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Positively charged lipids: structure, methods of synthesis, and applications

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Abstract. Data on the structure, methods of synthesis, and applications of positively charged lipids are presented. Their likely usefulness as antitumour and antiviral agents and also as antagonists of the platelet activating factor and transfection mediators is demonstrated. The bibliography includes 80 references.

I. Introduction

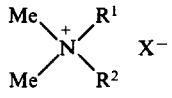
There has been a considerable growth of interest in recent years in a new type of modified glycerolipids containing a positively charged group (cationic lipids). Such interest in compounds of this class is due to the possibility of their wide scale employment in the transportation of genetic material and other macromolecules into cells of animal origin and plant protoplasts and also in the creation of artificial membranes with an affinity for negatively charged cell surfaces. Their antitumour activity, partly associated with the inhibition of protein kinase C and diacylglycerol kinase, is also known. Many representatives of cationic lipids inhibit effectively the replication of the HIV-1 virus. In addition, promising antagonists of the lipid bioregulator — the platelet activating factor (PAF) have been discovered among them. All these features lead to the possibility of devising chemotherapeutic preparations of a new type on the basis of positively charged lipids.

II. The structure and synthesis of positively charged lipids

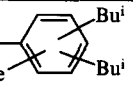
Among positively charged lipids, particular attention has been devoted to glycerol derivatives. Cationic glycerolipids have a wide variety of structures, but their common feature is the presence of a positively charged head, an intramolecularly uncompensated negatively charged group, and one or several long-chain substituents in the hydrophobic part of the molecule. It has been established that the length of the chains of the substituents at the C(1) and C(2) atoms of glycerol, the type of cationic head, and also the presence of a spacer group, separating it from the glycerol fragment, exert a significant influence on the biological activity exhibited by a particular compound.

1. Tetraalkylammonium derivatives

The quaternary ammonium detergents **1**–**4** in liposomes have found applications in the transfection of genetic material into cells of animal origin.[1–3]



1–4

Compound	Name[a]	R^1	R^2	X
1	DEBDA	$CH_2C_6H_5$	$(C_2H_4O)_2$–	OH
2	DTAB	Me	$(CH_2)_{11}Me$	Br
3	TTAB	Me	$(CH_2)_{13}Me$	Br
4	CTAB	Me	$(CH_2)_{15}Me$	Br

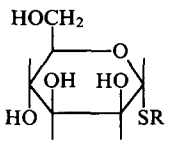
[a] DEBDA = diisobutylcresoxyethoxyethyldimethylbenzylammonium hydroxide, DTAB = dodecyltrimethylammonium bromide, TTAB = tetradecyltrimethylammonium bromide, CTAB = cetyltrimethylammonium bromide.

These cationic lipids are fairly widely distributed and are cheap. They can be synthesised by simple chemical reactions and can be incorporated in liposomes having different compositions in order to stabilise the bilayer. The tetraalkylammonium derivatives **5** and **6**, incorporating carbohydrates as the structural fragments (the so-called bolaamphiphiles) are regarded as promising amphiphiles for negatively charged polyelectrolytes such as nucleic acids. The presence of the electrically neutral carbohydrate fragment at one end of the long-chain residue and of an electropositive group at the other ensures their amphiphilic properties.[4]

The mannose bolaamphiphiles **5** have been synthesised by alkylating thio-α-D-mannopyranose with the corresponding ω-iodo(or bromo)-α-trimethylalkylammonio-derivatives.

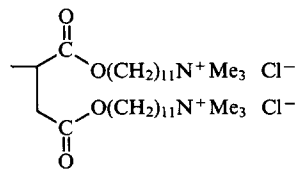
I D Konstantinova, G A Serebrennikova Department of Chemistry and Technology of Fine Organic Compounds, M V Lomonosov Moscow State Academy of Fine Chemical Technology, 86 prosp. Vernadskogo, 117571 Moscow, Russian Federation. Fax (7-095) 430 79 83. Tel. (7-095) 437 19 25 (I D Konstantinova)

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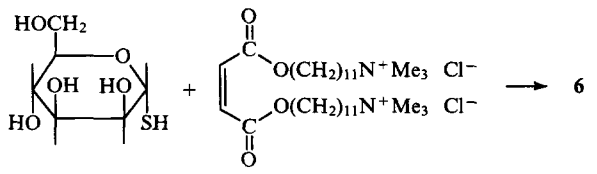
5a–d, 6

R = $(CH_2)_{11}C(O)O(CH_2)_2N^+Me_3\ Cl^-$ (**5a**),
$(CH_2)_{11}C(O)NH(CH_2)_2N^+Me_3\ Cl^-$ (**5b**),
$(CH_2)_{11}C(O)O(CH_2)_{11}N^+Me_3\ Cl^-$ (**5c**),
$(CH_2)_9C(O)O(CH_2)_{11}C(O)NH(CH_2)_2N^+Me_3\ I^-$ (**5d**),

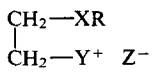


(**6**)

The bolaamphiphile **6**, containing two hydrocarbon chains, has been obtained by adding thiomannose to the corresponding maleic acid diester.



In order to investigate the influence of a series of quaternary ammonium derivatives of types **7–10** on normal and neoplastic cells, a series of such derivatives have been synthesised.[5–7]



7–10

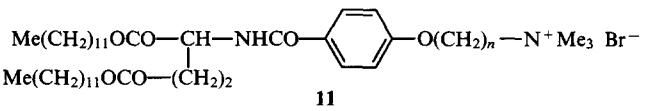
Compound	R	X	Y	Z
7	$(CH_2)_nMe$, n = 14, 16	OC(O)	$N(Me)_2(CH_2)_2OH$	Br
8	H, Ph, $(CH_2)_nMe$, n = 11, 13, 15, 17	O	$N(Me)_2(CH_2)_nOH$, n = 2–4	I
9	$(CH_2)_{15}Me$	S	$N(Me)_2(CH_2)_2OH$, NC_5H_5	Br
10	$(CH_2)_6Me$	OC(O)	NMe_3	I

The synthesis of these compounds is based on the quaternisation of tertiary amines or aromatic heterocyclic bases by β-haloethanolamine with subsequent alkylation or acylation of the free hydroxy-group.

2. Synthetic amphiphiles — aminoacid derivatives

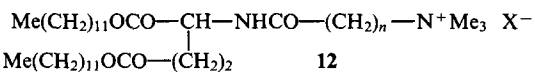
Lipophilic derivatives of aminoacids, peptides, and polyamines have proved fairly effective in the transfection of DNA into eukaryotic cells.

The new cationic amphiphiles **11** and **12** have been recently synthesised from glutamic acid.[8]



11

n = 2, 4, 6, 8



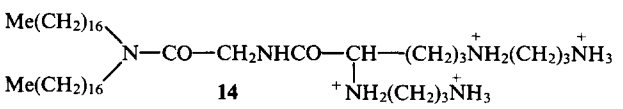
12

n = 1, X = Cl; n = 5, X = Br

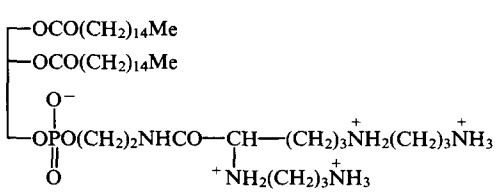
Lipopoly-L-lysine **13** (LPLL) has been attained in accordance with Scheme 1 by the reaction of poly-L-lysine and *N*-glutarylphosphatidylethanolamine in the presence of *N*-hydroxysuccinimide and dicyclohexylcarbodiimide (DCCD).[9]

Compound **13** usually includes two molecules of the phospholipid per poly-L-lysine molecule.

Lipophilic polyamines — the lipospermines **14** and **15** — have proved to be effective mediators of the transfection of DNA.[10]

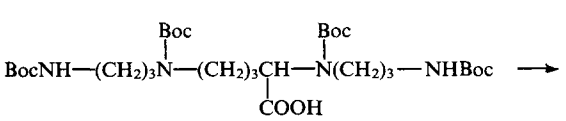


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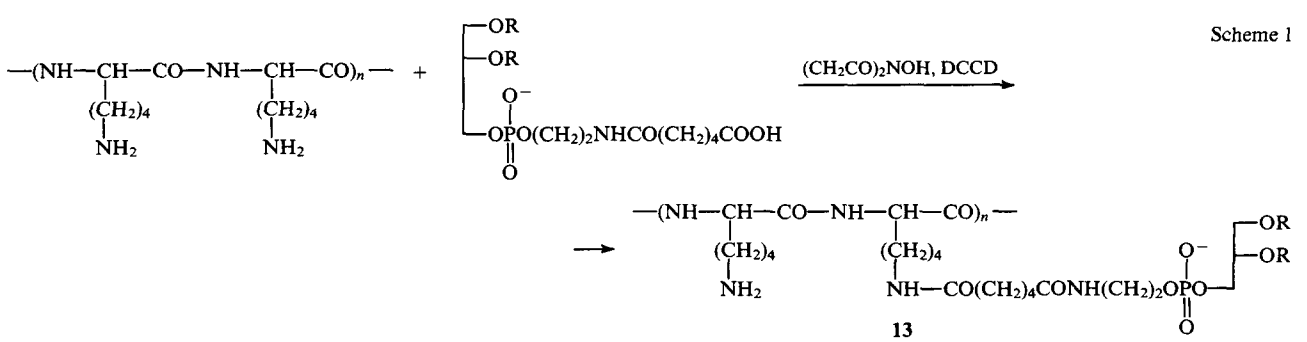


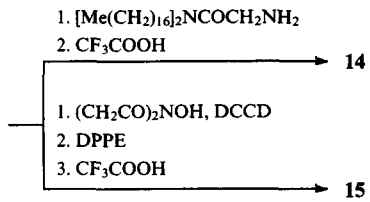
15

The lipospermines **14** and **15** have been synthesised by the reaction of protected L-5-carboxyspermine with the lipophilic glycine *N*,*N*-diheptadecylamide and dipalmitoylphosphatidylethanolamine (DPPE) respectively.



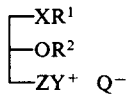
Scheme 1





3. Cationic lipids — glycerol derivatives

The commonest type of cationic lipids may be represented by the general formula **16**.[11-15]



16

X = O, S, or OCONH; R^1 = alkyl, alkenyl, and acyl ($C_{10}-C_{20}$), alkyls with aromatic and heterocyclic residues, etc.; R^2 = long-chain ($C_{10}-C_{20}$) or short-chain (C_1-C_8) alkyl and acyl substituents; Z = spacer groups of different types (their absence is also possible); Y^+ = ammonium and sulfonium groups or heterocyclic heads in which positively charged nitrogen or sulfur atoms are incorporated; Q = Hal, AcO, TsO, etc. counterions.

