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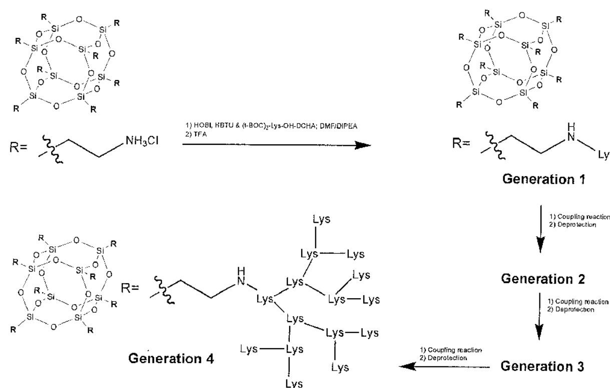

FIGURE 3

(57) **Abstract:** Described herein are dendrimers derived from polyhedral silsesquioxanes and methods of making/using thereof.

DENDRIMERS AND METHODS OF MAKING AND USING THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority upon U.S. provisional application Serial No. 60/822,671, filed August 17, 2006. This application is hereby incorporated by reference in its entirety for all of its teachings.

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BACKGROUND

For some time, silicon has been incorporated into materials to enhance their thermal and mechanical properties. Due to their chemical inertness and biocompatibility, silica-based materials are widely used in biomedical devices. In particular, polyhedral oligomeric silsesquioxane (POSS) cages are a popular silicon additive. Once considered a byproduct, POSS cages are now used in ceramics as well as creating highly porous materials. The applications of POSS cages have expanded to include surface catalysts, coatings on surgical instruments, and vascular stents.

Dendrimers grown from polyhedral oligomeric silsesquioxane cores have several advantages over traditional dendrimers with two-dimensional cores. In the case of cubic POSS, the POSS core has eight functional sites, which permits dendrimer branches to be grown divergently grown three-dimensionally, as opposed to two-dimensional cores, e.g. ethylene diamine, that have two nucleophilic amines. Dendrimers grown from cubic POSS cores exhibit a globular morphology at lower generations, which eliminates overcrowding of surface groups and improves the conjugation efficiency of additional generations. Conversely, the number of

imperfections increases at higher generations for two-dimensional dendrimers due to the sterically hindered surface.

Described herein are new dendrimers derived from polyhedral oligomeric silsesquioxane cores. The dendrimers exhibit a high conjugation efficiency due to
5 the rigidity and be further conjugated with a vast number of functionalities while still maintaining a small, discrete spherical morphology.

SUMMARY

Described herein are dendrimers derived from polyhedral silsesquioxanes and methods of making/using thereof. The advantages of the invention will be set
10 forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the aspects described below. The advantages described below will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed
15 description are exemplary and explanatory only and are not restrictive.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several aspects described below.

Figure 1 shows the structure of the cubic octa(3-aminopropyl)silsesquioxane
20 cage.

Figure 2 shows the Generation Four nanoscaffold of poly-L-lysine octa(3-aminopropyl)silsesquioxane dendrimer.

Figure 3 shows the synthetic scheme for the preparation of poly-L-lysine octa(3-aminopropyl)silsesquioxane dendrimers.

25 Figure 4 shows the ^{1}H-NMR spectra at 400 MHz of G_1 through G_4 dendrimers in D_2O at room temperature.

Figure 5 shows the ^{13}C-NMR spectra at 400 MHz of G_1 through G_4 in D_2O at room temperature.

Figure 6 shows the MALDI-TOF Mass Spectrum of G_1.

Figure 7 shows the MALDI-TOF Mass Spectrum of G_2.

Figure 8 shows the ESI Mass Spectrum of G_3.

Figure 9 shows the MALDI-TOF Mass Spectrum of G_4.

5 Figure 10 shows (A) the gel electrophoresis assay of the G_4 dendrimer and a plasmid DNA (the N/P ratios are labeled above each well) and (B) the TEM image of the nanoparticles formed by the G_4 dendrimers and a plasmid DNA (the scale bar indicates 50 nm).

Figure 11 shows the regular light images (left) and fluorescent (right) images 10 of MDA-MB-231 breast carcinoma cells acquired 4 hours after the incubation with G3-$[FITC]_2$-$[NH_2]_{62}$/DNA polyplexes at an N/P ratio of 40.

Figure 12 shows the *in vitro* transfection efficiency of the globular dendrimers at different N/P ratios with SuperFect (SF) as a control in MDA-MB-231 breast carcinoma cells.

15 Figure 13 shows confocal microscopic images showing cell uptake of the complexes of FITC labeled G3 lysine globular dendrimers and Cy3 labeled siRNA in MDA-MB-231 breast carcinoma cells.

Figure 14 shows the schematic synthetic procedure of Gd-DO3A L-lysine OAS dendrimer conjugates as MRI contrast agents.

20 Figure 15 shows the structure of a Generation 3 nanoglobule MRI contrast agent.

Figure 16 shows the size exclusion chromatography (SEC) spectrum of generations 1 to 3 nanoglobular MRI contrast agents; superose 12 column.

Figure 17 shows the 3D MIP MR images of the mice after intravenous 25 injection of generation 1-3 nanoglobule MRI contrast agents at a dose of 0.03 mmol Gd/kg body weight.

Figure 18 shows the 3D MIP MR images (top) and 2D axial tumor images (bottom) of mice bearing MDA-MB-231 xenografts after intravenous injection of generation 3 nanoglobule MRI contrast agents at a dose of 0.01 mmol Gd/kg body weight.

5 Figure 19 shows the MTT cytotoxicity assay of the nanoglobules of G_1-G_4, as well as the OAS core and poly-*L*-lysine (10 kDa) in MDA-MB-231 cells (N=3).

DETAILED DESCRIPTION

Before the present compounds, compositions, and/or methods are disclosed and described, it is to be understood that the aspects described below are not limited 10 to specific compounds, synthetic methods, or uses as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting.

In this specification and in the claims that follow, reference will be made to a number of terms that shall be defined to have the following meanings:

15 It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

"Optional" or "optionally" means that the subsequently described event or 20 circumstance can or cannot occur, and that the description includes instances where the event or circumstance occurs and instances where it does not. For example, the phrase "optionally substituted lower alkyl" means that the lower alkyl group can or cannot be substituted and that the description includes both unsubstituted lower alkyl and lower alkyl where there is substitution.

25 References in the specification and concluding claims to parts by weight, of a particular element or component in a composition or article, denotes the weight relationship between the element or component and any other elements or components in the composition or article for which a part by weight is expressed. Thus, in a compound containing 2 parts by weight of component X and 5 parts by 30 weight component Y, X and Y are present at a weight ratio of 2:5, and are present in

such ratio regardless of whether additional components are contained in the compound.

A weight percent of a component, unless specifically stated to the contrary, is based on the total weight of the formulation or composition in which the component is included.

A residue of a chemical species, as used in the specification and concluding claims, refers to the moiety that is the resulting product of the chemical species in a particular reaction scheme or subsequent formulation or chemical product, regardless of whether the moiety is actually obtained from the chemical species. For example, a polyhedral silsesquioxane that contains at least one –OH group can be represented by the formula Z-OH, where Z is the remainder (*i.e.*, residue) of the silsesquioxane.

The term “alkylene group” as used herein is a group having two or more CH_2 groups linked to one another. The alkylene group can be represented by the formula $-(CH_2)_n-$, where n is an integer of from 2 to 25.

The term “aromatic group” as used herein is any carbon-based aromatic group including, but not limited to, benzene, naphthalene, etc. The term “aromatic” also includes “heteroaryl group,” which is defined as an aromatic group that has at least one heteroatom incorporated within the ring of the aromatic group. Examples of heteroatoms include, but are not limited to, nitrogen, oxygen, sulfur, and phosphorus. The aryl group can be substituted or unsubstituted. The aryl group can be substituted with one or more groups including, but not limited to, alkyl, alkynyl, alkenyl, aryl, halide, nitro, amino, ester, ketone, aldehyde, hydroxy, carboxylic acid, or alkoxy.

The term “ether group” as used herein is a group having the formula $-[(CHU)_nO]_m-$, where U is hydrogen or a lower alkyl group, n is an integer of from 1 to 20, and m is an integer of from 1 to 100.

The term “thioether group” as used herein is a group having the formula $-[(CHU)_nS]_m-$, where U is hydrogen or a lower alkyl group, n is an integer of from 1 to 20, and m is an integer of from 1 to 100.

The term "imino group" as used herein is a group having the formula $-[(CHU)_nNU]_m$-, where each R is, independently, hydrogen or a lower alkyl group, n is an integer of from 1 to 20, and m is an integer of from 1 to 100.

The term "peptide group" as used herein is a group that is produced by the
5 reaction between a plurality of natural and/or non-natural amino acids.

Variables such as R, U, X, Y, n, m, p, q, and r used throughout the application are the same variables as previously defined unless stated to the contrary.

I. Dendrimers and Methods of Making Thereof

10 Described herein are dendrimers derived from a polyhedral silsesquioxane. Dendrimers have been studied extensively due to their unique structures and functional groups. Dendrimers are generally manufactured from a core material. Described herein, the core material used to produce the dendrimers is a "polyhedral silsesquioxane." The polyhedral silsesquioxane is reacted with a compound to
15 produce the first generation dendrimer. This compound is referred to herein as a "dendrimeric arm precursor." The first generation dendrimer has one or more "branches" attached to the core. A second generation dendrimer can be formed by reacting the same or different dendrimeric arm precursor with the branch of the first generation dendrimer. Similar reactions can be performed to produce third, fourth,
20 fifth generations, and so on.

In one aspect, the dendrimer is made by the method comprising reacting a linker-modified polyhedral silsesquioxane, wherein the linker comprises at least one reactive group, with a first dendrimeric arm precursor, wherein the first dendrimeric arm precursor comprises three or more functional groups, wherein at least one
25 functional group on the first dendrimeric arm precursor is capable of reacting with the reactive group on the linker to produce a first generation dendrimer.

In another aspect, described herein are dendrimers comprising a core comprising a polyhedral silsesquioxane, a plurality of linkers covalently attached to the polyhedral silsesquioxane, and a plurality dendrimeric arms covalently attached
30 to the linkers, wherein the dendrimeric arms comprise at least two functional groups.

The linker-modified polyhedral silsesquioxane is a polyhedral silsesquioxane that has a linker covalently attached to the silsesquioxane. Linker-modified polyhedral silsesquioxanes are easily prepared by the hydrolytic condensation of trifunctional organosilicon monomers. The techniques disclosed in U.S. Patent Nos. 5,047,492 and 6,927,270 as well as those disclosed in U.S. Published Application No. 2006/0040103, which are incorporated by reference, can be used to make linker-modified polyhedral silsesquioxanes useful herein. The linker-modified polyhedral silsesquioxanes disclosed in these patents and published application can also be used herein.

In one aspect, the linker-modified silsesquioxane comprises the formula $(RSiO_{1.5})_p$, wherein R comprises a residue of the linker and p is 6, 8, 10, or 12. In another aspect, the linker-modified silsesquioxane is $R_8Si_8O_{12}$, where R comprises a residue of the linker. This class of polyhedral silsesquioxanes is also referred to as a T_8 cage.

The linker of the linker-modified silsesquioxane can be a variety of different groups possessing one or more different reactive groups. In one aspect, the linker comprises an alkylene group, an ether group, an aromatic group, a peptide group, a thioether group, or an imino group comprising at least one reactive group. It is contemplated that the linker can be the same or different in the linker-modified polyhedral silsesquioxane.

The linker possesses one or more reactive groups. The reactive group is a group that is capable of forming a covalent bond when it reacts with the functional group present on the dendrimeric arm precursor. It is contemplated that the reactive groups can be the same or different on each linker. In one aspect, the reactive group can be an electrophilic group or a nucleophilic group. It is contemplated that the electrophilic group can be converted to a nucleophilic group, and vice-versa using techniques known in the art. In one aspect, the linker comprises one or more nucleophilic groups, wherein the nucleophilic group comprises a hydroxyl group, a thiol group, or a substituted or unsubstituted amine. In another aspect, the linker comprises one or more electrophilic groups. Examples of electrophilic groups

include, but are not limited to, a halogen, a carboxylic group, an ester group, an acyl halide group, a sulphonate group, or an ether group. In one aspect, the linker-modified polyhedral silsesquioxane has the formula $(RSiO_{1.5})_p$, wherein p is 6, 8, 10, or 12, R comprises $-(CH_2)_qY$, where q is from 1 to 10, and Y comprises an
5 electrophilic group, wherein the electrophilic group comprises a halogen (*e.g.*, Cl, Br, I, F), a carboxylic group (COOH), an ester group (*e.g.*, COOMe, COOEt), an acyl halide group (e.g., COCl), a sulphonate group (*e.g.*, p-toluenesulfonate group or p-bromobenzenesulfonate group), an electron-deficient aromatic group (e.g., $O-C_6H_4-NO_2$) or an ether group.

10 In one aspect, the linker-modified silsesquioxane is $R_8Si_8O_{12}$, where R comprises $-(CH_2)_rXH$, r is from 1 to 10, and X is O, S or NH. In another aspect, the linker-modified silsesquioxane is $R_8Si_8O_{12}$, where each R comprises $-(CH_2)_3NH_2$. Referring to Figure 1, this cubic nanoparticle has a siloxane core surrounded by a propyl amine functional group attached to each of the eight silicon atoms. The core
15 volume is approximately 6 $Å^3$, with a diameter of approximately 1 nm. These cubic nanoparticles are stable in various environments and exhibit high *in vivo* stability.

One or more functional groups present on the linker can be reacted with a first dendrimeric arm precursor to produce a first generation dendrimer. It is contemplated that the first dendrimeric arm precursor can be the same compound or
20 different compounds. The dendrimeric arm precursors used herein comprise at least three functional groups. The term "functional group" is defined as any group that is capable of forming a covalent bond with the reactive group present on the linker. The selection of the functional group can vary depending upon the identity of the reactive group on the linker. For example, if the reactive group is electrophilic, then
25 one of the functional groups present on the dendrimeric arm precursor is nucleophilic. In one aspect, the first dendrimeric arm precursor comprises three functional groups, wherein the functional group comprises one electrophilic group and two or more nucleophilic groups. In another aspect, the first dendrimeric arm precursor comprises three functional groups, wherein the functional group
30 comprises one nucleophilic group and two or more electrophilic groups.

In one aspect, the dendrimeric arm precursor comprises an alkylene compound, an ether compound, an aromatic compound, or a thioether compound comprising at least three functional groups. In one aspect, the first dendrimeric arm precursor comprises an amino acid, a derivative of an amino acid, or the
5 pharmaceutically acceptable salt or ester of the amino acid as defined below, wherein the amino acid comprises three functional groups. Several amino acids contain a carboxylic acid group (an electrophile) and two amino groups (a nucleophile). Examples of such natural amino acids include, lysine, tryptophan, serine, threonine, cysteine, tyrosine, asparagine, glutamine, 4-hydropxyproline,
10 aspartic acid, glutamic acid, or histidine. Non-natural amino acids are also contemplated. Derivatives of amino acids can include substitution of the amino group with, for example, an alkyl group. It is also contemplated that the first dendrimeric arm precursor comprises an amino acid, a derivative of an amino acid, or the pharmaceutically acceptable salt or ester of the amino acid as defined below,
15 wherein the amino acid comprises two functional groups. Examples of such amino acids include glycine and 3-aminopropionic acid.

The reaction between the linker-modified polyhedral silsesquioxane and the dendrimeric arm precursor can be conducted using techniques known in the art. The linker-modified polyhedral silsesquioxane can be neutral or used as the
20 corresponding salt. The selection of solvents and reaction parameters will vary depending upon the selection of starting materials. The amount of dendrimeric arm precursor used can also vary. In certain aspects, it is desirable to use an excess of dendrimeric arm precursor such that all of the reactive groups present on the linker react with the dendrimeric arm precursor.

25 In one aspect, a first generation dendrimer can be made by reacting a linker-modified silsesquioxane having the formula $R_8Si_8O_{12}$, where each R is $-(CH_2)_3NH_2$, with a first dendrimeric arm precursor comprising lysine. In this aspect, conjugation of the eight amines with a dendrimeric arm precursor results in a framework with eight equivalent groups, in relatively good yield, which may be further reacted with
30 additional functionalities. Referring to Figure 3, a first generation dendrimer is

produced by reacting a protected form of lysine with the ammonium salt of the linker-modified polyhedral silsesquioxane followed by deprotection with trifluoroacetic acid (TFA).

