Synthesis and Polymerization of Heterobifunctional Amphiphiles to Cross-Link Supramolecular Assemblies

Sanchao Liu, Thomas M. Sisson, and David F. O'Brien*

Department of Chemistry, C. S. Marvel Laboratories, University of Arizona, Tucson, Arizona 85721

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ABSTRACT: The cross-linking of supramolecular assemblies of hydrated amphiphiles is an effective method to stabilize the assembly. A well-known strategy for cross-linking of lipids in lyotropic phases is the inclusion of identical reactive groups in each hydrophobic tail of the lipids. An alternative approach is to incorporate two similar but distinct groups into different locations within a single hydrophobic tail of the amphiphile. In principle, these heterobifunctional amphiphiles could react to form ladderlike polymers or cross-linked polymers. This report describes the synthesis, characterization, and polymerization in lipid vesicles of two series of heterobifunctional phosphatidylcholine (PC) lipids with different separation distances between the two reactive groups. One of the groups, i.e., dienoyl (Den), is attached to the secondary oxygen of the glycerol backbone of the lipid, and the other is either an acryloyl (Acryl) or sorbyl (Sorb) functional group located at the *sn-2* chain terminus. Polymerization of both reactive groups in the Acryl/DenPC or the Sorb/DenPC was achieved by either redox polymerization or direct photoirradiation. The degree of polymerization depends on the initiation chemistry. Photoirradiation yields oligomers that are insufficient to cross-link the vesicles, whereas redox-initiated radical polymerization affords cross-linked polymeric vesicles. Under radical polymerization conditions a spacer length of seven or more atoms between the two reactive groups was long enough to ensure that each reactive group can follow an independent reaction path.

Introduction

The polymerization of supramolecular assemblies of hydrated amphiphiles is an effective way to modify the chemical and physical properties of the assembly.[1] The polymerization in a lipid assembly can proceed in a linear or cross-linked manner depending on the number of polymerizable groups in the monomeric lipid. It is known that polymerization of hydrated lipids with a single reactive moiety in either the hydrophobic tails or the lipid headgroup yields linear polymers, whereas polymerization of lipids with reactive groups in both tails yields cross-linked polymers.[2−6] The formation of cross-linked polymeric assemblies can be ascertained by the physical properties of the resulting assembly, including the chemical stability of the polymerized assembly toward added surfactant, general insolubility in organic solvents including hexafluoro-2-propanol (HFIP), and the rate of lateral diffusion of the lipids in the assembly.[7,8]

Heterobifunctional lipid monomers have two reactive moieties with distinct reactivities, which could result in differences in the rate and degree of polymerization. Moreover, if the two reactive groups are located in the same tail of the lipid, then there is the additional possibility that the groups could form cross-linked or linear-ladder polymers. Cross-linking was observed in the polymerization of rodlike mesogens with acryloyl and cyano groups separated by 13 atoms.[9,10] On the other hand, linear-ladder polymers appear to be formed in the polymerization of hydrated bilayers of 1-palmitoyl-2-(2,4,12,14-tetraenehexadecanoyl) phosphatidylcholine, where the two diene groups were separated by only six methylenes.[11,12] Several factors could influence the preferred course of polymerizations in organized media, such as thermotropic and lyotropic liquid crystals. These include the relative reactivity of the monomers, the percent conversion of the monomers, the distance of separation between the reactive groups, and the flexibility of the spacer group that separates the monomers, among others. In this study, we have examined the nature of the polymerization of hydrated bilayers, at high conversion, of two types of heterobifunctional lipids, each bearing a pair of reactive groups in the *sn-2* tail that are separated by either 7, 9, or 11 atoms. The properties of the resulting polymers were determined by polymer solubility and bilayer vesicle lysis techniques reported previously.

Results and Discussion

The key considerations in the design of a heterobifunctional lipid is the choice and location of the polymerizable groups in the lipid molecule. Reactive lipids may have polymerizable moieties located in the hydrophilic headgroup, near the lipid backbone, or in the hydrophobic lipid tail. The heterobifunctional lipids described here contain two polymerizable groups in the *sn-2* acyl chain of the lipid. One of the groups, i.e., dienoyl, is attached to the secondary oxygen of the glycerol backbone of the lipid, and the other is an acryloyl or sorbyl functional group located at the *sn-2* chain terminus. These lipids can be polymerized in a bilayer by either radical initiation or direct photoactivation. Since the two reactive groups are in regions of different polarity, selective polymerization of one reactive group in the presence of a second is possible if the initiation chemistry is only effective in regions of low polarity or in regions of moderate to high polarity. The dienoyl (Den) group located near the lipid backbone can be polymerized by polar initiators, whereas the acryloyl (Acryl) or sorbyl (Sorb) groups located in the interior of the bilayer can be selectively polymerized by hydrophobic initiators.[12] The reactive groups were selected to yield one series of monomeric lipids with groups having similar reactivity, i.e., Sorb/Den, and a second series where the reactivity of the groups differ significantly,

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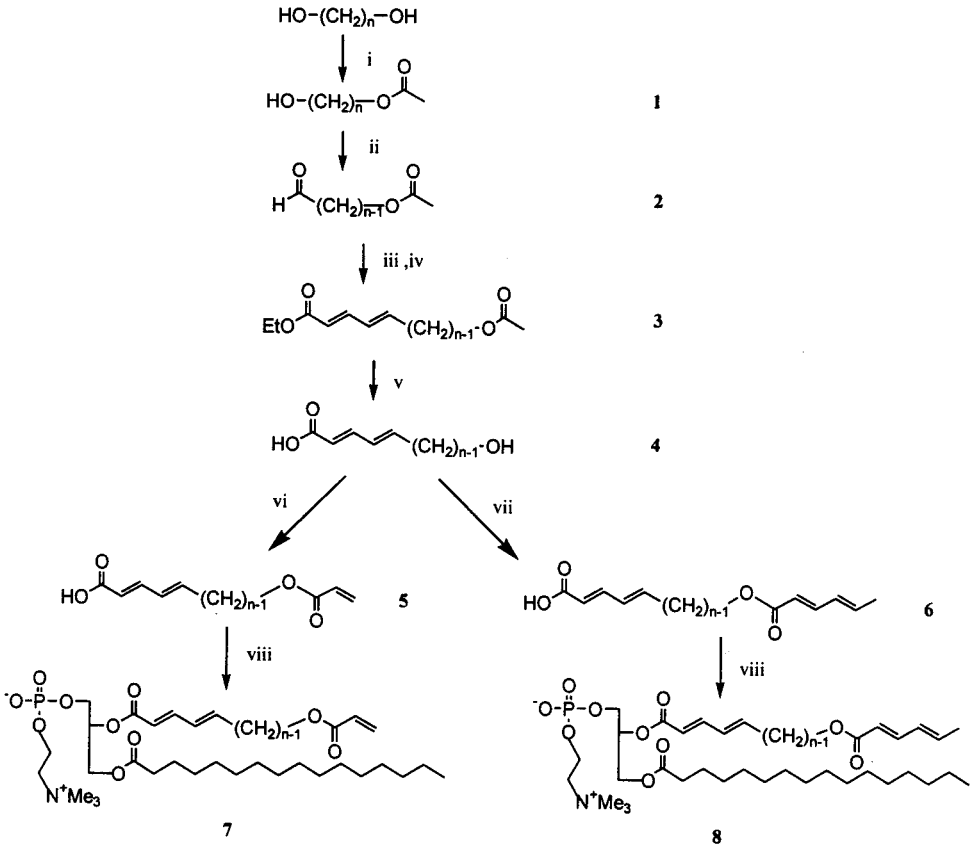
Scheme 1[a]



a: n = 6

b: n = 8

c: n = 10

[a] Key: (i) acetyl chloride, pyridine; (ii) oxalyl chloride, DMSO; (iii) LiOH, triethylphosphonocrotonate; (iv) urea inclusion; (v) KOH, MeOH; (vi) acryloyl chloride, pyridine; (vii) sorbyl chloride, pyridine; (viii) LysoPC, DCC, DMAP.

i.e., Acryl/Den.[13,14] These variations in molecular structure may result in differences in the rate and degree of polymerization. This report focuses on the effect of monomer structure on the architecture (linear or crosslinked) of the polymers formed.

