papers and notes on methodology

# Synthesis of phosphatidylcholine analogs with an alkyl group at C1 or C3 of the glycerol moiety

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**Abstract** The syntheses of 1,2-di-(*O*-hexadecyl)-*rac*-glycero-3-phosphocholines containing a methyl group at either C1 or C3 of the glycerol moiety are described. The methyl group was introduced at C1 in a synthetic scheme beginning with the hydroxylation of methyl vinyl ketone. The primary hydroxyl was protected by tritylation and the carbonyl group was reduced with sodium borohydride. Di-*O*-alkylation with 1-bromohexadecane was accomplished using finely powdered potassium hydroxide in refluxing toluene. Detritylation afforded two diastereomers of 2,3-di-(1-hexadecyloxy)butanol. Reaction with the simple phosphorylating agent, dimethylphosphoryl chloride (Bittman, R., A. F. Rosenthal, and L. A. Vargas. 1984. *Chem. Phys. Lipids.* **34:** 201–205), followed by conversion to the phosphatidic acid and condensation with choline tosylate in the presence of trichloroacetonitrile afforded the diastereomers of 1-methyl-1,2-di-(*O*-hexadecyl)-*rac*-glycero-3-phosphocholine. The analog bearing a methyl group at C3 was prepared in a synthetic scheme beginning with the hydroxylation of acrolein dimethyl acetal. After di-*O*-alkylation with 1-bromohexadecane and sodium hydride in dimethyl sulfoxide/toluene, the acetal was converted to the aldehyde. Reaction with methylmagnesium bromide afforded the diastereomers of 1,2-di-(1-hexadecyloxy)-3-butanol, which were converted to the phosphocholine derivatives. These diether phosphatidylcholine analogs may be useful for investigating the effect of steric bulk at C1 and C3 of the glycerol moiety on the interactions with membrane components. —**Witzke, N. M., and R. Bittman.** Synthesis of phosphatidylcholine analogs with an alkyl group at C1 or C3 of the glycerol moiety. *J. Lipid Res.* 1985. **26:** 623–628.

**Supplementary key words** phospholipid synthesis • ether-linked lipid

Systematic alterations in chemical structure have been introduced into phospholipids, increasing our understanding of the effects of structure on their physical and biological properties (1–6). Studies with synthetic phospholipid analogs have also contributed to our knowledge of the structural requirements in phospholipid-cholesterol and phospholipid-protein interactions. For example, replacement of the acyl carboxyl group by the *O*-alkyl linkage allowed examination of the role of the ester carbonyl groups in the interactions of phospholipids with cholesterol (7–12) and membrane proteins (11, 13). Similarly, the role of the glyceryl ether oxygen atoms has been studied using an alkyl analog of PC having neither a diacylglycerol nor a glycerol diether moiety (8, 9, 13). Isosteric and nonisosteric analogs of PC bearing C–P (phosphonate) and C–P–C (phosphinate) linkages have been used to examine the role of the oxygen atoms on either side of the phosphate ester of PC in the interactions with cholesterol (14) and proteins (13, 15, and references cited therein). A number of PC analogs were synthesized for the study of the structural requirements for phospholipase activity (1, 2, 16). The influence of the hydrocarbon region on the interaction with cholesterol and proteins has been studied using phospholipids differing in chain length, unsaturation, and branching. Natural and synthetic phospholipids with different head-group structures have been used to examine the involvement of specific interactions in the polar region (6, 13, 17). However, very few studies have been directed toward structural alterations at specific sites of the glycerol group of glycerophospholipids (with the exception of changes in stereochemical configuration). Severe restriction of the extent of conformational freedom in this region of the phospholipid molecule was achieved by introducing a ring into the glycerol moiety. Blume and Eibl (18) incorporated the oxygens of the 1 and 2 positions of glycerol into a five-membered ketal (dioxolane) ring, and Hancock and co-workers (19, 20) synthesized cyclopentanoid analogs. We report here the first type of structural derivatives containing a *C*-alkyl branch within the glycerol moiety of glycerophospholipids without introducing a ring. The synthetic procedures we describe for the

Abbreviations: PC, phosphatidylcholine; TLC, thin-layer chromatography; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran.

