Nonpeptide Renin Inhibitors Employing a Novel 3-Aza(or oxa)-2,4-dialkyl Glutaric Acid Moiety as a P_2/P_3 Amide Bond Replacement

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A new series of renin inhibitors has been developed. The inhibitors feature a novel replacement for the P_2/P_3 dipeptide moiety normally associated with renin inhibitors. The dipeptide replacement was a (2*S*,4*S*)-3-aza(or oxa)-2,4-dialkylglutaric acid amide. Extensive structure–activity relationship studies determined that optimum potency was achieved when inhibitors employed a benzyl and butyl group at the C(4) and C(2) carbon position, respectively. In addition, maximum in vitro potency was obtained when the N-terminus was functionalized by incorporating a 4-(1,3-dioxabutyl)piperidine amide. SAR data suggested that the 1,3-dioxabutyl group (methoxymethyl ether) interacted by hydrogen bonding to groups in the S_4 domain of renin. This hypothesis was strengthened when a 4-butylpiperidine amide was substituted and inhibitor potency decreased dramatically. Inhibitors employing this novel dipeptide mimic were prepared by coupling the glutaric acid amides with either the transition-state mimic (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (18) or the hydroxyethylene dipeptide isostere. The glutaric acid amides were prepared by two general procedures. The first procedure involved the reductive amination of α-amino acid esters with α-keto esters. The second procedure involved the displacement reaction of α-bromo esters or acids with α-amino acid amides.

Introduction

Modulation of the renin–angiotensin–aldosterone system (RAAS) has proven to be a viable approach for treating various cardiovascular disorders (Figure 1). The effector hormone of the RAAS cascade, angiotensin II (ANG II), regulates blood pressure and fluid balance. The major pharmacological effects of ANG II are vasoconstriction and stimulation of the adrenal cortex to release aldosterone, a hormone that causes sodium retention, thereby increasing blood volume. Inhibiting the production of ANG II with enzyme inhibitors of angiotensin-converting enzyme (ACE) has been shown as an effective means of controlling blood pressure.[1-3] The blockade of ANG II production by inhibition of renin has also received considerable attention by many research groups.[4] The impetus for developing renin inhibitors as antihypertensive drugs resides in the specificity of the enzyme. Renin's only role is to cleave the first 10 amino acids from the amino terminal of the enzyme substrate angiotensinogen. Therefore, a possible advantage of a therapeutic agent that inhibits renin is that it may have fewer side effects due to its specific nature.

In 1986, we launched a clinical program to evaluate the activity of our dipeptide renin inhibitor, enalkiren, in patients with hypertension and congestive heart failure (CHF). Enalkiren demonstrated clinical efficacy when administered intravenously to patients with essential hypertension and chronic CHF.[5-10] Encourgaged by these clinical findings, we sought to develop an orally active and bioavailable renin inhibitor.[11] We hypothesized that the

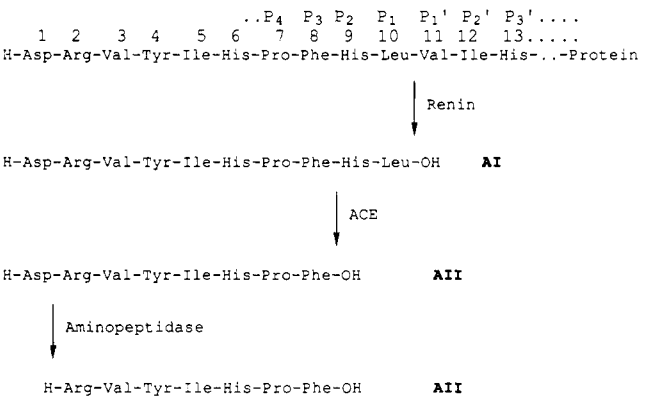

Figure 1. Renin–angiotensin–aldosterone (RAAS) system.

(1) Case, D. B.; Atlas, S. A.; Laragh, J. H.; Sealy, J. E.; Sullivan, P. A.; McKinstry, D. N. Clinical Experience with the Blockade of the Renin-Angiotensin-Aldosterone System by an Oral Converting-Enzyme Inhibitor (SQ-14225, Captopril) in Hypertensive Patients. *Prog. Cardiovasc. Dis.* **1978**, *21*, 195–206.

(2) Brunner, H. R.; Gavras, H.; Waeber, B.; Oral Angiotensin-Converting-Enzyme Inhibitor in Long-Term Treatment of Hypertensive Patients. *Ann. Intern. Med.* **1979**, *90*, 19–23.

(3) Gavras, H.; Brunner, H. R.; Turini, G. A.; Kershaw, G. R.; Tifft, C.; Cuttelod, S.; Gavras, I.; Vukovich, R. A.; McKinstry, D. N. Antihypertensive Effect of the Oral Angiotensin Converting Enzyme Inhibitor, SQ 14225, in Man. *N. Engl. J. Med.* **1978**, *298*, 991–995.

(4) For a recent review of the renin–angiotensin–aldosterone system, see: Kleinert, H. D.; Baker, W. R.; Stein, H. H. Renin Inhibitors. *Adv. Pharmacol.* **1991**, *22*, 207–250 and references cited therein.

(5) Weber, M. A.; Neutel, J. M.; Essinger, I.; Glassman, H. N.; Boger, R. S.; Luther, R. R. Assessment of Renin Dependency of Hypertension with a Dipeptide Renin Inhibitor. *Circulation* **1990**, *81*, 1768–1774.

(6) Boger, R. S.; Glassman, H. N.; Cavanaugh, J. H.; Schmitz, P. J.; Lamn, J.; Moyse, D.; Cohen, A.; Kleinert, H. D.; Luther, R. R. Prolonged Duration of Blood Pressure Response to Enalkiren, the Novel Dipeptide Renin Inhibitor, in Essential Hypertension. *Hypertension* **1990**, *15* (6, part 2), 835–840.

(7) Glassman, H. N.; Kleinert, H. D.; Boger, R. S.; Moyse, D. M.; Griffiths, A. M.; Luther, R. R. Clinical Pharmacology of Enalkiren, a Novel, Dipeptide Renin Inhibitor. *J. Cardiovas. Pharmacol.* **1990**, *16* (Suppl. 4), S76–81.

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(9) Neuberg, G. W.; Kukin, M. L.; Penn, J.; Yusak, M.; Medina, N.; Packer, M. Renin Inhibition (but not Plasma Renin Activity) Predicts the Response to Converting-Enzyme Inhibitors in Chronic Heart Failure. *J. Am. College Cardiol.* **1990**, *15* (2, Suppl A), 172A.

(10) Neuberg, G. W.; Penn, J.; Kukin, M. L.; Yushak, M.; Medina, N.; Packer, M. Hemodynamic Effects of Enalkiren, a Direct Inhibitor of Plasma Renin, in Patients with Chronic Heart Failure. *Clin. Res.* **1990**, *38* (2), 253A.

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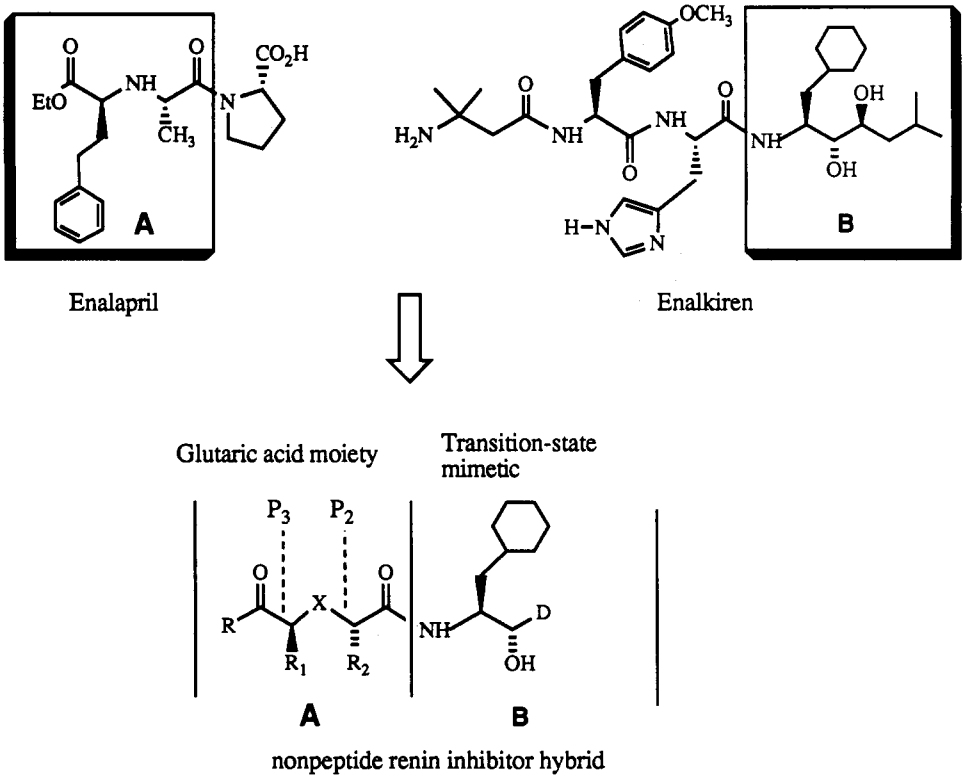

Figure 2. Structure of the ACE inhibitor Enalapril, renin inhibitor Enalkiren, and a general structure of a nonpeptide renin inhibitor hybrid.

poor oral bioavailability associated with enalkiren resided in its peptidic structure. We speculated that an inhibitor without the P_2/P_3 amide linkage would be more stable to first pass metabolism and be better absorbed through the gastrointestinal tract. A heteroatom substitution was selected as an amide bond surrogate. Thus, our nonpeptide renin inhibitor design incorporated two fragments: (1) a 3-aza or -oxa-2,4-dialkyl glutaric acid moiety (A), a substructure found in the ACE inhibitor enalapril, and (2) the transition state mimic (B) of enalkiren.[12] A general description of this hybrid nonpeptide inhibitor is shown in Figure 2. Substituent X is either nitrogen or oxygen, R is an ester or amide, R_1 and R_2 are alkyl or arylalkyl groups, and D is that portion of the transition-state mimic that binds to the $S_{1'}$ subsite of the enzyme. An extensive series of nonpeptide renin inhibitors were synthesized according to this general formula. The inhibitors were evaluated for in vitro potency, in vivo efficacy, and intraduodenal bioavailability. Initial results of our study are now reported.

Chemistry

The inhibitors prepared for this study are listed in Tables I and II. The syntheses of intermediates are shown in Schemes I–III. Esters **1a–d** were synthesized from α-amino acids by literature methods.[13] Amides **6a–n** were prepared by coupling the appropriate amine with either *N*-Cbz-L-phenylalanine or L-phenyllactic acid using EDC and HOBT in DMF (−23 °C to room temperature, 18 h). The *N*-Cbz protecting group for compound **6a** was removed by hydrogenolysis (H_2, Pd/C). Two general protocols were followed for the synthesis of the glutaric acid moieties (Schemes I and II). The first synthetic protocol involved the reductive amination ($NaCNBH_3$, sodium acetate, absolute EtOH, room temperature, 18 h) of amino acid esters **1a–d** with α-keto esters **2a–d** to produce the 3-aza-2,4-dialkylglutaric acid derivatives **3a–l** as mixtures of diastereomers in overall yields of 6–52%. Hydrogenolysis of the benzyl esters **3a–f** (H_2, Pd/C, 4 atm, CH_3OH) gave the carboxylic acids **4a–f** as white amorphous solids.

The absolute stereochemistry of **3e** and **3f** prepared by the reductive amination protocol (Scheme I) was determined by correlation and TLC mobility. Thus, compound **3f** (more polar isomer) was identical (TLC and ^1H NMR) to ester **8a**. Assuming S_N2 displacement with inversion of stereochemistry, the reaction of amine **1c** and triflate **7a** gave ester **8a** with the 2*S*,4*S* configuration. It was assumed that the more polar diastereomers obtained from related reductive alkylation reactions were also 2*S*,4*S*. In addition, the absolute stereochemistry of **3k** (2*S*,4*S*) was determined by hydrolysis of the *tert*-butyl ester (HCl), coupling the resultant acid to amine **13c**, and hydrogenolysis of the benzyl ester to give acid **8c** which was identical to acid **8c** prepared by alkylation.

The second protocol utilized an S_N2 displacement of an amine or alkoxide on a (2*R*)-2-bromo- or 2-trifluoromethanesulfonate carboxylic acid or ester. A typical example was as follows. Reaction of the piperidine amide

(11) Oral bioavailability of enalkiren in man is <1%, see: Lamon, J. E.; Luther, R. R.; Boger, R. S.; Hoyos, P.; Cavanaugh, J. H. Pharmacokinetics and Safety of Enalkiren (Abbott A-64662) Following Oral and Intravenous Doses in Normal Subjects. *Clin. Res.* **1991**, *39*, 420A.

(12) A portion of this work has been presented in preliminary form: Fung, A. K. L.; Baker, W. R.; Stein, H. H.; Kleinert, H. D.; Plattner, J. J.; Armiger, Y.-L.; Condon, S. L.; Cohen, J.; Egan, D. A.; Barlow, J. L.; Verburg, K. M.; Martin, D. L.; Young, G. A.; Polakowski, J. S.; Hutchins, C. W.; Donner, B. G.; Perun, T. J. Part II. Design, Synthesis and Biological Activity of Potent Nonpeptide Renin Inhibitors. *Abstracts of Papers*, 201st ACS National Meeting, Atlanta, GA; American Chemical Society: Washington, DC, 1991; MEDI 54.

