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The stereospecific synthesis of mixed-acid phospholipids with polyunsaturated fatty acid from D-mannitol

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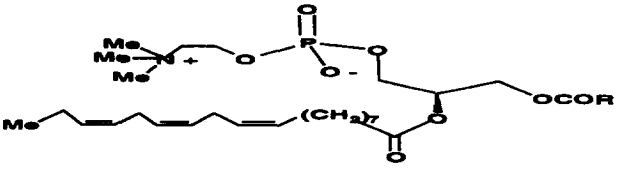
Abstract: The polyunsaturated mixed-acid phosphatidylcholine, 1-palimoyl-2-linolenoyl-sn-glycerophosphocholine **1a** and 1-stearoyl-2-linolenoyl-sn-glycerophosphocholine **1b** prepared from D-mannitol as an optically active starting material is described. © 1997 Published by Elsevier Science Ltd. All rights reserved.

Introduction

Current investigations of the structure of biological membranes require efficient methods for the preparation of phospholipids. In particular, there is a growing need for mixed acid phospholipids with defined fatty acid composition,[1] for example as components of artificial membranes for physiochemical studies,[2] fluorescent,[3] radiolabelled[4] and spin-labelled probes[5] for the study of membrane motion, and as photoactivatable probes for investigation of protein–lipid interactions.[4a,c,d,6] A commonly employed method for synthesis involves specific deacylation of phospholipase A_2 and reacylation of the resulting 2-lysophospholipids with the desired acid.[7] At the same time, liposome technology has provided a powerful tool for efficient drug delivery and targeting,[8] as a number of pharmaceuticals have been encapsulated in liposome form or attached to the surface of liposomes by a labile bond,[9] and (z,z,z)-9,12,15-linolenic acid has been known as an essential fatty acid for humans; its deficiency will alter membrane function, both in the brain as well as in the peripheral nerve,[10,11] and furthermore, it has also been reported capable of reducing the incidence and multiplicity of chemically-induced colon tumors,[12–14] decreasing the yield of chemically-induced mammary tumors[15–17] and inhibiting the hairlessness of mice.[18] These exciting resulting inspired us to re-investigate efficient methods for preparation of mixed-acid phosphocholines, with polyunsaturated fatty acid in the 2-position of glycerophospholipid containing glycerol, in which polyunsaturated fatty acid is desired to be released by hydrolysis with phospholipase A_2. Herein, we report the total synthesis of mixed-acid glycerophosphocholines with α-linolenic acid in the 2-position of glycerophospholipids containing glycerol from D-mannitol as the optically active starting material.



(1a) R: $C_{15}H_{31-n}$
(1b) R: $C_{17}H_{35-n}$

Results and discussion

As natural phospholipids are in general compounds with optical activity, we synthesized the key intermediate compounds **12a-b** through 9 reaction steps using D-mannitol as the optically active

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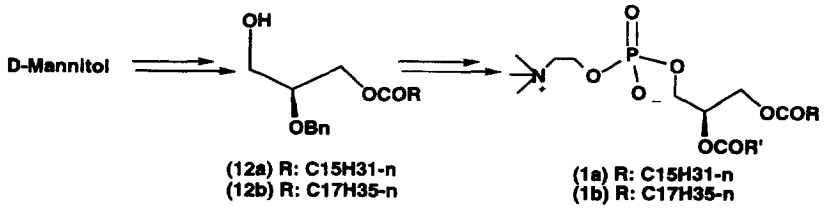


Scheme 1.

starting material. The strategy for synthesis of the key intermediate compounds **12a-b**,[19] 1-acyl-2-benzyl-sn-glycerol **12a-b**, and target molecules is outlined in Scheme 1.

Thus, we attempted to ditritylate 3,4-isopropylidene-D-mannitol **3**, with 1,2;3,4;5,6-triisopropylidene-D-mannitol **2** prepared from D-mannitol by reaction with acetone/H_2SO_4 being converted into compound **3** with dry trityl chloride in anhydrous pyridine, but the main product we obtained was monotrityl derivate **4**, which was then benzylated resulting in compound **5**. The trityl group was selectively removed from compound **5** in methanol/2-propanol/H_2SO_4 to obtain 2,5,6-tribenzyl-3,4-isopropylidene-D-mannitol **6**, which was then esterified in dry dichloromethane with saturated fatty acid–dicyclohexylcarbodiimide (DCC) and catalytic amount of 4-(dimethylamino)pyridine to produce 2,5,6-tribenzyl-1-acyl-3,4-isopropylidene-D-mannitol **7a-b**. Acidic deacetonation of 2,5,6-tribenzyl-1-acyl-3,4-isopyropylidene-D-mannitol **7a-b** in dichloromethane–trifluoroacetic acid 70% $HClO_4$ afforded 2,5,6-tribenzyl-1-acyl-D-mannitol **8a-b**, the vicinal diol moiety of which, in positions 3 and 4, was cleaved with lead tetraacetate in dry ethyl acetate, and the resulting aldehyde **10a-b** was then reduced with $NaBH_4$ to the key intermediate compounds **12a-b** (Scheme 2), with chloroethylphosphoric acid dichloride in dry dichloromethane and anhydrous triethylamine yielding the sn-3-phosphoric acid **13a-b** which had the natural configuration, outlined in Scheme 3. The key intermediate compounds **13a-b** were aminated by trimethylamine in chloroform–ethanol to obtain the compounds **14a-b**, which were debenzylated to lysophosphocholine **15a-b** with Pd–C(10%)–H_2. Esterification of lysophosphocholine **15a-b** in dry dichloromethane with linolenic acid anhydride/DMAP[20] afforded the target molecule **1a-b**.

