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 ± 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 signigicant challenge to medicinal chemists. In the case of inhibitors of renin, the enzyme responsible for the rate-limiting 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 P_2-P_4 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 id[4] bioavailability of 27 ± 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 ^{14}C-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*, 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 ^{14}C-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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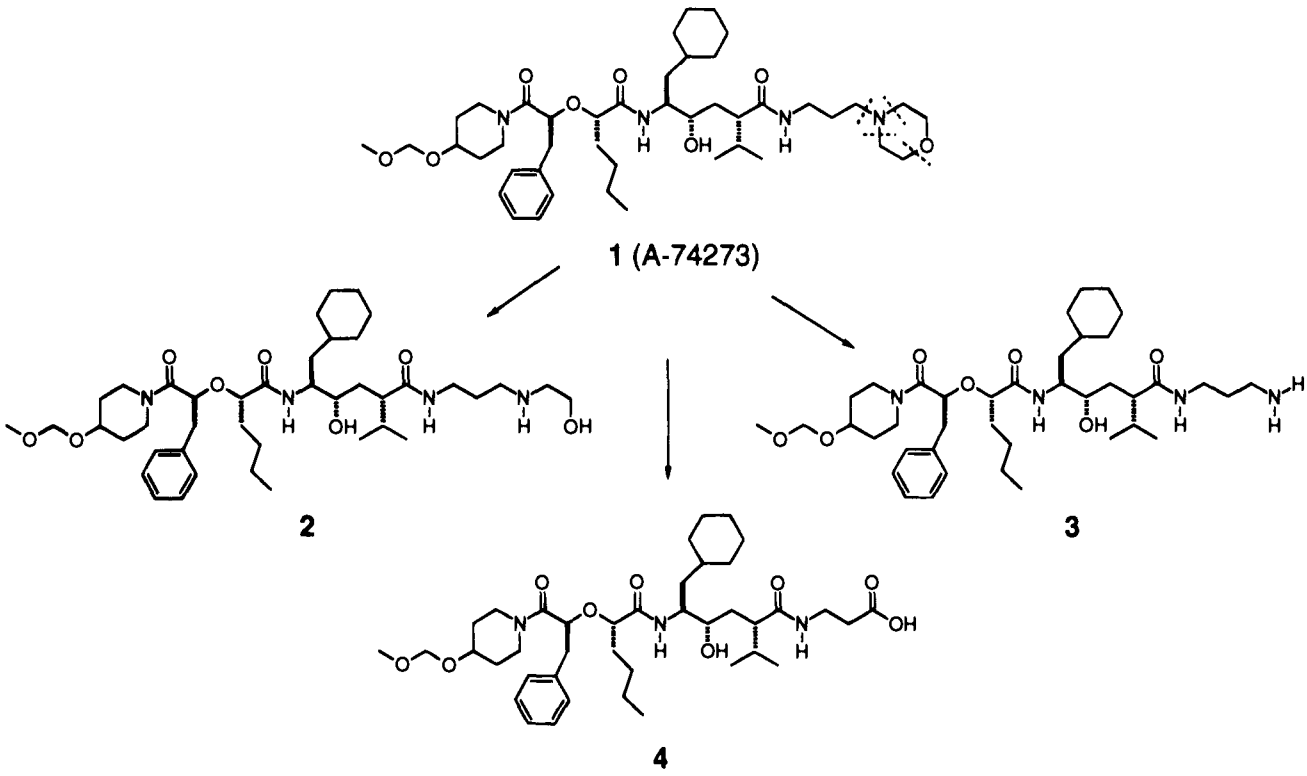
Scheme 1. Metabolic Pathways for A-74273 (1)



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 model[6] 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 simulations[7] 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 hydroxyethylene 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 doubly-protected 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

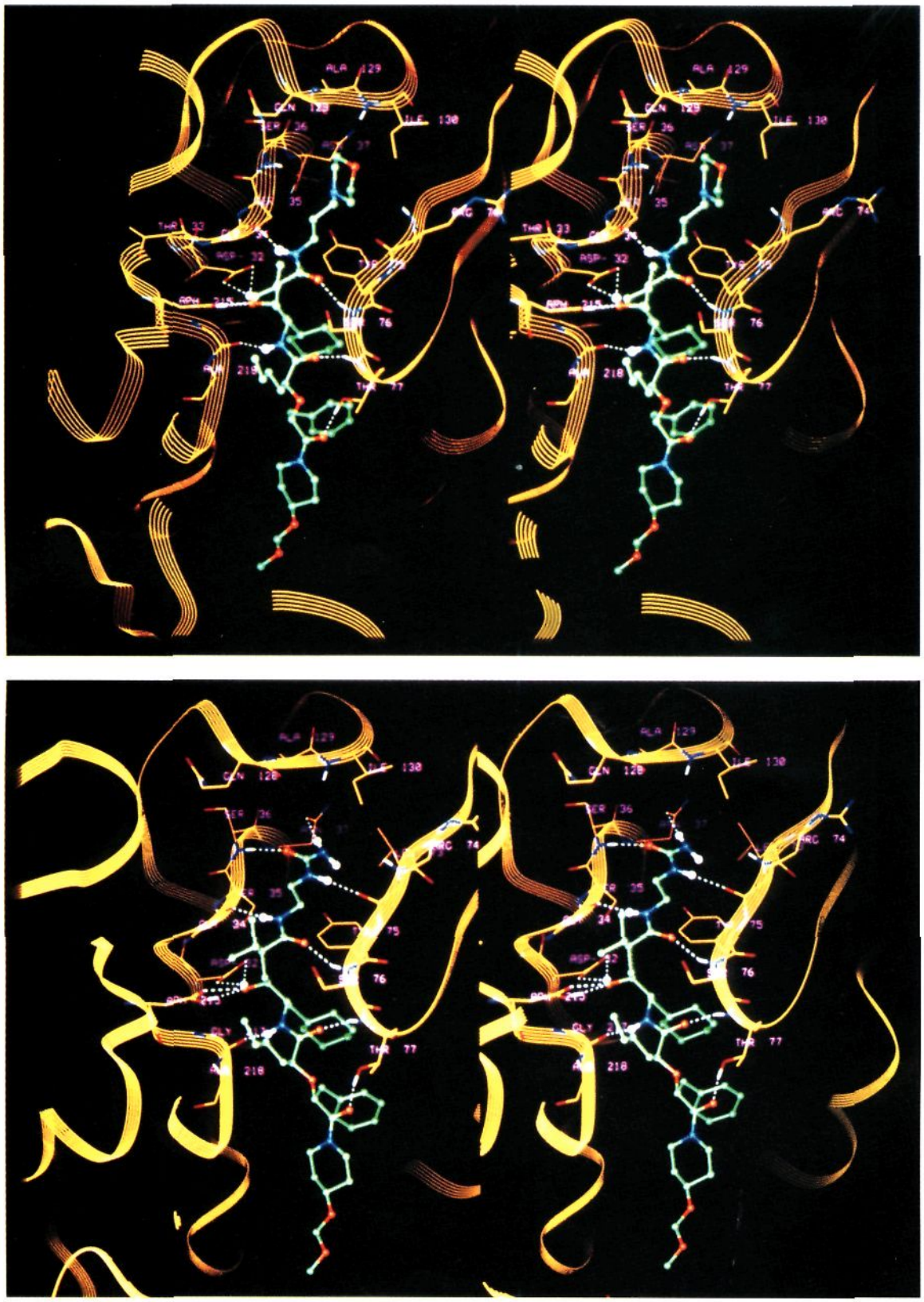
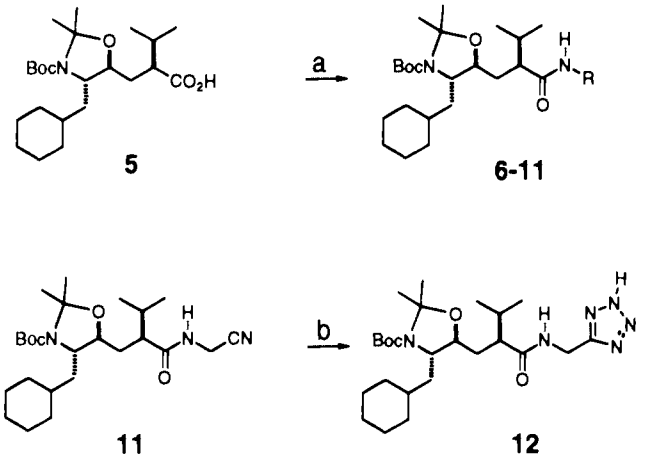


