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# Articles

## Synthesis of Polymerized Vesicles with Hydrolyzable Linkages†

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ABSTRACT: A novel polymerized vesicular system, which contains a cyclic α-alkoxy acrylate as the polymerizable group on the amphiphilic structure, has been synthesized. These lipids can be easily polymerized through a free-radical process. It has been shown that polymerization improved the stabilities of the synthetic vesicles. In an aqueous system the cyclic acrylate group, which connects the polymerized chain and the amphiphilic structure, can be slowly hydrolyzed to separate the polymer chain and the vesicular system and generate a water-soluble biodegradable polymer. Furthermore, in order to retain the fluidity and to prepare the polymerized vesicles directly from prepolymerized lipids, a hydrophilic spacer has been introduced.

### Introduction

Phospholipids or similar water-insoluble amphiphilic natural substances aggregate in water to form bilayer liquid crystals which rearrange when exposed to ultrasonic waves to give spherical vesicles.[1,2] Natural product vesicles are also called liposomes. Vesicles are sealed, extremely thin, often spherical membranes which enclose aqueous volumes of approximately 1–1000 $\mu m^3$. Natural liposomes are very stable for a long time and can be subjected to chromatography and centrifugation. Liposomes, as well as synthetic vesicles, can entrap substances in the inner aqueous phase, retain them for extended periods, and release them by a function of the phase transition of the bilayers or other outside effects.[3-7]

Unlike the naturally occurring membranes, the synthetic vesicles have very limited stability. In the 1980s, several groups reported the synthesis of polymerizable lipids and their incorporation into and subsequent polymerization in synthetic vesicles.[8-11] The polymerized vesicles showed both high stability and very low permeability of compounds encapsulated in inner space. However, to be useful, it is also necessary that the synthetic polymerized vesicles have controllable time-release properties. Generally there are two approaches to this requirement: one is to remove the polymer chain from the vesicular system and let the vesicle finally become polymer-free; the other is to cleave the polymer chain in the vesicular system. An important development for the first approach was made by Tirrell et al.; they synthesized the synthetic vesicles which form complexation with certain polymers which can associate with the amphiphile head groups.[12,13] These polymers can be removed from the vesicular system through environmental changes, such as pH or electrovoltage. There also have been a few reports of the second approach. For example, Regen et al. reported the synthesis and characterization of phospholipids bearing thiol groups.[14,15] These phospholipids were used to construct vesicles that could be oxidatively polymerized. The resulting disulfide in the backbone of the polymer could also be reductively depolymerized. Since the disulfide moiety is common to many biopolymers, they suggested that the disulfide-based polymerized vesicles may be biodegradable. Another important example was reported by Ringsdorf et al. They synthesized amphiphilic lipids which contained amino acid groups.[16] The amino acid groups on vesicles could be polymerized through peptide bonds. They also suggested that these peptide liposomes should have the advantage of being biodegradable. In this paper we describe the synthesis of a novel vesicular system based upon the concept of the first approach.

In our laboratories, a series of new cyclic α-alkoxy acrylates has been synthesized and studied.[17] These monomers are very reactive in free-radical polymerizations and could be easily polymerized to give polymers containing cyclic acetal groups. Since these cyclic acetal groups can be hydrolyzed to give a water-soluble poly(α-hydroxyacrylic acid) which has been found to be biodegradable,[18,19] it has been reasoned that introduction of this monomer as the monomeric group and utilization of the resulting hydrolyzable acetal as a connecting group between the amphiphilic structure and the polymer chain would give the vesicle reasonable stability and make it possible to remove the polymer chain from the vesicular system. For these ideas, two polymeric lipids containing the cyclic acrylate groups as the polymerizable groups have been synthesized. The principle is shown in Scheme I.

### Results and Discussion

**Synthesis of Lipid 1.** This polymeric lipid contains two alkyl chains attached to a quaternary nitrogen atom which serves as a polar head group. The major advantage of using a quaternary nitrogen as the polar head group is its simplicity in its chemical synthesis. The polymeric group is placed at the end of one of the alkyl chains through an ester linkage. The synthetic route for the lipid is outlined in Scheme II.

In the first step of the synthesis, esterification of 4-carboxybenzaldehyde with 11-bromo-1-undecanol by dicyclohexylcarbodiimide (DCC) as a coupling reagent with a catalytic amount of 4-(dimethylamino)pyridine (DMAP)

† Dedicated to the memory of Professor William J. Bailey and on the occasion of his 70th birthday.

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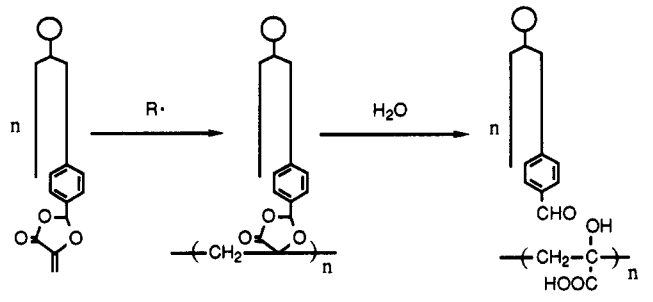
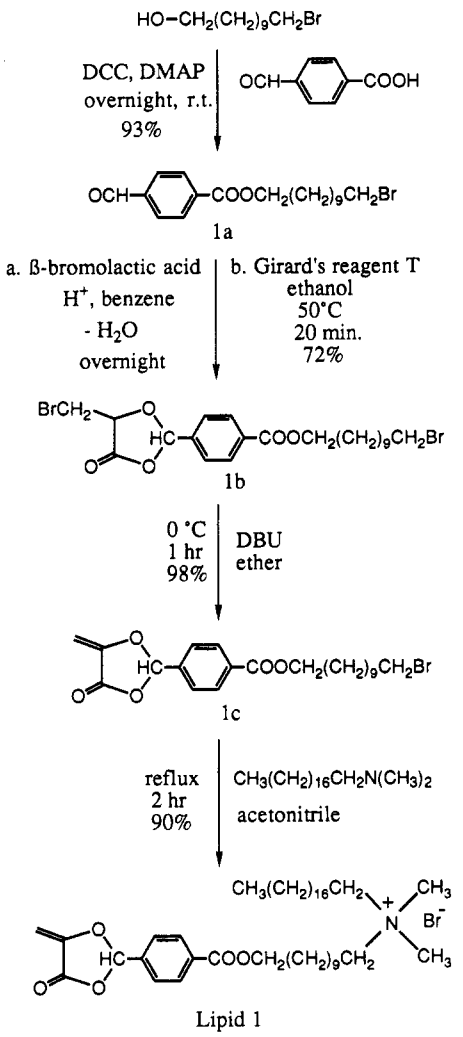
**Scheme I**




**Scheme II**




Lipid 1

gave a 93% yield of the product. It was found that the catalytic amount of DMAP was very important in this reaction, and without it the reaction could not be successfully completed. The bromine atom at one end of the long chain was introduced to eventually serve as the source of the counterion in the positive hydrophilic head.

Bromolactic acid was made according to the procedure of Baer and Robinson.[20] Then the bromolactic acid was cyclized with **1a** under acidic conditions in refluxing benzene which was used as the azeotropic solvent to remove water from the reaction. This reaction was an equilibrium reaction and was very difficult to complete under these conditions. There was always about 25% of the starting material left in the reaction mixture. The bromolactic acid was easy to remove because of its low solubility in organic solvents at low temperature. Chromatography was not effective here, because the product which contained a cyclic acetal group could be hydrolyzed back to the starting material on silica gel. After trying many conditions, it was found that Girard's reagent T (trimethylammonium acethydrazide chloride), which can react with aldehydes to form hydrazones which usually have very low solubility in organic solvents, could separate the starting material from the product.