Lipid Syntheses. The heterobifunctional lipids **7** and **8** of varying chain lengths were prepared by acylation of the appropriate fatty acid with either 1-palmitoyl-2-hydroxy-*sn*-glycerol-3-phosphocholine (LysoPC$_{16}$) or 1-oleoyl-2-hydroxy-*sn*-glycerol-3-phosphocholine (LysoPC$_{18}$) in the presence of 4-(dimethylamino)pyridine (DMAP) and dicyclohexylcarbodiimide (DCC). Fatty acids **5** and **6** were synthesized from the commercially available α, ω-diol as shown in Scheme 1. The diol was monoprotected with acetyl chloride. The resulting alcohol **1** was oxidized to aldehyde **2** using a Swern oxidation.[15] The Horner−Wadsworth−Emmons olefination[16] of **2** with triethyl phosphonocrotonate gave ethyl dienoate as a mixture of isomers (95% (*E*,*E*)). Urea inclusion was used to separate the (*E*,*E*) isomer **3** from the corresponding (*E*,*Z*) isomer.[17] The hydrolysis of the ethyl ester and the acetyl protecting group was accomplished with KOH in MeOH to afford the hydroxy dienoic acid **4**. Compound **4** was then reacted with either acryloyl chloride or sorbyl chloride to form the Acryl/Den acid **5** (30% overall yield for **5c**) or the Sorb/Den acid **6** (21% overall yield for **6c**).

Differential Scanning Calorimetry (DSC). DSC heating curves were obtained for hydrated bilayers of each of the synthesized lipids **7** and **8** (Figure 2). The main phase transition temperature T_m, peak width at half-height $T_{1/2}$, calorimetric enthalpy ΔH, and cooperative unit (cu) are reported in Table 1. The T_m values range from less than 5 to 32 °C depending on chain length. Figure 2 shows that bilayers of Acryl/DenPC$_{16,18}$, Sorb/DenPC$_{16,17}$, or Sorb/DenPC$_{16,19}$ each exhibited a single sharp main transition. Acryl/DenPC$_{16,16}$ exhibited a broad main transition endotherm, which could be either a function of the lipid-phase behavior or due to the presence of isomer impurities. A phase transition was not observed between 6 and 60 °C for Acryl/DenPC$_{16,14}$. Sorb/DenPC$_{18,21}$ did not have a phase transition above 5 °C, presumably because of the cis double bond at the center of the *sn-1* chain, which substantially lowers T_m values.

The main phase transition temperatures of Acryl/DenPCs and Sorb/DenPCs are plotted as a function of acyl chain length on *sn-2* (*sn-1* is 16 atoms long) in Figure 3. These data indicate that the T_m for Sorb/DenPC$_{16,15}$ will be near 10 °C. Similarly the T_m value

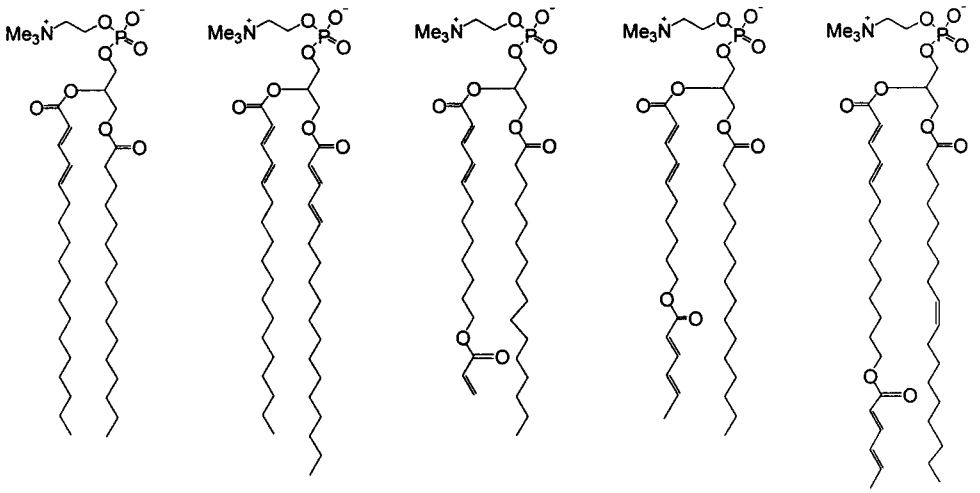


Figure 1. Structures of polymerizable monolipid, homobifunctional lipid, and heterobifunctional lipids. The subscripts at the end of the name represent the *sn-1* and *sn-2* chain length, respectively.

Table 1. Thermodynamic Characteristics of the Heating Endotherms of Hydrated Bilayers of Acryl/DenPC and Sorb/DenPC

lipid	T_m (°C)	$T_{1/2}$	ΔH (kcal/mol)	CU
Acryl/DenPC$_{16,16}$	9.23	1.57	6.83 ± 0.15	53 ± 3
Acryl/DenPC$_{16,18}$	27.7	0.99	9.86 ± 0.22	46 ± 2
Sorb/DenPC$_{16,17}$	20.3	1.04	8.14 ± 0.13	47 ± 5
Sorb/DenPC$_{16,19}$	31.9	1.08	8.48 ± 0.11	47 ± 3
Sorb/DenPC$_{18,21}$	none (>5 °C)			
monoAcrylPC$_{16,16}$[a]	31.8		8.90 ± 0.42	71 ± 10
monoSorbPC$_{16,17}$[a]	36.1	0.24	10.5 ± 0.3	202 ± 22
monoDenPC$_{16,18}$[b]	26.2	1.36	7.02 ± 0.29	71 ± 3

[a] All data are from ref 8. [b] LUV sample.

for Acryl/DenPC$_{16,14}$ is far below zero. A comparison of T_m values for Acryl/DenPC and Sorb/DenPC and the corresponding saturated chain acylPCs of the same chain length[18] reveals a notable depression in T_m for all of these heterobifunctional lipids. The T_m values of monosubstituted PCs are also shown in Table 1.[19] Comparison of the T_m for the monosubstituted PCs to the heterobifunctional lipids shows that the incorporation of the first acryloyl or sorbyl group into the terminus of the *sn-2* chain decreases the T_m by ca. 10 °C. However, the incorporation of a dienoyl group at the top of an acyl chain causes a large decrease (23 °C) in the T_m. Thus, the effect of the unsaturated group is mainly dependent on its position. When it is near the headgroup, it disturbs the well-ordered packing of the lipid bilayer and decreases the phase transition. On the other hand, when it is on the relatively disordered chain termini, its effect on the chain packing is attenuated.

Polymerization of Bilayer Vesicles. Each of the lipids were hydrated and then extruded to give large unilamellar bilayer vesicles (LUV) with an average diameter of ca. 100 ± 10 nm as determined by quasielastic light scattering (QELS). The polymerization was performed under positive Ar(g) pressure with use of different initiation conditions described in the Experimental Section. During each polymerization, the average LUV size did not change, indicating that only intravesicle polymerization took place. The percent conversion of the monomer was determined by absorption spectroscopy monitoring the decrease in monomer absorbance at various time intervals for both the dienoyl and sorbyl functionalities.

(a) Redox Polymerization. The redox polymerizations were performed at 60 °C with a 1:1 mole ratio of $KBrO_3$/L-cysteine, and a 1:1 mole ratio of monomer to oxidant. The nature of the resulting polymers, linear or cross-linked, could then be investigated by vesicle dissolution with Triton X-100 (TX-100) or by measuring the solubility of the isolated polymer. Under the above conditions, both functional groups were expected to be polymerized, since the redox system generates hydroxyl radicals, which can diffuse across the lipid bilayer and initiate the polymerization of reactive groups regardless of their location in the bilayer. In Sorb/DenPC, both dienoyl and sorbyl group showed the same absorbance peak at 260 nm, and it was not possible to distinguish them by their UV/vis absorption. However, the lack of an isosbestic point (Figure 4a) indicates that both functional groups were polymerized at the same time since the existence of an isosbestic point indicates the formation of a single product (as shown in Figure 5 for UV polymerization with filter of Acryl/DenPC). The reaction was fast and reached 99% conversion in 2 h (Figure 4b).

