Thermotropic Liquid Crystals Based on Chito-Oligosaccharides III. Effect of the Molecular Shape of N,O-Acylated Chito-Oligosaccharides on the Discotic Mesomorphism

Makoto SUGIURA, Masahiko MINODA, Junji WATANABE,*
and Takeaki MIYAMOTO

*Institute for Chemical Research, Kyoto University,
Uji, Kyoto 611, Japan*

(Received March 11, 1994)

ABSTRACT: Acylated derivatives of chitosan oligomers with a relatively high degree of polymerization ($DP=4, 5$, or 6) were synthesized and their mesophase properties were studied in comparison with those of the lower- DP homologues ($DP=2$ and 3) previously reported. By differential scanning calorimetry (DSC), all the compounds were found to show thermotropic mesomorphism in a temperature region below 150°C . The isotropization temperatures decreased with increasing DP , while the melting point remained at around 65°C , independent of DP . In addition to the narrowing of the mesomorphic region, the optical anisotropy as observed under a polarizing microscope remarkably decreased with increasing DP , indicating that the mesophase becomes less stable and ordered with an increase in DP . An X-ray diffraction analysis revealed that the tetramer as well as the dimer and trimer form a hexagonal ordered columnar phase (D_{ho}), while the pentamer forms a disordered one (D_{hd}). On the other hand, the phase formed by the hexamer was not a columnar but a discotic nematic phase (N_D). This is attributable to the highly anisotropic shape of the oligosaccharide core in the hexamer.

KEY WORDS Chito-Oligosaccharide / Discotic Liquid Crystals / Degree of Polymerization (DP) / Anisotropic Molecular Shape / Hexagonal Columnar Phase /

The geometric shape of a mesogen is closely related to the mode of molecular arrangement in the liquid crystalline state, as is clearly observed in the structural difference between the calamitic mesophases formed by rigid-rod molecules and the discotic mesophases formed by disc-shaped molecules. In the discotic liquid crystals so far reported,¹ the existence of an aromatic, rigid, flat and/or symmetric disc-like mesogen has been believed to be essential for generating some class of discotic mesophases. In fact, highly symmetric discogenic compounds often show a hexagonal-ordered discotic columnar phase (D_{ho}), the most ordered structure in discotics known so far. However,

it should be noted that a few compounds with a lower symmetry, *e.g.*, a two-fold rotational symmetry can form, like disc-shaped molecules, a discotic columnar phase (D_h). For example, alkyloxy substituted nitrophenylhydrazones with semi-discoidal molecular shape were found to induce a D_h phase due to both the intramolecular hydrogen-bond formation and the intermolecular donor-acceptor interaction.² We have also demonstrated that acylated cello- and chito-oligosaccharides can form an enantiotropic D_{ho} phase.³⁻⁶ Compared to the mesophase of the cello-versions, those of the chito-oligosaccharide derivatives were characterized by an enhanced thermal stability, in

* On leave from Faculty of Engineering, Tokyo Institute of Technology, Ookayama, Meguro-ku, Tokyo 152, Japan.

which the intermolecular hydrogen-bond interaction derived from the secondary amido functions at the C-2 position of the repeating pyranose ring plays an important role.

Previous studies dealt with the chitobiose and chitotriose derivatives.^{5,6} In this study, we synthesized a series of acylated chito-oligosaccharides with a higher degree of polymerization ($DP=4, 5$, or 6). Because of the rigid and linear structure due to the β -1,4 linkage between the repeating units, the axial ratio of the chito-oligosaccharide core proportionally increases with an increase in DP . The increase in this axial ratio is expected to make the discotic mesophase less stable, compensating the strong intermolecular interaction and eventually rendering the discotic columnar phase utterly unstable. We examined the thermal properties and mesophase structure of these acylated chito-oligosaccharides to study their discogenic character and the stability of the mesophase.

