A Stereoselective and Highly Practical Synthesis of Cytosolic Phospholipase A_2 Substrate, 2-*S*-Arachidonoyl-1-*O*-hexadecyl-*sn*-2-thioglycero-3-*O*-phosphocholine

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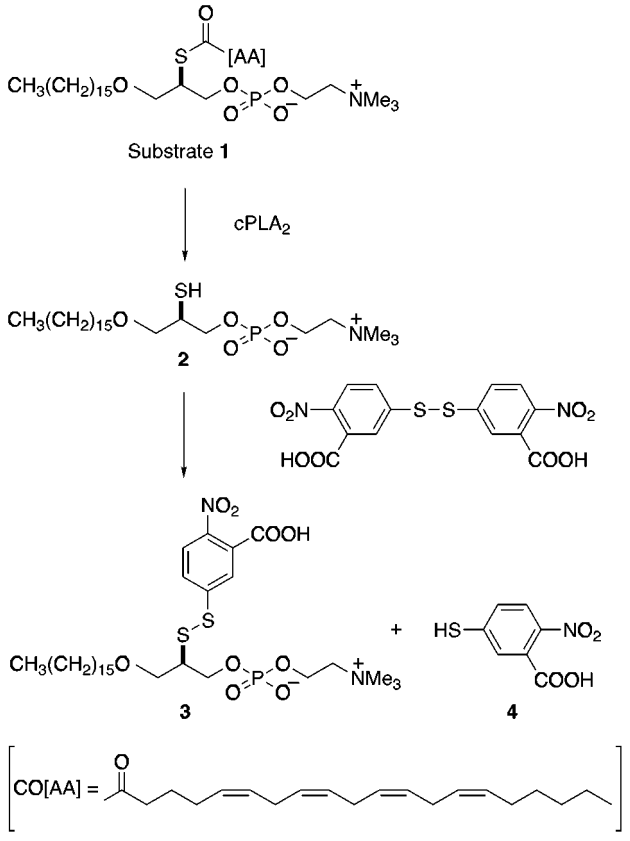
The substrate **1** of cytosolic phospholipase A_2 ($cPLA_2$) is an ether-type thiophospholipid with arachidonic acid at the C-2 position and is required for the chromogenic assay for reliable and convenient high throughput screening. The original method of synthesis of **1** has significant problems, resulting in extremely low overall yield and purity. We developed a novel and highly practical method of preparing sufficient quantities of pure **1** for assay. Our synthetic sequence is started with commercially available 1,2-*O*-isopropylidene-*sn*-grycerol (**5**) and is based on the following key steps: trityl migration reaction of **10** with boron trifluoride etherate to form **13**, phosphocholine-forming reaction of **13** to yield **15**, and efficient conversion of **15** into **1** by deprotection of a trityl group and condensation with arachidonic acid. Our method offers a practical means of large-scale production of **1** with excellent high chemical purity, because of the introduction of arachidonic acid at the last step of the synthetic sequence.

Introduction

Human cytosolic phospholipase A_2 ($cPLA_2$, 85 kDa)[1] is an enzyme which specifically cleaves arachidonic acid from membrane phospholipids and which is thought to catalyze the rate-limiting step in the cellular generation of biologically active eicosanoid products. Due to the significant role of $cPLA_2$ in the formation of a variety of proinflammatory lipid mediators such as prostaglandins, leukotrienes, and platelet activating factors (PAF),[2] selective and effective inhibitors of this enzyme have been attracting much attention as potential antiinflammatory agents. Despite considerable effort to develop inhibitors of this enzyme, no inhibitors have been reported to be in the clinical stage.[3]

Recently, Dennis and co-workers reported a nonradioactive, spectrophotomeric, microtiterplate assay for $cPLA_2$ utilizing 2-*S*-arachidonoyl-1-*O*-hexadecyl-*sn*-2-thioglycero-3-*O*-phosphocholine (**1**)[4] as a substrate (Scheme 1).[5] Dennis demonstrated that this assay is specific for $cPLA_2$ and is much better suited for high throughput screening of $cPLA_2$ inhibitors than are conventional radioactive assays.[6] The structurally notable feature of the substrate employed in this assay is that it has the arachidonoyl thioester at the *sn*-2 position and the alkyl ether at the *sn*-1 position. Since $cPLA_2$ also possesses lysophospholipase activity,[7] the use of *sn*-1 alkyl ether can prevent the lysothiophospholipid **2** produced from being a substrate for its activity. The free thiol group of **2** formed by $cPLA_2$, which has a marked preference for the *sn*-2

Scheme 1



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arachidonoyl group, is allowed to react with the thiol-sensitive reagent, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), resulting in the formation of the disulfide **3** and 5-mercapto-2-nitrobenzoic acid (**4**), which is a chromophore with an absorption maxima of 410 nm. $cPLA_2$ inhibitory activity can be determined by detection of **4** spectrophotomerically in 96-well microtiter plates.

While the reliably high throughput screening of cPLA2 inhibitors achieved with this chromogenic assay is particularly noteworthy, no method has been established for large-scale production of **1**.[8] Therefore, we concentrated our efforts on exploration of a method of synthesis of **1**. We describe here a highly enantioselective and practical synthesis of **1** that makes possible over 10-gram scale production in an extremely pure form.[9] Establishment of a method of synthesis of **1** should contribute to general and widely applicable methods of synthesis of phospholipids with unstable unsaturated substituents and also those which cannot undergo phosphocholine formation.

