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STUDIES OF MIXED LIPOSOMES WITH NOVEL SORBYL FUNCTIONALIZED HEAD GROUP LIPIDS

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Abstract

Three novel polymerizable amphiphiles with a sorbyl-substituted head group were synthesized and systematically characterized. These amphiphiles are neutral in charge. None of these molecules forms vesicles by itself, presumably due to lack of amphiphilicity and/or extensive head group interaction. Therefore, mixed vesicles were formed with other fluid lipids such as DPenPC, eggPC, or DOPC. We investigated the properties of these mixtures in both vesicles and Langmuir films. The Langmuir isotherms show formation of monolayers by all three molecules. However, the isotherms for mixed monolayers suggest that two components are largely immiscible to the mixing lipid. Under polymerization conditions, mixed vesicles of these amphiphiles form oligomers, suggesting that in spite of a larger head group, they form mesophases.

Keywords

Sorbyl groups; polymerizable lipids; Langmuir isotherms; mixed liposomes

1. Introduction

The polymerization of hydrated lipid amphiphiles is an effective way to modify the chemical and physical properties of a supramolecular assembly. This can be achieved by introducing reactive groups to either the tail or head group region of the amphiphile. A diverse assortment of reactive groups, including diacetylenyl, methacryloyl, acryloyl, dienoyl, sorbyl, and styryl have been commonly introduced into the tail region and are well characterized (Bader et al., 1985; Blume, 1991; Mueller and O'Brien, 2002). However, introducing reactive groups in the tail region may change properties inherent to fluid lipid assemblies upon polymerization. For example, a reactive group in the tail region is likely to perturb the lipid packing in mesophases (Blume, 1991) and may lead to changes in physical properties such as permeability (Roach et al., 2004). In addition, polymerization along the tail region has a notable effect on the lipid membrane fluidity (Bader et al., 1985; Gros et al., 1981; Ross et al., 2003b). Hence, understanding of structure-property parameters that affect lipid assembly and properties is a critical feature necessary for the design of novel and improved functional lipids to form micellar, or vesicular aggregates.

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Supplementary Data Available: Detailed synthesis procedures and spectral data.

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The incorporation of polymerizable groups in the head region may minimally perturb the lipid packing as the reactive groups are sufficiently spaced from the hydrophobic core (Ringsdorf et al., 1988). In addition, incorporating flexible spacers between reactive groups and the head group region imparts conformational entropy between the alkyl chains and the main polymer chain, which is required to enable both efficient assembly and connectivity. These types of reactive amphiphiles can be useful in applications that require retention of some of the physical properties of natural fluid lipids while enhancing the mechanical stability of the bilayer structure.

Many types of reactive groups have been incorporated into the head group region of lipids as well, including methacryloyl, acryloyl, styryl, nitrostyryl, vinyl groups which can be polymerized via free radical processes (Bader et al., 1985; Mueller and O'Brien, 2002; Ringsdorf et al., 1988). Most of these molecules are cationic or anionic while few of them are neutral (phospholipids) or uncharged. In addition to the compositional characterization of polymerized lipid assemblies, examination of Langmuir monolayer properties, studies of vesicle fusion mechanism, and mechanical and structural properties of these vesicles have been reported. These structural properties include phase behavior, electrical resistance, packing efficiency and self-organization, permeability behavior, and spacers to isolate molecular motions of the hydrophobic tails (see reviews above).

The synthetic lipids used in this study (Fig. 1) differ from the previously reported head group polymerizable lipids in that they contain sorbyl group(s) attached to the hydrophilic head group. Sorbyl groups inserted into the hydrophobic chains of polymerizable lipids have been well characterized (see review (Mueller and O'Brien, 2002)). The extent of the polymerization and the nature of the polymers are well known. The sorbyl group can be polymerized in both gel (L_{β}) and liquid-crystalline (L_{α}) phases (Lei et al., 1999) by several different initiation methods as well as on different scaffolds (Conboy et al., 2003; Mueller and O'Brien, 2002; Ratnayaka et al., 2008; Ross et al., 2003a). These reasons led us to synthesize a series of novel lipid molecules containing sorbyl moieties attached to the head group. These lipids are neutral in charge. The synthesis and characterization of three of these lipids: 2-[3-(2,3-dielaidoyloxypropylcarbamoyl)propanoyloxy]ethyl 2,4-hexadienoate (**1**), 2-(2-[3-(2,3-dielaidoyloxypropylcarbamoyl)propanoyloxy]ethoxy)ethoxyethyl 2,4-hexadienoate (**2**), and N-[2,3-(dioleoyloxy)-1-propyl]succinamide 1,3-(disorboylamino)-2-propyl ester (**3**) are reported herein (Fig. 1). **1** and **2** are mono-substituted with sorbyl groups while **3** is bis-substituted and has amide-linked sorbate groups.

