at δ 1.7 was assigned to C_2H and the multiplet at δ 2.35 was assigned to C_1HCO. Irradiation at δ 1.7 yielded a doublet of doublets at δ 2.35 with J = 4.9 and 9.3 Hz. This was due to axial-equatorial and axial-axial coupling with C_6H. Therefore, the proton geminal to the carboxaldehyde is in the axial position. This results in an equatorial configuration for the carboxaldehyde C-1. In **15a** decoupling was not possible, so this was assigned the axial configuration due to the assignment of **15b**. **15a**: ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6 H, J = 8 Hz, CH_3), 1.25 (s, 20 H, CH_2), 1.4-2.0 (m, 6 H, ring CH_2), 2.2 and 2.3 (t, 2 H, J = 8.5 Hz, CH_2CO), 2.6 (m, 1 H, $CHCO$), 4.8 and 5.3 (m, 1 H, $CHOCO$), 9.6 (s, 1 H, COH). Anal. ($C_{23}H_{40}O_5 \cdot 1.25H_2O$) C, H. Compound **15b** was assigned the S configuration. The differences in the ^{1}H NMR for **15b** are δ 2.35 (m, 1 H, $CHCO$), 5.0 and 5.2 (m, 1 H, $CHOCO$). Anal. ($C_{23}H_{40}O_5 \cdot 1.75H_2O$) C, H: calcd, 10.75; found, 9.65. All subsequent **a** compounds are in the axial configuration at C-1 and **b** compounds are in the equatorial configuration at C-1.

3-[3,4-Bis(octanoyloxy)cyclohexyl]-2-propen-1-al (16a and 16b). In 10 mL of toluene were combined 0.15 g (0.4 mmol) of **15a** and 0.13 g (0.44 mmol) of (triphenylphosphoranylidene)-acetaldehyde. This was refluxed for 12 h and then poured into 10 mL of water. The organic layer was separated and the water was extracted with 20 mL of ether. The combined organic layers were dried ($MgSO_4$), evaporated to a residue, and chromatographed with 98:2 CH_2Cl_2/CH_3OH to yield 0.13 g (0.3 mmol, 75% yield) of **16a** as a clear oil: ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6 H, J = 6 Hz, CH_3), 1.3 (s, 20 H, CH_2), 1.5-2.1 (m, 7 H, ring CH_2 and CHC=C), 2.25 and 2.35 (t, J = 8 Hz, 2 H, CH_2CO), 4.85-5.35 (m, 2 H, $CHOCO$), 6.1 (m, 1 H, C=CH), 6.75 (dt, 1 H, J = 7 Hz, J = 14 Hz, CH=C), 9.5-9.7 (m, 1 H, COH). Anal. ($C_{25}H_{42}O_5 \cdot H_2O$) C, H. Compound **15b** was treated in the same manner to yield 0.11 g of **16b** as a clear oil (0.25 mmol, 64% yield). There were no differences in the ^{1}H NMR compared to that of **16a**. Anal. ($C_{25}H_{42}O_5 \cdot 0.5H_2O$) C, H.

3-[3,4-Bis(octanoyloxy)cyclohexyl]-2-propen-1-ol (17a and 17b). Compounds **16a** and **16b** were treated as for **5** to yield 75 mg of **17a** and **17b** (0.4 mmol, 100% yield). **17a**: ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6 H, J = 6 Hz, CH_3), 1.3 (s, 20 H, CH_2), 1.5-2.0 (m, 7 H, ring CH_2), 2.25 and 2.35 (t, 2 H, J = 7.5 Hz, CH_2CO), 3.5 (dd, J = 6 Hz, J = 16 Hz, 2 H, CH_2OH), 4.8 and 5.3 (m, 1 H, $CHOCO$), 5.6 (m, 2 H, CH=CH). Anal. ($C_{25}H_{44}O_5$) C, H: calcd, 10.44; found, 11.86. ^{1}H NMR for **17b** is the same as that for **17a**. Anal. ($C_{25}H_{44}O_5$) C, H.

4-[3,4-Bis(octanoyloxy)cyclohexyl]-1-*O*-(dimethyl-*tert*-butylsilyl)-3-buten-1-ol (18a and 18b). These compounds were synthesized in the same manner as **8**, starting with 500 mg of **15a** (1.3 mmol) to yield 460 mg of **18a** (0.8 mmol, 63% yield): ^{1}H NMR ($CDCl_3$) δ 0.0 (s, 6 H, $SiCH_3$), 0.8 (s, 15 H, $C(CH_3)_3$ and CH_3), 1.25 (m, 20 H, CH_2), 1.5-1.8 (m, 9 H, ring CH_2 and CHC=C, CH_2C=C), 2.25 and 2.35 (t, 2 H, J = 7.5 Hz, CH_2CO), 3.6 (t, 2 H, J = 7 Hz, CH_2OSi), 4.75 (m, 1 H, $CHOCO$), 5.15-5.3 (m, 3 H, CH=CH and $CHOCO$). Anal. ($C_{32}H_{60}O_5Si$) H, C: calcd, 69.51; found, 70.31. Starting with 750 mg of **15b** (1.9 mmol) yielded 650 mg of **18b** (1.2 mmol, 62% yield). Differences in ^{1}H NMR from that of **18a**: 4.8 and 5.2 (m, 1 H, $CHOCO$), 5.3 (m, 2 H, CH=CH). Anal. ($C_{32}H_{60}O_5Si \cdot H_2O$) C, H.

4-[3,4-Bis(octanoyloxy)cyclohexyl]-3-buten-1-ol (19a and 19b). These compounds were synthesized in the same manner as **10**, starting with 200 mg of **18a** (0.36 mmol) to yield 150 mg of **19a** (0.34 mmol, 95% yield): ^{1}H NMR ($CDCl_3$) δ 0.9 (m, 6 H, CH_3), 1.3 (m, 20 H, CH_2), 1.6-2.0 (m, 9 H, ring CH_2 and CHC=C, CH_2C=C), 2.25 and 2.35 (t, 2 H, J = 7.5 Hz, CH_2CO), 3.7 (dd, 2 H, J = 10.5 H, J = 5.5 Hz, CH_2OH), 4.85 and 5.3 (m, 1 H, $CHOCO$), 5.35 (m, 2 H, CH=CH). Anal. ($C_{26}H_{46}O_5 \cdot 0.5H_2O$) C, H. Starting with 200 mg of **18b** (0.36 mmol) yielded 130 mg of **19b** (0.29 mmol, 82% yield). Differences in ^{1}H NMR from that of **19a**: ($CDCl_3$) δ 5.35-5.5 (m, 2 H, CH=CH). Anal. ($C_{26}H_{46}O_5 \cdot 0.75H_2O$) C, H.

3,4-Bis(octanoyloxy)cyclohexanemethanol (20a and 20b). Compounds **15a** and **15b** were treated as for **6** to yield 100 mg of **20a** and **20b** (0.3 mmol, 100% yield). **20a**: ^{1}H NMR ($CDCl_3$) δ 0.9 (t, 6 H, CH_3), 1.3 (m, 20 H, CH_2), 1.6-2.0 (m, 7 H, ring CH_2 and CHC=C), 2.25 and 2.35 (t, 2 H, J = 7.5 Hz, CH_2CO), 3.5 (d, 2 H, J = 6.5 Hz, CH_2OH), 4.8 and 5.35 (m, 1 H, $CHOCO$). Anal. ($C_{23}H_{41}O_5 \cdot 1.5H_2O$) C, H. Differences in ^{1}H NMR of **20b**: ($CDCl_3$) δ 4.85 and 5.3 (m, 1 H, $CHOCO$). Anal. ($C_{23}H_{41}O_5 \cdot 1.5H_2O$) C, H.

Molecular Modeling. Molecular modeling was performed with the program Alchemy II developed and distributed by Tripos, Inc., St. Louis, MO. The coordinates for phorbol were from the published crystal structure.[32] Coordinates for cyclohexane, benzene, and other portions of the compounds synthesized were from the internal data base. Conformational analysis and energy minimization were performed with internal programs. The lowest energy conformation of each isomer was determined independently.

Binding Assays. The binding assays were done as described before.[15] Essentially, murine brain fractions (50 μg of protein), [^{3}H]PDBu (5 nM), and the compounds at selected concentrations, or TPA at 5 μM, were placed into 200 μL of binding buffer. This was incubated for 1 h at 23 °C. This mixture was then filtered through GF/B glass-fiber filters with a Brandel filtration apparatus and washed with 5 mL of cold 10% polyethylene glycol in 1 mM Tris, pH 7.4. The filters were counted in a scintillation counter, and the percent bound was determined.

Acknowledgment. We express sincere thanks to James Medley for determining the configuration in compounds **15a** and **15b**.

Water-Soluble Renin Inhibitors: Design of a Subnanomolar Inhibitor with a Prolonged Duration of Action[1]

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Incorporation of nonreactive polar functionalities at the C- and N-termini of renin inhibitors led to the development of a subnanomolar compound (**21**) with millimolar solubility. This inhibitor demonstrated excellent efficacy and a long duration of action upon intravenous administration to monkeys. While activity was also observed intraduodenally, a comparison of the blood pressure responses indicated low bioavailability. Subsequent experiments in rats showed that, although the compound was absorbed from the gastrointestinal tract, extensive liver extraction severely limited bioavailability.

