Helical Structures

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Condensation Approach to Aliphatic Oligourea Foldamers: Helices with *N*-(Pyrrolidin-2-ylmethyl)ureido Junctions**

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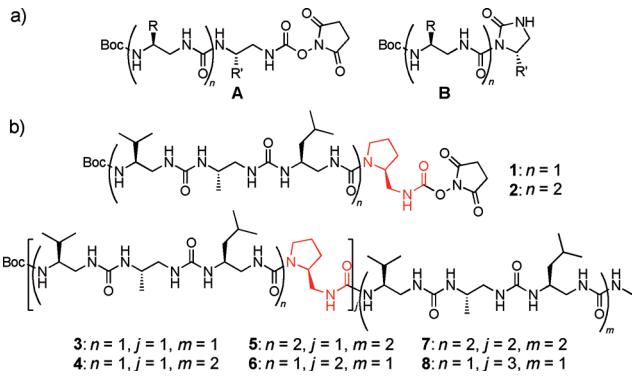
Dedicated to Dr. Michel Marraud on the occasion of his 70th birthday

The design and synthesis of large and complex folded structures resembling those of natural biopolymers is one of the current challenges in the field of foldamers.[1] Despite the difficulty, significant progress in this direction has been made over the last few years. Remarkably long helical segments, tertiary-type structures, and quaternary arrangements (helix bundles) constructed from aliphatic β peptides,[2] α/β-peptide hybrids,[3] or aromatic oligoamides,[4] have been characterized at atomic resolution. Long helical foldamers also show promise as α-helical mimics to inhibit protein–protein interactions. For example, 33-residue-long helical α/β peptides designed to mimic the heptad repeat 2 domain of the HIV protein gp41 are potent inhibitors of virus fusion and display significant improvement in proteolytic stability over corresponding α peptides.[5] Also promising is the use of proteins in which elements of the secondary structure have been replaced by synthetic foldamers to address the role of individual folded segments and to replicate or modulate protein topology and function.[6] Nevertheless, a prerequisite to accessing high-molecular-weight foldamers is the development of a robust synthetic methodology. In the case of aliphatic and aromatic oligoamides, optimized procedures involving convergent condensation of activated segments,[6,7,8a] and stepwise solid-phase synthesis (eventually assisted by microwave irradiation),[8] have proven particularly useful. However, such methods have hardly been applied to the construction of long non-oligoamide segments.

Aliphatic oligoureas of the general formula $[NHCH(R)CH_2NHCO]_n$ represent an interesting class of peptidomimetic foldamers with potential for interacting with bio-macromolecules.[9] High resolution structural studies in solution and in the crystal state have shown that these aza analogues of γ^4 peptides form well-defined 2.5-helical structures stabilized by three-centered hydrogen bonds.[10] Although aliphatic oligoureas can be prepared by solid-phase techniques, the need for long coupling times and the limitations imposed by the choice of the N-protecting group have so far limited the synthesis of oligourea helices to short segments of about 10 units long.[11,12] To decrease the number of synthetic steps and thus evolve more rapidly towards longer oligomers, we now introduce an iterative segment condensation approach to oligourea foldamers.

Our initial plan was to activate short oligoureas bearing an amino terminus with succinimidyl carbonate to yield the corresponding activated segment **A**. However, the competitive formation of cyclic biuret (**B**) resulting from the attack of the activated succinimidyl carbamate by the nearest urea NH was found to significantly reduce the yield of **A** (Scheme 1 a).



Scheme 1. A segment condensation approach to aliphatic oligourea foldamers. a) Oligomer activated as a succinimidyl carbamate (**A**) and competitive biuret formation (**B**). b) The sequences of the oligoureas **3**–**8**, which were prepared by convergent condensation of the activated segments **1** and **2** bearing a terminal pyrrolidine unit. Boc = *tert*-butoxycarbonyl.

This side reaction was also found to be problematic in the segment coupling step.[13] Although the installation of a temporary protecting group to block the reactivity of the NH of the neighboring urea was considered,[14] we felt that it would compromise the versatility of the method for rapid access to long helical segments.

