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The Synthesis of Curved and Linear Structures from a Minimal Set of Monomers

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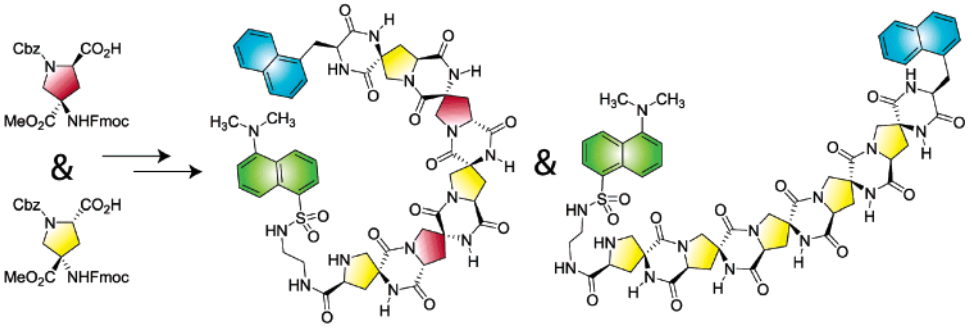

Spiro-ladder oligomers of designed shape were assembled from a set of two enantiomeric bis-amino acid monomers. Two tetramers of differing monomer sequence were synthesized to study the effect of monomer stereochemistry upon macromolecular shape. Two-dimensional NMR experiments were used to determine the conformational preference of the monomers within the context of the oligomers. The results of this structural study were used to design two pentamers: one resembling a rod and another with a curved shape. The pentamers were end-labeled with naphthyl and dansyl groups. The design hypothesis was confirmed by measuring the efficiency of fluorescence resonance energy transfer between the naphthyl and dansyl fluorophore pair.

Introduction

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.[6−14] Nevertheless, the development of a systematic approach to oligomers with designed tertiary structures is still elusive[15] because of the immense complexity involved in predicting the folded structure of molecules with even a few rotatable bonds.[16]

Our long-term objective is to design, rapidly synthesize, and study macromolecules that have compact tertiary

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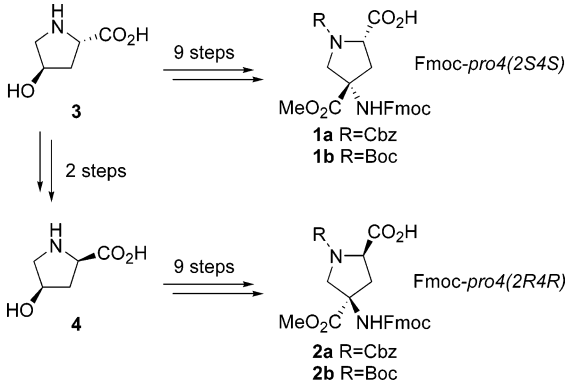
SCHEME 1. Structures of the *pro4(2S4S)* 1a and 1b and *pro4(2R4R)* 2a and 2b Monomers



structures and contain small molecule-sized cavities. Toward this goal, we have developed 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 conformational preferences of their fused rings.[17,18] We have previously reported monomer **1**, named *pro4(2S4S)*, and demonstrated that it is easily assembled into homooligomers that form water-soluble molecular rods of controlled lengths.[17] We present the synthesis of monomer **2** that we have named *pro4(2R4R)* and demonstrate the synthesis of heterosequences containing both monomers **1** and **2**. We also demonstrate that we can create oligomers that have different three-dimensional shapes by assembling specific sequences of monomers.

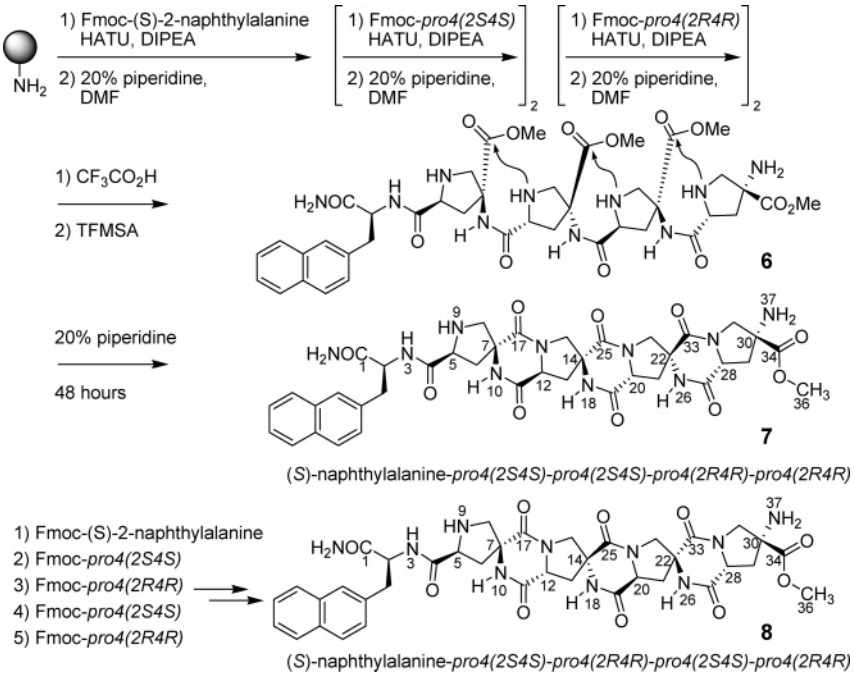
Results and Discussion

Monomer Synthesis. The two building blocks Fmoc-*pro4(2S4S)* **1a** and Fmoc-*pro4(2R4R)* **2a** are synthesized from the same stereochemically pure starting material, *trans*-4-hydroxy-l-proline **3** (Scheme 1). The synthesis of Fmoc-*pro4(2S4S)* **1a** has been described previously.[17] To access the Fmoc-*pro4(2R4R)* **2a** monomer, we first epimerize *trans*-4-hydroxy-L-proline **3** at the 2 position in a two-step process[19] to form *cis*-4-hydroxy-D-proline **4** and then carry this material through the synthesis developed for the Fmoc-*pro4(2S4S)* monomer.[17] The benzyl carbamate (Cbz) protecting group of monomers **1a** and **2a** can be converted to the *tert*-butyl carbamate (Boc) using a one-pot procedure[20] to afford monomers **1b** and **2b**.

