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# Polymerization of Supramolecular Assemblies: Comparison of Lamellar and Inverted Hexagonal Phases

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**Abstract:** Since the first reports of the polymerization of hydrated bilayers in the early 1980s, a wide variety of polymerizable groups and lipids has been successfully employed. Among the various strategies explored for the polymerization of lamellar phases, a particularly useful method relies on the design of suitable polymerizable amphiphiles, which upon hydration form assemblies that can then be polymerized with retention of structure. We have recently extended this strategy to successfully polymerize the inverted hexagonal ($H_{II}$) phase. This report is the first comparison of radical chain polymerizations in lamellar and $H_{II}$ phases. The number average degree of polymerization of polymers obtained in both lamellar and $H_{II}$ phases depended strongly on the initiation chemistry, but were insensitive to the lipid phases. The immediate benefit of these studies is the knowledge that polymer size can be varied extensively in both phases in order to obtain different materials properties. © 1997 Elsevier Science Ltd.

The hydration of polar natural and synthetic lipids yields a variety of lipid phases depending on the concentration, temperature, and pressure. These include lamellar phases (bilayers), the inverted hexagonal ($H_{II}$) phase, and various inverted cubic ($Q_{II}$) phases.[1-3] The tendency of hydrated lipids to form lamellar or nonlamellar phases is a consequence of forces across a lipid layer.[4] When the repulsive lateral pressure in the lipid hydrophobic region is greater than the repulsive forces acting at the hyrophilic head group, a lipid layer assumes a negative curvature. Lipids with cis-double bonds or branching substitutents in the tails, and relatively small, poorly hydrated head groups such as phosphoethanolamines form the $H_{II}$ phase at moderate to low temperatures.[5] On the other hand, lamellar phases are more likely with phosphocholines that have larger, well-hydrated head groups.[6] The $H_{II}$ phase can be considered as aqueous columns encased within a monolayer of lipid arranged in a hexagonal pattern. The polar head groups are well-ordered at the water interface, whereas the lipid tails are disordered to fill the volume between the columns of water.

Since the first reports of the polymerization of hydrated bilayers in the early 1980s, a wide variety of polymerizable groups and lipids has been successfully employed (see reviews).[7-10] Polymerized ensembles of molecules are likely to find utility in diagnostics, in the controlled delivery of reagents and drugs, in the design and preparation of stable assemblies for the efficient transduction of light energy, in surface modification, and in the preparation and stabilization of organic analogs of zeolites. Among the various strategies explored for the

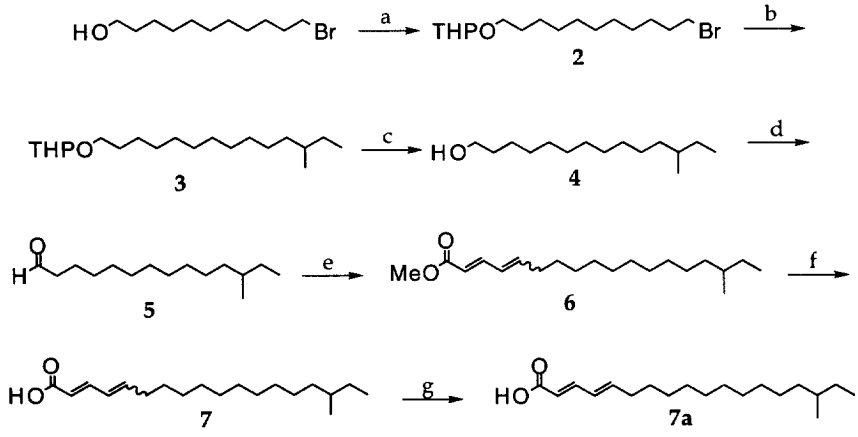
polymerization of lamellar phases, a particularly useful method relies on the design of suitable polymerizable amphiphiles, which upon hydration form assemblies that can then be polymerized with retention of structure. Recently we extended this strategy for the successful polymerization of both $Q_{II}$ and $H_{II}$ phases.[11,12] In addition we probed the nature of the radical chain polymerization process in the lamellar phase.[13-16] Here we report the first comparison of the radical chain polymerizations in lamellar and $H_{II}$ phases by analysis of the rate and degree of polymerization for the same reactive lipid in each phase.

**Results and Discussion**

A phosphoethanolamine that forms lamellar and $H_{II}$ phases at temperatures convenient for radical chain polymerization of the lipid assemblies was designed and synthesized for this study. 1-Oleoyl-2-[16-methyl-(E,E)-2-4-octadecadienoyl]-sn-glycero-3-phosphoethanolamine (**1**) was selected because it has 1) a branched methyl group at the acyl chain end to favor the formation of nonlamellar phases at reasonable temperatures, 2) a polymerizable dienoyl group located near the glycerol backbone of lipid in order to minimize the effect of the polymer chain on the lipid chain packing in the $H_{II}$ phase, and 3) a single polymerizable group per lipid thereby producing linear polymers to facilitate polymer size characterization.

*Synthesis*

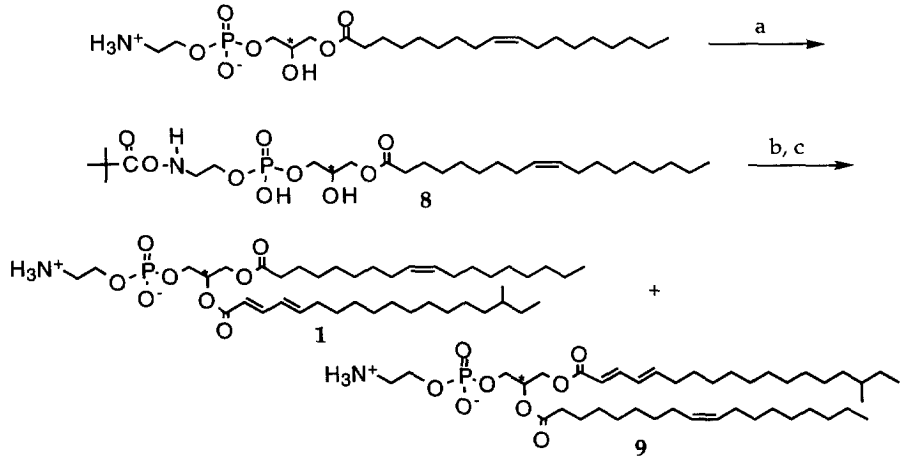
Lipid **1** was obtained from the acylation of N-protected-1-oleoyl-sn-3-phosphatidylethanolamine **8** with 16-methyl-(E,E)-2,4-octadienoic acid **7a**. The dienoic fatty acid was prepared from commercially available 11-bromo-1-undecanol.




