Renin Inhibitors Containing Conformationally Restricted P_1-P_1' Dipeptide Mimetics[1]

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A series of renin inhibitors containing lactam-bridged P_1-P_1' dipeptide mimetics based on the ACHPA (4(*S*)-amino-5-cyclohexyl-3(*S*)-hydroxypentanoic acid) design was studied. The inhibitors were obtained by aldol addition of various lactams with N^{α}-Boc-L-cyclohexylalaninal, followed by Boc group removal and acylation with Boc-Phe-His. The aldol diastereomer having the *S* configuration at the two newly generated stereogenic centers gave optimal enzyme inhibition. Potency was further enhanced in the γ-lactam ring series by substitution with small hydrophobic groups to mimic the P_1' side chain of the renin substrate. Thus, 2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxyl-1-(1,5,5-trimethyl-2-oxopyrrolidin-3(*S*)-yl)propane (**34**) has an IC_{50} of 1.3 nM in the human plasma renin assay. A variety of substituents on the lactam nitrogen are tolerated and can be used to vary the physical properties of the inhibitor. By using a model of the human renin active site, the conformation of **34** in the enzyme–inhibitor complex is proposed. This modeled conformation is very similar to the solid-state conformation of 2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxyl-1-(1-methyl-2-oxopyrrolidin-3(*S*)-yl)propane (**36**), the structure of which was determined by single-crystal X-ray diffraction analysis. The most potent ACHPA–lactam renin inhibitors show good selectivity when assayed against other types of aspartic proteinases. By varying the lactam ring substituents, potent and selective inhibitors of cathepsin D and cathepsin E can be obtained.

Introduction

The renin–angiotensin system (RAS) is a proteolytic cascade which occurs in the circulation and other tissues and plays an important role in the regulation of blood pressure and electrolyte and fluid homeostasis by the production of the potent pressor and aldosteronogenic peptide angiotensin II. The clinical efficacy of angiotensin converting enzyme inhibitors[2] for the treatment of hypertension and congestive heart failure has aroused considerable interest in developing agents which interrupt the RAS by other mechanisms, such as inhibition of the renin enzyme[3] and antagonism of the angiotensin II receptor.[4] Unlike angiotensin converting enzyme, which has several different physiological substrates, renin is a highly specific enzyme with its only known function being the cleavage of angiotensinogen to angiotensin I. Inhibitors of renin thus offer the possibility of being highly selective agents for interruption of the RAS.

Potent inhibitors of renin[3] have been developed by utilizing small peptides which correspond to the sequence around the cleavage site of angiotensinogen and in which the scissile Leu^{10}-Val^{11} dipeptide moiety is replaced with a nonhydrolyzing group which may mimic the tetrahedral transition-state geometry for amide hydrolysis. Although certain renin inhibitors of this general type have been shown to produce a hypotensive response upon iv infusion in man,[5] a therapeutically useful agent has not yet emerged because of a short duration of action and low oral bioavailability,[6] problems often encountered with peptidic drug targets. Therefore, inhibitors which minimize the peptide character and molecular size and allow for adjustments in physical properties such as aqueous solubility, lipophilicity, and ionic state are being pursued in an attempt to overcome these pharmacokinetic limitations.

One class of potent peptidic renin inhibitors utilizes the γ-amino acid statine (4(*S*)-amino-3(*S*)-hydroxy-6-methylheptanoic acid) as a replacement for the scissile Leu^{10}-Val^{11} dipeptide moiety.[7] The improved statine analogue ACHPA[9] (4(*S*)-amino-5-cyclohexyl-3(*S*)-hydroxypentanoic acid) provided an important advance by enhancing potency such that peptidic character could

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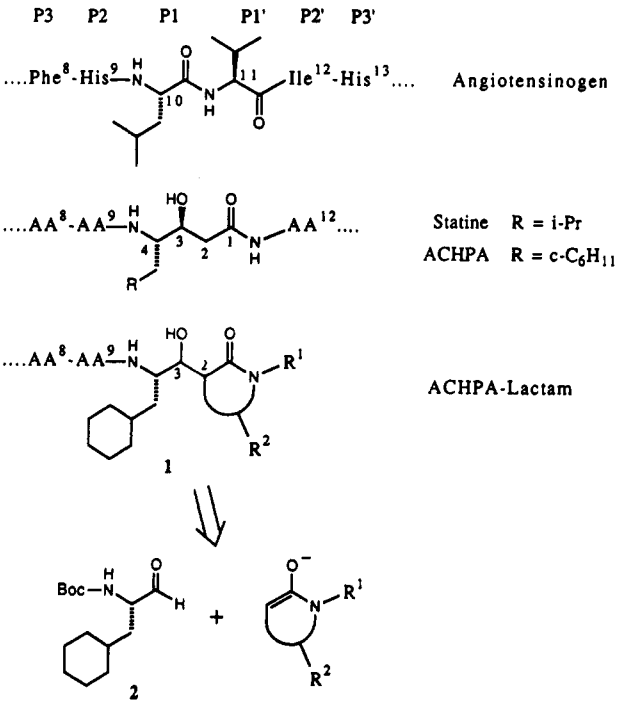


Figure 1. Lactam-constrained ACHPA as a dipeptide mimetic.

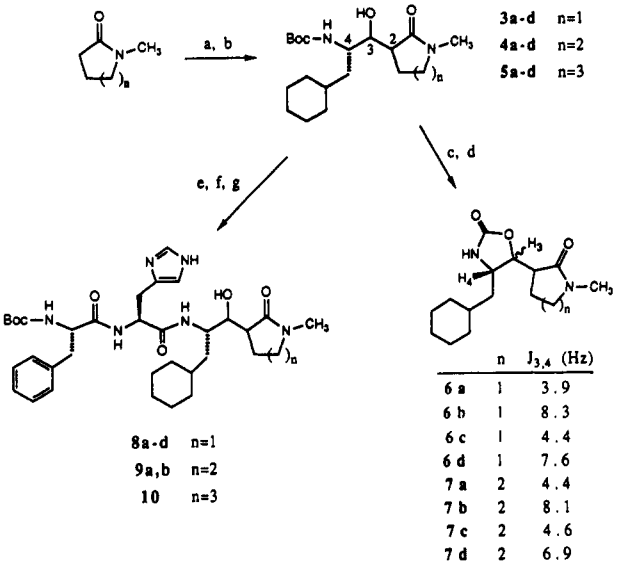
be removed from other parts of the inhibitor structure. We sought to further enhance potency in this class by introducing a group to mimic the hydrophobic P_1' side chain, a binding element which is absent in the statine/ACHPA design. A previous attempt to improve potency using 2-alkyl statine analogues met with limited success.[10] Herein we report lactam-constrained ACHPA analogues as a means to introduce a P_1' side chain mimic, an approach which produces potent inhibitors and allows for a reduction in peptide character and molecular size.

The design concept for the conformationally constrained ACHPA analogues described herein involves a formal cyclization between the ACHPA C1 amide nitrogen and C2 backbone positions, giving an "ACHPA–lactam" derivative represented generically as **1** (Figure 1). These ACHPA–lactams are synthetically accessible by aldol addition of a lactam enolate to N^{α}-(*tert*-butyloxycarbonyl)-L-cyclohexylalaninal (**2**[9]). Utilization of substituted lactams (e.g., R^1 and/or R^2) in the aldol addition gives rise to inhibitors which can be used to probe the enzyme in a spatially defined manner for additional binding sites. Of particular interest in this regard is the region which accommodates the hydrophobic P_1' side chain of the natural substrate.

Chemistry

Initial structure–activity studies were needed in order to determine which of the four ACHPA lactam aldol diastereomers (**1**; R^2 = H) produces the best enzyme inhibition. Aldol addition of the lithium enolate of 1-methyl-2-pyrrolidinone to N^{α}-Boc-L-cyclohexylalaninal[9] (**2**) in THF solution at −78 °C followed by an aqueous quench at that temperature provided an essentially equal mixture of four diastereomers, **3a–d**, which were readily separated by silica gel column chromatography[12] (Scheme I). Assignment of the C3 configuraion[13] for each of these diastereomers was facilitated by formation of the corresponding oxazolidinones **6a–d** and examining the H_3–H_4 coupling constant, the smaller values indicative of a trans relationship between H_3 and H_4 for the 3*S* epimers, and the larger values indicative of a cis relationship for the 3*R* epimers.[14] Additionally, the third-eluting diasteromer **3c** was found to have the *S* configuration at all three of its stereogenic centers as determined by X-ray crystallographic analysis (Figure 2). By inference, then, the other 3*S* epimer **3a** has the 2*R* configuration. The C2 configuration for the two 3*R* epimers **3b** and **3d** was not established. Each of the aldol isomers **3a–d** was then elaborated to its corresponding Boc-phenylalanyl-histidyl derivative by mixed-

Scheme I.[a] Synthesis of ACHPA Lactam Containing Renin Inhibitors



	n	$J_{3,4}$ (Hz)
6a	1	3.9
6b	1	8.3
6c	1	4.4
6d	1	7.6
7a	2	4.4
7b	2	8.1
7c	2	4.6
7d	2	6.9

[a] (a) LDA, THF, −78 °C; **2**; (b) chromatographic separation of diastereomers; (c) TFA, CH_2Cl_2; (d) 1,1'-carbonyldiimidazole, imidazole, THF; (e) TFA; Boc-(DNP)His-$OCO_2CH_2CH(CH_3)_2$, *N*-methylmorpholine; (f) TFA; Boc-Phe-$OCO_2CH_2CH(CH_3)_2$, *N*-methylmorpholine; (g) PhSH, Et_3N, CH_2Cl_2.

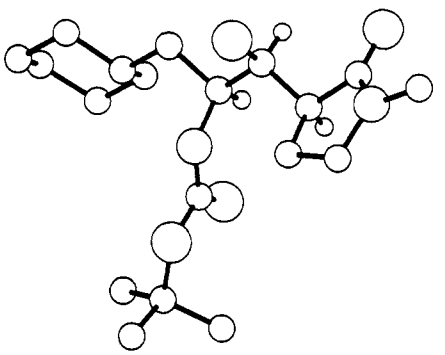


Figure 2. X-ray crystal structure of **3c**. Hydrogens have been omitted except at stereogenic centers.

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(12) The letter suffix associated with each aldol product refers to the order in which the isomer eluted during chromatographic separation on silica gel, isomer "*a*" being the first to elute and isomer "*d*" being the last to elute.

(13) The ACHPA numbering scheme is used for the "backbone" atoms in the ACHPA lactam analogues.

(14) Coupling constant criteria for assignment of *cis*- and *trans*-4,5-disubstituted-2-oxazolidinones derived from 1,2-amino alcohols has been previously described: Rich, D. H.; Sun, E. T. O. *J. Med. Chem.* **1980**, *23*, 27.

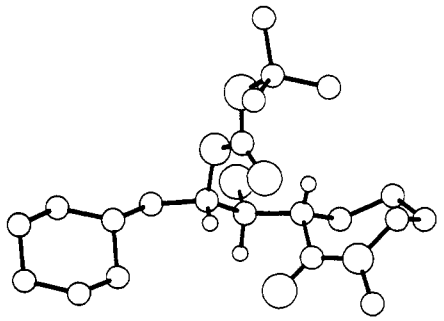


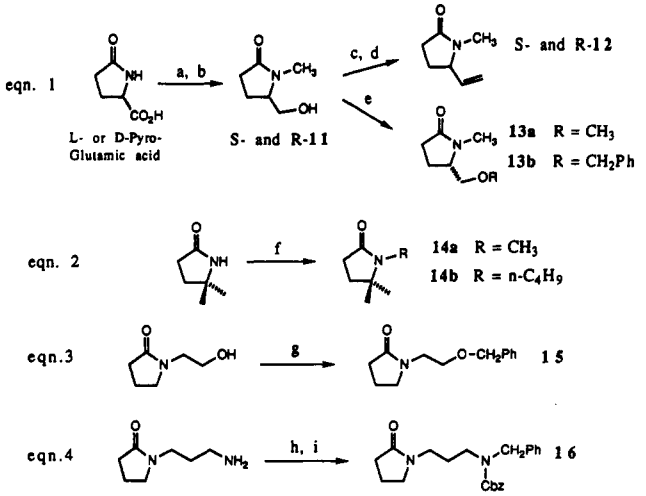
Figure 3. X-ray crystal structure of **5d**. Hydrogens have been omitted except at stereogenic centers.

anhydride coupling of the Boc-protected L-amino acids, giving **8a–d**. The 2,4-dinitrophenyl side chain protecting group on histidine used during the coupling sequence was removed in the final step with thiophenol. Acylation of the amines derived from **3a–d** with the homochiral amino acid derivatives provided the desired inhibitor sequences and also served to establish that the aldol condensation step with N^{α}-Boc-L-cyclohexylalaninal proceeded with negligible (<5%) loss of optical purity.

To investigate the effect of lactam ring size on enzyme inhibition, 1-methyl-2-piperidinone and 1-methylcaprolactam were added to aldehyde **2** by using the aldol conditions described above. In each case, a nearly statistical mixture of four diastereomers was obtained. The C3 configuration of the aldol products obtained from 1-methyl-2-piperidinone, **4a–d**, was determined by measuring the H_3–H_4 coupling constant of the derived oxazolidinones **7a–d**. From this analysis, the two 3*S* alcohol epimers **4a** and **4c** were converted to the corresponding Boc-Phe-His analogues **9a** and **9b** with mixed-anhydride couplings as before. On the basis of the relative TLC mobilities of **4a** and **4c** compared to the relative mobilities of **3a** and **3c**, the five-membered ring analogues of known configuration, and the inhibitory properties of the derived peptides **9a** and **b** compared to those of **8a** and **8c** (vide infra), the C2 configuration for **4a** and **4c** is inferred to be *R* and *S*, respectively. From the aldol mixture obtained with 1-methylcaprolactam, the 2*S*,3*S* diastereomer **5d** was identified by X-ray crystallographic analysis (Figure 3) and was converted to its Boc-Phe-His derivative, **10**.

