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Synthesis and Structure of AzAsx-Pro-Containing Aza-Peptides

Frédéric André, Michel Marraud, Guy Boussard*

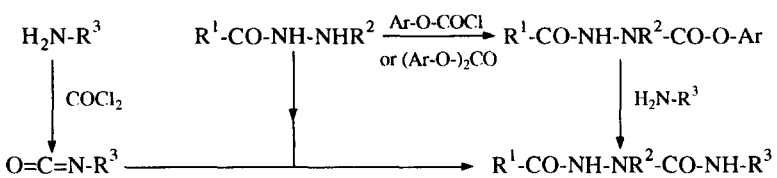
Laboratoire de Chimie Physique Macromoléculaire, associé au CNRS,
ENSIC-INPL, BP 451, 54001 Nancy, France

Claude Didierjean and André Aubry

Laboratoire de Cristallographie et Modélisation des Matériaux Minéraux et Biologiques, associé
au CNRS, Université de Nancy I, BP 239, 54506 Vandœuvre, France

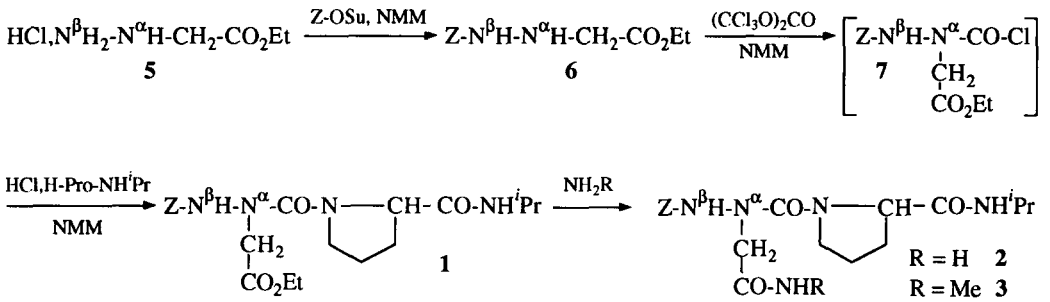
Abstract: *One possible α-modification in peptides is the substitution of a nitrogen for the $C^{\alpha}H$ group. We propose triphosgene as a carbonylating agent for coupling the properly substituted hydrazide to the proline nitrogen to obtain the AzAsx-Pro or AzAla-Pro aza-sequence (Az denotes the N/$C^{\alpha}H$ replacement, and Asx stands for Asn or Asp). The structure of three Z-AzAsx-Pro-$NH^{i}Pr$ and of the Boc-AzAla-Pro-$NH^{i}Pr$ aza dipeptides has been studied in solution by ^{1}H-NMR and IR spectroscopy, and three of them have been investigated in the crystalline state by X-ray diffraction.*

C^{α}-modifications reported in peptide analogues are much more limited than the different types of amide surrogates[1] or the numerous possibilities of side chain modification. In addition to α,β-unsaturation,[2] α-alkylation,[3] or α-hydroxymethylation,[4] substitution of a nitrogen for the $C^{\alpha}H$ group has been also proposed as a way to preserve in the peptide analogues the side chains eventually required for biological activity. The resulting aza-peptides are generally prepared either by condensing a hydrazide with a N-terminus isocyanate, or by action of an aryl chloroformate or carbonate on an urethane-protected hydrazine, before reacting with an amine (Scheme 1).[5,6] The former procedure cannot be applied when proline, in the N-terminus position, must be coupled to an aza-residue, and the latter is not really efficient in this case due to the restrained accessibility of the proline nitrogen.



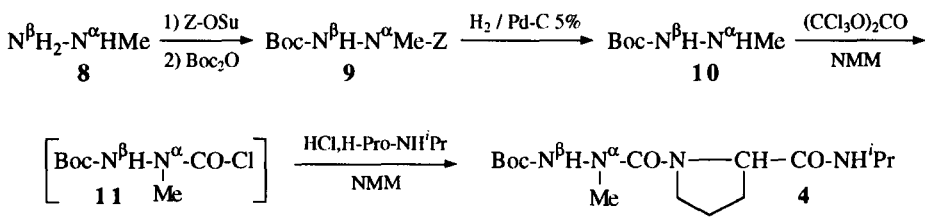
Scheme 1. Synthesis of an aza-peptide fragment using either the isocyanate or the activated ester procedure (Ar : 4-nitro-phenyl in Ar-O-COCl or 2,4-dinitrophenyl in $(Ar\text{-}O\text{-})_2CO$);[5,6] R^1,R^3 : adequate peptide backbone; R^2 : aza-residue side chain

