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Tetrahedron Letters
Tetrahedron Letters 45 (2004) 2077–2079

Phosphoramidite approach for the synthesis of cardiolipin☆

U. Murali Krishna, Moghis U. Ahmad and Imran Ahmad*

NeoPharm Inc., Waukegan, IL 60085, USA

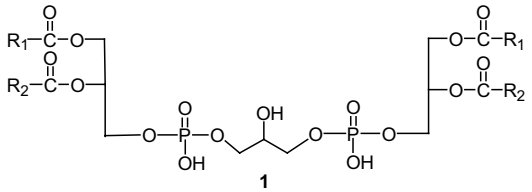
Received 10 November 2003; revised 16 January 2004; accepted 16 January 2004

Abstract—A phosphoramidite approach was utilized for the first time to synthesize cardiolipin. Optically active 1,2-di-*O*-acyl-*sn*-glycerol was coupled with 2-O-protected glycerols utilizing mono- and bifunctional phosphitylating agents to yield, after final removal of protecting groups, the title compound.

Cardiolipin (**1**) is a unique phospholipid with a dimeric structure having four acyl groups has long been associated with the mitochondria[2,3] and specifically with the proteins that conduct oxidative phosphorylation. It amounts to about 10% of the phospholipids of bovine heart muscle and 20% of the phospholipids of the mitochondrial membrane and appears to have a functional role in the regulation of gene expression.[4] Cardiolipin can be considered as a family of compounds depending on the type and distribution of fatty acids. In animal tissues it contains almost exclusively 18 carbon fatty acids (linoleic acid 18:2), yeast differs in having more 16:1 and 18:1 fatty acids, while the bacterial lipid contains saturated and monoenoic fatty acids with 14–18 carbons. Saturation and modification of precursors to mitochondrial phospholipids can lead to cardiolipin loss and suggests a general mechanism for cytochrome *c* release[5] in stress-induced apoptosis. The several synthetic approaches described for cardiolipin in literature utilize phosphorylating agents,[6–8] condensation reagents such as 2,4,6-triisopropyl benzenesulfonylchloride[9,10] and silver salts of phosphatidic acids.[11]

We have developed a novel method for the synthesis of cardiolipin and its analogues with varying fatty acid/alkyl chain with or without unsaturation. These compounds will be used for the development of novel liposome formulations containing active agents that will have more defined composition than those in clinical studies.[12] The serological specificity of cardiolipin in reacting with Wassermann antibody did not limit its application in liposomal drug delivery as evident by the number of liposomal products entrapped in cardiolipin liposomes.[13] The phosphoramidite approach[14] described herein could be applied to a broad spectrum of cardiolipins including saturated and unsaturated derivatives. Such an approach has not been used ever for cardiolipin synthesis.

Saturated analogues can be prepared much more easily than the unsaturated version since the synthetic procedures allow the use of common benzyl protecting groups and ease of debenzylation. However, the choice of protecting groups for alcohol and phosphate functionalities was the most crucial in unsaturated cardiolipin synthesis. We herein describe that benzyl and levulinoyl[15] groups can be efficiently utilized as protecting groups for the phosphate and hydroxyl functionalities in the synthesis of saturated (myristoyl) and unsaturated (oleoyl) versions of cardiolipin.



1

Cardiolipin

R_1, R_2= alkyl chain

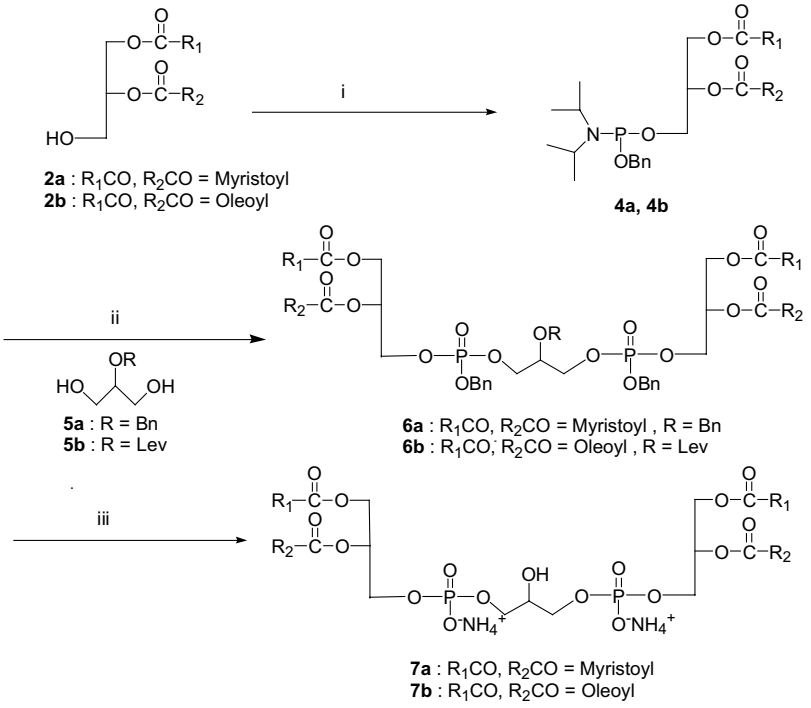
The formation of phosphotriester linkage between 1,2-*O*-diacyl-*sn*-glycerol and 2-O-protected glycerol via phosphoramidite coupling approach is outlined in Scheme 1. In the first step, treatment of 1,2-diacyl-*sn*-glycerol (**2**) with the bifunctional phosphitylating

☆ See Ref. 1.