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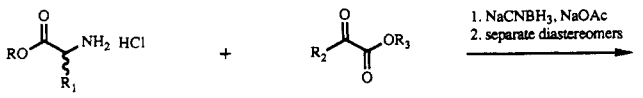
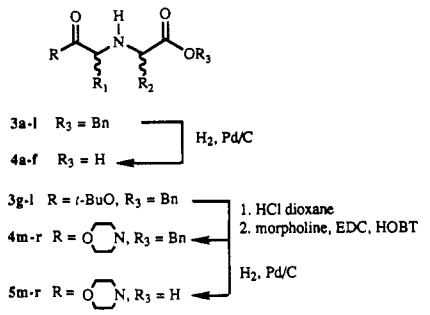
Scheme I



1 2

R	R_1	R_3	R_2
a: Bn	(S) CH_3	a: Et	CH_2CH_2Ph
b: t-Bu	(S) CH_2CH_2Ph	b: Bn	$CH_2CH(CH_3)2$
c: t-Bu	(S) CH_2Ph	c: Bn	CH_3
d: t-Bu	(R) CH_2Ph	d: Bn	$CH_2(CH_2)2CH_3$



R	R_1	R_2
a: EtO	CH_2CH_2Ph (less polar)	(S) CH_3
b: EtO	CH_2CH_2Ph (more polar)	(S) CH_3
c: t-BuO	(S) CH_2CH_2Ph	$CH_2CH(CH_3)2$ (less polar)
d: t-BuO	(S) CH_2CH_2Ph	$CH_2CH(CH_3)2$ (more polar)
e: t-BuO	(S) CH_2Ph	(R) $CH_2CH(CH_3)2$
f: t-BuO	(S) CH_2Ph	(S) $CH_2CH(CH_3)2$
g: t-BuO	(S) CH_2Ph	CH_3 (less polar)
h: t-BuO	(S) CH_2Ph	CH_3 (more polar)
i: t-BuO	(R) CH_2Ph	CH_3 (less polar)
j: t-BuO	(R) CH_2Ph	CH_3 (more polar)
k: t-BuO	(S) CH_2Ph	(S) $CH_2(CH_2)2CH_3$
l: t-BuO	(S) CH_2Ph	(R) $CH_2(CH_2)2CH_3$
m: 4-morpholino	(S) CH_2Ph	CH_3
n: 4-morpholino	(S) CH_2Ph	CH_3 (diastereomer)
o: 4-morpholino	(R) CH_2Ph	CH_3
p: 4-morpholino	(R) CH_2Ph	CH_3 (diastereomer)
q: 4-morpholino	(S) CH_2Ph	(S) $CH_2(CH_2)_2CH_3$
r: 4-morpholino	(S) CH_2Ph	(R) $CH_2(CH_2)CH_3$

of L-phenylalanine **6a** with (2*R*)-ethyl 2-bromohexanoate in nitromethane/aqueous ammonium carbonate at 48 °C for 3 days afforded as a single diastereomeric ester **8b** in 79% yield (Scheme II).[14] Hydrolysis of ester **8b** gave acid **8c** as a white solid which was readily purified by recrystallization from ethyl acetate. However, the alkylation of the phenyllactic acid amides was more problematic. For example, treatment of the piperidine amide of phenyllactic acid **6b** with NaH in DMF/THF at 45 °C for 3 h and then adding of 1.1 equiv of (2*R*)-2-bromohexanoic acid and stirring for an additional 2.5 h gave a 69% yield of acid **8r** and its C(2) diastereomer in a ratio of 6:1.

The preparation of the 4-substituted piperidines used for the synthesis of various target molecules is outlined in Scheme III. 4-Hydroxypiperidine (**9**) and 4-(2-hydroxyethyl)piperidine (**14**) were protected as their *N*-formyl derivatives. Alkylation of **10** with methyl iodide, allyl bromide, chloromethyl methyl ether, methoxyethyl bromide, or dimethyl sulfide–benzoyl peroxide[15] gave compounds **12a–c** and **12e–f** in 53–85% yield. Mitsunobu displacement of **10** (Ph_3P, DEAD, thioacetic acid)[16] gave the thioacetate which was hydrolyzed using LiOH to give thiol **11**. Reaction of **11** with chloromethyl methyl ether gave the thioacetal isomer **12d**. *N*-Formyl-4-(2-methoxyethyl)piperidine (**16**) was prepared by alkylation of **15** with sodium hydride and methyl iodide in 74% yield. The *N*-formyl protecting groups were removed using aqueous KOH at room temperature to furnish the desired 4-substituted piperidines **13a–f** and **17**.

The inhibitors **19a–b,e–v,x–y,za–f,zh–j**, **20**, and **21** were prepared by coupling acids **4a–f**, **5m–r**, and **8c–u** with the dihydroxyethylene **18**[17] and **8c** and **8r** with the hydroxyethylene dipeptide isostere[18] using EDC and HOBT, in DMF at −23 °C to room temperature, 18 h (Tables I and II, **20** and **21**). Inhibitors **19c,d** were prepared by reductive alkylation of ester **2a** with **18** (Y = Leu).[17a] Compound **19w** was synthesized by deprotection of **19ze** with TMSBr; inhibitor **19zg** was prepared by coupling the carboxylic acid derived from **4k** to **18** (Y = H), deprotection of the *tert*-butyl ester (HCl, dioxane), and coupling the resulting acid to amine **13f**. Inhibitors **19zk** and **19zl** were obtained by oxidation of **19zi** with OXONE and MCPBA, respectively. All inhibitors were tested as optically pure diastereomers.

Results and Discussion

In Vitro Activity of Nonpeptide Renin Inhibitors. Data for the in vitro potency of the nonpeptide renin inhibitors against purified and plasma renin are shown in Tables I and II and physical data in Table IV. The initial in vitro data obtained in the purified renin assay verified our inhibitor design that a heteroatom substitution (nitrogen) served as a P_2/P_3 amide bond replacement (Table I). The enalapril/enalkiren hybrid inhibitor **19a** had an IC_{50} of 130 nM. The data also showed that a benzyl substitution at the P_3 position, in the *tert*-butyl ester series,

(14) Iwasaki, G.; Kimura, R.; Kondo, S.; Numao, O. Preparation of N-Substituted Amino Acid Derivatives as Angiotensin-Converting Enzyme Inhibitors. Japanese patent 01121297, May 1989.

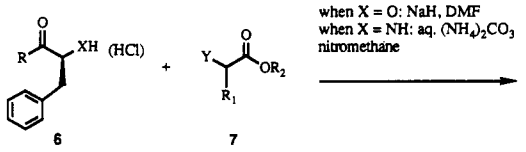
(15) Medina, J. C.; Salomon, M.; Kyler, K. S. A Mild Method for the Conversion of Alcohols to Methylthiomethyl Ethers. *Tetrahedron Lett.* **1988**, *29*, 3773–3776.

(16) Mitsunobu, O. The Use of Diethyl Azodicarboxylate and triphenylphosphine in Synthesis and Transformation of Natural Products. *Synthesis* **1981**, 1–28.

(17) For a synthesis of the amine **18** (Y = Boc) from *N*-Boc-L-phenylalanal, see: (a) Luly, J. R.; BaMaung, N.; Soderquist, J.; Fung, A. K. L.; Stein, H.; Kleinert, H. D.; Marcotte, P. A.; Egan, D. A.; Bopp, B.; Mertis, I.; Bolis, G.; Greer, G.; Perun, T. J.; Plattner, J. J. Renin Inhibitors. Dipeptide Analogues of Angiotensinogen Utilizing a Dihydroxyethylene Transition-State Mimic at the Scissle Bond to Impart Greater Inhibitory Potency. *J. Med. Chem.* **1988**, *31*, 2264–2276. (b) From *N*-Boc-L-phenalanal cyanohydrin, see: Luly, J. R.; Hsiao, C. N.; BaMaung, N.; Plattner, J. J. A Convenient Stereoselective Synthesis of 1,2,3-Aminodiols from α-Amino Acids. *J. Org. Chem.* **1988**, *53*, 6109–1612. (c) From (*E*)-5-methyl-2-hexen-1-ol using Sharpless epoxidation, see: Wood, J. L.; Jones, D. R.; Hirschman, R.; Smith, A. B., III. A Versatile, Efficient Synthesis of (−)-(2S,3R,4S)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane. The Abbott Pseudodipeptidyl Insert. *Tetrahedron Lett.* **1990**, *31*, 6329–6330.

(18) Herold, P.; Duthaler, R.; Rihs, G.; Angst, C. A Versatile and Stereocontrolled Synthesis of Hydroxyethylene Dipeptide Isosteres. *J. Org. Chem.* **1989**, *54*, 1178–1185.

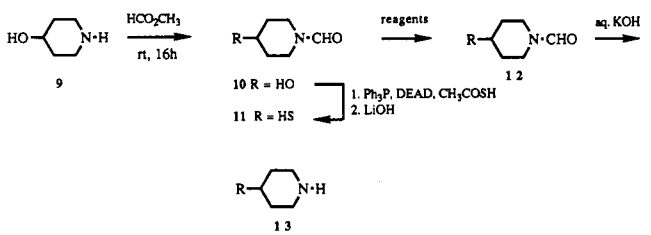
Scheme II



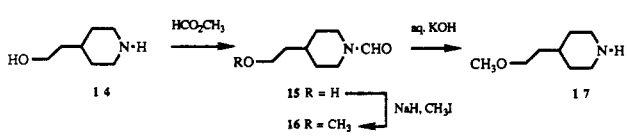
6 7 8

6		7			8			
R	X	R_2	R_1	Y	R	X	R_2	R_1
a: 4-(1,3-dioxabutyl)piperidinyl	NH	a: Bn	(R) $CH_2CH(CH_3)_2$	OTf	a: t-BuO	NH	Bn	$CH_2CH(CH_3)_2$
b: 4-(1,3-dioxabutyl)piperidinyl	O	b: H	(R) $CH_2(CH_2)_2CH_3$	Br	b: 4-(1,3-dioxabutyl)piperidinyl	NH	Et	$CH_2(CH_2)_2CH_3$
c: ethylamino	O	c: Et	(R) $CH_2(CH_2)_2CH_3$	Br	c: 4-(1,3-dioxabutyl)piperidinyl	NH	H	$CH_2(CH_2)_2CH_3$
d: diethylamino	O	d: H	(R,S) CH_2CH_3	Br	d: ethylamino	O	H	CH_3
e: azetidinyl	O	e: H	(R,S) $CH_2CH_2CH_3$	Br	e: diethylamino	O	H	CH_3
f: pyrrolidinyl	O	f: H	(R) CH_3	Br	f: azetidinyl	O	H	CH_3
g: piperidinyl	O				g: pyrrolidinyl	O	H	CH_3
h: morpholino	O				h: morpholino	O	H	CH_3
i: 4-methoxypiperidinyl	O				i: morpholino	O	H	$CH_2(CH_2)_2CH_3$
j: 4-(2-propenoxy)piperidinyl	O				j: piperidinyl	O	H	CH3
k: 4-(2-methoxyethyl)piperidinyl	O				k: piperidinyl	O	H	$CH_2(CH_2)_2CH_3$
l: 4-butylpiperidinyl	O				l: 4-methoxypiperidinyl	O	H	$CH_2(CH_2)_2CH_3$
m: 4-(1-oxa-3-thiabutyl)piperidinyl	O				m: 4-(2-propenoxy)piperidinyl	O	H	$CH_2(CH_2)_2CH_3$
n: 4-(3-oxa-1-thiabutyl)piperidinyl	O				n: 4-(2-methoxyethyl)piperidinyl	O	H	$CH_2(CH_2)_2CH_3$
					o: 4-(1,3-dioxabutyl)piperidinyl	O	H	CH_3
					p: 4-(1,3-dioxabutyl)piperidinyl	O	H	CH_2CH_3 (from **7d**, diastereomers)
					q: 4-(1,3-dioxabutyl)piperidinyl	O	H	$CH_2CH_2CH_3$ (from **7e**, diastereomers)
					r: 4-(1,3-dioxabutyl)piperidinyl	O	H	$CH_2(CH_2)_2CH_3$
					s: 4-butyltylpiperidinyl	O	H	$CH_2(CH_2)_2CH_3$
					t: 4-(1-oxa-3-thiabutyl)piperidinyl	O	H	$CH_2(CH_2)_2CH_3$
					u: 4-(3-oxa-1-thiabutyl)piperidinyl	O	H	$CH_2(CH_2)_2CH_3$

Scheme III



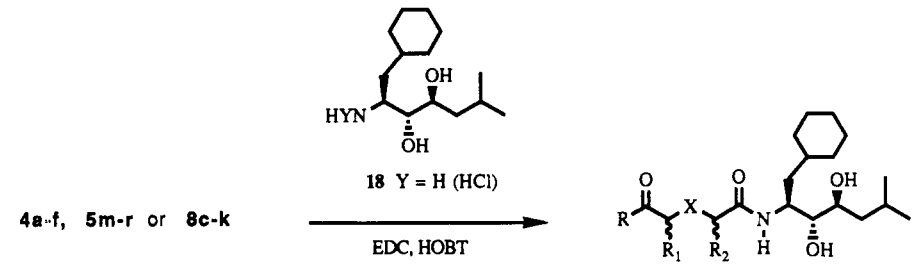
	R	Reagents
a.	CH_3O	NaH, CH_3I
b.	CH_2=$CHCH_2O$	NaH, allyl bromide
c.	CH_3OCH_2O	DIEA, MOMCl
d.	CH_3OCH_2S	DIEA, MOMCl
e.	CH_3SCH_2O	$(CH_3)_2S$, $(PhCO_2)_2$
f.	$CH_3OCH_2CH_2O$	NaH, $CH_3OCH_2CH_2Br$



imparted increased potency over the phenethyl analogue (**19h**, IC_{50} = 340 nM, vs **19f**, IC_{50} = 1000 nM). Ethyl ester analogues demonstrated reasonable potency with IC_{50} values between 150–240 nM (**19b–d**). The most noticeable effects on inhibitor potency were revealed in the morpholine amide series **19i–p** in which both the stereochemistry and the groups at the P_2 and P_3 positions were varied. Inhibitor **19i** with an (*S*)-benzyl group at the P_3 position was more potent than the (*R*)-benzyl isomer **19k** (85 vs 1600 nM). Similarly, the (*S*)-butyl substitution at the P_2 position in compound **19m** (13 nM) was more potent than inhibitor **19n** (750 nM) with (*R*)-butyl group at P_2. Increasing the chain length at the P_2 position from methyl, in inhibitor **19i**, to butyl, in inhibitor **19m**, increased potency over 6-fold. The most potent inhibitors in this group were the morpholine amides with an (*S*)-benzyl group at P_3 and a (*S*)-butyl side chain at P_2. Inhibitor potency did not depend on the heteroatom replacement. Inhibitors possessing the oxygen heteroatom as the P_2/P_3 amide bond replacement were equivalent in potency to the nitrogen series (compare **19i** to **19o** and **19m** to **19p**). Further structure–activity relationship studies were performed at the N-terminus of the nonpeptide renin inhibitors.

