Studies Directed toward the Design of Orally Active Renin Inhibitors. 1. Some Factors Influencing the Absorption of Small Peptides

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A systematic evaluation of structure–absorption relationships using a high throughput intraduodenal rat screening model has led to the delineation of a set of structural parameters that appear to govern bioavailability in a series of peptide-based renin inhibitors. Optimum structures, exemplified by **25** and **41**, incorporated a single, solubilizing substituent at the C- or N-terminus combined with a lipophilic P_2-site residue. Both inhibitors gave unprecedented plasma drug levels upon intraduodenal administration to monkeys, and the calculated bioavailability for **41** (14 ± 4%) is the highest reported for any peptidic renin inhibitor.

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 antihypertensive agents.[1] In order to become a viable drug, an orally active renin inhibitor must possess two attributes: it must be well and predictably absorbed from the gastrointestinal tract into the systemic circulation, and it must elicit a dose-related lowering of blood pressure when given orally. While several peptidic renin inhibitors have produced pharmacologic responses following oral dosing, these and related inhibitors have been subject to poor absorption and substantial hepatic elimination resulting in low (<10% in rats, <5% in primates) bioavailability.[2] This limited bioavailability has been attributed to the intrinsic nature of a peptide drug.[3] (In contrast, improved bioavailability has been demonstrated for a series of nonpeptide renin inhibitors.[4]) We have recently described the novel renin inhibitor A-72517.[5] This compound is the first peptide-based renin inhibitor to be both orally efficacious and significantly bioavailable in several species. Herein we describe the work that led to the discovery of A-72517.

We have shown, in a series of renin inhibitors incorporating a C-terminal oxazolidinone,[6] that modification of the amino acid occupying the P_2 site can affect absorption.[7] Thus inhibitor **25**, containing a P_2-site thiazol-4-ylalanine residue, displayed enhanced absorption upon intraduodenal administration to both rats and salt-depleted monkeys. Unfortunately, this compound caused only a moderate pharmacologic response in the primate model.[7] Table I and Figure 1 reproduce these data. That statistically significant hypotension was not observed was puzzling. Peak and 2-h plasma drug levels were greater than 40 times the IC_{50} value, and as expected these drug levels completely suppressed plasma renin activity (PRA). Dissociation between blood pressure and PRA has been observed by others,[8] and one explanation is that a renin inhibitor must affect a second, extraplasma, pool of renin in order to elicit a blood pressure response.[9] This suggested to us that perhaps **25** was not efficiently reaching additional putative renin pools and that an inhibitor with the same absorption characteristics as **25** but with different physicochemical properties might prove more efficacious. Thus we chose to design an inhibitor incorporating the equally potent but more lipophilic dipeptide glycol transition state mimic[10] in place of the C-terminal oxazolidinone.

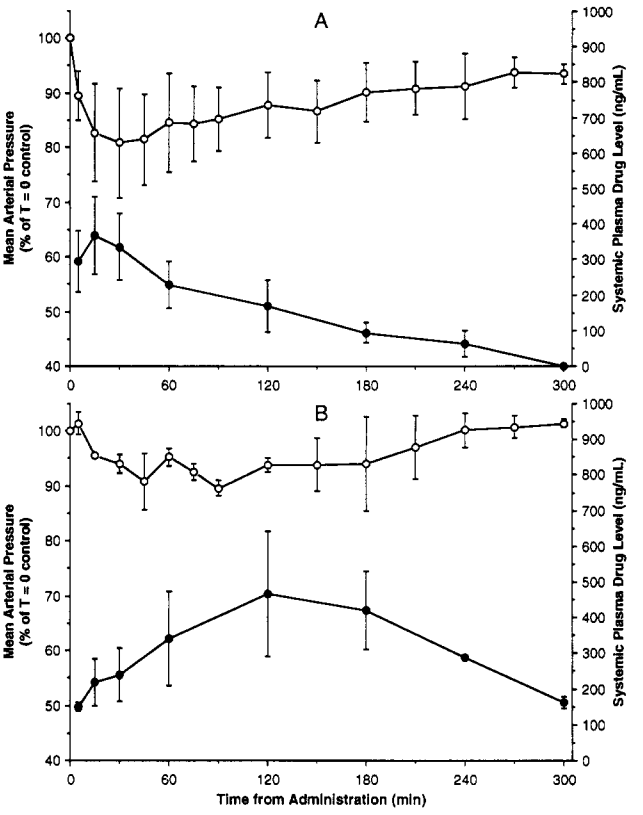

Figure 1. Plasma drug levels (●) and pharmacologic effects (○) from 10 mg/kg intraduodenal doses of inhibitors **25** (A, $n = 4$) and **41** (B, $n = 2$) in anesthetized, salt-depleted cynomolgus monkeys. Results are shown as mean ± SEM.

Results and Discussion

Synthesis. The C-terminal transition-state mimics **2** and **5** were attached to various protected P_2-site residues **1** using either carbodiimide or mixed-anhydride coupling methods (Scheme I). It was previously shown that HCl-mediated cleavage of the *tert*-butyloxycarbonyl group on peptides containing fragment **4a** led to the formation of side products that appeared to result from *N*- to *O*-acyl transfer reactions.[11] This problem proved to be more pronounced with peptides such as **3d** that incorporated

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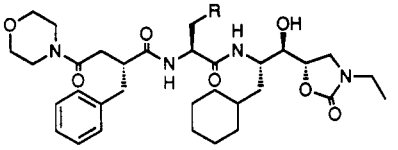
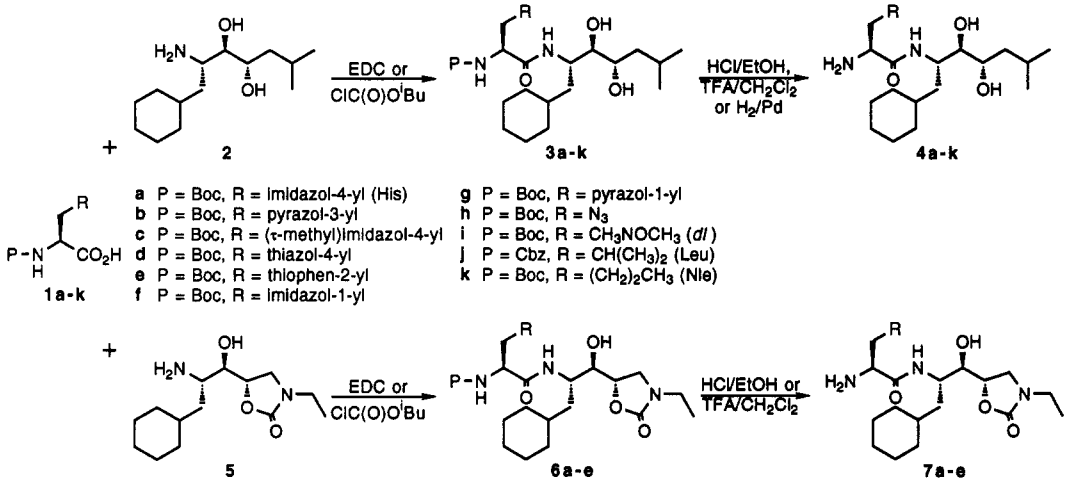
Table I. C-Terminal Oxazolidinone Renin Inhibitors Containing Heterocycle-Substituted Alanine Residues at the P_2 Histidine Site



					10 mg/kg id rat experiments plasma level (ng/mL)[a]			
					10 min		30 min	
no.	R	IC_{50} (nM)[b]	log P[c]	solubility[d]	systemic	portal	systemic	portal
22	imidazol-4-yl (His)[e]	1.3	2.20	2.8	55 ± 29	280 ± 80	34 ● 20[f]	390 ● 140[f]
23	pyrazol-3-yl	21	2.49	2.5	7 ± 2	28 ± 16[g]	7 ± 2	58 ± 23
24	1-methylimidazol-4-yl	58	2.16	2.1	93 ± 34	950 ± 710	43 ± 17	350 ± 230
25	thiazol-4-yl	8.1	2.83	0.46	320 ± 100[h]	1900 ± 700[h]	300 ± 50[i,k]	1100 ● 500[k]
26	thiophen-2-yl	43	3.59	0.067	340 ± 60[i]	810 ± 70[i]	160 ± 30[i]	570 ± 230

[a] Mean ± SEM, $n = 3$, determined by a renin inhibition assay. [b] Human plasma renin, pH 7.4. [c] Octanol/water, pH 7.4. [d] mg/mL, pH 7.4. [e] See ref 25. [f] $n = 5$. [g] Differs significantly from value for **22** ($P < 0.05$). [h] Significantly greater than value for **22** ($P < 0.05$). [i] Significantly greater than value for **22** ($P < 0.01$). [k] $n = 6$.

Scheme I. Peptide Coupling of P_2-Site Residues to C-Terminal Transition-State Mimics



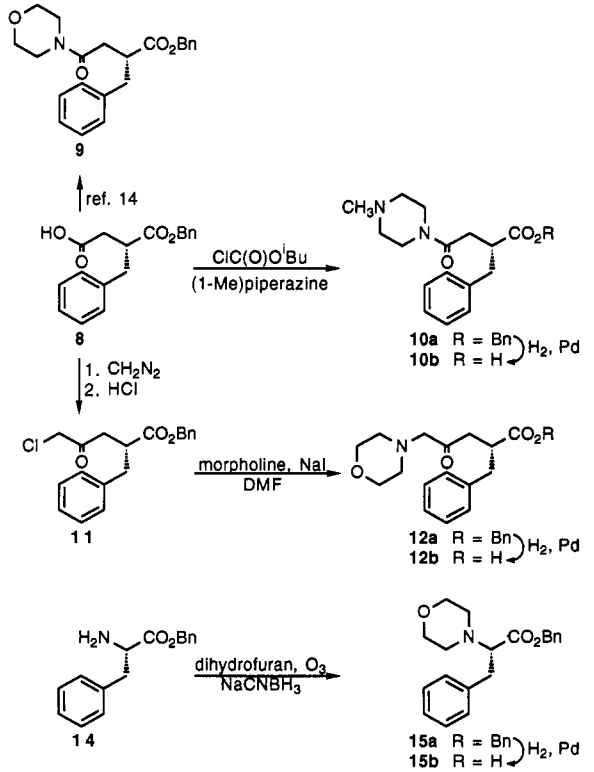
the less basic heterocycle-substituted alanine derivatives. Consequently, we examined a number of reagents for Boc deprotection and found that cold trifluoroacetic acid produced negligible amounts of these byproducts. Due to complications resulting from the presence of trifluoroacetic acid in subsequent reactions, the products **4** and **7** were neutralized and isolated as the free amines.

The syntheses of the various N-terminal residues are outlined in Schemes II and III. These were coupled to fragments **4** and **7** using a water-soluble carbodiimide (EDC). Final products were purified by chromatography on silica gel after an extractive isolation.

