ORGANIC LETTERS

2005
Vol. 7, No. 18
3837−3840

Concise Synthesis of Ether Analogues of Lysobisphosphatidic Acid

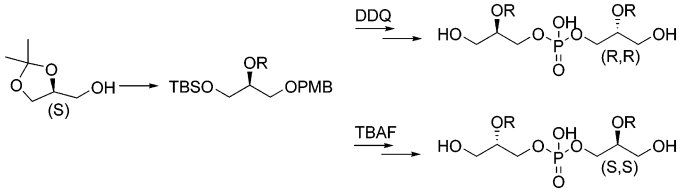
Guowei Jiang,[†] Yong Xu,[†] Thomas Falguières,[‡] Jean Gruenberg,[‡] and Glenn D. Prestwich*,[†]

Department of Medicinal Chemistry, The University of Utah, 419 Wakara Way, Suite 205, Salt Lake City, Utah 84108-1257, and Department of Biochemistry, Sciences II, 30 quai E. Ansermet, 1211-Geneva-4, Switzerland

gprestwich@pharm.utah.edu

Received May 20, 2005

ABSTRACT



We describe a versatile, efficient method for the preparation of ether analogues of (*S*,*S*)-lysobisphosphatidic acid (LBPA) and its enantiomer from (*S*)-solketal. Phosphorylation of a protected *sn*-2-*O*-octadecenyl glyceryl ether with 2-cyanoethyl bis-*N*,*N*-diisopropylamino phosphine and subsequent deprotection generated the bisether LBPA analogues. By simply changing the sequence of deprotection steps, we obtained the (*R*,*R*)- and (*S*,*S*)-enantiomers of 2,2′-bisether LBPA. An ELISA assay with anti-LBPA monoclonal antibodies showed that the bisether LBPAs were recognized with the same affinity as the natural 2,2′-bisoleolyl LBPA.

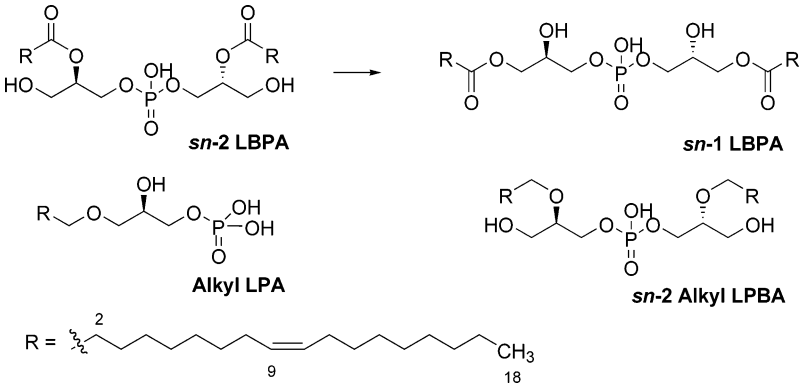
Lysobisphosphatidic acids (LBPAs), known also as bis-(monoacylglycerol)phosphates (BMPs), are natural phospholipids with unusual, poorly understood structure and activity.[1,2] LBPA was first discovered in pig lung homogenate in 1967 and has now been found in most tissues and cell types. It usually represents less than 1% of the total phospholipid mass,[3] but increased LBPA titers have been found in several lipidoses and in response to certain pharmaceutical agents.[4,5] Biochemical, immunocytochemical, and labeling studies have shown that LBPA is a major phospholipid of late endosomes (≈15 mol %), an obligatory station in the pathway followed by all down-regulated signaling receptors, and is not detected in other subcellular compartments.[6,7] This lipid is involved in cholesterol transport[8] and protein/receptor trafficking.[6] Recent studies also indicated that LBPA may regulate the sorting of the multifunctional receptor (IGF2/MPR), which may influence vesicular trafficking, lysosome biogenesis, cell growth, and angiogenesis.[6] LBPA has also been shown to be an antigen in the antiphospholipid syndrome, which can lead to changes in the endosomal sorting and multivesicular endosome formation.[9] Interfering with LBPA functions phenocopies the

[†] The University of Utah.
[‡] Sciences II.

