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PROCESS FOR PREPARING FARNESOL-BASED CATIONIC LIPIDS FOR CARRYING NUCLEIC ACIDS IN TO DESIRED CELL LINES

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ABSTRACT

The present invention generally relates to a process for preparing farnesol-based cationic lipids for carrying nucleic acids in to desired cell lines comprises synthesizing and characterizing number of farnesols based cationic lipids; and formulating synthesized farnesol based cationic lipids with helper lipids thereby optimizing their physicochemical properties.

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PROCESS FOR PREPARING FARNESOL-BASED CATIONIC LIPIDS FOR CARRYING NUCLEIC ACIDS IN TO DESIRED CELL LINES

FIELD OF THE INVENTION

The present disclosure relates to development of lipid aggregates comprising of farnesol based cationic lipids and co-lipids (DOPE/DOPC/Cholesterol). In more details, a process for preparing farnesol-based cationic lipids for carrying nucleic acids in to desired cell lines.

BACKGROUND OF THE INVENTION

Because of its apparent benefits over its viral cousin, non-viral gene delivery has emerged as a promising method in gene therapy. Although viral gene delivery methods are superior delivery vehicles, they have numerous disadvantages, including the danger of recombination with wild-type viruses, inherent immunogenicity, and limited nucleic acid packing capacity. As a result, non-viral gene delivery methods have been used as a safer and more promising platform for gene delivery. The use of cationic lipids as gene delivery vehicles has been deemed more promising than other non-viral vectors such as polymers and dendrimers because of its several advantages, including ease of production, repeatability, biodegradability, and potential economic value.

In the last several decades, a slew of cationic lipids and their formulations have been described, most of them include a backbone of glycerol, cholesterol, aliphatic hydrocarbon-tail, or tocopherol. Despite the various benefits of these cationic lipid systems for in vitro gene delivery, due to the complicated biological context in vivo, the performance of their nucleic acid treatments in clinical trials is far from ideal. However, significant progress has been made in identifying the unique obstacles to cationic lipid vector performance.

The fundamental variables in gene delivery are regulated by the configuration of the hydrophobic area in cationic lipid. The structure of the hydrophobic moiety influences factors such as liposome phase transition temperature, bilayer fluidity, liposome stability, endosomal escape, DNA protection from nucleases, DNA release from lipoplex, and nuclear uptake. Hydrophobic domains, on the other hand, have a role in determining lipid toxicity. Despite

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the fact that single chain and triple chain lipids have been described in the literature, double chain lipids have been shown to be the most efficient in controlling transfection and cytotoxicity. The limits of these cationic lipids, on the other hand, are well known. In comparison to longer aliphatic chains, studies on hydrocarbon chain length have shown that 14-carbon aliphatic chains are optimal for gene transfer.

In vitro and in vivo studies have shown that farnesol inhibits tumour cell growth. Farnesol also possesses chemopreventative, anti-inflammatory, antioxidant, analgesic, and neuroprotective effects, according to pharmacological investigations. Several patents on farnesol's medicinal potential have been filed in the last two decades. It's worth designing farnesol-based cationic lipids for gene delivery applications because of the vast range of pharmacological uses and intriguing structural characteristics.

In the view of the forgoing discussion, it is clearly portrayed that there is a need to have a process for preparing farnesol-based cationic lipids for carrying nucleic acids in to desired cell lines.

SUMMARY OF THE INVENTION

The present disclosure seeks to a process of synthesizing various cationic lipids and formulating lipid compositions for delivery of nucleic acids and therapeutic drugs.

In an embodiment, a process for preparing farnesol-based cationic lipids for carrying nucleic acids in to desired cell lines is disclosed. The process includes synthesizing and characterizing number of farnesols based cationic lipids. The process further includes formulating synthesized farnesol based cationic lipids with helper lipids thereby optimizing their physicochemical properties.

In an embodiment, the physicochemical properties of cationic lipids are optimizing in respect with size, zeta potential, morphology and DNA binding ability.

In an embodiment, structure activity function of various cationic lipids synthesized in terms of their ability is elucidated to deliver genetic material and anti-cancer effect.

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In an embodiment, the cationic lipids contains quaternary or primary amine head groups with two farnesol moieties as hydrophobic tail or two quaternary amine head group with one farnesol moieties are linked through different spacer varieties.

In an embodiment, the different spacer varieties are disulphide, alkyl and the like.

In an embodiment, each cationic lipid is different from others in terms of the linker group (R-groups) in case of normal lipid or spacer between two monomeric lipids in case of gemini lipids. R groups are methyl, hydroxylethyl, simple hydrogen atoms or amino acid residue.

In an embodiment, synthesis 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N,N- trimethylethanaminium comprises:

synthesizing tert-butyl (2-(dimethylamino)ethyl)carbamate;

synthesizing 2-((tert-butoxycarbonyl)amino)-N,N,N- trimethylethanaminium;

synthesizing 2-amino-N,N,N-trimethylethanaminium;

synthesizing farnesyl 2-bromoacetate;

synthesizing bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate; and

synthesizing 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl) amino) ethyl) amino) -N,N,N-trimethylethanaminium.

In an embodiment, synthesis of 2-((tert-butoxycarbonyl)amino)-N,N,N-trimethylethanaminium after synthesis of tert-butyl (2-(dimethylamino)ethyl)carbamate comprises:

dissolving 3.093 g, 14.4 mmol di-tert-butyl dicarbonate in dichloromethane (15 mL) thereby collecting solution to 0 °C;

adding solution of 1.0 g, 11.3 mmol N1,N1-dimethylethylenediamine in 10 mL of dichloromethane dropwise to the solution at 0 °C;

warming the mixture up to room temperature and continued to stir for 16 hours;

washing the reaction mixture trice with brine, thereafter dried over sodium sulfate and concentrated to give pure target product with 97 % yield;

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adding 10mL acetonitrile, 0.732 g, 5.3 mmol K2CO3 into a solution of 0.5 g, 2.6 mmol tert-butyl (2-(dimethylamino)ethyl)carbamate and stirring the reaction mixture at room temperature for 30 min;

adding 0.25 mL, 3.9 mmol methyl iodide to the above mixture and increasing reaction temperature to reflux condition;

stirring the reaction under reflux condition for 24 hrs, thereby the reaction temperature is decreased to room temperature and K2CO3 is filtered off;

removing organic solvent under reduced pressure to yield an oily crude product; and

washing the crude with diethyl ether for three times to yield the target product as white solid (yield, 93 %).

In an embodiment, synthesis of 2-amino-N,N,N-trimethylethanaminium comprises:

adding 9 mL dichloromethane and 1 mL trifluoroacetic acid (TFA) into a mixture of 0.76 g, 3.7 mmol 2-((tert-butoxycarbonyl)amino)-N,N,N-trimethylethanaminium dropwise at 0 °C and stirring the mixture stirred for 30 min;

increasing the reaction temperature to room temperature and continuously stirring for another 3 hours; and

evaporating the solvent under reduced pressure thereby washing resultant oily crude with diethyl ether thrice to produce target product as light yellow solid (yield, 80%).

In an embodiment, synthesis of farnesyl 2-bromoacetate comprises:

taking 5.0 g, 22.5 mmol farnesol and .125 g, 22.5 mmol bromoacetic acid in a round bottom flask thereby stirring the mixture at 65 ºC for 22 hours under solvent free conditions;

adding Hexane to the reaction mixture and hexane layer was transferred to separating funnel;

washing the residue with hexane twice and combined hexane layers are washed trice with water, separated, dried under sodium sulfate and concentrated under reduced pressure in a rotary evaporator; and

purifying the resultant crude by silica gel column chromatography using 2 % Ethyl acetate in hexane as mobile phase which yielded pure farnesyl-2-bromoacetate in 82% isolated yield.

