

Hydrophilic Spacer Groups in Polymerizable Lipids: Formation of Biomembrane Models from Bulk Polymerized Lipids

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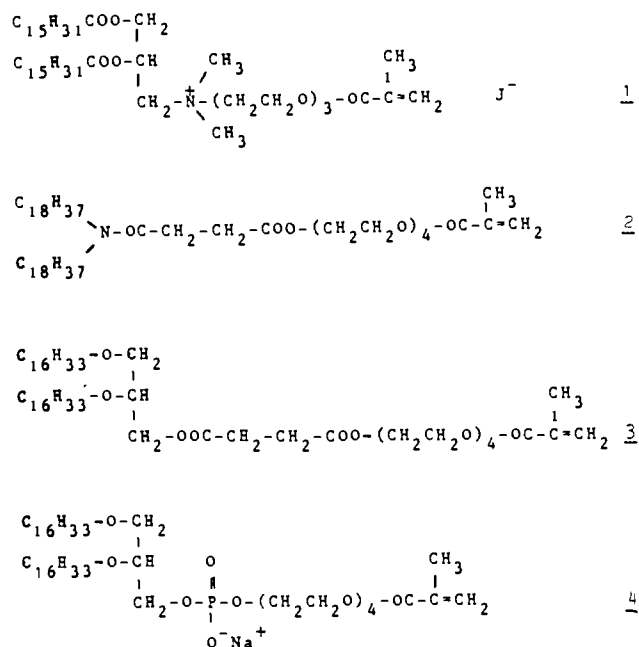
Abstract: A variety of polymerizable lipids containing a hydrophilic spacer group between the reactive group and the main amphiphilic structure have been synthesized. They were investigated in monolayers, liposomes, and multilayers. When the spacer concept was used, efficient decoupling of the motions of the polymeric chain and the amphiphilic side groups is achieved. Thus, the often found loss of the important fluid phases by polymerization is avoided. Polymeric monolayers of the spacer lipid, prepared either by polymerization in the monolayer or by spreading of prepolymerized lipid, exhibit nearly identical surface pressure-area diagrams. Most distinctly, the successful decoupling of the motions of the polymer main chain and the membrane forming amphiphilic side groups is demonstrated by the self-organization of bulk polymerized spacer lipids to polymeric liposomes. In addition, spacer lipids are able to build polymeric Langmuir-Blodgett multilayers. The decoupling of the polymer main chain and the membrane-forming amphiphilic side groups enables the deposition of already polymeric monolayers onto supports to form defined multilayers. If, alternatively, monomeric monolayers are deposited and polymerized on the support, defects in the layers due to structural changes during the polymerization are avoided by the flexible spacer group.

Biomembrane Models and the Function of Spacers. The self-organization of amphiphiles in aqueous systems results in the formation of biomembrane models. Such models include monolayers, liposomes (or vesicles), BLM's, and Langmuir-Blodgett (LB) multilayers. In general, these types of aggregates show poor stability compared to the biomembrane. The lack of stability can be overcome by the polymerization of reactive groups within the amphiphiles,^{1,2,3} but the resulting polymer chain interferes with the motion of the side chains and usually causes a decrease or even the loss of the fluid phases.^{1,2,4} More drastically, the reduced mobility due to the polymer backbone hinders efficient self-organization of polymerized lipids. Particularly, the formation of liposomes is prevented. Except for some hydrophilic copolymers with lipid side chains,⁵ polymeric liposomes were prepared only by polymerization of preformed monomer vesicles.^{3,6,7,23,24}

Because effective biomembrane mimetic chemistry depends on the combination of both stability and mobility of the model membranes, several attempts have been reported in the literature to decrease the antagonism between polymer-enhanced stability and polymer-reduced mobility. Liposomes in a net,⁸⁻¹¹ polymerizable-depolymerizable vesicles,¹² and phase-separated mixed vesicles containing monomeric functional arrays in a polymeric matrix⁴ have been investigated.

An alternative concept is presented by the introduction of a hydrophilic spacer group between the main amphiphilic part and the reactive group of polymerizable lipids. The importance of spacer groups for the function of reactive centers fixed to carriers

Chart I. Synthesized Polymerizable Lipids with Hydrophilic Spacer Groups



is well-known. Enzymes attached directly to polymers often lose their activity.^{13,14} A special spacer model has been discussed for polymeric drugs also.¹³ In addition the use of a spacer has been demonstrated for liquid crystalline side-chain polymers. They can be built systematically by the introduction of a spacer between the polymer backbone and the mesogenic side groups.¹⁵ Thus, the motion of the random polymer coil and the anisotropic mesogenic units is partially decoupled to allow the formation of liquid crystalline phases.

With that in mind, the use of the spacer concept for polymeric model membranes was investigated. In an attempt to decouple

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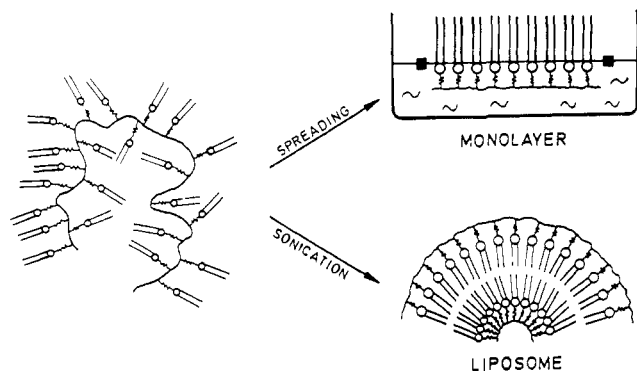
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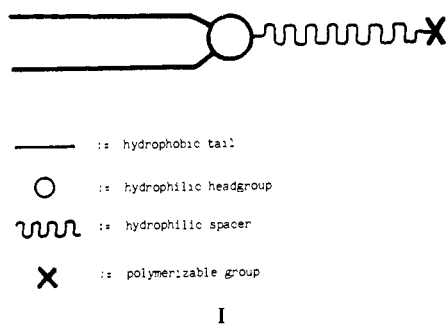
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Scheme 1. Principle of the Decoupling of the Polymer Main Chain Motion and the Self-Organization of the Amphiphilic Side Chains in Polymeric Spacer Lipids



the motions of the polymeric chain and the amphiphilic side groups, hydrophilic spacer groups were introduced into polymerizable lipids. The structure of such polymerizable lipids and



the desired decoupling effect of the hydrophilic spacer group is illustrated in Scheme I. Chart I summarizes the spacer lipids synthesized. Cationic, nonionic, and anionic head groups were investigated.

