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

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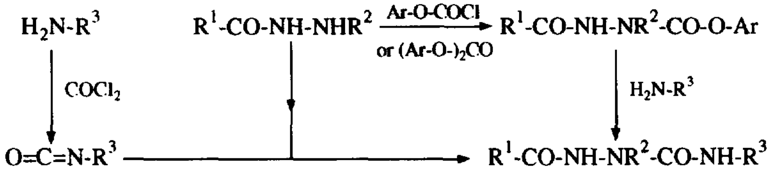
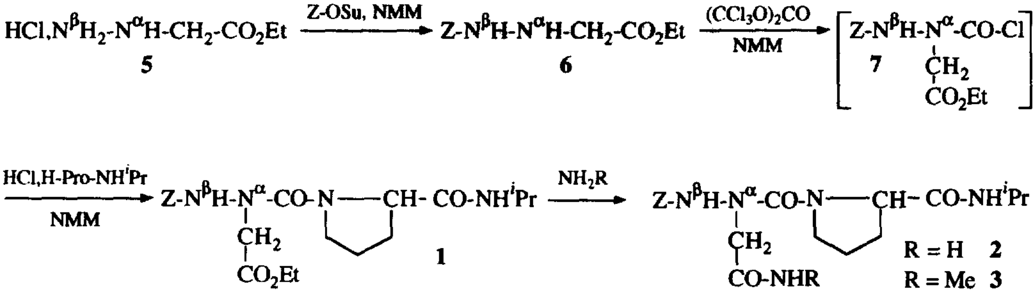
Abstract: *One possible α-modification in peptides is the substitution of a nitrogen for the* $C^{\alpha}H$ *group. We propose triphosgene as a carbonylating agent for coupling the properly substituted hydrazide to the proline nitrogen to obtain the AzAsx-Pro or AzAla-Pro aza-sequence (Az denotes the N/*$C^{\alpha}H$ *replacement, and Asx stands for Asn or Asp). The structure of three Z-AzAsx-Pro-NH*

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.



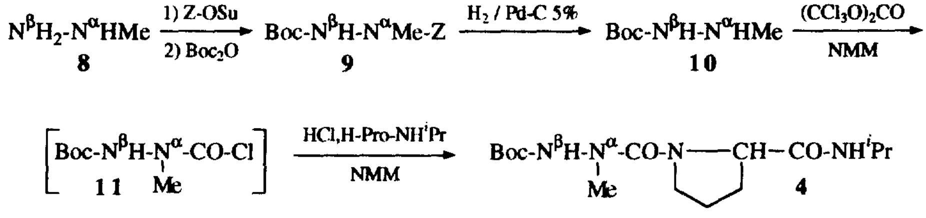
Scheme 1. Synthesis of an aza-peptide fragment using either the isocyanate or the activated ester procedure (Ar : 4-nitro-phenyl in Ar-O-COCl or 2,4-dinitrophenyl in $(Ar$-O-$)_2CO$);[5,6] R^1,R^3 : adequate peptide backbone; R^2 : aza-residue side chain

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$^{\alpha}$-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$^{\alpha}$-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).

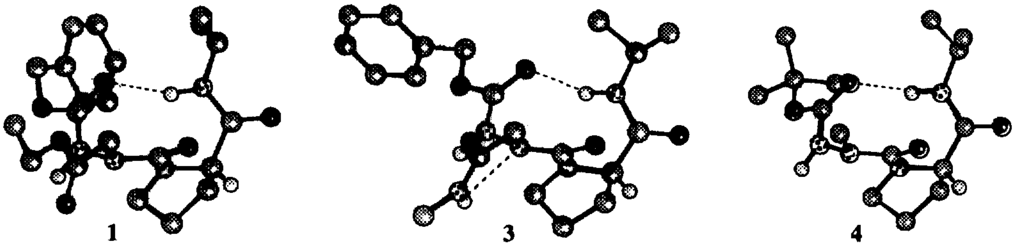


Fig. 1. Crystal molecular structures of Z-AzAsp(OEt)-Pro-NH^{i}Pr (**1**), Z-AzAsn(Me)-Pro-NH^{i}Pr (**3**) and Boc-AzAla-Pro-NH^{i}Pr (**4**) showing the βII'-like backbone folded with an intramolecular i+3 → i hydrogen bond. In **3**, the α-nitrogen is the accepting site from the [AzAsn(Me)]-N$^{\delta}$H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

1. Spatola, A. F. Peptide Backbone Modifications: a Structure-Activity Analysis of Peptides Containing Amide Bond Surrogates, Conformational Constraints, and Related Backbone Replacements. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins;* Weinstein, B. Ed.; Marcel Dekker, Inc.: New York and Basel, 1983; Vol. 7, pp. 267-357.
2. Singh, T. P.; Narula, P.; Patel, H. C. *Acta Crystallogr., Sect. B* **1990**, *46*, 539-545.
3. Toniolo, C. *Janssen Chimica Acta* **1993**, *11*, 10-16.
4. Wieczorek, W.; Bukowska-Strzyzewska, M.; Leplawy, M. T.; Olma, A. *J. Crystallogr. Spectrosc. Res.* **1991**, *21*, 209-215.
5. Gante, J. *Synthesis* **1989**, 405-413.
6. Gray, C. J.; Quibell, M.; Baggett, N.; Hammerle, T. *Int. J. Peptide Protein Res.* **1992**, *40*, 351-362.
7. Tzartos, S. J.; Barkas, T.; Cung, M. T.; Kordossi, A.; Loutrari, H.; Marraud, M.; Papadouli, I.; Sakarellos, C.; Sophianos, D.; Tsikaris, V. *Autoimmunity* **1991**, *8*, 259-270.
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.
9. Dutta, A.S.; Morley, J.S. *J. Chem. Soc. Perkin Trans.1* **1975**, 1712- 1720.
10. Benedetti, E. Structure and Conformation of Peptides as Determined by X-Ray Crystallography. In *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*, Weinstein, B. Ed.; Marcel Dekker, Inc.: New York and Basel, 1983; Vol. 6, pp. 105-184.
11. Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1-109.
12. Abbadi, A.; Mcharfi, M.; Aubry, A.; Prémilat, S.; Boussard, G.; Marraud, M. *J. Am. Chem. Soc.* **1991**, *113*, 2729-2735.

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