to give 17 (0.97 g, 62%).

General Method B. (1*S-cis*)-5-Amino-1-cyclopropyl-6,8-difluoro-7-(2,5-diazabicyclo[2.2.1]hept-2-yl)-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (8). 5-Amino-1-cyclopropyl-6,7,8-trifluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid[15] (4; 2.98 g, 10.0 mmol), (1*S*,4*S*) (1*S*,-*cis*)-2,5-diazabicyclo[2.2.1]heptane dihydrochloride[7] (A_{SS}; 3.36 g, 12.9 mmol), and triethylamine (6.0 g, 60.0 mmol) were added to acetonitrile (75 mL), and the mixture was heated to reflux for 7.5 h and then cooled to room temperature. The precipitated solid was collected by filtration and washed successively with ethanol, acetonitrile, and ether to give the crude product. This solid was dissolved in water, made basic (pH = 11), and filtered, and the pH slowly adjusted to pH = 7.2 with an HCl solution. The precipitated solid was collected and washed successively with water, 2-propanol, and ether to give 8 (2.40 g, 64%).

General Method C. *exo*-1-Cyclopropyl-6-fluoro-7-[3-(ethylamino)-8-azabicyclo[3.2.1]oct-8-yl]-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (39). 1-Cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid[13] (2; 0.66 g, 2.5 mmol), *exo*-3-(ethylamino)-8-azabicyclo[3.2.1]octane dihydrochloride[11,12] (E; 0.62 g, 2.75 mmol), and 1,8-diazabicyclo[5.4.0]undec-7-ene (1.12 mL, 7.5 mmol) were added to pyridine (10 mL), and the mixture was heated to reflux for 4 h and then cooled to room temperature. The precipitated solid was collected by filtration and washed with ethanol to give 39 (0.43 g, 43%).

General Method D. 7-(4-Amino-1-piperidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (29). 1-Cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid[13] (2; 1.20 g, 4.6 mmol), 4-[(trifluoroacetyl)amino]piperidine trifluoroacetic acid salt (1.84 g, 6.0 mmol), and triethylamine (3.8 mL, 27.3 mmol) were added to acetonitrile (40 mL), and the mixture was heated overnight at reflux, then cooled to room temperature and diluted with diethyl ether (50 mL). The precipitate formed was collected by filtration and dissolved in a solution of ethanol (150 mL), 2 N HCl (150 mL), and acetic acid (200 mL) and heated at reflux for 36 h. The reaction was cooled and concentrated to a solid. The solid was crystallized from methanol–diethyl ether to give a solid. This solid was recrystallized from methanol to give 0.30 g (17%) of the title compound; 29.

General Method E. (1*S-cis*)-1-Cyclopropyl-6,8-difluoro-1,4-dihydro-7-(5-methyl-2,5-diazabicyclo[2.2.1]hept-2-yl)-4-oxo-3-quinolinecarboxylic Acid (10). A solution of (1*S-cis*)-1-cyclopropyl-6,8-difluoro-7-(2,5-diazabicyclo[2.2.1]hept-2-yl)-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid (7; 3.60 g, 9.5 mmol), 85% formic acid (50 mL), and 37% formaldehyde solution (50 mL) was heated to reflux for 4.25 h. After cooling, the reaction mixture was evaporated and the residue was dissolved in hot ethanol and 6 N HCl in 2-propanol (3 mL) added. Upon cooling, a solid precipitated from the solution and was collected and dried to give 10 (3.05 g, 86%).

General Method F. *exo*-1-Cyclopropyl-7-[3-(ethylamino)-8-azabicyclo[3.2.1]oct-8-yl]-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid (33). The difluoroborate ester of 1-cyclopropyl-6,7-difluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid[19] (1.50 g, 4.8 mmol) and *endo*-3-[[(1,1-dimethylethoxy)carbonyl]amino]-8-azabicyclo[3.2.1]octane (E) (2.71 g, 12 mmol) were added to acetonitrile (10 mL), and the mixture was heated to reflux for 4 h and then cooled to room temperature and allowed to stand for 48 h. The precipitated solid was collected by filtration to give the protected intermediate (1.17 g, 47%). This intermediate was dissolved in ethanol (95%, 80 mL) containing triethylamine (0.5 mL) and heated to reflux for 24 h. The reaction was cooled and evaporated to a solid residue. This residue was dissolved in trifluoroacetic acid (10 mL) and stirred for 1.5 h, then evaporated to a solid. This crude product was dissolved in 5 N HCl (200 mL) and the pH adjusted to 7 with 50% solution of NaOH. The precipitated solid was collected by filtration and washed with water to give 33 (0.59 g, 61% for the deprotection steps).

Supplementary Material Available: MIC data, regression data, and analytical analysis for all compounds (18 pages). Ordering information is given on any current masthead page.

Configuration and Preferential Solid-State Conformations of Perindoprilat (S-9780). Comparison with the Crystal Structures of Other ACE Inhibitors and Conclusions Related to Structure–Activity Relationships

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The conformation of perindoprilat, an antihypertensive drug, is studied in the solid state by X-ray analysis. The resolution of its structure reveals important analogies between its observed conformation and that of several ACE inhibitors of the same family. This comparison points out a constant relative orientation of the functional groups, regardless of the molecular environment. This angular constancy appears to us as not being accidental and is a good argument for the spatial design of the ACE binding site. Although ACE is a carboxydipeptidase, the binding site may not contain two but one unique hydrophobic pocket receiving the C-terminal end of the inhibitors.

Introduction

Perindopril (S-9490) (1) (Figure 1) belongs to the class of antihypertensive drugs, acting through the inhibition of angiotensin converting enzyme (EC 31.15.1, ACE), a zinc metalloenzyme involved in the control of blood pressure. Perindopril is an acid–ester prodrug. It is well absorbed through the oral route and desesterified in the liver by esterases, resulting in perindoprilat (2), its active diacid form.

