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# Synthesis and Transfecting Properties of a Glycosylated Polycationic DNA Vector[1]

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**Abstract**—A glycosylated analogue of dialkylamidoglycylcarboxyspermine was synthesized. The physico-chemical and transfecting properties of **11** were evaluated indicating that this cationic lipid forms stable particles at low charge ratio and is efficient for gene delivery. 

## Introduction

The use of DNA as a drug to cure inherited and acquired diseases will depend on the ability to achieve efficient introduction of a therapeutic nucleic acid into target cells. For this reason much effort has been focused on the development of viral[2] and non-viral gene delivery systems which self-associate to DNA. Basically, synthetic DNA vectors are positively charged peptides, polymers and lipids. Among those, cationic lipids have been extensively studied.[3,4] These amphipathic compounds are made from a hydrophobic anchor connected to a cationic domain by a spacer. Owing to their importance, numerous modifications have been introduced to improve their transfecting properties. Thus, new structures allowing the biodegradability,[5–9] reducing their toxicity[10,11] and possessing a targeting moiety[12] have been reported with results.

Because of the net negative charge exhibited by the membrane, it has been observed that cationic lipid–DNA complexes must be positive for optimal transfection.[13,14] However, in vivo and physicochemical studies have shown that this positive charge induced several problems. First, lipoplexes interact with serum proteins, and are removed from the circulation by complement.[11,15,16] Second, less charged slightly positive or neutral particles are unstable and aggregate, while using a large excess of cationic lipid affords stable small complexes but leads to an increased toxicity.[17] In designing new vectors, we have planned to covalently connect a non-charged hydrophilic moiety at the end of the hydrophobic, in order to decrease the surface charge density of lipoplexes which would harbor on their surface these non-charged hydrophilic domains. With this concept in mind, we were attracted by carbohydrates that offered, in addition, anti-aggregation[18] and targeting properties.[19] Several groups have reported that glycosylation of cationic polymers like polylysine[20,21] or polyethylenimine[22] promotes the transfection. In the same fashion Remy et al.[23] have used dialkylamidoglycylspermine/DNA complex targeted to hepatoma cells by adding a triantennary galactose ligand to a lipid associated to the complex. Logically, the next step was to connect by a covalent bond a saccharide to the hydrophobic anchor. Recent reports in this field[19] prompted us to disclose our ongoing results. In this report, we describe the synthesis and the biological properties of a glycosylated lipopolyamine. In order to evaluate the different properties of the carbohydrate, we have synthesized a rhamnosyl derivative. This last choice was made to evaluate the physicochemical influence of a carbohydrate linked to the lipopolyamine, because L-rhamnose is not recognized by mammal lectins and thus does not possess any potential targeting capacity.

## Chemical Synthesis

Recent reports have described the glycosidation of carbohydrates with long chain diols using enolates,[24] trichloroacetamidate[25] or anhydro sugars[26] as glycosyl donors. We

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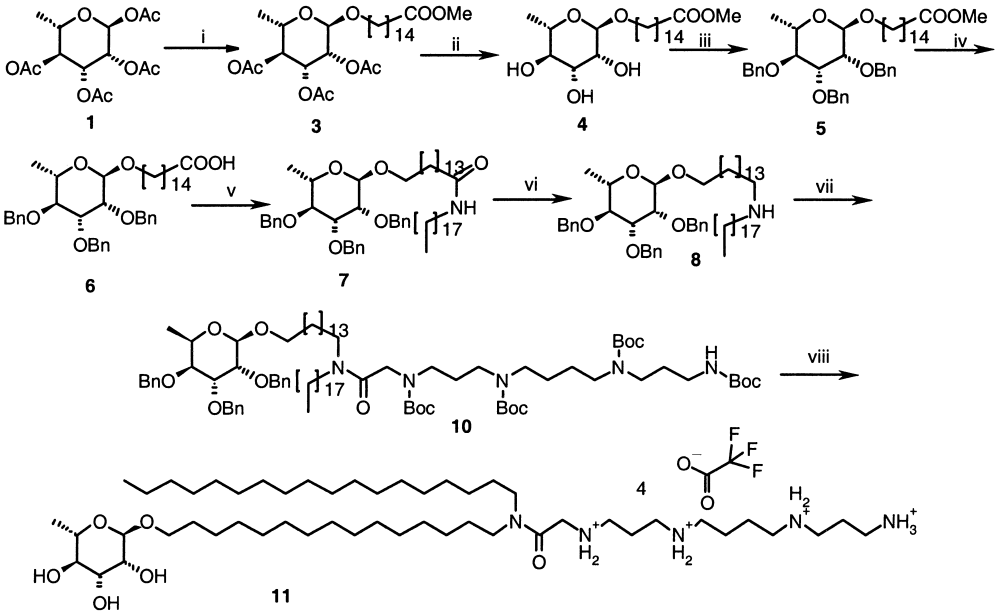


