ORGANIC LETTERS

2004
Vol. 6, No. 19
3369−3371

Synthesis of a Bis-amino Acid that Creates a Sharp Turn

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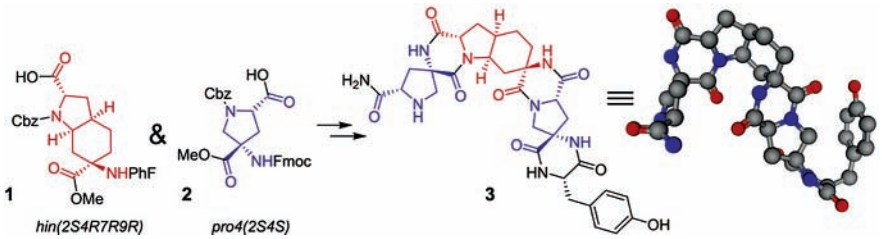
Received July 21, 2004

ABSTRACT



The synthesis of a new bis-amino acid 1 is presented. This monomer is designed to create a tightly curved structure when assembled into oligomers. The monomer is demonstrated to couple to the previously developed monomer 2 through pairs of amide bonds to create a strongly bent spiro-ladder oligomer. The structure of oligomer 3 was determined in aqueous solution using two-dimensional NMR.

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] Some of these oligomers have strong tendencies to form well-defined secondary structures through the influence of weak noncovalent interactions[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 that form rods and turns and are easily coupled to each other. We have previously synthesized monomer **2**, named *pro4(2S4S)*, and demonstrated that it is easily assembled into homooligomers that form water-soluble molecular rods of controlled lengths.[13] We present here a new monomer **1** that we have named *hin(2S4R7R9R)*. This monomer is designed to form a sharp turn, necessary for constructing a compact tertiary structure. We present the

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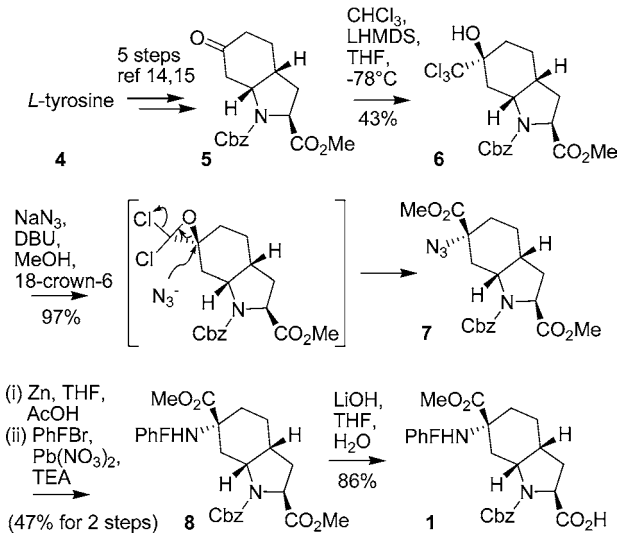
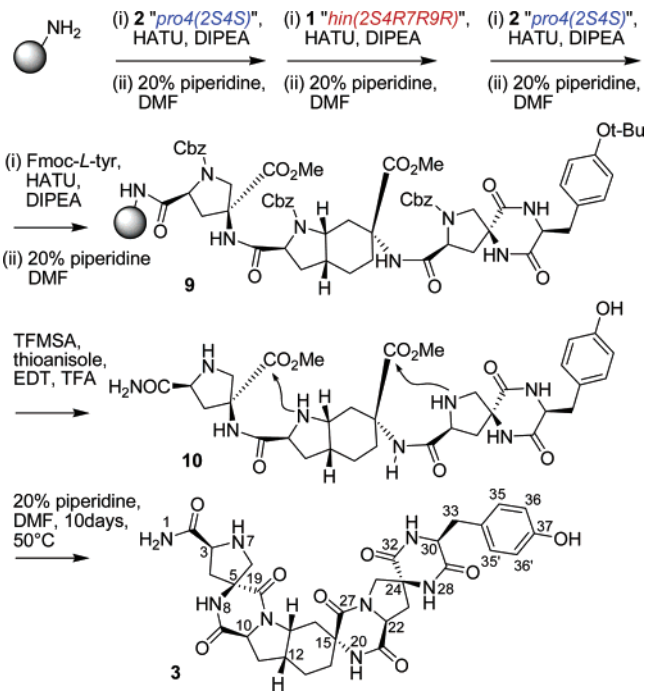
10.1021/ol048585f CCC: $27.50
Published on Web 08/20/2004