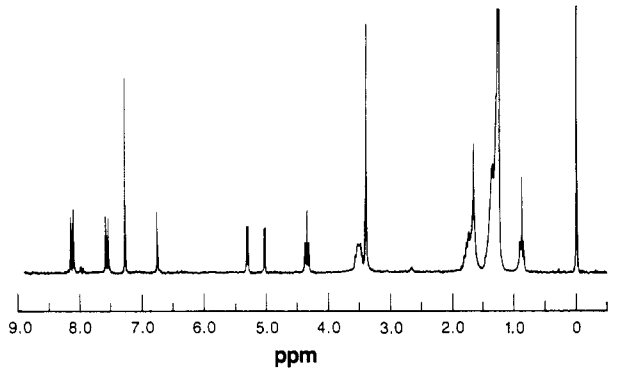



**Figure 1.** $^1$H NMR spectrum of the monomeric lipid 1.

The elimination of hydrogen bromide to produce the cyclic alkoxy acrylate **1c** was performed with 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) in ether solution at 0 °C. The isolated product was quite pure, and no further purification was needed after the reaction.

In the final step, *N*,*N*-dimethyloctadecylamine was quaternized with compound **1c** in acetonitrile to give the monomeric lipid 1. The pure product was obtained by recrystallization from acetone solutions. The $^1$H NMR spectrum of lipid 1 is shown in Figure 1. This monomeric lipid was found to be quite stable when kept in a refrigerator. Because of its amphiphilic structure, it can dissolve in many common organic solvents.

**Formation of Vesicles.** The most frequently used method for the formation of vesicles is by ultrasonication of the monomeric lipid in water. In the first step of the procedure lipid 1 was coated on the wall of a glass tube through removal of chloroform from the solution at room temperature. The tube was then evacuated at 0.1 mmHg overnight to make sure there was no solvent left in the system, since any organic solvent would interfere with the formation of the vesicles. After highly purified water was added to the tube and the mixture was heated at 60 °C for about 3 min, the mixture was vortex mixed for about 1 min. This procedure was repeated at least three times to give a milky suspension. When a sample of this suspension was placed under an optical microscope, big vesicles could be easily observed. This milky suspension was then further dispersed in a bath-type sonicator at 55 °C for about 10 min. A very optical clear suspension was then obtained. The electron microscope picture of a sample stained by 1% of uranyl acetate showed that small homogeneous vesicles were formed by the ultrasonication. The average diameter of the vesicles was about 350 Å (Figure 2).

**Polymerization.** Usually polymerization of the vesicles can be performed either by UV irradiation or by thermal free-radical initiators, such as AIBN. However, in the present case, thermal polymerization of the vesicles was not successful because hydrolysis of the monomeric acetal group proceeded very quickly at high temperatures. The hydrolysis during polymerization was monitored by the UV spectra of the polymerizing system as shown in Figure 3. The absorption around 240 nm, which is attributed to the cyclic ketene acetal group and the neighboring phenyl ester group, shifted to higher wavelength with increasing time; this shift was due to the hydrolysis of the cyclic

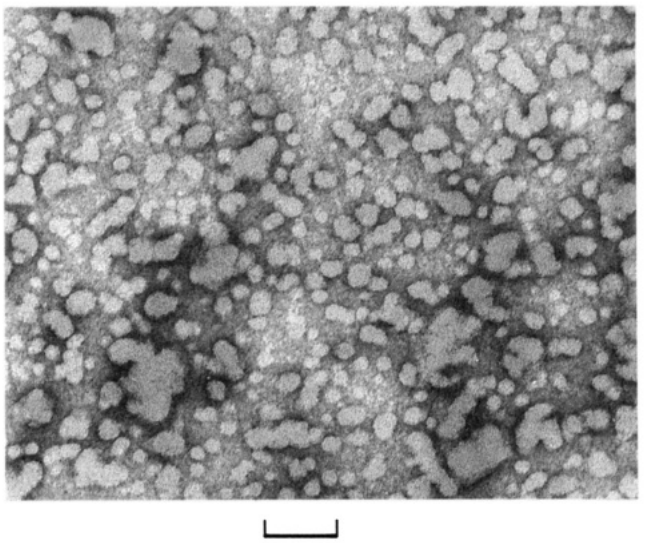


**Figure 2.** Electron micrograph of the vesicles of lipid 1 before UV irradiation (stained by 1% uranyl acetate; the bar represents 1000 Å).

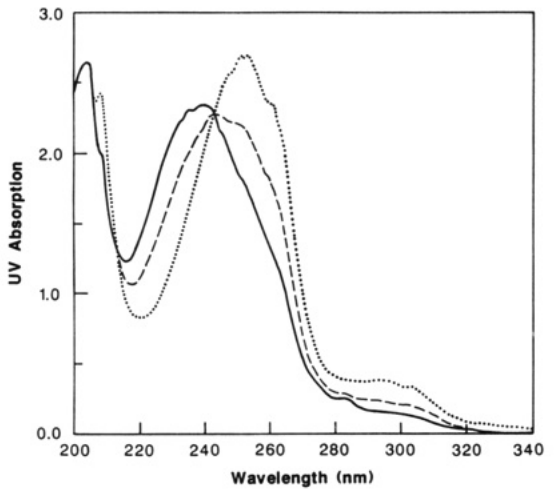



**Figure 3.** UV spectra of the thermal polymerization system of the vesicles of lipid 1 at 70 °C: (—) 0 h, (- - -) 3 h; (· · ·) 8 h.

acetal groups to the benzaldehyde ester functional group. Therefore, polymerization via UV irradiation was the only convenient route to these polymeric vesicles.

Polymerization of the prepared vesicle sample was carried out by direct UV irradiation in a miniphotoreactor. After the irradiation the sample had no visible change and remained optically clear. The electron microscope picture showed that the shape and size of the vesicles were the same as those before irradiation (Figure 4).

The polymerization through the cyclic acrylate carbon–carbon double bond was confirmed by both the UV spectra of the vesicle suspension and the IR spectrum of the polymerized lipids obtained by removal of the water through evaporation at low temperatures. As can be seen in Figure 5, the UV absorption peak at 238 nm, which is attributed to the cyclic ketene acetal group and the neighboring phenyl ester group, decreased about 35% after 3 min of irradiation of the vesicles, as a result of the polymerization of the carbon–carbon double bonds of cyclic ketene acetal. Further irradiation did not decrease the absorption significantly. In the IR spectra, the monomeric lipid had a typical absorption at 1670 $cm^{-1}$ for the cyclic acrylate carbon–carbon double bond; this absorption totally disappeared as the result of 3 min of irradiation, as shown in Figure 6.

**Stabilities of Vesicles.** The polymerized vesicles showed much greater stability over the unpolymerized ones. On standing at room temperature, the unpolymerized vesicles remained stable only for about 2 days, after which time precipitation of some of the lipid was noted; substantial precipitation of the lipid was observed after 6 days of standing. By comparison, there was only a trace of precipitation found in the polymerized vesicle system after standing 6 days at room temperature, and no substantial precipitation was observed after a 2-week period. The electron microscope pictures of Figure 4 showed that most of the polymerized vesicles retained their initial sizes and shapes after 6 days.

The UV absorption of the aqueous vesicular systems, which provides information on the relative concentration of lipids in an aqueous system, also proved the enhanced stability of the polymerized vesicles. The absorptions at 238 nm of the unpolymerized vesicles showed a sharp decrease, as seen in Figure 7, as a result of the precipitation of the lipid in the system. However, the absorptions at the same wavelength of the polymerized system showed a relatively steady trend that meant the polymerized lipid had a longer suspension life in the aqueous system.

