Azido Glycols: Potent, Low Molecular Weight Renin Inhibitors Containing an Unusual Post Scissile Site Residue[1,2]

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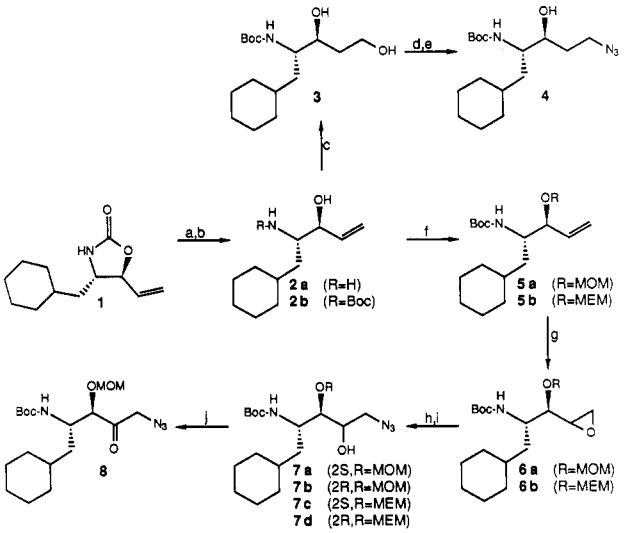
Azidomethyl-substituted 1,2- and 1,3-diols were prepared from Boc-cyclohexylalanal and evaluated as transition state analogue renin inhibitors, leading to the development of a small (MW < 600), nanomolar inhibitor. Remarkable aqueous solubility enhancement followed the incorporation of an N-terminal urea functionality. Evaluation of selected compounds both in vivo and in vitro demonstrated that while transport across the intestine occurred upon id administration, extensive liver extraction resulted in low systemic levels.

Renin, an aspartic proteinase, is the first and rate-limiting enzyme in the well-known renin–angiotensin cascade, an important system for the regulation of blood pressure. Disruption of this system by inhibition of angiotensin converting enzyme (ACE) has already proven to be a viable therapy for controlling hypertension, and renin inhibitors have demonstrated hypotensive activity in vivo.[3] Although significant progress has been made in the design of renin inhibitors, the search for an orally active inhibitor remains a challenge. One potential solution involves a reduction in the size of these molecules.

Currently, the most potent renin inhibitors are based on analogues of the natural substrate angiotensinogen in which the Leu–Val scissile bond has been replaced by a modified statine[4,5] or Leu-Val hydroxyethylene isostere residue.[6] The strong binding to the enzyme is attributed to a mimicking of the presumed tetrahedral transition state for enzymatic cleavage by these inhibitors.[7,8] Often, most of the postscissile site portion of a hydroxyethylene isostere based inhibitor can be eliminated and replaced with a small, nonpeptidyl residue without significant changes in in vitro potency.[9–11]

Scheme I. Synthesis of Azido Monol and Azido 1,2-Diols[a]



[a] Key: (a) $Ba(OH)_2 \cdot (H_2O)_8$, dioxane, H_2O. (b) $(t\text{-}BuO_2C)_2O$, CH_2Cl_2. (c) 9-BBN, THF; H_2O_2. (d) CH_3SO_2Cl, Et_3N, CH_2Cl_2. (e) NaN_3, DMF. (f) $CH_3OCH_2CH_2OCH_2Cl$ or CH_3OCH_2Cl, $(i\text{-}Pr)_2NEt$, CH_2Cl_2. (g) MCPBA, CH_2Cl_2. (h) NaN_3, NH_4Cl, CH_3OH. (i) Isomer separation. (j) $(COCl)_2$, CH_3SOCH_3, CH_2Cl_2; Et_3N.

The putative transition state for the renin mediated hydrolysis of angiotensinogen is a tetrahedral hydrated amide bond. Although the single hydroxyl in the above-mentioned inhibitors is a good analogue of this species, several attempts have been made to further enhance binding by incorporating a second hydroxyl, via either a diol or a hydrated carbonyl, to more closely mimic the proposed transition state. Hydrated carbonyls increased activity over the corresponding alcohols[12,13] while diols exhibited enhanced activity in certain cases[14,15] and no

(1) Presented in part at the 10th American Peptide Symposium, 1987.

(2) Abbreviations follow IUPAC–IUB Joint Commission on Biochemical Nomenclature for amino acids and peptides (*Eur. J. Biochem.* **1984**, *158*, 9–31). Additional abbreviations are as follows: THF, tetrahydrofuran; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; MCPBA, metachloroperbenzoic acid; MEM, (methoxyethoxy)methoxy; MOM, methoxymethoxy; TBS, *tert*-butyldimethylsilyl; 9-BBN, 9-borabicyclo-[3.3.1]nonane; PEG, polyethylene glycol; EDTA, ethylenediaminetetracetic acid.

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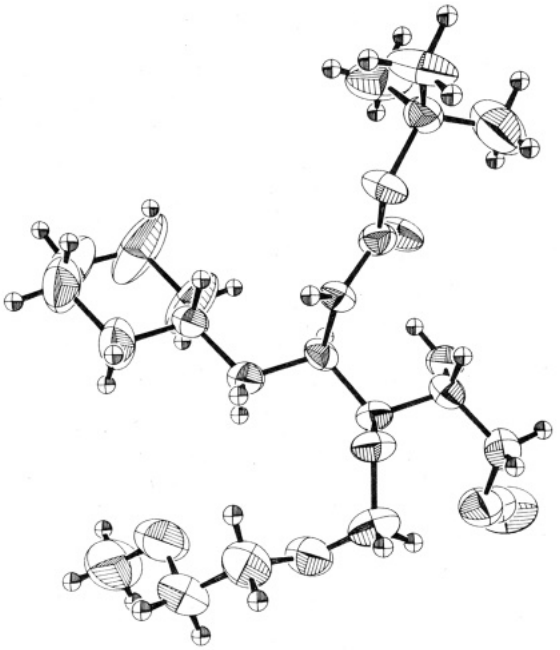


Figure 1. X-ray structure of glycol **7c**.

effect in others[16] (when compared to the parent monohydroxy inhibitors).

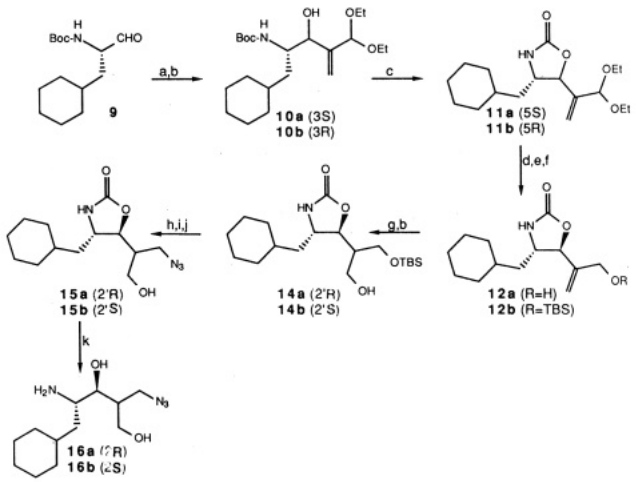
In conjunction with another study[11] we have found that the entire postscissile site portion of an inhibitor can be replaced by a simple azidomethyl group while maintaining full potency. Herein are described the results of a structure–activity examination that began with this novel azide structure and that, through the incorporation of 1,2- and 1,3-diols of various configurations, led to the discovery of a small (MW < 600), nanomolar renin inhibitor. Minor modifications of the N-terminus that have a profound effect on aqueous solubility are also discussed.

Results

Synthesis: Azido Monols. The synthesis of the C-terminal fragment of inhibitor **23** has already been described.[11] The homologue **4** was prepared from vinyl oxazolidinone **1**[17] (Scheme I). Basic hydrolysis followed by nitrogen protection produced allylic alcohol **2b**. Hydroboration to diol **3** followed by selective mesylation of the primary hydroxyl and subsequent displacement by azide completed the synthesis of fragment **4**.