In conclusion, we, for the first time, described a convenient method to totally synthesize the mixed-acid phosphatidylcholine **1a** and **1b** using D-mannitol as optically starting material.

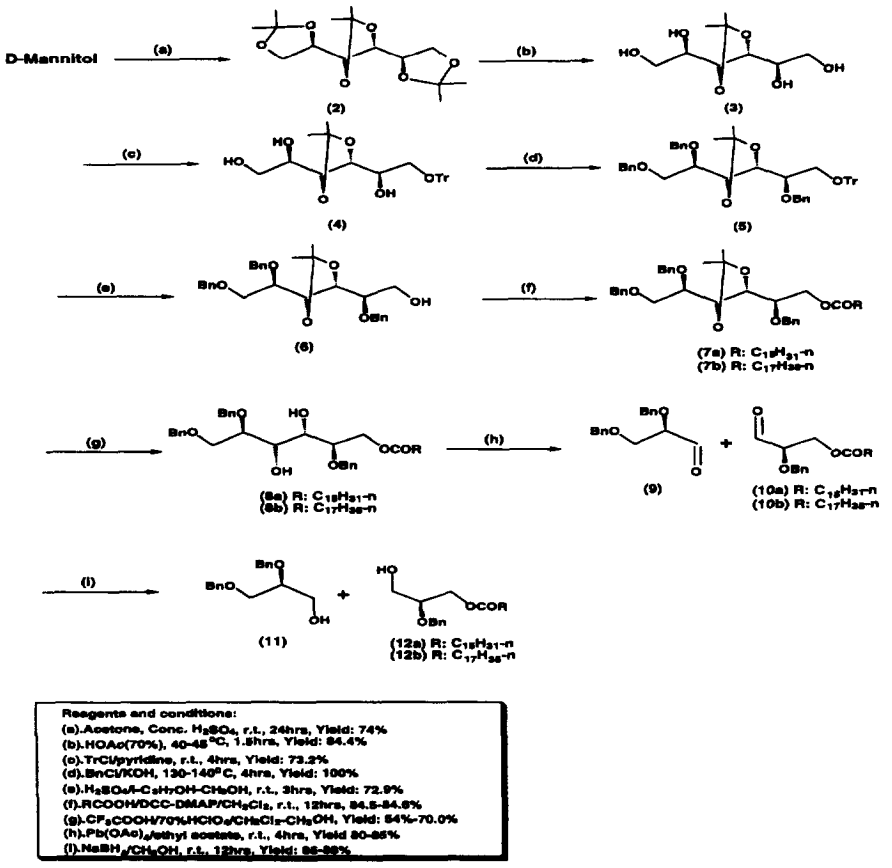
Experimental

General methods and materials

Dichloromethane was distilled from calcium hydride, triethylamine was distilled from calcium hydride, glass-backed silica gel TLC plates (silica gel F254, 0.2 mm thickness) were supplied by Qing Dao, China. Chromatography was performed on silica gel H, 400 mesh, from Qing Dao, China. ^1H NMR spectra were measured on a Bruker AMX-300 MHz spectrometer with tetramethylsilane as internal standard, hexadeuterioacetone and deuteriochloroform as solvent. EI mass spectra were obtained on a VG Quattro-MS/MS spectrometer. IR was measured on Shimadzu IR-440, optical rotations were measured on Perkin-Elmer 241c Polarimer.

1,2;3,4;5,6-Triisopropylidene-D-mannitol 2[21]

To a stirred solution of D-mannitol (40 g) in dry acetone (500 ml), conc. H_2SO_4 (4 ml) was added. The reaction mixture was stirred overnight at room temperature. The reaction was then poured into potassium hydroxide solution. The acetone was removed under reduced pressure resulting in a solution which was extracted with dichloromethane. The organic phase was separated and washed with water, dried with anhydrous sodium sulfate. Organic phase was then concentrated under reduced pressure to give a crude white solid which was crystallized with petroleum ether–ethyl ether to give a white solid



Scheme 2.