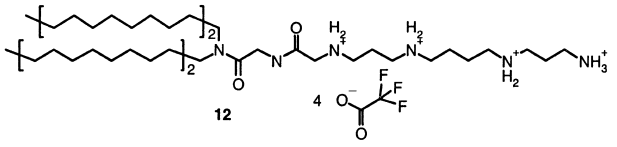
**Scheme 1.** Reagents and conditions. (i) Methyl 15-hydroxy-pentadecanoate **2**, $SnCl_4$ 0 °C, 30 min then rt 3 h, 60%; (ii) 2 N MeONa in MeOH quantitative; (iii) KI, NaH, BnBr, DMF overnight 70%; (iv) 25% NaOH reflux 30 min 73%; (v) octadecylamine, DIPEA, BOP, $CHCl_3$ 83%; (vi) $LiAlH_4$ 2 equiv reflux 10 h THF 93%; (vii) Boc protected carboxyspermine **9**, BOP, DIPEA, $CHCl_3$ rt 65%; (viii) (a) Pd/C 10% $H_2$ MeOH, (b) trifluoroacetic acid 2 steps 100%.

were attracted by another procedure, i.e., the method described by Banoub and Bundle[27] for the glycosylation of peracetylated carbohydrate with a mono-protected diol. The synthesis of the glycosylated vector **10** is outlined in Scheme 1. The synthesis was started by the condensation of the peracetylated rhamnopyranoside **1** with the methyl 15-hydoxy-pentadecanoate **2**. First, **1** was stirred at 0 °C in the presence of tin(IV) chloride (1.5 equiv) for 30 min, then **2** was added, and the reaction mixture was stirred for 3 h at rt to yield the β-glycoside **3** (60%). Removal of the acetyls was achieved by the Zemplen method (MeONa 2 N in MeOH, 2 equiv; 30 min) to give the triol **4**. Benzylation of **4** was conducted by adding successively to a DMF solution of the glycoside 4 equiv of potassium iodide, sodium hydride and benzyl bromide. After one night, the perbenzylated glycoside **5** was isolated in 70% yield. Saponification of **5** (25% NaOH in water, reflux 30 min) afforded **6** in 73% yield. The acid **6** was condensed with octadecylamine in the presence of BOP to gave the amide **7** (83%). Reduction of **7** was performed with $LiAlH_4$ (2 equiv; reflux overnight) and led to the desired amine **8** in 93% yield. With **8** in hand, the preparation of the cationic amphiphile was straightforward. Thus **8** was condensed with the BOC protected carboxy-spermine **9**.[12] The reaction was promoted by the BOP reagent and gave **10** in 65% yield. Next, **10** was fully deprotected, first by removal of the benzyl groups by catalytic hydrogenation (Pd/C 10%), then by treating the resulting product with trifluoroacetic acid. The glycosylated lipopolyamine **11** was recovered quantitatively.[28]

## Evaluation of the Rhamno Lipopolyamine 11

Compound **11** was essayed in vitro and the results were compared with those obtained for the non-glycosylated structural analogue RPR 120535 **12**. First, the size of the cationic lipid/DNA complexes[29,30] was analyzed by measuring the hydrodynamic diameter by dynamic light scattering.[31] The samples were diluted 20-fold in order to avoid multiple scattering (Scheme 2).