EXPERIMENTAL

Materials

Chito-oligosaccharides (Yaizu Suisan Co., Ltd., Japan) were obtained as a hydrochloric acid salt (purity, 95–100%) and employed without further purification. Acid chlorides (Tokyo Kasei Kogyo Co., Ltd., Japan) were used as received. Chloroform and pyridine (Nacalai Tesque, Inc., Japan) were distilled over calcium hydride prior to use. N,O-Acylated chitotetraose, chitopentaose and chitohexaose (Figure 1) will be abbreviated as CHT m An ($m=4$ – 6 , $n=10$ – 18), in which m refers to the degree of polymerization and n to the total number of the carbon atoms in the substituents.

Acylation of chito-oligosaccharides was carried out by reacting them with a long-alkyl fatty acid chloride in the presence of a base according to the previously reported procedure⁵ with some modifications made to cope with the decreased reactivity and solubility of

Degree of polymerization (m)	Sample code	n
2	CHT2An	10,12,14,18
3	CHT3An	10,12,14,18
4	CHT4An	10,12,14,18
5	CHT5An	10,14,18
6	CHT6An	14,

Figure 1. Structures of acylated chito-oligosaccharides.

the chito-oligosaccharides with larger DP .

Methods

In a typical preparation, chitopentaose hydrochloride (80 mg; 97.1 mmol) was soaked in pyridine (5 ml) for 1 h and then the pyridine was evaporated off under a reduced pressure. The liberated chitopentaose was dispersed in a mixture of pyridine (5 ml), dimethylaminopyridine (2.0 g), and chloroform (30 ml) with a magnetic stirring under nitrogen atmosphere. To this solution, myristoyl chloride (1.35 ml; 50 equivalents to the chitopentaose) was added dropwise at room temperature in the dark. The suspension was stirred for 18 h at 40°C. The reaction mixture changed from a heterogeneous colorless solution to a homogeneous orange solution. After the solution was cooled and concentrated to *ca.* 10 ml under a reduced pressure, it was poured into methanol to remove the excess acid chloride, and the precipitate was purified by reprecipitation with a chloroform/methanol system and dried *in vacuo* overnight. Other derivatives were prepared in the same manner, excepting that the synthesis of CHT6A14 required a more moderate condition to suppress the formation of the undesirable *N,N*-diacylated by-product. The overall yield decreased with an increase in DP : it was *ca.* 40% for CHT4Ans; *ca.* 15% for CHT5Ans; *ca.* 10% for CHT6A14. All of the derivatives were white or slightly yellow

powder and soluble in common organic solvents such as chloroform, benzene, and tetrahydrofuran.

Measurement

Infrared (IR) spectra were obtained with a JASCO FT/IR spectrometer Model 8800, Japan, by regulating the sample temperature with a Mettler Model FP-82 hot-stage, Switzerland. ^1H 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) with a Rigaku Denki Model DSC-8230, Japan, at a constant heating/cooling rate of $10^\circ\text{C min}^{-1}$. Thermal optical microscopy was performed on a Nikon Model Optiphot-Pol, Japan, employing a Mettler Model FP-82 hot-stage. X-Ray diffraction patterns of the samples in a glass capillary were recorded at different temperatures, using Ni-filtered Cu-K_α radiation. The sample temperatures were regulated by use of a Mettler Model FP-80 hot-stage.

having different *DP* were synthesized. IR and ^1H NMR analyses revealed that the obtained products possess the expected structure in which the amino groups of the starting chito-oligosaccharides were *N*-monoacylated (not *N,N*-diacylated). For example, the ^1H NMR spectra of the acylated chitotetraose, gave an overlapped absorption ($\delta = 5.65\text{--}5.95$) of the $\text{N}'\text{-H}$, $\text{N}''\text{-H}$, and $\text{N}''' \text{-H}$ groups together with a doublet peak ($\delta = 5.55$) of the N-H group in the reducing monomer unit. In addition, a doublet peak at $\delta = 6.10$ assignable to the α -anomeric proton predominated, showing an excellent stereoselectivity of the present acylation. The results of elemental analysis are listed in Table I. The observed elemental contents were in good agreement with those calculated for the expected structure, in all cases. Thus the tetra-, penta-, and hexa-oligosaccharide derivatives possess 14, 17, and 20 acyl pendants, respectively. This was also confirmed by ^1H NMR. Their purity was further checked by size exclusion chromatography (purity $\sim 100\%$).

