ORGANIC LETTERS

2000
Vol. 2, No. 13
1859−1861

Rapid Access to Synthetic Lysobisphosphatidic Acids Using P^{III} Chemistry

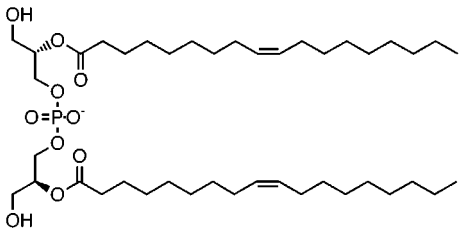
Julien Chevallier,[‡] Naomi Sakai,[†] Fabien Robert,[†] Toshihide Kobayashi,[‡,§] Jean Gruenberg,*[,‡] and Stefan Matile*[,†]

Departments of Organic Chemistry and Biochemistry, University of Geneva, CH-1211 Geneva, Switzerland

stefan.matile@chiorg.unige.ch

Received April 11, 2000

ABSTRACT



S,S-**1**

An expeditious route to synthetic lysobisphosphatidic acid *S,S*-1, its enantiomer, and regioisomers is reported. Synthetic difficulties concerning lipid stability and stereochemistry are bypassed using a phosphite triester approach in combination with multiple silyl protection. Spectroscopic studies evidence that acyl group migration in *S,S*-1 is accelerated by nonpolar solvents and inhibited by pyridine.

Lysobisphosphatidic acids (LBPAs), known also as bis-(monoacylglycerol)phosphates (BMPs), are natural phospholipids with unusual, poorly understood structure and activity (Scheme 1).[1] LBPAs have attracted scientific attention because of their proposed role in intracellular protein and lipid transport in healthy cells, their accumulation in pathologic liver tissues, and their function as antigens for human antibodies associated with the antiphospholipid syndrome.[1] The accumulation of LBPAs in intracellular, often multilamellar membranes is of particular interest because it relates these phospholipids to biomembrane polymorphism.[1d] Structural similarities between LBPA and the polymorphic, fusogenic cardiolipin[2] may be implicated in the supramolecular diversity of LBPA.[3]

The structure of natural LBPA of broadest acceptance (i.e., *S,S*-**1**, Scheme 1) is peculiar for two reasons. Most importantly, the molecule is not stable; intramolecular acyl chain migration,[4] expected to be facilitated under acidic and basic conditions, may yield LBPA *S,S*-**2**.[5] This instability of LBPA hampers isolation and reliable structure determination of the

[†] Department of Organic Chemistry.
[‡] Department of Biochemistry.
[§] Present address: Sphingolipid Functions Laboratory, RIKEN Frontier Research System, Saitama, Japan.

(1) (a) Kobayashi, T.; Beuchat, M.-H.; Lindsay, M.; Frias, S.; Palmiter, R. D.; Sakuraba, H.; Parton, R.; Gruenberg, J. *Nat. Cell Biol.* **1999**, *1*, 113. (b) Shinozaki, K.; Waite, M. *Biochemistry* **1999**, *38*, 1669. (c) Kobayashi, T.; Stang, E.; Fang, K. S.; de Moerloose, P.; Parton, R. G.; Gruenberg, J. *Nature* **1998**, *392*, 193. (d) Kobayashi, T.; Gu, F.; Gruenberg, J. *Sem. Cell Devel. Biol.* **1998**, *9*, 517. (e) Amidon, B.; Brown, A.; Waite, M. *Biochemistry* **1996**, *35*, 13995. (f) Huterer, S. J.; Hostetler, K. Y.; Gardner, M. F.; Wherrett, J. R. *Biochim. Biophys. Acta* **1993**, *1167*, 204. (g) Somerharju, P.; Renkonen, O. *Biochim. Biophys. Acta* **1980**, *618*, 407. (h) Body, D. R.; Gray, G. M. *Chem. Phys. Lipids* **1967**, *1*, 254.