Figure 1. Panel A (top) shows a computer model of nonpeptide inhibitor **1** bound to the active site of human renin (crossed-stereoview). The enzyme backbone is shown in ribbon representation, with only the side chains involved in hydrogen bonding (dashed lines) displayed. The active-site aspartic acids 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 hydrogen-bonding 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 **5**[a]



[a] 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.

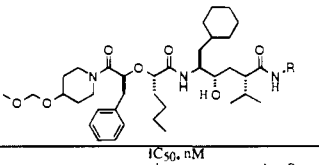
fashion. Primary amine **16** was converted to 1,2,4-oxadiazole **30** by a known procedure.[11]

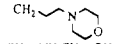
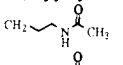
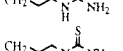
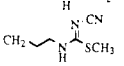
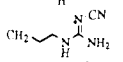
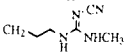
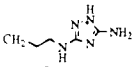
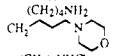
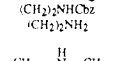
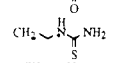
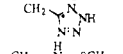
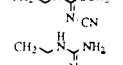
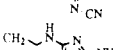
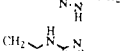
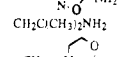
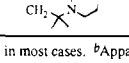
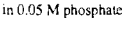
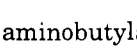
As Scheme 4 illustrates, the protected dipeptide isosteres were deprotected in the appropriate manner to provide the free amino alcohols **31**, which were not purified. The isolated crude amino alcohols were coupled to ether-containing carboxylic acid **32** under standard carbodiimide conditions to 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 initally 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 *N*-cyanodithioiminocarbonate to give the isothioureas **68**, **69**, and **70**, respectively. The isothioureas were further substituted with amines to produce guanidines **71–74** and 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 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.

Table 1. In Vitro Potency against Human Plasma Renin, Partition Coefficients, and Aqueous Solubilities for 3-Oxaglutaramide Inhibitors



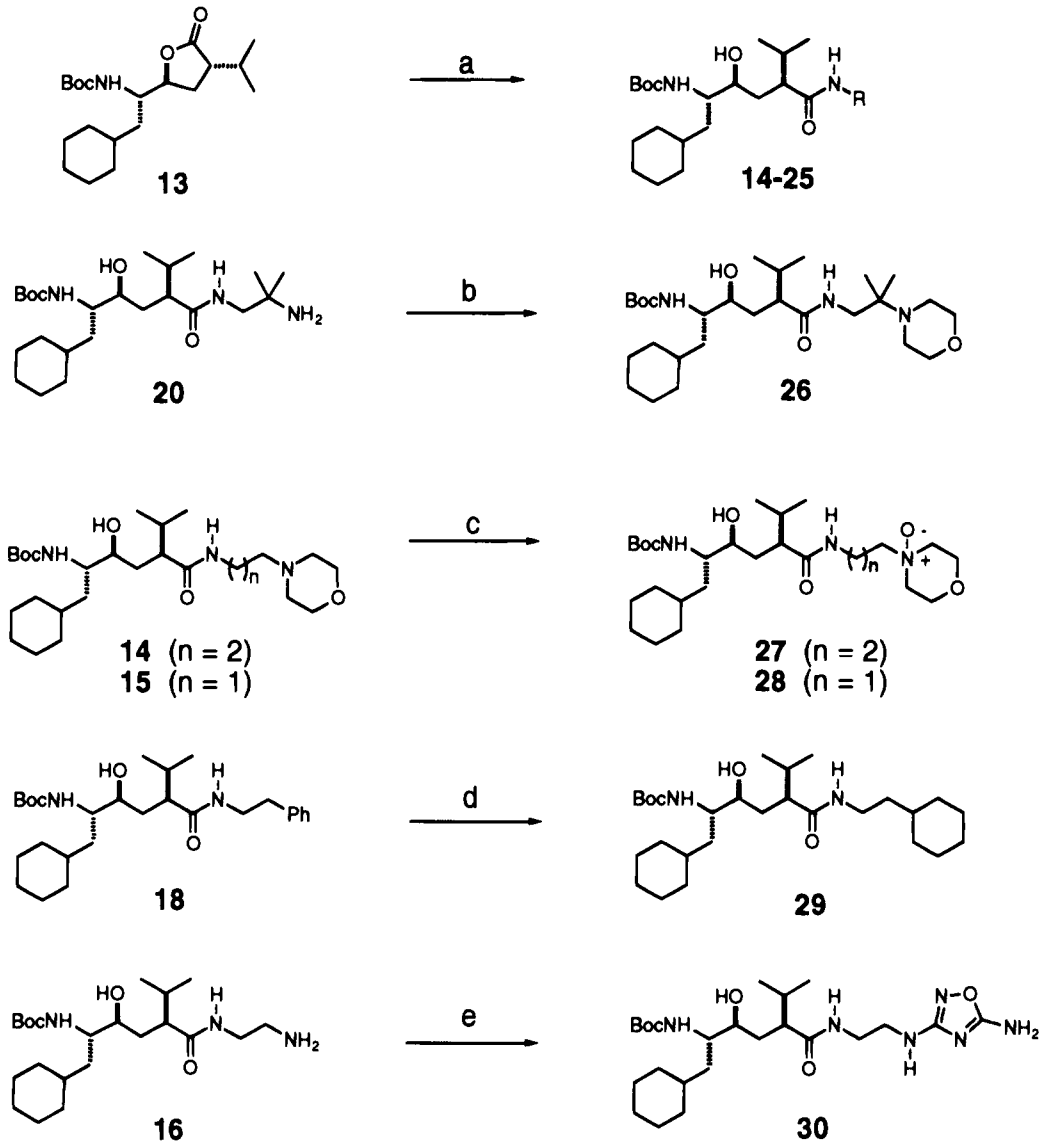
inhibitor	R	IC_{50}, nM human plasma renin, pH 7.4[a]	log *P* pH 7.4 (pH 6.5)[b]	aqueous solubility pH 7.4, mg/mL[c]
1		2.8	4.3 (3.3)	0.210
2	$(CH_2)_3NH(CH_2)_2OH$	6.0	1.8 (2.5)	1.84
34	$(CH_2)_3NHCbz$	4.3	nd[d]	nd
3	$(CH_2)_3NH_2$	4.5	1.8 (2.4)	1.87
4	$(CH_2)_2CO_2H$	6.8	2.8 (1.9)	nd
35	$(CH_2)_2CO_2Et$	12	nd	nd
62		2.4	4.3 (4.3)	0.180
63		2.0	4.2 (4.2)	0.015
64		2.5	nd	nd
68		1.3	nd	nd
71		1.5	4.5 (4.6)	nd
72		1.3	4.4 (4.4)	nd
75		1.7	4.2 (4.3)	nd
84	$(CH_2)_4NH_2$	4.3	nd	nd
83		3.3	nd	nd
36	$(CH_2)_2NHCbz$	11	nd	nd
60	$(CH_2)_2NH_2$	3.9	nd	nd
65		2.6	nd	nd
66		2.8	nd	nd
67		2.2	>5 (>5)	nd
37		8.4	2.0 (2.9)	nd
69		9.2	>5 (>5)	nd
73		1.8	>5 (>5)	nd
76		1.1	4.0 (3.9)	0.285
40		3.1	4.4 (4.5)	0.047
38	$CH_2O(CH_2)_2NH_2$	4.7	3.5 (2.7)	0.660
39		5.9	>5 (>5)	nd

[a] Single determination in most cases. [b] Apparent octanol-aqueous phosphate buffer partition coefficient. [c] Determined at 37 °C in 0.05 M phosphate containing 0.15 M NaCl. [d] nd = Value not determined.

