JOC*Article*

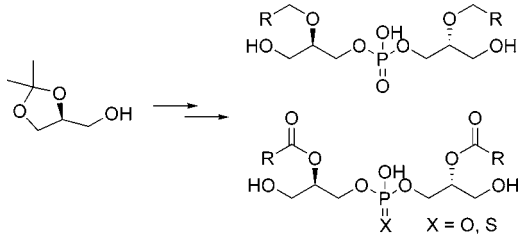
Practical Enantiospecific Syntheses of Lysobisphosphatidic Acid and Its Analogues

Guowei Jiang, Yong Xu, and Glenn D. Prestwich*

Department of Medicinal Chemistry, The University of Utah, 419 Wakara Way, Suite 205, Salt Lake City, Utah 84108-1257

gprestwich@pharm.utah.edu

Received September 8, 2005



We describe a versatile, efficient, and practical method for the preparation of enantiomerically pure lysobisphosphatidic acid (LBPA), bisether analogues, and phosphorothioate analogues of LBPA from solketal. Phosphorylation of a protected *sn*-2-*O*-oleoyl glycerol with 2-cyanoethyl bis(*N*,*N*-diisopropylamino)phosphite, followed by oxidation and deprotection, generated the enantiomers of 2,2′-LBPA. The corresponding phosphorothioate analogues were obtained by oxidation with sulfur. The (*R*,*R*) and (*S*,*S*) enantiomers of both LBPA and phosphorothioate LBPA were synthesized from (*S*)- and (*R*)-solketal, respectively. The ether analogue of (*S*,*S*)-lysobisphosphatidic acid (LBPA) and its enantiomer were synthesized from the same enantiomer (*S*)-solketal by simply changing the sequence of deprotection steps.

Introduction

Lysobisphosphatidic acid (LBPA) is a natural yet unusual phospholipid found in most tissues and cell types.[1,2] Although LBPA represents less than 1% of the total phospholipid mass,[3] increased LBPA titers have been found in several lipid disorders and as the result of certain therapeutic drugs.[4,5] Recent biochemical and immunocytochemical studies have shown that LBPA is highly enriched in late endosomes (≈15 mol %), but is not detected in other subcellular compartments.[6,7] This lipid is involved in cholesterol transport[8] and receptor trafficking.[7] LBPA has also been shown to be an antigen in the antiphospholipid syndrome, a condition in which endosomal sorting and multivesicular endosome formation is disrupted.[9] The trafficking defects observed in the cholesterol storage disorder Niemann−Pick type C can be recapitulated by disruption of LBPA function.[10,11] As a result, versatile synthetic access to enantiomerically and regiochemically defined LBPA and its analogues will further physiological research in the treatment of antiphospholipid syndrome lipid storage disorders.

The naturally occurring LBPA has a peculiar and interesting structure—two acyl chains at each of the *sn*-2 positions of

* Address correspondence to this author. Phone: 801-585-9051. Fax: 801-585-9053.

(1) Kobayashi, T.; Gu, F.; Gruenberg, J. *J. Sem. Cell Devel. Biol.* **1998**, *9*, 517−526.
(2) Amidon, B.; Brown, A.; Waite, M. *Biochemistry* **1996**, *35*, 13995−14002.
(3) Mason, R. J.; Stossel, T. P.; Vaughan, M. *J. Clin. Invest.* **1972**, *51*, 2399−2407.
(4) Cochran, F. R.; Connor, J. R.; Roddick, V. L.; Waite, M. *Biochem. Biophys. Res. Commun.* **1985**, *130*, 800−806.
(5) Brotherus, J.; Renkonen, O.; Fischer, W.; Hermann, J. *Chem. Phys. Lipids* **1974**, *13*, 178−182.
(6) Kobayashi, T.; Beuchat, M.; Chevallier, J.; Makino, A.; Mayran, N.; Escola, J.; Lebrand, C.; Cosson, P.; Kobayashi, T.; Gruenberg, J. *J. Biol. Chem.* **2002**, *277*, 32157−32164.
(7) Kobayashi, T.; Stang, E.; Fang, K. S.; Moerloose, P. D.; Parton, R. G.; Gruenberg, J. *Nature* **1998**, *392*, 193−197.
(8) Kobayashi, T.; Beuchat, M.; Lindsay, M.; Frias, S.; Palmiter, R. D.; Sakuraba, H.; Parton, R. G.; Gruenberg, J. *Nature Cell Biol.* **1999**, *1*, 133−138.
(9) Matsuo, H.; Chevallier, J.; Mayran, N.; Blanc, I. L.; Ferguson, C.; Faure, J.; Blanc, N. S.; Matile, S.; Dubochet, J.; Sadoul, R.; Parton, R. G.; Vilbois, F.; Gruenberg, J. *Science* **2004**, *303*, 531−534.
(10) Lebrand, C.; Corti, M.; Goodson, H.; Cosson, P.; Cavalli, V.; Mayran, N.; Faure, J.; Gruenberg, J. *EMBO J.* **2002**, *21*, 1289−1300.
(11) Mayran, N.; Parton, R. G.; Gruenberg, J. *EMBO J.* **2003**, *22*, 3242−3253.

10.1021/jo051894e CCC: $33.50
Published on Web 01/04/2006

glycerol backbones. However, this *sn*-2 acyl structure is very labile during isolation or preparation conditions.[12] Intramolecular acyl migration, which is facilitated by both acidic and basic conditions, results in an equilibrium between the 1-acyl- and 2-acyl-*sn*-glycerol 3-phosphates that favors the biologically inactive 1-acyl isomer. The instability of 2-acyl-*sn*-glycerol thus seriously compromises both isolation of the naturally occurring species as well as purification of chemically synthesized materials. The complex and fascinating structure and biology of LBPA motivated the development of a facile and practical synthetic route to LPBA and its analogues.

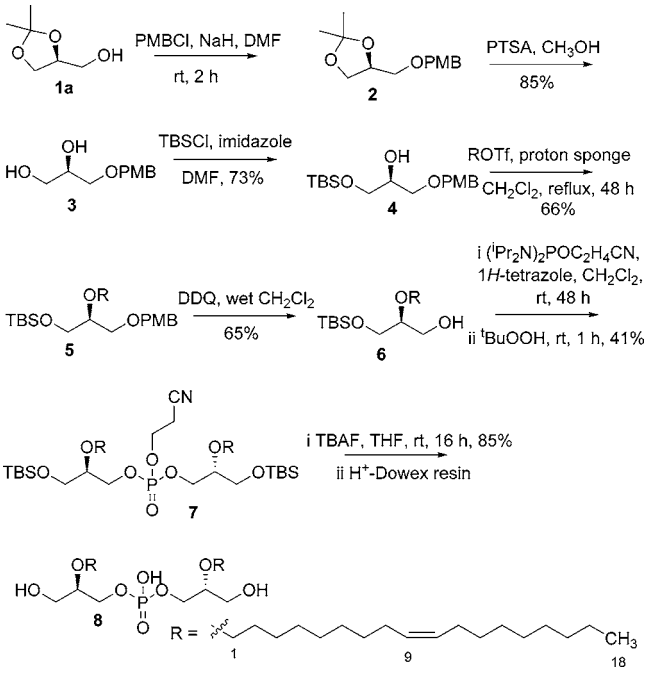
Replacement of acyl groups by alkyl chains in phospholipid compounds has afforded many valuable analogues.[13−15] For example, we found that alkyl analogues of lysophosphatidic acid (LPA) were equipotent with the natural acyl LPAs for three G-protein coupled LPA receptors.[16] Moreover, a strategic substitution of acyl by alkyl chain can enhance biological activity by altering pharmacokinetics and metabolism; the resulting alkyl analogues are useful probes for determining the mechanism of action. Since the alkyl chains cannot be hydrolyzed by phospholipase A,[13] alkyl substitution can introduce unexpected biological activity. Besides acyl chain hydrolysis, the phosphate diester group at the LBPA could be hydrolyzed in the presence of phosphatases.[17] One common approach for stabilization of phosphate diesters is the use of phosphothioate analogues in place of the phosphates. Phosphorothioates thus have potential applications for the selective manipulation of fundamental cellular responses that could validate new therapeutic approaches for human diseases and elucidate cellular signaling pathways.[18] We decided to test the hypothesis that bisether analogues of LBPA and phosphorothioate analogues of LBPA might mimic the 2,2′-bisacyl-LBPA as a biological ligand, but would lack the propensity to undergo intramolecular acyl migration or phosphate hydrolysis.

