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A Macrocyclic Triproline-Derived Template for Helix Nucleation

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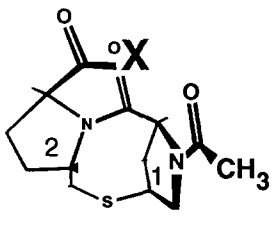
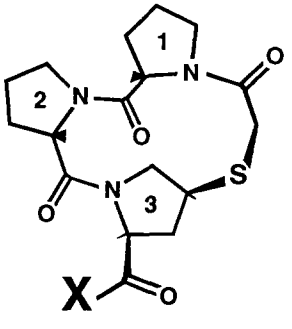
Abstract: The synthesis of **2** is reported in 11 steps from proline, 4-mercaptoproline, and iodoacetate. X-ray structural analysis is used to assign the cct amide orientation to **2** in the crystal.

Previously we have reported the synthesis and study of **1**, a conformational template[1] that induces helicity in a linked polypeptide under appropriate conditions.[2,3] Energetic characterization of helices formed by peptide conjugates of **1** will be reported elsewhere. To ensure that energetics observed for conjugates of **1** are not the result of special structural features of the template, other N-terrminal nucleating sites must be examined. Helices have been nucleated from linkages between side chain residues[4], and by electrostatic effects,[5] although nucleation from the N-terminus offers unusual design flexibility. Here we report synthesis and X-ray structural characterization of a potential macrocylic N-terminal helical template **2**.



1



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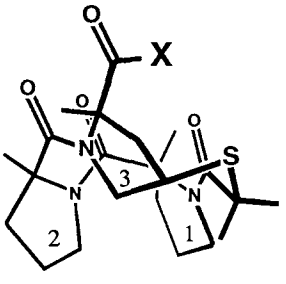
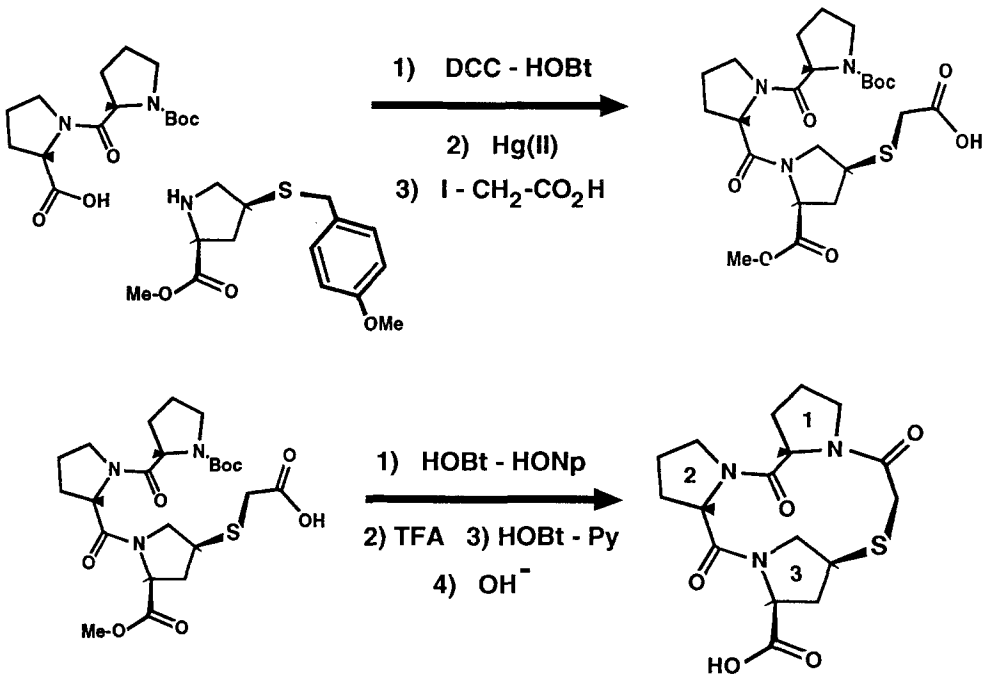


