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(71) Applicant: **ANGIOCHEM INC.** [CA/CA]; 201 President-Kennedy Avenue, Suite PK-2880, Montréal, Québec H2X 3Y7 (CA).

(72) Inventors; and
(71) Applicants : **DEMEULE, Michel** [CA/CA]; 343 Preston Drive, Beaconsfield, Québec H9W 1Z2 (CA). **LAROCQUE, Alain** [CA/CA]; 2911 rue Guy-Hoffman, Saint-Laurent, Québec H4R 2R1 (CA). **YANG, Gaoqiang** [CA/CA]; 318-4950 de la Savane, Montréal, Québec H4P 1T7 (CA). **TRIPATHY, Sasmita** [CA/CA]; 4451 King Street, Pierrefonds, Québec H9H 2G2 (CA).

(74) Agent: **GOUDREAU GAGE DUBUC**; 2000, McGill College, #2200, Montréal, Québec H3A 3H3 (CA).

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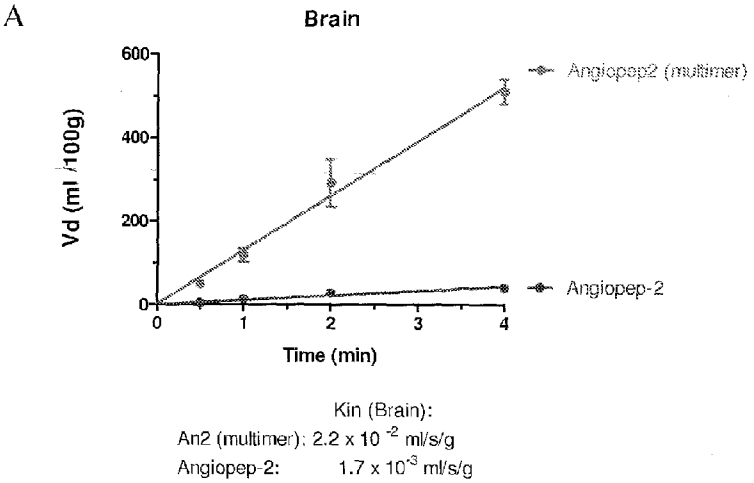
(54) Title: PEPTIDE-DENDRIMER CONJUGATES AND USES THEREOF

Figure 1



(57) Abstract: The invention relates to dendrimers conjugated to multiple targeting peptides and one or more therapeutic, diagnostic, or imaging agents for delivery of such agents across the blood-brain barrier and into certain cell types including, cells expressing the LRP-1 receptor. Also described are methods of making compounds that comprise dendrimers conjugated to targeting peptides and therapeutic, diagnostic, or imaging agents.

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PEPTIDE-DENDRIMER CONJUGATES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of U.S. Serial No. 61/682,991, filed August 14, 2012, the contents of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

The invention relates to compounds including dendrimers conjugated to targeting peptides and one or more therapeutic, diagnostic, or imaging agents and uses of such compounds.

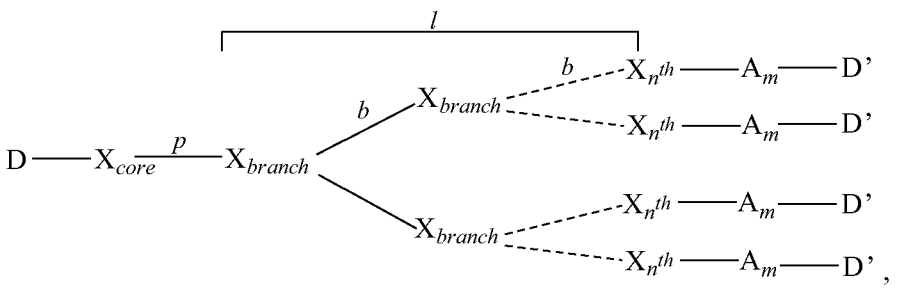
The brain is shielded against potentially toxic substances by the presence of two barrier systems: the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB). The BBB is considered to be the major route for the uptake of serum ligands since its surface area is approximately 5000-fold greater than that of BCSFB. The brain endothelium, which constitutes the BBB, represents the major obstacle for the use of potential drugs against many disorders of the CNS. As a general rule, only small lipophilic molecules may pass across the BBB, i.e., from circulating systemic blood to brain. Many drugs that have a larger size or higher hydrophobicity show promising results in animal studies for treating CNS disorders. Thus, peptide and protein agents such as those used as therapeutics are generally excluded from transport from blood to brain, owing to the negligible permeability of the brain capillary endothelial wall to these agents.

Therapy of brain diseases can be impaired by the inability of otherwise effective agents such as therapeutic agents to cross the BBB. Thus, new strategies for transporting agents into the brain with high efficiency are desired.

SUMMARY OF THE INVENTION

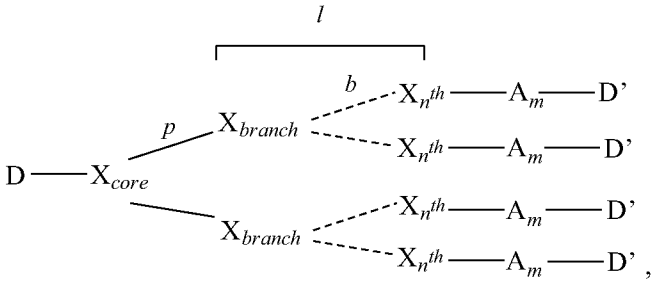
We have now developed compounds including a dendrimer conjugated to multiple targeting peptides. These compounds are capable of crossing the blood-brain barrier (BBB) or entering particular cell types (e.g., liver, lung, spleen, kidney, and muscle) with enhanced efficiency. When these compounds are joined with (e.g., conjugated to) one or more agents (e.g., therapeutic or diagnostic agents), efficiency of transport of the agent across the BBB or into particular cell types is increased compared to transport of the agent directly linked to a targeting peptide. The present invention also features methods of producing such compounds and the use of such compounds in treatment of disease.