A modular strategy for the expedient preparation of enantiopure LBPA was described by using solketal and a protected dichlorophosphite.[12] This method demonstrated some advantages of P^{III} relative to the P^{V} chemistry commonly used in classical phospholipid synthesis. In a preliminary communication, we developed a general method for the preparation of migration-resistant bisether LBPA analogues in which the commercially available reagent 2-cyanoethyl bis(*N*,*N*-diisopropylamino)phosphite was employed for phosphorylation.[19] Herein, we describe the extension of this methodology to the multimilligram scale synthesis of both enantiomers of 2,2′-bisacyl LPBA and the corresponding phosphorothioate analogues. In addition, full experimental details of the bisether LBPA analogues synthesis are included.

Results and Discussion

The strategy for the synthesis of LBPA and its analogues was designed on the basis of the following considerations. First, the alkyl or acyl chains were installed early in the synthesis. Second, a commercially available phosphatidylating reagent (2-cyanoethyl-bis-*N*,*N*-diisopropylphosphordiamidite) was used to introduce both glycerol backbones simultaneously. It would be extremely difficult to install alkyl chain once the phosphate moiety was constructed. Both enantiomers of LBPA and its analogues could be synthesized from the same starting material, *S*-solketal, by phosphorlyation of either the 1- or the 3-position of glycerol backbone. Third, revealing the charged phosphodiester of the enantiomeric LBPA and its analogues at the end of the synthesis facilitated the purification of synthetic precursors. Conventional silyl protection of the hydroxyl groups coupled with the cyanoethyl ester protection of the phosphate was selected as the most promising approach; all protecting groups could thus be removed under mild conditions in a single final step to give the desired LBPAs.

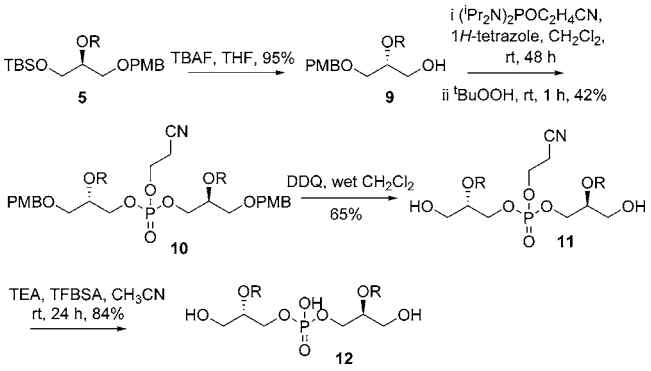
SCHEME 1. Synthesis of (*R*,*R*)-2,2′-Bisether-LBPA



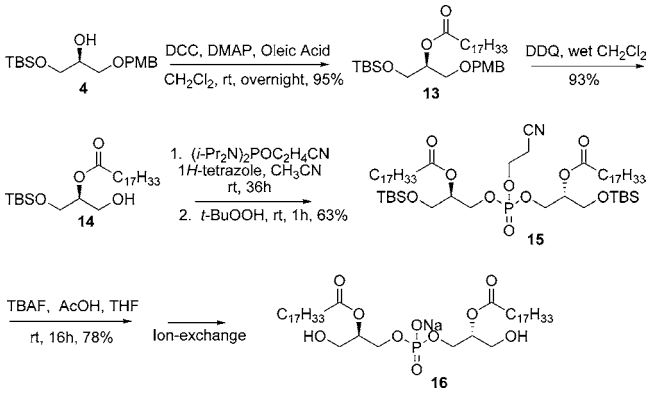
We selected *S*-solketal ((2*S*)-dimethyl-1,3-dioxolane-4-methanol) as our chiral starting material. Using the phosphoramidite methodology, widely exploited in nucleic acid chemistry, the alcohol was efficiently phosphorylated by using a variety of trivalent phosphorus reagents. The resulting phosphite triester could be oxidized in situ to yield the corresponding phosphate triester or phosphorothioate if desired. As shown in Scheme 1, protection of (*S*)-1,2-*O*-isopropylidene-*sn*-glycerol was performed with *p*-methoxybenzyl chloride (PMB-Cl) to give PMB ether, which was transketalized (10 mol % of *p*-TsOH in methanol) to the 1,2-diol in 83% isolated yield.[20] After silylation at the primary alcohol with *tert*-butyldimethylsilyl (TBDMS) chloride,[21] the secondary alcohol was alkylated with octadecenyl ((*Z*)-9-octadecen-1-yl) triflate in the presence of the hindered base "proton sponge" (1,8-bis(dimethylamino)naphthalene)[22] to give ether **5**. The octadecenyl triflate was prepared by a

(12) Chevallier, J.; Sakai, N.; Robert, F.; Kobayashi, T.; Gruenberg, J.; Matile, S. *Org. Lett.* **2000**, *2*, 1859−1861.
(13) Tyagi, S. R.; Burnham, D. N.; Lambeth, J. D. *J. Biol. Chem.* **1989**, *264*, 12977−12982.
(14) Kondapaka, S. B.; Singh, S. S.; Dasmahapatra, G. P.; Sausville, E. A.; Roy, K. K. *Mol. Cancer Ther.* **2003**, *2*, 1093−1103.
(15) Samadder, P.; Bittman, R.; Byun, H.; Arthur, G. *J. Med. Chem.* **2004**, *47*, 2710−2713.
(16) Xu, Y.; Tanaka, M.; Arai, H.; Aoki, J.; Prestwich, G. D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5323−5328.
(17) Lee, S.; Lynch, K. R. *Biochem. J.* **2005**, *391*, 317−323.
(18) Qian, L.; Xu, Y.; Hasegawa, Y.; Aoki, J.; Mills, G. B.; Prestwich, G. D. *J. Med. Chem.* **2003**, *46*, 5575−5578.
(19) Jiang, G.; Xu, Y.; Falguières, T.; Gruenberg, J.; Prestwich, G. D. *Org. Lett.* **2005**, *7*, 3877−3840.
(20) Chen, J.; Profit, A. A.; Prestwich, G. D. *J. Org. Chem.* **1996**, *61*, 1, 6305−6312.
(21) Qian, L.; Xu, Y.; Arai, H.; Aoki, J.; McIntyre, T. M.; Prestwich, G. D. *Org. Lett.* **2003**, *5*, 4685−4688.

