Reviews

Cationic amphiphiles of both lipid and nonlipid nature in gene therapy

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The synthesis and biological activity of cationic lipids and cationic amphiphiles of different natures are considered. The general factors influencing the transfection efficiency are identified and summarized for various classes of compounds.

Key words: cationic lipids, cationic liposomes, lipofection, gene therapy.

1. Introduction

The rapid development of the recombinant DNA technology and understanding of the molecular basis of many diseases resulted in the appearance of a new field of medicine, gene therapy. At present, gene therapy can be considered as a combined method for the treatment of hereditary and nonhereditary diseases, which involves introduction of correcting genes into the cells for targeted treatment of genetic defects. The main conditions for successful correction of a genetic disease include efficient delivery of the exogenic gene into the target cells and creation of conditions for its long-term expression.[1]

Genetic modification of somatic cells can be performed by either *in vivo* or *ex vivo* methods. Several methods of transfection have been developed and extensively studied for exogene delivery; they include coprecipitation of DNA with calcium phosphate, electroporation, charged particle bombardment, DNA microinjection, and methods using various recombinant viruses, liposomes, and receptor-mediated endocytosis. Each of the above-listed transfection methods has its restrictions and fields of application.

The lipofection method making use of cationic liposomes for the delivery of exogenic genes has been widely studied among other methods in the last decade.[1,2] Cationic liposomes possess a number of advantages over virus vectors, namely, they protect DNA, mRNA, or oligonucleotide molecules from inactivation induced by cell enzymes, they are not infectious or immunogenic, and are suitable for cell-targeted transfer into specific types of cells. Cationic liposomes can be easily prepared; they are stable during storage and do not cost much. All these properties of cationic liposomes make them promising as gene delivery systems.

Cationic liposomes are formed from a cationic amphiphile, which is referred to as cytofectin (*cyto* for cell and *fectin* for transfection) and a "structural" lipid (a helper lipid). However, in some cases, the use of the helper lipid is not necessary.

For the targeted search for new cationic lipids, structural requirements based on the understanding of the cell mechanisms of the genetic transport and on the determination of the limiting steps of lipofection should be developed. This is also necessary for the development of rational approaches to gene transfer and for verification of hypotheses related to cellular and molecular mechanisms of transfection.

To perform transfection mediated by cationic lipids, liposomes should be formed at the first stage and then

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their complexes with the plasmid DNA (genetic construction), which have been called genosomes or lipoplexes, should be produced. These complexes are formed due to electrostatic interaction of the positively charged hydrophilic part of the cationic lipid with the negatively charged phosphate groups of nucleic acids. These complexes are diversified in structure and size, which is determined by the type of the cationic lipid used, the procedure of preparation of cationic liposomes, and the DNA-to-liposome quantitative ratio.

The second stage of transfection is interaction of the complexes with a cell membrane followed by their entry into and distribution over the intracellular matrix. At this stage, the cationic lipid ensures a positive residual charge of the genosome, which allows it to interact with a negatively charged cell membrane. The complexes aggregated on the cell surface can subsequently either fuse with the cell membrane or, most frequently, enter the cell through endocytosis; besides, some of the complexes can remain unclaimed on the cell surface. It was found that after endocytosis, the complexes are accumulated in the perinuclear compartment, where they form large aggregates, which often have highly ordered tubular structures.

The next stage is escape of the genosome into the cytosole. At this stage, the helper lipid performs its function, namely, destabilizes the endosomal membrane. This is a rate-limiting step in the gene transfer; therefore, attempts are being made to solve this problem by designing new synthetic cationic lipids which could efficiently destabilize the endosomal membrane, by using agents preventing hydrolysis of complexes in the endosomes, by using pH-sensitive agents, and by treating the cell with adenoviruses.

At the final stage, the DNA—cationic liposome complex dissociates and the exogenic DNA enters the cell nucleus and is then expressed.

It is noteworthy that the most severe problem associated with the use and practical implementation of lipofection technology for the treatment of genetic diseases is the difference between the results of *in vitro* and *in vivo* expression. This is due to the inconsistency of the physicochemical characteristics of cell membranes *in vitro* and *in vivo*, for example, the membrane charge, charge density, and the structure and accessibility of membrane receptors. In addition, many therapeutic complexes can be changed and destroyed *in vivo* under the influence of blood components, the reticuloendothelial system, and the complement system.

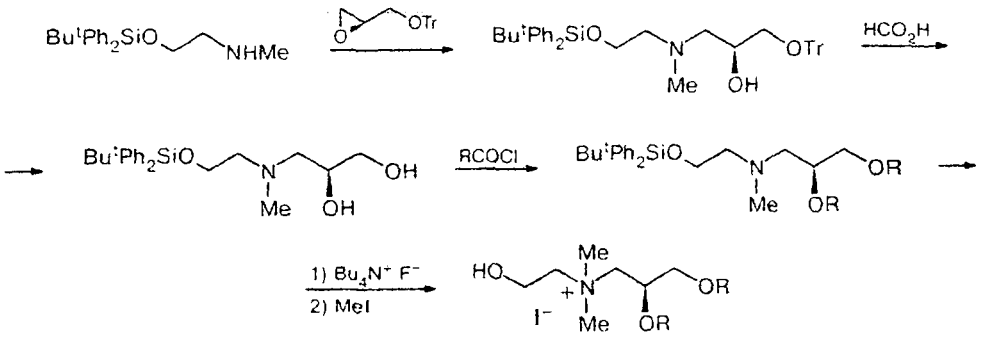
2. Structure and chemical synthesis of cationic amphiphiles

An ample body of data on the structure and synthesis of cationic lipids and the structure—biological activity relationship have been accumulated to date. The main pathways and methods for the preparation of cationic lipids have been surveyed in our earlier review.[3] In this review, we summarize the recent data on cationic lipids, consider their classification in terms of structure, and present syntheses of individual compounds.

2.1. Cationic glycerolipids with long-chain alkyl or acyl substituents

Synthesis of most cationic glycerolipids is mainly based on a small number of chemical transformations, giving the target compounds in acceptable yields. Thus in the synthesis of products **1**—**12**, the hydroxy groups of 3-(*N*,*N*-dimethylamino)propane-1,2-diol, which was used as the starting compound, were modified with long-chain alkyl or acyl substituents followed by exhaustive alkylation with methyl iodide (**1**, **2**), 2-bromoethanol (DORIE (**5**) and DMRIE (**10**)), or 3-bromopropanol (DORIE-HP (**6**)).[4—6] In the case of compound **13**, 1,2-dialkoxy-3-(*N*,*N*-dimethylamino)propane is made to react with *N*-(3-bromopropyl)phthalimide, which is followed by phthalyl deprotection with hydrazine.[7,8] The replacement of the hydroxy group in the polar head of compound **10** by a primary amino group creates some advantages, in particular, it becomes possible to vary the DNA—cationic lipid ratio and, hence, the concentration introduced into cells over wide limits. The activity of lipofection exhibited by lipids **13a,b** in *in vitro* experi-

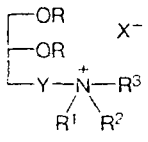
Scheme 1



ments was at least 10 times higher than that of lipid **10** and some other commercial formulations.

A convenient method for the preparation of diacylated cationic glycerolipids has been proposed (Scheme 1).[9] This method was used to synthesize tetraalkylammonium derivatives **2**, **9**, **14a—g**, and **15a—i**[10] with various sets of hydrophobic and hydrophilic substituents. These compounds were used to study the relationship between the structure of cationic lipids, the degree of lipid hydration, and the efficiency of the transfection *in vivo*.

Delivery of the plasmid DNA[4] and RNA[11] into eucaryotic cells was first accomplished using the cationic glycerolipid *N*-[1-(2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride (DOTMA (**1**)) in combination with 1,2-dioleoylphosphatidylethanolamine (DOPE) (1 : 1); a possible mechanism for the process was proposed.[12] The transfection efficiency was 5 to 100 times higher than that attained using the calcium phosphate precipitation method. The 1 : 1 DOTMA/DOPE composition, which received the name Lipofectin (GIBCO BRL), started to be used in studies of the structures of its complexes with DNA[13—15] and in experiments *in vivo*.[16,17] Compound **2** (DOTAP, Boehringer Mannheim) is a commercially available preparation, which is used to study the morphology of complexes with DNA[18—20] in *in vivo* experiments[19,21] and undergoes clinical trials for treatment of cystic fibrosis.[22] At present, composition **10** (DMRIE/DOPE) is widely used to study the mechanism of lipofection[23,24] and successfully undergoes clinical trials.[25]

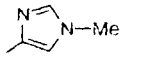
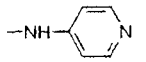
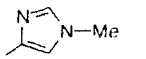


1—17

The ether lipids containing a cationic group linked to the glycerol skeleton either directly (**16**) or through a spacer group (**17**) have been prepared by quaternization of cyclic or acyclic amines by either 3-*O*-tosyl, 3-*O*-mesyl, and 3-bromo-3-deoxy derivatives[26] or by highly reactive α-bromo ethers[27] of 1,2-di-*O*-alkylglycerols; these products have been further employed for transfection of cell lines.[28]

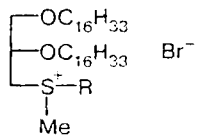
The charge of pH-sensitive liposomes changes upon variation of the pH value; thus, fusion with cell structure membranes can occur, which ensures entry of DNA into the cell.[29] Tertiary amines (**18a—c**), containing pH-sensitive polar methylimidazole and aminopyridine residues, have been prepared.[30] They are supposed to be more efficient at the stage of liberation of DNA from endosomes.

