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Synthesis and thermotropic characterization of a homologous series of racemic β-D-glucosyl dialkylglycerols

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Abstract

The phase behaviour of aqueous dispersions of a series of synthetic 1,2-di-*O*-alkyl-3-*O*-(β-D-glucosyl)-*rac*-glycerols with both odd and even hydrocarbon chain lengths was studied by differential scanning calorimetry and low angle X-ray diffraction (XRD). Thermograms of these lipids show a single, strongly energetic phase transition, which was shown to correspond to either a lamellar gel/liquid crystalline (L_β/L_α) phase transition (short chain compounds, $n \leq 14$ carbon atoms) or a lamellar gel/inverted hexagonal (L_β/H_{II}) phase transition (longer chain compounds, $n \geq 15$ carbon atoms) by XRD. The shorter chain compounds may exhibit additional transitions at higher temperatures, which have been identified as lamellar/nonlamellar phase transitions by XRD. The nature of these nonlamellar phases and the number of associated intermediate transitions can be seen to vary with chain length. The thermotropic phase properties of these lipids are generally similar to those reported for the corresponding 1,2-*sn*-diacyl α- and β-D-glucosyl counterparts, as well as the recently published 1,2-dialkyl-3-*O*-(β-D-glycosyl)-*sn*-glycerols. However, the racemic lipids studied here show no evidence of the complex patterns of gel phase polymorphism exhibited by the above mentioned compounds. This suggests that the chirality of the glycerol molecule, by virtue of its position in the interfacial region, may significantly alter the phase properties of a lipid, perhaps by controlling the relative positions of hydrogen bond donors and acceptors in the polar region of the membrane.

Keywords: Glycolipid; Synthesis; Differential scanning calorimetry; X-Ray diffraction; Lipid nonlamellar phase; Thermotropic phase behavior

1. Introduction

There is presently much interest in the thermotropic properties of glycosyl diglycerides in which a simple carbohydrate is attached via an α or β linkage to either a diacyl- or dialkylglycerol. The diacyl compounds are important structural elements in the membranes of a wide variety of photosynthetic plants and microorganisms [1–4]. For the most part, physical studies of these compounds have been confined to a few native lipids isolated from photosynthetic organelles [5–9], their hydrogenated derivatives [10–13] and the native glucolipids isolated from *Acholeplasma laidlawii* B [14–18]. More recently we have reported the synthesis of both α- and β-D-

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glucosyldiacylglycerols [19,20] and details of their physical properties [21–25], which show a rich pattern of lamellar and nonlamellar polymorphism.

The corresponding dialkyl glucolipids have been isolated from a relatively small number of sources, most of which are halotolerant archaebacteria [3]. The chirality of the glycerol backbone in these native glycosyl dialkylglycerols is exclusively 2,3-*sn*, whereas that of their diacyl counterparts from eucaryotic cells are exclusively 1,2-*sn* [3]. However, it is interesting that some primitive organisms contain diacyl glycolipids with the 2,3-*sn* chirality at glycerol while others may have a racemic glycerol configuration [3]. This raises the question of whether there is any biological significance in such differences in glycerol configuration.

The situation is further complicated in these glycolipids by the presence of a second chiral centre in the sugar portion of the molecule (D- or L-glucose). Thus, two lipids containing identical sugar headgroups (D-glucose), but glycerols of opposite chirality, are not enantiomers but diastereomers. There have been several recent studies that show that molecules differing in their chirality in the headgroup and interfacial region may also have different physical properties [26–29]. This has prompted us to ask whether the recent reports of the thermotropic properties of a small number of 1,2-dialkyl-3-*O*-(β-D-glucosyl)- and (β-D-galactosyl)-*sn*-glycerols [30–35] are also representative of the natural 2,3-*sn* diastereomers, as has been implied [30,31]. Our own model building studies suggested that differences in the physical properties of the diastereomers were indeed likely [36]. In order to investigate this possibility, we have made several improvements to a previously published synthetic procedure [37] to facilitate the synthesis of a series of compounds in which the configuration of both the carbohydrate headgroup and dialkylglycerol can be systematically varied. In the first paper of this series, we wish to report the synthesis and thermotropic phase behaviour of a series of racemic dialkyl-β-D-glucosyl glycerols, with alkyl chain lengths ranging from 10 to 20 carbon atoms, using calorimetry and X-ray diffraction. Similar studies on the 1,2-*sn* and 2,3-*sn* compounds will follow shortly.

2. Materials and methods

2.1. Synthesis

Racemic dialkylglycerols were prepared according to the procedure of Baumann and Mangold [38]. All other materials were prepared as reported earlier [19,20]. All analytical measurements were consistent with the assigned structures and were in agreement with literature values (see below).

The synthetic procedures available in the literature for the synthesis of glycosyl dialkylglycerols [37,39], although convenient for the preparation of a limited range of chain lengths, proved troublesome in our hands for the preparation of longer chain compounds. In some cases the proportion of orthoester formation was high [39] or a significant amount of the α anomer was formed [37]. Therefore we decided to modify a procedure which we have found useful for the preparation of the β-D-glucosyl diacylglycerols using 1,2-dibenzyl-*sn*-glycerol [40]. This was high yielding and successful in all cases, including the preparation of several β-D-galactosyl compounds (D. Mannock, unpublished data). However, it was necessary to warm the reaction mixtures containing longer chain dialkylglycerols before addition of the sugar bromide. The procedure utilized is as follows.

2.1.1. 1,2-Di-tetradecyl-3-O-(2, 3, 4, 6-tetraacetyl-β-D-glucopyranosyl)-rac-glycerol

The dialkylglycerol (1 mmol), $HgBr_2$ (1 mmol) and $Hg(CN)_2$ (1 mmol) were dissolved in 10 ml dry acetonitrile:dichloroethane (1:1, v/v). Two grams of Drierite were then added and the solution left to stand for 30 min. After this time, 1 mmol acetobromoglucose was added, the tube was sealed under nitrogen, placed in the dark and shaken for 5–6 h, when a further 1 mmole acetobromoglucose was added. The shaking was then continued for a further 12 h. After this time, thin layer chromatography (TLC, hexane:ethyl acetate, 3:1 v/v) showed the complete disappearance of the dialkyl glycerol (R_f 0.6) and the formation of a product, dialkyl tetraacetyl-β-D-glucosyl-*rac*-glycerol (R_f 0.4). The reaction mixture was filtered under vacuum and the solid residue washed with warm chloroform:methanol (1:1, v/v). The solvents were evaporated, the syrup redissolved in 60 ml chloroform and this organic phase

was then washed with 1 M aqueous KBr (3×30 ml) and water (1×30 ml). The combined organic phases were dried over $MgSO_4$, filtered on a Buchner funnel, evaporated to dryness and applied to a column of silica gel (40 g, 230–400 mesh) slurried in hexane. The crude material was added in CCl_4/hexane (2:1, v/v) and the column eluted with 2 column volumes hexane, 8 column volumes hexane:ethyl acetate 12:1 (v/v) and 5 column volumes hexane:ethyl acetate 10:1 which eluted the product. The purified peracetylated β-D-glucosyl-dialkyl-*rac*-glycerol was subsequently crystallized from ethanol. Yields were typically 75%.

