Expedited Articles

Nonpeptide Renin Inhibitors with Good Intraduodenal Bioavailability and Efficacy in Dog

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The aim of this study was the discovery of nonpeptide renin inhibitors with much improved oral absorption, bioavailability, and efficacy, for use as antihypertensive agents. Our prior efforts led to the identification of A-74273 [1, R = 3-(4-morpholino)propyl], with a bioavailability of $26 \pm 10\%$ [10 mg/kg intraduodenally (id), dog]. In vivo metabolism studies of A-74273 showed that the morpholino moiety underwent metabolic degradation. Computer modeling of A-74273 bound to renin indicated that the C-terminus was involved in a hydrogen-bonding network. New C-terminal groups were examined in two series of nonpeptides for effects on renin binding potency, lipophilicity (log P), and aqueous solubility. Those groups which possessed multiple hydrogen-bonding ability (3,5-diaminotriazole, cyanoguanidines, morpholino) provided particularly potent renin binding. Intraduodenal bioavailabilities of selected compounds, evaluated in rats, ferrets, and dogs, were higher for inhibitors with moderate solubility as well as moderate lipophilicity, in general. Although the absolute values varied substantially among species, the relative ordering of the inhibitors in terms of absorption and bioavailablity was reasonably consistent. Such well absorbed inhibitors (e.g. 41, 44, and 51) were demonstrated as highly efficacious hypotensive agents in the salt-depleted dog. We report here the discovery of a series of efficacious nonpeptide renin inhibitors based on the 3-azaglutaramide P_2 - P_4 replacement, the best of which showed id bioavailabilities >50% in dog.

Introduction

The process of transforming a peptidic molecule into an orally active therapeutic agent remains a significant challenge to medicinal chemists. In the case of inhibitors of renin, the enzyme responsible for the ratelimiting step in the renin-angiotensin cascade, many approaches have been investigated with modest success.² Our efforts toward an orally active renin inhibitor were recently reported for our two nonpeptide renin inhibitor series, based on a 3-oxa- or 3-azaglutaramide replacement for the P2-P4 positions.³ That report^{3b} highlighted our inhibitor A-74273 (1), which was shown to be an efficacious antihypertensive agent in dog and ferret, with an id⁴ bioavailability of $27 \pm 14\%$ (10 mg/ kg) in dog.⁵ We were encouraged by these results and undertook additional studies to improve the in vitro binding affinity, oral absorption, bioavailabilty, and efficacy of both of these novel series.

To help guide our inhibitor design, the major mode of in vivo metabolism of ¹⁴C-labeled A-74273 was elucidated. In addition, we utilized computer modeling of A-74273 bound to renin to propose possible binding interactions. We previously had found that the presence of a basic nitrogen atom at the C-terminus of our inhibitors correlated with higher in vitro renin binding affinity. Taken together, this information suggested potentially beneficial structural modifications for the C-terminal portion of the molecule. We sought to identify C-terminal solubilizing groups that would improve potency and avoid the A-74273 metabolic pathway, and thus confer increased in vivo stability and oral bioavailability to such derivatives. The new inhibitors were designed to examine the following parameters: (1) the solubilizing group tether length, (2) the basicity of the nitrogen group, (3) the effect of steric hinderance around the nitrogen atom, and (4) the effect of solubility and lipophilicity on absorption and bioavailability. Where possible, the potential metabolites were synthesized and characterized, in order to assist with the metabolism studies to be discussed below. Analogues bearing both 3-oxa- and 3-azaglutaramides were synthesized, the former due to their greater in vitro potency, and the latter due to the relative ease of synthesis of the enalapril-like backbone. We then attempted to correlate id absorption and bioavailability of the inhibitors with their physicochemical properties $(\log P, \text{ solubility})$. In this article, we report the discovery of highly efficacious new nonpeptide renin inhibitors with id bioavailabilities >50% in the dog.

Results from Metabolism Study of ¹⁴C-Labeled A-74273

We were alerted to the possibility that A-74273 produced active metabolites in vivo by discrepencies between HPLC and bioassay quantification of plasma drug concentrations. Three major metabolites of 14 C-labeled A-74273 were isolated from rat and dog bile after

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Scheme 1. Metabolic Pathways for A-74273 (1)



intravenous or intraduodenal dosing. Purification of the extracts by HPLC, characterization by LCMS, and synthesis of the proposed structures (based on molecular weight and chromatographic mobility) allowed the positive identification of the metabolic intermediates shown in Scheme 1. The morpholino group undergoes oxidative removal of two ring carbons to produce ethanolamine 2 or of the diethyleneoxy group to give primary amine 3. Alternatively, oxidative cleavage of the morpholino group would give carboxylic acid 4. Note that all of these metabolites are themselves potent inhibitors of human renin. Similar results were obtained upon oral dosing of 1 in conscious, salt-depleted dogs.⁴ Further details of the metabolism of 1 will be reported elsewhere in due course.

Computer Modeling

We utilized computer modeling to help define possible binding modes of A-74273, in order to guide the design of more potent analogues. We previously had found that the presence of a basic nitrogen atom at the C-terminus of our inhibitors correlated with higher in vitro binding affinity.^{3b} To begin to understand the reasons for the enhanced binding of the amine-bearing inhibitors to renin, computer modeling of 1 in our human renin model⁶ was performed. Inhibitor 1 was placed in the active site of the renin model, and iterative cycles of molecular mechanics energy minimization and molecular dynamics simulations⁷ placed the C-terminal morpholinopropyl group in a shallow pocket, presumably not normally occupied by substrate or extended inhibitors. As can be seen in Figure 1a, this pocket is lined with numerous hydrogen-bond donors and acceptors. We then proposed that incorporation of strong hydrogen bonding elements into the C-terminus of our nonpeptide inhibitors would lead to even more potent compounds. As an early example of such a hydrogen-bonding moiety, Figure 1b illustrates the calculated binding mode of a C-terminal urea-bearing inhibitor (**66**). Note the three hydrogen bonds which "stitch" together the urea moiety with the two enzyme regions. In particular, Ser 36, Asn 37, and Arg 74 of renin are involved in a hydrogen-bonding network with the urea group, and the hydroxy-ethylene C-terminal amide is bridged by hydrogen bonds with Gly 34 and Ser 76. This hypothesis led us to investigate a variety of such hydrogen-bonding moieties.

Chemistry

The synthesis of the inhibitors followed the general plan described earlier.^{2b} A protected hydroxyethylene dipeptide isostere was constructed and then deprotected and coupled to the previously described carboxylic acids. In many cases, further manipulation of the C-terminal functionality was required to produce the desired inhibitors. Scheme 2 delineates the synthesis of doublyprotected hydroxyethylene amides 6-12 from acetonide carboxylic acid 5,⁸ using a carbodiimide coupling method. The corresponding structures for the C-terminal portion of these amides are shown in Tables 1 and 2. Tetrazole 12 was synthesized from cyanomethylamide 11 by a standard method.⁹ The remaining protected hydroxyethylene isosteres were conveniently obtained by aminolysis of the known lactone 13,10 as shown in Scheme 3. Thus, hydroxyamides 14-25 were obtained by warming a solution of lactone in neat amine for extended periods of time. In the cases where reaction was sluggish, elevated temperatures or longer reaction times led to increasing epimerization of the isopropyl-bearing carbon. This lactone opening reaction was substantially accelerated by the addition of acetic acid to the reaction medium, which also alleviated the problem of epimeriation. Sterically hindered morpholine 26 was produced by a double reductive alkylation of primary amine 20 with 3-oxaglutaric dialdehyde (synthesized and used



Figure 1. Panel A (top) shows a computer model of nonpeptide inhibitor **1** bound to the active site of human renin (crossedstereoview). The enzyme backbone is shown in ribbon representation, with only the side chains involved in hydrogen bonding (dashed lines) displayed. The active-site aspartic acids 32 and 215 provide the critical binding to the hydroxyl group of the inhibitor. The C-terminal morpholine is positioned in a pocket lined with hydrogen-bonding groups. Panel B (bottom) depicts the calculated binding mode of the urea-bearing inhibitor **66** (see Table 1). Serine 36, asparagine 37, and arginine 74 are involved in a hydrogenbonding network with the urea group, and the hydroxyethylene C-terminal amide is bridged by hydrogen bonds with the backbone of glycine 34 and serine 76.

in situ).¹¹ The amine oxides 27 and 28 were produced via hydrogen peroxide oxidation of amines 14 and 15,

respectively. Phenethylamide 18 was hydrogenated to yield the saturated derivative 29 in straightforward

Scheme 2. Synthesis of Hydroxyethylene Amides via Carboxylic Acid $\mathbf{5}^{a}$



 $^{\alpha}$ Reaction conditions: (a) EDC, HOBT, 4-methylmorpholine, DMF, 0 °C, 24–48 h, then H_2NR, 0–25 °C; (b) NaN_3, Et_3NHCl, DMF, 100 °C.

fashion. Primary amine 16 was converted to 1,2,4-oxadiazole 30 by a known procedure.¹¹

As Scheme 4 illustrates, the protected dipeptide isosteres were deprotected in the appropriate manner to provide the free amino alcohols **31**, which were not purified. The isolated crude amino alcohols were coupled to ether-containing carboxylic acid **32** under standard carbodiimide conditions to afford the 3-oxaglutaramide inhibitors **34–40** or with amino acid **33** to afford 3-azaglutaramide inhibitors **41-57** (see Tables 1 and 2 for R group structures).

As mentioned above, several inhibitors were synthesized by further modification of the C-terminus. Scheme 5 shows the hydrogenolysis of benzyl carbamates 34, 36, and 46 into the versatile primary amines 3, and 60, and 61. Scheme 5 also summarizes the transformation of amines 3 and 60 into acetamides 62 and 65, ureas 63 and 66, and thioureas 64 and 67 by straightforward methods. Scheme 6 illustrates the conversion of these amines into a variety of guanidine-based derivatives.¹² We initially had planned to produce these derivatives of the protected hydroxyethylene isosteres but found that these amides (with the exception of 30) were not stable to the TFA-deprotection conditions. Thus, amines 3, 60, and 61 were each reacted with dimethyl Ncyanodithioiminocarbonate to give the isothioureas 68, 69, and 70, respectively. The isothioureas were further substituted with amines to produce guanidines 71-74and with hydrazine to produce 1,2,4-triazoles 75-77. The C-terminal carboxylic acids 4, 78, and 79 were obtained from the corresponding esters 35, 43, and 47, as shown in Scheme 7. The hindered primary amine 52 was transformed into a potential metabolite of morpholine 51 as depicted in Scheme 8. Amine 52 was reductively alkylated with (benzyloxy)acetaldehyde, and the intermediate benzyl ether was hydrogenolyzed to provide ethanolamine 80.

In a few examples, it proved more expedient to synthesize the inhibitors by aminolysis of lactones 81 or 82, thus minimizing protection/deprotection (Scheme 9). Lactone 13 was deprotected and coupled to acids 32 and 33 by the carbodiimide method, smoothly providing amides 81 and 82, respectively. Lactone 81 was opened with 4-(aminobutyl)morpholine to give morpholinobutylamide 83, and with 1,4-diaminobutane Bovd et al.

Table 1. In Vitro Potency against Human Plasma Renin,Partition Coefficients, and Aqueous Solubilities for3-Oxaglutaramide Inhibitors

Oxagluta	aramide Inhibitors			
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		10		
		buman	log P	aqueous
		plasma renin,	pH 7.4	solubility
inhibitor	R	pH 7.44	(pH 6.5) ^b	pH 7.4, mg/mL ^c
1	CHARANA	2.8	4.3 (3.3)	0.210
	L N			
2	(CH2)3NH(CH2)2OH	6.0	1.8 (2.5)	1.84
34	(CH ₂) ₃ NHChz	4.3	ndd	nd
3	(CH ₂) ₃ NH ₂	4.5	1.8 (2.4)	1.87 nd
35	(CH ₂) ₂ CO ₂ H	12	nd 2.8 (1.9)	nd
62	0 0	2.4	4.3 (4.3)	0.180
	CH2 N CH2			
63	ю	2.0	4.2 (4.2)	0.015
	CH2 NH			
6.4	н,	25	nd	nd
04	CH2	2.5	110	nu
	H NH2			
68	N ^{.CN}	1.3	nd	nd
	CH2 N SCH3			
71	n N CN	1.5	4.5 (4.6)	nd
	CH5 A.			
	H NH2			
72	N ^{CN}	1.3	4.4 (4.4)	nd
	CH2 NHCH3			
75	н	1.7	4.2 (4.3)	nd
	N [™] ≻ NHa			
	CH2 N N			
84	(CH ₂) ₄ NH ₂	4.3	nd	nd
83	CH ₂ N	3.3	nd	nd
36		n	nd	nd
60	(CH2)2NHC02 (CH2)2NH2	3.9	nd	nd
		2.6		nd
0.5	CH2 V N CH3	2.0	nu	nu
	0			
66	CH ₂ N, NH ₂	2.8	nd	nd
67	CH. N. NH.	2.2	>5 (>5)	nd
37	CH2 , N	8.4	2.0 (2.9)	nd
	V NR N'N			
69	CHA. H SCH.	9.2	>5 (>5)	nd
73	H H	1.8	>5 (>5)	nd
	$CH_2 \sim N \sim NH_2$			
	N. CN			
76	CH ₂ N N	1.1	4.0 (3.9)	0.285
	N NH2			
40	្រំដំ	2.1	44 (4 5)	0.017
40	CH2 N N	3.1	4,4 (4.2)	0.047
	$\stackrel{ }{\sim} \stackrel{\sim}{\rightarrow} \stackrel{\rm NH_2}{\sim}$			
38	CH2C(CH3)2NH2	4.7	3.5 (2.7)	0.660
39	<u>^</u> 0	5.9	>> (>>)	na
	^{CH2} × ^N			

^aSingle determination in most cases. ^bApparent octanol-aqueous phosphate buffer partition coefficient. ^cDetermined at 37 ^oC in 0.05 M phosphate containing 0.15 M NaCl. ^dnd = Value not determined.

to produce aminobutylamide **84**. Similarly, lactone **82** was opened with 3-[(2-hydroxyethyl)amino]propylamine and 2-[(2-hydroxyethyl)amino]ethylamine to give amides **86** and **87**, respectively. Unfortunately, this route suffered from a greater degree of epimerization of the isopropyl-bearing carbon than the corresponding reaction of carbamate lactone **15**, requiring careful purification of the products.

