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Foldamers of bifunctional diketopiperazines displaying a β-bend ribbon structure

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ABSTRACT

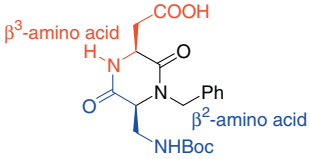
The synthesis of two oligomers, of a bifunctional diketopiperazine scaffold **DKP-1**, formally derived from the cyclization of L-aspartic acid and (*S*)-2,3-diaminopropionic acid, is reported. A trimeric and a tetrameric structure were prepared by solution-phase peptide synthesis (Boc strategy). Conformational analysis of these derivatives was carried out by a combination of ^{1}H NMR spectroscopy, CD spectroscopy, and molecular modeling, and revealed the formation of β-bend ribbon involving 10-membered H-bonded rings and a reverse turn of the growing peptide chain.

Peptides and peptidomimetics composed of repeating units have been used to stabilize conformationally ordered structures (so called foldamers)[1] and reproduce the secondary structures of proteins and more specifically α- and 3_{10}-helices. In the latter case the establishment of a network of hydrogen bonds promotes the formation of 10-membered rings. A particular type of 3_{10}-helix is the β-bend ribbon, which is characterized by a succession of β-turns forming a linear peptide with a ribbon-like shape.[2] β-Bend ribbons are found in several natural peptides containing repeating $(Pro\text{-}X)_n$ units, such as Zervamicin IIA and other peptaibol antibiotics[3] (membrane active peptide antibiotics). Its crystal structure exhibits a final spiral of three β-bends in a ribbon, containing Pro (or hydroxyproline)—Aib (or isovaline) sequence motifs.[4] Toniolo and co-workers have investigated the conformational preferences of several peptides containing an alternation of Pro (or substituted Pro) residues and helix-forming residues (such as Aib) and have shown by X-ray diffraction, NMR, and CD studies that these compounds indeed adopt a β-bend ribbon structure both in solution and in the solid state.[5] A different approach was followed by other authors, using conformationally constrained bifunctional peptidomimetics such as 5-carboxy oxazolidin-2-one derivatives[6] or tetrahydrofuran[7] and oxetane amino acids.[8]

We have recently reported the synthesis of a new bifunctional diketopiperazine scaffold (**DKP-1**, Fig. 1), formally derived from L-aspartic acid and (*S*)-2,3-diaminopropionic acid, and bearing a carboxylic acid and an amino functionality.[9] When inserted into an oligopeptide sequence, the **DKP-1**-scaffold acts as a reverse turn inducer. In addition, **DKP-1**, while being derived from α-amino acids, can be seen as a conformationally constrained dipeptide formed by two β-amino acids and in particular a (*S*) β^2- and a (*S*) β^3-amino acid (following Seebach's nomenclature).[10]

Prompted by these observations, we decided to explore the behavior of oligomers of **DKP-1**. In this Letter we describe the synthesis and a conformational study of two oligomers: namely the trimer Boc-(**DKP-1**)$_3$-NH*n*Bu **2**, and the tetramer Boc-(**DKP-1**)$_4$-NH*n*Bu **3**.

The synthesis of the trimer **2** and tetramer **3** was realized in solution (Boc strategy) starting from the C terminus. All the coupling reactions were performed using Carpino's reagent (HATU) and *i*-Pr_2NEt or collidine in DMF or acetonitrile. The highest yields



DKP-1

Figure 1. Structure of the bifunctional diketopiperazine scaffold **DKP-1**, highlighting the conformationally constrained β^2–β^3 dipeptide sequence.

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in the coupling of the 3rd and 4th **DKP-1** residues were obtained using collidine in CH_3CN.

The conformational preferences of these compounds in solution were investigated by ^1H NMR and CD spectroscopy and molecular modeling. Characteristic differences in the NMR spectral parameters for unstructured peptides and peptides in extended and intramolecularly hydrogen-bonded conformations have been reported in different organic solvents. In particular, concentration and temperature dependence of the amide protons can be indicative of aggregation phenomena, while NOE contacts can be highly indicative of the formation of secondary structures, such as β-hairpin,[11] when inter-strand contacts are visible.

The dimeric compound Boc-(**DKP-1**)$_2$-NH*n*Bu was analyzed first, but it showed some degree of aggregation already at the lowest 2 mM concentration in $CDCl_3$ and no significant NOE contacts. These data are in agreement with an equilibrium between a non-hydrogen-bonded and an intermolecularly H-bonded status.

The trimer Boc-(**DKP-1**)$_3$-NH*n*Bu **2** (Fig. 2) was studied next. In this case no concentration dependence of the NH signals was found up to 5 mM in $CDCl_3$, CD_3OH, and DMSO-d_6 and, for this reason, all the subsequent conformational studies were performed at this concentration. The amide proton chemical shifts in $CDCl_3$ were rather deshielded (7.77–8.63 ppm and 6.16 for the NH-Boc) indicating possible intramolecular hydrogen bonds.