In Acryl/DenPC, the percent conversion of the dienoyl group was greater than 95% as determined by UV/vis spectroscopy. However, the extent of conversion for the acryloyl group could not be determined due to the overlap of the absorption of the isolated double bond of the poly(dienoyl) as well as the absorption of the initiator. It is reasonable to assume that the conversion was at least 95%, since acryloyl is a more reactive group than dienoyl, and under similar conditions the extent of polymerization of mono-AcrylPC LUV was >95%.

(b) UV Polymerization. LUV of each lipid were irradiated at 40 °C with a low-pressure Hg vapor lamp and a CS9-54 filter (cutoff of wavelengths < 230 nm). For Acryl/DenPC, a clean isosbestic point was observed and retained until almost the end of the polymerization. After 90 min, 98% conversion of dienoyl group was reached, but a slight increase on the acryloyl peak at 195 nm was seen (Figure 5). When the same sample was irradiated by UV without a filter, the peak at 195 nm also decreased, suggesting that some polymerization of the acryloyl group occurred. For Sorb/DenPC, the peak at 260 nm reached 97% conversion at 90 min (Figure 6). UV irradiation of the sample without a cutoff filter

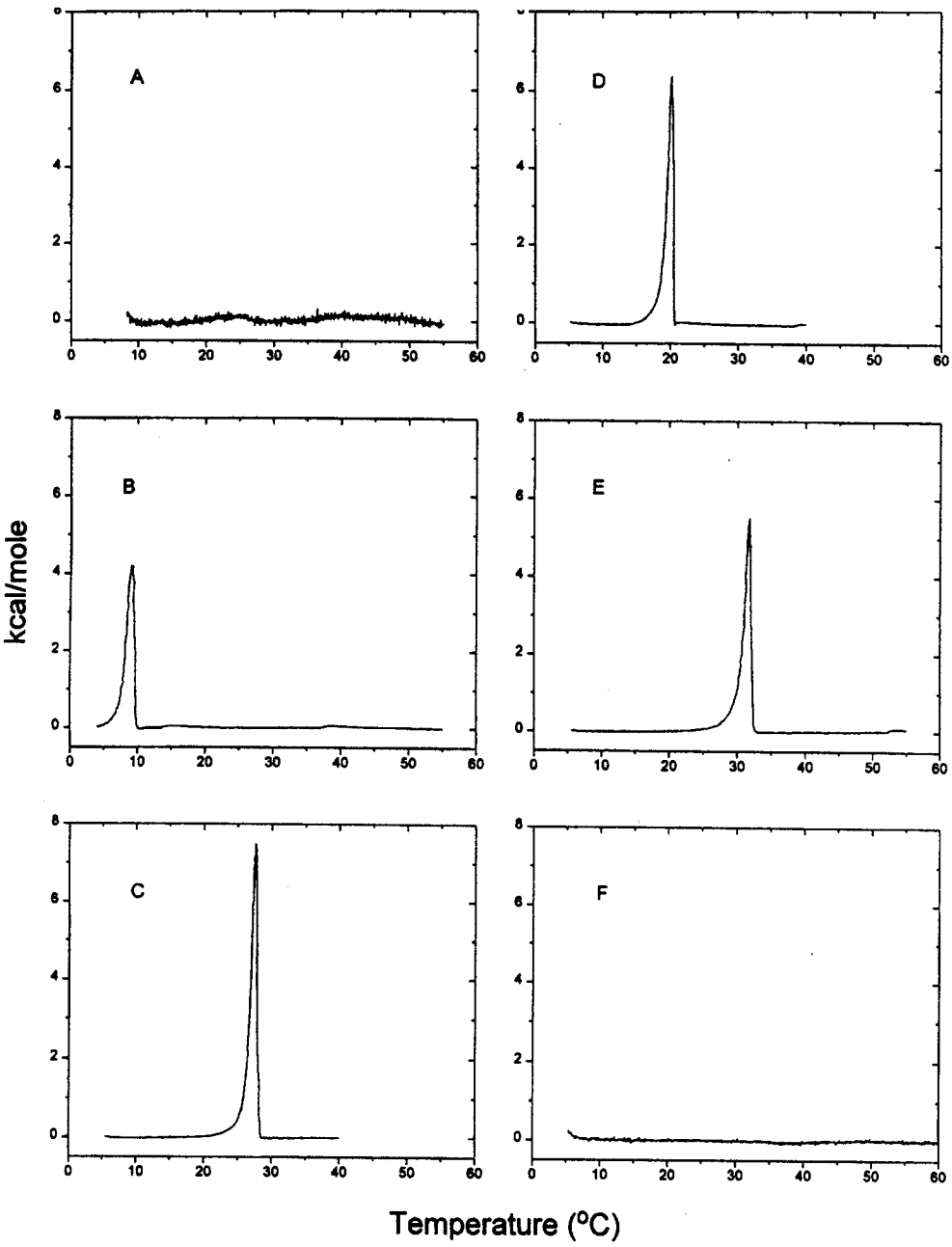


Figure 2. DSC heating thermograms of aqueous dispersions of Acryl/DenPCs and Sorb/DenPCs: (A) Acryl/DenPC$_{16,14}$; (B) Acryl/DenPC$_{16,16}$; (C) Acryl/DenPC$_{16,18}$; (D) Sorb/DenPC$_{16,17}$; (E) Sorb/DenPC$_{16,19}$; (F) Sorb/DenPC$_{18,21}$.

eventually caused the disappearance of the peak at 195 nm, which suggests the occurrence of a second slower reaction of the nonconjugated double bond. Photoirradiation of dienoyl and sorbyl groups in lipid bilayers has previously been reported to yield oligomers (X_n of 3−10).[14,20] The direct UV polymerization of heterobifunctional double diene PC gave oligomers with X_n about 10.[12] The UV polymerization of lipids **8** were performed under argon, in air, and in oxygen and they all showed the same polymerization rate (Figure 6).

MALDI−TOF mass spectrometry was used to determine the molecular weight of the UV (with filter) polymers. The MALDI−TOF spectra showed only oligomers ($n \leq 5$) for samples polymerized in Ar, in air, or in O_2 (Figure 7). For Acryl/DenPC$_{16,18}$ peaks at 772.2 (monomer), 1545.4 (dimer), 2318.3 (trimer), 3091.3 (tetramer), and 3864.5 (pentamer) amu were found. For Sorb/DenPC$_{18,21}$ peaks at 838.3 (monomer), 1678.2 (dimer), 2517.8 (trimer), 3357.6 (tetramer), and 4193.2 (pentamer) amu were found. These results indicate that the polymerization is insensitive to oxygen. It suggests that the excited state is a singlet and the photoreaction proceeds by photoactivated addition of the monomer.

Stability of the Vesicles toward Surfactant. Figure 8 shows the average diameter of Sorb/DenPC (**8a,b,c**) LUV in comparison to the molar ratio of the surfactant TX-100 to lipid as measured by QELS. After addition of 2−6 equiv of TX-100 per lipid to the UV polymerized Sorb/DenPC LUV, a sharp decrease in the average mean diameter of the suspended vesicles was observed. On the other hand, the vesicles polymerized by a redox initiator were essentially unchanged in size by the addition of up to 12 equiv of TX-100. Polymerized vesicles of Acryl/DenPC **7b,c** showed similar trends (Figure 9).

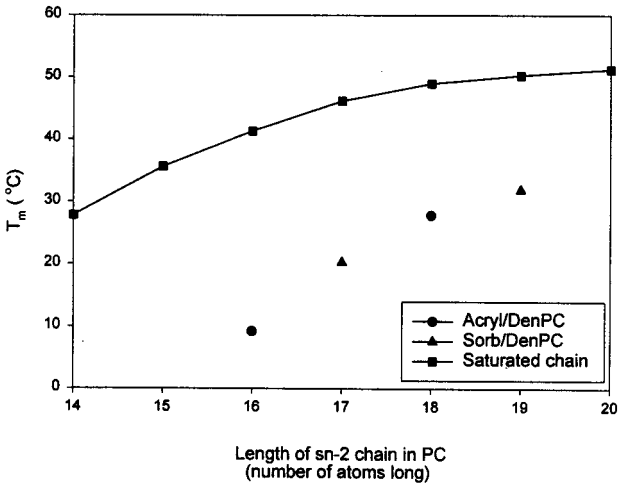


Figure 3. Chain-length dependence of the main phase transition temperature T_m of Acryl/DenPC and Sorb/DenPCs.