In Vitro Results for 3-Oxaglutaramide-Containing Inhibitors

Table 1 contains inhibitory potencies against human plasma renin, experimental octanol/aqueous buffer partition coefficients $(\log P)$, and solubilities measured in aqueous isotonic buffer for the new analogs of **1**. Most of these C-terminal groups were chosen on the basis of their ability to engage in hydrogen-bonding arrays, to test our computer-modeling hypothesis discussed above. Scheme 3. Synthesis of Hydroxyethylene Dipeptide Isosteres via Lactone 15^a



^a Reaction conditions: (a) H_2NR neat, 60–80 °C, 2–5 days; or H_2NR (9 equiv), HOAc (3 equiv), 65 °C, 1–2 h. See Table 2 for R-group structures; (b) (1) 3,4-dihydrofuran, CH₂Cl₂, MeOH, O₃, -70 to -65 °C; (2) NaBH₃CN; (c) H_2O_2 , MeOH, 25 °C; (d) 4 atm H_2 , 5% Rh/C, MeOH; (e) (1) (MeS)₂C=N(CN), CH₃CN, reflux, (2) H_2NOH , Et₃N, EtOH, 80 °C.

We found that such compounds did indeed provide very potent binding to renin (1.1 nM for 76), similar in potency to inhibitor 1. Thus ureas, thioureas, cyanoguanidines, and 1,2,4-triazoles were particularly good binding groups. The larger benzyl carbamates 34 and 37 and ester 35 were less effective inhibitors. The fourcarbon tethered amines 83 and 84 were of equivalent potency to 1, despite the extra methylene group. The series containing a two-carbon spacer was in general modestly more potent than the three-carbon spacer versions. However, the differences in binding potency among these various inhibitors were minimal when compared to those devoid of hydrogen-bonding C-terminal groups.^{3b} As computer modeling was not done for each of these compounds, it is not clear how this lack of geometric specificity relates to our proposed binding mode. In any case, substantial differences were evident in the lipophilicities and solubilities measured: the twocarbon series was more lipophilic than the three-carbon series, and the amides and urea derivatives were less soluble than amine-bearing inhibitors, as expected from previous results.^{3b}

In Vitro Results for 3-Azaglutaramide Inhibitors

Inhibitory Potencies and Physicochemical Properties. Analogues similar to those discussed above were examined for the 3-azaglutaramides, with the goal of identifying equally high affinity members of this synthetically accessible series. Table 2 presents the in vitro data for these compounds. Inhibitory activity against plasma renin was determined in human as well as three model animal species (rat, ferret, and dog). Inhibitor 41, disclosed previously,^{3b} served as the benchmark for comparisons of binding affinities within this group. In surveying the activities against human plasma renin, it was apparent that there were few significant differences among the hydrogen-bonding C-terminal groups. Only the hydrocarbon-based 49 (isosteric with 44 and 48) had significantly less affinity for renin, with an IC_{50} of 40 nM. In contrast, benzyl amide 56 had only slightly less binding affinity than the isosteric pyridinylmethylamides 53-55, despite the absence of a heteroatom. For the morpholine-bearing amides, the potential metabolites were of roughly Scheme 4. Coupling of Hydroxyethylene Dipeptide Isosteres 6-12, 14, and 16-30 to Carboxylic Acids 32 and 33^{a}



^a Reaction conditions: (a) (1) TFA, CH₂Cl₂ (1:1), 0 °C; (2) H₂O, THF, TFA, 20 °C; (3) Na₂CO₃; (b) (1) TFA, CH₂Cl₂ (1:1), 0 °C; (2) Na₂CO₃; (c) **32** or **33**, EDC, HOBT, 4-methylmorpholine, DMF, -20 to 20 °C.

Scheme 5. Preparation and Acylation of Primary Amines **3**, **60**, and **61** To Produce Inhibitors Containing Hydrogen-Bond-Donor/-Acceptor C-Terminal Groups^{*a*}



^a Reaction conditions: (a) H₂ (4 atm), 10% Pd/C, EtOAc; (b) N-acetoxy-5-norbornene-2,3-dicarboximide, CH₂Cl₂, 25 °C; (c) N-trimethylsilyl isocyanate, THF, 0-25 °C; (d) (1) N,N'-thiocarbonyldiimidazole, CH₂Cl₂, 0-25 °C; (2) concentrated aqueous NH₃, 25 °C.

equivalent potency to the parent: e.g. **41** vs **42**, **85**, **86**, and **78**. The two new analogues with the best binding affinities were morpholinoethylamide **44** ($IC_{50} = 3.9 \text{ nM}$) and 5-amino-1,2,4-oxadiazole **58** ($IC_{50} = 3.8 \text{ nM}$), both of which satisfied our goal of achieving an in vitro potency equivalent to that of A-74273.

None of the analogues exhibited $<1 \mu$ M inhibition of rat renin. Modest activity (<300 nM) against ferret plasma renin was detected for the morpholine-bearing **41**, **44**, and **51**. Interestingly, many of these compounds were quite effective inhibitors of dog plasma renin, with binding affinities in the range of 14–75 nM. This finding allowed us to examine the in vivo blood pressure efficacy of these analogues in the dog, as will be discussed below.

Table 2 also lists the physical parameters of log *P* at physiologic and mildly acidic pH, plus solubility in aqueous buffer at physiologic pH. The compounds fall into three general categories: (a) moderately lipophilic (log *P* < 4) and soluble (>1 mg/mL), (b) lipophilic (log *P* ~ 4) and modestly soluble (~0.1 mg/mL), and (c) very lipophilic (log *P* > 5) and poorly soluble (<0.1 mg/mL). These classifications will be referred to in the discussion of in vivo results.

Scheme 6. Preparation of Inhibitors Containing Guanidine-Based Hydrogen-Bond-Donor/-Acceptor C-Terminal Groups from Primary Amines 3, 60, 61, and 85^a



^a Reaction conditions: (a) (CH₃S)₂C=N(CN), CH₃CN, reflux; (b) concentrated aqueous RNH₂, EtOH, 90 °C (sealed tube); (c) H₂NNH₂H₂O, EtOH, reflux; (d) H₂NOH·HCl, Et₃N, EtOH, reflux.

Scheme 7. Synthesis of C-Terminal Carboxylic Acids 4, 78, and 79^{a}



 a Reaction conditions: (a) LiOH, THF, H_2O, 0–20 °C, then NaHSO4; (b) H_2 (1 atm), 10% Pd/C, EtOAc.

Microsomal Metabolism Results. As mentioned above, 1 has been shown to undergo cytochrome P-450mediated hepatic biotransformation in animals primarily at the morpholino terminus of the molecule, affording 2-4.¹³ In an attempt to identify structural features which would reduce the propensity for biotransformation of this terminus without negatively affecting solu-

bility or potency, the rates of in vitro microsomal metabolism of nine azaglutaramide analogues were determined in microsomal preparations from two different human livers. The mean rates of metabolism (normalized for cytochrome P-450 content) and HPLC retention times of parent and metabolites for each are listed in Table 3. Comparison of the mean rates of Scheme 8. Synthesis of C-Terminal Hindered Amines 39 and 80^a



^a Reaction conditions: (a) (1) 3,4-dihydrofuran, CH_2Cl_2 , MeOH, O₃, -70 to -65 °C; (2) NaBH₃CN; (3) 38, -60 to 20 °C; (b) (benzyloxy)acetaldehyde, NaBH₃CN, ⁱPrOH, HOAc, NaOAc, 0-20 °C; (c) H₂ (4 atm), Pd(OH)₂/C, EtOAc, HOAc.

Scheme 9. Synthesis of Inhibitors 83-87 via Lactones 81 and 82^a



^a Reaction conditions: (a) TFA, CH₂Cl₂ (1:1), 0 °C, then 1 M aqueous Na₂CO₃; (b) **32** or **33**, EDC, HOBT, 4-methylmorpholine, DMF, -20 to 20 °C; (c) H₂NR neat, 60-80 °C, 2-4 days, or H₂NR (9 equiv), HOAc (3 equiv), HOAc (3 equiv), 65 °C, 1-2 h. See Tables 1 and 2 for R-group structures.

conversion of each parent compound to those of the metabolites indicated that 1, 41, 44, 48, and 51 were all metabolized at nearly identical rates by the in vitro system. A comparable rate of metabolism was also observed for the 2-substituted pyridine 53, while noticeably slower rates were seen for the 3- and 4-substituted pyridines 54 and 55. Imidazolylpropylamide 59 was metabolized at a rate 4-5-fold slower than that of the morpholine-substituted compounds.

Metabolites of some of the nine microsomal substrates could be putatively identified by HPLC coelution with synthetic standards. Compound 1 was converted to the expected products of morpholino ring oxidative degradation, ethanolamine 2, amine 3, and carboxylic acid 4, in agreement with the in vivo metabolism results discussed above. Similarly, 41 and 51 apparently also underwent morpholino ring degradation analogous to that for 1. Thus, microsomal metabolism of 41 generated UV peaks that coeluted with ethanolamine 86, primary amine 85, and carboxylic acid 78, and 51 generated peaks that coeluted with ethanolamine 80 and primary amine 52. Curiously, 44, the one-carbonshorter homologue of 41, apparently underwent oxidative opening of the morpholino ring to afford the corresponding ethanolamine metabolite 87, yet the metabolite corresponding to the carboxylic acid standard 79 was not observed. Compound 48, bearing a piperidine substituent in place of the morpholino moiety, afforded primarily one somewhat more polar metabolite. No standards (e.g. *N*-oxides) were prepared to evaluate metabolites of 48, the substituted pyridine compound series 53-55, or imidazole 59.

Table 2. In Vitro Potency against Plasma Renins, Partition Coefficients, and Aqueous Solubilities for 3-Azaglutaramide Inhibitors



IC 50 in plasma, nM ^a							aqueous solubility,
inhibitor	R	human	rat	ferret	dog	pH 7.4 (pH 6.5) ^b	pH 7.4, mg/mL ^c
41		7.3	5300	290	16	4.5 (3.9)	0.167
42		4.3	6400	420	19	3.6 (3.6)	1.927
86	(CH ₃) ₃ NH(CH ₂) ₂ OH	7.9	ndd	nd	nd	3.5 (2.9)	1.606
85	(CH2)3NH2	9.5	nd	nd	nd	3.3 (2.7)	1.322
43	(CH ₂) ₂ CO ₂ CH ₂ Ph	23	nd	nd	nd	nd	nd
78	(CH ₂) ₂ CO ₂ H	8.4	nd	nd	nd	3.3 (2.8)	10.78
44	γ γ	3.9	3800	170	14	>5 (>5)	0.141
	CH ₂ N	<u> </u>				4.05 (4.05)	
45	CH ₂ N:	8.4	na	na	na	4.07 (4.07)	1.510
87	(CH2)2NH(CH2)2OH	11	nd	nd	nd	nd	nd
46	(CH ₂) ₂ NHCbz	17	nd	nd	nd	nd	nd
61	(CH ₂) ₂ NH ₂	9.7	nd	nd	nd	3.5 (2.7)	0.632
47	CH ₂ CO ₂ CH ₂ Ph	21	nd	nd	nd	nd	nd
79	CH ₂ CO ₂ H	12	nd	nd	nd	2.8 (2.3)	>10
48	$CH_2 \sim N$	14	nd	nd	nd	>> (>>)	0.093
49	СН, О	40	nd	nd	nd	>5 (>5)	0.006
50		13	nd	nd	nd	>5 (>5)	0.817
51		9.1	2100	120	14	>5 (>5)	0.023
80	CH2C(CH3)2NH(CH2)2OH	6.3	nd	nd	nd	nd	nd
52	CH2C(CH3)2NH2	12	nd	nd	nd	3.8 (3.2)	0.180
53	CH ₂ N	12	>10000	1000	75	>5 (>5)	0.0126
54	CH ₂ N	9.6	9100	550	44	>5 (>5)	0.006
55	CH ₂	7.8	10000	940	59	>5 (>5)	0.020
56	CH ₂ Ph	14	nd	nd	nd	>5 (>5)	< 0.001
57	CH ₂	9.4	7000	510	33	>5 (>5)	0.004
58	$CH_2 \sim N \sim NH_2$	3.8	nd	nd	nd	4.11 (4.07)	0.026
59	CH ₂ N N	7.6	7300	330	24	>5 (4.9)	0.060
77	$CH_2 \sim \overset{H}{\underset{N \sim N}{\overset{N}{\underset{N \sim N}{\overset{N}{\underset{N \sim N}{\overset{H}{\underset{N \sim N}{\overset{H}{N}{\overset{H}{N}{\underset{N}{\overset{H}{\underset{N \sim N}{\overset{H}{\underset{N \sim N}{\overset{H}{N}{\overset{H}{\underset{N \sim N}{\overset{H}{\underset{N \sim N}{\overset{H}{\underset{N \sim N}{\overset{H}{\underset{N \sim N}{\overset{H}{\underset{N \sim N}{\overset{H}{\underset{N \sim N}{\overset{H}{\underset{N \sim N}{\overset{H}{N}{\overset{H}{\underset{N \sim N}{\overset{H}{\underset{N \sim N}{\overset{H}{N \atopN}{N {N N}{\overset{H}{N}{\overset{H}{N N}{\overset{H}{\underset{N N}{\underset{N}{N}{N}{\overset{H}{N}{N}{N}{N}{N}{N}{N}{N}{N}{N}{N}{N}{N}$	6.1	nd	nd	nd	4.2 (4.1)	0.157

^aSingle determination in most cases. ^bApparent octanol-aqueous phosphate buffer partition coefficient. ^cDetermined at 37 °C in 0.05 M phosphate containing 0.15 M NaCl. ^dnd = Value not determined.

