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# Short Oligourea Foldamers as N- or C-Caps for Promoting $\alpha$ -Helix Formation in Water

Laura Mauran,<sup>[a, b]</sup> Coralie Assailly,<sup>[a]</sup> Sébastien R. Goudreau,<sup>[b]</sup> Benoît Odaert,<sup>[c]</sup> Gilles Guichard,<sup>\*[a]</sup> and Morgane Pasco<sup>\*[a]</sup>

While foldamers have been extensively studied as protein mimics and especially as  $\alpha$ -helix mimics, their use as capping motif to enhance  $\alpha$ -helix propensity remains comparatively much limited. In this study, we leverage the structural similarities between urea-based helical foldamers and  $\alpha$ -helix to investigate the efficacy of oligoureas as N- or C-caps for reinforcing  $\alpha$ -helical structures in water. Short oligoureas, comprising 3 to 4 residues, were strategically introduced at the N- or C-terminus of two peptide sequences (S-peptide and an Ala-rich model sequence). The impact of these foldamer

insertions on peptide conformation was examined using electronic circular dichroism (ECD) and solution NMR. This research identifies specific foldamer sequences capable of promoting  $\alpha$ -helicity when incorporated at either terminus of the peptides. Not only does this work broaden the application scope of foldamers, but it also provides valuable insights into novel strategies for modulating peptide conformation in aqueous environments. The findings presented in this study may have implications for peptide design and the development of bioactive foldamer-based peptide mimics.

## Introduction

$\alpha$ -Helices mediate numerous protein-protein interactions (PPIs) involved in critical biological processes and associated disease pathways. They thus represent ideal candidates to develop new pharmaceutical tools and drugs<sup>[1]</sup> as well as build bio-inspired nanoarchitectures resembling coil-coiled assemblies.<sup>[2]</sup> Short isolated peptides when not buried within the hydrophobic environment of a protein pocket generally do not form thermodynamically stable secondary structures in water due to H-bonding competition with the solvent. Introducing supplementary conformational constraints to a peptide scaffold emerges as a mean to enhance both its structural and biological stability, while concurrently increasing cell permeability. The stabilization of peptide  $\alpha$ -helices in their active conformation to target PPIs is thus an appealing strategy to increase potency and develop promising therapeutic candidates.<sup>[3]</sup>

During the last decades, several approaches aimed at mimicking or reinforcing stabilizing interactions used by Nature

in protein scaffolds have been developed to increase the helical content of short peptides. Strategies that require chemical modifications of the helical segment have been widely used, such as the insertion of constrained  $\alpha,\alpha$ -disubstituted amino acids<sup>[4]</sup> or non-natural scaffolds with high helix propensity,<sup>[5]</sup> as well as peptide stapling – i.e. side-chain to side-chain cross-linking via disulfide,<sup>[6]</sup> thioether,<sup>[7]</sup> lactam,<sup>[8]</sup> or hydrocarbon bridges.<sup>[9]</sup> An alternative successful approach involves the incorporation of helix-nucleation elements to mimic the first turn of a helix.<sup>[10]</sup> This includes the utilization of N-terminal templates that pre-organize the first amide bonds,<sup>[11]</sup> such as rigid organic scaffolds,<sup>[12]</sup> or Lys-Asp lactam bridges.<sup>[13]</sup> Additionally, hydrogen-bond surrogates<sup>[14]</sup> can be employed, wherein the intramolecular ( $i, i+4$ ) hydrogen-bond is substituted with a covalent linkage at the N-terminus. These capping effects are particularly attractive as they provide extra-helical stabilization without directly influencing the recognition surface of the helix involved in interactions with other partners. However, their application remains largely limited to the N-terminal extremity.<sup>[15]</sup>

Helically folded oligomers (i.e. foldamers)<sup>[16]</sup> with various degrees of similarity to  $\alpha$ -peptides have been described in the literature and significant achievements in terms of  $\alpha$ -helix mimicry have been obtained for the inhibition of key protein-protein interactions.<sup>[17]</sup> To our knowledge, however, the possibility for a foldamer to promote an  $\alpha$ -helical conformation in a contiguous  $\alpha$ -peptide segment has not been specifically documented thus far, despite the general high folding ability of these synthetic oligomers. Specific foldamers with suitable hydrogen bond donor and acceptor moieties in their backbone could be used to exert efficient capping at both N- or C-terminal extremities of a peptide. Aliphatic N,N'-linked oligoureas are a class of peptidomimetic foldamers developed in our group that adopt robust helical folds stabilized by a three-centered H-bond network (12,14-helix).<sup>[18]</sup> Heterogeneous back-

