

Cholesterol-Linked β-Cyclodextrin— A Thermotropic Liquid-Crystalline Derivative

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Liquid-crystalline derivative of β-cyclodextrin (BCD) was prepared by covalent linking of monocholesteryl succinate (ChMS) with β-cyclodextrin. To the best of our knowledge, this was first ever attempt so far of its kind, in which BCD has been converted into its liquid-crystalline derivative through covalent linkage of a mesogen. The degree of substitution (DS) obtained was ≈2.00. The product was characterized by various techniques, such as FT-IR, NMR, DSC, hot-stage-coupled optical polarizing microscopy (OPM), microanalysis and chemical methods. Cholesterol-linked β-cyclodextrin (CDCh) derivative was found to exhibit thermotropic liquid-crystalline behavior. The product exhibited birefringence during first heating above 130 °C, and it became isotropic at about 180 °C, whereas the parent compound BCD decomposed without melting above 250 °C. A comparison of CDCh derivative to similar liquid-crystalline polysaccharide derivatives is presented.

Semirigid- and rigid-structured polymers have been reported to form ordered phases in concentrated solutions.[1,2] Also, there are reports, in which they form thermotropic liquid-crystalline phases. Cellulose and cellulose derivatives exhibiting ordered phases have been reported.[1] Hydroxypropyl cellulose[3] and its derivatives[4–6] have been reported as thermotropic materials. Bhadani and Gray[6] have prepared a series of esters, namely, propionic, butyric, isobutyric, and phthalic acid esters, of hydroxypropyl cellulose that display thermotropic liquid-crystalline behavior. Guo and Gray have investigated chiroptical properties of lyotropic solutions of mixed ester–ether derivatives of cellulose.[7–10] Shaikh et al. have recently shown the liquid-crystalline behavior of some polysaccharide derivatives, in which a steroidal mesogen is linked covalently to the polysaccharides. In a typical reaction, an excess of succinic anhydride is reacted with cholesterol in pyridine as a solvent at 90 °C for 24 h. The product is then linked covalently to various polysaccharides, e.g., cellulose acetate, ethyl cellulose, hydroxyethyl cellulose, starch, and hydroxyethyl starch, in our previous work. It has been reported that the cholesterol-linked polysaccharides do not show clear liquid-crystalline transitions due to polydispersity, high molecular weight and molecular weight distribution, hydrogen-bonding, etc.[11–13] However, there is ample evidence for the formation of thermotropic and lyotropic liquid-crystalline phases in polysaccharides with or without covalently attached mesogen.

Several oligosaccharide-based liquid-crystalline derivatives have been reported.[14–17] These derivatives of oligosaccharides do not utilized a mesogen as a substituent to confer liquid crystallinity in the molecule. However, it is conferred by preparing higher alkyl[14,15,17] or acyl[16] derivatives of respective oligosaccharides. There are reports, in which the steroidal molecule has been covalently linked to the molecules like crown ethers.[18] Cholesterol-based liquid-crystalline derivatives of low molecular weight compounds have been reviewed in detail.[19] There is no report, so far, in which β-cyclodextrin has been utilized as a backbone material to confer the liquid crystallinity using a steroidal mesogen, especially cholesterol, through covalent linkage.

β-Cyclodextrin and other oligosaccharides have been known for about one hundred years.[20] The first patent on their complexes was registered in 1953.[21] In the pharmaceutical industry, cyclodextrins (CD) and their derivatives have mainly been used as complexing agents to increase the solubility of poorly water-soluble drugs, due to its relatively hydrophobic central cavity and hydrophilic outer surface. In addition, CD can also be used for several other application, like elimination of unpleasant smells or tastes, and to prevent drug–drug, drug–additive interactions, etc.