2. Experimental

2.1. Materials

L- α -Phosphatidylcholine (eggPC), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine (DEPC), and 1,2-dipentadecanoyl-*sn*-glycero-3-phosphocholine (DPenPC) were purchased from Avanti Polar Lipids (www.avantilipids.com) and used without further purification. All other chemicals and solvents were analytical grade and obtained from Sigma-Aldrich. The water used in polymerization and making lipid vesicles was obtained from a Barnstead Nanopure system with a measured resistivity of 18.3 M cm and a total organic content specified as less than 10 ppb.

2.2. Synthesis of 1–3

Syntheses of **1–3** are shown in Scheme 1 and 2. The purity of the lipids was evaluated by thin-layer chromatography (TLC) and visualized with a UV lamp. Compounds containing

UV-sensitive groups were handled under yellow lights. Detailed synthesis procedures and spectral information are provided in the Supplementary data.

2.3. Apparatus

^1H NMR spectra were measured on a Bruker spectrometer at 250 MHz in CDCl_3 . Mass spectroscopy data were obtained using HP 5988A GC-MS, Finnigan LCQ HPLC-MS, and JEOL HX110A FAB-MS instruments at the Mass Spectroscopy Facility in the Department of Chemistry, University of Arizona. UV-vis absorbance spectra were recorded on a Spectral Instruments (Tucson, AZ) model 440 UV-vis spectrometer. Size exclusion chromatography (SEC) was performed in a THF mobile phase with a Waters 1515 isocratic pump running three 5 μm PLgel columns (Polymer Labs, pore sizes 10^4 , 10^3 , and 10^2 Å) with a Waters 2414 differential refractometer and Waters 2487 dual wavelength UV-vis spectrometer. Molar masses were calculated using Empower software (Waters) calibrating against low polydispersity linear polystyrene standards. Dynamic light scattering (DLS) was performed with a BI-8000-autocorrelator with a 5 mW He-Ne polarized laser source from Brookhaven Instruments Corp., and vesicle size distributions were calculated with the accompanying software.

2.3.1. Phase transition measurements—Differential scanning calorimetry (DSC) data were obtained using a 2920 Modulated DSC (TA Instruments) running Thermal Solutions 1.4E (TA Instruments) software. DSC measurements were run from 10 to 80 °C at a ramp rate of 5 °C per minute. Thermotropic phase transitions were measured using a method adapted from Torchilin and Weissig (Torchilin and Weissig, 2003). Lipids were dried from the stock benzene solution into an aluminum DSC pan (10 mg). DI water (25 μL) was placed into the pan dropwise using a microsyringe. The pan was sealed hermetically with an aluminum lid. Thermal transitions of the sample were measured against a reference of 25 μL water sealed in an aluminum pan prepared similarly to the sample.

2.3.2. Langmuir isotherms—Langmuir isotherms were measured in a Nima model 611D LB trough. The spreading solvent was either the original stock solution or, in the case of mixed monolayers, CHCl_3 . The subphase was water. All measurements were carried out at 20 °C.

2.4. Methods

2.4.1. Preparation of vesicles—Lipids from stock solution were dried by passing a stream of argon over the sample followed by applying high vacuum for ca. 4 hours. The film was hydrated with deoxygenated water. Multilamellar vesicles were prepared by vortexing several times and ultra-sonication of the mixture for 1 min above the phase transition temperature (T_m) of lipids, followed by 10 freeze-thaw cycles (−77 °C in dry ice/2-propanol and then warmed to 40 °C).