In a first aspect, the invention features a compound including the formula:



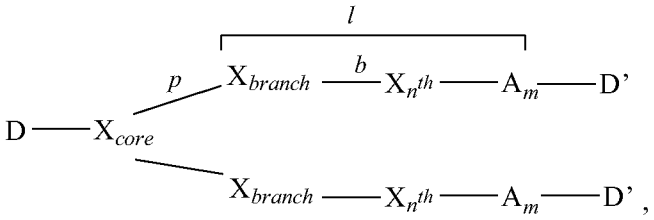
where D is a first agent; X_{core} is a core moiety of a dendrimer with *p* number of branches where *p* is an integer from 1 to 12 (e.g., 1, 2, 3, 4, 6, 8, 10, and 12 branches); X_{branch} is a branch moiety of said dendrimer, each X_{branch} is attached to a branch of X_{core} or to a branch of another X_{branch}, each X_{branch} has *b* branches where *b* is an integer from 2 to 8 (e.g., 2, 4, 6, and 8 branches); *l* is the number of successive layers of X_{branch} branches of said dendrimer and is an integer from 2 to 10 (e.g., 2, 4, 6, 8, and 10); X_n^{th} is one of *n* surface branches of said dendrimer and is attached to a *b* branch of a X_{branch} moiety, where $n = p(b)^l$, and where *n* is ≤ 512 (e.g., ≤ 500, ≤ 400, ≤ 300, ≤ 200, ≤ 50, ≤ 10, or ≤ 8 branches); A_m is a targeting peptide attached to an X_n^{th} and comprises an amino acid sequence substantially identical to a sequence selected from the group consisting of SEQ ID NOs:1-105 and 107-117, or a functional fragment thereof, or is a peptide having a formula selected from the group consisting of formulae Ia, Ib, IIa, IIb, and IIc; *m* is a positive integer ≤ *n*; D' is a second agent that is optional and is attached to one or more A_m or may replace one or more A_m and attach directly to one or more X_n^{th}, and wherein the number of D' in said compound is ≤ *n*; and the molecular weight of the dendrimer, excluding D, D' and A_m, is ≤ 500 kilodalton (e.g., ≤ 500, ≤ 400, ≤ 300, ≤ 200, ≤ 100, ≤ 50, or ≤20 kilodaltons).

The compound may also include the formula:



where the compound shares all properties with the above formula with the exceptions that *p* is an integer between 2 and 6 (e.g., 2, 3, 4, or 6 branches); *b* is an integer from 2 to 4 (e.g., 2, 3, or 4 branches); and *l* is an integer from 2 to 5 (e.g., 2, 3, 4, or 5 branches).

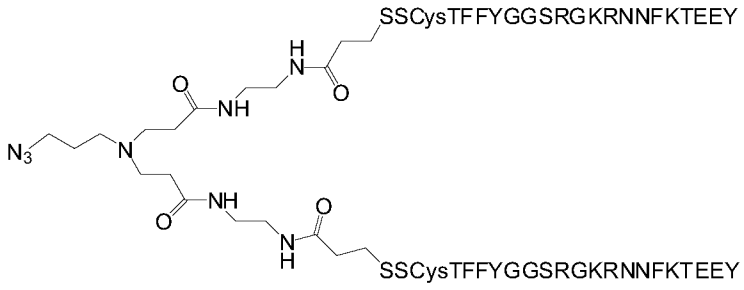
In another aspect, the invention features a compound including the formula:



where D is a first agent; X_{core} is a core moiety of a dendrimer with *p* number of branches where *p* is an integer from 2 to 6 (e.g., 2, 3, 4, or 6 branches); and where the core moiety is selected from the group consisting of propargylamine, ethylenediamine, triethanolamine, pentaerythritol, azido-propyl(alkyl)amine, hydroxyethyl(alkyl)amine, tetraphenyl methane , trimesoylchloride, diamino hexane, diaminobutane, cystamine, propylenediamine, lysine, and propyleneamine; X_{branch} is a branch moiety of the dendrimer, where each X_{branch}, is attached to a branch of X_{core} or to a branch of another X_{branch}, and where each X_{branch} has *b* branches, and where *b* is an integer from 1 to 4 (e.g., 1, 2, 3, or 4 branches); *l* is the number

of successive layers of X_{branch} branches of the dendrimer and is an integer from 0 to 4 (e.g., 0, 1, 2, 3 or 4 branches); X_n^{th} is one of *n* surface branches of the dendrimer and is attached to a *b* branch of a X_{branch} moiety where $n = p(b)^l$, and where *n* is ≤ 256 (e.g., ≤ 256, ≤ 200, ≤ 50, ≤ 10, or ≤ 8 branches); A_m is a targeting peptide attached to an X_n^{th} and comprises an amino acid sequence substantially identical to a sequence selected from the group consisting of SEQ ID NOs:1-105 and 107-117, or a functional fragment thereof, or is a peptide having a formula selected from the group consisting of formulae Ia, Ib, IIa, IIb, and IIc; *m* is a positive integer ≤ *n*; D' is a second agent that is optional and is attached to one or more A_m or may replace one or more A_m and attach directly to one or more X_n^{th}, and wherein the number of D' in said compound is ≤ *n*; and the molecular weight of the dendrimer, excluding D, D' and A_m, is ≤ 500 kilodalton (e.g., ≤ 500, ≤ 400, ≤ 300, ≤ 200, ≤ 100, ≤ 50, or ≤20 kilodaltons).

The compound may include the formula:



where Cys is cysteine and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine.

In one embodiment, *n*, which is the number of surface branches, can be ≤ 128 (e.g., ≤ 64 or ≤ 32 or ≤ 16 or ≤ 8). In another embodiment, the dendrimer part of the compound, excluding the first and second agents and the targeting peptides, of the invention can have a molecular weight of ≤ 100 kilodaltons (e.g., ≤ 50 kilodaltons, ≤ 25 kilodaltons, or ≤ 10 kilodaltons).

