_6H_{11}	2	$CH(CH_3)_2$	3.5	0	0
43	Etoc	Phe	His	C_6H_{11}	2	$CH(CH_3)_2$	5.0	–	–
44	Ibu	(Bn)Thr	His	C_6H_{11}	2	$CH(CH_3)_2$	10	–	–

[a] $IC_{50} > 10^{-6}$ M were not determined. The data in parentheses for these compounds are the % inhibitions measured at the noted concentrations. [b] The LVR bears a 2(*S*)-hydroxy group in this inhibitor.

tablished by compound **15**, in which the opposite 2(*R*)-hydroxy configuration led to a dramatic reduction in the potency of the resulting inhibitor.



15

In an attempt to assess the role(s) of the imidazole side chain in the His^9 site,[10] compounds **16–28** were prepared. With respect to size, this site is tolerant of large size deviations (**20** versus **23**). However, it is clear that size is not the only contributing factor, since the Phe^9 replacement (**19**) should be sterically similar to His^9 (**10**), yet there is a factor of 8 difference in their binding potencies. Reasoning that hydrogen bonding might be important, we prepared compounds **20–22**. The His^9 replacements Ala^9 (**17**), Ser^9 (**21**), and Thr^9 (**20**) provided a series of compounds where the side chain was systematically increased. In going from Ala^9 (**17**) to Ser^9 (**21**) the exchange of a hydrogen for a hydroxyl group led to a nearly twofold increase in potency (IC_{50} = 70 vs 40, respectively). Exchange of a hydrogen for a methyl group in going from Ser^9 (**21**) to Thr^9 (**20**) provided a fivefold increase in inhibitory potency. Increasing the side-chain length in $Hser^9$ (**22**) improved the IC_{50} by a factor of 2 and nearly 4 relative to Ser^9 (**21**) and Ala^9 (**17**), respectively. These results indicate that hydrogen bonding plays a small role in effective binding at this site but that a lipophilic interaction is more important (Thr^9 vs Ser^9). This is further demonstrated by the Leu^9 (**18**) inhibitor, which is twofold more potent than Thr^9 (**20**).

The charge preference at His^9 was explored by preparing the protected and deprotected forms of ornithine ($(CH_2)_3NH_2$) and lysine ($(CH_2)_4NH_2$). In each instance, revealing the basic nitrogen led to a large drop in potency (**24** versus **25**; **26** or **28** versus **27**), indicating that a positive charge (testing was performed at pH 6; see the Experi-

(10) Other work from these laboratories has investigated various heterocyclic replacements for the imidazole residue; see: 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.

Table II. Chemical Characterizations of the Inhibitors

no.	TLC[a] R_f	formula[b]
7	0.44 (A)	$C_{36}H_{49}N_5O_5S$
8	0.23 (B)	$C_{36}H_{55}N_5O_5S{\cdot}0.5H_2O$
9	0.30 (A)	$C_{34}H_{53}N_5O_5S{\cdot}0.5H_2O$
10	0.28 (A)	$C_{33}H_{51}N_5O_5S$[d]
11	0.18 (B)	$C_{31}H_{47}N_5O_5S$
12	0.36 (A)	$C_{36}H_{55}N_5O_7S{\cdot}0.5OH_2O$
13	0.30 (A)	$C_{33}H_{51}N_5O_7S{\cdot}0.75H_2O$
14	0.40 (A)	$C_{31}H_{47}N_5O_7S{\cdot}1.0H_2O$
15	0.27 (A)	$C_{36}H_{55}N_5O_5S{\cdot}1.25H_2O$[c,d]
16	0.39 (B)	$C_{30}H_{49}N_3O_5S{\cdot}0.25H_2O$
17	0.53 (A)	$C_{30}H_{49}N_3O_7S{\cdot}1.25H_2O$[c]
18	0.29 (A)	$C_{33}H_{55}N_3O_7S{\cdot}1.5H_2O$
19	0.34 (B)	$C_{36}H_{53}N_3O_7S{\cdot}0.5H_2O$
20	0.28 (A)	$C_{31}H_{51}N_3O_8S{\cdot}1.0H_2O$
21	0.18 (B)	$C_{30}H_{49}N_3O_8S$
22	0.16 (D)	$C_{31}H_{51}N_3O_8S$
23	0.42 (A)	$C_{38}H_{57}N_3O_8S$
24	0.33 (B)	$C_{40}H_{60}N_4O_9S$
25	0.24 (D)	$C_{32}H_{54}N_4O_7S$[d]
26	0.42 (B)	$C_{41}H_{62}N_4O_9S$[c]
27	0.17 (C)	$C_{33}H_{56}N_4O_7S{\cdot}5.5H_2O$[c,d]
28	0.28 (B)	$C_{35}H_{58}N_4O_8S$
29	0.30 (A)	$C_{33}H_{51}N_5O_7S$
30	0.45 (A)	$C_{34}H_{53}N_5O_8S$[d]
31	0.23 (B)	$C_{35}H_{55}N_5O_8S$
32	0.38 (A)	$C_{33}H_{51}N_5O_8S$
33	0.23 (B)	$C_{34}H_{53}N_5O_8S$
34	0.40 (A)	$C_{30}H_{44}N_4O_4S$
35	0.24 (A)	$C_{27}H_{46}N_4O_6S$
36	0.23 (B)	$C_{38}H_{52}N_4O_3S$[d]
37	0.45 (C)	$C_{38}H_{52}N_4O_5S{\cdot}0.25H_2O$
38	0.38 (B)	$C_{35}H_{52}N_2O_5S$
39	0.49 (C)	$C_{35}H_{48}N_4O_5S$[d]
40	0.15 (B)	$C_{30}H_{47}N_5O_6S{\cdot}1.1H_2O$
41	0.43 (C)	$C_{34}H_{53}N_5O_6S{\cdot}2.5H_2O$
42	0.59 (E)	$C_{34}H_{53}N_5O_6{\cdot}HCl{\cdot}1.4H_2O$
43	0.48 (C)	$C_{31}H_{47}N_5O_7S{\cdot}1.3H_2O$
44	0.59 (C)	$C_{34}H_{53}N_5O_7S{\cdot}2.5H_2O$