[a] L. Mauran, C. Assailly, G. Guichard, M. Pasco  
 Univ. Bordeaux, CNRS, Bordeaux INP, CBMN, UMR5248, IECB, 2 rue Robert  
 Escarpit, F-33600 Pessac, France  
 E-mail: g.guichard@iecb.u-bordeaux.fr  
 m.pasco@iecb.u-bordeaux.fr

[b] L. Mauran, S. R. Goudreau  
 IMMUPHARMA BIOTECH SAS, 15 rue de Bruxelles, 75009 Paris, France

[c] B. Odaert  
 Univ. Bordeaux, CNRS, Bordeaux INP, CBMN, UMR 5248, F-33600 Pessac,  
 France

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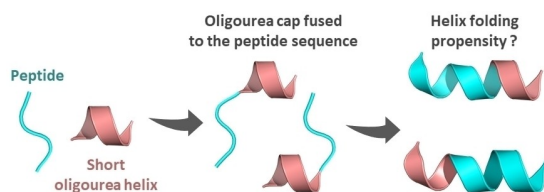
bones in which peptide segments are connected to oligoureas to form chimeric helices have been reported.<sup>[19]</sup> X-ray structure analyses of model sequences consisting of hydrophobic side chains revealed a continuous H-bond network at the fused junction, providing evidence of the remarkable compatibility between the peptide and oligourea scaffolds. Exploiting the similarity between these two backbones, we have generated effective peptide mimics with improved duration of action by strategically replacing consecutive amino acid residues in the peptide sequence with short oligourea segments.<sup>[17h,i,20]</sup>

Here we wish to report the first detailed study about the consequences of introducing short oligoureas (comprising 3 or 4 residues) at either the N- or the C-terminus of a peptide sequence on the helix folding propensity in aqueous solution. Two water-soluble peptide sequences with finely tunable helical characteristics, have been selected. The impact of the capping mode on  $\alpha$ -helix folding propensity, contingent on the initial level of helix stabilization (weak, medium or strong), was systematically evaluated at both extremities (Figure 1).

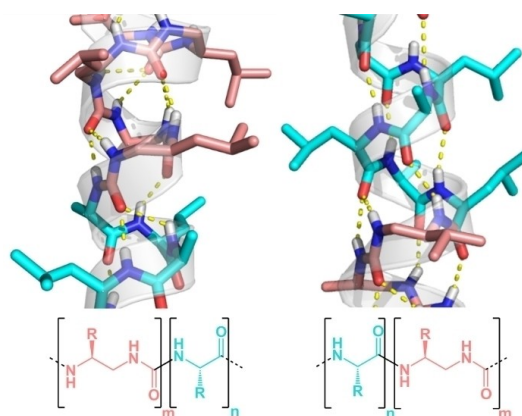
## Results and Discussion

### Selection of Peptide Models and General Design Principles of Peptides with Oligourea Caps

Detailed conformational analyses have shown that aliphatic N,N'-linked oligoureas as short as four or five residues adopt a well-defined 2.5-helical fold in various solvents.<sup>[21]</sup> As three residues constitute the minimum number required to form a complete 2.5-helix turn, we thus hypothesized in this work that a short segment of three or four urea units might be sufficient to initiate the first turn of an  $\alpha$ -helix. Several structures at atomic resolution of chimeric oligourea/peptide sequences with the oligourea sequence replacing a peptide segment at either the N- or the C-terminus extremity have been previously obtained from crystals grown in polar organic solvent.<sup>[19a]</sup> These structures revealed that the hydrogen bond donor and acceptor moieties of the oligourea at the junction with the peptide segment are correctly placed to exert a capping effect. Indeed, the two terminal carbonyl groups of the oligourea at the N-terminus of the peptide segment are hydrogen bonded to the three unpaired amino groups of the peptide, whereas the four available NH of the two urea functions are hydrogen bonded to the three unpaired CO groups of the peptide when the oligourea is placed at the C-terminus (Figure 2).



