Stable Helical Secondary Structure in Short-Chain *N*,*N*′-Linked Oligoureas Bearing Proteinogenic Side Chains**

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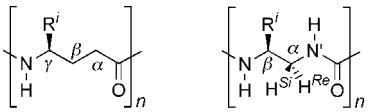
Dedicated to Professor Dieter Seebach on the occasion of his 65th birthday

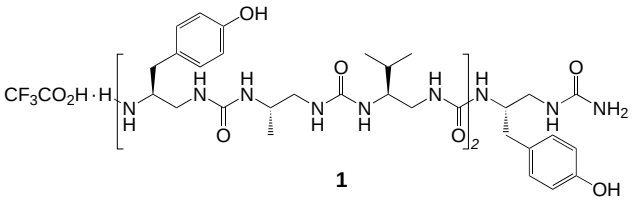
The functional diversity in proteins, although mediated by sophisticated tertiary and quaternary structures, relies on a small set of distinct secondary structural elements: sheets, helices, and turns. Unnatural oligomeric scaffolds designed to reproduce or mimic these essential protein features while retaining the chemical diversity of amino acid side chains have gained considerable interest in recent years, with potential application in drug discovery.[1] Work published by the research groups of Seebach, Gellman, and Hanessian has revealed that short-chain peptides consisting exclusively of enantiopure β- or γ-amino acids with defined substitution patterns can form stable helical or pleated-sheet-type structures in solution and in the solid state.[2–4] In particular, γ-peptides such as **A**, with chirality centers from an L-amino



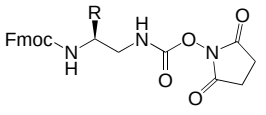
A **B**

acid form a right-handed (*P*)2.6$_{14}$ helix of about 5 Å pitch in which both ethane bonds are in a (+)-synclinal (si) conformation.[4a, 4c] Enantiopure *N*,*N*′-linked oligoureas of general formula **B**, obtained by formal replacement of the $^{\alpha}$C atom of γ-amino acid residues in **A** by a nitrogen atom, were originally described in 1995 by Burgess et al. and used as novel peptide backbone mimetics.[5]

Although *N*,*N*′-linked oligoureas are readily accessible by solid-phase synthesis using appropriate monomers,[5, 6] their conformational preferences and their folding propensities have not been elucidated so far. We reasoned that, subject to an antiperiplanar arrangement of the N′H and $^{\alpha}$CH^{Si} atoms (in **B**), the substitution of NH for the $^{\alpha}$CH$_2$ group of γ-amino acid residues (**A** → **B**), should be sterically compatible with an approximate 2.6-helical backbone. Herein, we report the solution structure of heptaurea **1** bearing side chains of natural amino acids Ala, Val, and Tyr.



1



2

2	R
a	Me
b	*i*Pr
c	Bn(O*t*Bu)

Oligourea **1** was prepared as described previously by solid-phase synthesis using succinimidyl carbamate derivatives **2a–c** (Fmoc = 9-fluorenylmethoxycarbonyl, Bn = benzyl) as activated monomers.[6d] The structure of **1** was investigated by 1D and 2D NMR spectroscopy in [D$_5$]pyridine. This solvent was used because of the larger dispersion of chemical shifts compared to those found in nonaromatic solvents such as CD$_3$OH or (CD$_3$)$_2$SO as a result of the ring current effect in the former. The spin systems of all seven residues were unambiguously identified from DQF-COSY and TOCSY experiments. The sequence was assigned on the basis of ROESY experiments (mixing time = 300 ms) and was deduced from the strong N′H$_i$-NH$_{i+1}$ NOE connectivities observed along the urea backbone. A number of data relevant to the structure determination were extracted from 1D and COSY spectra. The *J*(NH,$^{\beta}$CH) values for residues 2–7 were large ($J > 9$ Hz), which indicates a restricted rotation around the N–$^{\beta}$C bond and a nearly antiperiplanar arrangement of the corresponding protons. Analysis of 1D spectra recorded between 288 and 368 K at 10 K increments revealed that the coupling values are only weakly affected by temperature changes and remain large over the temperature range studied.[7]