We are engaged in the synthesis of aza-analogues of the Asn-Pro-containing decapeptide sequence which is the main immunogenic region of the acetylcholin receptor in *myasthenia gravis* disease.[7] To this end, we have examined different ways to obtain the AzAsx-Pro sequence where Az denotes the $N^{\alpha}/C^{\alpha}H$ substitution and Asx stands for asparagine (Asn) or aspartic acid (Asp).[8] We propose trichloromethyl carbonate or triphosgene ($Cl_3CO\text{-}CO\text{-}OCCl_3$) as an efficient and easy way to handle carbonylating agent for the synthesis of the AzAsx-Pro sequence (Scheme 2). We have prepared the model dipeptides **1-3** having the general formula Z-AzAsx-Pro-NH^{i}Pr where Asx = Asp(OEt) (**1**), Asn (**2**), or Asn(Me) (**3**). Their structure has been investigated in solution by using NMR and IR spectroscopy, and X-ray diffraction experiments have been carried out on single crystals of derivatives **1** and **3**. Boc-AzAla-Pro-NH^{i}Pr (**4**) has been also examined for comparison.



Scheme 2. Synthesis of the three aza-dipeptides containing an AzAsx-Pro sequence by using triphosgene as the carbonylating agent.

The β-nitrogen of the commercially available derivative **5** (Aldrich, 12,827-9) was selectively protected by action of Z-OSu.[9] Triphosgene reacted with **6** to give an activated intermediate, probably the acid chloride **7**, which was not isolated but rapidly coupled to the amino terminus of a peptide to give the desired aza-peptide motif. Coupling of HCl, H-Pro-NH^{i}Pr resulted in the aza-dipeptide **1** which was transformed into the AzAsn derivative **2** or **3** by action of ammoniac or methylamine. Similarly, **4** was obtained from methylhydrazine, but neither Boc nor Z-introducing agent was found to induce β-regioselectivity.[5,9] Thus it was necessary to protect first and selectively the α-nitrogen by action of Z-OSu (75% yield for the purified $HCl,H_2N\text{-}N(Me)\text{-}Z$ attested by NMR) before treatment by Boc_2O, and then to hydrogenolyse the Z group on Pd-C 5 % (Scheme 3).



Scheme 3. Synthesis of the aza-dipeptide **4** containing the AzAla-Pro sequence

Resolution of the crystal structures of **1**, **3** and **4** by X-ray diffraction shows that, in all three cases, the α-nitrogen is not planar, with a distance of 0.28 Å (**1**), 0.26 Å (**3**) and 0.32 Å (**4**) from the plane defined by the three atoms bonded to it, so that the aza-residue exhibits a D-like chirality. Moreover, the N^{α}-CO bond is 0.3 - 0.6 Å longer than the standard distance of the peptide amide bond, but 0.11 - 0.14 Å shorter than the standard distance of the peptide C^{α}-CO bond.[10] The three molecules adopt very similar structures, of the βII'-like type,[11] folded by an intramolecular hydrogen bond of the i+3 → i type between the ^{i}Pr-NH and (Z/Boc)CO groups, and closing a 10-membered cycle (Fig. 1).

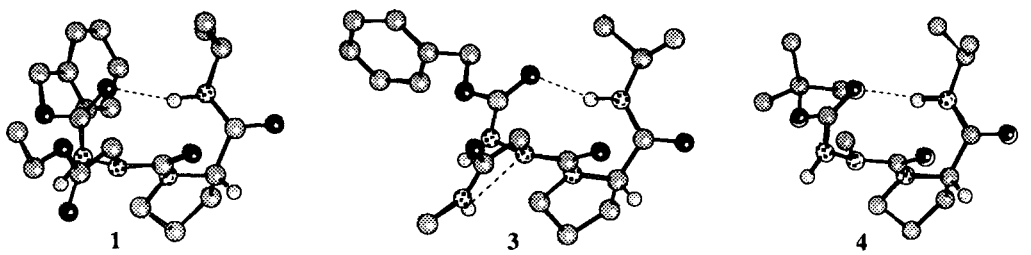


Fig. 1. Crystal molecular structures of Z-AzAsp(OEt)-Pro-NH^{i}Pr (**1**), Z-AzAsn(Me)-Pro-NH^{i}Pr (**3**) and Boc-AzAla-Pro-NH^{i}Pr (**4**) showing the βII'-like backbone folded with an intramolecular i+3 → i hydrogen bond. In **3**, the α-nitrogen is the accepting site from the [AzAsn(Me)]-N^{δ}H donating group.

