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Synthesis of New Cationic Lipids from an Unsaturated Glycoside Scaffold

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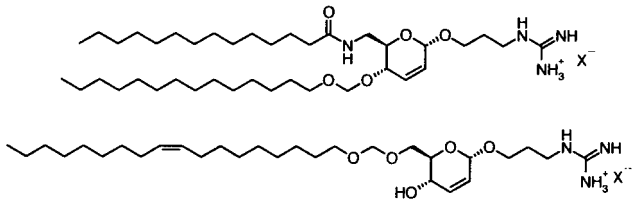
ABSTRACT



We report the synthesis of new cationic lipids. These amphiphiles present a hydrophobic domain connected to a guanidinium entity by an unsaturated glycoside scaffold. The synthetic strategy using amide or acetal linkage led to various mono- and bicatenar derivatives. Investigation of their physicochemical properties indicated that these new compounds compact DNA.

Positively charged polymers, peptides, and lipids have emerged as promising alternatives to viral-based technology for gene therapy.[1] Since the first report about plasmid transfection,[2] many cationic lipids have been investigated[3] for their ability to transfer foreign genes and oligonucleotides into cells. However, there is a continuous need for the development of new cationic lipids because of their low in vivo transfection efficiency and some toxicity. To improve the delivery of therapeutic DNA, recent efforts have focused on structure activity relationship,[4] linkages modification between the polar and hydrophobic parts of the vector,[5] biodegradable cationic lipids,[6] template oligomerization,[7] and introduction of targeting ligands.[8] As a part of our program aimed toward the development of new vehicles for gene delivery, we report a new approach for the synthesis of DNA vectors, based on an unsaturated carbohydrate scaffold.

Unsaturated glycosides offer several advantages for the design of gene delivery systems. First, each of the ring substituents can be functionalized with a high selectivity leading to a wide range of structures. Second, the unsaturated acetal shows a moderate stability in acidic media, which could facilitate the intracellular DNA release and lipid biodegradability. Finally, the ring could allow the formation of liposomes from single chain derivatives that usually led to micelles.[9]

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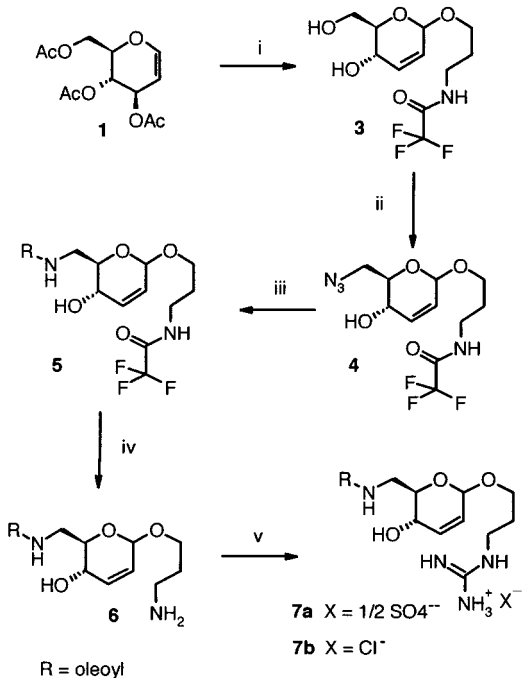
Thus, we present herein our preliminary results regarding the synthesis of cationic lipids, based on an unsaturated glycoside scaffold. Our first approach involved the condensation of an unsaturated glycosyl-amine with a BOC-protected carboxyspermine. This first choice was made in order to compare the properties of the new synthesized compounds with those previously described by our group.[4] However, preliminary observation demonstrated the high sensitivity of the unsaturated acetal to the conditions needed to remove the BOC groups. Thus, among the possible cationic head, we selected guanidines[10] that could be directly prepared from amines without any protection. In this letter we describe the preparation of a family of cationic lipids in which the hydrophobic domain, made from various combinations of alkyl chains, was connected by an unsaturated glycosyl linker to a guanidinium salt.

