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Synthesis and conformational preferences in solution and crystalline states of an aza-tripeptide

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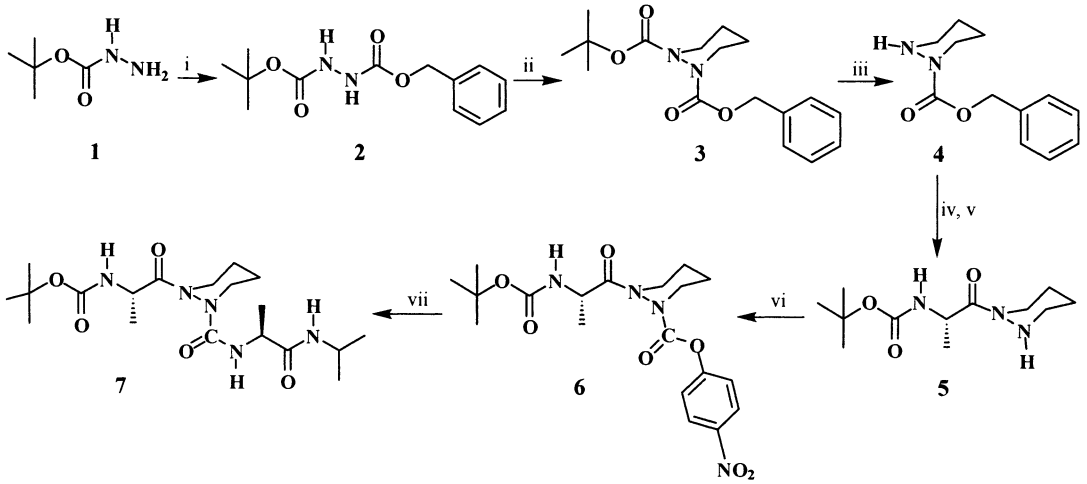
Abstract—The aza-tripeptide Boc-Ala-AzPip-Ala-NH*i*Pr (AzPip: 2-aza pipecolyl residue) was synthesized in seven steps using preferentially the diisopropylcarbodiimide/1-hydroxy-7-aza-benzotriazole (DIPCDI/AtOH) coupling method and via the Boc-AzPip-OBzl pivotal intermediate. Its crystal molecular structure is characterized by the presence of a βVI-like turn around the N-terminal Ala-AzPip sequence and stabilized by two intramolecular hydrogen bonds sharing the same (Boc)CO carbonyl acceptor. In solution (chloroform, DMSO), two isomers are in equilibrium, one of them resembling the stereoisomer found in the crystal, the other being unfolded, but keeping the *cis*-isomerism of the Ala-AzPip bond and the pronounced degree of pyramidicity of the axial 2-amide N^{α} nitrogen atom.

Peptidomimetics[1] have gained enormous popularity and relevance in recent years because they can mimic a natural peptide without changing its main biological effect,[2] but at the same time improve its undesirable therapeutic characteristics such as poor bioavailability, metabolic stability and receptor selectivity by modifying its primary structure.[3] From a 3D structural point of view, the foldamer concept has simultaneously emerged by the design and incorporation of unnatural constrained oligomers (building blocks) endowed with rather well-defined conformational propensities[4,5] and interesting biological features.[6]

In the course of our studies aimed at controlling, at least partially, the main peptidic torsional angles (ϕ, ψ, ω, χ),[7–10] we have reported that, considering the Pro/



Scheme 1. *Reagents and conditions*: (i) ZCl, TEA, THF; (ii) NaH then Br-$(CH_2)_4$-Br, DMF; (iii) HCl/AcOEt (≈3N); (iv) Boc-Ala-OH (1.0 equiv.), diisopropylcarbodiimide (1.0 equiv.), AtOH (1.0 equiv.), DCM; (v) H_2, Pd/C 5%, MeOH; (vi) 4-nitrophenylchlorocarbonate, anhydrous AcOEt; (vii) H-Ala-NH*i*Pr (1.5 equiv.), TEA (1.5 equiv.), DMAP (cat.), 80°C, 3 days.

