Solid-Phase Synthesis and Structural Analysis of Bicyclic β-Turn Mimetics Incorporating Functionality at the *i* to *i* + 3 Positions

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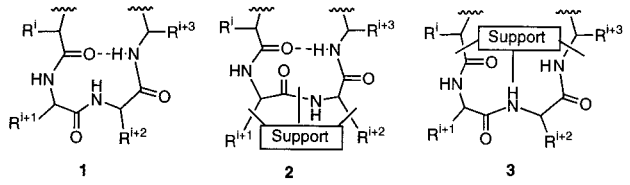
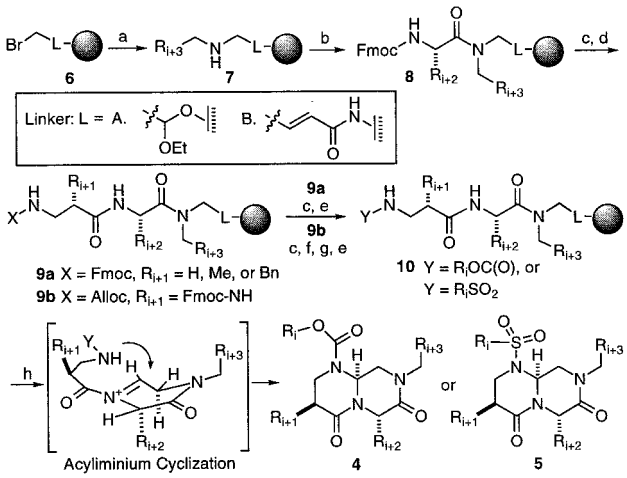
β-Turns, **1**, in biologically active peptides or proteins often play an important role in their interactions with receptors, enzymes, or antibodies.[1] In principle, such peptide−protein or protein−protein interactions can be mimicked by a small molecule bearing similar local structural features.[2] Therefore, libraries of such small molecules can serve as useful probes for drug discovery efforts and elucidation of biological events at the molecular level. Significant effort has been devoted to the development of monocyclic and bicyclic β-turn mimetics with the aim of discovering potent enzyme inhibitors and peptide hormone agonists and antagonists.[3] Such mimetics are constrained to form a β-turn peptide backbone structure by either external, **2**, or internal, **3**, support. For the interaction of the mimetics with a targeted protein,



1 **2** **3**

side chains of the internally supported β-turn mimetics are more accessible than those of the externally supported mimetics due to freedom from steric hindrance from external support. Several types of internal β-turn mimetics have been developed, a few of which have been generated in a combinatorial fashion using solid-phase synthesis.[4] These libraries of compounds, based on 9−16-membered cyclic lactams with secondary amide bonds, have been successfully applied to biologically significant targets.[4a,5] One concern with such macrocycles is that the geometry of the relatively flexible peptide backbone may not best reproduce a particular type of β-turn as accurately as that of a more rigid bicyclic scaffold. Here we report the solid-phase synthesis of conformationally constrained internal bicyclic β-turn mimetics, **4** and **5**, and the conformational analysis of these templates based on molecular mechanics, solution-phase NMR spectroscopy, and X-ray crystallography.

Scheme 1[a]



6 **7** **8** **4** **5**

[a] (a) 2M primary amine in DMSO; (b) Fmoc-α-amino acid, DIC/HOAT in NMP; (c) 20% piperidine in DMF; (d) Fmoc-β-amino acid or Fmoc-Dpr(Alloc), DIC/HOBT; (e) $R_iOC(O)ONp$ or R_iSO_2Cl, DIEA; (f) RCO_2H, DIC/HOBT; (g) cat. $Pd(Ph_3P)_4$, $PhSiH_3$; (h) formic acid at rt or cat. OsO_4/$NaIO_4$ then cat. TFA in CH_2Cl_2.

