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Synthesis and Liquid Crystals Properties of α-Methylated Galactosides

N. Z. B. M. Rodzi[a], T. Heidelberg[b], R. Hashim[b,c*] and A. Sugimura[c],

H. Minamikawa[d]

[a]Centre For Foundation Studies, University of Malaya, 50603 Kuala Lumpur.
[b]Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur
[c]Department of Information Systems Engineering, Faculty of Engineering, Osaka Sangyo University, 3-1-1 Nakagaito, Daito-shi, Osaka 574, 8530 Japan
[d] Nanotube Research Center (NTRC), National Institute of Advanced Industrial Science and Technology, AIST, Tsukuba Central 5, 1-1-1 Higashi, Tsukuba, Ibaraki, 305 8565, Japan

Abstract

Due to the amphiphilicity nature of glycolipids, some are known to exhibits liquid crystals phases both in thermotropic and lyotropic phases. Six different glycolipids have been synthesized using three steps process and their structures have been characterized by ^{1}H-NMR and ^{13}C-NMR in acetylated and deacytelated forms. Their liquid crystals properties were studied using optical polarising microscopy (OPM) and differential scanning calorimetry (DSC). The effect of α-methylated tails is compared with those of the straight chain glycolipids. The epimeric effect of the hydroxyl group at the C-4 of the sugar group was also commented.

Keywords: *Glycolipids; Galactosides; Branched chain lipid; Self-assembly; Amphitropic liquid crystals; Smectic A; Anomer*

1. Introduction

The focus on environmentally-friendly technologies leads to the demand for biologically degradable materials that are cheap and can be synthesized from renewable resources. This in turns becomes the major driving force for the development of synthetic glycolipids in recent years[1]. The amphiphilic characteristic of glycolipids enables a wide range of applications based on the molecules' ability to assemble in monolayers, bilayers, micelles, lyotropic mesophases or vesicles [2]. Applications using the self-assembly properties of these materials have been applied for both nano- and biotechnology[3], for example in meso crystallization of membrane protein[4]. Simple glycolipids such as alkyl polyglucosides (APGs) have been used in detergent industries [5], cosmetics and personal care products for their dermatological compatibility [6]. Natural glycolipids are known to exhibit biological activities that

* *corresponding author*
Tel.: +603-79674009; fax: +603-7967-4193.
E-mail address: rauzah@um.edu.my.

make them also useful in the pharmaceutical industries for antimicrobial, antifungal, antiviral, neurotoxin, hormones, and agents that act as agonists or antagonists to biological signals [7].

Six α-methylated galactosides, whose lipid domains contain 8, 9 and 11 carbons, were synthesized and investigated for their liquid crystalline properties to study influence of the anomeric linkage's configuration. Some α-methylated glycolipids have been synthesized [8] but their liquid crystals properties have not been studied. Although galactolipid plays important role in nature [9], there are not many records found in the literature for synthetic galactolipids and especially to compare their anomeric behaviours.

2. Material and methods

Synthesis

The starting sugar, β-D-galactose pentaacetate (98%), the secondary alcohols (≈ 98%) and boron trifluoride diethyl etherate were obtained from Sigma-Aldrich. Anhydrous dichloromethane was purchased from ACROS, while cGR grade solvents, i.e. dichloromethane, ethyl acetate, hexane and methanol were supplied by Fischer Scientific and were used without further purification.

The synthesis of these compounds was achieved using a two steps procedure [9]. Glycosylations were operated under nitrogen atmosphere, while no special precaution was required for the final deprotection step. Instead of using $SnCl_4$ as a Lewis acid catalyst for α-glycosidation, boron trifluoride diethyl etherate was used for both the anomers during glycosidation to confirm its efficiency [10]. The rate of reaction was monitored using thin layer chromatography. 72 hours was needed to obtain reasonable yields for α-galactosides, while only 48 hours are reported for $SnCl_4$ [9]. For purification of peracytelated products, flash chromatography on silica gel 230-400 mesh using either hexane /ethyl acetate mixtures in varying ratios was applied. The final glycolipids were dried in a vacuum oven at 50 ℃ for several hours over phosphorus pentoxide.