Keywords: Cardiolipin; Phosphitylation; Phosphoramidites.

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

0040-4039/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.tetlet.2004.01.072



Scheme 1. Reagents and conditions: (i) (BnO)P(NPr$_2$-*i*) **3**, CH_2Cl_2, 1*H*-tetrazole, 1 h; (ii) **5a/5b**, 1*H*-tetrazole, 1 h, then TBHP, −40 to 20 °C; (iii) For **7a**. Pd/C, THF, H_2, 50 psi, NH_4OH; For **7b**. NaI/2-butanone, NH_4OH, NH_2NH_2, pyridine.

reagent benzyloxy bis-(diisopropylamino) phosphine (**3**) in CH_2Cl_2 in the presence of 1*H*-tetrazole for 1 h afforded 1,2-diacyl glyceryl (*N*,*N*-diisopropylamino) phosphoramidite (**4**), which was used as such for next reaction without any purification. The required phosphoramidite **4** was also prepared by benzylchloro (*N*,*N*-diisopropylamino) phosphoramidite and diisopropylethylamine in CH_2Cl_2. Subsequent coupling of **4** with 2-O-protected glycerol (**5**) in the presence of 1*H*-tetrazole gave intermediate phosphite triester, which was oxidized in situ with *tert*-butylhydroperoxide to desired cardiolipin precursor (**6**) in 73% yield. Finally the hydrogenolysis (H_2/Pd/C) of compound (**6a**) at 50 psi in tetrahydrofuran at room temperature for 6 h furnished tetramyristoyl cardiolipin (**7a**), which was isolated as the ammonium salt in excellent yield (90%). The benzyl groups of the phosphate moiety in **6b** were cleaved with sodium iodide in refluxing 2-butanone and converted into ammonium salt. The hydrazinolysis[16] was smoothly accomplished by treatment with hydrazine to obtain tetraoleoyl cardiolipin (**7b**). The products **7a** and **7b** were soluble in $CHCl_3$ and showed clear ^{1}H NMR signals. The synthetic cardiolipins[17] were compared to the natural product by NMR, IR, and Mass spectra and found to be similar. Our synthetic strategy exploited the inexpensive diacyl glycerols and common benzylic protecting groups eschewing the enediol pyrophosphates[6] and phosphatidic acids[10,11] for the synthesis of cardiolipin.

References and notes

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17. All new compounds were fully characterized by ^{1}H NMR, Mass spec, IR, and TLC. Selected data of **7a**: ^{1}H NMR δ

($CDCl_3$, 300 MHz) 0.88 (t, $J = 7.0$ Hz, 12H), 1.22–1.34 (br s, 80H), 1.52–1.66 (m, 8H), 2.26–2.34 (m, 8H), 3.06 (br s, 1H), 3.82–3.98 (m, 9H), 4.12–4.18 (m, 2H), 4.35–4.42 (m, 2H), 5.14–5.24 (m, 2H), 7.41 (br s, 8H). FTIR (ATR) 3207, 3035, 2956, 2918, 2850, 1737, 1467, 1378, 1206, 1092, 1067 cm^{-1}. ESI-MS (negative), m/z 1240.2 ($M-2NH_4^+ + H^+$), 1011.9 ($M-2NH_4^+ - RCOO^-$), 619.9 $(M-2NH_4^+)^{2-}$. **7b**: ^1H NMR δ ($CDCl_3$, 500 MHz) 0.88 (t, $J = 7.0$ Hz, 12H), 1.22–1.39 (m, 80H), 1.52–1.65 (m, 8H), 1.82 (br s, 1H), 1.96–2.07 (m, 16H), 2.23–2.35 (m, 8H), 3.83–3.94 (m, 7H), 4.12–4.23 (m, 4H), 4.33–4.39 (m, 2H), 5.17–5.23 (m, 2H), 5.28–5.39 (m, 8H), 7.41–7.59 (br s, 8H). ESI-MS (negative), m/z 1478 $(M-2NH_4^+ + Na^+)^-$, 1456 $(M-2NH_4^+)$, 1174.2 $(M-2NH_4^+ - RCOO^-)$, 727.5 $(M-2NH_4^+)^{2-}$.