Based on this information, we can make the following observations. Shortening of the chain length between the amide moiety and the morpholino group (e.g. 44) or adding steric hindrance via a gem-dimethyl group near the morpholino nitrogen (51) failed to produce any significant decrease in the rate of morpholine biotransformation, compared to 1 and 41. Although replacement of the morpholino group with a piperidine ring (48)apparently succeeded in limiting oxidative ring opening, no decrease in metabolism rate was observed, presumably due to facile N-oxidation of the piperidine moiety. The replacement of the morpholino group with pyridine did result in decreased rates of microsomal metabolism, with the magnitude of the decrease dependent on the position of pyridine substitution. The 2-substituted analogue 53 was metabolized at a rate (3.52 nmol/min per nmol of cytochrome P-450) roughly comparable to that of 1 and 41. However, 3-substitution of the pyridine ring (54, 2.27 nmol/min per nmol of cytochrome P-450) resulted in a metabolism rate of roughly half that of the morpholino compounds and 4-substitution (55, 0.78 nmol/min per nmol of cytochrome P-450) afforded a metabolism rate 5-6 times slower than that of the 2-substituted pyridine compound and the morpholino analogues. Finally, replacement of the morpholino group with an imidazole moiety (59) resulted in a dramatic decrease in metabolism rate, presumably due to the resistance of this moiety toward cytochrome P-450-mediated oxidation.

In Vivo Results for 3-Azaglutaramide Inhibitors

The id bioavailabilities of selected members of this series were evaluated in three species (rat, ferret, and dog). Plasma drug concentrations were determined by a renin activity assay, which treated any active metabolites as parent drug.³ The integrated areas under

Table 3. Metabolism of Renin Inhibitors by Human LiverMicrosomes

inhibitors	HPLC retention time, min	mean rate of metabolism, nmol converted/min per nmol of cytochrome P-450 ^a	HPLC retention time of putative metabolites, min	HPLC standard
1	39.1	4.26	36.4	2
			35.6	3
			33.6	4
41	42.4	4.14	38.3	85
			36.3	96
			35.4	78
48	44.9	4.52	36.0	ns^b
51	46.8	4.32	43.3	52
			41.2	80
44	42.8	3.71	38.6	87
59	38.8	0.98	ns	
53	49.0	3.52	ns	
54	45.7	2.27	ns	
55	45.4	0.78	ns	

 a Mean of two assays done with human liver microsomal samples FGL-852 and EGF-426. b ns = No standards available for HPLC cochromatography.

the curves (AUC) were obtained by fitting the data to a biexponential decay model,¹⁴ and the id bioavailabilities were calculated as the dose-corrected ratio of AUC_{id} to AUC_{iv} for each experiment. In addition, their efficacy in reducing mean arterial pressure (MAP) following iv (1 mg/kg) and id dosing (10 mg/kg) was evaluated in dog. Table 4 lists data obtained in the rat, including peak plasma drug concentrations and total drug (integrated AUC's) following iv and id dosing, as well as the calculated id bioavailabilities. Table 5 lists the analogous data obtained in the ferret. In general, compounds which were well absorbed and bioavailable in rat were also well absorbed in ferret, with the latter showing higher drug concentrations in most cases. For some of the compounds (e.g. 50, 54, 78) there was considerable standard error, likely due to the limited number of experiments performed. No simple correlation existed between $\log P$ or solubility and drug level or bioavailability; however, the following observations are of interest. Compounds of category a above (e.g. 42, 61, 85) were poorly absorbed and gave low bioavailabilities, while compounds of category b (e.g. 51, 52, 48, 41) were generally well absorbed and had moderate to good bioavailabilities. Curiously, the poorly soluble compounds of class c had widely varying absorption characteristics, from the poorly bioavailable 49 to the well absorbed and bioavailable pyridinyl amides 54 and 57. As a comparison, similar results were observed in a peptidic series of orally-active renin inhibitors from our labs.15

A select group of these compounds was also evaluated for absorption, bioavailability and efficacy in saltdepleted dogs. These data are presented in Table 6. Morpholine *N*-oxide **42**, the most soluble and hydrophilic compound of the group, was not well absorbed upon id administration (10 mg/kg). The three moderately soluble and lipophilic compounds (**41**, **44**, and **51**) all provided high drug levels and bioavailabilities after id dosing. Despite having lipophilicity and solubility similar to **51**, the previously reported imidazole-bearing **59** gave good drug levels after iv administration (1 mg/kg), but had very low drug levels after id administration in the dog. The listed measure of efficacy (AUC_{bp}) shows that all of these compounds were efficacious at a dose of 10 mg/ kg id. As a comparison to oral dosing, similar bioavailability (56%) and efficacy were found for **41** in conscious salt-depleted dogs, administered at 10 mg/kg po (data not shown).¹⁶

Figure 2 graphically illustrates this information in greater detail for compound **51**. The upper panel shows mean arterial pressure response to a bolus of 51, administered either at 1 mg/kg iv or 10 mg/kg id. In either case, a consistent, marked fall in blood pressure was observed (maximum hypotensive response = -38.8 \pm 8.0% after 10 mg/kg id, n = 3), which was maintained for the 5 h duration of the experiment. This fall correlated with a nearly complete suppression of plasma renin activity, shown in the middle panel of Figure 2. A slight return to baseline PRA was observed in the 1 mg/kg iv dose. The bottom panel shows the corresponding plasma drug concentrations as determined by bioassay. Note that after the id dosing, drug levels rose over a 60 min period and were essentially maintained throughout the course of the experiment.

Conclusions

We have shown that the proper choice of the Cterminal solubilizing group in our nonpeptide renin inhibitors can provide significant improvements in potency, absorption, and id bioavailability. We also have demonstrated that potent inhibitors which balance solubility and lipophilicity can be very efficacious hypotensive agents in the salt-depleted dog. Inhibitors **41**, **44**, and **51** are among the most well absorbed and bioavailable compounds from our renin inhibitor program.

Experimental Section

Synthetic Methods. General. All reactions were performed under an inert atmosphere (argon or nitrogen). Reactions which required anhydrous conditions were carried out in flame-dried glassware which was cooled under dry nitrogen or argon. Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Solvents were distilled under nitrogen prior to use as follows: acetonitrile, dichloromethane, diisopropylethylamine, and triethylamine from calcium hydride; tetrahydrofuran (THF) and toluene from sodium benzophenone ketyl. The following compounds were synthesized according to literature procedures: 3-[2-(hydroxyethyl)amino]propylamine,¹⁷ 4-(4-morpholino)butylamine,¹⁸ N-(2-aminoethyl)benzylcarbamate.¹⁹ All other reagents were purchased commercially and used without purification. Reaction products were placed under high vacuum (<0.05 mmHg) for several hours prior to weighing. Thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F-254 glass-backed plates of 250 μ m thickness (analytical) and 2000 μ m thickness (preparative); UV light and phosphomolybdic acid stain were used for visualization. Flash chromatography was performed using E. Merck Kieselgel 60 (230-400 mesh), and eluent systems are listed as volume/ volume percents or ratios. Melting points are given for all solids except glasses; other compounds were isolated as lowmelting waxes or oils. Proton magnetic resonance (¹H NMR) spectra were recorded on a General Electric Model QZ-300 spectrometer (300 MHz). Chemical shifts are reported as ppm downfield from tetramethylsilane (δ). For resonances which include H₂O together with a large number of protons, approximate values for the integration are given. Integrations for overlapping broad resonances are listed as totals. Spectra displaying distinct amide rotameric forms are assigned total integrations for the set of corresponding resonances. Infrared spectra were recorded in CDCl₃ on a Nicolet 5SXC FTIR spectrometer. Mass spectra were determined on Kratos MS50 (FAB⁺, FAB⁻) and Finnigan SSQ-700 (DCI⁺ (NH₃)) spectrometers. Spectra were determined by the Structural Chemistry

 Table 4. In Vivo Results in Rat: Plasma Drug Concentrations and Bioavailabilities of 3-Azaglutaramide Inhibitors following

 Intravenous and Intraduodenal Administration^a

	1 mg/kg iv ^b dose		10 mg/kg id		
inhibitor	ave peak iv drug concentration, µg/mL	ave AUC _{iv} , µg min/mL	ave peak id drug concentration, µg/mL	ave AUC _{id} , µg min/mL	average % bioavailability
41		0.004^{d}	0.19 ± 0.02	14.1 ± 1.3	36 ± 3
42	1.13 ± 0.18	12.7 ± 2.40	0.14 ± 0.07	8.43 ± 3.69	6 ± 3
44	0.68 ± 0.31	14.5 ± 4.16	0.91 ± 0.23	73.2 ± 19.9	50 ± 19
48	1.00 ± 0.27	21.1 ± 5.20	0.36 ± 0.12	$34.2\pm19.1^{\circ}$	15 ± 9
49	0.14 ± 0.08	$4.92\pm0.84^{\circ}$	0.03 ± 0.03	$2.22\pm2.22^{\circ}$	4 ± 4
50	1.31 ± 0.54	20.4 ± 2.97	$>0.48 \pm 0.32$	$>51.0 \pm 37.7$	$>25\pm18$
51	0.34 ± 0.11	12.9 ± 3.37	0.60 ± 0.17	45.8 ± 7.34	35 ± 10
52	5.48 ± 0.87	84.1 ± 12.5	2.36 ± 0.39	207 ± 50.5	24 ± 7
53	6.25 ± 0.45	188 ± 34.9	4.57 ± 2.13	412 ± 213	21 ± 12
54	0.56 ± 0.01	$13.7 \pm 1.22^{\circ}$	2.32 ± 0.35	$220 \pm 43.8^{\circ}$	160 ± 35
55	2.48 ± 0.62	39.2 ± 3.50	0.46 ± 0.13	45.6 ± 11.0	11 ± 3
56	1.66 ± 0.30	$92.4 \pm 2.22^{\circ}$	0.93 ± 0.11	66.1 ± 2.13	7 ± 0.3
57	0.72 ± 0.32	29.2 ± 4.55	0.46 ± 0.22	$33.9\pm16.3^{\circ}$	11 ± 5
59	0.33 ± 0.16	5.69 ± 1.36	0.04 ± 0.02	0.92 ± 0.52	1 ± 1
61	0.66 ± 0.49	9.75 ± 2.48	0.012 ± 0.007	0.35 ± 0.20	0 ± 0
77	0.81 ± 0.19	19.1 ± 1.24	0.19 ± 0.19	5.69 ± 5.69	2 ± 2
78	0.34 ± 0.06	11.3 ± 3.14	0.207	16.0 ± 15.9	14 ± 14
80	3.48 ± 0.51	28.6 ± 2.09	0.08 ± 0.03	3.98 ± 2.19	1 ± 1

^a Values reported as mean \pm standard deviation of the mean. ^b n = 3 unless otherwise noted. ^c n = 2. ^d n = 1.