SCHEME 2. Synthesis of (*S,S*)-2,2′-Bisether-LBPA



SCHEME 3. Synthesis of (*R,R*)-2,2′-Bisoleoyl-LBPA



modification to the literature protocol;[23] specifically, the use of 2,6-lutidine in place of pyridine significantly increased the yield by minimizing the *N*-alkylation of pyridine. Removal of the PMB group with DDQ in wet CH_2Cl_2 (0.5% water in volume) afforded the primary alcohol **6** in 65% yield without migration of the alkyl group from the 2-position to the 3-position. Coupling of two molecules of this alkyl glyceryl intermediate **6** with 2-cyanoethyl-bis-*N*,*N*-diisopropylphosphordiamidite in the presence of 1*H*-tetrazole followed by *t*-BuOOH oxidation gave the fully protected LBPA precursor **7** in medium yield. However, the use of the more reactive phosphatidylating reagent (2-cyanoethyl-bis-*N*,*N*-diisopropylphosphordiamidite) gave a disappointingly low yield (20%). The most frequently used reagent for the deprotection of the TBS group is tetra(*n*-butyl)ammonium fluoride, or TBAF. Since the cyanoethyl ester protective group is base labile, the basicity of TBAF was harnessed to simultaneously deprotect the cyanoethyl ester and TBS groups. The final deprotection was carried out in THF containing 10 equiv of TBAF at room temperature overnight. The final product (*R,R*)-2,2′-octadecenyl LBPA was readily purified on silica gel with use of CH_2Cl_2 and methanol (10:1, v:v) as the eluent.

The enantiomeric (*S,S*)-2,2′-octadecenyl LBPA was prepared from intermediate **5** as shown in Scheme 2. First, TBAF was used to remove the TBS group and gave the 2*R* configuration primary alcohol **9**. The 2*R* configuration alcohol **9** reacted with 2-cyanoethyl-bis-*N*,*N*-diisopropylphosphordiamidite in the presence of 1*H*-tetrazole, and subsequently was oxidized by *tert*-butyl hydrogen peroxide to give the fully protected (*S,S*)-LBPA **10** in high yield. Next, DDQ in wet CH_2Cl_2 (overnight, room temperature) completely removed both PMB protective groups to give the primary alcohol **11**. Under basic aprotic conditions in the presence of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide, deprotection of cyanoethyl ester occurred at room temperature and without any side reactions to yield the final bisether LBPA analogue **12**.[24] Both natural and unnatural enantiomers of LBPA can thus be obtained in optically pure form from the (*S*)-solketal. The routes are short and efficient and proceed in good overall yields.

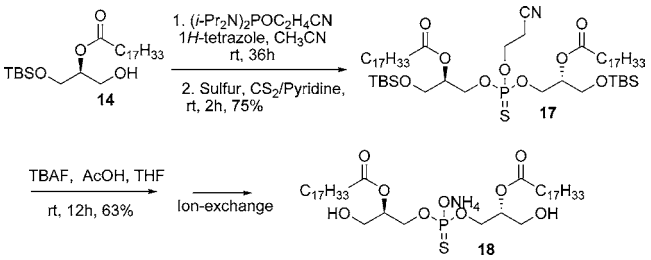
The syntheses of (*S,S*)-2,2′-bisoleoyl LBPA and its analogues were designed by using the same strategy as the synthesis of bisether LBPA. The acyl chain was installed instead of an alkyl chain on the 3-*O*-TBS-1-*O*-PMB-*sn*-glycerol building block. As shown in Scheme 3, 3-*O*-TBS-1-*O*-PMB-(2S)-*sn*-glycerol **4** was acylated with oleic acid in the presence of the DCC and DMAP to give ester **13**. Removal of the PMB group with DDQ in wet CH_2Cl_2 afforded the primary alcohol **14** in 93% yield without migration of the acyl group. Rapid elution with silica gel flash chromatography was necessary. Although the *sn*-2 acyl glycerol **14** was reasonably stable, extended contact with silica gel facilitated acyl chain migration. Coupling of two molecules of this acyl glyceryl intermediate **14** with 2-cyanoethyl-bis-*N*,*N*-diisopropylphosphordiamidite in the presence of 1*H*-tetrazole followed by *t*-BuOOH oxidation gave the fully protected LBPA precursor **15** in 63% yield.[25]

Since the migration of the acyl group from the 2-position to the 3-position is facilitated under basic conditions, TBAF neutralized with HOAc was employed for deprotection. As expected, the TBS group and cyanoethyl ester protective group were both removed. The final (*R,R*)-2,2′-bisoleoyl-LBPA **16** was readily purified on silica gel with cold (0 °C) CH_2Cl_2:methanol (6:1, v:v) as the eluent. No acyl migration was detectable by proton NMR. Importantly, the LBPA generated and purified in this fashion was the tetra-*n*-butylammonium salt, which was stable to purification on silica gel without acyl migration. The tetra-*n*-butylammonium LBPA salt was then converted to the sodium or ammonium salt by passage through a Dowex sodium or ammonium ion exchange column. The sodium and ammonium LBPA salts were stable for further NMR analysis, and optimal for biological studies. In this way, we have circumvented the instability[12] of acyl LBPA during its deprotection and purification. The product was soluble in CH_3OH and showed clear ^1H NMR signals, consistent with those reported previously.[12] The acyl chain migration can be monitored and determined accurately by proton NMR. For 2,2,-bisoleyl-LBPA, the *sn*-2 proton has the distinct resonance at 4.9 ppm. If acyl chain migration had occurred, the chemical shift of *sn*-2 proton would move to 4.0 ppm. Similarly, the chemical shift of *sn*-1 proton would change significantly if acyl chain migration had occurred.[12]

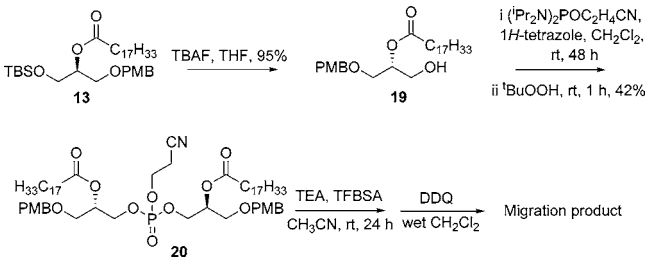
Coupling of two molecules of this acyl glyceryl intermediate **14** with 2-cyanoethyl-bis-*N*,*N*-diisopropylphosphordiamidite in the presence of 1*H*-tetrazole followed by elemental sulfur oxidation gave the fully protected phosphorothioate LBPA precursor **17** in 75% yield.[21] The phosphorothioate LBPA was obtained in 63% yield after deprotection of TBS and cyanoethyl groups by TBAF. The tetra-*n*-butylammonium salt was then converted to the ammonium salt by ion exchange for spectroscopic and biological studies.