Our starting point was Ferrier condensation[11] of triacetyl-D-glucal **1** with 2,2,2-trifluoro-acetamidopropanol **2** (Scheme 1). The resulting glycoside was immediately deacetylated

Scheme 1[a]

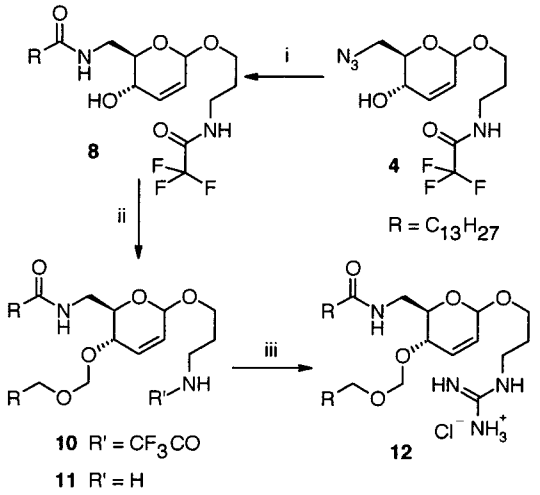


[a] (i) (a) CH_2Cl_2, 0 °C, 2,2,2-trifluoro-acetamidopropanol **2**, $BF_3{\cdot}Et_2O$, 1 h; (b) 2 N MeONa in MeOH, rt, 15 min (100%, two steps). (ii) (a) CH_2Cl_2, 0 °C, TsCl 1 equiv, pyridine overnight, 70%; (b) DMF, 60 °C, NaN_3, NaI, 18-crown-6, overnight, 70%. (iii) (a) THF, 40 °C, PPh_3, H_2O, 1 h; (b) CH_2Cl_2, rt, DCC oleic acid, 50% (two steps). (iv) MeOH/THF 3:1, 40 °C, K_2CO_3, 96%. (v) MeOH, 40 °C, Et_3N, *O*-methylisourea hydrogen sulfate or chloride, overnight, 70%.

to give quantitatively the diol **3**. Selective tosylation of the primary hydroxyl furnished the 6-*p*-toluenesulfonyloxy glycoside. Overnight treatment of this tosylate with NaN_3 and NaI in the presence of 18-crown-6[12] afforded the azide **4** in 70% yield. Reduction of **4** using the Staudinger procedure[13] (PPh_3/water) led to the amino glycoside, which was used without purification. Oleic acid was reacted with the crude mixture in the presence of DCC to yield the amide **5** (50%, two steps). Deprotection of **5** with a solution of potassium carbonate in methanol/THF gave the amine **6** in 96% yield. Finally, treatment of **6** with *O*-methylisourea hydrogen sulfate or hydrochloride[14] gave, respectively, the guanidines **7a** and **7b** (yields 70%).

Next, we investigated the preparation of bicatenar amphiphiles. To solve this issue, we selected an acetal strategy to connect the secondary hydroxyl to a fatty alcohol. This process offered several advantages. First, this group is orthogonal to the trifluoroacetamide. Second, the use of an acetal could increase the acid sensitivity of the cationic lipid. Thus, we choose to prepare the bis C-14 guanidine **12** (Scheme 2). First, the myristamide **8** was prepared from azide

Scheme 2[a]



[a] (i) (a) THF, 40 °C, PPh_3/H_2O; (b) CH_2Cl_2, rt, myristic acid, DCC, two steps. 70%. (ii) THF, 60 °C, DIPEA, tBu$_4$NI, $C_{14}H_{29}OCH_2Cl$ **(9)** 3 equiv, 70%. (iii) (a) MeOH/THF, 40 °C, K_2CO_3, 99%; (b) MeOH, 40 °C, *O*-methylisourea hydrochloride 50%.

4 by our reduction-condensation procedure. The methoxy-tetradecane synthesis was carried out by adding 3 equiv of the chloromethoxy-tetradecane[15] **9** to a THF solution of **8** in the presence of diisopropylethylamine and tetrabutylammonium iodide. After 2 h at 60 °C, the methoxy-acetal **10** was isolated in 80% yield. Removal of the trifluoroacetamide and treatment with *O*-methylisourea hydrochloride yielded the guanidine **12** (50%).

The methoxy alkyl chemistry offers a new approach for the design of mono- and bicatenar amphiphiles. Examination of the literature[16] showed that at room temperature chlo-