2.1.2. 1,2-Di-tetradecyl-3-O-(β-D-glucopyranosyl)-rac-glycerol

The above peracetylated compound was subsequently deprotected. Although the method of Ogawa and Beppu [37] was successful, impurities from the ion exchange resin proved difficult to remove. Therefore we modified a method which we had tried earlier on the diacyl compounds [19].

The peracetylated dialkyl glucolipid (400–500 mg) was added to 30 ml of triethylamine:methanol:water (2:4:1, v/v/v) in a screw cap tube. This was heated at 50–70°C in an oven for up to 12 h. TLC (chloroform:methanol, 5:1, v/v) then showed a single spot (R_f 0.8) which was the product dialkyl-β-D-glucosyl-*rac*-glycerol. The solvent was removed under vacuum and the resulting syrup repeatedly dried by evaporation with benzene. The crude product was redissolved in chloroform and applied to a column of silica gel (30 g, 230–400 mesh) slurried in the same solvent. This was eluted with chloroform (2 column volumes) and then with a gradient of either chloroform:methanol or chloroform:acetonitrile. The fractions containing the derived product were combined and evaporated to dryness and the material was crystallized first from acetone or acetone:water, and again from methanol or methanol:water, depending on the chain length. The purified compounds were collected and lyophilized from benzene:methanol (9:1, v/v) over P_2O_5. Analytical data for the unprotected glycolipids (unless stated otherwise) are as follows:

^{1}H-NMR: (200 MHz, di-14:0 peracetate in $CDCl_3$, ppm) 0.83, 6H (CH_3); 1.23, 48H (CH_2); 1.5, 4H (OCH_2); 2.0, 12H (CH_3 acetates); 3.32–5.22, 12H (H_1, H_2, H_3, H_4, H_5, $H_{6A,B}$, $H_{1'A,B}$, $H_{2'}$, $H_{3'A,B}$); H1 4.54 (8 Hz). The diastereomeric mixture can be distinguished from the individual diastereomers by the presence of a double doublet for the anomeric proton (4.5–4.6 ppm) and two overlapping triplets for H_2 (4.92–5.0 ppm) of the glucose headgroup. There are also small differences in the coupling constants arising from $H_{1'A}$ and $H_{1'B}$ and $H_{3'A}$ and $H_{3'B}$ as reported for the diphytanyl glycerols [41].

^{1}H-NMR: (500 MHz, di-16:0 in DMSO/deuterium exchanged, ppm) 0.85, 6H (CH_3); 1.23, 56H (CH_2); 1.5, 4H (OCH_2); 2.92–4.92, 12H ($H_{1-6A,B}$ of glucose, $H_{1'-3'}$ glycerol); H_1, 4.42 (DD, 6.5 Hz). El. anal. di-16:0: expected C 70.04%, H 11.75%, O 18.20%; observed C 69.93%, H 11.84%, O 18.55%.

2.2. Physical measurements

High sensitivity DSC measurements were performed using a Calorimetry Sciences Corporation multicell DSC (Provo, UT, USA). The samples were prepared by weighing the dry lipid into the cell using a Fisher/Mettler M3 microgrammatic microbalance and 0.5 ml de-ionized water as added and the cell was sonicated in a water bath for 20 min at room temperature. The cells were then manually dried and placed into the DSC, whereupon they were cycled between 10 and 90°C to ensure uniform mixing of the sample prior to measurement. Data were collected over the range of 5–95°C at a rate of 10°C/h and imported into Microcal (Northampton, MA, USA) Origin version 5.0 for analysis.

Samples for X-ray diffraction were prepared by transferring 3–5 mg of dry lipid into a thin-walled glass capillary (1.5 mm). Deionized water (1–2 times the dry lipid weight) was added and the capillaries were either flame sealed or sealed with 5 min epoxy. The samples were mixed by repeatedly heating and cooling the capillaries with a hot air gun and by centrifuging the material back and forth in the capillary. The samples were determined to be uniformly

Chain length	10:0	11:0	12:0	13:0	14:0	15:0	16:0	17:0	18:0	19:0	20:0
Soft. pt. (°C)	–	(45–6)	(49–50)	(50–2)	(53–4)	(56–8)	(60–2)	(65–7)	(71–3)	(74–5)	(77–9)
mp (°C)	–	106–7	114–5	116–7	117–8	115–6	112–3	113–4	110–1	107–8	101–2

dispersed when different parts of the X-ray capillary all yielded the same diffraction. The X-ray diffraction experiments were performed over the range −20 to 90°C.

The XRD camera was attached to a Rigaku RU200 rotating anode generator and used a linear 512 channel, 28.3 mm metal oxide semiconductor multichannel photodiode to detect the low angle scattering patterns, which were then digitally recorded on a computer [42]. The sample temperature was regulated by means of a sample holder connected to a computer controlled circulating bath, the temperature of the sample was controlled with a precision of ±0.5°C. Measurements were taken at approx. 2–3°C intervals following a 5–10 min equilibration period. Exposure times were typically of the order of 10 min.

3. Results

3.1. Calorimetric measurements

Heating and cooling thermograms of a homologous series of dialkyl-β-D-glucosyl-*rac*-glycerols containing hydrocarbon chains with odd and even numbers of carbon atoms obtained using the CSC DSC are shown in Fig. 1. (The corresponding thermodynamic parameters are listed in Table 1.) The pattern of phase behaviour is consistent with the occurrence