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In an embodiment, synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N,N-trimethylethanaminium upon synthesizing bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate comprises:

adding 0.4 g, 1.9 mmol of 2-Bromoethylamine in 1 mL DMF dropwise to the mixture of 2 g, 5.8 mmol Farnesyl 2-bromoacetate and 1 g, 7.8 mmol DIPEA in 2 mL DMF;

stirring the mixture at room temperature overnight thereafter adding 100 mL EA to the reaction mixture and washing with 60 mL X 3 water and with 60 mL X 2brine;

drying organic layer over sodium sulfate and concentrating under reduced pressure in a rotary evaporator thereby residue is subjected to silica gel column chromatography and the pure product is eluted with 2-3 % EA in hexane (yield, 49 %);

dissolving 0.1 g, 0.154 mmol bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate in 1 mL of acetonitrile and adding 0.07 g, 0.46 mmol K2CO3;

adding 0.067 g, 0.308 mmol 2-amino-N,N,N-trimethylethanaminium in 2 mL of acetonitrile dropwise;

stirring the reaction at room temperature for 24 hours thereby filtering K2CO3 using acetonitrile as solvent;

removing solvent under reduced pressure and washing resultant crude with hexane; and

adding residue with chloroform and filtered thereby filtrate is concentrated to afford pure target lipid with 50 % yield.

An object of the present disclosure is to design, synthesize and characterize a number of farnesols based cationic lipids.

Another object of the present disclosure is to formulate the synthesized farnesol based cationic lipids.

Yet another object of the present invention is to deliver an expeditious and cost-effective process for preparing farnesol-based cationic lipids for carrying nucleic acids in to desired cell lines.

To further clarify advantages and features of the present disclosure, a more particular description of the invention will be rendered by reference to specific embodiments thereof,

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which is illustrated in the appended drawings. It is appreciated that these drawings depict only typical embodiments of the invention and are therefore not to be considered limiting of its scope. The invention will be described and explained with additional specificity and detail with the accompanying drawings.

BRIEF DESCRIPTION OF FIGURES

These and other features, aspects, and advantages of the present disclosure will become better understood when the following detailed description is read with reference to the accompanying drawings in which like characters represent like parts throughout the drawings, wherein:

Figure 1 illustrates a flow chart of a process for preparing farnesol-based cationic lipids for carrying nucleic acids in to desired cell lines in accordance with an embodiment of the present disclosure;

Figure 2 illustrates a molecular structure of newly designed cationic lipids in accordance with an embodiment of the present disclosure;

Figure 3 illustrates synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N,N-trimethylethanaminium in accordance with an embodiment of the present disclosure;

Figure 4 illustrates synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N-(2-hydroxyethyl)-N,N-dimethylethanaminium in accordance with an embodiment of the present disclosure;

Figure 5 illustrates synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N-bis(2-hydroxyethyl)-N-methylethanaminium in accordance with an embodiment of the present disclosure; and

Figure 6 illustrates synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N,N-tris(2-hydroxyethyl)ethanaminium in accordance with an embodiment of the present disclosure.

Further, skilled artisans will appreciate that elements in the drawings are illustrated for simplicity and may not have necessarily been drawn to scale. For example, the flow charts illustrate the method in terms of the most prominent steps involved to help to improve understanding of aspects of the present disclosure. Furthermore, in terms of the construction

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of the device, one or more components of the device may have been represented in the drawings by conventional symbols, and the drawings may show only those specific details that are pertinent to understanding the embodiments of the present disclosure so as not to obscure the drawings with details that will be readily apparent to those of ordinary skill in the art having benefit of the description herein.

DETAILED DESCRIPTION:

For the purpose of promoting an understanding of the principles of the invention, reference will now be made to the embodiment illustrated in the drawings and specific language will be used to describe the same. It will nevertheless be understood that no limitation of the scope of the invention is thereby intended, such alterations and further modifications in the illustrated system, and such further applications of the principles of the invention as illustrated therein being contemplated as would normally occur to one skilled in the art to which the invention relates.

It will be understood by those skilled in the art that the foregoing general description and the following detailed description are exemplary and explanatory of the invention and are not intended to be restrictive thereof.

Reference throughout this specification to “an aspect”, “another aspect” or similar language means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment of the present disclosure. Thus, appearances of the phrase “in an embodiment”, “in another embodiment” and similar language throughout this specification may, but do not necessarily, all refer to the same embodiment.

The terms "comprises", "comprising", or any other variations thereof, are intended to cover a non-exclusive inclusion, such that a process or method that comprises a list of steps does not include only those steps but may include other steps not expressly listed or inherent to such process or method. Similarly, one or more devices or sub-systems or elements or structures or components proceeded by "comprises...a" does not, without more constraints, preclude the existence of other devices or other sub-systems or other elements or other

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structures or other components or additional devices or additional sub-systems or additional elements or additional structures or additional components.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The system, methods, and examples provided herein are illustrative only and not intended to be limiting.

Embodiments of the present disclosure will be described below in detail with reference to the accompanying drawings.

Referring to **Figure 1**, illustrates a flow chart of a process for preparing farnesol-based cationic lipids for carrying nucleic acids in to desired cell lines is illustrated in accordance with an embodiment of the present disclosure. The process is developed to design cationic lipids based on farnesol.

At step 102, the process 100 includes synthesizing and characterizing number of farnesols based cationic lipids.

At step 104, the process 100 includes formulating synthesized farnesol based cationic lipids with helper lipids thereby optimizing their physicochemical properties.

In an embodiment, the physicochemical properties of cationic lipids are optimizing in respect with size, zeta potential, morphology and DNA binding ability.

In an embodiment, structure activity function of various cationic lipids synthesized in terms of their ability is elucidated to deliver genetic material and anti-cancer effect.

In an embodiment, the cationic lipids contains quaternary or primary amine head groups with two farnesol moieties as hydrophobic tail or two quaternary amine head group with one farnesol moieties are linked through different spacer varieties.

In an embodiment, the different spacer varieties are disulphide, alkyl and the like.

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In an embodiment, each cationic lipid is different from others in terms of the linker group (R-groups) in case of normal lipid or spacer between two monomeric lipids in case of gemini lipids. R groups are methyl, hydroxylethyl, simple hydrogen atoms or amino acid residue.

In an embodiment, synthesis 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N,N- trimethylethanaminium includes synthesizing tert-butyl (2-(dimethylamino)ethyl)carbamate. Then, synthesizing 2-((tert-butoxycarbonyl)amino)-N,N,N- trimethylethanaminium. Then, synthesizing 2-amino-N,N,N-trimethylethanaminium. Then, synthesizing farnesyl 2-bromoacetate. Then, synthesizing bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate. Thereafter, synthesizing 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl) amino) ethyl) amino) -N,N,N-trimethylethanaminium.

In an embodiment, synthesis of 2-((tert-butoxycarbonyl)amino)-N,N,N-trimethylethanaminium after synthesis of tert-butyl (2-(dimethylamino)ethyl)carbamate includes dissolving 3.093 g, 14.4 mmol di-tert-butyl dicarbonate in dichloromethane (15 mL) thereby collecting solution to 0 °C. Then, adding solution of 1.0 g, 11.3 mmol N1,N1-dimethylethylenediamine in 10 mL of dichloromethane dropwise to the solution at 0 °C. Then, warming the mixture up to room temperature and continued to stir for 16 hours. Then, washing the reaction mixture trice with brine, thereafter dried over sodium sulfate and concentrated to give pure target product with 97 % yield. Then, adding 10mL acetonitrile, 0.732 g, 5.3 mmol K2CO3 into a solution of 0.5 g, 2.6 mmol tert-butyl (2-(dimethylamino)ethyl)carbamate and stirring the reaction mixture at room temperature for 30 min. Then, adding 0.25 mL, 3.9 mmol methyl iodide to the above mixture and increasing reaction temperature to reflux condition. Then, stirring the reaction under reflux condition for 24 hrs, thereby the reaction temperature is decreased to room temperature and K2CO3 is filtered off. Then, removing organic solvent under reduced pressure to yield an oily crude product. Thereafter, washing the crude with diethyl ether for three times to yield the target product as white solid (yield, 93 %).

