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Thermotropic Liquid Crystals Based on Chito-Oligosaccharides. 1. Synthesis of Chitobiose Octaalkanoates and Chitotriose Undecaalkanoates and Their Thermal Properties

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This paper deals with the preparation of two series of chito-oligosaccharide derivatives, chitobiose octaalkanoates, and chitotriose undecaalkanoates, in which the amino- and hydroxyl groups were blocked with alkyl pendants of differing lengths via an ester linkage. By differential scanning calorimetry(DSC) and optical microscopic observations, these compounds were found to exhibit an enantiotropic mesophase at temperatures ranging from ca. 50 °C to ca. 200 °C. The texture of the mesophases were similar to those of the discotic columnar liquid crystals formed by the cellobiose and cellotriose equivalents. However, the amido group in the chito-derivatives seems to stabilize the mesophase to an appreciable extent, bringing about higher temperatures and a longer temperature span of the mesophase than those of the cello-equivalents.

It has long been recognized that some long-chain *n*-alkyl pyranosides exhibit thermotropic mesomorphism;[1] however, it is not until recently that the fine structures of the mesophase formed by such carbohydrate derivatives have attracted much interest.[2-7] Most of the carbohydrate-based liquid crystals examined so far consist of a monosaccharide skeleton and one or two long-alkyl substituents, and generally show a smectic type of [2,4,8] or, in a special case, a columnar hexagonal type of mesophases,[6] in which the ordered packing of molecules is stabilized by the intermolecular hydrogen bonding originating from the unblocked multiple hydroxyl groups.[2,4] These derivatives, thus, can be viewed as molecules consisting of a polar and nonpolar moiety and this amphiphilic character results in the formation of the mesophases.

On the other hand, partially or fully acylated inositols have been shown to give rise to discotic liquid crystals.[9] The closest relatives of the inositol derivatives are the acylated glucopyranose and glycosides which have been found to show a discotic columnar mesophase.[10] In this case, the occurrence of the thermotropic mesomorphism is attributed to the discogenic character of the inositol ring skeleton rather than a hydrogen bonding. Oligosaccharides such as cellobiose and cellotriose have been expected to be potential sources of discogens due to the planarity and rigidity of the molecules. In fact, we recently demonstrated that the fully-acylated cellobiosides,[11] cellobiose, and cellotriose[12] gave a new family of thermotropic columnar liquid crystals. In particular, as for the latter two derivatives, an X-ray analysis revealed that, despite their highly-asymmetric molecular structures, they form a hexagonal ordered columnar (D_{ho}) phase, in which columns built up by a periodic stacking of the oligosaccharide skeleton are packed into a two-dimensional hexagonal lattice.

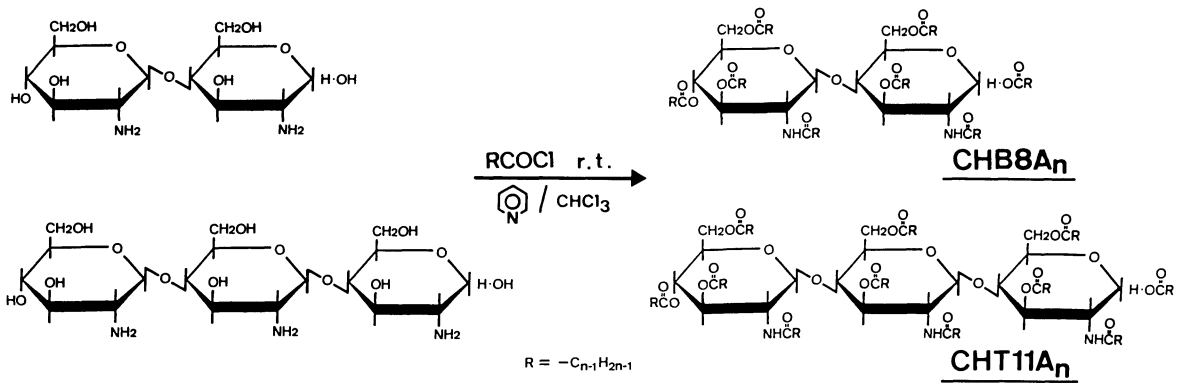
These features of cello-oligosaccharide derivatives have urged us to synthesize acylated chito-oligosaccharides, as it is very interesting to see the differences in the thermal behavior between these two types of compounds. Chito-oligosaccharides such as chitobiose and -triose, which consist of repeating D-glucosamine units with 1,2-*trans* glycosidic linkage, are distinguished from the cello-equivalents by the presence of a primary amino function at the C2 position. The amido group of the acylated products is, therefore, expected to bring about a rather strong interaction, e.g., hydrogen-bonding, which would affect the mesomorphic behavior.