Materials and Methods

Materials. Tetrahydrofuran (THF), dichloromethane, and triethylamine (reagent grade) were liberated from water by percolation through a column of basic alumina (70–230 mesh; Merck). *N,N'*-Dicyclohexylcarbodiimide, carbonyldiimidazole, 4-(dimethylamino)pyridine, and succinic anhydride were obtained from Aldrich. Tetraethylene glycol and eosin was purchased from Fluka. The synthesis of hexacosyl-10,12-dioic acid, **5**, was described previously.¹⁶

Synthesis of the Lipids with Hydrophilic Spacers 1–4. **2,3-Bis(hexadecanoyloxy)propyl-9-methacryloyl-3,6,9-trioxanonyldimethylammonium Iodide (1).** [2,3-Bis(hexadecanoyloxy)propyl]dimethylamine (1.26 g, 4.9 mmol) was quarternized with excess triethylene glycol monoiodohydrin (2 g, 3.4 mmol) in 50 mL of acetonitrile/acetone (9:1, by v/v) by stirring at 60 °C for 4 days. After cooling the reaction mixture precipitate was isolated and recrystallized from acetone: yield, 1.2 g (41%); mp 79–81 °C.

Dicyclohexylcarbodiimide (0.29 g, 1.4 mmol) dissolved in dry dichloromethane (30 mL) was added to a solution of the obtained alcohol (1 g, 1.17 mmol), methacrylic acid (0.15 g, 1.76 mmol), and 4-(dimethylamino)pyridine (4 mg) in dry dichloromethane (50 mL) at 10 °C. After the solution was stirred for 12 h at room temperature, the precipitated urea was separated by filtration. The filtrate was washed twice with saturated sodium bicarbonate solution and with water. The crude reaction product was purified by liquid chromatography on silica gel using chloroform/methanol/aqueous ammonia (starting with 100/15/1 over to 100/30/2 by volume) as eluent: yield, 0.35 g (32%), waxy solid; ¹H NMR (CDCl₃) δ 0.9 (t, 6 H, CH₃(CH₂)₁₃), 1.1–1.7 (m, 52 H, CH₃(CH₂)₁₃), 1.9 (t, 3 H, CH₂=CCH₃), 2.25 (t, 4 H, CH₂COO), 3.4 (d, 6 H, N(CH₃)₂), 3.7–4.4 (m, 14 H, CH₂CH₂O, CH₂NCH₂), 5.6–6.1 (m, 2 H, CH₂=C); IR (KBr) 2915, 2860 (CH₃, CH₂), 1730, 1745 (CO), 1635 (C=C), 1125 (COC) cm⁻¹. Anal. Calcd for C₄₇H₉₀INO₈: C,

61.09; H, 9.82; N, 1.57; I, 13.73. Found: C, 60.85; H, 10.04; N, 1.75; I, 13.64.

12-Methacryloyl-3,6,9,12-tetraoxadodecyl 3-(*N,N'*-Diocetadecyl-carbamoyl)propionate (2). *N,N'*-Diocetadecylsuccinamide (6 g, 9.6 mmol) was dissolved in dry THF (50 mL). After addition of carbonyldiimidazole (2.1 g, 13 mmol), the solution was refluxed for 1 h. After the solution was cooled to room temperature, a 4-fold excess of tetraethylene glycol (7.46 g, 3.84 mmol) and 1 mg of Na were added. The mixture was refluxed for 2 h to obtain the esterified alcohol. The residue was dissolved in ether (150 mL) and washed with water. After the mixture was dried with magnesium sulfate and the solvent evaporated the crude reaction product was purified by liquid chromatography on silica gel using ethyl acetate/hexane (1/1 to 5/1, by volume) as eluent: yield, 4.3 g (56%); mp 89–91 °C.

The methacrylic ester was obtained from the alcohol in the same manner as described for **1**: yield, 0.7 g (32%), waxy solid; ¹H NMR (CDCl₃) δ 0.87 (t, 6 H, CH₃(CH₂)₁₆), 1.1–1.7 (m, 64 H, CH₃(CH₂)₁₆), 2.0 (t, 3 H, CH₂=CCH₃), 2.65 (t, 4 H, N(CH₂)₂), 3.6–3.8 (m, 12 H, CH₂CH₂O), 3.1–3.4 (m, 4 H, NOCCH₂CH₂COO), 4.25 (t, 4 H, COOCH₂), 5.5–6.0 (m, 2 H, CH₂=C).

Anal. Calcd for C₅₂H₉₉NO₈: C, 72.09; H, 11.52; N, 1.62. Found: C, 71.85; H, 11.41; N, 1.71.

2,3-Bis(hexadecyloxy)propyl 12-Methacryloyl-3,6,9,12-tetraoxadodecyl Succinate (3). The synthesis of 1,2-*O*-dihexadecylglycerol has been described elsewhere.¹⁷

1,2-*O*-Dihexadecylglycerol (5 g, 9.3 mmol) was reacted with succinic anhydride (1.39 g, 13.9 mmol) and 1.5 g of pyridine in chloroform at 55 °C for 40 h. The solution was washed with 1 N HCl, saturated NaHCO₃ solution, and water. After drying with sodium sulfate and evaporation of the solvent, the reaction product was recrystallized from ether: yield, 5.3 g (89%); mp 52 °C.

Esterification with tetraethylene glycol was performed as described for **2**: yield, 3 g (47%); mp 43–44 °C.

The methacrylic ester was obtained from the alcohol by the same method as described for **1**: yield, 0.35 g (32%); waxy solid; ¹H NMR (CDCl₃) δ 0.88 (t, 6 H, CH₃(CH₂)₁₄), 1.2–1.7 (m, 56 H, CH₃(CH₂)₁₄), 1.9 (t, 3 H, CH₂=CCH₃), 3.3–3.8 (m, 19 H, CH₂O), 3.46 (t, 4 H, OOCCH₂CH₂COO) 4.0–4.3 (m, 6 H, COOCH₂) 5.6–6.1 (m, 2 H, CH₂=C); IR (KBr) 2915, 2825 (CH₃, CH₂), 1720 (CO), 1630 (C=C), 1140 (COC) cm⁻¹.

Anal. Calcd for C₅₁H₉₆O₁₁: C, 69.19; H, 10.93. Found: C, 69.05; H, 10.71.

Sodium 2,3-Bis(hexadecyloxy)propyl-12-methacryloyl-3,6,9,12-tetraoxadodecylphosphate (4). Freshly distilled phosphorus oxychloride (0.86 g, 5.6 mmol) was stirred and cooled to 0 °C. After the addition of triethylamine (0.56 g, 5.6 mmol) in THF (5 mL), a solution of 1,2-*O*-dihexadecylglycerol (2 g, 3.37 mmol) dissolved in THF (50 mL) was added dropwise. The mixture was stirred for 1 h at 0 °C and for 2 h at room temperature. Thin-layer chromatography showed complete conversion into the phosphoric acid dichloride. To the reaction mixture, a solution of tetraethylene glycol (1.96 g, 10.1 mmol) and triethylamine (0.35 g, 3.5 mmol) in THF (50 mL) was added at 0 °C. After stirring for 14 h at 20 °C, the reaction mixture was filtered by suction to remove the precipitated triethylamine hydrochloride. The solvent was evaporated to a final volume of 30 mL. Hydrolysis was performed by addition of 30 mL of diluted acetic acid (10 vol %). After 15 min, the pH of the reaction mixture was adjusted at pH 8–9 by addition of 0.1 M NaOH. The product was extracted with 100 mL of dichloromethane and dried over sodium sulfate. After evaporation of the solvent to a final volume of 10 mL, acetone (200 mL) was added and the white precipitate was collected: yield, 1.2 g (43%); mp 49–51 °C.

