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Constrained α/γ-peptides: a new stable extended structure in solution without any hydrogen bond and characterized by a four-fold symmetry†

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Small α/γ-peptides alternating α-aminoisobutyric acid and cyclic γ-amino acid residues are described. NMR studies together with restrained simulated annealing revealed that an extended backbone conformation largely dominates in solution for as short as 4-residues long oligomers. This new fold type is devoid of any hydrogen bond and characterized by a four-fold symmetry.

Construction of unnatural new peptidic architectures that mimic α-peptides with increased stability is an important challenge for organic chemists.[1] Among these peptidomimetic compounds, many β-peptides,[2] and more recently γ-peptides[3] or hybrid peptides[4] have been proved to efficiently mimic the various H-bond-stabilized secondary structures found in proteins (helices, turns and linear strands). Non hydrogen-bonded secondary structures also play important roles in proteins like extended PPII domains which are involved in protein–protein recognition processes.[5] However, obtaining conformationally stable foldamers is more challenging when no hydrogen bond is present. Within the β-peptide family, Gellman *et al.* have studied by circular dichroism or by restrained simulated annealing several oligomers based on pyrrolidine-3-carboxylic acid or nipecotic acids. Folded and unfolded conformations in equilibrium were observed for these oligomers lacking amide protons.[6] Independently, a few groups described oligomers incorporating constrained β-amino acids which form secondary structures without any hydrogen bond.[7] Within the γ-peptide family, only one example was reported where, according to CD spectra, rigid bicyclic residue oligomers adopt partially ordered structures without hydrogen bonding.[8] Here we report that hybrid α/γ oligomers of four residues or longer alternating sufficiently rigid units are able to adopt an extended structure without any hydrogen bond. This extended structure shows a four-fold symmetry, a fold that, to our knowledge, is unique.

Over the past few years we have developed an asymmetric synthesis of cyclic or acyclic β,γ-diamino acids starting from natural α-amino acids.[9] These compounds are promising substrates for elaboration of a large number of original peptidic oligomers, the second nitrogen providing a source of molecular diversity or a hydrophilic substituent.[10] Among the accessible β,γ-diamino acids, the cyclic compound **1** (Fig. 1), obtained from L-aspartic acid, appeared to be a valuable candidate for the preparation of extended structures because of its intrinsic rigidity. To further increase this rigidity, and because we are also very much interested in quaternary α-amino acids,[11] we decided to alternate this lactam **1** (LAC) and α-aminoisobutyric acid (AIB). Using standard solid phase synthesis (on Rink amide resin), we thus synthesized several hetero-oligomers and we embarked on detailed structural studies in DMSO-d_6 of tetramer **2**, hexamer **3** and octamer **4**, which were isolated as TFA salts[12] (Fig. 1).

Oligomers **2–4** are soluble in water, methanol and DMSO. We decided to perform ^{1}H NMR in DMSO-d_6 because the larger spectral dispersion observed in the NH region compared to other solvents was interpreted as a probable structure stabilization. Extensive NMR measurements (TOCSY, ROESY, HSQC, CH_2-TROSY,[13] and HMBC) allowed complete proton and carbon resonance assignments as well as homonuclear and heteronuclear coupling constants determination.[12] Chemical shift dependence on concentration was monitored for compound **3**. The absence of a significant change between 0.8 mM and 20 mM excludes aggregation. When applied to peptides, the use of two separate ranges of temperature coefficients $\Delta\delta/\Delta T$ to discriminate

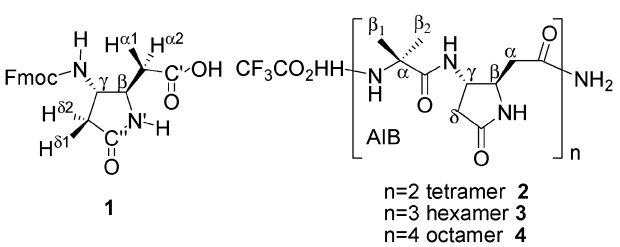

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Fig. 1 Structures and nomenclature of lactam **1** used as a monomer and hybrid α/γ oligomers.

