papers and notes on methodology

Convenient synthesis of racemic mixed-chain ether glycerophosphocholines from fatty alkyl allyl ethers: useful analogs for biophysical studies

Niels M. Witzke and Robert Bittman[1]

Department of Chemistry, Queens College of the City University of New York, Flushing, NY 11367

Abstract Diether glycerophosphocholines containing one long and one short chain were synthesized in moderate yield. The synthesis of a diether glycerophosphocholine containing a branched chain and a straight chain is also described. Alkylation of fatty alcohol with allyl bromide and dimethyl sulfoxide anion gave fatty alkyl allyl ether, which was hydroxylated and tritylated. By procedures described previously, 1-*O*-alkyl-3-*O*-triphenylmethylglycerol was alkylated, detritylated, and converted into the phosphocholine product. This method is of general utility for the preparation of a variety of mixed-chain racemic glycerophosphocholines whose physical properties are of interest in studies of biological membranes. —**Witzke, N. M., and R. Bittman.** Convenient synthesis of racemic mixed-chain ether glycerophosphocholines from fatty alkyl allyl ethers: useful analogs for biophysical studies. *J. Lipid Res.* 1986. **27:** 344–351.

Supplementary key words phospholipid synthesis • ether-linked lipid

Ether phospholipids are distributed widely in natural membranes (1). Diether-linked glycerophospholipids occur in the membranes of archaebacteria (2), and monoether-linked lipids exist in normal and malignant mammalian cells (3). They are also involved in a wide variety of physiological processes such as aggregation of blood platelets, vasodilation, and tumor cytotoxicity. Since ether glycerolipids are quite stable toward enzymatic and chemical hydrolysis of the alkyl moiety, ether analogs of ester lipids are potentially useful as markers in biosynthetic studies of glycerolipid metabolism (4, 5) and in structural studies of the interactions of phospholipids with other lipids or with proteins in membranes. Phospholipids with hydrocarbon chains of equal or approximately equal lengths have been used in most studies of the physical properties of ether phospholipids in membranes.

In this report we describe the chemical syntheses of racemic mixed-chain diether glycerophosphocholines containing *a*) one long (20:0) and one short (12:0) chain and *b*) two medium-length (16:0) chains, one of which bears an α-methyl branch. Since the methyl group is approximately the same size as oxygen but very different in electronic character, the latter diether phospholipid may be useful for the study of steric effects on phospholipid interactions with membrane components and for a comparison of the properties of acyl/alkyl- and diether-glycerolipids. The diether lipids bearing chains of unequal lengths also have potential application in studies of lipid-lipid interactions. Since phospholipids with highly asymmetric acyl chain moieties form interdigitated regions in the gel phase (6, 7), the availability of these synthetic ether analogs will make it possible to examine whether the presence of the carbonyl group in the interfacial region plays an important role in the interdigitation process.

EXPERIMENTAL PROCEDURES

Materials and methods

Allyl bromide, trityl chloride, trifluoroacetic acid, and 1-eicosanol (arachidyl alcohol) were purchased from Sigma. 1-Bromohexadecane, 2-hexadecanol, and *m*-chloroperoxybenzoic acid were obtained from Aldrich. Dodecanol was from Baker, 1-bromoeicosane was from Pfaltz and Bauer, and 1-bromododecane was from ChemService, Media, PA. Dimethyl sulfoxide (Fisher), toluene (MCB), pyridine (Fisher), triethylamine (Eastman), and tetrahydrofuran (Baker) were dried over calcium hydride (Ventron). Alcohol-free chloroform (hydrocarbon-stabilized from

Abbreviation: TLC, thin-layer chromatography.

[1]To whom correspondence and reprint requests should be addressed.

MCB) was distilled and stored over molecular sieves (5 Å, Fisher). 1,2-Dichloroethane (Aldrich) was stored over anhydrous K_2CO_3. Choline *p*-toluenesulfonate was dried overnight over phosphorus pentoxide at 1 torr before use. Silica gel was from either Baker (60–200 mesh) or Merck (230–400 mesh).

Thin-layer chromatography (TLC) was performed on Analtech (Newark, DE) silica gel GF glass plates. Detection of the compounds on the plates was by spraying and heating as described previously (8). ^1H NMR spectra were recorded on a Varian EM 360 60-MHz spectrometer, using tetramethylsilane as internal standard. The spectra at 200 MHz were recorded on an IBM/Bruker WP200 SY FT-NMR spectrometer.