Table 5. In Vivo Results in Ferret: Plasma Drug Concentrations and Bioavailabilities of 3-Azaglutaramide Inhibitors FollowingIntravenous and Intraduodenal Administration^a

	1 mg/kg iv ^b dose		10 mg/kg		
inhibitor	ave peak iv drug concentration, µg/mL	ave AUC _{iv} , µg min/mL	ave peak id drug concentration, µg/mL	ave AUC _{id} , μg min/mL	average % bioavailability
41 42	$\begin{array}{c} 1.55 \pm 0.31 \\ 1.83 \pm 0.82 \end{array}$	54.1 ± 3.27 40.1 ± 14.9	$\begin{array}{c} 4.62 \pm 0.85 \\ 1.18 \pm 0.56 \end{array}$	408 ± 64.1 81.0 ± 36.2	$75 \pm 13 \\ 20 \pm 12 \\ 12$
44 48 50	$2.84 \pm 0.31 \\ 3.60^{f} \\ 3.90 \pm 1.78$	$112 \pm 35.8 \\ 101^{f} \\ 165 \pm 67.0$	$egin{array}{c} 6.50 \pm 0.56 \ 3.73 \pm 0.73^{s} \ 5.50 \pm 1.03^{s} \end{array}$	585 ± 9.51 341 ± 41.5^{s} 493 ± 94.4^{s}	51 ± 16 33 ± 4 29 ± 13
51 52	2.98 ± 0.58 7.15 ± 0.95	158 ± 32.4 190 ± 55.8	$\frac{11.5 \pm 2.51}{2.96 \pm 0.40}$	999 ± 209 230 ± 44.7	$63 \pm 18 \\ 12 \pm 4$
53 54 57	10.0 ± 2.66 3.48 ± 1.61 6.10 ± 0.14	$182 \pm 84.3 \\ 49.6 \pm 26.0^d \\ 89.8 \pm 18.6$	$9.12 \pm 3.58'$ > 4.92 ± 0.81 2 00 + 0 10	$846 \pm 331'$ >490 ± 89.6 133 ± 48.6	$46 \pm 23 \\ > 98 \pm 54 \\ 14 \pm 6$
58 59	$\begin{array}{c} 0.32 \pm 0.06 \\ 2.10 \pm 0.10 \\ \end{array}$	$\begin{array}{c} 4.28 \pm 0.99 \\ 42.6 \pm 4.20 \end{array}$	0.11 ± 0.06 >4.93 ± 0.40 ^e	3.29 ± 1.09 488 ± 77.5^{e}	7 ± 3 >114 ± 21
59 61	$\begin{array}{c} 0.02 \pm 0.00 \\ 2.10 \pm 0.10 \\ 2.45 \pm 0.51 \end{array}$	42.6 ± 4.20 42.6 ± 4.20 74.7 ± 7.70	$>4.93 \pm 0.40^{e}$ 0.92 ± 0.41	$ \begin{array}{r} 3.25 \pm 1.05 \\ 488 \pm 77.5^{e} \\ 77.2 \pm 26.1 \end{array} $	$>114 \pm 21$ 10 ± 3

^a Values reported as mean \pm standard deviation of the mean. ^b n = 2, unless otherwise noted. ^c n = 3, unless otherwise noted. ^d n = 3. ^e n = 2. ^f n = 1. ^g n = 4.

Table 6. In Vivo Results in Salt-Depleted Dog: Plasma Drug Concentrations, Bioavailabilities, and Mean Arterial PressureResponses for 3-Azaglutaramide Inhibitors following Intravenous and Intraduodenal Administration^a

	1 mg/kg iv dose		10 mg/kg id ^c dose			ave AUC _{bp} , % mmHg min	
inhibitor	ave peak iv drug concentration, μ g/mL	ave AUC _{iv} , μg min/mL	ave peak id drug concentration, μg/mL	ave AUC _{id} , μg min/mL	average % bioavailability	(1 mg/kg iv)	(10 mg/kg id)
41	4.70 ± 0.20	41.6 ± 2.80	1.26 ± 0.52	240 ± 76.2^d	57 ± 18	8356 ± 638	10803 ± 743
42	>5.53	$>43.9 \pm 4.4$	0.42 ± 0.13	86.0 ± 32.6	<19	3707 ± 1235	6881 ± 1402
44	7.00 ± 0.73	75.5 ± 1.39	1.83 ± 0.8	392 ± 176	52 ± 19	2849 ± 1235	6449 ± 300
51	7.56 ± 1.20	110.4 ± 21.1	1.60 ± 0.62	418 ± 117	37 ± 12	7589 ± 1012	8525 ± 1263
59	4.51 ± 0.23	109.9 ± 19.2	0.13 ± 0.03	23.8 ± 2.15	2 ± 0	6177 ± 1929^{e}	7134 ± 708

^a Values reported as mean \pm standard deviation of the mean. ^b n = 2. ^c n = 3, unless otherwise noted. ^d n = 4. ^e Dosed 10 mg/kg iv.

Department, Pharmaceutical Products Division, Abbott Laboratories. Combustion analyses were performed by the Analytical Research Department, Pharmaceutical Products Division, Abbott Laboratories.

N-(3-((Benzyloxycarbonyl)amino)propyl)-(2S,4S,5S)-2-((3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4-(cyclohexylmethyl)-5-oxazolidinyl)methyl)-3-methylbutanamide (6). Part 1. Preparation of N-(3-Aminopropyl)benzylcarbamate. 1,3-Diaminopropane (35.5 g, 0.48 mol) was dissolved in 300 mL of CHCl₃, and the solution was cooled to 0 °C. A solution of N-((benzyloxycarbonyl)oxy)succinimide (4.5 g, 0.018 mol) in 150 mL of CHCl₃ was added dropwise over 6 h, with the internal temperature being maintained below 10 °C. After addition was complete, the reaction solution was washed with water, dried (Na₂SO₄), filtered, and concentrated in vacuo to provide 3.0 g (80%) of 3-((benzyloxycarbonyl)amino)propylamine as a low-melting solid: ¹H NMR (CDCl₃) δ 1.25 (br s, 2 H), 1.63 (quintet, J = 7 Hz, 2 H), 2.77 (t, J = 7 Hz, 2 H), 3.2– 3.34 (br m, 2 H), 5.09 (s, 2 H), 5.37 (br m, 1 H), 7.28–7.40 (m, 5 H); MS m/e 209 ((M + H)⁺).

Part 2. Coupling to Carboxylic Acid 5. Carboxylic acid 5 (1.50 g, 3.64 mmol), HOBt hydrate (837 mg, 5.47 mmol), 3-((benzyloxycarbonyl)amino)propylamine (949 mg, 4.56 mmol), and 4-methylmorpholine (601 mg, 5.47 mmol) were dissolved in 36.5 mL of dry DMF, and the solution was cooled to -20 °C under a nitrogen atmosphere. EDC (978 mg, 5.10 mmol) was added as a solid, and the resulting mixture was stirred at -20 °C for 4 h, then the resulting solution was stirred for 16 h at room temperature. The volatiles were removed by high-vacuum distillation, the residue was dissolved in 150 mL of CH₂Cl₂ and extracted with 2 × 300 mL of 80% saturated aqueous NaHCO₃, water (300 mL), and brine (300 mL), and then the organic phase was dried (Na₂SO₄) and concentrated



Figure 2. Effects of inhibitor **51** in salt-depleted dogs: mean arterial pressure (panel A), plasma renin activity (panel B), and plasma drug concentrations (panel C). Standard error is shown by the error bars in the positive direction only.

to a foam. Purification by flash chromatography (silica gel, 10% EtOAc–CH₂Cl₂) provided 1.98 g (3.28 mmol, 90%) of amide **6** as a white foam: R_f 0.11 (25% EtOAc–hexane); ¹H NMR (CDCl₃) δ 0.80–1.05 (br m, 8 H), 1.05–1.31 (br m, 4 H), 1.48 (s) and 1.31–1.50 (br m, 10 H total), 1.52–1.90 (m, 14 H), 2.05 (m, 1 H), 3.25 (m, 2 H), 3.34 (m, 2 H), 3.65 (br m, 1 H), 3.71 (m, 1 H), 5.10 (s, 2 H), 5.48 (br t, J = 6 Hz, 1 H), 6.00 (m, 1 H), 7.3–7.36 (m, 5 H); MS m/e 602 ((M + H)⁺), 619 ((M + NH₄)⁺). Anal. (C₃₄H₅₅N₃O₆) C, H, N.

Compounds 7-10 were synthesized in an analogous manner.

N-(Cyanomethyl)-(2S,4S,5S)-2-((3-(tert-butyloxycarbonyl)-2,2-dimethyl-4-(cyclohexylmethyl)-5-oxazolidinyl)methyl)-3-methylbutanamide (11). Carboxylic acid 5 (500 mg, 1.22 mmol), HOBt (279 mg, 1.82 mmol) and 4-methylmorpholine (200 mL, 184 mg, 1.82 mmol), were dissolved in 12 mL of dry DMF, and the solution was cooled to 0 °C under a nitrogen atmosphere. EDC (326 mg, 1.70 mmol) was added as a solid, and the resulting mixture was stirred at 0 °C for 1 h and then sealed and stored in a refrigerator at 0 °C for 22 h. The resulting solution of active ester was cooled to -23°C, then an additional portion of 4-methylmorpholine (347 mL, 319 mg, 3.16 mmol) was added, followed by aminoacetonitrile hydrochloride (146 mg, 1.58 mmol). The resultant solution was stirred at -23 °C and allowed to slowly warm to room temperature as the ice bath melted. After the reaction had stirred at ambient temperature for 20 h, the volatiles were removed by high vacuum distillation, and the residue was dissolved in 50 mL of CH_2Cl_2 and extracted sequentially with 2×50 mL of 80% saturated aqueous NaHCO₃, water (100 mL), and brine (50 mL). The organic phase was dried (Na₂SO₄) and concentrated to a foam (570 mg, 104% crude). Purification by flash chromatography (EtOAc-hexane 1:3) provided 513 mg (1.14 mmol, 94%) of amide **11** as a white foam: R_f 0.20 (EtOAc-hexane 1:3); ¹H NMR (CDCl₃) δ 0.94 (d, 3 H), 0.97 (d, 3 H), 0.85–1.05 (br m, 2 H), 1.05–1.53 (several br m, 5 H), 1.48 (s, 9 H), 1.49 (s, 3 H), 1.59 (s, 3 H), 1.55–1.85 (several br m, 8 H), 1.85–1.98 (m, 1 H), 2.1-2.2 (br m, 1 H), 3.55–3.75 (br m, 2 H), 4.21 (ABX, 2 H), 6.06 (bt, 1 H); MS m/e 450 ((M+H)⁺), 467 ((M+NH₄)⁺). Anal. (C₂₅H₄₃N₃O₄•0.5H₂O) C, H, N.

N-(5-(Tetrazolyl)methyl)-(2S,4S,5S)-2-((3-(tert-butyloxycarbonyl)-2,2-dimethyl-4-(cyclohexylmethyl)-5-oxazolidinyl)methyl)-3-methylbutanamide (12). A solution of cyanomethylamide 11 (150 mg, 0.334 mmol), triethylamine hydrochloride (68.9 mg, 0.500 mmol), and sodium azide (65.1 mg, 1.00 mmol) in 3.3 mL DMF was warmed to 125 °C for 24 h. The dark solution was concentrated under high vacuum, and the resulting oil was poured into a mixture of 10 mL of water and 10 mL of CH₂Cl₂. The mixture was acidified to pH 1 with 1 N HCl. The layers were stirred and then separated, and the aqueous phase was extracted with 2×20 mL of CH₂-Cl₂. The combined organic phases were washed with brine, dried (Na₂SO₄), filtered, and concentrated in vacuo to produce 168 mg (0.341 mmol, 102%) of crude tetrazole 12: $R_f 0.32$ (5%) MeOH–0.5% HOAc–CH₂Cl₂); ¹H NMR (CDCl₃) δ 0.91 (d, J = 7 Hz), 0.96 (d, J = 6 Hz), and 0.80-1.01 (br m, 8 H total), 1.49 (s, 9 H), 1.55 (br s), 1.60 (br s) and 1.01–1.98 (several br m, approximately 20 H total), 2.16 (br m, 1 H), 3.65-3.77 (m, 2 H), 4.62 (dd, J = 5, 16 Hz, 1 H), 5.03 (dd, J = 7, 15 Hz, 1 H), 6.55 (m, 1 H); MS m/e 515 ((M + Na)⁺); HRMS calcd for $C_{25}H_{45}N_6O_4Na\;((M{+}Na)^{-})\;516.3400,\;found\;516.3333$

N-(3-(4-Morpholino)propyl)-(2S,4S,5S)-5-((tert-butyloxycarbonyl)amino)-4-hydroxy-2-isopropylhexanamide (14). Lactone 13 (4.0018 g, 11.32 mmol) and 4-(3aminopropyl)morpholine (16.5 mL, 0.1124 mmol) were warmed at 60 °C for 96 h. The reaction mixture was partitioned between water (150 mL) and CH_2Cl_2 (120 mL). The organic phase was washed with water (3 \times 100 mL) and brine (100 mL), dried over sodium sulfate, and concentrated under reduced pressure to afford a solid which was recrystallized from hot ethyl acetate to afford the title compound (4.719 g, 84%): mp 161-2 °C; R_f 0.18 (7.5% MeOH-CHCl₃); ¹H NMR $(\text{CDCl}_3) \ \bar{\delta} \ 0.73 - 1.04 \ (\text{br m}) \text{ and } 0.93 \ (2 \text{ overlapping d}, J = 7)$ Hz, 8 H total), 1.05–1.50 (br m) and 1.45 (s, 17 H total), 1.58– 1.77 (br m, 10 H), 1.79-2.03 (m, 4 H), 2.41-2.52 (m, 6 H), 3.25-3.45 (m, 2 H), 3.43-3.59 (br m, 2 H), 3.68-03.74 (m, 4 H), 3.90-4.10 (br m, 1 H), 4.65 (br d, J = 9 Hz, 1 H), 7.04 (br m, 1 H); MS m/e 498 ((M + H)⁺). Anal. (C₂₇H₅₁N₃O₅·0.25H₂O) C, H, N.

