A General Synthetic Route for Preparing Ether Phospholipids Suitable for Immobilization: A Phosphotriester Approach

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A synthetic route was developed to prepare ether phospholipid (PL) ligands suitable for immobilization. PL ligand design included an ω-carboxyl functional group to assure proper molecular orientation during immobilization; i.e., the polar lipid head group protrudes from the surface. However, during immobilization, PL ligands required protecting groups to eliminate the possibility of the PL binding upside down. Four synthetic PL ligands were prepared that contain both ω-carboxyl groups for immobilization and protecting groups in the polar head group; these carboxyl-PL ligands are analogs of phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylglycerol (PG), and phosphatidic acid (PA). The critical synthetic step during PL synthesis is the phosphorylation step which usually has the lowest yield of all other steps. This is the first report demonstrating that *o*-chlorophenyl dichloro phosphate (CPDCP) can be used as a mild phosphorylation reagent for the preparation of PL analogs. Phosphorylation with CPDCP is routinely 50–90% efficient depending on the analog, but more important is that the protecting groups associated with PE, PS, PG, and PA are stable during this critical synthetic step. After immobilization of the carboxyl-PL ligands, acidic or basic solution conditions are needed for deprotection and generation of free PE, PG, PS, and PA polar lipid head groups which protrude from the surface. This work demonstrates that CPDCP is an excellent synthetic reagent for all ether PL analogs either with or without ω-carboxyl functional groups.

Introduction

Our laboratory is developing stable surfaces that emulate the lipid environment of artificial membranes. These surfaces denoted as immobilized artificial membranes (IAM), are a solid-phase membrane mimetic whereby cell membrane phospholipid (PL) molecules are covalently bonded to silica particles.[1-4] IAM surfaces have been used to predict the transport of solutes across human skin[5] and other biological barriers,[6] purify membrane proteins,[7-9] and stabilize the conformations of functional enzymes (e.g., trypsin, α-chymotrypsin, and lipase).[10-12] A most interesting and recent application of IAMs is their ability to predict pathophysiological effects of bile salts using the IAM membrane binding enthalpy of each bile salt.[13] In addition to these applications we have found IAM surfaces to be very useful for accelerating reactions between polar and nonpolar molecules, and this synthetic application of IAM is denoted as solid-phase adsorption synthesis. The solid-phase adsorption synthetic method, using IAM surfaces, has been used to prepare antiviral phospholipids.[14] We also note that the PL ligands discussed below required the use of the solid-phase adsorption synthetic method prior to bonding the ligands to silica. Thus, it is clear that many branches of surface chemistry require PL ligands that can be immobilized on mechanically stable solid surfaces.[3,4,15,16]

Naturally occurring endogenous phospholipids are not suitable for immobilization because their fatty acid chains contain nonreactive ω-methyl groups. In the preparation of PLs for immobilization only phosphatidylcholine (PC) analogs have been prepared because the PC head group does not contain reactive functional groups that require protection during immobilization. In contrast to PC ligands, phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylglycerol (PG), and phosphatidic acid (PA) ligands contain amines, carboxyls, hydroxyls, or phosphate groups that require protection prior to immobilization.

Our main objective is to bond PLs at approximately a monolayer density to any solid surface, and therefore, PL ligand design incorporated ideas that facilitate high surface coverage. Molecular design criteria for the PL analogs (Scheme 1) indicate that (i) an ω-carboxyl group is used for PL immobilization, (ii) a single chain is used to assure high bonding density,[17] (iii) ether linkages at the glycerol backbone increase the ligand stability during both syn-

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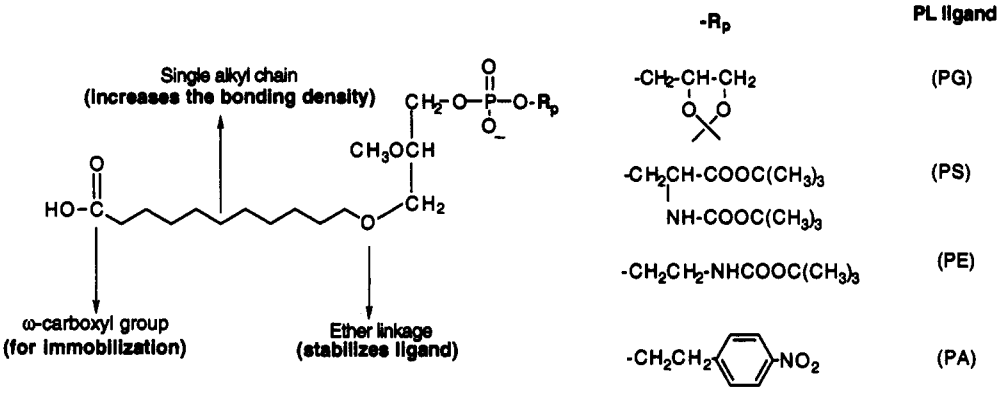
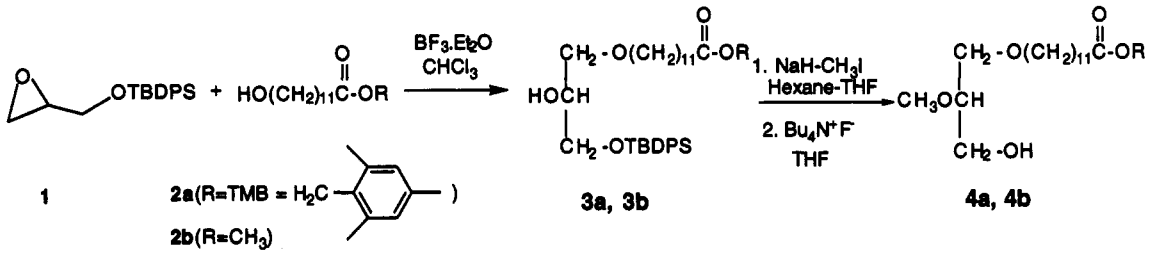
Scheme 1. Design Criteria for PL Ligands Suitable for Immobilization. Ligands Are Shown with Protecting Groups $-R_p$ in the Polar Head Group Region. After Immobilization Followed by Deprotection, These PL Ligands Have a Polar Head Group Corresponding to Endogenous Lipids (PG, PS, PE, or PA)



Scheme 2. Synthesis of Monoalkylglycerides 4a and 4b. The ω-Carboxyl for Compound 4a Contains a TMB Protecting Group and for Compound 4b a Methyl Protecting Group



thesis and after immobilization, and (iv) functional groups in the polar head groups are protected with either acid-labile (PE, PS, and PG analogs) or base-labile (PA analog) protecting groups. Although these PL ligands are designed for high ligand density after bonding to silica propylamine (SPA), these ligands are also suitable for bonding to many other chromatographic surfaces including soft gels and synthetic polymers.