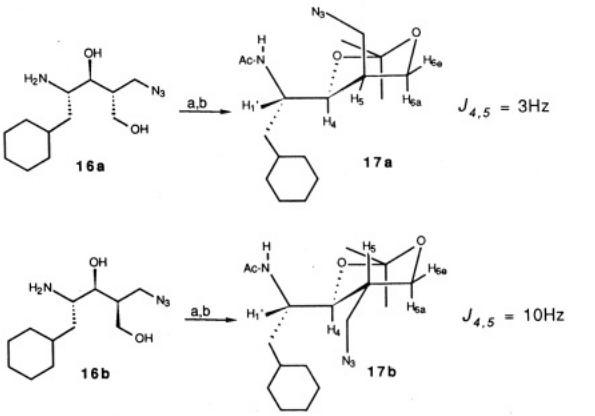
Synthesis: Azido 1,2-Diols. Oxazolidinone **1** is the starting point for the azido glycol fragments as outlined in Scheme I. Protection of allylic alcohol **2b** as either the MEM or MOM derivative followed by epoxidation produced **6** as a 2:1 mixture of diastereomers.[18] Reaction with azide produced azido diols **7a** (**7c**) and **7b** (**7d**) as the major and minor products, respectively. Oxidation of either isomer produced azido ketone **8**. Crystals of Mem derivative **7c** proved suitable for single-crystal X-ray analysis, and the results shown in Figure 1 confirmed the predicted stereochemistry.

Scheme II. Synthesis of Azido 1,3-Diols[a]



[a] Key: (a) $LiC(CH_2)CH(OEt)_2$, THF. (b) Isomer separation. (c) NaH, DMF. (d) $(HO_2C)_2$, SiO_2, CH_2Cl_2. (e) $NaBH_4$, CH_3OH. (f) TBS-Cl, imidazole, DMF. (g) BH_3 in THF; H_2O_2. (h) CH_3SO_2Cl, $(i\text{-}Pr)_2NEt$, CH_2Cl_2. (i) NaN_3, DMF. (j) Bu_4NF, THF. (k) $Ba(OH)_2{\cdot}(H_2O)_8$, dioxane, H_2O.

Scheme III. Stereochemistry of the 1,3-Diols[a]



[a] Key: (a) AcCl, TEA, CH_2Cl_2. (b) $CH_2C(CH_3)OCH_3$, TsOH, CH_2Cl_2.

Synthesis: Azido 1,3-Diols and Related Structures. The synthesis of the azido 1,3-diol fragments is outlined in Scheme II. Condensation of Boc-cyclohexylalanal[5,19] (**9**) with α-lithioacrolein diethyl acetal[20] afforded a separable 3:2 mixture of allylic alcohols **10a** and **10b**. Stereochemistry at C-3 was determined by conversion of **10a** and **10b** into the corresponding oxazolidinones **11a** and **11b**. Ring coupling constants of 4.8 and 7.3 Hz for **11a** and **11b**, respectively, are consistent with the stereochemistry shown.[21] Careful acetal hydrolysis and subsequent aldehyde reduction of isomer **11a** provided allylic alcohol **12a**. At this point the stereochemical integrity resulting from the initial anion addition was established by hydrolyzing oxazolidinone **12a** (barium hydroxide) and converting the resulting amine to the corresponding (–)- and (+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid amides.[22] Examination of both the 300-MHz proton and fluorine NMR spectra of the resulting diastereomers indicated that no detectable racemization had occurred.

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(18) The alcohol protecting group directs epoxidation in favor of the 2*S* isomer. Epoxidation of the intermediate Boc-allylic alcohol under identical conditions leads to an 8:1 mixture favoring the 2*R* isomer.
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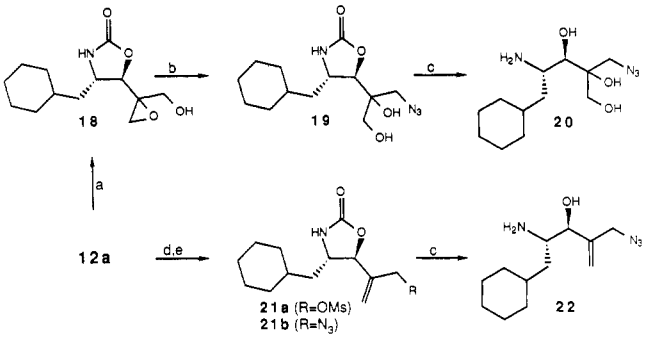
Scheme IV. Synthesis of Structures Related to the Azido Diols[a]



[a] Key: (a) MCPBA, CH_2Cl_2. (b) NaN_3, NH_4Cl, CH_3OH. (c) $Ba(OH)_2 \cdot (H_2O)_8$, dioxane, H_2O. (d) CH_2SO_2Cl, $(i\text{-}Pr)_2NEt$, CH_2Cl_2. (e) NaN_3, DMF.

Protection (*tert*-butyldimethylsilyl) of alcohol **12a** followed by hydroboration produced separable alcohols **14a** and **14b** in a 3:2 ratio which were converted to the mesylates and then to the azides. Deprotection with fluoride produced the azido alcohols **15a** and **15b**. Final deprotection to the free amino diols **16a** and **16b** was accomplished by barium hydroxide mediated hydrolysis.

Absolute stereochemistry at C-2 was established by first N-acetylating **16a** and **16b** and then converting the 1,3-diols into the corresponding cyclic dimethyl ketals **17a** and **17b** as illustrated in Scheme III. Coupling constants between protons H_4 and H_5 indicate an axial–equatorial relationship in isomer **17a** and a diaxial relationship in isomer **17b**. These relationships translate into the stereochemistries shown, but an absolute determination must await an X-ray structure.

Allylic alcohol **12a** was converted into allylic azide **22** by using procedures already described (Scheme IV). Epoxidation of **12a** to **18** followed by opening with azide provided oxazolidinone diol **19** as a 1:1 isomer mixture which was hydrolyzed to amino triol **20** without isomer separation.

Synthesis: Extension at the N-Terminus. Initial elaboration of the various fragments into renin inhibitors involved the coupling of Boc-Phe-His-OH to the N-termini as previously described[11] (fragments **4**, **7a**, **7b**, and **8** required prior acidic deprotection). Other inhibitors described were prepared by the stepwise coupling of two amino residues. Final peptides were purified by silica gel chromatography.

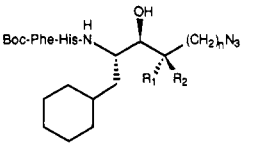
Discussion

The inhibitory potencies for the compounds in this paper are shown in Table I. Compound **23**, in which the entire postscissile site portion of the inhibitor has been replaced with an azidomethyl group, was found to be significantly active with an IC_{50} of 7.5 nM. The homologue **24** was somewhat less potent than the parent compound. Incorporation of a 2(*S*)-hydroxyl increased potency 50-fold to provide inhibitor **25**, while diol **26**, which includes a 2(*R*)-hydroxyl, was essentially equipotent with monohydroxy compound **24**. The increase in potency derived from the incorporation of a second hydroxyl in the *S* configuration has been observed in a similar series of inhibitors,[14,15] while incorporation of a second hydroxyl in the *R* configuration has been shown to either have no effect[16] or a slight deleterious effect[14] on potency. The increased potency of compound **25** is attributed to additional hydrogen bonding of the 2(*S*)-hydroxyl with the active site. Ketone **30** more firmly delineates the role of this second hydroxyl. This compound is more potent than monohydroxy compound **24**, suggesting that the second hydroxyl acts as a hydrogen bond acceptor. The loss in potency for **30** thus might be due to either the possible decreased hydrogen bonding ability of a carbonyl compared to a hydroxyl or the equally possible unfavorable geometry of an sp^2 center at this site (compare compounds **24** and **31**).

Replacement of the 2(*S*)-hydroxyl with a hydroxymethyl group[23] (**27**) decreased potency 25-fold, indicating that the 1,2-diol provides the optimum configuration for binding to the active site. Inhibitor **27** is a slightly more potent than the parent **24**, indicating that perhaps the 2(*R*)-hydroxymethyl does contribute to binding. In contrast, replacement of the 2(*R*)-hydroxyl with a hydroxymethyl significantly decreased binding (**28**). Reintroduction of the 2-hydroxyl (**29**, as an *RS* mixture) increased potency, but not to that of diol **25**.

The acid-labile Boc group was replaced with the smaller isobutyryl to provide inhibitor **32** (MW = 596). This compound was equipotent with **25** when tested against purified human renal renin and was more active than **25** against human plasma renin (Table II). Furthermore, compound **32** demonstrated intravenous efficacy in both sodium deplete and normal monkeys.[24] Stabilization of the Phe–His bond toward chymotrypsin cleavage was accomplished by replacing phenylalanine with *O*-methyltyrosine,[11] and this change was accompanied by a modest

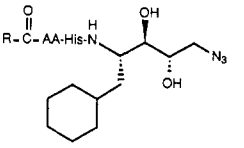
Table I. C-Terminal Azido Diol and Azido Monol Renin Inhibitors



no.	R_1	R_2	*n*	IC_{50},[a] nm
23	H	H	0	7.5
24	H	H	1	20
25	OH	H	1	0.40
26	H	OH	1	26
27	CH_2OH	H	1	10
28	H	CH_2OH	1	370
29	(OH, CH_2OH)[b]		1	4.8
30	O		1	5.4
31	CH_2		1	55

[a] Purified human renal renin, pH 6.0. [b] 1:1 2*R*/2*S*.