In an embodiment, synthesis of 2-amino-N,N,N-trimethylethanaminium includes adding 9 mL dichloromethane and 1 mL trifluoroacetic acid (TFA) into a mixture of 0.76 g, 3.7 mmol 2-((tert-butoxycarbonyl)amino)-N,N,N-trimethylethanaminium dropwise at 0 °C and stirring the mixture stirred for 30 min. then, increasing the reaction temperature to room

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temperature and continuously stirring for another 3 hours. Then, evaporating the solvent under reduced pressure thereby washing resultant oily crude with diethyl ether thrice to produce target product as light yellow solid (yield, 80%).

In an embodiment, synthesis of farnesyl 2-bromoacetate includes taking 5.0 g, 22.5 mmol farnesol and .125 g, 22.5 mmol bromoacetic acid in a round bottom flask thereby stirring the mixture at 65 ºC for 22 hours under solvent free conditions. Then adding Hexane to the reaction mixture and hexane layer was transferred to separating funnel. Then, washing the residue with hexane twice and combined hexane layers are washed trice with water, separated, dried under sodium sulfate and concentrated under reduced pressure in a rotary evaporator. Thereafter, purifying the resultant crude by silica gel column chromatography using 2 % Ethyl acetate in hexane as mobile phase which yielded pure farnesyl-2-bromoacetate in 82% isolated yield.

In an embodiment, synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N,N-trimethylethanaminium upon synthesizing bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate includes adding 0.4 g, 1.9 mmol of 2-Bromoethylamine in 1 mL DMF dropwise to the mixture of 2 g, 5.8 mmol Farnesyl 2-bromoacetate and 1 g, 7.8 mmol DIPEA in 2 mL DMF. Then, stirring the mixture at room temperature overnight thereafter adding 100 mL EA to the reaction mixture and washing with 60 mL X 3 water and with 60 mL X 2brine. Then, drying organic layer over sodium sulfate and concentrating under reduced pressure in a rotary evaporator thereby residue is subjected to silica gel column chromatography and the pure product is eluted with 2-3 % EA in hexane (yield, 49 %). Then, dissolving 0.1 g, 0.154 mmol bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate in 1 mL of acetonitrile and adding 0.07 g, 0.46 mmol K2CO3. Then, adding 0.067 g, 0.308 mmol 2-amino-N,N,N-trimethylethanaminium in 2 mL of acetonitrile dropwise. Then, stirring the reaction at room temperature for 24 hours thereby filtering K2CO3 using acetonitrile as solvent. Then, removing solvent under reduced pressure and washing resultant crude with hexane. Thereafter, adding residue with chloroform and filtered thereby filtrate is concentrated to afford pure target lipid with 50 % yield.

Figure 2 illustrates a molecular structure of newly designed cationic lipids in accordance with an embodiment of the present disclosure. The process offers a new class of farnesol based cationic lipids for carrying nucleic acids in to desired cell lines. The cationic

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lipids generally contain quaternary or primary amine head groups with two farnesol moieties as hydrophobic tail (Figure 2) or two quaternary amine head group with one farnesol moieties are linked through different spacer varieties viz di-sulphide, alkyl etc., The general molecular structure of newly designed cationic lipids in this invention are as follows.

Wherein, R groups represent methyl, hydroxylethyl, simple hydrogen atoms or amino acid residue. Each cationic lipid is different from others in terms of the R groups or the linker group in the case of normal lipid or spacer between the two monomeric lipids in case of gemini lipids. It is an object of the instant invention to provide a novel class of cationic lipids to be used as anticancer gene delivery vehicles.

The process facilitates in development of lipid aggregates comprising of these farnesols based cationic lipids and co-lipids (DOPE/DOPC/Cholesterol). The developed lipid aggregates having utility for the delivery of biologically active agents such as nucleic acids (DNA/RNA/siRNA), therapeutic proteins/peptides and therapeutic drugs into a variety of cells and tissues in vitro and/or in vivo. The process facilitates in synthesizing various new cationic lipids, formulating lipid compositions, as well as methods of delivery of nucleic acids and therapeutic drugs by means of these lipid formulations.

Figure 3 illustrates synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N,N-trimethylethanaminium in accordance with an embodiment of the present disclosure. To facilitate a further comprehensive understanding of the current invention, the following description presents various specific details, for instance particular configurations, examples, parameters, and so on. It is to be noted, though, such description is not proposed as a limitation upon the scope of the current invention, nonetheless intended to offer a better explanation of the typical embodiments.

The current invention affords a new class of cationic lipids to be used in developing nucleic acid complexes called lipoplexes for transfection of the nucleic acid. This class of compounds, contain two farnesol moieties as hydrophobic tails connected to different cationic head groups. Cationic head groups are designed by varying number of methyl, hydroxyethyl groups or hydrogen molecules on a positively charged nitrogen atoms. The rationale for this design is to assess the effect of increasing or decreasing number of hydroxyethyl groups on cationic head which are known to be beneficial in transfection. The

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number of cationic charge on each example lipid is constant, however, only differing by the moieties attached to the cationic nitrogen. The cationic charge on the example lipids is independent of pH, except for one example lipid where all the R groups on the nitrogen are represented by hydrogen atoms. It is to be noted that there are additional nitrogen atoms on the cationic lipids which may contribute to the charge on the lipid depending on the pH. Therefore, broadly stated, the newly designed cationic lipid of the current invention has two farnesol moieties connected to positively-charged head group comprising at least one ammonium group, which may be substituted by different number of methyl and hydroxyethyl groups.

The cationic lipids of the current invention can be synthesized by multistep reaction scheme as described as follows. The synthesis procedure can be divided into three broad segments, i.e., 1) synthesis of active cationic head groups, 2) Synthesis of intermediate hydrophobic tail group and 3) Conjugation of hydrophobic tail to the cationic head groups. The sesquiterpene farnesol is found to be unstable in presence of acid reagents such as trifluoroacidic acid. In order to avoid reactivity of unsaturated portion of farnesol, the current reaction is designed so as to minimize the number of reactions with farnesol leading to final cationic lipids. Thus, one aspect of the current invention is a method of efficient synthesis of cationic lipids comprising farnesol.

The newly developed cationic lipids of the invention can be used as drug/gene carriers when formulated with neutral lipids of other cationic lipids. The applications of the catioinic lipids in the current invention include, without out limitation, gene delivery, drug delivery, prodrug based therapy and anti-biotic treatment. In a preferred use, the farnesol based cationic lipids of the current invention are used in the formulation of liposomes which bind with nucleic acids and the resultant liposome-necleic acid complexes called as lipoplex can be incorporated in to preferred cell lines through a process called transfection.

Transfection is achieved using well-known model methods in the prior art for transfection with different types of cationic lipid based DNA complexes. Since farnesol structurally fulfilling basic requirements of an ideal hydrophobic tail and quarternary ammonium based head groups are known to bind with DNA efficiently, the new cationic lipids in the current invention may provide new class of efficient gene delivery vectors.

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The invention will now be illustrated with regards to the following, non-limiting examples.

Synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N,N-trimethylethanaminium comprises:

A. Synthesis of tert-butyl (2-(dimethylamino)ethyl)carbamate

Di-tert-butyl dicarbonate (3.093 g, 14.4 mmol) was dissolved in dichloromethane (15 mL) and the solution was colled to 0 °C. A solution of N1,N1-dimethylethylenediamine (1.0 g, 11.3 mmol) in 10 mL of dichloromethane was added dropwise to the above solution at 0 °C. The mixture was warmed up to room temperature and continued to stir for 16 hours. The reaction mixture was washed trice with brine, dried over sodium sulfate and concentrated to give pure target product with 97 % yield.