a) 2,3-dihydro-4-pyran, p-TsOH.$H_2O$; b) sec-butylmagnesium chloride, $CuLi_2Cl_4$;
c) p-TsOH.$H_2O$, MeOH; d) PDC, $CH_2Cl_2$; e) NaH, trimethylphosphonocrotonate;
f) KOH, MeOH; g) urea inclusion

After protection of the alcohol with 2,3-dihydro-4-pyran, the bromide was coupled with sec-butylmagnesium chloride in the presence of $CuLi_2Cl_4$ catalyst,[17] to yield the THP protected 12-methyltetradecanol **3**. Deprotection afforded the free alcohol **4**, which was oxidized to the corresponding aldehyde **5** using pyridinium dichromate in dichoromethane.[18] The Wittig-Horner reaction of this aldehyde and trimethyl 4-phosphonocrotonate gave methyl 16-methyl-2,4-octadecadienoate **6** as a mixture of (E,E)- and (E,Z)-isomers.[19] $^1H$ NMR of this product mixture indicated that it was composed of ca. 80% (E,E)-isomer and 20% (E,Z)-isomer. The doublet of doublet resonances with coupling constants 15.34 and 11.40 Hz at 7.55-7.68 ppm is characteristic of the vinyl proton of (E,Z)-isomer, while the vinyl proton of (E,E)-isomer shows coupling constants 15.39 and 9.97 Hz for the doublet of doublets at 7.29-7.40 ppm. The hydrolysis of the methyl ester was catalyzed by 1.5 molar equivalent of potassium hydroxide in methanol at reflux for 4-5 h to afford the dienoyl fatty acid **7**.[20] Urea inclusion complexation was utilized to separate the (E,E)-dienoic acid **7a** from its (E,Z)-isomer.[21] Urea molecules in methanol orient in a helical lattice leaving a narrow cylindrical channel with the diameter of 5.3 Å,[22] that can only accomodate the more linear (E,E)-isomer. Upon filtration and then extraction of the urea-fatty acid inclusion complex with ether (E,E)-dienoic acid was predominantly obtained judging from the absence of the characteristic vinyl proton resonance of (E,Z)-isomer at 7.55-7.68 ppm in the $^1H$ NMR.

t-Butyloxycarbonyl anhydride (Boc) was employed to protect the amino group on 1-oleoyl-sn-3-phosphoethanolamine (lysoPE), which was then acylated with 16-methyl-(E,E)-2,4-octadienoic acid **7a** in the presence of 4-(dimethylamino)pyridine (DMAP) and dicyclohexylcarbodiimide (DCC). The use of excess lysoPE relative to fatty acid afforded the acylated product in high yield.[12,23] The removal of the Boc-protecting group was accomplished with 10% sulfuric acid in dioxane.




a) t-$Boc_2O$, $Et_3N$; b) DCC, DMAP, $CHCl_3$; c) $H_2SO_4$, dioxane

The isomerization of 1-oleoyl-sn-3-phosphoethanolamine to the corresponding 2-oleoyl-sn-3-phosphoethanolamine is known to take place during base catalyzed acylation.[24] The final products obtained were therefore a mixture of the desired 1-oleoyl-2-dienoyl-sn-phosphoethanolamine (**1**) and its positional isomer, 1-dienoyl-2-oleoyl-sn-phosphoethanolamine (**9**). Lipid **1** was separated from isomeric **9** using enzymatic hydrolysis. Phospholipase $A_2$ selectively catalyzes the hydrolysis of the sn-2-acyl ester bond in glycerolphospholipids.[25,26] The rate of this enzymatic hydrolysis depends strongly on the nature of the ester acyl chain. The enzymatic hydrolysis of the glycerophospholipid containing saturated ester took place completely within 3 hours at 37 °C, whereas that containing the dienoyl group proceeded at much slower rate. The slow nature of the enzymatic hydrolysis of dienoyl phospholipids have been investigated by measuring the amount of the fatty acid generated,[27] by following the enzymatic destabilization of lipsomes via the release of entrapped dyes,[28] and by changes in the the pressure-area isotherm of LB films of dienoyl amphiphiles.[7] The flexibility of the acyl chain is known as one of the requirements for the efficient hydrolysis of phospholipid molecules by phospholipase $A_2$,[29] and the rigidity of the conjugated double bond next to the ester linkage on the dienoyl group appears to retard the hydrolysis. Upon treating the mixture of **1** and **9** with enzyme phospholipase $A_2$ for 3 hours, the undesired lipid **9** was hydrolyzed to afford lysoPE (**10**) and oleoic acid, which were readily separated from the desired lipid **1** (90% yield) by flash column chromatography. The enzymatic hydrolysis of lipid **1** over 3 h results in only a limited loss if any of lipid **1**. The purity of isolated lipid **1** was confirmed by FAB-MS, $^1$H NMR, and elemental analysis.

***Phase Behavior of Lipid 1***

The phase behavior of lipid **1** was investigated at low concentration (6 mg/ml) by differential scanning calorimetry (DSC), and at high concentration (100 mg/mL) using $^{31}$P NMR spectroscopy. The DSC of lipid over the temperature range of 5° to 70°C showed a thermotropic endotherm of at 55°C, with an enthalpy of 12 kcal/mol. The proton decoupled $^{31}$P-NMR spectra were obtained at a constant temperature of 45±0.2 °C during the first acquisition, then at 65±0.2 °C for the second acquisition. At 45°C, hydrated lipid **1** showed the characteristic line shape of lamellar phase with the width of 48 ppm.[30] The chemical shift anisotropy with the corresponding peak maximum at -10 ppm was indicative of a lamellar phase. The hydrated lipid at 65 °C showed a characteristic line shape of an inverted hexagonal assembly with the width of 26 ppm.[31] The observed line shape was consistent with a multidomain hexagonal phase which is expected for a sample at this concentration because the excess water edge effects distort the line shape slightly. The chemical shift anisotropy with the corresponding peak maximum at 8 ppm indicates an inverted hexagonal phase (Figure 1).

