Potent, Low Molecular Weight Renin Inhibitors Containing a C-Terminal Heterocycle: Hydrogen Bonding at the Active Site[1,2]

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A series of low-nanomolar renin inhibitors containing novel C-terminal heterocycles has been designed by formally cyclizing the C-terminus of a glycol-based inhibitor to the second hydroxyl. Molecular modeling suggests that the heterocyclic oxygen hydrogen bonds as an acceptor to the flap region of renin and that the second hydroxyl in the glycol-based inhibitors behaves similarly.

Dipeptide glycols[3,4] and azidoglycols[5] are representatives of a new class of renin inhibitors which contain a novel *erythro*-1,2-diol functionality designed to mimic the tetrahedral transition state of the enzyme-mediated amide bond hydrolysis. These compounds demonstrate remarkable in vitro[3-5] and in vivo[6,7] potencies, and one has proven active in man.[8] Structure–activity investigations have delineated the optimum stereochemical[3] and regiochemical[5] relationships for the two hydroxyls; however, the exact nature of their interactions with the active site of the enzyme is not known. Molecular modeling studies[3] suggest that the first hydroxyl[9] acts as a donor in a hydrogen bond to unprotonated Asp 32 and as an acceptor in a hydrogen bond to Asp 215. The second hydroxyl hydrogen bonds to Asp 215 as a donor. In contrast, structure–activity relationships within the azidoglycol series suggested that the second hydroxyl was in fact acting as a hydrogen-bond acceptor.[5]

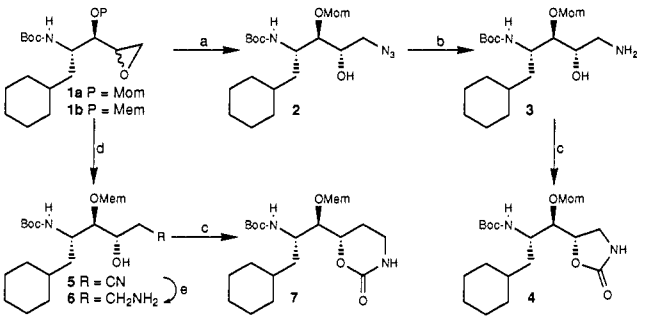
While examining a series of compounds related to the azidoglycols, we realized that these compounds could be used to probe the interactions of the second hydroxyl. Specifically, cyclizing the amine derived from the azide and the second hydroxyl through a carbonyl linkage would provide inhibitors in wich this oxygen could only participate as an acceptor. Herein are described the results of a structure–activity investigation that began within this novel cyclic structure and led to the development of a series of renin inhibitors containing substituted cyclic carbamates, ethers, and related groups, which maintains high potency against the target enzyme.

Results

Synthesis: Cyclic Carbamates and Related Structures. The cyclic carbamates unsubstituted at nitrogen (position 3) were prepared from epoxides **1a,b**[5] (2:1 2*S*/2*R*) as shown in Scheme I. Known protected azidoglycol **2**[5] was reduced with 1,3-propanedithiol[10,11] to amine **3** which was cyclized with phosgene to oxazolidinone **4**. Similarly, epoxide **1b** was opened with cyanide to nitrile **5**, which, after diastereomer separation, was reduced to amine **6**, the homologue of **3**. Cyclization with phosgene completed the synthesis of tetrahydrooxazinone **7**.

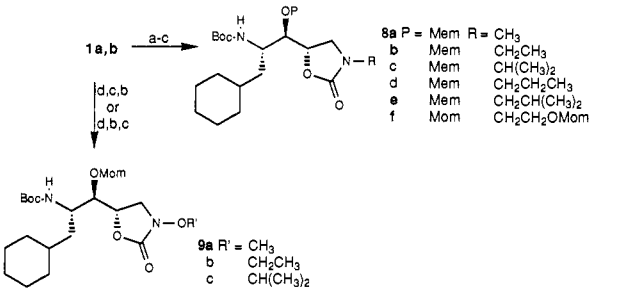
Oxazolidinones substituted at position 3 were also synthesized from epoxides **1a,b** (Scheme II). Reaction in neat primary alkyl amines followed by ring closure with phosgene and careful diastereomer separation produced oxazolidinones **8a–f** in moderate yields. Similarly, condensation of **1a** with primary alkoxyamines in 2-propanol and then ring closure either after or prior to diastereomer separation provided structures **9a–c**. An improved synthesis of oxazolidinone **8b** is shown in Scheme III. Epoxide **1b** was reacted with ethyl amine in aqueous meth-

Scheme I. Synthesis of Unsubstituted Cyclic Ureas[a]



[a] Key: (a) ref 5; (b) $HS(CH_2)_3SH$, TEA, CH_3OH; (c) ClCOCl, TEA, CH_2Cl_2; (d) KCN, CH_3OH, H_2O; (e) H_2, Raney Ni, NH_3, CH_3OH.

Scheme II. Synthesis of Substituted Oxazolidinones[a]



[a] Key: (a) RNH_2 (**8a–e**) or RNH_2, *i*-PrOH (**8f**); (b) ClCOCl, TEA, CH_2Cl_2; (c) isomer separation; (d) $R'ONH_3^+Cl^-$, Na_2CO_3, *i*-PrOH.

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(1) Presented in part at the 11th American Peptide Symposium, 1989.
(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: CDI, carbonyldiimidazole; DMF, dimethylformamide, DEAD, diethyl azodicarboxylate; LDA, lithium diisopropylamide; Mem, (methoxyethoxy)methyl; Mom, methoxymethyl, MPLC, medium-pressure liquid chromatography; Ms, methanesulfonyl; PPTS, pyridinium *p*-toluenesulfonate; TEA, triethylamine; THF, tetrahydrofuran; TMS, trimethylsilyl; Ts, *p*-tolylsulfonyl.
(3) Luly, J. R.; BaMaung, N.; Soderquist, J.; Fung, A. K. L.; Stein, H.; Kleinert, H. D.; Marcotte, P. A.; Egan, D. A.; Bopp, B.; Merits, I.; Bolis, G.; Greer, J.; Perun, T. J.; Plattner, J. J. *J. Med. Chem.* **1988**, *31*, 2264.
(4) Hanson, G. J.; Baran, J. S.; Lowrie, H. S.; Sarussi, S. J.; Yang, P.-C.; Babler, M.; Bittner, S. E.; Papaioannou, S. G.; Walsh, J. M. *Biochem. Biophys. Res. Commun.* **1987**, *146*, 959.

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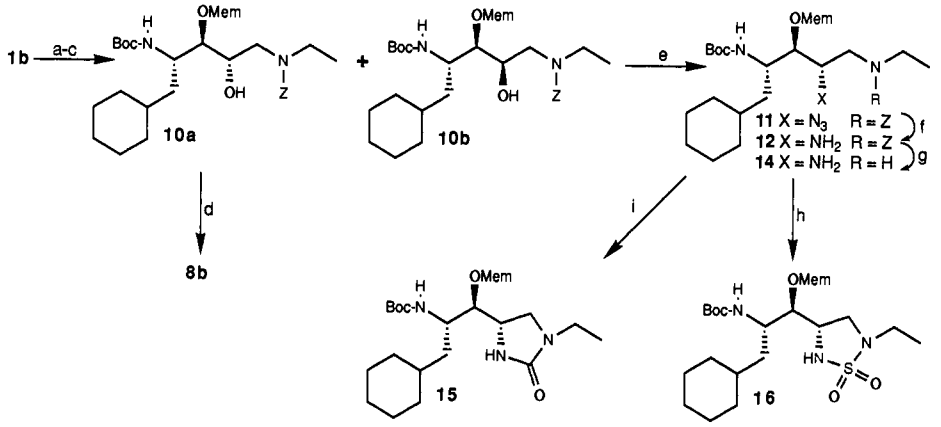
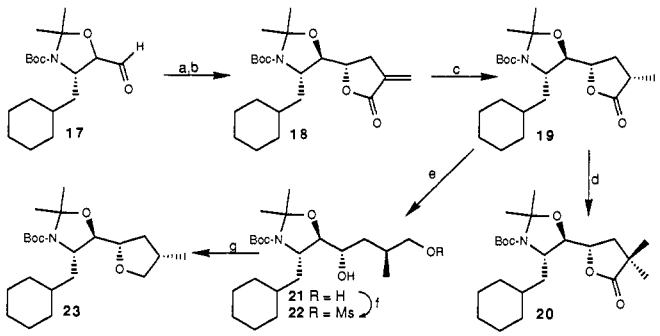
Scheme III. Synthesis of Structures Related to Oxazolidinone **8b** and Improved Synthesis of **8b**[a]



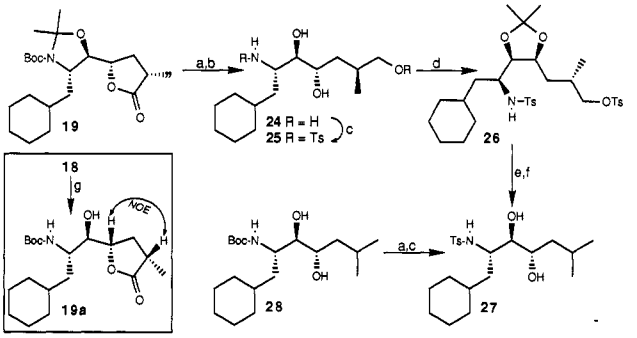
[a] Key: (a) $EtNH_2$, H_2O, CH_3OH; (b) Z-Cl, TEA, CH_2Cl_2; (c) isomer separation; (d) $NaN(TMS)_2$, THF; (e) HN_3, DEAD, $P(C_6H_5)_3$, THF; (f) $HS(CH_2)_3SH$, TEA, CH_3OH; (g) H_2, Pd/C, AcOH; (h) SO_2Cl_2, TEA, CH_2Cl_2; (i) CDI, TEA, CH_2Cl_2.

Scheme IV. Synthesis of Lactone and Tetrahydrofuran Structures[a]



[a] Key: (a) $BrCH_2C(CH_2)CO_2CH_3$, Zn, THF; (b) isomer separation; (c) H_2, Pd/C, EtOAc; (d) LDA, THF; CH_3I; (e) $NaBH_4$, THF; (f) CH_3SO_2Cl, TEA, CH_2Cl_2; (g) NaH, THF.

Scheme V. Stereochemistry of the Lactone and Tetrahydrofuran Structures[a]



[a] Key: (a) HCl, dioxane, ethanol; (b) $LiAlH_4$, THF; (c) TsCl, TEA, CH_2Cl_2; (d) acetone, PPTS, molecular sieves; (e) $LiEt_3BH$, THF; (f) TsOH, CH_3OH; (g) H_2, Pd/C, CH_3OH.

anol[12] and then with benzyl chloroformate. Chromatography afforded an excellent yield of the easily separable isomers **10a** and **10b**. Cyclization of **10a** under basic conditions provided **8b** in 47% overall yield from epoxide **1b**.

