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Chiral Self-Assembly of Designed Amphiphiles: Optimization for Nanotube Formation

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Supporting Information

ABSTRACT: Four amphiphiles with L-aspartic acid headgroups (Asp) and a diphenyldiazenyl group (Azo) contained within the hydrophobic tails were designed and synthesized for self-assembly into helically based nanotubes. The amphiphiles of the form R'-{4-[(4-alkylphenyl)diazenyl]phenoxy}alkanoyl-L-aspartic acid (where R' is 10 or 11) varied only in alkyl chain lengths either side of the azo group, having 4, 7, or 10 carbon distal chains and 10 or 11 carbon proximal chains (R-Azo-R'-Asp, where R denotes the number of carbons in the distal chain and R' denotes the number of carbons in the proximal chain). Despite the molecular similarities, distinct differences were identified in the chiral order of the structures self-assembled from hot methanolic aqueous



solutions using microscopy and spectroscopic analyses. This was reflected in dominant thermodynamic aggregate morphologies that ranged from amorphous material for 10-Azo-10-Asp, through twisted ribbons ($196 \pm 49 \text{ nm pitch}$) for 7-Azo-11-Asp, to the desired helically based nanotubes for 4- and 7-Azo-10-Asp (81 ± 11 and 76 ± 6 nm diameters, respectively). Another key variable in the self-assembly of the amphiphiles was the use of a second method to precipitate aggregates from solution at room temperature. This method enabled the isolation of thermodynamically unstable and key transitional structures. Helical ribbons were precursor structures to the nanotubes formed from 4- and 7-Azo-10-Asp as well as the wide, flattened nanotube structures ($587 \pm 85 \text{ nm width}$) found for 4-Azo-10-Asp. Overall, the results highlighted the interplay of influence of the headgroup and the hydrophobic tail on self-assembly, providing a basis for future rational design of self-assembling amphiphiles.

INTRODUCTION

A very useful structure in nanotechnology is the onedimensional container or nanotube. One type of nanotube with flexible utility is constructed through the helical wrapping of bilayer ribbons self-assembled from amphiphiles. Such nanotubes generally have hydrophilic surfaces, hosting a large range of different chemical functionalities, and also have an array of internal diameters.¹⁻³ This flexibility in chemistry and size, along with their shape, provides a range of useful applications including (i) encapsulating other substances for subsequent controlled release, such as in drug delivery,^{4,5} (ii) acting as templates for the organization of metals,^{6,7} semiconductors,⁸ proteins,⁹ and synthetic polymers,¹⁰ and (iii) in combination with other materials as nanocomposites.¹¹⁻¹³ Given the utility of these nanotubes, it is important to improve the efficiency in the design of precursor amphiphiles and assembly conditions by increasing the understanding of the influence that each variable has on the self-assembled morphology. To do this, we applied a rational design approach for constructing novel chiral amphiphiles (Chart 1), and by systematically varying the molecular parameters of the amphiphiles, we were able to study the effect of structuredirecting parameters on the self-assembly of the desired aggregates.

Self-assembly of nanotubes through the helical twisting of bilayer ribbons is driven largely by the molecular structure of the amphiphile.¹⁴ Herein we report the synthesis and characterization of a series of newly designed amphiphilic compounds (*R*-Azo-*R'*-Asp, where R = 4, 7, or 10 and is the number of alkyl units in the distal chain and R' = 10 or 11 and is the number of alkyl units in the proximal chain; Chart 1). The profound effect of the headgroup and interfacial region on the self-assembly of amphiphiles is known and is usually the focus of efforts in the rational design of self-assembled amphiphilic systems.^{1,15–17} Amino acids have often been used as a headgroup or in a linking role as they provide multiple functionality and high chiral fidelity in a cost-effective manner.^{18–21} Despite limited use in the extant literature,²² single residue aspartic acid was selected for the headgroup, as its polar chemistry provides multiple opportunities for hydrogen bonding, while the amide linkage to the hydrophobic

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Chart 1. Molecular Structures of the Synthesized Amphiphiles^a



