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Synthesis and transfection activity of novel galactosylated polycationic lipid

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ABSTRACT

In this study, we synthesized a new galactosylated cationic lipid and investigated its biological activity. The structure of lipid combines both spermine residue for DNA compaction and galactose moiety for the improvement of aggregation behavior of lipoplexes. Lipid was low toxic for different mammalian cells, and was able both to compact plasmid DNA and to mediate cellular accumulation of various nucleic acids (ODN, pDNA and siRNA) exhibiting biological activity (transgene expression, gene silencing).

Gene and antisense oligonucleotides based therapies have been developed for the treatment of both acquired and inherited diseases.[1] These approaches are based on the correction of the basis of diseases by therapeutic nucleic acids, namely DNA, oligonucleotides, small interfering RNA etc. Naked nucleic acids degrade easily within the organism, therefore special transport systems were developed to protect and to deliver nucleic acids into the targeted cells. Cationic lipids are widely used as non-viral delivery systems for the improvement of nucleic acid delivery into eukaryotic cells.[2] However, lipids used for the gene delivery often display both the high cytotoxicity and insufficient transfection efficiency. Hence, intensive development and study of the new non-toxic and efficient cationic lipids for the gene delivery are currently in progress.[3,4] Cationic lipids have common structural features such as the presence of positively charged and hydrophobic domains connected by a spacer. Numerous chemical modifications have been made to augment the delivering activity of cationic lipids.[2,5,6]

One promising strategy for the improvement of nucleic acids delivery is the design of cationic agents for the cell specific gene targeting, with the aid of covalently bounded ligands for specific cell receptors. Recently, cationic lipids with incorporated carbohydrate residues were under intensive investigation as targeted systems for the delivery of nucleic acids to hepatocytes, macrophages, and into the nucleus.[7–9] Several groups have reported that carbohydrate fragments increase the colloid stability of nucleic acid–lipid complexes (lipoplexes) in a blood serum and decrease the toxicity of cationic lipids.[10,11] Some polycationic single and double long-alkyl chain galactospermine bolaamphiphile have been synthesized and appeared as a promising non-viral synthetic vector for specific gene delivery.[12,13] Here we describe the new and convenient route to obtain a galactosylated polycationic lipid **1** (Fig. 1) and assess its biological activity in terms of cytotoxicity and transfection activity, in respect to different nucleic acids.

The structure of lipid **1** combines both spermine residue for DNA compaction and galactose moiety for improvement of aggregation behavior of lipoplexes. Galactosyl residue may then serve as a molecular signal for hepatocytes targeting. 1,2-di-*O*-tetradecyl-*rac*-glycerol was used as the hydrophobic anchor for incorporation into a lipid bilayer. It is known that cationic lipids containing tetradecyl substituents possess higher in vitro transfection efficacy

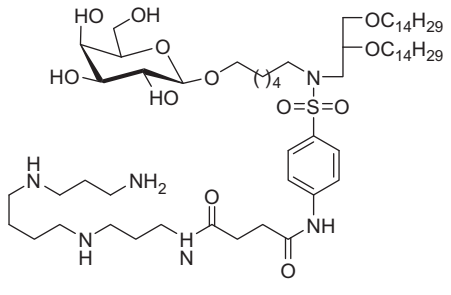

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Figure 1. Galactose-containing polycationic lipid.