RESULTS AND DISCUSSION

Synthesis of *N,O*-Acylated Chito-Oligosaccharides

A series of chito-oligosaccharide alkanoates

Thermal Properties

To investigate the thermal properties, differential scanning calorimetric (DSC) measurements were carried out. Like the dimer and trimer counterparts,⁵ all of the compounds

Table I. Characterization of the acylation products

Sample	<i>DP</i>	Molecular weight ^a	Molecular Formula	Elemental analysis		
				%C	%H	%N
				Found (Calcd)		
CHT4A10	4	2.82×10^3	$\text{C}_{164}\text{H}_{298}\text{N}_4\text{O}_{31}$	69.55 (69.80)	10.34 (10.64)	1.96 (1.99)
CHT4A12	4	3.22×10^3	$\text{C}_{192}\text{H}_{354}\text{N}_4\text{O}_{31}$	71.99 (71.73)	11.24 (11.10)	1.68 (1.74)
CHT4A14	4	3.60×10^3	$\text{C}_{220}\text{H}_{410}\text{N}_4\text{O}_{31}$	72.97 (73.24)	11.45 (11.45)	1.50 (1.55)
CHT4A18	4	4.39×10^3	$\text{C}_{276}\text{H}_{522}\text{N}_4\text{O}_{31}$	75.88 (75.46)	12.33 (11.98)	1.32 (1.28)
CHT5A10	5	3.44×10^3	$\text{C}_{200}\text{H}_{363}\text{N}_5\text{O}_{38}$	69.15 (69.71)	10.45 (10.62)	2.02 (2.03)
CHT5A14	5	4.40×10^3	$\text{C}_{268}\text{H}_{499}\text{N}_5\text{O}_{38}$	73.42 (73.16)	11.15 (11.43)	1.57 (1.59)
CHT5A18	5	5.35×10^3	$\text{C}_{336}\text{H}_{635}\text{N}_5\text{O}_{38}$	75.11 (75.38)	11.68 (11.96)	1.31 (1.31)
CHT6A14	6	5.19×10^3	$\text{C}_{316}\text{H}_{588}\text{N}_6\text{O}_{45}$	72.93 (73.10)	11.23 (11.41)	1.64 (1.62)

^a Calculated from the molecular formula.

Figure 2. Heating DSC traces of CHT m A14 ($m=2-6$).

Table II. Transition temperatures T_m and T_i and enthalpy changes at T_i (ΔH) obtained for CHT4A n , CHT5A n , and CHT6A14

Product	Transition temp ^a /°C		ΔH kcal/mru
	T_m	T_i	
CHT4A10	53	160	4.20
CHT4A12	56	152	1.00
CHT4A14	76	145	0.75
CHT4A18	77	143	2.43
CHT5A10	61	140	0.65
CHT5A14	52	130	0.25
CHT5A18	65	120	0.45
CHT6A14	40 ^b	120	0.36

^a Determined by DSC.

^b Determined by microscopic observation under a polarizing microscope.

prepared here reproducibly showed two transition peaks both in heating and cooling mode, indicating the induction of an enantiotropic mesomorphism. The representative heating DSC traces of CHT m A14 ($m=2-6$) are presented in Figure 2. The transition temperatures and enthalpies obtained in the heating mode are listed in Table II. Since CHT6A14, which has largest oligosaccharide core, showed

Figure 3. Transition temperatures as a function of the degree of polymerization (DP): Open symbols, isotropization point (T_i); closed symbols (T_m). I, M, and C stand for the isotropic phase, mesophase and crystalline phase, respectively.

no distinct transition from the crystal to the mesophase, microscopic observations under a polarizing microscope were instead performed to determine the transition temperature. The ΔH values at the isotropization point T_i of CHT4A n s were comparable to those of the lower- DP derivatives (2.6–4.2 kcal/mru for the dimers and 1.5–4.8 kcal/mru for the trimers⁵). However, much smaller ΔH values were obtained for CHT5A n s and CHT6A14. No systematic dependence of ΔH on the pendant length was observed for the series of derivatives having common DP , whereas the isotropization temperature slightly decreased with an increase in the pendant length.

