Macromolecules
ARTICLE

Macromolecules **2010**, *43*, 4453–4459 **4453**
DOI: 10.1021/ma100444b

Self-Assembly of Supramolecular Polymers from β-Strand Peptidomimetic−Poly(ethylene oxide) Hybrids

Tayirjan T. Isimjan,[†] John R. de Bruyn,[‡] and Elizabeth R. Gillies*[,†,§]

[†]*Department of Chemistry,* [‡]*Department of Physics and Astronomy, and* [§]*Department of Chemical and Biochemical Engineering, The University of Western Ontario, 1151 Richmond St., London, Canada N6A 5B7*

Received May 27, 2009; Revised Manuscript Received April 14, 2010

ABSTRACT: The use of hydrogen-bonding β-strand peptidomimetics for the preparation of supramolecular polymers is described here. The β-strand mimics were selected for their ability to form hydrogen bonds on only one face of the strand, allowing for controlled assembly into linear polymers. Alkyne-functionalized peptidomimetics with the capacity to form either four, six, or eight self-complementary hydrogen bonds were synthesized and conjugated to both termini of low molecular weight (MW) α,ω-diazidopoly(ethylene glycol). The assembly of these polymers into higher MW supramolecular polymers was investigated by multiangle light scattering, dynamic light scattering, and circular dichroism. It was found that the eight-hydrogen-bonding system was required for the significant formation of high MW assemblies and that the degree of assembly was dependent on the polymer concentration as well as the solvent. Thus, these peptidomimetics provide a new platform for the development of supramolecular polymers with the promise to tune their properties using functionalities on the amino acid side chains.

Introduction

In natural systems, noncovalent intramolecular and intermolecular interactions are exploited to impart biomaterials with exceptional properties. For example, in dragline silk relatively weak intermolecular forces drive the assembly of nanocomposites composed of β-sheet nanocrystals embedded in an amorphous matrix, imparting silk with exceptional strength, toughness, and elasticity.[1] In recent years there have been significant advances in the development of synthetic polymer systems that exploit noncovalent interactions in order to modulate the properties of materials.[2,3] Noncovalent interactions such as metal−ligand coordination,[4−8] hydrogen bonding,[9−13] π−π stacking,[14−17] inclusion complexes,[18−22] or a combination of these interactions[23−28] have all been explored for the development of supramolecular polymers. Through this work, useful properties such as temperature-dependent physical properties[11,29,30] and self-healing capabilities[31,32] have been introduced to synthetic polymer systems. In biomedical applications, supramolecular polymers have been used to form hydrogels that effectively direct the growth of cells,[33,34] strong sacs for the encapsulation of human stem cells for cell therapy,[35] and tissue engineering scaffolds with tunable mechanical and cell adhesive properties.[36]

Of the available noncovalent interactions used in nature, hydrogen bonding is of particular interest for the development supramolecular polymers. It combines reversibility, directionality, excellent strength depending on the surrounding environment and can be readily tuned by altering the number of bonds involved. While the development of synthetic hydrogen-bonding systems that operate in aqueous solution is still a challenge, hydrogen-bonding systems are common in nature, existing in DNA as well as in protein β-sheets. Despite this, there are relatively few examples involving the use of β-sheets and their peptidomimetics in supramolecular polymers. Several poly(ethylene oxide) (PEO)−peptide hybrids have been developed based on amyloid-β,[37] silk-based,[38] or de novo amino acid sequences.[39−42] These structures have generally been demonstrated to assemble into fibrils based on β-sheets. Most amino acid sequences are not amenable to the preparation of supramolecular linear polymers due to their propensity to hydrogen bond on both edges of the strand, leading to fibers or uncontrolled aggregation.

With the aim of forming high MW linear polymers in solution, we report here for the first time the use of a β-strand peptidomimetic that is designed to undergo hydrogen bonding on only one edge. Oligomers based on alternating α-amino acids and azacyclohexenone (Ach) units, developed and termed @-tides by Bartlett and co-workers, were selected as the hydrogen-bonding β-strand peptidomimetics for this work.[43] The replacement of alternating amino acids with the Ach units provides conformational restriction that favors elongated conformations, and the tertiary amide limits hydrogen bonding to one edge of the strand. Phillips et al. have reported detailed studies to demonstrate that @-tides of various lengths and compositions assemble in organic and aqueous solutions to form β-sheets.[43,44] The design strategy for this work involved the coupling of two @-tide molecules to the termini of relatively low MW (∼3400 g/mol) PEO in order to induce the formation of higher MW polymers as shown in Figure 1. PEO was selected due to the ease with which its end groups can be derivatized as well as its high solubility in a wide range of aqueous and organic systems. In contrast to the more conventional hydrogen-bonding systems that have previously been used for the preparation of linear supramolecular polymers,[9−13] this system provides the advantage that the length of the peptidomimetic can readily be tuned to control the strength of the association, and the amino acid side chains have the potential to later introduce chemical functionalities to the polymers for a diverse range of properties and applications. Furthermore, the use of peptide based hydrogen-bonding moieties provides the potential for the eventual formation of supramolecular polymers in water. The syntheses of polymers comprising varying numbers of hydrogen-bonding groups, and the characterization of their assemblies

*Corresponding author. E-mail: egillie@uwo.ca.

