Synthesis of conformationally restricted acidic lipids. I. Cyclopentanoid analogs of phosphatidylserine

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Abstract A series of six analogs of phosphatidylserine (PS) was synthesized in which the conformational mobility of the backbone was limited. Each analog was a derivative of one of the three diastereoisomeric cyclopentane-1,2,3-triols, so that the glycerol moiety of the PS was, in effect, replaced by each cyclopentan-1,2,3-triol. Four of the members of the series were vicinal dipalmitates [1,2/3-(1P); 1,2,3/0-(1P); 1,3/2-(1P)] while the other two members were “unnatural” in that they contained a 2-phosphate group. The two 2-phosphate derivatives were meso-forms and each of the other four derivatives was a dl-pair. Each PS analog was obtained as a stable microcrystalline precipitate analyzing for the mono-
hydrate of a mixture of mono- and di-sodium salts. The infrared spectra, melting behavior, and chromatographic mobility of each sodium salt mixture resembled those obtained for bovine (glycero-)phosphatidyl-L-serine; the stereochemical differences in the ring caused only marginal variation in these properties. The optical rotation values of the compounds varied with the stereochemistry of the ring. The all-trans-2-phosphate isomer 8d exhibited a negative rotation value, in contrast to each of the other isomers. The all-trans isomer [1,3/ 2-(1P)] was shown to undergo diazomethylation with diazomethane to give the dimethyl ester of cyclopentano-phosphatidic acid.—Pajouhesh, H., and A. J. Hancock. Synthesis of conformationally restricted acidic lipids. I. Cyclopentanoid analogs of phosphatidylserine. J. Lipid Res. 1983. 24: 645–651.

Supplementary key words cyclopentano-lipids • restricted conformational mobility • cyclitols

This communication describes the synthesis of a series of phosphatidylserine analogs whose conformationally restricted nature allows study of the relationship between the conformation of the molecules and their biochemical and biophysical properties. The analogs are derivatives of the three diastereoisomeric cyclopentan-1,2,3-triols (3). Since each triol closely mimics a particular rotameric state of the glycerol molecule, studies of the properties of these phospholipids and comparison of their behavior with that of the natural glyceryl counterpart should give some definition of the conformational state (rotameric state) of the glyceryl backbone during an interaction with other lipids or proteins. This rationale has been presented in detail in earlier communications (2, 4, 5).

We have described the synthesis of cyclopentano-analogs of triglycerides (2), phosphatidic acid (4–6), and lecithin (7), and have shown that information about the rotameric state of the glycerol backbone of natural lipids may indeed be obtained by using cyclopentano-lipid analogs in both physical and biochemical studies. For example, we have found in an electron diffraction study (8) that the all-trans-cyclopentano-triglyceride analog [1,3/2-(1P)] gives diffraction data resembling those of the “natural” (glycero)triglyceride, which offered strong support for the assignment of an analogous all-trans arrangement of substituents about the glycerol backbone. In a separate study, we have shown that both the permeability and thermal properties of the cyclopentano-lecithins are related to the configurational geometry of the cyclopentane ring backbone, which allows us to begin to estimate the conformational aspects of the glycerol backbone in “natural” lecithin. In biological studies, we have demonstrated the influence of the conformation of cyclopentano-lecithins on their susceptibility to enzymatic hydrolysis using phospholipase A2, and find that activity is confined to the all-trans isomer [1,3/2-(1P)] (9). In another study involving the phosphohydrolase activity of canine lung surfactant material, we have shown that only the all-trans [1,3/2-(1P)] cyclopentano-phosphatidic acid is an effective substrate for the en-

Abbreviations: PA, phosphatidic acid; PS, phosphatidylserine; DPPS, dipalmitoyl phosphatidylserine; TLC, thin-layer chromatography; TPS, triisopropylbenzene sulfonyle chloride; DMF, N,N-dimethylformamide; CBz, carbobenzyloxy.

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.
2 To whom correspondence and reprint requests should be addressed.
3 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). 1-Phosphates, 2-phosphates, and 3-phosphates are denoted, respectively, by -(1P), -(2P), and -(3P).
zyme. This kind of enzymatic discrimination was also found to be exhibited in water-soluble cyclopentanetriol phosphates as well as in amphipathic molecules. For example (10), rabbit muscle cytosolic sn-glycerol-3-P dehydrogenase was found effectively to catalyze the oxidation of only one of the six cyclopentano-analogs of glycerol-3-phosphate (1,2/3-1P isomer).

In view of the promise shown by the cyclopentano-analogs in aiding definition of the conformational state of both natural phosphoglycerides and glycerol phosphate during interaction with other biomolecules, we wished to extend the range of analogs to include acidic membrane phosphoglycerides so that studies may be made in systems where the anionic polar head region may make special contribution to their biological properties. This article therefore describes the synthesis and characterization of six such acidic phospholipid analogs (see Fig. 1).

**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. Optical rotation values were measured with a Perkin-Elmer 141 polarimeter. Microanalyses 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: one-dimensional, 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); two-dimensional, solvent 1, chloroform–methanol–ammonium hydroxide (25%–water 90:54:5:5:5.5 (by volume); solvent 2, chloroform–methanol–acetic acid–water 90:40:12:2 (by volume) (11). Purification of lipid products was done by column chromatography on silicic acid buffered with triethylamine, essentially as described by Aneja, Chadha, and Davies (12). Phosphates were detected after analytical chromatography with the modified reagent (13) of Dittmer and Lester (14). Bovine phosphatidylserine was obtained from Sigma Chemical Co., St. Louis, MO. The condensing agent 2,4,6-tri-isopropylbenzenesulfonyl chloride and N-carbobenzyloxy-L-serine were obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI). N-carbobenzyloxy-L-serine benzyl ester was prepared from carboxibenzyloxy-L-serine and benzyl bromide in DMF as described by Baer and Maurukas (15); the product was recrystallized from carbon tetrachloride–petroleum ether 1:1 (v/v). The six isomeric cyclopentano-phosphatic acids were synthesized from cyclopentadiene as described previously (4–6). Pyridine used for condensation reactions generating the blocked cyclopentano-phosphatidylserines was distilled from potassium hydroxide and stored over molecular sieves (4 Å) and calcium hydride. The Cbz and benzyl protecting groups were removed by hydrogenolysis in glacial acetic acid using palladium-on-charcoal (10%) as catalyst at room temperature and a pressure of 50 psi. Diazomethane was prepared from Diazald as described by the Aldrich Chemical Co., Inc. (Milwaukee, WI).

![Fig. 1. Configuration of diastereoisomeric cyclopentanetriols. 1, (1,2,3/0)-cyclopentane-1,2,3-triol; 2, DL-(1,2/3)-cyclopentane-1,2,3-triol; 3, (1,3,2)-cyclopentane-1,2,3-triol. Configuration of cyclopentano-phosphatic acid, diphenyl esters (4a–9a); cyclopentano-phosphatic acids (4b–9b); N-carbobenzyloxy-cyclopentano-phosphatidylserine, benzyl esters (4c–9c); cyclopentano-phosphatidylserines (4d–9d); 4, 1,2,3/0-(1P); 5, 1,2,3/0-(3P); 6, 1,2,3/0-(1P); 7, 1,3/2-(1P); 8, 1,3/2-(2P); 9, 1,2,3/0-(2P).](https://www.jlr.org/article-graphics/646/1/1.jpg)

**EXPERIMENTAL**

Each of the isomeric N-carbobenzyloxy-cyclopentano-phosphatidylserine benzyl esters was synthesized by the condensation of the corresponding cyclopentano-phosphatic acid 4b–9b with N-carbobenzyloxy-L-serine

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*Before use, DMF was dried over anhydrous calcium sulfate (Drierite) and fractionally distilled at normal pressure.*
benzyl ester in the presence of TPS in pyridine at room temperature. The experimental conditions described

below for the all-trans (1,3/2) isomer 7b were appropriate for each of the other isomers, 4b–9b (see Scheme 1).

Synthesis of (1,3/2)-(1,2-dipalmitoyl cyclopentano-3-phosphoryl)-N-carbobenzyloxy-L-serine benzyl ester (7c)

Freshly recrystallized N-carbobenzyloxy-L-serine benzyl ester (975 mg; 2.0 mmol) and TPS (896 mg; 2.0 mmol) in anhydrous pyridine (ca. 10 ml) were stirred for 25 min at room temperature and (1,3/2)-1,2-dipalmitoyl cyclopentanetriol-3-phosphate 7b (1000 mg; 1 mmol) in anhydrous pyridine (15 ml), was added under anhydrous conditions to the stirred solution. After 20 hr, the solution was diluted with water (1 ml) and evaporated under reduced pressure to give a light brown oil. Traces of pyridine and water were removed by evacuation (1 torr) over phosphorus pentoxide (15 hr). The residue was triturated with anhydrous diethyl ether (ca. 100 ml) and the suspension was allowed to stand for 20 hr at 4°C. The solution was filtered, the residue of the triisopropylbenzene sulfonic acid (pyridinium salt) was washed with cold diethyl ether, and the solvent was removed under reduced pressure to give a tan colored oil. TLC analysis of the oil showed a major phosphate-positive spot ($R_f$ 0.42) in CHCl$_3$-CH$_3$OH 4:1 (v/v) and a minor unidentified spot at the solvent front. The lipid product at this stage was relatively free of triisopropylbenzene sulfonate. The lipid (500 mg) was further purified by column chromatography on silicic acid, with triethylamine as a buffering agent, as described by Aneja et al. (12). The silicic acid (35 g) was slurried with chloroform containing 0.5% freshly distilled triethylamine, and the column was washed with CHCl$_3$-Et$_3$N 100:0.5, v/v (100 ml). A solution of the lipid product in CHCl$_3$ (500 mg in 5 ml) was applied to the column, and elution done as follows: CHCl$_3$-Et$_3$N 100:0.5 v/v (100 ml), CHCl$_3$-CH$_3$OH-Et$_3$N 99:1:0.5 v/v/v (150 ml), CHCl$_3$-CH$_3$OH-Et$_3$N 194:6:1, v/v/v (200 ml). The product was eluted by the latter solvent mixtures; it gave a single spot ($R_f$ 0.42) in CHCl$_3$-CH$_3$OH 4:1 (v/v). Removal of the solvent from the combined eluates gave a colorless gum. For analytical purposes, a small quantity of the gum (50–100 mg) was freed of cations and converted to the sodium salt according to a modification (16) of the procedure of Bligh and Dyer (17) as follows. The dried material was partitioned in a two-phase system consisting of CHCl$_3$–CH$_3$OH–H$_2$O (0.1 N HCl) 5:5:4.5 (v/v/v). The lower chloroform phase was removed and washed with CH$_3$OH–H$_2$O 10:9, v/v (10 ml). The cation-free solution was titrated with 0.2 N methanolic NaOH to a faint pink color (phenolphthalein internal indicator) and back-titrated to a colorless solution. This solution was evaporated to a small volume (1 ml) and diluted with ten volumes of cold acetone. The precipitated N-carbobenzyloxy-cyclopentano-phosphatidylserine benzyl ester was centrifuged at 4°C, and the solid was dried in vacuo at room temperature. The precipitation procedure generally yielded chromatographically pure lipid representing 70–75% by weight of the original gum employed. Elemental analyses for each of the six diastereoisomers are given in Table 1.

Synthesis of (1,3/2)-(1,2-dipalmitoyl-cyclopentano-3-phosphoryl)-L-serine (7d) [(1,3/2)-cyclopentano-phosphatidylserine]

(1,3/2)-(1,2-dipalmitoyl-cyclopentano-3-phosphoryl)-N-carbobenzyloxy-L-serine benzyl ester (7c; 900 mg) in glacial acetic acid (15 ml) was hydrogenolyzed at room temperature at a hydrogen pressure of 50 psi (20 hr) in the presence of palladium-C catalyst (200 mg). The solution was diluted with CHCl$_3$ (10 ml), filtered over

Scheme 1. Reaction sequence for 1,3/2-(1P)-cyclopentano-phosphatidylserine.
TABLE 1. Analytical data for isomeric cyclopentano-analogs of phosphatidylserine (N-carbobenzyloxy-benzyl esters sodium salts)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Configuration</th>
<th>Found:</th>
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<tbody>
<tr>
<td>4c</td>
<td>1,2,3/0-(1P)</td>
<td>65.40</td>
</tr>
<tr>
<td>5c</td>
<td>1,2/3-(3P)</td>
<td>65.63</td>
</tr>
<tr>
<td>6c</td>
<td>1,2/3-(1P)</td>
<td>64.81</td>
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<td>7c</td>
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<td>9c</td>
<td>1,2,3/0-(2P)</td>
<td>65.38</td>
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\(a\) N/P is ratio gram-atoms N/gram-atoms P. 
\(b\) Na/P is ratio gram-atoms Na/gram-atoms P.