In vitro data for a series of amide analogues **19q–v** are shown in Table I. In the oxygen series, the ethyl amide **19q** and the diethyl amide **19r** were less potent than the cyclic amide (morpholine) inhibitor **19o**. However, potency increased substantially when other cyclic amides were incorporated into the molecules. Of the cyclic amides that were evaluated, the azetidine analogue **19s** was the most potent, IC_{50} 5.5 nM. When compared to the azetidine analogue **19s**, the pyrrolidine **19t** and the piperidine **19u** amide-containing inhibitors were two and four times less potent, respectively. The butyl side-chain substitution in compound **19v** showed no improved activity over the

Table I. Synthesis of Nonpeptide Renin Inhibitors **19a–v** from Glutaric Acid Intermediates and Their in Vitro Potency against Purified and Plasma Renin



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19	R	X	R_1	R_2	IC_{50}, nM purified, pH 6.0	IC_{50}, nM plasma, pH 7.4
a	EtO	NH	$CH_2CH_2Ph^b$	CH_3 (*S*)	130	nd
b	EtO	NH	$CH_2CH_2Ph^a$	CH_3 (*S*)	150	nd
c	EtO[c]	NH	$CH_2CH_2Ph^a$	$CH_2CH(CH_3)_2$ (*S*)	240	nd
d	EtO[c]	NH	$CH_2CH_2Ph^b$	$CH_2CH(CH_3)_2$ (*S*)	160	nd
e	*t*-BuO	NH	CH_2CH_2Ph (*S*)	$CH_2CH(CH_3)_2{}^b$	4000	nd
f	*t*-BuO	NH	CH_2CH_2Ph (*S*)	$CH_2CH(CH_3)_2{}^a$	1000	nd
g	*t*-BuO	NH	CH_2Ph (*S*)	$CH_2CH(CH_3)_2$ (*R*)	2700	nd
h	*t*-BuO	NH	CH_2Ph (*S*)	$CH_2CH(CH_3)_2$ (*S*)	340	nd
i	morpholino	NH	CH_2Ph (*S*)	$CH_3{}^a$	85	nd
j	morpholino	NH	CH_2Ph (*S*)	$CH_3{}^b$	1000	nd
k	morpholino	NH	CH_2Ph (*R*)	$CH_3{}^a$	1600	nd
l	morpholino	NH	CH_2Ph (*R*)	$CH_3{}^b$	1000	nd
m	morpholino	NH	CH_2Ph (*S*)	$CH_2(CH_2)_2CH_3$ (*S*)	13	1200
n	morpholino	NH	CH_2Ph (*S*)	$CH_2(CH_2)_2CH_3$ (*R*)	750	nd
o	morpholino	O	CH_2Ph (*S*)	CH_3 (*S*)	45	4300
p	morpholino	O	CH_2Ph (*S*)	$CH_2(CH_2)_2CH_3$ (*S*)	15	1000
q	ethylamino	O	CH_2Ph (*S*)	CH_3 (*S*)	130	nd
r	diethylamino	O	CH_2Ph (*S*)	CH_3 (*S*)	>1000	nd
s	azetidinyl	O	CH_2Ph (*S*)	CH_3 (*S*)	5.5	1000
t	pyrrolidinyl	O	CH_2Ph (*S*)	CH_3 (*S*)	12	3700
u	piperidinyl	O	CH_2Ph (*S*)	CH_3 (*S*)	25	8100
v	piperidinyl	O	CH_2Ph (*S*)	$CH_2(CH_2)_2CH_3$ (*S*)	24	nd

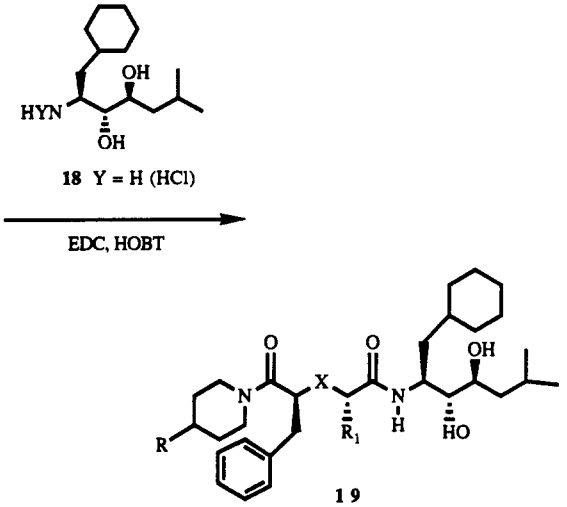
[a] Less polar diastereomer. [b] More polar diastereomer. [c] Prepared by reductive alkylation of **2a** with amine **18**, Y = H-Leu.

methyl analogue **19u**. This result was surprising, since inhibitors in the morpholino series (**19p** and **19m**) were three and six times more potent with a butyl group at P_2, than with a methyl group at the same position. The enhanced potency of inhibitors employing cyclic amides at the P_4 position suggested that groups at the N-terminus of the nonpeptide inhibitors bind to the S_4 site of the enzyme in a pocket suitable for a small hydrophobic ring. All of the inhibitors in Table I were 60–300-fold less potent in the plasma renin, pH 7.4 assay. The reason for this large difference in potency was unclear. To further probe the S_4 binding domain, a series of 4-substituted piperidine amides was synthesized and evaluated for enzyme activity.

IC_{50} values for a series of 4-substituted piperidine amide analogues are shown in Table II. Although this series of compounds is not inclusive, the data is representative. Inhibitors employing the hydroxy **19w**, allyloxy **19y**, and propyloxy **19z** substitutions were 5–10-fold less potent than the parent inhibitor **19v** and only the methoxy analogue **19x** was equivalent in potency. Since the IC_{50} values varied between 44–290 nM for compounds **19x–z**, it appeared that a lipophilic tail on the piperidine ring produced unfavorable interactions with the enzyme and was detrimental for binding renin. However, further modification of the hydrocarbon chain produced dramatic results. Placement of an oxygen atom at the C(3) position in the chain afforded inhibitor **19za** which was 16 times more potent than the propyloxy analogue **19z**. The C(3) and C(1) bis-oxygen analogue **19ze** was the most potent inhibitor in the series with an IC_{50} of 1 nM, and for the first time, potent inhibition of plasma renin at pH 7.4 was achieved (**19ze**, IC_{50} 13 nM). Additional SAR data demonstrated the importance of the 1,3-dioxa moiety for enzyme inhibition. Thus, increasing the distance between the two oxygen atoms in compound **19zg** (IC_{50} = 31 nM) reduced the potency 30-fold. Replacement of the C(3) oxygen with sulfur (**19zi**, IC_{50} = 130 nM), sulfoxide (**19zk**, IC_{50} = 770 nM), or sulfonyl (**19zl**, IC_{50} = 780 nM) reduced potency 100–700-fold. Finally, the all-carbon analogue **19zh** was 1000 times less potent than inhibitor **19ze**. Potent enzyme inhibition was recovered however, when the C(3) oxygen was returned to the chain, as demonstrated by inhibitor **19zj** (IC_{50} = 1.8 nM).

This data demonstrates that the nonpeptide inhibitors, with hydrophobic side chains at the 4 position of a (hydrophobic) piperidine amide at the N-terminus of the molecule, do not bind effectively with the enzyme. However, nonpeptide inhibitors that possessed 4-substituted hydrophilic side chains on the piperidine ring were very potent. We hypothesized that the increased potency (binding energy) was attributed to the oxygen atom(s) which hydrogen bonded to groups (possibly histidine or water) in the S_4 site. From the SAR data, the S_4 domain of the enzyme is most likely composed of a hydrophilic and hydrophobic region (Figure 3). Inhibitors with an oxygen atom at the C(3) position of the chain were more potent than inhibitors with the C(1) oxygen only (compare **19za** to **19z**). Thus, the C(3) oxygen atom formed a stronger hydrogen bond in the hydrophilic region. The most potent inhibitors had both oxygen atoms on the chain, one at C(3) and the other at C(1), in a 1,3 relationship. The increased potency observed for the bis-oxygen inhibitor **19ze** was due

Table II. Synthesis of Nonpeptide Renin Inhibitors, **19v–zl**, Employing a 4-Substituted Piperidine at the N-Terminus and Modifications at the P_2 Position and Their in Vitro Potency against Purified and Plasma Renin



19	R			IC_{50}, nM: purified, pH 6.0	IC_{50}, nM: plasma, pH 7.4
v	H	O	butyl	24	nd
w	HO[a]	O	butyl	130	nd
x	CH_3O	O	butyl	44	nd
y	CH_2=$CHCH_2O$	O	butyl	160	nd
z	$CH_3CH_2CH_2O$[b]	O	butyl	290	nd
za	$CH_3OCH_2CH_2$	O	butyl	18	1300
zb	CH_3OCH_2O	O	methyl	1.3	340
zc	CH_3OCH_2O[c]	O	ethyl	0.9	120
zd	CH_3OCH_2O[c]	O	propyl	1.4	31
ze	CH_3OCH_2O	O	butyl	1.0	13
zf	CH_3OCH_2O	NH	butyl	1.1	17
zg	$CH_3OCH_2CH_2O$[d]	NH	butyl	31	1800
zh	$CH_3CH_2CH_2CH_2$	O	butyl	1,100	nd
zi	CH_3SCH_2O	O	butyl	130	nd
zj	CH_3OCH_2S	O	butyl	1.8	120
zk	CH_3SOCH_2O[e]	O	butyl	770	nd
zl	$CH_3SO_2CH_2O$[e]	O	butyl	780	nd

[a] Prepared from **19ze**, see Experimental Section. [b] Prepared from **19y**, see Experimental Section. [c] Absolute stereochemistry tentatively assigned on the basis of in vitro potency. [d] Prepared from **4k**. [e] Prepared from **19zi**, see Experimental Section.

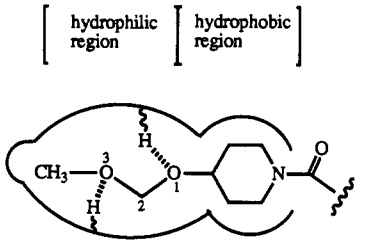
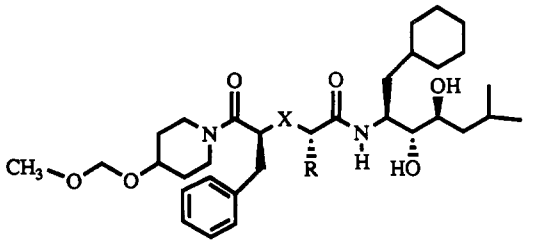


Figure 3. Interactions of the 4-(1,3-dioxabutyl)piperidine amide of a nonpeptide renin inhibitor with the hydrophilic and hydrophobic regions of the S_4 domain of renin.

to the following. Both oxygen atoms accept critical hydrogen bonds from the enzyme (S_4 hydrophilic region) and require precise positioning on the chain to achieve maximum interaction. The synergy produced by the hydrogen-bonding interactions of both oxygen atoms was responsible for the increased potency of the nonpeptide inhibitors.

The 4-(1,3-dioxabutyl)piperidine amide pharmacophore imparted nanomolar potency to nonpeptide renin inhibitors. We were interested in evaluating inhibitors employing this unique pharmacophore with other P_2 modifications. To gain additional insight into how the novel 4-(1,3-dioxabutyl)piperidine substituent affected renin inhibition, assays were performed using plasma renin at pH 6.0 and 7.4 and purified renin at pH 6.0 and 7.4 (Table III). These studies revealed that within the series of

Table III. Nonpeptide Renin Inhibitors, **19zb–zf**, Employing a 4-(1,3-Dioxabutyl)piperidine at the N-Terminus and Modifications at the P_2 Position and Their in Vitro Potency against Purified and Plasma Human Renin at pH 6.0 and 7.4



			IC_{50}, nM: purified		IC_{50}, nM: plasma	
19	X	R	pH 6.0	pH 7.4	pH 7.4	pH 6.0
zb	O	methyl	1.3	7.0	340	27
zc	O	ethyl	0.9	5.4	120	7.9
zd	O	propyl	1.4	5.5	31	3.3
ze	O	butyl	1.0	3.2	13	3
zf	NH	butyl	1.1	2.8	17	3.9

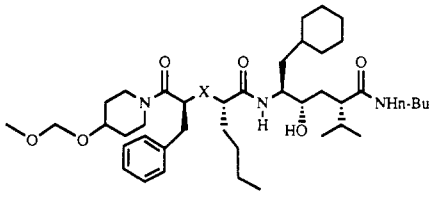
Table IV. Melting Point and Formula of Nonpeptide Renin Inhibitors **19a–zl**