1 was synthesized through a stereospecific method,[1,2] and 2 by saponification of 1. Nevertheless, it was desirable to

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(1) Vincent, M.; Rémond, G.; Portevin, B.; Serkiz, B.; Laubie, M. *Tetrahedron Lett.* **1982**, *23*, 1677–1680.
(2) Robert, F.; Jeannin, Y.; Vincent, M.; Laubie, M. *Acta Crystallogr.* **1984**, *C40*, 1219–1220.
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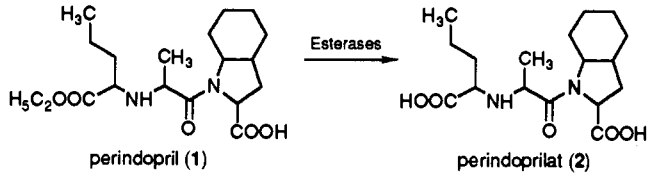

Figure 1. Structures of 1 and 2.

Table I. Structures of ACE Inhibitors Compared with 2

	rel lit.
captopril (3)	3, 4
enalapril (4)	5, 6
enalaprilat (5)	7, 5
ramiprilat (6)	8
seven-membered ring (Merck) 7	9
eight-membered ring (Merck) (8)	9
cilazapril (9)	10, 11

confirm by crystallographic studies the *S* configuration of the five chiral carbon atoms present in the active form 2.

We took advantage of this study to compare the solid-state conformation of 2 with the crystal structures of seven known ACE inhibitors structurally related to 2. They are listed in Table I. Some structure–activity relationships have been deduced from these comparisons.

Solid-State Studies

2 crystallizes with two ethanol molecules. The structure was refined to an *R* factor of 6.1%. All the crystallographic data and refinement details are gathered in the Experimental Section. The atomic coordinates are listed in Table II, and the short intermolecular hydrogen bonds in Table III.

Crystallographic Results on 2. The configuration of the molecule, based on the assumed (*S*)-alanine and represented on the stereo diagram Figure 2, is *all-S*. Atom numbering is given in Figure 3.

(6) (a) In, Y.; Shibata, M.; Doi, M.; Ishida, T.; Inoue, Y.; Sasaki, Y.; Morimoto, S. J. C. S. *Chem. Commun.* **1986**, 473. (b) Précigoux, G.; Geoffre, S.; Leroy, F. *Acta Crystallogr.* **1986**, *C42*, 1022–1024.

Table II. Atomic Coordinates of 2 (×10⁴) and Equivalent Isotropic Thermal Parameters (×10³)[a]

	X	*Y*	*Z*	*U*
O1	1906 (1)	−1191 (2)	3156 (4)	63 (3)
O2	−69 (2)	563 (3)	2229 (4)	76 (4)
O3	532 (1)	1050 (3)	451 (4)	64 (3)
O4	3609 (1)	−498 (3)	3748 (3)	56 (3)
O5	2933 (2)	−632 (3)	5470 (4)	70 (4)
N1	2069 (1)	535 (3)	3851 (4)	47 (3)
C2	2713 (2)	441 (3)	3418 (5)	45 (3)
C3	2950 (2)	1623 (4)	3516 (6)	59 (4)
C3A	2382 (2)	2313 (4)	3345 (6)	57 (4)
C4	2446 (3)	3507 (4)	3782 (7)	76 (6)
C5	2471 (3)	3650 (5)	5374 (8)	88 (7)
C6	1923 (3)	3116 (5)	6055 (8)	94 (7)
C7	1946 (3)	1899 (4)	5757 (6)	72 (5)
C7A	1911 (2)	1680 (4)	4161 (5)	52 (4)
C8	1722 (2)	−320 (4)	3617 (5)	47 (3)
C9	1050 (2)	−248 (3)	4001 (3)	48 (3)
N10	716 (1)	−994 (3)	3051 (4)	45 (3)
C11	693 (2)	−677 (4)	1544 (5)	55 (4)
C12	341 (4)	−1612 (5)	754 (6)	101 (9)
C13	252 (4)	−1489 (7)	−820 (8)	82 (8)
C13′	−148 (1)	−1843 (1)	405 (1)	104 (9)
C14	−60 (5)	−2517 (9)	−1400 (12)	136 (17)
C14′	−211 (1)	−2732 (1)	−505 (1)	135 (17)
C15	3078 (2)	−304 (4)	4347 (5)	47 (3)
C16	945 (3)	−581 (5)	5526 (6)	72 (5)
C17	358 (2)	412 (4)	1409 (5)	54 (4)
OEA	363 (2)	3274 (3)	694 (6)	86 (4)
E2A	943 (5)	3735 (12)	764 (28)	237 (31)
E3A	1084 (11)	4432 (22)	−296 (24)	208 (37)
E3′A	1357	3685	801	353
OEB	4000 (2)	3148 (3)	8362 (6)	99 (5)
E2B	3562 (3)	3762 (6)	9130 (11)	125 (12)
E3B	3803 (7)	3911 (14)	10630 (14)	186 (5)
E3′B	3152	3449	10069	199

[a] Occupation factor for C13 and C14, 80%; for C13′ and C14′, 20%; for the ethanol methyl groups E3A and E3B, and E3′A and E3′B, 60% and 40%, respectively.

Table III. Short Intermolecular Hydrogen Bonds (Å) of 2, 5, and 6

atom	ramiprilat (6)	enalaprilat (5)	perindoprilat (2)
acceptor	donor	donor	donor
O3	O4–H, 2.56	O4–H, 2.58	O4–H, 2.60
O2	MeB–H, 2.75	N10–H, 2.80	E3B-H, 2.66
O3	MeA–H, 2.65	WB–H, 2.74	E3A-H, 2.75
donor	acceptor	acceptor	acceptor
N10–H	MeA, 2.80	WA, 2.83	E3A, 2.82
N10–H	MeB, 2.75	O2, 2.80	E3B, 2.74

2 is observed here in the zwitterionic form: the negative charge is spread on the carboxyl group (both C–O distances are equal: 1.2 Å) bonded to proline, and the proton is fixed on the alanine nitrogen.

Geometrical Features of 2. The observed rotamer around the proline is trans (ω close to 180°).

The conformation of the proline ring is an envelope, with C3 endo, and the six-membered ring is in the chair conformation.

