An Efficient Synthesis of Unsymmetrical Optically Active Phosphatidyl Glycerol

Kazuo Murakami, Erich J. Molitor, and Hung-wen Liu*

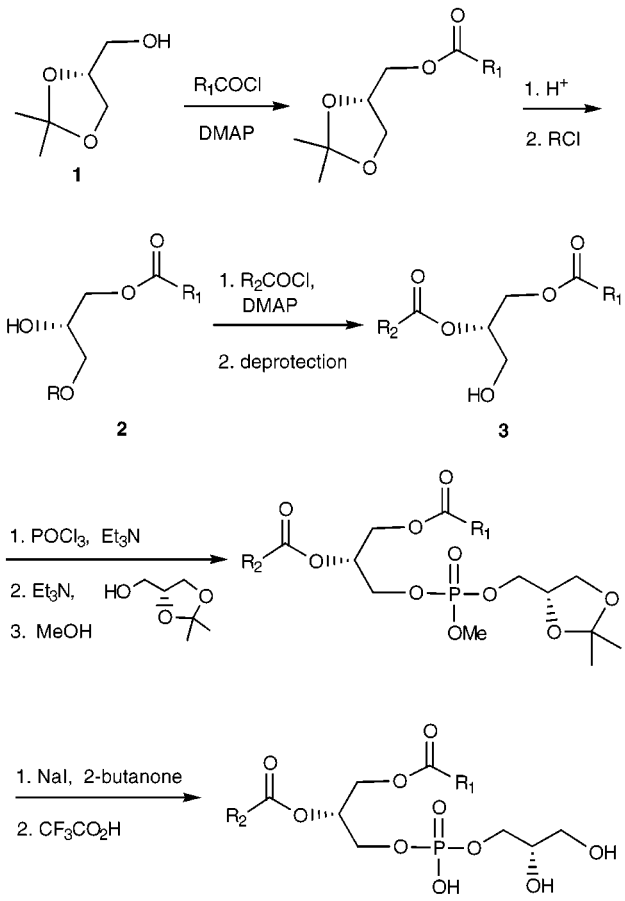
Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455

Received August 14, 1998

The availability of optically active phospholipids containing unsymmetrical fatty acids of defined regiochemistry has become increasingly important for the research of enzymes involved in signal transduction and membrane biochemistry. An eminent example of an enzyme using phospholipids as substrates is phospholipase A_2[1] which catalyzes the hydrolysis of phosphatidyl cholines at the *sn*-2 position to generate a variety of proinflammatory lipid mediators, such as prostaglandins,[2] leukotrienes,[3] and platelet activating factors.[4] Another notable example is cyclopropane fatty acid synthase, which catalyzes the transfer of a methylene bridge, derived from the methyl group of *S*-adenosylmethionine, to the cis double bond of an unsaturated fatty acid chain at the *sn*-2 position of a phosphatidyl glycerol (PG) substrate to form a cyclopropane ring.[5] The unique structure of the resultant cyclopropane-containing phospholipid has been postulated to contribute to the drug resistance and survival of mycobacteria in the hostile intracellular environment of macrophages by the formation of an impermeable asymmetric lipid bilayer.[6]

In support of our ongoing research to study cyclopropane fatty acid synthase, it has been necessary for us to prepare a series of PGs with various oleic acid derivatives at the *sn*-2 position as enzyme substrates and/or mechanistic probes. The most general strategy to prepare PGs begins with acylation of the *R*-isomer of isopropylidene glycerol (**1**), which is commercially available and can be synthesized,[7] if necessary, from ascorbic acid,[8] L-mannitol,[9] L-arabinose,[10] or L-erythrulose.[11] Removal of the acetonide protecting group of 1-acyl-isopropylidene glycerol followed by protection of the primary alcohol with the trityl or other bulky protecting group gives 1-acyl-3-protected glycerol (**2**) (Scheme 1). Acylation with the same or a different fatty acid yields 1,2-diacyl-3-protected glycerol, which, after removal of the protecting group, affords 1,2-diacylglycerol (**3**). The phosphoglycerol headgroup is then installed by a three-step reaction series involving treatment of **3** with phosphorus oxychloride and triethylamine, with **1** and triethylamine, and with methanol in sequence.[7] The synthesis is completed by deprotection of the methyl ester and acetonide group. This method, although quite commonly used, has a few notable drawbacks including multiple protection and deprotection steps and the use of easily hydrolyzable and corrosive phosphorus oxychloride as the phosphorylating reagent. The most serious problem with this synthesis is the high likelihood of acyl migration during acid-catalyzed deprotection of the acetonide group. To minimize the above complications, we have developed an efficient and convenient alternate strategy to make this type of molecule. Herein we report the versatility of this method as exemplified by the synthesis of 1-lauroyl-2-oleoyl-*sn*-glycero-3-[phospho-1-glycerol] (**4**).