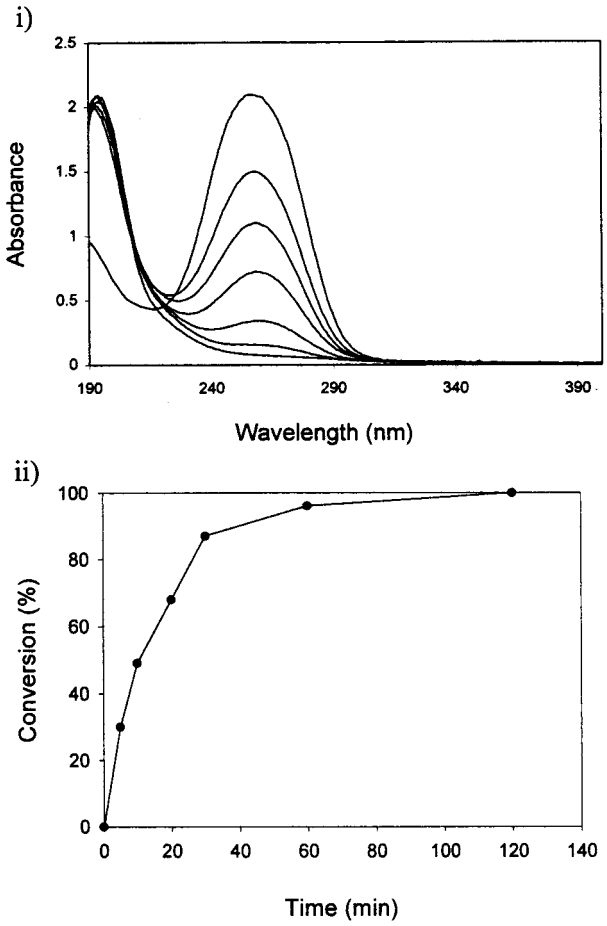


Figure 4. (a) Absorption spectra of Sorb/DenPC$_{18,21}$ LUV in water at different polymerization times of 0, 5, 10, 20, 30, 60, and 120 min. (b) Percent conversion of Sorb/DenPC$_{18,21}$ as a function of time for redox polymerization with $KBrO_3$/L-cysteine (1/1), [M]/[O] = 1:1. [M] = 2 mM, 60 °C.

Previous studies demonstrated that cross-linked lipid vesicles were stable in the presence of excess surfactant, whereas unpolymerized or linearly polymerized vesicles were dissolved by surfactant.[7,21] Further studies also showed that the surfactant solubilization was dependent on the degree of polymerization, extent of cross-linking, and location of the reactive groups in the lipid. Vesicles composed of oligomers were efficiently dissolved with TX-100, while vesicles composed of longer linear polymeric lipids were less readily solubilized. In this study, UV polymerization of vesicles prepared from **7** or **8** did not stabilize the vesicles to TX-100, indicating the formation of oligomeric lipids. On the other hand, the stabilization of LUV toward addition of TX-100 after redox polymerization may indicate cross-linking or a higher degree of polymerization. To exclude the possibility that the effect was caused by a high degree of polymerization, the same redox condition was performed with mono-DenPC$_{16,18}$ vesicles, which generates linear polymers. As shown in Figure 8, the polymerized mono-DenPC LUV were not stable in the presence of TX-100. This demonstrates that the polymers obtained by redox initiation of heterobifunctional amphiphiles cannot be linear polymers.

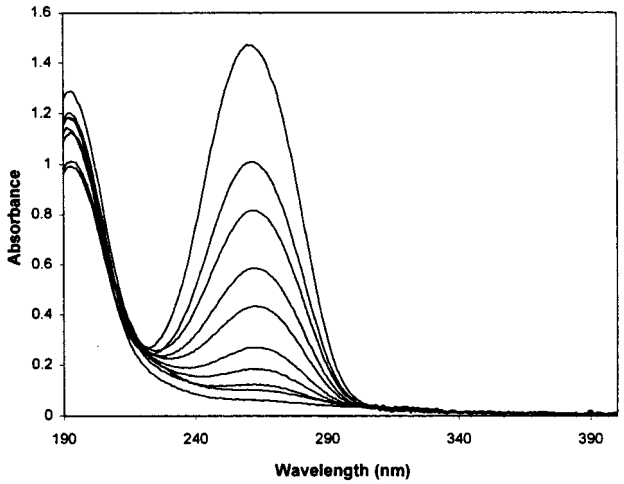


Figure 5. Absorption spectra of Acryl/DenPC$_{16,18}$ LUV in water at different photopolymerization times of 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 min. [M] = 4 mM, 40 °C.

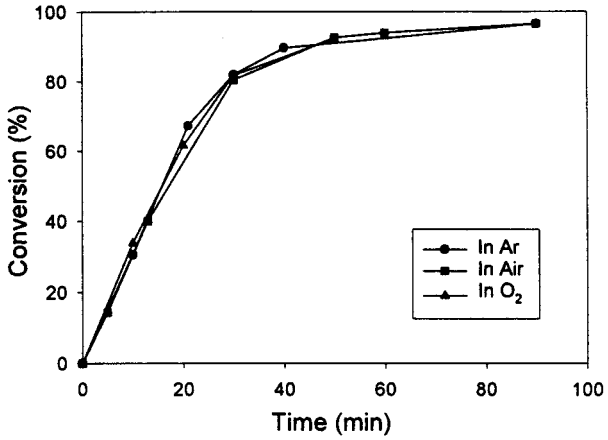
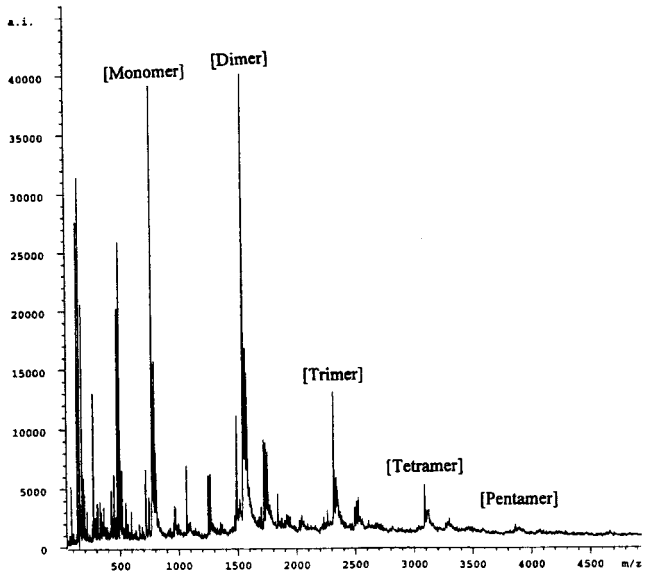


Figure 6. Percent conversion of Sorb/DenPC$_{18,21}$ as a function of time for UV polymerization in Ar, air, and O_2 with a low-pressure Hg vapor lamp and a CS9-54 filter (cutoff wavelength shorter than 230 nm). [M] = 2 mM, 40 °C.

Solubility of Polymerized Lipids. Another effective way to distinguish between the formation of cross-linked polymer from an non-cross-linked one is to measure the polymer solubility. The polymerized lipids were recovered from the hydrated bilayer by freeze-drying, and then the polymeric solid was dispersed in different organic solvents. UV polymerized Acryl/DenPC and Sorb/DenPC, as well as redox polymerized mono-DenPC, were soluble in various organic solvents, e.g. chloroform and benzene, whereas redox polymerized Sorb/DenPC and Acryl/DenPC were insoluble in most

a) Acryl/DenPC$_{16,18}$



b) Sorb/DenPC$_{18,21}$

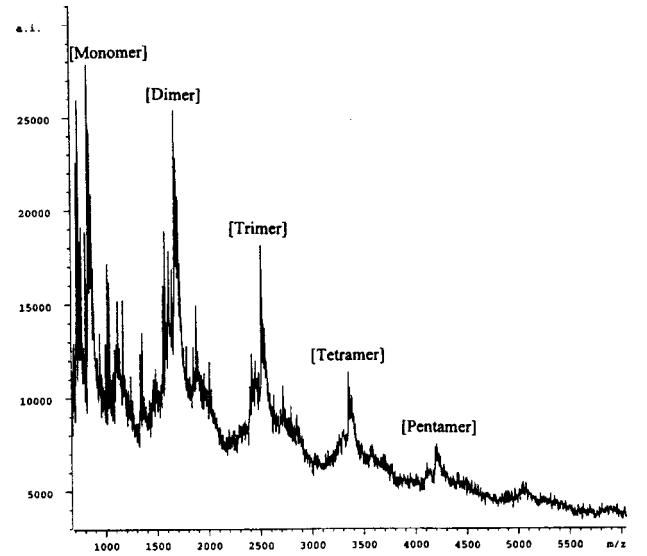


Figure 7. MALDI−TOF spectra for UV polymerized Acryl/DenPC$_{16,18}$ and Sorb/DenPC$_{18,21}$ with 2,5-dihydroxylbenzoic acid (DHB) as a matrix on addition of sodium salt.