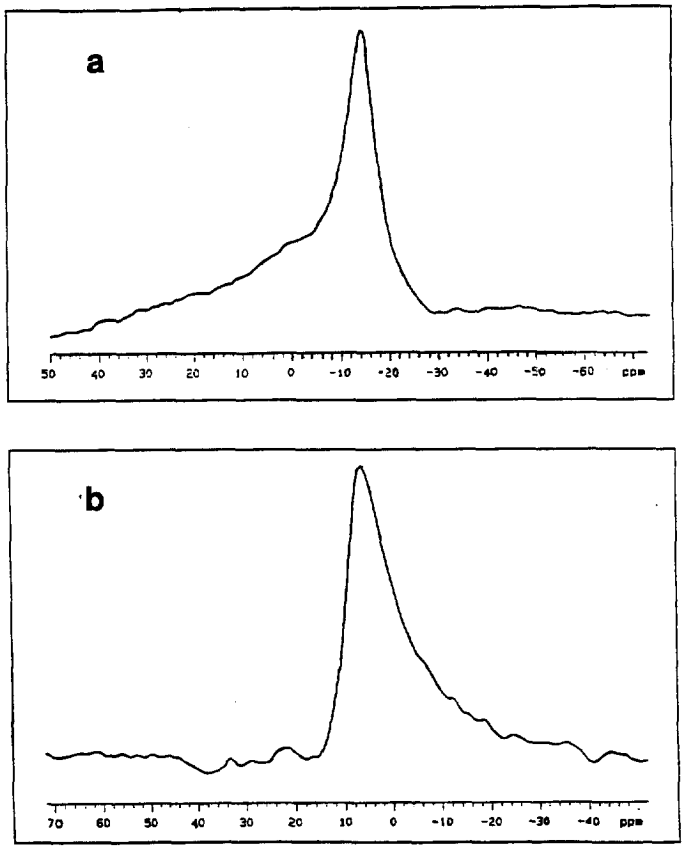
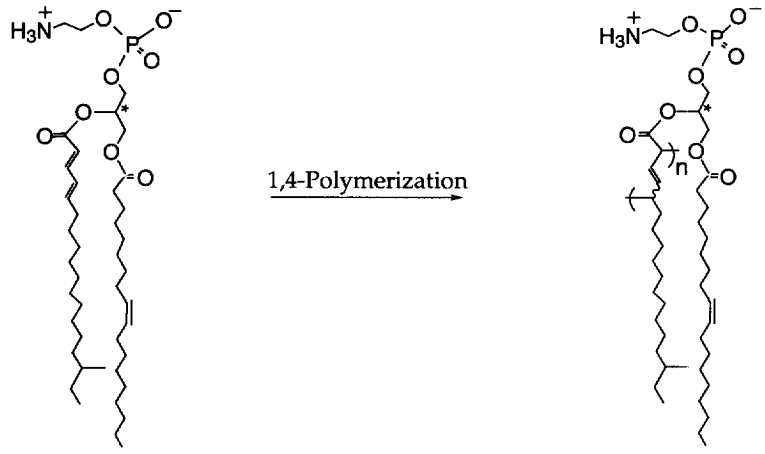



**Figure 1.** $^{31}P$ NMR spectra of hydrated lipid **1** at a) 45 °C, and b) 65 °C.

***Polymerization of Lipid 1 in Lamellar and $H_{II}$ Phases***

Azobis(2-amidinopropane)dihydrochloride (AAPD), hydrogen peroxide and redox initiators were used for the chain radical polymerization of the dienoyl group at the mole ratio of monomer to initiator or oxidant (in case of redox initiator) of 10/1. Hydrogen peroxide and redox initiators generate hydroxyl radicals which were free to diffuse into the lamellar and $H_{II}$ phases to react with the polymerizable dienoyl groups. The redox initiator systems were $KBrO_3$/L-cysteine, $K_2S_2O_8$/L-cysteine, and $K_2S_2O_8$/$NaHSO_3$. The polymerization of bilayers of a dienoyl phosphocholine similar to lipid **1** was reported to proceed through 1,4-addition giving a polymeric mixture of cis-and trans- isolated double bond at the 2,3-position.[32] The absorption peak of dienoyl group at $\lambda_{max}$ of 270 nm decreased as the polymerization proceeded. A control free radical polymerization with 1,2-dioleoyl-3-sn-phosphoethanolamine (DOPE) showed that the cis-double bond in each acyl chain did not participate in the polymerization process.[12]




1 poly-1

*Comparative Rates of Polymerization in the Lamellar and $H_{II}$ Phases*

Samples of lipid **1** in the lamellar phase were prepared as large unilamellar vesicles (LUV) with diameters of ca. 100 nm by the extrusion method at a concentration of 6 mg/ml in water. The polymerization was performed at 45 °C using various initiators, and the loss of monomer was followed with the aid of UV/Vis spectroscopy. The samples were taken at different time intervals, and the percent conversion was calculated from the decrease of the dienoyl absorption peak (Figure 2).

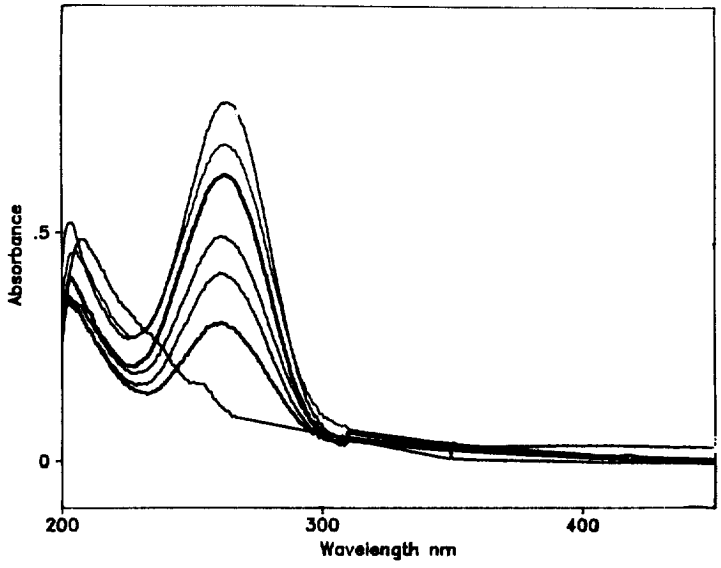



**Figure 2.** UV/Vis spectra at various times of polymerization of lipid **1** (6 mg/ml) in LUV at 45 °C. Initiator: $KBrO_3$/L-cysteine (1/1); [M]/[O] = 10/1.

The rate of polymerization of LUV of **1** was strongly dependent on the choice of initiators, and on the oxidant to reductant ratio in case of redox initiation (Figure 3). The rate decreased from $KBrO_3$/L-cysteine (1/1) to $H_2O_2$ to $KBrO_3$/L-cysteine (10/1). Both $KBrO_3$/L-cysteine, and $H_2O_2$ are thought to generate hydroxyl free radicals, but the activation energy for the decomposition of $H_2O_2$ is larger than that of equimolar $KBrO_3$/L-cysteine resulting in a lower rate of polymerization. The ratio of oxidant to reductant had a dramatic effect on the rate of polymerization. Decreasing the amount of reductant relative to the oxidant resulted in a slower generation of initiator radicals thereby slowing the polymerization.[33]

