C-Terminal Modifications of Nonpeptide Renin Inhibitors: Improved Oral Bioavailability via Modification of Physicochemical Properties[1]

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We describe the development of a series of soluble, potent, and bioavailable nonpeptide renin inhibitors. These inhibitors derived from a series of novel nonpeptide renin inhibitors which were recently identified in our laboratories, by alteration of the nature of the C-terminus (P_2') of the molecules. Introduction of basic substituents into modified hydroxyethylene dipeptide isosteres gave inhibitors with improved solubility as well as improved potency against human plasma renin. In addition, these modifications produced inhibitors which displayed markedly improved intraduodenal bioavailability in both the ferret and cynomolgus monkey. We also present data which demonstrate excellent efficacy in the monkey for A-74273 (**65**), with an intraduodenal bioavailability of 16 ± 4% in the monkey, compared to 1.7 ± 0.5% for the dipeptide renin inhibitor enalkiren (A-64662, **75**). A-74273 is an example of a nonpeptide inhibitor which possesses a good balance of the desirable properties of potency, solubility, and lipophilicity and which is well absorbed into the intestine.

Introduction

In the preceding article, a detailed account of the design and discovery of a new and novel class of renin inhibitors was described. Inhibitors **1**–**4** (see Table I) possessed excellent in vitro potency against human purified and plasma renin and, when administered intravenously and intraduodenally (id) to salt-depleted cynomolgus monkeys, reduced blood pressure. However, compounds **1**–**4** had two major liabilities, low aqueous solubility and limited intraduodenal bioavailability.

The process of intestinal absorption involves a complex sequence of events. Two physicochemical properties that influence absorption of drug molecules from the gastrointestinal tract are lipophilicity and water solubility. In order for a drug to traverse the epithelial layer in the gastrointestinal tract, it must first cross an aqueous diffusion layer to approach the mucosal membrane and then penetrate the lipid membrane. Therefore, for optimal absorption, the molecule must possess both lipid and aqueous solubility. Achieving this balance may determine the extent of intestinal absorption and oral bioavailability.

We undertook a program to improve the water solubility of the nonpeptide renin inhibitors. Our approach was to incorporate polar (basic) groups into the inhibitors in such a way that in vitro potency was not sacrificed. Similar strategies for improving the water solubility of renin inhibitors have been reported recently.[2] As described in the previous paper, extensive SAR studies were performed at the P_4, P_3, and P_2 positions on the nonpeptide renin inhibitors, leading to optimized in vitro potency for these positions.[3] Since the inhibitors employing the hydroxyethylene dipeptide isostere as a transition-state mimic were the most potent, we decided to focus our SAR studies on the modification of the C-terminus of inhibitors **3** and **4**. Analogues incorporating P_1'-amides and P_1'-retroinverted amino derivatives with water-solubilizing groups at the P_2' positions were prepared. Results of our studies of these analogues are now disclosed.

Chemistry

The synthesis of the protected retroinverted hydroxyethylene dipeptide isosteres **7**–**19** is delineated in Scheme I.[4] Curtius rearrangement of the previously reported carboxylic acid **5**,[5] followed by in situ trapping of the intermediate isocyanate **6** with a range of nucleophiles (alkyl magnesium halide, amine and alcohols) provided diprotected retroinverted amide **7**, urea **8**, and carbamates **9**–**16**. Benzyl carbamate **10** served as a convenient source of primary amine **17**, which was converted to sulfonamides **18** and **19** by reaction with the appropriate sulfonyl chloride.

(1) A portion of this work has been presented in preliminary form: Boyd, S. A.; Fung, A. K. L.; Baker, W. R.; Mantei, R. A.; Armiger, Y.-L.; Stein, H. H.; Cohen, J.; Egan, D. A.; Barlow, J. L.; Klinghofer, V.; Kleinert, H. D.; Verburg, K. M.; Martin, D. L.; Young, G. A.; Polakowski, J. S.; Hoffman, D. J.; Garren, K. W.; Perun, T. J. C-Terminal Modifications of Non-peptide Renin Inhibitors: Improved Oral Bioavailability via Modification of Physicochemical Properties. *Abstracts of Papers*, 201st ACS National Meeting, Atlanta, GA; American Chemical Society, Washington, DC, 1991; MEDI 53.

(2) (a) Bradbury, R. H.; Major, J. S.; Oldham, A. A.; Rivett, J. E.; Roberts, D. A.; Slater, A. M.; Timms, D.; Waterson, D. 1,2,4-Triazolo[4,3-a]pyrazine Derivatives with Human Renin Inhibitory Activity. 2. Synthesis, Biological Properties and Molecular Modeling of Hydroxyethylene Isostere Derivatives. *J. Med. Chem.* **1990**, *33*, 2335–2342. (b) Foxton, M. W.; Ayres, B. E.; Cooper, A. W. J. United States Patent 4,985,407. (c) Hodges, J. C.; Connolly, C. J.; Kornberg, B. E.; Repine, J. T.; Bernabei, A. A.; Klinkefus, B. A.; Batley, B. L.; Painchaud, C. A.; Ryan, M. J. Renin Inhibitors: Reduction of Lipophilicity with Retention of Activity. *Abstracts of Papers*, 199th ACS National Meeting, Boston, MA; American Chemical Society, Washington, DC, 1990; MEDI 129. (d) Hiwada, K.; Kokubu, T.; Murakami, E.; Muneta, S.; Morisawa, Y.; Yabe, Y.; Koike, H.; Iijima, Y. A. Highly Potent and Long Acting Oral Inhibitor of Human Renin. *Hypertension* **1988**, *11*, 708–16.

(3) For a description of subsite nomenclature, see: Schechter, L.; Berger, A. On The Size of the Active Site in Proteases. I. Papain. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157.

(4) (a) Similar retroinverted hydroxyethylene dipeptide isosteres have been investigated by workers at Upjohn: Thaisrivongs, S. PCT Patent Application WO 88/02374. (b) Simplified retroinverted hydroxyethylene dipeptide-containing renin inhibitors have been report by workers at Abbott: Rosenberg, S. H.; Plattner, J. J.; Woods, K. W.; Stein, H. H.; Marcotte, P. A.; Cohen, J.; Perun, T. J. Novel Renin Inhibitors Containing Analogues of Statine Retro-Inverted at the C-Termini: Specificity at the P_2 Histidine Site. *J. Med. Chem.* **1987**, *30*, 1224–1228.

(5) Boyd, S. A.; Mantei, R. M.; Hsaio, C.-N.; Baker, W. R. Stereoselective Syntheses of Hydroxyethylene Dipeptide Isosteres. *J. Org. Chem.* **1991**, *56*, 438–442.

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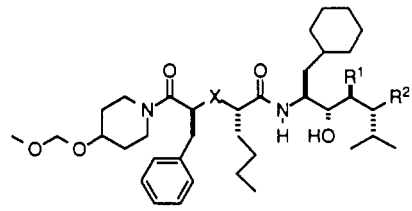
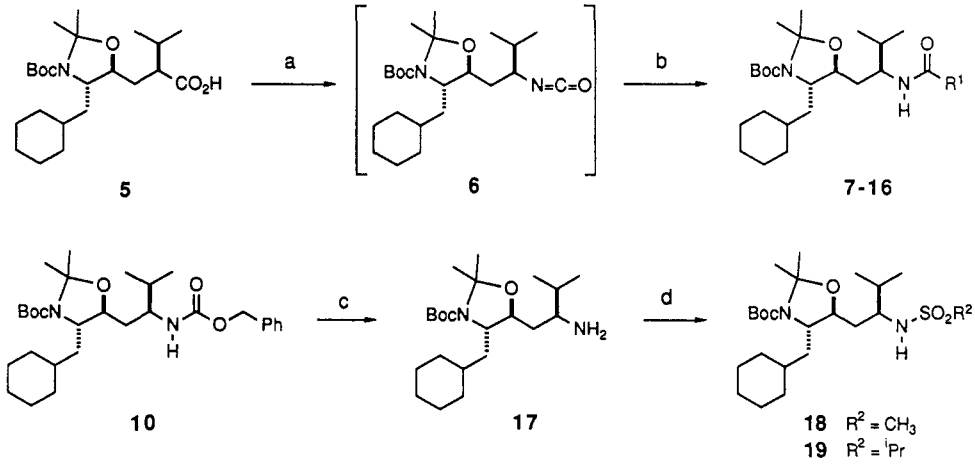
Table I. In Vitro Potency against Human Purified and Plasma Renin, Partition Coefficients, Solubility, and Intraduodenal Bioavailability in Monkey for the Lead Nonpeptide Renin Inhibitors



inhibitor	X	R^1	R^2	IC_{50}, nM[a] purified human renin, pH 6.0	IC_{50}, nM[a] human plasma renin, pH 7.4	log P[b] pH 6.5	log P[b] pH 7.4	aqueous solubility pH 7.4, mg/mL	bioavailability, 10 mg/kg id, monkey
1	O	OH	H	1.0	13[d]	4.8	4.8	nd[e]	2.5 ± 0.4% (n = 5)
2	NH	OH	H	1.1	17[d]	nd	nd	nd	2.9 ± 2.2% (n = 2)
3	O	H	CONHnBu	1.5	8.2	4.7	4.7	0.0008	2.5 ± 0.4% (n = 5)
4	NH	H	CONHnBu	1.8	8.7	>4.5	>4.5	0.00074	7.1 ± 2.3% (n = 2)

[a] Average of 2–4 determinations unless otherwise noted. [b] Apparent octanol/aqueous phosphate buffer partition coefficient. [c] Determined at 37 °C in 0.05 M phosphate containing 0.15 M NaCl. [d] Single determination. [e] nd = Value not determined.

Scheme I. Synthesis of Retroinverted Hydroxyethylene Dipeptide Isosteres[a]



[a] Reaction conditions: (a) $(PhO)_2P(O)N_3$, Et_3N, toluene, 55–60 °C; (b) R^1MgBr, 25 °C, R^1NH_2, 80 °C or R^1OH, 85 °C. See Tables II and III for R^1 structures; (c) H_2 (4 atm), 10% Pd/C, EtOAc; (d) CH_3SO_2Cl or $(CH_3)_2CHSO_2Cl$, Et_3N, CH_2Cl_2, 0–25 °C.

These diprotected isosteres were elaborated into the corresponding renin inhibitors according to Scheme II. A two-stage deprotection was employed: trifluoroacetic acid treatment cleanly removed the Boc group, and then the *N,O*-acetal was cleaved under mildly acidic aqueous conditions to provide the amino alcohols **20** after basic workup. The nonpeptide inhibitors **23–36** were obtained by coupling the derived amino alcohols **20** with either acid **21** or **22**, using a water-soluble carbodiimide method (EDC[6] and HOBT in DMF), in high yields. Retroinverted amine **27** was prepared by catalytic hydrogenolysis of benzyl carbamate **26**. Table II gives the structure of the C-terminal groups (R) for these inhibitors and their corresponding precursors.

We were interested in comparing these nonpeptide inhibitors with the corresponding substrate-based dipeptide inhibitors. The lower part of Scheme II shows the synthesis of Boc-Phe-His derivatives **38–48**, using a stepwise coupling method. Amino alcohols **20** were acylated with diprotected histidine *N*-hydroxysuccinimide ester **37** in CH_2Cl_2, then the Boc group on the imidazole moiety was removed with aqueous acetic acid. The α-amino group of the resulting histidinamide was deprotected with TFA and then acylated with Boc-phenylalanine *N*-hydroxysuccinimide ester. Table III gives the structures of the C-terminal groups (R) for these substrate-based inhibitors.