Therefore, we envisioned the introduction of an N-alkylated unit at the terminus that would not be prone to biuret formation and would thus facilitate segment activation. To expand our collection of building blocks with proteino-

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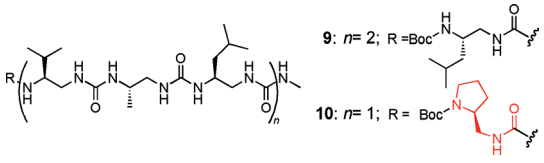
genic side chains, we selected and prepared a monomer with a pyrrolidine ring, resembling proline, without prejudging the conformational outcome. The compatibility of the 2.5-helix geometry of oligoureas for noncanonical substitution patterns such as N-alkylated residues has not been investigated previously. In α peptides and proteins, proline is frequently found at the ends of α-helical and β-sheet structures but rarely at their center.[15,16] This location preference is due in part to the lack of an amide proton at the Xaa-Pro bond that could participate in hydrogen-bond stabilization of regular secondary structures, and to steric constraints imposed by the pyrrolidine ring. In oligoureas, one donor site still remains in the trisubstituted urea formed by insertion of a proline-type residue, and could eventually participate in helix stabilization through intramolecular hydrogen bonding.

Activated oligomers **1** and **2** bearing a succinimidyl (pyrrolidin-2-ylmethyl)carbamate terminus were readily prepared in good overall yields. Segment condensation of the tetramer **1** and heptamer **2** with short oligoureas (trimer and hexamer) bearing the side chains of Val, Ala, and Leu in DMF using 3 equivalents of diisopropylethylamine (DIEA) gave the corresponding oligoureas **3**–**5** with a *N*-(pyrrolidin-2-ylmethyl)ureido unit at the segment junctions (Scheme 1) in yields ranging from 66–89%. Iterative segment coupling starting from **3** and **5** readily afforded the 11-mer **6**, 20-mer **7**, and 15-mer **8** with two to three pyrrolidine units in good to high coupling yields (70–93%).

The secondary structure propensity of oligoureas containing *N*-pyrrolidin-2-ylmethyl)ureido units was first examined by circular dichroism (CD) in 2,2,2-trifluoroethanol (TFE). Oligoureas forming right-handed 2.5 helices (i.e. consisting of units with an *S* configuration) have been shown previously to exhibit a typical CD signature in TFE with an intense positive band at $\lambda = 203$ nm, zero crossing around $\lambda = 193$ nm, and a trough at $\lambda = 188$ nm.[17] Remarkably, all spectra of oligoureas **3**–**7** having one and two pyrrolidine units were found to display the hallmarks of the canonical 2.5-helical structure (Figure 1). However, the difference in per residue molar ellipticity (PRME) observed at $\lambda = 203$ nm for the spectra of oligoureas of equal length, with and without a pyrrolidine unit (e.g. 7-mers **3** and **9** (see Scheme 2 for structures); Figure 1a), suggests partial destabilization of the helical structure caused by the proline-type residue.

However, the finding that the 7-mer **3** exhibits a much higher PRME than the 4-mer **10** (Scheme 2) bearing a pyrrolidine unit at the terminus (Figure 1a) suggests that an internal pyrrolidine unit is not acting as a helix breaker and that the helix spans the entire sequence. This conclusion is additionally supported by the finding that PRME is gradually increasing upon elongation of **3** to **6** and **8** (Figure 1b). To determine the impact of a pyrolidine residue insertion on the



Scheme 2. Structures for **9** and **10**.

