Two consecutive aza-amino acids in peptides promote stable β-turn formation in water

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Chenghui Shi,[a &] Isabelle Correia,[b &] Nicolo Tonali,[a] Sandrine Ongeri,*[a] Olivier Lequin*[b]

Studies on the synthetic methodologies and the structural propensity of peptides containing consecutive aza-amino acids are still in their infancy. Here, the synthesis and conformational analysis of tripeptides containing two consecutive aza-amino acids are provided. The demonstration that the type I β-turn folding is induced, even in aqueous media, by the introduction of one or two lateral chains on the diaza-peptide unit is of particular importance for the design of peptidomimetics of biological interest.

Introduction

Currently, more than 80 peptides have been approved as drugs for the treatment of various diseases, showing that peptide drugs have gained astounding interest in pharmaceutical industry.[1–3] As one type of drug entities, peptides have medium molecular weights between small molecules and antibodies as well as unique pharmacological properties including high affinity, high selectivity and low immunogenicity. However, the lower proteolytic stability, membrane permeability and oral bioavailability of natural peptides still prevent their rapid development in drug discovery despite notable recent advances in peptide delivery technology.[1] Moreover, small peptides are rather flexible and only rarely adopt stable conformations, which may preclude their ability to interact with protein targets or to inhibit protein-protein interactions (PPIs), which involve generally well-defined secondary structures in the hot-spot sequences of interaction. Modified peptides and peptidomimetics represent an alternative to avoid these limitations of peptides.[4] The field of peptide-based foldamers adopting stable conformations able to mimic secondary structures represent also recent promising therapeutic opportunities in particular to target PPIs.[5,6]

Azapeptides represent one type of peptidomimetics in which one or more of the residues are substituted by semicarbazide. It has been proven that azapeptides usually show higher selectivity and proteolytic stability than that of parent peptides.[7–9]

Given the repulsion between adjacent nitrogen lone pairs and the planar structure of the urea moiety, the preferential dihedral angle values of aza-amino acid residue (ϕ, ψ) are close to (±90±30°, 0±30°) or (±90±30°, 180±30°).[10,11] This narrow range of dihedral angle values explains why peptides containing a single aza-amino acid adopt a β-turn structure.[12–17] It has also been demonstrated that this non-extended structure can disrupt the β-hairpin conformation of peptides even containing a D-Pro-Gly template in their sequence.[18] The few reported computational and experimental studies of peptides (mainly di and tripeptides) containing one aza-amino acid residue have shown that this class usually adopts a type II β-turn conformation in solid-state or in organic solvent ($CDCl_3$ or DMSO).[15,16,19,20] However, only non-classical azapeptides encompassing a *N*-amidothiourea moiety adopt a type II β-turn structure in water thanks to the enhanced H bond donor ability of the thioureido NH group.[21] To our knowledge, only one statement, made by us, on the more challenging synthesis and conformation study of azapeptides containing two sequential aza-amino acids has been reported so far.[22,23] In this conformational study, we observed that diaza-tripeptide **1** containing two aza-amino acids at its *N*-terminus (aza/aza/α pseudotripeptide, Fig. 1) is prone to adopt a single type I β-turn or two consecutive type I β-turn conformations in methanol,[23] whereas the corresponding natural tripeptide shows highly disordered conformation. We exploited this property to synthesize the aza/aza/α/aza/aza/α pseudohexapeptide **2** composed of two diaza-tripeptide units (Fig. 1) which was shown to adopt highly folded conformations made of repeated β-turn conformations or even a full-length 3_{10}-helix structure. This suggests that the repeat mode aza/aza/α in longer peptide sequences has the propensity to induce helical structuration in peptides. Notably, we found that the insertion of side chains on both aza-amino acids had a beneficial effect to stabilize the turn conformation of the diaza-tripeptides. Indeed, diaza-tripeptide **3** with two azaGly at its *N*-terminus (Fig. 1) showed higher conformational flexibility by NMR and MM/DFT calculations than diaza-tripeptide **1** bearing azaVal-azaAla, oscillating from a few helical populations to extended structures. On the basis of these findings, we have the aim to use these diaza-peptide units in peptidomimetic foldamers targeting protein-protein interactions involving turn or helical secondary structures. For that purpose, we had to further investigate two new factors: (1) the ability of these units to retain their turn propensity in water, which is an even more challenging solvent than methanol for maintaining intramolecular hydrogen bonding and stable conformations, because solubility in water is mandatory for biological evaluations; and (2) the influence

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Electronic Supplementary Information (ESI) available: experimental section and characterization, NMR data, LC-MS spectra.

of the nature of the side chain of the aza-amino acid residues on the conformation.

Because of the low aqueous solubility of the diaza-tripeptides **1** and **2** mentioned above, their NMR analysis in water was not possible. Herein we propose to introduce more hydrophilic aza- and natural amino acids in order to improve the aqueous solubility of the target diaza-tripeptides, thereby making possible their NMR analysis in water. Thus, azaLys was used instead of azaVal at the *N*-terminus and, the natural amino acid Val was replaced by Ser at the *C*-terminus. The *N*-Boc protection was replaced by an acetyl group to further decrease the hydrophobic character and better mimic a neighboring amino acid residue. Meanwhile, we used three different aza-amino acids, including azaGly, azaAla and azaLeu as the central residue in diaza-tripeptides **4**, **5**, and **6** respectively, in order to investigate the influence of the presence or not, and the nature of the side chain on the conformation of diaza-tripeptides.