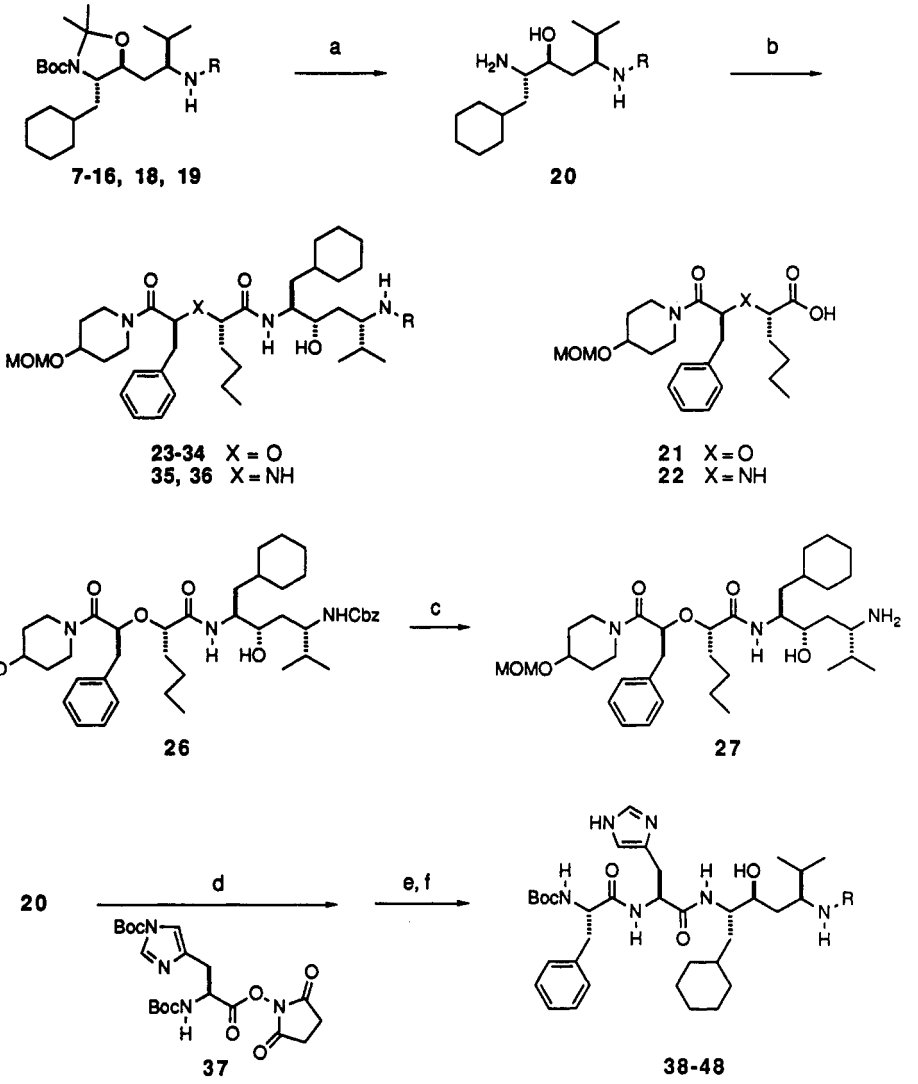
The nonpeptide renin inhibitors bearing hydroxyethylene dipeptide isosteres[7] were prepared according to the route outlined in Scheme III. Acid **5** was coupled to the appropriate amine using a modification of the procedure reported by workers at Ciba-Geigy,[8] in which greater

(6) The following abbreviations are used: AUC, integrated area under the curve; DMF, *N,N*-dimethylformamide; EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; HOBT, 1-hydroxybenzotriazole hydrate; HPLC, high-performance liquid chromatography; id, intraduodenal; OSu, *N*-oxysuccinimide; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

(7) Szelke, M.; Jones, D. M.; Atrash, B.; Hallett, A. *Peptides: Structure and Function. Proceedings of the Eighth American Peptide Syposium*; Hruby, V. J.; Rich, D. H.; Eds.; Pierce Chemical Co.: Rockford, IL, 1983; pp 579–582.

(8) Bühlmayer, P.; Caselli, A.; Fuhrer, W.; Göschke, R.; Rasetti, V.; Rüeger, H.; Stanton, J. L.; Criscione, L.; Wood, J. M. Synthesis and Biological Activity of Some Transition-State Inhibitors of Human Renin. *J. Med. Chem.* **1988**, *31*, 1839–46.

Scheme II. Synthesis of Inhibitors Containing Retroinverted Hydroxyethylene Dipeptide Isosteres[a]



[a] Reaction conditions: (a) (1) TFA–CH_2Cl_2 (1:1), 0 °C; (2) THF–H_2O (3:1), TFA, 0–25 °C; (3) aq Na_2CO_3; (b) EDC, HOBT, 4-methylmorpholine, **21** or **22**, −20 to 25 °C; (c) H_2 (4 atm), EtOAc, 10% Pd/C; (d) (1) **37**, CH_2Cl_2, 25 °C; (2) HOAc, THF, H_2O, 50 °C, then Na_2CO_3; (e) TFA/CH_2Cl_2, 0 °C, then aq Na_2CO_3; (f) Boc-Phe-OSu, CH_2Cl_2, 25 °C.

yields were obtained by addition of the amine to preformed hydroxybenzotriazole ester. The diprotected amides **49–57** were then deprotected by the two-stage method (TFA–CH_2Cl_2; H_2O–THF) described above. The crude amino alcohol intermediates were then coupled with acids **21** or **22** as described above to afford the inhibitors **58–71** in high yields. Use of harsher conditions (HCl–MeOH) for the deprotection of amides **49–57** led to the formation of substantial amounts of the amino lactone **72**. The presence of the basic substitutent appeared to exacerbate this problem.[9] The structures of the C-terminal groups (R^1 and R^2) for these hydroxyethylene dipeptide inhibitors are given in Table V. We also synthesized the substrate-based inhibitor **74** as a model for comparison to the above nonpeptide inhibitors.[10] The known amino alcohol **73**[8] was converted to the dipeptide amide using the stepwise method described above for peptides **38–48**.

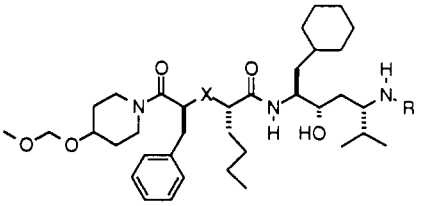
Results and Discussion

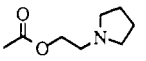
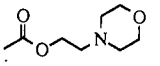
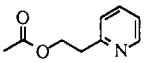
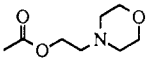
In Vitro Data. P_1'-Retroinverted Hydroxyethylene Dipeptide Isosteres. The in vitro potencies for the nonpeptide inhibitors with retroinverted hydroxyethylene transition-state mimics are listed in Table II. We examined these transition-state mimics initially with the expectation that the retroinverted isosteres would be less prone to proteolytic degradation in vivo. With the exception of primary amine **33**, all of these derivatives were very potent in the purified human renin assay, with IC_{50} values ranging from 0.9 nM for pyridinyl carbamate **32** to 5.0 nM for carbamate **27**. However, in the human plasma

(9) The tendency for lactone formation has been noted by others: (a) Chakravarty, P. K.; de Laszlo, S. E.; Sarnella, C. S.; Springer, J. P.; Schuda, P. F. The Synthesis of (2*S*,4*S*,5*S*)-5-(N-Boc)-Amino-6-cyclohexyl-4-hydroxy-2-isopropylhexanoic acid Lactone, an Hydroxyethylene Dipeptide Isostere Precursor. *Tetrahedron Lett.* **1989**, *30*, 415–8. (b) Evans, B. E.; Rittle, K. E.; Homnick, C. F.; Springer, J. P.; Hirshfield, J.; Veber, D. F. A Stereocontrolled Synthesis of Hydroxyethylene Dipeptide Isosteres Using Novel, Chiral Aminoalkyl Epoxides and γ-(Aminoalkyl) γ-Lactones. *J. Org. Chem.* **1985**, *50*, 4615–25.

(10) A very similar inhibitor has been reported by workers at Ciba-Geigy: Rüeger, H.; Bühlmayer, P.; Fuhrer, W.; Göschke, R.; Rasetti, V.; Stanton, J.; Wood, J. Orally Active Renin Inhibitors. *Abstracts and Slides*, 21st National Medicinal Chemistry Symposium, Minneapolis, MN; American Chemical Society, Washington, DC, 1988; pp 69–76.

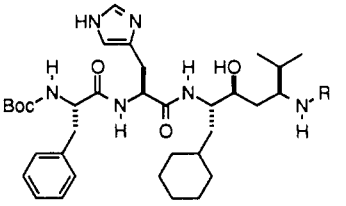
Table II. In Vitro Potency against Human Purified and Plasma Renin and Partition Coefficients of Nonpeptide Renin Inhibitors Employing P_1'-Retroinverted Hydroxyethylene Dipeptide Isostere Derivatives

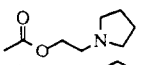
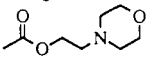
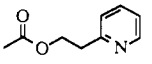


inhibitor	protected amino alcohol precursor	X	R	IC_{50}, nM[a] purified human renin, pH 6.0	IC_{50}, nM[a] human plasma renin, pH 7.4	log P[b] pH 6.5	log P[b] pH 7.4
23	7	O	COBu	3.2	53	nd[c]	nd
24	8	O	CONHPr	2.4	16	nd	nd
25	9	O	CO_2Pr	1.5	14	nd	nd
26	10	O	Cbz	2.2	83	nd	nd
27	11	O	$CO_2CH_2CH_2CH(CH_3)_2$	5.0	56	nd	nd
28	12	O	$CO_2CH_2CH_2N(CH_3)_2$	2.7	8.3	3.6	>4.0
29	13	O		2.5	13	nd	nd
30	14	O		1.4	7.5	3.6	3.7
31	15	O	$CO_2CH_2CH_2CH_2N(CH_3)_2$	2.4	12	2.9	3.8
32	16	O		0.9	40	2.7	3.4
33	*d*	O	H	110	nd	nd	nd
34	19	O	SO_2CH_3	4.3	61	nd	nd
35	12	NH	$CO_2CH_2CH_2N(CH_3)_2$	1.5	12	3.8	4.6
36	14	NH		1.8	9.5	3.9	4.3

[a] Single determination. [b] Apparent octanol/aqueous phosphate buffer partition coefficient. [c] nd = Value not determined. [d] Derived from **26**.