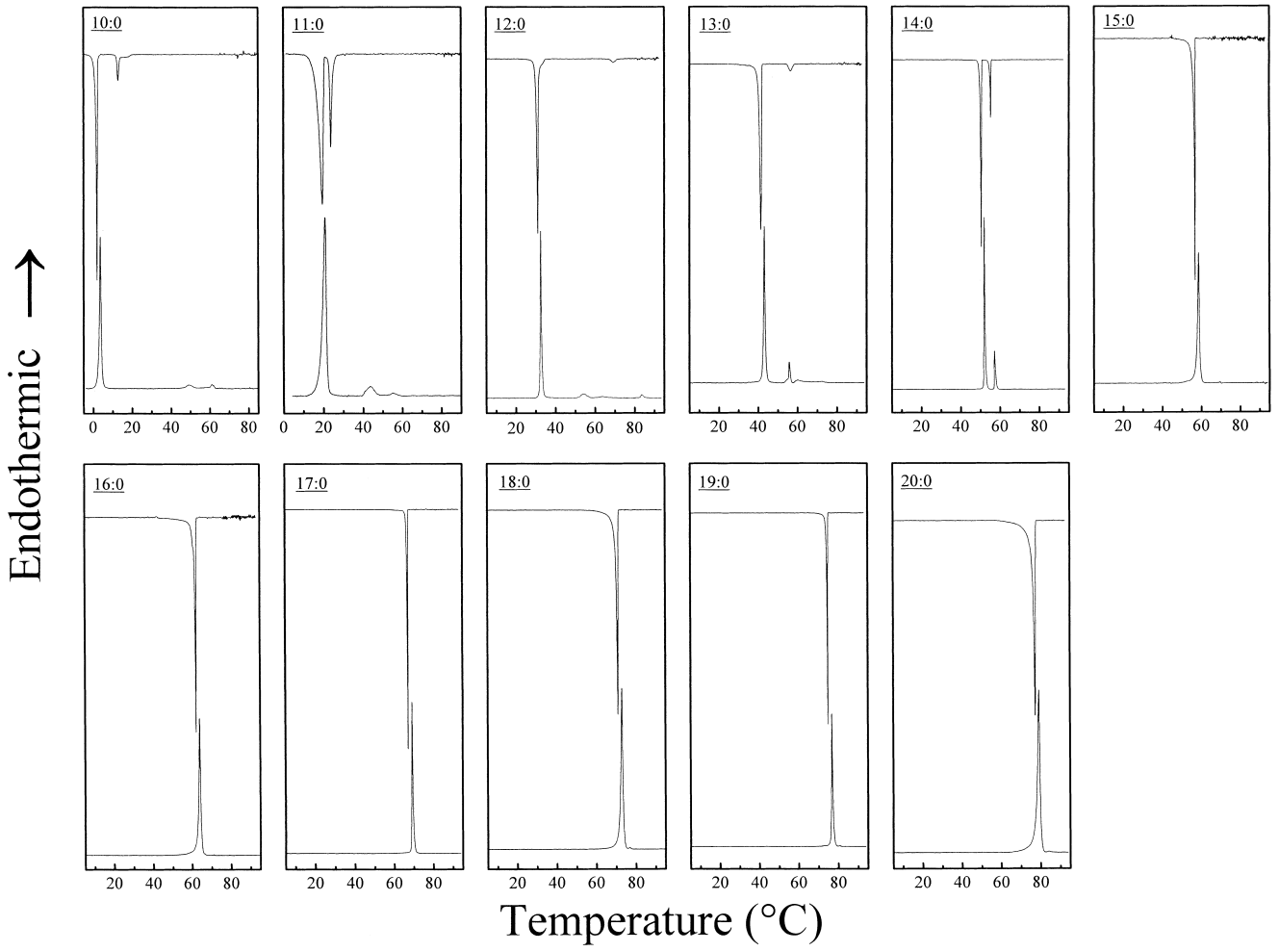

Fig. 1. High sensitivity DSC thermograms of a series of synthetic racemic dialkyl-β-D-glucosyl glycerols. The heating and cooling rates are 10°C/h.

Table 1
Phase transition properties of a series of synthetic 1,2-di-*O*-alkyl-3-*O*-(β-D-glucopyranosyl)-*rac*-glycerols

Lipid	Phase transition temperature (°C)						Enthalpy ΔH (kcal mole^{-1})					
	L_β/L_α	L_β/H_{II}	L_α/Q_{II}	Q_{II}/Q_{II}	Q_{II}/H_{II}	L_α/H_{II}	L_β/L_α	L_β/H_{II}	L_α/Q_{II}	Q_{II}/Q_{II}	Q_{II}/H_{II}	L_α/H_{II}
10:0	3.66	–	49.2	61.1	–	–	2.7	–	0.28	0.18	–	–
11:0	20.9	–	43.7	55.2	96.0	–	3.2	–	0.33	0.08	–	–
12:0	32.6	–	54.2	64.2	83.8	–	4.7	–	0.57	0.29	0.23	–
13:0	43.2	–	54.4	60.3	72.6	–	5.5	–	0.74	0.46	0.27	–
14:0	51.9	–	–	–	–	57.1	6.1	–	–	–	–	1.5
15:0	–	58.6	–	–	–	–	–	9.7	–	–	–	–
16:0	–	63.9	–	–	–	–	–	11.3	–	–	–	–
17:0	–	69.1	–	–	–	–	–	12.1	–	–	–	–
18:0	–	72.8	–	–	–	–	–	13.3	–	–	–	–
19:0	–	76.7	–	–	–	–	–	14.6	–	–	–	–
20:0	–	79.2	–	–	–	–	–	15.7	–	–	–	–

of both lamellar and type II nonlamellar phases, but there is no evidence of the gel state polymorphism reported for the 1,2-*sn* compounds [30,36] even after substantial annealing times (3 years). In the *rac*-β-GlcDAGs studied here, two distinct patterns of phase behaviour are apparent. The shorter chain compounds ($n = 10$–14) all exhibit a highly energetic, lower temperature event and one or more weakly energetic, higher temperature events. On the basis of our earlier studies of glycolipid phase properties, these events were tentatively assigned to a lamellar gel/lamellar liquid crystalline (L_β/L_α) phase transition, a lamellar liquid crystalline/inverted cubic (L_α/Q_{II}) phase transition, and to an inverted cubic/inverted hexagonal (Q_{II}/H_{II}) phase transition, respectively. As might be expected, the chain melting, L_β/L_α phase transition is strongly chain length dependent and is reversible on cooling. The poorly energetic, higher temperature events are fairly broad and are most easily discernible at fast heating rates. Whereas the transitions involving the H_{II} phase are reversible on cooling, the Q_{II} phases remain stable over a wide range of temperature and their transitions back to the lamellar phase tend to be substantially supercooled. Although these weakly energetic thermal events show some evidence of chain length dependence, the pattern of behaviour is not straightforward. Generally, as the chain length is increased, so the temperature intervals between the L_β/L_α and L_α/Q_{II} events (ΔT_1) and the L_α/Q_{II} and Q_{II}/H_{II} events (ΔT_2) decrease at similar rates. Thus with increasing chain length, the chain melting phase transition temperature (T_m) increases whereas the temperature of the transition from the lamellar liquid crystalline to the nonlamellar phase (T_{NL}) decreases. At a chain length of 14 carbon atoms, the separate L_α/Q_{II} and Q_{II}/H_{II} transitions are replaced by a single reversible L_α/H_{II} phase transition as ΔT_2 becomes zero (Fig. 1) and ΔT_1, the temperature window in which the L_α phase is stable, is reduced to only 5°C. At still longer chain lengths ($n = 15$–20), only a single highly energetic event is observed by DSC, probably corresponding to a reversible L_β/H_{II} phase transition.

Despite repeated attempts over a period of 3 years to induce the L_β phases in these *rac*-β-GlcDAGs to form crystalline (L_c) phases under a wide variety of nucleation and incubation conditions, we were unable to identify conditions under which the L_β/L_c conversion process occurred. In the absence of solid state phase polymorphism, there are no easily visible scan rate-dependent effects in the chain melting phase transitions. However, the inverted bicontinuous cubic phases show substantial hysteresis on cooling, confirming previous suggestions that such structures are likely to be extremely stable once they have been formed. A relative comparison of the rate of L_c phase formation and nonlamellar preference for the diastereomeric and *rac*-β-GlcDAGs is shown in Table 2.

3.2. X-Ray diffraction measurements

The X-ray diffraction patterns obtained for this

series of glucosyl dialkylglycerol diastereomeric mixtures are consistent with the thermotropic phase behaviour observed by DSC and confirm the transition temperatures measured by DSC. A plot of the long spacings of the *rac*-β-GlcDAG series as a function of temperature is shown in Fig. 2. For all *rac*-β-GlcDAGs studied, the X-ray measurements at temperatures below the major endothermic event seen in DSC show a series of low angle reflections consistent with a lamellar gel L_β phase. Fig. 3 shows a plot of the first order spacings for the L_β phases of this series of dialkyl glucolipids. The slope of the regression line is approx. 0.26, which translates into an L_β phase in which the chains are untilted.