Dipeptide isosteres 15 and 17-25 were synthesized in an analogous manner. The following preparation of 16 illustrates the use of acetic acid to markedly facilitate the lactone-opening reaction.

N-(2-Aminoethyl)-(2S,4S,5S)-5-((tert-butyloxycarbonyl)amino)-4-hydroxy-2-isopropylhexanamide (16). Lactone 13 (10.0 g, 28.3 mmol) and 1,2-diamino-2-methylpropane $(17\ mL,\,15.3\ g,\,0.255\ mol)$ were combined, then glacial acetic acid (4.86 mL, 5.10 g, 0.085 mol) was added. The solution was warmed to 85 °C for 30 min, at which time TLC (15% EtOAchexane) indicated complete consumption of the starting lactone. The solution was dissolved in 250 mL of EtOAc and extracted with 3×250 mL of H₂O and then with 250 mL brine. The organic phase was dried (Na_2SO_4) and filtered, and the filtrate was concentrated in vacuo to provide a white foam. The crude was recrystallized from hot EtOAc to give 5.20 g (12.6 mmol, 45%) of white needles of amide 16, mp 133-6 °C. The mother liquor was flash chromatographed (10% MeOH-1% concentrated aqueous $NH_4OH-CH_2Cl_2$) to provide an additional 4.12 g (10.0 mmol, 35%) of white foamy solid. Both materials had identical spectra as follows: $R_f 0.24$ (10%) MeOH-1% NH₄OH-CH₂Cl₂); ¹H NMR (CDCl₃) & 0.69-1.06 (br m) and 0.93 (d, J = 7 Hz, 8 H total), 1.06–1.33 (br m, 5 H), 1.33-1.50 (br m) and 1.45 (s, 10 H total), 1.50-1.98 (br m, 10 H), 1.99-2.09 (m, 1 H), 2.74-2.89 (m, 1 H), 2.90-3.03 (br m, 1 H), 3.03-3.19 (br m, 1 H), 3.40-3.75 (br m, 3 H), 4.68 (br d, J = 9 Hz, 1 H), 6.00-6.09 (br m) and 6.21-6.33 (br m, 1 H total); HRMS calcd for $C_{28}H_{53}N_2O_4$ ((M + H)⁺) 414.3332, found 414.3332.

N-(2-Amino-2-methylpropyl)-(2S,4S,5S)-5-((tert-butyloxycarbonyl)amino)-4-hydroxy-2-isopropylhexanamide (20). Lactone 13 (5.74 g, 16.2 mmol) and 1,2-diamino-2-methylpropane (7.16 g, 81.2 mmol) were combined, and the solution was warmed to 50 °C for 48 h and then at 55 °C for 5 days. TLC (15% EtOAc-hexane) indicated the presence of a spot, $R_f 0.23$, slightly more polar than starting lactone, plus a baseline material. The solution was partitioned between 100 mL of EtOAc and 50 mL of H_2O , then the organic phase was washed $(2 \times 50 \text{ mL H}_2\text{O}, 50 \text{ mL brine})$, dried (Na₂SO₄), and filtered, and the filtrate was concentrated in vacuo to provide a white foam (2.383 g, 95% crude). The crude was recrystallized from hot EtOAc (two crops), to give 1.79 g (4.06 mmol, 42%) of white needles of amide 20: mp 133-6 °C; $R_f 0.25 (10\%)$ MeOH-0.5% NH₄OH-CH₂Cl₂); ¹H NMR (CDCl₃) δ 0.70-1.03(br m) and 0.94 (d, J = 6 Hz, 8 H total), 1.03-1.50 (br m), 1.12 (s), 1.17 (s) and 1.44 (s, 21 H total), 1.52-2.00 (br m, 9 H), 2.01-2.12 (m, 1 H), 2.96 (br dd, J = 4, 13 Hz, 1 H), 3.40(br dd, J = 7, 13 Hz, 1 H), 3.47-3.59 (br m, 2 H), 4.66 (br d, J)J = 10 Hz, 1 H), 6.04–6.13 (br m, 1 H); MS m/e 442 ((M + $(C_{24}H_{47}N_{3}O_{4}O_{25}H_{2}O) C, H, N.$

N-(2-Methyl-2-(4-morpholino)propyl)-(2S,4S,5S)-5-((tertbutyloxycarbonyl)amino)-4-hydroxy-2-isopropylhexanamide (26). 3,4-Dihydrofuran (1.60 mL, 1.48 g, 21.2 mmol) was dissolved in 60 mL of CH₂Cl₂ and 20 mL of MeOH, the solution was cooled to -78 °C, and a stream of ozone in oxygen was passed through the solution using a gas dispersion tube (internal temperature -70 to -65 °C) until the solution was saturated. Excess ozone was removed by passing a stream of oxygen through the solution until the blue color faded, then sodium cyanoborohydride (800 mg, 12.7 mmol) was added as a solid. The resulting mixture was stirred at -65 °C for 45 min. Amino amide 20 (2.004 g, 3.92 mmol) was added as a solid, then the solution was stirred at - 65 °C for 3 h and then at room temperature for 72 h, at which time TLC (10% MeOH-0.5% NH₄OH-CH₂Cl₂) indicated the consumption of **20** and the presence of a product spot ($R_f 0.47$). The reaction solution was concentrated in vacuo, and the residue was partitioned between 100 mL of CH₂Cl₂ and 50 mL of 1 M aqueous Na₂CO₃. The aqueous phase was extracted (2×50) mL CH_2Cl_2), and the combined organic phases were washed with 50 mL of brine, dried (Na₂SO₄), filtered, rotoevaporated, and dried under vacuum to produce 1.90 g of white foamy solid. The crude was purified by filtration through silica gel (4×50) mL of 1% MeOH-EtOAc), and the partially purified material was recrystallized from hot EtOAc to afford the desired compound as white needles: mp 138-9 °C; R_f 0.26 (5%) MeOH-CH₂Cl₂); ¹H NMR (CDCl₃) δ 0.76-1.01 (br m), 0.94 (d, J = 7 Hz) and 0.97 (d, J = 7 Hz, 8 H total), 1.03 (s) and 1.04 (s, 6 H total), 1.09-1.50 (br m) and 1.46 (s, 16 H total), 1.52-2.00 (br m, approximately 8 H), 2.04-2.15 (br m, 1 H), 2.49-2.56 (m, 4 H), 3.19 (d, J = 4 Hz, 2 H), 3.45-3.60 (br m,2 H), 3.67-3.77 (m, 4 H), 4.65 (br d, J = 9 Hz, 1 H), 6.31 (br m, 1 H); MS m/e 512 ((M + H)⁺). Anal. (C₂₈H₅₃N₃O₅) C, H, N.

N-(3-(4-Oxido-4-morpholino)propyl)-(2S,4S,5S)-5-((tertbutyloxycarbonyl)amino)-4-hydroxy-2-isopropylhexanamide (27). Morpholine 14 (1.50 g, 3.01 mmol) was dissolved in 12 mL of MeOH and treated with 30% aqueous H_2O_2 (0.93 mL, 310 mg H_2O_2 , 9.10 mmol), and the solution was warmed to 50 °C for 48 h. The reaction was concentrated in vacuo to an aqueous mixture, which was partitioned between 100 mL of $CHCl_3$ and 50 mL of H_2O . The aqueous phase was extracted with 2×30 mL of CHCl₃, and the combined organic phases were washed with brine, dried (Na₂SO₄), filtered, and concentrated in vacuo. The foamy solid was purified by flash chromatography $(10-15\% \text{ MeOH}-\text{CH}_2\text{Cl}_2)$ to provide 1.43 g (2.78 mmol, 92%) of morpholine N-oxide 27 as a white powder: mp 154-7 °C; ¹H NMR (CDCl₃) δ 0.69-1.03 (br m), 0.91 (d, J = 6 Hz) and 0.93 (d, J = 6 Hz, 8 H total), 1.03-1.40(br m, 6 H), 1.43 (s, 9 H), 1.50-1.75 (br m, 6 H), 1.80-1.95 (brm, 3 H), 1.97-2.30 (br m, 6 H), 3.05-3.38 (br m, 6 H), 3.36-3.50 (m, 2 H), 3.50-3.71 (br m, 2 H), 3.75-3.86 (br m, 2 H), $4.30-4.44~(m,~2~{\rm H}),~4.81~({\rm br}~{\rm d},~J=9~{\rm Hz},~1~{\rm H}),~7.96\text{-}8.05~({\rm br}~{\rm s},~1~{\rm H});~{\rm MS}~m/e~514~(({\rm M}~+~{\rm H})^+),~498~({\rm M}~-~16~+~{\rm H})^+.$ Anal. $({\rm C}_{27}{\rm H}_{51}{\rm N}_3{\rm O}_6{\rm H}_2{\rm O}),~{\rm C},~{\rm H},~{\rm N}.$

Morpholine-N-oxide 28 was prepared in the same manner as that described for 27.

N-(2-Cyclohexylethyl)-(2S,4S,5S)-5-((*tert***-butyloxycarbonyl)amino)-4-hydroxy-2-isopropylhexanamide (29).** Phenylethylamide **18** (556 mg, 1.18 mmol) was hydrogenated (4 atm) over 110 mg of 5% Rh/C in 50 mL of MeOH for 48 h. Filtration through Celite provided 539 mg (1.12 mmol, 96%) of cyclohexane **29** as a pale yellow foamy solid: mp 151-5 °C; $R_f 0.47$ (EtOAc-hexane 1:1); ¹H NMR (CDCl₃) δ 0.75-1.04 (br m), 0.93 (d, J = 7 Hz) and 0.95 (d, J = 7 Hz, 10 H total), 1.05-1.42 (br m, 11 H), 1.43 (s, 9 H), 1.52-2.07 (br m, 15 H), 3.16-3.40 (m, 2 H), 3.46-3.57 (br m, 2 H), 3.69 (br m, 1 H), 4.62 (br d, J = 9 Hz, 1 H), 5.60 (br t, J = 6 Hz, 1 H); HRMS calcd for $C_{28}H_{53}N_2O_4$ ((M + H)⁺) 481.4005, found 481.4003.

N-(2-((5-Amino-1,2,4-oxadiazol-3-yl)amino)ethyl)-(2S,4S,5S)-5-((tert-butyloxycarbonyl)amino)-4-hydroxy-2-isopropylhexanamide (30). Primary amine 16 (5.00 g, 12.1 mmol) and dimethyl N-cyanothioiminocarbonate (2.06 g, 12.7 mmol) were dissolved in CH₃CN and the resulting solution was warmed to reflux for 32 h. The cloudy solution was concentrated in vacuo to give 6.0 g of crude N-cyano-Smethylisothiourea as a white powder. A portion of this crude material (1.25 g, 2.44 mmol) was combined with hydroxylamine hydrochloride (679 mg, 9.77 mmol) in 25 mL of absolute EtOH, then triethylamine (1.7 mL, 1.23 mmol, 12.2 mmol) was added, and the resulting solution was heated to reflux for 18 h. The reaction mixture was concentrated in vacuo, and the residue was dissolved in 100 mL of CH₂Cl₂ and washed with 2×100 mL of H₂O. The organic phase was dried (Na₂SO₄), filtered, and concentrated in vacuo. Flash chromatography (6% MeOH-1% concentrated aqueous-CH₂Cl₂) provided 494 mg (0.964 mmol, 40%) of oxadiazole **30** as a white foamy solid: mp 99-107 °C; R_f 0.40 (10% MeOH-CH₂Cl₂); ¹H NMR $(CDCl_3) \delta 0.70-1.05 (br m), 0.91 (d, J = 6 Hz) and 0.94 (d, J)$ = 6 Hz, 8 H total), 1.05–1.40 (br m, 6 H), 1.45 (s, 9 H), 1.54– 1.90 (br m, 9 H), 1.90-3.00 (vbr s) and 2.07-2.17 (m, 4 H total), 3.13-3.60 (br m, 5 H), 3.65-3.90 (br m, 1 H), 4.81 (br d, J =9 Hz) and 5.10-5.26 (br s, 1 H total), 6.42-6.80 (br m, 2 H); MS m/e 497 ((M + H)⁺). Anal. (C₂₄H₄₄N₆O₅·0.75 H₂O) C, H, N.