The dendrimer part of the compound may include a core moiety selected from the group consisting of propargylamine, ethylenediamine, triethanolamine, pentaerythritol, azido-propyl(alkyl)amine, hydroxyethyl(alkyl)amine, tetraphenyl methane , trimesoylchloride, diamino hexane, diaminobutane, cystamine, and propylenediamine. These cores are typically used to synthesize the poly(amido amine) (PAMAM) dendrimer. Lysine can also be used as a core moiety to synthesize a polylysine dendrimer. Alternatively the compound can include a propyleneimine to synthesize a POPAM dendrimer.

The compound of the invention can have branch moieties selected from the group consisting of propargylamine, ethylenediamine, triethanolamine, pentaerythritol, propylamine, propyleneimine, azido-propyl(alkyl)amine, hydroxyethyl(alkyl)amine, tetraphenyl methane, trimesoylchloride, diamino hexane, diaminobutane, cystamine, propylenediamine, and lysine. Alternatively, the branch moieties can be derivatives of any one of propargylamine, ethylenediamine, triethanolamine, pentaerythritol, propylamine, propyleneimine, azido-propyl(alkyl)amine, hydroxyethyl(alkyl)amine, tetraphenyl methane, trimesoylchloride, diamino hexane, diaminobutane, cystamine, propylenediamine, and lysine.

One or more terminal branches on the surface of the dendrimer can be functionalized to attach various numbers of targeting peptides (e.g., 2, 4, 6, 8, 12, 16, 32, or 64 targeting peptides). Some or all

of the surface branches of the dendrimer can have a targeting peptide attached. The linkage between the targeting peptide can be a cleavable linkage (e.g., a thioester linkage) or a non-cleavable linkage (e.g., a maleimide linkage). The targeting peptide can be attached to the surface branches of the dendrimers via linkers described herein.

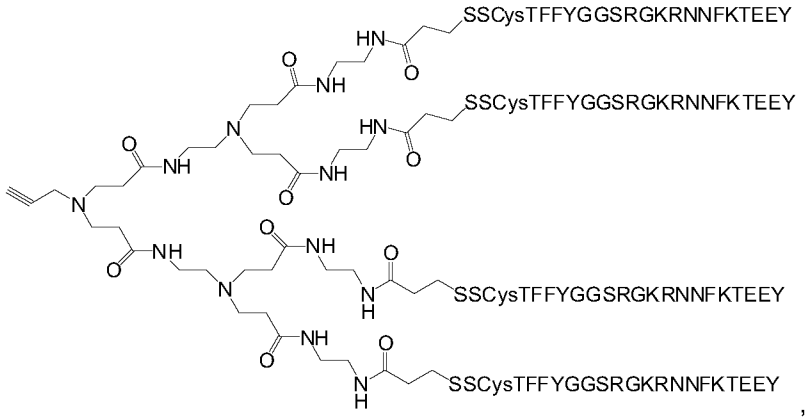
The targeting peptides attached to the dendrimer can have an amino acid sequence at least 85% identical to a sequence selected from the group consisting of SEQ ID NOs:1-105 and 107-117 or a fragment thereof. For example, the target peptide can have an amino acid sequence selected from the group consisting of Angiopep-1 (SEQ ID NO:67), Angiopep-2 (An_2) (SEQ ID NO:97), cys-Angiopep-2 ($CysAn_2$) (SEQ ID NO:113), Angiopep-2-cys (SEQ ID NO:114), and reversed Angiopep-2 (SEQ ID NO:117). Alternatively, the targeting peptide can have an amino acid sequence selected from the group consisting of Angiopep-1 (SEQ ID NO:67), Angiopep-2 (An_2) (SEQ ID NO:97), cys-Angiopep-2 ($CysAn_2$) (SEQ ID NO:113), Angiopep-2-cys (SEQ ID NO:114), and reversed Angiopep-2 (SEQ ID NO:117).

The compound also includes a first agent, D (e.g., a protein, a peptide, a nucleic acid, or a small molecule), attached to the dendrimer via a reactive group (e.g., maleimide, a hydrazide, an azide, a haloacetamide, or an alkoxyamine). The first agent can be attached to the dendrimer via a linker, e.g., pyridinedisulfide, thiosulfonate, vinylsulfonate, isocyanate, NHS ester, imidoester, diazine, hydrazine, thiol, carboxylic acid, a multi-peptide linker, acetylene linkers, cleavable linkers, non-cleavable linker, or a covalent bond. The first agent can be selected from the group consisting of a protein, a peptide, a small molecule, a nucleic acid, a diagnostic agent, an imaging agent, and a therapeutic agent.

The compound also includes an optional second agent, D' (e.g., a protein, a peptide, a nucleic acid, or a small molecule), attached to the dendrimer via a reactive group (e.g., maleimide, a hydrazide, an azide, a haloacetamide, or an alkoxyamine). The second agent can be attached to the dendrimer via a linker, e.g., pyridinedisulfide, thiosulfonate, vinylsulfonate, isocyanate, NHS ester, imidoester, diazine, hydrazine, thiol, carboxylic acid, a multi-peptide linker, acetylene linkers, a cleavable linker, a non-cleavable linker, or a covalent bond. The second agent can be selected from the group consisting of a protein, a small molecule, a nucleic acid, a diagnostic agent, an imaging agent, and a therapeutic agent. The second agent, when present, can be attached to one or more of the A_m peptides, or is attached to one or more of the X_n^{th} branches. The first and second agents maybe identical or maybe different types of molecules.