(22) Heyes, J. A.; Niculescu-Duvaz, D.; Cooper, R. G.; Springer, C. J. *J. Med. Chem.* **2002**, *45*, 99−114.
(23) Aoki, T.; Poulter, C. D. *J. Org. Chem.* **1985**, *50*, 5634−5636.
(24) Heeb, N. V.; Nambiar, K. P. *Tetrahedron Lett.* **1993**, *34*, 6193−6196.
(25) Sekine, M.; Tsuruoka, H.; Iimura, S.; Kusuoku, H.; Wada, T. *J. Org. Chem.* **1996**, *61*, 4087−4100.

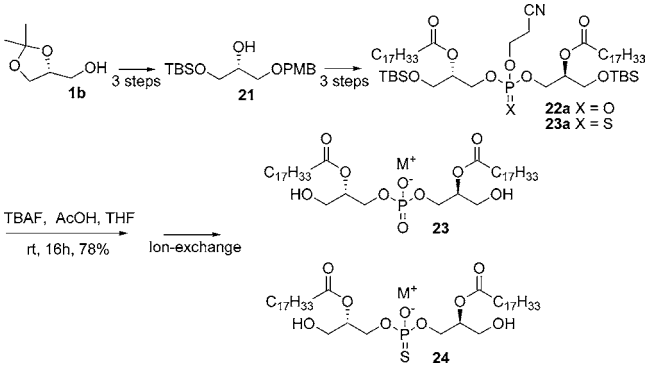
SCHEME 4. Synthesis of the Phosphorothioate Analogue 18 of (*R*,*R*)-2,2′-Bisoleoyl-LBPA



SCHEME 5. Synthesis of the (*S*,*S*)-2,2′-Bisoleoyl-LBPA



SCHEME 6. Synthesis of (*S*,*S*)-2,2′-Bisoleoyl-LBPA 23 and Its Phosphorothioate Analogue 24



Initially, we attempted to synthesize the enantiomeric (*S*,*S*)-2,2′-bisoleyl LBPA from intermediate **13** as shown in Scheme 5. First, TBAF was used to remove the TBS group and gave the 2*R* configuration primary alcohol **19**. The 2*R* configuration alcohol **19** reacted with 2-cyanoethyl-bis-*N*,*N*-diisopropylphosphordiamidite in the presence of 1*H*-tetrazole, and subsequently was oxidized by *tert*-butyl hydrogen peroxide to give the fully protected (*S*,*S*)-LBPA **20** in high yield. The protective groups were removed sequentially by TEA followed by DDQ. Unfortunately, the deprotection of PMB with DDQ in wet CH_2Cl_2 proved problematic, as substantial acyl chain migration occurred indicated by both TLC and NMR analysis. Deprotection of the PMB group with BBr_3 or TMSBr at low temperature (−78 to −20 °C) failed and afforded a complicated mixture.[26,27] From these results, we determined that it was unsuitable for preparation of 2,2′-bisoleoyl-LBPA. Therefore, we circumvented this problem for synthesis of (*S*, *S*)-2,2′-bisoleoyl-LBPA by starting from (*R*)-solketal and following the same procedure as for (*R*,*R*)-2,2′-bisoleoyl-LBPA.

Both the (*S*,*S*)-LBPA and phosphorothioate (*S*,*S*)-LBPA were prepared following the same procedures but starting from *R*-solketal as outlined in Scheme 6. The availability of both the natural *S*,*S* (as the biologically active configuration) and the unnatural *R*,*R* enantiomers (as experimental control) is crucial for in vitro structure−function studies with LBPA.

In summary, we have described a general and practical enantiospecific method for the preparation of LBPA, its bisether analogues, and its phosphorothioate analogue from commercially available reagents. Our synthetic strategy utilized TBAF for deprotection and neutralization of the final product, thereby solving the problem of obtaining and purifying LBPA without acyl migration. In addition, both enantiomers of bisether LBPA were synthesized from the same starting material, *S*-solketal, by phosphorlyation of either the 1- or the 3-position of the glycerol backbone.

Experimental Section

3-*O*-*tert*-Butyldimethylsilyl-1-*O*-methoxybenzyl-(2*S*)-glycerol (4). To an ice-cooled solution of 1.36 g (6.41 mmol) of 3-*O*-methoxybenzyl-(2*S*)-glycerol and 0.98 g (14.2 mmol) of imidazole in 5 mL of DMF was added 1.17 g (7.45 mmol) of *tert*-butyldimethylsilyl chloride in 3 mL of DMF over 1 h. Then the reaction mixture was kept at 4 °C overnight and stirred at room temperature for 8 h. Next, 5 mL of water was added to quench the reaction, and the mixture was extracted with 100 mL of ethyl acetate/hexane = 1/4 and 80 mL of diethyl ether. The combined organic extracts were washed with ice-cold 0.5 N HCl, saturated $NaHCO_3$ solution, followed by a saturated NaCl solution. Solvent was removed at reduced pressure and residue was purified by FC (ethyl acetate/ hexane, 1/5, v/v) to afford a colorless oil **4** (1.52 g, 73%): R_f 0.20 (ethyl acetate/hexane, 1/5, v/v); $[\alpha]^{20}_D$ −6.25 (*c* 3.04, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.20 (m, 2H), 6.82 (m, 2H), 4.42 (s, 2H), 3.66−3.80 (m, 4H), 3.60 (m, 2H), 3.42 (m, 2H), 2.44 (s, 1H), 0.83 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 159.2 (s), 130.1 (s), 129.3 (d, J = 91.6 Hz), 113.8 (s), 73.1 (s), 70.7 (s), 70.6 (s), 64.0 (s), 55.2 (s), 25.83 (s), 18.24 (s), −5.45 (s); MS (CI) *m*/*z* 325.2 ($M^+ - 1$). HRMS (CI) for $C_{17}H_{30}O_4Si$ (M^+): found 326.1936, calcd 326.1913.

3-*O*-*tert*-Butyldimethylsilyl-1-*O*-methoxybenzyl-(2*S*)-*O*-((*Z*)-9-octadecen-1-yl)-*sn*-glycerol (5). To a mixture of **4** (800 mg, 2.45 mmol), serachyl triflate (3.00 g, 7.20 mmol) and 1,8-bis(dimethylamino)naphthalene (proton sponge, 1.54 g, 7.20 mmol) under argon was added anhydrous distilled CH_2Cl_2 (12 mL). The yellow solution was refluxed under argon for 48 h. CH_2Cl_2 was then removed under reduced pressure to give dark brown oil, and hexane was added (60 mL). Then, the mixture was sonicated to ensure dissolution of product and filtered through a 1-in. bed of Celite 521, and the filtrate was evaporated under reduced pressure to yield orange oil. Silica gel FC (ethyl acetate/hexane, 1/100, v/v) gave **5** as a colorless oil (925 mg, 66%): R_f 0.23 (ethyl acetate/hexane, 1/15, v/v); $[\alpha]^{20}_D$ −1.28 (*c* 0.57, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.21 (m, 2H), 6.82 (m, 2H), 5.32 (m, 2H), 4.42 (m, 2H), 3.75 (s, 3H), 3.60 (d, J = 4.8 Hz, 2H), 3.52 (m, 3H), 3.42 (m, 2H), 1.96 (m, 4H), 1.50 (m, 2H), 1.16−1.36 (m, 24H), 0.83 (m, 12H), 0.01 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 159.1 (s), 130.5 (s), 129.9 (s), 129.8 (s), 129.2 (s), 113.7 (s), 79.5 (s), 73.0 (s), 70.6 (s), 69.7 (s), 70.6 (s), 62.9 (s), 55.2 (s), 35.6 (s), 31.9 (s), 30.1 (s), 29.8 (s), 29.7 (s), 29.6 (s), 29.5 (s), 29.5 (s), 29.3 (s), 29.3 (s), 29.2 (s), 27.2 (s), 26.1 (s), 25.9 (s), 22.7 (s), 18.2 (s), 14.1 (s), −5.4 (s), −5.5 (s); MS (CI) *m*/*z* 577.5 ($M^+ + 1$). HRMS (CI) for $C_{35}H_{64}O_4Si$ ($M^+ + 1$): found 577.4631, calcd 577.4652.