19	mp, °C	formula[a]
a	107–10	$C_{29}H_{48}N_2O_5$
b	110–13	$C_{29}H_{48}N_2O_5$[b]
c	72–3	$C_{32}H_{54}N_2O_5$
d	108–110	$C_{32}H_{54}N_2O_5 \cdot {}^1/_4H_2O$
e	130–32	$C_{34}H_{58}N_2O_5$[b]
f	108–10	$C_{34}H_{58}N_2O_5$
g	100–02	$C_{33}H_{56}N_2O_5 \cdot {}^1/_4H_2O$
h	94–6	$C_{33}H_{56}N_2O_5 \cdot {}^1/_4H_2O$
i	65–8	$C_{30}H_{49}N_3O_5$
j	70–2	$C_{30}H_{49}N_3O_5$[b]
k	156–8	$C_{30}H_{49}N_3O_5 \cdot {}^1/_4H_2O$
l	127–9	$C_{30}H_{49}N_3O_5 \cdot {}^1/_4H_2O$
m	104–6	$C_{33}H_{55}N_3O_5$[b]
n	103–5	$C_{33}H_{55}N_3O_5$
o	82–5	$C_{30}H_{48}N_2O_6$
p	140–3	$C_{33}H_{54}N_2O_6 \cdot {}^1/_4H_2O$
q	145–7	$C_{28}H_{46}N_2O_5$
r	55–60	$C_{30}H_{50}N_2O_5$[b]
s	140–2	$C_{29}H_{46}N_2O_5$
t	50–3	$C_{30}H_{48}N_2O_5 \cdot {}^1/_4H_2O$
u	65–8	$C_{31}H_{50}N_2O_5$
v	52–5	$C_{34}H_{56}N_2O_5$[b]
w	82–4	$C_{34}H_{56}N_2O_6$[b]
x	55–8	$C_{35}H_{58}N_2O_6$[b]
y	*d*	$C_{37}H_{60}N_2O_6 \cdot H_2O$
z	88–92	$C_{37}H_{62}N_2O_6 \cdot H_2O$
za	84–91	$C_{37}H_{62}N_2O_6$
zb	55–60	$C_{33}H_{54}N_2O_7 \cdot {}^1/_2H_2O$
zc	97–100	$C_{34}H_{56}N_2O_7$[b]
zd	56–60	$C_{35}H_{58}N_2O_7 \cdot {}^1/_2H_2O$
ze	*d*	$C_{36}H_{60}N_2O_7 \cdot {}^1/_4H_2O$
zf	50–5	$C_{36}H_{61}N_3O_6$
zg	*d*	$C_{37}H_{63}N_3O_6$[b]
zh	50–3	$C_{38}H_{64}N_2O_5$[c]
zi	53–7	$C_{36}H_{60}N_2O_6S \cdot {}^1/_4H_2O$
zj	*d*	$C_{36}H_{60}N_2O_6S \cdot {}^1/_3H_2O$
zk	65–73	$C_{36}H_{60}N_2O_7S$[b]
zl	65–71	$C_{36}H_{60}N_2O_8S$

[a] Analyses for C, H, N were correct within ±0.4% unless otherwise noted. [b] High-resolution mass spectra (±5 ppm) were obtained. [c] Compound exhibited ^1H NMR and mass spectrum consistent with assigned structure. [d] Compound was obtained as a gummy solid.

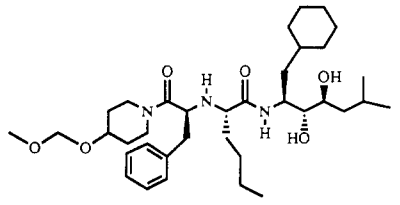
compounds **19zb–ze** (methyl to butyl), the inhibitory activity did not significantly change for either the purified, pH 6.0 or the pH 7.4 assays. However, between the two assays (purified, pH 6.0 and pH 7.4) there was a 3–5-fold decrease in potency. The results in the plasma renin assay were much different. At pH 7.4 a 3-fold increase in potency was observed for each inhibitor in the series methyl **19zb**, ethyl **19zc**, propyl **19zd**, and butyl **19ze**. The butyl analogue **19ze** (IC_{50} = 13 nM) was 26 times more potent than the methyl compound **19zb** (IC_{50} = 340 nM). At pH 6.0 in the plasma renin assay, the inhibitory activity increased from 4 to 15 times. Again, an increase in potency was observed within the series (**19zb**, 27 nM to **19ze**, 3.0 nM). There was no significant difference in potency between the nitrogen analogue **19zf** and the oxygen analogue **19ze**. The data suggests that inhibitors **19zb–zf** lost potency due to two effects. The primary effect on inhibitor potency was the increase in pH. The decrease of inhibitor potency at neutral pH reflects the inefficiency of renin at higher pH than its pH optimum of 5.5–6.0. The second effect concerned the influence of plasma or plasma-related artifacts (plasma binding proteins) on the inhibitors to decrease potency. There was a degree of synergism between the two effects.

With the glutaric acid moiety optimized for in vitro potency in the plasma renin (pH 7.4) assay, we further optimized the potency by preparing compounds with different transition-state mimics. The hydroxyethylene dipeptide isostere was selected for evaluation and coupled to glutaric acid moieties **8c** and **8r**. The potencies of inhibitors **20** and **21** were equivalent if not more potent than their glycol counterparts, **19ze** and **19zf**. They were the most potent nonpeptide inhibitors prepared. As a result, compounds **19zf**, **20**, and **21** were selected for further pharmacologic studies.



20 X = O
21 X = NH

X	IC_{50} (nM), human renin purified pH 6.0	plasma pH 7.4	Solubility (μg/mL) pH 7.4, 37 °C	Bioavailability monkey (%) id (10 mg/kg) iv (0.3 mg/kg)
O	1.2	8.2	0.8	2.5 ± 0.4
NH	0.9	8.7	0.74	7.1 ± 2.3



19zf

	0.9	17	nd	2.0 ± 0.8

In Vivo Activity of Nonpeptide Renin Inhibitors. Due to the primate-selective nature of renin inhibitors, in vivo studies were conducted in cynomologus monkeys. Compounds **19zf**, **20**, and **21** were administered intravenously to anesthetized salt-depleted monkeys at a standard dose of 0.3 mg/kg. Vehicle treated, time-control animals (n = 3, data not shown) demonstrated the hemodynamic stability of this preparation. Baseline mean arterial pressure (MAP) was 74 ± 8 mmHg (mean ± SE) versus 72 ± 5 mmHg at 180 min post-dosing. Baseline control MAP values for **21**, **19zf**, and **20** were 77 ± 16, 75 ± 11, and 83 ± 4 mmHg, respectively. The absolute fall in MAP is shown in Figure 4 for compounds **21**, **19zf**, and **20**. Each compound was studied in two monkeys. The results indicated that all three inhibitors reduced MAP and the peak falls in MAP were comparable. However, the duration of action was variable.

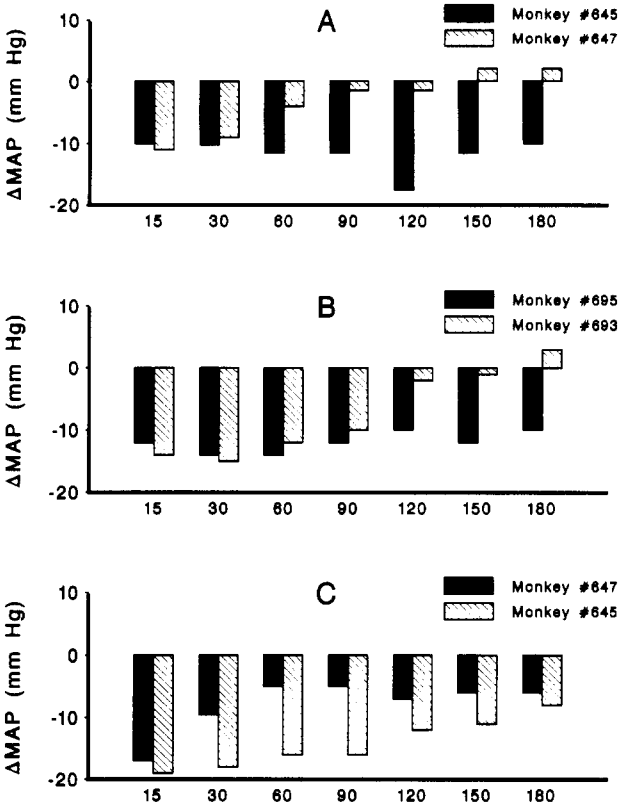


Figure 4. Mean arterial blood pressures in salt-depleted, anesthetized, cynomologus monkeys after iv (0.3 mg/kg) administration of (A) compound **21**, (B) compound **19zf**, and (C) compound **20**.

Intraduodenal (id) dosing of 1 and 10 mg/kg of inhibitor **21** in the same monkey model resulted in a dose-related reductions in the peak fall in MAP (% change from baseline) and prolonged duration of the hypotensive activity (Figure 5a,b). The hemodynamic response to the 10 mg/kg dose was more consistent than that observed with the 1 mg/kg dose. No remarkable effects on heart rate were noted. Plasma renin activity (PRA) was completely suppressed during the course of the experiments (data not shown).

Plasma Drug Concentrations of Nonpeptide Renin Inhibitors. Bioavailability studies were performed on early lead compounds to assess our progress toward identifying a bioavailable renin inhibitor. One inhibitor, **19i** (85 nM) which was prepared early in the study was selected for bioavailability screening. Plasma drug concentrations for compound **19i** is shown in Figure 6a. We were gratified to discover that after intraduodenal administration of a 10 mg/kg dose of **19i** to anesthetized cynomologus monkeys, a peak plasma drug level of 1 μg/mL was achieved after 2 h. Approximately 600 ng/mL of compound **19i** was circulating after 6 h. A 0.1 mg/kg dose of **19i** was administered intravenously and produced a peak drug concentration of 500 ng/mL after 5 min. A steady decline of plasma drug concentration to less than 100 ng/mL after

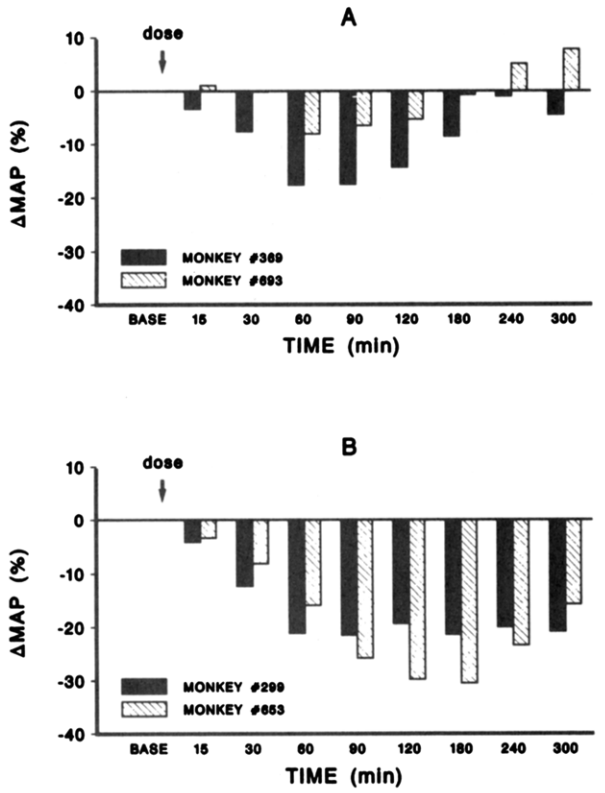


Figure 5. Mean arterial blood pressures in salt-depleted, anesthetized, cynomologus monkeys after id administration of inhibitor **21** at (A) 1 and (B) 10 mg/kg.

3 h was observed. Intraduodenal bioavailability for **19i** was calculated to be greater than 15%. This result encouraged us to evaluate other nonpeptide inhibitors for id bioavailability. The id absorption profile for the more efficacious inhibitors **20** (1.2 nM) and **21** (0.9 nM), was much different. Peak plasma blood levels for compounds **20** and **21** were 150 and 200 ng/mL, respectively and these levels were achieved 3–4 h post dosing (Figure 6b). The id absorption profile for **19zf** was similar to inhibitor **20**. The plasma drug concentration maximum for **20** and **21** after iv dosing was 4 and 1.5 μg/mL, respectively and, like compound **19i**, was achieved after 1–5 min (Figure 6c). Again, a rapid decline of plasma drug concentration to nanogram per milliliter levels over 3 h was observed. Bioavailability for **20**, **21**, and **19zf** was 2.5, 7.1, and 2.0%, respectively. Although there was considerable variability in absorption among the four compounds, they all attained a peak plasma drug concentration maximum between 2 to 3 h post id dosing.

Conclusion

We have developed a new series of renin inhibitors which incorporated a novel structural replacement for the P_2/P_3 amino acids, a (2*S*,4*S*)-3-aza(or oxa)-4-benzyl-2-butylglutaric acid amide moiety. Structure–activity relationship studies revealed that the compounds employing a 4-(1,3-dioxabutyl)piperidine amide at the N-terminus were the most potent inhibitors of purified renin at pH 6.0. In addition, maximum in vitro potency against plasma renin at pH 7.4 was achieved when both the 4-(1,3-dioxabutyl)piperidine amide and a butyl group at the P_2 position were present in the molecule. Analogues of the nonpeptide inhibitors were prepared in order to further improve plasma renin inhibition. As a result, analogues **19zf**, **20**, and **21** were identified as potent and efficacious nonpeptide renin inhibitors. Compound **21** showed a MAP reduction of 20 mmHg when administered id accompanied by a long