At the ratio of 3 nmoles lipid/μg DNA a mean diameter of 130 nm was recorded for lipoplex (lipopolyamine/plasmid complex) made from micelles.[29] When the plasmid was complexed with liposome formulations containing either cholesterol (25 mol%) or DOPE (dioleyl phosphatidyl ethanolamine 50 mol%) the diameters were 137 and 153 nm respectively (Table 1). Next, the colloidal stability of the lipoplex was studied at various charge ratios. Pitard et al.[17] have observed three zones



**Scheme 2.** RPR 120535 **12**.

**Table 1.** Size of the cationic lipid/DNA complexes in function of the cationic lipid/DNA ratio

| Compounds | 0.5 | 0.75 | 1 | 1.5 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|---|---|
| **11**[a] | A | B | B | C | C | C (130[d]) | C | C | C |
| **11**[b] | A | B | B | C | C | C (153[d]) | C | C | C |
| **11**[c] | A | B | B | C | C | C (137[d]) | C | C | C |
| **12**[a] | A | | A | | B | B | B | C | C |
| **12**[b] | A | | A | | B | B | B | B | |
| **12**[c] | A | | A | | B | B | B | B | B |

[a]Lipoplex made from micelles.
[b]Cationic lipid/DNA complex formulated with cholesterol.
[c]Cationic lipid/DNA complex formulated with DOPE.
[d]Size in nm.

namely A, B and C. In zones A and C, the complexes are stable. A corresponds to low cationic/DNA charge ratio thus leads to negatively charged lipoplex.

On the other hand, C corresponds to higher charge ratios, thus to lipoplex of net positive charges. In zone B, where the charged lipid/DNA complex is near neutrality, the complexes are colloidally unstable and precipitate after a few minutes.

Comparison with the RPR 120535 **12** shows that the precipitation zone is very small, allowing the formation of stable particles for a charge ratio of 1.5 instead of 5–6 for the non-glycosylated cationic lipid RPR 120535 **12** (Table 1). The glycosylated lipopolyamine **11** was essayed for transfecting activity in vitro on HeLa cells.[31] Results presented in Figure 1 indicate that the cationic lipid shows the highest level of transfection efficiency for a 1.5–4 charge ratio (500 μg luciferase/$10^5$ cells). An interesting fact is that micelles are more active than DOPE containing complexes.

Finally, the transfection efficiency between the RPR 120535 **12** and the RPR 204686 **11** were compared using M109 cells.[31] These data indicate that **11** is slightly more efficient than **12** (Fig. 2).

In conclusion, glycosylated polyamino cationic lipids are prepared from carbohydrate alkylamino lipids. The introduction of the carbohydrate led to vectors with a very small precipitation zone at low charge ratio. In addition we have shown that the presence of the sugar did not affect the transfecting properties of the polyamino vector. The galacto- and the manno- derivatives are now under investigation and will be published in due course.

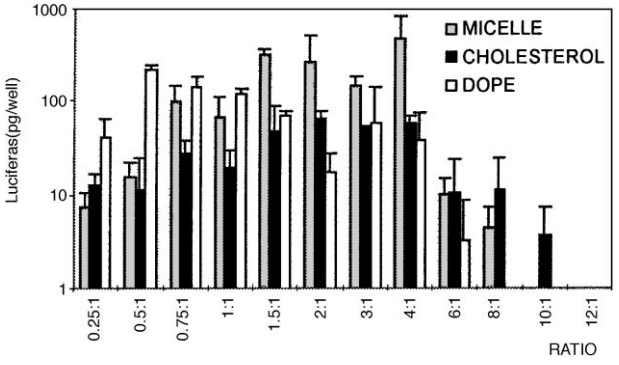



**Figure 1.** Transfection efficiency of **11**/DNA lipoplex as a function of the charge ratio. The transfection was studied on HeLa cells with and without DOPE.

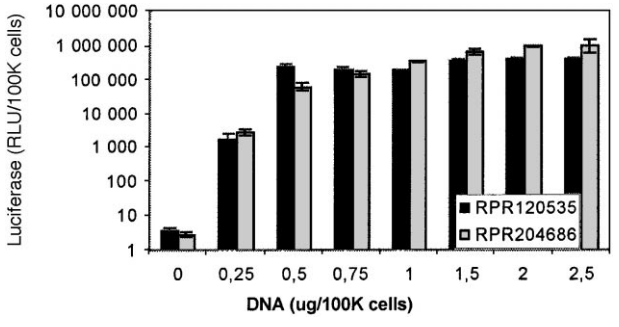



**Figure 2.** Comparison of the transfection efficiency between **11** and **12**. The transfection was studied on M109 cells with DOPE.