Table III. In Vitro Potencies for Substrate-Based Inhibitors with P_1'-Retroinverted Hydroxyethylene Dipeptide Isostere Derivatives

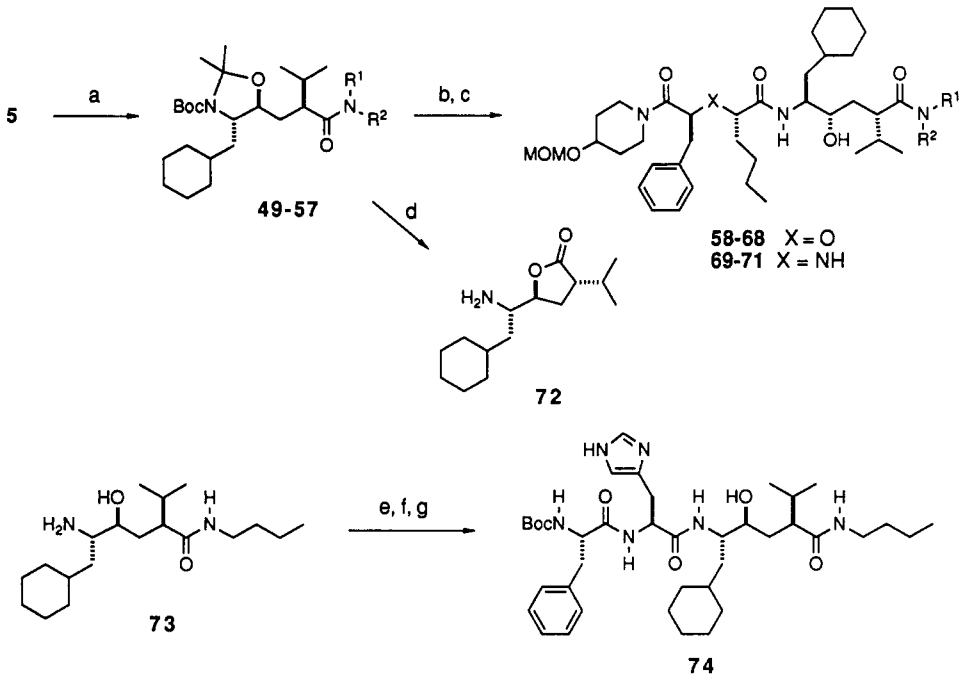


inhibitor	protected amino alcohol precursor	R	IC_{50}, nM[a] purified human renin, pH 6.0	IC_{50}, nM[a] human plasma renin, pH 7.4
38	7	COBu	6.0	200
39	8	CONHPr	2.7	83
40	9	CO_2Pr	1.7	16
41	10	Cbz	11	nd[b]
42	12	$CO_2CH_2CH_2N(CH_3)_2$	2.3	12
43	13		2.7	28
44	14		1.3	4.7
45	15	$CO_2CH_2CH_2CH_2N(CH_3)_2$	2.8	17
46	16		1.3	17
47	18	SO_2CH_3	28	nd
48	19	$SO_2CH(CH_3)_2$	67	nd

[a] Single determination. [b] nd = Value not determined.

renin assay at pH 7.4, which presumably is more pertinent to the in vivo situation, the potencies varied considerably. The retroinverted amide **23** was approximately 3-fold less potent than the isosteric propyl urea **24**, which in turn was slightly less potent than the isosteric carbamate **25**. The substitution of a sulfonamide group for the P_1' carbox-

Scheme III. Synthesis of Inhibitors Bearing Hydroxyethylene Dipeptide Isosteres[a]



[a] Reaction conditions: (a) EDC, HOBT, 4-methylmorpholine, DMF, 0 °C, 24–48 h, then HNR^1R^2, 0–25 °C; (b) (1) TFA–CH_2Cl_2 (1:1), 0 °C; (2) THF–H_2O (3:1), TFA, 0–25 °C; (3) aq Na_2CO_3; (c) **21** or **22**, EDC, HOBT, 4-methylmorpholine, DMF, −20 to 25 °C, then Na_2CO_3; (d) AcCl, MeOH, 0–25 °C, then solid $NaHCO_3$; (e) (1) **37**, CH_2Cl_2, 25 °C; (2) HOAc, THF, H_2O, 50 °C, then Na_2CO_3; (f) TFA/CH_2Cl_2, 0 °C, then aq Na_2CO_3; (g) Boc-Phe-OSu, CH_2Cl_2, 25 °C.

Table IV. Aqueous Solubilities for Selected Renin Inhibitors Bearing C-Terminal Amines

inhibitor	aqueous solubility at pH 7.4, mg/mL[a]
28	0.385
35	0.131
58	0.725
60	2.546
65	0.210
70	0.154
71	0.060

[a] Determined at 37 °C in 0.05 M phosphate buffer containing 0.15 M NaCl.

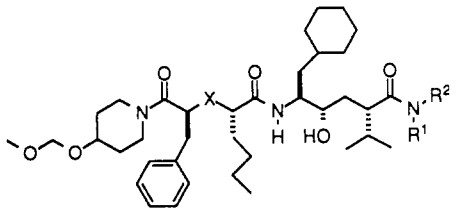
amide proved detrimental to potency, as shown by the weak inhibitory activity of **34**. On the basis of these results, we introduced solubilizing groups, in the form of tertiary amines, into the carbamate structure. We were gratified to discover that not only was the amino group tolerated by the enzyme in inhibitors **28–36**, but actually improved the plasma renin potency for these analogues. In the case of the two isosteric carbamates **27** and **28**, the (dimethylamino)ethyl carbamate **28** was nearly 7-fold more potent than its hydrocarbon analogue **27** in the plasma renin assay, implicating the existence of a specific interaction of the amino group with the enzyme. The morpholinylethyl carbamate **30** was the most potent of this series, with an IC_{50} of 7.5 nM. Increasing the distance between the P_1' amide and the amino group decreased the plasma potency. The potency of the (dimethylamino)propyl carbamate **31** and the larger pyridinylethyl carbamate **32** dropped to 12 and 40 nM, respectively. Primary amine **33**, which represents an attempt to place the solubilizing group closer to the active site, was only weakly active in the purified assay.

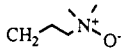
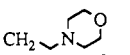
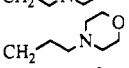
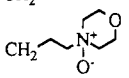
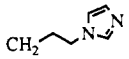
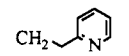
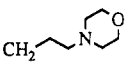
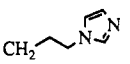
The basic amine C-termini of **28** and **30** were also incorporated into the nitrogen-linked nonpeptide inhibitor series. The (dimethylamino)ethyl carbamate **35** and morpholinylethyl carbamate **36** were very potent in the purified assay and were only slightly less potent than **4** in the plasma renin assay. Neither **35** nor **36** were as potent as their ether analogues **28** and **30**, but these four inhibitors demonstrate that the retroinverted carbamates containing P_2'-tertiary amino groups were nearly equipotent to the hydroxyethylene isostere-containing leads **3** and **4**.

In order to assess the water solubility and lipophilicity of the new analogues, the octanol–aqueous buffer partition coefficients (P) were measured at pH 6.5 and 7.4. This data is listed in Table II as log P values. These two values, which provide an indication of the basicity of the molecule, varied from 2.7 (pH 6.5) and 3.4 (pH 7.4) for the pyridinyl-containing inhibitor **32**, to 3.8 (pH 6.5) and 4.6 (pH 7.4) for the more lipophilic **35**. Aqueous solubilities in pH 7.4 buffer were determined for inhibitors **28** and **35** (see Table IV). Both **28** and **35** were considerably more soluble than the lead compounds **3** and **4**, with inhibitor **28** representing a 480-fold increase in solubility over **3**. Thus, these amine-bearing inhibitors exhibit both of the desirable properties of aqueous solubility and lipophilicity. It is interesting that the series with X = NH was more lipophilic, despite the presence of an additional amino functionality.

We evaluated the retroinverted hydroxyethylene dipeptide isosteres in substrate-based inhibitors containing Boc-Phe-His at the P_2–P_3 position. The in vitro potencies for the corresponding dipeptide-based P_1'-retroinverted inhibitors are listed in Table III. Assay results for inhibition of purified human renin (pH 6.0) and human plasma renin (pH 7.4) are given as IC_{50} values. As a benchmark, these compounds may be compared to the hydroxyethylene dipeptide-containing angiotensinogen analogue **74**, which had a potency of 0.40 nM against purified human renin and 1.2 nM against human plasma renin. Dipeptides **38–46** had excellent potencies against purified human renin, with IC_{50} values ranging from 1.3 to 11 nM. Sulfonamides **47** and **48** were 1 order of magnitude weaker inhibitors, displaying IC_{50} values of 28 and 67 nM, respectively. Once again, a broader range of potencies against human plasma

Table V. In Vitro Potency against Human Purified and Plasma Renin and Partition Coefficients for Nonpeptide Renin Inhibitors with Hydroxyethylene Dipeptide Isosteres Possessing C-Terminal (P_2') Amino Groups



inhibitor	protected amino alcohol precursor	X	R^1	R^2	IC_{50}, nM[a] purified human renin, pH 6.0	IC_{50}, nM[a] human plasma renin, pH 7.4	log P[b] pH 6.5	log P[b] pH 7.4
3	*c*	O	H	nBu	1.5	8.2	4.6	4.7
58	49	O	H	$CH_2CH_2N(CH_3)_2$	2.9	5.7	3.3	4.3
59	50	O	CH_3	$CH_2CH_2N(CH_3)_2$	30	290	3.3	>5.0
60	51	O	H	$CH_2CH_2CH_2N(CH_3)_2$	2.5	5.9	2.3	3.1
61	*d*	O	H		1.4	5.0	nd[e]	nd
62	52	O	CH_3	$CH_2CH_2CH_2N(CH_3)_2$	57	270	2.5	3.7
63	53	O	NR^1R^2 =		17	79	3.7	>5.0
64	54	O	H		1.0	3.7	>5.0	>5.0
65	55	O	H		2.4	2.8	3.3	4.3
66	*f*	O	H		1.3	3.5	nd	nd
67	56	O	H		1.3	3.0	4.0	4.0
68	57	O	H		1.0	5.1	>5.0	>5.0
4	*c*	NH	H	nBu	1.8	8.7	>4.5	>4.5
69	51	NH	H	$CH_2CH_2CH_2N(CH_3)_2$	2.1	6.1	2.2	2.8
70	55	NH	H		3.3	7.3	3.9	4.5
71	56	NH	H		2.0	7.6	4.9	>5.0

[a] Single determination. [b] Apparent octanol/aqueous phosphate buffer partition coefficient. [c] See previous article in this issue. [d] Derived from **60**. [e] nd = Value not determined. [f] Derived from **65**.

renin was observed. In an isosteric series, the P_1'-retroinverted amide **38** was the least potent (IC_{50} = 200 nM), urea **39** somewhat more potent (IC_{50} = 83 nM), and carbamate **40** more potent still (IC_{50} = 16 nM), analogous to the trend in the nonpeptide inhibitors. One should note that butyl amide **74** is an order of magnitude more potent than carbamate **40**, attesting to the excellent binding affinity of the hydroxyethylene dipeptide isostere for renin. The potencies of the amine-bearing carbamates also followed the trend found with the nonpeptides: morpholinylethyl carbamate **44** was the most potent (IC_{50} = 4.7 nM), (dimethylamino)ethyl carbamate **42** next most potent, followed by pyrrolidinylethyl carbamate **43**. Inhibitors **45** and **46**, with three-carbon tethers between the basic nitrogen and carbamate oxygen, were less potent than the ethylene-linked amines **42** and **44**. These data demonstrate that the nonpeptide inhibitors and the dipeptide based inhibitors bind to renin similarly.

