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# RDC Enhanced NMR Spectroscopy in Organic Solvent Media: The Importance for the Experimental Determination of Periodic Hydrogen Bonded Secondary Structures

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$\beta$-Peptides that exhibit discrete secondary folds ('foldamers'),[1] such as helices, strands, and turns similar to proteins, have been the subject of enormous interest for the past decade.[2] Recent seminal reports by the research groups of Gellman, Seebach, and Schepartz unveiled $\beta$-peptide based biofunctional foldamers (HIV-inhibitors,[3] BH3 and MHC recognition ligands,[4] and quaternary helical bundles[5]) and have attributed further impetus to this field. Precise solution state structural characterization of these foldamers is very important, which, however, has been carried out so far only by using conventional short-range NMR constraints, viz., distances from NOESY/ROESY cross-peak intensities and dihedral angles from $^3J_{HH}$ couplings.

As has been shown recently,[6] the measurement of residual one-bond dipolar couplings (RDCs) in small organic molecules with its access to coherent and long-range structural information can lead to profound improvements in the determination of relative configuration[7] and especially the conformation of small organic molecules. The recent advent of stretched polymer gel media[8,9] in organic solvents, with tunable alignment features, has disclosed new opportunities for accurate structural elucidation of such compounds. However, a logical extension of RDC-enhanced NMR spectroscopy to peptidomimics that exhibit periodic secondary structures and hydrogen bonded compact foldings has not been explored so far. Here, we demonstrate the power of RDCs in structure verification and their necessity for obtaining a purely experimentally derived accurate secondary fold, even in the cases of highly rigidified $\beta$-peptidic foldamers in solution.

The foldamers studied here are the two homo-oligomers **1** and **2**, comprised of $\beta$-amino acid building blocks *cis*-$\beta$-norbornene and *trans*-$\beta$-norbornene, respectively (Figure 1). It is known from earlier ROE studies that **1** adopts a 6-strand,[10a] while a nucleation of 8-helical folding[10b] is predicted based on NOE-supported DFT calculations, for a trimer analogue[10c] of **2**. As the effects of c*is/trans* configuration around the $C_\alpha-C_\beta$ bond on the overall secondary folding are profound, these molecules serve as good examples for exploring residue based conformational preferences.[11]

Initially, we examined the highly constrained strand conformer of tetramer **1**. $^1J_{CH}$ and $^1J_{NH}$ coupling constants were measured in $CDCl_3$ as the isotropic solvent by using the CLIP-HSQC approach.[12] Corresponding one-bond $D_{CH}$ and $D_{NH}$ RDCs were derived from identical measurements in a stretched poly(dimethylsiloxane) (PDMS)/$CDCl_3$ gel.[8] Altogether 22 $D_{CH}$ and 3 $D_{NH}$ RDCs could be determined and fitted to the ROE-derived (Supporting Information (SI)) structure using the program PALES.[13] The fit showed an excellent agreement of experimental RDCs vs back-calculated RDCs with a correlation factor of $R = 0.982$ and the Cornilescu quality factor,[14] $Q = 0.135$ (SI). A subsequent RDC-enhanced structure refinement with the program XPLOR-NIH[15] using the susceptibility anisotropy (sani) potential only led to a marginal improvement of the fit ($R = 0.992$ and $Q = 0.081$) (SI). Apparently, conventional NMR restraints in this case contain already sufficient information for the determination of the correct fold, and RDCs can at least be used for an independent structure verification.

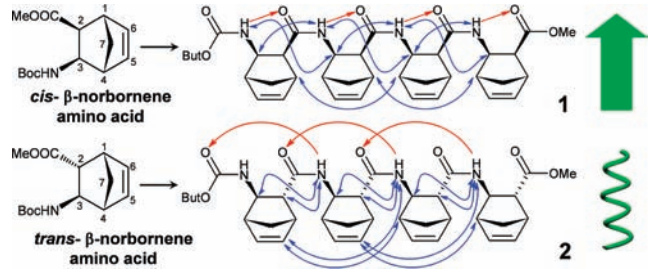


***Figure 1.*** Schematic view of *cis*- and *trans*-$\beta$-norbornene amino acid monomers and the tetramers **1** and **2** synthesized from them, respectively, with representation of hydrogen bonding (red curves) and characteristic ROEs (blue curves).

The situation changes for tetramer **2**. Highly downfield NH proton resonances (>9.0 ppm) with good dispersion are consistent with the presence of secondary folding and their plausible involvement in intramolecular hydrogen bonding in $CDCl_3$. This has been confirmed by DMSO titration studies (SI). The explicitly observed cross-peaks $^i$NH$-^{(i-1)}$H3, $^i$NH$-^{(i-1)}$H2, $^i$NH$-^{(i-2)}$H6, and $^i$H4$-^{(i-2)}$H6 in ROESY spectra are characteristic of either 8-helical or $2_8$-ribbon folding. The superimposed minimum energy structures (15 frames) obtained from restrained (4 $^3J_{HH}$ derived dihedral angles and 16 ROE-derived distances) MD simulations in isotropic $CDCl_3$ solvent have shown a uniform, seemingly well-defined pleated ribbon-like backbone conformation with inter-residue ($^i$NH to $^{(i-2)}$CO) 8-membered hydrogen bonded rings (SI).

