A Novel Peptide Fold: A Repeating βII′ Turn Secondary Structure

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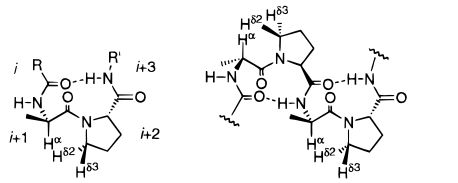
The synthesis of both natural and unnatural peptides that are designed to possess well-ordered structures in solution has been the focus of much effort for a number of years.[1] This interest is in part due to the intellectual challenge of understanding and controlling the many factors which contribute to the protein folding process, as well as the potential applications to the design of artificial proteins and bioactive agents. Within the context of seeking peptides which form ordered structures in solution, this paper describes conformational studies of RCO−(D-Xxx-Pro)$_n$-NHMe peptides that are designed to populate the as of yet unreported poly(βII′-turn) secondary structure in CD_2Cl_2. Central to the design strategy is the stability of the 10-membered cyclic hydrogen bond between residue *i* and *i* + 3 defining a βII′ turn in R-CO-D-Xxx-Pro-NHR′ (**1**) species.[2] In these structures, the minimization of pseudo A(1,3)-strain between the D-residue H^{α} and the proline δ-carbon restricts rotation of $\psi(i+1)$ to ∼−130°, as required for the formation of the critical hydrogen bond.[3] Examination of structure **1** suggested that poly(D-Xxx-Pro) peptides (**2**) could thus potentially populate a conformation characterized by a repeating βII′ turn unit.



1; βII′-turn **2**; poly(βII′-turn) secondary structure

Initial investigations began with CH_3CO-D-Ala-Pro-NHMe (**3**) since this motif has been shown by IR spectroscopy to preferentially populate a βII′-turn in chlorinated hydrocarbons.[2a] Specifically, the identification of critical conformational parameters by NMR spectroscopy including NOEs in this minimal turn unit would serve as a basis for the study of multiple-turn peptides. Accordingly, the NOESY spectrum of **3** exhibits NOEs consistent with the population of a βII′ turn.[4] It should first be noted that the NOEs between H^{α}(D-Ala2) and $H^{\delta 2}$(Pro3)/$H^{\delta 3}$(Pro3) are the strongest interresidue NOEs, consistent with an amide *trans* conformation. These strong NOEs also indicate that analogue **3** preferentially populates a conformation in which H^{α}(D-Ala2) and $H^{\delta 2}$(Pro3)/$H^{\delta 3}$(Pro3) are in close spatial proximity as expected for $\psi \sim -130°$, consistent with the minimization of pseudo A(1,3)-strain. The NOEs specifically critical to the assignment of a turn structure are a medium NOE between NH(Me) and $H^{\delta 2}$(Pro3) and a weak NOE between NH(Me) and H^{α}(D-Ala2) (Figure 1).[5] NH chemical shift perturbation trends observed from DMSO-d_6 titration of **3** are also consistent with the presence of the $i+3 \rightarrow i$ hydrogen bond (Table 1). Accordingly, the NH(Me) chemical shift exhibits less dependence on added DMSO-d_6 than that of NH(D-Ala2). To rule out the possibility that this solvent dependence and these NOEs are due to the formation of the cyclic seven-membered hydrogen bond characteristic of a γ-turn, the analogue **7** was also investigated. Compound **7** represents a suitable reference molecule since pseudo A(1,3)-strain should constrain the geometry between the isobutyryl H^{α} and the pyrrolidine δ-hydrogens as H^{α}(D-Ala2) is to the proline δ-hydrogens in **3**. Furthermore, analogue **7** has been reported by Gellman to be 75% H-bonded in a γ-turn geometry (25% non-H-bonded).[6] Interestingly, the NOESY spectrum of **7** shows neither the presence of the NH(Me)−H^{α}-isobutyryl NOE nor the NH(Me)−$H^{\delta 2}$(Pro) NOE.[7] These data thus support that the NOEs in **3** do not arise from a γ-turn or a non-H-bonded conformation but do arise from the population of a βII′ turn and may be used in the characterization of multiple-turn peptides.

