Novel Polymer Architectures via the Selective Polymerization of Lyotropic Liquid Crystals of Heterobifunctional Amphiphiles

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Received September 25, 1997

Abstract: The reactivity of polymerizable amphiphiles in supramolecular assemblies, such as lipid bilayers, is dependent on the mode of initiation, the polymerizable group, and the position of the reactive group in the amphiphile. This report describes the synthesis, characterization, and polymerization of a novel heterobifunctional lipid, 1-palmitoyl-2-(2,4,12,14-tetraenehexadecanoyl)phosphatidylcholine (**1**), that contains a diene and a dienoyl group in the *sn*-2 acyl chain. The difference in polarity of local sites of each group within the bilayer makes it possible to perform simultaneous or selective polymerizations of these groups. Simultaneous polymerization of both reactive groups was achieved by either redox polymerization or direct photoirradiation. Selective polymerization of the dienoyl was accomplished by photoirradiation with filtered light from a high-pressure Hg/Xe lamp. The diene was selectively initiated by thermal decomposition of AIBN. The degree of polymerization depended strongly on the initiation chemistry. Photoirradiation gave oligomers, whereas radical polymerization with redox or AIBN afforded polymers with relative number average degrees of polymerization of 200 and 350, respectively. Both diene and dienoyl groups formed 1,4-poly(diene) regardless of the mode and order of the polymerization applied. The polymers obtained from hydrated bilayers of **1** by either simultaneous polymerization of both reactive groups or sequential selective polymerization of each group were not cross-linked. The absence of cross-linking at high conversions to polymer and the size of the solubilized surfactant-polymer micelles are consistent with the formation of ladder-like polymers with parallel chains tethered together by a hexa-methylene spacer.

Introduction

Recent studies have highlighted the differences between polymerization reactions in organized media and the corresponding reactions in isotropic solutions. For example, the inherent orientational order in smectic liquid crystals was successfully employed in bulk polymerizations to create two-dimensional polymers.[1,2] Analysis of rates of polymerization in smectic mesophases shows significant effects on the rates of propagation and termination compared to isotropic systems.[3,4] In hydrated lamellar assemblies, i.e., lipid bilayers, the reactivity of two otherwise identical groups was observed to depend on the polarity of their individual site in the assembly.[5] In addition, radical chain polymerizations in the constrained environment of hydrated amphiphiles were found to terminate by reaction with initiator fragments, i.e., primary termination.[6−8] In each of these examples, i.e., cross-linking, reactivity, and mechanistic control of polymer size, the polymerization process in organized media is distinctive due in part to the anisotropic orientation and restricted motion of monomers in the two-dimensional local environment.

Lyotropic mesophases offer the possibility of selective initiation of polymerization by careful attention to the solubility of the initiating species.[5,9] It is also known that polymerizations of certain amphiphilic monomers, e.g. sorbyl, acryloyl, and methacryloyl derivatives, in hydrated lamellar phases can yield relatively large polymers.[6,8,10] Polymerization in these lipid assemblies proceeds in a linear or cross-linked manner depending on the number of polymerizable groups per amphiphile.[11−17] Lipids that contain a single reactive moiety in either of the hydrophobic chains or are associated with the hydrophilic headgroup yield linear polymers. Polymerization of lipids with reactive groups in each hydrophobic chain yields cross-linked polymers, which can be distinguished by their physical properties, e.g., general insolubility in organic solvents including

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S0002-7863(97)03371-4 CCC: $15.00
Published on Web 02/26/1998

hexafluoro-2-propanol (HFIP), chemical stability toward added surfactant, and the rate of lateral diffusion of amphiphiles in the mesophase, among others.[17]

The previous observations, that the polymerization of bilayers composed of lipids that possess reactive groups in each hydrophobic tail yield cross-linked polymers, are insufficient to distinguish whether cross-linking is simply a consequence of two reactive groups per lipid or also depends on their location in each lipid. It is especially interesting to consider the polymerization characteristics of an amphiphilic monomer with two reactive groups in a single chain. The behavior of such monomers might shed light on the competitive pathways for polymer chain growth for reactive groups located in different regions of an organized assembly. Moreover, there is the intriguing possibility of selective control of the polymerization process in lyotropic mesophases. Here we examine this question through the synthesis, characterization, and polymerization of a novel heterobifunctional lipid, 1-palmitoyl-2-(2,4,12,14-tetraenehexadecanoyl) phosphatidylcholine (**1**), that contains two polymerizable diene groups in the *sn*-2 acyl chain.[9] Heterobifunctional polymerizable lipids, which are amphiphiles with two different reactive functionalities, have been previously described with two chemically different groups: diacetylene and acetylene[18] or diacetylene and either vinyl or methacrylate.[19] In lipid **1** a dienoyl group is conjugated with the ester carbonyl of the fatty acid chain, and a 1,3-diene is located near the acyl chain terminus. The location of the reactive groups in regions of different polarity within a bilayer permits either simultaneous or selective and sequential polymerization, depending on the choice of initiation chemistry. The mechanism of polymerization for both reactive moieties was examined by using UV and ^{1}H NMR spectroscopy. Polymer sizes were estimated by size exclusion chromatography (SEC), and cross-linking of the bilayers was tested by the solubility and surfactant lysis techniques reported previously.[17]

Results and Discussion

Synthesis. The lipid (**1**) designed for these studies was prepared by acylation of 1-palmitoyl-2-hydroxy-*sn*-phosphatidylcholine (LysoPC) with 2,4,12,14-tetraenehexadecanoic acid **2a**, which was accessible in three steps from commercially available (*E*,*E*)-8,10-dodecadien-1-ol through intermediates **3** and **4** (see Experimental Section).[20−23] The stereochemical purity of the diene was determined by using ^{1}H NMR spectroscopy. The vinyl protons on the starting material (*E*,*E*)-8,10-dodecadien-1-ol appeared in the range of 6.03−5.53 ppm as multiplet peaks with the characteristic coupling constant for an (*E*)-isomer of 10.05−15.25 Hz. These vinyl protons on acid **3** retained their chemical shift and characteristic (*E*)-configuration coupling constant, confirming that the (*E*,*E*)-diene functionality at the end of the hydrophobic chain did not isomerize during the multiple-step synthesis. The synthesized dienoyl group at the 2-and 4-positions was a mixture of (*E*,*E*)- and (*E*,*Z*)-isomers. These were separated by urea inclusion to give the desired 2,4-(*E*,*E*)-acid **2a**.[20−23]

The acylation of LysoPC with fatty acid **2a** was performed in the presence of 4-(dimethylamino)pyridine and dicyclohexylcarbodiimide. The use of excess LysoPC affords the acylated product in high yield relative to the fatty acid. Isomerization of 1-palmitoyl-2-acyl-*sn*-phosphatidylcholine to the corresponding 1-acyl-2-palmitoyl-*sn*-phosphatidylcholine might proceed during the acylation of LysoPC.[24] To the extent that this occurs, the product mixture would contain the desired lipid **1** and its positional isomer. Since the dienoyl group in the *sn*-2 chain of lipid **1** renders it less reactive to phospholipase A_2 hydrolysis than lipids with saturated esters at the *sn*-2 position, enzymatic hydrolysis was used to separate lipid **1** from the positional isomer.[22] The structure and purity of lipid **1** was determined by TLC, high-resolution FAB-mass spectroscopy, and ^{1}H NMR spectroscopy.

