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The Synthesis of Functionalized Nanoscale Molecular Rods of Defined Length

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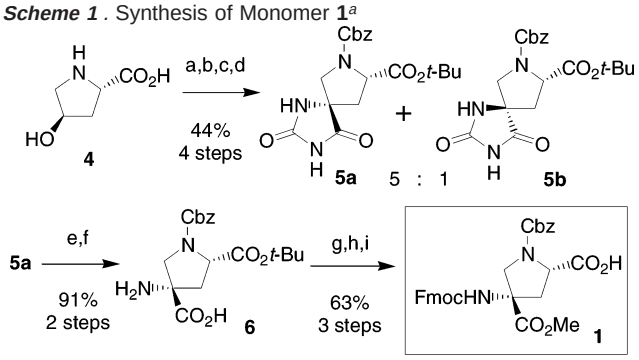
The development of systematic approaches to the synthesis of nanometer scale molecules of discrete size, shape, and mechanical properties is a long-term goal of macromolecular chemistry.[1−10] Molecular rods are macromolecules that have many exciting potential applications in nanoscale science as spacers, wires, and construction elements; yet as Michl's authoritative review[11] points out, the poor solubility of many current examples of molecular rods is the primary obstacle to their study and application. We have developed a synthetic approach to water-soluble molecular rods that are of defined length and contain multiple chiral centers. These molecules can be easily synthesized and functionalized on both ends. We present here the synthesis of a prototype molecular building block **1** and demonstrate the solid-phase synthesis of two molecular rods **2** and **3** containing three and five monomers, respectively. Molecular mechanics was used to predict the conformation of the scaffold **2**, and the prediction was confirmed by 2D NMR analysis.

Scheme 1. Synthesis of Monomer **1**[a]

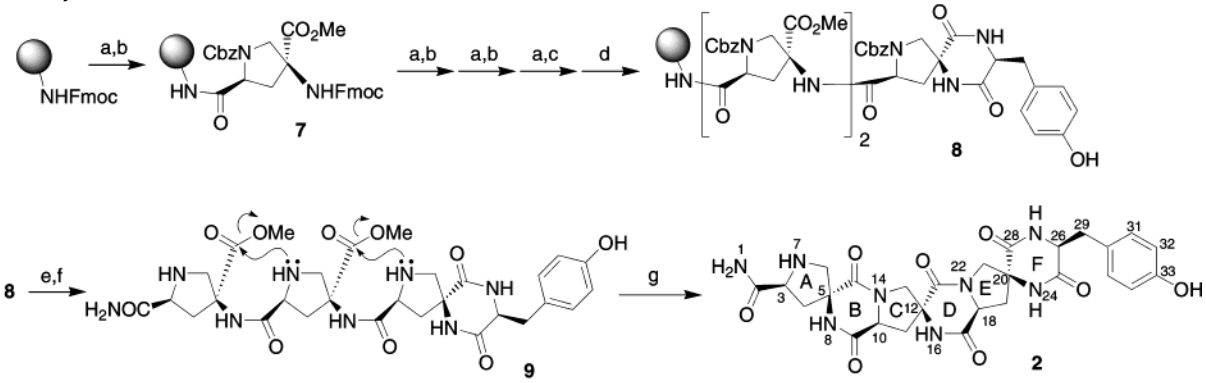


[a] Reagents and conditions: (a) (i) TMS−Cl, NEt(*i*Pr)$_2$, CH_2Cl_2, reflux; (ii) Cbz-Cl, 0 °C to room temperature. (b) Jones reagent, acetone, 20 °C. (c) Isobutylene, H_2SO_4 (cat.), CH_2Cl_2, room temperature. (d) (i) $(NH_4)_2CO_3$, KCN, 1:1 DMF/H_2O, 60 °C, sealed tube; (ii) silica gel chromatography, 99:1 CH_2Cl_2/MeOH. (e) $(Boc)_2O$, DMAP, THF, room temperature. (f) KOH, 1:1 H_2O/THF, room temperature. (g) (i) TMS−Cl, NEt(*i*Pr)$_2$, CH_2Cl_2, reflux; (ii) Fmoc−Cl, 0 °C to room temperature. (h) (i) MeOH, DCC, DMAP, CH_2Cl_2, 0 °C to room temperature; (ii) silica gel chromatography 2:1 EtOAc/hexanes. (i) CF_3CO_2H/CH_2Cl_2.

