Fourth International Electronic Conference on Synthetic Organic Chemistry (ECSOC-4), www.mdpi.org/ecsoc-4.htm, September 1-30, 2000

[C0014]

Synthesis and Structural Properties of Neoglycolipids Anchored at Membrane Surfaces

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Received: 7 August 2000 / Uploaded: 10 August

Aims and Scope

Glycolipids cover the surface of living cells and determine their properties in cell-adhesion processes. The extremely complex and heterogeneous native cell membrane forbids the functional characterization of individual glycolipid components. Homogeneous neoglycolipids on model membranes reduce the complex native glycocalix to its essential features.

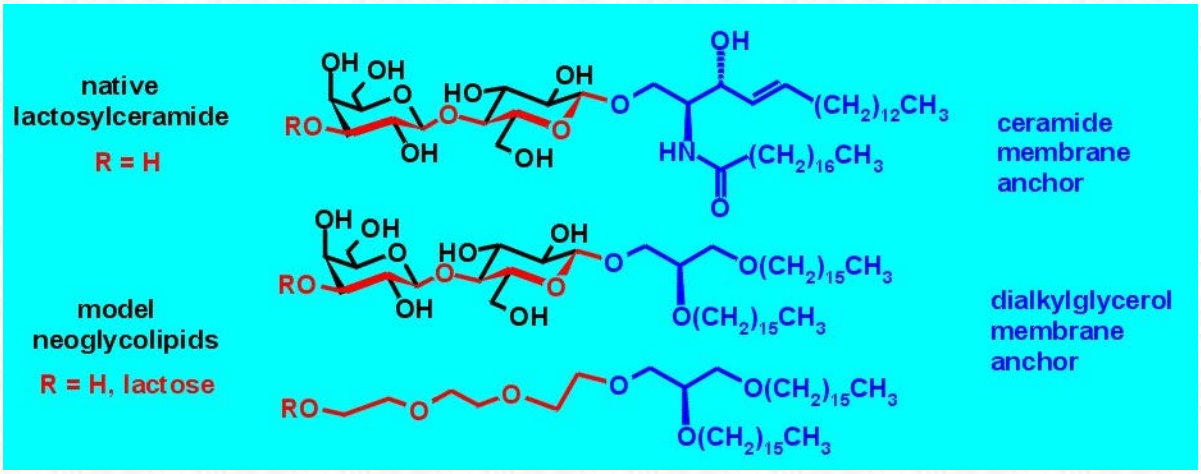

Figure 1. 1,2-di-*O*-alkyl-*sn*-glycerol serves as the membrane anchor for lactose building blocks. The triethyleneglycol spacer mimics one lactose disaccharide. Although the number of bonds are the same (red), the flexibility of the triethyleneglycol spacer is much higher.

Synthesis of the 1,2-di-*O*-alkyl-*sn*-glycerol membrane anchor

Fig. 2 gives an outline of the synthetic route to the 1,2-di-*O*-hexadecyl-*sn*-glycerol derivatives ***Peg$_n$*** (n = 3, 6, and 9).[3] Benzyl protected triethylene glycol derivative **1** was synthesized in one step from commercially available triethylene glycol monochlorohydrin. The spacered lipid ***Peg$_3$*** was synthesized in high overall yield by the coupling of **1** (2.5 equivalents) and 1,2-*O*-isopropylidene-*sn*-glycerol in the presence of sodium hydride as base and tetrabutylammonium iodide (TBAI) as activator, followed by acid catalyzed de-*O*-isopropylidenation, di-*O*-alkylation with hexadecyl bromide under the above described conditions, and finally hydrogenolytic *O*-debenzylation. Repetitive coupling of ***Peg*** with the building block **1** (2.5 equivalents) under the above described conditions yielded, after de-*O*-benzylation,

the elongated lipids ***Peg$_6$*** and ***Peg$_9$***.