organic solvents. The weight percent solubility of redox polymerized lipids in HFIP was determined. Previous studies showed that HFIP was an excellent solvent for polymerized zwitterionic PC lipids.[22,23] In this study, the weight percent solubility of poly(Sorb/DenPC) and poly(Acryl/DenPC) in HFIP was no more than 30−40%. The weight percent solubilities for bis-DenPC$_{18,18}$ and bis-SorbPC$_{17,17}$, as well as mono-DenPC$_{16,18}$ were also measured as a comparison. The bis-substituted PCs form cross-linked polymers whereas mono-substituted PCs form linear polymers.[7] The solubility of the two bis-substituted PCs is 40% and 30% respectively, whereas it was 86% for mono-DenPC (Table 2). These data indicate that the majority of the polymers formed from the Sorb/DenPC and Acryl/DenPC were cross-linked.

Conclusions

Redox-initiated polymerization of bilayers of each of the compounds studied here resulted in the formation of cross-linked polymeric bilayers, regardless of whether the spacer length was 7, 9, or 11 atoms. Similar results were observed when the reactive groups had similar reactivity (Sorb/DenPC) or varied in reactivity (Acryl/DenPC). These data indicate that lipids of this general design can be employed to cross-link lipid assemblies of various types, including lamellar and perhaps non-lamellar phases,[24,25] as well as bilayers on solid supports. The failure of photopolymerization of bilayers of the same monomeric lipids to yield cross-linked polymeric assemblies appears to be due to the formation of oligomers of insufficient length to create a polymeric network. The differences between the two modes of polymerization offer opportunities to control the materials properties of polymerized lipid assemblies. Moreover, it suggests that selective polymerization of one group to yield a prepolymerized assembly, which is not cross-linked, could have valuable properties that a second cross-linking reaction could change. We plan to report on this possibility more fully in due time.

Previously we reported that the redox-initiated polymerization of hydrated bilayers of 1-palmitoyl-2-(2,4,-12,14-tetraenehexadecanoyl) phosphatidylcholine (double diene PC), where the two diene groups are separated by six methylene groups, did not yield cross-linked polymers.[11,12] These data suggested that a lipid monomer with a short spacer would prefer to react with the same neighboring lipid in a linear, ladderlike fashion. The contrasting behavior bilayers of the double diene PC and bilayers of Sorb/DenPC, **8a**, with a seven-atom spacer, is quite interesting. The current results suggest that at least one factor other than spacer length is crucial to the polymerization reaction course. It is quite possible that the distinctive behavior, which warrants further examination, may be due to the greater flexibility of a short spacer when the segment contains an oxygen as well as six methylenes (**8a**) vs six methylenes (double diene PC).

Table 2. Weight Percent Solubility of Polymerized Lipids in HFIP

sample	wt % solubility
mono-DenPC$_{16,18}$	86
Sorb/DenPC$_{16,17}$	35
Sorb/DenPC$_{16,19}$	34
Sorb/DenPC$_{18,21}$	40
Acryl/DenPC$_{16,16}$	40
Acryl/DenPC$_{16,18}$	38
bisDenPC$_{18,18}$	40
bisSorbPC$_{17,17}$	30

Experimental Section

Methods and Materials. All chemicals were obtained from Aldrich Chemical Corp., except for triethyl 4-phosphonocrotonate (Lancaster Inc.), 1-palmitoyl-2-hydroxy-*sn*-glycerol-3-phosphocholine (LysoPC$_{16}$), and 1-oleoyl-2-hydroxy-*sn*-glycerol-3-phosphocholine (LysoPC$_{18}$) (Avanti Polar Lipids Co.). Solvents were dried and distilled prior to use. 4-(Dimethylamino)-pyridine (DMAP) was recrystallized from $CHCl_3$/ether (1:1). All other chemicals were used without further purification. The reactions were monitored by TLC visualized by an UV lamp and/or phosphomolybdic acid. The lipids were hydrated in Milli-Q water, Millipore Inc.

Compounds containing UV-sensitive groups were handled under yellow light. ^{1}H NMR spectra were acquired on a Bruker AM-250 magnetic resonance spectrometer. High-resolution mass spectrometry was carried out on a JEOL HX110A sector instrument. UV/vis absorption spectra were recorded on a Varian DMS 200 spectrophotometer. Quasi-elastic light scat-

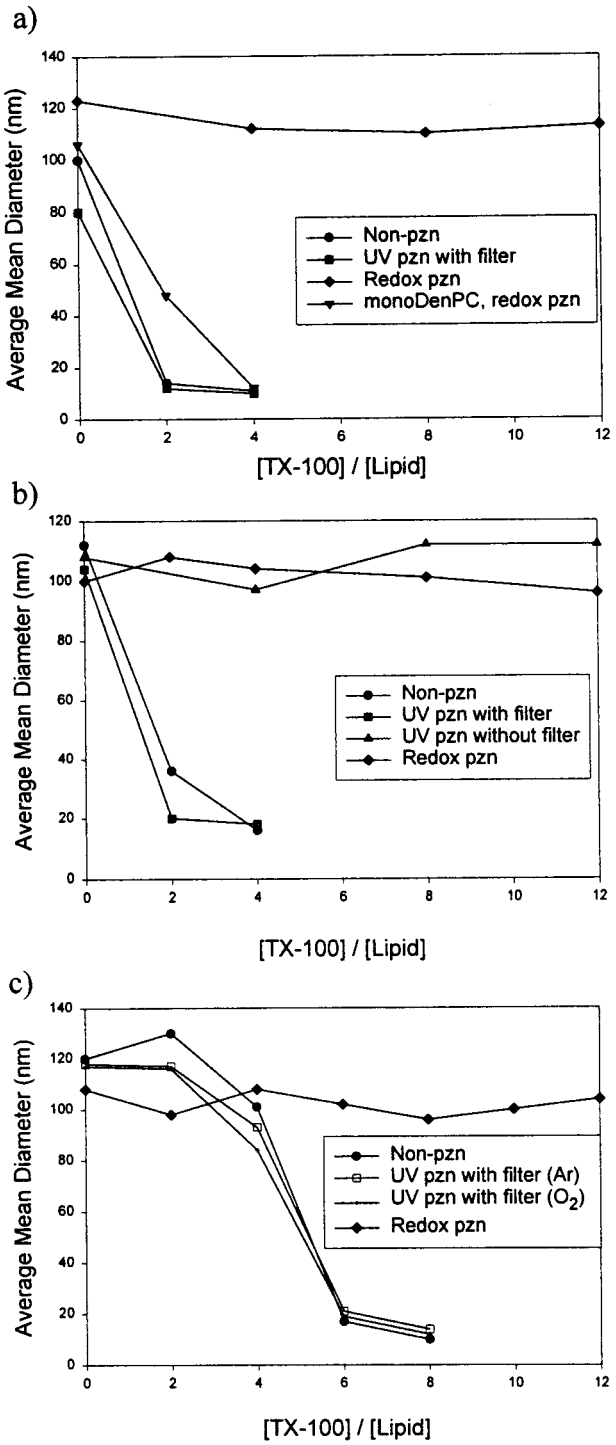


Figure 8. Average mean diameter of LUV composed of unpolymerized or polymerized Sorb/DenPC or mono-DenPC$_{16,18}$ as a function of added equivalents of Triton X-100: (a) Sorb/DenPC$_{16,17}$; (b) Sorb/DenPC$_{16,19}$; (c) Sorb/DenPC$_{18,21}$.