The relationships, between the transition temperature and DP are summarized in Figure 3. Irrespective of the pendant length, the isotropization temperature decreases with increasing DP , while the melting point remains constant at around 65 °C. Thus, an increase in DP brings about a narrowing of the mesophase temperature range.

The textures and optical anisotropy of the mesophases observed under a polarizing microscope also gave a similar indication. The

leaf-like texture, which is typical of highly ordered discotic columnar phases, was clearly observed for CHT2An ($DP=2$)⁵ but with increasing DP , the texture became less and less clear, and finally, CHT6A14 showed little birefringence. Thus an increase in shape anisotropy of the molecules reduces the stability and the degree of order of the mesophase. A similar result has been reported for acylated cello-oligosaccharides.⁸

In Figure 4, the DP dependence of the transition temperatures of the acylated chito-oligosaccharides is compared with that of the cello-versions,⁸ in which the pendant length is fixed at $n=10$. Both series of the derivatives have a similar structure except for the substituent at the C-2 position. However, this small structural difference brings about a surprisingly large difference in the thermal stability of the mesophase: CHTmA10s possess a mesophase in a much wider range of temperature than the cello-versions (CELmA10s). For example, the chito-pentamer (CHT5A10) exhibits a mesophase from about 60°C up to as high as 140°C, while the cello-pentamer (CEL5A10) has virtually no mesophase. These results demonstrate that the intermolecular

hydrogen-bonding in the chito-derivatives makes a large contribution to the formation of the stable mesophases. The mesophases induced by chito-oligosaccharides are presumed to be analogous to the ones formed by the cello-versions, *i.e.*, discotic columnar phases.^{3,4}

Mesophase Structures

By X-ray diffraction analysis, the mesophase structures of CHT4An, CHT5An, and CHT6A14 were determined precisely. The spacings corresponding to the observed reflections are listed in Table III. The diffraction patterns of CHT4An specimen contain one strong and two or three weak diffraction rings in the small angle region together with a sharp one in the wide angle region. When oriented by shearing, all reflections at small angles lie on the equatorial line perpendicular to the shearing direction, while the reflection at wide angles appears on the meridional line. In the case of CHT4A14, for example, peaks at small angles, which are indexed as $hk0$, correspond to real spacings of 31.8 Å, 18.5 Å, 15.7 Å, and 11.9 Å, respectively, the ratios being very close to $1:1/3^{1/2}:1/2:1/7^{1/2}$. This result indicates a hexagonal symmetry of the column packing, as in the case of the dimeric (CHT2An) and the trimeric (CHT3An) counterparts.⁶ The same packing features were found also for CHT4An and CHT5An even though the intensities of the 110 reflection for them are weaker than those for CHT2Ans and CHT3Ans. On the other hand, CHT6A14 showed only an ill-defined broad halo at small angles, meaning the lack of columnar structure in this derivative.

In Figure 5a, the lattice spacing of the 100 reflection, which can be considered as the diameter of the column, is plotted against the side chain length n . An increase in the 100 spacing in proportion to n is observed for both CHT4Ans and CHT5Ans as in the case of CHT2Ans and CHT3Ans.⁶ In addition, the 100 spacing increases with increasing DP for a

Figure 4. The DP dependence of the transition temperatures for chito-oligosaccharide (○, ●) and cello-oligosaccharide derivatives (△, ▲): ○ and △, isotropization point; ● and ▲, melting point.