## Acknowledgements

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28. Mp 54 °C, $[\alpha]_D$ −10.77 (*c* 0.9, MeOH), MS (FAB), *m*/*z* 884.9 ($MH^+$). $^1H$ NMR (DMSO-$d_6$, 400 MHz): δ 0.88 (t, 3H, *J* = 6.36 Hz, octadecyl), 1.13 (d, 3H, *J* = 6.45 Hz, H-6), 1.27 (m, 52 H, $(CH_2)_{11}$ *N*-pentadecyl and $(CH_2)_{15}$ *N*-octadecyl), 1.5 (m, 4H, $\underline{CH_2}CH_2N$ amide), 1.58 (m, 2H, $\underline{CH_2}\ \underline{CH_2}O$), 1.66 (m, 4H, $\underline{CH_2}CH_2NH^+$ butyl), 1.92–2 (m, 4H, $\underline{CH_2}CH_2NH^+$ propyl), 2.92–3 (m, 12H, $CH_2NH^+$), 3.14 (m, 1H, H-4), 3.18 (m, 4H, $CH_2N$ amide), 3.22 (m, 1H, $OCHaCH_2$), 3.39 (m, 1H, H-5), 3.41 (m, 1H, H-3), 3.52 (m, 1H, $OCHbCH_2$), 3.59 (m, 1H, H-2), 4.06 (m, 2H, NCC=O), 4.5 (br s, 1H, H-1). $^{13}C$ NMR (DMSO-$d_6$ + $CD_3COOD$, 400 MHz): δ 14.38 (C-18 *N*-octadecylamine), 18 (C-6), 22.5, 23 and 24.01 ($\underline{C}CNH^+$), 29 ($(\underline{C}H_2)_{11}$ *N*-pentadecyl and $(\underline{C}H_2)_{15}$ *N*-octadecyl), 36 ($\underline{C}NH_3^+$), 42 (CN amide), 44–46 ($\underline{C}NH_2^+$), 47.5 ($NCC{=}O$), 66.5 ($\underline{C}H_2O$), 68.5 (C-5), 70.5 (C-2), 71 (C-3), 72 (C-4), 100 (C-1), 158.40 (C=O).
29. The cationic micelles (particles from less than 5 nm diameter) were prepared by drying the lipid from organic solvent with argon or nitrogen to form a thin film on the bottom of a glass tube. The film is vacuum-desiccated for 1 h. The lipid film is allowed to hydrate in 5% dextrose and 20 mM NaCl at 4 °C overnight. Subsequently, the films are sonicated for 5 min and heated to 50 °C for 30 min. Liposomes formulation contain 'helper lipids', i.e., either 50 mol% dioleyl phosphatidyl ethanolamine (DOPE) or 25 mol% cholesterol. The appropriate amount of helper lipid ($CHCl_3$ stock) was added to a solution of **11** in chloroform. The solvent was removed under argon and the sample was dried for 1 h then the lipid was hydrated with a solution made of 5% dextrose and 10 mM sodium chloride. After one night the solution was heated at 60 °C (5 min) and sonicated (1 min). The process was repeated several times until the size of the lipidic particles was stable. Plasmid pXL3031 encoding the luciferase gene under control of the P/E CMV promoter of the cytomegalovirus was diluted at the concentration of 0.5 mg/mL or 1.0 mg/mL in a solution made of 5% dextrose and 10 mM sodium chloride. The lipoplexes were prepared by mixing various volumes of the plasmid DNA and the lipid **11** solutions at rt.
30. Size determination of the lipoplex was determined using a Coulter N4+ particle analyzer. Fifty μL of the lipid/DNA complex suspension is diluted to 1 mL in (5% Dextrose + 20 mM NaCl) and placed in the N4 + cuvette.
31. HeLa cells are plated at 60,000 cells per well of a 24-well plate, and allowed to grow overnight. The medium (DMEM + 10% FBS) is removed and replaced by 0.5 mL DMEM without serum, containing 1 μg DNA complex. The cells are transfected for 4 h. Subsequently, the complex containing medium is replaced with DMEM + 10% FBS. The cells are allowed to grow for another 24 h, after which they are harvested with 100 μL luciferase lysis buffer per well. M109 cells are lung carcinoma cells that are derived from Balb/C mice. The transfection protocol is identical to that used for HeLa cells except for the fact that we plate them at 40,000 cells/well because they grow slightly faster (so on the day of transfection the number of cells per well is approximately 100,000 for both HeLa and M109).