**Figure 1.** Representation of the potential capping effect of a short oligourea helix fused at either the C- or N-terminus of a peptide segment.



**Figure 2.** X-ray crystal structure representations of chimeric oligourea/peptide (left, CCDC 1040761) and peptide/oligourea (right, CCDC 1040765) sequences showing the specific H-bonding networks at the junction between the oligourea segment (light pink) and the  $\alpha$ -peptide segment (turquoise). Ureido residues will be denoted Xaa<sup>n</sup> or (X<sup>n</sup>) by analogy to the three (or one) letter code of corresponding  $\alpha$ -amino acids.

To explore the extent of  $\alpha$ -helix capping that can be achieved with oligoureas in aqueous solutions, we have now evaluated a series of triurea and tetraurea segments varying in terms of sequence that we fused at either the N- or the C-terminus of model peptide sequences.

The initial model sequence selected is biologically-relevant and corresponds to a truncated version of the S-peptide of the ribonuclease A (i.e. residues 1–15, with the segment 3–13 being  $\alpha$ -helical in the structure of the protein). This short sequence (1: KETAAAKFERQHMDN-NH<sub>2</sub>) is partially helical in aqueous conditions at 0.1 M NaCl concentration, with a helix content (% helix) around 16% that appears to be sensitive to the pH of the buffered solution as determined by electronic circular dichroism (ECD) (1, Table 1). However, this peptide contains different functional amino acids, and several ion-pair and charge-helix dipole interactions are involved in helix stabilization, which makes the effect of any substitution in the sequence complex to analyze.<sup>[22]</sup> To expand the scope of this study, we also included sequences derived from the alanine-based peptide originally designed by Baldwin in the 1990's and used as a reference to determine the helix propensity of non-polar amino acid residues.<sup>[23]</sup> This 17-mer sequence (2A: Ac-YKAAAAKAAAA-KAAAAK-NH<sub>2</sub>) contains four Lys residues to make the peptide soluble in water, as well as one Tyr residue for concentration determination using UV measurement at 280 nm. The mutation of Ala to another non-polar residue (Phe: 2F, Ile: 2I or Leu: 2L) at three specific positions in this sequence allows the helix content to vary from low (18%, 2F) to moderate (40%, 2I) and high (70%, 2L) respectively, based on molar ellipticity values obtained by ECD at 222 nm. By selecting one or the other variant, it is thus possible to investigate the capping effect of oligoureas on peptides of similar sequences and lengths but having different helix-forming abilities. ECD spectra of helically folded oligourea sequences (*P*-helices) bearing aliphatic side chains give rise to two characteristic Cotton effects at ca 188 nm (negative) and ca 203 nm (positive) and generally show ellipticity values close to zero around 220 nm and above this

**Table 1.** Sequences of peptides 1, 2I, 2F, 2L, and of all chimeric derivatives bearing an oligourea cap either at the N-terminus or at the C-terminus. Mean residue molar ellipticities (MRE, deg cm<sup>2</sup> dmol<sup>-1</sup> res<sup>-1</sup>) measured at 222 nm by ECD are shown for each sequence as well as the calculated percentage of helix content. In the peptide 1 series, the original Phe8 was replaced by a Tyr residue to enable precise determination of the peptide concentration using UV measurement at 280 nm.