Hydrated Bilayers of Lipid 1. The purified lipid **1** readily formed translucent suspensions upon hydration. Differential scanning calorimetry (DSC) of hydrated bilayers of lipid **1** (5 mg/mL) showed an endotherm at 18 °C, with a calorimetric enthalpy of 4.7 kcal/mol. The cooperative phase transtion indicates bilayer formation. Lipid bilayer vesicles were formed with use of conventional sonication or extrusion protocols. Vesicles of lipid **1** showed diene and dienoyl absorption peaks with λ_{max} of 230 and 260 nm, respectively. The extinction coefficients of diene and dienoyl chromophores were 1.17×10^4 and 1.03×10^4 L/mol cm in water and 2.58×10^4 and 2.34×10^4 L/mol cm in methanol, respectively.

Polymerization of Bilayer Vesicles of 1. The two polymerizable groups on lipid **1** are located in different regions of polarity within a bilayer, thereby permitting either simultaneous or selective sequential polymerizations depending on the initiation chemistry (Scheme 1). Lipid **1** was hydrated and then extruded to give large unilamellar bilayer vesicles (LUV) with an average diameter of ca. 100 nm as determined by quasielastic light scattering. The polymerizations were performed under positive Ar(g) with use of the selected initiators and conditions given in the Experimental Section. In all polymerizations, no change in LUV size was observed, indicating that only intravesicle polymerization took place. Kinetic studies were performed by using UV/vis spectroscopy to monitor the decrease in monomer absorbance at various time intervals for both dienoyl and diene functionalities. Number average molecular weights (M_n) and degree of polymerization (X_n) of the polymers were obtained by using size exclusion chromatography (SEC). The lipid polymers were lyophilized and then modified by transesterification with methanolic HCl solution to remove the phosphatidylcholine headgroups to increase their solubility in SEC solvents (THF, dichloromethane).[8]

(a) Simultaneous Polymerizations. Simultaneous polymerization of both reactive groups was achieved by either redox or direct UV photoirradiations. The term "simultaneous" means reaction of diene and dienoyl groups in the bilayer were concurrent, but does not necessarily imply that a given lipid possesses two propagating species at the same time.

(i) Redox Polymerization. The polymerization was carried out at 40 °C with $K_2S_2O_8$/L-cysteine (10/1),[25,26] with [monomer]/[oxidant] equal to 2. This redox system generates hydroxyl radicals,[5] which can diffuse across the lipid bilayer and initiate the polymerization of both reactive groups regardless of their locations in the bilayer. The diene reacted faster, reaching 95%

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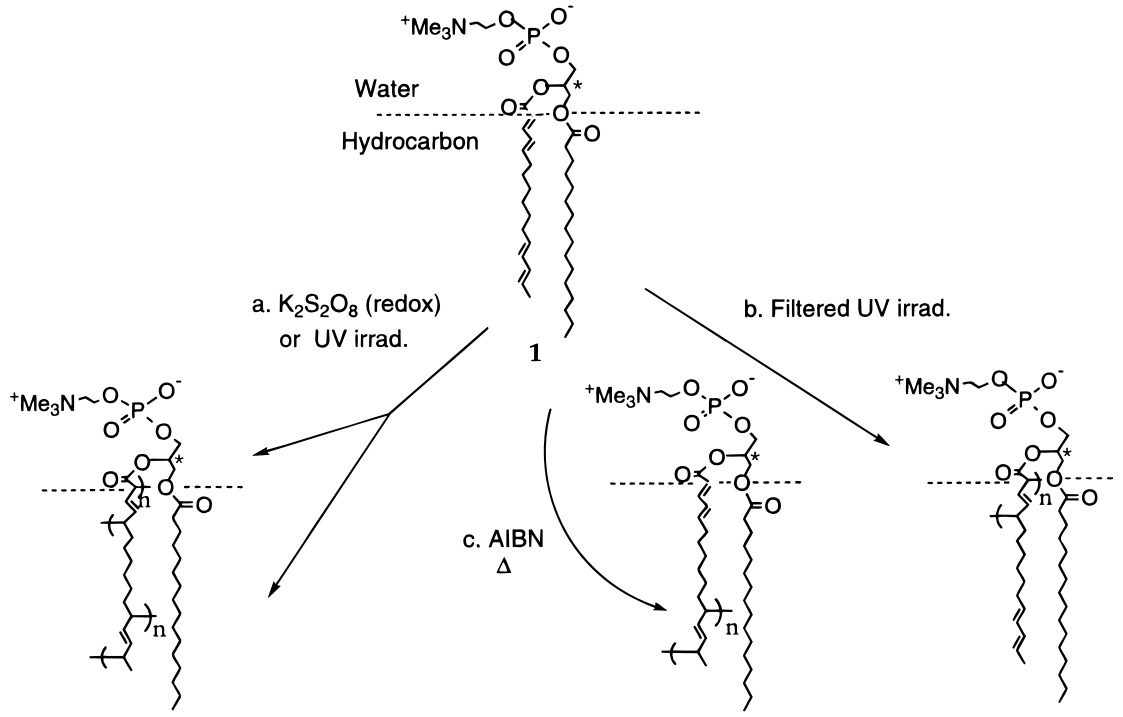
Scheme 1



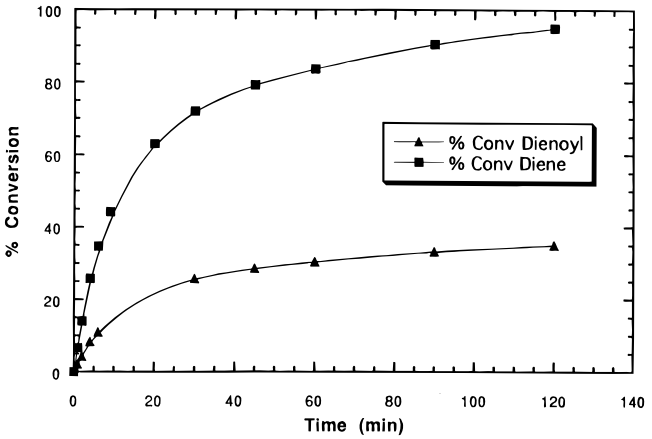


Figure 1. Percent conversion of lipid **1** as a function of time for redox polymerization with $K_2S_2O_8$/L-cysteine (10/1). [M] = 80 μM, [M]/[O] = 2, and 40 °C.

