The Design and Synthesis of the Angiotensin Converting Enzyme Inhibitor Cilazapril and Related Bicyclic Compounds

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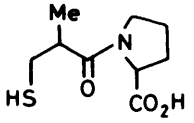
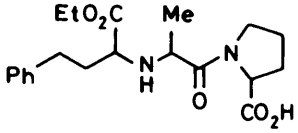
The postulated binding functions for the active site of Angiotensin Converting Enzyme (A.C.E.), derived in an earlier study, have made possible the design of improved inhibitors. Consequently, (1*S*,9*S*)-9-[(1*S*)-(ethoxycarbonyl)-3-phenylpropylamino]octahydro-10-oxo-6*H*-pyridazo[1,2-*a*][1,2]diazepine-1-carboxylic acid (Cilazapril), and related compounds, have been synthesized. They are very active inhibitors of A.C.E. and are highly potent antihypertensives *in vivo*.

Modulation of the renin–angiotensin system, in particular through inhibition of angiotensin converting enzyme (A.C.E., E.C.3.4.15.1.), has assumed increasing importance in the therapy of hypertension and of congestive heart failure.[1] Clinical investigations, initially with nonapeptide SQ20881[2] and later with Captopril (**1**)[3] and Enalapril (**2**),[4] have established that these potent A.C.E. inhibitors are effective antihypertensives in man.



(**1**)



(**2**)

Structure–activity studies[5–7] of these inhibitors and related compounds established a two-dimensional model of the molecular features which were important for binding of inhibitors to the active site of the enzyme. It was deduced[6] that the active centre of A.C.E. included a cationic binding site for the terminal carboxyl function, an H-bond donor which interacts with the carbonyl group of the amide function of the inhibitor, and a Zn atom to which the Zn ligand group of the inhibitor is bound. Since A.C.E. has not yet been crystallised, the model of the active site has been inferred from the enzyme's substrate specificity and by analogy with other Zn-containing peptidases such as thermolysin[8] and carboxypeptidase A,[9] the structures of which have been determined using *X*-ray diffraction.

In these investigations we have undertaken to define the three-dimensional relationship of the three binding sites. Using this approach it has been possible to design a new family of very active A.C.E. inhibitors.

At the outset, we postulated that more potent inhibitors could be expected if the binding functions of the inhibitor were orientated very specifically in space by attachment to a rigid framework; the rigidity of the system would favour 'entropy-gain'.[10] Moreover, it is reasonable to expect that a framework which was very different from Captopril and Enalapril would provide scope for the improvement of characteristics relating to specificity, metabolism, tissue distribution, and half-life *in vivo*. Our previous paper[11] demonstrated the application of molecular graphics studies to conformationally-restricted analogues of Captopril in the development of a 3-dimensional model of active-site topography. Correlation of the enzyme-inhibitory activity of these compounds with the spatial array of their three enzyme binding groups resulted in a three-dimensional representation of the favoured array for enzyme binding.

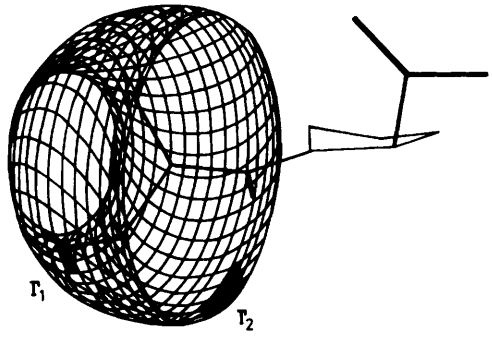


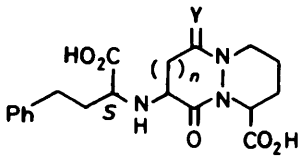
Figure 1. Parts of the captopril sulphur atom locus associated with most potent biological activity.[11]

The part of the locus of the zinc-binding ligand associated with high potency was limited to two areas, Γ_1 and Γ_2 (Figure 1). Recent work (to be published) has further restricted this to the left hand region, Γ_1, for thiol interactions. This paper describes an extension of the model, which has enabled the identification of potent, non-thiol-containing inhibitors.

There have been earlier studies investigating alternatives to the thiol function as zinc ligand. Structure–activity relationships in a series of peptide inhibitors, both natural and synthetic, indicated markedly higher potencies for inhibitors with the general formula [-(aromatic amino acid residue)-Ala-Pro].[12] Further data for simple dicarboxylic acid derivatives of Ala-Pro contributed to the selection by Patchett *et al.*[7] and others[13–15] of the homophenylalanine moiety, suitably orientated, for zinc binding. This choice can be related to the cases of other zinc-containing peptidases, *e.g.* thermolysin and carboxypeptidase A, for which *X*-ray crystallography data are available. The binding of 1-carboxy-3-phenylpropyl-Leu-Trp, a good inhibitor of thermolysin, to the active site of this enzyme has been elucidated on the basis of *X*-ray crystallographic evidence.[16] The binding of the homophenylalanine moiety in this compound can be correlated with the binding of the thiol function of (2-benzyl-3-mercaptopropanoyl)-L-alanylglycinamide to the zinc atom in thermolysin.[17] Consequently, we have investigated the use of homophenylalanine and related structures as A.C.E. zinc ligands which are alternatives to thiol.

The 7,6- *and* 8,6-*Bicyclic Series.*—Although particular 5,6- and 6,6-bicyclic compounds (**3**) and (**4**), described in our earlier paper, had substantial activities as inhibitors of A.C.E., they were less effective than Captopril and Enalapril. Investigations of the conformations of the most active members of the two series, by n.m.r. spectroscopy and *X*-ray diffraction, provided an

Table 1. Variation of A.C.E. IC_{50} values with ring size and angle



	Y = H_2			Y = O	
Compd. *n*	ψ[a]	IC_{50} (nM)	Compd. *n*	ψ	IC_{50} (nM)
0	254.9	8 000	0	237.6	
1	245.6	28	1	196.6	20
2	163.9	1.6	2	165.3	4
3	142.1[b]	4.5	3	146.4[b]	15

[a] ψ Values obtained from crystal structures of model bicyclic compounds. [b] Values calculated by MNDO.[25]

Note: The ψ value *ca.* 165° seems optimal for A.C.E. The value 316° was found for the thermolysin–*N*-(1-carboxy-3-phenylpropyl)-L-leucyl-L-tryptophan complex.[16] These are within the ranges of the two energy minima for alanylproline.