^{*a*}All compounds have an aspartic acid headgroup linked by an amide bond to the hydrophobic tail, which comprises an azo group between two alkyl chains. The key compositional variables are the lengths of the alkyl chains, the proximal chain being 10 or 11 carbons long, and the distal chain being 4, 7, or 10 carbons long.

chain provides a strongly directional interaction between neighboring molecules. Importantly, this directional interaction is adjacent to the chiral center and within the interfacial region between the hydrophilic head and hydrophobic tail of the amphiphile.^{23,24} Such organization of the headgroup and its connection to the bent, hydrophobic tail was designed to drive the formation of helically based nanotubes through molecularchirality-directed packing within the bilayer ribbons. In this mechanism neighboring molecules pack at a consistent nonzero angle, with the direction of propagation of the packing angle controlled by the molecular chirality, such chiral molecular packing being expressed in the macroscopic helices that form the tubes.^{25,26}

Overall, the influence of the hydrophobic tail on the selfassembly of amphiphilic molecules is less well understood than the influence of the hydrophilic headgroup and frequently more subtle. Azo groups are often used in self-assembled systems to exploit their photoresponsive nature.²⁷ In our molecular design the azo group was incorporated within the hydrophobic tail to provide a rigid kinked structure that provides the offset in molecular packing that can be chirally controlled. Such strategic molecular design supports both molecular-chirality-directed packing and another potentially complementary helical tube forming mechanism based on chiral symmetry breaking, previously observed in well-researched diacetylenic amphi-philes.^{28–30} Additionally, the azo group affords opportunities for $\pi - \pi$ stacking during self-assembly, thereby strengthening the noncovalent interactions between hydrophobic tails in the resulting aggregate. The azo linkages also provide the prospect of using light absorbance spectroscopy to probe the organization of molecules in the nanostructure.^{31,32}

Given that the amphiphiles vary only in the alkyl chain lengths either side of the azo group, we demonstrate how, for this system, the length of the distal and proximal chains is of great importance in determining the overall aggregate structure. This finding is in contrast to diacetylenic systems where the length variations in proximal and distal chains were found to be of more limited importance.^{33,34} This particular effect of the hydrophobic tail is difficult to predict, especially when considered as part of the entire complex amphiphile interaction, and illustrates why such empirical research is still important in the optimization of the design of amphiphilic molecules for self-assembly into helically based nanotubes.

EXPERIMENTAL SECTION

Materials. All materials were used as received. *O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate (HATU) and 1-hydroxy-7-azabenzotriazole (HOAt) were purchased from GL Biochem (Shanghai) Ltd. (China); AR grade methyl cyclohexane, hexane, and thionyl chloride were obtained from Merck Pty. Ltd. (Australia), and hydrochloric acid (37% v/v) was purchased from Ajax Finechem Pty. Ltd. (Australia). All other reactants and reagents were purchased from Sigma-Aldrich Pty. Ltd. (Australia) and were AR grade where available. Deuterated solvents for NMR analysis were manufactured by Cambridge Isotopes Laboratories, Inc. Other solvents were AR grade obtained from Ajax Finechem Pty. Ltd. (Australia). All water used for self-assembly was deionized using a Barnstead E-pure water purification system operating at a resistance of at least 18.0 M Ω .

Synthesis. Detailed synthetic procedures and molecular identification (¹H NMR, ¹³C NMR, FTIR, and elemental analysis) for the four amphiphiles 4-Azo-10-Asp, 7-Azo-10-Asp, 10-Azo-10-Asp, and 7-Azo-11-Asp (Chart 1) and their precursors are in the Supporting Information. An example of the synthetic procedure for 4-Azo-10-Asp is presented here.