Scheme 1



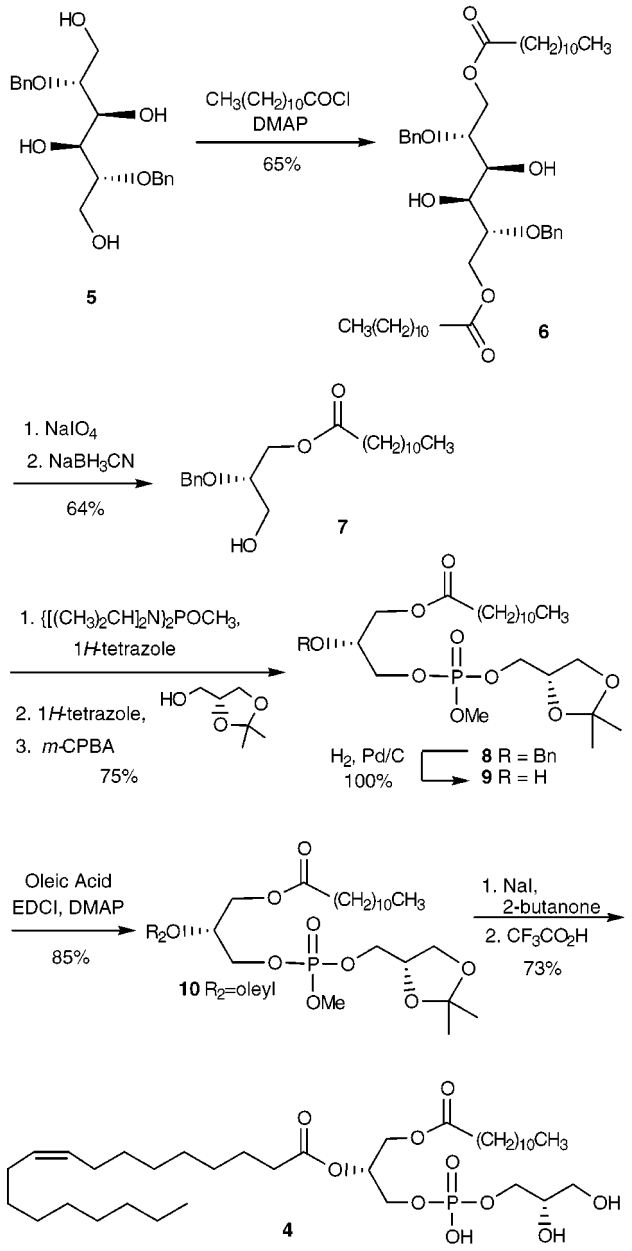
The starting material, 2,5-dibenzyl-D-mannitol (**5**), was prepared from D-mannitol by a known procedure consisting of 1,3−4,6-dibenzylidenylation of D-mannitol, benzyl protection of the 2,5-hydroxyls, and acid hydrolysis of the

* To whom correspondence should be addressed. Tel: (612)-625-5356. Fax: (612)-626-7541. E-mail: liu@chem.umn.edu.