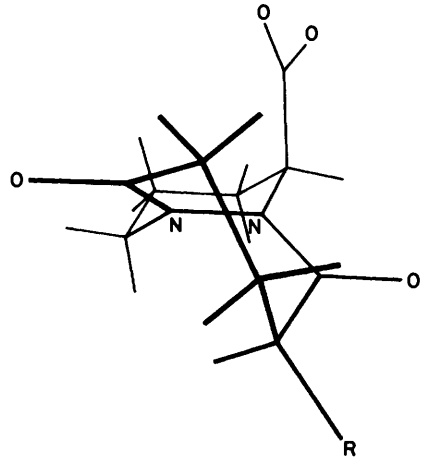


Figure 2. The crystal structure of the 6,6-bicyclic system (R = CH_2SAc)

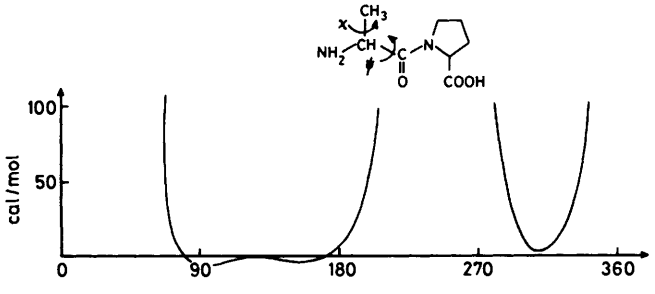
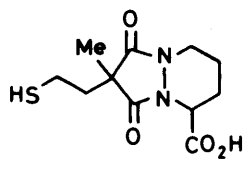


Figure 3. The ECEPP energy profile of the H-Ala-Pro-OH fragment (χ optimised)

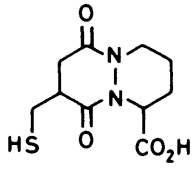
explanation. The substituted octahydro-6,9-dioxopyridazino-[1,2-*a*]pyridazine carboxylic acids in the 6,6-series[18] were all shown to have a rigid chair/twist boat conformation which was independent of the substituents present in the dioxopyridazine ring; the carboxyl function was axial in all cases. This is similar to the case of acylpiperazic acids[19] and of pipecolic acid.[20]

It was possible to assign, with confidence, the rigid conformation (Figure 2) to the 6,6-bicycle (**4**). Similar considerations apply to the related 5,6-bicyclic series. In both cases the conformations correspond to relatively high energy forms of alanylproline in which the methyl group almost eclipses the δ-carbon atom of the proline residues. This is reflected in the energy profile (ECEPP[21]) of alanylproline which has two rather broad minima (Figure 3), both relating to ψ-values outside the range deduced for the 5,6-and 6,6-bicyclic systems (Table 1).

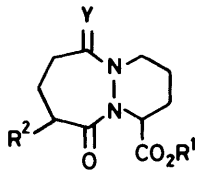
Consequently, we have synthesized the related 7,6- and 8,6-bicyclic systems (**5**) and (**6**), both of which, in molecular



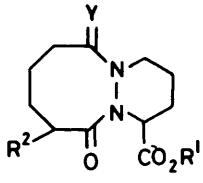
(**3**)



(**4**)



(**5**)



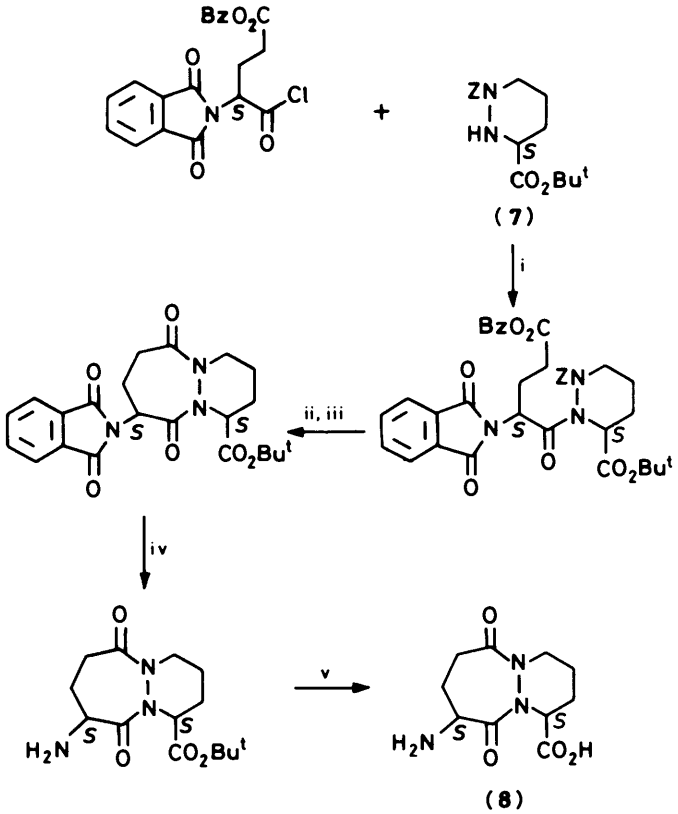
(**6**)

graphics simulations based on the 6,6-models, afforded better prospects of conformations which correspond more closely to the favoured forms of alanylproline (Table 1); they retained the appropriate orientation of their carboxyl, carbonyl, and zinc-ligand groups for binding to A.C.E. according to Figure 1.

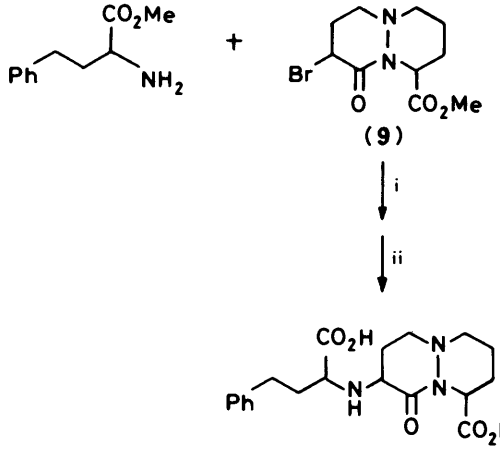
The synthesis of a representative member of the 7,6-bicyclic series is set out in Scheme 1. The t-butyl ester (**7**) of (3*S*)-(N-1-protected-hexahydropyridazine-3-carboxylic acid [(*S*)-piperazic acid] was acylated at N-2 and deprotected. Intramolecular acylation at N-1 gave, after deprotection, the 9-aminopyridazinodiazepine (**8**), in excellent yield. Optically pure materials were obtained most conveniently by using two optically active intermediates, but using one racemic starting material and separating the pure diastereomers was also practicable. Similar procedures were used for the 8,6-bicyclic series.

The mono-oxo systems, of which the compound (**16**) is an example, were prepared readily by reduction of the corresponding 6-oxo-compounds with borane in tetrahydrofuran. The reduction is highly selective, presumably because the second amide function is in a crowded environment.

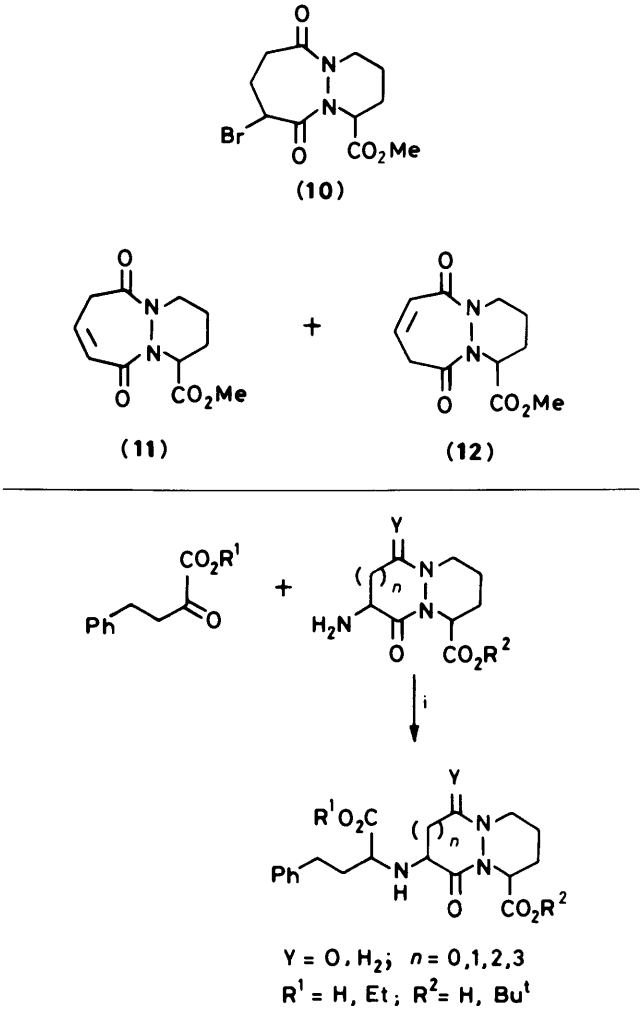
Three methods for attaching homophenylalanyl or a related sidechain were investigated. Alkylation of a suitably protected homophenylalanine, *e.g.* using the 7-bromo-bicycle (**9**), could be used for the preparation of substituted pyridazopyridazines (Scheme 2) but a similar sequence was not practicable for the 5,6-, 7,6-, or 8,6-series because of competing elimination. For example, the pyridazinodiazepine (**10**) gave the unsaturated products (**11**) and (**12**). The preparation of related alkylated amines has been achieved by reductive alkylation using oxo-acids or esters.[7] In our hands, yields were generally poor and there was no evidence of significant asymmetric induction at the newly formed chiral centre. Reductive alkylation was employed for the synthesis of particular homophenylalanine-substituted compounds and some related compounds[22] of the 5,6-, 6,6-, and 7,6-series, but the procedure (Scheme 3) was less convenient