For the preparation of the 1,3,6,8-substituted tetrahydro-2H-pyrazino[1,2-a]pyrimidine-4,7-diones, **4** and **5**, the key transformation involves the acid-catalyzed tandem cyclization of the corresponding acetal or aldehyde.[6] The solid-phase generation of the intermediates was carried out by employing two types of linker, i.e., acetal type[6b] (A), or olefin type (B) (Scheme 1). Nucleophilic displacement of the bromide, **6**, with a number of primary amines gave the corresponding secondary amine, **7**, which was then coupled with the appropriate Fmoc-α-amino acids with HOAT/DIC in NMP.[7] Treatment of **8** with 20% piperidine in DMF followed by coupling with Fmoc-β-amino acids[8] or Fmoc-Dpr(Alloc)-OH afforded **9a** or **9b**, respectively. To introduce functionality at the *i* + 1 position of **9b**, the Fmoc group on the diaminopropionic acid (Dpr) residue was removed with 20%

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Table 1. β-Turn Mimetics **4** and **5**

	L	R_i	R_{i+1}	R_{i+2}	R_{i+3}	yield[a]
4a	A	Bn	H	Bn	*p*-Cl-Ph	71
4b	A	Bn	Ac-NH	Me	H	42
4c	A	Bn	Bz-NH	Me	H	31
4d	A	Bn	Me	Me	H	47
4e	A	*p*-OH-PhEt	H	Bn	*n*Bu	36
4f	A	Bn	Bn	Bn	*n*Bu	42
4g	B	Bn	H	Me	H	33
4h	B	Bn	H	*t*BuO$_2$C-Et	H	22
5a	A	Ph	H	Bn	c.Hexyl	57
5b	A	Ph	Ac-NH	Me	H	33
5c	A	*p*-Tolyl	H	Bn	*p*-Cl-Ph	62

[a] Isolated yield (%) for chromatographically purified compounds based on the initial loading of the solid support (ArgoGelOH or NH_2).

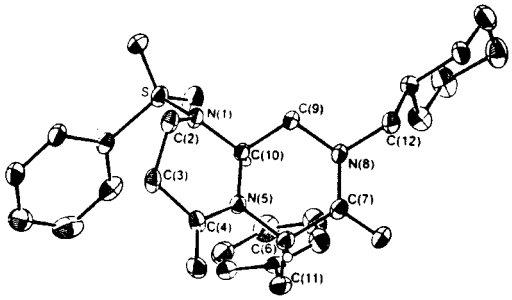

Figure 1. ORTEP illustration of **5a**.

piperidine and subsequently acylated with a carboxylic acid in the presence of DIC/HOBT. After deprotection of the Alloc group of **9b** with catalytic Pd(Ph$_3$P)$_4$/PhSiH$_3$[9] or the Fmoc group of **9a** with 20% piperidine, the resin was treated with alkyl or aryl sulfonyl chlorides or alkyl *p*-nitrophenylformates in the presence of DIEA to produce **10**. Cleavage from the acetal resin (Linker A) followed by acyliminium cyclization was performed by treatment with formic acid at room temperature.[10] Cleavage of the olefin linkage (Linker B) was effected by oxidation with a catalytic amount of osmium tetroxide and sodium periodate to liberate a mixture of aldehyde and hemiaminal. The product was then cyclized via an acyliminium intermediate with a catalytic amount of TFA in dichloromethane. This oxidation procedure for the Linker B is mild enough to retain acid labile functional groups, such as a *tert*-butyl ester. With this synthetic scheme, four functional side chains were introduced from four components: alkyl or aryl sulfonyl chloride or alkyl *p*-nitrophenylformate (R_i), β-amino acid derivatives (R_{i+1}), α-amino acid derivatives (R_{i+2}), and primary amines (R_{i+3}). Alkyl *p*-nitrophenylformates were generated in situ by the reaction of various alcohols with *p*-nitrophenyl chloroformate in the presence of 2,6-lutidine. These readily available components and simple synthetic procedures facilitate the introduction of diversity (Table 1). HPLC analysis of the crude products with monitoring at 214 nm showed the bicyclic mimetics as the major product in all cases. 2D-NMR experiments indicate the formation of only a single diastereomer,[11] in which the hydrogen at the ring junction is *trans* to the α-hydrogen at the $i + 2$ position. The stereochemistry was confirmed by X-ray crystallograpy for **5a** (Figure 1).[12]

Monte Carlo conformational analysis for the bicyclic template, **5a**, using the MMFF force field in vacuo as implemented in MacroModel,[13] revealed a dominant axial orientation of R_{i+2} for

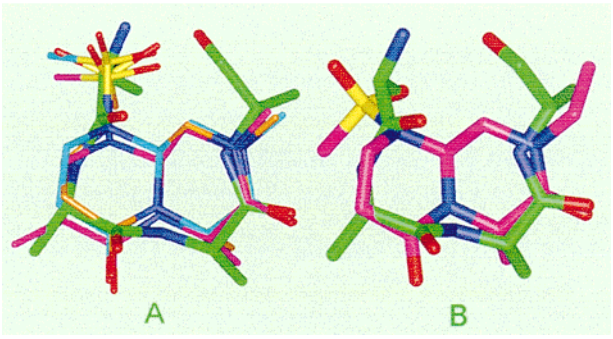

Figure 2. A. Overlay of the dominant low-energy conformers of **5a** (cyan and orange) and **5** (magenta) against type I β-turn (green). B. Overlay of the X-ray structure (magenta) against type I β-turn (green). The core structures of the template **5a** and **5** are shown for clarity.