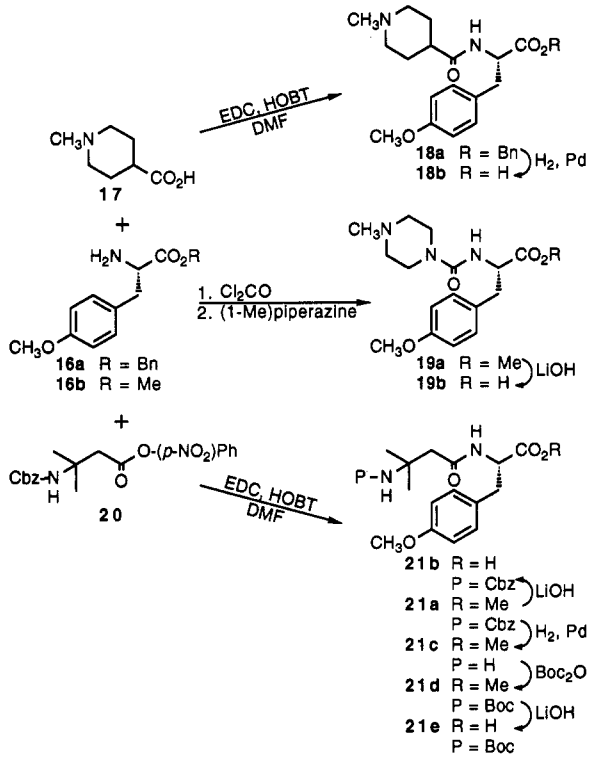
Structure–Absorption Relationships. Table II outlines the properties and absorption characteristics of a series of dipeptide glycol-containing renin inhibitors that vary at the P_2-site residue. Data from the corresponding oxazolidinone inhibitors are summarized in Table I. Measurement of partition coefficients and aqueous solubility confirmed that the physicochemical properties of the two sets of compounds differed significantly. As determined by partition coefficient, the oxazolidinone is a polar group. The contribution to lipophilicity of replacing the oxazolidinone with the dipeptide glycol was of the same magnitude as substituting thiophene for a P_2-site imidazole (compare **22**, **26**, and **27**). Thus these compounds represented two distinct classes of inhibitors with which the effects of the P_2-site residue on absorption could be studied.

As an initial test of absorption, compounds were

Scheme II. Synthesis of Benzylsuccinate and Phenylalanine Derived N-Terminal Groups



Scheme III. Synthesis of *O*-Methyltyrosine Derived N-Terminal Groups



administered intraduodenally (id) to anesthetized rats. Plasma drug levels were determined by HPLC or a renin inhibition assay[12] from samples taken at 10 and 30 min from both the systemic and portal circulation in the same animals. While this model was insufficient for the determination of bioavailability, the data provided an estimate of both absorption from the intestine and extraction by the liver. The results were largely similar for the two classes of inhibitors. In both series, the histidine containing compounds (**22** and **27**) were moderately absorbed from the intestine but were subject to extensive first-pass hepatic extraction. In contrast, compounds incorporating thiazol-4-ylalanine (**25** and **30**) or other lipophilic, weakly basic residues lacking an exchangeable proton (**26** and **31**) gave both portal and systemic drug levels that were significantly higher than were observed for the parent histidine derivatives. Thus this modification of the P_2-site residue, diminishing the basicity and eliminating the potential reactivity of histidine, enhanced intestinal absorption in both series of inhibitors and demonstrably reduced hepatic extraction in the C-terminal oxazolidinone series.

The P_2-site residues 1-methylhistidine and pyrazol-3-ylalanine represent intermediate species. The former amino acid maintains the basicity of histidine but lacks the potentially reactive NH, while the pyrazole is weakly basic yet contains the exchangeable proton. It is with these two P_2-site residues that differences in absorption were observed between the oxazolidinone and glycol series. Both oxazolidinone-based inhibitors gave low systemic drug levels: the pyrazol-3-ylalanine inhibitor **23** was poorly absorbed while the moderate absorption of 1-methylhistidine analog **24** was offset by enhanced hepatic extraction as was observed with histidine derivative **22**. In contrast, the corresponding glycol derivatives **28** and **29** showed enhanced intestinal absorption and reduced but variable hepatic extraction compared to the parent histidine-containing compound **27**. Thus the absorption data for compounds **22–31** can be summarized as follows: histidine at the P_2 site resulted in low systemic drug levels when combined with either C-terminus; with the polar oxazolidinones, good absorption and low hepatic extraction were observed only when a nonpolar P_2-site residue was present; and good absorption with the nonpolar glycol occurred with either a polar or a nonpolar P_2-site residue.[13]

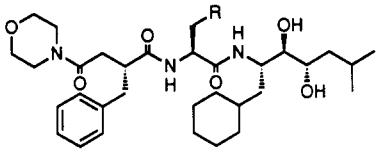
Although inhibitor **30** possessed good in vitro potency and was well absorbed in the id rat screening model, this compound proved difficult to formulate due to its low aqueous solubility and the lack of a group suitable for salt formation. We therefore decided to incorporate an ionizable group and to place this residue at the N-terminus since a non-histidine basic group at the P_2 site was detrimental to in vitro activity (**24**, **29**, **32**, and **34**). Our intravenous clinical candidate enalkiren (A-64662, **35**)[2i] contains such an N-terminal basic residue in the form of a dimethyl-β-alanine (DMBA), with *O*-methyltyrosine replacing the benzyl succinate of **22–34** as the group responsible for stabilizing the P_2–P_3 bond toward chymotrypsin degradation.[14,15] Results from the id screening model for enalkiren and derivatives modified at the P_2 site are summarized in Table III. Neither the thiazol-4-ylalanine (**36**) nor thiophen-2-ylalanine (**37**) analogs were well absorbed from the intestine. Enalkiren itself, while exhibiting moderate portal drug levels, was subject to high liver extraction, and previous oral rat experiments have shown that it is poorly absorbed.[2i] DMBA inhibitors **38–40**, incorporating the oxazolidinone C-terminus, behaved similarly.

At this point it was unclear whether a basic group at the N-terminus was compatible with absorption. Since inclusion of the enalkiren N-terminus represented a significant structural permutation, inhibitors **41** (Table IV), **48**, and **49** (Table V) were designed to mimic as closely as possible the structure of **30** while still incorporating an N-terminal basic residue. All three compounds gave excellent plasma drug levels in the id rat screening model. Furthermore, **41**, in which an *N*-methylpiperazine replaced the morpholine, and **49** maintained good potency. Thus a basic group at the N-terminus was not inherently detrimental to absorption, and inhibitor **41** (the more synthetically accessible) became the new lead structure.

A short study was undertaken to determine which P_2-site residues provided the best combination of potency and absorption for the N-terminal *N*-methylpiperazine series. These results are outlined in Table IV. As was expected from the previous two series, the neutral or weakly basic P_2-site residues of **41–45** led to significantly enhanced absorption, but lower in vitro potency, compared to histidine derivative **46**. Inhibitor **47**, containing the polar pyrazol-3-ylalanine residue and the only other compound more potent than **41**, was poorly absorbed.

That the pyrazol-3-ylalanine glycol **28** was well absorbed from the intestine, while **47** and **23** (the oxazolidinone analog of **28**) were not, suggested that the combination of a polar residue both at the P_2 site and at either terminus was incompatible with good absorption. In contrast, good systemic plasma drug levels were attainable with compounds incorporating a nonpolar P_2-site residue and a polar group at either the C-terminus (Table I) or N-terminus (Table IV). Dimethyl-β-alanine (DMBA) inhibitors **36** and **37** appeared to contradict these rules. Both incorporated a single basic substituent at the N-terminus and

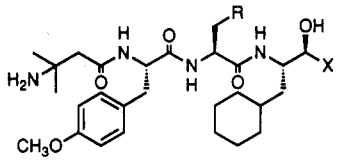
Table II. C-Terminal Glycol Renin Inhibitors Containing Substituted Alanine Residues at the P_2 Histidine Site and a Neutral N-Terminus



					10 mg/kg id rat experiments plasma level (ng/mL)[a]			
					10 min		30 min	
no.	R	IC_{50} (nM)[b]	log P[c]	solubility[d]	systemic	portal	systemic	portal
27	imidazol-4-yl (His)[e]	1.6	3.51	nd[f]	13 ± 6	190 ± 60	11 ± 1	170 ± 30
28	pyrazol-3-yl	3.5	4.01	0.12	88 ± 30[g]	770 ± 470	170 ± 80	1110 ± 500
29	1-methylimidazol-4-yl	110	3.71	0.095	470 ± 310	4900 ± 300[h]	320 ± 240	3300 ± 500[h]
30	thiazol-4-yl	8.3	4.27	0.016	130 ± 60	1400 ± 400[g]	210 ± 20[h]	2500 ± 400[h]
31	$N(CH_3)(OCH_3)$	88	>4.6	nd	340 ± 110[g]	3500 ± 400[h]	170 ± 40[h]	1400 ± 400[g]
32	imidazol-1-yl	93	3.79	nd	nd	nd	nd	nd
33	N_3	11	4.10	nd	nd	nd	nd	nd
34	$N(CH_3)_2$	210	nd	nd	nd	nd	nd	nd

[a] Mean ± SEM, n = 3, determined by a renin inhibition assay. [b] Human plasma renin, pH 7.4. [c] Octanol/water, pH 7.4. [d] mg/mL, pH 7.4. [e] See ref 15. [f] Not determined. [g] Significantly greater than value for **27** ($P < 0.05$). [h] Significantly greater than value for **27** ($P < 0.01$).

Table III. Renin Inhibitors Related to Enalkiren



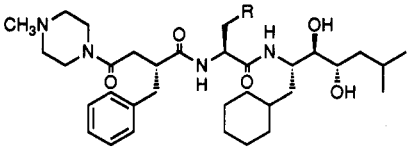
					10 mg/kg id rat experiments plasma level (ng/mL)[a]			
					10 min		30 min	
no.	R	X	IC_{50} (nM)[b]	assay	systemic	portal	systemic	portal
35	imidazol-4-yl (Enalkiren)[c]		14	RI[d]	170 ± 70	1100 ± 500	82 ± 44	540 ± 80
36	thiazol-4-yl		33	HPLC	17 ± 17	93 ± 9	13 ± 13	53 ± 3
37	thiophen-2-yl		85	RI	9 ± 5	23 ● 16	6 ± 4	23 ± 11
38	imidazol-4-yl		17	HPLC	93 ± 48	140 ± 80	110 ± 40	250 ± 130
39	thiazol-4-yl		39	RI	83 ± 29	880 ± 250	24 ● 6	240 ± 40
40	thiophen-2-yl		69	HPLC	16 ± 12	13 ± 5	32 ± 13	45 ± 8

[a] Mean ± SEM, n = 3. [b] Human plasma renin, pH 7.4. [c] See ref 2i. [d] Renin inhibition assay.

a nonpolar P_2-site residue, yet were poorly absorbed. To understand this behavior, we prepared compounds **50**, in which the benzyl succinate of **41** was replaced with *O*-methyltyrosine, and **51**, in which the acylated piperazine of **50** was replaced with the more basic[16] isonipecotic acid residue (Table V). Results from the id rat screening model for **41** and **50** were similar, consequently the presence of the DMBA, and not the *O*-methyltyrosine residue, was responsible for the low absorption of **36** and **37**. Inhibitor **51** was less well absorbed and showed greater hepatic extraction than **50**, although the differences were not statistically significant. These results indicate that it is in part the enhanced basicity of the DMBA residue[17] that adversely affects absorption (although other structural features, such as the presence of exchangeable NH protons, may also be factors). An inverse relationship between basicity and absorption is well documented, suggesting that the intestinal mucosa is not permeable to protonated species.[18] Hence the N-terminal basic residue should have a pK_a not significantly greater than physiologic pH in order to maintain a balance between solubility and absorption.

