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Synthesis of 1-palmitoyl-2-hexadecyl-*sn*-glycero-3-phosphocholine (PHPC)

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Abstract

A general method for the chirospecific synthesis of 1-acyl-2-alkyl-*sn*-glycero-3-phosphocholines is described. 1-Palmitoyl-2-hexadecyl-*sn*-glycero-3-phosphocholine (PHPC) was synthesized in 18% overall yield in ten steps via five new synthetic intermediates, and 1-acetyl-2-hexadecyl-*sn*-glycero-3-phosphocholine (AHPC) was also synthesized. 1-Acyl-2-alkyl-*sn*-glycero-3-phosphocholines, which have not been found to exist in nature, are ether lipid analogs of 1,2-diacyl-*sn*-glycero-3-phosphocholines, which are important components of cell membranes. Biophysical studies of hydrated bilayers of PHPC will be of interest in probing the critical importance of the central region of these amphiphilic molecules to the molecular assemblies that are formed.

Key words: 1-Acyl-2-alkyl-*sn*-glycero-3-phosphocholine; 1-Palmitoyl-2-hexadecyl-*sn*-glycero-3-phosphocholine; PHPC; 1-Acetyl-2-hexadecyl-*sn*-glycero-3-phosphocholine; AHPC; Palmitic anhydride

1. Introduction

Ether lipid analogs of 1,2-diacyl-*sn*-glycero-3-phosphocholines include 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholines, 1,2-dialkyl-*sn*-glycero-3-phosphocholines and 1-acyl-2-alkyl-*sn*-glycero-3-phosphocholines. There are many examples, including platelet-activating factor and plasmalogen, of 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholines that occur naturally [1]. Only a few studies have suggested the natural occurrence of 1,2-dialkyl-*sn*-glycero-3-phosphocholines [2]. 1-Acyl-2-alkyl-*sn*-glycero-3-phosphocholines, however, have not been found to exist in nature as membrane components. The biophysical properties of hydrated bilayers of these 1-acyl-2-alkyl-*sn*-glycero-3-phosphocholines remain unexamined and are of interest, especially in relation to the other structurally closely related lipids.

Abbreviations: AHPC, 1-acetyl-2-hexadecyl-*sn*-glycero-3-phosphocholine; br, broad; bT, bath temperature; d, doublet; DHPC, 1,2-dihexadecyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; FAB, fast atom bombardment; HPPC, 1-hexadecyl-2-palmitoyl-*sn*-glycero-3-phosphocholine; m, multiplet; p, pentet; PHPC, 1-palmitoyl-2-hexadecyl-*sn*-glycero-3-phosphocholine (properly abstracted alphabetically as 2-hexadecyl-1-palmitoyl-*sn*-glycero-3-phosphocholine); q, quartet; s, singlet; t, triplet; TLC, thin layer chromatography.

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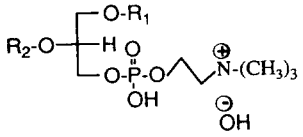
The series of four structurally closely related *sn*-glycero-3-phosphocholines (Fig. 1), including 1,2-dipalmitoyl-*sn*-glycero-2-phosphocholine (DPPC) [3–6], 1-hexadecyl-2-palmitoyl-*sn*-glycero-3-phosphocholine (HPPC) [7–9], 1,2-dihexadecyl-*sn*-glycero-3-phosphocholine (DHPC) [10,11] and 1-palmitoyl-2-hexadecyl-*sn*-glycero-3-phosphocholine (PHPC) [12,13] have all been synthesized. The hydrated bilayer structures of DPPC [14,15], HPPC [9] and DHPC [16–19] have been elucidated using differential scanning calorimetry (DSC) and X-ray diffraction.

rac-PHPC [20–22] and *rac*-AHPC [22] have been synthesized, and 1-palmitoyl-2-hexadecyl-*sn*-glycero-3-phosphocholine (PHPC) has been synthesized by a method [13] that gave a product with a low specific rotation and an unacceptable elemental analysis. I now detail a new and efficient chirospecific synthesis of the ether lipid 1-palmitoyl-2-hexadecyl-*sn*-glycero-3-phosphocholine (PHPC), starting from 2,3-isopropylidene-*sn*-glycerol (**1**), which proceeds through five new synthetic intermediates. This synthesis was performed to provide PHPC for biophysical studies of this last compound in the series of ester- and ether-linked *sn*-glycero-3-phosphocholines. This general method can be used to synthesize other nearly optically pure 1-acyl-2-alkyl-*sn*-glycero-3-phosphocholine analogs by using the appropriate alkyl bromide and acid anhydride, and the synthesis of 1-acetyl-2-hexadecyl-*sn*-glycero-3-phosphocholine (AHPC) is also reported. This synthetic methodology via protected glycerol derivatives [23] unambiguously gives 1-acyl-2-alkyl-*sn*-glycero-3-phosphocholines of superior optical purity in good overall yields.



	$-R_1$	$-R_2$
DPPC	$-O-C(=O)-(CH_2)_{14}-CH_3$	$-O-C(=O)-(CH_2)_{14}-CH_3$
HPPC	$-(CH_2)_{15}-CH_3$	$-O-C(=O)-(CH_2)_{14}-CH_3$
DHPC	$-(CH_2)_{15}-CH_3$	$-(CH_2)_{15}-CH_3$
PHPC	$-O-C(=O)-(CH_2)_{14}-CH_3$	$-(CH_2)_{15}-CH_3$

Fig. 1. Series of four structurally closely related *sn*-glycero-3-phosphocholines