3-*O*-*tert*-Butyldimethylsilyl-(2*S*)-*O*-((*Z*)-9-octadecen-1-yl)-*sn*-glycerol (6). To a solution of glyceryl ether **5** (364 mg, 0.612 mmol) in 10 mL of wet CH_2Cl_2 was added DDQ (290 mg, 1.27 mmol). The mixture was stirred at room temperature overnight, diluted with CH_2Cl_2, washed with 10% $NaHCO_3$, dried over Na_2SO_4, concentrated, and purified on silica gel FC (ethyl acetate/hexane, 1/10,

(26) Imai, H.; Uehara, H.; Inoue, M.; Oguri, H.; Oishi, T.; Hirama, M. *Tetrahedron Lett.* **2001**, *2001*, 6219−6222.

(27) Wakabayashi, T.; Mori, K.; Kobayashi, S. *J. Am. Chem. Soc.* **2001**, *123*, 1372−1375.

v/v) to give compound **6** (188 mg, 65%): R_f 0.18 (ethyl acetate/hexane, 1/10, v/v); $[\alpha]^{20}_D$ +1.11 (*c* 0.48, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.28 (m, 2H), 3.66 (m, 2H), 3.53 (m, 3H), 3.32−3.47 (m, 2H), 2.14 (s, 1H), 1.94 (m, 4H), 1.48 (m, 2H), 1.16−1.32 (m, 24H), 0.81 (m, 12H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 129.9 (d, J = 13 Hz), 79.7 (s), 70.4 (s), 63.0 (s), 62.8 (s), 31.9 (s), 30.1 (s), 29.8 (s), 29.7 (s), 29.7 (s), 29.6 (s), 29.5 (s), 29.5 (s), 29.4 (s), 29.3 (s), 29.2 (s), 27.2 (s), 26.1 (s), 25.8 (s), 22.7 (s), 18.1 (s), 14.1 (s), −5.5 (s), −5.5 (s); MS (CI) *m*/*z* 457.2 (M^+ + 1). HRMS (CI) for $C_{27}H_{56}O_3Si$ (M^+): found 456.3983, calcd 456.3999.

2-Cyanoethyl Bis(3-*O*-*tert*-butyldimethylsilyl-(2*R*)-*O*-((*Z*)-9-octadecen-1-yl)-*sn*-glycer1-yl) Phosphate (7). To a solution of 2-cyanoethyl-bis(*N*,*N*-diisopropylamino)phosphine (51 mg, 0.167 mmol, 1.0 equiv) and 1*H*-tetrazole (70 mg 1.0 mmol, 6.0 equiv) in dry CH_2Cl_2 was added a solution of ether **6** (168 mg, 0.367 mmol, 2.2 equiv) in CH_2Cl_2. The mixture was stirred under argon for 48 h. Then *t*-BuOOH (0.075 mL, 0.67 mmol, 4.0 equiv) was added, and the reaction was stirred for 1 h. The reaction mixture was diluted with CH_2Cl_2, poured into saturated $Na_2S_2O_3$, and extracted with 2 × 20 mL of CH_2Cl_2. The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo, and the crude product was purified on silica gel FC (ethyl acetate/hexane, 1/5, v/v) to give 70 mg (0.068 mmol, 41%) of phosphate **7**. R_f 0.33 (ethyl acetate/hexane, 1/5, v/v); $[\alpha]^{20}_D$ −5.71 (*c* 0.41, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.33 (m, 4H), 4.20 (m, 4H), 4.05 (m, 2H), 3.37−3.67 (m, 10H), 2.72 (t, J = 6 Hz, 2H), 1.97 (m, 8H), 1.54 (m, 4H), 1.25 (m, 44H), 0.85 (m, 24H), 0.04 (s, 12H); ^{13}C NMR ($CDCl_3$) δ 129.8 (d, J = 11.5 Hz), 116.2 (s), 78.7 (s), 78.6 (s), 70.7 (s), 67.1 (s), 66.2 (s), 61.8 (s), 61.7 (s), 51.6 (s), 51.0 (s), 31.9 (s), 30.2 (s), 29.8 (s), 29.7 (s), 29.7 (s), 29.5 (s), 29.5 (s), 29.3 (s), 27.2 (s), 26.1 (s), 25.8 (s), 22.7 (s), 19.6 (s), 18.2 (s), 14.1 (s), −5.4 (s); ^{31}P NMR ($CDCl_3$) −0.91 (s); HRMS (MALDI) for $C_{57}H_{114}NNaO_8PSi_2$ (M^+ + Na): found 1050.7713, calcd 1050.7718.

Bis(3-Hydroxy-(2*R*)-*O*-((*Z*)-9-octadecen-1-yl)-*sn*-glyceryl) Phosphate (8). To a solution of protected **7** (50 mg, 0.0486 mmol) in anhydrous THF (10 mL) was added TBAF (90 mg, 0.285 mmol, 6 equiv). The reaction mixture was stirred at room temperature overnight. Concentration and silica gel FC (CH_2Cl_2:/MeOH, 6/1, v/v) furnished colorless oil. The oil was purified on H^+ Dowex ion-exchange resin to give compound **8** (31 mg, 85%): R_f 0.17 (CH_2Cl_2/MeOH, 5/1, v/v); $[\alpha]^{20}_D$ +2.33 (*c* 0.14, MeOH); ^{1}H NMR (CD_3OD) δ 5.26 (m, 4H), 3.97 (m, 4H), 3.40−3.60 (m, 10H), 1.93 (m, 8H), 1.48 (m, 4H), 1.21 (m, 44H), 0.80 (t, J = 7.2 Hz, 6H); ^{13}C NMR (CD_3OD) δ 128.9 (s), 128.8 (s), 78.6 (s), 78.6 (s), 69.5 (s), 63.4 (s), 63.3 (s), 59.9 (s), 31.1 (s), 29.3 (s), 28.9 (s), 28.9 (s), 28.8 (s), 28.1 (s), 28.5 (s), 28.4 (s), 28.4 (s), 26.2 (s), 26.2 (s), 25.3 (s), 21.8 (s), 12.5 (s); ^{31}P NMR (CD_3OD) 0.55 (s). HRMS (MALDI) for $C_{42}H_{83}NaO_8P$ (M^+ + Na): found 769.5745, calcd 769.5723.