While these empirical rules were a guide for the design of renin inhibitors with oral activity, extremely subtle structural variations had pronounced effects upon both transport across the intestine and hepatic extraction. Compounds **41** and **42** have similar structures and physicochemical properties. In the id rat screening model, the two inhibitors produced nearly identical portal drug levels, yet significantly disparate systemic levels. While these results could be rationalized on the basis of hepatic metabolism at the P_2 site, the results from compounds **43** and **44** were more enigmatic. These two inhibitors, isomeric at a hydrocarbon side chain, exhibited significantly different transport across the intestine. Thus while

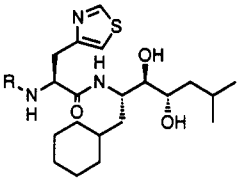
Table IV. C-Terminal Glycol Renin Inhibitors Containing Substituted Alanine Residues at the P_2 Histidine Site and a Basic N-Terminus

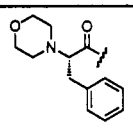
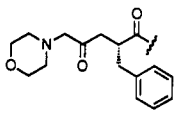
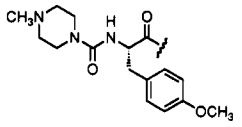
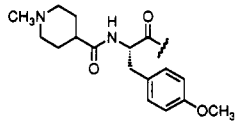


						10 mg/kg id rat experiments plasma level (ng/mL)[a]			
						10 min		30 min	
no.	R	IC_{50} (nM)[b]	log P[c]	solubility[d]	assay	systemic	portal	systemic	portal
41	thiazol-4-yl	18	4.12	0.17	RI[e]	240 ± 60[f]	1500 ± 500[f]	390 ± 70[g,h]	2500 ± 500[g]
42	pyrazol-1-yl	18	4.39	0.18	RI	86 ± 19	2000 ± 200[g]	140 ± 30[f]	2300 ± 300[g]
43	$CH(CH_3)_2$ (Leu)	37	nd[i]	nd	HPLC	380 ± 60[g]	880 ± 340[f,j]	620 ± 100[g]	1200 ± 300[g,j]
44	$(CH_2)_2CH_3$ (Nle)	25	>4.5	0.021	HPLC	260 ± 80[f]	3200 ± 600[g]	650 ± 220[f]	4600 ± 700[g]
45	thiophen-2-yl	110	4.44	0.028	HPLC	300 ± 60[g]	2900 ± 1200[f]	530 ± 90[g]	2900 ± 600[g]
46	imidazol-4-yl (His)	5.4	1.66	nd	HPLC	63 ± 18	67 ± 18	40 ± 6	30 ± 20
47	pyrazol-3-yl	2.8	4.02	nd	RI	21 ± 9	53 ± 23	22 ± 18	27 ± 7

[a] Mean ± SEM, $n = 3$. [b] Human plasma renin, pH 7.4. [c] Octanol/water, pH 7.4. [d] mg/mL, pH 7.4. [e] Renin inhibition assay. [f] Significantly greater than value for **46** ($P < 0.05$). [g] Significantly greater than value for **46** ($P < 0.01$). [h] DIffers significantly from value for **42** ($P < 0.05$). [i] Not determined. [j] Differs significantly from value for **44** ($P < 0.05$).

Table V. C-Terminal Glycol Renin Inhibitors Incorporating a Variety of Basic Residues at the N-Terminus



					10 mg/kg id rat experiments plasma level (ng/mL)[a]			
					10 min		30 min	
no.	R	IC_{50} (nM)[b]	log P[c]	solubility[d]	systemic	portal	systemic	portal
48		1600	nd[e]	nd	420 ± 160	2600 ● 500	890 ± 440	3300 ± 900
49		25	3.91	nd	190 ± 130	2500 ± 600	380 ± 80	1800 ± 500
50		120	3.38	0.48	160 ± 30	1300 ± 300	250 ± 80	2000 ± 900
51		170	3.03	nd	160 ± 90	1200 ± 500	90 ± 50	770 ± 170

[a] Mean ± SEM, $n = 3$, determined by a renin inhibition assay. [b] Human plasma renin, pH 7.4. [c] Octanol/water, pH 7.4. [d] mg/mL, pH 7.4. [e] Not determined.

a set of empirical rules can describe the absorption behavior of the inhibitors in this report, it is clear that the absorption process is complicated and not well understood.

Absolute Bioavailability. Compound **41** was dosed intraduodenally at 10 mg/kg to anesthetized, salt-depleted monkeys (Figure 1, Table VI). Companion intravenous experiments were also performed. Bioavailability, the dose-normalized ratio of the integrated plasma drug level–time curves from the intravenous and intraduodenal routes of administration, was estimated to be 14 ± 4%. This is the highest absorption reported for any peptidic renin inhibitor. As was observed with inhibitor **25** (which has similar potency against monkey plasma renin),[19] the hypotensive response, which did not appear to parallel the observed drug levels, was only moderate. Blood pressure was reduced a maximum of 10 ± 1%, returning to control by the end of the experiment (in contrast, blood pressure was reduced 39 ± 4% following a 10 mg/kg dose of the angiotensin-converting enzyme inhibitor captopril, $n = 3$, data not shown).

Thus we have conducted a systematic examination of structure–absorption relationships using a high throughput id rat screening model, allowing us to define a set of structural parameters that appears to govern bioavailability in this class of peptide-based renin inhibitors. Importantly, physicochemical properties alone, as measured by partition coefficient and solubility, did not correlate with absorption. Inhibitors **25** and **41** exemplify

Table VI. Plasma Drug Levels of Compounds 25 and 41 Dosed Intraduodenally at 10 mg/kg in Salt-Depleted Cynomolgus Monkeys[a]

			plasma level[b]				
			systemic		portal		
no.[c]	n	assay	AUC[d]	peak (ng/mL)	AUC[d]	peak (ng/mL)	bioavailability (%)[e]
25	4	HPLC	770 ± 230	360 ± 100	3800 ± 1000	3200 ± 1400	nd[f]
41	2	RI[g]	1700 ± 400	480 ± 170	13000 ± 4000	6300 ± 3900	14 ± 4

[a] 5-h experiments. [b] Mean ± SEM. [c] See Table I and IV for structures. [d] Integrated area under the plasma drug level–time curve, ng h/mL. [e] Compared to 0.3 mg/kg iv dose. [f] Not determined. [g] Renin inhibition assay.

Table VII. Physical Data and Synthetic Methods for Renin Inhibiting Compounds

no.[a]	formula[b]	method[c]	chromatography solvent[d]
23	$C_{35}H_{50}N_6O_7$	B	1.5–3
24	$C_{36}H_{52}N_6O_7 \cdot 0.25H_2O$	A	2
25	$C_{35}H_{49}N_5O_7S \cdot 0.5H_2O$	B	1.5
26	$C_{36}H_{50}N_4O_7S$	A	1.5
28	$C_{35}H_{53}N_5O_6$	B	2–5
29	$C_{36}H_{55}N_5O_6$	A	2
30	$C_{35}H_{52}N_4O_6S$	B	1.5
31	$C_{34}H_{56}N_4O_7$	B	1.5
32	$C_{35}H_{53}N_5O_6 \cdot 0.25H_2O$	B	3–3.5
33	$C_{32}H_{50}N_6O_6$	B	1.4
34	$C_{34}H_{56}N_4O_6 \cdot 0.5H_2O$	e	3
36	$C_{35}H_{55}N_5O_6S \cdot 0.5H_2O$	C	not required
37	$C_{36}H_{56}N_4O_6S \cdot H_2O$	C	3
38	$C_{39}H_{61}N_7O_{11} \cdot 1.39H_2O$	e	not required
39	$C_{35}H_{52}N_6O_7S \cdot 0.25H_2O$	C	not required
40	$C_{36}H_{53}N_5O_7S$	C	not required
41	$C_{36}H_{55}N_5O_5S$	B	3.5–4
42	$C_{36}H_{56}N_6O_5 \cdot 0.5H_2O$	B	3
43	$C_{36}H_{60}N_4O_5 \cdot 0.5H_2O$	B	2
44	$C_{36}H_{60}N_4O_5$	B	2.5
45	$C_{37}H_{56}N_4O_5S$	B	2
46	$C_{36}H_{56}N_6O_5 \cdot 0.5H_2O$	B	8–10
47	$C_{36}H_{56}N_6O_5 \cdot H_2O$	B	3–6
48	$C_{33}H_{50}N_4O_5S$	B	1.5
49	$C_{36}H_{54}N_4O_6S \cdot 0.25H_2O$	B	1.6
50	$C_{36}H_{56}N_6O_6S$	B	3–5
51	$C_{37}H_{57}N_5O_6S \cdot 0.5H_2O$	B	4–5

[a] See Tables I–V for structures. [b] Analyses for C, H, N were ± 0.4% of expected values for formulae shown. [c] See the following preparations in the Experimental Section: method A, 3a; method B, 3b; method C, 4a. [d] % Methanol in chloroform. [e] See the Experimental Section for this preparation.

these findings. Their physicochemical properties, as measured by log *P* values, differ significantly with 41 being considerably more lipophilic, yet these two renin inhibitors of differing polarity and aqueous solubility produced unprecedented plasma drug levels upon intraduodenal administration to both rats and monkeys.

Experimental Section

Synthetic methods for final compounds are outlined in Table VII. 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 CaH_2. NMR spectra were recorded at 300 MHz and are expressed in parts per million (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 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: 85% chloroform/15% methanol (I), 25% acetic acid/25% 1-butanol/25% ethyl acetate/25% water (II), 1% acetic acid/79% chloroform/20% methanol (III), 50% ethyl acetate/50% hexane (IV), 90% chloroform/10% methanol (V), ethyl acetate (VI), 95% chloroform/5% methanol (VII), 70% chloroform/30% ethyl acetate (VIII), 20% ethyl acetate/80% hexane (IX). Boc-His-OH (1a), Cbz-Leu-OH (1j), and Boc-Nle-OH (1k) were purchased from Sigma Chemical Company; and Boc-(τ-CH_3)-His-OH (1c), Boc-(thiophen-2-yl)Ala-OH (1e), and Boc-(Ome)-Tyr-OH were purchased from Bachem Bioscience, Inc.

***N*-(*tert*-Butyloxycarbonyl)-3-imidazol-1-yl-L-alanine Methyl Ester.** Imidazole (250 mg, 3.67 mmol) and *N*-(*tert*-butyloxycarbonyl)-L-serine β-lactone[20] (350 mg, 1.87 mmol) in CH_3CN (9 mL) were stirred at ambient temperature for 24 h. The mixture was cooled to 0 °C and treated with diazomethane in ether. After evaporation of the solvent, the residue was chromatographed with 3% methanol in chloroform to afford 305 mg (61%) of an oil: TLC (I) R_f = 0.48; ^{1}H NMR ($CDCl_3$) δ 7.39 (s, 1 H), 7.05 (s, 1 H), 6.82 (s, 1 H), 5.18 (br d, 1 H), 4.63–4.54 (m, 1 H), 4.42 (d, 2 H), 3.79 (s, 3 H), 1.47 (s, 9 H); MS *m/e* (M + H)$^+$ 270.