4-[4-(Butylphenyl)diazenyl]phenol. 4-Butylaniline (4.30 g, 28.8 mmol) and hydrochloric acid (1.0 M, 96 mL) were stirred for 1 h at room temperature before cooling to less than 5 °C. A solution of sodium nitrite (2.19 g, 32 mmol) in water (~4 mL) was then added dropwise. The resultant cold diazonium solution was then added dropwise to a solution of phenol (3.0 g, 32 mmol) and sodium acetate

(4.7 g, 58 mmol) in water (48 mL) that was also stirring at less than 5 $^{\circ}$ C. The mixture was stirred for a further 3 h at less than 5 $^{\circ}$ C before neutralization with aqueous sodium hydroxide (2.0 M), precipitating the crude product. The solid was then filtered and washed with copious amounts of water before being allowed to dry thoroughly under suction. It was then washed with methylcyclohexane. Final purification occurred by dissolution in a minimum quantity of chloroform followed by precipitation with methylcyclohexane. Orange platelike crystals (mp 78.5 $^{\circ}$ C) were isolated in 73% yield.

Methyl 10-Bromodecanoate. 10-Bromodecanoic acid (2.46 g, 9.79 mmol) was dissolved in methanol (67 mL) containing *p*-toluenesulfonic acid (1% w/v), and the mixture was left to stir overnight at room temperature. The methanol was then removed by evaporation and the residue dissolved in diethyl ether. This solution was then neutralized with aqueous sodium bicarbonate solution (1% w/v), and the organic fraction was washed three times with water, before drying over sodium sulfate and subsequent filtration. The solvent was then removed *in vacuo* to reveal a colorless oil (mp -8.3 °C), isolated in 92% yield.

10-{4-[4-(Butylphenyl)diazenyl]phenoxy}decanoate. A mixture of 4-[4-(butylphenyl)diazenyl]phenol (16.2 mmol), methyl 10-bromodecanoate (4.31 g, 16.2 mmol), potassium carbonate (4.49 g, 32.4 mmol), potassium iodide (0.166 g, 1 mmol), and acetone (80 mL) was stirred at reflux under nitrogen for 1 day. The solution was cooled to room temperature, and then water (80 mL) was added. The precipitate was collected by filtration and washed with water. The resulting crude product was purified by recrystallization from acetonitrile producing a yellow-orange solid (mp 64.9 °C) that was isolated in 86% yield.

10-{4-[4-(Butylphenyl)diazenyl]phenoxy}decanoic Acid. 10-{4-[4-(Butylphenyl)diazenyl]phenoxy}decanoate (2.22 g, 5.1 mmol) was stirred in tetrahydrofuran (9 mL) under nitrogen and heated until the solid dissolved. To this solution was added potassium hydroxide (1.43 g, 26 mmol) dissolved in water (9 mL), and the mixture was refluxed overnight. The product was extracted with a mixture of diethyl ether and tetrahydrofuran (1:1 v/v) and acidified by washing with aqueous hydrochloric acid (1.0 M). The organic phase was then washed once with water and then once with brine before drying over magnesium sulfate, filtration, and subsequent evaporation of the solvents. The isolated crude product was purified by dissolution in the minimum quantity of hot ethyl acetate and then precipitated with hexane. The solid was filtered and washed with hexane, revealing orange crystals (mp 113.7 °C), isolated in 91% yield.

L-Aspartic Acid Dimethyl Ester Hydrochloride. Thionyl chloride (30 mL, 150 mmol) was added dropwise to methanol (120 mL) which was rapidly stirred at -10 °C. To this solution was added L-aspartic acid (6.66 g, 50 mmol), and then the reaction mixture was allowed to warm to room temperature before being left to stir overnight. The solvent was removed *in vacuo*, and the residue was dissolved in methanol and then reduced *in vacuo* on two further occasions to remove residual thionyl chloride. Purification was via dissolution in a minimum quantity of methanol followed by precipitation with diethyl ether and then filtration. This purification procedure was then repeated revealing a white solid in 95% yield.