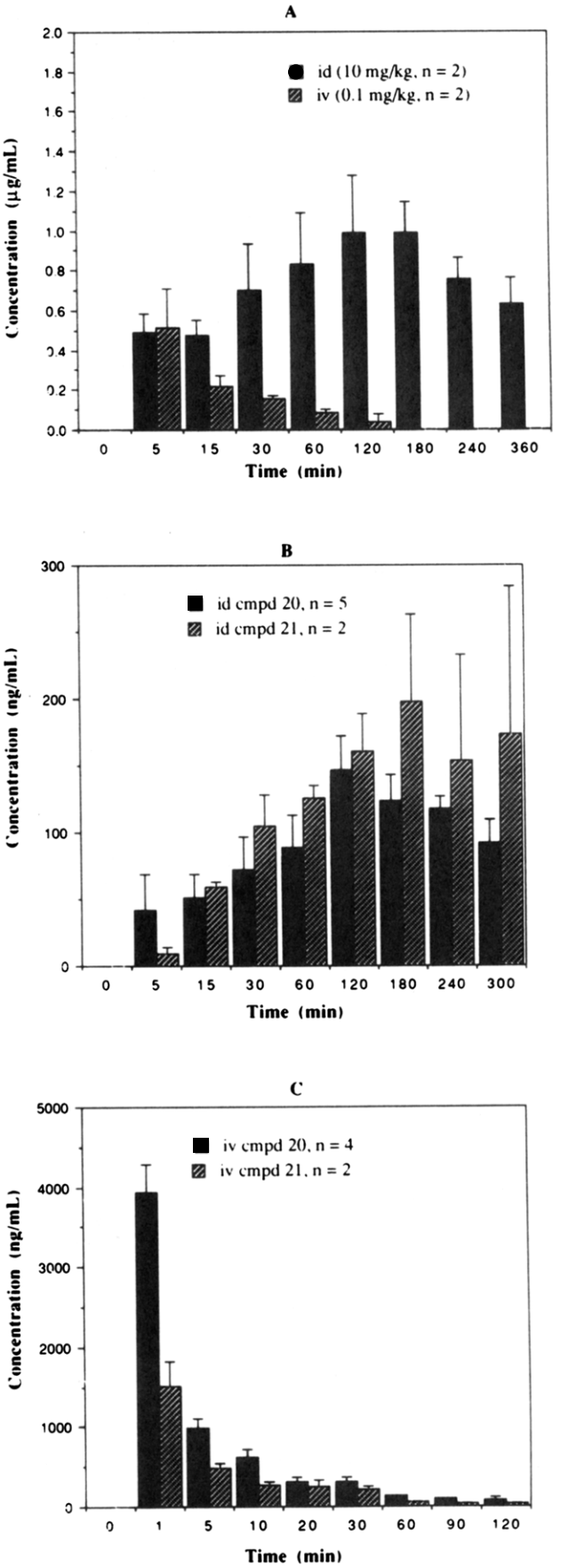


Figure 6. (A) Plasma drug concentrations of inhibitor **19i** after id (10 mg/kg) and iv (0.1 mg/kg) administration to salt-depleted monkeys (drug levels determined by HPLC) and plasma drug concentrations of inhibitors **20** and **21** after (B) id (10 mg/kg) and (C) iv (0.3 mg/kg) administration (drug levels determined by bioassay).

duration of action of greater than 300 min. The apparent long biological half-life of **21** and complete suppression of PRA may be attributed to the stabilization of the molecule by the heteroatom replcement of the P_2/P_3 amide bond. However, id bioavailability was low for compounds **20**, **21**, and **19zf**. Structure–activity studies directed toward improving the bioavailability of **20** and **21** by modifying the physicochemical properties of these nonpeptide inhibitors is reported in the following paper.

Experimental Section

Reagents were used without further purification. (2*R*)-Ethyl 2-bromohexanoate, (2*R*)-2-bromohexanoic acid,[19] and (2*R*)-2-bromopropionic acid[20] were prepared by literature methods. Tetrahydrofuran was freshly distilled (over sodium benzophenone ketyl). All other solvents were used without further purification unless otherwise noted. Solvent evaporations were performed at or below 40 °C using a Buchi rotary evaporator. Reactions were conducted under a positive pressure of dry nitrogen. Inhibitors were dried at 30–40 °C under vacuum.

Proton magnetic resonance spectra were measured on a Nicolet QE-300 (300 MHz). Chemical shifts are reported as values (parts per million) relative tetramethylsilane (TMS) as an internal standard. Mass spectra and elemental analyses were performed by the Analytical Chemistry Department of Abbott Laboratories. Thin-layer chromatography was performed on Merck precoated plate (silica gel 60, F 254). Column chromatography used 70–230 mesh silica gel 60 and for flash chromatography, 230–400 mesh grade. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected.

General Procedure for the Reductive Alkylation of α-Keto Esters and Amines. (2*S*,4*R* and *S*)-Benzyl 3-Aza-4-(ethoxycarbonyl)-2-methyl-6-phenylhexanoate (3a, Less Polar Diastereomer, and 3b, More Polar Diastereomer). A solution of L-alanine benzyl ester hydrochloride (431 mg, 2.0 mmol), ethyl 2-oxo-4-phenylbutyrate (494 mg, 2.4 mmol), and 328 mg (4 mmol) of anhydrous NaOAc in 50 mL of absolute ethanol was cooled and stirred in an ice–water bath while 138 mg (2.4 mmol) of $NaCNBH_3$ in 5 mL of EtOH was added portionwise. The reaction was stirred at ice–water bath temperature for 1 h and then warmed to room temperature over 15 h. The reaction mixture was filtered, the filtrate was concentrated under reduced pressure, and the residue was redissolved in chloroform, washed with 5% $NaHCO_3$, water, and saturated NaCl. The chloroform solution was dried ($MgSO_4$), filtered, and evaporated to give the crude amine. Purification of the amine by silica gel chromatography (1:9, ethyl acetate/hexane) gave 69.9 mg of **3a** (less polar isomer, 18%) and 88.7 mg of **3b** (more polar isomer, 20%). **3a**: ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3 H), 1.32 (d, 3 H), 1.94 (m, 2 H), 2.37 (bs, 1 H), 2.71 (t, 2 H), 3.27 (bt, 1 H), 3.38 (q, 1 H), 4.12 (m, 2 H), 5.13 (dd, 2 H); MS *m/e* 370 $(M + H)^+$. **3b**: ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3 H), 1.35 (d, 3 H), 1.93 (m, 2 H), 2.71 (m, 2 H), 3.35 (dd, 1 H), 3.43 (q, 1 H), 4.15 (m, 2 H), 5.15 (dd, 2 H); MS *m/e* 370 $(M + H)^+$.

(2*S* and *R*,4*S*)-Benzyl 3-Aza-4-(*tert*-butoxycarbonyl)-2-isobutyl-6-phenylhexanoate (3c, Less Polar Diastereomer, and 3d, More Polar Diastereomer). Chromatography (15:85, ethyl acetate/hexane) gave **3c** (less polar isomer, 14%) and **3d** (more polar isomer, 12%). **3c**: ^{1}H NMR ($CDCl_3$) δ 0.9 (dd, 6 H), 1.45 (s, 9 H), 1.8 (dd, 1 H), 1.9 (m, 2 H), 2.69 (m, 2 H), 3.25 (t, 1 H), 3.39 (t, 1 H), 5.11 (d, 2 H), 7.27 (m, 5 H); MS *m/e* 440 $(M + H)^+$. **3d**: ^{1}H NMR ($CDCl_3$) δ 0.89 (d, 3 H), 0.92 (d, 3 H), 1.46 (s, 9 H), 2.64 (m, 2 H), 3.15 (t, 1 H), 3.36 (dd, 1 H), 7.26 (m, 5 H); MS *m/e* 440 $(M + H)^+$.

(2*R*,4*S*)-Benzyl 3-Aza-4-(*tert*-butoxycarbonyl)-2-isobutyl-5-phenylpentanoate (3e) and (2*S*,4*S*) Diastereomer 3f. Chromatography (15:85, ethyl acetate/hexane) gave **3e** (less polar isomer, 13%) and **3f** (more polar isomer, 13%). **3e**: ^{1}H NMR ($CDCl_3$) δ 0.8 (dd, 6 H), 1.33 (s, 9 H), 1.43 (bt, 2 H), 1.62 (m, 1 H), 2.87 (bt, 2 H), 3.22 (bt, 1 H), 3.37 (bt, 1 H), 5.1 (s, 2 H); MS *m/e* 426 $(M + H)^+$. **3f**: ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 6 H), 1.33 (s, 9 H), 1.46 (d, 2 H), 1.67 (m, 1 H), 2.89 (bt, 2 H), 3.37 (bt, 1 H), 3.41 (bt, 1 H), 5.11 (d, 2 H); MS *m/e* 426 $(M + H)^+$.

(2*S* and *R*,4*S*)-Benzyl 3-Aza-4-(*tert*-butoxycarbonyl)-2-methyl-5-phenylpentanoate (3g, Less Polar Diastereomer, and 3h, More Polar Diastereomer). Chromatography (1:9, ethyl acetate/hexane) gave **3g** (less polar epimer, 32%) and **3h** (more polar epimer, 20%). **3g**: ^{1}H NMR ($CDCl_3$) δ 1.27 (d, 3 H), 1.33 (s, 9 H), 2.91 (dd, 2 H), 3.35 (dd, 1 H), 3.45 (bt, 1 H), 5.12 (d, 2 H); MS *m/e* 384 $(M + H)^+$. **3h**: ^{1}H NMR ($CDCl_3$) d 1.3 (d, 3 H), 1.34 (s, 9 H), 2.91 (d, 2 H), 3.44 (dd, 1 H), 3.42 (bt, 2 H), 5.12 (s, 2 H); MS *m/e* 384 $(M + H)^+$.

(2*S* and *R*,4*R*)-Benzyl 3-Aza-4-(*tert*-butoxycarbonyl)-2-methyl-5-phenylpentanoate (3i, Less Polar Diastereomer, and 3j, More Polar Diastereomer). Chromatography (1:4, ethyl acetate/hexane) gave **3i** (less polar epimer, 21%) and **3j** (more polar epimer, 11%). **3i**: ^{1}H NMR ($CDCl_3$) δ 1.27 (d, 3 H), 1.32 (s, 9 H), 2.91 (dd, 2 H), 3.34 (dd, 1 H), 3.45 (t, 1 H), 5.12 (d, 2 H); MS *m/e* 384 $(M + H)^+$. **3j**: ^{1}H NMR ($CDCl_3$) δ 1.3 (d, 3 H), 1.33 (s, 9 H), 2.91 (d, 2 H), 5.12 (s, 2 H); MS *m/e* 384 $(M + H)^+$.

(2*S*,4*S*)-Benzyl 3-Aza-4-(*tert*-butoxycarbonyl)-2-butyl-5-phenylpentanoate (3k) and (2*R*,4*S*) Diastereomer 3l. Chromatography (1:9, ethyl acetate/hexane) gave **3k** (more polar isomer, 9%) and **3l** (less polar isomer, 16%). **3k**: ^{1}H NMR ($CDCl_3$) δ 0.85 (m, 3 H), 1.33 (s, 9 H), 2.9 (d, 2 H), 3.31 (t, 1 H), 3.41 (t, 1 H), 5.11 (s, 2 H); MS *m/e* 426 $(M + H)^+$. **3l**: ^{1}H NMR ($CDCl_3$) δ 0.80 (bt, 3 H), 1.18 (m, 2 H), 1.34 (s, 9 H), 2.89 (bt, 2 H), 3.19 (t, 1 H), 3.37 (t, 1 H), 5.12 (d, 2 H); MS *m/e* 426 $(M + H)^+$.

(2*S* and *R*,4*S*)-3-Aza-4-(ethoxycarbonyl)-2-isobutyl-6-phenylhexanamide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (19c, Less Polar Diastereomer, and 19d, More Polar Diastereomer). Chromatography (3:7, ethyl acetate/hexane) gave **19c** (less polar epimer, 7%) and **19d** (more polar isomer, 13%). **19c**: ^{1}H NMR ($CDCl_3$) δ 0.76 (d, 3 H), 0.84 (m, 9 H), 1.1–2.0 (m, 21 H), 1.15 (t, 3 H), 2.65 (m, 2 H), 2.95 (m, 1 H), 3.1 (m, 1 H), 3.17 (bt, 1 H), 4.06 (q, 2 H), 4.1 (m, 2 H), 7.2 (m, 5 H); MS *m/e* 547 $(M + H)^+$. **19d**: ^{1}H NMR ($CDCl_3$) δ 0.90 (d, 3 H), 0.95 (m, 9 H), 1.28 (t, 3 H), 1.1–2.05 (m, 21 H), 2.73 (m, 2 H), 3.15 (m, 2 H), 3.2 (bd, 1 H), 4.16 (q, 2 H), 4.32 (m, 1 H), 4.49 (bs, 1 H), 7.2 (m, 5 H); MS *m/e* 547 $(M + H)^+$.

(2*S*,4*S*)-Benzyl 3-Aza-4-(*tert*-butoxycarbonyl)-2-isobutyl-5-phenylpentanoate (8a, More Polar Diastereomer). Triethylamine (TEA, 0.14 mL, 0.94 mmol) was added to the cooled suspension of L-phenylalanine *tert*-butyl ester hydrochloride (**1c**, 0.243 g, 0.94 mmol) in 3 mL of methylene chloride at 0 °C, and the mixture was stirred at 0–5 °C for 0.5 h. The mixture was added to a solution of the trifluoromethanesulfonate of (2*R*)-benzyl-4-methyl-2-hydroxypentanoate (0.288 g, 0.81 mmol) and TEA (0.12 mL, 0.82 mmol) in 2 mL of methylene chloride, and the mixture was stirred at 15 °C for 2 h, warmed to room temperature, and stirred for 1 h. The clear liquid was allowed to stand in a refrigerator for 18 h and concentrated. The clear liquid was dissolved in EtOAc and washed with water, dried ($MgSO_4$), and filtered. The filtrate was evaporated to an oil which was purified by chromatography (1:9, ether/hexane) to yield amine **8a** in 25% yield. The physical data (TLC, ^{1}H NMR, and mass spectrum) for the amine prepared by this method was identical to the more polar isomer **3f** prepared in the previous experiment.

General Procedure for the Coupling of Amines to the Glutaric Acid Intermediates. (2*S* or *R*,4*S*)-Benzyl 3-Aza-4-(morpholinocarbonyl)-2-methyl-5-phenylpentanoate (4m). A solution of *tert*-butyl ester **3g** (770 mg, 2.00 mmol) in 7 mL of 4 M HCl/dioxane was stirred for 16 h. The solvent was evaporated under reduced pressure to give 600 mg (82% crude yield) of the carboxylic acid. A 400-mg portion (0.95 mmol assuming 100% pure) of the crude acid, morpholine (82.7 mg, 0.95 mmol), and 1-hydroxybenztriazole hydrate (HOBT, 385 mg, 2.85 mmol), in 5 mL of DMF were cooled to −23 °C; 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDC, 210.9 mg, 1 mmol) was added, and the reaction was stirred to room temperature over 18 h. Ethyl acetate was added, and the organic solution was washed with dilute $NaHCO_3$, water, saturated NaCl, and dried ($MgSO_4$). The filtered organic solution was concentrated and the crude amide purified by chromatography using 6:4 ethyl

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acetate/hexane. The amide **4m** was obtained in 70% yield: ^{1}H NMR ($CDCl_3$) δ 1.35 (d, 3 H), 2.5 (m, 2 H), 2.8 (m, 2 H), 3.1 (m, 1 H), 3.3 (m, 1 H), 3.5 (m, 2 H), 3.8 (q, 1 H), 5.2 (s, 2 H), 7.25 (m, 5 H). (2*S* or *R*,4*S*)-benzyl 3-aza-4-(morpholinocarbonyl)-2-methyl-5-phenylpentanoate (**4n**) was obtained in 59% yield; (2*S* or *R*,4*R*)-benzyl 3-aza-4-(morpholinocarbonyl)-2-methyl-5-phenylpentanoate (**4o**), 32% yield; (2*S* or *R*,4*R*)-benzyl 3-aza-4-(morpholinocarbonyl)-2-methyl-5-phenylpentanoate (**4p**), 47% yield; (2*S*,4*S*)-benzyl-3-aza-4-(morpholinocarbonyl)-2-butyl-5-phenylpentanoate (**4q**), 73% yield; (2*R*,4*S*)-benzyl-3-aza-4-(morpholinocarbonyl)-2-butyl-5-phenylpentanoate (**4r**), 65% yield.