***N*-(*tert*-Butyloxycarbonyl)-3-imidazol-1-yl-L-alanine (1f).** *N*-(*tert*-Butyloxycarbonyl)-3-imidazol-1-yl-L-alanine methyl ester (301 mg, 1.12 mmol) in dioxane (6 mL) at 0 °C was treated with LiOH monohydrate (64.0 mg, 1.53 mmol) in water (4 mL). After 1 h the reaction was quenched with 2.0 M HCl (0.75 mL, 1.5 mmol) and evaporated to a white foam which was used without further purification: TLC (II) R_f = 0.49; ^{1}H NMR (CD_3OD) δ 8.37 (s, 1 H), 7.37 (s, 1 H), 7.30 (s, 1 H), 4.60 (dd, 1 H), 4.43–4.27 (m, 2 H), 1.41 (s, 9 H).

***N*-(*tert*-Butyloxycarbonyl)-3-pyrazol-1-yl-L-alanine (1g).** This material was prepared according to the method described for the corresponding benzyloxycarbonyl derivative.[20] Pyrazole (700 mg, 10.3 mmol) and *N*-(*tert*-butyloxycarbonyl)-L-serine β-lactone[20] (1.707 g, 9.117 mmol) in CH_3CN (75 mL) were heated at 52 °C for 72 h. The solvent was evaporated, and the residue was dissolved in hot methanol (8 mL), and then water (24 mL) was added with heating until the mixture became turbid. The mixture was cooled to room temperature with rapid stirring, and after the mixture stirred overnight, 745 mg (32%) of a white solid was collected by filtration: mp 130–134 °C; TLC (III) R_f = 0.38; ^{1}H NMR ($CDCl_3$) δ 7.65 (d, 1 H), 7.41 (d, 1 H), 6.30 (dd, 1 H), 5.48 (br d, 1 H), 4.82 (dd, 1 H), 4.67 (dd, 1 H), 4.52–4.44 (m, 1 H), 1.47 (s, 9 H); MS *m/e* (M + H)$^+$ 256. Anal. ($C_{11}H_{17}N_3O_4$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-3-azido-L-alanine Methyl Ester and *N*-(*tert*-Butyloxycarbonyl)-2,3-dehydroalanine Methyl Ester.** To sodium azide (1.62 g, 24.9 mmol) in water (2.0 mL) was added benzene (25 mL), and the mixture was cooled to 0 °C. Concentrated sulfuric acid (0.70 mL, 13 mmol) was added dropwise with rapid stirring. The solution of HN_3 was transferred via cannula to a flask charged with anhydrous Na_2SO_4 at 0 °C and allowed to stand for 1 h. To triphenyl phosphine (5.75 g, 21.9 mmol) in THF (20 mL) at −78 °C was added diethyl azodicarboxylate (3.40 mL, 21.6 mmol) in THF (10 mL) followed by the HN_3 solution and Boc-serine methyl ester (4.00 g, 18.2 mmol) in THF (16 mL). After the mixture stirred at −78 °C for 1 h and at ambient temperature 16 h, the solvent was evaporated and the residue was chromatographed with 33% ether in hexane to provide 0.33 g (9%) of the dehydroalanine [oil; TLC (IV) R_f = 0.72; ^{1}H NMR ($CDCl_3$) δ 7.02 (br, 1 H), 6.17 (s, 1 H), 5.73 (d, 1 H), 3.83 (s, 3 H), 1.49 (s, 9 H)] followed by 3.25 g (73%) of the azide as a mobile oil: TLC (IV) R_f = 0.60; ^{1}H NMR ($CDCl_3$) δ 5.38 (br d, 1 H), 4.52–4.44 (m, 1 H), 3.80 (s, 3 H), 3.72 (d, 2 H), 1.47 (s, 9 H); MS *m/e* (M + H)$^+$ 245.

***N*-(*tert*-Butyloxycarbonyl)-3-azido-L-alanine (1h).** *N*-(*tert*-Butyloxycarbonyl)-3-azido-L-alanine methyl ester (350.0 mg, 1.433 mmol) in dioxane (6 mL) at 0 °C was treated with LiOH monohydrate (84.0 mg, 2.00 mmol) in water (4 mL). After 1 h the mixture was concentrated, diluted with water, washed with ether, acidified with 0.5 M H_3PO_4, and extracted into chloroform

which was dried and evaporated to provide 250.4 mg (76%) of a colorless oil: ^{1}H NMR ($CDCl_3$) δ 5.37 (br d, 1 H), 4.57–4.48 (m, 1 H), 3.80 (d, 2 H), 1.47 (s, 9 H); MS *m/e* $(M + H)^+$ 231.

***N*-(*tert*-Butyloxycarbonyl)-3-(*N*-methoxy-*N*-methylamino)-D,L-alanine Methyl Ester.** *N,O*-Dimethylhydroxylamine hydrochloride (600 mg, 6.16 mmol), $NaHCO_3$ (560 mg, 6.60 mmol), and *N*-(*tert*-butyloxycarbonyl)-2,3-dehydroalanine methyl ester (570 mg, 2.84 mmol) in CH_3CN (14 mL) were heated at 100 °C for 65 h. The mixture was filtered and evaporated, and the residue was chromatographed with 20% ethyl acetate in hexane to afford 112.1 mg (15%) of an oil: TLC (IV) R_f = 0.46; ^{1}H NMR ($CDCl_3$) δ 5.50 (br d, 1 H), 4.42–4.33 (m, 1 H), 3.72 (s, 3 H), 3.39 (s, 3 H), 3.11 (dd, 1 H), 2.88 (dd, 1 H), 2.57 (s, 3 H), 1.46 (s, 9 H).

***N*-(*tert*-Butyloxycarbonyl)-3-(*N*-methoxy-*N*-methylamino)-D,L-alanine (1i).** *N*-(*tert*-Butyloxycarbonyl)-2-(*N*-methoxy-*N*-methylamino)-D,L-alanine methyl ester (109 mg, 0.416 mmol) in dioxane (3 mL) at 0 °C was treated with LiOH monohydrate (24.0 mg, 0.572 mmol) in water (2 mL). After 1 h the reaction was quenched with 2.0 M HCl (0.29 mL, 0.28 mmol) and evaporated to a white foam which was used without further purification: ^{1}H NMR (CD_3OD) δ 4.33–4.24 (m, 1 H), 3.44 (s, 3 H), 2.95 (d, 2 H), 2.58 (s, 3 H), 1.45 (s, 9 H); MS *m/e* $(M + H)^+$ 249.

(2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (2). To 4 M HCl in dioxane (100 mL) was added (2*S*,3*R*,4*S*)-2-[(*tert*-butyloxycarbonyl)amino]-1-cyclohexyl-3,4-dihydroxy-6-methylheptane[10] (10.00 g, 29.11 mmol). The mixture was stirred at ambient temperature for 1 h. The solvent was evaporated, and the residue was dissolved in water which was washed with ether and then was brought to pH 8–9 with solid Na_2CO_3. The mixture was extracted with chloroform which was dried and evaporated to afford 7.09 g (100%) of a white solid, mp 108.5–110 °C. A portion of this product (0.75 g) was recrystallized from ethyl acetate (6 mL) and hexane (24 mL) to afford 0.66 g of fine plates: mp 110.5–111 °C; ^{1}H NMR ($CDCl_3$) δ 3.81 (dt, 1 H) 3.27 (dd, 1 H), 3.07 (dt, 1 H), 1.90–0.75 (envelope, 16 H), 0.97 (d, 3 H), 0.92 (d, 3 H); MS *m/e* $(M + H)^+$ 244; $[\alpha]_D^{23}$ −30.7° (*c* 1.21 EtOH). Anal. ($C_{14}H_{29}NO_2$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)histidine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (3a). Method A.** To 4 M HCl in dioxane (213 mL) was added (2*S*,3*R*,4*S*)-2-[(*tert*-butyloxycarbonyl)amino]-1-cyclohexyl-3,4-dihydroxy-6-methylheptane[10] (30.0 g, 87.3 mmol). The mixture was stirred at ambient temperature for 1 h. The solvent was evaporated followed by two portions of toluene, and the residue was dissolved in dimethylformamide (870 mL). Acid **1a** (22.3 g, 87.3 mmol), 1-hydroxybenzotriazole (17.7 g, 131 mmol), and *N*-methylmorpholine (19.2 mL, 175 mmol) were added. The mixture was cooled to −23 °C and treated with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC, 16.7 g, 87.3 mmol). After the mixture stirred for 2 h at −23 °C and overnight at ambient temperature, the dimethylformamide was evaporated, and the residue was taken up in ethyl acetate which was washed with half-saturated brine and saturated $NaHCO_3$, and then was dried and evaporated. Recrystallization (2×) of the residue from acetone afforded 16.7 g (40%) of a white solid: mp 182–184 °C; TLC (I) R_f = 0.36; ^{1}H NMR ($CDCl_3$) δ 9.40–9.00 (br, 1 H), 7.58 (d, 1 H), 6.93 (s, 1 H), 4.42–4.25 (m, 3 H), 3.34–3.23 (m, 1 H), 3.23–3.18 (m, 1 H), 3.00 (dd, 1 H), 1.95–1.80 (m, 1 H), 1.80–0.75 (envelope, 15 H), 0.93 (d, 1 H), 0.85 (d, 3 H). Anal. ($C_{25}H_{44}N_4O_5$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-3-pyrazol-3-yl-L-alanine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (3b). Method B.** To compound **2** (1.04 g, 4.29 mmol) in dimethylformamide (8 mL) were added acid **1b**[21] (1.10 g, 4.29 mmol), 1-hydroxybenzotriazole (1.58 g, 11.7 mmol), and *N*-methylmorpholine (0.62 mL, 5.6 mmol). The mixture was cooled to −23 °C and treated with 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride (EDC, 1.18 g, 6.16 mmol). After stirring 2 h at −23 °C and 15 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 1.5–7% methanol in chloroform afforded 1.31 g (64%) of a white solid: mp 175–180 °C; TLC (I) R_f = 0.54; ^{1}H NMR ($CDCl_3$) δ 7.52 (d, 1 H), 6.98 (br d, 1 H), 6.22 (d, 1 H), 5.60 (br d, 1 H), 4.63–4.53 (m, 1 H), 4.33–4.22 (m, 1 H), 3.22–2.98 (m, 3 H), 1.93–1.76 (m, 1 H), 1.76–0.75 (envelope, 15 H), 1.46 (s, 9 H), 0.92 (d, 3 H), 0.82 (d, 3 H); MS *m/e* $(M + H)^+$ 481. Anal. ($C_{25}H_{44}N_4O_5 \cdot 0.25H_2O$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-τ-methyl-L-histidine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (3c).** Prepared according to the method for **3b** in 79% yield following chromatography with 4% methanol in chloroform: mp 148–150 °C; TLC (I) R_f = 0.50; ^{1}H NMR ($CDCl_3$) δ 7.35 (s, 1 H), 6.79 (s, 1 H), 6.37 (br d, 1 H), 6.07 (br d, 1 H), 4.43–4.30 (m, 1 H), 4.30–4.20 (m, 1 H), 3.64 (s, 3 H), 3.32–3.21 (m, 1 H), 3.21–3.12 (m, 1 H), 3.05 (dd, 1 H), 2.94 (dd, 1 H), 1.95–1.80 (m, 1 H), 1.80–0.75 (envelope, 15 H), 1.46 (s, 9 H), 0.93 (d, 3 H), 0.83 (d, 3 H); MS *m/e* $(M + H)^+$ 495. Anal. ($C_{26}H_{46}N_4O_5 \cdot 0.75H_2O$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-3-thiophen-2-yl-L-alanine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (3e).** To acid **1e** (381 mg, 1.40 mmol) in dichloromethane (7 mL) at −10 °C was added *N*-methylmorpholine (0.165 mL, 1.50 mmol) followed by isobutyl chloroformate (0.185 mL, 1.41 mmol). After 3 min amine **2** (341 mg, 1.40 mmol) was added, and the mixture was stirred at −10 °C for 10 min and at ambient temperature for 2 h. The solvent was evaporated, and the residue was taken up in ethyl acetate which was washed with 0.5 M H_3PO_4, saturated $NaHCO_3$, and brine, and then was dried and evaporated. Chromatography of the residue with 30% ethyl acetate in hexane afforded 702 mg (100%) of a foam: TLC (IV) R_f = 0.55; ^{1}H NMR ($CDCl_3$) δ 7.22 (dd, 1 H), 6.97 (dd, 1 H), 6.90 (dd, 1 H), 6.00 (br d, 1 H), 4.98 (br, 1 H), 4.40–4.25 (m, 3 H), 4.12–4.03 (m, 1 H), 3.25–3.06 (m, 4 H), 1.96–1.82 (m, 1 H), 1.80–0.75 (envelope, 15 H), 1.43 (s, 9 H), 0.95 (d, 3 H), 0.90 (d, 3 H); MS *m/e* $(M + H)^+$ 497.

