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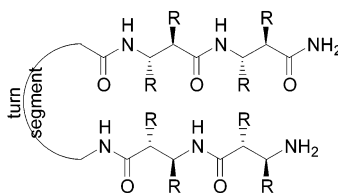
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Received December 16, 2003

## ABSTRACT



We describe a series of  $\beta$ -peptide hexamers that allow us to explore relationships between sequence and hairpin folding. Different reverse turn segments are compared at the central two positions, and the outer residues allow a variety of interstrand side chain–side chain pairings. NMR analysis in methanol demonstrates that several reverse turn and side chain pairing arrangements are compatible with antiparallel  $\beta$ -peptide sheet structure; however, none of the  $\beta$ -peptides folds in water.

In proteins, two classes of secondary structure display long-range order, helix and sheet.  $\beta$ -Peptide foldamers have been shown to adopt stable helical conformations in organic solvents and in water.<sup>1</sup> Parallel<sup>2</sup> and antiparallel<sup>3,4</sup>  $\beta$ -peptide sheet secondary structure has also been investigated, although less extensively, and folding has been clearly established only in organic solvents and in the solid state. Here we describe studies that explore how antiparallel  $\beta$ -peptide sheets tolerate variations in the reverse turn segment and in the arrangement of interstrand side chain pairs.

A hairpin (i.e., a strand-loop-strand motif) is the smallest increment of autonomously folding sheet secondary structure. The hairpin motif has been widely employed to generate  $\beta$ -sheet model systems among conventional peptides<sup>5</sup> and to explore sheet secondary structure involving nonnatural units.<sup>6</sup>

Hairpin designs have been used to elucidate some of the factors that govern the stability of antiparallel  $\beta$ -peptide sheets.<sup>3,4</sup> We used a hairpin motif to show that *syn*- $\alpha,\beta$ -dialkyl  $\beta$ -amino acid residues ( $\beta^{2,3}$  residues) have a higher

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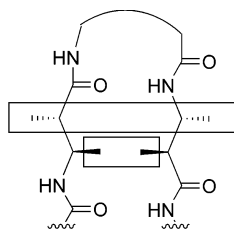
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sheet-forming propensity than do  $\beta$ -homoglycine residues or  $\beta$ -substituted ( $\beta^3$ ) residues.<sup>3a</sup> In subsequent work we employed hairpins to study how the stereochemistry of a dinipectic acid reverse turn segment affects sheet formation.<sup>3b,c</sup>

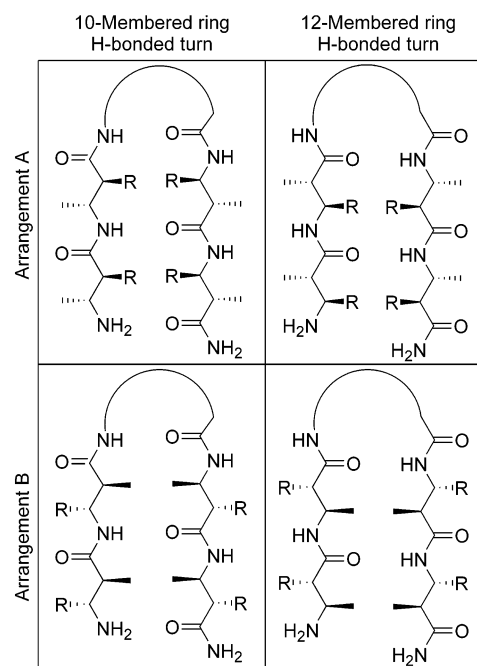
In antiparallel sheets, spatial juxtaposition (and therefore the potential for noncovalent interaction) occurs between the side chain at the  $\alpha$ -position of one residue and the side chain at the  $\beta$ -position of an appropriately aligned residue on a neighboring strand. For each interstrand residue pair there are two possible side chain juxtapositions, as shown in Figure 1. Known  $\beta$ -peptide sheet model systems<sup>2–4</sup> that are well-



**Figure 1.** General structure of an antiparallel  $\beta$ -peptide hairpin, with “side chain pairs” boxed.

folded in organic solvents share a limitation that may be significant with regard to folding in water. Each of the side chain pairs in each hairpin includes at least one methyl or ethyl group. Hairpin formation in water, a long-range goal in our laboratory, likely requires a hydrophobic driving force that would arise from noncovalent contacts between side chains from neighboring strands; methyl and ethyl groups probably do not have enough surface area to provide this driving force. In response to these limitations, we synthesized a set of *syn*- $\alpha,\beta$ -dialkyl  $\beta$ -amino acid residues that contain larger side chains, including polar lysine-like side chains, at both the  $\alpha$ - and  $\beta$ -positions.<sup>7</sup> The polar side chains were expected to confer water-solubility on  $\beta$ -peptides.

