Synthesis and structural characterisation as 12-helix of the hexamer of a β-amino acid tethered to a pyrrolidin-2-one ring†

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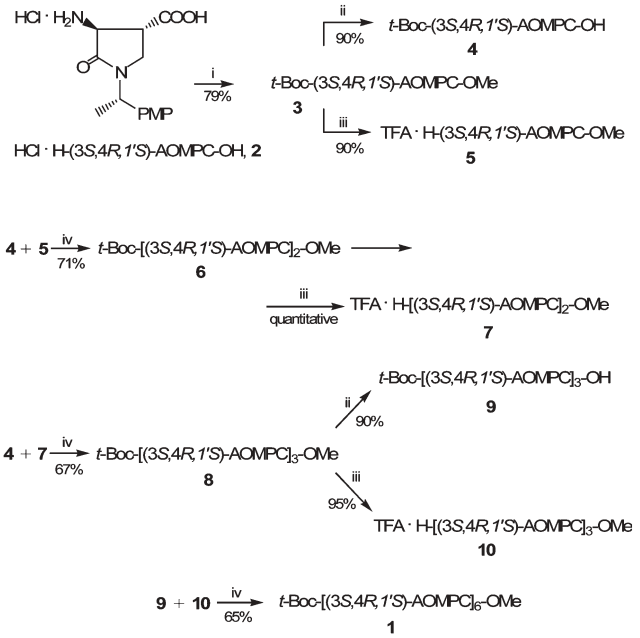
Starting from (3*S*,4*R*,1′*S*)-3-amino-2-oxo-1-[1′-(4-methoxyphenylethyl)]pyrrolidine carboxylic acid (2), the first synthesis of a β-foldamer containing pyrrolidin-2-one rings is described, whose 12-helix conformation is assigned by NMR analysis and confirmed by molecular dynamics (MD) simulations.

Non-natural polymers able to form organized secondary structures in solution ("foldamers"), can be a useful alternative to peptides.[1] Among them, oligomers of β-amino acids ("β-peptides") are of particular interest, since they display a high tendency to give defined secondary structures[2] and are unaffected by proteases, thus being very attractive for molecular biology and drug discovery.[3] Although many pyrrolidine ring-based foldamers have been studied,[4] to the best of our knowledge, amino acids tethered to a pyrrolidin-2-one (γ-lactam) ring were not used to build β-foldamers. We therefore planned to investigate the effect of the lactam moiety on the conformational behaviour of such molecules.[5] In addition, if the group attached to the nitrogen of the lactam ring can be easily removed, subsequent alkylation carried out with appropriate functionalised alkyl halides could afford a new class of foldamers where the conformational constriction due to the lactam ring affects the orientation of the side chain. Here, we report both the synthesis and the structural characterization of hexamer **1**, built from the β-amino acid (3*S*,4*R*,1′*S*)-3-amino-2-oxo-1-[1′-(4-methoxyphenylethyl)]pyrrolidine carboxylic acid [(3*S*,4*R*,1′*S*)-AOMPC], **2**, recently synthesized in our laboratory.[5] First, the (3*S*,4*R*,1′*S*)-AOMPC **2** hydrochloride was converted into the key intermediate, **3**, precursor of both building blocks **4** and **5** (Scheme 1).

The synthesis of the homo-oligomers of (3*S*,4*R*,1′*S*)-AOMPC **2** was carried out according to standard peptide synthesis in the homogeneous phase. Starting from the monoprotected derivatives **4** and **5**, the dimer *t*-Boc-(AOMPC)$_2$-OMe, **6**, was first isolated. The trimer *t*-Boc-(AOMPC)$_3$-OMe, **8**, was subsequently synthesized by coupling the dimer **7**, which has a free amino group, and **4**. Starting from **8**, the trimers **9** and **10** were prepared, with free



Scheme 1 Preparation of hexamer **1**. *Reagents and conditions*: (*t*-Boc = *tert*-butyloxycarbonyl; PMP = 4-methoxyphenyl): (i) (Boc)$_2$O, TEA, MeOH, rt, then CH_2N_2; (ii) 0.5 M NaOH, MeOH, then 2 M HCl; (iii) TFA, DCM, rt. (iv) EDAC, TEA, DCM, 0 °C.

carboxy and amino groups, respectively. Finally, coupling of **9** and **10** led to the hexamer *t*-Boc-(AOMPC)$_6$-OMe, **1** (Scheme 1 and Fig. 1).