We have found that the traditional $POCl_3$ synthetic route can be used to efficiently prepare zwitterionic PC ligands. However, during the phosphorylating step, this reagent causes significant loss of the PE, PG, PA, and PS protecting groups shown in Scheme 1 (Pidgeon, unpublished results). This encouraged us to identify a phosphorylating reagent that is chemically less destructive than $POCl_3$, and we have found that the bifunctional phosphorylating reagent *o*-chlorophenyl dichloro phosphate (CPDCP) works very well for phospholipid synthesis. CPDCP is an established reagent in nucleotide synthesis capable of reacting with two alcohols in a stepwise manner to form an unsymmetric phosphotriester.[18-20] The aryl group in CPDCP serves as a protecting group for the phosphorus alcohol; after triester formation the aryl protecting group can be selectively removed to generate the desired phosphodiester.[20,21] We have found that the protected alcohols needed to prepare PE, PA, PG, and PS analogs react virtually quantitatively with 1 equiv of CPDCP. In other words, only one of the chlorine atoms of CPDCP is substituted with the alcohol, and disubstitution to form the symmetric triester byproduct can easily be minimized if it occurs at all. More importantly, all protected polar lipid head groups shown in Scheme 1 are stable during the phosphorylation step; therefore, converting CPDCP into an arylphosphorodi(1,2,4-triazolide) or arylphosphorobis(hydroxybenzotriazole) (HOBT phosphate analog) is unneccessary.[22-25]

This facile reactivity of CPDCP to protected alcohols in the absence of HOBT allowed us to develop a general and efficient synthetic strategy for the preparation of PLs suitable for immobilization. The synthetic method is based on phosphotriester formation using a CPDCP. This is also the first report describing the immobilization of PE, PS, PG, and PA analogs on a solid matrix. Previous immobilizations with PC analogs containing -COOH, -OH, and -SH groups substituted for the ω-methyl have been reported.[1,2,4,17,26-29] Herein, we focus on the synthesis of the carboxyl-PL ligands themselves that can be directly immobilized on many different surfaces or polymers. Efficient bonding to SPA is verified by FTIR microscopy for all PL ligands, and the characterization of the surfaces will be published separately.

Results and Discussion

Scheme 2 shows the synthetic route to prepare the bifunctional monoalkylglycerides which are the precursors needed for the preparation of the ether PL ligands with

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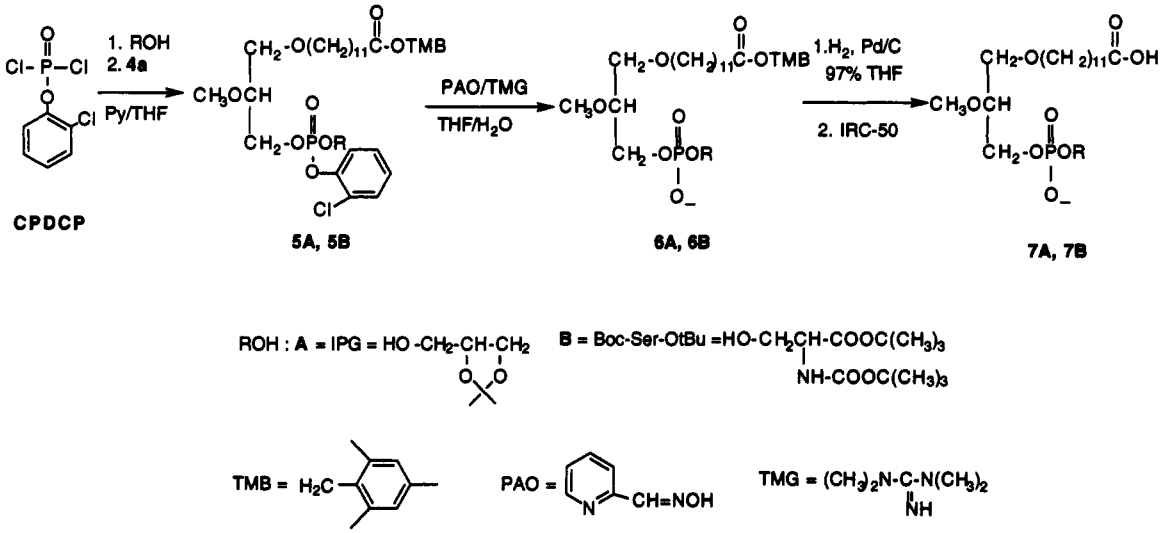
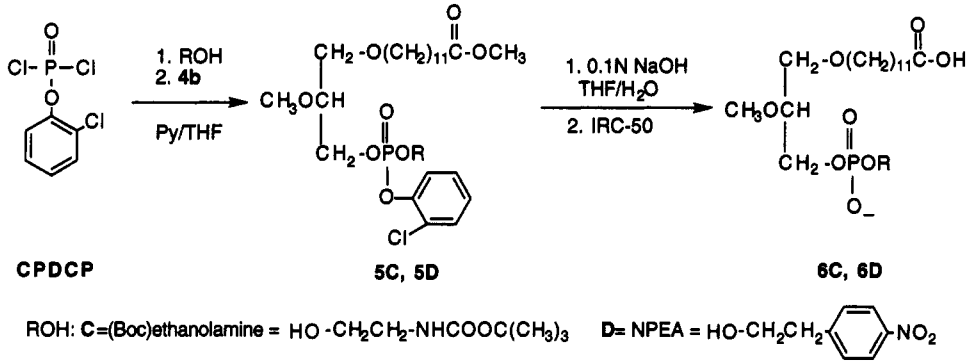
Scheme 3. Synthesis of PG (7A) and PS (7B) Analogs with a Free Carboxyl Group



Scheme 4. Synthesis of PE (6C) and PA (6D) Analogs Containing ω-Carboxyl Group in the Alkyl Chain



different head groups. The intrinsic molecular structure of most phospholipid molecules utilizes a three-carbon chiral glycerol bridge between the polar lipid head group and hydrocarbon chain(s). On the basis of Bittman's synthetic strategy,[30,31] a three-carbon chiral glycerol was incorporated into the PL(s) using chiral glycidol. The hydroxyl group of glycidol must first be protected, and we found that *tert*-butyldiphenylsilane (TBDPS) was the best protection group for glycidol because **1** is stable to the methylation conditions of the next step. The glycidol was reacted with *tert*-butylchlorodiphenylsilane at −50 °C to give **1** in 85–90% yield. This corresponds to an approximately 2-fold increase in product yield compared to similar reactions performed using a freezer to control the temperature.[32] Lower temperatures may prevent polymerization of the glycidol during reaction.

Fatty acid alcohols with the carboxyl protected with either a 2,4,6-trimethylbenzyl (TMB) (**2a**) or methyl (**2b**) group were also prepared in 85–95% yield. Epoxide ring opening of **1** by **2a** or **2b** was catalyzed by BF_3–etherate as described[30,31] to give **3a** and **3b**. This ring-opening reaction linked a fatty acid chain to the glycerol backbone through an ether bond. Earlier epoxide ring opening reactions of **1** utilized monofunctional alkanols that do not require protection groups.[30,31] Compounds **2a** and **2b** containing a methyl ester or a 2,4,6-trimethylbenzyl protecting group were stable during the epoxide ring opening of **1**; for **3b** both the yield (74%) and the ratio (90/10) of the desired regioisomer **3** (C_3 attack) to the undesired regioisomer (C_2 attack) are very similar to Bittman's results using monofunctional alkanols.[30] However, for **3a** both the yield (60%) and the regioisomer ratio (60/10) are less than **3b**, which may be due the relative larger TMB protecting goup.

