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Tetrahedron Letters 45 (2004) 2743–2746
Tetrahedron Letters

Synthesis of novel cationic cardiolipin analogues for the optimal delivery of therapeutic agents ☆

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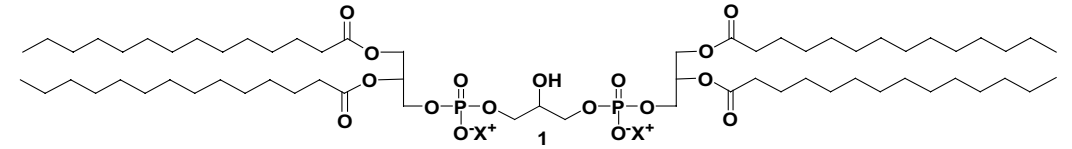
Received 26 November 2003; revised 4 February 2004; accepted 10 February 2004

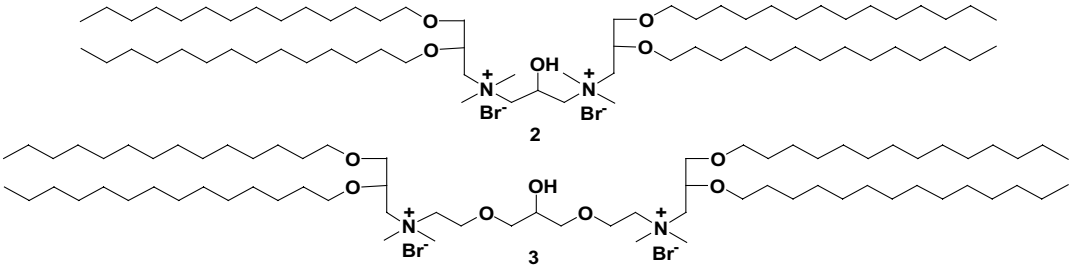
Abstract—A novel approach was developed to synthesize cardiolipin analogues containing two quaternary ammonium groups with tetraalkyl chain retaining 'glycerol' moiety, the central core of the molecule. The analogues were synthesized with or without spacer and/or lipid chain length with saturation to tailor lipid-based formulations of therapeutic agents for optimal delivery to target sites.

Felgner et al.[1] first reported the use of a cationic lipid as a synthetic vector for gene delivery into cells. Since then several groups reported the synthesis and uses of cationic lipids as gene carriers.[2] Cationic lipids[3] are also useful in liposomal formulations for the intracellular delivery of negatively charged biomolecules such as DNA,[4] mRNA,[5] antisense oligonucleotides,[6] proteins,[7] and antiviral drugs.[8]

Cardiolipin **1** (glycerol-bridged dimeric phosphatidic acid)[9] constitutes a class of complex phospholipids that occur mainly in the heart and skeletal muscles, showing high metabolic activity. There are few reports[10] in the literature on the synthesis of dimeric cationic lipids. Cationic lipid such as *N*-[1-(2,3-dioleyloxy)-*N*,*N*,*N*-trimethylammonium chloride (DOTMA),[11] which contains an ether linkage was shown to have much greater



Cardiolipin



Cationic cardiolipin analogues

Keywords: Cationic cardiolipin; Drug delivery; Gene transfer; Lipid based formulations.
☆ Presented in part at the 226th ACS National Meeting, New York, NY, September 7–11, 2003: ORGN 557.
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0040-4039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2004.02.044

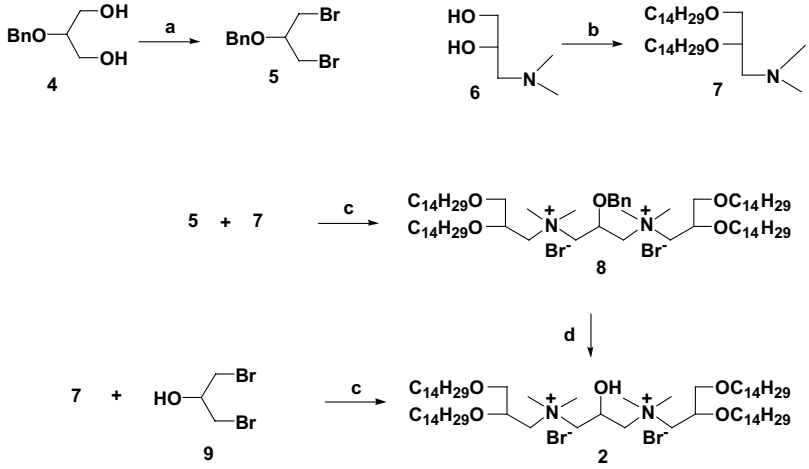
in vivo transfection efficiency than the corresponding ester analogue *N*-[1-(2,3-dioleyl)-*N*,*N*,*N*-trimethylammonium chloride (DOTAP).[11]