Heterooligomer Synthesis. To investigate the ability of the Fmoc-*pro4(2R4R)* monomer **2a** to couple with the Fmoc-*pro4(2S4S)* monomer **1a** we synthesized two heterosequences, **7** and **8**. The synthetic route to **7** is illustrated (Scheme 2). To 100 mg (0.64 mmol/g loading) of Rink amide resin was coupled Fmoc-(*S*)-2-naphthylalanine, followed by the sequence Fmoc-*pro4(2S4S)* **1a**, Fmoc-*pro4(2S4S)* **1a**, Fmoc-*pro4(2R4R)* **2a**, and Fmoc-*pro4(2R4R)* **2a**. Each coupling was carried out using double coupling (30 min each) with 2 equiv of protected amino acid activated with HATU. The oligomer was cleaved from the resin using trifluoroacetic acid (TFA) and then subjected to 8% trifluoromethanesulfonic acid in TFA for 20 min to remove the carboxybenzoyl protecting group from each monomer producing the flexible oligomer **6**. Compound **6** was then rigidified by dissolving it in 20% piperidine in dimethylformamide for 2 days at room temperature.[21] During this time, the free secondary amine of the second, third, and fourth monomer attack the methyl ester moieties preceding them in an intramolecular aminolysis reaction to form a second amide bond between each adjacent pair of monomers. The resulting oligomer **7** is a spiro-fused ring system from atom 5 to atom 30 (Scheme 2). The same process was carried out using a different sequence of monomers to create the spiro-fused oligomer **8**.

NMR Structure Characterization. Our goal is to accurately predict the structures of large oligomers to

SCHEME 2. Synthesis of the Two Heterosequences 7 and 8



enable rational design of macromolecules. Our approach toward this end is to model large structures based on the conformational preference of each monomer in the middle of a trimer. If we call the *pro4(2S4S)* monomer "A" and the *pro4(2R4R)* monomer "B", then all possible sequences of three monomers are: AAA, AAB, BAA, BAB (and their respective enantiomers BBB, BBA, ABB, and ABA). The conformation of the central monomer of an AAA sequence was determined in our previous work.[17] To determine the conformation of the central monomer in the context of the sequences AAB, BAA, and BAB, we determined the conformation of the monomers within oligomers **7** (sequence AABB) and **8** (sequence ABAB) in solution using two-dimensional nuclear magnetic resonance. In the ROESY spectrum of oligomer **7**, we observe a strong ROESY correlation between 20H and 15Hβ and a medium strength correlation between 20H and 15Hα. In addition, there is a weak correlation between 13Hα and 15Hα and no correlation between 13Hβ and 15Hβ. These data are consistent with the pyrrolidine ring containing nitrogen 16 existing in an envelope conformation that avoids a 1,3 interaction between carbon 11 and nitrogen 18, and the diketopiperazine existing in a boat conformation. This gives us the conformational preference of the central monomer of an AAB sequence (Figure 1, top). We also observe a strong ROESY correlation between 28H and 21Hβ, no observable correlation between 28H and 21Hα, a weak correlation between 21Hα and 23Hα, and no correlation between 21Hβ and 23Hβ. These observations are consistent with the pyrrolidine ring containing nitrogen 24 existing in a conformation that avoids a 1,3 interaction between carbon 19 and nitrogen 26. This provides the conformational preference of the central monomer of an ABB sequence (Figure 1, top).

The conformation of the central monomer of a BAB sequence was determined by examining the ROESY spectrum of oligomer **8**. In the ROESY spectrum of **8**, there was overlap between the correlation peaks of α-protons 28H and 20H. Although it is not possible to integrate the peaks directly, by taking a one-dimensional slice through the correlation peaks of 20H we were able to determine that the cross-peak between 20H and 15Hβ is stronger than the cross-peak between 20H and 15Hα. In addition, there is a weak cross-peak between 13Hα and 15Hα and no observed correlation between 13Hβ and 15Hβ. These observations suggest that the second pyrrolidine ring, containing atoms 12 through 16, is in an envelope conformation that avoids a 1,3 interaction between atoms 11 and 18 (Figure 1, bottom).