Large unilamellar vesicles of lipids (LUV) were prepared using an extruder (Northern Lipids Inc., Vancouver, BC). The suspension was extruded through two stacked Nuclepore polycarbonate filters (pore size 600($\times$ 3), 400($\times$ 3), and 100($\times$ 4) nm, sequentially) at 45 °C. The average diameter of the vesicles was determined by DLS.

2.4.2. Polymerization—UV polymerization was performed by exposure to light from a high-pressure Hg/Xe arc lamp (Newport Corporation, CT) for ca. 2 h at 45 °C. The lamp was equipped with a 260–400 nm FWHM band-pass filter (U-330, Edmund Optics) to remove shorter wavelengths of UV that was found to cause degradation of the lipid polymer (Meier et al., 1994). In addition, the lamp was equipped with a water filter to remove IR radiation to prevent excessive heating of the sample. Polymerization was monitored by UV

absorbance spectroscopy of aliquots withdrawn periodically during the course of the reaction and diluted with MeOH.

Redox-initiated polymerization was performed with degassed, Ar purged solutions of 39 mM $K_2S_2O_8$ and 39 mM $NaHSO_3$. The two solutions were mixed with the vesicle suspension to have a final molar concentration ratio of 1:1 between the polymerizable lipid and the oxidant/reductant (Sisson et al., 1996). Polymerizations were performed at ca. 45 °C under a positive argon pressure for 20 h. All polymerizations were carried out in the dark or under yellow light. After the polymerization, the vesicle suspension was dialyzed (MWCO 6000–8000) to remove the salts using water as the medium. Polymerizations were monitored by UV absorption spectroscopy as described above.

2.4.3. Analyses of lipid polymers—Following the polymerization of lipid vesicles, water was removed by lyophilization to leave a dry, fine powder that was resuspended in THF: MeOH: benzene (10:1:1 v/v) for analyses by SEC. Peak integration, number- and weight-average molecular weights ($\bar{M}_n$ and $\bar{M}_w$), and polydispersity index ($PDI = \bar{M}_w / \bar{M}_n$) were analyzed for each chromatogram using the supplied software. The number-average relative degree of polymerization ($\bar{X}_n$) was calculated by dividing $\bar{M}_n$ by the molecular weight of the monomer lipid.

3. Results and Discussion

A key consideration in the design of polymerizable lipids is the choice and the location of the reactive groups. Lipids **1–3** have sorbyl groups in the head region of the molecule. When located in the tail region of lipids, these groups are known to form polymers by radical- and photo-initiated polymerization (Mueller and O'Brien, 2002). **1–3** are neutral and synthetically simpler to prepare than corresponding zwitterionic lipids. **1** has a single sorbyl group in the head region separated by a short spacer group while **2** has a spacer group that is three times longer than that of **1** (Fig. 1). This increased spacer length is expected to further decouple the motion of the random polymer coil and the mesogenic tail groups of **2**. In addition, it is expected to increase the hydrophilicity of the head group region of the neutral lipid molecule with added ethyleneoxy groups, which may increase the likelihood of bilayer formation (Evans, 1988; Evans and Ninham, 1986; Neumann and Ringsdorf, 1986). While **1** and **2** are expected to generate linear polymers, **3** has bis-substituted, amide-linked sorbate groups at the head region that may generate cross-linked polymers (Fig. 1). All three molecules contain single unsaturations in the tails. **1** and **2** are trans lipids while **3** is a cis lipid.

3.1. Absorbance properties of the lipids

The absorbance properties of each molecule are listed in Table 1. All the lipids show an absorbance maximum at 258 nm, characteristic of a sorbyl group. **1** and **2** show a molar absorptivity (ϵ) of 23,600 $\text{cm}^{-1} \text{M}^{-1}$ at 258 nm in methanol, which is consistent with the values reported for mono-SorbPC lipids (Lee and O'Brien, 1992; Tyminski et al., 1987). The bis-substituted **3** has a ϵ of 50,000 $\text{cm}^{-1} \text{M}^{-1}$ in methanol.