Using NMR spectroscopy, we examined how different pairing arrangements between two large side chains affect antiparallel hairpin formation in methanol. Methanol promotes secondary structure formation in both  $\alpha$ - and  $\beta$ -peptides,<sup>8</sup> and we chose this solvent for initial studies to determine whether interstrand pairing of large side chains led to steric destabilization of hairpin formation. Two possible side chain arrangements, shown in Figure 2, were examined. The side chain groups in both arrangements are roughly perpendicular to the plane occupied by the amide groups. Arrangement A shows all of the large side chain groups (“R”) oriented above the plane of the amide groups, whereas arrangement B shows all of the large side chain groups oriented below the plane of the amide groups. Inspection of CPK models suggests that both arrangements could accommodate large side chain pairs and could poten-



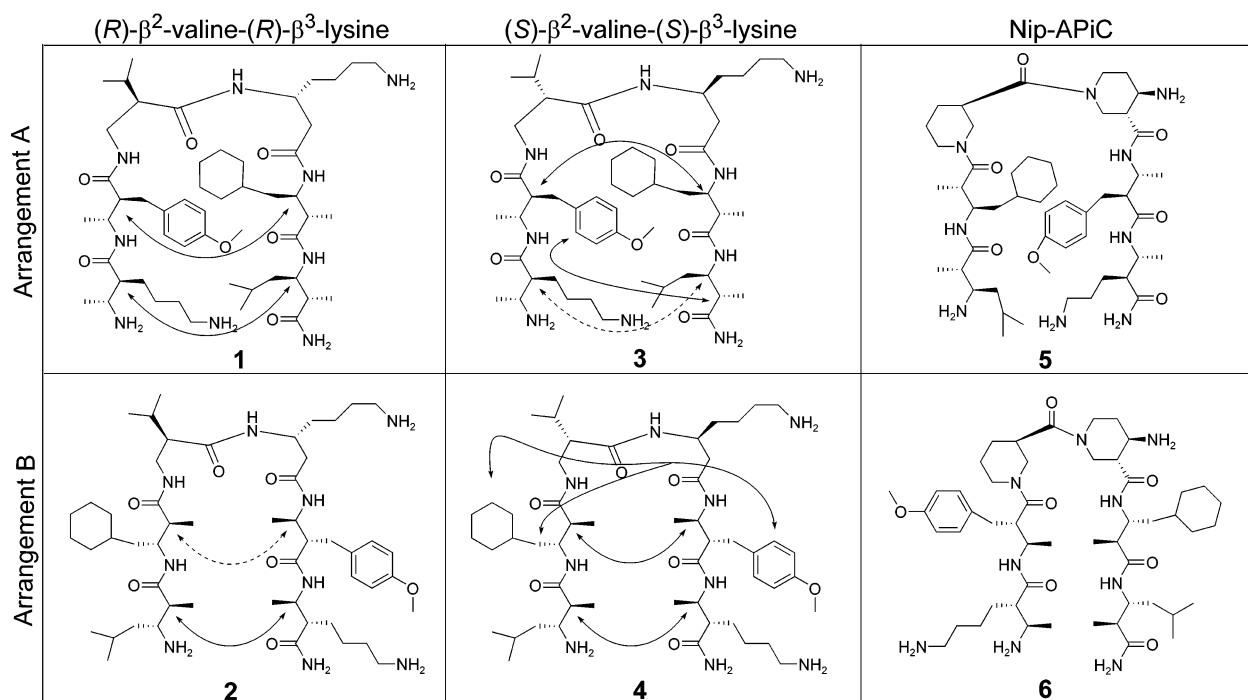
**Figure 2.** Definition of the two possible side chain arrangements for antiparallel  $\beta$ -peptide hairpins (R = large side chain group). Whether the turn segment is characterized by a 10- or 12-membered hydrogen bonded ring, arrangement A has large side chains paired above the plane of the hairpin, and arrangement B has large side chains paired below the plane.