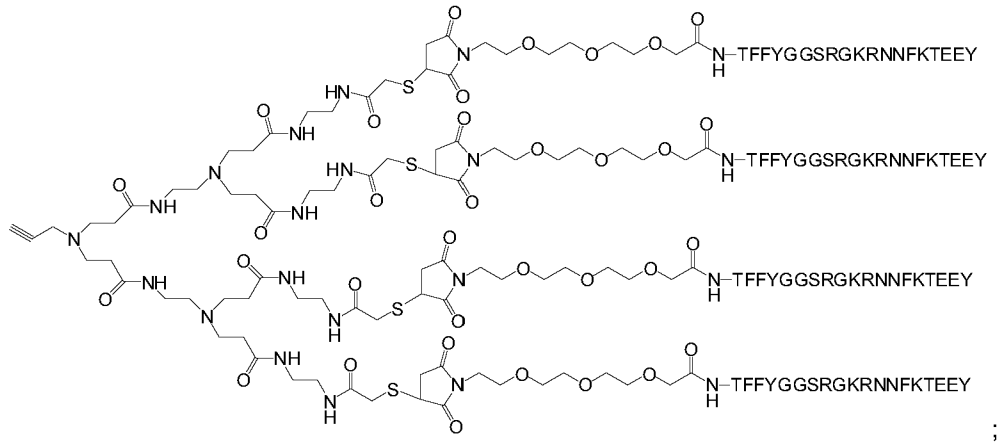
The invention includes compounds that may include one or more linkers used to attach the targeting peptide, first agent, and second agent to the dendrimer, wherein the reactive group is present on the linkers.

The compound of the invention includes:



,

wherein Cys is cysteine and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine;



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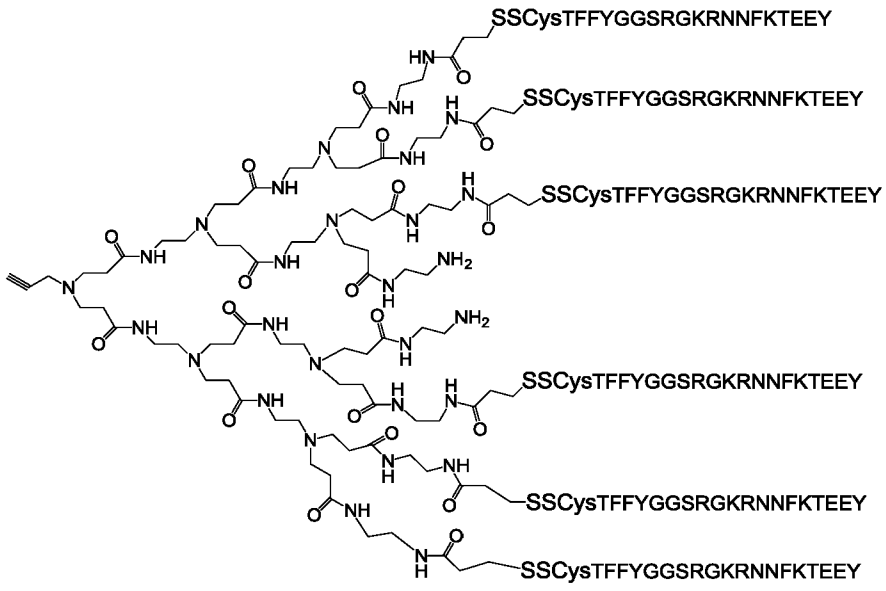
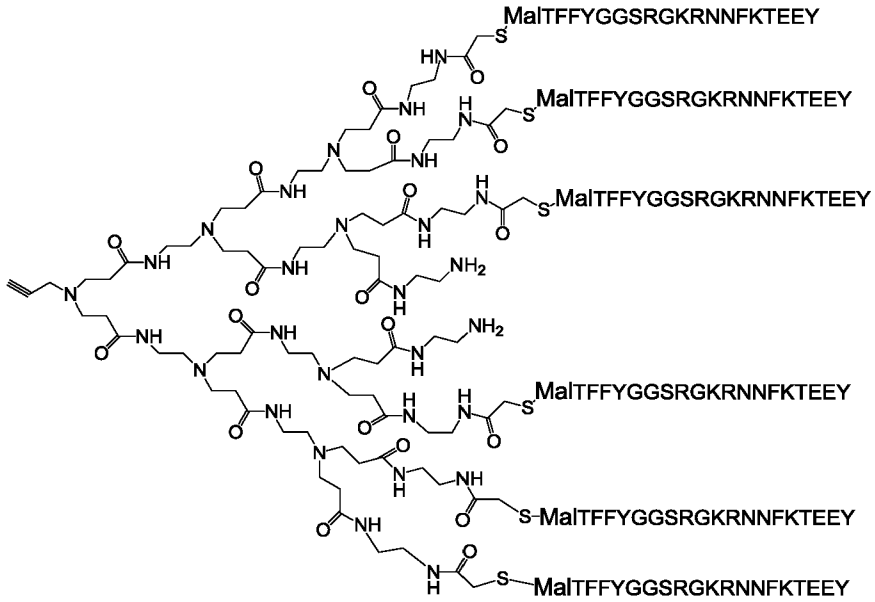