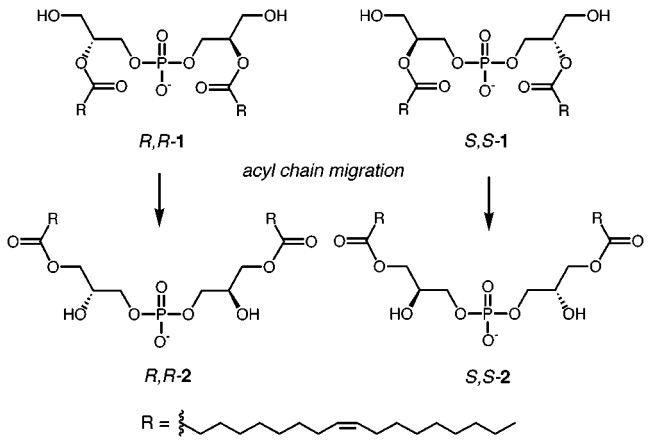
(2) (a) Ortiz, A.; Killian, J. A.; Verkleij, A. J.; Wilschut, J. *Biophys. J.* **1999**, *77*, 2003. (b) De Kruijff, B.; Verkleij, A. J.; Leunissen-Bijvelt, J.; Van Echtelt, C. J. A.; Hille, J.; Rijnbout, H. *Biochim. Biophys. Acta* **1982**, *693*, 1. (c) Cullis, P. R.; Verkleij, A. J.; Ververgaert, P. H. J. T. *Biochim. Biophys. Acta* **1978**, *513*, 11. (d) Rand, R. P.; Sengupta, S. *Biochim. Biophys. Acta* **1972**, *255*, 484.

(3) (a) Fuhrhop, J.-H.; Köning, J. *Membranes and Molecular Assemblies: The Cytokinetic Approach*; The Royal Society of Chemistry: Cambridge, U.K., 1994. (b) Yeagle, P. L. *The Structure of Biological Membranes*; CRC Press: Boca Raton, FL, 1992. (c) Kunitake, T. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 709. (d) Israelachvili, J. *Intramolecular & Surface Forces*, 2nd ed.; Academic Press Ltd.; London, U.K.; 1991.

(4) Fischer, E. *Ber. Dtsch. Chem. Ges.* **1920**, *53*, 1621.

10.1021/ol0059246 CCC: $19.00
Published on Web 05/27/2000

Scheme 1[a]



[a] The counterion of all phosphates (usually Na^+) is not shown; the lipid tail R may vary in nature. Common abbreviations: *S,S*-**1**, *sn*-1:*sn*-1′-BMP or 2,2′-LBPA; *R,R*-**1**, *sn*-3:*sn*-3′-BMP or 2,2′-LBPA; *S,S*-**2**, *sn*-1:*sn*-1′-BMP or 3,3′-LBPA; *R,R*-**2** *sn*-3:*sn*-3′-BMP or 3,3′-LBPA.

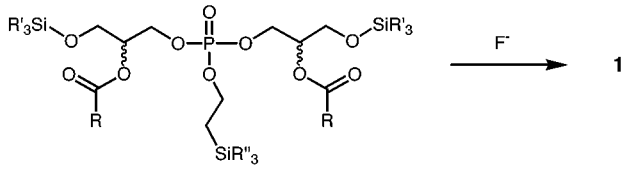
natural product(s) with regard to number and location of the acyl chains.[5,6]

Second, the absolute configuration of both chiral centers in *S,S*-**1** is rare, if not unique for natural lipids.[3b,7] The absolute configuration of *S,S*-**1** has been assigned by clever biochemical degradation procedures that operate on the stereoselectivity of *sn*-3-glycerophosphate dehydrogenase and various phospholipases.[7] However, it appears that the "unnatural" stereochemistry of LBPA has not yet been confirmed by spectroscopic methods.