Renin is the first and rate-limiting enzyme in the well-known renin-angiotensin cascade that produces the pressor hormone angiotensin II, thus inhibition of this enzyme could lead to the introduction of a new class of

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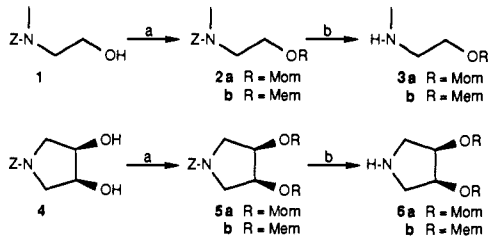
Scheme I. Synthesis of N-Terminal Amine Fragments[a]



[a] Key: (a) Mem-Cl or Mom-Cl, $(i\text{-Pr})_2EtN$, CH_2Cl_2. (b) H_2, Pd/C, CH_3OH.

antihypertensive agents. Disruption of the renin–angiotensin system by blocking the action of angiotensin-converting enzyme (ACE), the second enzyme in the cascade, has already proven to be a viable therapy for controlling hypertension. Although many potent renin inhibitors have been described,[2] the dual barriers of limited oral absorption and a short duration of action have prevented the development of therapeutically useful agents. One approach that has been applied toward solving the latter problem has been to design compounds with increased aqueous solubility by incorporating polar groups or amino acid residues at the C- or N-termini of the inhibitors.[2c,e,3] Two of these compounds, U-71038[4] and A-64662,[5] have dem-

(1) Abbreviations follow IUPAC–IUB Joint Commission on Biochemical Nomenclature for amino acids and peptides (*Eur. J. Biochem.* **1984**, *158*, 9–31). Additional abbreviations are as follows: AUC, integrated area under the curve; DMF, dimethylformamide; HPLC, high-pressure liquid chromatography; Mem, (methoxyethoxy)methyl; Mom, methoxymethyl; NOS, *N*-oxysuccinamide; TEA, triethylamine; THF, tetrahydrofuran.

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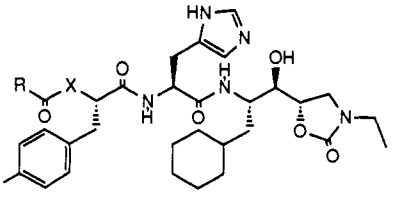
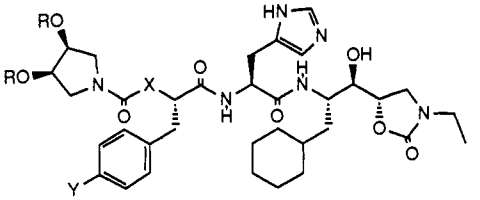
Table I. Renin Inhibitors Containing Acyclic N-Terminal Substituents and Related Structures



no.	R	X	Y	IC_{50}, nM: purified,[a] pH 6.5	IC_{50}, nM: plasma,[b] pH 7.4
11	$(CH_3)_2CH$	NH	OCH_3	1.7	9.9
12	morpholin-4-yl	NH	OCH_3	3.4	9.6
14	morpholin-4-yl	CH_2	H	0.99	1.3
15	$HO(CH_2)_2N(CH_3)$	CH_2	H	0.85	2.5
16	$HOCH_2CH(OH)CH_2N(CH_3)$	NH	OCH_3	7.9	46
17	$CH_3O(CH_2)_2N(CH_3)$	CH_2	H	0.59	1.5
18	$MomO(CH_2)_2N(CH_3)$	NH	OCH_3	4.6	27
19	$MomO(CH_2)_2N(CH_3)$	CH_2	H	0.49	1.1
20	$MemO(CH_2)_2N(CH_3)$	NH	OCH_3	1.7	14
21	$MemO(CH_2)_2N(CH_3)$	CH_2	H	0.37	0.57
	A-64662[c]				14

[a] Purified human renal renin. [b] Human plasma renin. [c] [*N*-(3-Amino-3-methyl-1-oxobutyl)-4-methoxy-L-phenylalanyl]-*N*-[(1*S*,2*R*,3*S*)-1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]-L-histidinamide; data taken from ref 5.

Table II. Renin Inhibitors Containing Dioxygenated Pyrrolidine N-Terminal Substituents



no.	R	X	Y	IC_{50}, nM: purified,[a] pH 6.5	IC_{50}, nM: plasma,[b] pH 7.4
22	H	NH	OCH_3	6.9	8.7
23	Mom	NH	OCH_3	12	59
24	Mom	CH_2	H	2.7	11
25	Mem	NH	OCH_3	8.5	26
26	Mem	CH_2	H	1.6	7.3

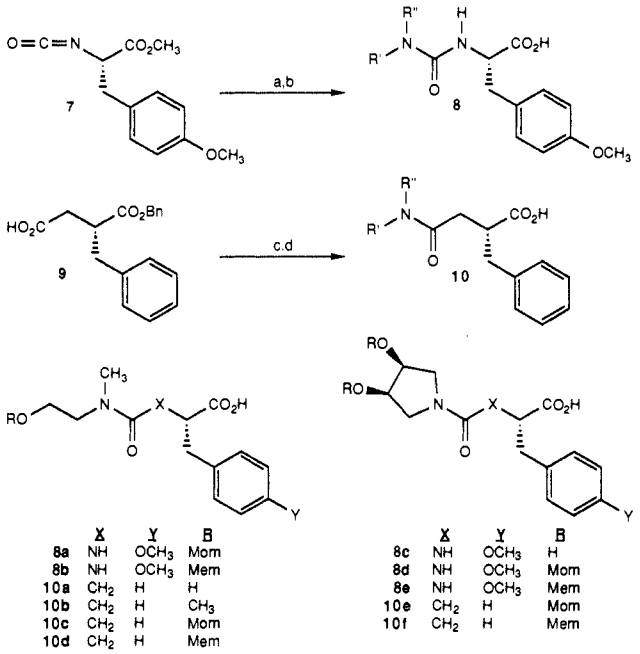
[a] Purified human renal renin. [b] Human plasma renin.

Table III. Physical Data for Renin-Inhibiting Compounds

no.[a]	formula[b]	log P[c] pH 6.5	log P[c] pH 7.4	solubility, mM[d]
11	$C_{34}H_{50}N_6O_6 \cdot {}^2/_3H_2O$	2.67	2.85	0.37[e]
12	$C_{35}H_{51}N_7O_8 \cdot 1.5H_2O$	1.91	2.11	5.9[f]
14	$C_{35}H_{50}N_6O_7 \cdot 1.5H_2O$	1.90	2.20	4.2
15	$C_{34}H_{50}N_6O_7 \cdot 1.6H_2O$[g]	1.74	1.94	5.2
16	$C_{35}H_{53}N_7O_9 \cdot 2H_2O$[h]	1.20	1.42	
17	$C_{35}H_{52}N_6O_7 \cdot 0.5H_2O$	2.29	2.45	3.4
18	$C_{36}H_{55}N_7O_9 \cdot 0.5H_2O$	2.08	2.30	2.1
19	$C_{36}H_{54}N_6O_8 \cdot 0.75H_2O$	2.28	2.49	
20	$C_{38}H_{59}N_7O_{10} \cdot 0.5H_2O$	1.83	2.03	
21	$C_{38}H_{58}N_6O_9 \cdot H_2O$	1.89	2.21	5.4
22	$C_{37}H_{55}N_7O_{11} \cdot H_2O \cdot AcOH$[h]	0.91	1.22	
23	$C_{39}H_{59}N_7O_{11} \cdot 0.5H_2O$	1.94	2.06	
24	$C_{39}H_{58}N_6O_{10} \cdot 0.3H_2O$[i]	2.08	2.31	4.5
25	$C_{43}H_{67}N_7O_{13} \cdot 2H_2O$	1.58	1.67	
26	$C_{43}H_{66}N_6O_{12} \cdot 0.5H_2O$	1.56	1.87	8.7

[a] See Tables I and II for structures. [b] Analyses for C, H, N were ±0.4% of expected values (for formulae shown) unless otherwise noted. [c] Octanol/water. [d] pH 7.4; 37 °C. [e] 0.70 mM at pH 6.5. [f] 7.2 mM at pH 6.5. [g] N: calcd, 12.29; found, 11.80; exact mass calcd for $C_{34}H_{51}N_6O_7$ (M + H) 655.3819, found 655.3813. [h] Special isolation procedure, see the Experimental Section. [i] N: calcd, 10.82; found, 10.39.