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1- and 3-methyl-1,2-di-(*O*-hexadecyl)glycerophosphocholines may be used to prepare derivatives with larger alkyl groups at C1 or C3. These compounds have potential for studying the effect of steric bulk at C1 and C3 of the glycerol moiety and consequent changes in rotational isomerizations around the C1–C2 or C2–C3 bonds on interactions between phospholipids and various membrane components.

## MATERIALS AND METHODS

Elemental analyses were performed by MicAnal (Tucson, AZ) and Schwarzkopf (Woodside, NY). Proton NMR spectra were recorded on a Varian EM 360 60-MHz spectrometer, using tetramethylsilane as internal standard. TLC was carried out on silica gel G glass plates (Analtech, Newark, DE). The purity of intermediates and products was checked on 0.25-mm-thick plates; preparative TLC was carried out on 1-mm and 2-mm-thick plates. General detection was by spraying the plates with 10% sulfuric acid in ethanol, followed by charring on a hot plate. Trityl compounds were visualized by their immediate yellow color after spraying. Phospholipids were detected with the molybdate spray (21). Purification of the products by column chromatography was performed with the same solvent used for analytical TLC. Trityl-containing compounds were purified by column chromatography using solvent systems containing 1,2-dichloroethane; we found that chloroform caused cleavage of the trityl group. Residual silicic acid was removed by passage of the chloroform solution of the product through $\alpha$-Metricel (0.45 $\mu$m) filters.

Toluene, triethylamine, DMSO, and pyridine were dried and stored over $CaH_2$ prior to use. Choline *p*-toluenesulfonate was prepared as described by Rosenthal (22) and dried thoroughly over $P_2O_5$ under vacuum before use. Dimethylphosphoryl chloride was synthesized as described previously (23). Methylmagnesium bromide was purchased from Alfa. Trichloroacetonitrile, trityl chloride, 1-bromohexadecane, and acrolein dimethyl acetal were purchased from Aldrich. Phosphorus oxychloride and alcohol-free chloroform were obtained from Matheson, Coleman, and Bell. Methyl vinyl ketone was from Sigma.