In solution, all of the azapeptides **1-4** exhibit quite similar IR and ^{1}H-NMR data suggesting very similar structural properties. First, the ROESY correlation between the (AzXaa)NH and (Pro)$C^{\delta}H_2$ proton signals reveals the trans conformation of the AzXaa-Pro amide bond in both $CDCl_3$ and DMSO-d_6 solution. The different sensitivities to solvation of the ^{i}Pr-N<u>H</u> and (AzXaa)N<u>H</u> proton resonances (the shift is 0.32 and 2.97 ppm, respectively, for **4** when going from $CDCl_3$ to DMSO-d_6) argue for the involvement of the former in a stable intramolecular hydrogen bond, and for the free character of the latter. This is also confirmed by the clearly different stretching frequencies of the C-terminal N-H (3361 cm^{-1}) and (AzAla)N-H bond (3419 cm^{-1}) for **4** in DCM. Moreover, the low stretching frequency of (Boc)C=O (1725 cm^{-1} for **4**, instead of 1739 cm^{-1} for Boc-AzAla-NMe_2 in DCM) suggests the existence of the ^{i}Pr-NH to (Boc)CO intramolecular hydrogen bond of the i+3 → i type. This conclusion also applies to the other aza-dipeptides **1-3**.

The moderate downfield shift (1.21 ppm) of the NMR signal for the [AzAsn(Me)]N^{δ}<u>H</u> proton in **3** when going from $CDCl_3$ to DMSO-d_6, together with the existence of two IR absorptions at 3441 and 3290 cm^{-1} for **3** in DCM, indicate the existence of two conformational states for the AzAsn(Me) side chain in which the [AzAsn(Me)]N^{δ}H site is either free or hydrogen bonded. Due to the fact that all of the carbonyl stretching frequencies, except (Z)C=O, are typical of free vibrators, the only possible accepting site is the AzAsn(Me) α-nitrogen, as already observed in the crystal molecular structure of **3**. The same holds true for **2**.

The above experiments show that a proline-preceding aza-residue AzXaa induces a folded structure of the AzXaa-Pro sequence. The acylated AzXaa α-nitrogen is not planar but adopts the D-like chirality so that, with reference to the turns in peptides, the folded structure of the AzXaa-Pro derivatives is comparable with the βII'-turn which is particularly favored for D-Xaa-L-Xbb sequences.[11] The non-planar structure of the AzXaa α-nitrogen allows it to participate in hydrogen bonding as an accepting group.

In peptides, proline is known to favor β-folded structures where it is almost exclusively located in position i+1, as in the crystal structures of many Pro-containing peptides.[11] It is only present in position i+2 of a turn, as it is the case for the folded AzXaa-Pro-containing aza-peptides, when it is preceded by a D-amino acid residue (βII'-turn) or by a cis amide bond (βVI-turn).[11] Moreover, the Asn-Pro sequence is often found to adopt the folded Asx-turn structure where (Asn)C$^{\gamma}$O in position i interacts with the peptide NH in position i+2.[12] Therefore, an aza-residue appears to be capable of inducing a local folded structure, and particularly of prevailing over the strong structural preferences of the proline residue.

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8. The following abbreviations are used: AzXaa, aza analogue of Xaa α-amino acid (N substituted for C$^{\alpha}$H); Boc, *tert*-butyloxycarbonyl; DCM, dichloromethane; DMSO, dimethylsulfoxide; DMSO-d_6, hexadeuterated dimethylsulfoxide; NMM, N-methylmorpholine; Su, succinimidyl; TFA, trifluoroacetic acid; Xaa and Xbb, amino acid residues; Z, benzyloxycarbonyl.
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