In this study, we have synthesized acylated chito-oligosaccharides to show, by polarized microscopic and a differential scanning calorimetric (DSC) analyses, that chito-oligosaccharides provide a new family of carbohydrate-based liquid crystals.

Results and Discussion

Synthesis of Chitobiose Octaalkanoates and Chitotriose Undecaalkanoates (CHB8A$_n$'s and CHT11A$_n$'s). Acylation of chitobiose and chitotriose was carried out according to the previously reported procedure[13] with a few modifications. As shown in Scheme 1, chito-oligosaccharides were reacted with long-alkyl fatty acid chlorides in the presence of pyridine as a catalyst at room temperature. The acid chlorides that were used in this study were hexanoic, decanoic, tetradecanoic, and octadecanoic versions. The acylation products, chitobiose octaalkanoates and chitotriose undecaalkanoates, will be abbreviated as CHB8A$_n$'s and CHT11A$_n$'s, respectively, in which 8 and 11 refer to the number of alkyl substituents and *n* to the number of the total carbon atoms in the substituents.

On addition of an acid chloride to the colorless dispersion solution of the chito-oligosaccharide, the reaction mixture immediately turned yellow and then



Scheme 1. Synthesis of chitobiose octaalkanoates (CHB8A$_n$'s) and chitotriose undecaalkanoates (CHT11A$_n$'s).

gradually changed to an orange homogeneous solution with proceeding acylation. After purified by reprecipitation with methanol and subsequent recrystallization with diethyl ether, the end products were recovered as a white waxy solid or a white powder, with yields of ca. 70%. (Derivatives with short side chains, CHB8A$_6$, CHB8A$_{10}$, and CHT11A$_6$, were purified by means of preparative size exclusion chromatography as well as recrystallization, with final yields around 20%. These low yields are ascribed presumably to the formation of by-products whose hydroxyl groups were not completely blocked with alkyl substituents. Furthermore, because of the small differences in solubility among the products acylated to differing degrees, a considerable portion of the end product might have been lost during the isolation process.) In contrast to the results of the synthesis of the polymeric analogues,[13] the *N*,*N*-diacylated derivatives were formed in no appreciable amount even after a long reaction.

Figure 1 shows the IR spectra of the reaction product CHB8A$_n$ (n=14) and its precursor chitobiose hydrochloride. A comparison of the two spectra proves that the hydroxyl and the primary amino groups were quantitatively converted to the corresponding ester and amido functions. For example, the O–H and N–H stretching bands (3000—3500 cm^{-1}), and the N–H bending band (1630 and 1510 cm^{-1}) in spectrum B for the starting material completely disappeared in spectrum A for CHB8A$_{14}$, and new strong bands appeared at ca. 2900, 1740, 1655, and 1525 cm^{-1} relevant to the C–H stretching, C=O(–O) stretching, C=O(–NH) stretching, and N–H bending modes, respectively.