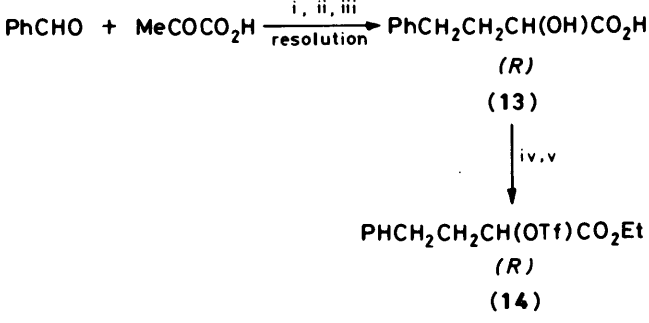
Scheme 1. *Reagents and Conditions:* i, toluene–H_2O–$NaHCO_3$; ii, H_2, Pd/C; iii, $SOCl_2$–CH_2Cl_2; iv, N_2H_4·H_2O–EtOH; v, HBr–HOAc or TFA



Scheme 2. *Reagents and Conditions:* i, Et_3N, MeCN, Heat; ii, NaOH, EtOH, H_2O



Scheme 3. *Reagents:* i, $NaBH_3CN$

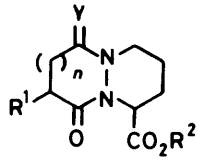


Scheme 4. *Reagents:* i, KOH–MeOH; ii, $NaBH_4$; iii, H_2, Pd–C; iv, EtOH–H^+; v Tf_2O, pyridine, CH_2Cl_2

than that for direct alkylations. The third procedure, based on direct alkylation of the aminobicycle, proved most successful. Initial trials with either optically active, or racemic, ethyl 2-bromo-4-phenylbutanoate gave 1:1 mixtures of epimers. Chiral control was, however, achieved by using triflate (trifluoromethanesulphonate) as the leaving group. The triflate ester (**14**) was prepared from the optically active hydroxy acid (**13**) by conventional methods (Scheme 4). Alkylation of the bicyclic amines proceeded rapidly at room temperature, in the presence of base, to give single, optically active products resulting from clean S_N2 inversion. Related studies using triflates have been reported recently.[23] We have utilised alkylation with a triflate for the preparation of the homophenylalanine derivatives in both the 7,6 and 8,6 series (Scheme 5). By these means, a range of substituted bicyclic compounds has been prepared (Tables 2 and 3).

The dicarboxylic acid (**18**; $n = 2$, R = H) was the most active A.C.E. inhibitor (Table 3). The corresponding mono-ester (**18**; $n = 2$, R = Et, Cilazapril) is used for *in vivo* inhibition of A.C.E. This prodrug has better biological characteristics as an

Table 2.



						Analysis (%)[a]					
n	Y	R^1	R^2	Stereochem.	Formula	C	H	N	M.p. (°C)	Solvent	$[\alpha]_D^{20}$ [*c*(%), solvent]
1	0	FtN	Bu^t	*SS*	$C_{21}H_{23}N_3O_6$	60.8 (61.0)	5.6 (5.6)	9.9 (10.2)	131—132	EtOAc–hexane	−191° (0.4, MeOH)
1	0	FtN	Bu^t	*RS*	$C_{21}H_{23}N_3O_6$	61.0 (61.0)	5.7 (5.6)	10.1 (10.2)	151—152	EtOAc–hexane	−58° (0.5, MeOH)
2	0	FtN	Bu^t	*SS*	$C_{22}H_{25}N_3O_6$	61.65 (61.8)	5.8 (5.9)	9.75 (9.85)	182—185	EtOAc–hexane	−80° (0.5, MeOH)
2	0	FtN	Bu^t	*SR*	$C_{22}H_{25}N_3O_6$	61.7 (61.8)	5.95 (5.9)	9.7 (9.85)	80—100[b]	Et_2O	−7.7° (0.5, MeOH)
2	H_2	FtN	Bu^t	*SS*	$C_{22}H_{27}N_3O_3$	64.05 (63.9)	6.6 (6.6)	10.2 (10.2)	137—138	EtOAc–hexane	−66.7° (1.0, MeOH)
2	H_2	FtN	Bu^t	*RS*	$C_{22}H_{27}N_3O_3$	63.6 (63.9)	6.5 (6.6)	10.1 (10.2)	171—172	EtOAc–hexane	−24.7° (1.0, MeOH)
2	H_2	FtN	Bu^t	*SR*	$C_{22}H_{27}N_3O_3$	63.65 (63.9)	6.5 (6.6)	10.1 (10.2)	171—172	EtOAc–hexane	+23.8° (1.0, MeOH)
2	H_2	H_2N	Bu^t	*SS*	$C_{14}H_{25}N_3O_3$	59.1 (59.3)	8.8 (8.9)	14.8 (14.8)	75—77	Hexane	−77.7° (1.0, EtOH)
3	0	FtN	Bu^t	*SS*	$C_{23}H_{27}N_3O_6$	62.8 (62.6)	6.2 (6.2)	9.4 (9.5)	174—175	Et_2O	−43.2° (1.0, MeOH)
3	H_2	FtN	Bu^t	*SS*	$C_{23}H_{29}N_3O_5$	64.85 (64.6)	6.8 (6.8)	9.55 (9.8)	181—183	Et_2O	−72.9° (1.0, MeOH)
1	0	FtN	H	*SS*	$C_{17}H_{15}N_3O_6$[d]	55.8 (55.8)	4.3 (4.4)	10.9 (10.8)	275—277	AcOH–Et_2O	−221.0° (0.5, AcOH)
2	0	FtN	H	*SS*	$C_{18}H_{17}N_3O_6$	58.3 (58.2)	4.8 (4.6)	11.15 (11.3)	307—310[b]	Me_2CO–H_2O	−138.9° (0.5, DMF)
2	0	FtN	H	*SR*	$C_{18}H_{17}N_3O_6$	58.05 (58.2)	4.65 (4.6)	11.15 (11.3)	235—237[c]	Me_2CO–H_2O	−48.4° (0.5, DMF)
2	H_2	FtN	H	*SS*	$C_{18}H_{19}N_3O_5$	60.2 (60.5)	5.65 (5.4)	11.7 (11.8)	255—257	EtOAc–hexane	−49.2° (1.0, MeOH)
2	H_2	FtN	H	*SR*	$C_{18}H_{19}N_3O_5$	60.4 (60.5)	5.5 (5.4)	11.8 (11.8)	241—244	EtOAc–hexane	+16.2° (0.5, MeOH)
1	0	H_2N	H	*SS*	$C_9H_{13}N_3O_4$	47.8 (47.6)	5.8 (5.8)	18.4 (17.85)	262—265[b]	EtOH–H_2O	−220.9° (0.5, H_2O)
2	0	H_2N	H	*SS*	$C_{10}H_{15}N_3O_4$[e]	46.0 (46.3)	6.45 (6.6)	16.15 (16.2)	204—207[b]	EtOH–H_2O	−174.6° (0.5, 2M HCl)
2	H_2	H_2N	H	*SS*	$C_{10}H_{17}N_3O_3$	52.8 (52.85)	7.6 (7.5)	18.5 (18.5)	247—249[b]	EtOH–H_2O	−121.9° (0.7, H_2O)
1	H_2	Br	Me	*R*R**	$C_{10}H_{15}BrN_2O_3$	41.5 (41.25)	5.05 (5.2)	9.6 (9.6)	102—103	EtOAc–hexane	
2	0	Br	Me	*SS*	$C_{11}H_{15}BrN_2O_4$	41.1 (41.4)	4.7 (4.7)	8.6 (8.8)	135—138	Et_2O–hexane	−152.8° (0.5, EtOH)