conversion in 2 h compared to ca. 30% for dienoyl (Figure 1). The dienoyl group may have polymerized slower than the diene because of the greater stability of the dienoyl radical. Note that the diene moiety is located at the chain end, whereas the dienoyl is close to the glycerol backbone. The diene group therefore has more freedom of movement that may facilitate polymerization. To further examine which of these explanations is more responsible for the observed faster polymerization rate of the diene group, the relative rate of redox polymerization of dienoyl and sorbyl reactive moieties was determined under the same conditions. The sorbate in mono-sorbyl-substituted PC is the same functional group as a dienoyl group in lipid **1** except that it is located at the chain end. The observation that the polymerization of the sorbyl group was only slightly faster than that of the dienoyl group indicates the location of the group has only a moderate effect on the reactivity. Therefore the higher rate of polymerization of diene moiety appears to be primarily due to the higher reactivity of the propagating species of the diene.

The effect of oxygen on the redox polymerization was examined by hydration of lipid in nondeoxygenated MilliQ water. An induction period in the polymerizations of both reactive moieties was observed.[27] The polymer had a M_n of ca. 5×10^4 and X_n of 200 with a polydispersity of 2, as determined by SEC.

(ii) Direct UV Polymerization. LUV of **1** were irradiated at 40 °C with a low-pressure Hg vapor lamp, resulting in 100% conversion of both reactive groups within 0.5 h or less. In this experiment the intensity of light from the lamp was 10 times greater at 260 nm than 230 nm; therefore, under these conditions the dienoyl polymerized faster than the diene. SEC of the transesterified polymers indicated the X_n was about 10. Photoirradiation of dienoyl and sorbyl groups in lipid bilayers has previously been reported to yield polymers with low molecular weight compared to other methods of initiation.[8]

(b) Selective Polymerizations. (i) Selective Polymerization of the Dienoyl Moiety. The dienoyl group of lipid **1** was selectively polymerized by using photoirradiation with filtered light from a high-pressure Hg/Xe lamp. A Corning CS-056 cutoff filter and Pyrex glass (300 nm) were placed between the sample and light source. The dienoyl absorption peak at 260 nm decreased 50% during the first hour (Figure 2). Although greater than 85% conversion was accomplished with longer irradiation times, the diene absorption at shorter wavelength did not change because the cutoff filter prevented absorption of shorter wavelength light necessary for the photopolymerization of the diene. Polymers with X_n of about 10 were obtained.

(ii) Selective Polymerization of the Diene Moiety. Selective polymerization of the diene of lipid **1** was achieved with thermally generated radicals from AIBN. The polymerization was carried out at 60 °C with [M]/[I] equal to 5. The absorption of the dienoyl group at 260 nm remained unchanged over the course of the 7 h reaction. A slight decrease in absorbance at 260 nm after 24 h of irradiation may be due to a decrease of the diene absorption (Figure 3). The absorption peak of the

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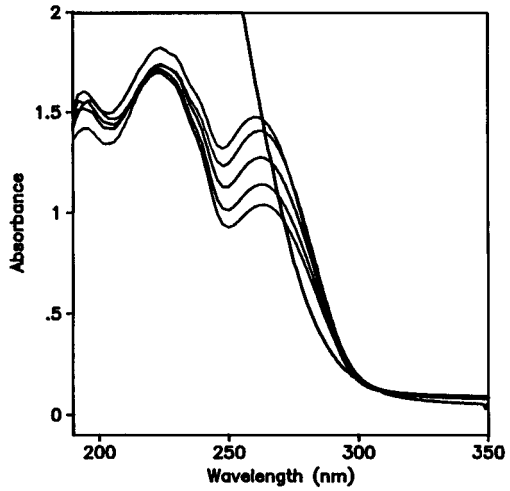


Figure 2. The absorption spectra of LUV of **1** before and after selective photoirradiation of the dienoyl group with a high-pressure Hg/Xe lamp and a CS-056 filter (cutoff curve from 270 to 350 nm). [M] = 80 μM and 40 °C.

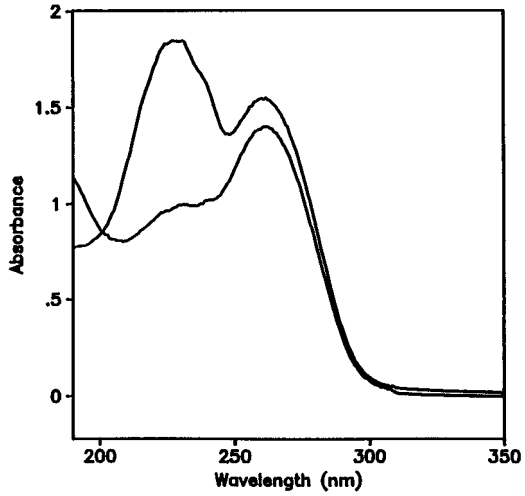


Figure 3. The absorption spectra of LUV of **1** before and after selective AIBN polymerization of the diene group. [M] = 80 μM, [M]/[I] = 5, and 60 °C.

isolated double bond, obtained from the 1,4-polymerization of the diene, at 195 nm overlaps with the absorption peak of the diene at 230 nm. Although this made deconvolution of the absorption peak for the diene difficult, the conversion was estimated to be 80%. SEC of the modified polymers indicated the number average degree of polymerization was ca. 350 with a polydispersity of 2.

The diene group is located at the end of the *sn*-2 chain in the hydrophobic region of the bilayer, a suitable environment for polymerization with a hydrophobic initiator. In contrast, the dienoyl group is at the top of the *sn-2* chain, which near the lipid water interface could not be polymerized by a hydrophobic initiator. This provides further support for the hypothesis that the position of the polymerizable group governs the type of initiation that can be usefully employed.[5]

(c) Sequential Polymerizations. The sequential polymerization of LUV of **1** was demonstrated with two protocols: (1) selective photopolymerization of the dienoyl group (70% conversion) followed by redox polymerization of the diene group (90% conversion) (Figure 4) and (2) selective AIBN polymerization of the diene group (95% conversion) followed by redox polymerization of the dienoyl group (95% conversion).

