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Factors Affecting Conformation in Proline-Containing Peptides

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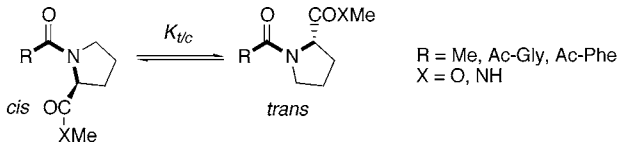
ABSTRACT



NMR was used to study the thermodynamics of the cis → trans isomerization for prolyl amide bonds in the compounds shown. The magnitude of $K_{t/c}$ for *C*-terminal esters is greater than for the corresponding amides, signifying stronger backbone stereoelectronic effects in esters. Increasing the steric bulk of the *N*-terminal residue from Ac- to Ac-Gly- favors the trans conformation. Incorporation of a Phe residue *N*-terminal to Pro, however, shifts the equilibrium in favor of the cis conformation, via a stabilizing aromatic–proline interaction.

Amide bonds, including peptide bonds, have partial double bond character, and this is often explained by invoking the resonance contributors I and II, as illustrated for Ac-Pro-OMe (**1**) (Figure 1a). In recent years, Wiberg has asked us

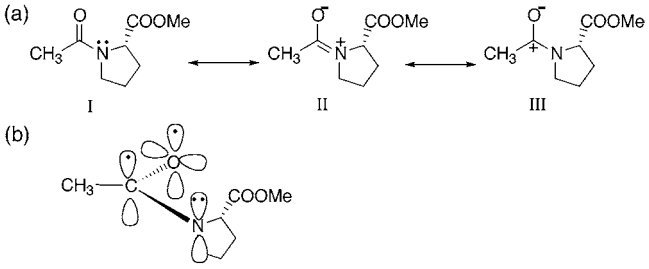


Figure 1. Electron distribution in prolylamides.

to consider structure III as well.[1] While these representations illustrate the distribution of π-electrons, they neglect the fact that the nitrogen atom inductively withdraws electron density back through the σ-bond network and, in fact, bears a partial negative charge.[2] It is perhaps more useful to view the planarity of the amide bond as a result of π-orbital overlap (Figure 1b).

As a result of this π-orbital overlap, and concomitant restricted rotation about the amide bond, there are two conformations which correspond to energy minima attained when the dihedral angle about the C(=O)-N bond (ω) is 0° (cis) and 180° (trans) as illustrated in the graphical abstract. For these two conformations to interconvert, the nitrogen must become transiently sp^3-hybridized, i.e., pyramidalized.[3,4]

Proline is arguably the most important amino acid, vis-à-vis the determination of protein structure and function.[5] The cyclic nature of the side chain means that cis and trans conformations are closer in energy for prolyl amides[6] than

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(1) Wiberg, K. B.; Laidig, K. E. *J. Am. Chem. Soc.* **1987**, *109*, 5935−5943.

(2) Milner-White, E. J. *Protein Sci.* **1997**, *6*, 2477−2482.

(3) Fischer, S.; Dunbrack, R. L., Jr.; Karplus, M. *J. Am. Chem. Soc.* **1994**, *116*, 11931−11937.

(4) Kang, Y. K. *THEOCHEM* **2002**, *585*, 209−221.

(5) (a) Vanhoof, G.; Goossens, F.; De Meester, I.; Hendriks, D.; Scharpé, S. *FASEB* **1995**, *9*, 736−744. (b) Reiersen, H.; Rees, A. R. *TIBS* **2001**, *26*, 679−684.

(6) Lubell has defined the term *prolyl amide* as "a tertiary amide composed of the pyrrolidine nitrogen of the prolyl residue and the carbonyl of the *N*-terminal residue." Halab, L.; Lubell, W. D. *J. Am. Chem. Soc.* **2002**, *124*, 2474−2484.