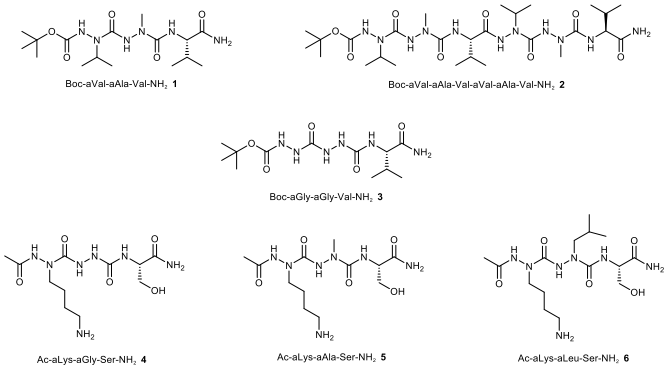
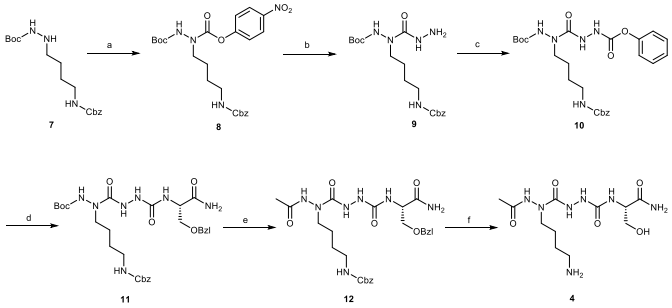


Fig. 1 Structure of the previously reported aza/aza/α pseudotripeptides and aza/aza/α/aza/aza/α pseudohexapeptide **1**-**3**[23] and of the newly synthesized aza/aza/α pseudotripeptides **4**-**6**. Aza-amino acids are designated with the "a" letter preceding the three-letter code.

Results and discussion

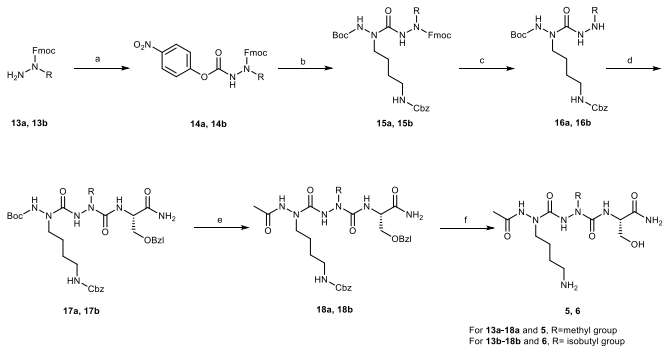
For the synthesis of peptide **4** without side chain on the central aza-amino acid, we used the same procedure described in our previous work (Scheme 1).[22] Compound **7** was activated by 4-nitrophenyl chloroformate to give compound **8** (89% yield), which reacted with hydrazine monohydrate to give compound **9** with a yield of 68%. **9** was activated by phenyl chloroformate to give compound **10** (98% yield), which was coupled with Ser(Bzl)-NH_2 (synthesis described in ESI) to provide compound **11** in a satisfactory yield of 71%. The Boc group of **11** was removed using a solution of HCl in dioxane. The free amine was immediately acetylated using acetic anhydride in the presence of pyridine as base to afford compound **12** in a good yield of 75%. Finally, both Bzl and Cbz groups were simultaneously removed from **12** by catalytic hydrogenation using Pd/C as catalyst to get compound **4** with a yield of 64%.

As reported in our previous work,[22] the active carbamate of protected 2-alkyl-hydrazines could not couple with protected 1-alkyl-hydrazines but the active carbamate of protected 1-alkyl-hydrazine is able to react with protected 2-alkyl-hydrazine, so we changed the strategy to synthesize compounds **5** and **6** which have side chains on their central aza-amino acid (Scheme 2). First, the activation of the protected 1-alkyl-hydrazines **13a** and **13b** using 4-nitrophenyl chloroformate afforded compounds **14a** and **14b** (76% and 59% yields respectively), which reacted with **7** to give diaza-peptide moieties **15a** and **15b** with a good yield of 79%. Fmoc groups were cleaved from **15a** and **15b** using 20% piperidine/DMF to provide **16a** and **16b** in satisfactory yields (91%).



Scheme 1 Synthesis of diaza-tripeptide **4**. *Reagents and conditions:* (a) 4-nitrophenyl chloroformate, pyridine, DCM, r.t, overnight, 89%; (b) hydrazine monohydrate, MeOH, r.t, overnight, 68%; (c) phenyl chloroformate, pyridine, THF, r.t, 1 h, 98%; (d) Ser(Bzl)-NH_2, TEA, ACN, r.t, 24 h, 71%; (e) i: 4 M HCl in dioxane, r.t, 3 h; ii: acetic anhydride, pyridine, DCM, r.t, overnight, 75%; (f) H_2, Pd/C 10%, acetic acid/H_2O, r.t, overnight, 64%.

Given the bulkiness of alkyl groups on the nitrogen which must be activated, active carbamates of **16a** and **16b** were less active to react with Ser(Bzl)-NH_2. Here, triphosgene was used to activate **16a** and **16b** to provide more active acyl chloride intermediates, followed by the addition of Ser(Bzl)-NH_2 to get tripeptides **17a** and **17b** in 46% and 54% yields respectively. Compounds **18a** and **18b** were then obtained by the replacement of the Boc group by an acetyl group using the same operating protocol as for the synthesis of **12** (75% and 76% yields respectively). Finally, the target diaza-tripeptides **5** and **6** were afforded by the simultaneous cleavage of the Bzl and Cbz groups using catalytic hydrogenation (58% and 59% yields respectively).