General Procedure for the Coupling of the Carboxylic Acids 4a–f and 5m–r to the Amino Diol 18. (2*S*,4*R* or *S*)-3-Aza-4-(ethoxycarbonyl)-2-methyl-6-phenylhexanamide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (19a, More Polar Isomer). Benzyl ester **3a** (less polar isomer, 69 mg, 0.19 mmol) in 10 mL of methanol and 35 mg of 10% Pd/C were stirred under an atmosphere of hydrogen for 2 h. After filtration of the catalyst through Celite, the filtrate was concentrated to give 44 mg of the crude acid **4a**. The acid (40 mg, 0.14 mmol), 2(*S*)-amino-1-cyclohexyl-3(*R*),4(*S*)-dihydroxy-6-methylheptane hydrochloride (**18**, 40 mg, 0.14 mmol), HOBT (56.8 mg, 0.42 mmol), and *N*-methylmorpholine (NMM, 17.2 mg, 0.17 mmol) in 2 mL of DMF were cooled to −23 °C. EDC (32.6 mg, 0.17 mmol) was added and the reaction stirred to room temperature over 18 h. Ethyl acetate was added, and the organic solution was washed with dilute $NaHCO_3$, water, saturated NaCl, and dried ($MgSO_4$). The filtered organic solution was concentrated and the crude amide purified by chromatography using 35:65 ethyl acetate/hexane gave 56.7 mg of compound **19a** in 66% overall yield: ^{1}H NMR ($CDCl_3$) δ 0.9 (dd, 6 H), 1.1.27 (t, 3 H), 1.29 (d, 3 H), 2.3 (bd, 1 H), 2.69 (bt, 2 H), 3.12 (m, 3 H), 3.34 (t, 1 H), 4.15 (m, 2 H), 4.3 (m, 1 H), 4.63 (bd, 1 H), 7.67 (bd, 1 H); MS *m*/*e* 505 $(M + H)^+$. (2*S*,4*R* or *S*)-3-Aza-4-(ethoxycarbonyl)-2-methyl-6-phenylhexanamide of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (**19b**, more polar isomer was obtained in 30% yield; (2*S* or *R*,4*S*)-3-aza-4-(*tert*-butoxycarbonyl)-2-isobutyl-6-phenylhexanamide of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (**19e**), 45% yield; (2*S* or *R*,4*S*)-3-aza-4-(*tert*-butoxycarbonyl)-2-isobutyl-6-phenylhexanamide of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (**19f**), 54% yield; (2*R*,4*S*)-3-aza-4-(*tert*-butoxycarbonyl)-2-isobutyl-5-phenylpentanamide of (24S,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (**19g**), 28% yield; (2*S*,4*S*)-3-aza-4-(*tert*-butoxycarbonyl)-2-isobutyl-5-phenylpentanamide of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (**19h**), 18% yield; (2*S* or *R*,4*S*)-3-aza-4-(morpholinocarbonyl)-2-methyl-5-phenylpentanamide of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (**19i**), 53% yield; (2*S* or *R*,4*S*)-3-aza-4-(morpholinocarbonyl)-2-methyl-5-phenylpentanamide of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (**19j**), 54% yield; (2*S* or *R*,4*R*)-3-aza-4-(morpholinocarbonyl)-2-methyl-5-phenylpentanamide of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (**19k**), 56% yield; (2*S* or *R*,4*R*)-3-aza-4-(morpholinocarbonyl)-2-methyl-5-phenylpentanamide of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (**19l**), 46% yield; (2*S*,4*S*)-3-aza-4-(morpholinocarbonyl)-2-butyl-5-phenylpentanamide of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (**19m**), 48% yield; (2*R*,4*S*)-3-aza-4-(morpholinocarbonyl)-2-butyl-5-phenylpentanamide of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (**19n**), 44% yield.

***N*-Formyl-4-(1,3-dioxabutyl)piperidine (12c).** A solution of 4-hydroxypiperidine (**9**, 200 g, 1.98 mol) and methyl formate (160 mL, 2.59 mol) was stirred at ice–water bath temperature for 30 min and then at room temperature for 16 h. Excess methyl formate and methanol were removed under vacuum. After 18 h at 0.5 mmHg, the crude formamide **10** was obtained as a yellow oil and used in the next step without purification.

A solution of the crude formamide in 1 L of CH_2Cl_2 and 700 mL of diisopropylethylamine (DIEA) was cooled in an ice–water bath. Chloromethyl methyl ether (MOMCl, 200 g, 2.48 mol) was added dropwise and the reaction stirred to room temperature over 8 h. Thin-layer chromatography (TLC) analysis of the reaction mixture indicated the presence of unreacted starting material. An additional 250-mL portion of diisopropylethylamine and chloromethyl methyl ether (100 g, 1.24 mol) was added. The reaction mixture was stirred to room temperature over 18 h. Saturated $NaHCO_3$ was added (2 L) and the CH_2Cl_2 layer separated. The aqueous layer was extracted once with CH_2Cl_2. The CH_2Cl_2 solutions were combined, dried ($MgSO_4$), and evaporated at 65 °C. The residue was submitted to high vacuum (0.5 mmHg) for 1 h, affording 290 g (84%) of crude ether. A sample was purified by chromatography using 2:98 $CH_3OH/CHCl_3$ as eluent to give the pure ether **12c** as an oil: ^{1}H NMR ($CDCl_3$) δ 1.54–1.70 (bm, 2 H), 1.80–1.90 (bm, 2 H), 3.18–3.38 (bm, 2 H), 3.52–3.62 (m, 1 H), 3.40 (s, 3 H), 3.78–3.90 (bm, 2 H), 4.70 (s, 2 H), 8.02 (s, 1 H); MS *m*/*e* 174 $(M + H)^+$.

***N*-Formyl-4-(2-propenoxy)piperidine (12b).** Crude *N*-formyl-4-hydroxypiperidine (**10**) (95.4 mmol, 12.3 g) and allyl bromide (113 mmol, 9.78 mL) were dissolved in 140 mL of anhydrous tetrahydrofuran (THF) and cooled to −60 °C. Sodium hydride (60% dispersion, 105 mmol, 4.2 g) was added, and the reaction was warmed slowly to room temperature. TLC (1:9, CH_3OH/CH_2Cl_2) analysis after 1.5 h indicated incomplete reaction. The reaction was then recooled to −60 °C and additional allyl bromide (34.9 mmol, 3.02 mL) and sodium hydride (17.5 mmol, 700 mg) were added. The mixture was then allowed to warm gradually overnight to room temperature. TLC showed only a trace of starting material present. The THF solution was concentrated to give 12.0 g of crude ether. Chromatography (2:98, CH_3OH/CH_2Cl_2) gave 10.1 g (69%) of a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.63 (m, 3 H), 1.86 (m, 2 H), 3.18 (m, 1 H), 3.35 (m, 1 H), 3.52–3.67 (m, 2 H), 3.77 (m, 1 H), 4.02 (m, 2 H), 5.19 (dd, 1 H, J = 10.5, 1 Hz), 5.29 (dd, J = 3 Hz, 1 H), 5.94 (m, 1 H), 8.03 (s, 1 H); MS *m*/*e* 170 $(M + H)^+$.

***N*-Formyl-4-(1-oxa-3-thiabutyl)piperidine (12e).** To a cooled (0 °C) solution of *N*-formyl-4-hydroxypiperidine (**10**, 3 g, 23.2 mmol) in 92 mL of acetonitrile was added methyl sulfide (8 equiv, 13.7 mL). Benzoyl peroxide (4 equiv, 22.5 g) was added to the solution portionwise over 20 min. After stirring at 0 °C for 4 h, the solution was mixed with ether and the organic solution was washed with saturated $NaHCO_3$ and saturated NaCl, dried (Na_2SO_4), filtered, and evaporated to give 20 g of a crude residue. The residue was chromatographed using 5:95 CH_3OH/CH_2Cl_2 as eluent to give 3.49 g (80%) of an oil. The oil was dissolved in ethyl acetate, washed with saturated $NaHCO_3$ and 1 M NaOH, dried, and evaporated to give 0.924 g (21%) of the sulfide **12e**: ^{1}H NMR ($CDCl_3$) δ 1.62 (m, 2 H), 1.82 (m, 2 H), 2.18 (s, 3 H), 3.23 (m, 1 H), 3.35 (m, 1 H), 3.56 (m, 1 H), 3.78 (m, 1 H), 3.98 (m, 1 H), 4.69 (s, 1 H), 8.03 (s, 1 H); MS *m*/*e* 190 $(M + H)^+$.

***N*-Formyl-4-(3-oxa-1-thiabutyl)piperidine (12d).** A solution of crude *N*-formyl-4-hydroxypiperidine (**10**, 3 g, 23.2 mmol) and triphenylphosphine (7.30 g, 27.8 mmol) in 23 mL THF was cooled to −78 °C while 588 mg of diethyl azodicarboxylate (DEAD) in 12.5 mL of THF was added (10 min). Thioacetic acid (2.16 mL, 30.2 mmol) dissolved in 12 mL of THF was added over a period of 10 min. After 1 h at −78 °C, the reaction mixture was warmed to room temperature and stirred for 18 h. The THF was evaporated to give 16 g of a yellow solid which was redissolved in 1:1 ethyl acetate/hexane and filtered. The filtrate was concentrated to a yellow oil and purified by chromatography (2:98, CH_3OH/CH_2Cl_2) to give 588 mg of the thioacetate: yield 14%; ^{1}H NMR (DMSO-d_6) δ 1.21 (m, 3 H), 1.8–1.93 (m, 2 H), 2.32 (major) and 2.39 (minor) (s, 3 H), 2.93–3.01 (m, 1 H), 3.5–3.7 (m, 3 H), 3.82 (m, 1 H), 4.08 (m, 1 H), 4.2 (m, 1 H); MS *m*/*e* 188 $(M + H)^+$.

Crude thioacetate (1.50 g, 8.02 mmol) was dissolved in 30 mL of THF and cooled to 5 °C. LiOH (370 mg, 8.81 mmol) in cold THF was added, and after 75 min, an additional 370 mg of LiOH was added to the mixture. The reaction mixture was treated with 1 M H_3PO_4 after 15 min (pH of 6.0) and the THF was evaporated. The residue was extracted with ethyl acetate, washed with aqueous NaCl, dried (Na_2SO_4), and evaporated. The crude compound **11** was dissolved in 21 mL of CH_2Cl_2 and cooled to 5 °C. *N*,*N*-Diisopropylethylamine (2.10 mL, 16 mmol) and chloromethyl methyl ether (0.688 mL, 12 mmol) were added, and the reaction mixture was warmed to room temperature overnight, washed with saturated $NaHCO_3$ and saturated NaCl, dried (Na_2SO_4), and evaporated to give 1.5 g of an oil. Purification by chromatography (75:25, ethyl acetate/hexanes) gave 568 mg of product: yield 38%; ^{1}H NMR ($CDCl_3$) δ 1.5–1.67 (m, 2 H), 2.04 (td, 2 H, J = 4.5, 13.5

Hz), 2.95–3.21 (m, 2 H), 3.37 (s, 3 H), 3.61 (dt, 1 H, J = 3, 13.5 Hz), 4.13 (dt, 1 H, J = 4.5, 12.5 Hz), 4.7 (s, 2 H), 8.01 (s, 1 H); MS m/e 190 $(M + H)^+$.

***N*-Formyl-4-(1,4-dioxapentyl)piperidine (12f).** Compound **10** (0.5 g, 3.88 mmol) in 6 mL of THF was cooled to 0 °C, NaH (60% dispersion, 0.16 g, 3.88 mmol) was added, and the mixture was stirred at room temperature for 2 h. The mixture was recooled to 0 °C, and methoxyethyl bromide (0.539 g, 3.88 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 16 h, quenched cautiously with cold water, and extracted with ethyl acetate, dried over sodium sulfate, and filtered. The filtrate was evaporated to an oily residue which was chromatographed eluting with 2:98 $CH_3OH/CHCl_3$ to give the desired product (**12f**) as an oil (380 mg, 53% yield): ^1H NMR ($CDCl_3$) δ 1.50–1.70 (bm, 2 H), 1.82–1.92 (bm, 2 H), 3.10–3.32 (bm, 2 H), 3.40 (s, 3 H), 3.55 (m, 2 H), 3.65 (m, 4 H), 3.80 (m, 1 H), 8.20 (s, 1 H); MS m/e 187 $(M + H)^+$.

***N*-Formyl-4-(2-methoxyethyl)piperidine (16).** 4-(2-Hydroxyethyl)piperidine (**14**, 5.49 g, 42.5 mmol) and methyl formate (3.41 mL, 5.53 mmol) were mixed at 0–5 °C, warmed to room temperature over 1 h, and poured into CH_2Cl_2. The solution was dried (Na_2SO_4), filtered, and evaporated to give 6.37 g product (**15**) in 95% yield: ^1H NMR (DMSO-d_6) δ 0.95 (m, 2 H), 1.36 (m, 2 H), 1.68 (m, 3 H), 2.58 (dd, 1 H, J = 13.5, 3 Hz), 2.98 (td, 1 H, J = 12, 3 Hz), 3.44 (m, 2 H), 3.63 (sp d, 1 H, J = 16 Hz), 4.13 (sp d, 1 H, J = 16 Hz), 4.39 (t, 1 H, J = 4.5 Hz), 7.96 (s, 1 H); MS m/e 158 $(M + H)^+$.