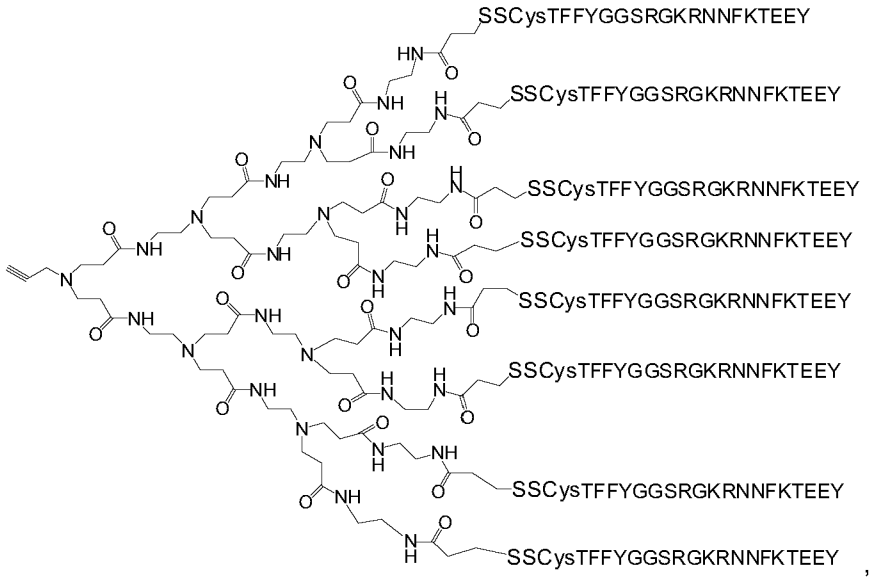
5 ,

wherein Cys is cysteine and the “SS” adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine;



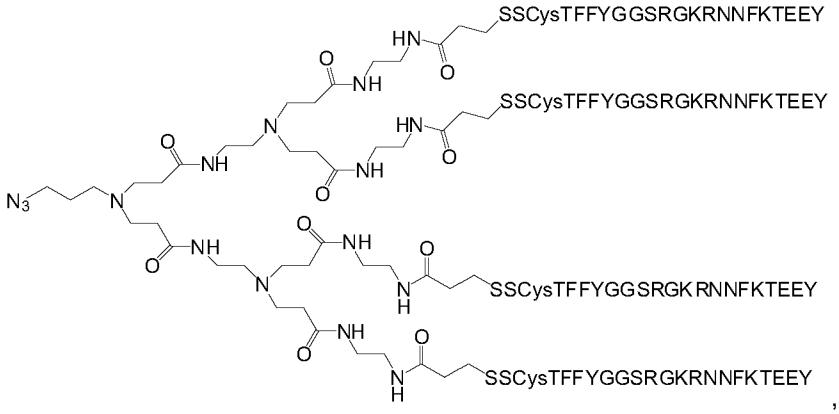
,

wherein Mal is maleimide;



,

wherein Cys is cysteine and the “SS” adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine;



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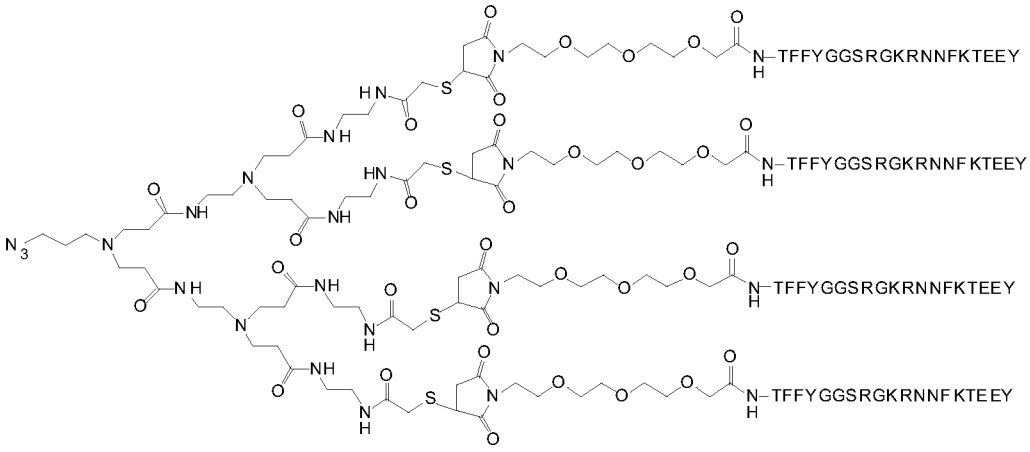
wherein Cys is cysteine and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine;



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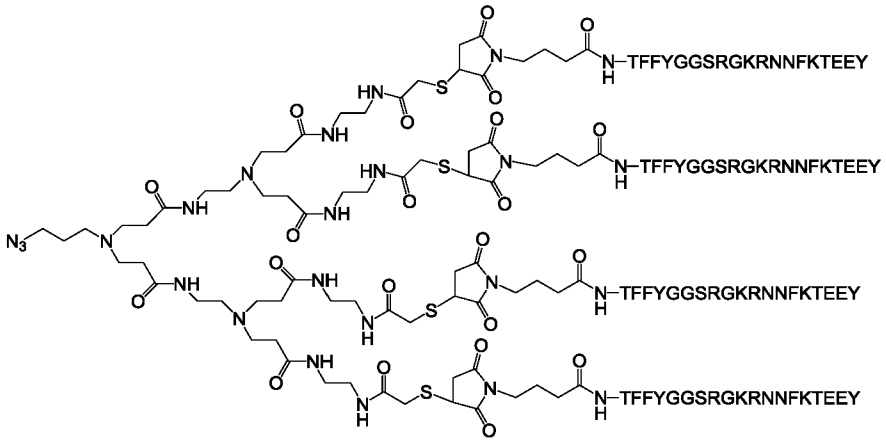