**Hydrolysis of the Connecting Groups.** The hydrolysis of the connecting groups actually happened both on the monomeric groups and in the polymerized system. Although hydrolysis of the monomeric group in the vesicular system was rapid at high temperature, the rate of hydrolysis at room temperature was quite moderate. The IR spectrum of the recovered monomeric lipid showed that most of the acetal linkages on the monomeric group still survived in an aqueous system after standing in a vesicular system at room temperature for 3 days. However, the IR spectrum of the recovered lipid after 2 weeks in an aqueous system showed the hydrolysis of the acetal linkages was almost complete.

The polymerized system gives similar results. The IR spectrum of polymerized lipid shows two absorption peaks at 1805 and 1735 $cm^{-1}$ which correspond to the lactone carbonyl and ester groups, respectively. After the polymerized vesicle had been allowed to stand in an aqueous system for 2 weeks, the lactone carbonyl absorption peak at 1805 $cm^{-1}$ disappeared as seen in Figure 6, which indicated the hydrolysis of the connecting acetal linkages had been complete.

**Synthesis of Lipid 2.** In the study of polymerizations of the first vesicular system of lipid 1 it has been learned that thermal polymerization in an aqueous medium was not possible because of the hydrolysis of the acetal group. UV irradiation seemed the only practical initial way to perform the polymerization. However, it is difficult to follow and manipulate the polymerization, and therefore is not possible to control the molecular weight of the polymer chain in the vesicular system.

The fluidity is one of the most vital properties of biological membranes. It relates to many functions involved in biological systems, and effective biomembrane mimetic chemistry depends on the combination of both stability and mobility of the model membranes. However, in the polymerized vesicles the polymer chain interferes with the motion of the side groups and usually causes a decrease or even the loss of the fluid phases inside the polymerized vesicle.[21,22]

In order to retain the fluidity which is a fundamental property of the biological membrane and to prepare the polymerized vesicles directly from the prepolymerized lipids, the incorporation of hydrophilic spacer groups, which decouple the motion of the polymer main chain from the

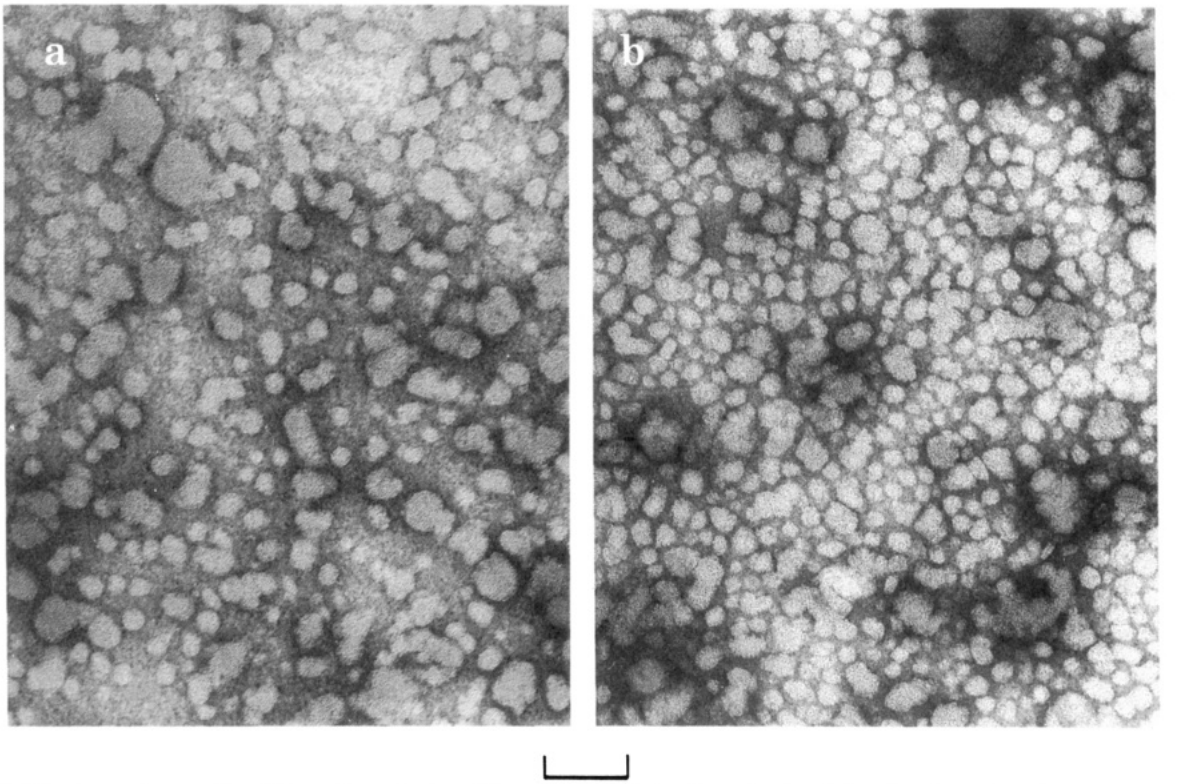



**Figure 4.** Electron micrographs of the polymerized vesicles of lipid 1: (a) polymerized vesicles; (b) after 6 days in an aqueous system (stained by 1% uranyl acetate; the bar represents 1000 Å).

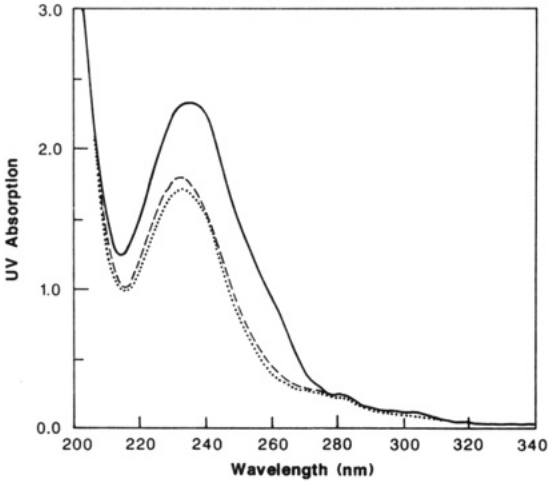



**Figure 5.** UV spectra of the polymerization system of the vesicles of lipid 1 with UV irradiation: (—) 0 min; (- - -) 1 min; (· · ·) 3 min.

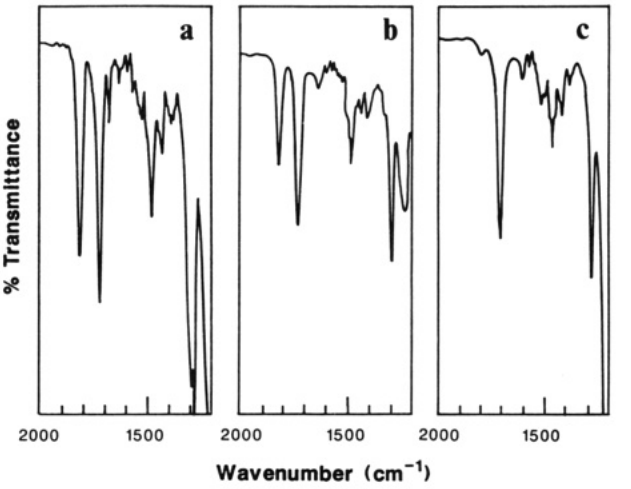



**Figure 6.** IR spectra of lipid 1: (a) monomeric lipid; (b) after UV irradiation in a vesicular system; (c) hydrolyzed lipid recovered from an aqueous system.

membrane-forming side groups, has been studied.[23,24] The advantage of this concept is that loss of mobility and structural changes of the membranes induced by the polymer chain can be avoided, and thus polymerized vesicles can be formed directly from the prepolymerized lipids. Based upon this concept, another new polymeric lipid which contains a hydrophilic spacer group derived from ethylene glycol units between the monomeric group of cyclic acrylate and the main amphiphilic structure was synthesized. The synthesis is outlined in Schemes III and IV.

