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A Thermotropic Study of 1-Deoxy-1-(*N*-methyloctanamido)-D-glucitol (MEGA-8) Using Microscopy, Calorimetry and X-Ray Diffraction

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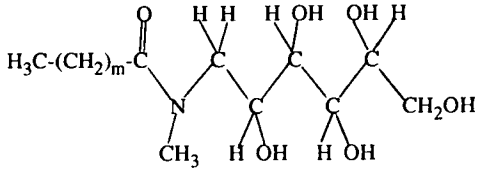
A detailed thermotropic study of 1-deoxy-1-(*N*-methyloctanamido)-D-glucitol (MEGA-8) revealed three crystalline forms of MEGA-8, formed at different conditions of solvent and temperature. Thermal polarizing microscopy was used to visualize birefringent solvent crystals (C_S), needle crystals (C_N), and terraced crystals (C_T) and to study their nucleation requirements. Differential scanning calorimetry (DSC) determined the melting temperatures (C_T = 78.4°C, C_S = 81.0°C, C_N = 87.5°C) and enthalpies of each crystalline phase transition to the isotropic phase. DSC was also used to study the kinetics of the phase transformations, and a Gibbs free diagram was constructed that shows C_N and the isotropic phase as the lowest free energy forms over the temperature range 25–100°C. Powder X-ray diffraction confirmed three crystalline forms that melt to the isotropic phase at their characteristic temperatures. When the isotropic melt was cooled to room temperature, a smectic liquid crystalline intermediate appeared before recrystallization of C_T, as observed by both X-ray diffraction and polarizing microscopy.

1. INTRODUCTION

The 1-deoxy-1-(*N*-methylalkanamido)-D-glucitols have been shown to possess lyotropic and thermotropic liquid crystalline properties.[1]



These compounds (called MEGA-*n*, where $n = 2 + m$ in the structure above)[2] are valuable as non-ionic detergents for crystallizing membrane proteins.[3,4] Thermotropic studies indicate that short chain-length members of this series of amphi-

philic compounds melt with a single transition to the isotropic phase, whereas longer chain-length members go through an intermediate phase identified as smectic A liquid crystals.[5]

In an initial effort to reproduce the work of Goodby *et al.*,[5] we measured the melting transitions by differential scanning calorimetry for one member of this series, 1-deoxy-1-(*N*-methyloctanamido)-D-glucitol (MEGA-8), which we found to be higher than reported. In a subsequent detailed thermotropic study, thermal polarizing microscopy revealed several different crystal morphologies upon heating to and cooling from the isotropic phase. Differential scanning calorimetry (DSC) showed that these changes in morphology corresponded to phase transitions and gave the temperatures and enthalpies of these transitions. The kinetics of the phase transformations were further studied by DSC permitting the construction of a Gibbs free energy diagram. This shows one of the crystal phases (C_N) and the melted isotropic phase as those with the lowest free energy over the temperature range 25 to 100°C. Powder X-ray diffraction patterns confirmed that there are three different crystal structures, one of which (C_S) is identical to that calculated from the single crystal structure analysis of MEGA-8.[6] A transient smectic liquid crystal intermediate was observed by both polarizing microscopy and X-ray diffraction prior to recrystallization from the melt at room temperature.

2. EXPERIMENTAL

The MEGA-8 used in these studies was purchased from Sigma Chemical Co. and used without further purification. This polycrystalline powder had been recrystallized from ethanol and diethylether, and is referred to herein as C_S.

Microscopy. The microscopy of MEGA-8 was carried out on a Leitz Wetzlar Laborlux 12 Pol polarized-light microscope equipped with Leitz Heated Stage 350 and a West 2050 programmable temperature controller (Gulton Industries). The microscope temperatures are accurate to ±0.5°C. Crystals of MEGA-8 were placed on a microscope slide either with or without a coverslip. Photographs were taken with crossed polars and a 1 λ retardation plate between the sample and the upper polar.

Calorimetry. A high sensitivity differential scanning calorimeter, Microcal MC-1 (Microcal, Inc., Amherst, MA), was used for the calorimetric measurements. MEGA-8 crystals were loaded into the sample cell using a plastic pipettor tip and a wire pusher, and the reference cell contained 50 μliters deionized, distilled water. The data were collected and converted to specific heat using an Intel System 310 microprocessor interfaced to the Microcal. In addition to the temperature calibrations described previously,[7] further calibrations in the high temperature range using melting point standards compared to a National Bureau of Standards thermometer were carried out. The calorimetry temperatures are accurate to ±0.1°C.

X-Ray Diffraction. X-ray diffraction studies at room temperature were carried out using a Norelco Water-Cooled X-Ray Diffraction Unit Type 12045 (Philips

Electronic Instruments, Mount Vernon, New York) in the CMU Department of Biological Sciences X-Ray Facility. This instrument produces nickel filtered, pin-hole collimated Cu Kα radiation, and typically operates at 20 kV and 10 mA. The diffraction photographs presented here resulted from exposure times of 18–20 hours at 24°C.

X-ray diffraction studies of the thermally induced phase behavior of MEGA-8 were made at the University of Pittsburgh Shared X-Ray Diffraction Facility which is equipped with a Rigaku RU200 rotating copper anode X-ray generator and a Siemens area detector controlled by a Periphere Computer System (PCS) Cadmus 9000/16 Microcomputer. 5 kilowatt double mirror focused Cu Kα (λ = 1.5418 Å) radiation was used. The diffraction patterns were recorded in 20 to 360 seconds. Each sample was contained in a capillary tube and heated in a small furnace. Temperature control on the furnace was provided by a West 2050 programmable controller (Gulton Industries) with an accuracy of ±0.5°C at the sample.

3. RESULTS

Polarizing Microscopy. When the MEGA-8 powder (C_S) was placed on the microscope stage and heated to 81°C, the sample melted with the transient appearance of birefringent crystals floating in the clear isotropic phase (Figure 1a). On cooling this isotropic sample to room temperature, a variety of crystalline textures appeared as shown in Figure 1b. (Figures 1a-f are reproduced in color at the end of this volume.)

On reheating to 78°C, needle crystals (C_N) grew out from the periphery of fan-like, terraced crystals (C_T) (Figure 1c). On further heating to 85°C, C_T melted to an isotropic liquid while C_N remained unmelted. At 87°C, C_N then melted to the clear, isotropic phase.