(1) Jain, M. K.; Gelb, M. H.; Rogers, J.; Berg, O. G. *Methods Enzymol.* **1995**, *249*, 567.
(2) Willis, A. L. *Handbook of Eicosanoids: Prostaglandins and Related Lipids*; CRC Press, Inc.: Boca Raton, 1987.
(3) Piper, P. J. *The Leukotrienes Their Biological Significance*; Raven Press: New York, 1986.
(4) Snyder, F. *Platelet-Activating Factor and Related Lipid Mediators*; Plenum Press: New York, 1987.
(5) (a) Wang, A.-Y.; Grogan, D. W.; Cronan, J. E. *Biochemistry* **1992**, *31*, 11020. (b) Grogan, D. W.; Cronan, J. E. *J. Bacteriol.* **1984**, *158*, 286. (c) Buist, P. H.; MacLean, D. B. *Can. J. Chem.* **1981**, *59*, 828. (d) Cronan, J. E.; Reed, R.; Taylor, F. R.; Jackson, M. B. *J. Bacteriol.* **1979**, *138*, 118. (e) Taylor, F. R.; Cronan, J. E. *Biochemistry* **1979**, *18*, 3292. (f) Cronan, J. E.; Nunn, W. D.; Batchelor, J. G. *Biochim. Biophys. Acta* **1974**, *348*, 63. (g) Polacheck, J. W.; Tropp, B. E.; Law, J. H. *J. Biol. Chem.* **1966**, *241*, 3362.
(6) George, K. M.; Yuan, Y.; Sherman, D. R.; Barry, C. E., III. *J. Biol. Chem.* **1995**, *270*, 27292.
(7) For reviews on phospholipid synthesis see the following: (a) Paltauf, F.; Hermetter, A. *Prog. Lipid Res.* **1994**, *33*, 239. (b) Eibl, H. *Chem. Phys. Lipids* **1980**, *26*, 405.
(8) Jung, M. E.; Shaw, T. J. *J. Am. Chem. Soc.* **1980**, *102*, 6304.
(9) (a) Eibl, H. *Chem. Phys. Lipids* **1981**, *28*, 1. (b) Hirth, G.; Walther, W. *Helv. Chim. Acta* **1985**, *68*, 1863.
(10) Kodali, D. R. *J. Lipid Res.* **1987**, *28*, 464.
(11) DeWilde, H.; DeClero, P.; Vandewalle, M. *Tetrahedron Lett.* **1987**, *28*, 4757.

10.1021/jo981653p CCC: $18.00
Published on Web 01/05/1999

Scheme 2



benzylidene groups.[12] Acylation of both primary hydroxyls of **5** to give 2,5-dibenzyl-1,6-dilauroyl-D-mannitol (**6**) was accomplished using lauric acid chloride and (dimethylamino)pyridine (DMAP) (Scheme 2). The desired alcohol, 2-benzyl-1-lauroyl-*sn*-glycerol (**7**), was isolated in 64% yield after $NaBH_3CN$ reduction of the nascent aldehyde generated directly from periodate cleavage.[13]

With compound **7** in hand, phosphorylation was conveniently and efficiently carried out using tetrazole and methyl tetraisopropylphosphorodiamidite. The diisopropylphosphoramidite product generated in situ was treated with (*R*)-isopropylidene glycerol and another equivalent of tetrazole followed by *m*-CPBA to give 2-benzyl-1-lauroyl-*sn*-glycerol-3-[phospho-1-isopropylideneglycerol] methyl ester (**8**) bearing the fully assembled phosphatidyl glycerol headgroup. With the 1-acyl and 3-phosphoryl portions of the molecule attached, the benzyl protecting group was removed to expose the *sn*-2 hydroxyl for further derivatization. Acylation at the *sn*-2 position of 2-hydroxy-1-lauroyl-*sn*-glycero-3-[phospho-1-isopropylideneglycerol] methyl ester (**9**) with oleic acid was accomplished under standard coupling conditions with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and DMAP to give 1-lauroyl-2-oleoyl-*sn*-glycero-3-[phospho-1-isopropylideneglycerol] methyl ester (**10**) in satisfactory yield. Deprotection of the methyl ester and the glycerol acetonide, in sequence, using sodium iodide in 2-butanone[14] and trifluoroacetic acid (TFA), respectively, afforded target phospholipid **4**. The overall yield of **4** from **5** through this 10-step synthesis was nearly 19%.