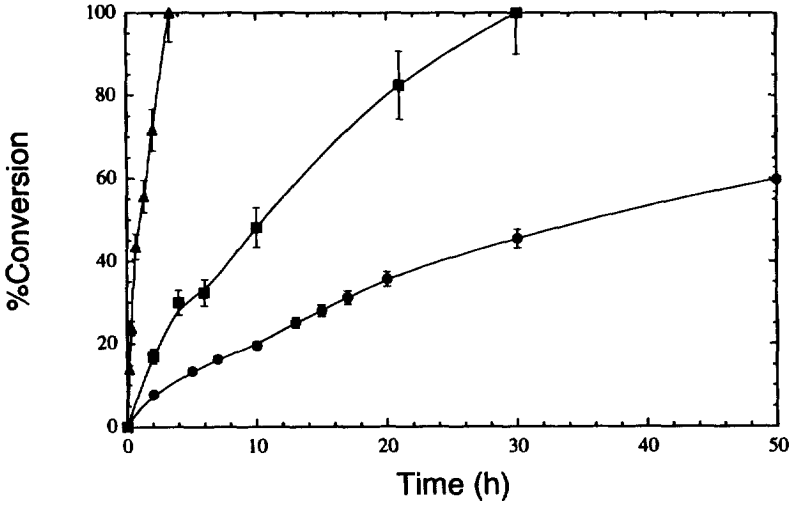



**Figure 3.** Polymerization of lipid **1** (6 mg/ml) in LUV at 45 °C: $KBrO_3$/L-cysteine (10/1), ●; $KBrO_3$/L-cysteine (1/1), ▲; $H_2O_2$, ■. [M]/[I] or [M/O] = 10/1.

Lipid **1** was hydrated (6 mg/ml) and incubated at 65 °C for 24 h to ensure the formation of the $H_{II}$ phase. The UV spectrum of the lipid showed no decrease in the absorbance of dienoyl peak, therefore no polymerization took place during the incubation period. The polymerization was then performed at 65 °C after the addition of the selected initiators. After an appropriate time, the polymerization of a sample was quenched and the sample freeze-dried. The conversion to polymer was determined by methanol extraction of a known weight of freeze-dried lipid, followed by determination of the absorbance of the dienoyl group.

Hydrogen peroxide and $KBrO_3$/L-cysteine redox initiator were employed for the radical polymerization of lipid **1** in lamellar and $H_{II}$ phases at the mole ratio of monomer/initiator of 10/1, and monomer/oxidant/reductant of 10/1/0.1, respectively. The polymerization of lipid **1** proceeded at about the same rate in both phases (Figure 4). The comparable rate of the polymerization of lipid **1** in both phases suggests that the polymerizable lipids have similar

rates of lateral diffusion in both. This supposition agrees well with earlier reports, that show that the diffusion of lipids is about four times faster in the $H_{II}$ than in lamellar phases.[3,34,35]

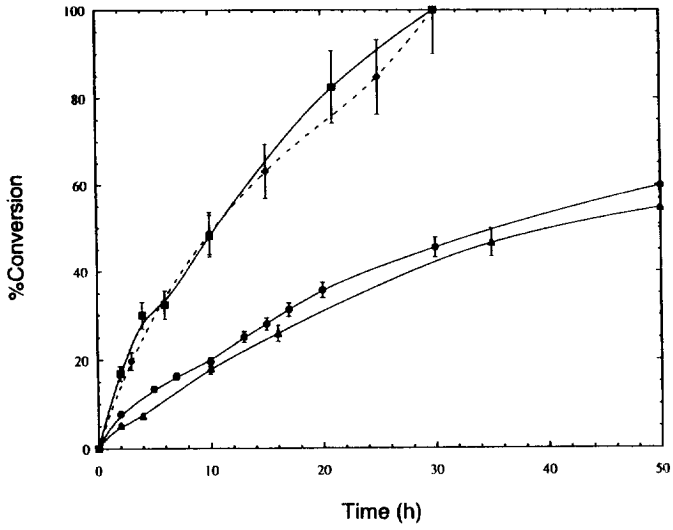
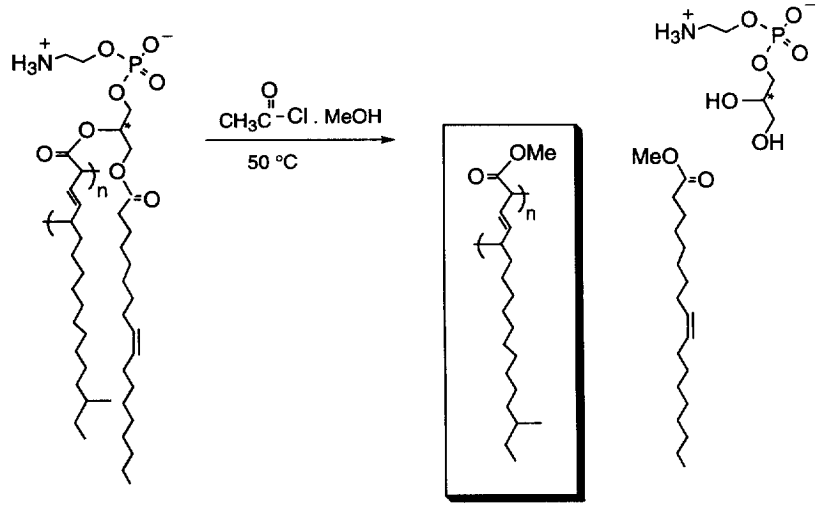



**Figure 4.** Polymerization of lipid **1** (6 mg/ml) in two different phases with $KBrO_3$/L-cysteine (10/1): lamellar, ● ; and $H_{II}$, ▲ ; and with $H_2O_2$: lamellar, ■ ; $H_{II}$, ◆ . [M/O] = 10/1.

*Degree of Polymerization of Poly-1*

Lipid **1** was polymerized using selected initiators and conditions in either lamellar or $H_{II}$ phases to high conversion (> 95%), prior to determination of the polymer molecular weight. Since poly-**1** contained polar zwitterionic head groups in each repeat unit, they were therefore modified to remove the head group by transesterification with methanolic HCl solution.[15]



Acetolysis using MeOH-HCl removed the phosphoethanolamine moiety while retaining the polymer acyl chain structure. The modified polymers were soluble in THF and/or dichloromethane, and their molecular weights were determined by size exclusion chromatography relative to poly(methyl methacrylate) standards.

The relative number average degree of polymerization ($X_n$) of polymers obtained from lipid **1** in both lamellar and $H_{II}$ phases depended strongly on the initiation chemistry, but were insensitive to the lipid phases (Table 1). When $H_2O_2$ or the redox initiators with an equimolar ratio of oxidant to reductant were employed, oligomers of lipid **1** were obtained in both phases. However large polymers of lipid **1** with number-average molecular weights ($M_n$) of $2x10^5$ to $3x10^5$ and degrees of polymerization ($X_n$) of at least 200 were formed in both phases when redox initiators with 10 molar equivalent excess of oxidant to reductant were used. The nature of reductant and oxidant also affected the observed polymer size.[36] The degree of polymerization in both phases increased in the following order: $KBrO_3$/L-cysteine < $K_2S_2O_8$/L-cysteine < $K_2S_2O_8$/$NaHSO_3$. The polydispersity of poly-**1** obtained from the lamellar and $H_{II}$ phases was 1.5±0.3 and 1.4±0.3, respectively.