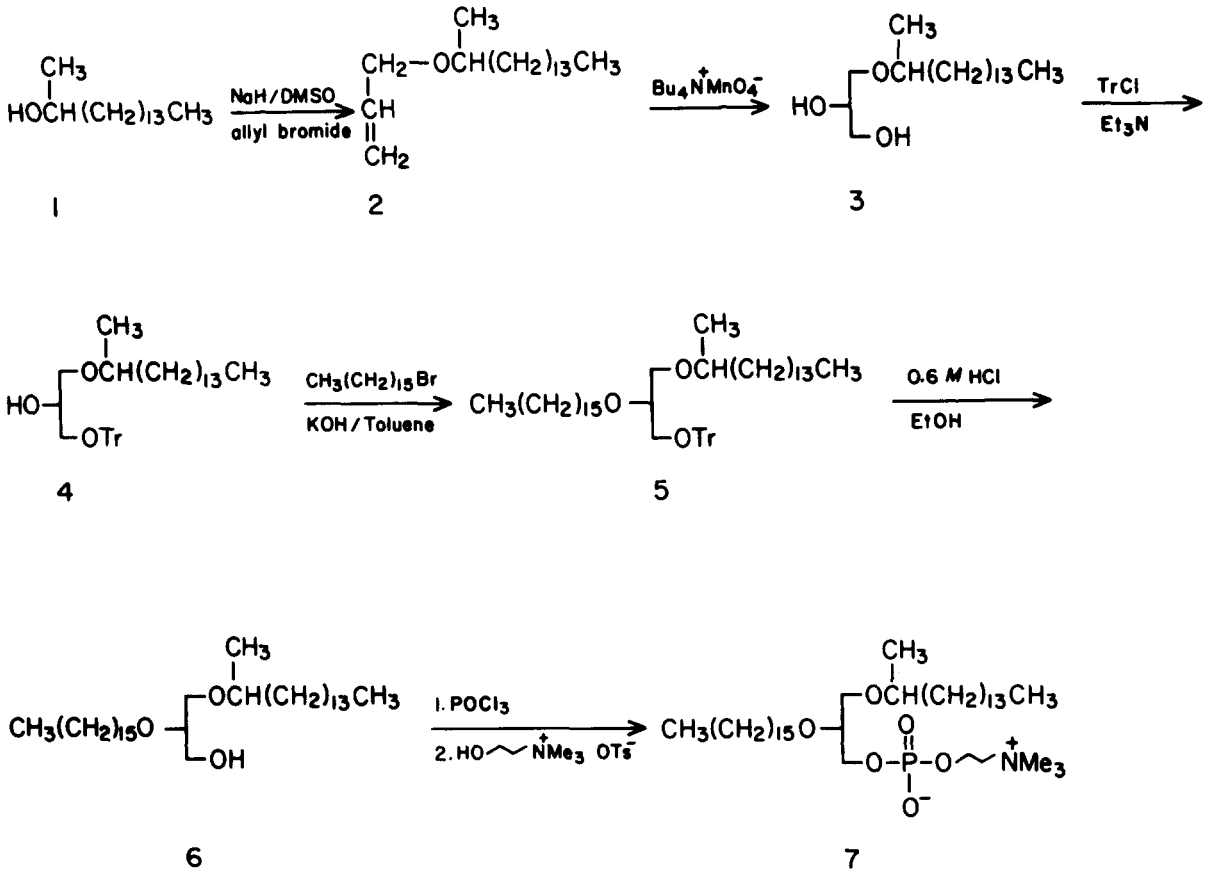
RESULTS

d,l-Allyl 2-hexadecyl ether (Scheme 1, *2*)

Dry sodium hydride (0.60 g, 25 mmol) was placed in a 250-ml three-necked flask equipped 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 and the mixture was stirred for 20 min under N_2. A solution of *d,l*-2-hexadecanol (Scheme 1, *1*) (2.52 g, 10.4 mmol) in 8 ml of toluene was injected, and the syringe was washed with 2 ml of toluene. The evolution of hydrogen subsided after 30 min. An excess of allyl bromide (2.2 ml, 25 mmol) was injected over a 5-min period and the mixture was stirred under N_2 for 18 hr at room temperature. Hexane (80 ml) and water (100 ml) were added, and the hexane layer was separated, washed with water (2 × 40 ml), and dried ($MgSO_4$). Removal of the solvent under reduced pressure left 3.19 g of a yellow-brown oil, which was applied to a column of silica gel (200 g, Baker) packed in toluene. Elution with toluene gave 2.45 g (83%) (after removal of the solvent) of ether *2* as a chromatographically pure colorless oil; the oil solidified on cooling below −10°C. Anal. Calc. for $C_{19}H_{38}O$ (282.51) C, 80.78; H, 13.56. Found: C, 80.65; H, 13.22.

1-*O*-(2'-Hexadecyl)-*rac*-glycerol (diastereomeric mixture) (Scheme 1, *3*)

A mixture of the allyl 2-hexadecyl ether (Scheme 1, *2*) (1 g, 3.5 mmol), tetra-*n*-butylammonium hydrogen sulfate (2.4 g, 7 mmol), anhydrous sodium carbonate (0.75 g, 7



Scheme 1. Reaction sequence for the preparation of 1-*O*-(2'-hexadecyl)-2-*O*-hexadecyl-*rac*-glycero-3-phosphocholine.

mmol), water (20 ml), alcohol-free $CHCl_3$ (25 ml), and benzene (25 ml) was cooled to 5°C. Finely powdered potassium permanganate (0.84 g, 5.3 mmol) was added, and the mixture was stirred vigorously for 1 hr at 5°C. Then 20 ml of saturated sodium hydrogen sulfite was added to dissolve the precipitate of manganese oxides. The organic phase (upper layer) was separated, washed with 60 ml of water, and dried with Na_2SO_4. Evaporation of the solvents under reduced pressure gave 1.43 g of an oil, which was diluted with 2 ml of chloroform and applied to a column of silica gel (90 g, Merck) packed in chloroform–ethanol 95:5 (v/v). Elution with chloroform–ethanol 95:5 (v/v) afforded, after evaporation of the solvents, 0.65 g (59%) of the 1-*O*-alkylglycerol (Scheme 1, *3*) as a chromatographically pure oil which crystallized slowly, mp 29–30°C. Anal. Calc. for $C_{19}H_{40}O_3$ (316.53) C, 72.10; H, 12.74. Found: C, 71.87; H, 12.87. ^{1}H NMR ($CDCl_3$) δ ppm: 0.5–2.3 (31H, m, $-CH_3$ and $-C(CH_2)_{13}C-$) and 2.7–4.0 (8H, m, $-OCH_2-$, $-CHO-$, and $-OH$). The expected integral ratio is 32H:8H.

1-*O*-(2'-Hexadecyl)-3-*O*-trityl-*rac*-glycerol (diastereomeric mixture) (Scheme 1, *4*)

A mixture of 1-*O*-(2'-hexadecyl)glycerol (Scheme 1, *3*) (0.38 g, 1.27 mmol) and trityl chloride (0.40 g, 1.45 mmol) was stirred in 3.8 ml of dry toluene and 0.20 ml (1.40 mmol) of dry triethylamine for 3 days at 50–55°C. The mixture was cooled to room temperature, the precipitate of triethylammonium chloride was removed by filtration and washed with hexane, and the filtrate was evaporated to dryness under reduced pressure. The oily residue was applied to a column of silica gel (80 g, Merck) and eluted with 1,2-dichloroethane–ethyl acetate 98:2 (v/v). Evaporation of the solvents under reduced pressure gave 0.38 g (62%) of the 1-*O*-alkyl-3-*O*-tritylglycerol *4* as an oil, which was used in the next step of the reaction without further purification. TLC analysis (solvent system, 1,2-dichloroethane–ethyl acetate, 98:2, v/v) showed a trace of trityl alcohol and two major spots (*4* as a pair of diastereomers) that were barely separated (R_f = 0.46 and 0.50). In this system, the R_f values of 1,2-dipalmitin and trityl alcohol are 0.18 and 0.68, respectively. ^{1}H NMR (CCl_4) δ (ppm): 0.6–2.4 (32H, m, $-CH_3$ and $-C(CH_2)_{13}C-$, 2.8–4.0 (6H, m, $-CH_2OC-$ and $-CHO-$), 5.9 (1H, broad s, $-OH$), and 6.8–7.5 (15H, m, C_6H_5).