Analysis of the NOESY spectrum in CD_3OH revealed the most interesting contacts, while the experiment in $CDCl_3$ was not conclusive for the absence of relevant long-range contacts and in DMSO-d_6, signal overlap hampered the full characterization and assignment of long-range contacts. Experiments in D_2O could not be performed due to the insolubility of **2** in water or aqueous solvents. In CD_3OH, several long-range interactions could be detected which revealed the formation of two β-hairpin conformations. In particular, NH^3_A (the carbamate proton) showed a medium NOE contact with the protons H^4_A, and NH^3_C showed a weak NOE contact with the protons H^4_C (Fig. 3). These contacts are indicative of the formation of a pseudo-reverse turn and of a 10-membered ring hydrogen bond between the NH^3_A and NH^3_C and the C=O of the carboxy-groups of the diketopiperazine residues A and C, respectively. A 10 ns stochastic dynamic (SD) simulation[12] was performed for compound **2** applying the NH^3_A/H^4_A and NH^3_C/H^4_C distance restraints derived from NOE contacts. During the simulation, the 10-membered ring hydrogen bond[13] was formed in 40% (DKP-A) and 7% (DKP-C) of the sampled structures. In summary, in the case of the trimeric structure **2**, no β-bend ribbon structure could be observed by NMR, although molecular modeling results suggest the possible formation of two turns for the first and third residues (A and C in Fig. 2).

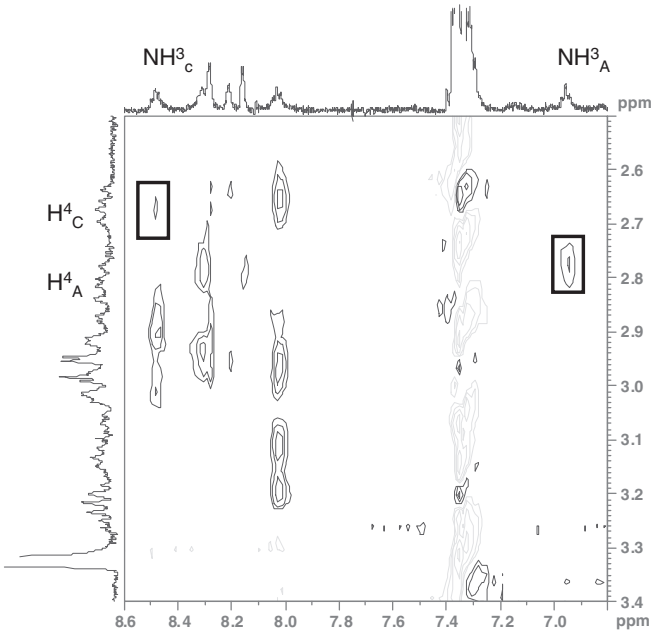

Figure 3. Section of the NOESY spectrum of **2** (5 mM in CD_3OH) showing inter-strand NOEs for H^4 and NH^3 protons.

The tetrameric structure **3** was then investigated (Fig. 4). Titration of the amide protons was performed by addition of CD_3OD to a solution of **3** in DMSO-d_6, and the rate of exchange was measured. The endocyclic amide protons exchanged completely in 7 min, while the exocyclic amide protons disappeared within 48 min from the addition of CD_3OD. The carbamate NH lasted for 169 min before complete disappearance. Apparently the endocyclic NH^2s are not involved in hydrogen bonding, while the exocyclic amide pro-

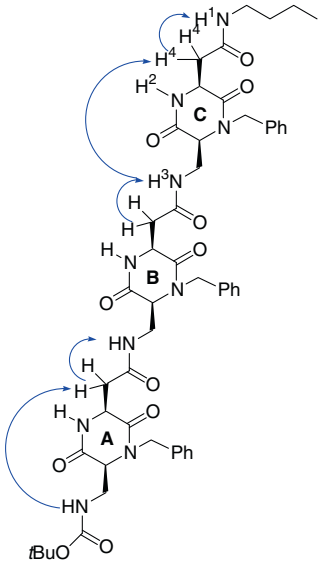

Figure 2. Structure of trimer **2**. Blue arrows indicate the NOE contacts typical of the hairpin conformation.

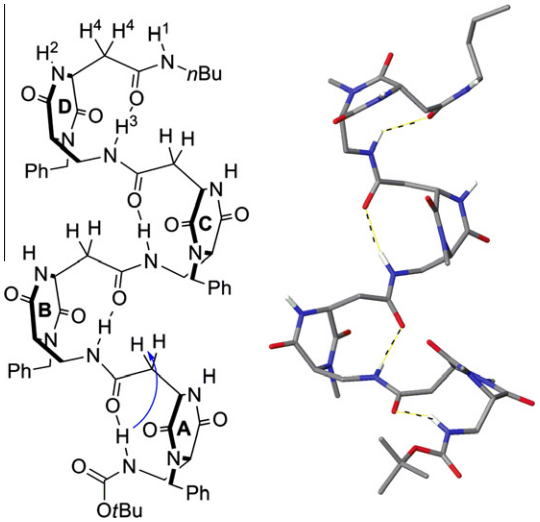

Figure 4. Structure of tetramer **3**: (a) on the left, sequence of hairpins and formation of the β-bend ribbon (the blue arrow indicates the NOE contact typical of the hairpin conformation); (b) on the right, snapshot taken from SD simulation (non polar H and benzyl groups have been omitted for clarity).

