

A New Turn Structure for the Formation of β -Hairpins in Peptides

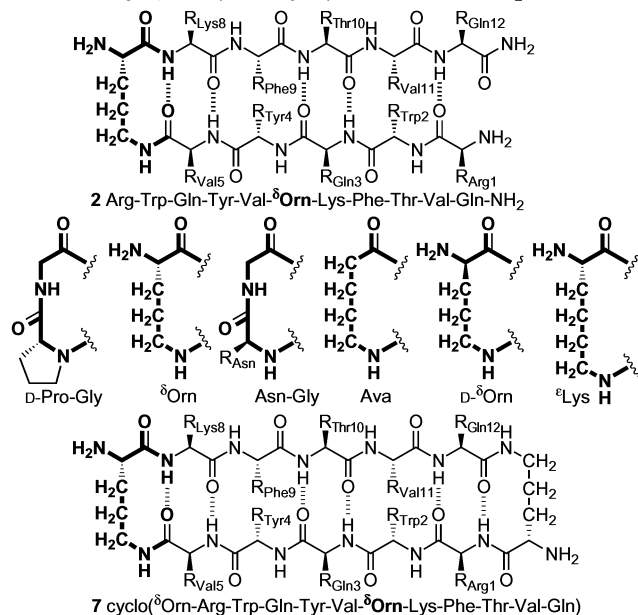
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Good turn structures are critical for the formation of β -hairpins in peptides.¹ Gellman and co-workers have elegantly shown that mirror-image β -turns based upon D-Pro-Gly are especially good at stabilizing β -hairpins.² We recently discovered that peptides containing the unnatural amino acid *Hao*³ linked to the side chain of the amino acid ornithine (Orn) also fold into well-defined β -hairpins.⁴ In this work, we establish that ornithine turns are especially good at stabilizing β -hairpins by comparing Gellman's D-Pro-Gly turn peptide **1**⁵ to Orn-containing analogue **2**.⁶

- 1 Arg-Trp-Gln-Tyr-Val-D-Pro-Gly-Lys-Phe-Thr-Val-Gln-NH₂
- 2 Arg-Trp-Gln-Tyr-Val- δ Orn-Lys-Phe-Thr-Val-Gln-NH₂
- 3 Arg-Trp-Gln-Tyr-Val-Asn-Gly-Lys-Phe-Thr-Val-Gln-NH₂
- 4 Arg-Trp-Gln-Tyr-Val-Ava-Lys-Phe-Thr-Val-Gln-NH₂
- 5 Arg-Trp-Gln-Tyr-Val-D⁸Orn-Lys-Phe-Thr-Val-Gln-NH₂
- 6 Arg-Trp-Gln-Tyr-Val- ϵ Lys-Lys-Phe-Thr-Val-Gln-NH₂



In the current study, we compare Orn-containing peptide **2** to D-Pro-Gly-containing peptide **1** by means of ¹H NMR chemical shift and NOE studies. We also compare it to peptides **3–6**, which contain other amino acids in the turn region, and to cyclic peptide **7**. Peptide **3** contains Asn-Gly,⁷ which is known to favor β -hairpin formation, albeit not as strongly as D-Pro-Gly.^{2c,5b} Peptide **4** contains δ -aminovaleric acid (Ava), which is identical to Orn, with the exception that it lacks an α -amino group. With a flexible chain in the turn region, this peptide provides a negative control for chemical shift and NOE studies. Peptide **5** contains D-Orn and is designed to probe the effect of the turn stereochemistry, which Gellman has shown to be critical in β -hairpin formation.^{2,5} Peptide **6** contains Lys in the turn region, to probe whether the length of the Orn side chain imparts a unique ability to stabilize a β -hairpin conformation. Cyclic peptide **7** contains two Orn turns, to lock it into a β -hairpin