[a] Solvent systems A–D were the following mixtures of methanol/chloroform: (A) 10%, (B) 5%, (C) 15%, and (D) 25%. Solvent system E was 4:1:4 toluene/1-butanol/acetic acid. [b] Elemental analyses within ±0.4% of the calculated values for C, H, N were obtained for these compounds unless noted otherwise. [c] **15**, N: calcd, 10.11; found, 8.65. **17**, N: calcd, 6.79; found, 6.33. **26**, N: calcd, 7.12; found, 6.54. **27**, H: calcd, 8.98; found, 7.47. **44**, H: calcd, 8.11; found, 7.37. [d] Exact mass determinations within ±4 ppm were obtained for these compounds: **10** ($C_{33}H_{52}N_5O_5S$; M + H); **15** ($C_{36}H_{56}N_5O_5S$; M + H); **25** ($C_{32}H_{56}N_4O_7S$; M + H); **27** ($C_{33}H_{57}N_4O_7S$; M + H); **30** ($C_{34}H_{54}N_5O_8S$; M + H); **36** ($C_{38}H_{53}N_4O_3S$; M + H); **39** ($C_{35}H_{49}N_4O_5S$; M + H).

mental Section for details) was detrimental at this site. However, the protected forms **24**, **26**, and **28** once again demonstrated an ability of this pocket to accommodate large side chains.

Substitutions at the Phe[8] site were made to investigate the size and topological requirements of this position. Replacement of L-Phe with D-Phe resulted in a 75-fold loss in potency (**13** versus **29**), revealing the importance of the side-chain spatial orientation. Larger side chains carried by *O*-benzylthreonine derivatives and *O*-methyltyrosine were well-tolerated. Interestingly, the *O*-benzylserine replacement **30** was unique in suffering a 38-fold loss in potency relative to the parent compound **13**. The deletion of Phe[8] (**35**) resulted in the virtual loss of binding potency. Finally, two achiral replacements for Boc-Phe were prepared. The dibenzylacetyl (Dba) moiety provided inhibitors that suffered only 10–35-fold losses in potency (**36–39**) in contrast to the phenoxyacetyl (Poa) replacement **34**, which lost 2 orders of magnitude in binding potency.

An abbreviated excursion was made to investigate the N-terminal protecting group. The presence of an N-protecting group appears to be necessary, as deprotection

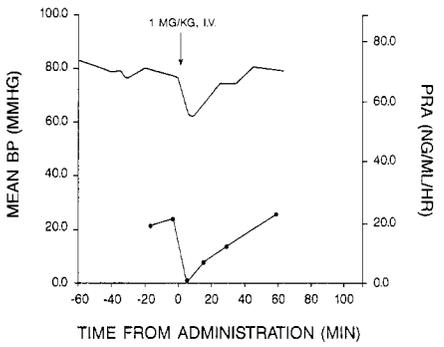


Figure 1. Representative intravenous effect of **12** in a salt-depleted, anesthetized monkey. (—) Mean bp; (·–·) PRA.

affords a less potent inhibitor (**31** versus **40**) by a factor of 55. Introduction of the *tert*-butylacetyl in lieu of the *tert*-butyloxycarbonyl protecting group (**41**, **42**) preserved activity and afforded an acid stabilized form of the inhibitor. The ethoxycarbonyl (Etoc) and isobutyryl (Ibu) protecting groups (**43** and **44**) suffered slight losses in potency, ca. twofold in each case, in exchnage for less lipophilicity. These findings revealed that a N-terminal protecting group at the Phe[8] position appears to be reasonably tolerant to size considerations and is necessary for good inhibitory potency.

The specificity of selected inhibitors toward renin over bovine cathepsin D and porcine pepsin, two related aspartic proteinases, is summarized in Table I. In all but one instance (**23**), these inhibitors demonstrated insignificant inhibition at 10^{-5} M toward these two latter enzymes. Compounds that have small and/or lipophilic side chains (**17**, **19**, **21**, and **23**) at the His[9] site tend to have low but measurable inhibition at this concentration. Compounds **12** and **23** were evaluated against human cathepsin D and pepsin. Virtually no inhibitory activity for **12** was observed at 10^{-5} M for either enzyme, 2% and 0%, respectively. On the other hand, **23** provided 74% and 54% inhibition against cathepsin D at 10^{-6} and 10^{-7} M, respectively, and 18% inhibition at 10^{-5} M for pepsin. Thus, these compounds are potent inhibitors of renin and exhibit excellent enzyme specificity over other related aspartic proteinases.

Pharmacological Evaluation. On the basis of the SAR results, compound **12** was chosen for further evaluation in whole animal models for efficacy as an antihypertensive agent and the associated metabolic patterns. These inhibitors were found to be highly species specific; thus an intravenous (iv) administration monkey model was developed to demonstrate the blood pressure lowering ability of this compound. Subsequently a metabolism study was undertaken in rats to provide preliminary indications on the oral bioavailability, gi stability, and plasma stability of this class of compounds.