Furthermore, the geminal protons at the $^{\alpha}$C atom exhibited significant differences in their chemical shift ($1.3 < \Delta\delta < 1.6$ ppm for central residues 3–6 and $\Delta\delta = 1.0–1.1$ ppm for residues 2 and 7). This chemical shift difference, which reflects a defined and distinct spatial arrangement for both diastereotopic $^{\alpha}$CH protons along the backbone, remained constant over the range 271–368 K. Careful examination of the $^3J(^{\alpha}\mathrm{CH_2},\mathrm{N'H})$ and $^3J(^{\beta}\mathrm{CH},^{\alpha}\mathrm{CH_2})$ values for residue 3[8] at 332 K (Table 1) revealed that the downfield diastereotopic $^{\alpha}$CH proton has a large coupling constant with the N′H proton

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Table 1. Chemical shifts and coupling constants for backbone protons of residue 3.

Proton	δ	J [Hz]
NH	6.64	$^{3}J(\mathrm{NH},{}^{\beta}\mathrm{CH}) = 10$
${}^{\beta}$CH	4.02	$^{3}J({}^{\beta}\mathrm{CH},{}^{\alpha}\mathrm{CH}^{Si}) = 2.5$, $^{3}J({}^{\beta}\mathrm{CH},{}^{\alpha}\mathrm{CH}^{Re}) = 11$
${}^{\alpha}\mathrm{CH}^{Re}$	2.53	$^{2}J({}^{\alpha}\mathrm{CH}^{Re},{}^{\alpha}\mathrm{CH}^{Si}) = -14$
${}^{\alpha}\mathrm{CH}^{Si}$	3.96	
N′H	6.66	$^{3}J(\mathrm{N'H},{}^{\alpha}\mathrm{CH}^{Si}) = 10$, $^{3}J(\mathrm{N'H},{}^{\alpha}\mathrm{CH}^{Re}) = 3.5$

(ca. 10 Hz) but a small one with the corresponding ${}^{\beta}$CH proton (ca. 2.5 Hz), which implies that this ${}^{\alpha}$CH proton is nearly antiperiplanar to N′H and synclinal to ${}^{\beta}$CH.[8]

These data strongly suggested that oligourea **1** adopts a well-defined and stable secondary structure in solution. To gain more information about the three-dimensional structure of heptaurea **1** ROESY experiments were acquired at 332 K with a mixing time of 300 ms. A total of 106 NOE interactions were extracted and classified in three distance categories according to their cross-peak volume. Out of the 26 inter-residue NOE interactions, 17 are from residue i to residue $i+2$. Particularly noteworthy were the medium-to-strong NOE interactions observed between ${}^{\beta}\mathrm{CH}_i$ and NH_{i+2} for $i = 2-5$ and between ${}^{\beta}\mathrm{CH}_i$ and $\mathrm{N'H}_{i+2}$ for $i = 2-5$. Unambiguous assignment of the diastereotopic ${}^{\alpha}$CH protons was possible for all residues by direct comparison of intraresidue NOE interactions between the NH and both ${}^{\alpha}$CH protons. The downfield ${}^{\alpha}$CH proton displayed a weaker NOE correlation with NH than did the corresponding upfield proton, and could thus be assigned to ${}^{\alpha}\mathrm{CH}^{Si}$.

The NOE interactions and J values were used as distance and dihedral angle restraints, respectively, in a 100-ps simulated-annealing protocol using the AMBER6 suite of programs.[9] Calculations converged well and yielded a set of 20 structures with no NOE violation greater than 0.3 Å and no dihedral angle violation greater than 5°. A bundle of the best 20 structures chosen to be representative of the structure in solution is depicted in Figure 1.

The secondary structure of heptaurea **1** is a right-handed 2.5 helix with a pitch of approximately 5.1 Å that is well defined for residues 2–6, with residue 1 and, to a lesser extent, residue 7 being flexible. Although the structure shares overall similarity with the helical backbone of γ-peptides, the helix of **1** displays a more complicated intramolecular hydrogen-bonding pattern that is characterized by the simultaneous presence of 12- and 14-membered pseudocycles resulting from $\mathrm{C{=}O}_i \cdots \mathrm{HN'}_{i+2}$ and $\mathrm{C{=}O}_i \cdots \mathrm{HN}_{i+3}$ hydrogen bonds (Figure 2).

