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Syntheses of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and analogs with ^{13}C- and ^{2}H-labeled choline head groups

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Abstract

The syntheses of four head group labeled analogs of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (**6**) by a general method from 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphatidic acid (**5**) have been performed. The syntheses of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[α-^{13}C]choline (**6a**) and 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[β-^{13}C]choline (**6b**) were performed from labeled [1-^{13}C]glycine (**1a**) in 52% overall yield and from [2-^{13}C]glycine (**1b**) in 56% overall yield, respectively. 1,2-Di-*O*-palmitoyl-*sn*-glycero-3-phospho[N(C^2H_3)$_3$]choline (**9**) was prepared from 2-aminoethanol in 39% overall yield. 1,2-Di-*O*-palmitoyl-*sn*-glycero-3-phospho[α-C^2H_2]choline (**12**) was prepared from *N*,*N*-dimethylglycine ethyl ester in 50% overall yield. © 1997 Elsevier Science Ireland Ltd.

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1. Introduction

We are interested in probing the role of lipid components on membrane structure and function using a variety of biophysical techniques including differential scanning calorimetry (DSC), low angle X-ray diffraction, as well as solid-state ^{2}H- and ^{13}C-nuclear magnetic resonance (NMR) spectroscopy. We have utilized a general synthetic

Abbreviations: DPPC, 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphocholine; DSC, differential scanning calorimetry; NMR, nuclear magnetic resonance; PA, phosphatidic acid; TPS, 2,4,6-triisopropylbenzenesulfonyl chloride; PC, 1,2-di-*O*-acyl-*sn*-glycero-3-phosphocholine; DMAP, 4-dimethylaminopyridine; DPPA, 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphatidic acid; TLC, thin layer chromatography; FAB, fast atom bombardment; HRMS, high-resolution mass spectrum.

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methodology to obtain the various appropriately labeled 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphocholines (DPPC) necessary for some of these biophysical studies from the corresponding labeled cholines. These four head group labeled lipids will be extremely useful probes for solid-state nuclear magnetic resonance (NMR) studies. The solid-state ^{2}H-NMR and solid-state ^{13}C-NMR techniques have been previously demonstrated to be powerful methods for obtaining information on the conformational and dynamical properties of model and biological membranes (Davis, 1983; Watts and van Gorkom, 1992). The use of solid-state ^{2}H-NMR has also been extended successfully to study drug and membrane interactions (Browning and Akutsu, 1982; Forrest et al., 1984; Auger et al., 1988; Lee et al., 1989; Makriyannis et al., 1990; Yang et al., 1992).

The synthesis of glycero-3-phospholipids has often involved the coupling of a phosphatidic acid (PA) with an alcohol in the presence of a condensing agent, such as 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) (Aneja et al., 1970). The synthesis of 1,2-di-*O*-acyl-*sn*-glycero-3-phosphocholines (PC) with this method has been difficult, because of the insolubility of the choline salts used during the coupling reaction. The superior solubility of the tetraphenylborate (Ph_4B^+) salt of choline in pyridine and its subsequent reactivity in 1,2-di-*O*-acyl-*sn*-glycero-3-phosphocholine (PC) synthesis has been demonstrated (Harbison and Griffin, 1984) and was based on the first use of tetraphenylborate salts for *sn*-glycero-3-phospholipid synthesis (Hintze and Gercken, 1975). Glycerophospholipid syntheses have been comprehensively reviewed (Eibl, 1980). Unlabeled DPPC had previously been synthesized by a variety of methods (Robles and Van Den Berg, 1969; Gupta et al., 1977; Patel et al., 1979; Hermetter and Paltauf, 1981; Singh, 1990), and a variety of isotopically labeled glycero-3-phosphocholines (Watts and van Gorkom, 1992; Browning and Akutsu, 1982; Forrest et al., 1984; Lee et al., 1989; Makriyannis et al., 1990; Yang et al., 1992; Harbison and Griffin, 1984; Gupta et al., 1977; Eibl and Westphal, 1967; Stockton et al., 1974; Gally et al., 1975; Wood et al., 1976; Oldfield et al., 1978; Kingsley and Feigenson, 1979; Laube and Kurreck, 1983; Mangroo and Gerber, 1988; Ulrich and Watts, 1994) and analogs (Seelig and Gally, 1976; Loffredo et al., 1990) have previously been reported. This methodology is the most convergent and efficient approach to head group labeled 1,2-di-*O*-acyl-*sn*-glycero-3-phosphocholines (PC), and the syntheses and characterizations of1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (**6**) and four head group labeled analogs, 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[α-^{13}C] choline (**6a**), 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[β-^{13}C]choline (**6b**), 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[N(C^2H_3)$_3$]choline (**9**) and 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[α-C^2H_2] choline (**12**), are detailed.

2. Experimental

2.1. General

Glycine (99%), formaldehyde (37% w/v in water), palladised charcoal (10% Pd), hydrogen chloride (>99%), lithium aluminum hydride (>99%), iodomethane (99.5%), sodium tetraphenylborate (>99.5%), 4-dimethylaminopyridine (DMAP) (99%), 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) (97%), thionyl chloride (>99%), ethanolamine (>99%), [C^2H_3]iodomethane (>99.5 atom% D), *N*,*N*-dimethylglycine ethyl ester (98%), and lithium aluminum deuteride (98 atom% D) were purchased from Aldrich, Milwaukee, WI. [1-^{13}C]Glycine (99.4 atom% ^{13}C) and [2-^{13}C]glycine (99.5 atom% ^{13}C) were purchased from Isotec Inc., Miamsburg, OH. 1,2-Di-*O*-palmitoyl-*sn*-glycero-3-phosphatidic acid (DPPA) (>99%) was purchased from Avanti Polar Lipids, Inc., Alabaster, AL. Acetonitrile (from CaH_2, bp 81–82°C), chloroform (from P_2O_5, bp 61°C), ethyl ether (from benzophenone ketyl, bp 35°C), methylene chloride (from P_2O_5, bp 40°C), methanol (bp 65°C), and pyridine (from CaH_2, bp 89°C) were distilled prior to use. Reactions that required anhydrous conditions were carried out under a N_2 atmosphere with magnetic stirring at room temperature, in flame-dried glassware and

anhydrous solvents. Reaction temperatures were reported as bath temperatures (b*T*). Solutions containing products were dried over anhydrous sodium sulfate or magnesium sulfate. Solvents were removed *in vacuo* using a Buchi rotary evaporator at a maximum temperature of approximately 40°C. All solvent ratios were by volume. Silica gel (E. Merck silica gel 60, 230–400 mesh) used for column chromatography was purchased from New England Scientific, Bridgewater, CT. Purity of the products was checked by analytical thin layer chromatography (TLC) on silica gel 60 plates. Iodine staining was used to visualize all chromatograms. Phosphorus containing products were also checked by molybdic acid reagent which was purchased from Sigma Chemical Company, St. Louis, MO. Melting points were determined in open pyrex capillaries using a Hoover apparatus and were uncorrected. All solution NMR spectra were recorded on an IBM/Bruker WP-200SY 200 MHz FT spectrometer unless indicated to be on a Bruker DMX at 500 MHz ^{1}H and at 125 MHz ^{13}C. Tetramethylsilane (TMS) or sodium 3-trimethylsilyl[2,3-(C^2H_2)$_2$]propionate (TSP) were used as internal references for organic and aqueous samples, respectively. The use of dimethylsulfoxide (δ 2.50) as an internal reference for aqueous samples is recommended by us for the ease of recovery of pure sample materials. In $CDCl_3/CD_3OD$ (2:1), residual protons from the deuterated chloroform were observed at δ 7.47 and from the perdeuterated methanol at δ 3.36 in the proton spectra, and the $CDCl_3$ triplet was centered at δ 77.68 while the CD_3OD multiplet appeared at δ 48.95 in the carbon spectra. Exchangeable peaks were not reported, although a DOH/ROH peak around δ 4.4 was generally observed in ^{1}H spectra in $CDCl_3/CD_3OD$ (2:1). Multiplicities were reported as either broad (b), singlet (s), doublet (d), triplet (t), quartet (q) or multiplet (m). Fast atom bombardment (FAB) mass spectra and FAB high-resolution mass spectra (HRMS) were obtained with a MAT 731 mass spectrometer fitted with an Ion Tech gun for FAB. Elemental analyses were obtained by Baron Consulting Co., Orange, CT, using a Perkin Elmer Elemental 2400.