Scheme 1. Synthesis of *hin(2S4R7R9R)* Monomer



Scheme 2. Synthesis of a Short Oligomer Containing *hin(2S4R7R9R)* and *pro4(2S4S)* Monomers



synthesis of monomer **1** and demonstrate that it assembles into a heterosequence with our previous monomer **2**. We also present the solution structure of a three-mer of the sequence *pro4(2S4S)-hin(2S4R7R9R)-pro4(2S4S)-(L)-tyrosine* and demonstrate that monomer **2** forms a turn.

Monomer **1** was synthesized on a 1.5 g scale from inexpensive L-tyrosine in 10 steps (Scheme 1). The synthesis uses chemistry developed by Wipf and co-workers[14,15] to convert L-tyrosine to ketone **5**. Ketone **5** was reduced with trichloromethyl anion to alcohol **6** with 10:1 stereoselectivity. The major diastereomer **6** was crystallized from EtOAc/hexanes and the stereochemistry confirmed using X-ray crystallography. The trichloromethyl carbinol **6** was then converted in a single step, with inversion of configuration, to the azido methyl ester **7** using the modified Corey−Link reaction developed by Dominguez and co-workers.[16,17] This remarkable reaction is considered to proceed via the *gem*-dichloro-oxirane intermediate shown in Scheme 1.[18] The azide **7** was then reduced with Zn to the amine and protected using the phenylfluorenyl (PhF) group[19] to form **8**. Finally, the tertiary methyl ester was regioselectively hydrolyzed, in the presence of the quaternary methyl ester, to carboxylate **1** due to the protective effect of the phenylfluorenyl-protected amine. Monomer **1** contains two orthogonally protected amino acid moieties suitable for sequential solid-phase coupling.

We incorporated **1** into a heterosequence of three monomers (Scheme 2) using standard solid-phase synthesis techniques on a 16.5 μmol scale utilizing a MBHA·LL HCl resin.[20] The sequence consisted of *pro4(2S4S)-hin(2S4R7R9R)-pro4(2S4S)-(L)-tyrosine*. The role of the tyrosine is to provide a UV-active chromophore and to enhance the lipophilicity of the oligomer to allow C_{18} reverse-phase purification. We have previously observed that oligomers that do not carry lipophilic groups are too water soluble and refuse to bind to a C_{18} reverse-phase column. Each monomer was activated as the 1-hydroxy-7-azabenzotriazole (HOAt) ester.[21] Quantitative coupling to the previous monomer was 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 three monomers were coupled, the oligomer was functionalized with an Fmoc-L-tyrosine residue. An extended, 2 h deprotection step was then carried out to remove the N-terminal Fmoc group and to accelerate the attack of the terminal amine on the methyl ester of the *pro4(2S4S)* monomer that precedes it to form the diketopiperazine **9**. The oligomer was then cleaved from the MBHA resin with simultaneous removal of all of the carboxybenzoyl (Cbz) groups using a trifluoroacetic/trifluoromethane sulfonic acid mixture. The flexible oligomer **10** was then converted into the rigidified scaffold **3** by exposure to 20% piperidine/DMF over 10 days at 50 °C.[22]

The molecular mechanics package MOE[23] was used to carry out a stochastic conformational search[24] of compound **3** to locate the lowest AMBER94[25] energy minima in vacuo. The modeled structure of the global energy minimum shows

