Conformationally Restricted Inhibitors of Angiotensin Converting Enzyme: Synthesis and Computations

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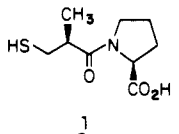
A series of inhibitors of angiotensin converting enzyme (ACE, dipeptidyl carboxypeptidase, EC 3.4.15.1) is described which addresses certain conformational aspects of the enzyme–inhibitor interaction. In this study the alanylproline portion of the potent ACE inhibitor enalaprilat (2) is replaced by a series of monocyclic lactams containing the required recognition and binding elements. In order to more fully assess the lactam ring conformations and the key backbone angle ψ as defined in 3 with respect to possible enzyme-bound conformations, a series of model lactams was investigated with use of molecular mechanics. The results point to a correlation between inhibitor potency (IC_{50}) and the computed ψ angle for the lowest energy conformation of the model compounds. Thus the ψ angle as defined in 3 is an important determinant in the binding of inhibitors to ACE. The inhibition data in conjunction with the computational data have served to define a window of ψ angles from 130° to 170° which seems to be acceptable to the ACE active site.

Introduction.[1-3] The general question of drug design based on peptide structure–activity relationships is an area of rapidly growing interest.[4-10] Our entry into the field grew out of an interest in the design of inhibitors of angiotensin converting enzyme (dipeptidyl carboxypeptidase, EC 3.1.15.1, ACE), a zinc metalloenzyme intimately involved in the control of blood pressure. ACE catalyzes the cleavage of angiotensin I to the potent vasopressor hormone angiotensin II.[11] The enzyme has also been shown to deactivate the vasodepressor substance bradykinin.[12] The development of ACE inhibitors for the therapy of hypertension beginning with basic studies on peptides found in a South American snake venom[13] has been described in a number of reviews.[14-16]

The demonstration that ACE inhibition has clinical importance stimulated interest in the design and synthesis of orally active inhibitors.[17] Captopril (1), developed by Ondetti and co-workers at Squibb,[18,19] was the first of these. Our efforts made use of structure–activity data for peptide ACE inhibitors[17] and the biproduct concept of inhibitor design proposed by Wolfenden.[20] This strategy resulted in a new class of potent, non-sulfhydryl inhibitors exemplified by enalaprilat (MK-422, 2).[21]



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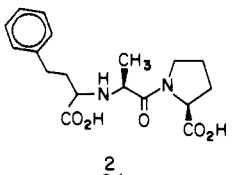

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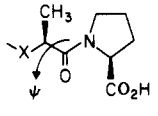
Part structure 3 has been shown to contribute significantly to the high ACE inhibitory potency of 1 and 2.[17,19,21] The methyl group of 3 is not required for binding, but its presence adds to inhibitory potency,[19,21] perhaps via a hydrophobic interaction with the enzyme. However, we felt it to be likely that the methyl group was more important for promoting a favorable conformer population



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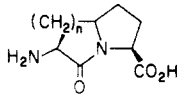
for **3** as a result of its interaction with the proline ring. Indeed, the conformational restriction of an alanyl residue succeeded by proline has previously been recognized.[22,23] After we began our investigations Ondetti and Cushman[24] also pointed out possible conformational restriction in **3**. In order to proceed with additional modeling and inhibitor design studies, we assumed that substructure **3** was bound to ACE with the trans configuration about the peptide bond (**3** as shown). Experimental data indicate the trans configuration to be preferred by alanylproline dipeptides in solution.[25-30] However, this preference is pH dependent. Around neutrality a significant amount of the cis isomer is present so binding of *cis*-**3** to ACE cannot be excluded a priori.

Once the amide bond of **3** was set trans in our binding model, the dihedral angle remained as the major source of conformational freedom. We explored this variable with energy minimization calculations using the Merck Molecular Modeling System[6] on a number of likely inhibitor structures incorporating **3**. These calculations indicated a preferred ψ angle in the 130–140° range and confirmed the inherent conformational restriction of the alanylproline unit recognized previously.[22,23] From the computational results as well as the examination of physical models, it is evident that insertion of a polymethylene chain between C-5 of proline and the methyl group would allow for variable restriction of ψ. Thus **4** was recognized as a conformationally constrained family of alanylproline analogues. For subsequent computational and synthetic manipulations we chose to reduce our initial targets to the simplified lactam structures **5** and **6–10**.



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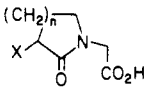


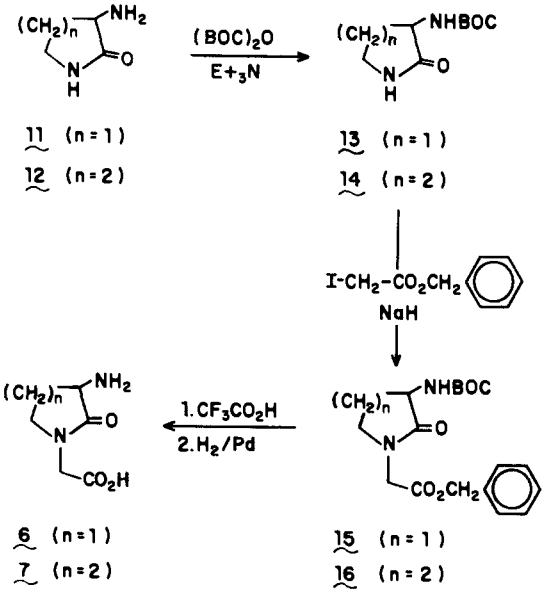
5: n = 1-4, X = CH_2SH

6-10: n = 1-5, X = NH_2

The usefulness of the lactam concept for design of ACE inhibitors was demonstrated by the mercaptomethyl inhibitors **5**.[2] These compounds established an order of inhibitory effectiveness based on ring size. The results also pointed to the ψ angle as an important determinant of inhibitor potency.

This report extends the lactam concept to **2** and shows that the replacement of the mercaptomethyl group by the *N*-alkylcarboxymethyl side chain does not alter the previously observed ring size preferences. Secondly, the biological activity data prompted us to undertake extensive conformational analyses of small- and medium-size lactams with the aim of securing a better understanding of inhibitor binding. Finally, this work along with that of Freidinger and co-workers[10] reveals one avenue of drug design starting from a peptide lead.

Scheme I



Results

Chemistry. Our first objective was to synthesize a series of 1-[(alkoxycarbonyl)methyl]-3-amino lactams for further elaboration to the entire inhibitor structure. A recent report by Freidinger and co-workers[31] details the syntheses of five- to seven-membered lactams. We have developed alternative syntheses which are complimentary to those described above.

The five- and six-membered lactam intermediates **15** and **16** were prepared from readily available[32] 3-amino-2-pyrrolidone (**11**) and 3-amino-2-piperidone (**12**), respectively (Scheme I). We reasoned that selective alkylation of the amide anion with an iodoacetate ester should occur cleanly in the presence of the amino group. In practice the 3-amino lactams proved to be so insoluble (THF or THF/DMF) that the alkylation reaction was very sluggish. The products isolated, though, were alkylated only at the amido nitrogen. Conversion of the amino lactams to their 3-BOC derivatives (**13**, **14**) provided soluble intermediates. In spite of the presence of the two amide functions, only alkylation of the lactam nitrogen occurs to afford **15** and **16**.