It is contemplated that the first generation dendrimers produced herein can be subsequently reacted with additional dendrimeric arm precursors to produce a high generation dendrimers. A high generation dendrimer is defined herein as a second generation or greater dendrimer. In one aspect, the high generation dendrimer comprises a second generation dendrimer to a generation 10 dendrimer. Figure 3 depicts one aspect described herein, wherein a generation two, three, and four dendrimer is produced. Using the techniques to produce the first generation dendrimer, subsequent coupling/deprotection with lysine as the dendrimeric arm precursor produces generation two-four dendrimers. The structure of the generation four dendrimer is depicted in Figure 2.

The advantages of using a dendrimeric arm precursor having at least three reactive groups can be seen in Figure 2. As shown in Figure 2, it is possible to produce highly branched dendrimers with multiple functional groups. If the dendrimeric arm precursor only had two functional groups, then it would only be possible to produce linear dendrimeric arms and not branched arms. This can significantly limit the use of the dendrimer, particularly if a high number of functional groups on the dendrimer are desired. As will be discussed below, the highly functionalized dendrimers described herein have numerous medical applications.

Any of the dendrimers described herein can exist or be converted to the salt thereof. In one aspect, the salt is a pharmaceutically acceptable salt. The salts can be prepared by treating the free acid with an appropriate amount of a chemically or pharmaceutically acceptable base. Representative chemically or pharmaceutically acceptable bases are ammonium hydroxide, sodium hydroxide, potassium hydroxide, lithium hydroxide, calcium hydroxide, magnesium hydroxide, ferrous hydroxide, zinc hydroxide, copper hydroxide, aluminum hydroxide, ferric hydroxide, isopropylamine, trimethylamine, diethylamine, triethylamine,

tripropylamine, ethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, lysine, arginine, histidine, and the like. In one aspect, the reaction is conducted in water, alone or in combination with an inert, water-miscible organic solvent, at a temperature of from about 0 °C to about 100 °C such as at room temperature. The
5 molar ratio of the compound to base used is chosen to provide the ratio desired for any particular salts. For preparing, for example, the ammonium salts of the free acid starting material, the starting material can be treated with approximately one equivalent of base to yield a salt.

In another aspect, any of the dendrimers described herein can exist or be
10 converted to the salt with a Lewis base thereof. The dendrimers can be treated with an appropriate amount of Lewis base. Representative Lewis bases are ammonium hydroxide, sodium hydroxide, potassium hydroxide, lithium hydroxide, calcium hydroxide, magnesium hydroxide, ferrous hydroxide, zinc hydroxide, copper hydroxide, aluminum hydroxide, ferric hydroxide, isopropylamine, trimethylamine,
15 diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, lysine, arginine, histidine, THF, ether, thiol reagent, alcohols, thiol ethers, carboxylates, phenolates, alkoxides, water, and the like. In one aspect, the reaction is conducted in water, alone or in combination with an inert, water-miscible organic solvent, at a temperature of from about 0 °C to about 100 °C
20 such as at room temperature. The molar ratio of the dendrimer to base used is chosen to provide the ratio desired for any particular complexes. For preparing, for example, the ammonium salts of the free acid starting material, the starting material can be treated with approximately one equivalent of chemically or pharmaceutically acceptable Lewis base to yield a complex.

25 If the dendrimers possess carboxylic acid groups, these groups can be converted to pharmaceutically acceptable esters using techniques known in the art. Alternatively, if an ester is present on the dendrimer, the ester can be converted to a pharmaceutically acceptable ester using transesterification techniques.

II. Methods of Use

The dendrimers described herein have numerous medical applications. In one aspect, the dendrimers described herein can be used to deliver pharmaceuticals to a subject. In another aspect, the dendrimers described herein can be used in gene 5 therapy to deliver genetic materials to cells and tissues. In a further aspect, the dendrimers can be used to deliver chemotherapeutics to a subject in need of such therapy. Any of the dendrimers described above (generation one and greater) can be used as a delivery device of bioactive agents. In one aspect, a bioactive agent can be complexed to the dendrimer. For example, the functional group present on the 10 dendrimeric arm can interact with the bioactive agent so that the dendrimer can be administered to a subject with the bioactive agent. The term "complexed" as defined herein includes any type of chemical or physical interaction between the dendrimer and the bioactive agent. For example, the interaction can involve the formation of covalent or non-covalent bonds (*e.g.*, electrostatic, hydrogen bonding, 15 dipole-dipole, ionic, and the like). The methods for delivering bioactive agents to a subject generally involve contacting the cells with the dendrimers described herein, wherein the bioactive agent is complexed to the dendrimer. The contacting step can be performed *in vitro*, *in vivo*, or *ex vivo* using techniques known in the art.

In one aspect, the bioactive agent comprises, a natural or synthetic 20 oligonucleotide, a natural or modified/blocked nucleotide/nucleoside, a nucleic acid, a peptide comprising natural or modified/blocked amino acid, an antibody or fragment thereof, a hapten, a biological ligand, a virus, a membrane protein, a lipid membrane, or a small pharmaceutical molecule.

In one aspect, the bioactive agent can be a protein. For example, the protein 25 can include peptides, fragments of proteins or peptides, membrane-bound proteins, or nuclear proteins. The protein can be of any length, and can include one or more amino acids or variants thereof. The protein(s) can be fragmented, such as by protease digestion, prior to analysis. A protein sample to be analyzed can also be subjected to fractionation or separation to reduce the complexity of the samples.

Fragmentation and fractionation can also be used together in the same assay. Such fragmentation and fractionation can simplify and extend the analysis of the proteins.

In one aspect, the bioactive agent is a virus. Examples of viruses include, but are not limited to, Herpes simplex virus type-1, Herpes simplex virus type-2, Cytomegalovirus, Epstein-Barr virus, Varicella-zoster virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Variola virus, Vesicular stomatitis virus, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, Rhinovirus, Coronavirus, Influenza virus A, Influenza virus B, Measles virus, Polyomavirus, Human Valley fever virus, West Nile virus, Rift Valley fever virus, Rotavirus A, Rotavirus B, Rotavirus C, Sindbis virus, Simian Immunodeficiency cirus, Human T-cell Leukemia virus type-1, Hantavirus, Rubella virus, Simian Immunodeficiency virus, Human Immunodeficiency virus type-1, Vaccinia virus, SARS virus, Human Immunodeficiency virus type-2, lentivirus, baculovirus, adeno-associated virus, or any strain or variant thereof.

In one aspect, the bioactive agent comprises a nucleic acid. The nucleic acid can be an oligonucleotide, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or peptide nucleic acid (PNA). The nucleic acid of interest introduced by the present method can be nucleic acid from any source, such as a nucleic acid obtained from cells in which it occurs in nature, recombinantly produced nucleic acid, or chemically synthesized nucleic acid. For example, the nucleic acid can be cDNA or genomic DNA or DNA synthesized to have the nucleotide sequence corresponding to that of naturally-occurring DNA. The nucleic acid can also be a mutated or altered form of nucleic acid (e.g., DNA that differs from a naturally occurring DNA by an alteration, deletion, substitution or addition of at least one nucleic acid residue) or nucleic acid that does not occur in nature.

In one aspect, the nucleic acid can be present in a vector such as an expression vector (e.g., a plasmid or viral-based vector). In another aspect, the nucleic acid selected can be introduced into cells in such a manner that it becomes integrated into genomic DNA and is expressed or remains extrachromosomal (i.e., is expressed episomally). In another aspect, the vector is a chromosomally integrated

vector. The nucleic acids useful herein can be linear or circular and can be of any size. In one aspect, the nucleic acid can be single or double stranded DNA or RNA.

In one aspect, the nucleic acid can be a functional nucleic acid. Functional nucleic acids are nucleic acid molecules that have a specific function, such as binding a target molecule or catalyzing a specific reaction. Functional nucleic acid molecules can be divided into the following categories, which are not meant to be limiting. For example, functional nucleic acids include antisense molecules, aptamers, ribozymes, triplex forming molecules, RNAi, siRNA, and external guide sequences. The functional nucleic acid molecules can act as affectors, inhibitors, modulators, and stimulators of a specific activity possessed by a target molecule, or the functional nucleic acid molecules can possess a de novo activity independent of any other molecules. Functional nucleic acids can be a small gene fragment that encodes dominant-acting synthetic genetic elements (SGEs), e.g., molecules that interfere with the function of genes from which they are derived (antagonists) or that are dominant constitutively active fragments (agonists) of such genes. SGEs can include, but are not limited to, polypeptides, inhibitory antisense RNA molecules, ribozymes, nucleic acid decoys, and small peptides. The small gene fragments and SGE libraries disclosed in U.S. Patent Publication No. 2003/0228601, which is incorporated by reference, can be used herein.

The functional nucleic acids of the present method can function to inhibit the function of an endogenous gene at the level of nucleic acids, e.g., by an antisense or decoy mechanism, or by encoding a polypeptide that is inhibitory through a mechanism of interference at the protein level, e.g., a dominant negative fragment of the native protein. Alternatively, certain functional nucleic acids can function to potentiate (including mimicking) the function of an endogenous gene by encoding a polypeptide, which retains at least a portion of the bioactivity of the corresponding endogenous gene, and may in particular instances be constitutively active.

Functional nucleic acid molecules can interact with any macromolecule, such as DNA, RNA, polypeptides, or carbohydrate chains. Often functional nucleic acids are designed to interact with other nucleic acids based on sequence homology between the target molecule and the functional nucleic acid molecule. In other situations, the specific recognition between the functional nucleic acid molecule and the target molecule is not based on sequence homology between the functional nucleic acid molecule and the target molecule, but rather is based on the formation of tertiary structure that allows specific recognition to take place.

In another aspect, a targeting agent is covalently attached to at least one dendrimeric arm. Examples of targeting agents include, but are not limited to, a protein, antibody, or peptide. The targeting agent can facilitate the delivery of a bioactive agent or an imaging agent into a cell. In one aspect, the targeting agent can be used to deliver a chemotherapeutic or nucleic acid into a cell.

In another aspect, an imaging agent can be complexed to the dendrimer (*e.g.*, one of the dendrimeric arms). The term "imaging agent" is defined herein as any agent or compound that increases or enhances the ability of cells or tissue to be imaged or viewed using techniques known in the art when compared to visualizing the cells or tissue without the imaging agent. The imaging agent can be covalently or non-covalently attached to the dendrimer.

In one aspect, at least one chelating agent is covalently attached to the dendrimeric arm. A chelating agent is any agent that can form non-covalent bond (*e.g.*, complexation, electrostatic, ionic, dipole-dipole, Lewis acid/base interaction) with the imaging agent. The chelating agent can possess a group that can react with one or more functional groups on the dendrimeric arm to form a covalent bond. For example, if the dendrimeric arm has an amino group, the amino group can react with a carboxylic group on the chelating agent to produce an amide bond.

A number of different chelating agents known in the art can be used herein. In one asepct, the chelating agent comprises an acyclic or cyclic compound comprising at least one heteroatom (*e.g.*, oxygen, nitrogen, sulfur, phosphorous) that has lone-pair electrons capable of coordinating with the imaging agent. An example of an acyclic chelating agent includes ethylenediamine. Examples of cyclic chelating agents include diethylenetriaminepentaacetate (DTPA) or its derivatives, 1,4,7,10-tetraazadodecanetetraacetate (DOTA) and its derivatives, 1,4,7,10-tetraazadodecane-1,4,7-triacetate (DO3A) and its derivatives, ethylenediaminetetraacetate (EDTA) and its derivatives, 1,4,7,10-tetraazacyclotridecanetetraacetic acid (TRITA) and its derivatives, 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA) and its derivatives, 1,4,7,10-tetraazadodecanetetramethylacetate (DOTMA) and its derivatives, 1,4,7,10-tetraazadodecane-1,4,7-trimethylacetate (DO3MA) and its derivatives, N,N',N",N"'-tetraphosphonatomethyl-1,4,7,10-tetraazacyclododecane (DOTP) and its derivatives, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylene methylphosphonic acid) (DOTMP) and its derivatives, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylene phenylphosphonic acid) (DOTPP) and its derivatives. The term "derivative" is defined herein as the corresponding salt and ester thereof of the chelating agent.

Imaging agents known in the art can be used herein. In one asepct, the imaging agent comprises a denoptical dye, a MRI contrast agent, a PET probe, a SPECT probe, a CT contrast agent, or an ultrasound contrast agent. In one aspect, imaging agents useful in magnetic resonance imaging include Gd^{+3}, Eu^{+3}, Tm^{+3}, Dy^{+3}, Yb^{+3}, Mn^{+2}, or Fe^{+3} ions or complexes. In another aspect, imaging agents useful in PET and SPECT imaging include ^{55}Co, ^{64}Cu, ^{67}Cu, ^{47}Sc, ^{66}Ga, ^{68}Ga, ^{90}Y, ^{97}Ru, ^{99m}Tc, ^{111}In, ^{109}Pd, ^{153}Sm, ^{177}Lu, ^{186}Re, ^{188}Re. The complexing of the imaging agent to the dendrimer having one or more chelating agents can be performed using routine techniques. For example, a salt of the imaging agent can be dissolved in a solvent and admixed with the dendrimer.

The dendrimers complexed with bioactive or imaging agents can be administered to a subject using techniques known in the art. For example, pharmaceutical compositions can be prepared with the dendrimers alone or complexed with bioactive/imaging agents. It will be appreciated that the actual preferred amounts of the dendrimer in a specified case will vary according to the specific compound being utilized, the particular compositions formulated, the mode of application, and the particular situs and subject being treated. Dosages for a given host can be determined using conventional considerations, e.g. by customary comparison of the differential activities of the subject compounds and of a known agent, e.g., by means of an appropriate conventional pharmacological protocol Physicians and formulators, skilled in the art of determining doses of pharmaceutical compounds, will have no problems determining dose according to standard recommendations (Physicians Desk Reference, Barnhart Publishing (1999).

Pharmaceutical compositions described herein can be formulated in any excipient the biological system or entity can tolerate. Examples of such excipients include, but are not limited to, water, saline, Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, vegetable oils such as olive oil and sesame oil, triglycerides, propylene glycol, polyethylene glycol, and injectable organic esters such as ethyl oleate can also be used. Other useful formulations include suspensions containing viscosity-enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer and Tris buffer, while examples of preservatives include thimerosol, cresols, formalin and benzyl alcohol.

Pharmaceutical carriers are known to those skilled in the art. These most typically would be standard carriers for administration to humans, including solutions such as sterile water, saline, and buffered solutions at physiological pH.

Molecules intended for pharmaceutical delivery can be formulated in a

pharmaceutical composition. Pharmaceutical compositions can include carriers, thickeners, diluents, buffers, preservatives, surface-active agents and the like in addition to the molecule of choice. Pharmaceutical compositions can also include one or more active ingredients such as antimicrobial agents, antiinflammatory
5 agents, anesthetics, and the like.

The pharmaceutical composition can be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration can be topically (including ophthalmically, vaginally, rectally, intranasally). In the case of contacting cells with the dendrimers described
10 herein, it is possible to contact the cells *in vivo* or *ex vivo*.

Preparations for administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles, if needed for collateral use of the disclosed
15 compositions and methods, include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles, if needed for collateral use of the disclosed compositions and methods, include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives can also be present such
20 as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

Formulations for topical administration can include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like can
25 be necessary or desirable.

Dosing is dependent on the severity and responsiveness of the condition to be treated, but will normally be one or more doses per day, with course of treatment lasting from several days to several months or until one of ordinary skill in the art determines the delivery should cease. Persons of ordinary skill can easily determine
30 optimum dosages, dosing methodologies and repetition rates.

The dendrimers described herein are effective in delivering bioactive agents into cells, which can ultimately be used to treat or prevent a number of different diseases. The term “treat” as used herein is defined as reducing the symptoms of the disease or maintaining the symptoms so that the symptoms of the disease do not become progressively worse. The term “treat” is also defined as the prevention of any symptoms associated with the particular disease. The term “effective amount” as used herein is the amount of dendrimer and bioactive agent sufficient to treat the disease in the subject upon administration to the subject. In one aspect, the dendrimers described herein can be used as carriers to deliver bioactive agents to treat or prevent cancer in a subject. Examples of different types of cancers include, but are not limited to, breast cancer, liver cancer, stomach cancer, colon cancer, pancreatic cancer, ovarian cancer, lung cancer, kidney cancer, prostate cancer, testicular cancer, glioblastoma, sarcoma, bone cancer, head-and-neck cancers, and skin cancer.

