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Conformationally Homogeneous Cyclic Tetrapeptides: Useful New Three-Dimensional Scaffolds

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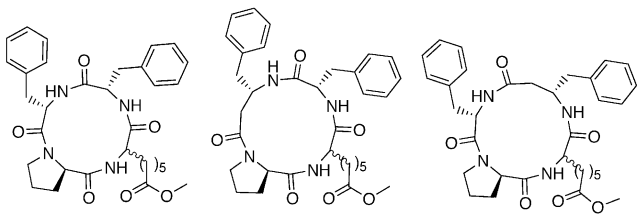
β-turns in proteins are defined by four consecutive amino acids. Cyclic tetrapeptides (CTPs) derived therefrom as minimalist turn mimetics have limited uses as biological probes or drug leads due to inefficient synthesis, instability to hydrolysis/metabolism, and conformational heterogeneity in water.[1] These disadvantages stem from unfavorable strain in a 12-membered ring containing four *trans*-amide bond restraints. The few known naturally occurring CTPs usually possess turn-inducing constraints (e.g., Pro, D-amino acids) that facilitate cyclization and enable access to a *cis*-amide.[1b,2] Some CTPs exhibit one conformation in nonpolar solvents or when a *cis*-amide is present, but usually CTPs exist as multiple conformations in polar solvents (H_2O, DMSO).[3] We demonstrate here that CTPs, with 13-membered rings and a β-amino acid, are easier to synthesize, chemically more stable, conformationally homogeneous, and are novel three-dimensional scaffolds.

To investigate the influence of a β-amino acid (e.g., βhPhe, β-*S*-homophenylalanine) in CTPs, we synthesized the tetrapeptides H_2N-Phe-(*R*)Pro-Asu-Phe-OH (**1**), H_2N-βhPhe-(*R*)Pro-Asu-Phe-OH (**2**), and H_2N-Phe-(*R*)Pro-Asu-βhPhe-OH (**3**) featuring the racemic amino acid Asu (*R*,*S*-amino suberic acid methyl ester) present in some known CTPs.[2] Cyclization of **1** by slow addition (syringe pump) to BOP (10^{-3} M) in DMF over 24 h gives ~50% **4** as two diastereomers, plus octapeptide cyclodimer. By contrast **2** and **3** with a β-amino acid cyclized efficiently (>95%) under the same conditions to **5** and **6**, respectively, each as two separable (rp-HPLC) diastereomers.



4 **5** **6**

^{1}H NMR spectra for **4R** and **5R** (*R*-Asu, Figure 1a) and **4S** and **5S** (*S*-Asu) in d_6-DMSO were consistent with multiple (≥3) solution conformations that interconvert slowly on the NMR time scale. However each diastereomer of **6**, where βhPhe was inserted between Phe and Asu, displayed only one set of sharp resonances (**6R** in Figure 1b,c; **6R** and **6S**, Figure S2) in DMSO or H_2O (+10% MeCN), indicating just one stable solution conformation for **6R** or **6S**. The contrast between conformational homogeneity for **6** and heterogeneity for **5** reflects the cooperative effect of separating two turn-inducing constraints (*R*-Pro and βhPhe) in **6** by an amino acid. **6R** and **6S** were also chemically more stable to hydrolysis in 0.3 M LiOH ($t_{1/2} \approx 50$ h, 20 °C) than the 12-membered **4R** and **4S** ($t_{1/2} < 10$ h, 20 °C), as assessed by rp-HPLC (Supporting Information).

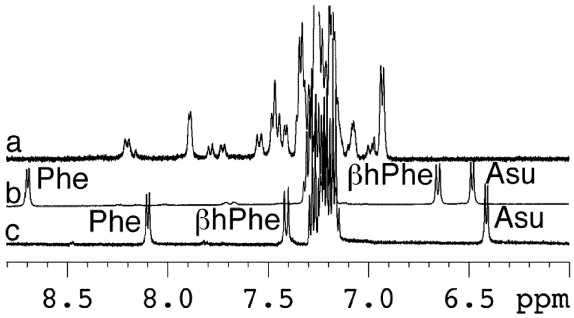


Figure 1. ^{1}H NMR spectra (amide NH region) for (a) **4R** in d_6-DMSO, (b) **6R** in d_6-DMSO, and (c) **6R** in 90% H_2O:10% CD_3CN.

