J|A|C|S
COMMUNICATIONS

Published on Web 10/19/2004

Large Structural Modification with Conserved Conformation: Analysis of Δ^3-Fused Aryl Prolines in Model β-Turns

Guillaume Jeannotte and William D. Lubell*

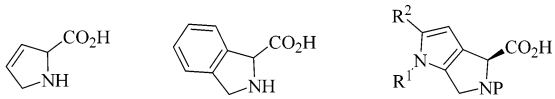
Département de chimie, Université de Montréal, C.P. 6128, Succursale CentreVille, Montréal, Québec, Canada H3C 3J7

Received May 17, 2004; E-mail: lubell@chimie.umontreal.ca

Proline serves as a turn inducer in natural peptides and proteins because of the pyrrolidine ring, which restricts motion about the ϕ dihedral angle and reduces the energy difference between the prolyl amide *cis*- and *trans*-isomers.[1a] Steric and stereoelectronic effects of alkyl- and heteroatom-substituted prolines have thus been used to induce specific conformations for studying factors that effect prolyl peptide activity and biology.[1] In contrast, Δ^3-dehydroproline **1** acts as a conservative proline replacement in peptides and proteins. For example, little conformational change was observed after the replacement of Pro by **1** in a 14-residue cyclic analogue of gramicidin S that exhibited a similarly stable β-sheet structure with two type II′ β-turns, as shown by spectroscopic and computational analysis.[2] Moreover, replacement of Pro by **1** has provided peptide and protein analogues with similar and improved biological activities.[3]

Δ^3-Fused arylprolines, such as **2**, have yet to be examined in peptides primarily because of the difficulties in their synthesis.[4] Like **1**, Δ^3-fused arylprolines may serve as conservative proline surrogates; moreover, structural modifications may be added without influencing conformation. Recently, we introduced an effective methodology for synthesizing enantiopure Δ^3-fused pyrrol−prolines (PyPro) of general structure **3** (Figure 1: R^1, R^2 = H, alkyl; P = protection).[4] To examine the influence of the aryl moiety and flattened pyrrolidine ring on conformation, Fmoc−PyPro **3** (R^1 = CH_3, R^2 = H, P = Fmoc) has now been introduced into peptides **4b** and **5b** for comparison with Pro in model β-turns, **4a** and **5a**, respectively (Table 1). Peptides **4a** and **4b** were selected to examine if PyPro would accommodate itself at the $i + 1$ position of a β-hairpin because studies of the enantiomeric sequence of **4a** have shown that the D-Pro-Gly residue adopted the central position of a β-hairpin.[5] Peptides **5a** and **5b**, analogues of the β-turn portion of gramicidin S, were synthesized to examine PyPro at the $i + 2$ position because spectroscopic analysis revealed a significant β-turn population in a peptide related to **5a**.[6] Sequences **4** and **5**, possessing Pro and PyPro, were synthesized in solution and examined by NMR spectroscopy to assess the influence of the pyrrole moiety on the hydrogen-bonding network, the prolyl amide equilibrium, and the turn conformation.

Peptides possessing Pro (**4a** and **5a**) and PyPro (**4b** and **5b**) were synthesized in solution using N^{α}-Boc and N^{α}-Fmoc strategies, respectively, as described in the Supporting Information (SI). The NMR spectra of peptides **4** and **5** were measured in CD_2Cl_2 at concentrations ≤10 mM because aggregation was observed at higher concentrations.[7a] Intramolecular hydrogen bonding was evaluated by plotting the change in the chemical shift of the amide and the pyrrole protons as a function of DMSO-d_6 added to the peptide in CD_2Cl_2 (Figure 2) because exchangeable protons engaged in intramolecular hydrogen bonds are typically not influenced by strong hydrogen-bonding solvents, such as DMSO-d_6 relative to exposed protons.[7b,8] The prolyl amide isomer equilibrium was measured in CD_2Cl_2, 2:1 CD_2Cl_2/DMSO, and DMSO by integration of the signals for the isomeric amide and α-protons for **4a** and **5a** and the pyrrole protons for **4b** and **5b**. Finally, sequential and long distance NOEs were measured in the NOESY spectrum of each peptide to assess the presence of a turn conformation.



1 **2** **3** (PyPro)

Figure 1. Representative unsaturated, aryl, and heteroaryl prolines.

