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Shape-Programmable Macromolecules

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Acc. Chem. Res., **2008**, 41 (10), 1387-1398 • DOI: 10.1021/ar700283y • Publication Date (Web): 29 July 2008

ACCOUNTS
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Shape-Programmable Macromolecules

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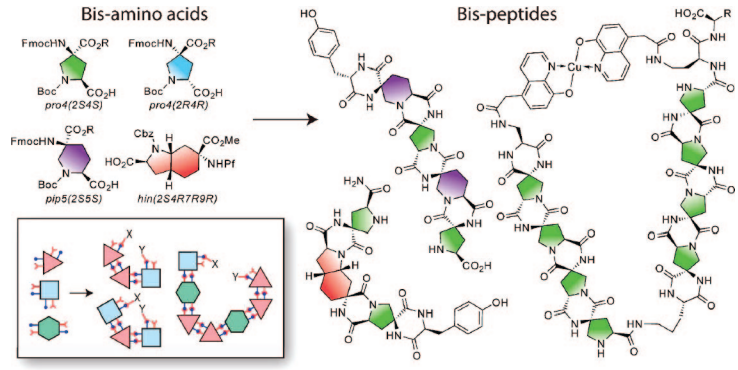
RECEIVED ON DECEMBER 21, 2007

CONSPECTUS



Proteins catalyze specific chemical reactions and carry out highly selective molecular recognition because they adopt well-defined three-dimensional structures and position chemically reactive functional groups in specific constellations. Proteins attain these well-defined structures through the complex process of protein folding. We seek to emulate these protein functions by constructing macromolecules that are easier to engineer by avoiding folding altogether.

Toward that goal, we have developed an approach for the synthesis of macromolecules with programmable shapes. As described in this Account, we have constructed synthetic building blocks called bis-amino acids that we then couple through pairs of amide bonds to create water-soluble, spiroladder oligomers (bis-peptides) with well-defined three-dimensional structures. Bis-peptides use the conformational preferences of fused rings, stereochemistry, and strong covalent bonds to define their shape, unlike natural proteins and synthetic foldamers, which depend on noncovalent interactions and an unpredictable folding process to attain structure.

Using these bis-amino acid monomers, we have built and characterized a number of bis-peptide nanostructures. We also constructed a molecular actuator that undergoes a large change in conformation under the control of metal exchange; the first application of bis-peptides. We are currently developing further approaches to functionalize bis-peptides as scaffolds to present well-defined constellations of functional groups. Such macromolecules could facilitate multifunctional catalysis and molecular recognition and lead to nanoscale molecular devices.

Introduction

I have been fascinated by the catalytic and molecular recognition capabilities of proteins since I first learned of them as an undergraduate student. Over the years, my fascination has grown into a drive to develop the ability to construct macromolecules that have the capabilities of proteins but that are easier to engineer. Proteins achieve their remarkable catalytic and molecular recognition abilities because they adopt well-defined three-dimensional structures. Proteins act as scaffolds to position multiple chemically active groups in three-dimensional space either inward, in the case of enzymes, to catalyze chemical reactions or outward to create recognition elements to bind other proteins. It is this ability, to position multiple functional groups in three-dimensional space that my group seeks to emulate. We use organic synthesis to synthesize a collection of cyclic, abiotic building blocks that we couple through pairs of

Published on the Web 07/29/2008 www.pubs.acs.org/acr
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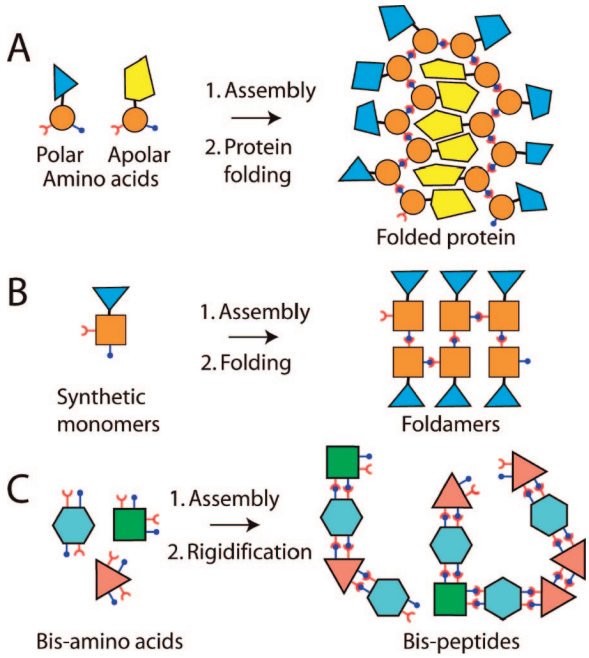


FIGURE 1. A comparison of bis-peptides to other approaches to forming structured macromolecules: (A) In proteins, widely separated apolar amino acids fold together to avoid water, and intraresidue hydrogen bonds help to stabilize the folded structure. (B) Foldamers are synthetic oligomers that fold into well-defined secondary structures due to the conformational preferences of their monomers, local inter-residue hydrogen bonds, and solvophobic tendencies of their monomers. (C) Bis-peptides are synthetic oligomers assembled from cyclic, stereochemically pure monomers coupled through pairs of amide bonds to form rigid spiroladder oligomers with predefined and programmable three-dimensional structures.

bonds to create ladder oligomers with programmable three-dimensional structures. We are currently developing functionalized bis-peptides to explore a variety of applications in catalysis, molecular recognition, nanoscience, and medicinal chemistry.

The idea of creating unnatural building blocks is not a new idea. In 1995, the Iverson group at the University of Texas at Austin developed unnatural oligomers that utilize donor–acceptor interactions between aromatic groups to fold into pleated structures.[1] At the same time, the Gellman group[2,3] and the Seebach group[4,5] were developing β-peptides, short sequences of β-amino acids, which adopt helical and sheet-like structures. In a radical departure from peptide-like structures, the Moore and Wolynes groups developed meta-linked phenylacetylene oligomers that undergo solvophobic collapse to form helical structures containing a central cavity.[6] Over the past decade there have been many examples of folding oligomers developed by other groups that have been summarized in excellent reviews.[7,8]

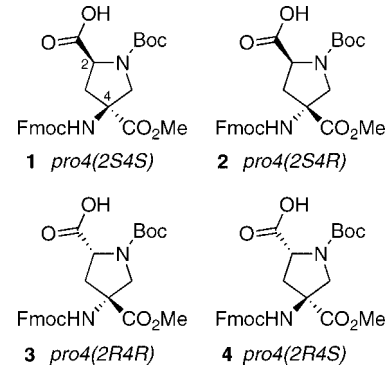
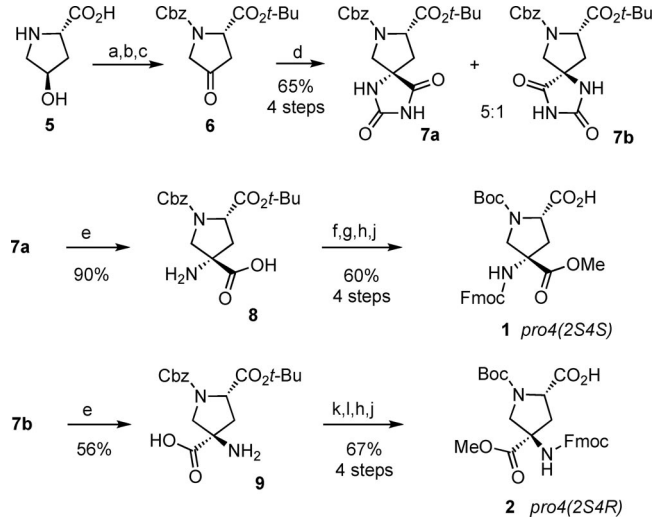


FIGURE 2. The chemical structure of the "pro4" class of bis-amino acid monomers. The "pro4" name indicates that they resemble proline with substitution at the 4 position and the characters in parentheses indicate the stereochemistry (e.g., for compound **2**, pro4(2S4R) indicates "*S*" and "*R*" stereochemistry at the 2 and 4 positions, respectively). These monomers share common characteristics with all bis-amino acid monomers. They consist of two α-amino acids mounted on a cyclic core. The amino acids are suitably protected for solid-phase synthesis of oligomers. Each monomer has a distinct stereochemistry that defines its shape and the shape that it imparts on oligomers into which it is incorporated.