The methacrylic ester of **4** was obtained in the same manner as described for **1**: yield, 0.72 g (55%); mp 38 °C; ¹H NMR (CDCl₃) δ 0.88 (t, 6 H, CH₃(CH₂)₁₄), 1.2–1.7 (m, 56 H, CH₃(CH₂)₁₄), 1.9 (m, 3 H, CH₂=CCH₃), 3.2–4 (m, 19 H, CH₂O), 4.3 (m, 6 H, POCH₂, CH₂OOC), 5.6–6.1 (m, 2 H, CH₂=C); IR (KBr) 3420 (PO), 2910, 2850 (CH₃, CH₂), 1710 (CO), 1630 (C=C), 1100 (COC).

Anal. Calcd for C₄₇H₉₂O₁₁NaP: C, 63.63; H, 10.45; P, 3.49. Found: C, 63.25; H, 10.38; P, 3.30.

Methods. The infrared spectra were recorded with a Beckman IR 4220 spectrometer. The IR bands are reported in wavenumbers (cm⁻¹). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker WH-90 (90 MHz) spectrometer. Chemical shifts are reported in ppm (δ) downfield relative to tetramethylsilane. Ultraviolet spectra were recorded with a Beckman DU-6 spectrometer. For DSC measurements, Model DSC-2C (Perkin-Elmer) was used. Transmission electron micrographs were taken with Model EM 300 (Philips).

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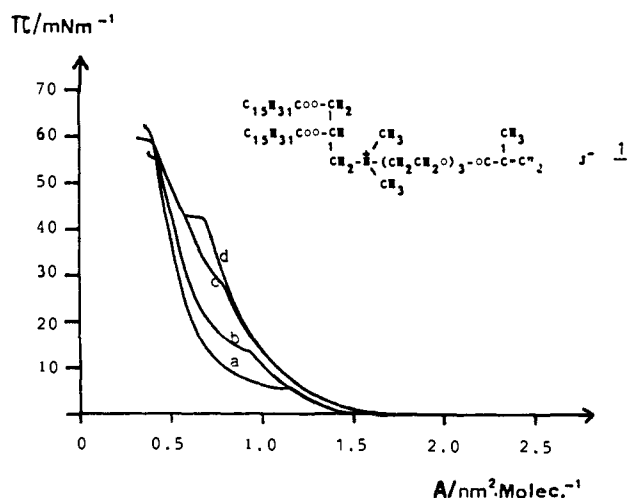


Figure 1. Surface pressure/area isotherms of the spacer lipid **1** on water (pH 5.5) at different temperatures: a = 1 °C, b = 10 °C, c = 20 °C, d = 30 °C.

For scanning electron microscopy, Model Cambridge Mark II A was used. The samples were sputtered with gold. X-ray wide-angle scattering was performed with a wide-angle diffractometer Type D 500 (Siemens). A Cu K line was used. Microanalysis were performed by Microanalysis Laboratories, Universität Mainz.

Monolayer Experiments. For the characterization of the monolayers, a computer-controlled film balance was used.¹⁸ The monomeric and polymeric lipids were spread from CHCl₃ solutions having concentrations of about 0.2 mg/mL. The subphase was water-purified by a Milli Q water purification system (Millipore). The water quality exceeds the quality of triply distilled water.¹⁸

Liposome Formation. The monomeric liposome samples were prepared by ultrasonating dispersions (1 mg/mL) of the lipids in water or phosphate buffer solution at a temperature above the phase transition (5 min, 20 W, Branson sonifier Model B 15).

Liposomes of prepolymerized lipid were prepared by ultrasonication of dispersions of the polymer of 0.1 mg/mL at 60 °C (30 min, 20 W).

Polymerization. Polymeric monolayers and liposomes were obtained by following methods: (a) The monomeric monolayers were flushed with nitrogen for 1 h and irradiated for 1 h with UV light. During the polymerization a surface pressure of 10 mN m⁻¹ was kept. (b) 2,2'-Azoisobutyronitrile (0.02 mg) was added to an opaque suspension of the lipid which was then sonicated for 3–5 min and flushed with argon for 30 min. Polymerization of the liposomes was carried out at 60 °C for 20 h.

The lipids were polymerized in solution by the following method: To the lipid dissolved in toluene (10 mg/mL), 0.2 mg of 2,2'-azoisobutyronitrile was added. After the mixture was flushed with argon for 30 min, polymerization was carried out at 60 °C for 20 h. The polymeric lipid was precipitated with methanol and dissolved in CHCl₃ for monolayer studies and spread. Liposomes were prepared from the precipitated polymer as described above.

Leakage. About 2 mg of the lipid was ultrasonicated in 2 mL of 0.1 M eosin in 0.1 M Tris/0.1 M NaOH. Free eosin was removed by passing 500 μL of the sonicated solution through a short column (13 × 180 mm) of Sephadex G-50 at 20 °C, with 0.1 M Tris-HCl/0.1 N KCl as eluent. The void volume fraction ($V_e = 7$ –10 mL) contained the marked liposomes. For determination of total entrapped eosin, the dye was released from the vesicles by ultrasonication for 10 min and the fluorescence was measured.

Preparation of Multilayers. As support materials, polypropylene foils (Celgard 2400/Celanese, USA, and Trespaphan PED 6/Kalle, Federal Republic of Germany), poly(tetrafluoroethylene) (2-mm foil/Huth, Germany), polyester (Hostaphan RE 3,0/Kalle, Germany), and silanized quartz slides (Suprasil/Heraeus-Schott, Germany) were used. The flexible polymer sheets were attached to a Teflon sample holder.¹⁹

Multilayers of **4** were built up by using pure water subphases. The transfer of the monolayers occurred at 45 mN m⁻¹ surface pressure at 20 °C with a dipping speed of 5 cm/min downward and 0.5 cm/min upward. The samples were allowed to stay 5 min in the air before the next dip. This was necessary because otherwise the last deposited monolayer was retransferred to the water surface. LB multilayers of poly-

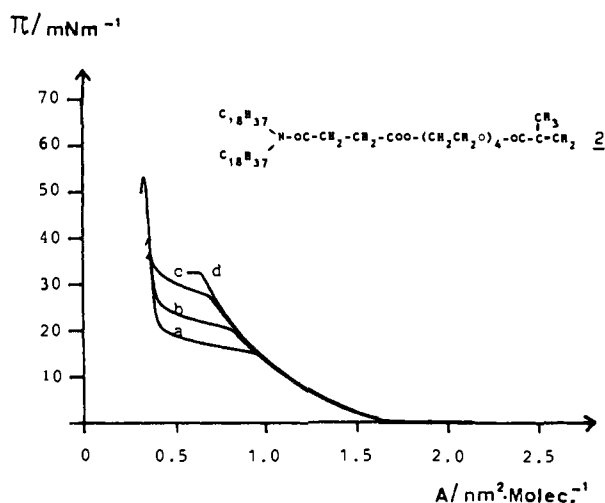


Figure 2. Surface pressure/area isotherms of the spacer lipid **2** on water (pH 5.5) at different temperatures: a = 1 °C, b = 10 °C, c = 20 °C, d = 30 °C.

