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Microtubes self-assembled from a cholesterol-modified nucleoside

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We describe the formation of lipid microtubes from a novel cholesterol-modified nucleoside in binary mixture with phospholipids. Stable cylindrical structures with an outer diameter of 2–3 μm and a length of 20–40 μm were formed. By varying the preparation conditions, thinner tubules with nanometre-scale diameters could also be obtained.

Self-assembled supramolecular structures formed from amphiphilic molecules are of fundamental interest with regard to self-organization processes. Further, these membrane -based structures offer a large potential for technological applications in a variety of fields from electronics to biomedicine.[1] Several different amphiphiles can self-assemble into micro- or nanotubes in aqueous environments.[2] Most of the theoretical models describing the mechanism of tubule formation are based on chiral elastic properties of membranes : the basic idea is that a tilt of the molecules induces a twist of the membrane that leads to the growth of helical and tubular structures.[3] In particular, cholesterol and other natural sterols (also chiral molecules) have been reported to form helical ribbons and microtubes in multicomponent mixtures with surfactants and phospholipids.[4] These ribbons and tubules are single crystals that represent metastable intermediates in a crystallization pathway proceeding from filaments to plate-like crystals.[4] Nucleobase -derived amphiphiles can also form helical and tubular structures, as described for phospholipid –nucleoside conjugates[5] as well as for different single- and double-chain amphiphilic nucleobase derivatives prepared either via covalent modification[6] or by electrostatic complexation.[7] The formation of these supramolecular structures is attributed to the combination of the aggregation properties of the lipid part (hydrophobic effect) with the hydrogen bonding and π–π stacking of the nucleobase moieties.[8]
Here we report on the self-assembly of microtubes from mixtures of simple synthetic phospholipids with the novel lipophilic nucleoside **1** (see Scheme 1). This new hybrid molecule, with cholesterol as hydrophobic moiety, combines several of the above-mentioned properties giving rise to the formation of supramolecular structures. Moreover, through the incorporation of a uridine moiety this compound offers a molecular recognition function, which could be exploited in nanotechnological applications.[9] The incorporation of cholesterol-modified nucleosides and nucleotides into phospholipid bilayers, as well as their applications as building blocks for nanostructured materials, have been previously described.[10] Vesicle formation has been shown for several cholesteryl derivatives of antiviral nucleoside analogues, e.g. cholesteryl-succinyl acyclovir.[11] To the best of our knowledge, however, the present work is the first report on the self-assembly of microtubes from a cholesteryl-nucleoside .

The lipophilic nucleoside 2′-N-(2-(cholesteryl)-succinyl)-2′-deoxy-2′-aminouridine, **1**, was obtained by attaching a cholesterol moiety to the 2′-position of the nucleoside 2′-deoxy-2′-aminouridine (see ESI†).12 In binary mixtures of **1** and the unsaturated phospholipid dioleoylphosphatidylcholine (DOPC), we observed the formation of microtubes after rehydration of dry lipid films in aqueous solution, followed by heating the samples to 70 °C and cooling them slowly to room temperature. In confocal fluorescence microscopy images (Fig. 1a) the tubes appear as open-ended cylindrical structures with outer diameters between 2–3 μm and lengths between 20–40 μm. Initially a significant

amount of vesicles was found after tube formation ; more homogeneous tube preparations were obtained by addition of small amounts of the nonionic surfactant Triton X-100 after the cooling step. This eliminates most of the vesicles , while the tubes withstand the treatment and can be collected by centrifugation . Different molar ratios of **1** and DOPC were tested, and we found tube formation in mixtures ranging between 20 and 50 mol% of **1** with maximum yields for 30 : 70 mol% **1** : DOPC. Below 20 mol% of **1** only vesicular structures were observed by light microscopy , whereas above 50 mol% of **1** the lipid films could not be dispersed in aqueous solvent . At 20 : 80 mol% **1** : DOPC most of the material formed vesicles , but some tubes could be found; at 30 : 70, 40 : 60 and 50 : 50 mol% **1** : DOPC a second and increasingly large population of thinner tubules with diameters below 1 μm appeared along with vesicles and thicker tubes (Fig. 1b and 2).

