

Thermotropic Properties of Hemicholinium and Related Lipids

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Cationic morpholine lipids **1** and **6** undergo complex phase transition patterns and exhibit metastable phases; lipids **1**, **2** and **6** become thermotropic liquid crystals through the first phase transition temperatures, whereas **5** and **7** lose birefringence and become opaque solids.

Hemicholinium, **1**, and related lipids, **2–7**, are new synthetic, biologically active compounds. These compounds inhibit protein kinase C,¹ inhibit aggregation of erythrocytes by clusterin or fibrinogen² and potently kill sperm and other cells. These cationic lipids structurally resemble the aryl hemicholiniums, which inhibit acetylcholine synthesis by blocking the uptake of choline,³ serve as substrates for choline acetyltransferase⁴ and inhibit acetylcholine esterase.⁵ As a part of an effort to understand the biological and physical behaviour of these lipids, we have investigated the thermotropic properties by differential scanning calorimetry (DSC) and optical microscopy in polarized light. In this communication, we report the thermotropic properties of **1–7**.

Mixing 1-bromooctadecan-2-one and 2-(*N,N*-dimethylamino)ethanol in nitromethane produced crystalline hemicholinium, **1** (Scheme 1). Condensation of 3-(*N,N*-dimethylamino)propanol with 1-bromooctadecan-2-one produced **2**. Condensation of 2-(*N*-methylamino)ethanol with 1-bromooctadecan-2-one gave **3**. Reaction of **3** with acidic methanol produced **4**, which reacted with methyl bromide and methyl iodide to produce **5** and **7**, respectively. Treatment of methyl iodide with **3** gave **6**. All products were characterized by ¹H NMR, ¹³C NMR, IR and mass spectroscopy, and elemental analyses.

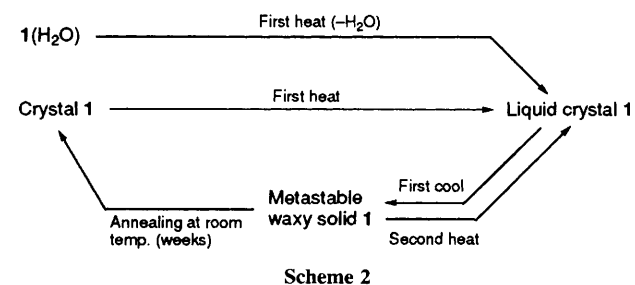
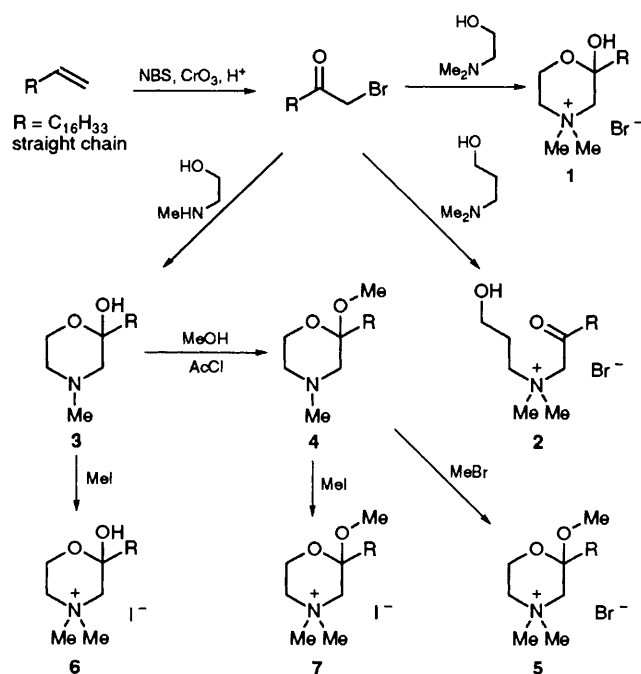
The DSC‡ analysis (Table 1) of **1** reveals that, during the first heating, it undergoes a phase transition at 102.9°C with an enthalpy change of 80.5 kJ mol⁻¹ and becomes an

anisotropic liquid (microscopic observations, see below). The cooling thermogram exhibits an exotherm at 33.4°C with an enthalpy change of -15.8 kJ mol⁻¹. The lipid is transformed into a waxy solid. In this phase the supercooled liquid crystal pattern is 'frozen' and the compound is not yet crystalline as illustrated by the powder X-ray pattern.⁶§ When the waxy solid remains at room temperature for several weeks, it anneals to the starting crystalline form. Subsequent heating, performed immediately after cooling, displays only an endotherm at 44.3°C with an enthalpy change of 15.2 kJ mol⁻¹. The heating of the waxy solid above 45°C restores the liquid crystalline phase obtained by melting crystalline **1**. This indicates that **1** exhibits a persistent metastable phase at ambient temperature.