Scheme 2 Synthesis of diaza-tripeptide **5** and **6**. *Reagents and conditions:* (a) 4-nitrophenyl chloroformate, pyridine, DCM, r.t, overnight, 76% (**14a**) and 59% (**14b**); (b) compound **7**, DIPEA, ACN, r.t, 5 h, 79%; (c) 20% piperidine/DMF, r.t, 30 min, 91%; (d) i: triphosgene, DIPEA, DCM, r.t, 20 min; ii: Ser(Bzl)-NH_2, DIPEA, DCM, r.t, overnight, 46% (**17a**) and 54% (**17b**) for two steps; (e) i: 4 M HCl in dioxane, r.t, 3 h; ii: acetic anhydride, pyridine, DCM, r.t, overnight, 75% (**18a**) and 76% (**18b**) for two steps; (f) H_2, Pd/C 10%, acetic acid/H_2O, r.t, overnight, 58% (**5**) and 59% (**6**).

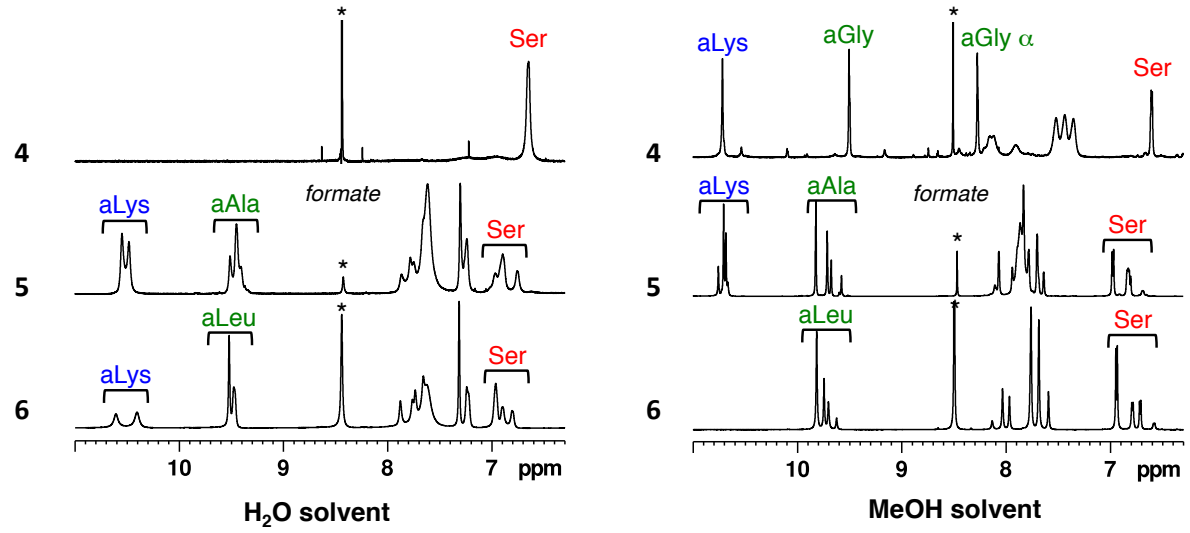


Fig. 2 1D ^{1}H NMR spectra of diaza-tripeptides **4**-**6** in water and in methanol, showing the region of HN resonances. Spectra were recorded at 278 K in water and at 228 K in methanol. The peak labelled with an asterisk corresponds to formate impurity. Unlabelled peaks correspond to carboxamide NH_2, side chain aLys NH_3^+ or impurities.

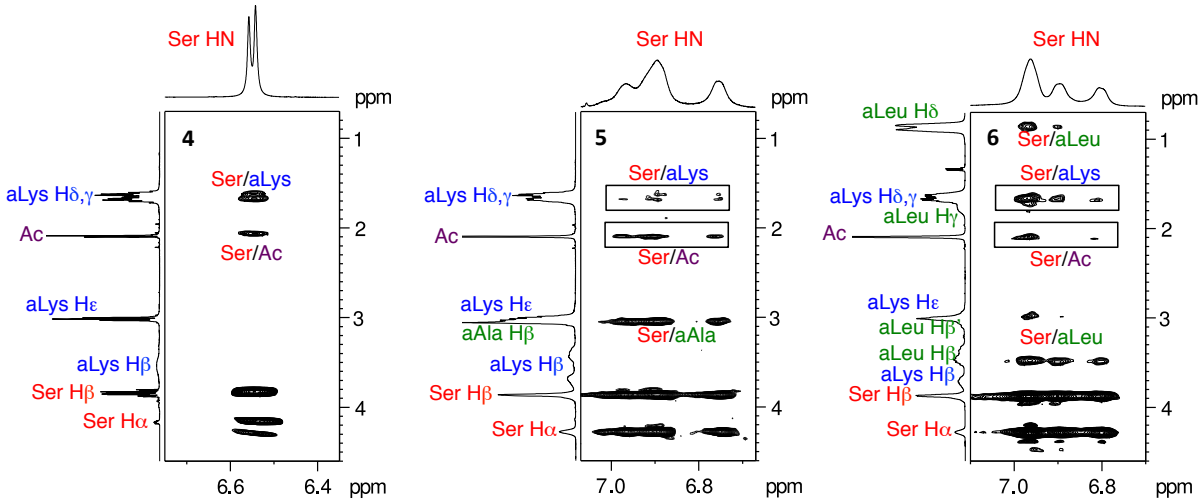


Fig. 3 2D ^{1}H-^{1}H ROESY spectra of diaza-tripeptides **4**-**6** in water showing the correlations of Ser HN proton with side chain aliphatic protons. Spectra were recorded at 308 K for **4**, and 278 K for **5** and **6**.