What these approaches have in common is that they involve flexible oligomers that adopt well-defined structures through a complex process of folding (Figure 1). An alternative approach would be to avoid folding altogether and to develop cyclic building blocks that can be coupled through pairs of bonds to create ladder oligomers that use strong covalent bonds and the conformational preferences of rings to determine their tertiary structures rather than subtle noncovalent interactions and folding. This also is not a completely new idea. In the 1980s, the Stoddart group developed the concept of the "Molecular Lego"; these were building blocks that they coupled through pairs of bonds using Diels–Alder reactions to create large cyclic molecules.[9,10] A drawback of the Diels–Alder reaction is that it produces mixtures of products. In our approach, we use pairs of amide bonds in a 1,4-diketopiperazine motif, formed in a two-stage process with complete regioselectivity, to connect cyclic monomers to form ladder oligomers. We have developed a collection of chiral, cyclic building blocks, called "bis-amino acids" (Figure 2, Figure 10) that display two suitably protected α-amino acids and assemble them in different sequences to create water-soluble, rigid, spiroladder oligomers (bis-peptides) with programmable three-dimensional structures (Figure 6, 8, 11, and 12). We are currently developing functionalized monomers that have side chains just as amino acids do to combine with structural monomers to create constellations of functional groups that could mimic active sites of proteins and protein binding surfaces. We have developed computer software that can rapidly build low-energy models of billions of synthetically acces-

SCHEME 1. The Synthesis of the Monomers pro4(2S4S) (**1**) and pro4(2S4R) (**2**)[a]



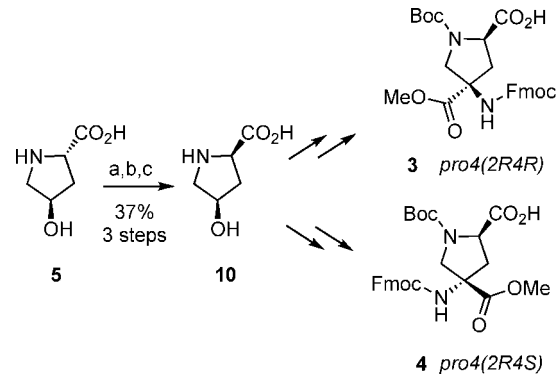
[a] Reagents and conditions: (a) $NaHCO_3$, Cbz-Cl, 1:1 dioxane/water; (b) Jones reagent, acetone; (c) isobutylene, H_2SO_4 (cat.), CH_2Cl_2; (d) $(NH_4)_2CO_3$, KCN, 1:1 EtOH/H_2O, 60 °C, sealed tube; (e) (i) $(Boc)_2O$, DMAP, THF; (ii) 2 M KOH; (f) Fmoc-OSu, Na_2CO_3, 1:1 dioxane/water; (g) MeOH, DCC, DMAP, CH_2Cl_2, 0 °C to room temperature; (h) 3:7 CF_3CO_2H/CH_2Cl_2; (j) H_2, 10% Pd/C, $(Boc)_2O$, THF; (k) (i) TMS-Cl, NEt(*i*Pr)$_2$, CH_2Cl_2, reflux; (ii) Fmoc-Cl, 0 °C to rt; (l) TMS-CHN_2, MeOH, Et_2O.

sible oligomers from these monomers and identify those that could present desired functional group constellations. With such a system, the problem of rationally designing functional macromolecules may be considerably easier than designing functional macromolecules based on proteins or foldamers because the structures of bis-peptides will be predetermined and programmable.

Monomer Synthesis

Bis-amino acid syntheses need to satisfy several simultaneous requirements: ideally, these syntheses need to be able to produce gram quantities of material, they need to produce stereochemically pure products, and they need to be reasonably short and inexpensive. Currently, the synthesis for the pro4(2S4S) monomer **1** is nine steps, it uses only two chromatographic column purifications, and 10 g of material can be prepared by one student in two weeks time. The synthesis was first demonstrated by my student Christopher Levins[11] and refined by other students within my laboratory, most notably Sharad Gupta (Scheme 1). The synthesis begins with inexpensive *trans*-4-hydroxy-L-proline **5** and the key step in the synthesis is a Bucherer–Bergs reaction on the ketone **6**, which forms two diastereomeric hydantoins, **7a** and **7b**, which we separate using silica gel chromatography. Each diastereomer **7a** and **7b** goes on to form a separate, valuable building block **1** (pro4(2S4S)) and **2** (pro4(2S4R)), respectively. The material cost of synthesizing the pro4(2S4S) monomer **1** using this synthesis is only $21/gram, this includes reagents, solvents, and chromatography silica. The synthesis of the diastereomeric bis-amino acids **3** and **4** also starts from *trans*-4-hydroxy-L-proline **5**, which we epimerize at the 2-position[12] to form *cis*-4-hydroxy-D-proline, which we carry through the synthetic steps described in Scheme 1 to produce the pro4(2R4R), **3**, and pro4(2R4S), **4**, monomers (Scheme 2).

SCHEME 2. The Synthesis of the pro4(2R4R) (**3**) and pro4(2R4S) (**4**) Monomers Follows the Route Shown in Scheme 1 Substituting *cis*-4-Hydroxy-D-proline, **10**, for *trans*-4-hydroxy-L-proline, **5**[a]



[a] Reagents and conditions: (a) Ac_2O, AcOH, reflux; (b) 2 M HCl (aq), reflux; (c) (i) 40:2:1 EtOH/H_2O/Et_3N; (ii) recryst. from EtOH/H_2O.

Oligomer Synthesis

The synthesis of a bis-peptide occurs in two stages, the "assembly" stage followed by the "rigidification" stage. The assembly stage follows the protocols of solid-phase fluorenylmethoxy-carbonyl (Fmoc)-based peptide synthesis (Figure 3). Each building block is activated as the 1-hydroxy-7-azabenzotriazole (HOAt) ester,[13] and quantitative coupling to the previous building block is achieved using 3 equiv of activated monomer in less than 30 min at room temperature, a surprising result given the hindered nature of the nucleophile. Fmoc deprotections are carried out with 20% piperidine in dimethylformamide for 30 min at room temperature. At the end of the assembly stage, the oligomer **11** is cleaved from the resin and globally deprotected to form the flexible oligomer **12**. In the rigidification stage, the flexible oligomer **12** is subjected to catalytic conditions that promote an intramolecular aminolysis reaction in which the secondary amine of each monomer attacks the ester of the previous monomer to form a diketopiperazine (DKP) ring between each adjacent pair of monomers and the rigidified bis-peptide **13**. The resulting spiroladder oligomer **13** has no rotatable bonds in its backbone, and its structure is determined by the specific sequences of monomers defined in the assembly stage.