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for other peptide bonds.[7] The population of molecules in the cis conformation is significant.

Herein, we describe the investigation of six proline derivatives (Figure 2) to probe the relative importance of various

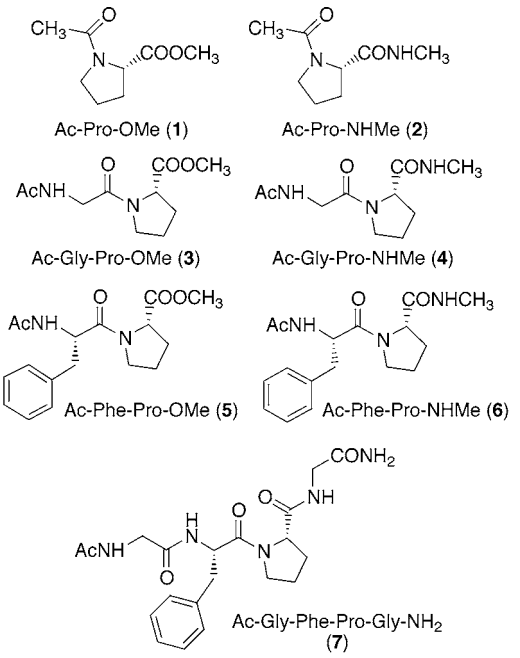

Figure 2. Proline derivatives.

factors in determining the position of the cis → trans equilibrium.

We prepared *N*-acetyl-L-proline methyl ester (**1**) and the corresponding methyl amide (**2**). These proline derivatives have been widely investigated[8] and serve as an important baseline. We next wanted to consider the impact of replacing the *N*-terminal acetyl group with an amino acid. Eberhardt et al. have prepared the *C*-terminal methyl ester **3**,[9] and we can compare their data with the *C*-terminal amide **4**. Finally, we will consider the impact of an aromatic amino acid in the so-called (i − 1) position, by looking at dipeptides **5** and **6**. It is also instructive to consider the tetrapeptide **7** which was studied by Wu and Raleigh.[10]

NMR spectra were recorded for 0.01−0.04 M solutions of each compound in D_2O over the temperature range 25−85 °C. The equilibrium constants were calculated by integration of as many well-resolved signals as possible in each spectrum.[11,12] The Van't Hoff plots for compounds **1** and **2** are given in Figure 3. A slight positive gradient was

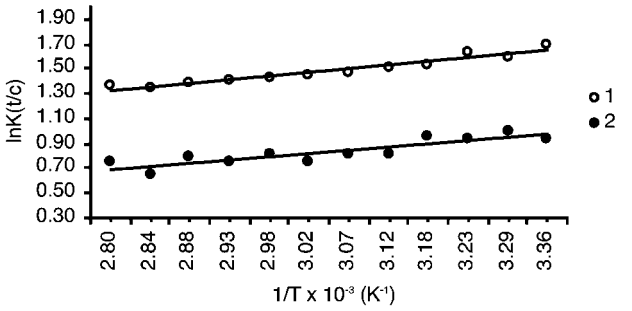

Figure 3. Van't Hoff plots for Ac-Pro-OMe (**1**) and Ac-Pro-NHMe (**2**) in D_2O.

seen for Ac-Pro-OMe (**1**), as reported by others.[9c,13] The trans conformation is favored ($K_{t/c}$ = 5.2 at 298 K), and $K_{t/c}$ decreases with increasing temperature ($K_{t/c}$ = 3.9 at 358 K), as more energy is available to populate the higher energy cis species. The replacement of the methyl ester with a methyl amide led to a significant reduction in the magnitude of the equilibrium constant. This effect was observed consistently in the other ester/amide pairs of compounds that we studied (vide supra).