(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.; Stang, E.; Fang, K. S.; Moerloose, P. D.; Parton, R. G.; Gruenberg, J. *Nature* **1998**, *392*, 193−197.
(7) 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.
(8) Kobayashi, T.; Beuchat, M.; Lindsay, M.; Frias, S.; Palmiter, R. D.; Sakuraba, H.; Parton, R. G.; Gruenberg, J. *Nat. 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/ol051194w CCC: $30.25 © 2005 American Chemical Society
Published on Web 08/02/2005

Scheme 1. Alkyl Analogues of LPA and LBPA



trafficking defects observed in the cholesterol storage disorder Niemann−Pick type C.[8,10,11] Therefore, LBPA analogues with agonistic effects may have potential therapeutic effects in treating antiphospholipid syndrome and possibly storage disorders, or analogues with antagonistic effects could be useful in cancer therapy.

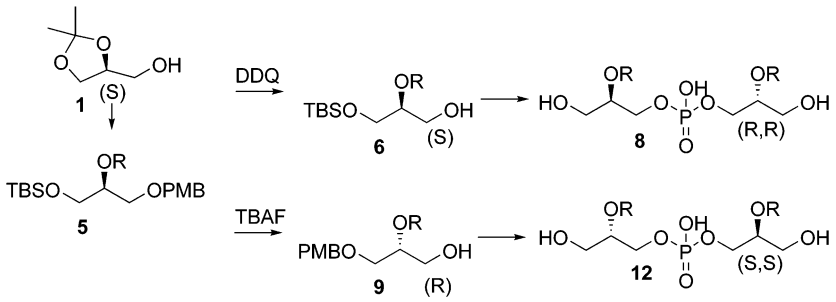
The naturally occurring LBPA has a peculiar and interesting structure: two acyl chains at each of the *sn*-2 positions of glycerol backbones. However, this *sn*-2 acyl structure is very labile during isolation and structural determination conditions.[12] Intramolecular acyl migration, which is facilitated by both acidic and basic conditions, affords an equilibrium mixture of 1-acyl- and 2-acyl-*sn*-glycerol 3-phosphates that favors the 1-acyl isomer (Scheme 1). The instability of 2-acyl-*sn*-glycerol thus seriously compromises both isolation of the naturally occurring species and determination of the activating ligand in structure−activity studies.

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. We decided to test the hypothesis that bisether analogues of LBPA might mimic 2,2′-bisacyl-LBPA as a biological ligand but would lack the propensity to undergo intramolecular acyl migration.

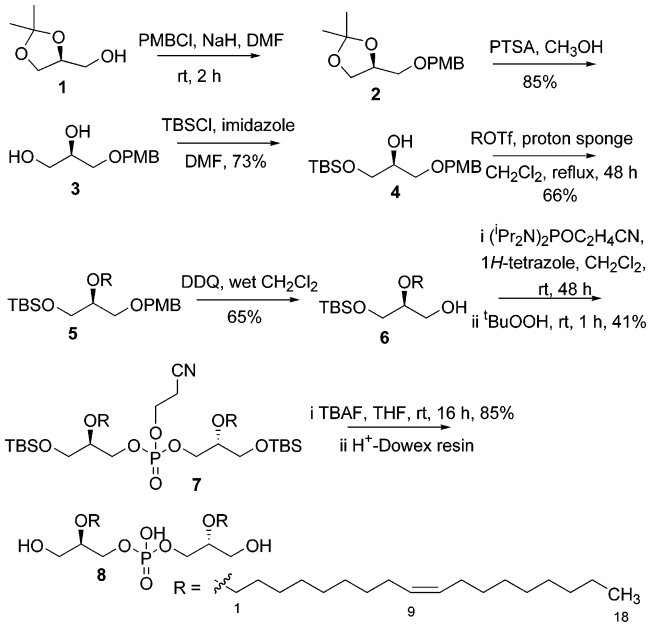
We describe herein a flexible, modular strategy for the expedient preparation of enantiopure alkyl LBPA analogues from a common intermediate (Scheme 2). The strategy for the synthesis of (*S*,*S*)-2,2′-bisether LBPA analogues was designed on the basis of the following considerations. First, the alkyl chains were installed early in the synthesis. Second, a commercially available phosphatidylating reagent (bis-*N*,*N*-diisopropylamino-cyanoethylphosphine) was used to introduce both glycerol backbones simultaneously. Both enantiomers of alkyl LBPA 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 2,2′-bisether LBPA 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 bisether LBPAs.