In the present work, BCD was converted into its liquid-crystalline derivative with the anticipation that the property of "complexation" can be enhanced several times further by the virtue of organized structure formation, if the BCD is made liquid crystalline. In addition, the possibility of complexation should further be improved due to the possibility of mixed mesophase[11,12] formation along with its own ability to form complexes. Thus the material should have applications in the field of biomedical or pharmaceutics as well as its usual applications as liquid crystals. However, for the present paper, we restricted our work to the synthesis and characterization of liquid-crystalline derivatives of BCD. In a work to be published separately, we will emphasize the effect of liquid-crystalline BCD on complex formation.

Experimental

BCD was donated by Yangzhou Pharmaceutical Co., Ltd.,

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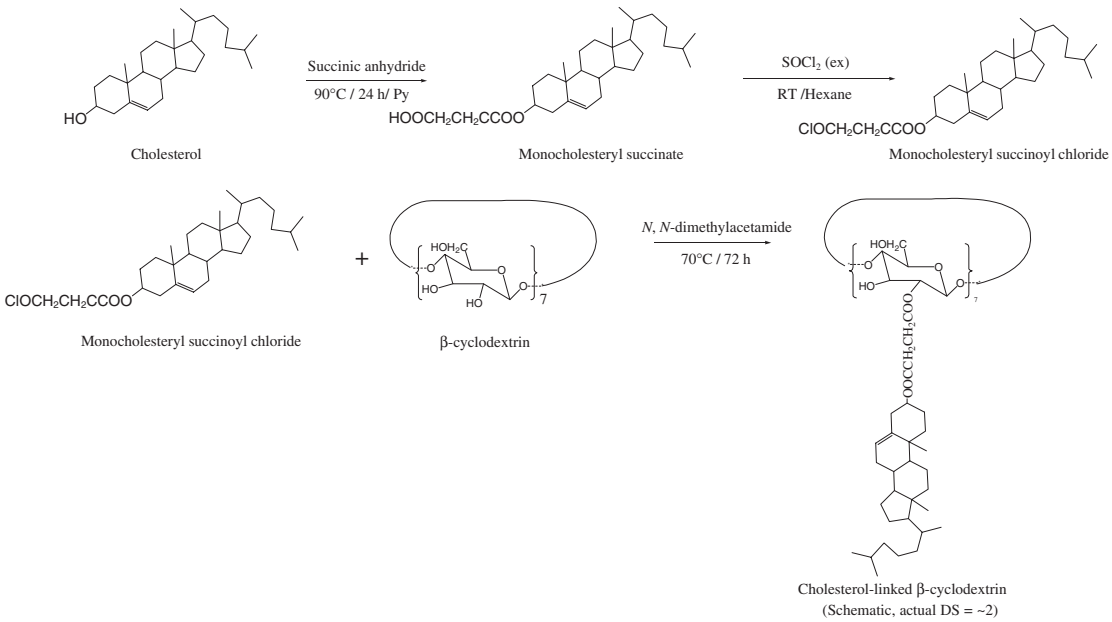

Fig. 1. Synthesis scheme for cholesterol-linked β-cyclodextrin.

China with a purity of 99.30%. This was further purified by recrystallizing from distilled water and drying under vacuum at 110 °C for 24 h before use.[22] Cholesterol and succinic acid were obtained from S. D. fine chemicals, India and were used as received. Other solvents and reagents obtained from commercial sources were purified according to reported procedures.[22]

3-Cholesteryloxycarbonylpropionic acid (monocholesteryl succinate) (ChMS) was prepared according to a published procedure.[11] Various characterization methods, such as IR, NMR, DSC, hot-stage-coupled optical polarizing microscopy (OPM), elemental analysis, and few chemical methods, were employed to ascertain the synthesis and liquid-crystalline properties of the products. IR spectra were recorded on a Perkin-Elmer FT-IR Spectrum GX Model calibrated with polystyrene. The samples were taken in the form of KBr pellet, and spectra were recorded in the range of 4000–400 cm^{-1}. ^1H NMR spectra were recorded on Bruker AV 200 MHz NMR spectrometer at 303 K in $CDCl_3$ (concentration, 40 mg mL^{-1}) using a 5-mm diameter NMR tube. The thermal properties of the samples were investigated by using differential scanning calorimeter TA series instrument, Model DSC Q10, U.S.A. Heating and cooling rate was 10 °C min^{-1} unless otherwise mentioned. In addition, the samples were also characterized by using optical polarizing microscope (OPM)—Olympus Electron Microscope, Model—BX50F4 equipped with a Mettler Toledo heating stage type: FP90.