In view of the interesting structural features of LBPAs, their biological importance, and apparent difficulties in isolating (or "biochemically" synthesizing) intact LBPAs in appreciable quantities, the development of rapid synthetic routes to LPBAs was of interest. In the following, we report a modular strategy for the expedient preparation of enantiopure lysobisphosphatidic acids *S,S*-**1** and *R,R*-**1** (Scheme 1). The chosen approach demonstrates some advantages of P^{III} compared to the P^V chemistry employed in classical phospholipid synthesis, bypasses acyl group migration using multiple silyl protection, and is compatible with variability of the hydrophobic tails. We further show that acyl group migration in LBPA **1** depends strongly on solvent polarity and can be prevented by the presence of pyridine.

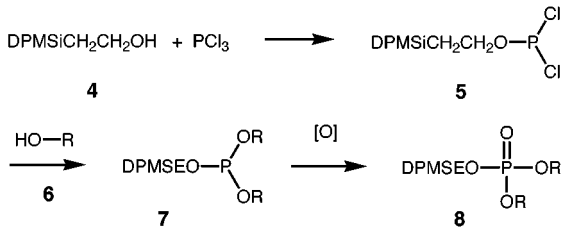
The strategy for the synthesis of *S,S*-**1** was designed on the basis of the following considerations. First, the primary alcohols in *S,S*-**1** were to be installed in the last step without the use of acid or base to avoid acyl chain migration (Scheme 1). Second, introduction of the phosphate anion in *S,S*-**1** at the end of the synthesis was desirable to facilitate purification of synthetic intermediates. Conventional silyl protection of the hydroxyl groups coupled with the more recent silylethyl protection of the phosphate[8] was selected as the most promising approach; all three protecting groups in **3** can be removed at once under mild conditions to give LBPA **1** (Scheme 2).

Scheme 2



Our silylethyl protecting group of choice, the 2-(diphenylmethylsilyl)ethyl group (DPMSE), is most conveniently introduced by the reaction of phosphorus trichloride and DPMS-ethanol **4** (Scheme 3).[8a] For the synthesis of LBPAs,

Scheme 3



this implied construction of the phosphate diester backbone of **1** using P^{III} chemistry that has been extensively refined for automated gene synthesis (i.e., condensation of the initial DPMSE-dichlorophosphines **5** with alcohols **6** followed by oxidation of the phosphite triesters **7** to give DPMSE-phosphate triesters **8**).[8,9] Compared to the P^V chemistry used in classical lipid synthesis, two additional advantages of a P^{III}-approach to LBPA were noted: the number of synthetic steps is reduced, and the acyl tails can be attached after construction of the bisglycerolphosphate scaffold.[10]

LBPA *S,S*-**1** was prepared along these lines (Scheme 4). Commercially available L-α,β-isopropylideneglycerol *R*-**6**

(5) Varying sensitivity of natural LBPA toward PLA assays during purification can be associated with such acyl chain migration (or loss): Kobayashi, T.; Chevallier, J.; Beuchat, M.-H.; Emery, G.; Kobayashi, T.; Gruenberg, J., unpublished results.

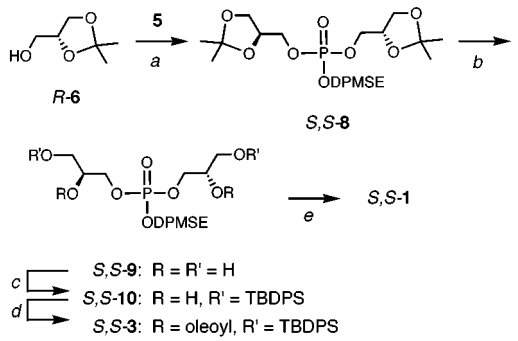
(6) Controversial results from NMR spectroscopic attempts to determine the acyl chain locations in natural LBPA are not surprising for this reason: (a) Mason, R.; Stossel, T.; Vaughn, M. *J. Clin. Invest.* **1972**, *51*, 2399. (b) Wherret, J.; Huterer, S. *Lipids* **1972**, *8*, 531.

