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The Couple Pro/AzaPro : a Means of β-Turn Formation Control Synthesis and Conformation of Two AzaPro-containing Dipeptides

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Abstract: *On the basis of two synthesized AzaPro-containing dipeptides, the conformational influence of the azaproline residue (a nitrogen atom is substituted for the Pro-CH$^{\alpha}$) on the β-turn occurrence was tested according to its relative position in the azadipeptide sequence.*

Modulation of the action of bioactive peptides can be achieved chemically by insertion of amide bond isosteres in the peptide backbone [1-3]. Among the large number of peptide bond surrogates, the substitution of a nitrogen atom for the CH$^{\alpha}$ group, which gives rise to an α-aza-aminoacid [4], has been encountered rather rarely and no in depth conformational study has appeared yet.

On the other hand, proline, which is unique among the coded amino acids because of its pyrrolidine ring, is recognized as being of special significance in its effect on chain conformations and on the process of protein folding[5].

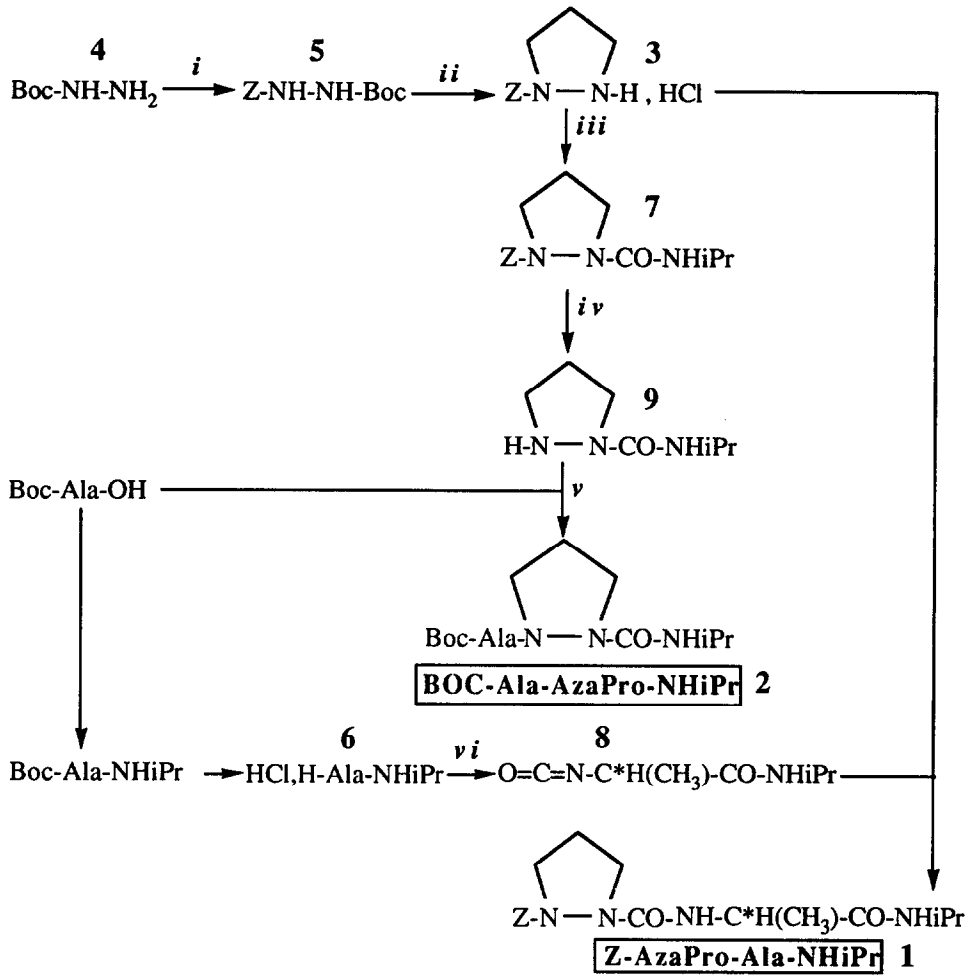
Therefore, on the basis of the two azadipeptides :

1 Z-AzaPro-L-Ala-NHiPr (Z : benzyloxycarbonyl)

2 Boc-L-Ala-AzaPro-NHiPr (Boc : *tert*-butyloxycarbonyl)

we have tackled the study of the conformational influence of AzaPro, in comparison with the native Pro, when incorporated in the simplest dipeptide sequence able to accomodate a β-turn, a spatial arrangement characterized by the N-H···O=C intramolecular hydrogen bond closing a 10-membered cycle and known to be very important in some biological processes [6].