With the exception of theoretical studies,[14,15] *C*-terminal amides have rarely been used in conformational studies,[16] to avoid complications arising from hydrogen bonding. Evidence has been presented for hydrogen bonds in both cis[17] and trans[18] conformations (Figure 4a) in non-hydrogen-bonding solvents.[19] Such effects are less significant in an aqueous environment and are unlikely to explain the difference in $K_{t/c}$ between compounds **1** and **2**.

Zimmerman and Scheraga proposed that restricted rotation about the N−Cα bond leads to a favorable C═O- - -C═O electrostatic interaction.[20] Maccallum et al. have demonstrated that this is almost as strong (80%) as a typical backbone hydrogen bond in a protein.[21] This stabilizing n → π^* interaction involves electron donation from the

(7) There are also issues concerning the conformation of the pyrrolidine ring, although this is beyond the scope of this discussion.

(8) For example, an extensive NMR analysis of Ac-Pro-NHMe: Higashijima, T.; Tasumi, M.; Miyazawa, T. *Biopolymers* **1977**, *16*, 1259−1270.

(9) Specifically, the doubly ^{13}C-labeled compound Ac-Gly-[β,δ-^{13}C)-Pro-OMe]: (a) Eberhardt, E. S.; Loh, S. N.; Hinck, A. P.; Raines, R. T. *J. Am. Chem. Soc.* **1992**, *114*, 5437−5439. (b) Eberhardt, E. S.; Loh, S. N.; Raines, R. T. *Tetrahedron Lett.* **1993**, *34*, 3055−3056. (c) Eberhardt, E. S.; Panasik, N., Jr.; Raines, R. T. *J. Am. Chem. Soc.* **1996**, *118*, 12261−12266.

(10) Wu, W.-J.; Raleigh, D. P. *Biopolymers* **1998**, *45*, 381−394.

(11) Others have utilized integration of $COOCH_3$ or $CONHCH_3$ singlets, or in the case of 5-^{t}Bu-Pro derivatives, the $C(CH_3)_3$ signal. Our experience has been that resolution is not adequate to give good results.

(12) The trans/cis ratio in D_2O has been shown to be independent of concentration (ref 8).

(13) Renner, C.; Aldefelder, S.; Bae, J. H.; Budisa, N.; Huber, R.; Moroder, L. *Angew. Chem., Int. Ed.* **2001**, *40*, 923−925.

(14) Kang, Y. K.; Jhon, J. S.; Han, S. J. *J. Peptide Res.* **1999**, *53*, 30−40.

(15) Zimmerman, S. S.; Scheraga, H. A. *Biopolymers* **1977**, *16*, 811−843.

(16) For other studies on Ac-X-Pro-NHMe derivative, see: (a) ref 6. (b) Halab, L.; Lubell, W. D. *J. Org. Chem.* **1999**, *64*, 3312−3321. (c) Halab, L.; Lubell, W. D. *J. Peptide Sci.* **2001**, *7*, 92−104.

(17) Cox, C.; Lectka, T. *J. Am. Chem. Soc.* **1998**, *120*, 10660−10668.

(18) (a) Mizushima, S.; Shimanouchi, T.; Tsuboi, M.; Sugita, T.; Kurosaki, K.; Mataga, N.; Souda, R. *J. Am. Chem. Soc.* **1952**, *74*, 4639−4641. (b) Matsuzaki, T.; Iitaka, Y. *Acta Crystallogr.* **1971**, *B27*, 507−516.

(19) Liang, G. B.; Rito, C. J.; Gellman, S. H. *Biopolymers* **1992**, *32*, 507−516.

(20) Zimmerman, S. S.; Scheraga, H. A. *Macromolecules* **1976**, *9*, 408−416.