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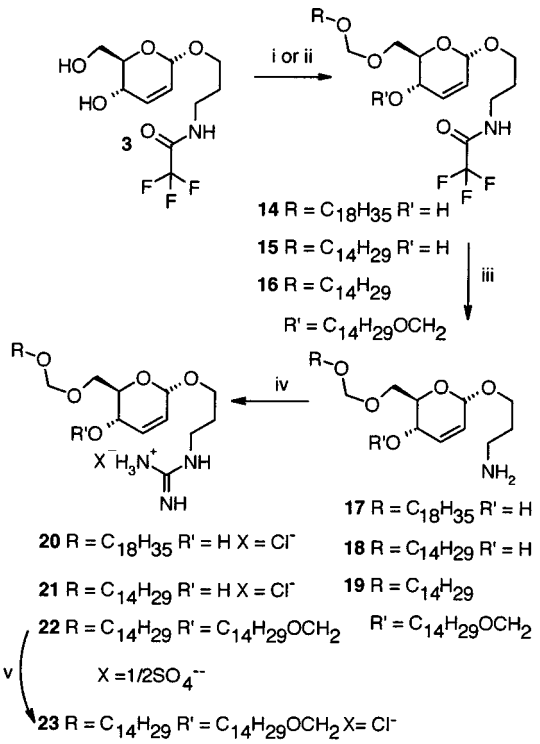
romethoxy-alkanes react selectively with a primary hydroxyl, whereas reaction with a secondary alcohol requires heating. Scheme 3 illustrates the preparation of guanidines from the

Scheme 3[a]



[a] (i) THF, rt, DIPEA, *t*Bu$_4$NI, $C_{14}H_{29}OCH_2Cl$ (**9**) or $C_{18}H_{35}OCH_2Cl$ (**13**) 2 equiv. (ii) THF, 60 °C, DIPEA, *t*Bu$_4$NI, **9** 5 equiv, 70%. (iii) MeOH/THF, 40 °C, K_2CO_3, overnight. (iv) MeOH, 40 °C, *O*-methylisourea hydrogen sulfate or hydrochloride overnight, 70%. (v) Dowex 21K, THF/H_2O 8:2, 43%.

diol **3**. Addition of 2 equiv of 1-chloromethoxy-*cis*-undec-9-ene **13** to a solution of **3** at room temperature gave the 6′-*O*-methoxy acetal **14** in 70% yield. In the same conditions, reaction of 1-chloromethoxy-tetradecane **9** with **3** led to **15**, whereas treatment of the diol **3** with 5 equiv of **9** afforded the bis acetal **16**. Removal of the trifluoroacetyl group with potassium carbonate afforded the amines **17**−**19**. Reaction of the amines with *O*-methylisourea hydrochloride or hydrogen sulfate gave, after purification by reverse phase HPLC, the guanidines **20**−**22** in 70% yield. Finally hydrochloride **23** was prepared from sulfate **22** by ion-exchange chromatography (Dowex 21K, 20% water in THF, 43% yield).

The capacity of the unsaturated guanidine glycosides (UGG) **7a**, **7b**, **12**, **20**, **21**, **22**, and **23** (Figure 1) to compact DNA and to form stable lipid−DNA complexes (lipoplexes)

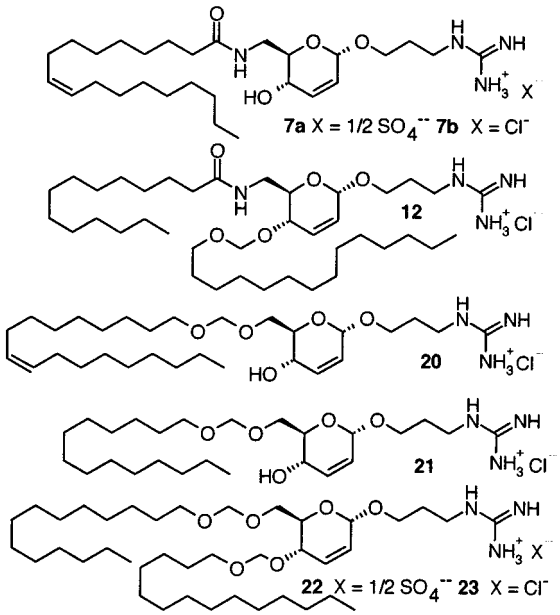


Figure 1. Unsaturated guanidine glycosides.

was then investigated. Lipoplex colloidal stability was first studied. The UGG/DNA complexes were prepared at different UGG/DNA ratios by mixing the glycoside with pXL3031 (3600 bp) plasmid[17] in the presence (liposomes) or in the absence (micelles) of dioleyl phosphatidylethanolamine (DOPE).

As a model for the physicochemical characterization of the UGG, we have used **23**, which exhibited a three-zone model, termed A, B and C, of colloidal stability.[18] In zone A, lipoplexes have a mean diameter of 100 nm (Figure 2).