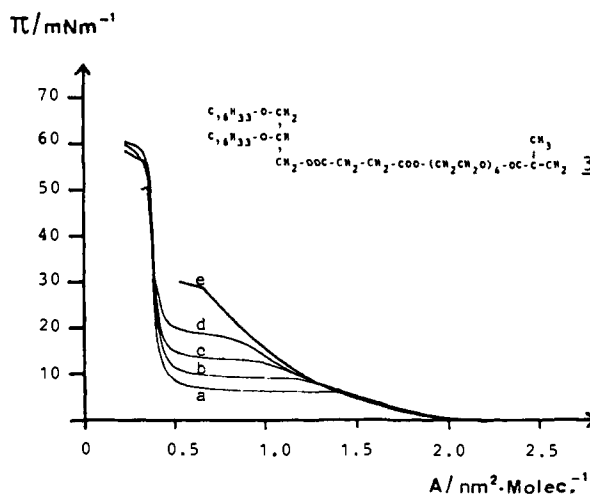


Figure 3. Surface pressure/area isotherms of the spacer lipid **3** on water (pH 5.5) at different temperatures: a = 1 °C, b = 10 °C, c = 20 °C, d = 30 °C, e = 40 °C.

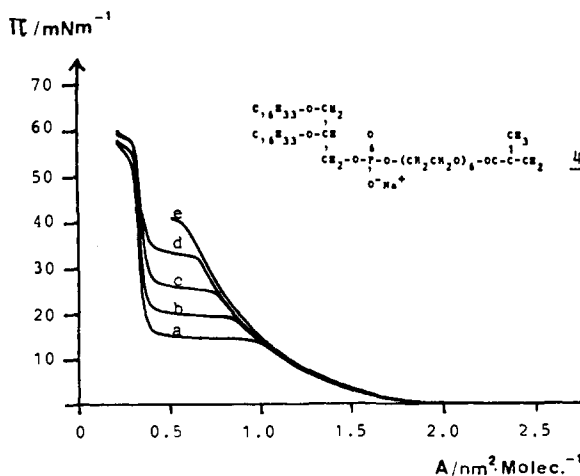


Figure 4. Surface pressure/area isotherms of the spacer lipid **4** on water (pH 5.5) at different temperatures: a = 1 °C, b = 10 °C, c = 20 °C, d = 30 °C, e = 40 °C.

meric lipid **4** were built by using pure water subphases, at 20 °C, 30 mN m⁻¹ surface pressure, and with dipping speeds of 2.5 cm min⁻¹ downward and 0.5 cm min⁻¹ upward. The samples were allowed to stay 5 min in the air before the next dip, as discussed for the monomeric lipid. Multilayers of **5** were built up by using subphases containing 1 g of Cd-Cl₂·H₂O per liter.¹⁹ A surface pressure of 25 mN m⁻¹ was applied at

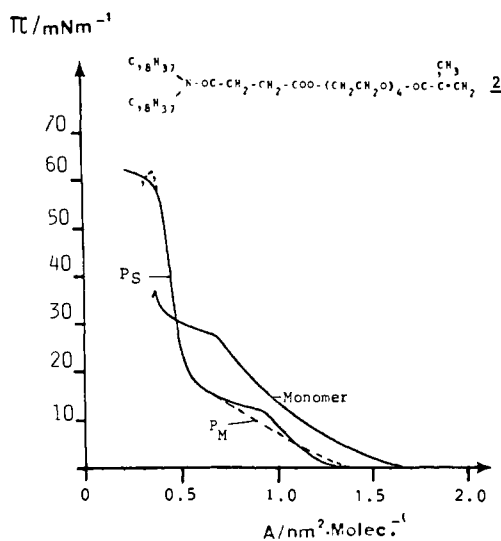


Figure 5. Surface pressure/area isotherms of the spacer lipid **2** on water (pH 5.5): P_M = polymerized in monolayer; P_S = prepolymerized in solution.

20 °C. The dipping speed was 5 cm/min downward and 2 cm/min upward.

The multilayers were polymerized at room temperature in air by a pen ray UV lamp Model 937-002 (Hamamatsu Corp.). The light intensity was 0.5 mW cm⁻², major intensity at 254 nm.

Results and Discussion

Spreading Behavior of the Monomers. Figures 1–4 show the spreading behavior of the monomeric spacer-lipids **1–4** at different temperatures. The surface pressure–area isotherms of all four lipids exhibit striking similarities. A liquid analogous phase is always observed, which stretches over a wide temperature range. The area occupied per molecule in the fluid phases are exceedingly large for all the lipids. This is caused by the large ethylene oxide containing spacer groups. At higher temperatures, only liquid analogous phases are observed. At lower temperatures, coexistence of liquid analogous and solid analogous phases is found. Whereas in the fluid phases large areas are occupied, the solid analogous phase is characterized by tight packing of the hydrophobic chains. Collapse areas down to 0.38 nm²·molecule⁻¹ and high collapse pressures are found. This shows that the mobility of the spacer groups in the solid analogous state is reduced and cannot interfere with the packing of the chains, pointing to a partially stretched or helical orientation of the ethylene oxide spacer. Thus, the spreading behavior of the lipids **1–4** is dominated by the spacer groups in the fluid phases and by the packing of the hydrophobic chains in the solid analogous phases.

Spreading Behavior of the Polymers. Two pathways to ordered polymeric monolayers are possible. The usual method is the polymerization of monomeric monolayers.^{1,20,21} The alternative method, the spreading of polymerized lipids, usually leads to less ordered polymeric monolayers.^{21,22} Because the motions of the polymer chain interfere with the self-organization of the amphiphilic side groups, perfect packing is hindered.