The methyl group of the alanine is in a direction nearly perpendicular to the amide plane and parallel to the C7–C7a bond.

The alkyl chain exists in two conformations: one (80%) fully extended, and the other (20%) folded.

Interactions with the Environment in the Crystal Cell. Cohesion in the crystal cell (see Figure 4c) is maintained by a hydrogen-binding net between the ethanol molecules, both carboxyls, and the protonated nitrogen.

The hydroxyl of the C15 terminal carboxylate is bound through its hydrogen to the second carboxylate bearing the minus charge (2.60 Å) of another molecule displaced along the helicoidal binary axis parallel to c. Each ethanol

Table IV. Observed Molecular Conformation of Some Representative ACE Inhibitors[a]

compounds	$\Psi 2$[b]	$\Phi 2$	ω	$\Psi 1$	$\Phi 1$	$\tau 1$	$\tau 2$	$\tau 3$	$\theta 1$[c]	$\theta 2$[d]
3, captopril[3,4]	163	−67	173	138	−71					
4, enalapril[6]	140	−57	−178	157	175	58	−173			180[e]
5, enalaprilat[7]	175	−88	179	143	−59	168	64	179	−17	−69
6, ramiprilat[8]	167	−81	175	131	−57	158	67	174	−12	−79
2, perindoprilat	169	−71	178	153	−69	176	178	−178	−36	−64
7, seven-membered ring[9]				166	−70[e]	180[e]	60[e]	180[e]		−70[e]
8, eight-membered ring[9]				159	−70[e]	160[e]	60[e]	180[e]		−70[e]
9, cilazapril[10,11]				165	−60[e]	180[e]	180[e]	180[e]		−70[e]
mean values	168	−73	178	152	−70[f]	170[f]		180	−20	−70[f]
calculated values[9,12,13]		−60		130–170	−60					

[a] Torsion angles (deg) as indicated Figure 3; angle notations as defined by Andrews.[13] [b] $\Psi 2$: HO–C15–C2–N1. [c] θ_1: N10–C11–C17–O. [d] θ_2: C9–N10–C11–C17. [e] Estimated from the published figure. [f] Enalapril not included.

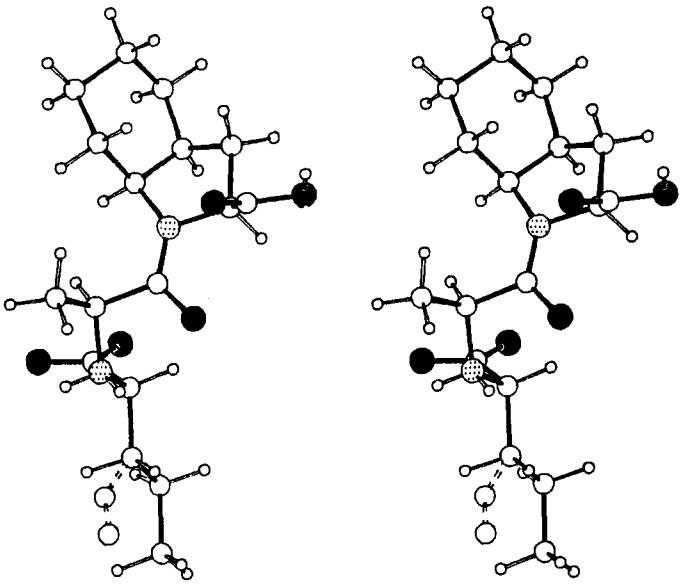

Figure 2. Stereo representation of **2**.

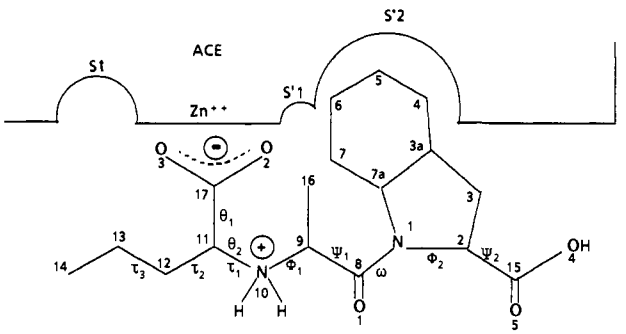

Figure 3. Site recognition of ACE by **2** (Atomic numbering of the inhibitor, and torsion angle notation).

molecule receives a hydrogen from the protonated nitrogen of the alanine and binds through its hydroxylic hydrogen to one of the negatively charged carboxylic oxygens of different molecules (see Figure 4c and Table III).

Comparison with Other ACE inhibitors. Discussion. Figure 3 gives a very schematic representation of the active site of ACE and its main links with **2** through the zinc atom and through the three hydrophobic pockets S1, S'1, and S'2.

In Table I are listed several ACE inhibitors structurally related to **2** and with published X-ray determined structure. Their conformational angles are compared in Table IV. For some of them (structures **7**–**9**), the numerical values not being accessible, we have estimated the angular values from published sawhorse perspectives.

We noted that the published structures of enalaprilat (**5**)[7] and ramiprilat (**6**)[8] are comparable to that of perindoprilat (**2**), the latter being isostructural with **6**; consequently, we transformed the crystal cells of **5** and **6** by permuting the axes to make easier comparison of the structures with that of **2** (see Figure 4a,b).

In all three structures, one finds the inhibitors as zwitterions, surrounded by solvent molecules (ethanol in **2**, methanol in **6**, and water in **5**). The general orientation of the hydrogen bonds is remarkably maintained. Figure 5 shows the superimposition of the three molecules with the directions toward their binding sites. The hydrophobic chain is in extended conformation in the three structures and the lengthening of the chain by an aromatic group in **5** and **6** does not change its conformation.

It is noteworthy to find, in these crystal structures of three slightly different molecules but with identical anchoring sites, a similar geometrical pattern of interactions with the environment.