B. Synthesis of 2-((tert-butoxycarbonyl)amino)-N,N,N-trimethylethanaminium

To a solution of tert-butyl (2-(dimethylamino)ethyl)carbamate (0.5 g, 2.6 mmol) in acetonitrile (10 mL), K2CO3 (0.732 g, 5.3 mmol) was added and the reaction mixture was stirred at room temperature for 30 min. Then, methyl iodide (0.25 mL, 3.9 mmol) was added to the above mixture and the reaction temperature was increased to reflux condition. The reaction was continued to stir under reflux condition for 24 hrs. Subsequently, the reaction temperature was decreased to room temperature and K2CO3 was filtered off. Organic solvent was removed under reduced pressure to yield an oily crude product. The crude was washed with diethyl ether for three times to yield the target product as white solid (yield, 93 %).

C. Synthesis of 2-amino-N,N,N-trimethylethanaminium

To a mixture of 2-((tert-butoxycarbonyl)amino)-N,N,N-trimethylethanaminium (0.76 g, 3.7 mmol) in dichloromethane (9 mL), trifluoroacetic acid (TFA) (1 mL) was added dropwise at 0 °C and the mixture was stirred for 30 min. After that, the reaction temperature was increased to room temperature and continuously stirred for another 3 hours. The solvent was evaporated under reduced pressure and the resultant oily crude was washed with diethyl ether thrice to give the target product as light yellow solid (yield, 80%).

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D. Synthesis of farnesyl 2-bromoacetate

farnesol (5.0 g, 22.5 mmol) and bromoacetic acid (3.125 g, 22.5 mmol) were taken in round bottom flask and the mixture was stirred at 65 °C for 22 hours under solvent free conditions. After that, Hexane was added to the reaction mixture and hexane layer was transferred to separating funnel. The residue was further washed with hexane twice and combined hexane layers were washed trice with water, separated, dried under sodium sulfate and concentrated under reduced pressure in a rotary evaporator. The resultant crude was further purified by silica gel column chromatography using 2 % Ethyl acetate in hexane as mobile phase which yielded pure farnesyl-2-bromoacetate in 82% isolated yield.

E. Synthesis of bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate

2-Bromoethylamine (0.4 g, 1.9 mmol) in DMF (1 mL) was dropwise added to the mixture of Farnesyl 2-bromoacetate (2 g, 5.8 mmol) and DIPEA (1 g, 7.8 mmol) in DMF (2 mL). The mixture was stirred at room temperature overnight. Then, EA (100 mL) was added to the reaction mixture and washed with water (60 mL X 3) and with brine (60 mL X 2). The organic layer was dried over sodium sulfate and concentrated under reduced pressure in a rotary evaporator. The residue was subjected to silica gel column chromatography and the pure product was eluted with 2-3 % EA in hexane (yield, 49 %).

F. Synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N,N-trimethylethanaminium

bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate (0.1 g, 0.154 mmol) was dissolved in 1 mL of acetonitrile and K2CO3 (0.07 g, 0.46 mmol) was added. Then, 2-amino-N,N,N-trimethylethanaminium (0.067 g, 0.308 mmol) in 2 mL of acetonitrile was added dropwise. The reaction was stirred at room temperature for 24 hours. K2CO3 was filtered off using acetonitrile as solvent. Solvent was removed under reduced pressure and the resultant crude was washed with hexane. The residue was added with chloroform and filtered. Filtrate was concentrated to afford pure target lipid with 50 % yield.

Figure 4 illustrates synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N-(2-hydroxyethyl)-N,N-dimethylethanaminium in accordance with an embodiment of the present disclosure. Synthesis of 2-((2-(bis(2-oxo-2-

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((farnesyl)oxy)ethyl)amino)ethyl)amino)-N-(2-hydroxyethyl)-N,N-dimethylethanaminium comprises:

A. Synthesis of 2-((tert-butoxycarbonyl)amino)-N-(2-hydroxyethyl)-N,N-dimethylethanaminium

To a solution of tert-butyl (2-(dimethylamino)ethyl)carbamate (0.5 g,2.6 mmol) in Acetonitrile (10 mL), K2CO3 (0.732 g, 5.3 mmol) was added and the reaction mixture was stirred at room temperature for 30 min. Then, Iodoethanol (0.413 mL, 5.3 mmol) was added to the above mixture and the reaction temperature was increased to reflux condition. The reaction was continued to stir under reflux condition for 24 hrs. Subsequently, the reaction temperature was decreased to room temperature and K2CO3 was filtered off. Organic solvent was removed under reduced pressure to yield an oily crude product. The crude was washed with diethyl ether for three times to yield the target product as oily liquid (yield, 87 %).

B. Synthesis of 2-amino-N-(2-hydroxyethyl)-N,N-dimethylethanaminium

To a mixture of 2-((tert-butoxycarbonyl)amino)-N-(2-hydroxyethyl)-N,N-dimethylethanaminium (0.8 g, 3.3 mmol.) in dichloromethane (9 mL), trifluoroacetic acid (TFA) (1 mL) was added dropwise at 0 °C and the mixture was stirred for 30 min. After that, the reaction temperature was increased to room temperature and continuously stirred for another 3 hours. The solvent was evaporated under reduced pressure and the resultant oily crude was washed with diethyl ether thrice to give the target product as light yellow solid (yield, 93 %).

C. Synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N-(2-hydroxyethyl)-N,N-dimethylethanaminium

bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate (0.1 g, o,154 mmol) was dissolved in 1 mL of acetonitrile and K2CO3 (0.07 g, 0.46 mmol) was added. Then, 2-amino-N-(2-hydroxyethyl)-N,N-dimethylethanaminium (0.076 g, 0.308 mmol) in 2 mL of DMF was added dropwise. The reaction was stirred at room temperature for 24 hours. K2CO3 was filtered off using acetonitrile as solvent. Solvent was removed under reduced pressure and the resultant crude was washed with hexane. The residue was added with chloroform and filtered. Filtrate was concentrated to afford pure target lipid with 50 % yield.

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Figure 5 illustrates synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N-bis(2-hydroxyethyl)-N-methylethanaminium in accordance with an embodiment of the present disclosure. Synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N-bis(2-hydroxyethyl)-N-methylethanaminium comprises:

A. Synthesis of tert-butyl (2-(methylamino)ethyl)carbamate

Di-tert-butyl dicarbonate (2.0 g, 9.2 mmol) was dissolved in dichloromethane (15 mL) and the solution was cooled to 0 °C. A solution of N-Methylethelenediamine (1.36 g, 18.33 mmol.) in 10 mL of dichloromethane was added dropwise to the above solution at 0 °C. The mixture was warmed up to room temperature and continued to stir for 16 hours. The reaction mixture was washed with brine, dried over sodium sulfate and filtered. The filtrate was concentrated to give pure product with 73 % yield. The resultant product was used without further purification.

B. Synthesis of 2-((tert-butoxycarbonyl)amino)-N,N-bis(2-hydroxyethyl)-N-methylethanaminium

To a solution of tert-butyl (2-(methylamino)ethyl)carbamate (0.3 g, 1.7 mmol) in Acetonitrile (3 mL), K2CO3 (0.7 g, 5.1 mmol) was added and the reaction mixture was stirred at room temperature for 30 min. Then, Bromoethanol (0.64 g, 5.1 mmol) was added to the above mixture and the reaction temperature was increased to reflux condition. The reaction was continued to stir under reflux condition for 24 hrs. Subsequently, the reaction temperature was decreased to room temperature and K2CO3 was filtered off. Organic solvent was removed under reduced pressure to yield an oily crude product. The crude was washed with diethyl ether for three times to yield the target product as oily liquid (yield, 76 %).

C. Synthesi of 2-amino-N,N-bis(2-hydroxyethyl)-N-methylethanaminium

To a mixture of 2-((tert-butoxycarbonyl)amino)-N,N-bis(2-hydroxyethyl)-N-ethylethanaminium (0.2 g, 0.75 mmol) in dichloromethane (9 mL), trifluoroacetic acid (TFA) (1 mL) was added dropwise at 0 °C and the mixture was stirred for 30 min. After that, the reaction temperature was increased to room temperature and continuously stirred for another 3 hours. The solvent was evaporated under reduced pressure and the resultant oily

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crude was washed with diethyl ether thrice to give the target product as light yellow oil (yield, 90%).

D. Synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N-bis(2-hydroxyethyl)-N-methylethanaminium

bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate (0.1 g, o,154 mmol) was dissolved in 1 mL of acetonitrile and K2CO3 (0.07 g, 0.46 mmol) was added. To this mixture 2-amino-N,N-bis(2-hydroxyethyl)-N-methylethanaminium (0.085 g, 0.308 mmol) in 2 mL of DMF was added dropwise. The reaction was stirred at room temperature for 24 hours. K2CO3 was filtered off using acetonitrile as solvent. Solvent was removed under reduced pressure and the resultant crude was washed with hexane. The residue was added with chloroform and filtered. Filtrate was concentrated to afford pure target lipid with 43 % yield.

Figure 6 illustrates synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N,N-tris(2-hydroxyethyl)ethanaminium in accordance with an embodiment of the present disclosure. Synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N,N-tris(2-hydroxyethyl)ethanaminium comprises:

A. Synthesis of tert-butyl (2-((2-hydroxyethyl)amino)ethyl)carbamate

Di-tert-butyl dicarbonate (4.3g, 20 mmol) was dissolved in dichloromethane (15 mL) and the solution was cooled to 0 °C. A solution of ethelenediamine (12.02 g, 200 mmol) in 30 mL of dichloromethane was added dropwise to the above solution at 0 °C. The mixture was warmed up to room temperature and continued to stir for 16 hours The reaction mixture was washed with brine, dried over sodium sulfate and filtered. The filtrate was concentrated to give pure product with 78 % yield. The resultant product was used without further purification.

B. Synthesis of 2-((tert-butoxycarbonyl)amino)-N,N,N-tris(2-hydroxyethyl)ethanaminium

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To a solution of tert-butyl (2-aminoethyl)carbamate (1.3 g, 8.12 mmol) in Acetonitrile (30 mL), K2CO3 (4.5 g, 32.5 mmol) was added and the reaction mixture was stirred at room temperature for 30 min. Then, Bromoethanol (4.06 g, 32.5 mmol) was added to the above mixture and the reaction temperature was increased to reflux condition. The reaction was continued to stir under reflux condition for 24 hrs. Subsequently, the reaction temperature was decreased to room temperature and K2CO3 was filtered off. Organic solvent was removed under reduced pressure to yield an oily crude product. The crude was washed with diethyl ether for three times to yield the target product as oily liquid (yield, 66 %).

C. Synthesis of 2-amino-N,N,N-tris(2-hydroxyethyl)ethanaminium

To a mixture of 2-((tert-butoxycarbonyl)amino)-N,N,N-tris(2-hydroxyethyl)ethanaminium (0.53 g, 1.7 mmol) in dichloromethane (9 mL), trifluoroacetic acid (TFA) (1 mL) was added dropwise at 0 °C and the mixture was stirred for 30 min. After that, the reaction temperature was increased to room temperature and continuously stirred for another 3 hours. The solvent was evaporated under reduced pressure and the resultant oily crude was washed with diethyl ether thrice to give the target product as light yellow oil (yield, 71%).

D. Synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N,N-tris(2-hydroxyethyl)ethanaminium

bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate (0.1 g, 0.154 mmol) was dissolved in 1 mL of acetonitrile and K2CO3 (0.07 g, 0.46 mmol) was added. To this mixture 2-amino-N,N,N-tris(2-hydroxyethyl)ethanaminium (0.095 g, 0.308 mmol) in 2 mL of DMF was added dropwise. The reaction was stirred at room temperature for 24 hours. K2CO3 was filtered off using acetonitrile as solvent. Solvent was removed under reduced pressure and the resultant crude was washed with hexane. The residue was added with chloroform and filtered. Filtrate was concentrated to afford pure target lipid with 45 % yield.

The drawings and the forgoing description give examples of embodiments. Those skilled in the art will appreciate that one or more of the described elements may well be combined into a single functional element. Alternatively, certain elements may be split into multiple functional elements. Elements from one embodiment may be added to another embodiment. For example, orders of processes described herein may be changed and are not

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limited to the manner described herein. Moreover, the actions of any flow diagram need not be implemented in the order shown; nor do all of the acts necessarily need to be performed. Also, those acts that are not dependent on other acts may be performed in parallel with the other acts. The scope of embodiments is by no means limited by these specific examples. Numerous variations, whether explicitly given in the specification or not, such as differences in structure, dimension, and use of material, are possible. The scope of embodiments is at least as broad as given by the following claims.

Benefits, other advantages, and solutions to problems have been described above with regard to specific embodiments. However, the benefits, advantages, solutions to problems, and any component(s) that may cause any benefit, advantage, or solution to occur or become more pronounced are not to be construed as a critical, required, or essential feature or component of any or all the claims.

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WE CLAIM:

1. A process for preparing farnesol-based cationic lipids for carrying nucleic acids in to desired cell lines, the process comprises:

synthesizing and characterizing number of farnesols based cationic lipids; and

formulating synthesized farnesol based cationic lipids with helper lipids thereby optimizing their physicochemical properties.

2. The process as claimed in claim 1, wherein the physicochemical properties of cationic lipids are optimizing in respect with size, zeta potential, morphology and DNA binding ability.

3. The process as claimed in claim 1, wherein structure activity function of various cationic lipids synthesized in terms of their ability is elucidated to deliver genetic material and anti-cancer effect.

4. The process as claimed in claim 1, wherein the cationic lipids contains quaternary or primary amine head groups with two farnesol moieties as hydrophobic tail or two quaternary amine head group with one farnesol moieties are linked through different spacer varieties, wherein the different spacer varieties are disulphide, alkyl and the like.

5. The process as claimed in claim 4, wherein each cationic lipid is different from others in terms of the linker group (R-groups) in case of normal lipid or spacer between two monomeric lipids in case of gemini lipids, wherein R groups are methyl, hydroxylethyl, simple hydrogen atoms or amino acid residue.

6. The process as claimed in claim 1, wherein synthesis 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N,N- trimethylethanaminium comprises:

synthesizing tert-butyl (2-(dimethylamino)ethyl)carbamate;

synthesizing 2-((tert-butoxycarbonyl)amino)-N,N,N- trimethylethanaminium;

synthesizing 2-amino-N,N,N-trimethylethanaminium;

synthesizing farnesyl 2-bromoacetate;

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synthesizing bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate; and

synthesizing 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl) amino) ethyl) amino) -N,N,N-trimethylethanaminium.

7. The process as claimed in claim 6, wherein synthesis of 2-((tert-butoxycarbonyl)amino)-N,N,N-trimethylethanaminium after synthesis of tert-butyl (2-(dimethylamino)ethyl)carbamate comprises:

dissolving 3.093 g, 14.4 mmol di-tert-butyl dicarbonate in dichloromethane (15 mL) thereby collecting solution to 0 °C;

adding solution of 1.0 g, 11.3 mmol N1,N1-dimethylethylenediamine in 10 mL of dichloromethane dropwise to the solution at 0 °C;

warming the mixture up to room temperature and continued to stir for 16 hours;

washing the reaction mixture trice with brine, thereafter dried over sodium sulfate and concentrated to give pure target product with 97 % yield;

adding 10mL acetonitrile, 0.732 g, 5.3 mmol K2CO3 into a solution of 0.5 g, 2.6 mmol tert-butyl (2-(dimethylamino)ethyl)carbamate and stirring the reaction mixture at room temperature for 30 min;

adding 0.25 mL, 3.9 mmol methyl iodide to the above mixture and increasing reaction temperature to reflux condition;

stirring the reaction under reflux condition for 24 hrs, thereby the reaction temperature is decreased to room temperature and K2CO3 is filtered off;

removing organic solvent under reduced pressure to yield an oily crude product; and

washing the crude with diethyl ether for three times to yield the target product as white solid (yield, 93 %).