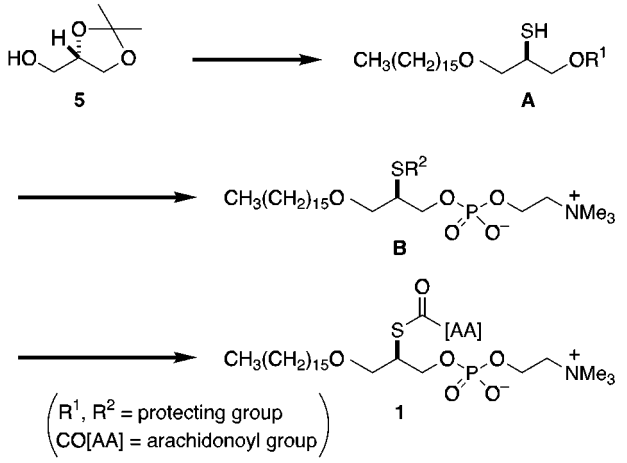
Results and Discussion

A series of ether-type 2-thiophospholipids[10] are considered congeners of 2-thioPAF (1-hexadecyl-2-thioacetyl-2-deoxy-*sn*-glycero-3-phosphocholine) and have been well investigated in studies of PAF-metabolizing enzymes and promising substrates for PAF acetylhydrolase and PLA_2. However, as mentioned above there are no reports describing details of the synthesis of arachidonoyl-containing ether-type 2-thiophospholipids.

In reported procedures,[4,5] the chemically unstable arachidonoyl thioester is incorporated midway through these procedures. Preliminary experiments we performed using these methods revealed that extremely unstable 1,4,7,10-*cis*-tetraene system of arachidonoyl group was damaged during the subsequent reactions, eventually leading to decrease in the purity of **1**. Multiple trials were run,[11] but pure **1** was not obtained. We therefore planned to introduce this labile group at the last step of the synthetic sequence,[12] with the expectation that this would yield **1** efficiently.

Scheme 2 shows our strategy for synthesis of **1**. Commercially available optically active 1,2-*O*-isopropylidene-*sn*-glycerol **5** was chosen as a starting material,

Scheme 2



and it was expected that **1** would be synthesized from **5** *via* the key intermediates **A** and **B**. In particular, the choice of a thiol-protecting group (R^2) in **B** appeared to be crucial since R^2 would need to tolerate the reaction conditions needed for formation of phosphocholine and to be removed easily prior to the introduction of an arachidonoyl group. Although it was presumed that highly hydrophilic phosphocholine intermediates would be handled in organic solvents in the usual manner because of their long-chain hexadecyl substituent at the *sn*-1 position, introduction of a phosphocholine moiety (**A** → **B**) without neighboring S group participation from the *sn*-2 position and reproduction of the unstable thiol compound with an intact phosphocholine structure (**B** → **1**) were major problems for our planned synthetic strategy. Moreover, the optical purities of **1** were not clear from the literature.[4,5] Thus, a highly enantioselective synthesis of **1** was required for $cPLA_2$ enzymatic reaction.

Synthesis of Intermediate 10. 1-*O*-Hexadecyl-3-*O*-trityl-*sn*-2-thioglycerol (**10**) was chosen as an intermediate **A**, since the thiol group of **10** was presumed not to be susceptible to oxidation due to the steric bulkiness of the neighboring triphenylmethyl (trityl) group (Scheme 3). Initially, **5** was alkylated to **6**[13] with hexadecyl methanesulfonate in the presence of sodium hydride. Acidic hydrolysis of **6** followed by selective protection of the primary hydroxyl group of the resulting diol **7**[14] as a trityl ether yielded the alcohol **8**[10i,15]. After mesylation of **8** with methanesulfonyl chloride and triethylamine, the mesylate compound[16] was converted to the thioacetate **9** with inversion at the C-2 position[17] by treatment with potassium thioacetate in *N*,*N*-dimethylformamide.

(8) In accordance with Dennis' procedure, **1** was prepared in nine steps from (*R*)-glycidol at about 2% overall yield by a sequence of reactions involving deprotection of the tetrahydropyranyl group and phosphocholine-forming reactions at the *sn*-3 position while maintaining the thioarachidonoyl ester functionality at the *sn*-2 position. Achiwa's method is similar to that of Dennis and gave similar results.

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(11) When arachidonoylthio ester existed at the *sn*-2 position, reactions for deprotection of the tetrahydropyranyl group at the *sn*-3 position were not accomplished. Trityl and *p*-nitrobenzyl groups were tested as hydroxyl-protecting groups, but we failed to obtain pure **1** efficiently.

(12) For acylation (including arachidonoylation) at the *sn*-2 position of lysophosphatidylcholine, see: (a) Baran, J. S.; Langford, D. D.; Laos, I. *J. Org. Chem.* **1977**, *42*, 2260. (b) Wolf, R. A.; Gross, R. W. *J. Lipid Res.* **1985**, *26*, 629. (c) Mena, P. L.; Djerassi, C. *Chem. Phys. Lipids* **1985**, *37*, 257. (d) Kallury, R. K.; Krull, U. J.; Thompson, M. *J. Org. Chem.* **1987**, *52*, 5478. (e) Hébert, N.; Beck, A.; Lennox, R. B.; Just, G. *J. Org. Chem.* **1992**, *57*, 1777. (f) Martin, S. F.; Josey, J. A.; Wong, Y.-L.; Dean, D. W. *J. Org. Chem.* **1994**, *59*, 4805. (g) Xia, J.; Hui, Y. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1919.

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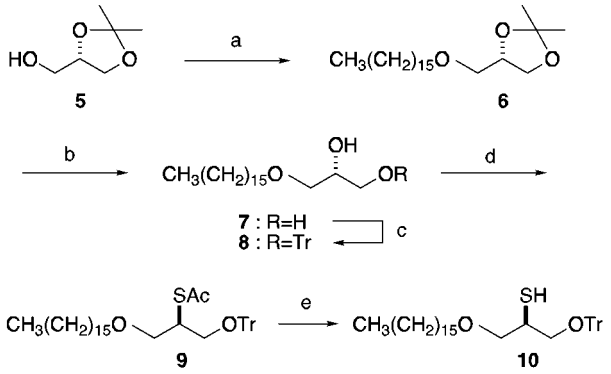
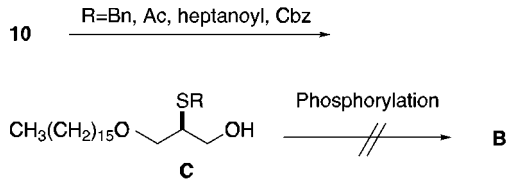
Scheme 3[a]



[a] (a) $CH_3(CH_2)_{15}OMs$, NaH (97%); (b) aq. HCl (97%); (c) TrCl, Et_3N; (d) i) MsCl, Et_3N, ii) AcSK (82% for c, d); (e) NaOMe (95%).

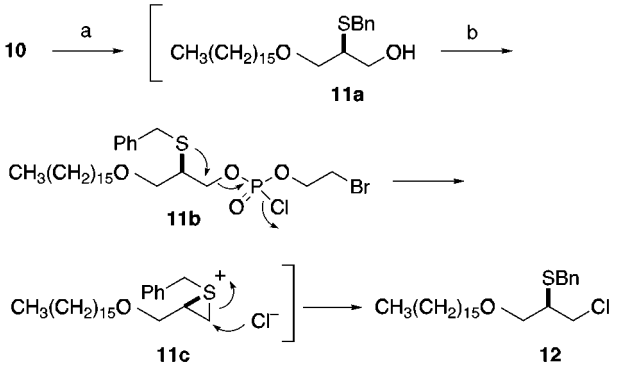
Scheme 4



The enantiomeric purity of **9** was clearly determined by measuring the ^{1}H NMR spectra of both (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid ((*R*)-(+)-MTPA) ester and (*S*)-(−)-MTPA ester of **13**, as mentioned below. Deprotection of the acetyl group of **9** using sodium methoxide in methanol and tetrahydrofuran yielded the desired thiol **10**, which was a crystalline compound and stable against oxidation to its disulfide.

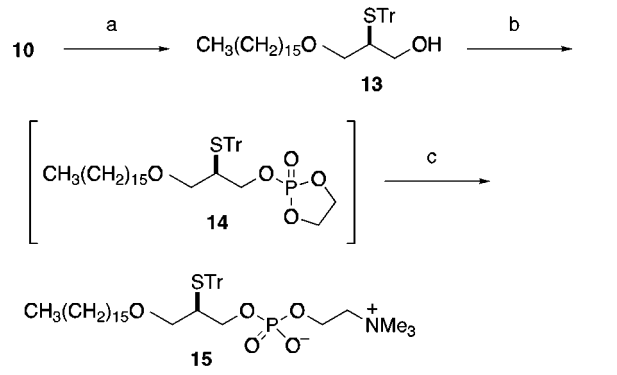
Synthesis of Intermediate 15. The next goal is synthesis of intermediate **B** from **10** through protection of the thiol group, deprotection of the trityl group, and formation of a phosphocholine group (Scheme 2). Initially, we attempted to protect the thiol group in **10** with groups such as benzyl, acetyl,[18] heptanoyl, and benzyloxycarbonyl groups, but derivatization to **B** was not achieved with any of them, as shown in Scheme 4. In all cases the phosphorylation steps (**C** → **B**) were unsuccessful. For instance, when a benzyl group was employed, the attempt to introduce a phosphocholine group to the alcohol **11a** using (2-bromoethyl)phosphodichloridate resulted in predominant formation of the labile chloride **12** (Scheme 5). The similar result was obtained when 2-chloro-2-oxo-1,3,2-dioxaphospholane[19] was used in place of (2-bromoethyl)phosphodichloridate, giving the same chloride **12**. Consideration of the mechanism of this reaction indicates that it proceeds *via* attack of chloride anion on the carbon adjacent to the episulfonium cation **11c**, which is formed by nucleophilic attack of the sulfur atom on the adjacent C-3 position in the chlorophosphate **11b**. On the other hand, when acetyl, heptanoyl, or benzyloxycarbonyl protecting groups were used, the thiocarbonyl groups were decomposed by treatment with trimethylamine during phosphocholine formation.

Scheme 5[a]



[a] (a) i) $PhCH_2Br$, NaOMe, ii) BF_3·MeOH; (b) (2-bromoethyl)-phosphodichloridate, Et_3N

Scheme 6[a]



[a] (a) BF_3·Et_2O (85%); (b) 2-chloro-2-oxo-1,3,2-dioxaphospholane, Et_3N; (c) Me_3N (70% for 2 steps)

To prevent participation of the neighboring sulfur, we next tested the trityl group, which was expected to eliminate the nucleophilicity of the sulfur atom due to its steric bulkiness. In order to accomplish tritylation of the thiol in **10**, sulfur participation, in turn, was utilized, i.e., O → S migration of the trityl group. As shown in Scheme 6, tritylation was very efficiently carried out, accompanied by deprotection of the hydroxyl group. Thus, treatment of **10** with 1 equiv of boron trifluoride etherate (BF_3·Et_2O) in dichloromethane at low temperature resulted in excellent yield of the alcohol **13** as a consequence of intramolecular migration of the trityl group from oxygen to sulfur. This reaction can be explained by the fact that capture of the cationic trityl group by the thiol is induced by coordination of BF_3 with the oxygen atom at the *sn*-3 position of **10**. The enantiomeric purity of compound **9** prepared by displacement reaction using potassium thioacetate from the corresponding mesylate was determined at this stage using the crude **13**. Compound **8** was transformed by successive straightforward reactions to **13** without any purification procedure. The enantiomeric excess (ee) of the crude **13** was determined by the MTPA ester method[20] to be >99.8:<0.2 by 600-MHz ^{1}H NMR analysis, indicating that the enantiomeric purity of **13** was >99.6% ee. It is clear that the stereochemistry at the C-2 position of **9** is *R*, since the displacement reaction of activated hydroxyl derivatives with thioacetate is known to proceed with inversion.[10h,17] Thus, the transformation from **8** to **9** *via* the mesylate was demonstrated to be highly stereoselec-