***N*-(*tert*-Butyloxycarbonyl)-3-imidazol-1-yl-L-alanine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (3f).** Prepared according to the method for **3b** in 47% yield following chromatography with 3–4% methanol in chloroform: mp 123–127 °C; TLC (I) R_f = 0.42, (V) R_f = 0.26; ^{1}H NMR ($CDCl_3$) δ 7.47 (s, 1 H), 7.08 (d, 1 H), 6.97 (d, 1 H), 6.41 (br d, 1 H), 6.12 (br d, 1 H), 4.47 (dd, 1 H), 4.44 (dd, 1 H), 4.35–4.22 (m, 2 H), 3.27–3.20 (m, 2 H), 1.98–1.82 (m, 1 H), 1.80–0.75 (envelope, 15 H), 1.45 (s, 9 H), 0.96 (d, 3 H), 0.91 (d, 3 H); MS *m/e* $(M + H)^+$ 481.

***N*-(*tert*-Butyloxycarbonyl)-3-pyrazol-1-yl-L-alanine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (3g).** Prepared according to the method for **3b** in 78% yield following chromatography with 0.5–1% methanol in chloroform: mp 161–163 °C; TLC (V) R_f = 0.55; ^{1}H NMR ($CDCl_3$) δ 7.53 (d, 1 H), 7.43 (d, 1 H), 6.28 (t, 1 H), 6.19 (br d, 1 H), 6.15 (br d, 1 H), 4.66 (dt, 1 H), 4.49–4.38 (m, 2 H), 4.32–4.19 (m, 1 H), 3.93 (br s, 1 H), 3.32–3.22 (m, 1 H), 3.22–3.12 (m, 1 H), 2.15 (br s, 1 H), 1.96–1.82 (m, 1 H), 1.73–0.75 (envelope, 15 H), 1.47 (s, 9 H), 0.95 (d, 3 H), 0.89 (d, 3 H); MS *m/e* $(M + H)^+$ 481. Anal. ($C_{25}H_{44}N_4O_5$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-3-azido-L-alanine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (3h).** Prepared according to the method for **3b** in 59% yield following chromatography with 25% ethyl acetate in hexane: TLC (IV) R_f = 0.51; ^{1}H NMR ($CDCl_3$) δ 6.32 (br d, 1 H), 5.25 (br d, 1 H), 4.36 (dt, 1 H), 4.30–4.22 (m, 1 H), 3.90–3.80 (m, 2 H), 3.60 (dd, 1 H), 3.37–3.25 (m, 1 H), 3.25 (dd, 1 H), 2.00–0.75 (envelope, 16 H), 1.47 (s, 9 H), 0.95 (d, 3 H), 0.89 (d, 3 H); MS *m/e* $(M + H)^+$ 456.

***N*-(*tert*-Butyloxycarbonyl)-3-(*N*-methoxy-*N*-methylamino)-D,L-alanine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (3i).** Prepared according to the method for **3b** in 77% yield following chromatography with 1.5% methanol in chloroform: TLC (V) R_f = 0.63; ^{1}H NMR ($CDCl_3$) δ 6.63, 6.53 (2 br d, total 1 H), 5.63, 5.58 (2 br d, total 1 H), 4.44–4.04 (envelope, 3 H), 3.51, 3.50 (2 s, total 3 H), 3.40–2.80 (envelope, 4 H), 2.62, 2.60 (2 s, total 3 H), 2.00–0.75 (envelope, 16 H), 1.46 (s, 9 H), 0.95, 0.93, 0.90, 0.88 (4 d, total 6 H); MS *m/e* $(M + H)^+$ 474.

***N*-(Benzyloxycarbonyl)leucine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (3j).** Prepared according to the method for **3e** in 99% yield. The crude product was used without further purification: TLC (VIII)

R_f = 0.44; ^{1}H NMR ($CDCl_3$) δ 7.42–7.28 (m, 5 H), 6.20 (br d, 1 H), 5.18–5.03 (m, 3 H), 4.32 (dt, 1 H), 4.22–4.07 (m, 2 H), 3.32–3.18 (m, 2 H), 2.10–0.75 (envelope, 19 H), 0.95 (d, 9 H), 0.88 (d, 3 H); MS *m/e* (M + H)$^+$ 491.

***N*-(*tert*-Butyloxycarbonyl)norleucine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (3k).** Prepared according to the method for **3b** in 91% yield following recrystallization from ethyl acetate in hexane: mp 126–129 °C; TLC (VI) R_f = 0.64, (VII) R_f = 0.54; ^{1}H NMR ($CDCl_3$) δ 6.16 (br d, 1 H), 4.87 (br, 1 H), 4.34 (dt, 1 H), 4.18 (d, 1 H), 4.02 (dd, 1 H), 3.34–3.22 (m, 1 H), 3.22 (dd, 1 H), 2.00–0.75 (envelope, 22 H), 1.45 (s, 9 H), 0.95 (d, 3 H), 0.91 (t, 3 H), 0.89 (d, 3 H); MS *m/e* (M + H)$^+$ 457. Anal. ($C_{25}H_{48}N_2O_5$) C, H, N.

H-Histidine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (4a). Method C. Compound **3a** (197.6 mg, 0.4111 mmol) in dichloromethane (4 mL) at 0 °C was treated with cold trifluoroacetic acid (4 mL) over 2 min. After the mixture was stirred at 0 °C for 2.5 h, the solvent was evaporated (30 °C bath) and the residue was dissolved in water. The mixture was made basic with solid K_2CO_3, saturated with NaCl, and extracted with chloroform which was dried and evaporated to afford 155.0 mg (99%) of a foam: TLC (II) R_f = 0.58; ^{1}H NMR ($CDCl_3$) δ 7.58 (d, 1 H), 7.23 (br, 1 H), 6.90 (s, 1 H), 4.28 (dt, 3 H), 3.67 (t, 3 H), 3.29–3.18 (m, 2 H), 3.08 (dd, 1 H), 2.98 (dd, 1 H), 1.97–1.82 (m, 1 H), 1.75–0.75 (envelope, 15 H), 0.93 (d, 3 H), 0.85 (d, 3 H).

H-3-Pyrazol-3-yl-L-alanine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (4b). Prepared according to the method for **4a**: TLC (I) R_f = 0.08; ^{1}H NMR ($CDCl_3$) δ 7.52 (d, 1 H), 7.35 (br d, 1 H), 6.19 (d, 1 H), 4.29 (dt, 1 H), 3.75 (dd, 1 H), 3.32–3.17 (m, 2 H), 3.16 (dd, 1 H), 3.07 (dd, 1 H), 2.00–1.82 (m, 1 H), 1.80–0.75 (envelope, 15 H), 0.93 (d, 3 H), 0.85 (d, 3 H); MS *m/e* (M + H)$^+$ 381. Anal. ($C_{20}H_{36}N_4O_3 \cdot 0.5H_2O$) C, H, N: calcd, 14.38; found, 13.76.

H-3-Thiophen-2-yl-L-alanine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (4e). Prepared according to the method for **4a** in 97% yield: TLC (I) R_f = 0.63, (V) R_f = 0.45; ^{1}H NMR ($CDCl_3$) δ 7.40 (br d, 1 H), 7.21 (dd, 1 H), 6.97 (dd, 1 H), 6.88 (dd, 1 H), 4.55–4.48 (m, 1 H), 4.30 (dt, 1 H), 3.68 (br, 1 H), 3.36 (dd, 1 H), 3.28–3.17 (m, 2 H), 3.16 (dd, 1 H), 2.02–1.85 (m, 1 H), 1.80–0.75 (envelope, 15 H), 0.96 (d, 3 H), 0.89 (d, 3 H); MS *m/e* (M + H)$^+$ 397. Anal. ($C_{21}H_{36}N_2O_3S \cdot 0.25H_2O$) C, H, N.

H-3-Imidazol-1-yl-L-alanine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (4f). Prepared according to the method for **4a** in 100% yield: mp 82–86 °C; TLC (I) R_f = 0.28; ^{1}H NMR ($CDCl_3$) δ 7.53 (s, 1 H), 7.42 (br d, 1 H), 7.10 (s, 1 H), 6.95 (s, 1 H), 4.46 (dd, 1 H), 4.30 (dt, 1 H), 4.23 (dd, 1 H), 3.74 (dd, 1 H), 3.30–3.15 (m, 2 H), 2.00–1.84 (m, 1 H), 1.85–0.75 (envelope, 15 H), 0.96 (d, 3 H), 0.89 (d, 3 H); MS *m/e* (M + H)$^+$ 381. Anal. ($C_{20}H_{36}N_4O_3$) C, H, N.

H-3-Pyrazol-1-yl-L-alanine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (4g). Prepared according to the method for **4a** in 100% yield: mp 120–123 °C; TLC (I) R_f = 0.52; ^{1}H NMR ($CDCl_3$) δ 7.52 (d, 1 H), 7.46 (br d, 1 H), 7.43 (d, 1 H), 6.27 (dd, 1 H), 4.51 (dd, 1 H), 4.42 (dd, 1 H), 4.22 (dt, 1 H), 3.79 (dd, 1 H), 3.26–3.12 (m, 2 H), 2.00–0.75 (envelope, 16 H), 0.95 (d, 3 H), 0.88 (d, 3 H); MS *m/e* (M + H)$^+$ 381. Anal. ($C_{20}H_{36}N_4O_3 \cdot 0.25H_2O$) C, H, N.

H-3-Azido-L-alanine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (4h). Prepared according to the method for **4a** in 95% yield: TLC (V) R_f = 0.35.