Depending on the nature of the substituents R^1 and R^2 in the hydrophobic part of the molecule, the cationic lipids of a given type can be subdivided into several varieties, which determine the methods of their synthesis and their applications:

(a) positively charged lipids with long-chain alkyl substituents [$R^1 = R^2$ = Alkyl(C_{10} – C_{20})]

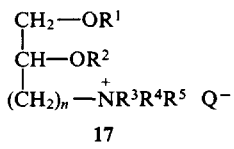
(b) lipids with long-chain and short-chain alkyl substituents [R^1 = Alkyl($C_{10}-C_{20}$), R^2 = Alkyl(C_1-C_8)];

(c) cationic glycerolipids with long-chain acyl substituents;

(d) mixed type lipids containing alkyl, acyl, acylcarbamoyl, and other types of long-chain and short-chain substituents in various combinations.

a. Cationic lipids with long-chain alkyl substituents

The compounds having the general formula **17** are used for the transfection of various biologically active substances (nucleosides, polynucleotides, peptides, hormones, etc.), containing a negatively charged section, into cells of animal origin and plant protoplasts.[11-15]

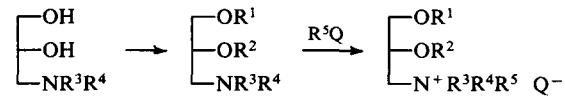


17

$R^1 = R^2$ = alkenyl or alkyl ($C_{10}-C_{20}$); $R^3 = R^4 = R^5$ = Me or Et; n = 1–4 (predominantly n = 1); Q = Hal

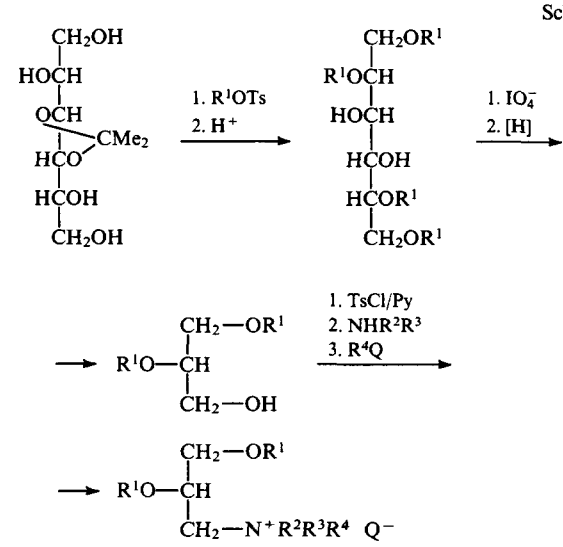
These lipids are usually incorporated in liposomes produced from phosphatidylcholine (PC) or phosphatidylethanolamine (PE) in the molar proportions cationic lipid : phospholipid = 1 : 1. This ratio may vary depending on the type of the transfected substance and the target cells.[11,12,14]

The scheme used in the synthesis of the lipids **17** is fairly simple and includes stages involving the alkylation of the aminodiol system with subsequent quaternisation of the amino-group.[11,12]



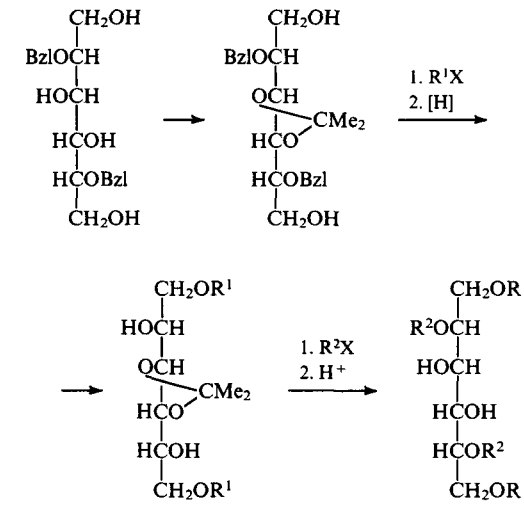
Racemic mixtures are usually obtained via this scheme with $R^1 = R^2$, while R^3, R^4, and R^5 are different or the same but are not incorporated into aromatic systems. Optically active compounds of this type [(*S*)-isomers] are synthesised via Scheme 2 from 3,4-isopropylidene-D-mannitol.[14]

Scheme 2



18

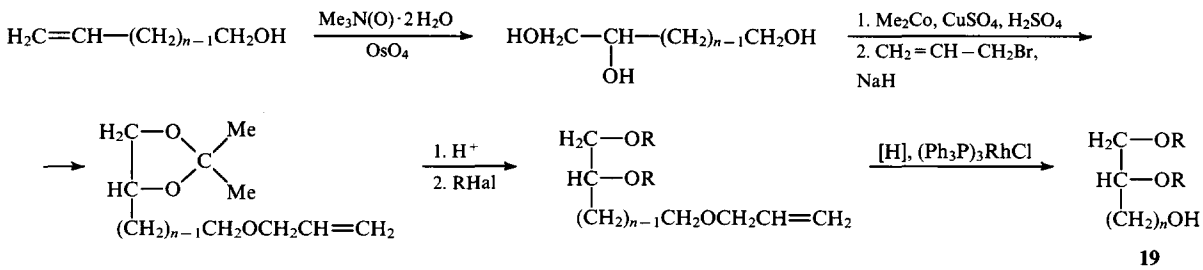
Optically active compounds with various alkyl residues R^1 and R^2 are synthesised from 2,5-dibenzyl-D-mannitol.



Positively charged glycerolipids of the alkyl type with various substituents in the hydrophobic part of the molecule are then synthesised similarly to Scheme 2.

In order to obtain compounds containing a spacer group comprising 2–8 methylene groups, the derivative **19** is synthesised initially (Scheme 3) and is then converted into cationic lipids by the method described above.

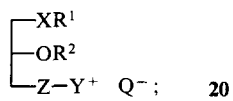
Scheme 3



19

b. Positively charged lipids with long-chain and short-chain substituents of the alkyl type

This group of lipids has been investigated most vigorously because its representatives exhibit an inhibiting activity on the growth of neoplastic cells and are also effective antagonists of the lipid bioregulator of the platelet activating factor. Certain compounds are capable of suppressing the development of the HIV-1 virus. In a general form, the structure of such glycerolipids can be represented as follows:



20

X = O or S;

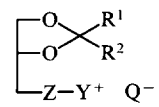
R^1 = alkenyl or alkyl ($C_{10}-C_{20}$), a branched alkyl, an alkyl with aromatic substituents, naphthylaryl, or aryl;

R^2 = alkyl (C_1-C_4), a cyclic alkyl, heterocycles (pyridine, triazoline, pyrazine, etc.), H, or CH_2CF_3;

Z = bond, $O(CH_2)_n$ (n = 1–7), $(OCH_2CH_2)_n$ (n = 1–3), $S(CH_2)_n$ (n = 1, 2), a spacer group of the alkyl or arylalkyl type, etc.;

Y = $NR^1R^2R^3$, where $R^1 = R^2 = R^3$ = Me or Et, $R^1 = R^2$ = Me and $R^3 = (CH_2)_2OH$ or $(CH_2)_3OH$, or R^1, R^2, and R^3 may be combined into heterocycles; Q = Hal, MsO, TsO, etc.

The groups R^1 and R^2 may be combined into an acetal ring with a long-chain alkyl group (for example, $R^1 = C_{17}H_{35}$ and R^2 = H).[16,17]

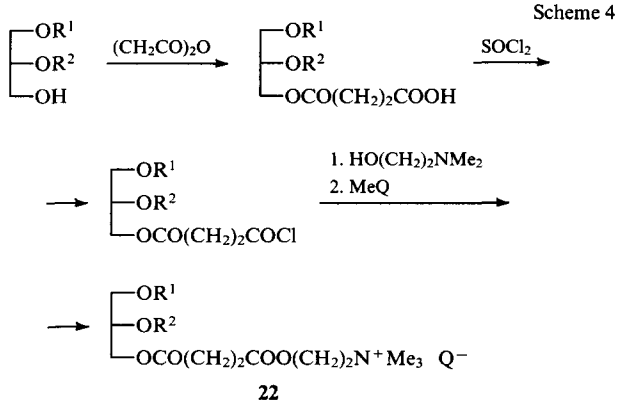


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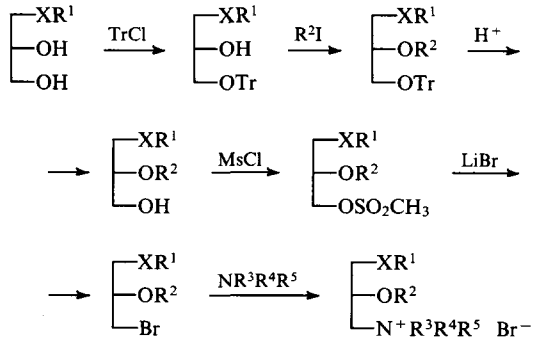
The cationic glycerolipids **22**, containing a spacer group of the succinyl type, are synthesised in accordance with Scheme **4**.[18,19]

The synthesis of compound **23**, containing a cationic group attached directly to the glycerol fragment, involves the preliminary preparation of disubstituted glycerols using a trityl protecting group.[16]

Scheme 4



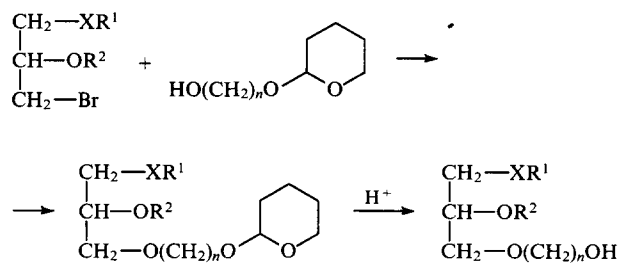
22



23

Various approaches are used in the preparation of compounds containing a spacer group depending on the structure of the latter.

