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Maximizing the Stereochemical Diversity of Spiro-Ladder Oligomers

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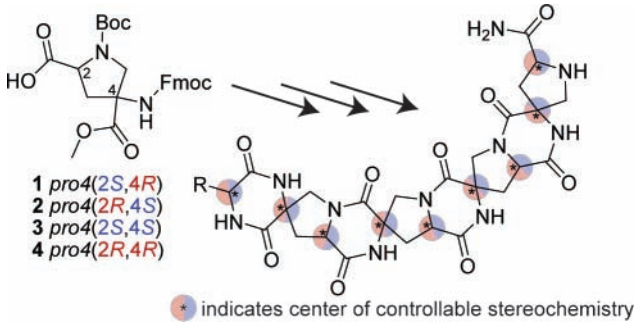
ABSTRACT



We introduce all stereoisomers of a bis-amino acid building block derived from *trans*-4-hydroxy-L-proline. This small library of monomers allows arbitrary stereochemical configuration at any chiral center within our spiro-ladder oligomers. Three tetramer oligomers containing several combinations of the monomers 1–4 were synthesized; we explored the effect of monomer sequence on scaffold conformation by NMR.

The ability to fashion nonnatural macromolecules that have designed tertiary structure and that precisely align chemical functionality in three-dimensional space would be valuable for biomimetic and nanotechnology applications.[1] Oligomer synthesis represents one strategy toward this end, as diverse sequences may be compiled from a small library of monomers. Foldamers are oligomers that adopt well-defined secondary structures with short sequences.[2] Most foldamers are assembled from monomers connected through single bonds and rely on hydrogen bonding and other noncovalent interactions to define their secondary structure.[3,4] Despite intense effort, there still is no systematic way to create oligomers with defined tertiary structure.[4]

We have devised a modular strategy toward the synthesis of water-soluble oligomeric molecules of designed shape.[5−7] We synthesize heterocyclic bis-amino acid monomers and couple them through pairs of amide bonds to form fused-ring spiro-ladder oligomers that lack rotational flexibility. Our long-term goal is to develop a library of monomers that can be assembled into any sequence and to develop functional oligomers by programming the sequence of monomers.

Previously, we described the synthesis of monomers **3** and **4** (Figure 1) gram-scale.[7] Both are derived from inexpensive

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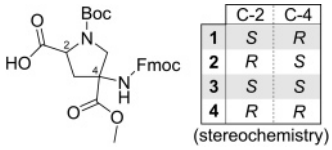


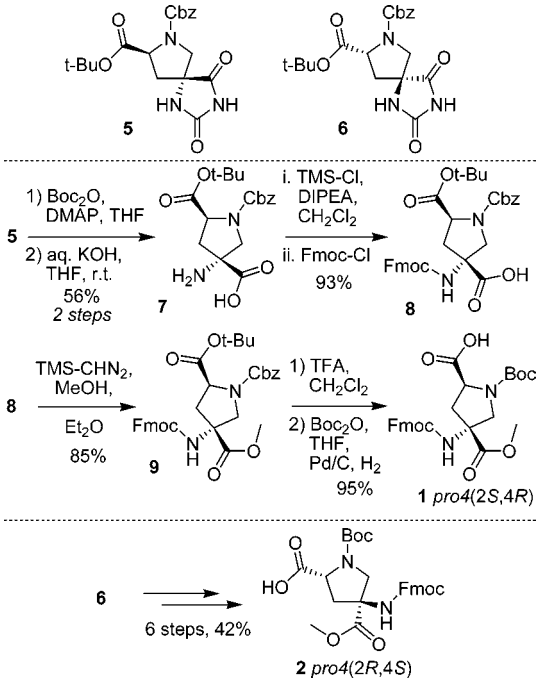
	C-2	C-4
1	S	R
2	R	S
3	S	S
4	R	R

Figure 1. Stereochemistry of monomers **1**−**4**.

and commercially available *trans*-4-hydroxy-L-proline; we have named **3** *pro4*(2*S*,4*S*) and **4** *pro4*(2*R*,4*R*). Herein, we report the gram-scale synthesis of **1** and **2** (the *pro4*(2*S*,4*R*) and *pro4*(2*R*,4*S*) monomers, respectively), the final stereoisomers of the *pro4* monomer class. This library of four monomers enables complete control over the stereochemistry at any chiral center of the oligomer and provides the means to direct hydrogen bonding groups in three-dimensional space along the length of the molecule.

We synthesized three tetramers; each sequence was a permutation of all four members of the *pro4* monomer class. The composition of each oligomer was verified by HRMS, whereas connectivity and stereochemistry were confirmed unambiguously through NMR experiments. We examined the three-dimensional structure of these oligomers in aqueous solution using two-dimensional NMR spectroscopy.