P_1'-Hydroxyethylene Dipeptide Isosteres. Improving the solubility and increasing the plasma renin potency of our nonpeptide inhibitors was gratifying, however, our objective was to identify inhibitors with nanomolar potencies. On the basis of our result in the peptide series described above, we felt that hydroxyethylene dipeptide isosteres with P_2' tertiary amino groups should give rise to inhibitors with improved potency and good aqueous solubility. Table V summarizes the in vitro data for compounds employing hydroxyethylene dipeptide isosteres. Within the ether series (X = O), the secondary amides **58**, **60**, **61**, and **64–68** (R^1 = H) were all more potent against human plasma renin than compound **3**. Tertiary amides at P_1' (**59**, **62**, and **63**) were less potent. Inhibitors **69**, **70**, and **71** (X = NH) were more potent than **4** against human plasma renin, but were slightly less potent than the corresponding ether series analogues (**58**, **65**, and **67**, respectively). Varying the chain length had little effect on potency, and a wide variety of nitrogen-bearing groups on the chain was accommodated: dimethylamino (**58**, **60**, and **69**), *N*-oxido (**61** and **66**), morpholinyl (**64**, **65**, and **70**), imidazolyl (**67** and **71**), and pyridinyl groups (**68**) all imparted excellent plasma renin potency, with the morpholinylpropyl amide **65** and imidazolylpropyl amide **66** being the most potent (IC_{50} = 2.8 and 3.0, respectively). Inhibitor **65** was nearly 3-fold more potent than the isosteric retroinverted morpholinyl carbamate **30**, again demonstrating the superior potency against plasma renin of the hydroxyethylene dipeptide isosteres. It is particularly noteworthy that inhibitors **58** and **64–68** showed very little difference between the purified and plasma renin potencies, in marked contrast to most other renin inhibitors.

log *P* values at pH 6.5 and 7.4 were determined for the hydroxyethylene series (see Table V). There was a larger

Table VI. Plasma Drug Concentrations and Intraduodenal Bioavailability in Ferrets for Nonpeptide Renin Inhibitors with C-Terminal (P_2') Amines

inhibitor	average peak drug concentration after iv administration,[a] μg/mL	average AUC_{iv},[b] μg mL/min	n_{iv}	average peak drug concentration after id administration,[c] μg/mL portal	systemic	average AUC_{id}, μg mL/min	n_{id}	average bioavailability[d]
4	2.23 ± 0.74	40.4 ± 20	2	3.22 ± 0.42	1.54 ± 0.26	212 ± 36.8	2	16 ± 8%
28	1.01 ± 0.18	13.9 ± 0.2	2	1.54 ± 0.23	1.19 ± 0.23	167 ± 30.0	2	36 ± 7%
30	0.92 ± 0.17	15.5 ± 0.5	2	5.01 ± 1.60	2.84 ± 1.40	363 ± 240	2	70 ± 46%
35	0.93 ± 0.16	14.7 ± 4.4	2	5.25 ± 1.67	2.07 ± 0.66	303 ± 88.1	2	62 ± 26%
60	2.99 ± 0.70	34.4 ± 0.7	2	4.99 ± 3.53	5.05 ± 2.95	580 ± 391	2	50 ± 36%
64	1.32 ± 0.38	28.3 ± 2.4	2	4.35 ± 0.59	2.77 ± 0.44	425 ± 55.6	2	45 ± 7%
65	2.11 ± 0.38	35.3 ± 2.4	2	6.01 ± 0.40	4.83 ± 1.01	391 ± 78.1[e]	6	28 ± 6%
67	3.39 ± 0.76	24.6 ± 9.4	2	2.42 ± 1.29	2.79 ± 0.92	203 ± 1.8	2	25 ± 9%
70	1.31 ± 0.15	40.9 ± 11	3	4.35 ± 0.76	3.49 ± 0.79	462 ± 94.0	4	34 ± 11%
71	2.21 ± 0.71	16.3 ± 4.3	2	2.16 ± 0.53	1.76 ± 0.52	245 ± 82.6	4	45 ± 19%

[a] Dosed 0.3 mg/kg iv (8 arterial blood samples collected over 120 min). [b] See Experimental Section for method of calculation of AUC. [c] Dosed 10 mg/kg id (6 portal and 7 arterial blood samples collected over 180 min). [d] Defined as the dose-corrected ratio of average AUC_{id} to average AUC_{iv}, reported as mean ± standard error (SEM). SEM accounts for variability in both iv and id data. [e] Two-hour id duration.

range of log *P* values for these compounds compared with the retroinverted series, from a lipophilic >5 (pH 6.5 and 7.4) for **64** and **68** to a hydrophilic 2.3 (pH 6.5) and 3.2 (pH 7.4) for **60**. The nonpeptide inhibitor **3** had high log *P* values for both pH 6.5 and 7.4 as expected for a nonionizable compound. Contrary to expectation, the amino group in inhibitor **4** did not contribute to a lower log *P* value at pH 6.5. The two electron-withdrawing carboxamides rendered the amino group only weakly basic. In contrast, the presence of a C-terminal amino group contributed to a considerable lowering of the log *P*, especially at the acidic pH. The basicity of the amino group was attenuated by the electron-withdrawing effect of the P_1' amide group. Increasing the length of the tether decreased the magnitude of the inductive effect, resulting in a more basic amine and hence a lower log *P* value. Compare the rather lipophilic log *P* values of 3.3 (pH 6.5) and 4.3 (pH 7.4) for (dimethylamino)ethyl amide **58** with the more hydrophilic values of 2.3 (pH 6.5) and 3.1 (pH 7.4) for (dimethylamino)propyl amide **60**. Similar results were obtained in the case of morpholinyl amides **64** and **65**, in which the extension of the chain by one methylene group dropped the resulting log *P* values from >5.0 to 3.3 (pH 6.5) and 4.3 (pH 7.4). In the latter case, the ether oxygen of the morpholine also decreased the basicity of the amino group relative to the simple dialkyl amine (compare **58** and **64**).

Aqueous solubilities for the most potent hydroxyethylene isostere-containing compounds were determined at physiologic pH, and are listed in Table IV. The polar inhibitor **60** was very soluble (2.5 mg/mL), and the solubilities of inhibitors **58**, **65**, **70**, and **71** ranged from 0.060 mg/mL up to 0.725 mg/mL. Again, the analogues of **3** were more soluble, and generally more hydrophilic, than the analogues of **4**. All of the amine-containing inhibitors gave homogeneous solutions when formulated as hydrochloride salts. For example, the hydrochloride salt of inhibitor **65** had a solubility in excess of 20 mg/mL.

Proteinase Specificity. The nonpeptide inhibitors exhibited a very high specificity for primate renin, and in general did not inhibit other animal or human proteinases. Representative values for percent inhibition of typical proteinases by compound **65** at a test concentration of 1×10^{-5} M were as follows: bovine cathepsin D (3%), human cathepsin D (6%), human cathepsin G (0%), human gastricsin (11%), human pepsin (2%), and porcine pepsin (13%).

In Vivo Results. Ferret Model. The absorption and bioavailability of the more promising inhibitors were evaluated in the ferret. The ferret model was chosen as a bioavailability screen for the following reasons:[11] (1) the ferret gastrointestinal tract closely mimics that of humans; (2) the ferret is a small animal model, and therefore requires less test compound for each experiment; and (3) ferrets are commercially available. The test inhibitors were administered at doses of 0.3 mg/kg iv and 10 mg/kg id. In the id experiments, plasma samples were obtained both from the portal vein, to evaluate absorption of the drug from the intestine, and the carotid artery, to estimate first pass metabolism and to assess systemic drug levels. The plasma drug concentrations were determined by a bioassay (see Experimental Section).[12] The integrated area under the curves were obtained by fitting the data to a biexponential decay model,[13] and the id bioavailabilities were calculated as the dose-corrected ratio of AUC_{id} to AUC_{iv} for each experiment. The peak drug levels in both portal and systemic plasma, AUC values and average bioavailabilities of the potent analogues and inhibitor **4** are summarized in Table VI. The peak values reported are the average of the maximum plasma concentrations for each animal, regardless of time from administration. The first point to note is that the average AUC_{iv}, which ranged from a low of 13.9 ± 0.2 μg min/mL for **28** to a high of 40.9 ± 11.0 μg min/mL for **70**, were equivalent to or below that found for **4**. However, with the exception of **28**, the average AUC_{id} for the new analogues was higher than that for **4** (212 ± 36.8 μg min/mL), ranging from a low of 245 ± 82.6 μg min/mL for **71** to a high of 580 ± 391 μg min/mL for **60**, resulting in higher calculated bioavailabilities. The roughly equivalent values for average peak portal and average peak systemic levels for all of the analogues suggested that hepatic clearance for the ferret was much less of a factor than in other animal models. The data also suggested that absorption in the ferret was quite fascile, with a wide variety of compounds showing high drug levels. This model allowed us to identify compounds with high circulating drug levels and high bioavailability, together with low variability, relative to the test drug group as a

(11) (a) Fox, J. G.; Otto, G.; Taylor, N. S.; Rosenblad, W.; Murphy, J. C. Helicobacter mustelae-Induced Gastritis and Elevated Gastric pH in the Ferret (Mustela putorius furo). *Infect. Immun.* **1991**, *56*, 1875–1880. (b) Fox, J. G. *Biology and Diseases of the Ferret*; Lea and Febiger: Philadelphia, PA, 1988.

(12) The bioassay method cannot distinguish between parent drug and any active metabolites, and thus the concentrations reported here treat any active metabolites as parent drug.

(13) Sedman, A.; Wagner, J. *AUTOAN-A Decision-Making Pharmacokinetic Computer Program.* Publication Distribution Service: Ann Arbor, MI, 1974.

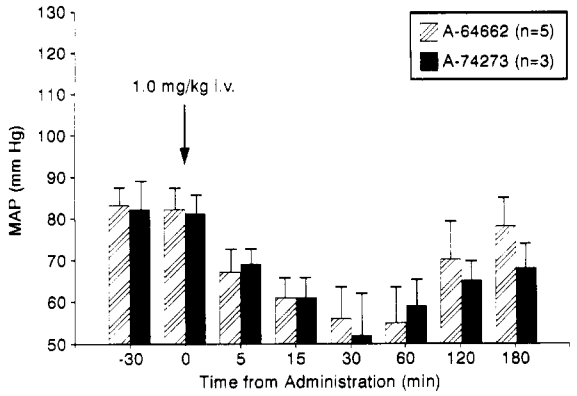
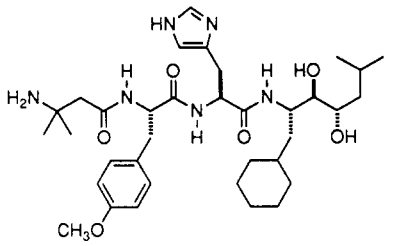


Figure 1. Comparison of blood pressure responses following intravenous administration of enalkiren (A-64662, **75**) and inhibitor **65** (A-74273) in anesthetized salt-depleted monkeys (1.0 mg/kg). Values shown are mean ± SEM.

whole. Compounds for further study were considered on the basis of bioavailability and the pharmacokinetic parameters (AUC_{id}, AUC_{iv}, $T_{1/2(terminal)}$, and average peak systemic drug levels) that contributed to the calculated bioavailability. Thus, compounds **65**, **67**, **70**, and **71** all possessed the desirable qualities of high circulating drug levels, low variability, and good id bioavailability in the ferret. It should be noted that oral administration of **65** yielded results (plasma drug concentration, bioavailability) which closely parallel those of the intraduodenal experiments (data not shown).[1]

Pharmacokinetics and Pharmacodynamics for Inhibitor 65 in the Monkey. Inhibitor **65**, one of the most promising of the foregoing compounds in terms of its overall profile, was chosen for further in vivo evaluation. Since the nonpeptide renin inhibitors are primate selective, the salt-depleted cynomolgus monkey was used to simultaneously evaluate efficacy as well as gastrointestinal absorption and potential oral bioavailability reflected by the id model. Figure 1 compares the mean arterial pressure (MAP) response to a single bolus of either 1.0 mg/kg iv of enalkiren[14] (**75**) or **65** in anesthetized salt-depleted