In other aspects, the dendrimers described herein can be used to image a cell or tissue in a subject. In one asepct, the method comprises (1) administering to the subject an imaging agent conjugated to a dendrimer described herein, and (2) detecting the imaging agent. The methods can be used to image healthy cells and tumor cells. A variety of different tissues and organs can be imaged using the methods described herein including, but not limited to, liver, spleen, heart, kidney, lung, esophagus, bone marrow, lymph node, lymph vessels, nervous system, brain, spinal cord, blood capillaries, stomach, ovaries, pancreas, small intestine, and large intestine. Techniques known in the art for detecting the imaging agent once incorporated into the cells or tissue are known in the art. For example, magnetic resonance imaging (MRI) can be used to detect the imaging agent. Additionally, the dendrimers described can be indispensable tools in a variety of other medical procedures, including, but not limited to, angiography, plethysmography, lymphography, mammography, cancer diagnosis, and functional and dynamic MRI.

In other aspects, dendrimers with pharmaceutical agents and imaging agents can be administered to a subject concurrently or sequentially. For example, a first

dendrimer with a pharmaceutical compound complexed to it can be admixed with a second dendrimer having an imaging agent complexed to it. The first and second dendrimer can be the same or different. It is also contemplated that a mixture of two or more different dendrimers with the same or different bioactive agent or imaging agent can be administered to the subject.

It is understood that any given particular aspect of the disclosed compositions and methods can be easily compared to the specific examples and embodiments disclosed herein, including the non- polysaccharide based reagents discussed in the Examples. By performing such a comparison, the relative efficacy of each particular embodiment can be easily determined. Particularly preferred compositions and methods are disclosed in the Examples herein, and it is understood that these compositions and methods, while not necessarily limiting, can be performed with any of the compositions and methods disclosed herein.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, and methods described and claimed herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.) but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric. There are numerous variations and combinations of reaction conditions, e.g., component concentrations, desired solvents, solvent mixtures, temperatures, pressures and other reaction ranges and conditions that can be used to optimize the product purity and yield obtained from the described process. Only reasonable and routine experimentation will be required to optimize such process conditions.

Materials and Methods. All reagents and solvents for dendrimer preparation were used without further purification. D_2O was purchased from Cambridge Isotope

Laboratories Inc. (Anclover, MA). Octa(3-aminopropyl)silsesquioxane HCl (OAS)-HCl was purchased from Hybrid Plastics (Hattiesburg, MS), 2-(1H-Benzotriazole-1yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 1-Hydroxy benzotriazole hydrate (HOBt), and N_α,N_ε-di-t-BOC-L-lysine
5 dicyclohexylammonium salt ((di-t-BOC)$_2$-Lys-OH•DCHA) were purchased from Nova Biochem (Darmstadt, Germany). N,N-diisopropylethyl amine (DIPEA) and N,N-dimethylformamide anhydrous (DMF) were purchased from Alfa Aesar (Ward Hill, MA). Citric Acid was purchased from J.T. Baker (Phillipsburg, NJ). Trifluoroacetic acid (TFA) was purchased from ACROS Organics (Morris Plains,
10 NJ).

^{1}H & ^{13}C-NMR spectra were acquired on a Varian INOVA 400 at 400 MHz at 25 °C. Electrospray ionization mass spectra were acquired on a Quattro-II mass spectrometer (Micromass, Inc.). Matrix-assisted laser desorption time of flight (MALDI-TOF) mass spectra were acquired on a Voyager DE-STR spectrometer
15 (PerSpeptive BioSystems) in linear mode with α-cyano-4-hydroxycinnamic acid as a matrix. Sample purification was performed using high pressure liquid chromatography (HPLC) on an Agilent 1100 equipped with a ZORBAX 300SB-C18 PrepHT column. The mobile phase was a mixture of H_2O (0.05% TFA) and acetonitrile (0.05% TFA).

20 **Preparation of Generation 1.** Referring to Figure 3, generation 1 (G_1) was first prepared by dissolving 0.70 grams of OAS-HCl (0.597 mmol, OAS, Figure 1), 7.60 grams 2-(1H-benzotriazole-1yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (20.0 mmol, HBTU), 2.70 grams 1-Hydroxy benzotriazole hydrate (20.0 mmol, HOBt), and N_α,N_ε-di-t-BOC-L-lysine dicyclohexylammonium
25 salt (20.0 mmol, (t-BOC)$_2$-Lys-OH•DCHA) in 30 ml of dimethylformamide (DMF). The reaction flask was stirred at room temperature for ca. 20 min. 10 ml of diisopropylethyl amine (63.1 mmol, DIPEA) was added to the reacting solution, and after ca. 20 min of stirring, the N_2 flush was discontinued and the solution was allowed to stir at room temperature for two days. The reaction was stopped by
30 precipitating out the solution in 0.5 M ice-cold citric acid. A white sticky precipitate

was present within the aqueous solution as well as adhering to the sides of the beaker; the aqueous solution turned slightly yellowish. The water was decanted, and the white precipitate was collected by dissolving in methanol, then concentrated under vacuum to a clear oil. The clear oil was dissolved in acetonitrile, after which a cloudy white precipitate was obtained. The cloudy white precipitate was collected by vacuum filtration, resulting in 1.29 grams (61.6 % yield) of $[(t\text{-BOC})_2\text{-L-lysine}]_8$-POSS as a solid white precipitate.

t-BOC protection was removed by dissolving 1.29 grams of $[(t\text{-BOC})_2\text{-L-lysine}]_8$-OAS in 5 ml of ice-cold trifluoroacetic acid (TFA) while stirring at room temperature for approximately one hour. The solution was concentrated under vacuum to a yellow viscous oil. The white crude product was precipitated out by the addition of ice-cold anhydrous diethyl ether, washed again with ether, dissolved in deionized H_2O (DI-H_2O) and lyophilized. 1.04 grams (47.1 % yield) of G_1 was present as a clear TFA salt. ^1H and ^{13}C-NMR, and MALDI-TOF spectra were acquired after HPLC purification using a ZORBAX 300SB-C18 PrepHT column (H_2O and acetonitrile both containing 0.5% TFA).

^1H NMR (400 MHz, D_2O, δ): 3.82 (t, 8H, -C$\underline{H}$), 3.40-2.98 (dm, 16H, -$Si(CH_2)_2C\underline{H}_2$), 2.84 (t, 16H, -$(CH_2)_3C\underline{H}_2$), 1.74 (m, 16H, -$CHC\underline{H}_2$), 1.58 (m, 16H, -$(CH_2)_2C\underline{H}_2CH_2$), 1.47 (m, 16H, -$SiCH_2C\underline{H}_2$), 1.31 (m, 16H, -$CH_2C\underline{H}_2(CH_2)_2$), 0.54 (t, 16H, -$SiC\underline{H}_2$); ^{13}C NMR (100 MHz, D_2O, δ): 169.4 (8C, $\underline{C}O$), 53.2 (8C, -$\underline{C}H$), 42.0 (8C, -$Si(CH_2)_2\underline{C}H_2$), 39.1 (8C, -$(CH_2)_3\underline{C}H_2$), 30.6 (8C, -$(CH\underline{C}H_2$), 26.5 (8C, -$CH_2)_2\underline{C}H_2CH_2$), 21.8 (8C, -$SiCH_2\underline{C}H_2$), 21.5 (m, 16H, -$CH_2\underline{C}H_2(CH_2)_2$), 8.39 (t, 16H, -$Si\underline{C}H_2$); MALDI-TOF (*m/z*): $[M+H]^+$ calcd. for $C_{72}H_{160}N_{24}O_{20}Si_8$, 1907.88; found 1907.04.

Preparation of Generation 2. Referring to Figure 3, generation 2 (G_2) was prepared similar to G_1. 0.497 grams (0.133 mmol) of G_1, 6.76 grams (17.8 mmol) HBTU, 2.40 grams (17.8 mmol) HOBt, and 9.40 grams (17.8 mmol) $(t\text{-BOC})_2$-Lys-OH•DCHA in 20.0 ml of DMF. The reaction flask was stirred at room temperature for ca. 20 min. 10 ml of (57.4 mmol) DIPEA was added to the reacting solution, and

after ca. 20 min of stirring, the N_2 flush was discontinued and the solution was allowed to stir at room temperature. The purification procedure was similar to that above, however, due to the increasing solubility of $[(t\text{-BOC})_2\text{-L-lysine}]_{16}$-OAS in acetonitrile, the crude product was purified by size exclusion chromatography (LH-
5 20, eluted with methanol) to yield a white precipitate, 0.794 grams (83.1 % yield).

t-BOC protection was removed by dissolving 0.794 grams of $[(t\text{-BOC})_2\text{-L-Lysine}]_{32}$-POSS in 3 ml of ice-cold TFA. 0.821 grams (80.1 % yield) of G_2 was present as a clear salt. ^{1}H and ^{13}C-NMR, and MALDI-TOF spectra were acquired after HPLC purification.

10 ^{1}H NMR (400 MHz, D_2O, δ): 4.12 (t, 8H, -C$\underline{H}$), 3.91 & 3.80 (t, 16H, -C$\underline{H}$), 3.20-2.91 (br 16H, -$(CH_2)_3C\underline{H}_2$, -$Si(CH_2)_2C\underline{H}_2$ 16H,), 2.90 (br t, 32H, -$(CH_2)_3C\underline{H}_2NH_2$), 1.84-1.68 (br m, 16H, -$CHC\underline{H}_2$ & 32H, -$CHC\underline{H}_2$), 1.66-1.48 (br m, 16H, -$(CH_2)_2C\underline{H}_2CH_2$ & 32H, -$(CH_2)_2C\underline{H}_2CH_2$), 1.48-1.18 (br m, 16H, -$SiCH_2C\underline{H}_2$, 16H, -$CH_2C\underline{H}_2(CH_2)_2$ & 32H, -$CH_2C\underline{H}_2(CH_2)_2$), 0.51 (br t, 16H, -$SiC\underline{H}_2$); ^{13}C NMR
15 (100 MHz, D_2O, δ): 173.2 (8C, $\underline{C}$O), 169.6-169.4 (16C, $\underline{C}$O), 54.3 (8C, -$\underline{C}$H), 53.2-52.9 (16C, -$\underline{C}$H), 42.0 (8C, -$Si(CH_2)_2\underline{C}H_2$), 39.5 (8C, -$(CH_2)_3\underline{C}H_2$), 39.1 (16C, -$(CH_2)_3\underline{C}H_2$), 31.1 (8C, -$(CH\underline{C}H_2)$), 30.5 (16C, -$(CH\underline{C}H_2)$), 28.1 (8C, -$(CH_2)_2\underline{C}H_2CH_2$), 26.5-26.4 (16C, -$(CH_2)_2\underline{C}H_2CH_2$), 22.8 (8C, -$CH_2\underline{C}H_2(CH_2)_2$), 21.9 (8C, -$SiCH_2\underline{C}H_2$), 21.5-21.4 (16C, -$CH_2\underline{C}H_2(CH_2)_2$), 8.39 (8C, -$Si\underline{C}H_2$);
20 MALDI-TOF (*m/z*): $[M+H]^+$ calcd. for $C_{168}H_{352}N_{56}O_{36}Si_8$ 3958.64; observed 3958.49.

Preparation of Generation 3. Referring to Figure 3, generation 3 (G_3) was prepared similar to G_2. 0.363 grams (0.0476 mmol) of G_2, 4.45 grams (11.7 mmol) HBTU, 1.58 grams (11.7 mmol) HOBt, and 6.17 grams (11.7 mmol) $(t\text{-BOC})_2$-Lys-
25 OH•DCHA in 12.0 ml of DMF. The reaction flask was stirred at room temperature for ca. 20 min. 4.0 ml of (23.0 mmol) DIPEA was added to the reacting solution, and after ca. 20 min of stirring, the N_2 flush was discontinued and the solution was allowed to stir at room temperature for three days. The purification procedure was similar to that above. The crude product was purified by size exclusion

chromatography (LH-20, eluted with methanol) to yield a white precipitate, 0.483 grams (70.0 % yield).

t-BOC protection was removed by dissolving 0.483 grams of $[(\text{t-BOC})_2\text{-L-lysine}]_{32}$-OAS in 4 ml of ice-cold TFA; 0.450 grams (61.3 % yield) of $(\text{L-Lysine})_{32}$-POSS was present as a clear salt. ^{1}H and ^{13}C-NMR, and MALDI-TOF spectra were acquired after HPLC purification.

^{1}H NMR (400 MHz, D_2O, δ): 4.25-4.08 (br, 8H, -C$\underline{H}$ & 16H, -C$\underline{H}$), 3.88 & 3.76 (br m, 32H, -C$\underline{H}$), 3.20-2.79 (br, 32H, -$(CH_2)_3C\underline{H}_2$, 16H, -$(CH_2)_3C\underline{H}_2$, 16H, -$Si(CH_2)_2C\underline{H}_2$, & 64H, -$(CH_2)_3C\underline{H}_2NH_2$), 1.95-1.10 (br, 16H, -$CHC\underline{H}_2$, 32H, -$CHC\underline{H}_2$, 64H, -$CHC\underline{H}_2$, 16H, -$(CH_2)_2C\underline{H}_2CH_2$, 32H, -$(CH_2)_2C\underline{H}_2CH_2$, 64H, -$(CH_2)_2C\underline{H}_2CH_2$, 16H, -$SiCH_2C\underline{H}_2$, 16H, -$CH_2C\underline{H}_2(CH_2)_2$, 32H, -$CH_2C\underline{H}_2(CH_2)_2$ & 64H, -$CH_2C\underline{H}_2(CH_2)_2$), 0.43 (t, 16H, -$SiC\underline{H}_2$); ^{13}C NMR (100 MHz, D_2O, δ): 174.0-173.3 (8C, $\underline{C}$O & 16, $\underline{C}$O), 169.7-169.4 (32C, $\underline{C}$O), 54.5-53.9 (8C, -$\underline{C}$H & 16C, -$\underline{C}$H), 53.3-52.8 (32C, -$\underline{C}$H) 42.7 (8C, -$Si(CH_2)_2\underline{C}H_2$), 41.8 (8C, -$(CH_2)_3\underline{C}H_2$), 39.4-39.1 (16C, -$(CH_2)_3\underline{C}H_2$ & 32C, -$(CH_2)_3\underline{C}H_2$) 31.0-30.1 (8C, -$CH\underline{C}H_2$, 16C, -$CH\underline{C}H_2$ & 32C, -$CH\underline{C}H_2$), 28.1-27.9 (8C, -$(CH_2)_2\underline{C}H_2CH_2$ & 16C, -$(CH_2)_2\underline{C}H_2CH_2$), 26.5 (32C, -$(CH_2)_2\underline{C}H_2CH_2$), 23.1-22.2 (8C, -$CH_2\underline{C}H_2(CH_2)_2$, 16C, -$CH_2\underline{C}H_2(CH_2)_2$ & 8C, -$SiCH_2\underline{C}H_2$), 21.5-21.3 (32C, -$CH_2\underline{C}H_2(CH_2)_2$), 8.39 (8C, -$Si\underline{C}H_2$); MALDI-TOF (*m/z*): $[M\text{-}H]^+$ calcd. for $C_{360}H_{736}N_{120}O_{68}Si_8$, 8057.97, observed 8057.80.