Synthesis of Monocholesteryl Succinoyl Chloride. Succinic acid (S. D. fine) was converted to succinic anhydride as per the literature procedure.[23] Cholesterol was converted to its cholesteryl hydrogen succinate as per our earlier reported procedure.[11] A predetermined quantity (4 g) of monofunctional acid derivative of cholesterol (ChMS) was placed in a three-neck round-bottomed flask equipped with reflux condenser, calcium chloride guard tube and magnetic stirrer. Dry and distilled hexane (10 mL) was added to the flask followed by addition of excess of thionyl chloride (10 mL approx.). The reaction mixture was stirred at room temperature for 24 h. After 24 h, dry and distilled benzene (10 mL) was added to the reaction mixture, and the mixture was distilled under reduced pressure to remove excess of thionyl chloride. The complete removal of thionyl chloride was ensured by repeated addition of benzene and distillation under reduced pressure. The obtained dark brown colored, low melting, sticky mass was utilized directly (without further purification and storing) for the modification of oligosaccharides.

Cholesterol-Linked β-Cyclodextrin Derivatives. Monocholesteryl succinoyl chloride (obtained from 4 g of ChMS), the product obtained from the above reaction was dissolved in minimum quantity of dry and distilled *N*,*N*-dimethylacetamide (DMAc) stored over molecular sieves for several days. Previously recrystallized and dried BCD (0.5 g) was added to the above solution in a three-neck round-bottomed flask equipped with magnetic stirrer, condenser, calcium chloride guard tube, and nitrogen inlet. The reaction mixture was stirred at about 70–72 °C for 72 h. (Please see synthesis scheme, Fig. 1). The heterogeneous reaction mixture turned into thick, viscous, and dark brown-colored homogeneous product. After of 72 h, the reaction mixture was precipitated in methanol. The precipitate was stirred vigorously to convert the lumps into a fine powder. The product obtained was then filtered through a sintered funnel to obtain a light brown-colored fine powder, which was purified by Soxhlet extraction with methanol. The purity of the product was confirmed by thin layer chromatography. The product was dried under vacuum at 40–50 °C for several hours.

Results and Discussion

Cholesterol-Linked β-Cyclodextrin (CDCh). Cholesteryl hydrogen succinate was converted into its acid chloride and was subsequently reacted with β-cyclodextrin to obtain the cholesteryl hydrogen succinate derivative of β-cyclodextrin. The reaction conditions were chosen based on our earlier work.[11]

The overlay of FT-IR spectrum of CDCh along with ChMS and BCD is shown in Fig. 2. A substantial decrease in the intensity of hydroxy stretching band appearing between 3350 and 3500 cm^{-1} was observed, indicating very high DS (degree of substitution per AHG unit of BCD) ($\approx$2.00 in the present