In the shorter chain compounds upon heating from the L_β phase, there is a decrease in the repeat distance as the L_β phase converts to an L_α phase. Further heating produces additional changes in the diffraction pattern as the L_α phase transforms into a Q_{II} phase. At lower temperatures in this phase region, the diffraction patterns appear to suggest a mixed phase region, probably consisting of at least two Q_{II} phases whose structures we have been unable to identify at this time. At higher temperatures in the cubic phase region, a diffraction pattern which is characterized by low angle XRD spacings in the ratio $\sqrt{2}:\sqrt{3}:\sqrt{4}:\sqrt{6}:\sqrt{8}:\sqrt{9}:\sqrt{11}$ appears. This phase has been identified as belonging to space group Q^{224} ($Pn\bar{3}m$) on the basis of similar studies in phosphatidylethanolamines [43–48] and a phase diagram of β-GlcDAG previously reported [49].

In the di-12:0 and di-13:0 compounds at temperatures above the transition to the Q^{224} phase, a transition to the reversed hexagonal (H_{II}) phase is observed with a characteristic series of low angle spacings in the order $1:1/\sqrt{3}:1/\sqrt{4}:1/\sqrt{7}):1/\sqrt{9}$ [5,6]. The cylinder axis to cylinder axis distance of this hexagonal phase, given by $d_a = 1/\sqrt{3}$) and $d_{1,0}$, is 61 Å for both the di-14:0- and di-16:0-β-GlcDAG at 68°C and 73°C, respectively, which is in good agreement with the value obtained for a native β-D-galactosyl diacyl glycerol with polyunsaturated chains [6]. In the XRD measurements of the di-14:0-β-GlcDAG, there is no cubic phase and the higher temperature thermal event seen on heating of these compounds can be assigned to a simple L_α/H_{II} phase transition.

At longer chain lengths ($n \geq 15$), the complex pattern of thermograms arising from lamellar/cubic and cubic/cubic phase transitions are replaced by a single highly energetic transition. At temperatures below this transition, the diffraction pattern is characteristic of a lamellar gel (L_β) phase. Immediately above the transition a pattern characteristic of an H_{II} phase is evident. Thus, the L_α and cubic phases must be absent in these longer chain compounds and the single thermal event seen by DSC can be confidently assigned to a direct L_β/H_{II} phase transition.

Table 2
A comparison of the phase properties of dialkyl glycosyl glycerols containing either a chiral or a racemic glycerol backbone[a]

Headgroup		β-D-Glucopyranoside			β-D-Galactopyranoside		
#C At./Chiral	Phase	1,2-*sn*	1,2-*rac*	2,3-*sn*	1,2-*sn*	1,2-*rac*	2,3-*sn*
12:0	L_c	****	0	0	*****	****	***
14:0		***	0	0	****	***	**
12:0	Q_{II}^a	***	**	0	0	*	**
14:0		0	0	0	0	0	0
12:0	Q_{II}^b	***	***	****	****	***	***
14:0		0	0	0	0	0	0
12:0	H_{II}	***	***	***1/2	**	**	**
14:0		****	****	****	***	***	***

[a]In the case of the L_c phases the number of * symbols indicates the rate of L_c phase formation. More * symbols represents a faster rate of conversion to the L_c phase. For the nonlamellar phases, the number of stars represents the temperature range over which that phase is stable and the temperature at which that phase is formed on heating. The greater the range over which the phase is stable and the lower the temperature of the transition to that phase, the greater the number of * symbols. Zero indicates that the phase did not exist under the conditions specified in the table margins. These estimates can only be qualitative.

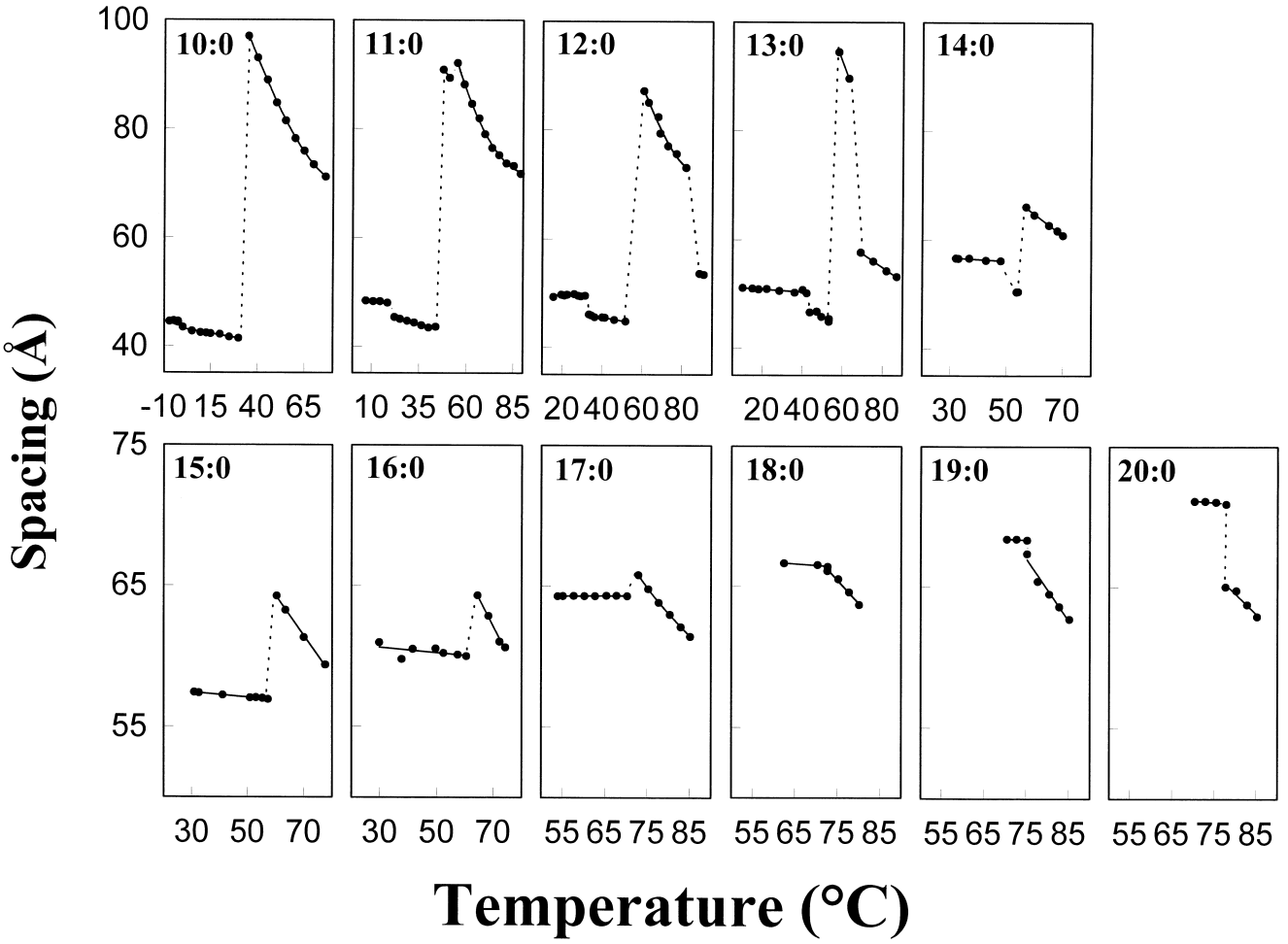

Fig. 2. Low angle X-ray diffraction measurements of the racemic dialkyl-β-D-glucosyl glycerols, showing plots of the cell lattice parameter (*a*, Å) as a function of temperature (°C).