Interestingly, the formation and morphology of the tubes shows also a strong dependence on temperature. For samples of equal composition, namely 30 : 70 mol% **1** : DOPC, the temperature during the film hydration process was varied between 20 and 90 °C. When the samples were kept below 60 °C, a heterogeneous mixture of uni- and multilamellar vesicles , tethers and short, straight filament-like structures was observed by optical microscopy (see ESI†). In samples hydrated between 60 and 75 °C, mostly tubes with micrometre-scale diameters were formed upon cooling; however, in samples heated to temperatures exceeding 75 °C only elongated tubules with nanometre-scale diameters were found (Fig. 1b). For a given temperature, longer incubation times before the cooling step also led to longer and thinner structures (not shown). Therefore, a certain degree of control over the final morphology of the tubes can be achieved by varying the composition of the initial lipid films and the conditions of preparation. The temperature dependence of the tube diameter has been reported before for tubular structures formed from 12-hydroxystearic acid.[13] In contrast to that system, however, the transition from larger to smaller diameters of the **1** : DOPC tubes is not reversed upon decreasing the temperature. It should also be noted that the main gel-to-liquid transition of DOPC takes place at temperatures well below 0 °C;[13] therefore, the mechanism of tube formation cannot be related to the phase transition of the phospholipid , as is the case for diacetylenic phosphocholines and several other tube-forming lipids.[4,14] Another difference to previously described cholesterol-based systems is the quick formation and the long-term stability of the tubules made from **1** and DOPC. Once formed, the **1** : DOPC microtubes are stable at least for six months. In contrast, cholesterol-based tubes are transient intermediates that take several days to form and then convert into non-tubular crystalline structures over a short time.[4]

The surface structure and the morphology of the tubules were studied using electron microscopy . A scanning electron microscopy (SEM) image of a microtubule (Fig. 2) reveals a straight structure with an almost uniform outer diameter of about 300 nm. One end of the tubule appears somewhat thicker due to several extra layers of material rolled on. A second, much thinner tubule can be seen sticking out of this open end, also showing a multilayered structure. Negative staining transmission electron microscopy images (TEM , ESI†) revealed tubular structures filled with uranyl acetate. These observations indicate that the thin tubules withstand air drying, are multilayered and have open ends.

We also performed high resolution ^{1}H NMR experiments to measure the chemical composition of both micrometre- and nanometre-diameter tubes formed from 30 : 70 mol% **1** : DOPC mixtures at 70 and 80 °C, respectively (see ESI†). From the quantification of the ^{1}H NMR spectra , the estimated content of compound **1** was 80 ± 5 and 92 ± 4 mol% for the micrometre- and nanometre-diameter tubes, respectively. These values indicate a significant difference in the composition of both kinds of tubules. Even more surprising is the strong deviation from the initial content of 30 mol% of **1**. Together with our previous observations for mixtures of different compositions, this result suggests that the tubes may form only above a specific ratio of the two components. High molar fractions of **1** may be required to induce a molecular tilt that causes the membranes to twist and convert into tubular structures. The rather high temperature would be necessary for the solubilization of **1**, allowing its progressive incorporation into the DOPC membranes and the resulting self-assembly of micrometre-diameter tubes. Higher temperatures and/or longer incubation times might then increase the amount of incorporated cholesteryl-uridine, leading to the formation of nanometre-diameter tubes.

Phospholipid -containing structures show a tendency to orient in external magnetic fields. This effect was also observed for the **1** : DOPC microtubes, and is due to the anisotropic magnetic susceptibility tensor of the lipid tubules.[15] The ^{31}P NMR spectrum (see ESI†) of the tubes shows spectral intensity almost entirely at −13.4 ppm, which corresponds to those phospholipid molecules that are oriented with their long axis perpendicular to the magnetic field, whereas vesicles made of the same mixture show the typical powder spectrum . This could also be confirmed by ^{2}H NMR spectra of lipid

microtubes that contained deuterated POPC-d_{31} (see ESI†). The order parameter of the lipid chains calculated from ^{2}H NMR spectra showed no significant differences, suggesting similar lipid packing in a microtube sample and in multilamellar vesicles prepared from the same material (see ESI†). Interestingly, the quadrupolar splittings of ^{2}H NMR spectra of the microtubes are characteristic for a rather unperturbed lamellar liquid-crystalline lipid phase. It is well known that cholesterol has a pronounced condensation effect, which is, however, strongly attenuated for cholesterol analogues.[16] This indicates that the linkage of the nucleoside to the cholesterol moiety probably results in a significant alteration of the membrane properties of the sterol .