**Table 1.** Number average degree of polymerization ($X_n$) of transesterified poly-**1**.

| Initiator | Degree of polymerization ($X_n$) | |
|---|---|---|
| | Lamellar | $H_{II}$ |
| $H_2O_2$ | 10 ± 3 | 10 ± 5 |
| $BrO_3^-$ / L-cysteine (10/1) | 200 ± 20 | 200 ± 30 |
| $S_2O_8^{2-}$ / L-cysteine (10/1) | 225 ± 35 | 237 ± 20 |
| $S_2O_8^{2-}$ / L-cysteine (1/1) | 10 ± 2 | 10 ± 3 |
| $S_2O_8^{2-}$ / $NaHSO_3$ (10/1) | 250 ± 30 | 293 ± 40 |

The results on $M_n$ and $X_n$ of the polymers correlated well with the rate of the polymerization. When the rate of the free radical polymerization of lipid **1** in both lamellar and $H_{II}$ phases were similar, the $X_n$ of the polymers were comparable in both phases. For redox initiation, the polymerization proceeded at much slower rates when excess oxidant was used, resulting in larger polymers compared to that obtained with equimolar oxidant and reductant. Each redox initiator pair possess a different activation energy ($E_a$) for the generation of radicals, i.e. $KBrO_3$/L-cysteine, 9.8 kcal/mol for $K_2SO_8$/L-cysteine, and 12.5 kcal/mol for

$K_2SO_8/NaHSO_3$.[32] The rate of polymerization decreased and the degree of polymerization increased as the initiators were changed in this order.

Besides the redox initiators, AAPD was employed for the polymerizations at 65°C of lipid **1** in the $H_{II}$ phase. It forms the hydrophilic aminidopropane radical which has been shown to effectively initiate the polymerization of a dienoyl group located in the sn-2 chain of phosphocholines in bilayers.[27] Our experiments showed that it was an effective initiator for the $H_{II}$ phase, yielding poly-**1** of a number average molecular weight of $3.7x10^4$ and a $X_n$ of 125. These experiments indicate that the dienoyl group in the lipid sn-2 chain is situated in a water accessible region of in both the lamellar and $H_{II}$ phases. Experiments in the lamellar phase at 45°C were largely ineffective because of the slow rate of thermal decomposition of AAPD at this temperature.

In this study, polymerizations of lipid **1** in lamellar and $H_{II}$ phases were performed at 45°C and 65°C, respectively. These temperatures were selected to span the lamellar to nonlamellar phase transition temperature, yet to minimize the temperature difference and the effect of this difference on the rates and degrees of polymerization. In order to assess the effect of temperature on the polymerization in a single phase we examined the polymerization of lipid **12** in the lamellar phase at both 45° and 65°C. 1-Palmitoyl-2-[(E,E)-2-4-hexadecadienoyl]-sn-glycero-3-phosphocholine (**12**) was selected as the control lipid to study the effect of temperature. It was prepared via the same methodology used for lipid **1**, i.e. (E,E)-2-4-hexadecadienoic acid was synthesized from dodecanal, and then used to acylate 1-palmitoyl-sn-3-phosphocholine (lysoPC). LUV of lipid **12** were prepared and polymerized at 45° and 65°C using $KBrO_3$/L-cysteine (10/1) and the same conditions described above for lipid **1** (Figure 5).

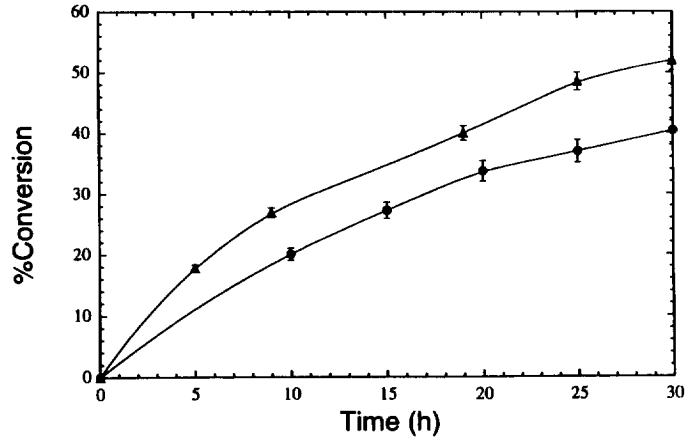



**Figure 5.** Polymerization of lipid **12** (6 mg/ml) in LUV with $KBrO_3$/L-cysteine (10/1) at 45 °C ●; and 65 °C ▲. [M]/[O] = 10/1.

The observed reaction rate was ca. 30% faster at 65°C than at 45 °C (Table 2). The effect of temperature on the polymer size was determined after lipid **12** was polymerized to 95% conversion, then transesterified as described for poly-**1**. The $X_n$ of poly-**12** was ca. 30% less at 65°C than at 45°C. The relative small effect of increased temperature on the rate and degree of polymerization in the lamellar phase is consistent with previous studies of redox polymerizations in solution.

**Table 2.** Rate and degree of polymerization of **12** in lamellar phase initiated by $KBrO_3$/L-cysteine (10/1) at 45 °C and 65 °C.

| Property | Temperature | |
|---|---|---|
| | 45 °C | 65 °C |
| Rate (L/mol sec) | $4.63 \times 10^{-8}$ | $6.11 \times 10^{-8}$ |
| Degree of Polymerization | 185 ± 15 | 125 ± 25 |

The observed temperature effect on the redox polymerization of lipid **12** in the lamellar phase shows the overall activation energy ($E_R$) for the rate of polymerization was 3 Kcal/mole. These values are obtained from the initial rate of reaction where lamellar polymerizations behave in a conventional mode, i.e. bimolecular termination.[14] In this case the $E_R$ is equal to $E_p + 1/2E_d - 1/2E_t$.[37] Since the value for the activation energy for decomposition ($E_d$) of $KBrO_3$/L-cysteine is ca. 9 Kcal/mole or less, then the difference between the activation energy for propagation ($E_p$) and termination ($E_t$) is small. The size of the polymers was determined after the polymerization reaction was run to high conversion where primary termination is the dominant mechanism.[13-15] The overall activation energy for the degree of polymerization of lipid **12** was -4 Kcal/mole, a reasonable value for polymerizations where $E_d$ is relatively small.