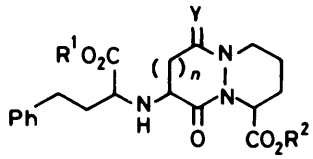
[a] Required values in parentheses. [b] Decomp. [c] Resolidifies and melts again 292—295 °C. [d] Crystallises with 0.5 equiv. $MeCO_2H$. [e] Crystallises with 1 equiv. H_2O.

antihypertensive in mammals than the corresponding dicarboxylic acid, which is formed from Cilazapril *in vivo* as a result of the action of esterases.

Stereochemistry, Conformation, and A.C.E. Inhibition.—The conformations of the 5,6- and 6,6-bicyclic thiols with the formulae (**3**) and (**4**) have been described. It may be inferred that replacing thiol with homophenylalanyl as the zinc-ligand function will not lead to a change in the conformational requirements of binding of the bicyclic compounds to the A.C.E. receptor. The 7,6-bicyclic compounds were prepared with (*S*)-configurations at each of the three chiral centres, since it was suggested with the aid of molecular graphics simulations that this stereochemistry favoured high levels of A.C.E. inhibition; this was confirmed by comparison with the inhibitory activities of alternative diastereoisomers (Table 3).

The conformations of the two potent inhibitors (**17**; *n* = 2, R = H) and (**18**; *n* = 2, R = H) have been investigated in detail using n.m.r. spectroscopy (proton and homonuclear shift-correlated 2-D methods).[24] These studies indicated that the 6,10-dioxo-compound (**17**; *n* = 2, R = H) has a very rigid conformation in which the seven-membered ring is very similar to that in isocolchicine. This chair–chair representation of the 7,6-bicyclic system also describes both the phthalimido and homophenylalanine derivatives (**15**) and (**17**; *n* = 2, R = Et), respectively, in the solid state, as determined by *X*-ray diffraction of a related compound (Figure 4). The rigidity of this dioxo-7,6-bicyclic system is attributed to the large A(1,3)-effect holding the carboxyl group in the axial position, the involve-

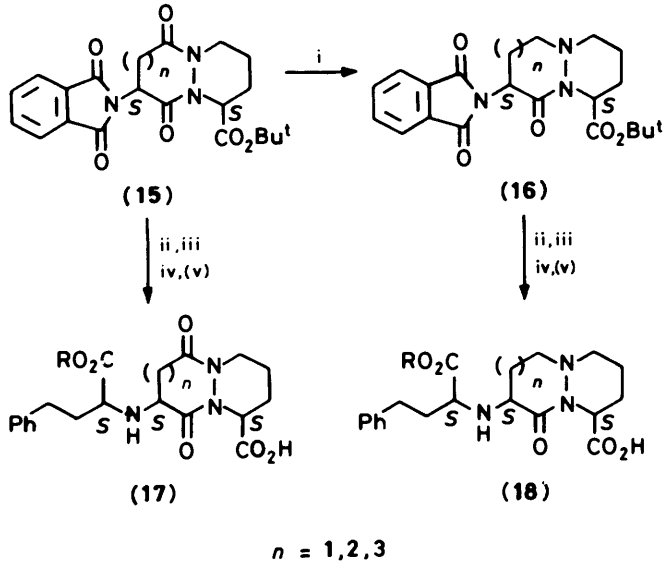
Table 3.



Analysis (%)[a]

n	Y	R^1	R^2	Stereochem.	Formula	C	H	N	Hal	M.p. (°C)	Solvent	$[\alpha]_D^{20}$ [c(%), solvent]	IC_{50}^{26} (nM)
1	0	Et	Bu^t	*RSS*	$C_{25}H_{35}N_3O_6$	63.5 (63.4)	7.4 (7.45)	8.7 (8.9)		122—123	EtOAc–hexane		
2	0	Et	Bu^t	*SSS*	$C_{26}H_{37}N_3O_6$	64.0 (64.05)	7.7 (7.65)	8.55 (8.6)		55—58	Hexane	−105.4° (1.0, MeOH)	
2	H_2	Et	Bu^t	*SSS*	$C_{26}H_{39}N_3O_5$	65.8 (65.95)	8.1 (8.3)	8.8 (8.9)		62—63	Hexane	−71.7° (1.0, EtOH)	
2	0	Et	H	*SSS*	$C_{22}H_{29}N_3O_6$[e]	51.2 (51.6)	5.9 (5.9)	8.2 (8.2)	15.7 (15.6)	216—218[b]	EtOH–Et_2O	−62.6° (0.5, EtOH)	4[d]
2	0	Et	H	*RSS*	$C_{22}H_{29}N_3O_6$[f]	56.1 (55.9)	6.2 (6.4)	8.8 (8.9)	8.7 (8.5)	210—213[b]	EtOH–EtOAc	−100.6° (0.5, EtOH)	250[d]
2	H_2	Et	H	*SSS*	$C_{22}H_{31}N_3O_5$[g]	60.4 (60.7)	7.55 (7.6)	9.65 (9.65)		94—97	EtOH–H_2O	−62.5° (1.0, EtOH)	1.6[d]
2	H_2	Et	H	*RSS*	$C_{22}H_{31}N_3O_5$	63.0 (63.3)	7.4 (7.5)	10.05 (10.1)		*c*		−116.5° (0.5, 1M HCl)	36[d]
2	H_2	Et	H	*SRS*	$C_{22}H_{31}N_3O_5$[h]	58.2 (58.2)	7.1 (7.1)	8.95 (9.3)		*c*		+38.8° (0.5, H_2O)	300[d]
2	H_2	Et	H	*SSR*	$C_{22}H_{31}N_3O_5$[i]	49.2 (49.0)	5.9 (6.1)	7.6 (7.8)	22.1 (22.2)	*c*		−12.8° (0.5, H_2O)	68[d]
3	0	Et	H	*SSS*	$C_{23}H_{31}N_3O_6$	61.95 (62.0)	6.9 (7.0)	9.2 (9.4)		*c*		−76.5° (0.5, EtOH)	15[d]
3	H_2	Et	H	*SSS*	$C_{23}H_{33}N_3O_5$[g]	61.5 (61.45)	7.7 (7.9)	9.1 (9.35)		105—108	EtOH–H_2O	−108.1° (0.5, EtOH)	4.5[d]
1	0	H	H	*SSS*	$C_{19}H_{23}N_3O_6$	58.5 (58.6)	6.0 (5.95)	10.7 (10.8)		214[b]	H_2O	−114.6° (1.0, 1M-NaOH)	20
1	H_2	H	H	*R*R*R**	$C_{19}H_{25}N_3O_5$	60.6 (60.8)	6.6 (6.7)	11.1 (11.2)		198—199[b]	H_2O		56
1	H_2	H	H	*S*R*R**	$C_{19}H_{25}N_3O_5$	60.6 (60.8)	6.6 (6.7)	11.1 (11.2)		209—210[b]	H_2O		1 400
2	0	H	H	*SSS*	$C_{20}H_{25}N_3O_6$	59.5 (59.55)	6.15 (6.25)	10.5 (10.4)		*c*			4
2	H_2	H	H	*SSS*	$C_{20}H_{27}N_3O_5$	61.4 (61.7)	7.1 (7.0)	10.6 (10.8)		242	H_2O	−74.7° (0.5, 1M-NaOH)	1.6