2a

Structure **2** is built from pyrrolidine rings and in fact includes much of the domain of **1**; thus rings labeled 1 and 2 in **2a** correspond to pyrrolidines of 1.. The conformation **2a** of **2** differs from **1** in providing four amide carbonyls as potential hydrogen bonding sites. The three amide carbonyls of **1** generate a hybrid 3_{10}-α-helix at the first hydrogen bonding site.[2] The four helically disposed amide carbonyls of **2a** define the structure at the peptide-template junction unambiguously as α–helical, and the versatile structure of **2** was

expected to permit conformational "tuning" through introduction of local constraints. In the accompanying paper the two conformations of **2** and its peptide derivatives in solution are assigned by ^1H NMR spectroscopy.

Scheme I



Scheme I outlines a synthesis of **2** proceeding in eleven steps from readily available starting materials.The required 4-thiaproline derivative is prepared as reported previously from hydroxyproline with minor modifications.[6] Formation of the macrocyclic lactam by cyclization of the p-nitrophenyl ester is optimal at 100°C in dry pyridine containing 0.1 M 1-hydroxy-benzotriazole (HOBt). at moderate dilution (< 1 mM) for 18 h. The yield of pure macrocyclic ester from the carboxylic acid is *ca.* 30 %, and the acid **2b** is readily prepared by this route on a scale of decigrams.[7] Parallel studies to be reported elsewhere with chiral thiolactate analogs suggest that complete exchange of the thioglycolate α-hydrogens occurs during the ring closure step. The products of these reactions are stable to epimerization under cyclization conditions. Though not proven, cyclization following fragmentation to a ketene is consistent with these observations.

Space-filling models of **2** reveal steric strain and suggest that the helical **2a** is a likely conformation. The stabilities of the conformations of **2** were estimated using molecular mechanics.[8] The tertiary amides each can assume either cis or trans orientations, generating $2^3 = 8$ major states that exhibit large energy differences. The thioether function can be oriented up or down, the carboxyamido function at ring 3 can assume peptide ψ angles of ca -10 to 0 ° or + 90 to 120 °, and the three pyrrolidine rings may assume alternative CH_2 puckers (left or right viewed from the top of 2a). More than 30 structures corresponding to relatively stable states were computer-generated and energy minimized, with results summarized in Table I. Part A compares the major

cis/trans amide orientations (signified by three-letter triplets), and the energy value shown corresponds to the most stable of the sub-states. (The ttc and ctc states were unstable to minimization).

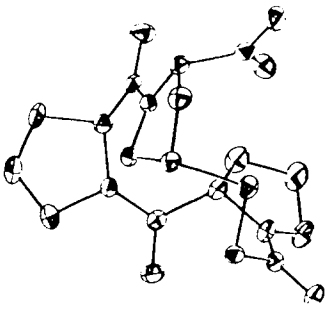
Table I Molecular Mechanics-Derived Energies of Potential Conformations of 2

A.	Amide Orientation	Helix Nucleating Potential	Calc Energy
	ccc	none	20.4 kcal/mol
	tcc	none	16.9
	tct	none	7.6
	cct	none	2.8
	ttt	α-helical	0.2
	ctt	3_{10} helical	- 1.4

B.	Amide Orientation	Sub-Conformation	Calc. Energy
	cct	S-up rll ψ +125°	2.84
	cct	S-up lrl ψ +125°	3.26
	cct	S-up rll ψ 0°	4.55
	cct	S-up rrl ψ +126°	4.70
	cct	S-up lll ψ +126°	4.84
	cct	S-down rrl ψ +120°	5.83
	cct	S-down lrl ψ +118°	6.48