2. Experimental procedures

2.1. Materials and methods

Unless otherwise stated, all materials were obtained from commercial suppliers and were used without further purification. The palmitic acid, as the methyl ester derivative, was demonstrated to be greater than 99.8% pure by gas chromatographic analysis on 5% DEGS-PS on 100/120 supelcoport at 175°C with flame ionization detection. The 2,3-isopropylidene-*sn*-glycerol (**1**) had been used in a previous ether lipid synthesis and had been demonstrated to be greater than 99.0% enantiomeric excess [24]. The trityl chloride (m.p. 113°C) was freshly recrystallized from toluene/hexane. The 4-pyrrolidinopyridine was purified [25] and was observed to melt at 57°C (lit. [26] m.p. 57°C). The CCl_4 (from P_2O_5, b.p. 76°C), CH_2Cl_2 (from P_2O_5, b.p. 40°C), $(i\text{-Pr})_2NEt$ (from CaH_2, b.p. 126°C), PhMe (after azeotropic removal of water, from benzophenone ketyl, b.p. 110°C), Et_2O (from benzophenone ketyl, b.p. 34°C) and $CHCl_3$ (twice from P_2O_5, b.p. 62°C) were distilled prior to use. Reactions were carried out under an argon atmosphere with magnetic stirring at ambient temperature, unless reported as bath temperatures (bT). Organic phases were dried over Na_2SO_4 and rotary-evaporated under reduced pressure. Silica gel (grade 60, 230–400 mesh, E. Merck, Darmstadt, Germany) was used for column chromatography unless Biosil A (100–200

mesh, Biorad, Richmond, CA) or Rexyn I-300 (Fisher, Fairlawn, NJ) was specified. All compounds were demonstrated to be homogeneous by analytical TLC on precoated silica gel TLC plates (grade 60, F254, E. Merck, Darmstadt, Germany), and chromatograms were visualized with cupric sulfate/phosphoric acid followed by charring [27]. Phosphorus-containing products were also checked by staining duplicate chromatograms with molybdic acid reagent [28]. Phospholipid solutions were filtered through 0.5 μm teflon membranes (Alltech, Deerfield, IL). Analytical samples were prepared by dissolving the lipids in *tert*-butanol and lyophylization (100 μm for 24 h), followed by drying (0.5 μm for 48 h in the presence of P_2O_5). Melting points of all solids were determined in a micro melting apparatus, and were determined in an argon atmosphere for the hygroscopic phospholipids. All ^{1}H- (200 MHz) and ^{13}C- (50.3 MHz) NMR spectra were run on a Bruker WP-200SY spectrometer in $CDCl_3$ solutions with tetramethylsilane as an internal standard. Exchangeable proton resonances were identified and, unless noted, are not reported. In addition, the spectra of the phosphocholines were run in $CD_3OD/CDCl_3$ 1:2, where the $CHCl_3$ resonance was then observed at δ 7.51. Optical rotations were taken on a Autopol-II polarimeter (Rudolph Research, Flanders, NJ) in a 1.00 dm cell. All solvent ratios are by volume.

2.2. Palmitic anhydride

A solution of 5.0 g (20 mmol) of anhydrous palmitic acid in 75 ml anhydrous CCl_4 and a solution of 2.0 g (9.7 mmol) dicyclohexylcarbodiimide in 25 ml anhydrous CCl_4 was shaken in a separatory funnel, then allowed to stand under argon for 16 h, during which time the dicyclohexylurea byproduct floated towards the top. The CCl_4 solution was filtered through a 20–25 μm fritted glass filter, the solvent evaporated and then the crude anhydride was recrystallized twice from acetone to give 4.0 g (8.1 mmol, 81%) palmitic anhydride [29,30] as white plates: m.p. 64–65°C (lit. [29] 64°C); ^{1}H-NMR ($CDCl_3$) δ 2.44 (t, 4 H, J = 7.4 Hz, $C2H_2$), 1.45–1.75 (m, 4 H, $C3H_2$), 1.26 (br s, 48 H, $C4H_2$—$C15H_2$), 0.88 (t, 6 H, J = 6.3 Hz, $C16H_3$); ^{13}C-NMR ($CDCl_3$) δ 169.64 (Cl), 35.32 (C2), 31.95 (C14), 28.91–29.69 (C4-C13), 24.27 (C3), 22.71 (C15), 14.11 (C16).

2.3. 1-Bromohexadecane

Stirred 1-hexadecanol (5.17 g, 21.3 mmol) was heated to 100°C (bT) and anhydrous hydrogen bromide gas was bubbled through the reaction mixture for 5 h until the disappearance of starting alcohol by TLC (hexane, R_f 0.05). The reaction mixture was cooled, then partitioned between 50 ml Et_2O and 20 ml H_2O. The organic phase was washed with H_2O, saturated aqueous $NaHCO_3$, H_2O, then dried, concentrated and chromatographed (hexane) to give 5.91 g (19.4 mmol, 91%) of a clear, colorless liquid which was homogeneous by TLC (hexane, R_f 0.54). The 1-bromohexadecane was demonstrated to be greater than 99.8% pure by gas chromatographic analysis on 5% DEGS-PS on 100/120 supelcoport at 150°C: ^{1}H-NMR ($CDCl_3$) δ 3.40 (t, 2 H, J = 6.8 Hz, $C1H_2$), 1.85 (p, 2 H, J = 7.0 Hz, $C2H_2$), 1.26 (br s, 26 H, $C3H_2$-$C15H_2$), 0.88 (t, 3 H, J = 6.4 Hz, $C16H_3$); ^{13}C-NMR ($CDCl_3$) δ 33.99 (C1), 32.88 (C2), 31.95 (C14), 28.21–29.70 (C3-C13), 22.71 (C15), 14.13 (C16).