Needle crystals of the C_N phase above were obtained when the C_S powder was heated to 94°C, and then held in the temperature range between 80°C and 85°C. Needle crystals grew quickly from the isotropic phase when seeded with crystals of MEGA-8 (any form) or D-glucitol (Figure 1d).

To produce the terraced crystals (C_T) shown in Figure 1e, the powder C_S was melted to the isotropic phase by heating to 94°C, and quickly lowered in temperature to 45°C where it was maintained for 30 minutes. This allowed the formation of homogeneous crystals of C_T (Figure 1e). If the temperature was then raised to 70°C before all the isotropic phase had converted to C_T, pebbly terraced crystals were formed (seen in the lower right of Figure 1e).

The transient liquid crystal intermediate shown in Figure 1f was observed as numerous small, birefringent blue and yellow spherulites within 10 minutes after cooling the clear isotropic phase to room temperature (Figure 1f).

Differential Scanning Calorimetry. The transition of each crystal phase, C_S, C_N and C_T, into the isotropic phase occurs at a characteristic temperature with transition peaks labeled I, II and III in the DSC thermograms shown in Figures 2, 3 and 4. Two variables involved in differential scanning calorimetry are presented:

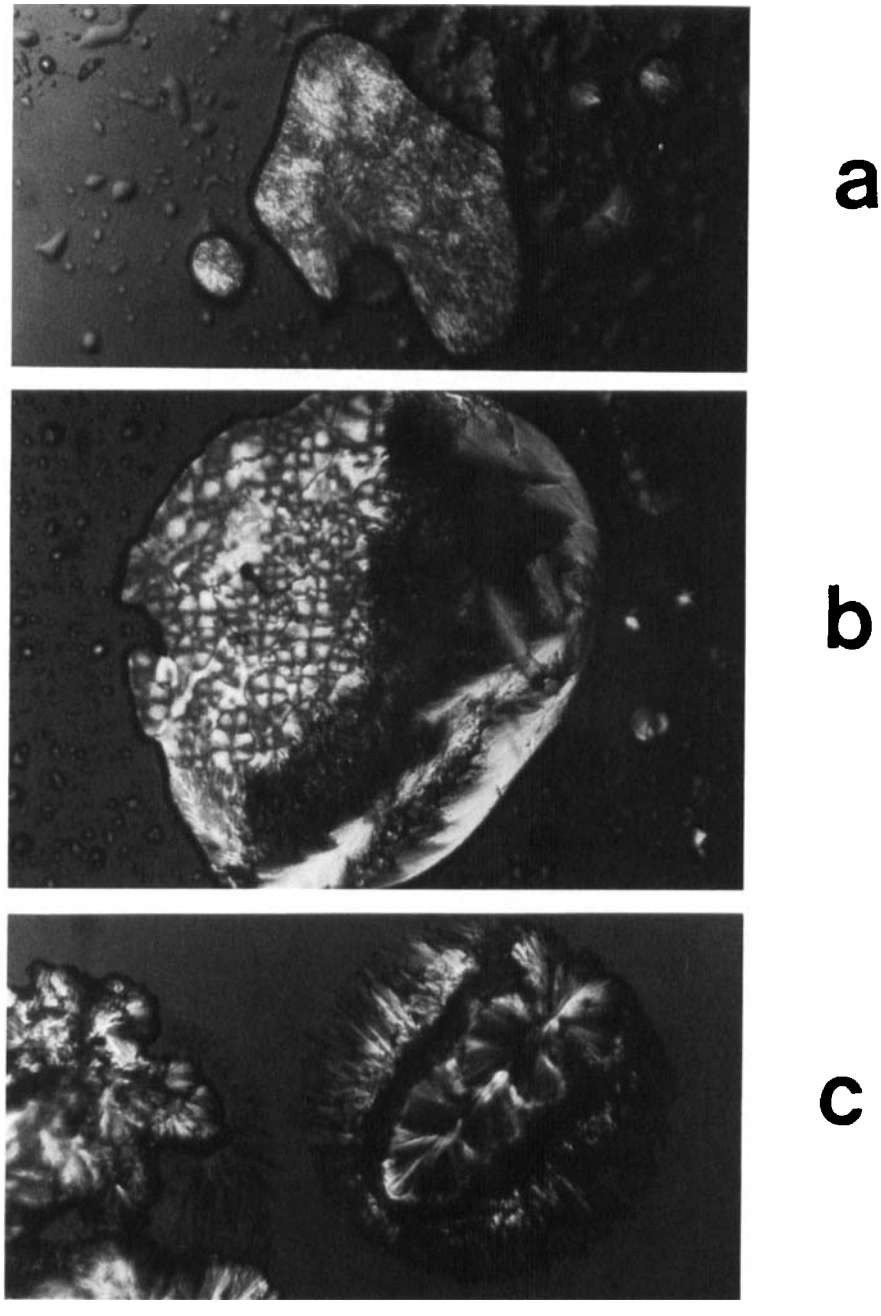


FIGURE 1 Polarizing micrographs of MEGA-8: (a) C_S and Isotropic (b) Varied C_T (c) C_T and C_N (d) C_N (e) C_T (f) Liquid Crystal. Details of the samples are given in Results. The black outlines are coverslip artifacts. See Color Plate I.

