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Synthesis and Biological Properties of New Glycosidic Cationic Lipids for DNA Delivery

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Abstract—Lipidic glycosides with amino alkyl pendent groups were synthesized and evaluated for in vitro DNA transfection activity. The first representative of this new class of cationic lipids showed good gene delivery and low toxicity to HeLa and 3T3 cells.

Nucleic acid transfer into eukariotic cells represents a considerable therapeutic challenge. The elaboration of non-viral synthetic vectors have engendered wide interest.[1] Various synthetic molecules have been proposed, among which cationic lipids seem to be particularly attractive.[2] Since the first report of the use of a cationic lipid as a synthetic vector for gene delivery into cells by Felgner and co-workers,[3] a number of articles have described the synthesis of cationic lipids as gene carriers.[4] It is generally accepted that cationic lipids form complexes, called lipoplexes, with DNA by electrostatic interaction with the negatively charged phosphates of the nucleic acid. The structure of the DNA in lipoplexes is highly compacted, which allows its cellular uptake and its protection from degradation by endo-nucleases. These supra-molecular structures are then supposed to collapse in the cell, thus permitting the expression of the transfected gene. However, the actual cationic lipids suffer so far from severe drawbacks, such as cell toxicity and poor efficiency in vivo. In the course of our studies in the field of synthetic carriers for gene delivery, we describe in this letter the synthesis and biological properties of a new class of cationic lipids bearing a glycosidic cationic head. The rationale for this synthesis was an organized spatial density of positive charges, by the glycosidic core, which could give a tighter interaction with DNA, and an eventual lower toxicity than the previously published cationic lipids.[4]

This synthesis is outlined in Scheme 1. Substrate 1,2-3,4-di-*O*-isopropylidene-D-galactose was reacted with benzyl bromide in a Williamson type etherification reaction,[5] to give the corresponding 6-*O*-benzyloxy derivative **1**. Methanolysis of the isopropylidene protecting groups in **1** gave the methyl glycoside **2**. This compound was then either etherified with bromoethylacetate to give **3**, or esterified with N-Boc glycine to give **4**. It is noteworthy that in the case of compound **3**, only the triacid was isolated, the same reaction was actually performed with bromoacetic acid, leading to the same product. From that point the synthesis followed two divergent protocols: boron hydride reduction of **3** led to the corresponding triol **5**, which was converted to the triazide **6** by the Mitsunobu reaction.[6] The azides were further reduced with concomitant cleavage of the benzyloxy protecting group (**7**). Compound **8** was then obtained after selective protection of the amino groups with di-*tert*-butyl dicarbonate. On the other hand, **4** was hydrogenolyzed to the corresponding 6-unprotected glycoside **9**. Compounds **8** and **9** were reacted with either succinic or phtalic anhydride to give, respectively, products **10**, **11** and **12**, **13**. Condensation of the latter compounds with dioctadecylamine lead to **14**, **15**, **16** and **17**, which were subsequently deprotected to the final products **18**, **19**, **20** and **21**.

Compounds **18**–**21** were then evaluated for their ability to deliver a plasmid bearing the sequence of the luciferase reporter gene on two different cell types. The pCMV-Luc plasmid used included the luciferase reporter gene under the dependence of the human cytomegalovirus promoter. It was constructed from pGL2-basic Vector (Promega) by insertion of the Mlu I-Hind III fragment containing the CMV promoter, which was extracted from the pcDNA3 plasmid (Invitrogen). The DNA saline solutions (NaCl

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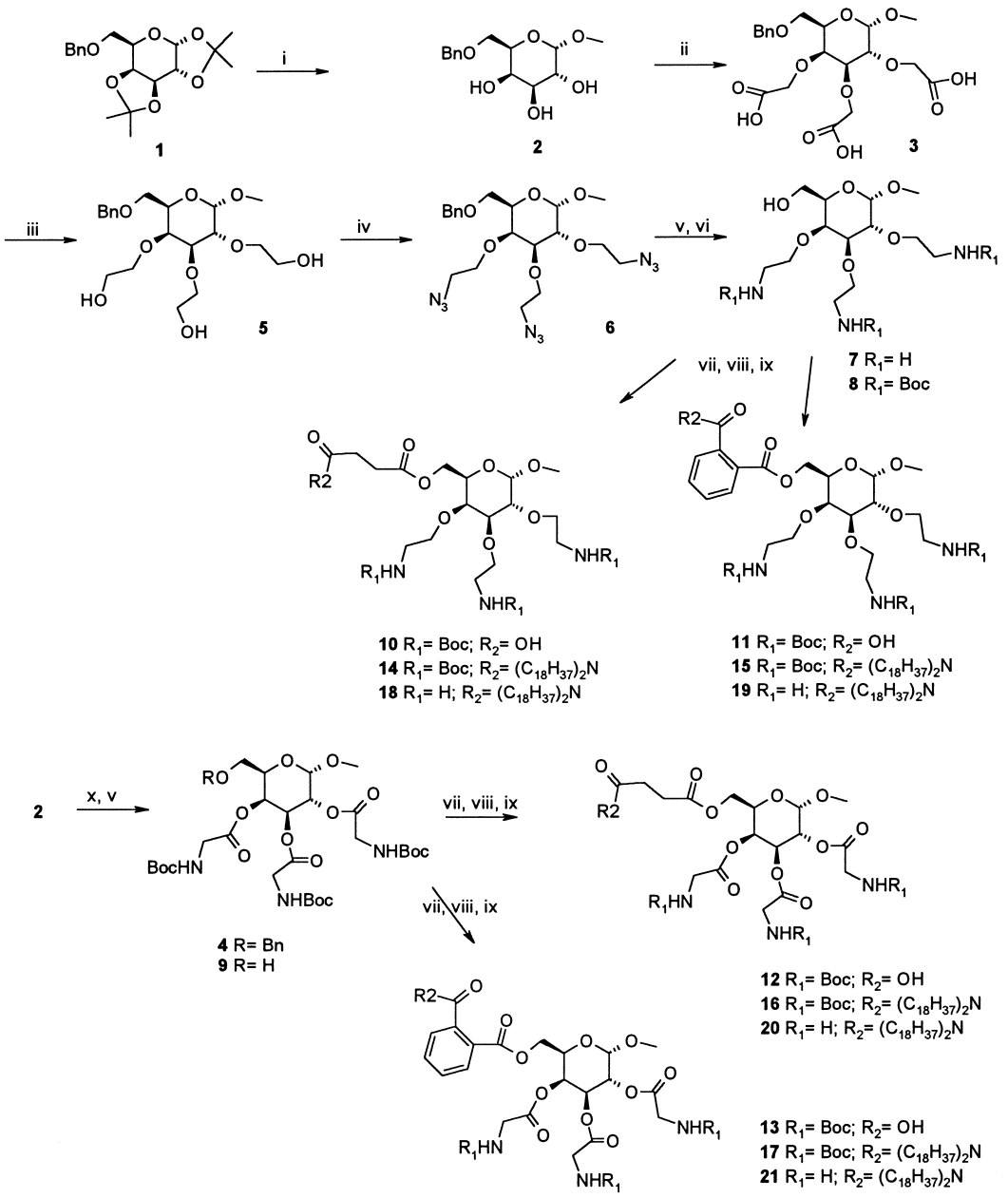