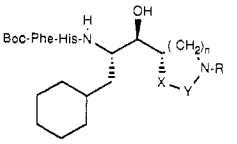
1-Aza analogues were prepared by displacing the hydroxyl of **10b** with azide under Mitsunobu conditions[13] to give **11**, which was reduced to amine **12** and then deprotected to provide diamine **14**. Reaction of **14** with CDI or sulfuryl chloride[14] produced imidazolidinone **15** and thiadiazolidinedioxide **16**, respectively.

Synthesis: Lactone and Tetrahydrofuran Structures. Acetonide-protected aldehyde **17** (8:1 5*R*/5*S*) was prepared as described for the corresponding leucine-derived product.[15] Condensation of this compound with methyl α-(bromomethyl)acrylate under Reformatskii conditions[16] produced α-methylenelactone **18** as the major product (Scheme IV). Catalytic hydrogenation to 3-(*S*)-methyl derivative **19** followed by carbonyl reduction gave diol **21**. The choice of solvent for the hydrogenation was critical. When methanol was employed, significant loss of the acetonide to give **19a** was observed (Scheme V). Selective activation of the primary hydroxyl and ring closure under basic conditions afforded tetrahydrofuran **23**. 3,3-Dimethyl analogue **20** was derived from **19** via enolization and alkylation.

The stereochemistry within the tetrahydrofuran–lactone series was determined by a combination of chemical transformations and spectroscopic analyses (Scheme V). Thus, an observed nuclear Overhauser enhancement between H_3 and H_5 in **19a** indicated that the two substituents were present in a cis relationship about the lactone ring.[17]

(5) Rosenberg, S. H.; Woods, K. W.; Kleinert, H. D.; Stein, H.; Nellans, H. N.; Hoffman, D. J.; Spanton, S. G.; Pyter, R. A.; Cohen, J.; Egan, D. A.; Plattner, J. J.; Perun, T. J. *J. Med. Chem.* **1989**, *32*, 1371.
(6) Kleinert, H. D.; Martin, D.; Chekel, M. A.; Kadam, J.; Luly, J. R.; Plattner, J. J.; Perun, T. J. *Hypertension* **1988**, *11*, 613.
(7) Kleinert, H. D.; Martin, D.; Chekel, M. A.; Young, G.; Rosenberg, S.; Plattner, J.; Perun, T. *J. Pharmacol. Exp. Ther.* **1988**, *246*, 975.
(8) Delabays, A.; Nussberger, J.; Porchet, M.; Waeber, B.; Danekas, L.; Boger, R.; Glassman, H.; Kleinert, H.; Luther, R.; Brunner, H. R. *Hypertension* **1988**, *12*, 360.
(9) The first hydroxyl refers to that hydroxyl which lies closest to the N-terminus and which occupies the same position as the single hydroxyl in statine containing inhibitors.
(10) Bayley, H.; Standring, D. N.; Knowles, J. R. *Tetrahedron Lett.* **1978**, 3633.
(11) Other reduction conditions (H_2, Pd/C; $NaBH_4$; $P(C_6H_5)_3$, NH_3) afforded mixtures of products.
(12) Hsiao, C.-N., Abbott Laboratories, unpublished results.
(13) Loibner, H.; Zbiral, E. *Helv. Chim. Acta* **1976**, *59*, 2100.
(14) The amino alcohol derived from **10a** could also be cyclized with sulfuryl chloride, however the resulting compound proved unstable toward acidic deprotection.
(15) Thaisrivongs, S.; Pals, D. T.; Kroll, L. T.; Turner, S. R.; Han, F.-S. *J. Med. Chem.* **1987**, *30*, 976.
(16) Pantaleo, N. S.; Satyamurthy, N.; Ramarajan, K.; O'Donnell, D. J.; Berlin, K. D.; van der Helm, D. *J. Org. Chem.* **1981**, *46*, 4284.

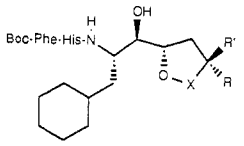
Table I. Renin Inhibitors Containing C-Terminal Oxazolidinones and Related Structures



no.	X	Y	n	R	IC50, nM purified[a]	IC50, nM plasma[b]
29	O	CO	1	H	4.7	25
30	O	CO	2	H	3.1	29
31	O	CO	1	CH_3	1.4	8.8
32	O	CO	1	CH_2CH_3	0.63	2.8
33[c]	O	CO	1	OCH_3	0.90	9.2
34[d]	O	CO	1	OCH_3	43	nd[e]
35	O	CO	1	$CH(CH_3)_2$	0.84	8.2
36	O	CO	1	$(CH_2)_2CH_3$	1.0	12
37	O	CO	1	OCH_2CH_3	1.3	17
38	O	CO	1	$(CH_2)_2OH$	0.70	7.4
39	O	CO	1	$CH_2CH(CH_3)_2$	3.0	21
40	O	CO	1	$OCH(CH_3)_2$	1.4	26
41	HN	CO	1	CH_2CH_3	1.2	11
42	HN	SO_2	1	CH_2CH_3	1.4	13

[a] Purified human renal renin, pH 6.0. [b] Human plasma renin, pH 7.4. [c] 5*S* isomer. [d] 5*R* isomer. [e] Not determined.

Table II. Renin Inhibitors Containing C-Terminal Tetrahydrofuran and Lactone Fragments



no.	X	R	R'	IC50, nM purified[a]	IC50, nM plasma[b]
43	CO	$=CH_2$		0.71	21
44	CO	CH_3	H	0.32	2.3
45	CO	CH_3	CH_3	0.89	2.8
46	CH_2	CH_3	H	1.2	3.0

[a] Purified human renal renin, pH 6.0. [b] Human plasma renin, pH 7.4.

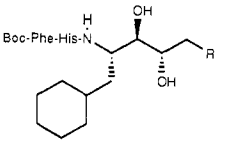
The absolute stereochemistry at C_3 (and hence at C_5) was established through a correlation between lactone **19** and known amine diol **28**.[18] Deprotection and carbonyl reduction gave free amino triol **24**, which was selectively tosylated on nitrogen and the primary hydroxyl (**25**). Protection of the remaining hydroxyls, displacement of the tosylate with hydride, and subsequent deprotection afforded tosylamide diol **27**, which was identical in all respects with material prepared from **28**.

Synthesis: Extension at the N-Terminus. Elaboration of the various fragments into renin inhibitors involved acidic deprotection followed by carbodiimide-mediated coupling of Boc-Phe-His-OH to the N-termini as previously described.[5] Final peptides were purified by silica gel chromatography.

Discussion

In Vitro Activities. The structures and inhibitory potencies of the compounds described in this paper are shown in Tables I–III. Unsubstituted oxazolidinone **29** (Table I) was significantly less active against purified human renal renin than the parent azidoglycol **47**[5] (Table III), while the difference in potencies against human plasma renin was not as great. Oxazolidinone **29**, however, lacks the lipophilic C-terminal group that is present as the azide in **47**. Thus the direct acyclic analogue of **29**, formamidoglycol **48** (Table III), has essentially the same activity as **29** in both renin assays. This result suggested that one or both of the oxazolidinone oxygens was contributing to binding to the same extent as the second hydroxyl in the glycol-containing renin inhibitors.

Table III. Related Acyclic Renin Inhibitors



no.	R	IC50, nM purified[a]	IC50, nM plasma[b]
47[c]	N_3	0.40	9.0
48[d]	NHCHO	8.0	63
49[e]	$CH(CH_3)_2$	1.5	nd[f]

[a] Purified human renal renin, pH 6.0. [b] Human plasma renin, pH 7.4. [c] See ref 5. [d] See ref 33. [e] See ref 3. [f] Not determined.

Since it appeared that a lipophilic C-terminal substituent was necessary for optimum binding, the series of *N*-alkyl and *N*-alkoxy oxazolidinones (**31–40**, Table I) was evaluated. Within the *N*-alkyl series (**31**, **32**, **35**, **36**, **39**) *N*-ethyl derivative **32** was the most potent against plasma renin while the unsubstituted (**29**) and largest (**39**) members were 7–10-fold less active. A similar, although less pronounced, trend was observed for the purified renin assay. In general, changes in activity were greater in the more physiologically relevant plasma renin assay, and unless otherwise mentioned it is this assay that is discussed. The *N*-alkoxyoxazolidinones (**33**, **37**, **40**) were equipotent with their isosteric *N*-alkyl counterparts with the exception of *N*-methoxyl compound **33**, which was 3-fold less potent than **32**. *N*-hydroxyethyl analogue **38** was only slightly less active than **32** and provides a site for potential prodrug attachment. These results indicate the presence of a small but nonspecific pocket which accepts the C-terminal group.

The oxazolidinone-ring oxygens in the compounds described possess the same absolute configuration as the hydroxyl in azidoglycol **47**. That this configuration is also superior for the present series is demonstrated by the 5*R* isomer of the *N*-methoxyoxazolidinone (**34**) which is 50-fold less potent in the purified renin assay than **33**, the corresponding 5*S* isomer (a compound containing a basic nitrogen in the same position as the oxazolidinone-ring oxygen but bonded to an sp^2 center has been described[19] and has essentially the same activity as the inhibitors reported herein). The fact that the same configuration is optimal in both the acyclic and cyclic series suggests that one or both of the oxazolidinone oxygens is binding in the same fashion as the second hydroxyl in **47**.

Further structure–activity examination within the cyclic carbamate series revealed that the ring oxygen could be replaced by nitrogen with only a moderate loss in potency (compare **32** and **41**) and that a sulfonyl group could replace the carbonyl without loss in activity (**41**, **42**). Finally, one six-membered cyclic carbamate was prepared (**30**) and

(17) The proton NMR spectrum of **19** was not sufficiently well resolved to perform the NOE analysis.

(18) Luly, J. R.; Hsiao, C.-N.; BaMaung, N.; Plattner, J. J. *J. Org. Chem.* **1988**, *53*, 6109.

(19) DeForrest, J. M.; Waldron, T. L.; Oehl, R. S.; Scalese, R. J.; Free, C. A.; Weller, H. N.; Ryono, D. E. *J. Hypertens.* **1989**, *7* (suppl. 2), S15.

shown to be equipotent to its oxazolidinone counterpart (29).

Lactone 44 is an analogue of oxazolidinone 31 in which the nitrogen has been replaced by carbon (Table II). The lower potency of 31 (compared to 44) may be due to the partial double bond character of the oxazolidinone nitrogen since a full sp^2 center at this position results in significantly lower activity (43).

The compounds described above all contain a carbonyl at the 2-position of the heterocycle, hence it was unclear whether it was this carbonyl or the ring heteroatom that was responsible for the enhanced binding. Tetrahydrofuran 46 and lactone 44 have identical potency, demonstrating that the carbonyl is not necessary for activity against renin. Furthermore, 46 is equipotent with its direct acyclic analogue dipeptide glycol 49,[3] a compound in which the importance of the second oxygen has been well-established.