Intravenous Monkey Model. The potential of compound **12** as an antihypertensive agent was demonstrated by iv administration to anesthetized, sodium-depleted, male cynomolgus monkeys (*Macaca fascicularis*). The studies were carried out at doses of 1.0 and 10.0 mg/kg. The drug was administrated as a single iv bolus injection (n = 3 each group). A representative experiment at the 1.0 mg/kg dose is shown in Figure 1. The summary of all experiments (mean ± SE) is presented in Figure 2, with the corresponding base-line values tabulated in Table III. Data are expressed as percent control, since base-line measurements are variable in monkeys. Each dose level provided subsequent hypotensive responses. The magnitude of the initial drop in blood pressure and the duration of the hypotensive action were greater at the higher

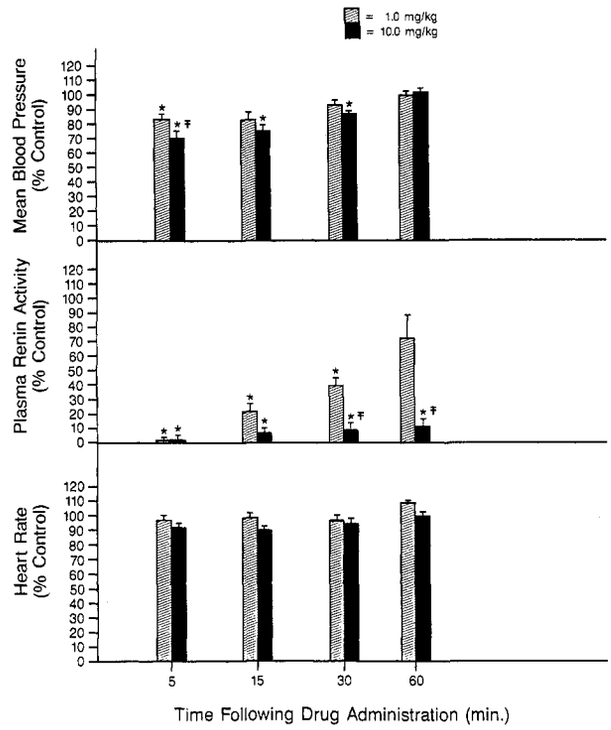


Figure 2. Physiological effects of **12** after iv administration in salt-depleted, anesthetized monkeys. Effects of two doses of **12** administered as iv bolus injections to sodium-depleted, anesthetized monkeys. Data are expressed as mean ± SE (n = 3, each group). (★) Significance was accepted at $P < 0.05$ as compared to control. ∓ represents differences between groups at $P < 0.05$.

Table III. Control Values in Salt-Depleted, Anesthetized Monkeys[a]

	mean blood pressure, mmHg	plasma renin act., ng mL^{-1} h^{-1}	heart rate, beats/min
group 1 = 1.0 mg/kg (n = 3)	68 ± 3	35.3 ± 3.6	140 ± 6
group 2 = 10.0 mg/kg (n = 3)	73 ± 2	107.7 ± 29.7	197 ± 4[b]

[a] Data are expressed as mean ± SE. [b] Differences between groups, $P < 0.05$.

dose. Simultaneous inhibition of plasma renin activity (PRA) was observed. The effect on PRA was prolonged at the higher dose level (10 mg/kg), despite the return to base-line blood pressure. It is notable that the initial drop in PRA was comparable at each dose. The heart rate was not affected by **12**. Thus, this renin inhibitor induced hypotension in association with plasma renin inhibition without causing reflex tachycardia at the doses tested.

Pharmacological Evaluation: Metabolism. The monkey iv results for **12** encouraged us to undertake preliminary metabolism studies to investigate the plasma and gi stabilities and the potential for oral bioavailability of this compound. A series of experiments were conducted with **12**, which was nonspecifically radiolabeled with tritium in the His[9] residue. These studies were carried out with male, Sprague–Dawley rats. In the first set of experiments, a 0.3 mg/kg dose of [^{3}H]-**12** was administered in a single iv injection to three rats (18–20 μCi/rat), and the urine and feces were collected for 24 h. A second set of experiments was carried out by following the same protocol, but the drug was introduced as a bolus intraduodenal (id) injection. All samples were assayed for total radioactivity by liquid scintillation spectroscopy and were corrected for tritiated water by lyophilization and radioassay of the distillates. The results in this paper represent the final percentage of total administered label after correcting for tritiated water. In all experiments, 80–90% of the administered radiolabel could be accounted for in the urine, feces, carcass, and tritiated water.

Table IV. Percent Radioactivity[a] in Excreta of Rats 24 h following Intravenous and Intraduodenal Administration of [^{3}H]-**12**

	intravenous	intraduodenal
urine	5.0	0.9
feces	34.5	26.6

[a] Radioactivity is corrected for tritiated water and represents the percent of radioactivity administered.

Table V. Metabolic Patterns and Amount of [^{3}H]-**12** in 24-h Urine Samples

	intravenous	intraduodenal
% radiolabel found[a]	5.0	~1.0
% parent drug	70	30
% [^{3}H]histidine	30	70

[a] Radioactivity is corrected for tritiated water and represents the percent of radioactivity administered.