In summary, the route to make phosphatidyl glycerol derivatives outlined here offers several advantages over the common strategy used for the preparation of this class of compounds. First, and quite importantly, is that this synthesis eliminates the possibility of the acyl migration which is a problem in the alternative synthesis beginning with isopropylidene glycerol. Second, our synthesis, beginning with readily available D-mannitol, generates two equivalents of the glycerol backbone from each mannitol equivalent. Third, acylation of the two primary hydroxyls of **5** prior to the diol cleavage step eliminates at least two protection and deprotection steps required in most previous syntheses in which **5** was used as a starting material for phospholipid synthesis.[7] Installation of the polar headgroup has also been made easy in this work by using phosphoramidite chemistry[15] instead of phosphorus oxychloride, which is more difficult to handle. Compared to the traditional scheme where the *sn*-2 acylation is performed with five reaction steps remaining, this sequence performs the acylation in the third to the last step of the synthesis with only two deprotections remaining. Efficient incorporation of the *sn*-2 fatty acid is important to our study of cyclopropane fatty acid (CFA) synthase since the enzymatic transformation occurs on the *sn*-2 fatty acid of the substrate. These fatty acid analogues are themselves products of multiple-step syntheses, and hence it is critical to carry out the *sn*-2 acylation near the end of the synthesis to most effectively utilize these valuable fatty acid derivatives. This general and efficient route presented herein could be applied to the synthesis of similar compounds such as phosphatidyl choline, phosphatidyl ethanolamine, cardiolipins,[16] and platelet activating factors.

Experimental Section

General Procedures. Melting points are uncorrected. Elemental analysis was carried out by National Chemical Consulting, Inc. (Tenafly, NJ). ^1H, ^{13}C, and ^{31}P NMR spectra were recorded at 300, 75, and 121 MHz, respectively, and all chemical shifts are in hertz (Hz). Flash chromatography was performed in columns of various diameters with Lagand Chemical (230−400 mesh) silica gel by elution with the specified solvents. Thin-layer chromatography (TLC) was carried out on Merck silica gel 60 F_{254} plates and developed with the appropriate solvents. The

(12) Massing, U.; Eibl, H. *Chem. Phys. Lipids* **1995**, *76*, 211.
(13) A (Nishio, Y.; Murata, M.; Achiwa, K. *Tetrahedron Lett.* **1988**, *29*, 5173, Shimizu, C.; Ohta, N.; Achiwa, K. *Chem. Pharm. Bull.* **1990**, *38*, 3347.) It is worth mentioning that the optical rotation ($[\alpha]_D$) of **7** prepared in our current work is more than twice the value reported in this reference.
(14) McMillen, D, A.; Volwerk, J. J.; Ohishi, J.; Erion, M.; Keana, J. F. W.; Jost, P. C.; Griffith, O. H. *Biochemistry* **1986**, *25*, 182.
(15) Hébert, N.; Just, G. *J. Chem. Soc., Chem. Commun.* **1990**, 1497.
(16) Ioannou, P.; Golding, B. T. *Prog. Lipid Res.* **1979**, *17*, 279.

TLC spots were visualized with either UV light or by heating plates previously stained with a solution of phosphomolybdic acid (7% ethanolic solution). All chemicals were products of Aldrich (Milwaukee, WI) except for isopropylidene glycerol which was from Oakwood (West Columbia, SC). The drying agent used in the routine workup was anhydrous magnesium sulfate. Solvents were of analytical-reagent grade or of the highest quality commercially available. Dichloromethane was purified by passage through a column of activated alumina. Compounds **8**−**10** were isolated as 1:1 mixtures of diastereomers based on ^{31}P NMR. No attempt was made to assign the ^{13}C NMR peaks for diastereotopic carbons which have different chemical shifts.