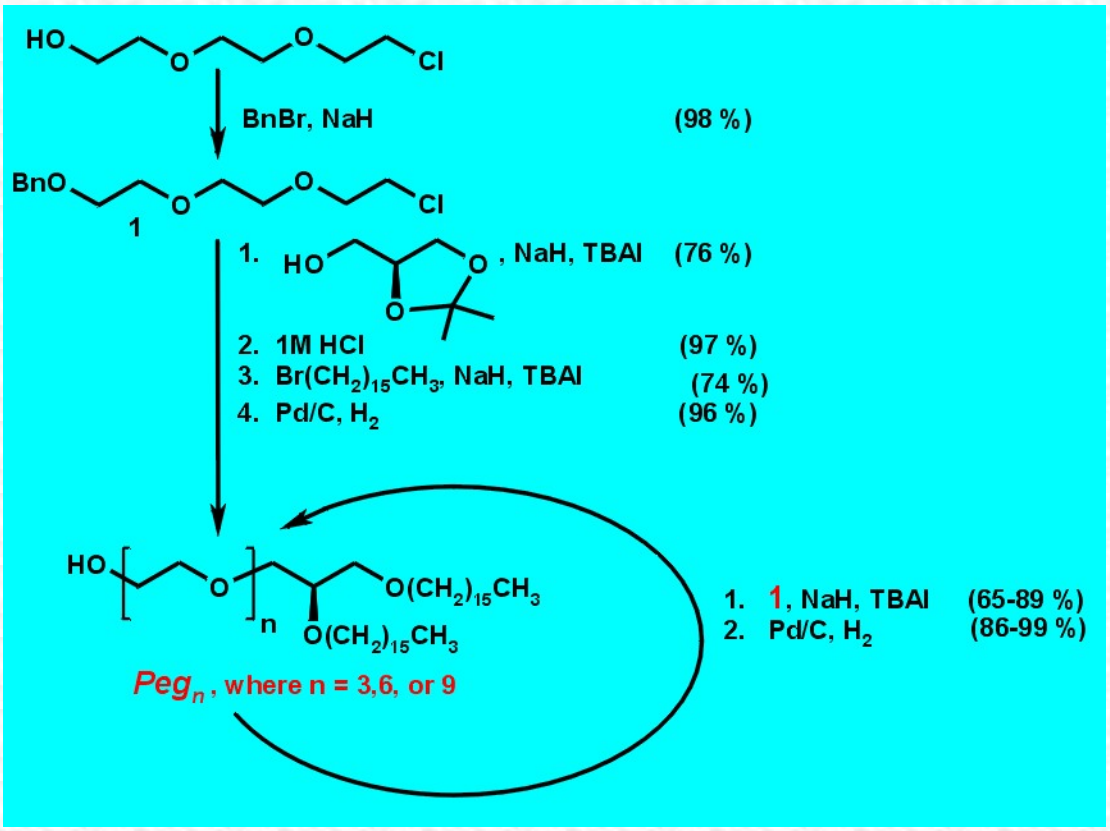

Figure 2. Synthesis of ethylene glycol spacered 1,2-Di-*O*-hexadecyl-*sn*-glycerol derivatives ***Peg$_n$*** (*n* = 3,6, and 9).

Attachment of lactose or oligolactose

The synthetic route to these glycolipids is shown in Fig. 3. Glycosylation of known lactose trichloroacetimidate **2**[1] with 1,2-di-*O*-hexadecyl-*sn*-glycerol[2,3] as lipid anchor was performed in the presence of $BF_3 \cdot Et_2O$ as catalyst to afford the lactose intermediate **3** in 94 % yield. The b-configuration of the newly formed glycosidic bond was confirmed in the ^1H NMR spectrum ($J_{1,2}$ = 7.9 Hz). Acid-catalyzed cleavage of the isopropylidene group furnished **4**, which served as acceptor for the next glycosylation. Removal of the *O*-benzoyl protective groups with sodium methoxide in CH_2Cl_2/CH_3OH afforded ***Lac$_1$*** in almost quantitative yield after purification on reversed phase (RP-18) silica gel.

Elongation of **4** with donor **2** in the presence of $BF_3 \cdot Et_2O$ as catalyst at – 20°C afforded exclusively the expected b(1®3)-connected bis-lactose intermediate **5 (**$J_{1,2}$ = 7.9 Hz for 1c-H; shift of the 4b-H-signal from 3.85 ppm to 5.29 ppm after acetylation). Again, de-*O*-isopropylidenation furnished the following acceptor **6**, and de-*O*-benzoylation the desired ***Lac$_2$*** derivative under the conditions described above. Repetitive coupling of **6** with donor **2** yielded the elongated protected glycolipid **7**, which was de-*O*-isopropylidenated in the same manner. Due to the low solubility of ***Lac$_3$*** the de-*O*-benzoylation and purification was performed in dimethyl sulfoxide (DMSO) mixtures.