Compound (abbreviation)	R	Y	R^1	R^2	R^3	X
1 (DOTMA)	(9*Z*)-$C_{18}H_{35}$	—	Me	Me	Me	Cl
2 (DOTAP)	(9*Z*)-$C_{17}H_{33}C(O)$	—	Me	Me	Me	Cl
3 (DOTB)	(9*Z*)-$C_{17}H_{33}C(O)$	$OC(O)(CH_2)_2$	Me	Me	Me	Cl
4 (DOSC)	(9*Z*)-$C_{17}H_{33}C(O)$	$OC(O)(CH_2)_2COO(CH_2)_2$	Me	Me	Me	Cl
5 (DORIE)	(9*Z*)-$C_{18}H_{35}$	—	Me	Me	CH_2CH_2OH	Br
6 (DORIE-HP)	(9*Z*)-$C_{18}H_{35}$	—	Me	Me	$(CH_2)_3OH$	Br
7 (DORIE-HB)	(9*Z*)-$C_{18}H_{35}$	—	Me	Me	$(CH_2)_4OH$	Br
8 (DORIE-HPe)	(9*Z*)-$C_{18}H_{35}$	—	Me	Me	$(CH_2)_5OH$	Br
9 (DORI)	(9*Z*)-$C_{17}H_{33}C(O)$	—	Me	Me	CH_2CH_2OH	Br
10 (DMRIE)	*n*-$C_{14}H_{29}$	—	Me	Me	CH_2CH_2OH	Br
11 (DPRIE)	*n*-$C_{16}H_{33}$	—	Me	Me	CH_2CH_2OH	Br
12 (DSRIE)	*n*-$C_{18}H_{37}$	—	Me	Me	CH_2CH_2OH	Br
13a	*n*-$C_{12}H_{25}$	—	Me	Me	$CH_2CH_2CH_2NH_2$	—
13b	*n*-$C_{14}H_{29}$	—	Me	Me	$CH_2CH_2NH_2$	—
14a	(9*Z*)-$C_{17}H_{33}C(O)$	—	Me	Me	Et	Cl
14b	(9*Z*)-$C_{17}H_{33}C(O)$	—	Me	Me	Pr	Cl
14c	(9*Z*)-$C_{17}H_{33}C(O)$	—	Me	Me	CH_2CH_2OMe	Cl
14d	(9*Z*)-$C_{17}H_{33}C(O)$	—	Me	Et	Et	Cl
14e	(9*Z*)-$C_{17}H_{33}C(O)$	—	Me	Et	Et	Cl
14f	(9*Z*)-$C_{17}H_{33}C(O)$	—	Me	CH_2CH_2OH	CH_2CH_2OH	Cl
14g	(9*Z*)-$C_{17}H_{33}C(O)$	—	Me	CH_2CH_2OMe	CH_2CH_2OMe	Cl
15a	$C_{13}H_{27}C(O)$	—	Me	Me	Me	Cl
15b	$C_{13}H_{27}C(O)$	—	Me	Me	Et	Cl
15c	$C_{13}H_{27}C(O)$	—	Me	Me	Pr	Cl
15d	$C_{13}H_{27}C(O)$	—	Me	Me	CH_2CH_2OH	Cl
15e	$C_{13}H_{27}C(O)$	—	Me	Me	CH_2CH_2OMe	Cl
15f	$C_{13}H_{27}C(O)$	—	Me	Et	Et	Cl
15g	$C_{13}H_{27}C(O)$	—	Et	Et	Et	Cl
15h	$C_{13}H_{27}C(O)$	—	Me	CH_2CH_2OH	CH_2CH_2OH	Cl
15i	$C_{13}H_{27}C(O)$	—	Me	CH_2CH_2OMe	CH_2CH_2OMe	Cl
16	*n*-$C_{18}H_{37}$	—	Me	Me	CH_2CH_2OH	Cl
17	*n*-$C_{16}H_{33}$	$OCH_2O(CH_2)_2$	Me	Me	Me	Br

Compound		Abbre-viation	R	X
	**18a**	DPIm	$C_{13}H_{27}C(O)$	
	18b	DPAPy	$C_{13}H_{27}C(O)$	
	18c	DOIm	$(9Z)$-$C_{17}H_{33}C(O)$	

Within the physiological range of pH (5.3—7.1), cationic lipids **18a—c** as complexes with DOPE (1 : 1) formed liposomes, which were assayed in the NIH 3T3 cells. It was found that incorporation of DNA into liposomes is more efficient under acidic conditions and that genosomes are more stable against DNAases. Of the compositions presented, the best transfection was observed for composition **18a**/DOPE, which proved to be 5 times as efficient as DOTMA/DOPE. Other liposomal formulations **18b,c**/DOPE were also conductive for efficient transfection.

The area of search for cationic lipids has been extended by the preparation of new less toxic glycerol thiocationic lipids (**19a,b**) with a polar group containing a sulfonium ion.[31]



19a: R = $(CH_2)_5COOH$
19b: R = $(CH_2)_6OH$

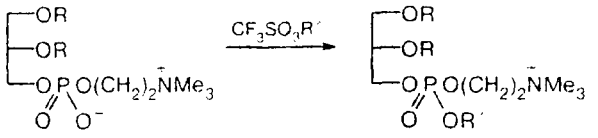
The ability of liposomal formulations comprising thiolipids **19a,b**, DOPE, and cholesterol or vitamin D_3 in 10 : 5 : 2 ratio to transfer phosphothioate oligonucleotides into the U 937 cells appeared to be close to that of the ammonium analogs, 1,2-dihexadecyloxy-3-[*N*-(6-hydroxyhexyl)-*N*,*N*-dimethylammonium]propane and 1,2-dihexadecyloxy-3-[*N*-(5-carboxypentyl)-*N*,*N*-dimethylammonium]propane. However, the toxicity of the sulfur-containing cationic lipids was somewhat lower. For instance, compound **19a** exhibited toxicity at a concentration of 90 μmol L^{-1}, while the lipid 1,2-dihexadecyloxy-3-[*N*-(6-hydroxyhexyl)-*N*,*N*-dimethylammonium]propane was toxic at a concentration of 10 μmol L^{-1}.

Apart from phosphorus-free derivatives of glycerolipids, cationic phospholipids **20a—e**, which are triesterified analogs of natural compounds, were synthesized (Scheme 2).[32]

Cationic lipids **20a—e** mediated the plasmid DNA transfer into eucaryotic cells; they were close to Lipofectin in efficiency.[33,34] A successful transfection of pulmonary tissues in experiments *in vivo* was performed with the aid of compound **20e**.[35]

The delivery of the genetic material was carried out using several cationic phosphonolipids (**21**), prepared by the Mannich reaction followed by quaternization of tertiary amines (Scheme 3).[36,37]

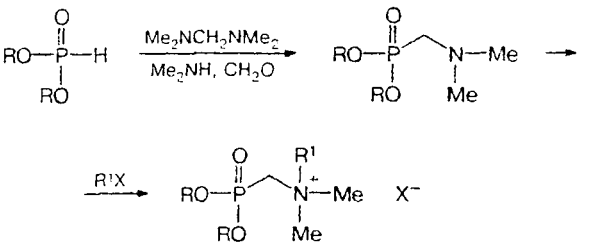
Scheme 2



20a—e

Compound	R	R′
20a	$(9Z)$-$C_{17}H_{33}C(O)$	Me
20b	$(9Z)$-$C_{17}H_{33}C(O)$	Et
20c	$(9Z)$-$C_{17}H_{33}C(O)$	$(CH_2)_6Br$
20d	$C_{13}H_{27}C(O)$	Me
20e	$C_{13}H_{27}C(O)$	Et

Scheme 3



21

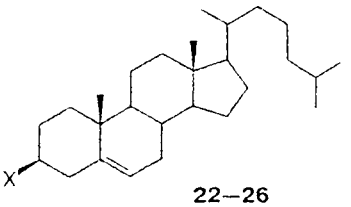
R = *n*-$C_{14}H_{29}$, *n*-$C_{16}H_{33}$, *n*-$C_{18}H_{37}$, $(9Z)$-$C_{18}H_{35}$;
R^1 = Me, $CH_2CH{=}CH_2$, $CH_2C{\equiv}CH$, $CH_2C{\equiv}N$;
X = Cl, Br, I

2.2. Cholesterol derivatives

To transfer the genetic material and to study the influence of the spacer group, the type of bond, and the type of the amino group on the transfer efficiency and on the toxicity, a number of compounds have been synthesized; some of them were 3-deoxycholesterol derivatives[5,38—40].