Molecular modeling studies suggested that a 3(*R*)-methyl group might contribute more to binding than the 3(*S*)-methyl group present in 44 and 46 (Figure 2, vida infra). *gem*-Dimethyl lactone 45 was prepared and was found to be equipotent to monomehyl compound 44, further demonstrating the nonspecific nature of the C-terminal binding region.

Enzyme Specificity. Tetrahydrofuran 46 and *N*-ethyl- and *N*-methoxyoxazolidinones 32 and 33 were tested for activity against bovine cathespin D and porcine pepsin (Table IV). All exhibited IC_{50}'s greater than 10 000 nM as was observed for acyclic glycols 47 and 49; however, 32 did show measurable activity with cathepsin D.

Molecular Modeling. Comparative modeling methods[20] were used to build a new model of human renin. The crystal structures of the fungal enzymes penicillopepsin,[21] rhizopuspepsin,[22] and endothiapepsin[23] and of the mammalian enzyme porcine pepsin[24] were superimposed, and the sequence of human renin was aligned with these aspartic proteinases. The residues of the structurally conserved regions were taken from the pepsin structure, while the residues of the variable regions were chosen from the structure that best fit the human renin sequence in that region. The residues of the model were mutated to the sequence of human renin and the side-chain torsion angles were altered interactively to relieve bad contacts. Inhibitor 32 was modeled in the active site of the renin model with the coordinates of the inhibitor in rhizopuspepsin.

The full model was then minimized with the program DISCOVER,[25] first tethering the main chain atoms to their initial positions in order to adjust the hydrogen atoms and relieve any major problems arising from the building procedure. The tethering was gradually removed during subsequent minimizations. The full model of the complex was subjected to molecular dynamics for 5 ps and then minimized to give a structure that was used as the initial structure for models of other renin:inhibitor complexes (Figure 1). This model is different from earlier models[26] since the current model is based on a recent high-resolution crystal structure of porcine pepsin.[24] This model is also in good agreement with a partially refined crystal structure of a porcine pepsin:inhibitor complex.[27] The common residues of the inhibitors 32, 46, 47, and 49 were superimposed, and the complex of each inhibitor in the renin model was subjected to energy minimization, molecular dynamics, and further energy minimization.

The results of the modeling show that the P_3–P_1 residues remain superimposed (Figure 2). The conformation of the C-terminal residue varies to achieve the best fit. The hydroxyl of the P_1 residue is bound between the active-site aspartic acids, accepting a hydrogen bond from protonated Asp 215 and donating a hydrogen bond to Asp 32. In the glycol inhibitors 47 and 49, the second hydroxyl accepts a hydrogen bond from the amide hydrogen of Ser 76. Occasionally the side-chain hydroxyl group of Ser 76 forms a hydrogen bond with the second hydroxyl of the inhibitors as well. The amide hydrogen of Ser 76 also forms a hydrogen bond with the ring oxygen of oxazolidinone 32 and with the oxygen of tetrahydrofuran 46. The *N*-alkyl group of the oxazolidinones lies in a pocket formed by the side chains of Val 189, Leu 213, Leu 291, and Pro 292. The ethyl group of 32 appears to optimally fit this pocket, which does not completely accommodate the larger groups. Residues with the opposite stereochemistry at the second hydroxyl or at the ring carbon atom could not simultaneously hydrogen bond with the flap residues and direct the alkyl group into the lipophilic pocket. Thus the important characteristics of these C-terminal residues are a hydrogen-bond-acceptor atom appropriately placed to in-

Table IV. Enzyme Specificity

no.[b]	% inhibition[a] cathepsin D[c]	pepsin[d]
32	21	0
33	0	0
46	9	2
47	0	0
49[e]	0	0

[a] At 1×10^{-5} M. [b] See Tables I–III for structures. [c] Bovine, pH 3.0. [d] Porcine, pH 2.0. [e] See ref 3.

Table V. Physical Data for Renin-Inhibiting Compounds

no.[a]	formula[b]	TLC, R_f[c]
29	$C_{32}H_{46}N_6O_7 \cdot 1.1H_2O$[d]	0.23
30	$C_{33}H_{48}N_6O_7$	0.11
31	$C_{33}H_{48}N_6O_7 \cdot 0.45H_2O$[e]	0.46
32	$C_{34}H_{50}N_6O_7 \cdot 0.75H_2O$	0.49
33	$C_{33}H_{48}N_6O_8 \cdot 1.5H_2O$	0.38[f]
34	$C_{33}H_{48}N_6O_8 \cdot 0.5H_2O$	0.33[f]
35	$C_{35}H_{52}N_9O_7 \cdot 0.5H_2O$	0.51
36	$C_{35}H_{52}N_6O_7 \cdot 0.5H_2O$	0.50
37	$C_{34}H_{50}N_6O_8 \cdot 1.25H_2O$	0.45
38	$C_{34}H_{50}N_6O_8 \cdot 1.75H_2O$	0.33
39	$C_{36}H_{54}N_6O_7 \cdot 0.5H_2O$	0.53
40	$C_{35}H_{52}N_6O_8 \cdot 0.25H_2O$	0.49
41	$C_{34}H_{51}N_7O_6 \cdot 1.0H_2O$	0.42
42	$C_{33}H_{51}N_7O_7S \cdot 0.5H_2O$	0.33
43	$C_{34}H_{47}N_5O_7 \cdot 1.5H_2O$	0.52
44	$C_{34}H_{49}N_5O_7 \cdot 1.75H_2O$[g]	0.53
45	$C_{35}H_{51}N_5O_7 \cdot H_2O$	0.49
46	$C_{34}H_{51}N_5O_6 \cdot 1.8H_2O$	0.57
48	$C_{32}H_{48}N_6O_7 \cdot 0.5H_2O$	0.33

[a] See Tables I–III for structures. [b] Analyses for C, H, N were ±0.4% of the expected values (for formulae shown) unless otherwise noted. [c] 15% methanol/85% chloroform. [d] N: calcd, 13.00; found 12.01. Exact mass calcd for $C_{32}H_{47}N_6O_7$ (M + H) 627.3506, found 627.3488. [e] N: calcd, 12.95; found 12.44. Exact mass calcd for $C_{33}H_{49}N_6O_7$ (M + H) 641.3663, found 641.3666. [f] 10% methanol/90% chloroform. [g] H: calcd, 7.88; found, 7.42.

(20) (a) Greer, J. *J. Mol. Biol.* **1981**, *153*, 1027. (b) Greer, J. *J. Mol. Biol.* **1981**, *153*, 1043. (c) Greer, J. *Science* **1985**, *228*, 1055.
(21) James, M. N. G.; Sielecki, A. R. *J. Mol. Biol.* **1983**, *163*, 299.
(22) Bott, R.; Subramanian, E.; Davies, D. R. *Biochemistry* **1982**, *21*, 6956.
(23) Pearl, L.; Blundell, T. *FEBS Lett.* **1984**, *174*, 96.
(24) Abad-Zapatero, C.; Andreeva, N. S.; Rydel, T.; Erickson, J., Unpublished results.
(25) Biosym Technologies, Inc.
(26) Sham, H. L.; Bolis, G.; Stein, H. H.; Fesik, S. W.; Marcotte, P. A.; Plattner, J. J.; Rempel, C. A.; Greer, J. *J. Med. Chem.* **1988**, *31*, 284.
(27) Abad-Zapatero, C.; Erickson, J., unpublished results.

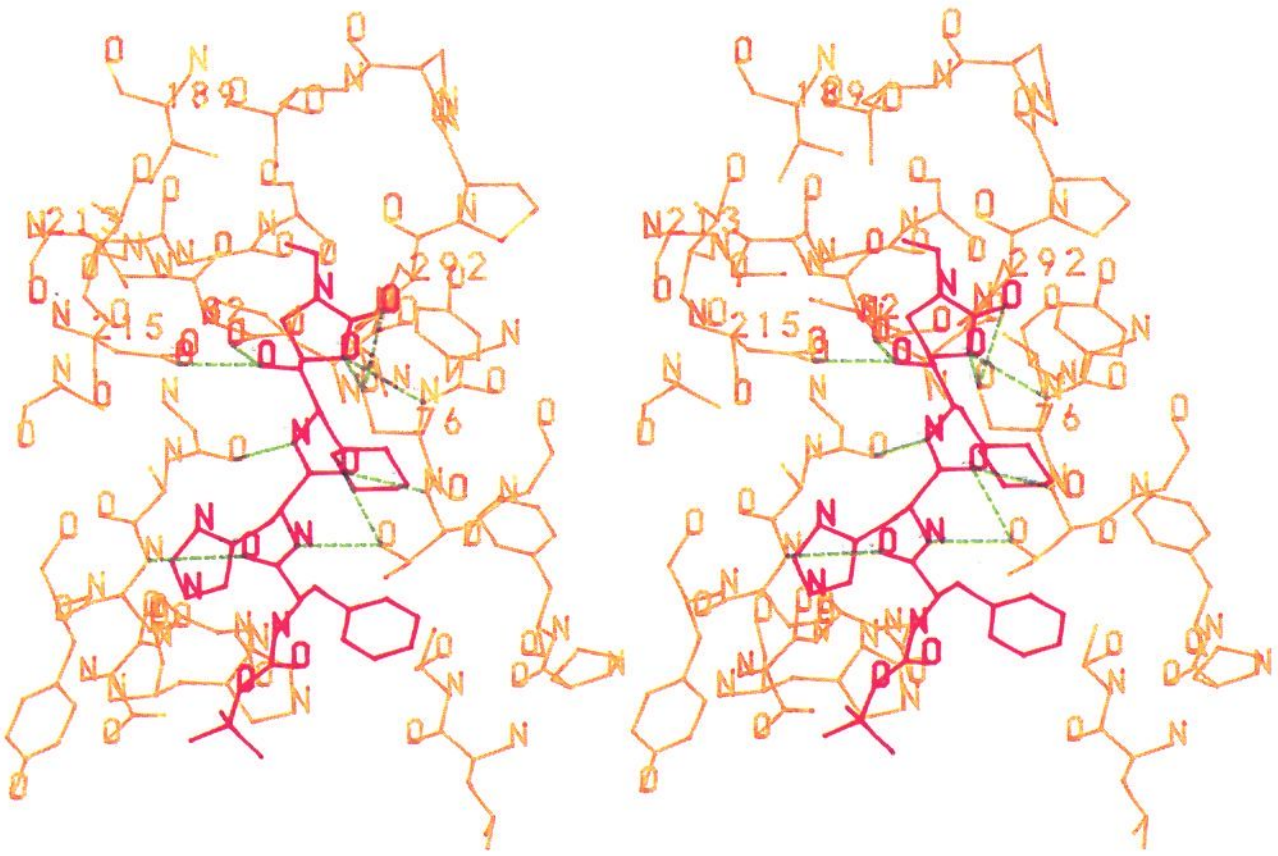


Figure 1. Model of oxazolidinone **32** (red) complexed with renin showing neighboring residues (orange) and hydrogen bonds (green).