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]



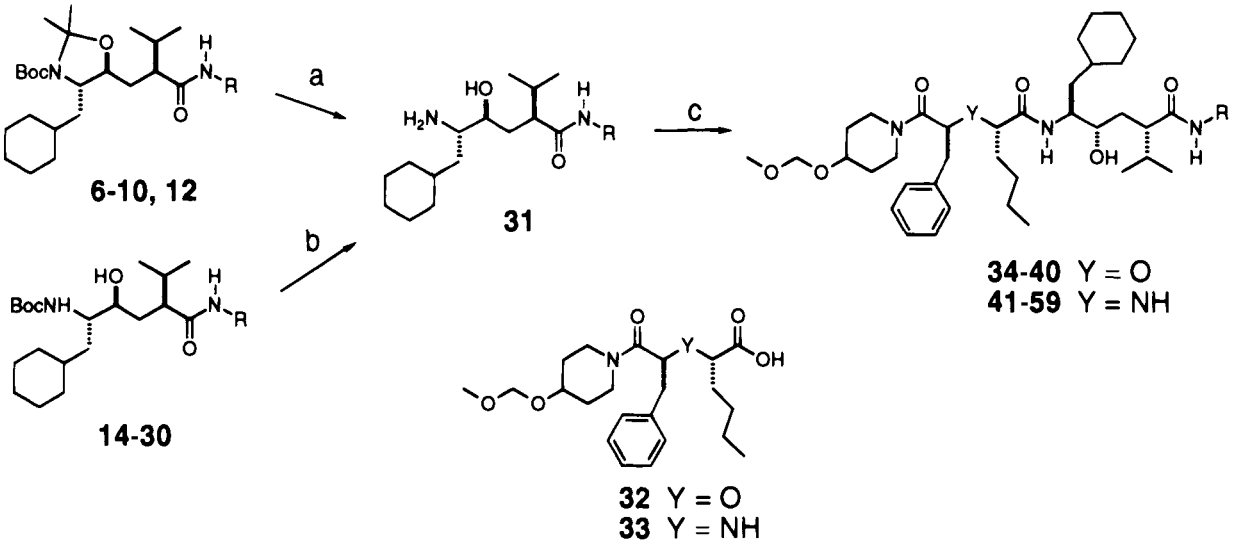
[a] Reaction conditions: (a) H_2NR neat, 60–80 °C, 2–5 days; or H_2NR (9 equiv), HOAc (3 equiv), 65 °C, 1–2 h. See Table 2 for R-group structures; (b) (1) 3,4-dihydrofuran, CH_2Cl_2, MeOH, O_3, −70 to −65 °C; (2) $NaBH_3CN$; (c) H_2O_2, MeOH, 25 °C; (d) 4 atm H_2, 5% Rh/C, MeOH; (e) (1) $(MeS)_2C{=}N(CN)$, CH_3CN, reflux, (2) H_2NOH, Et_3N, 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 four-carbon 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 two-carbon 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

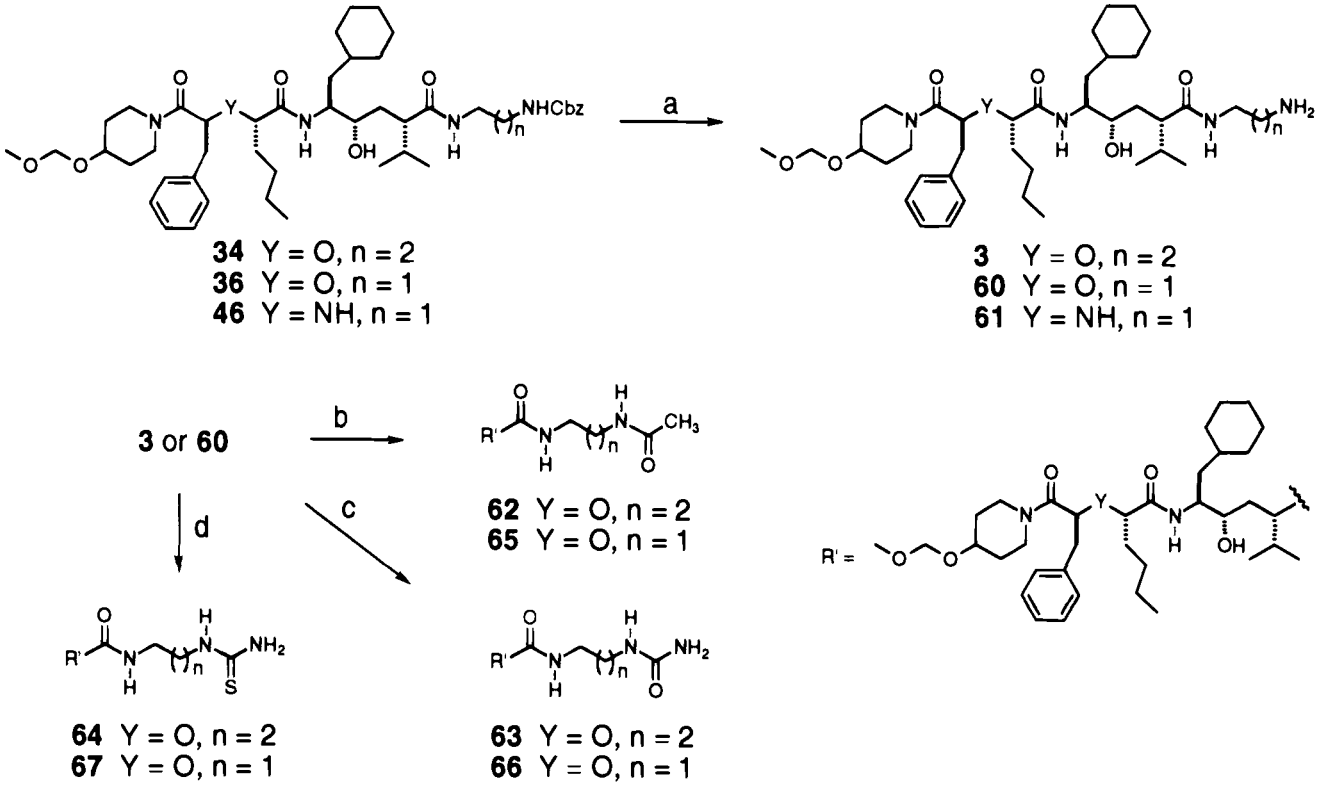
Inhibitory Potencies and Physicochemical Properties. Analogues similar to those discussed above were examined for the 3-azaglutaramides, with the goal of identifying equally high affinity members of this synthetically accessible series. Table 2 presents the in vitro data for these compounds. Inhibitory 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, CH_2Cl_2 (1:1), 0 °C; (2) H_2O, THF, TFA, 20 °C; (3) Na_2CO_3; (b) (1) TFA, CH_2Cl_2 (1:1), 0 °C; (2) Na_2CO3; (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]