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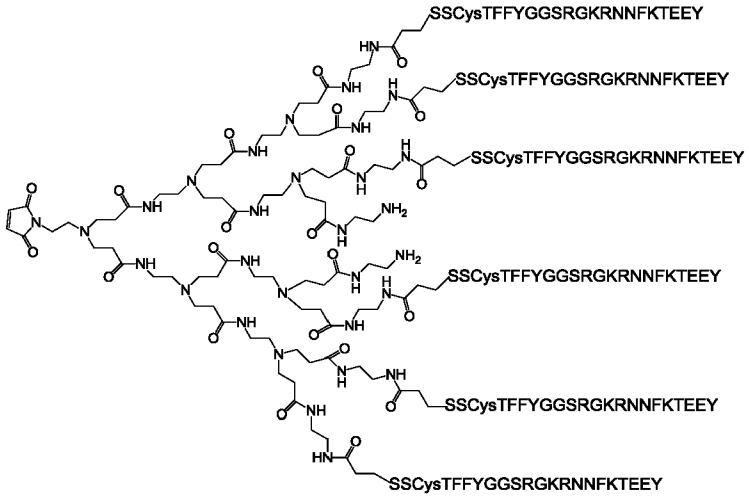
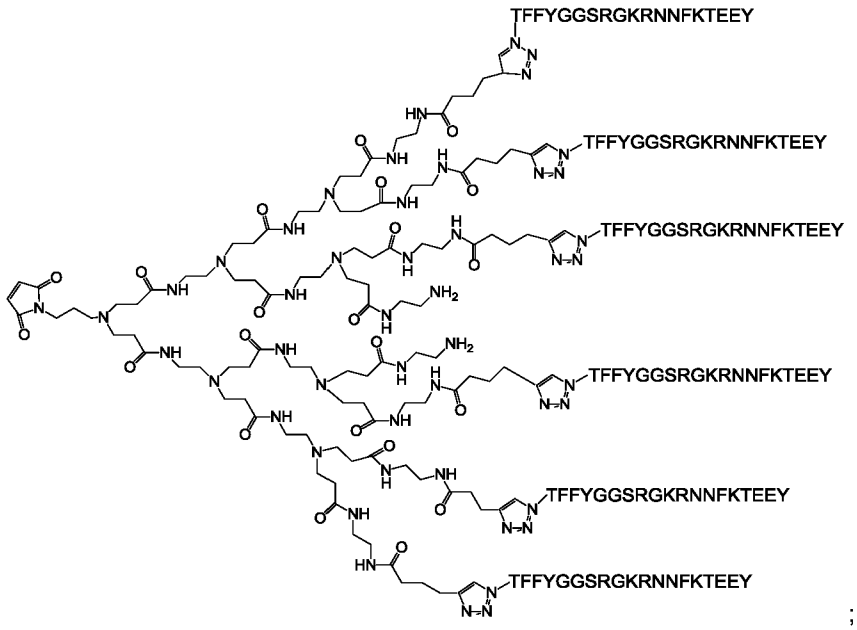


,

wherein Cys is cysteine and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine;



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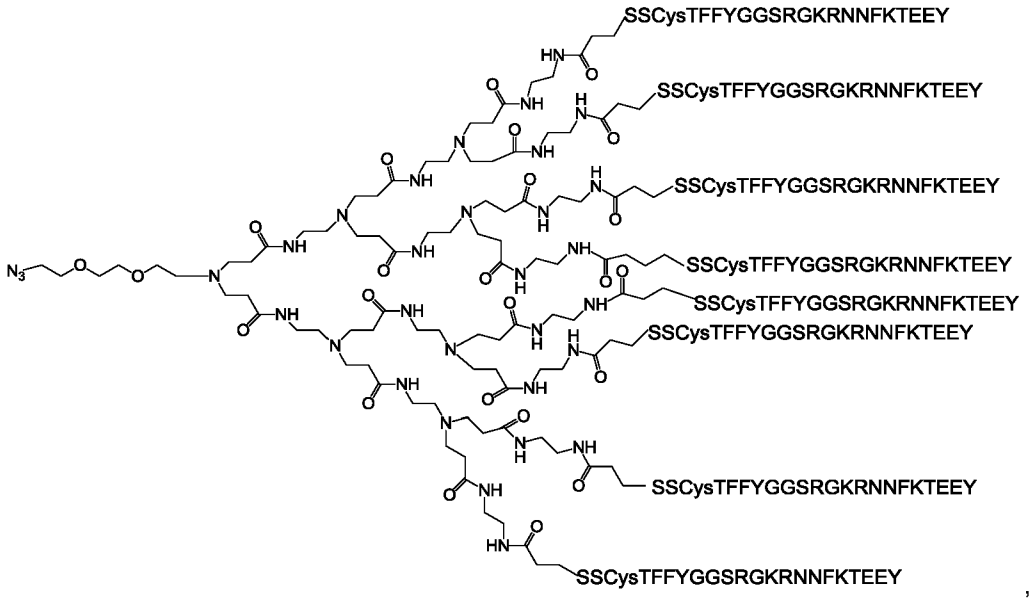
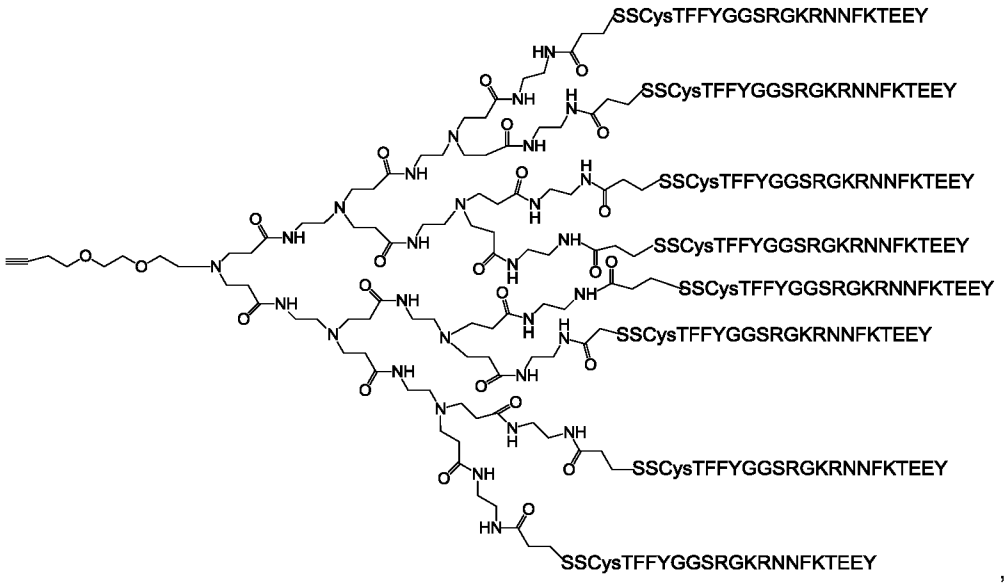
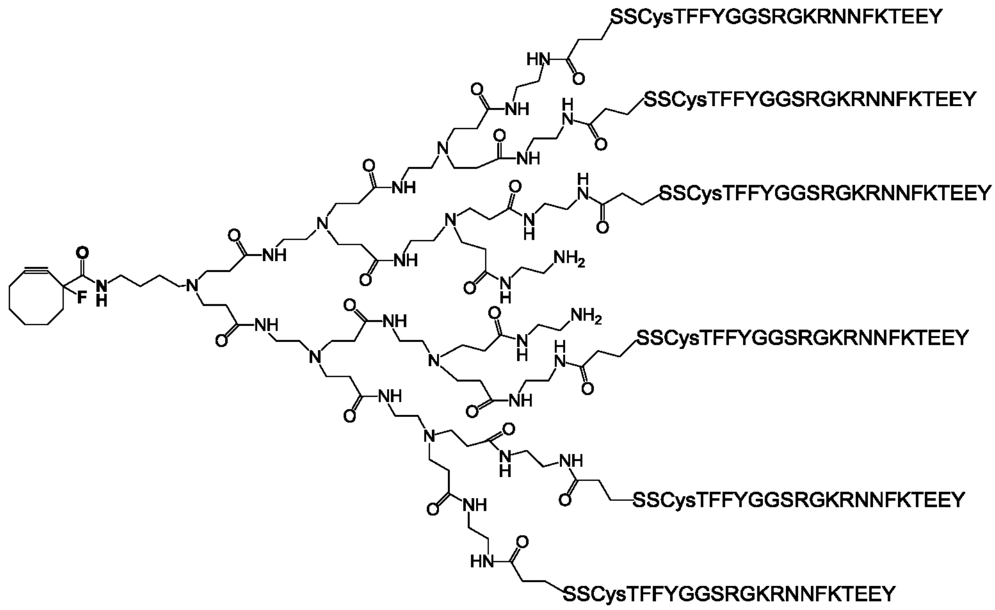