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 using

Scheme 2. Common Intermediate for the Enantiomeric LBPA Ethers



Scheme 3. Synthesis of (*R*,*R*)-2,2′-Bisether-LBPA

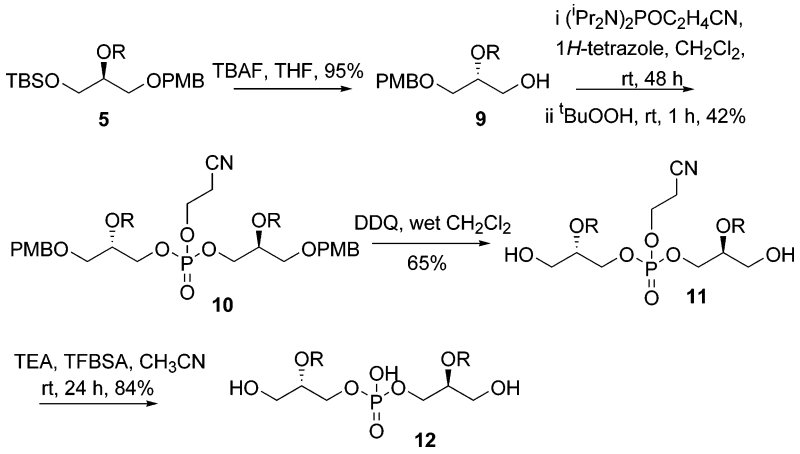


a variety of trivalent phosphorus reagents. The resulting phosphite triester could be oxidized in situ to yield the corresponding phosphate triester. Furthermore, this approach allows the introduction of a phosphorothioate moiety if desired. As shown in Scheme 3, protection of (*S*)-1,2-*O*-isopropylidene-*sn*-glycerol was performed with *p*-methoxybenzyl chloride (PMB-Cl) to give PMB ether, which was transketalized (10 mol % *p*-TsOH in methanol) to the 1,2-diol in 83% isolated yield.[17] After silylation at the primary alcohol with *tert*-butyldimethylsilyl (TBDMS) chloride,[18] 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)[19] to give ether **5**. The octadecenyl triflate was prepared by a modification to the literature protocol;[20] 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 (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 bis-*N*,*N*-diisopropylamino-cyanoethyl-phosphine in the presence of 1*H*-tetrazole followed by *t*-BuOOH oxidation gave the fully protected LBPA precursor **7** in medium yield. It is worth noting that the use of the more reactive phosphatidylating reagent (cyanoethyldichlorophosphine) gave a disappointingly low yield (20%). The most frequently used reagent for the deprotection of 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 using 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 4. First, TBAF was used to remove the TBS group and gave the 2*R*-configured primary alcohol **9**. The 2*R*-configured alcohol **9** reacted with bis-*N*,*N*-diisopropylamino cyanoethyl phosphine in the presence of 1*H*-tetrazole and was subsequently 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, rt) 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**.[21] Both natural and unnatural enantiomers of LBPA can thus be obtained in optically pure form from (*S*)-solketal. The routes are short and efficient and proceed in good overall yields.

Previous results indicated that LBPA was one of the physiological antigens that is recognized by sera from patients with antiphospholipid syndrome, which suggests that