Table 1. *cis*-Isomer N-terminal to Pro and PyPro (concn ≅ 10 mM)

peptide	% cis (±1%) 2:1 CD_2Cl_2/DMSO	% cis (±1%) DMSO
4a: Ac-D-Val-Pro-Gly-D-Leu-NMe_2	12	19
4b: Ac-D-Val-PyPro-Gly-D-Leu-NMe_2	15	18
5a: Ac-Leu-D-Phe-Pro-Val-NMe_2	26	43
5b: Ac-Leu-D-Phe-PyPro-Val-NMe_2	44	47

The influence of DMSO on the amide proton chemical shifts was similar in peptides **4** and **5** (Figure 2). In **4**, the amide protons for Val and Leu were downfield from that for Gly in CD_2Cl_2 and were unaffected by the addition of DMSO relative to that for Gly, which was downfield shifted ($\Delta\delta$ = 1.29 ppm), indicative of its exposure to solvent. In peptide **5**, the Leu amide proton was unaffected by the solvent change, indicative of a solvent-shielded NH. The Phe and Val amide protons were downfield shifted with the addition of DMSO, indicative of their exposure to the effects of the solvent. In peptides **4b** and **5b**, the pyrrole−NH was influenced most by the solvent change ($\Delta\delta$ = 1.95 and 2.1 ppm, respectively). The most significant difference between the Pro and PyPro peptides was the magnitude of the chemical shift variation of the Phe amide proton, which was larger for **5a** ($\Delta\delta$ = 1.1 ppm) than for **5b** ($\Delta\delta$ = 0.6 ppm), potentially due to a different orientation of the phenyl ring over the peptide backbone.

The population of the amide *cis*-isomer, N-terminal to Pro and PyPro (Table 1), was very similar in peptides **4** and **5** and undetectable in CD_2Cl_2. In comparison with 20% *cis*-isomer in DMSO-d_6 observed in a related β-hairpin octapeptide containing a central D-Pro-Gly residue,[8] peptides **4a** and **4b** exhibited similar amounts of *cis*-isomer (19 and 18%, respectively). Alternatively, relative to 39% *cis*-isomer observed in Ac-D-Phe-Pro-NH(Me) in DMSO,[1b] peptides **5a** and **5b** exhibited slightly higher *cis*-isomer populations (43 and 47%, respectively). When favored β-turn conformations are assumed, the lower amount of *cis*-isomer in **4** is consistent with the stabilization of the *trans*-isomer by an intramolecular hydrogen bond.[9] The significantly higher *cis*-isomer in **5b** may be due to a staggered $\pi-\pi$ interaction between the phenyl and pyrrole ring in this conformer.[1a,10] The greatest difference in

10.1021/ja0471222 CCC: $27.50

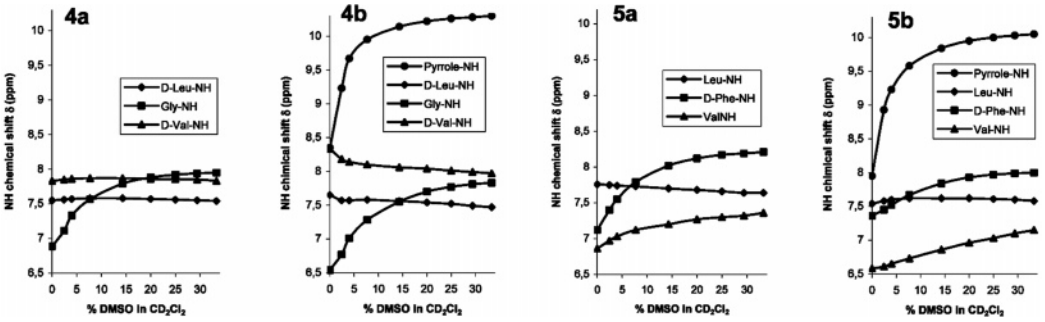


Figure 2. Amide proton NMR chemical shift (δ) as a function of the percentage of DMSO in CD_2Cl_2 for peptides **4** and **5** (concn $\cong$ 10 mM).

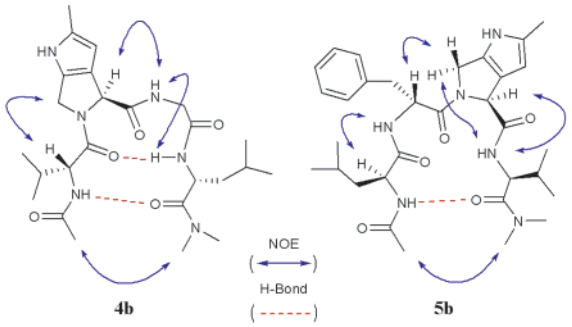


Figure 3. Selected ^{1}H-^{1}H NOESY cross-peaks in CD_2Cl_2 as well as potential hydrogen bonds from DMSO titration curves for peptides **4b** and **5b**.

the *cis*-isomer population of Pro relative to PyPro was detected in 1:2 DMSO/CD_2Cl_2, in which **5a** exhibited 26% and **5b** 44% *cis*-isomer, which may be due to a combination of aromatic stacking, as described above, and enhanced steric effects from DMSO coordination to the pyrrole nitrogen which disfavored the *trans*-isomer.

