Liquid Crystallinity and Biological Activity of a Novel Amphiphilic Compound

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We have designed an amphiphilic compound in which phenylpyrimidine and D-glucamine are connected via a flexible spacer. The compound shows two thermotropic lamellar bilayer mesophases with different layer spacings. The liquid-crystalline compound showed cytostatic activity against A549 human lung carcinoma cell lines, however an amphiphilic phenylpyrimidine derivative, D-glucamine, and an equimolar mixture of them were not active on cell lines under identical conditions.

Biological structure has links with liquid crystallinity.[1] Cell membranes are lamellar bilayer mesophases of phospholipids, glycolipids, and cholesterol. Liquid-crystalline materials find a new order in biomedical applications.[2] Some lyotropic liquid-crystalline materials that have structural affinity with cell membranes have been applied to develop novel drug delivery systems. Recently, Tang, Du, et al. developed a novel polyethylene glycol-phosphatidylethanolamine-based nanocarrier of doxorubicin that increased cytotoxicity in vitro and enhanced anticancer activity in vivo with low systematic toxicity.[3] The results support the authors' inference that 10-nm to 20-nm nanoassemblies of phospholipids containing doxorubicin would improve the drug's penetration, accumulation, and anticancer activity.

Many amphiphilic polyhydroxy derivatives have been prepared and their physical properties investigated.[1,4–11] A relationship between liquid crystallinity and immunosuppressive ability of some amphiphilic compounds has been reported.[12] However, to our knowledge, the actions of liquid-crystalline compounds against human cancer cells have never been reported. We have investigated liquid crystal oligomers with a hierarchical structure,[13] and recently, we found a lamellar to lamellar phase transition of an amphiphilic liquid crystal.[14] We surmise that a liquid-crystalline molecule that reorganizes its lamellar bilayer structure according to the circumstances can penetrate layers of tumor cells. For this study, we designed an amphiphilic compound **1** in which phenylpyrimidine and D-glucamine are connected via a flexible methylene spacer (Figure 1). We report here its liquid crystallinity and anticancer activity.

Compound **1** showed the following phase sequence on the first heating: Cry-136 °C (56 kJ mol^{-1})-unidentified smectic X-141 °C (5.3 kJ mol^{-1})-smectic A-194 °C (1.2 kJ mol^{-1})-isotropic liquid. On cooling the smectic A changed to the smecic X at 135 °C, which was accompanied by appearance of a fine *Schlieren* texture in the homeotropically aligned sample as shown in Figure 2. This optical microscopic observation revealed that the unidentified smectic X phase is a tilted smectic phase. The relatively large enthalpy change of the SmA to SmX transition indicates that the SmX phase is a higher ordered smectic phase. It should be noticed that appearance of a tilted smectic phase is not usual in amphiphilic liquid crystals with a polyhydroxy unit, except in a few cases where some amphiphilic propane-1,2-diol derivatives show the tilted smectic phase.[4,15]

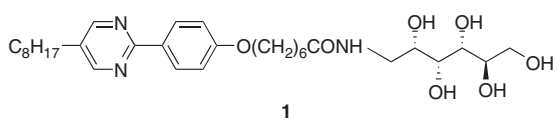

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Figure 1. Molecular structure of compound **1**.

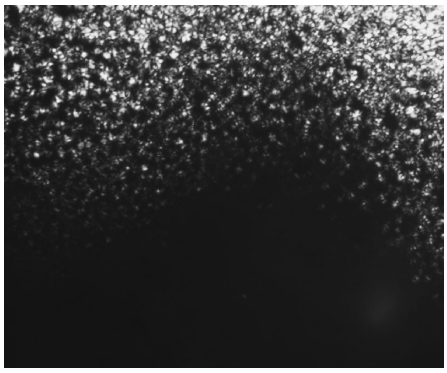

Figure 2. Photomicrograph of the SmA to SmX phase transition of a homeotropically aligned sample of compound **1**.

The layer spacing obtained by X-ray diffraction studies increased from 43 to 49 Å with decreasing temperature in the smectic A phase, although it was not dependent on the temperature in the smectic X phase and the layer spacing was 55 Å.[16] The molecular length was estimated to be about 37 Å for the elongated structure using the MM2 model. The longer layer spacings in the SmA and SmX phases than the molecular length suggest that both smectic phases have an interdigitated structure. Figure 3 portrays molecular organization models for the uniaxial smectic A phase and the tilted smectic X phase. We assumed intermolecular interactions between phenylpyrimidine units for the smectic A phase (Figure 3a) and those between polyhydroxy units for the smectic X phase (Figure 3b).