Scheme 4. Synthesis of (*S*,*S*)-2,2′-Bisether-LBPA



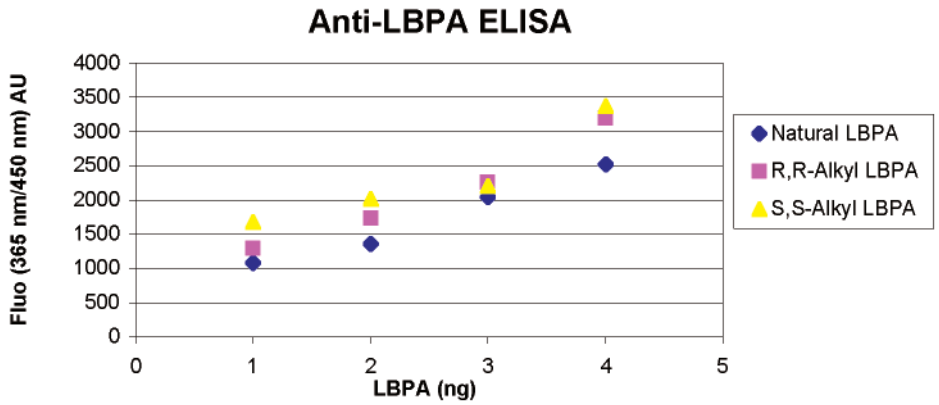


Figure 1. Recognition of bisether LBPAs and natural LBPA by anti-LBPA antiserum.

these antibodies exert some pathological effects intracellularly by altering endosomal sorting and/or trafficking of IGF2/MPR.[6] As IGF2/MPR is multifunctional, changes in its transport cycle may have multiple effects, including in vesicular traffic, lysosome biogenesis,[22] cell growth,[23] and angiogenesis.[24] The antiphospholipid syndrome, particularly fetal loss, may be at least partly related to the role of IGF2/MPR in endothelial cell migration and neovascularization.[24]

To determine whether the synthetic bisether LBPA analogues possessed the same physical and biological activities as natural LBPA, we initially tested these compounds using TLC analysis and ELISA. Purified LBPA was chromatographed on silica gel 60 HPTLC plates with chloroform/methanol/32% ammonia (65/35/5, v/v) as the developing solvent. The TLC analysis showed that alkyl-LBPAs and natural LBPA had the same R_f values (data not shown), which indicates that they share similar physical properties. Both (*R*,*R*)- and (*S*,*S*)-bisether analogues **8** and **12** were highly immunoreactive toward the 6C4 murine monoclonal antibody using ELISA. The comparative ELISA assay showed that analogues **8** and **12** had essentially equivalent immunoreactivity relative to natural (*S*,*S*)-2,2′-bisoleoyl LBPA (Figure 1).

In summary, we have described a general and efficient method for the preparation of LBPA bisether analogues from a common intermediate. The resulting compounds had comparable immunoreactivity relative to natural bisoleoyl LBPA. The evaluation of the bisether LBPA analogues in rescuing cells with a cholesterol storage disorder will be described elsewhere in due course.

Acknowledgment. We thank Dr. H. Zhang (University of Utah) for many helpful discussions. We acknowledge the NIH (NS29632 to G.D.P.) and the Swiss National Science Foundation (3100A0-104251 to J.G.) for financial support of this work. T.F. was supported by a fellowship from the Fondation pour la Recherche Médicale.

Supporting Information Available: Experimental procedures and characterization for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL051194W

(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) Chen, J.; Profit, A. A.; Prestwich, G. D. *J. Org. Chem.* **1996**, *61*, 6305−6312.
(18) Qian, L.; Xu, Y.; Arai, H.; Aoki, J.; McIntyre, T. M.; Prestwich, G. D. *Org. Lett.* **2003**, *5*, 4685−4688.
(19) Heyes, J. A.; Niculescu-Duvaz, D.; Cooper, R. G.; Springer, C. J. *J. Med. Chem.* **2002**, *45*, 99−114.
(20) Aoki, T.; Poulter, C. D. *J. Org. Chem.* **1985**, *50*, 5634−5636.
(21) Heeb, N. V.; Nambiar, K. P. *Tetrahedron Lett.* **1993**, *34*, 6193−6196.
(22) Kornfeld, S. *Annu. Rev. Biochem.* **1992**, *61*, 307−330.
(23) Wang, Z. Q.; Fung, M. R.; Barlow, D. P.; Wagner, E. F. *Nature* **1994**, *372*, 454−467.
(24) Volpert, O.; Jackson, D.; Bouck, N.; Linzer, D. I. *Endocrinology* **1996**, *137*, 3871−3876.