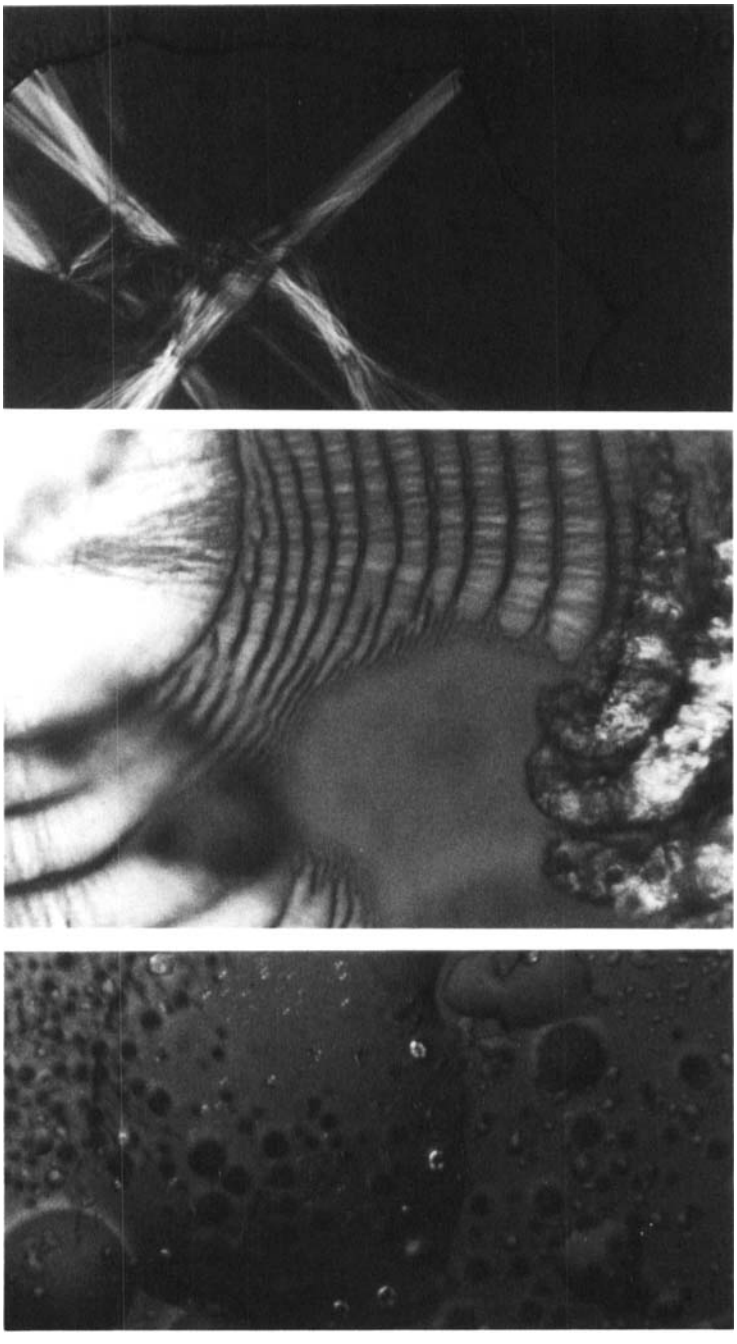


FIGURE 1 (*continued*)

See Color Plate II.

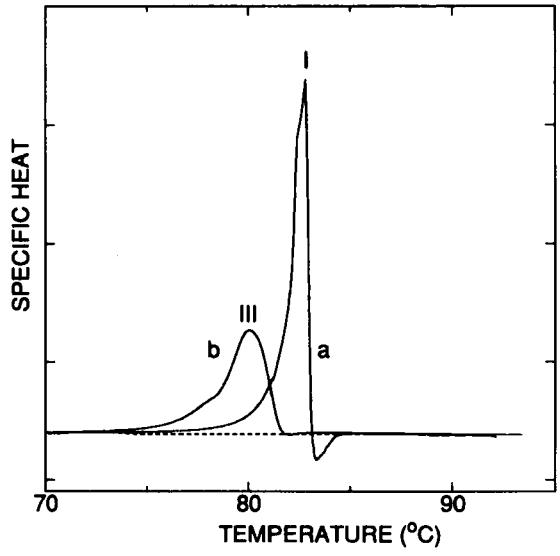


FIGURE 2 Specific heat (cal/deg · g) vs. temperature (°C) obtained at a scan rate = 36.7 ± 0.5°C/h. (a) The crystalline phase from solvent (C_S) was scanned, ΔH_I = 33.3 cal/g. (b) Sample in (a) was allowed to cool slowly to room temperature and then was rescanned at the same rate, ΔH_{III} = 26.4 cal/g. Sample weight = 4.7 mg. One division on the *y*-axis is 10 cal/deg · g.

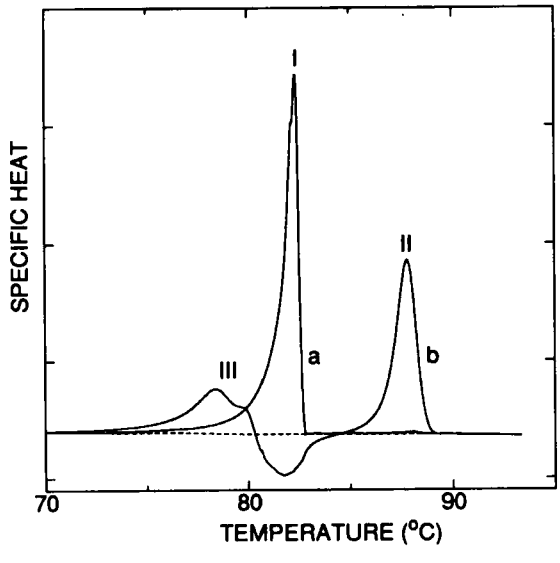


FIGURE 3 Specific heat (cal/deg · g) vs. temperature (°C) obtained at a scan rate = 13.0 ± 0.5°C/h. (a) C_S was scanned, ΔH_I = 33.3 cal/g. (b) Sample in (a) was allowed to cool slowly to room temperature and then was rescanned at the same rate. ΔH_{III} = 12.1 cal/g, $\Delta H_{exotherm}$ = −6.3 cal/g, ΔH_{II} = 20.6 cal/g. Sample weight = 7.4 mg. One division on the *y*-axis is 10 cal/deg · g.