2.4. 1-Benzyl-sn-glycerol (3)

The 2,3-isopropylidene-*sn*-glycerol (**1**) (α_D^{25} −14.0° (neat), d_4^{25} 1.05, $[\alpha]_D^{25}$ −13.3° (neat)) was converted via 1-benzyl-2,3-isopropylidene-*sn*-glycerol (**2**) to 1-benzyl-*sn*-glycerol (**3**) by the reported method [31] in 86% yield and was a clear, colorless liquid which was homogeneous by TLC (EtOAc/hexane 50:50, R_f 0.09): ^{1}H-NMR ($CDCl_3$) δ 7.26 (br s, 5 H, Ar), 4.45 (s, 2 H, CH_2Ar), 3.75–3.90 (m, 1 H, *sn*-2 CH), 3.70 (dd, 1 H, J = 11.4, 3.8 Hz, *sn*-3 CH), 3.62 (dd, 1 H, J = 11.4, 5.4 Hz, *sn*-3 CH), 3.41 (br d, 2 H, J = 5.5 Hz, *sn*-1 CH_2); ^{13}C-NMR ($CDCl_3$) δ 137.65, 128.36, 127.75 and 127.67 (Ar), 73.44 (CH_2Ar), 71.62 (*sn*-1 C), 70.67 (*sn*-2 C), 63.91 (*sn*-3 C); α_D^{25} −5.5° (neat), d_4^{25} 1.1, $[\alpha]_D^{25}$−5.0° (neat), $[\alpha]_D^{25}$−1.7° (c 7.5, $CHCl_3$) (lit. [31,32] $[\alpha]_D$−5.85° (neat); lit. [33] α_D −6.5° (neat), $[\alpha]_D$−5.7° (neat); lit. [34] α_D−5.73° (neat), $[\alpha]_D$−5.01° (neat); lit. [35,36] $[\alpha]_D^{20}$ −5.9° (neat)).

2.5. 1-Benzyl-3-trityl-sn-glycerol (**4**)

To a solution of 1.96 g (7.03 mmol) of trityl chloride in 10 ml dry CH_2Cl_2 was added 1.22 g (6.70 mmol) 1-benzyl-*sn*-glycerol (**3**), and then a solution of 1.74 ml (1.29 g, 9.98 mmol) of $(i\text{-}Pr)_2NEt$ in 5 ml CH_2Cl_2 was added dropwise. The reaction mixture was stirred overnight, then concentrated, and two reaction batches were combined. Chromatography ($EtOAc/CH_2Cl_2$ 1:99) followed by crystallization (diisopropylether/hexane) gave 4.10 g (9.66 mmol, 72%) of 1-benzyl-3-trityl-*sn*-glycerol (**4**) [37,38] as white crystals which were homogeneous by TLC ($EtOAc/CH_2Cl_2$ 5:95, R_f 0.40): m.p. 65°C (lit. [39] enantiomer m.p. 73°C; lit. [40] enantiomer m.p. 71–73.5°C; lit. [41] enantiomer m.p. 71.5–72.5°C); ^{1}H-NMR ($CDCl_3$) δ 7.15–7.45 (m, 20 H, Ar), 4.51 (s, 2 H, CH_2Ar), 3.90–4.05 (m, 1 H, *sn*-2 CH), 3.60 (dd, 1 H, J = 9.6, 4.4 Hz, *sn*-1 CH), 3.53 (dd, 1 H, J = 9.6, 6.0 Hz, *sn*-1 CH), 3.24 (dd, 1 H, J = 9.3, 5.6 Hz, *sn*-3 CH), 3.19 (dd, 1 H, J = 9.3, 5.4 Hz, *sn*-3 CH); ^{13}C-NMR ($CDCl_3$) δ 143.89, 138.06, 128.70, 128.40, 127.84, 127.68 and 127.07 (Ar), 86.71 ($C\text{-}Ar_3$), 73.38 (CH_2Ar), 71.59 (*sn*-1 C), 69.96 (*sn*-2 C), 64.63 (*sn*-3 C); $[\alpha]_D^{25}$ +6.3° (c 5.05, C_6H_6) (lit. [39] enantiomer $[\alpha]_D^{25}$ −6.37° (c 5.05, C_6H_6)). Elemental analysis calculated for $C_{29}H_{28}O_3$: C, 82.05; H, 6.65. Found: C, 82.06; H, 6.81.

2.6. 1-Benzyl-2-hexadecyl-3-trityl-sn-glycerol (**5**)

To 1.03 g (2.43 mmol) 1-benzyl-3-trityl-*sn*-glycerol (**4**), 1.85 g (6.06 mmol) 1-bromohexadecane and 0.078 g (0.24 mmol) $(n\text{-}Bu)_4NBr$ in 10 ml anhydrous PhMe was added 0.409 g (3.64 mmol) *tert*-BuOK. The heterogeneous reaction mixture was refluxed at 104°C (bT) for 2 h, then cooled and concentrated. The residue was redissolved in CH_2Cl_2, washed with H_2O, dried and then concentrated. Chromatography (CH_2Cl_2/hexane 50:50) gave 1.24 g (1.91 mmol, 79%) of 1-benzyl-2-hexadecyl-3-trityl-*sn*-glycerol (**5**) as a clear, colorless liquid which was homogeneous by TLC (CH_2Cl_2, R_f 0.63): ^{1}H-NMR ($CDCl_3$) δ 7.20–7.50 (m, 20 H, Ar), 4.52 (s, 2 H, C-H_2Ar), 3.50–3.65 (m, 3 H, *sn*-1 CH_2, *sn*-2 CH), 3.53 (t, 2 H, J = 6.6 Hz, C1″H_2), 3.15–3.25 (m, 2 H, *sn*-3 CH_2), 1.40–1.65 (m, 2 H, C2″H_2), 1.25 (br s, 26 H, C3″H_2-C15″H_2), 0.88 (t, 3 H, J = 6.1 Hz, C16″H_3); ^{13}C-NMR ($CDCl_3$) δ 144.16, 138.50, 128.77, 128.26, 127.72, 127.50, 127.41 and 126.89 (Ar), 86.58 ($C\text{-}Ar_3$), 78.36 (*sn*-2 C), 73.27 (CH_2Ar), 70.72 and 70.57 (*sn*-1 C and C1″), 63.55 (*sn*-3 C), 31.92 (C14″), 29.36–30.16 (C3″–C13″), 26.18 (C2″), 22.68 (C15″), 14.10 (C16″); $[\alpha]_D^{25}$ +6.0° (c 5.00, CH_2Cl_2), $[\alpha]_D^{25}$ +9.0° (c 5.00, C_6H_6). Elemental analysis calculated for $C_{45}H_{60}O_3$: C, 83.28; H, 9.32. Found: C, 83.14; H, 9.03.

