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## REAGENTS FOR INTRACELLULAR DELIVERY OF MACROMOLECULES

### CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. patent application Ser. No. 09/326,106, filed Jun. 4, 1999, which is a continuation of U.S. patent application Ser. No. 08/195,866, filed Feb. 11, 1994, now U.S. Pat. No. 6,075,012 and which is incorporated by reference in its entirety herein to the extent not inconsistent herewith.

### FIELD OF THE INVENTION

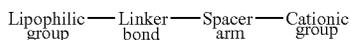
Cationic lipid compounds are disclosed, having utility in lipid aggregates for delivery of macromolecules and other compounds into cells. Also disclosed are compositions of cationic lipids and viral components or non-viral fusogenic compounds useful for enhancing transfection.

### BACKGROUND OF THE INVENTION

Lipid aggregates such as liposomes have been found to be useful as agents for delivery to introduce macromolecules, such as DNA, RNA, protein, and small chemical compounds such as pharmaceuticals, to cells. In particular, lipid aggregates comprising cationic lipid components have been shown to be especially effective for delivering anionic molecules to cells. In part, the effectiveness of cationic lipids is thought to result from enhanced affinity for cells, many of which bear a net negative charge. Also in part, the net positive charge on lipid aggregates comprising a cationic lipid enables the aggregate to bind polyanions, such as nucleic acids. Lipid aggregates containing DNA are known to be effective agents for efficient transfection of target cells.

The structure of various types of lipid aggregates varies, depending on composition and method of forming the aggregate. Such aggregates include liposomes, unilamellar vesicles, multilamellar vesicles, micelles and the like, having particle sizes in the nanometer to micrometer range. Methods of making lipid aggregates are by now well-known in the art. The main drawback to use of conventional phospholipid-containing liposomes for delivery is that the material to be delivered must be encapsulated and the liposome composition has a net negative charge which is not attracted to the negatively charged cell surface. By combining cationic lipid compounds with a phospholipid, positively charged vesicles and other types of lipid aggregates can bind DNA, which is negatively charged, can be taken up by target cells, and can transfect target cells. (Felgner, P. L. et al. (1987) Proc. Natl. Acad. Sci. USA 84:7413-7417; Eppstein, D. et al., U.S. Pat. No. 4,897,355.)

Cationic lipids useful for transfection and intracellular delivery of macromolecules generally contain the following four structural elements:

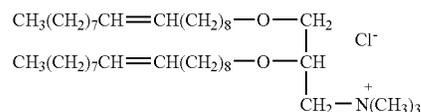


The lipophilic group is a hydrophobic moiety which facilitates the insertion of the cationic amphiphile into the membranes of the cell or liposome. The lipophilic group serves as an anchor for the cationic group (usually ammonium) which is positively charged at neutral Ph, to attach to the surface of the cell or liposome. The spacer arm is typically a hydro-

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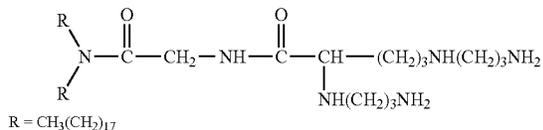
philic, 2 to 15-atom moiety which connects the cationic group to the lipophilic group via the linker bond. The linker bond is either an ether, ester, amide or other hydrolyzable bond.

A well-known cationic lipid disclosed in the prior art is N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA). The structure of DOTMA is:



DOTMA by itself or in 1:1 combination with dioleoylphosphatidyl-ethanolamine (DOPE) is formulated into liposomes using standard techniques. Felgner, et al. supra demonstrated that such liposomes provided efficient delivery of nucleic acids to some types of cells. A DOTMA:DOPE (1:1) formulation is sold under the trade name LIPOFECTIN (Gibco/BRL: Life Technologies, Inc., Gaithersburg, Md.). Another commercially available cationic lipid is 1,2-bis(oleoyloxy)-3-(3-(trimethylammonia) propane (DOTAP), which differs from DOTMA only in that the oleoyl moieties are linked via ester, rather than ether bonds to the propylamine. DOTAP is believed to be more readily degraded by target cells. A related group of prior art compounds differ from DOTMA and DOTAP in that one of the methyl groups of the trimethylammonium group is replaced by a hydroxyethyl group. Compounds of this type are similar to the Rosenthal Inhibitor (RI) of phospholipase A (Rosenthal, A. F. and Geyer, R. P. (1960) J. Biol. Chem. 235:2202-2206) which has stearoyl esters linked to the propylamine core. The dioleoyl analogs of RI are commonly abbreviated as DORI-ether and DORI-ester, depending on the linkage of the fatty acid moieties to the propylamine core. The hydroxy group can be used as a site for further functionalization, for example by esterification to carboxyspermine.

Another class of prior art compounds has been disclosed by Behr et al. (1989) Proc. Natl. Acad. Sci. USA 86:6982-6986; EPO publication 0 394 111 (Oct. 24, 1990), in which carboxyspermine has been conjugated to two types of lipids. The structures of 5-carboxyspermylglycine dioctadecylamide (DOGS) is:



The structure of dipalmitoylphosphatidylethanolamine 5-carboxy-spermylamide (DPPES) is:

