papers and notes on methodology

Synthesis of polar head group homologs of all-*trans*-*cyclopentano*-phosphatidylcholine, phosphatidyl-N,N-dimethylethanolamine, and phosphatidylethanolamine[1,2]

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Abstract The polar head group region of a conformationally restricted analog of phosphatidic acid (diacylglycero-phosphate) has been systematically modified to give analogs of phosphatidylcholine, phosphatidylethanolamine, and phosphatidyl-N,N-dimethylethanolamine. These analogs differ from their natural counterpart in both the backbone region and in the polar head region, respectively, as follows: the diacylglyceryl moiety has been replaced by an all-*trans* diacylcyclopentane-1,2,3-triol moiety and the phosphorus-nitrogen separation has been increased incrementally from two to nine methylene units. The synthesis of these homologous series involved phosphorylation of (1,3/2)-2,3-dipalmitoylcyclopentane-1,2,3-triol with each of a series of homologous bromoalkylphosphoric acid dichlorides, which were themselves obtained by phosphorus oxychloride treatment of the homologous bromoalkanols. The resulting bromoalkyl esters of 2,3-dipalmitoylcyclopentane-1,2,3-triol-1-phosphoric acid were reacted with trimethylamine, dimethylamine, or ammonia to give the *cyclopentano*-phosphatidylcholines, *cyclopentano*-N,N-dimethylethanolamines, and *cyclopentano*-phosphatidylethanolamine, respectively. All the compounds were obtained as stable microcrystalline solids. The yields of *cyclopentano*-phosphatidylethanolamines and of *cyclopentano*-N,N-dimethylethanolamines were reduced by the formation of compounds which analyzed as monoacyl (lyso) derivatives.—**Pajouhesh, H., and A. J. Hancock.** Synthesis of polar head group homologs of all-*trans*-*cyclopentano*-phosphatidylcholine, phosphatidyl-N,N-dimethylethanolamine, and phosphatidylethanolamine. *J. Lipid Res.* 1984. **25:** 294–303.

Supplementary key words *cyclopentano*-phosphatidylethanolamine homologs • *cyclopentano*-lecithin homologs • *cyclopentano*-phosphatidyl-N,N-dimethylethanolamine homologs • modified head group • restricted conformational mobility • cyclitols

Recent communications from this laboratory have described the synthetic routes to a variety of conformationally restricted phospholipids. These lipids are derivatives of the three isomeric cyclopentane-1,2,3-triols (**Fig. 1, 1–3**) and differ from their natural glyceryl counterparts in that free rotation along C–C single bonds of the phospholipid backbone is not possible. A *cyclopentano*-phospholipid, therefore, mimics a narrow range of conformational (rotameric) states of the glycerol backbone in a given type of glycerophospholipid.

We have demonstrated that the *cyclopentano*-phospholipids generally share common properties with their *glycero*-counterparts (3–6), but in certain physical or biochemical experiments there are observed subtle differences in behavior. These differences appear to support our conviction that, at least for some lipid interactions, the conformational disposition of the lipid backbone is critical to the effectiveness of the interaction. Thus, the degree of cooperativity of the lipid-lipid interaction in a

Abbreviations: 1,3/2-*cyclopentano*-PE-diMe, 1,3/2-*cyclopentano*-N,N-dimethyl-phosphatidylethanolamine (dipalmitoyl ester); 1,3/2-*cyclopentano*-PE, 1,3/2-*cyclopentano*-phosphatidylethanolamine (dipalmitoyl ester); 1,3/2-*cyclopentano*-PC, 1,3/2-*cyclopentano*-phosphatidylcholine; EDTA, ethylene diaminotetraacetic acid; DMF, dimethylformamide; GLC, gas–liquid chromatography; THF, tetrahydrofuran; TLC, thin-layer chromatography; DPPC, dipalmitoylphosphatidylcholine.

[1] This work forms part of a dissertation submitted by Hassan Pajouhesh to the Department of Chemistry, University of Missouri, Kansas City, for the Ph.D. degree. Present address (HP): Department of Chemistry, Southern Methodist University, Dallas, TX 75275.

[2] Cyclic compounds described in this paper are named according to the tentative rules for nomenclature of cyclitols (1). The names are derived from those of the parent cyclanes of which they are formal derivatives. A summary of these rules has been presented in an earlier communication (2).

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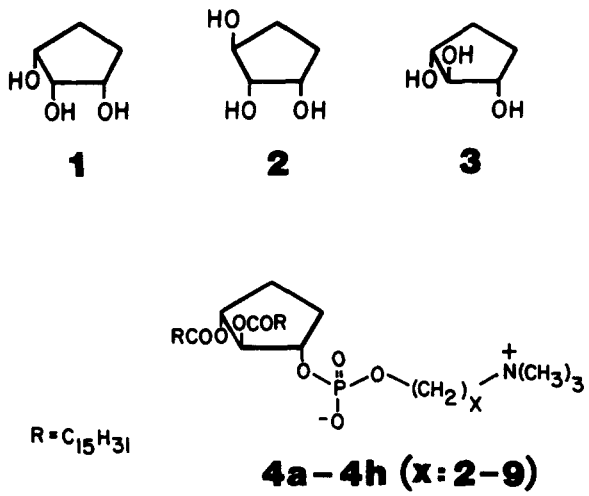


Fig. 1. Diastereoisomeric cyclopentane-1,2,3-triols. 1, All-*cis* isomer, 1,2,3/0; 2, *trans-cis* isomer, 1,2/3; 3, all-*trans* isomer, 1,3/2; **4a–4h,** all-*trans*-(1,3/2-)-*cyclopentano*-phosphatidylcholine homologs.

bilayer membrane aggregate or the catalytic ability of a membrane-bound enzyme is likely to depend on the conformational flexibility of the lipid backbone. For example, we have recently shown that both the permeability characteristics (7) and the thermal behavior (7) of liposomes prepared from the six isomeric *cyclopentano*-lecithins (5) are strongly dependent on the configurational and positional isomerism in the members of the series. In particular, we observed that two members of the series, the all-*trans* isomer (1,3/2-1P) and the *cis-trans* isomer (1,2/3-3P) were anomalous with respect to their permeability. Vesicles formed from these isomers were highly "leaky" at temperatures above or below their transition temperature. These results contrasted sharply with those of the other configurational isomers, each of which showed, like dipalmitoylphosphatidylcholine (DPPC), the increase in permeability now known to be associated with the main transition phenomenon (8). These two isomers were also anomalous in that each of them exhibited a value for both enthalpy (ΔH) and entropy of transition (ΔS) which was more than double that of DPPC. The permeability characteristics and organization (as measured by fluorescence depolarization of diphenylhexatriene) suggested that these large values for ΔH and ΔS result either from a reorganization of the polar head group region during the transition, or from an interdigitation of the acyl chains of opposing monolayers (7). We plan to explore the possibility that the polar head group region is involved in the anomalous behavior by synthesizing a series of *cyclopentano*-phospholipids which are configurationally identical, but which are homologous in the polar head group and which also have a variety of bases within the group. The compounds should also further our understanding of the substrate susceptibility of *cyclopentano*-phospholipids to phospholipase A_2, an enzyme known to catalyze the hydrolysis of only one of the six diastereoisomeric *cyclopentano*-lecithins viz., all-*trans*-(1,3/2-1P)-*cyclopentano*-lecithin (9).

We therefore wish to report the synthesis of three series of all-*trans*-(1,3/2)-*cyclopentano*-phospholipids viz., *cyclopentano*-phosphatidylcholines, *cyclopentano*-phosphatidylethanolamines, and *cyclopentano*-N,N-dimethylphosphatidylethanolamines. Each series consists of a group of homologs in which the number of methylene groups intervening between the phosphorus atom and the nitrogen atom (denoted as x in Fig. 1) is varied between two and nine. The new compounds are analogous to the homologs of *glycero*-lecithin which have been synthesized and studied by Diembeck and Eibl (10) and Bach et al. (11).

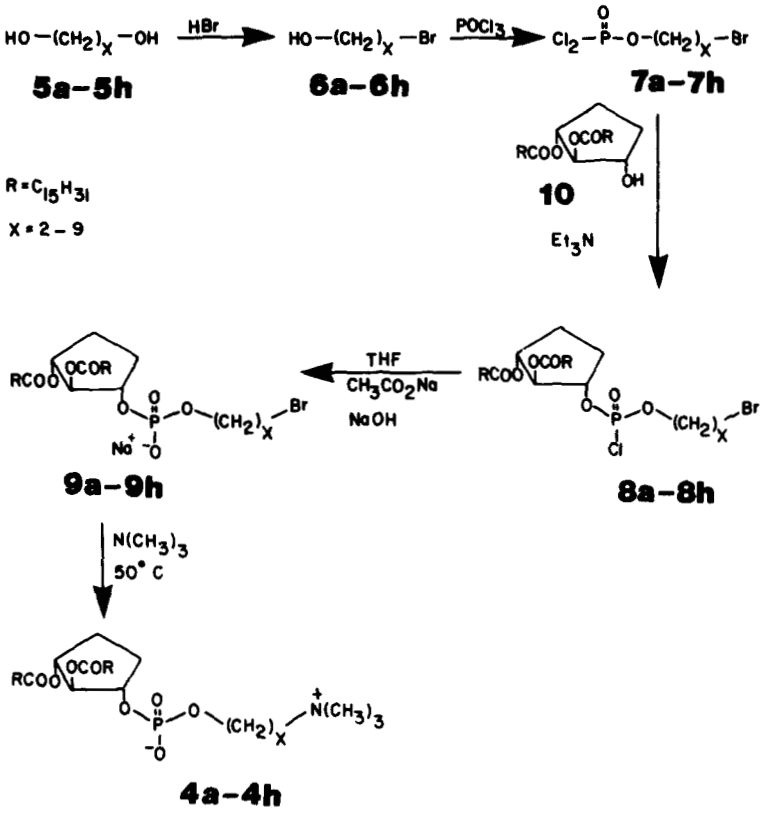
MATERIALS AND METHODS

Melting points were measured on a Thomas Hoover Unimelt capillary melting point apparatus and are uncorrected. Infrared spectra were measured for KBr dispersions with a Perkin-Elmer 621 spectrometer (Perkin-Elmer Corp., Norwalk, CT) and were calibrated with polystyrene. Micro-analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Reactions were monitored by thin-layer chromatography on silica gel G (absorbent thickness, 250 μm; EM Laboratories, Inc., Elmsford, NY). Solvents used for chromatography were: solvent A, chloroform–methanol–water 65:25:4 (v/v/v); solvent B, chloroform–acetone–methanol–acetic acid–water 6:8:2:2:1 (by volume). Purification of lipid products was by column chromatography on silicic acid buffered with triethylamine, essentially as described by Aneja, Chadha, and Davies (12). Fractions containing polar phospholipids were detected by TLC, solvents were removed by evaporation under reduced pressure, and the residue was dissolved in chloroform. The solutions were diluted with ten volumes of acetone and cooled to 4°C. Precipitated salts of phospholipids were removed by centrifugation, washed with acetone, and dried in vacuo. Phosphates were detected after analytical chromatography with modified reagent (13) of Dittmer and Lester (14). Alkane diols and aqueous solutions of dimethylamine (40%) and trimethylamine (40%) were obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI). Phosphorus oxychloride was obtained from Fisher Scientific Co., Chicago, IL. All-*trans*-(1,3/2)-dipalmitoylcyclopentane-1,2,3-triol was synthesized from cyclopentadiene as described previously (3, 4).

EXPERIMENTAL

Bromination of alkanediol

The general procedure adopted for conversion of the alkanediols (**5c–5h**) to the bromoalkanols (**6c–6h**)



Scheme 1. Reaction scheme for synthesis of all-*trans*-(1,3/2)-*cyclopentano*-phosphatidylcholine homologs.

was essentially that described by Diembeck and Eibl (10), except that ligroin (bp 90–110°C) was used as follows (**Scheme 1**).

*4-Bromobutanol (**6c**) and 5-bromopentanol (**6d**).* A solution of 1,4-butanediol (**5c**) or 1,4-pentanediol (**5d**) (0.12 mol), hydrogen bromide 40 g (0.24 mol; 47% aqueous solution), benzene (250 ml), and ligroin (5 ml) was gently refluxed (4 hr, 6c; 3.5 hr, 6d).

*6-Bromohexanol (**6e**), 7-bromoheptanol (**6f**), 8-bromooctanol (**6g**), and 9-bromononanol (**6h**).* A solution of alkanediol (**5e–5h;** 0.12 mol), hydrogen bromide 40 g (0.24 mol; 47% aqueous solution), and ligroin (750 ml) was gently refluxed (6e, 6f, 1.5 hr; 6g, 6h, 1.0 hr).

The bromination was followed by TLC (chloroform-diethyl ether 3:1, v/v) of the upper phase of the biphasic system. The upper phase was removed when each reaction was complete and was concentrated under reduced pressure to give a yellow oil; purification of the oil by vacuum distillation gave a colorless liquid in yields ranging from 80 to 90% (based on the diol). Each bromoalkanol was thus obtained in purity of >98% (GLC analysis: OV-17 on Chromosorb W, 80/100; 120°C).

All-*trans*-(1,3/2)-2,3-dipalmitoylcyclopentane-1,2,3-triol, bromoalkylesters (9a–9h)

The reaction conditions for the phosphorylation of all-*trans*-(1,3/2)-2,3-dipalmitoylcyclopentanetriol by each of the homologous bromoalkyl ester intermediates (**9a–9h**) were essentially identical. We thus report in detail the method leading to the synthesis of the lowest homolog **9a** (x = 2, Scheme 1).

2-Bromoethylphosphoric acid dichloride (7a)

This compound was prepared from 2-bromoethanol by a procedure based on that described by Diembeck and Eibl (10). To a cool (0–5°C) well-stirred solution of phosphorus oxychloride (0.2 g, 0.06 mol) in trichloroethylene (20 ml) was added 2-bromoethanol (**6a**) (5.0 g; 0.04 mol), and the resulting mixture was stirred overnight at room temperature. Toluene (20 ml) was added, and the solution was concentrated under vacuum to give a brown oil (**7a**), which was dried in vacuo (0.1 torr) at room temperature (12 hr). TLC analysis of the oil (chloroform–diethyl ether 3:1, v/v) showed a major phosphate-positive spot (R_f 0.80) and a minor unidentified spot near the origin. This compound was used in the next step without further purification.

2-Bromoethylester of all-*trans*-(1,3/2)-2,3-dipalmitoylcyclopentane-1,2,3-triol-1-phosphoric acid (9a)

The bromoethyl intermediate **9a** was prepared by reaction between all-*trans*-(1,3/2)-2,3-dipalmitoylcyclopentanetriol (10) and 2-bromoethyl-phosphoric acid dichlo-

ride (**7a**). A mixture of (**7a**) (0.967 g; 0.004 mol) in trichloroethylene (12 ml) was cooled (0°C) and stirred vigorously. Freshly distilled triethylamine (1.61 g; 0.016 mol) was added and the temperature of the reaction mixture was allowed to rise slowly to room temperature. A solution of all-*trans*-(1,3/2)-2,3-dipalmitoylcyclopentanetriol (10) (1.66 g; 0.0028 mol) in trichloroethylene (12 ml) was added dropwise and stirring was continued at room temperature for an additional 2 hr. Toluene (25 ml) was added and the precipitate of triethylammonium chloride was removed by filtration. Evaporation of the solvent under reduced pressure gave the 2-bromoethylester of all-*trans*-(1,3/2)-2,3-dipalmitoylcyclopentane-1,2,3-triol-1-phosphoric acid monochloride (**8a**). This compound was not isolated, but was immediately hydrolyzed to give **9a** as follows. The monochloride was dissolved in tetrahydrofuran (20 ml) and its hydrolysis (as well as that of excess 2-bromoethylphosphoric acid dichloride) was initiated by the addition of sodium acetate solution (20 ml; 0.5 M; pH 5.0; pH adjusted by dilute HCl). The resulting mixture was stirred for an additional 16 hr at room temperature. EDTA (1 ml; 0.5 M; pH 9.5) was added to the mixture and the pH of the solution was adjusted to 9.0 by the addition of 6 M NaOH. The resulting solution was extracted with diethyl ether (2 × 25 ml). The combined ethereal extract was concentrated to give a yellow gum, which was dissolved in chloroform (2 ml) and diluted with ten volumes of acetone to precipitate **9a** as a white solid. TLC analysis (chloroform–methanol–water 65:25:4, v/v/v) showed a major phosphate-positive spot (R_f 0.80) and a minor unidentified phosphorus-free spot near the solvent front (R_f 0.95).

Purification of an analytical sample was performed by column chromatography on silicic acid and elution was done with 100 ml of chloroform–methanol 99:1 (v/v) and 200 ml of chloroform–methanol 98:2 (v/v). Final elution with chloroform–methanol 95:5 (v/v) gave material that was chromatographically homogeneous. Removal of solvent under reduced pressure gave a residual gum which was dissolved in chloroform (1 ml). The bromoethylester intermediate **9a** was precipitated at 0°C by the addition of acetone (10 vol) giving the sodium salt as a white solid. The yields obtained for **9a** and each of the other homologs (**9b–9h**) were between 80–90%. Elemental analyses for (**9a**) and each of the other homologs (**9b–9h**) are given in **Table 1.** IR(KBr) cm^{-1}: 3700–3600 (broad), 2920, 1740, 1620, 1470, 1360, 1240, 1180, 1115, 1090, and 1020.

All-*trans*-(1,3/2)-2,3-dipalmitoyl-*cyclopentano*-1-phosphocholine [(1,3/2)-*cyclopentano*-phosphatidylcholine; (1,3/2)-*cyclopentano*-PC] (4a–4h)

The experimental procedures described below for the 2-bromoethylester of all-*trans*-(1,3/2)-2,3-dipalmitoylcyclopentane-1,2,3-triol-1-phosphoric acid (**9a**) were appropriate for each of the other homologs (**9b–9h**) (Scheme 1). The 2-bromoethylester intermediate **9a** (803 mg; 1.0 mmol) was dissolved in 13 ml of chloroform–isopropanol–dimethylformamide 3:5:5 (v/v/v) and the solution was heated to 50°C. To this solution trimethylamine (7 ml;

TABLE 1. Analytical data for homologous series of bromoalkylesters of all-*trans*-(1,3/2)-2,3-dipalmitoylcyclopentane-1,2,3-triol-1-phosphoric acid, mono sodium salts

Compound[a]	Homolog[b] (x)	Formula (mol wt)		C	H	Br	P	Br/P[c]
9a	2	$C_{39}H_{73}O_8BrPNa$	calc:	58.27	9.15	9.94	3.85	1.00
		(803.74)	found:	58.11	9.20	10.14	3.97	0.99
9b	3	$C_{40}H_{75}O_8BrPNa$	calc:	58.74	9.24	9.77	3.79	1.00
		(817.77)	found:	58.47	8.85	9.69	3.92	0.96
9c	4	$C_{41}H_{77}O_8BrPNa$	calc:	59.19	9.33	9.60	3.72	1.00
		(831.79)	found:	59.03	9.41	9.41	3.84	0.95
9d	5	$C_{42}H_{79}O_8BrPNa$	calc:	59.63	9.41	9.44	3.66	1.00
		(845.82)	found:	59.43	9.37	9.40	3.65	1.00
9e	6	$C_{43}H_{81}O_8BrPNa$	calc:	60.06	9.49	9.29	3.60	1.00
		(859.84)	found:	60.30	8.93	9.25	3.61	0.99
9f	7	$C_{44}H_{83}O_8BrPNa$	calc:	60.47	9.57	9.14	3.54	1.00
		(873.87)	found:	60.09	9.71	8.37	3.55	0.92
9g	8	$C_{45}H_{85}O_8BrPNa$	calc:	60.86	9.65	8.00	3.48	1.00
		(887.90)	found:	60.68	9.84	8.97	3.34	1.04
9h	9	$C_{46}H_{87}O_8BrPNa$	calc:	61.25	9.72	8.85	3.43	1.00
		(901.92)	found:	60.70	9.68	8.39	3.33	0.98

[a] Dried over phosphorus pentoxide for 24 hr at room temperature (0.2 torr).
[b] x indicates the number of methylene groups that separate phosphate group and bromine atom.
[c] Br/P is the ratio of g-atoms bromine to g-atoms phosphorus.

TABLE 2. Analytical data for homologous series of all-*trans*-(1,3/2-1P)-*cyclopentano*-analogs of phosphatidylcholine

Compound[a]	Homolog[b] (x)	Formula (mol wt)		C	H	N	P	N/P[c]	H_2O[d]
4a	2	$C_{42}H_{82}O_8NP \cdot \frac{1}{2}H_2O$	calc:	65.60	10.88	1.82	4.03	1.00	1.17
		(768.97)	found:	65.71	10.70	1.87	3.94	1.04	1.53
4b	3	$C_{43}H_{84}O_8NP$	calc:	66.72	10.94	1.81	4.00	1.00	0.00
		(773.99)	found:	66.35	10.87	1.67	4.20	0.88	0.51
4c	4	$C_{44}H_{86}O_8NP \cdot 3H_2O$	calc:	62.76	11.01	1.66	3.68	1.00	6.40
		(842.03)	found:	62.73	11.32	1.66	3.47	1.05	2.49
4d	5	$C_{45}H_{88}O_8NP \cdot 3H_2O$	calc:	63.13	11.07	1.63	3.62	1.00	6.31
		(856.06)	found:	63.29	11.37	1.58	3.81	0.92	3.63
4e	6	$C_{46}H_{90}O_8NP \cdot 3H_2O$	calc:	63.49	11.12	1.61	3.56	1.00	6.21
		(870.09)	found:	63.10	11.32	1.57	3.60	0.96	6.93
4f	7	$C_{47}H_{92}O_8NP \cdot 2\frac{1}{2}H_2O$	calc:	64.50	11.17	1.60	3.54	1.00	5.14
		(875.11)	found:	64.55	11.27	1.56	3.23	1.06	3.77
4g	8	$C_{48}H_{94}O_8NP \cdot 3H_2O$	calc:	64.18	11.22	1.56	3.45	1.00	6.01
		(898.14)	found:	64.13	11.50	1.55	3.49	0.98	2.94
4h	9	$C_{49}H_{96}O_8NP \cdot 4H_2O$	calc:	63.27	11.27	1.50	3.33	1.00	7.74
		(930.17)	found:	63.09	11.45	1.69	3.43	1.08	4.45

[a] Dried over phosphorus pentoxide for 24 hr at room temperature (0.2 torr).
[b] x indicates the number of methylene groups that separate phosphate and quaternary nitrogen.
[c] N/P is ratio gram-atoms N/gram-atoms P.
[d] Karl Fischer analysis.

25% aqueous solution) was added, and the resulting mixture was maintained at 50°C (water bath) for 6 hr. After cooling to room temperature, the solution was concentrated under reduced pressure (25 torr) and DMF was removed in vacuo (0.2 torr) at 25°C, to give a white solid. TLC analysis of the solid showed a major phosphate-positive spot (R_f 0.23 in $CHCl_3$–CH_3OH–H_2O 65:25:4 (v/v/v) and a minor unidentified spot (R_f 0.95) at the solvent front. The crude product was purified by slow-elution chromatography (0.2 ml min^{-1} of eluant containing 0.5% triethylamine) on silicic acid (35 g, buffered with triethylamine) using the eluting solvents: 200 ml of $CHCl_3$–CH_3OH 95:5 (v/v); 200 ml of $CHCl_3$–CH_3OH 90:10 (v/v); 150 ml of $CHCl_3$–CH_3OH 85:15 (v/v); 100 ml of $CHCl_3$–CH_3OH 80:20 (v/v); 100 ml of $CHCl_3$–CH_3OH 75:25 (v/v); and 300 ml of $CHCl_3$–CH_3OH 70:30 (v/v). Pure *cyclopentano*-lecithin was eluted by the latter solvent; the prior stepwise eluting procedures were found necessary to preclude contamination of the product by an unidentified phosphorus-free component. The eluted product was freed of solvent under vacuum, taken up in a minimum volume of chloroform, and precipitated by the addition of ten volumes of acetone (0°C) (620 mg, 82% based on bromoethyl intermediate **9a**). Elemental analyses for each of the homologs of all-*trans-cyclopentano*-phosphatidylcholine **4a–4h** are given in **Table 2.** Chromatographic mobilities and melting points are given in **Table 3.**