(18) **1** was synthesized at only 1% yield with 96% chemical purity from 2-*S*-acetyl-1-*O*-hexadecyl-3-*O*-trityl-*sn*-2-thioglycerol *via* five steps: deprotection of the trityl group, formation of 2-bromoethylphosphate by treatment with (2-bromoethyl)phosphodichloridate, cleavage of the acetyl group by lithium borohydride, condensation with arachidonic acid, and complete assembly of a phosphocholine moiety by treatment with dry trimethylamine.

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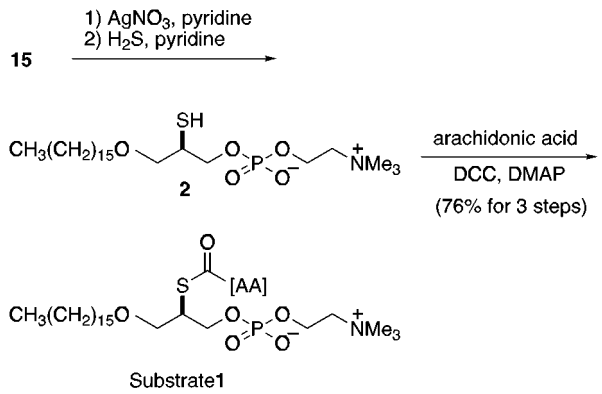
Scheme 7



tive. Use of compound **13**, aided by the bulky trityl group, enabled us to obtain the desired compound **15** as the intermediate **B**. The phosphocholine function was introduced by reaction of **13** with 2-chloro-2-oxo-1,3,2-dioxaphospholane,[19] followed by reaction of the resulting cyclic phosphate intermediate **14** with anhydrous trimethylamine at 60 °C in a sealed vessel, giving **15** in 70% yield for two steps. After trials using several phosphorylating reagents,[21] this cyclic phosphochloridate, which has been reported[22] to be more reactive than the commonly used (2-bromoethyl)phosphodichloridate, was found to be the most suitable reagent for the phosphorylation of **13**. Compound **15** is a stable crystalline solid, as we expected, and can be stored unchanged at room temperature for a long period. This is a very important characteristic for a precursor closest to the final product in large-scale preparation.

Synthesis of cPLA$_2$ Substrate 1. After several conditions were examined for deprotection of the trityl group in **15**, reaction of **15** with silver nitrate followed by treatment of the resulting silver thiolate with hydrogen sulfide[23] in the presence of pyridine yielded the thiol **2** very efficiently (Scheme 7). The use of 1 equiv of pyridine is critical, since **2** is very sensitive to acid and decomposes by reaction with the nitric acid generated from excess silver nitrate and hydrogen sulfide.[24] Mild deprotection of the *S*-trityl group using silver nitrate and hydrogen sulfide in slightly basic conditions is a very useful method for complex molecules possessing many reactive functional groups. **2** was used for the next step without any purification. Finally, condensation reaction with arachidonic acid using dicyclohexylcarbodiimide (DCC) resulted in complete synthesis of the cPLA$_2$ substrate **1** in 76% yield for three steps. All spectroscopic properties of **1** prepared by the present method were identical with those originally reported in the literature.[4,5] It should be noted that we were able to obtain **1** of high chemical purity by simple purification with silica gel chromatography since all of the last three successive steps were extremely mild and did not affect the phosphocholine and very labile arachidonic acid moiety, and that no byproducts with a structural similarity to **1** were observed in these reactions. Moreover, the optically high purity of **1** was achieved by derivatization from **13**, the enantiomeric excess of which was extremely high (>99.6% ee).

(21) For a recent review of methods for phosphorylation of glycerol derivatives, see: Bittman, R. In *Phospholipids Handbook*; Cevc, G., Ed.; Marcel Dekker: New York, 1993; pp141−232.

(22) Bonsen, P. P. M.; de Haas, G. H.; Pieterson, W. A.; van Deenen, L. L. M. *Biochem. Biophys. Acta* **1972**, *270*, 364.

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(24) Treatment of the isolated crystalline silver thiolate with hydrogen sulfide in the absence of pyridine and condensation with arachidonic acid yielded only structurally similar byproducts instead of the desired substrate **1**.

Conclusions

The original method of synthesis of **1** had two drawbacks, low total yield and purity of **1**, and it was unsuitable for large-scale production of **1**. These problems have been completely solved by our synthetic strategy, which involves introduction of chemically unstable arachidonic acid at the last step and effective utilization of a trityl group. We thus achieved highly enantioselective and practical synthesis of substrate **1** which involved 12 operations and featured a 33% overall yield from the commercially available glycerol derivative **5**. The availability of over 10-gram quantities of substrate **1** made possible performance of high throughput screening for cPLA$_2$ inhibitors. It is also noteworthy that the *S*-trityl phosphocholine **15** is a potentially useful intermediate for preparing various 2-thiophospholipids bearing labile unsaturated fatty acids at the C-2 position. Moreover, the concept established in the current method of synthesis should also be applicable to the synthesis of various phospholipid analogs bearing phosphocholine, phosphoethanolamine, phosphoinositol, or phosphoserine.