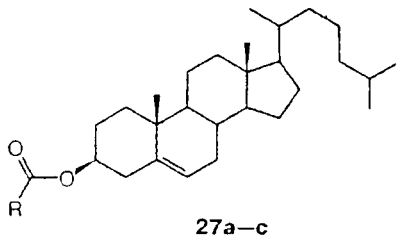
3-*O*-[*N*-(*N*′,*N*′-Dimethylaminoethyl)carbamoyl]-cholesterol (DC-Chol, **24**), containing a hydrolyzable dimethylaminoethylcarbamoyl group, mixed with DOPE (1 : 1) is a commercially available formulation used for transfection of a broad range of eucaryotic cells,[41,42] especially nerve cells both *in vitro* and *in vivo*[43,44] and for investigation of the structures of cationic liposome—DNA complexes.[45] This compound is also used in gene delivery for tumor immunotherapy[46] and artificial vaccination[21]; in addition, it undergoes extensive clinical trials for cystic fibrosis treatment.[47,48]

3-Deoxycholesterol derivative **26** with a hydroxyethylamino group proved to be more efficient in the transfection of the NIH 3T3 cells than compound **25d** with a dimethylamino group. This modification also increased significantly the efficiency of the cationic

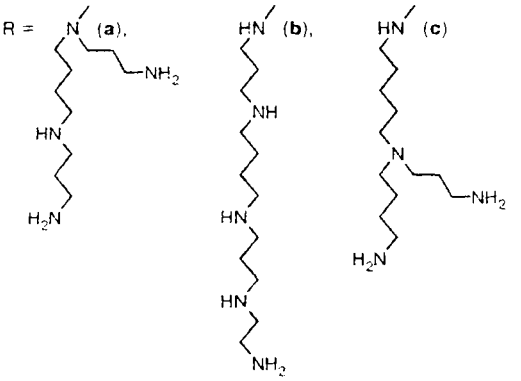


22—26

Compound	X
22 (ChoTB)	—OC(O)$CH_2CH_2N^+Me_3$
23 (ChoSC)	—OC(O)CH_2CH_2C(O)$OCH_2CH_2N^+Me_3$
24 (DC-Chol)	—OC(O)$NHCH_2CH_2NMe_2$
25a	—OC(O)$CH_2CH_2CONHCH_2CH_2N^+Me_3$ I^-
25b	—C(O)$NHCH_2CH_2N^+Me_3$ I^-
25c	—OC(O)$CH_2CH_2CONHCH_2CH_2NMe_2$
25d	—C(O)$NHCH_2CH_2NMe_2$
25e	—C(O)$NHCH_2CH_2NEt_2$
25f	—C(O)$NHCH_2CH_2CH_2NMe_2$
25g	—OC(O)$NHCH_2CH_2NEt_2$
25h	—OC(O)$NHCH_2CH_2NPr^i_2$
25i	—OC(O)$NHCH_2CH_2CH_2NMe_2$
26	—C(O)$NHCH_2CH_2NHCH_2CH_2OH$



27a—c



liposome-mediated DNA transfer in the presence of blood serum.[40]

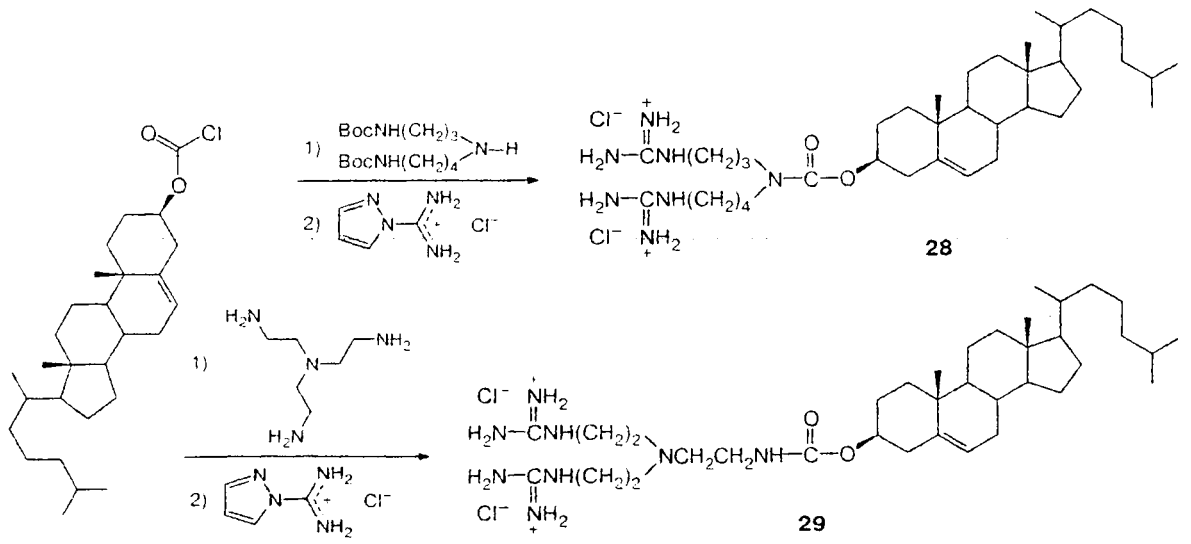
Further modification of cationic lipids containing the cholesterol residue as the hydrophobic moiety was aimed at changing the nature of the positively charged part of the molecule; thus, new analogs of cholesterol **27a—c** were synthesized by covalent binding of the corresponding polyamine to cholesterol.[49—51]

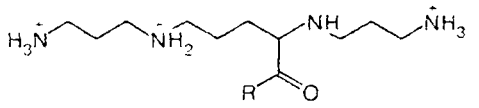
The data of performed transfection pointed out that the activity of liposome **27a**/DOPE is approximately 100 times as high as that of DC-Chol/DOPE for the delivery *in vivo* of the plasmid encoding chloramphenicolacetyltransferase gene[49]; **27a**/DOPE liposomal composition is currently under clinical trials.[52]

Cationic lipid **27c** (Transfectall, Apollon Inc.) is used without a helper lipid; it can be stored for long periods in the frozen state. Moreover, there is a possibility that it would form conjugates with compounds carrying cell-specific ligands or endosomal escape or nuclear localization ligands, which could facilitate the development of gene delivery systems.[51]

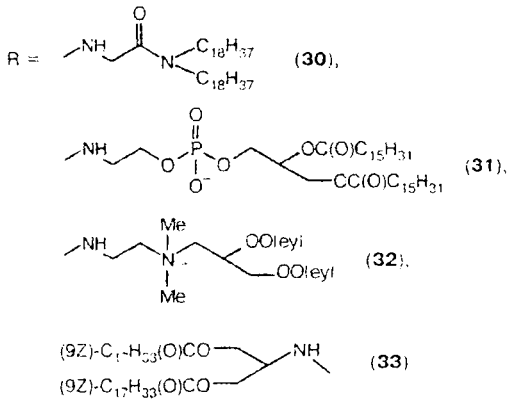
As a development of studies on the variation of the polar domain structure, cationic conjugates of guanidine

Scheme 4





30—33



Oleyl = (9Z)-$C_{18}H_{35}$

with cholesterol **28**, **29** have been synthesized in yields of up to 61% (Scheme 4).[53] Guanidine groups can form hydrogen bonds with the phosphate groups of polynucleotides, which thus become protected more efficiently from degradation in late endosomes and lysosomes. These lipids can be used for lipofection *in vitro* without DOPE. They form micellar solutions, which allows one to avoid the stage of liposome preparation.