Scheme II. Synthesis of N-Terminal Fragments[a]



	X	Y	B
8a	NH	OCH_3	Mom
8b	NH	OCH_3	Mem
10a	CH_2	H	H
10b	CH_2	H	CH_3
10c	CH_2	H	Mom
10d	CH_2	H	Mem

	X	Y	B
8c	NH	OCH_3	H
8d	NH	OCH_3	Mom
8e	NH	OCH_3	Mem
10e	CH_2	H	Mom
10f	CH_2	H	Mem

[a] Key: (a) amine, CH_2Cl_2. (b) LiOH, dioxane, water. (c) amine, isobutyl chloroformate, *N*-methylmorpholine, CH_2Cl_2. (d) H_2, Pd/C, CH_3OH.

onstrated enhanced in vivo efficacy and have been shown to be useful tools for the pharmacologic investigation of this class of enzyme inhibitors.

The polar groups employed to date have included amines, carboxylic acids, pyridines, guanidines, sulfonic acids, and related species. These are potentially reactive groups that may promote clearance through conjugation or metabolic oxidation, hence any beneficial effects arising from greater aqueous solubility might be partially offset by increased elimination. The use of less reactive hydrophilic groups, on the other hand, offers the possibility of enhancing efficacy without augmenting clearance.

We have found that our recently disclosed *N*-ethyloxazolidinone C-terminal fragment[6] imparts significant aqueous solubility when incorporated into renin inhibitors. Herein is described the development of a series of compounds which utilize this C-terminal oxazolidinone. Systematic incorporation of one to six N-terminal ether linkages as nonreactive solubilizing groups led to the discovery of a subnanomolar renin inhibitor with enhanced aqueous solubility and excellent in vivo activity.

Results and Discussion

Synthesis: N-Terminal Fragments. Various polyether-substituted secondary amines were synthesized from the corresponding (benzyloxy)carbonyl-protected hydroxyalkyl amines as illustrated in Scheme I. These and related amines were converted into ureas of *O*-methyltyrosine or amides of α-benzylsuccinic acid as shown in Scheme II.

Synthesis: Conversion into Renin Inhibitors. Final compounds were prepared by the stepwise carbodiimide-mediated coupling of Boc-histidine, and then the appropriate left hand fragment, to the amine of the *N*-ethyloxazolidinone. Final products were purified by chromatography on silica gel after an extractive isolation. Two inhibitors, **16** and **22**, were too hydrophilic to be extracted from an aqueous phase and were isolated instead by size-exclusion chromatography followed by chromatography on silica gel.

Solubility. Solubility and partition coefficient data are shown in Table III. The log *P* values correlated well with the measured aqueous solubilities when both were determined at pH 7.4 ($R = 0.88$). Isobutyryl amide **11** was taken as our standard. This compound was 15-fold more soluble than the corresponding azido glycol containing inhibitor.[2d,7] The related morpholino urea **12** was 10-fold (at pH 6.5) to 16-fold (at pH 7.4) more soluble than **11**, and was 5-fold more soluble than the corresponding azido glycol.[2d,8] Morpholino benzylsuccinate analogue **14** had essentially the same solubility as **12**, as did the related methyl and Mem ethers (**17**, **21**), while the Mom ether (**18**) was only $^1/_2$ as soluble. The most soluble ether-containing inhibitor (**26**), with six ether linkages, was twice as soluble as **14** and 24 times more soluble than **11**. For comparison, monohydroxy and dihydroxy compounds **15**, **16**, and **22** were evaluated. High aqueous solubility was observed for **15**, and the dihydroxy compounds appeared to be even more soluble (on the basis of upon their low log *P* values and our inability to isolate them through an extractive workup), indicating that a single hydroxyl contributes as much to aqueous solubility as several ether linkages.

In Vitro Activity. The structures and in vitro activities of the renin inhibitors described as shown in Tables I and II (data for our current clinical candidate A-64662 are included in Table I for comparison). All contain either *O*-methyltyrosine[9] or α-benzylsuccinic acid[2b] at the P_3 phenylalanine site in order to stabilize the P_3–P_2 bond toward chymotrypsin cleavage. Potencies ranged from 0.57 to 59 nM against human plasma renin (pH 7.4), while the range was not as great in the purified human renal renin (pH 6.5) assay (all discussions of relative potencies describe activity against human plasma renin). The compounds containing the isobutyryl amide (**11**) and morpholino urea (**12**) of *O*-methyltyrosine were equipotent and had essentially the same activity as the corresponding azido glycol containing inhibitors,[2d,7,8] while **12** was 4-fold less potent than its dipeptide glycol counterpart.[2b,10] Replacing the *O*-methyltyrosine of **12** with α-benzylsuccinic acid to give **14** increased potency 7-fold, resulting in a compound with activity identical with that of the analogous dipeptide glycol.[2b,11] A similar trend was observed for the other pairs of inhibitors, with those containing *O*-methyltyrosine showing 4–25-fold less activity than the α-benzylsuccinamides. Those inhibitors which incorporated 2-substituted ethylamine amides of the α-benzylsuccinate residue (**15**, **17**, **19**, and **21**) exhibited low nanomolar activities, with **21** being one of the most potent renin inhibitors reported to date. The nature of the N-terminal end group did not

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(7) Isobutyryl-(*O*-Me)Tyr-His amide of (2*S*,3*R*,4*S*)-1-Azido-2,3-dihydroxy-4-amino-5-cyclohexylpentane.

(8) (Morpholinocarbonyl)-(*O*-Me)Tyr-His amide of (2*S*,3*R*,4*S*)-1-Azido-2,3-dihydroxy-4-amino-5-cyclohexylpentane.

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(10) (Morpholinocarbonyl)-(*O*-Me)Tyr-His amide of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane.

(11) [3-(Morpholinocarbonyl)-2(*R*)-benzylpropionyl]-His amide of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane.

Table IV. In Vivo and in Vitro Monkey Data for Selected Renin-Inhibiting Compounds

no.	IC_{50}, nM: plasma, pH 7.4	iv efficacy[a] maximum % change[b]	iv efficacy[a] AUC, % mmHg min[c]	solubility, mM, pH 7.4
21	0.33	43 ± 8[d]	3400 ± 600[d]	5.4
A-62198[e]	0.29	18 ± 4	~460	0.045

[a] 0.1 mg/kg dose. [b] From $T = 0$ base-line value; mean ± SE. [c] Integrated mean blood pressure response from 0–120 min normalized for $T = 0$ blood pressure; mean ± SE. [d] $n = 5$. [e] Isobutyryl-Phe-His amide of (2S,3R,4S)-1-Azido-2,3-dihydroxy-4-amino-5-cyclohexylpentane; data taken from ref 12; $n = 4$.

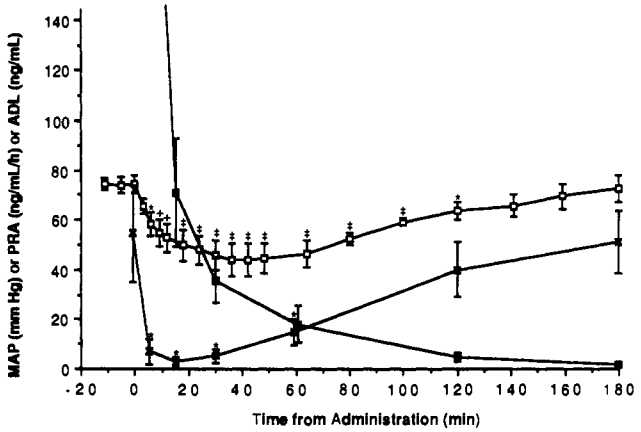

Figure 1. Effects of a 0.1 mg/kg iv bolus dose of inhibitor **21** in anesthetized, salt-depleted cynomolgus monkeys ($n = 5$). Results are shown as mean ± SE and were considered significantly different from $T = 0$, if $P \leq 0.005$ (‡), $P \leq 0.01$ (+), or $P \leq 0.05$ (*). MAP = mean arterial pressure (□), PRA = plasma renin activity (×), ADL = arterial drug level (■, value at 5 min is 280 ± 120 ng/mL).

significantly effect binding for these four compounds. Replacement of the substituted ethyl amine residue with dioxygenated pyrrolidines (**24**, **26**) resulted in a loss of potency. Dihydroxypyrrolidine *O*-methyltyrosine derivative **22**, however, was more potent than its acyclic analogue **16**.

Inhibitor **21** was also tested against monkey plasma renin (Table IV). The activity was similar to that determined in the human plasma renin assay. In order to determine proteolytic specificity, compounds **12**, **14**, and **21** were tested for inhibition of bovine cathepsin D and porcine pepsin. No activity was observed in the pepsin assay for the three compounds to 10^{-5} M or for **12** and **14** against cathepsin D at the same concentration. Compound **21** did show measurable activity (25% inhibition at 10^{-5} M) in the cathepsin D assay.

In Vivo Activity: Monkey. Compound **21**, the most potent in the series, was given intravenously at 0.1 mg/kg to anesthetized, salt-depleted monkeys. The blood pressure lowering and the drug level–time profiles are illustrated in Figure 1. A significant response was observed at 2 h after drug administration, which is longer than for A-62198,[12] a previously described azido glycol based inhibitor with similar potency but lower solubility than **21** (vide infra). The blood pressure response closely paralleled the suppression of plasma renin activity. Plasma drug levels decreased rapidly from 280 ± 120 ng/mL at 5 min to 4.8 ± 2.0 ng/mL at 120 min. The distribution and clearance phase half-lives were calculated to be 3.3 ± 0.2 and 31 ± 2 min, respectively.