The formation of the desired products was further confirmed by ^{1}H NMR spectroscopy. Figure 2 illustrates the ^{1}H NMR spectrum of CHB8A$_{14}$, in which all signals are consistent with the expected structure. For example, the four intense absorptions assignable to the amido- and the ester pendants appear: peak **a** (–CH$_2$COO– and –CH$_2$CONH–, δ=2.05—2.40); peak **b** (–CH$_2$CH$_2$CO–, δ=1.55); peak **c** (–(CH$_2$)$_{10}$CH$_3$, δ=1.25); peak **d** (–CH$_3$, δ=0.85). Along with these absorptions,

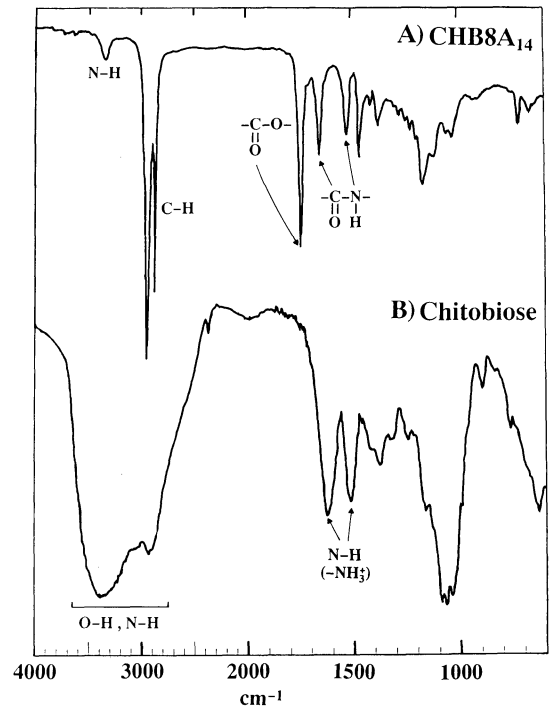


Fig. 1. Infrared spectra of (A) CHB8A$_n$ (n=14) and (B) Chitobiose hydrochloride.

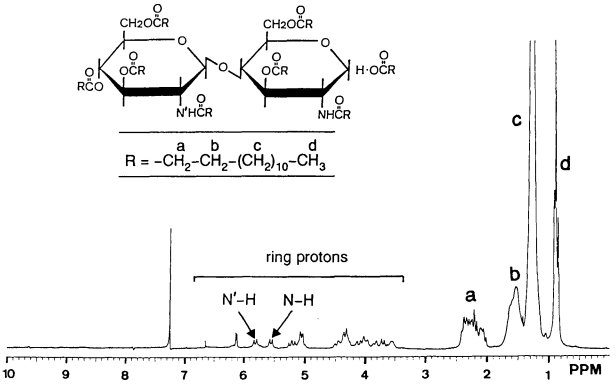


Fig. 2. ^{1}H NMR spectrum of CHB8A$_{14}$ (in CDCl$_3$ at 30°C).

Table 1. Characterization of the Acylation Products

Sample	Elemental analysis			$N^{a)}$	Appearance
	%C	%H found (calcd)	%N		
$CHB8A_6$	64.6 (64.2)	9.01 (9.10)	2.79 (2.50)	7.2	Waxy solid
$CHB8A_{10}$	70.1 (70.3)	10.7 (10.6)	1.81 (1.87)	8.0	Waxy solid
$CHB8A_{14}$	72.7 (73.6)	11.5 (11.5)	1.42 (1.39)	8.0	White powder
$CHB8A_{18}$	74.3 (75.7)	11.9 (11.9)	1.25 (1.23)	8.0	White powder
$CHT11A_6$	64.3 (63.8)	9.41 (9.24)	2.60 (2.66)	10.9	Waxy Solid
$CHT11A_{10}$	68.9 (69.9)	10.6 (10.7)	1.95 (1.91)	11.0	White powder
$CHT11A_{14}$	73.0 (73.3)	11.4 (11.5)	1.51 91.49)	11.0	White powder
$CHT11A_{18}$	75.3 (75.6)	11.9 (12.0)	1.22 (1.22)	11.0	White powder

a) Number of acyl pendants determined by ^1H NMR.

complicated signals attributable to the pyranose ring protons can also be observed. Among them, the two characteristic peaks at δ=5.55 and 5.80 were assigned, respectively, to the secondary amido functions (N–H and N′–H),[14] which supported that the product to be the *N*,*N*′-diacylated (not *N*,*N*,*N*′,*N*′-tetraacylated) derivative. In addition, the doublet at δ=6.12 indicates the α-anomeric configuration.[14] Thus, the acylation reaction occurred with an excellent stereoselectivity to give α-anomers predominantly.