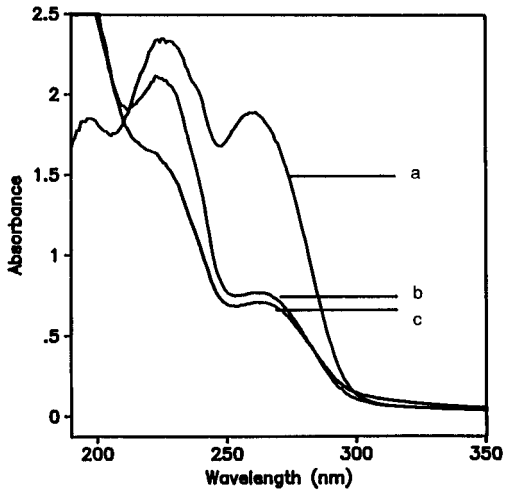


Figure 4. The absorption spectra of LUV of **1** before and after sequential polymerization: (a) lipid **1** monomer, (b) selective photopolymerization of dienoyl moiety, and then (c) polymerization of diene and unreacted dienoyl moieties with (10/1) $K_2S_2O_8$/L-cysteine ([M]/[O] = 2, 40 °C). [M] = 80 μM.

1,4-Polymerization of Lipid 1. Theoretically both the diene and dienoyl groups could be polymerized through the 1,2-, 3,4-, and/or 1,4-addition mechanism. ^{1}H NMR and UV spectroscopy were employed to elucidate the structure of the polymers and to provide information on the probable polymerization mechanism for each mode of initiation chemistry used here.

The ^{1}H NMR spectrum of lipid **1** showed vinyl protons of the dienoyl group at 7.19−7.28 (1H), 6.14−6.16 (2H), and 5.73−5.79 (1H) ppm. The diene vinyl proton resonances occurred at 5.97−6.02 (2H) and 5.50−5.60 (2H) ppm. The broad peak at 5.25 ppm is due to the single proton at the 2-position of the glycerol backbone. Methylene protons on the 1- and 3-positions of the glycerol and headgroup yield multiplet peaks in the range of 3.78−4.39 ppm. Trimethyl protons on the headgroup occur as a sharp singlet peak at 3.34 ppm, and the rest of the methylene protons on the hydrophobic chain are at 1.25−2.30 ppm. The signals of the terminal methyl protons of the *sn*-1 chain are at 0.85−0.90 ppm, whereas the terminal methyl protons of the *sn*-2 chain (conjugated to the diene group) give two peaks at 1.71−1.74 ppm.

(a) Selective Polymerization of the Diene Moiety. The polymers obtained from either 1,2-, 3,4-, and/or 1,4-polymerization can be distinquished by NMR, but not absorption spectroscopy (Scheme 2).

The ^{1}H NMR spectrum of the diene-polymerized lipid **1**, with the use of AIBN, showed a decrease in the vinyl protons on the diene group at 5.97−6.02 ppm, compared to those of the dienoyl group at 6.14−6.16 ppm (Figure 5b). The ratio of these diene and dienoyl vinyl protons in the monomer was 2:2 (Figure 5a), but only ca. 1:5 in the polymer sample, an 80% loss of the diene moiety. Polymerization of the dienoyl was not observed, since the ratio of peak integration of dienoyl vinyl protons at 6.14−6.16 ppm and trimethyl protons of the headgroup (Me_3N) at 3.34 ppm remained unchanged at 2:9 for both monomer and polymer.

For monomer **1**, the ratio of peak integrals between terminal methyl protons of the *sn*-2 chain at 1.71−1.74 ppm and the *sn*-1 chain at 0.85−0.90 ppm was equal to 3:3 (Figure 6a). If the polymerization proceeded through a 3,4- or 1,4-addition mechanism, the terminal methyl protons of the diene would not be adjacent to a double bond, and would have a similar chemical shift as the terminal methyl protons of the saturated *sn*-1 chain.

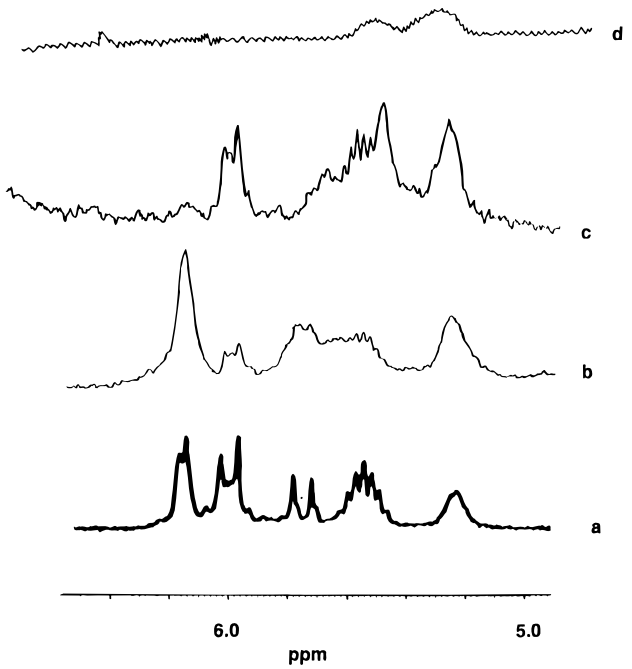
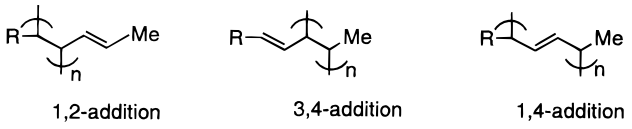


Figure 5. ^{1}H NMR spectra from 5 to 6.5 ppm of (a) lipid **1** monomer, (b) diene- selectively polymerized poly(lipid **1**), (c) dienoyl-selectively polymerized poly(lipid **1**), and (d) poly(lipid **1**) obtained from sequential polymerization of first the diene and then the dienoyl moiety.

Scheme 2



A decrease in integral ratio of the doublet peaks at ca. 1.74 ppm and triplet peaks at ca. 0.95 ppm in the polymers indicates 1,4- and/or 3,4-addition mechanisms. The ratio of terminal methyl protons would remain unchanged, if the polymerization proceeded via a 1,2-addition mechanism. The percent conversion calculated from the change in the ratio of these peaks integrals can be compared to that determined by the decrease in diene vinyl protons. After polymerization, the integral ratio of terminal methyls changed from 3:3 for the monomer to 1:6 for the polymer (Figure 6b). This corresponded to a percent conversion of 83% assuming that 1,2-addition did not occur. The activation energy of a 1,2- or 3,4-addition will be almost the same for this diene.[28] If any 1,2-addition occurred, then one would expect a similar amount of 3,4-polymerization because of the similar energetics for the two processes. However, if both 1,2- and 3,4-additions occurred to an equal extent, the percent conversion calculated by the decrease of diene protons at 5.97−6.02 ppm and that determined by the ratio of the terminal methyl integrals would not be similar. Since they are similar the polymerization of the diene in lipid **1** proceeds predominantly via a 1,4-addition mechanism.