Design and Synthesis of Shaped Oligomers. On the basis of the conformational preferences determined above, we created two sequences designed to form a molecular rod **10** (an AAAAA sequence) and a curved shape **12** (an ABABA sequence) and used fluorescence resonance energy transfer to test our design. Intermedi-

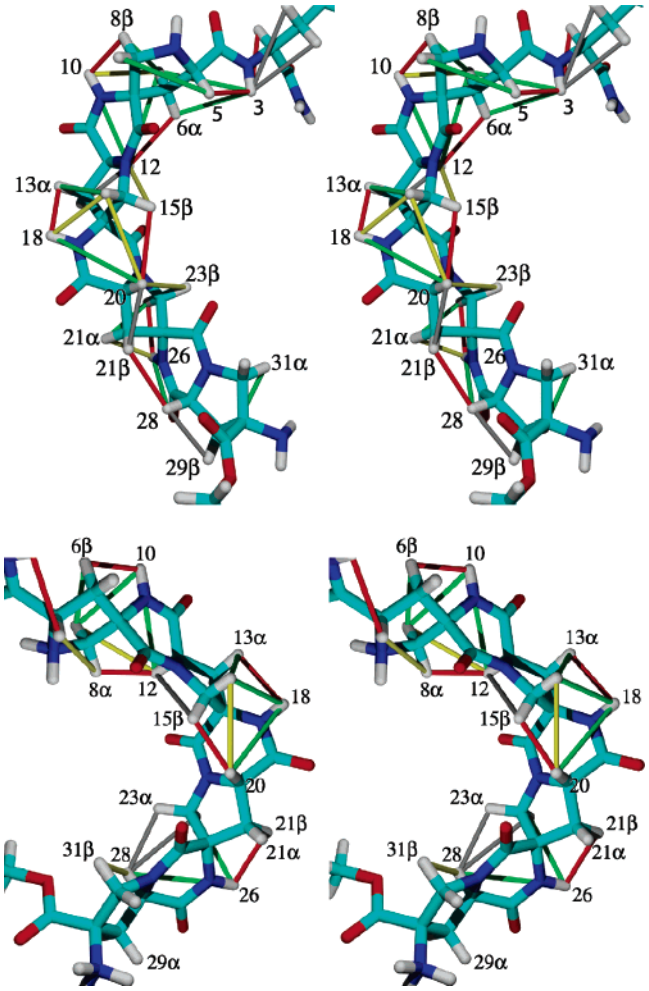


FIGURE 1. Stereo images of the modeled structures of oligomers **7** (top) and **8** (bottom). The ROESY correlations are superimposed in color (red: strong correlation, yellow: medium, green: weak, and gray: intensity is unassigned). Methylene hydrogens are labeled α if they are below the plane of the page and β if they are above the plane of the page as drawn in Scheme 2.

ates **9** and **11** were synthesized using methodology similar to that described for the synthesis of **7** and **8**, with the exception that Boc protected monomers **1b** and **2b** were utilized because we observed decomposition of the dansyl labeled scaffolds with the conditions that we use for Cbz group removal. Oligomers **10** and **12** were expected to form when intermediates **9** and **11** were dissolved in a solution of 20% piperidine in dimethylformamide for 2 days (Scheme 3).[21] In dimethylformamide alone, the rate of diketopiperazine formation is undetectable. In 20% piperidine/DMF at room temperature, the half-life of diketopiperazine formation is approximately 1 h. Diketopiperazines are known to epimerize under basic conditions,[22] and we were concerned that upon formation, the diketopiperazine rings within these longer oligomers might epimerize in the 20% piperidine solution. To partially address this question, we monitored the diketopiperazine formation as a function of time by reverse-phase high-performance liquid chromatography

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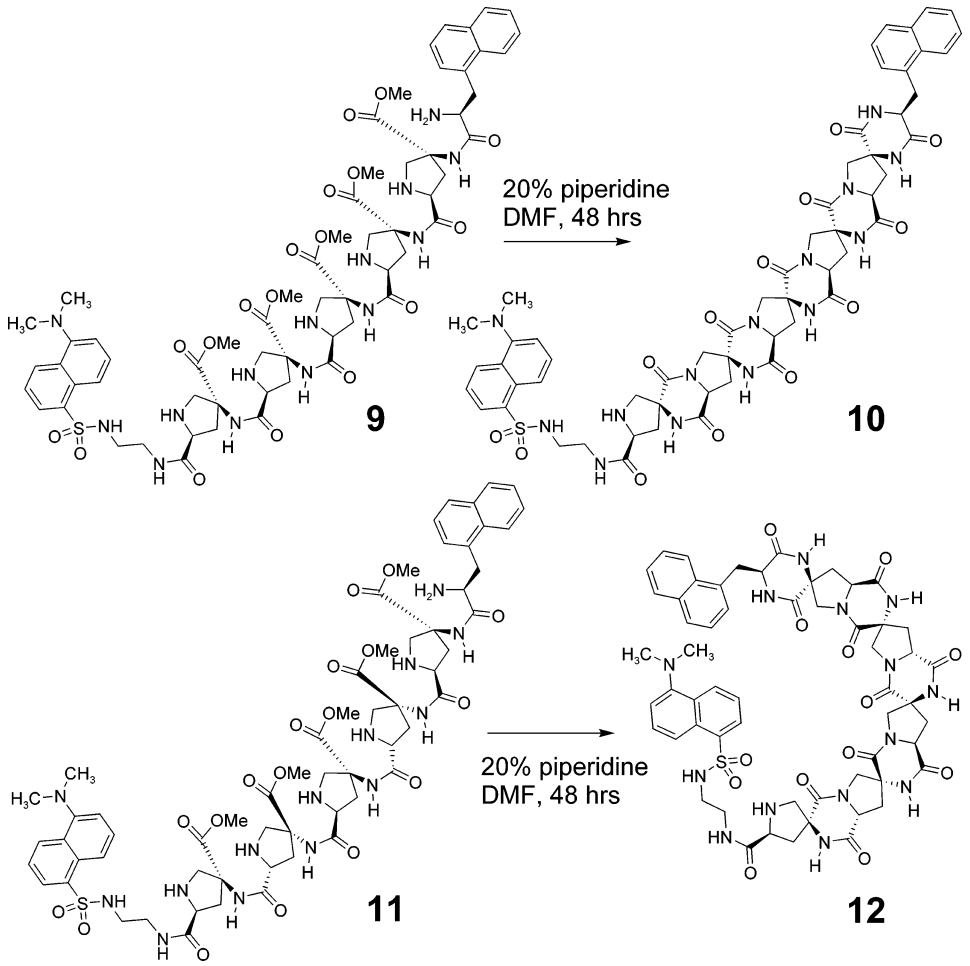
SCHEME 3. Rigidification of Oligomers 10 and 12