Published on Web 04/29/2010

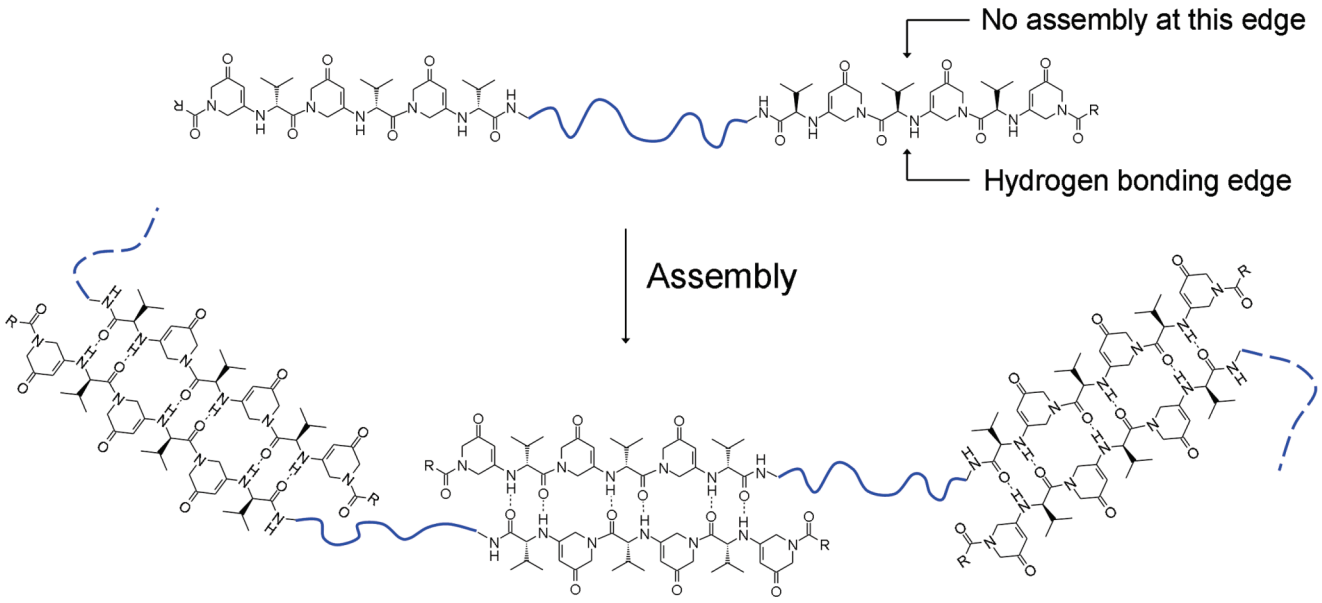


Figure 1. Schematic of supramolecular polymer formation.

by static and dynamic light scattering as well as circular dichroism (CD) spectroscopy is described here.

Experimental Section

Materials and General Procedures. Solvents were purchased from Caledon Laboratories (Georgetown, ON). All other chemicals were purchased from Sigma-Aldrich (Milwaukee, WI). Unless noted otherwise, all chemicals were used as received. All dry solvents were obtained from a solvent purification system. Column chromatography was performed using silica gel (0.063−0.200 mm particle size, 70−230 mesh). ^{1}H NMR data were obtained at 400 or 600 MHz, and ^{13}C NMR data were obtained at 100 or 150 MHz. All chemical shifts are reported in ppm and are calibrated against residual solvent signals of $CDCl_3$ (δ 7.26, 77.2) or CD_3OD (δ 3.31, 48.9). All coupling constants (J) are reported in hertz. Mass spectrometry data were obtained using a Finnigan MAT 8200 instrument in TOF ES+ mode. HPLC was performed at a flow rate of 1 mL/min on a Waters 2695 separations module with a Waters 2998 photodiode array detector at a wavelength of 285 nm. A Luna C18 3 μm (150 mm × 4.6 mm) column from Phenomenex equipped with the corresponding guard column was used. The HPLC gradient was linear from either 20/80 or 30/70 MeCN/H_2O to 95/5 MeCN/H_2O over 20 min. Solvent mixtures for all chromatographic analyses contained 0.1% TFA. Circular dichroism (CD) spectroscopy was performed at a concentration of 0.25 mM using a Jasco J-810 spectrometer.

Dimer Acid (2). Compound **1**[45] (1.0 g, 4.1 mmol, 1.0 equiv) and L-valine (0.54, 4.1 mmol, 1.0 mmol) were dissolved in 40 mL of MeOH, and the reaction mixture was heated at 60 °C overnight. The solution was then concentrated, redissolved in EtOAc/MeOH, and basified with 1 M NaOH (20 mL). The organic layer was separated and washed with 1 M NaOH (3 × 10 mL). The aqueous layers were combined and acidified with 1 M $KHSO_4$. The resulting solution was extracted with EtOAc. The organic layers were combined, dried over $MgSO_4$, and concentrated in vacuo generating 0.80 g (57%) of **2** as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ (2/1)): δ 0.97 (dd, 6H, J = 13.1, 6.5), 2.06−2.20 (m, 1H), 3.67−3.73 (m, 1H), 3.94−4.12 (m, 2H), 4.23−4.43 (m, 2H), 5.11 (s, 2H), 5.15 (s, 1H), 7.26−7.40 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.4, 18.7, 30.5, 43.9, 50.0, 61.4, 67.8, 94.5, 127.8, 128.2, 128.4, 135.7, 154.8, 163.0, 173.2, 192.1. MS calcd for $[M]^+$ ($C_{18}H_{22}N_2O_5$): 346.2. Found (ES+): 345.9. HPLC tR 4.2 min (gradient from 30/70 MeCN/H_2O).