(7) (a) Thornburg, T.; Miller, C.; Thuren, T.; King, L.; Waite, M. *J. Biol. Chem.* **1991**, *266*, 6834. (b) Joutti, A. *Biochim. Biophys. Acta* **1979**, *575*, 10. (c) Brotherus, J.; Renkonen, O.; Herrmann, J.; Fischer, W. *Chem. Phys. Lipids* **1974**, *13*, 178.

(8) (a) Ravikumar, V. T.; Wyrzyliewicz, T. K.; Cole, D. L. *Tetrahedron Lett.* **1994**, *35*, 9255. (b) Wada, T.; Sekine, M. *Tetrahedron Lett.* **1994**, *35*, 757. (c) Ravikumar, V. T.; Sasmor, H.; Cole, D. L. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2637. (d) Chao, H.-G.; Bernatowicz, M. S.; Klimas, C. E.; Matsueda, G. R. *Tetrahedron Lett.* **1993**, *34*, 3377. (e) Celebuski, J. E.; Chan, C. *J. Org. Chem.* **1992**, *57*, 5535. (f) Sawabe, A.; Filla, S. A.; Masamune, S. *Tetrahedron Lett.* **1992**, *33*, 7685. (g) Honda, S.; Hata, T. *Tetrahedron Lett.* **1981**, *22*, 2093.

(9) (a) Letsinger, R. L.; Finnan, J. L.; Heavner, G. A.; Lunsford, W. B. *J. Am. Chem. Soc.* **1975**, *97*, 3278. (b) Letsinger, R. L.; Lunsford, W. B. *J. Am. Chem. Soc.* **1976**, *98*, 3655.

Scheme 4[a]



[a] (a) (1) DPMS-ethanol **4**, PCl_3, rt, 3 h; (2) *R*-**6**, pyridine, rt, 1 h; (3) mCPBA, rt, 30 min, 63% (compare Scheme 3). (b) 80% aq. AcOH, 60 °C, 84%. (c) TBDPSCl, imidazole, DMF, 4 °C, 82%. (d) Oleic anhydride, DMAP, pyridine, 79%. (e) TBAF, AcOH, 89%.

was phosphitylated with dichlorophosphine **5** and oxidized to give DPMSE-phosphate triester *S*,*S*-**8**. Hydrolysis of *S*,*S*-**8** afforded tetraol *S*,*S*-**9**. Conventional, chemoselective TBDPS-protection of the primary hydroxyls in *S*,*S*-**9** followed by esterification of the secondary hydroxyls in *S*,*S*-**10** with oleic anhydride gave the trisilylated LBPA *S*,*S*-**3**.

As expected, initial attempts to deprotect *S*,*S*-**3** with TBAF in THF caused complete acyl chain migration to give *S*,*S*-**2** (Figure 1a). Neutralization of TBAF with acetic acid,[11]

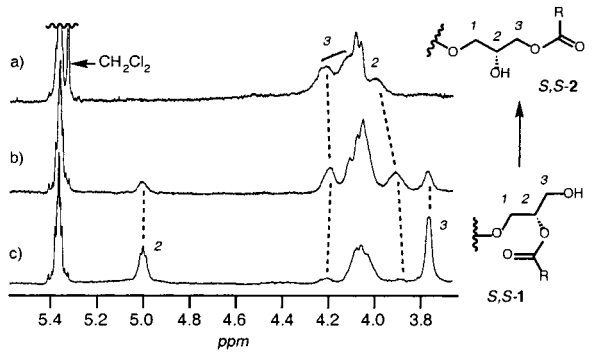


Figure 1. Parts of the 400 MHz ^{1}H NMR spectra of (a) *S*,*S*-**2** in $CDCl_3$, (b) the ~1:4 mixture of *S*,*S*-**1** and *S*,*S*-**2** obtained from TLC purification of *S*,*S*-**1**, and (c) *S*,*S*-**1** in $CDCl_3$ containing 0.1% v/v pyridine.

however, permitted desilylation of *S*,*S*-**3** without isomerization. Purification of synthetic LBPA *S*,*S*-**1** without significant acyl group migration was possible using DEAE-Sephadex ion exchange chromatography (Figure 1c) instead of silica columns or plates (Figure 1b).