Our prototype building block **1** was synthesized on a 1.8 g scale from inexpensive, commercially available *trans*-4-hydroxy-L-proline **4** in nine steps with an overall yield of 25% (Scheme 1). The synthesis uses a Bucherer−Bergs reaction[12] to install a quaternary stereocenter with a diastereoselectivity of 5:1.[13] Using a single chromatographic column, we isolated 16.5 g of pure diastereomer **5a** from a 20 g mixture of **5a** and **5b**. The intermediate **5a** leads to the differentially protected bis amino acid **1**.

We assembled three building blocks to form the molecular rod **2** using sequential solid-phase synthesis on a 46 μmol scale (Scheme 2). Each building block was activated as the 1-hydroxy-7-azabenzotriazole (HOAt) ester,[14] and quantitative coupling to the previous building block was achieved in less than 10 min, a surprising result given the apparent hindered nature of the nucleophile. After three monomers were coupled, the oligomer was functionalized with a tyrosine residue to facilitate characterization. The flexible oligomer **8** was cleaved from the resin, and high-performance liquid chromatography with mass spectrometry (HPLC-MS) analysis revealed a single peak with the expected *m*/*z* ratio of 1061.2 (M + H^+). The carboxybenzyl (Cbz) groups were then removed by hydrogenolysis to form **9**. The flexible oligomer **9** was converted into the rigidified scaffold **2** through the parallel formation of two diketopiperazine[15] rings by exposure to 20% piperidine/DMF over 48 h at 4 °C.[16] The product **2** precipitated from the 20% piperidine solution, and filtration was used to recover 5 mg (∼15% yield based on resin loading). HPLC-MS analysis of this material reveals a single peak with the expected *m*/*z* ratio of 595.2 (M + H^+) for **2**. The scaffold **2** is soluble in water to more than 5 mg/mL and stable in neutral and acidic aqueous solution at room temperature for weeks.

Scheme 2. Synthesis of Scaffold **2**[a]



[a] Reagents and conditions: (a) 20% piperidine/DMF, 40 min, room temperature. (b) Two times 2 equiv of **1**−OAt ester, 4 equiv of NEt(*i*Pr)$_2$, 20% CH_2Cl_2/DMF, 60 min, room temperature. (c) 3 equiv of *N*-α-Fmoc-*O*-*tert*-butyl-L-Tyr-OAt ester, 6 equiv of NEt(*i*Pr)$_2$, 20% CH_2Cl_2/DMF, 60 min, room temperature. (d) 20% piperidine/DMF, 120 min, room temperature. (e) 95% CF_3CO_2H/2.5% $(iPr)_3SiH$/2.5% H_2O, 120 min, room temperature. (f) H_2 (∼1 atm), 10% Pd/carbon, 7:2:1 MeOH/H_2O/AcOH, 60 min, room temperature. (g) 20% piperidine/DMF, 48 h, 4 °C.

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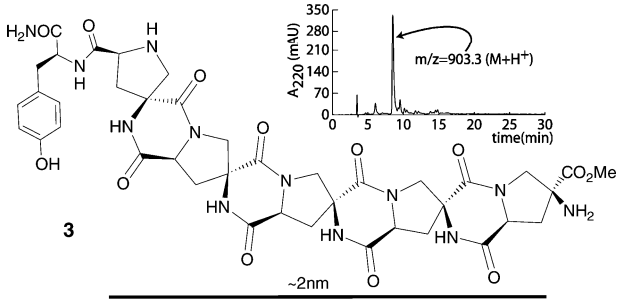

Figure 1. The chemical structure of scaffold **3**. Modeling indicates that the spiro-fused ring structure forms a narrow left-handed helical rod with approximately four residues per turn and a pitch of ~20 Å. Inset: the C_{18} reverse phase HPLC chromatogram of the unpurified ether precipitated product containing **3** (0−20% MeCN/H_2O, 0.1% TFA).