tons NH^3 appear to be intramolecularly bound. A study of the NOESY in CD_3OH and DMSO-d_6 (D_2O and other aqueous solvents were not considered due to the insolubility of **3** in these solvents) showed a medium contact between the carbamate proton NH^3_A and H^4_A. Unfortunately, the same NH^3/H^4 contacts could not be identified for the DKP-units B–D, due to the overlap of the H^4 protons. In these units, the strong contacts between H^4 of one DKP-unit and the closer NH^3 of the subsequent DKP-unit hid the weaker NH^3/H^4 contacts of the same unit. For compound **3** SD simulation was performed applying distance restraints between the NH^3/H^4 proton of each DKP-units. The 10-membered H-bonded ring was formed for the 75% and 44% of the simulation by the DKP-A and DKP-C, respectively, and less than the 20% by the DKP-B and DKP-D. According to experimental data and SD analysis, compound **3** is likely to adopt a β-bend ribbon conformation with 10-membered H-bonded ring on each DKP-unit (Fig. 4).

The ability of oligopeptides **2** and **3** to adopt an ordered secondary structure in solution was also evaluated by CD spectroscopy (Fig. 5). The spectra were measured in methanol (0.2 mM) and showed a rather different behavior for the two compounds. Oligopeptides containing the **DKP-1** scaffold which formed 10-membered H-bonded hairpins displayed typical Cotton effects in their circular dichroism (CD) spectra.[9a] In particular, two negative minima, a principal one at 200–205 nm and a second one, less intense, at about 220 nm, and a negative maximum at 209–215 nm were found. This pattern is also commonly found in 3_{10}-helices and in particular in β-bend ribbons.

In the case of the trimeric compound **2**, the CD spectrum showed a rather weak minimum at 198 nm and two strong minima at 208 and 220 nm. On the contrary, the tetramer **3** showed a rather strong minimum at 200 nm and a weaker one at 225 nm with a negative maximum at 215 nm.

This is very similar to the CD spectrum of oligopeptides containing a central **DKP-1** unit, which adopted a 10-membered H-bonded hairpin conformation,[9a] and also to other peptidomimetic structures showing a β-bend ribbon conformation.[3b,6b,14] In addition, the molar ellipticities of the Cotton effects displayed by the tetramer **3** (>130,000 deg cm^2 mol^{-1} for the peak at 200 nm) are much more intense than the analogous peaks shown by the hexapeptide-mimic structure containing **DKP-1** and four regular amino acids Boc-ValAla**DKP-1**-ValAla-NH*n*Bu (about 8,400 deg cm^2 mol^{-1} for the peak at 201 nm at a 0.5 mM concentration in methanol)[9a]

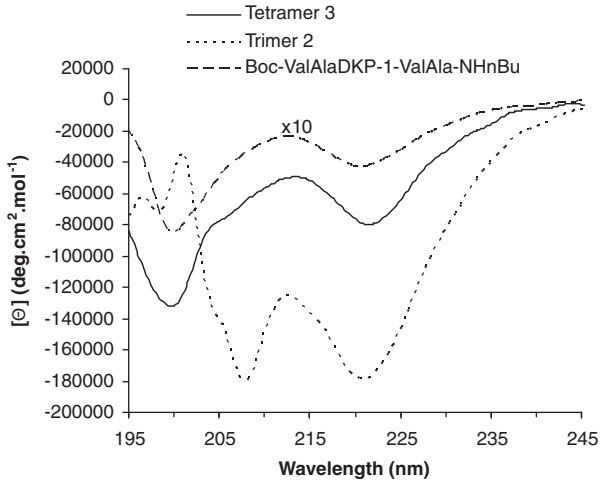

Figure 5. CD spectrum of tetramer **3**, trimer **2**, and the hairpin peptidomimetic Boc-ValAla**DKP-1**-ValAla-NH*n*Bu (Ref. 9a). The data of the latter compound have been multiplied by a factor 10 to magnify the appearance of the curve.

and are indicative of an organized secondary structure involving repeated turn-inducing units.[14]

In summary, we have described the synthesis of oligomeric bifunctional diketopiperazines **DKP-1** bearing a carboxylic acid and an amino functionality and formally derived from the cyclization of L-aspartic acid and (*S*)-2,3-diaminopropionic acid. A conformational study of these new foldamers showed that these diketopiperazines are well pre-organized to induce a reverse turn of the growing peptide chain with the formation of a 10-membered hydrogen-bonded cycle and that oligomers of **DKP-1** adopt a β-bend ribbon conformation starting from four units of **DKP-1**.

Acknowledgments

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Supplementary data

Supplementary data (general experimental methods, typical reaction conditions and spectroscopic characterization of representative compounds, ^1H and ^{13}C NMR spectra and conformational studies for compounds **2** and **3**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.06.043.

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