H-3-(*N*-Methoxy-*N*-methylamino)-L-alanine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (4i). Prepared according to the method for **4a**. The crude product was chromatographed with 3% methanol in chloroform to afford 47% of the D isomer [TLC (I) R_f = 0.46; ^{1}H NMR ($CDCl_3$) δ 7.60 (br d, 1 H), 4.30 (dt, 1 H), 3.66 (dd, 1 H), 3.50 (s, 3 H), 3.27–3.20 (m, 2 H), 2.97 (dd, 1 H), 2.72 (dd, 1 H), 2.60 (s, 3 H), 1.99–1.85 (m, 1 H), 1.85–0.75 (envelope, 15 H), 0.95 (d, 3 H), 0.87 (d, 3 H); MS *m/e* (M + H)$^+$ 374] and 43% of the desired L isomer:[22] TLC (I) R_f = 0.52; ^{1}H NMR ($CDCl_3$) δ 7.52 (br d, 1 H), 4.30 (dt, 1 H), 3.68 (dd, 1 H), 3.52 (s, 3 H), 3.29–3.17 (m, 2 H), 2.98 (dd, 1 H), 2.72 (dd, 1 H), 2.62 (s, 3 H), 1.99–1.85 (m, 1 H), 1.85–0.75 (envelope, 15 H), 0.95 (d, 3 H), 0.88 (d, 3 H); MS *m/e* (M + H)$^+$ 374.

H-Leucine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (4j). Compound **3j** (2.50 g, 5.09 mmol) and 10% Pd/C (0.25 g) in methanol (100 mL) were hydrogenated at 4 atm for 18 h. The mixture was filtered and evaporated, and the residue was chromatographed with 2.5% methanol in chloroform to afford 1.01 g (56%) of a white solid: mp 142–143.5 °C; TLC (V) R_f = 0.34; ^{1}H NMR ($CDCl_3$) δ 7.40 (br d, 1 H), 4.63–4.50 (m, 1 H), 4.29 (dt, 1 H), 3.48 (dd, 1 H), 3.28–3.16 (m, 2 H), 2.00–1.83 (m, 1 H), 1.83–0.75 (envelope, 18 H), 1.04–0.90 (m, 9 H), 0.88 (t, 3 H); MS *m/e* (M + H)$^+$ 357. Anal. ($C_{20}H_{40}N_2O_3 \cdot 0.30H_2O$) C, H, N.

H-Norleucine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (4k). Prepared according to the method for **4a** in 69% yield after recrystallization from dichloromethane in hexane: mp 146–148 °C; TLC (I) R_f = 0.49; ^{1}H NMR ($CDCl_3$) δ 7.41 (br d, 1 H), 4.29 (dt, 1 H), 3.47 (dd, 1 H), 3.28–3.16 (m, 2 H), 2.00–0.75 (envelope, 22 H), 0.95 (d, 3 H), 0.92 (t, 3 H), 0.88 (d, 3 H); MS *m/e* (M + H)$^+$ 357. Anal. ($C_{20}H_{40}N_2O_3$) C, H, N.

(1′*R*,2′*S*,5*S*)-5-(2′-Amino-3′-cyclohexyl-1′-hydroxypropyl)-3-ethyloxazolidin-2-one (5). (1′*R*,2′*S*,5*S*)-5-[2′-[(*tert*-Butyloxycarbonyl)amino]-3′-cyclohexyl-1′-(methoxymethoxy)propyl]-3-ethyloxazolidin-2-one[6] (4.64 g, 11.2 mmol) was stirred for 1 h at ambient temperature in 4.5 M ethanolic HCl. The solvent was evaporated, and the residue was dissolved in saturated $NaHCO_3$ solution. The mixture was extracted with chloroform which was dried and evaporated to afford 2.87 g (95%) of a white solid: mp 92–94 °C; TLC (I) R_f = 0.15; ^{1}H NMR ($CDCl_3$) δ 4.28 (dt, 1 H), 3.61 (dd, 1 H), 3.56 (dd, 1 H), 3.38–3.25 (m, 1 H), 3.325 (q, 1 H), 3.320 (q, 1 H), 3.23–3.13 (m, 1 H), 1.83–0.70 (envelope, 13 H), 1.18 (t, 3 H); MS *m/e* (M + H)$^+$ 271. Anal. ($C_{14}H_{26}N_2O_3$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-3-pyrazol-3-yl-D,L-alanine Amide of (1′*R*,2′*S*,5*S*)-5-(2′-Amino-3′-cyclohexyl-1′-hydroxypropyl)-3-ethyloxazolidin-2-one (6b).** Prepared according to the method for **3b**, using D,L-**1b**,[23] in 47% yield following chromatography with 2–2.5% methanol in chloroform: TLC (V) R_f = 0.36; ^{1}H NMR ($CDCl_3$) δ 7.47 (s, 1 H), 6.17, 6.14 (2 d, total 1 H), 5.88, 5.58 (2 br, total 1 H), 4.65, 4.50 (2 br, total 1 H), 4.10–3.00 (envelope), 1.80–0.70 (envelope, 13 H), 1.44 (s, 9 H), 1.13, 1.11 (2 t, total 3 H); MS *m/e* (M + H)$^+$ 508.

***N*-(*tert*-Butyloxycarbonyl)-τ-methyl-L-histidine Amide of (1′*R*,2′*S*,5*S*)-5-(2′-Amino-3′-cyclohexyl-1′-hydroxypropyl)-3-ethyloxazolidin-2-one (6c).** Prepared according to the method for **3b** in 86% yield following chromatography with 3% methanol in chloroform: TLC (V) R_f = 0.40; ^{1}H NMR ($CDCl_3$) δ 7.34 (d, 1 H), 6.83 (br d, 1 H), 6.72 (d, 1 H), 6.48 (br d, 1 H), 4.92–4.80 (m, 1 H), 4.12 (ddd, 1 H), 3.95–3.82 (m, 1 H), 3.63 (s, 3 H), 3.63–3.46 (m, 3 H), 3.305 (q, 1 H), 3.300 (q, 1 H), 3.11 (dd, 1 H), 2.92 (dd, 1 H), 1.80–0.70 (envelope, 13 H), 1.47 (s, 9 H), 1.16 (t, 3 H); MS *m/e* (M + H)$^+$ 522.

***N*-(*tert*-Butyloxycarbonyl)-3-thiazol-4-yl-L-alanine Amide of (1′*R*,2′*S*,5*S*)-5-(2′-Amino-3′-cyclohexyl-1′-hydroxypropyl)-3-ethyloxazolidin-2-one (6d).** Prepared according to the method for **3b**, using L-**1d**,[24] in 96% yield following chromatography with 0.5–1% methanol in chloroform: mp 135–140 °C; TLC (I) R_f = 0.61; ^{1}H NMR ($CDCl_3$) δ 8.79 (d, 1 H), 7.12 (d, 1 H), 6.79 (br d, 1 H), 6.58 (br d, 1 H), 4.95 (br, 1 H), 4.61–4.52 (m, 1 H), 3.73–3.60 (m, 1 H), 3.60–3.48 (m, 4 H), 3.31 (q, 1 H), 3.29 (q, 1 H), 3.15 (dd, 1 H), 1.80–0.70 (envelope, 13 H), 1.50 (s, 9 H), 1.17 (t, 3 H); MS *m/e* (M + H)$^+$ 525. Anal. ($C_{25}H_{40}N_4O_6S$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-3-thiophen-2-yl-L-alanine Amide of (1′*R*,2′*S*,5*S*)-5-(2′-Amino-3′-cyclohexyl-1′-hydroxypropyl)-3-ethyloxazolidin-2-one (6e).** Prepared according to the method for **3e** in 71% yield following chromatography with 50% ethyl acetate in hexane: TLC (IV) R_f = 0.14; ^{1}H NMR ($CDCl_3$) δ 7.20 (dd, 1 H), 6.96 (dd, 1 H), 6.88 (dd, 1 H), 6.63 (br d, 1 H), 4.97 (br d, 1 H), 4.58 (br, 1 H), 4.38 (dd, 1 H), 4.18 (dd, 1 H), 3.87–3.22 (envelope, 7 H), 1.80–0.70 (envelope, 13 H), 1.44 (s, 9 H), 1.16 (t, 3 H); MS *m/e* (M + H)$^+$ 524. Anal. ($C_{26}H_{41}N_3O_6S \cdot 0.25H_2O$) C, H, N.

H-Histidine Amide of (1′*R*,2′*S*,5*S*)-5-(2′-Amino-3′-cyclohexyl-1′-hydroxypropyl)-3-ethyloxazolidin-2-one (7a). Compound **6a**[25] (47 mg, 0.093 mmol) was stirred for 1 h at ambient temperature in 4.5 M ethanolic HCl. The solvent was evaporated,

and the residue was dissolved in saturated $NaHCO_3$ solution. The mixture was extracted with 25% 2-propanol in chloroform which was dried and evaporated to afford 38 mg (100%) of a white solid: ^1H NMR (CD_3OD) δ 7.61 (d, 1 H), 6.90 (s, 1 H), 4.25–4.10 (m, 2 H), 3.67–3.45 (m, 4 H), 3.00 (dd, 1 H), 2.80 (dd, 1 H), 1.80–0.80 (envelope, 13 H), 1.14 (t, 3 H). Exact mass calcd for $C_{20}H_{33}N_4O_5$ (M + H) 408.2611, found 408.2607.

H-3-Pyrazol-3-yl-L-alanine Amide of (1′*R*,2′*S*,5*S*)-5-(2′-Amino-3′-cyclohexyl-1′-hydroxypropyl)-3-ethyloxazolidin-2-one (7b). Prepared according to the method for **7a**. The crude product was chromatographed with 5–8% methanol in chloroform to afford 44% of the D isomer [TLC (I) R_f = 0.04; ^1H NMR ($CDCl_3$) δ 7.96 (br d, 1 H), 7.48 (s, 1 H), 6.18 (s, 1 H), 1.15 (t, 3 H); MS *m/e* (M + H)$^+$ 408] and 43% of the desired L isomer:[22] TLC (I) R_f = 0.09; ^1H NMR ($CDCl_3$) δ 7.99 (br d, 1 H), 7.49 (d, 1 H), 6.18 (d, 1 H), 4.12 (dd, 1 H), 3.85–3.72 (m, 2 H), 3.63–3.50 (m, 3 H), 3.30 (q, 1 H), 3.29 (q, 1 H), 3.22–3.06 (m, 2 H), 1.80–0.70 (envelope, 13 H), 1.14 (t, 3 H); MS *m/e* (M + H)$^+$ 408.

H-3-Thiazol-4-yl-L-alanine Amide of (1′*R*,2′*S*,5*S*)-5-(2′-Amino-3′-cyclohexyl-1′-hydroxypropyl)-3-ethyloxazolidin-2-one (7d). Prepared according to the method for **4a** in 98% yield: TLC (I) R_f = 0.31; ^1H NMR ($CDCl_3$) δ 8.77 (d, 1 H), 8.09 (br d, 1 H), 7.11 (d, 1 H), 5.59 (br d, 1 H), 3.90 (dd, 1 H), 3.76 (t, 3 H), 3.73–3.60 (m, 1 H), 3.60–3.45 (m, 3 H), 3.31 (q, 1 H), 3.30 (q, 1 H), 3.30–3.22 (m, 2 H), 2.00–0.70 (envelope, 13 H), 1.17 (t, 3 H); MS *m/e* (M + H)$^+$ 425. Anal. ($C_{20}H_{32}N_4O_4S$) C, H, N.