	Sequence	pH	MRE	Helix		Sequence	MRE	Helix		
1	H <sub>2</sub> N-KETAAAKFERQHMS-NH <sub>2</sub>	3	4823	19%	2I	Ac-YKAAIAKAAIAKAAIAK-NH <sub>2</sub>	13638	40%		
		5	3699	16%			2I-1	Ac-A <sup>u</sup> A <sup>u</sup> A <sup>u</sup> YKAAIAKAAIAKAAIAK-NH <sub>2</sub>	16200	48%
		7	2636	13%			2I-2	Ac-YKAAIAKAAIAKAAIAK <sup>u</sup> A <sup>u</sup> A <sup>u</sup> -NH <sub>2</sub>	18702	54%
1-Ac	Ac-KETAAAKYERQHMS-NH <sub>2</sub>	3	10299	34%	2I-3	Ac-V <sup>u</sup> V <sup>u</sup> V <sup>u</sup> YKAAIAKAAIAKAAIAK-NH <sub>2</sub>	26014	73%		
		5	9056	30%			2I-4	Ac-YKAAIAKAAIAKAAIAK <sup>u</sup> V <sup>u</sup> V <sup>u</sup> -NH <sub>2</sub>	21640	62%
		7	7075	25%			2I-5	Ac-A <sup>u</sup> A <sup>u</sup> A <sup>u</sup> YKAAIAKAAIAKAAIAK-NH <sub>2</sub>	23940	68%
1-1	Ac-A <sup>u</sup> A <sup>u</sup> A <sup>u</sup> KETAAAKYERQHMS-NH <sub>2</sub>	3	8459	29%	2I-6	Ac-YKAAIAKAAIAKAAIAK <sup>u</sup> A <sup>u</sup> A <sup>u</sup> -NH <sub>2</sub>	22934	65%		
		5	7620	26%			2I-7	Ac-E <sup>u</sup> A <sup>u</sup> K <sup>u</sup> YKAAIAKAAIAKAAIAK-NH <sub>2</sub>	25468	72%
		7	5641	21%			2I-8	Ac-YKAAIAKAAIAKAAIAK <sup>u</sup> E <sup>u</sup> K <sup>u</sup> -NH <sub>2</sub>	20236	58%
1-2	Ac-KETAAAKYERQHMSA <sup>u</sup> A <sup>u</sup> A <sup>u</sup> -NH <sub>2</sub>	3	11175	36%	2I-9	Ac-K <sup>u</sup> A <sup>u</sup> E <sup>u</sup> YKAAIAKAAIAKAAIAK-NH <sub>2</sub>	22819	65%		
		5	10829	35%			2I-10	Ac-YKAAIAKAAIAKAAIAK <sup>u</sup> A <sup>u</sup> E <sup>u</sup> -NH <sub>2</sub>	20292	58%
		7	8460	29%						
1-3	Ac-V <sup>u</sup> V <sup>u</sup> V <sup>u</sup> KETAAAKYERQHMS-NH <sub>2</sub>	3	7966	27%	2F	Ac-YKAAF <sup>u</sup> AKAAFAKAAFAK-NH <sub>2</sub>	6132	18%		
		5	7288	26%			2F-1	Ac-A <sup>u</sup> A <sup>u</sup> A <sup>u</sup> YKAAF <sup>u</sup> AKAAFAKAAFAK-NH <sub>2</sub>	8011	24%
		7	5417	20%			2F-2	Ac-YKAAF <sup>u</sup> AKAAFAKAAFAK <sup>u</sup> A <sup>u</sup> A <sup>u</sup> -NH <sub>2</sub>	7753	23%
1-4	Ac-KETAAAKYERQHMSV <sup>u</sup> V <sup>u</sup> V <sup>u</sup> -NH <sub>2</sub>	3	12063	39%	2L	Ac-YKAALAKAALAKAALAK-NH <sub>2</sub>	23219	70%		
		5	11497	37%			2L-1	Ac-A <sup>u</sup> A <sup>u</sup> A <sup>u</sup> YKAALAKAALAKAALAK-NH <sub>2</sub>	26950	82%
		7	9435	31%			2L-2	Ac-YKAALAKAALAKAALAK <sup>u</sup> A <sup>u</sup> A <sup>u</sup> -NH <sub>2</sub>	33628	100%

value. We thus assumed that the contribution of the oligourea chromophore to the ECD signal at 222 nm, characteristic of the formation of an  $\alpha$ -helix, would be negligible and that we could use  $[\Theta]_{222}$  to monitor  $\alpha$ -helix formation and obtain a rough estimate of the  $\alpha$ -helical content in chimeras.

### Effect of Ala<sup>u</sup>Ala<sup>u</sup>Ala<sup>u</sup> Triurea Caps on $\alpha$ -Helix

We first prepared chimeric versions of peptides 1, 2F, 2I and 2L with an Ala<sup>u</sup>Ala<sup>u</sup>Ala<sup>u</sup> triad (i.e. triureas with methyl side chains) linked either at the N- or the C-terminus of the peptide (Table 1). Chimeras were obtained using standard solid-phase synthesis methods on a Rink amide resin. The elongation of the oligourea segment was performed by successive couplings of succinimidyl (2-azidoethyl)carbamate derivatives under microwave assistance followed by the reduction of the azido group under Staudinger conditions as previously described.<sup>[24]</sup> After cleavage from the resin, resulting oligomers were purified using C18 RP-HPLC and their identity was confirmed by mass spectrometry.