Table 1 HN proton temperature coefficient $\Delta\delta_{HN}/\Delta T$ (ppb/K) for compounds **4**-**6** in methanol and water. The range of temperature coefficients obtained for the different conformational isomers before signal coalescence is indicated in brackets.

Compound	Solvant	T (K)	aLys1	aGly/aAla/aLeu2	Ser3	NH_2 Z	NH_2 E
4	MeOH	228–251	–5.8	–4.6, –7.9 (α)	–1.4	n.d.	n.d.
	H_2O	278–308	n.d.	n.d.	–3.4	n.d.	n.d.
5	MeOH	228–251	[–5.5, –4.8]	[–4.8, –4.0]	[–3.3, –2.2]	[–7.8, –6.8]	[–5.8, –4.9]
	H_2O	278–288	–7.3	–5.9	[–4.7, –3.2]	–5.5	-
6	MeOH	228–251	n.d.	[–5.0, –4.3]	[–2.9, –2.2]	[–7.4, –6.4]	[–5.3, –4.8]
	H_2O	278–298	[–8.8, –7.1]	–7.3	[–7.4, –4.3]	[–6.6, –5.8]	[–7.8, –4.0]

n.d., non-detected proton due to exchange

As expected, the three diaza-tripeptides **4**-**6** showed good solubility in aqueous solution (greater than 10 mM). We thus investigated their structures in water and compared them when in methanol solvent (Fig. 2), serving as a reference to our previous published work.[23] Different NMR parameters were analysed to assess the hydrogen bonding and folding propensities of diaza-tripeptides, in particular the temperature dependency of the amide proton chemical shift (temperature coefficient $\Delta\delta_{HN}/\Delta T$) and the through-space dipolar ^{1}H-^{1}H ROE correlations.

Diaza-tripeptide Ac-aLys-aGly-Ser-NH_2 (**4**) shows evidence of intramolecular hydrogen bond formation in both methanol and water solvents. Indeed, the Ser amide proton chemical shift exhibits a low temperature dependency in comparison with other NH protons, the temperature coefficient being –1.4 ppb/K and –3.4 ppb/K in methanol and water, respectively (Table 1). These coefficients are well below the threshold value of –4.6 ppb/K, which is usually an indicator to consider hydrogen bond engagement.[24] In addition, a ROE correlation is observed in both solvents between the amide proton of Ser and the methyl protons of the N-terminal acetyl group (Fig. 3, S3, S7), supporting the formation of a β-turn folded structure, stabilized by a *i*, *i*+3 hydrogen bond between the acetyl CO group and the Ser NH group. NMR structure calculations show that two turn conformations are compatible with the NMR data. Indeed, the diaza-peptide block can adopt either negative or positive ϕ angle values around ± 90°, leading to type I or I' β-turns, respectively (Fig. 4A). Such conformational equilibrium between these two β turn types had been previously described for diaza-tripeptide **1** in methanol,[23] and was found to occur in a slow exchange regime on the NMR time scale. Given that a single set of chemical shifts is observed for diaza-tripeptide **4** in both solvents at room temperature (Fig. 2), it is likely that the absence of side chain in the azaGly residue enables faster rotation around the N–Nα bond, leading to a fast exchange regime on the NMR time scale at room temperature. The appearance of slowly interconverting conformational isomers can only be detected for diaza-tripeptide **4** when cooling down to very low temperatures (228 K) in methanol (Fig. S5).

Additional *i*, *i*+2 ROEs were also observed between the Ser amide proton and the side chain methylenic protons (CH_2 γ, δ, ε) of azaLys (Fig. 3). These correlations can be accounted for a compact conformation in which azaLys side chain folds back onto Ser residue. The folding of aLys side chain is further suggested by the observation of a significant chemical shift difference between the two diastereotopic CH_2 ε protons in methanol (0.08 ppm difference), which is usually not the case when a Lys side chain is fully exposed to solvent. Structure calculations indicate that this compact orientation could be stabilized by a hydrogen bond between the ammonium group of azaLys and either the hydroxyl or carbonyl group of Ser (Fig. 4A).

The NMR spectra of diaza-tripeptides Ac-aLys-aAla-Ser-NH_2 (**5**) and Ac-aLys-aLeu-Ser-NH_2 (**6**) displayed much higher complexity than diaza-tripeptide **4**, owing to the presence of chemical shift heterogeneity. As a matter of fact, different sets of resonances could be observed for the residues of **5** and **6**, in water and in methanol (Fig. 2), a situation reminiscent of that observed for diaza-tripeptide **1** in methanol. The NMR spectra of the previously studied diaza-tripeptide **1** in methanol showed two major conformational isomers at room temperature (~45% population) which were ascribed to sign inversion of the ϕ dihedral angle, as aforementioned. Two other minor isomers (~5% population) were ascribed to N-terminal Boc *cis*/*trans* isomerization. The situation differs in diaza-tripeptides **5** and **6**, in so far as four forms with closer populations could be evidenced. This prompted us to run temperature variation experiments to further investigate the origin of the chemical shift heterogeneity. Experiments in water at low temperature show that the different forms have close chemical shifts. The coalescence of proton signals arises around 298 K for diaza-tripeptide **5** (Fig. S12) and above 308 K for diaza-tripeptide **6** (Fig. S20). Experiments in methanol enabled us to work at much lower temperatures, cooling down to 228 K. At this temperature, proton spectra exhibited much sharper and resolved peaks, providing better discrimination of the 4 forms. The peak integrations on 1D ^{1}H spectra yielded populations of 42%, 27%, 19% and 12% for diaza-peptide **5** (Fig. S13) and 55%, 22%, 17% and 6% for diaza-peptide **6** (Fig. S21). Exchange cross-peaks were observed on 2D ROESY spectra between the different forms (Fig. S15 and S23), unambiguously proving that the four forms correspond to conformational isomers that interconvert during the mixing time of 2D experiments (250 ms). The chemical shifts of NH protons show linear temperature dependency over the 228–251 K temperature range and the amide protons of Ser residues in the 4 different isomers have the lowest temperature coefficients, between –2.2 and –3.3 ppb/K, supporting their engagement in intramolecular hydrogen bonds (Table 1). The temperature coefficients where much more difficult to measure in water owing to spectral overlap. Partial data could be obtained either after peak coalescence or for isolated HN peaks before coalescence. They indicate that Ser HN proton has the smallest temperature dependency in comparison with other HN protons.