Nuclear magnetic resonance spectra were recorded on various spectrometers (270MHz-JOEL, 300MHz-Mercury, 400 MHz-JOEL, 400MHz-Bruker, 500MHz-Bruker) in chloroform-d (for protected glycolipids) and methanol-d_4 (after deacetylation). The reference set for chloroform-d was set to 7.26 ppm for ^{1}H-NMR and 77.0 ppm for ^{13}C-NMR. For methanol-d_4 the reference was set to 3.30 ppm for ^{1}H-NMR and 49.0 ppm for ^{13}C-NMR.

The assignment of each proton for these series was achieved through coupling constant and proton-proton correlation. For ^{13}C-NMR, no assignment of peracytelated and deacytelated glycolipids for each carbon of these series was made, and only signal position was noted. The ^{1}H-NMR enables the both, the structural elucidation and the detection and quantification of anomeric impurities, which otherwise are difficult to determine. Due to the chromatographic work-up, a high purity of the compounds could be achieved; NMR analysis indicates that the purity of most of the compounds exceeds 95%. The names and structures of these glycolipids are summarised in the appendix.

Physical characterization

The thermotropic phase behavior was obtained using optical polarizing microscopy (OPM) by controlling the temperature on a hot stage. Differential scanning calorimetry measurements were carried out by using a Mettler Toledo, DSC 822[e] equipped with a Haake EK90/MT intra-cooler. Software used for analysis is STAR[e] Thermal Analysis System. The sample was heated from the temperature of -55 ℃ at a constant rate of 5 ℃ per minute to about 10 degree above the clearing temperature detected previously by optical polarizing microscopy for each material. The sample used for measurement were weighed between 4-8mg and placed in standard aluminum pans of 40μl size and sealed with a pinhole cover. DSC was run for the individual α-methylated galactosides after deacytelation for the thermotropic phase behavior.
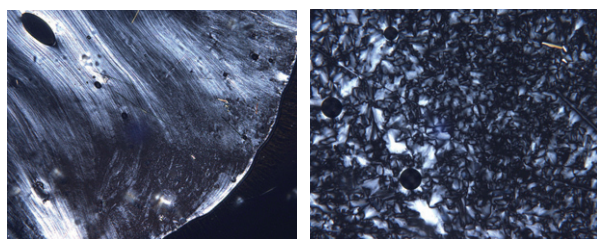
3. Results and Discussions

All compounds showed thermotropic properties and give a smectic A (S_A) texture (see Fig.1), when investigated under the OPM as tabulated in Table 1. These phase behaviours are similar to those of the straight chain analogues, which show exclusively L_α (or in the thermotropic nomenclature a smetic A) phase with systematic increase of the melting and clearing temperatures as the homologue series increases. In this manuscript we shall use the nomenclature adopted by Shearman et al., [12]; hence smectic A (S_A), columnar (Col) and cubic (Cub) correspond to L_α, H and Q respectively. While the former is more convenient for those familiar with thermotropic phases; the latter, originated from the lyotropic liquid crystal studies, is more consistently defined and able to include other
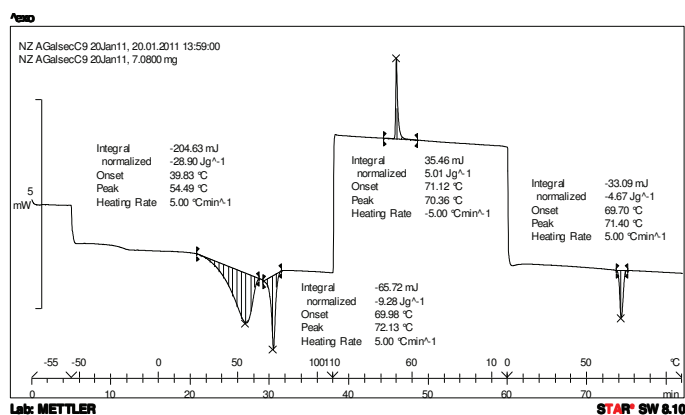
more complex phases like the reversed and gel found recently [13]; and therefore it will be adopted in the present manuscript which confines itself to the thermotropic investigation.