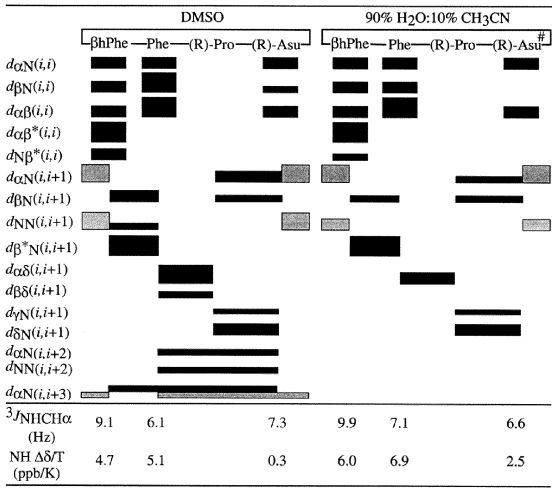


Figure 2. Summary of ROESY, $^3J_{NHCH\alpha}$ and variable-temperature ^{1}H NMR data for **6R** in d_6-DMSO and 90% H_2O:10% CD_3CN. Bars represent observed ROEs with intensities (strong, ≤2.5 Å; medium, ≤3.5 Å; weak, ≤5.0 Å) proportional to thickness. β* represents βhPhe backbone-CH_2. #Data acquired using the more soluble free acid.

^{1}H NMR resonances for **6R** and **6S** in d_6-DMSO were assigned from 2D TOCSY and ROESY spectra. ROEs ($d_{\alpha\alpha(i,i+1)}$) characteristic of cis peptide bonds were not observed, but strong ROEs between Phe CHα and Pro CHδ indicated a *trans*-amide bond at Pro. Multiple interresidue ROEs for both **6R** (Figure 2) and **6S** suggested a well populated, stable conformation defined by (i, i + 2) correlations. A temperature-independent chemical shift for Asu NH ($\Delta\delta/\Delta T \leq 3$ ppb)[4] in both **6R** (Figure 2) and **6S** supported its involvement in a structure-stabilizing H-bond. Large $^3J_{NHCH\alpha} > 9$ Hz for residues in **6R** (βhPhe, Asu) and **6S** (βhPhe) also provided strong evidence for a stable conformation in DMSO.

Two-dimensional ^{1}H NMR data (Figure 2) were similar for **6R** in DMSO and water (+ 10% MeCN), suggesting similar structures. Structures were calculated from DMSO, where there were more

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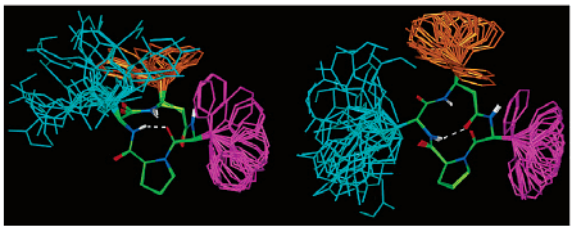

Figure 3. Superimposition of the 30 lowest-energy calculated NMR structures for **6S** (left, backbone RMSD 0.053 Å) and **6R** (right, RMSD 0.047 Å) in DMSO. White dashes indicate hydrogen bonds.

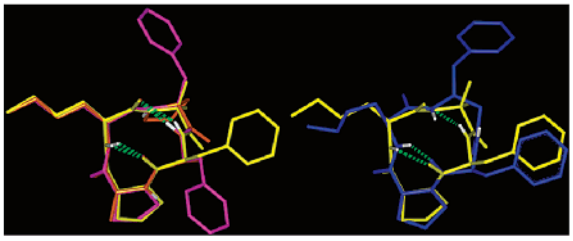

Figure 4. Superposition of X-ray structure of **7** (yellow)[7] on (a, left): modeled **8** (orange) and **9** (pink); (b, right): **6S** from Figure 3 (blue, RMSD: 0.55 Å (4 C$\alpha-$Cβ vectors), 0.48 Å (12 common backbone atoms)).