Preparation of Generation 4. Referring to Figures 2 and 3, generation 4 (G_4) was prepared similar to G_3. 0.176 grams (0.0114 mmol) of G_3, 2.12 grams (5.50 mmol) HBTU, 0.708 grams (5.60 mmol) HOBt, and 2.95 grams (5.60 mmol) $(\text{t-BOC})_2$-Lys-OH•DCHA in 10.0 ml of DMF. The reaction flask was stirred at room temperature for ca. 20 min. 4.0 ml of (23.0 mmol) DIPEA was added to the reacting solution, and after ca. 20 min of stirring, the N_2 flush was discontinued and the solution was allowed to stir at room temperature for 3 days. The purification procedure was similar to that above. The crude product was purified by size exclusion chromatography (LH-20, eluted with methanol) to yield a white precipitate, 0.250 grams (75.0 % yield).

t-BOC protection was removed by dissolving 0.250 grams of $[(t\text{-BOC})_2\text{-L-lysine}]_{64}$-OAS in 4 ml of ice-cold TFA; 0.249 grams (70.2 % yield) of G_4 was present as a clear salt. ^1H and ^{13}C-NMR, and MALDI-TOF spectra were acquired after HPLC purification. ^1H NMR (400 MHz, D_2O, δ): 4.21-4.02 (br, 8H, -C$\underline{H}$, 16H, -C$\underline{H}$ & 32H, -C$\underline{H}$), 3.90 & 3.78 (br 64H, -C$\underline{H}$), 3.15-2.76 (br, 64H, -$(CH_2)_3C\underline{H}_2$, 32H, -$(CH_2)_3C\underline{H}_2$, 16H, -$(CH_2)_3C\underline{H}_2$, 16H, -$Si(CH_2)_2C\underline{H}_2$, & 128H, -$(CH_2)_3C\underline{H}_2NH_2$), 1.83-1.00 (br, 16H, -$CHC\underline{H}_2$, 32H, -$CHC\underline{H}_2$, 64H, -$CHC\underline{H}_2$, 128H, -$CHC\underline{H}_2$, 16H, -$(CH_2)_2C\underline{H}_2CH_2$, 32H, -$(CH_2)_2C\underline{H}_2CH_2$, 64H, -$(CH_2)_2C\underline{H}_2CH_2$, 128H, -$(CH_2)_2C\underline{H}_2CH_2$, 16H, -$SiCH_2C\underline{H}_2$, 16H, -$CH_2C\underline{H}_2(CH_2)_2$, 32H, -$CH_2C\underline{H}_2(CH_2)_2$, 64H, -$CH_2C\underline{H}_2(CH_2)_2$ & 128H, -$CH_2C\underline{H}_2(CH_2)_2$), 0.43 (br, 16H, -$SiC\underline{H}_2$); ^{13}C NMR (100 MHz, D_2O, δ): 174.0-173.3 (8C, $\underline{C}O$, 16C, $\underline{C}O$ & 32C, $\underline{C}O$), 170.0-169.4 (64C, $\underline{C}O$), 54.5-52.9 (8C, -$\underline{C}H$, 16C, -$\underline{C}H$, 32C, -$\underline{C}H$ & 64C, -$\underline{C}H$), 40.1-38.5 (8C, -$(CH_2)_3\underline{C}H_2$, 16C, -$(CH_2)_3\underline{C}H_2$, 32C, -$(CH_2)_3\underline{C}H_2$ & 64C, -$(CH_2)_3\underline{C}H_2$), 31.0-29.6 (8C, -$CH\underline{C}H_2$, 16C, -$CH\underline{C}H_2$, 32C, -$CH\underline{C}H_2$ & 64C, -$CH\underline{C}H_2$), 29.3 (16C, -$(CH_2)_2\underline{C}H_2CH_2$), 28.0 (32C, -$(CH_2)_2\underline{C}H_2CH_2$ & 64C, -$(CH_2)_2\underline{C}H_2CH_2$), 23.6-22.2 (8C, -$CH_2\underline{C}H_2(CH_2)_2$, 16C, -$CH_2\underline{C}H_2(CH_2)_2$, 8C, -$SiCH_2\underline{C}H_2$, 32C, -$CH_2\underline{C}H_2(CH_2)_2$), 21.5 (64C, -$CH_2\underline{C}H_2(CH_2)_2$; MALDI-TOF (*m/z*): $[M]^+$ calcd. for $C_{744}H_{1504}N_{248}O_{152}Si_8$, 16262.18, observed 16314.96.

Transmission Electron Microscopy. G_4 (5 mg/ml) in 10 mM NaCl solution was added onto carbon mesh and allowed to dry overnight. The carbon mesh was further treated with uranyl tungstate for ca. 30 seconds. Conventional TEM micrographs of G_4 were obtained at 120 kV with a Philips Tecnai T12 Electron Microscope operating at a magnification of 595,000x or less.

Dendrimer Preparation. OAS was conjugated to L-lysine resulting in four novel, poly-L-lysine starburst dendrimers of generation one through four. The synthetic scheme is shown in Figure 3. These starburst dendrimers were first prepared by adding an excess of N_α, N_ε-di-t-BOC-L-lysine, HBTU, and HOBT in DMF to form the active ester N_α, N_ε-di-t-BOC-L-lysine-OBt, which then reacted with the eight corner amines of OAS-HCl. Diisopropylethyleneamine (DIPEA) was added to the solution to neutralize the hydrochloride salt of OAS during preparation of G_1, and

later added to neutralize the α- and ε- trifluoroacetic acid (TFA) amine salts on the surface of G_1, G_2, and G_3 during the preparation of higher generations. The hydrophobicity of the t-BOC protected dendrimers increased at higher generations, as was evident from its increasing solubility in anhydrous diethyl ether.

5 $[(t\text{-BOC})_2\text{-L-lysine}]_8$-OAS and $[(t\text{-BOC})_2\text{-L-lysine}]_{16}$-OAS were separated by precipitation in anhydrous diethyl ether; the product trickled out as a cloudy, white precipitate that eventually settled upon storing at a lower temperature. Precipitation of $[(t\text{-BOC})_2\text{-L-lysine}]_{32}$-OAS and $[(t\text{-BOC})_2\text{-L-lysine}]_{64}$-OAS in anhydrous diethyl ether resulted in much lower yields (ca. 10%) than $[(t\text{-BOC})_2$-L-
10 lysine$]_8$-OAS and $[(t\text{-BOC})_2\text{-L-lysine}]_{16}$-OAS. This could be attributed to the increased hydrophobicity of t-BOC protected G_3 and G_4, resulting in an increased solubility in organic solvents due to the greater number of t-BOC protection groups. Consequently, t-BOC protected G_3 and G_4 were purified using a LH-20 size exclusion chromatography eluted with methanol. The collected fractions resulted in
15 significantly greater yields (ca. 70%) with comparable purity to that of HPLC purification. t-BOC protection was removed by dissolving in excess of TFA. The product was precipitated out by dropping the clear, viscous oil in anhydrous diethyl ether, then dissolved in water and lyophilized to a clear salt.

G_3 and G_4 were prepared in much greater yields than G_1 and G_2, which most
20 likely was attributed to the separation of the crude product from the impurities by size exclusion chromatography. $[(t\text{-BOC})_2\text{-L-lysine}]_{32}$-OAS and $[(t\text{-BOC})_2$-L-lysine$]_{64}$-OAS are large enough to elute through the column at a faster rate than the impurities. The low yields for G_1 and G_2 were a result of t-BOC protected G_1 and G_2 being slightly soluble in anhydrous diethyl ether during the purification step. To
25 increase the yields of G_1 and G_2, $[(t\text{-BOC})_2\text{-L-lysine}]_8$-OAS and $[(t\text{-BOC})_2$-L-lysine$]_{16}$-OAS were passed through a LH-20 size exclusion column eluted with methanol, however, their yields were not as high as G_3 and G_4 due to their negligible size difference from the impurities.

The cationic and hygroscopic character of the dendrimers increased as the
30 number of surface amines increased from 16 (G_1) to 128 (G_4). Each generation was

prepared in relatively good yield with excellent purity by HPLC, as was evident by the ^{1}H and ^{13}C-NMR spectra, as well as mass spectrometry.

Dendrimer Characterization. Each generation was fully characterized by ^{1}H & ^{13}C-NMR in D_2O, and ESI or MALDI-TOF mass spectrometry after HPLC
5 purification. Some interesting trends were observed by ^{1}H-NMR as the dendrimer generation increased from one to four, Figure 4. The peak at ca. 0.54 ppm is characteristic of the methylene proton adjacent to the silicon atoms of the OAS core. This peak was used to identify the product during each purification step. Because a large excess of N_{α}, N_{ε}-di-t-BOC-L-lysine was added during each conjugation
10 reaction, separation of the product from N_{α}, N_{ε}-di-t-BOC-L-lysine was difficult due to the similar ^{1}H-NMR spectra. The position of this peak moved slightly upfield on the ^{1}H-NMR spectrum and became broader as the generation number increased. This was expected since the number of methylene protons adjacent to the silicon atoms remained constant upon repeated conjugations, as well as being buried within
15 the dendrimer core, which results in a lower signal to noise ratio. The methylene protons of the L-lysine side chain are represented by three multiplets at ca. 1.31, 1.58, and 1.77 ppm. These peaks became much broader at higher generations. The peak at ca. 1.47 ppm is the chemical shift value for the methylene protons of the OAS core, but became masked by the increasing methylene protons of the lysine
20 side chains of higher dendrimer generations. The triplet at ca. 2.88 ppm for G_1 represents the methylene protons adjacent to the ε-amine of the lysine side chain. This peak becomes much broader and larger for higher dendrimer generations.

An interesting observation was the presence of two multiplets at 3.00 and 3.13 ppm for G_1; integration resulted in a value of ca. 8 for each peak. These peaks
25 were assigned to the two methylene protons adjacent to the nitrogen atom of the OAS core. Although a single peak was expected for these two protons, because electron resonance exists between the oxygen-carbon and nitrogen-carbon of the peptide bond, the nitrogen orbitals exhibit a planar shape, therefore, the methylene protons may become partially fixed in space resulting in different chemical shifts.
30 Upon further addition of a second generation of L-lysine, the methylene protons

adjacent to the nitrogen atom of the OAS core shifted upfield to 3.00 ppm, while the methylene protons adjacent to the newly formed ε-amide bond of the first generation L-lysine were split in a similar fashion as the methylene protons of the OAS core. This phenomenon was observed for all the subsequent generations. In other words, 5 the methylene protons adjacent to the former peptide bond would no longer be observed as split peaks, but shifted upfield, while the methylene protons adjacent to the newly formed peptide bond would be represented by two peaks. Two-dimensional ^{1}H-NMR as well as pulsed-field-gradient heteronuclear multiple quantum correlation (gHMQC) NMR aided in clarifying the ^{1}H-NMR spectra.

10 The presence of these two peaks for the methylene protons adjacent to the newly formed peptide bond is indicative of a rigid dendrimer surface. Although these methylene protons of the lower L-lysine generations are no longer represented by two chemical shifts, it is proposed that the rigidity of the inner branches does not change after further conjugation of lysine, however the NMR instrument is 15 incapable of differentiating these methylene protons once they are buried within the dendrimer core. A ^{1}H-NMR with greater magnetic strength may be capable of differentiating the methylene protons within the dendrimer.

The peak at 3.83 ppm in the ^{1}H-NMR spectrum of G_1 was assigned to the α-carbon proton adjacent to the peptide bond. This peak moves downfield to ca. 4.15 20 ppm as a second generation of lysine is conjugated to the dendrimer surface. The α-carbon proton of the second generation of L-lysine is represented by two peaks, 3.80 and 3.91 ppm. Again, these two peaks are shifted downfield to ca. 4.14 and 4.09 ppm upon the third conjugation of L-lysine, while the α-carbon protons of the newly conjugated L-lysine have split chemical shift values of 3.80 and 3.91 ppm. This 25 trend is also observed after the fourth generation of L-lysine is conjugated to the outer surface; the α-carbon protons of the fourth lysine generation are represented by two peaks at 3.80 and 3.90 ppm, while the third generation α-carbon protons are shifted downfield to ca. 4.10 ppm.

^{13}C-NMR was also used to characterize G_1 through G_4. Figure 5 shows the 30 ^{13}C-NMR spectra of G_1 through G_4. The peak at ca. 8.39 ppm is representative of

the methylene carbon adjacent to the silicon atoms, which retains the same chemical shift from G_1 to G_2. However, this peak is faintly shown in the G_3 spectrum, and not observed in the G_4 spectrum. This observation is a result of a lower carbon signal to noise ratio, as well as the methylene carbon atoms becoming buried within the
5 dendrimer. This is analogous to the ^{1}H-NMR spectrum of G_4 (discussed above), in which the peak from the methylene protons adjacent to the silicon atoms become much smaller and broader. The methylene carbons of the L-lysine side chains positioned at 39.0, 30.6, 26.4, and 21.5 ppm, and similar to the ^{1}H-NMR spectra of G_1 through G_4, do not change chemical shifts but are represented by multiple peaks
10 positioned close to each other at higher generations. The inner methylene carbon of the OAS core has a chemical shift value of 26.4 ppm, but is not observed in the spectra of G_3 and G_4 due to the lower signal to noise ratio. The α-carbon of the first L-lysine generation is represented by a chemical shift value of 53.2 ppm; it then shifts downfield to ca. 54.2 ppm after the second L-lysine generation is conjugated
15 to the surface. The α-carbons on the newly conjugated L-lysine of G_2 are represented by two peaks, 53.2 and 52.8 ppm. This trend is similar to the α-carbon protons in the ^{1}H-NMR spectra; the chemical shift value of the α-carbon protons of the first generation move downfield, while the α-carbon protons of the newly conjugated lysine is represented by two peaks. The α-carbons of the surface L-lysine
20 branches of G_3 are shown as two peaks at ca. 53.3 and 52.8 ppm, while the α-carbons of G_1 and G_2 shift downfield to 54.4 and 53.9 ppm, respectively. Again, the α-carbons of the lysine branches of G_3 shift downfield to ca. 54.0 ppm, while α-carbons of the lysine branches of G_4 appear as two peaks at ca. 53.2 and 52.9 ppm. In the ^{13}C-NMR spectrum of G_1, the peak at 169 ppm was assigned to the carbonyl
25 carbon of the peptide bond, which is the most deshielded carbon. The carbonyl carbon of the first generation of lysine moves downfield to ca. 173 ppm upon further conjugation of L-lysine, which is represented by a chemical shift at ca. 169 ppm. This trend is repeated through G_4.

The ^{1}H and ^{13}C-NMR spectra of G_1 through G_4 showed similar trends: upon
30 further addition of L-lysine, the chemical shifts of the previous L-lysine generation

moved downfield, while the surface lysine branches had chemical shift values of the former generation. As the lysine generation number increased the peak height of earlier generations decreased, or disappeared. Another interesting observation discussed above was the presence of two peaks represented by the methylene
5 protons adjacent to the nitrogen of the OAS core. It was proposed that these protons are partially fixed in spaced due to the planarity of the amide bond. This hypothesis is supported by the ^{13}C-NMR spectra. These spectra show only a single peak for this specific carbon, while the ^{1}H-NMR spectra show two peaks. In other words, the methylene carbons are chemically equivalent, but the methylene protons are not.
10 This also supports the assumption that the dendrimer maintains a rigid structure, which may prove beneficial when conjugating additional moieties to the surface.

The OAS core is susceptible to hydrolysis. Hydrolysis of the core would result in a nonspherical dendrimer morphology. ^{29}Si-NMR has been used to identify cleavage of the oxygen-silicon bonds. Previous attempts to detect the ^{29}Si isotope
15 signal of G_1 through G_4 were unsuccessful, however, the ^{13}C-NMR spectra proved beneficial to ascertain the structural integrity of the OAS core. As discussed above, the methylene carbons adjacent to the silicon atoms of the OAS core were represented by a distinctive chemical shift value of ca. 8.39 ppm. Hydrolysis of the oxygen-silicon bond would place the adjacent methylene carbon in a different
20 chemical environment and, therefore, would be observed in the ^{13}C-NMR spectrum as a different chemical shift value. As shown in the ^{13}C-NMR spectra of G_1 through G_3, all of the methylene carbons adjacent to the silicon atoms are represented by one chemical shift value, 8.39 ppm. Therefore, we are certain the OAS core is not hydrolyzed during dendrimer preparation. Although this peak is not present in the
25 ^{13}C-NMR spectrum of G_4 due to the low signal to noise ratio, preparation and purification of the fourth generation was similar to the former generations.

G_1 through G_4 were further characterized by either electrospray ionization (ESI) or matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. The data are summarized in Table 1. Figure 6 shows the
30 MALDI-TOF mass spectrum of G_1. This spectrum has a single peak at 1907.05 m/z

and corresponds to the $[M+H]^+$ molecular ion (calcd. 1907.88 m/z). G_2 was also characterized by MALDI-TOF mass spectrometry, Figure 7, and shows three major peaks. The mass at 3958.49 m/z corresponds to the $[M+H]^+$ molecular ion (calcd. 3958.63 m/z). The masses at 3979.34 and 3996.31 m/z correspond to the $[M+Na-H]^+$ (calcd. 3979.60 m/z) and $[M+K]^+$ (calcd. 3996.72 m/z) molecular ions, respectively. ESI mass spectrometry was used to characterize G_3, Figure 8. The peak at 8057.8 m/z corresponds to the $[M-H]^+$ (calcd. 8057.97) molecular ion and, like the previous mass spectra, shows that a fully substituted, monodisperse G_3 was prepared. Figure 9 shows the MALDI-TOF mass spectrum of G_4. The major peak is ca. 16323.24 m/z (calcd. $[M+H]^+$ 16262.17) and is broader than the previous mass spectra of the earlier generations. This is expected due to the difficulty in preparing a completely monodispersed dendrimer of higher generations in the solution phase, as well as the possible difficulty of a high molecular weight dendrimer ionizing from the matrix. Although the major peak is greater than the calculated $[M]^+$ molecular ion, it is less than the mass of an additional L-lysine residue, therefore, we were confident that G_4 was prepared with relatively good monodispersity and in excellent purity.

Table 1. Dendrimer characteristics: theoretical M.w, observed M.w, difference between theoretical and observed M.w, number of surface amines, and percent yield.