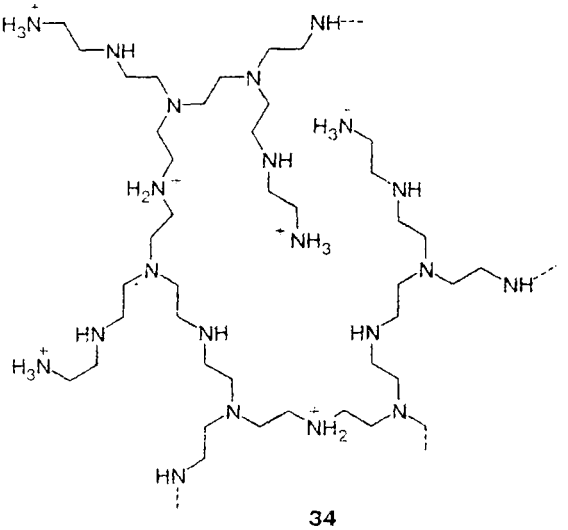
Transfection of a broad range of cell lines indicates that the process efficiency is fairly high, being comparable with that of commercial compositions and 10—20 times higher than the efficiency of the method in which calcium phosphate is employed.[53,54]

2.3. Lipophilic derivatives of polyamines, amino acids, and peptides

The ability of lipoamines to bind DNA[55] was used to transfect eucaryotic cells[56] with the aid of compounds **30—31**. Commercialization of dioctadecylamino-*N*-(6-spermylcarbonyl)glycine* (**30**) (DOGS, Transfectam, Promega) was followed by the preparation of *N*-[1-(2,3-dioleyloxy)propyl]-*N*-[2-(6-spermylcarbonylamino)-ethyl]-*N*,*N*-dimethylammonium trifluoroacetate (**32**) (DOSPA)[57] (1 : 3 mixture of this compound with DOPE has received the name Lipofectamine, GIBCO BRL) and 1,3-dioleoyloxy-2-(6-spermylcarbonylamino)propane (**33**) (DOSPER), which is used without a helper lipid. These lipopolyamines are widely used in the transfection of a number of cell cultures[58,59] and are more efficient than monocationic lipids.

DOGS is widely used to study the morphology of cationic lipid—DNA complexes[60,61] and to deliver genetic material into mammalian embryos and brain tissues.[62,63] DOSPA is used to deliver antisense oligonucleotides[25,64] and genes responsible for the biosynthesis of immunostimulating proteins,[65] which can be used for therapy of oncological diseases.

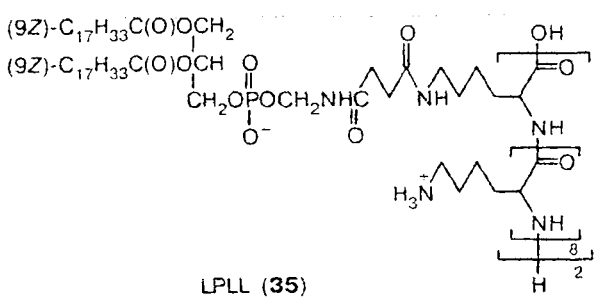
* Spermine is *N*,*N*′-bis(3-aminopropyl)-1,4-diaminobutane.



34

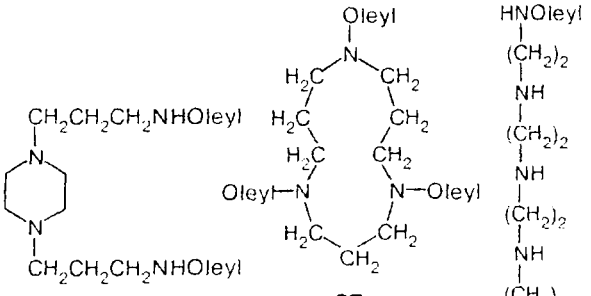
Polyethyleneimine (**34**)[66] is an efficient mediator of transfer of genetic material operating as a "proton sponge" over a broad range of pH values. Polyethyleneimine (**34**) is regarded as an perfect vector for the *in vivo* transfer of antisense oligonucleotides into brain cells of mice, because it ensures high levels of transfection over a broad range of DNA concentrations.[67] No cases of necrosis or mortality of laboratory animal cells were observed during the experiments; the integrity of cell membranes was not deteriorated either. In addition, polyethylenimine improves the delivery of DNA to dense tissues such as adult mammalian brain tissues, in which cationic lipids are of low efficiency. Polyethyleneimines possess relatively low cytotoxicity, which allowed them to be used, for example, in gene transfer into mammal embryos.[68]

The lipophilic derivative of poly-L-lysine (LPLL (**35**)) proved to be 3 times as efficient as Lipofectin in experiments with transformed fibroblasts.[69,70] This compound is stable in the presence of blood serum (30—40%).



LPLL (**35**)

New lipopolyamines (**36**—**38**) were synthesized and found to mediate the transfection of the NIH 3T3, HeLa, and COS-7 cells. The results obtained for these compounds were close to those for DOSPA/DOPE but much better than those for DOTMA/DOPE. In addition, these polyamines were found to be less toxic than the DOSPA/DOPE or DOTMA/DOPE formulations.[71]

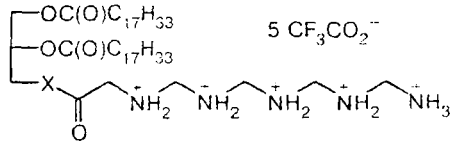


36 **37** **38**

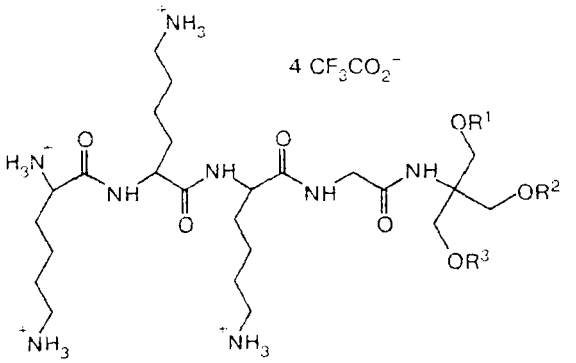
In order to elucidate the relationship between the structure and capacity to deliver genetic materials mediated by lipophilic derivatives of polyamines, modified lipopolyamines of various structures were synthesized by the solid-phase method (Scheme 5)[72].

Experiments *in vitro* showed that, although all the modified polyamines exhibited high levels of transfection activity, compound **39c** with the nonbranched polyamine fragment was still more efficient than compounds **39e,f** with branched structures. An increase in the distance between the hydrophobic and hydrophilic parts of the lipopolyamine molecule had virtually no influence on the transfection efficiency, while a decrease in the length of the aliphatic chains resulted in lower efficiency.

Two new types of cationic amphiphiles of peptide and polyamine natures (**40**, **41**) have been prepared for gene therapy.[73,74]



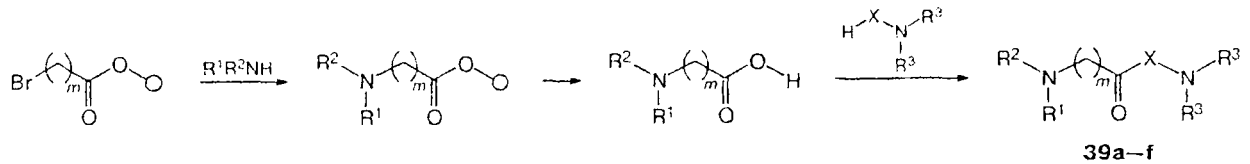
40a: X = O
40b: X = NH



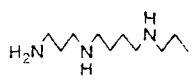
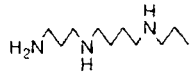
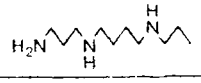
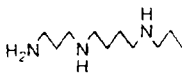
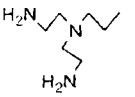
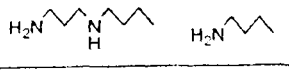
41a: $R^1 = R^2 = H$, $R^3 = C_{15}H_{31}CO$
41b: $R^1 = R^2 = C_{15}H_{31}CO$, $R^3 = H$
41c: $R^1 = R^2 = R^3 = C_{15}H_{31}CO$

Lipid **40b** was found to be more stable during storage[73] and to demonstrate higher activity as mediator of gene transfer[75] than the analog **40a**.

Scheme 5

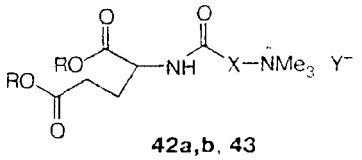


O — solid phase support

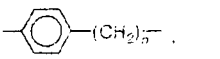
39	R^1	R^2	m	X	R^3
a		H	1	—	$(CH_2)_{17}Me$
b		H	3	—	$(CH_2)_{17}Me$
c		H	1	$NHCH_2CO$	$(CH_2)_{17}Me$
d		H	1	$NHCH_2CO$	$(CH_2)_{13}Me$
e		H	1	$NHCH_2CO$	$(CH_2)_{17}Me$
f			1	$NHCH_2CO$	$(CH_2)_{17}Me$

Among lipids **41**, containing identical cationic groups and different numbers of acyl groups in the hydrophobic domain, compound **41b** with two acyl substituents was the most active in transfection; these compounds are relatively nontoxic and do not require a helper lipid.[74]

Substances of amino acid nature present considerable interest. Cationic amphiphiles **42** and **43** have been synthesized using diester of glutamic acid.[76]