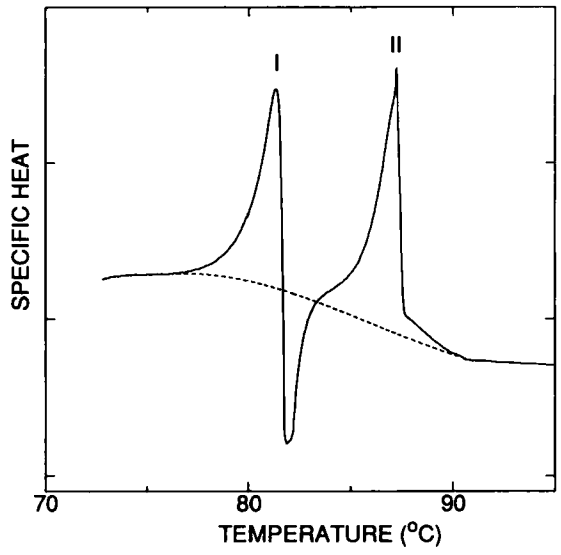


FIGURE 4 Specific heat (cal/deg · g) vs. temperature (°C) obtained at a scan rate = 2.6 ± 0.1°C/h. C_S was scanned quickly to 70°C and then heated slowly. ΔH_I = 21.4 cal/g, $\Delta H_{exotherm}$ = −7.5 cal/g, ΔH_{II} = 28.8 cal/g. Sample weight = 8.0 mg. One division on the *y*-axis is 10 cal/deg · g.

the effect of rescanning a fresh sample, and the effect of scan rate. The results are summarized in Table I. In Figure 5, recrystallization from the melt is investigated. Prior to scan 5a, C_S was heated to 94°C and then cooled to 83°C. Prolonged incubation of the isotropic melt at 83°C did not result in peak II occurring upon a subsequent scan. However, in 5b, seeding of the melted isotropic phase at 83°C with a crystal of D-glucitol did yield peak II in the rescan. In Figure 6 formation of C_T was investigated as a function of incubation time at 45°C after cooling the melt of C_S. As shown, 2-1/2 hours incubation at 45°C was required for complete transformation to C_T, since in the rescan C_T melted to the isotropic phase with a single, maximal endotherm. At 55°C, formation of C_T did not occur spontaneously within 2-1/2 hours. The DSC results were used to construct a qualitative Gibbs free energy diagram shown in Figure 8 and discussed below.

X-Ray Diffraction. Using the information from polarizing light microscopy and DSC, homogeneous samples of C_N and C_T were prepared in thin-walled glass capillaries as described in the legend to Figure 7. The d-spacings from the room temperature X-ray diffraction of C_S, C_N and C_T in Figure 7 and those calculated from the single crystal structure are summarized in Table II.

In Figure 9, X-ray diffraction patterns were obtained during heating of C_S, C_N and C_T to the isotropic phase (100°C) and after quick cooling of each sample to room temperature. The isotropic phase is characterized by a diffuse low angle band centered at 24 Å, and a very broad, diffuse wide angle band centered at 4.8 Å. After the isotropic phase is cooled rapidly to room temperature, the low angle diffuse band sharpens, yielding a d-spacing of 26.7 Å, while the wide angle band

TABLE I

Temperatures and enthalpies of MEGA-8 transformations. Transition temperatures are the extrapolation to zero scan rate of transition temperatures at scan rates between 2 and 75°C/h. Numbers in parentheses refer to number of scans used to average data.

Peak	Transition	T_t (°C)	ΔH (cal/g)
I	C_S[1] → Iso[2]	81.0	33.2 ± 0.2 (4)
II	C_N[3] → Iso	87.5	28.3 ± 0.6 (4)
III	C_T[4] → Iso	78.4	28.2 ± 1.2 (2)

[1]Crystals from the solvents ethanol and diethyl ether (C_S) were from Sigma Chemical Co.
[2]The melted isotropic phase was produced by heating above 87.5°C.
[3]Needle crystals (C_N) were formed in several ways as described in the text.
[4]Terraced crystals (C_T) with a single endotherm were produced as in Figure 6.C.

remains diffuse at 4.8 Å. This pattern suggests the formation of a smectic liquid crystal.[8] For each sample, this liquid crystal pattern disappears with increasing time and is replaced by the C_T pattern. The rate of recrystallization of C_T from the melt varied, probably due to impurities. The slowest rate of recrystallization (Figure 9b) may have been caused by sample degradation at the high temperatures required to form C_N. In Figure 9c, there is one new d-spacing at 9.7 Å in addition to the d-spacings for C_T. The new reflection may also have been caused by an impurity.

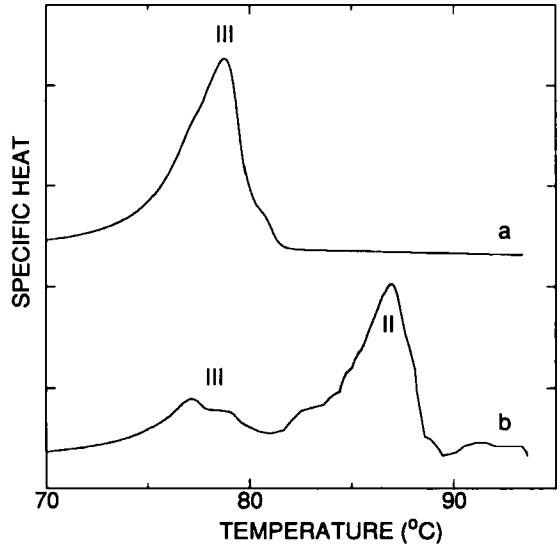


FIGURE 5 Effect of crystal seeding. (a) C_S was rapidly scanned to 94°C (not shown), then cooled to 83°C and incubated for 2 h, and then cooled to 45°C and incubated for 1-1/2 h. The trace shown is the specific heat (cal/deg · g) vs. temperature (°C) obtained at 36.2 ± 0.6°C/h. ΔH_{III} = 20 cal/g. Sample weight = 7.8 mg. (b) C_S was rapidly scanned to 94°C (not shown), then cooled to 83°C, seeded with a small grain of D-glucitol and incubated at 83°C for 2 h, then cooled to 45°C and incubated for 1-1/2 h. The trace shown is the specific heat (cal/deg · g) vs. temperature (°C) obtained at 34.8 ± 0.6°C/h. ΔH_{III} = 6.5 cal/g, ΔH_{II} = 15.2 cal/g. Sample weight = 8.6 mg. One division on the y-axis is 2.5 cal/deg · g.