ROEs, for **6R** (30 ROE distance restraints: 14 intraresidue, 14 sequential, 2 medium range, and 2 ϕ-angle restraints: βhPhe ϕ $-120 \pm 30°$, Asu ϕ $-120 \pm 30°$) and for **6S** (32 ROE distance restraints: 20 intraresidue, 10 sequential, 2 medium range, and 1 ϕ-restraint: βhPhe ϕ $-120 \pm 30°$) using a dynamic simulated annealing and energy minimization protocol in XPLOR.[5] Initial structures indicated a likely H-bond in both **6R** (Asu NH$\cdots$OC βhPhe) and **6S** (Asu NH$\cdots$OC Phe), consistent with VT-NMR data (Figure 2).

The H-bond restraints improved structural convergence (Figure 3), well-defined backbones being consistent with stabilization by four *trans*-amides, D-proline, a trans-annular H-bond and steric constraints imposed by the 13-membered ring. The backbones and three side-chain projections (βhPhe, Phe, *R*-Pro) were similar for **6R** and **6S**, the epimeric Asu side chain being directed into or above the plane of the cycle, respectively. ϕ-Angles agreed with measured $^3J_{\mathrm{NHCH}\alpha}$ constants. (Supporting Information).

6S is homologous to bioactive CTPs such as chlamydocin (**7**), HC toxin (**8**), and trapoxin B (**9**).[2,3] Modeled[6] structures for **7**$-$**9** (Figure 4a) and structures in $CDCl_3$[3] for **7** and **8** have two γ-turns and a backbone conformation very similar to those of **6S** (Figure 4b). Inserting a methylene into the 12-membered ring rotates the Asu-βhPhe amide bond in **6**, preventing formation of one of the γ-turn H-bonds and redirecting the C$\alpha-$Cβ vector at βhPhe. Inversion of stereochemistry at Asu causes the Pro CO to rotate 69° in **6R** relative to **6S** (Figure 3), restoring a gauche relationship between the Pro carbonyl and the C$\alpha-$Cβ vector of Asu, permitting a β turn-defining Asu NH$\cdots$OC βhPhe H-bond.

To illustrate the potential of 13-membered CTPs as peptidomimetic scaffolds, the four C$\alpha-$Cβ vectors of **6S** and **6R** (Figure 3) were used as constraints to search for matching compounds in the Interbioscreen database with Catalyst.[8] Their unique side-chain projections matched a structurally diverse range of useful nonpeptidic templates, including sugars and spirocyclic compounds (Figure 5), found in natural products. For example, C$\alpha-$Cβ atoms of **6S** superimposed well (RMSD = 0.40 Å over eight atom pairs) on the indicated bonds (boxed) of carbohydrate **10**, which did not match nearly so well to **6R** (1.07 Å) or **7** (0.59 Å). On the otherhand **6R** superimposed well on the indicated bonds (boxed) of the tricyclic spiro-compound **11** (RMSD 0.51 Å), which did not match so well to **6S** or **7** (RMSDs 1.08, 1.20 Å). Thus CTPs with a β-amino acid could provide a useful link between protein architecture and nonpeptidic molecules. Libraries of such CTPs may be useful screening tools to rapidly identify pharmacophore space that can then be computer-matched to nonpeptidic structures.

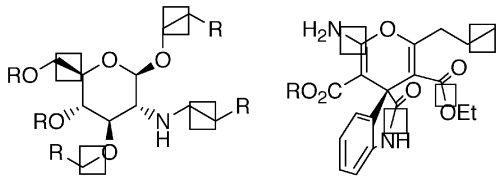

Figure 5. Sugar analogue **10** and spirocyclic compound **11** showing bonds (boxed) that match side chain C$\alpha-$Cβ vectors in **6S** and **6R**, respectively.

Thus selective incorporation of a β-amino acid can make CTPs easier to synthesize, more resistant to hydrolysis, conformationally homogeneous, and more useful as chiral scaffolds that can be derived from protein sequence alone. These advantages may expand the utility of CTPs as pharmacophoric probes which mimic both protein turns and natural products.

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Supporting Information Available: Syntheses, structures, characterization data for **4**$-$**6** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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