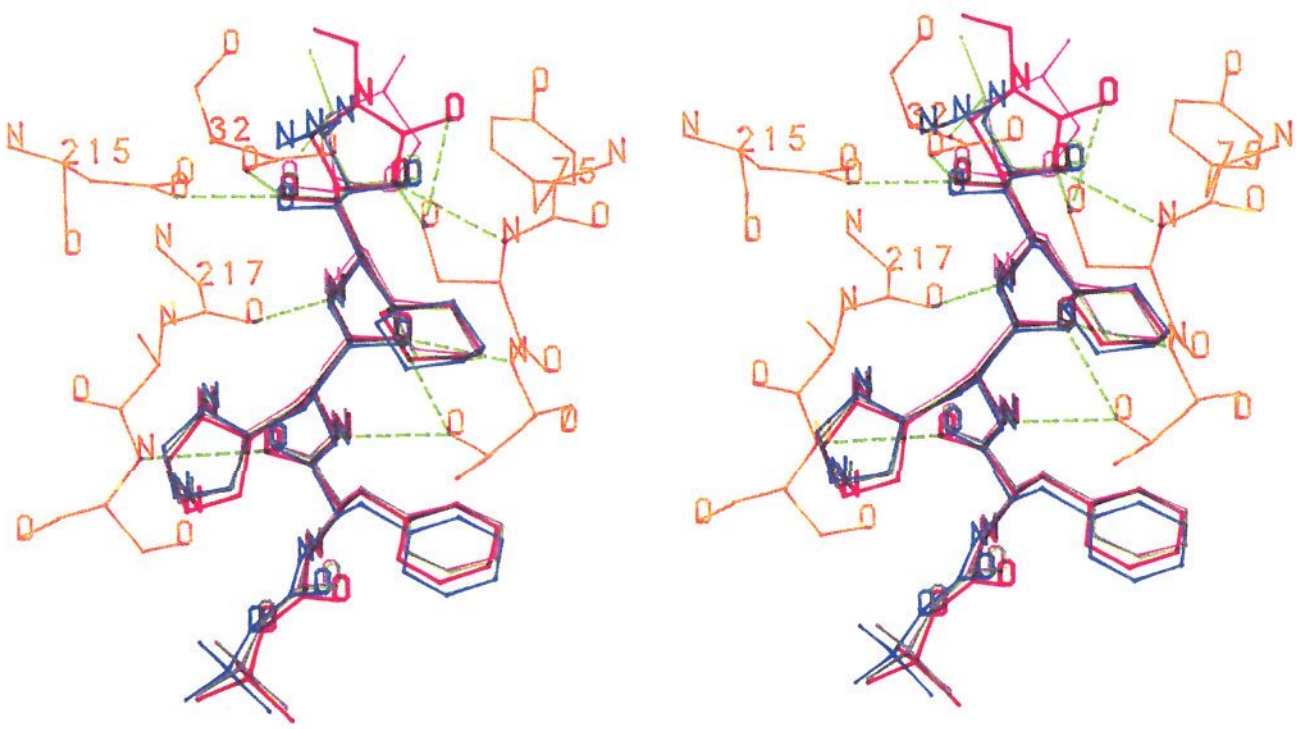


Figure 2. Model of inhibitors **32** (red), **46** (violet), **47** (blue), and **49** (green) complexed with renin (orange) showing hydrogen bonds (dashed green).

teract with the amide hydrogen of Ser 76 and a small lipophilic group able to interact with the lipophilic pocket.

In conclusion we have developed a series of small, nanomolar renin inhibitors based upon novel C-terminal heterocycles. Structure–activity relationships and computer-modeling studies suggest a hydrogen-bond-accepting role for the heterocyclic-ring oxygen and hence for the second hydroxyl in glycol-based renin inhibitors. Studies are currently underway to explore the in vivo and physicochemical characteristics of these novel renin inhibitors.

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 (unless otherwise noted) 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), eluting with 5–10 psi air pressure. Thin-layer chromatography was done on silica gel plates (E. Merck) and components were visualized with ninhydrin or phosphomolybdic acid reagents. The following solvent systems were used: 25% acetic acid/25% 1-butanol/25% ethyl acetate/25% water (I), 50% ethyl acetate/50% hexane (II), 100% ethyl acetate (III), 95% chloroform/5% methanol (IV), 90% chloroform/10% methanol (V), 20% ethyl acetate/80% hexane (VI), 40% ethyl acetate/60% hexane (VII), 10% ethyl acetate/90% hexane (VIII), 33% ethyl acetate/67% chloroform (IX), 33% ethyl acetate/67% hexane (X), 50% ethyl acetate/50% chloroform (XI), 20% ethyl acetate/80% chloroform (XII).

(2*S*,3*R*,4*S*)-1-Amino-4-[[(*tert*-butyloxy)carbonyl]-amino]-5-cyclohexyl-2-hydroxy-3-(methoxymethoxy)pentane (3). Azide **2**[5] (1.640 g, 4.24 mmol) in methanol (22 mL) was treated with TEA (1.80 mL, 12.8 mmol) and 1,3-popanedithiol (1.30 mL, 12.9 mmol). After 48 h at ambient temperature the milky suspension was poured into 0.5 M H_3PO_4 (150 mL) and washed with ether (4×, insoluble polysulfides were removed with the organic layers). The aqueous phase was made basic with K_2CO_3 and extracted with ethyl acetate which was dried and evaporated to

afford 1.380 g (90%) of a white solid: mp 97–98 °C; TLC R_f = 0.63 (I). Anal. ($C_{18}H_{36}N_2O_5$) C, H, N.

(1′*R*,2′*S*,5*S*)-5-[2′-[[(*tert*-Butyloxy)carbonyl]amino]-3′-cyclohexyl-1′-(methoxymethoxy)propyl]oxazolidin-2-one (4). Amine **3** (151.0 mg, 0.419 mmol) in dichloromethane (5 mL) at 0 °C was treated with TEA (0.180 mL, 1.28 mmol) and phosgene in toluene (0.52 mL, 0.59 mmol, 12.5% solution). After 1 h at 0 °C and 1 h at ambient temperature the mixture was evaporated. The residue was taken up in ethyl acetate which was washed with 0.5 M H_3PO_4, saturated $NaHCO_3$ solution, and brine and then was dried and evaporated to afford 161 mg (100%) of a colorless oil: TLC R_f = 0.10 (II); ^{1}H NMR ($CDCl_3$) δ 4.90 (1 H, s), 4.81–4.66 (1 H, m), 4.78 (1 H, d), 4.72 (1 H, d), 4.56 (1 H, d), 3.98–3.82 (2 H, m), 3.73 (1 H, dd), 3.52 (1 H, dd), 3.40 (3 H, s), 1.43 (9 H, s).

(3*S*,4*R*,5*S*)-5-[[(*tert*-Butyloxy)carbonyl]amino]-6-cyclohexyl-3-hydroxy-4-[(methoxyethoxy)methoxy]hexanenitrile (5). To epoxide **1b**[5] (627.0 mg, 1.62 mmol) in methanol (9 mL) was added KCN (320 mg, 4.91 mmol) in water (3 mL). After 32 h the mixture was concentrated, poured into ethyl acetate which was washed with water and brine, and then was dried and evaporated. Chromatography of the residue with 2:1 hexane/ethyl acetate afforded 208.8 mg (31%) of the desired product as a white solid followed by 149.9 mg (22%) of the 3*R* isomer as an oil.

3*S* isomer **5**: mp 69–71 °C; TLC R_f = 0.35 (II). Anal. ($C_{21}H_{38}N_2O_6$) C, H, N.

3*R* isomer: TLC R_f = 0.27 (II); ^{1}H NMR ($CDCl_3$) δ 4.92 (1 H, d), 4.75 (1 H, d), 4.69 (1 H, d), 3.40 (3 H, s), 2.80 (2 H, d), 1.45 (9 H, s).

(3*S*,4*R*,5*S*)-1-Amino-5-[[(*tert*-butyloxy)carbonyl]amino]-6-cyclohexyl-3-hydroxy-4-[(methoxyethoxy)methoxy]hexane (6). Nitrile **5** (197.0 mg, 0.475 mmol) and Raney Ni (200 mg) in methanol (50 mL) and ammonia (10 mL) were shaken under 4 atm of H_2 for 18 h. The mixture was evaporated, taken up in ethyl acetate, filtered, and evaporated to provide 240 mg of a green oil which was used without further purification: TLC R_f = 0.73 (I); ^{1}H NMR ($CDCl_3$) δ 4.98 (1 H, d), 4.80 (1 H, d), 4.75 (1 H, d), 4.10–4.00 (1 H, m), 3.76–3.63 (3 H, m), 3.58–3.53 (2 H, m), 3.39 (3 H, s), 3.07–2.85 (2 H, m), 1.43 (9 H, s).

(1′*R*,2′*S*,6*S*)-6-[2′-[[(*tert*-Butyloxy)carbonyl]amino]-3′-cyclohexyl-1′-[(methoxyethoxy)methoxy]propyl]tetrahydro-1,3-oxazin-2-one (7). This compound was prepared in 86% yield from amine **6** as described for oxazolidinone **4**: TLC R_f = 0.10 (III); ^{1}H NMR ($CDCl_3$) δ 5.08 (1 H, br s), 4.90 (1 H, d), 4.74 (2 H, s), 4.40–4.32 (1 H, m), 4.00–3.88 (1 H, m), 3.79–3.69 (3 H, m), 3.56 (2 H, dd), 3.39 (3 H, s), 1.43 (9 H, s).

(1′*R*,2′*S*,5*S*)-5-[2′-[[(*tert*-Butyloxy)carbonyl]amino]-3′-cyclohexyl-1′-[(methoxyethoxy)methoxy]propyl]-3-methyloxazolidin-2-one (8a). Epoxide **1b** (308 mg, 0.795 mmol) was stirred with $MeNH_2$ (5 mL) in a sealed tube for 18 h. The solvent was evaporated and the residue was dissolved in dichloromethane (10 mL), cooled to 0 °C, and treated with triethylamine (0.34 mL, 2.4 mmol) and phosgene in toluene (1.0 mL, 1.1 mmol, 12.5% solution). After 1 h the mixture was evaporated and taken up in ethyl acetate which was washed with 0.5 M H_3PO_4, saturated $NaHCO_3$ solution, and brine and then was evaporated. Chromatography of the residue with 3:2 ethyl acetate/hexane afforded 35.2 mg (10%) of the 5*R* isomer followed by 107.4 mg (31%) of the desired 5*S* isomer, both as oils.

5*S* isomer **8a**: TLC R_f = 0.41 (III); ^{1}H NMR ($CDCl_3$) δ 4.84 (1 H, d), 4.80 (1 H, d), 4.69 (1 H, d), 4.58 (1 H, ddd), 3.95–3.82 (1 H, m), 3.83–3.60 (4 H, m), 3.56 (2 H, dd), 3.46 (1 H, dd), 3.39 (3 H, s), 2.86 (3 H, s), 1.43 (9 H, s).