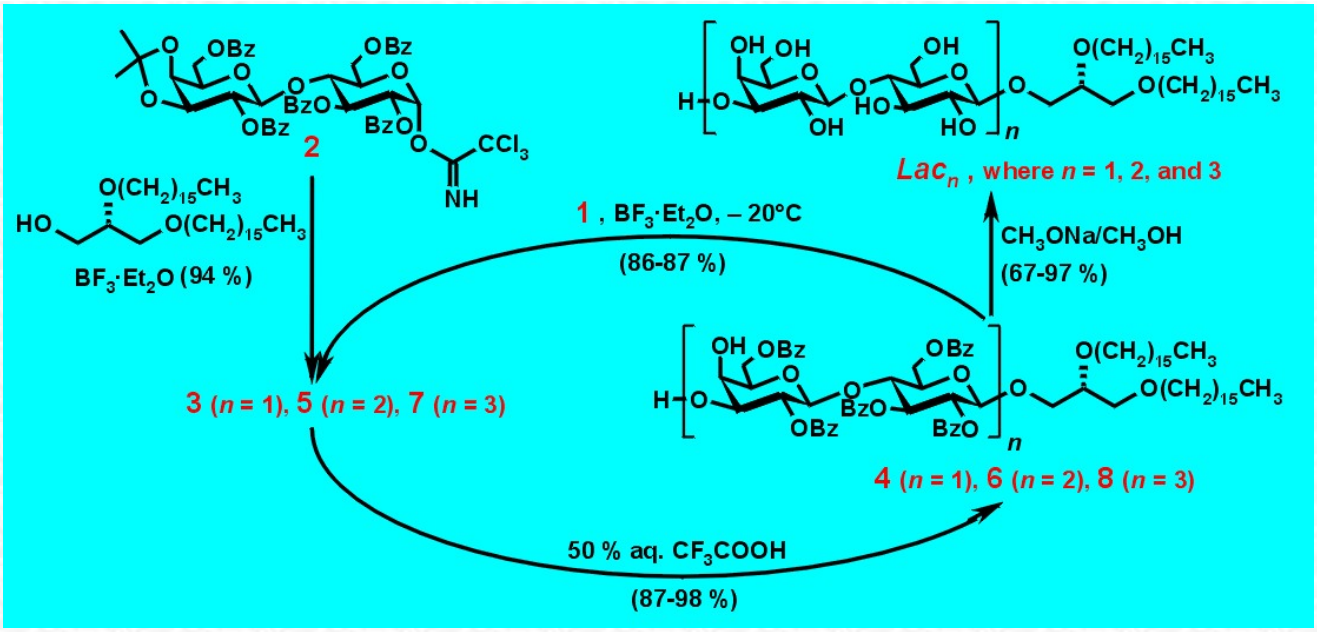

Figure 3. Synthesis of ***Lac$_n$*** (*n* = 1, 2, and 3) by repetitive glycosylation.

Fig. 4 shows mixed neoglycolipids characterized by triethylene spacers of various lengths inserted between lactose and the membran anchor. They were named ***LacPeg$_n$***, with n corresponds to the number of ethylene glycol units (n = 3, 6, or 9). The benzoylated lactose trichloroacetimidate **9** is available from lactose in a three-step procedure.[4] Again, the benzoyl protecting group next to the anomeric activation directs the formation of b-linked glycolipids without formation of undesired orthoester.

Glycosidation of 1,2-di-*O*-hexadecyl-*sn*-glycerol derivatives of ***Peg$_n$*** (n = 3, 6, 9) as acceptors was performed with donor lactose trichloroacetamidate **9** in the presence of TMSOTf as catalyst to afford the protected products **10**, **11**, and **12**, respectively. The newly formed glycosidic bond was found exclusively b -oriented in the ^{1}H-NMR spectrum. Removal of the benzoyl groups with sodium methoxide in $CHCl_3/CH_3OH$ afforded the ***LacPeg$_n$*** derivatives in quantitative yields after purification by flash chromatography.