3.2. Thermotropic transitions by DSC

A modified procedure (Torchilin and Weissig, 2003) was used to measure the thermotropic phase behavior, since pure **1–3** do not form vesicles (see section 3.4). Even though the aqueous content is relatively small in this method, lipids with sufficiently long chains can still form lamellar phases with a small aqueous content (Blume, 1991). The method was validated using DPenPC with known DSC transitions. The thermotropic transitions were observed as maxima in the excess heat capacity vs. temperature endotherms (Fig. 2).

The T_m values of **1**, **2**, **3**, monomers and DPenPC are reported in Table 2.¹ The measured T_m is 32 °C for DPenPC, which is consistent with reported values (31–34 °C) (Koynova and Caffrey, 1998; www.avantilipids.com; www.lipidat.chemistry.ohio-state.edu) and shows that the method is valid despite the low water content. Although DPenPC shows a single sharp transition, all other lipids tested show broad transitions. This behavior is not unexpected. It has been reported that the thermotropic transition is broadened when there are functional groups in the tails, as well as with bulkier head groups, by reducing the cooperative transitions (Blume, 1991; Kusumi et al., 1983). It is thought that the tail chains become tilted more to minimize the influence from bulky head groups, reducing the interactions between them and producing broader peaks.

1, **2**, and **3** have T_m values of 11, 13, and 7 °C respectively with standard deviations of less than 1. Replicate temperature scans did not influence the DSC curves. The T_m values of trans lipids **1** and **2** are very close to that of the corresponding PC trans lipid, DEPC (12 °C) (www.avantilipids.com). They have the same tail structure. This suggests that head groups of these polymerizable lipids exert less influence on T_m than the trans structure of the tails. Usually the T_m of a trans lipid is higher than that of the corresponding cis lipid due to the larger perturbations to lipid packing from cis double bonds than trans double bonds (Roach et al., 2004). This is consistent with the results for the lipids measured in this study.

Increasing the length of the head group spacer in **2** vs. **1** slightly increased the T_m . Similar results were also reported by Blume and Walle for polymerizable lipids having methacryloyl head groups (Blume, 1991). They found that when the methacryloyl group is attached to the PC head group via a short spacer, the T_m is slightly shifted to lower values relative to the unfunctionalized lipid. Increasing the length of the spacer slightly increased the T_m . The stabilization of the gel phase by the increased interactions between the chains of the bulky molecule is assumed as a result of the increased length of the spacer.

3.3. Langmuir isotherms

Measurements of surface pressure/area ($\pi - A$) isotherms provide a method for studying the miscibility of two components in mixed lipid monolayers (Bader et al., 1985) as well as studying the influence of parameters such as head group charge and size, chain unsaturation, and chain packing on membranes. Therefore, $\pi - A$ isotherms of **1–3**, DPenPC, and mixed monolayers of **1–3** with DPenPC were studied (Fig. 3).

The shapes of the $\pi - A$ isotherms of pure **1–3** are comparable to each other. They show a single transition point corresponding to a collapse from the liquid-expanded (LE) state (Fig. 3a–c). The pressure at which the collapse occurs is comparable among the three lipids and close to 35 mN/m (Table 3). In contrast, DPenPC exhibits two transitions: a phase transition between LE and liquid-condensed (LC) states at 17 mN/m and a film collapse at 55 mN/m. As suggested by previous authors, it is possible that the lower collapse pressure for **1–3**, relative to DPenPC, is due to the presence of an uncharged head group (Elbert et al., 1984) and packing perturbations that arise from unsaturation as well as tail disturbances from the bulky head group (Rice et al., 1987).

In contrast to DPenPC, the **1–3** isotherms show more expanded films at lower surface pressures. Presumably these could be due to less hydrophilicity and bulkier groups as suggested by Cadenhead and coworkers based on studies of esters and corresponding acidic

¹A characteristic feature of lipids with polymerizable groups attached to the head group via spacers is that the phase transition is not abolished after polymerization, as the motions of the hydrophobic chains are decoupled from the polymer backbone [Blume, 1991]. This was not tested with lipids mentioned here due to the difficulty of separating poly(lipids) from mixed lipid vesicles in which polymerization was performed.

hydrocarbons (Matuo et al., 1982). The area per molecule at the collapse pressure for **1–3** is 82, 142, and 130 Å² respectively. The larger increments for **2** and **3** compared to **1** are anticipated because these are expected to occupy a larger area and pack less efficiently than **1**. It is possible that **2**, with an extended spacer occupies more space and/or creates additional tail perturbations relative to **3**.