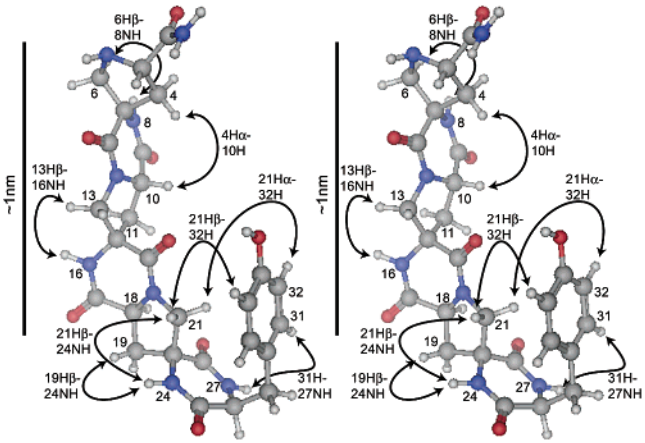

Figure 2. A stereoimage of the predicted third lowest energy conformer of scaffold **2** most consistent with NMR data. The arrows indicate that strong and medium ROESY cross-peaks are seen between the indicated pairs of protons. The molecule forms a molecular rod about 1.5 nm in length.

To demonstrate the generality of this synthetic approach, the five-mer scaffold **3** (Figure 1) was synthesized in a fashion similar to that of scaffold **2**. The resin (49 mg, 31.4 umol loading) was first charged with an Fmoc-protected tyrosine residue, and then five cycles of coupling using monomer **1** were performed. Roughly 13 mg of resin was removed and subjected to cleavage conditions. The Cbz groups were removed and the scaffold was rigidified by exposure to 20% piperidine/DMF over 24 h. In this case, about 3 mg of the scaffold **3** (~33% from initial resin loading) was isolated by precipitation with ether and centrifugation. After all of these manipulations, this unpurified material was remarkably homogeneous (Figure 1), and HPLC-MS analysis confirmed that the major peak has the expected *m*/*z* ratio of 903.3 ($M + H^+$). This material was soluble in 10% D_2O/H_2O at 5 mg/mL.

To construct a model of the scaffold **2**, we carried out an *in vacuo* conformational search using the AMBER95 force field[17] within the molecular mechanics package MOE.[18] The conformational search revealed a cluster of five lowest energy conformations all within 0.4 kcal/mol of each other separated from the next highest energy cluster by a gap of 2.2 kcal/mol. This energy gap predicts that the molecule will spend roughly 98% of its time collectively in the five lowest energy minima (Figure 2). A superposition of these predicted lowest energy conformations reveals that rings B, C, D, E, F and the folded tyrosine conformation are identical. The differences between the five conformations involve combinations of rotamers around the C2−C3 bond, rotamers around the tyrosine OH bond, and two envelope conformations of ring A. The 2D ROESY spectra display cross-peaks consistent with those from the predicted rigid B−F ring system. The NMR data are more consistent with ring A existing predominately in the single envelope conformation depicted in Figure 2 based upon a strong cross-peak between 4Hα and 10H and a weak or nonexistent cross-peak between 4Hβ and 10H.

We have demonstrated the synthesis of two nanoscale molecular rods using a novel building block approach. The synthesis takes place in two phases: a flexible chain is first assembled through amide coupling on solid support, and then the scaffold is rigidified by the selective formation of a second set of amide bonds. We have also demonstrated that the three-mer scaffold has a rodlike three-dimensional structure. The scaffolds were rapidly assembled using solid-phase synthesis and were easily functionalized on either of their two ends. Among molecular rods, these scaffolds exhibit the rare property of water solubility,[11] making them compatible with biological buffers. Because of their fused ring structure, we expect them to be more inflexible than spacers such as poly-proline helices.[19,20] This synthetic approach may also be extended to incorporate other building blocks to construct more complex shapes and ultimately functional macromolecules.

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Supporting Information Available: Synthesis and characterization of **1**, **2**, and **3**. HPLC-MS and 2D NMR data for **2** and HPLC-MS data for **3** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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