However, $\mathrm{C{=}O}_i$ is not equidistant from $\mathrm{N'H}_{i+2}$ and NH_{i+3}, and statistics performed on the calculated 20 structures

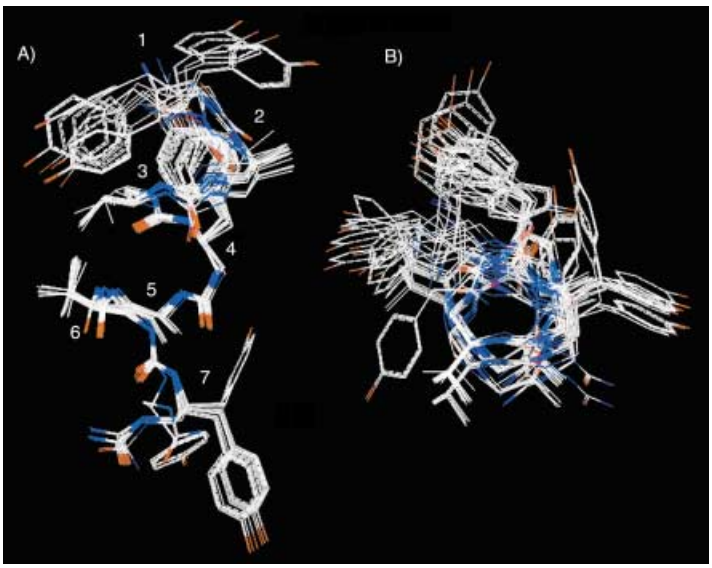

Figure 1. Bundle of the 20 best structures of lowest energy A) viewed along the helix axis, and B) top view. Root-mean-square (rms) differences of bond and angle deviations from ideality were less than 0.02 Å and 3°, respectively. Rms deviations for all heavy backbone atoms from a mean structure were 0.56 ± 0.18 for residues 1–7 and 0.36 ± 0.19 Å for residues 2–7.

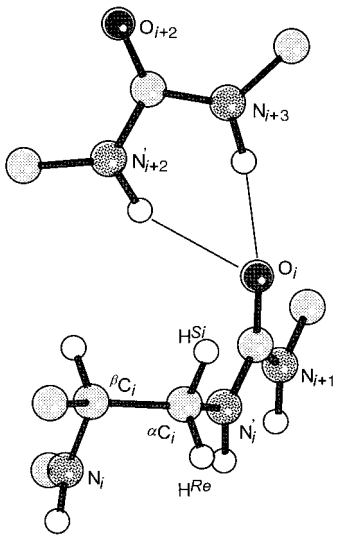

Figure 2. Atom numbering and schematic representation of the hydrogen-bonding pattern as found in the 2.5 helix of oligourea **1**.

(Table 2) suggest that the 14-membered H-bonded rings are more populated than the corresponding 12-membered rings, at least in the inner part of the helix.[10]

Table 2. Estimated $\mathrm{C{=}O}_i \cdots \mathrm{HN'}_{i+2}$ and $\mathrm{C{=}O}_i \cdots \mathrm{HN}_{i+3}$ hydrogen-bonding pattern for $i = 1-4$ based on statistical analysis of the final 20 structures of lowest energy.[a]

	$\mathrm{C{=}O}_i \cdots \mathrm{HN'}_{i+2}$			$\mathrm{C{=}O}_i \cdots \mathrm{HN}_{i+3}$		
	H-bond occurence [%]	Average N-H-O angle	Average d(N-O) [Å]	H-bond occurence [%]	Average N-H-O angle	Average d(N-O) [Å]
$i = 1$	50	142	3.10	55	157	2.98
$i = 2$	35	137	3.41	90	166	3.13
$i = 3$	50	139	3.46	100	160	2.91
$i = 4$	50	156	3.24	100	167	2.98

[a] The geometry of a hydrogen bond is defined by d(N-O) < 3.25 Å and N-H-O angle > 120°.