The crude *N*-formyl-4-(2-hydroxyethyl)piperidine (**15**, 6.0 g, 38.2 mmol) in 57 mL of THF was cooled to −60 °C. Methyl iodide (2.37 mL, 50 mmol) and NaH (60% dispersion, 1.83 g, 46 mmol) was added. The mixture was warmed to room temperature over 18 h, quenched with water, and concentrated under reduced pressure, and the residue was redissolved in CH_2Cl_2, dried (Na_2SO_4), and filtered. The filtrate was concentrated to give 12 g of crude ether which was chromatographed (2:98, MeOH/CH_2Cl_2) affording 4.86 g (74%) of **16**: ^1H NMR (DMSO-d_6) δ 0.95 (m, 2 H), 1.44 (m, 2 H), 1.65 (m, 1 H), 2.58 (dd, 1 H, J = 12, 3 Hz), 2.98 (td, 1 H, J = 12, 3 Hz), 3.21 (s, 3 H), 3.63 (sp d, 1 H, J = 12 Hz), 4.12 (sp d, 1 H, J = 12 Hz), 7.96 (s, 1 H); MS m/e 172 $(M + H)^+$.

General Procedure for the Hydrolysis of the *N*-Formylpiperidines. 4-(1,3-Dioxabutyl)piperidine (13c). A heterogenous mixture of the crude MOM ether **12c** (290 g, 1.6 mol) and 300 g of KOH in 1.5 L of water was stirred rapidly at room temperature for 24 h. The aqueous suspension was extracted four times with diethyl ether, dried ($MgSO_4$), and concentrated under reduced pressure. The amine product was purified by short-path distillation to give a water white liquid **13c**: yield 190 g, 78% (66% from 4-hydroxypiperidine); bp 68–70 °C (1 mmHg); ^1H NMR ($CDCl_3$) δ 1.47 (m, 2 H), 1.90 (m, 2 H), 2.65 (m, 2 H), 3.10 (m, 2 H), 3.37 (s, 3 H), 3.64 (m, 1 H), 4.70 (s, 2 H); MS m/e 146 $(M + H)^+$. 4-(2-Propenoxy)piperidine (**13b**), from crude *N*-formylpiperidine **12b**, was obtained in 99% overall yield; 4-(1-oxa-3-thiabutyl)piperidine (**13e**), from crude *N*-formylpiperidine **12e**, was obtained in 81% overall yield; 4-(3-oxa-1-thiabutyl)-piperidine (**13d**), from crude *N*-formylpiperidine **12d**, was obtained in 75% overall yield; 4-(1,4-dioxapentyl)piperidine (**13f**), from crude *N*-formylpiperidine **12f**, was obtained in 23% overall yield; 4-(2-methoxyethyl)piperidine (**17**), from crude *N*-formylpiperidine **16**, was obtained in 75% overall yield.

General Procedure for the Coupling of 4-Substituted Piperidines and Amines to *N*-Cbz-L-Phenylalanine and L-Phenyllactic Acid. 4-(1,3-Dioxabutyl)piperidine Amide of L-Phenylalanine (6a). 4-(1,3-Dioxabutyl)piperidine (**13c**, 50.0 g, 0.344 mol), *N*-Cbz-L-phenylalanine (113 g, 0.379 mol), and HOBT (106 g, 0.690 mol) were dissolved in 300 mL of DMF and cooled to −20 °C. EDC (86 g, 0.448 mol) in 300 mL of DMF was added. After the mixture was warmed to room temperature overnight, DMF was removed under reduced pressure at 35 °C. The crude product was partitioned between EtOAc and 10% citric acid. The organic phase was washed with 10% citric acid, 5% $NaHCO_3$, and saturated NaCl, dried ($MgSO_4$), and evaporated to give 132 g of crude amide (90%): ^1H NMR ($CDCl_3$) δ 1.01 (m, 1 H), 1.18 (m, 1 H), 1.42 (m, 1 H), 1.65 (m, 1 H), 2.99 (m, 2 H), 3.13 (m, 1 H), 3.31 (m, 2 H), 3.33 (s, 3 H), 3.64 (m, 1 H), 3.81 (m, 1 H), 4.62 (s, 2 H), 4.91 (m, 1 H), 5.09 (s, 2 H), 5.71 (t, 1 H, J = 9 Hz), 7.29 (m, 10 H); MS m/e 427 $(M + H)^+$.

The crude amide (122 g, 0.286 mol) was hydrogenated under 4 atm of H_2 in 2 L of CH_3OH using 24.5 g of 20% Pd/C (48 h) followed by another 25 g of 10% Pd/C (16 h) to give upon filtration of the catalyst and evaporation of the filtrate 75.4 g of crude amine **6a** (90%): ^1H NMR ($CDCl_3$) δ 1.08 (m, 1 H), 1.27 (m, 1 H), 1.45 (m, 1 H), 1.70 (m, 1 H), 3.07 (dd, 2 H), 3.2 (m, 2 H), 3.35 (m, 2 H), 3.35 (s, 3 H), 4.67 (s, 2 H), 7.29 (m, 5 H); MS m/e 293 $(M + H)^+$.

4-(1,3-Dioxabutyl)piperidine Amide of L-Phenyllactic Acid (6b). Using the general coupling procedure described above and L-3-phenyllactic acid (80 g, 0.48 mol), HOBT (176 g, 1.3 mol), amine **13c** (76 g, 0.52 mol), DMF (800 mL), and EDC (132 g, 0.68 mol) gave the crude amide. The product was isolated by chromatography (60:40, ethyl acetate/hexane): yield 120 g (79%); ^1H NMR ($CDCl_3$) δ 1.61 (m, 2 H), 1.81 (m, 2 H), 2.89 (m, 2 H), 3.38 (s, 3 H), 3.5 (m, 2 H), 3.79 (m, 2 H), 3.96 (m, 1 H), 4.62 (t, 1 H), 4.68 (s, 2 H); MS m/e 294 $(M + H)^+$. Piperidine amide of L-phenyllactic acid (**6g**) was obtained in yield of 1.17 g (84%) (MS m/e 234 $(M + H)^+$); 4-butylpiperidine amide of L-phenyllactic acid (**6l**), yield of 3.32 g (96%) (MS m/e 290 $(M + H)^+$); 4-methoxypiperidine amide of L-phenyllactic acid (**6i**), yield of 0.1 g (35%) (MS m/e 264 $(M + H)^+$); 4-(2-propenoxy)piperidine amide of L-phenyllactic acid (**6j**), yield of 0.3 g (74%) (MS m/e 290 $(M + H)^+$); 4-(1-oxa-3-thiabutyl)piperidine amide of L-phenyllactic acid (**6m**), yield of 1.13 g (92%) (MS m/e 310 $(M + H)^+$); 4-(3-oxa-1-thiabutyl)piperidine amide of L-phenyllactic acid (**6n**), yield of 0.41 g (77%) (MS m/e 310 $(M + H)^+$); 4-(2-methoxyethyl)piperidine amide of L-phenyllactic acid (**6k**), yield of 1.66 g (82%) (MS m/e 292 $(M + H)^+$); morpholine amide of L-phenyllactic acid (**6h**), yield of 1.09 g (85%) (MS m/e 236 $(M + H)^+$); *N*-ethyl amide of L-phenyllactic acid (**6c**), yield of 1.04 g (99%) (MS m/e 193 $(M + H)^+$); *N,N*-diethyl amide of L-phenyllactic acid (**6d**), yield of 0.47 g (35%) (MS m/e 222 $(M + H)^+$); azetidine amide of L-phenyllactic acid (**6e**), yield of 1.23 g (99%) (MS m/e 206 $(M + H)^+$); pyrrolidine amide of L-phenyllactic acid (**6f**), yield of 1.21 g (92%) (MS m/e 220 $(M + H)^+$).

Alkylation of L-Phenylalaninamide, General Procedure. (2*S*,4*S*)-3-Aza-2-butyl-4-[[4-(1,3-dioxabutyl)piperidin-1-yl]carbonyl]-5-phenylpentanoic Acid (8c). To a heterogeneous mixture of amine (**6a**, 75 g, 0.2568 mol) and $(NH_4)_2CO_3$ (27.26 g, 0.29 mol) in 200 mL of water at 35 °C was added with stirring (2*R*)-ethyl 2-bromohexanoate (**7c**, 53.52 g, 0.24 mol) in 100 mL of nitromethane. The reaction mixture was stirred at 48 °C for 3 days whereupon the color of the mixture turned light dark (TLC indicated trace of starting material still present). The reaction mixture was cooled to room temperature, extracted with ethyl acetate, dried ($MgSO_4$), and filtered. The filtrate was evaporated under reduced pressure to give an oil which was purified by a short-path silica gel column chromatography eluting with 2:98 $CH_3OH/CHCl_3$ to give 81.4 g of **8b** as a single diastereomer plus 5 g of recovered starting material (79% based on recovered starting material **6a**): ^1H NMR (DMSO-d_6) δ 0.82 (t, 3 H), 1.15 (q, 3 H), 1.2 (m, 4 H), 1.3 (m, 2 H), 1.4 (m, 2 H), 1.6 (m, 2 H), 2.7 (br d, 2 H), 2.6–3.15 (several m, 4 H), 3.22 (d, 3 H, rotomer), 3.85 (m, 1 H), 4.03 (m, 2 H), 4.57 (d, 2 H, rotomer), 7.2 (m, 5 H).

Ester **8b** (35 g, 0.081 mol) was stirred in 112.5 mL of 2 N NaOH (0.227 mol) at room temperature for 24 h. The mixture was acidified with citric acid to pH 5.5, and a white solid precipitated. After the mixture stood at room temperature for 2 h, the solid was filtered. The precipitate was washed with cold water and 1:4 ether/hexane and dried ($MgSO_4$) to afford 28.4 g of acid **8c**. Recrystallization from hot ethyl acetate gave pure **8c** (27 g, 82%): mp 155–157 °C; $[\alpha]^{25}_D$ = +26° (*c* 0.28, CH_3OH); ^1H NMR (DMSO-d_6) δ 0.85 (t, 3 H), 1.25 (br m, 6 H), 1.5 (br m, 4 H), 2.75 (dd, 2 H), 2.82 (m, 2 H), 3.02 (m, 2 H), 3.22 (d, 3 H, rotomer), 3.45 (m, 1 H), 3.58 (m, 1 H), 4.0 (m, 1 H), 4.55 (d, 2 H, rotomer), 7.25 (m, 5 H); MS m/e $(M + H)^+$ 407. Anal. ($C_{22}H_{34}N_2O_5 \cdot {}^1/_4H_2O$) Calcd C, 64.31, H, 8.40, N, 6.82. Found: C, 63.98, H, 8.06, N, 6.90.

Alkylation of L-Phenyllactic Acid Amides, General Procedure. (2*S*,4*S*)-3-Oxa-2-butyl-4-[[4-(1,3-dioxabutyl)-piperidin-1-yl]carbonyl]-5-phenylpentanoic Acid (8r) and the (2*R*,4*S*) Diastereomer. 6b, (43.13 g, 147.2 mmol) in 200 mL of dry THF was added dropwise to the suspension of sodium hydride (60% dispersion in oil, 12.36 g, 309.1 mmol) in 136 mL

of dry THF and 22.7 mL of DMF at an oil bath temperature of 45 °C. The addition took approximately 1 h. The mixture was allowed to stir at 45 °C for additional 3 h. The gray suspension turned white after stirring for 1 h and became very viscous. An additional 36 mL of dry THF was added to facilitate stirring. A solution of (2*R*)-2-bromohexanoic acid (31.57 g, 161.92 mmol) in 180 mL of THF was added dropwise to the thick, white suspension at 45 °C. The addition took approximately 1.75 h. The suspension was removed from the oil bath 45 min after addition was completed and quenched immediately with careful addition of 120 mL of pH 7 phosphate buffer (0.3 M). The solution was then concentrated under reduced pressure at 35 °C and the resulting liquid extracted with 3 × 100 mL of diethyl ether to remove the unreacted alcohol. The aqueous phase was mixed with 300 mL of CH_2Cl_2 and acidified to pH 2 with 200 mL of 1 M sodium hydrogen sulfate. The layers were shaken and separated, and then the aqueous phase was extracted with 2 × 300 mL of CH_2Cl_2. The combined organic phase was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (1.5:5:35:65, HOAc/iPrOH/THF/hexane) to obtain 36.35 g (88.32 mmol, 60%) of the desired acid, R_f 0.30 (1.5:5:35:65, HOAc/iPrOH/THF/hexane). **8r**: mp 63–66 °C; $[\alpha]^{25}_D$ = −10° (*c* 0.30, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.9 (t, 3 H), 1.1–1.5 (bm, 6 H), 1.7–1.82 (bm, 4 H), 3.05 (dd, 2 H), 3.35 (s, 3 H), 3.5–3.85 (several m, 4 H), 3.95 (t, 2 H), 4.6 (m, 1 H), 4.65 (s, 2 H), 7.25 (m, 5 H); MS *m/e* 408 (M + H)$^+$. Anal. ($C_{22}H_{33}NO_6$) Calcd C, 64.84, H, 8.16, N. 3.44. Found: C, 64.95, H, 8.36, N, 3.42. **8r** C(2) diastereomer: mp 108–10 °C; $[\alpha]^{25}_D$ = +2.22 (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.75 (t, 3 H), 0.9–1.1 (bm, 4 H), 1.55–90 (bm, 6 H), 2.95 (dd, 2 H), 3.40 (s, 3 H), 3.60 (m, 4 H), 3.87 (m, 2 H), 4.45 (m, 1 H), 4.70 (s, 2 H), 7.25 (m, 5 H); MS *m/e* 408 (M + H)$^+$. Anal. ($C_{22}H_{33}NO_6$) Calcd C, 64.84; H, 8.16; N, 3.44. Found: C, 65.50; H, 8.04; N, 3.55.