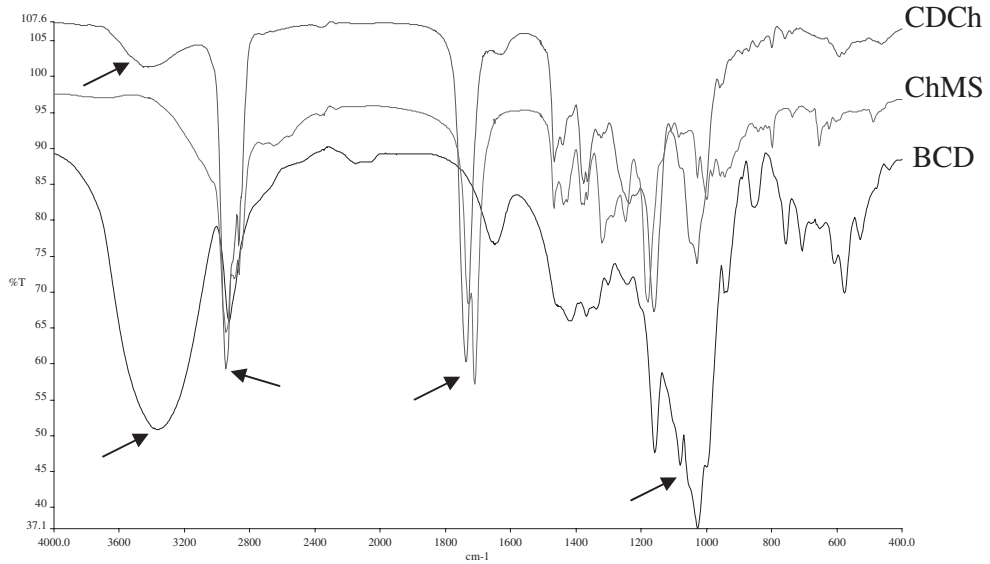

Fig. 2. Overlay of FT-IR spectrum of CDCh, ChMS, and BCD.

investigation). A very sharp peak due to >C=O stretching vibrations was seen at 1737 cm^{-1}, which confirms esterification. A significant increase in the intensity of $-CH_2-$ stretching vibrations between 2800 and 2900 cm^{-1} is due to the large number of methylene groups of the ChMS moiety. In addition, CDCh also showed peaks due to cholesterol and anhydroglucose units, confirming the product–cholesterol-linked β-cyclodextrin.

The degree of substitution of cholesterol was estimated by measuring the weight increase of the sample and also by using chemical analysis.[11] In a typical run, about 0.1–0.3 g of the dried polymer sample was dissolved in 20-mL pyridine, and then, 20 mL of standard NaOH was added. The hydrolysis was carried out for 1 h at reflux. After cooling to room temperature, the excess alkali was back-titrated conductometrically with standard hydrochloric acid. A blank titration was carried out in a similar manner using the corresponding oligosaccharide and cholesterol in a weight ratio nearly the same as that of the product, which was estimated from the weight increase.

The estimated DS, which was equal to 2 in the present synthesis, is comparatively towards higher side. This observation was further supported by the marked decrease in the hydroxy-stretching band intensity appearing at 3350–3500 cm^{-1} and substantial increase in the intensity at about 1730 cm^{-1} due to ester >C=O in the IR spectrum (Fig. 2). The maximum possible DS is 3.

NMR spectrum of CDCh is shown in Fig. 3. In addition to the multiplet at 2.65 ppm, which is due to adjacent methylene groups of succinic acid moiety in ChMS, peaks in the range 3.0–5.2 ppm were also observed confirming the glucopyranose ring protons. It should be noted that the discernible peaks were not seen except in the region 3.0–4.0 ppm, which is due to relatively poor resolution of cellulosic and similar class of materials in NMR spectra.[11] The overall presence of peaks in the range 3.0–5.2 ppm and at 2.65 ppm in proton NMR spectrum of CDCh confirms the structure of cholesterol-linked β-cyclodextrin. Other peak assignments were similar to those reported earlier.[24]

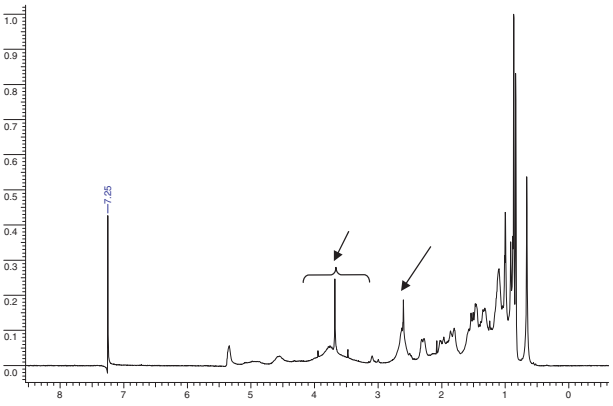

Fig. 3. ^{1}H NMR spectrum of CDCh.