Despite numerous investigations of cationic lipids in gene therapy, most of the known cationic lipids possess certain toxicities. Recognizing the need for development of new cationic lipids to improve gene therapy and drug delivery, we undertook a program to design and synthesize a new class of cationic lipids. We developed synthetic methods for ether-linked cationic cardiolipin (dimeric cationic lipid) analogues. Cardiolipin has two negatively charged phosphate groups, which are replaced with quaternary ammonium groups to give cationic cardiolipin **2** (Scheme 1). We also prepared analogues of the cationic cardiolipin **2** by introducing oxyethylene (CH_2CH_2O) unit on both side of the central glycerol moiety to give spacer cationic cardiolipin **3** (Scheme 4).

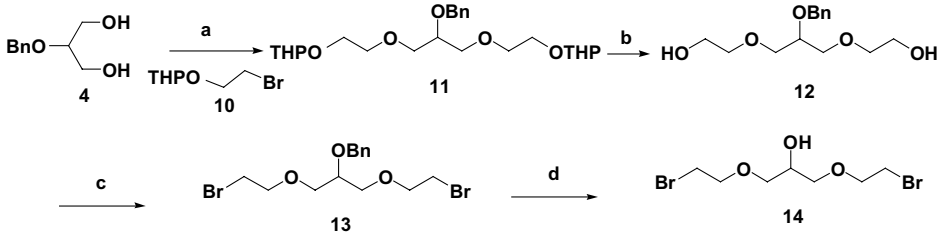
The synthesis of cationic cardiolipin **2** is outlined in Scheme 1. Commercially available 2-*O*-benzyl glycerol **4** was brominated with Ph_3P/CBr_4 in CH_2Cl_2 to afford 1,3-dibromo-2-*O*-benzyl glycerol **5** in yield of 87%. Williamson type etherification of racemic diol **6** with tetradecyl bromide in presence of NaH in DMF with slight modification of a literature procedure[12] gave dialkylated derivative 1,2-bis-(tetradecyloxy)-3-dimethylamine propane **7** in 68% yield. Cationic cardiolipin **2** was synthesized via quanternization of tertiary amine **7** with dibromo compound **5** in ethanol at reflux temperature for 5 days to afford **8** in low yield (8%) that on debenzylation gave quantitative yield of **2**. Since the reaction of **5** with **7** gave low yield of **8**, we improved the yield of cationic cardiolipin **2** by using 1,3-dibromo glycerol **9** without benzyl protecting group. This gave compound **2** in 77% yield.

Synthesis of spacer cationic cardiolipin **3** is outlined in Schemes 2–4. Alkylation of 2-*O*-benzyl glycerol **4** with 2-(2-bromoethoxy) tetrahydro-2*H*-pyran **10** in presence of NaH in DMF afforded 1,3 bis[(2-ethoxy tetrahydro-2*H*-pyran)]-2-*O*-benzyl glycerol **11** (71%). The THP derivative **11** on deprotection with catalytic amount of HCl (1 M in diethyl ether) in methanol at room temperature for 2 h gave the corresponding diol derivative 3,7-dioxa-5-*O*-benzyl-1,9-nonanediol **12** (88%). The diol **12** on bromination using triphenylphosphine and carbon tetrabromide (Ph_3P/CBr_4) in CH_2Cl_2 yielded 1,9-dibromo-3,7-dioxa-5-*O*-benzyl nonane **13** (91%). Debenzylation of **13** via hydrogenation gave 1,3-bis-(2-bromoethoxy) propane-2-ol **14** in 94% yield.

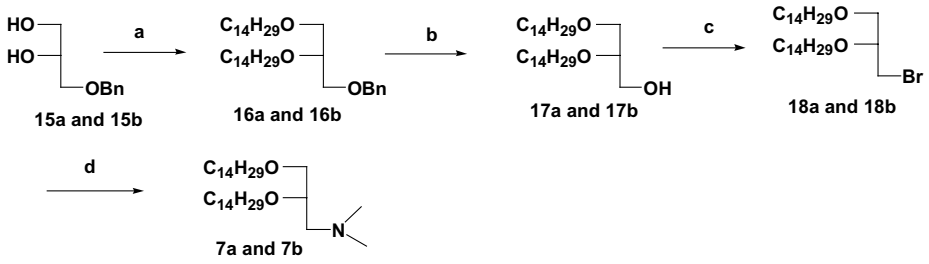
The cationic cardiolipin analogue **3** was synthesized in both optically pure (*R*) and (*S*) form, as well as racemic mixture (*R*,*S*). Since optically pure **6** was not commercially available, the synthesis of chiral (*R*) and (*S*) isomers of 1,2-bis-(tetradecyloxy)-3-dimethylamine



