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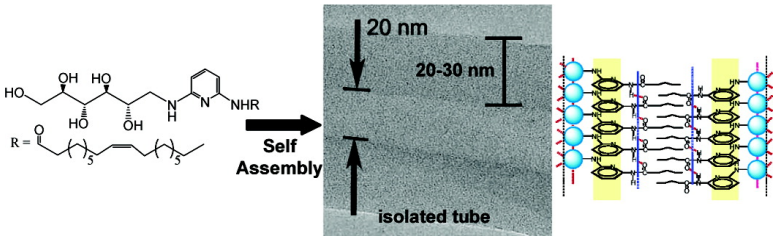

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Lipid-Based Nanotubes as Functional Architectures with Embedded Fluorescence and Recognition Capabilities

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Soft nanotubular structures represent a potentially powerful architecture generated through self-assembly of amphiphilic molecules.[1−5] Several classes of amphiphiles are known to provide these materials, including lipid-modified peptides[2], bola-amphiphiles[3], and sugar-lipid conjugates.[4] An example of the latter is the sugar-derivatized cardanols, which consist of a carbohydrate headgroup and an aliphatic alkyl chain connected through a phenyl moiety.[5a] Under optimal solution conditions, these alkylphenyl-glucopyranosides form fibrous aggregates and nanotubes upon dispersion in water, particularly when the alkyl chain is unsaturated leading to a bent structure that induces supramolecular chirality.[6] Nevertheless, despite the ability of a wide range of compounds to assemble into coiled fibers,[5,6] no clear design rules have been formulated. Such information is critical to advance applications in medicine,[7] chemical and biological sensing,[8] and sub-micro-total analysis system (sub-μ-TAS) designs.[9] For this reason, we embarked on a limited combinatorial strategy to synthesize lipid-based nanotubes. Specifically, by combining simple monosaccharides, fatty acids, and diamino-aromatic linkers, we generated a library of products ranging from fibers that lack structural regularity to highly uniform nanotubes. These results represent a systematic strategy to design functional lipid nanotubes with precise structural and functional features.

Three monosaccharides (β-D-glucose, β-D-galactose, and β-D-mannose), three C-18 fatty acids (stearic, oleic, and linoleic), and six diamino-aromatic linkers (2,3-; 2,5-; and 2,6-diaminopyridine (DAP) and 1,2-; 1,3-; and 1,4-diaminobenzene) were reacted (see Supporting Information) according to Figure 1a to give the monomer components used in this study. Of particular interest were compounds **1** and **2**, which formed fibrous assemblies upon dispersing in water following vortexing at 100 °C for 30 min, slow cooling to room temperature, and incubation for 12 h.

Visible and fluorescence microscopy showed bundles of self-assembled structures, with the latter showing fluorescence properties identical to those of free DAP (λ_{ex} = 263 nm; λ_{em} = 535 nm). TEM images after 4 h showed that the precipitates from **1** formed helical ribbon morphologies, which upon aging for an additional 12 h yielded nanotubes with an outer diameter of 60−80 nm and an inner diameter of ca. 20 nm (Figure 1b). DSC analysis of the nanotubes from **1** showed a gel-to-liquid crystalline-phase transition temperature (T_m) of 70 °C, which was far higher than the T_m of 42 °C for the unsaturated cardanyl glucoside system.[5a,b] Despite the relatively high T_m, the nanostructures from **1** were not highly crystalline. The lack of the double bond in the alkyl chain to give **2** had a significant effect on the morphology of the resulting nanostructure. Instead of nanotubes, the precipitates from **2** formed