Dried over phosphorus pentoxide for 24 hr at room temperature (0.2 torr).

celite, and evaporated under reduced pressure to give a residual gum; TLC analysis showed a major ninhydrin-positive and phosphate-positive spot \(R_f\) 0.27 in CHCl_3–CH_3OH–H_2O 65:35:5 (v/v/v) and a minor unidentified spot near the solvent front. The cyclopentano-analog (500 mg) was further purified by column chromatography on silicic acid, with triethylamine as a buffering agent, as follows. The silicic acid (35 g) was slurried with CHCl_3 containing 0.5% freshly distilled triethylamine, and the column was washed with CHCl_3–Et_3N 100:0.5 v/v (100 ml). A solution of the cyclopentano-PS isomer in CHCl_3 (500 mg in 5 ml) was applied to the column, and eluted as follows: CHCl_3–CH_3OH 95:5 v/v (150 ml), CHCl_3–CH_3OH 90:10 v/v (100 ml), CHCl_3–CH_3OH 85:15 v/v (100 ml), CHCl_3–CH_3OH 80:20 v/v (250 ml). The product was eluted by the latter solvent mixture; it gave a single spot on TLC in neutral and acidic solvent systems (Table 4). Removal of solvent gave a residual gum. The lipid was freed of cations and converted to its sodium salt which was precipitated by acetone in a manner analogous to that described for the protected derivative. The precipitation procedure yielded chromatographically pure lipid representing 85–90% by weight of the lipid originally eluted from the column. Elemental analyses, melting ranges, and chromatographic mobilities for each of the six diastereoisomeric analogs of PS are given in Table 2, Table 3, and Table 4, respectively.

Diazomethylation of (1,3/2)-(1,2-dipalmityloctylcyclopentano-3-phosphoryl)-l-serine

To a solution (1,3/2)-cyclopentano-PS \((7d; 500 \text{ mg})\) in CHCl_3 (10 ml) a freshly prepared solution of diazomethane in diethyl ether was slowly added until the yellow color persisted. After standing overnight at room temperature, the solution was freed of a small amount of suspended polymethylene and brought to dryness under reduced pressure. The residual solid was dis-

TABLE 2. Analytical data for isomeric cyclopentano-analogs of phosphatidylserine

<table>
<thead>
<tr>
<th>Compound</th>
<th>Configuration</th>
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<tr>
<td>(4d)</td>
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<td>(5d)</td>
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<td>(6d)</td>
<td>1,2/3-(1P)</td>
<td>58.09</td>
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<td>(7d)</td>
<td>1,3/2-(1P)</td>
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<td>(8d)</td>
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<td>57.51</td>
</tr>
<tr>
<td>(9d)</td>
<td>1,2,3/0-(2P)</td>
<td>58.09</td>
</tr>
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</table>

\(a\) N/P is ratio gram-atoms N/gram-atoms P. 
\(b\) Na/P is ratio gram-atoms Na/gram-atoms P.

Dried over P_2O_5 for 24 hr at room temperature (0.2 torr).
solved in diethyl ether and treated with excess ethereal diazomethane. After standing at room temperature for 4 hr, the solution was concentrated and the products were chromatographed on silicic acid using CHCl₃ as eluting solvent. The lipid product gave a single spot on TLC (Rf 0.75, CHCl₃-diethyl ether 3:1 (v/v) and was spectrally identical with the dimethyl ester of cyclopentano-phosphatidic acid.