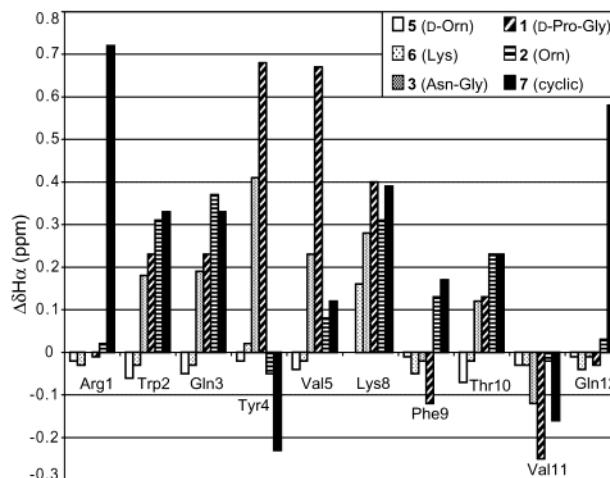


Figure 1. ¹H NMR chemical shifts of the α -protons of peptides **1–3** and **5–7** relative to those of Ava peptide **4**.¹⁰

conformation, and provides a positive control for chemical shift and NOE studies.⁸

Comparison of the chemical shifts of the α -protons of peptide **2** to those of controls **4** and **7** suggests that it is largely folded in D₂O at 276 K (Figure 1).^{9,10} Consistent with a β -hairpin structure, the nonterminal α -proton resonances from the β -strand regions of both **2** and **7** are generally downfield of those of control **4**, with the α -proton resonances of Trp, Gln3, Lys, Phe, and Thr exhibiting significant (>0.10 ppm) and comparable downfield shifting.¹¹ Gellman and co-workers have reported that the Gln3, Val5, Lys, and Thr α -proton resonances are reliable reporters of the degree of folding of peptide **1**.⁵ If Val5 is excluded from this set to reflect structural differences between the Orn and D-Pro-Gly turns, then Gln3, Lys, and Thr constitute an appropriate set for comparison. Orn-turn peptide **2** exhibits 0.30 ppm average downfield shifting of these resonances, while cyclic peptide **7** exhibits 0.32 ppm average downfield shifting, suggesting that **2** is largely folded.¹² D-Pro-Gly peptide **1** exhibits 0.25 ppm average downfield shifting, suggesting that it is also largely folded, albeit possibly slightly less so. Asn-Gly peptide **3** exhibits 0.20 ppm average downfield shifting, suggesting folding to a lesser degree. Lys peptide **6** exhibits little downfield shifting of the α -proton resonances (0.04 ppm), suggesting little or no folding into a β -hairpin. D-Orn peptide **5** exhibits slight (0.04 ppm) upfield shifting relative to Ava control **4**, suggesting that it is not folded into a β -hairpin and that control **4** may have a very small degree of β -hairpin conformation. Collectively, these data indicate that Orn is comparable or slightly better in turn-forming propensity than D-Pro-Gly and is significantly better than Lys.

¹H NMR transverse-ROESY (Tr-ROESY)¹³ experiments corroborate the chemical shift results. The Orn peptide **2**, D-Pro-Gly peptide **1**, and cyclic peptide **7** exhibit relatively strong NOEs between the Tyr and Phe α -protons and between the Trp and Val11

