Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Tetrahedron Letters 45 (2004) 6923–6925
Tetrahedron Letters

A new convenient method for the synthesis of cardiolipin☆

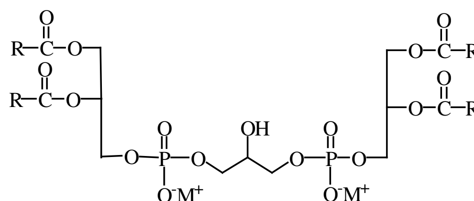
Zhen Lin, Moghis U. Ahmad, Shoukath M. Ali and Imran Ahmad*

NeoPharm Inc., 1850 Lakeside Drive, Waukegan, IL 60085, USA

Received 24 May 2004; revised 15 July 2004; accepted 19 July 2004
Available online 7 August 2004

Abstract—A novel dichlorophosphate coupling method is developed for the synthesis of the title compound. *O*-Chlorophenyl dichlorophosphate can be used as a mild phosphorylating reagent to effectively couple with optically active 1,2-*O*-diacyl-*sn*-glycerol and 2-*O*-protected glycerol to assemble cardiolipin bearing different fatty acid chains.
© 2004 Elsevier Ltd. All rights reserved.

Naturally occurring cardiolipin **1** is considered a family of complex phospholipids having two phosphatidyl moieties linked by a central glycerol group. Cardiolipin (CL) was first isolated from beef heart by Pangborn in 1942[2] and was later found to be ubiquitously present in mitochondria from mammalian tissues and eukaryotes, that is in all cells where mitochondria occur.[3,4] For decades, the biomembrane function of cardiolipin has not been well resolved, although it is widely believed that its function is related to its unique ability to interact with proteins.[4] Synthetic modifications of cardiolipin were generated with the intention to study cardiolipin–protein interaction and membrane structure. As the specific lipid component of mitochondria, CL plays major and diverse roles in the regulation of various mitochondrial processes including apoptosis, electron transport, and mitochondrial lipid and protein import. Many experiments revealed a correlation between cardiolipin content and diseases.[4,5] It was found that a reduction in the specific mitochondrial cardiolipin is an underlying biochemical cause of Barth Syndrome, a rare and often fatal X-linked genetic disease that is associated with cardiomyopathy.[5,6] Besides the numerous biomedical studies and applications, drug-delivery using CL are now the most widely investigated area of their practical applications.



1 Cardiolipin, R = fatty acid chain, $M^+ = H^+$
1a Tetramyristoyl cardiolipin, $M^+ = NH_4^+$

Liposomes formed by synthetic tetramyristoyl cardiolipin **1a** were found to be a unique way to deliver drugs to tumor cells.[7,8] This communication described a novel and effective method to the synthesis of optically active diphosphatidylglycerol (DPG) having the configuration of naturally occurring cardiolipin. The need for substantial amounts of DPG with different fatty acid residues for biological membrane research, identification of the role for cardiolipin involvement in human diseases, as well as for the development of novel liposome formulations containing active agents to improve cancer therapies provided an incentive for the present work.

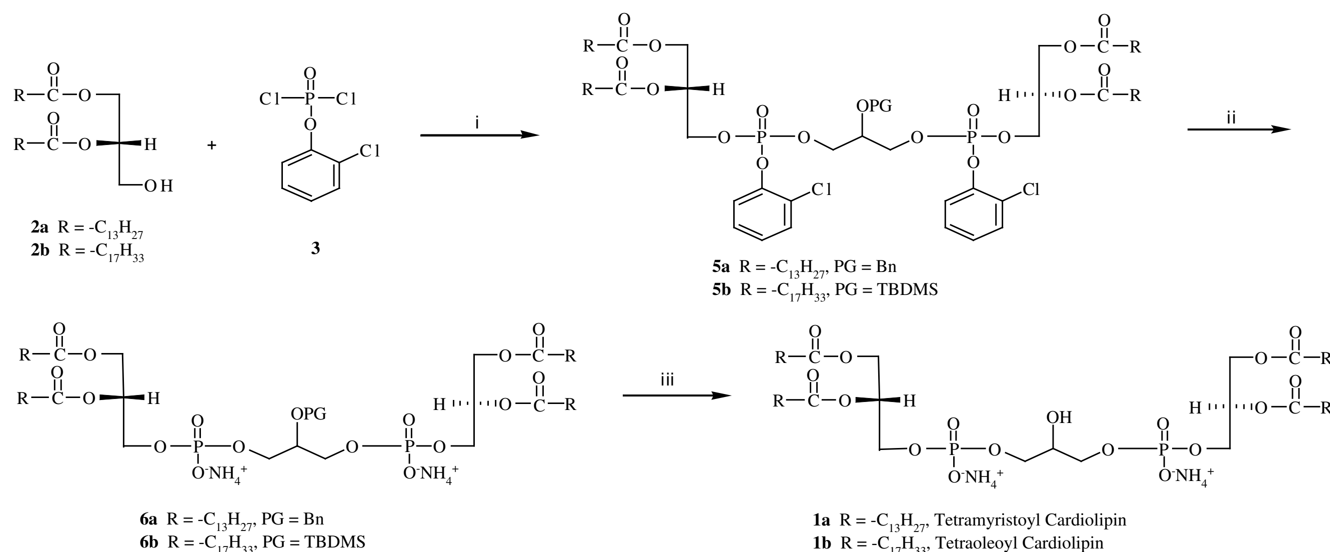
The synthetic approaches described to date for the synthesis of cardiolipin involve multi-steps along with difficulties in purifications, which result in poor yields.[9] In the course of our investigation, we have developed new synthetic approaches to synthesize cardiolipin and its analogues using phosphoramidite method.[10,11] Herein, we report a novel dichlorophosphate coupling method to prepare cardiolipin with or without unsaturation. In the phosphotriester approach shown in Scheme 1, the bifunctional phosphorylating reagent, *o*-chlorophenyl dichlorophosphate (CPDCP) **3**, is used to sequentially