Scheme 1. Monomer Synthesis



Hydantoins **5** and **6** (Scheme 1) are the minor products of the Bucherer−Bergs reaction[8] performed during the synthesis of **3** and **4**.[7] Hydantoin **5** was hydrolyzed to amino acid **7** using a mild two-step procedure described previously.[9] The amine of **7** was converted to the Fmoc derivative **8** by the method described by Bolin,[10] and the carboxylic acid was converted efficiently to the methyl ester **9** using TMS−CHN_2. The *tert*-butyl ester **9** was cleaved with TFA in CH_2Cl_2, and the Cbz protecting group was exchanged for a Boc group,[11] yielding the desired monomer **1**. Monomer **2** was generated from **6** by the same route and with comparable yield.

The three oligomers described in this work are shown in Figure 2. For oligomer **13**, monomers **3**, **1**, **2**, and **4** were

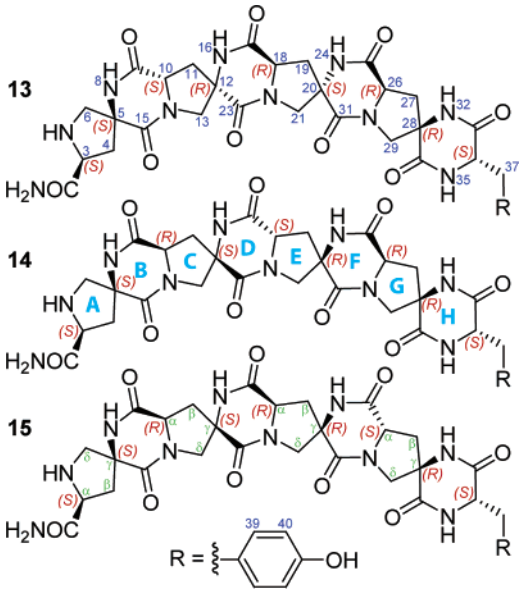


Figure 2. Structure of oligomers **13**−**15**. Backbone C−H−N atom numbering is labeled on **13** in blue (the same system is used for **14** and **15**). Stereochemistry is indicated in red. Heterocycle naming is labeled on **14** in cyan; relative pyrrolidine ring atom naming is illustrated in green on **15**.

coupled sequentially to solid support, followed by Fmoc-L-tyrosine. The synthesis was carried out on 100 mg of Rink Amide AM resin (0.64 mmol/g loading) using standard Fmoc solid-phase peptide synthesis methodology (Scheme 2). Each coupling was achieved using 2 equiv of monomer, 2 equiv of HATU,[12] and 4 equiv of diisopropylethylamine; double coupling was used to achieve quantitative yield. Tyrosine was added as a UV tag and to ease purification by increasing the lipophilicity of the oligomer.

The product was cleaved from the resin by treatment with 8% triflic acid in TFA for 1.5 h in an ice bath. The crude "open form" product **10** was precipitated from diethyl ether and dissolved in a solution of 20% piperidine in *N*-

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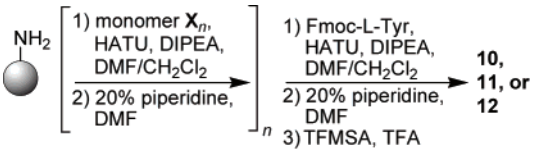
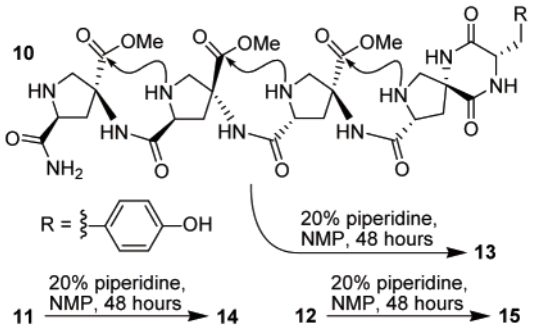
Scheme 2. Oligomer Synthesis



Oligomer	*pro4* monomer X_n for coupling *n*			
	n = 1	*n* = 2	*n* = 3	*n* = 4
13 (from **10**)	**3** (2*S*,4*S*)	**1** (2*S*,4*R*)	**2** (2*R*,4*S*)	**4** (2*R*,4*R*)
14 (from **11**)	**3** (2*S*,4*S*)	**2** (2*R*,4*S*)	**1** (2*S*,4*R*)	**4** (2*R*,4*R*)
15 (from **12**)	**3** (2*S*,4*S*)	**2** (2*R*,4*S*)	**4** (2*R*,4*R*)	**1** (2*S*,4*R*)



methylpyrrolidinone to accelerate the closure of the second amide bond between adjacent residues. After approximately 48 h, the desired product **13** was precipitated in ether and purified by reverse-phase HPLC on a C_{18} column. Oligomers **14** and **15** were prepared in a similar fashion, as described in Scheme 2.