10-{4-[(4-Butylphenyl)diazenyl]phenoxy}decanoylaspartic Acid Dimethyl Ester. 10-{4-[4-(Butylphenyl)diazenyl]phenoxy}decanoic acid (0.850 g, 2.0 mmol) was stirred under nitrogen in a minimum quantity of ethyl acetate/tetrahydrofuran (1:1 v/v) to dissolve the acid at room temperature. Once dissolved, N,N-disopropylethylamine (DIEA) (0.85 mL, 5 mmol), 1-hydroxy-7-azabenzotriazole (HOAt) (0.27 g, 2 mmol), and O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) (950.5 mg, 2.5 mmol) were added, and this mixture was stirred for a further hour. Then Laspartic acid dimethyl ester hydrochloride (0.79 g, 4 mmol) was added, and the stirring under nitrogen continued overnight at room temperature. The resultant solution was diluted in ethyl acetate/ tetrahydrofuran (1:1 v/v, 100 mL), washed with aqueous solutions of sodium hydrogen sulfate (5% w/v, 2×50 mL) and sodium hydrogen carbonate (5% w/v, 2×50 mL), and then finally washed with brine (2 \times 50 mL). The volume of the organic phase was maintained during the

washing process by adding tetrahydrofuran. The organic phase was then dried over magnesium sulfate and filtered before the solvent was removed by evaporation. Purification was by recrystallization from acetonitrile, revealing an orange solid (mp 97.4 $^{\circ}$ C), isolated in 87% yield.

 $10-\{4-[(4-Butylphenyl)diazenyl]phenoxy\}decanoylaspartic Acid (4-Azo-10-Asp). 10-\{4-[(4-Butylphenyl)diazenyl]phenoxy\}$ decanoylaspartic acid dimethyl ester (0.622 g, 1.1 mmol) was dissolved in tetrahydrofuran (2 mL) and stirred under nitrogen. To this was added aqueous potassium hydroxide (5.5 M, 2 mL), and the mixture was refluxed overnight. Ethyl acetate/tetrahydrofuran (1:1 v/ v, 100 mL) was added, and the mixture was acidified with hydrochloric acid (1.0 M, 2 × 100 mL). The organic phase was then washed once with water and then once with brine before drying over magnesium sulfate, filtration, and then subsequent evaporation of the solvents. The isolated product was dissolved in the minimum quantity of ethyl acetate and then precipitated with hexane. The precipitate was then filtered under vacuum, revealing an orange solid in 74% yield.

Self-Assembly. Heated Self-Assembly Procedure. The amphiphile was dissolved in methanol (2.5 mL, 0.8 mM) by sonication at 63 °C. To this was added heated pure water (7.5 mL; 18.0 M Ω ; 63 °C), and sonication continued at 65 °C while the solution slowly became cloudy. After 1 h sonication was ceased, but heating continued for a further 5 h. The dispersion was then slowly cooled to room temperature.

Room Temperature Self-Assembly Procedure (RT). The amphiphiles were dissolved (0.4 mM) in methanol (100 mL). Aliquots of this solution (300 μ L) were then diluted 10-fold with mixtures of methanol and pure water (2700 μ L; water = 18.0 MΩ) such that the final solutions ranged in methanol concentration relative to water from 10% to 100% in increments of 10%. The solvent mixture for each amphiphile having the largest blue-shift using UV–vis spectroscopy was selected for subsequent imaging by TEM.

Instrumentation. Ultraviolet and Visible Light Spectroscopy. Ultraviolet and visible light (UV–vis) spectroscopy was conducted on methanolic aqueous dispersions of nanotubes, as prepared in the room temperature self-assembly procedure. Spectra were recorded with a Varian Cary 50 Scan UV–vis spectrophotometer using a quartz cuvette with a 10 mm path length.

Circular Dichroism. The amphiphiles were self-assembled using the heated procedure, and the dispersion was analyzed by circular dichroism (CD) using a JASCO J-725 spectropolarimeter equipped with a Peltier accessory to maintain a temperature of 25 °C. The experiment was conducted using a SQ-grade cuvette, with a path length of 10 mm, at a scanning rate of 100 nm/min, a bandwidth of 1 nm, and a response time of 1 s, using a single accumulation.