In the case of the lipids **1–4** with hydrophilic spacers, the mobility of the amphiphilic groups is retained after the polymerization. This is exemplified by the surface pressure–area diagrams and the isobars of the lipids **2** and **4** in Figures 5–8. As shown, all the polymeric monolayers still exhibit fluid phases after the polymerization. Figures 6 and 8 show that the prepolymerized lipids have nearly the same spreading behavior as those lipids polymerized in the monolayer. This demonstrates that the spacer

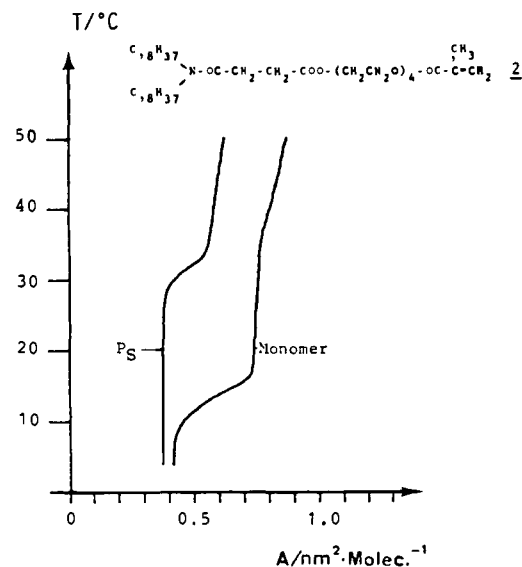


Figure 6. Temperature/area isobars of the spacer lipid **2** on water (pH 5.5) at $\pi = 25 \text{ mN m}^{-1}$: P_S = prepolymerized in solution.

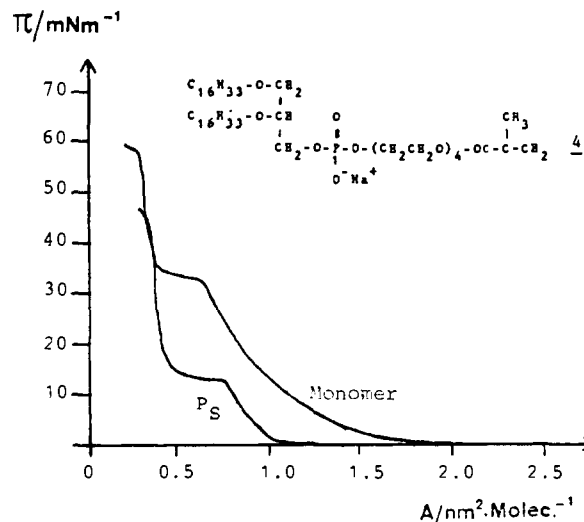


Figure 7. Surface pressure/area isotherms of the spacer lipid **4** on water (pH 5.5): P_S = prepolymerized in solution. $T = 30 \text{ }^\circ\text{C}$.

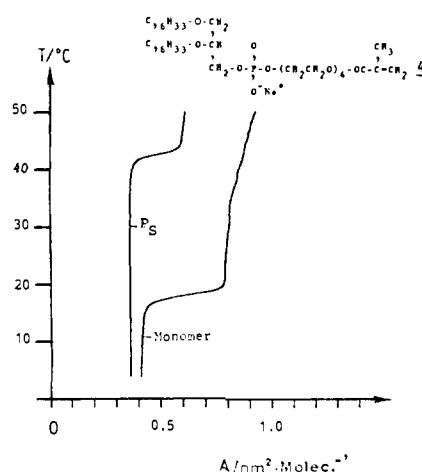


Figure 8. Temperature/area isobars of the spacer lipid **4** on water (pH 5.5) at $\pi = 25 \text{ mN m}^{-1}$: P_S = prepolymerized in solution.

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groups allow a polymer chain independent orientation of the amphiphilic side groups.

The isotherms in Figures 5 and 7 and the isobars in Figures 6 and 8 show some characteristic distinctions between monomers and polymers. The phase transition of the polymeric monolayers

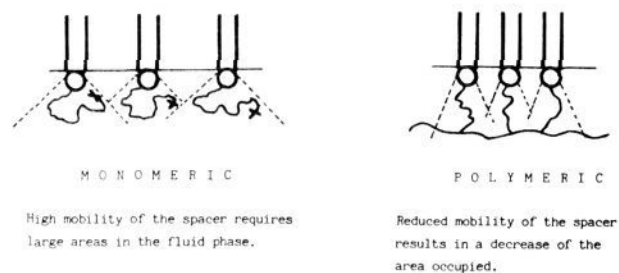


Figure 9. Schematic representation of the influence of the hydrophilic spacer on the area occupied by the lipids in the monolayer.



Figure 10. Freeze-fracture electron micrograph of liposomes from pre-polymerized spacer lipid 4.

is shifted to higher temperatures. Of special interest is the fact that the areas occupied by the amphiphilic side groups of the polymer in the fluid phases are significantly lower than the areas occupied by the monomers, e.g., at 5 mN m^{-1} , 30°C , for lipid 2, monomer, $1.35 \text{ nm}^2/\text{molecule}$, polymer, $1.10 \text{ nm}^2/\text{molecule}$. For lipid 4, monomer, $1.50 \text{ nm}^2/\text{molecule}$, polymer, $0.95 \text{ nm}^2/\text{molecule}$. This might be caused by the reduced independent mobility of the spacers after polymerization as shown schematically in Figure 9. However, in the solid analogous phase, the monomeric and polymeric monolayers show the same packing (Figure 7). In this case, the properties are controlled by the chain packing which is scarcely affected by the polymerization.

Formation of Liposomes. Of all biomembrane models, liposomes are the closest to cell membranes and therefore of a special interest. As discussed above, the stability of the low molecular weight liposomes is improved by the polymerization of reactive groups in the lipids.^{2,3,7,23,24} Similar to polymeric monolayers (Figure 5), there are two pathways of the formation of polymeric vesicles. The polymerization of preformed liposomes was first reported in 1979⁶ and is now a widely used technique.¹ The alternative method is the formation of vesicles of prepolymerized lipids. Up to now, there is only one report about liposomes from polymers using hydrophilic copolymers with lipid side groups attached.⁵ It was interesting to see if the spacer concept, as discussed in the introduction, could lead to polymeric liposomes from prepolymerized lipids as shown in Scheme I.

Indeed, it could be shown that polymeric vesicles from the spacer lipids 1–4 can be produced by both methods, the polymerization of preformed vesicles and the ultrasonication of polymeric lipids prepolymerized in solution. In the latter case, the isolated polymeric lipids were ultrasonicated in water to yield opaque solutions. The formation of liposomes was shown by scanning electron microscopy and freeze-fracture electron microscopy (Figures 10 and 11).