Intermediates **26–28** were produced from the unsubstituted parent lactams as outlined in Scheme II. Halogenation of the parent lactams with phosphorus pentabromide[33] afforded bromo lactams **17–19**. Reaction of **17–19** with lithium azide in DMF or sodium azide in aqueous ethanol[34] provided azido lactams **20–22**. Subsequent alkylation of the amide nitrogen with *tert*-butyl

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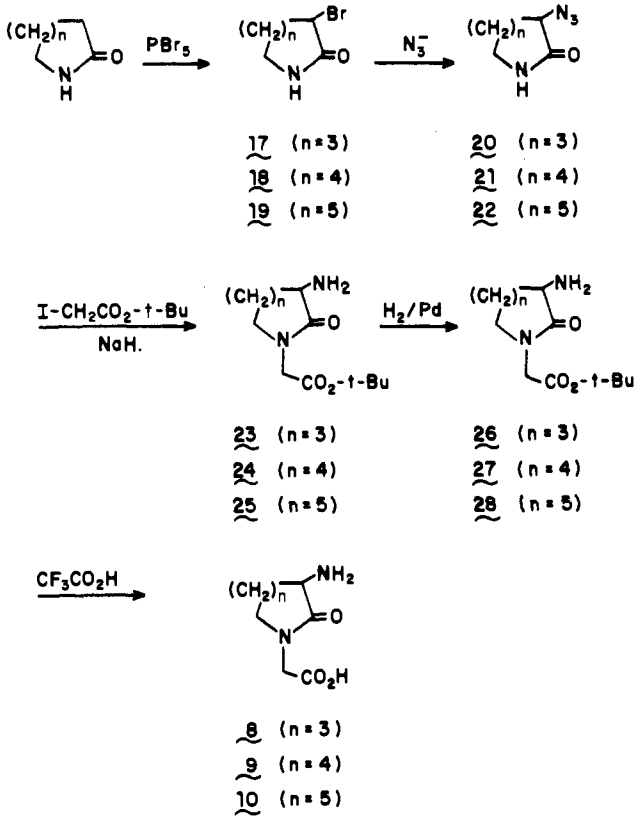
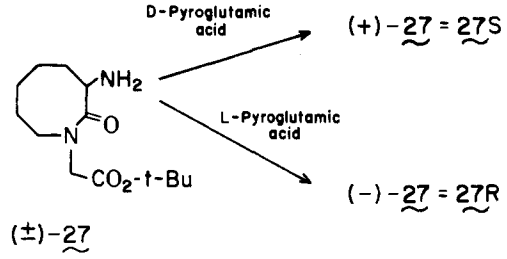
Scheme II



Scheme III



iodoacetate (NaH, THF) afforded **23–25**, which after azide reduction (Pd–C) yielded the lactam intermediates **26–28**. The racemic eight-membered lactam **27** obtained in this way was readily resolved via its pyroglutamic acid salts (Scheme III). The assignment of chirality of the enantiomers of **27** was based on the X-ray crystal structure of an intermediate in combination with biological activity from the derived inhibitors (see below).

With amino lactam intermediates **15**, **16**, and **26–28** in hand, the diacid inhibitor structures **34–38** were readily obtained as outlined in Scheme IV. The reductive amination of ethyl 2-oxo-4-phenylbutyrate proceeded in good yield with use of either Pd–C/H_2 or sodium cyanoborohydride[35] as the reducing agent. The reaction afforded a mixture of diastereomeric diesters (**29–33**) which were separated by using medium-pressure chromatography over silica gel. Hydrolysis of the separated diastereomeric diesters of **29–33** (isomers A and B; B more polar) gave the desired diacids **34–38** (isomers A and B), which were tested for in vitro ACE inhibition (Table **I**).[21]

ACE Inhibition and Stereochemistry. The IC_{50} values for ACE inhibition by **34–38** are reported in Table I. In agreement with the 3-mercaptomethyl lactam series

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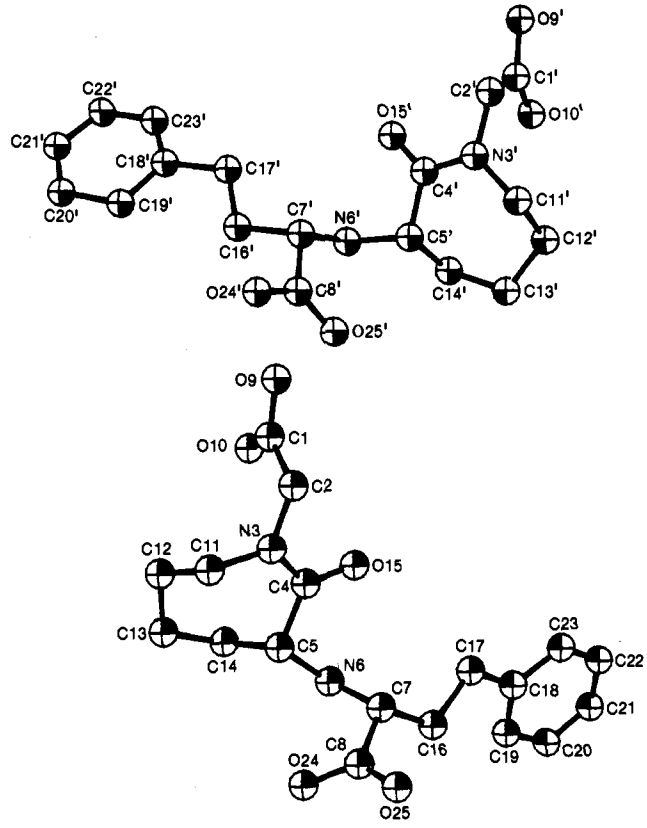


Figure 1. Computer-generated perspective drawing of asymmetric unit for structure **36** with crystallographic numbering system.

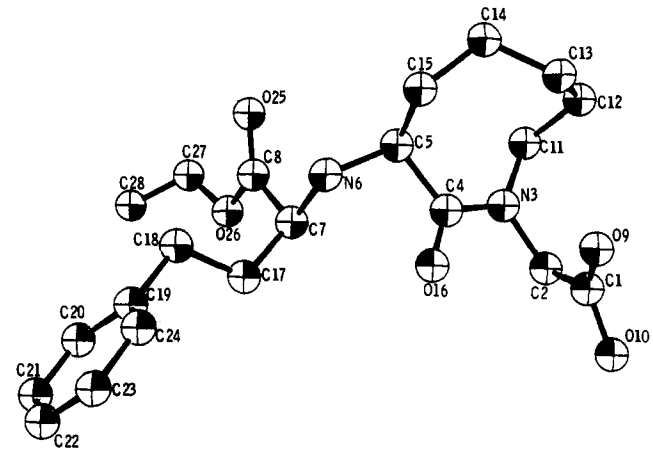
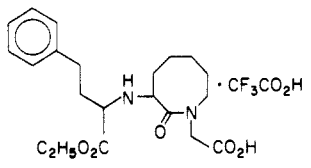


Figure 2. Computer-generated perspective drawing of structure **39** with crystallographic numbering system.

5,[2] there is good correlation of ring size with inhibitory potency. Analogous to earlier examples,[2] optimum activity is reached at the eight-membered ring **37**. The maximum inhibitory potency for the medium rings was found in the more polar isomer B. For the racemic five- and six-membered lactams **34** and **35**, the diastereomers A and B show similar activity.

Stereochemical studies demonstrate that the highest activity of **2** is achieved when all the chiral centers possess the *S* configuration.[21] It is reasonable to expect the same stereochemical assignment to prevail for **34–38**. Confirmatory X-ray crystal structure determinations were performed on the more polar B isomers of both the seven- and eight-membered lactams (Table I). For the former ring satisfactory crystals were obtained from **36**. The latter ring was represented by the racemic monoethyl ester trifluoroacetate salt **39**, the precursor of **37**. Each of the



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crystals proved to share the same relative configuration at both chiral centers (i.e., *S,S* or *R,R*; Figures 1 and 2). Since the resolved eight-membered aminolactam (+)-**27** leads to the most active inhibitor (+)-**37**, the *S* and *S,S* configurations may be respectively assigned. This follows from the X-ray data and by analogy with the highly active (*S,S*)-enalaprilat (**2**).

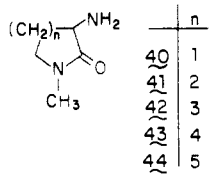
Corroborative support comes from force field derived dipole moments for the various configurational isomers. (See next section for computational details.) The MM2 values for the *S,S*/*R,R* isomers of the seven- and eight-membered lactams **36** and **37** modeled with *N*-methyl replacing *N*-carboxymethyl are calculated to be 0.7 and 1.3 D (μ; Table III) greater than the *S,R*/*R,S* diastereomers. The *S,S* diastereomer is thus predicted to be more polar in accord with the higher activity found in the B isomers. For the nine-membered lactam, the corresponding difference is 2.0 D. The more active B isomer (Table I) is thus assigned the *S,S* stereochemistry.

The relative stereochemical assignments for the racemic five- and six-membered lactams **34** and **35** are not straightforward. The diminished differences in biological activity between diastereomers A and B is a complicating factor. In addition, the persistent lack of suitable crystals prevented unequivocal chirality determination by X-ray crystallography. The force field calculations predict the *S,S*/*R,R* enantiomers to be more polar than the *R,S*/*S,R* pair by 1.6 D. Again the more polar species is suggested to have the same relative configuration at the two centers in question.