Dimer Methyl Ester (3). Compound **2** (1.0 g, 2.9 mmol, 1.0 equiv) was dissolved in 80 mL of THF, and 8 mL of H_2O was added. The solution was adjusted to pH 7 with 8 mL of 20% Cs_2CO_3 and evaporated to dryness to give the cesium salt of the acid. This salt was then stirred with methyl iodide (0.13 mL, 3.5 mmol, 1.2 equiv) in 25 mL of DMF for 1 h. The solvent was then removed, and the resulting mixture was purified by silica gel chromatography using EtOAc/hexanes 2/1 as an eluent to provide 0.95 g (93%) of **3** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (d, J = 6.8, 3H), 0.95 (d, J = 6.8, 3H), 2.07−2.15 (m, 1H) 3.68 (s, 3H), 3.84 (dd, J = 8.1, 6.0, 1H), 4.01 (dd, J = 16.0, 32, 1H), 4.28 (dd, J = 16.2, 40, 1H), 5.09 (s, 2H), 5.16 (s, 1H), 6.45 (br s, 1H), 7.21−7.32 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.6, 31.1, 44.1, 50.6, 52.3, 60.9, 67.7, 95.3, 127.8, 128.5, 135.9, 154.9, 161.2, 171.6, 191.0. MS calcd for $[M]^+$ ($C_{19}H_{24}N_2O_5$): 360.2. Found (ES+): 359.9.

Dimer Amine (4). To a solution of compound **3** (0.40 g, 1.1 mmol) in 20 mL of MeOH was added 10 wt % Pd/C (40 mg). The mixture was stirred under H_2 in a Parr shaker apparatus at a pressure of 30 psi overnight. The resulting mixture was filtered through Celite, and the filtrate was concentrated in vacuo to provide a quantitative recovery of the product, which was taken to the next step without further purification.

Tetramer (5). The amine **4** (65 mg, 0.29 mmol. 1.0 equiv), the acid **2** (0.11 g, 0.32 mmol, 1.1 equiv), *O*-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HATU) (0.12 g, 0.32 mmol, 1.1 equiv), and diisopropylethylamine (DIPEA) (60 μL, 0.35 mmol, 1.2 equiv) were dissolved in 3 mL of DMF, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was then diluted with H_2O and was extracted with EtOAc. The organic layers were combined, dried over $MgSO_4$, and evaporated in vacuo. The resulting mixture was purified by silica gel chromatography using a gradient from CH_2Cl_2/MeOH 96/4 to CH_2Cl_2/MeOH 94/6 as an eluent to provide 0.13 g (81%) of **5** as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86−1.02 (m, 12H), 2.01−2.06 (m, 1H), 2.10−2.21 (m, 1H), 3.77 (s, 3H), 3.85−4.39 (m, 8H), 4.65 (d, J = 8.0, 1H), 4.85 (d, J = 8.0, 1H), 5.16 (s, 1H), 5.17 (s, 2H), 5.39 (s, 1H), 6.29 (br s, 1H), 6.99 (br s, 1H), 7.27−7.37 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.91, 18.6, 18.7, 19.1 31.3, 32.5, 42.9, 44.4, 52.4, 54.8, 56.6, 61.1, 61.3, 67.7, 95.0, 94.7, 127.7, 127.9, 128.2, 128.5, 135.8, 155.1, 162.0, 162.1, 170.1, 171.6, 189.3, 191.2. MS calcd for $[M]^+$ ($C_{29}H_{38}N_4O_7$): 554.3. Found (ES+): 554.3. HPLC tR 8.7 min (gradient from 20/80 MeCN/H_2O).

Tetramer Acid (6). Tetramer **5** (0.10 g, 0.18 mmol, 1.0 equiv) was dissolved in 2 mL of THF/H_2O 1/1, and LiOH (15 mg, 0.36 mmol, 2.0 equiv) was added. The reaction mixture was stirred at room temperature for 1 h, then was diluted with 1 M $KHSO_4$, and extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to provide 82 mg (82.0%) of **6** as a glassy solid. This product was taken to the next step without further purification.

Tetramer Propargylamide (7). The acid **6** (0.21 g, 0.39 mmol, 1.0 equiv), propargylamine hydrochloride (33 mg, 0.78 mmol, 2.0 equiv), HATU (0.27 g, 0.78 mmol, 2.0 equiv), and DIPEA (0.14 mL, 0.78 mmol, 2.0 equiv) were dissolved in 10 mL of DMF, and the reaction mixture was stirred at 0 °C for 3 h. The reaction mixture was then diluted with H_2O (60 mL) and was extracted with EtOAc. The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated in vacuo. The product was purified by silica gel chromatography using EtOAc/MeOH 95/5 as the eluent to provide 0.12 g (54%) of **7** as a pale yellow solid. NMR spectra are complicated by rotamers. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.75−1.10 (m, 12H), 1.85−2.25 (m, 3H), 3.75−5.20 (m, 14H), 5.70−5.95 (m, 2H), 7.10−7.40 (m, 5H), 8.42 (br s, 1H), 8.64 (br s, 1H). MS calcd for $[M + H]^+$ ($C_{31}H_{40}N_5O_6$): 578.3. Found (ES+): 578.4. HPLC tR 7.9 min (gradient from 30/70 MeCN/H_2O).

Tetramer Amine (8). The same procedure as described above for the preparation of the dimer amine **4** was used to provide a quantitative recovery of the product, which was taken to the next step without further purification.