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In conclusion, by determining the solution structure of **1**, we have demonstrated that *N*,*N*′-linked oligoureas of general formula **B** belong to the growing family of non-natural non-peptide oligomers with defined and predictable secondary structures. Although heptaurea **1** forms a (*P*)2.5 helix of approximately 5.1 Å pitch that is closely related to the $(P)2.6_{14}$ helix of approximately 5 Å pitch of corresponding γ^4-peptides,[4a] it is worth noting that both NH groups within the same urea linkage may participate in intramolecular hydrogen bonding to the same C=O group. The knowledge of the three-dimensional structure of **1** is likely to be useful for the de novo design of oligoureas with controlled shape and defined biological activities.

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[7] Similarly, Seebach and co-workers have shown in the case of a β-heptapeptide that $J(\mathrm{NH},{}^{\beta}\mathrm{CH})$ values decrease only slowly upon an increase of the temperature from 298 to 353 K. A decrease of about 0.4 Hz was observed for the central residues (3 – 6) and about 0.7 Hz for the flanking residues 2 and 7: K. Gademann, B. Jaun, D. Seebach, R. Perozzo, L. Scapozza, G. Folkers, *Helv. Chim. Acta* **1999**, *82*, 1 – 11.

[8] In the case of residue 3, $^3J({}^{\alpha}\mathrm{CH}_2,\mathrm{N}'\mathrm{H})$ values were extracted directly from the 1D NMR spectrum, but decoupling experiments were required to access precise $^3J({}^{\beta}\mathrm{CH},{}^{\alpha}\mathrm{CH}_2)$values. Severe overlaps in the ${}^{\alpha}\mathrm{CH}$ region precluded measurement of the $^3J({}^{\beta}\mathrm{CH},{}^{\alpha}\mathrm{CH}_2)$ values for other residues. However, qualitative examination of the COSY spectrum reveals similar vicinal coupling patterns.

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[10] Further experimental evidence for $\mathrm{C{=}O}_i\cdots\mathrm{HN}'_{i+2}$ and $\mathrm{C{=}O}_i\cdots\mathrm{HN}_{i+3}$ hydrogen bonds is gained from the determination of temperature coefficients. $\mathrm{N}'\mathrm{H}_{i+2}$ for $i=1-4$ and NH_{i+3} for $i=1-4$ have temperature coefficients with absolute values <4 ppb K^{-1}, which indicates limited solvent accessibility. In addition, temperature coefficients of $\mathrm{N}'\mathrm{H}_{i+2}$ for $i=2-4$ ($3.5\ \mathrm{ppb\,K^{-1}} < -\Delta\delta/\Delta T < 3.8\ \mathrm{ppb\,K^{-1}}$) have higher absolute values than those of NH_{i+3} for $i=2-4$ ($1.5\ \mathrm{ppb\,K^{-1}} < -\Delta\delta/\Delta T < 2.5\ \mathrm{ppb\,K^{-1}}$). This trend could suggest an increased solvent accessibility for $\mathrm{N}'\mathrm{H}_{i+2}$ compared to NH_{i+3}.

Self-Assembling Organic Nanotubes from Enantiopure Cyclo-*N*,*N*′-Linked Oligoureas: Design, Synthesis, and Crystal Structure

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Assembly of self-complementary cyclo-oligomeric subunits through noncovalent processes (for example, hydrogen bonding, aromatic stacking) has emerged as a powerful strategy to generate artificial organic nanotubular structures.[1, 2] Highly functionalized tubular assemblies based on peptides have attracted much interest recently in this area. Ghadiri and co-workers have compellingly demonstrated that 24- and 30-membered-ring cyclo-α-peptides with an even number of alternating D- and L-amino acids stack in an antiparallel β-sheet-like arrangement to form hydrogen-bonded tubular structures, that is, "peptide nanotubes".[2–4] Remarkably, related cyclic peptides consisting exclusively of β-amino acids[5, 6] (16- and 12-membered ring), of alternating α- and β-amino acids[7] (14-membered ring), or of vinylogous δ-amino acids[8] (18-membered ring) also form tubular stacks.

We have shown previously[9] that linear *N*,*N*′-linked oligoureas **A** consisting of homochiral residues adopt a stable 2.5-helical secondary structure in solution. The helix is characterized by the simultaneous presence of 12- and 14-membered hydrogen-bonded rings resulting from the capacity of the urea group to establish self-complementary bidirec-

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