1-*O*-(2'-Hexadecyl)-2-*O*-(1-hexadecyl)-3-*O*-trityl-*rac*-glycerol (diastereomeric mixture) (Scheme 1, *5*)

A mixture of 1-*O*-(2'-hexadecyl)-3-*O*-trityl-*rac*-glycerol (Scheme 1, *4*) (0.64 g, 1.14 mmol), 86% powdered potassium hydroxide (0.21 g, 3.2 mmol), and toluene (10 ml) was refluxed for 45 min under a Dean-Stark trap that was previously filled with toluene. 1-Bromohexadecane (0.35 ml, 1.14 mmol) was added, and the mixture was refluxed and stirred under the water trap for 5 hr. Then an additional 0.40 ml (1.31 mmol) of 1-bromohexadecane was added and reflux was continued for 5 hr more. Finally, additional portions of powdered potassium hydroxide (0.19 g, 2.9 mmol) and 1-bromohexadecane (0.40 ml, 1.31 mmol) were added. After 5 hr of additional refluxing and stirring, the mixture was diluted with 60 ml of hexane and 25 ml of water. The organic phase was washed twice with 30 ml of water, dried (K_2CO_3), and evaporated under reduced pressure. The waxy solid obtained (1.38 g) was dissolved in toluene–hexane 1:1 (v/v), and the solution was applied to a column of silica gel (120 g, Baker) packed in toluene–hexane 1:1 (v/v). Elution with toluene–hexane 1:1 (v/v) yielded 0.62 g (69%) of the 1,2-di-*O*-alkyl-3-*O*-trityl-glycerol (Scheme 1, *5*) as a white solid, mp 42–44°C. Anal. Calc. for $C_{54}H_{86}O_3$ (783.28) C, 82.81; H, 11.07. Found: C, 82.91; H, 11.30.

1-*O*-(2'-Hexadecyl)-2-*O*-(1-hexadecyl)-*rac*-glycerol (diastereomeric mixture) (Scheme 1, *6*)

A mixture of the trityl di-*O*-alkyl ether (Scheme 1, *5*) (0.70 g, 0.89 mmol) in 95% ethanol (28 ml) and concentrated HCl (1.4 ml) was refluxed for 1 hr. Water (14 ml) and hexane (140 ml) were added. The hexane layer was separated, washed with 85% methanol (3 × 40 ml), and dried with Na_2SO_4. The residue obtained (0.54 g) after removal of the solvents was applied to a column of silica gel (120 g, Merck). Elution with chloroform yielded 0.42 (85%) of the di-*O*-alkyl-glycerol (Scheme 1, *6*), mp 45–47°C. Anal. Calc. for $C_{35}H_{72}O_3$ (540.96) C, 77.71; H, 13.42. Found: C, 77.47; H, 13.46. ^{1}H NMR (200 MHz) ($CDCl_3$) δ (ppm): 0.88 (6H, t, J = 6.5 Hz, ω $-CH_3$), 1.13 (3H, d, J = 6.1 Hz, α $-CH_3$), 1.2–1.4 (50H, m, $-(CH_2)_n-$), 1.5–1.8 (4H, m, $-OCH_2CH_2-$ in each chain), 2.27 (1H, broad s, $-OH$), and 3.3–3.8 (8H, m, $-OCH_2-$ and $-CHOC-$).

1-*O*-(2'-Hexadecyl)-2-*O*-(1-hexadecyl)-*rac*-glycero-3-phosphocholine (diastereomeric mixture) (Scheme 1, *7*)

A solution of 194 mg (0.359 mmol) of 1-*O*-(2'-hexadecyl)-2-*O*-(1-hexadecyl)-*rac*-glycerol (Scheme 1, *6*) in 2 ml of alcohol-free chloroform containing 63 μl (0.45 mmol) of dry triethylamine was added over a 5-min period with stirring to phosphorus oxychloride (67 mg, 0.44 mmol). An additional 1 ml of chloroform was added and the mixture was stirred for 30 min at room temperature. Then dry pyridine (0.24 g, 3.1 mmol) and choline *p*-toluenesulfonate (0.15 g, 0.54 mmol) were added. The reaction mixture was stirred overnight at room temperature. Water (0.1 ml) was added, and the mixture was stirred for an additional 30 min. The mixture was diluted with 15 ml of chloroform, and the chloroform layer was separated and washed with water (5 ml), 4% aqueous potassium carbonate (5 ml), 5% HCl (5 ml), and again with water (5

ml). The chloroform layer was dried with Na_2SO_4 and evaporated, affording 235 mg of a residue that was applied to a column of silica gel (49 g, Baker). The column was eluted with with chloroform–methanol 65:25 (v/v) and then with chloroform–methanol–water 65:25:4 (v/v/v). The phosphocholine product (Scheme 1, *7*) was obtained from the column, and the solvents were removed by evaporation under reduced pressure. Acetone and acetonitrile precipitation of a concentrated solution of *7* in chloroform afforded 137 mg (51%) of the pure phospholipid. Anal. Calc. for $C_{40}H_{84}NO_6P \cdot 2H_2O$ (742.12) C, 64.74; H, 11.95; N, 1.89; P, 4.17. Found: C, 64.18; H, 11.40; N, 2.02; P, 4.15.

Dodecyl allyl ether (Scheme 2, *8*)