tially allow favorable side chain–side chain interactions between neighboring strands in the two-stranded sheet. In conjunction with our examination of different side chain arrangements, we compared two  $\beta$ -peptide reverse turns, (*R*)-nipecotic acid-(3*S*,4*R*)-4-aminopiperidine-3-carboxylic acid<sup>9</sup> (Nip-APiC), and  $\beta^2$ -valine- $\beta^3$ -lysine. Nip-APiC is a derivative of (*R,S*)-dinipectic acid, a reverse turn that promotes sheet formation in CD<sub>2</sub>Cl<sub>2</sub>.<sup>3b,c</sup> This type of  $\beta$ -peptide reverse turn contains a 12-membered ring hydrogen bond (Figure 2, right column). A  $\beta$ -peptide containing the (*S*)- $\beta^2$ -valine-(*S*)- $\beta^3$ -lysine reverse turn segment was shown by Seebach et al. to adopt a hairpin conformation in methanol.<sup>4</sup> This type of  $\beta$ -peptide reverse turn contains a 10-membered ring hydrogen bond (Figure 2, left column). Because changes in reverse turn residue configuration can exert a large effect on hairpin folding among  $\alpha$ -peptides,<sup>5a</sup> we compared the (*S*)- $\beta^2$ -valine-(*S*)- $\beta^3$ -lysine turn segment with the (*R*)- $\beta^2$ -valine-(*R*)- $\beta^3$ -lysine segment. Hexa- $\beta$ -peptides **1–6** (Figure 3) were designed to probe various combinations among the three turn segments and the two side chain orientations outlined above.  $\beta$ -Peptide pairs **1/2** vs **3/4** vs **5/6** differ from one another in the reverse turn segments. The members of each pair, e.g., **1** vs **2**, differ from one another in the juxtaposition of the large nonpolar side chains on the strand residues. We wished to compare  $\beta$ -peptides that have large side chains above the plane of the hairpin (arrangement A) with  $\beta$ -peptides that

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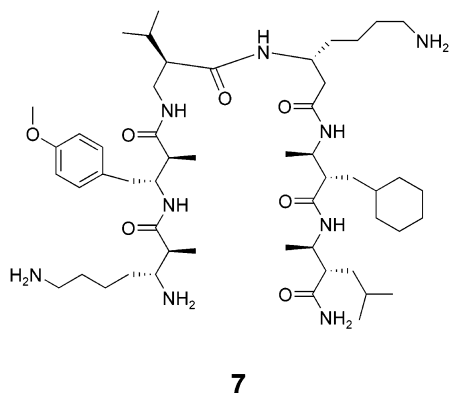
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**Figure 3.** Six antiparallel hairpins with different reverse turns and side chain arrangements. Structures **1**, **3**, and **5** have large side chains paired above the plane of the hairpin, whereas **2**, **4**, and **6** have large side chains paired below the plane. Turn segments are indicated at the top of each column. Nonsequential NOEs observed for  $\beta$ -peptides **1–4** in methanol are indicated. NOEs that could not be unambiguously assigned because of resonance overlap are dashed.

have the same large side chains below the plane (arrangement B). The most direct such comparison for  $\beta$ -peptide **1** would have been **7**. However, to move the *p*-methoxybenzyl and



lysine side chains of  $\beta$ -peptide **1** from the  $\alpha$ -carbons to the  $\beta$ -carbons of the N-terminal strand and correspondingly to move the cyclohexyl and leucine side chains from the  $\beta$ -carbons to the  $\alpha$ -carbons of the C-terminal strand would require the synthesis of three additional *syn*- $\alpha,\beta$ -dialkyl  $\beta$ -amino acid monomers. As an alternative that would not require the synthesis of new monomers, we designed  $\beta$ -peptides **2**, **4**, and **6**, in which the strand residues from **1**, **3**, and **5** are interchanged with their interstrand partners. This design has the dual effect of changing the overall side chain arrangement from A to B and placing side chains that were

formerly on the N-terminal strand on the C-terminal strand and vice versa.

A characteristic set of backbone–backbone NOEs was identified between sequentially nonadjacent residues in previous NMR studies of  $\beta$ -peptide hairpins.<sup>3,4</sup>  $C_\beta H-C_\alpha H$  NOEs are observed between *syn*- $\alpha,\beta$ -dialkyl  $\beta$ -residues that are expected to engage in interstrand hydrogen bonding. For compounds that contain a 12-membered ring hydrogen bonded reverse turn (possible in **5** and **6**),<sup>3</sup> these NOEs are in the N-to-C direction. For hairpins containing a 10-membered ring hydrogen bonded reverse turn (possible in **1–4**),<sup>4</sup> these NOEs are in the C-to-N direction.

The sharp amide proton signals in the  $^1H$  NMR spectra of hexamers **1–6** in  $d_3$ -methanol (<5 mM) suggest that little or no aggregation occurs in this solvent. Most of the  $^1H$  resonances of  $\beta$ -peptides **1–4** could be assigned using COSY,<sup>10</sup> TOCSY,<sup>11</sup> and NOESY<sup>12</sup> data. In addition to the expected sequential NOEs, several NOEs between residues not adjacent in sequence were observed for **1–4** (Figure 3).  $\beta$ -Peptides **1–4** displayed the two  $C_\beta H-C_\alpha H$  backbone–backbone NOEs characteristic of antiparallel sheet structure, albeit for **2** and **3** assignment of one of these NOEs was ambiguous because of resonance overlap.  $\beta$ -Peptides **3** and **4** also showed additional nonsequential NOEs involving side chain protons.