The synthetic procedures for preparing **1** and **2** are summarized in Scheme 1. The key synthon is the benzyl-azaprolinate hydrochloride **3**, a stable crystalline compound which was obtained by slight modifications of the previously described procedures [7]. The N'-benzyloxycarbonyl-*tert*-butylcarbazate **5** was prepared in



Scheme 1. (i) Z-Cl / NMM / THF; (ii) NaH / Br-$(CH_2)_3$-Br / DMF; HCl / AcOEt; (iii) O=C=NiPr / THF; (iv) H_2/Pd/C 10% / iPrOH; (v) iBuOCOCl / NMM / THF; (vi) $(CCl_3O)_2CO$ / NMM / $CHCl_3$.

90% yield after chromatographic purification by condensation of benzyl chlorocarbonate with *tert*-butylcarbazate **4** (Aldrich) using N-methylmorpholine as a tertiary base in THF solution. **3** was synthesized by treatement of **5** with NaH and then 1,3-dibromopropane in DMF solution followed by selective acidic cleavage of the Boc-protecting group. Starting from alanyl-isopropylamide hydrochloride **6**, prepared by usual peptide synthetic methods [8,9], the transformation into its corresponding isocyanate **8** was achieved by treatment with triphosgene (bis(trichloromethyl) carbonate, Fluka) in chloroformic medium. Compound **3** was coupled to **8** in the presence of N-methylmorpholine to get **1**. Compound **9** was obtained after hydrogenolysis of the Z-protecting group of **7** prepared by reaction of **3** with isopropylisocyanate in THF solution. Compound **2** was prepared by coupling **9** with Boc-L-Ala-OH using isobutyl chlorocarbonate mixed-anhydride method [8]. All derivatives in Scheme 1 are chromatographically pure and give satisfactory spectroscopic data.

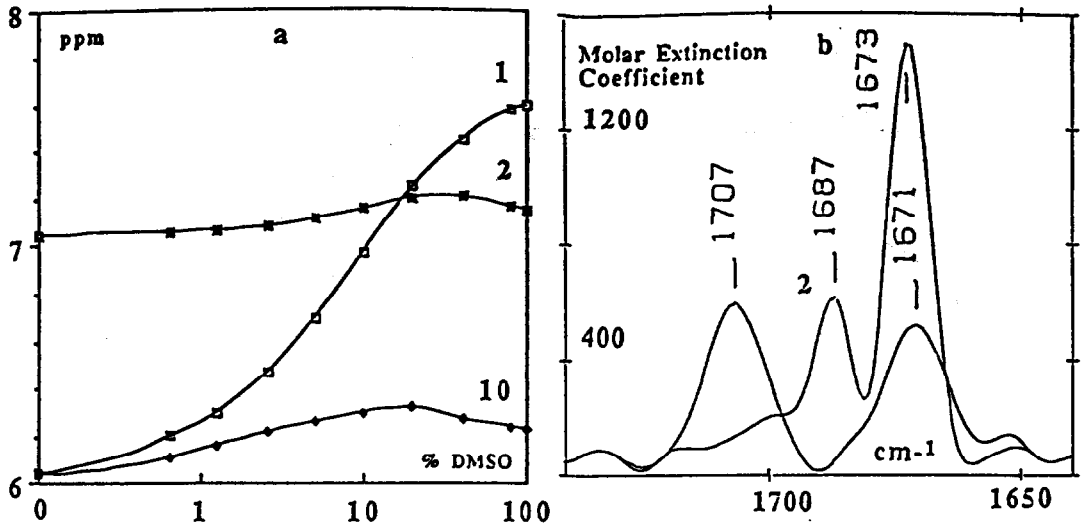


Figure 1. (a) Titration versus solvent composition (Chloroform / DMSO) of the C-terminal NHiPr of the aza-dipeptides Z-AzaPro-L-Ala-NHiPr **1**, Boc-L-Ala-AzaPro-NHiPr **2**, tBuCO-Pro-AzaAla-NHiPr **10**
(b) Deconvoluted FTIR spectra of Boc-L-Ala-AzaPro-NHiPr **2** and Boc-L-Ala-NHiPr in the carbonyl stretching absorption domain.