For the NMR experiments, the oligomers were dissolved in 10% D_2O/H_2O; the aqueous solution contained 0.01 M acetic acid (d_4)/ammonium acetate (d_8) to neutralize TFA salts and to raise the pH to slow amide hydrogen/deuterium exchange. Experiments were performed at 4 °C. The two-dimensional NMR spectra (COSY, ROESY, HMQC, HMBC) were assigned using SPARKY;[13] the NMR data confirmed the expected connectivity and stereochemistry of **13**−**15**. Compared with less stereochemically-diverse *pro4* oligomers we have synthesized,[7] there is good chemical shift dispersion in the proton spectra of these molecules, particularly for the δ protons of the pyrrolidine rings.

The ROESY data were used to select oligomer conformations generated by an *in vacuo* stochastic search with the Amber94 force field[14] within MOE.[15] ROESY correlations between non-*J*-coupled protons were sorted by intensity, classified as strong, medium, and weak, and superimposed upon the energy-minimized conformers.

In the lowest-energy conformers, diketopiperazine (DKP) rings are shallow boats, whereas the pyrrolidine rings assume an envelope conformation where atom Cβ is positioned out of the plane defined by Cα, Cγ, Cδ, and the pyrrolidine N atom. In this conformation, Hβb and Hδb (Hβ and Hδ atoms on the face of the pyrrolidine ring opposite to Hα) are closer to one another than Hβa and Hδa (on the same face as Hα). This encodes a unique pattern of transannular ROESY interactions that enables us to distinguish this conformation from other pyrrolidine conformations. The minimized conformations we judged to be most consistent with the ROESY correlations are depicted in Figure 3. **13** and **15** are bent; **14** has a linear shape.

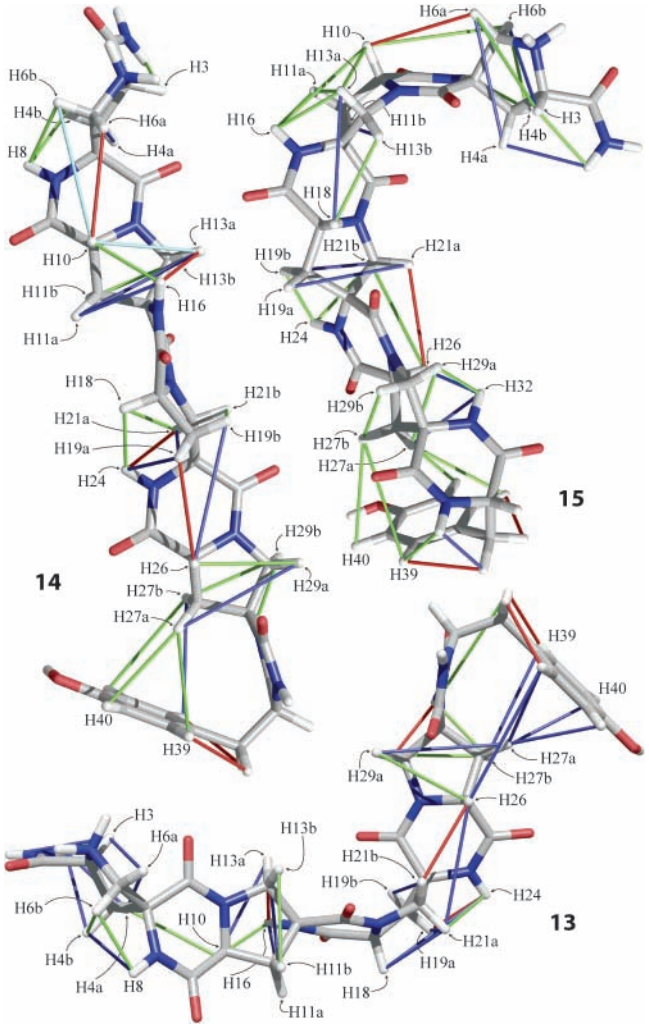


Figure 3. Conformations of **13**−**15** generated by molecular mechanics calculations that agree with observed ROESY correlations. The thin red, green, blue, and cyan cylinders represent strong, medium, weak, and undefined ROESY correlations, respectively.

