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Synthesis and Conformational Studies of Zabicipril (S 9650-3), a Potent Inhibitor of Angiotensin Converting Enzyme

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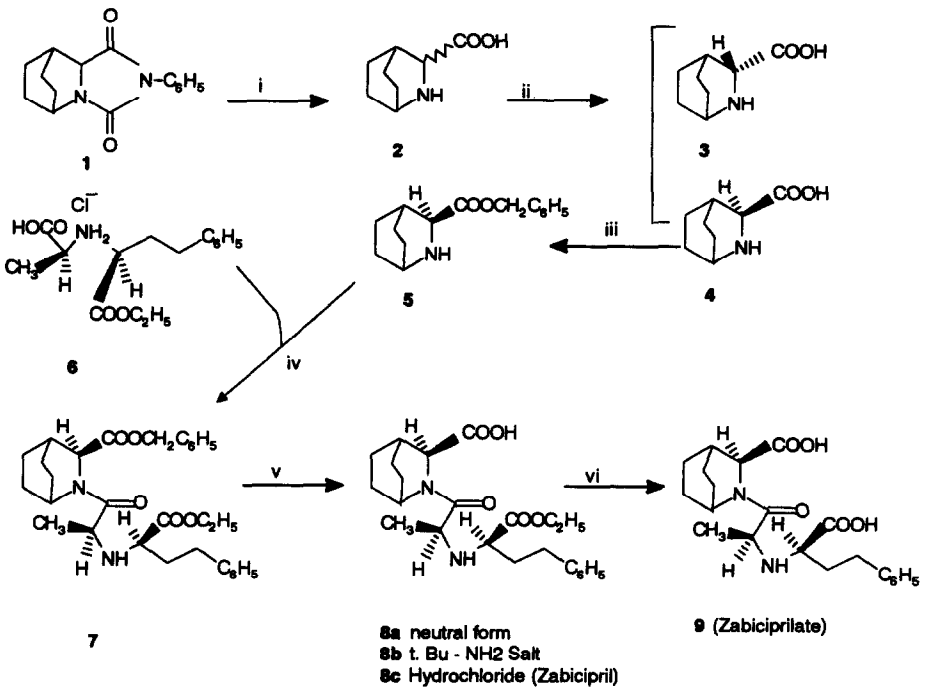
Abstract : The synthesis of the title compound **8c** is described. This inhibitor of Angiotensin Converting Enzyme (ACE) contains aza-2 bicyclo [2, 2, 2] octane-carboxylic acid, a bulky cyclic amino acid replacing the proline moiety present in most ACE inhibitors described in the literature. Structural analysis of **8c** supports the hypothesis of preferred conformations for this type of pseudo peptidic molecule, in solution (^1H, ^{13}C NMR) and in the solid state (X- Ray).

Angiotensin Converting Enzyme (ACE) has long been the target of therapeutic agents designed to lower blood pressure in man[1]. The results of the clinical studies of (3S) 2-[[(S) 1 - carboethoxy 3-phenyl propyl] (S) alanyl] 2-aza 3-carboxy bicyclo [2, 2, 2] octane hydrochloride **8c** (Zabicipril, S 9650-3) suggest that **8c** is a well tolerated, powerful and long acting inhibitor of ACE, with maximal inhibitory effect at 2.5 mg per day when given *via* the oral route[2]. The synthesis of **8c** has been briefly described in a patent[3]. **8c** is an acid-ester devoid of activity *per se*, which is transformed by liver esterases into its active diacid form Zabiciprilate **9**. *In vitro*, **9** is a very active compound (IC_{50} = 1.8 nmol, substrate : hippuryl-histidyl-leucine).

Synthesis

The synthesis of **8** and **9** was performed *via* coupling of the two chiral intermediates **5** and **6** following the route outlined in scheme 1. The chiral intermediate **5** was prepared as follows : the racemic aminoacid **2** was obtained by alkaline hydrolysis (24 h reflux in 4N NaOH/MeOH 3/1. yield 78 %) of the hydantoïn **1** obtained according to Benishai[4]. Starting from **2**, the (-) tartaric acid salt of **4** crystallized from EtOH (yield 87 %). **4** was obtained from this salt by ion-exchange on Dowex 50 WX 8 (H^+ form) and elution with 0.3 N NaOH. (yield 98 %, ee = 97 %) (GLC after esterification with CH_2N_2 and amidation with (-) camphanyl chloride). Using (+) tartaric acid **2** yielded **3** (85 %, ee = 93 %). **4** was esterified to **5** with benzyl alcohol in toluene using *p*-toluenesulfonic acid as catalyst. The second chiral intermediate **6** was prepared according to Kaltenbronn[5]. The coupling of the benzyl ester **5** with the iminodiacid ester **6** was performed with DCC-HOBT-Et_3N in DMF[6], thus producing the benzyl ester **7** (yield 97 %). The high yield of this coupling is probably due to the high reactivity of the rigid bulky nucleophile **5** and to the low reactivity of the sterically hindered secondary amine of **6**[7] so that the formation of by-products[8] (racemates, diketopiperazine from two mol of **6**, acylurea by addition of **6** on DCC) was not observed (TLC). **7** was submitted to hydrogenolysis on palladium charcoal in EtOH at room temperature (yield 98 %) and crystallized as its *t*-butylamine salt **8b** from Et_2O, and finally transformed to its more stable hydrochloride **8c**[9] (yield 95 %). Careful saponification of **8b** with 1N NaOH at room temperature gave crude **9** which was purified by ion-exchange on Dowex 50 W X 8 (H^+ form) and elution with water / pyridine 9:1, then crystallization from 2-propanol (yield 80 %). Note 10 gathers the data of new compounds.



