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SYNTHESIS OF DIETHER-LINKED CATIONIC LIPIDS FOR GENE DELIVERY

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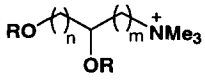
Abstract: Quaternary ammonium lipids **1b-d**, with diether linkages between hydrocarbon chains and butane or hexane backbone, were synthesized for cationic liposome-mediated gene delivery. The synthetic strategy of using C-4 or C-6 synthon permits the achievement of the variation of the hydrophobic domain as well as changes of space between the quaternary ammonium head and the hydrophobic domain in the diether-linked cationic lipids.

In 1987, Felgner and coworkers first reported the utilization of unnatural diether-linked cationic lipid (DOTMA) as a synthetic carrier to deliver gene into cells.[1] Since then, a number of published reports have described strategies for synthesis of versatile cationic lipids for gene delivery.[2,3] Cationic lipids, with prominent non-immunogenic character and low cellular toxicity in delivering gene, have engendered considerable interest by the gene therapy community.[4] It is generally believed that electrostatic interaction brings cationic lipids and polyanion DNA together to form DNA/liposome complexes. These complexes, once exposed to cells, are then taken up by the cells and the inserted gene expressed. In the course of our studies on cationic lipid-mediated gene delivery systems, we found that diether-linked cationic lipid DOTMA gave a higher in vivo transfection activity than did its diester analog.[5] Moreover, this trend was also found in our recent observation that diether-linked cationic lipid **1a** gave better activity in vivo over its diester cationic derivatives.[6] The unique in vivo gene delivery behavior of unnatural diether-linked cationic lipids has triggered us to synthesize more potential diether cationic lipids for the requirement ofestablishing a structure-function relationship between the lipids and the gene transfection activity. Thus, we recently developed a strategy for synthesis of lipid **1a**, in which the two-alkyl chains are in a 1,3-relationship.[7] In this communication, we focus our attention on the synthesis of new diether-linked cationic lipid analogs **1b-d** bearing different hydrophobic domain and variable length of linker between the quaternary ammonium head and the hydrophobic domain.



DOTMA: n=m=1, R= oleyl
1a: m=1, n=2, R= oleyl
1b: m=1, n=4, R= oleyl
1c: m=2, n=1, R= oleyl
1d: m=4, n=1, R= oleyl

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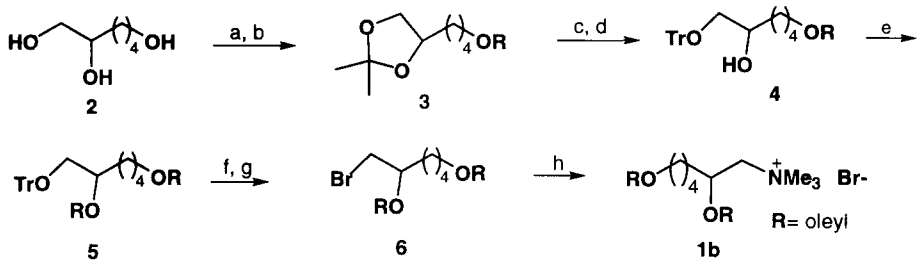
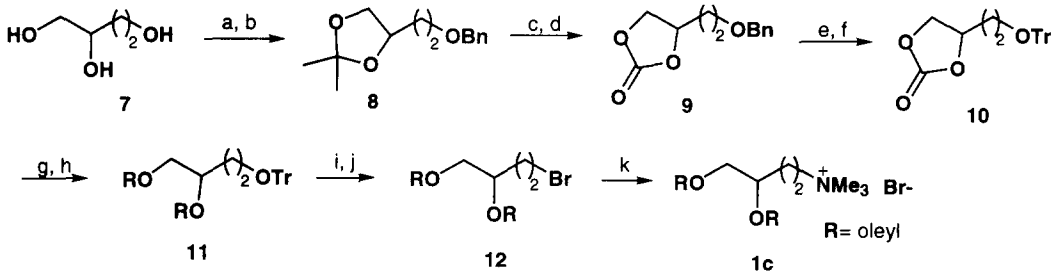
The synthesis of diether-linked cationic lipid **1b** is outlined in Scheme 1. The synthesis was started by acetonidation of 1,2,6-hexanetriol **2** with a catalytic amount of *p*-toluenesulfonic acid, followed by alkylation the primary alcohol to give dioxolane **3**. Acidic cleavage of ketal and selective mask the primary hydroxyl group as trityl ether furnished the secondary alcohol **4**. Subsequent alkylation of the secondary alcohol provided dialkyl compound **5**. Removal of the trityl-protecting group under acidic condition and bromination of the released primary alcohol with Appel reagent (CBr_4/Ph_3P) afforded bromide **6**. Finally, Menshutkin's type quaternarization[8] of the bromide **6** afforded the desired ammonium salt **1b** with the two-alkyl chains in a 1,5-relationship.