Transmission Electron Microscopy. Transmission electron microscopy (TEM) was conducted on methanolic aqueous dispersions, as prepared in the self-assembly procedures. The dispersion was diluted 4-fold using the same solvent mixture used in self-assembly, and then 4 μ L of the dilution was deposited onto Formvar-covered copper grids and air-dried before imaging using a JEOL JEM-1200EX instrument operated at 80 kV with a spot size of 3.

RESULTS AND DISCUSSION

The basic amphiphile design utilized aspartic acid headgroup interactions and the geometry and intermolecular interactions of the azo group within the hydrophobic tail to drive self-assembly into nanotubes. The influence of these two components on the self-assembled morphology was elucidated by synthesizing amphiphiles that varied in distal (4, 7, and 10 carbons long) and proximal (10, and 11 carbons long) chain lengths. The distal chain length was set by the choice of 4-alkylaniline precursors, which were converted to aryldiazonium salts before coupling to phenol to create the azo group. The proximal chain was generated by the addition of R'-bromoalkyl carboxylic acid (where R' is 10 or 11) to the azo hydroxyl group using Williamson ether synthesis, before the final



Table 1. Summary of Characteristics of Structures Self-Assembled from the Synthesized Amphiphiles

attachment of the aspartic acid headgroup using an amide coupling to the carboxylic acid. The self-assembly of the amphiphiles was conducted in aqueous methanolic solutions using two methods, viz., heated method and room temperature (RT) method. In the heated method each amphiphile (0.2 mM in the final self-assembly mixture) dissolved in methanol was self-assembled at 65 °C by the addition of water (MeOH/H₂O, 25/75 v/v), and the structures were annealed through heat and sonication. For the RT method of self-assembly the compounds were dissolved in methanol at RT and precipitated by adding water. In the final mixture, the amphiphile concentration was 0.04 mM and the amount of methanol relative to water varied from 10% to 100% in increments of 10%. TEM, UV-vis, and CD analyses were used to study the influence of the compositional variables and the effect of temperature (65 °C and RT) as well as the methanol concentration (10–100% v/v for RT method) on the self-assembly.

Transmission Electron Microscopy. The results of the self-assembly for all four amphiphiles are summarized in Table 1 and in further detail in the Supporting Information (Table S1). Using the heated procedure, the aggregates self-assembled from 4-Azo-10-Asp were dominated by nanotubes (Figure 1a), with some amorphous aggregate and right-handed helical



Figure 1. TEM images of helically based tubes self-assembled from 4-Azo-10-Asp using the heated method.

ribbons also found. The nanotubes had an average outer diameter (o.d.) of 81 ± 11 nm and an average wall thickness of 28 ± 6 nm, giving a calculated internal diameter (i.d.) of 25 nm. The helical ribbons had an average ribbon width of 71 ± 14 nm and an average pitch of 472 ± 72 nm. The helical basis of the tubes was confirmed by many instances of hybrid structures exhibiting both tubular and helical morphologies (Figure 1b).

Using the RT self-assembly method with a solvent mixture containing 80% water, precipitation of 4-Azo-10-Asp produced nanotubes (o.d. 87 ± 8 nm, wall 32 ± 3 nm, i.d. 23 nm) that closely matched those self-assembled using the heated procedure. However, flattened tubes with very large external tube widths (587 ± 85 nm) and very thin tube walls were predominant in the RT self-assembly of 4-Azo-10-Asp, and these were also assembled via helical ribbon intermediates (width 671 ± 129 nm). Figure 2 exhibits examples of all of these structures including the denser, narrow diameter tubes



Figure 2. TEM image of tubes and helices self-assembled from 4-Azo-10-Asp using the RT method and having 80% water in the solvent mixture.

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(see the top left corner of Figure 2), a wide helical ribbon, and wide flattened tubes. A couple of the flattened tubes also showed the growth of new helical ribbon layers. These ribbons had varying widths and consistent pitch angles, indicating that new layers formed by ribbon widening rather than pitch compression (the most prevalent process for these types of self-assembled tubes¹).

