A β-Peptide Reverse Turn that Promotes Hairpin Formation

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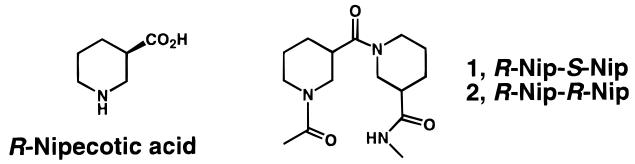
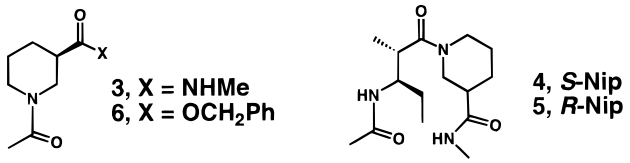
The β-turn represents a particularly favorable way for α-amino acid backbones to reverse direction, as required for compact folding of proteins.[1,2] Identification of unnatural polymer backbones that display compact folding patterns ("foldamers") is a goal in many laboratories.[3] Recently, we have shown that β-amino acid oligomers ("β-peptides") composed of appropriately chosen residues can adopt helix[4] or sheet[5] secondary structures; complementary findings have been reported by Seebach et al.[6−8] Here we demonstrate that nipecotic acid residues can form a β-peptide reverse turn and that a heterochiral dinipecotic acid segment promotes antiparallel sheet secondary structure.

One- and two-residue β-peptide segments were evaluated computationally for the capacity to form reverse turns.[9] Segments composed of acyclic[6] and/or cycloalkane-based[4] β-amino acid residues did not display strong reverse turn propensities, but dinipecotic acid segments were predicted to form very stable reverse turns. In particular, a heterochiral dinipecotic acid β-peptide unit, as in **1**, was predicted to form a 12-membered ring hydrogen bond, which is analogous to the 10-membered ring hydrogen bond commonly observed in β-turns of α-peptides. In contrast, the homochiral diastereomer, **2**, was predicted not to



favor the 12-membered ring hydrogen bond. These studies suggested that a tertiary amide was necessary at the center of the turn-forming segment because this amide adopts an *E* configuration in the reverse turn conformation (as shown), while secondary amides are largely confined to *Z* configurations.

We tested these predictions by preparing *R,S* β-peptide **1** and *R,R* diastereomer **2**,[10] and evaluating internal hydrogen bonding in dilute CH_2Cl_2 solutions with IR spectroscopy (Figure 1).[11] As predicted, **1** displays extensive internal hydrogen bonding (major N−H stretch band at 3350 cm^{-1}, consistent with a N−H- -O═C hydrogen bond), while **2** displays little hydrogen bonding (major band at 3455 cm^{-1}, consistent with solvent-exposed N−H). Diamide **3** also displays a small amount of internal hydrogen bonding, which shows that formation of an 8-membered ring hydrogen bond across a single nipecotic acid residue is neither favored nor completely precluded. Since only the second of the two residues need be nipecotic acid to provide a tertiary amide group at the center of the reverse turn segment, we examined di-β-peptide **4**, which contains an acyclic residue in the first



position.[12] The configurations at the α- and β-positions of this first residue favor an anti $NC_\beta-C_\alpha C(=O)$ torsion angle, which mimics the $NC_\beta-C_\alpha C(=O)$ torsional preference of a nipecotic acid residue. Indeed, modeling suggested that **4** would display a modest tendency for reverse turn formation; IR data (Figure 1), however, indicate that both N−H groups are largely free of hydrogen bonding in dilute solution. Similar behavior was observed for diastereomer **5**.

Simple *N*-acylated derivatives of nipecotic acid have little or no intrinsic rotamer preference, as indicated by the observation that **6** displays a 1:1 ratio of rotamers in $CDCl_3$, according to ^1H NMR. This behavior presumably arises from the lack of a steric bias for one rotamer relative to the other. ^1H NMR data suggest that three of the four possible rotamers are present for both **1** and **2** in CD_2Cl_2 because there are three broad resonances in the amide NH region (δ 5.8 to δ 7.5) in each case (1 mM). For **1**, the major NH resonance appears at δ 7.33, while minor NH resonances appear at δ 6.40 and δ 6.26. For **2**, the major NH resonance appears at δ 6.42, while the minor rotamers appear at δ 5.96 and δ 5.85. Since interconversion between hydrogen bonded and non-hydrogen bonded forms is rapid on the NMR time scale (in contrast to interconversion between amide rotamers), each δ NH value represents a weighted average of contributions

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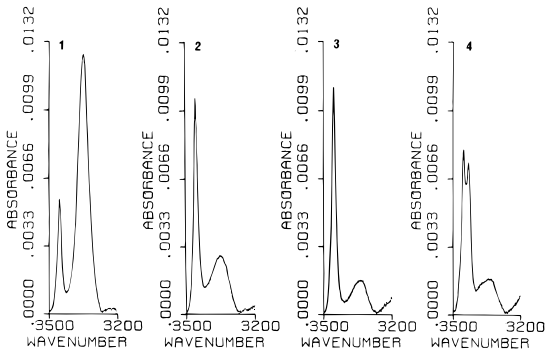
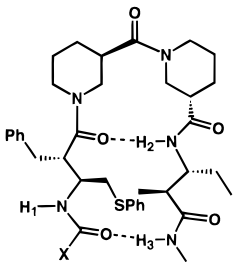