Table IV summarizes the distribution of the radiolabel in the 0–24-h excreta after iv and id administration. The results in each series of experiments were very similar in that much more of the radiolabel was excreted in the feces than the urine and significant amounts of label remained in the carcass even 5 days after drug administration. The metabolic patterns in the urine are summarized in Table V. There are several points worthy of note from this study. First, a significant amount of radioactivity appeared in the feces after iv administration (34.5%), indicating that **12** and/or its metabolites were being secreted by the liver into the bile[12] and ultimately into the intestine. Second, a small but quantifiable amount of radiolabel appeared in the urine after id administration; since 70% of the radiolabel in the urine was accounted for as intact [^{3}H]-**12** (Table V), this provided direct evidence for intestinal absorption of **12**. The small amount of ^{3}H-**12** found in the urine represents the lower limit for the amount of compound absorbed; experiments are under way to more accurately assess the bioavailability of [^{3}H]-**12**. Finally, attempts to establish metabolic patterns in the feces after iv or id administration revealed several radioactive peaks representing unidentified metabolites as the major components in addition to small amounts of [^{3}H]-**12** and [^{3}H]His.

A final metabolism experiment was carried out in the iv rat model to determine the amount of drug in the blood at 1 h. The 1-h time point was chosen on the basis of the duration of action in the iv monkey experiment. At 1 h, the ^{3}H levels in plasma averaged 12 ng equiv/mL, with 64% and 34% of the radioactivity associated with [^{3}H]-**12** and [^{3}H]His, respectively, thus indicating good plasma stability for **12**.

(11) Attempts to determine the metabolic patterns in the feces were thwarted by overlapping peaks which did not correspond to any of the authentic fragments of **12**.

(12) Preliminary experiments were carried out where radiolabeled **12** was administered in the iv model with the bile duct cannulated. In agreement with these results, 36% of the radioactivity administered appeared in the bile after 2 h and nearly 50% after 24 h. Attempts to analyze the metabolic patterns revealed small amounts of intact **12** and [^{3}H]His with the majority of the radioactivity appearing as components that did not satisfactorily match any of the authentic fragments of **12**. Bopp, B. A., et al., unpublished results.

Conclusions. We have delineated further SAR on the general structure 1, which has led to the identification of the potent inhibitor **12**, a nanomolar inhibitor of renin. In addition, the SAR work demonstrated the increase in potency derived from increasing the size and lipophilicity of the leucine portion of the LVR; provided indications for the influence of size, shape, and charge of the side chain at the His^9 position; and revealed the necessity for a Phe^8 residue that bears a N-protecting group. Furthermore, the SAR work revealed that His^9 replacements, Leu or (Bn)-Thr, and Phe^8 replacements, (Me)Tyr and (Bn)Thr, predominantly maintained the inhibitory potency observed for the sequence (Phe^8-His^9) found in the native substrates angiotensinogen. The pharmacological evaluation of **12** demonstrated that inhibitors of this type are indeed capable of iv antihypertensive activity in primates. Metabolism studies in rats indicated that **12** was cleared to a significant extent by the liver and that the compound was absorbed in the gi tract upon id administration. Future SAR work will be undertaken to reduce the hepatic clearance and improve the intestinal uptake of these compounds.

Experimental Section

Proton magnetic resonance spectra were obtained on a Nicolet QE-300 (300 MHz) instrument. Chemical shifts are reported as δ values (ppm) relative to Me_4Si as the internal standard. Mass spectra were obtained with Hewlett-Packard HP5985 (CI, EI), Varian CH7 (EI), and Dratos MS50 (FAB, HRMS) spectrometers. Elemental analysis and the above determinations were performed by the Analytical Research Department at Abbott Laboratories, Abbott Park and North Chicago.

Thin-layer chromatography (TLC) was carried out by using E. Merck precoated silica gel F-254 plates (thickness, 0.25 mm). Chromatographic purification was carried out by either medium-pressure liquid chromatography (MPLC) employing columns packed with EM silica gel 60 (40–63 μm) at 30–50 psi or by forced-air chromatography employing the previously described silica gel at 5–10 psi of air pressure.

Protected amino acids were purchased from Bachem (Torrance, CA). Tetrahydrofuran was distilled from sodium/benzophenone ketyl, and dichloromethane was distilled from P_2O_5. All other solvents and reagents were reagent grade and used without further purification.

Preparation of the LVR. (2*R*,3*S*)-3-(*t*-Boc-amino)-1-(cyclohexylthio)-2-hydroxy-4-phenylbutane (5a, R_2 = Cyclohexyl). Epoxide **3**[7] (225.2 mg, 8.56 mmol), triethylamine (156 μL, 10.28 mmol), and cyclohexyl mercaptan (109 μL, 8.91 mmol) were dissolved in absolute methanol (3.5 mL) and heated at reflux for 2 h. After cooling, the volatiles were removed in vacuo and the resulting slurry was purified by forced-air chromatography (55 g of sg; 15% ethyl acetate/hexane) to provide, in order of elution, 240.9 mg (74%) of **5a** and 32.4 mg (10%) of the 2(*S*)-hydroxy isomer and **5a**: ^{1}H NMR ($CDCl_3$) δ 7.15–7.33 (m, 5 H), 4.88 (br d, J = 9 Hz, 1 H), 3.82 (d, d, d, J = 9, 9, 9 Hz, 1 H), 3.57 (d of p, J = 9, 1.8, 1.8, 1.8, 1.8, 1.8 Hz, 1 H), 2.85–3.02 (m, 3H), 2.70 (AB, J = 14.7, 3.6 Hz, 1 H), 2.53 (br AB, J = 14.7, 10.7 Hz, 2 H), 1.82–1.95 (br m, 2 H), 1.68–1.78 (br m, 2 H), 1.55–1.61 (br m, 1 H), 1.40 (s, 9 H), 1.15–1.45 (m, 10 H). Anal. ($C_{21}H_{33}NO_3S$) C, H, N.