Both the narrow tubes and the flattened tubes self-assembled from 4-Azo-10-Asp at RT exhibited a further organizational level; they aggregated into star-shaped bundles (Figure 3). This



Figure 3. TEM images of bundles of narrow tubes (a) and wide, flattened tubes (b) self-assembled from 4-Azo-10-Asp using the RT method and having 80% water in the solvent mixture. The arrow in (b) illustrates the possible transition from wide tubes to narrow tubes.

suggests they are related structures. Indeed, what appears to be a conversion from a wide, flat tube to a thinner tube is seen in the top left of Figure 3b. Previous research on the nanotubes self-assembled from a diacetylenic lipid suggested that selfassembly of the final helically based tube occurred from a similar flattened tube intermediate.³⁵ This transitional structure was only found using a novel electron microscopy specimen protocol³⁵ and had not been found using standard techniques. While observation of transitional structures is rare,^{35,36} the selfassembly of helically based tubes is suggested to proceed through intermediates that are not always seen due to the rapid kinetics of the self-assembly.³⁶ In the case of 4-Azo-10-Asp we deduce, similarly to other related amphiphiles,^{19,37} the rapid precipitation of self-assembled structures at RT enabled the isolation of a higher energy intermediate, which was not found when the sample was cured at elevated temperature and thermodynamic equilibrium was approached.

Amphiphile 7-Azo-10-Asp self-assembled into similar morphologies found for 4-Azo-10-Asp except that 7-Azo-10-Asp produced significantly more right-handed helical ribbons (heated method: width 75 \pm 14 nm, pitch 357 \pm 50 nm; RT method using 90% water: width 67 \pm 23 nm, pitch 386 \pm 54 nm [Figure 4b]) and less nanotubes (heated method: o.d. 76 \pm 6 nm, wall 24 \pm 3 nm, i.d. 28 nm [Figure 4a]; RT method: o.d.



Figure 4. TEM images of structures self-assembled from 7-Azo-10-Asp, using the heated method (a) and using the RT method with 90% water in the solvent mixture (b).

 83 ± 26 nm.) and amorphous material. Indeed, helical ribbons became the preponderant self-assembled structure for the RT method using 90% water in the solvent mixture. The changing relative populations of self-assembled morphologies between RT and heated self-assembly methods support that the helical ribbons are a kinetic product, isolated in greater populations by the rapid precipitation at RT. Meanwhile, nanotubes are the thermodynamic product, found in higher population under better-optimized annealing conditions at 65 °C. Despite a lack of observed wide ribbons self-assembled from 7-Azo-10-Asp using the RT procedure, the organized aggregates for 7-Azo-10-Asp were also often bundled together in star-shaped morphologies, another similarity to the structures of 4-Azo-10-Asp.

Organized aggregates self-assembled from 10-Azo-10-Asp were predominantly right-handed helical ribbons (Figure 5)



Figure 5. TEM image of helical ribbons self-assembled from 10-Azo-10-Asp using the heated method.

and distorted nanotubes, but in much lower populations than for 4-Azo-10-Asp and 7-Azo-10-Asp, and amorphous aggregates were dominant. Shimomura et al.³⁸ previously demonstrated a link between the distal chain length and the aggregate morphology for bilayer assemblies of azo amphiphiles, finding a shift from fully interdigitated bilayers to partially interdigitated bilayers when the distal chain became long enough to interfere with headgroup organization.³⁸ Therefore, it is proposed that the distal chain for 10-Azo-10-Asp interferes with headgroup packing, thus preventing the formation of fully interdigitated bilayers and leading to its relatively disorganized self-assembly.