Keywords: hexahydropyridazine 2-carboxylic acid; pipecolic acid; azapeptides; beta-turn; conformational studies; X-ray diffraction; 2D-NOESY.
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AzPro couple[8,9] (AzPro means that a nitrogen atom has been substituted for the CH^{α} in the pyrrolidine ring), AzPro induces a βVI-like reverse turn with the preceding residue when incorporated in a peptide sequence, whereas Pro is well-known to favor a classical β-turn with the following residue. In the past few years, we moved to the couple Pip/AzPip,[10] where AzPip represents the Pip (H-Pip-OH, pipecolic acid, homoproline, piperidine 2-carboxylic acid) residue, the CH^{α} of which has been changed for a nitrogen atom, and we have just reported the conformational features in the solid state of the AzPip motif included in an aza-dipeptide sequence. To confirm the observed main conformational propensities,[10] we studied the aza-tripeptide Boc-Ala-AzPip-Ala-NH*i*Pr, which is more adapted to mimic a modified aza-peptide backbone. It was prepared in seven steps according to a classical liquid-phase synthesis, but going from N- to C-terminus (Scheme 1). The pivotal intermediate molecule was Boc-AzPip-OBzl **3**, which was obtained, similarly to Boc-AzPro-OBzl,[9] first by benzyloxycarbonylation of the commercial *t*-butylcarbazate **1**, to give the *N*,*N*′-diacylhydrazine Boc-NH-NH-Z **2**, followed by bi-metallation and in situ intramolecular cyclization after treatment with 1,4-dibromobutane, to give the *N*,*N*′ orthogonally protected hexahydropyridazine **3**. After elimination of the Boc protective group to get the hydrazide **4**, and then taking into account the poor nucleophilicity of the involved hydrazine nitrogen, we made use of the highly efficient coupling method DIPCDI/AtOH to react **4** with Boc-Ala-OH to form the protected aza-dipeptide Boc-Ala-AzPip-OBzl, which was hydrogenolyzed to obtain the hexahydropyridazide **5**. This latter compound was condensed with 4-nitrophenylchlorocarbonate to get the mandatory 'activated' 4-nitrophenylcarbazate **6** able to react with H-Ala-NH*i*Pr to get the desired crude aza-tripeptide isopropylamide **7**. A final silica gel flash chromatography (AcOEt) furnished the pure Boc-Ala-AzPip-Ala-NH*i*Pr **7**, monocrystals of which, suitable for X-ray diffraction, were then grown from a slowly cooled AcOEt solution.

A stereo drawing of the three-dimensional crystal molecular structure of the aza-tripeptide **7** (deposited at the Cambridge Crystallographic Data Center) is shown in Fig. 1. The saturated hexahydropyridazine heterocycle adopts a quasi *chair* conformation in which the two nitrogen atoms deserve special attention: on the one hand, X-ray experimental data show the N atom of the tertiary amide function Ala-AzPip in a planar arrangement while the nitrogen N^{α}, substituted for CH^{α}, takes a pyramid one with a S(L) nitrogen absolute configuration.[10,11] The axial disposition of the pyramidal N^{α} amide function and the *cisoid* form of the Ala-AzPip tertiary amide can be explained by the concept of pseudo allylic strains which favors the 1,2 diaxial relationship between the two *N*-substituted carbonyls over what would be strong 1,2 diequatorial repulsions. The ϕ and ψ torsion angles[12] of Ala^1 and AzPip residues in addition with the *cis*-configured Ala^1–AzPip bond are characteristic of the observed pseudo βVI turn, which is doubly stabilized by the two strong intramolecular hydrogen bonds: the expected (Boc)C=O···HN(Ala^3) one of type $i+3\rightarrow i$ in addition to the (Boc)C=O···HN(*i*Pr) of type $i+4\rightarrow i$ closing a 13-membered ring and sharing the same carbonyl acceptor group.