Benzyl (2*R*)-2-Benzyl-3-[(4-methylpiperazin-1-yl)carbonyl]propionate (10a). To acid **8**[15] (500 mg, 1.7 mmol) in dichloromethane (7 mL) at −10 °C was added *N*-methylmorpholine (0.20 mL, 1.8 mmol) followed by isobutyl chloroformate (0.22 mL, 1.7 mmol). After 5 min, 1-methylpiperazine (0.20 mL, 1.8 mmol) was added, and the mixture was stirred at −10 °C for 15 min and at ambient temperature for 2 h. The solvent was evaporated, and the residue was taken up in ethyl acetate which was washed with saturated $NaHCO_3$, water, and brine and then was dried and evaporated. Chromatography of the residue with 2% methanol in chloroform afforded 0.58 g (91%) of a foam: TLC (VII) R_f = 0.20; ^1H NMR ($CDCl_3$) δ 7.40–7.10 (m, 10 H), 5.16 (d, 1 H), 5.05 (d, 1 H), 3.70–3.25 (m, 5 H), 3.03 (dd, 1 H), 2.82 (dd, 1 H), 2.72 (dd, 1 H), 2.42–2.28 (m, 5 H), 2.28 (s, 3 H); MS *m/e* (M + H)$^+$ 381.

(2*R*)-2-Benzyl-3-[(4-methylpiperazin-1-yl)carbonyl]propionic Acid (10b). Compound **10a** (0.58 g, 1.5 mmol) and 10% Pd/C (0.30 g) in methanol (30 mL) were stirred under a hydrogen atmosphere for 3 h. The mixture was filtered and evaporated to afford 0.40 g (90%) of a solid: mp 135–140 °C; ^1H NMR ($CDCl_3$) δ 7.15–7.35 (m, 5 H), 3.88–3.48 (m, 4 H), 3.22–3.10 (m, 2 H), 2.80–2.50 (m, 7 H), 2.47 (s, 3 H), 2.30 (dd, 1 H); MS *m/e* (M + H)$^+$ 291.

Benzyl (2*R*)-2-Benzyl-3-[(chloromethyl)carbonyl]propionate (11). Acid **8**[15] (500 mg, 1.68 mmol) in CH_2Cl_2 (8 mL) at 0 °C was treated with oxalyl chloride (0.160 mL, 1.83 mmol) and dimethylformamide (6.5 μL, 0.084 mmol). After 2 h at 0 °C, the solvent was evaporated and the residue was dissolved in ether (6 mL), which was cooled to 0 °C and treated with an ether solution of CH_2N_2. After 2 h at 0 °C, the solvent was evaporated, and the residue was dissolved in ether (6 mL), cooled to −10 °C, and treated with 4.0 M HCl in dioxane (0.60 mL, 2.4 mmol). After 1 h, the solvent was evaporated, and the residue was chromatographed with 10% ethyl acetate in hexane to afford 477 mg (83%) of a colorless oil: TLC (IX) R_f = 0.31; ^1H NMR ($CDCl_3$) δ 7.40–7.08 (m, 10 H), 5.12 (d, 1 H), 5.08 (d, 1 H), 4.02 (s, 2 H), 3.37–3.23 (m, 1 H), 3.10 (dd, 1 H), 2.97 (dd, 1 H), 2.78 (dd, 1 H), 2.55 (dd, 1 H); MS *m/e* (M + H)$^+$ 331.

Benzyl (2*R*)-2-Benzyl-5-morpholin-4-yl-4-oxopentanoate (12a). Compound **11** (470 mg, 1.42 mmol) in dimethylformamide (10 mL) was treated with NaI (33 mg, 0.22 mmol) and morpholine (0.60 mL, 6.9 mmol). After 2 h the mixture was diluted with ethyl acetate, washed with water and brine, and then was dried and evaporated. Chromatography of the residue with 60% ethyl acetate in hexane afforded 460 mg (85%) of an oil: TLC (IV) R_f = 0.12, (V) R_f = 0.65; ^1H NMR ($CDCl_3$) δ 7.40–7.05 (m, 10 H), 5.11 (d, 1 H), 5.06 (d, 1 H), 3.68 (t, 4 H), 3.32–3.22 (m, 1 H), 3.15 (d, 1 H), 3.08 (d, 1 H), 3.10–3.00 (m, 1 H), 2.87 (dd, 1 H), 2.77 (dd, 1 H), 2.50–2.35 (m, 5 H); MS *m/e* (M + H)$^+$ 382.

(2*R*)-2-Benzyl-5-morpholin-4-yl-4-oxopentanoic Acid (12b). Prepared according to the method for **10b** in 90% yield: TLC (V) R_f = 0.26; ^1H NMR ($CDCl_3$) δ 7.35–7.15 (m, 5 H), 3.75–3.65 (m, 4 H), 3.25–3.10 (m, 2 H), 2.90–2.40 (m, 8 H), 2.17 (dd, 1 H); MS *m/e* (M + H)$^+$ 292.

Benzyl (2*S*)-2-Morpholin-4-yl-3-phenylpropionate (15a). Using the procedure of Kanamoto et al.[26] 2,5-dihydrofuran (0.78 mL, 10 mmol) in methanol (4 mL) and CH_2Cl_2 (16 mL) at −60 °C was treated with ozone until a blue color persisted. The excess ozone was removed under a stream of N_2, and $NaCNBH_3$ (456 mg, 7.26 mmol) was added. After 15 min at −60 °C, H-Phe-OBn *p*-toluene sulfonic acid salt (**14**, 2.22 g, 5.19 mmol) in methanol (20 mL) was added over 5 min, and the mixture was stirred at −60 °C for 15 min and at 0 °C for 20 h. The reaction was quenched with acetic acid (0.30 mL, 5.2 mmol), and after stirring at 0 °C for 30 min the solvent was evaporated. The residue was taken up in saturated $NaHCO_3$ solution and extracted into CH_2Cl_2 which was dried and evaporated. Chromatography of the residue with 20% ethyl acetate in hexane afforded 1.37 g (81%) of an oil: TLC (VI) R_f = 0.62), (IX) R_f = 0.12; ^1H NMR ($CDCl_3$) δ 7.35–7.10 (m, 10 H), 5.03 (s, 2 H), 3.75–3.62 (m, 4 H), 3.48 (dd, 1 H), 3.08 (dd, 1 H), 2.97 (dd, 1 H), 2.80–2.58 (m, 4 H); MS *m/e* (M + H)$^+$ 326.

(2*S*)-2-Morpholin-4-yl-3-phenylpropionic Acid (15b). Prepared according to the method for **10b** in 74% yield, after employing hot methanol to remove the product from the catalyst: mp 193–195 °C; TLC (I) R_f = 0.19; ^1H NMR (CD_3OD) δ 7.35–7.15 (m, 5 H), 3.78 (t, 4 H), 3.57 (dt, 1 H), 3.12 (d, 2 H), 3.10–2.98 (m, 4 H); MS *m/e* (M + H)$^+$ 236. Anal. ($C_{13}H_{17}NO_3 \cdot 0.25H_2O$) C, H, N.

1-Methylpiperidine-4-carboxylic Acid (17). To piperidine-4-carboxylic acid (1.00 g, 7.74 mmol, TLC (II) R_f = 0.36) in water (50 mL) was added aqueous formaldehyde (2.5 mL, 33 mmol, 37 wt %) and 10% Pd/C (1 g). The reaction was stirred under a hydrogen atmosphere overnight and then was filtered and evaporated with water (3×) and ethanol (2×) chasers. Recrystallization from ethanol in ethyl acetate afforded 0.68 g (61%) of a white solid: mp 164–168 °C (lit.[27] mp 172.5 °C); TLC (II) R_f = 0.27.

***N*-(*tert*-Butyloxycarbonyl)-*O*-methyltyrosine Benzyl Ester.** To Boc-Tyr-OH (4.76 g, 16.9 mmol) in dimethylformamide (70 mL) at 0 °C was added NaH (1.57 g, 41.0 mmol, 60% in oil). After 1 h iodomethane (1.05 mL, 16.9 mmol) was added and the reaction was stirred at 0 °C for 3 h. Benzyl bromide (2.20 mL, 18.5 mmol) was added, and the reaction was allowed to warm to ambient temperature and was stirred for 2 h. The reaction was quenched with acetic acid (1.0 mL, 17 mmol), poured into saturated $NaHCO_3$ solution and extracted into ethyl acetate which was washed with water and brine, then dried, and evaporated. Chromatography of the residue with 10% ethyl acetate in hexane afforded 3.61 g (55%) of a white solid: mp 54–57 °C; TLC (IX) R_f = 0.27; ^1H NMR ($CDCl_3$) δ 7.45–7.25 (m, 5 H), 6.94 (d, 2 H), 6.76 (d, 2 H), 5.18 (d, 1 H), 5.09 (d, 1 H), 4.96 (br d, 1 H), 4.63–4.52 (m, 1 H), 3.78 (s, 3 H), 3.03 (d, 2 H), 1.42 (s, 9 H); MS *m/e* M$^+$ 385. Anal. ($C_{22}H_{27}NO_5$) C, H, N.

***N*-[(1-Methylpiperidin-4-yl)carbonyl]-*O*-methyltyrosine Benzyl Ester (18a).** Prepared from **16a** (hydrochloride salt, from deprotection of Boc-(OMe)Tyr-OBn with HCl/dioxane) and **17** according to the method for **3b** in 63% yield: TLC (V) R_f = 0.10; ^1H NMR ($CDCl_3$) δ 7.43–7.30 (m, 5 H), 6.87 (d, 2 H), 6.73 (d, 2 H), 5.89 (br d, 1 H), 5.20 (d, 1 H), 5.12 (d, 1 H), 4.89 (dt, 1 H), 3.77 (s, 3 H), 3.13–3.02 (m, 2 H), 2.93–2.82 (m, 2 H), 2.28 (s, 3 H), 2.15–1.65 (m, 7 H); MS *m/e* (M + H)$^+$ 411.

***N*-[(1-Methylpiperidin-4-yl)carbonyl]-*O*-methyltyrosine (18b).** Prepared according to the method for **10b** in 91% yield: TLC (II) R_f = 0.42; ^1H NMR (CD_3OD) δ 7.12 (d, 2 H), 6.81 (d, 2 H), 4.55 (dd, 1 H), 3.74 (s, 3 H), 3.52–3.38 (m, 1 H), 3.19 (dd, 1 H), 3.10–2.82 (m, 3 H), 2.82 (s, 3 H), 2.57–2.42 (m, 1 H), 2.07–1.65 (m, 4 H); MS *m/e* (M + H)$^+$ 321.

***N*-[(4-Methylpiperazin-1-yl)carbonyl]-*O*-methyltyrosine Methyl Ester (19a).** To α-isocyanato-L-*O*-methyltyrosine methyl ester[15] (250 mg, 1.06 mmol) in CH_2Cl_2 (5 mL) was added 1-methylpiperazine (0.12 mL, 1.1 mmol). After 2 h, the solvent was evaporated, and the residue was chromatographed with 2% methanol in chloroform to afford 331 mg (93%) of a white solid: mp 86–89 °C; TLC (V) R_f = 0.26; ^1H NMR ($CDCl_3$)

δ 7.02 (d, 2 H), 6.83 (d, 2 H), 4.84 (br d, 1 H), 4.76 (t, 1 H), 3.79 (s, 3 H), 3.73 (s, 3 H), 3.43–3.30 (m, 4 H), 3.07 (d, 2 H), 2.37 (t, 4 H), 2.31 (s, 3 H); MS *m/e* (M + H)$^+$ 336.