Cholesterol-linked β-cyclodextrin, CDCh, was also checked for thermotropic mesomorphic behavior using an optical polarizing microscope. Like cellulosic polymers, β-cyclodextrin also has a semi rigid structure and chirality due to anhydroglucose units, which is a primary requirement (sufficient stiffness of the backbone and asymmetric interactions) for realizing a liquid-crystalline phase[6,7] in such class of materials. Moreover, the β-cyclodextrin molecule is more rigid due to cyclic structure, in which seven anhydroglucopyranose units are linked in a cyclic manner. There is no report, so far, in which β-cyclodextrin is converted into its liquid-crystalline derivative in particular by covalent linking of mesogens. The present investigation, to the best of our knowledge, is the first attempt of its kind, in which β-cyclodextrin was successfully converted into its liquid-crystalline derivative. In our earlier work, we have attempted to synthesize liquid-crystalline derivatives of polysaccharides through covalent linkage of mesogens.[11–13]

BCD is not a thermotropic material, and it decomposes without melting above 250 °C. However, the BCD derivative was found to melt clearly at about 180 °C, which is even below the decomposition temperature of BCD. BCD has several hydroxy functional groups in its structure, which lead to strong

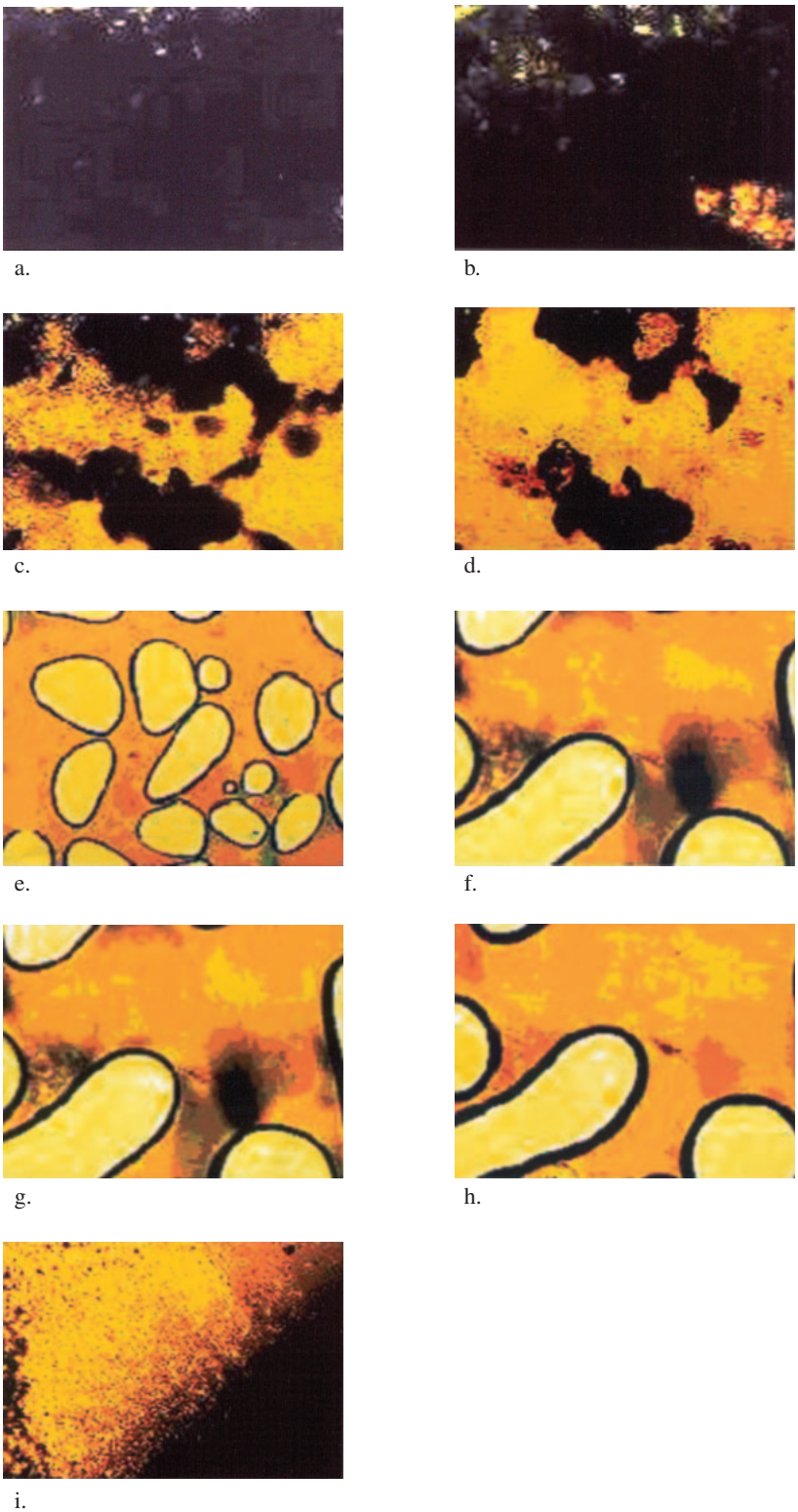

Fig. 4. Photomicrographs of CDCh (Magnification—a, b, c, d, e, i 200×, f, g, h 500×).

hydrogen bonding that causes the molecule to decompose before melting. CDCh melts due to disruption of the H-bonds due to covalent linking of the monocholesteryl succinate, i.e., –OH groups in BCD are replaced with cholesteryl moieties. While checking for thermotropic mesomorphic behavior, a very small amount of initially vacuum dried sample at room temperature was sandwiched between a clean glass plate and a cover glass. The cover glass was slightly pressed so as to make a thin film of the sample on the glass slide. The sample was slightly yellowish in color. The sample was heated at a programmed heating rate of 10 °C per minute from room temperature. The initial dark field (Fig. 4a) under cross-polars started