In **4** and **5** the NOESY spectra for the Pro and PyPro peptides exhibited similar correlations for β-turns in CD_2Cl_2 (Figure 3). The amide *trans*-isomer, N-terminal to the Pro and PyPro residues, was identified by NOEs between their δ-protons and the α-proton of the N-terminal residue. A long-range NOE between the Gly and Leu amide protons was indicative of a turn conformer in **4**. Similarly, long-range NOEs between the δ-proton of Pro/PyPro and the Val amide hydrogen were indicative of a turn conformer in **5**. The presence of a turn geometry in **4** and **5** was supported by long-range NOEs between the acetyl and dimethyl amide singlets. Finally, sequential NOEs were observed between the neighboring Leu-C$^\alpha$H and Phe-NH as well as the Pro/PyPro-C$^\alpha$H and Val-NH in **4** and between the Pro/PyPro-C$^\alpha$H and Gly-NH in **5**, indicative of their proximity in the major conformer.

For the first time, the influence of a fused Δ^3-arylproline on peptide conformation has been studied by the synthesis of model peptides, **4b** and **5b**, containing PyPro **3**. The DMSO titration curves, the *cis*/*trans*-isomer ratios, and the NOESY spectra all demonstrate an overall conservation of β-turn conformation on replacement of Pro by PyPro in peptides **4** and **5**. In light of the importance of turns in recognition events,[11] future efforts may employ PyPro analogues to create interactions for enhancing biological activity without perturbing conformation.

Acknowledgment. This research was supported, in part, by Merck Frosst Canada, NSERC Canada, and FQRNT Québec. We thank Ms. Sylvie Bilodeau of the Regional High-Field NMR Laboratory for her assistance, and Mr. Dalbir Sekhon for performing LC−MS experiments.

Supporting Information Available: Details on the syntheses, concentrations, and conformational analyses, ^{1}H and ^{13}C NMR, IR, DEPT, COSY, and NOESY spectra for **4a**,**b** and **5a**,**b**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Halab, L.; Lubell, W. D. *J. Am. Chem. Soc.* **2002**, *124*, 2474−2484 and references therein. (b) Halab, L.; Lubell, W. D. *J. Pept. Sci.* **2001**, *7*, 92−104. (c) Beausoleil, E.; Sharma, R.; Michnick, S. W.; Lubell, W. D. *J. Org. Chem.* **1998**, *63*, 6572−6578 and references therein. (d) Cavelier, F.; Vivet, B.; Martinez, J.; Aubry, A.; Didierjean, C.; Vicherat, A.; Marraud, M. *J. Am. Chem. Soc.* **2002**, *124*, 2917−2923. (e) DeRider, M. L.; Wilkens, S. J.; Wadell, M. J.; Bretscher, L. E.; Weinhold, F.; Raines, R. T.; Markley, J. L. *J. Am. Chem. Soc.* **2002**, *124*, 2497−2505. (f) Montelione, G. T.; Hughes, P.; Clardy, J.; Scheraga, H. A. *J. Am. Chem. Soc.* **1986**, *108*, 6765−6773.

(2) Gibbs, A. C. G.; Bjorndahl, T. C.; Hodges, R. S.; Wishart, D. S. *J. Am. Chem. Soc.* **2002**, *124*, 1203−1213.

(3) Felix, A. M.; Wang, C.-T.; Liebman, A. A.; Delaney, C. M.; Mowles, T.; Burghardt, B. A.; Charnecki, A. M.; Meienhofer, J. *Int. J. Pept. Protein Res.* **1977**, *10*, 299−310 and references therein. (b) Zhao, Z.; Liu, X.; Shi, Z.; Danley, L.; Buang, B.; Jiang, R.-T.; Tsai, M.-D. *J. Am. Chem. Soc.* **1996**, *118*, 3535−3536.

(4) Jeannotte, G.; Lubell, W. D. *J. Org. Chem.* **2004**, *69*, 4656−4662 and references cited therein. See, also, refs 8, 11−13, and references cited therein.

(5) Haque, T. S.; Little, J. C.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 6975−6985.

(6) Sato, K.; Higashijima, T.; Sugawara, R.; Nagai, U. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 3699−3702.

(7) (a) See details in the Supporting Information. (b) Hydrogen bonding was also examined in the NH stretch region of the IR spectra of **4** and **5**; see SI.

(8) Awasthi, S. K.; Raghothama, S.; Balaram, P. *Biochem. Biophys. Res. Commun.* **1995**, *216*, 275−381.

(9) Ac-D-Ala-Pro-NH(Me) exhibits 36% *cis*-isomer in DMSO; see ref 1b.

(10) Yao, J.; Bruschweiler, R.; Dyson, H. J.; Wright, P. E. *J. Am. Chem. Soc.* **1994**, *116*, 12051−12052. (b) Wu, W.-J.; Raleigh, D. P. *Biopolymers* **1998**, *45*, 381−394.

(11) Rose, G. D.; Gierash, L. M.; Smith, J. A. *Adv. Prot. Chem.* **1985**, *27*, 1−109.

JA0471222