Hexamer (9). The amine **8** (50 mg, 0.12 mmol, 1.0 equiv), the acid **2** (39 mg, 0.16 mmol, 1.5 equiv), HATU (56 mg, 0.16 mmol, 1.5 equiv), and DIPEA (50 μL, 0.24 mmol, 2.0 equiv) were dissolved in 2 mL of DMF, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was then diluted with H_2O and was extracted with EtOAc. The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated. The product was purified by silica gel chromatography using a gradient from CH_2Cl_2/MeOH 95/5 to CH_2Cl_2/MeOH 90/10 to provide 54 mg (70%) of **9** as a yellow solid. NMR spectra are complicated by rotamers. ^{1}H NMR: δ 0.76−1.05 (m, 18H), 1.95−2.20 (m, 3H), 3.65−4.60 (m, 18H), 4.90−5.55 (m, 5H), 7.11 (br s, 1H), 7.22−7.28 (m, 5H), 7.52 (br s, 1H), 7.61 (br s, 1H). MS calcd for $[M + H]^+$ ($C_{39}H_{53}N_6O_9$): 749.4. Found (ES+): 750.4. HPLC tR 6.8 min (gradient from 30/70 MeCN/H_2O).

Hexamer Acid (10). The same procedure as described above for the preparation of the tetramer acid **6** was used to provide a quantitative recovery of the product, which was taken to the next step without further purification.

Hexamer Propargylamide (11). The acid **10** (50 mg, 80 μmol, 1.0 equiv), propargylamine hydrochloride (10 mg, 0.24 mmol, 3.0 equiv), HATU (42 mg, 0.12 mmol, 1.5 equiv), and DIPEA (0.05 mL, 0.24 mmol, 3.0 equiv) were dissolved in 2 mL of DMF, and the reaction mixture was stirred at 0 °C for 3 h. The reaction mixture was then diluted with H_2O and was extracted with EtOAc. The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated in vacuo. The product was purified by silica gel chromatography using a gradient from CH_2Cl_2/MeOH 95/5 to CH_2Cl_2/MeOH 90/10 as the eluent to provide 37 mg (70%) of **11** as a pale yellow solid. NMR spectra are complicated by rotamers. ^{1}H NMR: δ 0.76−1.11 (m, 18H), 1.91−2.20 (m, 4H), 3.74−5.22 (m, 19H), 5.60−6.02 (m, 3H), 7.14−7.31 (m, 5H), 7.41 (br s, 1H), 7.61 (br s, 1H), 7.75 (br s, 1H). MS calcd for $[M + H]^+$ ($C_{41}H_{54}N_7O_8$): 772.4. Found (ES+): 772.4. HPLC tR 6.2 min (gradient from 20/80 MeCN/H_2O).

Octamer (12). The amine **8** (82 mg, 0.20 mmol, 1.1 equiv), acid **6** (95 mg, 0.18 mmol, 1.0 equiv), HATU (85 g, 0.22 mmol, 1.2 equiv), and DIPEA (40 μL, 0.22 mmol, 1.2 equiv) were dissolved in 3 mL of DMF, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was then diluted with H_2O and was extracted with EtOAc. The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated in vacuo. The product was purified by silica gel chromatography using a gradient from CH_2Cl_2/MeOH 95/5 to CH_2Cl_2/MeOH 90/10 to provide 0.11 g (77% yield) of **12** as a pale yellow solid. NMR spectra are complicated by rotamers. ^{1}H NMR: δ 0.77−1.09 (m, 24H), 1.96−2.22 (m, 4H), 3.62−4.72 (m, 21H), 4.95−5.82 (m, 8H), 7.05 (br s, 1H), 7.21−7.37 (m, 5H), 7.45 (br s, 1H), 7.63 (br s, 2H). MS calcd for $[M + Na]^+$ ($C_{49}H_{66}N_8NaO_{11}$): 965.5. Found (ES+): 965.5. HPLC tR 8.5 min (gradient from 20/80 MeCN/H_2O).

Octamer Acid (13). The same procedure as described above for the preparation of the tetramer acid **6** was used to provide a 64% recovery of the product, which was taken to the next step without further purification.

Octamer Propargylamide (14). The acid **13** (45 mg, 50 μmol, 1.0 equiv), propargylamine hydrochloride (6.0 mg, 0.14 mmol, 2.8 equiv), HATU (26 mg, 75 μmol, 1.5 equiv), and DIPEA (30 μL, 0.15 mmol, 3.0 equiv) were dissolved in 2 mL of DMF, and the reaction mixture was stirred at 0 °C for 3 h. The reaction mixture was then diluted with H_2O and was extracted with EtOAc. The organic layers were combined, dried over $MgSO_4$, filtered, and evaporated in vacuo. The product was purified by silica gel chromatography using a gradient from CH_2Cl_2/MeOH 95/5 to CH_2Cl_2/MeOH 90/10 to provide 40 mg (88%) of **14** as a pale yellow solid. NMR spectra are complicated by rotamers. MS calcd for $[M + Na]^+$ ($C_{51}H_{67}N_9NaO_{10}$): 988.5. Found (ES+): 989.5. HPLC tR 6.2 min (gradient from 30/70 MeCN/H_2O).

Tetramer−PEO−Tetramer (15). α,ω-Diazidopoly(ethylene glycol),[46] MW ~3400 g/mol (0.14 g, 41 μmol, 1.0 equiv), and the alkyne **7** (58 mg, 0.10 mmol, 2.4 equiv) were dissolved in 2 mL of tBuOH/H_20 1/1, and the solution was degassed using the freeze−pump−thaw technique. To the solution were added sodium ascorbate (2.0 mg, 10 μmol, 0.25 equiv) and $CuSO_4$ (1.2 mg, 7.5 μmol, 0.18 equiv), and the reaction mixture was stirred under nitrogen at room temperature overnight. 0.2 mL of 28% aqueous NH_4OH was added, then the solution was evaporated in vacuo. The product was purified by silica gel chromatography using a gradient from EtOAc/MeOH 95/5 to CH_2Cl_2/MeOH 90/10 to CH_2Cl_2/MeOH 50/50 as the eluent to provide 0.13 g (74%) of **15** as a white solid. MS calcd for $[M + Na]^+$ based on conjugation to the starting polymer $N_3CH_2(CH_2OCH_2)_nCH_2N_3$ with a peak MW of 3370 g/mol (n = 74): 4548. Found (MALDI-TOF+): 4550. HPLC tR 6.9 min (gradient from 30/70 MeCN/H_2O).