Looking now through Table IV, we find a surprising constancy in the torsion angles: (i) the torsion angles, from $\psi 2$ to $\tau 1$, have very close values for all structures, except for enalapril (**4**), where the esterified carboxylate and the hydrophobic chain have permuted their positions (see angles $\phi 1$, $\tau 1$, $\theta 2$), (ii) the mean value of ω is very close to 180° (proline always trans), (iii) the dihedral angle $\psi 1$, which governs the alanine position, ranges between 140° and 166°, (iv) $\phi 1$ and $\theta 2$ exist in a very narrow range, 168°

(7) Wyvratt, M. J.; Tristram, E. E.; Ikeler, T. J.; Lohr, N. S.; Joshua, H.; Spinger, J. P.; Arison, B. H.; Patchett, A. A. *J. Org. Chem.* **1984**, *49*, 2816.
(8) Paulus, E. F.; Hennings, R.; Urbach, H. *Acta Crystallogr.* **1987**, *C43*, 941.

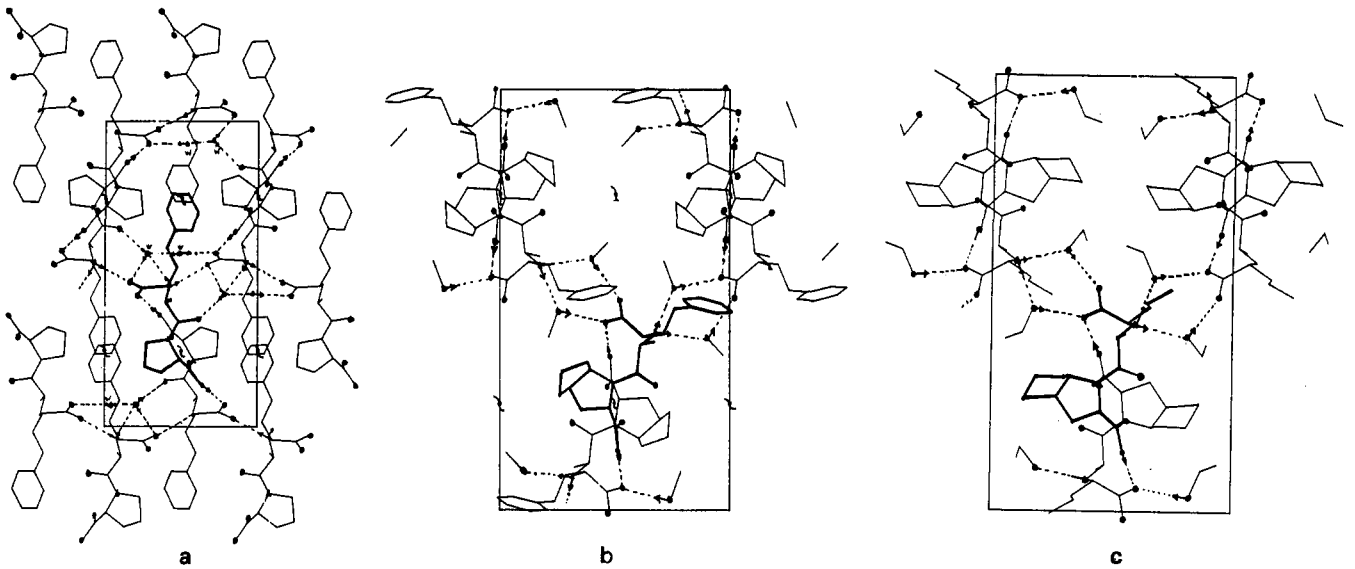

Figure 4. Projection $(x,y,0)$ of the crystal cell of **2**. H bonds are shown as dotted lines. (a) Enalsprilat (**5**), (b) ramiprilat (**6**), (c) perindoprilat (**2**).

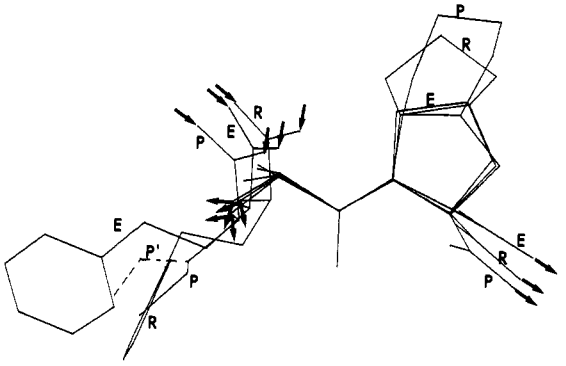

Figure 5. Comparative binding directions of (P) **2**, (E) **5**, and (R) **6**, superimposed on their common amido plane. The dotted line represents the second position adopted by the alkyl chain of **2**.

$< \phi 1 <$ 180° and −70° $< \theta 2 <$ −64°, and command the side chain and the carboxyl positions, (v) $\theta 1$ gives the orientation of the COO^-, and (vi) $\tau 2$ and $\tau 3$ are relatively consistent but with more expected variations. This calls for the following comments.

The amido bond is planar and in the trans conformation. X-ray studies of several inhibitors and energy calculations by Thorsett,[9] Wyvratt,[12] and Marshall,[14] have shown that this rotamer would be the bioactive form of this (L-Ala-L-Pro) inhibitor.

The C15-terminal carboxyl is axial, and the value of $\phi 2$ depicts the gauche conformation of the carboxyl with respect to the amide bond. This is coherent with Andrews's conclusions.[13] The orientation of the COOH group is constant with a 168° average and agrees with Marshall's calculations[14] of the carboxyl orientation in the proline ring.

The alanyl methyl is oriented nearly perpendicular to the amide plane, with a mean $\psi 1$ value of 152°, which falls in the range 130°–170° defined as being essential to the activity (Thorsett[9]) and agrees with the value proposed by Andrews (165°) from an energy-map computation.[13]

The alanine nitrogen can be considered as a H-bond donor and the direction of the H bonds is given by the torsion angle $\phi 1$ (average −70° enalapril excepted). This value was noted for captopril (**3**) and derivatives from energy calculations (Andrews[13]).

The Zn binding group is represented in all the structures, except **3** and **4**, by a carboxylate group, the position of which is determined by angles $\phi 1$ and $\tau 1$ (or $\theta 2$). From Andrews' E map for **5**,[13] the mean values of Table IV ($\Phi 1$ = −70° and $\tau 1$ = 170°) agree well with the energy minima.