The 12-helical conformation of **1** was elucidated by NMR analysis. Assignment of the backbone proton resonances of each monomer was achieved with standard ^{1}H-homonuclear spectra[6] and assignment of the carbon backbone resonances was achieved using ^{1}H/^{13}C HMQC and HMBC experiments.[7] Long-range couplings between *C*ONH(*i*) and *H*4(*i*) and between *C*O(*i*) and N*H*(*i* + 1) detected in the HMBC spectrum provided the sequence-specific assignment (Fig. 2). The complete backbone assignment is reported in the electronic supplementary information (ESI).†

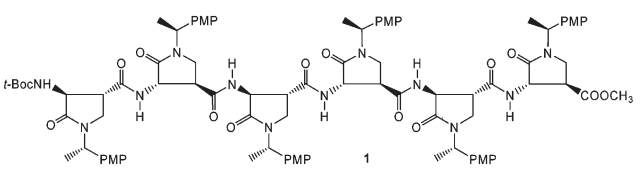


Fig. 1 The structure of hexamer **1**.

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† Electronic supplementary information (ESI) available: Detailed synthetic procedures, characterization data for all compounds, spectra for compounds **1**, **2**, **4**, and **5** and molecular dynamics (MD) simulations. See DOI: 10.1039/b612071g

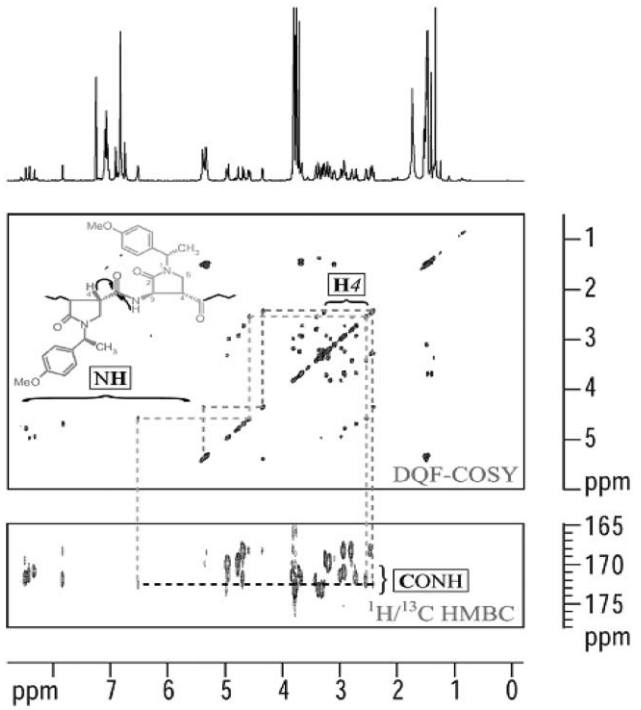

Fig. 2 Example of spectral assignment of *t*-Boc-(AOMPC)$_6$-OMe, **1**, in $CDCl_3$ at 298 K.

Medium-range correlations detected in the ROESY spectrum (see ESI†) strongly suggested a 12-helical conformation: H3(*i*)–NH(*i* + 2), H3(*i*)–H4(*i* + 2), H3(*i*)–NH(*i* + 3), and H5(*i*)–NH(*i* + 3). The conformation of **1** was also studied by molecular dynamics (MD) simulations[8–10] and at first ROESY distance restraints were used.[11–13]

A detailed cluster analysis was carried out and the results, given in the form of torsion angles ϕ, θ, and ψ population distributions (clustering level 42, RMS 0.53), showed 48 structures grouped into 7 families (clusters). The lowest energy structures for **1** all provided a 12-helical conformation and the differences in energy between the conformations mainly arose from the side chain arrangements which strongly differ from each other. In Fig. 3, the lowest energy conformer is represented and its 12-helix like geometry is clearly visible, whereas the superposition of the lowest energy structures of **1** from cluster 1 is reported in Fig. 4.