Generation	Theoretical M.w.	Observed M.w.	M.w. Difference	No. of Surface Amines	% Yield
1	1906.87	1907.05	0.18	16	47.1
2	3957.61	3958.5	0.89	32	80.1
3	8058.97	8057.8	-1.17	64	61.3
4	16262.17	16314.96	52.79	128	70.2

From the NMR and mass spectrometry spectra, it is apparent that the spherical poly-L-lysine dendrimers were synthesized in excellent purity and have uniform molecular weight distribution; the observed molecular weight of G_4 was only 52.79 Da greater than the calculated molecular weight. In addition, the preparation of these novel nanoglobules required common conjugate chemistry and

instrumentation to achieve a purity suitable for further addition of L-lysine, or possibly other surface moieties. The rigidity of the dendrimer surface most likely contributed to the high conjugation efficiency. These novel, poly-L-lysine dendrimers would make excellent scaffolds for further addition of surface moieties,
5 such as, for example, paramagnetic chelating agents, i.e. DOTA, targeting moieties, i.e. RGD, or therapeutic drugs, i.e. doxorubicin. In addition, the rigid surface would improve interaction of the dendrimer surface moieties with the surrounding environment. Linear polymers are susceptible to folding, which may bury hydrophobic moieties. A rigid dendrimer surface, where every conjugate is
10 completely exposed to the outside, would result in greater interactions with the surrounding environment.

Plasmid DNA complexation and transfection with lysine-OAS dendrimers. The DNA complexation and *in vitro* transfection of lysine-OAS dendrimers were investigated with a plasmid DNA encoding luciferase. Generation 2 through 4
15 nanoglobules had a similar capacity to retard plasmid DNA. These molecules resulted in partial plasmid retardation at N/P ratios of approximately 0.5 and complete retardation at N/P ratios equal to or higher than 0.75 in gel electrophoresis assay. Figure 10(A) shows the DNA retardation of plasmid DNA with G_4 nanoglobules. G_1 dendrimers showed complete DNA retardation at an N/P ratio of
20 1.5, while the OAS core was incapable of retarding DNA at the N/P ratio of 1.5. In addition, at N/P ratios of 1.0 or higher, nanoglobules of generations 2 through 4 were capable of fully complexing DNA, which lead to the exclusion of ethidium bromide, and a complete loss of fluorescence bands. Figure 10(B) shows the transmission electron microscopic image of the complexes formed by the G_4
25 dendrimer and the plasmid DNA at an N/P ratio of 5 after they were incubated with plasmid DNA for 30 min in PBS. The particle size of the complexes was in the range of 60 to 80 nm in diameter.

Cellular uptake of DNA complexes with the FITC labeled G_3 globular dendrimer was preliminarily studied with MDA-MB-231 breast carcinoma cells.
30 Figure 11 shows that regular light and fluorescent images of the MDA-MB-231

breast carcinoma cells acquired 4 hours after the incubation with G3-$[FITC]_2$-$[NH_2]_{62}$/DNA polyplexes at an N/P ratio of 40. Green fluorescence was observed within the cells, which is indicative of cellular uptake of FITC labeled dendrimer/DNA complexes. The labeled dendrimer complexes were present in the early endosomal or lysosomal compartments (small, intensive circular spots) and in the cytoplasm. The results demonstrated that the compact globular dendrimers were efficient to facilitate cellular uptake of nucleic acids.

In vitro transfection efficiency of the globular dendrimers was studied in MDA-MB-231 cells using gWiz plasmid DNA encoding luciferase as a reporter gene and SuperFect (PAMAM dendrimers) as a control. The DNA complexes were incubated with cells for 48 hours. The transfection efficiency was determined by the standard luciferase assay. Figure 12 shows the transfection efficiency of the lysine globular dendrimers. The transfection efficiency of all globular dendrimers is much higher than that of SuperFect ($p < 0.05$). At N/P = 2 and 5, all globular dendrimers had comparable transfection efficiency. At N/P = 10, the G_4 dendrimers showed much higher transfection efficiency than other dendrimers ($p < 0.05$).

***In vitro* siRNA delivery with lysine-OAS dendrimers.** The siRNA delivery efficiency of globular lysine-OAS dendrimers G_1 through G_4 was preliminarily evaluated with luciferase expressing U373 cells using an anti-luciferase siRNA. Linear poly-*L*-lysine (Mw 10,000), which was studied as a carrier for siRNA delivery, and the OAS and polylysine core were used as controls. The cells were incubated with the dendrimer/siRNA polyplexes at N/P ratios of 10, 20, 30, and 40 for 4 hours, after which the medium was removed, replaced with fresh medium and incubated for additional 48 hours. Figure 13 shows the gene silencing efficiency of the globular dendrimers and controls. The luciferase expression efficiency is shown as the percentage of the expression with the dendrimers or polylysine relative to the average expression with the OAS core. The gene silencing efficiency of the dendrimers increased with increasing the size of the dendrimers. G_4 dendrimers showed the highest gene silencing efficiency among the dendrimers with silencing efficiency up to 50%. Low gene transfection efficiency was observed for polylysine

because of its cytotoxicity. The examination of cell viability with microscopy showed that the cells incubated with polylysine/siRNA complexes had very low viability. Their viability decreased with increasing the N/P ratio of PLL/siRNA complexes. No change of cell viability was observed for the cells incubated with the
5 core and dendrimers at all N/P ratios. The luciferase silencing efficiency mediated by the dendrimer/siRNA complexes was not greatly affected by their N/P ratios in the tested range.

The particle formation of the lysine globular dendrimers with siRNA was investigated using dynamic light scattering. An N/P of 10 was selected to study the
10 particle formation. The OAS core, polylysine (10 KDa), PAMAM-G_5 (128 surface amino groups, 28,8826 Da) and PAMAM-G_6 (256 surface amino groups, 58,048 Da) dendrimers were used as controls. The lysine dendrimers G_1-G_4 and controls were dissolved in Millipore 2x filtered water at 1.0 nM of positive charges. siRNA (0.5 μg) was added in RNase free water, and then complexed with the lysine
15 dendrimers G_1-G_4 and the controls at an N/P ratio of 10 for 30 min at room temperature. The polyplex solutions were diluted to a volume of 0.5 ml 2x filtered Millipore water. The sizes of siRNA complexes were measured by dynamic light scattering for 2 min. The sizes of the siRNA complexes with OAS, globular lysine dendrimers and PAMAM dendrimers are listed in Table 2. As shown in the Table 2,
20 globular lysine dendrimers G_2-G_4 formed smaller nanoparticles (~50 nm) than the OAS core, globular lysine dendrimer G_1 and PAMAM G_5 and G_6 dendrimers. The result suggests that even though the PAMAM dendrimers have larger molecular weights and more cationic charges than the globular lysine dendrimers G_2-G_4, the latter are more effective to form stable condensed complexes with siRNA than the
25 PAMAM dendrimers. The size of the complexes did not change in 24 hours in the solution.

Table 2. Particle sizes of the siRNA complexes.

siRNA complex	OAS	Globular lysine dendrimers				PAMAM	
		G_1	G_2	G_3	G_4	G_5	G_6
Size (nm)	1073	165	50	52	49	109	59

Cellular uptake of the siRNA complexes with the globular dendrimers was evaluated by confocal microscopy in MDA-MB-231 breast carcinoma cells. The complexes of Cy3 labeled siRNA with FITC labeled lysine globular dendrimers (N/P = 10) were incubated with the cells for 2 hours. The cells were fixed with 2% paraldehyde in PBS at 37 °C for 50 min. Cellular uptake was observed on a dual channel FV1000 Olympus IX81 confocal microscope. A Z-series image acquisition was performed to achieve the correct cell slice and to ensure the observed complexes were located within the cell and not attached to the cell surface. Figure 13 shows the representative confocal images of the cell uptake of the siRNA complexes with the G_3 dendrimer, indicating that both the carrier and siRNA accumulated in the endosomal-lysosomal compartments. The result suggested that G_3 globular lysine dendrimer formed stable complexes with siRNA and intact complexes were taken by the cells. The globular dendrimers are able to prevent siRNA from enzymatic degradation and to effectively deliver siRNA into target cells.

Synthesis and Characterization of [Gd-DO3A]-Poly-L-Lysine OAS Dendrimers as MRI Contrast Agents. Generation 1-3 [Gd-DO3A]-Poly-L-lysine OAS MRI contrast agents were synthesized using standard liquid phase peptide synthesis. The synthetic procedure is shown in Figure 14 and described as follows: $(\text{L-lysine})_8$-OAS (G_1) trifluoroacetate (0.0185 mmol), HBTU (1.5 mmol), HOBt (1.5 mmol), and 1,4,7,10-Tetraazacyclododecane-1,4,7-tris-tert-butyl acetate-10-acetic acid (DOTA-tris(t-bu)) (0.52 mmol; Macrocyclics) were dissolved in DMF and stirred at room temperature for 20 min. Diisopropylethylamine (4.0 mmol) was added to the solution and was stirred at room temperature for two days. The reaction solution was concentrated under vacuum, dissolved in methanol, then precipitated out in anhydrous diethyl ether. The protective t-butyl groups were removed by dissolving the crude product in neat trifluoracetic acid for 18 hours at room temperature. The solution was then concentrated under vacuum to a viscous oil. The residue was treated with ice-cold diethyl ether to give a colorless solid product. The crude

[DO3A]-poly-L-lysine OAS generation 1 dendrimers were further purified by HPLC. Yield of pure [DO3A]-poly-L-lysine OAS generation 1 dendrimers was 35.0 %. Complexation of Gd^{3+} to [DO3A]-poly-L-lysine OAS generation 1 conjugates was achieved by dissolving both [DO3A]-nanogobular conjugates
5 (0.0065 mmol) and $Gd(OAc)_3$ (0.30 mmol) in water, and stirring for 2 days at room temperature. Free Gd^{3+} ions were removed by the addition of EDTA, as well as passing the reaction solution through a PD-10 desalting column and lyophilization. Pure [Gd-DO3A]-Poly-L-lysine OAS MRI contrast agents were obtained after HPLC purification: yield 15.1%. Generation 2 (15.5% yield) and generation 3
10 (18.8%) [Gd-DO3A]-Poly-L-lysine OAS MRI contrast agents were synthesized according to a similar procedure (Figure 15).

The Gd^{3+} content was measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES) and from these data the number of [Gd-DO3A] chelates conjugated to the nanoglobular surface, as well as molecular weight was
15 approximated; T_1 relaxivity (r_1) of the conjugate was determined at 3T; nanoglobular MRI contrast agents' apparent molecular weight was measured by size exclusion chromatography (SEC) using a superose 12 column, calibrated by 2-hydroxypropropyl methacrylate (HPMA); and the particle size was measured by dynamic light scattering (DLS) in water. The physicochemical properties are
20 summarized in Table 3.

Table 3. Gd^{3+} content measured by ICP-OES, T_1 relaxivity measured at 3T, MW obtained by ICP-OES, apparent MW measured by SEC, particle size measured by DLS and percent step yield.

MRI Conjugate	Gd^{3+} Content (mmols Gd/g polymer)	r_1 ($mM^{-1}sec^{-1}$)	Mw ICP-OES (kDa)	Apparent Mw SEC (kDa)	Particle Size (nm)	% Step Yield
Gen 1	0.97	6.42	7.3	7.7	~2.0	15.1
Gen 2	0.93	7.18	14.8	13.2	~2.4	15.5
Gen 3	1.01	10.05	34.7	27.0	~3.2	18.8

5 The agents were prepared with approximately 60-76% conjugation efficiency of [Gd-DO3A] onto the poly-L-lysine dendrimer surface. The T1 relaxivity and particle size increased with generation size. In addition, the SEC spectrum in Figure 16 shows that the agents where prepared with excellent purity, and that the hydrodynamic volume of the nanoglobular MRI contrast agents 10 increases with dendrimer generation.

***In Vivo* MR Imaging.** *In vivo* MR imaging was performed in female nu/nu athymic mice bearing MDA-MB-231 human breast carcinoma xenografts. Groups of three mice (n=3) each were used for each conjugate and the mice were anesthetized with an I.P. injection of a mixture of 2 mg/kg xylazine and 80 mg/kg ketamine. 15 Generation 1-3 nanoglobule MRI contrast agents were administered via a tail vein at a dose of 0.03 mmol Gd/kg body weight. The mice were placed in a human wrist coil and scanned in a Siemens Trio 3T MRI scanner at pre-injection and at 2, 5, 10, 15, 30, 60, and 120 min post-injection using a fat suppression 3D FLASH sequence (TR=7.8 ms, TE=2.74 ms, 25° flip angle, 0.5 mm slice thickness). Figure 17 shows 20 the 3D maximum intensity projection images (3D MIP).

Generation 1 and 2 nanoglobule MRI contrast agents showed a similar contrast enhancement profile on MR images. These agents were excreted via renal filtration and accumulated within the bladder at approximately 10-min post-injection as shown by MRI. The generation 3 nanoglobular MRI contrast agent showed

significantly greater blood pool enhancement at 2-min post-injection, and up to 60-min post-injection. In addition, this agent was eventually excreted via renal filtration and showed accumulation within the bladder at approximately 15-min post-injection. All agents showed slight enhancement within the liver, which is
5 indicative of minimal liver up-take often observed with high molecular weight MRI contrast agents. Generation 3 nanoglobular MRI contrast agents most likely provided greater contrast enhancement as compared to the lower nanoglobular generations due to its higher relaxivity, as well as its larger hydrodynamic volume.

Figure 18 shows the representative 3D MIP and axial 2D spin-echo images
10 enhanced with the G_3 nanoglobular contrast agent at a dose of 0.01 mmol-Gd/kg, $1/10^{th}$ of the regular clinical dose. The agent resulted in a significant contrast enhancement in the blood pool for at least 60 minutes at such a low dose. The contrast enhancement gradually decreased over time in the blood pool. At the same time, contrast enhancement in the bladder increased gradually, indicating that the G3
15 nanoglobular contrast agent gradually excreted via renal filtration and accumulated in the bladder. At two hours post-injection, the contrast enhancement in the body returned to the pre-injection level except for the bladder, which had strong enhancement.

Cytotoxicity. Cytotoxicity of the dendrimers and the octa(3-
20 aminopropyl)silsesquioxane core was evaluated with MTT assay by incubation with MDA-MB-231 human breast carcinoma cells with poly-*L*-lysine (10 kDa) as a control. Figure 19 shows the concentration dependent cell viability of the OAS core, nanoglobule dendrimers and poly-*L*-lysine. The dendrimers and the core exhibited much lower cytotoxicity than poly-*L*-lysine. The cytotoxicity of the
25 dendrimers gradually increases with their size. The IC_{50} values for G_3 and G_4 dendrimers were 134.8 μg/ml and 97.9 μg/ml, respectively, which is much higher than that of poly-*L*-lysine (12.0 μg/ml). IC_{50} values for the OAS core, G_1, and G_2 could not be calculated because the highest tested concentration (200 μg/ml) did not result in 50% inhibition of cell growth. The results indicate that these nanoglobules
30 have low toxicity for biomedical applications.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the compounds, compositions and methods described herein.

5 Various modifications and variations can be made to the compounds, compositions and methods described herein. Other aspects of the compounds, compositions and methods described herein will be apparent from consideration of the specification and practice of the compounds, compositions and methods disclosed herein. It is intended that the specification and examples be considered as
10 exemplary.

What is claimed:

1. A dendrimer made by the method comprising reacting a linker-modified polyhedral silsesquioxane, wherein the linker comprises at least one reactive group, with a first dendrimeric arm precursor, wherein the first dendrimeric arm precursor comprises three or more functional groups, wherein at least one functional group on the first dendrimeric arm precursor is capable of reacting with the reactive group on the linker to produce a first generation dendrimer.

2. The dendrimer of claim 1, wherein the linker comprises an alkylene group, an ether group, an aromatic group, a peptide group, a thioether group, or an imino group.

3. The dendrimer of claim 1, wherein the reactive group comprises one or more nucleophilic groups, wherein the nucleophilic group comprises a hydroxyl group, a thiol group, or a substituted or unsubstituted amine.

4. The dendrimer of claim 1, wherein the reactive group comprises one or more electrophilic groups.

5. The dendrimer of claim 4, wherein the electrophilic group comprises a halogen, a carboxylic group, an ester group, an acyl halide group, a sulphonate group, or an ether group.

6. The dendrimer of claim 1, wherein the linker-modified silsesquioxane comprises the formula $(RSiO_{1.5})_p$, wherein R comprises a residue of the linker and p is 6, 8, 10, or 12.

7. The dendrimer of claim 1, wherein the linker-modified silsesquioxane is $R_8Si_8O_{12}$, where R comprises a residue of the linker.