This gradual change in the pattern of phase behaviour on heating can be seen in the plots of cell lattice parameter (*a*) as a function of temperature in Fig. 2. These data clearly demonstrate the decrease in the temperature range over which both the L_α and Q_{II} phases can exist as the chain length increases. This window is 80°C broad in the di-10:0 *rac*-β-GlcDAG and is reduced to nothing at a chain length of 15 carbon atoms. These measurements also show that the cubic phases evident in compounds with a chain length less than 12 carbon atoms are stable over a very wide temperature range (approx. 60°C in the di-10:0 compound). Inverted cubic phases have recently been observed in a wide range of PCs [50–52] at lower water contents and in di-dodecyl-PE (DDPE) [47,48] and *N*-methylated DOPE [46] in excess water. More recently, studies of diacyl [21,23,24] and dialkyl [30,34–36,49] glycolipids have also reported the existence of cubic phase structures. However, this work is the first occasion on which a sufficient number of shorter chain members of a homologous series of dialkyl glycolipids have been available to permit the changes in both the L_α/Q_{II} and Q_{II}/H_{II} phase transition temperatures as a function of chain length to be fully appreciated. What is immediately obvious from this data (Figs. 1,2 and 4, upper panel) is the discrepancy in the L_α/Q_{II} phase transition temperatures of the odd and even chain compounds. The L_α/Q_{II} phase transition temperatures of di-11:0 and di-13:0-β-GlcDAGs are lower than those of di-10:0 and di-12:0, respectively. Why this should be the case is not immediately apparent and is suggestive of some type of odd-even alternation, which has previously been attributed to end group effects in the formation

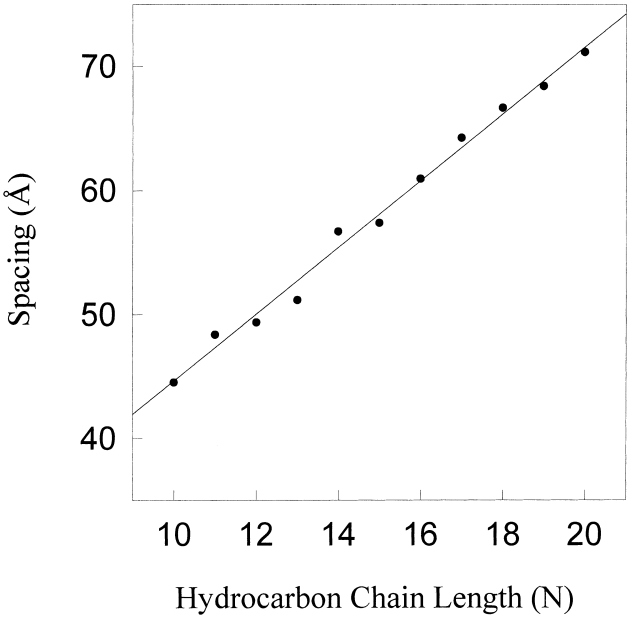

Fig. 3. A plot of the *d*-spacing versus chain length for a series of racemic dialkyl-β-D-glucosyl glycerols.

of crystalline phases ([21,22] and refs. cited therein). However, it is possible that it simply reflects differences in the kinetics of formation of the Q_{II} phases in these compounds and perhaps the nature of the Q_{II} phases formed at this transition.

The temperature-dependent variation in the lattice dimensions of the nonlamellar phases in these compounds is quite considerable (Fig. 2). The long spacings for the H_{II} phases in several of these lipids can be seen to decrease by approx. 5 Å over a temperature interval of 20°C. A similar variation in the repeat distance of a H_{II} phase as a function with temperature and water content has been reported for a polyunsaturated β-D-galactosyl diacylglycerol [6]. In comparison, the lattice parameter of the $Pn\bar{3}m$ cubic phase decreases by almost 20 Å over a range of 50°C in the di-10:0 compound. Although the change in the latter lipid appears to be smooth and continuous, the decrease in this parameter in the cubic phase regions of the di-11:0 and di-12:0 compounds may be discontinuous. The deviation of the lattice parameter as a function of temperature can be correlated with a small, broad thermal event in the corresponding DSC thermogram, suggesting that more than one cubic phase exists in this region in some members of this series, in agreement with earlier measurements of PEs [48,53] and glycolipids [36,49]. Thus, the weakly energetic event seen in this region by DSC probably corresponds to a Q_{II}^{a}/Q_{II}^{b} transition. These observations also suggest that increasing the chain length effects the kinetics of Q_{II} phase formation. Additional support for this idea can be found in the sample of di-13:0, where additional lines are visible in the cubic phase region which cannot be attributed to the L_α phase.

4. Discussion

The calorimetric and XRD studies of this series of synthetic *rac*-β-D-GlcDAGs show the existence of a single L_β lamellar gel phase in all compounds which, depending on chain length, may convert to either an L_α phase ($n \leq 14$) or an H_{II} phase ($n \geq 15$) on heating. In compounds where L_α phases exist, a transition to a Q_{II} mixed-phase region is seen at approx. 60°C, which then converts to a single Q_{II} (Q^{224}, space group $Pn\bar{3}m$) phase at higher temperatures. At still higher temperatures, the cubic phase may convert to an H_{II} phase. On cooling, the transition from the H_{II} phase is reversible, but those from the Q_{II} to the L_α phase are hysteretic. Our earlier studies of the di-12:0-β-GlcDAGs demonstrated the existence of two cubic phases on heating whereas only $Pn\bar{3}m$ was visible on cooling [49]. The one exception to this is the di-14:0 compound, where the L_α phase converts directly to a H_{II} phase without forming a cubic phase intermediate. On nucleation and extended annealing of samples of the *rac*-β-GlcDAGs, there was no evidence of more highly ordered lamellar crystalline phases like those observed in the 1,2-*sn*-β-GlcDAGs and β-GalDAGs [30,34–36,54,55]. This absence of crystalline phases and the number of chain length variants available in this series has greatly simplified the interpretation of the thermotropic phase behaviour and has made it possible to view the complete range of lamellar and nonlamellar mesophases which would otherwise be obscured in the above mentioned chiral compounds.