The ^{1}H NMR spectrum of *S*,*S*-**1** in $CDCl_3$, characterized by distinct resonances at 5.0 and 3.8 ppm for the 2,2′- and 3,3′-hydrogens, respectively, changed into that of a stable ~1:4 mixture of *S*,*S*-**1** and *S*,*S*-**2** within 8 h (Figure 1c vs 1b). However, acyl group migration did not proceed to completion (Figure 1a). Identical observations made in benzene-d_6 were inconsistent with the expected catalysis of LBPA isomerization by the acidity of chloroform. The presence of strong acids such as TFA, however, caused rapid and complete conversion of *S*,*S*-**1** into *S*,*S*-**2** as expected. In contrast to possible catalysis by mild bases, the presence of pyridine in $CDCl_3$ (or benzene-d_6) strongly reduced acyl chain migration (Figure 1c). Indeed, *S*,*S*-**1** was stable at room temperature for weeks under these basic conditions. The improved resolution of the ^{1}H NMR signals under these conditions suggested that the increased solubility of **1** as the pyridinium salt accounts for near inhibition of acyl group migration in nonpolar solvents by pyridine.

The enantiomeric LBPA *R*,*R*-**1** ($[\alpha]^{20}_D$ +3.27 (*c* 0.49, $CHCl_3$)) was prepared along the route developed for *S*,*S*-**1** ($[\alpha]^{20}_D$ -3.60 (*c* 0.50, $CHCl_3$)) using alcohol *S*-**6** instead of *R*-**6** (Scheme 4). Preliminary results from immunological tests[1c] and conventional TLC assays for lipid identification[1c] support identity between synthetic *S*,*S*-**1** and natural LBPA isolated from late endosomes of baby hamster kidney (BHK) cells.

In summary, we report a rapid, modular synthesis of enantiopure lysobisphosphatidic acids (LBPAs) and show how to control acyl chain migration in vitro. This protocol is currently applied to the synthesis of LBPAs with various hydrophobic tails containing fluorescent tags and radiolabels to facilitate future biological studies of these peculiar lipids.

Acknowledgment. We thank A. Pinto and J.-P. Saulnier, the group of Prof. Gülaçar, and Dr. H. Eder for NMR, MS, and elemental analyses, respectively, and the Swiss NSF (31/55325.98 to J.G. and 21-57059.99 to S.M.), the International Human Frontier Science Program (RG0260/19999-M, J.G.), and Suntory Institute for Bioorganic Research (SUNBOR grant, S.M.) for financial support of this work.

Supporting Information Available: Experimental procedure and characterization for all new compounds and ^{1}H and ^{13}C NMR spectra of *S*,*S*-**1**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0059246

(10) (a) Sommerdijk, N. A. J. M.; Hoeks, T. H. L.; Booy, K. J.; Feiters, M. C.; Nolte, R. J. M.; Zwanenburg, B. *Chem. Commun.* **1998**, 743. (b) Sommerdijk, N. A. J. M.; Hoeks, T. H. L.; Synak, M.; Feiters, M. C.; Nolte, R. J. M.; Zwanenburg, B. *J. Am. Chem. Soc.* **1997**, *119*, 4338. (c) Kluba, M.; Zwierzak, A. *Synthesis* **1978**, 770. (d) Bauman, R. A. *Synthesis* **1974**, 870.

(11) Smith, A. B., III; Ott, G. R. *J. Am. Chem. Soc.* **1996,** *118,* 13095.