Short synthetic routes are described to produce structurally related phospholipids that are either conformationally restricted or flexible. The conformationally restricted structures have a cyclopropyl ring in the interfacial region of the phospholipid. The key synthetic step is a cyclopropanation reaction between 2(5H)-furanone and a stabilized chloroallylic phosphonate anion. The synthetic routes enable the incorporation of different polar headgroups as well as nonpolar tails at late stages in the sequence. The phosphoethanolamine derivatives 1b and 2b readily form encapsulating vesicles, however, dye leakage from vesicles composed of the restricted phospholipid 1b is significantly slower than from vesicles composed of flexible analogue 2b. Physicochemical analyses using 31P NMR, differential scanning calorimetry, fluorescence anisotropy, and Langmuir—Blodgett films suggest that the decreased permeability of membranes composed of conformationally restricted 1b is due to its ability to pack more closely in a bilayer assembly.

Results and Discussion

Retrosynthetic Analysis. Despite their biochemical importance, there are relatively few general methods for preparing unnatural phospholipids.1 In the case of meso-
phospholipids 1 and 2, a new synthetic approach had to be developed. The stereospecific syntheses are shown in Schemes 1 and 2. Both schemes allow the preparation of phospholipids bearing different headgroups and/or non-polar tails from a late-stage intermediate. In the case of conformationally restricted phospholipid 1, the key step in the sequence is cyclopropanation of 2(SH)-furanone 8 using the methodology developed by Hanessian and coworkers. Since our target compounds, possess meso stereochemistry, we can use the achiral phosphonate 7. At the time of our studies, the precursor 1,3-dichloropropane, 9, was only available as a mixture of cis and trans isomers. Thus, we expected the formation of cyclopropane derivative 6 as a mixture of diastereomers. However, there was literature precedent to suggest that after transformation to 5, the undesired diastereomer could be converted to the desired one via equilibration.5

\textit{Synthesis of Conformationally Restricted Phospholipids} 1. Chloroallyl phosphonate 7 was prepared via Arbuzov reaction in 88% yield on a multigram scale (Scheme 3). The compound was roughly a 3:2 mixture of two inseparable geometric isomers. The (2)-7 isomer was expected to give the cyclopropane derivative with desired trans stereochemistry, whereas in the case of (E)-7 all three substituents on the cyclopropane ring would have undesired cis stereochemistry.4 Treatment of 7 with n-BuLi at −78 °C followed by rapid addition of furan 8 afforded the cyclopropane derivative 6 in 55–60% yield as a mixture of two diastereomers (diastereoselectivity ca. 85:15). A reason that the diastereoselectivity is higher than the starting E/Z ratio of 7 might be that (Z)-7 reacts faster than (E)-7.

Treatment of alkene 6 with ozone, followed by the reduction of the ozonide with Me2S, afforded the aldehyde 5 as a single diastereomer in 61% yield.6 The product 5 was isolated together with an unidentified phosphorus-containing byproduct (the ratio of 5:byproduct ca. 2:1).7 Subsequently, the aldehyde was reduced to the corresponding alcohol by sodium borohydride and the hydroxyl group protected as a silyl ether (Scheme 4).

\textbf{SYNTHESIS OF CONFORMATIONALY RESTRICTED PHOSPHOLIPIDS \textit{1}}

![Scheme 1](image1)

![Scheme 2](image2)

![Scheme 3](image3)

\textbf{SCHEME 3}\textsuperscript{a}

- Reaction conditions: (a) reflux, 20 h, 88% (b) n-BuLi, 2-(SH)-furanone, THF, −78 °C, 2–3 h, 55–60% (c) ozone, −78 °C, then Me2S, 61% (d) NaBH4, THF/MeOH, 0 °C, 15 min; (e) TBDPSCl, imidazole, DMF, rt, 68% (two steps); (f) DIBALH, CH2Cl2, −78 °C, 97%. (g) NaBH4, ETOH, rt, 1.5 h, 87%; (h) 19a: palmityl chloride, Et3N, CH2Cl2, rt, 40 min, 99% 19b: palmiticyl chloride, Et3N, CH2Cl2, 1 h, 99% (i) TBAF, THF, rt, 14 h, 93% (20a), >99% (20b).

