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Synthesis of Cationic Lipids from 1,2,4-Butanetriol

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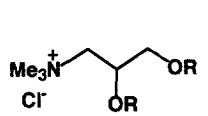
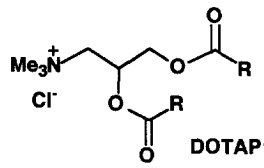
Abstract: Starting from 1,2,4-butanetriol, a series of quaternary ammonium lipids with diether, ether/ester, and diester bonds as linkage between the hydrocarbon chains and butane backbone were synthesized for gene delivery.

Cationic lipids are finding increasing applications in the field of gene therapy.[1] These compounds are considered to be capable of binding with DNA by means of electrostatic interaction, and carrying DNA into cells. Many cationic lipids have been synthesized in the past and shown to be active in transfecting cells *in vitro* and some of them have been tested *in vivo*. These include glycerol based cationic lipids,[2,3,4] carnitine based cationic lipids,[5] cholesterol based polyamine,[6,7] and lipopolyamine.[8] Recently, we found that the widely used glycerol based cationic lipid DOTMA gave 10 fold higher transfection activity than that of DOTAP *in vivo*.[9] The structural difference between the two types of cationic lipids is the linkage between the glycerol backbone and the hydrocarbon chains. Such difference has prompted us to study what effect varying the type of linkage has on the activity of cationic lipid-mediated gene transfection. For this purpose, we have synthesized a new series of cationic lipids with different linkage bonds starting from 1,2,4-butanetriol.



DOTMA: R= n-(9z)-$C_{18}H_{35}$



DOTAP: R= n-(9z)-$C_{17}H_{33}$

The synthesis of cationic lipids with diether linkages is outlined in Scheme 1. The synthesis was started by acetonidation of 1,2,4-butanetriol **1** with a catalytic amount of p-toluenesulfonic acid, affording the five membered acetonide **2**. As determined by ^{1}H and ^{13}C NMR observation, there was no detectable amount of a six-membered species, which is consistent with the literature.[10] Alkylation of the primary alcohol **2**, followed by acidic cleavage of ketal afforded diol **3** in a yield of 76% for the two steps. Selective tritylation of the primary hydroxyl group of the diol **3** gave trityl compound **4**. Subsequent alkylation of the secondary alcohol of compound **4** gave dialkyl compound **5**. Removal of the trityl-protecting group by the

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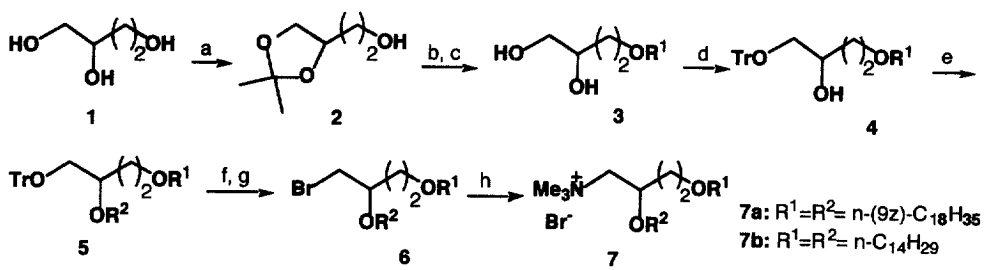
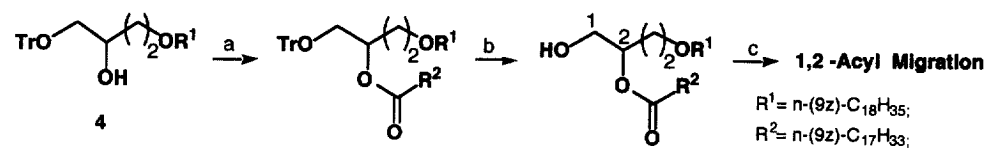
standard method yielded the primary alcohol, which was subsequently transformed into bromide **6** with Appel reagents (CBr_4/PPh_3). Treatment of bromide **6** with trimethyl amine in dry dimethyl sulfoxide gave the desired quaternary ammonium salt **7a-7b** in moderate yields.



Scheme 1. (a) Acetone, cat p-TsOH, rt, 2h, 82%; (b) R^1I, NaH, DMF, rt, 6h; (c) THF-2N HCl, reflux, 2h, 76% for two steps; (d) TrCl, Py, 60 °C, 3h, 83%; (e) R^2I, NaH, DMF, 60 °C, 12h, 73%; (f) THF-MeOH-2N HCl, reflux, 2h, 87%; (g) CBr_4, PPh_3, 4h, 93%; (h) Me_3N-DMSO, rt, 72h, pressure tube, 50-55%.