showed birefringence at about 130 °C (Fig. 4b), which continued to increase until the sample became completely birefringent (Figs. 4c and 4d). The birefringence started flowing and disappearing (isotropization) from 170 °C onwards. The sample turned totally isotropic at about 180 °C. It was held at this temperature for about five minutes and then cooled. Slight birefringent regions were seen upon cooling from 125 °C (Fig. 4e), and it increased slightly on further cooling and remained until room temperature (Figs. 4f and 4g). The same sample was reheated (2nd heating) at the same rate of 10 °C per minute. The birefringence that formed on cooling started to disappear from 135 °C onwards, and the sample became isotropic (Fig. 4h). The possibility of a homeotropic phase formation was ruled out, because birefringence could not be seen even after the sample was deformed by slight moving and pressing the cover glass, while heating as well as cooling. It was found to melt at the same temperature as above.

In an another attempt to characterize the CDCh under OPM, the sample was heated directly to 145 °C at a programmed heating rate of 10 °C per minute. It was held at this temperature for 10 min and cooled to room temperature. The birefringence developed at 145 °C was retained at room temperature, indicating formation of "liquid-crystal glass" (Fig. 4i). Clearly identifiable textures were not seen in any of the case under OPM.

Differential scanning calorimetric (DSC) (Fig. 5) was conducted to establish the liquid-crystalline behavior of the CDCh. The heating and cooling rate was programmed at 10 °C per minute. Here, the sample was heated from −50 °C instead of room temperature, as the later was featureless during first heating, cooling and second heating run. The DSC study was in reasonable agreement with that of OPM study. However, the structure of BCD plays a role in the final properties of the product. It was anticipated that, due to oligomeric (small) structure of β-cyclodextrin, CDCh would show clear thermal transitions in contrast to the corresponding polysaccharide derivatives,[11–13] which is due to their very high molecular weight, polydispersity, non-solubility of the backbone, non-melting character, non-uniform substitution of the mesogens, etc. However, no clear transitions were observed in the DSC thermograms. This is further evidenced by the fact that these polysaccharide derivatives also did not have any clearly identifiable textures under OPM. In the present investigation, even