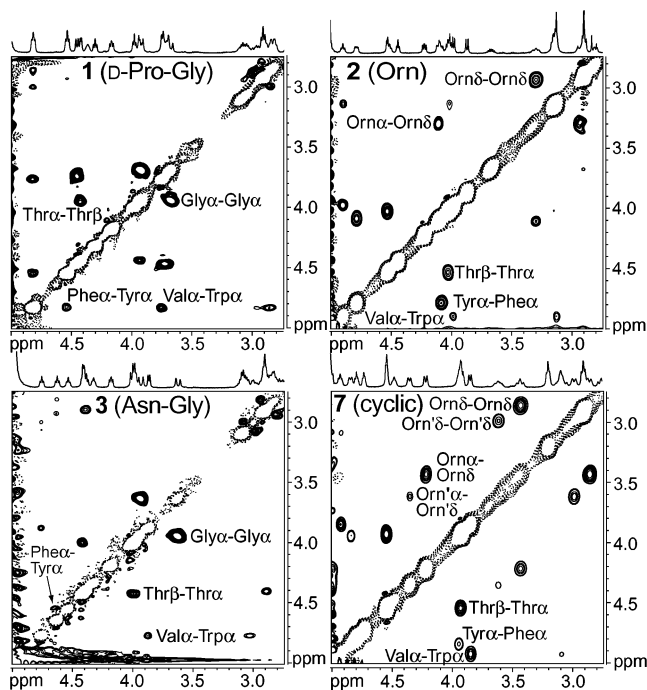


Figure 2. ^1H NMR Tr-ROESY 13 spectra of peptides 1–3 and 7.⁹

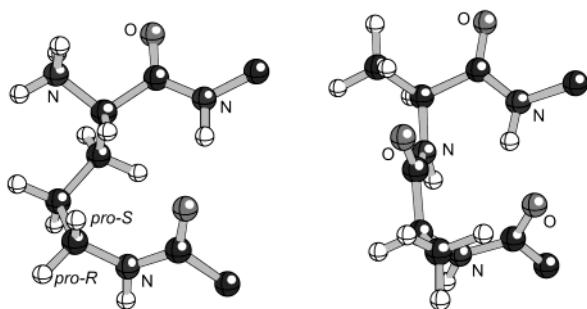


Figure 3. Models of the Orn turn ($\text{Ac}^{\delta}\text{Orn-NHMe}$, global minimum; MacroModel V7.0/AMBER*/ H_2O) and a type I' mirror-image β -turn.

α -protons, which are characteristic of the main-chain close contacts of β -sheets (Figure 2). Asn-Gly peptide 3 exhibits significantly weaker interstrand NOEs, suggesting that it is less well-folded than peptides 1, 2, and 7. Peptides 4–6 exhibit no long-range NOEs, suggesting little or no folding.

^1H NMR studies, in conjunction with molecular modeling, indicate that the Orn turn adopts a well-defined conformation similar to a mirror-image β -turn (Figure 3). Consistent with this model, Orn peptide 2 exhibits an NOE, which is strong, between the Orn α -proton and only one of the two diastereotopic Orn δ -protons (Figure 2). This proton (*pro-S*) appears 0.37 ppm downfield of the other δ -proton (*pro-R*), reflecting the magnetic anisotropy of the adjacent (Val5) carbonyl group. Differences between the coupling patterns of the two protons also support this model; in D_2O solution the downfield resonance resembles a broad triplet ($J \approx 14$ Hz), and the upfield resonance resembles a broad doublet ($J \approx 14$ Hz). The diastereotopic δ -protons of the Orn turn in cyclic peptide 7 exhibit 0.60 ppm separation and similar coupling patterns and NOEs. If 0.60 ppm is taken as a limit for complete folding, and the absence of magnetic anisotropy (0.00 ppm) in Ava peptide 4 is

taken as a limit for complete unfolding, then the 0.37 ppm value associated with Orn peptide 2 reflects 62% folding, which is identical to that which Gellman estimates for D-Pro-Gly peptide 1.⁵ Comparison of the Orn turn to a type I' mirror-image β -turn (Figure 3) reveals that both turns have the same twist, suggesting that only the L-Orn enantiomer promotes β -hairpin formation because the twist of the L-Orn turn matches that of a β -sheet, while that of a D-Orn turn opposes it.^{2,14}

In summary, the Orn turn is comparable to the D-Pro-Gly turn in promoting β -hairpin formation in peptides. This novel δ -peptide turn may offer practical advantages for NMR studies, because the separation of the diastereotopic δ -proton resonances reflects the degree of folding of peptides containing it and because poorly folded structures will not exhibit complex spectra from tertiary amide rotamers.

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- (8) Peptides 1–6 were prepared by automated solid-phase peptide synthesis on PAL resin (Fmoc chemistry), purified by reverse-phase HPLC, and isolated and studied as the trifluoroacetate salts. Peptides 2 and 7 were prepared in a similar fashion using Boc-Orn(Fmoc)-OH. Peptide 7 was assembled on trityl resin, starting with Boc-Orn(Fmoc)-OH, and was efficiently cyclized in solution with HBTU while still protected.
- (9) All peptides were studied by ^1H NMR spectroscopy at 500 MHz in 4 mM solution in pH 3.8 (uncorrected) 100 mM deuterioacetate-buffered D_2O solutions at 276 K by one-dimensional, TOCSY (150 ms mixing time), and transverse-ROESY (Tr-ROESY) 13 experiments (250 ms mixing time) and are referenced against sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).
- (10) α -Proton resonances were assigned by TOCSY and Tr-ROESY 13 experiments and by comparison to published assignments.^{5b} Gln3 and Gln12 were assigned by side-chain magnetic anisotropy. Val5 and Val11 were assigned by NOEs in peptides 1–3 and 7 and were assigned tentatively by chemical shifts in peptides 4–6.
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