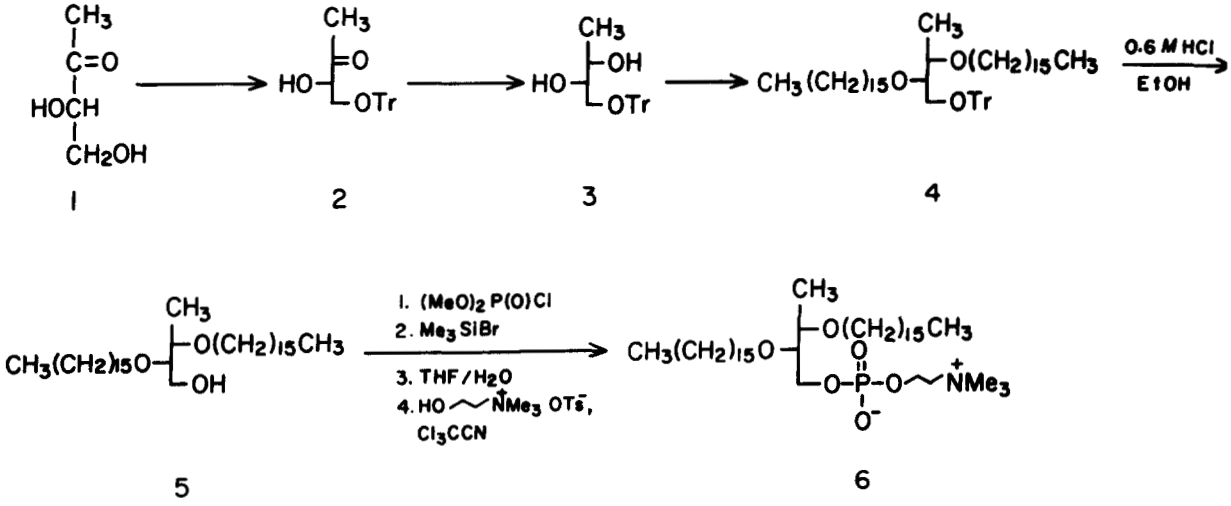
## CHEMICAL SYNTHESES

### *rac*-Butane-3,4-diol-2-one (Scheme 1, *1*)

This compound was prepared from methyl vinyl ketone and sodium chlorate/osmium tetroxide according to the procedure of Fischer et al. (24). The keto glycol (Scheme 1, *1*) was obtained in 35% yield, bp 81–84°C (0.2 torr), mp 36–37°C.

### *rac*-4-Trityloxy-3-hydroxybutan-2-one (Scheme 1, *2*)

A solution of *rac*-butane-3,4-diol-2-one (Scheme 1, *1*) (1.00 g, 9.6 mmol) and trityl chloride (3.00 g, 10.7 mmol) in 8 ml of dry pyridine was stirred for 3 days at 30°C. The reaction mixture was diluted with ether (100 ml) and the precipitate was removed by filtration and washed with 20 ml of ether. The filtrate was washed with water (4 × 50 ml), dried with $K_2CO_3$, and evaporated under reduced pressure. The residual oil (3.01 g), which contained the product and trityl alcohol, was used in the next step without further purification.




**Scheme 1.** Reaction sequence for preparation of 1,2-di-(*O*-hexadecyl)-1-methyl-*rac*-glycero-3-phosphocholine (two diastereomers).

### 1-*O*-Tritylbutane-1,2,3-triol (diastereomeric mixture) (Scheme 1, *3*)

The crude ketone (Scheme 1, *2*) (3.01 g, 8.6 mmol) was dissolved in a mixture of 2-propanol (30 ml) and 95% ethanol (75 ml). The solution was cooled to 2°C and sodium borohydride (0.60 g, 15.8 mmol) was added. The mixture was stirred for 1 hr at 2–5°C, and then for 30 min at room temperature. The excess borohydride was decomposed by dropwise addition of glacial acetic acid (1.5 ml). Then ether and water were added (400 ml of each), and the ether layer was separated, washed with water (2 × 100 ml), and dried with $K_2CO_3$. The aqueous phases were combined and re-extracted with 150 ml of ether. After the ether extract was washed with 50 ml of water and combined with the previous ether extract, the solvents were removed under reduced pressure. A glassy solid (2.60 g) remained, which was dissolved in 6 ml of 1,2-dichloroethane and applied to a column of silica gel (240 g, 230–400 mesh, Merck). Elution with 1,2-dichloroethane–ethanol 96:4 (v/v) afforded 1.47 g (43% based on *rac*-butane-3,4-diol-2-one) of the triol bearing the protected primary hydroxyl group (Scheme 1, *3*). $^1$H NMR ($CCl_4$) $\delta$ (ppm):0.95 (d, J = 6Hz) and 1.15 (d, J = 6Hz) [3H, $CH_3$], 2.9–4.1 (6H, m, $-CH_2OTr$ and $-CHOH$), and 6.9–7.5 (15H, m, $C_6H_5$).