Hexamer−PEO−Hexamer (16). The same procedure as described above for the preparation of polymer **15** was used to provide 70 mg (75%) of polymer **16** as a white solid. MS calcd for $[M + Na]^+$ based on conjugation to the starting polymer $N_3CH_2(CH_2OCH_2)_nCH_2N_3$ with a peak MW of 3370 g/mol (n = 74): 4936. Found (MALDI-TOF+): 4937. HPLC tR 6.8 min (gradient from 30/70 MeCN/H_2O).

Octamer−PEO−Octamer (17). The same procedure as described above for the preparation of polymer **15** was used to provide 50 mg (60%) of polymer **17** as a white solid. MS calcd for $[M + Na]^+$ based on conjugation to the starting polymer $N_3CH_2(CH_2OCH_2)_nCH_2N_3$ with a peak MW of 3370 g/mol (n = 74): 5324. Found (MALDI-TOF+): 5327. HPLC tR 6.8 min (gradient from 30/70 MeCN/H_2O).

Multiangle Light Scattering Analyses. Polymers **15**, **16**, and **17** were dissolved in spectral grade $CHCl_3$ at a concentration of 1 mM. Polymer **17** was also analyzed at 0.5 mM. The samples were filtered through a 0.2 μm PTFE filter into an injection loop of ~0.9 mL, and care was taken to void the loop of air bubbles. The samples were then injected at a flow rate of 1 mL/min through a miniDAWN Treos detector (Wyatt Technology, Santa Barbara, CA). Each sample was injected at least three times. The data were analyzed using Astra software (Wyatt) using the following equation[47]

$$R(\theta) = K^*McP(\theta)[1 - 2A_2McP(\theta)] \quad (1)$$

where $R(\theta)$ = excess Rayleigh ratio, the ratio of scattered and incident light intensity (corrected for scattering volume and distance from scattering volume), $K^* = (4\pi n_0^2/N_A\lambda_0^4)(dn/dc)^2$, n_0 = solvent refractive index, N_A = Avogadro's number, λ_0 = vacuum wavelength of incident light, dn/dc = specific refractive index of the material, M = molar mass, c = solute concentration (g/mL), $P(\theta)$ = scattering function which relates the angular variation in scattering intensity to the mean square radius of the particle, and A_2 = second viral coefficient which is a measure of solute−solvent interactions (usually very small). A dn/dc of 0.135 for PEO in $CHCl_3$ was used, and the reported errors on the measurements were generated in the software based on the fits. The Debye plots are shown in Figures 2, S18, S19, and S20.

Dynamic Light Scattering Analyses. Polymers **16** and **17** were dissolved in 2 mL of spectral grade $CHCl_3$ at a concentration of 2 mM and were filtered through a 0.2 μm filter into a quartz cuvette. The measurements were taken using a Zetasizer Nano ZS from Malvern Instruments. Multiple runs on each sample were performed to check the reproducibility of the measurements. To investigate the effect of MeOH on the hydrodynamic diameter, aliquots of filtered MeOH were added to provide solutions containing 2 or 5% MeOH in $CHCl_3$. The autocorrelation functions measured by the Zetasizer were analyzed using a regularized inverse Laplace transform method,[48] as discussed in more detail below. The autocorrelation functions are shown in Figure S21 to illustrate the reproducibility of the measurements and the differences in correlation times between the polymers. An example illustrating the quality of the fits to the data produced by the data analysis is shown in Figure S22.

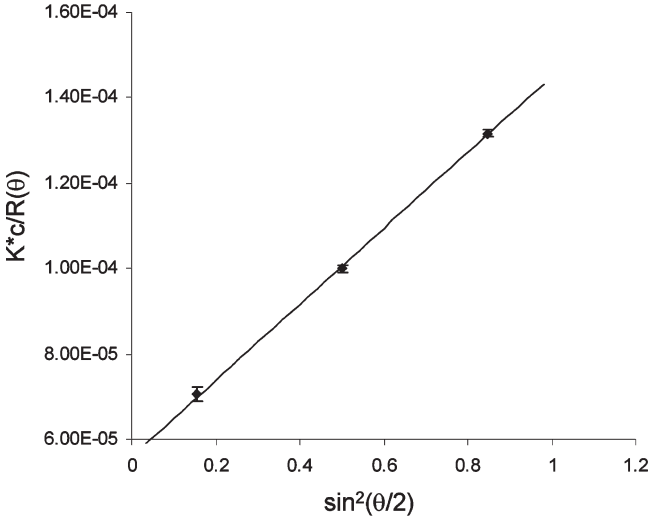


Figure 2. Multiangle light scattering Debye plot for polymer **17** at a concentration of 1 mM in $CHCl_3$ (K^*, c, θ, and $R(\theta)$ are defined in the Experimental Section).