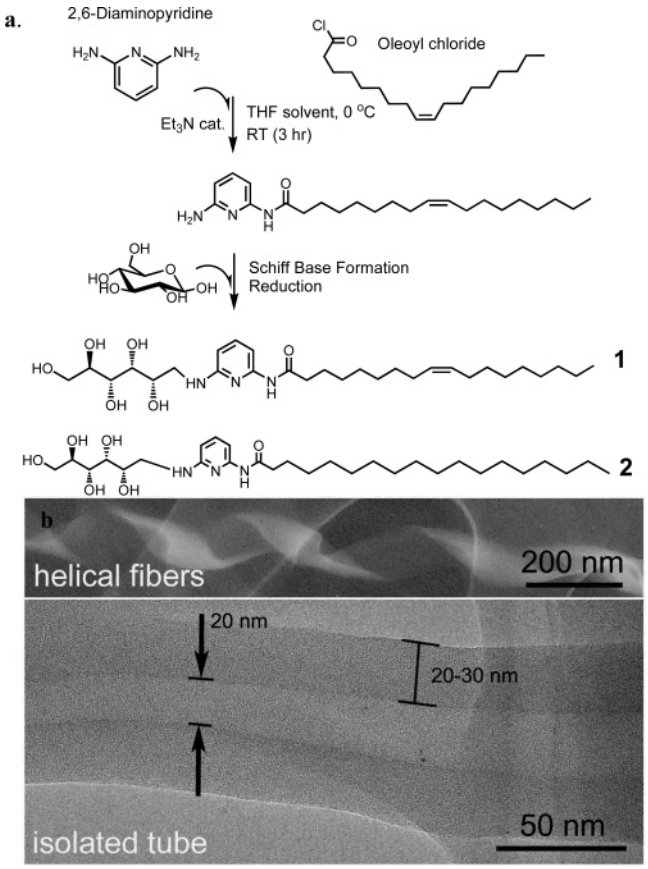

Figure 1. (a) Synthetic scheme for sugar-containing DAP alkylamides. (b) TEM images of self-assembled morphologies of **1** in water, helical coiled fibers, and nanotubes. The dimensions of the nanotubes were uniform and reproducible using 0.16 mM of **1** at pH 7.

fibrous structures with a thickness of ca. 80−150 nm. These fibers had a relatively high T_m of 90 °C, indicating more significant crystalline packing compared to the unsaturated systems from **1**. Hence, the unsaturated oleic acid moiety appears to be critical in nanotube formation.

To gain insight into the molecular orientation and packing profile within the assembled morphologies from **1** and **2** and to understand why the two similar monomers gave strikingly different self-assembled morphologies, we examined the wet and dry forms of the self-assembled nanofibers via small-angle X-ray scattering. The molecular length of the amphiphiles was calculated by CPK modeling on the basis of single-crystalline data of oleic acid,[10] and then X-ray diffraction patterns were obtained. The small-angle diffraction patterns of the nanotubes from **1** revealed ordered reflection peaks with a long period of 3.5 nm, which is substantially

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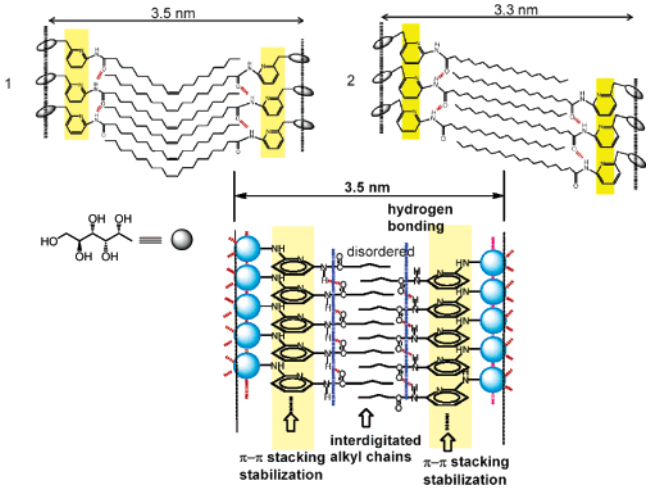

Figure 2. Proposed self-assembled nanostructures from **1** and **2**. The unsaturation in **1** results in a kink and a slightly less layered interdigitation. The planarity of DAP is shown (as a side view) in the bottom picture.

smaller than twice the extended molecular length of **1** (*d*-spacing of 3.03 nm by the CPK molecular modeling).