### 2,3-Di-(1-hexadecyloxy)-1-*O*-tritylbutanol (diastereomeric mixture) (Scheme 1, *4*)

A mixture of 1-*O*-tritylbutane-1,2,3-triol (Scheme 1, *3*) (0.38 g, 1.06 mmol) and 90% powdered potassium hydroxide (0.38 g, 6.06 mmol) in 11 ml of toluene was refluxed with stirring for 45 min under a Dean-Stark water trap (about 2.5-ml capacity). Then 1-bromohexadecane (0.70 g, 2.3 mmol) was added, and reflux and stirring were continued for 5 hr. An additional 0.60 g (2.0 mmol) of 1-bromohexadecane was added and reflux was continued for 5 hr. The trap was emptied and 2.5 ml of toluene was removed by distillation. The residue was cooled and treated with water (20 ml) and hexane (30 ml). The organic phase was separated, washed with water (2 × 10 ml), and dried with $K_2CO_3$. Removal of the solvents under reduced pressure gave 1.22 g of an oil, which was subjected to column chromatography on silica gel (80 g, 60–200 mesh, Baker). Elution with toluene–hexane 1:1 (v/v) yielded, after evaporation of the solvents, 0.55 g (65%) of the dialkyltritylbutanetriol (Scheme 1, *4*) as a viscous oil. $^1$H NMR ($CCl_4$) $\delta$ (ppm):0.8–1.8 (65H, m, $-C-CH_2-C$, $-CH_3$), 2.9–3.7 (8H, m, $OCH_2$, $-CHO-$), and 6.9–7.5 (15H, m, $C_6H_5$).

### 2,3-Di-(1-hexadecyloxy)butanol (two diastereomers) (Scheme 1, *5*)

Detritylation of the dialkyltritylbutanetriol (Scheme 1, *4*) was carried out by refluxing a solution of 2,3-dihexadecyloxy-1-*O*-tritylbutanol (0.65 g, 0.815 mmol) in 95% ethanol (26 ml) with concentrated HCl (1.3 ml) for 1.5 hr. The mixture was cooled, and water (13 ml) and hexane (130 ml) were added. The hexane layer was separated, washed with 85% methanol (3 × 30 ml) to remove most of the trityl alcohol, dried with $Na_2SO_4$, and evaporated under vacuum. The residue was applied to a column of silica gel (95 g, 230–400 mesh, Woelm). The column was eluted with chloroform. Upon evaporation of the solvent, the faster-moving (120 mg, 27%, mp 58.5–60°C) and the slower-moving (250 mg, 55%, mp 43.0–43.5°C) diastereomers of the deprotected dialkylbutanetriol (Scheme 1, *5*) were obtained as crystalline residues. Anal. Calc. for $C_{36}H_{74}O_3$ (554.99) C, 77.91; H, 13.44. Found: faster-moving diastereomer, C, 77.46; H, 12.71; slower-moving diastereomer, C, 77.68; H, 13.32.

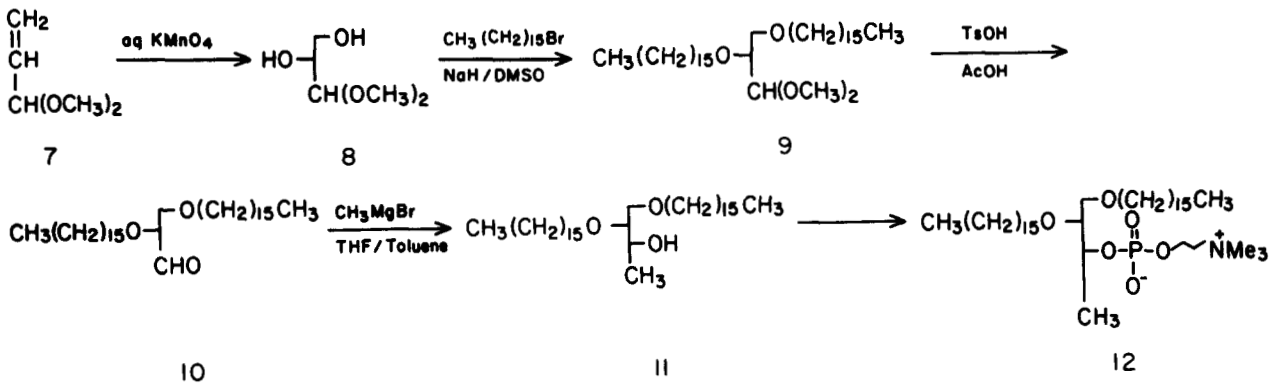
### 1,2-Di-(*O*-hexadecyl)-1-methyl-*rac*-glycero-3-phosphocholine (two diastereomers) (Scheme 1, *6*)