To verify the obtained structure of **2**, we measured RDCs in two independent polymer gel-based alignment media, PS/$CDCl_3$[9a] and PDMS/$CDCl_3$.[8] Out of the 32 possible $D_{CH}$ RDCs 17 and 27 couplings could unambiguously be derived in a stretched PS/$CDCl_3$ gel and PDMS/$CDCl_3$ gel, respectively, whereas all of the 4 possible $D_{NH}$ couplings could be derived in both gel media. Unexpectedly, the PALES fit for the ROE-based structure showed a poor correlation of back-calculated vs experimental RDCs for the PS/$CDCl_3$ ($R = 0.665$, $Q = 0.640$) as well as the PDMS/$CDCl_3$ gel ($R = 0.805$, $Q = 0.429$) (Figure 2a). On the other hand, a dramatic improvement in the PALES fit (Figure 2b), with $R = 0.994$ and $Q = 0.056$, has been obtained for a refined structure (XPLOR-NIH) that includes RDCs from the PS/$CDCl_3$ gel as angular restraints. Similar improvement is observed for the refined structure with RDCs measured in the PDMS/$CDCl_3$ gel ($R = 0.992$ and $Q = 0.081$) (Figure 2b). Although none of the obtained

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structural models violate any ROE-derived distance restraints (>0.2 Å), the superposition of conventionally obtained (ROE-derived) and RDC-enhanced (i.e., ROE+RDC) minimum energy structures of **2** yields a poor rmsd larger than 1.74 Å (averaged over all atoms). The comparison of the top views of the ROE-derived (Figure 2c) and RDC-enhanced structures (Figure 2d) finally reveals a considerable deviation from a ribbon-like fold (Figure 2c) to the predicted left-handed 8-helix (Figure 2d), with 2.2 residues per turn.[10b,c] The measured dihedral angles (SI) are also consistent with the values reported earlier for 8-helical folding.[10c]

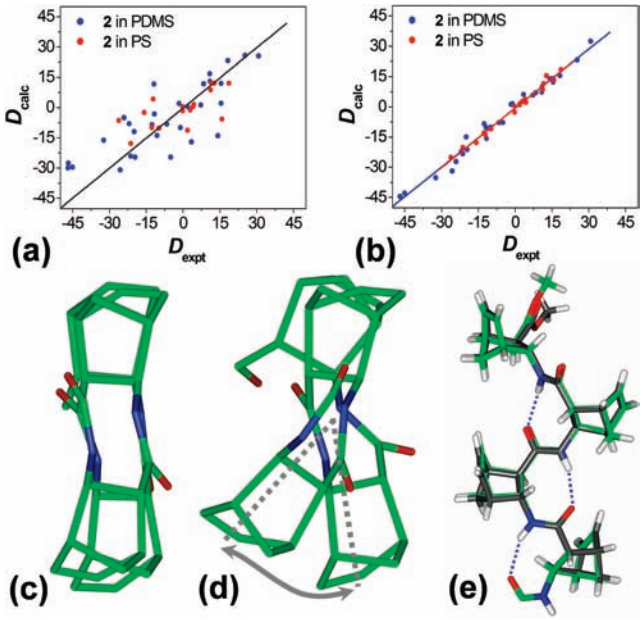


***Figure 2.*** Plot of experimental vs back-calculated $D_{CH}$ and $D_{NH}$ RDCs of **2** for the ROE-derived structure (a) and the RDC enhanced structure (b). The corresponding axial views of the ROE-derived and RDC-enhanced (i.e., ROE+RDC-derived) structures are shown in (c) and (d), respectively. Overlay of side view of **2** for the (ROE+RDC) structure (carbons in green) and the structure based only on RDCs (without ROEs) measured in PDMS/$CDCl_3$ (carbons in gray) is shown in (e).

The attempt for structural elucidation based on RDCs alone (without ROEs) for **2** has also resulted in an 8-helical structure that is practically identical to the ROE+RDC structure (rmsd ~0.1 Å), as evident in the overlay of these two structures (Figure 2e). The findings clearly reinstate the impact and predominance of the experimentally obtained RDCs on the precision of the obtained structure. It also suggests a scope for RDC-based structural design of compact secondary folds,[16] at least for the rigidified peptides. In contrast, the amount and quality of ROE-derived distance restraints for tetramer **2** alone do not allow the determination of a precise secondary structure.

In summary, we have employed RDC-enhanced NMR spectroscopy to unnatural peptidic secondary structures in organic solvent media. While the classically $^3J_{HH}$ and ROE-derived structure of tetramer **1** could be validated by experimentally obtained RDCs, such an exercise for the equally complex tetramer **2** did not lead to the correct fold, which could only be obtained by the inclusion of RDC-derived structural information. These findings revealed the sensitivity of RDCs to distinct hydrogen-bonded secondary folds, led by the residue level stereochemical changes, and also to the overall conformational changes even in short oligomers. The results clearly demonstrate the immense need of anisotropic NMR parameters as an independent source for structure validation in solution and also as restraints for precise structure calculations of periodic hydrogen bonded structures of unnatural peptides in particular and small organic compounds in general. It can be expected that their importance will further increase for more dynamic systems where RDCs already have shown their potential in the structure determination of proteins[17] and the configurational analysis of natural products.[7]

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**Supporting Information Available:** Experimental details, NMR, RDC data and alignment tensor parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

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