Our initial approach for the synthesis of cationic lipids with ether/ester linkage was started from the acylation of the secondary alcohol **4** (Scheme 2). Attempts to remove the trityl group by using the classic trityl cleavage reagents such as aqueous hydrochloric acid, acetic acid, or trifluroacetic acid gave rise to a 1,2-acyl migration, which has been well documented.[3a,11] Boron trichloride is a widely used *O-C* bond cleavage reagent which has been successfully utilized for the removal of both the acetonide protecting group[12] and the benzyl group.[13] In our case, the detritylation was successfully achieved by using boron

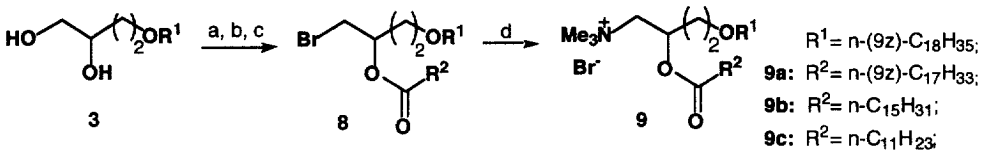


Scheme 2. (a) R^2COCl, TEA, DMAP, rt, 6h, 87%; (b) BCl_3, -78 °C ~ -28 °C, 40 min, 74%; (c) CBr_4-PPh_3.

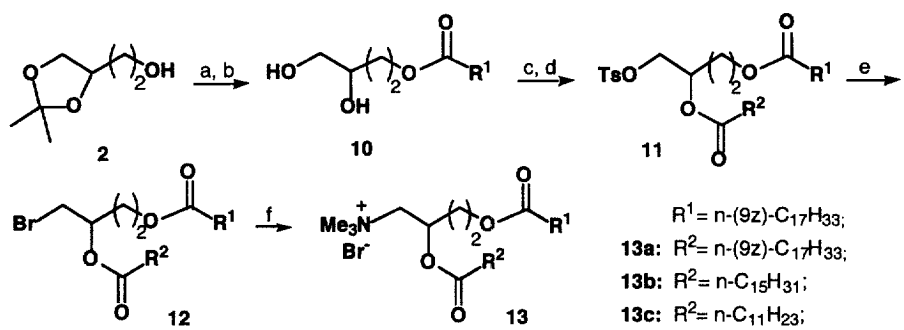
trichloride at low temperature without 1,2-acyl migration. It is believed that an intermediate in which boron chelates with the ether oxygen was formed during the process of detritylation and this intermediate effectively prevent the acyl migration.[12] Unfortunately, attempts to activate the terminal hydroxyl under Appel condition gave the undesired 1,2-acyl migration product. It is obvious that acidic species such as hydrobromic acid generated under the Appel condition accelerated the intramolecular 1,2-acyl migration.

To circumvent the problems arising from 1,2-acyl migration in the bromination step, we turned to an alternative route for the synthesis of cationic lipids with ether/ester linkage (Scheme 3). Selective tosylation

of the vicinal diol **3**, in the presence of a catalytic amount of DMAP (0.05eq), afforded the primary tosylate in a yield of 65%. Refluxing the tosylate with lithium bromide in methyl ethyl ketone (MEK) yielded the corresponding bromide, which upon acylation of the secondary hydroxyl group furnished the ester/ether **8** in a satisfactory yield. Reaction of compound **8** with trimethyl amine in dry dimethyl sulfoxide generated the desired quaternary ammonium salt **9a-9c** in yields of 33-36%.



Scheme 3. a) TsCl, Py, cat DMAP, rt, 65%; (b) LiBr, MEK, reflux, 30min, 92%; (c) R^2COCl, TEA, DMAP, rt, 6h, 80-87%; (d) Me_3N-DMSO, rt, 72h, pressure tube, 33-36%.



Scheme 4. (a) R^1COCl, TEA, cat DMAP, rt, 6h, 88%; (b) BCl_3, -78 °C~ -28 °C, 30min, 74% (c) TsCl, Py, cat DMAP, rt, 6h, 65%; (d) R^2COCl, TEA, cat DMAP, rt, 12hr, 80-87%; (e) LiBr, MEK, reflux, 1hr, 92%; (f) Me_3N-DMSO, rt, 72h, pressure tube, 30-36%.

The synthesis of cationic lipids with diester linkages is shown in Scheme 4. Acylation of acetonide primary alcohol **2** was complete by the standard method. Removal of isopropylidene with boron trichloride in methylene chloride at low temperature furnished diol **10**. Selective tosylation of the diol **10** followed by acylation of the secondary hydroxyl group gave diester **11.** Bromination of diester **11** afforded the cationic lipid precursor **12** in a yield of 92%. As shown in Scheme 4, the cationic lipids with diester linkage were processed in a similar way as described above for preparation of the cationic lipids bearing ether/ester bond (Scheme 3). However, considering the possibility of intramolecular 1,3-acyl migration[14] during the formation of compound **10**, boron trichloride was thus used as *O-C* bond cleavage reagent for the acetonide. Selective tosylation was not followed by S_N2 type bromination reaction but rather directly acylation of the secondary hydroxyl group, again in order to prevent 1,3-acyl migration. It is noteworthy to point out that

the reaction of bromide **12** (or bromide **8**) with trimethyl amine in methyl alcohol was painfully sluggish and therefore resulted in the quaternary ammonium salt in a very low yield. Increase of the reaction temperature resulted in an increase of byproducts. However, reaction of bromide **12** (or bromide **8**) with trimethyl amine in dry DMSO or DMF gave the quaternary ammonium salt in acceptable yields. Compared with the hydroxylic solvent (methanol), the polar aprotic solvents (DMSO or DMF) facilitated the nucleophilic displacement reaction.

In summary, we herein report the synthesis of cationic lipids with different carbon chain length as well as different functional linkages.

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