5*R* isomer: TLC R_f = 0.46 (III); ^{1}H NMR ($CDCl_3$) δ 4.98 (1 H, d), 4.82 (1 H, d), 4.71 (1 H, d), 4.48 (1 H, dd), 3.84–3.68 (4 H, m), 3.63–3.52 (3 H, m), 3.38 (3 H, s), 3.23 (1 H, dd), 2.87 (3 H, s), 1.44 (9 H, s).

(1′*R*,2′*S*,5*S*)-5-[2′-[[(*tert*-Butyloxy)carbonyl]amino]-3′-cyclohexyl-1′-[(methoxyethoxy)methoxy]propyl]-3-ethyloxazolidin-2-one (8b) was prepared as described for **8a** and chromatographed with 1:1 ethyl acetate/hexane to afford 4% of the 5*R* isomer [TLC R_f = 0.57 (III), R_f = 0.15 (II)] followed by 17% of the desired 5*S* isomer (along with mixed fractions), both as oils: TLC R_f = 0.53 (III), R_f = 0.13 (II); ^{1}H NMR ($CDCl_3$) δ 4.84 (1 H, d), 4.80 (1 H, d), 4.70 (1 H, d), 4.57 (1 H, ddd), 3.95–3.83 (1 H, m), 3.83–3.60 (4 H, m), 3.55 (2 H, dd), 3.46 (1 H, dd), 3.39 (3 H, s), 3.40–3.20 (2 H, m), 1.43 (9 H, s), 1.14 (3 H, t); $[\alpha]^{23}_D$ −39.4° (*c* 1.06, EtOH). Anal. ($C_{23}H_{42}N_2O_7$) C, H, N.

(1′*R*,2′*S*,5*S*)-5-[2′-[[(*tert*-Butyloxy)carbonyl]amino]-3′-cyclohexyl-1′-[(methoxyethoxy)methoxy]propyl]-3-(2-propyl)oxazolidin-2-one (8c) was pepared as described for **8a** (168 h amine reaction) and chromatographed with 3:2 hexane/ethyl acetate to afford the 5*R* isomer [TLC R_f = 0.24 (II)] followed by 22% of the desired 5*S* isomer as an oil: TLC R_f = 0.21 (II); ^{1}H NMR ($CDCl_3$) δ 4.83 (1 H, d), 4.79 (1 H, d), 4.72 (1 H, d), 4.56 (1 H, ddd), 4.08 (1 H, septet), 3.95–3.83 (1 H, m), 3.82–3.66 (4 H, m), 3.64–3.52 (3 H, m), 3.38 (3 H, s), 1.43 (9 H, s), 1.16 (6 H, d).

(1′*R*,2′*S*,5*S*)-5-[2′-[[(*tert*-Butyloxy)carbonyl]amino]-3′-cyclohexyl-1′-[(methoxyethoxy)methoxy]propyl]-3-propyloxazolidin-2-one (8d) was prepared as described for **8a** (112 h amine reaction) and chromatographed with 3:2 hexane/ethyl acetate to afford the 5*R* isomer [TLC R_f = 0.26 (II)] followed by 39% of the desired 5*S* isomer as an oil: TLC R_f = 0.21 (II); ^{1}H NMR ($CDCl_3$) δ 4.84 (1 H, d), 4.80 (1 H, d), 4.71 (1 H, d), 4.58 (1 H, ddd), 3.95–3.83 (1 H, m), 3.83–3.59 (4 H, m), 3.55 (2 H, dd), 3.45 (1 H, dd), 3.39 (3 H, s), 3.31–3.09 (2 H, m), 1.43 (9 H, s), 0.93 (3 H, t).

(1′*R*,2′*S*,5*S*)-5-[2′-[[(*tert*-Butyloxy)carbonyl]amino]-3′-cyclohexyl-1′-[(methoxyethoxy)methoxy]propyl]-3-(2-methylpropyl)oxazolidin-2-one (8e) was prepared as described for **8a** (112 h amine reaction) and chromatographed with 2:1 hexane/ethyl acetate to afford the 5*R* isomer [TLC R_f = 0.32 (II)] followed by 32% of the desired 5*S* isomer: TLC R_f = 0.28 (II); ^{1}H NMR ($CDCl_3$) δ 4.85 (1 H, d), 4.80 (1 H, d), 4.72 (1 H, d), 4.58 (1 H, ddd), 3.93–3.85 (1 H, m), 3.85–3.60 (4 H, m), 3.55 (2 H, dd), 3.45 (1 H, dd), 3.39 (3 H, s), 3.08 (1 H, dd), 2.97 (1 H, dd), 1.43 (9 H, s), 0.92 (6 H, d).

8b from 10a. To compound **10a** (127.0 mg, 0.224 mmol) in THF (4 mL) at 0 °C was added $NaN(TMS)_2$ in THF (0.38 mL, 0.38 mmol, 1.0 M). After 2 h at ambient temperature the mixture was poured into brine and extracted into ethyl acetate which was dried and evaporated. Chromatography with 1:1 ethyl acetate/hexane afforded 95.8 mg (93%) of a colorless oil identical in all respects with the material prepared from **1b**.

1-[[(Benzyloxy)carbonyl]amino]-2-(methoxymethoxy)ethane. To 2-[[(benzyloxy)carbonyl]amino]ethanol[28] (12.90 g, 66.1 mmol) in dichloromethane (100 mL) was added diisopropylethylamine (24 mL, 140 mmol) and chloromethyl methyl ether (10.0 mL, 130 mmol). After 4 h the mixture was evaporated, taken up in ethyl acetate, washed with 0.5 M H_3PO_4, saturated $NaHCO_3$ solution, and brine, and then dried and evaporated to afford 15.27 g (97%) of a colorless oil: TLC R_f = 0.35 (II); ^{1}H NMR ($CDCl_3$) δ 7.40–7.30 (5 H, m), 5.15 (1 H, br s), 5.11 (2 H, s), 4.62 (2 H, s), 3.62 (2 H, t), 3.46–3.38 (2 H, m), 3.35 (3 H, s).

1-Amino-2-(methoxymethoxy)ethane. 1-[[(Benzyloxy)carbonyl]amino]-2-(methoxymethoxy)ethane (7.60 g, 31.2 mmol) and 10% Pd/C (3.0 g) in methanol (60 mL) were stirred under a H_2 atmosphere for 34 h. The mixture was filtered, evaporated, and distilled to afford 2.02 g (60%) of a colorless liquid; bp 60–70 °C (45 mm); ^{1}H NMR ($CDCl_3$) δ 4.63 (2 H, s), 3.55 (2 H, t), 3.37 (3 H, s), 2.88 (2 H, t).

(1′*R*,2′*S*,5*S*)-5-[2′-[[(*tert*-Butyloxy)carbonyl]amino]-3′-cyclohexyl-1′-(methoxymethoxy)propyl]-3-[2-(methoxymethoxy)ethyl]oxazolidin-2-one (8f). Epoxide **1a** (555 mg, 1.62 mmol) and 1-amino-2-(methoxymethoxy)ethane (510 mg, 4.85 mmol) in 2-propanol (10 mL) were heated at 60–70 °C for 14 h. The solvent was evaporated and the residue was dissolved in dichloromethane, cooled to 0 °C, and treated with TEA (1.10 mL, 7.85 mmol) and phosgene in toluene (2.00 mL, 2.2 mmol, 12.5% solution). After 30 min at 0 °C and 1 h at room temperature the product was isolated as described for **8a** and was chromatographed with 11:9 hexane/ethyl acetate to afford the 5*R* isomer [TLC R_f = 0.21 (II)] followed by 190 mg (25%) of the desired 5*S* isomer as an oil: TLC R_f = 0.16 (II); ^{1}H NMR ($CDCl_3$) δ 4.76 (1 H, d), 4.69 (1 H, d), 4.62 (2 H, s), 3.39 (3 H, s), 3.36 (3 H, s), 1.45 (9 H, s).

(2*S*,3*R*,4*S*)-4-[[(*tert*-Butyloxy)carbonyl]amino]-5-cyclohexyl-2-hydroxy-1-(methoxyamino)-3-(methoxymethoxy)-

(28) Berntsson, P.; Brandstrom, A.; Junggren, U.; Palmer, L.; Sjostrand, S. E.; Sundell, G. *Acta Pharm. Suec.* **1977**, *14*, 229.

pentane and (2*R*,3*R*,4*S*)-4-[[(*tert*-Butyloxy)carbonyl]amino]-5-cyclohexyl-2-hydroxy-1-(methoxyamino)-3-(methoxymethoxy)pentane. Epoxide **1a** (4.01 g, 11.7 mmol), *O*-methylhydroxylamine hydrochloride (2.93 g, 35.0 mmol), and sodium carbonate (3.71 g, 35.0 mmol) were dissolved in 2-propanol (40 mL) and water (5 mL) and heated in a resealable tube at 115 °C until the reaction was judged complete by TLC (typically 6–10 h). The volatiles were removed under vacuum, and the resulting slurry was stirred with 50% ethyl acetate in chloroform to give a suspension which was filtered. The filtrate was concentrated and the resulting residue was chromatographed (MPLC) by eluting with a mixture of ethyl acetate in chloroform to provide the more mobile, major 2*S* isomer (1.51 g, 33%), mixed fractions (0.35 g, 8%), and the less mobile, minor 2*R* isomer (0.75 g, 16%). The major isomer crystallized upon standing for a few hours, while the minor isomer became partially crystalline after 3 months. Recrystallization of the 2*S* isomer from ether/hexanes provided an analytical sample.

2*S* isomer: mp 68.5–69.5 °C; TLC R_f = 0.45 (XI); ^{1}H NMR ($CDCl_3$) δ 6.06 (1 H, br s), 4.65–4.7 (3 H, m), 4.28 (1 H, d, *J* = 4.5 Hz), 4.07 (1 H, dt, *J* = 4.5, 9.5, 9.5 Hz), 3.69 (1 H, dddd, *J* = 9, 9, 3.5, 3.5 Hz), 3.52 (3 H, s), 3.41 (3 H, s), 3.27–3.33 (2 H, m), 2.37 (1 H, dd, *J* = 9, 13 Hz), 1.6–1.85 (7 H, m), 1.45 (9 H, s), 1.10–1.43 (4 H, m), 0.78–1.03 (2 H, m); MS (DEI) $(M + H)^+$ = 491. Anal. ($C_{19}H_{38}N_2O_6 \cdot 0.25H_2O$) C, H, N.

2*R* isomer: TLC R_f = 0.32 (XI); ^{1}H NMR ($CDCl_3$) δ 4.8 (1 H, *AB*, *J* = 6.5 Hz), ca. 4.65–4.70 (1 H, m), 4.67 (1 H, A*B*, *J* = 6.5 Hz), 3.97 (1 H, br dt, *J* = 4.5, 9, 9 Hz), 3.83 (1 H, dt, *J* = 3, 7, 7 Hz), 3.51 (3 H, s), 3.45 (3 H, s), 3.40–3.45 (1 H, m), 3.03 (1 H, *ABX*, *J* = 3, 13.5 Hz), 2.98 (1 H, A*BX*, *J* = 7.5, 13.5 Hz), 1.43 (9 H, s), 0.78–1.90 (13 H, m); MS (DEI) $(M + H)^+$ = 491. Anal. ($C_{19}H_{38}N_2O_6$) C, H, N.