2.7. 1-Benzyl-2-hexadecyl-sn-glycerol (**6**)

To a solution of 1.22 g (1.88 mmol) of 1-benzyl-2-hexadecyl-3-trityl-*sn*-glycerol (**5**) in 19 ml of 1,4-dioxane was added 9.4 ml of 1 N HCl, and the reaction mixture was refluxed at 110° (bT) for 2 h, then cooled to 0°C (bT), and 0.790 g (9.40 mmol) of $NaHCO_3$ was cautiously added. The reaction mixture was concentrated, 10 ml of H_2O was added, and then the mixture was extracted with CH_2Cl_2 (4 × 20 ml). The organic extracts were combined and then dried and the solvent evaporated. Chromatography (Et_2O/PhMe 50:50) gave the crude product, which was rechromatographed ($EtOAc/CH_2Cl_2$ 15:85) to give 0.760 g (1.87 mmol, 99%) of 1-benzyl-2-hexadecyl-*sn*-glycerol (**6**) as a clear, colorless liquid which was homogeneous by TLC ($EtOAc/CH_2Cl_2$ 15:85, R_f 0.46): ^{1}H-NMR ($CDCl_3$) δ 7.33 (br s, 5 H, Ar), 4.55 (s, 2 H, CH_2Ar), 3.45–3.80 (m, 5 H, *sn*-1 CH_2, *sn*-2 CH, *sn*-3 CH_2), 3.56 (t, 2 H, J = 6.3 Hz, C1″H_2), 1.40–1.65 (m, 2 H, C2″H_2), 1.24 (br s, 26 H, C3″H_2—C15″H_2), 0.88 (t, 3 H, J = 6.4 Hz, C16″H_3); ^{13}C-NMR ($CDCl_3$) δ 138.09, 128.43, 127.71 and 127.65 (Ar), 78.53 (*sn*-2 C), 73.55 (CH_2Ar), 70.47 and 70.06 (*sn*-1 C and C1″), 62.89 (*sn*-3 C), 31.95 (C14″), 29.38–30.11 (C3″–C13″), 26.13 (C2″), 22.71 (C15″), 14.12 (C16″); $[\alpha]_D^{25}$ −10.2° (c 5.00, CH_2Cl_2), $[\alpha]_D^{25}$ −4.6° (c 5.00, C_6H_6). Elemental analysis calculated for $C_{26}H_{46}O_3$: C, 76.79; H, 11.40. Found: C, 76.71; H, 11.69.

2.8. 1-Benzyl-2-hexadecyl-sn-glycero-3-phospho-β-bromoethanol (**7**)

To a stirred solution of 1.39 g (5.75 mmol) of β-bromoethyl dichlorophosphate [42–44] in 10 ml anhydrous Et_2O was added 2.33 ml pyridine dropwise over 5 min. After 15 min a solution of 0.781

g (1.92 mmol) of 1-benzyl-2-hexadecyl-*sn*-glycerol (**6**) in 10 ml anhydrous Et_2O was added dropwise over 20 min. The reaction mixture was warmed to 55°C (bT) for 4 h. The reaction mixture was then cooled to 0°C (bT), 5.5 ml of H_2O was added and the reaction mixture was stirred overnight to give a clear mixture which was partitioned between 30 ml of MeOH/$CHCl_3$ 10:90 and 11 ml of 4 N HCl. The aqueous phase was extracted with additional MeOH/$CHCl_3$ 10:90 (4 × 25 ml), and the organic extracts were all combined, dried and evaporated. Chromatography (MeOH/$CHCl_3$ 7:93 gradually increased to MeOH/$CHCl_3$ 30:70) gave 0.766 g (1.29 mmol, 67%) of 1-benzyl-2-hexadecyl-*sn*-glycero-3-phospho-β- bromoethanol (**7**) as a clear, colorless viscous semi-solid which was homogeneous by TLC ($CHCl_3$/MeOH/H_2O 60:30:4, R_f 0.50): ^{1}H-NMR (CD_3OD/$CDCl_3$ 1:2) δ 7.40–7.70 (m, 5 H, Ar), 4.25–4.65 (m, 2 H, *sn*-3 CH_2), 4.50 (s, 2 H, CH_2Ar), 4.05–4.25 (m, 2 H, α-CH_2), 3.55–4.05 (m, 3 H, *sn*-1 CH_2, *sn*-2 CH), 3.40–3.60 (m, 2 H, C1″H_2), 2.32 (dt, 2 H, J = 9.4, 7.6 Hz, β-CH_2), 1.40–1.65 (m, 2 H, C2″H_2), 1.27 (br s, 26 H, C3″H_2—C15″H_2), 0.89 (t, 3 H, J = 6.4 Hz, C16″H_3); ^{13}C-NMR (CD_3OD/$CDCl_3$ 1:2) δ 138.30, 128.64 and 127.94 (Ar), 78.21 (d, $^3J_{CP}$ = 6.1 Hz, *sn*-2 C), 73.76 (CH_2Ar), 71.02 and 69.93 (*sn*-1 C and C1″), 65.64 and 65.39 (d, $^2J_{CP}$ = 4.7 Hz, and d, $^2J_{CP}$ = 5.1 Hz; *sn*-3 C and α-C), 32.19 (C14″), 31.24 (d, $^3J_{CP}$ = 7.8 Hz, β-C), 29.63–30.29 (C3″–C13″), 26.34 (C2″), 22.93 (C15″), 14.21 (C16″); $[\alpha]_D^{25}$ +4° (c 1.0, MeOH/$CHCl_3$ 1:2). Elemental analysis calculated for $C_{28}H_{50}BrO_6P$: C, 56.66; H, 8.49. Found: C, 56.39; H, 8.19.