The synthesis of this lipid started with the conversion of triethylene glycol chlorohydrin to triethylene glycol iodohydrin. Triethylene glycol iodohydrin was then coupled with 4-carboxybenzaldehyde by use of DCC as a coupling reagent with DMAP as a catalyst. The cyclization of the aldehyde **2b** with β-bromolactic acid was performed with *p*-toluenesulfonic acid as the catalyst in a procedure similar to that of lipid 1. The elimination to form the cyclic acrylate **2d** was essentially quantitative; no side reactions were observed with the iodide. Since the eliminated product **2d** from the reaction was quite pure, no further purification was needed.

Palmitic acid was allowed to react with (dimethylamino)-propanediol in the presence of DCC and DMAP under conditions similar to that described above to give compound **2e**. Finally the monomeric lipid 2 was prepared by combination of compounds **2d** and **2e** in a mixture solvent of acetonitrile and acetone in a high-pressure reaction tube. The pure monomeric lipid could be obtained by recrystallization from acetone or a mixture of acetone and acetonitrile. The pure monomeric lipid is very stable if it is kept in a refrigerator and can be dissolved in chloroform, methanol, and hot benzene. The $^1$H NMR spectrum and

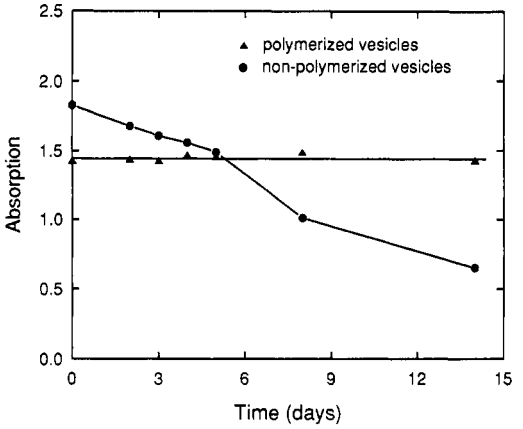
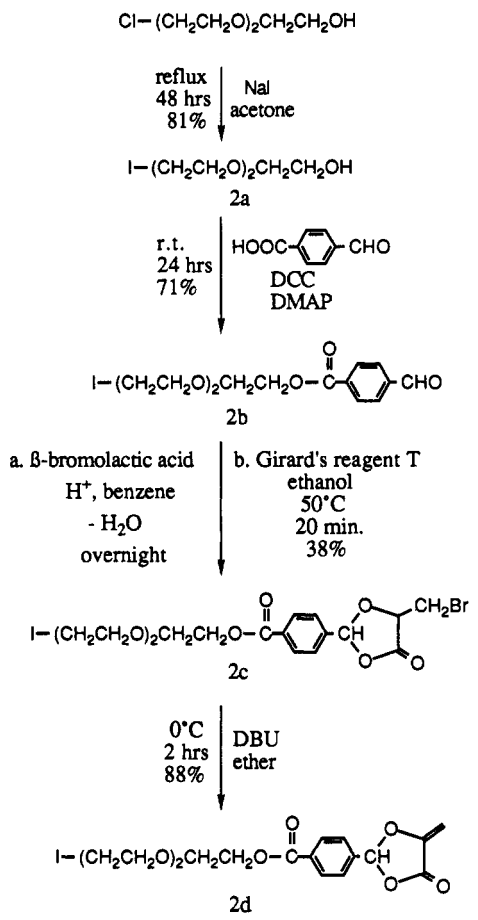



**Figure 7.** UV absorption at 238 nm of the vesicle systems of lipid 1.

**Scheme III**




FT-IR spectrum of the lipid are shown in Figures 8 and 9.

**Polymerization.** As described before, one of the advantages of this new lipid is that the monomeric lipid can be polymerized first before the making of vesicles. The polymerization of lipid 2, with benzene as the solvent and 5% AIBN as the initiator, was carried out at 60 °C in a sealed tube. After the polymerization, the resulting polymer was precipitated in a large amount of methanol. The molecular weight of the polymer was found to be around 50 000 by GPC.

The $^1$H NMR spectrum (Figure 8) of the polymerized lipid shows that vinyl protons of the cyclic acrylate between 5.00 and 6.00 ppm disappeared from the spectrum, compared with that of monomeric lipid. Also in the IR spectrum (Figure 9) the absorption peak at 1670 cm$^{-1}$ for

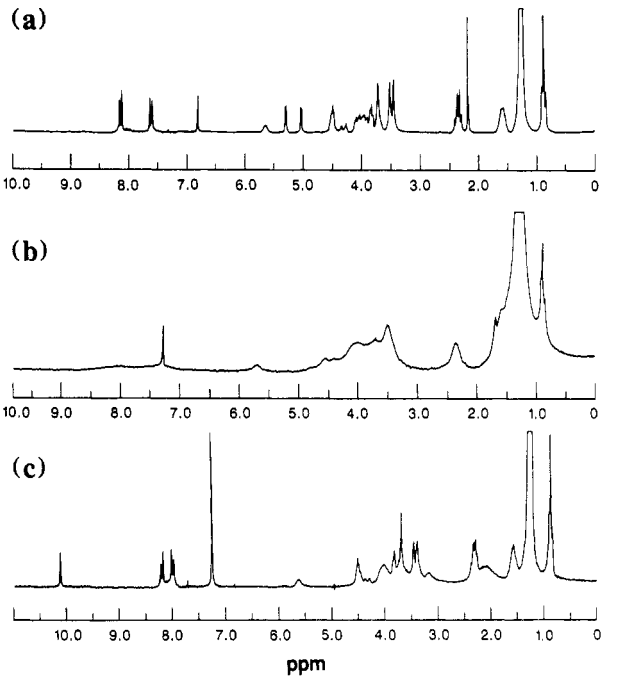
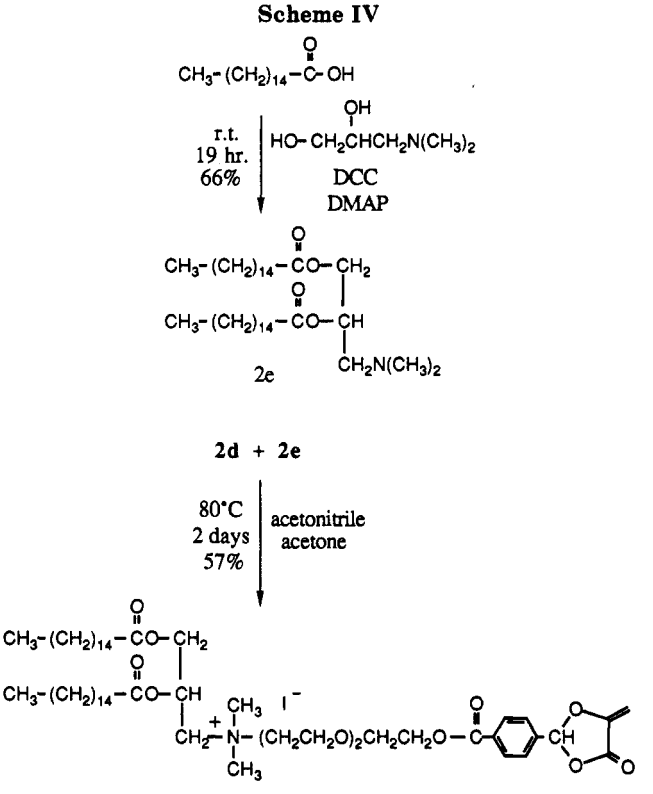



**Figure 8.** $^1$H NMR spectra of lipid 2: (a) monomeric lipid; (b) polymerized lipid; (c) recovered lipid after 21 days in an aqueous system.