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† Electronic supplementary information (ESI) available: Crystallographic data of a derivative of **1**, experimental details and characterization of all new monomeric compounds; general procedures for peptide synthesis, ^{1}H NMR, ^{13}C NMR, ROE constrains, coupling constant values, temperature and concentration studies of oligomers **2**, **3** and **4**. CCDC 798420. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2cc16852a

Table 1 $-\Delta\delta/\Delta T$ (ppb/K) of amide protons H^N and $H^{N'}$ in tetramer **2**, hexamer **3** and octamer **4** (DMSO-d_6 between 289 K and 317 K)

	Residue						
Proton (comp.)	LAC2	AIB3	LAC4	AIB5	LAC6	AIB7	LAC8
H^N (**2**)	3.7	4.9	3.3				
$H^{N'}$ (**2**)	5.8	—	6.4				
H^N (**3**)	3.7	5.0	3.5	5.0	3.5		
$H^{N'}$ (**3**)	6.2	—	5.6	—	6.4		
H^N (**4**)	3.8	4.8	3.5	4.8	3.2	4.7	3.2
$H^{N'}$ (**4**)	6.0	—	5.6	—	5.3	—	6.2

between H-bonded exchange-protected and solvent-exposed NH is hazardous.[14] However, we noticed that for a given amide proton type (backbone LAC H^N, cyclic LAC $H^{N'}$ or backbone AIB H^N), temperature coefficients $\Delta\delta/\Delta T$ were almost identical whatever the length of the oligomer or the position of the amino acid in the sequence, suggesting that no hydrogen bond was present (Table 1). NMR spectra recorded on compounds **2**, **3** and **4** displayed similarities in terms of chemical shifts, J couplings and ROE correlations. Markedly, within each residue type, chemical shifts of backbone amide protons were found to decrease from the N-terminus to the C-terminus ($\delta H^N_i > \delta H^N_{i+2}$). The same behavior was observed for the LAC cyclic amide $H^{N'}$. Furthermore, chemical shift differences between diastereotopic H^α protons in LAC residues, or diastereotopic $CH_3{}^\beta$ methyl in AIB residues grew larger when approaching the carboxamide terminus. These general features together with observations of non-averaged backbone $^3J_{H^\alpha H^\beta}$ and $(i) - (i + 2)$ ROE correlations were indicative of a common fold for compounds **2**, **3** and **4**.

In γ-amino acids, the number of possible conformers is increased as a direct consequence of the addition of two tetrahedral carbon atoms. However, it was shown that the conformations obtained in γ-peptides are inherently more stable than those in α-peptides.[15] In compound **1**, the dihedral angle about the C^β–C^γ bond is restricted to about 120° by the cyclic lactam as observed in the crystallographic structure of a derivative of compound **1**.[12] Extraction of precise $^3J_{H^\alpha H^\beta}$ from the ^{1}H homodecoupled CH_2-TROSY spectra[13] made it possible to further constrain the C^α–C^β bond (Fig. 2).

Indeed, $^3J_{H^{\alpha 1}H^\beta}$ was found stable along the oligomer sequences with an average value of 8.2 Hz whereas $^3J_{H^{\alpha 2}H^\beta}$ was lower at 4.2 Hz. Using proper Karplus parameters,[16] rotameric populations can be estimated, provided that ideal staggered conformations are adopted. This would lead to an excess of the *anti* rotamer of ~70%. Interestingly, among the three oligomers, the first LAC2 residues were characterized by more dynamics, since values of 7.6 Hz and 5.4 Hz were measured for $^3J_{H^{\alpha 1}H^\beta}$ and $^3J_{H^{\alpha 2}H^\beta}$, respectively. In the same manner, inspection of $^3J_{H^\gamma H^\delta}$ couplings revealed that the LAC2 C^β–C^γ–C^δ–C'' dihedral angles were experiencing fast exchange between the two possible values within the cycle (±15°) whereas other LAC residues were blocked with a +15° puckering.

In the α/γ oligomers, the observation of high $^3J_{H^{N'}C^\delta}$ values (*ca.* 5.8 Hz) in the lactam ring was consistent with the *cis* conformation of the cyclic amide bond. In contrast, the absence of correlations between all backbone $H^N_{(i)}$ and preceding $C^\alpha_{(i-1)}$ resonances in the HMBC spectra revealed that the corresponding $^3J_{H^N C^\alpha}$ couplings were very weak which was

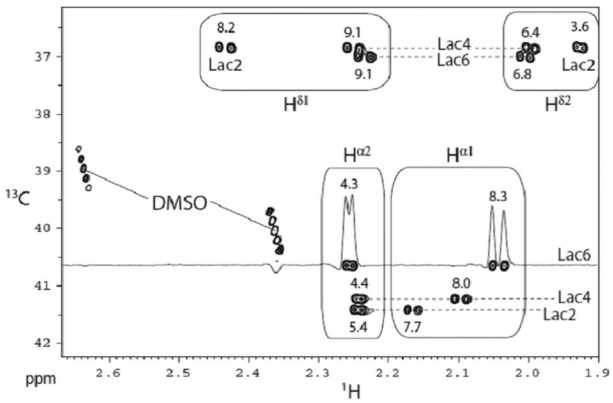

Fig. 2 The CH_2-TROSY spectrum of CH_2 groups in hexamer **3** (500 MHz, 20 °C). Pure doublets obtained are labelled with corresponding coupling values in Hertz. In this experiment, chemical shifts of methylene groups are downfield-shifted by $^1J_{CH}$ in the ^{13}C dimension and upfield-shifted by $(^1J_{CH} + {}^2J_{HH})/2$ in the ^{1}H dimension.[13] Lorentz–Gauss window functions were applied in both indirect and direct detected dimensions with extensive zero-filling prior to Fourier transformation to yield high digital resolution.