(1′*R*,2′*S*,5*S*)-5-[2′-[[(*tert*-Butyloxy)carbonyl]amino]-3′-cyclohexyl-1′-(methoxymethoxy)propyl]-3-methoxyoxazolidin-2-one (9a). (2*S*,3*R*,4*S*)-4-[[(*tert*-Butyloxy)carbonyl]amino]-5-cyclohexyl-2-hydroxy-1-(methoxyamino)-3-(methoxymethoxy)pentane (1.44 g, 3.69 mmol) and triethylamine (1.54 mL, 11.1 mmol) were dissolved in dichloromethane (20 mL) and cooled to −23 °C (CO_2(s)/CCl_4) under a nitrogen atmosphere. A benzene solution of phosgene (4.26 g, 5.16 mmol, 12% by wt) was added dropwise, and after the addition was complete, the cooling bath was removed and the reaction was stirred for 2 h. The reaction mixture was diluted with ethyl ether, washed with 10% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, and then was dried and evaporated. The resulting oil was purified by MPLC (20% ethyl acetate in chloroform) to provide title compound **9a** (1.44 g, 94%): TLC R_f = 0.32 (XII); ^{1}H NMR ($CDCl_3$) δ 4.77 (1 H, *AB*, *J* = 10 Hz), 4.68 (1 H, A*B*, *J* = 10 Hz), 4.5–4.6 (2 H, m), 3.80–3.95 (2 H, m), 3.80 (3 H, s), 3.68–3.75 (2 H, m), 3.39 (3 H, s), 1.55–1.87 (5 H, m), 1.44 (9 H, s), 0.77–1.5 (8 H, m). Anal. ($C_{20}H_{36}N_2O_7$) C, H, N.

(1′*R*,2′*S*,5*R*)-5-[2′-[[(*tert*-Butyloxy)carbonyl]amino]-3′-cyclohexyl-1′-(methoxymethoxy)propyl]-3-methoxyoxazolidin-2-one (5*R* isomer of 9a). The 5*R* isomer of **9a** was prepared as described for **9a**. Thus (2*R*,3*R*,4*S*)-4-[[(*tert*-butyloxy)carbonyl]amino]-5-cyclohexyl-2-hydroxy-1-(methoxyamino)-3-(methoxymethoxy)pentane (0.59 g, 1.5 mmol) was converted to the title compound by treatment with phosgene (1.94 mL, 2.12 mmol, 12.5 wt % in toluene) in the presence of triethylamine (0.61 mL, 4.3 mmol) in dichloromethane (8 mL). The purified product was obtained by chromatography (15% ethyl acetate in chloroform) to provide 0.51 g (81%) of an oil: R_f = 0.48 (XII); ^{1}H NMR ($CDCl_3$) δ 4.90 (1 H, *AB*, *J* = 7 Hz), 4.68 (1 H, A*B*, *J* = 7 Hz), 4.67 (1 H, br d, *J* = 10.5 Hz), 4.47 (1 H, q, *J* = 9 Hz), 4.01 (1 H, t, *J* = 7.5 Hz), 3.82 (3 H, s), 3.72 (1 H, br q, *J* = 7.5 Hz), 3.61 (1 H, dd, *J* = 9, 1.5 Hz), 3.42 (3 H, s), 3.30 (1 H, dd, *J* = 9, 7.5 Hz), 1.45 (9 H, s), 0.70–1.83 (13 H, m); MS (DCI) $(M + NH_4)^+$ = 434 (strong), $(M + H)^+$ = 417 (weak).

(1′*R*,2′*S*,5*S*)-5-[2′-[[(*tert*-Butyloxy)carbonyl]amino]-3′-cyclohexyl-1′-(methoxymethoxy)propyl]-3-ethoxyoxazolidin-2-one (9b). As previously described, epoxide **1a** (0.990 g, 2.88 mmol) was reacted with *O*-ethylhydroxylamine hydrochloride (0.86 g, 8.6 mmol) in the presence of Na_2CO_3 (0.92 g, 8.6 mmol) to provide 1.12 g of the ring-opened products as a gummy solid [TLC R_f = 0.11, 0.18 (II); MS (DCI) $(M + H)^+$ = 405]. The unpurified product (1.10 g, 2.72 mmol) was converted into the title compound by treatment with phosgene (3.5 mL, 3.8 mmol, 12.5 wt % solution in toluene) and triethylamine (1.1 mL, 7.9 mmol) in dichloromethane (14 mL). Purification by chromatography (25% ethyl acetate in chloroform) provided the 5*R* isomer [TLC R_f = 0.56 (XII)] followed by 0.37 g (32%) of **9b**: TLC R_f = 0.52 (XII); ^{1}H NMR ($CDCl_3$) δ 4.76 (1 H, *AB*, *J* = 6 Hz), 4.67 (1 H, A*B*, *J* = 6 Hz), 4.5–4.6 (2 H, m), 3.96–4.05 (2 H, m), 3.90 (1 H, br d, *J* = 7 Hz), 3.85 (1 H, br s), 3.73 (1 H, dd, *J* = 7.5, 9 Hz), 3.40 (3 H, s), 1.47 (9 H, s), 1.28 (3 H, t, *J* = 7.5 Hz), 0.80–1.87 (13 H, m); MS (DCI) $(M + NH_4)^+$ = 448, $(M + H)^+$ = 431.

(1′*R*,2′*S*,5*S*)-5-[2′-[[(*tert*-Butyloxy)carbonyl]amino]-3′-cyclohexyl-1′-(methoxymethoxy)propyl]-3-(2-propyloxy)-oxazolidin-2-one (9c). As previously described, epoxide **1a** (1.15 g, 3.35 mmol) was reated with *O*-isopropylhydroxylamine hydrochloride (0.56 g, 5.0 mmol) and Na_2CO_3 (0.53 g, 5.0 mmol) to provide (1.40 g (100%) of the unpurified amino alcohol products as a thick oil [TLC R_f = 0.35, 0.27 (XI); MS (FAB) $(M + H)^+$ = 419]. This material (0.91 g, 2.2 mmol) was converted to the title compound by treatment with phosgene in toluene (2.80 mL, 3.05 mmol, 12.5 wt %) and triethylamine (0.88 mL, 6.3 mmol) in dichloromethane (11 mL). Purification by chromatography (20% ethyl acetate in chloroform) provided the 5*R* isomer [TLC R_f = 0.56 (XII)] followed by 0.29 g (30%) of **9c**: TLC R_f = 0.49 (XII); ^{1}H NMR ($CDCl_3$) δ 4.77 (1 H, *AB*, *J* = 7 Hz), 4.67 (1 H, A*B*, *J* = 7 Hz), 4.5–4.61 (2 H, m), 4.17 (1 H, septet, *J* = 6 Hz), 3.90 (1 H, br q, *J* = 7.5 Hz), 3.85 (1 H, br s), 3.73 (1 H, dd, *J* = 7.5, 9 Hz), 3.61 (1 H, t, *J* = 7.5 Hz), 3.39 (3 H, s), 1.45 (9 H, s), 1.27 (3 H, d, *J* = 6 Hz), 1.23 (3 H, d, *J* = 6 Hz), 0.80–1.87 (m, 13 H); MS (DEI/DIP) $(M - C_4H_8)^+$ = 388, $(M - C_4H_9O)^+$ = 371.

(2*S*,3*R*,4*S*)-1-[[(Benzyloxy)carbonyl]ethylamino]-4-[[(*tert*-butyloxy)carbonyl]amino]-5-cyclohexyl-2-hydroxy-3-[(methoxyethoxy)methoxy]pentane (10a) and (2*R*,3*R*,4*S*)-1-[[(Benzyloxy)carbonyl]ethylamino]-4-[[(*tert*-butyloxy)carbonyl]amino]-5-cyclohexyl-2-hydroxy-3-[(methoxyethoxy)methoxy]pentane (10b). Epoxide **1b** (509.5 mg, 1.315 mmol) in methanol (4 mL) was treated with 70% aqueous ethylamine (3.0 mL, 37 mmol). After 18 h the mixture was evaporated with methanol chasers, dissolved in dichloromethane, cooled to 0 °C, and treated with TEA (0.28 mL, 2.0 mmol) and benzyl chloroformate (0.23 mL, 1.6 mmol). After 15 min at 0 °C and 30 min at ambient temperature, the mixture was evaporated and taken up in ethyl acetate, which was washed with 0.5 M H_3PO_4, saturated $NaHCO_3$ solution, and brine and then was dried and evaporated. Chromatography with 25–33% ethyl acetate in hexane afforded 371.0 mg (50%) of **10a** followed by 218.6 mg (29%) of **10b**, both as oils.

10a: TLC R_f = 0.42 (II); $[\alpha]^{22}_D$ −51.8° (*c* 0.93, EtOH). Anal. ($C_{30}H_{50}N_2O_8$) C, H, N.

10b: TLC R_f = 0.29 (II); $[\alpha]^{22}_D$ +0.23° (*c* 0.86, EtOH). Anal. ($C_{30}H_{50}N_2O_8 \cdot 0.25H_2O$) C, H, N.

(2*S*,3*R*,4*S*)-2-Azido-1-[[(benzyloxy)carbonyl]ethylamino]-4-[[(*tert*-butyloxy)carbonyl]amino]-5-cyclohexyl-3-[(methoxyethoxy)methoxy]pentane (11). To triphenylphosphine (2.45 g, 9.34 mmol) in THF (14 mL) at −78 °C was added a solution of DEAD (1.45 mL, 9.21 mmol) in THF (24 mL). A solution of hydrazoic acid in benzene[29] [prepared from 877 mg (13.5 mmol) of NaN_3, and 0.35 mL (6.6 mmol) of H_2SO_4 in 9 mL of benzene] was then added followed by a solution of **10b** (4.189 g, 7.392 mmol) in THF (34 mL). After 1 h the mixture was warmed to room temperature, stirred for 16 h, evaporated, and chromatographed with 20–25% ethyl acetate in hexane to afford 3.562 g (82%) of an oil: TLC R_f = 0.55 (II); ^{1}H NMR ($CDCl_3$) δ 7.42–7.28 (5 H, m), 5.18 (1 H, d), 5.13 (1 H, d), 4.85–4.65 (3 H, m), 3.38 (3 H, s), 1.43 (9 H, s), 1.23–1.08 (3 H, m).