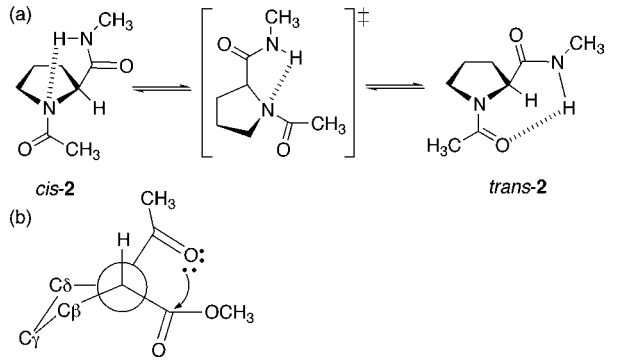

Figure 4. Intramolecular forces: (a) hydrogen bonding and (b) n → π^* interaction (looking down the Cα−N bond).

oxygen lone pair of the (i − 1) amide C═O, to the antibonding orbital of the C═O bond belonging to the Pro (i) residue. Raines and co-workers have recently provided evidence for the significance of this interaction, which they describe as quantum mechanical rather than electrostatic, and estimate a contribution of 0.7 kcal mol^{-1} to the stability of the trans conformation of compound **2** at 298 K.[22] Wiberg et al. have shown that there is greater positive charge density on the C═O carbon of esters than amides.[23] We suggest cautiously that a stronger n → π^* interaction in the trans conformation of esters (cf., amides) accounts for the difference in $K_{t/c}$ values. Indeed, Raines and co-workers have acknowledged that an amide carbon is less electron-deficient than an ester carbon,[22b] thereby predicting the difference in the strength of the n → π^* interactions which we have observed experimentally.

We next consider the dipeptides Ac-Gly-Pro-OMe (**3**) and Ac-Gly-Pro-NHMe (**4**). A dipeptide represents the smallest unit which enables us to look at a true peptide bond (i.e., between two amino acid residues) and is the next step up in complexity from a proline derivative. Early studies of cis → trans isomerism of X-Pro dipeptides were carried out with free amino and carboxy termini. This work demonstrated that pH, and therefore the degree of ionization, has a significant impact on the conformation of the peptide bond.[24] The incorporation of amide blocking groups at the *N*- and *C*-termini has therefore been employed to eliminate electrostatic interactions, making it possible to focus on more subtle effects.

The addition of a Gly residue *N*-terminal to the Pro residue leads to an increase in $K_{t/c}$ (Figure 5). This significant shift

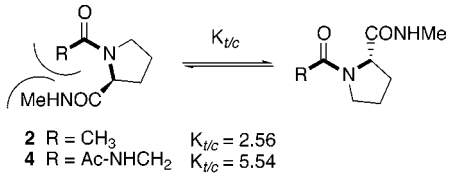

Figure 5. Steric interactions and $K_{t/c}$ values at 298 K.

in favor of the trans conformation is a consequence of the increase in steric bulk. The equilibrium constants are larger for Ac-Gly-Pro-OMe (**3**) than for Ac-Gly-Pro-NHMe (**4**), since the trans conformation is again stabilized to a greater extent by the n → π^* interaction in the ester.

The Van't Hoff plots for dipeptides **3**,[9] **4**, **5**, **6**, and tetrapeptide **7**[10] are presented in Figure 6. As observed for

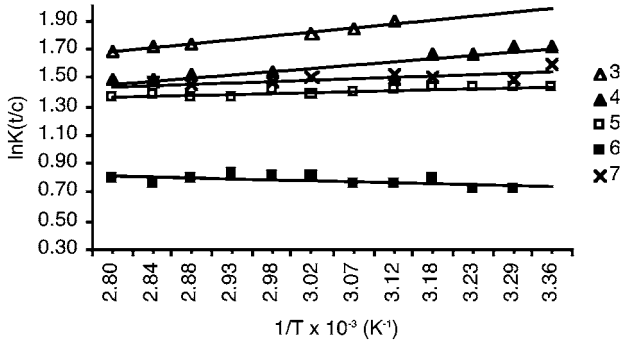

Figure 6. Van't Hoff Plots for Ac-Gly-Pro-OMe (**3**),[9] Ac-Gly-Pro-NHMe (**4**), Ac-Phe-Pro-OMe (**5**), Ac-Phe-Pro-NHMe (**6**), and Ac-Gly-Phe-Pro-Gly-NH_2 (**7**)[10] in D_2O.

the single residue Pro derivatives (Figure 3), the dipeptides containing glycine have a positive slope, with the two lines running parallel. For the peptides containing a Phe-Pro amide bond, especially dipeptide **6**, there is a reduction in the magnitude of $K_{t/c}$; there is also less temperature-dependence.