As shown in Table 1, the number of the acyl pendants, *N*, which was determined by the peak intensity ratio of the pendant methyl protons and the pyranose ring protons, is very close to the expected value (8 and 11 for di- and trisaccharide derivatives, respectively), which also evidences the absence of by-products. The results of an elemental analysis are listed in Table 1. Irrespective of the acyl length *n* and the kind of precursors, the observed elemental contents are in fair agreement with those calculated for the expected structure. Chitobiose and chitotriose hydrochlorides are soluble only in water and methanol, while both $CHB8A_n$ and $CHT11A_n$ are soluble in a number of common organic solvents such as tetrahydrofuran, chloroform, and benzene.

Thermal Behavior. Figure 3 shows two respresentative DSC thermograms observed for $CHB8A_{14}$ and $CHT11A_{14}$ on heating. Both exhibit two transitions between the room temperature and 200°C. The higher transitions can be ascribed to the isotropization of a mesophase and the lower ones to the melting of the crystal to the mesophase, as was confirmed by an optical microscopic observation (see below). Other specimens, except ones with n=6, showed similar transition behavior. The thermal data are listed in Table 2. As the table shows, the mesophases appear at temperatures between 65—75°C and 170—207°C. Some narrowing of the mesomorphic region can be observed with increasing alkyl length. $CHB8A_6$ and $CHT11A_6$ decompose at around 200°C without reaching an isotropic temperature. The temperature regions of mesophase of the chito-derivatives are appreciably higher and longer

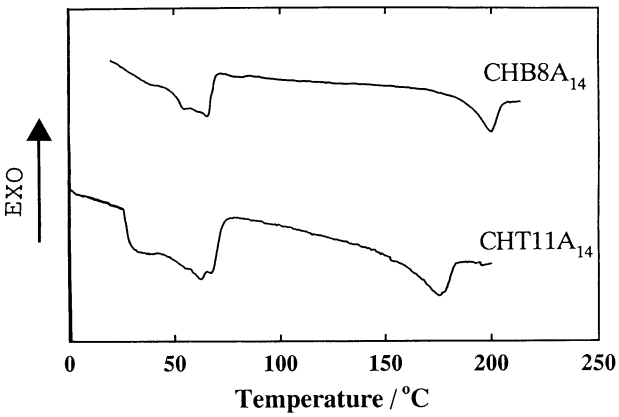


Fig. 3. DSC traces of (A) $CHB8A_{14}$ and (B) $CHT11A_{14}$ recorded on heating at a rate of 10°C min^{-1}.

Table 2. Phase Transition Temperatures[a] (°C) and Enthalpy Changes ΔH (kcal/mru[b]) at the Isotropization Point of the Peracylated Chito-Oligosaccharides

Sample	C	M	I	ΔH
$CHB8A_6$[c]	—	—		—
$CHB8A_{10}$	65	207		2.67
$CHB8A_{14}$	70	196		2.83
$CHB8A_{18}$	73	185		4.20
$CHT11A_6$[c]	—	—		—
$CHT11A_{10}$	60	195		4.76
$CHT11A_{14}$	65	175		1.50
$CHT11A_{18}$	75	170		2.97

a) C stands for the crystalline phase, M for mesophase, and I for the isotropic phase. b) mru=mole-repeat-unit. c) Decomposed at around 200°C.