***N*-[(4-Methylpiperazin-1-yl)carbonyl]-*O*-methyltyrosine (19b).** To compound **19a** (375.0 mg, 1.12 mmol) in dioxane (6 mL) at 0 °C was added LiOH monohydrate (56.3 mg, 1.34 mmol) in H_2O (3 mL). After 70 min the reaction was quenched with 2.0 M HCl (0.67 mL, 1.3 mmol), concentrated, diluted with brine, and extracted into chloroform which was dried and evaporated. The residue was suspended in ethanol and filtered, and the filtrate was evaporated to afford 344.8 mg (96%) of a white foam: ^{1}H NMR (CD_3OD) δ 7.13 (d, 2 H), 6.81 (d, 2 H), 4.47–4.37 (m, 1 H), 3.75 (s, 3 H), 3.55–3.46 (m, 4 H), 3.20–3.10 (m, 1 H), 2.97–2.75 (m, 5 H), 2.61 (s, 3 H); MS *m/e* (M + H)$^+$ 322.

***N*-[3-[(Benzyloxycarbonyl)amino]-3,3-dimethylpropionyl]-*O*-methyltyrosine Methyl Ester (21a).** Compound **20**[28] (2.86 g, 7.68 mmol), **16b**[29] (hydrochloride, 1.99 g, 8.10 mmol), *N*-methylmorpholine (0.98 mL, 8.9 mmol), and DMAP (0.10 g, 0.82 mmol) in dichloromethane (80 mL) and dimethylformamide (20 mL) were stirred at ambient temperature for 36 h. The mixture was concentrated and taken up in ethyl acetate which was washed with 2 M HCl (3×), 2 M NaOH (7×), and brine (1×) and then dried and evaporated. Chromatography of the residue with 33–50% ethyl acetate in hexane afforded 2.36 g (69%) of an oil: TLC (IV) R_f = 0.27; ^{1}H NMR ($CDCl_3$) δ 7.37–7.13 (m, 5 H), 6.99 (d, 2 H), 6.80 (d, 2 H), 6.11 (br d, 1 H), 5.32 (s, 1 H), 5.02 (d, 1 H), 4.97 (d, 1 H), 4.83–4.75 (m, 1 H), 3.76 (s, 3 H), 3.68 (s, 3 H), 3.02 (dd, 1 H), 2.92 (dd, 1 H), 2.63 (d, 1 H), 2.45 (d, 1 H), 1.36 (s, 3 H), 1.35 (s, 3 H); MS *m/e* (M + H)$^+$ 443.

***N*-[3-[(Benzyloxycarbonyl)amino]-3,3-dimethylpropionyl]-*O*-methyltyrosine (21b).** Prepared as described for **1h** in 93% yield: ^{1}H NMR ($CDCl_3$) δ 7.40–7.29 (m, 5 H), 7.07 (d, 2 H), 6.80 (d, 2 H), 6.38 (br d, 1 H), 5.25 (br s, 1 H), 5.03 (d, 1 H), 4.92 (d, 1 H), 4.77 (dd, 1 H), 3.72 (s, 3 H), 3.08 (dd, 1 H), 2.95–2.80 (m, 1 H), 2.72–2.60 (m, 1 H), 2.49 (d, 1 H), 1.33 (s, 3 H), 1.29 (s, 3 H); MS *m/e* (M + H)$^+$ 429. Anal. ($C_{23}H_{28}N_2O_6 \cdot 0.25H_2O$) C, H, N.

***N*-[3-Amino-3,3-dimethylpropionyl]-*O*-methyltyrosine Methyl Ester (21c).** Compound **21a** (2.35 g, 5.31 mmol) and 10% Pd/C (2.35 g) in ethyl acetate (60 mL) were stirred under a hydrogen atmosphere for 2.5 h. The reaction was filtered and evaporated to afford 1.43 g (87%) of an oil: ^{1}H NMR ($CDCl_3$) δ 8.38 (br d, 1 H), 7.07 (d, 2 H), 6.80 (d, 2 H), 4.88–4.79 (m, 1 H), 3.78 (s, 3 H), 3.72 (s, 3 H), 3.10 (dd, 1 H), 2.99 (dd, 1 H), 2.22 (s, 2 H), 1.18 (s, 3 H), 1.11 (s, 3 H); MS *m/e* (M + H)$^+$ 309.

***N*-[3-[(*tert*-Butyloxycarbonyl)amino]-3,3-dimethylpropionyl]-*O*-methyltyrosine Methyl Ester (21d).** Compound **21c** (1.42 g, 4.60 mmol) and di-*tert*-butyl dicarbonate (1.06 g, 4.86 mmol) in dichloromethane (50 mL) were stirred at ambient temperature for 16 h. Evaporation of the solvent afforded 1.97 g of an oil that was used without further purification: TLC (IV) R_f = 0.32; ^{1}H NMR ($CDCl_3$) δ 7.03 (d, 2 H), 6.82 (d, 2 H), 6.13 (br d, 1 H), 5.00 (s, 1 H), 4.87–4.78 (m, 1 H), 3.79 (s, 3 H), 3.70 (s, 3 H), 3.07 (dd, 1 H), 2.98 (dd, 1 H), 2.60 (d, 1 H), 2.51 (d, 1 H), 1.42 (s, 9 H), 1.33 (s, 3 H), 1.30 (s, 3 H); MS *m/e* (M + H)$^+$ 409.

***N*-[3-[(*tert*-Butyloxycarbonyl)amino]-3,3-dimethylpropionyl]-*O*-methyltyrosine (21e).** Compound **21d** (4.60 mmol) in dioxane (50 mL) at 0 °C was treated with LiOH monohydrate (0.30 g, 7.1 mmol) in water (17 mL). After stirring 90 min at 0 °C, the reaction was quenched with 2.0 M HCl (4.0 mL, 8.0 mmol) and concentrated. The mixture was taken up in ethyl acetate which was washed with water and brine and then dried and evaporated to a white solid. Recrystallization from ethyl acetate in hexane afforded 1.50 g (83% from **21c**) of fine needles: mp 157–158 °C; ^{1}H NMR ($CDCl_3$) δ 7.09 (d, 2 H), 6.81 (d, 2 H), 6.75–6.50 (br, 1 H), 4.80 (br, 1 H), 3.74 (s, 3 H), 3.20–3.06 (m, 2 H), 2.60–2.44 (m, 2 H), 1.46 (s, 9 H), 1.32 (s, 3 H), 1.25 (s, 3 H); MS *m/e* (M + H)$^+$ 395. Anal. ($C_{20}H_{30}N_2O_6$) C, H, N.

(2*R*)-*N*-[2-Benzyl-3-(morpholin-4-ylcarbonyl)propionyl]-3-(dimethylamino)-L-alanine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (34). Compound **33** (45.0 mg, 0.073 mmol), aqueous formaldehyde (0.033 mL, 0.44 mmol, 37 wt %) and 10% Pd/C (45 mg) in methanol (1.5 mL) were stirred under a hydrogen atmosphere for 24 h. The mixture was filtered and evaporated, and the residue was chromatographed with 3% methanol in chloroform to afford 27 mg (60%) of a solid: TLC (V) R_f = 0.28. Anal. ($C_{34}H_{56}N_4O_6 \cdot 0.5H_2O$) C, H, N.

[*N*-[3-[(*tert*-Butyloxycarbonyl)amino]-3,3-dimethylpropionyl]-*O*-methyltyrosinyl]-3-thiazol-4-yl-L-alanine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (Boc-36). Prepared from amine **4d**[30] according to the method for **3b** in 95% yield following chromatography with 1.5% methanol in chloroform: TLC (V) R_f = 0.49. Anal. ($C_{40}H_{63}N_5O_8S$) C, H, N.

Also prepared in this manner were the Boc-derivatives of **37**, **39**, and **40**. BOC-37: TLC (VI) R_f = 0.58. Anal. ($C_{41}H_{64}N_4O_8S$) C, H, N. Boc-39: TLC (V) R_f = 0.46. Anal. ($C_{40}H_{60}N_6O_9S$) C, H, N. Boc-40: TLC (V) R_f = 0.49. Anal. ($C_{41}H_{61}N_5O_9S$) C, H, N.

[*N*-[3-[(Benzyloxycarbonyl)amino]-3,3-dimethylpropionyl]-*O*-methyltyrosinyl]-L-histidine Amide of (1′*R*,2′*S*,5*S*)-5-[2′-Amino-3′-cyclohexyl-1′-hydroxypropyl]-3-ethyloxazolidin-2-one (Cbz-38). Prepared according to the method for **3a** in 48% yield following chromatography with 5% methanol in chloroform: TLC (V) R_f = 0.17. Anal. ($C_{43}H_{59}N_7O_9 \cdot 0.25H_2O$) C, H, N.

[*N*-(3-Amino-3,3-dimethylpropionyl)-*O*-methyltyrosinyl]-L-histidine Amide of (1′*R*,2′*S*,5*S*)-5-[2′-Amino-3′-cyclohexyl-1′-hydroxypropyl]-3-ethyloxazolidin-2-one Bisacetic Acid Salt (38). CBz-38 (178.5 mg, 0.218 mmol) and 10% Pd/C (160 mg) in acetic acid (6 mL) were stirred under a hydrogen atmosphere for 2.5 h. The mixture was filtered with methanol washes and evaporated. The residue was dissolved in water and lyophilized to afford 158 mg (90%) of a solid. Anal. ($C_{39}H_{61}N_7O_{11} \cdot 1.39H_2O$) C, H, N.

Physicochemical Properties. log *P* values and aqueous solubilities were determined as described previously.[25]

In Vitro and in Vivo Data. Potencies against human plasma renin[15] and monkey plasma renin[25] were determined as described previously. Compounds for id testing were formulated as follows: the test compound was dissolved (40 mg/mL) in ethanol, 110 mol % of HCl was added, and the solution was diluted with an aqueous solution of hydroxypropyl methylcellulose to a final ethanol concentration of 25% (10 mg/mL in the test compound). Compounds for iv testing were formulated as follows: The test compound was dissolved (30 mg/mL) in ethanol, 110 mol % of HCl was added, and the solution was diluted with an aqueous solution of 5% dextrose to a final ethanol concentration of 10% (3 mg/mL in the test compound). Dosing via the intraduodenal or intravenous routes was performed as described previously.[15] Plasma drug levels were determined via a renin inhibition assay[12] or by HPLC as follows: Plasma samples (0.40 mL) were mixed with 10% Na_2CO_3 and extracted with ethyl acetate. An aliquot was evaporated to dryness and reconstituted in mobile phase (40% CH_3CN in water containing 0.1% CF_3CO_2H). The solution was assayed by HPLC utilizing a Waters μ Bondpack C_{18} reverse-phase column eluting with mobile phase at a flow rate of 1.5 mL/min with detection at 214 nm. The recoveries of known concentrations of inhibitors added to plasma ranged from 75% to 85%.

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