To evaluate the effect of the oligourea cap on the fused peptide segment, we first recorded ECD spectra of compound 1 at 3 °C in a 1 mM phosphate-citrate buffer containing 0.1 M NaCl (at pH 3, 5 and 7; 30–50  $\mu$ M oligomer concentration) (Figures S1–S3). The % helix values calculated from ECD spectra are reported in Table 1.<sup>[13a]</sup> When 1 is capped by a triurea

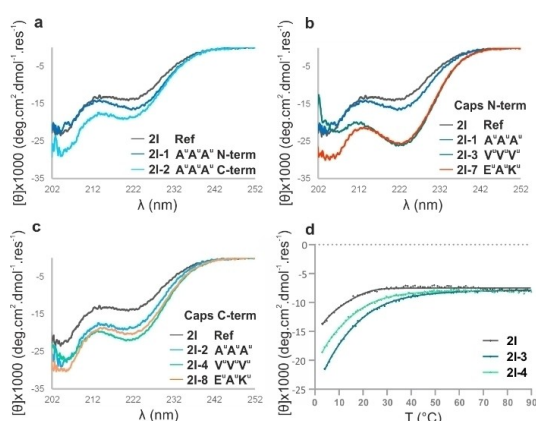
bearing Ala side chains at its N-terminus (compound 1-1), the stabilization of the sequence increases by 10 percent points (p.p.), slightly less than an acetyl cap according to the CD spectra obtained at pH 3, 5 and 7. One of the crucial interactions to maintain helicity in the segment 3–13 of the isolated S-peptide is the Glu2-Arg10 salt bridge that makes the first amino terminal residues twisted out of the helix.<sup>[25]</sup> Any template that would force the N-terminal extremity to be  $\alpha$ -helical is expected to break the kink after the Thr3 residue and thus prevent the salt bridge formation which may impact the overall helicity. The N-terminal triurea cap seems therefore to be able to compensate quite efficiently this loss of interaction and propagate further the helix. When the oligourea cap is placed at the C-terminus (compound 1-2), a substantial increase of the helix content (up to 120% increase) is also obtained at all pH values, meaning that the helix stabilization effect can also develop from this extremity despite the presence of the natural “helix stop” signal at Met13 and the overall low propensity helix propensity of residues 13 to 15. It is striking that this effect is even more pronounced than when the foldamer cap is placed at the N-terminus. This finding if general is of particular interest as to our knowledge, examples of C-terminal capping motifs for effective  $\alpha$ -helix induction are rare.<sup>[13b,26]</sup>

To confirm these observations on another peptide of low helix content (2F), and to evaluate the impact on peptides with a higher helix content (2I and 2L), we recorded ECD spectra of compounds 2X-1 and 2X-2 (X = I, F, L) at 0 °C in a 1 mM pH 7.0

phosphate buffer containing 1 M NaCl (30–50  $\mu$ M oligomer concentration). The results support the conclusion that the Ala<sup>u</sup>Ala<sup>u</sup>Ala<sup>u</sup> triurea cap installed at the peptide N- or C-terminus substantially enhances helicity in all three sequences from low to high helix content (Table 1, Figures 3a, S4 and S5). However, the effect is more pronounced when the  $\alpha$ -helix content increases. Indeed, for peptide 2F which is characterized by a low helix propensity, the gain is only around 5 p.p., whereas it reaches 14 p.p. and 30 p.p. for peptide 2I and 2L respectively. Remarkably, in the case of peptide 2I and 2L, the capping effect is maximized when the triurea cap is placed at the C-terminus versus the N-terminus (compare 54% with 48% in the case of 2I and 100% with 82% in the case of 2L).