The faster-moving diastereomer of 2,3-(di-1-hexadecyloxy)butanol (Scheme 1, *5*) (60 mg, 0.108 mmol) was converted to the phosphocholine (Scheme 1, *6*) (39 mg, 50%), after recrystallization from methyl ethyl ketone (50 ml/g), using the procedure outlined by Bittman, Rosenthal, and Vargas (23); see also Scheme 2, *12*. Similarly, 62 mg (0.112 mmol) of the slower-moving diastereomer of 2,3-(di-1-hexadecyloxy)butanol was converted to the corresponding phosphocholine (42 mg, 52%). Anal. Calc. for $C_{41}H_{86}NO_6P \cdot H_2O$ (738.13) C, 66.72; H, 12.02; N, 1.90; P, 4.20. Found: C, 66.69; H, 11.62; N, 1.96; P, 4.11. The $R_f$ value of the slower-moving diastereomer *6* was identical to that of dipalmitoyl-PC (0.34) in chloroform–methanol–7 N ammonium hydroxide 65:27:5 (v/v/v), and 0.27 in chloroform–methanol–water 65:25:4 (v/v/v). $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 0.90 (6H, apparent triplet, $(CH_2)_{15}CH_3$), 1.3 (59H, m, $-C-CH_2-C$, $CH_3-CHO-$), and 3.2–4.0 (21H, m, $OCH_2$, $-OCH-C$, $-CH_2N$, $-NCH_3$); the $N-CH_3$ appeared at $\delta$ 3.4 ppm.

### *rac*-Glyceraldehyde dimethyl acetal (Scheme 2, *8*)

This compound was prepared by a modification of the synthesis of *rac*-glyceraldehyde diethyl acetal from acrolein diethyl acetal (25).

A solution of potassium permanganate (16 g, 0.10 mol) in 300 ml of water was added dropwise with stirring over a period of 1 hr to a cold (5°C) solution of acrolein dimethyl acetal (Scheme 2, *7*) (10.2 g, 0.10 mol) in 120 ml of water. During the addition the temperature was maintained between 5° and 7°C. On standing in an ice bath for 2 hr the reaction mixture set to a gel. The mixture was then heated on a boiling water bath until the precipitate coagulated (30–40 min). The precipitate was removed by filtration and washed with water (50 ml). Anhydrous potassium carbonate (300 g) was added to the filtrate (380




**Scheme 2.** Reaction sequence for preparation of 1,2-di-(*O*-hexadecyl)-3-methyl-*rac*-glycero-3-phosphocholine (two diastereomers).

ml) and the solution was extracted with 1-butanol (4 × 25 ml). The combined butanol extract was dried with $K_2CO_3$ and evaporated under reduced pressure. The product was isolated (5.5 g, 40%) by vacuum distillation, bp 133–133.5°C (22 torr). $^1H$ NMR ($D_2O$) $\delta$ ppm (relative to external tetramethylsilane in $CDCl_3$): 2.6 (6H, s, $-OC\underline{H}_3$), 2.75 (3H, m, $-OC\underline{H}_2$ and $-C\underline{H}O-$), and 3.6 (1H, d, J = 5Hz, $-C\underline{H}(OCH_3)_2$).