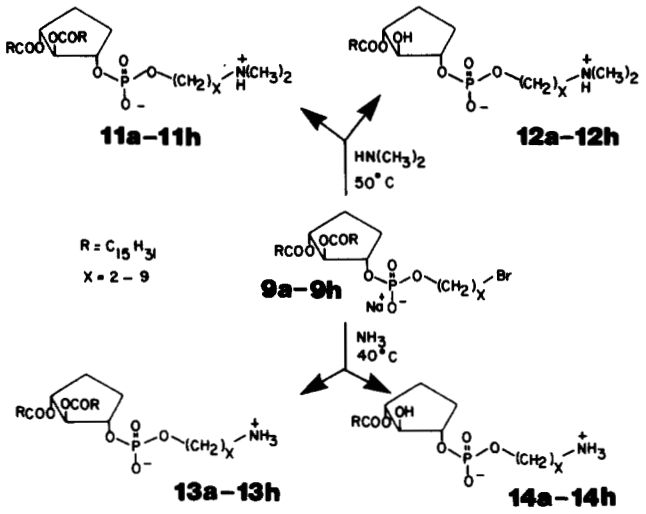
All-*trans*-(1,3/2)-2,3-dipalmitoyl-*cyclopentano*-1-phosphoryl-N,N-dimethylethanolamine homologs [(1,3/2)-*cyclopentano*-PE-diMe] (11a–11h)

The experimental procedures described below for the 2-bromoethylester of all-*trans*-(1,3/2)-2,3-dipalmitoylcy-

TABLE 3. Chromatographic mobilities and melting ranges of homologs of all-*trans*-(1,3/2-1P)-*cyclopentano* analogs of phosphatidylcholine

Compound	Homolog[a] (x)	R_f in Solvent[b] A	R_f in Solvent[b] B	Melting Range (°C)[c]
4a	2	0.23	0.20	197–199
4b	3	0.19	0.14	208–210
4c	4	0.21	0.13	191–193
4d	5	0.21	0.13	189–191
4e	6	0.18	0.17	216–218
4f	7	0.21	0.21	217–219
4g	8	0.20	0.23	223–225
4h	9	0.22	0.27	229–231
DPPC	2	0.15	0.17	227–229[d]

[a] x indicates the number of methylene groups that separate the pentacovalent phosphorus atom and the substituted ammonium group.
[b] Solvent A: chloroform–methanol–water 65:25:4 (v/v/v). Solvent B: chloroform–acetone–methanol–acetic acid–water 6:8:2:2:1 (by volume).
[c] The temperatures given correspond to the temperature range from initial liquefaction to final liquefaction.
[d] Taken from Ref. 17 (DL-DPPC).



Scheme 2. Reaction scheme for synthesis of all-*trans*-(1,3/2)-*cyclopentano*-phosphatidyl-N,N-dimethylethanolamine homologs (**11a–11h**) and all-*trans*-(1,3/2)-*cyclopentano*-phosphatidylethanolamine homologs (*13a–13h*).

clopentane-1,2,3-triol-1-phosphoric acid (**9a**) were appropriate for each of the other homologs (**9b–9h**) (**Scheme 2**).

The 2-bromoethylester intermediate (**9a**) (803 mg; 1.0 mmol) was dissolved in 13 ml of chloroform–isopropanol–dimethylformamide 3:5:5 (v/v/v). To this solution dimethylamine (7 ml; 40% solution in water) was added, and the resulting mixture was maintained at 50°C (water bath) for 6 hr. After cooling to room temperature, some solvent was removed under reduced pressure (25 torr) and DMF was removed in vacuo (0.2 torr) at 25°C, to give a yellowish solid. TLC analysis of this solid showed two major phosphate-positive spots corresponding to the diacyl and monoacyl products (R_f 0.16, 0.43 in $CHCl_3$–CH_3OH–H_2O 65:25:4 (v/v/v) and a minor unidentified spot (R_f 0.95) at the solvent front. Purification of this mixture by column chromatography (0.2 ml min^{-1} of eluant containing 0.5% triethylamine) gave two chromatographically pure phospholipids as follows: 200 ml of $CHCl_3$–CH_3OH 95:5 (v/v) unidentified compound; 200 ml of $CHCl_3$–CH_3OH 90:10 (v/v); 100 ml of $CHCl_3$–CH_3OH 85:15 (v/v); 50 ml of $CHCl_3$–CH_3OH 80:20 (v/v) *cyclopentano*-PE-diMe; 250 ml of $CHCl_3$–CH_3OH 75:25 (v/v) *cyclopentano*-PE-diMe; and 200 ml of $CHCl_3$–CH_3OH 65:35 (v/v) *cyclopentano*-lyso-PE-diMe. Note: The chromatographic procedure described above was appropriate for homologs X = 2–6. The higher homologs (X = 7, 8, 9) required less polar eluant mixtures, i.e., $CHCl_3$–CH_3OH 85:15 (v/v) eluted the *cyclopentano*-PE-diMe and $CHCl_3$–CH_3OH 80:20 (v/v) eluted *cyclopentano*-lyso-PE-diMe. The diacyl and monoacyl products were obtained as solids by acetone precipitation (see Materials and Methods) to give **11a,** 400 mg (53.6%) and **12a,** 107 mg (20.3%). Chromatographic mobilities and melting points for each of the homologs of all-*trans-cyclopentano*-phosphatidyl-N,N-dimethylethanolamine (**11a–11h**) are given in **Table 4.** Elemental analysis data for homologs **11a–11h** are given in **Table 5.** Analysis: Calc. for $C_{25}H_{50}O_7NP \cdot H_2O$ (525.55) (**12a**) C, 57.13; H, 9.97; N, 2.66; P, 5.89; N/P ratio 1.00. Found: C, 56.99; H, 9.94; N, 2.60; P, 5.91; N/P ratio 0.97.

All-*trans*-(1,3/2)-2,3-dipalmitoyl-*cyclopentano*-1-phosphoethanolamine homologs (1,3/2-*cyclopentano*-PE) (13a–13h)

The experimental procedures described below for the 2-bromoethylester of all-*trans*-(1,3/2)-2,3-dipalmitoylcyclopentane-1,2,3-triol-1-phosphoric acid (**9a**) were appropriate for each of the other homologs (**9b–9h**) (Scheme 2).