### Effects of Sequence Variations in the Foldamer Capping Box

We next turned our attention to the nature and the length of the oligourea cap. We first tested sequence variations and thought to replace Ala<sup>u</sup> residues by Val<sup>u</sup> residues. The idea is that oligourea side chain branching, similar to what has been reported for beta amino acids, could increase oligourea helix stability and indirectly  $\alpha$ -helix content.<sup>[27]</sup> We thus prepared capped peptides derived from sequences 1 and 2I with a Val<sup>u</sup> triad added at either the N- or the C-terminus of the peptide. In the case of the ribonuclease S-peptide, CD spectra showed again a greater effect with the cap at the C-terminus (1-4) to reach effective stabilization, similar to 1-2. In the case of the model peptide 2I, we observed a stronger capping effect of the (Val<sup>u</sup>)<sub>3</sub> compared to (Ala<sup>u</sup>)<sub>3</sub>, with a gain of 33 p.p. vs 8 p.p. at the N-terminus (2I-3 vs 2I-1), and of 22 p.p. vs 14 p.p. at the C-terminus (2I-4 vs 2I-2) (Figure 3b and c). The CD thermal-melting curves of 2I, 2I-3 and 2I-4 measured at 222 nm confirmed that the oligourea cap increases the thermodynamic stability of the peptide, with a more pronounced effect at the N-terminus than the C-terminus (Figure 3d).



**Figure 3.** ECD spectra of compounds derived from peptide 2I comparing the effect of (a) the position of the Ala<sup>u</sup>Ala<sup>u</sup>Ala<sup>u</sup> triurea cap at the N-terminus (2I-1) vs C-terminus (2I-2); (b) the sequence of the triurea cap at the N-terminus: Ala<sup>u</sup>Ala<sup>u</sup>Ala<sup>u</sup> (2I-1) vs Val<sup>u</sup>Val<sup>u</sup>Val<sup>u</sup> (2I-3) and Glu<sup>u</sup>Ala<sup>u</sup>Lys<sup>u</sup> (2I-7) and (c) the sequence of the triurea cap at the C-terminus: Ala<sup>u</sup>Ala<sup>u</sup>Ala<sup>u</sup> (2I-2) vs Val<sup>u</sup>Val<sup>u</sup>Val<sup>u</sup> (2I-4) and Glu<sup>u</sup>Ala<sup>u</sup>Lys<sup>u</sup> (2I-8). (d) CD thermal-melting curves of the peptides 2I, 2I-3 and 2I-4 at 222 nm.

We then decided to evaluate the influence of the length of the oligourea cap. A tetraurea Ala<sup>u</sup> segment has been added at each of the two termini of the compound 2I in order to initiate a second helix turn. The level of stabilization observed was very similar to that obtained with the triurea Val<sup>u</sup> segment – with even a slightly stronger effect at the C-terminus but a slightly lower efficiency at the N-terminus. The additional Ala<sup>u</sup> residue thus compensate for the lower capping effect of Ala<sup>u</sup> triurea sequence compared to the cognate Val<sup>u</sup> sequence. We wondered finally if the nature of the oligourea could be tuned from a hydrophobic to a more water-soluble sequence bearing ionizable side chains for further stabilization of the urea helix. A triurea cap able to form a salt bridge between residues *i* and *i* + 2 has thus been designed, and the two palindromic triads Glu<sup>u</sup>Ala<sup>u</sup>Lys<sup>u</sup> and Lys<sup>u</sup>Ala<sup>u</sup>Glu<sup>u</sup> investigated. An increase of respectively 32 and 25 p.p. has been obtained when the two ionizable caps are fused at the N-terminus (2I-7 and 2I-9) and of 18 p.p. for both caps at the C-terminus (2I-8 and 2I-10) (Table 1, Figures 3b, c and S6).

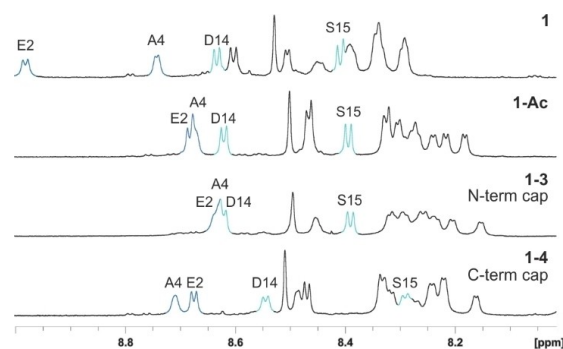
### Further Evidence of the $\alpha$ -Helix Stabilization Through Structural Characterization of Peptide Sequences by NMR