8. The process as claimed in claim 6, wherein synthesis of 2-amino-N,N,N-trimethylethanaminium comprises:

adding 9 mL dichloromethane and 1 mL trifluoroacetic acid (TFA) into a mixture of 0.76 g, 3.7 mmol 2-((tert-butoxycarbonyl)amino)-N,N,N-trimethylethanaminium dropwise at 0 °C and stirring the mixture stirred for 30 min;

increasing the reaction temperature to room temperature and continuously stirring for another 3 hours; and

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evaporating the solvent under reduced pressure thereby washing resultant oily crude with diethyl ether thrice to produce target product as light yellow solid (yield, 80%).

9. The process as claimed in claim 6, wherein synthesis of farnesyl 2-bromoacetate comprises:

taking 5.0 g, 22.5 mmol farnesol and .125 g, 22.5 mmol bromoacetic acid in a round bottom flask thereby stirring the mixture at 65 ºC for 22 hours under solvent free conditions;

adding Hexane to the reaction mixture and hexane layer was transferred to separating funnel;

washing the residue with hexane twice and combined hexane layers are washed trice with water, separated, dried under sodium sulfate and concentrated under reduced pressure in a rotary evaporator; and

purifying the resultant crude by silica gel column chromatography using 2 % Ethyl acetate in hexane as mobile phase which yielded pure farnesyl-2-bromoacetate in 82% isolated yield.

10. The process as claimed in claim 6, wherein synthesis of 2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N,N,N-trimethylethanaminium upon synthesizing bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate comprises:

adding 0.4 g, 1.9 mmol of 2-Bromoethylamine in 1 mL DMF dropwise to the mixture of 2 g, 5.8 mmol Farnesyl 2-bromoacetate and 1 g, 7.8 mmol DIPEA in 2 mL DMF;

stirring the mixture at room temperature overnight thereafter adding 100 mL EA to the reaction mixture and washing with 60 mL X 3 water and with 60 mL X 2brine;

drying organic layer over sodium sulfate and concentrating under reduced pressure in a rotary evaporator thereby residue is subjected to silica gel column chromatography and the pure product is eluted with 2-3 % EA in hexane (yield, 49 %);

dissolving 0.1 g, 0.154 mmol bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate in 1 mL of acetonitrile and adding 0.07 g, 0.46 mmol K2CO3;

adding 0.067 g, 0.308 mmol 2-amino-N,N,N-trimethylethanaminium in 2 mL of acetonitrile dropwise;

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stirring the reaction at room temperature for 24 hours thereby filtering K2CO3 using acetonitrile as solvent;

removing solvent under reduced pressure and washing resultant crude with hexane; and

adding residue with chloroform and filtered thereby filtrate is concentrated to afford pure target lipid with 50 % yield.

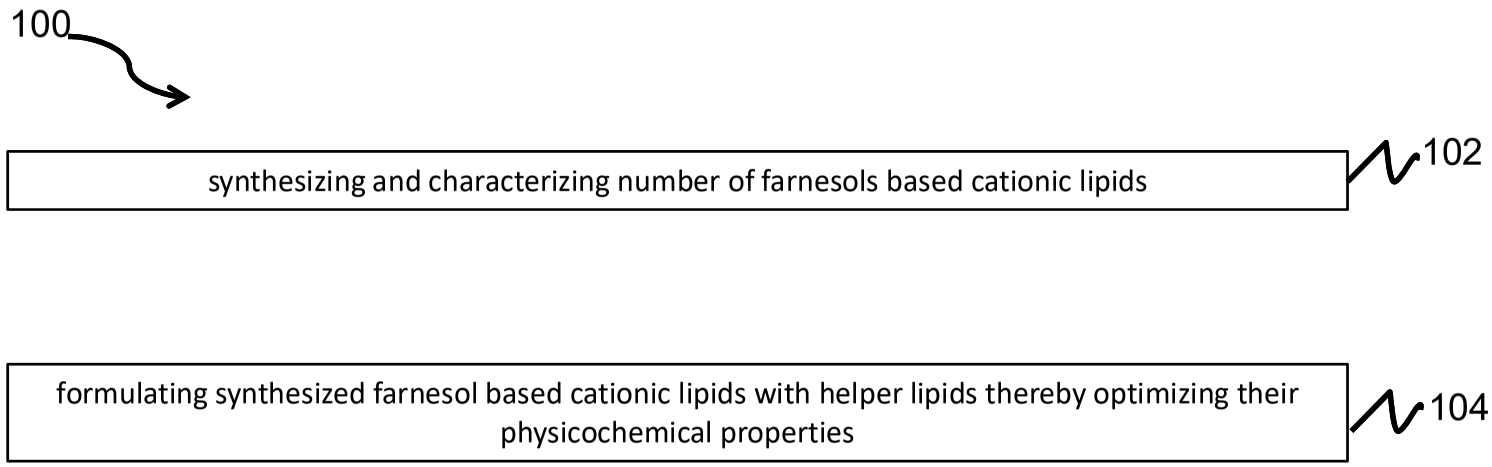

100
synthesizing and characterizing number of farnesols based cationic lipids
102
formulating synthesized farnesol based cationic lipids with helper lipids thereby optimizing their physicochemical properties
104