2.2. *N,N*-Dimethylglycine (**2**)

A mixture of 2.00 g (26.6 mmol) of glycine (**1**), 6.4 ml of formalin, and 0.8 g of palladised charcoal (containing 10% of Pd) in 30 ml of absolute EtOH was mechanically shaken under 40 PSI of H_2 on a Parr apparatus for 10 h at room temperature. The reaction mixture was filtered through a celite pad and the residue was washed with warm $CHCl_3$. The filtrate was evaporated *in vacuo* to dryness, and the product was crystallized from acetone-ethanol to give 2.70 g (26.2 mmol, 98% yield) of *N,N*-dimethylglycine (**2**) as a white solid: TLC ($CHCl_3$/MeOH/H_2O (50:50:5)) R_f 0.19; mp 179–180°C (Lit. (Bowman and Stroud, 1950) mp 182–183°C); ^{1}H NMR (D_2O) δ 3.96 (s, 2 H, C2H_2), 2.80 (s, 6 H, N(CH_3)$_2$).

2.3. *N,N*-Dimethyl[1-^{13}C]glycine (**2a**)

N,N-Dimethyl[1-^{13}C]glycine (**2a**) was prepared from 1.00 g (13.1 mmol) of [1-^{13}C]glycine (**1a**), 3.2 ml of formalin and 0.5 g of palladised charcoal following the procedure described for unlabeled **2** to give 1.36 g (13.1 mmol, 100% yield) of **2a** as a white solid: TLC ($CHCl_3$/MeOH/H_2O (50:50:5)) R_f 0.19; mp 179–181°C; ^{1}H NMR (D_2O) δ 3.73 (d, 2 H, $J_{HC} = 5.1$ Hz, C2H_2), 2.92 (s, 6 H, N(CH_3)$_2$).

2.4. *N,N*-Dimethyl[2-^{13}C]glycine (**2b**)

N,N-Dimethyl[2-^{13}C]glycine (**2b**) was prepared from 1.00 g (13.1 mmol) of [2-^{13}C]glycine (**1b**), 3.2 ml of formalin and 0.5 g of palladised charcoal following the procedure described for unlabeled **2** to give 1.36 g (13.1 mmol, 100% yield) of **2b** as a white solid: TLC ($CHCl_3$/MeOH/H_2O (50:50:5)) R_f 0.19; mp 178–180°C; ^{1}H NMR (D_2O) δ 3.69 (dm, 2 H, $^{1}J_{HC} = 140$ Hz, C2H_2), 2.90 (s, 6 H, N(CH_3)$_2$).

2.5. *N,N*-Dimethylglycine methyl ester hydrochloride (**3**)

A stream of dry HCl gas was bubbled through a magnetically stirred solution of 200 mg (1.94 mmol) of *N,N*-dimethylglycine (**2**) in 10 ml of

MeOH at 80°C (b*T*) for 30 min. The reaction mixture was allowed to stand overnight. The solvent was then removed *in vacuo* to give 216 mg (1.41 mmol, 73% yield) of *N*,*N*-dimethylglycine methyl ester hydrochloride (**3**) as a white solid: TLC ($CHCl_3$/MeOH/H_2O (50:50:5)) R_f 0.60; ^1H NMR (D_2O) δ 4.16 (s, 2 H, $C2H_2$), 3.85 (s, 3 H, OCH_3), 2.99 (s, 6 H, $N(CH_3)_2$).

2.6. *N*,*N*-Dimethyl[1-^{13}C]glycine methyl ester hydrochloride (**3a**)

N,*N*-Dimethyl[1-^{13}C]glycine methyl ester hydrochloride (**3a**) was prepared from 1.20 g (11.5 mmol) of *N*,*N*-dimethyl[1-^{13}C]glycine (**2a**) in MeOH following the procedure described for unlabeled **3** to give 1.30 g (8.41 mmol, 73% yield) of **3a** as a white solid: TLC ($CHCl_3$/MeOH/H_2O (50:50:5)) R_f 0.60; ^1H NMR (D_2O) δ 4.20 (d, 2 H, $J_{HC} = 5.3$ Hz, $C2H_2$), 3.92 (s, 3 H, OCH_3), 3.02 (s, 6 H, $N(CH_3)_2$).

2.7. *N*,*N*-Dimethyl[2-^{13}C]glycine methyl ester hydrochloride (**3b**)

N,*N*-Dimethyl[2-^{13}C]glycine methyl ester hydrochloride (**3b**) was prepared from 1.20 g (11.5 mmol) of *N*,*N*-dimethyl[2-^{13}C]glycine (**2b**) in MeOH following the procedure described for unlabeled **3** to give 1.30 g (8.41 mmol, 73% yield) of **3b** as a white solid: TLC ($CHCl_3$/MeOH/H_2O (50:50:5)) R_f 0.60; ^1H NMR (D_2O) δ 4.18 (d, 2 H, $^1J_{HC} = 140$ Hz, $C2H_2$), 3.88 (s, 3 H, OCH_3), 3.01 (s, 6 H, $N(CH_3)_2$).

2.8. Choline tetraphenylborate (**4**)

To a magnetically stirred solution of 26 mg (0.68 mmol) of $LiAlH_4$ suspended in 20 ml of Et_2O was added dropwise 80 mg (0.52 mmol) of *N*,*N*-dimethylglycine methyl ester hydrochloride (**3**) in 20 ml of Et_2O at 0°C (b*T*). After refluxing 1 h, the mixture was decomposed by careful dropwise addition of 1 ml of H_2O at 0°C (b*T*). After the hydrogen evolution had ceased, 241 mg (1.70 mmol) of CH_3I was added and the mixture was stirred at ambient temperature overnight. The reaction mixture was filtered and the precipitate was washed with four 20 ml portions of 10% aqueous NaOH. The combined filtrate and washings were backwashed with 100 ml of Et_2O, and to the aqueous phase was added 582 mg (1.70 mmol) of sodium tetraphenylborate in 20 ml of H_2O at room temperature. The white gelatinous precipitate was collected by filtration, washed with H_2O (2×50 ml), pressed dry on the filter, and then dried azeotropically with EtOH and benzene. The product was crystallized from MeCN and gave 217 mg (0.512 mmol, 98% yield) of choline tetraphenylborate (**4**) (Harbison and Griffin, 1984) as large hexagonal prisms: TLC ($CHCl_3$/MeOH (70:30)) R_f 0.50; mp 220–222°C; ^1H NMR (($D_3C)_2SO$) δ 7.05–7.25 (m, 8 H, Ar), 6.90–7.00 (m, 8 H, Ar), 6.75–6.85 (m, 4 H, Ar), 3.75–3.80 (m, 2 H, $C1H_2$), 3.25–3.35 (m, 2 H, $C2H_2$), 3.04 (s, 9 H, $N(CH_3)_3$).

2.9. [1-^{13}C]Choline tetraphenylborate (**4a**)

[1-^{13}C]Choline tetraphenylborate (**4a**) was prepared from 1.00 g (6.47 mmol) of *N*,*N*-dimethyl[1-^{13}C]glycine methyl ester hydrochloride (**3a**), 0.31 g (8.2 mmol) of $LiAlH_4$, 2.50 g (17.6 mmol) of CH_3I in 100 ml of Et_2O, and then 5.80 g (16.9 mmol) of sodium tetraphenylborate in 100 ml of H_2O following the procedure described for unlabeled **4** to give 2.58 g (6.08 mmol, 94% yield) of **4a** as large hexagonal prisms: TLC ($CHCl_3$/MeOH (70:30)) R_f 0.50; mp 224–226°C; ^1H NMR (($D_3C)_2SO$) δ 7.05–7.25 (m, 8 H, Ar), 6.90–7.00 (m, 8 H, Ar), 6.75–6.85 (m, 4 H, Ar), 3.80 (dm, 2 H, $^1J_{HC} = 140$ Hz, $C1H_2$), 3.35–3.45 (m, 2 H, $C2H_2$), 3.09 (s, 9 H, $N(CH_3)_3$).

2.10. [2-^{13}C]Choline tetraphenylborate (**4b**)

[2-^{13}C]Choline tetraphenylborate (**4b**) was prepared from 1.00 g (6.47 mmol) of *N*,*N*-dimethyl[2-^{13}C]glycine methyl ester hydrochloride (**3b**), 0.31 g (8.2 mmol) of $LiAlH_4$, 2.50 g (17.6 mmol) of CH_3I in 100 ml of Et_2O, and then 5.80 g (16.9 mmol) of sodium tetraphenylborate in 100 ml of H_2O following the procedure described for unlabeled **4** to give 2.63 g (6.20 mmol, 96% yield) of **4b** as large hexagonal prisms: TLC ($CHCl_3$/MeOH (70:30)) R_f 0.50; mp 223–225°C; ^1H NMR

MeOH at 80°C (b*T*) for 30 min. The reaction mixture was allowed to stand overnight. The solvent was then removed *in vacuo* to give 216 mg (1.41 mmol, 73% yield) of *N*,*N*-dimethylglycine methyl ester hydrochloride (**3**) as a white solid: TLC ($CHCl_3$/MeOH/H_2O (50:50:5)) R_f 0.60; ^1H NMR (D_2O) δ 4.16 (s, 2 H, $C2H_2$), 3.85 (s, 3 H, OCH_3), 2.99 (s, 6 H, $N(CH_3)_2$).

2.6. *N*,*N*-Dimethyl[1-^{13}C]glycine methyl ester hydrochloride (**3a**)

N,*N*-Dimethyl[1-^{13}C]glycine methyl ester hydrochloride (**3a**) was prepared from 1.20 g (11.5 mmol) of *N*,*N*-dimethyl[1-^{13}C]glycine (**2a**) in MeOH following the procedure described for unlabeled **3** to give 1.30 g (8.41 mmol, 73% yield) of **3a** as a white solid: TLC ($CHCl_3$/MeOH/H_2O (50:50:5)) R_f 0.60; ^1H NMR (D_2O) δ 4.20 (d, 2 H, $J_{HC} = 5.3$ Hz, $C2H_2$), 3.92 (s, 3 H, OCH_3), 3.02 (s, 6 H, $N(CH_3)_2$).

2.7. *N*,*N*-Dimethyl[2-^{13}C]glycine methyl ester hydrochloride (**3b**)

N,*N*-Dimethyl[2-^{13}C]glycine methyl ester hydrochloride (**3b**) was prepared from 1.20 g (11.5 mmol) of *N*,*N*-dimethyl[2-^{13}C]glycine (**2b**) in MeOH following the procedure described for unlabeled **3** to give 1.30 g (8.41 mmol, 73% yield) of **3b** as a white solid: TLC ($CHCl_3$/MeOH/H_2O (50:50:5)) R_f 0.60; ^1H NMR (D_2O) δ 4.18 (d, 2 H, $^1J_{HC} = 140$ Hz, $C2H_2$), 3.88 (s, 3 H, OCH_3), 3.01 (s, 6 H, $N(CH_3)_2$).

2.8. Choline tetraphenylborate (**4**)

To a magnetically stirred solution of 26 mg (0.68 mmol) of $LiAlH_4$ suspended in 20 ml of Et_2O was added dropwise 80 mg (0.52 mmol) of *N*,*N*-dimethylglycine methyl ester hydrochloride (**3**) in 20 ml of Et_2O at 0°C (b*T*). After refluxing 1 h, the mixture was decomposed by careful dropwise addition of 1 ml of H_2O at 0°C (b*T*). After the hydrogen evolution had ceased, 241 mg (1.70 mmol) of CH_3I was added and the mixture was stirred at ambient temperature overnight. The reaction mixture was filtered and the precipitate was washed with four 20 ml portions of 10% aqueous NaOH. The combined filtrate and washings were backwashed with 100 ml of Et_2O, and to the aqueous phase was added 582 mg (1.70 mmol) of sodium tetraphenylborate in 20 ml of H_2O at room temperature. The white gelatinous precipitate was collected by filtration, washed with H_2O (2×50 ml), pressed dry on the filter, and then dried azeotropically with EtOH and benzene. The product was crystallized from MeCN and gave 217 mg (0.512 mmol, 98% yield) of choline tetraphenylborate (**4**) (Harbison and Griffin, 1984) as large hexagonal prisms: TLC ($CHCl_3$/MeOH (70:30)) R_f 0.50; mp 220–222°C; ^1H NMR (($D_3C)_2SO$) δ 7.05–7.25 (m, 8 H, Ar), 6.90–7.00 (m, 8 H, Ar), 6.75–6.85 (m, 4 H, Ar), 3.75–3.80 (m, 2 H, $C1H_2$), 3.25–3.35 (m, 2 H, $C2H_2$), 3.04 (s, 9 H, $N(CH_3)_3$).

2.9. [1-^{13}C]Choline tetraphenylborate (**4a**)

[1-^{13}C]Choline tetraphenylborate (**4a**) was prepared from 1.00 g (6.47 mmol) of *N*,*N*-dimethyl[1-^{13}C]glycine methyl ester hydrochloride (**3a**), 0.31 g (8.2 mmol) of $LiAlH_4$, 2.50 g (17.6 mmol) of CH_3I in 100 ml of Et_2O, and then 5.80 g (16.9 mmol) of sodium tetraphenylborate in 100 ml of H_2O following the procedure described for unlabeled **4** to give 2.58 g (6.08 mmol, 94% yield) of **4a** as large hexagonal prisms: TLC ($CHCl_3$/MeOH (70:30)) R_f 0.50; mp 224–226°C; ^1H NMR (($D_3C)_2SO$) δ 7.05–7.25 (m, 8 H, Ar), 6.90–7.00 (m, 8 H, Ar), 6.75–6.85 (m, 4 H, Ar), 3.80 (dm, 2 H, $^1J_{HC} = 140$ Hz, $C1H_2$), 3.35–3.45 (m, 2 H, $C2H_2$), 3.09 (s, 9 H, $N(CH_3)_3$).

2.10. [2-^{13}C]Choline tetraphenylborate (**4b**)

[2-^{13}C]Choline tetraphenylborate (**4b**) was prepared from 1.00 g (6.47 mmol) of *N*,*N*-dimethyl[2-^{13}C]glycine methyl ester hydrochloride (**3b**), 0.31 g (8.2 mmol) of $LiAlH_4$, 2.50 g (17.6 mmol) of CH_3I in 100 ml of Et_2O, and then 5.80 g (16.9 mmol) of sodium tetraphenylborate in 100 ml of H_2O following the procedure described for unlabeled **4** to give 2.63 g (6.20 mmol, 96% yield) of **4b** as large hexagonal prisms: TLC ($CHCl_3$/MeOH (70:30)) R_f 0.50; mp 223–225°C; ^1H NMR

($(D_3C)_2SO$) δ 7.05–7.25 (m, 8 H, Ar), 6.90–7.00 (m, 8 H, Ar), 6.75–6.85 (m, 4 H, Ar), 3.70–3.85 (m, 2 H, C1H_2), 3.35 (dm, 2 H, $^1J_{HC} = 140$ Hz, C2H_2), 3.07 (s, 9 H, N(CH_3)$_3$).

2.11. 1,2-Di-O-palmitoyl-sn-glycero-3-phosphocholine (6)

A mixture of 180 mg (0.263 mmol) of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphatidic acid (**5**) and 95 mg (0.78 mmol) of dimethylaminopyridine (DMAP) was dried by repeated evaporation of anhydrous pyridine (3 × 10 ml) according to the literature report (Kingsley and Feigenson, 1979). The residue was then dissolved in 10 ml of pyridine and warmed to 40°C (b*T*) for 30 min until fully dissolved. To this mixture was added 314 mg (1.04 mmol) of 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) (freshly recrystallized before use from *n*-pentane containing 1% thionyl chloride) followed by 110 mg (0.260 mmol) of choline tetraphenylborate (**4**) and the reaction mixture was stirred at 35°C (b*T*) for 4 h. The excess TPS was decomposed by the addition of 1 ml of H_2O, and the solvent was evaporated *in vacuo* to dryness. Chromatography with silica gel (elution was begun with $CHCl_3$/MeOH/H_2O (60:30:0) and the solvent polarity was increased stepwise to $CHCl_3$/MeOH/H_2O (60:30:4)), evaporation, followed by microfiltration (0.5-μm Teflon membrane) of a CH_2Cl_2 solution of the product then gave 152 mg (0.202 mmol, 77% yield) of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphocholine (**6**) (Robles and Van Den Berg, 1969; Gupta et al., 1977; Patel et al., 1979; Hermetter and Paltauf, 1981; Singh, 1990) as a white solid: TLC ($CHCl_3$/MeOH/H_2O (60:30:4)) R_f 0.20; ^{1}H NMR (500 MHz) ($CDCl_3$/CD_3OD (2:1)) δ 5.24 (m, 1 H, *sn*-2-CH), 4.42 (dd, 1 H, $J = 12$, 2.8 Hz, *sn*-1-CHa), 4.26 (m, 2 H, α-CH_2), 4.16 (dd, 1 H, $J = 12$, 7.0 Hz, *sn*-1-CHb), 4.01 (dd, 2 H, $J_{HH} = {}^3J_{HP} = 6$ Hz, *sn*-3-CH_2), 3.62 (m, 2 H, β-CH_2), 3.23 (s, 9 H, N(CH_3)$_3$), 2.33 and 2.32 (t, 2 H, $J = 8.1$ Hz, and t, 2 H, $J = 8.1$ Hz; C2′H_2 and C2″H_2), 1.61 (m, 4 H, C3′H_2, C3″H_2), 1.27 (br s, 48 H, C4′H_2–C15′H_2, C4″H_2–C15″H_2), 0.89 (t, 6 H, $J = 6.8$ Hz, C16′H_3, C16″H_3); ^{13}C NMR (125 MHz) ($CDCl_3$/CD_3OD (2:1)) δ 174.24 (C1′), 173.87 (C1″), 70.64 (d, $^3J_{CP} = 7.8$ Hz, *sn*-2-C), 66.74 (br s, β-C), 63.96 (d, $J_{CP} = 5.1$ Hz, *sn*-3-C), 62.95 (*sn*-1-C), 59.36 (d, $J_{CP} = 4.8$ Hz, α-C), 54.37 (N-Me_3), 34.51 and 34.36 (C2′, C2″), 32.21 (C14′, C14″), 29.40–30.00 (C4′-C13′ and C4″-C13″), 25.20 and 25.15 (C3′, C3″), 22.94 (C15′, C15″), 14.21 (C16′, C16″); mass spectrum *m*/*z* 734 (MH^+); HRMS calcd. for $C_{40}H_{81}NO_8P$ (MH^+) *m*/*z* 734.5700, found *m*/*z* 734.5704. Anal. Calcd. for $C_{40}H_{80}NO_8P \cdot H_2O$: C, 63.88; H, 10.99; N, 1.86. Found: C, 63.80; H, 11.14; N, 1.79.

2.12. 1, 2 -Di - O - palmitoyl - sn - glycero - 3 - phospho[α-^{13}C]choline (6a)

1,2-Di-*O*-palmitoyl-*sn*-glycero-3-phospho[α-^{13}C] choline (**6a**) was prepared from 1.00 g (1.46 mmol) of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphatidic acid (**5**), 400 mg (3.27 mmol) of DMAP, 1.32 g (4.36 mmol) of TPS, and 1.20 g (2.83 mmol) of [1-^{13}C]choline tetraphenylborate (**4a**) following the procedure described for unlabeled **6** to give 820 mg (1.09 mmol, 75% yield) of **6a** as a white solid: TLC ($CHCl_3$/MeOH/H_2O (60:30:4)) R_f 0.20; ^{1}H NMR (500 MHz) ($CDCl_3$/CD_3OD (2:1)) δ 5.24 (m, 1 H, *sn*-2-CH), 4.42 (dd, 1 H, $J = 12$, 2.8 Hz, *sn*-1-CHa), 4.26 (dm, 2 H, $^1J_{HC} = 145$ Hz, α-CH_2), 4.16 (dd, 1 H, $J = 12$, 7.0 Hz, *sn*-1-CHb), 4.01 (dd, 2 H, $J_{HH} = {}^3J_{HP} = 6$ Hz, *sn*-3-CH_2), 3.62 (m, 2 H, β-CH_2), 3.23 (s, 9 H, N(CH_3)$_3$), 2.33 and 2.32 (t, 2 H, $J = 8.1$ Hz, and t, 2 H, $J = 8.1$ Hz; C2′H_2 and C2″H_2), 1.61 (m, 4 H, C3′H_2, C3″H_2), 1.27 (br s, 48 H, C4′H_2-C15′H_2, C4″H_2-C15″H_2), 0.89 (t, 6 H, $J = 6.8$ Hz, C16′H_3, C16″H_3); ^{13}C NMR (125 MHz) ($CDCl_3$/CD_3OD (2:1)) δ 174.27 (C1′), 173.89 (C1″), 70.61 (d, $^3J_{CP} = 7.6$ Hz, *sn*-2-C), 66.73 (d, $^1J_{CC} = 39$ Hz, β-C), 64.03 (d, $J_{CP} = 5.5$ Hz, *sn*-3-C), 62.93 (*sn*-1-C), 59.42 (d, $J_{CP} = 4$ Hz, α-C), 54.39 (N-Me_3), 34.51 and 34.36 (C2′, C2″), 32.20 (C14′, C14″), 29.40–30.00 (C4′–C13′ and C4″–C13″), 25.20 and 25.15 (C3′, C3″), 22.94 (C15′, C15″), 14.21 (C16′, C16″); mass spectrum *m*/*z* 735 (MH^+); HRMS calcd. for $C_{39}^{13}CH_{81}NO_8P$ (MH^+) *m*/*z* 735.5733, found *m*/*z* 735.5728. Anal. Calcd. for

($(D_3C)_2SO$) δ 7.05–7.25 (m, 8 H, Ar), 6.90–7.00 (m, 8 H, Ar), 6.75–6.85 (m, 4 H, Ar), 3.70–3.85 (m, 2 H, C1H_2), 3.35 (dm, 2 H, $^1J_{HC} = 140$ Hz, C2H_2), 3.07 (s, 9 H, N(CH_3)$_3$).

2.11. 1,2-Di-O-palmitoyl-sn-glycero-3-phosphocholine (6)

A mixture of 180 mg (0.263 mmol) of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphatidic acid (**5**) and 95 mg (0.78 mmol) of dimethylaminopyridine (DMAP) was dried by repeated evaporation of anhydrous pyridine (3 × 10 ml) according to the literature report (Kingsley and Feigenson, 1979). The residue was then dissolved in 10 ml of pyridine and warmed to 40°C (b*T*) for 30 min until fully dissolved. To this mixture was added 314 mg (1.04 mmol) of 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) (freshly recrystallized before use from *n*-pentane containing 1% thionyl chloride) followed by 110 mg (0.260 mmol) of choline tetraphenylborate (**4**) and the reaction mixture was stirred at 35°C (b*T*) for 4 h. The excess TPS was decomposed by the addition of 1 ml of H_2O, and the solvent was evaporated *in vacuo* to dryness. Chromatography with silica gel (elution was begun with $CHCl_3$/MeOH/H_2O (60:30:0) and the solvent polarity was increased stepwise to $CHCl_3$/MeOH/H_2O (60:30:4)), evaporation, followed by microfiltration (0.5-μm Teflon membrane) of a CH_2Cl_2 solution of the product then gave 152 mg (0.202 mmol, 77% yield) of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphocholine (**6**) (Robles and Van Den Berg, 1969; Gupta et al., 1977; Patel et al., 1979; Hermetter and Paltauf, 1981; Singh, 1990) as a white solid: TLC ($CHCl_3$/MeOH/H_2O (60:30:4)) R_f 0.20; ^{1}H NMR (500 MHz) ($CDCl_3$/CD_3OD (2:1)) δ 5.24 (m, 1 H, *sn*-2-CH), 4.42 (dd, 1 H, $J = 12$, 2.8 Hz, *sn*-1-CHa), 4.26 (m, 2 H, α-CH_2), 4.16 (dd, 1 H, $J = 12$, 7.0 Hz, *sn*-1-CHb), 4.01 (dd, 2 H, $J_{HH} = {}^3J_{HP} = 6$ Hz, *sn*-3-CH_2), 3.62 (m, 2 H, β-CH_2), 3.23 (s, 9 H, N(CH_3)$_3$), 2.33 and 2.32 (t, 2 H, $J = 8.1$ Hz, and t, 2 H, $J = 8.1$ Hz; C2′H_2 and C2″H_2), 1.61 (m, 4 H, C3′H_2, C3″H_2), 1.27 (br s, 48 H, C4′H_2–C15′H_2, C4″H_2–C15″H_2), 0.89 (t, 6 H, $J = 6.8$ Hz, C16′H_3, C16″H_3); ^{13}C NMR (125 MHz) ($CDCl_3$/CD_3OD (2:1)) δ 174.24 (C1′), 173.87 (C1″), 70.64 (d, $^3J_{CP} = 7.8$ Hz, *sn*-2-C), 66.74 (br s, β-C), 63.96 (d, $J_{CP} = 5.1$ Hz, *sn*-3-C), 62.95 (*sn*-1-C), 59.36 (d, $J_{CP} = 4.8$ Hz, α-C), 54.37 (N-Me_3), 34.51 and 34.36 (C2′, C2″), 32.21 (C14′, C14″), 29.40–30.00 (C4′-C13′ and C4″-C13″), 25.20 and 25.15 (C3′, C3″), 22.94 (C15′, C15″), 14.21 (C16′, C16″); mass spectrum *m*/*z* 734 (MH^+); HRMS calcd. for $C_{40}H_{81}NO_8P$ (MH^+) *m*/*z* 734.5700, found *m*/*z* 734.5704. Anal. Calcd. for $C_{40}H_{80}NO_8P \cdot H_2O$: C, 63.88; H, 10.99; N, 1.86. Found: C, 63.80; H, 11.14; N, 1.79.

2.12. 1, 2 -Di - O - palmitoyl - sn - glycero - 3 - phospho[α-^{13}C]choline (6a)

1,2-Di-*O*-palmitoyl-*sn*-glycero-3-phospho[α-^{13}C] choline (**6a**) was prepared from 1.00 g (1.46 mmol) of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphatidic acid (**5**), 400 mg (3.27 mmol) of DMAP, 1.32 g (4.36 mmol) of TPS, and 1.20 g (2.83 mmol) of [1-^{13}C]choline tetraphenylborate (**4a**) following the procedure described for unlabeled **6** to give 820 mg (1.09 mmol, 75% yield) of **6a** as a white solid: TLC ($CHCl_3$/MeOH/H_2O (60:30:4)) R_f 0.20; ^{1}H NMR (500 MHz) ($CDCl_3$/CD_3OD (2:1)) δ 5.24 (m, 1 H, *sn*-2-CH), 4.42 (dd, 1 H, $J = 12$, 2.8 Hz, *sn*-1-CHa), 4.26 (dm, 2 H, $^1J_{HC} = 145$ Hz, α-CH_2), 4.16 (dd, 1 H, $J = 12$, 7.0 Hz, *sn*-1-CHb), 4.01 (dd, 2 H, $J_{HH} = {}^3J_{HP} = 6$ Hz, *sn*-3-CH_2), 3.62 (m, 2 H, β-CH_2), 3.23 (s, 9 H, N(CH_3)$_3$), 2.33 and 2.32 (t, 2 H, $J = 8.1$ Hz, and t, 2 H, $J = 8.1$ Hz; C2′H_2 and C2″H_2), 1.61 (m, 4 H, C3′H_2, C3″H_2), 1.27 (br s, 48 H, C4′H_2-C15′H_2, C4″H_2-C15″H_2), 0.89 (t, 6 H, $J = 6.8$ Hz, C16′H_3, C16″H_3); ^{13}C NMR (125 MHz) ($CDCl_3$/CD_3OD (2:1)) δ 174.27 (C1′), 173.89 (C1″), 70.61 (d, $^3J_{CP} = 7.6$ Hz, *sn*-2-C), 66.73 (d, $^1J_{CC} = 39$ Hz, β-C), 64.03 (d, $J_{CP} = 5.5$ Hz, *sn*-3-C), 62.93 (*sn*-1-C), 59.42 (d, $J_{CP} = 4$ Hz, α-C), 54.39 (N-Me_3), 34.51 and 34.36 (C2′, C2″), 32.20 (C14′, C14″), 29.40–30.00 (C4′–C13′ and C4″–C13″), 25.20 and 25.15 (C3′, C3″), 22.94 (C15′, C15″), 14.21 (C16′, C16″); mass spectrum *m*/*z* 735 (MH^+); HRMS calcd. for $C_{39}^{13}CH_{81}NO_8P$ (MH^+) *m*/*z* 735.5733, found *m*/*z* 735.5728. Anal. Calcd. for

$C_{39}^{13}CH_{80}NO_8P \cdot H_2O$: C, 63.93; H, 10.98; N, 1.86. Found: C, 64.11; H, 11.07; N, 1.92.

2.13. 1,2-Di-O-palmitoyl-sn-glycero-3-phospho[β-^{13}C] choline (**6b**)

1,2-Di-*O*-palmitoyl-*sn*-glycero-3-phospho[β-^{13}C]choline (**6b**) was prepared from 400 mg (0.584 mmol) of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphatidic acid (**5**), 200 mg (1.64 mmol) of DMAP, 531 mg (1.75 mmol) of TPS, and 484 mg (1.14 mmol) of [2-^{13}C]choline tetraphenylborate (**4b**) following the procedure described for unlabeled **6** to give 350 mg (0.465 mmol, 80% yield) of **6b** as a white solid: TLC ($CHCl_3$/MeOH/H_2O (60:30:4)) R_f 0.20; ^{1}H NMR (500 MHz) ($CDCl_3$/CD_3OD (2:1)) δ 5.24 (m, 1 H, *sn*-2-CH), 4.42 (dd, 1 H, $J = 12$, 2.8 Hz, *sn*-1-CHa), 4.26 (m, 2 H, α-CH_2), 4.16 (dd, 1 H, $J = 12$, 7.0 Hz, *sn*-1-CHb), 4.01 (dd, 2 H, $J_{HH} = {}^3J_{HP} = 6$ Hz, *sn*-3-CH_2), 3.62 (dm, 2 H, $^1J_{HC} = 145$ Hz, β-CH_2), 3.23 (d, 9 H, $^3J_{HC} = 2.0$ Hz, $N(CH_3)_3$), 2.33 and 2.32 (t, 2 H, $J = 8.1$ Hz, and t, 2 H, $J = 8.1$ Hz; C2′H_2 and C2″H_2), 1.61 (m, 4 H, C3′H_2, C3″H_2), 1.27 (br s, 48 H, C4′H_2–C15′H_2, C4″H_2–C15″H_2), 0.89 (t, 6 H, $J = 6.8$ Hz, C16′H_3, C16″H_3); ^{13}C NMR (125 MHz) ($CDCl_3$/CD_3OD (2:1)) δ 174.26 (C1′), 173.89 (C1″), 70.63 (d, $^3J_{CP} = 7.6$ Hz, *sn*-2-C), 66.74 (br s, β-C), 64.03 (d, $J_{CP} = 5.5$ Hz, *sn*-3-C), 62.94 (*sn*-1-C), 59.41 (dd, $^1J_{CC} = 39$ Hz, $J_{CP} = 4$ Hz, α-C), 54.38 (N-Me_3), 34.52 and 34.37 (C2′, C2″), 32.21 (C14′, C14″), 29.40–30.00 (C4′–C13′ and C4″–C13″), 25.20 and 25.16 (C3′, C3″), 22.95 (C15′, C15″), 14.21 (C16′, C16″); mass spectrum m/z 735 (MH^+); HRMS calcd. for $C_{39}^{13}CH_{81}NO_8P$ (MH^+) m/z 735.5733, found m/z 735.5749. Anal. Calcd. for $C_{39}^{13}CH_{80}NO_8P \cdot H_2O$: C, 63.93; H, 10.98; N, 1.86. Found: C, 63.91; H, 10.79; N, 1.96.

2.14. [N(C^2H_3)$_3$]Choline tetraphenylborate (**8**)

To a magnetically stirred solution of 500 mg (8.19 mmol) of ethanolamine (**7**) and 1.60 g (40.0 mmol) of NaOH in 50 ml of 75% aqueous EtOH was added 5.90 g (40.7 mmol) of CD_3I at room temperature. After 10 h the solution was concentrated to a white gelatinous precipitate *in vacuo*. The precipitate was dissolved in 50 ml of H_2O and a solution of 5.60 g (16.4 mmol) of sodium tetraphenylborate in 100 ml of H_2O was added. A white precipitate formed immediately. The reaction mixture was stirred at ambient temperature for an additional 2 h. The precipitated solid was then collected by filtration, washed with H_2O (3×50 ml), and dried in a desiccator *in vacuo*. Crystallization from MeCN gave 1.70 g (3.93 mmol, 48% yield) of [N(C^2H_3)$_3$]choline tetraphenylborate (**8**) (Harbison and Griffin, 1984) as white prisms: TLC ($CHCl_3$/MeOH (70:30)) R_f 0.50; mp 205–207°C; ^{1}H NMR ($(D_3C)_2SO$) δ 7.05–7.25 (m, 8 H, Ar), 6.90–7.00 (m, 8 H, Ar), 6.80–6.85 (m, 4 H, Ar), 3.83 (m, 2 H, C1H_2), 3.37 (m, 2 H, C2H_2).

2.15. 1,2-Di-O-palmitoyl-sn-glycero-3-phospho[N(C^2H_3)$_3$]choline (**9**)

1,2-Di-*O*-palmitoyl-*sn*-glycero-3-phospho[N(C^2H_3)$_3$]choline (**9**) was prepared from 100 mg (0.146 mmol) of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphatidic acid (**5**), 35 mg (0.29 mmol) of DMAP, 133 mg (0.439 mmol) of TPS, and 125 mg (0.289 mmol) of [N(C^2H_3)$_3$]choline tetraphenylborate (**8**) following the procedure described for unlabeled **6** to give 90 mg (0.12 mmol, 82% yield) of **9** (Lee et al., 1989; Yang et al., 1992; Wood et al., 1976) as a white solid: TLC ($CHCl_3$/MeOH/H_2O (60:30:4)) R_f 0.20; ^{1}H NMR (500 MHz) ($CDCl_3$/CD_3OD (2:1)) δ 5.24 (m, 1 H, *sn*-2-CH), 4.42 (dd, 1 H, $J = 12$, 2.5 Hz, *sn*-1-CHa), 4.26 (m, 2 H, α-CH_2), 4.16 (dd, 1 H, $J = 12$, 7.0 Hz, *sn*-1-CHb), 4.00 (dd, 2 H, $J_{HH} = {}^3J_{HP} = 6$ Hz, *sn*-3-CH_2), 3.61 (m, 2 H, β-CH_2), 3.23 (s, 0.4 H, residual $NCHD_2(CD_3)_2$), 2.33 and 2.32 (t, 2 H, $J = 8.1$ Hz, and t, 2 H, $J = 8.1$ Hz; C2′H_2 and C2″H_2), 1.61 (m, 4 H, C3′H_2, C3″H_2), 1.27 (br s, 48 H, C4′H_2–C15′H_2, C4″H_2–C15″H_2), 0.89 (t, 6 H, $J = 6.8$ Hz, C16′H_3, C16″H_3); mass spectrum m/z 743 (MH^+); HRMS calcd. for $C_{40}H_{72}D_9NO_8P$ (MH^+) m/z 743.6265, found m/z 743.6260. Anal. Calcd. for $C_{40}H_{71}D_9NO_8P \cdot H_2O$: C, 63.13; H, 12.04; N, 1.84. Found: C, 63.01; H, 12.14; N, 1.97.

2.16. [1-C^2H_2]Choline tetraphenylborate (**11**)

[1-C^2H_2]Choline tetraphenylborate (**11**) was prepared from 500 mg (3.80 mmol) of *N*,*N*-dimethylglycine ethyl ester (**10**) and 606 mg (14.4 mmol) of $LiAlD_4$ in 40 ml of Et_2O, followed by 2.70 g (19.0 mmol) of CH_3I and 2.60 g (7.60 mmol) of sodium tetraphenylborate in 100 ml of H_2O following the procedure described for unlabeled **4** to give 976 mg (2.29 mmol, 60% yield) of **11** (Harbison and Griffin, 1984) as a white solid: TLC ($CHCl_3$/MeOH (70:30)) R_f 0.50; mp 232–234°C; ^1H NMR ($(D_3C)_2SO$) δ 7.05–7.25 (m, 8 H, Ar), 6.90–7.00 (m, 8 H, Ar), 6.80–6.85 (m, 4 H, Ar), 3.35 (m, 2 H, $C2H_2$), 3.10 (s, 9 H, $N(CH_3)_3$).

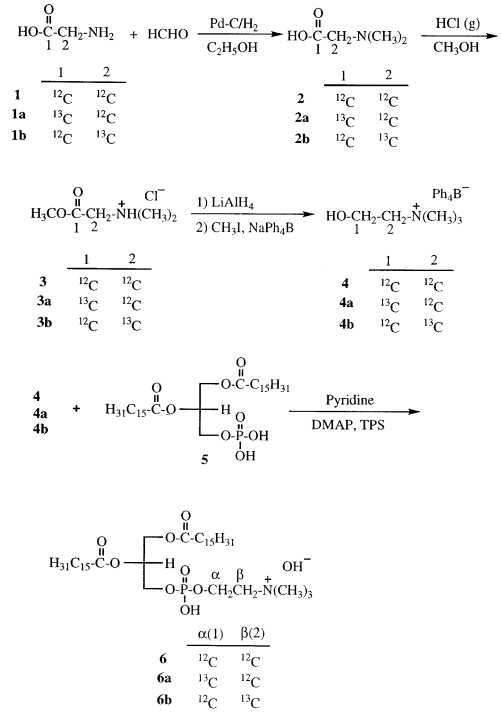
2.17. 1,2-Di-*O*-palmitoyl-*sn*-glycero-3-phospho-[α-C^2H_2]choline (**12**)

1,2-Di-*O*-palmitoyl-*sn*-glycero-3-phospho[α-C^2H_2]choline (**12**) was prepared from 250 mg (0.365 mmol) of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphatidic acid (**5**), 88 mg (0.72 mmol) of DMAP, 332 mg (1.10 mmol) of TPS, and 311 mg (0.731 mmol) of [1-C^2H_2]choline tetraphenylborate (**11**) in pyridine following the procedure described for unlabeled **6** to give 230 mg (0.305 mmol, 84% yield) of **12** (Browning and Akutsu, 1982; Gally et al., 1975) as a white solid: TLC ($CHCl_3$/MeOH/H_2O (60:30:4)) R_f 0.20; ^1H NMR (500 MHz) ($CDCl_3$/CD_3OD (2:1)) δ 5.24 (m, 1 H, *sn*-2-CH), 4.42 (dd, 1 H, $J = 12$, 2.8 Hz, *sn*-1-CHa), 4.16 (dd, 1 H, $J = 12$, 7.0 Hz, *sn*-1-CHb), 4.01 (dd, 2 H, $J_{HH} = {}^3J_{HP} = 6$ Hz, *sn*-3-CH_2), 3.60 (br s, 2 H, β-CH_2), 3.23 (s, 9 H, $N(CH_3)_3$), 2.33 and 2.32 (t, 2 H, $J = 8.1$ Hz, and t, 2 H, $J = 8.1$ Hz; C2′H_2 and C2″H_2), 1.61 (m, 4 H, C3′H_2, C3″H_2), 1.27 (br s, 48 H, C4′H_2–C15′H_2, C4″H_2–C15″H_2), 0.89 (t, 6 H, $J = 6.8$ Hz, C16′H_3, C16″H_3); mass spectrum *m*/*z* 736 (MH^+); HRMS calcd. for $C_{40}H_{79}D_2NO_8P$ (MH^+) *m*/*z* 736.5825, found *m*/*z* 736.5831. Anal. Calcd. for $C_{40}H_{78}D_2NO_8P \cdot H_2O$: C, 63.71; H, 11.23; N, 1.86. Found: C, 63.59; H, 11.37; N, 1.78.

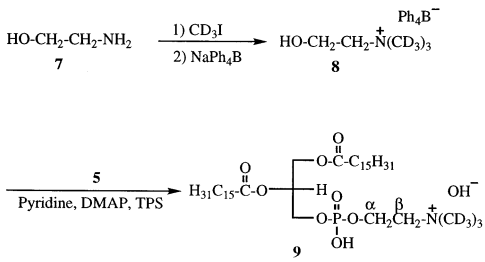
3. Results and discussion

Unlabeled 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (**6**) and a variety of ^{13}C- and ^2H-labeled choline head group analogs were synthesized by a general method. The two possible isotopically labeled analogs of DPPC having ^{13}C enrichment at each of the methylene carbons of the choline head group (Scheme 1) were synthesized from the correspondingly labeled glycine starting materials. In addition, two isotopically labeled analogs of DPPC with ^2H isotopic substitution at the three methyls of the choline group (Scheme 2) and at the α-methylene group (Scheme 3) were synthesized.

Glycine (*1*) was converted to *N*,*N*-dimethylglycine (**2**) by reductive methylation (Bowman and Stroud, 1950) with formaldehyde and hydrogen gas in the presence of palladium on activated

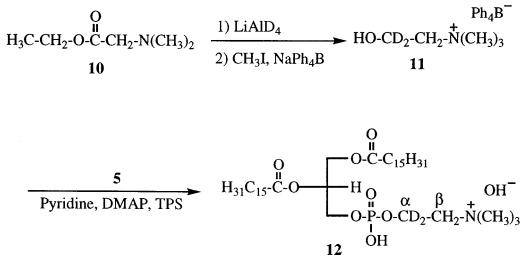


Scheme 1. Synthesis of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphocholine (**6**), 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[α-^{13}C]choline (**6a**) and 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho-[β-^{13}C]choline (**6b**).



Scheme 2. Synthesis of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[$N(C^2H_3)_3$]choline (**9**).

carbon in quantitative yield. Esterification of **2** was carried out by bubbling dry hydrogen chloride gas through a solution of **2** in methanol at an elevated temperature to give *N*,*N*-dimethylglycine methyl ester hydrochloride (**3**) in 73% yield. Choline tetraphenylborate (**4**) was prepared (Harbison and Griffin, 1984) by reduction of **3** with lithium aluminum hydride in diethyl ether followed by quaternization of the *N*,*N*-dimethylethanolamine intermediate with methyl iodide and the addition of sodium tetraphenylborate to precipitate the product salt. Choline tetraphenylborate (**4**) was isolated in 98% yield after recrystallization from acetonitrile to give large hexagonal prisms which were highly soluble in dimethylformamide or dimethylsulfoxide, slightly soluble in methanol, pyridine, or cold acetonitrile, and insoluble in water, chloroform or methylene chloride. The unlabeled parent choline tetraphenylborate (**4**) was then coupled to the anhydrous 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphatidic acid (**5**) salt of dimethylaminopyridine (DMAP) (Kingsley and Feigenson, 1979) in anhydrous pyridine with triisopropylbenzenesulfonyl chloride (TPS) according to the reported general method (Harbison and Griffin, 1984). The use of equal molar amounts of choline tetraphenylborate (**4**) and phosphatidic acid (**5**) is recommended, and anhydrous reaction conditions are critically important. Chromatography with silica gel gave unlabeled 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (**6**) in 77% yield, and the overall yield for **6** was 55%.



Scheme 3. Synthesis of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[α-C^2H_2]choline (**12**).

The syntheses of the two DPPC analogs with the ^{13}C isotopic labels specifically at each of the two methylene carbons of the choline head group were carried out by using the appropriately labeled [1-^{13}C]glycine (**1a**) and [2-^{13}C]glycine (**1b**) starting materials. Following the general procedure described for the unlabeled parent compound **6**, 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[α-^{13}C]choline (**6a**) was synthesized in a 52% overall yield, and 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[β-^{13}C]choline (**6b**) was synthesized in a 56% overall yield.

The synthesis of the known deuterated DPPC analog 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[$N(C^2H_3)_3$]choline (**9**) (Lee et al., 1989; Yang et al., 1992; Wood et al., 1976) by this novel method is shown in Scheme 2. Starting from ethanolamine (**7**), which is a reduced form of glycine, [$N(C^2H_3)_3$]choline tetraphenylborate (**8**) (Harbison and Griffin, 1984) was synthesized with the three perdeuterated methyl groups. Coupling with 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphatidic acid (**5**) following the procedure described for unlabeled **6** gave 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[$N(C^2H_3)_3$]choline (**9**) in 39% overall yield.

The synthesis of the deuterated DPPC analog, 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[α-C^2H_2] choline (**12**) (Browning and Akutsu, 1982; Gally et al., 1975), is shown in Scheme 3. [1-C^2H_2]Choline tetraphenylborate (**11**) (Harbison and Griffin, 1984) was first prepared from *N*,*N*-dimethylglycine ethyl ester (**10**) by lithium alu-

minum deuteride reduction of the ester group followed by quaternization of the amino group and precipitation of the product with sodium tetraphenylborate. 1,2-Di-*O*-palmitoyl-*sn*-glycero-3-phospho[α-C^2H_2]choline (**12**) was then prepared from the [1-C^2H_2]choline tetraphenylborate (**11**) and 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphatidic acid (**5**) following the procedure described for the unlabeled compound **6** in 50% overall yield.

Most of the possible head group ^{13}C and ^{2}H isotopically labeled analogs of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (**6**) have been synthesized in good overall yields, characterized, and are reported here in detail not previously published. This general methodology would certainly be appropriate for multiply isotopically labeled DPPC analogs. The solution NMR spectra of the phosphocholines in $CDCl_3/CD_3OD$ (2:1) were assigned based on previously reported data for DPPC and related phospholipids (Harbison and Griffin, 1984; Laube and Kurreck, 1983; Birdsall et al., 1972; Hauser et al., 1980; Burns and Roberts, 1980; Murari et al., 1982; Sparling et al., 1989; Basti and LaPlanche, 1990) and confirmed by our proton correlation spectroscopy and the inverse-heterocorrelation spectroscopy experiments with unlabeled DPPC (**6**). In the proton spectra of the labeled phosphocholines (Fig. 1), a $^1J_{CH}$ coupling of 145 Hz was observed for the α-protons of **6a** and for the β-protons of **6b**, a small $J_{CC} = 2$ Hz coupling to the choline methyls of **6b** was observed, a small peak for residual protons in the choline methyls was observed for **9**, and the β-methlyene was observed as a singlet for **12**. In the corresponding carbon spectra (Fig. 2), enrichment of the α-carbon with coupling to the β-carbon was observed for **6a** and enrichment of the β-carbon with coupling to the α-carbon was observed for **6b**. The choline methyls resonances were broadened and greatly attenuated in the ^{13}C spectrum of **9** and the α-carbon resonance was broadened and greatly attenuated in the ^{13}C spectrum of **12** due to coupling to deuterium and the absence of nuclear Overhauser enhancement. The definitive assignments of C1′ and C1″ in the carbon spectra were based on our unpublished syntheses of 1,2-di-*O*-acyl-*sn*-glycero-3-phosphocholines (PC) labeled in these positions. In addition, C2″ and C3″ have been reported (Burns and Roberts, 1980; Basti and LaPlanche, 1990) to resonate downfield from C2′ and C3′, respectively. The complex melting behavior of dried DPPC was recently detailed (Small, 1986). Hydrated multilamellar bilayers of these labeled analogs of DPPC **6a, 6b, 9** and **12,** which are model membranes, are currently being used for a variety of solid-state NMR experiments to probe membrane structure and function.

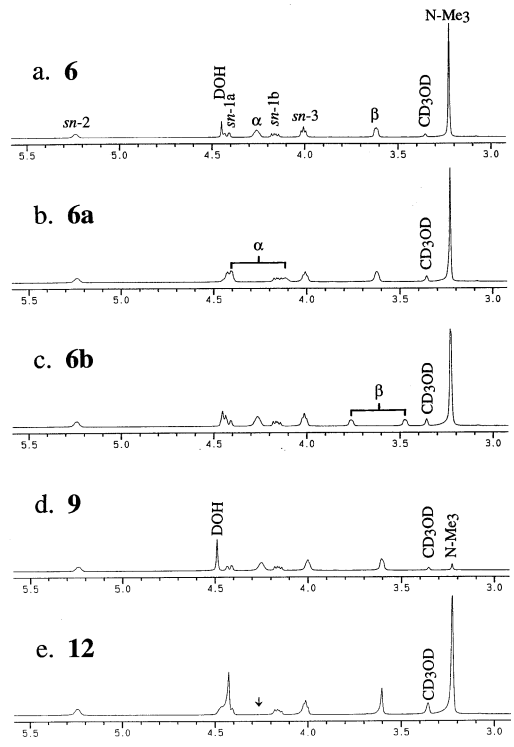


Fig. 1. 500-MHz ^1H NMR spectra in $CDCl_3/CD_3OD$ (2:1): (a) 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphocholine (**6**); (b) 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[α-^{13}C]choline (**6a**); (c) 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[β-^{13}C]choline (**6c**); (d) 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[$N(C^2H_3)_3$]choline (**9**); (e) 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[α-C^2H_2]choline (**12**).

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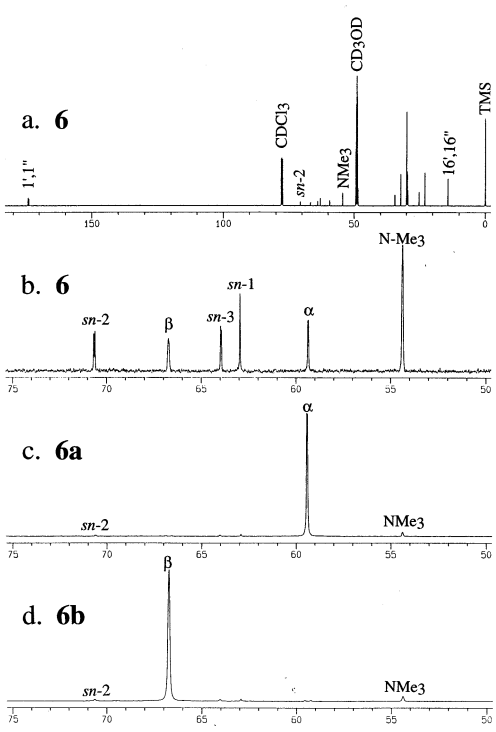

Fig. 2. 125-MHz ^{13}C NMR spectra in $CDCl_3/CD_3OD$ (2:1): (a,b) 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphocholine (**6**); (c) 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[α-^{13}C]choline (**6a**); (d) 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phospho[β-^{13}C]choline (**6c**).

3801). The FAB mass spectral and high-resolution FAB mass spectral analyses were performed by Catherine E. Costello at the Massachusetts Institute of Technology Mass Spectrometry Facility, which is currently moving to the Mass Spectrometry Resource in the Department of Biophysics at the Boston University School of Medicine.

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$C_{39}^{13}CH_{80}NO_8P \cdot H_2O$: C, 63.93; H, 10.98; N, 1.86. Found: C, 64.11; H, 11.07; N, 1.92.

2.13. 1,2-Di-O-palmitoyl-sn-glycero-3-phospho[β-^{13}C] choline (**6b**)

1,2-Di-*O*-palmitoyl-*sn*-glycero-3-phospho[β-^{13}C]choline (**6b**) was prepared from 400 mg (0.584 mmol) of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphatidic acid (**5**), 200 mg (1.64 mmol) of DMAP, 531 mg (1.75 mmol) of TPS, and 484 mg (1.14 mmol) of [2-^{13}C]choline tetraphenylborate (**4b**) following the procedure described for unlabeled **6** to give 350 mg (0.465 mmol, 80% yield) of **6b** as a white solid: TLC ($CHCl_3$/MeOH/H_2O (60:30:4)) R_f 0.20; ^{1}H NMR (500 MHz) ($CDCl_3$/CD_3OD (2:1)) δ 5.24 (m, 1 H, *sn*-2-CH), 4.42 (dd, 1 H, $J = 12$, 2.8 Hz, *sn*-1-CHa), 4.26 (m, 2 H, α-CH_2), 4.16 (dd, 1 H, $J = 12$, 7.0 Hz, *sn*-1-CHb), 4.01 (dd, 2 H, $J_{HH} = {}^3J_{HP} = 6$ Hz, *sn*-3-CH_2), 3.62 (dm, 2 H, $^1J_{HC} = 145$ Hz, β-CH_2), 3.23 (d, 9 H, $^3J_{HC} = 2.0$ Hz, $N(CH_3)_3$), 2.33 and 2.32 (t, 2 H, $J = 8.1$ Hz, and t, 2 H, $J = 8.1$ Hz; C2′H_2 and C2″H_2), 1.61 (m, 4 H, C3′H_2, C3″H_2), 1.27 (br s, 48 H, C4′H_2–C15′H_2, C4″H_2–C15″H_2), 0.89 (t, 6 H, $J = 6.8$ Hz, C16′H_3, C16″H_3); ^{13}C NMR (125 MHz) ($CDCl_3$/CD_3OD (2:1)) δ 174.26 (C1′), 173.89 (C1″), 70.63 (d, $^3J_{CP} = 7.6$ Hz, *sn*-2-C), 66.74 (br s, β-C), 64.03 (d, $J_{CP} = 5.5$ Hz, *sn*-3-C), 62.94 (*sn*-1-C), 59.41 (dd, $^1J_{CC} = 39$ Hz, $J_{CP} = 4$ Hz, α-C), 54.38 (N-Me_3), 34.52 and 34.37 (C2′, C2″), 32.21 (C14′, C14″), 29.40–30.00 (C4′–C13′ and C4″–C13″), 25.20 and 25.16 (C3′, C3″), 22.95 (C15′, C15″), 14.21 (C16′, C16″); mass spectrum m/z 735 (MH^+); HRMS calcd. for $C_{39}^{13}CH_{81}NO_8P$ (MH^+) m/z 735.5733, found m/z 735.5749. Anal. Calcd. for $C_{39}^{13}CH_{80}NO_8P \cdot H_2O$: C, 63.93; H, 10.98; N, 1.86. Found: C, 63.91; H, 10.79; N, 1.96.

2.14. [N(C^2H_3)$_3$]Choline tetraphenylborate (**8**)

To a magnetically stirred solution of 500 mg (8.19 mmol) of ethanolamine (**7**) and 1.60 g (40.0 mmol) of NaOH in 50 ml of 75% aqueous EtOH was added 5.90 g (40.7 mmol) of CD_3I at room temperature. After 10 h the solution was concentrated to a white gelatinous precipitate *in vacuo*. The precipitate was dissolved in 50 ml of H_2O and a solution of 5.60 g (16.4 mmol) of sodium tetraphenylborate in 100 ml of H_2O was added. A white precipitate formed immediately. The reaction mixture was stirred at ambient temperature for an additional 2 h. The precipitated solid was then collected by filtration, washed with H_2O (3×50 ml), and dried in a desiccator *in vacuo*. Crystallization from MeCN gave 1.70 g (3.93 mmol, 48% yield) of [N(C^2H_3)$_3$]choline tetraphenylborate (**8**) (Harbison and Griffin, 1984) as white prisms: TLC ($CHCl_3$/MeOH (70:30)) R_f 0.50; mp 205–207°C; ^{1}H NMR ($(D_3C)_2SO$) δ 7.05–7.25 (m, 8 H, Ar), 6.90–7.00 (m, 8 H, Ar), 6.80–6.85 (m, 4 H, Ar), 3.83 (m, 2 H, C1H_2), 3.37 (m, 2 H, C2H_2).

2.15. 1,2-Di-O-palmitoyl-sn-glycero-3-phospho[N(C^2H_3)$_3$]choline (**9**)

1,2-Di-*O*-palmitoyl-*sn*-glycero-3-phospho[N(C^2H_3)$_3$]choline (**9**) was prepared from 100 mg (0.146 mmol) of 1,2-di-*O*-palmitoyl-*sn*-glycero-3-phosphatidic acid (**5**), 35 mg (0.29 mmol) of DMAP, 133 mg (0.439 mmol) of TPS, and 125 mg (0.289 mmol) of [N(C^2H_3)$_3$]choline tetraphenylborate (**8**) following the procedure described for unlabeled **6** to give 90 mg (0.12 mmol, 82% yield) of **9** (Lee et al., 1989; Yang et al., 1992; Wood et al., 1976) as a white solid: TLC ($CHCl_3$/MeOH/H_2O (60:30:4)) R_f 0.20; ^{1}H NMR (500 MHz) ($CDCl_3$/CD_3OD (2:1)) δ 5.24 (m, 1 H, *sn*-2-CH), 4.42 (dd, 1 H, $J = 12$, 2.5 Hz, *sn*-1-CHa), 4.26 (m, 2 H, α-CH_2), 4.16 (dd, 1 H, $J = 12$, 7.0 Hz, *sn*-1-CHb), 4.00 (dd, 2 H, $J_{HH} = {}^3J_{HP} = 6$ Hz, *sn*-3-CH_2), 3.61 (m, 2 H, β-CH_2), 3.23 (s, 0.4 H, residual $NCHD_2(CD_3)_2$), 2.33 and 2.32 (t, 2 H, $J = 8.1$ Hz, and t, 2 H, $J = 8.1$ Hz; C2′H_2 and C2″H_2), 1.61 (m, 4 H, C3′H_2, C3″H_2), 1.27 (br s, 48 H, C4′H_2–C15′H_2, C4″H_2–C15″H_2), 0.89 (t, 6 H, $J = 6.8$ Hz, C16′H_3, C16″H_3); mass spectrum m/z 743 (MH^+); HRMS calcd. for $C_{40}H_{72}D_9NO_8P$ (MH^+) m/z 743.6265, found m/z 743.6260. Anal. Calcd. for $C_{40}H_{71}D_9NO_8P \cdot H_2O$: C, 63.13; H, 12.04; N, 1.84. Found: C, 63.01; H, 12.14; N, 1.97.