To evaluate the local  $\alpha$ -helix stabilization effect of the oligourea cap within the fused peptide sequence, comprehensive characterizations of the reference compounds from both series, 1 and 2I, as well as the corresponding peptides capped with the triurea Val<sup>u</sup>, at either the N- or the C-terminus, were performed using 1D and 2D <sup>1</sup>H, <sup>13</sup>C and <sup>15</sup>N NMR spectroscopies. Experiments have been conducted at a concentration of 1 mM in buffered solution (either a 20 mM phosphate-citrate buffer at pH 5.0 containing 0.1 M NaCl at 276 K, or a 20 mM phosphate buffer at pH 7.4 containing 1 M NaCl at 273 K for the peptides of the two series 1 and 2 respectively). 2D-TOCSY spectra at 700 MHz were used to assign the spin system of each amino acid and ROESY spectra allowed the determination of the sequential residue connectivity. Spectral overlap on compound 2I prevented unambiguous assignment of certain Ala residue positions in the sequence. Thus, the peptide 2I containing <sup>15</sup>N-labelled Ala residues at positions 3, 8 and 13 was synthesized for precise sequence identification. NMR chemical shift values of <sup>1</sup>H and <sup>13</sup>C nuclei were computed to probe secondary structure propensities using POTENCI (Prediction Of TEMperature, Neighbor and pH Corrected shifts for Intrinsically disordered proteins), a library developed by the Mulder lab to generate the random coil chemical shifts as reference values.<sup>[28]</sup> Local structural information can be extracted when comparing the observed chemical shift of a folded peptide segment with its predictive random coil value, since the measured deviation gives the relative tendency for each residue of the primary sequence to adopt either a transient helical or extended conformation. The POTENCI database is derived from the nuclear shielding observed for protein regions that are not involved in regular secondary structure elements and is thus based on experimental data. This prediction method considers intrinsic factors, such as the influence of the nearest and next-

nearest neighbor residues, but also the pH, temperature or ionic strength dependency that affect the protonation state of the side chains, and thus, the predicted random coil values. The most reliable chemical shifts to assess  $\alpha$ -helical secondary structure in decreasing order are  $^{13}\text{C}_\alpha$ ,  $^{13}\text{CO}$  (which give a positive shift) and  $^1\text{H}_\alpha$  (which gives a negative shift). Carbon atoms are indeed less sensitive than protons to hydrogen-bonding or other local environment perturbations likely to interfere with the chemical shift value. Analysis of the  $^{13}\text{C}_\alpha$  and  $^1\text{H}_\alpha$  secondary chemical shifts (i.e.  $\Delta\delta = \delta_{\text{obs}} - \delta_{\text{random coil}}$ ) for the compounds in the two series, **1** and **2I**, confirms in both cases the increase in helicity for the capped-peptides (Figure 4). In the Baldwin peptide series, the  $\Delta\delta^{13}\text{C}_\alpha$  values show higher stabilization effect when the triurea Val<sup>U</sup> cap is placed at the C-terminus. But since this peptide is an artificial construct, it does not show the classical behavior of biological peptides, with helix fraying at both termini, which may impair the interpretation of the data. With respect to the sequences derived from the Ribonuclease A, the bell-shaped graphs are more consistent with protein-like behavior and the helix stabilization due to the oligourea capping more convincing. Consistent with the CD results, the NMR data demonstrate significant  $\alpha$ -helix stabilization occurring from the C-terminus, as evidenced by the distinctly higher  $\Delta\delta^{13}\text{C}_\alpha$  values observed for **1-4** compared to those of S-peptide **1** or the acetylated compound **1-Ac**. The  $^1\text{H}$  NMR spectra in the 8–9 ppm range also support  $\alpha$ -helical stabilization for the capped peptide sequences derived from **1**. These spectra exhibit an upfield shift and broadening of the signals of the amide NHs, characteristic features indicative of a well-defined and less dynamic structure (Figure 5). NOE signals within the Val<sup>U</sup>Val<sup>U</sup>Val<sup>U</sup> triad of compound **1-4** and at the peptide-oligourea junction, e.g. between the His12 and Val16 side chains, are consistent as well, with the C-terminus cap adopting a helical conformation (Figure S9).