IC_{50} values were likewise obtained for amino lactams **6–10** (Table II) for comparison to alanylproline. This was done in order to obtain inhibitory data independent of side-chain effects. The IC_{50} values for these simple lactams are remarkably similar and do not correspond with the trends shown in Table I.

For inhibitors **34–38** the enhancement of biological activity on passage from the small-ring lactams to the medium-sized rings is consistent with our initial assumptions and preliminary modeling studies.[1-3] As will be discussed below, this enhancement is believed to be dependent on the accessibility of an acceptable binding conformation in **36–38** which is not available in **34** and **35**.

Computations and Modeling. In order to assess the lactam ring conformations and ultimately the key backbone angle ψ, amino lactam models **40** (n = 1)–**44** (n = 5) were investigated. Several facilities were used to construct



	n
40	1
41	2
42	3
43	4
44	5

and evaluate the structures under consideration. In all cases a final geometry refinement was carried out with Allinger's MM2 procedure[36] using amide parameters developed largely by Marshall's group.[37] The amino groups carried the MM2 force field lone pair.[36,38]

The five- and six-membered ring conformations (**40**, **41**) were obtained by light pen input via the programs DRAWMOL and MOLEDIT, features of the Merck Molecular Modeling System. The resulting standardized structures were then geometry optimized with MM2. Relative energies and ψ values are given in Table IV.

An intuitive analysis of the seven- to nine-membered lactams is insufficient to explore fully the conformational energy surface. Our present approach utilized the RINGMAKER/BAKMOD procedures of Still and Galynker.[39] RINGMAKER accepts a linear chain of atoms without substituents or hydrogens and seeks all possible ring-closure solutions within a set of input constraints. Applied to the nine-membered cis lactam,[40] for example, 407 rough starting conformations were generated. In a second step BAKMOD adds substituents, hydrogens, and lone pairs and then carries out an MM2 optimization on the initial conformations. The final optimized structures are sorted by relative strain energy. For the cis isomer of **44** and a 10-kcal upper energy limit, this led to 76 optimized conformations. The computations consumed 14 h on a VAX 11/780.

Since there are often a variety of local minima involving small coupled differences in the dihedral angles as well as a subset of identical structures arising from the BAKMOD treatment, a second conformational filtration was performed using FILTERD.[41] This program matches and compares dihedral angles for all structures in a collection and extracts those which are unique for a user specified angle tolerance. Thus, if all comparable dihedral angles for a given pair of conformations are within the specified limit, they are considered identical. For a 30° tolerance the 76 optimized nine-membered cis-lactam geometries of one of the C-3 epimers of **44** were reduced to a total of 32. Ten of these are within the lowest 4 kcal of energy. The same overall procedure was carried out a second time on the other C-3 epimer of **44**. Thus, the initial conformers arising from the RINGMAKER search are coupled to both NH_2 orientations prior to optimization. The most complete exploration of conformation space is thereby assured. in this way an additional five cis conformations for **44** below 4 kcal were found. A set of four trans conformations for **44** was located in subsequent runs which sought specifically the trans isomers. Thus a total of 19 conformations of **44** were identified within 4 kcal of the minimum-energy conformer. The conformations listed for the seven- and eight-membered rings (Table IV) were produced in similar fashion, leading to six and four conformations, respectively, within 4 kcal of the minimum-energy conformer. The second epimeric search produced no change in the number in conformations below the 4-kcal cutoff.

Representative dipole moments for the A and B isomers of **34–38** (Scheme IV) were obtained as follows. To each of the lowest energy ring shapes given in Table IV was attached a 4-phenylbutyric acid side chain to give the

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(40) When referring to lactam rings the designations cis and trans usually imply the configuration associated with the ring carbons. If the lactam is part of a peptide, the carbon to which the exocyclic nitrogen is attached dominates the configurational terminology. Thus for a small NR_2 substituted lactam, the ring is cis but the peptide is trans. For the sake of clarity, the discussion of lactams will utilize the ring nomenclature.
(41) FILTERD has been programmed and installed on the Merck Molecular Modeling System by Dr. G. M. Smith.

Scheme IV

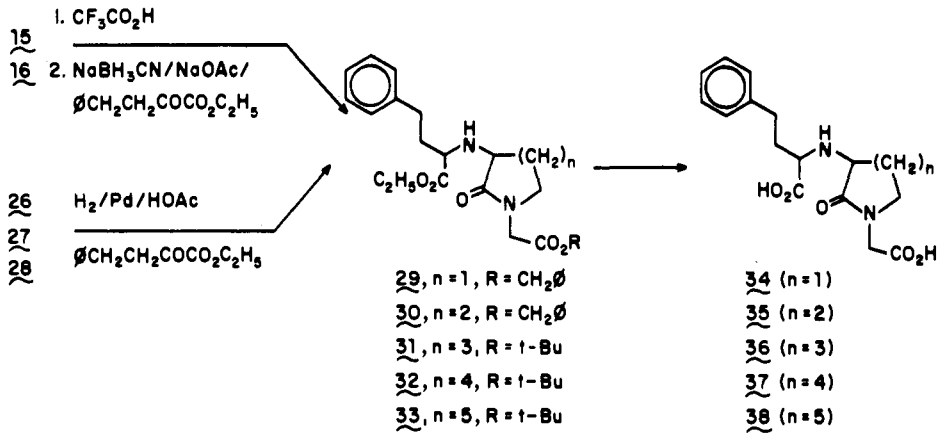


Table I. Comparison of ACE Inhibitory Activities and Minimum Energy ψ Angles for Lactam Inhibitors 34–38 and Enalaprilat (2)

compd	*n*	ring size	IC_{50} (isomer A)[a]	IC_{50} (isomer B)[a]	model[b]	ψ_{calcd}	$\psi_{X\text{-ray}}$
34	1	5	1.2×10^{-5}	1.9×10^{-5}	40	−132 (132)[c]	
35	2	6	4.3×10^{-7}	1.7×10^{-6}	41	−138 (138)[c]	
36	3	7	7.0×10^{-7} [d]	1.9×10^{-8}	42	166	166[e]
37	4	8	1.7×10^{-6}	4.8×10^{-9}	43	145	159[f]
37S,S	4	8		2.0×10^{-9} [g]			
37R,R	4	8		9.2×10^{-8} [h]			
38	5	9	1.3×10^{-6}	8.1×10^{-9}	44	135	
2				1.2×10^{-9} [i]		140	143

[a] IC_{50} concentrations are molar. Isomer A is the first diester isomer to elute from silica gel and B is the second. All compounds tested as racemic mixtures unless otherwise noted. [b] Model lactam used to obtain ψ_{calcd}. [c] Values in parentheses are for lactams having *R* configuration at C-3. [d] Approximately 5% Isomer B present by HPLC. [e] From crystal structure of **36**. [f] From crystal structure of **39**. [g] Single enantiomer from **27S**. [h] Single enantiomer from **27R**. [i] Value from *S,S,S* enantiomer.

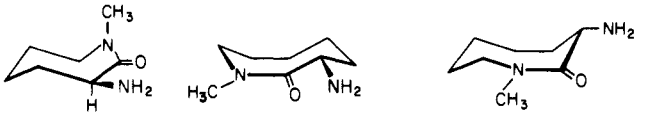
Table II. ACE Inhibition by Racemic 1-(Carboxymethyl)-3-amino Lactams **6**–**8** Compared to Alanylproline

compd	*n*	IC_{50}, M
6	1	inactive[a]
7	2	1.7×10^{-3}
8	3	4.8×10^{-4}
9	4	4.4×10^{-4}
alanylproline		4.2×10^{-5}

[a] 0% Inhibition at 3.3×10^{-4} M.

N-methyl analogues of **34**–**38**. A low-energy extended side-chain conformation was adopted for the *S,S* enantiomers and the constructs thus obtained energy optimized with MM2. The resulting structures were then converted to the *R,S*, *S,R*, and *R,R* forms by separate inversion at each of the chiral centers. An extended conformation was once again adopted with each of the C-3 invertomers now bearing an axial NHR substituent. The structures were reoptimized, giving two separate conformations for each of the *S,S*/*R,R* pairs. The individual dipole moments calculated within the force field framework are provided in Table III. A dipole moment (μ) for each diastereomer was estimated from a Boltzmann distribution based on relative MM2 energies for the optimized equatorial and axial conformations (Table III).

