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Synthesis of a Pipecolic Acid-Based Bis-amino Acid and Its Assembly into a Spiro Ladder Oligomer

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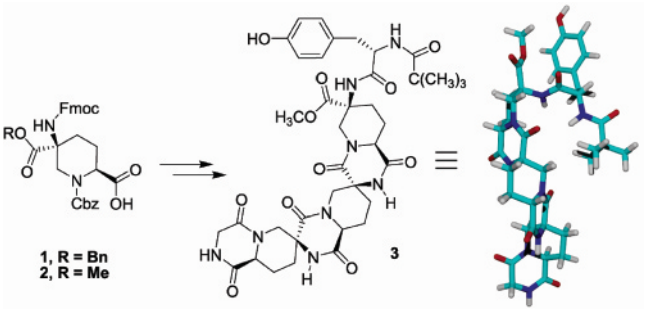
ABSTRACT



The synthesis of a new pipecolic acid-based bis-amino acid building block 1 (and 2) is presented. Assembly of this monomer into a spiro ladder oligomer 3 utilizing solid-phase synthesis followed by in situ activation by dicyclohexylcarbodiimide and *N*-hydroxysuccinimide has been demonstrated. The structure of oligomer 3, determined in aqueous solution using two-dimensional NMR, reveals that the oligomer forms a left-handed helix and that each monomer unit adopts a chair conformation.

A systematic approach to the rapid synthesis of macromolecules with designed shapes and functions would greatly facilitate the development of biomimetic chemistry[1] and nanotechnology.[2] Oligomer synthesis is an efficient approach to macromolecules because it is modular and allows the rapid assembly of large structures from a collection of small monomers. Many groups are developing unnatural monomers that are assembled through single bonds to form oligomers.[3−5] Foldamers are oligomers that contain a small number of monomers and adopt well-defined secondary structures in solution.[6−10] The development of a systematic approach to oligomers with designed tertiary structures is still elusive,[11] however, because of the immense complexity involved in predicting the folded structure of molecules with even a few rotatable bonds.[12]

We are developing stereochemically pure, cyclic, bis-amino acid monomers that couple through *pairs of amide bonds* to form spiro ladder oligomers that do not fold but, instead, display complex shapes by virtue of their rich stereochemistry and the well-defined conformations of their fused rings. Our long-term goal is to rapidly design, synthesize, and study macromolecules that have compact tertiary structures and contain small-molecule-sized cavities. Toward this goal we have developed synthetic access to bis-amino acid monomers **4** *pro4(2S4S)*[13] and **5** *hin(2S4R7R9R)*[14]

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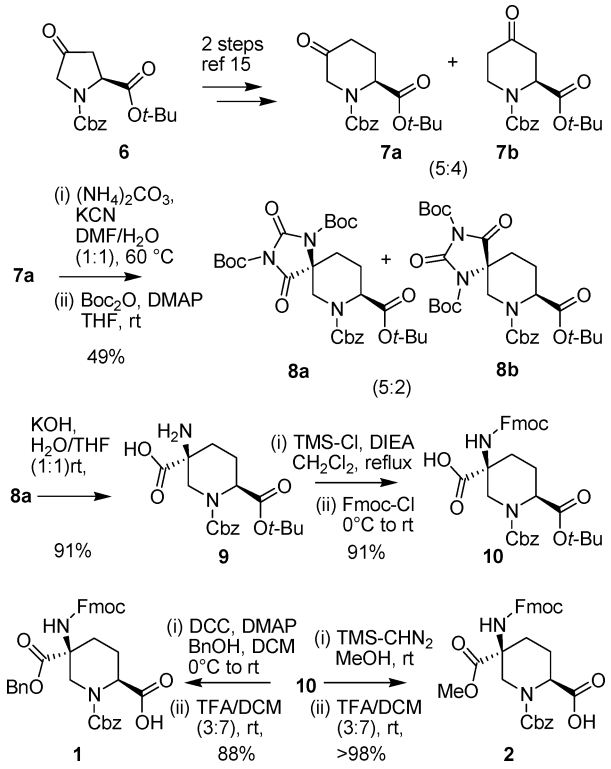
Scheme 1. Synthesis of *pip5(2S5S)* Monomer