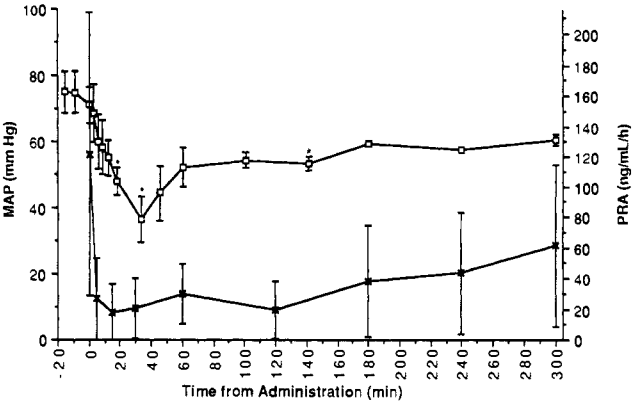

Figure 2. Effects of a 10 mg/kg id dose of inhibitor **21** in anesthetized, salt-depleted cynomolgus monkeys ($n = 2$). Results are shown as mean ± SE and were considered significantly different from $T = 0$, if $P \leq 0.05$ (*). MAP = mean arterial pressure (□), PRA = plasma renin activity (×).

Table V. Excretion of Radioactivity and Metabolic Profile in Rats Dosed Orally or Intravenously with [^{14}C]-**21**[a]

zone	% ^{14}C dose[b] oral urine	oral feces	intravenous urine	intravenous feces
aminooxazolidinone	0.1	3.2 ± 0.8	0.2	3.2 ± 0.8
des-Mem-ethanolamine-**21**	<0.1	4.4 ± 1.8	0.1	25 ± 5
21	1.9 ± 0.8	73 ± 6	8.5 ± 3.6	35 ± 4
total ^{14}C[c]	9.8 ± 2.0	84 ± 5	18 ± 1	68 ± 1

[a] 1.0 mg/kg. [b] Mean ± SE; $n = 2$. [c] Includes unidentified metabolites.

Table IV is a comparison of the effects of a 0.1 mg/kg iv bolus of compounds **21** and A-62198[12] in anesthetized, salt-depleted monkeys. This comparison was made in order to assess the possible effects of increased aqueous solubility on intravenous efficacy. These two compounds possess equal potency against monkey plasma renin, while their aqueous solubilities differ 100-fold. Inhibitor **21**, the more soluble compound, caused twice the maximum blood pressure lowering and 7 times the integrated blood pressure response as was observed for A-62198. Thus, from the data presented, it appears that higher aqueous solubility can lead to increased efficacy. Because the two compounds have significant structural differences, however, other factors cannot be ruled out as the cause of the differing biological responses.

Inhibitor **21** was given id at 10 mg/kg to two anesthetized, salt-depleted cynomolgus monkeys, and the results are shown in Figure 2. The blood pressure response was essentially the same as was seen with the 0.1 mg/kg iv dose, suggesting poor absorption into the systemic circulation.

In Vivo Results: Rat. Compound **21** labeled with ^{14}C at the oxazolidinone carbonyl[13] was administered orally and intravenously to rats at 1 mg/kg. Urine and feces were collected over 72 h and analyzed for total radioactivity, parent drug, and metabolites (Table V). Within three days after intravenous administration, 18% of the labeled dose was excreted in the urine and 68% was eliminated in the feces. Following the oral dose, urinary and fecal excretion averaged 9.8% and 84%, respectively. The unmetabolized parent drug accounted for 75% of the oral dose and 44% of the intravenous dose and was primarily

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(13) This material was prepared using [^{14}C]phosgene as described in ref 6.

Table VI. Plasma Levels of 21 Given Intraduodenally to Anesthetized Rats[a]

time, min	plasma levels,[b] ng/mL systemic	portal
10	1.6 ± 0.4	360 ± 160
30	2.5 ± 0.1	420 ± 260

[a] 10 mg/kg id dose. [b] Mean ± SE, n = 4.

eliminated in the feces. The free acid resulting from cleavage of the ethanolamine residue represented 4.4% of the oral dose and 25% of the intravenous dose and was also found mainly in the feces. This suggests that the ethanolamine–benzylsuccinate bond was labile and that the cleavage occurred primarily after absorption from the digestive tract. A small amount of the aminooxazolidinone arising from cleavage after the histidyl residue was detected, following both routes of administration. No free amino compound resulting from cleavage between histidine and the benzylsuccinate residue was detected, indicating that this bond was stable or that the resulting fragment underwent rapid metabolism.

In a related experiment, bile was collected for 4 h following oral and intravenous dosing. Thirty-four percent of the radioactivity from the intravenous dose compared to 7% for the oral dose was found in the bile, suggesting an upper limit of 20% absorption from the gastrointestinal tract. Comparison of the amount of parent in the urine after both routes of administration supports this estimate, as does the ratio of the levels of the desethanolamine metabolite.

In order to determined the fate of the fraction of the dose that was absorbed, four rats were dosed intraduodenally at 10 mg/kg with unlabeled **21**, and drug levels in the portal and systemic circulation were determined at 10 and 30 min (Table VI). High levels were found in the portal samples, confirming that absorption was occurring; however, only very low levels reached the systemic circulation, indicating that **21** was efficiently extracted by the liver.

In conclusion we have designed a subnanomolar renin inhibitor with millimolar aqueous solubility without the incorporation of potentially reactive polar groups. This compound demonstrated excellent efficacy and a long duration of action upon intravenous administration to monkeys. While **21** was also active intraduodenally, a comparison of the blood pressure responses indicated low bioavailability. Subsequent experiments in rats showed that although the compound was absorbed from the gastrointestinal tract, extensive liver extraction severely limited bioavailability, a phenomenon which has been observed for other renin inhibitors.[2b,d,14] Studies are currently underway to determine which attributes of **21** and related inhibitors are responsible for the hepatic clearance.

Experimental Section

Solvents and other reagents were reagent grade and were used without further purification unless otherwise noted. Final product solutions were dried over anhydrous Na_2SO_4 (unless otherwise noted) prior to evaporation on a rotary evaporator. Tetrahydrofuran was distilled from sodium/benzophenone and methylene chloride was distilled from P_2O_5. NMR spectra were recorded at 300 MHz and are expressed in ppm downfield from tetramethylsilane as an internal standard. Column chromatography was performed on silica gel 60, 0.04–0.063 mm (E. Merck), eluting with 5–10 psi of air pressure. Thin-layer chromatography was done on silica gel plates (E. Merck), and components were visualized with ninhydrin or phosphomolybdic acid reagents. The following solvent systems were used: 50% ethyl acetate/50% hexane (I), 95% chloroform/5% methanol (II) 100% ethyl acetate (III), 90% chloroform/10% methanol (IV), 85% chloroform/15% methanol (V).

2-[[(Benzyloxy)carbonyl]methylamino]-1-(methoxymethoxy)ethane (2a). To alcohol 1[15] (6.60 g, 31.5 mmol) in CH_2Cl_2 (50 mL) was added diisopropylethylamine (12.0 mL, 68.9 mmol) and chloromethyl methyl ether (4.80 mL, 63.2 mmol). After 4 h at ambient temperature the mixture was evaporated, taken up in ethyl acetate, washed with 0.5 M H_3PO_4, saturated $NaHCO_3$ solution, and brine, and then was dried and evaporated to afford 7.76 g (97%) of a colorless oil: TLC R_f = 0.34 (I); ^{1}H NMR ($CDCl_3$) δ 7.40–7.27 (m, 5 H), 5.14 (s, 2 H), 4.60 (br d, 2 H), 3.75–3.60 (m, 2 H), 3.55–3.45 (m, 2 H), 3.35–3.30 (m, 3 H), 3.02 (s, 3 H).

2-[[(Benzyloxy)carbonyl]methylamino]-1-[(methoxyethoxy)methoxy]ethane (2b). To alcohol 1[15] (11.22 g, 53.6 mmol) in CH_2Cl_2 (50 mL) was added diisopropylethylamine (28.0 mL, 161 mmol) and (2-methoxyethoxy)methyl chloride (15.0 mL, 131 mmol). After 3 h at ambient temperature the mixture was evaporated, taken up in ethyl acetate, washed with 0.5 M H_3PO_4, saturated $NaHCO_3$ solution, and brine, and then was dried and evaporated. Bulb to bulb distillation afforded 15.75 g (99%) of a colorless oil: bp 150–170 °C (0.3 mm); TLC R_f = 0.22 (I); ^{1}H NMR ($CDCl_3$) δ 7.42–7.27 (m, 5 H), 5.13 (s, 2 H), 4.70 (br d, 2 H), 3.75–3.61 (m, 4 H), 3.58–3.45 (m, 4 H), 3.38 (s, 3 H), 3.00 (s, 3 H).