Comparison of X-ray and MM2 Conformations. The X-ray structures and force field conformations of **42**–**44** in terms of dihedral angles are compared in Table V. In the crystalline state the seven-membered lactam assumes the chair shape **42a** irrespective of the presence of substituents on the amide nitrogen or at C-3. The ring dihedral angles for caprolactam and **36** are provided in Table V. The MM2 global minimum for **42** (conformer **42a**) is qualitatively identical. In accord with structure **36** by X-ray crystallography (Figure 1) the amino group is situated pseudoequatorial.



42a **43a** **43b**

In the X-ray determined structure of the eight-membered lactam **39** (Figure 2), the C-3 side chain is equatorial in a distorted boat/chair form (**43a**). The lowest MM2 conformation for model lactam **43** is the same in all respects. Interestingly, 2-azacyclooctanone and its hydrochloride salt in the solid state both adopt the alternate

Table III. MM2 Calculated Relative Steric Engergies and Dipole Moments for Various Conformations of Diastereomers of Congeners of Lactams 34–37[a]

		R,S			*S,R*				*S,S*			*R,R*				
no.[a]	*n*	Erel	(%)[b]	μ[c]	Erel	(%)[b]	μ[c]	$\bar{\mu}_d$	Erel	(%)[b]	μ[c]	Erel	(%)[b]	μ[c]	$\bar{\mu}$[d]	$\Delta\mu$[e]
34	1	0.0	(76)	1.2	0.7	(24)	3.5	1.7	0.0	(98)	3.4	2.3	(2.0)	1.6	3.3	1.6
35	2	0.2	(41)	1.7	0.0	(59)	2.7	2.3	0.0	(83)	3.3	1.0	(17.0)	1.7	3.0	0.7
36	3	0.0	(62)	1.7	0.3	(38)	2.5	2.0	0.0	(99.7)	3.3	3.4	(0.3)	1.9	3.3	1.3
37	4	0.0	(99.9)	1.2	3.9	(0.1)	2.7	1.2	0.0	(100)	3.2	8.2	(0.0)	2.1	3.2	2.0

[a] The NCH_2CO_2H moiety of **34**–**37** has been simulated by NCH_3 in the MM2 calculations. [b] Erel (kcal/mol) refers to the energy difference between different conformations within the *R,S/S,R* or *S,S/R,R* manifold. The parenthetical value is the equilibrium percentage assuming only two conformations for each enantiomer and T = 25 °C. [c] MM2 dipole moments (debye). [d] The weighted average of $\mu(R,S/S,R)$ and $\mu(S,S/R,R)$ obtained from a Boltzmann distribution, T = 25 °C. [e] $\Delta\mu = \bar{\mu}(S,S/R,R) - \bar{\mu}(R,S/S,R)$.

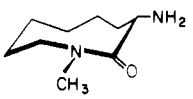
Table IV. Relative Energies, ψ Values, and Relative Populations[a] for Low-Energy Conformations of Five- to Seven-Membered *N*-Methyl-3-amino Lactams 40-44 Derived from MM2 Calculations

	E^b	ψ	%[c]		E^b	ψ	%[c]
	40 (n = 1)				44 (n = 5)		
a	0.0	−132	52.0	a	0.0	135	42.5
b	0.04	−106	48.0	b	0.6	−64	15.4
	41 (n = 2)			c	0.8	143	11.0
				d	0.9	116	9.3
a	0.0	−138	88.4	e	1.1	129	6.6
b	1.2	−111	11.6	f	1.5	−45	4.0
				g	1.5	116	3.4
	42 (n = 3)			h	1.6	131	2.9
a	0.0	166	75.0	i	1.8	−60	2.2
b	0.7	−72	23.0	j	2.1	135	1.2
c	2.3	−98	1.5	k	2.4	169	0.7
d	3.3	−109	0.3	l	3.0	124	0.3
e	3.9	−149	0.1	m	3.4	146	0.1
f	3.9	−155	0.1	n	3.4[d]	145	0.1
				o	3.7	169	0.08
	43 (n = 4)			p	3.8[d]	139	0.07
				q	3.9[d]	120	0.06
a	0.0	145	69.4	r	4.0[d]	130	0.05
b	0.5	144	29.8	s	4.0	−80	0.05
c	2.7	−48	0.7				
d	3.9	−89	0.1				

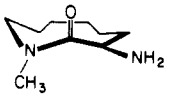
[a] Relative populations were calculated from the discrete Boltzmann distribution function: $P_i = 1/\exp[(E_i - E_j)/KT]$ as the mole ratio. [b] Relative energies, kcal/mol. [c] Population distribution. [d] Trans isomers, see ref 40.

conformation **43b**[43,44] corresponding to a shift of groups around the boat/chair frame. The second lowest MM2 conformation, only 0.5 kcal above **43a** (cf. Table IV), corresponds to the latter as indicated by the near-perfect match of dihedral angles presented in Table V.

At a ring size of nine the presence of both cis and trans amide isomers must be taken into account. The X-ray structure of 2-azacyclononanone hydrochloride is in the cis amide configuration.[47] By contrast the unprotonated lactam in the crystal is the trans isomer sustaining a nonplanar amide group with a torsion angle of 148°.[48] The MM2 global minima for the cis and trans isomers of **44** are **44a** and **44n**, respectively. The structures match the X-ray geometries closely as indicated in Table V.



44a

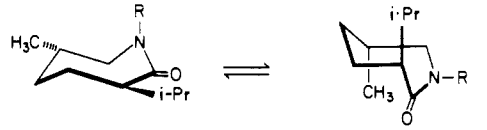


44n

The computed structures of the five- and six-membered lactams **40** and **41** are unexceptional in their mimicry of the stereochemical features of the corresponding monosubstituted olefins cyclopentene and cyclohexene. Both display nearly isoenergetic pseudoequatorial and pseudoaxial conformations (Table IV). The relative stability of the axial isomers can be attributed to the allylic effect,[49] a steric influence which destabilizes the equatorial form as found for hydrocarbons.

In short, the Still/Galynker conformation generating procedure in the MM2 framework appears capable not only of locating energy minima but also of providing a quantitative measure of geometric detail with the present parameters. However, analysis of our biological data by comparison with the computational results requires that the computed lactam conformations be representative of those which obtain in solution. For this reason the calculated conformer populations have been evaluated by comparison with solution measurements.

Lactam Equilibria and MM2 Conformations. In solution ($CDCl_3/CD_2Cl_2$) caprolactam derivatives with or without C-3 substituents appear to exist exclusively in the rigid chair conformation (e.g., **42a**) ring inverting over a barrier of around 10 kcal/mol.[50] However, circular dichroism studies on the caprolactam derivatives **45** derived from menthone (R = H or CH_3) indicate the presence of chair conformers.[51] The present force field treatment of 1-methyl-3-aminocaprolactam implies the presence of six conformers within 4 kcal of the lowest (**42a-f**). Two of these are predicted to comprise 98% of the equilibrium mixture (Table IV). Both are chairs differing only in the equatorial/axial orientation of the amino group. The theoretical result is completely compatible with the experimental findings for **45**.



45a 45b

Very little is known about the conformational freedom of 2-azacyclooctanone. Its infrared spectrum in dilute chloroform solution suggests the presence of two conformers of nearly equal free energy.[52] The MM2 calculations are in agreement. The computations suggest the most stable isomer pair (**43a,b**) exists in 69:30 population ratio (Table IV).