Pressure-area isotherms of mixed monolayers prepared from 50% (mol) **1–3** and DPenPC were studied in a manner similar to prior reports of immiscible and miscible mixed monolayers (Bader et al., 1985; Buschl et al., 1982; Chen et al., 2000; Elbert et al., 1984; Fukuda et al., 1979; Lee et al., 2006; Petty, 1996; Wu and Huntsber.Jr, 1969). These mixed isotherms fall between those of the two pure monolayers (Fig. 3a–c) and are similar in shape. All three isotherms exhibit three distinct transition points, corresponding to the presence of two different phases. The first and the last transition pressures are nearly identical to the collapse pressures found for pure monolayers, i.e. head group lipids and DPenPC, respectively (Fig. 3a–c). The first transition points for the **1**/DPenPC and **3**/DPenPC mixed monolayers occur slightly above the collapse pressures of pure **1** and **3**, approximately at 42 and 37 mN/m, respectively. However, the first transition point for **2**/DPenPC mixed monolayer is no different than the collapse pressure of pure **2**, which is 34 mN/m (Table 3). According to the phase rule, the $\pi - A$ isotherm behavior for the mixed monolayers indicates that the two components are immiscible with component **1** or **2** or **3** being “squeezed out” at the first transition point (Crisp, 1949).

The immiscibility observed in the mixed monolayer isotherms is likely due to monolayer formation from different film states (Cadenhead, 1972). At 20 °C, amphiphiles **1–3** are in the L_α phase and DPenPC is in the L_β phase (Table 2). It has been reported that in a two-component mixture, complete miscibility occurs if the acyl chain lengths of the two lipids differ by less than 4 methylene units and the difference in their T_m values is less than 20° (Lee, 1978; Lee, 1983). The acyl chain length difference is limited to 3 methylene units in **1–3** and DPenPC mixtures, but not the T_m values except for **2** (Table 2). However, the first transition pressures in the **1**/DPenPC and **3**/DPenPC mixed isotherms are somewhat higher than the collapse pressures of pure **1** and **3**. In addition, a slight deviation of parallelism in the **3**/DPenPC mixed isotherm after the second transition point compared to that of pure DPenPC is observed. These can be ascribed to slight miscibility of **1** and **3** in the DPenPC monolayers.

The acyl chain length and the T_m differences are within the suggested limits for complete miscibility (Lee, 1978; Lee, 1983) between **2** and DPenPC. However, the two components are not miscible as observed by the Langmuir isotherm as well as the absence of vesicle formation at higher temperatures, even above T_m where both lipids are in the L_α phase. We speculate that the large head and spacer groups in **2** fold back toward the tail region, creating unfavorable interactions for mixing.

3.4. Preparation of vesicles

In all cases, suspended particles or emulsions were visually observed in aqueous suspensions of **1–3**. Ultrasonication for extended periods above the T_m did not render clear solutions. Extruded aqueous solutions of these lipids produced population distributions of structures that were too small to measure reliably using DLS. Similar observations were reported for polymeric amphiphilic monomers that showed difficulty in making vesicles (Johnston et al., 1983; Kunitake et al., 1981; Regen et al., 1980; Ringsdorf et al., 1988). The reason was presumed to be insufficient amphiphilicity (Blume, 1991; Evans and Ninham, 1986; Israelachvili, 1991; Ringsdorf et al., 1988) and/or extensive intermolecular head group interactions that decrease hydration (Litzinger and Huang, 1992). Attempts were then made

to form mixed vesicles using fluid lipids having tail lengths close to those in **1–3** (Lee, 1978; Lee, 1983).

The commercially available lipids DPenPC, eggPC, or DOPC were combined with **1–3** in various molar ratios to form vesicles.² The hydrodynamic mean diameter of the mixed vesicles was measured using DLS. Attempts to form mixed vesicles with **2** in any composition were not successful and suspended particles were visually observed. Homogeneous populations were observed by DLS up to 50% in mole composition for both **1** and **3** with the mixing lipid with a mean value of ca. 120 nm. Above 50% for **1** and 65% for **3**, two population distributions were observed. For example, when the composition was 70% of **1** and 30% DPenPC, two populations with mean diameters of 50 nm and 180 nm were observed (Fig. 4). We speculate that after a threshold concentration is reached, the vesicles rearrange to form more stable mixed structures with appropriate hydrophilic-lipophilic balance. These results were comparable for **1** and **3** mixed with all three fluid lipids.