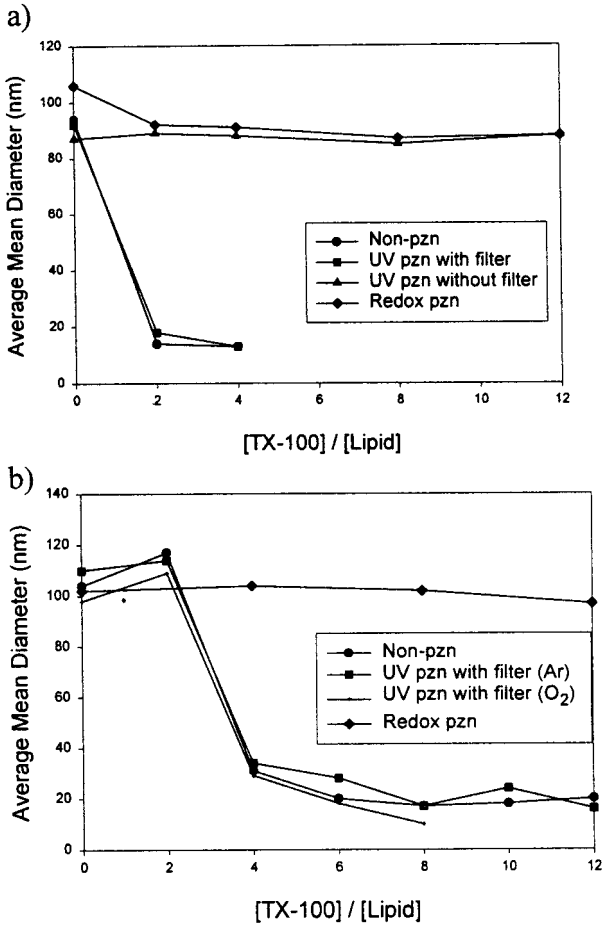


Figure 9. Average mean diameter of LUV composed of unpolymerized or polymerized Acryl/DenPC as a function of added equivalents of Triton X-100: (a) Acryl/DenPC$_{16,16}$; (b) Acryl/DenPC$_{16,18}$.

tering (QELS) was performed with a BI 8000 autocorrelator from Brookhaven Instrument Corp., and particle sizes were calculated with the software accompanying the instrument. A Microcal, Inc., model MC-2 differential scanning calorimeter (DSC) was used for thermotropic studies.

Synthesis. A general outline of the synthetic procedures is shown in Scheme 1 and a representative example is described for **c**.

10-(Acetyloxy)decan-1-ol (1c). Acetyl chloride (3.6 mL, 0.05 mol) in THF (100 mL) was added dropwise to a solution of 1,10-decanediol (17.4 g, 0.1 mol) and pyridine (4.1 mL, 0.05 mol) in THF (250 mL) at 0 °C under argon. The reaction mixture was warmed slowly to room temperature and stirred overnight under a positive argon atmosphere. The mixture was filtered, and the solvent was removed by rotary evaporation. The crude product was dissolved in CH_2Cl_2 and cooled to −30 °C for 3 h. The unreacted diol was recrystallized and removed by vacuum filtration. After evaporation of CH_2Cl_2, the crude ester was purified by column chromatography using Hex/EtOAc (8/2), affording the 10-(acetyloxy)decan-1-ol. The yield was 7.7 g (71%). ^{1}H NMR ($CDCl_3$): 4.08−4.02 (t, J = 6.74 Hz, 2H, −OCOCH_2−), 3.64−3.59 (t, J = 6.60 Hz, 2H, −CH_2OH), 2.19 (br s 1H, −OH), 2.05 (s, 3H, CH_3-), 1.64−1.53 (m, 4H, −COCH$_2$CH_2, −CH_2CH$_2$OH), 1.30 (br s, 12H, −CH_2−) ppm.

10-(Acetyloxy)decan-1-al (2c). Dimethyl sulfoxide (5.6 mL, 78.4 mmol) in CH_2Cl_2 (60 mL) was added to the stirred oxalyl chloride (2.0 M in CH_2Cl_2, 19.6 mL, 39.2 mmol) solution in CH_2Cl_2 (140 mL) at −70 °C. The reaction mixture was stirred for 5 min, and the 10-(acetyloxy) decan-1-ol **1c** (7.7 g, 35.6 mmol) in CH_2Cl_2 (100 mL) was added within 5 min. The mixture was stirred for an additional 30 min. TEA (24.8 mL, 178 mmol) was added, and the reaction mixture was stirred for 10 min and then allowed to warm to room temperature. Water (200 mL) was then added and the aqueous layer was re-extracted with additional CH_2Cl_2 (60 mL) two times. The organic layers were combined and washed successively with dilute HCl (2%), brine, dilute Na_2CO_3 (5%), and brine until they were neutral. The solution was dried over anhydrous $MgSO_4$. After filtration, the solvent was evaporated, giving a slightly yellow crude aldehyde, which was used without further purification. TLC with Hex/EtOAc (8/2) showed the major product at R_f = 0.61 with trace impurities. ^{1}H NMR showed less than 5% impurity. The yield was 7.4 g (97%). ^{1}H NMR ($CDCl_3$): 9.78−9.76 (t, J = 1.83 Hz, 1H, −CHO), 4.08−4.02 (t, J = 6.74 Hz, 2H, −OCOCH_2−), 2.46−2.39 (dd, J = 7.31,

1.82 Hz, 2H, $-CH_2CHO$), 2.05 (s, 3H, $-CH_3$), 1.64−1.60 (m, 4H, $-OCH_2CH_2-$, $-CH_2CH_2CHO$), 1.30 (br s, 10H, $-CH_2-$) ppm.

Ethyl 10-Acetyloxy-(*E*,*E*)-2,4-tetradecadienoate (3c). A suspension of the aldehyde **2c** (7.4 g, 34.6 mmol), triethyl 4-phosphonocrotonate (9.5 g, 38.0 mmol), $LiOH·H_2O$ (1.6 g, 38.0 mmol), and activated 4 Å molecular sieves (10 g, 0.3 g/mmol of aldehyde) in THF (360 mL) was stirred at room-temperature overnight under argon. The crude reaction mixture was filtered through a short plug of silica gel, eluting with ether. The mixture was concentrated, and the residue was purified by column chromatography with Hex/EtOAc (9/1). The yield was 8.0 g (75%). The ratio of (*E*,*E*)-2,4-dienoyl ester to its (*E*,*Z*) isomer was determined by ^{1}H NMR for peaks at 7.65−7.52 (*E*,*Z*) and 7.28−7.18 (*E*,*E*) (92/8), as well as by GC/MS (95/5).

A well-stirred solution of urea (13.8 g, 230.8 mmol) in methanol (140 mL) was treated with the above ester isomer (5.3 g, 17.1 mmol). The solution was kept at 0 °C overnight. The crystals of **3c** were filtered, washed with cold methanol, and them dried under vacuum. These crystals were dissolved in water and extracted several times with ether. The organic layer was combined and dried with anhydrous $MgSO_4$. After evaporation of ether, pure (*E*,*E*) isomer **3c** was obtained. ^{1}H NMR (CD_2Cl_2): 7.28−7.18 (m, 1H, $-CH=CHCOOH$), 6.24−6.13 (m, 2H, $-CH_2CH=CH-$), 5.80−5.73 (d, J = 15.31 Hz, 1H, $=CHCOOH$), 4.19−4.10 (q, J = 7.20 Hz, 2H, CH_3CH_2O-), 4.04−3.98 (t, J = 6.72 Hz, 2H, $-OCH_2CH_2-$), 2.20−2.12 (m, 2H, $=CHCH_2-$), 2.00 (s, 3H, CH_3CO-), 1.62−1.54 (m, 2H, $-OCH_2CH_2CH_2-$), 1.45−1.23 (m, 15H, $-CH_2-$, $-CH_3$) ppm. FAB−MS *m*/*z*: calcd for $C_{18}H_{31}O_4$, 311.2222; found, 311.2219.