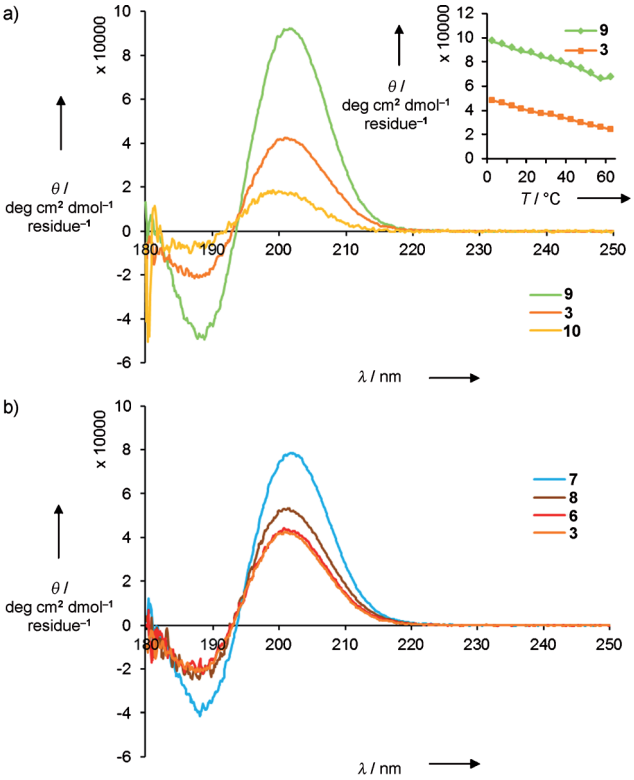


Figure 1. Circular dichroism spectra of oligoureas with pyrrolidine junction in TFE. a) Spectra for the 4-mer **10** and 7-mers **3** and **9**. Inset: temperature dependence of the CD signal of **3** and **9** at $\lambda = 203$ nm between 0°C and 60°C in TFE. b) Spectra for the 7-mer **3**, 11-mer **6**, 15-mer **8**, and 20-mer **7**. Samples were studied at 0.1 or 0.2 mM.

thermal stability of helices formed by oligoureas, we conducted comparative temperature experiments between **3** and **9** (Figure 1a, inset). A linear and gradual decrease in helicity measured at $\lambda = 203$ nm was observed in both cases between 0°C and 60°C. The slope is similar for both oligomers irrespective of the presence of a pyrrolidine unit, thus suggesting that the insertion of a proline-type residue does not exacerbate thermal unfolding in an organic solvent such as TFE.

^{1}H NMR spectra of oligoureas **3**–**6** and **8** recorded in [D_3]methanol and [D_5]pyridine show features characteristic of 2.5-helical oligoureas, including dispersion of the urea NH groups, large vicinal coupling constants between NH and CH(R) protons (>9 Hz), and strong differentiation between vicinal coupling constants of main chain diasterotopic CH_2 protons. Additional insight into the helical conformation of oligoureas containing pyrrolidine units was gained by monitoring ^{1}H NMR diastereotopicity ($\Delta\delta$) of the main chain CH_2 protons. We reported previously that $\Delta\delta$ values are useful descriptors of the conformational homogeneity of helical N,N′-linked oligoureas.[17]

Diastereotopicity measurements for the main chain CH_2 protons in the sequence of the 7-mer **3** revealed $\Delta\delta$ values in the range 0.82–1.23 ppm, which are indicative of 2.5-helical folding (Table 1). However, comparison with the $\Delta\delta$ values measured for **9** suggests that the insertion of a central pyrrolidine unit has a local destabilizing effect at the

Table 1: [1]H NMR diastereotopicity ($\Delta\delta$) values for main chain methylene protons in **3** [one pyrrolidine unit at position 4 (P4)] and **9** (no pyrrolidine unit).[a]

Compound	P7	P6	P5	P4	P3	P2	P1[b]
3	0.82	1.01	0.95	0.94	1.12	1.23	0.92
9	0.94	1.16	1.24	1.37	1.22	1.21	0.96

[a] The $\Delta\delta$ values are in ppm. [b] In oligomers **1**–**10**, the P1 position corresponds to the terminal residue coupled to methyl amine.

pyrrolidine unit ($\Delta\delta$ 0.94 < 1.37 ppm) and on the following residue with a Leu side chain ($\Delta\delta$ 0.95 < 1.24 ppm). All together, these data support the view that the incorporation of noncontiguous pyrrolidine units at various ratios is compatible with 2.5-helical folding although it brings some flexibility.

Atomic details of the structural consequences of pyrrolidine junction insertion were gained from X-ray diffraction analyses of single crystals of oligoureas **3**–**7**.[18] As shown in Figure 2a, all five oligoureas adopt a regular helical structure with no apparent break or kink at the *N*-(pyrrolidin-2-ylmethyl)ureido junctions and almost perfect repeat of 2.5 residues per turn. Overlay of the X-ray structure of the 7-mer **3** with that of a previously described 8-mer (CCDC 750017 in Ref.[10d]) shows a very good match with the canonical 2.5 helix (Figure 2b).