Figure 1. N−H stretch FT-IR data for 1 mM samples in CH_2Cl_2 at room temperature after subtraction of the spectrum of pure CH_2Cl_2. From left to right: **1**, maxima at 3454 (minor) and 3350 (major) cm^{-1}; **2**, maxima at 3455 (major) and 3348 (minor) cm^{-1}; **3**, maxima at 3454 (major) and 3339 (minor) cm^{-1}; **4**, maxima at 3454 (major), 3430 (major) and 3350 (minor) cm^{-1}. Data in the Supporting Information indicate that there is little or no aggregation in the 1 mM samples.

from the hydrogen bonded and non-hydrogen bonded states of the rotamer in question. Previous data,[5] and data reported below, suggest that δ NH $\leq$ 5.9 for a completely non-hydrogen bonded β-peptide amide proton, and δ NH $\geq$ 7.4 for a completely hydrogen bonded β-peptide amide proton, in the absence of unusual secondary effects. Therefore, the major rotamer of **1** appears to be internally hydrogen bonded to a large extent, as expected if this rotamer has the *Z* configuration at the N-terminal amide group and the *E* configuration at the amide between the two nipecotic acid residues. The minor rotamers of **1** and all three rotamers of **2**, on the other hand, seem to experience little or no internal hydrogen bonding. These conclusions are consistent with the IR data in Figure 1.

We are particularly interested in β-peptide reverse turns that can promote formation of antiparallel sheet secondary structure in attached β-peptide strand segments. The analogous strand-turn-strand motif in conventional peptides and proteins (α-amino acid residues) is referred to as a "β-hairpin" supersecondary structure.[13] Previously, we have shown that acyclic β-amino acid residues bearing one α- and one β-substituent, with relative stereochemistry as in the first and fourth residues of **7**, are very



7, X = CH_3
8, X = $OC(CH_3)_3$

well suited as strand residues for sheet secondary structure.[5] We prepared tetra-β-peptide **7** in order to determine whether the *R*,*S* dinipecotic acid segment would induce formation of a mini-hairpin, with just one residue in each strand.

Figure 2 shows the crystal structure of **8**, the immediate precursor to **7**; the tetra-β-peptide backbone adopts a hairpin conformation in the solid state. As anticipated, the amide between the two nipecotic acid residues has the *E* configuration, while the other tertiary amide is *Z*. Both cross-strand hydrogen bonds are present, and all four carbonyl groups associated with the hydrogen bonding partners are oriented in approximately the same direction. Thus, this type of β-peptide sheet has a net dipole, in contrast to β-sheets formed by α-peptides.[5]

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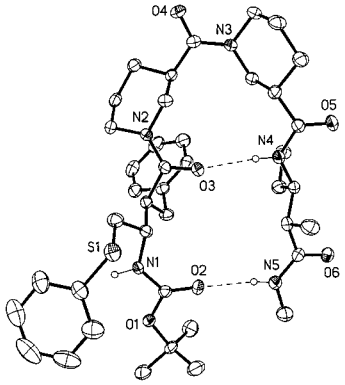

Figure 2. Ball-and-stick representation of the solid-state conformation of **8**. For clarity, all hydrogen atoms, except those attached to nitrogen, have been omitted. Hydrogen bonds are indicated with dotted lines. The nitrogen, oxygen, and sulfur atoms are labeled; note that the atom numbering in the crystal structure differs from the numbering in the text.

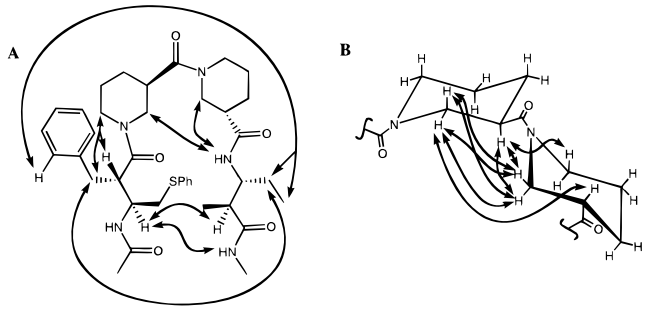

Figure 3. Summary of long-range NOEs observed in ROESY experiments for 1 mM **7** in CD_2Cl_2 (room temperature). Data in the Supporting Information indicate that there is little or no aggregation at 1 mM.

ROESY[14] data acquired for **7** in CD_2Cl_2 (1 mM) indicated that the hairpin conformation seen in the crystal structure is highly populated in this solvent (Figure 3a). The observed long-range NOEs are consistent with the folded conformation observed for analogue **8** in the solid state, and no long-range NOEs inconsistent with this structure were observed. NOEs between the nipecotic acid residues indicate that these two residues are linked by an amide in the *E* configuration (Figure 3b). High population of the hairpin folding pattern is also indicated by δ NH data for **7** (data not shown), which show that the N-terminal NH experiences relatively little hydrogen bonding, while the C-terminal NH and the NH of residue 4 are extensively hydrogen bonded.

We have shown that a heterochiral dinipecotic acid segment forms a stable reverse turn that promotes antiparallel sheet secondary structure in attached β-peptide strand segments. This result, along with previous reports on helix and sheet formation by properly designed β-peptides,[4−6] demonstrates that β-amino acid oligomers can display all three of the regular secondary structure types that are observed in proteins. β-Peptides displaying well-defined tertiary structure should also be within reach.[15]

Supporting Information Available: Variable concentration NMR data for **1**−**3** and **7**, ROESY data for **7**, and crystallographic data for **8** (27 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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