Table II. N-Terminus Modified Azido Glycols



no.	R	AA	IC_{50}, nM: purified[a]	IC_{50}, nM: plasma[b]	solubility,[c] mg/mL
25	$(CH_3)_3CO$	Phe	0.40	9.0	
32	$(CH_3)_2CH$	Phe	0.55	3.3	0.013
33	$(CH_3)_2CH$	(Me)Tyr[d]	2.0	10	0.029
34	morpholin-4-yl	Phe	0.67	2.4	2.6
35	tetrahydropyran-4-yl	Phe	0.70	7.6	0.023
36	morpholin-4-yl	(Me)Tyr	4.0	20	1.0
37	pepstatin		3200	41000	

[a] Purified human renal renin, pH 6.0. [b] Human plasma renin, pH 7.4. [c] pH 6.5 buffer. [d] *O*-Methyltyrosine.

(23) Because of hierarchy changes this group is in the 2*R* configuration.

(24) Kleinert, H. D.; Martin, D.; Chekal, M.; Young, G.; Rosenberg, S. H.; Plattner, J. J.; Perun, T. J. *J. Pharmacol. Exp. Ther.* **1988**, *246*, 975.

loss in potency in both assays (**33**). When compound **33** was administered id (10 mg/kg) to Na-deplete monkeys, no significant effect on blood pressure and only a transient fall in plasma renin activity were observed (results not shown).

Reasoning that these results were probably caused by limited absorption and that this might be due to the low water solubility of **33** (Table II), we examined groups to replace the N-terminal isobutyryl. The study resulted in morpholinourea **34**. It has already been demonstrated that this N-terminal modification preserves in vitro potency,[25] and we now show that this simple change increases aqueous solubility 100-fold. That this effect is due to the urea linkage and not the accompanying ether functionality is demonstrated by compound **35**, which possesses the low water solubility of compounds **32** and **33**. The enhanced solubilities of the urea derivatives over their amide counterparts are due in part to decreased crystal lattice energies as reflected in the lower melting points for these compounds (Table IV). Introduction of *O*-methyltyrosine (**36**) maintained aqueous solubility but was accompanied by a loss in in vitro potency against both purified renal and plasma renin comparable to that observed for inhibitor **33**.

Upon id administration of Na-deplete monkeys (10 mg/kg, data not shown), compound **36** produced similar results to those observed for **33**, again indicating negligible bioavailability. To determine the underlying cause of the low bioavailability, compounds **33** and **36** were examined in both an in vitro and an in vivo rat model. The in vitro intestinal permeability for these compounds was determined in isolated perfused segments of rat intestine and are shown in Table III. The value obtained for **33** was low and for **36** was moderate compared to the value determined for the well-absorbed drug theophylline,[26] indicating that compound **36** had the potential for transport across the intestine in vivo. These compounds were next administered id to anesthetized rats. After 10 min blood samples were drawn from both the carotid artery and the portal vein and were then assayed for drug levels with the results shown in Table III.

For both compounds systemic levels were low, confirming the limited absorption observed in monkeys. The levels of compounds **33** and **36** in the portal blood samples paralleled the in vitro intestinal permeabilities. The portal levels for **33** were low, signifying poor absorption. The more soluble **36** produced significantly higher portal levels, demonstrating that this compound is transported across the intestine in vivo; however, the majority of the compound is extracted by the liver, resulting in the low observed systemic levels.

In conclusion we have developed a series of small, nanomolar renin inhibitors based upon a novel C-terminal azido diol residue. We have demonstrated that solubility is easily modulated through simple chemical changes and we have shown that, although transport across the intestine does occur in vivo, the primary barrier to oral absorption appears to be liver extraction, a problem we are currently examining.

Experimental Section

Solvents and other reagents were reagent grade and were used without further purification unless otherwise noted. Final product solutions were dried over anhydrous Na_2SO_4 prior to evaporation on a rotary evaporator. Tetrahydrofuran was distilled from sodium/benzophenone and methylene chloride was distilled from P_2O_5. NMR spectra were recorded at 300 MHz and are expressed in ppm downfield from tetramethylsilane as an internal standard. Column chromatography was performed on silica gel 60, 0.04–0.063 mm (E. Merck), and components were visualized with ninhydrin or phosphomolybdic acid reagents. The following systems were used: 25% ethyl acetate/25% water/25% 1-butanol/25% acetic acid (I), 20% ethyl acetate/80% hexane (II), 10% methanol/90% chloroform (III), 50% ethyl acetate/50% hexane (IV), 50% ether/50% hexane (V), 80% ether/20% hexane (VI), 5% methanol/95% chloroform (VII), 8% methanol/92% chloroform (VIII), 100% ethyl acetate (IX), 7% methanol/93% chloroform (X), and 15% methanol/85% chloroform (XI).

(3*S*,4*S*)-4-Amino-5-cyclohexyl-3-hydroxy-1-pentene (2a). To (4*S*,5*S*)-4-(cyclohexylmethyl)-5-vinyloxazolidin-2-one (**1**)[17] (8.83 g, 42.2 mmol) in dioxane (420 mL) and water (280 mL) was added $Ba(OH)_2{\cdot}(H_2O)_8$ (26.8 g, 84.9 mmol), and the mixture was heated to reflux for 4 h. The mixture was coolled, filtered through Celite, and concentrated in vacuo to approximately 50 mL. This residue was partitioned between ether and water and extracted with ether which was dried and evaporated to afford 7.49 g (97%) of a pale yellow solid: mp 59–61 °C; TLC R_f 0.66 (I); ^{1}H NMR ($CDCl_3$) δ 5.82 (1 H, ddd), 5.33 (1 H, ddd), 5.20 (1 H, ddd), 3.74 (1 H, dd), 2.75 (1 H, m). Anal. ($C_{11}H_{21}NO$) C, H, N.

(3*S*,4*S*)-4-[(*tert*-Butyloxycarbonyl)amino]-5-cyclohexyl-3-hydroxy-1-pentene (2b). Amine **2a** (1.64 g, 8.84 mmol) in CH_2Cl_2 (20 mL) at 0 °C was treated with di-*tert*-butyl dicarbonate (1.93 g, 8.84 mmol). After stirring at ambient temperature for 4 h, the mixture was evaporated, dissolved in ethyl acetate, washed with 0.5 M H_3PO_4 and brine, dried, and evaporated to afford 2.51 g (100%) of an oil: TLC R_f = 0.27 (II); ^{1}H NMR ($CDCl_3$) δ 5.90 (1 H, ddd), 5.29 (1 H, ddd), 5.19 (1 H, ddd), 4.57 (1 H, br d), 4.05 (1 H, m), 3.70 (1 H, m). Anal. ($C_{16}H_{29}NO_3$) C, H, N.

(3*S*,4*S*)-4-[(*tert*-Butyloxycarbonyl)amino]-5-cyclohexyl-1,3-dihydroxypentane (3). Allylic alcohol **2b** (327.6 mg, 1.16 mmol) in THF (3 mL) at 0 °C was stirred dropwise with a solution of 9-BBN in THF (4.6 mL, 2.3 mmol, 0.5 M). After 3 h at ambient temperature the reaction was cooled to 0 °C and water (0.1 mL), then NaOH in H_2O (0.28 g, 7.0 mmol in 1 mL) and finally H_2O_2 (0.70 mL, 6.9 mmol, 30% solution) were added, and the mixture was heated at 50 °C for 90 min. The mixture was concentrated, dissolved in ethyl acetate, washed with brine, dried, and evaporated. Chromatography of the residue on silica gel with 2% CH_3OH in $CHCl_3$ afforded 351.1 mg (100%) of a colorless oil: TLC R_f = 0.41 (III); ^{1}H NMR ($CDCl_3$) δ 4.67 (1 H, br d), 3.85–3.95 (2 H, m), 3.81 (1 H, m), 3.63 (1 H, m).