2.9. *1-Benzyl-2-hexadecyl-sn-glycero-3-phosphocholine* (**8**)

To a stirred solution of 680 mg (1.15 mmol) of 1-benzyl-2-hexadecyl-*sn*-glycero-3-phospho-β-bromoethanol (**7**) in 11 ml of chloroform/isopropanol/dimethylformamide 2:2:1 in a glass pressure bottle was added excess trimethylamine, and the reaction mixture was heated at 50°C (bT) for 48 h. The reaction mixture was cooled to ambient temperature, the excess trimethylamine was evaporated with a stream of argon, and then the reaction mixture was concentrated. Chromatography ($CHCl_3$/MeOH/H_2O 60:30:0 increased stepwise to $CHCl_3$/MeOH/H_2O 60:30:4) gave 498 mg (0.844 mmol, 73%) of 1-benzyl-2-hexadecyl-*sn*-glycero-3-phosphocholine (**8**) as a hygroscopic white solid which was homogeneous by TLC ($CHCl_3$/MeOH/H_2O 60:30:4, R_f 0.11): m.p. (argon) soften 62°C, melt 64–66°C; ^{1}H-NMR (CD_3OD/$CDCl_3$ 1:2) δ 7.34 (br s, 5 H, Ar), 4.56 (s, 2 H, CH_2Ar), 4.15–4.25 (m, 2 H, α-CH_2), 3.95 (dd, 2 H $^3J_{HH}$ = 5 Hz, $^3J_{HP}$ = 5 Hz, *sn*-3 CH_2), 3.45–3.75 (m, 7 H, *sn*-1 CH_2, *sn*-2 CH, C1″H_2, β-CH_2), 3.16 (s, 9 H, N-$(CH_3)_3$), 1.40–1.65 (m, 2 H, C2″H_2), 1.27 (br s, 26 H, C3″H_2—C15″H_2), 0.89 (t, 3 H, J = 6.3 Hz, C16″H_3); ^{13}C-NMR (CD_3OD/$CDCl_3$ 1:2) δ 138.67, 128.69, 128.08 and 128.00 (Ar), 78.38 (d, $^3J_{CP}$ = 8.1 Hz, *sn*-2 C), 73.82 (CH_2Ar), 70.94 and 70.19 (*sn*-1 C and C1″), 66.84 (m, β-C), 65.25 (d, $^2J_{CP}$ = 5.6 Hz, *sn*-3 C), 59.23 (d, $^2J_{CP}$ = 5.0 Hz, α-C), 54.39 (br s, N-$(CH_3)_3$), 32.23 (C14″), 29.64–30.40 (C3″-C13″), 26.39 (C2″), 22.95 (C15″), 14.17 (C16″); $[\alpha]_D^{25}$ +5° (c 1.0, $CHCl_3$/MeOH/H_2O 60:30:4); FAB mass spectrum *m/z* 572 $(M + H)^+$, 480, 224, 184, 166. Elemental analysis calculated for $C_{31}H_{60}NO_7P$: C, 63:13; H, 10.25; N, 2.37. Found: C, 64.34; H, 10.11; N, 3.27.

2.10. *2-Hexadecyl-sn-glycero-3-phosphocholine* (**9**)

To a stirred solution of 262 mg (0.444 mmol) of 1-benzyl-2-hexadecyl-*sn*-glycero-3-phosphocholine (**8**) in 15 ml of CH_3OH/H_2O 90:10 was added 12 mg (0.086 mmol) of PdO, and the reaction mixture was mechanically shaken under 50 psi of H_2 on a Parr apparatus for 24 h. The catalyst was removed by filtration through a 0.5 μm filter, and the solvent was removed. Chromatography ($CHCl_3$/CH_3OH/2.5 M NH_4OH 60:35:8) gave 202 mg (0.404 mmol, 91%) of 2-hexadecyl-*sn*-glycero-3-phosphocholine (**9**) as a white solid which was homogeneous by TLC ($CHCl_3$/CH_3OH/2.5 M NH_4OH 60:35:8, R_f 0.16; $CHCl_3$/CH_3OH/H_2O 60:30:4, R_f 0.07): m.p. (argon) (49–50°C; ^{1}H-NMR (CD_3OD/$CDCl_3$ 1:2) δ 4.20–4.35 (m, 2 H, α-CH_2), 3.97 (dd, 2 H, $^3J_{HH}$ = 5 Hz, $^3J_{HP}$ = 5 Hz, *sn*-3 CH_2), 3.40–3.75 (m, 7 H, *sn*-1 CH_2, *sn*-2 CH, C1″H_2, β-CH_2), 3.22 (s, 9 H, N-$(CH_3)_3$), 1.40–1.65 (m, 2 H, C2″H_2), 1.27 (br s, 26 H, C3″H_2-C15″H_3), 0.89 (t, 3 H, J = 6.1 Hz, C16″H_3); ^{13}C-NMR (CD_3OD/$CDCl_3$ 1:2) δ 79.22

(d, $^3J_{CP}$ = 7.7 Hz, *sn*-2 C), 70.64 (C1″), 66.80 (m, β-C), 64.08 (d, $^2J_{CP}$ = 5.3 Hz, *sn*-3 C), 60.83 (*sn*-1 C), 59.34 (d, $^2J_{CP}$ = 4.6 Hz, α-C), 54.39 (br s, N-$(CH_3)_3$), 32.22 (C14″), 29.65–30.30 (C3″–C13″), 26.32 (C2″), 22.95 (C15″), 14.19 (C16″); $[\alpha]_D^{25}$ +6° (c 1.0, $CHCl_3$/MeOH/H_2O 60:30:4); FAB mass spectrum *m/z* 482 $(M + H)^+$. Elemental analysis calculated for $C_{24}H_{54}NO_7P$: C, 57.69; H, 10.89; N, 2.80. Found: C, 57.40; H, 11.03; N, 3.06.