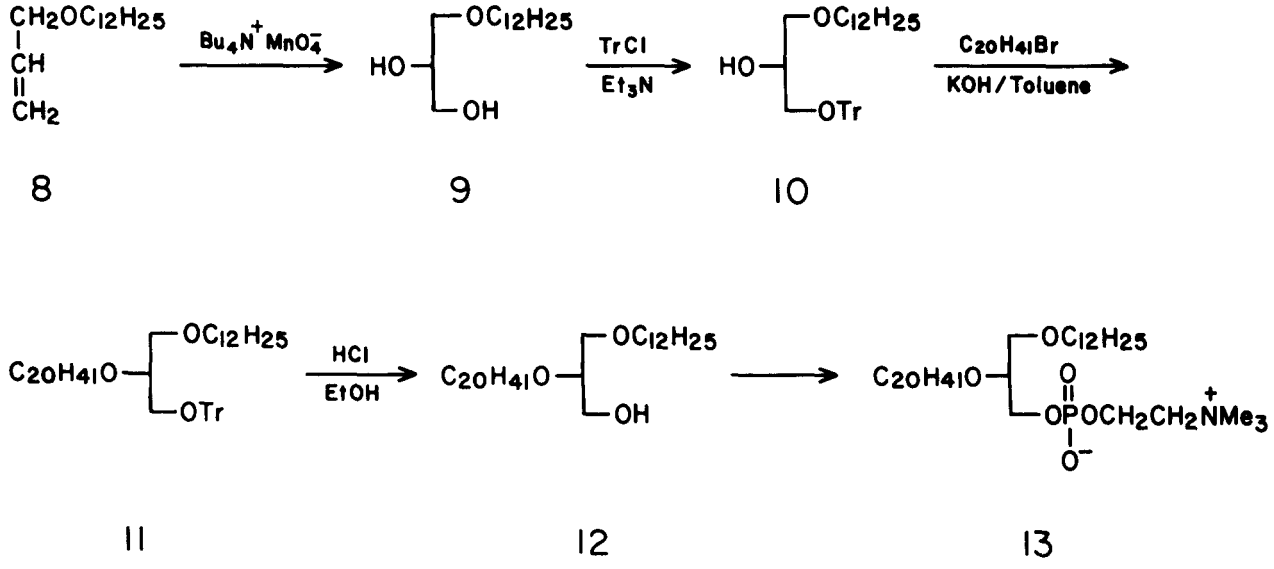
This compound was prepared in 53% yield from 1-dodecanol, allyl bromide, and sodium hydride in dimethyl sulfoxide according to the procedure used to prepare *d,l*-2-hexadecyl allyl ether (Scheme 1, *2*). ^{1}H NMR (CCl_4) δ (ppm): 0.90 (3H, t, J = 5 Hz, $-CH_3$), 1.1–1.9 (20H, m, dodecyl chain CH_2 groups, C_2–C_{11}), 3.4 (2H, t, J = 5.5 Hz, $-OCH_2$ of dodecyl chain), 3.9 (2H, d of d, J = 4.5 and 1.5 Hz, $-OCH_2$ of allyl group), 5.0–5.5 (2H, m, $=CH_2$), and 5.6–6.2 (1H, m, $=CH-$).

1-*O*-Dodecyl-*rac*-glycerol (Scheme 2, *9*)

A mixture of dodecyl allyl ether (2.38 g, 10.5 mmol), tetra-*n*-butylammonium hydrogen sulfate (7.2 g, 21 mmol), and anhydrous sodium carbonate (2.25 g, 21 mmol) in 60 ml of water, 75 ml of benzene, and 75 ml of alcohol-free chloroform was cooled to 2°C. Finely powdered potassium permanganate (2.55 g, 16.1 mmol) was added to the mixture, which was stirred for 1 hr at 2–4°C. The reaction was worked up as described under the preparation of 1-*O*-(2'-hexadecyl)-*rac*-glycerol (Scheme 1, *3*). The solvents were evaporated under reduced pressure, and the residue (3.35 g) was applied to a column of silica gel (250 g, Baker) packed in chloroform–ethanol 95:5 (v/v). Elution with this solvent mixture afforded, after evaporation of the solvents, 1.13 g (41%) of 1-*O*-dodecyl-*rac*-glycerol (Scheme 2, *9*) as a white crystalline residue, mp 47–48°C. Anal. Calc. for $C_{15}H_{32}O_3$ (260.42) C, 69.18; H, 12.39. Found: C, 69.04; H, 12.43. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.90 (3H, t, J = 5 Hz, $-CH_3$), 1.1–1.9 (20H, m, $-C(CH_2)_{10}C-$), and 3.4–4.1 (9H, m, $-OCH_2-$, $-CHOH-$, and $-OH$).

1-*O*-Dodecyl-3-*O*-trityl-*rac*-glycerol (Scheme 2, *10*)

A solution of 1-*O*-dodecyl-*rac*-glycerol (Scheme 2, *9*) (0.66 g, 2.5 mmol) and trityl chloride (0.80 g, 2.9 mmol) in 7.6 ml of dry toluene and 0.40 ml (2.9 mmol) of dry triethylamine was kept for 3 days at 50–55°C. The reaction mixture was cooled to room temperature, and the precipitate was removed by filtration and washed with hexane. The filtrate was evaporated under reduced pressure. The residue was applied to a column of silica gel (50 g, Baker) packed in 1,2-dichloroethane–ethyl acetate 98:2 (v/v). The column was eluted with this solvent mixture, and 1.01 g (79%) of the product was obtained as a light yellow oil after evaporation of the solvents. Anal. Calc. for $C_{34}H_{46}O_3$ (502.74) C, 81.23; H, 9.22. Found: C, 81.16; H, 8.92. ^{1}H NMR (CCl_4) δ (ppm): 0.90 (3H, t, J = 5 Hz, $-CH_3$), 1.1–1.8 (20H, m, $-C(CH_2)_{10}C-$), 2.2 (1H, broad s, $-OH$), 3.15 (2H, d, J = 5 Hz, $-CH_2OTr$), 3.4 and 3.5



Scheme 2. Reaction sequence for the preparation of 1-*O*-dodecyl-2-*O*-eicosyl-*rac*-glycero-3-phosphocholine.

(4H, d, J = 4.5 Hz, and t, J = 5.5 Hz, $-CH_2OCH_2-$), 3.7–4.0 (1H, m, $-CHOH$), and 7.1–7.6 (15H, m, C_6H_5).

1-*O*-Dodecyl-2-*O*-eicosyl-3-*O*-trityl-*rac*-glycerol (Scheme 2, *11*)

A mixture of 1-*O*-dodecyl-3-*O*-trityl-*rac*-glycerol (Scheme 2, *10*) (0.50 g, 1.0 mmol), 86% powdered potassium hydroxide (0.25 g, 3.8 mmol), and 1-bromoeicosane (0.48 g, 1.3 mmol), in 12 ml of toluene was refluxed with stirring for 24 hr under a Dean-Stark water trap (about 2.5 ml capacity). An additional amount of 1-bromoeicosane (0.36 g, 1 mmol) was then added, and reflux was continued for 20 hr. The reaction mixture was cooled and partitioned between ether (50 ml) and water (30 ml). The organic phase was separated and dried over anhydrous potassium carbonate. Evaporation of the solvent under reduced pressure left a residue, which was subjected to column chromatography on silica gel (42 g, Baker). Elution with toluene–hexane 1:1 (v/v) gave, after evaporation of the solvents, 0.59 g (75%) of the 1,2-di-*O*-alkyl-3-*O*-tritylglycerol (Scheme 2, *11*) as a colorless oil. Anal. Calc. for $C_{54}H_{86}O_3$ (783.29) C, 82.81; H, 11.07. Found: C, 83.05; H, 11.13. ^1H NMR (CCl_4) δ (ppm): 0.95 (6H, broad t, $-CH_3$), 1.1–1.8 (56H, m, $-C(CH_2)_{10}C-$ and $-C(CH_2)_{18}C-$), 3.0–3.7 (9H, m, $-OCH_2-$ and $-CHOC-$), and 7.1–7.7 (15H, m, C_6H_5).