(3*S*,4*S*)-1-Azido-4-[(*tert*-butyloxycarbonyl)amino]-5-cyclohexyl-3-hydroxypentane (4). Alcohol **3** (297.3 mg, 0.986 mmol) in CH_2Cl_2 (5 mL) at −10 °C was treated with CH_3SO_2Cl (76.0 μL, 0.980 mmol) and Et_3N (200 μL, 1.43 mmol). After 15 min the mixture was poured into ethyl acetate which was washed with 0.5 M H_3PO_4, saturated $NaHCO_3$ solution, and brine and then dried and evaporated to afford 344.8 mg (92%) of the mesylate: TLC R_f = 0.24 (IV). To this mesylate in DMF (5 mL) was added sodium azide (130 mg, 2.00 mmol) and the reaction was heated at 55 °C for 12 h. The mixture was poured into ethyl acetate, washed with water and brine, and then dried and evaporated. Chromatography of the residue on silica gel with 10% ethyl acetate in hexane afforded 194.5 mg (66%) of a colorless oil: TLC R_f = 0.59 (IV);: IR ($CHCl_3$) 2102 cm^{-1}.

(3*S*,4*S*)-4-[(*tert*-Butyloxycarbonyl)amino]-5-cyclohexyl-3-(methoxymethoxy)-1-pentene (5a). Alcohol **2b** (13.81 g, 48.7 mmol) in methylene chloride (120 mL) at 0 °C was treated with chloromethyl methyl ether (17.0 mL, 234 mmol) and diisopropylethylamine (42 mL, 240 mmol). After 15 min at 0 °C and 14 h at ambient temperature, the solvent was evaporated and the residue was taken up in ethyl acetate and washed with 0.5 M H_3PO_4. The aqueous washes were extracted with ethyl acetate and the combined organic phases were washed with saturated $NaHCO_3$ solution and brine and then dried and evaporated. Chromatography of the residue on silica gel with 10% ethyl acetate in hexane afforded 13.29 g (83%) of a colorless oil: TLC R_f = 0.44 (II). Anal. ($C_{18}H_{33}NO_4$) C, H, N.

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Table III. Data from in Vivo and in Vitro Rat Experiments for Inhibitors **33** and **36**[a]

no.	in vitro intestinal permeability[b]	plasma levels,[c] ng/mL	
		systemic	portal
33	0.79 ± 0.35	12 ± 10	53 ± 29[d]
36	4.25 ± 0.12	15 ± 13	230 ± 90[d]
theophylline	22.35 ± 0.95		

[a] A description of the protocol can be found in the Experimental Section. [b] cm/h × 10^3; mean ± SE; n = 2. [c] Mean ± SE; 10 mg/kg id; 10-min sample; n = 3. [d] Values differ significantly, $p < 0.1$.

(3*S*,4*S*)-4-[(*tert*-Butyloxycarbonyl)amino]-5-cyclohexyl-3-[(methoxyethoxy)methoxy]-1-pentene (5b): prepared from alcohol **2b** as described for **5a** by replacing chloromethyl methyl ether with MEM chloride; TLC R_f = 0.25 (II); $[\alpha]^{23}_D$ +16.1° (*c* 1.0, EtOH). Anal. ($C_{20}H_{37}NO_5$) C, H, N.

(2*RS*,3*R*,4*S*)-4-[(*tert*-Butyloxycarbonyl)amino]-5-cyclohexyl-3-(methoxymethoxy)-1,2-epoxypentane (6a). Alkene **5a** (13.28 g, 40.55 mmol) in methylene chloride (150 mL) at 0 °C was treated with MCPBA (17.50 g, 80 mmol, 80–85% pure). After 41 h at ambient temperature the mixture was evaporated, dissolved in ethyl acetate, and washed with cold 10% aqueous Na_2SO_3 solution, saturated $NaHCO_3$ solution, and brine and then dried and evaporated to afford 13.17 g (95%) of an oil which was used without further purification. A portion was chromatographed (silica gel, 33% ethyl acetate in hexane) for combustion analysis: TLC R_f = 0.26 (II); ^{1}H NMR ($CDCl_3$) δ 3.41 (minor), 3.37 (major) (3 H total, 2 s). Anal. ($C_{18}H_{33}NO_5$) C, H, N.

(2*RS*,3*R*,4*S*)-4-[(*tert*-Butyloxycarbonyl)amino]-5-cyclohexyl-3-[(methoxyethoxy)methoxy]-1,2-epoxypentane (6b). Alkene **5b** was epoxidized as described for **6a**: TLC R_f = 0.12 (II). Anal. ($C_{20}H_{37}NO_6$) C, H, N.

(2*S*,3*R*,4*S*)-1-Azido-4-[(*tert*-butyloxycarbonyl)amino]-5-cyclohexyl-2-hydroxy-3-(methoxymethoxy)pentane (7a) and (2*R*,3*R*,4*S*)-1-Azido-4-[(*tert*-butyloxycarbonyl)amino]-5-cyclohexyl-2-hydroxy-3-(methoxymethoxy)pentane (7b). Epoxide **6** (14.59 g, 42.49 mmol) in methanol (300 mL) was treated with sodium azide (6.65 g, 102 mmol) and ammonium chloride (4.10 g, 76.6 mmol), and the mixture was heated at reflux for 48 h. The mixture was concentrated, taken up in ethyl acetate, washed with water and brine, and then dried and evaporated. Chromatography on silica gel with 20% ether in hexane afforded 5.95 g (36%) of the 2*S* isomer as a white solid followed by 2.92 g (18%) of the 2*R* isomer as a colorless oil.

2*S* isomer: TLC R_f = 0.47 (V); mp 81–82 °C; $[\alpha]^{23}_D$ −42.7° (*c* 0.96 EtOH); ^{1}H NMR ($CDCl_3$) δ 4.65 (1 H, d), 4.62 (1 H, d), 4.57 (1 H, br d), 4.03–4.15 (1 H, m), 3.50–3.63 (2 H, m), 3.40 (3 H, s), 3.30–3.45 (2 H, m); IR ($CHCl_3$) 2100 cm^{-1}. Anal. ($C_{18}H_{34}N_4O_5$) C, H, N.

2*R* isomer: TLC R_f = 0.32 (V); $[\alpha]^{23}_D$ −3.57° (*c* 0.62, EtOH); ^{1}H NMR ($CDCl_3$) δ 4.78 (1 H, d), 4.66 (1 H, d), 4.61 (1 H, br d), 3.70–3.90 (3 H, m), 3.60 (1 H, dd), 3.40–3.50 (1 H, m), 3.46 (3 H, s); IR ($CHCl_3$) 2100 cm^{-1}. Anal. ($C_{18}H_{34}N_4O_5$) C, H, N.

(2*S*,3*R*,4*S*)-1-Azido-4-[(*tert*-butyloxycarbonyl)amino]-5-cyclohexyl-2-hydroxy-3-[(methoxyethoxy)methoxy]pentane (7c) and (2*R*,3*R*,4*S*)-1-Azido-4-[(*tert*-butyloxycarbonyl)amino]-5-cyclohexyl-2-hydroxy-3-[(methoxyethoxy)methoxy]pentane (7d). Prepared from epoxide **6b** as described for **7a** and **7b**. A sample of **7c** was recrystallized from CH_2Cl_2/hexane for single-crystal X-ray analysis.

2*S* isomer: TLC R_f = 0.30 (V); mp 75–76 °C; $[\alpha]^{23}_D$ −43.0° (*c* 1.0, EtOH); IR ($CHCl_3$) 2100 cm^{-1}. Anal. ($C_{20}H_{38}N_4O_6$) C, H; N: calcd, 13.01; found, 12.57.

2*R* isomer: TLC R_f = 0.15 (V).

(3*R*,4*S*)-1-Azido-4-[(*tert*-butyloxycarbonyl)amino]-5-cyclohexyl-3-(methoxymethoxy)-2-oxopentane (8). To oxalyl chloride (70 μL, 0.82 mmol) in methylene chloride (2 mL) at −69 °C was added CH_3SOCH_3 (85 μL, 1.3 mmol) in methylene chloride (2 mL). After 5 min compound **7b** (105.0 mg, 0.272 mmol) in methylene chloride (3 mL) was added followed 15 min later by Et_3N (300 μL, 2.1 mmol). After 20 min at −69 °C the reaction was quenched with 20% saturated $KHSO_4$ solution (10 mL), poured into hexane, washed with water and brine, and then dried and evaporated. Chromatography on silica gel with 20% ether in hexane afforded 79.7 mg (76%) of an oil: TLC R_f = 0.43 (V); ^{1}H NMR ($CDCl_3$) δ 4.69 (1 H, br d), 4.66 (2 H, s), 4.31 (1 H, d), 4.10–4.25 (2 H, m), 4.04 (1 H, d), 3.38 (3 H, s).