2-Azacyclononanone has been subjected to a series of configurational and conformational studies. Infrared spectroscopic work from several laboratories is corroborative in assigning two cis and two trans or skew conformations in a cis–trans ratio of around 70:30.[52-54] The most recent infrared study determined the percentage of each conformer in dilute CCl_4 solution (cis, 24/47%; trans, 8/21%).[54] From variable-temperature ^{15}N NMR measurements two trans isomers are likewise observed.[55] In tetrachloroethylene and dimethyl sulfoxide the free energy of activaton for cis/trans interconversion amounts to ΔG = 17 kcal/mol. The protic solvents, ^{15}N NMR additionally indicates the cis configuration to be strongly favored over the trans form. This observation is in accord with the

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Table V. Dihedral Angles for Lactams from X-ray Structure Determinations and MM2 Calculations

	ring size										
	seven			eight				nine			
	MM2(**42a**)	**36**	parent[a]	MM2(**43a**)	**41**	MM2(**43b**)	parent[b]	MM2(**44a**)	parent salt[c]	MM2(**44n**)	parent[d]
C-N-CO-C	7	5	-4	13	3	6	-1	-13	-9	139	148
N-CO-C_2-C_3	-73	-72	-63	-91	-84	93	94	-103	-100	-100	-89
C_1O-C_2-C_3-C_4	81	85	82	74	77	-51	-49	48	50	68	66
C_2-C_3-C_4-C_5	-61	-60	-64	-69	-70	-53	-57	76	78	-109	-109
C_3-C_4-C_5-C_6	62	59	61	102	96	101	100	-61	-66	65	64
C_4-C_5-C_6-C_7				-55	-50	-68	-69	-58	-55	63	67
C_5-C_6-C_7-C_8								100	103	-117	-109
C-C-C-N	-82	-79	-77	-52	-56	72	73	-87	-86	68	58
C-C-N-CO	64	64	68	87	95	-86	-82	93	93	-80	-91
N-CO-C-N	166	166	175[f]	145	159	144	146[f]	135	143[f]	145	151[f]
rms dev[e]			7		8		3		4		7

[a] Reference 42. [b] Reference 43, 47. [c] Reference 47. [d] Reference 48 [e] Rms deviation $[\sum\Delta\phi(\text{exp} - \text{calcd})^2/N]^{1/2}$. [f] The N-CO-C-H angles for the unsubstituted parent compounds.

X-ray studies on 2-azacyclononanone and its hydrochloride cited above. Ordinarily, trans amides are on the order of 2 kcal/mol more stable than the cis isomer.[56] The differential strain imposed by the nine-membered ring, 3–4 kcal/mol,[46] clearly offsets the trans preference in the 2-azacyclononanone. Ring-closure searches for conformations of the nine-membered lactam were conducted on the *N*-methyl derivatives (**44**) as for the smaller rings. Methyl substitution of the lactam nitrogen can be expected to eliminate configurational energy distinctions and to permit ring-strain domination of isomers ratios. Thus, the 13 lowest energy conformations calculated for **44** are cis (**a**–m) followed by four trans (**n**, **p**–**r**) and two cis isomers below the 4-kcal cutoff (Table IV). The trans rings have been lifted well above the MM2 global minimum and the two lowest cis conformations are predicted to exist in a ratio of around 3:1 and to account for nearly 60% of the equilibrium mixture. Correspondence with the interpretation of the infrared and NMR studies is striking.

Discussion

At the outset we assumed that the interaction of the alanine methyl group with the proline ring is of primary importance for controlling conformational equilibria. The literature is supportive in suggesting significant conformational restriction in alanylproline itself.[22,23] MM2 calculations performed on structures incorporating **3** served to reinforce this view. We further hypothesized that these inhibitors, especially in the molecular fragment defined by **3**, bind to the enzyme at or very close to their low-energy conformations. For the purpose of specifying and simplifying the synthetic design, the presence of a trans peptide bond was likewise assumed. As pointed out earlier, a significant amount of alanylproline cis isomer exists around neutral pH. Likewise, NMR measurements demonstrate a cis–trans ratio of 1:4 for the peptide bond in **2** in D_2O at room temperature.[57] The adoption of a trans target both fulfilled our assumption of employing the low-energy species and providing a definitive means for testing this hypothesis. Accordingly, we focused on structures **4** and **5** as likely candidates for conformationally restricted analogues of **3**. The data in Table I confirmed our assumption that the peptide bond in inhibitors such as **1** and **2** is in the trans configuration when bound to the enzyme. While our work was in progress, two reports appeared describing lactam analogues of captopril (**5**, X = CH_2SH, n = 1, 2) as inhibitors of ACE.[58,59] These results also pointed to the trans amide as the enzyme-bound configuration.

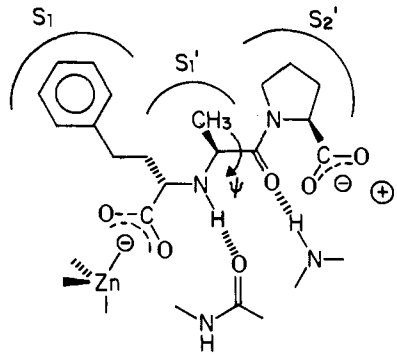

Figure 3. Enalaprilat (**2**) in an idealized ACE active site which exemplifies four putative chemical binding interactions and three hydrophobic S sites.

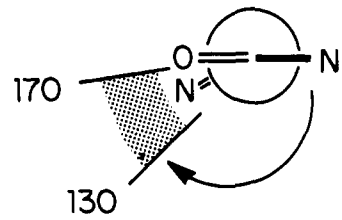

Figure 4. Window of ψ angles for **2** and the lactam inhibitors **34**–**38** which leads to the highest degree of ACE inhibition.

A phenomenological ACE active site model has been proposed by Ondetti and co-workers.[18,19] It has been adapted in Figure 3 to show **2** interacting with the proposed active site. In order to maximize the simultaneous orientation of the seven illustrated enzyme–inhibitor interactions, a specific geometric arrangement at the active site is necessary. Clearly, from Figure 3, the ψ angle plays

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a crucial role in the orientation of the bound inhibitor.

Inspection of the data in Table I points to a correlation between inhibitor potency (IC_{50}) and the calculated ψ angle for the lowest energy conformation of the respective model compounds. If the assumption that the enzyme binds a low-energy conformation is adopted, it is reasonable to conclude that a window of ψ angles from 130° to 170° (Figure 4) leads to tight binding of the inhibitor. The extremes of this window are represented by the computed ψ angles of **42** and **44** in Table IV. The existence of a range of acceptable ψ angles accommodates the notion that binding energy may be distributed among a set of binding interactions in a variety of ways. Variations of up to 20° in either direction from the mean value of 150° leads to little loss in activity. The similar activity shown by inhibitors with different ψ values implies that the ACE active site exercises an adaptive capability. Thus, modest conformational change in inhibitor structure can be accommodated through compensatory variations in the collection of binding interactions.

The small-ring inhibitors **34** and **35** are of interest from the standpoint of the inhibitory behavior of the diastereomers A and B. Unlike the most potent inhibitors **37** and **38**, there is an insignificant difference between the IC_{50} values of the A and B diastereomers. For the inhibitors having the *S* configuration at the lactam C-3 position, our computations yielded ψ angles of −132° for the five-membered lactam and −138° for the six-membered lactam. Note that these values are of the correct magnitude (but opposite sign) to fall at the lower end of the proposed ψ-angle window of 130–170°. Inhibitors with the *R* configuration at the lactam C-3 position (but retaining the *S* configuration at the phenethyl bearing carbon) possess a ψ angle of correct sign and magnitude and therefore might have higher affinity for the enzyme. Thus the slight enhancement in activity of the A isomer of **35** over its B isomer may be due to such a preference of the *R* over the *S* configuration at C-3. In the final analysis, both isomers A and B in the five- and six-membered lactam series (**34**, **35**) inhibit ACE both nearly equally and relatively poorly (Table I).

The importance of the phenylbutyric acid side chain for good binding of the inhibitor to the enzyme is illustrated by the 1-(carboxymethyl)-3-amino lactams **6**–**10** (Table II). Rather surprisingly, changes in ring size have almost no effect on the IC_{50} values. This is in marked contrast to the effect of ring size on the entire inhibitor structure (Table I). Furthermore, the inhibitor potencies of the simple lactams **6**–**10** were 10-fold less than that of alanylproline. The latter result indicates that even the eight-membered lactam **9**, which yields an inhibitor nearly equipotent with **2**, does not fully mimic the alanylproline structure. This could be due in part to the absence of a fused five-membered ring.[60] Lack of the proline ring could be responsible for nonoptimal orientation of the carboxyl group in **9**. It is also possible that adverse interactions of the lactam ring with the enzyme active site might also contribute to the lower binding affinity of **9** with respect to alanylproline. Large differences in inhibitor potency arise only when the phenylbutyric acid side chain is appended onto each of the relatively equipotent simple lactams. This indicates again that an acceptable ψ angle is a necessary but not sufficient condition for high inhibitory potency. Other torsion angles must also be considered. In particular, angles which mediate the spatial relationship of the phenylbutyric acid side chain with the lactam ring are undoubtedly important. Consideration of the conformational outcome of this intramolecular interaction leads to a more complete understanding of the binding of the inhibitors to the ACE active site.[61]

It appears that the key binding events are shared in detail by the lactam inhibitors **34**–**38** and inhibitors such as enalaprilat (**2**).[62] In this context and in view of the experimental data currently available, we belive that the ψ angle defined in **3** is an important determinant in the binding of inhibitors to ACE. The inhibitors **34**–**38** have served to define a window of ψ angles from 130° to 170° which seems to be acceptable to the ACE active site.[63] The presence of such a window implies a certain amount of active site flexibility capable of optimizing the hydrophobic, electrostatic, and hydrogen-bonding interactions believed to be important for substrate and inhibitor binding.