8. The dendrimer of claim 7, wherein R comprises $-(CH_2)_qY$, where q is from 1 to 10, and Y comprises an electrophilic group, wherein the electrophilic group comprises a halogen, a carboxylic group, an ester group, an acyl halide group, a sulphonate group, or an ether group.

9. The dendrimer of claim 7, wherein R comprises $-(CH_2)_rXH$, where r is from 1 to 10, and X is O, S or NH.

10. The dendrimer of claim 1, wherein the linker-modified silsesquioxane is $R_8Si_8O_{12}$, where each R is $-(CH_2)_3NH_2$.

11. The dendrimer of claim 1, wherein the first dendrimeric arm precursor comprises three functional groups, wherein the functional group comprises one electrophilic group and two or more nucleophilic groups.

12. The dendrimer of claim 1, wherein the first dendrimeric arm precursor comprises three functional groups, wherein the functional group comprises one nucleophilic group and two or more electrophilic groups.

13. The dendrimer of claim 1, wherein the first dendrimeric arm precursor comprises an amino acid, a derivative of an amino acid, or the pharmaceutically acceptable salt or ester of the amino acid, wherein the amino acid comprises three functional groups.

14. The dendrimer of claim 13, wherein the amino acid comprises glycine, 3-aminopropionic acid, tryptophan, serine, threonine, cysteine, tyrosine, asparagine, glutamine, 4-hydropxyproline, aspartic acid, glutamic acid, or histidine.

15. The dendrimer of claim 1, wherein the first dendrimeric arm precursor comprises lysine, a derivative of lysine, or the pharmaceutically acceptable salt or ester of lysine.

16. The dendrimer of claim 1, wherein the amount of the first dendrimeric arm precursor is sufficient to react with every reactive group on each linker.

17. The dendrimer of claim 1, wherein the linker-modified silsesquioxane is $R_8Si_8O_{12}$, where each R is $-(CH_2)_3NH_2$, and the first dendrimeric arm precursor comprises lysine.

18. The dendrimer of claim 1, wherein the first generation dendrimer is subsequently reacted with additional dendrimeric arm precursor to produce a high generation dendrimer.

19. The dendrimer of claim 18, wherein the high generation dendrimer comprises a second generation dendrimer to a generation 10 dendrimer.

20. The dendrimer of claim 1, further comprising complexing a bioactive agent to the dendrimer.

21. The dendrimer of claim 20, wherein the bioactive agent comprises, a natural or synthetic oligonucleotide, a natural or modified/blocked nucleotide/nucleoside, a nucleic acid, a peptide comprising natural or modified/blocked amino acid, an antibody or fragment thereof, a virus, a hapten, a biological ligand, a membrane protein, a lipid membrane, or a small pharmaceutical molecule.

22. The dendrimer of claim 20, wherein the bioactive agent comprises DNA or a fragment thereof.

23. The dendrimer of claim 20, wherein the bioactive agent comprises RNA or a fragment thereof.

24. The dendrimer of claim 1, further comprising a targeting agent covalently attached to at least one dendrimeric arm.

25. The dendrimer of claim 24, wherein the targeting agent comprises a protein, antibody, or peptide for the delivery of a bioactive agent or an imaging agent.

26. The dendrimer of claim 25, wherein the bioactive agent comprises a chemotherapeutic or nucleic acid.

27. The dendrimer of claim 1, further comprising complexing an imaging agent to the dendrimer.

28. The dendrimer of claim 27, wherein the imaging agent comprises a denoptical dye, a MRI contrast agent, a PET probe, a SPECT probe, a CT contrast agent, or an ultrasound contrast agent.

29. A dendrimer comprising a core comprising a polyhedral silsesquioxane, a plurality of linkers covalently attached to the polyhedral silsesquioxane, and a plurality dendrimeric arms covalently attached to the linkers, wherein the dendrimeric arms comprise at least two functional groups.

30. The dendrimer of claim 29, wherein a bioactive agent or an imaging agent is complexed to at least one dendrimeric arm.

31. The dendrimer of claim 29, wherein at least one chelating agent is covalently attached to at least one dendrimeric arm, and the chelating agent complexes with an imaging agent.

32. The dendrimer of claim 31, wherein the chelating agent comprises a cyclic or acyclic compound comprising at least one heteroatom.

33. The dendrimer of claim 31, wherein the chelating agent comprises diethylenetriaminepentaacetate (DTPA) or its derivatives, 1,4,7,10-tetraazadodecanetetraacetate (DOTA) and its derivatives, 1,4,7,10-tetraazadodecane-1,4,7-triacetate (DO3A) and its derivatives, ethylenediaminetetraacetate (EDTA) and its derivatives, 1,4,7,10-tetraazacyclotridecanetetraacetic acid (TRITA) and its derivatives, 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA) and its derivatives, 1,4,7,10-tetraazadodecanetetramethylacetate (DOTMA) and its derivatives, 1,4,7,10-tetraazadodecane-1,4,7-trimethylacetate (DO3MA) and its derivatives, N,N',N",N"'-tetraphosphonatomethyl-1,4,7,10-tetraazacyclododecane (DOTP) and its derivatives, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylene methylphosphonic acid) (DOTMP) and its derivatives, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylene phenylphosphonic acid) (DOTPP) and its derivatives.

34. The dendrimer of claim 31, wherein the imaging agent comprises a denoptical dye, a MRI contrast agent, a PET probe, a SPECT probe, a CT contrast agent, or an ultrasound contrast agent.

35. The dendrimer of claim 31, wherein the MRI contrast agent comprises a Gd^{+3}, Eu^{+3}, Tm^{+3}, Dy^{+3}, Yb^{+3}, Mn^{+2}, or Fe^{+3} ion or complex.

36. The dendrimer of claim 31, wherein the PET and SPECT probe comprises ^{55}Co, ^{64}Cu, ^{67}Cu, ^{47}Sc, ^{66}Ga, ^{68}Ga, ^{90}Y, ^{97}Ru, ^{99m}Tc, ^{111}In, ^{109}Pd, ^{153}Sm, ^{177}Lu, ^{186}Re, or ^{188}Re.

37. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the dendrimer of claims 1-36.

38. A method for delivering a bioactive agent to a cell, comprising contacting the cell with the bioactive agent complexed or conjugated to the dendrimer of claims 1-36.

39. The method of claim 38, wherein the contacting step is *in vitro*, *in vivo*, or *ex vivo*.

40. A method for treating a subject with cancer comprising administering to the subject an effective amount of bioactive agent complexed or conjugated to the dendrimer of claims 1-36.

41. The method of claim 40, wherein the cancer comprises breast cancer, liver cancer, stomach cancer, colon cancer, pancreatic cancer, ovarian cancer, lung cancer, kidney cancer, prostate cancer, testicular cancer, glioblastoma, sarcoma, bone cancer, head-and-neck cancers, and skin cancer.

42. The method of claim 40, wherein the bioactive agent comprises a nucleic acid or a pharmaceutical molecule.

43. A method for imaging a cell or tissue in a subject comprising (1) administering to the subject an imaging agent complexed to the dendrimer of claims 1-36, and (2) detecting the imaging agent.

44. A method for imaging a cell or tissue in a subject comprising (1) contacting the cell or tissue with an imaging agent complexed to the dendrimer of claims 1-36, and (2) detecting the imaging agent.