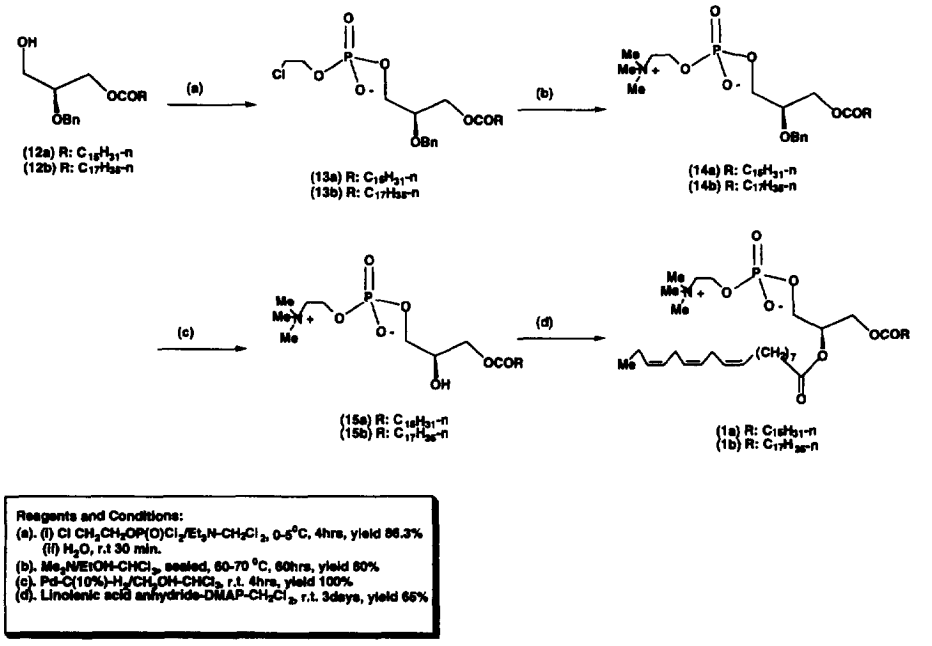
(47.2 g), yield 74%, m.p. 74–75°C (lit: 60–70°C). EIMS (m/e): 287 (M^+–OCH_3), 43 (100%). ^1H NMR ($CDCl_3$): δ 4.30–4.15 (m, 2H, CH_2O), 4.10–4.00 (m, 2H, CH_2O), 3.95–3.80 (m, 4H, $2CH_2O$), 1.40–1.30 (m, 18H, $6CH_3$).

*3,4-Isopropylidene-*D*-mannitol 3*[21]

To a stirred solution of 70% HOAc (956 ml) at 40°C was added 1,2;3,4;5,6-isopropylidene-D-mannitol (48 g) and kept at this temperature for 1.5 h. The reaction mixture was then concentrated under reduced presure to give a crude oil which was crystallized with anhydrous benzene resulting in compound **3**, yield: 84.5%. ^1H NMR ($CDCl_3$): δ 4.00–3.90 (m, 2H, 2CHO), 3.80–3.70 (dd, 2H, J=2.9 Hz, J=10.8 Hz, CH_2O), 3.70–3.62 (m, 2H, CH_2O), 3.62–3.50 (dd, 2H, J=5.1 Hz, J=10.8 Hz, CH_2O), 1.40–1.30 (dd, 6H, $2CH_3$).

*1-Trityl-3,4-isopropylidene-*D*-mannitol 4*

To a stirred solution of 3,4-isopropylidene-D-mannitol (1.947 g, 8.77 mmol) in anhydrous pyridine (58 ml) was added trityl chloride (5.383 g, 19.29 mmol). The reaction mixture was stirred for 4 h at room temperature. The solution was then concentrated under reduced pressure to give a crude product which was purified on silica gel column eluting with petroleum ether–ethyl acetate–methanol (4:1:0.6) resulting in compound **4** (1.43 g), yield: 73.3%. $[\alpha]_D^{20}$=+18.0 (C=1.10 CH_3OH). IR (film): 3500–3400 cm^{-1}. ^1H NMR (acetone-d_6): δ 7.60–7.50 (m, 15H, 3Ph), 4.40–4.20 (dd, 1H, J=5.1 Hz, J=6.9 Hz,



Scheme 3.