2,5-Dibenzyl-D-mannitol (5). Compound **5** was prepared from D-mannitol by known procedures with minor modifications.[12] The $[\alpha]^{24}_D$ −7.8 (*c* 0.48, ethanol) compared well with the literature value.[12]

2,5-Dibenzyl-1,6-dilauroyl-D-mannitol (6). To a stirred solution of 2,5-dibenzyl-D-mannitol **5** (1.0 g, 2.76 mmol) and (dimethylamino)pyridine (DMAP, 0.775 g, 6.35 mmol) in DMF (16 mL) cooled to 5 °C was added lauroyl chloride (1.21 g, 5.52 mmol) dropwise under argon. The reaction was stirred at 5 °C for 45 min, quenched by the addition of water, and extracted with ethyl acetate (3 × 35 mL). The combined extracts were washed with brine, dried, and concentrated. Purification by silica-gel column chromatography (20% then 33% ethyl acetate in hexanes) gave **6** as a white solid (1.3 g, 65%): mp 49 °C (hexanes); $[\alpha]^{24}_D$ −15.9 (*c* 0.56, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.25 (m, 10 H), 4.71 (2 H, d, J = 11.6), 4.52 (2 H, d, J = 11.6), 4.46 (2 H, dd, J = 12.2, 3.3), 4.25 (2 H, dd, J = 12.2, 4.7), 3.88 (2 H, d, J = 7.2), 3.72 (2 H, m), 2.31 (2 H, t, J = 7.7), 1.60 (2 H, m), 1.24 (16 H, bs), and 0.88 (3 H, t, J = 6.8); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 174.1, 137.7, 128.5, 128.1, 128.17, 78.0, 72.8, 68.7, 62.8, 34.3, 31.9, 29.7, 29.5, 29.4, 29.3, 29.2, 25.0, 22.7, and 14.2. Anal. Calcd for $C_{44}H_{70}O_8$: C, 72.69; H, 9.70. Found: C, 72.83; H, 9.88.

2-Benzyl-1-lauroyl-*sn*-glycerol (7). To a stirred solution of **6** (2.48 g, 3.41 mmol) in methylene chloride (125 mL), 95% ethanol (50 mL), and saturated aqueous $NaHCO_3$ (2.5 mL) was added $NaIO_4$ (2.325 g, 10.87 mmol), and the resulting solution was then stirred at room temperature for 2.5 h. The reaction mixture was dried and concentrated, and the resulting residue was dissolved in methanol (25 mL). To this solution was added $NaBH_3CN$ (975 mg, 15.5 mmol, in water (2.5 mL)), followed by acetic acid (3 mL), and then the solution was stirred at room temperature for 1.5 h. The reaction was quenched with saturated $NaHCO_3$ and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried, and concentrated. Chromatography on silica gel (stepwise elution with 5%, 10%, 20%, and then 33% ethyl acetate in hexanes) yielded **7** as a colorless oil (1.60 g, 64%): TLC (33% ethyl acetate in hexanes) R_f = 0.47; $[\alpha]^{25}_D$ −15.6 (*c* 1.17, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36−7.26 (5 H, m), 4.62 (1 H, d, J = 11.7), 4.55 (1 H, d, J = 11.7), 4.18 (1 H, dd, J = 24.6, 11.7), 4.17 (1 H, dd, J = 24.6, 11.7), 3.64−3.55 (3 H, m), 3.03 (1 H, bs), 2.26 (2 H, t, J = 7.7), 1.57 (2 H, m), 1.24 (16 H, bs), 0.87 (3 H, t, J = 6.6); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 173.8, 137.8, 128.5, 127.9, 127.8, 77.2, 72.1, 62.7, 62.0, 34.2, 31.9, 29.6 (2 C), 29.4, 29.3, 29.2, 29.1, 24.9, 22.7, 14.1; high-resolution MS *m*/*z* calcd for $C_{22}H_{40}NO_4$ (M + NH_4^+) 382.2957, found 382.2964.