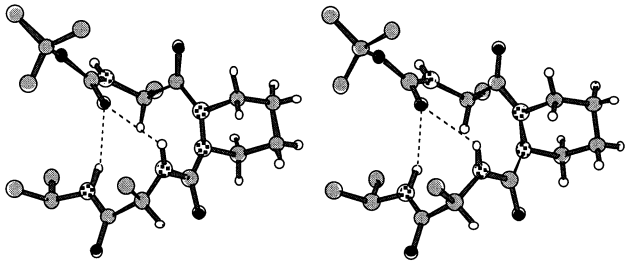

Figure 1. Stereo drawing of the crystal molecular structure of Boc-L-Ala-(N^{α}S)-AzPip-L-Ala-NH*i*Pr derived from X-ray diffraction.

In solution, FTIR spectra (DCM, $c = 5$ mM) present, in the more informative ν(N–H) domain, broad bands highlighting the presence of free (≈ 3428 cm^{-1}) and bonded (≈ 3369 cm^{-1}) N–H vibrators. ^{1}H NMR signals (chloroform, DMSO) are split, thus evidencing the occurrence of two populations of stereoconformers. Isomer **M**,[13] which is 90% populated in $CDCl_3$ and about 45% in DMSO, and isomer **m**[14] have in common the *cis*-disposition of the Ala-AzPip tertiary amide function as attested, in NOESY spectra, by the absence of any spatial proximity between (Ala^1/Ala'^1)C$\underline{H}^{\alpha}$ and (AzPip/AzPip′)C$\underline{H}^{\varepsilon}_2$, but by a medium range connectivity between (Ala^1)C$\underline{H}^{\alpha}$ and (Ala^3)N$\underline{H}$ protons in the case of isomer **M**.

If we look (Fig. 2) at the N$\underline{H}$ proton chemical shift variation as a function of the solvent composition (increasing percentages of DMSO in $CDCl_3$/DMSO solution at constant concentration), both (Ala^3) N$\underline{H}$ and N$\underline{H}$*i*Pr amide protons of isomer **M** are shielded from solvation by solvent molecules, that corroborates the presence of the *i*PrN$\underline{H}$···O=C(Boc), [$i+4\rightarrow i$ type], and especially the stronger (Ala^3)NH···O=C(Boc), [$i+3\rightarrow i$ type], intramolecular hydrogen bonds which stabilize the βVI-like turn. Thus, rotamers of stereoisomer **M** in solution ($CDCl_3$, DMSO) are folded and resemble very closely to the 3D molecular structure observed in the crystalline state. As regards isomer **m**, all of its N$\underline{H}$ protons are freely accessible (solvent effect, Fig. 2) and NOESY spectrum data are in agreement on the *cis*-form of the Ala'^1–AzPip′ amide bond and the slow inversion at the NMR time scale and room temperature of the six-membered diazaheterocycle, partly driven by the pyramidal inversion of the rather sp^3 hybridized N^{α}. By taking into account the previous NMR data and the observed NOE connectivities with respect to the pseudoallylic constraint, which imposes the axial disposition of the N^{α} amide function with the largest distance between the carbonyl oxygen atoms, we can calculate (CS Chem 3D Pro™, CambridgeSoft Corp.) a model rotamer of stereoisomer **m** (Fig. 3). This fulfills, for the best, all of the latter constraints and releases the huge steric constraints between (Ala'^1)Me and N^{α} if a βVI-like turn were present.