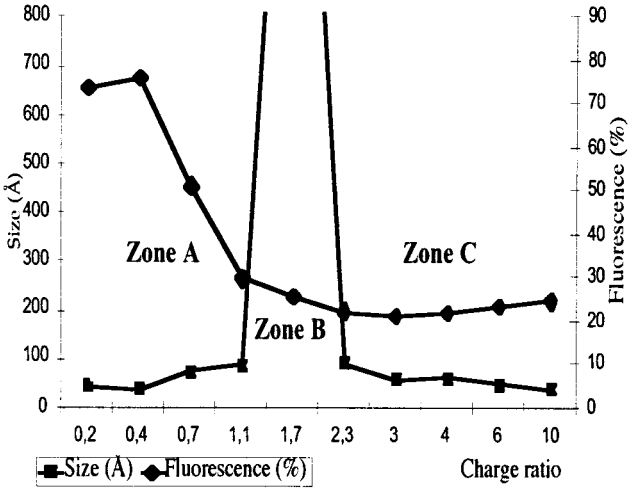


Figure 2. Colloidal stability and fluorescence experiments for **23**-DNA lipoplexes. **23**-DNA lipoplexes of various charge ratios, at 10 μg DNA/mL in 150 mM NaCl, were obtained by mixing **23** micelles with the pXL 3031 plasmid. EtBr = ethidium bromide.

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Table 1. Size of the Cationic Lipid/DNA Complexes as a Function of the Lipid/DNA Ratio

	7b[a]	**20**[a]	**21**[a]	**7a**[b]	**7b**[b]	**20**[b]	**21**[b]	**23**[a]	**12**[b]	**23**[b]
0.2	A	A	A	A	A	A	A	A	A	A
0.4	A	A	A	A	A	A	A	A	A	A
0.7	A	A	A	A	B	A	A	A	B	A
1.1	A	A	A	A	C	A	B	B	B	B
1.7	B	A	B	B	C	B	C	C	B	C
2.3	C	B	C	B	C	C	C	C	C	C
3	C	C	C	C	C	C	C	C	C	C
	68[c]	92[c]	87[c]	222[c]	68[c]	85[c]	66[c]	50[c]	180[c]	66[c]
4	C	C	C	C	C	C	C	C	C	C
6	C	C	C	C	C	C	C	C	C	C

[a] Lipoplexes made from micelles. [b] Lipoplexes made from liposomes. [c] Size in nm.

In the B zone, the lipoplexes are unstable and precipitate. This precipitation is due to a charge ratio near neutrality, inducing lipoplex aggregation by reducing electrostatic repulsion. Finally, zone C lipoplexes were colloidally stable with a mean diameter around 60 nm. Ethidium bromide fluorescence experiments were performed to evaluate DNA entrapment in the lipoplexes. Ethidium bromide displays high fluorescence when intercalated between DNA bases. Fluorescence sharply decreased in zone A from 100% to about 25% in zone B and C, indicating ethidium bromide exclusion due to compaction in the lipoplexes. Gel retardation experiments confirmed these results, showing that all DNA was retained for a charge ratio above 1 (see Supporting Information Figure 1). Examination of Table 1 deserves further comments. All UGG that can be formulated behave like **23**, exhibiting the three-zone scheme. At the ratio of 3 nmoles lipid/μg of DNA, particles around 200 nm were obtained for the sulfate **7a**. In addition, attempts to formulate **22** and the sulfate analogue of **12** led to highly insoluble complexes. These results prompted us to synthesize hydrochloride UGG. For all the chloride except **12**, small particles of less than 100 nm sizes were observed. Comparison of the data for **7b**, **20**, and **21** indicated that neither the lipid linkage (amide or methoxy-acetal) nor the insaturation did significantly modify the UGG behavior. The bismethoxy-acetal **23** gave also small particles. However, the more hydrophobic amido-acetal **12** displayed an increase in the size of lipoplexes, as well as the broadness of the B zone. These results showed that either a single chain or two acetal bridges favored the formation of small and stable lipoplexes. This is consistent with the idea that hydrophilicity is the main factor controlling the formation of small size UGG lipoplexes.

Finally, **7b** and **12** have been used as models to evaluate the lipid stability in acid media. Reaction with strong acids such as trifluoroacetic acid or IR 120 led to the immediate destruction of the unsaturated glycoside. On the other hand **7b** and **12** were stable when dissolved in 0.01 N HCl.

In summary, we have prepared a series of new cationic lipids that compact DNA and form colloidally stable lipoplexes. Transfection experiments are underway and will be published in due course.

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Supporting Information Available: Experimental procedures and NMR data for compounds **5**−**23** and DNA complexation experiments (**7a**, **7b**, **12**, **20**, **21**, **23**). This material is available free of charge via the Internet at http://pubs.acs.org.

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