**Scheme IV**




the cyclic acrylate carbon–carbon double bond disappeared as the result of polymerization. The carbonyl absorptions of the esters at 1740 cm$^{-1}$ and the lactone at 1805 cm$^{-1}$ still remain in the spectrum.

**Formation of Vesicles.** The procedure for the formation of vesicles from this prepolymerized lipid was similar to that for lipid 1. However, the concentration of the lipid in this system was lower than that in the case of the monomeric lipid. Also the time of sonication for this polymerized lipid was longer than that for the monomeric lipid because of the decreased freedom of motion of the

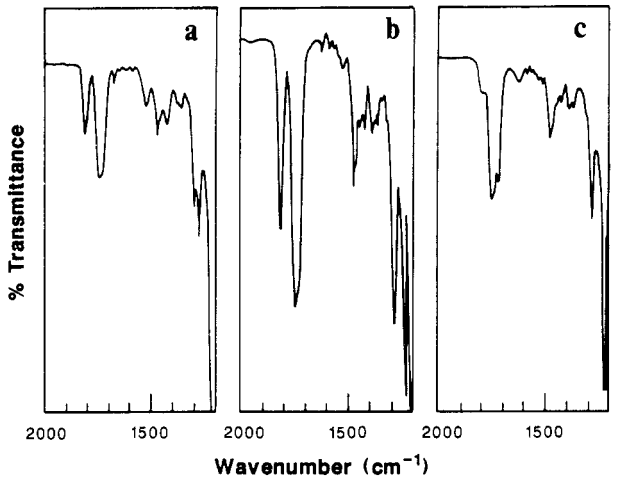



**Figure 9.** IR spectra of lipid 2: (a) monomeric lipid; (b) polymerized lipid; (c) hydrolyzed lipid.

amphiphilic structure in the polymerized system. The electron microscope pictures (Figure 10) show the formation of tiny and very homogeneous vesicles. The average diameter of the polymerized vesicles was about 300 Å.

**Freeze-Fracture Electron Microscopy.** If the polymer chain did not cross the bilayer inside the vesicle, the freeze-fracture plane should run through the middle of the bilayer and a good freeze-fracture electron microscopy picture should be obtained.[23] The freeze-fracture electron microscopy is also one of the best methods for the characterization of bilayer vesicles.[25,26]

In the vesicular system of lipid 1, the polymerizable group was attached to the end of the amphiphilic chain, and the growing polymer chain can cross the bilayer. Therefore, good freeze-fracture pictures could not be taken because the layer crossing prevented the bilayer from splitting. However, in the vesicular system of lipid 2 all the polymer chains were placed close to the polar head group and on the surface of the bilayer. There was no linkage between two layers inside the vesicle. Therefore, these polymerized vesicles gave a clear freeze-fracture electron microscope picture as seen in Figure 11.

**Stability.** The polymerized vesicles from this prepolymerized lipid showed enhanced stability as expected. In a 6-day period there was little precipitation of the lipid and no visible change of the aqueous system was observed. Even after 18 days, as seen in Figure 10, some of the vesicles could be seen in the electron micrograph.

**Differential Scanning Calorimetry (DSC) Diagrams.** The phase transition of bilayer lipids is related to the highly ordered arrangement of the lipids inside the vesicle. In the ordered gel state below a characteristic temperature, the lipid hydrocarbon chains are in an all-trans configuration. When the temperature is increased, an endothermic phase transition occurs, during which there is a trans–gauche rotational isomerization along the chains which results in a lateral expansion and decrease in thickness of the bilayer. This so-called gel to liquid-crystalline transition has been demonstrated in many different lipid systems, and the relationship of the transition to molecular structure and environmental conditions has been studied extensively.

The DSC diagrams confirm that the fluid phase of the polymerized vesicles of lipid 2 remains and the phase transitions are retained with the introduction of the spacer group. As can be seen in Figure 12 of the DSC diagram of the monomeric lipid 2, there is a peak around 28 °C which corresponds to the phase transition of monomeric lipid. As a result of the presence of the spacer group, a similar phase transition can also be observed clearly in the diagram of the polymerized lipid 2 as shown in Figure 13, but the transition temperature is increased to 36 °C by the presence of the polymer chains.

**Hydrolysis.** The hydrolysis of the cyclic acetal, which was used as the connecting group between the polymer chain and the lipid, was confirmed by both the IR and the $^1$H NMR spectra of the lipid recovered from the vesicular system after standing for 3 weeks at room temperature. The lactone absorption at 1805 cm$^{-1}$ disappeared from the IR spectrum (Figure 9) as a result of hydrolysis. Furthermore, a new aldehyde absorption band at 1705 cm$^{-1}$ was observed in the spectrum, which was related to the substituted benzaldehyde group of the hydrolyzed product. The $^1$H NMR spectrum (Figure 8) also clearly showed the formation of the benzaldehyde, as indicated by the peak at 10.20 ppm.

## Experimental Section

**General Procedures.** Unless otherwise specified, all solvents were distilled and stored over molecular sieves. Melting points were determined on a Mel-Temp melting point apparatus in open air capillary tubes and are reported uncorrected. Infrared (IR) spectra were obtained on a Perkin-Elmer 298 spectrophotometer and on a Nicolet 5DXC FT-IR spectrophotometer. Ultraviolet (UV) spectra were recorded on a Hewlett Packard HP 8450 UV/vis spectrophotometer. Proton nuclear magnetic resonance ($^1$H NMR) spectra and carbon nuclear magnetic resonance ($^{13}$C NMR) spectra were recorded with Bruker IBM WP-200 (FT) and Bruker IBM AF-200 (FT) spectrometers. Electron microscopic pictures were recorded on a Zeiss EM-10CA electron microscope of the Department of Zoology at the University of Maryland, College Park, MD.

**Highly Purified Water.** Distilled water was passed through a Barnstead brand NANOpure system, and the highly purified water, which was collected when the reading was above 15 MΩ-cm, was stored in a polyethylene bottle.

**11-Bromoundecanyl 4-Formylbenzoate (1a).** To a solution containing 12.0 g (48 mmol) of 11-bromo-1-undecanol, 0.5 g (5 mmol) of (dimethylamino)pyridine (DMAP), 7.5 g (50 mmol) of 4-carboxybenzaldehyde, and 40 mL of methylene chloride was added 11.0 g (53 mmol) of dicyclohexylcarbodiimide (DCC) at 0 °C. After the solution was stirred at room temperature for 12 h, the precipitate was removed by filtration. Then, the solvent was removed from the filtrate by evaporation and the residue was added to a silica gel filled chromatograph followed by elution with ethyl acetate/hexane (1:9) to give 17.5 g (93%) of **1a** as a waxy solid: $R_f$ = 0.6 (ethyl acetate/hexane, 2:8); $^1$H NMR ($CDCl_3$, 200 MHz) δ 1.30 (m, 14 H), 1.83 (m, 4 H), 3.41 (t, 2 H), 4.36 (t, 2 H), 7.96 (m, 2 H), 8.20 (m, 2 H), 10.1 (s, 1 H); IR ($CDCl_3$) 2930, 2860, 1740, 1705, 1280, 1120, 1100, 1030 cm$^{-1}$.