3.5. Polymerization of vesicles

Mixed vesicles composed of **1** or **3** and DPenPC were subjected to redox-initiated radical polymerization or UV photopolymerization before measuring the molecular weight of the products. In order to more efficiently polymerize these vesicles, the temperature of the solution was held at 45 °C, above the higher of the two T_m values. This ensured that both lipids were in the L_α phase.

The mixed vesicles of both **1** and **3** show an absorbance maximum at 258 nm, which decreases following UV irradiation or redox polymerization. The similarity in λ_{max} in methanol and in vesicles (aqueous) indicates that the two sorbyl chromophores do not interact or aggregate at temperatures above the T_m (Lee and O'Brien, 1992; Tyminski et al., 1987). Fig. 5 shows a representative set of absorbance spectra obtained by sampling **1** and **3** mixed vesicle dispersions during UV irradiation. The disappearance of the absorbance band at 258 nm shows that molecules **1** and **3** undergo UV induced reactions and conveniently tracks the reaction progress.

The loss of monomer absorption was also accompanied by the progressive loss of migration of the lipid in TLC. For example, **1** and DPenPC showed 0.88 and 0.33 R_f values, respectively, with the solvent mixture 65:25:4 (v/v) $CHCl_3$:MeOH:H₂O. The UV irradiated **1**/DPenPC mixture showed three R_f values: two values corresponding to the pure lipids and one intermediate value (0.51). If there is an oligomer or a polymer formed during the UV illumination, the higher MW fragment is expected to display a slower migration than that of the monomer. Therefore, the 0.51 R_f value is presumed to be due to the migration of polymer. Similar observations were reported by Lee and O'Brien for the TLC of sorbyl ether glycerophosphocholine lipids (Lee and O'Brien, 1992).

The ¹H NMR spectra of both redox and UV polymerized samples of **1** showed that all of the proton resonances due to the sorbyl moiety disappeared. Losses of conjugated terminal methyl protons adjacent to the sorbyl unit (1.85 ppm, doublet) and the vinyl proton adjacent to the carbonyl (5.72–5.80 ppm, doublet) are consistent with the formation of a polymer product via 1,4-polymerization (Clapp et al., 1997; Lamparski et al., 1992; Lamparski and O'Brien, 1995). It has been reported that ester-linked sorbyl group polymerization usually follows a 1,4 mechanism (Mueller and O'Brien, 2002). However, in the ¹H NMR spectra of polymerized **3**, the loss of the 1.85 ppm signal was clear, but the disappearance of other

²It is possible that the isolated double bond in either DOPC or eggPC reacts to form oligomers during the polymerization, and this could interfere with the identification of polymer products of **1–3**. Therefore, mixed vesicle studies of **1–3** with the saturated lipid, DPenPC, were performed to eliminate this complication.

proton signals was not. Mueller and O'Brien suggested that an amide-linked sorbate monomer might follow a different polymerization mechanism (Mueller and O'Brien, 2002). Further studies are being done to identify the polymerization mechanism of **3**.

3.6. DLS of polymerized mixed vesicles

The sizes of the vesicles were not significantly changed as observed by DLS after they were subjected to polymerization conditions (data not shown). The absence of larger diameter aggregates for polymerized vesicles suggests that inter-vesicle cross-linking polymerization or inter-vesicle aggregation was not favored under the conditions used here.