**Analysis.** Theory for C₃₉H₇₅O₈P (702.28): C, 66.69; H, 10.68; P, 4.41. Found: C, 66.71; H, 10.70; P, 4.48.

**RESULTS AND DISCUSSION**

The protected cyclopentanoid analogs of PS were obtained by TPS-mediated condensation (12) of each in turn of the cyclopentano-PA isomers (6) and a suitably protected L-serine derivative (15). The principal lipid product in each reaction, Cbz-cyclopentano-phosphatidyl-L-serine benzyl ester, was isolated, in yields between 60 and 80%, by column chromatography on silicic acid buffered with triethylamine (12). After hydrogenolysis to remove the Cbz and benzyl protecting groups, the cyclopentano-PS isomers were obtained in good yield by chromatography on buffered silicic acid. Each PS analog was precipitated by acetone from chloroform solution as a microcrystalline mixture of mono- and di-sodium salts which was stored at -15°C. No decomposition could be detected by TLC after prolonged periods of storage at 4°C.

**Thin-layer chromatography**

Although the protected cyclopentano-PS isomers were well separated in TLC examination, only marginal differences in chromatographic mobility were observed between the unprotected cyclopentano-PS isomers in both neutral and acidic solvent systems (Table 4). In two-dimensional chromatography, each isomer gave one spot, but a mixture of the six isomers could not be completely resolved, three spots being observed. One-dimensional chromatography shows that the effect of the configurational differences in the cyclopentane ring on the mobility of the isomers also appears to be sensitive to the nature of the polar head group. Comparison of the Rf values of both the series of cyclopentano-PC isomers (7) and cyclopentano-PA isomers (6) with the cyclopentano-PS isomers reveals little correlation with ring configuration. In the case of the cyclopentano-PS series, the most striking and consistent feature is the high mobility of the cis-trans isomers 6c, 6d (1,2/3-(1P) and the low mobility of the all-cis isomers 9d and 4d (Table 4). The characteristically high apparent polarity of the all-cis isomer in cyclopentano-phospholipids has been observed in both the cyclopentano-PC and the cyclopentano-PA series (6, 7). The mobility of each of the cyclopentano-PS isomers was similar to that of bovine glycerophosphatidylserine (Table 4).

**Analytical data**

Analysis of the vacuum-dried (0.2 Torr) sodium salt precipitates of cyclopentano-PS isomers gave values for C, H, N, and P that are consistent with formulation as monohydrates of di-sodium salts. However, the sodium analysis values themselves varied from 1.37 to 1.88 g-atoms Na per mol (Table 2), and we must conclude that the use of phenolphthalein as an external indicator leads to the formation of a mixture of mono- and di-sodium salt forms. It is worthy of note that titration of the protected cyclopentano-PS isomers, under identical experimental conditions as described for the unprotected compounds, leads to the formation of anhydrous salts that contain one equivalent of sodium per phosphorus (Ta-
ble 1). These data are in accord with those anticipated for lipids with one ionizable acid functional group.

**Infrared spectra**

The infrared spectra of KBr dispersions of the sodium salts of the protected cyclopentano-PS derivatives (4c–9c) show the expected absorption bands as follows: P=O (1240–1270 cm⁻¹), P−O−C (1055 cm⁻¹ and P−O− 1100 cm⁻¹), −C ester (1745,1710 cm⁻¹),

−CH₂ (2940–2830 cm⁻¹), aromatic C−H (3040–3010 cm⁻¹), −N−H (broad, centered at 3350 cm⁻¹),

R−C−O−R (1260–1160 cm⁻¹), C−N (1342–1320 cm⁻¹),

<table>
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<th>Compound</th>
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<th>578</th>
<th>546</th>
<th>436</th>
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<td>+6.18</td>
<td>+6.75</td>
<td>+9.23</td>
</tr>
<tr>
<td>5d</td>
<td>1,3/3-(3P)</td>
<td>+1.00</td>
<td>+2.01</td>
<td>+3.80</td>
<td>+3.83</td>
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<tr>
<td>6d</td>
<td>1,2/3-(1P)</td>
<td>+1.00</td>
<td>+2.00</td>
<td>+4.24</td>
<td>+5.13</td>
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<tr>
<td>7d</td>
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<td>+9.16</td>
<td>+9.96</td>
<td>+11.30</td>
<td>+18.30</td>
</tr>
<tr>
<td>8d</td>
<td>1,3/2-(2P)</td>
<td>−7.47</td>
<td>−9.28</td>
<td>−11.00</td>
<td>−13.40</td>
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<td>+4.47</td>
<td>+5.10</td>
<td>+5.75</td>
<td>+9.52</td>
</tr>
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</table>

Since the differences in spectra obtained for each isomer are very slight, only a representative spectrum is shown in Fig. 2 [all-trans 1,3/2-(1P)].