For **2**, $\theta 2$ = −64°: this means a dihedral angle between the two carboxyl groups of −150°. The Zn putative binding group makes a dihedral angle of −166° with the peptide bond which makes a dihedral angle of −60° with the C15 terminal carboxyl.

From Table IV, six structures of inhibitors present the same value of torsion angles (average $\theta 2$: −70°), which results in definite orientation of the two carboxyl groups (on opposite side of the amido plane).

We think that this commonly observed conformation in six crystal structures, **5**, **6**, **2**, **7**, **8**, **9**, is not accidental, nor is it explained by the crystal packings (different crystalline cells with different solvent molecules). It is certain that the molecular conformation in the crystal formation corresponds to an energy minimum. Moreover, it does not seem logical to claim that the molecules will adapt their shape to the solicitations of the solvent molecules imposed by the crystal structures.

The hydrophobic chain is extended for most of the structures.

(9) Thorsett, E. D.; Harris, E. E.; Aster, S. D.; Petersen, E. R.; Snyder, J. P.; Springer, J. P.; Hirsfield, J.; Tristram, E. E.; Patchett, A. A.; Ulm, E. H.; Vassil, T. C. *J. Med. Chem.* **1986**, *29*, 251.
(10) Attwood, M. R.; Francis, R. J. *FEBS Lett.* **1984**, *165*, 201–206.
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(12) Wyvratt, M. J.; Patchett, A. A. *Med. Res. Rev.* **1985**, *5*, 483–531.
(13) Andrews, P. R.; Carson, J. M.; Caselli, A.; Spark, M. J.; Wood, R. *J. Med. Chem.* **1985**, *28*, 393.
(14) Mayer, D.; Naylor, C. B.; Motoc, I.; Marshall, G. R. *J. Comput. Aided Design.* **1987**, 3–16.
(15) Cushman, D. W.; Cheung, H. S. *Biochem. Pharmacol.* **1971**, *20*, 1637–1648.
(16) Vincent, M.; Schiavi, P. *Mécanismes de Reconnaissance Moléculaire*; INSERM, Ed.; Lavoisier: Paris, 1989; Vol. 181, pp 95–135.
(17) Cushman, D. W.; Cheung, H. S.; Ondetti, M. A. *Biochemistry* **1977**, *16*, 5484–5491.

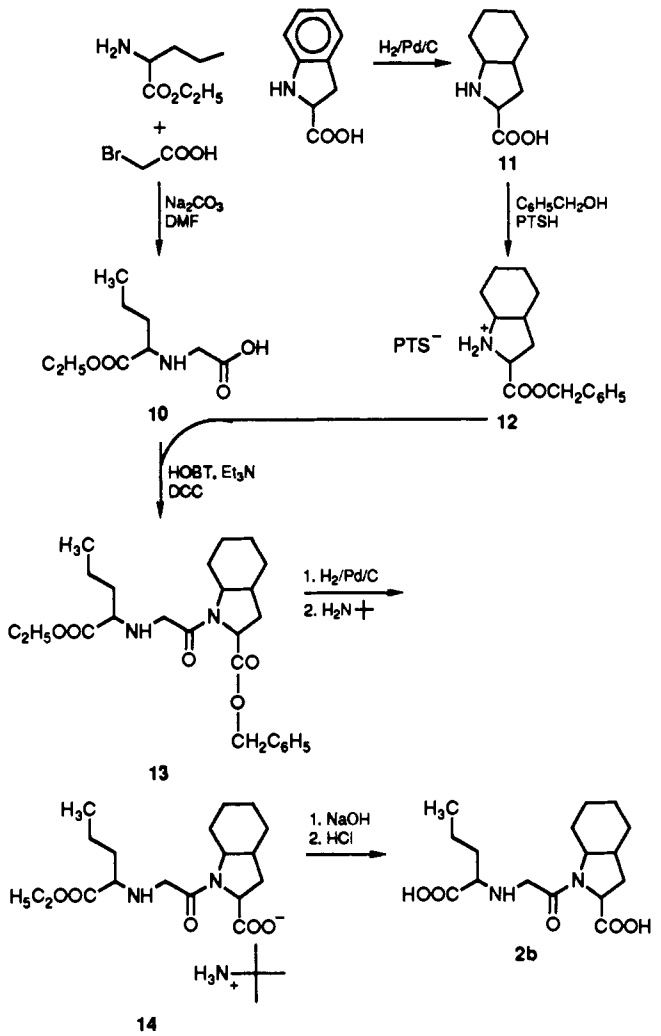


Figure 6. Synthesis of **2b**.

All the observed ω, $\phi 1$, and $\psi 1$, belong to the narrow range delimited by the activity tests.

Structure–Activity Relationships (SAR) Studies

This solid-state study gives us a conformation of **2** which offers binding capacities of its different functions with the active site of ACE.

Our SAR studies concern the binding of the alanine and perhydroindole (PHI) parts of **2** with the hydrophobic pockets S'1 and S'2.

In the perhydroindole inhibitor **2** the hydrogens bonded to the alanine α- and β–carbons (C9 and C16) are close to the six-membered ring: HC9···HC7a, 2.0 Å; and HC16··· H2C7, 2.51 Å. This observation led us to think that S'1 and S'2 are necessarily very close to each other, and might even be fused. These pockets could be filled by the alanine methyl together with the PHI ring, with the exclusive condition that alanine α-carbon (C9) was in the *S* configuration.

A question arose: would the 6-member ring PHI alone be able to fill the pockets S'1-S'2 so that it could give the right orientation to the chain for a good fit of the inhibitor with the receptor?

In other words, is the methyl of alanine really necessary for activity?

We have prepared the "C9-demethylated" perindoprilat **2b** and (9*R*)-perindoprilat **2a**, following the schemes of Figures 6 and 7, respectively.

By measuring their interactions with the receptor, i.e. their in vitro enzymatic activities (IC_{50}) against Hip-His-Leu,[15] we could compare the activities of **2**, **2a**, and **2b** with each other and with their corresponding 9*S*, 9*R*, and C9-demethylated derivatives of enalaprilat (**5b**, **5a**) and captopril (**3b**, **3a**). The results are gathered in Table V.