\textbf{SCHEME 4}

\textbf{SCHEME 3}\textsuperscript{a}

- Reaction conditions: (a) reflux, 20 h, 88% (b) n-BuLi, 2-(SH)-furanone, THF, −78 °C, 2–3 h, 55–60% (c) ozone, −78 °C, then Me2S, 61% (d) NaBH4, THF/MeOH, 0 °C, 15 min; (e) TBDPSCl, imidazole, DMF, rt, 68% (two steps); (f) DIBALH, CH2Cl2, −78 °C, 97%. (g) NaBH4, ETOH, rt, 1.5 h, 87%; (h) 19a: palmityl chloride, Et3N, CH2Cl2, rt, 40 min, 99% 19b: palmiticyl chloride, Et3N, CH2Cl2, 1 h, 99% (i) TBAF, THF, rt, 14 h, 93% (20a), >99% (20b).

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\textbf{SCHEME 3}\textsuperscript{a}

- Reaction conditions: (a) reflux, 20 h, 88% (b) n-BuLi, 2-(SH)-furanone, THF, −78 °C, 2–3 h, 55–60% (c) ozone, −78 °C, then Me2S, 61% (d) NaBH4, THF/MeOH, 0 °C, 15 min; (e) TBDPSCl, imidazole, DMF, rt, 68% (two steps); (f) DIBALH, CH2Cl2, −78 °C, 97%. (g) NaBH4, ETOH, rt, 1.5 h, 87%; (h) 19a: palmityl chloride, Et3N, CH2Cl2, rt, 40 min, 99% 19b: palmiticyl chloride, Et3N, CH2Cl2, 1 h, 99% (i) TBAF, THF, rt, 14 h, 93% (20a), >99% (20b).


Conformationally Restricted and Flexible Phospholipids

**SCHEME 4**

(a) **20a:**

![Diagram](Image)

(b) **20b:**

![Diagram](Image)

\( R = (\text{CH}_2)_n \text{CH}_3 \)
\( 1b: R = (\text{CH}_2)_{17} (\text{CH}_3)\text{CH}_3 \)
\( 1a: R^1 = \text{H} \)
\( 1c: R^1 = \text{CH}_3\text{CH}_2\text{NH}_2 \)

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\( ^a \) Reaction conditions: (a) **1a:** diphenylchlorophosphate, imidazole, CH\(_2\)Cl\(_2\), rt, 14 h, then cat. PtO\(_2\), H\(_2\), 8 h, rt, 91%.
\( 1c: 2\)-chloro-1,3,2-dioxaphospholane 2-oxide, Et\(_3\)N, benzene, rt, 20 h, then NH\(_3\), benzene/acetonitrile, 4 h, rt, 62%.
\( 1b: 2\)-chloro-1,3,2-dioxaphospholane-2-oxide, Et\(_3\)N, benzene, rt, 20–21 h, then NH\(_3\), benzene 5.5 h, rt, 62%.

**18** in 87% yield. 

Diol **18** is a versatile intermediate, since different nonpolar tails can be incorporated. For example, palmitoyl and palmitoleoyl tails were attached each in 99% yield. The diol **18** was first decarboxylated in 86% yield (Scheme 5). 11

**Synthesis of Flexible Phospholipids 2.** Alcohol **12** was prepared by modification of the method described by Nugent and co-workers. 10 Diethyl diallylmalonate was prepared by modification of the method described. 12 After the hydroxyl group was protected as a silyl ether in 78% yield, the terminal alkenes in **14** were cleaved by first treating the diene with ozone followed by reduction of the ozonide with NaBH\(_4\) to give diol **22** in quantitative yield. 

**SCHEME 5**

![Diagram](Image)

\( ^a \) Reaction conditions: (a) LiCl, DMSO, H\(_2\)O, reflux, 6 h, 86%.
\( b: \text{DIBAL, CH}_2\text{Cl}_2, -78 \, ^\circ\text{C}, \text{then NaBH}_4, \text{MeOH, rt, 30 min, 69%}.
\( c: \text{TBDPSCl, imidazole, DMF, rt, 20 h, 78%}.
\( d: \text{ozone, } -78 \, ^\circ\text{C}, \text{then NaBH}_4, 99\%.

**SCHEME 6**

![Diagram](Image)

\( ^a \) Reaction conditions: (a) **23a:** palmitoyl chloride, Et\(_3\)N, CH\(_2\)Cl\(_2\), 1 h, rt, 70%.
\( 23b: \text{palmitoleic acid, DCC, DMAP, CH}_2\text{Cl}_2, 20 \, h, 77\% \) (b) TBAF, THF, rt, 4–16 h, rt, 79% (24a), 60% (24b).

**SCHEME 7**

![Diagram](Image)

\( ^a \) Reaction conditions: (a) **24a:** diphenylchlorophosphate, imidazole, CH\(_2\)Cl\(_2\), 17 h, then cat. PtO\(_2\), H\(_2\), AcOH, rt, 6 h, 85%.
\( 24b: \text{2-chloro-1,3,2-dioxaphospholane-2-oxide, Et}_3\text{N, benzene, rt, 1 h, then NH}_3, \text{acetonitrile/benzene 4 h, rt, 62%}.

