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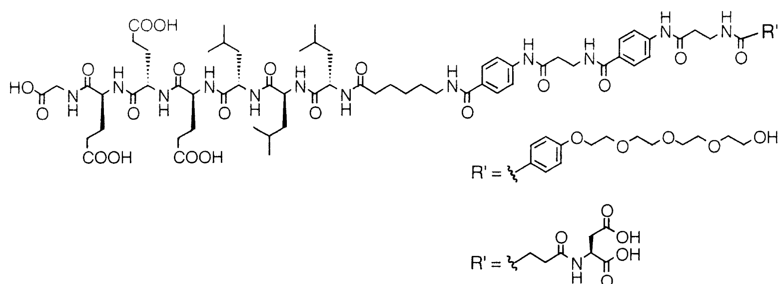

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Aqueous Self-Assembly of Unsymmetric Peptide Bolaamphiphiles into Nanofibers with Hydrophilic Cores and Surfaces

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Natural proteins and peptides adopt well-defined secondary structures such as α-helices and *β*-sheets through an interplay of hydrophobic collapse, hydrogen bonding, and electrostatic and van der Waal interactions.[1] Using these same forces, artificial peptides[2−4] and peptide amphiphiles[5−7] (PAs) have been used to form novel nanostructures. For example, peptide nanotubes are formed through sheetlike hydrogen bonding between cyclic D,L-peptides.[2] PAs with lipid tails have been shown to induce formation of stable triple-helices[7] and also nanofibers containing parallel *β*-sheets[5,6] and hydrophobic cores. Bolaamphiphiles, molecules containing two hydrophilic headgroups linked by a hydrophobic spacer, are receiving increased attention as building blocks for structures such as membranes, fibers, tubes, ribbons, and ropes.[8−12] Several groups have reported the self-assembly of unsymmetric bolaamphiphiles.[11,12] However, these molecules typically form lamellar or tubular structures in water, and in one instance they create monolayer rods.[12c] We report here the aqueous self-assembly of unsymmetric peptide bolaamphiphiles **1** and **2** (Figure 1) to give nanofibers with hydrophilic cores and surfaces, in our efforts to control the core chemistry within nanofibers.

Bolaamphiphiles **1** and **2** contain headgroups (L-glutamyl)$_3$glycine and tetraethylene glycol (EO_4) or succinyl-L-aspartic acid separated by a hydrophobic segment based on *β*-alanine (*β*-Ala), *p*-aminobenzoic acid (PABA), 6-aminohexanoic acid, and L-leucine. Control PA **3** is similar to **1** but lacks the second headgroup. These amphiphiles were synthesized by Fmoc solid phase peptide synthesis from commercially available amino acids with the exception of building blocks **6**, **9**, and **10**, which were prepared as outlined in Scheme 1. Dipeptide **6** is a semirigid *β*-peptide-like building block that combines the stiffness of PABA with the reactivity of the aliphatic amino group of *β*-alanine and can be readily prepared through the temporary silyl ester protection of PABA.

Self-assembly of the peptide amphiphiles was achieved by exposing 1 wt % solutions in 0.1 N KOH or NH_4OH to HCl vapors, resulting in the formation of translucent, birefringent, self-supporting gels. The peptides require a base to be soluble in water, and gelation occurs upon acidification to a pH of ca. 2.[13] Self-healing and self-supporting gels were formed from solutions of **1** as dilute as 0.5 wt %, whereas **2** and **3** gave weak gels at 0.5 wt %, and **3** partially precipitated. The solutions of **1**−**3** at a pH of 8 and their gels at a pH of 1 were analyzed by circular dichroism (CD) to show random structure in the solutions becoming *β*-sheet structure after gelation.[14] The solid-state FTIR amide I region of lyophylized 1 wt % gels showed predominately *β*-sheet character with peaks at 1634−1641 cm^{-1}, with possible random content at 1653−1662 cm^{-1}. The similarities between the amide I regions of **1** and **3** likely indicate the adoption of similar conformations.[14]

Self-assembled gels of 1 wt % were analyzed by transmission electron microscopy (TEM). The bright field image of a gel sample of **1** negatively stained with a 2 wt % aqueous solution of phosphotungstic acid shows one-dimensional objects that aggregate along their long axis.[14] Individual fiber components of these bundles are up to 5 nm in width, with lengths in excess of a micrometer. By preferentially staining the carboxylic acid groups of the (L-glutamyl)$_3$glycine with 2 wt % uranyl acetate, TEM shows that the outer edge of individual fibers has higher contrast than the interior (Figure 2a). This contrast is quantified by line profiles (Figure 2a, inset) taken normal to the long axis of individual fibers. Tilting experiments on the TEM samples support a fiber, not a flat ribbon, morphology. Negatively stained TEM samples of **2** and **3** also show fiber morphologies, with diameters of 5 nm and 6−8 nm, respectively, where **3** gives shorter fibers than **1** or **2**.[14] Positive staining of **2** with uranyl acetate for 1 h shows evidence for staining at the cores and the peripheries of the fibers (Figure 2b).