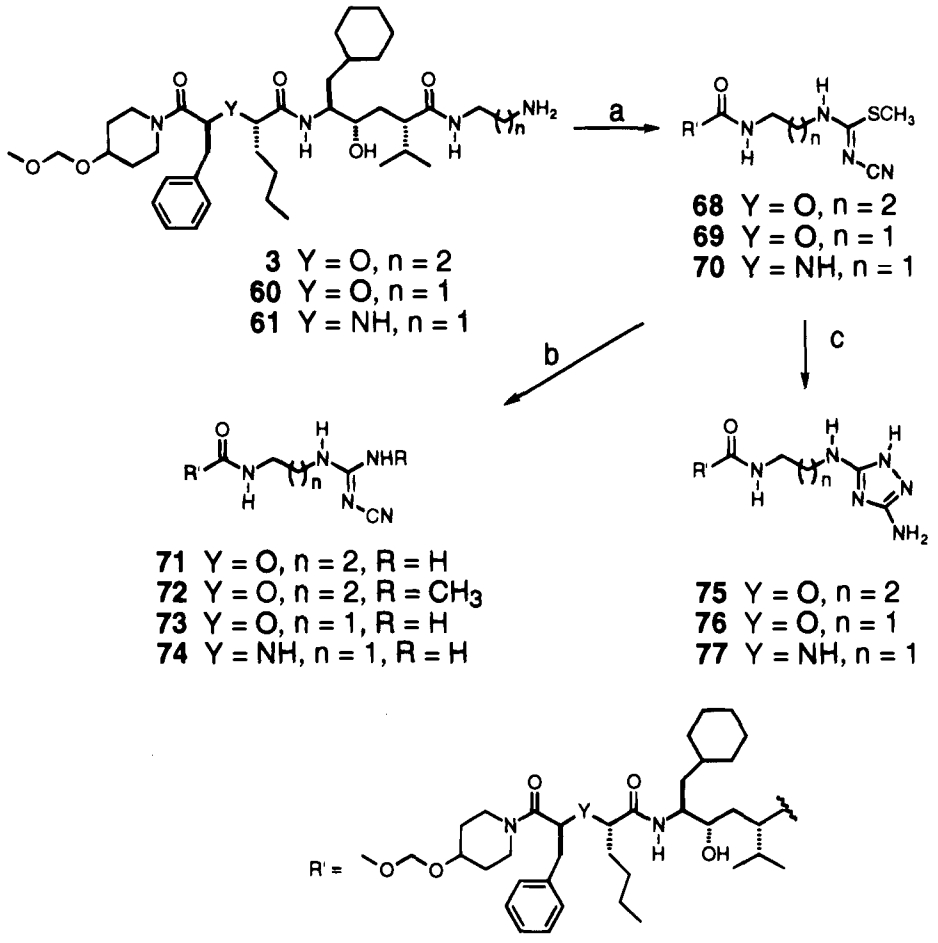
[a] Reaction conditions: (a) H_2 (4 atm), 10% Pd/C, EtOAc; (b) *N*-acetoxy-5-norbornene-2,3-dicarboximide, CH_2Cl_2, 25 °C; (c) *N*-trimethylsilyl isocyanate, THF, 0–25 °C; (d) (1) *N,N'*-thiocarbonyldiimidazole, CH_2Cl_2, 0–25 °C; (2) concentrated aqueous NH_3, 25 °C.

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 nM) and 5-amino-1,2,4-oxadiazole **58** (IC_{50} = 3.8 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 μM 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.

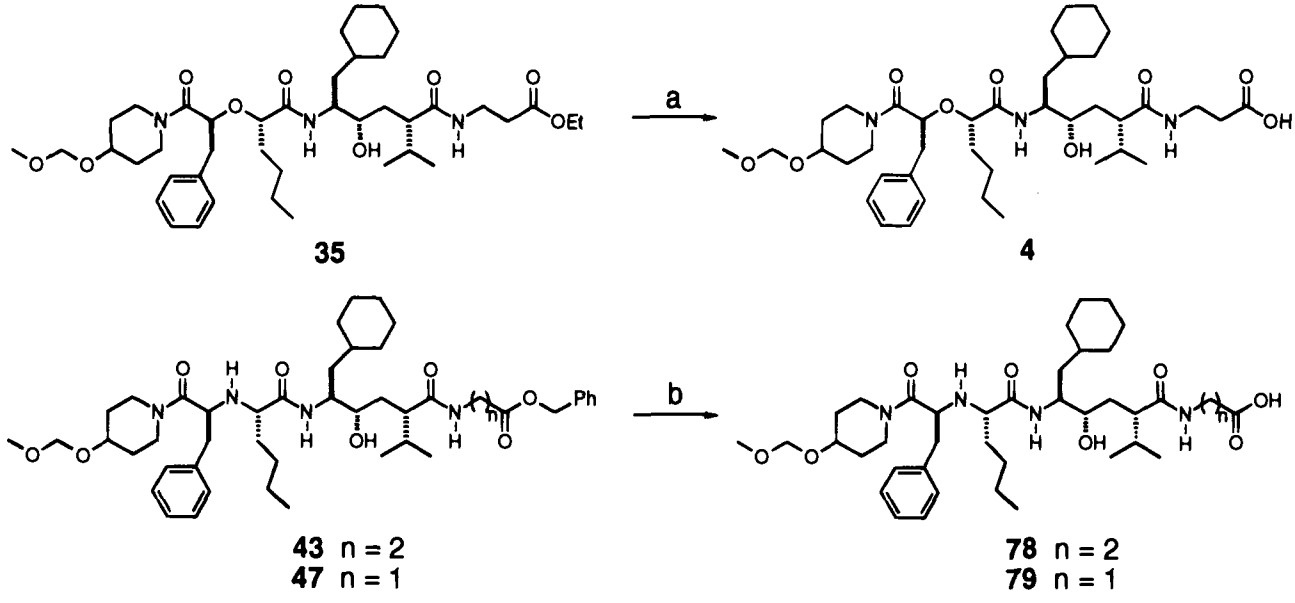
Table 2 also lists the physical parameters of log *P* at physiologic and mildly acidic pH, plus solubility in aqueous buffer at physiologic pH. The compounds fall into three general categories: (a) moderately lipophilic (log *P* < 4) and soluble (>1 mg/mL), (b) lipophilic (log *P* ~ 4) and modestly soluble (~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) $(CH_3S)_2C{=}N(CN)$, CH_3CN, reflux; (b) concentrated aqueous RNH_2, EtOH, 90 °C (sealed tube); (c) $H_2NNH_2{\cdot}H_2O$, EtOH, reflux; (d) $H_2NOH{\cdot}HCl$, Et_3N, EtOH, reflux.

