Self-Assembled Soft Nanomaterials from Renewable Resources

George John

Department of Chemistry
City College of the City University of New York
Convent Avenue at 138th Street, NY 10031
john@sci.ccny.cuny.edu

ABSTRACT

A set of amphiphilic glycolipids were synthesized from cardanol (a by-product of cashew industry) and diaminopyridine (DAP). These amphiphiles encompass self-assembling units such as long hydrophobic saturated or unsaturated chain, open or closed sugar as headgroup and aromatic (phenyl or DAP) as linker. Amphiphiles from both series (cardanyl and DAP) exhibited excellent self-assembling properties to produce various lipid based materials ranging from structurally unordered fibers to highly uniform nanotubes. Their self-assembling properties were investigated by various techniques including EF-TEM, SEM, XRD and DSC. The nanotubes are comprised of bilayer structure with interdigitated alkyl chains associated through hydrophobic interactions, hydrogen bonding and π–π stacking. The self-assembling behavior of cardanyl glucosides and the synthetic analogues from diaminopyridine were compared. The tubes derived from DAP amphiphiles contain accessible 2,6-diaminopyridine linker that can interact with thymidine and related nucleosides through multipoint hydrogen bonding, thereby quenching the intrinsic fluorescence of the aromatic linker. These results clearly showed that efficient molecular design, and synthesis of novel amphiphiles from renewable resources will lead to supramolecular nanostructures and nanomaterials, otherwise under-utilised.

***Keywords*:** organic soft materials, amphiphiles, self-assembly, lipid nanotube, renewable resources.

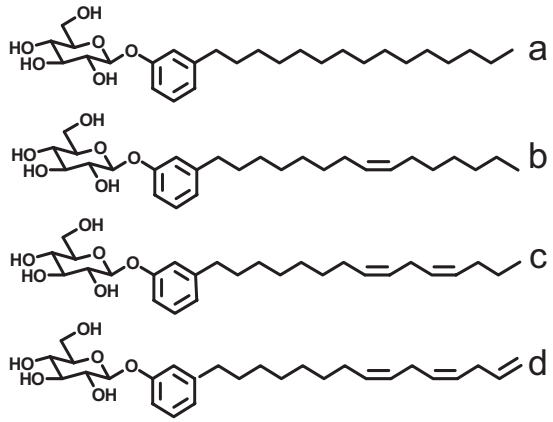
1 INTRODUCTION

The self-assembly of low molecular weight building blocks into nanoscale molecular objects has recently attracted considerable interest in terms of the bottom-up fabrication of nanomaterials [1]. Soft nanotubular structures represent a potentially powerful architecture generated through self-assembly of amphiphilic molecules [2-6]. Several classes of amphiphiles are known to provide these materials including lipid-modified peptides [3], bolaamphiphiles [4], and sugar-lipid conjugates [5]. The building blocks currently used in supramolecular chemistry are synthesized mainly from petroleum-based starting materials. However, bio-based organic synthesis presents distinct advantages for the generation of new building blocks since they are obtainable from renewable resources. An example of the latter is the sugar-derivatized cardanols [6], which consist of a carbohydrate head group and an aliphatic alkyl chain connected through a phenyl moiety [6a]. Under optimal solution conditions, these alkylphenylgluco-pyranosides 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 [7]. Despite the ability of a wide range of compounds to assemble into coiled fibers [6, 7], no clear design rules have been formulated. Such information is critical to advance applications in medicine [8], chemical and biological sensing [9], and sub-micro-Total Analysis System (sub-μ-TAS) designs [10]. Here we report the facile nanotube preparation from cardanol based glycolipids, easily available from plant crop-derived resources, and further the design, synthesis and utility of a fully synthetic analogue from diaminopyridine (DAP) derivatives. Specifically, by combining simple monosaccharides, fatty acids, and diamino-aromatic linkers, we generated an array of morphologies ranging from fibers that lack structural regularity to highly uniform nanotubes.