It is likely that a significant contribution to the propagation step is the diffusion of the monomeric lipid to the growing radical chain end. The experiments in the lamellar phase were performed in the fast diffusion regime, where lipids typically exhibit diffusion coefficients of ca. 1 $\mu m^2\ s^{-1}$.[38] A twenty degree increase in the sample temperature has been shown to increase the lipid lateral diffusion coefficient in the lamellar phase by a factor of 2.[39] This small change seems consistent with the moderate increase in rate and should be reflected in both the propagation ($E_p$) and termination ($E_t$) parameters. Reported comparisons of the motion of lipids lamellar and $H_{II}$ phases show that the lateral diffusion coefficient is ca. 4 times larger in the $H_{II}$ phase.[34,35] Thus the combined effect of temperature and phase change could result in an 8 fold increase in lipid lateral diffusion between a lamellar sample at 45°C and a $H_{II}$ phase at 65°C. If the increase in diffusion was reflected primarily in the propagation step, then the

overall rate of polymerization should increase. However rate of polymerization was similar in both phases. This indicates that either (1) the effect of increased diffusion is similar on both the propagation and termination steps; and/or (2) the change of phase from lamellar to hexagonal introduces another presently unidentified factor that moderates the polymerization process. For example the phase change could influence the permeation of the water phase redox generated radical into the lipid region of the $H_{II}$ phase.

Whatever the fundamental reasons for the similarity of rates and degrees of polymerization in the lamellar and $H_{II}$ phase, the immediate benefit of these studies is the knowledge that polymer size can be varied extensively in both phases. This enables one to create different materials properties in both lamellar and hexagonal assemblies. Furthermore the more comprehensive studies performed in the lamellar phase can be used as a reasonable starting point for the selection of polymerization conditions for the $H_{II}$ phase. It is important to note that large polymers can be formed in the unit cells of the $H_{II}$ phase. This coupled with our published observations of the crosslinking of the unit cells of the $H_{II}$ phase, opens the way for the preparation of stabilized nanotubes with well-defined diameters based on the $H_{II}$ unit cell.[12]

## Experimental

### *Solvents and Reagents*

Chloroform was distilled over calcium hydride prior to use. Benzene and tetrahydrofuran were distilled in the presence of sodium metal. 11-Bromo-1-undecanol (98%), 3,4-dihydro-2H-pyran (97%), p-toluenesulfonic acid monohydrate (99%), copper (II) chloride (99.99%), lithium chloride (99+%), pyridinium dichromate (98%), sodium hydride (60% dispersion in mineral oil), dicyclohexylcarbodiimide (99%), 4-dimethylaminopyridine (99+%), and 1,4-dioxane were purchased from Aldrich Chemical Co. (Milwaukee, WI). Triethyl 4-phosphonocrotonate (90%) was purchased from Lancaster Synthesis Inc. (Windham, NH). 1-Oleoyl-*sn*-glycero-3-phosphoethanolamine were purchased from Avanti Polar-Lipids, Inc. (Alabaster, AL) and the purity of these lipids was more than 99% as determined by HPLC and TLC. t-Butyloxycarbonyl anhydride (97%) was purchased from Bachem, Inc. (Torrance, CA). Phospholipase $A_2$ from rattle snake venom (*Crotalus Adamanteus)* was obtained from Sigma Chemical Company as a lyophilized powder.

### *Methods*

Compounds containing a UV-sensitive group were handled under yellow light. Thin layer chromatography was used to monitor each reaction and to check the purity of products. $^1H$ NMR spectra were obtained on a 250-MHz Bruker WM 250 spectrometer in chloroform-*d* with tetramethylsilane as an internal reference. Proton decoupled $^{31}P$ NMR spectra were acquired on a Varian-Unity 300 spectrometer operating at 121.4 MHz (7.0 T). The temperature was controlled to ±0.2 °C with the variable temperature controller. UV-visible absorption spectra were recorded on a Hewlett-Packard 8452A diode array spectrophotometer. Melting points were

not corrected. Elemental analysis data were obtained from the Desert Analytics, Tucson, AZ. The FAB mass spectra were obtained from the Nebraska Center for Mass Spectrometry.

***Syntheses***

*1-Tetrahydropyranyl-11-bromoundecane* (**2**). 11-Bromo-1-undecanol (17.0 g; 67.7 mmol) was dissolved in 100 ml of tetrahydrofuran, followed by addition of 3,4-dihydro-2H-pyran (8.5 g; 101 mmol) and p-toluenesulfonic acid monohydrate (80 mg). The solution was stirred for 4 h at rt. The reaction mixture was concentrated under reduced pressure, and the residue was purified by flash column chromatography (hexane/EtOAc, 95/5) to give 20.9 g of **2** as a viscous liquid (92% yield). $^1$H NMR : 4.59-4.58 (m, 1H, CH), 3.88-3.71 (m, 2H, $CH_2$), 3.52-3.36 (m, 4H, $CH_2$), 1.91-1.56 (m, 24H, $CH_2$).

*12-Methyl-1-tetradecanol* (**4**). Compound **2** (13.0 g; 38.8 mmol) was dissolved in 140 ml of freshly distilled tetrahydrofuran. The solution was cooled to 0 °C, followed by addition of sec-butylmagnesium chloride (25 ml, 2.0 M in diethyl ether) under nitrogen atmosphere. A $CuLi_2Cl_4$ solution (1.0 ml) was added dropwise to the above solution. The light brownish mixture was stirred for 3 h at 0 °C. If some of the starting bromide was still observed by TLC (hexane/EtOAc, 95/5), then additional sec-butylmagnesium bromide was used, and the reaction mixture was stirred for another 2 h. After flash column chromatography (hexane/EtOAc, 95/5) a viscous liquid **3** was obtained (11.0 g, 91% yield). $^1$H NMR : 4.59-4.56 (m, 1H, CH), 3.91-3.69 (m, 2H, $CH_2$), 3.54-3.33 (m, 2H, $CH_2$), 1.85-1.06 (m, 29H, CH and $CH_2$), 0.88-0.82 (m, 6H, $CH_3$). This liquid **3** (19.2 g; 61.5 mmol) was combined with p-toluenesulfonic acid monohydrate (0.6 g) in 300 ml of methanol and stirred overnight. The mixture was separated by flash column chromatograpy (hexane/EtOAc, 9/1, 8/2) to yield 11.3 g of the alcohol **4** (80% yield) as a colorless liquid. $^1$H NMR : 3.67-3.61 (t, J =6.59 Hz, 2H, $CH_2$), 1.57-1.26 (m, 23H, CH and $CH_2$), 0.88-0.82 (m, 6H, $CH_3$).