In order to explore the enzyme active site for additional binding subsites, it became of interest to prepare analogues of **8c** in which the five-membered lactam ring is substituted with groups of differing size and polarity. The availability of both enantiomers of pyroglutamic acid provided a convenient entry to homochiral 5-monosubstituted 2-pyrrolidinones as shown in eq 1 in Scheme II. Thus, treatment of either L- or D-pyroglutamic acid with 2 equiv of NaH in DMF followed by alkylation with excess iodomethane provided the correponding N-methylated methyl esters. Selective reduction of the ester group using 2 hydride equiv of LAH in THF solution at −78 °C followed by warming to ambient temperature produced the *S* and *R* enantiomers of alcohol **11**. Oxidation using the Swern procedure[15] afforded the corresponding aldehydes which were isolated by extractive workup and immediately olefinated with methylene triphenylphosphorane in THF at −78 to 0 °C to give the ethenyl lactams *S*- and *R*-**12**. The enantiomeric purity of these lactams was not rigorously established; however aldol condensation with homochiral **2** provided the expected mixture of four products in each case, with little evidence of other diastereomers which

Scheme II.[a] Synthesis of Substituted Lactam Precursors



[a] (a) NaH (2 equiv) DMF; CH_3I (excess); (b) LAH, THF, −78 °C; (c) DMSO/$(COCl)_2$; Et_3N; (d) $Ph_3P{=}CH_2$, THF, −78 °C; (e) NaH, THF; CH_3I or $PhCH_2Br$; (f) NaH, DMF; CH_3I or *n*-BuBr; (g) NaH, THF; $PhCH_2Br$; (h) Cbz-Cl, Et_3N, CH_2Cl_2; (i) NaH, DMF, $PhCH_2Br$.

would have resulted had the starting lactams not been of high enantiomeric purity. From *S*-**11**, the ethers **13a** and **13b** were prepared by deprotonation with NaH and alkylation with iodomethane or benzyl bromide in THF. Achiral lactams **14a** and **14b** (Scheme II, eq 2) were prepared by deprotonation of readily available 5,5-dimethyl-2-pyrrolidinone[16] using NaH in DMF, followed by alkylation with iodomethane or 1-bromobutane. Two other 1-substituted 2-pyrrolidinones were prepared in a straightforward manner from commercially available starting materials (Scheme II, eqs 3 and 4). Thus, 1-(2-hydroxyethyl)-2-pyrrolidinone was alkylated by treatment with NaH and benzyl bromide in THF to give benzyl ether **15**, and 1-(3-aminopropyl)-2-pyrrolidinone was protected with the carbobenzyloxy group and then N-alkylated by treatment with NaH and benzyl bromide in DMF to give **16**.

Aldol condensation of aldehyde **2** with lactams **12–16** and with commercially available 1-cyclohexyl-2-pyrrolidinone using the conditions described above gave four chromatographically separable diastereomers in each case. Compared to the prototype aldol reaction using 1-methyl-2-pyrrolidinone, diastereoselectivity was not greatly affected by the presence of substituents on the lactam ring, with the exception of **13b** in which case the relative abundance of the fourth-eluting isomer was greatly diminished. The relative TLC mobilities of the four diastereomers obtained from each reaction resembled the elution pattern observed for the **3a–d** mixture. In addition, the third-eluting diasteromer from each mixture exhibited NMR spectroscopic data for protons H_2, H_3, and H_4 which closely resembled those found for **3c** (Table I), the aldol isomer whose complete configurational assignment was established by X-ray crystallographic analysis. On the basis of these chromatographic and spectroscopic analogies, then, it is inferred that aldol products **17–24** have the 2*S*,3*S* configuration. The NMR data suggests that the substituted analogues **17–24** have essentially the same solution conformation as **8c**. Elaboration to their corresponding Boc-phenylalanyl-histidyl derivatives was accomplished using sequential mixed anhydride couplings as before.

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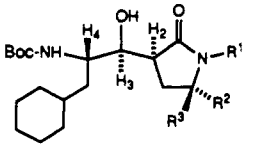
Table I. Comparison of ^1H NMR Spectroscopic Properties[a] of 2S,3S-ACHPA-Lactam Diastereomers



	R1	R2	R3	H_2, ppm	H_3, ppm	H_4, ppm	$J_{2,3}$,[b] Hz	$J_{3,4}$,[b] Hz
3c	CH_3	H	H	2.59	3.95	3.72	2.2	4.2
17	CH_3	$CH{=}CH_2$	H	2.67	3.9	3.71	–[c]	–[c]
18	CH_3	H	$CH{=}CH_2$	2.59	3.99	3.71	2.6	3.8
19	CH_3	CH_2OCH_3	H	2.68	3.96	3.7	1.8	3.9
20	CH_3	CH_2OCH_2Ph	H	2.71	3.94	3.7	2.2	4.4
21	CH_3	CH_3	CH_3	2.67	4.01	3.70	2.4	3.8
22	n-C_4H_9	CH_3	CH_3	2.65	4.01	3.70	2.5	3.8
23	$CH_2CH_2OCH_2Ph$	H	H	2.59	3.95	3.72	2.3	4.0
24	$CH_2CH_2CH_2N(Cbz)CH_2Ph$	H	H	–[d]	–[d]	–[d]		
25	c-C_6H_{11}	H	H	2.59	3.95	3.70	2.4	4.0

[a] Spectra were obtained at 300 MHz in CD_3OD solution. [b] Coupling constants were determined by decoupling experiments in which H_2 was irradiated. [c] Overlap of H_3 signal with other resonances obscures coupling constants. [d] cis–trans isomerization of the Cbz group makes proton assignment difficult due to line broadening.

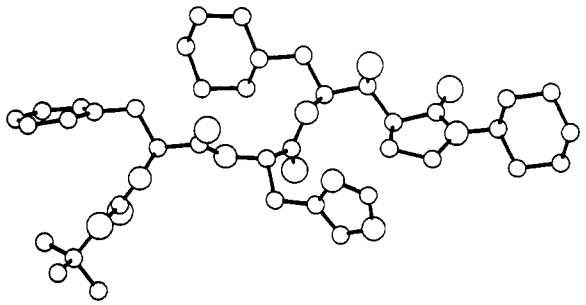


Figure 4. X-ray crystal structure of **36**. Hydrogens have been omitted for clarity.

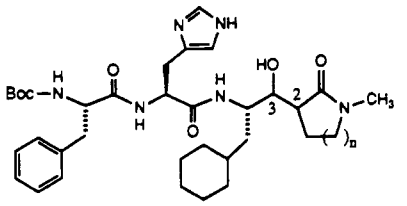
Single-crystal X-ray diffraction analysis of **36** confirmed its configurational assignment and provides, for the first time, the solid-state conformation of a potent renin inhibitor in the absence of enzyme (Figure 4).

Additional analogues were obtained through further chemical manipulations of several of the peptides (Table III). Hydrogenation of the ethenyl lactam diastereomers **26** and **28** provided the corresponding ethyl derivatives **27** and **29**. Alcohols **33** and **38** were obtained from the corresponding benzyl ether derivatives **30** and **37** by hydrogenolysis. Removal of the Cbz group in **39** using transfer hydrogenation conditions[17] afforded amino derivative **40**. Selective monoacetylation of **33** on the lactam hydroxymethyl group to give **31** was achieved by reaction with 2.5 equiv of acetic anhydride in DMF, followed by treatment with aqueous triethylamine to deacylate the histidine side chain.

Results and Discussion

In Vitro Activity. Renin inhibition in vitro was measured at pH 7.4 by using the previously reported human plasma renin assay.[9] A secondary in vitro assay utilizing purified human kidney renin and purified angiotensinogen[18] was employed for selected compounds.

Table II. Diastereomer and Ring Size Preference for ACHPA-Lactam Renin Inhibitors



	n	C2	C3	HPLC[a]	IC_{50},[b] nM
8a	1	R	S	94.1	>20000
8b	1	c	R	90.0	470
8c	1	S	S	98.2	9.3
8d	1	c	R	95.2	280
9a	2	R	S	95.1	>20000
9b	2	S	S	98.7	47
10	3	S	S	97.1	200

[a] Percent purity as determined by reverse-phase HPLC analysis; see the Experimental Section for details. [b] Human plasma renin assay, pH 7.4. [c] Configuration not determined.

Potencies are expressed as IC_{50} values for suppression of AI formation. As can be seen from the results for the diastereomerically related inhibitors **8a–d** (Table II), there is a marked preference for the 2S,3S isomer **8c**. The two 3R isomers **8b** and **8d** are both greater than 10-fold less potent than **8c**, and the 2R,3S isomer **8a** is greater than 1000-fold less potent. The dramatic difference in potency for the two 3S epimers **8a** and **8c** is also observed for the corresponding stereoisomers in the six-membered ACHPA-lactam series **9a** and **9b**. Preference for the 2S,3S ACHPA-lactam diastereomer contrasts previous findings[10a] wherein inhibitors containing 2R- or 2S-isobutylstatine showed a 70-fold potency advantage favoring the 2R,3S isomer. The reversed chirality preference at the 2-position for these two series of inhibitors is not clearly understood but is speculated to be related to the presence of additional amino acids at the C-terminus in the 2-alkyl statine series. Ring size is known to markedly affect the structure–activity profile of certain lactam-constrained peptides[11] and is addressed in this study with homologues **8c**, **9b**, and **10**, which clearly show a progressive deterioration of potency

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(18) Experimental details for the enzyme assays using purified human kidney renin, human pepsin, human gastricsin, endothiapepsin, cathepsin D, and cathepsin E are given: Jupp, R. A.; Dunn, B. M.; Jacobs, J. W.; Vlasuk, G.; Arcuri, K. E.; Veber, D. F.; Perlow, D. S.; Boger, J.; de Laszlo, S.; Chakravaraty, P. K.; tenBroeke, J.; Ondeyka, D.; Greenlee, W. J.; Kay, J. *Biochem. J.* **1990**, *266*, 871.

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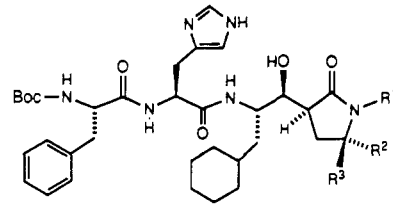
Table III. Effect of Lactam Ring Substitution on Renin Inhibition



no.	R^1	R^2	R^3	HPLC[a]	IC_{50},[b] nM: plasma renin assay	IC_{50},[b] nM: purified renin assay
8c	CH_3	H	H	98.2	9.3	5.5
26	CH_3	$CH{=}CH_2$	H	98.0	8.0	8.5
27	CH_3	CH_2CH_3	H	97.3	8.5	11
28	CH_3	H	$CH{=}CH_2$	96.7	2.5	1.5
29	CH_3	H	CH_2CH_3	97.7	5.3	3.3
30	CH_3	CH_2OCH_2Ph	H	98.0	4400	
31	CH_3	CH_2OAc	H	95.8	157	155
32	CH_3	CH_2OCH_3	H	93.8	71	40
33	CH_3	CH_2OH	H	95.6	14	10
34	CH_3	CH_3	CH_3	96.9	1.3	1.3
35	n-C_4H_9	CH_3	CH_3	95.8	2.4	1.9
36	c-C_6H_{11}	H	H	95.0	10	
37	$CH_2CH_2OCH_2Ph$	H	H	97.0	24	
38	CH_2CH_2OH	H	H	94.3	7.7	
39	$(CH_2)_3N(Cbz)CH_2Ph$	H	H	98.8	275	
40	$(CH_2)_3NHCH_2Ph$	H	H	97.7	14	

[a] Percent purity as determined by reverse-phase HPLC analysis, for details see the Experimental Section. [b] Assays performed at pH 7.4.

upon increasing lactam ring size. The optimal inhibitor, **8c**, exhibits an in vitro potency that is significantly improved over the structurally similar ACHPA amide **41** (Figure 5), and provides a reference point for evaluating the effect of additional substitution on the lactam ring.

Several analogues of **8c** in which the γ-lactam ring is substituted at the 1- and/or 5-positions were prepared in order to probe the enzyme for additional binding subsites (**26–40**, Table III). The ^1H NMR spectra (CD_3OD) of inhibitors **26–40** show chemical shifts and coupling constants for protons H_2, H_3, and H_4 that are similar to those in **8c**, and thus differences in potency are not ascribed to changes in the ground-state conformation. Comparison of 5-ethenyl epimers **26** and **28** indicates a preference for the latter isomer, the potency of which is improved over that of **8c**. Hydrogenation to the corresponding 5-ethyl analogues **27** and **29** was not beneficial. Disubstitution at the lactam 5-position provided the optimal inhibitor (**34**). The improved potency of **28** and **34** relative to **8c** is thought to be the result of having properly positioned substituents that can make favorable contacts with the S_1' subsite, the binding pocket that accommodates the hydrophobic P_1' side chain of angiotensinogen. Comparable potency in the ACHPA amide series is obtained only with higher molecular weight inhibitors in which the C-terminus includes structural elements corresponding to the P_2' and P_3' amino acids. Additional information on the environment around the lactam 5-position was provided by the monosubstituted analogues **30–33** derived from L-pyroglutamic acid. The progressive decrease in potency observed upon increasing substituent size in this series suggests that the enzyme cannot accommodate groups larger than ethyl.

The effect of lactam ring N-substitution was explored with inhibitors **35–40**. Substitution with linear (**35**) or cyclic (**36**) alkyl groups that could potentially mimic the hydrophobic P_2' side chain of angiotensinogen was found to have little effect on potency relative to their *N*-methyl counterparts **34** and **8c**, respectively. Relative to **8c**, large hydrophobic groups reduced potency (**37** and **39**), whereas

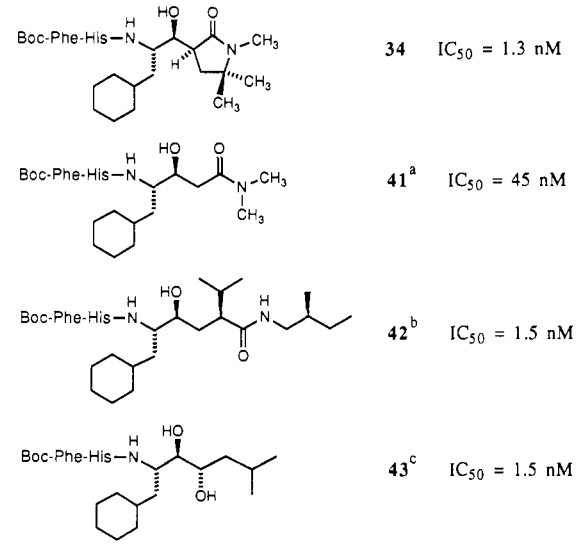


Figure 5. Comparison of **34** with other renin inhibitors which contain non-peptide C-termini. [a]L. S. Payne, unpublished result from these laboratories. [b]Unpublished result: S. de Laszlo, W. Greenlee; Merck Sharp and Dohme Research Laboratories, Rahway, NJ. [c]See ref 20j in the text.

polar and charged groups were tolerated (**38** and **40**). From these analogues it is concluded that the ACHPA-lactam N-position tolerates a range of substituents, including charged groups which can be used to influence physical properties. Notable in this series is inhibitor **36**, which has been obtained in crystalline form of sufficient quality to allow single-crystal X-ray diffraction analysis (Figure 4).

In Vivo Activity. The in vivo properties of **8c** were investigated by intravenous and oral administration to conscious, sodium-depleted rhesus monkeys and monitoring plasma renin activity (PRA) as a function of time. As shown in Figure 6, **8c** given orally at 50 mg/kg as a citric acid suspension produced a maximum effect of 40% inhibition of PRA at approximately 1 h, and after 3 h PRA had returned to baseline. Because of the low inhibition

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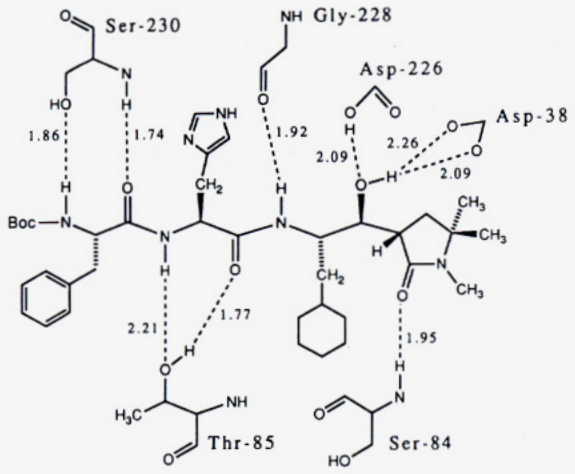

Figure 8. Schematic representation of the hydrogen-bond network of **34** in the human renin model. Distances are in angstroms.