This analysis generates only three plausible amide orientations for **2,** cct, ttt, and ctt. The margin of error for molecular mechanics-generated estimates of energy differences probably lies in the range of 5 kcal/mol for structures of these types, and a choice among these therefore cannot be made reliably. The results of an X-ray diffraction analysis [9] of a single crystal of **2** (x = OH) is depicted as an ORTEP diagram in **3.** The macrocycle is significantly strained. All but four of the twelve bond angles within the macrocyclic ring show distortions from normal values (average distortion: 4.5 °) with three angles showing deviations of +9 to +11°. This structure corresponds to the non-nucleating cct S-up CH_2-out conformation calculated by molecular mechanics to be the most stable of the cct sub-conformers, as seen in Table I Part B. A stereodiagram of this calculated state is shown for comparison in **4.** Strikingly, molecular mechanics does not identify the most stable orientation in the crystal or in solution from among the amide orientations of Part A , but it does select the most stable from the list of cct conformers. This difference probably reflects the errors in the analysis. The c-t amide conformers vary substantially in bond angle and dihedral distortions, but members of the cct group do not.

Among the stable calculated conformations of **2,** the S-up sub-states of the cct conformation have unique features. As is evident from **3** and **4,** none of the β, γ, or δ-protons of rings 1 or 2 are in van der Waals contact, and the α-hydrogen belonging to ring 1 is positioned within the tightly compressed span of the macrocycle, in proximity to the S and the carbonyl of the peptide linkage. No other conformation of **2** shares these features. These structural considerations provide the background necessary for interpretation of the solution-derived spectroscopic findings presented in the accompanying manuscript. They also provide the foundation for molecular redesign exercises in which incorporation of local constraints and additional substituents within the framework of **2** can be used to constrain and modify its structure.



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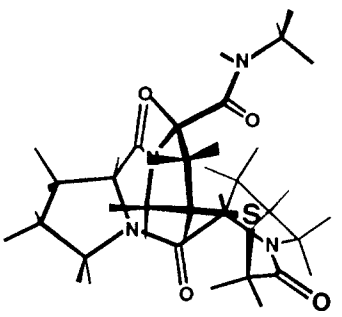

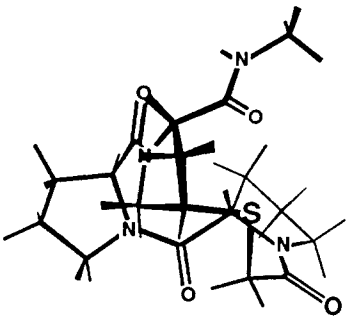

4 cct up rll

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5. Shoemaker, K.R.; Kim, P.S.; York, E.J.; Stewart, J.M.; Baldwin, R.L., *Nature*,**1987**, *326*, 563-567.
6. Slow addition of p-methoxybenzylthiolate to N-Boc-2(S)-methoxycarbonyl-4(R)-tosyloxypyrrolidine was required to avoid epimerization. Treatment of the product with HCl/dioxane generated the crystalline 2(S)-methoxycarbonyl-4(S)-(p-methoxybenzylthio)-pyrrolidine HCl salt, mp 105-106°C See also, Ref. 2. and Eschwarakrishnan, V.; Field, L., *J. Org. Chem.*, **1981**, *46*, 4182-4188.
7. The carboxylic acid precursor of the p-nitrophenyl ester required for the macrocyclic ring closure of Scheme 1 was obtained in an overall yield of 55 % for 3 steps from the thiaproline derivative.. Yield is 30% for overall conversion of this acid to the active ester, Boc cleavage with TFA, and cyclization to **2** as methyl ester, mp 119-122°C, MS: MH^+ 396.
8. Molecular modeling was carried out using the CHARMm QUANTA© 3.3 software of Molecular Dynamics Inc. Structures were generated systematically as described in the text and subjected to minimization; random conformational search and dynamics algorithms were used to check inclusiveness.
9. The acid **2b** crystallized from water by slow evaporation at 20 °C in the monoclinic system, lattice parameters a = 9.495 (5) Å, b = 7.361 (5) Å, c = 12.420 (2) Å , = 90.39 (2)°; space group $P2_1$ (#4).

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