Experimental Section

Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were determined at 200 and 101 MHz, respectively, unless otherwise stated. Liquid secondary ion mass spectra (LSIMS) and high resolution (HR)-LSIMS were determined using *m*-nitrobenzyl alcohol as a matrix. Reactions were carried out under a nitrogen atmosphere in anhydrous solvents (dried over type 4A molecular sieves). Organic extracts were dried over anhydrous sodium sulfate. Column chromatography was performed with Merck silica gel 60 (70−230 or 230−400 mesh).

3-*O*-Hexadecyl-1,2-*O*-isopropylidene-*sn*-glycerol (6). In a three-necked, round-bottomed flask equipped with a mechanical stirrer and a 500 mL dropping funnel was placed 7.26 g (0.182 mol) of sodium hydride (60% in mineral oil). The mineral oil was removed by washing with 25 mL (four times) of *n*-hexane, and to this was added 300 mL of *N*,*N*-dimethylformamide at room temperature. To this suspension was added a solution of 20.0 g (0.151 mol) of (*S*)-1,2-*O*-isopropylidene-*sn*-glycerol in 300 mL of *N*,*N*-dimethylformamide at 0 °C, and the mixture was vigorously stirred at the same temperature for 30 min. To this mixture was added a solution of 58.21 g (0.182 mol) of hexadecyl methanesulfonate in 300 mL of *N*,*N*-dimethylformamide at 0 °C, and the resulting mixture was stirred for 17 h at room temperature. The reaction mixture was poured into ice-cold water and was extracted with ethyl acetate. The organic solution was washed successively with water and brine, dried, and concentrated. The residue was purified by silica gel chromatography, eluting with hexane−EtOAc (95:5), to yield 52.37 g (97%) of **6**[13] as a colorless oil. $[\alpha]^{22}_D$ +8.2° (*c* 2.12, $CHCl_3$). IR ($CHCl_3$): 1465, 1381, 1372, 1236, 1133, 1114 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.88 (t, J = 6.4 Hz, 3H), 1.10−1.40 (m, 26H), 1.36 (s, 3H), 1.42 (s, 3H), 1.45−1.65 (m, 2H), 3.36−3.58 (m, 4H), 3.73 (dd, J = 8.2, 6.4 Hz, 1H), 4.06 (dd, J = 8.2, 6.4 Hz, 1H), 4.27 (tt, J = 6.1, 6.1 Hz, 1H). Anal. Calcd for $C_{22}H_{44}O_3$: C, 74.10; H, 12.40. Found: C, 73.97; H, 12.39.

3-*O*-Hexadecyl-*sn*-glycerol (7). To a solution of 52.37 g (0.147 mol) of **6** in 370 mL of 1,2-dimethoxyethane was added 150 mL of 0.5 N hydrochloric acid. The mixture was refluxed for 1.5 h. The reaction mixture was cooled and concentrated, and the residue was diluted with ethyl acetate. The organic solution was washed with water, saturated sodium hydrogen-

carbonate, and brine successively, dried, and concentrated to yield a crystalline residue. Recrystallization from *n*-hexane−ethyl acetate afforded 45.30 g (97%) of **7** as colorless plates. Mp 66−66.5 °C [lit.[14a] Mp 63−64 °C; lit.[14b] mp 62.5−63.5 °C]. $[\alpha]^{26}_{D}$ +2.5° (*c* 3.00, THF) [lit.[14a] $[\alpha]^{25}_{D}$ +2.69° (*c* 3.5, THF)]. IR (KBr): 3415, 3335, 3250, 1460, 1130, 1050 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.4 Hz, 3H), 1.10−1.45 (m, 26H), 1.45−1.65 (m, 2H), 2.20 (dd, J = 6.4, 5.4 Hz, OH), 2.63 (d, J = 5.0 Hz, OH), 3.40−3.93 (m, 7H). Anal. Calcd for $C_{19}H_{40}O_3$: C, 72.10; H, 12.74. Found: C, 71.95; H, 12.68.

3-*O*-Hexadecyl-1-*O*-trityl-*sn*-glycerol (8). A mixture of 42.98 g (0.136 mol) of **7**, 45.43 g (0.163 mol) of triphenylmethyl chloride, and 37.9 mL (0.163 mol) of triethylamine in 470 mL of tetrahydrofuran and 120 mL of acetonitrile was refluxed for 15 h. The reaction mixture was concentrated and filtered to remove the precipitated triethylamine hydrochloride. The salt was washed thoroughly with ethyl acetate. The filtrate and washings were combined and washed with water, 0.1 N hydrochloric acid, saturated sodium hydrogencarbonate, and brine successively. The solution was dried and concentrated to yield 85.36 g of crude compound **8** as pale-yellowish crystals, which were used for the next step without further purification. A small amount of crude **8** was purified by silica gel chromatography, eluting with *n*-hexane−EtOAc (9:1), to yield pure **6**[15] as colorless solids. Mp 54−55 °C. $[\alpha]^{24}_{D}$ −2.3° (*c* 1.00, $CHCl_3$) [lit.[15] $[\alpha]^{26}_{D}$ −2.25° (*c* 1.02, $CHCl_3$)]. IR (KBr): 2915, 2850, 1446, 1090 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 3H), 1.10−1.40 (m, 26H), 1.40−1.65 (m, 2H), 2.43 (d, J = 4.8 Hz, 1H), 3.10−3.25 (m, 2H), 3.35−3.60 (m, 4H), 3.88−4.03 (m, 1H), 7.17−7.55 (m, 15H). Anal. Calcd for $C_{38}H_{54}O_3$: C, 81.67; H, 9.74. Found: C, 81.66; H, 9.74.