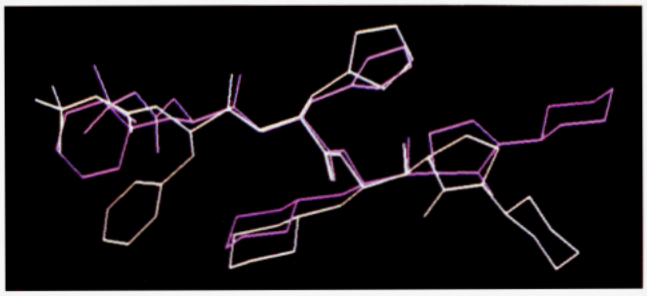

Figure 9. Superposition of the solid-state conformation of **36** (purple) and the modeled enzyme-bound conformation (yellow).

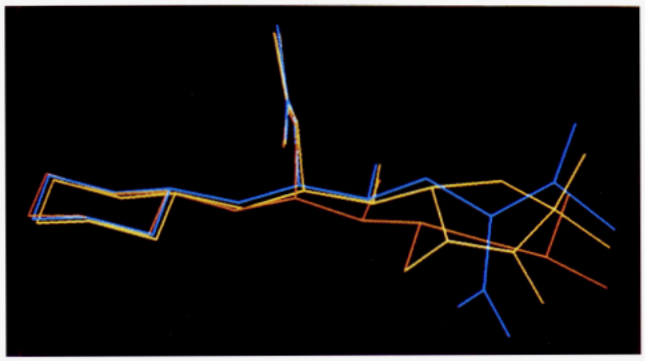

Figure 10. Superposition of the modeled enzyme bound conformations of **34** (yellow), alkyl diol **43** (red), and hydroxyethylene isostere **42** (blue) C-termini. The 2-methylbutyl side chain of **42** has been omitted for clarity.

with the sequences of the fungal proteases. The aligned human sequence was then mapped onto the superimposed X-ray structures of the fungal aspartyl proteases. The structurally conserved regions were taken from the rhizopuspepsin X-ray structure while the variable regions were taken from protease structure whose sequence best aligned with the human sequence in that region. Coordinates for the renin model are available in Protein Data Bank format as supplementary material. X-ray structures for several fungal enzyme–inhibitor complexes[25] provided a starting conformation for docking the ACHPA–lactam inhibitors in the human renin model. Molecular geometries for the docked inhibitors were created with the Merck molecular modeling system MOLEDIT[26] and were energy minimized in the static active site by using OPTIMOL, a modified MM2 force field program.[27]

Comparison of the docked conformations of **34** and residues 7–13 of angiotensinogen in which the Leu^{10} amide carbonyl is hydrated produced a good overlap of the *gem*-dimethyl group in **34** with the Val^{11} side chain, suggesting that the increased potency of **34** compared to that of **8c** is the result of incorporating a P_1' side chain mimic. Additionally, the lactam carbonyl oxygen in the docked conformation of **34** is within hydrogen bonding distance to the backbone N–H of Ser^{84} in the "flap" region of the enzyme. Graphic and schematic representations of the enzyme-bound form of **34** are given in Figures 7 and 8, respectively. This model of the enzyme–inhibitor complex is consistent with the experimental data obtained for the epimerically related inhibitors **8a** and **8c** in that the poorly binding epimer **8a** which has the "wrong" chirality at C2 (IC_{50} > 20 000 nM) cannot form the aforementioned hydrogen bond to the flap without the rest of the lactam ring encountering unfavorable van der Waals contacts with the enzyme. The substituent on the lactam nitrogen of **34** can be oriented in a direction away from the binding cleft, an arrangement which is consistent with the experimental results for inhibitors **35–40** that indicate a tolerance for a wide variety of substituents at this position. The binding model, however, does not adequately account for the trend in potency observed for inhibitors **30–33** in that the enzyme model provides a steric environment around the face of the lactam bearing the 5-position substituent that should accommodate groups larger than ethyl. This area in the human enzyme model contains a polyproline sequence (residues 306–311) that is not found in the fungal enzymes, and the results for inhibitors **30–33** suggest that this region of the model may need modification.

The solid-state conformation of inhibitor **36** obtained by single-crystal X-ray diffraction analysis shows a remarkable similarity to the enzyme-bound conformation obtained by using our renin model (Figure 9). Rotations of 60° and 95° about the Phe ψ and ACHPA–lactam C2–C3 bonds, respectively, in the solid-state conformer produces a backbone conformation which closely resembles that of the modeled bound conformer. To the extent that the observed solid-state conformation of **36** might resemble its solution conformation, our model of the enzyme–inhibitor complex suggests that only a minimal amount of torsional reorganization would be required to adopt a good conformation for binding to the enzyme.

The in vitro potency of the optimized ACHPA–lactam inhibitor **34** is very similar to inhibitors that contain hydroxyethylene isostere and alkyl diol[28j] non-peptide C-

(25) (a) Foundling, S. I.; Cooper, J.; Watson, F. E.; Cleasby, A.; Pearl, L. H.; Sibanda, B. L.; Hemmings, A.; Wood, S. P.; Blundell, T. L.; Valler, M. J.; Norey, C. G.; Kay, J.; Boger, J.; Dunn, B. M.; Leckie, B. J.; Jones, D. M.; Atrash, B.; Hallet, A.; Szelke, M. *Nature* **1987**, *327*, 349. (b) Davies, D.; Suguna, K. Preliminary coordinate sets for enzyme-inhibitor complexes of rhizopuspepsin were graciously provided.

(26) Gund, P.; Andose, J. D.; Rhodes, J. B.; Smith, G. M. *Science* **1980**, *208*, 1425. (b) Smith, G. M.; Hangauer, D. G.; Andose, J. D.; Bush, B. L.; Fluder, E. M.; Gund, P.; McIntyre, E. F. *Drug. Inf. J.* **1984**, *18*, 167.

(27) Halgren, T. A.; Merck, Sharpe and Dohme Research Laboratories, Rahway, NJ; unpublished work on the development of the force field program OPTIMOL. OPTIMOL differs from MM2 (Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127) mainly in the use of partial charges on atoms, instead of bond dipoles, and in the absence of unshared pairs of electrons on certain nitrogen and oxygen atoms.

termini[28] (42 and 43, respectively, Figure 5). Inhibitors 42 and 43 were energy minimized in the renin active site model by using a hydrogen-bonding scheme analogous to that of 34. Superposition of the C-termini in the three-docked structures (Figure 10) shows that it is possible to obtain a good spatial overlap of the P_1 side chain and transition-state hydroxyl common to each structure, as well as hydrophobic groups that could mimic the P_1' side chain and a polar group capable of accepting a hydrogen bond from the enzyme. This observation suggests that each of these non-peptide C-termini represents a unique structural motif for accomplishing the same set of interactions with the enzyme.[29]

Conclusion

A new series of renin inhibitors containing a lactam-constrained ACHPA group (ACHPA–lactam) at the P_1-P_1' site was developed. These lactams offer special advantage in that their conformational rigidity allows structural modifications which provide new insights about the active site of renin. Docking experiments with the most potent of these inhibitors, 34 (IC_{50} = 1.3 nM), in a model of human renin suggests that the hydrophobic portion of the γ-lactam ring can mimic the P_1' side chain of angiotensinogen, a binding element which is absent in the ACHPA design. Compared to ACHPA-containing inhibitors of similar potency, 34 represents a significant reduction in molecular size and peptide character. Improved pharmacokinetic properties, however, were not observed upon intravenous or oral administration of 8c to the rhesus monkey. The ACHPA–lactam variation thus represents another step on the path to designing true peptidomimetics. Perhaps modifications to the Phe-His portion of these molecules[21] will yield the long-sought renin inhibitor for testing as a therapuetic agent.

Experimental Section

^1H NMR spectra were obtained at 300 MHz on a Varian XL-300 or at 360 MHz on a Nicolet NT-360 spectrometer. Analytical HPLC data were obtained by either of two methods: method A used a Spectra-Physics instrument (SP8800 pump, SP8480 detector, SP4270 integrator) equipped with a Vydak C_{18} reverse-phase column (150 × 2.1 mm) at ambient temperature, a 95:5 to 0:100 A/B linear 15 min gradient, 2.00 mL/min flow rate (A = 0.1% TFA in H_2O, B = 0.1% TFA in CH_3CN), and detection at 210 nm; and method B used a Hewlett-Packard 1084B instrument equipped with a Waters C_{18} reverse-phase column (300 × 2.1 mm) at 40 °C, a 95:5 to 5:95 A/B linear 30 minute gradient, 3.00 mL/min flow rate (A = 0.1% H_3PO_4 in H_2O, B = 0.1% H_3PO_4 in CH_3CN), and detection at 210 nm. Preparative HPLC was performed on a SepTech 800B unit, using a C_{18} reverse-phase Waters Delta Pak prep cartridge (30 × 5 cm) with a 95:5 to 0:100 A/B linear 45 min gradient, 40 mL/min flow rate (A = 0.1% TFA in H_2O, B = 0.1% TFA in CH_3CN), and detection at 210 nm. Flash chromatography was performed on E. Merck silica gel (230–400 mesh). Thin-layer chromatography was performed on E. Merck 60F-254 precoated silica gel plates (0.25 mm). Visualization was accomplished with UV light and/or phosphomolybdic acid stain. FAB mass spectra were obtained on a VG MM Zab-HF spectrometer at 8 keV. Tetrahydrofuran was distilled from lithium aluminum hydride, dry dichloromethane was obtained by distillation from calcium hydride, and dioxane was passed through a column of activity I neutral alumina (70–230 mesh, E. Merck) to remove peroxides.

5(*R*)- and 5(*S*)-(Hydroxymethyl)-1-methyl-2-pyrrolidinone (5*S*-11 and 5*R*-11). To a suspension of NaH (19.4 g of a 60% suspension in mineral oil; 0.484 mol) in dry, degassed DMF (500 mL) under an atmosphere of N_2 was added D- or L-pyroglutamic acid (25 g, 0.19 mol) portionwise over a period of 15 min. The resulting suspension was stirred at ambient temperature for 8 h and cooled to 0 °C, and iodomethane (25 mL, 0.40 mol) was added. The mixture was stirred overnight, and the cooling bath was allowed to warm to ambient temperature. Acetic acid (3 mL) was added, and the DMF was removed at 30 °C under reduced pressure (2 Torr). The resulting thick precipitate was suspended in CH_2Cl_2 (200 mL), cooled to 0 °C, and filtered. The solvents were removed under reduced pressure, and residual DMF was removed by heating at 35 °C overnight at 0.1 Torr. The crude product was separated from immiscible mineral oil by pipet and was purified with flash chromatography (2%–5% MeOH/CH_2Cl_2). 5(*R*)- or 5(*S*)-carbomethoxy-1-methyl-2-pyrrolidinone was obtained as a pale yellow liquid (24 g; 80% yield). ^1H NMR ($CDCl_3$): δ 4.13 (dd, *J* = 3.4, 5.7 Hz, 1 H), 3.79 (s, 3 H), 2.86 (2, 3 H), 2.30–2.55 (m, 3 H), 2.09 (m, 1 H).

To a mechanically stirred −78 °C solution of 5(*R*)- or 5(*S*)-carbomethoxy-1-methyl-2-pyrrolidinone (22.0 g, 0.140 mol) in dry THF (400 mL) under an atmosphere of N_2 was added a solution of LAH (70 mL of a 1.0 M solution in THF, 70 mmol) dropwise over a period of 20 min. After being stirred for 1 h at −78 °C, the cooling bath was removed and stirring was continued for another 1.5 h. The reaction was cooled to 0 °C and quenched by the addition of 7 mL of 10% aqueous NaOH solution, followed by 7 mL of water. The resulting mixture was stirred for 2 h, until a white precipitate had formed. The precipitate was removed by filtration and was washed with EtOAc. The filtrate solvents were removed under reduced pressure, and the resulting oil was purified with flash chromatography (3%–7% MeOH/CH_2Cl_2). The product was obtained as a colorless oil (13.5 g, 75% yield). ^1H NMR (CD_3OD): δ 3.78 (dd, *J* = 3.3, 11.8 Hz, 1 H), 3.62 (sextet, *J* = 3.5 Hz, 1 H), 3.55 (dd, *J* = 3.3, 11.8 Hz, 1 H), 2.84 (s, 3 H), 2.43 (m, 1 H), 2.29 (m, 1 H), 2.13 (m, 1 H), 1.92 (m, 1 H). TLC R_f: 0.25 (5% MeOH/$CHCl_3$). FAB MS: *m/z* 130 (M^+ + H).

1-Methyl-5(*R*)- and 5(*S*)-vinyl-2-pyrrolidinone (5*S*-12 and 5*R*-12). To a −78 °C solution of oxalyl chloride (9.44 g, 74.3 mmol) in dry CH_2Cl_2 (200 mL) under an atmosphere of N_2 was added DMSO (8.80 g, 124 mmol) dropwise over a period of 5 min. After being stirred for 15 min, a solution of 5*R*- or 5*S*-11 (8.00 g, 62.0 mmol) in dry CH_2Cl_2 (15 mL) was added dropwise over a period

(28) While this work was in progress, a number of renin inhibitors containing structurally unique non-peptide C-termini were reported: (a) Thaisrivongs, S.; Pals, D. T.; Kroll, L. T.; Turner, S. R.; Han, F.-S. *J Med. Chem.* **1987**, *30*, 976. (b) Kempf, D. J.; DeLara, E.; Stein, H. H.; Cohen, J.; Plattner, J. J. *J. Med. Chem.* **1987**, *30*, 1978. (c) Luly, J. R.; Yi, N.; Sonderquist, J.; Stein, H.; Cohen, J.; Perun, T. J.; Plattner, J. J. *J. Med. Chem.* **1987**, *30*, 1609. (d) Bolis, G.; Fung, A. K. L.; Greer, J.; Kleinert, H. K.; Marcotte, P. A.; Perun, T. J.; Plattner, J. J.; Stein, H. H. *J. Med. Chem.* **1987**, *30*, 2137. (e) Dellaria, J. F.; Maki, R. G.; Bopp, B. A.; Cohen, J.; Kleinert, H. D.; Luly, J. R.; Merits, I.; Plattner, J. J.; Stein, H. H. *J. Med. Chem.* **1987**, *30*, 2137. (f) Rosenberg, S. H.; Plattner, J. J.; Woods, K. W.; Stein, H. H.; Marcotte, P. A.; Cohen, J.; Perun, T. J. *J. Med. Chem.* **1987**, *30*, 1224. (g) Sham, H. L.; Stein, H.; Rempel, C. A.; Cohen, J., Plattner, J. J. *FEBS Lett.* **1987**, *220*, 299. (h) Hanson, G. J.; Baran, J. S.; Lowrie, H. S.; Jarussi, S. J.; Yang, P.-C.; Babler, M.; Bittner, S. E.; Papaioannou, S. E.; Walsh *Biochem. Biophys. Res. Commun.* **1987**, *146*, 959. (i) Iizuka, K.; Kamijo, T.; Kubota, T.; Akahane, K.; Umeyama, H.; Kiso, Y. *J. Med. Chem.* **1988**, *31*, 704. (j) 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.; Green, J.; Perun, T. J., Plattner, J. J. *J. Med. Chem.* **1988**, *31*, 2264. (k) Buehlmayer, P.; Caselli, A.; Fuhrer, W.; Goeschke, R.; Rasetti, V.; Rueger, H.; Stanton, J. L.; Criscione, L.; Wood, J. M. *J. Med. Chem.* **1989**, *31*, 1839. (l) Rosenberg, S. H.; Dellaria, J. F.; Kempf, D. J.; Hutchins, C. W.; Woods, K. W.; Maki, R. G.; de Lara, E.; Spina, K. P.; Stein, H. H.; Cohen, J.; Baker, W. R.; Plattner, J. J.; Kleinert, H. D.; Perun, T. J. *J. Med. Chem.* **1990**, *32*, 1582.