We can monitor the progress of the diketopiperazine closure reaction using reverse-phase high-performance liquid

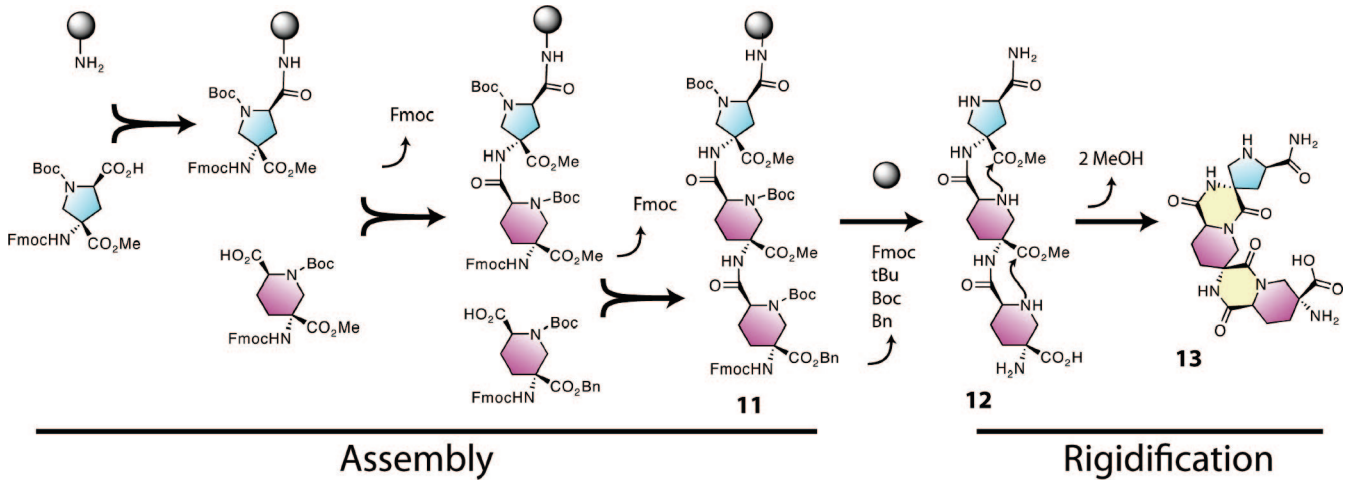
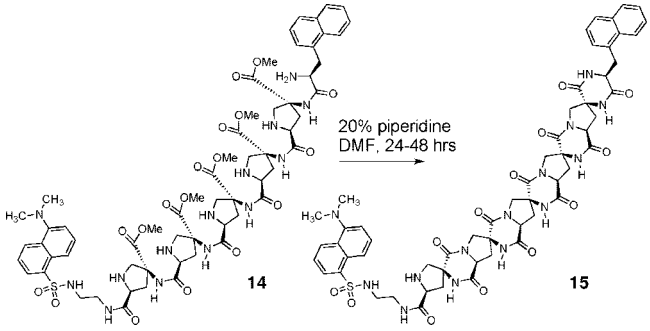


FIGURE 3. The synthesis of bis-peptides occurs in two stages, "assembly" followed by "rigidification". The assembly stage is Fmoc-based solid-phase peptide synthesis. In the rigidification stage, an oligomer such as **12** is subjected to catalytic conditions in which the free secondary amine of each building block attacks the ester of the preceding building block, closing a diketopiperazine ring (yellow) and forming a conformationally constrained bis-peptide such as **13**.

SCHEME 3. The Parallel Diketopiperazine Formation Reaction of Oligomer **14** To Form Bis-peptide **15**[a]



[a] The chromatograms of these compounds at different time points in the rigidification process are shown in Figure 4.

chromatography with mass spectrometry (HPLC-MS.) An example is shown in Scheme 3 and Figure 4 in which we synthesized oligomer **14** and treated it with 20% piperidine in dimethylformamide at room temperature (our first catalytic DKP closure conditions) and immediately injected it onto a C_{18} reverse-phase column and carried out HPLC-MS using a 5% to 95% acetonitrile (0.1% TFA) gradient over 30 min.[14] The chromatogram (Figure 4A) shows one main peak at ~8.5 min, and mass spectrometry indicates that the eluting compound has a mass to charge ratio (*m*/*z*) consistent with compound **14**. After three hours, a portion of the remaining sample in 20% piperidine/DMF was injected and many new peaks appeared (Figure 4B) that had an *m*/*z* consistent with compound **14** missing between 1 and 5 equiv of methanol, indicating that a mixture of diketopiperazine containing intermediates had formed. After 32 h in 20% piperidine, a sample was injected again and the chromatogram (Figure 4C) showed that compound **14** and all of the intermediates had disappeared and

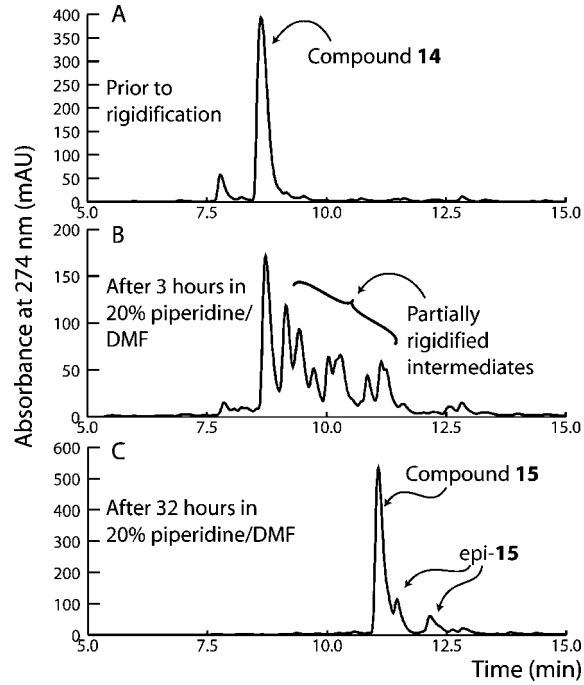


FIGURE 4. The progress of the rigidification of compound **14** to form **15** (Scheme 3) is shown at different time points.

the primary product that remained had a *m*/*z* consistent with product **15**. We observed that with prolonged exposure to piperidine new peaks appeared with the same *m*/*z* as product **15** but with different retention times. We believe that these are diastereomers of compound **15** that form when base-catalyzed epimerization takes place at the tertiary α-carbon of each monomer fused to a diketopiperazine. Diketopiperazines are known to epimerize under basic conditions,[15] and because of this, we avoid exposure of bis-peptides to strong bases for extended periods of time.

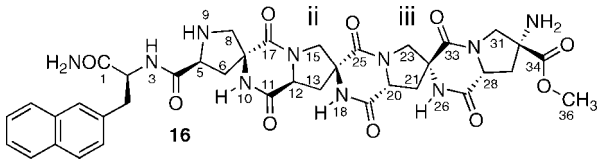


FIGURE 5. The chemical structure of oligomer **16**. Hydrogens have the same number as the heavy atoms that they are attached too. Diastereotopic hydrogens are labeled "α" if they go into the page and "β" if they come out of the page.

Oligomer Structure Determination

In order to test our ability to predict and design bis-peptide structures, we synthesized several oligomers and carried out two-dimensional nuclear magnetic resonance (2D-NMR) ROESY experiments to identify pairs of hydrogen atoms that are physically close to each other. We qualitatively classify the ROESY correlations as strong, medium, and weak intensity based on the integrated intensity of the ROESY cross-peaks. We have also carried out molecular mechanics calculations using the AMBER94 force field[16] to identify the preferred energy conformations of each oligomer. In almost every case, the global minimum energy conformation predicted by AMBER94 has been consistent with the ROESY correlations. The most valuable ROESY correlations are the *trans*-annular correlations, which provide information about the preferred conformations of individual rings.

We synthesized oligomer **16** containing the sequence pro4(2S4S)=pro4(2S4S)=pro4(2R4R)=pro4(2R4R) (here the "=" character represents a diketopiperazine linkage between the adjacent monomers to differentiate it from a single amide bond "−") to determine its solution structure using 2D-NMR.[14] In the ROESY spectrum of oligomer **16** (see Figure 5 for the structure of **16** and atom labels), we observe a strong ROESY correlation between H20 and H15β and a medium strength correlation between H20 and H15α (Figure 6ii). In addition, there is a weak correlation between H13α and H15α and no correlation between H13β and H15β. These data are consistent with the pyrrolidine ring containing nitrogen 16 existing in an envelope conformation that avoids a 1,3 interaction between carbonyl carbon 11 and nitrogen 18 and with the diketopiperazine containing carbon 20 existing in a boat conformation. We also observe a strong ROESY correlation between H28 and H21β, no correlation between H28 and H21α, a weak correlation between H21α and H23α, and no correlation between H21β and H23β (Figure 6iii). These observations are consistent with the pyrrolidine ring containing carbon 20 existing in a conformation that avoids a 1,3 interaction between carbonyl carbon 19 and nitrogen 26 (Figure 6iii). Compound **16** is one member of a set of 512 (2^9) synthetically accessible stereoisomers each with a different well-defined shape and presentation of hydrogen bonding groups and terminal groups.