The 2-bromoethylester intermediate (**9a**) (803 mg; 1.0 mmol) was dissolved in 60 ml of chloroform–isopropanol–dimethylformamide 1:1:4 (v/v/v) and the solution was heated to 40°C. To this solution ammonium hydroxide (30 ml; 28% aqueous solution) was added, and the resulting mixture was maintained at 40°C (water bath) for 16 hr. The solution was then cooled and the volatile solvent was removed under reduced pressure (25 torr), followed by removal of DMF in vacuo (0.2 torr) at room temperature, to give a tan-colored low-melting solid. TLC analysis of this residue showed two major spots, each of which was ninhydrin-positive and phosphate-positive (R_f 0.21 and 0.42 in $CHCl_3$–CH_3OH–H_2O 65:25:4 (v/v/v) corresponding to the monoacyl and diacyl products, respectively. Purification of this mixture by column chromatography on silicic acid (0.2 ml min^{-1} of eluant containing 0.5% triethylamine) was done as follows: 200 ml

TABLE 4. Chromatographic mobilities and melting ranges of homologs of all-*trans*-(1,3/2)-*cyclopentano*-phosphatidyl-N,N-dimethylethanolamine

Compound	Homolog[a] (x)	R_f in Solvent[b] A	R_f in Solvent[b] B	Melting Range °C
11a	2	0.43	0.41	128–130
11b	3	0.43	0.35	120–122
11c	4	0.35	0.26	110–112
11d	5	0.35	0.28	106–108
11e	6	0.47	0.35	101–103
11f	7	0.45	0.37	102–104
11g	8	0.56	0.48	105–107
11h	9	0.59	0.51	104–106

[a] x indicates the number of methylene groups that separate the pentacovalent phosphorus atom and the substituted ammonium group.
[b] Solvent A: chloroform–methanol–water 65:25:4 (v/v/v). Solvent B: chloroform–acetone–methanol–acetic acid–water 6:8:2:2:1 (by volume).

TABLE 5. Analytical data for homologous series of all-*trans*-(1,3/2-1P)-*cyclopentano*-analogs of phosphatidyl-N,N-dimethylethanolamine

Compound[a]	Homolog[b] (x)	Formula (mol wt)		C	H	N	P	N/P[c]
11a	2	$C_{41}H_{80}O_8NP$	calc:	66.01	10.81	1.87	4.15	1.00
		(745.95)	found:	66.40	10.71	1.75	3.90	0.99
11b	3	$C_{42}H_{82}O_8NP \cdot 2H_2O$	calc:	63.37	10.89	1.76	3.89	1.00
		(795.98)	found:	63.13	10.95	1.67	4.09	0.91
11c	4	$C_{43}H_{84}O_8NP \cdot H_2O$	calc:	65.20	10.94	1.77	3.91	1.00
		(791.99)	found:	65.15	10.67	1.79	3.85	1.02
11d	5	$C_{44}H_{86}O_8NP \cdot H_2O$	calc:	65.56	11.00	1.74	3.84	1.00
		(806.02)	found:	65.60	10.87	1.79	3.79	1.04
11e	6	$C_{45}H_{88}O_8NP \cdot \frac{1}{2}H_2O$	calc:	66.63	11.06	1.73	3.82	1.00
		(811.05)	found:	66.54	10.92	1.90	3.81	1.10
11f	7	$C_{46}H_{90}O_8NP$	calc:	67.70	11.11	1.71	3.79	1.00
		(816.07)	found:	67.81	10.70	1.64	3.73	0.97
11g	8	$C_{47}H_{92}O_8NP$	calc:	68.00	11.17	1.68	3.73	1.00
		(830.09)	found:	68.28	10.78	1.59	3.68	0.96
11h	9	$C_{48}H_{94}O_8NP$	calc:	68.29	11.22	1.66	3.67	1.00
		(844.12)	found:	68.30	10.99	1.63	3.68	0.98

[a] Dried over phosphorus pentoxide for 24 hr at room temperature (0.2 torr).
[b] x indicates the number of methylene groups that separate phosphate and dimethyl ammonium group.
[c] N/P is ratio gram-atoms N/gram-atoms P.

of $CHCl_3$–CH_3OH 90:10 (v/v); 100 ml of $CHCl_3$–CH_3OH 80:20 (v/v) *cyclopentano*-PE with minor unidentified impurity; 250 ml of $CHCl_3$–CH_3OH 70:30 (v/v) *cyclopentano*-PE; and 200 ml of $CHCl_3$–CH_3OH 65:35 (v/v) *cyclopentano*-lyso-PE. This procedure was suitable for homologs X = 2–6. The higher homologs (X = 7–9) required less polar eluant mixtures, i.e., $CHCl_3$–CH_3OH 80:20 (v/v) eluted *cyclopentano*-PE and $CHCl_3$–CH_3OH 75:25 (v/v) eluted *cyclopentano*-lyso-PE. The products were precipitated by acetone (Materials and Methods) to give **13a,** 370 mg (49.1%) and **14a,** 105 mg (20.5%).

Chromatographic mobilities and melting points for each of the homologs of all-*trans-cyclopentano*-phosphatidylethanolamine (**13a–13h**) are given in **Table 6.** Elemental analysis data for homologs **13a–13h** are given in **Table 7.** Analysis: Calc. for $C_{23}H_{46}O_7NP$ (488.50) (**14a**) C, 56.55; H, 9.70; N, 2.86; P, 6.34; N/P ratio 1.00. Found: C, 56.83; H, 9.83; N, 2.80; P, 6.30; N/P ratio 0.98.

RESULTS AND DISCUSSION

Conformationally restricted analogs of phosphatidylcholine, phosphatidylethanolamine, and phosphatidyl-N,N-dimethylethanolamine have been synthesized. The restriction has been achieved by means of the use of an all-*trans* configurational isomer of cyclopentane-1,2,3-triol [1,3/2 in the cyclitol notation (1)] as described in earlier communications (2). However, in this work, each of the three lipid classes has been synthesized as an homologous series in that the separation of phosphorus and nitrogen atoms in the polar head group has been increased by the incremental incorporation of methylene groups (Fig. 1).

The synthetic strategy was based on the work of Diembeck and Eibl (10) in which the synthesis of the corresponding phosphoglycerides was described. The key intermediate in our work was the all-*trans*-(1,3/2)-1,2-dipalmitoylcyclopentane-1,2,3-triol (**10,** Scheme 1) which was phosphorylated with a series of homologs of 2-bromoethylphosphoric acid dichloride to give the bromoalkyl phosphoric esters of the dipalmitoyltriol.