(b) Selective Polymerization of the Dienoyl Moiety. Selective polymerization of the dienoyl group of lipid **1** in water with use of photoirradiation with a cutoff filter diminished the dienoyl absorption peak at 254 nm with a corresponding increase in the absorption at 195 nm, while the diene absorption peak at 230 nm remained unchanged. The new absorption peak from the polymer at 195 nm indicates the presence of isolated double bonds, resulting form either 1,4- and/or 1,2-addition mechanisms. If 3,4-addition had occurred, the polymer would show an absorption peak of an acryloyl group (not seen) at ca. 210 nm (Scheme 3).

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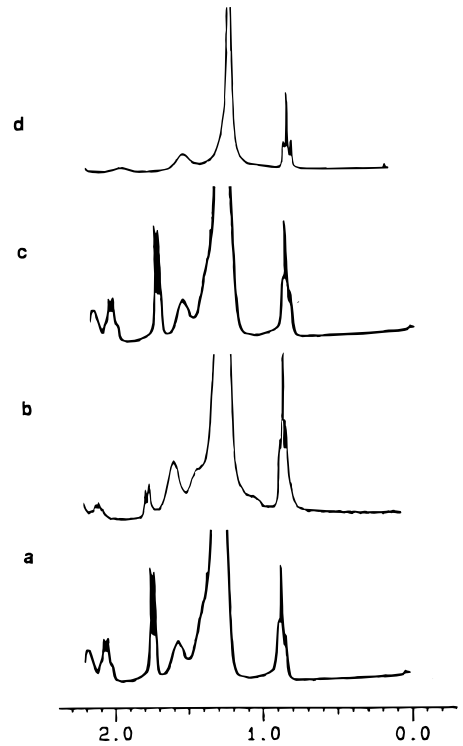
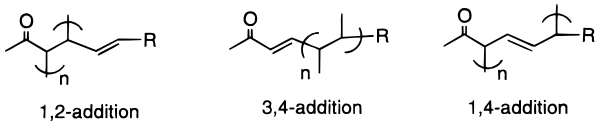


Figure 6. ^{1}H NMR spectra from 0 to 2.0 ppm of (a) lipid **1** monomer, (b) diene- selectively polymerized poly(lipid **1**), (c) dienoyl-selectively polymerized poly(lipid **1**), and (d) poly(lipid **1**) obtained from sequential polymerization of first the diene and then the dienoyl moiety.

Scheme 3



Analysis of the ^{1}H NMR spectrum of the polymer confirmed the polymerization was selective for the dienoyl group. The intensity of the dienoyl proton peaks at 6.14−6.12 ppm decreased relative to that of diene proton peaks at 5.79−6.02 ppm (Figure 5c). The ratio of the dienoyl and diene protons was 2:2 in the monomer, but less than 1:100 in the polymer. This corresponds to a loss of more than 95% of the dienoyl groups. Polymerization of the diene was not observed, since the integral ratio between diene protons at 5.79−6.02 ppm and trimethyl protons of the headgroup at 3.34 ppm remained unchanged during the polymerization. The same ratio between terminal methyl protons of the *sn*-1 and *sn*-2 acyl chains before and after polymerization also confirmed that the diene group was not polymerized under these conditions (Figure 6c). The polymer also showed new proton resonances from 5.50 to 5.70 ppm, which are consistent with the polymer product formed by the 1,4-addition mechanism (Figure 5c).[5]

(c) Sequential Polymerization of Lipid 1. The UV and ^{1}H NMR spectra of the polymers obtained from both strategies of sequential polymerizations of lipid **1** were identical regardless of the order of which polymerization moiety was polymerized first. The UV spectrum showed evidence of an increase in absorption at 195 nm characteristic of an isolated double bond from a 1,4-addition of the dienoyl and diene moieties. The disapperance of all dienoyl and diene protons in the vinyl region

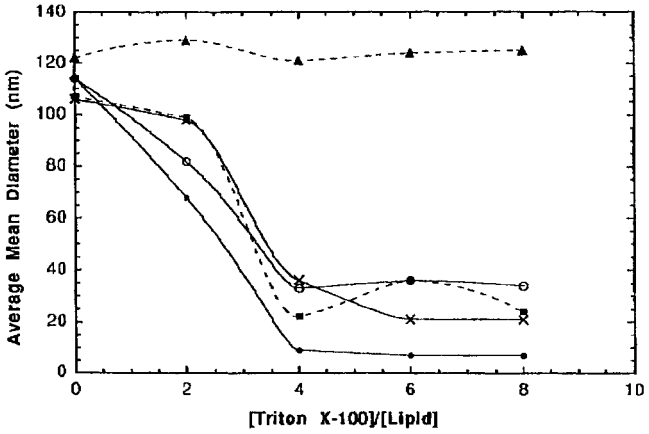
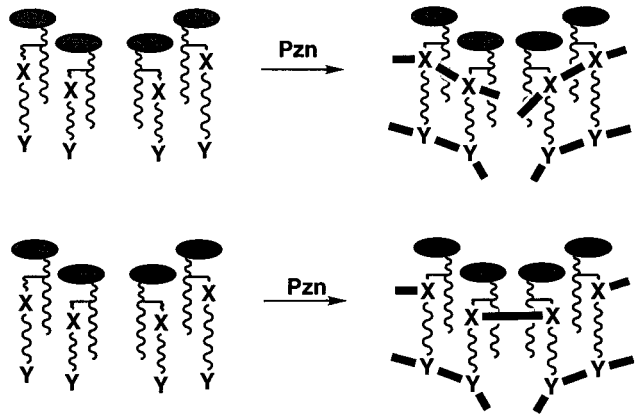


Figure 7. Average mean diameter of particles obtained from polymerized LUV of the indicated lipid (and polymerization method) as a function of surfactant to lipid molar ratio: (●) mono-DenPC (redox); (■) lipid **1** (AIBN/redox); (X) lipid **1** (UV/redox); (○) lipid **1** (redox); and (▲) cross-linked bis-SorbPC (UV). Particle sizes were determined by QELS.

(Figure 5d) and the doublet peaks of the terminal protons of the *sn*-2 acyl chain (Figure 6d) in the ^{1}H NMR spectra confirmed that both groups were polymerized to high conversion. Examination of the vinyl region of the spectra showed a new resonance at 5.42−5.48 ppm (Figure 5d). Previous studies by Tsuchida et al. of polymers formed from dienoyl-substituted PC ascribed a similar signal to the isolated double bond formed from a 1,4-addition mechanism.[5] On the basis of the UV and ^{1}H NMR analysis of the sequentially polymerized samples, the polymerizations of the dienoyl and diene groups proceed through a 1,4-addition mechanism, regardless of the order of the polymerization.