Keywords: Cardiolipin; Diphosphatidylglycerol; Drug delivery.

☆ See Ref. 1.

* Corresponding author. Tel.: +1 847-887-0800; fax: +1 847-887-9281; e-mail: imran@neophrm.com

0040-4039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2004.07.084



Scheme 1. Reagents and conditions: (i) (a) pyridine, CH_2Cl_2, 0 °C, 1 h; (b) 2-*O*-benzylglycerol **4a** or 2-*O*-*t*-butyldimethylglycerol **4b**, CH_2Cl_2, rt, 6 h; (ii) (a) 2-nitrobenzaldoxime, *N*,*N*,*N*,*N*-tetramethylguanidine, THF, rt, 4 h; (iii) for **1a**, H_2, 10% Pd/C, THF, for **1b**, THF/H_2O/1 N HCl (10:5:0.1), then aq NH_4OH.

phosphorylate alcohols in a straightforward manner to directly provide cardiolipin core structure in a simple one-pot process. CPDCP has been used in nucleotide synthesis capable of directly reacting with two different alcohols in a stepwise manner to form an unsymmetric phosphotriester.[12] Such approach has not been used in the synthesis of cardiolipin. In Scheme 1, the optically active 1,2-di-*O*-myristoyl-*sn*-glycerol **2a** (2.5 mole equiv) in CH_2Cl_2 was added slowly to CPDCP (2.56 mole equiv) in CH_2Cl_2 in the presence of pyridine (12 mole equiv) at 0 °C. The mixture was stirred at 0 °C for 1 h, then warmed up to rt to complete the first chloride substitution, followed by coupling with 2-*O*-benzylglycerol **4a** (1 mole equiv) to form phosphotriester of cardiolipin precursor **5a** in good yield of 72% after column purification using hexane/ethyl acetate (3:1, v/v). It was found that the reaction temperature is critical to the success of the coupling reaction. When the first phosphorylation was conducted at ambient temperature, the reaction failed to give desired product **5a**.

The *o*-chlorophenyl protecting group was removed from **5a** using 2-nitrobenzaldoxime and *N*,*N*,*N*,*N*-tetramethylguanidine as described in the previous literature[13] to provide ammonium salt of cardiolipin precursor **6a** with 87% yield after column purification using $CHCl_3$/MeOH/NH_4OH (65:15:1, v/v/v). Tetramyristoyl Cardiolipin **1a** was obtained after removing benzyl group by hydrogenolysis over 10% Pd/C at 50 psi. The synthetic sequence involves three steps with 55% overall yield. This method is superior to the traditional arylphosphorodi(1,2,4-triazolide) approach due to the unnecessary conversion of CPDCP to arylphosphorodi(1,2,4-triazolide) as well as avoiding the use of an additional activating reagent such as 2,4,6-triisopropylbenzenesulfonyl chloride.[13,14] The novel dichlorophosphate coupling protocol is also applied in the synthesis of unsaturated cardiolipin derivatives, for example, tetraoleoyl cardiolipin **1b** (Scheme 1).

The most notable feature of this phosphotriester approach is that the troublesome 1,2 to 1,3-diglyceride isomerization is largely prevented. High resolution NMR experiments confirm no acyl group migration took place. The chemical shift of glycerol methylene protons of 1,3-di-*O*-myristoylglycerol[15] appeared at 4.12–4.19 ppm. However, the chemical shift of methine proton adjacent to 2-acyl group of 1,2-di-*O*-myristoylglycerol shifted downfield at 5.08 ppm. In **1a** and **1b**, the chemical shift of acylated glycerol methine proton appears at 5.26 and 5.19 ppm, respectively, indicating them to be 1,2-diglycerides. The homonuclear COSY spectra also clearly shows 1,2-correlation of two acylated glycerol protons. Comparison of bovine heart cardiolipin (Sigma Chemical Co.) with the synthesized compounds reveals similarity in IR and Mass spectra. The highly characteristic 500 MHz ^1H NMR spectra[16] of **1a**,**b** were essentially identical with bovine heart cardiolipin except for the additional resonances seen in the latter due to vinyl and allylic protons. The synthetic method described here offers a rapid access to synthesize a variety of cardiolipins.