(3*S*,4*S*)-4-[(*tert*-Butyloxycarbonyl)amino]-5-cyclohexyl-2-(diethoxymethyl)-3-hydroxy-1-pentene (10a) and (3*R*,4*S*)-4-[(*tert*-Butyloxycarbonyl)amino]-5-cyclohexyl-2-(diethoxymethyl)-3-hydroxy-1-pentene (10b). To α-bromoacrolein diethyl acetal[20] (70.0 g, 335 mmol) in THF (200 mL) at −78 °C was added *n*-BuLi (125 mL, 313 mmol, 2.5 M in hexane). After 1 h this mixture was transferred via cannula to Boc-cyclohexylalanal[5,19] (**9**, 25.8 g, 101 mmol) in THF (250 mL) at −78 °C. After 1 h the mixture was quenched with methanol (100 mL), concentrated, taken up in ethyl acetate which was washed sequentially with saturated $NaHCO_3$ solution, water, and brine, and then dried and evaporated. A portion of the crude material (810 mg) was chromatographed on silica gel with 20% ether in hexane to afford 409 mg of **10a** followed by 188 mg of **10b** and 79 mg of mixed fractions as oils. Chromatography of the remainder afforded 13.25 g of **10a** for a total of 13.65 g (35%).

3*S* isomer: TLC R_f = 0.35 (V); ^{1}H NMR ($CDCl_3$) δ 6.55 (1 H, s), 6.12 (1 H, s), 5.39 (1 H, m), 5.33 (1 H, d), 3.73 (4 H, q), 1.48 (9 H, s), 1.25 (6 H, t). Anal. ($C_{21}H_{39}NO_5$) C, H, N.

3*R* isomer: TLC R_f = 0.24 (V); ^{1}H NMR ($CDCl_3$) δ 5.40 (1 H, s), 5.27 (1 H, s), 4.99 (1 H, s), 4.72 (1 H, s), 1.43 (9 H, s), 1.25 (3 H, t), 1.23 (3 H, t).

(4*S*,5*S*)-4-(Cyclohexylmethyl)-5-(3′,3′-diethoxy-2′-propenyl)oxazolidin-2-one (11a). To NaH (3.10 g, 77.5 mmol, 60% dispersion in oil, hexane washed) in DMF (30 mL) at 0 °C was added compound **10a** (13.60 g, 35.3 mmol) in DMF (65 mL). After stirring at room temperature overnight, the mixture was poured into ether which was washed with water and brine and then was dried and evaporated to afford 10.99 g (100%) of the product as an oil: TLC R_f = 0.78 (VI); ^{1}H NMR ($CDCl_3$) δ 5.72 (1 H, s), 5.40 (1 H, s), 5.38 (1 H, s), 4.88 (1 H, s), 4.72 (1 H, d, J = 4.8 Hz), 3.86 (1 H, m), 3.75–3.40 (4 H, m), 1.24 (3 H, t), 1.22 (3 H, t). Anal. ($C_{17}H_{29}NO_4 \cdot 0.25H_2O$) C, H, N.

(4*S*,5*R*)-4-(Cyclohexylmethyl)-5-(3′,3′-diethoxy-2′-propenyl)oxazolidin-2-one (11b): prepared in 82% yield from compound **10b** according to the procedure for **11a**; ^{1}H NMR ($CDCl_3$) δ 5.52 (1 H, s), 5.50 (1 H, s), 5.40 (1 H, s), 5.25 (1 H, d, J = 7.3 Hz), 4.76 (1 H, s), 3.90 (1 H, m), 3.85–3.40 (4 H, m), 1.225 (3 H, t), 1.220 (3 H, t). Anal. ($C_{17}H_{29}NO_4 \cdot 0.75H_2O$) C, H, N.

(4*S*,5*S*)-4-(Cyclohexylmethyl)-5-(3′-hydroxy-2′-propenyl)oxazolidin-2-one (12a). To silica gel (100 g) in CH_2Cl_2 (250 mL) was added 10% aqueous oxalic acid (10 mL). After the water droplets had disappeared, acetal **11a** (10.99 g, 35.3 mmol) in CH_2Cl_2 was added. After 1 h the mixture containing the intermediate aldehyde [TLC R_f = 0.58 (VI)] was diluted with methanol (900 mL), cooled to 0 °C, and treated with $NaBH_4$ (2.80 g, 74.0 mmol). After 1 h at 0 °C the mixture was evaporated to dryness, suspended in ethyl acetate, and washed with 0.5 M H_3PO_4. The aqueous phase was extracted with ethyl acetate. The combined organic phases were washed with water and brine and then dried and evaporated. Chromatography of the residue on silica gel with 5% methanol in chloroform afforded 4.37 g (52%) of the alcohol as an oil: TLC R_f = 0.48 (III), R_f = 0.42 (X); ^{1}H NMR ($CDCl_3$) δ 5.56 (1 H, s), 5.33 (1 H, s), 5.25 (1 H, s), 4.69 (1 H, d), 4.28 (1 H, s), 4.26 (1 H, s), 3.34 (1 H, m). Anal. ($C_{13}H_{21}NO_3 \cdot 0.5H_2O$) C, H, N.

(4*S*,5*S*)-5-[3′-[(*tert*-Butyldimethylsilyl)oxy]-2′-propenyl]-4-(cyclohexylmethyl)oxazolidin-2-one (12b). Alcohol **12a** (4.33 g, 18.1 mmol) in DMF (20 mL) was treated with *tert*-butyldimethylsilyl chloride (2.86 g, 19.0 mmol) and imidazole (1.29 g, 18.9 mmol). After 16 h at room temperature the mixture was diluted with ethyl acetate, washed sequentially with 0.5 M H_3PO_4, saturated $NaHCO_3$ solution, water, and brine, and then dried and evaporated to afford 5.81 g (91%) of a pale brown waxy solid: mp 105–107 °C; TLC R_f = 0.76 (VII). Anal. ($C_{19}H_{35}NO_3Si \cdot 0.25H_2O$) C, H, N.

(4*S*,5*S*,2′*R*)-5-[1′-[(*tert*-Butyldimethylsilyl)oxy]-3′-hydroxy-2′-propyl]-4-(cyclohexylmethyl)oxazolidin-2-one (14a) and (4*S*,5*S*,2′*S*)-5-[1′-[(*tert*-Butyldimethylsilyl)oxy]-3′-hydroxy-2′-propyl]-4-(cyclohexylmethyl)oxazolidin-2-one (14b). Olefin **12b** (5.80 g, 16.4 mmol) in THF (25 mL) at 0 °C was treated with BH_3 in THF (33 mL, 33 mmol, 1.0 M). After 90 min at room temperature the mixture was cooled to 0 °C and

Table IV. Physical Data for Renin-Inhibiting Compounds

no.[a]	formula[b]	TLC, R_f[c]	mp,[d] °C
23	$C_{30}H_{44}N_8O_5 \cdot H_2O$	0.50	
24	$C_{31}H_{46}N_8O_5 \cdot 0.5H_2O$	0.43	
25	$C_{31}H_{46}N_8O_6 \cdot 0.75H_2O$[e]	0.44	
26	$C_{31}H_{46}N_8O_6 \cdot 0.25H_2O$	0.32	
27	$C_{32}H_{48}N_8O_6 \cdot 0.5H_2O$	0.46	
28	$C_{32}H_{48}N_8O_6$[f]	0.36	
29	$C_{32}H_{48}N_8O_7$[g]	0.31, 0.35[h]	
30	$C_{31}H_{44}N_8O_6$[i]	0.39	
31	$C_{32}H_{46}N_8O_5 \cdot 0.25H_2O$	0.40	
32	$C_{30}H_{44}N_8O_5 \cdot 0.4H_2O$	0.40	202–206
33	$C_{31}H_{46}N_8O_6 \cdot 0.5H_2O$	0.40	201–204
34	$C_{31}H_{45}N_8O_6 \cdot 0.75H_2O$	0.41	95–100
35	$C_{32}H_{46}N_8O_6 \cdot 1.5H_2O$	0.32	207–210
36	$C_{32}H_{47}N_9O_7 \cdot H_2O$	0.44	85–90

[a] See Tables I and II for structure. [b] Analyses for C, H, N were ±0.4% of expected values (for formulae shown), unless otherwise noted. [c] 15% methanol/85% chloroform. [d] Decomposition. [e] N: calcd, 17.50; found, 15.66. Exact mass calcd for $C_{31}H_{47}N_8O_6$ (M + H) 627.3618, found 627.3605. [f] Exact mass calcd for $C_{32}H_{49}N_8O_6$ (M + H) 641.3775, found 641.3770. [g] Exact mass calcd for $C_{32}H_{49}N_8O_7$, (M + H) 657.3724, found 657.3728. [h] Diastereomers separable. [i] N: calcd, 17.94; found, 16.60.

treated sequentially with water (5.0 mL), NaOH solution (15 mL, 3 M), and then 30% H_2O_2 (15 mL). After 90 min at room temperature the mixture was concentrated, partitioned between saturated $NaHCO_3$ solution and ethyl acetate, and extracted into ethyl acetate which was washed with brine, dried, and evaporated. Chromatography on silica gel with 20–30% ethyl acetate in chloroform afforded 997.5 mg (16%) of the 2′*S* isomer as an oil followed by 1.3745 g (23%) of the 2′*R* isomer as a solid.