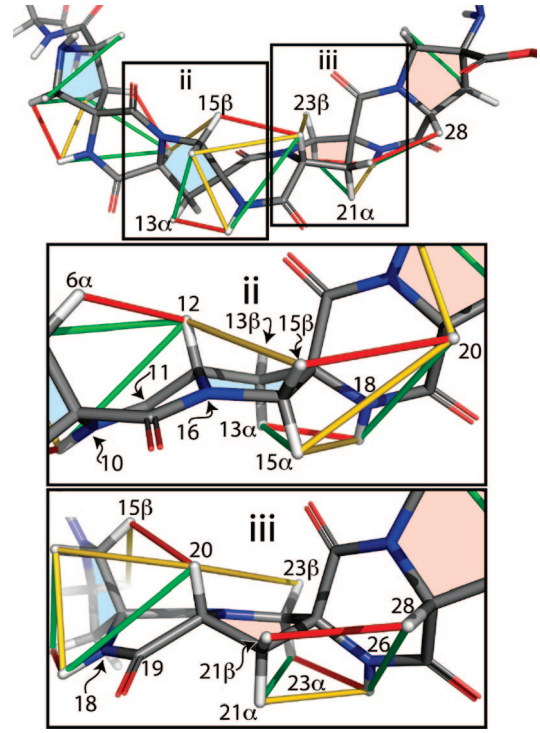


FIGURE 6. The minimum energy structure of bis-peptide **16** with superimposed ROESY correlations. The blue shaded pyrrolidines are pro4(2S4S) monomers, and the red shaded pyrrolidines are pro4(2R4R) monomers. The inset figures are close-ups of the second monomer (ii) and the third monomer (iii). The colors of the superimposed ROESY correlation lines are color coded by their intensity (red = strong, yellow = medium, green = weak).

Global Structures of Bis-peptides

The solution structures of bis-peptides determined using two-dimensional NMR experiments provided us a great deal of information about the conformations of the individual rings. However, small uncertainties in ring conformations and uncertainties about the prevalence of different pyrrolidine ring conformations will accumulate and lead to large uncertainties in the global structure. In order to begin to resolve some of these uncertainties, we constructed two bis-peptides that would have different shapes (a rod and a "C" shape) based on modeling and what we had learned from our 2D-NMR determined solution structures. We functionalized the two oligomers with a fluorescent group on each end and used fluorescence resonance energy transfer (FRET) to qualitatively determine whether the ends of the oligomer were positioned relative to each other in the manner predicted by modeling.[14] The first oligomer **15** was a sequence of five pro4(2S4S) monomers, which modeling suggested would form an extended molecular rod approx-

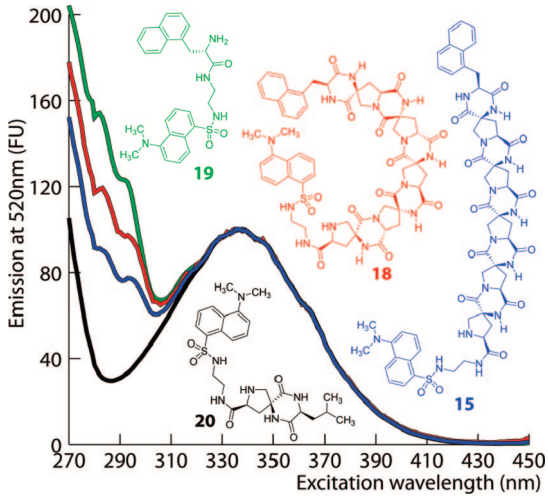


FIGURE 7. The emission spectrum of four dansylated oligomers **15**, **18**, **19**, and **20**. The structures are inset, and their colors correspond to their respective emission spectra. The more curved compound **18** shows more efficient FRET than the more extended compound **15** because the more curved structure of **18** holds the donor and acceptor closer together.

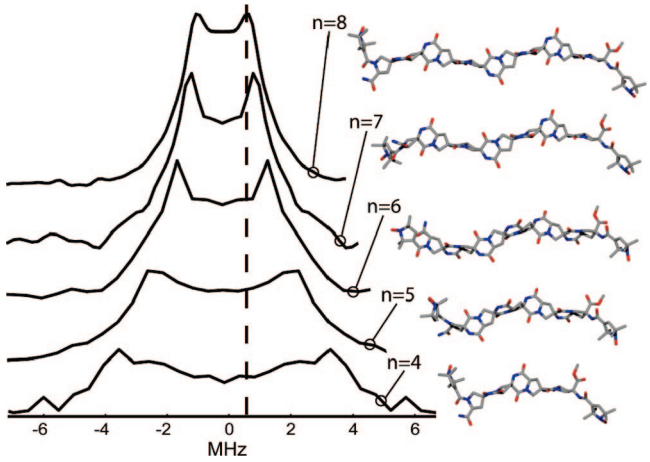
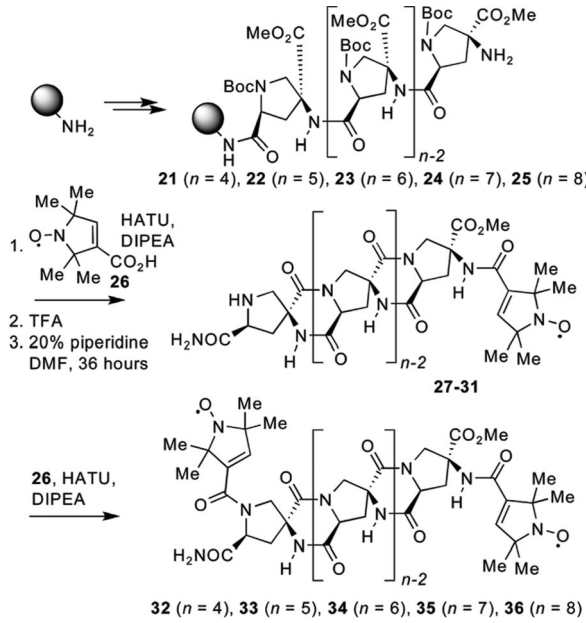


FIGURE 8. The DEER spectra of compounds **32** ($n = 4$), **33** ($n = 5$), **34** ($n = 6$), **35** ($n = 7$), and **36** ($n = 8$) alongside their modeled structures.

imately 30 Å long. The second bis-peptide contained the sequence pro4(2S4S)=pro4(2R4R)=pro4(2S4S)=pro4(2R4R)=pro4(2S4S) **18**, which modeling suggested would form a "C" shaped curved structure that would hold its ends closer together than **15**. On one end of each molecule, we coupled a naphthylalanine (donor), and to the other end, we coupled a dansyl group (acceptor) in order to use FRET to qualitatively determine if **15** was straight and **18** was curved. We also synthesized the molecule **19**, which would provide a control in which the donor and acceptor were very close to each other, and compound **20** where they are effectively infinitely far apart. The emission spectra and chemical structures of **15**, **18**, **19**, and **20** are shown (Figure 7), and the increase in fluorescence emission at 520 nm at the excitation wavelength of

SCHEME 4. The Synthesis and Structures of the Double Spin Labeled Bis-peptide Molecular Rods **32**–**36**



290 nm indicates that the curved compound **18** holds its donor/acceptor pair closer together than is seen with more extended compound **15**. The quantitative interpretation of FRET experiments is complicated by the dependence of the FRET efficiency on the angle between the transition dipoles of the two dyes,[17] so we turned next to electron spin resonance in order to quantitatively characterize the global structures of bis-peptides.