3.7. Analyses of lipid polymers by SEC

After polymerization, lyophilized mixed vesicles were partially soluble in organic solvents. To improve the solubility and enable SEC analysis, cosolvents (MeOH and benzene) were added to the lyophilized samples dissolved in THF (Lamparski and O'Brien, 1995). The SEC analysis of molecular weight data for polymerized mixed vesicles is summarized in Table 4. These molecular weights are apparent MWs measured relative to PS standards. These SEC data clearly reveal that **1** and **3** form polymers (oligomers) during UV and radical polymerizations. However, these results reflect only the soluble fraction rather than the complete $\bar{X}_n$ range for the polymer. O'Brien and coworkers reported that the $\bar{X}_n$ is 4–51 for the transesterified samples of redox poly(mono-SorbPC) (Lei et al., 1999) and 3–10 for UV poly(mono-SorbPC) (Lamparski and O'Brien, 1995). The $\bar{X}_n$ value for both UV and redox polymerization of the **1** is ca. 2–10. This suggests that the sorbyl group in the head group region of **1** behaves similarly to the sorbyl group in the tail region of mono-SorbPC during two-dimensional polymerizations. The reported $\bar{X}_n$ for redox poly(bis-SorbPC) is 100–600 and 5–10 for UV poly(bis-SorbPC) (Lamparski and O'Brien, 1995), compared to 5 for both redox and UV polymerized **3**. The lower $\bar{X}_n$ for **3** is likely due to using only the soluble fraction for SEC measurements.

It has been reported that sorbyl ester groups do not readily polymerize unless the molecules aggregate to form mesophases (Masuda et al., 2003; Matsumoto et al., 1991; Mueller and O'Brien, 2002). The identification of oligomers from **1** and **3** under typical polymerization conditions that have been used for other sorbyl lipids (Mueller and O'Brien, 2002; Sisson et al., 1996) indicates that, in spite of a relatively large head group, **1** and **3** form mesophases in mixed vesicles.

4. Summary and Conclusions

A series of neutral lipids with polymerizable sorbyl head groups were synthesized. Lipids **1** and **2** are mono-substituted and **3** is bis-substituted. There is a short spacer in **1** and longer spacer group in **2** separating the sorbyl group from the head group.

Pure solutions of all three lipids do not form stable vesicles. This is likely to be due to a lack of amphiphilic nature of the neutral molecules, extensive intermolecular head group interactions that decrease hydration, and/or bulky head groups that may reduce the cohesive tail interactions necessary for bilayer formation. However, **1** and **3** (but not **2**) form mixed vesicles with commercially available fluid lipids such as DPenPC, eggPC and DOPC.

Langmuir isotherms of mixed monolayers of **1–3** with DPenPC suggest that they are largely immiscible with **1**, **2**, or **3** at 20 °C. However, slight deviations in the isotherms for **1** and **3** with DPenPC in mixed monolayers are ascribed to a slight miscibility of the components. Thus mixed vesicles can be formed with **1** and **3** but not with **2**. However, the slight

miscibility of components restricts the molar fraction of **1** or **3** in the mixed vesicles to be 50%, even at temperatures above T_m .

The disappearance of the absorbance band at 258 nm, TLC migration changes, and ^1H NMR data suggest that the monomers react to form polymer products under polymerization conditions. The SEC studies of **1** and **3** mixed vesicles show that both form oligomers when subjected to either UV- or redox-initiated polymerization. These results indicate that sorbyl groups on bulky head groups aggregate sufficiently to be polymerized. Using ^1H NMR, polymerization of **1** was determined to be following the 1,4 mechanism. Further work is required to identify the polymerization mechanism of **3**.

This report describes the first known studies of sorbyl-substituted head group lipids. These results provide new insights into designing novel lipid molecules for supramolecular structures. The future possibilities include mixed lipid vesicles composed of head group lipids that can be usefully employed to phase-separate unreactive lipids from the newly formed polymeric domains.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Three novel polymerizable amphiphiles with sorbyl-substituted head group were synthesized.
- Physical characteristics of these molecules were studied.
- Mixing properties of these molecules with natural lipids were investigated using Langmuir isotherms.
- The sorbyl groups on the bulky head groups of these molecules form polymers.

Figure 1. Structures of lipids 1–3. Lipids 1 and 2 are trans structures while 3 is a cis lipid.

Figure 2.
DSC heating thermograms: (a) DPenPC, (b) **1**, (c) **2**, and (d) **3**.

Figure 3. Pressure-area isotherms for pure lipids and 50% (mol) mixtures of synthesized lipids and DPenPC at 20 °C: (a) **1**, (b) **2**, and (c) **3**, with DPenPC.