Competition between those intermolecular interactions allows compound **1** to exhibit a tilted phase and to reorganize from lamellar bilayer assemblies with hydrophilic parts in the outer shell of each layer to those with hydrophobic units in the outer shell.

Then we investigated whether compound **1** inhibits proliferation of A549 human lung cancer cells. The A549 cells (4×10^3 cells/mL) were placed at 1 mL per well in 24-well

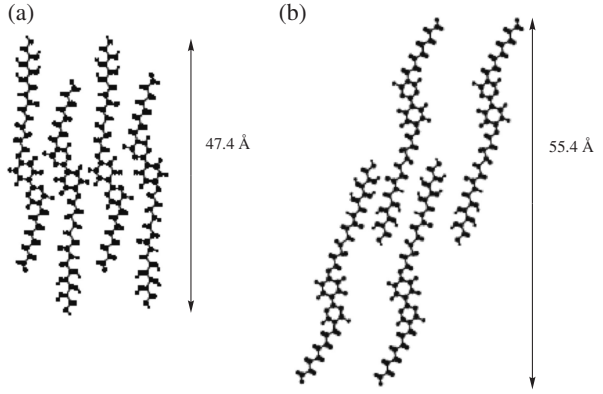

Figure 3. Molecular organization model for the SmA phase (a) and for the SmX phase (b).

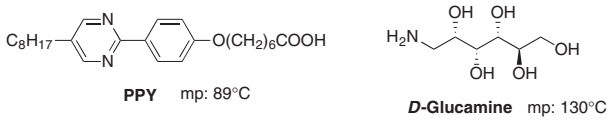

Figure 4. Molecular structures and melting temperatures of the phenylpyrimidine derivative (PPY) and D-glucamine.

plates at 37 °C in a humidified atmosphere with 5% CO_2 for 24 h. The cells were then exposed to various concentrations of compound **1** at 37 °C for 96 h. Cells attached to the plates were released by trypsinization and counted using a Coulter counter. Cell viability was expressed as a percentage relative to solvent (DMSO)-treated control incubations. We also investigated the anticancer activity of the phenylpyrimidine derivative (PPY), D-glucamine, and their equimolar mixture under identical conditions. The phenylpyrimidine derivative and D-glucamine showed no liquid crystallinity (Figure 4). The results are shown in Table 1.[16]

Compound **1** showed marked inhibition of cell proliferation at higher concentrations than 10 μM, although the precursors and the mixture showed no such pronounced inhibition.

Effects of amphiphilic liquid crystal **1** on cytostatic activity are discussed next. The non-liquid-crystalline materials, i.e., PPY, D-glucamine, and the equimolar mixture of them, showed no pronounced activity. We assume that the marked inhibition of cell proliferation by liquid-crystalline compound **1** is due to its high ability to penetrate cell membranes. Results of X-ray investigations show that compound **1** exhibits two different bilayer structures, as depicted in Figure 3. We infer that compound **1** can form two different nanoassemblies composed of bilayer structures in the tumor cells, i.e., a structure in which the hydrophobic phenylpyrimidine units mutually interact in the inner shell and another structure in which the hydrophilic glucamine units mutually interact in the inner shell. By transformation between these molecular assemblies depending on the circumstances, compound **1** can penetrate multiple layers of the tumor cells.

In summary, we designed an amphiphilic compound **1** that shows two smectic phases composed of bilayer structures. Compound **1** inhibited cell proliferation in A549 human lung cancer cells at a concentration higher than 10 μM, although the precursors of compound **1**, having no liquid crystallinity showed no pronounced inhibition. Our findings lead a new frontier field of liquid-crystalline materials beyond display applications and offer a novel approach for anticancer-drug design.

Table 1. Cell viability expressed as a percentage (%) relative to solvent (DMSO)-treated control incubations.

Conc./μM	**1**	PPY	Glucamine	Mixture[a]
1	102 ± 4	105 ± 4	110 ± 3	103 ± 5
10	66 ± 0	91 ± 5	93 ± 1	86 ± 6
20	31 ± 10	89 ± 2	101 ± 3	80 ± 5

[a]An equimolar mixture of PPY and D-glucamine.

Experimental procedure is shown in supporting information.[16]

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16 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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