(29) The Abbott group originally proposed a different conformation and mode of interaction for the alkyl diol portion of inhibitor 43 in their model of human renin (ref 28j). More recent work from Abbott (ref 28l) and also work from Searle (Hanson, G. H.; Baran, J. S.; Clare, M.; Williams, K.; Babler, M.; Bittner, S. E.; Russel, M. A.; Papaioannou, S. E.; Yang, P.-C.; Walsh, G. M. *Peptides: Chemistry, Structure, and Biology. Proceedings of the Eleventh American Peptide Symposium*; Rivier, J. E., Marshall, G. R., Eds.; ESCOM: Leiden, 1990; p 396) suggests an arrangement similar to what is proposed here.

of 5 min. The resulting solution was stirred at −78 °C for 30 min, at which time Et_3N (21.3 mL, 153 mmol) was added. The cooling bath was removed and the mixture was stirred for an additional 45 min. Ether (200 mL) was added, the mixture was cooled to 0 °C and filtered through Celite. The filter cake was washed with ether and the filtrate was concentrated at 20 °C under reduced pressure. Residual DMSO was removed at 0.1 Torr. The crude aldehyde was dissolved in dry THF (40 mL) and was added via cannula to a −78 °C solution of methylenetriphenylphosphorane, the preparation of which is given below. To a suspension of methyltriphenylphosphonium bromide (46.6 g, 131 mmol) in dry THF (250 mL) at −78 °C under an atmosphere of N_2 was added *n*-butyllithium (78 mL of a 1.6 M solution in hexane; 125 mmol). The yellow-orange solution was stirred at −78 °C for 30 min, at 0 °C for 1 h, and then cooled to −78 °C. After addition of the aldehyde solution, the reaction mixture was stirred at −78 °C for 4 h and was then allowed to warm to ambient temperature overnight. Acetic acid (4 mL) was added and the thick, pasty mixture was diluted with 2 volumes of ether and cooled to 0 °C. The solids were removed by filtration and the filter cake was washed with cold ether. The filtrate was concentrated under reduced pressure and additional triphenylphosphine oxide was removed by filtration. The filtrate solvents were removed under reduced pressure and the crude product was purified by flash chromatography (2:15:85 MeOH/ether/CH_2Cl_2). The product was further purified by bulb-to-bulb distillation (60 °C bath temperature, 0.3 Torr) to give a colorless liquid (3.40 g, 44% yield over two steps). ^1H NMR ($CDCl_3$): δ 5.68 (ddd, J = 8.3, 10.1, 17.0 Hz, 1 H), 5.27 (d, J = 10.1 Hz, 1 H), 5.23 (d, J = 17.0 Hz, 1 H), 3.92 (q, J = 7.9 Hz, 1 H), 2.75 (s, 3 H), 2.35–2.45 (m, 2 H), 2.26 (m, 1 H), 1.75 (m, 1 H). TLC R_f: 0.51 (5% MeOH/$CHCl_3$). HPLC (method A): 4.18 min. FAB MS: m/z 126 (M^+ + H).

5(*S*)-(Methoxymethyl)-1-methyl-2-pyrrolidinone (13a). To a suspension of NaH (1.53 g of a 60% suspension in mineral oil, 38.1 mmol) in dry THF (200 mL) under an atmosphere of N_2 was added a solution of 3*S*-11 (4.10 g, 31.8 mmol) in dry THF (15 mL). The resulting suspension was stirred at ambient temperature for 4 h and then cooled to 0 °C, at which time iodomethane (2.37 mL, 38.1 mmol) was added. The mixture was stirred overnight, and the cooling bath was allowed to warm to ambient temperature. Acetic acid (1 mL) was added, the mixture was diluted with 2 volumes of ether and cooled to 0 °C. The salts were removed by filtration and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography (2–4% MeOH/CH_2Cl_2). The product was further purified by bulb-to-bulb distillation (bath temperature 60 °C, 0.2 Torr), to give **13a** as a pale yellow liquid (3.72 g, 82% yield). ^1H NMR (CD_3OD): δ 3.72 (sextet, J = 4.1 Hz, 1 H), 3.59 (dd, J = 3.4, 10.3 Hz, 1 H), 3.41 (dd, J = 4.5, 10.3 Hz, 1 H), 3.36 (s, 3 H), 2.81 (s, 3 H), 2.40 (m, 1 H), 2.26 (m, 1 H), 2.12 (m, 1 H), 1.86 (m, 1 H). TLC R_f: 0.45 (5% MeOH/$CHCl_3$). HPLC (method A): 2.96 min. FAB MS: m/e 144 (M^+ + H).

5(*S*)-[(Benzyloxy)methyl]-1-methyl-2-pyrrolidinone (13b). The title compound was prepared in a manner analogous to that of **13a** with the exception that benzyl bromide was used in place of iodomethane. The crude product was purified by flash chromatography (25–40% EtOAc/CH_2Cl_2) to give **13b** as a colorless liquid (10.6 g, 96% yield). ^1H NMR ($CDCl_3$): δ 7.3–7.4 (m, 5 H), 4.51 (s, 2 H), 3.68 (sextet, J = 4.6 Hz, 1 H), 3.56 (dd, J = 3.9, 10.2 Hz, 1 H), 3.48 (dd, J = 4.9, 10.2 Hz, 1 H), 2.85 (s, 3 H), 2.47 (m, 1 H), 2.31 (m, 1 H), 2.12 (m, 1 H), 1.83 (m, 1 H). TCL R_f: 0.58 (5% MeOH/$CHCl_3$). HPLC (method A): 6.80 min. FAB MS: m/z 220 (M^+ + H).

1,5,5-Trimethyl-2-pyrrolidinone (14a). To a 0 °C solution of 5,5-dimethyl-2-pyrrolidinone[16] (21.0 g, 184 mmol) in dry degassed DMF (600 mL) under an atmosphere of N_2 was added iodomethane (23.2 mL, 372 mmol). NaH (8.80 g of a 60% suspension in mineral oil; 220 mmol) was added in portions over a period of 20 min. The mixture was stirred at 0 °C for 2 h and then at ambient temperature for 16 h. Acetic acid (3 mL) was added, and the bulk of the DMF was removed under reduced pressure. Ether (250 mL) was added and the suspension was cooled to 0 °C and filtered, and the filtrate solvents were removed under reduced pressure. The crude product was separated from immiscible mineral oil by pipet and was distilled under reduced pressure, giving 18.4 g of **14a** (78% yield) as a colorless liquid, bp 47–49 °C (1.0 Torr). ^1H NMR ($CDCl_3$): δ 2.73 (s, 3 H), 2.41 (t, J = 7.9 Hz, 2 H), 1.87 (t, J = 7.9 Hz, 2 H), 1.22 (s, 6 H). HPLC (method A): 4.16 min. FAB MS: m/z 128 (M^+ + H).

1-Butyl-5,5-dimethyl-2-pyrrolidinone (14b). The title compound was prepared in a manner analogous to that of **14a** with the exception that 1-bromobutane was used in place of iodomethane. The crude product was distilled under reduced pressure to give **13b** (12.7 g, 71% yield) as a colorless liquid, bp 72–75 °C (0.2 Torr). ^1H NMR ($CDCl_3$): δ 3.08 (t, J = 7.9 Hz, 2 H), 2.35 (t, J = 7.9 Hz, 2 H), 1.81 (t, J = 7.9 Hz, 2 H), 1.51 (m, 2 H), 1.32 (m, 2 H), 1.21 (s, 6 H), 0.90 (t, J = 7.2 Hz, 3 H). HPLC (method A): 6.99 min. FAB MS: m/z 170 (M^+ + H).

1-[2-(Benzyloxy)ethyl]-2-pyrrolidinone (15). To a suspension of NaH (9.30 g of a 60% suspension in mineral oil; 233 mmol) in dry THF (300 mL) under an atmosphere of N_2 was added a solution of 1-(2-hydroxyethyl)-2-pyrrolidinone (25.0 g, 194 mmol) in dry THF (25 mL) dropwise over a period of 30 min. The resulting mixture was stirred at ambient temperature for 3 h, at which time it was cooled to 0 °C and benzyl bromide (27.7 mL, 233 mmol) was added. The mixture was stirred overnight and the cooling bath was allowed to warm to room temperature. Acetic acid (2 mL) and an equal volume of ether were added. The suspension was cooled to 0 °C and filtered. The filtrate was concentrated under reduced pressure and the resulting oil was purified by flash chromatography (20% EtOAc/CH_2Cl_2) to give **15** as a colorless liquid (39.9 g, 94% yield). ^1H NMR ($CDCl_3$): δ 7.3–7.4 (m, 5 H), 4.50 (s, 2 H), 3.62 (t, J = 5.2 Hz, 2 H), 3.60 (m, 4 H), 2.38 (t, J = 7.5 Hz, 2 H), 2.00 (quintet, J = 7.2 Hz, 2 H). HPLC (method A): 6.67 min. FAB MS: m/z 220 (M^+ + H).

1-[3-[*N*-Benzyl-*N*-(benzyloxycarbonyl)amino]propyl]-2-pyrrolidinone (16). To a 0 °C solution of 1-(3-aminopropyl)-2-pyrrolidinone (25.0 g, 176 mmol) and Et_3N (37.2 mL, 264 mmol) in CH_2Cl_2 (500 mL) was added benzyl chloroformate (33.1 g, 194 mmol) dropwise over a period of 15 min. The mixture was stirred for 1.5 h at 0 °C and then for 15 h at ambient temperature. The mixture was diluted with CH_2Cl_2 and extracted with 5% aqueous HCl (200 mL), H_2O (200 mL), and saturated aqueous $NaHCO_3$ (200 mL). The organic phase was dried ($MgSO_4$) and filtered, and the solvents were removed under reduced pressure. The crude product was purified by flash chromatography (2–5% MeOH/CH_2Cl_2), giving 1-[3-[(benzyloxycarbonyl)amino]propyl]-2-pyrrolidinone as a colorless oil (42.7 g, 88% yield). ^1H NMR ($CDCl_3$): δ 7.3–7.4 (m, 5 H), 5.70 (br t, 1 H), 5.10 (2, 2 H), 3.36 (t, J = 7.1 Hz, 2 H), 3.33 (t, J = 6.2 Hz, 2 H), 3.15 (q, J = 6.2 Hz, 2 H), 2.38 (t, J = 8.0 Hz, 2 H), 2.01 (quintet, J = 7.6 Hz, 2 H), 1.69 (quintet, J = 6.2 Hz, 2 H).

To a suspension of NaH (3.62 g of a 60% suspension in mineral oil; 90.6 mmol) in dry degassed DMF (300 mL) was added a solution of 1-[3-[(benzyloxycarbonyl)amino]propyl]-2-pyrrolidinone (20.0 g, 72.5 mmol) in DMF (50 mL). The mixture was stirred at ambient temperature for 4 h, cooled to 0 °C, and to it was added benzyl bromide (10.4 mL, 87.0 mmol). The resulting mixture was stirred at 0 °C for 2 h, and then at ambient temperature for 15 h. The mixture was diluted with an equal volume of ether and cooled to 0 °C, and the salts were removed by filtration. The filtrate solvents were removed under reduced pressure, and the crude product was purified by flash chromatography (2–5% MeOH/CH_2Cl_2) to give **16** as a colorless oil (22.3 g, 84% yield). ^1H NMR ($CDCl_3$): δ 7.1–7.4 (m, 10 H), 5.17 (s, 2 H), 4.50 (s, 2 H), 3.1–3.4 (m, 6 H), 2.2–2.4 (m, 2 H), 1.6–2.0 (m, 4 H). HPLC (method A): 8.70 min. FAB MS: m/z 367 (M^+ + H).

General Procedure for the Preparation of ACHPA-Lactams 3a–d, 4a–d, 5d, and 17–25. ACHPA-lactams **17–25** are the third-eluting isomers obtained by chromatographic separation of the crude aldol mixture on silica gel.

To a 0 °C solution of diisopropylamine (3.39 mL, 31.3 mmol) in dry THF (100 mL) under an atmosphere of N_2 was added *n*-butyllithium (19.3 mL of a 1.6 M solution in hexane, 30.8 mmol). After being stirred for 10 min, the solution was cooled to −78 °C, at which time a solution of 1-methyl-2-pyrrolidinone (2.98 mL, 31.0 mmol) in dry THF (10 mL) was added dropwise over a period of 5 min. The resulting solution was stirred at −78 °C for 1.5 h, when a −78 °C solution of *N*-Boc-L-cyclohexylalaninal[9] (7.5 g, 29 mmol) in dry THF (50 mL) was added rapidly via cannula. After

being stirred for 5 min at 78 °C, the reaction was quenched by the addition of 5 mL of water. The cooling bath was removed, and more water (25 mL) and EtOAc (125 mL) were added. The layers were separated, and the organic phase was extracted with 5% aqueous HCl (150 mL) and saturated aqueous $NaHCO_3$ (150 mL), dried ($MgSO_4$), and filtered. Removal of the solvents under reduced pressure gave a viscous oil. Separation of the diastereomeric aldol products was accomplished by using flash chromatography (2–5% MeOH/CH_2Cl_2).

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*S*)-hydroxy-1-(1-methyl-2-oxopyrrolidin-3(*R*)-yl)propane (3a) was obtained as an oil (1.8 g, 17% yield). ^1H NMR (CD_3OD): δ 3.69 (m, 1 H), 3.58 (dd, J = 1.7, 9.3 Hz, 1 H), 3.3–3.4 (m, 2 H), 2.82 (s, 3 H), 2.49 (q, J = 9.3 Hz, 1 H), 2.23 (m, 1 H), 1.44 (s, 9 H). HPLC (method A): 10.32 min. FAB MS: m/z 355 (M^+ + H).

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*R*)-hydroxy-1-(1-methyl-2-oxopyrrolidin-3-yl)propane (3b) was obtained as a foam (1.5 g, 15% yield). ^1H NMR (CD_3OD): δ 3.69 (d, J = 6.1 Hz, 1 H), 3.7 (m, 1 H; overlaps with resonance at 3.69 ppm), 3.3–3.4 (m, 2 H), 2.82 (s, 3 H), 2.62 (ddd, J = 6.1, 8.9, 9.3 Hz, 1 H), 2.12 (m, 1 H), 1.85–2.05 (m, 2 H), 1.55–1.75 (m, 4 H), 1.43 (s, 9 H). HPLC (method A): 9.56 min. FAB MS: m/z 355 (M^+ + H).