CHO), 4.10–4.00 (m, 1H, CHO), 3.90–3.80 (m, 2H), 3.70–3.60 (m, 2H), 3.30–3.20 (d, 2H), 1.30–1.20 (2s, 6H, $2CH_3$). Anal. Calcd for $C_{28}H_{32}O_6$: 72.18 (C%), 7.36 (H%). Found: 72.59 (C%), 7.80 (H%).

1-Trityl-2,5,6-tribenzyl-3,4-isopropylidene-D-*mannitol* **5**

To a stirred solution of potassium hydroxide (13.48 g) in benzyl chloride (15 ml) was added compound **4** (1.7 g, 3.66 mmol). The reaction mixture was stirred for 4 h at 130–140°C and poured into iced water. The mixture was extracted with dichloromethane, organic phase was separated, washed with water and dried with anhydrous sodium sulfate. The dichloromethane was removed under reduced pressure to give a crude product which was purified on silica gel column (petroleum ether–ethyl acetate 20:1) resulting in a yellow oil (2 g), yield: 100%. $[\alpha]_D^{20}$=+15.0 (C=1.0 $CHCl_3$). EIMS (m/e): 491, 243 (Ph_3C) (100%), 91 ($PhCH_2$) (92%), 43. ^{1}H NMR (acetone-d_6): δ 7.60–7.50 (m, 5H, Ar), 7.40–7.20 (m, 35H, Ar), 4.85–4.70 (dd, 2H, J=11.7 Hz, J=12.0 Hz), 4.65–4.55 (dd, 2H, J=11.6 Hz, J=11.8 Hz), 4.50–4.40 (s, 2H), 4.30–4.20 (dd, 1H, J=5.1 Hz, J=6.9 Hz), 4.20–4.10 (dd, 1H, J=4.9 Hz, J=6.9 Hz), 4.00–3.85 (dt, 2H), 3.85–3.70 (dt, 2H), 3.70–3.60 (m, 1H), 3.40–3.30 (m, 2H), 1.30–1.20 (s, 3H, CH_3), 1.20–1.10 (s, 3H, CH_3). Anal. Calcd for $C_{49}H_{56}O_6$: 76.56 (C%), 8.57 (H%). Found: 76.40 (C%), 8.60 (H%).

2,5,6-Tribenzyl-3,4-isopropylidene-D-*mannitol* **6**

To a stirred solution of compound **5** (370 mg, 0.63 mmol) in *i*-propanol (50 ml) and methanol (50 ml) was added conc. H_2SO_4 (0.05 ml). The reaction mixture was stirred for 4–6 h at room temperature. The solution was then poured into a solution of potassium hydroxide and extracted with dichloromethane. The organic phase was separated, washed with water and dried with anhydrous sodium sulfate. The dichloromethane was removed under reduced pressure to give a crude product which was purified on silica gel column eluting with petroleum ether–ethyl acetate (4:1) resulting in compound **6** (270 mg), yield: 73%. $[\alpha]_D^{20}$=+16.0 (C=0.80 $CHCl_3$). IR (film): 3500–3400 cm^{-1}. EIMS (m/e): 475 (M^+–OH), 474 (M^+–H_2O), 91 ($PhCH_2$) (100%), 57, 43. ^{1}H NMR (acetone-d_6): δ 7.50–7.30 (m, 15H, Ar), 4.80–4.70 (dd, 2H, J=11.6 Hz, J=11.7 Hz), 4.75–4.55 (dd, 2H, J=11.6 Hz,

J=11.8 Hz), 4.50–4.40 (s, 2H), 4.40–4.30 (m, 2H), 3.90–3.80 (m, 3H), 3.75–3.40 (m, 3H), 1.40–1.30 (2s, 6H, $2CH_3$). HR EIMS (m/e): 475.3665 (M^+–OH) Calcd: 475.3655.

The general procedure for esterification of compound ***6*** *with fatty acid under DCC–DMAP system*

To a stirred solution of compound **6** (1 mmol), fatty acid (2.5–3 mmol) and DMAP (0.09 mmol) in dry dichloromethane was added DCC (2.5–3 mmol). The reaction mixture was stirred overnight at room temperature. The precipitate was filtered off and the filtrate was concentrated under reduced pressure to give a crude product which was purified on silica gel column eluting with petroleum ether–ethyl acetate (25:1 and 10:1) resulting in an oil.