### *rac*-2,3-Di-(1-hexadecyloxy)-1,1-dimethoxypropane (Scheme 2, *9*)

Sodium hydride (0.95 g, 40 mmol) was placed in a 250-ml three-necked flask fitted with a reflux condenser, $N_2$ inlet, and septum injection port. After the flask was flushed with $N_2$ for 20 min, dimethyl sulfoxide (40 ml) was added. The evolution of hydrogen ceased after about 40 min of stirring. Then a solution of *rac*-glyceraldehyde dimethyl acetal (Scheme 2, *8*) (1.40 g, 10.2 mmol) in 8 ml of toluene was injected into the flask. After the mixture was stirred under $N_2$ for 30 min, 1-bromohexadecane (6.7 g, 22 mmol) was injected over a 10-min period. The reaction mixture was stirred at room temperature under $N_2$ for 24 hr. Hexane (100 ml) and water (200 ml) were added, and the hexane layer was separated, washed with water (3 × 50 ml), dried with $K_2CO_3$, and evaporated under vacuum. The resulting crude oil (5.78 g) was dissolved in 10 ml of hexane. On cooling to 15°C, a precipitate formed (mono-hexadecyloxy-1,1-dimethoxypropanol), which was removed by filtration and washed with cold hexane. The filtrate was evaporated to dryness under reduced pressure. The residue (5.39 g) was applied to a column of silica gel (200 g, 60–200 mesh, Baker). Elution with chloroform yielded 2.43 g (40%) of the di-*O*-alkyl derivative of glyceraldehyde dimethyl acetal (Scheme 2, *9*) as a colorless oil, which crystallized slowly, mp 30–31°C. $^1H$ NMR ($C_2Cl_4$) $\delta$ ppm:0.6–2.3 (59H, m, $-C-C\underline{H}_2-C$, $-C\underline{H}_3$), 3.15–3.7 (12H, m, $-OC\underline{H}_2$, $-OC\underline{H}_3$, $-OC\underline{H}-C$), and 4.15 (1H, d, J = 5Hz, $-C\underline{H}(OCH_3)_2$). The expected integral ratio is 62H:13H:1H.

### *rac*-2,3-Di-(1-hexadecyloxy)propanal (Scheme 2, *10*)

The dialkylated acetal (Scheme 2, *9*) (1.84 g, 3.15 mmol) was dissolved in 18 ml of glacial acetic acid with warming. To this solution *p*-toluenesulfonic acid monohydrate (0.18 g, 0.95 mmol) was added, and the mixture was heated at 100°C for 5 min. The mixture was then cooled to below 50°C, and acetone (2 ml) was added. The product precipitated when the mixture was maintained at 5°C overnight. The precipitate was collected and washed with 30 ml of cold acetic acid–acetone 9:1 (v/v) and then with acetone. The crystalline aldehyde (Scheme 2, *10*) was dried at 0.1 torr over KOH, yielding 1.44 g (85%), mp 46.5–47.0°C. Anal. Calc. for $C_{35}H_{70}O_3$ (538.94) C, 78.00; H, 13.09. Found: C, 77.75; H, 13.25.

### 1,2-Di-(1-hexadecyloxy)-3-butanol (two diastereomers) (Scheme 2, *11*)

Methylmagnesium bromide (2.5 ml of 1.7 M in THF-toluene) and 6 ml of dry THF were added to a 100-ml three-necked flask (equipped with a reflux condenser, $N_2$ inlet, and septum injection port) that had been flushed with $N_2$. A solution of *rac*-2,3-di-(1-hexadecyloxy)propanal (0.80 g, 1.48 mmol) in 4 ml of dry toluene was injected slowly into the stirred mixture. After the mixture was stirred under $N_2$ for 2 hr at room temperature, the reaction was terminated by dropwise addition of a solution of 1.5 ml of concentrated HCl in 15 ml of water. Hexane (60 ml) and water (30 ml) were added, and the organic layer was separated, washed with water (3 × 30 ml), and dried with $MgSO_4$. The residual oil (0.61 g) obtained after evaporation of the solvent was dissolved in hexane and purified by preparative TLC on eight silica gel GF plates (1-mm thick). After the crude product was spotted onto the plates, the plates were dried for 30 min at 2 torr, and then eluted with chloroform–ethanol 95:5 (v/v). Two major bands were visualized using iodine vapor; they were scraped from the plates and eluted with chloroform–methanol 9:1 (v/v) (1 × 50 ml) and chloroform–methanol 1:1 (v/v) (6 × 25 ml). The solvents were removed under