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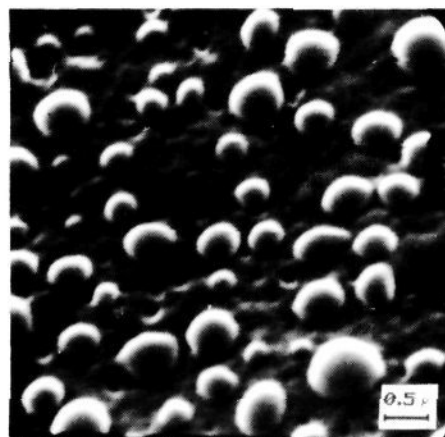


Figure 11. Scanning electron micrograph of liposomes from pre-polymerized spacer lipid 4, magnification 20000X.

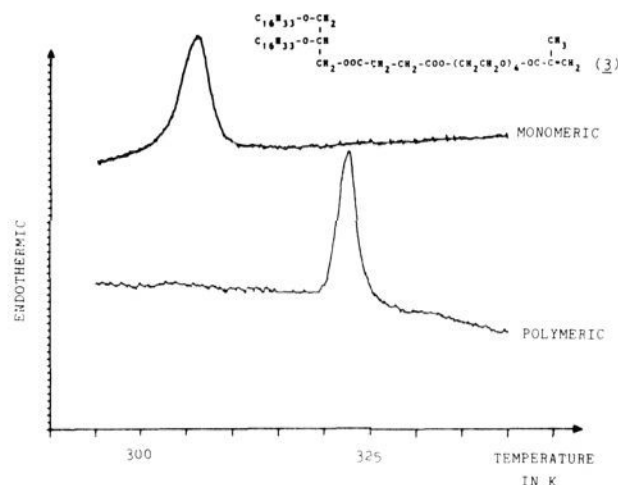


Figure 12. DSC diagrams of vesicles prepared from spacer lipid 3: a = monomeric, b = polymerized.

Table I. Phase Transition of Monomeric and Polymerized Liposomes Prepared from the Spacer Lipids 1–4 Determined by DSC

Compound	T_C Monomer in K	T_C Polymer in K
1	315	322
2	290	305
3	306	322
4	282	323

Usually the polymerization of lipids in liposomes causes a loss of the phase transition,²⁵ comparable to the behavior of polymeric monolayers.²⁶

Typical for the polymeric vesicles prepared from spacer lipid monomers is the existence of a phase transition. The phase-transition temperature, T_C , is shifted to higher temperatures compared to the monomeric vesicles (Table I and Figure 12). Further, the transition seems to be narrowed.

This is in contrast to the rare phase transitions found in polymerized vesicles previously^{4,5} which were broadened and shifted to lower temperatures T_C . The results were explained by distortion

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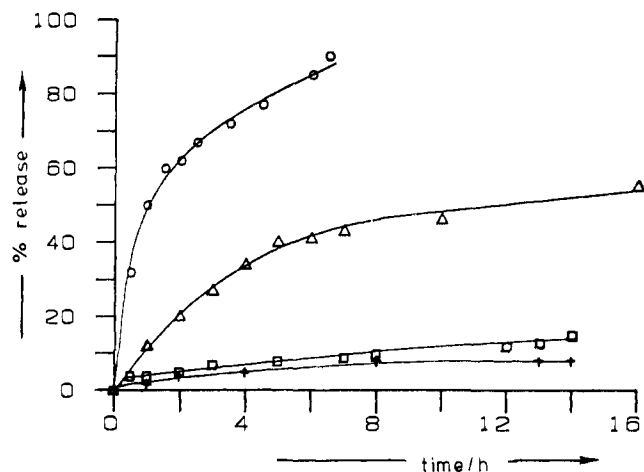


Figure 13. Leakage of monomeric and polymeric liposomes of lipid 4. Percent release of eosin dye vs. time at 20 and 60 °C. (O) Monomer, 20 °C. (+) Polymerized as vesicles, 20 °C. (□) Prepolymerized in solution, 20 °C. (Δ) Polymerized as vesicles, 60 °C.

of the membranes due to the polymeric chains. In case of the polymerized vesicles prepared from the spacer lipids, the spacer groups decouple the motions of the polymeric chain and the self-organization of the amphiphilic side chains. Hence, distortions of the membrane are avoided, and the enhanced cooperativity of the polymerized lipids leads to a narrowed phase transition shifted to higher temperatures T_C . Calorimetric investigations of polymeric vesicles derived from prepolymerized lipids could not be performed because the lipid content of vesicle dispersions is too low for measurements. In addition to electron microscopy, the formation of liposomes from prepolymerized lipids was shown by the entrapment of water-soluble dyes with the subsequent measurement of the leakage kinetics (Figure 13).

The leakage rates for polymeric vesicles prepared by both methods are comparable and are much lower than the leakage rates of the monomeric vesicles. Because the phase transition of polymerized vesicles is preserved, leakage rates of monomeric and polymeric vesicles in the fluid phase can be measured too. As seen in Figure 13, the leakage rate of the polymerized vesicles in the fluid phase at 60 °C is still much lower than the leakage rate of the monomeric vesicles at 20 °C. This demonstrates the reduced permeability of polymeric vesicles in the fluid phase also. The comparison of the fluid phase and the gel phase shows that above the phase transition, the leakage rate is increased by a factor of 5.

Multilayers. Recently, LB multilayers²⁷ have found widespread interest because of their highly ordered structure in molecular dimensions.²⁸⁻³³ Potential applications of the multilayer systems requires stability of the layers. This can be achieved by polymerization of the amphiphiles.³⁴⁻³⁶

Analogously to polymeric monolayers and vesicles, two pathways to polymeric multilayers exist. Either monomeric monolayers are transferred from the water surface onto the solid support and the amphiphiles are polymerized in the built-up multilayer or already polymeric monolayers are transferred onto the support. Both methods have inherent problems. The advantageous use of

Table II. Thicknesses of Lipid Sandwich Bilayers in Multilayers, Determined by X-ray Wide-Angle Scattering

Compound	Thickness of a Sandwich Bilayer in nm	Calculated Maximal Thickness of a Sandwich Bilayer* in nm
monomer	7.09	9.9
polymer		
a) polymerized in the multilayer	6.95	
b) polymerized in solution before spreading	6.9	
monomer	5.68	7.3
polymer (red form)	6.15	

* = hydration of the head groups not considered

the spacer lipids for polymeric multilayers prepared by either method was investigated.

The polymerization of built-up multilayers has been investigated for several years.³⁴⁻³⁶ The covalent linkage of the amphiphiles within the layers enhances the stability of the multilayers. However, normally the polyreaction causes disturbances in the layers or even formation of defects.^{37,44} This can be demonstrated by the example of the hexacosanoic acid, 5, a typical polymerizable lipid used for LB multilayers.¹⁹ The comparison of the measured thickness of a sandwich bilayer of 5.68 nm (see Table II) and the calculated value for the maximal stretched conformation of about 7.3 nm indicates a tilting of the lipids in the layers. During the polymerization, the tilting is reduced and the thickness of the bilayers enlarged to 6.15 nm. This structural change disturbs the order of the multilayer as can be seen by the decrease of the number of orders observed in X-ray scattering experiments.^{36,37} In bilayers, polymerization can even cause defects (see Figure 14).