wherein Cys is cysteine, and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine;

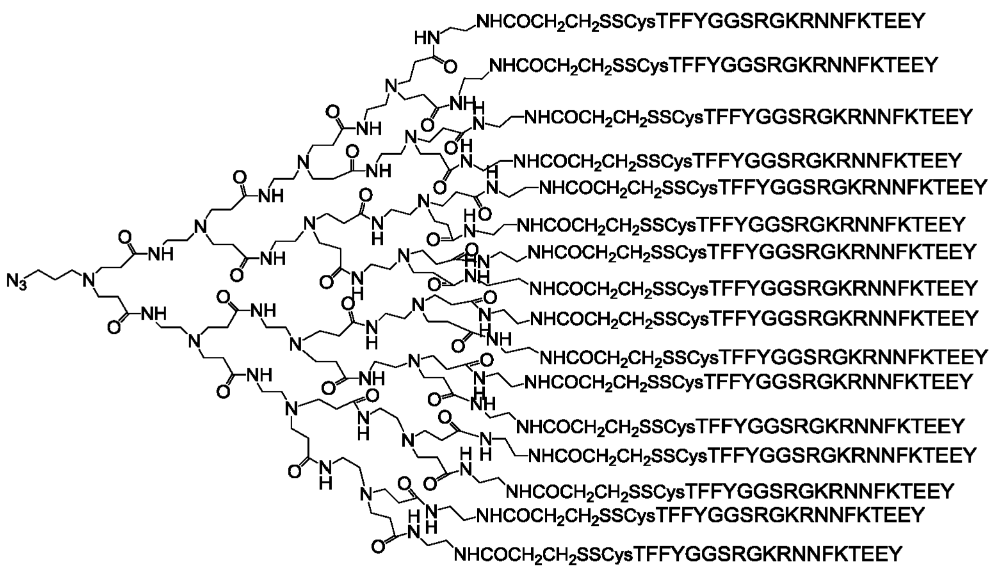


wherein Cys is cysteine, and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine;



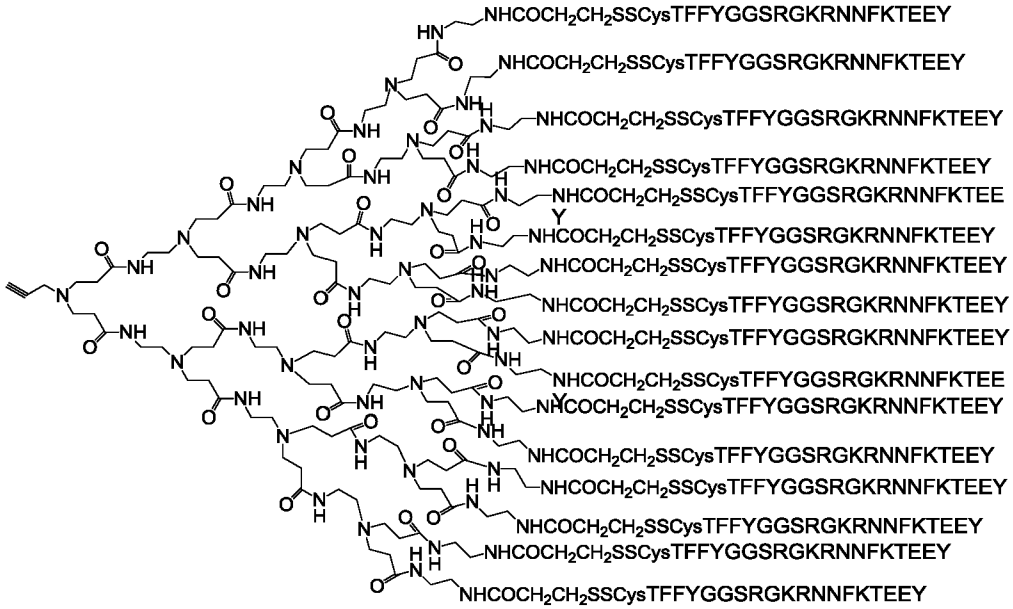
,

wherein Cys is cysteine, and the “SS” adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine;