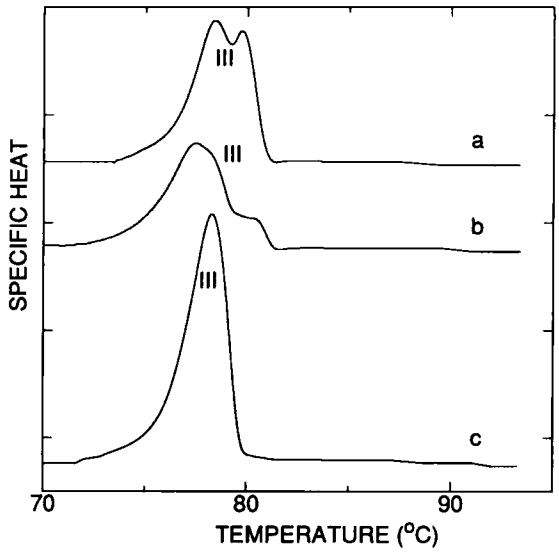


FIGURE 6 Specific heat (cal/deg · g) vs. temperature (°C), effect of incubation time at 45°(C). In (a), (b) and (c), fresh samples of C_S were rapidly scanned to 94°C (not shown), and quickly cooled to 45°C where they were incubated for: (a) 5 min, (b) 1 h, (c) 2-1/2 h. They were then scanned at 36 ± 1.5°C/h. ΔH_{III}: (a) 22.6 cal/g, (b) 19.4 cal/g and (c) 29.2 cal/g. Sample weights: (a) 5.2 mg, (b) 6.3 mg, (c) 5.3 mg. One division on the y-axis is 5 cal/deg · g.

4. DISCUSSION

The first major result of this study is that there are three crystalline forms of MEGA-8. While this result was discovered using polarizing microscopy and DSC, the confirmation was obtained by X-ray diffraction. Powder diffraction of pure C_S, C_T and C_N produced three different sets of crystal spacings shown in Figure 7 and summarized in Table II. C_S, which was recrystallized by Sigma Chemical Co. from diethyl ether and ethanol, has the same powder pattern as calculated from the crystal structure of a single crystal grown from water[6] (see Table II). In this crystal structure there are bimolecular layers in which the alditol groups are strongly hydrogen-bonded in a head-to-head arrangement, and the alkyl chains, which extend in both directions from the hydrogen-bonded region, interdigitate with alkyl chains of adjacent layers. This arrangement is commonly observed in mesogenic *N*-alkyl-1-O and 1-S pyranoside crystal structures.[9-13] C_T has a very distinct lamellar repeat with a first order reflection of 34.5 Å, twice the length of the MEGA-8 molecule calculated from the single crystal unit cell, suggesting a bilayer head-to-head molecular packing. (A sample that contained only pebbly C_T had the same powder pattern as smooth C_T confirming that smooth and pebbly C_T have the same crystal structure.) The low angle diffraction of C_N is not clearly indicative of a simple lamellar arrangement.

The second major emphasis of this work concerns the phase transitions between the three crystalline forms and the isotropic phase. The plethora of peaks in the DSC figures 2-6 shows that the phase transformations of MEGA-8 are complicated

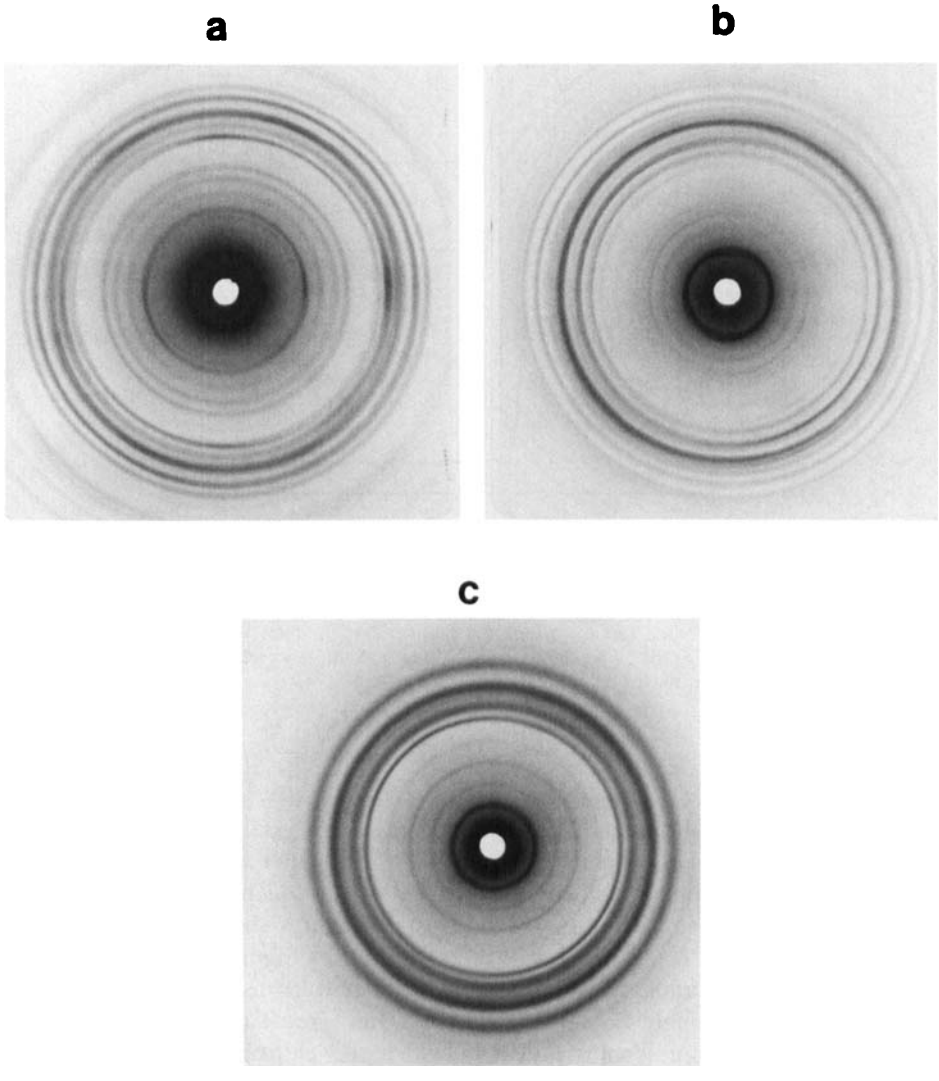


FIGURE 7 Powder X-ray diffraction of MEGA-8 at room temperature. (a) C_S was loaded into a capillary directly from the Sigma bottle. (b) C_N was formed by slowly heating C_S in a capillary in a water bath to 83°C and incubating for 2-1/2 hours at 83°C. (c) C_T was formed by first melting C_S in a capillary in boiling water and then incubating at 45°C for 2-1/2 hours in a water bath.