Figure 1

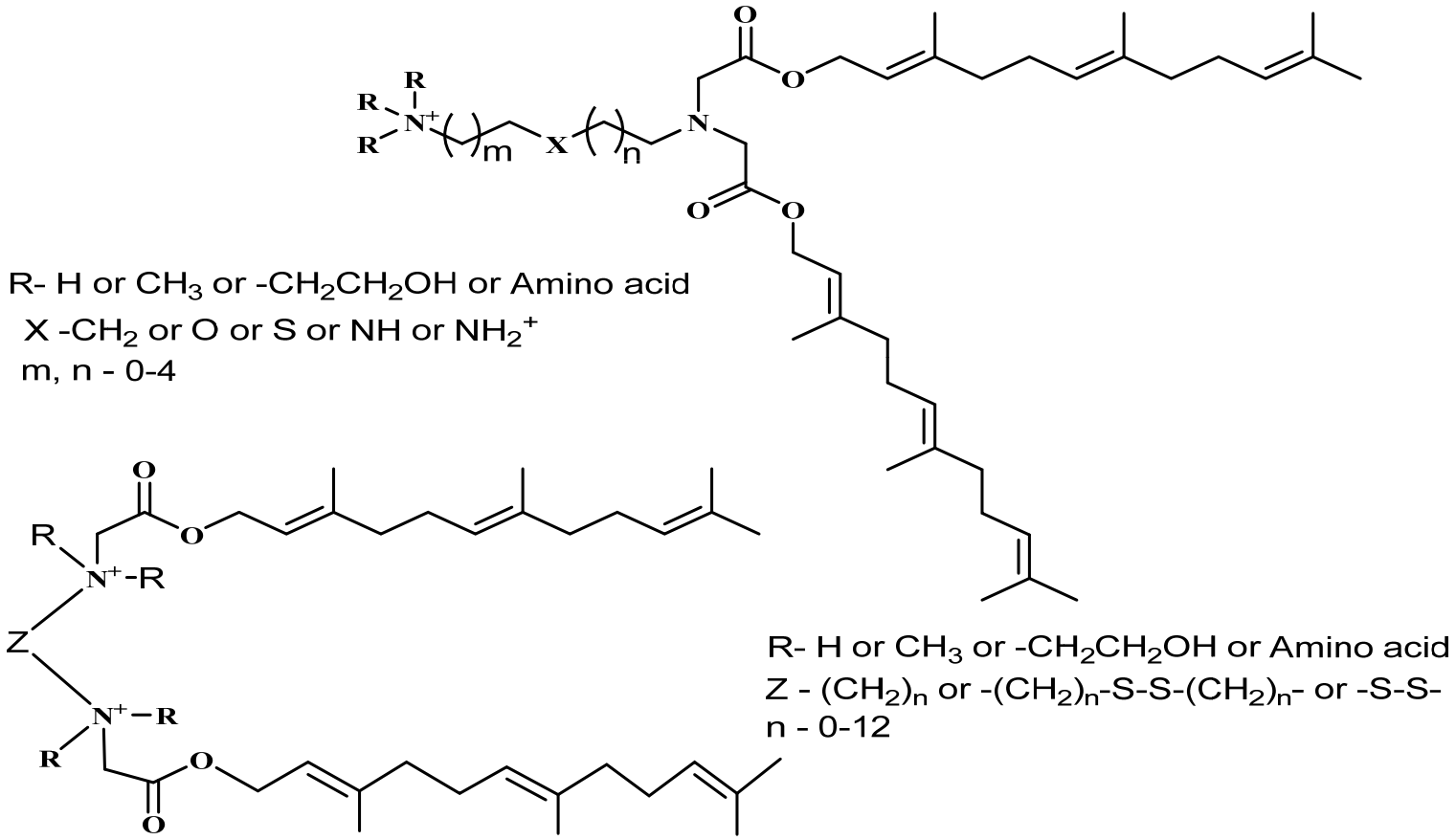

R- H or CH3 or -CH2CH2OH or Amino acid
X -CH2 or O or S or NH or NH2+
m, n - 0-4
R- H or CH3 or -CH2CH2OH or Amino acid
Z - (CH2)n or -(CH2)n-S-S-(CH2)n- or -S-S-
n - 0-12

Figure 2

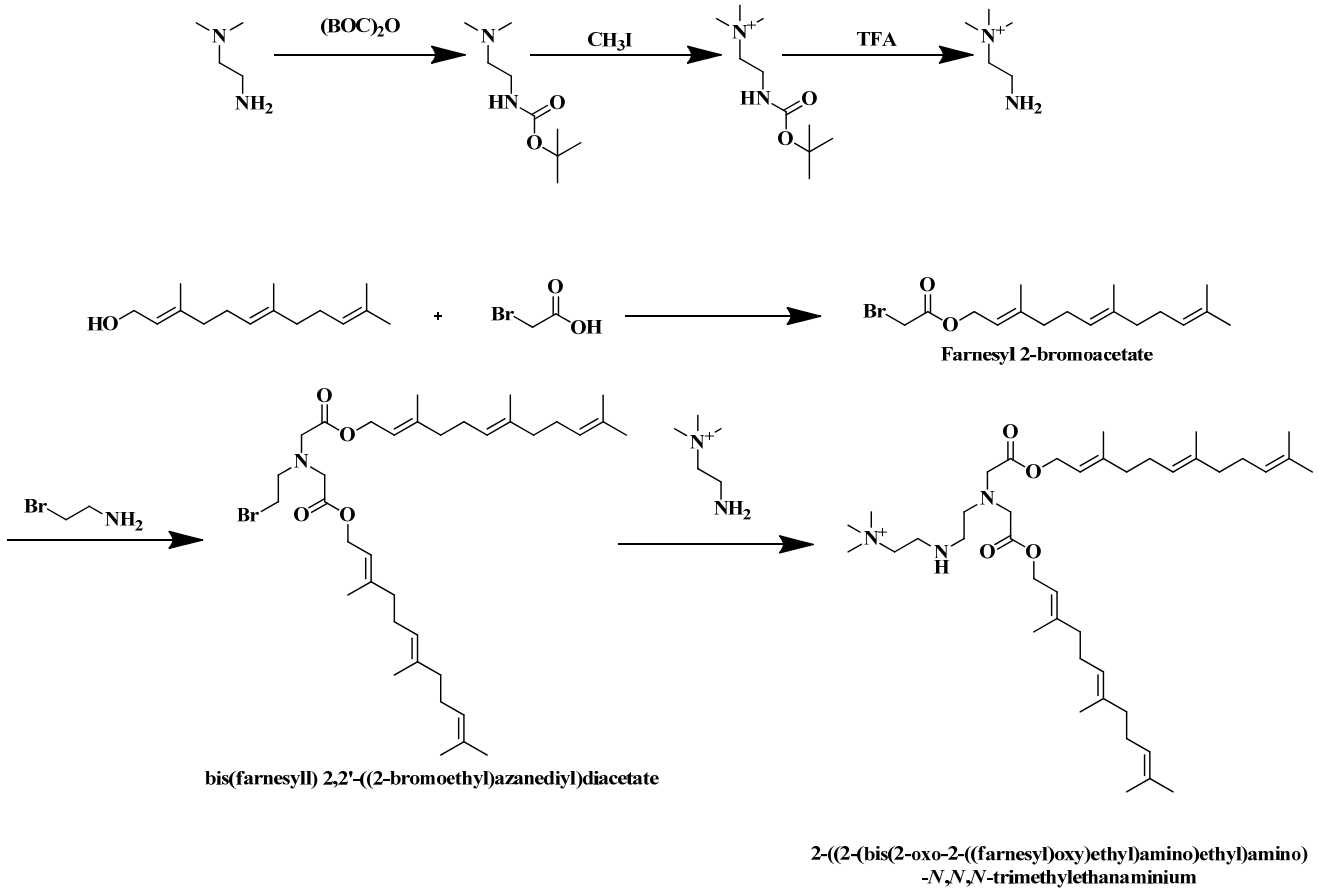

(BOC)2O
CH3I
TFA
HO
Br
OH
Farnesyl 2-bromoacetate
Br
NH2
bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate
2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)
-N,N,N-trimethylethanaminium