[a] Required values in parentheses. [b] Decomp. [c] Lyophilisate. [d] After esterase treatment. [e] Crystallises with 1 equiv. HBr. [f] Crystallises with 1.13 equiv. HCl. [g] Crystallises with 1 equiv. H_2O. [h] Crystallises with 1 equiv. HCl. [i] Crystallises with 1.5 equiv. HBr.



Scheme 5. *Reagents:* i, H_3B–THF; ii, N_2H_4·H_2O–EtOH; iii, (**14**)–Na_2CO_3–H_2O–CH_2Cl_2; iv, H^+–CH_2Cl_2; v, NaOH–EtOH–H_2O

ment of the two amide planes which allow interannular conformational transmission, and the limitation of the seven-membered ring to a single conformation for a given chair conformation of the piperazic acid moiety.

In the case of the corresponding 10-mono-oxo-system, the n.m.r. data suggested that there was some flexibility. A calculation using the MNDO technique indicated that there was a relatively small difference in the heat of formation values for the chair–chair (ΔH_p = 124 kcal/mol) and twist boat–chair (ΔH_p = 131 kcal/mol) represented by A and B, respectively, but that other forms were less favoured (Figure 7). The dominant conformation in solution and the conformation in the solid state both correspond to the chair–chair form. Using molecular graphics, it can be shown (Figure 5) that the orientation of the three groups determining the interaction with A.C.E. (CO_2^-, CONH, 9-NH) is the same in both the dioxo- and the mono-oxo-7,6-bicyclic systems (refer also to Table 1). It favours a ψ-value of approximately 165° for optimal interaction with the A.C.E. receptor.

If the information on the interaction of the homophenylalanine unit with zinc at the active site (as indicated for thermolysin) is combined with the new data presented here on the favoured orientation of the binding groups, it becomes possible to postulate a model for the interaction of these

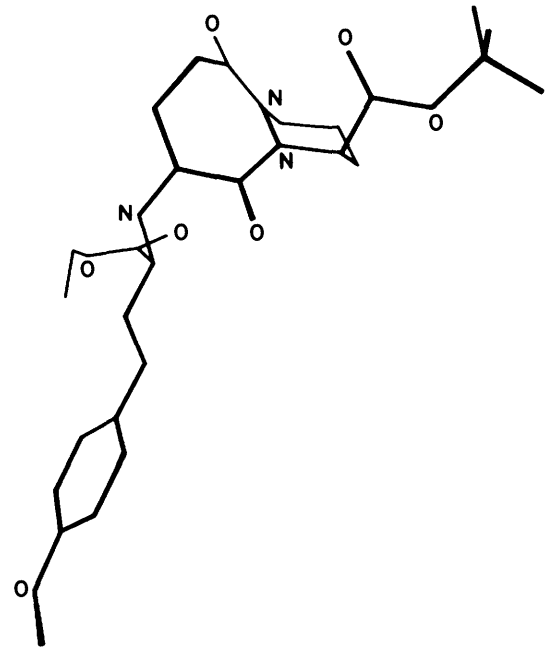


Figure 4. The crystal structure of the t-butyl-9-[1-ethoxycarbonyl-3-(4-methoxyphenyl)-1-propylamino]octahydro-6,10-dioxo-6*H*-pyridazo-[1,2-*a*][1,2]diazepine-1-carboxylate

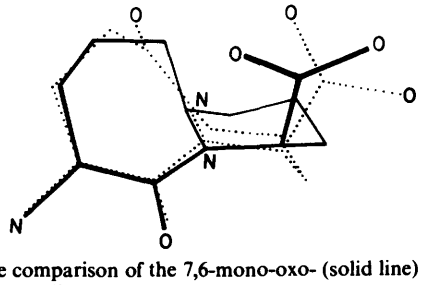


Figure 5. The comparison of the 7,6-mono-oxo- (solid line) and dioxo- (dotted line) bicyclic ring systems

potent inhibitors with A.C.E. Figure 6 is the computer drawn representation of the binding mode of the dicarboxylic acid (**18**; $n = 2$, R = H), formed *in vivo* from the prodrug, Cilazapril (**18**; $n = 2$, R = Et).

Experimental

^{1}H N.m.r. spectra were recorded on a Varian XL100/15 or a Bruker WM300 spectrometer; chemical shifts are expressed as δ-values in p.p.m. from internal $SiMe_4$. M.p.s were determined using a Büchi melting point apparatus and are uncorrected. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. Elemental analyses were performed by Mr. M. R. Cottrell using a Perkin-Elmer 240 instrument. Molecular graphics studies were carried out using a VAX11/750 computer with a Megatek 7000 terminal. *X*-Ray crystallographic data provided by Dr. J. J. Daly and Mr. P. Schoenholzer are gratefully acknowledged.

(1S,9S)-*t-Butyl* 1,2,3,4,7,8,9,10-*Octahydro*-6,10-*dioxo*-9-*phthalimidopyridazino*[1,2-a][1,2]*diazepine*-1-*carboxylate*.—(3*S*)-1-Benzyl 3-t-butyl hexahydropyridazine-1,3-dicarboxylate (24.6 g, 77 mmol) was dissolved in toluene (135 ml) and stirred at 10 °C with a solution of sodium hydrogen carbonate (20.2 g) in water (200 ml). To the stirred mixture was added a solution in toluene (150 ml) of the acid chloride derived from (2*S*)-4-benzyloxycarbonyl-2-phthalimidobutanoic acid (33.8 g, 90 mmol) by treatment with thionyl chloride (13.1 ml) in toluene. Stirring was continued at ambient temperature for 17 h and the layers were then separated. The organic solution was applied to a short column of Florisil and the eluate evaporated.