Amphiphile 7-Azo-11-Asp provided contrast to the other amphiphiles by increasing the proximal chain length by one methylene unit. This was reflected in the self-assembled structures, being dominated by high axial ratio twisted ribbons using both the heated and RT methods (heated method: pitch 196 \pm 49 nm, Figure 6; RT method using 90% water: width 35 \pm 1 nm, pitch 148 \pm 38 nm) (note: the relatively high standard deviation of the pitch is indicative of variation between ribbons rather than large variations within a single ribbon). In addition, 7-Azo-11-Asp formed low populations of both helical ribbons (heated method: width 81 \pm 7 nm, pitch 428 \pm 33 nm, Figure 6a; RT method: width 131 \pm 14 nm, pitch 607 \pm 12 nm) and nanotubes, the nanotubes being of two distinct diameters using each method (heated method: 1, o.d. 68 \pm 9 nm, wall 24 \pm 2 nm; 2, o.d. 166 \pm 16 nm, wall 51 \pm 7 nm, Figure 6b; RT



Figure 6. TEM images of structures self-assembled from 7-Azo-11-Asp using the heated method.

method: 1, o.d. 48 ± 5.4 nm, wall 21 ± 1.5 nm; 2, o.d. 92 ± 7.6 nm, wall 31 ± 2.1 nm).

Twisted ribbons and helical ribbons are closely related structures that can transform from one to the other in a non-first-order transition, $^{39-41}$ the helical morphology being found to have greater chiral order. 42,43 The twisted ribbons selfassembled from 7-Azo-11-Asp have a pitch roughly half that of the helical ribbons from the same amphiphile, which provides confirmation that these structures are related as full ribbon rotations come at approximately the same length. (The twist pitch is measured every half rotation of the ribbon, while the helical pitch is measured at every full rotation.) The change from the dominance of the helical aggregates for R-Azo-10-Asp amphiphiles to twisted aggregates for 7-Azo-11-Asp suggests that the difference in relative orientations of the headgroup and the azo group for 7-Azo-11-Asp compared to the R-Azo-10-Asp species33,44 has reduced the intensity of the chirality of the interactions without necessarily decreasing the order of the assemblies. Moreover, R-Azo-10-Asp amphiphiles self-assembled into right-handed chiral aggregates, whereas the chiral aggregates (twisted and helical ribbons) of 7-Azo-11-Asp were left-handed. Selinger et al.^{45,46} showed that the handedness of macroscopic shapes depends not only on the microscopic chiral parameters, but also on the orientation of the molecular tilt to the long edge of the ribbon. It is clear that a change in alignment between the headgroup and the azo group induced by the extra methylene unit in the proximal chain could alter the orientation of the molecular tilt and explains the different handedness of the aggregates for 7-Azo-11-Asp compared to R-Azo-10-Asp. Further, small changes in tilt direction relative to nearest neighbors can also alter the elastic properties of the bilayer and consequently the diameter and pitch of the final self-assembled nanostructure.^{45,46} This was used to elucidate why multiple, distinct pitch angles for helical ribbons can be found for a single amphiphile 45,47 and is a reasonable explanation for the two distinct tube diameters found for amphiphile 7-Azo-11-Asp. The effect of the proximal chain length on the self-assembled aggregates again illustrates the importance of the interplay between the influences of the azo group and the headgroup on self-assembly.

UV–vis and **CD Spectroscopy Characterization.** UV– vis was used to monitor the self-assembly of the amphiphiles using the RT procedure. There was a blue-shift and concomitant reduction of intensity of the main π – π * transition upon precipitation of all amphiphiles (example for 4-Azo-10-Asp in Figure 7, complete spectra of all amphiphiles in the Supporting Information, Figures S17–S20), indicative of Haggregation of the chromophores.⁴⁸ This side-by-side arrangement of azo groups makes for a strong, rigid, organized, noncovalent bond, providing a high degree of order to the aggregate. Another important feature of the spectra of all four



Figure 7. UV–vis spectra for 4-Azo-10-Asp monitoring the change in spectral profile for aqueous methanolic solutions containing varying percentages of methanol at RT (only 3 of 10 solvent ratios included for clarity).

amphiphiles is the reduction in intensity of the $n-\pi^*$ transition at 430 nm upon self-assembly. This band is formally forbidden for the planar organization of the azo group but exists in solution due to a twist in the group around the N=N-C plane induced by repulsive interactions between ortho hydrogens.⁴⁹ As such, it can be deduced that the aggregation of the chromophores in the organized self-assembled structures led to a reduction in twist in the azo groups compared to those in solution.