The analysis of ROESY spectra in methanol at low temperature (Fig. S15 and S23) shows diagnostic ROEs of β-turn folding, in particular the characteristic *i*, *i*+3 correlation between Ser HN proton and acetyl protons. Strong sequential *i*, *i*+1 HN-HN correlations also indicate that the aza-amino acids adopt (ϕ, ψ) angles values around (±90°, 0°). Finally, ROEs are observed between Ser HN proton and aLys methylenic side chain protons, as in diaza-tripeptide **4**. All these ROEs are also observed at higher temperature, in both methanol and water (Fig. 3, S11 and S19), where partial coalescence between the different forms occurred. Intriguingly, it was not possible to detect distinctive ROEs between the different forms at low temperature that would provide clues about the nature of the conformational isomerism affecting the different conformers. Importantly, exchange peaks are observed even at the lowest temperature of study in methanol. Therefore,

magnetization transfers due to conformational exchange induce some contamination of the through-space dipolar ROE correlations, which may complicate the discrimination of distinctive conformational features. Of note, the sign inversion of ϕ angle between +90 and –90° in the aza-amino acids yields mirror-imaged backbone conformations within the diaza-amino acid unit and consequently gives rise to similar sets of ROEs.

Structure calculations based on NMR restraints show actually two possible turn conformations for diaza-tripeptides **5** and **6** (Figures 4B, 4C) corresponding to types I and I', as for diaza-tripeptides **1**[23] and **4** (Figure 4A). However, we had previously shown for diaza-tripeptide **1** that a second 10-membered β-turn could be formed if the first turn adopts a type I conformation. This second turn was stabilized by a hydrogen bond involving the *C*-terminal primary amide group and the carbonyl group of the first aza-amino acid residue. Although this double turn conformation is compatible with the observed ROEs between Ser HN and carboxamide protons, the average temperature coefficient of carboxamide protons in the three diaza-tripeptides **4**-**6** suggest that this hydrogen bond is not as stable as the acetyl-Ser H-bond.

An unusual N_{amide} ⋯ H-N_{amide} hydrogen bond has been proposed to stabilize the backbone conformation of aza-amino acids with (ϕ, ψ) angles close to (±90±30°, 0±30°).[25,26] In the diaza-tripeptide β-turns, two N_{amide} ⋯ H-N_{amide} hydrogen bonds are formed between consecutive residues (N1⋯ H-N2 and N2⋯ H-N3) which could further contribute to the β-turn stabilization. It should be noted that these atypical hydrogen bonds do not seem to have a strong effect on amide proton temperature coefficients in methanol and water, in comparison with conventional C=O ⋯ H-N hydrogen bonds. This can be shown by comparing the temperature coefficients of HN2 and HN3 in the diaza-tripeptides **1**[23] and **4**-**6** (Table 1).

The particular feature of the studied diaza-tripeptides **5** and **6** is the presence of conformational isomerism that severely complicates NMR analyses, whatever the solvent. We show that the presence and the nature of the aza-amino acid side chain have an effect on the conformational exchange. Diaza-tripeptide **4** displays fast exchange at room temperature while diaza-tripeptides **5** and **6** bearing a side chain on the second aza-amino acid exhibit slow exchange under the same conditions. The bulkiness of the substituent (isobutyl *vs* methyl) also slows down the kinetics of interconversion, as indicated by differences in coalescence temperatures. One source of postulated conformational isomerism is the slow rotation around the N–Nα bond in substituted aza-amino acids (ϕ = ±90°), yielding 4 possible conformational isomers. Diaza-peptide units having opposite signs of their ϕ angles are not compatible with β-turn formation. As all conformational isomers show H-bonded β-turn features, this implies that the sign inversion of ϕ angle concerns both aza-amino acids simultaneously, yielding only two conformational isomers, i.e. (ϕ_1,ϕ_2) = (+90°,+90°) or (–90°,–90°). Another possibility of conformational exchange would be *E*/*Z* isomerism around amide bonds or Nα-C' bond (ψ angle). Indeed, we had previously suggested in the case of compound **1** that E/Z interconversion of Boc carbamate groups could be a source of conformational isomerism. In the case of *N*-terminal acetyl groups preceding aza-amino acids, *cis*-*trans* isomerism has also been reported for the Ac-azaGly amide bond.[25] Concerning the rotation around the Nα-C' bond, sequential HN/HN ROEs are only compatible with conformations having ψ angles around 0°. Finally, the Nα atom in aza-amino acids has a small pyramidal character, offering the possibility to dynamically orient the side chain in two opposite configurations. Lubell et al. have reported the adaptive chirality of Nα center in aza-amino acids that could be achiral or exhibit either L or D-like chirality.[27] The X-ray structure and DFT analyses of diaza-tripeptide **1** revealed that the pyramidal character of the Nα atom is very weak.[23] Of note, a slow inversion of Nα chiral center has not been observed in peptides incorporating one aza-amino acid unit by NMR analyses.[25,27,28] Nevertheless, it is possible that the situation may differ in peptides incorporating two consecutive aza-amino acids and which are conformationally stabilized.