Characterization of the Polymerized Vesicles of Lipid 1. The polymerization of lipid **1** in vesicles could yield either linear ladder-like or cross-linked polymers as represented in Scheme 4. If the reaction paths of both reactive groups are independent of each other, then a cross-linked bilayer will be formed. However, if the polymerization of the reactive groups occurred preferentially with an adjoining group in a repeating unit of the polymer formed, then a ladder-like polymer without cross-linking will be obtained.

Scheme 4



We previously reported several methods for characterizing the linear or cross-linked nature of polymers formed in bilayer vesicles, as well as to determine the critical composition for gelation in the copolymerization of mono- and bis-substituted lipids. The physical properties, e.g. permeability and solubility, of linearly polymerized bilayers are significantly different from those of cross-linked bilayers. The lateral diffusion of a small molecular probe in the lipid assembly,[29] the chemical stability of lipid vesicle in the presence of surfactant,[17,30,31] and the solubility of the lipid polymer[5,17] may be employed to characterize the polymer. In this study, the chemical stability and solubility of the polymerized lipid vesicles were employed.

The increased stability of vesicles to solubilization by surfactants is attributed to the covalent linkages in the polymeric vesicles. The mechanism of surfactant lysis involves incorporation of surfactant into the vesicle bilayer. Eventually this leads to the disruption of the vesicle, giving mixed micelles of surfactant and lipid. Since the size of vesicles, which range in diameter from 100 to 125 nm, is much larger than that of micelles, which are about 5−20 nm, QELS can be used to detect the solubilization process. The disruption of the vesicle is indicated by a sharp decrease in light scattering intensity. Both unpolymerized and linearly polymerized vesicles, the later from monosubstituted dienoylPC (mono-DenPC) (Figure 7), are solubilized by surfactant to yield mixed micelles of surfactant and lipid or poly(lipid). Cross-linked polymerized vesicles, e.g. vesicles from bis-substituted sorbylPC (bis-SorbPC),[8] are much more stable to surfactant and are not dissolved. The normalized light scattering intensity of the cross-linked polymerized vesicles is therefore nearly independent of the amount of surfactant added.[17]

Polymerized LUV of **1** obtained from both sequential and simultaneous polymerizations were characterized by surfactant lysis. Upon addition of Triton X-100 to the polymerized lipid vesicles, a sharp decrease in the normalized light scattering intensity and calculated average mean diameter of the suspended particles was observed (Figure 7). As shown in the figure, each mode of polymerization of lipid **1** gave poly-LUV that were not stable in the presence of Triton X-100. It is interesting to compare the average mean diameter of solubilized linear polymers from mono-DenPC, with the values measured for the solubilized poly-**1**. The data indicate the particles obtained from poly-**1** are 20−40 nm in diameter, which is significantly larger than the 5−10 nm diameter of surfactant solubilized linear poly(lipid), and the 5−7 nm diameter of pure Triton X-100 spherical micelles. These data are consistent with a nonrandom coil, ladder-like poly-**1** that is accommodated in rodlike micelles rather than smaller spherical micelles.

Further evidence for the lack of cross-linking of the polymerized LUV of **1** was obtained by removal of the sample water by freeze-drying and dissolving the resulting polymeric residue in organic solvents, e.g. chloroform. Both simultaneous and sequential polymerization afforded soluble polymers, thereby indicating poly(lipid) vesicles were not cross-linked. The possibility that some of the polymers obtained were highly branched is difficult to exclude. However, the formation of branched polymers in organized media, i.e. smectic or lamellar phases, will lead to a cross-linked network at low to moderate conversion. In our experiments all of the polymerizable functionalities were converted to polymer in excess of 70% conversion, a much larger conversion than is consistent with branching without cross-linking in the ordered liquid crystalline phase of the bilayer. These considerations suggest that the polymers formed in the bilayer vesicles are predominantly ladder-like in nature. In this context, the term ladder-like means

(29) Kölchens, S.; Lamparski, H.; O'Brien, D. F. *Macromolecules* **1993**, *26*, 398−400.
(30) Anikin, A.; Chupin, V.; Anikin, M.; Serebrennikova, G.; Tarahovsky, J. *Mackromol. Chem.* **1989**, *194*, 2663−2673.
(31) Takeoka, S.; Kimura, N.; Ohno, H.; Tsuchida, E. *Polym. J.* **1990**, *22*, 867−874.

that the polymers are formed by a correlated polymerization of the dienoyl and diene groups, i.e., both goups in one lipid molecule preferentially react with the dienoyl and diene groups, respectively, in the same neighbor lipid. Note that since the degree of the polymerization of the poly(diene) and poly(dienoyl) was not the same (the two polymer chains were not identical in length) the polymers obtained from lipid **1** were not true ladder polymers. An alternative interpretation that their reactions occurred in nonoverlapping domains of the bilayer can be excluded by the high conversion to polymer.

Many ladder polymers exhibit resticted rotation along the polymer backbone, and some of these structures can form liquid crystalline phases. The ladder-like polymers formed by polymerization of bilayers of lipid **1** are distinctive because of the relatively long aliphatic link between the two polymer chains. These results suggest that properly designed amphiphilic monomers could be used to prepare novel polymer architectures, such as copolymers where the two components are essentially parallel blocks. Lipid **1** yields two poly(1,4-diene)s. In a similar manner, it should be possible to form parallel polymers of poly(diene) and poly(acrylate), among others. The reactivity of diene radicals is quite different from that of less stabilized monomers. Although a large difference in radical reactivity makes it quite difficult to form certain conventional copolymers, the formation of parallel polymer blocks should effectively avoid the relative reactivity complications inherent in radical copolymerizations.

The preferred ladder-like mode of polymerization of lipid **1** is attributed at least in part to the relatively short spacer link between two reactive moieties, i.e., six methylenes. At some separation distance, the congruence of the preferred reactivity of the two groups will diminish to the point that the polymerization of both groups will be uncorrelated, resulting in the formation of cross-linked polymers instead of ladder-like polymers. The seminal studies of Stupp and co-workers revealed that acrylate and cyano groups separated by 13 atoms in rodlike mesogens react independently to yield cross-linked two-dimensional polymers.[1,2] The preferred reaction path for homologues of **1** may depend on several factors, including the length and nature of the spacer group, the extent of conformational and diffusional freedom within the molecular assembly, and the reactivity of the polymerizable groups. We have already obtained preliminary evidence that if the spacer length is 13 atoms in a lipid similar to **1**, then polymerization yields cross-linked vesicles. These studies will be reported in due course.