The residual gum was taken up in dimethylformamide (500 ml) and hydrogenated over 5% palladium-on-charcoal during 18 h. The catalyst was filtered off and the filtrate evaporated. The residue was treated with diethyl ether (150 ml) and the mixture was filtered. The resulting white solid was taken up in dichloromethane (590 ml) and stirred at 10 °C during the addition over 15 min of thionyl chloride (5.5 ml, 0.08 mol). The solution was stirred for 4 h at ambient temperature and then a

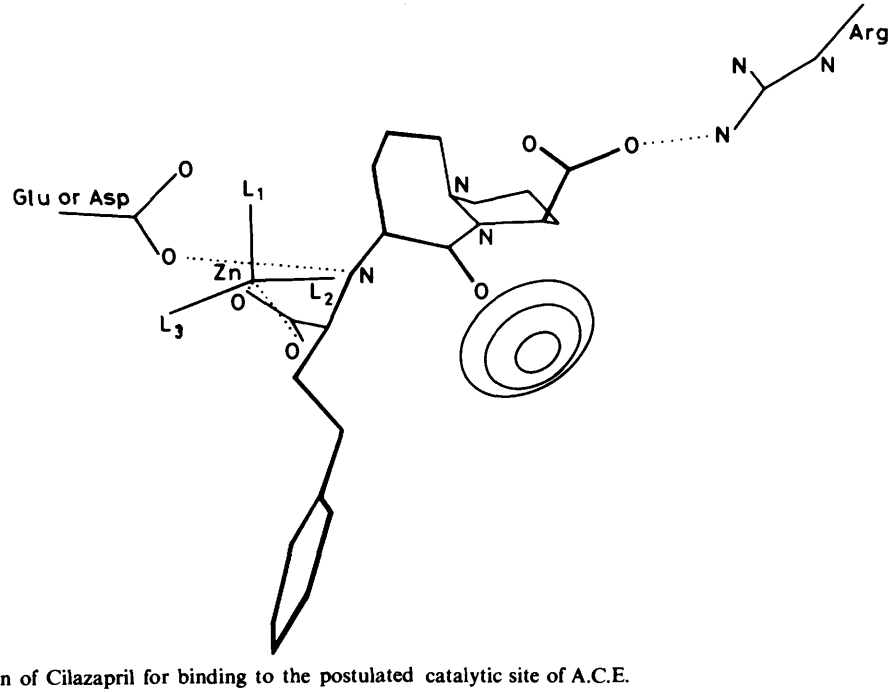
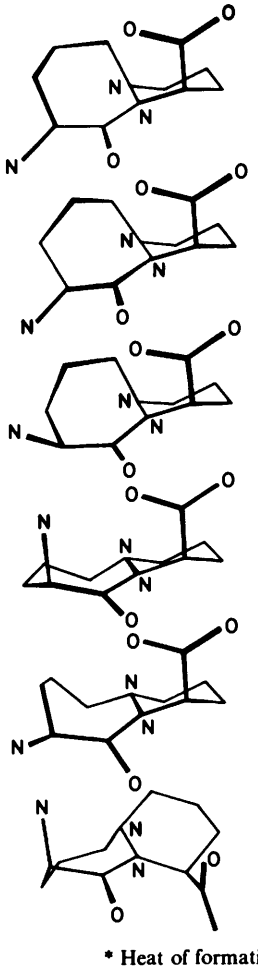


Figure 6. The orientation of Cilazapril for binding to the postulated catalytic site of A.C.E.



	Conformation	ΔH_f*
A	Chair–chair (N lone pair)	124.0
B	Chair–twist boat (N lone pair)	131.2
C	Chair–twist boat (N lone pair)	138.4
D	Chair–chair (N lone pair)	146.2
E	Chair–twist boat (N lone pair)	148.3
F	Chair–chair (N-axial COOH–equatorial)	142.0

* Heat of formation (calculated values, kcal/mol)

Figure 7. Conformations of the 7,6-bicyclic system calculated by the MNDO technique (ΔH_f values in kcal/mol)

solution of potassium hydrogen carbonate (15.5 g) in water (155 ml) was added. The organic layer was separated and evaporated to a gum which was chromatographed to give the *title compound* (25.1 g, 76%), m.p. 188—189 °C (from ethyl acetate–hexane), $[\alpha]_D^{20}$ −85.1° (*c* 0.5 in methanol) (Found: C, 62.0; H, 5.8; N, 9.8. $C_{22}H_{25}N_3O_6$ requires C, 61.8; H, 5.9; N, 9.8%); $\delta_H(CDCl_3)$ 1.5 (9 H, s), 1.5—2.0 (3 H, m), 2.2—2.5 (3 H, m), 2.92 (1 H, dt), 3.35—3.5 (1 H, m), 3.7—3.85 (1 H, m), 4.7 (1 H, br dt), 5.25—5.35 (2 H, m), and 7.7—7.9 (4 H, m).

(1R*,8R*)-8-[(1R*)-*Carboxy*-3-*phenylpropylamino*]*octahydro*-9-*oxopyridazino*[1,2-a]*pyridazine*-1-*carboxylic Acid*.—A solution of (1*R**,8*S**)-methyl 8-bromo-octahydro-9-oxopyridazino[1,2-*a*]pyridazine-1-carboxylate (1.6 g, 5.5 mmol), methyl 2-amino-4-phenylbutanoate (1.27 g, 6.6 mmol), and triethylamine (0.55 g, 5.5 mmol) in dimethylformamide (10 ml) was stirred at 60 °C for 24 h. The solvent was evaporated and the residue partitioned between dichloromethane and aqueous sodium hydrogen carbonate. Evaporation of the organic solution gave a gum which was chromatographed on silica gel to give (1*R**,8*S**)-methyl 8-[(1*R**)-methoxycarbonyl-3-phenylpropylamino]-octahydro-9-oxopyridazino[1,2-*a*]pyridazine-1-carboxylate (0.52 g, 23%), followed by (1*R**,8*R**)-methyl 8-[(1*R**)-methoxycarbonyl-3-phenylpropylamino]-octahydro-9-oxopyridazino[1,2-*a*]pyridazine-1-carboxylate (0.89 g, 40%).

A solution of the (*R**,*R**,*R**)-isomer (0.8 g, 2 mmol) in methanol (12 ml) was treated with 0.5M-aqueous sodium hydroxide solution (12 ml, 6 mmol), and the solution was stirred at 20 °C for 3.5 h and then applied to sulphonic acid ion exchange resin. Elution with 2% pyridine in water afforded the *title compound* (0.17 g, 23%), m.p. 198—199 °C (from water) (Found: C, 60.6; H, 6.6; N, 11.1. $C_{19}H_{25}N_3O_5$ requires C, 60.8; H, 6.7; N, 11.2%); $\delta_H(D_2O$–DCl) 1.62—2.01 (3 H, m), 2.07—2.51 (5 H, m), 2.75—3.08 (3 H, m), 3.22—3.39 (2 H, m), 3.56 (1 H, br d), 4.37 (1 H, dd), 4.46 (1 H, dd), 5.39 (1 H, br d), and 7.27—7.47 (5 H, m).