i :NaOH ; ii : resolution by (-) tartaric acid ; iii : C6H5CH2OH, PTSH ; iv : coupling ; v : H2Cat Pd/C ; vi : NaOH

Scheme 1

Conformational studies

NMR Studies (Fig. 1)

Two conformations for **8c** in D_2O. ***Trans*** : NOE at 4.42 ppm (CH_c) by irradiation at 3.87 ppm (CH_b). ***Cis*** : NOE at 4.02 ppm (CH_c) by irradiation at 4.38 ppm (CH_a). Results complemented by ^{13}C NMR (100 MHz ; D_2O ; pH = 2). ***Trans :*** CH_3 : 15.6 ; 17.8 ; CH_2 : 21.8 ; 26.9 ; 26.0 ; 27.8 ; 32.5 ; 33.0 ; 66.0 ; CH : 30.2 ; 48.6 ; 56.2 ; 61.0 ; 62.2 ; 128.7 ; 130.75 (2 carbons) ; 130.8 (2 carbons) ; C : 141.6 ; 169.4 ; 170.8 ; 175.3.***Cis*** CH_3 : 15.6 ; 17.6 ; CH_2 : 21.8 ; 25.7 ; 26.1 ; 26.2 ; 32.5 ; 33.7 ; 66.0 ; CH : 30.9 ; 45.6 ; 56.6 ; 60.6 ; 62.5 ; 128.6 ; (4 carbons of the phenyl were not attributed) ; C : 141.8 ; 170.7 ; 171.0 ; 176.0.

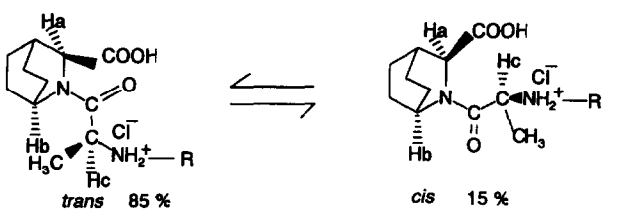


Fig. 1

Crystal Structure

An X-Ray crystal structure analysis[11] was undertaken to determine the absolute configuration of compound **8c**. Figure 2 shows its conformation in the crystal with the observed absolute configuration.

A study of the torsion angles of different ACE inhibitors with the same pseudopeptidic skeleton has been done in a previous work[7]. It had been shown that, in the crystal state, in the presence or absence of solvent molecules, the conformation of the skeleton remains constant.

The torsion angles values of **8c** (Table 1) agree completely with earlier studies in this area : proline *trans*, alanyl methyl *perpendicular* to the amido plane, the H-binding directions of the alanyl nitrogen identical with the previously observed ones, and the putative Zn binding group ($COOC_2H_5$) and the proline carboxylate end pointing in *opposite sides of the amido plane.*

Table 1

		8c	mean*			8c	mean*
$C_9C_3N_2C_1$'	$\phi 2$	- 63 °	- 73 °	N_2'C_1•C_2•C_3•	$\tau 2$	67	*g* or *t*
$C_3N_2C_1$'C_2'	ϖ	174	178	C_1•C_2•C_3•C_4•	$\tau 3$	- 178	180
N_2C_1'C_2'N_2'	$\psi 1$	163	152	C_2'N_2'C_1•C_{10}	$\theta 1$	- 28	- 20
C_1'C_2'N_2'C_1•	$\phi 1$	- 65	- 70	N_2'C_1•$C_{10}O_{10}$	$\theta 2$	- 66	- 70
C_2'N_2'C_1•C_2•	$\tau 1$	170	170				

* : Values taken from ref.7

These results support the hypothesis of a preferred conformation for this type of pseudo-peptidic chain, with the bicyclo-octane occupying the same volume as the perhydroindole group of perindoprilate[7] in the hydrophobic pocket S'2 of ACE. Also noteworthy is the relative position, remarkably constant all through this family of inhibitors, of the two carboxylic groups assumed to bind to corresponding positive sites of ACE, which suggests the spatial disposition of the enzymic groups.