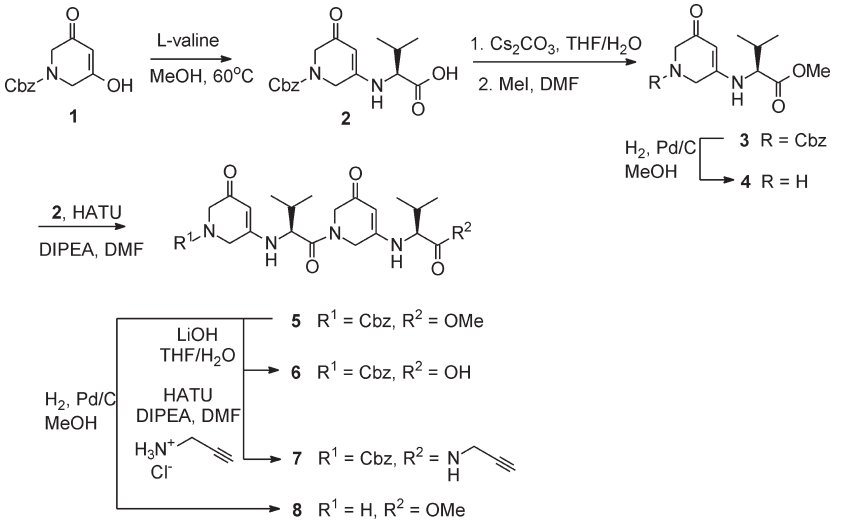
Results and Discussion

Syntheses of @-Tide−PEO Hybrids. In order to conjugate the @-tides to the termini of PEO in high yield, a Cu(I)-catalyzed "click" cycloaddition reaction was selected. Thus, α,ω-diazidopoly(ethylene glycol) was prepared as previously reported.[46] It was determined by matrix-assisted laser desorption ionization (MALDI) MS that this material had a peak MW of 3370 g/mol. @-Tides with carboxy-terminal propargylamides were prepared as the complementary click reaction partners. L-Valine was selected as the amino acid component of the @-tides due to its propensity to favor β-sheet formation,[49] and the target molecules were prepared by a convergent solution phase synthesis based on modifications to the previously reported @-tide synthesis.[45]

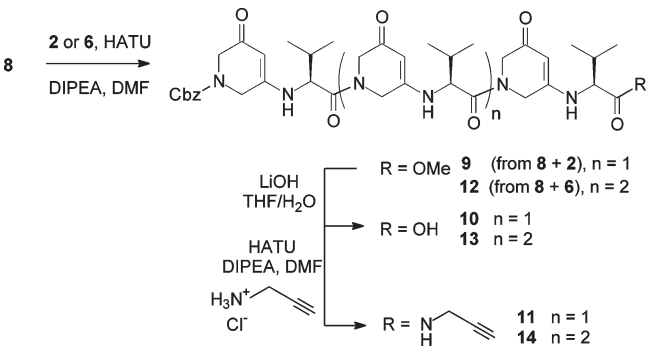
As shown in Scheme 1, the previously reported benzyl carbamate (Cbz) protected @-tide unit **1** was reacted with L-valine to provide the corresponding condensation product **2** which will be referred to as a dimer. This dimer was converted to the corresponding methyl ester **3** by reaction with Cs_2CO_3, followed by methyl iodide. After removal of the Cbz group by catalytic hydrogenolysis in methanol, the resulting amine **4** was coupled with the acid **2** using *O*-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HATU) in diisopropylethylamine (DIPEA) and DMF to provide the tetramer **5**. The methyl ester of **5** could be hydrolyzed to the acid **6** by treatment with LiOH, followed by coupling with propargylamine hydrochloride to provide the amide **7**. Alternatively, the Cbz group could be removed by catalytic hydrogenolysis to give the amine **8**. For the preparation of longer hydrogen-bonding oligomers, as shown in Scheme 2, the tetramer amine **8** was coupled to the dimer acid **2** to provide the hexamer, while coupling of **8** with the tetramer acid **6** gave the octamer. The hexamer and octamer were functionalized to form the respective propargylamides **11** and **14** using the same procedures described above.

Molecules up to the tetramer length (**5**) were characterized by the standard methods used for small molecules, including

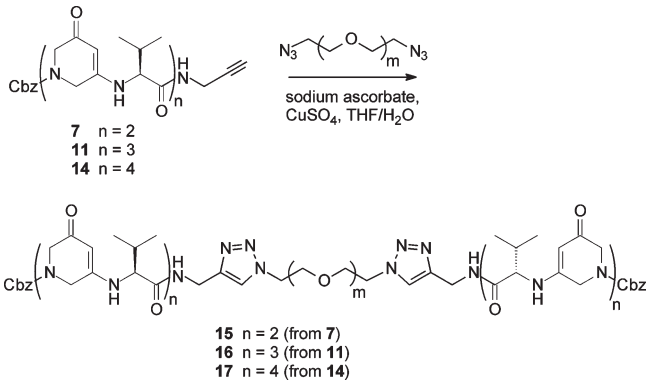
Scheme 1



Scheme 2



Scheme 3



^{1}H NMR, ^{13}C NMR, high performance liquid chromatography (HPLC), and mass spectrometry (HRMS). All characterization data were consistent with the proposed structures and high purities. Because of the increasing broadness and complexity of the NMR spectra with increasing length and solubility constraints, the propargyl amides as well as the hexamers and octamers were characterized mainly by HPLC and MS, techniques that are standard for the characterization of oligopeptides, along with ^{1}H NMR spectroscopy.

As shown in Scheme 3, the propargylamide-functionalized @-tides **7**, **11**, and **14** were then coupled to the PEO under click chemistry conditions consisting of $CuSO_4$ and sodium ascorbate in a mixture of THF/H_2O to provide the corresponding @-tide functionalized PEOs **15**, **16**, and **17**. The purity of these final @-tide−PEO hybrids was critical to their abilities to assemble as free @-tide impurities would inhibit the assembly, while monofunctionalized PEOs would provide end-caps, thus reducing the MWs of the assemblies. Therefore, following purification, HPLC was used to confirm the absence of excess @-tide or other impurities. In addition, MALDI MS was used to investigate the MWs of the hybrids, and it was found that the peaks corresponding to the mass of the starting PEO with two @-tide units added were found in each case.