TABLE 6. Chromatographic mobilities and melting ranges of homologs of all-*trans*(1,3/2)-*cyclopentano*-phosphatidylethanolamine

Compound	Homolog[a] (x)	R_f in Solvent[b] A	R_f in Solvent[b] B	Melting Range °C
13a	2	0.42	0.54	185–187
13b	3	0.42	0.51	177–179
13c	4	0.38	0.44	153–155
13d	5	0.40	0.49	129–131
13e	6	0.48	0.55	113–115
13f	7	0.47	0.56	139–141
13g	8	0.50	0.62	226–228
13h	9	0.54	0.65	230–232

[a] x indicates the number of methylene groups that separate the pentacovalent phosphorus atom and the substituted ammonium group.
[b] Solvent A: chloroform–methanol–water 65:25:4 (v/v/v). Solvent B: chloroform–acetone–methanol–acetic acid–water 6:8:2:2:1 (by volume).

TABLE 7. Analytical data for homologous series of all-*trans*-(1,3/2-1P)-*cyclopentano*-analogs of phosphatidylethanolamine

Compound[a]	Homolog[b] (x)	Formula (mol wt)		C	H	N	P	N/P[c]
13a	2	$C_{39}H_{76}O_8NP \cdot 2H_2O$	calc:	62.13	10.69	1.85	4.11	1.00
		(753.90)	found:	61.92	10.52	1.74	4.04	0.95
13b	3	$C_{40}H_{78}O_8NP \cdot 1\frac{1}{2}H_2O$	calc:	63.30	10.75	1.84	4.08	1.00
		(758.91)	found:	63.25	10.39	1.97	4.00	1.08
13c	4	$C_{41}H_{80}O_8NP \cdot H_2O$	calc:	64.45	10.82	1.83	4.05	1.00
		(763.94)	found:	64.37	10.88	1.86	4.01	1.02
13d	5	$C_{42}H_{82}O_8NP \cdot 1\frac{1}{2}H_2O$	calc:	64.10	10.88	1.78	3.94	1.00
		(786.97)	found:	64.07	10.42	1.76	3.90	0.99
13e	6	$C_{43}H_{84}O_8NP \cdot H_2O$	calc:	65.21	10.94	1.77	3.91	1.00
		(791.99)	found:	65.60	10.82	1.77	3.89	1.00
13f	7	$C_{44}H_{86}O_8NP \cdot H_2O$	calc:	65.56	11.00	1.73	3.84	1.00
		(806.02)	found:	65.51	11.30	1.69	3.74	0.99
13g	8	$C_{45}H_{88}O_8NP \cdot H_2O$	calc:	65.90	11.06	1.71	3.77	1.00
		(820.05)	found:	65.79	11.18	1.85	3.70	1.10
13h	9	$C_{46}H_{90}O_8NP \cdot H_2O$	calc:	66.24	11.11	1.68	3.71	1.00
		(834.07)	found:	66.39	10.81	1.63	3.69	0.98

[a] Dried over phosphorus pentoxide for 24 hr at room temperature (0.2 torr).
[b] x indicates the number of methylene groups that separate phosphate and amine groups.
[c] N/P is ratio gram-atoms N/gram-atoms P.

The homologous bromoalkyl derivatives were then treated with trimethylamine, dimethylamine, or ammonia to give the choline, dimethylethanolamine, and ethanolamine-based phospholipids, respectively (Schemes 1 and 2). In the case of the trimethylamine reaction, the amination proceeded smoothly and, unlike corresponding reactions performed with ammonia, monomethylamine (15), and dimethylamine, did not result in deacylation of the lipid moiety. The yields of *cyclopentano*-lecithin, based on diacyltriol, were in the range 75–80%. Thus, the amination method was superior to the method used previously in this laboratory in which *cyclopentano*-phosphatidic acid isomers were condensed with choline tosylate (5). No evidence of decomposition was observed during 1 month at room temperature for these solids or their solutions in chloroform, although they were normally stored at 4°C in the dry state after an acetone precipitation. As indicated above, corresponding reactions using other methylamines led to the formation of a more polar compound in each case. The likelihood that these compounds resulted from deacylation of starting material (or deacylation of final product) was supported by elemental analytical data and infrared spectroscopic data which were consistent with those required for a monoacyl-phospholipid, i.e., a *cyclopentano*-lyso derivative (Scheme 2).

The partial deacylation reactions catalyzed by both ammonia and dimethylamine could, in principle, give a mixture of the two lyso-derivatives (3-O-acyl and 2-O-acyl positional isomers) either by nonspecific nucleophilic attack or as a result of a subsequent acyl (or phosphate) migration in the ring. We believe that the latter possibility is unlikely since the substituents in the ring have an all-*trans* configuration (1,3/2). The fairly narrow melting range observed for each of the lyso-derivatives and the presence of a single spot in each thin-layer chromatographic analysis suggest, but do not unequivocally establish, that each of the lyso-derivatives is a unique compound rather than a mixture of two positional isomers. Further studies are required to establish the isomeric integrity of these compounds. An unequivocal synthesis of lyso-*cyclopentano*-lecithin is currently in progress[4] and it is hoped that ^{31}P-NMR comparison studies may aid the assignment of unequivocal structures for these by-products.

Analytical data

Cyclopentano-*PC homologs.* In general, the *cyclopentano*-lecithins gave elemental analysis data that were consistent with those expected for the hydrated compounds even after vacuum treatment (0.2 torr, 56°C, 24 hr). After several days storage in stoppered vials, elemental analysis indicated the presence of three molecules of water per molecule of *cyclopentano*-lipid in four out of eight of the compounds. Two compounds (homologs X = 2 and X = 3) gave analytical data corresponding to one-half and zero molecules of water per molecule of lipid, respectively

[4] Hancock, A. J., and M. D. Lister. Unpublished results.

(Table 2). Comparative data from the literature for synthetic *glycero*-phosphatidylcholine (DPPC) include those for an anhydrous compound (after recrystallization from dioxane) (16) and a hydrated compound (after recrystallization from diisobutylketone) (17), while data for a monohydrated synthetic sulfonium analog of lecithin have been reported (18). It appears that the higher homologs are able to hydrate more extensively. However, although the data (Table 2) generally support formulation as shown, these results are not substantiated by direct Karl Fischer water analysis. In most cases, the water content required by the C, H, N, P data is greater than that actually found by assay.

Cyclopentano-*PE-diMe homologs.* In the *cyclopentano*-PE-diMe series, analysis of the vacuum-dried compounds (0.2 torr) gave values for C, H, N, and P which are consistent with formulation for an anhydrous lipid in all but four compounds. These four compounds (homologs X = 3, 4, 5, and 6) gave analytical data corresponding to two, one, one, and one-half molecule of water per molecule lipid, respectively (Table 5). Comparative data from the literature for synthetic *glycero*-PE-diMe include those for an anhydrous compound after recrystallization from absolute ethanol (19).