*1-Palimoyl-2,5,6-tribenzyl-3,4-isopropylidene-*D*-mannitol* ***7a***

Yield: 84.6%. $[\alpha]_D^{20}$=+6.4 (C=0.25 $CHCl_3$). IR (film): 1730 cm^{-1}. EIMS (m/e): 401, 310, 239 ($C_{15}H_{31}CO^+$), 91 ($PhCH_2$) (100%), 57, 43. ^1H NMR (acetone-d_6): δ 7.50–7.30 (m, 15H, Ar), 4.90–4.60 (m, 6H), 4.40–4.20 (dd, 2H), 3.90–3.70 (m, 3H), 3.60–3.50 (m, 3H), 2.40–2.20 (t, 2H), 1.70–1.50 (m, 2H), 1.40–1.20 (s, 30), 1.00–0.80 (t, 3H, CH_3, J=7.5 Hz, J=7.6 Hz,). Anal. Calcd for $C_{46}H_{72}O_7$: 75.95 (C%), 9.30 (H%). Found: 76.20 (C%), 9.80 (H%).

*1-Stearoyl-2,5,6-tribenzyl-3,4-isopropylidene-*D*-mannitol* ***7b***

Yield: 84.5%. $[\alpha]_D^{20}$=+8.00 (C=0.70 $CHCl_3$). IR (film): 1750–1740 cm^{-1}. EIMS (m/e): 401, 310, 267 ($C_{17}H_{35}CO^+$), 91 ($PhCH_2$) (100%), 57, 43. ^1H NMR (acetone-d_6): δ 7.40–7.20 (m, 15H, Ar), 4.80–4.50 (m, 6H), 4.40–4.20 (dd, 2H), 3.80–3.70 (m, 3H), 3.60–3.50 (m, 3H), 2.40–2.20 (t, 2H, CH_2CO), 1.70–1.50 (m, 2H), 1.40–1.20 (s, 34H), 1.00–0.80 (t, 3H, CH_3). Anal. Calcd for $C_{48}H_{76}O_7$: 75.95 (C%), 9.30 (H%). Found: 76.20 (C%), 9.80 (H%).

The general procedure for removal of isopropylidene from compound ***7a*** *or* ***7b*** *with TFA and 70% $HClO_4$*

To a stirred solution of compound **7a** or **7b** (1 mmol) and TFA (6–7 ml) in dry dichloromethane (20 ml) at 0–5°C was added 70% $HClO_4$ (5 ml). The reaction mixture was stirred for 10 min at this temperature and poured into a mixture of methanol and water. The dichloromethane was separated, washed with water and dried with anhydrous sodium sulfate. The dichloromethane was removed under reduced pressure to give a crude product which was purified on silica gel column eluting with petroleum ether–ethyl acetate–methanol (4:1:0.6) resulting in compound **8a** or **8b**.

*1-Palimoyl-2,5,6-tribenzyl-*D*-mannitol* ***8a***

Yield: 70.0%. $[\alpha]_D^{20}$=+35.4 (C=0.25 $CHCl_3$). IR (film): 3500–3400, 1740 cm^{-1}. EIMS (m/e): 430, 339, 239 ($C_{15}H_{31}CO^+$), 181, 91 ($PhCH_2$) (100%), 57, 43. ^1H NMR (acetone-d_6): δ 7.40–7.20 (m, 15H, Ar), 4.80–4.60 (m, 6H), 4.30–4.20 (dd, 2H), 3.80–3.70 (m, 3H), 3.60–3.50 (m, 3H), 2.20 (t, 2H), 1.70–1.50 (m, 2H), 1.40–1.20 (s, 24), 1.00–0.80 (t, 3H, CH_3, J=7.5 Hz, J=7.6 Hz,). Anal. Calcd for $C_{43}H_{68}O_7$: 74.40 (C%), 9.58 (H%). Found: 75.00 (C%), 10.00 (H%).

*1-Stearoyl-2,5,6-tribenzyl-*D*-mannitol* ***8b***

Yield: 54.0%. $[\alpha]_D^{20}$=+8.00 (C=0.70 $CHCl_3$). IR (film): 1750–1740 cm^{-1}. EIMS (m/e): 451, 361, 267 ($C_{17}H_{35}CO^+$), 91 ($PhCH_2$) (100%), 57, 43. ^1H NMR (acetone-d_6): δ 7.40–7.20 (m, 15H, Ar), 4.80–4.60 (m, 6H), 4.40–4.20 (dd, 2H), 3.80–3.70 (m, 3H), 3.60–3.50 (m, 3H), 2.40–2.20 (t, 2H, CH_2CO), 1.70–1.50 (m, 2H), 1.40–1.20 (s, 28H), 1.00–0.80 (t, 3H, CH_3). Anal. Calcd for $C_{45}H_{72}O_7$: 75.17 (C%), 9.25 (H%). Found: 75.60 (C%), 9.65 (H%).