Characterization of the Assemblies by Light Scattering. The assembly of polymers based on the tetramer, hexamer, and octamer (**15**, **16**, and **17**, respectively) into higher MW polymers via hydrogen bonding of the @-tide units into β-sheet mimics was investigated by light scattering. Multiangle light scattering (MALS) was used to determine the weight-average molecular weights (M_ws) for the polymers in $CHCl_3$. At a concentration of 1 mM, polymers **15** and **16** exhibited low scattered light intensities, and M_ws of 11 610 ± 8700 and 7100 ± 2200 g/mol were calculated.[47] The errors on these measurements are high due to the low signal-to-noise

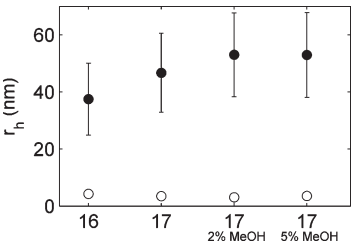


Figure 3. Mean hydrodynamic radii of scatterers determined from dynamic light scattering measurements on polymers **16** and **17** in $CHCl_3$ and polymer **17** in $CHCl_3$ + MeOH. The error bars indicate the standard deviation of the peak in the distribution due to the larger scatterers and give a measure of polydispersity. The corresponding width for the smaller scatterers is approximately the size of the symbols.

associated with the low scattering intensities, but these measurements indicate that there was not a significant degree of supramolecular assembly of these polymers. In contrast, at 1 mM, polymer **17** exhibited much greater scattered light intensities, resulting in much greater signal-to-noise, and an M_w of 61 200 ± 1300 g/mol was calculated. The Debye plot for polymer **17** is shown in Figure 2. This indicates that polymer **17**, containing octameric @-tides with the capacity to form eight hydrogen bonds, is capable of assembling to form higher MW polymers. In addition, when MALS experiments were performed on polymer **17** at a concentration of 0.5 mM, a decreased M_w of 33 500 ± 1800 g/mol was observed. This result indicates that the polymerization is concentration dependent, a result that is expected for an assembly based on noncovalent intermolecular bonding between the monomers.[3]

The assembly of polymers **16** and **17** was also investigated by dynamic light scattering (DLS) at a concentration of 2 mM in $CHCl_3$. The Zetasizer measures the intensity autocorrelation function $g^{(2)}(\tau)$ as a function of the delay time τ. These data were converted to the field autocorrelation function $g^{(1)}(\tau)$ using the Siegert relation.[50] $g^{(1)}(\tau)$ decays with time as a result of the diffusion of the scatterers in the solvent. We determined the distribution of hydrodynamic radii of the scatterers using an implementation of the "CONTIN" method[48,51] written in MATLAB.[52] A typical size distribution is shown in Figure S23. In all cases, the distributions displayed two prominent peaks, indicating the presence of two populations of scatterers with different characteristic sizes. Figure 3 shows the mean hydrodynamic radii of the scatterers and the widths (standard deviations) of the peaks determined for the two polymers. For both polymers, the field autocorrelation function had a rapidly decaying component corresponding to scatterers with a hydrodynamic radius r_1 of ~3.4 ± 1.2 nm, likely corresponding to unassembled polymer molecules, and a slower component of the decay due to larger particles which we assume to be supramolecular assemblies. These larger scatterers had a hydrodynamic radius r_2 of about 40 ± 13 nm. For polymer **16**, the two peaks in the size distribution are similar in strength, but the large-radius peak is much stronger for polymer **17.** The magnitude of the scattered electric field increases as the cube of the size of the scatterer. Taking the ratio of the areas of the peaks in the size distribution and dividing by $(r_2/r_1)^3$ thus gives an estimate of the relative number of scatterers of each size. This quantity is plotted in Figure 4, which shows that, while there is a small number of higher MW assemblies formed through hydrogen-bond-mediated assembly of polymer **16**, the relative number of large assemblies is about 20 times higher in polymer **17**. Assuming that the larger scatterers are uniform-density assemblies of the individual

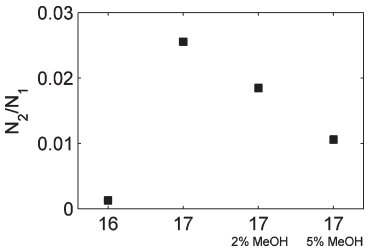


Figure 4. Ratio N_2/N_1 of number of large and small scatterers, respectively, estimated from the DLS data. Data are for polymers **16** and **17** in $CHCl_3$ and polymer **17** in $CHCl_3$ + MeOH.

polymer molecules, our results suggest that roughly 98% of the molecules of **17** are assembled into larger aggregates, compared to only 45% for **16**. Although a hydrodynamic radius around 40 nm still seems somewhat large for the M_w of 61 200 ± 1300 g/mol measured by MALS, these DLS experiments were carried out at a 2-fold higher concentration, which would be expected to lead to higher MW assemblies due to the concentration-dependent nature of the assembly.

The effect of methanol addition on the assemblies formed by polymer **17** was also investigated by dynamic light scattering. When 2% or 5% methanol was added to the chloroform solution, two populations of scatterers were still observed, as indicated in Figure 3. The hydrodynamic radii of these scatterers remained approximately the same as in the absence of methanol, but the relative number of large assemblies decreased substantially, as seen in Figure 4. These data are consistent with the dissociation of the high MW polymer assemblies due to the fact that MeOH is a competitive hydrogen bond donor and acceptor.