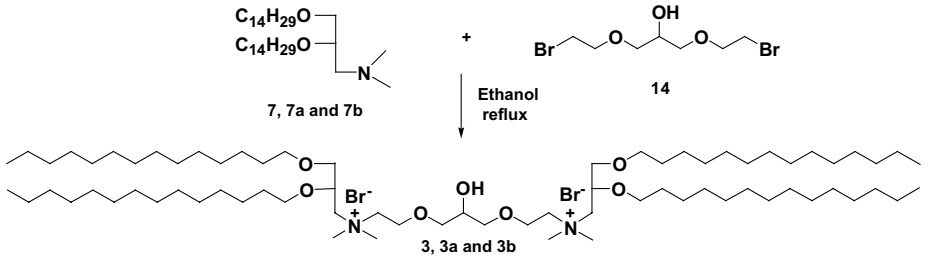
Scheme 1. Reagents and conditions: (a) Ph_3P, CBr_4, CH_2Cl_2, 0 °C to rt, 2 h; (b) NaH, $C_{14}H_{29}Br$, DMF, 60 °C, 2 days; (c) ethanol, reflux, 5 days; (d) 10% Pd/C, H_2, 50 psi, ethanol, 14 h.



Scheme 2. Reagent and conditions: (a) NaH, DMF, 0 ° to rt, 24 h; (b) 1 M HCl in diethyl ether, MeOH, rt, 2 h; (c) Ph_3P, CBr_4, CH_2Cl_2, 0 °C to rt, 2 h; (d) 10% Pd/C, H_2, 50 psi, ethanol, 14 h.



Scheme 3. Reagents and conditions: (a) NaH, $C_{14}H_{29}Br$, DMF, 60 °C, 10 h; (b) 10% Pd/C, H_2, 50 psi, ethyl acetate, 12 h; (c) Ph_3P, CBr_4, CH_2Cl_2, 0 °C to rt, 2 h; (d) 2 M dimethylamine in methanol, MeOH, pressure bottle, 90 °C, 60 h.



Scheme 4.

propane **7a** and **7b** were accomplished starting from commercially available (*S*) and (*R*)-1-*O*-benzyl glycerol **15a** and **15b**, respectively (Scheme 3). Williamson etherification of 1-*O*-benzyl glycerol **15a** and **15b** with tetradecyl bromide in presence of NaH in DMF afforded 1,2-bis-tetradecyloxy-3-*O*-benzylpropane desired products **16a** (75%) and **16b** (80%), respectively. Debenzylation of **16a** and **16b** via hydrogenation over 10% Pd/C catalyst in ethyl acetate gave 1,2-bis-tetradecyloxy propane-3-ol **17a** and **17b** in 92% yields, respectively. The alcohol **17a** and **17b** on reaction with Ph_3P/CBr_4 in CH_2Cl_2 afforded 1,2-bis-tetradecyloxy-3-bromopropane **18a** and **18b** in 90% and 87% yields, respectively. The bromo compound **18a** and **18b** on heating with 10-fold excess of 2 M methanolic dimethylamine solution in pressure bottle gave (*R*)-1,2-bis-tetradecyloxy-3-dimethylamine propane **7a** (88% yield) and (*S*)-1,2-bis-tetradecyloxy-3-dimethylamine propane **7b** (80% yield), respectively.

The optically pure (*R*) and (*S*) isomers **3a** (78% yield), **3b** (80% yield) as well as racemic (*R*,*S*) of spacer cationic cardiolipin **3** (69% yield) (Scheme 4) were obtained by quanternization of tertiary amine compounds **7a**, **7b**, and **7**, respectively, with dibromo derivative **14** in ethanol at reflux temperature for 5 days.

The method developed is novel and efficient to synthesize a series of cationic cardiolipin and its analogues. The solubility of compound **3** is increased in polar solvents compared to compound **2**, because of incorporation of oxyethylene group. The process is scalable from gram to kilogram scale. Cationic cardiolipin analogues will be used to formulate a broad range of therapeutic agents, including antisense oligonucleotode as well as gene transfection agents. Application of these cationic cardiolipin analogues to deliver antisense oligonucleotode (AON) and RNAi into the targeted cells in vitro and in vivo is under investigation and will be reported elsewhere.

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