2 EXPERIMENTS

2.1 Cardanyl Glucoside (1 and 2)

Cardanol (a mixture of long chain phenol differing in the degree of unsaturation in the side chain) was obtainable by double vacuum distillation of cashew nut shell liquid (CNSL) [11]. Cardanyl glucosides **1** and **2** were synthesized in two steps from penta-O-acetyl β-D-glucose and the corresponding phenol as reported earlier [6]. The glycosidic bond was formed in a Lewis acid-catalysed (borontrifluoride etherate) reaction at room temperature to give the β-product exclusively.



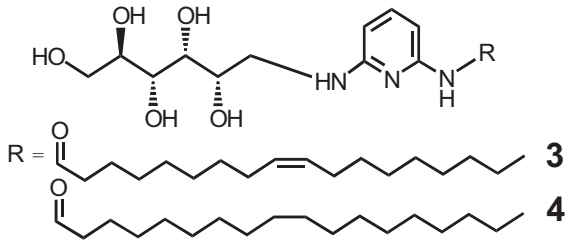
1: a (5%)+b (50%)+c (16%)+d (29%) **2:** a (100%)

Cardanyl glucosides

The acetylated β-glucopyranosides were purified by recrystallization from ethanol and deprotected quantitatively using trimethylamine in aqueous methanol. The crude products were purified by silica-gel column chromatography and recrystallization in methanol afforded a cardanyl glucoside **1** and its saturated homologue **2**. The self-assembled fibrous structure was prepared by dissolving 1-5 mg in 50-100 mL of water at boiling temperature. The clear solution obtained was cooled to room temperature at ambient conditions. The helical fibers obtained from compound **1** were aged to several days at ambient conditions yielded tubular structures.

2.2 Diaminopyridine (DAP) Amphiphiles (3 and 4)

1,3-diaminopyridine was coupled with β-D-glucose by reductive amination and further purification procedures afforded monomer components used in this study [12]. Of particular interest were compounds **3** and **4**, 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.



DAP amphiphiles

3 RESULTS AND DISCUSSION

The cardanyl glucoside mixture **1** self-assembled into characteristic helically-coiled ribbons after 12-24 h incubation [Fig. 1(a)], whereas the saturated analogue **2** gave a helically-twisted morphology [Fig. 1(c)]. Thus, we found that the difference in degree of unsaturation on the hydrophobic long chain phenols could generate variety of morphologies on self-assembly. This might be one of the first examples of morphological control of helical nanofibers by unsaturation of the hydrophobic segment in a single tail amphiphile. Interestingly, coiled ribbons formed from **1** were gradually converted into a tubular structure over several days, but the twisted ribbon remains intact even after many months. High resolution transmission microscopy (TEM) revealed that the obtained lipid nanotubes have uniform inner diameters of 10-15 nm, outer diameters of 50-60 nm, and extended lengths of 10-1000μm [Fig 1(b)] and the aspect ratio is ~1000. Nevertheless the cardanyl glucosides produced intriguing morphologies from self-assembly in water; those materials lack the ability of efficient binding of target molecules. This prompted us to design and synthesis of DAP amphiphiles capable of generating functional nanostructures, utilizing the chemistry of cardanyl glucosides.

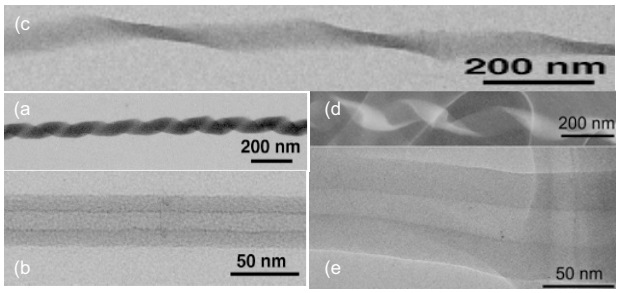


Figure 1. TEM images of (a) an individual helically coiled nanofiber from cardanyl glucoside mixtures, **1** (b) an individual nanotube from cardanyl glucoside mixtures, **1**. (c) a helically twisted nanofiber from saturated glucoside, **2,** and TEM images of self-assembled morphologies of DAP lipids, **3** in water, (d) helical coiled fibers and (e) nanotubes. The dimensions of the nanotubes were uniform and reproducible.