The results of this study illustrate two aspects of drug design utilizing peptide leads as a starting point. First, the very high inhibitory potency of MK-422 (**2**) can be interpreted to imply that a conformation of its Ala-Pro part structure near the global minimum is that which binds to the enzyme. Accordingly, the X-ray structure in tandem with the force field calculations have permitted both the identification and exploitation of one of the key torsional angles central to ACE inhibitor design. The result is a new structural class of ACE inhibitors equipotent with **2**.

The second aspect of drug design to emerge from this work is the potentially useful technique of using lactams as conformational probes in peptides. This concept was independently derived and used by Freidinger and coworkers.[10] Our work shows that medium-ring lactams of seven to nine members can be useful as replacements for alanylproline. Freidinger has shown that small ring lactams of five and six members are of value in promoting β-turn conformations in peptides. Lactams suitably integrated into a polyamide ribbon are thus complementary to other chemical modifications useful as conformational probes of peptides.

Experimental Section

General Procedures. NMR spectra data are tabulated in Table VI in the supplementary material. Melting points are uncorrected. Tetrahydrofuran (THF) was freshly distilled from sodium benzophenone. All reactions involving air-sensitive compounds were run in dried glassware under nitrogen.

(±)-1-(Carboxymethyl)-3-[(1-carboxy-3-phenylpropyl)-amino]-2-pyrrolidone (34). (±)-3-Amino-2-pyrrolidone (11; 2.04 g, 23.7 mmol) was dissolved in 25 mL of CH_2Cl_2 containing 3.4 mL of triethylamine. Di-*tert*-butyl dicarbonate (5.43 g, 24.9 mmol) was then added and the reaction was stirred for 3 h at room temperature. After concentration ether was added to the residue and the mixture filtered. Concentration of the filtrate yielded 4.0 g (85%) of 3-[(*tert*-butoxycarbonyl)amino]-2-pyrrolidone (**13**; mp 174–175 °C).

A solution of 4 g of **13** (20 mmol) and 5.79 g of benzyl iodoacetate (21 mmol) in 25 mL of tetrahydrofuran and 25 mL of dimethylformamide was added dropwise over 15 min to a suspension of 0.52 g of sodium hydride (22 mmol) in 10 mL of tetrahydrofuran. The reaction was stirred at room temperature

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(61) Snyder, J. P., unpublished results.

(62) Bull, H., unpublished observations.

(63) Andrews et al. (Andrews, P. R.; Carson, J. M.; Caselli, A.; Spark, M. J.; Woods, R. *J. Med. Chem.* **1985**, *28*, 393–399) have reported computed ψ angles in general agreement with our data for a variety of ACE inhibitors.

for 3.5 h at which time TLC (silica, ethyl acetate–hexane, 7:3) showed only a trace of **13** remaining. The reaction was treated with a few milliliters of saturated NH_4Cl solution and then concentrated in vacuo. The residue was partitioned between ethyl acetate and water. The organic phase was washed with water, dried ($MgSO_4$), and concentrated to an oily solid (5.79 g). Trituration with ether gave 3.2 g (46%) of 1-[(benzyloxycarbonyl)methyl]-3-[(*tert*-butoxycarbonyl)amino]-2-pyrrolidone (**15**; mp 131–133 °C). Anal. ($C_{18}H_{24}N_2O_5$) C, H, N.

Trifluoroacetic acid (10 mL) was added to 3.0 g (8.6 mmol) of **15** and the resulting solution kept for 2 h at room temperature. The reaction was then concentrated and the residue twice reconcentrated from water. 1-[(Benzyloxycarbonyl)methyl]-3-amino-2-pyrrolidone trifluoroacetate (**46**) was obtained as a gum and used directly in the following step.

To a solution of 2.97 g of **46** (8.47 mmol), 0.694 g of sodium acetate (8.47) mmol), and 7.0 g of ethyl 2-oxo-4-phenylbutyrate (33.9 mmole) in 10 mL of ethanol was added dropwise 1.72 g of sodium cyanoborohydride (25.4 mmol) in 15 mL of ethanol over 18 h. The reaction mixture was then concentrated and the residue partitioned between ethyl acetate and water. The organic phase was dried ($MgSO_4$) and concentrated to 9.21 g of an oil. Chromatography over silica gel (hexane–ethyl acetate, 3:2) afforded the two sets of racemates of (±)-1-[(benzyloxycarbonyl)-methyl]-3-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2-pyrrolidone (**29**). Isomer A (first to elute): 680 mg (18%), oil. Anal. ($C_{25}H_{30}N_2O_5$) C, H, N. R_f 0.63 (silica; ethyl acetate–hexane, 3:2). Isomer B (second to elute): 1.84 g (50%), oil. Anal. ($C_{25}H_{30}N_2O_5$) C, H, N. R_f 0.45 (silica; ethyl acetate–hexane, 3:2).

Each of the above diesters (**29**) was hydrolyzed in dilute aqueous methanolic sodium hydroxide. The hydrolysates were chromatographed over Dowex 50 (acid cycle), the desired diacids being eluted with 5% aqueous pyridine. Concentration of the eluates afforded the acids **34** as white solids. Isomer A: 50%. Anal. ($C_{15}H_{20}N_2O_5$) C, H, N. Isomer B: 62%. Anal. ($C_{15}H_{20}N_2O_5$) C, H, N.

(±)-1-(Carboxymethyl)-3-amino-2-pyrrolidone (6). A solution of 0.488 g of **46** in 15 mL of 2:1 methanol–water was hydrogenated over 150 mg of 10% Pd–C at 40 psi. The reaction was filtered and concentrated to afford a gum which was purified by chromatography over Dowex 50 (acid cycle). The desired **6** was eluted with 5% aqueous pyridine and isolated as the monohydrate (142 mg) after concentration of the eluate. Anal. ($C_6H_{10}N_2O_3 \cdot H_2O$) C, H, N.

Piperidone Derivatives. The piperidone-derived inhibitors were prepared from the corresponding 3-amino-2-piperidone derivatives by using the procedures described above. Dimethylformamide was required as a cosolvent for the alkylation of the 3-[(*tert*-butoxycarbonyl)amino]-2-piperidones.

(±)-3-[(*tert*-Butoxycarbonyl)amino]-2-piperidone (14): 80%, mp 108–110 °C (ether).

(±)-1-[(Benzyloxycarbonyl)methyl]-3-[(*tert*-butoxycarbonyl)amino]-2-piperidone (16): isolated as an oil (56%) after chromatography over silica (ether). R_f 0.6 (silica; ethyl acetate–hexane, 1:1).

(±)-1-[(Benzyloxycarbonyl)methyl]-3-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2-piperidone (30). Isomer A, 15%. Anal. ($C_{26}H_{32}N_2O_5$) C, H, N. Isomer B, 40%. NMR: Anal. ($C_{26}H_{32}N_2O_5$) C, H, N.

(±)-1-(Carboxymethyl)-3-[(1-carboxy-3-phenylpropyl)-amino]-2-piperidone (35). Isomer A. Anal. ($C_{17}H_{22}N_2O_5 \cdot {}^1/_3H_2O$) C, H, N. HPLC, t_R = 7.5 min.[64a] Isomer B. Anal. ($C_{17}H_{22}N_2O_5 \cdot {}^1/_2H_2O$) C, H, N. HPLC, t_R = 5.5 min.