If the quaternary ammonium group is separated from the glycerol fragment by several methylene groups, compound **24** is synthesised and then converted into cationic lipids by traditional methods.[16]

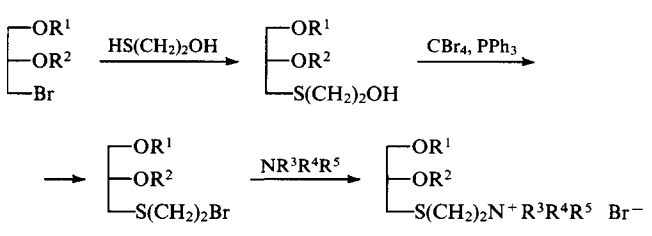


24

The 3-(2-hydroxyethoxy)- and 3-[2-(2-hydroxyethoxy)-ethoxy]-residues are also used as spacer groups. They are introduced into the molecule by treating the corresponding tosyl derivatives of mono- or di-alkylglycerols with ethylene glycol or diethylene glycol.[20]

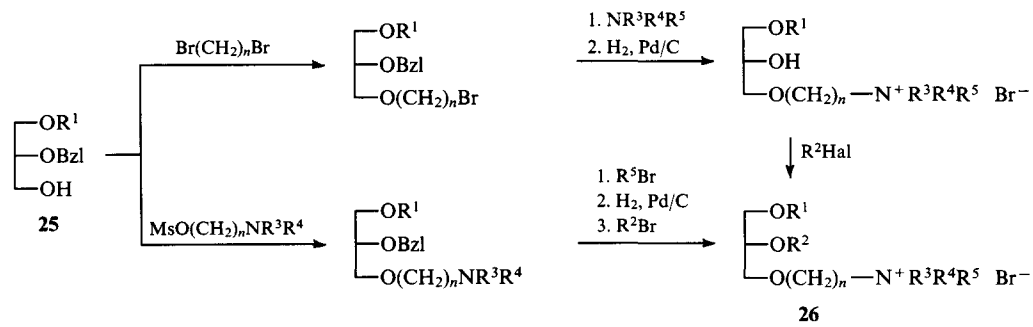
A series of cationic lipids **26** with a spacer group have been obtained from 1-*O*-alkyl-2-*O*-benzyl-*rac*-glycerols **25** (Scheme 5).[21,22]

The synthesis of positively charged lipids **27**, having an ethylthio spacer group, has been reported.[6,23]



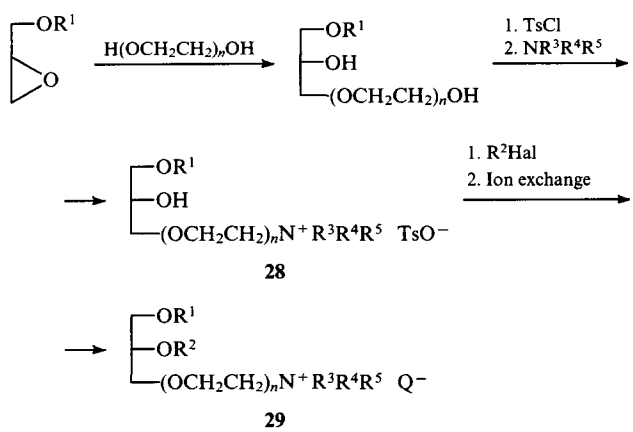
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Scheme 5

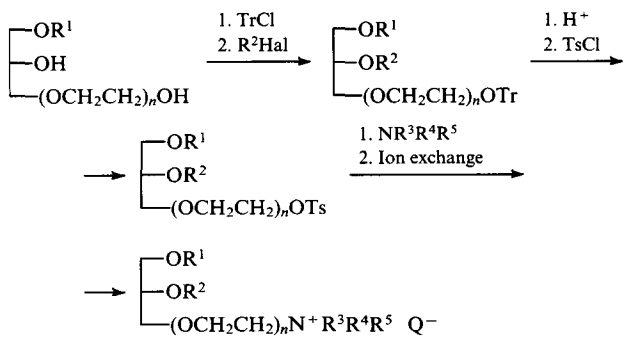


Compounds of types **28** and **29**, containing heterocycles as the substituent R^2 and the $(OCH_2CH_2)_n$ group as the spacer, are synthesised in accordance with Scheme 6.[24]

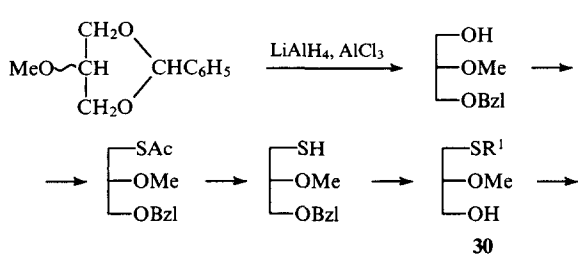
Scheme 6

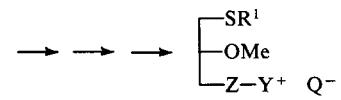


The scheme may be modified by employing the trityl protecting group.



The method involving the reductive cleavage of cyclic acetals has been used in the synthesis of the disubstituted thioglycerol derivative **30**, which is converted into cationic lipids by traditional methods.[25]

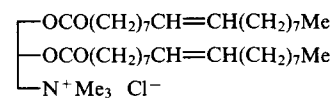




Together with lipids containing alkylammonium groups as the polar head, compounds incorporating various heterocycles have been obtained.[22,26,27]

c. Cationic lipids with long-chain acyl substituents

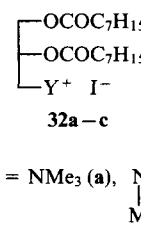
2,3-Dioleoyloxypropyltrimethylammonium chloride **31** (DOTAP) is one of the commonest glycerolipids of this series.

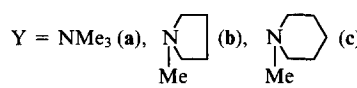


This lipid is used to investigate the processes involving the fusion of liposomes carrying opposite charges, the mixing of the biologically active substances incorporated in them, and their possible transport through a liposomal bilayer.

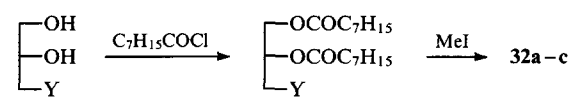
DOTAP is synthesised by acylating 3-bromopropane-1,2-diol with subsequent quaternisation of the resulting compound with trimethylamine.[28]

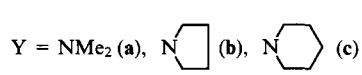
The synthesis of the positively charged lipids **32a–c** containing various cationic groups and higher aliphatic acid residues has been described.[7]





The general scheme for the synthesis of compounds **32** can be represented as follows:

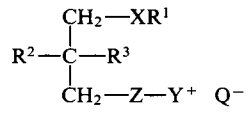




Compounds **32a–c** are inhibitors of the membrane-bound protein kinase C to the same extent as cationic lipids of the alkyl ether type having a short-chain substituent at the C(2) atom of the glycerol fragment.[7]

g. Cationic lipids of the mixed type

Among positively charged glycerolipids of the mixed acylalkyl ether, alkyl ether, or acylcarbamoyl types, a series of effective antagonists of the platelet activating factor as well as compounds with antitumour activity have been discovered. This stimulated the study of the structure–function relations in this class of lipids and led to the synthesis of a large group of compounds having the general formula **33**.



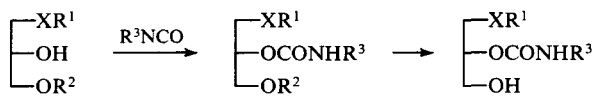
33

X = O, S, OCONH or SCONH;
R^1 = alkyl(C_8-C_{20}), $(CH_2)_3OC_{18}H_{37}$, $(CH_2)_{10}NHCONHMe$, $CH_2-C_6P_4-Ph$, etc.;
R^2 = OMe, OEt, OCOMe, $OCOC_7H_{15}$, OCH_2COMe, CH_2CN, NHCOOMe, OCOOMe, NHCONHMe, or heterocycles of the pyrrole, triazole, etc. type;
R^3 = H or Me;
Z = $O(CH_2)_n$, $(OCH_2CH_2)_n$, $OCO(CH_2)_n$, $(CH_2)_n$, $CH_2SO_2(CH_2)_n$, $NHCO(CH_2)_n$, $NHSO_2(CH_2)_n$, $N(COMe)SO_2(CH_2)_n$, or $OCONR(CH_2)_n$
(R = H, Me, COMe, COEt, or COOMe; n = 1–6);
Y = NMe_3, $NMe_2CH_2CH_2OH$, various heterocycles in which a quaternary nitrogen atom is incorporated, etc.;
Q = Hal, MsO, TsO, etc.

Compounds of the alkylacyl type are synthesised in accordance with the general strategy developed for the preparation of dialkyl lipids with various substituents in the glycerol skeleton using the same selective protecting groups (Bzl, Tr, Ts, benzylidene) and alkylation, acylation, and thioalkylation methods.

The syntheses of compounds containing alkylcarbamoyl and alkoxycarbonylamino-groups have specific features. They usually involve the preparation of the corresponding disubstituted glycerol derivatives with subsequent introduction of the cationic head by traditional methods.

Scheme 7



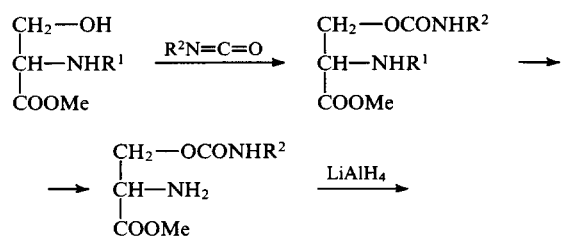
35 **34**

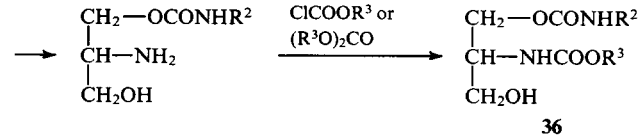
R^2 = protecting group; R^3 = alkyl(C_1-C_3).

The overall strategy for the synthesis of the initial compounds **34** in the preparation of cationic lipids with an alkylcarbamoyl residue at the C(2) atom may be represented by Scheme 7.[25]

The alkylcarbamoyl group was introduced by the interaction of compound **35** with alkyl isocyanate in the presence of an acid catalyst ($AlCl_3$) and a base (pyridine, triethylamine).

Compounds **36**, containing alkylcarbamoyloxy- and alkoxycarbonylamino-groups at the C(1) and C(2) atoms respectively, are synthesised in the following manner:[25]



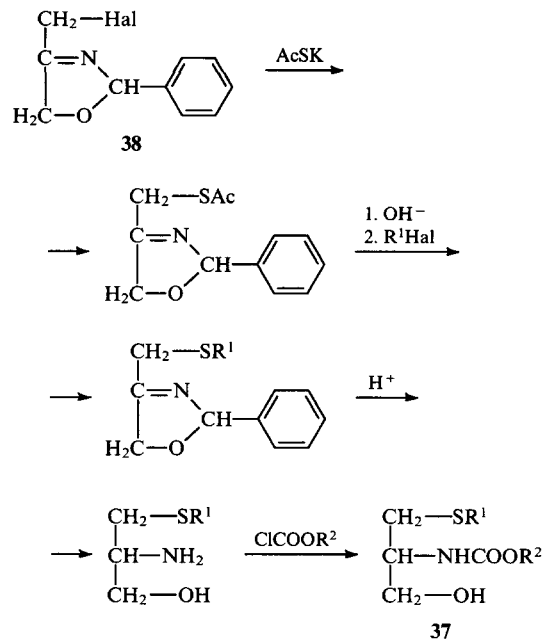


36

R^1 = protecting group; R^2 = alkyl($C_{12}-C_{20}$); R^3 = alkyl(C_1-C_4).