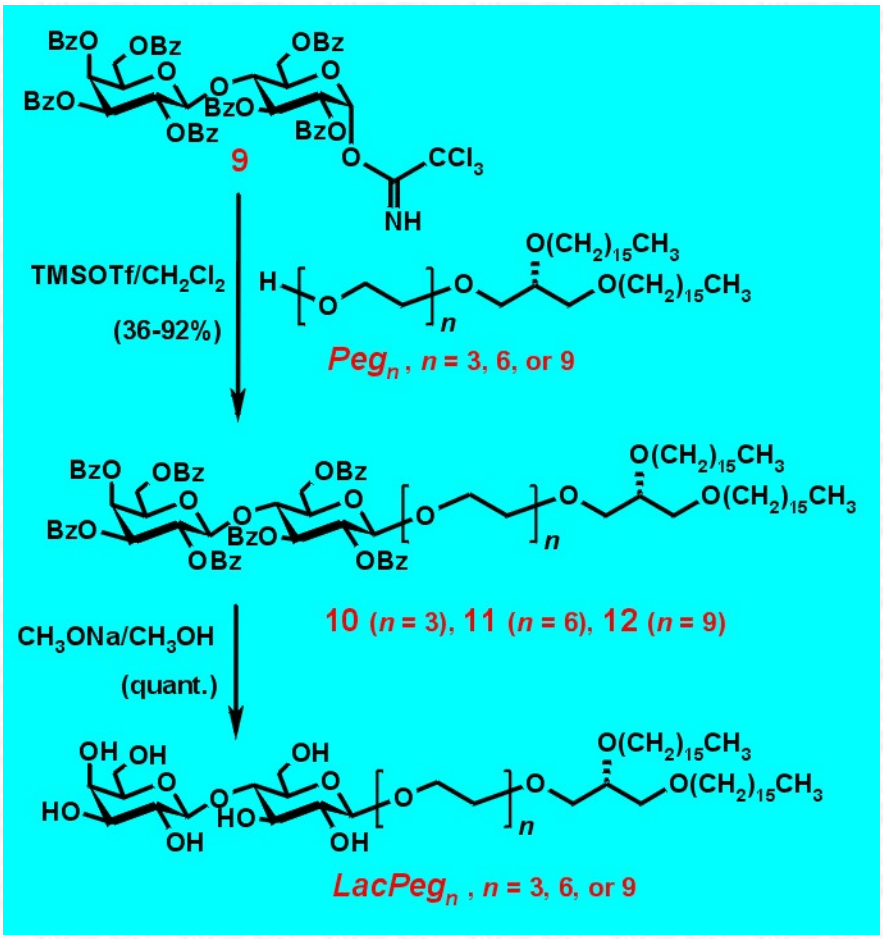

Figure 4. Synthesis of the spacered lactosyl-glycolipids.

Synthesis of a neoglycolipid with a deuterated acetyl group

Glycosylation of ***Peg*$_9$** with the glucosamine imidate[5] led to b-linked glycoside ($J_{1,2}$ = 8.6 Hz) in high yield. Treatment with activated zinc in deuterated acetic anhydride led to the *N*-acetyl derivative, which furnished on treatment with NaOMe in MeOH the desired glucosamine derivative ***GlcNAcPeg*$_9$**.[3]

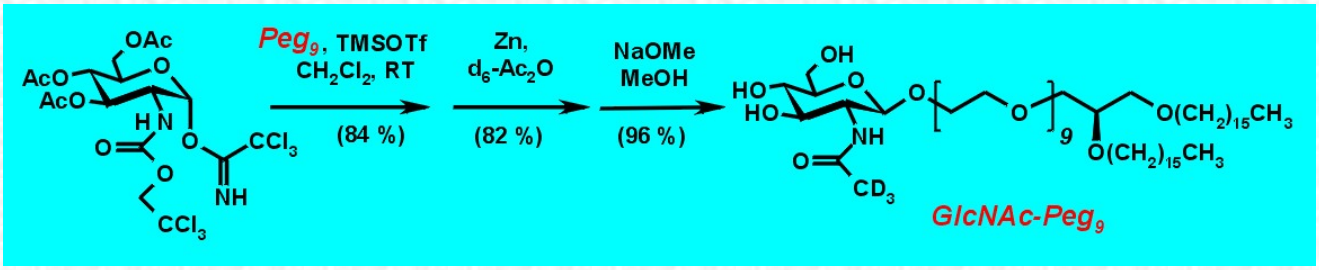

Figure 5: Synthesis of ***GlcNAcPeg*$_9$**.