2-*S*-Acetyl-1-*O*-hexadecyl-3-*O*-trityl-*sn*-2-thioglycerol (9). To a solution of 85.34 g of crude **8** in 880 mL of dichloromethane were added dropwise 31.9 mL (0.229 mol) of triethylamine and 11.8 mL (0.152 mol) of methanesulfonyl chloride at −5 °C. After stirring for another 1.5 h at the same temperature, the reaction mixture was poured into ice-cold water. The organic phase was separated, and the aqueous phase was extracted with dichloromethane. The organic phases were combined and washed with 0.1 N hydrochloric acid, saturated sodium hydrogencarbonate, and brine successively. The solution was dried and concentrated to yield 94.07 g of the mesylate[16] as a pale-yellowish oil.

To a solution of 94.07 g of the above mesylate in 800 mL of *N*,*N*-dimethylformamide was added 33.74 g (0.295 mol) of potassium thioacetate, and the mixture was stirred for 16 h while heating at 90 °C (bath temperature). An additional 16.87 g (0.148 mol) of potassium thioacetate was added, and the reaction mixture was stirred for 4 h under the same conditions. After cooling, the mixture was poured into ice-cold water and extracted with ethyl acetate. The organic solution was washed successively with water and brine, dried, and concentrated. The residue was purified by silica gel chromatography, eluting with *n*-hexane−toluene (from 5:1 to 1:2), to yield 68.75 g (82% from **7**) of **9** as a reddish-brown oil. EI-MS m/z 617 $(M + H)^+$. IR ($CHCl_3$): 1686, 1490, 1466, 1448, 1133, 1114 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.4 Hz, 3H), 1.10−1.40 (m, 26H), 1.40−1.60 (m, 2H), 2.31 (s, 3H), 3.22 (dd, J = 9.2, 5.6 Hz, 1H), 3.37 (t, J = 6.6 Hz, 2H), 3.39 (dd, J = 9.2, 3.8 Hz, 1H), 3.65 (d, J = 6.0 Hz, 2H), 3.80−3.97 (m, 1H), 7.15−7.50 (m, 15H).

1-*O*-Hexadecyl-3-*O*-trityl-*sn*-2-thioglycerol (10). To a solution of 68.75 g (0.111 mol) of **9** in 400 mL of MeOH and 200 mL of tetrahydrofuran was added dropwise 23.6 mL (0.123 mol) of 28 wt % sodium methoxide solution in methanol at 0 °C. The reaction mixture was stirred for 30 min at the same temperature and concentrated. The residue was diluted with ethyl acetate. The solution was washed with 0.1 N hydrochloric acid, saturated sodium hydrogencarbonate, and brine successively, dried and concentrated. The residue was purified by silica gel chromatography, eluting with *n*-hexane-toluene (3:1), to yield 60.68 g (95%) of **10** as colorless solids. Mp 61.5−63 °C. $[\alpha]^{25}_{D}$ +3.5° (*c* 1.01, $CHCl_3$). IR (KBr): 3430, 1490, 1468, 1450, 1443, 1118, 1093 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.4 Hz, 3H), 1.10−1.40 (m, 26H), 1.40−1.65 (m, 2H), 1.89 (d, J = 8.2 Hz, SH), 2.95−3.15 (m, 1H), 3.26 (dd, J = 9.2, 5.8 Hz, 1H), 3.32 (dd, J = 9.2, 5.4 Hz, 1H), 3.40 (t, J = 6.6 Hz, 2H), 3.55−3.70 (m, 2H), 7.15−7.50 (m, 15H). ^{13}C NMR ($CDCl_3$) δ 14.12, 22.70, 26.16, 29.38, 29.54, 29.62, 29.65, 29.68, 29.72, 31.95, 39.88, 64.86, 71.34, 72.57, 86.63, 127.07, 127.84, 128.77, 144.06. LSIMS m/z 597 $(M + Na)^+$. Anal. Calcd for $C_{38}H_{54}O_2S$: C, 79.39; H, 9.47; S, 5.58. Found: C, 79.28; H, 9.46; S, 5.55.

Attempt to Convert Compound 10 to Intermediate B (*S*-benzyl). To a solution of 491 mg (0.854 mmol) of **10** in 10 mL of *N*,*N*-dimethylformamide were added dropwise 0.11 mL (0.897 mmol) of benzyl bromide and 0.94 mL (0.940 mmol) of 1 N sodium methoxide at room temperature, and the mixture was stirred for 15 min. The reaction mixture was partitioned between ethyl acetate and 1 N hydrochloric acid, and the organic solution was washed with water and brine successively, dried, and concentrated. The residual oil (560 mg) was dissolved in 10 mL of dichloromethane. To this was added 0.87 mL of 12 wt % boron trifluoride−methanol complex in methanol at room temperature, and the mixture was stirred for 1 h. The reaction mixture was poured into ice-cold water and extracted with chloroform. The organic solution was washed with water and brine successively, dried, and concentrated to yield 342 mg (95%) of **11a** as a colorless oil. ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.4 Hz, 3H), 1.10−1.40 (m, 26H), 1.40−1.65 (m, 2H), 2.60 (t, J = 6.2 Hz, 1H), 2.85−3.00 (m, 1H), 3.33−3.44 (m, 2H), 3.48 (dd, J = 9.6, 9.6 Hz, 1H), 3.59 (dd, J = 9.6, 4.8 Hz, 1H), 3.64−3.82 (m, 2H), 3.78 (s, 2H), 7.20−7.30 (m, 5H).