Based on the data gathered so far, we propose that the DOPC in the microtubes is in liquid-crystalline state and that the tubules are assemblies of several layers of cholesteryl-uridine **1** and DOPC. These structures may qualify for various biological applications, as they consist of biocompatible molecules. In addition, the molecule that induces microtube formation contains a molecular recognition function that can be utilized for nanotechnological engineering based on DNA base pairing and host –guest chemistry. Additional experiments are underway to further clarify the nature and properties of these microtubes, as well as their potential for functionalization and future use in biotechnological applications.

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Figures

Scheme 1 Chemical structure of 2′-*N*-(2-(cholesteryl)-succinyl)-2′-deoxy-2′-aminouridine **1**.

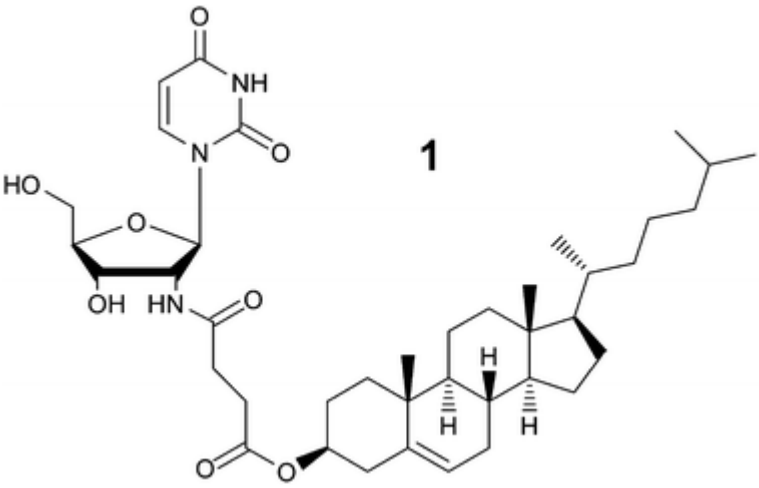

Figure 1. Fluorescence (top) and transmission microscopy images (bottom) of microtubes self-assembled from a 30 : 70 mol% **1** : DOPC mixture (labeled with 0.5 mol% NBD-phosphatidylethanolamine) heated to (a) 70 °C or (b) 80 °C.

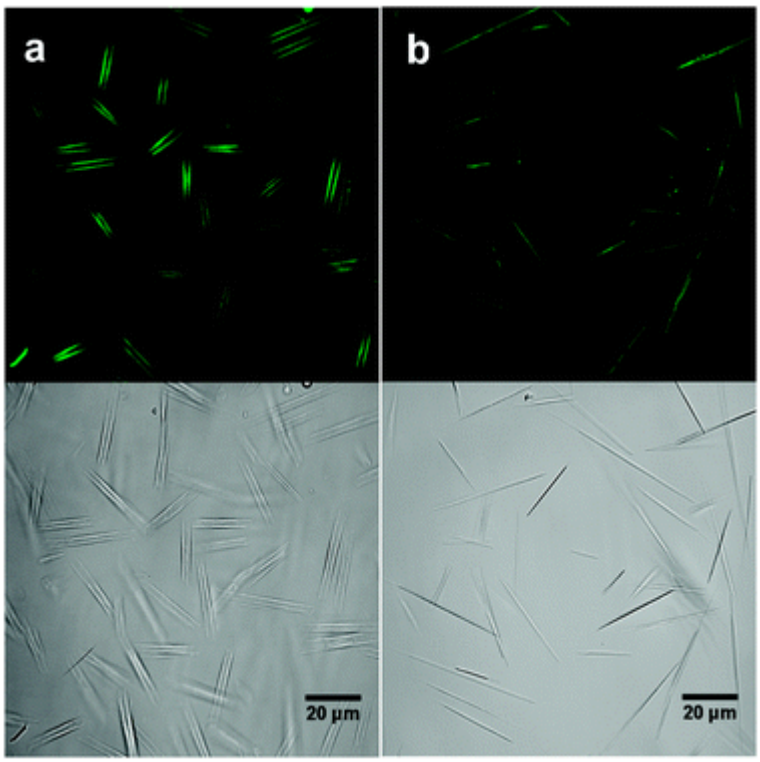

Figure 2. SEM image of a ~300 nm diameter microtubule with a thinner tubule sticking out of the open end (scale bar = 100 nm). The insert shows a lower magnification of the microtubule (scale bar = 300 nm).