Reaction of (1S,9S)-*Methyl* 9-*Bromo-octahydro*-6,10-*dioxo*-6H-*pyridazino*[1,2-a][1,2]*diazepine*-1-*carboxylate* (**10**) *with* (2S)-*methyl* 2-*amino*-4-*phenylbutanoate*.—A mixture of (1*S*,9*S*)-methyl 9-bromo-octahydro-6,10-dioxo-6*H*-pyridazino[1,2-*a*]-[1,2]diazepine-1-carboxylate (**10**) (636 mg, 2 mmol), (2*S*)-methyl 2-amino-4-phenylbutanoate (463 mg, 2.4 mmol), and triethylamine (202 mg, 2 mmol) in dimethylformamide (2 ml) was stirred at 80 °C for 72 h. The solvent was then removed by evaporation and the residue partitioned between water and dichloromethane. The organic solution was evaporated to a gum which was chromatographed on silica gel to afford (1S)-*methyl* 1,2,3,4,9,10-*hexahydro*-6,10-*dioxo*-6H-*pyridazino*[1,2-a]-[1,2]*diazepine*-1-*carboxylate* (**12**) (76 mg, 16%), m.p. 110—111 °C (from diethyl ether) (Found: C, 55.3; H, 5.9; N, 11.6. $C_{11}H_{14}N_2O_4$ requires C, 55.5; H, 5.9; N, 11.8%); $\delta_H(CDCl_3)$ 1.74 (2 H, m), 1.96 (1 H, m), 2.37 (1 H, dm), 2.89 (1 H, m), 3.08 (1 H, ddd), 3.53 (1 H, ddd), 3.70 (3 H, s), 4.72 (1 H, dm), 5.35 (1 H, dd), 6.19 (1 H, ddd), and 6.44 (1 H, ddd); *m*/*z* 238 (70%, M^+) and 179 (100); and *methyl* 1,2,3,4,7,10-*hexahydro*-6,10-*dioxo*-6H-*pyridazino*[1,2-a][1,2]*diazepine*-1-*carboxylate* (**11**) (86 mg, 18%), m.p. 115—116 °C (from diethyl ether) (Found: C, 55.2; H, 6.0; N, 11.7. $C_{11}H_{14}N_2O_4$ requires C, 55.5; H, 5.9; N, 11.8%); $\delta_H(CDCl_3)$ 1.71 (2 H, m), 1.98 (1 H, m), 2.38 (1 H, dm), 2.74 (1 H, ddd), 3.07 (1 H, ddd), 3.77 (3 H, s), 4.06 (1 H, d, m), 4.59 (1 H, dm), 5.62 (1 H, dd), 6.08 (1 H, ddd), and 6.59 (1 H, ddd); *m*/*z* 238 (28%, M^+) and 179 (100).

(1S,8S)-8-[(1R,S)-*Carboxy*-3-*phenylpropylamino*]*octahydro*-6,9-*dioxopyridazino*[1,2-a]*pyridazine*-1-*carboxylic Acid*.—(1*S*,8*S*)-8-Amino-octahydro-6,9-dioxopyridazino[1,2-*a*]pyridazine-1-carboxylic acid (900 mg, 4.07 mmol) and 2-oxo-4-phenylbutanoic acid (3.5 g, 19.7 mmol) were stirred with water (25 ml) and the pH was adjusted to 7 by addition of 2M sodium hydroxide solution. Sodium cyanoborohydride (720 mg, 11.4 mmol) was added and the solution stirred at 20 °C for 24 h. Sulphonic acid ion-exchange resin was added followed by diethyl ether (50 ml). The mixture was stirred for 30 min and then filtered. The resin was washed with water (2 × 250 ml). Elution with 2% pyridine in water gave the diastereoisomeric products which were separated by chromatography on Amberlyte XAD 2[7] and isolated by lyophilisation.

First eluted was (1S, 8S)-8-[(R)-*carboxy*-3-*phenylpropylamino*]-*octahydro*-6,9-*dioxo*-*pyridazino*[1,2-a]*pyridazine*-1-*carboxylic acid* (300 mg, 19%) (Found: C, 56.1; H, 6.1; N, 10.4; H_2O, 4.3. $C_{19}H_{23}N_3O_6 \cdot H_2O$ requires C, 56.0; H, 6.2; N, 10.3; H_2O, 4.4%); (8S,1S)-[(1S)-*Carboxy*-3-*phenylpropylamino*]*octahydro*-6,9-*dioxo*-*pyridazo*[1,2-a]*pyridazine*-1-*carboxylic acid* (320 mg, 20%) was eluted second [Found: C, 57.1; H, 6.1; N, 10.5; H_2O, 2.4. Calc. (dry): C, 58.5; H, 6.0; N, 10.7. $C_{19}H_{23}N_3O_6$ requires C, 58.6; H, 6.0; N, 10.8%].

(1S,9S)-*t*-*Butyl* 9-[(1R,S)-*Ethoxycarbonyl*-3-*phenylpropylamino*]-*octahydro*-6,10-*dioxo*-6H-*pyridazino*[1,2-a][1,2]*diazepine*-1-*carboxylate*.—(1*S*,9*S*)-t-Butyl 9-amino-octahydro-6,10-dioxo-6*H*-pyridazino[1,2-*a*][1,2]diazepine-1-carboxylate (13.7 g, 46 mmol) and ethyl 2-oxo-4-phenylbutanoate (29 g, 141

mmol) were stirred at 20 °C for 17 h in tetrahydrofuran (200 ml) in the presence of powdered 4A molecular sieves. Sodium cyanoborohydride (6.5 g, 102 mmol) was added in five portions at hourly intervals and stirring continued for a further 2 h after the final addition. Solvent was removed by evaporation and the residue was partitioned between ethyl acetate and 10% aqueous sodium carbonate. The organic layer was dried ($MgSO_4$) and evaporated and the residual oil chromatographed on silica gel (elution with 3:1 diethyl ether–hexane) to give (1S,9S)-*t-butyl* 9-[(1R)-*ethoxycarbonyl*-3-*phenylpropylamino*]*octahydro*-6,10-*dioxo*-6H-*pyridazino*[1,2-a][1,2]*diazepine*-1-*carboxylate* as a colourless oil (4 g, 21%) (Found: C, 64.2; H, 7.4; N, 8.7. $C_{26}H_{37}N_3O_6$ requires C, 64.05; H, 7.65; N, 8.6%); δ($CDCl_3$) 1.28 (3 H, t), 1.46 (9 H, s), 1.55—2.0 (7 H, m), 2.3—2.65 (6 H, m), 2.74 (2 H, t), 3.12 (1 H, t), 3.4 (1 H, dt), 3.5 (1 H, dd), 4.05—4.2 (2 H, m), 4.55 (1 H, br d), 5.29 (1 H, dd), and 7.1—7.3 (5 H, m); and (1S,9S)-*t-butyl* 9-[(1S)-*ethoxycarbonyl*-3-*phenylpropyl-amino*]*octahydro*-6,10-*dioxo*-6H-*pyridazino*[1,2-a][1,2]-*diaze-pine*-1-*carboxylate* as white crystals (4.1 g, 18%), m.p. 55—58 °C (from hexane) (Found: C, 64.0; H, 7.7; N, 8.55. $C_{26}H_{37}N_3O_6$ requires C, 64.05; H, 7.65; N, 8.6%); δ($CDCl_3$) 1.27 (3 H, t), 1.45 (9 H, s), 1.6—2.35 (9 H, m), 2.35—2.5 (1 H, m), 2.6—2.85 (3 H, m), 3.30 (1 H, t), 3.39 (1 H, dt), 3.63 (1 H, dd), 4.13 (2 H, q), 4.57 (1 H, br d), 5.28 (1 H, dd), and 7.1—7.3 (5 H, m).