1-Palimoyl-2-benzylglycerol ***12a***

To a stirred solution of compound **8a** (475 mg, 0.58 mmol) in dry ethyl acetate (20 ml) was added lead tetraacetate (301 mg, 0.69 mmol). The reaction mixture was stirred for 4 h at room temperature. The solution was filtered and filtrate was concentrated under reduced pressure to give a crude product which was purified on silica gel column eluting with petroleum ether–ethyl acetate (4:1) resulting in

compound **9** and compound **10a**. To a stirred solution of the compound **10a** (648 mg, 1.55 mmol) in methanol (15 ml) was added $NaBH_4$ (71 mg, 1.86 mmol). The mixture was stirred for 4 h at room temperature. The solution was concentrated under reduced pressure to give a crude product which was then purified on silica gel column eluting with petroleum ether–ethyl acetate (6:1 and 4:1) resulting in compound **12a** (538 mg), yield: 83%. $[\alpha]_D^{20}=-7.50$ (C=0.38 $CHCl_3$). IR (film): 3500–3300, 1740 cm^{-1}. EIMS (m/e): 449 (M^++1), 267 ($C_{17}H_{35}CO^+$), 91 ($PhCH_2$) (100%), 57, 43. ^1H NMR (acetone-d_6): δ 7.40–7.20 (m, 5H, Ar), 4.70–4.60 (s, 2H), 4.40–4.30 (dd, 1H, J=3.6 Hz, J=11.7 Hz), 4.20–4.10 (dd, 1H, J=5.6 Hz, J=11.7 Hz), 3.70–3.65 (m, 3H), 2.50–2.30 (t, 2H, J=7.5 Hz, J=7.5 Hz, CH_2CO), 1.60–1.40 (t, 2H), 1.40–1.20 (s, 28H), 1.00–0.80 (t, 3H, CH_3).

1-Stearoyl-2-benzylglycerol ***12b***

To a stirred solution of compound **8b** (1.44 g, 1.62 mmol) in dry ethyl acetate (20 ml) was added lead tetraacetate (2.87 g, 7.16 mmol). The reaction mixture was stirred for 4 h at room temperature. The solution was filtered and filtrate was concentrated under reduced pressure to give a crude product which was purified on silica gel column eluting with petroleum ether–ethyl acetate (4:1) resulting in compound **9** and compound **10b**. To a stirred solution of the compound **10b** (570 mg, 1.28 mmol) in methanol (15 ml) was added $NaBH_4$ (36 mg, 1.5 mmol). The mixture was stirred for 4 h at room temperature. The solution was concentrated under reduced pressure to give a crude product which was then purified on silica gel column eluting with petroleum ether–ethyl acetate (6:1 and 4:1) resulting in compound **12b** (484–502 mg), yield: 85–88%. $[\alpha]_D^{20}=-7.80$ (C=0.38 $CHCl_3$). IR (film): 3500–3300, 1740 cm^{-1}. EIMS (m/e): 449 (M^++1), 267 ($C_{17}H_{35}CO^+$), 91 ($PhCH_2$) (100%), 57, 43. ^1H NMR (acetone-d_6): δ 7.40–7.20 (m, 5H, Ar), 4.70–4.60 (s, 2H), 4.40–4.30 (dd, 1H, J=3.6 Hz, J=11.7 Hz), 4.20–4.10 (dd, 1H, J=5.6 Hz, J=11.7 Hz), 3.70–3.65 (m, 3H), 2.50–2.30 (t, 2H, J=7.5 Hz, J=7.5 Hz, CH_2CO), 1.60–1.40 (t, 2H), 1.40–1.20 (s, 28H), 1.00–0.80 (t, 3H, CH_3). HR EIMS (m/e): 449.4069 (M^++1) Calcd: 449.4062.

The general procedure for preparation of 1-acyl-2-benzylglycerophosphocholine ***14a*** *and* ***14b*** *from 1-acyl-2-benzylglycerol* ***12a*** *and* ***12b***

To a stirred solution of compound **12a** or **12b** (0.07–0.09 mmol) and triethylamine (1 ml) in dry dichloromethane (10 ml) at 0–5°C was added β-chloroethylphosphoric dichloride (0.3 ml). The reaction mixture was stirred overnight at room temperature. The reaction mixture was then poured into water and extracted with dichloromethane. The combined organic phase was washed with water and dried with anhydrous sodium sulfate. The dichloromethane was removed under reduced pressure to give a crude product which was purified on silica gel column eluting with trichloromethane–methanol–water (60:35:4) resulting in a little yellow oil.