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monkeys. Although there was a slight tendency for **65** to be more efficacious than enalkiren, both compounds elicited a comparable MAP response that peaked at 30 min following injection. The intravenous studies showed the similarity between the two compounds using this route of administration. Figure 2 graphs the average plasma drug concentrations for **65** for the same set of animals. After the initial bolus, levels of the drug decreased to 193 ± 20 ng/mL after 30 min, but even at 180 min, significant circulating levels remained (44 ± 1.2 ng/mL). This was borne out in the continued reduction in MAP at the longer

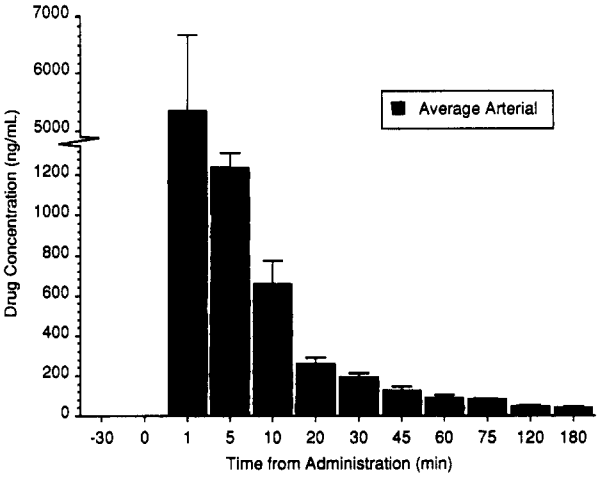

Figure 2. Plasma drug concentrations for inhibitor **65** (A-74273) following intravenous administration in anesthetized salt-depleted monkeys (1.0 mg/kg, n = 3). Values shown are mean ± SEM.

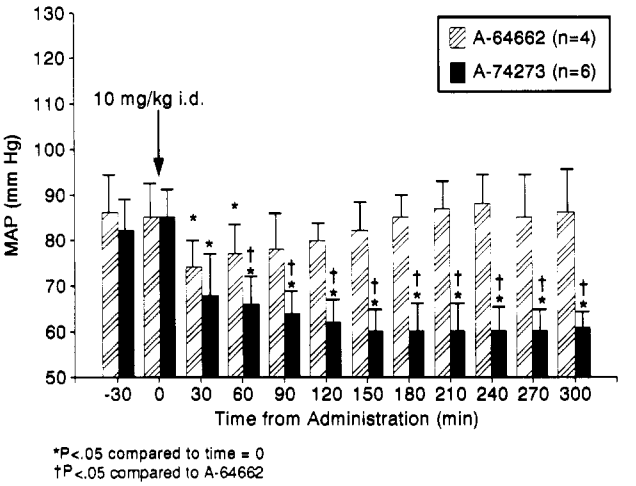

Figure 3. Comparison of blood pressure reponses following intraduodenal administration of enalkiren (A-64662, **75**) and inhibitor **65** (A-74273) in anesthetized salt-depleted monkeys (10 mg/kg). Values shown are mean ± SEM.

time points. Half-life ($T_{1/2(terminal)}$) ranged from 45–52 min, with average AUC_{iv} = 42.6 μg min/mL.

The studies employing intraduodenal dosing as a measure of oral activity clearly depicted the divergent profiles of these two compounds. Figure 3 shows the MAP response to a 10 mg/kg dose of either drug via the id route for a group of six animals. A statistical comparison between **65** and enalkiren data revealed that (1) compared to within group baseline values, statistically significant reductions in MAP were observed only at 30 and 60 min post-dosing for enalkiren, but at all intervals for **65** following drug administration and (2) differences between the two drugs were observed. Inhibitor **65** induced a significantly greater reduction in MAP than enalkiren at all time points, starting with 60 min following dosing throughout the 300-min observation period. Of particular note was the consistency and low variability of the hypotensive response to **65** and the delay to peak activity which occurred at 150 min, which was sustained at a plateau for the duration of the experiment. Figure 4 graphs the average plasma drug concentrations for **65** in the experiments, with values for both portal and systemic plasma. The portal levels rapidly climbed, and reached an average peak value of 2.07 ± 0.66 μg/mL at 120 min. These high levels were maintained for the duration of the experiment,

(14) Boger, R. S.; Glassman, H. N.; Cavanaugh, J. H.; Schmitz, P. J.; Lamm, J.; Moyse, D.; Cohen, A.; Keinert, H. D.; Luther, R. R. Prolonged Duration of Blood Pressure Response to Enalkiren, the Novel Dipeptide Renin Inhibitor, In Essential Hypertension. *Hypertension* **1990**, *16* (6, Part 2), 835–840.

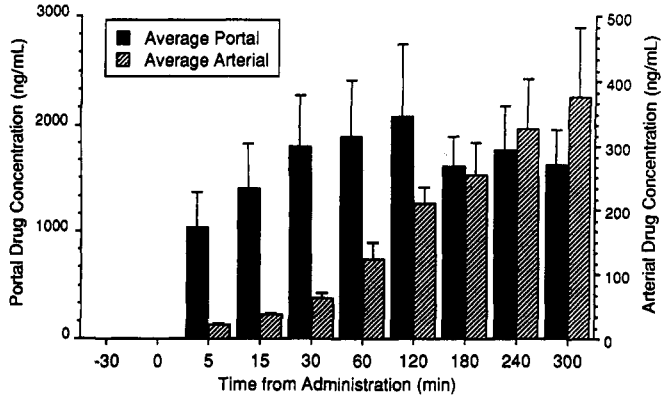

Figure 4. Drug concentrations in portal and systemic plasma for inhibitor **65** (A-74273) following intraduodenal administration in anesthetized salt-depleted monkeys (10 mg/kg, $n = 5$). Values shown are mean ± SEM.

consistent with enhanced absorption of **65** over **75**. The systemic levels increased slowly and steadily and attained an average peak value of 377 ± 104 ng/mL at 300 min, for an average AUC_{id} = 66.5 ± 12.4 mg min/mL. These levels corresponded nicely with the observed long-lasting reduction in MAP. Since the IC_{50} values in monkey plasma at pH 7.4 were similar for **65** and enalkiren (1.5 and 2.3 nM, respectively), the improved activity displayed by **65** was due to the improved bioavailability. Indeed, this was the case, as the calculated bioavailabilities based on bioassay of plasma drug levels were 16 ± 4% for **65**, but only 1.7 ± 0.5% for enalkiren (data not shown).

It is appropriate to comment on the relationship between the results from the above id monkey experiments and those from oral (po) monkey experiments. In general, the AUC's and bioavailabilities in monkeys dosed at 10 mg/kg po (7.7 ± 5.8 μg min/mL and 1.9 ± 1.5%, respectively, for **65**)[15] were lower than the corresponding id values. The reasons for these differences are not clear at this time. However, we presently cannot state which species or experimental model will best predict results in humans.

Conclusions

Our goal was to discover new compounds which combine the intravenous efficacy of enalkiren with oral activity. Our approach was to modify the C-terminal portion of our nonpeptide inhibitors in order to improve the solubility, hydrophilicity, potency, and bioavailability of this novel series. In large measure, we have achieved these goals, producing a series of renin inhibitors with these desirable characteristics. Compound **65** (A-74273) is a representative of the nonpeptide series which meets our original goal. A-74273 displayed high plasma renin potency, high solubility (especially as the hydrochloride salt), good balance of lipophilicity and hydrophilicity, high systemic drug levels after id dosing, and relatively high oral bioavailability in the salt-depleted monkey and thus make A-74273 one of the more promising potential orally active renin inhibitors reported to date. These data collectively represent the demonstration that improved bioavailability of renin inhibitors can result in enhanced efficacy and predictability of a consistent pharmacological response.

Experimental Section

Synthetic Methods. General. All reactions were performed under an inert atmosphere (argon or nitrogen). Reactions requiring anhydrous conditions were carried out in flame-dried glassware which was cooled under dry nitrogen or argon. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Solvents were distilled under nitrogen prior to use as follows: dichloromethane, diisopropylethylamine, and triethylamine from calcium hydride, and tetrahydrofuran (THF) and toluene from sodium benzophenone ketyl. Methanesulfonyl chloride was vacuum distilled from calcium hydride. All other reagents were purchased commercially and used without purification. Reaction products were placed under high vacuum (<0.05 mmHg) for several hours prior to weighing. Thin-layer chromatography (TLC) was performed using E. Merck silical gel 60 F-254 glass-backed plates, 250-μm thickness (analytical) and 2000-μm thickness (preparative); UV light and phosphomolybdic acid stain were used for visualization. Flash chromatography was performed using E. Merck Kieselgel 60 (230–400 mesh), and eluent systems are listed as v/v %. Melting points are given for all solids except glasses; other compounds were isolated as low-melting waxes or oils. Proton magnetic resonance (^{1}H NMR) spectra were recorded on a General Electric Model QZ-300 spectrometer (300 MHz). Chemical shifts are reported as parts per million (ppm) downfield from tetramethylsilane (δ). For resonances which include H_2O with large numbers of protons, approximate values for the integration are given. Infrared spectra were recorded in $CDCl_3$ on a Nicolet 5SXC FTIR spectrophotometer. Mass spectra (FAB^+ and DCI (NH_3)) were determined on a Kratos MS50 spectrometer. Combustion analyses were performed by the Analytical Research Department, Pharmaceutical Products Division, Abbott Laboratories.

(2*S*,4*S*,5*S*)-1-[3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4-(cyclohexylmethyl)-5-oxazolidinyl]-2-(pentanoylamino)-3-methylbutane (7). A solution of acid **5** (200 mg, 0.486 mmol) in 1.5 mL of dry toluene was treated with triethylamine (0.075 mL, 0.54 mmol) and diphenylphosphoryl azide (0.115 mL, 0.54 mmol). The solution was warmed to 65 °C for 2.5 h, then was cooled to 0 °C, and treated with a solution of butylmagnesium chloride (0.668 mL, 2.0 M in THF, 1.34 mmol). The solution was allowed to slowly warm to room temperature and stir for 14 h. The mixture was partitioned between EtOAc and water, and the organic phase was washed (1 × 25 mL of 1 N HCl, 2 × 75 mL of water, 1 × 75 mL of saturated aqueous $NaHCO_3$, 1 × 100 mL of brine), and then concentrated in vacuo. Flash chromatography (20% EtOAc–hexanes) gave 202 mg (84%) of amide **7** as a white solid: mp 124–125 °C; R_f 0.42 (25% EtOAc–hexane); ^{1}H NMR ($CDCl_3$) δ 0.93 (m) and 0.8–1.05 (br m, 11 H total), 1.48 (s) and 1.1–1.9 (several br m, ca. 35 H total), 2.16 (dd, 2 H), 3.63 (br m, 1 H), 3.95 (br m) and 4.0 (m, 2 H total), 5.25 (br m, 1 H); MS m/e 467 $(M + H)^+$.