There is a high propensity for cis amide bonds when Pro is preceded by an aromatic residue.[25] Surveys of crystallographic databases reveal that 5.7% of X-Pro peptide bonds are in the cis conformation. When X is Phe this percentage rises to 6.4% and leaps to 19.1% for Tyr;[26] there is limited data for Trp-Pro linkages.[27] This is attributed to a stabilizing Ar−Pro interaction in the cis conformation (Figure 7). Halab

(21) (a) Maccallum, P. H.; Poet, R.; Milner-White, E. J. *J. Mol. Biol.* **1995**, *248*, 361−373. (b) Maccallum, P. H.; Poet, R.; Milner-White, E. J. *J. Mol. Biol.* **1995**, *248*, 374−384.

(22) (a) DeRider, M. L.; Wilkens, S. J.; Waddell, M. J.; Bretscher, L. E.; Weinhold, F.; Raines, R. T.; Markley, J. L. *J. Am. Chem.. Soc.* **2002**, *124*, 2497−2505. (b) Hinderaker, M. P.; Raines, R. T. *Protein Sci.* **2003**, *12*, 1188−1194.

(23) Wiberg, K. B.; Hadad, C. M.; Rablen, P. R.; Cioslowski, J. *J. Am. Chem. Soc.* **1992**, *114*, 8644−8654.

(24) (a) Grathwohl, C.; Wütherich, K. *Biopolymers* **1976**, *15*, 2025−2041. (b) Grathwohl, C.; Wütherich, K. *Biopolymers* **1981**, *20*, 2623−2633. (c) Mariappan, S. V. S.; Rabenstein, D. L. *J. Org. Chem.* **1992**, *57*, 6675−6678.

(25) (a) MacArthur, M. W.; Thornton, J. M. *J. Mol. Biol.* **1991**, *218*, 397−412. (b) Stewart, D. E.; Sarkar, A.; Wampler, J. E. *J. Mol. Biol.* **1990**, *214*, 253−260.

(26) For NMR studies of cis−trans isomerism in model peptides containing Tyr-Pro linkages, see: (a) Stimson, E. R.; Montelione, G. T.; Meinwald, Y. C.; Rudolph, R. K. E.; Scheraga, H. A. *Biochemistry* **1982**, *21*, 5252−5262. (b) Juy, M.; Lam-Thanh, H.; Lintner, K.; Fermandjian, S. *Int. J. Peptide Protein Res.* **1983**, *22*, 437−449.

(27) Poznanski et al. have gone so far as to say that nonbonding interactions between pyrrolidine and indole rings govern the cis−trans equilibrium in peptides containing Trp-Pro fragments: Poznanski, J.; Ejchart, A.; Wierzchowski, K. L.; Ciurak, M. *Biopolymers* **1993**, *33*, 781−795.

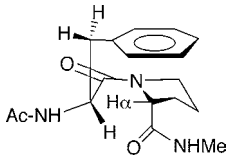

Figure 7. Ar-Pro Interaction.

and Lubell have suggested[6] that the Ar−Pro interaction is of a cationic-pi nature, as defined by Dougherty.[28] This seems unlikely if we acknowledge the net negative charge on the prolyl nitrogen discussed earlier.