Cyclopentano-*PE homologs.* In the *cyclopentano*-PE series, analysis of the vacuum-dried compounds (0.2 torr) indicated the presence of one molecule of water per molecule of *cyclopentano*-phospholipid in all but three compounds. These three compounds (homologs X = 2, 3, and 5) gave analytical data corresponding to two, or one and one-half molecules of water per molecule lipid (Table 7). Data from the literature for synthetic *glycero*-phosphatidylethanolamine (DPPE) include those for an anhydrous compound after recrystallization from chloroform–acetone (20).

Thin-layer chromatography

Cyclopentano-*PC homologs.* Chromatographic mobilities of each of the homologs of *cyclopentano*-PC were higher than that of DPPC (Table 3) both in neutral and acidic solvent systems. However, the effect of an increase in the value of X led to an unusual chromatographic behavior upon development in the acidic system. The R_f values for the homologs fall as the value of X increases, reaching a minimum (X = 4, 5). Further increase in the value of X leads to a progressive increase in R_f value (X = 5–9). We speculate that the low apparent polarity of the higher homologs (X = 7–9) may be due to an intramolecular association in the phospholipid which tends to preclude binding at the silica surface. Since the acidic solvent may protonate the zwitterionic species, this interaction may be chiefly due to a hydrogen-bonded association. In the case of the lower homologs (X = 3–5), the protonation may also occur, but a different association between the protonated molecules may permit more favorable binding to the silica.

Cyclopentano-*PE-diMe and* cyclopentano-*PE.* The chromatographic mobility of these lipid homologs also varied with the length of the chain in the polar head group both in neutral and acidic solvent systems. As is evident from Tables 4 and 6, it appears that the *cyclopentano*-analogs of PE-diMe and PE have three chromatographic features in common. First, successive incremental increases in chain length (X = 4–9) cause a progressive increase in the chromatographic mobility of the compound both in the neutral and in the acidic solvent systems. Second, for both of these classes of lipid, the R_f values for X = 2 homologs are identical with those for X = 3 homologs. Third, there is an overall resemblance between the pattern of R_f values in the neutral solvent system and that in the acidic solvent system. The R_f value for each homolog of *cyclopentano*-PE was smaller for the neutral solvent system (solvent A, Table 6) than the corresponding R_f value in the acidic solvent system (solvent B, Table 6). In this respect, *cyclopentano*-PE differed chromatographically from the other *cyclopentano*-phospholipids studied in this work, whose mobility in each case was greater for the neutral solvent system.

Spectroscopic analysis

Cyclopentano-*PC homologs.* Each infrared spectrum (KBr dispersion) was consistent with that expected for a hydrated zwitterionic lecithin analog. Absorption bands observed in each spectrum included those for P = O (1270 cm^{-1}), P–O–C (1175, 1060 cm^{-1}), $N^+(CH_3)_3$ (980 cm^{-1}). The value for the latter band absorption (trimethylammonium group) was consistently higher (980 cm^{-1}) than the value determined by Nelson (970 cm^{-1}) (21).

The strong $P–O^-$ absorption band (1100 cm^{-1}) and the absence of the P–OH absorption in the 2700 cm^{-1} region in all spectra measured suggests that, at least in the microcrystalline state, these *cyclopentano*-phospholipids exist as zwitterions. The broad absorption band centered at 3450 cm^{-1} probably indicates the presence of strongly bonded water in the *cyclopentano*-phospholipids. This finding is consistent with the analytical data for the compounds (Table 3).

Cyclopentano-*PE-diMe homologs and* cyclopentano-*PE homologs.* Each spectrum was consistent with that expected for the lipid class. In addition to the absorption bands found in common to each spectrum (P = O, 1240 cm^{-1} or 1210 cm^{-1}; P–O–C, 1165–1180 cm^{-1}; $P–O^-$, 1090–1100 cm^{-1}; C = O, 1175 cm^{-1}), we observed $–NH^+$ bands (2600–2200 cm^{-1}) for *cyclopentano*-PE-diMe and $–NH_3^+$ bands (3000 cm^{-1}, shoulder, 1600 cm^{-1}) for *cyclopentano*-PE. Like the *cyclopentano*-PC homologs, these compounds gave absorption bands indicating the zwitterionic form. Unlike the spectrum of the *cyclopentano*-

lecithin, the spectra of the *cyclopentano*-PE and *cyclopentano*-PE-diMe do not exhibit a distinct absorption band at 980 cm^{-1}, a finding which agrees with the observation of Nelson (21) for the corresponding glycerophospholipids.

Melting behavior

Each class of phospholipid exhibited a minimum melting point value as x (in $[CH_2]_x$) was increased from two to nine methylene units. The most dramatic change is exhibited by the homologs of *cyclopentano*-PE (Table 6). The value of the melting points of successive homologs decreases as methylene units are incorporated between the amine group and the phosphorus atom until a minimum is reached (X = 6). Then, further increase in chain length causes the melting point values to increase drastically until the maximum value is reached (X = 9). On the basis of molecular weight consideration alone, one might expect that successive increments in chain length within the polar head group of the lipids studied here would cause a progressive increase in melting point, an expectation that is not borne out in practice. The homologs of *cyclopentano*-PE-diMe and *cyclopentano*-PC also exhibit a minimum in melting point (X = 6) but unlike those of *cyclopentano*-PE, the higher homologs (X = 7–9) have approximately the same melting point. The observed variation of melting point with x (in $[CH_2]_x$) presumably arises from differences in intermolecular and intramolecular interactions of the cationic nitrogen and the phosphate anion. Our simple examination may have been insufficiently sensitive to detect subtle thermal phenomena. At present, we are examining the thermal properties of these *cyclopentano*-phospholipids by differential scanning calorimetry in order to detect, and then compare, polymorphism and phase transitions across the homologous series of each of these lipid classes.[5]

The authors thank Philip Engdahl, Mark D. Lister, Everett O. Lewis, Maureen O'Connell, Joseph Rima, and Kim C. Wade for assistance in the preparation of some intermediates used in this work. The work was supported by a grant from the Weldon Springs Endowment (AJH) and the Faculty Research Council, University of Missouri-Kansas City.

Manuscript received 27 June 1983.

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