2′*S* isomer: TLC R_f = 0.40 (VII), R_f = 0.61 (VIII). Anal. ($C_{19}H_{37}NO_4Si \cdot 0.5H_2O$) C, H, N.

2′*R* isomer: TLC R_f = 0.30 (VII), R_f = 0.58 (VIII); mp 119–120 °C. Anal. ($C_{19}H_{37}NO_4Si$) C, H, N.

(4*S*,5*S*,2′*S*)-5-[1′-[(*tert*-Butyldimethylsilyl)oxy]-3′-(methylsulfonoxy)-2′-propyl]-4-(cyclohexylmethyl)oxazolidin-2-one. To alcohol **14a** (1.475 g, 3.97 mmol) and diisopropylethylamine (1.25 mL, 7.18 mmol) in CH_2Cl_2 (12 mL) at 0 °C was added methanesulfonyl chloride (340 μL, 4.39 mmol). After 90 min at 0 °C the mixture was diluted with ethyl acetate and washed with 0.5 M H_3PO_4, saturated $NaHCO_3$ solution, and brine and then dried and evaporated to afford 1.620 g (91%) of a pale yellow solid: mp 93–95 °C; TLC R_f = 0.75 (VIII). Anal. ($C_{20}H_{39}NO_6SSi$) C, H, N.

(4*S*,5*S*,2′*R*)-5-[1′-[(*tert*-Butyldimethylsilyl)oxy]-3′-(methylsulfonoxy)-2′-propyl]-4-(cyclohexylmethyl)oxazolidin-2-one: prepared from alcohol **14b** in 92% yield as a pale yellow solid as described for the 4*S*,5*S*,2′*S* isomer; mp 119.5–120 °C; TLC R_f = 0.75 (VIII). Anal. ($C_{20}H_{39}NO_6SSi$) H, N; C: calcd, 53.42; found, 53.85.

(4*S*,5*S*,2′*R*)-5-[3′-Azido-1′-[(*tert*-butyldimethylsilyl)oxy]-2′-propyl]-4-(cyclohexylmethyl)oxazolidinone. Sodium azide (465 mg, 7.15 mmol) and (4*S*,5*S*,2′*S*)-5-[1′-[(*tert*-butyldimethylsilyl)oxy]-3′-(methylsulfonoxy)-2′-propyl]-4-(cyclohexylmethyl)oxazolidin-2-one (1.6045 g, 3.57 mmol) in DMF (7.5 mL) were heated at 80 °C for 14 h. The mixture was diluted with ethyl acetate, washed with water and brine, and then dried and evaporated to afford 1.342 g (95%) of a light yellow solid: mp 110–112 °C; ^{1}H NMR ($CDCl_3$) δ 5.38 (1 H, s), 4.25 (1 H, dd), 3.82 (1 H, m), 3.75–3.60 (3 H, m), 3.46 (1 H, dd), 1.94 (1 H, m), 0.90 (9 H, s), 0.08 (3 H, s), 0.07 (3 H, s); IR ($CHCl_3$) 2100 cm^{-1}. Anal. ($C_{19}H_{36}N_4O_3Si \cdot 0.1H_2O$) C, H, N.

(4*S*,5*S*,2′*S*)-5-[3′-Azido-1′-[(*tert*-butyldimethylsilyl)oxy]-2′-propyl]-4-(cyclohexylmethyl)oxazolidin-2-one. Prepared in 94% yield from (4*S*,5*S*,2′*R*)-5-[1′-[(*tert*-butyldimethylsilyl)oxy]-3-(methylsulfonoxy)-2′-propyl]-4-(cyclohexylmethyl)oxazolidin-2-one as described for the 4*S*,5*S*,2′*R* isomer: mp 94–96 °C; TLC R_f = 0.85 (VIII); ^{1}H NMR ($CDCl_3$) δ 5.26 (1 H, s), 4.24 (1 H, dd), 3.87 (1 H, m), 3.82 (1 H, dd), 3.70 (1 H, dd), 3.47 (2 H, d), 1.99 (1 H, m), 0.89 (9 H, s), 0.08 (3 H, s), 0.07 (3 H, s); IR ($CHCl_3$) 2100 cm^{-1}.

(4*S*,5*S*,2′*R*)-5-(3′-Azido-1′-hydroxy-2′-propyl)-4-(cyclohexylmethyl)oxazolidin-2-one (15a). (4*S*,5*S*,2′*R*)-5-[3′-Azido-1′-[(*tert*-butyldimethylsilyl)oxy]-2′-propyl]-4-(cyclohexylmethyl)oxazolidin-2-one (1.315 g, 3.32 mmol) was treated with $Bu_4N^+F^-$ in THF (10 mL, 1.0 M). After 90 min at room temperature the mixture was diluted with ethyl acetate, washed with water and brine, and then dried and evaporated. Chromatography on silica gel with 2.5% methanol in chloroform afforded 867 mg (93%) of the free alcohol as a white solid: mp 140–141 °C; TLC R_f = 0.46 (VIII); ^{1}H NMR (DMSO) δ 4.86 (1 H, dd), 4.08 (1 H, dd), 3.75 (1 H, dd), 3.56 (1 H, dd); IR (KBr) 2107 cm^{-1}. Anal. ($C_{13}H_{22}N_4O_3 \cdot 0.1H_2O$) C, H, N.

(4*S*,5*S*,2′*S*)-5-(3′-Azido-1′-hydroxy-2′-propyl)-4-(cyclohexylmethyl)oxazolidin-2-one (15b): prepared in 98% chromatographed yield from (4*S*,5*S*,2′*S*)-5-[3′-azido-1′-[(*tert*-butyldimethylsilyl)oxy]-2′-propyl]-4-(cyclohexylmethyl)oxazolidin-2-one as described for the 4*S*,5*S*,2′*R* isomer: mp 84.5–85.5 °C; ^{1}H NMR (DMSO) δ 4.80 (1 H, dd), 4.09 (1 H, dd), 3.77 (1 H, dd); IR ($CHCl_3$) 2100 cm^{-1}. Anal. ($C_{13}H_{22}N_4O_3 \cdot 0.1H_2O$) C, H, N.

(2*R*,3*S*,4*S*)-4-Amino-1-azido-5-cyclohexyl-3-hydroxy-2-(hydroxymethyl)pentane (16a). Oxazolidinone **15a** (835 mg, 2.96 mmol) in dioxane (30 mL) and water (20 mL) was treated with $Ba(OH)_2 \cdot 8H_2O$ (1.87 g, 5.93 mmol) and heated at reflux overnight. The mixture was evaporated to dryness, suspended in ethyl acetate, and filtered through Celite. The solids were rinsed with methanol, and the combined filtrate was evaporated, suspended in ethyl acetate, and filtered a second time through Celite. After a methanol wash the combined filtrate was evaporated to 710 mg (90%) of a waxy solid: mp 108.5–109.5 °C; IR ($CHCl_3$) 2100 cm^{-1}. Anal. ($C_{12}H_{24}N_4O_2$) C, H; N: calcd, 21.86; found, 19.47.

(2*S*,3*S*,4*S*)-4-Amino-1-azido-5-cyclohexyl-3-hydroxy-2-(hydroxymethyl)pentane (16b): prepared in 91% yield as an oil from oxazolidinone **15b** as described for **16a**; exact mass ($C_{12}H_{24}N_4O_2$) calcd 257.1977, found 257.1955.