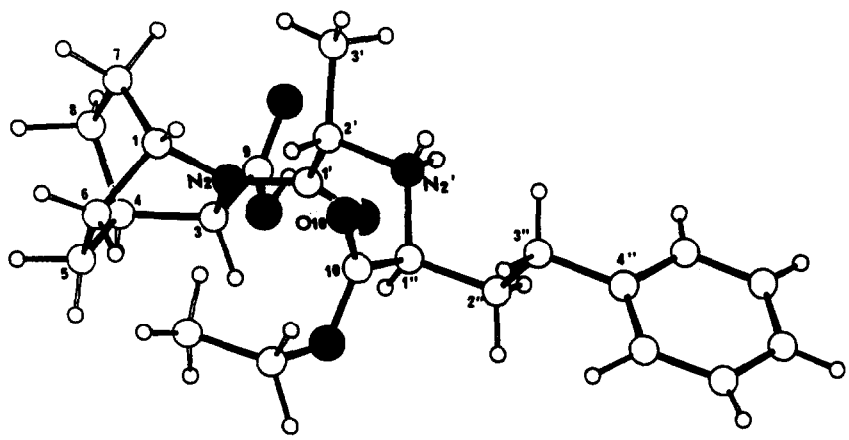


Fig. 2

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9. **8c** was obtained by dissolution of **8b** in CH_2Cl_2, addition of an equivalent of 1N aqueous HCl, extraction of t-Bu-NH_2 , HCl with water, drying of the organic layer ($MgSO_4$), filtration, acidification with 10N HCl, evaporation to dryness and crystallisation of the residue from CH_3CN.
10. Elementary analyses were within $\pm$ 0.4 % of the theoretical values.
    2. ^{1}HNMR (D_2O, ppm) : 3.85 (d.1H, J=2.5 Hz) ;3.6 (m, 1H) ; 2.1 (m, 1H) ; 2-1.8 (m, 8H).
    3. $[\alpha]_D^{25}$ = + 16 (C=1, H_2O). IR (Nujol, cm^{-1}) : 3400, 3450. ^{1}HNMR (D_2O, ppm) : 3.65 (d, 1H) ; 3.4-2.15 (m, 2H) ; 1.7 (m, 8H).
    4. $[\alpha]_D^{25}$ = -17(C=1, H_2O). IR and ^{1}HNMR idem **3**.
    5. $[\alpha]_D^{20.5}$ = -14 (C=1.0, EtOH/H_2O/1/3). IR (Nujol, cm^{-1}) : 1730, 3100-3500. ^{1}HNMR (D_2O, ppm) : 7.1 (s, 5H) ; 5.25 (s, 2H) ; 3.5 (d, 1H, J=2.4 Hz) ; 2.9 (m, 1H) ; 2.1-1.5 (m, 9H).
    7. IR (film, cm^{-1}) : 1635, 1735, 3200-3600. ^{1}HNMR ($CDCl_3$, ppm) : 7.4, 7.25 (2s, 10H) ; 5.2 (s, 2H) ;
    8b. IR (Nujol. cm^{-1}) : 1560, 1645, 1735, 3340.
    ^{1}HNMR (D_2O, ppm) : 7.3 (s, 5H) ; 4.2 (q, 2H, J=7 Hz) ; 6-7.5 (m.3H) ; 2.5-1 (m, 27H) ; 1.3 (s, 9H).
    8c. m.p. = 200 °C (Kofler). $[\alpha]_D^{20}$ = - 42 % (C=1, EtOH 95 %).
    9. IR (Nujol, cm^{-1}). 1650, 1710, 1800-3600. ^{1}HNMR (D_2O, NaOD, ppm) : 7.4 (s.5H) ; 5-3.4 (m, 4H) ; 1.35 (d, 3H, J=6Hz) ; 3.3-1 (m, 13H).
11. Crystal data. $C_{23}H_{32}N_2O_5$, HCl ; orthorhombic, space group $P2_12_12_1$; a = 19.317 (8), b = 12.637 (4), c=10.276 (4) A, V = 2508.46 A^3 ; dc = 1.19, Z = 4. A crystal of dimensions 0.3 x 0.3 x 0.4 mm was mounted on a 4-circle diffractometer with graphic monochromated CuKα radiation (λ = 1.5418 A). From 5138 measured reflections ($\pm$ h, k, l), 2100 independent were used [I > 3σ (I)]. Lorentz and polarisation, no absorption corrections. Structure solved by direct methods using a local programm [Riche C., (1982), 7th European Crystallographic Meeting, Jerusalem, Abstract 25]. Anisotropic least-squares refinement using SHELX76 [Sheldrick, G.M., (1976) ; SHELX76, Program for crystal structure determination, Univ. of Cambridge, England.] to R = 0.0499 and R_w = 0.0559 for 2094 reflections . The minimized function in the refinement was Σw (Fo - Fc)^2 with a final weighting scheme w=1/[σ^2 (Fo) + 0.007 Fo^2]. All hydrogen atoms located on difference Fourier maps and refined with isotropic thermal parameters. With regard of the high value of C-30 thermal parameters, the only H-atoms of methyl group C-30 were refined with constraints to the atom-atom distances and assigned the equivalent isotropic thermal parameter of the C bounded atom. The absolute configuration of every asymmetric carbon, determined unequivocally from comparison of the Bijvoet pairs, is S. The tables of structural data are available from the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, Great Britain.

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