Conformational studies were then extended to the peptides **4**−**6**, **8**, and **9**. Assignments of individual spin systems were accomplished from TOCSY or COSY spectra. Sequential assignments were obtained through analysis of NOESY (for **4**, **5**, **8**, and **9**) and ROESY (for **4**) spectra. Concentration dependence studies showed a stable chemical shift for the amide protons below 5 mM concentrations. The simplest extension of the βII′ motif is represented in the two-turn unit **4**, Ac-(D-Ala-Pro)$_2$−NHMe. The compilation of NOE data, H^{α} chemical shift data, and NH chemical shift solvent dependence for **4** provides compelling evidence for the population of the two-turn geometry when compared with the analogous data for **3**. The DMSO-d_6 titration data shows that NH(Me) and NH(D-Ala4) are significantly more solvent-shielded than NH(D-Ala2) as expected if NH(Me) and NH(D-Ala4) were involved in hydrogen bonds. That these hydrogen bonds aid in populating the respective turns is further supported by the presence of the NH(Me)−$H^{\delta 2}$(Pro5) and NH(Me)−H^{α}(D-Ala4) NOEs for the C-terminal turn and the NH(D-Ala4)−$H^{\delta 2}$(Pro3) and NH(D-Ala4)−H^{α}(D-Ala2) NOEs for the N-terminal turn (Figure 1). The two-turn structure **4** thus exhibits NOEs analogous to those found in one-turn structure **3**, which arise from a βII′ conformation. Further evidence that **4** populates the two-turn motif comes from comparison of H^{α} chemical shifts which are nearly identical for H^{α}(D-Ala2) in **3** and H^{α}(D-Ala2) in **4** (4.51 and 4.49 ppm, respectively) and also nearly identical for H^{α}(Pro3) in **3** and H^{α}-(Pro5) in **4** (4.43 and 4.45 ppm, respectively).

With the establishment that peptides **3** and **4** populate the one-turn and two-turn motifs respectively, some general statements

(1) For examples of secondary structure studies, see: (a) Tsang, K. Y.; Diaz, H.; Graciani, N.; Kelly, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 3988. (b) Diaz, H.; Espina, J. R.; Kelly, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 8316. (c) Seebach, D.; Overhand, M.; Kuhnle, F. N. M.; Martinoni; B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 913. (d) Seebach, D.; Ciceri, P. E.; Overhand, M.; Jaun, B.; Rigo, D.; Obere, L.; Hommel, U.; Amstutz, R.; Widmer, H. *Helv. Chim. Acta* **1996** *79*, 2043. (e) Appella, D. H.; Christianson, L. A.; Klein, D.; Powell, D. R.; Huang, X.; Barchi, J. J.; Gellman, S. H. *Nature* **1997**, *387*, 381. (f) Gung, B. W.; Zou, D.; Stalcup, A. M.; Cottrell, C. E. *J. Org. Chem.* **1999**, *64*, 2176. (g) Hanessian, S.; Luo, X.; Schaum, R.; Michnick, S. *J. Am. Chem. Soc.* **1998**, *120*, 8569. (h) Nowick, J. S.; Pairish, M.; Lee, I. Q.; Holmes, D. L.; Ziller, J. W. *J. Am. Chem. Soc.* **1997**, *119*, 5413. (i) Austin, R. E.; Maplestone, R. A.; Sefler, A. M.; Liu, K.; Hruzewicz, W. N.; Liu, C. W.; Cho, H. S.; Wemmer, D. E.; Bartlett, P. A. *J. Am. Chem. Soc.* **1997**, *119*, 6461.

(2) (a) Boussard G.; Marraud, M.; Aubry, A. *Biopolymers* **1979**, *18*, 1297. (b) Cung, M. T.; Vitoux, B.; Marraud, M. *New J. Chem.* **1987**, *11*, 503. (c) Kopple, K. D.; Go, A.; Schamper, T. J.; Wilcox, C. S. *J. Am. Chem. Soc.* **1973**, *95*, 6090. (d) Bean, J. W.; Kopple, K. D.; Peishoff, C. E. *J. Am. Chem. Soc.* **1992**, *114*, 5328.

(3) For examples of the use of pseudo A(1,3)-strain in the design of conformationally constrained peptides see: (a) Zhang, R.; Brownewell, F.; Madalengoitia, J. S. *J. Am. Chem. Soc.* **1998**, *120*, 3894. (b) Zhang, R.; Madalengoitia, J. S. *J. Org. Chem.* **1999**, *64*, 330.

(4) As a compromise between spin diffusion, zero quantum coherence, and signal-to-noise ratio a 400 ms mixing time was utilized in all NOESY experiments. More details are included in the Supporting Information.