The synthesis of DAP derivatives was accomplished by combining simple monosaccharides, fatty acids, and diamino-aromatic linkers, which on self-assembly in water generated an array of morphologies ranging from fibers that lack structural regularity to highly uniform nanotubes. Visible and fluorescence microscopy showed bundles of self-assembled structures, with the latter showing identical fluorescence properties as free DAP (λ_{ex}=263 nm; λ_{em}= 535 nm). TEM images after 4 h showed that the precipitates from **3** 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 (Fig. 1d, e). DSC analysis of the nanotubes from **3** 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 [6a,b]. Despite the relatively high T_m; the nanostructures from **3** were not highly crystalline. The lack of the double bond in the alkyl chain to give **4** had a significant effect on the morphology of the resulting nanostructure. Instead of nanotubes, the precipitates from **4** formed 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 **3**. 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 **3** and **4**, 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 modelling on the basis of single crystalline data of oleic acid [13], and then X-ray diffraction patterns were obtained. The small-angle diffraction patterns of the nanotubes from **3** revealed ordered reflection peaks with a long period of 3.5 nm, which is substantially smaller than twice the extended molecular length of **3** (*d*-spacing of 3.03 nm by the CPK molecular modelling).

These results strongly suggest that the nanotubes from **3** (nanotube from **1** also adopts similar morphology) form a bilayer structure with interdigitated alkyl chains associated through hydrophobic interactions (Fig. 2). Moreover, according to powder X-ray diffraction analysis, the

glucopyranoside moieties of the bilayer participate in strong intermolecular hydrogen bonding, which results in a highly ordered chiral packing structure.

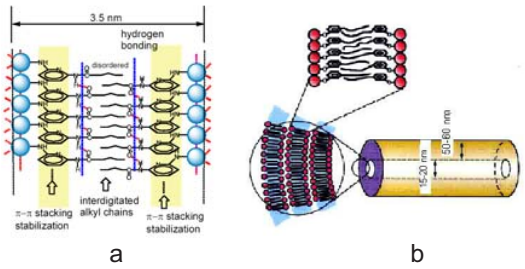


Figure 2. Shematic representation of (a) DAP, **3** solid bilayers (b) molecular packing within the lipid nanotube.

This combination of hydrophobic and hydrogen bonding interactions appears to favour the formation of the nanotubular structure. Conversely, the diffraction pattern of the nanofibers from **4** (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. The "kink" in self-assembled structures from **3** appears to reduce the crystallinity of the nanostructure enabling more facile formation of a nanotube.

Aminopyridine derivatives are known to function as artificial receptors that can bind various ligands through complementary multipoint H-bonding [14]. 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 **3**. Addition of up to 10 mM thymidine caused the nearly immediate quenching of fluorescence (Fig. 3a), with an apparent binding constant of ~ 2.5 x 10^3 M^{-1}. In addition, thymidine analogs such as uracil and the anticancer compounds 5-fluorouracil also quenched fluorescence.

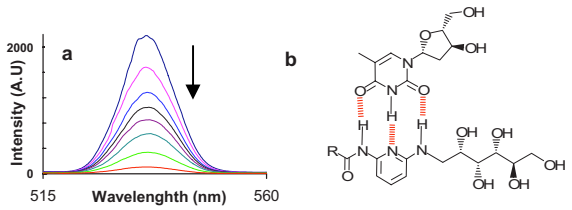


Figure 3. (a) Fluorescence quenching of DAP nanotubes by thymidine. The thymidine concentrations ranged from (top to bottom) 0 to 10 μM, emission (λmax = 535 nm), (b) schematic representation of the possible interaction between nanotubes of **3** and thymidine.