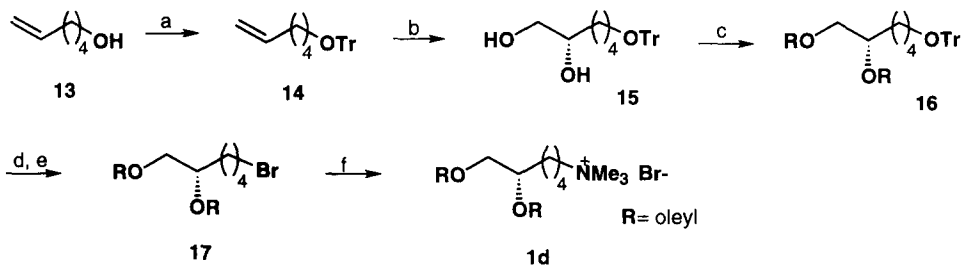
Scheme 1. *Reagents and conditions*: (a) Acetone, cat. *p*-TsOH, rt, 3 h, 92%; (b) NaH (1.5 equiv), RI (1.5 equiv), DMF, rt, 6 h, 76%; (c) THF-2 N HCl, reflux, 1 h, 92%; (d) TrCl (1 equiv), Py, 60 °C, 3 h, 83%; (e) NaH (1.5 equiv), RI (1.5 equiv), DMF, 60 °C, 12 h, 70%; (f) THF-MeOH-2 N HCl, reflux, 2 h, 90%; (g) CBr_4 (1.5 equiv), Ph_3P (1.5 equiv), CH_2Cl_2, rt, 4 h, 92%; (h) Me_3N-DMSO, rt, 72 h, pressure tube, 57%.



Scheme 2. *Reagents and conditions:* (a) Acetone, cat. *p*-TsOH, rt, 2 h, 82%; (b) BnCl (1.25 equiv), NaH (1.5 equiv), cat. NaI,DMF, rt, 12 h, 86%; (c) THF-2 N HCl, reflux, 1 h, 92%; (d) $CO(OEt)_2$ (4 equiv), THF, reflux, 6 h, 89%; (e) Pd-C, H_2, THF, 1 h, 98%; (f) TrCl (1.2 equiv), Py, 60 °C, 3 h, 89%; (g) KOH, MeOH-H_2O, reflux, 1 h, 98%; (h) NaH (2.5 equiv), RI (3 equiv), DMF, 80 °C, 4 h, 20%; (i) THF-2 N HCl, reflux, 1 h, 92%; (j) CBr_4 (1.5 equiv), Ph_3P (1.5 equiv), CH_2Cl_2, rt, 4 h, 92%; (k) Me_3N-DMSO, rt, 72 h, pressure tube, 55%.

The synthesis of diether quaternary ammonium **1c** (Scheme 2) began with acetonidation of 1,2,4-butanetriol **7** to furnish the corresponding ketal, *O*-benzylation the primary hydroxyl group in ketal afforded compound **8**. Acidic cleavage of ketal and introduction of the carbonate protection[9] of the corresponding vicinal diol afforded carbonate **9**. Deprotection of the benzyl function under catalytic

hydrogenolysis and tritylation of the released hydroxyl group gave the trityl ether **10**. Removal of carbonate protection under basic condition, followed by Williamson dialkylation to yield compound **11**. Detritylation under the acidic condition and Appel's type bromination of the liberated primary alcohol afforded bromide **12**. To the end, Menshutkin's type quaternarization of the bromide **12** produced the requisite ammonium salt **1c.** Comparison with DOTMA, compound **1c** has one more carbon inserted between the quaternary ammonium head group and its neighbor alkyl chain.



Scheme 3. *Reagents and conditions:* (a) TrCl (1.2 equiv), Py, 60 °C, 3 h, 89%; (b) AD-mix-α, *t*-BuOH-H_2O, 0 °C, 36 h, 90%; (c) RI (3 equiv), NaH (2.5 equiv), DMF, 80 °C, 4 h, 19%; (d) THF-2 N HCl, reflux, 1 h, 92%; (e) CBr_4 (1.5 equiv), Ph_3P (1.5 equiv), CH_2Cl_2, rt, 4 h, 87%; (f) Me_3N-DMSO, rt, 72 h, pressure tube, 55%.

In order to examine how variations in the distance between quaternary ammonium head group and its hydrophobic domain might affect the outcome of the gene transfer activity. We conducted the synthesis of compound **1d** (Scheme 3). Tritylation of commercial available 5-hexene-1-ol **13** in pyridine gave the trityl ether **14**. Sharpless asymmetric dihydroxylation[10] of the terminal olefin furnished (2*S*)-5-O-trityl-1,2-hexanediol **15** in an excellent yield with moderate enantioselectivity (62% ee). The enantiomeric purity of the diol was determined via ^1H NMR analysis of the bis Mosher ester prepared from (*R*)-(-)-MTPA chloride.[11] Williamson dialkylation afforded compound **16**. Detritylation and Appel's type bromination of the liberated primary alcohol afforded bromide precursor **17**. Eventually, Menshutkin's type quaternarization of the bromide **17** produced the resultant ammonium salt **1d.**

In summary, we have described the synthesis of diether-linked cationic lipids **1b-d**, with the structural feature of having different hydrophobic domain and variable length of linker between the quaternary ammonium head group and the hydrophobic domain. Application of these diether-linked cationic lipids as DNA carriers to deliver gene into cells in vitro and in vivo is now in progress.

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11. To a stirring solution of diol **15** (68 mg, 0.18 mmol) in dry pyridine (2 mL) was added (*R*)-(-)-α-methoxy-α-trifluoromethylphenylacetic acid chloride (MTPA-Cl, 100 mg, 0.396 mmol). After stirring at room temperature for 12 h, the reaction mixture was condensed in vacuo. The residue was taken up with ethyl acetate (20 mL) and washed with brine. The organic layer was dried over magnesium sulfate and concentrated under vacuo. The residue was purified by column chromatography (hexane/ ethyl acetate 100/15) to afford bis Mosher ester (127 mg, 87%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41-7.22 (m, 25H, Ar), 5.29 (m, H_c), 4.59 (dd, J = 12.2, 2.4 Hz, H_a= 0.81H), 4.26 (dd, J = 12.2, 6.5 Hz, H_b), 3.47 (s, 3H, CH_3O), 3.38 (s, 3H, CH_3O), 2.98 (t, J= 6.3 Hz, 2H), 1.53 (m, 4H), 1.22 (m, 2H) ppm.