1-*O*-Dodecyl-2-*O*-eicosyl-*rac*-glycerol (Scheme 2, *12*)

A solution of 1-*O*-dodecyl-2-*O*-eicosyl-3-*O*-trityl-*rac*-glycerol (Scheme 2, *11*) (500 mg, 0.64 mmol) in 10 ml of 95% ethanol and 0.5 ml of concentrated HCl was refluxed with stirring for 30 min. The mixture was cooled to about 50°C, diluted with 110 ml of acetonitrile, and allowed to stand at −20°C overnight. The precipitate was collected by filtration in a chilled Büchner funnel, washed thoroughly with cold acetonitrile, and dried in a desiccator over phosphorus pentoxide. The yield of the deprotected di-*O*-alkylglycerol (Scheme 2, *11*) was 328 mg (95%), mp 51–52°C. Anal. Calc. for $C_{35}H_{72}O_3$ (540.96) C, 77.71; H, 13.42. Found: C, 77.85; H, 13.38. ^1H NMR (200 MHz) ($CDCl_3$) δ (ppm): 0.88 (6H, apparent t, $-CH_3$), 1.26 (52H, m, $-C(CH_2)_9C-$ and $-C(CH_2)_{17}C-$), 1.57 (4H, m, $-OCH_2CH_2-$ in each chain), 2.23 (1H, broad s, $-OH$), and 3.35–3.80 (9H, m, $-OCH_2-$ and $-CHOC-$).

1-*O*-Dodecyl-2-*O*-eicosyl-*rac*-glycero-3-phosphocholine (Scheme 2, *13*)

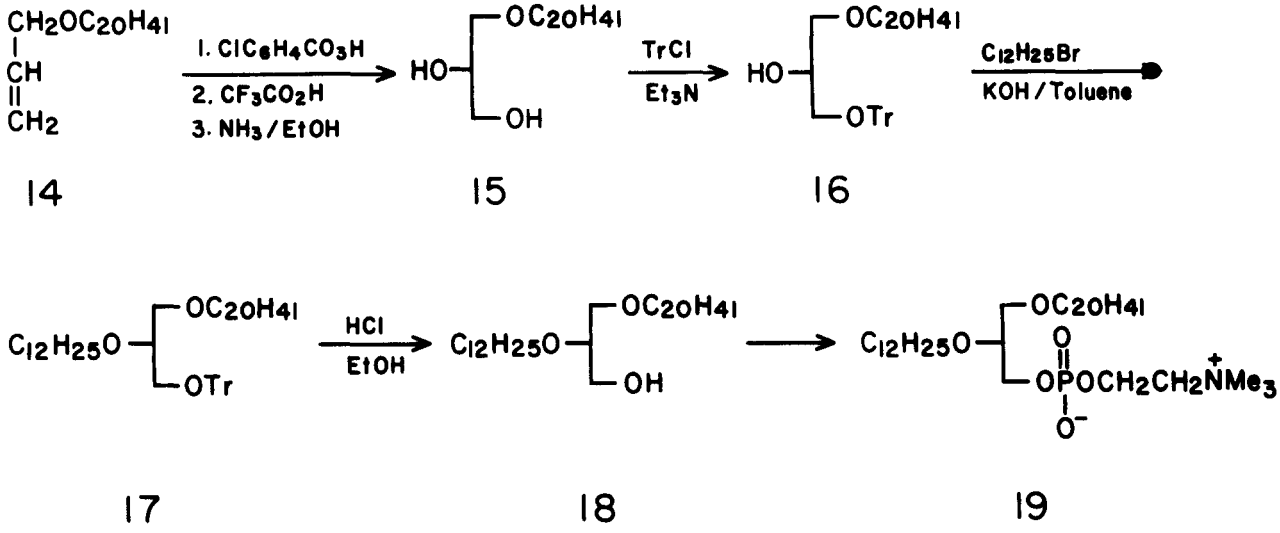
The procedure for the conversion of 1-*O*-dodecyl-2-*O*-eicosyl-*rac*-glycerol (Scheme 2, *12*) (200 mg, 0.37 mmol) into the phosphocholine product *13* was the same as that used to prepare the phosphocholine bearing the α-methyl branch (Scheme 1, *7*). The residue (0.26 g) obtained after workup of the reaction mixture was dissolved in 2 ml of chloroform and applied to a column of silica gel (50 g, Merck) packed in chloroform–methanol 65:25 (v/v). The column was first eluted with 150 ml of this solvent mixture, and then with chloroform–methanol–water 65:25:4 (v/v/v). Evaporation of the solvents afforded 145 mg (55%) of the phosphocholine product (Scheme 2, *13*) as a white powder. Anal. Calc. for $C_{40}H_{84}NO_6P$ (706.09) C, 68.04; H, 11.99; N, 1.98; P, 4.39. Found: C, 67.01; H, 11.60; N, 2.17; P, 4.29. ^1H NMR (200 MHz) ($CDCl_3$) δ (ppm): 0.88 (6H, t, J = 6 Hz, ω $-CH_3$), 1.25 (52H, m, $-C(CH_2)_9C-$ and $-C(CH_2)_{17}C-$), 1.52 (4H, m, $-OCH_2CH_2-$ in each chain), 3.2–3.6 (18H, m, $-OCH_2-$, $-CHOC-$, $-CH_2N$, $-N(CH_3)_3$; the $N-CH_3$ appeared as a singlet at δ 3.36); 3.81 (2H, m, $-CH_2OP$), and 4.26 (2H, m, $-POCH_2CH_2$).