(5) In the X-ray crystal structure of Piv-Pro-D-Pro-NHMe, which forms a βII′ turn in the solid state, the NH(Me)−$H^{\delta 2}$(D-Pro) distance is 3.0 Å: Aubry, A.; Vitoux, B.; Marraud, M. *Biopolymers* **1985**, *24*, 1089. In cyclo(D-Pro^1-Pro^2-Gly^3-Arg^4-Gly^5-Asp^6) the NOE derived lower and upper bounds for the NH(Gly3)−$H^{\delta 2}$(Pro2) distances are 2.46 and 3.14 Å respectively. In cyclo-(D-Pro^1-Pro^2-Arg^3-Gly^4-Asp^5-Gly^6) the lower and upper bounds for the NH-(Arg3)−$H^{\delta 2}$(Pro2) distances are 2.53 and 3.23 Å respectively (ref 2d). In as much as minimal spin diffusion is assumed in the present study, the analogous calculated distances for peptides in this study consistently ranged 2.8−3.2 Å.

(6) Liang, G.-B.; Rito, C. J.; Gellman, S. H. *Biopolymers* **1992**, *32*, 293.

(7) The NH−H^{α}(Pro) NOE exhibited by **7** is also exhibited in every potential turn unit in the multiple turn peptides.

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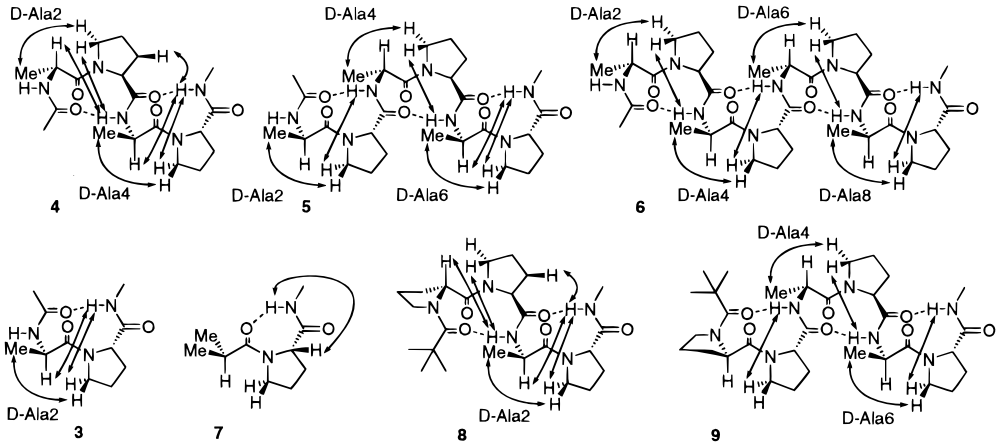

Figure 1. Selected NOEs.

Table 1. Change in NH Chemical Shift on Addition of DMSO-d_6 (100 μL) to 1.5 mM Peptide Solutions in 1 mL CD_2Cl_2 ($\Delta\delta_{solvent}$)

compd	NH	$\Delta\delta_{solvent}$ (ppm)
3	D-Ala2	1.26
Ac-D-Ala-Pro-NHMe	NHMe	0.18
4	D-Ala2	1.11
Ac-(D-Ala-Pro)$_2$-NHMe	D-Ala4	0.15
	NHMe	0.13
5	D-Ala2	0.92
Ac-(D-Ala-Pro)$_3$-NHMe	D-Ala4	0.34
	D-Ala6	−0.115
	NHMe	0.023
6	D-Ala2	1.02
Ac−(D-Ala-Pro)$_4$-NHMe	D-Ala4	0.30
	D-Ala6	0.11
	D-Ala8	0.01
	NHMe	0.00
8	D-Ala2	−0.04
Piv-D-Pro-Pro-D-Ala-Pro-NHMe	NHMe	−0.04
9	D-Ala2	−0.04
Piv-D-Pro-Pro-(D-Ala-Pro)$_2$-NHMe	D-ALA4	−0.03
	NHMe	−0.03