Table III. X-Ray diffraction data and lattice parameters obtained for the mesophase of CHT4A n , CHT5A n , and CHT6A14

Sample	Temp	d_{obs}	d_{calcd}^a	Index	Phase	Lattice parameter
	°C	Å	Å			Å
CHT4A10	100	26.3 (s)	26.4	100	D _{ho}	$a = 30.5$
		13.2 (m)	13.2	200		
		5.15 (w)		001		
CHT4A14	100	31.8 (s)	31.9	100	D _{ho}	$a = 36.8$
		18.5 (w)	18.4	110		
		15.7 (m)	15.9	200		
		11.9 (m)	12.0	210		
		5.18 (w)		001		
CHT4A18	100	35.6 (s)	35.2	100	D _{ho}	$a = 40.7$
		20.2 (w)	20.3	110		
		17.6 (m)	17.6	200		
		13.6 (w)	13.3	210		
		4.96 (vw)		001		
CHT5A10	100	28.0 (s)	28.1	100	D _{hd}	$a = 32.4$
CHT5A14	90	13.9 (w)	14.0	200	D _{hd}	$a = 38.8$
		33.6 (s)	33.6	100		
		17.0 (w)	16.8	200		
CHT5A18	90	12.4 (vw)	12.6	210	D _{hd}	$a = 44.0$
		38.1 (s)	38.1	100		
		18.8 (m)	19.0	200		
		14.4 (w)	14.4	210		

^a Calculation based on the lattice parameters obtained for the two-dimensional hexagonal lattice.

Figure 5. Plots of the lattice spacing of (a) 100 reflection and (b) 001 reflection versus the total number, n , of carbon in the acyl pendant: ○, CHT2A n ; △, CHT3A n ; □, CHT4A n ; ▽, CHT5A n .

fixed value of n .

The X-ray pattern of the CHT4A n specimen includes a sharp reflection, indexed as 001, at wide angles, indicating an ordered stacking of molecules within a column. The lattice spacing of this reflection, d_{001} , coincides with the intermolecular distance along the column axis. Figure 5b depicts the relationship between d_{001} and n for CHT4A n together with those for CHT2A n and CHT3A n . The d_{001} values for CHT4A10 and CHT4A14 are nearly equal to the constant value of *ca.* 5.15 Å obtained for the dimeric and the trimeric analogues, whereas that for CHT4A18 is slightly smaller. The lattice parameters a obtained for the most plausible hexagonal lattice for CHT4A n are summarized in Table III. The spacings calculated with this lattice parameters are in good agreement with the observed values (Table III). A further support for these lattices

can be obtained from a density consideration: Assuming that each molecule is included in a unit lattice volume, we calculate the specific gravities to be $0.9\text{--}1.0\text{ g ml}^{-1}$ for the CHT4An mesophases. These values are very plausible in light of those for the hexagonal ordered columnar mesophases of similar chemical compositions thus far reported.^{5,6} On the other hand, no 001 reflection can be observed for CHT5An, showing a disordered molecular stacking in the columns. As described above, the isotropization enthalpies ΔH of CHT5Ans are much smaller than those of CHT4Ans, which can be attributable to the less ordered mesophase structure.

The typical X-ray diffraction patterns and the possible structures of the mesophases for the tetrameric, pentameric, and hexameric alkanates are illustrated in Figure 6. The phases of CHT4An and CHT5An are both assignable to a hexagonal columnar phase in a class of discotics; furthermore, the former is distinguished from the latter by the presence of the ordered molecular stacking along the column axis. According to Destradé's terminology,⁷ the mesophase of CHT4An corresponds to a D_{ho} phase while CHT5An to a D_{hd} phase. The hexameric alkanate CHT6A14 exhibits no discotic columnar mesomorphism.

Figure 6. The typical X-ray diffraction patterns (upper) and the possible structures (lower) of the discotic mesophases: (a) D_{ho} for CHT2An, CHT3An, and CHT4An, (b) D_{hd} for CHT5An, and (c) N_D for CHT6A14.

The phase of this derivative is believed to be a discotic nematic (N_D). This difference presumably results from the difference in the axial ratios of the core saccharides; consequently, chitopentaose possesses the maximum axial ratio so as to behave as a discotic columnar mesogen. The intermolecular hydrogen-bonding is responsible for the enhanced stability of the chito-derivatives compared to that of the cello-counterparts.