(2*S*,3*S*,4*S*)-2-Amino-1-[[(benzyloxy)carbonyl]ethylamino]-4-[[(*tert*-butyloxy)carbonyl]amino]-5-cyclohexyl-3-[(methoxyethoxy)methoxy]pentane (12). Azide **11** (3.562 g, 6.02 mmol) in methanol (40 mL) was treated with TEA (2.60 mL, 18.6 mmol) and 1,3-propanedithiol (1.85 mL, 18.4 mmol). The mixture was stirred rapidly for 72 h and was then filtered and evaporated. Chromatography with 1:1 ethyl acetate/hexane followed by 3% methanol in chloroform afforded 3.150 g (93%)

(29) Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*; John Wiley and Sons, Inc.: New York, 1967; Vol. 1, p 446.

of an oil: TLC R_f = 0.31 (IV). Anal. ($C_{30}H_{51}N_3O_7 \cdot 0.25H_2O$) C, H, N.

(2*S*,3*S*,4*S*)-2-Amino-4-[[(*tert*-butyloxy)carbonyl]amino]-5-cyclohexyl-1-(ethylamino)-3-[(methoxyethoxy)methoxy]pentane (14). To compound **12** (1.35 g, 2.39 mmol) was added wet 20% Pd on activated carbon (0.68 g) followed by glacial acetic acid (100 mL). The mixture was shaken under hydrogen pressure (4 atm) for 23 h, filtered through Celite, and concentrated. The residue was taken up in water and basified to pH 10 with K_2CO_3. After saturating with NaCl, the aqueous layer was extracted with chloroform until the product was no longer detectable in extracts by TLC analysis. The combined extracts were dried and evaporated to afford 1.11 g (>100%) of a pale yellow oil which was of sufficient purity for subsequent steps: TLC R_f = 0.46 (I); ^{1}H NMR ($CDCl_3$) δ 4.86 (1 H, d), 4.76 (2 H, s), 4.10–3.99 (1 H, m), 3.78–3.70 (2 H, m), 3.59–3.52 (2 H, m), 3.39 (3 H, s), 3.27 (1 H, d), 2.94–2.84 (2 H, m), 2.76–2.50 (3 H, m), 1.43 (9 H, s), 1.13 (3 H, t).

(1′*R*,2′*S*,4*S*)-4-[2′-[[(*tert*-Butyloxy)carbonyl]amino]-3′-cyclohexyl-1′-[(methoxyethoxy)methoxy]propyl]-1-ethylimidazolidin-2-one (15). To diamine **14** (52 mg, 0.12 mmol) in methylene chloride (1 mL) was added carbonyldiimidazole (23 mg, 0.14 mmol) and the mixture was stirred at ambient temperature for 6 h. The solvent was evaporated, the mixture was taken up in ethyl acetate and then washed with saturated $NaHCO_3$ solution, 2 M HCl, and brine. After the organic layer was dried and evaporated, the crude product was chromatographed with 5% methanol in chloroform to afford 12.0 mg (22%) of a viscous oil: TLC R_f = 0.56 (V); ^{1}H NMR ($CDCl_3$) δ 5.35 (1 H, s), 4.78 (1 H, d), 4.74 (1 H, d), 4.57 (1 H, d), 3.99–3.88 (1 H, m), 3.83–3.73 (1 H, m), 3.68–3.59 (1 H, m), 3.60–3.50 (4 H, m), 3.45–3.33 (2 H, m), 3.39 (3 H, s), 3.33–3.12 (2 H, m), 1.43 (9 H, s), 1.11 (3 H, t). Anal. ($C_{23}H_{43}N_3O_6 \cdot 0.5H_2O$) C, N, H: calcd, 9.50; found, 8.98.

(1′*R*,2′*S*,4*S*)-4-[2′-[[(*tert*-Butyloxy)carbonyl]amino]-3′-cyclohexyl-1′-[(methoxyethoxy)methoxy]propyl]-2-ethyl-1,2,5-thiadiazolidine 1,1-Dioxide (16). To diamine **14** (130 mg, 0.30 mmol) in methylene chloride (5 mL) was added *N*,*N*-diisopropylethylamine (185 μL, 1.06 mmol). The solution was cooled to −23 °C, treated with sulfuryl chloride (31 μL, 0.31 mmol), and stirred for 1.5 h at −23 °C followed by 2 h at ambient temperature. After evaporation of the solvent, the mixture was taken up in ethyl acetate and the organic layer was washed with 0.5 M H_3PO_4, saturated $NaHCO_3$ solution, and brine and then was dried and evaporated. Chromatography with 40% ethyl acetate in hexane afforded 55.0 mg (37%) of a tacky foam: TLC R_f = 0.58 (III); ^{1}H NMR ($CDCl_3$) δ 5.83 (1 H, d), 4.77 (1 H, d), 4.71 (1 H, d), 4.59 (1 H, d), 3.98–3.87 (1 H, m), 3.84–3.76 (1 H, m), 3.70–3.60 (2 H, m), 3.60–3.50 (3 H, m), 3.45 (1 H, d), 3.39 (3 H, s), 3.27–3.09 (2 H, m), 3.00–2.87 (1 H, m), 1.44 (9 H, s), 1.26 (3 H, t).

(4*S*,5*R*)-3-[(*tert*-Butyloxy)carbonyl]-4-(cyclohexylmethyl)-2,2-dimethyloxazolidine-5-carboxaldehyde (17). According to the procedure of Thaisrivongs et al.,[15] a solution of (3*RS*,4*S*)-4-[[(*tert*-butyloxy)carbonyl]amino]-5-cyclohexyl-3-hydroxy-1-pentene[30] (40 g, 140 mmol) and 2-methoxypropene (102 g, 1.41 mol) in dichloromethane (250 mL) was stirred at ambient temperature. Solid pyridinium *p*-toluenesulfonate (177 g, 704 mmol) was added slowly to the reaction mixture. After the addition was complete, the reaction was stirred for 1 h and neutralized by addition of solid sodium bicarbonate. The solids were filtered and the filtrate was concentrated. Chromatography gave 3-[(*tert*-butyloxy)carbonyl]-4-(cyclohexylmethyl)-2,2-dimethyl-5-vinyloxazolidine (57 g, 100%), a portion of which (10 g, 31 mmol) was taken up in 2:1 dichloromethane/methanol (150 mL) and cooled to −78 °C. Ozone was bubbled through the solution until a blue color persisted (1 h) followed by dry nitrogen to remove excess dissolved ozone. The reaction mixture was transferred via cannula into a suspension of zinc dust (8.0 g, 120 mmol) in glacial acetic acid (8 mL), water (200 mL), and methanol (200 mL) precooled to −45 °C. After 5 min the bath was removed and the mixture was allowed to warm to ambient temperature overnight. The resulting mixture was treated with 100 mL of saturated sodium chloride, extracted with two 300-mL portions of dichloromethane, dried over $MgSO_4$, and concentrated. The crude aldehyde was purified by chromatography using 20% ethyl acetate in hexane to give 9.7 g (96%) of **17** as a mixture of diastereomers (3:1 trans/cis) as judged by the integrated resonances of the two aldehyde protons: ^{1}H NMR ($CDCl_3$) δ 9.73 (1 H, d, CHO, cis diastereomer), 9.83 (1 H, s, CHO, trans diastereomer). Anal. ($C_{18}H_{31}NO_4 \cdot 0.25H_2O$) C, H, N.

Equilibration of Aldehyde Isomers. A suspension of the above aldehyde (25 g, 77 mmol) and powdered potassium carbonate (10.7 g, 77 mmol) in methanol (300 mL) was stirred at ambient temperature for 6 h. The reaction mixture was cooled in an ice-water bath and treated with glacial acetic acid (9.3 g, 155 mmol) for 5 min. A solution of 0.5 M sodium dihydrogen phosphate (300 mL) was added to the mixture. After 30 min, the solution was concentrated to one-half the original volume and extracted with ether (600 mL). The combined ether extracts were dried over $MgSO_4$ and concentrated. The crude mixture was purified by chromatography using 20% ethyl acetate in hexane to give 19.5 g (89%) of **17** as an 8:1 mixture of trans/cis diastereomers.

(5*S*,4′*S*,5′*R*)-5-[3′-[(*tert*-Butyloxy)carbonyl]-4′-(cyclohexylmethyl)-2′,2′-dimethyloxazolidin-5′-yl]-3-methylenedihydrofuran-2(4*H*)-one (18). A solution of **17** (16.52 g, 51 mmol) in tetrahydrofuran (15 mL) was treated with freshly activated zinc dust (3.98 g, 61 mmol). With vigorous stirring, the mixture was treated with methyl 2-(bromomethyl)acrylate (10 g, 56 mmol) at a rate which maintained the temperature at 50–60 °C. Upon completion of the addition, the mixture was stirred at 50 °C for 1 h, allowed to cool, and poured into 100 mL of cold 1 M HCl. The mixture was extracted with dichloromethane (3 × 100 mL), washed successively with saturated aqueous $NaHCO_3$ and H_2O, dried, and concentrated. Chromatography using 10% ethyl acetate in hexane provided 10.83 g (61%) of **18**: TLC R_f = 0.58 (VII); mp 122–126 °C; $[\alpha]^{22}_D$ +21.8° (c 1.04, $CHCl_3$). Anal. ($C_{22}H_{35}NO_5$), C, H, N.

(3*S*,5*S*,4′*S*,5′*R*)-5-[3′-[(*tert*-Butyloxy)carbonyl]-4′-(cyclohexylmethyl)-2′,2′-dimethyloxazolidin-5′-yl]-3-methyldihydrofuran-2(3*H*)-one (19). A mixture of **18** (8.03 g, 20 mmol) and 0.81 g of 10% palladium on carbon in ethyl acetate (200 mL) was shaken under 4 atm of H_2. After filtration, concentration of the filtrate gave 7.58 g (94%) of **19**: TLC R_f = 0.59 (VII); mp 124–125 °C. Anal. ($C_{22}H_{37}NO_5$) C, H, N.

(3*S*,5*S*,1′*R*,2′*S*)-5-[2′-[[(*tert*-Butyloxy)carbonyl]amino]-3′-cyclohexyl-1′-hydroxypropyl]-3-methyldihydrofuran-2(3*H*)-one (19a). Using the above procedure with methanol as a solvent instead of ethyl acetate gave a 1.5:1 mixture of **19a** and **19** which was separated by chromatography using 20% ethyl acetate in hexane: TLC R_f = 0.10 (VI); ^{1}H NMR ($CDCl_3$) δ 0.8–1.0 (2 H, m), 1.1–1.4 (5 H, m), 1.38 (3 H, d, J = 7 Hz), 1.43 (9 H, s), 1.6–1.85 (6 H, m), 1.92 (1 H, td, J = 12, 10 Hz), 2.46 (1 H, m), 2.68 (1 H, ddq, J = 12, 9, 7 Hz; H_3), 3.49 (1 H, br d), 3.70 (1 H, m), 4.34 (1 H, dt, J = 10, 6 Hz; H_5), 4.78 (1 H, br d). Irradiation at δ 4.34 gave a strong NOE enhancement at δ 2.68.