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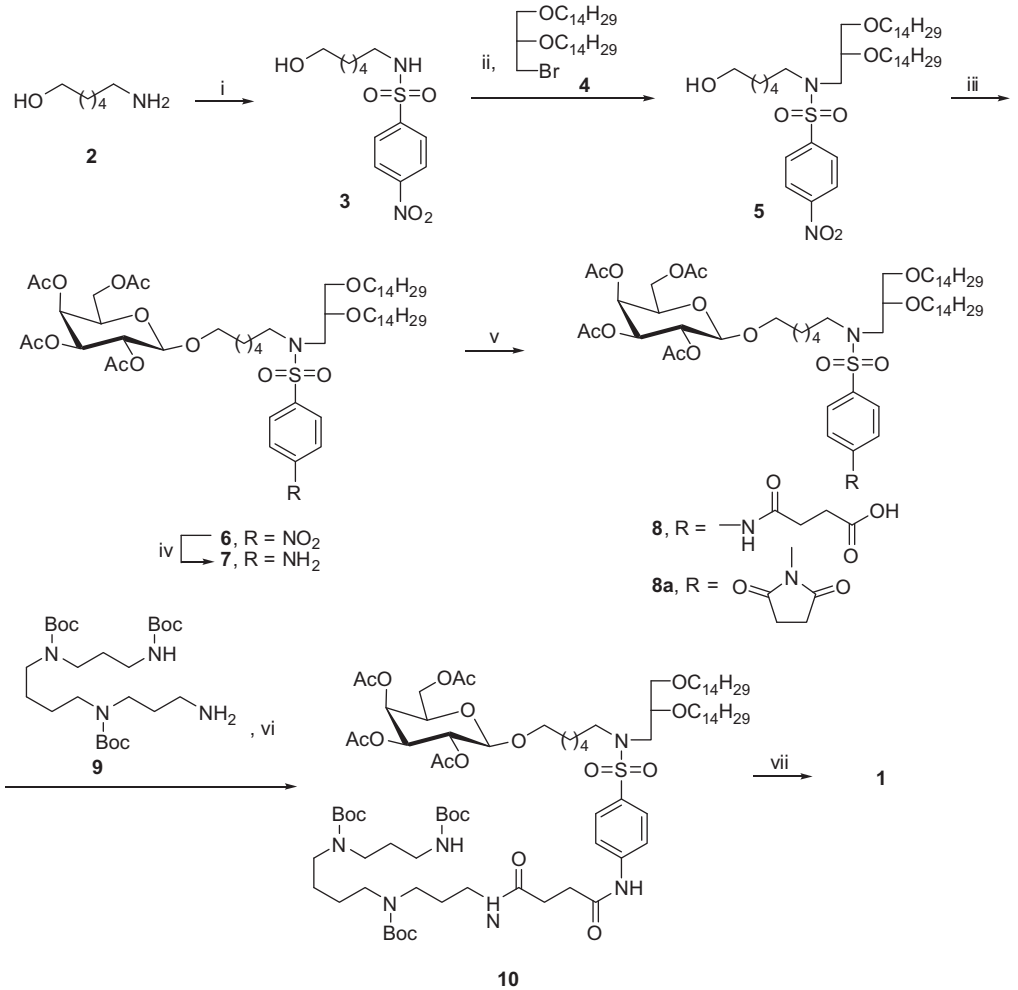
compared to other long-chain hydrocarbon analogues.[14,15] Natural polyamines such as spermine or spermidine are capable of binding and condensing DNA molecules into small, dense particles. Lipophilic derivatives of polyamines condense DNA more efficiently than polyamines themselves.[16] Among the cationic lipids tested lipophilic spermines are the most efficient synthetic gene delivery agents.[17] Therefore, for the creation of new polycationic lipid natural polyamine—spermine have to be covalently attached to the hydrophobic anchor by means of an amide bond. On the other hand it was necessary to link to the same anchor galactosyl residue. The attachment of hydrophobic, cationic, and carbohydrate domains was performed by using a Fukuyama amine synthesis—the reaction of 6-hydroxyhexyl 4-nitrobenzenesulfonamide with 1,2-ditetradecyl-3-bromo-*rac*-glyceride. This convenient approach permits to obtain a bifunctional molecule for the assembly of both spermine and galactose residues into a single structure of polycationic glycolipid.

According to the developed synthetic route the sulfonylation of 6-aminohexanol (**2**) with 2-nitrobenzenesulfonyl chloride in the presence of Et_3N afforded amide **3** in 92% yield (Scheme 1). Bromide **4** was prepared from 1,2-di-*O*-tetradecyl-*rac*-glycerol by treatment with CBr_4 in the presence of Ph_3P as described previously.[18] The alkylation of amide **3** with bromide **4** was carried out with a Fukuyama amine synthesis[19] and gave **5** in 64% yield.

The glycosylation of compound **5** with 2,3,4,6-tetra-*O*-acetyl-α-D-galactosyl bromide under the modified Königs–Knorr method in a Soxlet apparatus using $CdCO_3$ as the promoter gave galactoside **6a**. It is known that the ratio of the anomeric glycosides obtained is strongly dependent upon the reaction conditions. Upon optimising glycosylation conditions the best results were obtained by reflux of compound **5** (1 equiv) with galactosylbromide (3 equiv) in benzene for 1.5 h. Under these conditions β-glycoside **6** was formed in 65% yield accompanied by only 2% of α-galactoside. The preferable formation of β-glycoside **6** was a result of neighboring-group participation of a 2-*O*-acyl functionality in the glycosyl donor.[20] The reduction of nitro group by catalytic hydrogenolysis on Pd/C in the presence of ammonium formate as a hydrogen source afforded amine **7** with 90% yield. Acylation of lipid **7** with succinic anhydride in the presence of *N*,*N*-dimethyl-4-aminopyridine (DMAP) (2 equiv) and *N*-hydroxybenzotriazole (HOBT) at 70 °C for 24 h gave the corresponding succinate **8** in the very low yield (29%). The main product obtained in this reaction was cyclic imide **8a** (70%). In order to minimize the amount of undesirable **8a** we optimized ratios of reagents and temperature conditions and found that the treatment of compound **7** with succinic anhydride in the presence of Et_3N (2 equiv) and catalytic amount of DMAP (0.2 equiv) for 36 h at the room temperature gave succinate **8** in 73% yield.



Scheme 1. Synthesis of polycationic lipid **1**. Reagents and conditions: (i) 2-NO_2-$C_6H_4SO_2Cl$, TEA, CH_2Cl_2, 24 °C, 36 h, 73%; (ii) Cs_2CO_3, TBAI, DMF, 90 °C, 8 h, 65%; (iii) 2,3,4,6-tetra-*O*-acetyl–D-galactopyranosyl bromide, $CdCO_3$, C_6H_6, reflux, 1.5 h, 65%; (iv) $NH_4{\cdot}HCO_2$, Pd/C, MeOH/THF (2:1), 60 °C, 30 min, 90%; (v) succinic anhydride, TEA, DMAP, CH_2Cl_2, 24 °C, 36 h, 73%; (vi) tri-Boc-spermine (**9**), HBTU, DIEA, DMF, 4 °C, 40 min, 85%; (vii) (a) TFA, CH_2Cl_2, 24 °C, 3 h, 86%; (b) 0.04 N MeONa, MeOH, 24 °C, 4 h, 53%.