and are greatly influenced by the kinetics of the transformations between phases. We present an hypothesis for the underlying equilibrium phase behavior in Figure 8, which shows that the lowest free energy phases over the temperature range 40 to 90°C are C_N and the isotropic phase. This discussion will demonstrate that the equilibrium hypothesis presented in Figure 8 is consistent with the data presented in Figures 2-6 provided that some reasonable assumptions are made about the kinetics of the phase transformations. In particular, it is assumed that crystal-to-crystal phase transformations are very slow, so that the C_T and C_S phases in Figure 8 can exist metastably for very long times without transforming to C_N; similar

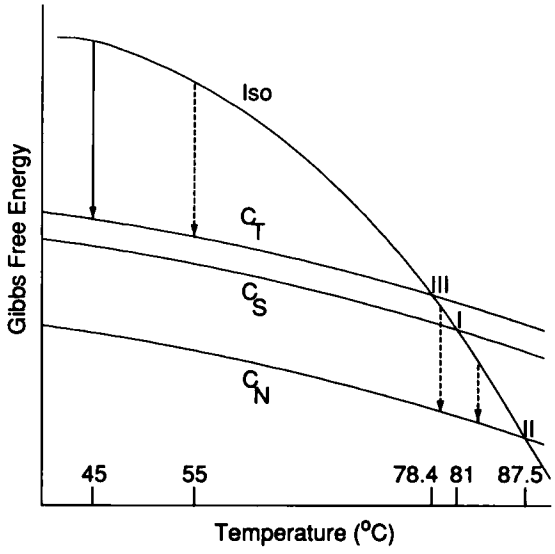


FIGURE 8 Qualitative Gibbs free energy diagram of MEGA-8 crystal transitions, Gibbs free energy vs. temperature (°C). The names of the phases are the same as in Table I. The transitions are indicated by the same Roman numerals used in Figures 2–6 to identify the DSC peaks.

TABLE II

Comparison of d-spacings (Å) from the single crystal structure with powder crystals of MEGA-8. D-spacings from the single crystal structure of MEGA-8 (Reference 6) were computed. Relative intensities of the reflections are represented by the symbols: vs, very strong; s, strong; m, medium; w, weak, vw, very weak; vvw, very very weak.

Single Crystal	C_S[1]	C_N[2]	C_T[3]	
19.55 (vs)	19.6 (vs)		34.5 (vs)	(h = 1)
9.77 (w)	9.7 (w)	17.1 (vs)	17.2 (s)	(h = 2)
8.94 (s)	8.9 (s)	10.9 (m)	11.4 (m)	(h = 3)
8.31 (vw)		9.1 (m)	8.6 (m)	(h = 4)
7.51 (w)	7.5 (w)	8.4 (w)	6.9 (w)	(h = 5)
6.70 (vw)		7.8 (w)	6.3 (vw)	
6.52 (m)	6.4 (m)	6.7 (vvw)	5.7 (s)	
5.95 (m)	5.9 (m)	5.7 (w)	5.3 (m)	
4.83 (s)	4.8 (s)	5.3 (m)	4.9 (w)	
4.58 (m)	4.5 (m)	5.2 (vw)	4.7 (s)	
4.24 (s)	4.2 (s)	4.9 (s)	4.3 (vw)	
3.95 (s)	3.9 (s)	4.8 (s)	4.2 (s)	
3.75 (s)	3.7 (s)	4.6 (m)		
		4.5 (s)		
		4.3 (s)		
		4.2 (m)		
		4.1 (m)		
		3.9 (s)		
		3.7 (s)		

[1]C_S was loaded into a thin-walled capillary directly from the Sigma bottle.

[2]C_N was formed by slowly heating C_S in a thin-walled capillary in a water bath to 83°C where it was maintained for 2-1/2 hours.

[3]C_T was formed by melting C_S in a thin-walled capillary in boiling water for 30 seconds and then quickly transferring it to a water bath at 45°C where it was maintained for 2-1/2 hours.