(HPLC) with mass spectrometry (Figure 2). After 3 h, a large number of intermediates containing less than five diketopiperazines were observed (Figure 2B). After 32 h, the reaction is essentially complete; the intermediates have disappeared and only the product **10** remains (Figure 2C). Two small peaks with later retention times and with the identical mass of compound **10** are visible; these minor impurities are likely epimers of compound **10**. Oligomer **10** was cleanly purified by preparative reverse-phase HPLC.

Fluorescence Measurements. Oligomer **10** was designed to form a molecular rod about 20 Å long, and curved oligomer **12** was designed to hold its two ends closer together. Modeling compound **10** using the Amber89[23] and Amber94[24] force fields predicts that the distance from the pyrrolidine nitrogen of the first monomer to the quaternary center of the last monomer will be 19 and 21 Å respectively. Modeling compound **12** using the Amber89 and Amber94 force fields predicts that the distance from the pyrrolidine nitrogen of the first monomer to the quaternary center of the last monomer will be 12 and 16 Å, respectively. Both oligomers were labeled on one end with a naphthylalanine fluorescent donor and

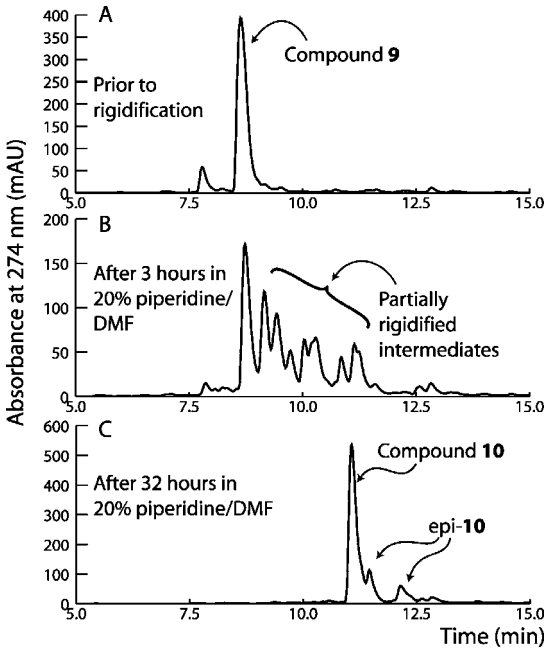


FIGURE 2. Progress of diketopiperazine formation as monitored by C_{18} reverse-phase HPLC, 5% to 95% acetonitrile, 30 min, 0.1% TFA.

on the other end with a dansyl acceptor. We also synthesized two controls: compound **13**, wherein the

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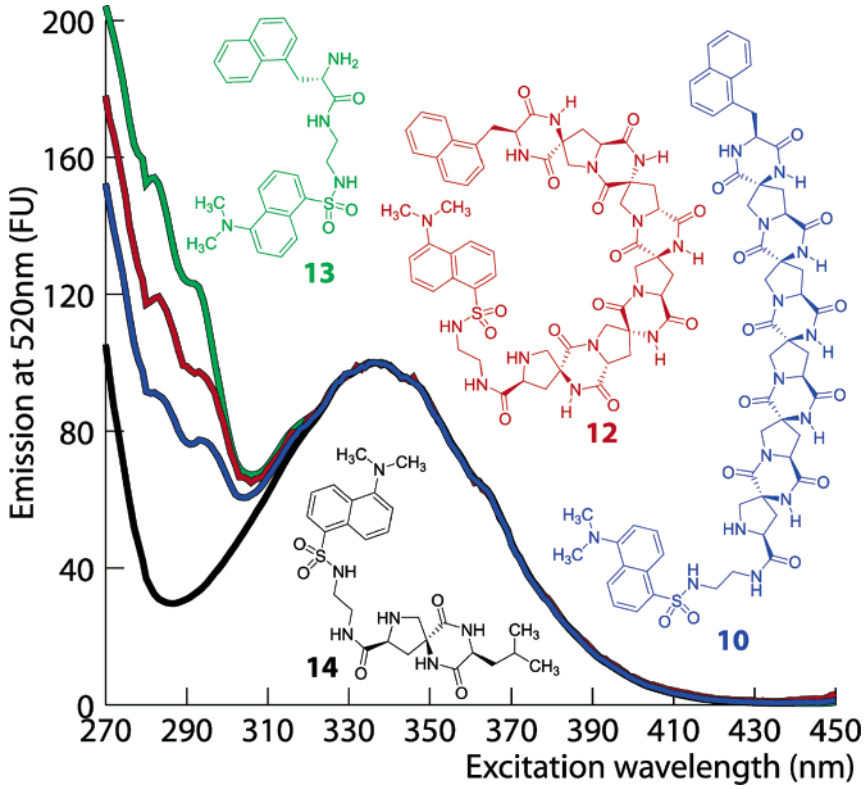

FIGURE 3. Excitation spectra of four dansylated molecules **10**, **12**, **13**, and **14**. The structures are inset, and their colors correspond to the color of their respective excitation spectrum.