N-(3-((Benzyloxycarbonyl)amino)propyl)-(2S,4S,5S,1'S,2'S)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (34). Part 1. Deprotection of tert-Butyl Carbamate 6. A solution of carbamate 6 (119 mg, 0.198 mmol) in 1.5 mL CH₂Cl₂ was cooled to 0 °C. and 1.5 mL of trifluoroacetic acid was added dropwise over 2 min. The resulting solution was stirred at 0 °C for 4 h and then concentrated in vacuo to give an oil. The crude aminal salt was dissolved in 3 mL of THF and 1 mL of H₂O and the solution was stirred at 0 °C for 18 h. The mixture was concentrated in vacuo to give an aqueous suspension, which was then partitioned between 20 mL of saturated aqueous $NaHCO_3$ and 20 mL of CH_2Cl_2 . The aqueous phase was extracted with 2×20 mL of CH₂Cl₂, then the combined organic phases were dried (Na₂SO₄), filtered, and concentrated in vacuo to give 95 mg (104%) of the crude amino alcohol N-(3-((benzyloxycarbonyl)amino)propyl)-(2S,4S,5S)-5-amino-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide: Rf 0.30 (10% MeOH- CH_2Cl_2 ; ¹H NMR (CDCl₃) δ 0.92 (m, 6 H), 0.7–1.9 (several br m, approximately 18 H), 2.15 (m, 1 H), 2.59 (m, 1 H), 3.10 (m, 1 H), 3.20-3.42 (br m, 5 H), 5.09 (s, 2 H), 5.43 (br m, 1 H), 6.19 (br m, 1 H), 7.28-7.40 (m, 5 H).

Part 2. Coupling to Carboxylic Acid 32. The above crude amino alcohol (90.5 mg, 0.196 mmol), carboxylic acid 32 (87.9 mg, 0.216 mmol), HOBt hydrate (34.5 mg, 0.225 mmol), and 4-methylmorpholine (22 mg, 0.22 mmol) were dissolved in 2.0 mL of DMF, and the resulting solution was cooled to -23 °C. EDC (52.6 mg, 0.275 mmol) was added, and the mixture was stirred at -23 °C for 4 h and allowed to warm to room temperature with stirring overnight (18 h). The solvent was partitioned between 25 mL of CH₂Cl₂ and 80% saturated aqueous NaHCO₃. The organic phase was washed sequentially

with H₂O (25 mL) and brine (25 mL) and then dried (Na₂SO₄), filtered, and concentrated in vacuo to a foam (174 mg). Flash chromatography (silica gel, 2.5% MeOH–CH₂Cl₂) provided 106 mg (0.125 mmol, 64%) of inhibitor **34** as a white foam: mp 64–8 °C; ¹H NMR (CDCl₃) δ 0.61–1.07 (br m, 11 H), 1.07–1.90 (several br m, approximately 26 H), 2.00–2.11 (m, 1 H), 2.90–3.10 (m, 2 H), 3.10-3.44 (br m) and 3.37 (s, approximately 20 H total), 3.45–3.58 (br m, 1 H), 3.58–4.00 (br m, 5 H), 4.46–4.55 (m, 1 H), 4.67 (s, 2 H), 5.10 (AB, 2 H), 5.48–5.56 (m, 1 H), 5.81 (d, J = 11 Hz) and 5.88 (d, J = 11 Hz, 1 H total), 6.09 (br t, J = 6 Hz, 1 H), 7.27–7.40 (m, 10 H); MS m/e 851 ((M + H)⁺). Anal. (C4₈H₇₄N₄O₉0.5H₂O) C, H, N.

Acetonide carbamates 7, 8, and 12 were deprotected and coupled to acid 32 in the same manner, to provide inhibitors 36, 35, and 37, respectively. Acetonide carbamates 7, 9, and 10 were deprotected and coupled to acid 33 in a similar manner, to provide inhibitors 46, 47, and 43, respectively.

N-(3-Aminopropyl)-(2S,4S,5S,1'S,2'S)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (3). Benzyl carbamate 34 (50 mg, 0.0587 mmol) was stirred with 10% palladium on carbon (13 mg) in 3 mL of EtOAc under 1 atmosphere of hydrogen for 3 days. The mixture was filtered, and the filtrate was concentrated to a foam. Thin-layer chromatography (silica gel, 10% MeOH-1% concentrated aqueous NH₄OH-CH₂Cl₂) gave 26.4 mg (0.0368 mmol, 63%) of amine 3 as a white foamy solid: mp 58-60 °C; $R_f 0.26 (10\% \text{ MeOH}-1\% \text{ concentrated aq. } \text{NH}_4\text{OH}-\text{CH}_2\text{Cl}_2);$ ^{1}H NMR (CDCl₃) δ 0.90–0.92 (m, 9 H), 0.65-2.0 (vbr m, approximately 28 H total), 2.05 (m, 1 H), 2.82 (t, J = 6 Hz, 2 H), 2.9-3.1 (m, 2 H), 3.13-3.28 (br m, 2 H), 3.37 (s, 3 H), 3.3-3.55 (br m, 5 H), 3.6-4.0 (vbr m, 5 H), 4.50 (dd, J = 5, 9 Hz)1 H), 4.67 (s, 2 H), 5.79 (d, J = 9 Hz) and 5.87 (d, J = 9 Hz, 1 H total), 6.52 (br m, 1 H), 7.30 (br s, 5 H); HRMS calcd for $C_{40}H_{69}N_4O_7 ((M + H)^+)$ 717.5166, found 717.5178.

Primary amines 60 and 61 were prepared from 36 and 46, respectively, in the same manner.

 $N\-(2-Carboxyethyl)\-(2S,\!4S,\!5S,\!1'S,\,2'S)\-5\-(2\-(1\-((4\-(methermath{metherm{methermath{methe$ oxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (4). To a solution of ethyl ester 35 (125.7 mg, 0.1654 mmol) in tetrahydrofuran (2.5 mL) cooled to 0 °C was added a solution of lithium hydroxide (13.9 mg, 0.3308 mmol) in water (0.3 mL). After stirring at 0 °C under nitrogen for 2 h, the cooling bath was removed and stirring was continued for 2 h at ambient temperature. The reaction mixture was diluted with water (10 mL) and concentrated under reduced pressure to remove the tetrahydrofuran. The aqueous solution was acidified to pH 2 with 1 N NaHSO $_4$ (3 mL) and extracted with CH_2Cl_2 (4 \times 20 mL). The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated under reduced pressure to afford crude acid as an amorphous solid (112.7 mg). Flash chromatography (5% MeOH-1.0% HOAc-CH $_2Cl_2$) afforded 80 mg of partially purified material. This material was further purified by preparative thin-layer chromatography (5% MeOH-0.5%HOAc-CH2Cl2) to afford the title compound as a white amorphous solid (53.1 mg, 44%): mp 115–124 °C; R_f 0.45 (5% MeOH-1.0% HOAc-CH₂Cl₂); ¹H NMR (CDCl₃) δ 0.33-2.05 (several br m, approximately 37 H), 2.05-2.80 vbr m, 4 H), 2.86-3.08 (br m, 3 H), 3.10-3.25 (br m, 2 H), 3.35 (s) and 3.39 (s, 3 H total), 3.45-4.02 (br m, 6 H), 4.05-4.27 (br m, 1 H), 4.69 (2 s) and 4.65-4.90 (br m, 3 H total), 5.35-5.70 (br m, 1 H), 7.27 - 7.48 (m, 5 H); MS m/e 754 ((M + Na)⁺); (FAB⁻) m/e730 ($(M - H)^{-}$); HRMS (FAB⁻) calcd for $C_{40}H_{64}N_3O_9Na_2$ ((M + 2Na - H)-) 776.4438, found 776.4443

N-(2-(4-Morpholinyl)-2-methylpropyl)-(2S,4S,5S,1'S,2'S)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2phenylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2isopropylhexanamide (39). Through a solution of dihydrofuran (15 μ L, 14 mg, 0.198 mmol) dissolved in methanol (0.1 mL) and CH₂Cl₂ (0.4 mL) and cooled to -65 °C under argon was passed a stream of ozone in oxygen for approximately 30 s to give a pale blue solution. Oxygen was bubbled through the solution to drive off the excess ozone, and then sodium cyanoborohydride (6.9 mg, 0.110 mmol) was added. The reaction mixture was stirred at -65 to -60 °C for 25 min and then a solution of amine 38 (33.4 mg, 0.0457 mmol) dissolved in methanol (0.6 mL) was added. The reaction mixture was stirred at -60 °C for 30 min, allowed to warm to 0 °C, and then stirred at 0 °C for 6 h. It was then allowed to warm to ambient temperature and stirred for 24 h. The reaction mixture was partitioned between 1 M Na₂CO₃ (10 mL) and CH_2Cl_2 (20 mL). The aqueous phase was extracted with CH_2Cl_2 (2 \times 10 mL). The combined organic extracts were washed with brine (15 mL), dried over sodium sulfate, and concentrated in vacuo to afford crude product (36.1 mg). Preparative TLC (4% MeOH-CH₂Cl₂) afforded pure morpholine 39 as a white amorphous solid (26.3 mg, 58%): mp 46-52 °C; $R_f 0.24 (5\% \text{ MeOH} - \text{CH}_2\text{Cl}_2)$; ¹H NMR (CDCl₃) $\delta 0.66 - \delta 0.66$ 1.48 (br m), 0.91 (t, J = 4 Hz), 0.91–0.97 (2 overlapping d, J= 6 Hz) and 1.04 (s, 31 H total), 1.49–1.90 (br m, $17 \text{ }\hat{\text{H}}), 2.11-$ 2.17 (m, 1 H), 2.47-2.54 (m, 4 H), 2.93-3.00 (m, 2 H), 3.06 (dd, J = 5.5, 8 Hz, 1 H), 3.11 (dd, J = 3, 8 Hz, 1 H), 3.17 - 3.29(br m, 3 H), 3.37 (s) and 3.30-3.49 (br m, 5 H), 3.64-3.84 (br m, 6 H), 3.84-3.97 (br m, 1 H), 4.49 (dd, J = 3, 6 Hz, 1 H), 4.69 (s, 2 H), 5.83 (d, J = 5 Hz) and 5.90 (d, J = 5 Hz, 1 H total), 6.28 (br m, 1 H), 7.38-7.48 (m, 5 H); MS m/e 801 ((M $(C_{45}H_{76}N_4O_8H_2O)$ C, H, N.

(2'S,3'S,5'S)-N-(5-(((2-Amino-2-methylpropyl)amino)carbonyl)-1-cyclohexyl-3-hydroxy-6-methylheptan-2-yl)- $(1S) \hbox{-} (1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2$ phenylethyl)-L-norleucinamide (52), Part 1. Deprotection of tert-Butyl Carbamate 20. A solution of carbamate 20 (1.20 g, 2.72 mmol) in 20 mL of CH₂Cl₂ was cooled to 0 °C, and 20 mL of trifluoroacetic acid was added dropwise over 5 min. The resulting solution was stirred at 0 °C for 4.5 h and then was concentrated in vacuo to give an oil. The crude product was partitioned between 50 mL of saturated aqueous NaHCO₃, 25 mL of 1 M aqueous NaOH, and 100 mL of CH₂-Cl2. The aqueous phase was saturated with NaCl and extracted with 4×75 mL of CH₂Cl₂, then the combined organic phases were dried (Na_2SO_4) , filtered, and concentrated in vacuo to give 0.898 g (97%) of the crude amino alcohol N-(2amino-2-methylpropyl) (2S,4S,5S)-5-amino-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide: ¹H NMR (CDCl₃) δ 0.74–1.00 (m) and 0.96 (2 overlapping d, 8 H total), 1.13 (s), 1.15 (s) and 1.10-1.40 (br m, 12 H total), 1.45-1.56 (m, 1 H), 1.57-2.10 (several br m, approximately 14 H), 2.15 (m, 1 H), 2.60 (m, 1 H), 3.10 (dd, J = 6, 14 Hz, 1 H), 3.10-3.17 (m, 1 H), 3.25 (dd, J) $J=7,\,14$ Hz, 1 H), 6.25 (br t, J=6 Hz, 1 H); MS m/e 342 ((M $(+ H)^{+}).$

Part 2. Coupling to Carboxylic Acid 33. The above crude amino alcohol (894 mg, 2.62 mmol), carboxylic acid 33 (1.18 g, 2.89 mmol), HOBt hydrate (526 mg, 3.42 mmol), and 4-methylmorpholine (0.41 mL, 377 mg, 3.68 mmol) were dissolved in 20 mL of DMF, and the resulting solution was cooled to -23 °C. EDC (756 mg, 3.94 mmol) was added, and the mixture was stirred at -23 °C for 4 h and allowed to warm to room temperature with stirring overnight (18 h). The solvent was removed by high-vacuum distillation, and the residue was partitioned between 75 mL of CH₂Cl₂ and 75 mL of 80% saturated aqueous NaHCO₃. The aqueous phase was extracted with 75 mL of CH₂Cl₂, and the combined organic phases were dried (Na₂SO₄), filtered, and concentrated in vacuo to a foam (1.98 g). Flash chromatography (5-6%)MeOH-0.5% concentrated aqueous $NH_4OH-CH_2Cl_2$) provided 948 mg (1.30 mmol, 50%) of inhibitor 52 as a white foam: mp 75-83 °C; Rf 0.26 (7.5% MeOH-0.75% concentrated aqueous $NH_4OH-CH_2Cl_2$; ¹H NMR (CDCl₃) δ 0.65-0.84 (br m, 1 H), $0.84-1.00 \ (m, 11 \ H), 1.00-1.40 \ (several \ br \ m) \ and \ 1.19 \ (s, 11 \ H)$ H total), 1.40–1.90 (several br m, 12 H), 2.13–2.67 (v br m, approximately 7 H), 2.67-2.90 (m, 3 H), 3.00-3.20 (m, 1 H), $3.20{-}3.43\,(m)$ and $3.35\,(2~s,\,6~H$ total), $3.45{-}3.60\,(br~m,\,2~H),$ 3.60-3.82 (br m, 2 H), 3.82-3.98 (br m, 1 H), 4.66 (2 s, 2 H), 6.46-6.75 (v br m, 1 H), 6.89 (br d, J = 8 Hz) and 6.96 (br d, J = 8 Hz, 1 H total), 7.25–7.40 (m, 5 H); MS m/e 730 ((M + $H)^+)$. Anal. (C₄₁ $H_{71}N_5O_6$ ·1.5 H_2O) C, H, N.