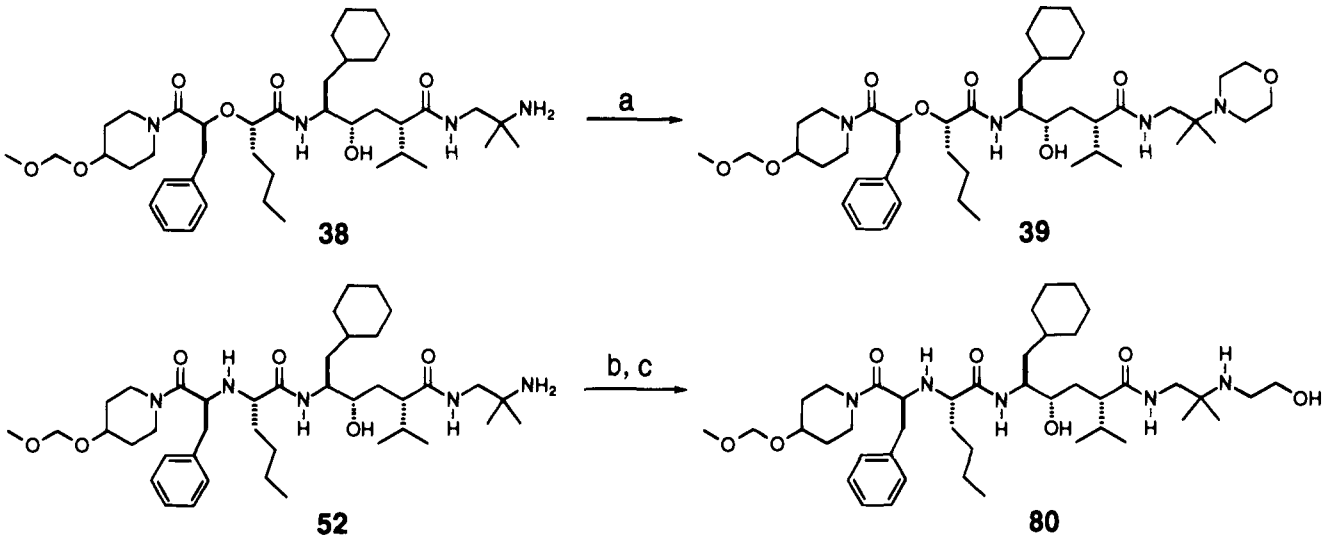
Scheme 7. Synthesis of C-Terminal Carboxylic Acids **4**, **78**, and **79**[a]



[a] Reaction conditions: (a) LiOH, THF, H_2O, 0–20 °C, then $NaHSO_4$; (b) H_2 (1 atm), 10% Pd/C, EtOAc.

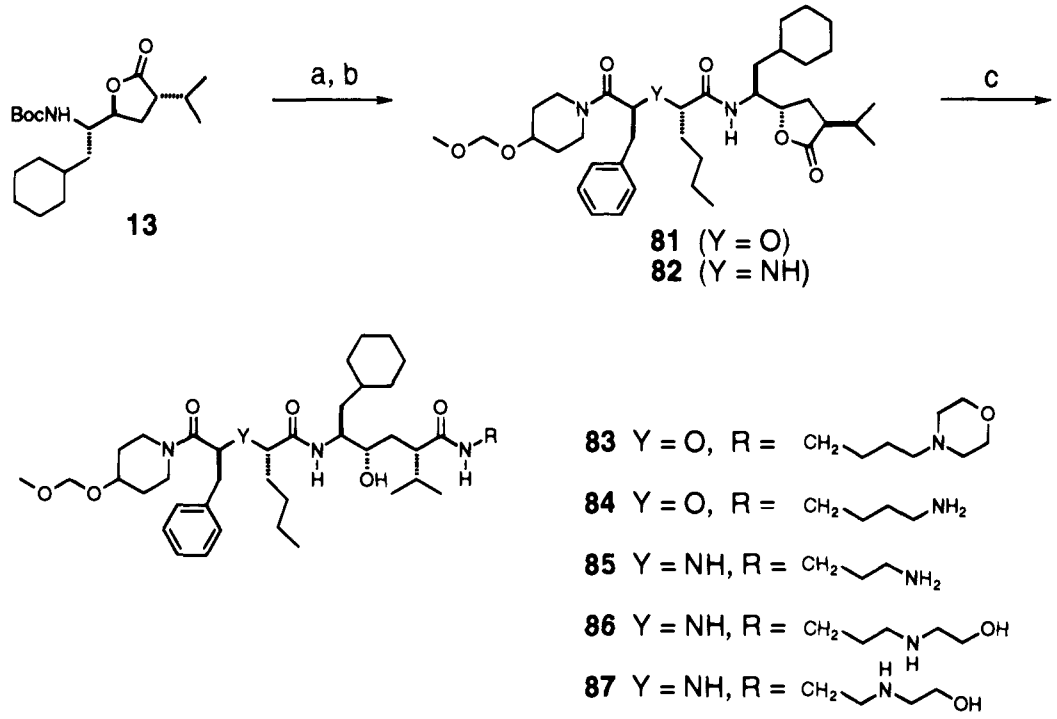
Microsomal Metabolism Results. As mentioned above, **1** has been shown to undergo cytochrome P-450-mediated hepatic biotransformation in animals primarily at the morpholino terminus of the molecule, affording **2**–**4**.[13] In an attempt to identify structural features which would reduce the propensity for biotransformation of this terminus without negatively affecting solubility 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, O_3, −70 to −65 °C; (2) $NaBH_3CN$; (3) **38**, −60 to 20 °C; (b) (benzyloxy)acetaldehyde, $NaBH_3CN$, ^iPrOH, HOAc, NaOAc, 0–20 °C; (c) H_2 (4 atm), $Pd(OH)_2/C$, EtOAc, HOAc.

Scheme 9. Synthesis of Inhibitors **83**–**87** via Lactones **81** and **82**[a]

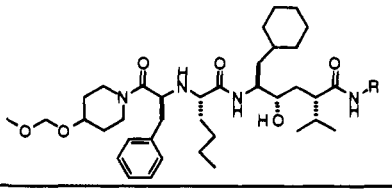


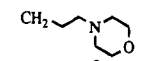
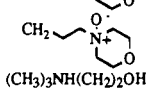
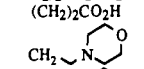
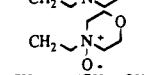
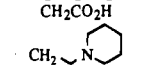
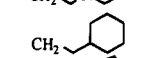
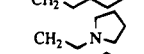
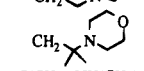
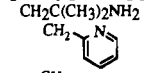
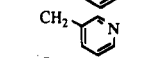
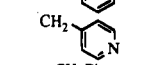
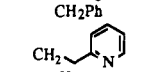
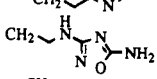
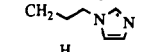
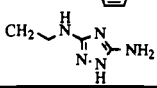
[a] Reaction conditions: (a) TFA, CH_2Cl_2 (1:1), 0 °C, then 1 M aqueous Na_2CO_3; (b) **32** or **33**, EDC, HOBT, 4-methylmorpholine, DMF, −20 to 20 °C; (c) H_2NR neat, 60–80 °C, 2–4 days, or H_2NR (9 equiv), HOAc (3 equiv), HOAc (3 equiv), 65 °C, 1–2 h. See Tables 1 and 2 for R-group structures.