A critical synthetic step for the efficient preparation of the target PL analogs was the stepwise methylation and then deprotection of **3a** and **3b** to form **4a** and **4b**, respectively. Although conversion of **3a** to **4a** and **3b** to **4b** was routinely performed in 60–75% yield, the key finding is that chromatography was not needed after methylation of the C2 carbon of **3a** and **3b** and the TBDPS could be directly removed (Scheme 2). Compound **4a** containing a TMB protecting group for the ω-carboxyl group was used to prepare PG and PS analogs (Scheme 3), whereas compound **4b** containing a methyl protecting group for the ω-carboxyl group was used to prepare PE and PA analogs (Scheme 4). The alcohols **4a** and **4b** are chemical intermediates that can be used to prepare many PLs in addition to the PLs shown in Scheme 1.

Scheme 3 shows that **4a** was linked to either (−)-2,3-isopropylideneglycerol (IPG) or *N*-(*tert*-butoxycarbonyl)-*O*-*tert*-butylserine [(HO)Boc-Ser-OtBu] via a phosphate bridge using CPDCP. Thus, **4a** was used to form the PG (**5A**) and PS (**5B**) phosphotriester analogs. The isopropylidene, *tert*-butyl ester, and Boc protecting groups R_p

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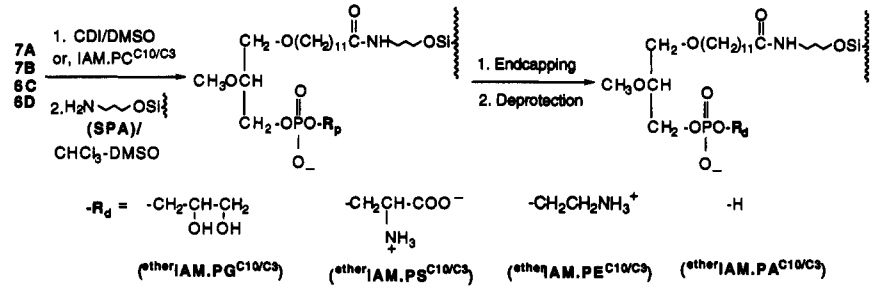
Scheme 5. Immobilization of PG (7A), PS (7B), PE (6C), and PA (6D) Ligands Followed by Endcapping and Deprotection of the Polar Lipid Head Groups[a]



SPA was used as the solid matrix for immobilization. Endcapping was performed in two steps, first with a long-chain alkyl anhydride (dodecanoic anhydride C10) and then a short-chain anhydride (proprioinc anhydride C3). Deprotection conditions for immobilized PG, PS, and PE utilized acidic solution conditions, whereas deprotection conditions for PA utilized 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU).[36]

were very stable during the phosphorylation step. Virtually no loss of these protecting groups was found while monitoring the reactions by TLC.

CPDCP contains two chlorides that are replaced stepwise with two different alcohols.[18,20] In general, the less expensive alcohol is used for the first chloride substitution reaction so that an excess (~2–4-fold) of the intermediate formed during the first phosphorylation step is present during the second substitution reaction. This permits very high product yields for the phosphorylation based on the more expensive alcohols, **4a** and **4b**. For instance, IPG was first phosphorylated with CPDCP followed by addition of **4a** to form the phosphotriester **5A**. The key condition for assuring high yields is to make sure that there is no unreacted CPDCP in the reaction mixture prior to the addition of the second alcohol. The *o*-chlorophenyl group was selectively removed from **5A** using PAO/TMG as described[21] to form **6A**. This deprotection step is virtually quantitative. Finally, the TMB protecting group was removed in ~90% yield by hydrogenolysis to form **7A** which is a PG analog suitable for immobilization with an ω-carboxyl group and protected polar head group. As shown in Scheme 3, the PS analog **7B** was prepared using the same strategy as that used to prepare the PG analog, except that the yield after deprotection of the *o*-chlorophenyl group of **5B** to generate **6B** was low (~40%). The main reason for the low yield was that a major side product, [1-*O*-[11-[[(2,4,6-trimethylbenzyl)oxy]carbonyl]-undecyl]-2-*O*-methyl-*sn*-glycero-3-phosphoryl]-*o*-chlorophenol, was generated because the Boc-Ser-OtBu was displaced instead of the *o*-chlorphenyl protecting group in **5B**. The side product is a PA analog that may be suitable for immobilization.

The synthesis of the PE (**6C**) and PA (**6D**) analogs from **4b** are shown in Scheme 4; initially, **4b** was linked to either *N*-Boc-ethanolamine or *p*-nitrophenethyl alcohols using CPDCP to form the phosphotriester compounds **5C** and **5D**, respectively. Subsequently, both the *o*-chlorophenyl and methyl protecting groups were removed in one step using NaOH to form **6C** and **6D**. This deprotection step did not cause any loss of the other protecting groups (i.e., the *N*-Boc or *p*-nitrophenethyl groups). Conversion of **4b** into **5C** was 47% efficient, and this reflects an inefficient use of the expensive synthetic alcohol **4b**. This low yield may be increased by using **4b** as the first alcohol that reacts with CPDCP instead of the second alcohol.

Bonding of PLs, **7A** (PG ligand), **7B** (PS ligand), **6C** (PE ligand), and **6D** (PA ligand) to SPA utilized carbonyldiimidazole (CDI) to activate the carboxyl groups.[1] All of these PLs have very poor solubility in chloroform, tetrahydrofuran (THF), and several other organic solvents, and therefore activation of the ω-carboxyl required either solid-phase adsorption synthesis[14] or dimethyl sulfoxide (DMSO). The solid-phase adsorption method obviates the need to use DMSO, which must be distilled immediately prior to use. The bonding density based on IR analysis[33–36] for each IAM surface is ~70 mg of PL ligand/g of silica. After deprotection (i.e., the coversion of R_p to R_d in Scheme 5), all IAMs were spontaneously wet with water, indicating that monolayer PL coverage occurred during bonding and that the polar head groups are confluent on the surface. After bonding and endcapping, all IAM surfaces were completely negative or slightly positive to ninhydrin analysis, indicating that very little residual surface amines exist on the IAM surface. However, after deprotection of the immobilized ligands, IAM·PS and IAM·PE surfaces are ninhydrin positive as expected because of the free amines generated on the surface of the IAM surface. The bonding chemistry and surface chemistry for the IAMs shown in Scheme 5 will be described in detail in a separate publication. The main purpose of this report is to demonstrate the synthetic versatility of CPDCP in the preparation of PL carboxyl ligands suitable for immobilization.