To learn more about the global structures of larger bis-peptides, we synthesized a series of molecular rods of different length and measured the distance across the ends using electron spin resonance experiments. My student Gregory Bird synthesized five bis-peptide oligomers containing between four and eight pro4(2S4S) monomers and attached 2,2,5,5-tetramethyl-3-pyrroline-1-oxyl-3-carboxylic acid (POAC) spin probes to each end (Scheme 4).[18] In collaboration with Sunil Saxena at the University of Pittsburgh, we carried out double electron–electron resonance (DEER)[19] experiments and obtained a DEER spectrum for each oligomer (Figure 8). From the DEER spectra, we were able to calculate population distributions and demonstrate that there was a linear relationship between the interspin-probe distance and the number of monomers in the oligomers (Figure 9). We also observed that the distribution of lengths increased as the number of monomers increased, which indicated that the oligomers become more flexible as they grow longer. It is important to note though that the oligomers are shape-persistent and that the longer oligomers did not collapse or fold back on themselves.

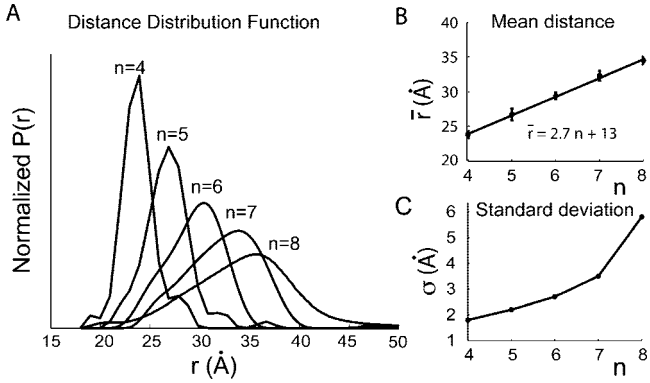


FIGURE 9. (A) The distance distribution function, $P(r)$ calculated from the electron spin resonance spectra of compounds **32** ($n = 4$), **33** ($n = 5$), **34** ($n = 6$), **35** ($n = 7$), and **36** ($n = 8$), (B) the mean distance calculated from each population distribution, and (C) the standard deviation for the compounds **32**–**36**.

The most probable distance between the spin probes and the population distributions derived from the DEER spectra could not be accurately modeled using molecular dynamics (MD) simulations in vacuum with the AMBER94 force field.[18] The AMBER94 force field underpredicted the length of the short oligomers and overpredicted the length of the longer oligomers. It also predicted that the oligomers would be stiffer with more narrow population distributions than those derived from the DEER spectra. We hypothesized that the lack of solvent in our first MD simulations might be the source of disagreement between theory and experiment, so we attempted to simulate the oligomers in explicit solvent. We quickly gave up on this idea because the simulations would have required months of computer time due to the large size of the solvent boxes required to contain the oligomers. To more rapidly simulate the dynamic behavior of bis-peptide nanostructures, we developed a simple dynamical model parametrized using the DEER spectra.[20] This model treats each monomer as a stiff segment that connects to the next through a flexible joint. The model provided end-to-end distribution functions for the oligomers that better fit the DEER spectra than those obtained from *in vacuo* molecular dynamics simulations.

Expanding the Monomer Set

The first four "pro4" bis-amino acid monomers that we developed allow us to create extended, rod-like structures. In order to create more complex bis-peptides, capable of curving back on themselves and able to hold functional groups close to each other, we needed additional building blocks that create tight turns. Driven by this need, we developed synthetic access to ten additional building blocks (Figure 10).

The synthesis of the hin(2S4R7R9R) monomer **37** was developed by my graduate student Stephen Habay; it is the

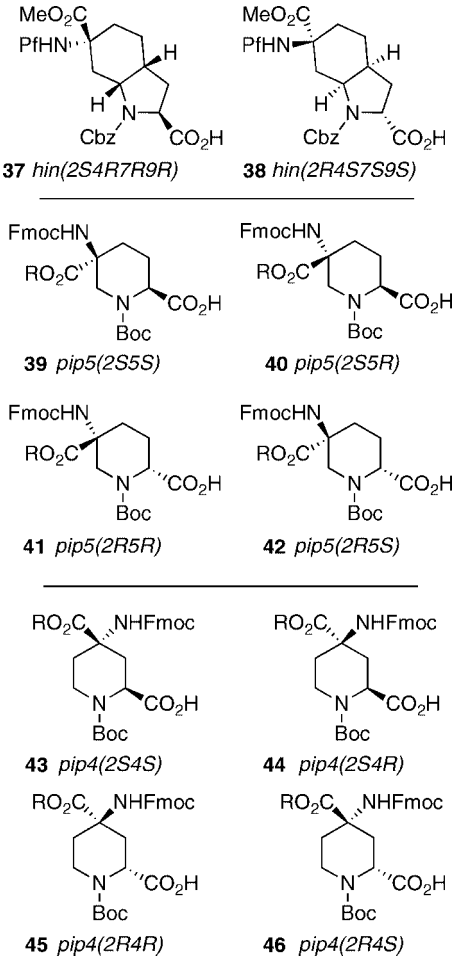


FIGURE 10. The structures of ten additional bis-amino acid building blocks to which we have developed synthetic access. Monomers hin(2S4R7R9R) (**37**) and pip5(2S5S) (**39**) have been published, monomers hin(2R4S7S9S) (**38**) and pip5(2R5R) (**41**) are enantiomers of these, and monomers **40**, **42**, **43**–**46** have been prepared and will be published in time. Pf = phenylfluorenyl, R = Me, $CH_2CF_2CF_2H$, and $CH_2C_6H_5$.

first monomer that creates a sharp turn.[21] The synthesis of hin(2S4R7R9R) uses oxidative cyclization chemistry developed by Peter Wipf's group[22,23] to convert tyrosine (**47**) into the protected amino-ester-ketone intermediate **48** (Scheme 5). Steve's attempts to carry out a Bucherer–Bergs reaction on the ketone **48** lead to inseparable mixtures of diastereomers, so he reduced the ketone **48** with trichloromethyl anion and carried out a modified Corey–Link reaction[24,25] to obtain the azido-ester **51**. This reaction is considered to proceed via the *gem*-dichloro-oxirane intermediate **50**.[26] Reduction of the azide **51** to the amine, followed by protection of the amine with a bulky phenylfluorenyl group, allowed us to selectively hydrolyze the methyl ester at position 2 to afford the completed hin(2S4R7R9R) monomer **37**. Steve assembled the heterosequence pro4(2S4S)=hin(2S4R7R9R)=pro4(2S4S)=(*S*)-Tyr using manual solid-phase peptide synthesis on an MBHA

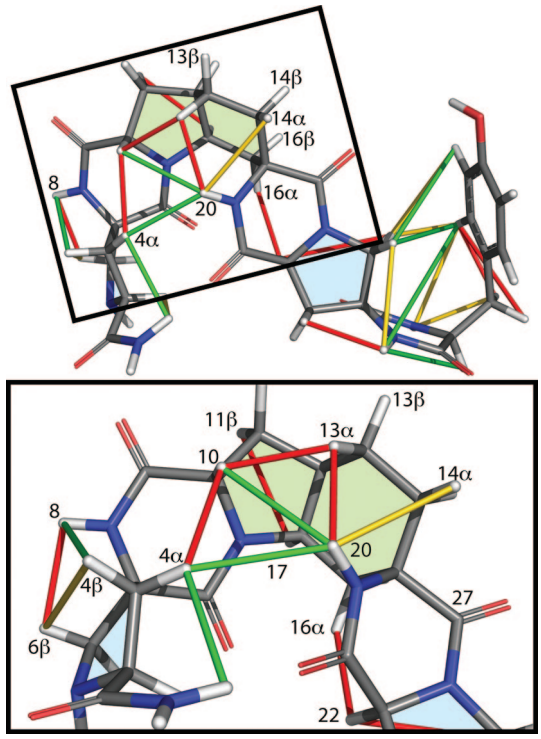


FIGURE 11. The lowest energy structure of compound **55** with the ROESY correlations superimposed. The ROESY correlations were most consistent with the minimum energy structure using the AMBER94 force field. The ROESY correlations between H20 and H4α and others suggest that this monomer creates a sharp turn. The colors of the ROESY correlation lines are related to the intensity of the ROESY correlation peak (red = strong, yellow = medium, green = weak).