The transition temperatures and enthalpy changes associated with endothermic transitions of the *rac*-β-GlcDAGs are listed in Table 1. The L_β/L_α (and L_β/H_{II}) phase transitions are strongly chain length dependent in both their transition temperatures and enthalpies and, when plotted as a function of chain

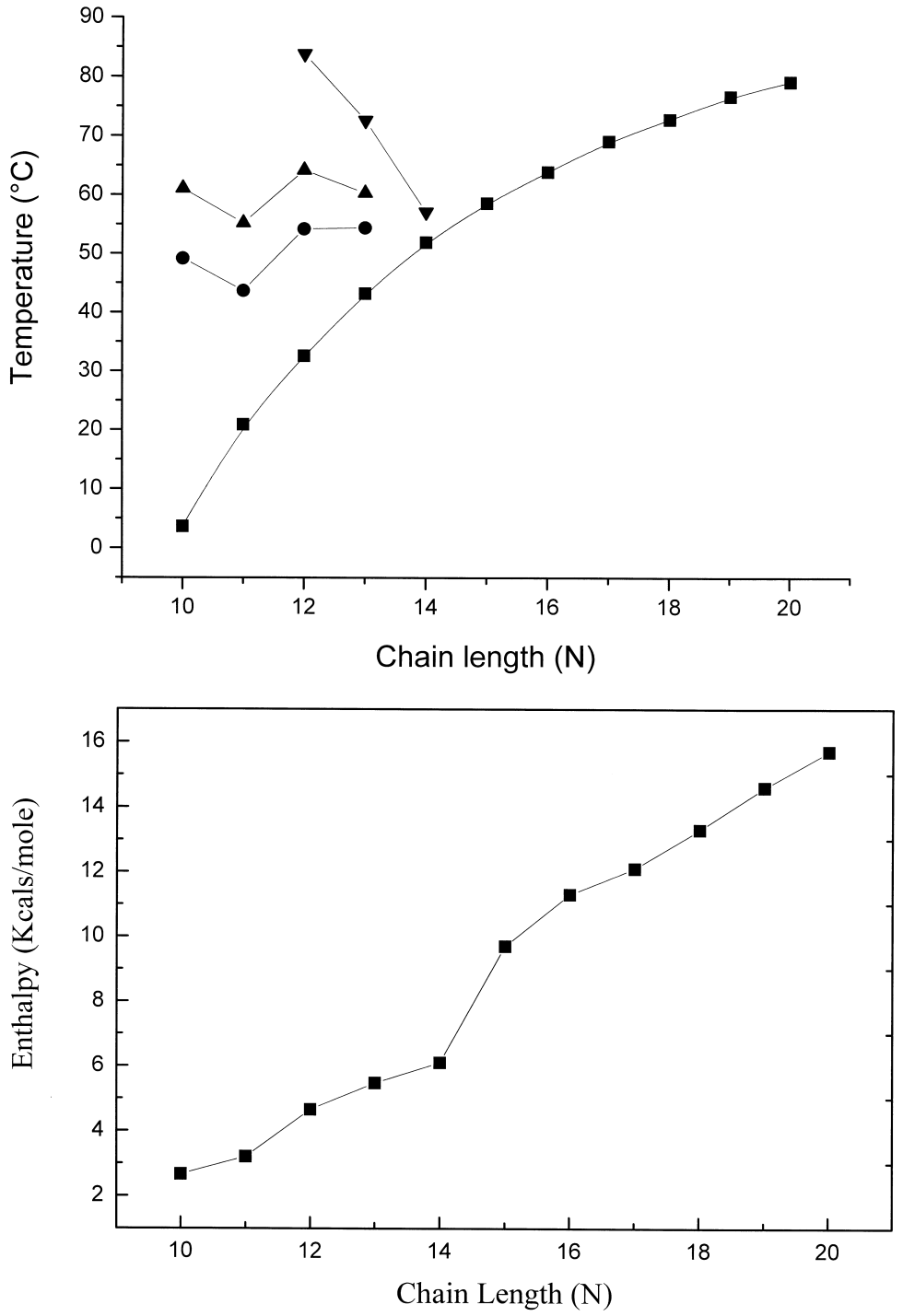

Fig. 4. Plots of the thermodynamic data obtained from DSC measurements of a series of synthetic racemic dialkyl-β-D-glucosyl glycerols as a function of chain length. (Upper panel) Transition temperatures; (lower panel) enthalpies. ■, L_β/L_α, L_β/H_{II}; ●, L_α/Q^a_{II}; ▲, Q^a_{II}/Q^{224}; ▼, L_α/H_{II}.

length (Fig. 4), describe a smooth curve characteristic of a simple chain-melting process. The Q_{II}/H_{II} and L_α/H_{II} phase transition temperatures decrease markedly with increasing chain length, reaching a minimum at a chain length of 14 carbon atoms. At longer chain lengths, the L_α phase is absent and the temperature of the transition to the H_{II} phase (now an L_β/H_{II} phase transition) increases slowly as a function of chain length, continuing as a smooth curve from the L_β/L_α transition temperatures seen in the shorter chain compounds. In contrast, the L_α/Q_{II} phase transition temperatures show relatively little chain length dependence. However, a comparison of the DSC and XRD data (Figs. 1 and 2) reveals significant differences in the L_α/Q_{II} phase transition temperatures which most likely arise from kinetic effects originating from the different thermal protocols used by each technique. A plot of the enthalpy values for the chain melting transitions of this series of *rac*-β-GlcDAGs versus hydrocarbon chain length is shown in Fig. 4 (lower panel). A discontinuity clearly exists in this plot at a chain length of 15 carbon atoms. This corresponds to the first occurrence of an L_β/H_{II} phase transition with increasing chain length and must represent the combined ΔH values originating from chain melting and the change from a lamellar to an H_{II} morphology.

The basic pattern of thermotropic phase behaviour described here for these *rac*-β-GlcDAGs is one which is similar to that seen in several other nonlamellar phase-preferring phospho- and glycolipid systems, such as the diastereomeric diacyl and dialkyl glycosyl glycerols [21–24,30–35] and the PEs [43–48]. Events such as the L_α/Q_{II} and Q_{II}/H_{II} phase transitions involving the double diamond cubic phase (previously termed 2-D-monoclinic in our earlier paper [21]) belonging to space group $Pn\bar{3}m$, have been observed in our own studies of 1,2-diacyl-3-*O*-(α- and β-D-glucosyl)-*sn*-glycerols [21,23,24] as well as in studies of DDPE [47,48,53] and *N*-methylated DOPE [46]. Transitions of the L_α/H_{II} type are more widespread, having been reported on numerous occasions in aqueous dispersions for both diacyl and dialkyl glycolipids [9–13,21,23,24,30–35] and PEs [47,48,56]. In contrast, reports of L_β/H_{II} phase transitions are somewhat fewer, but such transitions have been observed in a study of a PE at high salt concentrations [57]. These studies all suggest that the L_β/H_{II} phase transition is a characteristic of lipids containing long chains and small headgroups.