In addition, an unconstrained molecular dynamics simulation was carried out, and the cluster analysis (clustering level 38, RMS 0.52) showed 44 structures grouped into 7 families (see ESI†). For the set clusters, we could identify a backbone structure highly compatible with a 12-helical conformation, and the results were in total agreement with the observed ROE interproton distances.

In summary, we have reported the synthesis and the structural elucidation of hexamer **1** which displays a 12-helix secondary structure. The possibility of linking further substituents at the ring nitrogen, exploiting the rigid sp^2 moiety make this compound synthetically useful since a lot of cationic or anionic functionalities can be placed at specific sites along the helix. Thus, with the aim to synthesize foldamers useful for their antibiotic activity[14] and/or for generation of nanostructures,[15] work is currently in progress in our laboratory and will be reported in due course.

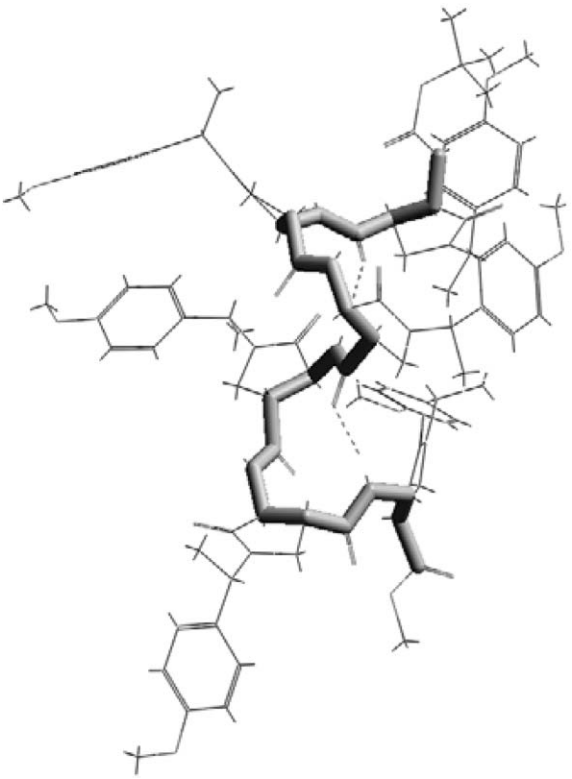

Fig. 3 The 12-helix structure for hexamer **1** arising from MD simulations showing intramolecular H-bonding.

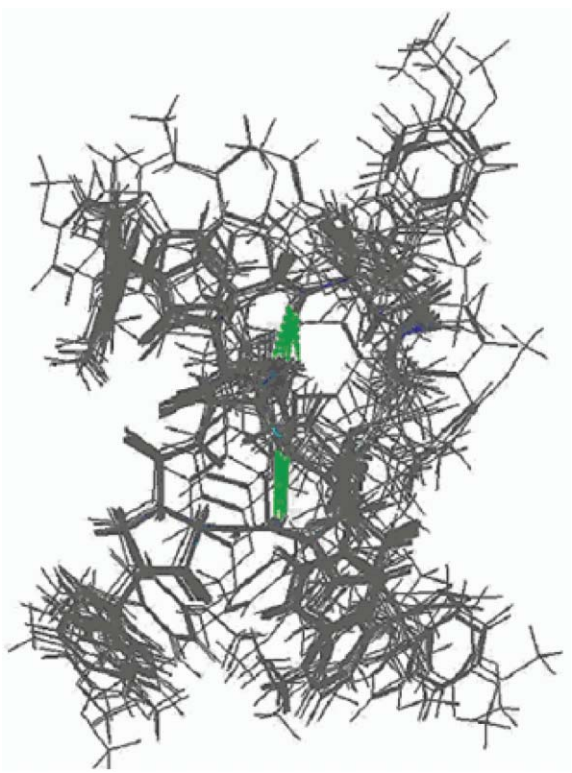

Fig. 4 The cluster for 12-helix structure of hexamer **1** arising from MD simulations (intramolecular H-bonding shown in green).

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