Of specific interest were the differences between the spectroscopy results monitoring the self-assembly of 7-Azo-10-Asp and those for 4-Azo-10-Asp. UV-vis results showed that initial assembly occurred using 10% less water in the solvent mixture, due to reduced solubility, and the blue-shift was only 29 nm, compared to 37 nm for 4-Azo-10-Asp. The reduced blue-shift is indicative of a differing interaction between the aggregated 7-Azo-10-Asp chromophores compared to those for 4-Azo-10-Asp.50 This was supported by circular dichroism (CD) spectra for 7-Azo-10-Asp and 4-Azo-10-Asp self-assembled using the heated procedure. Figure 8 shows an opposite sign and a reduced intensity of CD absorbance for 7-Azo-10-Asp compared to 4-Azo-10-Asp, respectively, indicative of a reduced intensity of chiral azo interactions and opposing chiral arrangements of the chromophore transition moments in the aggregates.⁵¹ However, this variation in molecular packing of the azo groups, caused by the difference in distal chain lengths, is not reflected in the consistently right-handed helical morphologies observed using TEM. Consequently molecularchirality-directed packing, driven by the chiral headgroup, sets the aggregate chirality for both of these species, in spite of the varying organization of the azo groups. Nonetheless, the effect of the azo group is significant, and there is interplay between the influence on the self-assembly driven by the interactions of the headgroups and the azo groups. This is demonstrated by the fact that the azo interactions for 7-Azo-10-Asp differ in character from those for 4-Azo-10-Asp, reflected by the spectroscopic data and the reduced number of helical ribbons wound sufficiently tightly to form tubes.²⁴ This suggests that the influence on self-assembly of the azo group and the



Figure 8. CD spectra of 4-Azo-10-Asp and 7-Azo-10-Asp self-assembled using the heated method.

headgroup was more complementary for 4-Azo-10-Asp than it was for 7-Azo-10-Asp.

CONCLUSION

An investigation was made into the differences in the selfassembly of four closely related amphiphiles with aspartic acid headgroups and an azo group contained within the hydrophobic tails, varying only in proximal and distal chain lengths. These amphiphiles were designed to self-assemble into nanotubes using molecular-chirality-directed packing, driven by the headgroup interactions and supported by the intermolecular interactions and geometry of the hydrophobic tail. 4-Azo-10-Asp and 7-Azo-10-Asp self-assembled most efficiently into nanotubes, having distal chains short enough to allow fully interdigitated assembly, and a proximal chain length that supported greater chiral order in the self-assembled structure.

The temperature of the self-assembly was another important variable, with the heated method used to highlight the thermodynamic products. The similarity in dimensions and character of the helical ribbon (357–472 nm pitch; 71–81 nm width) and nanotube (68–81 nm diameter; 24–28 nm wall) structures found for 4-Azo-10-Asp, 7-Azo-10-Asp, and 7-Azo-11-Asp using the heated method is indicative that the same forces drive their self-assembly. It is subtle changes in molecular packing driven by distal and proximal chain length that caused the variations in the populations of certain morphologies. The RT method of self-assembly allowed the identification of helical ribbons as a kinetic precursor in the thermodynamic formation of nanotubes from 4-Azo-10-Asp and 7-Azo-10-Asp and the isolation of a fascinating transitional intermediate nanotube with much larger dimensions for 4-Azo-10-Asp.

The variation in assembly between the four amphiphiles makes it clear that there is interplay between the interactions of the azo group in the hydrophobic tail and the hydrophilic headgroup. These influences must be balanced to provide the optimal assembly, and consequently, for azo-based amphiphiles, the lengths of the proximal and distal chains provide a means optimizing molecular structure for production of the desired self-assembled morphologies.

ASSOCIATED CONTENT

Supporting Information

Table summarizing self-assembly results; experimental procedures and analyses; additional TEM images and UV–vis spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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