The average backbone torsion angles ϕ, θ_1, and θ_2 for the pyrrolidine residues in **3**–**7** (−95.9°, +54.4°, +87.9°, respectively) do not differ much from the corresponding values previously reported for the canonical 2.5 helix (−103.8°, +57.8°, +80.8°, respectively).[19] Helices in both series have a pitch of 5.1 Å and a rise per residue of 2.1 Å. The three-centered hydrogen-bonding scheme between C=O(i) and urea HN′(i−2) and HN(i−3) is maintained in all structures except at the trisubstituted urea junction where only the 12-membered pseudoring between C=O(i) and HN′(i−2) remains. The presence of the pyrrolidine unit imposes some local rearrangements, including a shift of C=O(i) away from the pyrrolidine N(i−3). The distance between O(i) and pyrrolidine N(i−3) is in the range of 3.36–3.76 Å and is much longer than the average O(i),N(i−3) distance observed in **9** (2.8–3.0 Å). In contrast, the distance between O(i) and N′(i−2) in the range 2.8–2.9 Å is not affected. In proline residues, the pyrrolidine ring exhibits two predominant envelope (half-chair) conformers referred to as Cγ-exo and Cγ-endo which correspond to χ_1 dihedral angles of approximately −30° and +30°, respectively.[20] It is noteworthy that the pyrrolidine ring in the crystal structures of **3**–**7**, preferentially adopts the endo conformation with χ_1 values close to +30°.

Herein, we have reported a simple and efficient segment condensation approach to oligourea foldamers by insertion of proline-type units at segment junctions. Because the formation of the 2.5 helix is not impaired by the presence of multiple and nonadjacent pyrrolidine units, the approach enables iterative synthesis of remarkably long helical segments, such as the approximately 40 Å long helix formed by the 20-mer **7**. Our results also point to the lower 2.5-helix propensity of the pyrrolidine unit and suggest that by changing the ratio of proline-type residues to canonical units, it may be possible to tune the stability of the 2.5 helix. The possibility for the pyrrolidine unit to act as a molecular hinge similar to proline in transmembrane helices is another feature worth being investigated in future development of this work.

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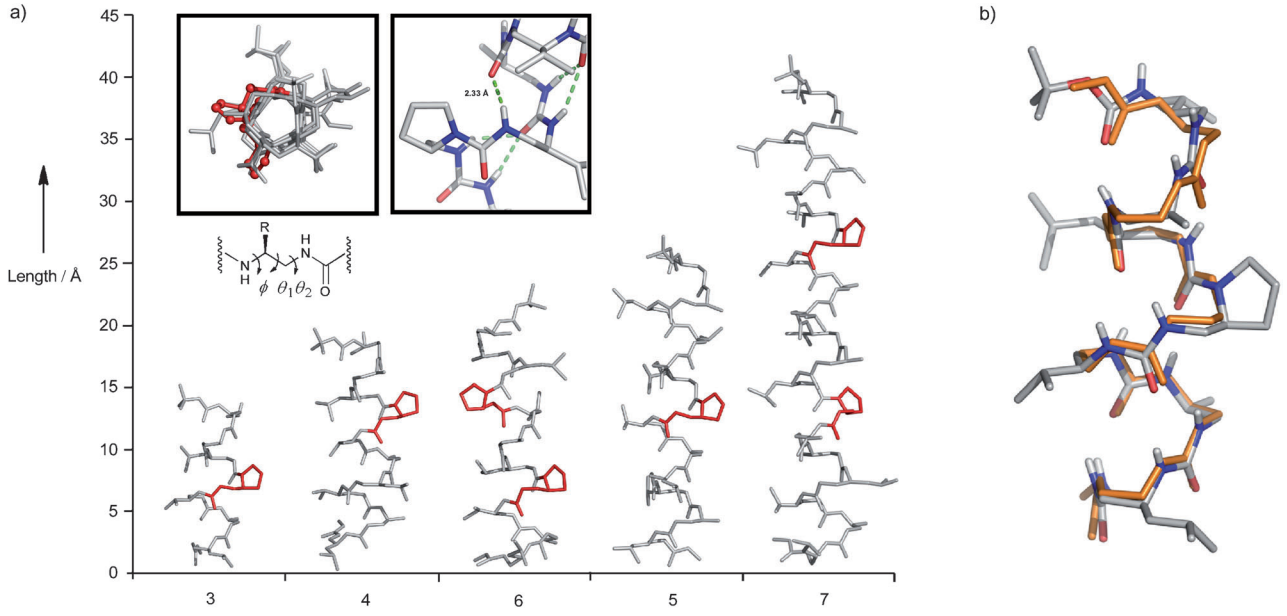

Figure 2. a) X-ray diffraction structures of 7- to 20-residue long oligoureas **3**–**7**. Pyrrolidine units are in red. Inset: top view of the helix **7** (left) and details of the noncanonical hydrogen-bond pattern at the pyrrolidine junction in **7** (right); b) Overlay of the crystal structures of **3** and a previously reported 8-mer (RMSD 0.41 Å).[10d]

Keywords: helical structures · peptidomimetics · synthetic methods · urea · x-ray diffraction

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