Acknowledgements

We thank the bioanalytical group at NeoPharm, Inc. for Mass spectral analysis of all compounds.

References and notes

1. Presented in Part at the *227th ACS National Meeting*, Anaheim, CA, March 28–April 1, 2004: ORGN 512.
2. Pangborn, M. C. *J. Biol. Chem.* **1942**, *143*, 247–256.
3. (a) McMillin, J. B.; Dowhan, W. *Biochim. Biophys. Acta* **2002**, *1585*, 97–107; (b) Ioannou, P.; Golding, B. T. *Prog. Lipid Res.* **1979**, *17*, 279–318.
4. Schlame, M.; Rua, D.; Greenberg, M. L. *Prog. Lipid Res.* **2000**, *39*, 257–288.

5. Hatch, G. M. *Biochem. Cell Biol.* **2004**, *82*, 99–112.
6. Schlame, M.; Towbin, J. A.; Heerdt, P. M.; Jehle, R.; DiMauro, S.; Blanck, T. J. J. *Ann. Neurol.* **2002**, *51*, 634–637.
7. Deborah, J. *Drug Discov. Dev.* **2002**, *5*, 28–32.
8. (a) Gokhale, P. C.; Radhakrishnan, B.; Husain, S. R.; Abernethy, D. R.; Sacher, R.; Dritschilo, A.; Rahman, A. *Br. J. Cancer* **1996**, *74*, 43–48; (b) Gokhale, P. C.; Pei, J.; Jhang, C.; Ahmad, I.; Rahman, A.; Kasid, U. *Anticancer Res.* **2001**, *21*, 3313–3322.
9. (a) Saunders, R. M.; Schwarz, H. P. *J. Am. Chem. Soc.* **1966**, *88*, 3844–3847; (b) Ramirez, F.; Ioannou, P. V.; Marecek, J. F.; Dodd, G. H.; Golding, B. T. *Tetrahedron* **1977**, *33*, 599–608; (c) Mishina, I. M.; Vasilenko, I. A.; Stepanov, A. E.; Shvets, V. I. *Bioorg. Khim.* **1987**, *13*, 1110–1115; (d) Inoue, K.; Nojima, S. *Chem. Pharm. Bull.* **1968**, *16*, 76–81.
10. Lin, Z.; Ahmad, M. U.; Ali, S. M.; Ahmad, I. *Lipids* **2004**, *39*, 285–290.
11. Ukkalam, U.; Ahmad, M. U.; Ahmad, I. *Tetrahedron Lett.* **2004**, *45*, 2077–2079.
12. Reese, C. B. *Tetrahedron* **1978**, *34*, 3143–3179.
13. Duralski, A. A.; Spooner, P. J. R.; Rankin, S. E.; Watts, A. *Tetrahedron Lett.* **1998**, *39*, 1607–1610.
14. (a) Ramirez, F.; Marecek, J. F. *Synthesis* **1985**, 449–489; (b) van Boeckel, C. A. A.; van der Marel, G. A.; Westerduin, P.; van Boom, J. H. *Synthesis* **1982**, 399–402.
15. 1,3-Di-*O*-myristoylglycerol was synthesized by reacting 2-*O*-benzylglycerol with myristoyl chloride in the presence of pyridine and DMAP at 40 °C followed by hydrogenation with 10% Pd/C in ethanol.
16. All compounds were fully characterized by ^{1}H NMR, Mass spectra, and IR. Selected data of **1a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (br s, 8H), 5.26 (m, 2H), 4.34 – 3.92 (m, 13H), 2.33 (m, 8H), 2.29 (t, J = 7.5 Hz, 1H), 1.58 (m, 8H), 1.30 (br s, 80H), 0.88 (t, J = 6.5 Hz, 12H); FTIR (ATR) 3231s, 2918s, 2850s, 1738s, 1467w, 1378w, 1203ms, 1067s cm^{-1} ; ESI-MS (negative), *m*/*z* 619.9 $(M-2NH_4)^{2-}$, 1011.9 $(M-2NH_4-RCOO)^-$, 1240.2 $(M-2NH_4+H)^-$. **1b**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.43 (br s, 8H), 5.34 (m, 8H), 5.19 (m, 2H), 4.38–3.91 (m, 13H), 2.29 (m, 8H), 2.17 (br s, 1H), 2.01 (m, 16H), 1.58 (m, 8H), 1.29 (br s, 80H), 0.88 (t, *J* = 6.5 Hz, 12H). ESI-MS (negative), *m*/*z* 727.6 $(M-2NH_4)^{2-}$, 1174.2 $(M-2NH_4-RCOO)^-$, 1456.6 $(M-2NH_4+H)^-$.