## Conclusions

In summary, the results obtained by ECD and NMR spectroscopy demonstrate the capability of short oligourea segments, fused at either end of a peptide sequence, to effectively stabilize the

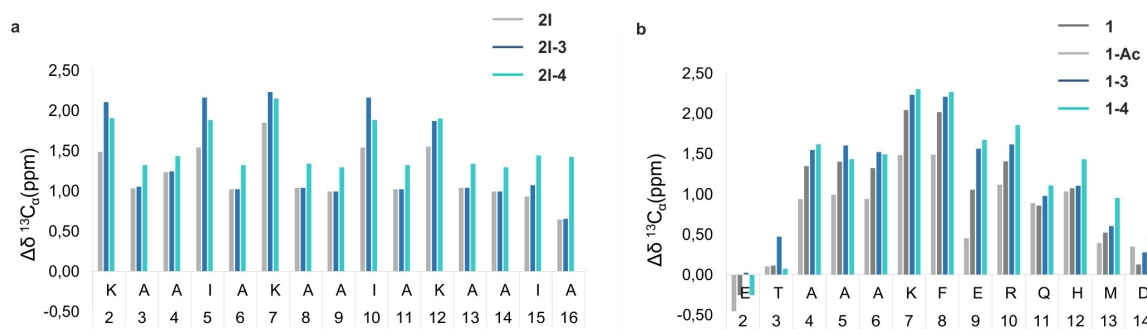


**Figure 5.** Superimposition of amide NH resonances in the  $^1\text{H}$  NMR spectra of compounds **1**, **1-Ac**, **1-3** and **1-4** (700 MHz, NMR buffer). Some characteristic signals of the C-terminal residues of the peptide are indicated in blue and of the N-terminal residues in cyan.

formation of an  $\alpha$ -helix conformation in aqueous solution. This foldamer capping box proved to be highly versatile, since efficient stabilization was obtained regardless of the position (at both C- and N-terminal extremities of the peptide), the length (triad vs tetrad) or the nature (hydrophobic vs hydrophilic, short vs hindered side chains) of the oligourea segment. This foldamer capping box, which is easily accessible and can be carefully tuned, is poised to be a valuable tool for many biologically relevant applications. It is particularly useful in situations where constrained peptides need to interact with proteins while minimizing perturbations, a potential advantage of helix capping over stapling. Our system compares favorably in terms of helix stabilization to the rare synthetic C-caps for peptide sequences of similar length reported in the literature,<sup>[15b,26c]</sup> providing a valuable alternative that does not require any cyclization step.

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**Figure 4.** Chemical shift changes ( $\Delta\delta$ , ppm) from random coil values for  $^{13}\text{C}_\alpha$  of peptides from (a) the Baldwin series (**2I**, **2I-3** and **2I-4**) and (b) the RNase A series (**1**, **1-Ac**, **1-3** and **1-4**).

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## Conflict of Interests

The authors declare no conflict of interest.

## Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

**Keywords:**  $\alpha$ -helix capping · Oligourea foldamer · Helical structures · Solid-phase synthesis

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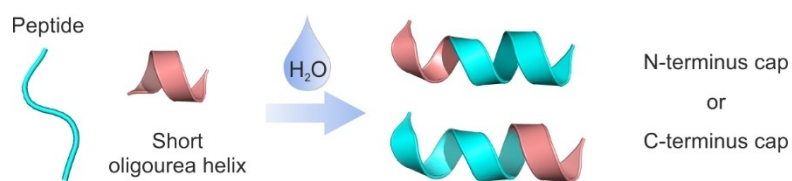
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## RESEARCH ARTICLE



Locked at either end: The high helix propensity of short oligourea sequences allows efficient capping at both the N- and C-terminus of water-

soluble peptide sequences. This extrahelical stabilization is a versatile tool for modulating the conformation of bioactive peptides.

*L. Mauran, C. Assailly, S. R. Goudreau, B. Odaert, G. Guichard\*, M. Pasco\**

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**Short Oligourea Foldamers as N- or C-Caps for Promoting  $\alpha$ -Helix Formation in Water**