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-carbon-shorter 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



inhibitor	R	IC_{50} in plasma, nM[a] human	rat	ferret	dog	log *P* pH 7.4 (pH 6.5)[b]	aqueous solubility, 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	$(CH_3)_3NH(CH_2)_2OH$	7.9	nd[d]	nd	nd	3.5 (2.9)	1.606
85	$(CH_2)_3NH_2$	9.5	nd	nd	nd	3.3 (2.7)	1.322
43	$(CH_2)_2CO_2CH_2Ph$	23	nd	nd	nd	nd	nd
78	$(CH_2)_2CO_2H$	8.4	nd	nd	nd	3.3 (2.8)	10.78
44		3.9	3800	170	14	>5 (>5)	0.141
45		8.4	nd	nd	nd	4.07 (4.07)	1.510
87	$(CH_2)_2NH(CH_2)_2OH$	11	nd	nd	nd	nd	nd
46	$(CH_2)_2NHCbz$	17	nd	nd	nd	nd	nd
61	$(CH_2)_2NH_2$	9.7	nd	nd	nd	3.5 (2.7)	0.632
47	$CH_2CO_2CH_2Ph$	21	nd	nd	nd	nd	nd
79	CH_2CO_2H	12	nd	nd	nd	2.8 (2.3)	>10
48		14	nd	nd	nd	>5 (>5)	0.093
49		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	$CH_2C(CH_3)_2NH(CH_2)_2OH$	6.3	nd	nd	nd	nd	nd
52	$CH_2C(CH_3)_2NH_2$	12	nd	nd	nd	3.8 (3.2)	0.180
53		12	>10000	1000	75	>5 (>5)	0.0126
54		9.6	9100	550	44	>5 (>5)	0.006
55		7.8	10000	940	59	>5 (>5)	0.020
56	CH_2Ph	14	nd	nd	nd	>5 (>5)	< 0.001
57		9.4	7000	510	33	>5 (>5)	0.004
58		3.8	nd	nd	nd	4.11 (4.07)	0.026
59		7.6	7300	330	24	>5 (4.9)	0.060
77		6.1	nd	nd	nd	4.2 (4.1)	0.157

[a]Single determination in most cases. [b]Apparent octanol-aqueous phosphate buffer partition coefficient. [c]Determined at 37 °C in 0.05 M phosphate containing 0.15 M NaCl. [d]nd = 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 Liver Microsomes

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 salt-depleted 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 C-terminal 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 μm thickness (analytical) and 2000 μm thickness (preparative); UV light and phosphomolybdic acid stain were used for visualization. Flash chromatography was performed using E. Merck Kieselgel 60 (230–400 mesh), and eluent systems are listed as volume/volume percents or ratios. Melting points are given for all solids except glasses; other compounds were isolated as low-melting waxes or oils. Proton magnetic resonance (^{1}H NMR) spectra were recorded on a General Electric Model QZ-300 spectrometer (300 MHz). Chemical shifts are reported as ppm downfield from tetramethylsilane (δ). For resonances which include H_2O 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 $CDCl_3$ on a Nicolet 5SXC FTIR spectrometer. Mass spectra were determined on Kratos MS50 (FAB^+, FAB^-) and Finnigan SSQ-700 (DCI^+ (NH_3)) 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[b] dose		
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 ± 19.1[c]	15 ± 9
49	0.14 ± 0.08	4.92 ± 0.84[c]	0.03 ± 0.03	2.22 ± 2.22[c]	4 ± 4
50	1.31 ± 0.54	20.4 ± 2.97	>0.48 ± 0.32	>51.0 ± 37.7	>25 ± 18
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 ± 1.22[c]	2.32 ± 0.35	220 ± 43.8[c]	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 ± 2.22[c]	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 ± 16.3[c]	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 ± 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 Following Intravenous and Intraduodenal Administration[a]

	1 mg/kg iv[b] dose		10 mg/kg id[c]		
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	1.55 ± 0.31	54.1 ± 3.27	4.62 ± 0.85	408 ± 64.1	75 ± 13
42	1.83 ± 0.82	40.1 ± 14.9	1.18 ± 0.56	81.0 ± 36.2	20 ± 12
44	2.84 ± 0.31	112 ± 35.8	6.50 ± 0.56	585 ± 9.51	51 ± 16
48	3.60[f]	101[f]	3.73 ± 0.73[g]	341 ± 41.5[g]	33 ± 4
50	3.90 ± 1.78	165 ± 67.0	5.50 ± 1.03[g]	493 ± 94.4[g]	29 ± 13
51	2.98 ± 0.58	158 ± 32.4	11.5 ± 2.51	999 ± 209	63 ± 18
52	7.15 ± 0.95	190 ± 55.8	2.96 ± 0.40	230 ± 44.7	12 ± 4
53	10.0 ± 2.66	182 ± 84.3	9.12 ± 3.58[f]	846 ± 331[f]	46 ± 23
54	3.48 ± 1.61	49.6 ± 26.0[d]	>4.92 ± 0.81	>490 ± 89.6	>98 ± 54
57	6.10 ± 0.14	89.8 ± 18.6	2.00 ± 0.10	133 ± 48.6	14 ± 6
58	0.32 ± 0.06	4.28 ± 0.99	0.11 ± 0.06	3.29 ± 1.09	7 ± 3
59	2.10 ± 0.10	42.6 ± 4.20	>4.93 ± 0.40[e]	488 ± 77.5[e]	>114 ± 21
61	2.45 ± 0.51	74.7 ± 7.70	0.92 ± 0.41	77.2 ± 26.1	10 ± 3

[a] Values reported as mean ± 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 Pressure Responses 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, μg/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 ± 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 ± 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)-(2*S*,4*S*,5*S*)-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 $CHCl_3$, 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 $CHCl_3$ was added dropwise over 6 h, with the internal temperature being maintained below 10 °C. After addition was complete, the reaction solution was stirred at room temperature overnight. The solution was washed with water, dried (Na_2SO_4), filtered, and concentrated in vacuo to provide 3.0 g (80%) of 3-((benzyloxycarbonyl)amino)propylamine as a low-melting solid: ^1H NMR ($CDCl_3$) 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 CH_2Cl_2 and extracted with 2 × 300 mL of 80% saturated aqueous $NaHCO_3$, water (300 mL), and brine (300 mL), and then the organic phase was dried (Na_2SO_4) and concentrated

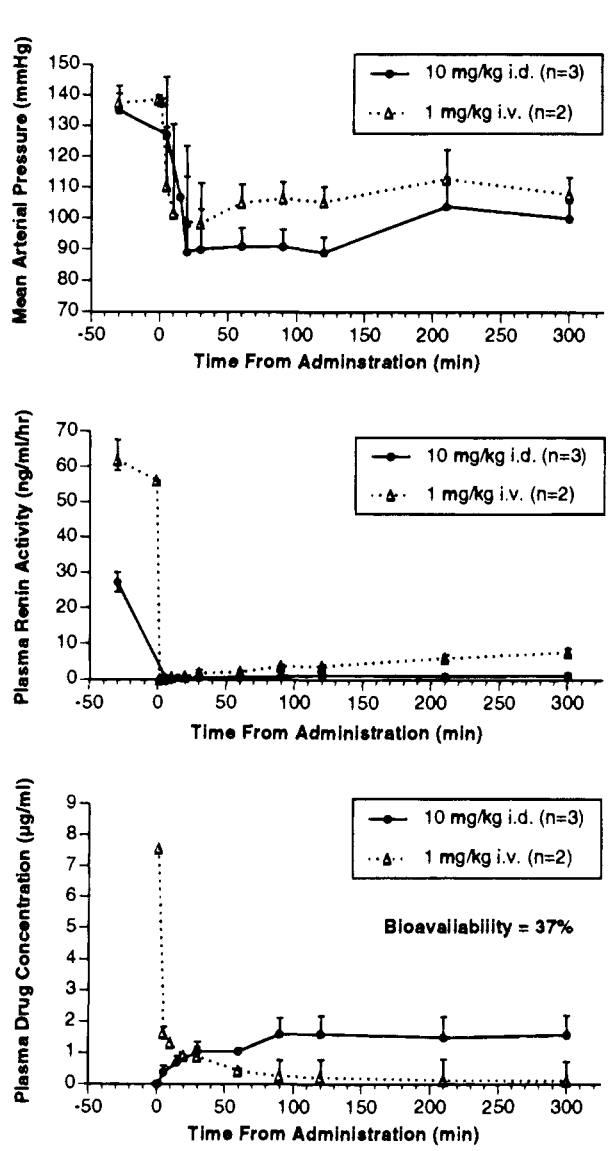


Figure 2. Effects of inhibitor **51** in salt-depleted dogs: mean arterial pressure (panel A), plasma renin activity (panel B), and plasma drug concentrations (panel C). Standard error is shown by the error bars in the positive direction only.

to a foam. Purification by flash chromatography (silica gel, 10% EtOAc–CH_2Cl_2) provided 1.98 g (3.28 mmol, 90%) of amide **6** as a white foam: R_f 0.11 (25% EtOAc–hexane); ^{1}H NMR ($CDCl_3$) 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 + NH_4)$^+$). Anal. ($C_{34}H_{55}N_3O_6$) C, H, N.