than those of the cello-equivalents, the latter ranging from about 50°C to about 100°C.[12] In addition, the chito-derivatives exhibit somewhat broader phase transition peaks. The enthalpy changes associated with the isotropization, ΔH, are also listed in Table 2. These values are much larger than those of the cello-derivatives (ΔH for $CEB8A_{10}$, $CEB8A_{14}$, and $CET11A_{10}$ were found to be 1.1, 0.64, and 1.2 kcal/mru, respectively,[12] where

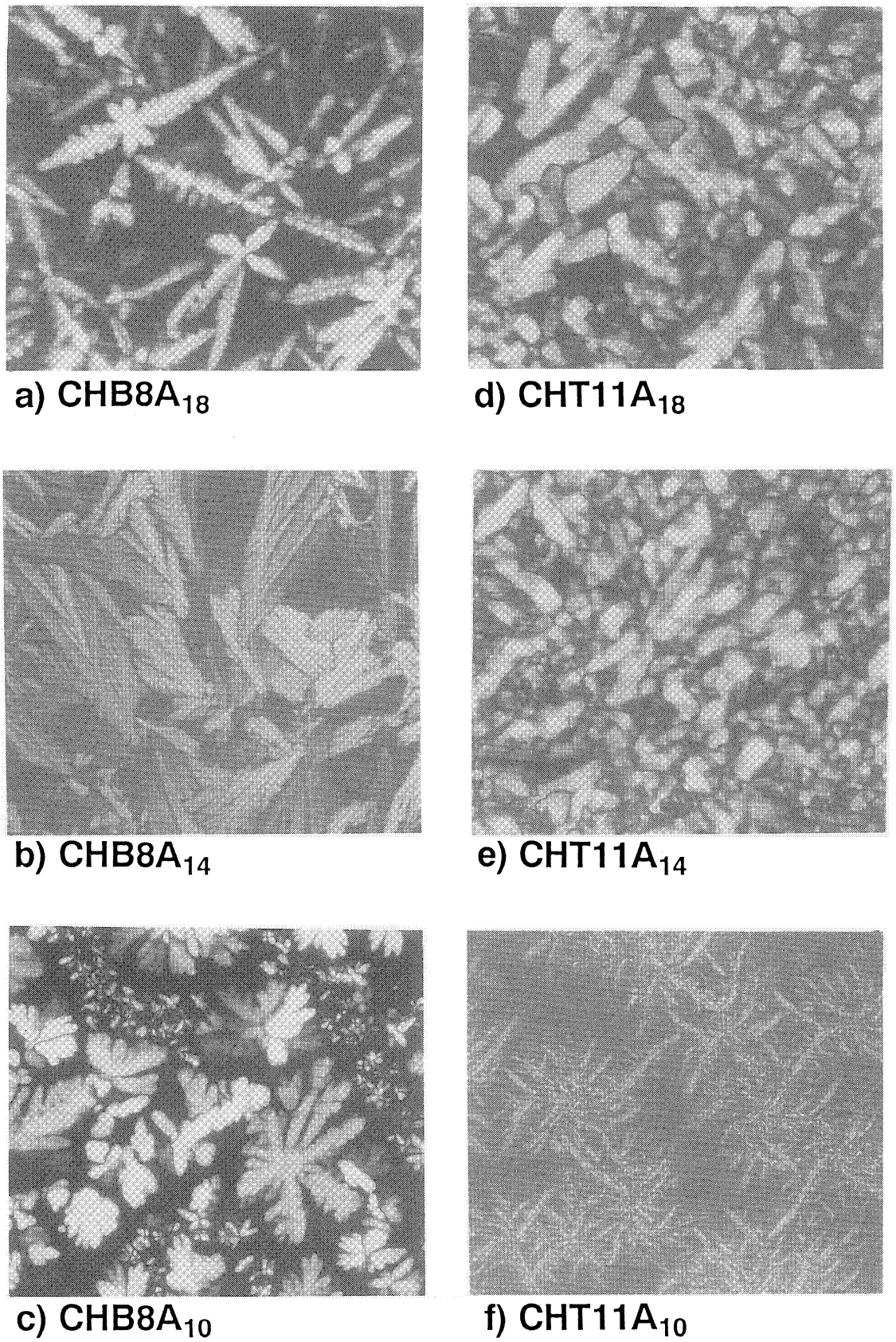


Fig. 4. Optical microscopic textures observed for acylated chito-oligosaccharides on cooling slowly; codes of the compounds are as indicated.