can be made regarding trends of the peptides included in this study. For the peptides **3**−**6**, DMSO-d_6 titration shows that all NH groups are solvent-shielded except that on the N-terminal alanine, NH(D-Ala2). For the oligomers **8** and **9**, possessing the N-terminal D-proline, all NH groups exhibit solvent-shielding on titration with DMSO-d_6. In the longer peptides **5**, **6**, and **9** the NH(i + 3)−H$^{\alpha}$(i + 1) weak NOE is difficult to detect for some turns due to the degree of overlap in the H$^{\alpha}$ region of the spectrum. The diagnostic NH(i + 3)−H$^{\delta 2}$(i + 2) NOE of medium intensity is uniformly present in every potential turn unit. This NOE is, however, somewhat weaker in the N-terminal turns of peptides **5** and **6**. Interestingly, the chemical shift of NH(D-Ala4) in compounds **5** and **6** shows an increased susceptibility to solvent (Table 1) and H$^{\alpha}$(D-Ala2) in **5** (three turn units) and **6** (four turn units) exhibit a downfield shift when compared with **3** (one turn unit) and **4** (two turn units). These data suggest that in peptides **5** and **6** the N-terminal turn might exhibit a different dynamic behavior.[8] The N-terminal turn of **8** (three turn units) does not exhibit this profile (this should not be surprising, considering the additional conformational constraints imparted by the D-Pro residue). Curiously, the C-terminus (NH(Me)) exhibits a decrease in solvent accessibility as the chain length increases in the series **3**−**6**. Evidence for the poly(βII′-turn)-forming potential of these peptides can also be obtained from IR spectroscopy. Peptides **8** and **9** were selected for this study because their respective IR spectra should exhibit only a H-bonded NH stretch band in the poly(βII′-turn) conformation. Indeed, IR spectroscopy of two-turn structure **8** (1 mM, CH_2Cl_2) reveals that the NH-stretch region exhibits one significant band at 3341 cm^{-1} (H-bonded), while

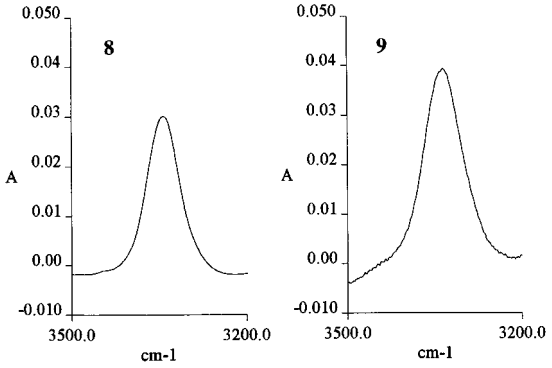

Figure 2. NH stretch FT-IR data for 1 mM peptides in CH_2Cl_2. **8**, Maximum at 3341 cm^{-1}; **9**, maximum at 3336 cm^{-1}.

the three-turn structure **9** exhibits one significant band at 3336 cm^{-1} (Figure 2). The presence of these strong bands attributed to a H-bonded state and the presence of non-H-bonded NH-stretch bands which are almost negligible (∼3450 cm^{-1}) indicates that peptides **8** and **9** are essentially locked in their H-bonded geometries.[2a] These IR data are complementary to the NOE and DMSO-d_6 titration data in that they provide evidence that the turns are populated simultaneously, giving rise to the repeating βII′-turn secondary structure.

Work is ongoing to evaluate the effects of amino acids other than D-alanine on this secondary structure and to evaluate these peptides in competitive solvents.

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Supporting Information Available: Representative experimental procedure for the synthesis of peptides, DMSO-d_6 titration curves, assignments, TOCSY spectra for **4**−**6**, **8**, NOESY spectra for **3**−**5**, **7**−**9**, and ROESY spectrum of **6** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) The reviewers have pointed out that this statement is puzzling considering that cooperativity should stabilize the longer structure. The author agrees that this is a curious statement; however, it is not clear at this juncture whether this fold is cooperative. Evidence for cooperativity comes from the increased resistance to solvent perturbation some NH resonances exhibit as the number of turns increases. In addition, *cis*/*trans* amide bond isomerism which is quite evident in the one turn unit **3** (∼10% *cis*) is less evident in the multiple turn peptides also suggesting cooperativity. However, some arguments could also be made that H-bond cooperativity may not necessarily play a dominant role. For example, Seebach has proposed that H-bonding in β-peptide helices is noncooperative and that ethane staggering (e.g., minimization of steric interactions) is the predominant stabilizing force. Considering minimization of pseudo A(1,3)-strain (a steric interaction) contributes significantly to the stability of a βII′ turn and considering that the formation of a hydrogen bond in one turn unit does not aid in bringing into closer proximity the groups which hydrogen bond in the adjacent turn unit, it *may* be that H-bond cooperativity is not a major stabilizing force. Gademann, K.; Jaun, B.; Seebach, D.; Perozzo, R.; Scapozza, L.; Folkers, G. *Helv. Chim. Acta* **1999**, *82*, 1.