To a solution of 338 mg (0.800 mmol) of **11a** and 0.16 mL (1.14 mmol) of triethylamine in 3 mL of chloroform was added dropwise a solution of 216 mg (0.893 mmol) of (2-bromoethyl)phosphodichloridate in 1.5 mL of chloroform at 0 °C. After stirring for 2 h at room temperature, 4 mL of ether was added and the mixture was refluxed for 2 h. The reaction mixture was poured into water and extracted with ether. The organic solution was washed successively with water and brine, dried, and concentrated. The residue was purified by silica gel chromatography, eluting with *n*-hexane−ethyl acetate (98:2), to yield 300 mg (85%) of **12** as a colorless oil. This compound was found to decompose on TLC and during silica gel chromatographic separation. IR (film): 1470, 1455, 1118 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.2 Hz, 3H), 1.10−1.45 (m, 26H), 1.45−1.65 (m, 2H), 2.76 (dd, J = 14.0, 6.2 Hz, 1H), 2.93 (dd, J = 14.0, 7.0 Hz, 1H), 3.44 (t, J = 6.6 Hz, 2H), 3.58−3.73 (m, 2H), 3.78 (s, 3H), 3.90−4.08 (m, 1H), 7.20−7.40 (m, 5H). LSIMS m/z 463 $(M + Na)^+$. Anal. Calcd for $C_{38}H_{54}O_2S$: C, 70.79; H, 10.28; S, 7.27. Found: C, 71.11; H, 10.26; S, 7.48.

1-*O*-Hexadecyl-2-*S*-trityl-*sn*-2-thioglycerol (13). To a solution of 60.68 g (0.106 mol) of **10** in 1.2 L of dichloromethane was added dropwise 14.3 mL (0.116 mol) of boron trifluoride−diethyl ether complex at −10 °C. After stirring for 45 min at the same temperature, the reaction mixture was poured into ice-cold saturated sodium hydrogencarbonate and extracted with dichloromethane. The organic solution was washed with brine, dried, and concentrated. The residue was purified by silica gel chromatography, eluting with *n*-hexane−toluene (1:1) and then ethyl acetate, to yield 51.86 g (85%) of **13** as a colorless oil. $[\alpha]^{23}_{D}$ −25.8° (*c* 2.28, $CHCl_3$). IR ($CHCl_3$): 3574, 3468, 1595, 1488, 1465, 1444, 1133, 1110 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.4 Hz, 3H), 1.10−1.35 (m, 26H), 1.35−1.55 (m, 2H), 2.54−2.72 (m, 2H), 3.00−3.58 (m, 6H), 7.15−7.35 (m, 9H), 7.40−7.50 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.13, 22.71, 26.05, 29.39, 29.44, 29.52, 29.59, 29.64, 29.69, 29.72, 31.95, 45.42, 64.92, 67.46, 71.47, 72.94, 126.80, 128.00, 129.58, 144.88. LSIMS m/z 597 $(M + Na)^+$. Anal. Calcd for $C_{38}H_{54}O_2S$: C, 79.39; H, 9.47; S, 5.58. Found: C, 79.23; H, 9.51; S, 5.52.

1-*O*-Hexadecyl-2-*S*-trityl-*sn*-2-thioglycero-3-*O*-phosphocholine (15). To a solution of 39.83 g (69.3 mmol) of **13** and 22.0 mL (157.8 mmol) of triethylamine in 950 mL of benzene was added a solution of 18.74 g (131.5 mmol) of 2-chloro-2-oxo-1,3,2-dioxaphospholane in 100 mL of benzene at room temperature. After stirring for 3.5 h at room temperature, the reaction mixture was filtered to remove the precipitated salts. The salts were washed with a small amount of benzene. The filtrate and washings were combined and

concentrated to yield **1-*O*-hexadecyl-2-*S*-trityl-3-*O*-(2-oxo-1,3,2-dioxaphospholan-2-yl)-*sn*-2-thioglycerol (14)** as a pale-brown oil. A solution of the resultant **14** in 96 mL of 3.8 M trimethylamine/acetonitrile solution was heated in a sealed tube at 50 °C for 16 h. The reaction solution was concentrated, and the residue was diluted with 2-butanone. The solution was washed with water and brine successively. After removing the solvent, the residue was purified by silica gel chromatography, eluting with chloroform−methanol−water (32:9:1) and then recrystallizing from chloroform−acetonitrile, to yield 35.95 g (70% from **13**) of **15** as colorless plates. Mp 132−136 °C. $[\alpha]^{25}_D$ +29.9° (*c* 2.00, $CHCl_3$). IR (KBr): 3393, 1488, 1468, 1445, 1257, 1091, 1064, 1004, 968 cm^{-1}. ^1H NMR (CD_3OD) δ 0.89 (t, J = 6.4 Hz, 3H), 1.10−1.50 (m, 28H), 2.50−2.65 (m, 1H), 2.84 (dd, J = 10.2, 4.4 Hz, 1H), 3.05−3.27 (m, 3H), 3.18 (s, 9H), 3.50−3.65 (m, 2H), 3.75−3.95 (m, 2H), 4.10−4.30 (m, 2H), 7.15−7.40 (m, 9H), 7.45−7.55 (m, 6H). ^{13}C NMR (150.8 MHz, $CDCl_3$) δ 14.15, 22.70, 26.07, 29.38, 29.56, 29.68, 29.74, 31.93, 44.93 (J = 9 Hz), 54.29, 59.18 (J = 5 Hz), 64.87 (J = 5 Hz), 66.22 (J = 6 Hz), 67.20, 69.35, 70.80, 126.70, 127.94, 129.65, 144.88. LSIMS m/z 740 $(M + H)^+$. Anal. Calcd for $C_{43}H_{66}NO_5PS \cdot H_2O$: C, 68.13; H, 9.04; N, 1.85; P, 4.09; S, 4.23. Found: C, 68.06; H, 8.97; N, 2.03; P, 4.35; S, 4.31.