CEB and CET denote cellobiose and cellotriose, respectively, and mru stands for mole-repeat-unit). These differences could be attributed to the possible hydrogen bonding formed between the secondary amido group at the C2 position of the glucopyranose unit and the ester group.

A polarized microscopic observation at regulated sample temperatures was made to confirm the mesomorphic behavior. Each sample was heated to above its isotropization temperature T_i, and cooled down very slowly to be observed under a microscope. At a temperature somewhat below T_i, CHB8A$_n$'s exhibited a germ with birefringence appearing from the isotropic phase. On further cooling, more and more germs appeared and grew into leaf- or flowerlike shapes, as shown in Figs. 4a to 4c. CHT11A$_n$'s also showed similar mesophase textures but their structures were smaller than those of CHB8A$_n$'s (Figs. 4d to 4f). Finally the germs coalesced into a not-well-defined texture, which turned to a fan-shaped texture upon shearing the specimens between glasses. These textural features are essentially similar to those observed for the cello-oligosaccharide equivalents, and suggest that the mesophase of the chito-derivatives is likely classified into the same type as that of the cello-versions, that is, a discotic columnar phase. A need is apparent for a more extensive study by, e.g., X-ray diffraction, before a final conclusion about the mesophase classification can be reached. Such a study will be reported elsewhere.

Experimental

Materials. CHB8A$_n$ and CHT11A$_n$ (n=6, 10, 14, and 18) were prepared by the acylation of chitobiose and chitotriose with corresponding fatty acid chlorides in chloroform/pyridine. Chitobiose and chitotriose (Seikagaku Kogyo Co., Ltd.) were obtained as a hydrochloric acid salt and employed without further purification. Acid chlorides (Tokyo Kasei Kogyo Co., Ltd.) were used as received. Chloroform and pyridine (Nacalai Tesque, Inc.) were distilled over calcium hydride prior to use.

Methods. In a typical preparation, chitobiose hydrochloride (200 mg; 0.59 mmol) was soaked in pyridine (10 ml) overnight and then the pyridine was evaporated off under a reduced pressure. The liberated chitobiose was dispersed in a mixture of pyridine (20 ml) and chloroform (20 ml) with magnetically stirring. To this solution, tetradecanoyl chloride (1.6 ml; 10 equiv to the chitobiose) was added dropwise by use of a syringe at room temperature. After an hour of the reaction, the solution was poured into methanol to remove the excess acid chloride. The precipitate was filtered off, reprecipitated repeatedly with diethyl ether, and dried in vacuo to obtain CHT8A$_{14}$ in a white powder. All other derivatives were prepared in a similar way, except derivatives with short side chains (CHB8A$_6$, CHB8A$_{10}$, and CHT11A$_6$), which were isolated and purified by preparative size exclusion chromatography and recrystallization.

Measurements. Infrared (IR) spectra were obtained with a JASCO FT/IR spectrometer Model 8800, Japan. ^{1}H NMR spectra were recorded in $CDCl_3$ on a Varian VXR-200 (200 MHz) instrument, the United States. Thermal phase behavior was studied by differential scanning calorimetry (DSC) on a Rigaku Denki Model DSC-8230, Japan, at a constant heating/cooling rate of 10 °C min^{-1}. Thermal optical microscopy was performed on a Nikon Model Optiphoto-Pol, Japan, employing a Mettler Model FP-82 hot-stage, Switzerland.

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