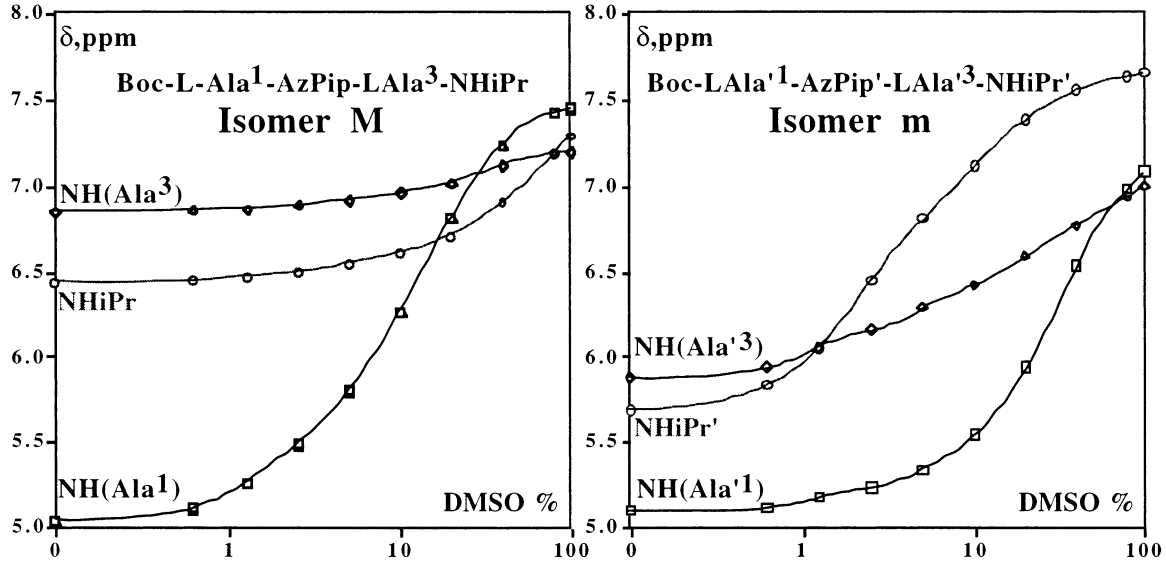

Figure 2. Plots of NH proton chemical shifts in the ^{1}H NMR spectrum of isomer **M** (left) and isomer **m** (right) as a function of increasing percentages of DMSO-d_6 added to the $CDCl_3$ solution (v/v).

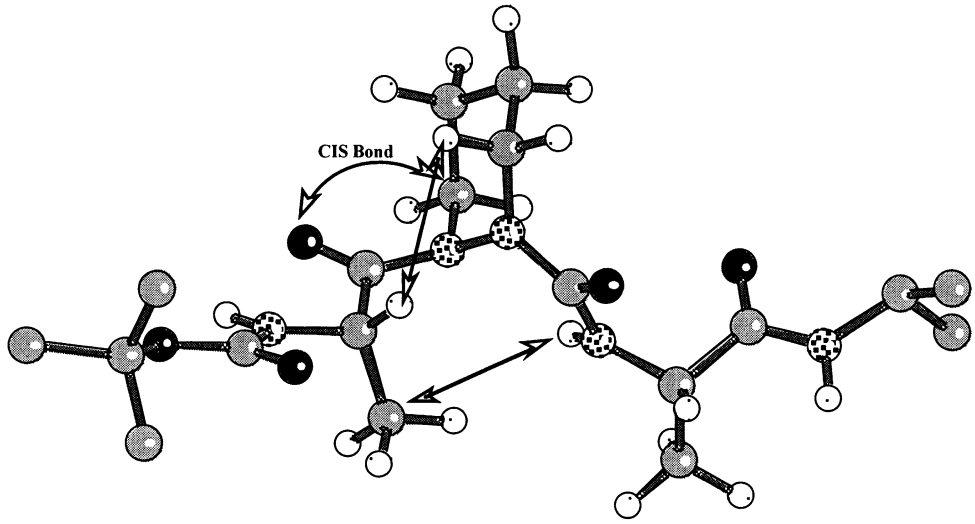

Figure 3. Computer-generated model rotamer of isomer **m** built on the experimental arrowed NOESY constraints.

To sum up, the introduction of an AzPip residue, as a new building block with ϕ and ψ well-defined torsion angles and in a selected place within a peptide sequence, should locally induce a βVI-like turn with the preceding residue as reported for AzPro,[9,10] but with a distinct bulkiness of the cyclic side chain. The synthesis of the parent Pip-tripeptides of different chiral sequences designed with the aim of comparison with the present reported conformational findings is currently being performed and will be reported in due course.