The hydrate of **1**, *i.e.* **1** (H₂O), shows two endothermic transitions on the first heating, at 70.0 and 97.7°C, respectively. Thermogravimetric analysis (TG) performed under nitrogen indicates that at 70.0°C, the hydrate loses water. After this first transition the dehydrated material behaves as **1**. These processes are schematically summarized in Scheme 2.

Table 1 DSC transition temperatures (*T*/°C) and associated enthalpic changes (ΔH /kJ mol⁻¹) for compounds **1–7**

Compound	First heating		First cooling		Second heating	
	<i>T</i>	ΔH	<i>T</i>	ΔH	<i>T</i>	ΔH
1	102.9	80.5	33.4	-15.8	26.9	0.8
1 (H ₂ O)	70.0	109.5	32.0	-13.8	44.3	15.2
	97.7	30.1			47.0	15.5
2	87.9	84.2	50.5	-70.0	46.6	-12.3
3	56.7	86.4	42.5	-80.0	47.8	-6.6
					58.2	80.7
4	33.1	80.9	12.2	-70.0	21.1	-4.6
					27.0, 33.8	72.2
5	103.4	51.7	63.5	-23.5	65.6	22.8
					60.5	-36.1
6	102.7	55.6	35.0	-10.9	45.5	16.7
					94.5	47.9
					102.6	22.7
7	101.3	37.2	93.1	-17.9	102.6	22.7
					55.9	66.7
					48.1	-1.1
					59.3	1.3



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‡ DSC measurements were performed with a Seiko 220C DSC instrument (scanning rate, 2°C min⁻¹).

§ The X-ray pattern of the waxy solid shows four weak lines at $d = 6.7$, 5.6, 4.7 and 4.1 Å. The latter two lines might indicate a mixture of a disordered packing for a glass and a regular alkane packing for a gel, respectively. It also contains an intense diffraction line at $d = 32.1$ Å, closely related to the liquid crystal phase and three moderate lines at $d = 16.4$, 11.0, 8.3 Å.

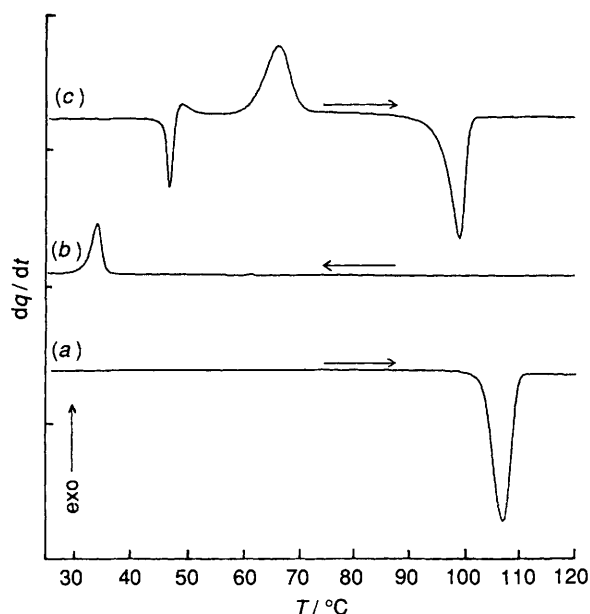
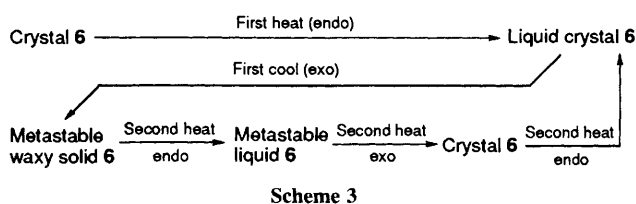


Fig. 1 DSC thermograms of lipid 6: (a) first heating; (b) first cooling; (c) second heating



When the bromide counter ion is replaced by the larger iodide ion, the compound, **6**, undergoes a more complex melting–crystallization process (Fig. 1). On the first heating, **6** becomes an anisotropic liquid at 102.7°C ($\Delta H = 55.6$ kJ mol⁻¹). The anisotropic phase is transformed on cooling to the metastable waxy solid. However, as soon as the lipid is reheated it shows an endothermic transition (melting) at 45.5°C, which is followed by an exothermic one (crystallization) at 60.5°C. Further heating results in the same high temperature (94.5°C), high enthalpy ($\Delta H = 47.9$ kJ mol⁻¹) transition exhibited in the first heating and the compound becomes an anisotropic liquid. These transformations are depicted in Scheme 3.