**β-Bromolactic Acid.** To 220 mL of nitric acid in a 3-L round-bottomed flask immersed in an ice-bath was added 68.5 g (500 mmol) of epibromohydrin over a period of 30 min. The flask was placed on a boiling water bath until reaction started. The water bath was then immediately removed, and the reaction was allowed to proceed spontaneously. After the vigorous reaction had subsided, the flask was returned to the boiling water bath for about 40 min. The reaction mixture was concentrated by removal of the water through evaporation under reduced pressure, as far as possible with the temperature not exceeding 50 °C. After the remaining crystalline material was dissolved in 185 mL of distilled water and the resulting solution was made neutral to litmus with sodium carbonate, the sodium oxalate was then removed by filtration. The remaining oxalic acid was removed by adding to the filtrate cautiously a concentrated solution of calcium chloride until no further precipitate was formed, and the calcium oxalate was removed by filtration. To the ice-cold filtrate was added 21.2 mL of concentrated sulfuric acid, and the aqueous solution was extracted with eight 125-mL portions of ether. The ether was removed from the combined extracts by evaporation under reduced pressure, and the residue was kept for 3 h at 60 °C and

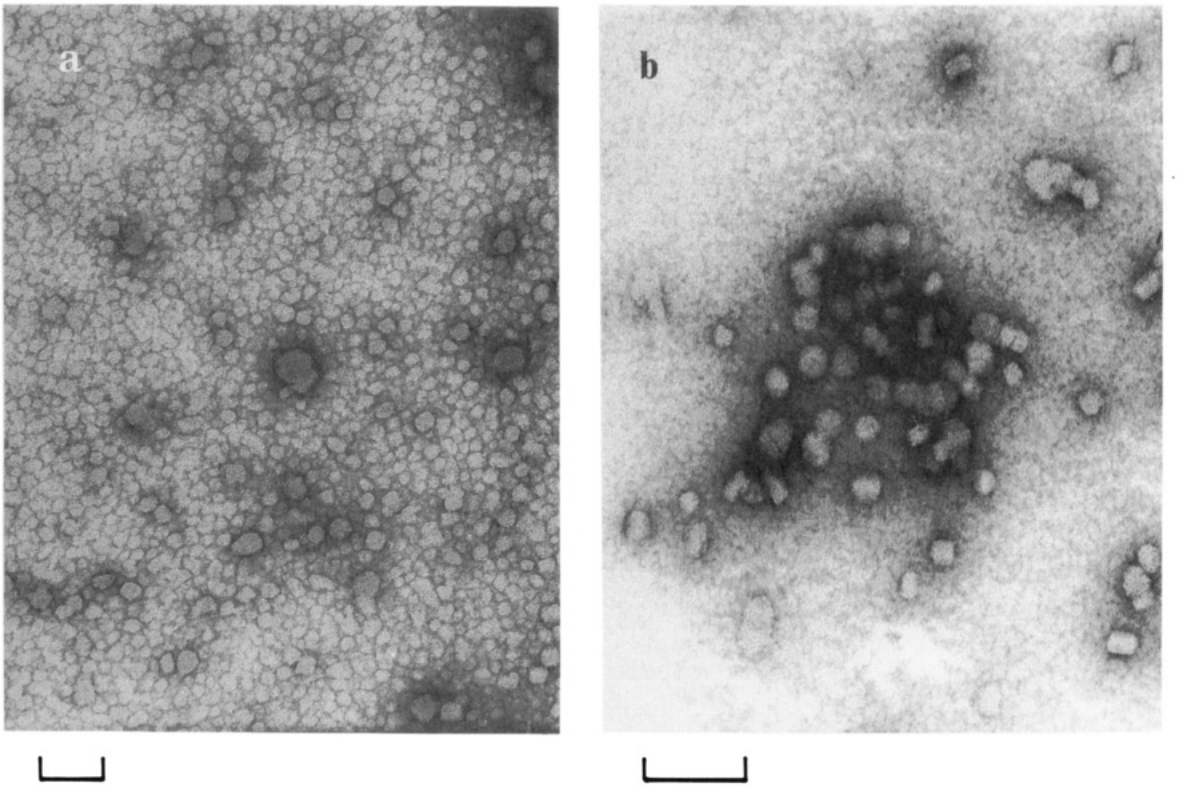



**Figure 10.** Electron micrographs of the vesicles of the polymerized lipid 2: (a) polymerized vesicles; (b) after 18 days in an aqueous system (stained by 1% uranyl acetate; the bar represents 1000 Å).

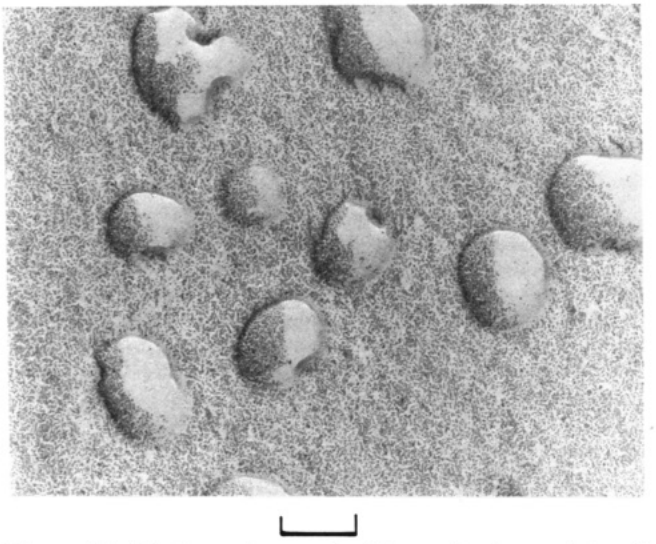


**Figure 11.** Electron micrograph of freeze-fracture vesicles of the polymerized lipid 2 (the bar represents 1000 Å).

a pressure of 1 mmHg. The crude product was recrystallized from a hot benzene solution, and about 34 g (40%) of the product was obtained, mp 83.0–84.6 °C (lit. mp 88–89 °C).

**11-Bromoundecanyl *p*-[5-(Bromomethyl)-4-oxo-1,3-dioxolan-2-yl]benzoate (1b).** In 30 mL of benzene were placed 7.91 g (20 mmol) of **1a**, 3.84 g (23 mmol) of bromolactic acid, and 0.10 g of *p*-toluenesulfonic acid (PTSA). While the mixture was heated under reflux overnight, the water from reaction was collected in a Dean–Stark trap. After the cooled mixture was diluted with 50 mL of ether, the resulting solution was washed with 40 mL of 0.1 M $Na_2CO_3$ solution and 40 mL of saturated saline and the organic solution was dried over anhydrous $Na_2SO_4$. After most of the solvent was removed by evaporation, the residue was added to 50 mL of ethanol containing 2.0 g of Girard's reagent T. After the mixture had been heated at 50 °C for 20 min, the ethanol was removed by evaporation and 100 mL of anhydrous ether was added. The resulting precipitate was then removed by filtration,