These problems might be overcome by the use of lipids containing the hydrophilic spacers. The separation of the polymer chain and the membrane-forming part of the molecule should minimize the disturbing influence of the polymer backbone. Based on these considerations, we investigated the possibility to build up LB multilayers with lipid 4. In fact, we succeeded to produce nearly defect-free multilayers, as electron micrographs show (see Figure 15). Monolayer transfer takes place during the downward and the upward dips of the support (Y-type deposition).²¹ It is interesting that the large spacer group does not interfere with the deposition process. The surface pressure-area isotherms (Figure 4) suggest that the spacer group must be oriented to a high degree in the solid analogous phase, extending into the subphase. This orientation might enhance the transfer of the monolayers. The built-up multilayers can be readily polymerized by UV light. Figures 16 and 17 show the polymerization behavior of multilayers of 4, as followed by UV spectroscopy. In contrast to topochemically controlled reactions,^{39,40} complete conversion can be achieved. The electron micrographs in Figure 15 show that no defects caused by the polymerization can be observed even after complete conversion of the monomer. This fits well with the data obtained by X-ray experiments. Wide angle scattering experiments show that the thickness of the sandwich bilayers is only slightly reduced by polymerization from 7.09 to 6.95 nm (Table II). How the spacer groups are arranged in the multilayers is not clear. The

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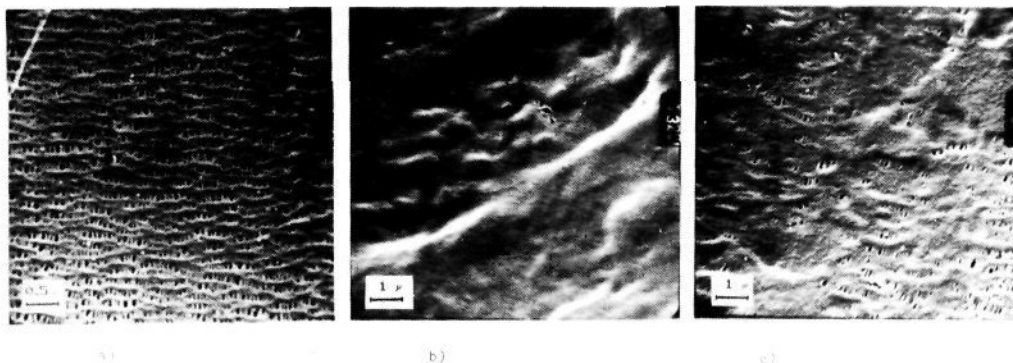


Figure 14. Scanning electron micrographs of bilayers of lipid 5 on Celgard 2400: (a) naked support material, magnification 20.000X; (b) monomeric bilayer, magnification 10.000X; (c) polymeric bilayer (red form), magnification 10.000X.

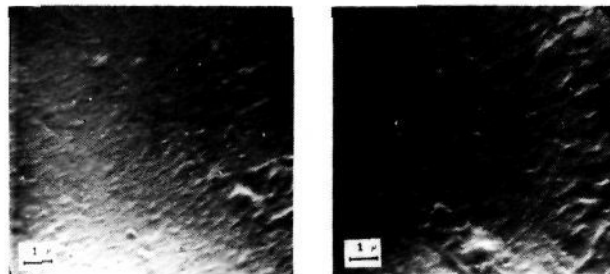


Figure 15. Scanning electron micrographs of bilayers of lipid 4 on Celgard 2400: (a) monomeric bilayer, magnification 10.000X; (b) polymeric bilayer (100% conversion), magnification 10.000X.

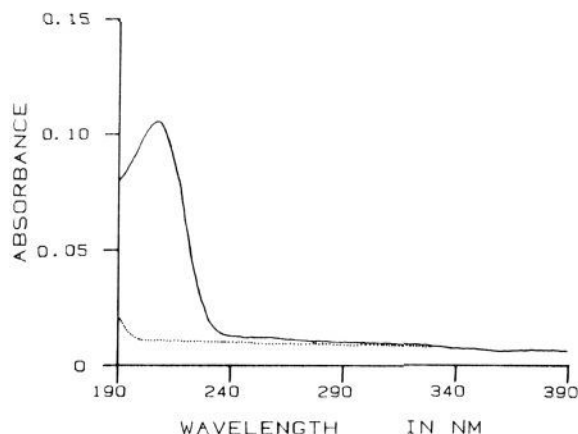


Figure 16. UV spectra of multilayers of spacer lipid 4 on quartz, 40 layer deposited (—) 0-min irradiation, (---) 40-min irradiation.

measured thickness of a sandwich bilayer of 7.09 nm lies between the maximal stretched conformation of the amphiphile of about 9.9 nm and the maximal stretched length of the membrane-forming part (up to the phosphate group) of 6.0 nm (Table II). A possible explanation might be the tilting of the lipids which would mean a tilting angle of 45° against the layer plane. Alternatively, a nonstretched conformation of the spacer may be considered. For example, an U-turn in the middle of the spacer would reduce the spacing to about 7.5 nm, which is nearly the value found. Also a helical structure of the ethylene glycol can be discussed. A nonstretched conformation could also explain the hydrophilic surface of the multilayer during the upward dip. In the case of the fully stretched conformation, the methacrylate group would be exposed at the surface and, thus, a hydrophobic surface would be expected.

The second method to obtain polymeric multilayers, the transfer of polymeric monolayers onto solid supports, should void the problems connected with the polyreaction in the multilayer. Further, a larger variety of polyreactions and monomers can be used, especially if the polymeric monolayers are prepared by the

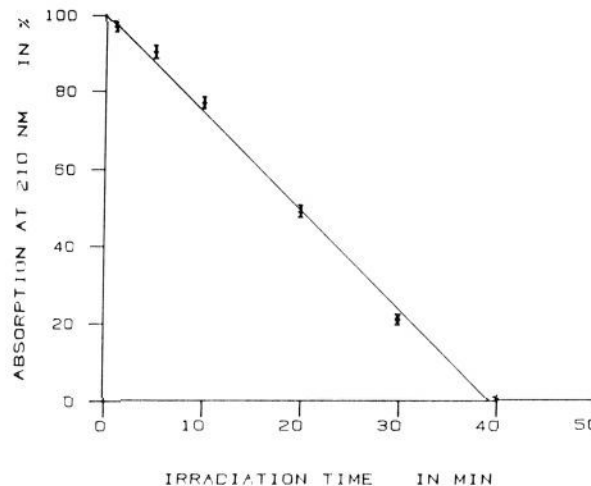


Figure 17. Polymerization of multilayers of spacer lipid 4 by UV irradiation. Polymerization followed by the decrease of monomer absorption at 210 nm.

spreading of prepolymerized amphiphiles.