dansyl group is directly connected to the naphthylalanine donor, and compound **14**, labeled only with the dansyl group. The excitation spectra of all four molecules are shown (Figure 3). Strong 520-nm emission results from 293-nm excitation when the dansyl and naphthyl groups are in close proximity, as resonance energy transfer increases with decreasing donor−acceptor distance.[25] The excitation spectrum of **14** (black) is that of a dansyl group infinitely far away from a naphthyl donor. The excitation spectrum of **13** (green) demonstrates the strong absorbance by the naphthyl group and highly efficient resonance energy transfer to the dansyl group when they are coupled through a short linker. The spectrum of **10** (blue), the oligomer designed to form the rod, demonstrates resonance energy transfer that is ~50% efficient (at 293 nm), which is reasonable considering that the Förster distance of the dansyl/naphthyl pair is 22 Å.[26] The excitation spectrum of the curved oligomer **12** shows resonance energy transfer at 293-nm excitation that is about 72% efficient, which suggests that **12** has a curved shape, as designed. Assuming that the angular relationship between the dansyl and naphthyl groups is substantially randomized during the excited-state lifetime,[25] the calculated distances between the donor acceptor pair are 22 and 18.5 Å for the rod-shaped compound **10** and the curved compound **12**, respectively. These values are more consistent with the predictions of the Amber94 force field. However, recent single-molecule fluorescence measurements reveal that distances measured using fluorescence resonance energy transfer are overestimated when the donor acceptor pair are closer to each other than the Förster distance.[27] This suggests that the curved oligomer **12** may hold its donor acceptor pair closer together than 18.5 Å. Interestingly, the excitation spectra (not shown) of the flexible precursors **9** and **11** (Scheme 3) are indistinguishable from one another and nearly identical to the excitation spectrum of the curved compound **12**. An explanation for this is that prior to rigidification of **10** and **12** the flexible oligomers **9** and **11** exist as an ensemble of conformations where the dansyl and naphthyl groups are close enough to one another to permit efficient resonance energy transfer. It is only after the molecules are rigidified that they exhibit the behavior inherent to their design.

Conclusion

We have demonstrated the synthesis of the enantiomeric bis-amino acid monomer *pro4(2R4R)* **2** and that we can construct heterosequences of a minimal set of two monomers, **1** and **2**. We have further demonstrated that the three-dimensional structure and physical properties of our bis-amino acid containing oligomers can be controlled by the sequence of monomers from which they are composed.

Experimental Section

Compound 2a. Compound **sc8** (shown in Supporting Information, 4.1 g, 6.8 mmol) was transferred to a 200-mL round-bottom flask with a magnetic stir bar and dissolved in

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a solution of 1:1 TFA/CH_2Cl_2 (70 mL). The reaction mixture was stirred for 4 h and then concentrated by rotary evaporation; the resulting oily residue was dissolved in toluene and concentrated again. Solvent was removed under vacuum overnight, yielding **2a** (3.4 g, 6.4 mmol, 94%) as a foam, used without further purification. An analytical sample was purified by chromatography on silica (gradient elution over 14 column volumes from $CHCl_3$ (0.1% AcOH) to 95:5 $CHCl_3$/MeOH (0.1% AcOH)). ^{1}H NMR (300 MHz, 75 °C, DMSO-d_6): δ 7.96 (br s, Fmoc-N*H*-, 1H), 7.86 (d, J = 9.3 Hz, 2H), 7.68 (d, J = 7.5 Hz, 2H), 7.43−7.31 (m, 9H), 5.11 (s, 2H), 4.36 (m, 3H), 4.22 (apparent t, J = 6.3 Hz, 1H), 4.08 (d, J = 9.0 Hz, 1H), 3.61 (s, 3H), 3.58 (overlap with $-CO_2CH_3$, 1H), 2.84 (m, 1H), 2.28 (dd, J = 12.8, 5.6 Hz, 1H); ^{13}C NMR (75.4 MHz, DMSO-d_6): mixture of rotamers δ 172.5, 172.2, 171.9, 155.6, 153.6 and 153.3, 143.5 and 143.4, 140.6, 136.5, 128.2, 128.1, 127.7, 127.4, 127.3, 126.9, 125.0, 119.9, 66.1 (CH_2), 65.6 (CH_2), 62.7 and 61.9, 57.7 and 57.4 (CH), 54.8 and 54.4 (CH_2), 52.5 (CH), 46.5 (CH_3), 37.6 and 37.5 (CH_2); IR (neat film) 3308, 3065, 2953, 1715, 1529, 1450, 1423, 1357, 1251, 1110, 1085, 1051, 973, 826, 760, 740, 699 cm^{-1}; $[\alpha]_D$ +31.3° (c 1.4, $CHCl_3$); HPLC: C_{18} column, mobile phase, MeCN (0.05% TFA)/water (0.1% TFA), 5% to 95% MeCN over 30 min, 1.00 mL/min, UV detection at 274 nm; t_R for **2a**, 23.80 min; ESI-MS *m/z* (relative intensity): 567.2 (100%), 501.2 (11%); HRESIQTOFMS calcd for $C_{30}H_{28}N_2NaO_8$ (M + Na$^+$) 567.1743, found 567.1743.