(2*R*,3*S*)- and (2*S*,3*S*)-3-(*t*-Boc-amino)-4-cyclohexyl-1-(cyclohexylthio)-2-hydroxybutane (6a and 2(*S*)-6a, R_2 = Cyclohexyl). Epoxide **4**[7] (1.26 g, 3.94 mmol) was converted to the title compound and the corresponding 2(*S*)-hydroxy isomer by following the procedure described for **5a**. Purification by forced-air chromatography (100 g of sg; 10% ethyl acetate/carbon tetrachloride) provided, in the order of elution, **6a** (0.76 g, 50%), a mixture of the two hydroxy isomers (0.18 g, 12%), and the pure 2(*S*)-hydroxy isomer (0.046 g, 3%). **5a**: mp 60–61 °C (cold hexanes); ^{1}H NMR ($CDCl_3$) δ 4.67 (br d, J = 9.3 Hz, 1 H), 3.70 (d, d, d, J = 9.3, 3.3, 3.3 Hz, 1 H), 3.56 (br d, J = 9.3 Hz, 1 H), 2.9 (br s, 1 H, D_2O exchangeable), 2.79 (AB, J = 14.4, 3.0 Hz, 1H), 2.65 (br m, 1 H), 2.52 (AB, J = 14.4, 9.3 Hz, 1 H), 1.2–2.05 (overlapping m, 21 H), 1.45 (s, 9 H), 0.78–1.03 (m, 2 H); mass spectrum, M^+ = 385 (weak). Anal. ($C_{21}H_{39}NO_3S$) C, H, N.

(2*R*,3*S*)-3(*t*-Boc-amino)-4-cyclohexyl-2-hydroxy-1-(isopropylthio)butane (6a, R_2 = Isopropyl). Following the procedure described for **5a** but employing isopropyl mercaptan in lieu of cyclohexyl mercaptan, epoxide **4**[7] (3.18 g, 11.8 mmol) was converted to a mixture of the 2(*S*)- and 2(*R*)-hydroxy isomers. Purification by forced-air chromatography (150 g of sg; 10% ethyl acetate/carbon tetrachloride) provided, in the order of elution, **6b** (2.42 g, 59%), a mixture of **6b** and the 2(*S*)-hydroxy isomer (1.02 g, 25%), and the pure 2(*S*)-hydroxy isomer (0.122 g, 3%). **6b**: ^{1}H NMR ($CDCl_3$) δ 4.67 (br d, J = 9.3 Hz, 1 H), 3.70 (br d, d, d, J = 9.3, 1.8, 1.8 Hz, 1 H), 3.58 (br d, J = 9.3 Hz, 1 H), 2.73 (s, J = 6.2 Hz, 1 H), 2.87 (br s, 1 H), 2.78 (br AB, J = 14.1, 33 Hz), 2.53 (br AB, J = 14.1, 9.3 Hz, 1 H), 1.85 (br d, J = 12.3 Hz, 1 H), 0.77–1.75 (overlapping m, 13 H), 1.45 (s, 9 H), 1.27 (d, d, J = 6.3, 1.4 Hz, 6 H). Anal. ($C_{18}H_{35}NO_3S$) C, H, N.

(2*R*,3*S*)-3-(*t*-Boc-amino)-4-cyclohexyl-1-(cyclohexylsulfonyl)-2-hydroxybutane (7a, R_2 = Cyclohexyl). To an ice-cooled dichloromethane (16 mL) solution of **6a** (1.05 g, 2.72 mmol) was added *m*-chloroperoxybenzoic acid (1.5 g, 6.8 mmol) in four portions. After the addition was completed, the cooling bath was removed and the reaction mixture stirred for 1 h. The reaction mixture was diluted with ethyl ether (200 mL); washed with saturated aqueous Na_2SO_3 (2×), saturated aqueous $NaHCO_3$, and brine; dried over Na_2SO_4; filtered; and concentrated in vacuo. Purification by forced-air chromatography (75 g of sg; 20% ethyl acetate/carbon tetrachloride) provided the title compound **7a** (1.10 g, 97%). **7a**: ^{1}H NMR ($CDCl_3$) δ 4.72 (br d, J = 9.3 Hz, 1 H), 4.31 (d, d, d, J = 8.7, 2.7, 2.7 Hz, 1 H), 3.67 (br m, 1 H), 3.54 (br d, J = 1.5 Hz, 1 H), ca. 3.1 (m, 1 H), 2.93 (d, d, d, d, J = 12.3, 12.3, 3.0, 3.0 Hz, 1 H), 2.17 (br d, J = 13 Hz 2 H), 0.78–2.0 (m, 22 H), 1.42 (s, 9 H); mass spectrum (EI), $(M + H)^+$ = 418 (weak). Anal. ($C_{21}H_{39}NO_5S \cdot {}^1/_2H_2O$) C, H, N.

(2*R*,3*S*)-3-(*t*-Boc-amino)-4-cyclohexyl-2-hydroxy-1-(isopropylsulfonyl)butane (7b, R_2 = Isopropyl). Following the procedure for **7a** but employing **6b** (1.10 g, 3.18 mmol) provided the title compound **7b** (1.16 g, 97%) after forced-air chromatography (25% ethyl acetate/carbon tetrachloride). **7b**: ^{1}H NMR ($CDCl_3$) δ 4.73 (br d, J = 9.3 Hz, 1 H), 4.37 (d, d, d, J = 8.4, 4.5, 2.1, 1 H), 3.67 (br m, 1 H), 3.56 (br s, 1 H), 3.20 (septet, J = 6.6 Hz, 1 H), ca. 3.1 (br m, 2 H), 0.85–1.85 (m, 13 H), 1.47 (s, 9 H), 1.42 (d, J = 6.6 Hz, 6 H). Anal. ($C_{18}H_{35}NO_5S$) C, H, N.