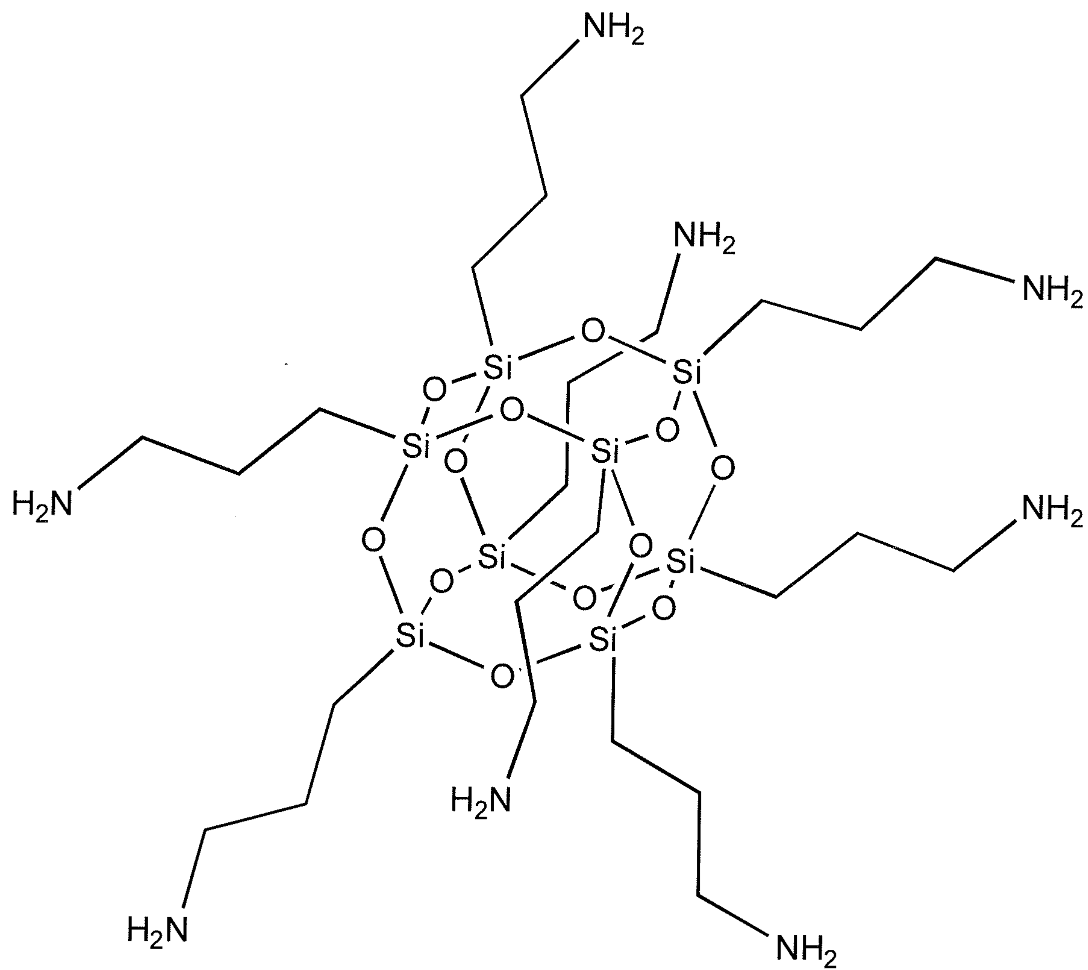

FIGURE 1

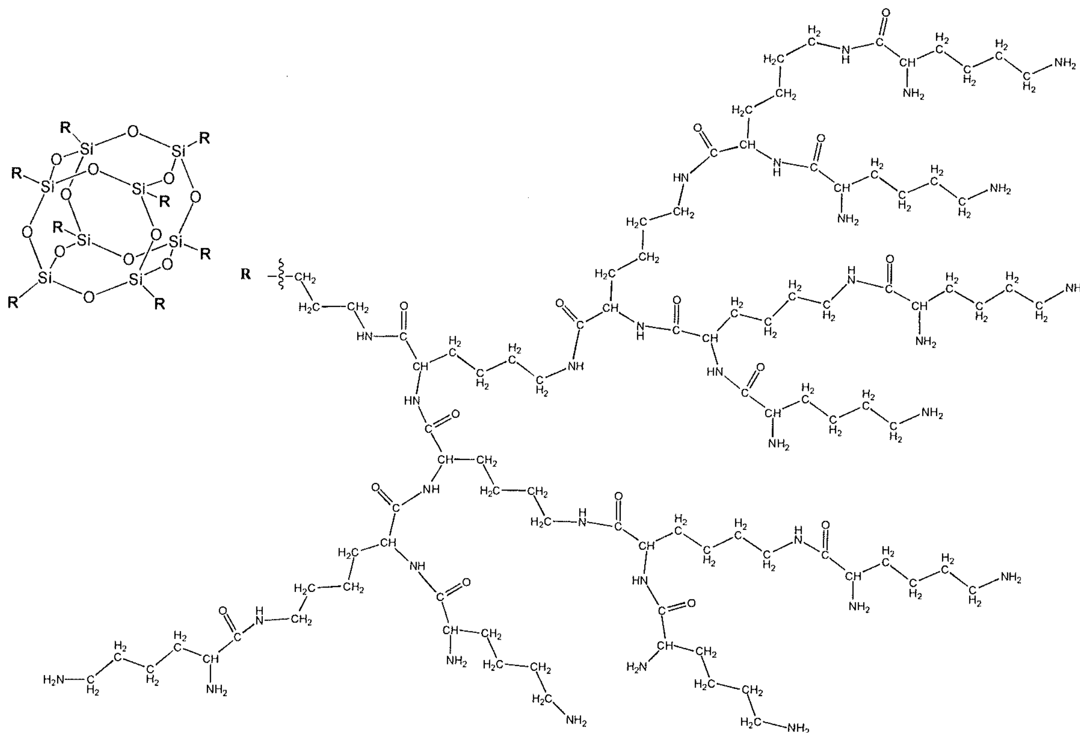

FIGURE 2

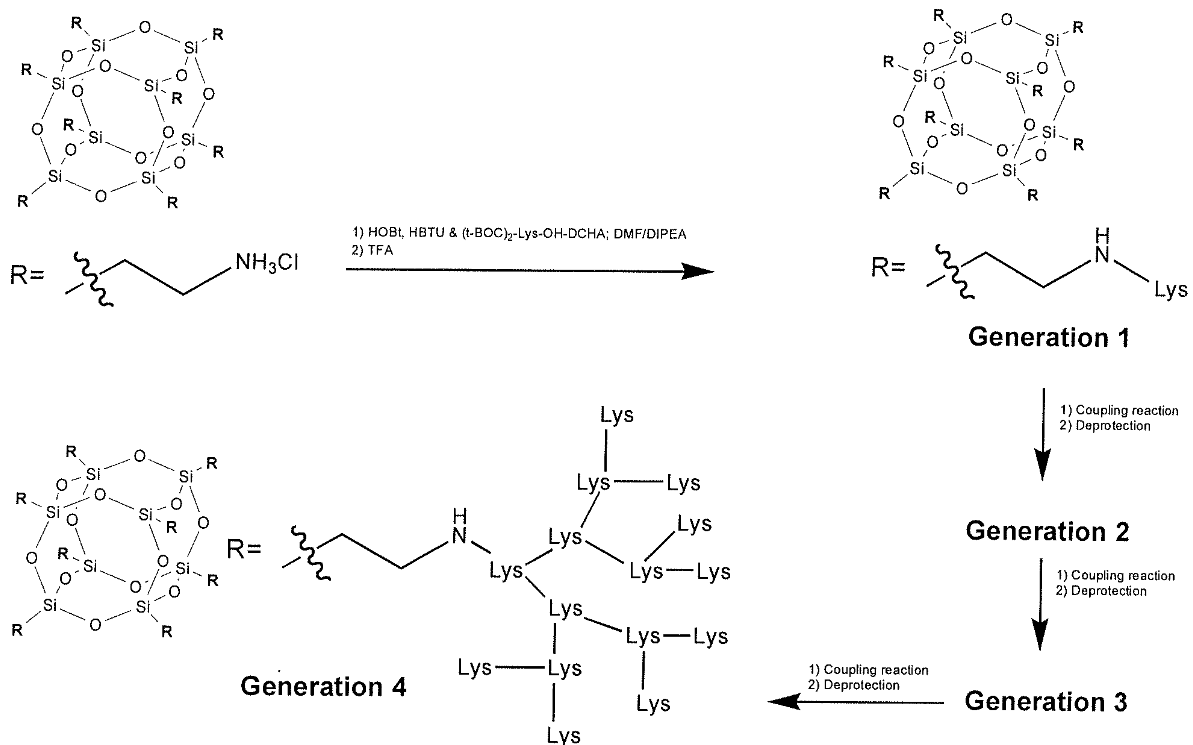

FIGURE 3

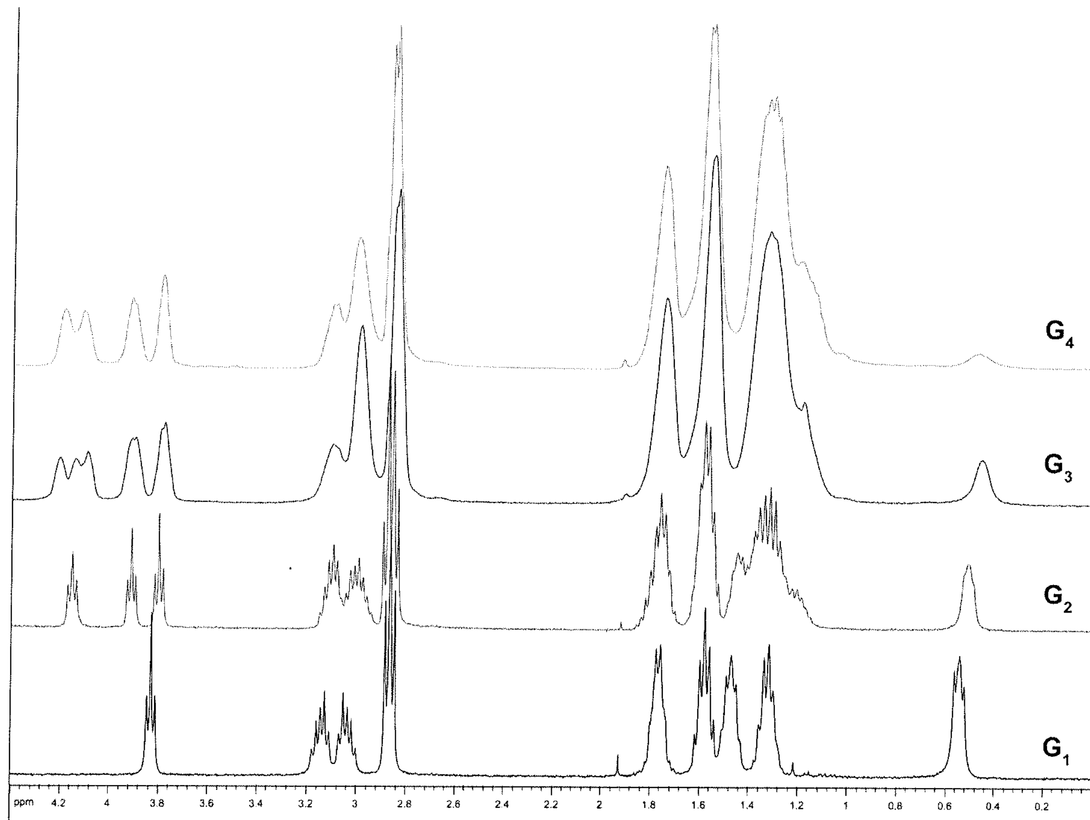

FIGURE 4

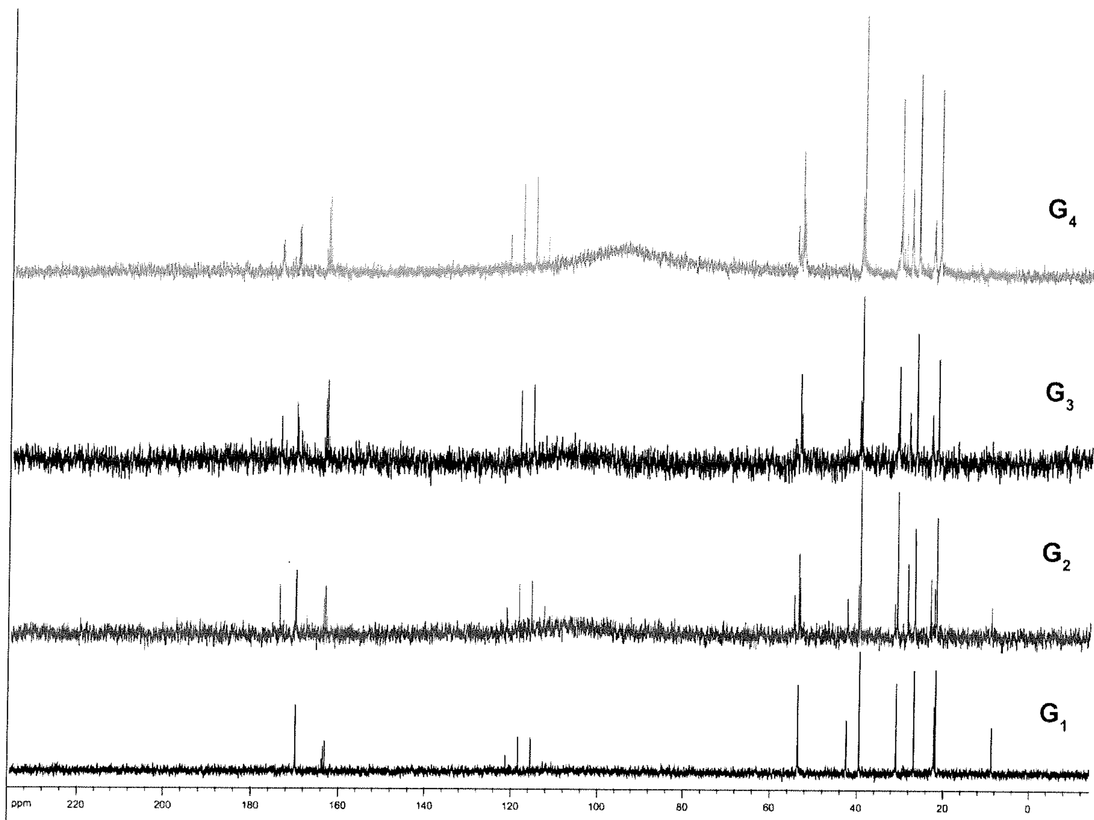

FIGURE 5

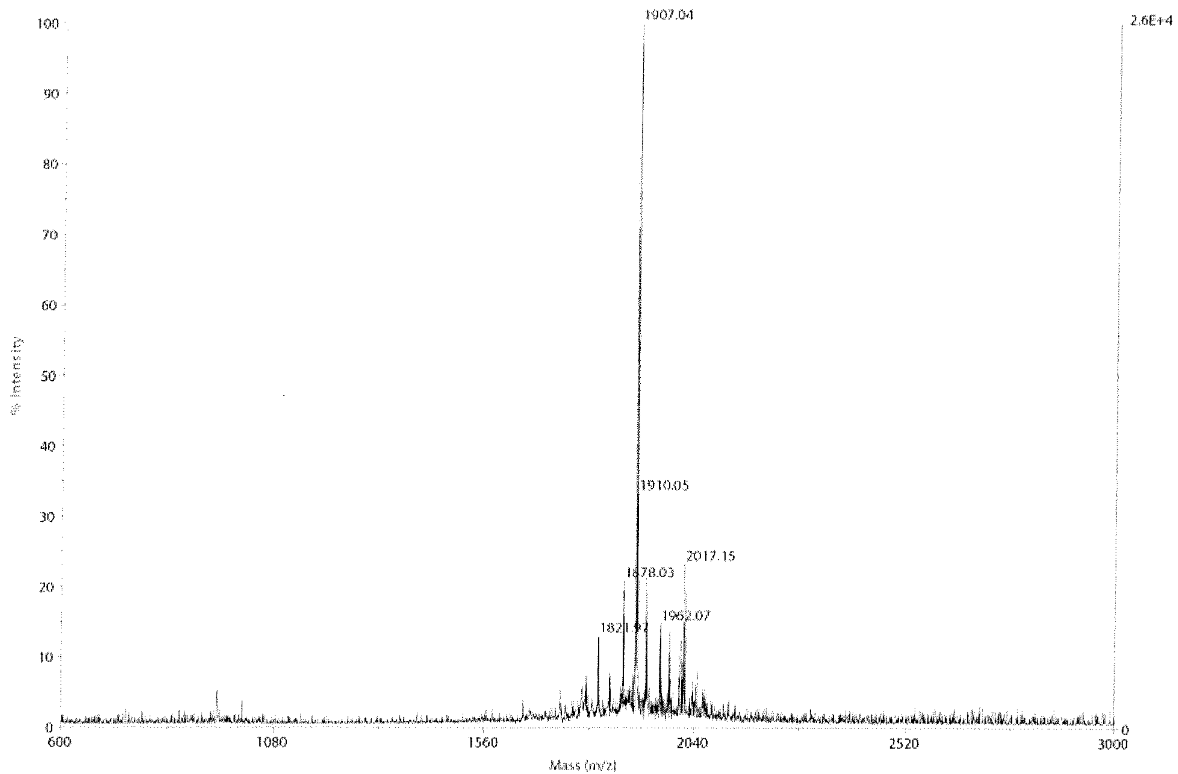

FIGURE 6

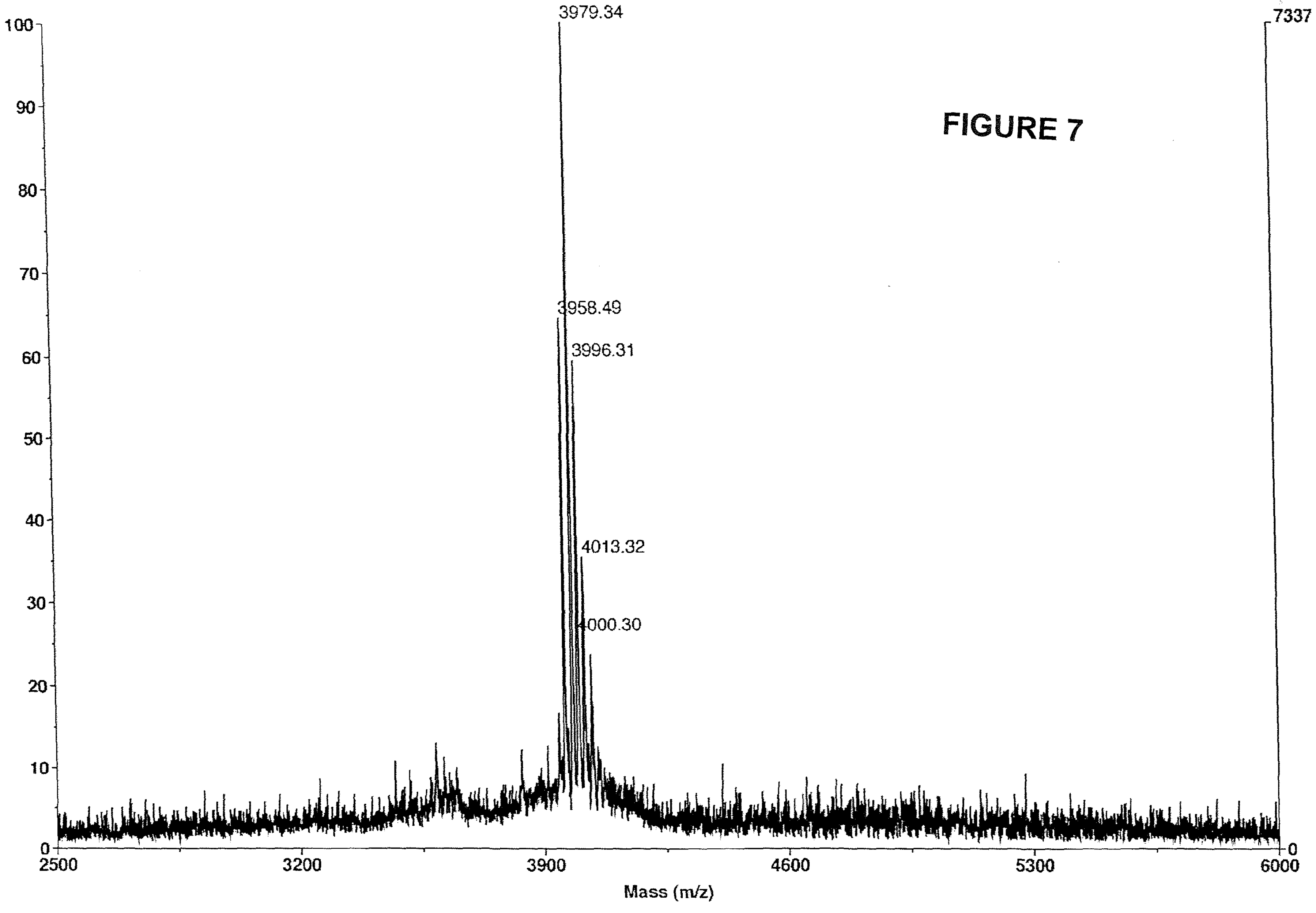
FIGURE 7
3979.34
3958.49
3996.31
4013.32
4000.30
7337
100
90
80
70
60
50
40
30
20
10
0
2500
3200
3900
4600
5300
6000
Mass (m/z)