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*S*)-hydroxy-1-(1-methyl-2-oxopyrrolidin-3(*S*)-yl)propane (3c) was obtained as a white solid (1.6 g, 16% yield) and was recrystallized from CH_2Cl_2/hexanes, mp 177–179 °C. ^1H NMR (CD_3OD): δ 3.95 (dd, J = 2.2, 4.2 Hz, 1 H), 3.72 (m, 1 H), 3.36 (m, 2 H), 2.83 (s, 3 H), 2.59 (br t, J = 9 Hz), 2.23 (dq, J_d = 12.8 Hz, J_q = 8.7 Hz, 1 H), 1.98 (m, 2 H), 1.44 (s, 9 H). HPLC (method A): 9.03 min. FAB MS: m/z 355 (M^+ + H).

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*R*)-hydroxy-1-(1-methyl-2-oxopyrrolidin-3-yl)propane (3d) was obtained as a foam (1.3 g, 13% yield). ^1H NMR (CD_3OD): δ 3.75 (dd, J = 1.8, 9.4 Hz, 1 H), 3.55 (m, 1 H), 3.3–3.4 (m, 2 H), 2.82 (s, 3 H), 2.66 (br t, J = 9 Hz, 1 H), 2.05–2.15 (m, 2 H), 1.92 (m 2 H), 1.6–1.8 (m, 4 H), 1.43 (s, 9 H). HPLC (method A): 8.69 min. FAB MS: m/z 355 (M^+ + H).

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*S*)-hydroxy-1-(1-methyl-2-oxopiperidin-3(*R*)-yl)propane (4a) was obtained as an oil (1.1 g, 21% yield). ^1H NMR (CD_3OD): δ 3.81 (m, 1 H), 3.67 (dd, J = 1.7, 9.0 Hz, 1 H), 3.3–3.4 (m, 2 H), 2.92 (s, 3 H), 2.30 (dd, J = 5.8, 9.0, 10.2 Hz, 1 H), 2.10 (m, 1 H), 1.90 (m, 2 H), 1.43 (s, 9 H). HPLC (method A): 10.89 min. FAB MS: m/z 369 (M^+ + H).

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*R*)-hydroxy-1-(1-methyl-2-oxopiperidin-3-yl)propane (4b) was obtained as a foam (0.90 g, 17% yield). ^1H NMR (CD_3OD): δ 3.97 (dd, J = 5.6, 6.8 Hz, 1 H), 3.72 (m, 1 H), 3.3–3.4 (m, 2 H), 2.91 (s, 3 H), 2.40 (m, 1 H), 1.95 (m, 2 H), 1.43 (s, 9 H). HPLC (method A): 10.12 min. FAB MS: m/z 369 (M^+ + H).

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*S*)-hydroxy-1-(1-methyl-2-oxopiperidin-3(*S*)-yl)propane (4c) was obtained as a foam (0.92 g, 18% yield). ^1H NMR (CD_3OD): δ 3.97 (dd, J = 3.7, 5.4 Hz, 1 H), 3.77 (m, 1 H), 3.32 (m, 2 H), 2.92 (s, 3 H), 2.47 (m, 1 H), 1.43 (s, 9 H). HPLC (method A): 9.44 min. FAB MS: m/z 369 (M^+ + H).

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*R*)-hydroxy-1-(1-methyl-2-oxopiperidin-3-yl)propane (4d) was obtained as a foam (0.80 g, 16% yield). ^1H NMR (CD_3OD): δ 4.02 (dd, J = 1.8, 9.9 Hz, 1 H), 3.60 (m, 1 H), 3.2–3.4 (m, 2 H), 2.92 (s, 3 H), 2.47 (br t, J = 8 Hz, 1 H), 1.42 (s, 9 H). HPLC (method A): 9.24 min. FAB MS: m/z 369 (M^+ + H).

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*S*)-hydroxy-1-(1-methyl-2-oxoperhydroazepin-3(*S*)-yl)propane (5d) was the fourth-eluting isomer obtained by flash chromatography (1–3% MeOH/CH_2Cl_2). (0.96 g, 13% yield). Crystallization from CH_2Cl_2/hexanes gave needles, mp 111–113 °C. ^1H NMR (CD_3OD): δ 3.89 (m, 1 H), 3.67 (d, J = 8.6 Hz, 1 H), 3.65 (m, 1 H), 3.19 (br dd, J = 3, 15 Hz, 1 H), 2.96 (s, 3 H), 2.63, (br t, J = 10 Hz, 1 H), 1.43 (s, 9 H). HPLC (method A): 9.41 min. FAB MS: m/z 383 (M^+ + H).

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*S*)-hydroxy-1-(1-methyl-5(*S*)-ethenyl-2-oxopyrrolidin-3-(*S*)-yl)propane (17). Crystallization from CH_2Cl_2/hexanes gave needles, mp 116–118 °C. ^1H NMR (CD_3OD): δ 5.72 (ddd, J = 8.3, 10.0, 17.1 Hz, 1 H), 5.23 (d, J = 17.1 Hz, 1 H), 5.20 (d, J = 10.0 Hz, 1 H), 3.9–4.0 (m, 2 H), 3.71 (m, 1 H), 2.74 (s, 3 H), 2.67 (br t, J = 9 Hz, 1 H), 2.51 (dt, J_t = 8.6 Hz, J_d = 12.6 Hz, 1 H), 1.44 (s, 9 H). HPLC (method A): 9.96 min. FAB MS: m/z 381 (M^+ + H).

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*S*)-hydroxy-1-(1-methyl-5(*R*)-ethenyl-2-oxopyrrolidin-3-(*S*)-yl)propane (18). ^1H NMR (CD_3OD): δ 5.71 (ddd, J = 9.0, 9.9, 17.1 Hz, 1 H), 5.35 (dd, J = 1.2, 17.1 Hz, 1 H), 5.25 (dd, J = 1.2, 9.9 Hz, 1 H), 3.99 (dd, J = 2.6, 3.8 Hz, 1 H), 3.90 (br q, J = 8 Hz, 1 H), 3.71 (m, 1 H), 2.71 (s, 3 H), 2.59 (br t, J = 9 Hz, 1 H), 2.16 (ddd, J = 7.5, 9.3, 12.9 Hz, 1 H), 1.95 (ddd, J = 8.2, 9.7, 12.9 Hz, 1 H), 1.46 (s, 9 H). HPLC (method A): 9.91 min. FAB MS: m/z 381 (M^+ + H).

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*S*)-hydroxy-1-[1-methyl-5(*S*)-(methoxymethyl)-2-oxopyrrolidin-3(*S*)-yl]propane (19). ^1H NMR (CD_3OD): δ 3.96 (dd, J = 1.8, 3.9 Hz, 1 H). 3.60–3.87 (m, 2 H), 3.55 (dd, J = 4.0, 10.0 Hz, 1 H), 3.40 (dd, J = 4.2, 10.0 Hz, 1 H), 3.34 (s, 3 H), 2.84 (s, 3 H), 2.68 (br t, J = 9 Hz, 1 H), 2.38 (dt, J_t = 9.0 Hz, J_d = 13.0 Hz, 1 H), 1.45 (s, 9 H). HPLC (method A): 9.23 min. FAB MS: m/z 399 (M^+ + H).

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*S*)-hydroxy-1-[1-methyl-5(*S*)-[(benzyloxy)methyl]-2-oxopyrrolidin-3(*S*)-yl]propane (20). ^1H NMR (CD_3OD): δ 7.25–7.35 (m, 5 H), 4.51 (s, 2 H), 3.94 (dd J = 2.2, 4.4 Hz, 1 H), 3.60–3.75 (m, 3 H), 3.48 (m, 1 H), 2.79 (s, 3 H), 2.71 (br t, J = 9 Hz, 1 H), 2.39 (dt, J_t = 9.3 Hz, J_d = 12.6 Hz, 1 H), 1.43 (s, 9 H). HPLC (method A): 10.95 min. FAB MS: m/z 475 (M^+ + H).

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*S*)-hydroxy-1-(1,5,5-trimethyl-2-oxopyrrolidin-3(*S*)-yl)-propane (21). An analytical sample was obtained by crystallization from ether, mp 85–87 °C. ^1H NMR (CD_3OD): δ 4.01 (dd, J = 2.4, 3.8 Hz, 1 H), 3.70 (m, 1 H), 2.72 (s, 3 H), 2.67 (br t, J = 9 Hz, 1 H), 2.06 (dd, J = 10.1, 12.5 Hz, 1 H), 1.87 (dd, J = 9.0, 12.5 Hz, 1 H), 1.44 (s, 9 H), 1.29 (s, 3 H), 1.17 (s, 3 H). HPLC (method A): 9.89 min. FAB MS: m/z 383 (M^+ + H). Anal. ($C_{21}H_{38}N_2O_4$): C, H, N.

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*S*)-hydroxy-1-(1-butyl-5,5-dimethyl-2-oxopyrrolidin-3-(*S*)-yl)propane (22). ^1H NMR (CD_3OD): δ 4.01 (dd, J = 2.6, 3.3 Hz, 1 H), 3.70 (m, 1 H), 3.22 (ddd, J = 6.1, 9.8, 13.9 Hz, 1 H), 3.05 (ddd, J = 6.1, 10.0, 13.9 Hz, 1 H), 2.65 (br t, J = 9 Hz, 1 H), 2.07 (dd, J = 0.4, 12.4 Hz, 1 H), 1.85 (dd, J = 9.1, 12.4 Hz, 1 H), 1.45 (s, 9 H), 1.31 (s, 3 H), 1.18 (s, 3 H), 0.93 (t, J = 7.3 Hz, 3 H). HPLC (method A): 11.20 min. FAB MS: m/e 425 (M^+ + H).

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*S*)-hydroxy-1-[1-[2-(benzyloxy)ethyl]-2-oxopyrrolidin-3-(*S*)-yl]propane (23). ^1H NMR (CD_3OD): δ 7.25–7.35 (m, 5 H), 4.50 (s, 2 H), 3.95 (dd, J = 2.4, 4.0 Hz, 1 H), 3.72 (m, 1 H), 3.3–3.6 (m, 6 H), 2.59 (dt, J_d = 2.3 Hz, J_t = 9.0 Hz, 1 H), 2.21 (dq, J_q = 8.8 Hz, J_d = 12.7 Hz, 1 H), 1.44 (s, 9 H). HPLC (method A): 10.98 min. FAB MS: m/z 475 (M^+ + H).

2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*S*)-hydroxy-1-[1-[3-[*N*-(carbobenzyloxy)-*N*-benzylamino]propyl]-2-oxopyrrolidin-3(*S*)-yl]propane (24). ^1H NMR (CD_3OD): δ 7.1–7.4 (m, 10 H), 5.17 (s, 2 H), 4.51 (s, 2 H), 3.90 (br m, 1 H), 3.70 (br m, 1 H), 3.1–3.4 (br m, 6 H), 2.50 (br m, 1 H), 1.45 (s, 9 H). HPLC (method A): 12.25 min. FAB MS: m/z 622 (M^+ + H).

2(*S*)-*tert*-[(Butyloxycarbonyl)amino]-3-cyclohexyl-1-(*S*)-hydroxy-1-(1-cyclohexyl-2-oxopyrrolidin-3(*S*)-yl)-propane (25). ^1H NMR (CD_3OD): δ 3.94 (dd, J = 2.4, 4.0 Hz, 1 H), 3.85 (m, 1 H), 3.72 (m, 1 H), 3.3–3.4 (m, 2 H), 2.60 (dt, J_d = 2.4 Hz, J_t = 9.0 Hz, 1 H), 1.44 (s, 9 H). FAB MS: m/z 423 (M^+ + H).

General Procedure for the Preparation of Oxazolidinones 6a–d and 7a–d. To a solution of the Boc-ACHPA–lactam (0.1 mmol) in dry CH_2Cl_2 (3 mL) was added TFA (1.5 mL). After being stirred at ambient temperature for 45 min, the solvent and excess TFA were evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 mL) and the solution was extracted twice with saturated aqueous $NaHCO_3$ (10 mL). The organic phase was dried ($MgSO_4$) and filtered, and the solvent was removed

under reduced pressure. The residue was dissolved in THF (5 mL) and to the solution was added imidazole (1.0 mmol) and 1,1'-carbonyldiimidazole (0.5 mmol). The mixture was stirred at ambient temperature for 18 h. The solvent was removed under reduced pressure and the residue was partitioned between $CHCl_3$ and H_2O. The organic phase was separated, washed with H_2O, dried ($MgSO_4$), filtered, and the solvent was removed under reduced pressure. The crude product was purified by passage through a short bed of silica gel using 2% $MeOH/CH_2Cl_2$, and the product was analyzed by ^1H NMR spectroscopy in $CDCl_3$ solution.

4(*S*)-(Cyclohexylmethyl)-5(*S*)-(1-methyl-2-oxopyrrolidin-3-yl)-1,3-oxazolidin-2-one (6a). ^1H NMR ($CDCl_3$): δ 6.08 (br s, 1 H), 5.22 (pentet, J = 4.6 Hz, 1 H), 4.49 (d, J = 3.9 Hz, 1 H), 3.3–3.4 (m, 2 H), 2.81 (s, 3 H), 2.35 (m, 1 H), 2.10 (m, 2 H).

4(*S*)-(Cyclohexylmethyl)-5(*R*)-(1-methyl-2-oxopyrrolidin-3-yl)-1,3-oxazolidinone (6b). ^1H NMR ($CDCl_3$): δ 5.48 (s, 1 H), 4.81 (dd, J = 4.2, 8.3 Hz, 1 H), 4.12 (dt, J_d = 3.4 Hz, J_t = 8.3 Hz, 1 H), 3.38 (m, 2 H), 2.96 (dt, J_d = 4.2 Hz, J_t = 8.8 Hz, 1 H), 2.84 (s, 3 H), 2.29 (m, 1 H), 2.10 (m, 1 H).

4(*S*)-(Cyclohexylmethyl)-5(*S*)-(1-methyl-2-oxopyrrolidin-3-yl)-1,3-oxazolidin-2-one (6c). ^1H NMR ($CDCl_3$): δ 5.60 (s, 1 H), 4.56 (t, J = 5.0 Hz, 1 H), 3.88 (dt, J_t = 6.8 Hz, J_d = 8.1 Hz, 1 H), 3.3–3.4 (m, 2 H), 2.85 (s, 3 H), 2.70 (dt, J_d = 4.8 Hz, J_t = 9.1 Hz, 1 H) 2.08 (m, 1 H).

4(*S*)-(Cyclohexylmethyl)-5(*R*)-(1-methyl-2-oxopyrrolidin-3-yl)-1,3-oxazolidin-2-one (6d). ^1H NMR ($CDCl_3$): δ 5.26 (s), 4.87 (t, J = 7.6 Hz, 1 H), 4.12 (dt, J_d = 2.9 Hz, J_t = 7.6 Hz, 1 H), 3.38 (m, 2 H), 2.82 (br t, J = 8 Hz, 1 H), 2.86 (s, 3 H), 2.27 (m, 1 H), 2.11 (m, 1 H).