Summary

The polymerization of the heterobifunctional lipid **1** that contains polymerizable diene and dienoyl groups in the *sn*-2 acyl chain was dependent on the mode of initiation, the polymerizable group, and the position of the reactive group in the amphiphiles. Moreover, since the two reactive groups of lipid **1** are in regions of different polarity, it was readily possible to perform either selective or simultaneous polymerizations by appropriate choices of initiation chemistry. The selective polymerization of either group could be used in a sequential manner. Both reactive groups were simultaneously polymerized via either redox polymerization or direct photoirradiation. The polymerizations of both diene and dienoyl groups proceeded through the 1,4-addition mechanism regardless of the mode and order of the polymerization. The polymerized vesicles obtained from either simultaneous or sequential polymerization were not cross-linked, as they were disrupted by Triton X-100, and the dried polymers were soluble in organic solvents. At the high conversions used in this study the majority of the lipid monomers preferentially react with the same lipid neighbor in a linear ladder-like fashion.

Experimental Section

Instrumental Methods. ^{1}H NMR spectra were recorded on a Bruker AM-250 magnetic resonance spectrometer in chloroform-*d* with TMS as an internal reference. Compounds containing the UV-sensitive groups were handled under yellow light. The reactions were monitored by TLC visualized by a UV lamp. FAB-mass spectrometry was performed at the Nebraska Center of Mass Spectrometer, Nebraska. A Microcal Inc., model MC-2 differential scanning calorimeter was used for the thermotropic phase study. UV−vis absorption spectra were recorded on a Varian DMS 200 UV−vis spectrometer. SEC chromatograms were obtained with a Waters Maxima 820 chromatography workstation equipped with a Waters Model R401 differential refractometer detector calibrated with poly(methacrylate). The data were analyzed with Maxima 820 version 3.31 software. Quasielastic light scattering (QELS) was performed with a BI-8000-autocorrelator from Brookhaven Instruments, and particle sizes were calculated with the accompanying software.

Solvents and Reagents. (*E*,*E*)-8,10-Dodecadien-1-ol, pyridinium dichromate, dicyclohexylcarbodiimide (DCC), and 4-(dimethylamino)-pyridine (DMAP) were obtained from Aldrich Chemical Co. Azobis(isobutyronitrile) (AIBN) was recrystallized three times from methanol. Trimethyl-4-phosphonocrotonate was purchased from Lancaster Synthesis Inc. 1-Palmitoyl-2-hydroxy-*sn*-3-phosphatidylcholine was ordered from Avanti Lipids Co. Phospholipase A_2 from rattle snake venom (*Crotalus Adamanteus)* was obtained from Sigma Chemical Co. as a lyophilized powder. THF was distilled from sodium benzophenone ketyl. Chloroform and CH_2Cl_2 were distilled from CaH_2 under Ar(g).

Synthesis. (a) (*E*,*E*)-8,10-Dodecadienal (4). Pyridinium dichloromate (9 g, 24 mmol) was added slowly to the solution of (*E*,*E*)-8,10-dodecadien-1-ol (3 g, 16 mmol) in CH_2Cl_2 under Ar(g). The reaction was stirred overnight, and followed by TLC with hexane/EtOAc (97/3) as the mobile phase. When the reaction was finished, the TLC spot from the alcohol at R_f 0.15 had disappeared. The mixture was filtered through silica gel until the filtrate was colorless. The filtrate was concentrated, and the crude product was purified by column chromatography with hexane/EtOAc (97/3) to afford the aldehyde **4** as colorless oil in 65% yield. ^{1}H NMR ($CDCl_3$): 9.77−9.75 (t, J = 1.85 Hz, 1H), 6.03−5.97 (m, 2H), 5.59−5.53 (m, 2H), 2.45−2.39 (dt, J = 7.32, 1.82 Hz, 2H), 2.06−2.03 (m, 2H), 1.74−1.72 (d, J = 6.32 Hz, 3H), 1.65−1.60 (m, 2H), 1.33 (b, 6H) ppm.

(b) Methyl 2,4,12,14-Tetraenehexadecanoate (3). Hexane (30 mL) was added to a flask containing 0.6 g (15 mmol) of NaH (60% in mineral oil) under argon. The mixture was stirred for 5 min and the hexane was removed under vacuum. Dry THF (100 mL) was transferred into the flask under argon and the solution of trimethyl-4-phosphonocrotonate (3 g, 15 mmol) in 200 mL of THF was then added dropwise at 0 °C. After 1 h, the solution of aldehyde **4** (2.0 g, 11 mmol) in 200 mL of THF was added slowly at 0 °C. The reaction was allowed to warm to room temperature and monitored by TLC with hexane/EtOAc (97/3) as the mobile phase. After the reaction was complete, excess NaH was quenched by slow addition of cold water to the reaction. The THF was evaporated and the residue was diluted with diethyl ether, then extracted with water and brine. The organic layer was dried with anhydrous $MgSO_4$ and concentrated. The product was purified by column chromatography with hexane/EtOAc (97/3) to give the methyl ester **3** in 70% yield. The ratio of (*E*,*E*)-2,4-dienoyl ester to its (*E*,*Z*)-isomer was determined by ^{1}H NMR to be 4. ^{1}H NMR ($CDCl_3$): 7.66−7.56 (dd, J = 15.36, 10.69 Hz, (*E*,*Z*)-isomer, 1H) and 7.32−7.21 (m, (*E*,*E*)-isomer, 1H), 6.17−5.51 (m, 7H), 3.75 (s, (*E*,*Z*)-isomer, 3H) and 3.74 (s, (*E*,*E*)-isomer, 3H), 2.31−2.15 (m, 2H), 2.05−2.03 (m, 2H), 1.74−1.71 (d, J = 6.39 Hz, 3H), 1.39−1.31 (b, 8H) ppm.

(c) 2,4,12,14-Tetraenehexadecanoic Acid (2). A methanolic solution of methyl ester **3** (2.0 g, 7.6 mmol in 100 mL) was treated with 1.5 mol equiv of an 85% aqueous solution of KOH. The mixture was refluxed gently until the reaction was finished (about 5 h) as determined

by TLC with hexane/ethyl acetate (97/3) as the mobile phase. The methanolic solution was concentrated and then diluted with ether. After the solution was acidified to pH 3 with dilute HCl solution, it was extracted several times with water. The organic layer was dried with anhydrous $MgSO_4$ and then concentrated, affording the crude carboxylic acid.