Experimental Section

General Procedures. $(Et)_3N$ and alcohol-free $CHCl_3$ were distilled over CaH_2. Ethyl acetate (EtOAc) was first dried over anhyd K_2CO_3 and then distilled over P_2O_5. Pyridine was dried by refluxing with CaH_2 for 16 h and then redistilled from *p*-tolunesulfonyl chloride (from Aldrich). THF was distilled over sodium. All other solvents were used as received. Toluene and HPLC-grade hexane were purchased from Fischer Scientific (Fair Lawn, NJ). HPLC-grade CH_3OH and $(Et)_3N$ and spectral-grade EtOAc were purchased from Mallinckrodt (Paris, KY). Spectral-grade C_6H_6 was purchased from J.T. Baker (Phillipsburg, NJ). Glycidol, *tert*-butylchlorodiphenylsilane (BDPS), 12-hydroxydodecanoic acid, 2,4,6-trimethylbenzyl chloride (TMB-Cl), NaH (60% oil dispersion), CH_3I, tetrabutylammonium fluoride (TBAF) 1 M in THF, *N*-(*tert*-butoxycarbonyl)ethanolamine, CDI, trifluoroacetic acid (TFA), anhyd $CHCl_3$, anhyd CH_2Cl_2, anhyd pyridine, CPDCP, NPEA, 2-pyridinealdoxime (PAO), 1,1,3,3-tetramethylguanidine (TMG), decanoic anhydride, propionic

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anhydride, 10% Pd/C and IRC-50 weakly acidic cation exchanger were purchased from Aldrich Chemical Co. (Milwaukee, WI). Boron trifluoride etherate (BF_3·Et_2O) and IPG were purchased from Sigma Chemical Co. (St. Louis, MO). Boc-Ser-OtBu was purchased from Bachem (Feinchemikalien AG, Switzerland). All alcohols used for phosphorylation were lyophilized from benzene and then further dried under high vacuo overnight at either rt or ~45 °C.

All reactions were performed using flame-dried flasks sealed under a nitrogen atmosphere and protected from light unless otherwise noted. All solid reactants were dried in a 40 °C vacuum oven for at least 4 h (but usually overnight). Residual water from compounds **1**, **2a**, **2b**, etc. was removed by a benzene/water azeotrope prior to further reaction of each compound. In general, extracted organic layers were pooled and dried over anhyd Na_2SO_4, and then the organic solvents were removed with a rotary evaporator. Silica gel 60 (230–400 mesh) from E. Merck was used for flash chromatography. Flash chromatography used fresh silica gel for every purification, and columns were 5 × 50 cm or 6 × 85 cm. Fractions containing the final products were usually dried by rotoevaporation and high vacuum. IAM·$PC^{C10/C3}$ used for solid-phase adsorption synthesis was purchased from Regis Chemical Co. IAM·$PC^{C10/C3}$ is a stable IAM surface that exhibits little or no phospholipid leaching when challenged with organic solvents.[35] SPA (12 μm, 300 Å, 107 m^2/g) was purchased from Regis Chemical Co.

Reactions were monitored on 0.25-mm-thick silica gel 60 F254 thin-layer chromatography (TLC) plates purchased from E. Merck (Darmstadt, FGR). Short-wavelength ultraviolet light, 50% H_2SO_4 in water, Phospray, and ninhydrin (Supelco Inc.) were routinely used to detect chemical intermediates on the TLC plates. ^{1}H-NMR spectra were obtained on a Varian VXR 500 spectrometer. Infrared spectra were recorded on a Nicolet Magna-IR spectrometer. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter.

Oxiranemethanol *tert*-Butyldiphenylsilyl Ether (1). The protected glycidol 1 was prepared from BDPS and racemic or chiral glycidol.[31,32] (Dimethylamino)pyridine (1.10 g, 0.009 mol), 350 mL of anhyd CH_2Cl_2, and 26.5 mL (0.190 mol) of distilled $(Et)_3N$ were cooled to −50 °C by a propylene glycol/dry ice bath. The temperature was maintained at −50 °C for the remainder of the reaction. Cold (5 °C) glycidol (15.05 mL, 13.48 g, 0.18 mol) was added, and cold (5 °C) BDPS (50.00 g, 50 mL, 0.18 mol) was slowly added over 15 min. After being stirred at −50 °C for 4 h, the reaction mixture was allowed to warm to rt, filtered, and then washed with additional CH_2Cl_2. The filtrate was washed with 10% NH_4Cl (200 mL × 2) followed by 10% NaCl (200 mL × 1) and dried by rotoevaporation and high vacuum to yield a viscous oil (60 g). The resulting oil was purified by flash chromatography using an isocratic mobile phase containing 2% EtOAc/hexane. Epoxide 1 47.70 g (85%) was a colorless viscous oil: TLC (EtOAc/hexane (1:5)) R_f 0.82; $[\alpha]_D$ +0.8°, −0.8° for the *S* and *R* enantiomers, respectively; FTIR (CaF_2, neat) 3050.1, 2998.6, 2958.6, 2857.9, 1617.5, 1589.3, 1472.3, 1463.4, 1427.9, 1390.5, 1254.4, 1112.7 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.67–7.71 (m, 4 H), 7.35–7.45 (m, 6 H), 3.84 (dd, 1 H, *J* = 3.2, 11.8 Hz), 3.68 (dd, 1 H, *J* = 4.7, 11.8 Hz), 3.13 (m, 1 H), 2.73 (dd, 1 H, *J* = 4.0, 5.2 Hz), 2.60 (dd, 1 H, *J* = 2.5, 5.2 Hz), 1.07 (s, 9 H); CI-MS *m/z* 313 (MH^+).

2,4,6-Trimethylbenzyl 12-Hydroxydodecanoate (2a). A solution of 48.47 g (0.224 mol) of 12-hydroxydodecanoic acid, 75.2 g (0.448 mol) of TMB-Cl, and 62 mL of distilled $(Et)_3N$ (0.4481 mol) in 500 mL of distilled anhyd EtOAc was refluxed under N_2 for 12 h. The reaction mixture was cooled to rt and the precipitate filtered through an M suction filtration funnel. The filtrate was washed with 1.2 N HCl (200 mL × 2), H_2O (200 mL), 5% $NaHCO_3$ (200 mL × 2), and 10% NaCl (200 mL) and dried by rotoevaporation to obtain 110.8 g of a yellowish solid. The 110.8-g reaction mixture was dissolved in 125 mL of EtOAc and then mixed with 265 mL of silica gel (Mesh 80–200). This mixture was dried at high vacuum overnight and loaded on top of the silica gel column and the product eluted with a gradient of 10% (2.5 L), 20% (2.5 L), and 30% (4 L) EtOAc/hexane to obtain 74.8 g (84% yield) of a slightly yellowish solid: TLC (EtOAc/hexane (1:1)) R_f 0.65; FTIR (CaF_2, neat) 3396.1, 2931.8, 2857.2, 1739.5, 1616.6, 1379.7, 1483.3, 1464.8, 1170.5 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 6.86 (s, 2 H), 5.13 (s, 2 H), 3.61 (t, 2 H, *J* = 6.9 Hz), 2.32 (s, 6 H), 2.27 (t, 2 H, *J* = 7.9 Hz), 2.25 (s, 3 H), 1.59 (m, 2 H), 1.54 (m, 2 H), 1.24 (br s, 14 H).

Methyl 12-Hydroxydodecanoate (2b). A solution of 40.10 g (0.1853 mol) of 12-hydroxydodecanoic acid and 25 mL of 1 M HCl solution in 3 L of MeOH was stirred at rt for 12 h and then was neutralized with NaOH to pH 7.0. After removal of MeOH, the residue was dissolved in 200 mL of EtOAc and extracted with 5% $NaHCO_3$ (200 mL). The solvent was dried to obtain 42.41 g for a 99% conversion of 12-hydroxydodecanoic acid to the methyl ester: TLC (EtOAc/hexane (1:1)) R_f 0.6; FTIR (CaF_2, neat) 3396.2, 2932.5, 2857.9, 1743.9, 1463.0, 1370.8, 1173.6 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 3.62 (s, 3 H), 3.58 (t, 2 H, *J* = 7 Hz), 2.25 (t, 2 H, *J* = 8 Hz), 1.57 (m, 2 H), 1.51 (m, 2 H), 1.24 (br s, 14 H).