reduced pressure, and the residues from each major band were dissolved in 2 ml of chloroform and precipitated from 40 ml of acetonitrile at −20°C. The yields of the faster- and slower-moving diastereomers were 0.23 g (28%), mp 36.5–37.5°C, and 0.17 g (21%), mp 37.5–38°C, respectively. Anal. Calc. for $C_{36}H_{74}O_3$ (554.99) C, 77.91; H, 13.44. Found: faster-moving diastereomer, C, 77.35; H, 13.96; slower-moving diastereomer, C, 77.95; H, 13.63. Mass spectrum of the faster-moving diastereomer: m/z 554 (0.16%, $M^+$), 536 (0.16%, $M-H_2O$), 510 (0.8%), 269 (21%), 222 (22%), 111 (12%), 97 (29%), 82 (40%), 69 (60%), 57 (95%), and 43 (100%). The slower-moving diastereomer had essentially the same mass spectrum, except that the small peak at m/z 536 was absent. The 200-MHz $^1H$ NMR spectra of the two diastereomers in $CDCl_3$ were very similar; e.g., for the faster-moving isomer: $\delta$ (ppm): 0.88 (7.83H, t, J = 6.4Hz, hexadecyl $CH_3$-), 1.02–1.43 (71.42H, m, hexadecyl chain $CH_2$ groups, $C_3$–$C_{15}$), 1.43–1.76 (9.61H, m, hexadecyl $CH_2$ of $C_2$ and $CHOHCH_3$), 2.66 (0.95H, d, J = 4.0Hz, -OH), 3.18 (1H, apparent hextet, apparent J = 5.2 Hz, CHOR), 3.29–3.59 (5.92H, m, $-CH_2OR$), and 3.59–4.01 (3.05H, m, CHOH). The expected ratios are 6H:52H:7H:1H:6H:1H.

**1,2-Di-(*O*-hexadecyl)-3-methyl-*rac*-glycero-3-phosphocholine (two diastereomers) (Scheme 2, *12*)**

A mixture of 1,2-di-(1-hexadecyloxy)-3-butanol (faster-moving diastereomer) (65 mg, 0.117 mmol) and dimethyl chlorophosphate (0.25 ml, 2.3 mmol) in 1.2 ml of alcohol-free chloroform and 0.25 ml of dry pyridine was kept at 5°C for 3 days. The solvents were removed under reduced pressure and the residue was evaporated twice with 5 ml of chloroform–methanol 1:1 (v/v) to assure removal of pyridine. After the solvents were removed and the residue (phosphatidic acid dimethyl ester) was dried for 2 hr at 2 torr, a solution of bromotrimethylsilane (1 ml, 7.6 mmol) in 5 ml of alcohol-free chloroform was added to the residue. The mixture was stirred for 30 min at room temperature, and then the volatiles were removed under reduced pressure. The residue was treated with THF (5 ml) and water (1 ml) for 2 hr at room temperature. The solvents were removed under vacuum, and the residue was dried by repeated azeotropic distillation with 2-propanol and by desiccation overnight at 2 torr. Cold (−20°C) acetonitrile precipitation afforded crude phosphatidic acid, which was dried over $P_2O_5$ and mixed with choline *p*-toluenesulfonate (250 mg, 0.91 mmol), pyridine (6 ml), and trichloroacetonitrile (5 ml). The reaction mixture was stirred at 55–65°C for 2 days. The solvents were removed under vacuum and the residue was dried by treatment with and evaporation of chloroform–methanol 1:1 (v/v) (2 × 10 ml). The crude product was dissolved in 4 ml of THF–water 9:1 (v/v) and applied to a column of Amberlite MB-3 (10 g) that had been equilibrated with THF–water 9:1. The column was eluted with the same solvent mixture. After evaporation of the solvents, the residue was dissolved in a small volume of chloroform. Precipitation of the product from acetonitrile at −20°C, followed by drying, gave 62 mg (74%) of the pure 3-methyl-1,2-di-*O*-alkyl phospholipid (Scheme 2, *12*). Anal. Calc. for $C_{41}H_{86}NO_6 \cdot 3H_2O$ (774.17) C, 63.56; H, 11.88; N, 1.85; P, 4.00. Found: C, 63.61; H, 11.31; N, 1.85; P, 3.96. The slower-moving diastereomer was prepared as described above. The two diastereomers were indistinguishable from each other by TLC in chloroform–methanol–7 N ammonium hydroxide 65:27:5 (v/v/v) and chloroform–methanol–water 65:25:4 (v/v/v). $^1H$ NMR ($CDCl_3$) $\delta$ (ppm): 0.95 (6H, apparent triplet, $(CH_2)_{15}CH_3$), 1.3 (59H, m, $-C-CH_2-C$, $CH_3-CHO-$), and 3.2–3.9 (21H, m, $-OCH_2$, $-OCH-C$, $-CH_2N$, $-NCH_3$); the $N-CH_3$ peak appeared at $\delta$ 3.4 ppm.