Eicosyl allyl ether (Scheme 3, *14*)

Since 1-eicosanol is not soluble in toluene, eicosyl allyl ether was prepared using a variation of the procedure we used to synthesize 2-hexadecyl allyl ether (Scheme 1, *2*) or dodecyl allyl ether (Scheme 2, *8*). Tetrahydrofuran was used as a co-solvent with dimethyl sulfoxide. Sodium hydride (0.90 g, 37 mmol) was placed in a three-necked flask fitted with a nitrogen inlet, septum port, and dropping funnel. After the flask was flushed with nitrogen for 20 min, dry dimethyl sulfoxide (60 ml) was added, and the mixture was stirred for 30 min. A warm solution of 1-eicosanol (4.5 g, 15 mmol) in dry tetrahydrofuran (45 ml) was added dropwise over a 15-min period. The mixture was stirred for 45 min. Allyl bromide (3.3 ml, 38 mmol) was injected slowly into the flask, and stirring was continued under nitrogen for 18 hr. The reaction mixture was diluted with hexane (150 ml) and water (150 ml), and the hexane layer was separated and washed with 85% methanol (3 × 75 ml) and water (1 × 75 ml). The hexane extract was dried with Na_2SO_4 and the solvent was removed under reduced pressure. The residue (2.9 g) was applied to a column of silica gel (250 g, Baker) packed in hexane. The column was eluted with 500 ml of hexane, and then with hexane–diethyl ether 96:4 (v/v). After evaporation of the solvents, the pure eicosyl allyl ether was obtained as an oil which crystallized slowly (2.15 g, 42%), mp 38–39°C. Anal. Calc. for $C_{23}H_{46}O$ (338.62) C, 81.58; H, 13.69. Found C, 81.33; H, 13.84. ^1H NMR (CCl_4) δ (ppm): 0.90 (3H, t, J = 5 Hz, $-CH_3$), 1.1–1.9 (36H, m, $-(CH_2)_{18}-$), 3.4 (2H, t, J = 5.5 Hz, $-OCH_2$ of eicosyl group), 3.9 (2H, d of d, J = 4.5 and 1.5 Hz, $-OCH_2$ of allyl group), 4.9–5.4 (2H, m, $=CH_2$), and 5.5–6.2 (1H, m, $=CH-$).

1-*O*-Eicosyl-*rac*-glycerol (Scheme 3, *15*)

A solution of eicosyl allyl ether (Scheme 3, *14*) (0.80 g, 2.4 mmol) and 80% *m*-chloroperoxybenzoic acid (0.70 g, 3.2 mmol) in 7 ml of dichloromethane was stirred for 2 days at 30°C. The reaction mixture was diluted with ether



Scheme 3. Reaction sequence for the preparation of 1-*O*-eicosyl-2-*O*-dodecyl-*rac*-glycero-3-phosphocholine.

(14 ml), and the solution was washed with 2 ml of saturated sodium hydrogen sulfite and 5% aqueous sodium carbonate (3 × 7 ml). After the solution was dried with K_2CO_3, the solvents were removed, leaving 0.91 g of the crude epoxide. According to TLC analysis (solvent, 1,2-dichloroethane), the product consisted of about 95% epoxide, R_f 0.80, and about 5% starting material, R_f 0.95. The crude epoxide was treated with 7 ml of trifluoroacetic acid at 5°C overnight. The solvents were evaporated under reduced pressure (finally at 1 torr) to give a residue which was stirred for 2 hr at 50°C with a mixture of 95% ethanol (10 ml), chloroform (4 ml), and 14 M ammonium hydroxide (0.5 ml). The reaction mixture was cooled and diluted with 50 ml of diethyl ether and 25 ml of chloroform. The organic layer was washed with water (3 × 25 ml) and dried with K_2CO_3, and the solvents were evaporated. The residue was recrystallized from acetone (20 ml) to give 0.66 g (75%) of 1-*O*-eicosyl-*rac*-glycerol (Scheme 3, *15*) as a white powder, mp 73.5–74°C. The product was identical to that obtained by permanganate oxidation of *14* under phase-transfer conditions, as judged by TLC (solvent system, chloroform–ethanol 95:5, v/v, R_f 0.55) and mixed melting-point analysis. Anal. Calc. for $C_{23}H_{48}O_3$ (372.64) C, 74.14; H, 12.98. Found: C, 74.52; H, 13.20.

1-*O*-Eicosyl-3-*O*-trityl-*rac*-glycerol (Scheme 3, *16*)

A mixture of 1-*O*-eicosyl-*rac*-glycerol (Scheme 3, *15*) 0.54 g, 1.45 mmol) and trityl chloride (0.47 g, 1.70 mmol) in 3.5 ml of dry toluene and 0.24 ml (1.68 mmol) of dry triethylamine was stirred for 3 days at 55–60°C. The cooled reaction mixture was diluted with ether (50 ml), and the precipitate (triethylammonium chloride) was washed with ether. The filtrate was evaporated under vacuum, and the residue was applied to a column of silica gel (55 g, Baker) packed in 1,2-dichloroethane-ethyl acetate 98:2 (v/v). Elution with this solvent mixture and evaporation of the solvents gave the product (0.49 g, 55%) as light yellow crystals, mp 63°C. Anal. Calc. for $C_{42}H_{62}O_3$ (614.96) C, 82.03; H, 10.16. Found: C, 82.02; H, 10.31. ^{1}H NMR (CCl_4) δ (ppm): 0.90 (3H, t, J = 5 Hz, $-CH_3$), 1.1–1.75 (36H, m, $-(CH_2)_{18}-$), 2.4 (1H, broad s, OH), 3.15 (2H, d, J = 5 Hz, $-CH_2OTr$), 3.5 and 3.4 (4H, d of t, J = 4.5 and 5 Hz, $-CH_2OCH_2-$), 3.7–4.0 (1H, m, –CHOH), and 7.1–7.6 (15H, m, C_6H_5).

1-*O*-Eicosyl-2-*O*-dodecyl-3-*O*-trityl-*rac*-glycerol (Scheme 3, *17*)

A mixture of 1-*O*-eicosyl-3-*O*-trityl-*rac*-glycerol (Scheme 3, *16*) (0.46 g, 0.75 mmol), 90% powdered potassium hydroxide (0.17 g, 2.7 mmol), and 1-bromododecane (216 μl, 0.9 mmol) in 8 ml of toluene was refluxed with stirring under a Dean-Stark trap that was previously filled with toluene. After 22 hr of reflux, an additional 108 μl (0.45 mmol) of 1-bromododecane was added, and refluxing was continued for 22 hr. The cooled reaction mixture was diluted with hexane (40 ml), and then washed with water (2 × 20 ml). The organic phase was dried with K_2CO_3 and evaporated under vacuum. The residue was purified by column chromatography on silica gel (55 g, Merck). Elution with 1,2-dichloroethane afforded 0.59 g (75%) of pure product, mp 16°C, which was used directly in the next step. ^{1}H NMR (CCl_4) δ (ppm): 0.90 (6H, broad t, $-CH_3$), 1.1–1.8 (56H, m, $-C(CH_2)_{10}C-$ and $-C(CH_2)_{18}C-$), 3.0–3.7 (9H, m, $-OCH_2-$ and –CHOC–), and 7.1–7.7 (15H, m, C_6H_5).