1-*O*-Methoxybenzyl-(2*R*)-*O*-((*Z*)-9-octadecen-1-yl)-*sn*-glycerol (9). To a solution of protected **5** (420 mg, 0.739 mmol) in anhydrous THF (20 mL) was added TBAF·3H_2O (690 mg, 2.22 mmol). The reaction mixture was stirred at room temperature overnight. Concentration and silica gel flash chromatography (ethyl acetate/hexane, 1/5, v/v) furnished **9** (320 mg, 95%): R_f 0.10 (ethyl acetate/hexane 1/5, v/v); $[\alpha]^{20}_D$ +1.44 (*c* 0.49, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.18 (m, 2H), δ 6.81 (m, 2H), 5.27 (m, 2H), 4.10 (m, 2H), 3.73 (s, 3H), 3.40−3.65 (m, 7H), 1.94 (m, 4H), 1.50 (t, J = 7.2 Hz, 2H), 1.18−1.22 (m, 22H), δ 0.81 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 159.1 (s), 129.9 (s), 129.8 (s), 129.3 (s), 113.8 (s), 78.4 (s), 73.1 (s), 73.4 (s), 69.4 (s), 62.9 (s), 55.2 (s), 31.9 (s), 30.0 (s), 29.7 (s), 29.7 (s), 29.5 (s), 29.5 (s), 29.4 (s), 29.3 (s), 29.2 (s), 27.2 (s), 26.1 (s), 22.7 (s), 14.1 (s). HRMS (MALDI) for $C_{29}H_{50}NaO_4$ (M^+ + Na): found 485.3601, calcd 485.3607.

2-Cyanoethyl Bis(1-*O*-methoxybenzyl-(2*S*)-*O*-((*Z*)-9-octadecen-1-yl)-*sn*-glycer-1-yl) Phosphate (10). To a solution of 2-cyanoethyl bis(*N*,*N*-diisopropylamino) phosphine (370 mg, 0.80 mmol, 2.4 equiv) and 1*H*-tetrazole (139 mg 1.98 mmol, 6.0 equiv) in 1.5 mL of CH_3CN was added a solution of ether **9** (100 mg, 0.33 mmol, 1.0 equiv). The mixture was stirred under argon for 48 h. Then *t*-BuOOH (0.148 mL, 1.32 mmol, 4.0 equiv) was added, and the reaction was stirred for a further 1 h. The reaction was diluted with CH_2Cl_2, poured into saturated $Na_2S_2O_3$, and extracted with 2 × 40 mL of CH_2Cl_2. Combined organic phases were dried (Na_2SO_4) and concentrated in vacuo, and the crude product was purified on silica gel FC (ethyl acetate/hexane, 1/3, v/v) to give 142 mg (0.137 mmol, 42%) of phosphate **10**. R_f 0.41 (ethyl acetate/hexane, 1/1, v/v); $[\alpha]^{20}_D$ +15.14 (*c* 0.12, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.18 (m, 4H), 6.80 (m, 4H), 5.29 (m, 4H), 4.39 (m, 4H), 4.00−4.15 (m, 6H), 3.73 (s, 6H), 3.40−3.65 (m, 10H), 2.60 (t, J = 6.4 Hz, 2H), 1.92 (m, 8H), 1.47 (t, J = 6.4 Hz, 4H), 1.18−1.22 (m, 44H), 0.81 (t, J = 6.8 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 159.2 (s), 130.4 (s), 130.2 (s), 130.0 (s), 129.9 (s), 129.8 (s), 129.3 (s), 116.3 (s), 113.7 (s), 73.1 (s), 70.6 (s), 68.4 (s), 67.3 (s), 61.7 (s), 61.6 (s), 55.2 (s), 32.6 (s), 31.9 (s), 30.0 (s), 29.7 (s), 29.7 (s), 29.7 (s), 29.6 (s), 29.5 (s), 29.5 (s), 29.4 (s), 29.3 (s), 29.2 (s), 29.1 (s), 27.2 (s), 26.0 (s), 22.6 (s), 19.5 (s), 19.4 (s), 14.1 (s); ^{31}P NMR ($CDCl_3$) −0.39 (s). HRMS (MALDI) for $C_{61}H_{102}NNaO_{10}P$ (M^+ + Na): found 1062.7120, calcd 1062.7139.

2-Cyanoethyl Bis(3-hydroxy-(2*S*)-*O*-((*Z*)-9-octadecen-1-yl)-*sn*-glycer-1-yl) Phosphate (11). To a solution of **10** (110 mg, 0.106 mmol) in 2 mL of wet CH_2Cl_2 was added DDQ (120 mg, 0.529 mmol). The mixture was stirred at room temperature overnight, diluted with CH_2Cl_2, washed with 10% $NaHCO_3$, dried over Na_2SO_4, concentrated, and purified on silica gel FC (ethyl acetate/hexane, 2/1, v/v followed by CH_2Cl_2/MeOH, 25/1, v/v) to give compound **11** (57 mg, 67%): R_f 0.34 (CH_2Cl_2/MeOH, 15/1, v/v); $[\alpha]^{20}_D$ +3.80 (*c* 0.36, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.28 (m, 4H), 4.10−4.23 (m, 6H), 3.40−3.70 (m, 10H), 2.71 (t, J = 6.0 Hz, 2H), 1.94 (m, 8H), 1.49 (m, 4H), 1.20 (m, 44H), 0.81 (t, J = 5.6 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 129.95 (s), 129.77 (s), 116.28 (s), 81.15 (s), 77.85 (s), 70.59 (s), 66.12 (s), 66.06 (s), 61.99 (s), 60.78 (s), 60.75 (s), 31.88 (s), 29.94 (s), 29.74 (s), 29.68 (s), 29.50 (s), 29.43 (s), 29.30 (s), 29.26 (s), 29.19 (s), 26.01 (s), 22.66 (s), 19.67 (s), 19.60 (s), 14.10 (s); ^{31}P NMR ($CDCl_3$) 0.35 (s). HRMS (MALDI) for $C_{45}H_{86}NNaO_8P$ (M^+ + Na): found 822.6010, calcd 822.5989.

Bis(3-hydroxy-(2*S*)-*O*-((*Z*)-9-octadecen-1-yl)-*sn*-glycer-1-yl) Phosphate (12). To a solution of **11** (12 mg, 0.015 mmol) in 0.5 mL of CH_3CN was added 0.1 mL of BSTFA, followed by 0.5 mL of triethylamine. The mixture was stirred at room temperature overnight. Solvent was removed at reduced pressure. Then 0.5 mL of MeOH/H_2O (20/1, v/v) was added, and the mixture was stirred for 30 min, concentrated, and purified on silica gel (200−300 mesh, 10 g) FC (ethyl acetate, followed by CH_2Cl_2/MeOH, 5/1, v/v) to give a colorless oil. The oil was purified on H^+ Dowex ion-exchange resin (5 g) to give compound **12**: R_f 0.17 (CH_2Cl_2/MeOH, 5/1, v/v); $[\alpha]^{20}_D$ −2.36 (*c* 0.17, MeOH); ^{1}H NMR (CD_3OD) δ 5.33 (m, 4H), 3.90−4.10 (m, 4H), 3.40−3.70 (m, 10H), 2.02 (m, 8H), 1.56 (m, 4H), 1.26 (m, 44H), 0.89 (t, J = 6.8 Hz, 6H); ^{13}C NMR (CD_3OD) δ 128.9 (s), 128.8 (s), 78.6 (s), 78.6 (s), 69.5 (s), 63.4 (s), 63.3 (s), 59.9 (s), 31.1 (s), 29.3 (s), 28.9 (s), 28.9 (s), 28.8 (s), 28.1 (s), 28.5 (s), 28.4 (s), 28.4 (s), 26.2 (s), 26.2 (s), 25.3 (s), 21.8 (s), 12.5 (s); ^{31}P NMR (CD_3OD) −1.01 (s). HRMS (MALDI) for $C_{42}H_{83}NaO_8P$ (M^+ + Na): found 769.5718, calcd 769.5723.