Characterization of the Assemblies by Circular Dichroism Spectroscopy. In order to further probe the nature of the polymer assemblies, circular dichroism (CD) spectroscopy was performed on polymers **16** and **17**. It has previously been demonstrated by Bartlett et al. that the assembly of @-tides into β-sheet-like structures led to a characteristic signal in the CD spectrum near 280 nm, attributable to the vinylogous amide absorbance.[44] The addition of methanol to @-tide solutions led to a significant attenuation of this signal as the oligomers became unstructured. As shown in Figure 5, at a concentration of 0.25 mM in $CHCl_3$, polymer **16** provided a weak signal near 280 nm. Upon the addition of 5% MeOH, the signal was attenuated to only a small degree. This result supports the light scattering data above, suggesting that this polymer does not assemble to a great extent. In contrast, at the same concentration polymer **17** exhibited a stronger peak near 280 nm, and this peak was significantly attenuated when 5% MeOH was added. These results are important as they suggest that the assemblies observed by light scattering were not randomly structured aggregates, but rather polymer assemblies formed at least predominantly by well-defined and structured β-sheet mimics. While the formation of branched structures formed by imperfect hydrogen bonding cannot be excluded, it is unlikely due to the short lengths of the @-tides and their relatively rigid structures.

Comparison of the Results with @-Tide Dissociation Constants. The results observed for the assembly of polymers **15**, **16**, and **17** can be compared with the dissociation constants previously reported for the @-tide oligomers.[43] @-Tides capable of forming only four hydrogen bonds were reported to exhibit dissociation constants (K_ds) on the order of 10^{-2} M in 1% CD_3OH in $CDCl_3$, so the formation of supramolecular assemblies was not expected for polymer **15** at the concentrations tested. Systems capable of forming six hydrogen bonds

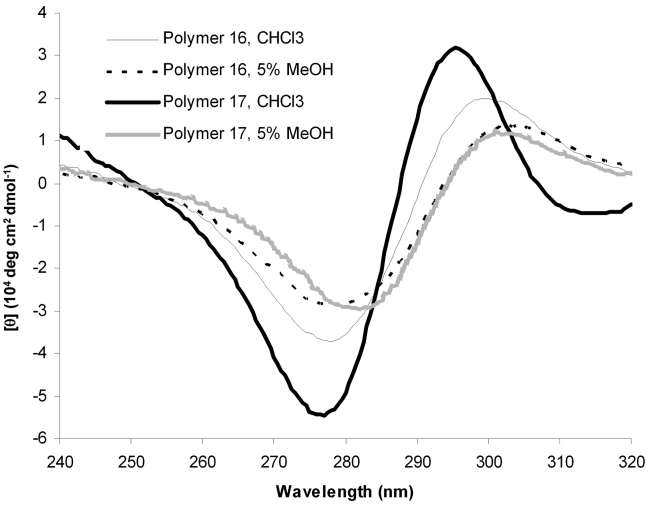


Figure 5. CD spectra of polymers **16** and **17** in pure $CHCl_3$ or 5% MeOH in $CHCl_3$.

were reported to have K_ds on the order of 10^{-4} M in $CDCl_3$, so only a small degree of association was expected. This is consistent with the light scattering and CD spectroscopy results. On the other hand, @-tides capable of forming eight hydrogen bonds had K_ds too large to measure in pure $CHCl_3$ but were reported to assemble with K_ds on the order of 10^{-3} M, even in 15% CD_3OH in $CDCl_3$. Therefore, as expected, the minimum @-tide length required for a significant degree of assembly in this study was the octamer. It is also possible that the PEO chains inhibit the degree of assembly to some degree due to their ability to backfold and stabilize the unimers via competing intramolecular hydrogen bonding.[53] In order to obtain assemblies at lower concentrations or to obtain higher MW assemblies, it will be necessary to prepare and conjugate longer @-tides. In addition, the effects of the methanol addition on the assemblies indicate that to obtain stable assemblies in water for biomedical applications it will be necessary to protect the hydrogen-bonding units from water by encapsulating them within the environment of a hydrophobic polymer, an approach that has previously been successful with supramolecular polymers based on the ureidopyrimidinone unit.[36]

Conclusions

In conclusion, alkyne-functionalized @-tide β-strand peptidomimetics of varying lengths were successfully synthesized and conjugated to PEO functionalized with azides at each terminus. Unlike previous examples of PEO-β-strand hybrids, hydrogen bonding in the @-tides was limited to one edge of the strand, making the formation of linear polymers rather than fibrillar structures possible. The assembly of the resulting low MW polymers into higher MW polymers was investigated by MALS, DLS, and CD spectroscopy. It was found that @-tides capable of forming a minimum of eight hydrogen bonds were necessary for the significant formation of high MW polymers in $CHCl_3$, and the sizes of the assemblies was sensitive to both concentration and the presence of MeOH in the solution. CD spectroscopy results supported the proposed mode of assembly via the formation of β-sheet mimics from the @-tide β-strand mimics. This study thus provides the first groundwork for the use of β-strand peptidomimetics for the development of supramolecular linear polymers and suggests that the strength of the association can be tuned by the length of the @-tide, an aspect that is not readily accessible in other hydrogen-bonding moieties. Upon further development, the potential utility of the amino acid side chains to introduce

chemically and biologically active functionalities makes these materials highly promising for the development of new functional materials.

Acknowledgment. The authors thank the Natural Sciences and Engineering Research Council of Canada and the Canada Research Chairs Program for support of the research.

Supporting Information Available: HPLC chromatograms/NMR spectra of compounds **2**, **3**, **5**, **7**, **9**, **11**, **12**, **14**, **15**, **16**, and **17** as proof of purity, Debye plots for polymers **15**, **16**, and **17**, correlograms for polymers **16** and **17**, fit of eq 1 to DLS data. This material is available free of charge via the Internet at http://pubs.acs.org.

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