The ¹H NMR spectrum of **1** in CDCl₃ suggests that approximately 3% is the open (keto) form. The IR spectrum of **1** in KBr recorded above the first transition temperature (110°C), has an absorption at 1730 cm⁻¹ (C=O stretching vibration), which belongs to the keto (open) form. Because this absorption is absent in the room temperature spectrum of the crystal, we may conclude that the anisotropic phase of **1** is a mixture of open and closed forms. In order to compare the thermotropic properties of **1** with that of the completely open and completely closed compounds, we have investigated **2** and **5**, respectively.

The open chain compound **2** undergoes a phase transition at 87.9°C with an enthalpy change of 84.2 kJ mol⁻¹ and becomes an anisotropic liquid (microscopy observation). It crystallizes at 50.5°C, but the crystallization is not complete. The difference is found partially as the heat of crystallization during the second heating process because the lipid starts to recrystallize at 46.6°C before the melting at 86.1°C. The enthalpic balance of the first cooling and the second heating process is satisfied.

The closed compound **5** also undergoes a phase transition at 103.4°C with an enthalpy change of 51.7 kJ mol⁻¹. It

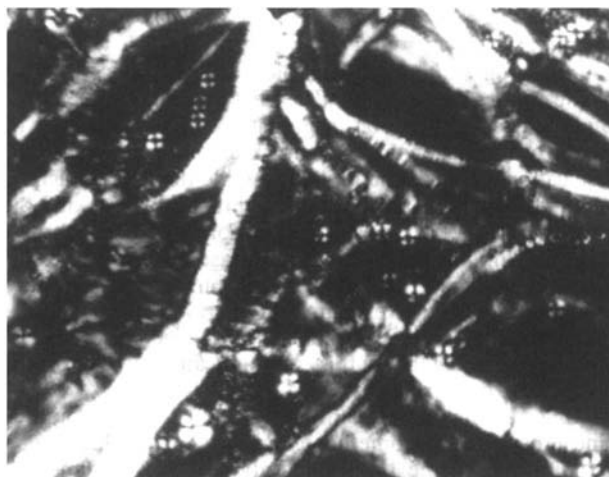


Fig. 2 Thermotropic texture of lipid **1** at 115°C. The bar length is 50 μ m

crystallizes at 63.5°C and on reheating, it shows an endothermic transition at 65.6°C ($\Delta H = 22.8$ kJ mol⁻¹). The supercooled solid anneals to the starting crystalline form on standing at room temperature for several days.

Microscopic observations[¶] of these compounds indicate that **1**, **2** and **6** on heating undergo a crystal-to-liquid-crystal transition. 'Maltese crosses', from a few μ m to ca. 50 μ m, develop immediately. They organize in threads and bands, which compose the mesomorphic structure (Fig. 2). The oily streak texture resembles that observed for some monosaccharide amphiphiles.⁷ X-Ray diffraction⁶ of **1** at 110°C shows an intense diffraction line corresponding to $d = 34.5$ Å and a broad, weak line centred at $d = 4.6$ Å in the liquid crystal phase. The length of the molecule obtained from single crystal X-ray⁶ of **1**(H₂O) is 23.9 Å. From these data, we propose that the observed mesophase is smectic A_d with a bilayer structure.⁷ The mesophase concentration varies with the liquid film thickness. Applying light pressure to the cover glass plate breaks the organized structures to elementary spherulites as the film becomes thinner. This mesophase is stable until the compound starts to decompose (150–180°C).

Compounds **3** and **4** melt at 56.7 and 33.1°C, respectively, forming an isotropic liquid. Crystal **5** and **7** lose their birefringence in the temperature range 100–110°C, and become opaque solids (a solid-to-solid transition, which needs further study, occurs at these temperatures). The compounds decompose at high temperatures (>180°C) before going to a liquid state. Some birefringence is restored on cooling.

In summary, cationic morpholine lipids **1** and **6** undergo complex phase transition patterns and exhibit metastable phases. Others have seen similar patterns in sphingolipids.^{8,9} The open chain compound **2** undergoes a crystal to anisotropic liquid phase transition but shows no metastable phase. Lipids **1** and **6** also become thermotropic liquid crystals through the first phase transition temperatures. Lipids **5** and **7** on heating

¶ Microscopic observations were performed using an Olympus BH microscope equipped with a Mettler FP82 hot stage, a Mettler FP80 central processor, a camera and an Olympus exposure control unit.

through the first phase transition temperatures become opaque solids. We conclude that (i) the *N,N*-dimethylmorpholinium head group enables the appearance of a metastable phase and (ii) both the hydroxy group and quaternary nitrogen are necessary for the formation of the thermotropic phase.

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