4(*S*)-(Cyclohexylmethyl)-5(*S*)-(1-methyl-2-oxopiperidin-3-yl)-1,3-oxazolidin-2-one (7a). ^1H NMR ($CDCl_3$): δ 6.01 (s, 1 H), 4.77 (t, J = 4.4 Hz, 1 H), 3.80 (dt, J_d = 8.06 Hz, J_t = 4.8 Hz, 1 H), 3.25–3.40 (m, 2 H), 2.92 (s, 3 H), 2.79 (m, 1 H), 2.04 (m, 2 H).

4(*S*)-(Cyclohexylmethyl)-5(*R*)-(1-methyl-2-oxopiperidin-3-yl)-1,3-oxazolidinon-2-one (7b). ^1H NMR ($CDCl_3$): δ 5.43 (s, 1 H), 5.31 (dd, J = 4.9, 8.1 Hz, 1 H), 4.17 (ddd, J = 2.9, 8.1, 10.9 Hz, 1 H), 3.25–3.35 (m, 2 H), 2.92 (s, 3 H), 2.9 (m, 1 H; overlaps with resonance at 2.92 ppm), 2.14 (m, 1 H), 2.00 (m, 1 H).

4(*S*)-(Cyclohexylmethyl)-5(*S*)-(1-methyl-2-oxopiperidin-3-yl)-1,3-oxazolidin-2-one (7c). ^1H NMR ($CDCl_3$): δ 5.58 (br s, 1 H), 4.79 (t, J = 4.9 Hz, 1 H), 3.82 (m, 1 H), 3.2–3.3 (m, 2 H), 2.93 (s, 3 H), 2.44 (m, 1 H), 2.00 (m, 2 H).

4(*S*)-(Cyclohexylmethyl)-5(*R*)-(1-methyl-2-oxopiperidin-3-yl)-1,3-oxazolidin-2-one (7d). ^1H NMR ($CDCl_3$): δ 5.32 (s, 1 H), 4.70 (dd, J = 6.9, 9.7 Hz, 1 H), 4.18 (ddd, J = 2.9, 6.9, 10.3 Hz, 1 H), 3.3–3.4 (m, 2 H), 2.92 (s, 3 H), 2.70 (ddd, J = 5.4, 9.7, 15.1 Hz, 1 H), 2.24 (m, 1 H), 2.00 (m, 1 H).

General Procedure for Amino Acid Couplings To Obtain Compounds 8a–d, 9a,b, 10, 26, 28, 30, 32, 34–37, and 39.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-(1-methyl-2-oxopyrrolidin-3(*S*)-yl)-propane (8c). Step 1. A solution of **3c** (1.50 g, 4.24 mmol) in CH_2Cl_2 (3 mL) and TFA (2 mL) was stirred for 45 min at ambient temperature. The solvents were removed under reduced pressure and a solid TFA salt (1.46 g, 93% yield) was obtained by trituration in ether. To a suspension of N^{α}-(*tert*-butoxycarbonyl)-N^{im}-(2,4-dinitrophenyl)-L-histidine (2.23 g, 5.30 mmol) in dry EtOAc (20 mL) under an atmosphere of N_2 was added *N*-methylmorpholine (0.64 mL, 5.8 mmol). The resulting solution was cooled to −23 °C when isobutyl chloroformate (0.66 mL, 5.1 mmol) was added, and the mixture was stirred at −23 °C for 30 min. At this time a solution of the TFA salt in dry, degassed DMF (3 mL) containing *N*-methylmorpholine (0.56 mL, 5.1 mmol) was added via cannula to the cold solution of mixed anhydride. The reaction was stirred at −23 °C for 1 h, at 0 °C for 1 h, and then at ambient temperature for 1.5 h. Water (20 mL) and EtOAc (75 mL) were added to the reaction, and the organic phase was washed with 5% aqueous HCl (100 mL), water (20 mL), and saturated aqueous $NaHCO_3$ (100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give an orange solid which was purified by flash chromatography (3–5% $MeOH/CH_2Cl_2$). The product was obtained as a yellow foam (2.08 g, 75% yield).

Step 2. A solution of the product from step 1 (2.08 g, 3.17 mmol) in CH_2Cl_2 (3 mL) and TFA (2 mL) was stirred for 45 min at ambient temperature. The solvents were removed under reduced pressure and a yellow solid (2.04 g, 96% yield) was obtained upon trituration in ether. To a −23 °C solution of N^{α}-(*tert*-butoxycarbonyl)-L-phenylalanine (1.05 g, 3.96 mmol) in dry EtOAc (15 mL) under an atmosphere of N_2 was added *N*-methylmorpholine (0.48 mL, 4.3 mmol) followed by isobutyl chloroformate (0.49 mL, 3.8 mmol) and the resulting solution was stirred at −23 °C for 30 min. At this time, a solution of the TFA salt in dry EtOAc (15 mL) containing *N*-methylmorpholine (0.38 mL, 3.5 mmol) was added via cannula to the cold solution of mixed anhydride. The reaction was stirred at −23 °C for 1 h, at 0 °C for 1 h, and then at ambient temperature for 1.5 h. Water (20 mL) and EtOAc (75 mL) were added to the reaction, and the organic phase was washed with 5% aqueous HCl (100 mL), water (20 mL), and saturated, aqueous $NaHCO_3$ (100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give an orange solid which was purified by flash chromatography (4–7% $MeOH/CH_2Cl_2$). The product was obtained as a yellow foam (2.24 g, 88% yield).

Step 3. The product obtained from step 2 (2.24 g, 2.79 mmol) was dissolved under an atmosphere of N_2 in dry CH_2Cl_2 (5 mL) and thiophenol (3 mL) and *N*-methylmorpholine (0.03 mL, 0.3 mmol) were added. The mixture was stirred at ambient temperature for 5 h, at which time the solvent and excess thiophenol were removed under reduced pressure (40 °C, 0.2 Torr). The residue was purified by flash chromatography (5–10% $MeOH/CH_2Cl_2$). Trituration in ether/EtOAc gave **8c** as an amorphous powder (1.53 g, 86% yield). ^1H NMR (CD_3OD): δ 7.60 (s, 1 H), 7.1–7.3 (m, 5 H), 6.91 (s, 1 H), 4.54 (t, J = 6.6 Hz, 1 H), 4.28 (dd, J = 4.3, 10.0 Hz, 1 H), 4.05 (m, 1 H), 3.93 (dd, J = 2.7, 5.7 Hz, 1 H), 3.33 (m, 2 H), 3.11 (dd, J = 4.3, 14.0 Hz, 1 H), 3.05 (m, 2 H), 2.82 (s, 3 H), 2.77 (dd, J = 10.0, 14.0 Hz, 1 H), 2.55 (br t, J = 9 Hz, 1 H), 2.16 (dq, J_d = 12.9 Hz, J_q = 8.5 Hz, 1 H), 1.35 (s, 9 H). HPLC (method B): 98.2%, 13.02 min. High-resolution FAB MS for $C_{34}H_{51}N_6O_6$: calcd 639.387009 (M^+ + H), found 639.386871. Anal. ($C_{34}H_{50}N_6O_6 \cdot 0.5H_2O$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-(1-methyl-2-oxopyrrolidin-3(*R*)-yl)-propane (8a). ^1H NMR (CD_3OD): δ 7.59 (s, 1 H), 7.2–7.3 (m, 5 H), 6.92 (s, 1 H), 4.65 (t, J = 6.8 Hz, 1 H), 4.32 (dd, J = 4.2, 10.1 Hz, 1 H), 3.98 (m, 1 H), 3.53 (dd, J = 1.6, 9.2 Hz, 1 H), 3.2–3.3 (m, 2 H), 3.05–3.15 (m, 2 H), 2.98 (dd, J = 6.6, 14.7 Hz, 1 H), 2.82 (s, 3 H), 2.79 (dd, J = 10.0, 13.7 Hz, 1 H), 1.95–2.10 (m, 2 H), 1.35 (s, 9 H). HPLC (method B): 94.1% 13.89 min. High resolution FAB MS for $C_{34}H_{51}N_6O_6$: calcd 639.387009 (M^+ + H), found 639.386551. Anal. ($C_{34}H_{50}N_6O_6 \cdot 1.25H_2O$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*R*)-hydroxy-1-(1-methyl-2-oxopyrrolidin-3-yl)-propane (8b). ^1H NMR (CD_3OD): δ 7.57 (s, 1 H), 7.2–7.3 (m, 5 H), 6.88 (s, 1 H), 4.57 (t, J = 6.3 Hz, 1 H), 4.29 (dd, J = 4.4, 10.0 Hz, 1 H), 4.14 (m, 1 H), 3.60 (t, J = 5.5 Hz, 1 H), 3.3–3.4 (m, 2 H), 3.12 (dd, J = 4.4, 14.3 Hz, 1 H), 3.07 (m, 2 H), 2.79 (s, 3 H), 2.59 (m, 1 H), 2.10 (m, 1 H), 1.85–1.95 (m, 2 H), 1.34 (s, 9 H). HPLC (method B): 90.0%, 14.02 min. High-resolution FAB MS for $C_{34}H_{51}N_6O_6$: calcd 639.387009 (M^+ + H), found 639.387436. Anal. ($C_{34}H_{50}N_6O_6 \cdot 1.5H_2O$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*R*)-hydroxy-1-(1-methyl-2-oxopyrrolidin-3-yl)-propane (8d). ^1H NMR (CD_3OD): δ 7.57 (s, 1 H), 7.2–7.3 (m, 5 H), 6.88 (s, 1 H), 4.59 (t, J = 6.6 Hz, 1 H), 4.28 (dd, J = 4.4, 10.2 Hz, 1 H), 3.86 (t, J = 10 Hz, 1 H), 3.77 (dd, J = 1.6, 9.6 Hz, 1 H), 3.2–3.3 (m, 2 H), 3.10 (dd, J = 4.1, 13.9 Hz, 1 H), 3.00 (m, 2 H), 2.82 (s, 3 H), 2.79 (dd, J = 10.2, 14.1 Hz, 1 H), 2.22 (br t, J = 7 Hz, 1 H), 1.35 (s, 9 H). HPLC (method B): 95.2%, 12.74 min. High-resolution FAB MS for $C_{34}H_{51}N_6O_6$: calcd 639.387009 (M^+ + H), found 639.386490. Anal. $C_{34}H_{50}N_6O_6 \cdot 2H_2O$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-(1-methyl-2-oxopiperidin-3(*R*)-yl)-propane (9a). ^1H NMR (CD_3OD): δ 7.57 (s, 1 H), 7.2–7.3 (m, 5 H), 6.89 (s, 1 H), 4.64 (t, J = 6.8 Hz, 1 H), 4.34 (dd, J = 4.5, 10.1 Hz, 1 H), 4.12 (m, 1 H), 3.62 (dd, J = 1.3, 9.1 Hz, 1 H), 3.3–3.4 (m, 2 H), 3.12 (m, 2 H), 2.99 (dd, J = 6.8, 14.9 Hz, 1 H), 2.92 (s, 3 H), 2.79 (dd, J = 10.1, 13.7 Hz, 1 H), 1.35 (s, 9 H). HPLC