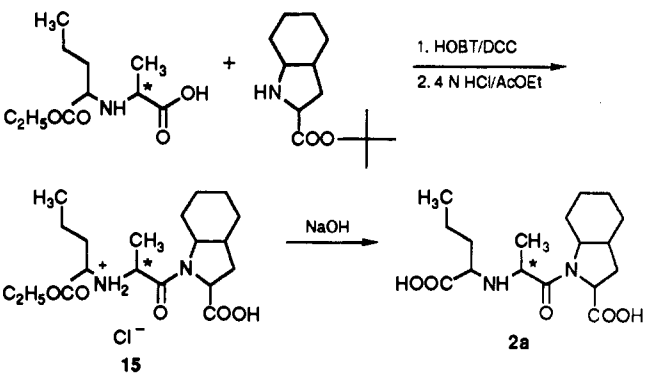


Figure 7. Synthesis of **2a**.

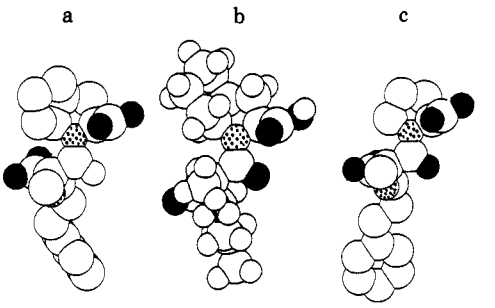


Figure 8. CPK representations of the X-ray structures of (a) **6**, (b) **2** with its hydrogens, and (c) **5**. The structures are presented with the amido plane in the projection plane. The similarities between the three molecules are striking. Note the different conformation of the proline ring.

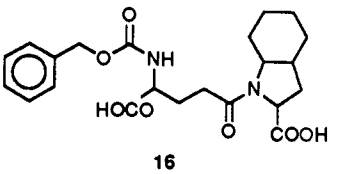


Figure 9. Structure of **16**.

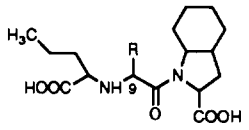
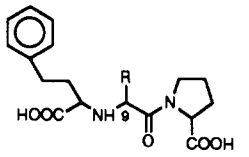
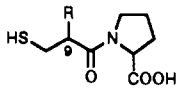
The comparison of the IC_{50}s shows that, as previously reported by Thorsett,[9] "the methyl group is not required for binding, but its presence adds to the inhibitory potency".

This seems to be true only if the "small" ring proline is present at the C-terminal end of the inhibitors (**3** and **5**). In compound **2** (see Figure 8) the C3 endo conformation of the proline ring, compared to that in **6** and **5** (C3 exo), gives to the six-membered ring fused to proline a chair conformation with close contacts between its hydrogens and the alanine Cα-hydrogen.

The PHI group alone thus appears to be sufficient for a good fit of the inhibitor with the receptor, so that a methyl does not add to the inhibitory potency: the activities (IC_{50}) of **2b** and **2** are not significantly different. Moreover, Ksander[18] has prepared a dipeptide **16** (Figure 9) devoid of a methyl and containing the ring PHI. This compound has a good affinity for the receptor of ACE (IC_{50} = 2.7 nM). This finding is interpreted as "the fused ring

(18) Ksander, G. M.; Yuan, A. M.; Diefenbacher, C. G.; Stanten, J. L. *J. Med. Chem.* **1985**, *28*, 1606–1611.
(19) Riche, C.; Devin, *Collected Abstracts*; 7th European Crystallographic Meeting; Jerusalem, 1976; p 25.

Table V. Enzymatic in Vitro Comparative Activities of 2 and Analogues

structures	R	no.	C_9 configuration[a]	IC_{50},[b] nM	rel lit.
 perindoprilat derivatives	CH_3	**2**	*S*	1.5	16
	CH_3	**2a**	*R*	1100	16
	H	**2b**	achiral	1.9	
 enalaprilat derivatives	CH_3	**5**	*S*	1.2	5
	CH_3	**5a**	*R*	2500	5
	H	**5b**	achiral	230	5
 captopril derivatives	CH_3	**3**	*S*	23	17
	CH_3	**3a**	*R*	1.5×10^6	17
	H	**3b**	achiral	200	17

[a] All other chiral C are *S*. [b] IC_{50} measured against Hip-His-Leu.[15]

(PHI) forces the side chain to occupy a more favorable position in space for interaction with the enzyme".[18]

The results of our structural studies are in good agreement with those of Thorsett[9] and Ksander[18] and open the way to the design of new ACE inhibitors in which bulky hydrophobic groups enhance the strength of enzyme–substrate interaction without modifying torsional angles required by the model we propose.

Experimental Section

X-ray Studies. The octahedral, colorless crystals were crystallized from absolute ethanol and sealed in a capillary with mother liquor to prevent deterioration by rapid desolvation.

Data collection: $C_{17}H_{28}N_2O_5 \cdot 2CH_3CH_2OH$; crystal dimensions, $0.4 \times 0.4 \times 0.3$ mm^3; capillary diameter, 0.5 mm; system orthorhombic; space-group, $P2_12_12_1$; a = 22.155 (5) Å, b = 12.263 (3) Å, c = 9.475 (2) Å, V = 2574.2 Å^3, T = 295 K, D_m = 1.12, D_x = 1.115; four asymmetric units in the unit cell; four-circle Philips automatic diffractometer; wavelength λ(CuKα) = 1.5418 Å; graphite monochromator; ω–2θ scan up to 2θ = 136°; 2574 measured reflections in the range $0 < h < 26$, $0 < k < 14$, $0 < l < 11$; 1842 independent reflections with $I > 3\sigma(I)$. Lorentz–polarization correction; no absorption correction; three standard reflections (-2-24, -33-3, 221) monitored every 3 h showed no significant change in intensity.