***t*-Boc Deprotection.** Deprotection of *t*-Boc-protected compounds was achieved by dissolving the desired compound in excess dry ethanolic hydrochloric acid (4.1–4.5 M HCl) and stirring at room temperature for 1–4 h. When analysis by TLC indicated complete reaction, the volatiles were removed in vacuo; the resulting slurry was chased with benzene (2–3×) and the salt dried under high vacuum. The salts thus obtained were used without further purification.

General Coupling Method. The subsequent coupling procedure is exemplary of the procedures employed to sequentially add the two amino acid residues to the LVR. While this approach is more tedius, it avoids the significant racemization (10–30%) often encountered when the dipeptide is coupled to the LVR.

To a −23 °C (CCl_4/dry ice bath) DMF solution (0.25 M) of the amine hydrochloride salt (1.0 equiv) were added, in order, the desired N-protected amino acid (1.0 equiv), 1-hydroxybenzotriazole monohydrate (3.0 equiv), *N*-methylmorpholine (2.0 equiv), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (1.0 equiv). The reaction mixture was stirred for 2 h at −23 °C and for 12–18 h at room temperature and quenched by being poured into saturated aqueous $NaHCO_3$. The resulting aqueous mixture was extracted with ethyl acetate (2×). The combined organic layers were washed (brine), dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by normal phase chromatography was performed by using a methanol/chloroform solvent system, which gave an R_f between 0.15 and 0.3. When the N-terminus piece contained more than one basic functionality (e.g., H-His-LVR·2HCl), an additional equivalent(s) of *N*-methylmorpholine was/were added to neutralize all basic moieties. The following procedure is provided as an example.

(2*R*,3*S*)-3-[[*N*-(*tert*-Butyloxycarbonyl)histidyl]-amino]-4-cyclohexyl-1-(cyclohexylsulfonyl)-2-hydroxybutane (35). To a −23 °C (CCl_4/dry ice) DMF solution (10 mL) of the HCl salt of the deblocked LVR-**7a** (prepared as described

by deprotecting LVR-6a, 0.985 g, 2.55 mmol) were added, in order, Boc-His-OH (0.650 g, 2.55 mmol), HOBt (1.03 g, 7.65 mmol), *N*-methylmorpholine (0.620 mL, 5.61 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.490 g, 2.55 mmol). After the reaction mixture was stirred for 2 h at −23 °C, the cooling bath was removed and the reaction mixture stirred overnight (12–18 h) at room temperature. The reaction solution was poured into saturated aqueous $NaHCO_3$ and extracted with ethyl acetate. The organic layer was washed with brine (2×), dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by forced-air chromatography (100 g of sg; 4% methanol/chloroform) provided the title compound **35** (1.08 g, 81%). **35**: ^1H NMR ($CDCl_3$) δ 7.67 (br s, 1 H), 6.87 (br s, 1 H), 6.58 (br d, J = 7.8, 1 H), 3.16 (*AB*X, J = 14.4, 4.2 Hz, 1 H), 2.97 (*AB*X, J = 14.4, 6.0 Hz, 1 H), 1.46 (s, 9 H). Anal. Calcd for $C_{27}H_{46}N_4O_4S·0.25H_2O$: C, 61.51; H, 8.89; N, 10.63. Found: C, 61.72; H, 8.89; N, 10.63.

Deprotection of Final Peptides. The inhibitors **24** and **26** were deprotected by adding a 1:1 ethanol/acetic acid solution of the inhibitor to an equal weight of 10% palladium on carbon under 1 atm of hydrogen and stirred until the reaction was complete (4–12 h). The mixture was filtered through Celite and the filtrate concentrated in vacuo. The resulting residue was dissolved in water, basified with saturated aqueous Na_2CO3, extracted into ethyl acetate, and concentrated in vacuo. Purification by chromatrography on silica gel (25% methanol/chloroform was employed as the eluant) provided the final products **25** and **27**.

Removal of benzyl ether protecting groups was performed in ethyl acetate by using an equal weight of 10% Pd/C. The catalyst was removed by filtration and the filtrate concentrated in vacuo. Purification by chromatography provided the final compounds **20** and **21**.

(2*R*,3*S*)-3-[[[(*tert*-Butyloxycarbonyl)phenylalaninyl]-*N*$^\epsilon$-acetyllysinyl]amino]-4-cyclohexyl-2-hydroxy-1-(isobutylsulfonyl)butane (28). Compound **26** (20.0 mg, 25.4 μmol) was hydrogenolyzed as described above. After removal of the catalyst by filtration, the filtrate was concentrated in vacuo and the resulting acetate salts were suspended in dry DMF (1 mL) and cooled to 0 °C in an ice bath. To this suspension were added sequentially ethyldiiospropylamine (9.0 μL, 50.8 μmol) and acetic anhydride (2.4 μL, 25.4 μmol). The resulting solution was stirred for 2 h at 0 °C and warmed to room temperature as it was stirred overnight. The reaction mixture was diluted with ethyl acetate and washed with 10% aqueous $KHSO_4$. After the layers were separated, the aqueous layer was back extracted with ethyl acetate. The combined organic layers were washed sequentially with saturated aqueous $NaHCO_3$ (2×) and brine (3×), dried over Na_2SO_4, filtered, and concentrated in vacuo to provide a crystalline powder. Trituration (ethyl ether, hexanes, methanol) provided an analytically pure sample of the title compound (13.3 mg, 75%) **28**, as a colorless amorphous solid. **28**: ^1H NMR ($CDCl_3$) δ 7.03 (br d, J = 5.7 Hz, 1H), 6.70 (br d, J = 9.0 Hz, 1 H), 5.76 (br m, $W_{1/2h}$ = 15.2 Hz, 1 H), 1.97 (s, 3 H), 1.42 (s, 9 H).