*12-Methyltetradecanal* (**5**). The alcohol **4** (3.3 g; 14.5 mmol) and pyridinium dichromate (8.2 g; 21.8 mmol) in 30 ml of dichloromethane was stirred at rt for 18 h. The reaction mixture was filtered through a silica gel column using hexane/EtOAc (80/20). The crude reaction mixture was separated by flash column chromatography (petroleum ether/EtOAc, 97/3) to yield 7.5 g of the aldehyde (60% yield). $^1$H NMR : 9.77-9.76 (d, J = 1.79 Hz, 1H, CHO), 2.46-2.39 (td, J = 7.35, 1.79 Hz, 2H, $CH_2$), 1.66-1.60 (m, 2H, $CH_2$), 1.29-1.09 (m, 19H, CH and $CH_2$), 0.88-0.82 (m, 6H, $CH_3$).

*Methyl 16-methyl-2,4-octadecadienoate* (**6**). Hexane (30 ml) was added into the flask containing 1.3 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 a solution of trimethyl 4-phosphonocrotonate (90% purity, 9.87 g; 35 mmol) in 200 ml THF was then added dropwise at 0 °C. After 1h, the solution of aldehyde **5** (6.7 g; 29 mmol) in 200 ml THF was added slowly at 0 °C. The reaction was allowed to warm up to rt, and

monitored by TLC using hexane/EtOAc (97/3) as the mobile phase. After the reaction was completed, excess NaH was killed by slow addition of cold water to the reaction. After evaporation of THF, the residue was diluted by diethyl ether and extracted with water and brine. The organic layer was dried with anhyd $MgSO_4$ and concentrated. The product was purified by column chromatography using hexane/EtOAc (97/3) to give methyl ester in 70% yield. The ratio of (E,E)- to its (E,Z)-isomer was determined to be 4 by $^1$H-NMR ($CDCl_3$) : 7.55-7.68 (m, 1H, CH=, (E,Z)-isomer), 7.29-7.40 (m, 1H, CH=, (E,E)-isomer), 6.16-6.18 (m, 2H, CH=), 5.74-5.80 (d, J = 15.28 Hz, 1H, CH=), 3.76 (s, $CH_3$), 2.14-2.19 (m, 2H, $CH_2$); 1.18-1.25 (m, 21H, CH and $CH_2$); 0.82-0.90 (t, J = 6.50 Hz, 6H, $CH_3$).

*Methyl 16-methyl-2-4-octadecadienoic acid* (**7**). A methanolic solution of methyl ester **6** (2.0 g, 7.6 mmol in 100 ml) was treated with 1.5 mol equivalent of 85% aqueous solution of KOH. The mixture was refluxed gently until the reaction was finished (about 5 h) as determined by TLC using 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 many times with water. The organic layer was dried with anhyd $MgSO_4$ and then concentrated, affording the crude dienoic acid.

*Methyl 16-methyl-(E,E)-2-4-octadecadienoic acid* (**7a**). A well-stirred solution of urea (1.39 g; 22.7 mmol) in methanol (100 ml) was treated with a solution of acid **7** (0.48 g; 1.6 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 anhyd $MgSO_4$. After concentration, the crude acid was purified by recrystallization from hexane at -30 °C, giving the (E,E) acid **7a** as colorless needle in 80% yield. M.P. 67-69 °C. The Rf value was 0.3 in hexane/EtOAc/formic acid (100/100/1), and 0.9 in toluene/pyridine/water (60/60/10) $^1$H NMR ($CDCl_3$): 7.40-7.29 (dd, J = 15.39, 9.97 Hz, 1H, CH=), 6.21-6.18 (m, 2H, CH=), 5.81-5.75 (d, J = 15.28 Hz, 1H, CH=), 2.22-2.14 (m, 2H, $CH_2$), 1.43-1.21 (m, 21H, CH and $CH_2$), 0.91-0.85 (t, J = 6.50 Hz, 6H, $CH_3$).

*1-Oleoyl-2-hydroxy-sn-glycero-3-(N-t-butyloxycarbonyl)phosphoethanolamine* (**8**). 1-Oleoyl-*sn*-glycero-3-phosphoethanolamine (1.0 g; 2.09 mmol) was dissolved in 50 ml of chloroform and cooled in an ice bath, then triethylamine (0.42 g; 4.2 mmol) was added, followed by dropwise addition of t-butyloxycarbonyl anhydride (0.55 g; 2.5 mmol) in 20 ml of chloroform. The reaction solution was stirred for 16 h at rt by which time the starting material was no longer observed by TLC. The solvent was evaporated under reduced pressure and the oily residue was separated by flash column chromatography ($CHCl_3$/MeOH/$H_2O$, 65/25/4) to obtain 0.96 g of the solid t-Boc-lysoPE (80% yield). $^1$H-NMR ($CDCl_3$) 0.88-0.93 (t, 3H, $CH_3$), 1.27-1.34 (m, 20H, $(CH_2)_4$ and $(CH_2)_6$), 1.43 (s, 9H, $(CH_3)_3CO$), 1.60-1.53 (m, 2H, $\underline{CH_2}CH_2CO_2$), 1.90-2.10 (m, 4H, $\underline{CH_2}$-CH=CH-$\underline{CH_2}$), 2.31-2.29 (t, 2H, $CH_2CO_2$), 3.08-3.05 (m, 1H, $OCH_2$-$\underline{CH}$(O)-$CH_2O$), 3.35-3.30 (m, 2H, $CH_2N$), 3.70-4.20 (m, 6H, $CH_2OPO_2$, O$\underline{CH_2}$-CH(O)-$\underline{CH_2}$O), 5.34-5.30 (m, 2H, CH=CH).

*1-Oleoyl-2-[17-methyl-(E,E)-2,4-octadecadienoyl]-sn-glycero-3-phosphoethanolamine* (**1**). A mixture of the t-Boc-lysoPE (**8**) (50 mg; 0.78 mmol), dienoic fatty acid **7a** (15 mg; 0.52 mmol), dicyclohexylcarbodiimide (160 mg; 0.78 mmol), and dimethylaminopyridine (98.5 mg; 0.78 mmol) in 20 ml of THF was stirred for 36 h at rt. The white precipitate was filtered, and the reaction mixture was separated by flash column chromatography ($CHCl_3$/MeOH, 9/1, 8/2). The crude product which still contained a trace amount of free dienoic fatty acid as indicated by TLC was dissolved in 10 ml of 1,4-dioxane, and then slowly added to 10 ml solution of 10% sulfuric acid in 1,4-dioxane. After 45 min, the reaction mixture was concentrated under reduced pressure. The solid residue was dissolved in 100 ml chloroform, which was washed with 100 ml water. After separation the aqueous phase was extracted with chloroform, and the combined chloroform solution was concentrated and separated by flash column chromatography ($CHCl_3$/MeOH, 8/2; $CHCl_3$/MeOH/$H_2O$, 65/25/2) to obtain 300 mg of the phosphoethanolamine (50% yield). The Rf value in $CHCl_3$/MeOH/water (65/25/4) was 0.7.