A comparison of the L_β/L_α phase transition temperatures of the *rac*-β-GlcDAGs with those for the corresponding 1,2-*sn*-β-GlcDAGs ([30,35,36]; D. Mannock, unpublished experiments) show close agreement. However, the values obtained for the 2,3-*sn* compounds tend to be slightly lower, especially in the shorter chain compounds ([54,55,58]; D. Mannock, unpublished observations), suggesting that the chirality of the glycerol moiety plays only a minor role in determining the T_m of these lipids (see below). The chain melting phase transition temperatures of the diastereomeric (1,2-*sn* and 2,3-*sn*) β-GalDAGs and their mixtures (*rac*) (D. Mannock, unpublished experiments) are also close to those reported here for the *rac*-β-GlcDAGs. These observations suggest that small changes in the carbohydrate isomeric configuration do not significantly alter T_m. Even more extensive headgroup modifications, such as the substitution of the sugar headgroup with a phosphorylethanolamine headgroup, tend to raise T_m [47], whereas insertion of two carbonyl groups into the polar/apolar interface lowers T_m in the corresponding diacyl glycolipids [21,23,24,60] and PEs [47].

A comparison of the L_α/NBL phase transition temperatures of the *rac*-β-GlcDAGs with those of the chiral β-GlcDAGs reveal similarities between the 1,2-*sn* and 1,2-*rac* lipids, whereas those of the 2,3-*sn* lipids appear to be shifted to slightly lower temperatures [36]. This is particularly evident with the transition to the H_{II} phase. XRD measurements also suggest that there is a difference in the number of cubic phases found in the 1,2-*sn*, 1,2-*rac* and 2,3-*sn*-β-GlcDAGs, or that there may be a difference in which the stability of the respective cubics with changing backbone configuration [36]. There are also notable differences in the L_α/H_{II} phase transition temperatures of the chiral and *rac*-β-GlcDAGs and comparable β-GalDAGs suggest that differences in glycerol chirality and headgroup configuration alter the lamellar to nonlamellar phase transition temperatures more than those of the chain-melting phase transitions (Table 2). The headgroup contributions to this process are even more pronounced in the corresponding dialkyl-pentopyranosyl-glycerols in which the 'effective size of the headgroup' is reduced

[59]. In such cases, the nature of the nonlamellar phase (and thus the nonlamellar phase preference) may shift in favour of H_{II} and micellar cubic phases, such as the Fd3m phase [59], without substantially changing the chain-melting phase transition temperature. This discontinuous relationship between 'headgroup size' and lamellar/nonlamellar phase preference suggests that the above headgroup and interfacial modifications play a role in regulating the ability of the polar region to counterbalance the volume of the hydrocarbon chains at the lamellar/nonlamellar phase transition. To what extent other variables such as the enthalpy and entropy of the above transitions are affected by these changes in headgroup and interfacial configuration is presently unclear and must await measurements on the new generation of nanocalorimeters.

A closer inspection of Fig. 4 (upper panel) shows that the phase boundary between the L_α and cubic phases increases gradually in temperature with increasing chain length, whereas that for the Q^{224}/H_{II} phase boundary decreases sharply and then gently approaches a minimum before meeting the L_β/H_{II} phase boundary. Interestingly, plots of the lamellar/nonlamellar phase transition temperatures as a function of chain length of lipids in which the headgroup, interface and hydrocarbon chain structure have been systematically varied, as well as plots of analogous PEs, show L_α/Q_{II} and Q_{II}/H_{II} phase boundaries whose linear dependence also varies. This supports earlier suggestions that the chemical diversity of lipids in biological membranes exists, in part, to regulate membrane curvature.

Recent investigations of 1,2-dialkyl-3-*O*-(β-D-glycosyl)-*sn*-glycerols [30,34,35] have reported the formation of highly ordered L_c phases under suitable annealing conditions. Such gel phase polymorphism is absent both here in the *rac*-β-GlcDAGs and in the 2,3-*sn*-β-GlcDAGs, suggesting that the chirality of the glycerol moiety is important in the hydrogen bonding interactions necessary to form such highly ordered phases. Our own studies of space filling models and molecular modelling using CHARM show that while the two diastereomers have similar molecular shapes, the relative positions of the hydrogen bonding groups in the interfacial regions of these diastereomers are different. Thus, the ability of each diastereomer to eliminate water from the interfacial region and rearrange to form hydrogen bonds with a neighbouring headgroup, and hence its ability to form L_c type phases, is not the same. In the diastereomeric mixture, the above differences are further compounded by the problem of packing two molecules with different conformations together (see [23] for a full discussion).

A combination of all these factors will determine whether or not a given lipid molecule is capable of forming a highly ordered L_c phase as well as the kinetics of transformation of such phases. In the di-14:0-β-GalDAGs, where transitions involving the stable L_c phases occur at different temperatures, the rate of formation of the L_c phase changes with the chirality of the glycerol backbone in the order 1,2-*sn* > *rac* > 2,3-*sn*. The absence of L_c phases in the racemic and 2,3-*sn*-β-GlcDAGs suggests that the two headgroups (β-D-Gal and β-D-Glc) have very different hydrogen bonding properties and that in the glucosyl series formation of L_c phases may not be so highly favoured. Thus, regulating the headgroup and interfacial properties of a glycolipid in a biological environment at lower temperatures would favour a well hydrated, less ordered membrane. In extreme environments, the absence of highly ordered phases would tend to produce a more stable membrane with a reduced tendency for phase separation that would also maintain its barrier properties under such circumstances.

The observations reported here, in combination with the recent reports on the physical properties of some chiral β-Glc- and β-GalDAGs, suggest that both the headgroup structure and the chirality at the glycerol offer a simple but effective means by which an organism can alter the physical properties of its membrane at higher temperatures by regulating its nonlamellar tendencies, and consequently, its membrane curvature. Further details of the physical properties of chiral and racemic glycolipids will follow shortly.