1-*O*-[11-[[(2,4,6-Trimethylbenzyl)oxy]carbonyl]undecyl]-3-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (3a). A mixture of a catalytic amount of BF_3·Et_2O (300 μL, 0.0021 mol), 38.48 g (0.1232 mol) of 1 and 53.64 g (0.2332 mol) of **2a** in 500 mL of distilled $CHCl_3$ was stirred at rt for 6 h. A 19.32-g (0.062 mol) portion of 1 and 200 μL (0.014 mol) of BF_3·Et_2O were added, and the reaction mixture was stirred at rt overnight. After removal of $CHCl_3$, the residue was pumped at high vacuum at 45 °C to obtain the crude product which was a yellow oil (~120 g). The crude product was purified in 60-g aliquots by silica gel flash chromatography with a gradient mobile phase containing 8% (4 L), 13% (4 L), and 50% (2 L) EtOAc/hexane. Chromatography was repeated with the pooled fractions contaminated with side product to obtain 59.4 g (60.6%) of oily product **3a** and 10.3 g (10.5%) of the regioisomer (C_2 attack): TLC (EtOAc/hexane (1:3)) R_f 0.65 for **3a** and 0.60 for the regioisomer; FTIR (CaF_2, neat) 3495.3, 3073.9, 3051.6, 2930.3, 2858.9, 1734.2, 1617.7, 1589.7, 1390.0, 1464.9, 1115.6 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.64 (dd, 4 H, *J* = 8.0, 1.6 Hz), 7.36–7.41 (m, 6 H), 6.85 (s, 2 H), 5.13 (s, 2 H), 3.86 (m, 1 H), 3.68 (d, 2 H, *J* = 5 Hz), 3.50–3.44 (m, 2 H), 3.41 (m, 3 H), 2.48 (d, 1 H, *J* = 5.0 Hz), 2.32 (s, 6 H), 2.27 (t, 2 H, *J* = 7.5 Hz), 2.25 (s, 3 H), 1.59 (m, 2 H), 1.52 (m, 2 H), 1.23 (br s, 14 H), 1.04 (s, 9 H).

1-*O*-[11-(Methoxycarbonyl)undecyl]-3-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (3b). Compound **3b** was prepared similarly to **3a**. Thus, 53.64 g (0.2332 mol) of **2b** was converted to **3b** and purified by chromatography on a 6- × 85-cm glass column packed with 1.700 L of silica gel on 8% EtOAc/hexane. Sample loading utilized 60 g of crude product dissolved in 40 mL of the mobile phase. The eluting gradient consisted of 8% (4 L), 13% (4 L), and 50% (2 L) EtOAc/hexane. Chromatography was repeated for fractions contaminated with side products. After chromatography 71.98 g (71.7%) of the oily product **3b** and 7.49 g (7.5%) of the regioisomer (C_2 attack) were obtained: TLC (EtOAc/hexane (1:3)) R_f 0.60 for **3b** and 0.55 for the regioisomer; FTIR (CaF_2, neat) 3492.4, 3072.4, 3050.7, 2928.9, 2856.9, 1740.6, 1427.3, 1472.7, 1467.1, 1113.9 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.64 (dd, 4 H, *J* = 8.0, 1.6 Hz), 7.36–7.41 (m, 6 H), 3.86 (m, 1 H), 3.68 (d, 2 H, *J* = 5 Hz), 3.64 (s, 3 H), 3.50–3.44 (m, 2 H), 3.41 (m, 3 H), 2.48 (br s , 1 H), 2.28 (t, 2 H, *J* = 7.5 Hz), 1.59 (q, 2 H, *J* = 7.0 Hz), 1.52 (q, 2 H, *J* = 7.0 Hz), 1.23 (br s, 14 H), 1.04 (s, 9 H).

1-*O*-[11-[[(2,4,6-Trimethylbenzyl)oxy]carbonyl]undecyl]-2-*O*-methyl-*sn*-glycerol (4a). A 2.27-g (0.0568 mol) portion of NaH was added to 300 mL of HPLC-grade hexane under N_2. After H_2 gas production stopped (~15 min), 30 mL of CH_3I (0.4820 mol) was added, and after 5 min, the methylation was initiated by the addition of 9.33 g (0.01412 mol) of compound **3a** dissolved in 30 mL of anhyd THF. The reaction was stirred for 28 h and filtered by suction through Whatman no. 1 filter paper and the reaction solvent evaporated to yield 10.16 g of crude methylated product. The 10.16 g (0.0150 mol) of crude methylated product was dissolved in 200 mL of THF, and then 30 mL of TBAF was added. After 30–45 min, complete deprotection of the BDPS was observed based on TLC, and the reaction was quenched with 125 mL of H_2O. THF was removed by rotoevaporation, and then the H_2O phase was extracted with Et_2O (200 mL × 2). The Et_2O phase containing the product was rotoevaporated to obtain 10.59 g of a yellowish oil. This synthetic procedure was repeated four times to obtain 42.98 g of crude product which was purified by silica gel chromatography. Purification was performed on 10.65-g aliquots of the crude reaction mixture loaded on a 5- × 40-cm glass column packed

with silica and eluted with a step gradient of 10% (1.2 L), 25% (1 L), and 40% (1.7 L) EtOAc/hexane. After chromatography, 5.05 g of **4a** was obtained as a clear oil representing a 72.6% yield based on compound **3a**: TLC (EtOAc/hexane (1:1)) R_f 0.45 for **4a** and 0.8 for the methylated intermediate; FTIR (CaF_2, neat) 3458.1, 2930.3, 2859.0, 1733.7, 1616.8, 1383.1, 1466.2, 1117.9 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 6.85 (s, 2 H), 5.13 (s, 2 H), 3.74 (dd, 1 H, J = 11.5, 2 Hz), 3.62 (dd, 1 H, J = 11.5, 8.0 Hz), 3.55–3.49 (m, 2 H), 3.45 (s, 3 H), 3.42 (m, 3 H), 2.32 (s, 6 H), 2.27 (t, 2 H, J = 7.5 Hz), 2.25 (s, 3 H), 1.59 (m, 2 H), 1.52 (m, 2 H), 1.22 (br s, 14 H).

1-*O*-[11-(Methoxycarbonyl)undecyl]-2-*O*-methyl-*sn*-glycerol (4b). Conversion of **3b** to **4b** was facile and efficient similar to the conversion of **3a** to **4a**. Thus, 22.27 g (0.041 05 mol) of **3b** was methylated using 6.6 g of NaH and 75 mL of CH_3I, and the tBDPS was deprotected with 90 mL of TBAF dissolved in 450 mL of THF. **4b** was purified by silica gel flash chromatography using the same EtOAc/hexane gradient used to purify **4a** except that a large silica gel column (6 × 85 cm) was used to purify **4b** in a single step to give 7.038 g (56.5% based on **3b**) of **4b** as a white solid: TLC (EtOAc/hexane (1:1)) R_f 0.45 for **4b** and 0.8 for the methylated intermediate; the configuration of **4b** is *R* and $[\alpha]_D$ −2.90°; FTIR (CaF_2, neat) 3465.6, 2927.3, 2854.8, 1740.4, 1462.5, 1438.2, 1361.6, 1251.2, 1195.8, 1117.6 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 3.73 (dd, 1 H, J = 4.2, 11.6 Hz), 3.64 (s, 3 H), 3.63 (dd, 1 H, J = 5.4, 11.6 Hz), 3.47–3.54 (m, 2 H), 3.43 (s, 3 H), 3.40 (m, 3 H), 2.27 (t, 2 H, J = 7.5 Hz), 2.06 (br s, 1 H), 1.53–1.58 (m, 4 H), 1.25 (br s, 14 H).

1-*O*-[11-[[(2,4,6-Trimethylbenzyl)oxy]carbonyl]undecyl]-2-*O*-methyl-*sn*-3-glyceryl *o*-Chlorophenyl 2′,3′-Isopropylidene-*sn*-glyceryl Phosphate (5A). To a stirring solution of CPDCP (2.70 g, 0.0110 mol) and distilled pyridine (5 mL, 5.11 g, 0.065 mol) in 5 mL of distilled THF was slowly added IPG (1.458 g, 0.0110 mol) in 15 mL of distilled THF over 30 min to initiate the phosphorylation of the first alcohol. The reaction was complete in about 1 h. The supernatant of the reaction mixture was then transferred under N_2 to a flask containing 1.010 g of **4a** (0.002 316 mol). The phosphate triester formation was complete within 4 h. The reaction was then quenched with 150 μL of water (0.15 g, 0.0083 mol) at rt for 30 min. The reaction mixture was filtered to remove pyridine hydrochloride precipitate and then rotoevaporated to remove THF. To the residue was added 40 mL of water, which was then extracted with Et_2O (40 mL × 4). The product **5A** was in the Et_2O layer, which was dried by rotoevaporation and purified by flash chromatography using 50% EtOAc/hexane to give 1.560 g (91.0% based on **4a**) of **5A** as a pale yellow oil. When this reaction was scaled up using 4 g of **4a**, the yield of **5A** was 90%: TLC (EtOAc/hexane (1:1)) R_f 0.55; $[\alpha]_D$ −1.67°; FTIR (CaF_2, neat) 2986.6, 2929.6, 2856.5, 1733.8, 1615.5, 1460.0, 1380.8, 1371.9 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.45 (d, 1 H, J = 8.0 Hz), 7.39 (d, 1 H, J = 8.0 Hz), 7.22 (t, 1 H, J = 8.0 Hz), 7.10 (t, 1 H, J = 8.0 Hz), 6.86 (s, 2 H), 5.13 (s, 2 H), 4.17–4.35 (m, 4 H), 4.12–4.15 (m, 1 H), 4.05 (m, 1 H), 3.82 (m, 1 H), 3.54 (br s, 1 H), 3.47 (d, 2 H, J = 5 Hz), 3.42 (s, 3 H), 3.38 (t, 2 H, J = 7 Hz), 2.31 (s, 6 H), 2.27 (t, 2 H, J = 8.0 Hz), 2.25 (s, 3 H), 1.59 (m, 2 H), 1.52 (m, 2 H), 1.39 (s, 3 H), 1.33 (s, 3 H), 1.23 (br s, 14 H).

1-*O*-[11-[[(2,4,6-Trimethylbenzyl)oxy]carbonyl]undecyl]-2-*O*-methyl-*sn*-3-glyceryl *o*-Chlorophenyl *N*-(*tert*-Butoxycarbonyl)serine *tert*-Butyl Ester Phosphate (5B). To a solution of CPDCP (0.5 g, 0.002 04 mol) and distilled pyridine (1.01 g, 0.013 mol) in 5 mL of distilled THF and was slowly added (Boc)Ser(OtBu) (0.552 g, 0.002 11 mol) in 6 mL of distilled THF over 30 min. The reaction was complete after 4 h. The supernatant of the reaction mixture was then transferred under N_2 to a flask containing 0.39 g of **4a** (0.000 89 mol). The phosphate triester formation was complete within 18 h. The reaction was then quenched by the addition of 20 μL of water (0.020 g, 0.0011 mol) at rt for 30 min. The reaction mixture was filtered to remove the pyridine hydrochloride precipitate. After removal of THF, 20 mL of water was added to residue. This aqueous phase containing the product was extracted with Et_2O (20 mL × 4). The pooled Et_2O phases containing **5B** were dried by rotoevaporation and purified by flash chromatography using a step gradient of 10% (0.1 L), 20% (0.1 L), 30% (0.2 L), 35% (0.2 L), and 40% (0.2 L) EtOAc/hexane to give 0.70 g (90.0% based on **4a**) of **5B** as a pale yellow oil: TLC (EtOAc/hexane (1:1)) R_f 0.70 for **5B** and 0.50 for **4a**. When this reaction was scaled using 10 g of **4a**, the yield of **5A** was 93%: FTIR (CaF_2, neat) 2978.2, 2928.2, 2854.4, 1729, 1617.5, 1460, 1394.2, 1369.2 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.38–7.43 (m, 2 H), 7.20 (t, 1 H, J = 8.0 Hz), 7.10 (t, 1 H, J = 8.0 Hz), 6.86 (s, 2 H), 5.40 (d, 1 H, J = 8.5 Hz), 5.13 (s, 2 H), 4.54 (m, 1 H), 4.41 (m, 2 H), 4.31 (m, 1 H), 4.18 (m, 1 H), 3.50 (m, 1 H), 3.46 (m, 2 H), 3.41 (s, 3 H), 3.37 (t, 2 H, J = 7 Hz), 2.31 (s, 6 H), 2.27 (t, 2 H, J = 8.0 Hz), 2.25 (s, 3 H), 1.59 (m, 2 H), 1.52 (m, 2 H), 1.44 (s, 9 H), 1.42 (s, 9 H), 1.23 (br s, 14 H).

1-*O*-[11-(Methoxycarbonyl)undecyl]-2-*O*-methyl-*sn*-3-glyceryl *o*-Chlorophenyl [*N*-(*tert*-Butoxycarbonyl)amino]ethyl Phosphate (5C). To a solution of CPDCP (15.6g, 0.0635 mol) and pyridine (40 mL, 0.5 mol) in 60 mL of THF was slowly added a solution of N-BOC-ethanolamine (12.29 g, 0.076 mol) in 60 mL of THF over 1 h. After the solution was stirred for another 6 h, the supernatant of the reaction mixture was transferred under N_2 to a solution of **4b** (7.36 g, 0.0229 mol) in 50 mL of the THF. After being stirred for 36 h, the reaction was quenched with 0.4 mL of water for 1 h. Then water (40 mL) was introduced to the reaction mixture followed by rotoevaporation to remove most of the THF. The residual aqueous phase was extracted with Et_2O (100 mL × 3). The Et_2O layers were concentrated, and the residue was dissolved in THF/H_2O (9:1) and passed through an AG501-X8D column using THF/H_2O (9:1). The eluants were concentrated and purified by flash chromatography using a step gradient of 10% (0.2 L), 20% (0.5 L), 30% (1.5 L), 40% (2 L), and then 50% (1 L) EtOAc/hexane, and **5C** (7 g, 47% yield based on **4b**) was obtained as a yellow oil: TLC (EtOAc/hexane (1:1)) R_f 0.35 FTIR (CaF_2, neat) 3346.5, 2978.7, 2930.4, 2857.6, 1736.8, 1716.0, 1586.5, 1482.9, 1519.2, 1175.5, 1236.0, 1118.5 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.38–7.41 (m, 2 H), 7.22 (t, 1 H, J = 8.0 Hz), 7.11 (t, 1 H, J = 8.0 Hz), 4.20–4.40 (m, 4 H), 3.72 (m, 2 H), 3.64 (s, 3 H), 3.54 (m, 1 H), 3.48 (m, 2 H), 3.42 (d, 3 H), 3.39 (m, 2 H), 2.28 (t, 2 H, J = 8.0 Hz), 1.83 (m, 2 H), 1.41 (br s, 9 H), 1.23 (br s, 14 H).

1-*O*-[11-(Methoxycarbonyl)undecyl]-2-*O*-methyl-*sn*-3-glyceryl *o*-Chlorophenyl (*p*-Nitrophenyl)ethyl Phosphate (5D). To a stirred solution of CPDCP (8.92 g, 0.035 98 mol) and distilled pyridine (20 mL, 0.2473 mol) in 50 mL of dry THF was added a solution of NPEA (7.988 g, 0.047 68 mol) in 50 mL of dry THF over 1 h. Stirring was continued for 2 h at rt until the reaction was complete based on TLC ($CHCl_3$/MeOH/H_2O (65:25:4)). The reaction supernatant was then transferred under N_2 to a solution of **4b** (5.30 g, 0.001 67 mol) in 20 mL of dry THF. The reaction mixture was stirred at rt for 30 h and then quenched with water (0.5 g, 0.027 mol). After the precipitate of pyridine hydrochloride was removed by filtration, the solvent was removed by rotoevaporation. The residue was dissolved in 200 mL of Et_2O, and 150 mL of 1 N HCl was added to the Et_2O solution. The Et_2O layer was separated, and the aqueous layer was extracted with Et_2O (200 mL × 2). The Et_2O layers were dried to give a oily yellow residue that was purified by flash chromatography using an isocratic mobile phase containing 50% EtOAc/hexane to give 8.0 g (74% based on **4b**) of **5D** as a yellow oil: TLC (EtOAc/hexane (1:1)) R_f 0.40; FTIR (CaF_2, neat) 2929.6, 2856.4, 1737.1, 1522.1, 1482.5, 1348.5 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 8.10 (d, 2 H, J = 8.5 Hz), 7.34 (d, 1 H, J = 8.0 Hz), 7.32 (d, 2 H, J = 8.5 Hz), 7.31 (d, 1 H, J = 8.0 Hz), 7.16 (t, 1 H, J = 8.0 Hz), 7.08 (t, 1 H, J = 8.0 Hz), 4.43 (q, 2 H, J = 6.5 Hz), 4.11–4.31 (m, 2 H), 3.64 (s, 3 H), 3.50 (m, 1 H), 3.44 (d, 2 H, J = 8.0 Hz), 3.40 (s, 3 H), 3.37 (t, 2 H, J = 7.0 Hz), 3.09 (t, 2 H, J = 6.5 Hz), 2.27 (t, J = 7.5 Hz), 1.48–1.61 (m, 4 H), 1.22 (br s, 14 H).

O-[1-*O*-[11-[[(2,4,6-Trimethylbenzyl)oxy]carbonyl]undecyl]-2-*O*-methyl-*sn*-glycero-3-phosphoryl]-2,3-isopropylidene-*sn*-glycerol (6A). A solution of **5A** (1.560 g, 0.002 11 mol), PAO (1.545 g, 0.012565 mol) and TMG (1.455 g, 012565 mol) in 240 mL of THF and 160 mL of water was stirred at rt for 20 h. After removal of THF, 160 mL of MeOH was added to the aqueous solution containing **6A**, which was then extracted with $CHCl_3$ (160 mL × 6). The $CHCl_3$ layer containing **6A** was dried and purified by flash chromatography using a gradient mobile phase of 0% (0.1 L), 10% (0.44 L), 20% (0.12 L), 30% (0.13 L), 40% (0.56 L), and 40% (0.3 L) MeOH in EtOAc/hexane (1:1) to give 1.200 g (90.5% based on **5A**) of **6A** as a pale yellow oil: TLC ($CHCl_3$/MeOH/H_2O (65:25:4)) R_f 0.45; FTIR (CaF_2,

neat) 2986.6, 2929.6, 2856.5, 1733.8, 1615.5, 1456.6, 1380.8, 1371.9 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 6.86 (s, 2 H), 5.13 (s, 2 H), 4.31 (m, 1 H), 3.80–4.05 (m, 6 H), 3.45–3.56 (m, 3 H), 3.41 (s, 3 H), 3.37 (t, 2 H, J = 7.0 Hz), 2.30 (s, 6 H), 2.27 (t, 2 H, J = 8.0 Hz), 2.25 (s, 3 H), 1.59 (m, 2 H), 1.52 (m, 2 H), 1.39 (s, 3 H), 1.33 (s, 3 H), 1.23 (br s, 14 H).

***O*-[1-*O*-[11-[[(2,4,6-Trimethylbenzyl)oxy]carbonyl]undecyl]-2-*O*-methyl-*sn*-glycero-3-phosphoryl]-*N*-(*tert*-butoxycarbonyl)serine *tert*-Butyl Ester (6B).** A solution of **5B** (0.700 g, 0.000 806 mol), PAO (0.590 g, 0.004 83 mol), and TMG (0.556 g, 0.004 83 mol) in 240 mL of THF and 160 mL of water was stirred at rt for 20 h. After removal of THF, 160 mL of MeOH was added to the aqueous solution containing **6B**, which was then extracted with $CHCl_3$ (60 mL × 6). The $CHCl_3$ layer containing **6B** was dried and purified by flash chromatography using the same gradient mobile phase used to purify **6A** to give 0.23 g (37.8% based on **5B**) of **6B** as a pale yellow oil and 0.18 g (36.8% based on **5B**) of the side product,[[1-*O*-[11-[[(2,4,6-trimethylbenzyl)oxy]carbonyl]undecyl]-2-*O*-methyl-*sn*-glycero]-3-phosphoryl]-*o*-chlorophenol: TLC ($CHCl_3$/MeOH/H_2O (65:25:4)) R_f 0.7 for **6B** and 0.65 for the side product; FTIR (CaF_2, neat) 2978.2, 2928.2, 2854.4, 1729, 1617.5, 1460, 1394.2, 1369.2 cm^{-1}; ^1H-NMR ($CDCl_3$) δ 6.85 (s, 2 H), 5.13 (s, 2 H), 4.26 (m, 1 H), 4.12 (m, 2 H), 3.95 (m, 1 H), 3.82 (m, 1 H), 3.50 (m, 1 H), 3.46 (m, 2 H), 3.44 (s, 3 H), 3.37 (t, 2 H, J = 7 Hz), 2.31 (s, 6 H), 2.27 (t, 2 H, J = 8.0 Hz), 2.25 (s, 3 H), 1.59 (m, 2 H), 1.52 (m, 2 H), 1.43 (s, 9 H), 1.41 (s, 9 H), 1.23 (br s, 14 H).