Methylated galactoside abbreviated name	Transition temperature T/ °C (± 5 °C) by OPM	Transition temperature T/ °C (± 5 °C) by DSC and enthalpy ΔH/ kJmol^{-1} (± 0.5 °C)				Chain hydrophobicity (v/l) / Å^2 [14]
		T_1 (L_C/L_β-L_α)	ΔH$_1$	T_2 (L_α-Iso)	ΔH$_2$	
α-Gal-C1C7	*Cr <25 L_α* 49 *Iso*	-2	0.5	34	0.5	27.49
α-Gal-C1C8	*Cr <25 L_α* 78 *Iso*	55	8.8	72	2.8	26.63
α-Gal-C1C10	*Cr <25 L_α* 105 *Iso*	66	0.6	93	1.3	25.97
β-Gal-C1C7	*Cr <25 L_α* 92 *Iso*	72	5.1	87	1.4	27.49
β-Gal-C1C8	*Cr <25 L_α* 116 *Iso*	108	1.5	124	0.6	26.63
β-Gal-C1C10	*Cr <25 L_α* 109 *Iso*	72	15.0	109	1.5	25.97
Straight chain	Clearing temperature T/ °C Ref	Chain hydrophobicity (v/l) / Å^2 [14]				
β-Gal-C7	*Cr 90 Iso*	[15]	20.75			Data extracted from LiqCyst [16]
β-Gal-C8	*Cr 98.3 L_α 133 Iso*	[17]	20.81			
β-Gal-C10	*Cr 93 L_α 157.3 Iso*	[17]	20.89			

Table 1: The thermotropic phase behaviour of methylated galactosides by OPM and DSC; comparison with similar single chain galatosides are given wherever possible. The chain hydrophobicity is also given here. L_α here refers to the S$_A$ phase used in the thermotropic conventional nomenclature.

Figure 1: Typical thermotropic texture of α-Gal-C1C8 using OPM showing (left) low birefringent with step textures at 35°C and (right) a fan shaped textures denoting L_α phase upon cooling from isotropic (20 x magnification)

Figure 2: A DSC thermogram of α-Gal-C1C8 showing typical transitions associated with L_C/L_β to L_α and L_α to Iso phase (scan rate 5°C/min). The L_C or L_β phase is not recovered during the cooling scan.

The identification of the textures was best achieved after the compounds was heated beyond the isotropic point and later were cooled down, which then gave better textures. The miscibility test using octyl-β-glucosides latter confirmed the assignment of the phase.

Table 1 shows peak positions of the DSC scans upon heating and cooling. Two phase transitions were observed. The first set of phase transitions T_1 (from DSC) with a higher molar enthalpy is attributed to the lamellar crystalline (L_C) or gel (L_β) phase to L_α (smectic A) since the associated enthalpy indicates such a transition [18]. But this must be confirmed by a proper structural determination which is beyond the scope of the present work. The second set of transition temperatures, T_2 are attributed to the L_α (smectic A) to isotropic phase transition [13] and these agree qualitatively (to within the error) to those measured by OPM, except for α-Gal-C1C7 and α-Gal-C1C10. These slight differences are attributed mainly to the kinetic phenomenon occur within the samples, which is probably due to the decomposition of the material at an elevated temperatures that depends on the rate of heating, the time spent and the nature of the supporting substrate (e.g. the materials decomposed more quickly in an aluminum pans than on the microscope slides). The determination of melting or clearing point are different in DSC and in OPM due to the rates of heating, where in DSC, the rates are kept constant throughout the experiments, while in the OPM, the rates are depends on the

physical transformations that can be observed with the naked eye. The enthalpy values for the clearing points of the compounds prepared are listed in the Table 1.