Compound **37**, incorporating an alkylthio- and an alkoxycarbonylamino-group at the C(1) and C(2) atoms respectively, was synthesised from the phenyloxazoline derivative **38** in accordance with Scheme 8.[25]

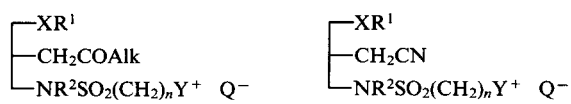
Scheme 8



38 **37**

R^2 = Alk(C_1-C_4).

The synthesis of the cationic lipids **39** and **40** with alkanoylmethyl and cyanomethyl substituents at the C(2) atom of the glycerol fragment has been described in detail.[20–22,29]

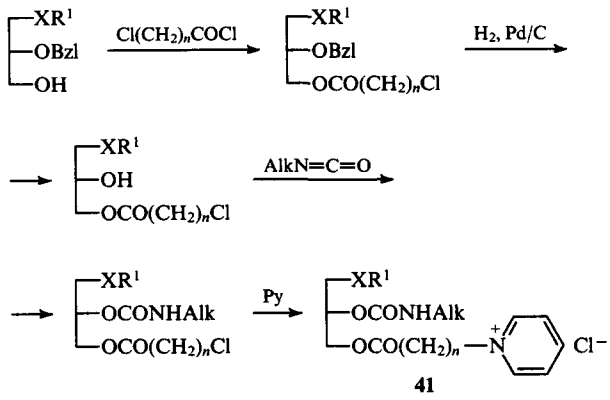


39 **40**

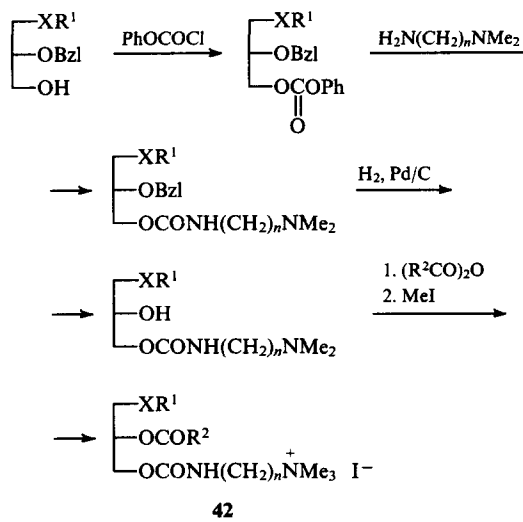
R^2 = H, Me, COMe, COEt, or COOMe; n = 1–6;
Y = NMe_3, $NMe_2CH_2CH_2OH$, various heterocycles in which a quaternary nitrogen atom is incorporated, etc.;
Q = Hal, MsO, TsO, etc.

The general approach to the synthesis of lipids **41** having the 1-alkyl-2-carbamoyl structure and containing a cationic head attached via a spacer group of the acyl type is presented in Scheme 9.[22,30] The cationic head with a spacer of the acyl type may be obtained via the acylation of the dialkylglyceride of a dimethylaminoalkanoic acid of a suitable type followed by the quaternisation of the dimethylamino-group with methyl iodide.[31,32]

Scheme 9



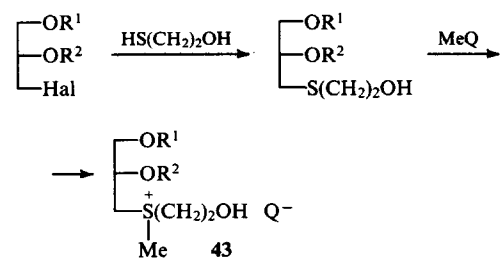
The positively charged lipids **42** — PAF antagonists with a carbamoyl structure — have been obtained by the following method.[29]



f. Positively charged alkyl ether lipids with a sulfonium cationic head

The type **43** lipids are of interest for the study of the mechanism of the inhibition of the membrane-bound protein kinase C (PKC), the growth of neoplastic cells, and the replication of the HIV-1 virus.[6,23] They are synthesised in accordance with Scheme 10.

Scheme 10

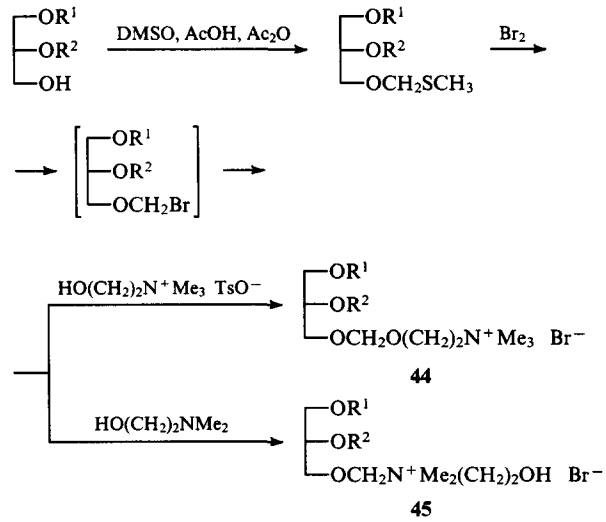


$R^1 = C_{16}H_{33}, C_{18}H_{37}$; R^2 = Me, Et; Q = I, TsO.

g. Positively charged lipids synthesised by the alkylthioalkylation reaction

Konstantinova et al.[33] were the first to demonstrate in lipid chemistry the possibility of using a modified Pummerer's rearrangement (the alkylthioalkylation reaction)[34] in the chemistry of lipids by introducing the $MeSCH_2$ group at a primary

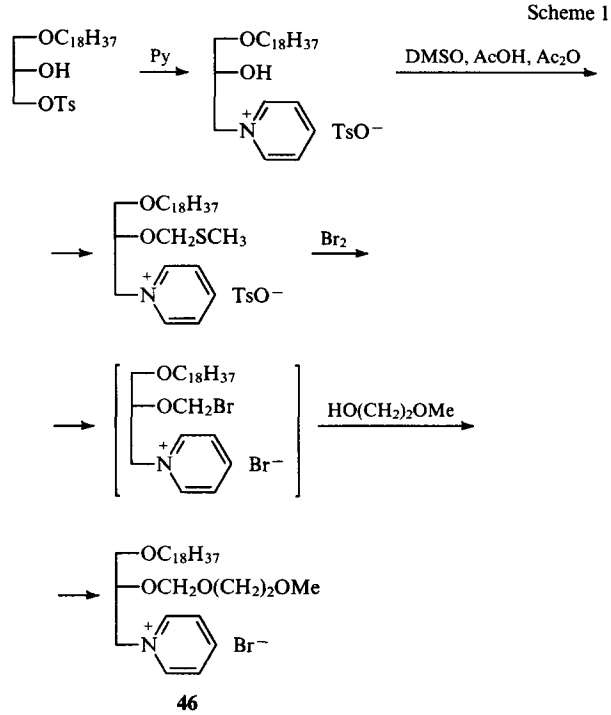
Scheme 11



$R^1 = C_{18}H_{37}$; R^2 = Me, Et; $R^1 = R^2 = C_{16}H_{33}$.

(Scheme 11) or secondary hydroxy-group (Scheme 12) of disubstituted glycerols **44–46**. Subsequent conversion of these highly reactive groups (asymmetric *O,S*-acetals) into α-bromoethers, capable of interacting readily with nucleophiles, leads to prospects for the synthesis of various modified lipids, including positively charged ones.

Scheme 12



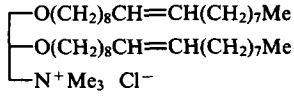
III. Applications of positively charged lipids

1. Cationic lipids in liposomology

Positively charged lipids form liposomes in an aqueous medium or in a buffer solution either individually or in a mixture with other natural phospholipids. For example, an active preparation, known as lipofectin, includes the cationic lipid DOTMA† **47** and

† *N*-[1-(2,3-dioleyloxy)propyl]-*N,N,N*-triethylammonium salt.

PE; in molar proportions ranging from 1 : 1 to 3 : 7 depending on the type of cultures of the cells used in transfection.[11,12,14,35]



47

Cationic liposomes are obtained similarly from the quaternary ammonium derivatives **1–4** (DEBDA, DTAB, TTAB, and CTAB), which exert a stabilising effect on the bilayers formed by PE.[1,36,37] DTAB stabilises the bilayers more effectively than the TTAB and CTAB analogues, the amount of which required to achieve a similar effect is twice as great.

The mechanism of the interaction of cationic liposomes with liposomes incorporating negatively charged or zwitterionic phospholipids has been widely investigated in recent years.[36–39] The elucidation of the details of this process may explain the interaction of the positively charged liposomes with the cell membrane during the transfection process. It has been shown that the populations of such liposomes may interact with one another in various ways which depend on the lipid composition of the membrane surface and on the ionic strength of the medium.

It has been established that liposomes containing DOTMA and PE interact with the negatively charged membrane lipids and fuse with the cell surface. Different anions can also induce the fusion of cationic liposomes. Thus, for the DOTMA/PE (1 : 1) liposomes, the influence of the anions on the degree of fusion decreases in the sequence citrate > ethylenediaminetetraacetate > phosphate. It is of interest that these liposomes were entirely resistant to fusion in the presence of polyanions. Liposomes of the DOTMA/PE and DOTMA/PC types may fuse at a high citrate concentration. The negatively charged phosphatidylserine (PS) exerts a similar effect even in the absence of polyanions. The interaction of the negatively charged groups in PS with the quaternary ammonium group of DOTMA or DOTAP **31** is apparently sufficient for the fusion of the membranes containing these lipids, while the zwitterionic phospholipid component of these membranes is an additional and decisive factor for membrane conductivity.[15]

It is essential to note that the fusion of liposomes in the presence of anions is influenced significantly by the size of the liposomes. For example, liposomes 300 nm in diameter, the composition of which includes didodecylammonium bromide, fuse in the presence of dipicolinic or toluene-*p*-sulfonic acid. On the other hand, if the diameter of the liposomes is <200 nm, no fusion occurs and only aggregation is observed.[15]

Fusion results in the formation of a complex between the liposomes and the anion, which is preceded by the dehydration of the surface of the liposome. Studies are planned in the future on the quantitative determination of the binding capacities of a wide variety of anions in relation to cationic lipids in liposomes and on the determination of the possible dehydration of liposomes in the presence of anions.[28]

It has been shown that the DOTMA/PC/PE mixed liposomes are entirely resistant to fusion in the presence of polyanions. However, when they were mixed with liposomes containing negative charges on the surface, aggregates were formed readily at the physiological or lower ionic strength. The PC/PE ratio in liposomes has a considerable influence on the degree of aggregation even under the conditions where it is not followed by coalescence (i.e. the mixing of the lipids of the bilayer and/or aqueous compositions). The degree of aggregation is higher the greater the PC/PE ratio. These observations may be useful for the study of the interaction between liposomes having different lipid compositions and also for providing investigators with systems in which the interaction between liposomes is independent of the bond between ions and the lipid surface. The tendency of liposomes to become associated with one another can be readily controlled by varying the ionic composition of the medium.[15,28]

The mechanism of the interaction of the DOTMA/PE vesicles with the plasma membranes of cultured cells has not been elucidated. It may be that the binding of DOTMA to the negatively charged sialic acid in gangliosides leads to the destabilisation of the plasma membrane and the coalescence of the liposomes with cells. It is essential to note that cationic liposomes containing cationic amphiphiles with ether and ester bonds interact equally readily with the cell membrane. Furthermore, liposomes containing fluorescent labels are readily disrupted after being incorporated in CV-1 cells.[28,40] One of the most interesting properties of positively charged liposomes with a high PE content is their ability to interact with membranes containing surface proteins. Liposomes formed from DOTMA/PE are readily adsorbed on erythrocytes in a medium with the physiological ionic strength.[28]

A fairly important property of the lipofectin preparation is its toxicity for cells in vitro following an increase in the concentration of the cationic lipid above a certain level. The toxicity of the preparations is different for different types of cells and depends on the duration of exposure and the density of the cell culture. It is therefore necessary to optimise the transfection parameters for each cell line.