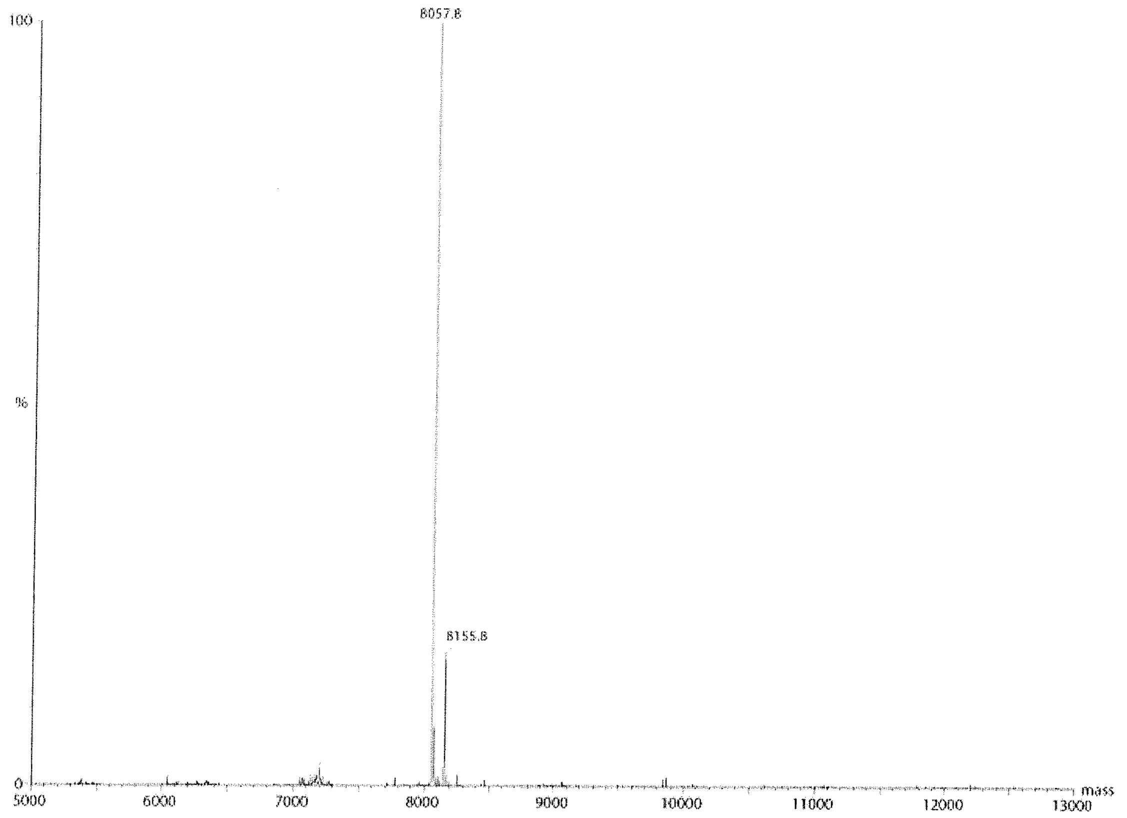

FIGURE 8

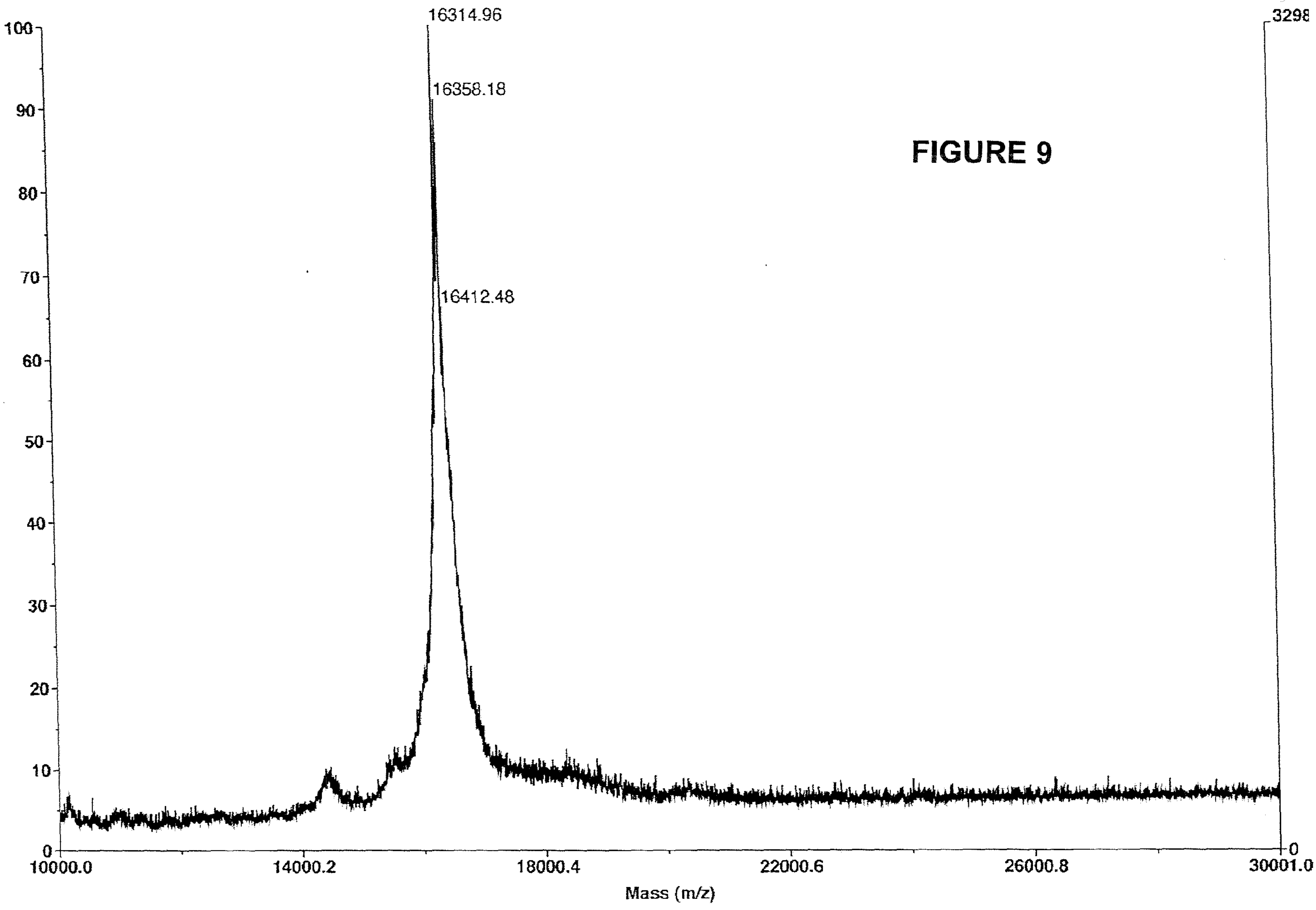
FIGURE 9
16314.96
16358.18
16412.48
3298
100
90
80
70
60
50
40
30
20
10
0
10000.0
14000.2
18000.4
22000.6
26000.8
30001.0
Mass (m/z)

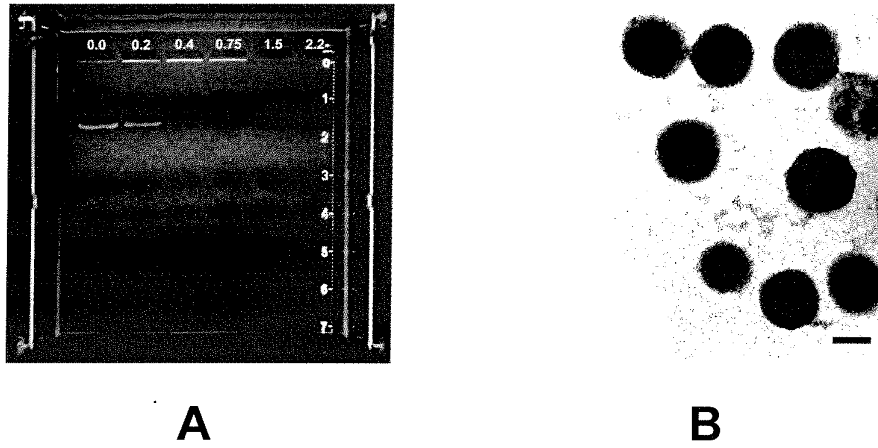

FIGURE 10

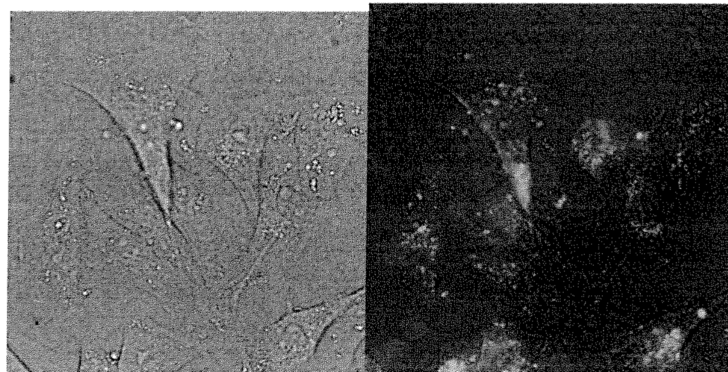

FIGURE 11

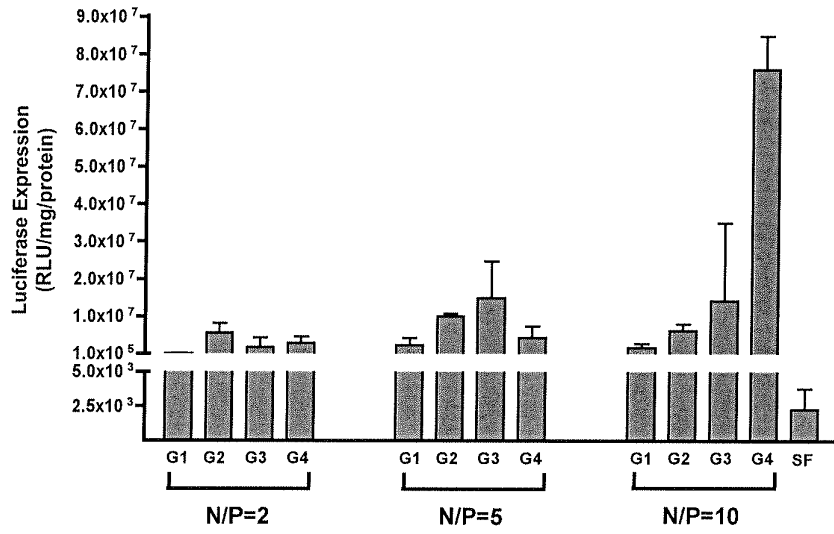

FIGURE 12

FITC Cy3 merged

FIGURE 13

TB-DOTA

1) HBTU, HOBt
2) CF_3COOH

$Gd(OAc)_3$

: —N(H)—C(=O)-DO3A
Gd : —N(H)—C(=O)-(Gd-DO3A)

FIGURE 14

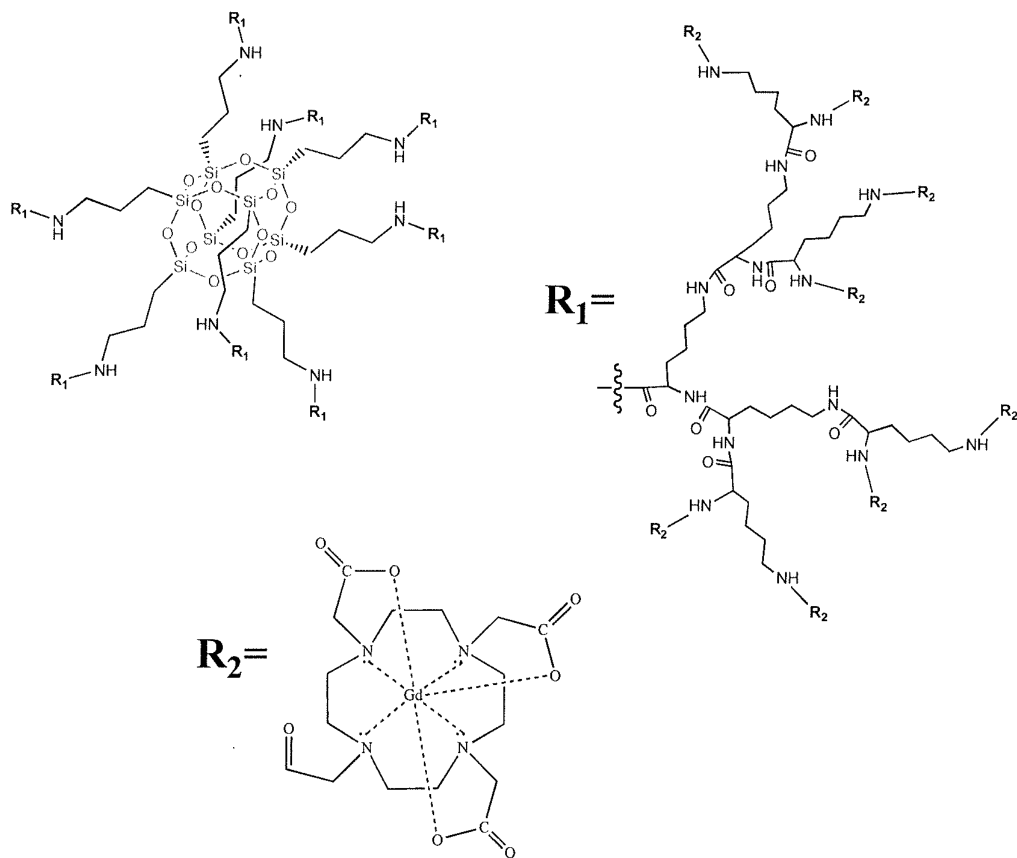

FIGURE 15

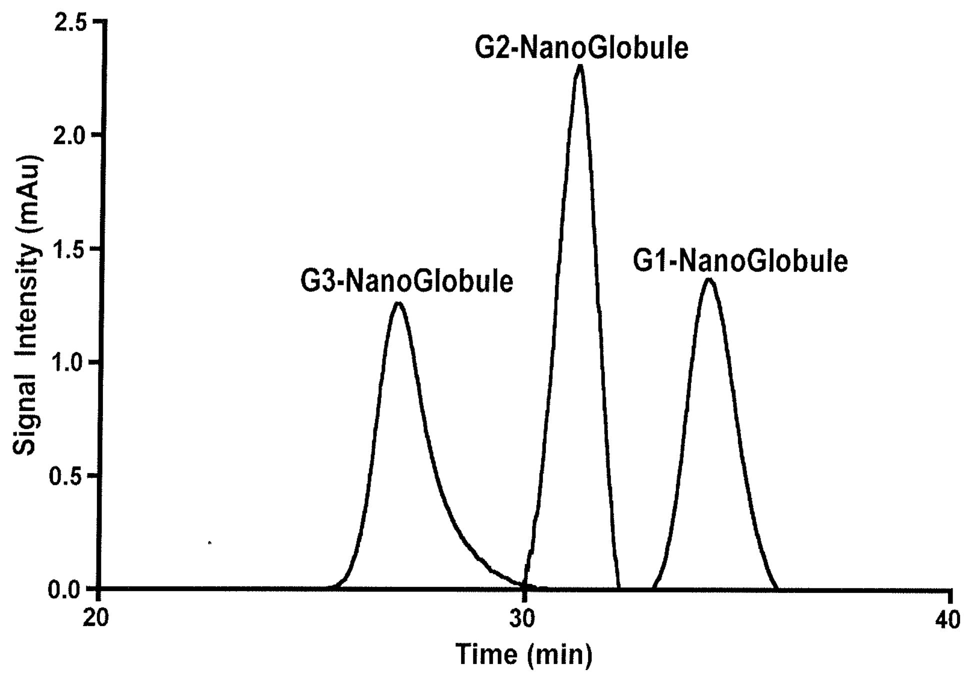

FIGURE 16

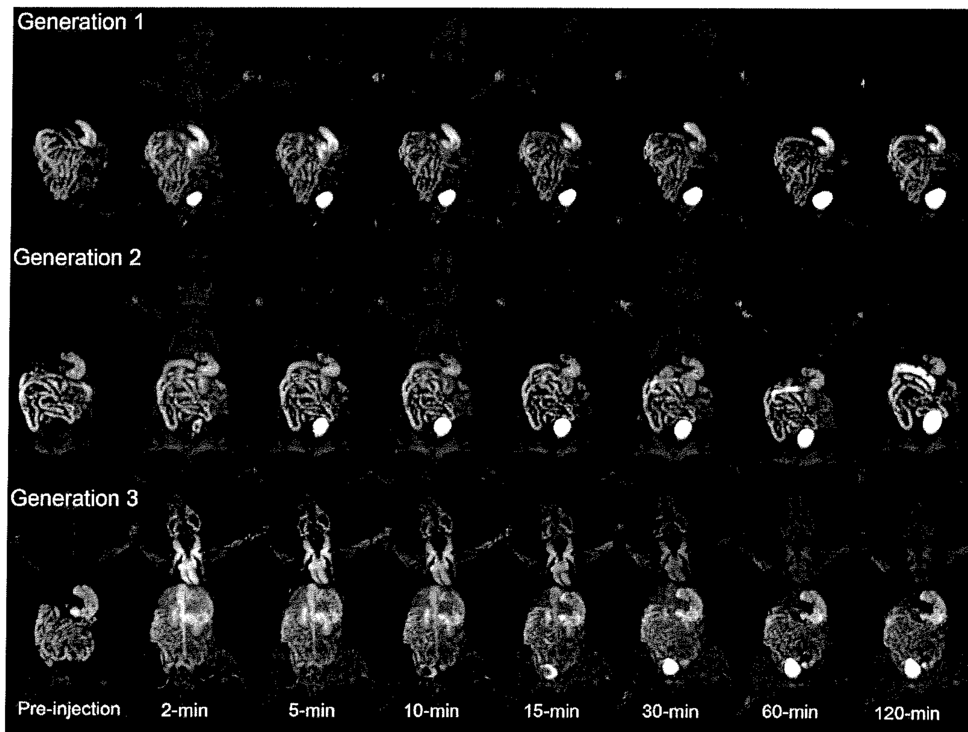

FIGURE 17

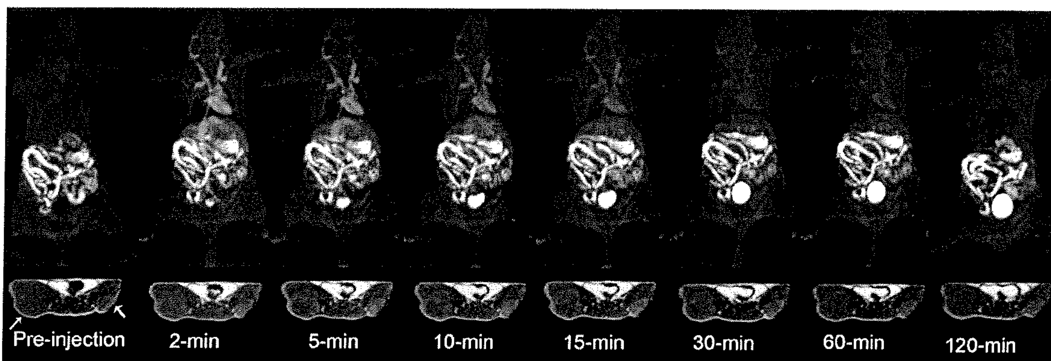

FIGURE 18

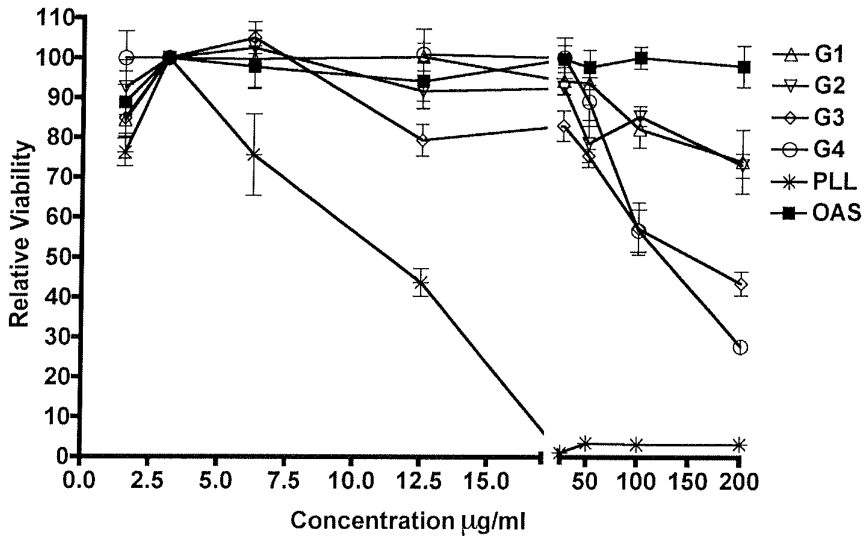

FIGURE 19