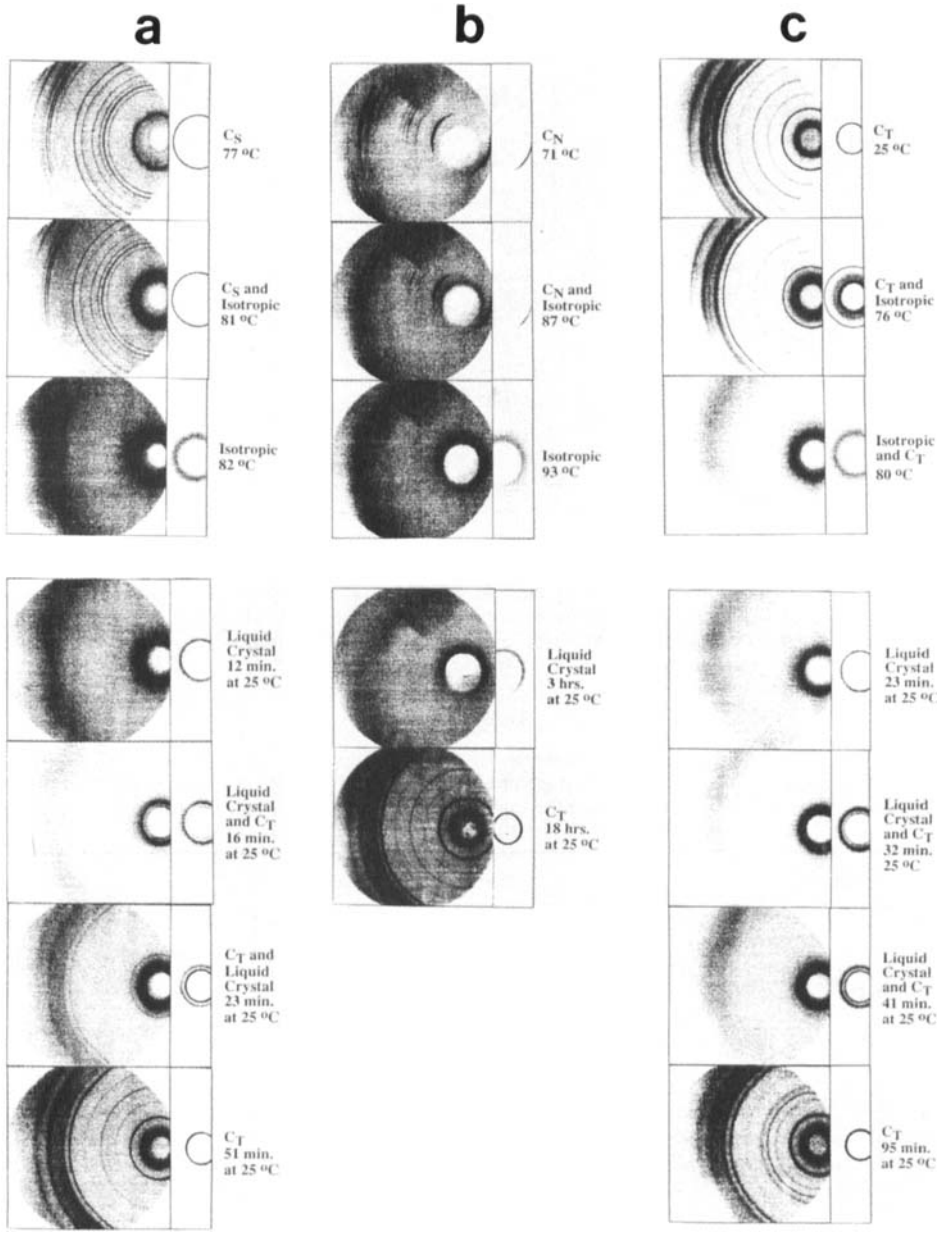


FIGURE 9 Powder X-ray diffraction of MEGA-8 crystals as a function of temperature. The samples were prepared as in Figure 7 and slowly heated to isotropic, then cooled within 10 minutes to room temperature and held at 25°C. The strip to the right of each pattern represents the top of the innermost ring which is overexposed in the left panel. (a) C_S (b) C_N (c) C_T.

metastability has been shown to occur for other polymorphic systems.[14] Furthermore, it is assumed, as has been shown for lyotropic phospholipid systems,[15] that nucleation of any of the crystal phases can be kinetically limited so that there can be considerable hysteresis upon cooling through the transition temperatures, T_I, T_{II} and T_{III} where the equilibrium phase transitions are supposed to occur according to the hypothesis of Figure 8.

As shown in Figures 2a and 3a, solvent crystals (C_S) first melt into the isotropic phase at T_I, and if the scan rate is sufficiently fast, little or no transformation of Iso to C_N (needle crystals) takes place. This is also shown by polarizing microscopy in Figure 1a. When the scan rate is slowed as in Figure 3a, a small, barely noticeable peak II occurs near 88°C, indicating that a small amount of C_N had been formed at lower temperatures. At the slowest scan rate, complete transformation of $C_S \rightarrow$Iso$\rightarrow C_N$ occurs. This is shown by the scan in Figure 4, obtained at 2.6°C/h, which shows that before the $C_S \rightarrow$Iso endothermic transition is complete, a large exotherm occurs, followed by peak II. We interpret these events in Figure 4 to be an initial $C_S \rightarrow$Iso transformation, in which small domains of C_N are nucleated by unmelted crystalline C_S. Once nucleated, formation of C_N is an exothermic event. At T_{II} the $C_N \rightarrow$Iso transition takes place and an endotherm is observed. The overall transformation of $C_S \rightarrow$Iso$\rightarrow C_N$ is a slow process relative to conventional DSC. We think that the transformation to C_N in Figure 4 is complete since the enthalpy obtained for peak II is 28.8 cal/g, which is the highest of our measurements for the $C_N \rightarrow$Iso transition. A similar transformation, $C_T \rightarrow$Iso$\rightarrow C_N$, occurs in Figure 3b. The difference here is that the seed crystals are C_T instead of C_S. At 13°C/h, Figure 3b shows that the Iso$\rightarrow C_N$ transformation is not complete since the ΔH_{II} of 20.6 cal/g is less than the maximum for peak II (see Table I). Another incomplete transformation, $C_T \rightarrow$Iso$\rightarrow C_N$, is also observed in Figure 1c by polarizing microscopy, where C_N is nucleated in the isotropic phase near the periphery of C_T.

In order to confirm the above hypothesis concerning C_N nucleation, C_S was heated only to 83°C so as not to melt any growing crystals of C_N. Then, incubation at 83°C for 2-1/2 hours was sufficient to completely transform the sample into C_N. This was revealed by a single peak II in the subsequent scan at 12.4 ± 0.5°C with ΔH_{II} = 28.5 cal/g (data not shown).

By contrast, when C_S was heated to 94°C and the melt incubated at 83°C for 2 hours, no C_N was formed. Presumably no crystalline nuclei remained after heating to 94°C. This result is shown in Figure 5a, where the $C_N \rightarrow$Iso transition at peak II is absent. In Figure 5b, the only change from the conditions in Figure 5a, is that a small grain of D-glucitol was introduced into the sample cell before the 83°C incubation. In Figure 5b, a prominent endotherm near 87.5°C occurred, indicating that the seed crystal had promoted the conversion of a large part of the sample to C_N at 83°C. Similarly, nucleation of C_N occurred on the microscope stage using a crystal of D-glucitol or any form of MEGA-8 (Figure 1d), whereas ground glass and NaCl were ineffective.

Nucleation of C_T was also investigated. In the temperature region between 55°C and T_{III}, polarizing microscopy revealed that seeding nucleates the Iso$\rightarrow C_T$ transformation. Without seeding, DSC showed no transformation at 55°C for 2-1/2 hours. At 45°C and lower temperatures, Iso$\rightarrow C_T$ was a spontaneous process. C_T was

formed after the isotropic liquid was allowed to cool slowly to room temperature (Figure 2b), and also by incubating the isotropic liquid at 45°C as shown in Figure 5a. (In Figure 5b only a minor part of the sample formed C_T since most of the sample had already crystallized to C_N due to seeding with D-glucitol at 83°C). We suggest that the morphologies shown in Figure 1b are all variants of C_T since they all melted before 80°C.