low energy conformers. All conformations with a Boltzmann population (Pop.) of more than 0.5% were compared with idealized β-turns[14] (type I, I′, II, II′, VIa, and VIb) at seven atom positions [S, N(1), C(3), C(6), C(11), N(8), and C(12) atoms of the mimetic, **5a**, and the corresponding $C_\alpha(i)$, $C(i)$, $C_\alpha(i + 1)$, $C_\alpha(i + 2)$, $C_\beta(i + 2)$, $N(i + 3)$, and $C_\alpha(i + 3)$ atoms of ideal β-turns]. Good RMSD values (0.38−0.50 Å) were obtained for a type I β-turn conformation. The two dominant low energy conformers (Pop. = 45 and 39%) of the bicyclic mimetic closely approximate the type I β-turn as shown in Figure 2A. The RMSD values for the X-ray structure of the mimetic with idealized β-turns (vide supra) are as follows; 0.55 Å for type I, 1.35 Å for type I′, 0.92 Å for type II, 1.06 Å for type II′, 1.07 Å for type VIa, and 1.25 Å for type VIb. It appears that mimetic **5a** best represents the most common type I β-turn actually observed in proteins[14] (Figure 2B). In solution ($CDCl_3$, CD_3OD, or d_6-DMSO/D_2O) the same ring conformation as seen in the crystal structure predominates as judged by the data from ROESY experiments for **5a** [observed the key ROEs:[15] H(2, *pro-R*) − H(9, *pro-R*); H(10) − H(11)]. In addition, the same patterns of ROE's [H(2, *pro-R*) − H(9, *pro-R*); H(10) − H(11 or methyl at 6)] for **4b**, **4f**, and **5b** suggest that the predominant ring conformation of **4b**, **4f**, and **5b** is the same as that of **5a**, in which the R_{i+1} and R_{i+2} take an equatorial and axial orientation, respectively. Monte Carlo conformational analysis followed by comparison of the resulting low-energy conformers with an idealized β-turn at 8 atom positions[16] showed that a dominant conformer of template **5** (Pop. = 60%) and dominant conformers of **4f** closely mimicked a type I β-turn (RMSD = 0.60 Å for **5**, Figure 2A, Boltzmann weighted average RMSD = 0.53 Å for **4f**).

The generation of libraries of this β-turn scaffold aimed at biologically significant targets and the evaluation of their pharmacokinetic and pharmacodynamic profiles are underway and the results will be reported in due course.

Supporting Information Available: Experimental and crystallographic information (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) The cyclization for **4f** was carried out at 55 °C for 4 h.

(11) Most of the derivatives of type **4** exist as a mixture of two rotamers about the urethane linkage.

(12) **5a**: prism/colorless, crystal system: monoclinic, space group: $P2_1$ (No. 4), a = 12.3452(2) Å, b = 7.85710(10) Å, c = 13.9644(2) Å, α = 90°, β = 112.1119(6)°, γ = 90°, V = 1254.89(3) Å^3, Z = 2, R = 0.0357, R_w = 0.1010, GOF = 1.084.

(13) MacroModel V 6.0: Mohanmadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.

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(15) (a) The key protons are numbered according to their attached carbon in Figure 1. (b) The ROEs between the two phenyl groups expected from the X-ray structure were observed in d_6-DMSO/D_2O (3:2). However, the same ROEs were not observed in d_6-DMSO/D_2O (4:1), $CDCl_3$, or CD_3OD, which suggests that, although the benzyl group is axial, both benzyl and phenylsulfonyl groups maintain some distance from each other in relatively hydrophobic solvent system.

(16) S, N(1), C(3), C_β(3), C(6), C(11), N(8), and C(12) atoms of the mimetic, **5**, and the corresponding $C_\alpha(i)$, $C(i)$, $C_\alpha(i + 1)$, $C_\beta(i + 1)$, $C_\alpha(i + 2)$, $C_\beta(i + 2)$, $N(i + 3)$, and $C_\alpha(i + 3)$ atoms of ideal β-turns.