3-*O*-*tert*-Butyldimethysilyl-1-*O*-methoxybenzyl-(2*S*)-*O*-oleoyl-*sn*-glycerol (13). To a solution of 3-*O*-*tert*-butyldimethysilyl-1-*O*-methoxybenzyl-2(*S*)-glycerol **4** (190 mg, 0.58 mmol) and oleic acid (0.22 mL, 0.70 mmol) in dry CH_2Cl_2 (1 mL) was added a solution of DCC (157 mg, 0.75 mmol) and DMAP (91 mg, 0.75 mmol) in dry CH_2Cl_2 (4 mL). The solution was stirred for 16 h at room temperature, filtered, and concentrated in vacuo, and the residue was purified by FC (ethyl acetate:hexane, 1:100, v:v) to afford 325 mg of **13** (95%) as a colorless oil: R_f 0.20 (ethyl acetate:hexane, 1:20, v:v); $[\alpha]^{20}_D$ −8.96 (*c* 0.44, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.10 (m, 2H), 6.83 (m, 2H), 5.30 (m, 2H), 5.00 (m, 1H), 4.42 (m, 2H), 3.76 (s, 3H), 3.69 (d, J = 4.8 Hz, 2H), 3.54 (m, 2H), 2.27 (t, J = 7.2 Hz, 2H), 1.96 (m, 4H), 1.58 (m, 2H), 1.16−1.36 (m, 20H), 0.83 (m, 12H), 0.01 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 173.3 (s), 159.2 (s), 130.1 (s), 130.0 (s), 129.7 (s), 129.2 (s), 113.7 (s), 72.9 (s),

72.8 (s), 68.0 (s), 61.6 (s), 55.2 (s), 34.4 (s), 31.9 (s), 29.7 (s), 29.7 (s), 29.5 (s), 29.3 (s), 29.2 (s), 29.1 (s), 27.2 (s), 27.2 (s), 25.8 (s), 25.0 (s), 22.7 (s), 18.2 (s), 14.1 (s), −5.5 (s), −5.5 (s); MS (ESI) *m*/*z* 591.59 ($M^+ + 1$). HRMS (MALDI) for $C_{35}H_{62}NaO_5Si$ ($M^+ +$ Na): found 613.4259, calcd 613.4264.

3-*O*-*tert*-Butyldimethysilyl-(2*S*)-*O*-oleoyl-*sn*-glycerol (14). To a solution of glyceryl ester **13** (420 mg, 0.713 mmol) in 20 mL of wet CH_2Cl_2 was added DDQ (247 mg, 1.09 mmol). The mixture was stirred at room temperature overnight, diluted with CH_2Cl_2, washed with 10% $NaHCO_3$, dried over Na_2SO_4, concentrated, and purified by FC quickly (ethyl acetate:hexane, 1:15, v:v) to give 311 mg of compound **14** (93%): R_f 0.12 (ethyl acetate:hexane, 1:10, v:v); $[\alpha]^{20}_D$ −7.06 (*c* 0.14, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.32 (m, 2H), 4.86 (m, 1H), 3.72−3.87 (m, 4H), 2.32 (t, J = 7.2 Hz, 2H), 2.00 (m, 4H), 1.59 (m, 2H), 1.20−1.35 (m, 20H), 0.85 (m, 12H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 173.7 (s), 129.9 (d, J = 28 Hz), 74.2 (s), 62.8 (s), 62.5 (s), 34.4 (s), 31.9 (s), 29.8 (s), 29.7 (s), 29.5 (s), 29.3 (s), 29.2 (s), 29.18 (s), 27.2 (s), 27.2 (s), 25.8 (s), 24.9 (s), 22.7 (s), 18.2 (s), 14.1 (s), −5.5 (s), −5.6 (s); MS (ESI) *m*/*z* 471.56 ($M^+ + 1$). HRMS (CI) for $C_{27}H_{55}O_4Si$ ($M^+ + 1$): found 471.3862, calcd 471.3869.

2-Cyanoethyl Bis(3-*O*-*tert*-butyldimethysilyl-(2*R*)-*O*-oleoyl-*sn*-glycer-1-yl) Phosphate (15). To a solution of 2-cyanoethyl bis-(*N*,*N*-diisopropylamino) phosphite (84 mg, 0.28 mmol, 1.0 equiv) and 1*H*-tetrazole (160 mg, 2.28 mmol, 8.0 equiv) in 0.5 mL of dry CH_3CN was added a solution of ester **14** (291 mg, 0.62 mmol, 2.2 equiv) in 1 mL of dry CH_3CN. The mixture was stirred under argon for 48 h. Then, *t*-BuOOH (0.126 mL, 1.12 mmol, 4.0 equiv) was added, and the reaction was stirred for 1 h. The reaction was diluted with CH_2Cl_2, poured into saturated $Na_2S_2O_3$, and extracted with 2 × 30 mL of CH_2Cl_2. The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo, and the crude product was purified by FC (ethyl acetate:hexane, 1:5, v:v) to give 181 mg of phosphate **15** (61%). R_f 0.18 (ethyl acetate:hexane, 1:3, v:v); $[\alpha]^{20}_D$ −9.21 (*c* 0.47, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.31 (m, 4H), 5.01 (m, 2H), 4.13−4.28 (m, 6H), 3.70 (m, 4H), 2.73 (t, J = 6.4 Hz, 2H), 2.29 (dt, J = 2.0 Hz, 7.6 Hz, 4H), 1.98 (m, 8H), 1.58 (m, 4H), 1.16−1.32 (m, 40H), 0.85 (m, 24H), 0.03 (s, 12H); ^{13}C NMR ($CDCl_3$) δ 173.0 (s), 129.9 (d, J = 13 Hz), 79.7 (s), 70.4 (s), 63.0 (s), 62.8 (s), 31.9 (s), 30.1 (s), 29.8 (s), 29.7 (s), 29.7 (s), 29.6 (s), 29.5 (s), 29.5 (s), 29.4 (s), 29.3 (s), 29.2 (s), 27.2 (s), 26.1 (s), 25.8 (s), 22.7 (s), 18.1 (s), 14.1 (s), −5.5 (s), −5.5 (s); ^{31}P NMR ($CDCl_3$) δ −0.42 (s); MS (ESI) *m*/*z* 1056.81 ($M^+ + 1$). HRMS (ESI) for $C_{57}H_{111}NO_{10}PSi_2$ ($M^+ + 1$): found 1056.7483, calcd 1056.7479.