NMR studies

So-called bicelles are a powerfull tool for the spectroscopic characterization of neoglycolipids. This membrane model was developed by Prestegard for NMR studies.[6] Bicelles combine several advantageous qualities. They form a flat and highly solvated bilayer model which is stable between 303 and 320 K and which can be studied with highfield NMR methods (Fig. 6). Fig. 7 shows typical phase transitions when glycolipid ***GlcNAcPeg*$_9$** is inserted into the membranes. In the temperature range of bicelle formation, two ^{31}P NMR signals are observed which belong to the edge (lowfield,

mainly dihexanoylphosphatidylcholine, DHPC) and the flat bilayer (highfield, mainly dimyristoylphosphatidylcholine, DMPC). The ^{1}H NMR shows excessive line-broadening. Also the carbohydrate protons resonances are broadened, proving the slowing down of the glycolipid tumbling. The temperture of phase transition is influenced by the admixed glycolipid. ^{31}P NMR spectra and ^{1}H NMR spectra are shown in Fig. 7 for ***GlcNAcPeg*$_9$**. Only in a very small region of less than 5 K the bicelles are formed. The ^{31}P NMR spectra exhibit two resonances: the broader highfield signal originates from the flat bilayer, while the sharper lowfiled signal comes from the DHPC at the edges of the bicelles. Only one isotropic ^{31}P resonance is detected above and below this temperature range. At 302 K all three signals are visible and indicate the existence of bicelles plus isotropic liposomes. A very sharp phase transition is observed between 305 K and 306 K, the bicelles `melt´.