2-*S*-Arachidonoyl-1-*O*-hexadecyl-*sn*-2-thioglycero-3-*O*-phosphocholine (1). To a solution of 20.0 g (26.4 mmol) of **15** in 20 mL of methanol and 200 mL of acetonitrile was added 4.69 mL (58.1 mmol) of pyridine at room temperature. A solution of 8.96 g (52.8 mmol) of silver nitrate in 30 mL of acetonitrile was added dropwise at 0 °C, and the resulting suspension was stirred for 30 min at the same temperature. After the addition of 250 mL of ether, the precipitated solid was collected by filtration, washed with acetonitrile−ether (1:1) and ether successively, and dried under reduced pressure to obtain 17.3 g of the silver salt.

To a suspension of 17.3 g of the above salt in 380 mL of dichloromethane was added 4.27 mL (52.8 mmol) of pyridine, and hydrogen sulfide gas (15 mL/min) was bubbled through the mixture for 30 min while vigorously stirring at 0 °C. As silver sulfide was generated, the suspension gradually became a black solution. After stopping the introduction of hydrogen sulfide gas, the mixture was stirred for 1 h at room temperature to obtain a solution of **2** in dichloromethane, which was immediately used for the next step.

After the reaction mixture was concentrated to about half of its original volume, it was added to a solution of 7.23 g (23.8 mmol) of arachidonic acid and 3.88 g (31.7 mmol) of 4-(dimethylamino)pyridine in 150 mL of dichloromethane at 0 °C. To the mixture was added a solution of 6.54 g (31.7 mmol) of dicyclohexylcarbodiimide in 15 mL of dichloromethane at 0 °C, and the reaction mixture was stirred for 3 h at room temperature. The reaction suspension was diluted with 500 mL of 2-butanone and stirred for 5 min at 0 °C, and the precipitated dicyclohexylurea was removed by filtration. To the filtrate was added 360 mL of 3% citric acid, and the mixture was stirred for 15 min at 0 °C. The resultant black precipitates were filtered off using powdered filter paper and washed thoroughly with 470 mL of 2-butanone. The filtrate and washings were combined, and the organic phase was separated and washed with 200 mL of 3% citric acid and 200 mL of water successively. Each aqueous phase was extracted with 2-butanone. All the organic phases were combined, dried, and concentrated. The residue was purified by silica gel chromatography, eluting with chloroform−methanol−water [from (64:9:1) to (32:9:1)], to yield 15.8 g (76% from **15**) of **1** as a colorless oil. $[\alpha]^{26}_D$ −3.1° (*c* 2.2, $CHCl_3$:MeOH (4:1)). [lit.[4] $[\alpha]^{22}_D$ −2.8° (*c* 2.45, $CHCl_3$:MeOH (4:1))] IR ($CHCl_3$): 3399, 1682, 1603, 1467, 1240, 1087 cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.88 (t, J = 6.4 Hz, 3H), 0.89 (t, J = 6.4 Hz, 3H), 1.20−1.42 (m, 32H), 1.42−1.60 (m, 2H), 1.60−1.80 (m, 2H), 1.96−2.18 (m, 4H), 2.54 (t, J = 7.6 Hz, 2H), 2.72−2.92 (m, 6H), 3.30−3.47 (m, 2H), 3.40 (s, 9H), 3.48−3.68 (m, 2H), 3.75−4.03 (m, 5H), 4.26−4.42 (m, 2H), 5.24−5.50 (m, 8H). ^{13}C NMR ($CDCl_3$) δ 14.09, 14.13, 22.59, 22.71, 25.46, 25.66, 26.11, 26.43, 27.24, 29.33, 29.38, 29.58, 29.70, 29.74, 31.53, 31.94, 43.63, 44.20 (J = 8 Hz), 54.51, 59.24 (J = 5 Hz), 64.24 (J = 6 Hz), 66.52 (J = 6 Hz), 69.20, 71.47, 127.53, 127.83, 128.07, 128.34, 128.65, 128.68, 129.10, 130.52, 198.98. HR-LSIMS m/z 784.5673 $(M + H)^+$ (calcd for $C_{44}H_{83}NO_6PS$ m/z 784.5674). TLC [$CHCl_3$:MeOH:H_2O (65:25:4)] R_f 0.41. The chemical purity was determined to be >99.0% by HPLC [column: Finepak SIL (Jasco, 5 μm, 4.6 × 150 mm), mobile phase: CH_3CN:H_2O (4:1), flow rate: 0.8 mL/min, detection: 225 nm, t_R 4.5 min].

Evaluation of Enantiomeric Excess of 13. The enantiomeric excess was determined by 600 MHz ^1H NMR of both the crude (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid [(*S*)-(−)-MTPA] ester and (*R*)-(+)-MTPA ester of the crude **13**, and proved to be >99.6% ee.

Compound **8** (196 mg, 0.350 mmol) was transformed to **13** (130 mg) by the procedure described above without any purification. To a solution of 115 mg (0.200 mmol) of the crude **13** and 117 mg (0.500 mmol) of (*S*)-(−)-MTPA in 2 mL of dichloromethane was added a solution of 103 mg (0.50 mmol) of dicyclohexylcarbodiimide in 0.5 mL of dichloromethane and 37 mg (0.300 mmol) of 4-(dimethylanimo)pyridine at 0 °C. The mixture was stirred for 5 h at room temperature, resulting in complete consumption of **13**. The precipitated materials were removed by filtration. The filtrate was washed with 0.1 N hydrochloric acid, 5% sodium hydrogencarbonate, and brine successively, dried, and concentrated to obtain crude (*S*)-(−)-MTPA ester (172 mg) of **13** for ^1H NMR analysis. Crude (*R*)-(+)-MTPA ester (165 mg) of **13** was prepared in a similar manner. The two singlet signals of OMe for each ester exhibited base line separation, and the signals at δ 3.52 for (*S*)-(−)-MTPA ester and at δ 3.48 for (*R*)-(+)-MTPA ester were major. Integration of the signals at δ 3.52 vs 3.48 for each ester indicated an enantiomeric excess of >99.6% for **13**.

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