Structure Resolution. The structure was solved by direct methods.[19] Many hydrogen atoms were localized on the Fourier difference series, in particular those bound to the alanine nitrogen, the carboxylic hydrogen of the C15-terminal, and two hydroxyl hydrogens of the ethanol molecules. The positional coordinates of these five hydrogens were allowed to refine. The remaining hydrogen atoms were introduced in the calculations at their theoretical positions (C–H = 1.08 Å), including the methyl hydrogens of alanine which had been observed on the difference series and tied by constraints to the carbon atom. All hydrogens were given fixed temperature factors of their parent atoms. Two disorder phenomenons were observed: the alkyl chain lies in two positions (80% and 20%), and the methyl end of each ethanol molecule occupies two positions (60% and 40%). The atoms corresponding to the minor occupancy were given isotropic temperature factors and not refined. All the other non-hydrogen atoms were treated anisotropically. The coordinates were refined by full-matrix least squares with SHELX76.[20] The final R value is 0.061, R_w = 0.072 with $w = 1/[\sigma 2(F) + 0.005F^2]$; δ/σ = 0.1; $\Delta\rho$ = 0.25Å^{-3}. The atomic coordinates are listed in Table II.

Tables of H coordinates, anisotropic temperature factors, interatomic distances and angles, torsion angles and structures factors are deposited as supplementary material.

Synthesis. All melting points were determined on a Kofler apparatus and are uncorrected. Analytical (C, H, N) results were within ±0.4% of the theorical values. Analytical thin-layer chromatography (TLC) was conducted on precoated glass plates of silica gel 60F-254 (E. Merck and Co.). IR spectra were recorded on a Perkin-Elmer 682 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Perkin-Elmer R12-B spectrometer at 60 MHz and a Bruker AM 400 spectrometer, respectively. Mass spectral analyses were determined on a Nermag R10-10C. Optical rotation values were measured on a Perkin-Elmer 241 polarimeter. HPLC were performed with a Waters 6000 A pump + 46K injector + M450 spectrophotometer.

(2*S*,3a*S*,7a*S*)-1-[*N*-[1(*S*)-Carboxybutyl]-(*S*)-alanyl]-2-carboxyperhydroindole (2). A solution of (2*S*,3a*S*,7a*S*)-1-[*N*-[1*S*)-carbethoxybutyl]-(*S*)-alanyl]-2-carboxyperhydroindole, *tert*-butylamine salt[1] (1; 5 g, 11.3 mmol), in 0.1 N NaOH (250 mL, 25 mmol) was allowed to stand at room temperature for 3 days. The reaction mixture was concentrated in vacuo to eliminate *tert*-butylamine. The residue was dissolved in 100 mL of water and was acidified (pH = 1) by addition of 4 N HCl. The solution was added to an ion-exchange-resin column (Dowex-50W × 8 H^+ form). The ion-exchange resin was washed with water then eluted with water–pyridine (9:1). The eluates were evaporated to dryness and the residue crystallized from 2-propanol to yield 2.9 g (75%) of title compound. Anal. ($C_{17}H_{28}N_2O_5$) C, H, N.

***N*-[1(*S*)-Carbethoxybutyl]glycine (10).** A mixture of (*S*)-norvaline ethyl ester hydrochloride (9.07 g, 50 mmol), triethylamine (6.9 mL, 50 mmol), and DMF (100 mL) was stirred for 15 min at room temperature. Triethylamine (6.9 mL, 50 mmol) and bromoacetic acid (6.95 g, 50 mmol) were added, and the reaction mixture was heated at 90 °C with stirring for 6 h. The DMF was removed in vacuo and the residue dissolved in water (150 mL). The solution was added to a column of Dowex-50W × 8 (H^+ form). The ion-exchange resin was washed with water, then eluted with 10% NH_4OH. The eluates were concentrated in vacuo, and the residue was triturated with 2-propanol to afford 2.3 g (23%) of title compound as a mixture of free acid and of its ammonium salt (80:20). Anal. C, H, N.

(2*S*,3a*S*,7a*S*)-2-Carboxyperhydroindole (11). A mixture of (*S*)-2-carboxyindoline[1] (6 g, 36.8 mmol) and Pd/C (10%, 0.5 g) in acetic acid was hydrogenated at 50 kg/cm^2 for 18 h at 50 °C. The catalyst was removed by filtration and the filtrate was evaporated. The residue was crystallized in diethyl ether. The product was collected by filtration and washed with 2-propanol to afford 4.1 g (66%) of title compound. Anal. ($C_9H_{15}NO_2$) C, H, N.

(20) Sheldrick, G. M. *SHELX 76. Program for Crystal Structure Determination*; University of Cambridge: Cambridge, England, 1976.

(2S,3aS,7aS)-2-[(Benzyloxy)carbonyl]perhydroindole, Tosylate (12). This compound was prepared by using the method described previously[21] for a mixture of isomers. A mixture of 11 (17 g, 0.1 mol), benzyl alcohol (40 g, 0.37 mol), *p*-toluenesulfonic acid (PTSH; 30 g, 0.16 mol), and toluene (300 mL) was stirred under reflux for 4 h. Water was collected in a Dean-Stark trap. Then toluene was removed by evaporation. The residue was triturated with diisopropyl oxide and the resulting solid was collected by filtration to yield 40 g (93%) of title compound melting at 162 °C. Anal. ($C_{23}H_{29}NO_5S$) C, H, N, S.

(2S,3aS,7aS)-1-[N-[1(S)-Carbethoxybutyl]glycinyl]-2-[(benzyloxy)carbonyl]perhydroindole (13). To a solution of 12 (4.31 g, 10 mmol), triethylamine (1.4 mL, 10 mmol), and 10 (2.03 g, 10 mmol) in DMF (50 mL) was added a solution of HOBT (1.35 g, 10 mmol) and DCC (2.06 g, 10 mmol) in DMF (10 mL). The reaction mixture was stirred for 20 h at room temperature. Dicyclohexylurea (DCU) was removed by filtration and DMF was evaporated in vacuo. The residue was dissolved in ethyl acetate and washed with a 5% solution of $NaHCO_3$ in water then with brine. The organic layer was dried on $CaSO_4$, filtered, and concentrated. The product was purified by silica gel chromatography with ethyl acetate as eluent to afford 2.5 g (56%) of title compound. Anal. ($C_{25}H_{36}N_2O_5$) C, H, N.

