PHOSPHONIC ACID-BASED CATIONIC LIPIDS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Pat. No. 08/665,055, filed Jun. 5, 1996 now U.S. Pat. No. 5,958,901, continuation-in-part of U.S. patent application Ser. No. 08/484,716, entitled "Novel Methyl Phosphonate-Based Cationic Lipids", filed Jun. 7, 1995.

TECHNICAL FIELD OF THE INVENTION

The present invention relates to the field of cationic lipid compounds and their uses, including the delivery of macromolecules into cells.

BACKGROUND OF THE INVENTION

None of the following discussion of the background of the invention is admitted to be prior art to the invention.

Lipid aggregates, such as liposomes, have been reported to be useful as agents for the delivery of macromolecules (such as DNA, RNA, oligonucleotides, proteins, and pharmaceutical compounds) into cells. In particular, lipid aggregates, which include charged as well as uncharged lipids, have been described as being especially effective for delivering polyanionic molecules to cells. The reported effectiveness of cationic lipids may result from charge inmteractions with cells which are said to bear a net negative charge. It has also been postulated that the net positive charge on the cationic lipid aggregates may enable them to bind polyanions, such as nucleic acids. For example, lipid aggregates containing DNA have been reported to be effective agents for efficient transfection of cells.

The structure of a lipid aggregate depends on factors which include composition of the lipid and the method of forming the aggregate. Lipid aggregates include, for example, liposomes, unilamellar vesicles, multilamellar vesicles, micelles and the like, and may have particle sizes in the nanometer to micrometer range. Various methods of making lipid aggregates have been reported in the art. One type of lipid aggregate includes phospholipid containing liposomes. An important drawback to the use of this type of aggregate as a cell delivery vehicle is that the liposome has a negative charge that reduces the efficiency of binding to a negatively charged cell surface. It has been reported that positively charged liposomes that are able to bind DNA may be formed by combining cationic lipid compounds with phospholipids. These liposomes then be utilized to transfer DNA into target cells. (See, e.g. Feigner et al., *Proc. Nat. Acad. Sci.* 84:7413–7417, 1987; Eppstein et al. U.S. Pat. No. 4,897,355; Felgner et al. U.S. Pat. No. 5,264,618; and Gebeyehu et al. U.S. Pat. No. 5,334,761).

Known cationic lipids include N[1-(2,3-dioleoyloxy) propyl]-N,N,N-trimethyl-ammonium chloride ("DOTMA") and combinations of DOTHA wit dioleoylphosphatidylethanolamine "DOPE") have been commercially available. Formulation of DOTMA, either by itself or in 1:1 combination with DOPE, into liposomes by conventional techniques has been reported. However, compositions comprising DOTMA have been reported to show some toxicity to cells.

Another commercially available cationic lipid, 1,2-bis (oleoyloxy)-3,3-(trimethylammonia)propane ("DOTAP") differs from DOTMA in structure in that the oleoyl moieties are linked by ester, rather than ether, linkages to the propylamine. However, DOTAP is reported to be more readily degraded by target cells. Other cationic lipids which represent structural modifications of DOTMA and DOTAP have also been reported.

Other reported cationic lipid compounds include those in which carboxyspermine has been conjugated to one of two types of lipids and includes compounds such as 5-carboxyspermylglycine dioctaoleoylamide ("DOGS") and dipalmitoyl-phosphatidylethanolamine 5-carboxyspermylamide ("DPPES") (See, e.g. Behr et al., U.S. Pat. No. 5,171,678).

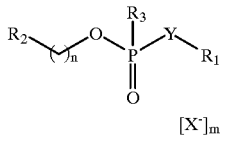
Another reported cationic lipid composition is a cationic cholesterol derivative ("DC-Chol") which has been formulated into liposomes in combination with DOPE. (See, Gao, X. and Huang, L., *Biochim. Biophys. Res. Coramun.* 179:280, 1991). For certain cell lines, these liposomes were said to exhibit lower toxicity and provide more efficient transfection than the DOTMA-containing compositions.

Lipopolylysine, made by conjugating polylysine to DOPE has been reported to be effective for transfection in the presence of serum. (Zhou, X. et al., *Biochim. Biophys. Acta* 1065:8, 1991).

However, of the cationic lipids which have been proposed for use in delivering macromolecules to cells, no particular cationic lipid has been reported to work well with a wide variety of cell types. Since cell types differ from one another in membrane composition, different cationic lipid compositions and different types of lipid aggregates may be effective for different cell types, either due to their ability to contact and fuse with target cell membranes directly or due to different interactions with intracellular membranes or the intracellular environment. For these and other reasons, design of effective cationic lipids has largely been empirical. In addition to content and transfer, other factors believed important include, for example, ability to form lipid aggregates suited to the intended purpose, toxicity of the composition to the target cell, stability as a carrier for the macromolecule to be delivered, and function in an in vivo environment. Thus, there remains a need for improved cationic lipids which are capable of delivering macromolecules to a wide variety cell types with greater effeciency.

SUMMARY OF THE INVENTION

In one aspect of the present invention novel phosphonic acid-based cationic lipids having the structure:



or a salt, or solvate, or enantiomers thereof are provided wherein; (a) R_1 is a lipophilic moiety; (b) R_2 is a positively charged moiety; (c) R_3 is a lipophilic moiety of about 1 to about 24 carbon atoms, a positively charged moiety, or a negatively charged moiety; (d) n is an integer from 0 to 8; (e) X^- is an anion or polyanion; (f) Y is N or 0, and (g) m is an integer from 0 to a number equivalent to the positive charge(s) present on the lipid.

In one embodiment RI may be selected from a variety of lipophilic moieties including a straight chain alkyl of 1 to about 24 carbon atoms, a straight chain alkenyl of 2 to about 24 carbon atoms, a symmetrical branched alkyl or alkenyl of about 10 to about 50 carbon atoms (preferably 25–40), an unsymmetrical branched alkyl or alkenyl of about 10 to