***O*-[1-*O*-(11-Carboxyundecyl)-2-*O*-methyl-*sn*-glycero-3-phosphoryl]-*N*-(*tert*-butoxycarbonyl)ethanolamine (6C).** A solution of **5C** (7 g, 0.0107 mol), 0.1 N NaOH (320 mL), and 480 mL of THF was stirred at rt for 16 h followed by concentration to remove most of the THF, leaving ~250 mL of H_2O. This aqueous phase was extracted with $CHCl_3$/MeOH (1:1, 500 mL) and $CHCl_3$ (200 mL). Only the aqueous layer was taken, neutralized with formic acid, and lyophilized to obtain the crude product. The crude product was passed through an IRC-50 column using THF/H_2O (9:1) as a mobile phase to give the product, **6C** (5 g, 89% yield), as a white solid: TLC ($CHCl_3$/MeOH/H_2O 65:25:4) R_f 0.47 $[\alpha]_D$ +3.10°; FTIR (CaF_2, neat) 2927.4, 2854.7, 1697.2, 1710.3, 1455.8, 1557.2, 1171.4, 1224.6, 1106.3 cm^{-1}; ^1H-NMR (D_2O) δ 3.84 (m, 1 H), δ 3.71 (m, 3 H), 3.52 (m, 2 H), 3.45–3.49 (m, 2 H), 3.36–3.42 (m, 3 H), 3.30 (s, 3 H), 3.17 (t, 2 H, J = 7 Hz), 2.17 (t, 2 H, J = 7.5 Hz), 1.42 (m, 4 H), 1.28 (br s, 9 H), 1.15 (br s, 14 H).

***O*-[1-*O*-(11-Carboxyundecyl)-2-*O*-methyl-*sn*-glycero-3-phosphoryl](*p*-nitrophenyl)ethanol (6D).** A solution of **5D** (8.0 g, 0.01242 mol) and 320 mL of 0.1 N NaOH in 480 mL of THF was stirred at rt for 16 h. After removal of THF, the aqueous phase was extracted with 300 mL of Et_2O to remove the deprotected *o*-chlorophenol and then acidified with 1 N HCl to pH 2. MeOH (300 mL) was added to the aqueous phase containing **6D**, and the aqueous phase was then extracted with $CHCl_3$ (300 mL × 4). The organic extract was combined and neutralized to pH 7 with NH_4OH. Removal of the solvents gave 6.0 g (90%) of **6D** as a yellowish oil: TLC ($CHCl_3$/MeOH/H_2O (65:25:4)) R_f 0.40; $[\alpha]_D$ +2.34°; IR (CaF_2, neat) 2927.6, 2856.1, 1711.3, 1522.6, 1347.9 cm^{-1}; ^1H-NMR (D_2O) δ 7.91 (d, 2 H, J = 9 Hz), 7.32 (d, 2 H, J = 9 Hz), 3.93 (q, 2 H, J = 7 Hz), 3.48–3.57 (m, 2 H), 3.28 (m, 3 H), 3.22 (s, 3 H), 3.16 (t, 2 H, J = 7 Hz), 2.88 (t, 2 H, J = 6 Hz), 2.08 (br s, 2 H), 1.28–1.34 (br d, 4 H), 1.11 (br s, 14 H).

***O*-[1-*O*-(11-Carboxyundecyl)-2-*O*-methyl-*sn*-glycero-3-phosphoryl]-2′,3′-isopropylidene-*sn*-glycerol (7A).** A mixture of **6A** (1.20 g, 0.00191 mol) and 1.0 g of 10% Pd/C in 194 mL of THF and 6 mL of water was stirred at rt under H_2 for 1 h. The reaction mixture was filtered through a Celite 545 pad in an F sintered glass suction filter and washed extensively with MeOH. The filtrate was dried and purified by flash chromatography using a mobile phase of $CHCl_3$/MeOH/H_2O (65:25:2) and then passed through an IRC-50 weak cation-exchange column using MeOH/H_2O (9:1) to give 0.860 g (90.4%) of **7A** as a white glassy solid: TLC ($CHCl_3$/MeOH/H_2O (65:25:4)) R_f 0.30; $[\alpha]_D$ −1.35°; FTIR (CaF_2, neat) 2986.6, 2929.6, 2856.5, 1705.6, 1460.0, 1380.8, 1371.9 cm^{-1}; ^1H-NMR (D_2O) δ 4.31 (m, 1 H), 4.05 (dd, 1 H, J = 8.5, 7.0 Hz), 3.70–3.90 (m, 5 H), 3.40–3.56 (m, 5 H), 3.33 (s, 3 H), 2.19 (t, 2 H, J = 7.5 Hz), 1.43–1.46 (m, 4 H), 1.34 (s, 3 H), 1.27 (s, 3 H), 1.16 (br s, 14 H).

***O*-[1-*O*-(11-Carboxyundecyl-2-*O*-methyl-*sn*-glycero-3-phosphoryl]-*N*-(*tert*-butoxycarbonyl)serine *tert*-butyl ester (7B).** **7B** was prepared following exactly the procedure of preparation of **7A**. From 0.230 g (0.000 303 mol) of **6B** was obtained 0.170 g (89.5%) of **7A** as a white glassy solid: TLC ($CHCl_3$/MeOH/H_2O (65:25:4)) R_f 0.35; $[\alpha]_D$ −4.75°; FTIR 2978.2, 2928.2, 2854.4, 1700–1750, 1460, 1394.2, 1369.2 cm^{-1}; ^1H-NMR (D_2O) δ 4.17 (br s, 1 H), 4.08 (m, 1 H), 3.91 (m, 1 H), 3.85 (m, 1 H), 3.72 (m, 1 H), 3.40–3.56 (m, 5 H), 3.33 (s, 3 H), 2.10 (t, 2 H, J = 7.5 Hz), 1.43–1.46 (m, 4 H), 1.35 (s, 9 H), 1.32 (s, 9 H), 1.16 (br s, 14 H).

Abbreviations: BDPS, *tert*-butylchlorodiphenylsilane; CDI, 1,1′-carbonyldiimidazole; CPDCP, *o*-chlorophenyl dichloro phosphate; DBU, 1,8-diazobicyclo[5.4.0]-undec-7-ene; EtOAc, ethyl acetate; (HO)Boc-Ser-OtBu, *N*-(*tert*-butoxycarbonyl)-*O*-*tert*-butylserine; HOBT, hydroxybenzotriazole; IAM(s), immobilized artificial membrane(s); IPG, (−)-2,3- isopropylidene-*sn*-glycerol; NPEA, (*p*-nitrophenyl)ethyl alcohol; PA, phosphatidic acid; PAO, 2-pyridinealdoxime; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL(s), phospholipid(s); PS, phosphatidylserine; SPA, silica propylamine; TMB, 2,4,6-trimethylbenzyl; TBDPS, *tert*-butyldiphenylsilane; TBAF, tetrabutylammonium fluoride; TMG, tetramethylguanidine.

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Supplementary Material Available: ^1H NMR spectra of all new compounds (16 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.