14-(Hydroxy)-2,4-tetradecadienoic acid (4c). An aqueous solution of 1 N KOH (50 mL, 50 mmol) was added to the methanol solution (50 mL) of ethyl ester **3c** (3.8 g, 12.25 mmol) and heated under refluxing conditions for 1 h. After the solution was acidified to pH 3 with dilute HCl solution, it was extracted several times with $CHCl_3$. The organic layer was dried with anhydrous $MgSO_4$ and then concentrated, affording the pure dienoic acid **4c** without further purification. The yield was 3.0 g (100%). ^{1}H NMR (methanol-d_4): 7.29−7.19 (dd, J = 15.38, 9.95 Hz, 1H, $-CH=CHCOOH$), 6.30−6.10 (m, 2H, $-CH_2CH=CH-$), 5.80−5.73 (d, J = 15.38 Hz, 1H, $=CHCOOH$), 3.55−3.50 (t, J = 6.56 Hz, 2H, $-CH_2OH$), 2.22−2.14 (m, 2H, $=CHCH_2-$), 1.54−1.32 (m, 14H, $-CH_2-$) ppm. FAB−MS *m*/*z*: calcd for $C_{14}H_{25}O_3$, 241.1804; found, 241.1803.

14-Acryloxy-2,4-tetradecadienoic Acid (Acryl/Den Acid) (5c). Acryloyl chloride (0.53 mL, 6.5 mmol) in THF (30 mL) was added dropwise to a solution of 14-hydroxy-2,4-tetradecadienoic acid **4c** (1.2 g, 5.0 mmol) with pyridine (0.61 mL, 7.5 mmol) and one crystal of 2,6-di-*tert*-butyl-4-methylphenol in THF (120 mL) at 0 °C. The solution was allowed to warm to room temperature and stirred for another 2 h. The pyridine chloride was removed by vacuum filtration and the mixture was concentrated by rotary evaporation. The crude acid was purified by column chromatography using $CHCl_3$/MeOH (95/5), affording 14-acryloxy-2,4-tetradecadienoic acid, **5c**. The yield was 0.89 g (60%). ^{1}H NMR ($CDCl_3$): 7.40−7.30 (m, 1H, $-CH=CHCOOH$), 6.44−6.36 (dd, J = 17.32, 1.62 Hz, 1H, $H_aCH_b=$), 6.21−6.06 (m, 3H, $-CH_2CH=CH-$, $H_aCH_b=CHCOO-$), 5.84−5.76 (m, 2H, $CH_2=CHCOO-$, $=CHCOOH$), 4.18−4.12 (t, J = 6.72 Hz, 2H, $-CH_2O-$), 2.22−2.14 (m, 2H, $=CHCH_2-$), 1.69−1.61 (m, 2H, $-CH_2CH_2O-$), 1.43−1.29 (m, 12H, $-CH_2-$) ppm. FAB−MS *m*/*z*: calcd for $C_{17}H_{27}O_4$, 295.1909; found, 295.1918.

14-Sorbyl-2,4-tetradecadienoic Acid (Sorb/Den Acid) (6c). Sorbyl chloride (0.75 g, 5.7 mmol) in THF (40 mL) was added dropwise to a solution of 14-hydroxy-2,4-tetradecadienoic acid **4c** (1.0 g, 4.2 mmol) and pyridine (0.46 mL, 5.7 mmol) in THF (100 mL) at 0 °C. The solution was allowed to warm to room temperature and stir overnight under argon. The reaction mixture was filtered and concentrated. The crude acid was purified by reverse phase C-18 column chromatography using a gradient of MeOH/H_2O (1/1 to 7/3). The yield was 0.58 g (42%). ^{1}H NMR ($CDCl_3$): 7.40−7.20 (m, 2H, $-CH=CHCOOH$, $CH_3CH=CHCH=$), 6.21−6.12 (m, 4H, $-CH_2CH=CH-$, $CH_3CH=CH-$), 5.82−5.74 (2d, J = 15.31 Hz, 2H, $=CHCO$), 4.15−4.10 (t, J = 6.72 Hz, 2H, $-CH_2O-$), 2.19−2.14 (m, 2H, $=CHCH_2-$), 1.86−1.84 (d, J = 5.34 Hz, 3H, CH_3-), 1.68−1.62 (m, 2H, $-CH_2CH_2O-$), 1.42−1.29 (br s, 12H $-CH_2-$) ppm. FAB−MS *m*/*z*: calcd for $C_{20}H_{31}O_4$, 335.2222; found, 335.2213.

1-Palmitoyl-2-[14-acryloxy-2,4-tetradecadienoic]-*sn*-glycerol-3-phosphocholine (Acryl/Den $PC_{16,18}$) (7c). Lyso PC_{16} (0.808 g, 1.63 mmol), Acryl/Den acid **5c** (0.40 g, 1.36 mmol), dicyclohexylcarbodiimide (DCC) (0.336 g, 1.63 mmol), 4-(dimethylamino)pyridine (DMAP) (0.332 g, 2.72 mmol), and one crystal of 2,6-di-*tert*-butyl-4-methylphenol were added into a flask with $CHCl_3$ (8 mL). The mixture was stirred at room temperature in the dark for 3 days under argon. The white suspension was filtered and the solution was concentrated. The residue was dissolved in MeOH (20 mL), and stirred with Bio-Rad AG 501-X8 ion-exchange resin (5 g) for 15 min. The resin was removed by vacuum filtration, and the filtrate was concentrated. The crude product was purified by column chromatography using $CHCl_3$/MeOH (9/1) followed by $CHCl_3$/MeOH/H_2O (65/25/4), giving 0.46 g of lipid (44%). ^{1}H NMR ($CDCl_3$): 7.29−7.19 (m, 1H, $-CH=CHCO-$), 6.44−6.36 (dd, J = 17.29, 1.59 Hz, 1H, $H_aCH_b=$), 6.17−6.06 (m, 3H, $-CH_2CH=CH-$, $H_aCH_b=CH-$), 5.84−5.73 (m, 2H, $=CHCOO-$), 5.27 (br s, 1H, $-POCH_2CHO-$), 4.42−4.19 (m, 4H, $-POCH_2CH-$, $-CHCH_2OCO-$), 4.17−4.12 (t, J = 6.71 Hz, 2H, $-OCH_2CH_2-$), 4.01 (br s, 2H, $-NCH_2CH_2-$), 3.82 (br s, 2H, $-NCH_2CH_2-$), 3.38 (s, 9H, $-NCH_3$), 2.30−2.24 (t, J = 7.50 Hz, 2H, $-CH_2-COO-$), 2.18−2.16 (m, 2H, $=CHCH_2-$), 1.70−1.56 (m, 4H, $-OCH_2CH_2-$, $-CH_2CH_2COO-$), 1.48−1.18 (m, 36H, $-CH_2-$), 0.90−0.85 (t, J = 6.56 Hz, 3H, $-CH_3$) ppm. FAB−MS *m*/*z*: calcd for $C_{41}H_{75}O_{10}PN$, 772.5129; found, 772.5110.

1-Oleoyl-2-[14-sorbyl-2,4-tetradecadienoic]-*sn*-glycero-3-phosphocholine (Sorb/Den $PC_{18,21}$) (8c). Following the same procedure as in Acryl/Den $PC_{16,18}$. LysoPC_{18} (0.56 g, 1.1 mmol), Sorb/Den acid **6c** (0.30 g, 0.9 mmol), DCC (0.22 g, 1.1 mmol) and DMAP (0.22 g, 1.8 mmol) afforded lipid **8c** with a yield of 0.55 g (73%). ^{1}H NMR ($CDCl_3$): 7.29−7.20 (m, 2H, $-CH=CHCOO-$), 6.19−6.12 (m, 4H, $-CH_2CH=CH-$, $CH_3CH=CH-$), 5.80−5.73 (d, J = 15.43 Hz, 2H, $=CHCO-$), 5.36−5.32 (m, 2H, $-CH_2CH=CHCH_2-$), 5.31−5.27 (br s, 1H, $-POCH_2-CHO$), 4.40−4.19 (m, 4H, $-POCH_2CH-$, $-CHCH_2OCO-$), 4.15−4.10 (t, J = 6.69 Hz, 2H, $-OCH_2CH_2-$), 3.99 (br s, 2H, $-NCH_2CH_2-$), 3.84 (br s, 2H, $-NCH_2CH_2-$), 3.38 (s, 9H, $-NCH_3$), 2.30−2.24 (t, J = 7.50 Hz, 2H, $-CH_2COO-$), 2.18−2.15 (m, 2H, $=CHCH_2-$), 2.02−1.98 (m, 4H, $-CH_2CH=CHCH_2-$), 1.86−1.84 (d, J = 5.28 Hz, 3H, $=CHCH_3$), 1.70−1.52 (m, 4H, $-OCH_2CH_2-$, $-CH_2CH_2COO-$), 1.27 (br s, 32H, $-CH_2-$), 0.90−0.85 (t, J = 6.50 Hz, 3H, $-CH_2CH_3$). FAB−MS *m*/*z*: calcd for $C_{46}H_{81}O_{10}PN$ 838.5598; found, 838.5607.