Scheme 1. Reagents and conditions: (i) MeOH, HCl; (ii) $BrCH_2CO_2Et$, KOH, 18-Cr-6, THF; (iii) BH_3/THF; (iv) Ph_3P, DEAD, N_3H, THF; (v) H_2, Pd/C, EtOH; (vi) Boc_2O, Et_3N, CH_2Cl_2; (vii) succinic anhydride (or phtalic anhydride), pyridine, DMAP; (viii) $(C_{18}H_{37})_2NH$, BOP, Et_3N, CH_2Cl_2; (ix) TFA, CH_2Cl_2; (x) Boc-glycine, BOP, Et_3N, CH_2Cl_2.

0.15M) were adjusted to 40μg/mL. Compounds **18**–**21** were solubilized in water (80–800μM) and mixed (v/v) to the nucleic acid solution to a final NaCl concentration of 75mM. Cells were grown in 24 wells microplaques ($2cm^2$/well) and transfected in their exponential phase, to 50–70% confluence. Transfection was evaluated 48 h after addition of DNA lipoplexes by measuring the luciferase expression using a commercial kit. Toxicity was estimated from a dosage of protein concentration in lysate cells.

Results and Discussion

Compounds **20** and **21** were found inactive in these conditions. Transfection efficiency with compounds **18** and **19** was optimum for a ratio of 8 nmol vector/μg DNA in the case of the HeLa cells (Figure 1, bars), and for a ratio of 12 nmol vector/μg DNA in the case of the NIH 3T3 cells (Figure 2, bars). The histograms relating the dose effects of the vectors **18** and **19** were superimposable in either cell type. The two compounds were non toxic to cells at optimal active ratio and only a 20% loss in cellular proteins was observed for ratios >8 nmol lipid/μg DNA (Figures 1 and 2, black dots).

In summary, we have described the synthesis and gene transfection properties of new glycosidic cationic lipids. Compounds **18** and **19** showed a transfection level comparable to previously described cationic lipids.[4j] Compounds **20** and **21** were inactive in these conditions,

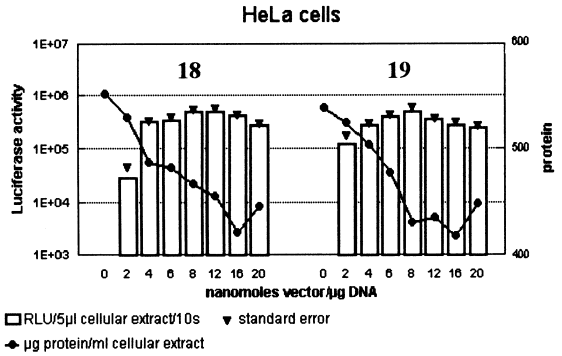

Figure 1.

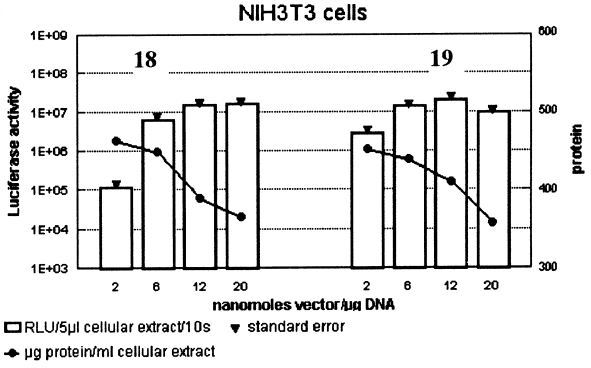

Figure 2.

suggesting the influence of the p*K* value of the amino groups on the interaction with the nucleic acids. All the four compounds tested were found non toxic, which could permit the use in vitro and eventually in vivo of higher vector/DNA ratio. Such high ratios usually lead to lipoplexes displaying favorable physicochemical and biological characteristics, such as size, stability and transfecting efficiency. Active work is in progress to develop this new class of promising cationic DNA vectors.

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