(2*R*,3*S*)-3-[[(Phenoxyacetyl)histidyl]amino]-4-cyclohexyl-1-(cyclohexylthio)-2-hydroxybutane (34). The bis(HCl salt) of 3-(histidylamino)-4-cyclohexyl-1-(cyclohexylthio)-2-hydroxybutane (218 mg, 0.44 mmol) was dissolved in dry DMF (2 mL), *N*-methylmorpholine added (97 μL, 0.88 mmol), and the solution cooled to 0 °C. To this was added the succinimido ester of phenoxyacetate [formed in situ by adding phenoxyacetyl chloride (61 μL, 0.44 mmol) to a 0 °C, dry DMF solution (0.5 mL) of *N*-methylmorpholine (48 μL, 0.44 mmol) and *N*-hydroxysuccinimide (56 mg, 0.48 mmol)]. The reaction mixture was stirred at 0 °C for 2 h, the cooling bath removed, and the reaction mixture stirred overnight at room temperature. The reaction mixture was poured into saturated aqueous $NaHCO_3$ and extracted with ethyl acetate (2×). The combined organic layers were washed (2×, brine), dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting substance was purified by MPLC (52 g of sg; 3% methanol/chloroform) to provide the title compound (99.3 mg, 41%). **34**: ^1H NMR ($CDCl_3$) δ 8.07 (br d, 1 H), 7.5 (s, 1 H), 7.3 (d, d, J = 8.1, 8.1, 2 H), 4.52 (br s, 2 H).

(2*R*,3*S*)-3-[[(*O*-Benzylthreoninyl)histidyl]amino]-4-cyclohexyl-2-hydroxy-1-(isopropylsulfonyl)butane (40). Compound **31** (6.8 mg, 9.63 μmol) was deblocked as described in the general procedure. The volatiles were removed in vacuo, and the resulting slurry was taken up in water. The aqueous solution was made basic (pH ≅10 by pH paper) by addition of saturated aqueous Na_2CO_3 and extracted with ethyl acetate (2×). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting colorless solid was purified by forced-air chromatography (3 g of sg; 3% methanol/chloroform) to provide the title compound **40** (3.5 mg, 60%). **40**: ^1H NMR ($CDCl_3$) δ 8.68 (d, J = 6.6 Hz, 1 H), 7.53 (s, 1H), 6.76 (s, 1 H), 4.60 (*AB*, J = 11.7 Hz, 1 H), 4.46 (*AB*, J = 11.7 Hz, 1 H), 1.28 (d, J = 6.0 Hz, 3 H).

In Vitro Enzyme Inhibition. Purified human renal renin[13] was assayed by utilizing pure human angiotensinogen[14] at pH 6.0 in maleate buffer. Test compounds were dissolved in DMSO and diluted so that prior to addition to the assay system the solutions were 10% in DMSO and 0.5% in BSA. The final incubation mixture (100 μL) contained the following: maleate buffer, pH 6.0, 0.135 M; EDTA, 3 mM; PMSF, 1.4 mM; angiotensinogen, 0.21 μM; renin, 0.24 mGU;[15] BSA, 0.44%; and DMSO, 1%. At least three different concentrations of inhibitor that bracketed the IC_{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 AI 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–AI generation curve, and at the highest concentrations tested, none of the compounds cross-reacted with the antibody to AI. The presence of 1% DMSO in the final incubation mixture caused no statistically significant effect on the renin activity.

Bovine cathepsin D (Sigma) and porcine pepsin (Sigma) activities were assessed by the hydrolysis of hemoglobin at pH 3.1 and 1.9, respectively, at 37 °C, and measurments of the absorbance at 280 nm of the supernatant after precipitation with trichloroacetic acid.[16]

Pharmacological Evaluation. Our compounds to date have been species specific and require pharmacological testing in primates. Male cynomolgus monkeys (*M. fascicularis*), weighing 4–6 kg, were maintained on a low salt chow and fruit diet, accompanied by treatments with furosemide (5 mg/kg, po) on day 8 and day 1 prior to experimentation. This regimen stimulates renin secretion and elevates base-line plasma renin activity.

On the morning of the experiment, each monkey was sedated (ketamine, 10 mg/kg, im) and an iv line was inserted into a leg vein for the administration of sample drugs and anesthesia, with sodium pentobarbital (Abbott Laboratories, North Chicago, IL) as a 15 mg/kg bolus plus 0.1 mg/kg per min maintenance infusion, iv.

The femoral artery was catheterized with PE-90 tubing for continuous monitoring of blood pressure and heart rate employing a Statham-Gould transducer (Model P23dB) and recording on a Grass polygraph (Grass Instrument, Quincy, MA). Blood samples were obtained for the measurement of plasma arterial renin activity during control and 5, 15, 30, 60, and 120 min after drug administration. Compound **12** was given as an iv bolus at two concentrations. Each animal received one dose, and there were three animals in each group. Each monkey served as its own control in order to minimize the number of primates studied. Data were analyzed by employing the paired "*t*" test to determine differences from control, and ANOVA was employed to compare differences between the two groups.