Protected hydroxyethylene isosteres 14, 15, and 17-30 were deprotected and coupled to amino acid 33 in a similar manner, to provide inhibitors 41-58.

N-(3-Acetamidopropyl)-(2S,4S,5S,1'S,2'S)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phen-ylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2-isopro-

pylhexanamide (62). A solution of amine 3 (23.4 mg, 0.0326 mmol) in 5.4 mL of CH₂Cl₂ was cooled to 0 °C, and N-acetoxynorbornene-2,3-dicarboximide (21.7 mg, 0.122 mmol) was added. The solution was stirred for 3 days at ambient temperature and then concentrated and purified by preparative thin-layer chromatography (silica gel, 10% MeOH-1% concentrated aqueous NH₄OH-CH₂Cl₂), which provided 19.6 mg (0.0258 mmol, 79%) of acetamide 62 as a white foam: mp 62-67 °C; $R_f 0.29 (10\% \text{ MeOH}-1\% \text{ concentrated aq. NH}_4\text{OH}-1\% \text{ concentrated aq. NH}_4\text{ concentrated an}-1\% \text{ concentrated aq. NH}_4\text{ concentrated an}-1\% \text{ concent$ CH₂Cl₂); ¹H NMR (CDCl₃) δ 0.65–1.95 (several br m, 29 H approximately), 0.90 (t, J = 7 Hz) and 0.92 (d, J = 6.5 Hz, 9 H total), 2.00 (s, 3 H), 2.0-2.11 (m, 1 H), 2.97 (m, 1 H), 3.06 (m, 1 H), 3.1-3.5 (br m, 6 H), 3.37 (s, 3 H), 3.5-4.0 (vbr m, 6 H), 4.52 (dd, J = 6, 9 Hz, 1 H), 4.68 (s, 2 H), 5.85 (d, J = 9 Hz)and 5.93 (d, J = 9 Hz, 1 H total), 6.30 (br t, J = 6 Hz, 1 H), 6.48 (br t, J = 6 Hz, 1 H), 7.33 (br m, 5 H); MS m/e 759 $((M+H)^+)$. Anal. $(C_{42}H_{70}N_4O_8 \cdot 1.25H_2O) C, H, N.$

Inhibitor 65 was prepared from primary amine 60 in a similar manner.

N-(3-(Carbamoylamino)propyl)-(2S,4S,5S,1'S,2'S)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (63). Primary amine 3 (40 mg, 0.0558 mmol) was dissolved in 1 mL of THF, trimethylsilyl isocyanate (15 μL, 0.11 mmol) was added, and the solution was stirred at ambient temperature for 2 days. The solution was concentrated and purified by flash chromatography (5% MeOH-CH₂Cl₂), which provided 31 mg (74%) of the title compound as a white powder: mp 87–92 °C; R_f 0.58 (15% MeOH-CH₂-Cl₂); ¹H NMR (CDCl₃) δ 0.65–2.10 (several m, 33 H), 0.93 (m, 9 H), 2.92–3.35 (several m, 6 H), 3.37 (s, 3 H), 3.41-3.63 (m, 3 H), 3.68–3.87 (m, 3 H), 3.87–4.05 (br m, 1 H), 4.60 (m, 1 H), 7.33 (m, 5 H); MS m/e 760 ((M + H)⁺). Anal. (C₄₁H₆₉N₅O₈·H₂O) C, H, N.

Inhibitor **66** was prepared from primary amine **60** in a similar manner.

N-(2-(Thioureido)ethyl)-(2S,4S,5S,1'S,2'S)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (67). Primary amine 60 (50.0 mg, 71.1 μ mol) was dissolved in 1.4 mL of THF, the solution was cooled to 0 °C, and N,N'-thiocarbonyldiimidazole (15.8 mg, 88.9 μ mol) was added. The yelllow solution was stirred and allowed to warm to room temperature over 18 h. The resultant thionoimidazolide was then treated with 30% aqueous NH₄OH (0.75 mL, 5.78 mmol), and the resulting mixture was allowed to react at ambient temperature for 48 h. The reaction mixture was concentrated to an oil, which was purified by flash chromatography (4% MeOH-CH₂Cl₂), that provided 28.2 mg (37.0 μ mol, 52%) of thiourea 67 as a white powder: mp 98-112 °C; $R_f 0.40 (5\% \text{ MeOH}-\text{CH}_2\text{Cl}_2); \ ^1\text{H} \ \text{NMR} \ (\text{CDCl}_3) \ \delta \ 0.51-1.96$ (several br m, approximately 37 H), 2.81-2.94 (m, 1 H), 3.01 (dd, J = 10, 13 Hz), 3.05-3.48 (br m) and 3.48 (2 s, 9 H total),3.455-4.26 (several br m, 8 H), 4.69 (s, 2 H), 4.68-7.80 (br m, 1 H), 5.63 (d, J = 9 Hz) and 5.68 (d, J = 9 Hz, 1 H total), 6.19 (br m, 1 H), 6.52 (br m, 1 H), 6.92 (br m, 1 H), 7.29-7.40 (m, 5 H); MS m/e 762 ((M + H)⁺). Anal. (C₄₀H₆₇N₅O₇S) C, H, N. Inhibitor 64 was prepared from primary amine 3 in a similar

manner.

N-(3-(S-Methyl-N'-cyanoisothioureido)propyl)-(2S,4S,5S,1'S,2'S)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (68). A solution of primary amine 3 (250 mg, 0.349 mmol), dimethyl N-cyanothioiminocarbonate (66 mg, 0.453 mmol) in 7 mL acetonitrile was refluxed for 2 days. The reaction was concentrated in vacuo, and the residue was purified by flash chromatography (4% MeOH-CH₂Cl₂) to provide 240 mg (85%) of isothiourea 68 as a white powder: mp 88-94 °C; $R_f 0.49 (10\% \text{ MeOH}-\text{CH}_2\text{Cl}_2)$; ¹H NMR (CDCl₃) δ 0.65–1.90 (several br m, approximately 37 H), 2.08 (m, 1 H), 2.59 (s, 3 H), 2.95 (dd, J = 5, 13 Hz, 1 H), 3.03 (dd, J = 9, 13 Hz, 1 H), 3.12-3.54 (br m) and <math>3.38 (2 s, 1)12 H total), 3.70-4.04 (several br m, 5 H), 4.50 (m, 1 H), 4.68 (s, 2 H), 5.79 (d, J = 9 Hz) and 5.85 (d, J = 9 Hz, 1 H), 6.21 (br s, 1 H), 7.27-7.39 (m, 5 H), 7.89 (br s, 1 H); MS m/e 815 ((M + H)⁺). Anal. (C₄₃H₇₀N₆O₇S·0.5H₂O) C, H, N.

Isothioureas 69 and 70 were prepared from primary amines 60 and 61, respectively, in a similar manner.

N-(3-(N-Cyano-N'-methylguanidino)propyl)-(2S,4S,5S,1'S,2'S)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (72). Isothiourea 68 $(50 \text{ mg}, 61.3 \,\mu\text{mol})$ was dissolved in 2 mL of EtOH in a screwcap vial, 40% aqueous methyl amine (132 μ L, 1.53 mmol) was added, and the vial was sealed and warmed to 90 °C for 48 h. The solution was concentrated and purified by flash chromatography (3.75% MeOH-CH₂Cl₂), which provided 39.3 mg $(49.2 \,\mu\text{mol}, 80\%)$ of guanidine 72 as a white powder: mp 101-7°C; $R_f 0.34 (7.5\% \text{ MeOH}-CH_2Cl_2)$; ¹H NMR (CDCl₃) $\delta 0.84-$ 0.99 (m) and 0.64-1.93 (several br m, approximately 37 H total), 2.13 (m, 1 H), 2.88 (d, J = 5 Hz, 3 H), 2.93-4.10 (several br m, 15 H), 3.38 (s) and (s, 3 H total), 4.61 (m, 1 H), 4.69 (s, 2 H), 5.72 (br d, J = 8 Hz) and 5.78 (br d, J = 8 Hz, 1 H total), 5.90 (br s, 1 H), 6.31 (br s, 2 H), 7.28-7.40 (br m, 5 H); MS m/e 798 ((M + H)⁺), 815 ((M + NH₄)⁺). Anal. (C₄₃H₇₁N₇O₇) C, H, N.

Guanidines **71**, **73**, and **74** were prepared from isothioureas **68**, **69**, and **70**, respectively, in a similar manner.

N-(3-((5-Amino-1,2,4-triazol-2-yl)amino)propyl)-(2S,4S,5S,1'S,2'S)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (75). Isothiourea 68 $(50 \text{ mg}, 61.3 \,\mu\text{mol})$ and hydrazine monohydrate (74 mL, 1.53 mmol) were dissolved in 2 mL of EtOH in a 2 dram screw-cap vial, and the vial was sealed and warmed to 90 °C for 48 h. The solution was concentrated to an oil, which was purified by flash chromatography (10% MeOH-1% concentrated NH₄-OH-CH₂Cl₂), which provided 44.6 mg (55.8 mmol, 91%) of triazole **75** as a white powder: mp $110-20^{\circ}$ C; $R_f 0.24$ (10%) MeOH-1% concentrated NH₄OH-CH₂Cl₂); ¹H NMR (CDCl₃) δ 0.58-1.90 (several br m, approximately 37 H), 2.11 (br m, 1 H), 2.90-3.09 (m, 2 H), 3.37 (s), 3.38 (s) and 3.11-4.04 (several br m, 15 H total), 4.54–4.65 (br m, 1 H), 4.68 (s) and 4.69 (s, 2 H total), 4.70-5.50 (v br m, 3 H), 5.67 (br d, J = 9 Hz) and 5.75 (br d, J = 9 Hz, 1 H), 7.13–7.43 (br m) and 7.29 (m, 7 H total); MS m/e 799 ((M + H)⁺). Anal. (C₄₂H₇₀N₈O₇·H₂O) C, H, N.

Triazoles **76** and **77** were prepared from isothioureas **69** and **70**, respectively, in a similar manner.

(2'S,3'S,5'S)-N-(5-(((2-((2-Hydroxyethyl)amino)-2-methylpropyl)amino)carbonyl)-1-cyclohexyl-3-hydroxy-6methylheptan-2-yl)-(1S)-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethyl)-l-norleucinamide (80). Part 1. Reductive Alkylation of Primary Amine 52. Amine 52 (130 mg, 0.179 mmol) and (benzyloxy)acetaldehyde (55.6 mg, 0.370 mmol) were dissolved in 1.8 mL of 2-propanol, and the pH was adjusted to ca. 6.0 with HOAc (12 μ L) and NaOAc (5 mg). The resulting solution was cooled to 0 °C and NaBH₃CN (14.3 mg, 0.228 mmol) was added. The mixture was stirred at 0 °C for 6 h and then at ambient temperature for an additional 14 h. The reaction was concentrated in vacuo and partitioned between 30 mL of EtOAc and 30 mL of 1 M aqueous Na_2CO_3 . The organic phase was washed (20 mL H₂O, 20 mL brine) and dried (Na_2SO_4) , and the filtrate was concentrated in vacuo to a colorless glass (163 mg, 105% crude yield). The crude secondary amine was purified by flash chromatography (4% MeOH-0.5% concentrated aqueous NH₄-OH-CH₂Cl₂) to provide 44.0 mg (0.051 mmol, 29%) of the benzyl ether as a colorless glass: $R_f 0.21 (5\% \text{ MeOH}-0.5\%)$ concentrated aqueous NH₄OH-CH₂Cl₂); ¹H NMR (CDCl₃) δ $0.64{-}1.00$ (m, 9 H), 1.10 (s) and $1.0{-}2.0$ (several br m, approximately 31 H), 2.07-2.17 (br m, 1 H), 2.25-2.43 (br m, 1 H), 2.70-3.00 (br m, 6 H), 3.36 (s) and 3.10-4.00 several br m (12 H total), 4.54 (s, 2 H), 4.65 (s, 2 H), 6.30 (br s, 1 H), 6.85 (br d, J = 9 Hz) and 6.93 (br d, J = 9 Hz, 1 H total), 7.20-7.40 (m, 10 H); MS m/e 864 (M + H)⁺

Part 2. Hydrogenolysis of Benzyl Ether. The above benzyl ether (39.5 mg, 45.7 μ mol) was hydrogenated over 10 mg of 10% Pd(OH)₂/C in 3 mL of EtOAc under 4 atm of H₂ for 7 days. The mixture was filtered through Celite, using 25 mL of EtOAc and 25 mL of MeOH to wash the catalyst. The combined filtrate and washes were concentrated in vacuo to give 31.4 mg of a yellow viscous oil, which was purified by preparative TLC (10% MeOH=0.5% concentrated aqueous NH₄OH=CH₂Cl₂) to produce 12.4 mg (16.0 mol, 35%) of pure amino alcohol **80** as a colorless glass: R_f 0.07 (5% MeOH=0.5% concentrated aqueous NH₄OH=CH₂Cl₂); ¹H NMR (CDCl₃) δ 0.68=1.00 (m, 12 H), 1.00=1.47 (several br m, approximately 20 H), 1.49=1.90 (several br m, 13 H), 2.0=2.6 (vbr m, H₂O), 2.63=2.90 (m, 5 H), 2.97=3.15 (m, 2 H), 3.20=3.47 (br m) and 3.35 (2 s, 5 H total), 3.48=4.05 (several br m, 8 H), 4.67 (2 s, 2 H), 6.88 (br d, J = 7.5 Hz) and 6.94 (br d, J = 7.5 Hz, 1 H total), 7.25=7.37 (m, 5 H); HRMS calcd for C₄₃H₇₆N₅O₇ ((M + H)⁺) 774.5745, found 774.5746.