(method B): 95.1%, 14.01 min. High-resolution FAB MS for $C_{35}H_{53}N_6O_6$: calcd 653.402659 ($M^+ + H$); found 653.402512. Anal. ($C_{35}H_{52}N_6O_6 \cdot H_2O$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-(1-methyl-2-oxopiperidin-3(*S*)-yl)propane (9b). ^1H NMR (CD_3OD): δ 7.60 (s, 1 H), 7.2–7.4 (m, 5 H), 6.90 (s, 1 H), 4.50 (br t, J = 6 Hz, 1 H), 4.29 (dd, J = 4.4, 9.8 Hz, 1 H), 4.10 (s, 1 H), 4.05 (m, 1 H), 3.25–3.45 (m, 2 H), 3.13 (dd, J = 4.4, 14.0 Hz, 1 H), 3.02 (m, 2 H), 2.94 (s, 3 H), 2.79 (dd, J = 9.8, 14.0 Hz, 1 H), 2.42 (br t, J = 7 Hz, 1 H), 1.35 (s, 9 H). HPLC (method B): 98.7%, 13.51 min. High-resolution FAB MS $C_{35}H_{53}N_6O_6$: calcd 653.402659 ($M^+ + H$), found 653.402609. Anal. ($C_{35}H_{52}N_6O_6 \cdot 0.5H_2O$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-(1-methyl-2-oxoperhydroazepin-3-(*S*)-yl)propane (10) was lyophilized from dioxane. ^1H NMR (CD_3OD): δ 7.61 (s, 1 H), 7.2–7.3 (m, 5 H), 6.92 (s, 1 H), 4.59 (t, J = 6.2 Hz, 1 H), 4.29 (dd, J = 4.2, 10.0 Hz, 1 H), 4.20 (m, 1 H), 3.67 (d, J = 9.6 Hz, 1 H), 2.95–3.20 (m, 5 H), 2.93 (s, 3 H), 2.80 (dd, J = 13.9, 10.0 Hz, 1 H), 2.55 (br t, J = 10 Hz, 1 H), 1.35 (s, 9 H). HPLC (method B): 97.1%, 14.01 min. High-resolution FAB MS for $C_{36}H_{55}N_6O_6$: calcd 667.418309 ($M^+ + H$), found 667.417664. Anal. ($C_{36}H_{54}N_6O_6 \cdot 0.5H_2O$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-(1-methyl-5(*S*)-ethenyl-2-oxopyrrolidin-3(*S*)-yl)propane (26) was precipitated from CH_2Cl_2 solution with ether. ^1H NMR (CD_3OD): δ 7.58 (d, J = 1.3 Hz, 1 H), 7.15–7.30 (m, 5 H), 6.90 (s, 1 H), 5.73 (ddd, J = 17.4, 9.8, 7.9 Hz, 1 H), 5.23 (d, J = 17.4 Hz, 1 H), 5.20 (d, J = 9.8 Hz, 1 H), 4.54 (t, J = 6.1 Hz, 1 H), 4.28 (dd, J = 4.3, 10.0 Hz, 1 H), 4.02 (m, 2 H), 3.95 (dd, J = 2.2, 5.8 Hz, 1 H), 3.11 (dd, J = 4.3, 14.0 Hz, 1 H), 3.03 (m, 2 H), 2.78 (dd, J = 10.0, 14.0 Hz, 1 H), 2.74 (s, 3 H), 2.65 (br t, J = 8 Hz, 1 H), 2.44 (dt, J_t = 8.4 Hz, J_d = 12.7 Hz, 1 H), 1.34 (s, 9 H). HPLC (method B): 98.0%, 13.92 min. High-resolution FAB MS for $C_{36}H_{53}N_6O_6$: calcd 665.402659 ($M^+ + H$), found 665.402267. Anal. ($C_{36}H_{52}N_6O_6 \cdot H_2O$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-(1-methyl-5(*R*)-ethenyl-2-oxopyrrolidin-3(*S*)-yl)propane (28) was lyophilized from dioxane. ^1H NMR (CD_3OD): δ 7.59 (d, J = 1.2 Hz, 1 H), 7.15–7.30 (m, 5 H), 6.91 (s, 1 H), 5.72 (dt, J_t = 9.9 Hz, J_d = 17.1 Hz, 1 H), 5.36 (dd, J = 1.4, 17.1 Hz, 1 H), 5.26 (dd, J = 1.4, 9.9 Hz, 1 H), 4.55 (t, J = 6.2 Hz, 1 H), 4.29 (dd, J = 4.4, 10.0 Hz, 1 H), 4.05 (m, 1 H), 3.97 (dd, J = 2.7, 5.8 Hz, 1 H), 3.88 (q, J = 8.5 Hz, 1 H), 3.11 (dd, J = 4.4, 13.9 Hz, 1 H), 3.03 (m, 2 H), 2.78 (dd, J = 10.0, 13.9 Hz, 1 H), 2.71 (s, 3 H), 2.57 (br t, J = 9 Hz, 1 H), 2.08 (dt, J_t = 9.0 Hz, J_d = 12.9 Hz, 1 H), 1.89 (ddd, J = 8.1, 9.8, 12.9 Hz, 1 H), 1.35 (s, 9 H). HPLC (method B): 97.3%, 14.00 min. High-resolution FAB MS for $C_{36}H_{53}N_6O_6$: calcd 665.402659 ($M^+ + H$), found 665.404434. Anal. ($C_{36}H_{52}N_6O_6 \cdot 0.5H_2O$) C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-[1-methyl-5(*S*)-[(benzyloxy)methyl]-2-oxopyrrolidin-3(*S*)-yl]propane (30). ^1H NMR (CD_3OD): δ 7.58 (d, J = 1.1 Hz, 1 H), 7.20–7.35 (m, 10 H), 6.90 (s, 1 H), 4.53 (t, J = 6.4 Hz, 1 H), 4.51 (s, 2 H), 4.28 (dd, J = 4.4, 9.7 Hz, 1 H), 4.00 (m, 1 H), 3.92 (dd, J = 2.2, 6.1 Hz, 1 H), 3.6–3.7 (m, 2 H), 3.49 (m, 1 H), 3.11 (dd, J = 4.4, 13.9 Hz, 1 H), 3.02 (m, 2 H), 2.80 (s, 3 H), 2.7–2.8 (m, 2 H), 2.35 (dt, J_t = 9.3 Hz, J_d = 12.8 Hz, 1 H), 1.34 (s, 9 H). HPLC (method A): 98.0%, 9.82 min. FAB MS: 759 ($M^+ + H$). Anal. ($C_{42}H_{58}N_6O_7 \cdot H_2O$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-[1-methyl-5(*S*)-(methoxymethyl)-2-oxopyrrolidin-3(*S*)-yl]propane (32) was lyophilized from dioxane. ^1H NMR (CD_3OD): δ 7.60 (s, 1 H), 7.15–7.3 (m, 5 H), 6.91 (s, 1 H), 4.54 (t, J = 6.2 Hz, 1 H), 4.28 (dd, J = 4.4, 10.0 Hz, 1 H), 4.01 (m, 1 H), 3.93 (dd, J = 2.5, 6.1 Hz, 1 H), 3.62 (m, 1 H), 3.57 (dd, J = 3.4, 10.2 Hz, 1 H), 3.40 (dd, J = 4.0, 10.2 Hz, 1 H), 3.35 (s, 3 H), 3.10 (dd, J = 4.4, 13.9 Hz, 1 H), 3.03 (m, 2 H), 2.84 (s, 3 H), 2.77 (dd, J = 10.0, 13.9 Hz, 1 H), 2.65 (br t, J = 9 Hz, 1 H), 2.31 (dt, J_t = 9.1 Hz, J_d = 12.8 Hz, 1 H), 1.77 (ddd, J = 2.7, 9.5, 12.8 Hz, 1 H), 1.35 (s, 9 H). HPLC (method A): 93.8%, 8.57 min. FAB MS: 683 ($M^+ + H$). Anal. ($C_{36}H_{54}N_6O_7 \cdot 0.5H_2O$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-(1,5,5-trimethyl-2-oxopyrrolidin-3-(*S*)-yl)propane (34) was lyophilized from dioxane. ^1H NMR (CD_3OD): δ 7.59 (d, J = 1.1 Hz, 1 H), 7.2–7.3 (m, 5 H), 6.93 (s, 1 H), 4.57 (t, J = 6.4 Hz, 1 H), 4.26 (dd, J = 4.5, 10.0 Hz, 1 H), 4.06 (m, 1 H), 3.98 (dd, J = 2.4, 4.5 Hz, 1 H), 2.9–3.1 (m, 3 H), 2.78 (s, 3 H), 2.73 (dd, J = 10.0, 13.8 Hz, 1 H), 2.65 (br t, J = 9 Hz, 1 H), 2.00 (dd, J = 10.2, 12.5 Hz, 1 H), 1.81 (dd, J = 9.0 Hz, 12.5 Hz, 1 H), 1.35 (s, 9 H), 1.31 (s, 3 H), 1.20 (s, 3 H). HPLC (method A): 96.9%, 8.89 min. High-resolution FAB MS for $C_{36}H_{55}N_6O_6$: calcd 667.18309 ($M^+ + H$), found 667.420288. Anal. ($C_{36}H_{54}N_6O_6 \cdot H_2O$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-(1-butyl-5,5-dimethyl-2-oxopyrrolidin-3(*S*)-yl)propane (35) was lyophilized from dioxane. ^1H NMR (CD_3OD): δ 7.62 (d, J = 1.0 Hz, 1 H), 7.2–7.3 (m, 5 H), 6.92 (s, 1 H), 4.56 (t, J = 6.2 Hz, 1 H), 4.28 (dd, J = 4.3, 10.0 Hz, 1 H), 4.04 (m, 1 H), 3.98 (dd, J = 2.7, 5.2 Hz, 1 H), 3.0–3.3 (m, 5 H), 2.78 (dd, J = 10.2, 13.9 Hz, 1 H), 2.62 (br t, J = 9 Hz, 1 H), 2.02 (dd, J = 10.5, 12.4 Hz, 1 H), 1.78 (dd, J = 9.1 Hz, 12.4 Hz, 1 H), 1.35 (s, 9 H), 1.32 (s, 3 H), 1.19 (s, 3 H), 0.93 (t, J = 7.3 Hz, 1 H). HPLC (method A): 95.8%, 9.84 min. High-resolution FAB MS for $C_{39}H_{61}N_6O_6$: calcd 709.465259 ($M^+ + H$), found 709.465240. Anal. ($C_{39}H_{60}N_6O_6 \cdot 0.5H_2O$) C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-(1-cyclohexyl-2-oxopyrrolidin-3(*S*)-yl)propane (36) was lyophilized from dioxane. A portion of this material was crystallized from MeOH, mp 110–112 °C. ^1H NMR (CD_3OD): δ 7.61 (s, 1 H), 7.1–7.3 (m, 5 H), 6.91 (s, 1 H), 4.56 (t, J = 6.3 Hz, 1 H), 4.28 (dd, J = 4.5, 10.2 Hz, 1 H), 4.04 (m, 1 H), 3.93 (dd, J = 2.9, 5.6 Hz, 1 H), 3.82 (m, 1 H), 3.2–3.4 (m, 2 H), 3.11 (dd, J = 4.1, 13.7 Hz, 1 H), 3.0–3.1 (m, 2 H), 2.78 (dd, J = 10.7, 13.7 Hz, 1 H), 2.56 (dt, J_d = 2.7 Hz, J_t = 9.1 Hz, 1 H), 1.35 (s, 9 H). HPLC (method A): 95.0%, 9.67 min. FAB MS: 707 ($M^+ + H$). Anal. ($C_{39}H_{58}N_6O_6 \cdot 1.5H_2O$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-[1-[2-(benzyloxy)ethyl]-2-oxopyrrolidin-3(*S*)-yl]propane (37). ^1H NMR (CD_3OD): δ 7.56 (s, 1 H), 7.1–7.35 (m, 10 H), 6.89 (s, 1 H), 4.56 (br t, J = 6 Hz, 1 H), 4.50 (s, 2 H), 4.28 (dd, J = 4.7, 10.0 Hz, 1 H), 4.06 (m, 1 H), 3.93 (dd, J = 2.3, 5.5 Hz, 1 H), 3.35–3.65 (m, 4 H), 3.11 (dd, J = 4.7, 13.9 Hz, 1 H), 3.02 (m, 2 H), 2.78 (dd, J = 10.0, 13.9 Hz, 1 H), 2.54 (dt, J_d = 2.4 Hz, J_t = 9.0 Hz, 1 H), 2.15 (dq, J_q = 8.9 Hz, J_d = 12.7 Hz, 1 H), 1.34 (s, 9 H). HPLC (method A): 97.0%, 9.73 min. FAB MS: 759 ($M^+ + H$). Anal. ($C_{42}H_{58}N_6O_7 \cdot H_2O$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-[1-[3-[*N*-benzyl-*N*-(carbobenzyloxy)amino]propyl]-2-oxopyrrolidin-3(*S*)-yl]propane (39) was precipitated from CH_2Cl_2 solution with ether. ^1H NMR (CD_3OD): δ 7.50 (br s, 1 H), 7.1–7.4 (m, 10 H), 6.90 (br s, 1 H), 5.17 (s, 2 H), 4.55 (br m, 1 H), 4.51 (s, 2 H), 4.30 (br m, 1 H), 4.10 (br m, 1 H), 3.90 (br m, 1 H), 3.1–3.4 (br m, 6 H), 2.50 (br m, 1 H), 1.35 (s, 9 H). HPLC (method A): 98.8%, 10.70 min. FAB MS: 906 ($M^+ + H$). Anal. ($C_{51}H_{67}N_7O_8 \cdot 1.5H_2O$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-(1-methyl-5(*R*)-ethyl-2-oxopyrrolidin-3(*S*)-yl)propane (27). To a solution of **26** (86 mg, 0.13 mmol) in MeOH (10 mL) was added 10% Pd on carbon (45 mg). The resulting suspension was stirred at ambient temperature under H_2 (1 atm) for 4 h. The catalyst was removed by filtration through Celite and was washed with MeOH. The filtrate solvent was removed under reduced pressure and the residue was passed through a short bed of SiO_2 using 7% MeOH/CH_2Cl_2. The product was lyophilized from dioxane (78 mg, 91% yield). ^1H NMR (CD_3OD): δ 7.59 (s, 1 H), 7.15–7.3 (m, 5 H), 6.90 (s, 1 H), 4.55 (br t, J = 6 Hz, 1 H), 4.27 (dd, J = 4.6, 10.0 Hz, 1 H), 4.04 (m, 1 H), 3.93 (dd, J = 2.7, 5.9 Hz, 1 H), 3.50 (m, 1 H), 3.11 (dd, J = 4.6, 14.1 Hz, 1 H), 3.04 (m, 2 H), 2.79 (s, 3 H), 2.62 (br t, J = 8 Hz), 2.33 (dt, J_t = 8.6 Hz, J_d = 12.8 Hz, 1 H), 1.35 (s, 9 H), 0.88 (t, J = 7.3 Hz, 3 H). HPLC (method B): 97.3%, 14.00 min. High-resolution FAB MS for $C_{36}H_{55}N_6O_6$: calcd 667.418309 ($M^+ + H$), found 667.419357. Anal. ($C_{36}H_{54}N_6O_6$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-(1-methyl-5(*S*)-ethyl-2-oxopyrrolidin-3(*S*)-yl)propane (29). Reduction of **28** (70 mg, 0.11 mmol) was accomplished by using the procedure given for the preparation of **27**. The product was lyophilized from dioxane (62 mg, 88% yield). ^1H NMR (CD_3OD): δ 7.57 (d, J = 1.1 Hz, 1 H),

7.15–7.3 (m, 5 H), 6.90 (s, 1 H), 4.54 (t, J = 6.1 Hz, 1 H), 4.28 (dd, J = 4.4, 10.0 Hz, 1 H), 4.05 (m, 1 H), 3.95 (dd, J = 2.7, 5.6 Hz, 1 H), 3.38 (m, 1 H), 3.11 (dd, J = 4.4, 13.9 Hz, 1 H), 3.03 (m, 2 H), 2.77 (s, 3 H), 2.53 (br t, J = 8 Hz, 1 H), 2.01 (dt, J_t = 8.6 Hz, J_d = 12.5 Hz, 1 H), 1.34 (s, 9 H), 0.93 (t, J = 7.4 Hz, 3 H). HPLC (method B): 97.7%, 14.34 min. High-resolution FAB MS for $C_{36}H_{55}N_6O_6$: calcd 667.418309 (M^+ + H), found 667.418045. Anal. ($C_{36}H_{54}N_6O_6 \cdot H_2O$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-[1-methyl-5(*S*)-(hydroxymethyl)-2-oxopyrrolidin-3(*S*)-yl]propane (33). To a solution of **30** (320 mg, 0.422 mmol) in 20 mL of 10:1 MeOH/HOAc was added 10% Pd on carbon (160 mg), and the resulting suspension was shaken on a Parr apparatus for 18 h under 4 atm of H_2. The catalyst was removed by filtration through Celite and was washed with MeOH. The filtrate solvents were removed under reduced pressure, and the residue was flash chromatographed (8–10% MeOH/CH_2Cl_2). The product was obtained as a white solid (191 mg; 68% yield) after trituration in EtOAc. ^{1}H NMR (CD_3OD): δ 7.62 (s, 1 H), 7.15–7.3 (m, 5 H), 6.92 (s, 1 H), 4.54 (t, J = 6.3 Hz, 1 H), 4.28 (dd, J = 4.5, 9.9 Hz, 1 H), 4.02 (m, 1 H), 3.93 (dd, J = 2.4, 6.4 Hz, 1 H), 3.77 (dd, J = 4.0, 12.0 Hz, 1 H), 3.50–3.60 (m, 2 H), 3.11 (dd, J = 9.9, 14.0 Hz, 1 H), 3.03 (m, 2 H), 2.86 (s, 3 H), 2.78 (dd, J = 9.9, 14.0 Hz, 1 H), 2.70 (br t, J = 9 Hz, 1 H), 2.32 (dt, J_t = 9.0 Hz, J_d = 12.4 Hz, 1 H), 1.34 (s, 9 H). HPLC (method A): 95.6%, 7.85 min. FAB MS: 669 (M^+ + H). Anal. ($C_{35}H_{52}N_6O_7 \cdot H_2O$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-[1-methyl-5(*S*)-(acetoxymethyl)-2-oxopyrrolidin-3(*S*)-yl]propane (31). To a solution of **33** (74 mg, 0.11 mmol) in dry DMF (0.60 mL) under an atmosphere of N_2 was added NaOAc (45 mg, 0.55 mmol) and acetic anhydride (28 μL, 0.30 mmol). The resulting suspension was stirred at ambient temperature for 6 h, when Et_3N (0.11 mL, 0.80 mmol) and H_2O (40 μL, 2.2 mmol) were added. This mixture was stirred at ambient temperature for 2 h. The solvents were removed under reduced pressure and the residue was passed through a short bed of SiO_2 with 6% MeOH/CH_2Cl_2. The product was lyophilized from dioxane (55 mg, 70% yield). ^{1}H NMR (CD_3OD): δ 7.61 (s, 1 H), 7.15–7.30 (m, 5 H), 6.91 (s, 1 H), 4.56 (t, J = 6.1 Hz, 1 H), 4.38 (dd, J = 3.4, 11.9 Hz, 1 H), 4.28 (dd, J = 4.4, 10.0 Hz, 1 H), 4.05 (m, 2 H), 3.95 (dd, J = 2.3, 5.5 Hz, 1 H), 3.75 (dq, J_q = 3.0 Hz, J_d = 9.6 Hz, 1 H), 3.11 (dd, J = 4.4, 13.9 Hz, 1 H), 3.04 (m, 2 H), 2.83 (s, 3 H), 2.78 (dd, J = 10.0, 13.9 Hz, 1 H), 2.67 (br t, J = 9 Hz, 1 H), 2.39 (dt, J_t = 9.1 Hz, J_d = 12.9 Hz, 1 H), 2.03 (s, 3 H), 1.35 (s, 9 H). HPLC (method A): 95.8%, 8.48 min. FAB MS: 711 (M^+ + H). Anal. ($C_{37}H_{54}N_6O_8 \cdot 1.25H_2O$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-[1-(2-hydroxyethyl)-2-oxopyrrolidin-3(*S*)-yl]propane (38). Hydrogenolysis of **37** (360 mg, 0.475 mmol) was accomplished by using the procedure given for the preparation of **33**. The product was obtained as a white solid (221 mg, 70% yield) upon trituration in EtOAc. ^{1}H NMR (CD_3OD): δ 7.60 (s, 1 H), 7.2–7.3 (m, 5 H), 6.91 (s, 1 H), 4.55 (t, J = 6.6 Hz, 1 H), 4.29 (dd, J = 4.6, 10.1 Hz, 1 H), 4.09 (m, 1 H), 3.94 (dd, J = 2.9, 5.7 Hz, 1 H), 3.68 (t, J = 5.5 Hz, 2 H), 3.35–3.50 (m, 4 H), 3.12 (dd, J = 4.6, 14.0 Hz, 1 H), 2.9–3.1 (m, 2 H), 2.78 (dd, J = 10.0, 14.0 Hz, 1 H), 2.57 (br t, J = 9 Hz, 1 H), 2.17 (dq, J_d = 2.4 Hz, J_q = 8.6 Hz), 1.35 (s, 9 H). HPLC (method A): 94.3%, 8.01 min. FAB MS: 669 (M^+ + H). Anal. ($C_{35}H_{52}N_6O_7$): C, H, N.