Evidence for this nonbonding interaction is found in the ^{1}H chemical shifts for Hα of the Pro residue. In the cis conformation, Hα is shielded by the aromatic ring and a dramatic upfield shift is observed. The data are summarized for compounds **4**, **6**, and **7** in Table 1.

Table 1. Parameters Derived from ^{1}H NMR (D_2O, 298 K)

parameter	**4**	**6**	**7**
δHα (trans)	4.23	4.38	4.42
δHα (cis)	4.41	3.47	3.82
$\Delta\delta\alpha$	+0.18	−0.91	−0.60
$K_{t/c}$	5.54	2.56	4.8

Wu and Raleigh advocate $\Delta\delta\alpha$,[29] the chemical shift difference for Pro Hα in the cis and trans conformations, as a measure of the strength of the Ar−Pro interaction. The low $K_{t/c}$ value for dipeptide Ac-Phe-Pro-NHMe (**6**) reflects the maximum impact of a preceding Phe residue. In the tetrapeptide **7**, described by Wu and Raleigh,[10] the effect of elongating the peptide chain is demonstrated. While the Ar-Pro interaction persists, the steric demands of the extended peptide backbone counteract it. A similar example was reported by Wütherich and Grathwohl: in DMSO-d_6, Thr-Phe-Pro was 60% cis with respect to the X-Pro peptide bond, while Phe-His-Thr-Phe-Pro was only 15% cis.[30] The isomerization of X-Pro bonds is normally enthalpy-driven.[31] However, the Ar-Pro interaction stabilizes the cis conformation and brings it closer in energy to its trans counterpart so that ΔH is small, accounting for the essentially flat line in the Van't Hoff plots for these compounds. Moreover, the conversion of cis → trans must disrupt the "organized" *cis* species and its inherent Ar-Pro interaction (Figure 7). This is reflected in positive ΔS values for compounds **5**−**7**.

In conclusion, we have identified significant differences in the strength of backbone n → π^* stabilizing effects between esters and amides. This difference is tentatively attributed to the difference in electron density at the acyl carbon. So long as these differences are borne in mind, we believe esters can still serve as useful model compounds.

The effect of noncovalent interactions on the relative energies of cis and trans conformations is considerable. An aromatic residue preceding the proline greatly stabilizes the cis conformation via a hydrophobic interaction between the aromatic and pyrrolidine rings. It is not clear what makes the pyrrolidine ring different from other nonpolar side chains (e.g., Val, Leu), other than its cyclic nature and the lack of an amide proton.[32] The impact of this Ar-Pro interaction is tempered by steric considerations which cause the peptide backbone to prefer an extended conformation (i.e., a trans peptide bond).

While the ultimate preferred conformation, or conformations, of proline-containing peptides remains the outcome of the interplay of a number of considerations, we hope that we have demonstrated how these work together.

Acknowledgment. This research was supported by the Marsden Fund, administered by the Royal Society of New Zealand (Grant No. 00-MAU-007). Jodie M. Johnston made early contributions to the synthesis of some dipeptides on a Health Research Council Summer Studentship (1997−98).

Supporting Information Available: Experimental procedures and ^{1}H and ^{13}C NMR spectra for compounds **1**, **2**, **4**−**6**, and intermediates in their synthesis. Tables of thermodynamic data derived from NMR experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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(28) (a) Dougherty, D. A. *Science* **1996**, *271*, 163−168. (b) Gallivan, J. P.; Dougherty, D. A. *J. Am. Chem. Soc.* **2000**, *122*, 870−874.

(29) Wu and Raleigh actually use the term "ΔΔα"; however, we believe that the second character should be a lower case δ since it refers to chemical shift.

(30) Wütherich, K.; Grathwohl, C. *FEBS Lett.* **1974**, *43*, 337−340.

(31) Kang, Y. K.; Park, H. S. *THEOCHEM* **2002**, *593*, 55−65.

(32) Kersteen, E. A.; Raines, R. T. *Biopolymers* **2001**, *59*, 24−28.