1-Palimoyl-2-benzylglycerophosphocholine ***14a***

Yield: 68.0%. $[\alpha]_D^{20}=-3.50$ (C=1.1 $CHCl_3$). IR (film): 3500–3300, 1740 cm^{-1}. EIMS (m/e): 449 (M^++1), 267 ($C_{17}H_{35}CO^+$), 91 ($PhCH_2$) (100%), 57, 43. ^1H NMR ($CDCl_3$): δ 7.40–7.20 (m, 5H, Ar), 4.70–4.50 (s, 2H), 4.40–3.4 (m, 18H), 2.40–2.20 (t, 2H), 1.70–1.50 (t, 2H), 1.40–1.20 (s, 24H), 1.00–0.80 (t, 3H, J=6.3 Hz, J=6.9 Hz, CH_3).

1-Stearoyl-2-benzylglycerophosphocholine ***14b***

Yield: 60.0%. $[\alpha]_D^{20}=-3.30$ (C=0.2 $CHCl_3$). IR (film): 1740 cm^{-1}. EIMS (m/e): 614 (M^+), 267 ($C_{17}H_{35}CO^+$), 91 ($PhCH_2$) (100%). ^1H NMR ($CDCl_3$): δ 7.40–7.20 (m, 5H, Ar), 4.70–4.50 (s, 2H, CH_2Ph), 4.40–3.4 [m, 18H, $OCH_2CHCH_2OPOCH_2CH_2N(CH_3)_3$], 2.40–2.20 (t, 2H), 1.70–1.50 (t, 2H), 1.40–1.20 (s, 28H), 1.00–0.80 (t, 3H, J=6.4 Hz, J=6.9 Hz, CH_3).

The general procedure for preparation of 1-acyl-2-[(z,z,z)-9,12,15-octadecatrienoyl]-glycerophosphocholine ***1a*** *and* ***1b*** *from 1-acyl-2-benzylglycerophosphocholine* ***14a*** *and* ***14b***

Hydrogen was flowed through a stirred solution of compound **14a** or **14b** (15–36 mg) in dry trichloromethane (10 ml) and Pd–C (10%) (15 mg) for 4 h at room temperature. The solution was

filtered and filtrate was concentrated under reduced pressure to give a crude product (10–24 mg) which was dissolved in dry dichloromethane (10 ml) to which were added linolenic anhydride (150–200 mg) and DMAP (8–10 mg). The reaction mixture was stirred for 3 days at room temperature. The precipitate was filtered off and the filtrate was concentrated under reduced pressure to give a crude product which was purified on silica gel column eluting with petroleum ether–ethyl acetate (4:1) and trichloromethane–methanol–water (60:35:4) resulting in a little yellow oil.

1-Palimoyl-2-[(z,z,z)-9,12,15-octadecatrienoyl]-sn-glycerophosphocholine ***Ia***

Yield: 46%. $[\alpha]_D^{20}=-2.9$ (C=0.9 $CHCl_3$). ^1H NMR ($CDCl_3$): δ 5.4–5.30 (m, 6H, 3CH=CH), 5.00–4.90 (m, 1H, CHOCO), 4.40–4.25 (m, 4H, CH_2CH_2O), 4.10–4.00 (m, 4H, CH_2CH_2N), 3.20–3.00 (s, 9H, NMe_3), 2.90–2.70 (m, 4H, 2=CCH_2C=), 2.40–2.30 (dt, 4H, RCH_2CO, $R'CH_2CO$), 2.20–2.00 (m, 4H, $2CH_2C$=), 1.70–1.20 (s, 38H), 1.00–0.90 (t, 3H, J=7.5 Hz, J=7.5 Hz, CH_3), 0.90–0.80 (t, 3H, J=6.5 Hz, J=6.8 Hz, CH_3) ^{31}P NMR ($CDCl_3$): −2.54 ppm.

1-Stearoyl-2-[(z,z,z)-9,12,15-octadecatrienoyl]-sn-glycerophosphocholine ***Ib***

Yield: 65%. $[\alpha]_D^{20}=-6.4$ (C=0.25 $CHCl_3$). ^1H NMR ($CDCl_3$): δ 5.4–5.30 (m, 6H, 3CH=CH), 5.00–4.90 (m, 1H, CHOCO), 4.40–4.20 (m, 4H, $2CH_2O$), 4.00–3.80 (m, 4H, CH_2CH_2N), 3.20–3.00 (s, 9H, NMe_3), 2.90–2.70 (m, 4H, 2=CCH_2C=), 2.40–2.30 (dt, 4H, RCH_2CO, $R'CH_2CO$), 2.20–2.00 (m, 4H, CH_2C=), 1.70–1.20 (s, 42H), 1.00–0.90 (t, 3H, J=7.4 Hz, J=7.5 Hz, CH_3), 0.90–0.80 (t, 3H, J=6.4 Hz, J=7.0 Hz) ^{31}P NMR ($CDCl_3$): −1.04 ppm.