1-(Methoxymethoxy)-2-(methylamino)ethane (3a). Compound **2a** (7.76 g, 30.6 mmol) and 10% Pd/C (7.5 g) in methanol (5 mL) were stirred under a hydrogen atmosphere for 12 h. The mixture was filtered, evaporated, and bulb to bulb distilled to afford 3.07 g (84%) of a colorless liquid: bp 60–80 °C (60 mm); ^{1}H NMR ($CDCl_3$) δ 4.65 (s, 2 H), 3.65 (t, 2 H), 3.37 (s, 3 H), 2.78 (t, 2 H), 2.46 (s, 3 H).

1-[(Methoxyethoxy)methoxy]-2-(methylamino)ethane (3b). This compound was prepared in 96% yield from **2b** as described for **3a**: bp 130–140 °C (45 mm); ^{1}H NMR ($CDCl_3$) δ 4.74 (s, 2 H), 3.75–3.65 (m, 4 H), 3.60–3.54 (m, 2 H), 3.40 (s, 3 H), 2.78 (t, 2 H), 2.46 (s, 3 H).

1-[(Benzyloxy)carbonyl]-3,4-*cis*-dihydroxypyrrolidine (4). To Cbz-NOS (17.0 g, 68.2 mmol) in CH_2Cl_2 (30 mL) was added 3-pyrroline (5.0 g, 72 mmol, 75% pure) dropwise at 0 °C. After stirring overnight at ambient temperature, the reaction mixture was washed with 0.5 M HCl and aqueous $NaHCO_3$ and was dried over $MgSO_4$. Evaporation of the solvent gave 1-[(benzyloxy)carbonyl]-3-pyrroline (13.2 g, 95%). This material (12.95 g, 63.8 mmol) in THF (100 mL) was treated with OsO_4 in *tert*-butanol (8.0 mL, 2.5% solution) followed by *N*-methylmorpholine *N*-oxide (7.46 g, 63.7 mmol). After 1 h, the solvent was evaporated and the residue was dissolved in ethyl acetate (300 mL) and washed with dilute Na_2SO_3 solution, saturated $NaHCO_3$ solution, and brine, and then was dried over $MgSO_4$. Evaporation of the solvent gave a gummy solid which was chromatographed with 5% methanol in CH_2Cl_2 to give 8.30 g (55%) of a white solid: mp 82–84 °C; TLC R_f = 0.15 (II). Anal ($C_{12}H_{15}NO_4$) C, H, N.

1-[(Benzyloxy)carbonyl]-3,4-*cis*-bis(methoxymethoxy)pyrrolidine (5a). To alcohol 4 (0.340 g, 1.43 mmol) in CH_2Cl_2 (5 mL) was added diisopropylethylamine (1.25 mL, 7.18 mmol) and chloromethyl methyl ether (0.54 mL, 7.11 mmol). After 22 h at ambient temperature the mixture was evaporated, taken up in ethyl acetate, washed with 0.5 M H_3PO_4, saturated $NaHCO_3$ solution, and brine, and then was dried and evaporated. Chromatography with 1:1 ethyl acetate/hexane afforded 0.420 g (90%) of a colorless oil: TLC R_f = 0.60 (II); ^{1}H NMR ($CDCl_3$) δ 7.40–7.28 (m, 5 H), 5.16 (d, 1 H), 5.13 (d, 1 H), 4.79–4.66 (m, 4 H), 4.27–4.16 (m, 2 H), 3.68–3.46 (m, 4 H), 3.40 (s, 3 H), 3.38 (s, 3 H).

1-[(Benzyloxy)carbonyl]-3,4-*cis*-bis[(methoxyethoxy)methoxy]pyrrolidine (5b). To alcohol 4 (0.310 g, 1.31 mmol) in CH_2Cl_2 (5 mL) was added diisopropylethylamine (1.25 mL, 7.18 mmol) and (2-methoxyethoxy)methyl chloride (0.74 mL, 6.5 mmol). After 90 h at ambient temperature the mixture was evaporated, taken up in ethyl acetate, washed with 0.5 M H_3PO_4, saturated $NaHCO_3$ solution, and brine, and then was dried and

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(15) Baer, E.; Pavanaram, S. K. *J. Biol. Chem.* **1961**, *236*, 1269.

evaporated. Chromatography with ethyl acetate afforded 0.500 g (93%) of a colorless oil: TLC R_f = 0.50 (II); ^{1}H NMR ($CDCl_3$) δ 7.40–7.28 (m, 5 H), 5.15 (d, 1 H), 5.12 (d, 1 H), 4.90–4.76 (m, 4 H), 4.30–4.22 (m, 2 H), 3.78–3.67 (m, 4 H), 3.67–3.47 (m, 8 H), 3.39 (s, 3 H), 3.36 (s, 3 H).

3,4-*cis*-Bis(methoxymethoxy)pyrrolidine (6a). Compound **5a** (0.420 g, 1.29 mmol) and 10% Pd/C (0.40 g) in methanol (6 mL) were stirred under a hydrogen atmosphere for 12 h. The mixture was filtered and evaporated to afford 0.250 g (100%) of a colorless liquid: ^{1}H NMR (CD_3OD) δ 4.73 (d, 2 H), 4.68 (d, 2 H), 4.22–4.06 (m, 2 H), 3.47 (s, 6 H), 3.09 (dd, 2 H), 2.89 (dd, 2 H).

3,4-*cis*-Bis[(methoxyethoxy)methoxy]pyrrolidine (6b). This compound was prepared in 85% yield from **5b** as described for **6a**: ^{1}H NMR (CD_3OD) δ 4.80 (d, 2 H), 4.76 (d, 2 H), 4.22–4.13 (m, 2 H), 3.78–3.63 (m, 4 H), 3.58–3.52 (m, 4 H), 3.36 (s, 6 H), 3.08 (dd, 2 H), 2.92 (dd, 2 H).

[[[2-(Methoxymethoxy)ethyl]methylamino]carbonyl]-*O*-methyltyrosine Methyl Ester (Ester of 8a). α-Isocyanato-*O*-methyltyrosine methyl ester[2b] (**7**, 405 mg, 1.72 mmol) in CH_2Cl_2 (2 mL) was added to amine **3a** (207 mg, 1.74 mmol) in CH_2Cl_2 (4 mL) at 0 °C. The reaction was stirred at 0 °C for 30 min and at ambient temperature overnight. Evaporation followed by chromatography with 75–100% ethyl acetate in hexane afforded 351.0 mg (58%) of a colorless oil: TLC R_f = 0.20 (III); ^{1}H NMR ($CDCl_3$) δ 7.06 (dd, 2 H), 6.82 (dd, 2 H), 5.52 (br d, 1 H), 4.69 (dd, 1 H), 4.53 (s, 2 H), 3.78 (s, 3 H), 3.72 (s, 3 H), 3.65–3.59 (m, 2 H), 3.53–3.33 (m, 2 H), 3.28 (s, 3 H), 3.07–3.00 (m, 2 H), 2.92 (s, 3 H).

[[[2-[(Methoxyethoxy)methoxy]ethyl]methylamino]carbonyl]-*O*-methyltyrosine Methyl Ester (Ester of 8b). This compound was prepared in 85% yield from **3b** as described for the ester of **8a**: TLC R_f = 0.12 (III); ^{1}H NMR ($CDCl_3$) δ 7.05 (dd, 2 H), 6.82 (dd, 2 H), 5.54 (br d, 1 H), 4.68 (dd, 1 H), 4.62 (s, 2 H), 3.78 (s, 3 H), 3.71 (s, 3 H), 3.68–3.57 (m, 4 H), 3.55–3.38 (m, 4 H), 3.38 (s, 3 H), 3.05–3.00 (m, 2 H), 2.91 (s, 3 H).

[(3,4-*cis*-Dihydroxypyrrolidino)carbonyl]-*O*-methyltyrosine Methyl Ester (Ester of 8c). To α-isocyanato-*O*-methyltyrosine methyl ester[2b] (**7**, 9.57 g, 40.7 mmol) in CH_2Cl_2 (100 mL) was added 3-pyrroline (3.10 g, 45 mmol, 75% pure) dropwise at 0 °C. After 15 min, the reaction mixture was washed with 0.5 M HCl and aqueous $NaHCO_3$ and was dried over $MgSO_4$. Evaporation of the solvent gave [(3-pyrrolinyl)carbonyl]-*O*-methyltyrosine methyl ester (12.0 g, 96%). This material (2.50 g, 8.21 mmol) in THF (50 mL) was treated with OsO_4 in *tert*-butanol (1.0 mL, 2.5% solution) followed by *N*-methylmorpholine *N*-oxide (1.15 g, 9.82 mmol). After 1 h, the solvent was evaporated and the residue was dissolved in ethyl acetate (150 mL) and washed with dilute Na_2SO_3 solution, saturated $NaHCO_3$ solution, and brine, and then was dried over $MgSO_4$. Evaporation of the solvent gave a gummy solid which was chromatographed with 5% methanol in CH_2Cl_2 to give 1.80 g (65%) of the desired product as an oil: TLC R_f = 0.28 (IV); ^{1}H NMR ($CDCl_3$) δ 7.03 (dd, 2 H), 6.82 (dd, 2 H), 4.76–4.63 (m, 2 H), 4.28–4.22 (m, 2 H), 3.79 (s, 3 H), 3.72 (s, 3 H), 3.58–3.47 (m, 2 H), 3.38–3.28 (m, 2 H), 3.03 (d, 2 H).