However, the transfer of polymeric monolayers has been scarcely used up to now because the polymeric chain within the membrane-forming part of the compounds leads to severe problems with deposition. Normally, using the LB technique, only incomplete transfer to the support is achieved. Additionally, due to the strongly reduced flexibility of the monolayers, the transfer causes defects in the layers. Often, no more defined layer structure is found.

Because of these problems, to date, the technique of Langmuir and Schaefer⁴¹ has been used to build multilayers from polymeric monolayers.^{42,43} But, this method is restricted to coat small supports, and hence the applications of such polymeric multilayers are limited.

Because of the efficient decoupling of the motion of the polymer main chain and the self-organization of the amphiphilic side groups found in polymeric monolayers and polymeric vesicles, we investigated the use of prepolymerized spacer lipids for the preparation of LB multilayers. For that purpose, polymeric lipid 4 was spread (see Figure 8) and the surface pressure adjusted at 30 mN m⁻¹ corresponding to the condensed phase of the monolayer. Applying slow dipping speeds downward and upward, the polymeric layers can be transferred onto various support materials as silanized quartz, polypropylene, poly(tetrafluoroethylene), or polyester. Regular Y-type deposition of the monolayers is observed. The thickness of the sandwich bilayer was determined by X-ray wide angle scattering experiments (Table II). As can be seen, the value corresponds to the thickness of multilayers of 4 polymerized on the support. The considerations about the arrangement of the lipid side chains in the multilayer are the same as pointed out above for the multilayers prepared from monomeric lipid. The good agreement of the thicknesses of the polymeric

multilayers built by the different methods suggests that the structure of the layers is independent of the preparation technique chosen. But, without additional investigations including such of other spacer lipid systems, the result should not be overestimated.

Conclusions

The spacer concept, that means the partial decoupling of the motions of the polymer backbone and the orientable, anisotropic side groups, proved to be useful for polymerizable lipids. The main problem of biomembrane models, that the stability achieved by polymerization will exclude the important mobility of the membrane, can thus be overcome. Polymeric model membranes of lipids with hydrophilic spacers still show the desired fluid phases. Moreover, the flexibility of polymerized lipids is sufficient to make possible monolayer spreading and vesicle formation of polymerized lipids. These unique properties offer wide possibilities in biomimetic systems. For example, proteins or enzymes can be incorporated in liposomes efficiently now, which before suffered either from poor stability in case of monomeric lipids or from

reduced fluid phases and damage by the polymerization process in case of polymerized vesicles. The multilayer systems, which are of special technical interest, also profit by the spacer concept. Distortions caused by the polymerization process are minimized, and thus the high quality of the layers is preserved. Furthermore, the flexibility of the polymeric lipids due to the spacer group allows one to build up multilayers from polymeric monolayers. Thus, potential distortions of the lipids created by the polyreaction are avoided, and the scope of polyreactions suited to polymerize amphiphiles is enlarged considerably.

Registry No. 1, 96326-70-4; 1 (alcohol), 96326-75-9; 2, 96326-71-5; 2 (alcohol), 96326-76-0; 3, 96326-72-6; 3 (alcohol), 96326-78-2; 4, 96326-73-7; 4 (phosphoric acid dichloride), 74123-29-8; 4 (dialcohol phosphate), 96326-79-3; 4 (hydrolysis product), 96326-80-6; [2,3-bis-(hexadecanoyloxy)propyl]dimethylamine, 96326-74-8; triethyleneglycol monoiodohydrin, 62573-16-4; methacrylic acid, 79-41-4; *N,N*-dioctadecylsuccinamide, 37519-63-4; tetraethyleneglycol, 112-60-7; 1,2-*O*-dihexadecylglycerol, 6076-35-3; 2,3-bis(hexadecyloxy)propyl succinate, 96326-77-1; phosphorus oxychloride, 10025-87-3.

Stereoelectronic Effect in Anionic 1,1-Addition to Isocyanides. An ab Initio Study of the $H^- + HNC$ System

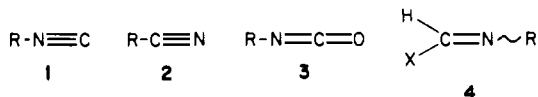
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Abstract: The stereochemistry of 1,1-addition to isocyanides initiated by nucleophilic attack has been modeled by using ab initio methods. The linear HNC molecule is surprisingly easily deformed: 3.9 kcal mol⁻¹ is sufficient to produce a bending of 30°. Addition of H⁻ to the carbon of HNC gives an energy surface in which a distinct valley favoring the *Z* (or *cis*) anion appears early in the reaction. The *Z* anion, then formed [HNCH]⁻, is, however, less stable by about 6.7 kcal mol⁻¹ than the *E* form. The energy barrier for *Z-E* interconversion of the anion is, however, high (38.2 kcal mol⁻¹ using MP4SDQ/6-31+G**//HF/4-31G) so that the *Z* form, although less stable, should be the kinetic product following rapid protonation of the carbanion. The favored route for *Z-E* interconversion of [HNCH]⁻ is by nitrogen inversion rather than inversion at the carbanionic center. The stereospecificity of the addition is consistent with the concerted electronic movement in the same direction about the isonitrile as the nucleophile approaches carbon.

Although isocyanides **1** were first discovered by Lieke in 1859,¹ this class of compounds has received relatively little attention by comparison with isomeric cyanides **2** or their close chemical parents isocyanates **3**.

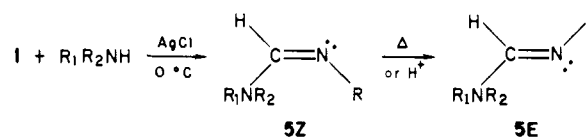
The rearrangement of **1** to **2** and the consequent preparative difficulties in obtaining pure and stable isocyanides was often considered as the main reason for this lack of interest. A ren-



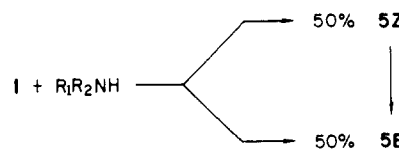
naissance of isocyanides has, however, occurred during the past 2 decades. Efficient and general synthetic routes for the laboratory preparation of isocyanides are now available, and these materials are shown to undergo a variety of reactions.^{2a}

In recent years, isocyanides attracted interest as complexing agents for transition-metal compounds,³ as synthons for a wide range of heterocycles through cycloaddition of their α -metalated

Scheme I



Scheme II



derivatives,⁴ or particularly as potential reagents for antibiotic synthesis via four-component condensations.⁵

Because of the presence of monocoordinated carbon atoms, 1,1-addition reactions of isocyanides to hydrogen compounds (HX)

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