The structure shown for **13** in Figure 3 is identical to the Amber94 minimum energy conformation except for pyrrolidine ring E. The integrated intensities of the Hβb−Hδb correlations across pyrrolidine rings A, C, and G (H4b−H6b, H11b−H13b, H19b−H21b, H28b−H29b) are 4−5 times greater than those for the corresponding Hβa−Hδa correlations, supporting the Cβ displaced conformation. The expected ROESY correlations across DKP ring D of **13** are occluded due to overlap, but the H10−H4a correlation

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suggests the predicted boat conformation of DKP ring B and provides additional evidence for the Cβ displaced conformation of pyrrolidine A. Correlations between H27a and H27b with H39 and H40 evince the predicted boat conformation of DKP ring H and suggest that the aromatic ring of the tyrosine residue is folded back against the backbone of the oligomer.

The conformation of pyrrolidine ring E of **13** (Cδ displaced rather than Cβ, with Hβa closer to Hδa than Hβb is to Hδb) is unexpected. The integrated intensity of the H19a−H21a correlation is 2 times greater than the intensity of the H19b−H21b correlation, and the H26−H21b correlation across DKP ring F is 4 times the integrated intensity of the H26−H21a correlation.

For compound **14**, the ROESY correlations were consistent with the minimum-energy Amber94 structure shown in Figure 3. The four Hβb−Hδb correlations (H4b−H6b, H11b−H13b, H19b−H21b, H27b−H29b) are 3−5 times greater in integrated intensity than the corresponding Hβa−Hδa correlations, suggesting the Cβ displaced conformation for every pyrrolidine ring. As with **13**, correlations across DKP ring D are overlapped. Nevertheless, the strong H10−H6a correlation supports the illustrated conformation of DKP ring B, and the strength of this correlation relative to H10−H6b (visibly weaker but overlapped, preventing integration) supports the depicted conformation of pyrrolidine ring A. Furthermore, the correlation H26−H19a, across DKP ring F, is 10 times the intensity of the correlation H26−H19b, which lends support to the conformation of DKP ring F and the envelope conformation of E shown in Figure 3.

The structure of **15** shown in Figure 3 is consistent with its Amber94 predicted minimum-energy structure, except for pyrrolidine ring C. The Hβb−Hδb correlations across rings A, E, and G (H4b−H6b, H19b−H21b, H27b−H29b) are visibly stronger than the corresponding Hβa−Hδa interactions (though the integrated intensities are greater only by factors of 1.2−2.1) supporting the predicted Cβ displaced conformations. For pyrrolidine ring C, the correlation H11a−H13a is slightly more intense (1.1 times) than H11b−H13b, suggesting the unexpected Cδ displaced conformation. The integrated intensities of the Hβa−Hδa correlations in **15** were similar to those between Hβb and Hδb, contrasting with the results for **13** and **14** and providing only weak support for the conformation of **15** shown. There are very clear ROESY correlations across all of the DKP rings of **15** (H10−H6a, H18−H13b, and H26−H21a which are 5, 9, and 2 times the intensity of H10−6Hb, H18−H13b, and H26−H21b, respectively) supporting the illustrated conformation of DKP rings B, D, and F and pyrrolidine rings A, C, and E.

In **14**, the *pro4*(2*R*,4*S*) residue is clearly in the Cβ displaced conformation; it precedes a *pro4*(2*S*,4*R*) residue in this oligomer. In both **13** and **15**, where *pro4*(2*R*,4*S*) precedes a *pro4*(2*R*,4*R*) residue, there is strong evidence that its pyrrolidine ring prefers the Cδ displaced conformation. This suggests that the preferred envelope conformation of a particular residue may be context dependent.

We carried out quantum mechanics calculations to calculate energy differences between the two envelope conformations of the pyrrolidine rings that would give rise to the different transannular ROESY correlation patterns that we observe. Density functional theory minimizations were performed on every contiguous trimer within oligomers **13**−**15** using Gaussian 03[16] (B3LYP/6-31G*, see Supporting Information). At this level of theory, we found that the differences in energy between pyrrolidine conformations were small (<1 kcal/mol), with no strong preferences for one ring conformation over the other. Our NMR observations suggest that the oligomers do have strong conformational preferences; the difference between experiment and theory may be due to solvent stabilization of one conformation over the other.

We synthesized all possible stereoisomers of the *pro4* monomer and demonstrated the assembly of oligomers incorporating all four monomers in different sequences. We solved the solution-phase NMR structures of three oligomers and established that the connectivity and stereochemistry for each was as designed. We are currently exploring new methods to probe the shape and rigidity of these oligomers, such as labeling the scaffolds with spin probes to study their shapes and dynamics by electron spin resonance.[17]

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Supporting Information Available: Experimental procedures for the synthesis of monomers **1** and **2**, oligomers **13**−**15**, characterization of new compounds, and 2D NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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