2-Bis(3-hydroxyl-(2*R*)-*O*-oleoyl-*sn*-glycer-1-yl) Phosphate (16).[12] A solution of protected **15** (14 mg, 0.0133 mmol, 1 equiv) in anhydrous THF (1 mL) was treated successively with acetic acid (15.1 μL, 0.265 mmol, 20 equiv) and TBAF (84 mg, 0.265 mmol, 20 equiv) in 3 mL of THF. The reaction mixture was stirred at room temperature overnight. After concentration in vacuo, the residue was dissolved in 30 mL of $CHCl_3$ and transferred to a separatory funnel, and MeOH (20 mL) and water (10 mL) were added. After mixing, the lower layer was concentrated in vacuo. The residue was loaded onto silica gel (200−300 mesh, 10 g) and eluted quickly with CH_2Cl_2: MeOH (8:1 then 6:1, v:v) solution (precooled to 0 °C) to furnish 8 mg of a colorless oil **16** (78%): R_f 0.19 (CH_2Cl_2/MeOH, 5/1, v/v); ^{1}H NMR ($CDCl_3$ /CD_3OD) δ 5.28 (m, 4H), 4.89 (m, 2H), 3.95 (m, 4H), 3.65 (m, 4H), 2.25 (t, J = 7.6 Hz, 4H), 1.94 (m, 8H), 1.53 (m, 4H), 1.21 (m, 40H), 0.81 (t, J = 6.8 Hz, 6H); ^{13}C NMR ($CDCl_3$/CD_3OD) δ 173.6 (s), 129.6 (s), 129.1 (s), 72.8 (s), 71.5 (d, J = 7.7 Hz), 68.1 (s), 63.9 (s), 34.1 (s), 31.7 (s), 29.6 (s), 29.4 (s), 29.2 (s), 29.1 (s), 29.0 (s), 27.1 (d, J = 1.5 Hz), 24.8 (s), 22.5 (s), 13.9 (s); ^{31}P NMR ($CDCl_3$/CD_3OD) δ 2.46 (s); MS (ESI) *m*/*z* 775.67 ($M^+ + 1$).

2-Cyanoethyl Bis(3-*O*-*tert*-butyldimethysily-(2*R*)-*O*-oleoyl-*sn*-glycer-1-yl) Phosphorothioate (17). To a solution of 2-cyanoethyl bis(*N*,*N*-diisopropylamino) phosphite (98 μL, 0.30 mmol, 1.0 equiv) and 1*H*-tetrazole (170 mg, 2.4 mmol, 8.0 equiv) in 0.5 mL of dry CH_3CN was added a solution of ester **16** (300 mg, 0.63 mmol, 2.1 equiv) in 1.5 mL of dry CH_3CN. The mixture was stirred under argon for 48 h. Then sulfur (48 mg, 1.5 mmol, 5.0 equiv) and CS_2/pyridine (75 μL, 1:1, v/v) were added, and the reaction was stirred for an additional 2 h. The reaction mixture was filtered and the filtrate was washed with brine, dried over Na_2SO_4, and concentrated in vacuo, and the crude product was purified by FC (ethyl acetate: hexane, 1:15, v:v) to give 242 mg of phosphorothioate **17** (75%). R_f 0.50 (ethyl acetate:hexane, 1:5, v/v); $[\alpha]^{20}_D$ −2.43 (*c* 2.33, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.30 (m, 4H), 4.99 (m, 2H), 4.11−4.30 (m, 6H), 3.69 (d, J = 6.8 Hz, 4H), 2.70 (t, J = 6.0 Hz, 2H), 2.29 (dt, J = 2.8 Hz, J = 6.8 Hz, 4H), 1.97 (m, 8H), 1.58 (m, 4H), 1.16−1.32 (m, 40H), 0.84 (m, 24H), 0.03 (s, 12H); ^{13}C NMR ($CDCl_3$) δ 172.9 (s), 129.8 (d, J = 27 Hz), 71.9 (s), 66.5 (s), 62.2 (s), 60.9 (s), 60.8 (s), 34.2 (s), 31.9 (s), 29.7 (s), 29.5 (s), 29.3 (s), 29.2 (s), 29.1 (s), 27.1 (s), 25.7 (s), 24.8 (s), 22.6 (s), 18.1 (s), 14.1 (s), −5.5 (s); ^{31}P NMR ($CDCl_3$) δ 70.00 (s); MS (ESI) *m*/*z* 1072.2 (M^+). HRMS (ESI) for $C_{57}H_{111}NO_9PSSi_2$ ($M^+ + 1$): found 1072.7240, calcd 1072.7250.

2-Bis(3-hydroxyl-(2*R*)-*O*-oleoyl-*sn*-glycer-1-yl)phosphorothioate (18). A solution of protected **7** (52 mg, 0.052 mmol, 1 equiv) in anhydrous THF (1 mL) was treated successively with acetic acid (59 μL, 1.04 mmol, 20 equiv) and TBAF (329 mg, 1.04 mmol, 20 equiv) in 3.5 mL of THF. The reaction mixture was stirred at room temperature for 12 h. After concentration in vacuo, the residue was dissolved in 40 mL of $CHCl_3$, transferred to separatory funnel, and treated with MeOH (10 mL) and 8 mM acetate ammonium solution (20 mL). After mixing, the lower layer was concentrated in vacuo. The residue was loaded onto silica gel (200−300 mesh, 15 g) and eluted quickly with hexane:acetone (2:1 to 1:2, v:v, with a drop of 2.0 M ammonium hydroxide) solution (precooled to 0 °C) to furnish 26 mg of a colorless oil **18** (63%): R_f 0.20 (hexane:acetone, 1:1, v:v); $[\alpha]^{20}_D$ 6.72 (*c* 0.08, MeOH); ^{1}H NMR ($CDCl_3$/CD_3OD) δ 5.17 (m, 4H), 4.80 (m, 2H), 3.92 (m, 4H), 3.57 (m, 4H), 2.18 (t, J = 7.2 Hz, 4H), 1.84 (m, 8H), 1.44 (m, 4H), 1.21 (m, 40H), 0.71 (t, J = 6.4 Hz, 6H); ^{13}C NMR ($CDCl_3$/CD_3OD) δ 173.6 (s), 129.7 (d, J = 29 Hz), 72.5 (s), 62.9 (s), 59.4 (s), 58.4 (s), 33.9 (s), 31.6 (s), 29.4 (s), 29.2 (s), 29.0 (s), 28.8 (s), 26.9 (s), 24.5 (s), 22.4 (s), 13.7 (s); ^{31}P NMR ($CDCl_3$/CD_3OD) δ 60.54 (s); MS (ESI) *m*/*z* 789.5 ($M^+ - 1$). HRMS (MALDI) for $C_{42}H_{79}O_9PS$ (M^+): found 790.5141, calcd 790.5132.

Acknowledgment. We thank the NIH (NS29632 and HL070231) for financial support of this work.

Supporting Information Available: NMR spectra for the synthetic molecules, experimental procedures, and compound characterization for the synthesis of (*S*,*S*)-2,2′-oleoyl-LBPA and its phosphorothioate analogue. This material is available free of charge via the Internet at http://pubs.acs.org.

JO051894E