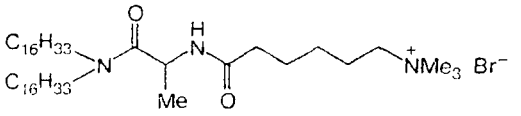
42a,b, 43

Compound	R	X	Y
42a	$C_{12}H_{25}$	 n = 2, 4, 6, 8	Cl, Br
42b	$C_{14}H_{29}$	$(CH_2)_n$, n = 1, 5	Cl, Br
43	$C_{12}H_{25}$	CH_2	Cl

The efficiency was found to decrease significantly with an augmentation in the distance between the lipid and cationic moieties of the molecule. The best results were obtained for the compound with two methylene groups. However, it was shown that these cationic lipids can change the DNA structure.[77]

Compound **43** (TMAG) in combination with DOPE is more efficient for the transfection of fibroblasts than $Ca_3(PO_4)_2$.[78] Later, it was shown that TMAG complexed with DOPE and dilauroylphosphatidylcholine (DLPC) exhibited activity comparable to that of DC-Chol/DOPE and higher than that of Lipofectin and DDAB/DOPE without a serum[79] and transfected not only cell monolayers but also suspensions in the presence of 10% serum. TMAG was used efficiently for the delivery of oligonucleotides[80] and the gene encoding the diphtheritic toxin A-chain *in vivo*[81] into eucaryotic cells and for the preparation of magnetic liposomes.[82] The above-mentioned approaches permit efficient therapy of cancer.[80–82]

Cationic amphiphile **43** containing an L-Ala residue between the polar head and the aliphatic segment has been synthesized and used to prepare liposomes.[83]



44 (NC_5-Ala-$2C_{16}$)

The sonication of lipid **44** results in the formation of monolayer vesicles, whose parameters are comparable with those of bilayer vesicles obtained from egg yolk phosphatidylcholine. They are highly stable in solution, apparently, due to strong intravesicular hydrogen interaction between amino acid residues. This agent proved to be more active and less toxic than Lipofectin or DOTAP/DOPE.

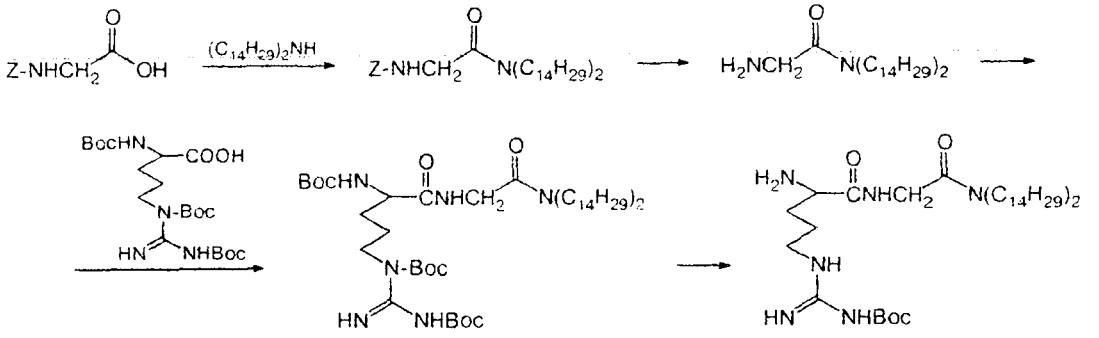
In order to deliver antisense oligonucleotides to eucaryotic cells, a compound (**45**) (GS 2888) of amino acid nature has been synthesized (Scheme 6).[84]

Compound **45** mediated efficiently the transfer of antisense oligonucleotides into eucaryotic cells and displayed several advantages over Lipofectin, Lipofectamine, and Transfectam; this agent can be used both in the presence and in the absence of blood serum with high reproducibility and is relatively nontoxic for cells.[84]

Cationic lipids used in the preparation of liposomes for *in vivo* application should exhibit low toxicity. In order to find new, more efficient, and less toxic cell-biodegradable cationic lipids, esters of carnitine (3-hydroxy-4-trimethylaminobutyric acid betaine) (**46a–f**) (Scheme 7)[85] and compounds **47a–c** based on *N,N,N*-trimethylglycine (Scheme 8)[86] have been synthesized.

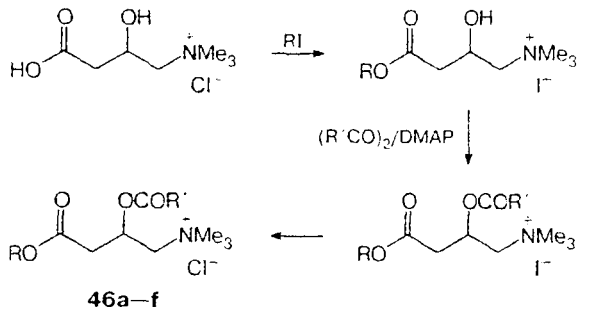
The results of CV-1 cell transfection showed that the transfection activity of carnitine esters mixed with DOPE decreases in the following sequence: **46d** > **46e** > **46b** >

Scheme 6



45

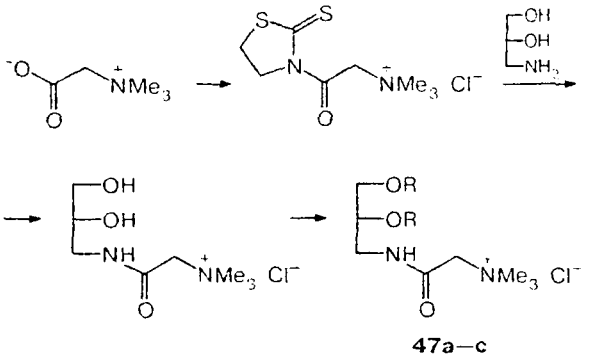
Scheme 7



46a—f

	R	R′		R	R′
a	*n*-$C_{18}H_{37}$	$C_{17}H_{35}$	**d**	*n*-$C_{14}H_{29}$	$C_{13}H_{27}$
b	*n*-$C_{18}H_{37}$	(9*Z*)-$C_{17}H_{33}$	**e**	*n*-$C_{12}H_{25}$	$C_{11}H_{23}$
c	*n*-$C_{16}H_{33}$	$C_{15}H_{31}$	**f**	(9*Z*)-$C_{18}H_{35}$	(9*Z*)-$C_{17}H_{33}$

Scheme 8



47a—c

R = $C_{12}H_{21}$ (**a**), $C_{14}H_{29}$ (**b**), $C_{16}H_{33}$ (**c**)

46f > **46c** > **46a**. Based on the results of experiments *in vitro*, compounds **46b**, **46d**, and **46f** were chosen for *in vivo* assays. Comparison of the *in vivo* efficiencies of cationic liposomes consisting of compounds **46b**, **46d**, **46f**, DOTMA, and DDAB as mixtures with cholesterol (1 : 1) revealed the following order of decrease in the efficiency: DOTAP > **46f** > DDAB > **46d** > **46b**.

An interesting fact has been noted when using compounds **47a—c**. It was found that lipids **47a,b** promoted efficient DNA transfer without a helper lipid (DOPE); however, lipofection in the presence of blood serum required the presence of DOPE.[86]

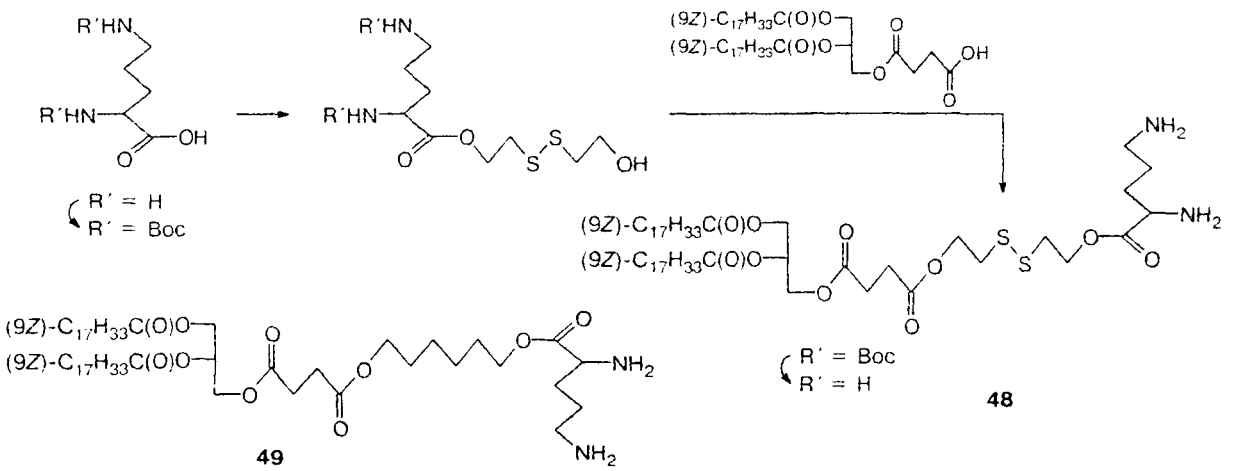
The escape of the DNA molecule from the liposomal compartment is known[23] to be the rate-limiting step. Hence, to facilitate the release of the DNA molecule, a new ornithine derivative **48** has been synthesized (Scheme 9). The disulfide bond linkage between the polar and aliphatic domains in this molecule can be easily cleaved under the action of the cell environment.[87]