2.11. *1-Acetyl-2-hexadecyl-sn-glycero-3-phosphocholine* (**AHPC**)

The acetylation of 2-hexadecyl-*sn*-glycero-3-phosphocholine (**9**) by the method used for **PHPC** gave 1-acetyl-2-hexadecyl-*sn*-glycero-3-phosphocholine (**AHPC**), which was purified by chromatography ($CHCl_3$/MeOH/H_2O 60:30:0 increased stepwise to $CHCl_3$/MeOH/H_2O 60:30:4) and gave a hygroscopic white solid which was homogeneous by TLC ($CHCl_3$/MeOH/H_2O 60:30:4, R_f 0.09): m.p. (argon) soften 40°C, melt 233°C, ^{1}H-NMR ($CDCl_3$) δ 4.30–4.45 (m, 2 H, α-CH_2), 4.27 (dd, 1 H, *J* = 11.7, 3.3 Hz, *sn*-1 CH), 4.11 (dd, 1 H, *J* = 11.7, 6.2 Hz, *sn*-1 CH), 3.85–4.05 (m, 2 H, *sn*-3 CH_2), 3.80–3.95 (m, 2 H, β-CH_2), 3.60–3.75 (m, 1 H, *sn*-2 CH), 3.45–3.65 (m, 2 H, C1″H_2), 3.40 (s, 9 H, N-$(CH_3)_3$), 2.06 (s, 3 H, C2′H_3), 1.40–1.60 (m, 2 H, C2″H_2), 1.26 (br s, 26 H, C3″H_2—C15″H_2), 0.88 (t, 3 H, *J* = 6.2 Hz, C16″H_3); ^{1}H-NMR (CD_3OD/$CDCl_3$ 1:2) δ 4.28 (dd, 1 H, *J* = 11.6, 3.7 Hz, *sn*-1 CH), 4.20–4.35 (m, 2 H, α-CH_2), 4.14 (dd, 1 H, *J* = 11.6, 5.9 Hz, *sn*-1 CH), 3.80–4.05 (m, 2 H, *sn*-3 CH_2), 3.60–3.80 (m, 1 H, *sn*-2 CH), 3.40–3.70 (m, 4 H, β-CH_2, C1″H_2), 3.23 (s, 9 H, N-$(CH_3)_3$), 2.09 (s, 3 H, C2′H_3), 1.40–1.65 (m, 2 H, C2″H_2), 1.27 (br s, 26 H, C3″H_2—C15″H_2), 0.89 (t, 3 H, *J* = 6.0 Hz, C16″H_3); ^{13}C-NMR (CD_3OD/$CDCl_3$ 1:2) δ 171.92 (C1′), 76.86 (d, $^3J_{CP}$ = 8.1 Hz, *sn*-2 C), 70.99 (C1″), 66.85 (m, β-C), 64.53 (d, $^2J_{CP}$ = 5.2 Hz, *sn*-3 C), 64.14 (*sn*-1 C), 59.31 (d, $^2J_{CP}$ = 4.8 Hz, α-C), 54.42 (br s, N-$(CH_3)_3$), 32.23 (C14″), 29.65–30.27 (C3″–C13″), 26.30 (C2″), 22.96 (C15″), 20.89 (C2′), 14.20 (C16″); IR (KBr) 1730 cm^{-1} (carbonyl stretch); $[\alpha]_D^{25}$ +11° (c, 1.0, $CHCl_3$/MeOH/H_2O 60:30:4), $[\alpha]_D^{25}$ +10° (c, 0.59, MeOH/$CHCl_3$ 50:50); FAB mass spectrum *m/z* 524 $(M + H)^+$, 224, 184, 166. Elemental analysis calculated for $C_{26}H_{56}NO_8P$: C, 57.65; H, 10.42; N, 2.59. Found: C, 57.37; H, 10.72; N, 2.53.

2.12. *1-Palmitoyl-2-hexadecyl-sn-glycero-3-phosphocholine* (**PHPC**)

To a stirred solution of 187 mg (0.374 mmol) of 2-hexadecyl-*sn*-glycero-3-phosphocholine (**9**) in 2 ml of alcohol-free anhydrous $CHCl_3$ was added a solution of 370 mg (0.748 mmol) of palmitic anhydride in 2 ml of pure $CHCl_3$, and then a solution of 111 mg (0.749 mmol) of freshly recrystallized 4-pyrrolidinopyridine in 2 ml of pure $CHCl_3$ was added dropwise. The reaction was complete by TLC after 20 h. The reaction mixture was concentrated and then chromatographed ($CHCl_3$/MeOH/H_2O 60:30:0 gradually increased to $CHCl_3$/MeOH/H_2O 60:30:4). Ion exchange chromatography (60 cc Rexyn I-300 dry packed, washed and eluted with $CHCl_3$/MeOH/H_2O 4:5:1) gave the product, which was rechromatographed (Biosil A 100–200 mesh, MeOH/$CHCl_3$ 25:75 gradually increased to MeOH/$CHCl_3$ 70:30) to give 228 mg (309 mmol, 83%) of 1-palmitoyl-2-hexadecyl-*sn*-glycero-3-phosphocholine (**PHPC**) [12,13] as a hygroscopic white solid which was homogeneous by TLC ($CHCl_3$/MeOH/H_2O 60:30:4, R_f 0.21): m.p. (argon) soften 75°C, melt 95–97°C; ^{1}H-NMR ($CDCl_3$) δ 4.20–4.40 (m, 2 H, α-CH_2), 4.28 (dd, 1 H, *J* = 11.6, 2.9 Hz, *sn*-1 CH), 4.05 (dd, 1 H, *J* = 11.6, 7.0 Hz, *sn*-1 CH), 3.70–4.00 (m, 2 H, *sn*-3 CH_2), 3.40–3.85 (m, 5 H, *sn*-2 CH, β-CH_2, C1″H_2), 3.35 (s, 9 H, N-$(CH_3)_3$), 2.30 (t, 2 H, *J* = 7.6 Hz, C2′H_2), 1.35–1.65 (m, 4 H, C3′H_2, C2″H_2), 1.26 (br s, 50 H, C4′H_2-C15′H_2, C3″H_2-C15″H_2), 0.88 (t, 6 H, *J* = 6.4 Hz, C16′H_3, C16″H_3); ^{1}H-NMR (CD_3OD/$CDCl_3$ 1:2) δ 4.30 (dd, 1 H, *J* = 11.6, 3.4 Hz, *sn*-1 CH), 4.20–4.35 (m, 2 H, α-CH_2), 4.13 (dd, 1 H, *J* = 11.6, 6.4 Hz, *sn*-1 CH), 3.80–4.05 (m, 2 H, *sn*-3 CH_2), 3.65–3.80 (m, 1 H, *sn*-2CH), 3.45–3.75 (m, 4H, β-CH_2, C1″H_2), 3.22 (s, 9 H, N-$(CH_3)_3$), 2.35 (t, 2 H, *J* = 7.4 Hz, C2′H_2), 1.40–1.65 (m, 4 H, C3′H_2, C2″H_2), 1.28 (br s, 50 H, C4′H_2-C15′H_2, C3″H_2—C15″H_2), 0.89 (t, 6 H, *J* = 6.4 Hz, C16′H_3, C16″H_3); ^{13}C-NMR (CD_3OD/$CDCl_3$ 1:2) δ 174.66 (C1′), 77.06 (d, $^3J_{CP}$ = 7.4 Hz, *sn*-2 C), 71.03 (C1″), 66.84 (m, β-C), 64.67 (d, $^2J_{CP}$ = 4.9 Hz, *sn*-3 C), 64.26 (*sn*-1 C), 59.41 (d,