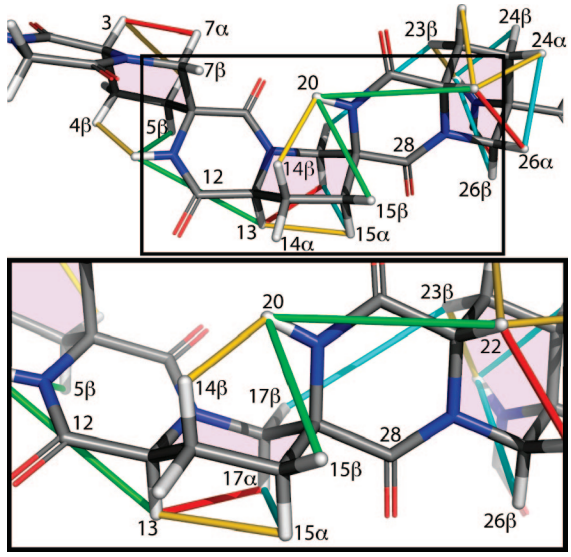
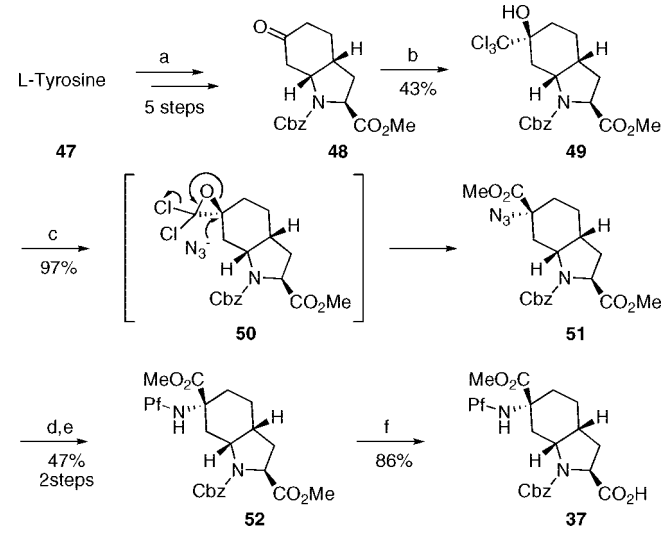


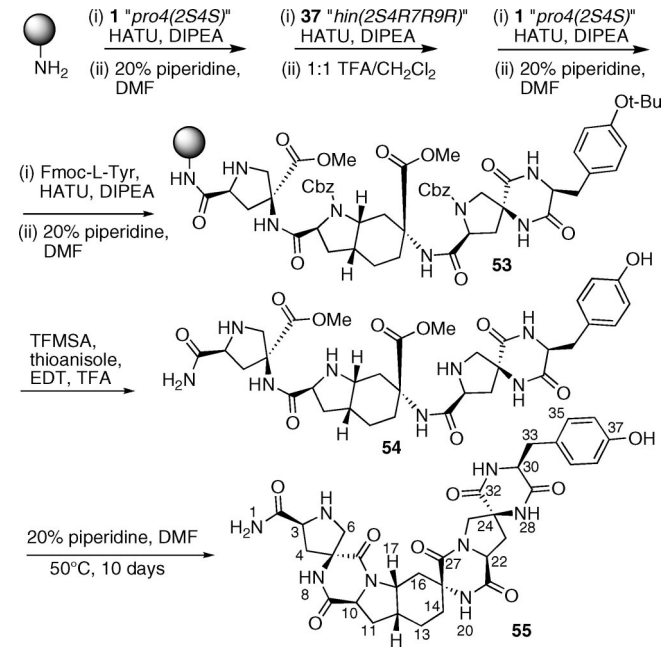
FIGURE 12. The global minimum energy structure of compound **64** with the ROESY correlations superimposed. The piperazine rings are shaded purple. The ROESY correlations were most consistent with the minimum energy structure using the Amber94 force field. The colors of the ROESY correlation lines are related to the intensity of the ROESY correlation peak (red = strong, yellow = medium, green = weak).

SCHEME 5. The Synthesis of the hin(2S4R7R9R) Bis-amino Acid **37**[a]



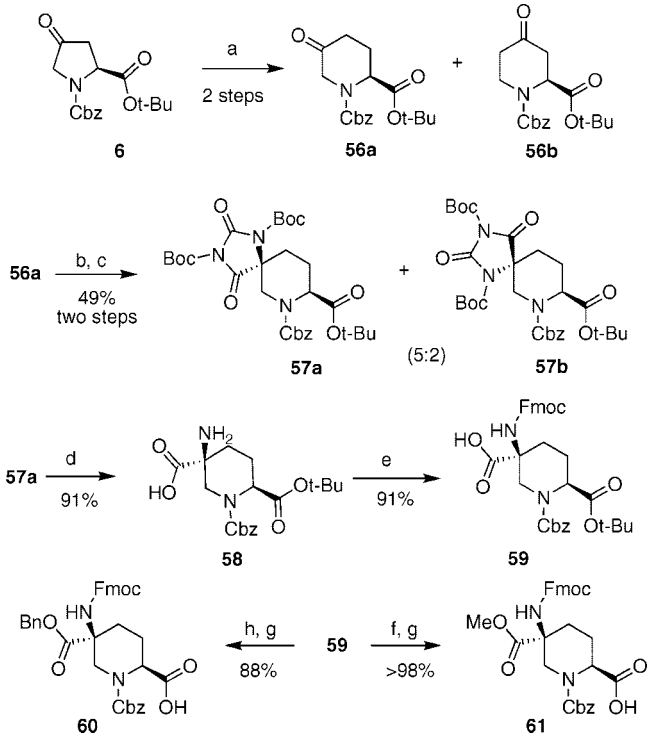
[a] Reagents and conditions: (a) 5 steps;[22,23] (b) $CHCl_3$, LHMDS, THF, −78 °C.; (c) NaN_3, DBU, MeOH, 18-crown-6; (d) Zn, THF, AcOH; (e) PfBr, $Pb(NO_3)_2$, TEA; (f) LiOH, THF, H_2O.