The quaternary ammonium derivatives **1–4** are even more toxic to the transfected cells. Therefore, out of all the above compounds, the least toxic CTAB is most often employed. It is a cheaper and readily available substitute for DOTMA and DOTAP. Similar observations have been made also for another cationic lipid, namely DEBDA, which is used in studies on plant protoplasts.[1]

2. Positively charged lipids as transfection mediators

One of the most interesting fields of medicine — genetic therapy — has been rapidly developing recently. It is essential for the correction of genetic cell defects and for the treatment of genetic diseases. In order to correct genetic defects, normal exogenic genes are introduced into the target cell, where they or the products of their vital activity act together with the defective molecules or replace the latter and hence assist the cell in its normal functioning.

One of the main tasks in genetic therapy is the search for methods which are suitable for the specific and effective introduction of genetic material. It has been shown that under exceptional conditions eukaryotic cells may receive DNA molecules, a proportion of which are localised in the nucleus. However, owing to its large size and charge, the spontaneous permeation of DNA into the cell and its subsequent expression into the nucleus (transfection) are difficult. For this reason, various methods facilitating this process, i.e. inducing cell fusion under specially selected conditions, or mediators of DNA gene transfection have been used. Employment of calcium phosphate, liposomes, retrovirus vectors, electroporation, and microinjection may be included among the effective methods for the transfection of genetic material. In certain cases, the application of polycations such as polylysine, DEAE-dextran, and polyornithine yields satisfactory results. However, all these methods are characterised by a low effectiveness, an unstable introduction of genes, and a poor reproducibility. Furthermore, the compounds employed in this procedure are frequently toxic to the target cells.[11,12]

The most modern DNA-transfection methods employ positively charged liposomes as mediators. The use of liposomes capable of transferring genetic material into cells via fusion with the cell membrane is of special interest. Cationic lipids with various structures (compounds **1–4**, **11–17**, and **47**) form together with PE and PC positively charged liposomes which spontaneously produce complexes with polynucleotides. These complexes are adsorbed by the cell surface, fuse with the plasma membrane, and transfer the genetic material to the cytoplasm. Further study of the positively charged liposomes can therefore lead to the creation of convenient and effective systems for the introduction of various biologically active substances into the cell:

nucleosides oligo- and poly-nucleotides, hormones, proteins, and other natural and synthetic macromolecules with a negatively charged section.[14]

The current break-through in transfection arose from the preparation lipofectin. It is convenient to use and is effective.[11-14,41,42] The DNA — lipofectin (DOTMA/PE) complex has a network of positive charges, which facilitate its interaction with the negative charges of glycolipids and glycoproteins on the surface of the cell membrane. [11]

The quaternary ammonium derivatives **1–4** are also sometimes used for DNA transfection. Although the transfecting activity of liposomes containing these compounds is somewhat lower than the activity of lipofectin, nevertheless they can be used as cheap readily available substitutes.[1,13,15,40,43]

Apart from DNA transfection, lipofectin is used for the effective introduction of RNA into cells of animal origin and also into the protoplasts of a number of plants. By employing this method, it is possible to introduce up to 70% of RNA.[2] The RNA–lipofectin complexes are used to investigate the stability of mRNA when they are introduced into cell tissues and embryos. This may lead to the development of a technology in which RNA is used as a medicinal preparation.[13]

Lipofectin is a simple and convenient preparation whereby one can obtain various specific tissue cells or cell lines infected by whole viruses or only by virus vectors in the absence of homologous virus receptors.[42]

Communications concerning the possibility of the transfection of the vector for the expression of cDNA luciferase into the brain cells of the Xenopus and mouse embryos, which permits the lipofection (transfection with the aid of lipofectin) of various functional genes into the cells of the central nervous systems of embryos, have appeared recently.[42,44]

The bolaamphiphiles **5** and **6** are also used as ideal amphiphiles for polyanions. The positively charged end of the bolaamphiphile molecule may bind a negatively charged polyanion in aqueous solutions even if it is only sparingly soluble in the latter. However, since these compounds do not possess aliphatic chains arranged in parallel, they are unable to form a stable bilayer. They are therefore more frequently used as models for the investigation of the interaction of liposomes and cell membranes with polyelectrolytes.[4]

Cationic liposomes, prepared by the sonication of aqueous dispersions of lipids or by employing solubilising detergents, are used to incorporate horseradish peroxidase into human erythrocytes in vitro. The stability of the modified erythrocytes has been investigated from the standpoint of photohemolysis.[35]

Behr et al.[10] developed a highly effective transfection method based on the ionic binding of plasmids to the lipopolyamines **14** and **15**. This procedure has been used for the transfection of lipopolyamines into the endocrine cells of the intermediate lobe of the pituitary gland. Such transfection did not entail changes in the membrane-dependent receptors and did not affect the physiological regulation of the biosynthesis of hormones. The cationic lipopolyamines **14** and **15** do not exert a toxic activity on cells and may be used for the transfection of chromaffine cells and the neurones of the central nervous system. It is of interest that the method does not affect the normal cell growth or the electrochemical characteristics of the peripheral neurones. The introduction of DNA into the epidermal human keratinocytes has also been achieved with the aid of lipopolyamines.[45]

The so called lipopolylysines **13** are some of the most effective mediators of DNA transfection.[9] Poly-L-lysine is used as the starting material for the synthesis of this preparation because of its biodegradability on entering the cell, in contrast to poly-D-lysine. This preparation is used in the individual state, since its liposomal form (with PC or PE) exhibits only 50% of the activity of the individual form in DNA transfection. Lipopolylysine preparations are at present some of the most active and relatively cheap mediators of the DNA transfection of type L929 mouse cells and human HeLa cells as well as the monkey Vero cells.

The data presented indicate the possibility of using lipofection under in vivo conditions. The method using DOTMA, or its analogues belonging to the series of dioleylalkyl derivatives containing a hydroxyalkyl chain in the quaternary ammonium head, as the cationic lipid has been recognised nowadays as optimal. These compounds enter into the composition of liposomes in a mixture with dioleoylphosphatidylethanolamine (50 mol %).[46-48]

An aerosol method for the introduction of plasmids for the expression of chloroamphenicol acetyltransferase, which makes it possible to achieve a high lung-specific level of its production in the mouse organism in vivo, has been developed recently.[49]

Promising results have been obtained in the lipofection of plasmids, coding the synthesis of the human growth hormone, via the introduction of the preparation intravenously into the mouse organism.[50]

It has been shown that the direct intravenous introduction of DNA–cationic liposome complexes into the organisms of adult animals transfects effectively all types of tissues (the endothelial tissues of blood vessels and the parenchymal cells of the lungs, spleen, lymph nodes, and bone marrow). The expression of the genes introduced remained at a stable level for 9 weeks after transfection. At the present time, these results are being considered from the standpoint of the study of ageing processes at a molecular level.[51]

Furthermore, the study of organ tissues and analysis of the cardiac functions of mice and pigs after lipofection in vivo has not revealed any pathologies following the employment of deliberately excess doses of the DNA–liposome complex.[52]

It is essential to note that the transfection method, employing cationic liposomes as mediators, may prove promising in the enzyme- and hormone-substitution therapy of a wide variety of diseases such as sugar diabetes, growth defects, and various blood affections (β-thalassemia, adenosine deaminase deficiency, sickle cell anaemia, etc.).[14,49,50,53]

3. The use of cationic lipids as inhibitors of the membrane-bound protein kinase C and diacylglycerol kinase

In connection with the study of the influence of lipids of the type of 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (ET-18-OMe) on metastases and the growth of a wide variety of neoplastic cell lines, the new quaternary ammonium derivatives of glycerolipids **20** are of great interest. These compounds proved to be effective inhibitors of the membrane-bound protein kinase C (PKC), which regulates the content of phospholipids and Ca^{2+} ions in the cell. The study of the mechanism of the action of this kinase, activated by phosphatidylserine, phorbol esters, and diacylglycerols, has shown that exogenic alkylacylglycerols and dialkyl derivatives are capable of inhibiting this kinase, which is present in HL-60 cells. A similar activity of compounds of types **23** and **32** has been observed in relation to diacylglycerol kinase (DAG).[7] In order to investigate the molecular interaction of these enzymes with their natural activators, inhibitors, and substrates, a series of dialkyl and diacyl quaternary ammonium derivatives with various structures have been synthesised (compounds **10**, **21**, **23**, **32**, etc.)

The study of the activity of PKC in the presence of the above compounds using *rac*-1,2-dioleoylglycerol–PS–Ca^{2+} mixtures as activators showed that the manifestation of inhibiting activities requires the presence in its composition of a relatively long-chain alkyl or acyl substituent at the C(1) atom of the glycerol fragment, a short-chain alkyl or acyl substituent at the C(2) atom, and also a functional group capable of forming hydrogen bonds at the C(3) atom. A similar conclusion was reached in relation to DAG when *rac*-1,2-dioleoylglycerol was used as the substrate in the investigations.

Compounds **32a,b** proved to be the most active under the experimental conditions, whereas the diol type lipid **10** proved to be inactive in this instance.