(1S,3'S,5'S)-N-(2-Cyclohexyl-1-(3-isopropyl-2-oxo-2,3,4,5tetrahydrofuran-5-yl)ethyl)-(1S)-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethyl)-l-norleucinamide (82). Part 1. Deprotection of Lactone Carbamate **13.** Lactone **13** (1.50 g, 4.24 mmol) was dissolved in 20 mL of CH₂Cl₂, the solution was cooled to 0 °C, and 20 mL of TFA was added rapidly dropwise. The solution was stirred for 4 h at 0 °C and then concentrated in vacuo to an oil. The crude material was made basic with 75 mL of 1 N Na₂CO₃, and the mixture was saturated with NaCl and extracted with 3×100 mL CH_2Cl_2 . The combined organic phases were dried (Na₂- SO_4), filtered, and concentrated in vacuo to provide (3S, 5S, 1'S)-5-(1-amino-2-cyclohexylethyl)-3-isopropyltetrahydrofuran-2one as a crystalline solid, 1.086 g (101% crude yield): ¹H NMR $(CDCl_3) \delta 0.73 - 0.93 (m, 1 H), 0.96 (d, J = 7 Hz, 3 H), 1.03 (d, J$ J = 7 Hz, 3 H), 1.05–1.55 (several m, 8 H), 1.60–1.84 (br m, 5 H), 2.02-2.24 (m, 3 H), 2.58-2.68 (m, 1 H), 2.81 (dt, J = 6, 8 Hz, 1 H), 4.20 (dd, J = 8, 12 Hz, 1 H); MS m/e 254 ((M + H)+).

Part 2. Coupling to Carboxylic Acid 33. Crude amine (1.07 g, 4.24 mmol maximum), acid 33 (1.90 g, 4.67 mmol), and HOBt hydrate (845 mg, 5.52 mmol) were dissolved in 40 mL of DMF, 4-methylmorpholine (653 mL, 601 mg, 5.94 mmol) was added, the solution was cooled to -23 °C, and EDC (1.22 g, 6.36 mmol) was added as a solid. The reaction mixture was stirred over 18 h, being allowed to warm slowly to ambient temperature. The solution was concentrated under high vacuum, and the residue was partitioned between 150 mL of CH₂Cl₂ and 100 mL of 1 M aqueous Na₂CO₃. The organic phase was washed with 100 mL of brine, dried (Na_2SO_4) , filtered, and concentrated in vacuo to give 3.21 g of a viscous oil. Flash chromatography (2% MeOH-CH₂Cl₂) provided 2.13 g (3.32 mmol, 78%) of lactone amide 82 as a waxy oil: $R_f 0.42$ $(5\% \text{ MeOH}-\text{CH}_2\text{Cl}_2)$; ¹H NMR (CHCl₃) $\delta 0.63-0.80$ (br m 1h), 0.80-0.97 (m), 1.01 (d, J = 7 Hz) and 1.04-1.45 (br m, approximately 20 H total), 1.45-1.90 (br m, 11 H), 1.90-2.20 (m, 3 H), 2.28 (br d, J = 12 Hz, 1 H), 2.45 - 2.55 (m, 1 H), 2.64 - 2.55 (m, 1 H), 2.552.90 (m, 3 H), 2.97-3.10 (br m, 1 H), 3.28-3.47 (m), 3.35 (s) and 3.36 (s, 5 H total), 3.54-4.02 (several br m, 6 H), 4.63-4.67 (m, 2 H), 6.48 (br d, J = 11 Hz) and 6.60 (br d, J = 11 Hz)1 H total), 7.20-7.35 (m, 5 H); MS m/e 642 ((M + H)⁺). Anal. $(C_{37}H_{59}N_3O_6)$ C, H, N.

Lactone 81 was prepared from acid 32 in the same manner.

(2'S,3'S,5'S)-N-(5-(((3-((2-Hydroxyethyl)amino)propyl) $amino) carbonyl) \hbox{-} 1-cyclohexyl-3-hydroxy-6-methyl heptan-$ 2-yl)-(1S)-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethyl)-l-norleucinamide (86). Lactone 82 (423 mg, 0.659 mmol), 3-((2-hydroxyethyl)amino)propylamine (700 mg, 5.93 mmol) and glacial acetic acid (113 μ L, 119 mg, 1.98 mmol) were combined, and the mixture was warmed to $85\ ^\circ C$ for 24 h. The mixture was dissolved in 75 mL of EtOAc and extracted with H_2O (4 \times 50 mL) and then 50 mL of brine. The organic phase was dried $(Na_2SO_4), \, filtered, \, and \, concentration of the second state of the sec$ trated in vacuo to a white foamy solid. The crude amide was partially purified by flash chromatography (7.5% MeOH-CH2- Cl_2) and further purified by preparative TLC (20% MeOH-2% concentrated aqueous NH₄OH) to give 272 mg (52%) of pure **86** as a foamy solid: mp 58-67 °C; $R_f 0.34 (15\% \text{ MeOH}-1.5\%)$ concentrated $NH_4OH-CH_2Cl_2$); ¹H NMR (CDCl₃) δ 0.65-1.00 (m, 11 H), 1.00-1.95 (several br m, approximately 25 H), 2.00-2.14 (m, 1 H), 2.32 (br dd, J = 5, 12 Hz, 1 H), 2.62-2.92 (br m,8 H), 3.03-3.31 (br m, 4 H), 3.31-3.60 (br m), 3.36 (s) and $3.37~(s,\,7~H$ total), $3.60{-}3.95~(br~m,\,6~H),\,3.95{-}4.06~(br~m,\,1$ H), 4.67 (2 s, 2 H), 6.89 (d, J = 8 Hz) and 6.96 (d, J = 8 Hz, 1 H total), 7.03-7.10 (br m) and 7.14-7.22 (br m, 1 H total),

7.24–7.36 (m, 5 H); MS m/e 760 ((M+H)⁺). Anal. (C₄₂H₇₃N₅O₇·H₂O) C, H, N.

Inhibitors 83 and 84 were prepared from lactone 81, and inhibitors 85 and 87 were prepared from lactone 82 in an analogous manner.

Biochemical Methods. Renin inhibitory activities against rat, ferret, dog, and human plasma renins were determined as previously described.^{2b} Plasma drug concentrations were measured by a renin inhibitory assay, and the resultant plasma drug level-time data were fitted to a biexponential decay model, as described previously.^{2b}

Physicochemical Methods. Octanol/water distribution coefficients $(\log P)$ were determined by the shake-flask method at the physiologically relevant pH's 6.5 and 7.4.²⁰ Approximately 0.5 mg of test compound was dissolved in 1.5 mL of 1-octanol. To a test tube containing 5 mL of 0.05 M PIPES (pH 6.5) or HEPES (pH 7.4) buffer was added 0.5 mL of the compound in 1-octanol and mixed for 4 h at room temperature and centrifuged for 15 min at 3000 rpm. Samples from both the 1-octanol and aqueous phase were appropriately diluted with mobile phase and assayed by HPLC on a reverse-phase ODS Little Champ column $(5 \times 0.4 \text{ cm})$ from Regis Chemical Co. Dilution of the 1-octanol phase by mobile phase must be at least 1:100 in order to completely dissolve the 1-octanol. The mobile phase was a mixture of CH₃CN-MeOH-0.02 M tetramethylammonium perchlorate-0.1% TFA. Flow rate was 1 mL/min with detection by UV absorption at 210 nm. After dilution factor adjustment, the distribution coefficient was calculated as the HPLC peak area ratio of the 1-octanol phase to the aqueous phase.

Solubility determinations were performed as follows. Approximately 2–5 mg of test compound was placed into a small glass vial and then 0.5–1.0 mL of buffer (0.05 M aqueous phosphate containing 0.15 M NaCl, adjusted to pH 7.4) was added. The mixtures were equilibrated by end-over-end rotation in a water bath at 25 °C for at least 24 h. The equilibrated suspension was filtered through a 0.45 μ m filter, and the filtrate was assayed by HPLC. Quantitation was achieved by comparison of sample peak area to a 3–4 point standard curve (peak area vs concentration). Standards were prepared from a portion of the original material.

Microsomal Metabolism Studies. Portions of two human livers, which could not be used for transplantation, were obtained from the International Institute for the Advancement of Medicine (Essington, PA). The livers were sectioned into 6 g portions, finely chopped with scissors or a razor blade, and each portion was homogenized in 0.01 M (pH 7.4) sodium phosphate buffer containing 1.15% KCl using a mechanical high-frequency homogenizer. Each homogenate was centrifuged at 9000g at 4 °C for 20 min, then the supernatant fraction was centrifuged at 105000g at 4 °C for 1 h. The resulting pellet was resuspended in buffer and recentrifuged at 105000g for an additional hour. The microsomal pellet was resuspended in 0.1 M (pH 7.4) potassium phosphate buffer containing 20% (v/v) glycerol and 10 mM EDTA to afford a protein concentration of 14-23 mg/mL. Microsomal protein content was determined with BCA reagent (Pierce), using bovine serum albumin as the standard, according to the supplier's method, while cytochrome P-450 content was measured according to a literature procedure.¹ Samples were designated EGF-426 (0.77 nmol of cytochrome P-450/mg of protein) and FGL-852 (0.41 nmol of cytochrome P-450/mg of protein).

Individual 1.0-mL microsomal incubation mixtures contained 20 μ M of renin inhibitor, 1 mM magnesium chloride, 0.14-0.35 mg/mL of human microsomal protein, and an NADPH generating system consisting of 5 mM glucose 6-phosphate, 1 mM NADP, and 1-2 units of glucose-6-phosphate dehydrogenase (Sigma) in 0.1 M potassium phosphate buffer (pH 7.4). After a 5 min preincubation period at 37 °C, reaction was started by addition of the NADPH generating system to each incubation mixture. Incubations were conducted for 5-20 min and were stopped either with 2-3 drops of 1 M sodium carbonate and 2 mL of ethyl acetate (53-55) or with 2 mL of acetonitrile (all other compounds). Ethyl acetate was removed from the extracted samples and the aqueous layer was acidified with 1 M HCl and extracted with a second 2-mL

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portion of ethyl acetate. The combined organic layers were concentrated to dryness under a nitrogen stream. Protein precipitated by acetonitrile addition was removed via centrifugation and the superantant was concentrated to dryness under nitrogen. All sample residues were then reconstituted in 200 μL of HPLC mobile phase for analysis. For each renin inhibitor, a zero-hour incubation (quenched immediately) and a 20 min no-NADPH control incubation were also conducted.

HPLC Analysis. Microsomal incubation samples were analyzed using a Perkin-Elmer Series 410 liquid chromatography pump, equipped with a Hitachi Model AS-2000 autosampler for automatic injection, and an Applied Biosystems Model 1000S photodiode array detector operated at 215 nm. Separations were achieved at ambient temperature with a Beckman Ultrasphere 5 μ m 4.6 imes 250 mm C₁₈ column connected to a Nucleosil 5 μ m C₁₈ cartridge guard column (Alltech). A linear gradient of 30-70% acetonitrile in 0.1% trifluoroacetic acid (adjusted to pH 4.6 with ammonium acetate)-0.01 M tetramethylammonium perchlorate over 55 min was used as column eluent at a flow rate of 1 mL/minute. Levels of unmetabolized parent drug in individual incubation samples were quantitated using an internal standard method. Metabolites were putatively identified on the basis of comparison of HPLC retention times to those of synthetic standards.

Pharmacological Methods. The pharmacological studies in the rat, ferret, and dog models were performed according to previously described methods.²

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Supplementary Material Available: Characterization data (nuclear magnetic resonance spectra, mass spectra, melting points and microanalytical) for all compounds contained in Tables 1 and 2 which do not appear in the Experimental Section (17 pages). Ordering information is given on any current masthead page.

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