Compound 2b. A 500-mL round-bottom flask was charged with a magnetic stir bar, **2a** (3.4 g, 6.4 mmol), and 10 wt % Pd/C (679 mg). THF (190 mL) was added to the flask, and the reaction mixture was degassed through repeated cycles of evacuation under reduced pressure followed by backfilling with H_2. The solution was stirred under a H_2 atmosphere (∼1 atm) overnight. The Pd/C was filtered from the solution and washed with EtOAc. The filtrates were concentrated by rotary evaporation. The resulting oil was purified by chromatography on silica (gradient elution over 10 column volumes from $CHCl_3$ to 10% MeOH/$CHCl_3$). Fractions containing the desired product were combined and concentrated by rotary evaporation and then under reduced pressure overnight yielding **2b** (2.7 g, 5.4 mmol, 80% from **sc8**) as a white foam. ^{1}H NMR (300 MHz, DMSO-d_6): mixture of rotamers δ 8.29 (s, Fmoc-N*H*-, 1H), 7.88 (d, J = 7.4, 2H), 7.71 (d, J = 7.1, 2H), 7.44−7.31 (m, 4H), 4.31−4.20 (m, 4H, rotameric), 3.98 (apparent dd, J = 24.3, 11.2 Hz, 1H), 3.60 (s, 3H), 3.48 (apparent t, J = 10.4 Hz, 1H), 2.89−2.76 (m, 1H), 2.29−2.20 (m, 1H), 1.41 and 1.36 (s, 9H, rotameric); ^{13}C NMR (75.4 MHz, DMSO-d_6): mixture of rotamers δ 173.5, 172.2, 155.7, 153.3, 152.9, 143.6 and 143.5, 140.7, 127.6, 127.0, 125.2, 120.0, 79.0, 65.6 (CH_2), 62.7 and 61.9, 58.1 and 57.7 (CH), 54.8 and 54.4 (CH_2), 52.5 (CH_3), 46.6 (CH), 38.8 and 37.9 (CH_2), 28.0 and 27.8 (CH_3, 3C); IR (neat film) 3307, 3016, 2979, 1744, 1530, 1477, 1450, 1409, 1368, 1297, 1253, 1217, 1160, 1088, 1050, 972, 913, 858, 759, 667 cm^{-1}; $[\alpha]_D$ +61.8° (c 1.9, $CHCl_3$); HPLC: C_{18} column, mobile phase, MeCN (0.05% TFA)/water (0.1% TFA), 5% to 95% MeCN over 30 min, 1.00 mL/min, UV detection at 274 nm, t_R for 2b, 23.20 min.; ESI-MS *m/z* (relative intensity): 533.2 (100%, M + Na$^+$), 477.1 (30%), 433.1 (90%); HRESIQTOFMS calcd for $C_{27}H_{30}N_2NaO_8$ (M + Na$^+$) 533.1900, found 533.1904.

Compound 1b. Compound **sc9** (shown in Supporting Information, 6.30 g, 11.5 mmol) was prepared as described previously,[17] transferred to a 500-mL round-bottom flask containing a magnetic stir bar, and dissolved in 1:1 CH_2Cl_2/TFA (210 mL). The reaction mixture was stirred at room temperature and monitored by TLC (5:1 $CHCl_3$/MeOH, starting material **sc9** R_f 0.7, **1b** R_f 0.4). When all of the starting material had been consumed (approximately 4 h), the solution was concentrated by rotary evaporation, and residual solvent was removed under reduced pressure overnight. Pd/C (10 wt %, 1.1 g), THF (300 mL), and Boc_2O (7.2 mL, 32 mmol) were added to the flask, and the solution was degassed under reduced pressure and then back-filled with H_2 gas. The mixture was stirred overnight under a hydrogen atmosphere (∼1 atm). The reaction mixture was filtered, and the filtrate was concentrated by rotary evaporation to an oil, which was purified by chromatography on silica (gradient elution over 10 column volumes from $CHCl_3$ to 10% MeOH/$CHCl_3$). Fractions containing the desired product were concentrated, and residual solvent was removed under reduced pressure to give **1b** (4.5 g, 8.8 mmol, 83% from **sc9**) as a white foam. ^{1}H NMR (300 MHz, DMSO-d_6): mixture of rotamers δ 8.32 (s, Fmoc-N*H*-, 1H), 7.93 (d, J = 7.4 Hz, 2H), 7.75 (d, J = 7.1 Hz, 2H), 7.48−7.35 (m, 4H), 4.29−3.94 (m, 5H), 3.58 (s, 3H), 3.41−3.32 (m, 1H), 2.76−2.59 (m, 1H), 2.18−2.08 (m, 1H), 1.39 and 1.34 (s, 9H, rotameric); ^{13}C NMR (75.4 MHz, DMSO-d_6): mixture of rotamers δ 174.9, 172.4, 155.6, 153.4 and 153.3, 143.6 and 143.5, 140.6, 127.5, 127.0, 125.1, 120.0, 78.7 and 78.5, 65.6 (CH_2), 62.6 and 61.9, 59.1 and 58.9 (CH), 55.0 and 54.5 (CH_2), 52.3 (CH_3), 46.5 (CH), 39.3 and 38.4 (CH_2), 28.0 and 27.9 (CH_3, 3C); IR (neat film) 3307, 3016, 2979, 1744, 1529, 1477, 1450, 1408, 1369, 1254, 1156, 1088, 1050, 972, 913, 858, 759, 667 cm^{-1}; $[\alpha]_D$ −61.8° (c 1.42, $CHCl_3$); ESI-MS *m/z* (relative intensity): 533.2 (100%, M + Na$^+$), 477.1 (35%), 433.1 (90%), 411.2 (20%); HRESIQTOFMS calcd for $C_{27}H_{30}N_2NaO_8$ (M + Na$^+$) 533.1900, found 533.1887.