Compounds **7–10** were synthesized in an analogous manner.

***N*-(Cyanomethyl)-(2*S*,4*S*,5*S*)-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 $NaHCO_3$, water (100 mL), and brine (50 mL). The organic phase was dried (Na_2SO_4) 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); ^{1}H NMR ($CDCl_3$) 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+NH_4)$^+$). Anal. ($C_{25}H_{43}N_3O_4$·0.5H_2O) C, H, N.

***N*-(5-(Tetrazolyl)methyl)-(2*S*,4*S*,5*S*)-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 CH_2Cl_2. 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 CH_2Cl_2. The combined organic phases were washed with brine, dried (Na_2SO_4), 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–CH_2Cl_2); ^{1}H NMR ($CDCl_3$) 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)-(2*S*,4*S*,5*S*)-5-((*tert*-butyloxycarbonyl)amino)-4-hydroxy-2-isopropylhexanamide** (**14**). Lactone **13** (4.0018 g, 11.32 mmol) and 4-(3-aminopropyl)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 × 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–$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.73–1.04 (br m) and 0.93 (2 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. ($C_{27}H_{51}N_3O_5$·0.25H_2O) 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)-(2*S*,4*S*,5*S*)-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% EtOAc–hexane) indicated complete consumption of the starting lactone. The solution was dissolved in 250 mL of EtOAc and extracted with 3 × 250 mL of H_2O 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% NH_4OH–CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 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)-(2*S*,4*S*,5*S*)-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 × 50 mL H_2O, 50 mL brine), dried (Na_2SO_4), 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% NH_4OH–CH_2Cl_2); ^1H NMR ($CDCl_3$) 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 = 10 Hz, 1 H), 6.04–6.13 (br m, 1 H); MS m/e 442 ($(M + H)^+$). Anal. ($C_{24}H_{47}N_3O_4 \cdot 0.25H_2O$) C, H, N.

***N*-(2-Methyl-2-(4-morpholino)propyl)-(2*S*,4*S*,5*S*)-5-((*tert*-butyloxycarbonyl)amino)-4-hydroxy-2-isopropylhexanamide (26).** 3,4-Dihydrofuran (1.60 mL, 1.48 g, 21.2 mmol) was dissolved in 60 mL of CH_2Cl_2 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% NH_4OH–CH_2Cl_2) 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 CH_2Cl_2 and 50 mL of 1 M aqueous Na_2CO_3. The aqueous phase was extracted (2 × 50 mL CH_2Cl_2), and the combined organic phases were washed with 50 mL of brine, dried (Na_2SO_4), 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–CH_2Cl_2); ^1H NMR ($CDCl_3$) 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. ($C_{28}H_{53}N_3O_5$) C, H, N.

***N*-(3-(4-Oxido-4-morpholino)propyl)-(2*S*,4*S*,5*S*)-5-((*tert*-butyloxycarbonyl)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 $CHCl_3$, and the combined organic phases were washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The foamy solid was purified by flash chromatography (10–15% MeOH–CH_2Cl_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 ($CDCl_3$) 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 (br m, 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 H), 4.81 (br d, J = 9 Hz, 1 H), 7.96-8.05 (br s, 1 H); MS m/e 514 ($(M + H)^+$), 498 ($(M - 16 + H)^+$. Anal. ($C_{27}H_{51}N_3O_6 \cdot H_2O$), C, H, N.

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

***N*-(2-Cyclohexylethyl)-(2*S*,4*S*,5*S*)-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 ($CDCl_3$) 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)-(2*S*,4*S*,5*S*)-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 CH_3CN 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-*S*-methylisothiourea 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 CH_2Cl_2 and washed with 2 × 100 mL of H_2O. The organic phase was dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography (6% MeOH–1% concentrated aqueous–CH_2Cl_2) 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–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 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. ($C_{24}H_{44}N_6O_5 \cdot 0.75\ H_2O$) C, H, N.

***N*-(3-((Benzyloxycarbonyl)amino)propyl)-(2*S*,4*S*,5*S*,1′*S*,2′*S*)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (34). Part 1. Deprotection of *tert*-Butyl Carbamate 6.** A solution of carbamate **6** (119 mg, 0.198 mmol) in 1.5 mL CH_2Cl_2 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 H_2O 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 CH_2Cl_2, then the combined organic phases were dried (Na_2SO_4), filtered, and concentrated in vacuo to give 95 mg (104%) of the crude amino alcohol *N*-(3-((benzyloxycarbonyl)amino)propyl)-(2*S*,4*S*,5*S*)-5-amino-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide: R_f 0.30 (10% MeOH–CH_2Cl_2); ^1H NMR ($CDCl_3$) 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 removed by high-vacuum distillation, and the residue was partitioned between 25 mL of CH_2Cl_2 and 80% saturated aqueous $NaHCO_3$. The organic phase was washed sequentially

with H_2O (25 mL) and brine (25 mL) and then dried (Na_2SO_4), filtered, and concentrated in vacuo to a foam (174 mg). Flash chromatography (silica gel, 2.5% MeOH–CH_2Cl_2) provided 106 mg (0.125 mmol, 64%) of inhibitor **34** as a white foam: mp 64–8 °C; ^1H NMR ($CDCl_3$) 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. ($C_{48}H_{74}N_4O_9 \cdot 0.5H_2O$) 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)-(2*S*,4*S*,5*S*,1′*S*,2′*S*)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)-hexanamido)-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (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 NH_4OH–CH_2Cl_2) 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% MeOH–1% concentrated aq. NH_4OH–CH_2Cl_2); ^1H NMR ($CDCl_3$) 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)-(2*S*,4*S*,5*S*,1′*S*, 2′*S*)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)-hexanamido)-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (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 × 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–CH_2Cl_2) 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–CH_2Cl_2); ^1H NMR ($CDCl_3$) 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/e 730 ($(M - H)^-$); 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)-(2*S*,4*S*,5*S*,1′*S*,2′*S*)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (39).** Through a solution of dihydrofuran (15 μL, 14 mg, 0.198 mmol) dissolved in methanol (0.1 mL) and CH_2Cl_2 (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 Na_2CO_3 (10 mL) and CH_2Cl_2 (20 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 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–CH_2Cl_2) afforded pure morpholine **39** as a white amorphous solid (26.3 mg, 58%): mp 46–52 °C; R_f 0.24 (5% MeOH–CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 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 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 ($(M + H)^+$). Anal. ($C_{45}H_{76}N_4O_8 \cdot H_2O$) 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)-(1*S*)-(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 CH_2Cl_2 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 $NaHCO_3$, 25 mL of 1 M aqueous NaOH, and 100 mL of CH_2Cl_2. The aqueous phase was saturated with NaCl and extracted with 4 × 75 mL of CH_2Cl_2, 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*-(2-amino-2-methylpropyl) (2*S*,4*S*,5*S*)-5-amino-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide: ^1H NMR ($CDCl_3$) 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 = 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 CH_2Cl_2 and 75 mL of 80% saturated aqueous $NaHCO_3$. The aqueous phase was extracted with 75 mL of CH_2Cl_2, and the combined organic phases were dried (Na_2SO_4), 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; R_f 0.26 (7.5% MeOH–0.75% concentrated aqueous NH_4OH–CH_2Cl_2); ^1H NMR ($CDCl_3$) 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 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. ($C_{41}H_{71}N_5O_6 \cdot 1.5H_2O$) 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)-(2*S*,4*S*,5*S*,1′*S*,2′*S*)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2-isopro-**