These results strongly suggest that the nanotubes from **1** form a bilayer structure with interdigitated alkyl chains associated through hydrophobic interactions (Figure 2). Moreover, according to powder X-ray diffraction analysis (see Supporting Information), the glucopyranoside moieties of the bilayer participate in strong intermolecular hydrogen bonding, which results in a highly ordered chiral packing structure. This combination of hydrophobic and hydrogen bonding interactions appears to favor the formation of the nanotubular structure. Conversely, the diffraction pattern of the nanofibers from **2** (molecular length of 3.2 nm) indicated a shorter *d*-spacing of 3.3 nm, which translates into a greater degree of interdigitation of the bilayer structure (Figure 2). The "kink" in self-assembled structures from **1** appears to reduce the crystallinity of the nanostructure, enabling more facile formation of a nanotube. Further differences between nanostructures from **1** and **2** were revealed by FT-IR spectroscopy. The C−H stretching peaks were slightly shifted between **1** and **2** (2854 vs 2850 cm^{-1}, respectively) and the C═O (amide I) stretching patterns were shifted (1651 and 1654 cm^{-1}, respectively), which are consistent with a more crystalline structure for the nanofibers derived from **2**.[5a,c]

Aminopyridine derivatives are known to function as artificial receptors that can bind various ligands through complementary multipoint H-bonding.[11] Hence, we reasoned that the DAP residue could serve as a functional recognition element, and in the process, its intrinsic fluorescence would be affected by selective interaction with external ligands. To test this hypothesis, we added water-soluble compounds that can undergo H-bonding to the nanotube from **1**. Addition of up to 10 mM thymidine caused the nearly immediate quenching of fluorescence (Figure 3a), with an apparent binding constant of $\sim 2.5 \times 10^3$ M^{-1}. In addition, thymidine analogues such as uracil and the anticancer compounds 5-fluorouracil and its prodrug derivative Tegafur also quenched fluorescence. The fluorescence quenching was selective for nucleosides; β-D-glucose and urea, while capable of undergoing extensive H-bonding, did not quench the fluorescence of the nanotubes from **1**, even at concentrations as high as 16 mM (100 fold higher than the nanotube concentration, based on the concentration of 1). These results suggest that the interaction between the nanotubes and thymidine may occur through a three-point hydrogen bonded network[11] (Figure 3b). Consistent with this hypothesis, adenosine, which cannot bind in this three-point manner, was at least 2-fold

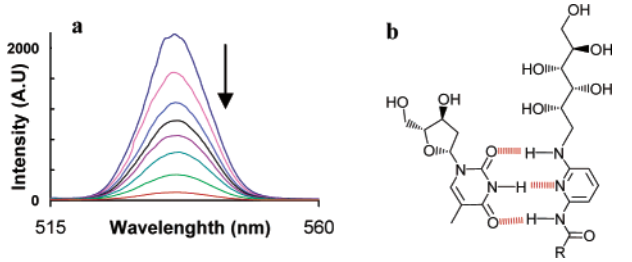

Figure 3. (a) Fluorescence quenching of DAP nanotubes by thymidine (0.16 mM nanotube based on concentration of **1**). The thymidine concentrations ranged from (top to bottom) 0 to 10 mM, emission $\lambda_{max} = 535$ nm. (b) Schematic representation of the possible interaction between nanotubes of **1** and thymidine.

less effective in quenching fluorescence. The interaction of the nanotubes from **1** with thymidine was extended to polyT (M_w 8 kDa; up to 0.16 mM and giving an apparent binding constant of 1.7×10^5 M^{-1}), which quenched fluorescence similar to that of thymidine itself.

On the basis of these results, the structural requirements for self-assembly of amphiphilic monomers into highly organized nanotubes have begun to be elucidated. These include the combination of strongly hydrophobic and hydrophilic moieties, hydrogen bonding interactions of hydrophilic groups that are favored in sugars with equatorial anomers at the 2 and 4 positions of a pyranose ring, and potentially a linker with suitable planarity. In addition, substantial bending of the monomers is required, which arises from the *meta* orientation of the linker, along with unsaturation of the alkyl chain. This information can be used to design single-chain amphiphiles that form high-axial-ratio nanostructures starting from simple molecules, which also contain molecular recognition groups that can be used to monitor the chemical selectivity of supramolecular aggregates toward guest binding.

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Supporting Information Available: Synthesis of a set of amphiphiles and the self-assembly procedure, optical microscopy images of the fluorescent nanotubes, and TEM images of nanotubes and fibers. This material is available free of charge via the Internet at http://pubs.acs.org.

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