Calorimetry. The lipid was freeze-dried overnight and then hydrated with deoxygenated water to a concentration of 8 mM. The hydration of the lipid was done by heating to 45 °C, vortexing, and then cooling to −78 °C 10 times. The fully hydrated lipid bilayers were then transferred to a DSC cell. Thermograms were obtained at a scan rate of 10 °/h, and the phase transition temperature was measured from the point of maximum excess heat capacity. The cooperative unit (number of lipids undergoing the phase transition at the same time) was calculated by dividing the van't Hoff enthalpy by the calorimetric enthalpy.

Vesicle Polymerization. (a) Redox Initiation. Large unilamellar vesicles (LUV) of polymerizable lipid were prepared as follows: Approximately 6 mg of polymerizable lipid from a benzene stock solution (10 mg/mL) were freeze-dried under high vacuum for at least 4 h. The dried lipid was then hydrated with deoxygenated Milli-Q water to a concentration of either 0.6 or 2 mM. Samples were vortexed to uniformity and subjected to 10 freeze−thaw-vortex cycles (−77 to +45 °C). The LUV with a diameter of ca. 100 nm were prepared by extrusion 10 times (4 × 0.2 μm + 6 × 0.1 μm) through two stacked Nuclepore polycarbonate filters at 45 °C using a stainless steel extruder from Lipex Biomembranes.[26]

The redox initiator was prepared from $KBrO_3$ (66.8 mg, 0.4 mmol) and L-cysteine hydrochloride hydrate (63.0 mg, 0.4

mmol), which were weighed into a 10 mL volumetric flask and diluted. An aliquot of $KBrO_3$/L-cysteine (1/1) was added to the vesicle suspension, giving the [M]/[I] ratio of 1. The sample was sealed in an ampule with a septum and flushed with argon for 0.5 h. Polymerization was performed at 60 ± 2 °C in a water-circulating bath under a positive argon pressure for 18 h. Polymerization was monitored by UV absorption spectroscopy of aliquots diluted with Milli-Q water to ca. 60 μM.

(b) Photopolymerization. The LUV, prepared as above, were placed into a 3 mL quartz cuvette equipped with a magnetic stir bar, and the cuvette was placed 1 cm in front of a low-pressure Hg vapor pen lamp with a CS9−54 filter, which has a cut off wavelength shorter than 230 nm. The polymerization was carried out at 40 °C and monitored by UV absorption spectroscopy as above.

Surfactant Dissolution of Vesicles. The LUV were prepared as described above. After polymerization, the LUV were characterized by QELS for a 2 mL sample with a lipid concentration of 300 μM. Aliquots of 50 mM TX-100 solution, each 2 equiv. with respect to lipid, were added until the vesicles dissolved or 12 equiv. were added. The light-scattering intensities were determined again by QELS. Measurements at each concentration of TX-100 were performed at least three times. The average mean diameter of vesicles/particles was calculated by nonnegatively constrained least-squares program.

Weight Percent Solubility. Only samples with greater than 90% monomer conversion, as determined by UV/vis spectroscopy, were used in the solubility studies. Samples were lyophilized after polymerization. The lipid was weighed and HFIP added to a concentration of 2 mg/mL. The samples were shaken for 2 min and allowed to stand for at least 4 h. The solution was filtered through an Acrodisc CR PTFE filter with 0.2 μm pore size. The solvent was removed by lyophilization overnight, leaving the soluble polymer. The weight of the polymer was used to calculate the percent solubility.

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References and Notes

(1) O'Brien, D. F.; Armitage, B.; Benedicto, A.; Bennett, D. E.; Lamparski, H. G.; Lee, Y.-S.; Srisiri, W.; Sisson, T. M. *Acc. Chem. Res.* **1998**, *31*, 861−868.
(2) Regen, S. L.; Singh, A.; Oehme, G.; Singh, M. *J. Am. Chem. Soc.* **1982**, *104*, 791−795.
(3) Hupfer, B.; Ringsdorf, H.; Schupp, H. *Chem. Phys. Lipids* **1983**, *33*, 355−374.
(4) Dorn, K.; Klingbiel, R. T.; Specht, D. P.; Tyminski, P. N.; Ringsdorf, H.; O'Brien, D. F. *J. Am. Chem. Soc.* **1984**, *106*, 1627−1633.
(5) Ohno, H.; Ogata, Y.; Tsuchida, E. *Macromolecules* **1987**, *20*, 929−933.
(6) Stefely, J.; Markowitz, M. A.; Regen, S. L. *J. Am. Chem. Soc.* **1988**, *110*, 7463−7469.
(7) Sisson, T. M.; Lamparski, H. G.; Kölchens, S.; Elyadi, A.; O'Brien, D. F. *Macromolecules* **1996**, *29*, 8321−8329.
(8) Kölchens, S.; Lamparski, H.; O'Brien, D. F. *Macromolecules* **1993**, *26*, 398−400.
(9) Stupp, S. I.; Son, S.; Lin, H. C.; Li, L. S. *Science* **1993**, *259*, 59−63.
(10) Stupp, S. I.; Son, S.; Li, L. S.; Lin, C.; Keser, M. *J. Am. Chem. Soc.* **1995**, *117*, 5212−5227.
(11) Srisiri, W.; Sisson, T. M.; O'Brien, D. F. *J. Am. Chem. Soc.* **1996**, *118*, 11327−11328.
(12) Sisson, T. M.; Srisiri, W.; O'Brien, D. F. *J. Am. Chem. Soc.* **1998**, *120*, 2322−2329.
(13) Sells, T. D.; O'Brien, D. F. *Macromolecules* **1994**, *27*, 226−233.
(14) Lamparski, H.; O'Brien, D. F. *Macromolecules* **1995**, *28*, 1786−1794.
(15) Mancuso, A. J.; Huang, S.-L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480−2482.
(16) Bonadies, F.; Cardilli, A.; Lattanzi, A.; Orelli, L. R.; Scettri, A. *Tetrahedron Lett.* **1994**, *35*, 3383−3386.
(17) Srisiri, W.; Lamparski, H.; O'Brien, D. F. *J. Org. Chem.* **1996**, *61*, 5911−5915.
(18) Koynova, R.; Caffrey, M. *Biochim. Biophys. Acta* **1998**, *1376*, 91−145.
(19) Lamparski, H.; Lee, Y.-S.; Sells, T. D.; O'Brien, D. F. *J. Am. Chem. Soc.* **1993**, *115*, 8096−8102.
(20) Tsuchida, E.; Hasegawa, E.; Kimura, N.; Hatashita, M.; Makino, C. *Macromolecules* **1992**, *25*, 207−212.
(21) Liu, S.; O'Brien, D. F. *Macromolecules* **1999**, *32*, 5519−5524.
(22) Pujol-Fortin, M.; Galin, J.-C. *Polymer* **1994**, *34*, 1462−1472.
(23) Ueda, T.; Oshida, H.; Kurita, K.; Ishihara, K.; Nakabayashi, N. *Polym. J.* **1992**, *24*, 1259−1269.
(24) Lee, Y.-S.; Yang, J.-Z.; Sisson, T. M.; Frankel, D. A.; Gleeson, J. T.; Aksay, E.; Keller, S. L.; Gruner, S. M.; O'Brien, D. F. *J. Am. Chem. Soc.* **1995**, *117*, 5573−5578.
(25) Srisiri, W.; Sisson, T. M.; O'Brien, D. F.; McGrath, K. M.; Han, Y.; Gruner, S. M. *J. Am. Chem. Soc.* **1997**, *119*, 4866−4873.
(26) Hope, M. J.; Bally, M. B.; Webb, G.; Cullis, P. R. *Biochem. Biophys. Acta* **1985**, *812*, 55−65.

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