(2*S*,4*S*,5*S*)-1-[3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4-(cyclohexylmethyl)-5-oxazolidinyl]-2-[[(phenylmethoxy)carbonyl]amino]-3-methylbutane (10). A solution of acid **5** (500 mg, 1.22 mmol) in 3.7 mL of dry toluene was treated with triethylamine (186 μL, 1.34 mmol) and diphenylphosphoryl azide (375 mg, 1.34 mmol). The solution was warmed to 70 °C for 1.5 h and then benzyl alcohol (2.46 g, 27.6 mmol) was added, and the resulting solution was refluxed for 72 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in 125 mL of EtOAc and extracted (1 × 125 mL of 1 N HCl, 1 × 125 mL of water, 1 × 125 mL of saturated aqueous $NaHCO_3$, 1 × 125 mL of brine). The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure. Flash chromatography (6% MeOH–CH_2Cl_2) of the residue provided 510 mg (81%) of carbamate **10** as a colorless semisolid glass: R_f 0.38 (15% EtOAc–hexane); ^{1}H NMR ($CDCl_3$) δ 0.91 (d, J = 6 Hz, 6 H), 1.47 (s, 9 H), 0.85–1.90 (several br m, ca. 22 H), 3.5–3.9 (br m, 2 H), 3.99 (m, 1 H), 4.92 (br m, 1 H), 5.10 (AB, 2 H), 7.3–7.4 (m, 5 H); MS m/e 517 $(M + H)^+$. Anal. ($C_{30}H_{48}N_2O_5$) C, H, N.

(2*S*,4*S*,5*S*)-1-[3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4-(cyclohexylmethyl)-5-oxazolidinyl]-2-[[[2-(4-morpholinyl)ethoxy]carbonyl]amino]-3-methylbutane (14). A solution of acid **5** (260 mg, 0.632 mmol) in 2 mL of dry toluene was treated with triethylamine (99 μL, 72 mg, 0.69 mmol) and diphenylphosphoryl azide (150 mg, 0.695 mmol). The solution was warmed to 65 °C for 2 h and then 4-(2-hydroxyethyl)-morpholine (382 μL, 414 mg, 3.15 mmol) was added, and the resulting solution was warmed to 85 °C for 24 h. The solvent was

(15) Kleinert, H. D. Private communication.

evaporated under reduced pressure and the residue was dissolved in 75 mL of EtOAc and extracted (1 × 50 mL of saturated aqueous $NaHCO_3$, 1 × 50 mL of water, 1 × 50, mL of brine). The organic phase was dried (Na_2SO_4) and filtered, and the filtrate was concentrated under reduced pressure to afford a yellow oil. Flash chromatography (2.5% MeOH–CH_2Cl_2) provided 221 mg (0.409 mmol, 65%) of carbamate **14** as a colorless glass: R_f 0.46 (5% MeOH–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.93 (m), 1.49 (s), and 0.84–1.90 (several br m, 37 H), 2.50 (m, 4 H), 2.61 (m, 2 H), 3.58–3.78 (br m, 6 H), 3.99 (m, 1 H), 4.20 (m, 2 H), 4.90 (m, 1 H); MS m/e 540 $(M + H)^+$. Anal. ($C_{29}H_{53}N_3O_6$) C, H, N.

Compounds **8**, **9**, **11–13**, **15**, and **16** were synthesized in an analogous manner by substituting the appropriate alcohol or amine for *N,N*-dimethylethanolamine.

(2*S*,4*S*,5*S*)-1-[3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4-(cyclohexylmethyl)-5-oxazolidinyl]-2-amino-3-methylbutane (17). A solution of benzyl carbamate **10** (481.6 mg, 0.929 mmol) was dissolved in 50 mL of EtOAc and hydrogenated (4 atm H_2) over 0.25 g 10% Pd–C for 18 h. The resultant solution was filtered through Celite, concentrated, and dried under high vacuum to produce 354.3 mg (0.926 mmol, 99%) of amine **17** as a colorless, viscous oil: R_f 0.22 (0.5 % concentrated aqueous NH_4OH–10% MeOH–EtOAc); ^1H NMR ($CDCl_3$) δ 0.89 (d, J = 6 Hz), 0.91 (d, J = 6 Hz) and 0.8–1.05 (br m, 8 H total), 1.48 (s), 1.50 (br s), 1.58 (br s), and 1.1–1.88 (several br m, ca. 29 H total), 2.81 (m, 1 H), 3.57–3.90 (br m, 2 H), 4.11 (ddd, J = 3, 4, 10.5 Hz, 1 H); MS m/e 383 $(M + H)^+$. Anal. ($C_{22}H_{42}N_2O_3 \cdot 0.5\ H_2O$) C, H, N.

(2*S*,4*S*,5*S*)-1-[3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4-(cyclohexylmethyl)-5-oxazolidinyl]-2-[(methylsulfonyl)amino]-3-methylbutane (18). A solution of amine **17** (182 mg, 0.476 mmol) in 3 mL of CH_2Cl_2 was cooled to −10 °C and treated sequentially with triethylamine (100 μL, 73 mg, 0.714 mmol) and methanesulfonyl chloride (55 μL, 82 mg, 0.714 mmol). The solution was stirred at −10 °C for 1.5 h and then partitioned between 40 mL pH 7.0 phosphate buffer and 80 mL of CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 (2 × 35 mL) and then the combined organic phases were washed (50 mL of saturated aqueous $NaHCO_3$, 50 mL of brine), dried ($MgSO_4$), filtered, and concentrated in vacuo to provide sulfonamide **18** as a white foam (199 mg, 0.432 mmol, 91%): mp 145–146 °C; R_f 0.66 (5% MeOH–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.95 (d, J = 7 Hz), 0.98 (d, J = 6 Hz) and 0.90–1.05 (br m, 8 H total), 1.1–1.35 (br m, 6 H), 1.48 (s, 9 H), 1.62 (br s), and 1.35–1.90 (several br m, ca. 13 H total), 1.90–2.02 (m, 1 H), 3.03 (s, 3 H), 3.44 (m, 1 H), 3.5–3.75 (br m, 1 H), 4.04 (m, 1 H), 4.53 (d, J = 11 Hz, 1 H); MS m/e 461 $(M + H)^+$, 478 $(M + NH_4)^+$. Anal. ($C_{23}H_{44}N_2O_5S$) C, H, N.

Sulfonamide **19** was synthesized from amine **17** in an analogous manner, by substituting 2-propanesulfonyl chloride for methanesulfonyl chloride.

(2*S*,3*S*,5*S*)-*N*-[1-Cyclohexyl-3-hydroxy-6-methyl-5-[[(phenylmethoxy)carbonyl]amino]heptan-2-yl]-(2*S*,1′*S*)-2-[[1-[4-(methoxymethoxy)piperidin-1-yl]carbonyl]-2-phenylethoxy]hexanamide (26). Part A. Biscarbamate **10** (268 mg, 0.519 mmol) was dissolved in 5 mL of dry CH_2Cl_2, the solution was cooled to 0 °C, and TFA (5 mL) was added slowly dropwise. The resulting solution was stirred at 0 °C for 3.5 h, at which time TLC (neutralized small aliquot, eluted with 25% EtOAc–hexane) indicated complete consumption of starting carbamate. The reaction was concentrated in vacuo, the residue was dissolved in 7 mL of THF and 3.5 mL of H_2O, the solution was cooled to 0 °C, and TFA (ca. 0.1 mL) was added. The mixture was stirred at 0 °C for 4 h, and at ambient temperature for an additional 12 h. The mixture was concentrated and the residue partitioned between 20 mL of 1 M Na_2CO_3 and 25 mL of CH_2Cl_2. The aqueous phase was extracted with CH_2Cl_2 (2 × 25 mL), and then the combined organic phases were dried (Na_2SO_4), filtered, and concentrated in vacuo to produce 190.2 mg (0.505 mmol, 97 %) of crude (2*S*,3*S*,5*R*)-2-amino-5-[[(phenylmethoxy)carbonyl]amino]-1-cyclohexyl-3-hydroxy-6-methylheptane as a waxy solid: R_f 0.38 (7.5% MeOH–0.75% concentrated aqueous NH_4OH–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.73–1.05 (br m), 0.92 (d, J = 6 Hz) and 0.95 (d, J = 6 Hz, 8 H total), 1.05–1.35 (br m, 6 H), 1.35–2.5 (several br m, ca. 14 H total), 2.64–2.72 (m, 1 H), 3.25–3.34 (m, 1 H), 3.67–3.79 (m, 1 H), 4.84 (br d, J = 9 Hz, 1 H), 5.10 (s, 2 H), 7.29–7.41 (m, 5 H); MS m/e 377 $(M + H)^+$.

Part B. A portion of the crude amino alcohol from part A (60.3 mg, 0.160 mmol) was combined with acid **21** (71.8 mg, 0.176 mmol) and HOBT (31.9 mg, 0.208 mmol), the solids were dissolved in 1.7 mL of DMF, and 4-methylmorpholine (25 μL, 23 mg, 0.224 mmol) was added. The solution was cooled to −23 °C (under N_2), and EDC (46 mg, 0.240 mmol) was added as a solid. The resulting mixture was stirred at −23 °C for 4 h and then allowed to warm slowly to ambient temperature and stir for 18 h. The solution was concentrated under high vacuum, and the residue partitioned between 25 mL of CH_2Cl_2 and 25 mL of 80% saturated aqueous $NaHCO_3$. The organic phase was washed with 25 mL of 80 % saturated aqueous $NaHCO_3$, and then the combined aqueous phase was back-extracted with 25 mL of CH_2Cl_2. The combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure to an oil, 149 mg. Flash chromatography (1.5% MeOH–CH_2Cl_2) gave 114.1 mg of amide **26** as a white foam: mp 43–59 °C; R_f 0.26 (3% MeOH–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.89 (m) and 0.66–1.88 (several br m, ca. 36 H total), 3.34 (s), and 2.95–3.98 (several br m, 14 H total), 4.50 (m, 1 H), 4.63 (s, 2 H), 4.78 (d, 1 H), 5.15 (m, 2 H), 6.02, 6.15 (2 d, 1 H), 7.19–7.40 (m, 10 H); MS m/e 766 $(M + H)^+$, 783 $(M + NH_4)^+$. Anal. ($C_{44}H_{67}N_3O_8 \cdot 0.5H_2O$) C, H, N.

(2*S*,3*S*,5*S*)-*N*-(1-Cyclohexyl-3-hydroxy-6-methyl-5-aminoheptan-2-yl)-(2*S*,1′*S*)-2-[1-[[4-(methoxymethoxy)-piperidin-1-yl]carbonyl]-2-phenylethoxy]hexanamide (33). Benzyl carbamate **26** (67 mg, 0.875 mmol) was dissolved in 9 mL of EtOAc and stirred with 10% Pd/C (14 mg) under 1 atm H_2 for 24 h. The mixture was filtered through Celite, the filtrate was concentrated in vacuo, and the residue was purified by flash chromatography (4% MeOH–0.5% concentrated aqueous NH_4OH–CH_2Cl_2) to provide 37.7 mg (0.590 mmol, 67 %) of amine **33** as low-melting waxy solid: R_f 0.57 (10% MeOH–1% concentrated aqueous NH_4OH–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.89 (m) and 0.63–2.30 (several br m, 38 H total), 3.36 (s) and 2.75–3.98 (several br m, 14 H total), 4.65 (s, 2 H), 4.70 (m, 1 H), 5.66, 5.76 (2 d, 1 H), 7.22–7.39 (m, 5 H); MS m/e 632 $(M + H)^+$. Anal. ($C_{36}H_{61}N_3O_6 \cdot 0.5\ H_2O$) C, H, N.