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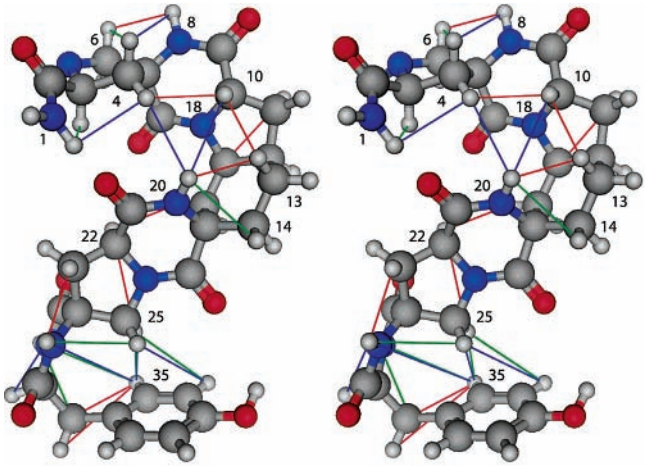

Figure 1. Stereoimage of the lowest energy conformation of **3**. Protons that are correlated in the 2D ROESY spectrum are connected with lines (strong, medium, and weak ROESY correlations are colored red, green, and blue, respectively).

that the molecule is strongly bent, with the cyclohexane ring of the central "*hin*" monomer adopting a chair conformation that places the amide nitrogen substituents N18 and N20 in a 1,3-diaxial arrangement (Figure 1). This 1,3-diaxial arrangement is reasonable because the planes of the two amides are parallel to each other and are locked in an extended fused ring system. The first low-energy minimum that displays a different cyclohexane chair conformation is 2.4 kcal/mol higher than the global energy minimum, indicating that molecular mechanics predicts that this oligomer has a 4 times greater kT preference for this conformation.

To test this molecular mechanics prediction we determined the solution structure of the three-mer **3** using two-dimensional (2D) NMR. The solution structure was determined in $H_2O/D_2O/CD_3CN$ 80:10:10 at a concentration of 4 mM at 4 °C. The ^1H and ^{13}C resonances were assigned through the interpretation of a collection of 2D spectra including a COSY, an HMQC, an HMBC and a ROESY. The assignment was carried out using the software package SPARKY.[26] The ROESY spectrum provided 26 cross-peaks that correlated non-*J*-coupled protons. The cross-peaks were ranked as strong, medium, and weak on the basis of their relative intensity. The ROESY correlations were superimposed on top of the minimum energy structure and are completely consistent with the model (Figure 1). The bent conformation of the *hin(2S4R7R9R)* monomer is supported by eight ROESY correlations, including a web of correlations between the proton on N20 and protons on C4, C10, C13, and C14, as well as strong correlations between the α-proton on C22 and the proton of C16 (Figure 1). The diketopiperazines between monomers form shallow boats with their substituents C11 and C23 occupying pseudoequatorial orientations. The proline ring-based *pro4(2S4S)* monomers adopt an envelope structure that avoids a 1,3-interaction between the two substituents that are syn to each other on the ring (C2 and N8, and C21 and N28). These conformations are identical to what was seen in a previous NMR structure that contained *pro4(2S4S)*.[13] The folded-back conformation of the tyrosine side chain is also a feature that was seen in a previous NMR structure and is probably due a hydrophobic interaction between the aryl ring and the methylene of the last *pro4(2S4S)* monomer.[13]

We have demonstrated the synthesis of monomer **1** and that it can be assembled into a heterosequence with another monomer **2**. We have determined the solution structure of this monomer in the context of a short oligomer, and, at 4 °C, it has a strong conformational preference to form a tight curve. Combining this monomer with others to form longer sequences should enable us to construct compact, water-soluble macromolecules that contain cavities with controlled size and shape for a variety of biomimetic and nanotechnology applications.

Acknowledgment. This work was supported in part by the NIH/NIGMS (GM067866) and by the Research Corporation. We thank Dr. Steven Geib for X-ray structural analysis. We thank Dr. Kasi Somayajula for assistance with LC/MS and mass spectroscopy. We thank Dr. Fu Tyan Lin for assistance with NMR.

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.

OL048585F

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