SCHEME 6. The Synthesis and Structure of the Oligomer **55**



resin (Scheme 6). After cleavage from the resin and removal of the carboxybenzyl protecting groups from the oligomer **53**, we ran into a serious problem. The diketopiperazine formation reaction of this oligomer was extremely slow using our initial 20% piperidine/dimethylformamide/room temperature conditions that we had used successfully with our oligomers that contained only pro4 monomers. Steve found that by heating oligomer **54** in 20% piperidine/dimethylformamide at 50 °C for ten days he could form the two diketopiperazines to obtain **55**; however, there was evidence of considerable epimerization by HPLC-MS. Steve purified **55** and carried out

SCHEME 7. Synthesis of Bis-amino Acid pip5(2S5S), Benzyl Ester Form **60**, and Methyl Ester Form **61**[a]

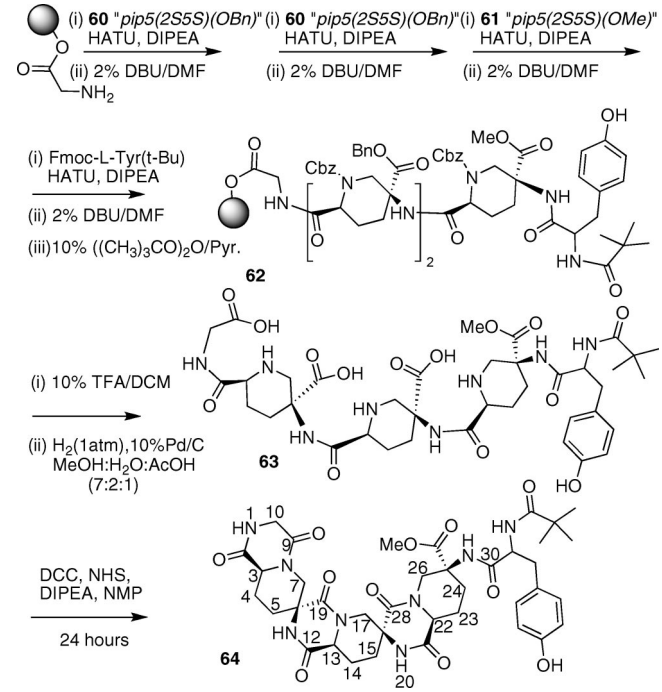


[a] Reagents and conditions: (a) 2 steps;[28] (b) $(NH_4)_2CO_3$, KCN, 1:1 DMF/H_2O, 60 °C, sealed tube, 4 h; (c) $(Boc)_2O$, DMAP, THF; (d) KOH, 1:1 H_2O/THF; (e) (i) TMS-Cl, NEt(*i*Pr)$_2$, CH_2Cl_2, reflux; (ii) Fmoc-Cl, 0 °C to rt; (f) TMS-CHN_2, MeOH; (g) 3:7 CF_3CO_2H/CH_2Cl_2; (h) DCC, DMAP, BnOH, DCM 0 °C to rt.

two-dimensional NMR experiments including a ROESY experiment that showed ROESY correlations between H20−H4α, H20−H10, H20−H13α, H10−H4α, and H13α−H10 that were consistent with a sharp hairpin turn structure just as we had desired (Figure 11). The observed ROESY correlations were consistent with the global minimum energy structure of **55** using the AMBER94 force field.

My graduate student Sharad Gupta and postdoctoral coworker Bhaskar Das developed the synthesis of the pip5 monomers (Scheme 7).[27] An interesting aspect of this synthesis is that it starts with *trans*-4-hydroxy-L-proline **5**, the same starting material that the pro4 monomers are made from. In the key step of the synthesis, the protected amino-ester-ketone **6** is ring expanded[28] to form two regioisomeric ketones **56a** and **56b** that both go on to form the pip5 monomers and the pip4 monomers. In total, we synthesized 12 stereochemically pure monomers from *trans*-4-hydroxy-L-proline; Sharad assembled an oligomer containing a sequence of three methyl-ester protected pip5(2S5S) monomers **61**, and we encountered the problem again that the diketopiperazine formation failed using 20% piperidine in DMF. Sharad switched to a benzyl ester protected monomer **60** and successfully closed the diketopiperazine rings using an *in situ* activation strategy in

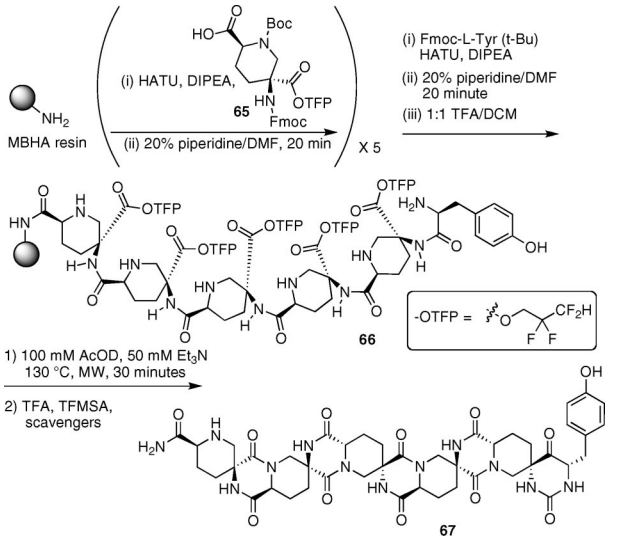
SCHEME 8. Synthesis and Chemical Structure of Oligomer **64**



which a free carboxylic acid on each building block of oligomer **63** is activated using dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (NHS) in the presence of base (DIPEA) in *N*-methylpyrrolidone to form bis-peptide **64** (Scheme 8). It was reassuring that a single product was obtained despite the numerous combinations of amines and activated esters that could react to form amide bonds.[29] Unfortunately, we have not been able to as yet to extend this *in situ* activation strategy to longer sequences. Sharad carried out ROESY experiments on **64** in water and found that the ROESY correlations were consistent with the AMBER94 minimum energy structure (Figure 12), which suggested that each piperazine ring was in a chair conformation.

The rigidification step of bis-peptide synthesis was becoming a serious problem as we sought to incorporate the hin and pip5 bis-amino acids into bis-peptide sequences. We hypothesize that the more "tied-back" nature of the pyrrolidine ring of the pro4 monomers is enabling them to attack a methyl ester of a preceding monomer at a reasonable rate ($t_{1/2}$ of 1−2 h at room temperature) using 20% piperidine as a general base catalyst. The more hindered hin monomers and pip5 monomers were reacting much more slowly under these conditions, and if we used basic conditions with higher temperatures or longer reaction times, we ran into the problem that diketopiperazines that did form epimerized, destroying our carefully constructed stereochemistry. In order to develop a general solution to this problem, Sharad substituted the

SCHEME 9. The Synthesis of Bis-peptide **67**



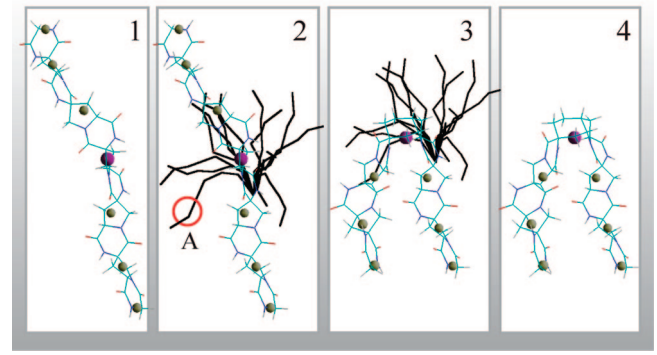


FIGURE 13. An illustration of how we interactively design bis-peptides using CANDO. The yellow balls represent the centers of each monomer and the purple ball represents the monomer currently being edited. (1) A chain of pro4(2S4S) monomers creates an extended helical molecular rod. (2) The black lines represent the directions in space that the chain will bend if one of the 14 building blocks is incorporated at the selected position. (2, 3) The circled line (A) is selected and the "hin(2S4R7R9R)" monomer is substituted at this position. (4) The resulting sequence has a hairpin turn identical to that seen in Figure 11.

methyl ester of our original monomers with a slightly more electron-withdrawing tetrafluoropropyl ester, replaced our original basic conditions with mildly acidic conditions (100 mM acetic acid in xylenes) at high temperature, and developed the methodology to close the diketopiperazine rings while the oligomer remained on solid support.[30] The tetrafluoropropyl ester is a compromise between sufficiently activating to accelerate DKP formation but not so activating that it is attacked by piperidine during repeated Fmoc deprotection. Forming the diketopiperazine rings on solid support had been a long-term goal of ours because it would allow us to use the solubilizing power of the solid support to avoid problems of rigidification intermediates crashing out of solution. This is not a final solution because preliminary attempts to synthesize longer sequences using this approach inexplicably stall with at least one diketopiperazine failing to close completely. We hypothesize that this might be due to intermolecular hydrogen bonding between oligomers on the resin, and we are continuing to develop better methods. Using this new approach, Sharad synthesized an oligomer consisting of a sequence of five of the new pip5(2S5S) monomers, **65** (Scheme 9). We are now confident that we can rigidify any sequence of pro4 monomers of intermediate length (less than 20 monomers) and any sequence containing five or fewer of all 14 monomers.