2(*S*)-[(Boc-L-phenylalanyl-L-histidyl)amino]-3-cyclohexyl-1(*S*)-hydroxy-1-[1-[3-(benzylamino)propyl]-2-oxopyrrolidin-3(*S*)-yl]propane, Bis(trifluoroacetic acid) Salt (40). To a solution of **39** (225 mg, 0.248 mmol) in 4% HCO_2H/MeOH solution under an atmosphere of N_2 was added 10% Pd on carbon (200 mg). The mixture was stirred at ambient temperature for 6 h and then filtered through Celite. The filtrate solvents were removed under reduced pressure and the residue was purified by preparative reverse-phase HPLC. The product was lyophilized from dioxane (176 mg, 71% yield). ^{1}H NMR (CD_3OD): δ 8.80 (d, J = 1.1 Hz, 1 H), 7.45–7.55 (m, 5 H), 7.35 (s, 1 H), 7.2–7.3 (m, 5 H), 4.66 (dd, J = 5.1, 8.5 Hz, 1 H), 4.21 (dd, J = 5.2, (.9 Hz, 1 H), 4.15 (s, 2 H), 4.1 (m, 1 H; overlaps with the resonance at δ 4.15), 3.98 (dd, J = 2.9, 5.4 Hz, 1 H), 3.35–4.00 (m, 4 H), 3.12 (dd, J = 8.5, 15.9 Hz, 1 H), 3.0–3.1 (m, 3 H), 2.79 (dd, J = 9.9, 14.1 Hz, 1 H), 2.70 (br t, J = 9 Hz, 1 H), 1.36 (s, 9 H). HPLC (method A): 97.7%, 8.23 min. FAB MS: 772 (M^+ + H). Anal. ($C_{43}H_{61}N_7O_6 \cdot 2.75TFA$): C, H, N.

Single-Crystal X-ray Diffraction Analysis of 3c, 5d, and 36. The following library of crystallographic programs was used: SHELXS-86 (G. M. Sheldrick, University of Gottingen, West Germany, 1986), PLUTO (W. D. S. Motherwell and W. Clegg, University of Cambridge, Cambridge, England, 1978), and a version of SDP V. 3 (Enraf-Nonius, Delft, The Netherlands, 1985) locally modified for a Sun Microsystems computer.

3c: Crystals formed from CH_2Cl_2/hexane mixtures in space group $P2_12_12_1$ with a = 6.406 (1) Å, b = 17.768 (3) Å, and c = 17.843 (4) Å for Z = 4 and a calculated density of 1.159 g/cm^3. An automatic four circle diffractometer equipped with Cu Kα radiation (λ = 1.5418 Å) was used to measure 1595 potential diffraction peaks of which 1398 were observed ($I \geq 3\sigma I$). Application of a multisolution tangent formula approach to phase solution gave an initial model for the structure which was subsequently refined by using least squares and Fourier methods. Hydrogens were added with isotropic temperature factors which were not refined. The function $\sum\omega(F_o-F_c)^2$ with $\omega = 1/(\sigma F_o)^2$ was minimized with full matrix least squares to give an unweighted residual of 0.042. Figure 2 is a PLUTO plot of **3c** showing its stereochemistry and conformation.

5d: Crystals formed from hexane in space group $P6_5$ with a = 11.603 (2) Å and c = 27.974 (5) Å for Z = 6 and a calculated density of 1.169 g/cm^3. An automatic four circle diffractometer equipped with Cu Kα radiation (λ = 1.5418 Å) was used to measure 1494 potential diffraction peaks of which 1206 were observed ($I \geq 3\sigma I$). Application of a multisolution tangent formula approach to phase solution gave an initial model for the structure which was subsequently refined by using least squares and Fourier methods. Hydrogens were added with isotropic temperature factors which were not refined. The function $\sum\omega(F_o-F_c)^2$ with $\omega = 1/(\sigma F_o)^2$ was minimized with full matrix least squares to give an unweighted residual of 0.034. Figure 3 is a PLUTO plot of **5d** showing its stereochemistry and conformation.

36: Crystals formed from methanol in space group $P1$ with a = 10.150 (4) Å, b = 21.606 (4) Å, c = 5.175 (2) Å, α = 96.05 (2)°, β = 101.45 (23)°, and γ = 77.78 (3)° for Z = 1 and a calculated density of 1.082 g/cm^3. An automatic four circle diffractometer equipped with Cu Kα radiation (λ = 1.5418 Å) was used to measure 2984 potential diffraction peaks at which 2368 were observed ($I \geq 3\sigma I$). Application of a multisolution tangent formula approach to phase solution gave an initial model for the structure which was subsequently refined using least squares and Fourier methods. Hydrogens were added with isotropic temperature factors which were not refined. The function $\sum\omega(F_o-F_c)^2$ with $\omega = 1/(\sigma F_o)^2$ was minimized with full matrix least squares to give an unweighted residual of 0.081. Figure 4 is a PLUTO plot of **36** showing its stereochemistry and conformation. The crystal structure contains disordered solvent molecules.

Oral and Intravenous Effects of 8c on Plasma Renin Activity in Sodium-Deficient Rhesus Monkeys. Male and female rhesus monkeys were surgically implanted with chronic arterial catheters with access ports (Access Technologies) for direct monitoring of mean arterial blood pressure and repeated collection of blood samples for determination of plasma renin activity (PRA). The monkeys were maintained on a low sodium diet (1.2 mmol sodium per day) supplemented with fruit for 1 week, and given furosemide (2.5 mg/kg, im) the evening before the experiment. Food was denied 18 h prior to and during the experiment (water ad lib). On the day of the experiment, the monkeys were placed in restraining chairs and a pediatric nasogastric catheter was inserted in the nostril and guided down the esophagus for oral administration. Blood samples (1.0 mL) were withdrawn from the arterial port (EDTA, 4.5 mmol), centrifuged cold (4 °C) at 1000g for 20 min, and the plasma was stored frozen (−70 °C) for assay of PRA. For intravenous testing, blood samples were collected at 30 min before and 5 min after administration of vehicle control solution (0.5% citric acid/5% dextrose/H_2O) at 0.4 mL/kg. Twenty minutes after vehicle control administration, **8c** was injected (0.22 mg/kg in control vehicle solution). Blood samples for PRA analysis were collected at various times after challenge with **8c**. A standard (either a renin inhibitor or an angiotensin converting enzyme inhibitor) was given at the end of the experiment to check the responsiveness of the animal to

inhibition of the RAS. For oral testing, 8c was crushed with a mortar and pestle, suspended in 0.1 M citric acid/H_2O, and given to two animals at dose of 50 mg/kg. Blood samples were collected at -30, -5, 15, 30, and 60 min, and every hour thereafter for 6 h. Averaged results are given in Figure 6. A standard was given at the end of the experiment as in the intravenous testing.

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Registry No. **3a**, 129941-26-0; **3c**, 122662-97-9; **3bd** (epimer 1), 129941-27-1; **3bd** (epimer 2), 129941-28-2; **4a**, 129941-29-3; **4c**, 123350-52-7; **4bd** (epimer 1), 129941-30-6; **4bd** (epimer 2), 129941-31-7; **5d**, 129941-34-0; **5abc** (diastereomer 1), 129896-98-6; **5abc** (diastereomer 2), 129941-32-8; **5abc** (diastereomer 3), 129941-33-9; **6ac** (epimer 1), 129896-99-7; **7ac** (epimer 2), 129897-01-4; **6bd** (epimer 1), 129897-00-3; **6bd** (epimer 2), 129897-02-5; **7ac** (epimer 1), 129897-03-6; **7ac** (epimer 2), 129897-05-8; **7bd** (epimer 1), 129897-04-7; **7bd** (epimer 2), 129897-06-9; **8a**, 129897-07-0; **8c**, 129941-36-2; **8bd** (epimer 1), 129941-35-1; **8bd** (epimer 2), 129941-37-3; **9a**, 130007-84-0; **9b**, 123350-46-9; **10**, 123350-47-0; (5*S*)-**11**, 122663-19-8; (5*R*)-**11**, 122663-22-3; (5*S*)-**12**, 122663-18-7; (5*R*)-**12**, 122662-87-7; **13a**, 122662-89-9; **13b**, 122663-21-2; **14a**, 119715-71-8; **14a** (R = H), 5165-28-6; **14b**, 129896-97-5; **15**, 103740-50-7; **16**, 122662-94-6; **17**, 129896-88-4; **18**, 129941-24-8; **19**, 129896-89-5; **20**, 129896-90-8; **21**, 129896-91-9; **22**, 129896-92-0; **23**, 129896-93-1; **24**, 129896-94-2; **25**, 122663-39-2; **26**, 122662-80-0; **27**, 122662-84-4; **28**, 122742-12-5; **29**, 122742-13-6; **30**, 122662-77-5; **31**, 122662-83-3; **32**, 122662-78-6; **33**, 122662-82-2; **34**, 129896-95-3; **35**, 129896-96-4; **36**, 122662-76-4; **37**, 122662-79-7; **638**, 122662-81-1; **39**, 122663-51-8; **40**·2TFA, 129941-25-9; H-D-pGlu-OH, 4042-36-8; H-pGlu-OH, 98-79-3; Me-pGlu-OMe, 42435-88-1; Me-D-pGlu-OMe, 122742-14-7; Boc-His(DNP)-OH, 25024-53-7; Boc-Phe-OH, 13734-34-4; 1-(2-hydroxyethyl)-2-pyrrolidinone, 3445-11-2; 1-(3-aminopropyl)-2-pyrrolidinone, 7663-77-6; 1-[3-[(benzyloxycarbonyl)amino]-propyl]-2-pyrrolidinone, 122662-93-5; *N*-methyl-2-pyrrolidinone, 872-50-4; *N*-methyl-2-piperidinone, 931-20-4; perhydro-*N*-methyl-2-azepinone, 2556-73-2; renin, 9015-94-5; cathepsin D, 9025-26-7; cathepsin E, 110910-42-4; *N*-Boc-L-cyclohexylalaninal, 98105-42-1.

Supplementary Material Available: Tables of atomic positional parameters, temperature parameters, and interatomic distances and angles for **3c**, **5d**, and **36** and coordinates for the model of human renin in Protein Data Bank format are available (36 pages). Ordering information is given on any current masthead page.

Syntheses and in Vitro Evaluation of Water-Soluble "Cationic Metalloporphyrin-Ellipticine" Molecules Having a High Affinity for DNA

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The synthesis of hybrid "cationic metalloporphyrin-intercalator" molecules is reported. These molecules are based on 9-methoxyellipticine as intercalator and tris-(4-*N*-methylpyridiniumyl)metalloporphyrins having a 4-aminophenyl or a 4-hydroxyphenyl group for the attachment of the linker. The effect of the length of linker (7-13 bonds), the chemical nature of the linking group (with a carboxamido or an ether function), the position of amino group between the two parts of hybrid molecules, the number of intercalator moieties (ellipticinium) covalently attached to the metalloporphyrin, and the nature of the central metal atom (Mn, Fe, Zn) on the biological activity of these hybrid molecules were studied. In addition, these molecules have a high affinity for double-stranded DNA (affinity constant of hybrid molecule **9Mn,Me** = 2.3×10^9 M^{-1} for poly[d(A-T)] and 2.8×10^8 M^{-1} for poly[d(G-C)] and are cytotoxic against murine leukemia cells L1210 in vitro (IC_{50} of **9Mn,Me** = 0.8 μM). Their cytotoxicities are dependent on the nature of central atom. Iron derivatives are less active than manganese analogues and the corresponding zinc derivatives are nearly inactive despite their same affinity for nucleic acids. These highly water-soluble hybrid molecules could be considered as efficient bleomycin models based on a cationic metalloporphyrin.

Introduction

Bleomycin has to be regarded as a paradigm for synthetic DNA cleavers. This antitumoral antibiotic is able to create single- and also double-strand breaks on duplex DNA in association with three cofactors: iron or copper salts, molecular oxygen, and an electron source.[1] The DNA cleavage occurs via the abstraction of an hydrogen atom at a C_4-position of a deoxyribose ring by "activated bleomycin" (see review articles 1a,b for the mechanism of DNA cleavage and refs 1b,f,g for the possible role of bleomycin-iron-oxo species as reactive intermediate). The structure of bleomycin has two domains: one part of the molecule is involved in the DNA binding mode and five nitrogen atoms from the peptidic chain are the chelating atoms of metal salts.[1] All the modeling studies of bleomycin have been used on this structure duality: the association of an intercalating agent to EDTA[2] or a metalloporphyrin.[3]

Since our group is involved in oxidation reactions catalyzed by metalloporphyrins,[4] DNA cleavage by high-va-

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