It was found, the addition of chain length influences the clearing temperature. For the α-galactosides showed increasing clearing temperature while for β-linked galactosides, the optimum plateau have been reached somewhere near to chain length of C9. The type of linkage between chain and head group (α versus β) affects the thermal stability. The clearing temperature of β-galactosides is much higher than α-galactosides indicating the higher stability of liquid crystalline phase due to the β-linkage. Different configuration of the hydroxyl group of the sugar residue gave different influence on the thermal stability. The increase in clearing temperatures is the increase in intramolecular hydrogen bonding since the axial C-4 of galactosides oriented out of the plane of the sugar ring and is more likely to interact with adjacent molecules, thus increasing the clearing point [19, 20].

The chain hydrophobicity (v/l) [14] was calculated for both the α-methylated galactosides as well as the straight chain analogs. Clearly, the chain hydrophobicity for the former is much bigger than the later. Moreover, the value for the shortest one is comparable to those of Guerbet based glycosides [21]. But the α-methylated series shows that the volume did not increase as much as chain length increases, hence the chain hydrophobicity decreases, and this behaviour will affect the lyotropic phases.
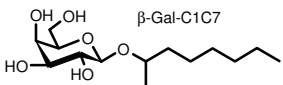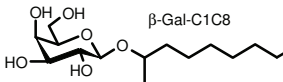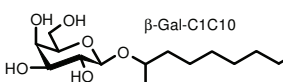
4. Conclusion

The present work is a preliminary study to explore the behaviour of galactosides and study their anomeric behaviour as well as the phases which can be found. Amphiphilic characteristic possessed by glycolipids give them the ability to exhibit liquid crystals properties. The introduction of a single α-methylated side branch significantly reduces the clearing temperatures of the glycosides. The anomeric pair α- and β-galactosides behave differently from each other; the transition L_C or L_β into the L_α phase, as well as the clearing transitions are much higher for the β-galactosides compare to the α-anomers. This trend is reversed for the anomeric pairs of glucosides. Even though, these epimeric and anomeric pairs essentially have the same chemical compositions, the small stereochemical difference in the sugar group have a profound effect in the self-assembly.
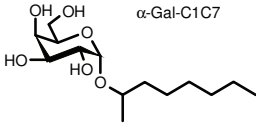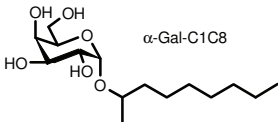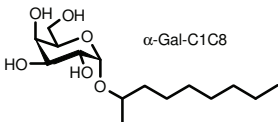
Appendix

Experimental Data

Compound name, abbreviation and structure	Experimental Details
(1)　1-methyl-heptyl -β-D-galactopyranoside　β-Gal-C1C7	**^{1}H-NMR (270MHz, CD$_3$OD):** 4.27 (d, 1H, H-1), 3.92-3.76 (m, 1H, CH), 3.84 (m$_c$, 1H, H-4), 3.72 (d, 2H, H-6a, H-6b), 3.53-3.43 (m, 2H, H-2, H-3), 3.48 (dt, 1H, H-5), 1.69-1.23 (m, 10H, -CH$_2$-), 1.22, 1.17 (each d, 3H, -CH$_3$), 0.90 (t, 3H, -CH$_3$) $^3J_{1,2}$ =7.5 Hz **^{13}C-NMR (100MHz, CD$_3$OD):** δ = 104.49, 102.81, 77.52, 76.44, 75.62, 75.12, 75.06, 72.78, 72.60, 70.22, 62.42, 62.30, 38.46, 37.66, 33.05, 30.62, 30.51, 26.51, 26.38, 23.70, 21.97, 19.81, 14.43
(2)　1-methyl-octyl -β-D-galactopyranoside　β-Gal-C1C8	**^{1}H-NMR (270MHz, CD$_3$OD):** ^{1}H-NMR (270MHz, CD$_3$OD): 4.23 (d, 1H, H-1), 3.84-3.65 (m, 1H, CH), 3.86 (m$_c$, 1H, H-4), 3.72 (d, 2H, H-6a, H-6b), 3.45-3.40 (m, 2H, H-2, H-3), 3.26 (dt, 1H, H-5), 1.69-1.23 (m, 10H, -CH$_2$-), 1.13, 1.09 (each d, 3H, -CH$_3$), 0.85 (t, 3H, -CH$_3$) $^3J_{1,2}$ = 7.5 Hz **^{13}C-NMR (100MHz, CD$_3$OD):** δ = 109.15, 106.94, 84.12, 83.85, 83.72, 83.66, 78.71, 78.61, 76.12, 73.55, 72.32, 64.92, 64.66, 38.43, 37.79, 33.06, 33.04, 33.01, 30.83, 30.79, 30.72, 30.48, 30.42, 26.77, 26.56, 26.42, 23.73, 22.09, 19.67, 14.45
(3)　1-methyl-decyl-β-D-galactopyranoside　β-Gal-C1C10	**1H-NMR (270MHz, CD$_3$OD):** 4.27 (d, 1H, H-1), 3.92-3.80 (m, 1H, CH), 3.84 (m$_c$, 1H, H-4), 3.72 (d, 2H, H-6a, H-6b), 3.53-3.40 (m, 2H, H-2, H-3), 3.48 (dt, 1H, H-5), 1.69-1.18 (m, 16H, -CH$_2$-), 1.22, 1.17 (each d, 3H, -CH$_3$), 0.90 (t, 3H, -CH$_3$) $^3J_{1,2}$ =7.5 Hz
(4)　1-methyl-heptyl-α-D-galactopyranoside	**^{1}H-NMR (400MHz, CD$_3$OD):** δ = 4.94, 4.89 (2d, 1H, H-1), 3.98-3.66 (m, 7H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-α), 1.50-1.25 (m, 10H, -CH$_2$-), 1.21 (d, 1½H, β-CH$_3$), 1.13 (d, 1½H, β-CH$_3$), 0.90 (t, 3H, -CH$_3$)