The occurrence in solution of the intramolecular (iPr)NH····O=C(urethane) hydrogen bond, typical of a β-folded structure in compounds **1** and **2** has been searched by considering (i) the solvent sensitivity of the (iPr)NH proton signal in DMSO-d_6 chloroform-d mixtures, (ii) the urethane C=O stretching vibration in pure DMSO solution. The relative invariance of **2** (iPr)NH proton chemical shift versus solvent composition, as that observed for tBu-CO-Pro-AzaAla-NHiPr taken as an informative exemple and known to be β-folded in crystalline state[10] and in DMSO solution (Figure 1a), shows that this NH is the donor site for the presence of an intramolecular hydrogen bond, the acceptor site being the Boc-urethane carbonyl group with an Amide I absorption band shifted to lower wavenumbers by 20 cm^{-1} in comparison with Boc-L-Ala-NHiPr (Figure 1b). Unlike **2**, compound **1** with a large sensitivity to the medium (Figure 1a) of the same (iPr)NH proton, in addition with no observed shift in wavenumbers for neither of the three carbonyls present in its molecule, adopts in DMSO solution an extended backbone conformation with no intramolecular hydrogen bond.

The above findings in solution are corroborated in the solid state by solving the crystal structures of **1** and **2** by x-ray diffraction[11] (Figure 2). Compounds **1** and **2** have in common (i) the pyramidal character of the two nitrogen atoms of their pyrazolidine ring, (ii) the pseudo cis-conformation of the urethane (**1**) or the tertiary amide function (**2**) preceding the AzaPro residue, (iii) the identical absolute values of the Azaproline residue "ϕ,ψ" torsion angles, ± 111° and ± 22° respectively. But they differ about the absolute configurations of the two nitrogen atoms of their pyrazolidine ring (R,R in **1** opposite to S,S in **2**) and about the extended backbone conformation observed in **1** whereas compound **2** is folded according to a pseudo type VI β-turn [12] with a weak stabilizing intramolecular i+3 → i hydrogen bond.

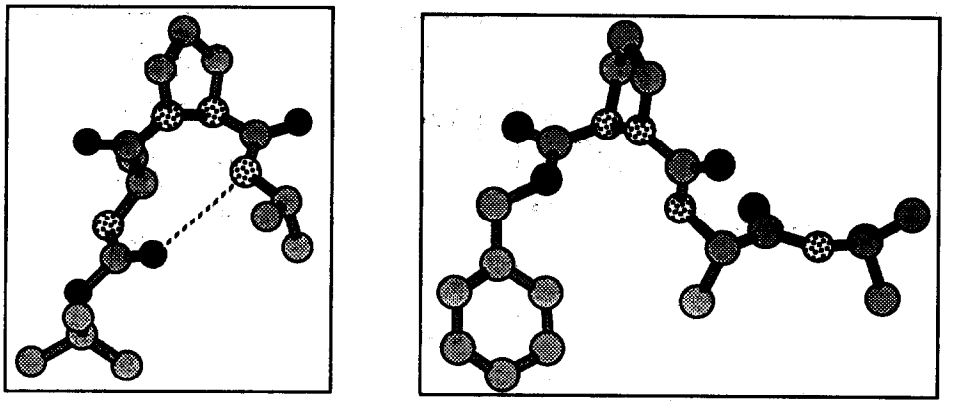


Figure 2. Crystal molecular structures of Boc-L-Ala-AzaPro-NHiPr **2** (left) and Z-AzaPro-L-Ala-NHiPr **1** (right)

Considering all these results, in a peptide chain, would AzaPro prevent the formation of a β-turn with the *following* residue contrary to the native Pro residue[13,14], but be compatible with a folded structure with the *preceding* residue ?

If this holds true, because of its unique geometric properties, the introduction of an AzaPro residue in a peptide chain would be of tremendous importance and interest in the design of bioactive peptide analogues.

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