Metabolism Study in Rats. Compound **12** was labeled with tritium in the histidine moiety and had a specific activity of 338 ±μCi/mg. Male Sprague–Dawley derived rats, weighing 0.18–0.20 kg, were given a 0.3 mg/kg dose of [^3H]-**12** (18–20 μCi/rat),

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dissolved in an ethanol/water mixture (1:1, volume), by iv or id injection. Plasma samples were obtained by cardiac puncture at 1 h after iv administration. Urine and feces were collected, from other rats, for 24 h after iv or id drug administration. Prior to radioassay, the feces and carcass were homogenized separately in 70% aqueous ethanol. Samples were assayed for total radioactivity by liquid-scintillation spectrometry and were corrected for tritiated water, as determined by lyophilization and radioassay of the distillates.

Plasma samples were lyophilized, and the residues were extracted with 50% aqueous ethanol or 20% aqueous acetonitrile. Metabolic patterns in clarified urine and fecal samples, and plasma extracts, were determined by thin-layer chromatography on silica gel GF plates (5 × 20 cm, 250-μm thick; Redi-Plates, Fisher Scientific Co.), which were developed in *n*-butanol/concentrated NH_4OH/water (20:1:1, by volume), *n*-butanol/chloroform/concentrated NH_4OH/water (20:5:1:1, by volume), or *n*-butanol/isopropyl alcohol/concentrated NH_4OH/water (10:7:2:4, by volume). Sections of the silica gel were sequentially scraped from the plates and radioassayed by liquid-scintillation counting. The radioactive zones were tentatively identified by comparison of their R_f values with those of authentic reference compounds, which were visualized by exposure of the plates to iodine vapors or spraying with ninhydrin.

Registry No. **3**, 98760-08-8; **4**, 107202-61-9; (2 *R*, 3 *S*)-**5a**, 110205-74-8; (2 *S*, 3 *S*)-**5a**, 110205-75-9; (2 *R*, 3 *S*)-**6a**, 110205-76-0; (2 *S*, 3 *S*)-**6a**, 110206-05-8; (2 *S*, 3 *S*)-**6b**, 110206-06-9; (2 *R*, 3 *S*)-**6b**, 110206-07-0; **7**, 110269-24-4; **7a**, 110206-08-1; **7a** (N-deblocked, HCl salt), 110206-10-5; **7b**, 110206-09-2; **8**, 110269-25-5; **9**, 110205-77-1; **10**, 110269-26-6; **11**, 110205-78-2; **12**, 110205-79-3; **13**, 110205-80-6; **14**, 110205-81-7; **15**, 110269-27-7; **16**, 110205-82-8; **17**, 110205-83-9; **18**, 110205-84-0; **19**, 110205-85-1; **20**, 110205-86-2; **21**, 110269-28-8; **22**, 110205-87-3; **23**, 110205-88-4; **24**, 110205-89-5; **25**, 110205-90-8; **26**, 110205-91-9; **27**, 110205-92-0; **28**, 110205-93-1; **29**, 110269-29-9; **30**, 110205-94-2; **31**, 110205-95-3; **32**, 110205-96-4; **33**, 110205-97-5; **34**, 110205-98-6; **34** (*N*-deblocked, 2 HCl salt), 110206-11-6; **35**, 110205-99-7; **36**, 110269-30-2; **37**, 110206-00-3; **38**, 110206-01-4; **39**, 110269-31-3; **40**, 110206-02-5; **41**, 110269-32-4; **42**, 110311-40-5; **43**, 110206-03-6; 44, 110206-04-7; BOC-His-OH, 17791-52-5; c-$C_6H_{11}SH$, 1569-69-3; *i*-PrSH, 75-33-2; $PhOCH_2COCl$, 701-99-5; renin, 9015-94-5.

Notes

3-Pyrroline *N*-Oxide Bis(carbamate) Tumor Inhibitors as Analogues of Indicine *N*-Oxide

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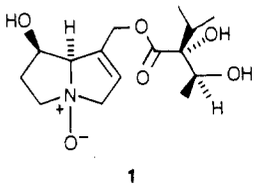
The 2,3-bis[[(*N*-methylcarbamoyl)oxy]methyl]-3-pyrroline 1-oxide **5** was synthesized and tested in the murine P388 lymphocytic leukemia model. The compound showed significant reproducible activity and was more potent than indicine *N*-oxide. 1-Methyl-2-phenyl-3,4-bis[[(*N*-2-propylcarbamoyl)oxy]methyl]-3-pyrroline *N*-oxide (**6**) was less active than **5**, and the 5,5-dimethyl analogue of **6**, the pyrroline *N*-oxide **7**, was inactive. The *N*-oxide **7** cannot be converted to a pyrrole in vivo because of the *gem*-dimethyl substitution at C-5.

Indicine *N*-oxide (NSC 132319), **1**, an alkaloid isolated from *Heliotropium indicum*, underwent human clinical trials as an antineoplastic agent.[1-4] The compound is a



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pyrrolizidine alkaloid *N*-oxide but lacks the significant hepatotoxicity[5-15] associated with many of the pyrrolizidine alkaloid free bases, **2**. Some of the free bases do possess antineoplastic activity,[16-19] but this is overshadowed by hepatotoxicity. The toxicity and antineoplastic activity

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