α-Gal-C1C7	$^3J_{1,2}$ =3.7 Hz **^{13}C-NMR (100MHz, CD$_3$OD):** δ = 99.98, 97.69, 76.10, 73.71, 72.41, 72.29, 71.63, 71.55, 71.07, 70.40, 70.11, 62.65, 62.60, 38.42, 37.47, 33.03, 33.01, 30.62, 30.53, 27.01, 26.42, 23.73, 23.70, 21.65, 19.20, 14.45
(5) 1-methyl-octyl α-D-galactopyranoside α-Gal-C1C8	**^{1}H-NMR (400MHz, CD$_3$OD):** δ = 4.92, 4.89 (2d, 1H, H-1), 3.91 - 3.66 (m, 7H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-α), 1.50-1.25 (m, 12H, -CH$_2$), 1.21 (d, 1½H, β-CH$_3$), 1.13 (d, 1½H, β-CH$_3$), 0.89 (t, 3H, -CH$_3$) $^3J_{1,2}$ =3.7 Hz **^{13}C-NMR (100MHz, CD$_3$OD):** δ = 99.98, 97.68, 76.09, 73.71, 72.41, 72.29, 71.62, 71.54, 71.08, 70.41, 70.12, 62.66, 38.41, 37.45, 33.06, 33.03, 30.91, 30.82, 30.44, 27.05, 26.46, 23.73, 21.64, 19.17, 14.42
(6) 1-methyl-decyl -α-D-galactopyranoside α-Gal-C1C8	**^{1}H-NMR (400MHz, CD$_3$OD):** δ = 4.92, 4.89 (2d, 1H, H-1), 3.90 - 3.64 (m, 7H, H-2, H-3, H-4, H-5, H-6a, H-6b, H-α), 1.48-1.25 (m, 12H, -CH$_2$), 1.21 (d, 1½H, β-CH$_3$), 1.13 (d, 1½H, β-CH$_3$), 0.89 (t, 3H, -CH$_3$) $^3J_{1,2}$ =3.7 Hz **^{13}C-NMR (100MHz, CD$_3$OD):** δ = 99.98, 97.70, 76.09, 73.70, 72.41, 72.29, 71.62, 71.54, 71.08, 70.41, 70.13, 62.62, 38.41, 37.45, 33.06, 30.94, 30.85, 30.74, 30.48, 27.06, 26.45, 23.73, 21.64, 19.18, 14.43

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