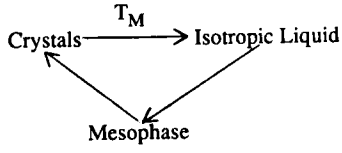
The results in the previous three paragraphs are consistent with the Gibbs free energy diagram. For those processes that required crystal seeding, there is a kinetic barrier that must be overcome in order to decrease in free energy from the isotropic phase. These transformations are shown by dotted arrows in Figure 8. When ascending slowly in temperature above T_{III}, this kinetic barrier is overcome by unmelted crystals of C_T or C_S that serve as nucleation sites for C_N. When descending in temperature, this barrier must be overcome by the introduction of crystals, either of MEGA-8 or D-glucitol, that serve as nucleation sites for C_N. Between 50°C and T_{III}, this kinetic barrier is overcome by the introduction of seed crystals that serve as nucleation sites for C_T. The only spontaneous nucleation we observed is the Iso$\rightarrow C_T$ transformation at 45°C and below, which is shown by a solid arrow in Figure 8. If the liquid crystal intermediate seen by polarizing microscopy at room temperature (Figure 1f) serves as a crystal seed (see discussion below), then the DSC results suggest that the liquid crystal transient is able to form between 25 and 45°C.

The melting of C_T often appears as a double endotherm. This feature was examined more closely by incubating at 45°C as a function of time, followed by scanning at 36°C/h as shown in Figure 6. The prominent right shoulder seen after only 5 minutes incubation at 45°C (Figure 6a), was reduced after incubation for one hour at 45°C (Figure 6b), and disappeared completely after incubation for 2-1/2 hours at 45°C (Figure 6c). The ratio of the shoulder to the main peak is sensitive to the kinetics of the incubation, as shown also in Figure 2b, where the sample was allowed to cool slowly and stand overnight after heating to 94°C. The rescan of this sample displayed only a small left shoulder on the right main peak. These two forms of C_T are probably the smooth and pebbly forms of C_T (Figure 1e) since the pebbly terraces melted at a slightly higher temperature on the microscope stage. They are probably closely related, as are the two subgel forms (C^* and C) of dipalmitoylphosphatidylcholine (DPPC) studied by Tristram-Nagle *et al.*[7]

Once melted, C_S was not reformed by any of the procedures described so far in this investigation, such as seeding the isotropic form or incubating at various temperatures. Also, briefly boiling C_S dissolved in water, then cooling to room temperature and evaporating the water under vacuum, produced mostly C_T and some C_N, but no C_S. As others have noted,[16] different conditions, such as polarity of the solvent or temperature of the melt, allow different intermolecular interactions to dominate, resulting in the growth of different crystalline polymorphs. A similar conclusion was reached by Yang *et al.*[17] in the case of the lyotropic DPPC lipid system. In that study, cold hydration of DPPC produced two new phases depending on the chloroform/water molar ratio from which the lipid was dried.

X-ray diffraction as a function of temperature confirmed that each crystal form

of MEGA-8 melts to the isotropic phase at its characteristic melting point (see Figure 9). In addition, the isotropic melt of all three forms recrystallizes at room temperature into C_T, via a transient liquid crystalline form. The liquid crystal has an X-ray pattern that consists of a sharp low angle band indicative of a layered structure and a diffuse wide angle band indicating disorder within the layers. The d-spacing of 26.7 Å is about 1-1/2 times the length of the MEGA-8 molecule but since the molecules may be tilted or less extended than in C_S no conclusions about interdigitation in the liquid crystal phase should be drawn. Although smectic mesophases are usually observed upon heating a mesogenic crystal before it clears to isotropic, quite frequently a mesophase may form when the isotropic liquid is supercooled, before crystallization occurs.[18] This can be summarized as:



Polarizing micrographs of isotropic MEGA-8 samples quickly cooled to room temperature show the appearance and growth of small isolated spherulites of blue and yellow birefringence (Figure 1f) when these structures are observed through a retardation plate. The arrangement of blue and yellow quadrants indicates that the orientation of the long axes of the molecules[19] is radial. C_T crystals, which appear immediately after the birefringent circles, present the opposite arrangement of blue and yellow quadrants (shown in Figure 1f and redrawn in Figure 10). This inversion of color indicates a conversion to a radial orientation of the molecules' *short* axes. We propose that the birefringent phase seen by polarizing microscopy before C_T crystallization can be identified as the liquid crystal phase observed by X-ray diffraction before the appearance of the C_T diffraction pattern (Figure 9a, b, c). Although this liquid crystal birefringent pattern is not typical of smectic mesophases, we have observed the same pattern in polarizing micrographs of hydrated lipid multilamellar vesicles which are radially smectic (unpublished data). It follows, therefore, that the periodicity of the liquid crystal phase corresponds to bimolecular layers which are circular, not flat. These liquid crystals, surrounded by the isotropic phase, apparently serve as nucleation sites for the growth of C_T crystals.

The polymorphic behavior of MEGA-8 revealed in this work may explain the anomalous melting behavior of MEGA-8 when compared to the other members of this glucitol series. In Figure 1 in Reference 5 an apparent odd-even effect predominates when the alkyl chains have 9 or fewer carbon atoms. In the even series, the melting points are lower which may be related to their inability to form the strong hydrogen bonds of the homodromic four-bond cycle.[21] The needle crystals (C_N), on the other hand, may be able to form the homodromic cycle of hydrogen bonding since they do not show the odd-even effect in the melting temperatures. (I.e., C_N crystals of MEGA-8 have a melting transition which corresponds well with MEGA-7 and MEGA-9.) Future X-ray diffraction studies of the single crystal

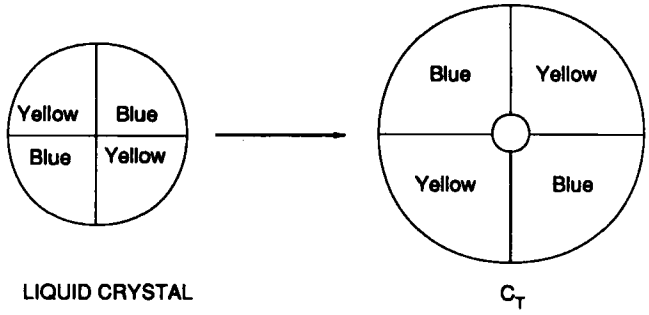


FIGURE 10 Drawing of spherulites in Figure 1f with proposed interconversion from liquid crystal to C_T shown by the arrow.

structure of C_N will be required to determine definitively the hydrogen bonding in C_N.

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