[[3,4-*cis*-Bis(methoxymethoxy)pyrrolidino]carbonyl]-*O*-methyltyrosine Methyl Ester (Ester of 8d). This compound was prepared in 84% yield from **6a** as described for the ester of **8a**: TLC R_f = 0.14 (III); ^{1}H NMR ($CDCl_3$) δ 7.03 (dd, 2 H), 6.82 (dd, 2 H), 4.82–4.67 (m, 5 H), 4.51 (br d, 1 H), 4.27–4.18 (m, 2 H), 3.79 (s, 3 H), 3.72 (s, 3 H), 3.57–3.37 (m, 4 H), 3.39 (s, 3 H), 3.38 (s, 3 H), 3.05 (d, 2 H).

[[3,4-*cis*-Bis[(methoxyethoxy)methoxy]pyrrolidino]carbonyl]-*O*-methyltyrosine Methyl Ester (Ester of 8e). This compound was prepared in 84% yield from **6b** as described for the ester of **8a**: TLC R_f = 0.06 (III); ^{1}H NMR ($CDCl_3$) δ 7.03 (dd, 2 H), 6.82 (dd, 2 H), 4.88–4.72 (m, 5 H), 4.63 (br d, 1 H), 4.32–4.23 (m, 2 H), 3.78 (s, 3 H), 3.77–3.67 (m, 4 H), 3.72 (s, 3 H), 3.58–3.43 (m, 8 H), 3.38 (s, 6 H), 3.05 (d, 2 H).

[[[2-(Methoxymethoxy)ethyl]methylamino]carbonyl]-*O*-methyltyrosine (8a). To [[[2-(methoxymethoxy)ethyl]-methylamino]carbonyl]-*O*-methyltyrosine methyl ester (348 mg, 0.982 mmol) in dioxane (3 mL) at 0 °C was added $LiOH·H_2O$ (49.4 mg, 1.18 mmol) in water (2 mL). After 1 h at 0 °C, the reaction was quenched with 2 M HCl, poured into saturated brine, and extracted into ethyl acetate which was dried and evaporated to afford 335 mg (100%) of an oil which slowly crystallized: mp 69–71 °C; ^{1}H NMR ($CDCl_3$) δ 7.13 (dd, 2 H), 6.84 (dd, 2 H), 5.78–5.66 (br, 1 H), 4.53 (d, 1 H), 4.51 (d, 1 H), 4.40–4.31 (m, 1 H), 3.79 (s, 3 H), 3.59 (dd, 2 H), 3.28 (s, 3 H), 3.24 (dd, 1 H), 3.01 (dd, 1 H), 2.89 (s, 3 H). Anal. ($C_{16}H_{24}N_2O_6$) C, H, N.

[[[2-[(Methoxyethoxy)methoxy]ethyl]methylamino]carbonyl]-*O*-methyltyrosine (8b). This compound was prepared in 100% yield from [[[2-[(methoxyethoxy)methoxy]-ethyl]methylamino]carbonyl]-*O*-methyltyrosine methyl ester as described for **8a**: ^{1}H NMR ($CDCl_3$) δ 7.14 (dd, 2 H), 6.83 (dd, 2 H), 5.80–5.63 (br, 1 H), 4.62 (d, 1 H), 4.59 (d, 1 H), 4.40–4.30 (m, 1 H), 3.38 (s, 3 H), 3.22 (dd, 1 H), 3.02 (dd, 1 H), 2.89 (s, 3 H). Anal. ($C_{18}H_{28}N_2O_7·0.25H_2O$) C, H, N.

[(3,4-*cis*-Dihydroxypyrrolidino)carbonyl]-*O*-methyltyrosine (8c). This compound was prepared in 73% yield from [(3,4-*cis*-dihydroxypyrrolidino)carbonyl]-*O*-methyltyrosine methyl ester as described for **8a**: mp 61–74 °C; ^{1}H NMR (CD_3OD) δ 7.15 (dd, 2 H), 6.83 (dd, 2 H), 4.47 (dd, 1 H), 3.76 (s, 3 H).

[[3,4-*cis*-Bis(methoxymethoxy)pyrrolidino]carbonyl]-*O*-methyltyrosine (8d). This compound was prepared in 100% yield from [[3,4-*cis*-bis(methoxymethoxy)pyrrolidino]-carbonyl]-*O*-methyltyrosine methyl ester as described for **8a**: ^{1}H NMR ($CDCl_3$) δ 7.12 (dd, 2 H), 6.86 (dd, 2 H), 3.38 (s, 3 H), 3.36 (s, 3 H), 3.21 (dd, 2 H), 3.09 (dd, 1 H). Anal. ($C_{19}H_{28}N_2O_8·0.25H_2O$) C, H, N.

[[3,4-*cis*-bis[(methoxyethoxy)methoxy]pyrrolidino]-carbonyl]-*O*-methyltyrosine (8e). This compound was prepared in 100% yield from [[3,4-*cis*-bis[(methoxyethoxy)methoxy]pyrrolidino]carbonyl]-*O*-methyltyrosine methyl ester as described for **8a**: ^{1}H NMR ($CDCl_3$) δ 7.12 (dd 2 H), 6.84 (dd, 2 H), 4.88–4.75 (m, 4 H), 4.50–4.40 (m, 1 H), 3.79 (s, 3 H), 3.38 (s, 3 H), 3.37 (s, 3 H), 3.21 (dd, 1 H), 3.08 (dd, 1 H). Anal. ($C_{23}H_{36}N_2O_{10}·0.75H_2O$) C, H, N.

Benzyl (2*R*)-2-Benzyl-3-[[(2-hydroxyethyl)methylamino]carbonyl]propionate (Ester of 10a). To benzyl (2*R*)-2-(carboxymethyl)-3-phenylpropionate[2b] (**9**, 512.0 mg, 1.72 mmol) in CH_2Cl_2 (7 mL) at −10 °C was added *N*-methylmorpholine (0.24 mL, 2.2 mmol) followed by isobutyl chloroformate (0.225 mL, 1.74 mmol). After 3 min, *N*-methylethanolamine (0.152 mL, 1.89 mmol) was added and the reaction was stirred at −10 °C for 15 min and at ambient temperature for 2 h. The mixture was concentrated, taken up in ethyl acetate, washed with 0.5 M H_3PO_4, saturated $NaHCO_3$ solution, and brine, and then was dried and evaporated. Chromatography with 80–100% ethyl acetate in hexane afforded 505.7 mg (83%) of an oil which slowly crystallized: mp 77–78 °C; TLC R_f = 0.23 (III); ^{1}H NMR ($CDCl_3$) δ 7.10–7.40 (m, 10 H), 5.20–5.00 (m, 2 H), 3.00, 2.92 (2 s, total 3 H). Anal. ($C_{21}H_{25}NO_4$) C, H, N.

Benzyl (2*R*)-2-Benzyl-3-[[(2-methoxyethyl)methylamino]carbonyl]propionate (Ester of 10b). This compound was prepared in 90% yield from 2-methoxy-*N*-methylethylamine[16] as described for the ester of **10a**: TLC R_f = 0.20 (I); ^{1}H NMR ($CDCl_3$) δ 7.40–7.10 (m, 10 H), 5.20–5.02 (m, 2 H), 3.31, 3.26 (2 s, total 3 H), 2.97, 2.91 (2 s, total 3 H), 2.47, 2.36 (2 dd, total, 1 H).

Benzyl (2*R*)-2-Benzyl-3-[[[2-(methoxymethoxy)ethyl]-methylamino]carbonyl]propionate (Ester of 10c). This compound was prepared in 72% yield from amine **3a** as described for the ester of **10a**: TLC R_f = 0.15 (I); ^{1}H NMR ($CDCl_3$) δ 7.37–7.11 (m, 10 H), 5.18–5.02 (m, 2 H), 4.58, 4.49 (2 s, total 2 H), 3.32, 3.27 (2 s, total 3 H), 3.00, 2.92 (2 s, total 3 H), 2.49, 2.35 (2 dd, total 1 H).

Benzyl (2*R*)-2-Benzyl-3-[[[2-[(methoxyethoxy)methoxy]ethyl]methylamino]carbonyl]propionate (Ester of 10d). This compound was prepared in 80% yield from amine **3b** as described for the ester of **10a**: TLC R_f = 0.33 (III). Anal. ($C_{25}H_{33}NO_6$) C, H, N.

Benzyl (2*R*)-2-Benzyl-3-[[3,4-*cis*-bis(methoxymethoxy)-pyrrolidino]carbonyl]propionate (Ester of 10e). This compound was prepared in 70% yield from amine **6a** as described for the ester of **10a**: TLC R_f = 0.33 (III); ^{1}H NMR ($CDCl_3$) δ 7.37–7.10 (m, 10 H), 5.18–5.00 (m, 2 H), 4.78–4.66 (m, 4 H), 4.22–4.12 (m, 2 H), 3.67–3.30 (m, 5 H), 3.38, 3.37, 3.36, 3.35 (4

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s, total 6 H), 3.04 (dd, 1 H), 2.88–2.77 (m, 1 H), 2.69–2.57 (m, 1 H), 2.36–2.24 (m, 1 H).