(±)-1-(Carboxymethyl)-3-amino-2-piperidone (7). Hydrogenation of (±)-1-[(benzyloxycarbonyl)methyl]-3-[(*tert*-butoxycarbonyl)amino]-2-piperidone afforded (±)-1-(carboxymethyl)-3-[(*tert*-butoxycarbonyl)amino]-2-piperidone (**49**), mp 125–126 °C (ethyl acetate–hexane). Treatment of **49** with anhydrous trifluoroacetic acid and subsequent purification over Dowex 50 resin yielded **7**. Anal. ($C_7H_{12}N_2O_3 \cdot {}^1/_2H_2O$) C, H, N.

(±)-1-(Carboxymethyl)-3-[(1-carboxy-3-phenylpropyl)-amino]perhydroazocin-2-one (37). 3-Bromoperhydroazocin-2-one[33] (**18**) was converted to 3-azidoperhydrozoacin-2-one (**21**) with sodium azide in aqueous ethanol[34] (70%). An analytical sample was obtained by chromatography (silica; ethyl acetate–acetonitrile, 9:1): mp 108–109 °C. Anal. ($C_7H_{12}N_4O$) C, H, N.

To a stirred suspension of 16.5 g of sodium hydride (0.687 mol) in 600 mL of tetrahydrofuran was added 100.6 g of 3-azidoperhydroazocin-2-one (0.63 mol) and 150.8 g of *tert*-butyl iodoacetate (0.673 mol) in 1.2 L of tetrahydrofuran at a rate to maintain gentle reflux. After the addition was completed the mixture was stirred for 1.5 h and then quenched with 100 mL of saturated ammonium chloride solution. The mixture was filtered and concentrated to afford 136 g (76%) 1-[(*tert*-butoxycarbonyl)methyl]-3-azidoperhydroazocin-2-one (**24**). An analytical sample was obtained by chromatography (silica; hexane–ethyl acetate, 7:3: mp 101–102 °C. Anal. ($C_{13}H_{22}N_4O_3$) C, H, N.

Hydrogenation of 9.5 g of **24** over 1.0 g of 10% Pd–C yielded 1-[(*tert*-butoxycarbonyl)methyl]-3-aminoperhydroazocin-2-one (**27**; 100%): mp 87–88 °C (ether–hexane). Anal. ($C_{13}H_{24}NO_3$) C, H, N.

A solution of 4.10 g of **27** (16 mmol), 4.95 g of ethyl 2-oxo-4-phenylbutyrate (24 mmol), and 0.96 g of acetic acid (16 mmol) in 150 mL of ethanol was hydrogenated over 0.5 g of 10% Pd–C at 40 psig and room temperature. After filtration and concentration the residue was chromatographed (silica; hexane–ethyl acetate, 3:2) to afford the two diastereomeric racemates of 1-[(*tert*-butoxycarbonyl)methyl]-3-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]perhydroazocin-2-one (**32**) as oils. Isomer A, 1.16 g (16%). R_f 0.5 (silica; hexane–ethyl acetate, 1:1). Anal. ($C_{25}H_{38}N_2O_5$) C, H, N. Isomer B, 2.48 g (35%. R_f 0.36 (silica, hexane–ethyl acetate, 1:1). Anal. ($C_{25}H_{38}N_2O_5$) C, H, N.

Diester isomer A was treated with anhydrous trifluoroacetic acid to remove the *tert*-butyl ester. The resulting monoester was then hydrolyzed in methanolic sodium hydroxide. Chromatography over Dowex 50(H^+) afforded **37** (isomer A). This compound eluted from the column as its monosodium salt. Anal. ($C_{19}H_{25}N_2O_5Na$) C, H, N.

Diester isomer B (500 mg) was treated with 5 mL of trifluoroacetic acid at room temperature for 2 h to afford (±)-1-(carboxymethyl)-3-[[1-(ethoxycarbonyl)-3-phenylpropyl]-amino]perhydroazocin-2-one trifluoroacetate (**39**), mp 211–213 °C dec. Anal. ($C_{21}H_{30}N_2O_5 \cdot CF_3CO_2H$) C, H, N.

A solution of 504 mg of **39** in 2.5 mL of methanol and 2.5 mL of 1 N NaOH was stirred overnight at room temperature. The reaction was reduced in volume by ca. 50% and chromatographed over Dowex 50(H^+). The product eluted with 5% aqueous pyridine. Concentration of the eluate afforded **37** (isomer B) as a white solid, mp 265–268 °C dec. Anal. ($C_{19}H_{26}N_2O_5 \cdot {}^1/_2H_2O$) C, H, N. HPLC, t_R = 6.2 min.[64b]

Resolution of (±)-1-[(*tert*-butoxycarbonyl)methyl]-3-aminoperhydroazocin-2-one (27). To a warm solution (60 °C) of 47.5 g of L-pyroglutamic acid in 2 L of acetonitrile was added 94.7 g of **27** in 600 mL of acetonitrile. The solution was stirred for 2 h at room temperature and then stored at 4 °C for 18 h. The solids were filtered and washed with acetonitrile (2 × 200 mL) to afford 56.4 g of **27R** as the L-pyroglutamate. Repeated slurrying with aceonitrile gave material of constant mp 183–185 °C. Treatment of the pure salt with aqueous ammonia gave pure amino ester **27R**, $[\alpha]_D$ −43.3, (*c* 0.71, ethanol).

The mother liquors from the above preparation were concentrated and the residue partitioned between ethyl acetate and aqueous ammonia. Concentration of the organic phase gave 61.1 g of the amine. This amine was dissolved in 600 mL of acetonitrile and added to 30.5 g of D-pyroglutamic acid in 2 L of acetonitrile. Workup as described above afforded 51.6 g of **27S**, $[\alpha]_D$ +43° (*c* 1.55, ethanol).

(±)-1-(Carboxymethyl)-3(*S*)-[(1(*S*)-carboxy-3-phenylpropyl)amino]perhydroazocin-2-one (37S,S). Ethyl 2-oxo-4-phenylbutyrate was reductively aminated with **27S** as described above to yield, after chromatography, the two diastereomeric diesters **32S,R** (isomer A) and **32S,S** (isomer B). Diester isomer B was treated with 20 mL of 4 N HCl in ethyl acetate to afford

(64) HPLC analyses were carried out on EM Labs RP-18 Hibar 0.43 × 25 cm columns using the following solvent systems at a flow rate of 2.0 mL/min: (A) 25% CH_3CN–75% 0.01 M tetrabutylammonium phosphate, pH 7, (B) 30% CH_3CN–70% 0.01 M tetrabutylammonium phosphate, pH 7, (C) 35% CH_3CN–65% 0.01 M tetrabutylammonium phosphate, pH 7.

(65) Isomer A is either *R* or *S* at the chiral center bearing the phenethyl group and isomer B is of opposite chirality.

(±)-1-(carboxymethyl)-3(*S*)-[(1(*S*)-(ethoxycarbonyl)-3-phenylpropyl]amino]perhydroazocin-2-one hydrochloride (**39S,S**), mp 171–173 °C dec, $[\alpha]_D$ +29.4° (*c* 1.8, ethanol). Anal. ($C_{21}H_{30}N_2O_5$) C, H, N. Subsequent alkaline hydrolysis and purification over Dowex 50(H) gave **37S,S**, mp 208–211 °C, $[\alpha]_D$ +47.2° (*c* 1.7, 1 N NaOH). Anal. ($C_{19}H_{26}N_2O_5 \cdot {}^1/_4H_2O$) C, H, N.

(−)-1-(**Carboxymethyl**)-3(***R***)-[[1(***R***)-carboxy-3-phenylpropyl]amino]perhydroazocin-2-one (**37R,R**). This compound was prepared from **27R** by using the procedure described for the preparaton of **32**. Chromatography afforded the diastereomers **32R,S** (isomer A) and **32R,R** (isomer B). Isomer B was converted to (−)-1-(carboxymethyl)-3(*R*)-[[1(*R*)-(ethoxycarbonyl)-3-phenylpropyl]amino]perhydroazepin-2-one hydrochloride (**39R,R**), mp 171–174 °C dec, $[\alpha]_D$ −25.7 (*c* 1.5, H_2O). Anal. ($C_{21}H_{31}N_2O_5$) C, H, N. Alkaline hydrolysis of **39R,R** and purification over Dowex 50(H^+) afforded **37R,R**, mp 215–219 °C dec, $[\alpha]_D$ −43.5 (*c* 1.4, 1 N NaOH). Anal. ($C_{19}H_{26}N_2O_5 \cdot {}^1/_4H_2O$) C, H, N.