Transfection of various eucaryotic cell lines (including those of brain cells) demonstrated that the **48**/DOPE composition is 6—10 times as active as DOTAP/DOPE and 30—50 times as active as the **49**/DOPE composition.[87,88]

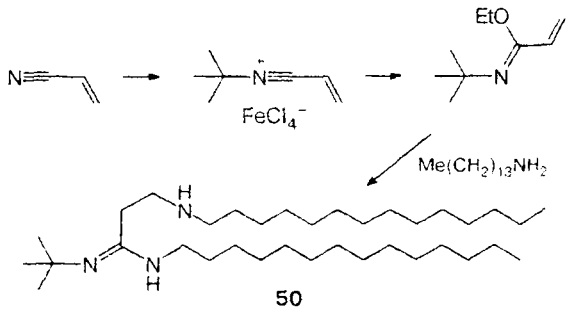
New cationic amphiphile (**50**) (Scheme 10), called Amidine (the 1 : 1 Amidine/DOPE composition was patented as Clonfectin (Clontech)), is capable of transferring DNA and mRNA into eucaryotic cells with high efficiency.[89—91]

Comparison of the data on transgene expression showed that in the case of Amidine, expression was higher than that for Lipofectin and comparable to that for the DMRIE/cholesterol composition, apparently, due to the ability of Amidine to destabilize and disrupt the endosomal membrane. Amidine, as well as DMRIE/DOPE, can fuse with the endosomal membrane, thus liberating the complex into the cytoplasm, but the mecha-

Scheme 9



Scheme 10

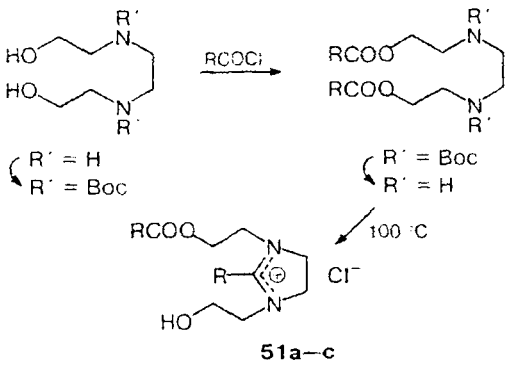


nism of this action differs from the mechanism characteristic of DOPE.[92] In all probability, this is due to the presence of short saturated ($C_{14}H_{29}$) hydrocarbon chains in the hydrophobic domain of the lipid. In addition, Amidine can favor DNA transfer in the presence of blood serum.[93]

2.4. Amphiphilic derivatives of imidazole and pyridine

Lipids **51a—c**, containing an imidazolinium ring,[94] were prepared according to Scheme 11.

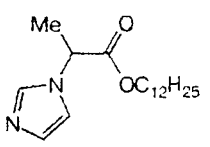
Scheme 11



R = (9*Z*)-$C_{17}H_{33}$ (**a**), $C_{15}H_{31}$ (**b**), $C_{13}H_{27}$ (**c**)

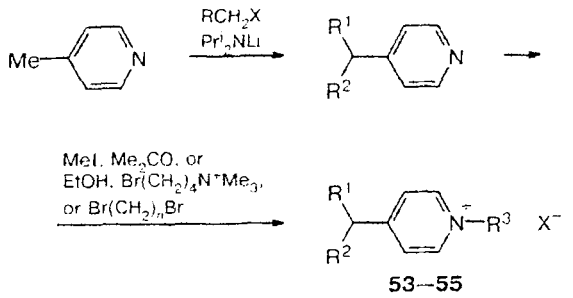
Compound **51a** (DOTIM) showed itself as the most efficient compound for transfection *in vivo*.[95] Cationic monolamellar vesicles consisting of a 1 : 1 **51a**—cholesterol mixture were used for direct intravenous injection of the pLG-CSF plasmid in animals suffering from limphopathologies and showed encouraging results.[96]

To form pH-sensitive liposomes, dodecyl 2-(imidazol-1-yl)propionate (**52**) has been synthesized[97] and transfection of HTB11 cells with DOTAP/DOPE and DOTAP/DOPE/**52** (1 : 1 : 1) liposomes was performed.[98] Liposomes containing compound **52** proved to be 5 times as efficient as DOTAP/DOPE, apparently, due to the influence of the imidazolium derivative on the escape of the genetic material from the endosome.



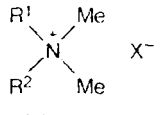
New pyridinium cationic amphiphiles **53—55** for gene transport have been synthesized. The I^- or Br^- counterions were subsequently replaced by Cl^- (Scheme 12).[99]

Scheme 12



Compound	R^1	R^2	R^3	X
53a	*n*-$C_{16}H_{33}$	*n*-$C_{16}H_{33}$	Me	Cl
53b	$C_{18}H_{35}$	$C_{18}H_{35}$	Me	Cl
53c	(9*E*)-$C_{18}H_{35}$	(9*E*)-$C_{18}H_{35}$	Me	Cl
53d	(9*Z*)-$C_{18}H_{35}$	(9*Z*)-$C_{18}H_{35}$	Me	Cl
53e	*n*-$C_{18}H_{37}$	*n*-$C_{18}H_{37}$	Me	Cl
54	*n*-$C_{16}H_{33}$	*n*-$C_{16}H_{33}$	$(CH_2)_4N^+(Me)_3$	Cl
55a	*n*-$C_{16}H_{33}$	*n*-$C_{16}H_{33}$	$-C_3H_6-N^+$ (R¹, R²)	Cl
55b	*n*-$C_{16}H_{33}$	*n*-$C_{16}H_{33}$	$-C_4H_8-N^+$ (R¹, R²)	Cl
55c	*n*-$C_{16}H_{33}$	*n*-$C_{16}H_{33}$	$-C_5H_{10}-N^+$ (R¹, R²)	Cl

Cationic lipids **53—55** were used as complexes with DOPE (1 : 1) for transfection of the COS-7 cell line with a plasmid DNA containing the β-Gal reporter gene. Comparative analysis of the activity of these lipids made it possible to formulate the following structural requirements. Elongation of the chains in the R^1 and R^2 substituents from $C_{16:0}$ to $C_{18:0}$ halves the efficiency: if $R^1 = C_{18:0}$ and $R^2 = C_{18:1}$, the efficiency is much higher than that observed for completely saturated compound **53e** and 2—3 times higher than that of Lipofectin; unsaturated analog **53d** with R^1, $R^2 = C_{18:1}$ is 10 times more efficient than saturated analog **53e** and isomers *cis*-**53d** and *trans*-**53c** are comparable in efficiency; the introduction of a polymethylene group between the pyridinium groups of **55a—c** does not change appreciа-



56, 57a—h

Compound	Abbreviation	R^1	R^2	X
56	DEBDA	$PhCH_2$	$(CH_2)_7O(CH_2)_2$–C(Me)(Me)–CH_2CMe_3	OH
57a	DTAB	n-$C_{12}H_{25}$	Me	Br
57b	TTAB	n-$C_{14}H_{29}$	Me	Br
57c	CTAC/CTAB	n-$C_{16}H_{33}$	Me	Cl/Br
57d	DHDAB	n-$C_{16}H_{33}$	n-$C_{16}H_{33}$	Br
57e	HODAB	n-$C_{16}H_{33}$	(9Z)-$C_{17}H_{33}(CH_2)_2(O)CO$	Br
57f	DDAB/DDAC	n-$C_{18}H_{37}$	n-$C_{18}H_{37}$	Cl/Br
57g	OSDAC	n-$C_{18}H_{37}$	(9Z)-$C_{18}H_{35}$	Cl
57h	DODAC/DODAB	(9Z)-$C_{18}H_{35}$	(9Z)-$C_{18}H_{35}$	Cl/Br

bly the transfection efficiency; all the amphiphiles except for **55c** showed low toxicity.

The above results also indicate that these lipids are much more efficient than DOTMA/DOPE and function at lower concentrations.

2.5. Surfactant quaternary ammonium salts

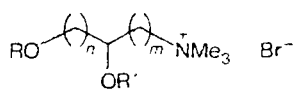
Surfactant quaternary ammonium salts (**56**, **57**) contained in liposomes are finding use in the introduction of gene into various cell lines.[100—104] These cationic lipids are widely distributed and relatively inexpensive; they can be synthesized *via* rather simple chemical transformations.[103—105]

Transfection of the RBC cells demonstrated that the activity decreases in the series **57h** > **57g** > DOTMA > **57f**; the sequence found for the transfection of the BHK cells[103] is **57h** > **57g** ≈ DOTMA ≈ **57f**.