The infrared spectra of KBr dispersions of the sodium salts of the cyclopentano-PS isomers (4d–9d) show the expected absorption bands as follows: for P=O (1240–1270 cm⁻¹), P−O−C (1055 cm⁻¹ and P−O− 1100 cm⁻¹), −C ester (1745–1710 cm⁻¹), −CH₂ (2940–2840 cm⁻¹), N−H and H₂O (3500–3100 cm⁻¹),

R−C−O−(1260–1160 cm⁻¹). A representative spectrum is shown in Fig. 2 [all-trans 1,3/2-(1P)].

**Optical rotations**

The optical rotation values are shown in Table 5. With one exception, each of the cyclopentano-PS isomers exhibited a positive rotation that increases with decreasing wavelength. The exception is the isomer of all-trans configuration [1,3/2-(2P)] which exhibited an increasingly negative rotation as the wavelength of the incident light was reduced. The chirality of the compounds is solely due to the contribution of the amino acid since no attempt has yet been made by us to resolve optical antipodes in the cyclic moiety.

**Melting behavior**

The sodium salts of the series of cyclopentano-PS isomers exhibit significant differences in melting point. These differences appear to originate from two kinds of structural feature in the compounds: 1) the configurational geometry of the cyclopentane ring in the isomer and 2) the position of substitution of the polar head group. The apparent effect of the ring configuration is shown by the decrease in melting point throughout the series: 1,3/2-(1P) > 1,2,3/0-(1P) > 1,2/3-(3P) > 1,2/3-(1P) > 1,3/2-(2P) > 1,2,3/0-(2P).

However, the difference in melting point between the all-trans isomer [1,3/2-(1P)] and the all-cis isomer [1,2,3/0-(1P)] is marginal (ΔT = 3°), in contrast to our earlier observation for the corresponding cyclopentano-lecithin analogs (7), for which ΔT = 36°. Furthermore, we observed that in the cyclopentano-lecithins, those isomers that had adjacent and "syn"-chains had higher melting points than those in which the acyl chains were
in an “anti”-disposition. We cannot make such a
generalization for the cyclopentano-PS analogs because the
highest melting compound is the all-trans [1,3/2-(1P)]
isomer. We should point out that correlations between
structure and melting point may be obscured by the
variation in cation content between the cyclopentano-PS
isomers (see Na/P ratios, Table 2), since the overall
melting point of phospholipids involves interactions at
the polar head region as well as associations within the
acyl chain aggregates. In support of this contention, we
observe that for the fully protected cyclopentano-PS deri-
atives (4c–9c, Table 1), in which the Na/P ratio is
lower, the trend of melting point values closely resembles
that observed for the zwitterionic cyclopentano-lec-
ithins.

Diazometatholysis of cyclopentano-PS

The characteristic diazomethane-mediated cleavage
reaction of glyceryl-phosphatidylserines, first reported by
Baer and Maurukas (18), was also demonstrated for the
all-trans-cyclopentano-PS [1,3/2-(1P)]. The lipid product,
obtained in quantitative yield, was shown to be the di-
methyl ester of cyclopentano-PA by comparison with an
authentic sample (IR, NMR, TLC) and by elemental
analysis. A control experiment using all-trans-cyclo-
 pentano-PC [1,3/2-(1P)] instead of cyclopentano-PS yielded
only unchanged starting material, so we conclude that,
at least for the all-trans isomer, the cyclopentano-phos-
pholipid analogs of PS and PC behave in an analogous
manner to glycerol-PS and glycerol-PC.11

The authors thank Paul A. Phillips and Kim C. Wade for
assistance in the preparation of some of the intermediates used
in this work and Barbara L. Rauscher for the photography.
The help of Dr. A. W. Burgstahler in obtaining optical ro-
tation measurements is gratefully acknowledged. The work
was supported by a grant from the Faculty Research Council,
University of Missouri (Kansas City), and was initiated with
funding from the American Heart Association (Missouri af-
filiate), and the National Institutes of Health (GM-21047-06).

Manuscript received 9 August 1982 and in revised form 6 January 1983.

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mimic the active rotameric state of the natural substrate.
synthesis of phosphatidylserine and purification by CM-
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