During tests of 2-*O*-acetyl-1-*O*-oleoylglycerol as one of the activators, a study was made of the ability of compounds of types **21**, **23**, and **26** to inhibit PKC (obtained from the bovine brain), which made it possible to discover the influence of the structure of these compounds on the inhibition process. Table 1 presents the concentrations corresponding to 50% inhibition of PKC by compounds having different structures.[16, 54]

The data presented permit the following conclusions:

(a) the replacement of an oxygen atom by a sulfur atom does not alter significantly the activity (cf. compounds **23a** and **23f**);

(b) the long alkyl chain at the C(1) atom of glycerol plays a decisive role in the manifestation of activity (compound **23e** is inactive);

(c) significant differences between the activities of compounds containing methoxy- and ethoxy-groups at the C(2) atom of the glycerol fragment have not been observed (compounds **23a** and **23b**);

(d)the activities are influenced by a change in the length of the chain of the *N*,*N*-dimethylalkylammonium alcohol; thus the propanol derivative **23g** exhibits a higher activity than compound **23a**;

(e) the replacement of the 'inverse' choline group by a trialkylammonium group improves the inhibition of the enzyme (a free hydroxy-group alters the activity insignificantly);

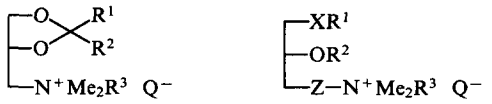
(f) the replacement of the quaternary ammonium group by a hydroxy-group does not influence the inhibition of PKC but fully abolishes the cytotoxicity of these analogues in relation to tumour cells;

(g) the migration of the quaternary nitrogen atom from the glycerol skeleton to the end of the alkyl chain made up of several methylene groups has only a slight influence on the PKC activity (compounds **26a–c** and **23h**).

In conclusion, one must note that the restriction of the mobility of the glycerol on incorporation of R^1 and R^2 into a structure of the acetal type does not diminish the activity. Furthermore, the stereospecificity of the compounds synthesised is not a decisive factor in the inhibition of PKC.[16] Preliminary experiments designed to elucidate the mechanism of the inhibition of PKC showed that the compounds quoted block its activation by phosphatidylserine.

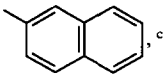
Studies which will help to discover for these compounds a correlation between the antineoplastic activity and the ability to inhibit PKC are being prosecuted at the present time.

Table 1. The concentrations corresponding to 50% inhibition (IC_{50}/ μmol litre^{-1}) of PKC.[16, 54]



21 **23, 26**

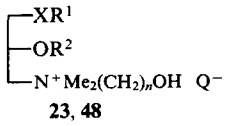
Compound	X	R^1	R^2	R^3	Q	IC_{50}
ET-18-OMe[a]	O	$C_{18}H_{37}$	Me	Me	–	12
21	–	$C_{17}H_{35}$	H	$(CH_2)_3OH$	Br	13
23a	S	$C_{16}H_{33}$	Me	$(CH_2)_2OH$	Br	27
23b	S	$C_{16}H_{33}$	Et	$(CH_2)_2OH$	Br	37
23c	S	$C_{16}H_{33}$	Et	$(CH_2)_3OH$	Br	26
23d	S	$C_{16}H_{33}$	Me	Me	Me	25
23e	O	Naphth[b]	Me	$(CH_2)_2OH$	Br	≫40
23f	O	$C_{16}H_{33}$	Me	$(CH_2)_2OH$	I	17
23g	S	$C_{18}H_{37}$	Me	$(CH_2)_3OH$	I	5
23h	O	$C_{16}H_{33}$	Me	Me	Br	>40
23i	O	$C_{16}H_{33}$	H (instead of OR^2)	Me	Br	≫40
26a[c]	O	$C_{16}H_{33}$	Me	Me	Br	31
26b[d]	O	$C_{16}H_{33}$	Me	Me	Br	>40
26c[e]	O	$C_{16}H_{33}$	Me	Me	Br	≫0
26d[d]	O	$C_{16}H_{33}$	Et	Me	Br	≫40

[a] Z = $OPO_3^-(CH_2)_2$, [b] Naphth = , [c] Z = $O(CH_2)_2$, [d] Z = $O(CH_2)_4$, [e] Z = $O(CH_2)_6$.

4. The antitumour activity of positively charged lipids

It is widely known that positively charged alkyl ether lipids influence the metabolism and growth of neoplastic cell lines. The compound ET-18-OMe and its phosphorus-free analogues

Table 2. Concentrations of lipids with a cationic head in the form of 'reversed' choline corresponding to 50% inhibition of the growth of neoplastic cells.



23, 48

Compound	X	R^1	R^2	*n*	Q	IC_{50}/ μmol litre^{-1}	Type of cell	Ref.
ET-18-OMe	O	$C_{18}H_{37}$	Me	–	–	2.50 ± 0.31	HL-60	6
23a	S	$C_{16}H_{33}$	Me	2	Br	4.66 ± 0.27	HL-60	54
23b	S	$C_{16}H_{33}$	Et	2	Br	3.72 ± 0.05	HL-60	54
23c	S	$C_{16}H_{33}$	Et	3	Br	2.95 ± 0.36	HL-60	54
23g	S	$C_{18}H_{37}$	Me	3	Br	3.55 ± 0.22	HL-60	54
23e	S	Napht	Me	2	Br	>10	HL-60	54
23j	S	$C_{18}H_{37}$	H	2	I	0.35 ± 0.05	KB	5
23k	S	$C_{16}H_{33}$	C_6H_{11}	2	Br	7.15	HL-60	6
48	S	$C_{16}H_{33}$	CH_2OMe (instead of OR^2)	3	Br	3.46	HL-60	6

accumulate on the surface of the plasma membrane, inhibiting PKC and certain other kinases, which may be associated with the antineoplastic activity which they exhibit in vitro (HL-60 and KB cells) and in vivo (S-180 and MM46 cells).[20] In addition, alkyl ether lipids may accumulate selectively in tumour cells by virtue of the low activity in the latter of the enzymes which cleave ether bonds.[28,55] This disturbs the normal biosynthesis of the lipids of the cell membrane and the functions and integrity of the latter.

A series of cationic lipids with an antineoplastic activity have been synthesised. They can be subdivided arbitrarily into five main series:

(1) Lipids with a cationic head in the form of 'inverse' choline (Table 2).

(2) Cationic lipids of the acetal type (Table 3).

(3) Lipids with an ammonium group attached directly to the glycerol fragment (Table 4).

(4) Lipids with a cationic head attached to the glycerol skeleton via a spacer group (Table 5).

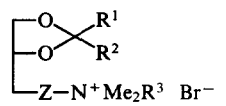
(5) Derivatives of cationic lipids of the diol type (Table 6).

All the compounds were investigated from the standpoint of their antineoplastic activity in relation to cells of different types (Tables 2–6). Comparison of the results of experiments performed under similar conditions permits a series of conclusions.

The 'inverse' cholines of the first series have largely proved less active than the compounds of the third and fourth series, the activities of which are comparable with that of the ET-18-OMe reference standard. The presence of the hydroxy-group in the cationic head reduces the activity somewhat. The influence of the heteroatom (S or O) at the C(1) atom of glycerol has not been accurately elucidated.[54]

A decrease in the length of the alkyl substituent at the C(1) atom of glycerol or its replacement by an aromatic system lowers the activity of the compound in relation to the HL-60 cells. Thus compound **23e** (Table 2) shows no activity within the limits of the measured concentrations.

Table 3. Concentrations of cationic lipids of the acetal type corresponding to 50% inhibition of the growth of neoplastic cells.[54]

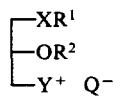


21

Compound	R^1	R^2	R^3	IC_{50}/ μmol litre^{-1}	Type of cells
21a	$C_{17}H_{35}$	H	$(CH_2)_3OH$	6.47 ± 0.24	HL-60
21b	Me	Me	$(CH_2)_2OH$	> 10	HL-60
21c[a]	$C_{17}H_{35}$	Me	Me	2.82 ± 0.42	HL-60

[a] $Z = O(CH_2)_4$.

Table 4. Concentrations of lipids with an ammonium group attached directly to the glycerol fragment corresponding to 50% inhibition of the growth of neoplastic HL-60 cells.



23, 43

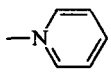
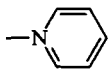
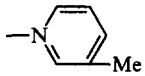
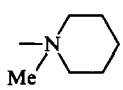
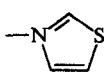
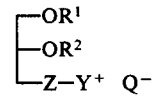
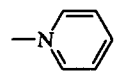
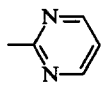
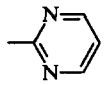
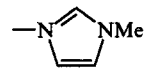
Compound	X	R^1	R^2	Y	Q	IC_{50}/ μmol litre^{-1}	Ref.
23h	O	$C_{16}H_{33}$	Me	NMe_3	Br	1.59 ± 0.17	54
23i	O	$C_{16}H_{33}$	H (instead of OR^2)	NMe_3	Br	1.61 ± 0.10	54
23l	O	$C_{16}H_{33}$	Et	NEt_3	Br	0.68 ± 0.11	54
23m	O	$C_{16}H_{33}$	Et		Br	1.01 ± 0.06	54
23n	O	$C_{16}H_{33}$	H (instead of OR^2)		Br	0.82 ± 0.24	54
23o	O	C_8H_{17}	Me	NMe_3	Br	21.15 ± 5.40	6
23p	O	$C_{16}H_{33}$	Me		Br	1.07 ± 0.30	6
23q	O	$C_{18}H_{37}$	Et		Br	2.92	6
23d	S	$C_{16}H_{33}$	Me	NMe_3	Br	2.20 ± 0.30	43
23r	S	$C_{16}H_{33}$	Me	$NMe_2CH_2CH(OH)CH_2OH$	Br	3.62 ± 0.04	6
23s	S	$C_{16}H_{33}$	Me		Br	8.75	6
23t	S	$C_{16}H_{33}$	C_5H_{11}	NMe_3	Br	6.14	6
43	O	$C_{16}H_{33}$	Me	$SMe(CH_2)_2OH$	TsO	2.49 ± 0.29	6

Table 5. Concentrations of lipids with a cationic head attached to glycerol via a spacer group corresponding to 50% inhibition of the growth of neoplastic cells.