The surfactant quaternary ammonium salts used most frequently are compounds **57f** (1 : 2.5 DDAB/DOPE composition was patented as Transfectam (Promega)) and the **57h**/DOPE composition. They are widely used as mediators of the delivery of plasmid DNA and antisense oligonucleotides both *in vitro*[59,64,65,106—108] and *in vivo*[19,85] and also in the studies of the mechanisms of formation and morphology of DNA—cationic lipid complexes.[109,110]

2.6. Cationic derivatives of polyols

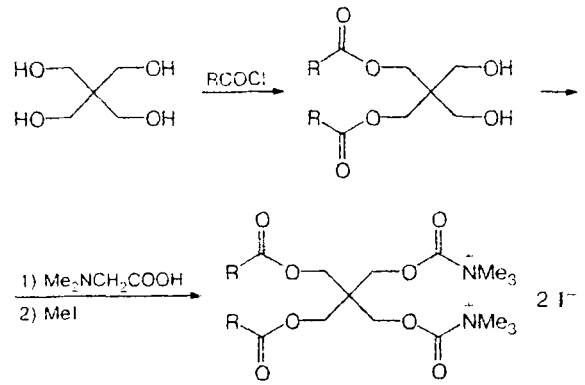
Development of cationic lipids possessing the minimum toxic side effect but ensuring efficient transgene delivery underlies lipofection. Cationic lipids (**58**) were prepared based on pentaerythritol (Scheme 13); their hydrolysis in the cell affords a zwitterion amino acid and pentaerythritol diester, which do not posses noticeable toxicity.[111] Moreover, this hydrolysis may promote more efficient entry of plasmid DNA into a cell.



59

R = (9Z)-$C_{18}H_{35}$, n-$C_{14}H_{29}$, (9Z)-$C_{17}H_{33}C(O)$;
R′ = n-$C_{14}H_{29}$, $C_{13}H_{27}C(O)$, (9Z)-$C_{18}H_{35}$, (9Z)-$C_{17}H_{33}C(O)$;
n, m = 1, 2, 4

Scheme 13



58a: R = $C_{13}H_{27}$
58b: R = (9Z)-$C_{17}H_{33}$

The most efficient lipofection of eucaryotic cells was observed for compound **58b**; moreover, both compounds **58a,b** are less toxic than DOTAP or DC-Chol.

Triols were used as the starting compounds to prepare dialkyl, alkyl acyl, and diacyl positively charged derivatives **59** with a potential transfection activity.[112,113]

3. Relationship between the structure and the activity

A great number of cationic lipids with diversified structures and different activities and toxicities in cell models have been synthesized by now. Therefore, it is fairly difficult to follow the exact relationship between the structure and the activity of a cationic lipid. Many complexes active *in vitro* proved to be inefficient *in vivo* because of their instability, interaction with blood components, and difference in biodistribution.

Structure—activity relationships are usually studied *in vitro* using different types of cell lines, which hampers substantially comparison of the results. The first investigations[5] of series of lipids containing cholesterol or long-chain hydrocarbon groups as the hydrophobic components showed dissimilar results. For this reason and

for the convenience of comparison, it is expedient to consider these groups of lipids separately.

3.1. Cholesterol-containing lipids

The molecular structure of these lipids can be represented conventionally by several structural units, which influence the transfection activity. These include the types of the amino and spacer groups and the type of linkage with cholesterol. In order to evaluate the influence of these structural units on the efficiency of DNA transfer and the toxicity, compounds **22**, **23**, and **25a—d**, some of which are derivatives of 3-deoxycholesterol (**25b,d**), have been studied.[38] Thus tertiary amines **25c,d** displayed a lower toxicity than quaternary ammonium derivatives. The latter showed relatively low activity in the transfection experiments, whereas the activity of compound **25c** exceeded that of Lipofectin by a factor of 3. Compound **25d** was less efficient. Thus, it was found[38,41] that successful transfection requires the presence of a tertiary amino group attached to a cholesterol by an ester or carbamoyl bond through a small spacer (three to six carbon atoms). These empirical correlations served as the grounds for preparing DC-Chol (**24**), which promoted efficient lipofection both *in vitro* and *in vivo*.[41—44]

In the case of compounds **24** and **25c—i**, the relationship between the structure, the liposome size, and the ζ-potential (the surface potential of the membrane), *i.e.*, factors determining the transfection efficiency, has been studied.[114,115]

The following sizes of liposomal aggregates were found by microscopic studies: 200—400 nm for **25c,h**, 0.4—1.4 μm for **25d—f,i**, and 1.4—2.0 μm for **24**, **25g**. Compound **25d** displayed high activity in the transfection of the NIH 3T3 cells, whereas **24**, **25f,g,i** were only moderately active. The values of the ζ-potential were correlated with the transfection efficiency of the HeLa, COS-7, and NIH 3T3 cells. Cationic liposomes formed from compound **25d**, having the highest ζ-potential, showed the best lipofection activity for all cell lines, whereas the activity of liposomes formed from **25c,g,h**, having the lowest ζ-potential, was less than 20% of the activity of compound **25d**. The replacement of the dimethylamino group in compounds **24** and **25d** by a diethylamine group giving compound **25e,g** or by a diisopropylamino group giving compound **25h** decreased the ζ-potential and, hence, the transfection efficiency. The activity of DC-Chol was comparable to that of compounds **25e,f,i**.

Compound	ζ-potential	Compound	ζ-potential
24	26.5±4.0	**25f**	27.5 ±1.1
25c	12.6±1.0	**25g**	9.0±1.4
25d	33.6±1.3	**25h**	11.1±1.3
25e	22.2±2.4	**25i**	27.5±2.5

The replacement of two methyl groups in compound **25d** by hydroxyethyl groups resulted in a compound that proved to be more active in the transfection of the NIH 3T3 cells. This structure modification also increased substantially the efficiency of DNA delivery mediated by cationic liposomes in the presence of blood serum.[40]

All the modifications listed above and studies on the influence of these modifications on the transfer of genetic material were performed with monocationic lipids; therefore, based on the use of compounds such as DOGS (**30**) or DOSPA (**32**), lipids **27a—c**, containing polycationic groups in the hydrophilic moiety of the molecule, have been synthesized.[49—51] The results of lipofection carried out for **27a** and related compounds showed that the site of attachment of the hydrophobic part to the polyamine is fairly significant. Linking of cholesterol to a secondary nitrogen atom resulted in the formation of the compound as the so-called T-shaped conformation. These compounds were found to be more active than amphiphiles with "linear" attachment, in which linking involved a primary nitrogen atom. The type of linking also influenced the activity, namely, a carbamoyl group is preferred over an amide or amine group.

3.2. Lipids containing aliphatic hydrocarbon substituents

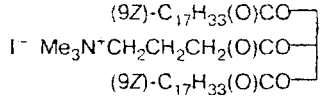
After DOTMA had been used for DNA transfer,[4] hundreds of other compounds obtained by modification of this structure appeared. These modifications involved both the hydrophobic and hydrophilic moieties of the molecule, changes in the backbone, and the presence or the absence of a spacer group. Studies on the transfection and toxicity gave ambiguous results. It was noted that the molecule of DOTAP (**2**), having aliphatic acyl substituents, is less toxic than the alkyl analog DOTMA. It was also found that an increase in the distance between the hydrophobic and positively charged moieties of molecules **3** and **4** decreases the transfection efficiency.[5] Data on the efficiency of *in vitro* transfection by means of cationic lipids **5—12** led to the following conclusions. First, the efficiency diminishes in the series of compounds with the following alkyl substituents: ditetradecyl > dioleyl > dihexadecyl > dioctadecyl; second, replacement of one methyl group in the polar head of DOTMA by a hydroxyethyl group giving compound **5** substantially increases the activity; third, the efficiency of transfection decreases with an increase in the number of methylene groups between the hydroxy group and the nitrogen atom in the cationic part of the molecule.[6] The established correlation between the length of the hydrophobic fragment and the activity was also followed for other classes of compounds, for example, for phosphonolipids[36] **21**, carnitine esters[85] **46**, and glycine[86] **47**. However, in the case of cationic imidazolium derivatives **51**[94] and pentaerythritol esters **58**,[111] the efficiency increased in the series dioleyl > ditetradecyl, while in the case of lipoamines **39**,[72] the most active compound was that containing dioctadecyl substituents.