Figure 3

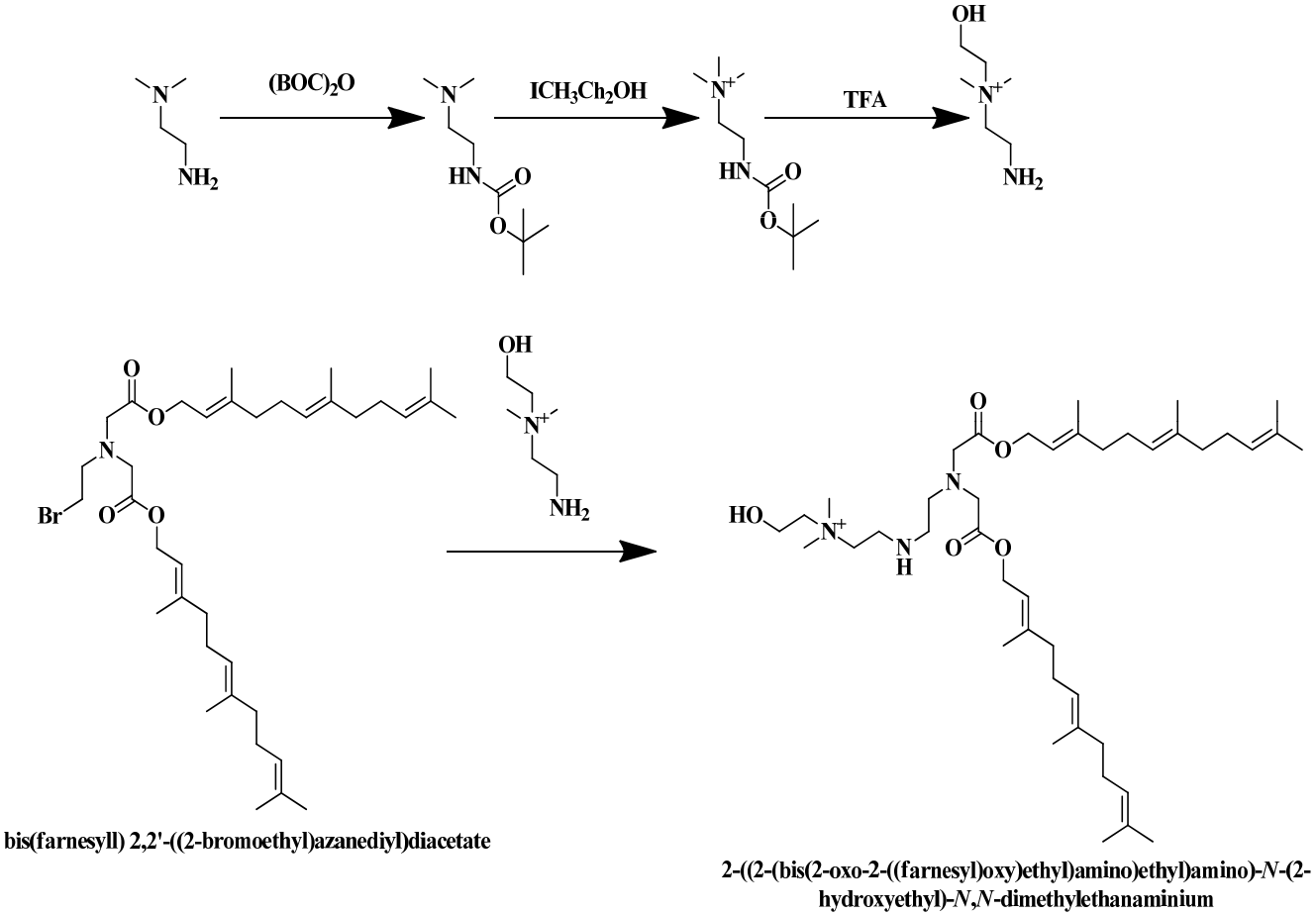
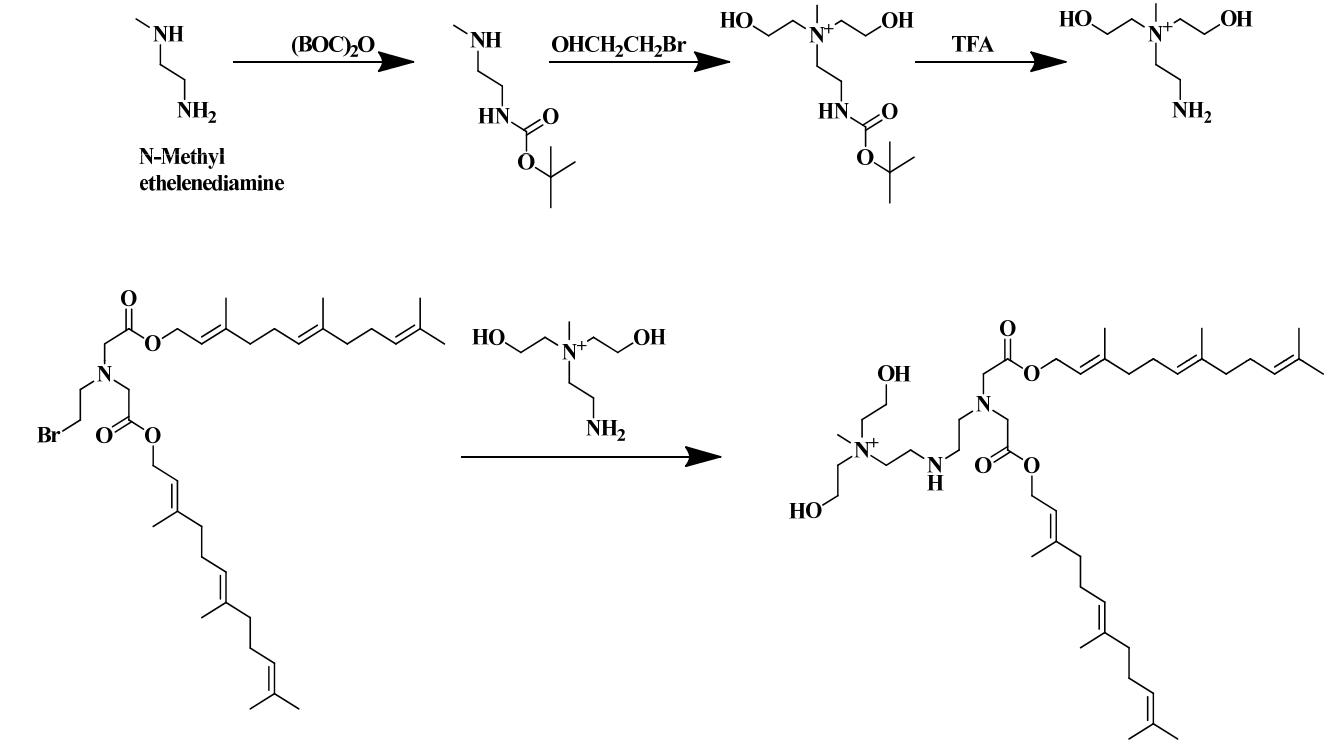

(BOC)2O
ICH3Ch2OH
TFA
bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate
2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-N-(2-hydroxyethyl)-N,N-dimethylethanaminium

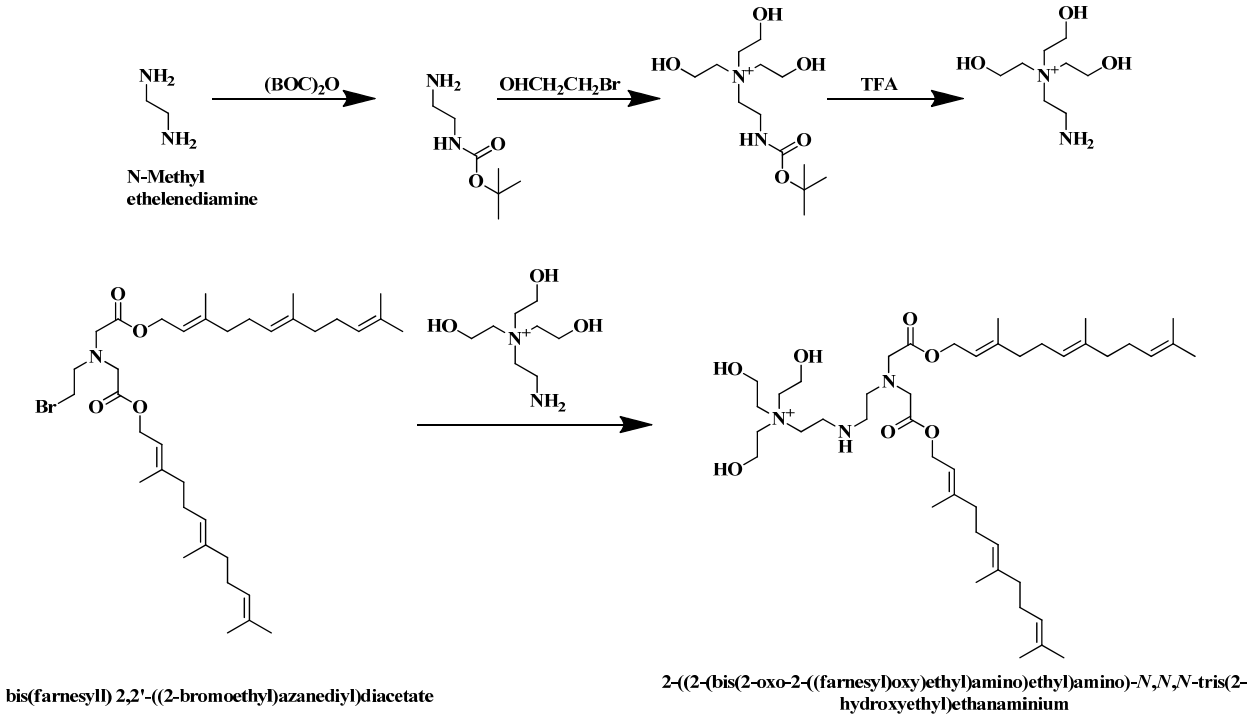
Figure 4



bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate

2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-*N*,*N*-bis(2-hydroxyethyl)-*N*-methylethanaminium

Figure 5



bis(farnesyll) 2,2'-((2-bromoethyl)azanediyl)diacetate

2-((2-(bis(2-oxo-2-((farnesyl)oxy)ethyl)amino)ethyl)amino)-*N*,*N*,*N*-tris(2-hydroxyethyl)ethanaminium

Figure 6