## DISCUSSION

*rac*-Diether-PC analogs with a methyl group at either C1 or C3 of the glycerol moiety have been synthesized. The possibility of isomerization via acyl migration during detritylation and phosphorylation was eliminated by using di-*O*-alkyl glycerol derivatives. It should be noted, however, that acyl-linked analogs of *6* and *12* could also be prepared from tritylbutanetriols or glyceraldehyde dimethyl acetal, provided that conditions minimizing acyl migrations are used.

The key intermediate in the preparation of the 3-methyl-1,2-dihexadecyl-PC, *rac*-2,3-di-(1-hexadecyloxy)propanal (Scheme 2, *10*), can serve as precursor to other 3-alkyl-1,2-di-(*O*-hexadecyl)glycerophosphocholines or to labeled derivatives, using the Grignard addition shown in **Fig. 1**. Such products offer potential for detailed investigation of the structural specificity of PC interactions with other membrane constituents.

The elemental analyses were in agreement with the proposed structures. It is of interest to note that variation in the number of water molecules tightly bound to PC and its analogs has been reported previously (e.g., 20).

Satisfactory conversion of a glycol into the di-1-hexadecyl ether using sodium hydride and 1-bromohexadecane in dimethyl sulfoxide was obtained with *rac*-glyceraldehyde

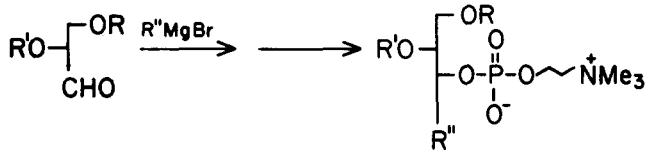



**Fig. 1.** Scheme for introduction of steric bulk into C3 of the glycerol moiety of PC analogs (R" = alkyl or aryl).

dimethyl acetal (Scheme 2, *9*); the di-*O*-alkyl ether was prepared in 40% yield. However, di-etherification of 1-*O*-tritylbutane-1,2,3-triol (Scheme 1, *3*) did not proceed well under these conditions; the major product with sodium hydride in dimethyl sulfoxide was a monohexadecyl ether even when 2.2 equivalents of 1-bromohexadecane were used. We obtained the desired dihexadecyl ether in 65% yield (Scheme 1, *4*) by using powdered potassium hydroxide and 1-bromohexadecane in refluxing toluene. Although benzene and xylene have been used as solvents for the introduction of long-chain alkyl groups into glycerol derivatives with powdered potassium hydroxide and alkyl halides or mesylates (2, 26, 27), we have found toluene to offer several advantages. The high boiling point of xylene led to extensive degradation of the trityl glycol (Scheme 1, *4*), whereas the use of benzene resulted in lower yields and required longer reaction times.

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