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References

1. (a) Eibl, H. Chem. Phys. Lipids **1980**, 26, 405. (b) Burgos, C. E.; Ayer, D. E.; Johnson, R. A. J. Org. Chem. **1987**, 52, 4973. (c) Menger, F. M.; Wang, Y.-L. J. Org. Chem. **1996**, 61, 7382–7390. (d) Martin, F. M.; Josey, J. A.; Wang, Y.-L.; Dean, D. E. J. Org. Chem. **1994**, 59, 4805–4820. (e) Yuan, W.; Berman, R. J.; Gelb. M. H. J. Am. Chem. Soc. **1987**, 109, 8071. (f) Eibl, H. Angew. Chem. Int. Ed. Engl. **1984**, 23, 257–271.
2. (a) Keough, K. M. W.; Davis, P. J. Biochemistry **1979**, 18, 1453. (b) Huang, C.-H.; Mason, J. T. Biochem. Biophys. Acta **1986**, 864, 423.
3. (a) Longuir, K. J.; Martin, O. C.; Pagano, R. E. Chem. Phys. Lipids **1985**, 36, 197. (b) Schmidt, N.; Gercken, G.; Chem. Phys. Lipids **1985**, 38, 309.
4. (a) Charabarti, P.; Khorana, H. G. Biochemistry **1975**, 14, 5021. (b) Warner, T. G.; Benson, A. A. J. Lipid Res. **1977**, 18, 548. (c) Radhakrishnan, R.; Robson, R. J.; Takagaki, T.; Khorana, H. G. Methods Enzymol. **1981**, 72, 408, 793. (d) Stofdel, W.; Salm, K. P.; Muller, M.; Hoppe-Seyer'SZ. Physiol. Chem. **1982**, 363, 1. (e) Perly, B.; Dufourc, E. J.; Jarrel, H. C. J. Labelled Compounds Radiopharm. **1984**, 21, 1.
5. (a) Hubbell, W. L.; MaConnrll, H. M.; J. Am. Chem. Soc. **1971**, 93, 314. (b) Boss, W. F.; Kelley, C. J.; Landsberger, F. R. Anal. Biochem. **1975**, 64, 289.
6. (a) Brunner, J.; Richards, F. M. J. Biol. Chem. **1980**, 255, 3319. (b) Richards, F. M.; Brunner, J. Ann. N.Y. Acad. Sci. **1980**, 346, 144.
7. Coolbear, K. P.; Berde, C. B.; Keough, K. M. W. Biochemistry **1983**, 22, 1466–1473.
8. (a) Ostro, M. J.; Ed. Liposomes, Marcel Dekker, Inc. New York, 1983. (b) Gregoriadis, G.; Ed. Liposome Technology, CRC Press, Inc. Boca Raton, FL, 1984, Vol. I–III.
9. (a) Gregoriadis, G.; Ed. Liposomes as Drug Carriers: Recent Trends and Progress, Wiley and Sons, New York, 1988 (b) Knight, C. C. G. Ed. Liposomes: From Physical Structures to Therapeutic Applications, Elsevier/North-Holland, Amsterdam, 1981.
10. Bourre, J. M.; Pascal, G.; Masson, Dumont, O.; Piciotii, M. J. Nurochem. **1984**, 43, 342–340.

11. Paoletti, R.; Gallic, C. In Lipid, Malnutrition and the Developing Brain p. 121–140, 1972, Ciba, Foundation Symposium.
12. Reddy, B. S.; Bullrill, C.; Rigotty, J. Cancer Res. **1991**, 51, 487–491.
13. Reddy, B. S.; Maruyymama, H. C.; Rigotty, J. Cancer Res. **1986**, 46, 3363–3370.
14. Minoura, T.; Takata, T.; Sakaguchi, M.; Takada, H.; Yamaura, M.; Hioki, K.; Yamaoto, M. Cancer Res. **1988**, 48, 4790–4794.
15. Karmali, R. A.; Marsh, J.; Fuchs, C. J. Natl. Cancer Inst **1981**, 73, 457–6.
16. Jurkowski, J. J.; Cave, W. T. J. Natl. Cancer Inst **1985**, 75, 1145.
17. Braden, L. M.; Carroll, K. K. Lipids **1986**, 21, 285–288.
18. Orengo, I. F.; Black, H. S.; Kettler, A. H.; Wolf, J. E. Photochem. Photobiol. **1989**, 49, 71–77.
19. Fukase, K.; Matsumoto, T.; Ito, N.; Yoshimura, T.; Kotani, S.; Kusumoto, S. Bull. Chem. Jpn. **1992**, 65, 2643–2654.
20. Selinger, Z.; Lapidot, Y. J. Lipid Res. **1966**, 7, 174–175.
21. Beving, H. Acta. Chem. Scand. **1967**, 21, 2083.

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