pylhexanamide (62). A solution of amine **3** (23.4 mg, 0.0326 mmol) in 5.4 mL of CH_2Cl_2 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 $NH_4OH-CH_2Cl_2$), 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% MeOH−1% concentrated aq. $NH_4OH-CH_2Cl_2$); ^1H NMR ($CDCl_3$) 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)-(2*S*,4*S*,5*S*,1′*S*,2′*S*)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (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−CH_2Cl_2), which provided 31 mg (74%) of the title compound as a white powder: mp 87−92 °C; R_f 0.58 (15% MeOH−CH_2Cl_2); ^1H NMR ($CDCl_3$) 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), 4.69 (s, 2 H), 5.75 (br m, 1 H), 5.80 (m, 1 H), 6.44 (m, 1 H), 7.33 (m, 5 H); MS *m/e* 760 ($(M + H)^+$). Anal. ($C_{41}H_{69}N_5O_8 \cdot H_2O$) C, H, N.

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

***N*-(2-(Thioureido)ethyl)-(2*S*,4*S*,5*S*,1′*S*,2′*S*)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (67).** Primary amine **60** (50.0 mg, 71.1 μmol) was dissolved in 1.4 mL of THF, the solution was cooled to 0 °C, and *N,N*′-thiocarbonyldiimidazole (15.8 mg, 88.9 μmol) 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 NH_4OH (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−CH_2Cl_2), that provided 28.2 mg (37.0 μmol, 52%) of thiourea **67** as a white powder: mp 98−112 °C; R_f 0.40 (5% MeOH−CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 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. ($C_{40}H_{67}N_5O_7S$) C, H, N.

Inhibitor **64** was prepared from primary amine 3 in a similar manner.

***N*-(3-(S-Methyl-*N*′-cyanoisothioureido)propyl)-(2*S*,4*S*,5*S*,1′*S*,2′*S*)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (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−CH_2Cl_2) to provide 240 mg (85%) of isothiourea **68** as a white powder: mp 88−94 °C; R_f 0.49 (10% MeOH−CH_2Cl_2); ^1H NMR ($CDCl_3$) 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 3.38 (2 s, 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. ($C_{43}H_{70}N_6O_7S \cdot 0.5H_2O$) 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)-(2*S*,4*S*,5*S*,1′*S*,2′*S*)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (72).** Isothiourea **68** (50 mg, 61.3 μmol) was dissolved in 2 mL of EtOH in a screw-cap vial, 40% aqueous methyl amine (132 μL, 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−CH_2Cl_2), which provided 39.3 mg (49.2 μmol, 80%) of guanidine **72** as a white powder: mp 101−7 °C; R_f 0.34 (7.5% MeOH−CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 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 ($(M + NH_4)^+$). Anal. ($C_{43}H_{71}N_7O_7$) 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)-(2*S*,4*S*,5*S*,1′*S*,2′*S*)-5-(2-(1-((4-(methoxymethoxy)piperidin-1-yl)carbonyl)-2-phenylethoxy)hexanamido)-6-cyclohexyl-4-hydroxy-2-isopropylhexanamide (75).** Isothiourea **68** (50 mg, 61.3 μmol) 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 $NH_4OH-CH_2Cl_2$), which provided 44.6 mg (55.8 mmol, 91%) of triazole **75** as a white powder: mp 110−20°C; R_f 0.24 (10% MeOH−1% concentrated $NH_4OH-CH_2Cl_2$); ^1H NMR ($CDCl_3$) 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. ($C_{42}H_{70}N_8O_7 \cdot H_2O$) 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-6-methylheptan-2-yl)-(1*S*)-(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 μL) and NaOAc (5 mg). The resulting solution was cooled to 0 °C and $NaBH_3CN$ (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 H_2O, 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 $NH_4OH-CH_2Cl_2$) to provide 44.0 mg (0.051 mmol, 29%) of the benzyl ether as a colorless glass: R_f 0.21 (5% MeOH−0.5% concentrated aqueous $NH_4OH-CH_2Cl_2$); ^1H NMR ($CDCl_3$) 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 μmol) was hydrogenated over 10 mg of 10% $Pd(OH)_2/C$ in 3 mL of EtOAc under 4 atm of H_2 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 NH_4OH–CH_2Cl_2) 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 NH_4OH–CH_2Cl_2); ^1H NMR ($CDCl_3$) 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, H_2O), 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 $C_{43}H_{76}N_5O_7$ ((M + H)$^+$) 774.5745, found 774.5746.

(1*S*,3′*S*,5′*S*)-*N*-(2-Cyclohexyl-1-(3-isopropyl-2-oxo-2,3,4,5-tetrahydrofuran-5-yl)ethyl)-(1*S*)-(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 CH_2Cl_2, 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 Na_2CO_3, and the mixture was saturated with NaCl and extracted with 3 × 100 mL CH_2Cl_2. The combined organic phases were dried (Na_2SO_4), filtered, and concentrated in vacuo to provide (3*S*,5*S*,1′*S*)-5-(1-amino-2-cyclohexylethyl)-3-isopropyltetrahydrofuran-2-one as a crystalline solid, 1.086 g (101% crude yield): ^1H NMR ($CDCl_3$) δ 0.73–0.93 (m, 1 H), 0.96 (d, J = 7 Hz, 3 H), 1.03 (d, 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 CH_2Cl_2 and 100 mL of 1 M aqueous Na_2CO_3. 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–CH_2Cl_2) provided 2.13 g (3.32 mmol, 78%) of lactone amide **82** as a waxy oil: R_f 0.42 (5% MeOH–CH_2Cl_2); ^1H NMR ($CHCl_3$) δ 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), 2.64–2.90 (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)-1-cyclohexyl-3-hydroxy-6-methylheptan-2-yl)-(1*S*)-(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 μL, 119 mg, 1.98 mmol) were combined, and the mixture was warmed to 85 °C for 24 h. The mixture was dissolved in 75 mL of EtOAc and extracted with H_2O (4 × 50 mL) and then 50 mL of brine. The organic phase was dried (Na_2SO_4), filtered, and concentrated in vacuo to a white foamy solid. The crude amide was partially purified by flash chromatography (7.5% MeOH–CH_2Cl_2) and further purified by preparative TLC (20% MeOH–2% concentrated aqueous NH_4OH) to give 272 mg (52%) of pure **86** as a foamy solid: mp 58–67 °C; R_f 0.34 (15% MeOH–1.5% concentrated NH_4OH–CH_2Cl_2); ^1H NMR ($CDCl_3$) 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. ($C_{42}H_{73}N_5O_7 \cdot H_2O$) 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 × 0.4 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 CH_3CN–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 μm 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 9000*g* at 4 °C for 20 min, then the supernatant fraction was centrifuged at 105000*g* at 4 °C for 1 h. The resulting pellet was resuspended in buffer and recentrifuged at 105000*g* 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 μM 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

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 μm 4.6 × 250 mm C_{18} column connected to a Nucleosil 5 μm C_{18} 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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