$^2J_{CP}$ = 4.9 Hz, α-C), 54.41 (br s, N-$(CH_3)_3$), 34.60 (C2′), 32.29 (C14′, C14″), 29.30–30.37 (C4′–C13′, C3″–C13″), 26.41 (C2″), 25.33 (C3′), 23.01 (C15′, C15″), 14.22 (C16′, C16″); IR (KBr) 1730 cm^{-1} (carbonyl stretch); $[\alpha]_D^{25}$ +10° (c 1.0, $CHCl_3$/MeOH/H_2O 60:30:4), $[\alpha]_D^{25}$ +10° (c 0.59, MeOH/$CHCl_3$ 50:50) (lit. [13] $[\alpha]_D^{25}$ +2.42° (c 0.59, MeOH/$CHCl_3$ 50:50)); FAB mass spectrum *m/z* 720 $(M + H)^+$, 224, 184, 166. Elemental analysis calculated for $C_{40}H_{84}NO_8P$: C, 65.09; H, 11.47; N, 1.90. Found: C, 64.80; H, 11.63; N, 1.92.

3. Results and discussion

The chirospecific synthesis of 1-acyl-2-alkyl-*sn*-glycero-3-phosphocholines of high optical purity was performed in ten steps (Fig. 2) from commercially available 2,3-isopropylidene-*sn*-glycerol (**1**) of high optical purity. The 2,3-isopropylidene-*sn*-glycerol (**1**) was demonstrated to be nearly enantiomerically pure by the quantitative NMR method that was previously reported [24]. Briefly, the 2,3-isopropylidene-*sn*-glycerol (**1**) was converted to 1-octadecyl-2,3-isopropylidene-*sn*-glycerol, transacetalated to 2,3-benzylidene-1-octadecyl-*sn*-glycerol, then reduced with lithium aluminum hydride-aluminum chloride. The 3-benzyl-1-octadecyl-*sn*-glycerol, the major product of the regioselective reduction, was converted to its Mosher's ester [45] derivative. We unambiguously determined the optical purity of this Mosher's ester to be greater than 99.0% enantiomeric excess from the integrations of the two AB quartets of the benzyl protons in the ^{1}H-NMR spectra. The corresponding Mosher's ester of 1-benzyl-3-octadecyl-*sn*-glycerol, which was prepared from 1,2-isopropylidene-*sn*-glycerol, was also prepared, and the appropriate doping experiments permitted us