(2*S*,3*S*,5*S*)-*N*-[1-Cyclohexyl-3-hydroxy-6-methyl-5-[[[2-(4-morpholinyl)ethoxy]carbonyl]amino]heptan-2-yl](*tert*-butyloxycarbonyl)-L-phenylalaninyl-L-histidinamide (44). Part A. Carbamate **14** (224.4 mg, 0.379 mmol) was dissolved in 2 mL of dry CH_2Cl_2, the solution was cooled to 0 °C, and TFA (2 mL) was added slowly dropwise. The resulting solution was stirred at 0 °C for 4 h, at which time TLC (neutralized small aliquot, eluted with 5% MeOH–CH_2Cl_2) indicated complete consumption of starting carbamate. The reaction was concentrated in vacuo, the residue was dissolved in 3 mL of THF and 1.5 mL of H_2O, the solution was cooled to 0 °C, and TFA (ca. 0.2 mL) was added. The mixture was stirred at 0 °C for 4 h and at ambient temperature for an additional 12 h. The mixture was concentrated and the residue partitioned between 50 mL of 1 M Na_2CO_3 and 25 mL of CH_2Cl_2. The aqueous phase was saturated with NaCl and extracted with CH_2Cl_2 (2 × 25 mL), and then the combined organic phases were dried (Na_2SO_4), filtered, and concentrated in vacuo to produce 157 mg (0.393 mmol, 104 %) of crude (2*S*,3*S*,5*R*)-2-amino-5-[[[2-(4-morpholinyl)ethoxy]-carbonyl]amino]-1-cyclohexyl-3-hydroxy-6-methylheptane as a waxy solid: R_f 0.36 (10% MeOH–1% concentrated aqueous NH_4OH–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.70–1.05 (br m, 8 H), 1.05–2.20 (several br m, ca. 18 H), 2.46–2.58 (br m, 4 H), 2.59–2.74 (m, 3 H), 3.25–3.50 (br m, 1 H), 3.65–3.90 (m, 5 H), 4.20 (t, J = 6 Hz, 2 H), 4.49–4.58 (br m) and 4.83 (br d, 1 H total).

Part B. Crude amino alcohol from part A (20.0 mg, 50.0 μmol) and active ester **37** (28.3 mg, 62.6 μmol) were combined in a 1-mL reaction vial, the vial was cooled in an ice bath, and 0.30 mL of CH_2Cl_2 was added. The resulting solution was stirred at 0 °C for 6 h and for an additional 14 h at ambient temperature. The solution was concentrated with a stream of N_2, and the residue was dissolved in 0.10 mL of THF, 0.15 mL of HOAc, and 0.05 mL of water. The vial was sealed and warmed to 50 °C for 5 h, at which time TLC (10% MeOH–CH_2Cl_2) indicated consumption of bis-Boc material (R_f 0.63). The solution was partitioned between 20 mL of CH_2Cl_2 and 10 mL of 1 M aqueous Na_2CO_3. The aqueous layer was extracted with 3 × 10 mL of CH_2Cl_2, then the combined organic phases were washed with 10 mL brine, dried (Na_2SO_4), and filtered, and the filtrate was concentrated to a glass, 32.1 mg. Flash chromatography (5% MeOH–CH_2Cl_2) provided

27 mg (42.4 μmol, 85%) of (2*S*,3*S*,5*S*)-*N*-[1-cyclohexyl-3-hydroxy-6-methyl-5-[[[2-(4-morpholinyl)ethoxy]carbonyl]-amino]heptan-2-yl](*tert*-butyloxycarbonyl)-L-histidinamide as a yellowish glass: R_f 0.38 (10% MeOH–1% concentrated aqueous NH_4OH–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.65–1.00 (m, 8 H), 1.00–1.36 (br m, 8 H), 1.37–1.85 (br m) and 1.46 (s, ca. 23 H total), 2.45–2.59 (m, 4 H), 2.59–2.67 (m, 2 H), 2.92–3.04 (m, 1 H), 3.12–3.24 (m, 1 H), 3.50–3.60 (br m, 2 H), 3.71 (t, J = 4.5 Hz) and 3.60–4.00 (br m, 7 H total), 4.10–4.20 (m, 1 H), 4.23–4.35 (m, 1 H), 4.32–4.45 (br m, 1 H), 4.50–4.62 (br m) and 4.90–5.05 (br m, 1 H total), 6.47–6.70 (br m, 1 H), 6.87 (s, 1 H), 7.57 (s, 1 H); MS m/e 637 $(M + H)^+$.

Part C. Boc-histadinamide from part B (24 mg, 37.7 μmol) was dissolved in 1 mL of CH_2Cl_2, and the solution was cooled to 0 °C. TFA (1 mL) was added slowly dropwise over 5 min, and the resulting solution was stirred for 4 h at 0 °C. The solution was concentrated in vacuo without warming, and the residue was partitioned between 10 mL of 1 M Na_2CO_3 and 20 mL of CH_2Cl_2. The aqueous phase was saturated with NaCl and further extracted with 2 × 15 mL of CH_2Cl_2, then the combined organic phases were dried (Na_2SO_4) and filtered and the filtrate concentrated in vacuo to give (2*S*,3*S*,5*S*)-*N*-[1-cyclohexyl-3-hydroxy-6-methyl-5-[[[2-(4-morpholinyl)ethoxy]carbonyl]amino]heptan-2-yl]-L-histidinamide as a foam, 14.7 mg (70% crude): R_f 0.25 (10% MeOH–1% concentrated aqueous NH_4OH–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.60–1.00 (br m, 9 H), 1.00–2.20 (several br m, ca. 20 H), 2.40–2.60 (br m, 4 H), 2.60–2.68 (m, 2 H), 2.87–3.00 (m, 1 H), 3.08–3.22 (br m, 1 H), 3.50–3.98 (several m, 10 H), 4.11–4.34 (m, 2 H), 4.43 (br d, J = 10 Hz) and 4.53–4.63 (br m, 1 H), 4.90–5.08 (br m, 1 H), 6.86 (s, 1 H), 7.40–7.50 (m, 1 H), 7.55 (s, 1 H); MS m/e 537 $(M + H)^+$.

Part D. Crude histidinamide from part C (10.9 mg, 20.3 μmol) and *N*-α-Boc-phenylalanine *N*-hydroxysuccinimide ester (8.5 mg, 23.4 mmol) were dissolved in 0.23 mL DMF, and the resulting solution was stirred at ambient temperature for 18 h. The reaction solution was concentrated under high vacuum, and the residue was partitioned between 10 mL of CH_2Cl_2 and 10 mL of a 1:1 (v/v) solution of 1 M aqueous Na_2CO_3 and water. The aqueous layer was extracted with 2 × 10 mL of CH_2Cl_2 and 1 × 10 mL of 10% EtOH–CH_2Cl_2. The combined organic phases were washed with 10 mL of brine and dried (Na_2SO_4), and the filtrate was concentrated under reduced pressure to produce the crude dipeptide as a glass (14.8 mg, 93%). The crude was purified by preparative TLC (5 × 20-cm plate, 0.25-μm thickness, 10% MeOH–CH_2Cl_2), providing 9.8 mg (12.5 μmol, 62%) of pure dipeptide **44** as a white powder: mp 106–112 °C; R_f 0.46 (15% MeOH–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.67–0.97 (br m, 8 H), 0.97–1.46 (br m) and 1.39 (s, ca. 19 H total), 1.45–1.80 (br m, 6 H), 2.46–2.77 (br m, 6 H), 2.80–3.00 (br m, 2 H), 3.17–3.35 (br m, 2 H), 3.40–4.01 (several br m, 8 H), 4.10–4.34 (m, 3 H), 4.52–4.71 (br m, 2 H), 4.94–5.07 (br m, 1 H), 5.50–5.70 (br m) and 6.53–6.61 (br m, 1 H total), 6.62–6.80 (br m, 1 H), 6.80 (br s, 1 H), 7.20–7.40 (br m, 5 H), 7.50 (br s, 1 H), 8.0–8.9 (vbr m, 1 H). HRMS calcd for $(M + H)^+$ for ($C_{41}H_{66}N_7O_8$) 784.4973, found 784.4980.

Peptide-based inhibitors **38–48** were synthesized in a similar manner from the corresponding acetonide carbamates **7–10** and **12–19**. Inhibitor **74** was synthesized according to parts B–D from the known butyl (2*S*,4*S*,5*S*)-5-amino-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (**73**).[8]

***N*-[3-(4-Morpholinyl)propyl]-(2*S*,4′*S*,5′*S*)-2-[[3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4-(cyclohexylmethyl)-5-oxazolidinyl]methyl]-3-methylbutanamide (55).** Carboxylic acid **5** (180 mg, 0.473 mmol), HOBT (100 mg, 0.653 mmol) and *N*-methylmorpholine (133 mg, 1.32 mmol) were dissolved in 2.5 mL of dry DMF, and the solution was cooled to −20 °C (under N_2). Solid EDC (128 mg, 0.668 mmol) was added, and the resulting mixture was stirred at −20 to 0 °C for 1 h. The vessel was sealed and allowed to react at 0 °C (in refrigerator) during 24 h. TLC indicated complete conversion to HOBT ester (R_f 0.45, EtOAc–hexane 1:3). To the resulting solution was added 4-(3-aminopropyl)morpholine (76 mg, 0.526 mmol). The resulting solution was stirred at 0 °C for 4 h and for a further 20 h, allowing it to warm slowly to room temperature. The volatiles were removed by high-vacuum distillation, and the residue was purified by flash chromatography (4% MeOH–CH_2Cl_2), yielding 244 mg (96%) of amide **55** as a colorless glass: R_f 0.23 (5% MeOH–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.92 (d, J = 6.5 Hz), 0.95 (d, J = 6.5 Hz) and 0.8–1.0 (br m, 8 H total), 1.46 (s) and 1.48 (s, 12 H total), 1.57 (br s) and 1.08–1.91 (several br m, ca. 11 H total), 2.01 (m, 1 H), 2.46 (br m, 6 H), 3.37 (m, 2 H), 3.64 (br m, 1 H), 3.75 (br m, 5 H), 6.80 (br m, 1 H). HRMS calcd for $(M + H)^+$ of $C_{30}H_{56}N_3O_5$ 538.4220, found 538.4220.

Compounds **49–54**, **56**, and **57** were produced in an analogous manner, substituting the appropriate amine for 4-(3-aminopropyl)morpholine.

***N*-[3-(4-Morpholinyl)propyl]-(2*S*,4*S*,5*S*,1′*S*,2′*S*)-5-[2-[1-[(4-(methoxymethoxy)piperidin-1-yl)carbonyl]-2-phenylethoxy]hexanamido]-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (65).** Carbamate **55** (85.2 mg, 0.158 mmol) was dissolved in 1.0 mL of dry CH_2Cl_2, the solution was cooled to −10 °C (N_2), and 1.0 mL of trifluoroacetic acid was added rapidly dropwise. The resulting solution was stirred at −10 to 0 °C for 4 h. The solvents were removed in large part with a stream of nitrogen, and the residue (as a concentrated solution in trifluoroacetic acid) was dissolved in 1.0 mL of THF and 0.3 mL of water at 0 °C. The solution was allowed to warm slowly to ambient temperature and stir for 18 h. The solution was made basic with an excess of 1.0 M aqueous Na_2CO_3, the solution was saturated with NaCl and then extracted with 5 × 10 mL of 5% EtOH–$CHCl_3$. The combined organic phases were washed with brine, dried (Na_2SO_4), and concentrated, and the residue placed under high vacuum overnight to yield 70.3 mg (0.178 mmol, 113%) of the crude amino alcohol as a yellow viscous oil: ^1H NMR ($CDCl_3$) δ 0.92 (d), 0.95 (d) and 0.75–1.07 (br m, 8 H total), 1.07–1.94 (several br m, ca. 20 H), 2.08 (m, 1 H), 2.45 (m, 6 H), 2.56 (m, 1 H), 3.07 (m, 1 H), 3.35 (m, 2 H), 3.71 (m, 4 H), 6.8 (br m, 1 H).