Supporting evidence for the contribution of the secondary amido groups to the stabilization of the mesophase comes from the infrared (IR) measurement conducted with regulating the sample temperature by a hot stage instrument. Here the pendant length is fixed at $n=14$ with DP ranging from 2 to 6. In the IR spectra of the acylated chito-oligosaccharides, N-H stretching band of the secondary amido group appears at around 3300 cm^{-1} , the variation of which band is a measure of the strength of the hydrogen-bonding interaction. Measurement was performed after 10 minutes aging at $2.5\text{--}10^\circ\text{C}$ intervals in a wide temperature range covering the crystalline, liquid crystalline, and isotropic liquid states. In Figure 7, the frequency of the amido absorption band was plotted against temperature.

In the crystalline and liquid crystalline states, the absorption frequency remains almost independent of temperature for all compounds.

Figure 7. Temperature dependence of the frequency of the N-H stretching band for the indicated specimens.

Considerable frequency differences are seen among different samples in the crystalline and liquid crystalline phases, but no systematic relationship between the absorption frequency and DP can be obtained. At the transition from the mesophase to the isotropic phase, the N–H absorption peak for the derivatives with DP of 2 to 5 is drastically shifted to higher frequencies above 3400 cm^{-1} , indicating that the intermolecular hydrogen-bonding relevant to the secondary amido groups either disappears or is remarkably weakened in the isotropic melt. In contrast to this, in the case of CHT6A14, which does not form a columnar structure, the peak is not shifted at the clearing point but just broadens and becomes hardly detectable. This confirms that the mesophase transition behavior of CHT6A14 is fundamentally different from those of the lower- DP derivatives.

In conclusion, the chitotetraose- and chitopentaose alkanoates can work as a discotic mesogen, forming a highly ordered discotic columnar phase, D_{ho} and D_{hd} . The hexameric derivatives do not form a columnar phase but a discotic nematic phase. The result shows that the chitopentaose possesses the maximum axial ratio so as to behave as a discotic mesogen, although the fact that the columns built up by such fairly asymmetric molecules are packed in a highly symmetric lattice, *i.e.*, a hexagonal lattice may be surprising and poses an interesting question as to the fashion of

molecular packing within each column. Compared with cello-oligosaccharide counterparts, the mesophase temperature range of chito-versions is remarkably expanded mainly due to the increase in T_i temperature. Furthermore, the stacking period along the column, d_{001} , decreased. These can be interpreted as resulting from the intermolecular hydrogen-bonding which is formed between the secondary amido group in the C-2 position and the ester group.

Acknowledgments. We are indebted to Yaizu Suisan Co., Ltd. for supplying chito-oligosaccharides.

REFERENCES

1. For a review, see: S. Chandrasekhar and G. S. Ranganath, *Rep. Prog. Phys.*, **53**, 57 (1990).
2. W. Paulus, H. Ringsdorf, S. Diele, and G. Pelzl, *Liq. Cryst.*, **9**, 807 (1991).
3. T. Itoh, A. Takada, T. Fukuda, T. Miyamoto, Y. Yakoh, and J. Watanabe, *Liq. Cryst.*, **9**, 221 (1991).
4. A. Takada, T. Fukuda, T. Miyamoto, Y. Yakoh, and J. Watanabe, *Liq. Cryst.*, **12**, 337 (1992).
5. M. Sugiura, M. Minoda, J. Watanabe, T. Fukuda, and T. Miyamoto, *Bull. Chem. Soc. Jpn.*, **65**, 1939 (1992).
6. M. Sugiura, M. Minoda, J. Watanabe, T. Fukuda, and T. Miyamoto, *Liq. Cryst.*, **12**, 603 (1992).
7. A. M. Levelut, J. Malthete, C. Destrade, and N. H. Tinh, *Liq. Cryst.*, **2**, 877 (1987).
8. A. Takada, K. Fujii, J. Watanabe, T. Fukuda, and T. Miyamoto, *Macromolecules*, **27**, 1651 (1994).