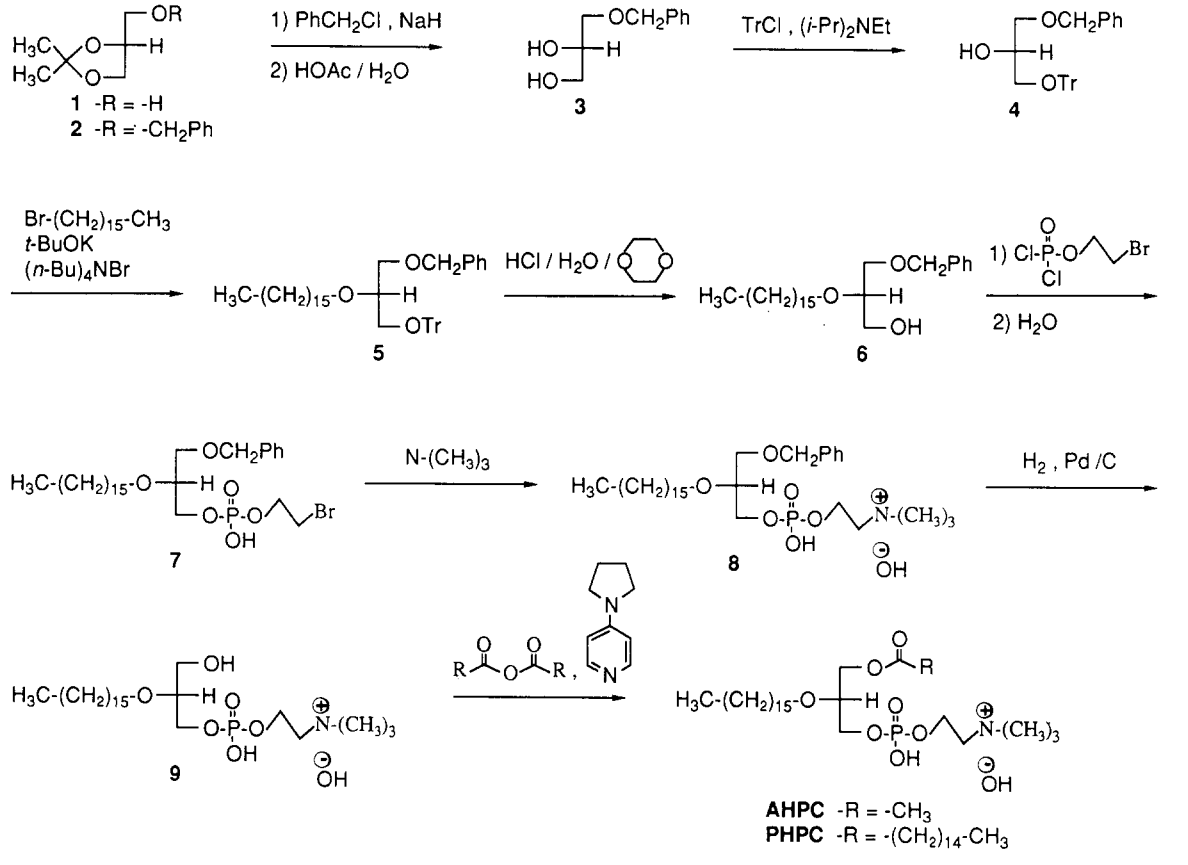


Fig. 2. Synthesis of 1-acyl-2-alkyl-*sn*-glycero-3-phosphocholines AHPC and PHPC.

to quantitatively determine optical purities. The optical purities of 2,3-isopropylidene-*sn*-glycerol (**1**) and its enantiomer, 1,2-isopropylidene-*sn*-glycerol, can unambiguously be determined by a capillary GC method for the separation of their diastereomeric Mosher's ester derivatives [46] or by the HPLC separation of their benzoate ester derivatives on a chiral stationary phase [47]. However, it is better to determine the optical purity of 1-benzyl-*sn*-glycerol derivatives, which must be completely stable to racemization. Preferably, the 1-benzyl-*sn*-glycerol (**1**) should be converted to the corresponding stearylaldehyde acetal and reduced [48] to give 1-benzyl-3-octadecyl-*sn*-glycerol, the precursor to the Mosher's ester derivative used to determine optical purity.

My synthetic methodology for the synthesis of 1-acyl-2-alkyl-*sn*-glycero-3-phosphocholines, via protected glycerol derivatives [23], requires acylation in the final synthetic step, which allows the flexibility to synthesize other 1-acyl analogs in addition to preventing racemization by acyl-rearrangement. This synthetic sequence avoids the phosphorylation of a 1-acyl-2-alkyl-*sn*-glycerol, although the two enantiomeric 2-octadecyl analogs, 1-acetyl-2-octadecyl-*sn*-glycero-3-phosphocholine (prepared by a route which risked 1 to 3 acyl-rearrangement) and 3-acetyl-2-octadecyl-*sn*-glycero-1-phosphocholine (prepared by a route which risked 1 to 3 phosphocholine-rearrangement) were reportedly [39] synthesized from a common intermediate and had opposite rotations of essentially the same magnitude.

The acylation of 2-hexadecyl-*sn*-glycero-3-phosphocholine (**9**) was performed by a slight variation of reported [3,25,49,50] methods using 200 mol% of palmitic anhydride and 200 mol% of freshly recrystallized 4-pyrrolidinopyridine. I have found that commercial palmitic anhydride, which was prepared from palmitoyl chloride and acetic anhydride according to an old report [51], was approximately 50% palmitic acetic mixed anhydride. Commercial quality control of methanolysis and gas chromatographic analysis cannot distinguish the solvent peak from the methyl acetate resulting from this impurity. I suggest that fatty acid anhydrides be prepared by the reported [29,30] dicyclohexylcarbodiimide method from well-characterized fatty acid, or the ^{1}H and ^{13}C spectra of commercial fatty acid anhydride products be examined carefully for the acetyl methyl singlet at δ 2.23 in the ^{1}H spectrum and the acetyl group at δ 166.61 and 22.16 in the ^{13}C spectrum. The ^{1}H and ^{13}C spectra of 1-acyl-2-alkyl-*sn*-glycero-3-phosphocholines PHPC and AHPC in both $CDCl_3$ and in $CD_3OD/CDCl_3$ 1:2 were assigned with the assistance of reported spectra of related glycero-3-phosphocholines [52–61]. The positive ion FAB mass spectra gave the expected $(M+H)^+$ molecular ions and characteristic fragments [24,62–65].

Optically active PHPC had been previously synthesized by a method [13] that gave a product with a specific rotation that was low by a factor of 4. The elemental analysis was unacceptably low in carbon, though it was reported to be low in hydrogen for a multi-hydrate. Also, the ^{1}H-NMR spectrum for this PHPC was not properly assigned. The authors had previously demonstrated that their synthetic method [8,66], via esterified 1,2-dihydroxy-3-iodopropanes, can proceed without racemization [66], although for the synthesis of optically active PHPC [13] the optical purity was determined prior to the critical phosphorylation step during which partial racemization is possible. The PHPC prepared by my chirospecific synthetic sequence via nearly optically pure 1-benzyl-*sn*-glycerol (**3**) was fully characterized and compared favorably with the previously reported [13] synthetic PHPC.

The acetyl analog AHPC, which was prepared by my same method, was the major isolable product from the acylation reaction of 2-hexadecyl-*sn*-glycero-3-phosphocholine (**9**) with poor-quality commercial palmitic anhydride containing palmitic acetic mixed anhydride. AHPC could best be synthesized by acylation with acetic anhydride. AHPC had not previously been prepared in a nearly optically pure form.

The ten-step synthesis of 1-palmitoyl-2-hexadecyl-*sn*-glycero-3-phosphocholine (PHPC) required nine purifications and gave nearly optically pure PHPC in 18% overall yield from 2,3-isopropylidene-*sn*-glycerol (**1**). The synthesis of other 1-acyl-2-alkyl-*sn*-glycero-3-phosphocholine analogs is possible by using the appropriate alkyl

bromides and acid anhydrides, and the synthesis and characterization of the acetyl analog 1-acetyl-2-hexadecyl-*sn*-glycero-3-phosphocholine (AHPC) is included as an example. The biophysical studies of hydrated bilayers of PHPC have been completed and will be reported in detail in a separate paper.

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