FTIR and CD data indicate *β*-sheet formation among peptide segments of the nanofibers. Because peptide segments can only be parallel to each other along the fiber axis, the fiber axis must lie within the plane of the *β*-sheets. Within these *β*-sheets, parallel alignment of molecules is promoted by designing two hydrogen-

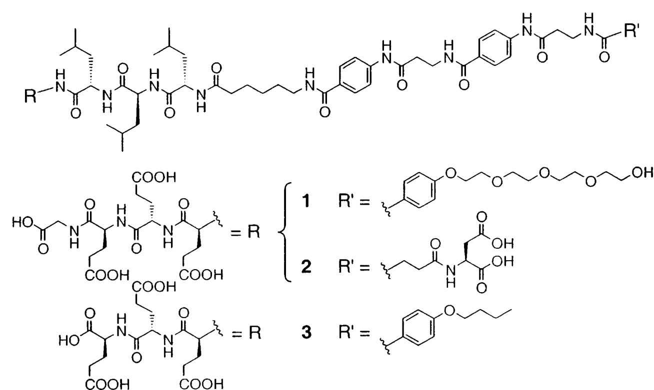
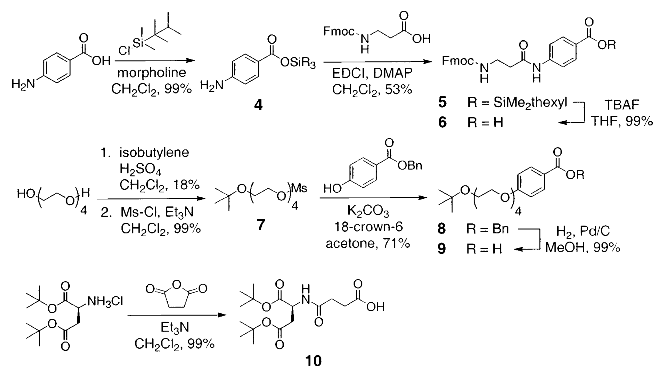


Figure 1. Structure of peptide bolaamphiphiles **1** and **2** and control PA **3**.

Scheme 1. Synthesis of Amphiphile Building Blocks **6**, **9**, and **10**



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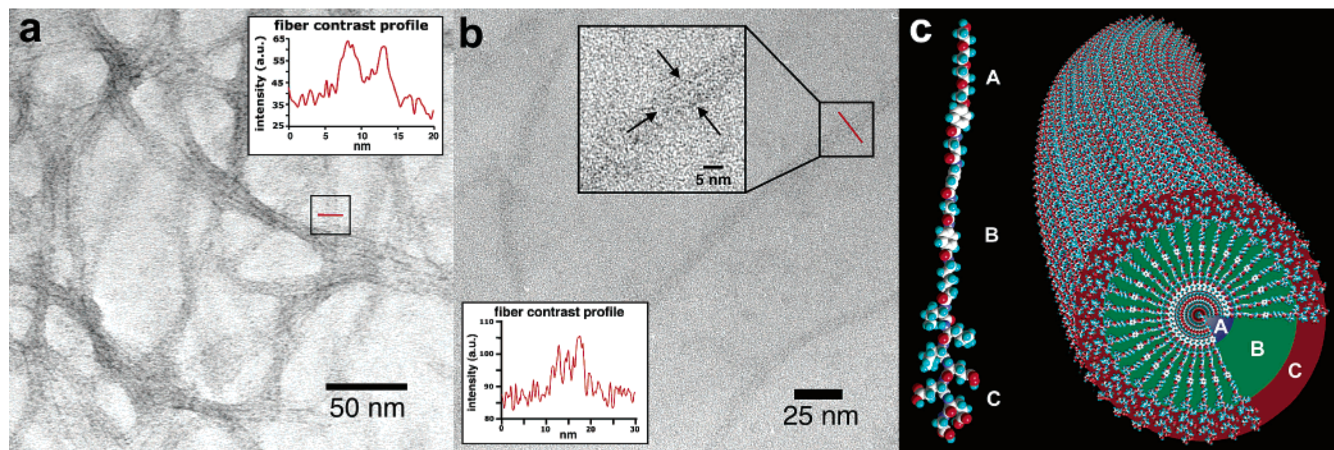

Figure 2. The TEM image of **1** positively stained with uranyl acetate (a) shows the preferential staining of fiber peripheries, with line profile inset, and the TEM image of **2** positively stained with uranyl acetate (b) shows staining at both cores and peripheries, with line profile and high magnification insets. The molecular graphics rendition of the cross section of the nanofibers of **1** (c) illustrates hydrophilic domains A and C separated by the hydrophobic section B.

bonding patterns into the hydrophobic midsection. These patterns lead to selective intermolecular hydrogen bonding of the α-amino acid regions and the β-Ala-PABA regions, resulting in parallel alignment of molecules along the length of the fiber. Even though the wedge shape of the molecule may favor nanofiber formation, we believe the driving force for self-assembly is β-sheet formation. In Figure 2c, we schematically show the proposed arrangement of amphiphiles viewed along the axis of the β-sheets. Additionally, this representation illustrates the fact that the hydrophilic peptide headgroup (L-glutamyl)$_3$glycine is at the outer periphery and the hydrophilic EO_4 segment of **1** (or the aspartic acid of **2**) is confined to the core of the fiber.

These results show the self-assembly of unsymmetric peptide bolaamphiphiles into cylindrical micelles that presumably bury one headgroup in their core and present the other at the surface. We believe this self-assembly is largely driven by the hydrogen-bonding patterns that lead to sheet formation along the axis of the fiber. Whereas we previously showed that the surface chemistry of the nanofiber can be varied by using hydrophilic peptide epitope sequences,[5] here we demonstrate peptide-based bolaamphiphiles self-assemble in water to form nanofibers with hydrophilic cores as well as hydrophilic surfaces. These nanofibers could be used as both bioactive structures as well as ion channels in biomedical applications. Further research on their functionality is currently in progress.

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Supporting Information Available: CD spectra for **1**−**3** in solution and as gelled. Solid-state FTIR amide I and II spectra for **1**−**3**. TEM images of negatively stained **1**−**3**. An additional molecular graphics image. Experimental procedures for compounds **1**−**10** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) The exact pH at which these gels form is difficult to determine given the gel nature of the materials.

(14) See Supporting Information.

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