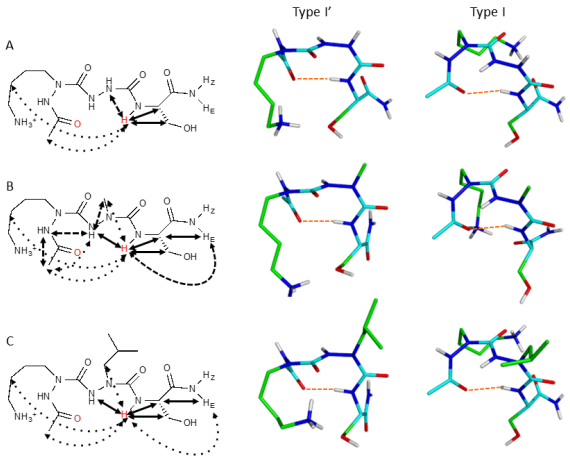


Fig. 4 NMR structures of diaza-tripeptides **4** (A), **5** (B) and **6** (C). For each peptide, two low-energy NMR structures were selected to represent type I' and type I β-turn conformations. The diagram of ROE correlations is shown on the left. Strong, medium and weak ROE intensities are represented by solid, dashed and dotted lines, respectively.

Conclusion

This work confirms our previous finding that unlike peptides containing exclusively α-amino acids, the conformational restriction conferred by the insertion of two consecutive aza-amino acids in aza/aza/α pseudotripeptides endows them with a strong tendency to adopt 10-membered type I β-turn conformations. The introduction of polar lateral chains on the *N*-terminus aza-amino acid (azaLys) and on the natural amino acid at the *C*-terminus (Ser), as well as the replacement of the N-Boc by an acetyl group dramatically increased the solubility in water of compounds **4**-**6** compared to compound **1**. Although the NMR conformational studies of azapeptides **4**-**6** were hampered by conformational exchange, they reveal striking similarities of the NMR spectra recorded in water and methanol conditions, in terms of HN chemical shifts, HN temperature coefficients, and sets of ROE correlations. Therefore, the conformational space of diaza-tripeptides is largely comparable in both solvents, indicating that stable turn conformations are populated even in the more

challenging aqueous solvent. Diaza-tripeptides **4**-**6** have a strong propensity to adopt a 10-membered β-turn, through a *i*, *i*+3 hydrogen bond between the carbonyl group of the acetyl moiety and the amide proton of Ser. Two backbone conformations can be adopted in the diaza-amino acid segment corresponding to types I and I' β-turns. This is of particular interest as the insertion of only one aza-amino acid induces a type II β-turn, that is less abundant in protein structures (19%) than the type I β-turn (46%)[29] and this conformation is stable only in solid-state or in organic solvent but unstable in aqueous solution.[15,16,19,20]

However, the nature of the side chain of the aza-amino acid and amino acid residues has an influence on the stability of the double turn conformation. Our previous conformational study[23] of compound **3** containing two azaGly units had shown that the presence of side chains on both aza-amino acids had a beneficial effect in stabilizing the turn conformation of the diaza-tripeptides. This new report on Ac-aLys-aGly-SerCONH$_2$ **4**, which has similar β-turn propensity as diaza-tripeptides **5** and **6**, indicates that the presence of a single side chain on the first aza-amino acid residue is sufficient to promote turn stabilization.

The presence of conformational isomerism with provision of four conformers observed in the case of diaza-tripeptides **5** and **6** could be explained by the restricted pyramidal inversion of a nitrogen adopting a sp3 hybridization state, that could thus be a source of chirality producing two diastereomers. While this chirality has not been observed in previously reported NMR studies of peptides having one aza-amino acid,[25,27,28] two consecutive aza-amino acids could further constrain and slow down the nitrogen inversion. The presence of polar side chains allowing hydrogen bond between the ammonium group of aLys and either the hydroxyl or carbonyl group of Ser could also further slow down the isomerization.

Overall, this work provides an additional proof that foldamers based on diaza-amino acids units might resolve a major issue in the use of peptides as drugs, by stabilizing turns and promoting helical conformations unlike natural peptides, while having the ability to maintain the selectivity thanks to the lateral chains. The next exciting step will be to investigate the ability of longer peptides containing these diaza-amino acids units to target PPIs involving turn or helical secondary structures[30,31] or to stabilize the helical conformation of intrinsically disordered proteins (IDPs) such as amyloid proteins.[32,33] PPIs[34] and IDPs[35,36] represent two of the most challenging drug discovery targets nowadays. Furthermore, our demonstration of the folding propensity in water of peptides incorporating diaza-peptide units suggest the ability of these new peptidomimetic foldamers to be active in biologically compatible media.