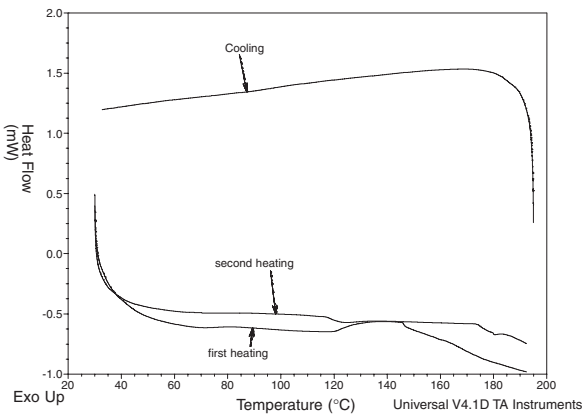

Fig. 5. DSC thermograms of CDCh.

though the backbone utilized was oligomeric, the textures were not clearly identifiable. Only birefringence could be seen under OPM. In addition, the melting character of the product was unique. The sample, when heated in a capillary, first underwent a volume contraction at about 130 °C and then it stuck to the sidewalls of the capillary as if showing the signs of melting at about 150–160 °C. On further heating the sample spread on the sidewalls of the capillary at about 170 °C and then underwent volume expansion above 175 °C. However, when observed under OPM, it was found that the sample starts exhibiting birefringence at about 130 °C that increased until it started melting above 170 °C. The melt was very viscous and sticky in nature, suggesting that the product is not of the typical low molecular weight material. The overall "abnormal" melting behavior of the CDCh can be attributed to the basic non-melting and cyclic structure of the parent BCD. Also, the higher DS (2.00 in the present work) of the bulky molecule–cholesterol molecule that causes "overcrowding" on the BCD makes the CDCh molecule "stodgy." The first heating DSC thermogram showed an exotherm in the range of 162–180 °C corresponding to the organized phase formation as seen under OPM. It continued melting and decomposing from about 200 °C onwards. At this temperature, the sample turned dark brown in color as observed under OPM. In addition, the sample was investigated for decomposition by IR spectroscopy after first heating. The peak due to hydroxy groups (free) disappeared, whereas other peaks remained intact, indicating partial decomposition. The cooling thermogram was featureless whereas the second heating showed a glass to liquid-crystal transition at about 120–125 °C. This is in contrast to the OPM observations, in which no birefringence was seen during the second heating. Slight birefringence was seen on cooling around 125 °C, which remained at room temperature, and on reheating, it disappeared at about 135 °C. No endotherm was seen during the second heating, most probably due to the fact that the sample did not crystallize on cooling. This is further supported by the observations made under OPM, during which crystallization was not observed on cooling. It can be interpreted that the bulky and complex nature of the molecule hinders the crystallization process. Also, the partial decomposition as studied by IR supports the non-reproducibility of LC phase formation on cooling and second heating.

It is very interesting to note here that the temperature of transition, i.e., crystal to liquid crystal and liquid crystal to isotropization, are comparable to that of the mesogen (ChMS) transitions. As anticipated, the mesogenic character overrides the oligomeric character. That is, the transition temperatures are more or less similar to those of the mesogen ($T_m = 180$ °C) than the oligomer (decomposes without melting). This can be attributed to the "excess" of ChMS (DS 2.00) on the oligomer, which causes the properties of ChMS to be more prominent and the properties of BCD to fade away. However, it is important to note that, in the case of ChMS, the mesogenic character was observed while cooling[25] whereas CDCh exhibits mesogenic character during the first heating.

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Supporting Information

IR of CDCh before and after heating at 200 °C. This material is available free of charge on the web at http://www.csj.jp/journals/bcsj/.

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