Compound 7. A quantity of 100 mg of Rink Amide AM resin (0.63 mmol/g substitution) was transferred to a solid-phase reaction vessel and swollen in DMF for several hours. The *N*-Fmoc protecting group of the resin was removed by treatment with a solution of 20% piperidine/DMF for 40 min. The resin was washed sequentially with DMF, CH_2Cl_2, *i*-PrOH, and DMF. (*S*)-*N*-Fmoc-2-naphthylalanine (56.4 mg, 128 μmol) and HATU (48.6 mg, 128 μmol) were added to a polypropylene microcentrifuge tube. The protected amino acid and coupling reagent were dissolved in 640 μL of 20% CH_2Cl_2/DMF. DIPEA (45.0 μL, 256 μmol) was added to the coupling solution; the solution was mixed and added to the resin immediately. The resin and coupling solution were agitated for 1 h and then washed. The coupling reaction was repeated one additional time. The resin was capped with a solution of 400:100:8 DMF/Ac_2O/DIPEA. The naphthylalanine *N*-terminal Fmoc protecting group was removed using a solution of 20% piperidine/DMF over 40 min, and the resin was washed sequentially with DMF, CH_2Cl_2, *i*-PrOH, and DMF.

Monomer **1a** (70.0 mg, 128 μmol) was coupled to the resin using HATU in a similar fashion. After the attachment of the first monomer to the solid phase, the resin was capped, the *N*-terminal Fmoc protecting group was removed, and the resin was washed as described above. The monomer coupling procedure was repeated three additional times using the appropriate monomers (**1a**, **2a**, then **2a**) and the final *N*-terminal Fmoc protecting group was removed. The resin was then washed with CH_2Cl_2, MeOH, and CH_2Cl_2 and then dried under reduced pressure overnight.

The dried resin was transferred to a 4-mL conical vial containing a magnetic spin vane. Triisopropylsilane (86 μL), water (86 μL), and TFA (3.25 mL) were added sequentially. This cleavage solution was mixed for 2 h, at which time the resin was filtered from the solution and washed with an additional volume of TFA. The filtrates were combined and concentrated by centrifugal evaporation at room temperature. Thioanisole (25 μL) and ethanedithiol (10 μL) were added to the resulting oily residue, followed by TFA (250 μL) and triflic acid (25 μL). This solution was stirred for 15 min and then added slowly to diethyl ether (∼80 mL); the precipitate (crude compound **6**) that evolved was pelleted by centrifugation. The ether was decanted from the pellet, and the pellet was dissolved in 20% piperidine/DMF (1.25 mL). After approximately 48 h at room temperature, the crude product was precipitated from ether. The precipitate was dissolved in a 90:10:1 H_2O/MeCN/TFA solution, and the product was purified by preparative HPLC (C_{18} column; 30 mm × 300 mm; mobile phase, MeCN (0.05% TFA)/water (0.1% TFA), 10% to 35% MeCN over 30 min; flow rate, 43 mL/min). The fractions containing the desired product were concentrated by lyo-

philization, yielding **7** (10.3 mg, 12.9 μmol, ~20% overall yield, based upon initial resin loading): HRESIQTOFMS calcd for $C_{38}H_{43}N_{10}O_{10}$ (M + H^+) 799.3158, found 799.3251.

Compound 8. 8 was prepared following a procedure similar to the one described for the synthesis of **7**. (*S*)-*N*-Fmoc-2-naphthylalanine, **1a**, **2a**, **1a**, and **2a** were coupled sequentially to 100 mg of Rink Amide AM resin. The resin was cleaved using TFA, and the Cbz groups of the resulting crude product were removed with exposure to triflic acid. The product was treated with 20% piperidine/DMF for 48 h, precipitated from ether, and purified by preparative HPLC. Fractions containing the desired product were concentrated by lyophilization, yielding **8** (10.4 mg, 13.0 μmol, ~20% overall yield, based upon initial resin loading): HRESIQTOFMS calcd for $C_{38}H_{43}N_{10}O_{10}$ (M + H^+) 799.3158, found 799.3190.

Compound 9. A quantity of 10 mg of Dansyl NovaTag resin (0.51 mmol/g substitution) was transferred to a solid-phase reactor and swollen in DMF. The *N*-terminal Fmoc protecting group was removed by mixing the resin with 20% piperidine/DMF for 40 min. The resin was then washed with DMF, CH_2Cl_2, *i*-PrOH, CH_2Cl_2, and DMF. **1b**, **1b**, **1b**, **1b**, **1b**, and (*S*)-*N*-Fmoc-1-naphthylalanine were coupled sequentially to the resin using the method described for the synthesis of compound **7**; each coupling to the resin (2 equiv of *N*-Fmoc protected amino acid, 2 equiv of HATU, 4 equiv of DIPEA, 0.2 M in 20% CH_2Cl_2/DMF, 30 min reaction time) was repeated an additional time and was followed by washing, resin capping with a 400:100:8 DMF/Ac_2O/DIPEA solution, and deprotection of the terminal *N*-Fmoc group with 20% piperidine/DMF. After careful washing, residual solvent was removed from the resin overnight under reduced pressure. The resin was cleaved using 95% TFA, 2.5% triisopropylsilane, and 2.5% water over 2 h with stirring. The cleavage solution was filtered, the resin was washed with an additional volume of TFA, and the filtrates were combined and concentrated by centrifugal evaporation. One-quarter of the resulting residue was purified by preparative HPLC (C_{18} column; mobile phase, MeCN (0.05% TFA)/H_2O (0.1% TFA), 5% to 95% MeCN over 30 min; flow rate, 15 mL/min, herein, the "standard method"). Fractions containing the desired product were pooled and concentrated to dryness by centrifugal evaporation, yielding compound **9**: HRESIQTOFMS calcd for $C_{61}H_{77}N_{14}O_{17}S$ (M + H^+) 1309.5312, found 1309.5281.