1-*O*-Eicosyl-2-*O*-dodecyl-*rac*-glycerol (Scheme 3, *18*)

A solution of 1-*O*-eicosyl-2-*O*-dodecyl-3-*O*-trityl-*rac*-glycerol (Scheme 3, *17*) (0.48 g, 0.61 mmol) in 20 ml of 95% ethanol and 1 ml of concentrated HCl was refluxed

with stirring for 1 hr. The cooled reaction mixture was diluted with 10 ml of water and 100 ml of hexane. The hexane layer was separated, washed with 85% methanol (4 × 30 ml), and dried with K_2CO_3. Evaporation of the solvent gave a residue, which was dissolved in 1.5 ml of chloroform and added to 30 ml of acetonitrile. The mixture was allowed to stand at −20°C for 2 hr. The precipitate was collected in a chilled Büchner funnel, washed thoroughly with cold acetonitrile, and dried over phosphorus pentoxide to give 0.28 g (84%) of pure 1,2-di-*O*-alkyl-*rac*-glycerol (Scheme 3, *18*), mp 41–42°C. Anal. Calc. for $C_{35}H_{72}O_3$ (540.96) C, 77.71; H, 13.42. Found: C, 77.51; H, 13.68. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.90 (6H, broad t, $-CH_3$), 1.1–1.9 (56H, m, $-C(CH_2)_{10}C-$ and $-C(CH_2)_{18}C-$), 2.1 (1H, broad s, $-OH$), and 3.3–3.8 (9H, m, $-OCH_2-$ and $-CHOC-$).

1-*O*-Eicosyl-2-*O*-dodecyl-*rac*-glycero-3-phosphocholine (Scheme 3, *19*)

This product was prepared in 34% overall yield by the procedure described for the isomer (Scheme 2, *13*). Anal. Calc. for $C_{40}H_{84}NO_6P \cdot 2H_2O$ (706.09) C, 64.74; H, 11.95; N, 1.89; P, 4.17. Found: C, 63.14; H, 11.93; N, 1.98; P, 4.14. ^{1}H NMR (200 MHz) ($CDCl_3$) δ (ppm): 0.88 (6H, t, J = 6.5 Hz, ω $-CH_3$), 1.26 (52H, m, $-C(CH_2)_9C-$ and $-C(CH_2)_{17}C-$), 1.52 (4H, m, $-OCH_2CH_2-$ in each chain), 3.25–3.62 (18H, m, $-OCH_2-$, $-CHOC-$, CH_2N, $-NCH_3$; the N-CH_3 appeared as a singlet at δ 3.36); 3.72–3.88 (2H, m, $-CH_2OP$ and 2H, H_2O), and 4.27 (2H, m, $-POCH_2CH_2$ and 2H, H_2O).

DISCUSSION

This report describes a general and straightforward chemical synthesis of racemic diether glycerophosphocholines of mixed-chain composition using modifications of previous procedures. The alkyl groups are introduced in a sequential fashion, resulting in products whose two alkyl groups differ in the degree of branching or in the chain length. The 1-*O*-alkyl chain is introduced by displacement of bromide ion from allyl bromide by the long-chain alkoxide ion. The double bond is then converted to the glycol, and the primary hydroxyl group is protected by tritylation. The 2-*O*-alkyl chain is added by etherification of 1-*O*-alkyl-3-*O*-tritylglycerol (*4*, *10*, and *16*). Deprotection of the 1,2-di-*O*-alkyl-3-tritylglycerols affords the mixed-chain glycerols, which are phosphorylated with phosphorus oxychloride and converted into the phosphocholine products. These procedures offer considerable flexibility, since they can be applied to the preparation of phospholipids in which one chain is isotopically labeled and the other chain is unlabeled, or to saturated and unsaturated 1,2-di-*O*-alkyl- or 1-*O*-alkyl-2-acylglycero-3-phosphocholines.

An advantage of this procedure is that the allyl alkyl ether starting material (*2*, *8*, or *14*) is prepared in satisfactory (42–83%) yield from readily available, inexpensive reagents (allyl bromide, long-chain alcohol, and sodium hydride in dimethyl sulfoxide–toluene or tetrahydrofuran). Alternatively, allyl alcohol can be alkylated with a long-chain alkyl halide (9). Other routes to glycerol-1- or -3-ethers involve more expensive starting materials (such as isopropylideneglycerol or its derivatives, which are readily alkylated, refs. 10–14); routes that start with naturally occurring *O*-alkylglycerols (such as batyl, chimyl, and selachyl alcohols, e.g., refs. 15 and 16) are limited in the structure of the alkyl group. On the other hand, the present procedure can be used to synthesize racemic mixtures of diether-linked glycerophospholipids with a wide range of alkyl chains from inexpensive starting materials.

An alternative route to the mixed-chain 1,2-di-*O*-alkyl lipids is via the glycerol iodohydrin, which is formed from allyl alkyl ether, zinc oxide, iodine, and excess long-chain alcohol (17). We found this approach to be unsuccessful, however. Although glycerol iodohydrins (usually 1,2-diacyl derivatives) have been converted to the corresponding esters of phosphatidic acid using the silver salt of a phosphate ester (reviewed in refs. 18 and 19), we found that the dialkyl iodohydrins were quite unreactive toward silver dibenzylphosphate in refluxing toluene. This finding is consistent with earlier reports of the relative unreactivity of 1-halo-2-alkoxy compounds in displacement reactions (20, 21). Attempts to convert the iodohydrins to the corresponding benzoates with tetra-*n*-butylammonium benzoate in refluxing toluene/water, and then to the 1,2-di-*O*-alkylglycerols also did not result in good yields of the desired compounds. Therefore, we used the synthetic route of tritylation of 1-*O*-alkylglycerol, since tritylation takes place preferentially at the primary hydroxyl group. Alkylation of 1-*O*-alkyl-3-*O*-tritylglycerol with the long-chain alkyl bromide and potassium hydroxide in refluxing toluene proceeded in good yield (69–75%). The hydrocarbon chains are introduced in separate reactions, rather than in a one-pot conversion of a protected glycerol to a di-*O*-alkylglycerol with identical chains (13, 22).