---

(8) On the basis of \(^1\)H NMR in CDCl\(_3\), the lactol/hydroxy-aldehyde ratio in **17** is about 95:5 at room temperature. We have not yet explored the possibility of converting **17** into differentially acylated phospholipids.


(12) Even if excess DIBAL was used, some intermediate aldehyde was always obtained. However, subsequent treatment with NaBH\(_4\) converted all material to desired alcohol **12**.

and polar headgroups. For example, the diol **22** was successfully acylated with palmitoyl chloride in 67% yield (Scheme 6). When palmitoleoyl chloride was used as an acylating agent, the diester **23b** was isolated in less that 50% yield. However, when the corresponding acid was used instead, the desired **23b** was obtained in 77% yield. Removal of silyl protecting groups gave alcohols **24a** and **24b** in 79% and 60% yield, respectively. Different head-
groups were introduced in analogous manner to the above cyclopropane derivatives. Reaction of alcohol 24a with diphenyl chlorophosphate (Scheme 7) followed by the catalytic hydrogenation afforded the phosphatidic acid 2a. The treatment of alcohol 24a with 2-chloro-1,3,2-dioxaphospholane-2-oxide, followed by ring opening with ammonia afforded phosphoethanolamine 2c in 43% yield. As was the case with corresponding cyclopropane-containing analogues, compounds 2a and 2c with saturated tails were practically insoluble in common organic solvents and could not be dispersed in water. However, the unsaturated phosphoethanolamine, 2b, prepared from 24b in 81% yield, was readily soluble in chloroform or methanol.

Physicochemical Studies. The leakage of encapsulated fluorescent dyes from vesicles composed of 1b or 2b was compared to leakage from control vesicles composed of dioleoylphosphatidylcholine (DOPC). Two sets of leakage experiments were conducted. The data in Figure 2 shows leakage of the fluorophore/quencher pair HPTS/DPX (1-hydroxypyrene-3,6,8-trisulfonic acid/p-xylylenebis(pyridinium) bromide) as a function of time. The fluorescence emission of HPTS after excitation at 413 nm is independent of pH, and increases as the HPTS/DPX is diluted upon escape from the vesicles. The leakage experiments were performed at 25, 37, and 50 °C. Vesicles composed of 2b release about 30% of their encapsulated HPTS/DPX over 3 days at 25 °C, while vesicles composed of 1b or DOPC were essentially impermeable (Figure 2A). At 37 °C, the leakage rate from vesicles composed of 2b is twenty-seven times greater that from vesicles composed of 1b which in turn is higher than leakage from vesicles composed of DOPC (Figure 2B). When the temperature is elevated to 50 °C, the vesicles composed of 2b release all of their entire aqueous contents within 5 h, compared to 50 h for vesicles composed of 1b, while DOPC vesicles remain relatively impermeable (Figure 2C). The same relative order of vesicle permeabilities is observed with leakage experiments using encapsulated carboxyfluorescein at neutral pH, although the absolute leakage rates are higher (Figure 3). As previously communicated, the leakage rates do not change with phospholipid concentration, and as leakage progresses there is no increase in vesicle size as judged by dynamic light scattering. Thus, it appears that leakage does not require vesicle collision.

Shown in Figure 4 are 31P NMR spectra of vesicle suspensions composed of 1b, 2b, or egg phosphatidyl-

Figure 2. Percent leakage of HPTS/DPX from 100 nm diameter unilamellar vesicles (50 µM phospholipid) composed of 1b (triangles), 2b (squares), or DOPC (circles). Vesicles contained 5 mM HPTS/1 mM DPX/100 mM NaCl and were dispersed in 5 mM TES/100 mM NaCl, pH 7.4. Experiments were conducted at (A) 25 °C; (B) 37 °C; (C) 50 °C.

Figure 3. Percent leakage of carboxyfluorescein from 100 nm diameter unilamellar vesicles (50 µM phospholipid) composed of 1b (triangles), 2b (squares), or DOPC (circles) at 25 °C. Vesicles contained 50 mM carboxyfluorescein/100 mM NaCl and were suspended in 5 mM TES/150 mM NaCl, pH 7.4.
choline at pH 7.4 and 25 °C. The spectrum for egg phosphatidylcholine vesicles exhibits a broad powder pattern that is typical of a glycerophospholipid in a liquid crystalline lamellar phase. In contrast, the spectra for 1b and 2b exhibit narrow, isotropic signals. Since all three vesicle systems were prepared by sample extrusion through filters with 100 nm pores, the difference in 31P NMR peak shape is unlikely to be due to differences in vesicle tumbling rates. Instead, we attribute the isotropic signals for vesicles composed of 1b or 2b to fast axial diffusion of the structurally symmetrical phospholipids, which leads to motional averaging of the chemical shift anisotropy and dipolar interactions.