(d) (*E*,*E*,*E*,*E*)-2,4,12,14-Tetraenehexadecanoic Acid (2a). A well-stirred solution of urea (6.5 g, 108 mmol) in methanol (100 mL) was treated with a solution of acid **2** (2.0 g, 8.0 mmol) in methanol (100 mL). The solution was then kept at 0 °C overnight. The needle crystals were filtered, washed many times with methanol, and then dried under vacuum. These crystals were dissolved in ether and washed several times with dilute HCl solution and water. The organic layer was combined and dried with anhydrous $MgSO_4$. After concentration, the crude acid was purified by recrystallization from hexane at −30 °C, giving the acid **2a** as colorless needles in 80% yield. ^{1}H NMR ($CDCl_3$): 7.34−7.25 (m, 1H), 6.19−6.16 (m, 2H), 6.01−5.91 (m, 2H), 5.80−5.74 (d, J = 15.33 Hz, 1H), 5.59−5.49 (m, 2H), 2.17−2.01 (m, 4H), 1.72−1.70 (d, J = 6.55 Hz, 3H), 1.38−1.29 (b, 8H) ppm.

(e) 1-Palmitoyl-2-(2,4,12,14-tetraenehexadecanoyl)-*sn*-phosphatidylcholine (1). Chloroform (20 mL) was added under Ar(g) to a flask containing 1-palmitoyl-2-hydroxy-*sn*-phosphatidylcholine (0.56 g, 1.1 mmol), acid **2a** (0.23 g, 0.95 mmol), and DMAP (0.12 g, 0.95 mmol). DCC (0.20 g, 0.95 mmol) in 10 mL chloroform was added slowly to the suspension. The reaction mixture was kept in the dark for 2 days, and followed occasionally by TLC with chloroform/MeOH/water (65/25/4) as the mobile phase. The urea side product was filtered and the filtrate concentrated. The crude product was purified by column chromatography with chloroform/MeOH/water (65/25/2), to give a 70% yield of lipid.

The lipid was further purified by enzymatic hydrolysis of any positional isomer of lipid **1**. Tris buffer (pH 9, 0.017M) containing $CaCl_2$ (83 μM) was prepared and 5 mL of this solution was added to the lipid mixture (30 mg, 36 μmol). Phospholipase A_2 from crude rattle snake venom (0.13 mg) in borate buffer solution (13 μL) was then added, and the reaction mixture was sonicated for 3 min. After addition of ether (10 mL), the mixture was vigorously shaken at 37 °C for 3 h. The reaction was quenched by addition of water, and the organic layer containing the hydrolyzed fatty acid and unreacted lipid **1** was separated. The aqueous layer was washed several times with ether. The organic layers were combined, dried with anhydrous $MgSO_4$, and concentrated. Lipid **1** was separated from any hydrolysis products by using column chromatography and obtained as white solid in 95% yield based on the starting lipid. ^{1}H NMR ($CDCl_3$): 7.28−7.19 (m, 1H), 6.16−6.14 (m, 2H), 6.02−5.97 (m, 2H), 5.79−5.73 (d, J = 15.28 Hz, 1H), 5.60−5.50 (m, 2H), 5.25 (b, 1H), 4.39−4.20 (b, 4H), 3.97−3.96 (b, 2H), 3.78 (m, 2H), 3.34 (s, 9H), 2.30−2.24 (m, 2H), 2.17 (b, 2H), 2.05−2.01 (b, 2H), 1.74−1.71 (d, J = 6.49 Hz, 3H), 1.56−1.25 (b, 34H), 0.90−0.85 (t, J = 4.89 Hz, 3H) ppm. MS: Calcd MW for $C_{42}H_{78}O_8$-PN 755.5. Found: *m*/*z* 755.3.

Calorimetry. Lipid **1** was freeze-dried overnight, then hydrated with deionized water to a concentration of 7 mM. Hydration of the lipid was done by heating to 45 °C, vortexing, and then cooling to −78 °C several times. The fully hydrated lipid bilayers were then transferred to a DSC cell. The phase transition temperature was measured from the point of maximum excess heat capacity. The calorimetric enthalpy was calculated from the peak area and concentration of the lipid with the aid of Microcal Corp. software.

Polymerization of Lipid 1 Vesicles. Simultaneous Polymerization. (1) Redox. Approximately 5 mg of lipid **1** was evaporated from a stock solution (10 mg/mL in benzene) by freeze-drying. The lipid was then hydrated with deoxygenated MilliQ water at a concentration of 80 μM through several freeze−thaw−vortex cycles. Large unilamellar lipid vesicles (LUV) with a diameter of ca. 100 nm were prepared by extrusion 10 times through two stacked 100 nm pore size polycarbonate filters. A solution of $K_2S_2O_8$/L-cysteine (10/1) was prepared and an aliquot was added to the hydrated lipid to give the [M]/[I] ratio of 2. Polymerization was performed at 40 °C under positive argon pressure. The UV absorption of the sample was taken at different time intervals, and the percent conversion was calculated from the decrease of the diene and dienoyl absorption peaks with the aid of the Spectral Calc Arithmetric program to minimize the effect of peak overlap.

(2) Direct Irradiation. UV irradiation of 100 nm LUV of lipid **1** was carried out in a quartz cuvette thermostated at 40 °C, using a low-pressure Hg vapor lamp. The distance between the sample and the light source was 1 cm.

(b) Selective Polymerization. (1) Filtered UV Irradiation. LUV of **1** were irradiated in a Pyrex tube thermostated at 40 °C, placed 10 cm from a high-pressure Hg/Xe light source. A Corning CS-056 cut-off filter (300 nm) was located between the sample and light source.

(2) AIBN Radical Polymerization. An AIBN solution (1−1.5 mg/mL in benzene) was prepared. An aliquot was added to the dry lipid **1** to give the [M]/[I] ratio of 5, and benzene was removed under vacuum. The sample was then hydrated with deoxygenated MilliQ water at a concentration of 80 μM through several freeze−thaw−vortex cycles. LUV were prepared through extrusion as described before. The sample was then placed in an ampule sealed with a septum and flushed with argon for 0.5 h. Polymerization was performed at 60 °C in the absence of light.

(c) Molecular Weight Determination. Samples of poly-**1** were transesterified and the resulting polymers were compared to poly(methyl methacrylate) standards by using an Ultrastyragel linear column as described previously.[8]

(d) Polymerized Vesicle Stability. LUV of lipid **1** were prepared as described before. The LUV sizes were determined by quasielastic light scattering. The light-scattering intensity was measured at 90° to the incident light, and each value reported was the average of at least three measurements. After polymerization of the LUV by one of the methods described above, aliquots of Triton X-100 were added to the polymerized vesicles in two equivalent increments. The particle sizes of the polymerized LUV after Triton X-100 treatment were then redetermined.

Acknowledgment. Financial support of this research by the National Science Foundation is appreciated.

JA973371X