Experimental section

General information

Usual solvents were purchased from commercial sources and DCM was dried and distilled over CaH_2. Thin-layer chromatography (TLC) analyses were performed on silica gel 60 F250 (0.26 mm thickness) plates. The plates were visualized with UV light (λ = 254 nm) or stained by a 4 % solution of phosphomolybdic acid or ninhydrin in EtOH. NMR spectra were recorded on an Ultrafield Bruker AVANCE 300 (^{1}H, 300 MHz, ^{13}C, 75 MHz) or on a Bruker AVANCE 400 (^{1}H, 400 MHz, ^{13}C, 100 MHz). Chemical shifts δ are in ppm with the solvent resonance as the internal standard (^{1}H NMR, residual protiated solvent in $CDCl_3$: δ = 7.26 ppm, in CD_3OD and CD_3OH: δ = 3.31 ppm; ^{13}C NMR, $CDCl_3$: δ = 77.16 ppm, CD_3OD and CD_3OH: δ = 49.00 ppm), and the following abbreviations are used: singlet (s), doublet (d), doublet of doublet (dd), triplet (t), quintuplet (qt), multiplet (m), broad multiplet (bm), and broad singlet (brs). Mass spectra were obtained using a Bruker Esquire electrospray ionization apparatus. HRMS were obtained using a TOF LCT Premier apparatus (Waters) with an electrospray ionization source.

The purity of compounds was determined by HPLC-MS on Agilent 1260 Infinity. Column: ATLANTIS T3 column (C18, 2.1 x 150mm-3µm), mobile phase: ACN/H_2O + 0.1% TFA (gradient 1-30% in 15 or 20 min). Preparative HPLC were performed on Agilent 1260 Infinity II. Column: Pursuit (C18 10 x 250µm-5µm), mobile phase: ACN/H_2O + 0.1% formic acid.

Compounds **7**, **8**, **9**, **10** and **22** were prepared as reported in our previous literature[22] and **13a** was synthesized according to published method.[37] The synthesis of **13b** and Ser(Bzl)-NH$_2$ are described in ESI. The protocols of synthesis and the characterization of intermediates **11**-**12**, **14a/b**-**18a/b** and of the final diaza-tripeptides **4**-**6** are detailed in ESI.

NMR conformational studies

NMR experiments conducted over a temperature range from 273 K to 308 K were acquired on a Bruker Avance 500 MHz NMR spectrometer equipped with a cryogenic TCI probe. ^{1}H and ^{13}C resonance assignments were obtained from the analysis of 2D ^{1}H-^{1}H TOCSY (mixing time of 60 ms), 2D ^{1}H-^{1}H ROESY (mixing time of 250 ms), 2D ^{1}H-^{13}C HSQC, and 2D ^{1}H-^{13}C HMBC spectra. Low temperature NMR experiments (225-250 K) were acquired on a Bruker Avance 600 MHz NMR spectrometer equipped with a room temperature TBI probe. NMR data were processed with TopSpin 3.6 and analysed with TopSpin 3.6 or NMRFAM-SPARKY program. For NMR studies in water, about 2.5 mg of compounds **4**-**6** were dissolved in 0.6 mL of H_2O/D_2O (90/10 v/v), pH=5.5. DSS (sodium 4,4-dimethyl-4-silapentane-1-sulfonate, 0.1 mM) was used for chemical shift calibration. For NMR experiments in methanol, 4 to 6 mg of compounds **4**-**6** were dissolved in 0.6 mL of CD_3OH. ^{1}H and ^{13}C chemical shifts were referenced to the methanol solvent signal (residual protonated CHD_2OH at 3.31 ppm and deuterated $^{13}CD_3OH$ at 49.1 ppm, respectively). We checked that the pseudopeptides **4**-**6** did not self-assemble in aqueous solution or in methanol in this concentration range, by assessing the absence of concentration dependency on chemical shifts upon a 10 folds dilution for samples in water or a 20 folds dilution for samples in methanol.

NMR structure calculations

Structures were calculated using Amber14 program and ff14SB forcefield, as previously described.[23] Structures were refined using GBSA solvation model. Aza-amino acids parameterization was made with Antechamber, using gaff forcefield atom types and partial charges were calculated with AM1-BCC. 2D ROESY cross-peaks were integrated using NMRFAM-SPARKY. Three classes of distance restraints were defined, corresponding to upper limit distances of 3.0, 3.8 and 5.0 Å.

Author contributions

C. Shi : Conducting the research and investigation process in the chemical synthesis, specifically performing the experiments, and data collection

I. Correia : Conducting the research and investigation process in the NMR conformational studies, specifically performing the experiments, and data collection

N. Tonali : Supervision and verification of the overall reproducibility of results and experiments in chemical synthesis

S. Ongeri : Oversight and leadership responsibility for the research activity planning and execution

O. Lequin : formulation and evolution of overarching research goals and aims. Supervision and verification of the overall reproducibility of results and NMR structure calculation

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 M. Muttenthaler, G. F. King, D. J. Adams and P. F. Alewood, *Nat. Rev. Drug Discov.*, 2021, **20**, 309–325.

2 A. F. B. Räder, M. Weinmüller, F. Reichart, A. Schumacher-Klinger, S. Merzbach, C. Gilon, A. Hoffman and H. Kessler, *Angew. Chem., Int. Ed.*, 2018, **57**, 14414–14438.

3 J. L. Lau and M. K. Dunn, *Bioorg. Med. Chem.*, 2018, **26**, 2700–2707.

4 E. Lenci and A. Trabocchi, *Chem. Soc. Rev.*, 2020, **49**, 3262–3277.

5 R. Gopalakrishnan, A. I. Frolov, L. Knerr, W. J. Drury and E. Valeur, *J. Med. Chem.*, 2016, **59**, 9599–9621.

6 I. M. Mándity and F. Fülöp, *Expert Opin. Drug Discov.*, 2015, **10**, 1163–1177.

7 M. Galibert, M. Wartenberg, F. Lecaille, A. Saidi, S. Mavel, A. Joulin-Giet, B. Korkmaz, D. Brömme, V. Aucagne, A. F. Delmas and G. Lalmanach, *Eur. J. Med. Chem.*, 2018, **144**, 201–210.