2-Benzyl-1-lauroyl-*sn*-glycero-3-[phospho-1-isopropylideneglycerol] Methyl Ester (8). To a solution of **7** (4.0 g, 10.9 mmol) and methyltetraisopropylphosphorodiamidite (4.03 g, 15.36 mmol) in methylene chloride (90 mL) cooled to 0 °C was added 1*H*-tetrazole (1.38 g, 19.75 mmol). The resulting mixture was stirred at 0 °C for 2 h. To this solution were added isopropylidene glycerol (2.55 g, 19.3 mmol) and additional tetrazole (1.38 g, 19.75 mmol), and the stirring was continued at 0 °C for another 2 h. The reaction was cooled to −20 °C, treated with *m*-CPBA (4.35 g, 25.2 mmol), and stirred from −20 to 0 °C over 0.5 h. To this solution were added Na_2SO_3 (1.6 g, 12.6 mmol) and saturated $NaHCO_3$, and the resulting mixture was stirred for 45 min as it warmed from 0 °C to room temperature. The reaction mixture was extracted with chloroform, dried, and concentrated. Chromatography on silica gel (stepwise elution with 10%, 20%, 33%, and then 50% ethyl acetate in hexanes) yielded **8** as a clear yellow oil consisting of a 1:1 mixture of diastereomers (4.7 g, 75%): TLC (33% ethyl acetate in hexanes) R_f = 0.31; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34−7.24 (5 H, m), 4.64 (2 H, s), 4.31−4.22 (2 H, m), 4.20−4.08 (3 H, m), 4.06−3.95 (3 H, m), 3.84−3.70 (2 H, m), 3.75, 3.72 (total 3 H, each d, J_{H-P} = 1.8), 2.28 (2 H, t, J = 7.5), 1.58 (2 H, m) 1.38 (3 H, s), 1.32 (3 H, s), 1.23 (16 H, bs), 0.85 (3 H, t, J = 6.8); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 173.4, 137.6, 128.4, 127.9, 127.8, 109.9, 75.1, 75.0, 74.0, 73.9, 72.2, 67.7, 67.6, 66.4, 66.3, 66.0, 62.3, 54.59, 54.52, 34.1, 31.9, 29.6, 29.4, 29.3, 29.2, 29.1, 26.7, 25.2, 24.8, 22.6, 14.1 (a mixture of diastereomers); ^{31}P NMR (121.4 MHz, $CDCl_3$) δ 0.7; high-resolution FABMS *m*/*z* calcd for $C_{29}H_{50}O_9P$ (M + H^+) 573.3194, found 573.3173.

2-Hydroxy-1-lauroyl-*sn*-glycero-3-[phospho-1-isopropylideneglycerol] Methyl Ester (9). A solution of **8** (400 mg, 0.698 mmol) in ethyl acetate (25 mL) and methanol (5 mL) containing Pd on charcoal (Pd/C, 70 mg) was stirred under H_2 at room temperature for 4 h. The Pd/C was removed by filtration through Celite, and the filtrate was concentrated to give a clear oil **9** which consisted of a 1:1 mixture of diastereomers (336 mg, quantitative): TLC (33% $CHCl_3$ in ethyl acetate) R_f = 0.19; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.31 (1 H, m), 4.18−4.00 (8 H, m), 3.79 (1 H, m), 3.80, 3.72 (total 3 H, each d, J_{H-P} = 0.6), 2.31 (2 H, t, J = 7.7), 1.59 (2 H, m), 1.41 (3 H, s), 1.33 (3 H, s), 1.22 (16 H, bs), 0.85 (3 H, t, J = 6.8); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 173.6, 109.88, 109.86, 74.0, 73.98, 73.91, 73.88, 68.8, 68.7, 68.2, 68.1, 67.8, 67.78, 67.71, 65.7, 64.1, 54.6, 54.5, 33.9, 31.8, 29.5, 29.3, 29.2, 29.1, 29.0, 26.5, 25.1, 24.7, 22.5, 14.0 (a mixture of diastereomers); ^{31}P NMR (121.4 MHz, $CDCl_3$) δ −0.01, −0.06.