The degree of control that we have over oligomer shape is illustrated in Figure 13. At any position in an oligomer, in principle, we can substitute any one of our 14 bis-amino acid monomers. Each monomer twists, turns, and translates the chain in a different direction. If we construct a sequence of 20 monomers, it will have one shape out of a universe of 14^{20} or 8.3×10^{22} different three-dimensional shapes (ignoring those that are not self-avoiding). As we develop functionalized monomers, we will be able to decorate these shapes with functional groups to allow them to carry out catalytic and molecular recognition functions.

At the same time that we have been developing the synthesis of our monomers and the chemistry for assembling them into oligomers, I have been writing a software package that will allow us to rapidly predict the structure of any oligomer given the primary sequence of monomers. This program is called "Computer Aided Nanostructure Design and Optimization" or CANDO; it is written in C++ and Python. It will allow the rapid, automated construction of low-energy conformations of any oligomeric molecules assembled from sequences of monomers including bis-peptides, peptides, β-peptides, oligosaccharides, phenylethynylenes, etc. It will allow us to score these conformations based on their modeled ability to present functional groups in desired constellations. We will use CANDO to search for bis-peptide sequences that can present functional groups in desired constellations.

We have recently developed a bis-peptide based molecular actuator, a molecule that undergoes a large change in conformation when it binds copper (Figure 14).[31] This molecule consists of two 2 nm rods joined by a flexible hinge and carrying two 8-hydroxyquinoline (Q) groups, one on each end. In the absence of metal ions, the molecule is disordered and spends a considerable amount of time in an extended conformation. In the presence of Cu^{2+} ions, the two Q groups bind the metal in a 2:1 (Q/Cu^{2+}) complex and cause the molecule to fold and lock into a more compact conformation. We have demonstrated this conformational change using sedi-

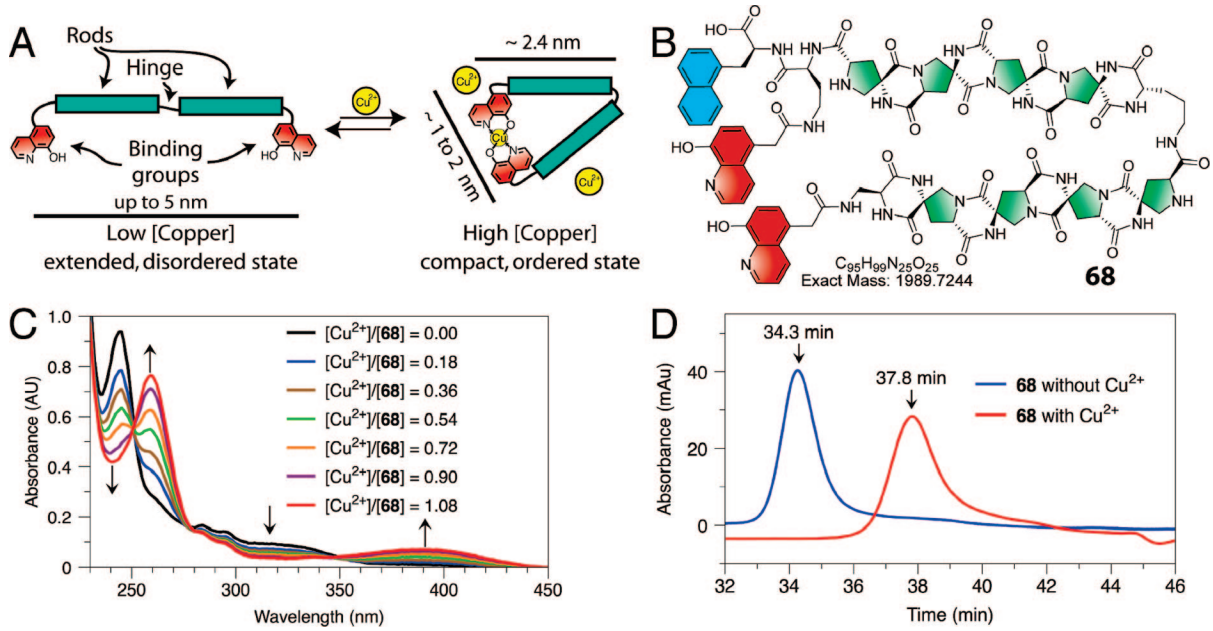


FIGURE 14. A bis-peptide based mechanical molecular actuator: (A) a cartoon illustrating the principle of a bis-peptide that undergoes a large, reversible conformational change upon binding and releasing Cu^{2+} ions; (B) the structure of the bis-peptide **68**; (C) titration of **68** with $CuCl_2$ produces changes in the UV−vis spectrum consistent with metal binding with 1:1 (**68**:Cu^{2+}) stoichiometry; the arrows indicate the direction that the peak moves with increasing copper and the presence of isosbestic points suggests that **68** binds copper in a two-state binding mode; (D) size exclusion chromatography traces of **68** in the absence and presence of copper are consistent with copper-free **68** existing in a disordered, extended conformation (blue trace, elutes early) and **68** with Cu^{2+} bound being smaller and more compact (red trace, elutes later).

mentation analysis and size exclusion chromatography.[31] We envision that we could harness this cooperative behavior and large conformational change to create new sensors and to create nanoscale valves.

In summary, bis-peptide methodology enables us to create water-soluble macromolecules with designed shapes. Solution structures determined using NMR demonstrate that bis-peptides have well-defined three-dimensional structures. The structure of each bis-peptide is defined by the sequence of monomers that compose it, and the shape can be easily predicted using molecular mechanics calculations. Bis-peptides are rapidly assembled using solid-phase synthesis and rigidified in one additional step after assembly. We have developed synthetic access to 14 bis-amino acid monomers, and we are currently developing monomers that present an additional element of functionality. Developing applications for bis-peptides is the next big challenge for us. We envision many applications in multifunctional catalysis, molecular recognition, and nanoscience toward which we can apply our unique ability to position two or three functional groups at controlled distances and orientations relative to each other on a water-soluble scaffold that can be rapidly assembled using solid phase synthesis.

This work has been supported by the National Institutes of Health, National Institute of General Medical Sciences (Grant GM067866), and the National Science Foundation (Grant No. 0348823), the Research Institute, and the Camille and Henry Dreyfus Foundation.

BIOGRAPHICAL INFORMATION

Christian E. Schafmeister was born in Westlock, Alberta, Canada, in 1964. He earned a B.Sc. in chemistry from Simon Fraser University in British Columbia, Canada, and a Ph.D. in biophysics from the University of California in San Francisco where he worked with Robert M. Stroud. He joined the faculty at the University of Pittsburgh in 2000 and moved to Temple University in 2007. His research interests are in the areas of chemical biology and nanoscience. He is the recipient of the Cottrell Scholar Award, the Research Corporation, Research Innovation Award, the Camille and Henry Dreyfus New Faculty Award, and the 2005 Feynman Prize for Experimental Nanotechnology.

Zachary Z. Brown received his B.S. in chemistry from the University of Wisconsin—Green Bay in 2004. He is currently enrolled in the chemistry Ph.D. program at the University of Pittsburgh under the supervision of Dr. C. E. Schafmeister. His graduate research includes the synthesis of functionalized bis-amino acid oligomers and sterically hindered amino acids.

Sharad Gupta received his M.Sc.(Int.) degree in chemistry from Indian Institute of Technology Kanpur (IITK), India, in 2003. He

enrolled in the Ph.D. program in chemistry at University of Pittsburgh (2003) and joined the research group of Dr. C. E. Schafmeister to pursue research in organic chemistry. His graduate research focuses on the development of synthesis strategies for new bis-amino acid building blocks.

FOOTNOTES

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