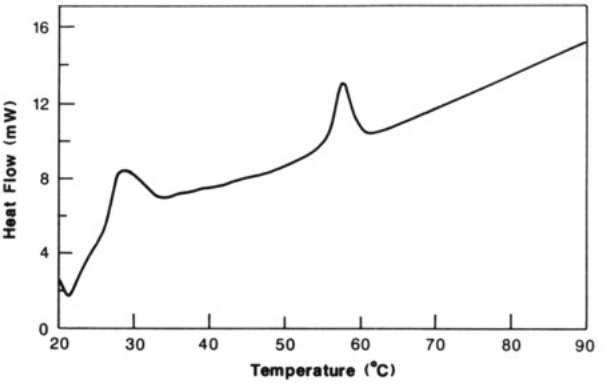



**Figure 12.** DSC diagram of the monomeric lipid 2 in an aqueous system.

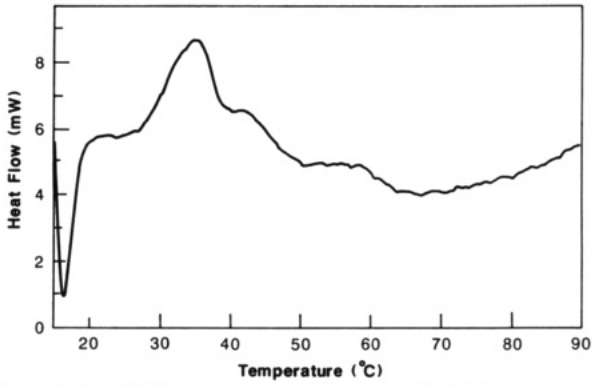



**Figure 13.** DSC diagram of the polymerized lipid 2 in an aqueous system.

and all the ether was removed by evaporation to give 6.7 g (72%) of **1b** as a viscous liquid: $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 1.30 (m, 14 H), 1.81 (m, 4 H), 3.41 (t, 2 H), 3.79 (m, 2 H), 4.34 (t, 2 H), 4.81 (t, 1 H), 6.51 (s, 1 H), 7.70 (d, 2 H), 8.15 (d, 2 H); IR ($CHCl_3$) 2920, 2850, 1808, 1735, 1275, 1220, 1195, 1105, 1020 cm$^{-1}$.

**11-Bromoundecanyl *p*-(5-Methylene-4-oxo-1,3-dioxolan-2-yl)benzoate (1c).** To a solution of 4.2 g (7.9 mmol) of **1b** in 50 mL of anhydrous ether was added dropwise at 0 °C a solution containing 1.2 g (7.9 mmol) of diazabicycloundecene (DBU) dissolved in 5 mL of anhydrous ether. After the mixture was stirred at 0 °C for 1 h, the precipitate was removed by filtration. After the solvent was removed from the filtrate by evaporation, 3.49 g (98%) of **1c** (viscous liquid) was isolated: $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 1.30 (m, 14 H), 1.81 (m, 4 H), 3.41 (t, 2 H), 4.34 (t, 2 H), 5.03 (d, 1 H), 5.32 (d, 1 H), 6.73 (s, 1 H), 7.55 (d, 2 H), 8.12 (d, 2 H); IR ($CHCl_3$) 2920, 2850, 1805, 1735, 1670, 1395, 1375, 1110, 1015, 860 cm$^{-1}$.

**Dimethyloctadecylamine.** Commercial dimethyloctadecylamine was purified by silica gel chromatography eluded with chloroform.

**[11-[[*p*-(5-Methylene-4-oxo-1,3-dioxolan-2-yl)benzoyl]-oxy]undecanyl]octadecyldimethylammonium Bromide (Lipid 1).** A solution of 0.50 g (1.1 mmol) of **1c** and 0.34 g (1.1 mmol) of dimethyloctadecylamine in 10 mL of acetonitrile was heated under reflux for 2 h. After the mixture was cooled to room temperature, the resulting precipitate was reprecipitated from acetone at 0 °C to give 0.75 g (90%) of lipid 1: $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 0.88 (t, 3 H), 1.25 (m, 42 H), 1.66 (m, 8 H), 3.40 (s, 6 H), 3.29 (m, 4 H), 4.34 (t, 2 H), 5.03 (d, 1 H), 5.31 (d, 1 H), 6.75 (s, 1 H), 7.55 (d, 2 H), 8.13 (d, 2 H); IR ($CHCl_3$) 2920, 2830, 1805, 1735, 1670, 1470, 1295, 1275, 1120, 1110, 1015, 980, 860 cm$^{-1}$. Anal. Calcd for $C_{42}H_{72}BrNO_5$: C, 67.18; H, 9.66; N, 1.87. Found: C, 66.48; H, 10.20; N, 1.97.

**Preparation of the Vesicles.** The tube which was used for preparation of vesicles was first washed with detergent and then rinsed consecutively with distilled water, highly purified water, BJ brand methanol, and BJ brand chloroform and finally dried in a oven overnight. In the tube 2 mg of lipid 1 was dissolved in 1 mL of chloroform (BJ brand). The solvent was slowly removed by evaporation at reduced pressure with the temperature not exceeding 40 °C to make sure there were no bubbles formed during the evaporation. After the solvent was removed, the lipid was coated on the wall of the tube, and the tube was evacuated at 0.1 mmHg overnight. After 2 mL of highly purified water was added, the suspension was placed in a heating bath with the temperature set at 60 °C for 3 min, and then the suspension was vortex mixed for about 1 min; this procedure was repeated three times. After that, the system became a milky suspension. This milky suspension was further dispersed in a bath-type sonicator for about 10 min to give an optical clear vesicle system.

**Polymerization by UV Irradiation.** The vesicle suspension prepared as described above was transferred into a quartz tube which was then flushed with nitrogen gas for about 20 min. After the tube was sealed with a rubber stopper, it was put on a rotator contained in a miniphotoreactor for UV irradiation for 3 min with slow rotation.

**Sample Preparation for an Electron Microscopic Study.** A drop of the vesicle suspension described above was placed on a copper grid and allowed to stand for about 1 min. Half of the drop was removed by a small piece of filter paper, and a drop of 1% of uranyl acetate solution was placed on the top of the suspension on the grid. After 1.5 min most the solution on the grid was removed by a piece of filter paper, and the coated grid was dried at room temperature.

**Triethylene Glycol Iodohydrin (2a).** A solution containing 16.8 g (65 mmol) of triethylene glycol chlorohydrin and 11.0 g (73 mmol) of sodium iodide in 50 mL of acetone was heated under reflux for 48 h. After the mixture was cooled, the resulting precipitate was removed by filtration and the solvent was removed from the filtrate by evaporation. To the residue, 100 mL of ether was added, and the resulting precipitate was removed by filtration. The product (13.7 g) was isolated after removal of the ether by evaporation to give **2a** as a viscous liquid in an 81% yield: $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 2.60 (b, 1 H), 3.28 (t, 2 H), 3.72 (m, 12 H).

**9-Ioda-3,6-dioxanonyl 4-Formylbenzoate (2b).** To a solution containing 12.0 g (46 mmol) of **2a**, 0.5 g of DMAP, and 7.5 g (50 mmol) of 4-carboxybenzaldehyde in 40 mL of $CH_2Cl_2$ was added 11.0 g (53 mmol) of DCC at 0 °C. The mixture was stirred at 0 °C for 0.5 h and then warmed to room temperature. After the mixture had been stirred at room temperature for 12 h, the precipitate was removed by filtration and the solvent was then removed from the filtrate by evaporation. The residue was purified by chromatography on a silica gel column by elution with a mixture of ethyl acetate and hexane (1:1) to furnished 13.2 g (71%) of **2b** as a viscous liquid: $R_f$ = 0.7 (ethyl acetate/hexane, 1:1); $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 3.25 (t, 2 H), 3.74 (m, 8 H), 3.88 (t, 2 H), 4.53 (t, 2 H), 7.96 (d, 2 H), 8.23 (d, 2 H), 10.11 (s, 1 H); IR ($CHCl_3$) 3020, 1724, 1707, 1277, 1212, 1016 cm$^{-1}$.