(4*S*,5*R*,1′*S*)-4-[1′-(Acetylamino)-2′-cyclohexylethyl]-5-(azidomethyl)-2,2-dimethyl-1,3-dioxane (17a). Amine **16a** (39.0 mg, 0.152 mmol) in CH_2Cl_2 (2 mL) and dioxane (1 mL) was treated with triethylamine (32 μL, 0.23 mmol) and acetyl chloride (11.0 μL, 0.155 mmol). After 15 min the mixture was diluted with ethyl acetate, washed with 2 M HCl, 2 M NaOH, and brine, and then dried and evaporated to give 37.0 mg (82%) of the crude acetamide: TLC R_f = 0.58 (III). This material (20 mg, 0.067 mmol) in CH_2Cl_2 (3 mL) was treated with *p*-toluenesulfonic acid (2 mg) and 2-methoxypropene (15 μL, 0.16 mmol). After 2 h the mixture was diluted with ethyl acetate, washed with saturated $NaHCO_3$ solution and brine, and then dried and evaporated. Chromatography on silica gel with 10% ethyl acetate in chloroform afforded 9.0 mg (40%) of the ketal: TLC R_f = 0.54 (VII); ^{1}H NMR (1:1 benzene-d_6/acetone-d_6) δ 6.63 (1 H, br, NH), 4.14–4.22 (1 H, m, H_1′), 4.06 (1 H, dd, H_4, $J_{4,5}$ = 3 Hz, $J_{4,1'}$ = 6 Hz), 3.79 (1 H, dd, H_{6a}, $J_{6a,5}$ = 11 Hz, $J_{6a,6e}$ = 12 Hz), 3.62 (1 H, ddd, H_{6e}, $J_{6e,5}$ = 5 Hz, J_{6e,CH_2N_3} = 1 Hz), 1.93 (3 H, s, CH_3CO), 1.75 (1 H, m, H_5), 1.46 (3 H, s), 1.38 (3 H, s).

(4*S*,5*S*,1′*S*)-4-[1′-(Acetylamino)-2′-cyclohexylethyl]-5-(azidomethyl)-2,2-dimethyl-1,3-dioxane (17b): prepared from amine **16b** in 27% overall yield as described for **17a**; ^{1}H NMR (1:1 benzene-d_6/acetone-d_6) δ 6.75 (1 H, br, NH), 4.34–4.48 (1 H, m, $H_{1'}$), 3.82 (1 H, d, H_4, $J_{4,5}$ = 10 Hz, $J_{4,1'}$ = 0 Hz), 3.66 (1 H, dd, H_{6e}, $J_{6e,5}$ = 3 Hz, $J_{6e,6a}$ = 12 Hz), 3.45 (1 H, dd, H_{6a}, $J_{6a,5}$ = 8 Hz), 1.97 (3 H, s, CH_3CO), 1.80 (1 H, m, H_5), 1.42 (3 H, s), 1.35 (3 H, s).

(4*S*,5*S*,2′*RS*)-4-(Cyclohexylmethyl)-5-(3′-hydroxy-1′,2′-epoxy-2′-propyl)oxazolidin-2-one (18): prepared from olefin **12a** in 84% yield as described for epoxide **6**; TLC R_f = 0.45 (IX); ^{1}H NMR ($CDCl_3$) δ 3.02, 3.01, 2.92, 2.73 (total 2 H, 4 d, epoxide CH_2).

(4*S*,5*S*,2′*RS*)-4-(Cyclohexylmethyl)-5-(1′-azido-2′,3′-dihydroxy-2′-propyl)oxazolidin-2-one (19). Epoxide **18** was opened with azide as described for epoxide **6** to provide the product in 50% yield as a 1:1 mixture of diastersomers: TLC R_f = 0.25, 0.37 (IX); ^{1}H NMR ($CDCl_3$) δ 4.23, 4.21 (total 1 H, 2 d), 4.10–4.00 (1 H, m).

(4*S*,5*S*)-4-(Cyclohexylmethyl)-5-[3′-(mesyloxy)-2′-propenyl]oxazolidin-2-one (21a). Alcohol **12a** (110 mg, 0.46 mmol) in CH_2Cl_2 (2 mL) at −10 °C was treated with CH_3SO_2Cl (36 μL, 0.46 mmol) and diisopropylethylamine (120 μL, 0.69 mmol). After 30 min the mixture was diluted with ethyl acetate, washed with 0.5 M H_3PO_4, saturated $NaHCO_3$ solution, and brine,

and then dried and evaporated to afford 126 mg (86%) of a yellow solid: TLC R_f = 0.54 (X); ^{1}H NMR ($CDCl_3$) δ 5.52 (1 H, s), 4.47 (1 H, s), 5.35 (1 H, br), 4.75–4.85 (2 H, m), 4.69 (1 H, d), 3.80–3.88 (1 H, m), 3.07 (3 H, s).

(4*S*,5*S*)-4-(Cyclohexylmethyl)-5-(3′-azido-2′-propenyl)oxazolidin-2-one (21b). Mesylate **21a** was converted to the azide as described for compound **4** in 90% yield after chromatography on silica gel with 1% $CH_3OH/CHCl_3$: TLC R_f = 0.63 (X); ^{1}H NMR ($CDCl_3$) δ 5.40 (1 H, s), 5.36 (1 H, s), 5.32 (1 H, br), 4.64 (1 H, d), 3.96 (1 H, d), 3.90 (1 H, d), 3.75–3.88 (1 H, m).

Amines 20 and 22. These compounds were prepared from oxazolidinones **19** and **21b** according to the procedure for compound **16a** and were used without purification.

General Procedure for Peptide Coupling: Boc-His Amide of (2*S*,3*R*,4*S*)-4-Amino-1-azido-5-cyclohexyl-2,3-dihydroxypentane. Compound **7a** (713 mg, 1.84 mmol) was dissolved in 4.0 M HCl/dioxane (5 mL) and stirred at room temperature for 1 h. The solvent was evaporated, and two portions of ether were added and evaporated to leave a white solid. To this residue was added Boc-His-OH (523 mg, 2.05 mmol) and 1-hydroxybenzotriazole (500 mg, 5.92 mmol). The mixture was dissolved in DMF (10 mL), treated with *N*-methylmorpholine (410 μL, 3.73 mmol), cooled to −23 °C, and treated with 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (390 mg, 2.03 mmol). After 2 h at −23 °C and 12 h at ambient temperature, the mixture was poured into saturated $NaHCO_3$ solution and extracted with ethyl acetate which was washed with water and brine and then dried and evaporated. Chromatography of the residue with 4.5% methanol in chloroform afforded 576 mg (65%) of a white solid. Anal. ($C_{22}H_{37}N_7O_5$) C, H; N: calcd, 20.44; found, 19.54.

Boc-Phe-His Amide of (2*S*,3*R*,4*S*)-4-Amino-1-azido-5-cyclohexyl-2,3-dihydroxypentane (25). This compound was prepared as described for the Boc-His amide replacing Boc-His-OH with the dipeptide Boc-Phe-His-OH. Chromatography of the crude product on silica gel with 3% methanol in chloroform afforded 49% of the desired product as a white solid: mp 158–163 °C; TLC R_f = 0.44 (XI); ^{1}H NMR ($CDCl_3$) δ 7.48 (1 H, s), 7.40–7.20 (5 H, m), 6.83 (1 H, s), 6.68 (1 H, br), 5.15 (1 H, br), 4.57 (1 H, br), 4.35–4.20 (2 H, m), 1.39 (9 H, s). Anal. ($C_{31}H_{46}N_8O_6 \cdot 0.75H_2O$) C, H; N: calcd, 17.50; found, 15.66.

Boc-Phe-His Amide of (2*R*,3*R*,4*S*)-4-Amino-1-azido-5-cyclohexyl-2,3-dihydroxypentane (26). This compound was prepared from compound **7b** as described for **25**: TLC R_f = 0.32 (XI); ^{1}H NMR (CD_3OD) δ 7.61 (1 H, d), 7.17–7.30 (5 H, m), 6.90 (1 H, s), 4.56 (1 H, dd), 4.28 (1 H, dd), 4.05 (1 H, m), 1.35 (9 H, s). Anal. ($C_{31}H_{46}N_8O_6 \cdot 0.25H_2O$) C, H, N.

Crystallography. Compound **7c**: $C_{20}H_{38}N_4O_6$, M_r = 430.54, orthorhomobic, $P2_12_12_1$, a = 10.881 (4) Å, b = 22.136 (3) Å, c = 10.274 (2) Å, V = 2474. (1) Å^3, Z = 4, D_{calc} = 1.15 g/cm^3, Cu Kα, λ = 1.5418 Å, $F(000)$ = 936, T = 296 K, R = 0.062 for 1135 unique reflections. A clear plate of dimensions 0.30 × 0.10 × 0.05 mm was used for intensity measurement. The data were collected on a Rigaku AFC5 diffractometer with Cu Kα radiation in the $\theta/2\theta$ scanning mode. Of the 2136 unique reflections measured ($2\theta_{max}$ = 120°) 1135 had intensities greater than 3σ. The data was corrected for absorption, Lorentz, and polarization factors. The structure was solved by SHELXS, a direct methods program. The structure was refined through the least-squares procedure with the complete matrix of normal equations. Non-hydrogen atoms were refined anisotropically. The hydrogen atom on N1 and O1 was found by Fourier difference; all other hydrogen positions were calculated.