(±)-1-(**Carboxymethyl**)-3-[(1-**carboxy-3-phenylpropyl**)-**amino**]**perhydroazepin-2-one** (**36**). These isomeric compounds were prepared via the following intermediates by using the procedure described for **37** starting from 3-azidoperhydroazepin-2-one[34] (**20**). 1-[(*tert*-Butoxycarbonyl)methyl]-3-azidoperhydroazepin-2-one (**23**), mp 69–70 °C. Anal. ($C_{12}H_{20}N_4O_3$) C, H, N. 1-(*tert*-Butoxycarbonyl)-3-aminoperhydroazepin-2-one (**26**): oil, NMR δ 1.5 (s, 9 H), 1.5–2.2 (m, 6 H), 2.9–4.0 (m, 5 H), 4.1 (s, 2 H). 1-[(*tert*-Butoxycarbonyl)methyl]-3-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]perhydroazepin-2-one (**31**). Isomer A, oil, 26%. Anal. ($C_{24}H_{36}N_2O_5$) C, H, N. Isomer B, oil, 40%. Anal. ($C_{24}H_{36}N_2O_5$) C, H, N. 1-(Carboxymethyl)-3-[[1-(carboxymethyl)-3-phenylpropyl]amino]perhydroazepin-2-one (**36**). Isomer A. Anal. ($C_{18}H_{24}N_2O_5 \cdot {}^1/_5H_2O$) C, H, N. HPLC, t_R 7.5 min.[64a] Isomer B. Anal. ($C_{18}H_{24}N_2O_5 \cdot {}^1/_5H_2O$): C, H, N. HPLC, t_R = 4.0 min.[64a]

(±)-1-(**Carboxymethyl**)-3-[(1-**carboxy-3-phenylpropyl**)-**amino**]**perhydroazonin-2-one** (**38**). A solution of 2.20 g of 3-bromoperhydroazonin-2-one[33] (**19**; 10 mmol) and 0.96 g of lithium azide (19.5 mmol) in 15 mL of dimethylformamide was heated at 80 °C for 96 h. The reaction was concentrated and the residue partitioned between water and dichloromethane. Drying and concentration of the organic extract afforded 1.60 g of the azide (**22**) as a solid which was purified by chromatography (silica; ethyl acetate–acetonitrile, 9:1). Sublimation (80 °C, 10 μ) afforded an analytical sample, mp 105–106 °C. Anal. ($C_8H_{14}N_4O$) C, H, N. With use of the procedures described for **37**, the above azide was converted through the following intermediates to the inhibitors **38**. 1-[(*tert*-Butoxycarbonyl)methyl]-3-azidoperhydroazonin-2-one (**25**), 47%, mp 111–112 °C (silica, hexane–ethyl acetate, 4:1). Anal. ($C_{14}H_{24}N_4O_3$) C, H, N. 1-[(*tert*-Butoxycarbonyl)methyl]-3-[[1-(ethoxycarbonyl)-3-phenylpropyl]-amino]perhydroazonin-2-one (**33**). Isomer A, R_f 0.6 (silica; hexane–ethyl acetate, 1:1); mass spectrum, 460 (M^+), 403 ($M^+ - C_4H_9$), 387 ($M^+ - CO_2C_2H_5$). Isomer B, R_f 0.5; mass spectrum, indistinguishable from isomer A. 1-(Carboxymethyl)-3-[(1-carboxy-3-phenylpropyl)amino]perhydroazonin-2-one (**38**). Isomer A. Anal. ($C_{20}H_{28}N_2O_5$) C, H, N. Mass spectrum, 376 (M^+), 358 ($M^+ - H_2O$), 331 ($M^+ - CO_2H$); HPLC, t_R 5.5 min.[64c] Isomer B. Anal. ($C_{20}H_{28}N_2O_5 \cdot 2H_2O$) C, H, N. Mass spectrum, indistinguishable from isomer A; HPLC, t_R = 4.2 min.[64c]

1-(**Carboxymethyl**)-**3-amino Lactams 8 and 9**. The 1-[(*tert*-butoxycarbonyl)methyl-3-amino lactams **26** and **27** were treated with anhydrous trifluoroacetic acid at room temperature for 3 h. Concentration and chromatography over Dowex 50(H^+), eluting first with water and then 5% aqueous pyridine, afforded **8** and **9**, respectively, as crystalline solids after evaporation of the solvent.

(±)-1-(**Carboxymethyl**)-**3-aminoperhydroazepin-2-one** (**8**): mp 288–290 °C dec. Anal. ($C_8H_{14}NO_3$) C, H, N.

(±)-1-(**Carboxymethyl**)-**3-aminoperhydroazocin-2-one** (**9**): mp 266–268 °C dec. Anal. ($C_9H_{16}NO_3$) C, H, N.

Crystal Structure of 36. Data for the single-crystal solution of structure **36** was collected on a fully automated Enraf-Nonium CAD4 X-ray diffractometer using Cu Kα radiation and employing a $2\theta/\omega$ scan at 50 kV/20 ma up to a maximum 2θ of 115°. The unit cell parameters are a = 12.868 (2) Å, b = 14.047 (3) Å, c = 11.508 (2) Å, α = 101.32 (1)°, β = 108.56 (1)°, c = 67.85 (1)°, and V = 1820 (1) Å in the centrosymmetric space group $P\bar{1}$ (Z = 4). The specimen was grown in dimethyl sulfoxide and mounted in air with epoxy. A total of 4995 symmetry independent reflections were collected of which 3054 (61.1%) were considered observed at the level $I \geq 3\sigma(I)$. Data reduction, least squares, electron-density synthesis, and other related calculations were performed by the Structure Determination Package (SDP) of computer programs on a DEC 11/60 computer (Okaya, Y.; Frenz, B.; Brice, M.; Corfield, P.; Hodgson, K.; Rohrer, D.; Sinn, E., Enraf-Nonius Structure Determination Package, Revision 3-B; April 1980, An Integrated Set of Crystallographic Computer Programs Written for Use on PDP-11 Series of Computers). A partial solution was found by MULTAN (Main, P.; Fiske, S. J.; Hull, S. E.; Lessinger, L.; Germain, C.; Declercq, J. P.; Woolfson, M. M., 1980, MULTAN "A System of Computer Programs for the Automatic Solution of Crystal Structures from X-Ray Diffraction Data"; Universities of York, England and Louvain, Belgium) and expanded into a complete structure by a series of difference electron density syntheses. Refinement was carried out with full-matrix least squares with anisotropic temperature factors for non-hydrogen atoms. Hydrogen atoms were assigned equivalent isotropic temperature factors for the atoms to which they were bound and refined for positional parameter variation only. The final residual index (R factor) was 0.0530. Three tables consisting of atomic fractional coordinates, bond lengths, and angles have been deposited as supplementary material. Figure 1 shows the asymmetric unit of a perspective drawing of **36** with the crystallographic numbering scheme.

Crystal Structure of 39. Crystals of **39** formed as the trifluoroacetate salt in space group $P\bar{1}$ with a = 8.995 (4) Å, b = 12.724 (7) Å, c = 13.576 (7) Å, α = 90.62 (4)°, β = 106.47 (4)°, and γ = 117.46 (4)° for Z = 2. Of the 3439 reflections measured with $2\theta \leq 114°$ using Cu Kα radiation, 2042 were observed at $I \geq 3\sigma I$. Data reduction, least squares, electron-density synthesis, and related calculations were performed by using the methods and procedures described for **36**. Figure 2 is a computer-generated drawing of **39** showing its relative configurations and crystal-state conformation. Surprisingly, there are no hydrogen bonds between the inhibitor structure and its trifluoroacetate counter ion. The only hydrogen bonds are two asymmetric ones [N(6)–O(9), 2.80 Å and N(6)–O(10), 2.85 Å] and two symmetric ones across a center of symmetry [O(10)–O(10′), 2.46 Å and O(30)–O(30′), 2.47 Å].

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Supplementary Material Available: Tables listing NMR data and the fractional coordinates, bond distances, and bond angles for **36** and **3** (12 pages). Ordering information is given on any current masthead page.