**9-Ioda-3,6-dioxanonyl *p*-[5-(Bromomethyl)-4-oxo-1,3-dioxolan-2-yl]benzoate (2c).** While a mixture of 9.4 g (24 mmol) of **2b**, 4.6 g (27 mmol) of $\beta$-bromolactic acid, and 0.1 g of *p*-toluenesulfonic acid in 40 mL of benzene was heated under reflux overnight, the water from the reaction was collected in a Dean–Stark trap. After the cooled mixture was washed with a 0.1 M $Na_2CO_3$ solution and then saline, the solvent was removed by evaporation in vacuo. The residue was then added to 50 mL of ethanol containing 2.5 g of Girard's reagent T, and the mixture was heated at 50 °C for 20 min. After most of the ethanol was removed by evaporation, 100 mL of anhydrous ether was added and the resulting precipitate was removed by filtration. After removal of the ether from the filtrate by evaporation, 4.9 g (38%) of **2c** (viscous liquid) was isolated; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 3.24 (t, 2 H), 3.74 (m, 8 H), 3.87 (t, 2 H), 4.51 (t, 2 H), 4.83 (t, 1 H), 6.52 (s, 1 H), 7.70 (d, 2 H), 8.16 (d, 2 H); IR ($CHCl_3$), 3020, 1805, 1729, 1275, 1112 cm$^{-1}$.

**9-Ioda-3,6-dioxanonyl *p*-(5-Methylene-4-oxo-1,3-dioxolan-2-yl)benzoate (2d).** To a solution of 4.9 g (9.0 mmol) of **2c** in 15 mL of anhydrous ether was added dropwise at 0 °C 1.36 g (9.0 mmol) of DBU in 10 mL of ether. After the mixture had been allowed to stand at 0 °C for 2 h, 100 mL of ether was added and the resulting precipitate was removed by filtration. After removal of the solvent from the filtrate by evaporation there was obtained 3.7 g (88%) of **2d** as a viscous liquid; $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 3.24 (t, 2 H), 3.74 (m, 8 H), 3.86 (t, 2 H), 4.51 (t, 2 H), 5.04 (d, 1 H), 5.32 (d, 1 H), 6.74 (s, 1 H), 7.55 (d, 2 H), 8.15 (d, 2 H); IR ($CHCl_3$) 3020, 1806, 1729, 1670, 1275, 1112 cm$^{-1}$.

**1,2-Bis(palmitoyloxy)-3-(dimethylamino)propane (2e).** In a solution containing 14 g (50 mmol) of palmitic acid, 6.0 g (50 mmol) of 3-(dimethylamino)-1,2-propanediol, and 0.7 g of DMAP in 60 mL of methylene chloride was added 12 g (58 mmol) of DCC at 0 °C. After the mixture was stirred at 0 °C for 0.5 h, it was allowed to warm to room temperature. After the reaction mixture had been stirred at room temperature for 19 h, the precipitate from the reaction was removed by filtration. The solvent was removed from the filtrate by evaporation, and the residue was purified by chromatography on a silica gel column by elution with a mixture of 40% ethyl acetate in hexane to give 13 g (66%) of the product (waxy solid); $R_f$ = 0.42 (ethyl acetate/hexane, 1:1); $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 0.88 (t, 6 H), 1.25 (s, 48 H), 1.60 (t, 4 H), 2.25 (s, 6 H), 2.31 (t, 4 H), 2.44 (m, 2 H), 4.08 (m, 1 H), 4.36 (m, 1 H), 5.20 (m, 1 H); IR ($CHCl_3$) 3021, 2928, 2855, 1734, 1468, 1255, 1223, 1210 cm$^{-1}$.

**[1,2-Bis(palmitoyloxy)propanyl][8-[[*p*-(5-methylene-4-oxo-1,3-dioxolan-2-yl)benzoyloxy]-3,6-dioxaoctyl]dimethylammonium Iodide (Lipid 2).** In 10 mL of acetonitrile/acetone (9:1, v/v), 0.41 (0.70 mmol) g of **2d** was quaternized with 0.38 g (0.70 mmol) of **2e** in a screw-capped tube at 80 °C for 2 days. After the mixture was cooled, the precipitate was isolated and reprecipitated three times from acetone to yield 0.42 g (57%) of lipid 2: $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 0.88 (t, 6 H), 1.25 (s, 48 H), 1.59 (m, 4 H), 2.34 (m, 4 H), 3.45 (s, 3 H), 3.51 (s, 3 H), 3.71 (s, 4 H), 3.83–4.13 (m, 11 H), 4.33 (d, 1 H), 4.49 (t, 2 H), 5.04 (d, 1 H), 5.31 (d, 1 H), 5.66 (m, 1 H), 6.81 (s, 1 H), 7.61 (d, 2 H), 8.14 (d, 1 H); IR ($CHCl_3$) 3020, 2928, 2856, 1806, 1740, 1729, 1670, 1521, 1467, 1423, 1297, 1146, 1112, 1062, 781, 773 cm$^{-1}$. Anal. Calcd for $C_{54}H_{92}INO_{11}$: C, 61.29; H, 8.76; N, 1.32. Found: C, 61.20; H, 9.20; N, 1.48.

**Polymerization of Lipid 2 by Free-Radical Initiation.** Into a polymerization tube was placed 110 mg of lipid 2, 2 mg of AIBN, and 1 mL of benzene. After the tube was evacuated and flushed three times with nitrogen gas and sealed under vacuo, the tube was heated overnight at 60 °C. After the sealed tube was opened the mixture was dissolved in a small amount of chloroform, and this chloroform solution was added to a large excess of methanol. The precipitated polymer was collected by

filtration. After the solid was dried in vacuo, 78 mg (71%) of polymer was obtained: $^1$H NMR ($CDCl_3$, 200 MHz) $\delta$ 0.88 (br, 6 H), 1.00–2.00 (br, 54 H), 2.34 (br, 4 H), 3.0–5.0 (br, 24 H), 5.70 (s, 1 H), and 7.0–8.0 (br, 4 H); IR ($CHCl_3$) 3019, 2927, 2855, 1805, 1740, 1468, 1278, 1180, 1154, 1209, 773, 741 $cm^{-1}$.

**Freeze-Fracture Electron Microscopy.** Freeze-fracture electron microscopy of the polymerized vesicles was performed by the faculty of the Electron Microscopy Laboratory in the Department of Zoology at the University of Maryland, College Park, MD.

**Differential Scanning Calorimetry (DSC) Spectra.** The DSC spectra were done with the help of Dr. Edward Chang of the Naval Research Laboratory in Washington, D.C.

## Conclusion

In this paper two new polymerized vesicle systems have been presented. The first lipid can be polymerized in vesicle through UV irradiation. Because the second lipid contains a flexible spacer group, it can be prepolymerized in benzene and then converted to vesicles by ultrasonication in water. The polymerization improves the stabilities of the synthetic liposomes. Since there is an acetal linkage between the polymer chain and the amphiphilic structure, this linkage can be slowly hydrolyzed in an aqueous system to separate the polymer chain from the lipid and finally generate a water-soluble biodegradable polymer.

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**Registry No.** 1, 117532-97-5; 1 (homopolymer), 117532-98-6; **1a**, 117684-94-3; **1b**, 117684-95-4; **1c**, 117684-96-5; **2**, 137494-52-1; **2** (homopolymer), 137494-63-4; **2a**, 62573-16-4; **2b**, 117685-44-6; **2c**, 117685-45-7; **2d**, 117685-46-8; **2e**, 137494-53-2; $HO(CH_2)_{11}Br$, 1611-56-9; $H(CH_2)_{18}N(CH_3)_2$, 124-28-7; $Cl(CH_2CH_2O)_3H$, 5197-62-6; $HO_2CC_6H_4$-*p*-CHO, 619-66-9; $H(CH_2)_{15}CO_2H$, 57-10-3; $HOCH_2CH(OH)CH_2N(CH_3)_2$, 623-57-4; $\beta$-bromolactic acid, 32777-03-0.