This sample of **9** was dissolved in UV/vis grade MeOH (1.5 mL) and filtered through a 0.2-μm Nylon frit. A sample for fluorescence spectroscopy was prepared by diluting an aliquot (22.6 μL) of this stock solution into MeOH (3 mL).

Compound 10. The remaining larger portion of the residue that had afforded **9** was dissolved in 20% piperidine/DMF (1.25 mL) and transferred to a sealed amber HPLC vial. Diketopiperazine closure was monitored by analytical HPLC-MS. After ~48 h, the solution was added slowly to diethyl ether (45 mL); the resulting precipitate was pelleted by centrifugation. After decanting the ether, the pellet was dissolved in a 50:50:1 H_2O/MeCN/TFA, and the desired product was purified by preparative HPLC using the standard method. The desired fractions were pooled and concentrated to dryness by centrifugal evaporation, affording compound **10**: HRESIQTOFMS calcd for $C_{57}H_{61}N_{14}O_{13}S$ (M + H^+) 1181.4263, found 1181.4136.

This sample of **10** was dissolved in UV/vis grade MeOH (1.5 mL) and filtered through a 0.2-μm Nylon frit. A sample for fluorescence spectroscopy was prepared by diluting an aliquot (10.0 μL) of this stock solution into MeOH (3 mL).

Compound 11. 1b, **2b**, **1b**, **2b**, **1b** and (*S*)-*N*-Fmoc-1-naphthylalanine were coupled sequentially to 10 mg of Dansyl NovaTag resin using the method described for the synthesis of compound **9**. Following removal of the terminal *N*-Fmoc group, resin cleavage, filtration of the resin, and concentration of the filtrates by centrifugal evaporation, one-quarter of the resulting residue was purified by preparative HPLC using the standard method. Fractions containing the desired product were pooled and concentrated to dryness by centrifugal evaporation, yielding compound **11**: HRESIQTOFMS calcd for $C_{61}H_{77}N_{14}O_{17}S$ (M + H^+) 1309.5312, found 1309.5237.

This sample of **11** was dissolved in UV/vis grade MeOH (1.5 mL) and filtered through a 0.2-μm Nylon frit. A sample for fluorescence spectroscopy was prepared by diluting an aliquot (7.4 μL) of this stock solution into MeOH (3 mL).

Compound 12. The remaining larger portion of the residue that had afforded **9** was dissolved in 20% piperidine/DMF (1.25 mL) and transferred to a sealed amber HPLC vial. After ~48 h, the solution was added slowly to diethyl ether (45 mL); the resulting precipitate was pelleted by centrifugation. After decanting the ether, the pellet was dissolved in a 50:50:1 H_2O/MeCN/TFA, and the desired product was purified by preparative HPLC using the standard method. The desired fractions were pooled and concentrated to dryness by centrifugal evaporation, affording compound **12**: HRESIQTOFMS calcd for $C_{57}H_{61}N_{14}O_{13}S$ (M + H^+) 1181.4263, found 1181.4182.

This sample of **12** was dissolved in UV/vis grade MeOH (1.5 mL) and filtered through a 0.2-μm Nylon frit. A sample for fluorescence spectroscopy was prepared by diluting an aliquot (150 μL) of this stock solution into MeOH (3 mL).

Compound 13. (*S*)-*N*-Fmoc-1-naphthylalanine was coupled to Dansyl NovaTag resin (10 mg) using the method described above. Following removal of the *N*-Fmoc group of the naphthylalanine, resin cleavage, and filtration of the resin, the cleavage solution was concentrated by centrifugal evaporation. The resulting residue was dissolved in 40:60:0.1 MeCN/H_2O/TFA (1 mL) and purified by preparative HPLC by the standard method. The desired fractions were pooled and concentrated to dryness by centrifugal evaporation, giving compound **13**: HRESIQTOFMS calcd for $C_{27}H_{31}N_4O_3S$ (M + H^+) 491.2117, found 491.2092.

This sample of **13** was dissolved in UV/vis grade MeOH (1.5 mL) and filtered through a 0.2-μm Nylon frit. A sample for fluorescence spectroscopy was prepared by diluting an aliquot (2.5 μL) of the stock solution into MeOH (3 mL).

Compound 14. 1b and (*S*)-*N*-Fmoc-leucine was coupled to Dansyl NovaTag resin (10 mg) sequentially using the method described above. After removal of the *N*-Fmoc group of the leucine, resin cleavage, and filtration of the resin, the cleavage solution was concentrated by centrifugal evaporation. The resulting residue was dissolved in 40:60:0.1 MeCN/H_2O/TFA (1 mL) and purified by preparative HPLC by the standard method. The desired fractions were pooled and concentrated to dryness by centrifugal evaporation, giving compound **14**: HRESIQTOFMS calcd for $C_{26}H_{37}N_6O_5S$ (M + H^+) 545.2546, found 545.2516.

This sample of **14** was dissolved in UV/vis grade MeOH (1.5 mL) and filtered through a 0.2-μm Nylon frit. A sample for fluorescence spectroscopy was prepared by diluting an aliquot (15 μL) of this stock solution into MeOH (3 mL).

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Supporting Information Available: General procedures, experimental details for synthesis of monomers **1b**, **2a**, and **2b**, and characterization of new compounds. ^1H and ^{13}C spectra of intermediates in synthesis of monomers. HPLC-MS chromatograms of compounds **7**, **8**, **9**, **10**, **11**, **12**, **13**, and **14**. Excitation spectra of compounds **9** and **11**. Two-dimensional NMR spectra for compounds **7** and **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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