(5*S*,4′*S*,5′*R*)-5-[3′-[(*tert*-Butyloxy)carbonyl]-4′-(cyclohexylmethyl)-2′,2′-dimethyloxazolidin-5′-yl]-3,3-dimethyldihydrofuran-2(3*H*)-one (20). A solution of dry diisopropylamine (0.21 mL, 1.5 mmol) in tetrahydrofuran (15 mL) was cooled under a nitrogen atmosphere to −78 °C and treated with *n*-butyllithium (0.56 mL, 1.4 mmol, 2.5 M in hexane). The resulting solution was allowed to warm for 5 min, and recooled to −78 °C, treated with a solution of **19** (500 mg, 1.26 mmol) in tetrahydrofuran (5 mL), stirred for 15 min, and treated with iodomethane (0.08 mL, 1.3 mmol). After being allowed to warm to ambient temperature, the solution was partitioned between ether and aqueous NH_4Cl, washed sequentially with 10% $Na_2S_2O_3$ and brine, dried over $MgSO_4$, and concentrated. Chromatography using 15% ethyl acetate in hexane afforded 456 mg (88%) of **20** as a white solid: mp 152–153 °C; TLC R_f = 0.34 (VI). Anal. ($C_{23}H_{39}NO_5$) C, H, N.

(4*S*,5*R*,1′*S*,3′*S*)-3-[(*tert*-Butyloxy)carbonyl]-4-(cyclohexylmethyl)-5-(1′,4′-dihydroxy-3′-methylbutyl)-2,2-dimethyloxazolidine (21). A mixture of **19** (0.50 g, 1.26 mmol) and sodium borohydride (0.15 g, 4 mmol) in tetrahydrofuran (50 mL) was heated at reflux under a nitrogen atmosphere for 48 h. After being allowed to cool, the mixture was treated cautiously

(30) Bolis, G.; Fung, A. K. L.; Greer, J.; Kleinert, H. D.; Marcotte, P. A.; Perun, T. J.; Plattner, J. J.; Stein, H. H. *J. Med. Chem.* 1987, *30*, 1729.

with aqueous NH_4Cl, extracted with ether, washed with brine, dried over $MgSO_4$, and concentrated. Chromatography using 33% ethyl acetate in chloroform gave 0.37 g (73%) of **21**: TLC R_f = 0.26 (VII); mp 45–52 °C. Anal. ($C_{22}H_{41}NO_5 \cdot H_2O$) C, H, N.

(4*S*,5*R*,2′*S*,4′*S*)-3-[(*tert*-Butyloxy)carbonyl]-4-(cyclohexylmethyl)-2,2-dimethyl-5-[4′-methyltetrahydrofuran-2′-yl]oxazolidine (23). A solution of **21** (51 mg, 0.13 mmol) and triethylamine (0.037 mL, 0.27 mmol) in dichloromethane (2 mL) was cooled to 0 °C under a nitrogen atmosphere and treated with methanesulfonyl chloride (0.012 mL, 0.15 mmol). After 1 h, the solution was diluted with dichloromethane, washed successively with 10% citric acid, water, and saturated aqueous $NaHCO_3$, dried, and concentrated to give 59 mg of crude **22** [TLC R_f = 0.45 (VII)], which was taken up in tetrahydrofuran (8 mL), treated with sodium hydride (20 mg, 0.50 mmol, 60% dispersion in oil), and heated at reflux for 2 h. After being allowed to cool, the solution was treated cautiously with saturated aqueous NH_4Cl, extracted with ether, dried over $MgSO_4$, and concentrated. Chromatography using 10% ethyl acetate in hexane gave 30 mg (75%) of **23**: TLC R_f = 0.29 (VIII); mp 68–70 °C. Anal. ($C_{22}H_{39}NO_4$) C, H, N.

(2*S*,3*R*,4*S*,6*S*)-1-Cyclohexyl-3,4-dihydroxy-6-methyl-2-[(*p*-tolylsulfonyl)amino]-7-[(*p*-tolylsulfonyl)oxy]heptane (25). A solution of **19** (0.44 g, 1.11 mmol) in ethanol (5 mL) was treated with 4 M hydrogen chloride in dioxane (20 mL). After being stirred for 30 min, the solution was concentrated and the residue was taken up in tetrahydrofuran (30 mL), cooled to 0 °C, and treated with lithium aluminum hydride (0.17 g, 4.5 mmol). After 30 min, the solution was quenched by sequential addition of 0.17 mL of water, 0.17 mL of 3 M NaOH, and 0.51 mL of water. The resulting mixture was diluted with ether and filtered. The residue was treated with chloroform, heated at reflux for 16 h, and filtered. The combined filtrates were concentrated to give 0.32 g of crude **24**, which was taken up in 20 mL of dichloromethane and treated sequentially with triethylamine (0.4 mL, 2.9 mmol) and *p*-toluenesulfonyl chloride (0.43 g, 2.2 mmol). The resulting solution was stirred at ambient temperature for 4 days, diluted with ether and aqueous $NaHCO_3$, stirred for 10 min, separated, and washed sequentially with 1 M HCl, water, aqueous $NaHCO_3$, and brine. The ethereal solution was dried over $MgSO_4$ and concentrated. Chromatography with 25% ethyl acetate in chloroform afforded 120 mg (19%) pure **25**: TLC R_f = 0.33 (IX); ^1H NMR ($CDCl_3$) δ 7.8–7.7 (4 H, m), 7.4–7.3 (4 H, m), 4.6 (1 H, br), 3.99 (1 H, dd, J = 9, 6 Hz), 3.90 (1 H, dd, J = 9, 6 Hz), 3.68 (2 H, m), 3.19 (1 H, d, J = 8 Hz), 2.45 (6 H, s), 2.43 (1 H, br d), 2.15 (2 H, m), 1.6–1.3 (8 H, m), 1.1–0.7 (6 H, m), 0.96 (3 H, d, J = 7 Hz), 0.7–0.5 (2 H, m).

(2*S*,3*R*,4*S*)-1-Cyclohexyl-3,4-dihydroxy-6-methyl-2-[(*p*-tolylsulfonyl)amino]heptane (27). A mixture of **25** (0.11 g, 0.19 mmol), pyridinium *p*-toluenesulfonate (0.05 g, 0.2 mmol), dichloromethane (3 mL), and acetone (2 mL) was treated with powdered 3A molecular sieves and stirred at ambient temperature. After four days, the mixture was diluted with ether, filtered, washed with aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated. Chromatography using 33% ethyl acetate in hexane gave 23 mg (20%) of pure **26** [TLC R_f = 0.25 (X)]. A solution of **26** (23 mg, 0.38 mmol) in tetrahydrofuran (1 mL) was cooled to 0 °C and treated with lithium triethylborohydride (0.4 mL, 0.4 mmol, 1.0 M in THF). The resulting solution was stirred at ambient temperature for 2.5 h, treated cautiously with 0.3 mL of 3 M NaOH, cooled to 0 °C, and treated cautiously with 0.3 mL of 30% hydrogen peroxide. After being stirred for 10 min, the solution was extracted with ether, washed sequentially with water and 10% aqueous sodium thiosulfate, dried, and concentrated. The crude residue was taken up in methanol (5 mL), treated with *p*-toluenesulfonic acid monohydrate (0.02 g), and allowed to stand at ambient temperature for 16 h. The resulting solution was concentrated, taken up in ether, washed sequentially with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated. A pure sample of **27** (2.6 mg, 17%) was obtained after chromatography using 33% ethyl acetate in hexane: TLC R_f = 0.31 (X); ^1H NMR ($CDCl_3$) δ 0.5–1.1 (br envelope), 1.25–1.6 (br envelope), 1.83 (1 H, d, J = 7 Hz), 1.92 (1 H, heptet, J = 7 Hz), 2.45 (3 H, s), 3.21 (2 H, m), 3.70 (2 H, m), 4.71 (1 H, d, J = 9 Hz), 7.34 (2 H, d, J = 8 Hz), 7.77 (2 H, d, J = 8 Hz). ^{13}C NMR ($CDCl_3$) δ 21.5, 21.7, 24.0, 24.5, 25.8, 26.0, 26.3, 33.0, 33.3, 33.5, 40.4, 42.3, 51.2, 69.2, 76.0, 127.1, 129.7, 137.7, 143.7.

27 from 28. Compound **28**[18] (10.00 g, 29.11 mmol) was stirred for 1 h at ambient temperature in 4 M HCl in dioxane. The mixture was evaporated and then dissolved in water which was washed with ether, basified with Na_2CO_3, saturated with NaCl, and extracted into chloroform which was dried and evaporated to afford 7.09 g (100%) of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (mp 110–111 °C). This compound (20 mg, 0.082 mmol) and triethylamine (0.028 mL, 0.2 mmol) in dichloromethane (1 mL) were cooled to 0 °C and treated with *p*-toluenesulfonyl chloride (19 mg, 0.1 mmol). The resulting solution was stirred at ambient temperature for 4 h, diluted with ether, washed sequentially with 10% citric acid, aqueous $NaHCO_3$, and brine and then dried over $MgSO_4$, and concentrated. Chromatography using 33% ethyl acetate in hexane gave 24.8 mg (76%) of **27** which was identical by TLC, ^1H NMR, and ^{13}C NMR with the sample of **27** prepared from **26**.

(2*S*,3*R*,4*S*)-4-[[(*tert*-Butyloxy)carbonyl]amino]-5-cyclohexyl-1-(formylamino)-2-hydroxy-3-(methoxymethoxy)pentane. Amine **3** (205 mg, 0.569 mmol) in dichloromethane (5 mL) at 0 °C was treated with TEA (0.175 mL, 1.26 mmol) and acetic formic anhydride[31] (0.25 mL, ~3 mmol). After 30 min at 0 °C and 30 min at ambient temperature the mixture was diluted with ethyl acetate, washed with 2 M HCl, 2 M NaOH, and brine, and then was dried and evaporated. Chromatography with 5% methanol in chloroform afforded 214.1 mg (97%) of a white foam: TLC R_f = 0.49 (IV). Anal. ($C_{19}H_{36}N_2O_6 \cdot 0.25H_2O$) C, H, N.

Biological Methods. Assays of purified human renal renin,[30] bovine cathepsin D,[30] pepsin,[30] and human plasma renin[32] were performed as previously described.

Acknowledgment. The assistance of Carl Nordeen with the preparation of **1a,b** is gratefully acknowledged.

(31) Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*; John Wiley and Sons, Inc.: New York, 1967; Vol. 1, p 4.
(32) Plattner, J. J.; Marcotte, P. A.; Kleinert, H. D.; Stein, H. H.; Greer, J.; Bolis, G.; Fung, A. K. L.; Bopp, B. A.; Luly, J. R.; Sham, H. L.; Kempf, D. J.; Rosenberg, S. H.; Dellaria, J. F.; De, B.; Merits, I.; Perun, T. J. *J. Med. Chem.* **1988**, *31*, 2277.
(33) The synthesis of the C-terminal fragment for **48** is not illustrated. See the Experimental Section.