The use of phase-transfer conditions for the hydroxylation of the C_{12}- and C_{16}-alkyl allyl ethers resulted in conversion to the corresponding glycols in moderate yield. However, the yield of glycol *15* by the same conditions as used to prepare glycols *3* and *9* was very low. This may suggest that the long chain makes the double bond less accessible, although other factors (such as solubility) may also be involved. We found, however, that 1-*O*-eicosyl-*rac*-glycerol could be prepared from 1-*O*-eicosyl allyl ether via the glycidol ether in overall yield of 75%.

In conclusion, the synthetic method described here is efficient and flexible, since a variety of hydrocarbon chains can be introduced in good yields. A variety of PC analogs

containing different chains will be useful in model systems for the study of the properties of biological membranes. Studies of the gel-phase interdigitation of ether-linked glycerophosphocholines are in progress.

This work was supported in part by National Institutes of Health grant HL-16660.

Manuscript received 29 August 1985.

REFERENCES

1. Horrocks, L. A., and M. Sharma. 1982. Plasmalogens and *O*-alkyl glycerophospholipids. *In* Phospholipids. J. N. Hawthorne and G. B. Ansell, editors. Elsevier, Amsterdam. 51–93.
2. Kates, M. 1978. The phytanyl ether-linked polar lipids and isopropenoid neutral lipids of extremely halophilic bacteria. *Prog. Chem. Fats Other Lipids.* **15:** 301–342.
3. Friedberg, S. J., and M. Halpert. 1978. Ehrlich ascites tumor cell surface membranes: an abnormality in ether lipid content. *J. Lipid Res.* **19:** 57–64.
4. Paltauf, F. 1983. Ether lipids as substrates for lipolytic enzymes. *In* Ether Lipids: Biochemical and Biomedical Aspects. H. K. Mangold and F. Paltauf, editors. Academic Press, New York. 211–227.
5. Weber, N., and H. Benning. 1985. Ether glycerolipids: novel substrates for studying specificity of enzymes involved in glycerolipid biosynthesis in higher plants. *Eur. J. Biochem.* **146:** 323–329.
6. McIntosh, T. J., S. A. Simon, J. C. Ellington, Jr., and N. A. Porter. 1984. New structural model for mixed-chain phosphatidylcholine bilayers. *Biochemistry.* **23:** 4038–4044.
7. Hui, S. W., J. T. Mason, and C-H. Huang. 1984. Acyl chain interdigitation in saturated mixed-chain phosphatidylcholine bilayer dispersions. *Biochemistry.* **23:** 5570–5577.
8. Witzke, N. M., and R. Bittman. 1985. Synthesis of phosphatidylcholine analogs with an alkyl group at C1 or C3 of the glycerol moiety. *J. Lipid Res.* **26:** 623–628.
9. Stegerhoek, L. J., and P. E. Verkade. 1956. Esters derived from batyl alcohol. *Rec. Trav. Chim. Pays-Bas.* **75:** 143–163.
10. Baer, E., L. J. Rubin, and H. O. L. Fischer. 1944. Naturally occurring glycerol ethers. II. Synthesis of selachyl alcohol. *J. Biol. Chem.* **155:** 447–457.
11. Baer, E., and H. O. L. Fischer. 1947. Naturally occurring glycerol ethers. III. Selachyl alcohol and its geometrical isomer. *J. Biol. Chem.* **170:** 337–342.
12. Baumann, W. J., and Mangold, H. K. 1964. Reactions of aliphatic methanesulfonates. I. Syntheses of long-chain glyceryl-(1) ethers. *J. Org. Chem.* **29:** 3055–3057.
13. Palameta, B., and M. Kates. 1966. Aliphatic diether analogs of glyceride-derived lipids. III. Synthesis of dialkenyl and mixed alkylalkenylglycerol ethers. *Biochemistry.* **5:** 618–625.
14. Hirth, G., and R. Barner. 1982. Herstellung von 1-*O*-Octadecyl-2-*O*-acetyl-*sn*-glyceryl-3-phosphorylcholin ("Platelet Activating Factor"), des Enantiomeren sowie einiger analoger Verbindungen. *Helv. Chim. Acta.* **65:** 1059–1084.
15. Chacko, G. K., and D. J. Hanahan. 1968. Chemical synthesis of 1-*O*-(D)- and 3-*O*-(L)-glyceryl monoethers, diethers and derivatives: glycerides, monoester phospholipids and diether phospholipids. *Biochim. Biophys. Acta.* **164:** 252–271.
16. Hirth, G., H. Saroka, W. Bannwarth, and R. Barner. 1983. Herstellung von 2-*O*- Acetyl-1-*O*-[(*Z*)-9-octadecenyl]-*sn*-glyceryl-3-phosphorylcholin ("Oleyl-PAF"), des Enantiomeren sowie einiger analoger, ungesättigter Verbindungen. *Helv. Chim. Acta.* **66:** 1210–1240.
17. Rosenthal, A. F., G. M. Kosolapoff, and R. P. Geyer. 1964. Nonhydrolyzable synthetic analogs of phosphatidic acids. *Rec. Trav. Chim. Pays-Bas.* **83:** 1273–1283.
18. Eibl, H. 1980. Synthesis of glycerophospholipids. *Chem. Phys. Lipids.* **26:** 405–429.
19. Ramirez, F., and J. F. Maracek. 1985. Synthesis of phosphodiesters: the cyclic enediol phosphoryl (CEP) method. *Synthesis.* 449–488.
20. Streitwieser, A. S., Jr. 1956. Solvolytic displacement reactions at saturated carbon atoms. *Chem. Rev.* **56:** 571–752.
21. Kreutzkamp, N., H. Meerwein, and R. Stroh. 1960. Reactivität und umwandlung von chlor-, brom- und jodverbindungen. *Methoden Org. Chem. (Houben-Weyl)* **5 (4):** 679–717.
22. Kates, M., T. H. Chan, and N. Z. Stanacev. 1963. Aliphatic diether analogs of glyceride-derived lipids. I. Synthesis of D-α,β-dialkyl glyceryl ethers. *Biochemistry.* **2:** 394–397.