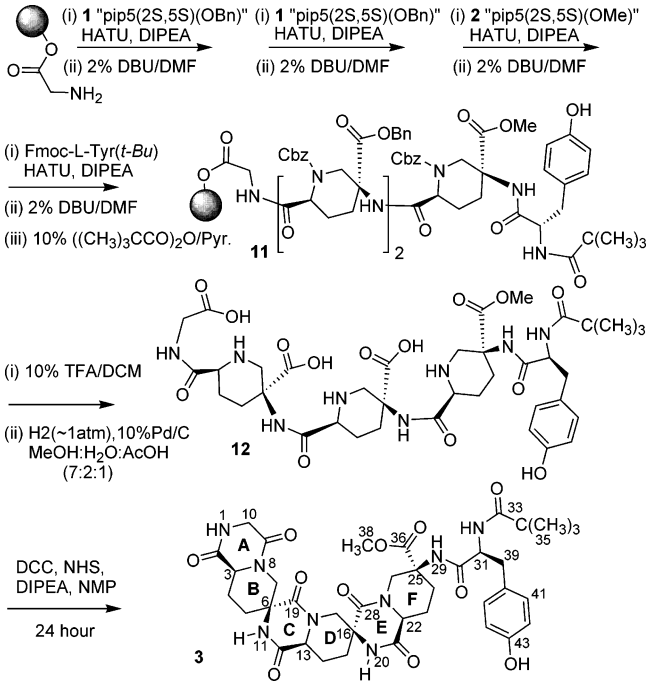
that form rods and turns and are easily coupled to each other. Herein we present the synthesis of a new monomer **1** that we have named *pip5(2S5S)*, its assembly into a homosequence of three monomers *gly-pip5(2S5S)-pip5(2S5S)-pip5-(2S5S)-(L)-tyrosine*, and its solution structure. We also demonstrate the use of in situ coupling as a new way to form diketopiperazine rings between adjacent monomers to rigidify scaffolds and create well-defined three-dimensional structure.

Synthesis of monomer **1** follows a route similar to the one developed for **4** *pro4(2S4S)*.[13] Ketone **6** is synthesized from inexpensive *trans*-4-hydroxy-L-proline, yielding a mixture of regioisomeric ketones **7a** and **7b** after homologation.[15] After chromatographic separation, ketone **7a** was subjected to a Bucherer−Bergs reaction,[16] thereby installing a quaternary stereocenter with a diastereoselectivity of 5:2 as determined by NMR. The mixture of diastereomeric hydantoins thus obtained was found to be inseparable. However, after protection with Boc groups, the two diastereomers **8a** and **8b** become easily separable by SiO_2 flash chromatography. After separation, the relative stereochemistry of **8a** and **8b** was determined.[17]

The major diastereomer **8a** was hydrolyzed using KOH to give the corresponding amino acid **9**,[18] which was subsequently protected with the Fmoc protecting group to produce **10**. Intermediate **10** was also treated with DCC/DMAP and benzyl alcohol followed by trifluoroacetic acid to form the benzyl ester version of the monomer **1**. Intermediate **10** was treated with trimethylsilyldiazomethane followed by trifluoroacetic acid to form the methyl ester version of the monomer **2**. Monomers **1** and **2** are suitable for sequential solid-phase coupling.

To test the ability of monomers **1** and **2** to form spiro ladder oligomers, a homooligomer consisting of three monomer units was synthesized on a 16.5 μmol scale on Rink acid resin using Fmoc solid-phase peptide synthesis techniques (Scheme 2).[19] The sequence consisted of *gly-pip5(2S5S)-pip5(2S5S)-pip5(2S5S)-(L)-tyrosine*. The role of the tyrosine is to provide a UV active group. A glycine residue was first attached to the resin followed by two benzyl ester monomers **1** and a methyl ester monomer **2**. After the three monomers were coupled, an Fmoc-L-tyrosine residue was added, and the α-amine of the tyrosine was capped with a trimethyl acetyl group. Each residue was activated as the 1-hydroxy-7-azabenzotriazole (HOAt) ester.[20] Near quantitative couplings to the previous monomer were achieved through double coupling of 2 equiv of activated monomer with respect to the resin loading. Couplings were carried out at room temperature for 90 min. After the trimethylacetyl group was coupled, the oligomer was cleaved from the Rink acid resin using 10% TFA/DCM. The carboxybenzoyl (Cbz) and benzyl groups were removed simultaneously by hydrogenolysis under a hydrogen atmosphere using 10% Pd/C as a catalyst. The

Scheme 2. Synthesis of a Short Oligomer with *pip5(2S5S)*



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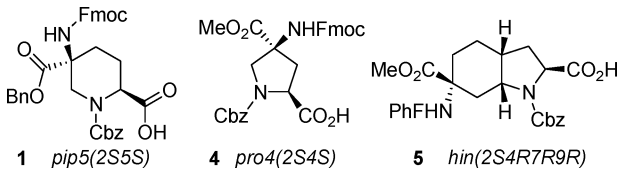
flexible oligomer **12** was then converted into the rigidified scaffold **3** by treatment with 1,3-dicyclohexylcarbodiimide (DCC) and *N*-hydroxy succinimide (NHS) in the presence of base (DIPEA) in NMP at room temperature for 24 h.