*Separation of lipid* **1** *from its positional isomer* **9** *via phospholipase* $A_2$ *hydrolysis.* 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 anhyd. $MgSO_4$, and concentrated. Lipid **1** was then separated from the hydrolyzed fatty acids using column chromatography, and obtained as white solid (27 mg). $^1H$ NMR ($CDCl_3$) 0.82-0.88 (m, 9H, $CH_3$), 1.27-1.34 (m, 41H, CH and $CH_2$), 1.52-1.60 (m, 2H, $CH_2$), 1.92-2.20 (m, 6H, $CH_2$), 2.35-2.28 (m, 4H, $CH_2$), 3.05-3.10 (m, 1H, CH), 3.38-3.40 (m, 2H, $CH_2$), 3.80-4.28 (m, 6H, $CH_2$), 5.30-5.34 (m, 2H, CH=), 5.72-5.80 (m, 1H, CH=), 6.10-6.23 (m, 2H, CH=), 7.35-7.20 (m, 1H, CH=). Mass spectrum; Calcd mol wt for $C_{42}H_{78}O_8PN$: 755.5, Found: m/z 755.3. Elemental analysis; Calcd for $C_{42}H_{78}O_8PN \cdot 1.5\ H_2O$: C, 64.42; H, 10.43; N, 1.79; P, 3.96; Found: C, 64.38; H, 10.31; N, 1.66; P, 3.38. Absorption in $CH_2Cl_2$: $\lambda_{max}$ 261 nm, ε 21,100 $M^{-1}cm^{-1}$.

***Phase Behavior of Lipid 1***

*DSC.* Lipid **1** was freeze-dried, and MilliQ water was added until the concentration of the lipid was 6 mg/ml. Hydration of the lipid was done by heating at 45°C, vortexing, and then cooling to -78°C several times. This fully hydrated lipid was then transferred to the DSC cell of a Microcal Inc. MC-2 differential scanning calorimeter. The phase transition temperature was measured from the point of the maximum excess heat capacity. The calorimetric enthalpy was calculated from the peak area and lipid concentration with the aid of Microcal Inc. software.

*$^{31}P$ NMR.* Lipid **1** was hydrated with MilliQ water at a concentration of 100 mg/ml and sealed in a 5 mm NMR tube. All spectra were referenced to phosphoric acid. The sample was

equilibarated at 45 °C for 4 h and at 65 °C for 1 h prior to aquisition. A phase cycled pulse sequence (90°-$t_1$-180°-$t_2$-acquisition) was used with a 90° pulse of 14 μs, the delay $t_1$ of 50 μs, the time before acquisition $t_2$ of 30 μs, and the delay between sequences of 0.5 s. The free induction decays ($10^4$) were multiplied by a line broadening of 100 Hz after Fourier transform.

***Polymerization Studies***

*Lamellar phase.* Freeze-dried lipid **1** (3 mg) was hydrated with deoxygenated MilliQ water at a concentration of 6 mg/ml 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 0.1 mm pore size polycarbonate filters. The solutions of hydrogen peroxide, oxidant, e.g. $K_2S_2O_8$ and $KBrO_3$, and reductant, e.g. L-cysteine and $NaHSO_3$, were prepared. Calculated amount of selected hydrogen peroxide or oxidant and reductant solutions were then added into the hydrates lipid to give the selected [monomer]/[initiator] mole ratio. The sample was sealed in an ampoule with a septum and flushed with argon for 0.5 h. The polymerization was performed at 45 °C in a water circulator bath under a positive argon pressure. Aliquots of sample at different time intervals were taken for the kinetic study. The absorption of dienoyl group was measured, and the percent conversion calculated. LUV from lipid **12** were prepared with a diameter of ca. 100 nm and polymerized in the same way as that for lipid **1.**

*$H_{II}$ phase.* Freeze-dried lipid **1** (3 mg) was hydrated with 0.5 ml of deoxygenated MilliQ water through several freeze-thaw-vortex cycles, and then incubated at 65 °C overnight. The solutions of selected initiators were prepared, and then added to the incubated hydrated sample to give the selected [monomer]/[initiator] mole ratio. The polymerization was performed at 65 °C in a water circulator bath under Ar(g). At the selected time, the polymerization was stopped, and the sample freeze-dried. The methanol extract of a known weight lipid was then characterized using UV spectroscopy to calculate the percent conversion to polymer.

*Control polymerization of DOPE.* DOPE (60 mg) was hydrated with argon flushed $K_2S_2O_8$ buffer solution (2 ml), and incubated at 60 °C for a day to ensure the formation of an equilibrated $H_{II}$ phase. Potassium persulfate solution ( 200 mM) and sodium bisulfite solution (200 mM) were then added to the incubated DOPE lipid to give [M]/[I] = 8. After 2 days at 60°C, the sample was freeze-dried, and then dissolved in chloroform. The chloroform extract was concentrated, and its $^1H$ NMR spectrum was taken in chloroform-d solvent. The ratio of integration area between vinyl and terminal methyl protons on the DOPE acyl chains was to 2:3, same as that obtained before addition of polymerization initiator. The chloroform extract therefore did not contain any polymerized DOPE lipids. The chloroform insoluble residue was tested for its solubility, and found to be totally soluble in water. This residue was a trace amount of salts from buffer and redox initiators. There was therefore no polymerization of DOPE lipids under the redox polymerization conditions, as no crosslinked polymers, linear polymers or oligomers were observed.

*Characterization of Polymerized Lipid*

Methanolic HCl was prepared freshly from slowly addition of 5 ml acetyl chloride to cold 50 ml anhydrous methanol. The polymerized lipid samples were lyophilized, then suspended in 4 ml benzene, and 2 ml of methanolic HCl was added. The reaction was heated at 50°C for 24 h. The acidic solution was neutralized with solid $NaHCO_3$ until the evolution of $CO_2$ stopped. The solid was filtered and the filtrate was concentrated. The residue was taken up in 5 ml of water and extracted several times with $CHCl_3$. The organic layer was dried over anhd. $MgSO_4$.

The transesterified polymer was examined by size exclusion chromatography using an Ultrastyragel linear column calibrated with poly(methyl methacrylate) standards. Chromatograms were obtained from a Waters Maxima 820 Chromatography Workstation (Milford, MA) equipped with a Waters R401 differential refractometer. The mobile phase ($CH_2Cl_2$ or THF) was filtered through 0.45 mm Waters nylon filters and purged with helium. Each chromatogram was obtained from a 100 µl injection of a polymer sample at a concentration of 2-3 mg/ml. The molecular weights are the average of at least two samples.

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