Benzyl (2*R*)-2-Benzyl-3-[[3,4-*cis*-bis[(methoxyethoxy)methoxy]pyrrolidino]carbonyl]propionate (Ester of 10f). This compound was prepared in 84% yield from amine **6b** as described for the ester of **10a**: TLC R_f = 0.10 (III); ^{1}H NMR ($CDCl_3$) δ 7.37–7.10 (m, 10 H), 5.18–5.01 (m, 2 H), 4.88–4.75 (m, 4 H), 4.28–4.19 (m, 2 H), 3.80–3.28 (m, 13 H), 3.38 (2 s, total 6 H), 3.03 (2 dd, 1 H), 2.88–2.77 (m, 1 H), 2.70–2.56 (m, 1 H), 2.29 (2 dd, 1 H).

(2*R*)-2-Benzyl-3-[[(2-hydroxyethyl)methylamino]carbonyl]propionic Acid (10a). Benzyl (2*R*)-2-benzyl-3-[[(2-hydroxyethyl)methylamino]carbonyl]propionate (0.502 g, 1.41 mmol) and 10% Pd/C (0.24 g) in methanol (5 mL) were stirred under a hydrogen atmosphere for 1 h. The mixture was filtered and evaporated to afford 0.374 g (100%) of a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.37–7.17 (m, 5 H), 2.95, 2.93 (2 s, total 3 H). Anal. ($C_{14}H_{19}NO_4 \cdot 0.1H_2O$) C, H, N.

(2*R*)-2-Benzyl-3-[[(2-methoxyethyl)methylamino]carbonyl]propionic Acid (10b). This compound was prepared in 94% yield from benzyl (2*R*)-2-benzyl-3-[[(2-methoxyethyl)-methylamino]carbonyl]propionate as described for **10a**: ^{1}H NMR ($CDCl_3$) δ 7.34–7.17 (m, 5 H), 3.32, 3.20 (2 s, total 3 H), 2.95, 2.88 (2 s, total 3 H). Anal. ($C_{15}H_{21}NO_4 \cdot 0.53H_2O$) C, N, H: calcd, 7.70; found, 7.25.

(2*R*)-2-Benzyl-3-[[[2-(methoxymethoxy)ethyl]methylamino]carbonyl]propionic Acid (10c). This compound was prepared in 100% yield from benzyl (2*R*)-2-benzyl-3-[[[2-(methoxymethoxy)ethyl]methylamino]carbonyl]propionate as described for **10a**: ^{1}H NMR ($CDCl_3$) δ 7.37–7.16 (m, 5 H), 3.31, 3.23 (2 s, total 3 H), 2.97, 2.91 (2 s, total 3 H). Anal. ($C_{16}H_{23}NO_5 \cdot 0.75H_2O$) C, H, N.

(2*R*)-2-Benzyl-3-[[[2-[(methoxyethoxy)methoxy]ethyl]methylamino]carbonyl]propionic Acid (10d). This compound was prepared in 100% yield from benzyl (2*R*)-2-benzyl-3-[[[2-[(methoxyethoxy)methoxy]ethyl]methylamino]carbonyl]-propionate as described for **10a**: ^{1}H NMR ($CDCl_3$) δ 7.36–7.17 (m, 5 H), 3.40 (s, 3 H), 2.97, 2.92 (2 s, total 3 H). Anal. ($C_{18}H_{27}NO_6 \cdot 0.75H_2O$) C, H, N.

(2*R*)-2-Benzyl-3-[[3,4-*cis*-bis(methoxymethoxy)pyrrolidino]carbonyl]propionic Acid (10e). This compound was prepared in 95% yield from benzyl (2*R*)-2-benzyl-3-[[3,4-*cis*-bis(methoxymethoxy)pyrrolidino]carbonyl]propionate as described for **10a**: ^{1}H NMR ($CDCl_3$) δ 7.37–7.14 (m, 5 H), 4.79–4.61 (m, 4 H), 4.23–4.12 (m, 2 H), 3.72–3.51 (m, 2 H), 3.29 (dd, 1 H), 3.22–3.05 (m, 1 H), 2.74 (dd, 1 H), 2.43–2.50 (m, 2 H). Anal. ($C_{19}H_{27}NO_7 \cdot H_2O$) C, H, N.

(2*R*)-2-Benzyl-3-[[3,4-*cis*-bis[(methoxyethoxy)methoxy]pyrrolidino]carbonyl]propionic Acid (10f). This compound was prepared in 96% yield from benzyl (2*R*)-2-benzyl-3-[[3,4-*cis*-bis[(methoxyethoxy)methoxy]pyrrolidino]carbonyl]propionate as described for **10a**: ^{1}H NMR ($CDCl_3$) δ 7.34–7.15 (m, 5 H), 4.87–4.72 (m, 4 H), 4.28–4.16 (m, 2 H), 3.38 (s, 6 H).

(2*RS*)-[[(2,3-Dihydroxypropyl)methylamino]carbonyl]-*O*-methyltyrosine Methyl Ester. To α-Isocyanato-*O*-methyltyrosine methyl ester[2b] (**7**, 1.53 g, 6.5 mmol) in dioxane (5 mL) at 0 °C was added racemic 3-(methylamino)-1,2-propanediol[17] (0.684 g, 6.5 mmol). The reaction was stirred at 0 °C for 1 h and at room temperature for 1 h and then was evaporated and chromatographed with 5% methanol in chloroform to afford 1.855 g (84%) of a colorless oil: TLC R_f = 0.47 (IV); ^{1}H NMR ($CDCl_3$) δ 7.02 (dd, 2 H), 6.83 (dd, 2 H), 5.13–5.00 (br m, 1 H), 4.76–4.65 (m, 1 H), 3.80 (s, 3 H), 3.73 (s, 3 H), 3.60–3.26 (m, 5 H), 3.13–2.98 (m, 2 H), 2.89, 2.88 (2 s, total 3 H).

(2*RS*)-[[(2,3-Dihydroxypropyl)methylamino]carbonyl]-*O*-methyltyrosine. (2*RS*)-[[(2,3-Dihydroxypropyl)methylamino]carbonyl]-*O*-methyltyrosine methyl ester (114 mg, 0.355 mmol) in dioxane (4 mL) and water (2 mL) at 0 °C was treated with LiOH·H_2O (42.0 mg, 1 mmol). After 90 min, 2 M HCl (0.6 mL, 1.2 mmol) was added and the mixture was evaporated to a foam which was used without further purification: ^{1}H NMR (CD_3OD) δ 7.15 (dd, 2 H), 6.83 (dd, 2 H), 4.43 (dd, 1 H), 3.77 (s, 3 H), 2.91 (s, 3 H).

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Boc-His Amide of (1′*R*,2′*S*,5*S*)-5-[2′-Amino-3′-cyclohexyl-1′-hydroxypropyl]-3-ethyloxazolidin-2-one. (1′*R*,2′*S*,5*S*)-5-[[2′-[(*tert*-Butyloxy)carbonyl]amino]-3′-cyclohexyl-1′-[(methoxyethoxy)methoxy]propyl]-3-ethyloxazolidin-2-one[6] (1.65 g, 3.48 mmol) was stirred for 1 h in 4.5 M ethanolic HCl (15 mL), and the solvent was evaporated followed by portions of ethanol and then ether to leave a white solid. To this residue, in dimethylformamide (11 mL), were added Boc-His-OH (0.976 g, 3.82 mmol), 1-hydroxybenzotriazole (1.41 g, 10.4 mmol), and *N*-methylmorpholine (0.40 mL, 3.64 mmol). The mixture was cooled to −23 °C and treated with 1-ethyl-3-[(dimethylamino)-propyl]carbodiimide hydrochloride (0.746 g, 3.89 mmol). After 2 h at −23 °C and 14 h at ambient temperature, the mixture was poured into saturated $NaHCO_3$ solution and was extracted into ethyl acetate which was washed with water and brine and then was dried and evaporated. Chromatography of the residue with 4–6% methanol in chloroform afforded 1.27 g (72%) of a white powder: TLC R_f = 0.36 (V). Anal. ($C_{25}H_{41}N_5O_6 \cdot 0.4H_2O$) C, H, N.