Coupling was achieved by combining carboxylic acid **21** (72 mg, 0.177 mmol), the above amino alcohol (63.8 mg, 0.168 mmol), HOBT (34 mg, 0.22 mmol), and *N*-methylmorpholine (25 mg, 0.25 mmol) in 1.0 mL of dry DMF. The resulting solution was cooled to −20 °C (under argon), and EDC (45 mg, 0.23 mmol) was added. The reaction was allowed to slowly warm to room temperature as the ice bath melted, and stirred for a total of 24 h. The solvent was removed by high-vacuum distillation, and the residue was partitioned between 15 mL of CH_2Cl_2, 9 mL of saturated aqueous $NaHCO_3$, and 1 mL of H_2O. The aqueous phase was further extracted (3 × 10 mL of CH_2Cl_2), and the combined organic phases were washed with 10 mL of brine, dried (Na_2SO_4), and concentrated. Purification by flash chromatography (6% MeOH–0.5% concentrated aqueous NH_4OH–CH_2Cl_2) yielded 85.1 mg (0.108 mmol, 68%) of amide **65** as a hygroscopic glassy solid: mp 49–51 °C; R_f 0.16 (5% MeOH–0.5% concentrated aqueous NH_4OH–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.90–0.92 (m, 9 H), 0.65–1.90 (several br m, ca. 28 H total), 2.02 (m, 1 H), 2.45 (br m, 6 H), 2.95 (m, 1 H), 3.05 (dd, J = 9, 13.5 Hz, 1 H), 3.20 (br m, 2 H), 3.36 (s, 3 H), 3.45 (m, 2 H), 3.6–4.0 (several br m) and 3.71 (m, 10 H total), 4.48 (dd, J = 5, 9 Hz, 1 H), 4.68 (s, 2 H), 5.80 (d, J = 9 Hz) and 5.88 (d, J = 9 Hz, 1 H total), 6.87 (br t, 1 H), 7.3 (br m, 5 H); MS m/e 787 $(M + H)^+$. Anal. ($C_{44}H_{74}N_4O_4 \cdot H_2O$) C, H, N.

Compounds **23–32**, **34**, **58–60**, **62–64**, **67**, and **68** were produced in a similar manner from acetonide carbamates **7–16**, **18**, **49–51**, **52–54**, **56**, and **57**, respectively.

***N*-[3-(4-Oxido-4-morpholinyl)propyl]-(2*S*,4*S*,5*S*,1′*S*,2′*S*)-5-[2-[1-[(4-(methoxymethoxy)piperidin-1-yl)-carbonyl]-2-phenylethoxy]hexanamido]-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (66).** Morpholinyl amide **65** (44.1 mg, 56 μmol) was dissolved in 0.22 mL of MeOH and treated with 30% aqueous H_2O_2 (17 μL, 5.7 mg H_2O_2, 0.17 mmol). The resulting solution was stirred at ambient temperature for 4 days and then at 50 °C for 18 h, at which time TLC (15% MeOH–CH_2Cl_2) indicated complete consumption of starting amine. The solution was concentrated with a stream of N_2, and the residue was partitioned between 20 mL of $CHCl_3$, 5 mL of brine, and 5 mL of H_2O. The aqueous phase was extracted with 3 × 10 mL of $CHCl_3$, and the combined organic phases were washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo to a solid. The crude was purified by preparative TLC (15% MeOH–$CHCl_3$) to give 24.8 mg (30.9 mmol, 55%) of amine *N*-oxide **66** as a white foam: mp 71–83 °C; R_f 0.13 (15% MeOH–$CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.85–0.98 (m) and 0.60–2.2 (several br m, ca. 49 H total), 2.88–3.1 (br m, 2 H), 3.1–3.25 (br m, 3 H),

3.35 (s), 3.37 (s) and 3.25–3.67 (several br m, 1 H total), 3.67–3.95 (br m, 4 H), 4.01–4.22 (br m, 1 H), 4.26–4.40 (br m, 2 H), 4.53–4.63 (br m, 1 H), 4.68 (s) and 4.69 (s, 2 H total), 5.80 (br t, J = 8.5 Hz, 1 H), 7.25–7.44 (br m, 5 H), 8.12–8.21 (br m, 1 H); MS m/e 803 $(M + H)^+$. Anal. ($C_{44}H_{74}N_4O_5 \cdot 1.5\ H_2O$) C, H, N.

Amine N-oxide **61** was produced in a similar manner from amino amide **60**.

(2′*S*,3′*S*,5′*R*)-*N*-[5-[[[3-(4-Morpholinyl)propyl]amino]carbonyl]-1-cyclohexyl-3-hydroxy-6-methylheptan-2-yl]-(1*S*)-[1-[(4-(methoxymethoxy)piperidin-1-yl)carbonyl]-2-phenylethyl]-L-norleucinamide (70). Acetonide carbamate **55** (100 mg, 0.186 mmol) was deprotected as described in part A. of the preparation of inhibitor **65**, and the resulting crude amino alcohol (73.9 mg, 0.186 mmol), amino acid **22** (82.8 mg, 0.204 mmol), HOBT (36.8 mg, 0.241 mmol), and N-methylmorpholine (27 mg, 0.26 mmol) in 1.0 mL of dry DMF. The resulting solution was cooled to −20 °C (under argon), and EDC (56.0 mg, 0.292 mmol) was added. The reaction was allowed to slowly warm to room temperature as the ice bath melted and then was stirred for 24 h. The solvent was removed by high-vacuum distillation, and the residue was partitioned between 40 mL of CH_2Cl_2, 18 mL of saturated aqueous $NaHCO_3$ and 2 mL of H_2O. The aqueous phase was further extracted (4 × 15 mL of CH_2Cl_2), and the combined organic phases were washed with 10 mL of brine, dried (Na_2SO_4), and concentrated to a yellow foam, 156 mg. Purification by flash chromatography (6% MeOH–0.5% concentrated aqueous NH_4OH–CH_2Cl_2) yielded 118 mg (0.150 mmol, 81%) of inhibitor **70** as a hygroscopic foamy solid: mp 66–71 °C; R_f 0.32 (7.5% MeOH–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 0.89 (t, J = 7.5 Hz), 0.93 (d, J = 6.5 Hz), and 0.65–1.0 (br m, 11 H total), 1.0–1.94 (several br m, ca. 20 H total), 2.06 (m, 1 H), 2.33–2.6 (br m, 7 H), 2.67–2.85 (m, 3 H), 3.0 (br m, 1 H), 3.25 (br m, 3 H), 3.35 (2 s, 3 H), 3.35–3.9 (several br m, 13 H total), 4.65 (2 s, 2 H), 6.75–6.91 (m, 2 H), 7.2–7.4 (br m, 5 H); MS m/e 786 $(M + H)^+$. Anal. ($C_{44}H_{75}N_5O_7$) C, H, N.

Compounds **35**, **36**, **69**, and **71** were synthesized in a similar manner from acetonide carbamates **12**, **14**, **51**, and **57**, respectively.

Biochemical Methods. The procedures for the following assays have been described previously: purified human renal renin (pH 6.0),[16] human plasma renin (pH 7.4),[17] bovine cathepsin D,[14] porcine pepsin,[14] human gastricsin,[18] and human pepsin.[16]

The IC_{50} values for animal renins at pH 7.4 were determined by the following modification of the human assay:[15] 1.3 mM 8-hydroxyquinoline was present in the final 100-μL incubation mixture, and the respective incubation times (37 °C) and fraction of incubate utilized for the radioimmunoassay of angiotensin I were 2 h and 50% for the monkey, 1 h and 50% for the ferret, 4 h and 100% for the dog, and 2 h and 50% for the rat. The dog samples were supplemented with 10% nephrectomized hog plasma with no detectable renin activity, in order to increase the plasma renin activity.

Human cathepsin G (Sigma Chemical Co.) was from leukocytes and inhibition studies were performed with N-succinyl-Ala-Ala-Pro-Phe-4-nitroanilide as substrate.[19] Human cathepsin D was isolated from spleen utilizing hemoglobin affinity chromatography,[20] and assayed with hemoglobin substrate similar to bovine cathepsin D.[14]

Blood levels in ferrets and monkeys were measured by a bioassay procedure similar to that previously utilized for rat samples,[21] except that the plasma was diluted serially with buffer prior to precipitation with acetonitrile, and both the angiotensinogen and renin solutions used for reconstitution of the evaporated samples contained 1.15 mM phenylmethanesulfonyl fluoride.

Plasma drug level–time data from intravenous experiments were fitted to a bisexponential decay model: $C_t = A_e^{-\alpha t} + B_e^{-\beta t}$ where t represents time from administration and C_t represents the plasma drug level at a given time.[12] Trapezoidal integration of the observed data and an extrapolated $C_{t=0}$ data point (A + B) provided the intravenous area under the curve (AUC_{iv}). Trapezoidal integration of plasma drug level–time data from intraduodenal experiments provided the intraduodenal areas under the curve (AUC_{id}). The per cent bioavailability value is the ratio of the AUC_{id} to the AUC_{iv} normalized for dose and is expressed as mean ± standard error of measurement (SEM, $\sigma/\sqrt{n}$):

$$\text{per cent bioavailability} = \frac{AUC_{id} \times \text{intravenous dose}}{AUC_{iv} \times \text{intraduodenal dose}} \times 100$$

Pharmacological Methods. Hemodynamic Assessments. Intravenous and intraduodenal activities of renin inhibitors were determined in male cynomolgus monkeys (*Macaca fascicularis*) weighing between 3 and 5 kg. Pretreatment (in order to elevate baseline plasma renin activities) consisted of furosemide treatment (5 mg/kg, po) on days 7 and 1 prior to the experiment accompanied by a low-salt chow and fresh fruit diet. The monkeys were fasted overnight and on the day of the study, anesthesia was induced with sodium pentobarbital, 15 mg/kg bolus sustained by a 0.10 mg/kg per min infusion. Blood pressure was measured directly from a femoral artery catheter connected to a Grass pressure transducer Model P23dB and Grass polygraph Model 7 (Grass Instruments, Quincy, MA). Compounds were administered through a leg vein to determine intravenous efficacy and through a catheter placed in the duodenum to assess intraduodenal activity. Each monkey received only one dose of a compound.

Pharmacokinetics in the Ferret. Plasma drug concentrations were determined following either intravenous (0.3 mg/kg) or intraduodenal (10.0 mg/kg) dosing in fasted male Fitch ferrets, weighing between 0.4–1.2 kg. Each animal under Inactin anesthesia (100–120 mg/kg, ip) underwent catheterizations of the carotid artery, jugular vein (iv injection), portal vein, and trachea. Blood samples were collected at various intervals simultaneously from the portal and carotid vessels and plasma drug levels quantitated.

Formulation. All compounds were tested as HCl salt solutions.

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Supplementary Material Available: Characterization data (nuclear magnetic resonance spectra, mass spectra, melting points and microanalytical) for all compounds contained in Tables II–IV which do not appear in the Experimental Section (13 pages). Ordering information is given on any current masthead page.

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