8 C. Proulx, D. Sabatino, R. Hopewell, J. Spiegel, Y. García Ramos and W. D. Lubell, *Future Med. Chem.*, 2011, **3**, 1139–1164.

9 Y. Tal-Gan, N. S. Freeman, S. Klein, A. Levitzki and C. Gilon, *Chem. Biol. Drug Des.*, 2011, **78**, 887–892.

10 H.-J. Lee, J.-W. Song, Y.-S. Choi, H.-M. Park and K.-B. Lee, *J. Am. Chem. Soc.*, 2002, **124**, 11881–11893.

11 M. Thormann and H.-J. Hofmann, *J. Mol. Struct.: THEOCHEM*, 1999, **469**, 63–76.

12 Z. Benatalah, A. Aubry, G. Boussard and M. Marraud, *Int. J. Pept. Protein Res.*, 1991, **38**, 603–605.

13 F. André, A. Vicherat, G. Boussard, A. Aubry and M. Marraud, *J. Pept. Res.*, 1997, **50**, 372–381.

14 F. André, G. Boussard, D. Bayeul, C. Didierjean, A. Aubry and M. Marraud, *J. Pept. Res.*, 1997, **49**, 556–562.

15 H.-J. Lee, K.-B. Lee, I.-A. Ahn, S. Ro, K.-H. Choi and Y.-S. Choi, *J. Pept. Res.*, 2000, **56**, 35–46.

16 H.-J. Lee, K.-H. Choi, I.-A. Ahn, S. Ro, H. G. Jang, Y.-S. Choi and K.-B. Lee, *J. Mol. Struct.*, 2001, **569**, 43–54.

17 S. Ro, H.-J. Lee, I.-A. Ahn, D.-K. Shin, K.-B. Lee, C.-J. Yoon and Y.-S. Choi, *Bioorg. Med. Chem.*, 2001, **9**, 1837–1841.

18 M. A. McMechen, E. L. Willis, P. C. Gourville and C. Proulx, *Molecules*, 2019, **24**, 1919.

19 H.-J. Lee, H.-M. Park and K.-B. Lee, *Biophys. Chem.*, 2007, **125**, 117–126.

20 C. Abbas, G. Pickaert, C. Didierjean, B. J. Grégoire and R. Vanderesse, *Tetrahedron Lett.*, 2009, **50**, 4158–4160.

21 X.-S. Yan, H. Luo, K.-S. Zou, J.-L. Cao, Z. Li and Y.-B. Jiang, *ACS Omega*, 2018, **3**, 4786–4790.

22 F. Bizet, N. Tonali, J.-L. Soulier, A. Oliva, J. Kaffy, B. Crousse and S. Ongeri, *New J. Chem.*, 2018, **42**, 17062–17072.

23 N. Tonali, I. Correia, J. Lesma, G. Bernadat, S. Ongeri and O. Lequin, *Org. Biomol. Chem.*, 2020, **18**, 3452–3458.

24 T. Cierpicki and J. Otlewski, *J Biomol NMR*, 2001, **21**, 249–261.

25 K. Baruah, B. Sahariah, S. S. Sakpal, J. K. R. Deka, A. K. Bar, S. Bagchi and B. K. Sarma, *Org. Lett.*, 2021, **23**, 4949–4954.

26 A. Lecoq, G. Boussard, M. Marraud and A. Aubry, *Biopolymers*, 1993, **33**, 1051–1059.

27 S. H. Bouayad-Gervais and W. D. Lubell, *Molecules*, 2013, **18**, 14739–14746.

28 P. A. Ottersbach, G. Schnakenburg and M. Gütschow, *Chem. Commun.*, 2012, **48**, 5772–5774.

29 N. Panasik Jr., P. J. Fleming and G. D. Rose, *Protein Sci.*, 2005, **14**, 2910–2914.

30 T. A. Edwards and A. J. Wilson, *Amino Acids*, 2011, **41**, 743–754.

31 X. Ran and J. E. Gestwicki, *Curr. Opin. Chem. Biol.*, 2018, **44**, 75–86.

32 S. Vivekanandan, J. R. Brender, S. Y. Lee and A. Ramamoorthy, *Biochem. Biophys. Res. Commun.*, 2011, **411**, 312–316.

33 Y. Bram, S. Peled, S. Brahmachari, M. Harlev and E. Gazit, *Sci. Rep.*, 2017, **7**, 14031.

34 H. Lu, Q. Zhou, J. He, Z. Jiang, C. Peng, R. Tong and J. Shi, *Signal Transduct. Target. Ther.*, 2020, **5**, 1–23.

35 J. Wang, Z. Cao, L. Zhao and S. Li, *Int. J. Mol. Sci.*, 2011, **12**, 3205–3219.

36 P. Joshi and M. Vendruscolo, in *Intrinsically Disordered Proteins Studied by NMR Spectroscopy*, eds. I. C. Felli and R. Pierattelli, Springer International Publishing, Cham, 2015, pp. 383–400.

37 C. Gibson, S. L. Goodman, D. Hahn, G. Hölzemann and H. Kessler, *J. Org. Chem.*, 1999, **64**, 7388–7394.