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 **3**, even at concentrations as high as 16 mM (100 fold excess the nanotube concentration, based on the concentration of **3**). These results suggest that the interaction between the nanotubes and thymidine might occur through a three-point hydrogen bonded network [14] (Fig. 3b).

These and above results showed that the structural requirements for self-assembly of amphiphilic monomers into highly organized and functional nanotubes have begun to be elucidated. These include the combination of strongly hydrophobic and hydrophilic moieties, a linker with suitable planarity, and hydrogen bonding interactions of hydrophilic groups that are favoured in sugars. 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 contains molecular recognition groups that can be used to monitor the chemical selectivity of supramolecular aggregates towards guest binding, and for bionanocomposites. Taking together, present study clearly demonstrates the utility of plant/crop-based resources and their industrial by-products as an alternate feedstock for existing and new chemicals, detergents and functional soft materials.

4 ACKNOWLEDGEMENTS

Thanks are due to Dr. T. Shimizu, NARC, AIST, Japan and Prof. J. S. Dordick, Dept. of Chemical Engineering, RPI, Troy, NY for their support and encouragements.

REFERENCES

[1] G. M. Whitesides, J. P. Mathias and C. T. Seto, *Science* (254), 1312, 1991.

[2] (a) N. Nakashima, S. Asakuma and T. Kunitake, *J. Am. Chem. Soc.* (107), 509, 1985. (b) P. Yager and P. E. Schoen, *Mol. Cryst. Liq. Cryst.* (106), 371, 1984. (c) B. N. Thomas, C. R. Safinya, R. J. Plano and N. A. Clark, *Science* (267), 1635, 1995.

[3] (a) J. M. Schnur, *Science* (262), 1669, 1993. (b) T. Kunitake, *Angew.Chem. Int. Ed. Engl*. (31), 709, 1992.

[4] (a) J. -H. Fuhrhop and W. Helfrich, *Chem. Rev*. (93), 1565, 1993. (b) T. Shimizu, M. Kogiso and M. Masuda, *Nature* (383), 487, 1996.

[5] (a) D. F. O'Brien, *J. Am. Chem. Soc*. (116), 10057, 1994. (b) H. Engelkamp, S. Middlebeck and R. J. M. Nolte, *Science* (284), 785, 1999.

[6] (a) G. John, M. Masuda, Y. Okada, K. Yase and T. Shimizu, *Adv. Mater*. (13), 715, 2001. (b) G. John, J. H. Jung, H. Minamikawa, K. Yoshida and T. Shimizu, *Chem. Eur. J.* (8), 5494, 2002.

[7] (a) J. V. Selinger, M. S. Spector and J. M. Schnur, *J. Phys. Chem. B*, (105), 7157, 2001. (b) D. Berthier, T. Buffeteau, J.-M. Leger, R.Oda and I. Huc, *J. Am. Chem. Soc*. (124), 13486, 2002.

[8] X. Guo and F. C. Szoka Jr., *Acc. Chem. Res*. (36), 335, 2003.

[9] B. E. Rothenberg, B. K. Hayes, D. Toomre, A. E. Manzi and A. Varki, *Proc. Natl. Acad. Sci. USA*, (90), 11939, 1993.

[10] T. Vilkner, D. Janasek and A. Manz, *Anal. Chem*.(76),3373, 2004.

[11] J. H. P. Tyman, *Chem. Soc. Rev*. (8), 499, 1979.

[12] G. John, M. Mason, J. S. Dordick and P. M. Ajayan, *J. Am. Chem. Soc*.(126), 15012, 2004.

[13] J. Ernst, W. S. Sheldrick and J. –H. Fuhrhop, *Naturforsch*. (34b), 706, 1979.

[14] R. Shenhar and V.M. Rotello, *Acc. Chem. Res.* (36), 549, 2003.