(2S,3aS,7aS)-1-[N-[1(S)-Carbethoxybutyl]glycinyl]-2-carboxyperhydroindole, *tert*-Butylamine Salt (14). A mixture of 13 (2.3 g, 52 mmol) and Pd/C (10%, 0.5 g) in absolute ethanol (50 mL) was shaken in a pressure bottle on a Parr hydrogenator at 3 kg/cm^2 for 18 h at room temperature. The catalyst was removed by filtration and the filtrate was evaporated to afford 1.7 g (92%) of the crude acid form of 14. Crystallization of *tert*-butylamine salt from diisopropyl oxide gave pure 14 as colorless crystals melting at 122 °C. Anal. ($C_{22}H_{41}N_3O_5$) C, H, N.

(2S,3aS,7aS)-1-[N-[1(S)-Carboxybutyl]glycinyl]-2-carboxyperhydroindole (2b). A solution of 14 (0.83 g, 2.5 mmol) in 0.2 N NaOH (37.5 mL, 7.5 mmol) was allowed to stand for 3 days. Hydrochloric acid (1 N, 7.5 mL, 7.5 mmol) was added and the reaction mixture evaporated to dryness. The residue was dissolved in absolute ethanol (50 mL) and sodium chloride was removed by filtration. After evaporation of ethanol the product was dissolved in water; the solution was filtered and lyophilized to afford 0.58 g (72%) of title compound. Anal. ($C_{16}H_{26}N_2O_5$) C, H, N.

(21) Pichat, L.; Tostain, J. M.; Gomis, M.; Moustier, A. M.; Vincent, M.; Rémond, G.; Portevin, B.; Laubie, M. *J. Labelled Compd. Radiopharm.* **1987**, *XXV*, 553-558.

(2S,3aS,7aS)-1-[N-[1(S)-Carbethoxybutyl]-(R)-alanyl]-2-carboxyperhydroindole Hydrochloride (15). To a mixture of *N*-[1(*S*)-carbethoxybutyl]-(*R*)-alanine hydrochloride[1] (9.9 g, 39.1 mmol), triethylamine (5.4 mL, 39.1 mmol), and (2*S*,3a*S*,7a*S*)-2-(*tert*-butoxycarbonyl)perhydroindole[1] (8.8 g, 39.1 mmol) in DMF (250 mL) was added a solution of HOBT (4.65 g, 39.1 mmol) in DMF (100 mL) then DCC (8.1 g, 39.1 mmol). The reaction mixture was stirred for 20 h at room temperature. The DCU was removed by filtration and the DMF was evaporated in vacuo. The residue was dissolved in ethyl acetate and washed with a 5% solution of $NaHCO_3$ in water then brine. The organic layer was dried on $CaSO_4$, filtered, and concentrated to afford 16 g of crude (2*S*,3a*S*,7a*S*)-1-[*N*-[1(*S*)-carbethoxybutyl]-(*R*)-alanyl]-2-(*tert*-butoxycarbonyl)perhydroindole as an oil. This oil was dissolved in a solution of hydrochloric acid in 4 N ethyl acetate (250 mL) and the solution stirred for 20 h at room temperature. The product was collected by filtration and washed three times with ethyl acetate (50 mL) to afford 8.2 g (51.8%) of title compound melting at 180 °C with decomposition. Anal. ($C_{19}H_{33}ClN_2O_5$) C, H, N, Cl.

(2S,3aS,7aS)-1-[N-[1(S)-Carboxybutyl]-(R)-alanyl]-2-carboxyperhydroindole (2a). A solution of 15 (5 g, 12.3 mmol) in 0.5 N NaOH (100 mL, 50 mmol) was allowed to stand at room temperature for 48 h. The solution was acidified (pH = 2) by addition of 4 N HCl and was added to a column of Dowex-50W × 8 (H^+ form). The ion-exchange resin was washed with water then eluted with water–pyridine (9:1). The eluates were evaporated to dryness. The residue was triturated with acetone and the solid was collected by filtration to yield 1.9 g (45%) of title compound. Anal. ($C_{17}H_{28}N_2O_5$) C, H, N.

Registry No. 2, 95153-31-4; **2a**, 130982-51-3; **2b**, 130933-17-4; 10, 130933-18-5; 11, 80875-98-5; 12, 94062-52-9; 13, 130933-19-6; 14, 130933-21-0; 15, 130982-52-4; perindopril, 82834-16-0; (*S*)-norvaline ethyl ester hydrochloride, 40918-51-2; bromoacetic acid, 79-08-3; (*S*)-2-carboxyindoline, 79815-20-6; benzyl alcohol, 100-51-6; angiotensin converting enzyme, 9015-82-1.

Supplementary Material Available: Tables of atomic coordinates for H atoms and isotropic thermal parameter, anisotropic thermal coefficients for non-hydrogen atoms, bond length, and valency angles for 2, IR, ^{1}H NMR, and TLC studies for all new compounds, optical rotation values for 2 and 11, ^{13}C NMR studies for 2, 2a, and 2b, HPLC studies for 2, 2a, 2b, and 14, MS studies for 2, 2b, and 14 (15 pages); a list of observed and calculated structure factors for 2 (11 pages). Ordering information is given on any current masthead page.

Dual-Action Cephalosporins: Cephalosporin 3′-Quaternary Ammonium Quinolones[1]

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When cephalosporins exert their biological activity by reacting with bacterial enzymes, opening of the β-lactam ring can lead to expulsion of the 3′-substituent. A series of cephalosporins was prepared in which antibacterial quinolones were linked to the 3′-position through a quaternary nitrogen. Like the 3′-ester-linked dual-action cephalosporins reported earlier, these compounds demonstrated a broad spectrum of antibacterial activity derived from cephalosporin-like and quinolone-like components, suggesting a dual mode of action.

When cephalosporins react with bacterial enzymes, opening of the β-lactam ring leads to liberation of the 3′-substituent, if that substituent can function as a leaving group.[2-8] When the leaving group possesses antibacterial activity of its own, the cephalosporin should exhibit a dual mode of action.[9-11] As a rationale for drug design, this

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