Figure 4.

Representative DLS data showing diameters of the vesicles prepared from 50:50 and 70:30 (mol/mol) **1**:DPenPC mixtures. For 70% lipid **1** mixture, the mean diameter of the smaller population was 50 nm and the mean of the larger population was ca. 180 nm. The mean diameter is ca. 120 nm for mixtures below 50% of lipid **1**.

Figure 5. Absorbance measurements of 50% (mol) mixtures of **1** and **3** in methanol before and after UV illumination: (a) **1**/DPePC and (b) **3**/DPePC.

Scheme 1.

Syntheses of lipids **1** and **2**. Reagents and conditions: (a) ethylene glycol or triethylene glycol, K_2CO_3 , THF, RT, 20 h, 73% and 82%; (b) succinic anhydride, N,N-diisopropylethylamine, THF, RT, 18 h, 88% and 93%; (c) $(\text{COCl})_2$, CH_2Cl_2 , RT, 16 h, 97% and 96%; (d) 3-amino-1,2-propanediol, Et_3N , THF/ H_2O , 13 °C, 20 h, 30% and 32%; (e) elaidic acid, DCC, DMAP, CH_2Cl_2 , RT, 20 h, 51% and 88%

Scheme 2.

Synthesis of lipid **3**. Reagents and conditions: (a) 1,3-diamino-2-propanol, CH₂Cl₂, RT, 91%; (b) succinic anhydride, N,N-diisopropylethylamine, THF, refluxed 5 days, 55%; (c) oleic acid, DCC, DMAP, CH₂Cl₂, RT, 48 h, 60%; (d) TFA, RT, 3 h; (e) **S2.3**, triethylamine, DEPC, CH₂Cl₂, RT, 7 h, 43%.

Table 1

Molecular formula, molecular weight, absorbance maximum (nm), and molar absorptivity ($\text{cm}^{-1}\text{M}^{-1}$) in methanol of **1–3**.

Molecule	Molecular formula	MW	Abs λ_{max} (nm)	ϵ at 258 nm ($\text{cm}^{-1}\text{M}^{-1}$)
1	$\text{C}_{51}\text{H}_{87}\text{O}_9\text{N}$	858.26	258	23,600
2	$\text{C}_{55}\text{H}_{95}\text{O}_{11}\text{N}$	946.34	258	23,600
3	$\text{C}_{58}\text{H}_{97}\text{O}_9\text{N}_3$	980.41	258	50,000

Table 2

T_m of the lipids measured from the DSC transitions.

Lipid	T_m (°C)
DPenPC	32
1	11
2	13
3	7

Table 3

Selected properties of pressure-area isotherms shown in Fig. 3: Surface pressure at the first transition and area per molecule at the first transition for mixed monolayers, collapse pressure, and area per molecule at the collapse pressure.

Lipid	Pressure at first transition (mN/m) ^a	Area per molecule (Å ²) at the first transition ^a	Collapse pressure (mN/m)	Area per molecule (Å ²) at the collapse pressure
DPenPC	17	62	55	41
1	-	-	35	82
2	-	-	34	142
3	-	-	35	130
50% (w/w) 1 /DPenPC	42	66	68	33
50% (w/w) 2 /DPenPC	34	97	55	31
50% (w/w) 3 /DPenPC	37	93	55	32

^aFor lipids **1–3** the first transition is the collapse pressure. Therefore, the pressure and the area per molecule at first transition are presented at the collapse pressure, and the area per molecule at the collapse pressure columns respectively.

Table 4

SEC data: number- and weight-average molecular weights ($\bar{M}_n$ and $\bar{M}_w$), polydispersity index (PDI), and number-average relative degree of polymerization ($\bar{X}_n$) for the soluble fractions of polymerized mixed vesicles of DPenPC and lipids **1** and **3**.

Lipid	Polymerization condition	$\bar{M}_n$ range	$\bar{M}_w$ range	$\bar{X}_n$	PDI
1	UV	1600–9700	1500–8300	2–10	1.1–1.2
1	redox	1600–27800	1500–9800	2–11	1.0–2.8
3	UV	6400	4900	5	1.3
3	redox	7100	5200	5	1.4