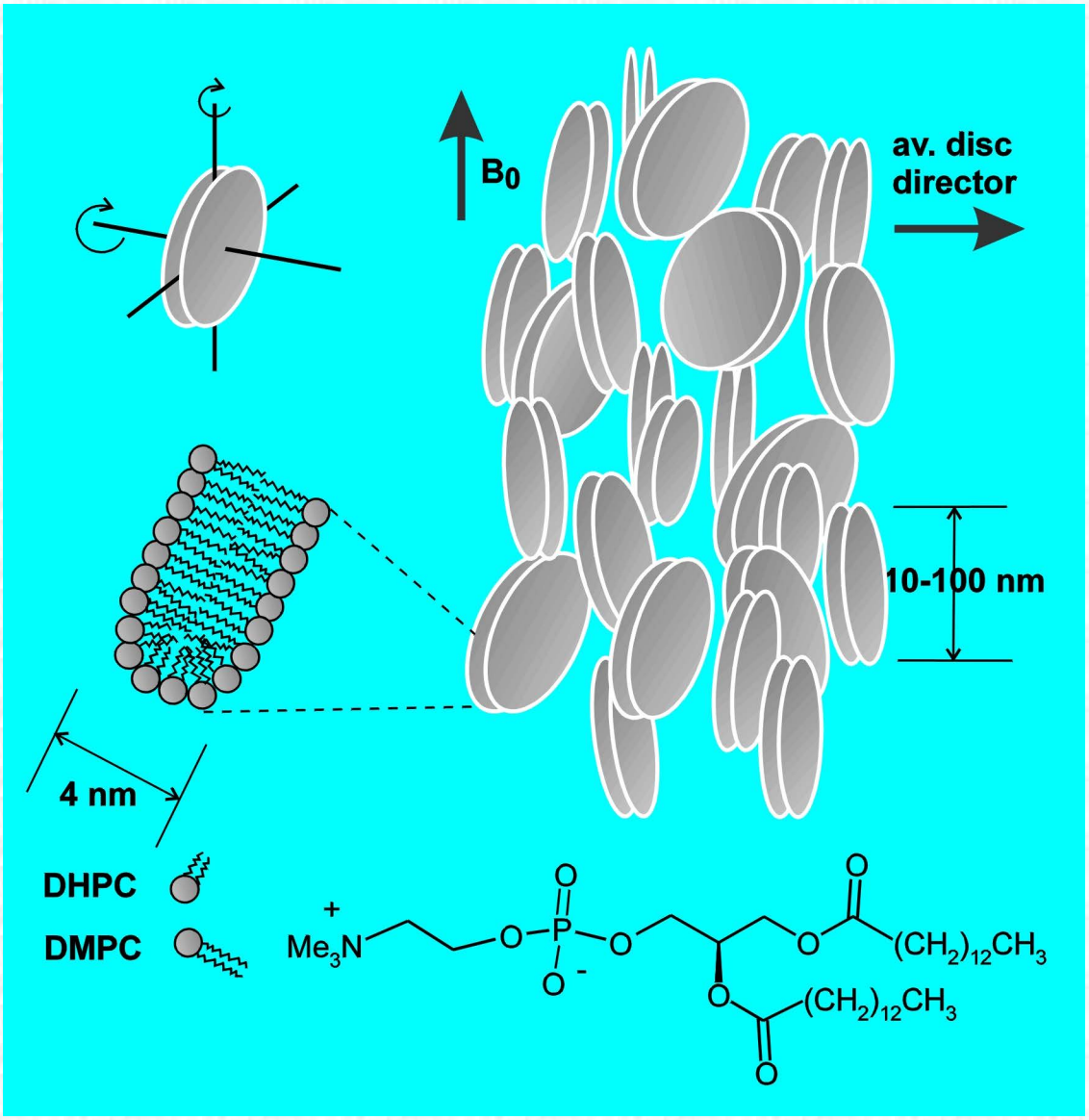

Figure 6: Disc-shaped micelles, so-called bicelles are formed by DMPC and DHPC. Flat lipid bilayers are formed by the DMPC fraction, while the DHPC molecules probably concentrate at the edges of the bicelles. The bicelles form a nematic fluid-crystalline phase with the average disc vector perpendicular to the direction of the magnetic field B_0. The diameter of the disc varies from 10 to 100 nm, the thickness is about 4 nm.

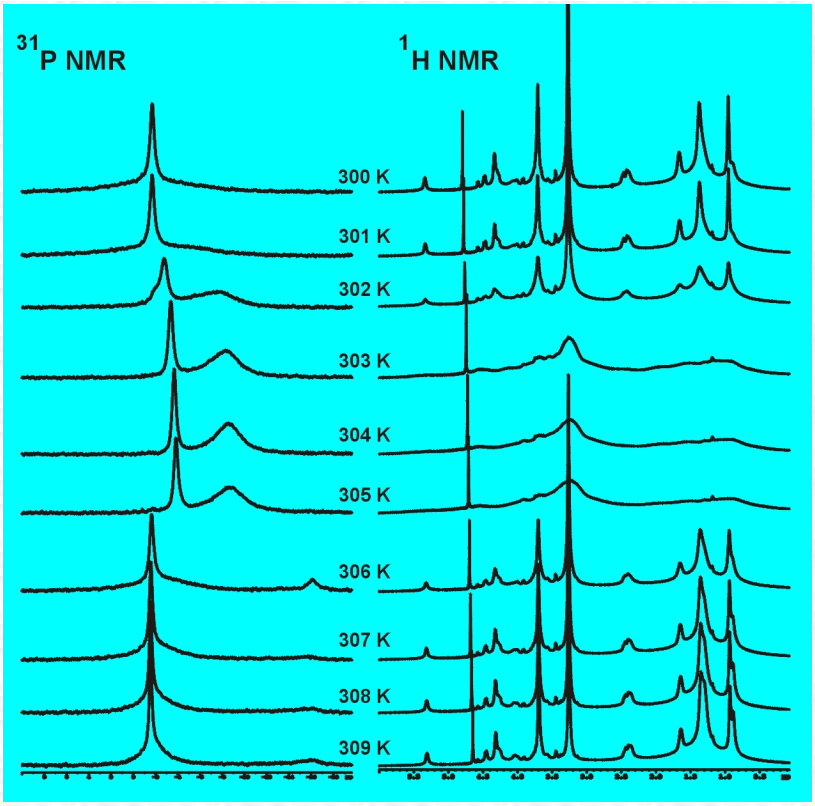

Figure 7: Temperature dependence of a 3:1 mixture of DMPC and DHPC containing the neoglycolipid ***GlcNAcPeg*$_9$** in a mM concentration.

Conclusion

The amphiphilic lactose-derived neoglycolipids described here exhibit poor solubilities in monolayered or bilayered membrane models. The triethylene spacer improves the solubilities. Although we described a straightforward synthetic strategy making the final compounds accessible in the gram scale, their NMR spectroscopic characterization in flat phospholipid bilayers remains a difficult task.

References

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