26, 27, 29, 49

Compound	R^1	R^2	Z	Y	Q	IC_{50}/ μg ml^{-1}	Type of cell	Ref.
ET-18-OMe	$C_{16}H_{33}$	Me	$O(CH_2)_2$	NMe_3	Br	2.5 ± 0.31 [a]	HL-60	6, 54
						0.63	KB	20
26a	$C_{16}H_{33}$	Me	$O(CH_2)_2$	NMe_3	Br	1.85 ± 0.15 [a]	HL-60	54
26b	$C_{16}H_{33}$	Me	$O(CH_2)_2$	NMe_3	Br	2.30 ± 0.37	HL-60	54
26e	$C_{16}H_{33}$	Me	$O(CH_2)_2$	NMe_3	Br	3.79 ± 0.13 [a]	HL-60	54
26d	$C_{16}H_{33}$	Et	$O(CH_2)_4$	NMe_3	Br	1.86 ± 0.04	HL-60	54
26f	$C_{16}H_{33}$	Et	$O(CH_2)_4$		Br	0.78 ± 0.02	HL-60	54
26g	$C_{16}H_{33}$	H (instead of OR^2)	$O(CH_2)_4$	NMe_3	Br	1.56 ± 0.10 [a]	HL-60	54
26h	$C_{18}H_{37}$	Me	$O(CH_2)_2$	NMe_3	Cl	1.25	HL-60	20
						0.16	KB	20
29a	$C_{18}H_{37}$	Me	$(OCH_2CH_2)_2$	NMe_3	Cl	1.25	HL-60	20
						0.32	KB	20
29b	$C_{18}H_{37}$	Me	$(OCH_2CH_2)_5$	NMe_3	Cl	1.25	HL-60	20
						0.16	KB	20
29c	$C_{18}H_{37}$	C_4H_9	$(OCH_2CH_2)_2$	NMe_3	Cl	2.5	HL-60	20, 24
29d	$C_{18}H_{37}$		$(OCH_2CH_2)_2$	NMe_3	Cl	0.31	HL-60	20, 24, 58
						0.32	KB	20, 24, 58
29e	$C_{18}H_{37}$	$COCH_2Ac$	$(OCH_2CH_2)_2$	NMe_3	Cl	20.0	HL-60	20, 24
29f	$C_{18}H_{37}$	CH_2CF_3	$(OCH_2CH_2)_2$	NMe_3	Cl	2.5	HL-60	20, 24
29g	$C_{18}H_{37}$		$(OCH_2CH_2)_2$		Cl	0.63	HL-60	24, 58
27	$C_{16}H_{33}$	Me	$S(CH_2)_2$	NMe_3	Br	2.4	HL-60	6
49a	$C_{16}H_{33}$	Me	$SO_2(CH_2)_3$	NMe_3	MsO	1.25	HL-60	56
49b	$C_{16}H_{33}$	Me	$SO_2(CH_2)_6$	NMe_3	MsO	5.0	HL-60	56

[a] Expressed in μmol $litre^{-1}$.

The restriction of rotational mobility in the presence of the acetal system at the C(1) and C(2) atoms of the glycerol fragment diminishes the antineoplastic activity of the compounds. The presence of methoxy- or ethoxy-groups at the C(2) atom does not affect the activity. However, the presence of an alkyl substituent with a long chain containing more than five carbon atoms reduces the activity of the compound sharply. The replacement of the methoxy- and ethoxy-groups by heterocyclic bases of the pyrimidine or pyridazine type induces a slight decrease in the antineoplastic activity.[24]

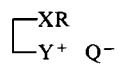
Among the compounds of the fourth group, the inhibition of the growth of neoplastic cells diminishes slightly with increase in the length of the spacer group but remains similar to the inhibiting activity of ET-18-OMe.

The presence of a quaternary ammonium or a tertiary sulfonium group is essential for the manifestation of activity, the sulfonium and pyridinium derivatives being the most active.[6]

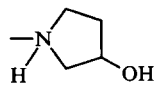
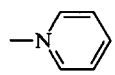
The degree of oligomerisation of the ethyleneoxy-group in compounds with the structure **29** does not play a decisive role for the activation in vitro (Table 5).[20] A similar conclusion can be reached also as regards compounds **49a** and **49b** with different numbers of methylene groups separating the sulfo-group from the cationic head.[56]

It is of interest that compound **29**, containing the acetoacetyl group at the C(2) atom of the glycerol fragment, proved to be fairly active against the S-180 and MM46 cells in studies carried out in vivo in contrast to in vitro investigations.[20]

Table 6. Concentrations of a series of derivatives of cationic lipids of the diol type corresponding to 50% inhibition of the growth of neoplastic cells.



7, 8, 19

Compound	X	R	Y	Q	IC_{50} / μmol litre^{-1}	Type of cell	Ref.
7	OCO	$C_{15}H_{31}$	$NMe_2(CH_2)_2OH$	Br	50[a]	KB	5
8a	O	$C_{12}H_{25}$	$NMe_2(CH_2)_2OH$	I	0.78 ± 0.03	KB	5
8b	O	$C_{16}H_{33}$	$NMe_2(CH_2)_2OH$	Br	0.33 ± 0.12	KB	5
8c	O	Ph	$NMe_2(CH_2)_2OH$	Br	> 100	HL-60	5
8d	O	H	$NMe_2(CH_2)_2OH$	Br	> 100	HL-60	5
8e	O	$C_{16}H_{33}$		Br	1.26 ± 0.36	HL-60	5
19a	S	$C_{16}H_{33}$		Br	2.28	HL-60	6
19b	S	$C_{16}H_{33}$	$NMe_2(CH_2)_2OH$	Br	3.95	HL-60	6

[a] Expressed in mmol litre^{-1}.

numbers of methylene groups separating the sulfo-group from the cationic head.[56]

It is of interest that compound **29**, containing the acetoacetyl group at the C(2) atom of the glycerol fragment, proved to be fairly active against the S-180 and MM46 cells in studies carried out in vivo in contrast to in vitro investigations.[20]

Some of the analogues synthesised have been tested in other systems. Thus for cells of the ChaGo and K562-4 lines, the inhibition was similar to that of the cell line 77. The inhibition of the P388 cells in mouse leukemia is comparable to the activity against the KB cells. The cells of the MHC rat hepatoma, obtained from the Morris 7795 hepatoma, were inhibited 2–3 times less effectively than the cells of the 77 line.[5]

There are data [57] demonstrating that increased concentration of cholesterol in the cell membrane plays a fairly important role in reducing the toxicity of the alkyl ether lipids (AL) to the HL-60 cells.

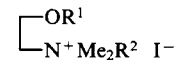
5. The antiviral activity of positively charged lipids

Synthetic lipids of the alkyl type exhibit a broad spectrum of biological activity, including the inhibition of replication of the HIV-1 virus. The exact mechanism of their action is not entirely clear. The HIV infection is a multistage process, which begins with the binding of the HIV to the CD4 receptors of the lymphocytes. After binding, the HIV permeates into the cell either via the direct fusion of the viral coating with the plasma membrane or by virtue of the receptor-dependent endocytosis of the CD4–HIV complex.[6,59] There are data demonstrating that the blocking of the HIV-dependent phosphorylation of the CD4 receptors by the introduction of a selective PKC inhibitor inhibits effectively the infection of the cell by the virus.

A new class of alkyl ether lipids — potential PKC inhibitors — has been investigated recently as regards their anti-HIV-1 activity. Monolayers or suspension cultures of human T-cells (CEM-ss) were used in these investigations to determine the activity of the structurally modified, phosphorus-free analogues of lipids of the alkyl ether type. The concentration corresponding to 50% inhibition of the growth of the CEM-ss cells and the formation of HIV-1 plaques as well as the decrease in viral budding (the capture by the virus of a portion of the cell membrane on permeation into the cell) were investigated.[17,60]

The experiments established that treatment of the cells infected by HIV-1 with compounds of this type is not accompanied by an increase in the activity of the reverse transcriptase, which implies different mechanisms of the action of 3′-azido-3′-deoxythymidine (AZT) and cationic lipids.

Electron microscope studies have shown [60] that there is no viral budding on the surface of a cell membrane in cells infected by HIV-1 and treated with positively charged lipids, but intracellular vacuolar viral particles are present. These data suggests that alkyl ether lipid analogues disturb the process involving the formation of cytoplasmic vacuolar forms of HIV in T-cells. The binding of HIV-1 to CD4 receptors of the T-lymphocytes induces their phosphorylation by means of the phospholipid-Ca^{2+}-dependent

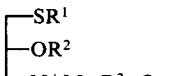


8

$R^1 = C_{18}H_{37}$: $R^2 = (CH_2)_2CH(OH)CH_2OH$ (**8f**);
$R^1 = C_{16}H_{33}$: $R^2 = (CH_2)_2OH$ (**8g**), $(CH_2)_3OH$ (**8h**);

PKC, whereupon cationic lipids, inhibiting the kinase, block the intracellular processes involving the development and multiplication of viral particles. The formation of HIV-1 plaques is inhibited via a concentration-dependent mechanism until the virus ceases to act on the growth of human T-cells.

The activities of the following compounds against HIV-1 have been investigated:



23, 50

Compound	R^1	R^2	R^3	Q
23a	$C_{16}H_{33}$	Me	$(CH_2)_2OH$	Br
23b	$C_{16}H_{33}$	Et	$(CH_2)_2OH$	Br
23d	$C_{16}H_{33}$	Me	Me	Br
23g	$C_{18}H_{37}$	Me	$(CH_2)_3OH$	I
23r	$C_{16}H_{33}$	Me	$CH_2CH(OH)CH_2OH$	Br
23u	$C_{16}H_{33}$	Et	$C_5H_5N^+$ (instead of $N^+Me_2R^3$)	Br
50	$C_{16}H_{33}$	Et	OH (instead of $N^+Me_2R^3$)	–

Analysis of the results (Table 7) shows that the formation of HIV-1 plaques in the CEM-ss cells is inhibited within the limits of

Table 7. Concentrations corresponding to 50% inhibition of the formation of HIV-1 plaques and the growth of CEM-ss cells.[17,60]

Compound	IC_{50} for the formation of HIV plaques / μmol litre^{-1}	IC_{50} for the growth of CEM-ss cells / μmol litre^{-1}	D[a]
AZT	0.004 ± 0.001	5.6 ± 0.8	1400
ET-18-OMe	0.92 ± 0.39	4.0 ± 0.7	4.3
23a	0.37 ± 0.02	2.1 ± 1.0	5.7
23b	0.63 ± 0.18	5.1 ± 0.4	8.1
23d	0.39 ± 0.24	5.3 ± 0.1	13.4
23g	0.41 ± 0.29	4.0 ± 2.7	9.8
23i	0.49 ± 0.23	2.0 ± 0.1	4.1
23k	0.21 ± 0.01	1.1	5.2
8f	0.20 ± 0.05	2.0 ± 0.5	14.5
8g	0.38 ± 0.29	6.0 ± 0.2	15.6
8h	0.24 ± 0.06	1.5 ± 0.1	6.1
50	> 5.0	> 20.0	–

[a] D is the differential selectivity defined as the ratio of the concentration corresponding to the inhibition of the growth of the CEM-ss cells to the concentration corresponding to the inhibition of the formation of HIV-1 plaques.

concentrations which are not toxic to the host cells. In order to exhibit anti-HIV-1 activity, compounds of this type must possess long-chain alkyl substituents at the C(1) atom, a short-chain substituent at the C(2) atom, and a quaternary ammonium group at the C(3) atom of glycerol.