1-Lauroyl-2-oleoyl-*sn*-glycero-3-[phospho-1-isopropylideneglycerol] Methyl Ester (10). To a solution of **9** (100 mg, 0.207 mmol) and oleic acid (87 mg, 0.308 mmol) in methylene chloride (1 mL), cooled to −6 °C under argon, was added dropwise over 110 min a solution of EDCI·HCl (48 mg, 0.25 mmol) and DMAP (30.5 mg, 0.25 mmol) in methylene chloride as the temperature was increased to 10 °C. After being stirred for an additional 19 h at room temperature, the reaction was quenched with water and extracted with chloroform (3 × 10 mL). The combined organic extracts were dried and concentrated. Chromatography on silica gel (stepwise elution with 25%, 33%, and then 50% ethyl acetate in hexanes) yielded **10** as a clear colorless oil consisting of a 1:1 mixture of diastereomers (131 mg, 85%): TLC (33% $CHCl_3$ in ethyl acetate) R_f = 0.71; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.33 (2 H, m), 5.23 (1 H, m), 4.35−4.28 (2 H, m), 4.21−4.12 (3 H, m), 4.10−4.00 (3 H, m), 3.84−3.78 (1 H, m), 3.79, 3.75 (total 3 H, each d, J = 2.1), 2.32 (2 H, t, J = 7.5), 2.29 (2 H, t, J = 7.5), 2.00 (4 H, m), 1.60 (4 H, m), 1.42 (3 H, s), 1.35 (3 H, s), 1.32−1.20 (34 H, m), 0.87 (6 H, m); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 173.2, 172.8, 130.0, 129.6, 109.9, 74.0, 73.9, 69.4, 69.3, 67.84, 67.80, 66.0, 65.5, 61.6, 54.64, 54.56, 34.1, 34.0, 31.9, 29.77, 29.72, 29.6, 29.5, 29.4, 29.3, 29.28, 29.21, 29.1, 29.0, 27.2, 27.1, 26.2, 25.3, 24.8, 22.6, 14.1 (a mixture of diastereomers); ^{31}P NMR (121.4 MHz, $CDCl_3$) δ 0.58, 0.53.

1-Lauroyl-2-oleoyl-*sn*-glycero-3-[phospho-1-glycerol] (4). To a solution of **10** (95 mg, 0.127 mmol) in 2-butanone (3.5 mL) was added NaI (124 mg, 0.824 mmol). The reaction was stirred as it was heated to 75 °C for 1 h. The solvent was removed under vacuum to give a yellow residue which was dissolved in chloroform/methanol (5:1). The resulting solution was washed with 10% aqueous HCl followed by brine. The combined aqueous solutions were extracted with chloroform/methanol (5:1) three times. The combined organic extracts were dried (Na_2SO_4) and concentrated. The resulting residue was taken up in CH_2Cl_2/TFA/MeOH (6:3:1) (6 mL) and stirred at room temperature for 1.5 h. The solvent was removed by vacuum, and residual TFA was removed by coevaporation with toluene. Chromatography on silica gel (stepwise elution with 0, 9%, and 17% methanol in chloroform) yielded a light yellow powder (66 mg, 73%): TLC (chloroform/ethyl acetate/methanol/acetic acid, 2:2:1:1) R_f = 0.50; $[\alpha]^{23}_D$ +1.75 (*c* 0.214, $CHCl_3$); ^{1}H NMR (300 MHz, CD_3OD) δ 5.34 (2 H, m), 5.22 (1 H, m), 4.43 (1 H, dd, J = 12.0, 3.0), 4.18 (1 H, dd, J = 12.0, 6.6), 4.00 (2 H, m, J = 5.4), 3.90 (2 H, m), 3.77 (1 H, m, J = 4.8), 3.60 (2 H, m), 2.34 (2 H, t, J = 7.2), 2.32 (2 H, t, J = 7.2), 2.03 (4 H, m), 1.60 (4 H, m), 1.35−1.25 (36 H, bs), 0.90 (6 H, m); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 172.4, 172.1, 129.4, 129.3, 70.9 (d, J_{C-P} = 6.4), 70.1 (d, J_{C-P} = 7.8), 66.1, 62.8, 62.5, 62.3, 33.5, 33.4, 31.30, 31.27, 29.1−28.3 (14 Cs), 26.60, 26.56, 24.4, 24.3, 22.1, 13.8; ^{31}P NMR (121.4 MHz, CD_3OD/$CDCl_3$) δ −2.47.

Acknowledgment. This work is supported by National Institutes of Health Grant GM40541.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **6** and **7** and ^{1}H, ^{13}C, and ^{31}P NMR spectra of compounds **8**−**10** and **4** (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.

JO981653P