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12. X-Ray crystallographic analysis data: Colorless orthorhombic crystal; crystal size = 0.4×0.3×0.3 mm; cell parameters: $a = 8.90050(12)$, $b = 14.479(3)$, $c = 18.451(3)$ Å; crystal system: $P2_12_12_1$ ($Z = 4$); final R indices [$I > 2\sigma(I)$]: $R^1 = 0.0543$ and $wR^2 = 0.1289$. Torsion angles: Ala[1] [$\phi = -57.8(5)$, $\psi = 136.6(4)$], Ala[3] [$\phi = -75.8(5)$, $\psi = -34.2(6)$], AzPip [$\phi = -113.2(4)$, $\psi = 22.1(5)$]; amide bonds: $\omega_0 = 172.5(4)$, $\omega_1 = 15.7(6)$ *cisoid* form, $\omega_2 = 166.1(4)$, $\omega_3 = -172.3(4)$. Intramolecular hydrogen bonds: (Boc)C=**O**···**N**[3]H(Ala[3]) 2.992(5) Å, (Boc)C=**O**···**N**[4]H(*i*Pr) 3.036(5) Å.
13. Stereoisomer **M** relative populations: 90% ($CDCl_3$), 45% (DMSO); ^{1}H NMR data (600 MHz, DMSO, δ, ppm): 7.42 [1H, d, $J = 5.4$ Hz, NH(Ala[1])], 7.27 [1H, d, $J = 7.2$ Hz, NH(*i*Pr)], 7.19 [1H, d, $J = 7.8$ Hz, NH(Ala[3])], 4.48 [1H, m CH$^{\alpha}$(Ala[1])], 4.30 [1H, br d, $J = 13.2$ Hz, H$^{\varepsilon}$eq(AzPip)], 4.19 [1H, br d, $J = 13.8$ Hz, H$^{\beta}$eq (AzPip)], 3.94 [1H, m, CH$^{\alpha}$(Ala[3])], 3.75 [1H, m CH(*i*Pr)], 2.74 [1H, br t, $J \approx 12$ Hz, H$^{\beta}$ax(AzPip)], 2.64 [1H, br m, H$^{\varepsilon}$ax(AzPip)], 1.67–1.42 [4H, br m, H$^{\gamma}{}_2$ and H$^{\delta}{}_2$ (AxPip′)], 1.37 [9H, s, 3Me(Boc)], 1.26 [3H, d, $J = 7.2$ Hz, Me(Ala[3])], 1.14 [3H, d, $J = 7.2$ Hz, Me(Ala[1])], 1.01 [3H, d, $J = 6.6$ Hz, Me(*i*Pr)], 0.98 [3H, d, $J = 6.6$ Hz, Me(*i*Pr)].
14. Stereoisomer **m** relative populations: 10% ($CDCl_3$), 55% (DMSO); ^{1}H NMR data (600 MHz, DMSO, δ, ppm): 7.63 [1H, d, $J = 7.8$ Hz, NH(*i*Pr′)], 7.05 [1H, d, $J = 7.2$ Hz, NH(Ala′[1])], 6.96 [1H, d, $J = 7.8$ Hz, NH(Ala′[3])], 4.44 [1H, m, CH$^{\alpha}$(Ala′[1])], 4.23 [1H, br d, $J = 12.6$ Hz, H$^{\varepsilon}$eq(AzPip′)], 4.15 [1H, m, CH$^{\alpha}$(Ala′[3])], 4.10 [1H, br d, $J = 13.2$ Hz, H$^{\beta}$eq(AzPip′)], 3.81 [1H, m, CH(*i*Pr′)], 3.11 [1H, br t, $J \approx 11.8$ Hz, H$^{\beta}$ax(AzPip′)], 2.80 [1H, br t, $J \approx 11.4$ Hz, H$^{\varepsilon}$ax(AzPip′)], 1.67–1.42 [4H, br m, H$^{\gamma}{}_2$ and H$^{\delta}{}_2$ (AxPip′)], 1.36 [9H, s, 3Me(Boc′)], 1.20 [3H, d, $J = 6.6$ Hz, Me(Ala′[3])], 1.11 [3H, d, $J = 7.2$ Hz, Me(Ala′[1])], 1.06 [3H, d, $J = 6.6$ Hz, Me(*i*Pr′)], 1.04 [3H, d, $J = 6.6$ Hz, Me(*i*Pr′)].