The changes in the type of cationic head and in the size of the substituents at the quaternary nitrogen atom do not influence significantly the activity of the compounds.[17,60] However, in its absence the compound becomes inactive (for example, compound **50**).

Unfortunately no data have been obtained which would make it possible to infer the inhibition of the formation of plaques of the type 2 herpes simplest virus (HSV-2) or the inactivation of the intracellular virus, which evidently indicates the selectivity of compounds of this type in their action against HIV-1. This apparently occurs because they interact with the cytoplasmic membrane (the HIV-1 budding site) and not with the nuclear membrane (the herpes simplest budding site). In addition, the herpes virus does not require PKC for infection and its multiplication is different from that of HIV-1 (Table 7).[17]

Studies designed to elucidate the mechanism of the action of alkyl ether lipid analogues, inhibiting infection by HIV-1 and inducing the formation of a defective form of the virus, are being carried out at the present time. It has been shown that lipid antagonists are not inhibitors of reverse transcriptase but disturb the replication of the virus in the last stage. The choice of optimum analogues may provide specialists with effective chemotherapeutic preparations which can be used in the treatment of AIDS either individually or in combination with DNA-interactive anti-HIV-nucleosidic analogues.[60]

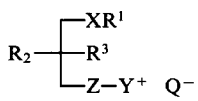
6. Cationic lipids — analogues and antagonists of the platelet activating factor

Numerous specific and nonspecific analogues and antagonists of the platelet activating factor (PAF) have been synthesised and investigated during the last decade. Synthetic derivatives permitting the solution of the problem of the relation between structure and biological activity and the possible therapeutic activity are of special interest.

The numerous PAF antagonists with a lipid structure can be subdivided into the following types: natural phospholipid PAF antagonists, their derivatives, and synthetic compounds. These antagonists are in the main active inhibitors of the binding of PAF to its receptors. Many of them are highly specific in their biological activity with the exception of certain phospholipid analogues, which do not exhibit any appreciable antagonistic activity at high concentrations.[26,61]

Two methods for the investigation of PAF antagonists under in vitro conditions are most widely used nowadays: inhibition of the PAF-induced aggregation of rabbit, human, and guinea pig platelets, as well as the inhibition of the specific binding of [^{1}H] PAF to cells or isolated membranes.[61] In the in vivo experiments, usually carried out on mice or rabbits, the effect of antagonists on the decrease in pressure, induced by PAF, and also the prevention of the lethal effect of high PAF doses are considered.[47,62–67]

One of the first phospholipid PAF antagonists, namely CV-3988 (Table 8), was synthesised initially as a powerful antifungal and cytotoxic agent.[68] The next structural phosphorus-free analogue, CV-6209, proved to be approximately 80 times more active in experiments in vitro.[26,61,63,69,70] A series of antagonists were synthesised in this connection by substituting the phosphocholine groups of PAF by quaternary ammonium groups of different types, attached to the glycerol fragment via spacer groups. In a general form, the structure of the PAF antagonists can be represented as follows:

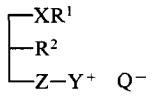


The strength of the antagonists in relation to the PAF-induced state depends on their structure and the experimental conditions. For each series of cationic lipids, representatives of which are listed in Table 8, the concentrations corresponding to the inhibition of the PAF-induced aggregation of platelets were obtained. For the majority of them, experiments designed to investigate the influence of the antagonists on the pathological states induced by the introduction of PAF under in vivo conditions were formulated.[25,35,61–64,71]

The principal requirements which must be met by the structure of the PAF antagonists are listed below:

(a) the long-chain substituent at the C(1) atom attached to glycerol via oxy-, thio-, carbamoyloxy-, and carbamoylthio-groups should have an optimum content of 16–18 carbon atoms;

Table 8. Concentrations corresponding to the 50% inhibition of the aggregation of blood platelets by PAF antagonists (for the experimental conditions and the type of platelets, see the literature sources).



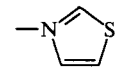
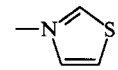
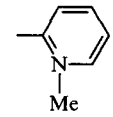
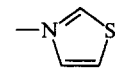
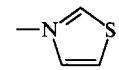
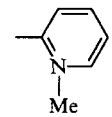
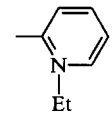
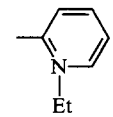
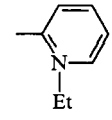
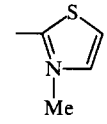
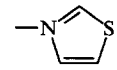
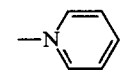
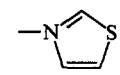
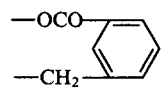
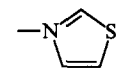
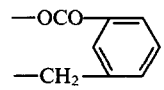
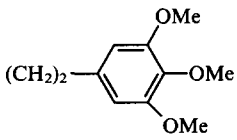
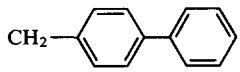
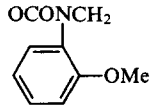
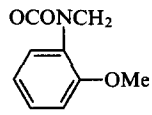
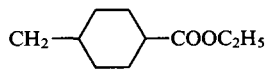
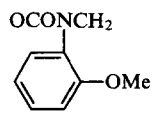
Compound	X	R^1	R^2	Z	Y	Q	IC_{50}/ μmol litre^{-1}	Ref.
CV-3988	OCONH	$C_{18}H_{37}$	OMe	$OPO_3^-(CH_2)_2$		–	1.6	26
ONO-6240	O	$C_{16}H_{33}$	OEt	$O(CH_2)_7$		OMs	0.1	61, 72, 73
CV-6209	OCONH	$C_{18}H_{37}$	OMe	$OCONAcCH_2$		OMs	0.2	35
Ro-18-8736	OCONH	$C_{18}H_{37}$	NHCOOMe	$OCO(CH_2)_3$		I	0.63	74
Ro-19-3704	OCONH	$C_{18}H_{37}$	OCOOMe	$O(CH_2)_4$		I	0.1 + 1	74, 75
Ru-45703	O	$C_{18}H_{37}$	OEt	$COO(CH_2)_4$	NMe_3	I	8.0	22, 76
33a	O	$C_{18}H_{37}$	OCOMe	$O(CH_2)_4$	NMe_3	I	> 100	77
42a	O	$C_{18}H_{37}$	OCOMe	$OCONAcCH_2CH_2$	NMe_3	I	0.88	35
42b	O	$C_{18}H_{37}$	OCOMe	$OCONH(CH_2)_2$	NMe_3	Br	8.4	35
42c	OCONH	$C_{18}H_{37}$	OMe	$OCONAc(CH_2)_3$	NMe_3	I	8.5	35
42d	OCONH	$C_{18}H_{37}$	OMe	$OCONH(CH_2)_2Me$	–	–	> 30	35
51a	OCONH	$C_{18}H_{37}$	OMe	$OCONAc(CH_2)_2$		Cl	0.2	35
51b	OCONH	$C_{18}H_{37}$	OMe	$OCONAcCH_2$		Cl	0.075	61
51c	OCONH	$C_{18}H_{37}$	OMe	$OCONAcCH_2$		Cl	0.084	61
51d	OCONH	$C_{18}H_{37}$	OMe	$OCONAcCH_2$		Cl	0.091	61
51e	OCONH	$C_{18}H_{37}$	OMe	$OCONAcCH_2$		I	0.41	61
52a	OCONH	$C_{18}H_{37}$	OMe	$NHSO_2(CH_2)_3$	NMe_3	Cl	48.1	25
52b	OCONH	$C_{18}H_{37}$	OMe	$NHSO_2(CH_2)_3$		I	1.89	25

Table 8 (continued).

Compound	X	R^1	R^2	Z	Y	Q	IC_{50} / μmol litre^{-1}	Ref.
52c	OCONH	$C_{18}H_{37}$	OMe	$NHSO_2(CH_2)_3$		I	5.20	25
52d	S	$C_{16}H_{33}$	OMe	$NHSO_2(CH_2)_3$		I	3.64	25
53a	O	$C_{16}H_{33}$	H			Br	3.6	78
53b	O	$C_{18}H_{37}$	H		NMe_3	Br	31	78
54a	O		–	$OCONAcCH_2$	–	I	0.38	62, 70
54b	O				–	I	0.025	62, 70
54c	OCONH	$(CH_2)_3OCONHC_{18}H_{37}$	–		–	Cl	0.044	62, 70
54d	OCONH		–		–	I	2.6	62, 70

(b) the configuration of the chiral centre must be analogous to that of PAF (*R*);
(c) the character and bulk of the substituent at the C(2) atom of glycerol must be analogous to those of acetyl;
(d) the replacement of the phosphate group at the C(3) atom by a spacer group of any kind is essential;
(e) the cationic head must have an acceptable structure.

It follows from the data presented in Table 8 that the replacement of the alkyl chain at the C(1) atom of glycerol by aromatic or heterocyclic groups with substituents of different lengths and structure did not lead to a sharp alteration of the antagonistic activity (cf. CV-6209 and **54a**).[35, 61, 62]

No appreciable differences between the in vitro activities of the (*RS*)- and (*R*)(−)- and (*S*)(+)-analogues have been observed (cf. **51b–d**). A similar situation has been noted for the compounds SRI 63-072 and SRI 63-119.[35, 79]

In the case where the spacer group Z is a carbamoyl derivative, the introduction of an acyl substituent into this group increases the blocking of the aggregation of platelets in vitro (cf. compounds **42a** and **42b**).[62, 64, 70] However, when amide bridges, linear imide bonds, and alkyl and certain carbamoyl substituents are used in the spacer group instead of the acyl substituent, the activity decreases fairly appreciably. An increase in the size of the spacer group as a whole induces a similar effect.[62, 70]

It is preferable to use thiazolinium and pyridinium groups as the cationic head. The presence of the ethyl substituent at the quaternary nitrogen atom in the heterocycle has then the most favourable effect on the antagonistic activity (compound **51b**).[35, 61, 70]

PAF antagonists help nowadays to elucidate the possible role of this natural bioregulator in various pathological changes in the organism and also its interaction with other causative agents and immunological mediators of these processes.[80] Many investigators are actively engaged in the assessment of their clinical activity. The data obtained suggest that it is possible to exert a definite therapeutic effect on asthma, allergic diseases, shock syndromes, the endointoxication of the organism, premature births, psoriasis, and many allergic diseases of the eyes.

In conclusion, it is essential to note that the creation and comprehensive investigation of new positively charged glycerolipids with the aim of discovering among them biologically active substances with an extended spectrum of therapeutic activity is an aspect of bioorganic chemistry and biotechnology of current interest.

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