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

Biological Methods. Purified human renal renin (pH 6.0)[11] and human plasma renin (pH 7.4)[25] assays have been described previously.

In Vitro Intestinal Permeability of 33, 36, and Theophylline. Intestinal permeabilities were measured in an in vitro preparation of rat jejunum. Following cervical dislocation, the small intestine was removed and washed free of contents with osmotically balanced electrolyte solution at 4 °C. The central 30 cm of intestine was mounted in a glass perfusion apparatus which allowed recirculation of lumenal medium (30 mL) while controlling temperature (37 °C) and oxygenation (95% O_2–5% CO_2). Renin inhibitors were added to the lumenal (mucosal) medium at 100 μM together with ^{3}H-PEG-900. Samples of both the mucosal and serosal baths were taken at 15-min intervals for 150 min. Both the appearance of compounds in the serosal bath as well as disappearance from mucosal bath were determined as a function of time. Renin inhibitor concentrations were determined by HPLC analysis with ultraviolet detection while PEG concentrations were determined by liquid scintillation spectrometry. Steady-state appearance rates (45–120 min) were determined by linear regression analysis, and the permeability, P (cm/h), was calculated as the ratio between the flux, J (nmol/cm^2 h) and the mean difference in concentration, ΔC (nmol/cm^3), between the mucosal and serosal media: $P = J/\Delta C$.

In practice, intestinal transport begins steady-state rates at 30–45 min after addition of compound and ΔC may be approximated by the concentration in the mucosal bath since the serosal concentration is typically 50–100-fold less than the mucosal concentration. Provision for variability in base-line permeation entails simultaneous measurement of PEG-900 permeability. Variability of more than 20% between tissues or more than 30% from the total population mean is considered cause for rejection. No more than 5% of studies have been discarded for failure to meet these criteria. Viability of the preparation is assessed by monitoring transepithelial electrical potential differences as a function of time. The potential difference is typically maintained within 30% of its initial value for up to 150 min, reflecting changes of less than 20% in net ion transport and 10% in tissue electrical conductance.

Intraduodenal Rat Experiments. Male Sprague–Dawley rats starved overnight and weighing at least 150 g were anesthetized with inactin (80 mg/kg, ip), and a PE50 catheter was surgically implanted into the carotid artery for the collection of systemic blood samples. A PE50 joined to PE10 catheter was inserted into the portal vein for the collection of portal blood samples. At this point the pyloric sphincter was ligated. At time zero, the id rats were dosed by injection (needle and syringe) into the duodenum with 10 mg/kg of the test compound. At the end of the protocol all rats were sacrificed by anesthesia overdose. Both systemic and portal blood samples were drawn simultaneously, before and at given intervals following drug administration.

Enzyme-Inhibition Assay of Rat Plasma Samples. Plasma samples (100 μL) were precipitated by addition of 300 μL of acetonitrile, followed by vigorous mixing. The supernatant from the precipitated, centrifuged samples was diluted sequentially in buffer (typically 1/4, 1/16, 1/64, 1/256) and then evaporated. To the dry residue was added one stock solution (50 μL) containing purified human angiotensinogen[27] (ca. 500 ng) and a second solution (50 μL) containing either purified human renin[28] (ca. 0.2 ng) or renin-absent buffer controls. The buffer used for both stock solutions was 0.135 M sodium maleate, pH 6.0, containing 0.0015 M EDTA, 0.046 M NaCl, and 1% bovine serum albumin. A 10-μL aliquot of the incubation, after 30 min at 37 °C, was subjected to radioimmunoassay for angiotensin I.[29] The control assay, which excludes renin, determined the angiotensin I level of the sample and any other immunoreactive substance in the extract or reagents. The extent of the inhibition of the assay was used to quantitate the concentration of renin inhibitor present in the extract, based on the following equation (which is derived from the kinetic equation of competitive enzyme inhibition): [inhibitor] = $(V_0/V_{inh} - 1) \times IC_{50}$. The concentration of compound in the blood sample was determined by multiplication of the calculated value in the assay by the overall dilution factor. The compounds

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were found to extract quantitatively from plasma with acetonitrile and to inhibit the reconstituted renin assay in a manner similar to the initial primary test system.

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Supplementary Material Available: Tables of fractional coordinates, bond angles, intramolecular distances, and thermal parameters (8 pages). Ordering information is given on any current masthead page.

Inhibition of Aminopeptidases by Peptides Containing Ketomethylene and Hydroxyethylene Amide Bond Replacements

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Inhibitors of aminopeptidase enzymes have been prepared by the synthesis of peptide substrate analogues in which the scissile amide bond has been replaced with the hydrolytically stable ketomethylene ($-COCH_2-$) and hydroxyethylene [$-CH(OH)CH_2-$] functionalities. Two synthetic strategies were used to prepare the inhibitors, and the advantages and disadvantages of each are discussed. The synthesis of peptides that contain the hydroxyethylene isostere was complicated by competing lactone and lactam formation, and attempts to prepare free N-terminal dipeptide hydroxyethylene isostere derivatives were unsuccessful. All ketomethylene isosteres examined were weak inhibitors of both leucine aminopeptidase and aminopeptidase M. However, the ketomethylene inhibitor $Lys^K(RS)Phe$ (**58**) (K_i = 4 nM) is a potent inhibitor comparable to the natural product, arphamenine A (Arg^KPhe; K_i = 2.5 nM). Normal Michaelis–Menten kinetics for inhibition of membrane leucine aminopeptidase are observed in the absence of magnesium ion, but nonlinear kinetics were obtained in the presence of Mg^{2+}.

The aminopeptidases are a diverse group of enzymes that catalyze the hydrolysis of amino-terminal residues from peptide substrates. Since these enzymes appear to be involved in important biological processes, compounds that inhibit the aminopeptidases may have therapeutic applications. Several naturally occurring aminopeptidase inhibitors have been isolated, and their structures are shown in Table I. Bestatin (1), a potent inhibitor of the three enzymes shown in Table I and of other aminopeptidases,[5-9] has been reported to produce important in vitro and in vivo effects. Studies in which bestatin enhances the delayed-type hypersensitivity response in mice to sheep red blood cells[10] suggest that bestatin modulates immune activity. Bestatin also stimulates polysome assembly in T-cell lymphoma (grown in suspension),[11] increases [^{3}H]thymidine incorporation into lymphocytes,[12] and shows promising results in clinical trials with human cancer patients.[13] Other studies have shown that bestatin inhibits metabolism of opioid peptides[14] and potentiates antinociceptive activity.[15] These results suggest that new, potent inhibitors of aminopeptidases may have important medicinal applications.

Our approach to new inhibitors of aminopeptidases was to replace the scissile amide bond in a substrate with a functionality that is hydrolytically stable. Different moieties have been used as isosteric amide bond replacements to provide inhibitors of proteolytic enzymes (Table II).[16-19] Compound **6** is from the work of Szelke and collaborators, who prepared renin inhibitors from substrates by replacing amide bonds with one of four isosteric linkages: the reduced amide bond or aminomethylene group ($-CH_2NH-$); the ketomethylene group ($-COCH_2-$); the hydroxyethylene group [$-CH(OH)CH_2-$]; and the ethylene group ($-CH_2CH_2-$).[17] The hydroxyethylene and ketomethylene compounds **8** and **9** had been prepared as inhibitors of pepsin.[18] When we began work on the preparation of aminopeptidase inhibitors with altered amide bonds, only one example of this approach had been

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(1) Abbreviations: APB, aminopeptidase B or arginyl aminopeptidase; APM, aminopeptidase M or membrane leucine aminopeptidase; Boc, *tert*-butyloxycarbonyl; Cbz, benzyloxycarbonyl; DMAP, (dimethylamino)pyridine; DMF, dimethylformamide; DTT, dithiothreitol; EDC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HMPA, hexamethylphosphoramide; HOBt, 1-hydroxybenzotriazole hydrate; IPA, isopropyl alcohol; LAP, leucine aminopeptidase; MOPS, 4-morpholinepropanesulfonic acid; MPLC, medium-pressure liquid chromatography; NMM, *N*-methylmorpholine; PDC, pyridinium dichromate; RP-HPLC, reversed-phase high-performance liquid chromatography; RT, retention time; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin-layer chromatography; Tris, tris(hydroxymethyl)aminomethane; TsOH, *p*-toluenesulfonic acid; UV, ultraviolet.

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