(R)-*Ethyl* 2-*Hydroxy*-4-*phenylbutanoate*.—A solution of (*R*)-2-hydroxy-4-phenylbutanoic acid (**13**) (10.66 g, 60 mmol) in ethanol (220 ml) containing concentrated sulphuric acid (0.4 ml) was boiled under reflux for 2 h. The mixture was basified with aqueous sodium hydrogen carbonate and evaporated. The residue was partitioned between water and ethyl acetate and the organic layer evaporated to give the *title compound* (12.0 g, 97%) as a colourless oil, $[\alpha]_D^{20}$ −7.8° (*c* 1 in ethanol) (Found: C, 69.1; H, 7.6. $C_{12}H_{16}O_3$ requires C, 69.2; H, 7.7%); δ_H($CDCl_3$) 1.29 (3 H, t), 1.9—2.2 (2 H, m), 2.7—2.9 (2 H, m), 2.95 (1 H, br s, exch.), 4.2 (1 H, m), 4.22 (2 H, q), and 7.15—7.35 (5 H, m).

(R)-*Ethyl* 2-*Trifluoromethylsulphonyloxy*-4-*phenylbutanoate* (**14**).—A solution of (*R*)-ethyl 2-hydroxy-4-phenylbutanoate (7.97 g, 40 mmol) and pyridine (3.1 ml, 40 mmol) in dichloromethane (15 ml) was added over 1 h to a cooled (< 10 °C) solution of trifluoromethanesulphonic anhydride (12.42 g, 40 mmol) in dichloromethane (30 ml) stirred in an atmosphere of dry nitrogen. The mixture was stirred for a further 2 h at 10 °C then washed with water. The organic layer was concentrated and the residue passed through a short column of silica gel giving the *title compound* (11.1 g, 85%) as a pale yellow oil, $[\alpha]_D^{20}$ +16° (*c* 1 in hexane) (Found: C, 45.65; H, 4.5. $C_{13}H_{15}O_5SF_3$ requires C, 45.9; H, 4.4); δ_H($CDCl_3$) 1.25 (3 H, t), 2.2—3.0 (4 H, m), 4.15 (2 H, q), 5.0 (1 H, t), and 7.1—7.3 (5 H, m).

(1S,9S)-*t-Butyl octahydro*-10-*oxo*-9-*phthalimido*-6H-*pyrid-azino*[1,2-a][1,2]*diazepine*-1-*carboxylate* (**16**; n = 2).—A solution of (1*S*,9*S*)-t-butyl octahydro-6-,10-dioxo-9-phthal-imido-6*H*-pyridazo[1,2-*a*][1,2]diazepine-1-carboxylate (**15**; *n* = 2) (36.5 g, 85 mmol) in dry tetrahydrofuran (85 ml) was cooled and stirred under a slow stream of nitrogen. 1M-Borane solution in tetrahydrofuran (75.2 ml, 75 mmol) was added at such a rate as to maintain the internal temperature at 10—15 °C for 1 h. The reaction mixture was stirred at 10—15 °C for 1 h and at ambient temperature for a further 3 h. Dichloromethane (170 ml) was then added and the reaction mixture stirred during the addition of 2M-hydrochloric acid (170 ml). After being stirred for 15 min, the reaction mixture was basified with anhydrous sodium carbonate and the organic layer separated, washed with brine, and evaporated to afford the *title compound* (31.7 g, 90%), m.p. 140.5—141.5 °C (from aqueous ethanol), $[\alpha]_D^{20}$ −68.5° (*c* 1 in methanol) (Found: C, 63.7; H, 6.5; N, 10.1. $C_{22}H_{27}N_3O_5$ requires C, 63.9; H, 6.6; N, 10.2%); δ_H($CDCl_3$) 1.36—1.48 (1 H, m), 1.53 (9 H, s), 1.56—1.98 (3 H, m), 2.00—2.20 (1 H, m), 2.20—2.62 (4 H, m), 3.04 (1 H, br d), 3.23 (1 H, dt), 3.48 (1 H, dt), 4.98 (1 H, d), 5.70 (1 H, dd), and 7.66—7.97 (4 H, m).

(1S,9S)-9-[(1S)-*Ethoxycarbonyl*-3-*phenylpropylamino*]*octa-hydro*-10-*oxo*-6H-*pyridazino*[1,2-a][1,2]*diazepine*-1-*carboxylic Acid Monohydrate*.—A suspension of (1*S*,9*S*)-t-butyloctahydro-10-oxo-9-phthalimido-6*H*-pyridazino[1,2-*a*][1,2]diazepine-1-carboxylate (**18**; *n* = 2) (8.26 g, 20 mmol) in ethanol (82.6 ml) was treated with hydrazine hydrate (2.2 g, 44 mmol). After the mixture had been stirred for 1 h at ambient temperature, the solvents were evaporated and the residue re-evaporated with toluene. 2M Aqueous acetic acid (82.6 ml) was added and the mixture stirred for 16 h and then filtered. The filtrate was basified with anhydrous sodium carbonate and extracted with dichloromethane. The organic solution was added to 10% aqueous sodium carbonate (40 ml) and the mixture stirred at ambient temperature during the addition of (2*R*)-ethyl 2-trifluoromethanesulphonyloxy-4-phenylbutanoate (7.48 g, 22 mmol) in dichloromethane (20 ml). After the mixture had been stirred for 5 h, the organic solution was separated, stirred for 30 min with Florisil (1.0 g), and then filtered. The resulting solution of (1*S*,9*S*)-t-butyl 9-[(1*S*)-ethoxycarbonyl-3-phenylpropylamino]octahydro-10-oxo-6*H*-pyridazino[1,2-*a*]-[1,2]diazepine-1-carboxylate (**18**; *n* = 2) was cooled to 0—5 °C and stirred slowly during the passage over 2 h of dry hydrogen chloride. The solution was allowed to stand at ambient temperature for 16 h and was then evaporated. The residue was partitioned between water and diethyl ether and the combined ethereal solutions were then extracted with 1M-hydrochloric acid. The combined aqueous solutions were adjusted to pH 4.4 with 5M-aqueous sodium hydroxide. The precipitated solid was filtered off to give (1S,9S)-9-[(1S)-*ethoxy-carbonyl*-3-*phenylpropylamino*]*octahydro*-10-*oxo*-6H-*pyrid-azino*[1,2-a][1,2]*diazepine*-1-*carboxylic acid monohydrate* (7.21 g, 83%). A second crop (0.72 g, 8%) was obtained by extracting the aqueous filtrate with dichloromethane. After recrystallisation from aqueous ethanol, the product had m.p. 95—97 °C, $[\alpha]_D^{20}$ −62.51 (*c* 1 in ethanol) (Found: C, 60.4; H, 7.55; N, 9.65; H_2O, 4.2. $C_{22}H_{31}N_3O_5 \cdot H_2O$ requires C, 60.7; H, 7.6; N, 9.65; H_2O, 4.1%); δ_H(CD_3OD) 1.30 (3 H, t), 1.30—1.42 (1 H, m), 1.58—1.90 (4 H, m), 1.96—2.26 (4 H, m), 2.44 (1 H, m), 2.55 (1 H, m), 2.76 (2 H, m), 3.03 (1 H, br t), 3.18 (1 H, m), 3.59 (1 H, dt), 3.72 (1 H, t), 4.24 (2 H, m), 4.52 (1 H, t), 4.81 (1 H, m), and 7.12—7.34 (5 H, m).

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