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Hexamers **5** and **6** differ from **1–4** in that **5** and **6** contain tertiary amide bonds.  $\beta$ -Peptides **5** and **6** both displayed multiple NMR resonances for each proton, suggesting the presence of multiple amide bond rotamers in  $d_3$ -methanol.<sup>13</sup> Because only the *Z/E* Nip-APiC rotamer depicted in Figure 3 supports sheet formation between the N- and C-terminal segments, other observed rotameric conformations must represent unfolded structures. Two-dimensional NMR analysis was attempted, but most of the resonances could not be assigned. These results are consistent with published observations for a related  $\beta$ -peptide in  $d_3$ -methanol.<sup>3b,c</sup>

Several conclusions can be drawn from the NMR data for **1–6**. First,  $\beta$ -peptides **1–4**, containing  $\beta^2$ -valine- $\beta^3$ -lysine turn segments, display at least partial population of the expected hairpin conformation (it is impossible to quantify hairpin folding from the data reported here or from any other readily available experimental information). In contrast, the complexity of the NMR spectra for **5** and **6**, containing the Nip-APiC turn segment, prevents us from drawing clear conclusions about partial hairpin formation by these molecules. Second, both configurations of the  $\beta^2$ -valine- $\beta^3$ -lysine turn segment support hairpin formation, although configurational differences may exert a subtle effect on the hairpin conformations adopted by **1–4**. This possibility is suggested by the fact that only backbone-backbone nonsequential NOEs were observed for  $\beta$ -peptides containing the (*R*)- $\beta^2$ -valine-(*R*)- $\beta^3$ -lysine turn segment (**1** and **2**), while other nonsequential NOEs in addition to the backbone-backbone NOEs were observed for  $\beta$ -peptides containing the (*S*)- $\beta^2$ -valine-(*S*)- $\beta^3$ -lysine turn segment (**3** and **4**). Third, pairing of large side chains on neighboring strands does not sterically disallow hairpin formation, as indicated by the NOE evidence for folding of **1–4**. Fourth, side chain arrangements A and B are both well tolerated in antiparallel  $\beta$ -peptide sheet (compare **1** with **2** and **3** with **4**).

We examined **1–6** for folding in water<sup>14</sup> using two-dimensional NMR spectroscopy. The <sup>1</sup>H NMR spectra of  $\beta$ -peptides **5** and **6** could not be assigned because of the presence of multiple tertiary amide bond rotamers. Most of the <sup>1</sup>H resonances of **1–4** could be assigned, but nonsequential NOEs could not be identified for any of these

$\beta$ -peptides. These results demonstrate that hexamers **1–6** do not adopt hairpin conformations to a significant extent in water. Driving forces for hairpin folding by  $\alpha$ -peptides in water include the intrinsic conformational preferences of the reverse turn and strand segments as well as hydrophobic interactions among side chains on neighboring strands.<sup>5</sup> The intrinsic conformational preferences of  $\beta$ -peptides **1–6**, plus the designed side chain pairs, evidently do not provide a large enough driving force to promote folding in water. Potential strategies to increase the driving force for  $\beta$ -peptide hairpin formation in water include synthesizing strand residues that contain larger hydrophobic side chains and designing  $\beta$ -peptides of greater length. Larger side chains could provide side chain–side chain interactions of increased strength, generating a large enough hydrophobic driving force to overcome the entropic cost associated with folding.<sup>15</sup> Increasing the length of the strands could lead to a more stable hairpin if the formation of antiparallel  $\beta$ -peptide sheet is cooperative, as is the case for formation of antiparallel  $\alpha$ -peptide sheet.<sup>16</sup> Although the synthesis of  $\beta$ -hexapeptides **1–6** was relatively straightforward, our efforts toward the generation of  $\beta$ -octapeptide hairpins have been complicated by synthetic difficulties.<sup>17</sup> Careful optimization of the synthetic conditions used to generate  $\beta$ -peptides that contain *syn*- $\alpha,\beta$ -dialkyl  $\beta$ -amino acid residues will be required before longer hairpin designs can be pursued.

**Acknowledgment.** This research was supported by the NIH (GM56414). J.M.L. was supported in part by an NSF Predoctoral Fellowship. We thank Bayard Huck, Jin-Seung Park, Emilie Porter, and Tami Raguse for contributing turn monomers. NMR equipment was purchased in part with funds from the NIH (1 S10 RR13866-01).

OL0364430

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