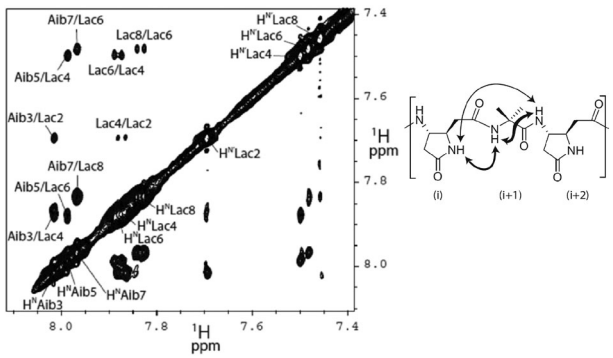

Fig. 3 Amide network observed in the HN–HN region of the ROESY spectrum in octamer **4** (500 MHz, 20 °C).

indicative of *trans* peptide bond conformations. ROESY spectra were characterized by numerous cross peaks that were converted into 47, 70 and 84 distance constraints for tetramer **2**, hexamer **3** and octamer **4**, respectively. Almost half of these correlations corresponds to inter-residue correlations (21, 33, and 43 for tetramer **2**, hexamer **3** and octamer **4**, respectively), which efficiently constrained the structures.[17] In particular, networks of amide protons were identified, involving AIB and two adjacent residues (Fig. 3).

For each oligomer, 200 structures have been calculated starting from extended folds.[18] Complete sets of NMR data including both homonuclear and heteronuclear vicinal coupling constants and ROE distance restraints were used in the simulated annealing protocol.[19] Superimposition of the 10 lowest energy structures of tetramer **2**, hexamer **3** and octamer **4** revealed a common extended fold (Fig. 4). In the three studied oligomers, AIB1 and LAC2 experienced more dynamics than the following residues. In contrast, C-terminal tails appeared as stable as the central region of the oligomers. The dihedral angle observed for non-starting AIB residues was on average $\phi = -168 \pm 5°$ and $\psi = +27 \pm 3°$.[20] To our knowledge, although these values fall in a favoured region of the AIB Ramachandran map,[21]

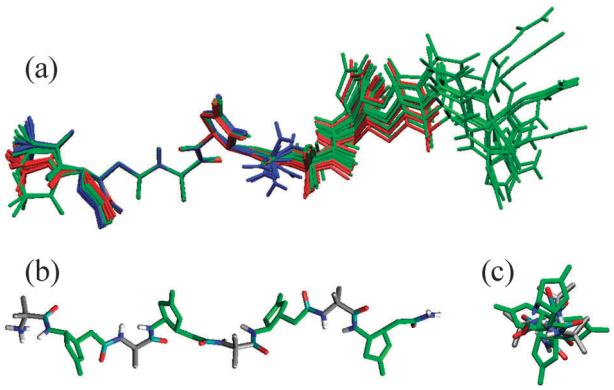

Fig. 4 (a) Overlay of the 10 lowest energy structures of tetramer **2** (blue), hexamer **3** (red) and octamer **4** (green) using the backbone atoms of residues 2 to 4 for superposition. (b,c) Orthogonal views of the lowest energy structure of the octamer **4**.

no such geometry has been yet described for AIB residues within α-peptides.

Structures obtained revealed thus a new stable extended fold characterized by a four-fold symmetry.[22] It is noteworthy that since oligomers as short as 4-residues adopt this extended conformation, the folding process is probably governed by local steric interactions, no hydrogen bond being required. These features recall the polyproline II helix properties, stable without hydrogen bonding, and showing a three-fold symmetry for the pyrrolidine ring.[23]

In conclusion, we have shown that oligomers alternating AIB and cyclic γ-amino acid **1** are able to adopt an extended structure in solution, without any hydrogen bond, regardless of the peptide length. Concerning the AIB residue, the dihedral ϕ and ψ angles observed in our structure are close to a local minimum in the Ramachandran map but have not been found in other structures. We have thus highlighted a new extended helix showing a four-fold symmetry. To our knowledge, there is no precedent for this type of extended structure in the β-peptide or γ-peptide families. Additional peptides with a functionalized nitrogen lactam or without the AIB residue are under investigation as well as potential biological applications of these compounds, such as inhibition of protein–protein interactions.

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