To our surprise, an earlier version of the oligomer assembled using only the methyl ester monomer **2** did not undergo intramolecular aminolysis between adjacent monomers on treatment with 20% piperidine in dimethylformamide. These are conditions that have proven to be effective in the synthesis of oligomers containing **4** *pro4(2S4S)*[13] and **5** *hin(2S4R7R9R)*[14] monomers. The secondary amines of the *pip5(2S5S)* monomers appear to be much less nucleophilic than the secondary amines of the *pro4(2S4S)* and *hin-(2S4R7R9R)* monomers. By replacing the methyl ester of the monomer with a benzyl ester, we were able to simultaneously deprotect the carboxybenzoyl groups and the benzyl esters of the oligomer to form a free amine and a free carboxylic acid between each adjacent pair of monomers (see compound **12**). When the carboxylic acids were activated in situ using DCC/NHS, intramolecular amide formation took place between each adjacent pair of monomers. This in situ coupling reaction simultaneously forms three diketopiperazine rings and produces a single pure product as determined by C_{18} reverse-phase HPLC and NMR. This indicates that the diketopiperazine rings form faster than any other macrocycles that would result from one of the three amines N8, N18, or N27 attacking one of the other activated carbonyl groups at C9, C19, or C28.

The molecular mechanics package MOE[21] was used to carry out a stochastic conformational search[22] of the three-mer sequence *gly-pip5(2S5S)-pip5(2S5S)-pip5(2S5S)-gly* in order to locate the lowest energy minima in vacuo using the AMBER94[23] force field. The modeled structure at the global energy minimum suggests that the sequence forms a helical rod and that each pipecolic acid ring has a strong preference for a chairlike conformation, placing the amide nitrogens N11, N20, and N29 in the axial positions (Figure 1).



1 *pip5(2S5S)* **4** *pro4(2S4S)* **5** *hin(2S4R7R9R)*

Figure 1. Structures of three synthetically accessible bis-amino acid monomers.

We determined the conformational preferences of the component rings of compound **3** using two-dimensional NMR in H_2O/D_2O 90:10 at 25 °C. The ^1H and ^{13}C resonances were assigned through the interpretation of a collection of two-dimensional spectra, including a DQF-COSY, a TOCSY, an HMQC, an HMBC and a ROESY spectrum. The assignment was carried out using the software package SPARKY.[24] For protons that were correlated to several other protons in the ROESY spectrum, the relative intensity of the cross-peaks was assigned as strong, medium, or weak on the basis of the integrated intensity. The chair conformation of each pipecolic acid ring was indicated by the correlation of three axial protons syn to each other on each ring. On the **B** ring of **3**, correlations are seen between H3, H5α, and H7α, and the axial orientation of the amine substituent N11 is indicated by the correlation between H11 and H4*β*. On the **D** ring of **3**, protons H13, H15α, and H17α are correlated, and the axial orientation of N20 is seen in the correlation between H20 and H14*β*. In the **F** ring of **3**, correlations are seen between H22, H24α, and H26α. The amine substituent N29 is not the part of a diketopiperazine ring and can rotate freely. However, we observe a correlation between H29 and H23*β*, suggesting that N29 is axial to ring **F**. These observations are consistent with rings **B**, **D**, and **F**

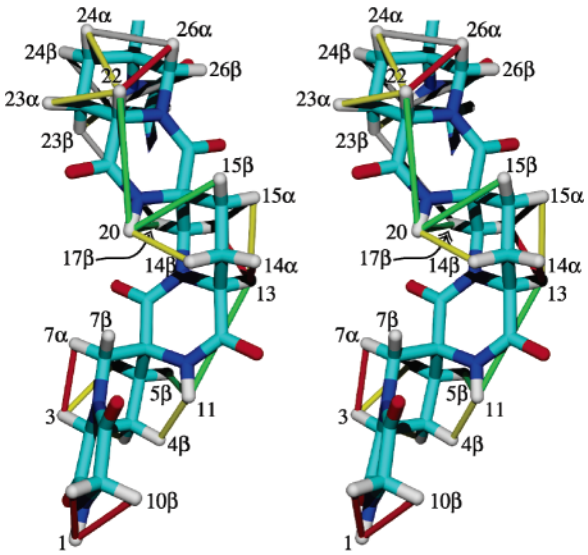


Figure 2. Stereoimage of the lowest energy conformation of **3**. Protons that are correlated in the two-dimensional ROESY spectra are connected by lines (strong, red; medium, yellow; weak, green; unassigned, gray). The tyrosine residue and pivalic group have been omitted for clarity.

each being in a chair conformation. The conformation of the diketopiperazine ring **C** is not clear because the only correlation seen across this ring is a very weak correlation between H11 and H13. A correlation is seen between H17*β* and H23*β*, suggesting that the diketopiperazine ring **E** is in a boat conformation that places C23 and C17 in a pseudo-axial orientation. Combining these conformational prefer-

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ences allowed us to construct a three-dimensional model of the spiro-fused ring structure of **3** shown in Figure 2. The model suggests that the molecule forms a left-handed helical structure. We have demonstrated the synthesis of monomer **1** and that it can be assembled into a spiro ladder oligomer. We have determined the conformational preferences of the monomers within the oligomer, and together they suggest that the oligomer forms a left-handed helix with only three monomers. We will combine this monomer with others to form longer sequences and test our ability to construct compact, water-soluble macromolecules that contain cavities with controlled size and shape for a variety of biomimetic and nanotechnology applications.

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Supporting Information Available: Experimental procedures, relevant NMR spectra, and characterization data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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