It has been found previously that even minor structural changes in the hydrophobic or polar domains of

lipids changes significantly the transfection activity.[6] The relationship between the structure of cationic lipids or the degree of hydration of the lipid surface and the efficiency of *in vivo* transfection has been studied[10] in relation to a number of tetraalkylammonium derivatives **2**, **9**, **15a—g**, and **16a—i**. Lipids were mixed with DOPE in 1 : 1 ratio and were administered intratracheally in mice as complexes with DNA. Data on the degree of hydration of cationic lipids, obtained by high-sensitivity differential scanning calorimetry, and on the efficiency of the delivery of the pNDCLux plasmid to lung permitted the following conclusions to be made: hydrophobic domains in dioleoyl derivatives **2**, **9**, and **15a—g** contain larger amounts of lipid-associated water than hydrophobic domains in compounds **16a—i** containing residues of myristic acid; consequently, the volume occupied by aliphatic chains decreases in the latter case: the introduction of hydroxy groups in the cationic fragment of lipids decreases the degree of its hydration and, hence, decreases the bulk of the polar domain. This, in turn, decreases the repulsion between the adjacent lipid molecules and increases the propensity of lipids to form intramolecular hydrogen bonds, which results in a better packing of polar heads of the lipids. Cationic lipids **15c**, **15f**, and **15g** possess the greatest imbalance in the areas occupied by polar and hydrophobic domains, which favored the formation of a cone-shaped molecular geometry and made them more prone to form nonbilayer structures; this can facilitate membrane fusion.[116] Compounds **15c**, **15f**, and **15g** were found to be the most active under the experimental conditions. Thus, according to the *in vivo* results, the activity decreases in the series dioleoyl > dimyristoyl, which has been confirmed subsequently by other *in vivo* assays.[85]

In order to evaluate the influence of the counterion on the lipofection efficiency for DOTAP (**2**), compounds with different anionic groups have been prepared.[117] It was found that some counterions improve the plasmid DNA delivery into cells by decreasing the degree of hydration of the polar domain of the lipid. The activity decreased in the following sequence of compounds with different counterions: $DOTAP \cdot HSO_4^-$ > $CF_3SO_3^-$ ≈ I^- ≈ Br^- > $H_2PO_4^-$ ≈ Cl^- ≈ CH_3COO^- > SO_4^{2-}.

The influence of the type of linkage between the hydrophilic and hydrophobic fragments is still unknown. It was found that the ether type of linking increases the stability and the service life of liposomes but it also increases toxicity.[5] Attachment through an ester bond decreases the liposome toxicity[85,86,111] but does not ensure proper stability. New acyl-type lipid **60**, containing the cationic function at the C(2) atom of the glycerol backbone, displayed high activity and stability and low toxicity despite the presence of ester groups.[118,119]



60

The polar head in most cationic lipids is represented by a quaternary ammonium salt, which is believed to be responsible for the certain toxicity of these compounds. The introduction of imidazole, methylimidazole, or aminopyridine into the hydrophilic moiety of the lipid molecule can substantially increase the activity, apparently, due to delocalization of the positive charge over the cyclic fragment of the molecule; this promotes more efficient formation and disruption of DNA complexes with liposomes.[7,8,30,94,98] The use of a polyamine cationic group enhances the lipid solubility in water and its ability to form micellar structures. However, the complexes formed upon the interaction of these structures with DNA possess low stability and low activity.[1]

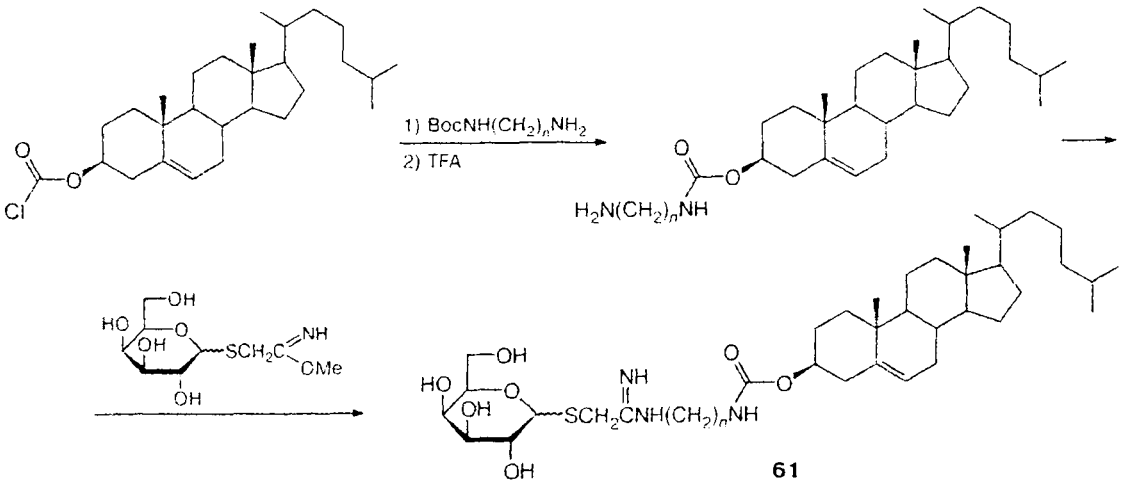
As noted above, complexes active *in vitro* are not always active *in vivo*. This depends not only on the molecular structure of the cationic lipid but also on the helper lipid and the method used to form the cationic liposomes and their complexes with DNA. Although it was shown that efficient *in vitro* transfection requires the presence of DOPE as the helper lipid,[42,120] the highest activity in the *in vivo* assays was exhibited by cholesterol-containing liposomes[1,19,85]; this might be due to the ability of cholesterol to form more ordered structures with cationic lipids. This, in turn, ensures compact packing of DNA, which protects it from degrading influences, and improves adsorption of the complexes by the cell surface. The *in vivo* activity of genosomes depends on characteristics of colloidal particles such as the size and stability rather than on the structure of the cationic amphiphile and the type of the helper lipid.

4. Conclusion

This review is an attempt to classify cationic lipids used as mediators of transfection. However, it should be noted that this classification is rather arbitrary because cationic lipids can also be classified according to other features and properties.[72] The aim of this review was to demonstrate the diversity of compounds employed in gene therapy as nonviral mediators of gene transfer, to note particular features of these compounds, and to follow the structure—activity relationship.

The cationic-liposome mediated transfer of nucleic acids is currently a well-developed method widely used both *in vitro* and *in vivo*, or *ex vivo*, owing to simplicity and ready availability. The properties of cationic liposomes that are worth noting are nonimmonogenicity and the ability to transfer plasmid DNA of virtually unrestricted sizes; in this respect, they are superior to virus vectors. However, the lipofection efficiency often proves to be relatively low for several reasons, which include toxicity of cationic lipids, cell nonspecificity of cationic liposomes, and the low percentage of genosome uptake by cells, clearance from the endosomal compartment, and entry into the nucleus needed for the required therapeutic effect to be manifested. These problems

Scheme 14



n = 2, 4, 6

could hardly be solved by the development of new cationic lipids that would be biodegradable, assimilable, and relatively nontoxic; thus, cationic liposomes should be provided with not only the appropriate lipid components but also with special ligands that would be responsible for cell-specific targeting (*i.e.*, would perform targeted transport of liposomes), for interaction with the cell and endosomal surface, and for DNA escape and interaction with the nucleus.[121]

The prospects for the design of biodegradable cationic lipids based on natural products, for example, carnitine or glycine, are worth noting.[85—87,111] For cell-specific targeting to hepatocytes, galactose ligands exposed on the surface of cationic liposomes[19,122,123] and glycosylated derivatives of poly(L-lysine) have been used.[124] Cationic lipids **61** with covalently attached galactose residues have been proposed (Scheme 14); the incorporation of these carbohydrate fragments into cationic liposomes afforded transfection of hepatocytes with high efficiency.[125]

Entry of complexes into the cells can be facilitated by using oligopeptide ligands of receptor-mediated endocytosis, fusogenic peptides,[126] nuclear localization peptides,[127] or combinations of cationic liposomes with inactivated Sendai viruses (fusogenic liposomes)[128] or with adenoviruses.[129] The problem of escape of DNA—cationic liposome complexes from the endosomal environment can be solved by using lipoamine molecules[58,59,66,69,70] or by using pH-sensitive compounds[28,98,130] including lipids[7,8,30] or peptides able to destabilize and break the endosomal membrane[125] as components of cationic liposomes.

Apart from the above-listed problems related to the selection of the composition of cationic liposomes, the use of cationic liposomes *in vivo* is also associated with certain difficulties, especially in the case of system administration; they include short time of circulation in an organism and interaction with proteins of the complement system and blood components, which can be eliminated by using so-called stabilized DNA/lipid particles. In addition to a cationic lipid and a helper lipid, these particles contain a small amount of polyethylene glycol linked to ceramides or DOPE by a covalent bond. Thus, the external surface of DNA—lipid complexes has flexible polyethylene glycol chains, which protect liposomes from early aggregation and inactivation.[131—133]

In conclusion, it should be noted that synthesis of new cationic lipids, study of the structure—activity relationship, and design of effective multicomponent cationic liposomes are promising for the development of gene therapy for treatment of hereditary and nonhereditary diseases.

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