The alkylation of L-phenyllactic acid amides **6b** and **6g–n** with (2*R*)-2-bromohexanoic acid (**7b**) using the general procedure gave the carboxylic acids **8r**, **8i**, **8k–n**, and **8s–u** as single diastereomers after chromatography, yields 20–60%. The alkylation of the L-phenyllactic acid amides **6b–h** with (2*R*)-2-bromopropionic acid (**7f**) using the general procedure gave the carboxylic acids **8o**, **8d–h**, and **8j** as mixtures of diastereomers in 70–95% yield and were used without further purification.

General Procedure for the Synthesis of Inhibitors 19o–v,x,y,za–zf,zh–zj. Carboxylic acid **8r** (125 mg, 0.31 mmol) was coupled to amine hydrochloride **18** (86.6 mg, 0.31 mmol) according to the general procedure previously described to give, after chromatographic purification using 1:1 ethyl acetate/hexanes, **19ze** in 42% yield (80 mg): ^1H NMR ($CDCl_3$) δ 0.80 (d, 3 H), 0.9 (m, 6 H), 1.1–1.9 (several m, 26 H), 2.98 (m, 2 H), 3.08 (m, 2 H), 3.25–3.80 (several m, 4 H), 3.35 (s, 3 H), 3.90 (m, 1 H), 4.15 (m, 1 H), 4.30 (m, 1 H), 4.45 (m, 1 H), 4.66 (s, 2 H), 5.98 (dd, 1 H); MS *m/e* 633 (M + H)$^+$.

(2*S*,4*S*)-3-Oxa-2-ethyl-4-[[4-(1,3-dioxabutyl)piperidin-1-yl]carbonyl]-5-phenylpentanamide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (19zc). The carboxylic acid **8p** was coupled to the amine hydrochloride **18** to give, after chromatography (45:55, ethyl acetate/hexane), **19zc** (22%): ^1H NMR ($CDCl_3$) δ 0.85 (d, 3 H), 0.95 (m, 6 H), 1.1–1.9 (several m, 22 H), 3.0 (m, 2 H), 3.05 (m, 2 H), 3.25–3.80 (several m, 4 H), 3.35 (s, 3 H), 3.90 (m, 1 H), 4.15 (m, 1 H), 4.30 (m, 1 H), 4.45 (m, 1 H), 4.66 (s, 2 H), 6.05 (dd, 1 H), 7.30 (m, 5 H); MS *m/e* 605 (M + H)$^+$. **19zc** C(2) diastereomer (23%): ^1H NMR ($CDCl_3$) δ 0.75 (q, 3 H), 0.85 (d, 3 H), 0.94 (d, 3 H), 1.10–1.90 (several m, 22 H), 3.0 (bd, 2 H), 3.20 (bt, 2 H), 3.30–3.75 (several m, 4 H), 3.35 (s, 3 H), 3.90 (m, 1 H), 4.15 (m, 1 H), 4.42 (m, 1 H), 4.50 (m, 1 H), 4.68 (s, 2 H), 7.25 (m, 5 H); MS *m/e* 605 (M + H)$^+$.

(2*S*,4*S*)-3-Oxa-2-propyl-4-[[4-(1,3-dioxabutyl)piperidin-1-yl]carbonyl]-5-phenylpentanamide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (19zd). The carboxylic acid **8q** was coupled to the amine hydrochloride **18** to give, after chromatography (35:65, ethyl acetate/hexane), **19zd** (29%): ^1H NMR ($CDCl_3$) δ 0.85 (d, 3 H), 0.94 (m, 6 H), 1.1–1.9 (several m, 24 H), 2.95 (m, 2 H), 3.05 (m, 2 H), 3.25–3.85 (several m, 4 H), 3.35 (s, 3 H), 3.90 (m, 1 H), 4.15 (m, 1 H), 4.28 (m, 1 H), 4.45 (m, 1 H), 4.69 (s, 2 H), 5.95 (dd, 1 H), 7.3 (m, 5 H); MS *m/e* 619 (M + H)$^+$. **19zd** C(2) diastereomer (41%): ^1H NMR ($CDCl_3$) δ 0.80 (q, 3 H), 0.85 (d, 6 H), 0.95 (d, 3 H), 1.10–1.90 (several m, 24 H), 3.00 (bd, 2 H), 3.20 (bt, 2 H), 3.30–3.65 (several m, 4 H), 3.35 (s, 3 H), 3.90 (m, 1 H), 4.15 (m, 1 H), 4.40 (m, 1 H), 4.50 (m, 1 H), 4.68 (s, 2 H), 7.25 (m, 5 H); MS *m/e* 619 (M + H)$^+$.

(2*S*,4*S*)-3-Oxa-2-butyl-4-[[4-(1,3-dioxabutyl)piperidin-1-yl]carbonyl]-5-phenylpentanamide of (2*S*,3*S*,6*S*)-2-Amino-1-cyclohexyl-3-hydroxy-6-(*n*-butylcarbamoyl)-6-methylheptane (20). Carboxylic acid **8r** was coupled to (2*S*,3*S*,6*S*)-2-amino-1-cyclohexyl-3-hydroxy-6-(*n*-butylcarbamoyl)-6-methylheptane hydrochloride[16] using the general coupling procedure to give, after chromatography (60:40, ethyl acetate/hexane), inhibitor **20** (47%): ^1H NMR ($CDCl_3$) δ 0.90 (m, 12 H), 1.1–1.9 (several m, 30 H), 2.03 (m, 1 H), 2.97 (m, 2 H), 3.17 (m, 2 H), 3.38 (s, 3 H), 3.40 (m, 4 H), 3.84 (m, 4 H), 4.47 (m, 1 H), 4.68 (s, 2 H), 5.7 (bm, 1 H), 5.80 (bd, 1 H), 7.35 (bs, 5 H); MS *m/e* 716 (M + H)$^+$.

(2*S*,4*S*)-3-Aza-2-butyl-4-[[4-(1,3-dioxabutyl)piperidin-1-yl]carbonyl]-5-phenylpentanamide of (2*S*,3*S*,6*S*)-2-Amino-1-cyclohexyl-3-hydroxy-6-(*n*-butylcarbamoyl)-6-methylheptane (21). Using acid **8c** and following the procedure for the synthesis of inhibitor **20** gave compound **21** (64%): ^1H NMR ($CDCl_3$) δ 0.90 (m, 12 H), 1.1–1.9 (several m, 30 H), 2.05 (m, 1 H), 2.75 (m, 2 H), 3.15 (m, 2 H), 3.36 (s, 3 H), 3.40–90 (several bm, 8 H), 4.67 (d, 2 H, rotomer), 7.25 (m, 5 H); MS *m/e* 715 (M + H)$^+$.

(2*S*,4*S*)-3-Aza-2-butyl-4-[[4-(1,3-dioxabutyl)piperidin-1-yl]carbonyl]-5-phenylpentanamide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (19zf). Carboxylic acid **8c** (80 mg, 0.2 mmol) was coupled to the amine hydrochloride **18** (55.9 mg, 0.2 mmol) according to the general procedure to give, after chromatography (35:65, ethyl acetate/hexane), 87 mg (69%) of **19zf**: ^1H NMR ($CDCl_3$) δ 0.82 (d, 3 H), 0.90 (bt, 3 H), 0.96 (d, 3 H), 1.10–1.70 (several m, 26 H), 2.80 (m, 2 H), 3.10–3.60 (several m, 4 H), 3.35 (s, 3 H), 3.70 (m, 1 H), 3.90 (m, 1 H), 4.19 (m, 1 H), 4.55 (m, 1 H), 4.65 (d, 2 H, rotomer), 7.30 (m, 5 H); MS *m/e* 632 (M + H)$^+$.

(2*S*,4*S*)-3-Oxa-2-butyl-4-[[4-(propyloxy)piperidin-1-yl]-carbonyl]-5-phenylpentanamide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (19z). Compound **19y** (98 mg, 0.156 mmol) was dissolved in 25 mL of methanol and combined with catalyst (10% Pd/C, dry, 50% load, 50 mg). The mixture was shaken under 4 atm of H_2 overnight and then filtered and the methanol evaporated to give 98.3 mg of **19z** (100%): mp 88–92 °C; ^1H NMR ($CDCl_3$) δ 0.84 (d, 1 H, *J* = 6 Hz), 0.95 (m, 6 H), 3.83 (m, 2 H), 4.3 (m, 1 H), 4.44 (m, 1 H), 5.91 (d, 1 H, *J* = 9 Hz), 6.06 (d, 1 H, *J* = 9 Hz), 7.3 (m, 5 H); MS *m/e* 631 (M + H)$^+$.

(2*S*,4*S*)-3-Oxa-2-butyl-4-[(4-hydroxypiperidin-1-yl)-carbonyl]-5-phenylpentanamide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (19w). Compound **19ze** (100 mg, 0.158 mmol) in 1 mL of methylene chloride was added trimethylsilyl bromide (97 mg, 0.634 mmol). The reaction mixture was stirred at room temperature for 16 h. The mixture was taken up with 10 mL of methylene chloride, washed with citric acid, dilute sodium bicarbonate, and brine, dried, and filtered. The filtrate was evaporated to a residue which was chromatographed eluting with 2:98 $CH_3OH/CHCl_3$ to give 60 mg of solid **19w** (64.5% yield): ^1H NMR ($CDCl_3$) δ 0.82 (d, 3 H), 0.90 (t, 3 H), 0.92 (d, 3 H), 1.05–1.90 (m, 26 H), 2.95 (m, 2 H), 3.05 (m, 2 H), 3.20–3.70 (several m, 4 H), 4.00 (m, 1 H), 4.15 (m, 1 H), 4.30 (m, 1 H), 4.45 (m, 1 H), 7.30 (m, 5 H); MS *m/e* 589 (M + H)$^+$.

(2*S*,4*S*)-3-Oxa-2-butyl-4-[[4-(1-oxa-3-sulfinylbutyl)-piperidin-1-yl]carbonyl]-5-phenylpentanamide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (19zk). **19zi** (25 mg, 0.039 mmol) was dissolved in 2 mL of THF, and OXONE (Aldrich, 2.0 equiv, 0.078 mmol, 48 mg) in 1 mL of THF was added. A white precipitate was formed immediately. The reaction mixture was stirred at room temperature for 4 h and partitioned between diethyl ether and water. The organic phase was dried with sodium sulfate, filtered, and evaporated to give 32 mg of crude product which was chromatographed (2:98, CH_3OH/CH_2Cl_2) to give 12.2 mg (45%) of **19zk**: ^1H NMR ($CDCl_3$) δ 0.84 (d, 3 H, *J* = 6 Hz), 0.92 (m, 6 H), 2.90 (s, 3 H), 3.1 (br m, 2 H), 3.8 (m, 1 H), 4.43 (m, 2 H), 5.94 (d, 1 H, *J* = 9 Hz), 6.03 (d, 1 H, *J* = 9 Hz), 7.3 (br m, 5 H).

(2*S*,4*S*)-3-Oxa-2-butyl-4-[[4-(1-oxa-3-sulfonylbutyl)-piperidin-1-yl]carbonyl]-5-phenylpentanamide of

(2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (19zl). **19zi** (25 mg, 0.039 mmol) was combined with 2 mL of CH_2Cl_2 and cooled to 5 °C. *m*-Chloroperoxybenzoic acid (85%, 9 mg, 0.043 mmol) was added, and the mixture was stirred at 5 °C for 25 min. At the end of this time, 15% aqueous sodium sulfite was added and the organic phase was separated, dried (Na_2SO_4), filtered, and evaporated to give 29 mg of a crude material. This was combined with 20 mg of crude material from a previous reaction performed in an identical manner. Chromatography (3:97, CH_3OH/CH_2Cl_2) gave compound **19zl** (22 mg, 47%): ^1H NMR ($CDCl_3$) δ 0.84 (d, 3 H, J = 6 Hz), 0.93 (m, 6 H), 1.86 (m, 1 H), 2.58 (s, 3 H), 3.81 (t, 1 H, J = 4.5 Hz), 3.9 (br m, 1 H), 4.4–4.52 (m, 3 H), 5.95 (m, 1 H), 6.06 (d, 1 H, J = 4.5 Hz), 7.26–7.35 (sev m, 5 H); MS m/e 665 $(M + H)^+$.

In Vitro Enzyme Inhibition Assays. Enzyme assays using purified human renin at pH 6.0[21] and plasma renin at pH 7.4 were performed as previously described.[22,23]

In Vivo Pharmacology. Intravenous and intraduodenal activities were assessed in male cynomolgus monkeys (*Macaca fasicularis*) weighing between 3 and 5 kg, since the compounds tested were primate selective. Pretreatment included maintenance on a low-salt chow and fresh fruit diet in conjuction with furosemide treatment (5 mg/kg, po) on days 7 and 1 prior to the experiment. This regimen elevates the baseline PRA values, but maintains or reduces baseline blood pressures. The monkeys are fasted overnight and anesthetized on the study day with sodium pentobarbital, 15 mg/kg bolus sustained by a 0.10 mg kg^{-1} min^{-1} constant infusion. Blood pressure and heart rate were measured directly through 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 activity and by direct catheter placement and delivery into the proximal segment of the duodenum subsequent to laparatomy for the determination of intraduodenal activity. Each monkey received only one dose of compound. All compounds were administered as HCl salt solutions.

Bioavailability Determinations. Plasma drug concentrations were determined by HPLC or bioassay. Blood samples were obtained at intervals for pharmacokinetic evaluation. The integrated area under the curves for plasma drug concentrations were obtained by fitting the data to a biexponential decay model.[24] Intraduodenal (id) and intravenous (iv) bioavailabilities were calculated as the dose-corrected ratio of AUC (id) to AUC (iv) X 100 and are expressed as percents. For a more detailed description of the bioavailability experiments see the following paper.

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Supplementary Material Available: Microanalytical and high-resolution mass spectra data for compounds **19a–z,za–g,i–l**, **20**, and **21** (3 pages). Ordering information is given on any current masthead page.

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