Compound 21. The Boc-His amide of (1′*R*,2′*S*,5*S*)-5-[2′-amino-3′-cyclohexyl-1′-hydroxypropyl]-3-ethyloxazoladin-2-one (390 mg, 0.768 mmol) was stirred for 1 h in 4.5 M ethanolic HCl (7 mL), and the solvent was evaporated followed by portions of ethanol and then ether to leave a white solid. To this residue, in dimethylformamide (5 mL), were added acid **10d** (280 mg, 0.792 mmol), 1-hydroxybenzotriazole (311 mg, 2.30 mmol), and *N*-methylmorpholine (0.150 mL, 1.36 mmol). The mixture was cooled to −23 °C and treated with 1-ethyl-3-[(dimethylamino)propyl]-carbodiimide hydrochloride (0.162 g, 0.845 mmol). After 2 h at −23 °C and 16 h at ambient temperature, the mixture was poured into a saturated $NaHCO_3$ solution and was extracted into ethyl acetate which was then washed with water and brine and then was dried and evaporated. Chromatography of the residue with 4% methanol in chloroform afforded 277 mg (49%) of a brittle foam: ^{1}H NMR ($CDCl_3$) δ 7.57 (s, 1 H), 7.36–7.16 (m, 5 H), 6.87 (s, 1 H), 3.38 (s, 3 H), 3.28 (q, 2 H), 3.02, 3.92 (2 s, total 3 H), 1.16 (t, 3 H); DCI-MS 743 (M + H), 699 (M + H − CO_2), 536 (M − CO_2 − $CH_3OCH_2CH_2OCH_2OCH_2CH_2NCH_3$).

Compounds 11–15, 17–20, and 23–26. These inhibitors were prepared and purified as described for **21**.

Compound 16. Couplings were performed as described above and the product was isolated as follows. The dimethylformamide was removed by bulb to bulb distillation [40 °C (bath), −78 °C (receiver), 0.1 mm] and the residue was dissolved in methanol and eluted through Sephadex LH 20 to remove the 1-hydroxybenzotriazole. After solvent evaporation, the residue was chromatographed on silica gel with 6–10% methanol in chloroform to provide the desired product: ^{1}H NMR ($CDCl_3$) δ 7.66 (s, 1 H), 7.19 (dd, 2 H), 6.96 (s, 1 H), 6.84 (dd, 2 H), 3.79 (s, 3 H), 3.27 (q, 2 H), 2.94 (s, 3 H), 1.15 (t, 3 H).

Compound 22. This compound was prepared and isolated as described for **16**. A sample was converted to the acetate salt for elemental analysis: ^{1}H NMR (CD_3OD) δ 7.90 (s, 1 H), 7.17 (dd, 2 H), 6.87 (s, 1 H), 6.85 (dd, 2 H), 3.77 (s, 3 H), 1.16 (t, 3 H).

Solubility Studies. Solubilities were determined by agitating in a side by side shaker ca. 10 mg of the test compound in 1 mL of 0.01 M, pH 6.5, phosphate buffer, or 0.01 M, pH 7.4, phosphate buffer containing 0.15 M NaCl, for 24 h at 37 °C. Suspensions were then filtered through glass wool, diluted with the mobile phase (49% 1% aqueous $HClO_4$/38% CH_3CN/13% CH_3OH) and assayed by HPLC (15-cm PRP Column, detection at 214 nm).

Partition Coefficients: Hydrophobic Compounds ($P > 50$). The inhibitor (0.3–0.5 mg) was dissolved in octanol (1.00 mL). To each buffer [5.00 mL; pH 6.5, 0.1 M disodium piperazine-*N*,*N*′-bis(2-ethanesulfonate); pH 7.4, 0.1 M sodium *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonate] was added 200 μL of the octanol solution. After shaking on a reciprocating shaker (~300 cpm) for 2 h, the mixtures were centrifuged at 1500 rpm for 10 min. A portion of the octanol phase (5.0 μL) was diluted with the HPLC mobile phase (1.00 mL). The octanol was removed by aspiration and the aqueous phase (400 μL) was diluted with the HPLC mobile phase (400 μL). Measured volumes (15.0–50.0 μL) were assayed by HPLC:

$$P = \frac{\text{peak area}_{\text{octanol}} \times \text{DF}_{\text{octanol}}}{\text{peak area}_{\text{aqueous}} \times \text{DF}_{\text{aqueous}}}$$

where $DF_{octanol}$ and $DF_{aqueous}$ are the respective octanol and aqueous dilution factors.

Partition Coefficients: Hydrophilic Compounds ($P < 50$). The inhibitor (0.3–0.5 mg) was dissolved in each buffer (2 mL, vide supra), octanol (2.00 mL) was added, and the mixture was shaken and centrifuged as described above. A portion of the octanol phase (50.0 μL) was evaporated at 40 °C under a stream of dry, filtered air and was reconstituted in the appropriate mobile phase (800 μL). The remaining octanol phase was removed by aspiration and the aqueous phase (50.0 μL) was diluted with the HPLC mobile phase (950 μL).

In Vitro Enzyme Assays. Assays of purified human renal renin,[18] bovine cathepsin D,[18] pepsin,[18] and human plasma renin[2b] were performed as previously described. Inhibition of monkey plasma renin was measured by the same procedure as for human plasma renin except that only one-half of the incubation volume was assayed.

Monkey Experiments. Monkeys were dosed intravenously ($n = 5$) and intraduodenally ($n = 2$) with compound **21** as described previously.[2b] Blood samples were withdrawn for measurement of plasma renin activity.[19] Plasma levels (P_L) of **21** were determined in the blood samples from two of the iv monkeys (5, 15, 30, 60, 120, and 180 min samples) by a renin inhibition assay as described previously for id rat experiments.[2d] The data were fitted to a bis-exponential decay model:

$$\text{monkey 1} \quad P_L = 289.1e^{-0.1991T} + 4.964e^{-0.02385T}$$

$$R = 0.99967$$

$$\text{monkey 2} \quad P_L = 1009e^{-0.2270T} + 8.581e^{-0.02093T}$$

$$R = 0.99996$$

Drug Metabolism Studies. Compound **21** was labeled with ^{14}C at the oxazolidinone carbonyl[13] and had a specific activity of 57 μCi/mg. Male Sprague–Dawley rats, weighing 180–300 g, were dosed at 1 mg/kg either orally by gavage or intravenously into the femoral vein. Urine and feces were collected over 3 days following drug administration. Bile was collected from another two rats (one each dosing procedure) after surgical implantation of a bile duct cannula under diethyl ether anesthesia. The feces were homogenized in 70% aqueous ethanol and aliquots were burned in a sample oxidizer. All samples were assayed for total radioactivity by liquid-scintillation spectrometry and corrected for quenching with an internal standard.

Metabolic patterns is urine, bile, and fecal samples were determined by HPLC on a C-18 column with a linear 15–50% aqueous acetonitrile gradient containing 0.1% trifluoroacetic acid. Some of the radioactive peaks in the samples were tentatively identified by comparison of their retention times with those of authentic reference standards, based on absorbance at 215 nm or radioactivity. A second set of HPLC conditions using 41.5% aqueous acetonitrile containing 0.01 M tetramethylammonium perchlorate, 0.01 M dodecylsulfate disodium salt, and 0.01 M sodium phosphate was employed to demonstrate the absence of the free amino compound resulting from cleavage between histidine and the benzylsuccinate residue.

Intraduodenal Rat Experiments. Rats were dosed id with compound **21** and plasma drug levels were determined by a renin inhibition assay as described previously.[2d]

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Inhibition of Ornithine Decarboxylase by the Isomers of 1,4-Dimethylputrescine

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1,4-Dimethylputrescine (2,5-hexanediamine) was separated into its racemic and meso isomers by fractional crystallization of its dibenzoyl derivative. The racemic form was resolved into its (+)- and (−)-isomers with (+)- and (−)-dibenzoyltartaric acids. None of the three isomers (meso, +, and −) inhibited ornithine decarboxylase (ODC) activity in vitro, while all the three were strongly inhibitory of ODC when assayed in vivo in rats or in H-35 hepatoma cells. In rat liver the three isomers also decreased the putrescine pool while only the (+)-isomer decreased spermidine content. In the H-35 cells the (−)- and (+)-isomers decreased the spermidine and spermine content. When ODC was induced in the latter by insulin it was found that the (−)-isomer strongly inhibited protein and ODC synthesis, while the (+)-isomer and the meso isomer were less inhibitory. The meso isomer was a good inducer of ODC antizyme in rat liver, while the (+)- and (−)-isomers were poor inducers of the former.

Ornithine decarboxylase (ODC, L-ornithine decarboxylase, EC 4.1.1.17) is a permanent target for studies on the inhibition of the proliferative and neoplastic processes.[1,2] The inhibitors include ornithine and polyamine (putrescine, spermidine, and spermine) derivatives and are usually of three types. To the first type belong the "suicide" or mechanism-based enzyme inhibitors such as the substance analogue (difluoromethyl)ornithine (DFMO), and the product analogues of the alkyne 1,4-diaminobutane type.[3] DFMO was found to be useful in the treatment of human parasitic diseases.[4] To the second type belong polyamine analogues such as alkylspermidines which do not directly inhibit the enzyme but exert a feed-back repression.[5] Finally, the third type of ODC inhibitors are the product analogues which are competitive inhibitors of ODC such as the *N*-alkyl, 1-alkyl, and 2-alkylputrescines.[6,7] It was shown that among the latter the methylputrescines are the best in vivo inhibitors of liver ODC in rats treated with thioacetamide or dexa-

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