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References

[1] P.J. Quinn, W.P. Williams, Prog. Biophys. Mol. Biol. 34 (1978) 109–179.
[2] P.J. Quinn, W.P. Williams, Biochim. Biophys. Acta 737 (1983) 223–266.
[3] C. Ratledge, S.G. Wilkinson (Eds.), Microbial Lipids, vol. 1, Academic Press, New York, 1988.
[4] W.P. Williams, P.J. Quinn, J. Bioenerg. Biomembr. 19 (1987) 605–624.
[5] E. Rivas, V. Luzzati, J. Mol. Biol. 41 (1969) 261–275.
[6] G.G. Shipley, J.P. Green, B.D. Nichols, Biochim. Biophys. Acta 311 (1973) 531–544.
[7] D. Oldani, H. Hauser, B.W. Nichols, M.C. Phillips, Biochim. Biophys. Acta 382 (1975) 1–9.
[8] D.A. Mannock, A.P.R. Brain, W.P. Williams, Biochim. Biophys. Acta 821 (1985) 153–164.
[9] P.W. Sanderson, W.P. Williams, Biochim. Biophys. Acta 1107 (1992) 77–85.
[10] A. Sen, D.A. Mannock, D.J. Collins, P.J. Quinn, W.P. Williams, Proc. R. Soc. London B218 (1983) 349–364.
[11] D.A. Mannock, A.P.R. Brain, W.P. Williams, Biochim. Biophys. Acta 817 (1985) 289–298.
[12] P.J. Quinn, L.J. Lis, J. Colloid Interface Sci. 115 (1987) 220–224.
[13] L.J. Lis, P.J. Quinn, Biochim. Biophys. Acta 862 (1986) 81–86.
[14] A. Wieslander, J. Ulmius, G. Lindblom, K. Fontell, Biochim. Biophys. Acta 512 (1978) 241–253.
[15] A. Wieslander, L. Rilfors, L.B.-A. Johansson, G. Lindblom, Biochemistry 20 (1981) 730–735.
[16] A. Khan, L. Rilfors, A. Wieslander, G. Lindblom, Eur. J. Biochem. 16 (1981) 215–220.
[17] J.R. Silvius, N. Mak, R.N. McElhaney, Biochim. Biophys. Acta 597 (1980) 199–215.
[18] A. Wieslander, A. Christiansson, L. Rilfors, A. Khan, L.B.-A. Johansson, G. Lindblom, FEBS Lett. 124 (1981) 273–278.
[19] D.A. Mannock, R.N.A.H. Lewis, R.N. McElhaney, Chem. Phys. Lipids 43 (1987) 113–127.
[20] D.A. Mannock, R.N.A.H. Lewis, R.N. McElhaney, Chem. Phys. Lipids 55 (1990) 309–321.
[21] D.A. Mannock, R.N.A.H. Lewis, A. Sen, R.N. McElhaney, Biochemistry 27 (1988) 6852.
[22] R.N.A.H. Lewis, D.A. Mannock, R.N. McElhaney, P.T.T. Wong, H.H. Mantsch, Biochemistry 29 (1989) 8933–8943.
[23] D.A. Mannock, R.N.A.H. Lewis, R.N. McElhaney, Biochemistry 29 (1990) 7790–7799.
[24] A. Sen, S.W. Hui, D.A. Mannock, R.N.A.H. Lewis, R.N. McElhaney, Biochemistry 29 (1990) 7799–7804.
[25] B. Asgharian, D.A. Cadenhead, D.A. Mannock, R.N.A.H. Lewis, R.N. McElhaney, Biochemistry 28 (1989) 7102–7106.
[26] D.A. Wisner, T. Rosarro-Jansen, M.D. Tsai, J. Am. Chem. Soc. 108 (1986) 8064–8068.
[27] P.M. Brain, T. Steers, S.W. Hui, P.L. Yeagle, J.R. Silvius, Biochemistry 25 (1986) 4259–4267.
[28] J.R. Silvius, P.M. Brain, T.J. O'Leary, Biochemistry 25 (1986) 4249–4258.
[29] K.S. Bruzik, M.D. Tsai, Biochemistry 26 (1987) 5364–5368.
[30] H.-J. Hinz, L. Six, K.L. Ruess, M. Lieflander, Biochemistry 24 (1985) 806–813.
[31] H.C. Jarrell, J.B. Giziewicz, I.C.P. Smith, Biochemistry 25 (1986) 3950–3957.
[32] H.C. Jarrell, A.T. Wand, J.B. Giziewicz, I.C.P. Smith, Biochim. Biophys. Acta 897 (1987) 69–82.
[33] H.C. Jarrell, P.A. Jorall, J.B. Giziewicz, L.A. Turner, I.C.P. Smith, Biochemistry 26 (1987) 1805–1811.
[34] R.D. Koynova, H.L. Kuttenreich, B.G. Tenchov, H.-J. Hinz, Biochemistry 27 (1988) 4612–4619.
[35] H. Kuttenreich, H.-J. Hinz, M. Inczedy-Marcsek, R. Koynova, B. Tenchov, P. Laggner, Chem. Phys. Lipids 47 (1988) 245–260.
[36] D.A. Mannock, R.N.A.H. Lewis, R.N. McElhaney, M. Akiyama, H. Yamada, D.C. Turner, S.M. Gruner, Biophys. J. 63 (1992) 1355–1368.
[37] T. Ogawa, K. Beppu, Agric. Biol. Chem. 46 (1982) 255–262.
[38] W.J. Baumann, H.K. Mangold, Biochim. Biophys. Acta 116 (1966) 570–576.
[39] L. Six, K.-P. Ruess, M. Lieflander, Tetrahedron Lett. 24 (1983) 1229–1232.
[40] C.A.A. van Boeckel, G.M. Visser, J.H. van Boom, Tetrahedron Lett. 41 (1985) 4557–4565.
[41] M. Yamagishi, K. Kakinuma, Agric. Biol. Chem. 53 (1989) 867–868.
[42] M. Akiyama, Y. Terayama, J. Pre-Med. Sapporo Med. Coll. 19 (1978) 59–64.
[43] E. Shyamsunder, S.M. Gruner, M.W. Tate, D.C. Turner, P.T.C. So, C.P.S. Tilcock, Biochemistry 27 (1988) 2332–2336.
[44] D.M. Anderson, S.M. Gruner, S. Leibler, Proc. Natl. Acad. Sci. USA 85 (1988) 5364–5368.
[45] P. Mariani, V. Luzzati, H. Delacroix, J. Mol. Biol. 204 (1988) 165–189.
[46] S.W. Gruner, M.W. Tate, G.L. Kirk, P.T.C. So, D.C. Turner, D.T. Keane, C.P.S. Tilcock, P.R. Cullis, Biochemistry 27 (1988) 2853–2866.
[47] J.M. Seddon, G. Cevc, D. Marsh, Biochemistry 22 (1983) 1280–1289.
[48] J.M. Seddon, G. Cevc, R.D. Kaye, D. Marsh, Biochemistry 23 (1984) 2634–2644.
[49] D.C. Turner, S.G. Gruner, Z.-G. Wang, D.A. Mannock, R.N. McElhaney, J. Phys. II (1992) 2039.

[50] D.M. Small, J. Lipid Res. 8 (1967) 551–557.
[51] V. Luzzati, T. Gulik-Krzywicki, A. Tardieu, Nature 218 (1968) 1031–1034.
[52] A. Tardieu, V. Luzzati, F.C. Reman, J. Mol. Biol. 75 (1973) 711–733.
[53] J.M. Seddon, J.L. Hogan, N.A. Warrender, A. Pebay-Peyroula, Prog. Colloid Polymer Sci. 81 (1990) 189–197.
[54] D.A. Mannock, R.N. McElhaney, P.E. Harper, S.M. Gruner, Biophys. J. 66 (1994) 734–740.
[55] H. Kuttenreich, H.-J. Hinz, R.D. Koynova, B.G. Tenchov, Chem. Phys. Lipids 66 (1993) 55–62.
[56] M. Caffrey, Biochemistry 24 (1985) 4826–4844.
[57] D. Marsh, J.M. Seddon, Biochim. Biophys. Acta 690 (1982) 117–123.
[58] R.N.A.H Lewis, D.A. Mannock, R.N. McElhaney, in: R. Epand (Ed.), Lipid Polymorphism and Membrane Properties, Current Topics in Membranes, vol. 44, Academic Press, New York, 1997, pp. 25–102.
[59] J.M. Seddon, N. Zeb, R.H. Templer, R.N. McElhaney, D.A. Mannock, Langmuir 12 (1996) 5250–5253.
[60] D.A. Mannock, R.N. McElhaney, Biochem. Cell Biol. 69 (1991) 863–867.