Previously communicated fluorescence anisotropy measurements using vesicles containing appropriate fluorescent probes indicate that membranes composed purely of 1b or 2b are fluid phase at 25 °C. Furthermore, analysis by differential scanning calorimetry (DSC) shows that the vesicles do not undergo a measurable phase transition between 10 and 90 °C. Additional DSC measurements were made on samples of dipalmitoleylphosphatidylethanolamine (DiPOPE), the glycerophospholipid version of 1b and 2b with the same palmitoleyl tails and phosphoethanolamine headgroup. The lamellar-inverse hexagonal phase transition temperature, T_H, for DiPOPE is known to be quite sensitive to amphiphilic additives that influence membrane curvature. For example, we recently reported that the presence of one mol % of various triple-chain amphiphiles can raise or lower the T_H for DiPOPE by up to 10 °C. However, in this present case we find that the presence of one mol % 1b or 2b has only a very minor effect on T_H (Figure 5) demonstrating that the nonglycerol interfacial regions in 1b and 2b do not significantly alter the effective size or hydration of the phosphoethanolamine headgroups.

A Langmuir film balance was used to compare the packing of monolayers composed purely of 1b, 2b, or DiPOPE. The compression isotherms shown in Figure 6 were recorded at 25.0 °C. The limiting area, A_∞, a parameter approximating the area occupied by one molecule on the monolayer surface at zero pressure, was 72, 82, and 90 Å² for compounds 1b, 2b, and DiPOPE, respectively. This allows calculation of A_L - A_∞ which reflects the change in molecular area in going from an expanded to a condensed state at the air-water interface.

(18) The presence of one mol % 1b or 2b in DiPOPE increases the transition enthalpy from 47 cal/mol to 114 cal/mol and 155 cal/mol, respectively. This allows calculation of A_L - A_∞ which reflects the change in molecular area in going from an expanded to a condensed state at the air-water interface.

FIGURE 4. 31P NMR spectra of 100 nm diameter unilamellar vesicles (50 mM in 5 mM TES/100 mM NaCl pH 7.4) composed of (A) 1b, (B) 2b, (C) egg phosphatidylcholine.

FIGURE 5. Differential scanning calorimetry. Lamellar to inverse hexagonal phase transitions of: (a) pure DiPOPE; (b) 99:1 mixture of DiPOPE/1b, and (c) 99:1 mixture of DiPOPE/2b. In each case, 14.5 mM phospholipid in 5 mM TES/100 mM NaCl, pH 7.4.
The higher values of $A_\infty - A_{\text{∞}}$ for $1b$ and $2b$ indicate that their expanded states are more "compressible" than DiPOPE which is reasonable since the structures of $1b$ and $2b$ have three additional carbons in their interfacial regions.

**Summary**

Practical and versatile routes have been developed to produce structurally related phospholipids that are conformationally restricted or flexible ($1$ and $2$, respectively). The conformationally restricted structures have a cyclopropyl ring in the interfacial region of the phospholipid. The phosphoethanolamine derivatives $1b$ and $2b$ readily form encapsulating vesicles, however, dye leakage from vesicles composed of the conformationally restricted $1b$ is significantly slower than from vesicles composed of flexible analogue $2b$. It is notable that such a subtle restriction on conformational flexibility can produce a large decrease in vesicle permeability; particularly, since the membranes are in the fluid phase which is characterized by high acyl chain mobility, as well as rapid rotational and translational motion. The monolayer compression data in Figure 6 demonstrates that $1b$ packs more closely in a condensed monolayer than $2b$. The observation that the rate of dye leakage is independent of vesicle concentration suggests that the leakage process is collision independent, and implies that passage of the relatively large, hydrophilic dyes through the membrane involves transient but substantial local reorganization of the bilayer. Thus, we conclude that membranes composed of the restricted phospholipid $1b$ are less likely to form local nonbilayer defects. In other words, the degree of defect formation in a bilayer membrane, and hence the membrane permeability, can be lowered by decreasing structural flexibility at the phospholipid interfacial region.

**Experimental Section.** Full experimental details are provided as Supporting Information.

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**Supporting Information Available:** Experimental methods, synthetic procedures, and NMR spectra of all compounds. This material is available free of charge via the Internet at [http://pubs.acs.org](http://pubs.acs.org).

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