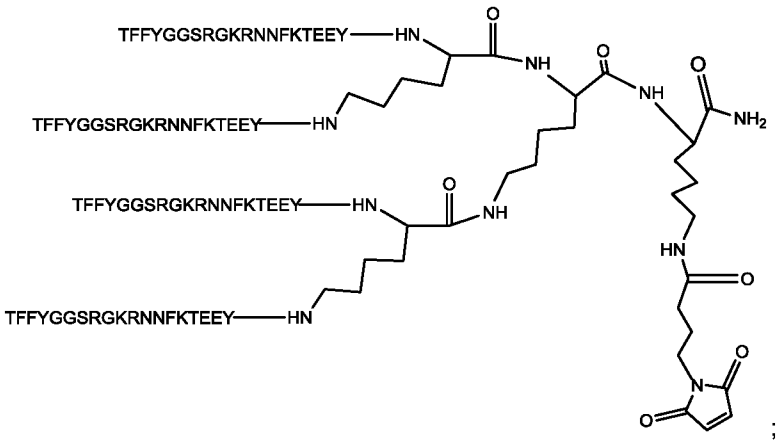
,

wherein Cys is cysteine and the “SS” adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine;



,

wherein Cys is cysteine, and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine;



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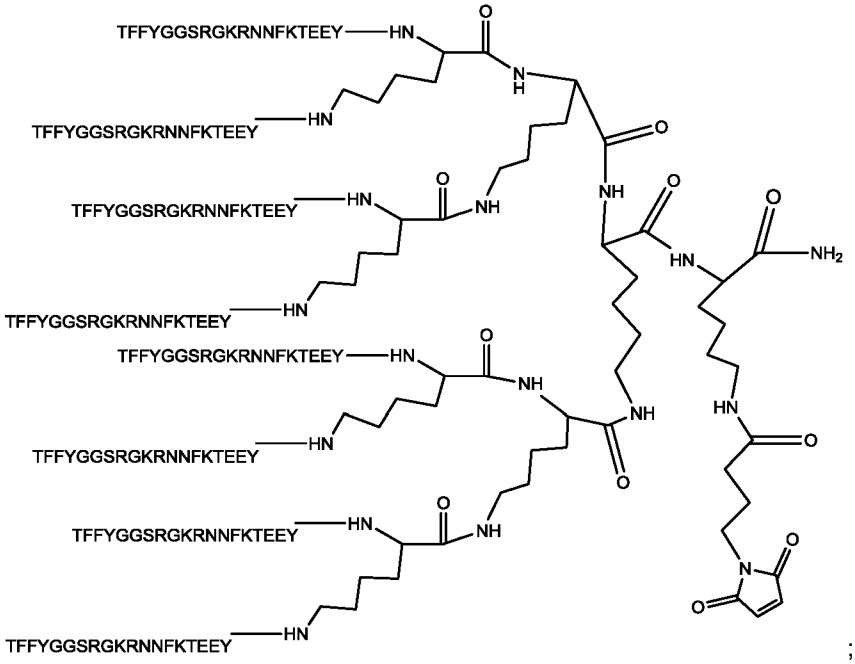

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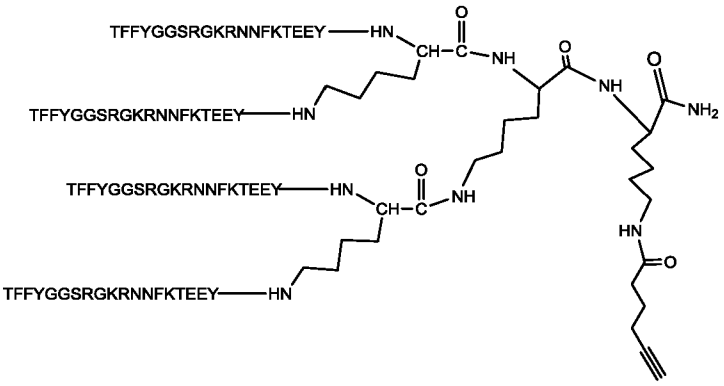

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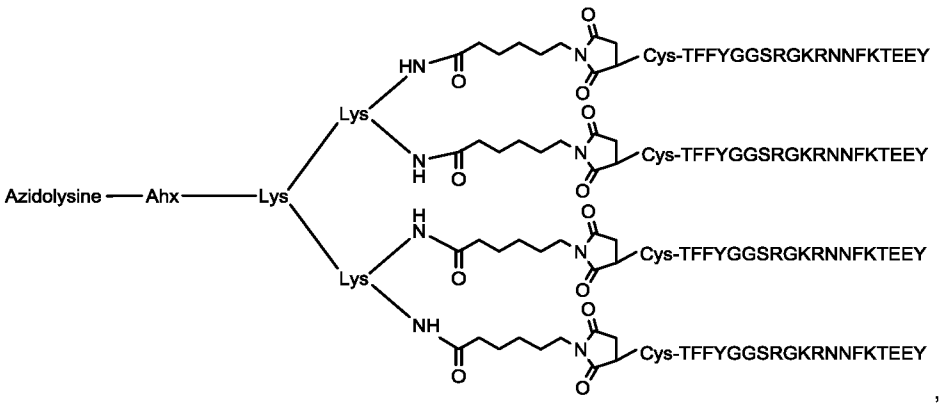
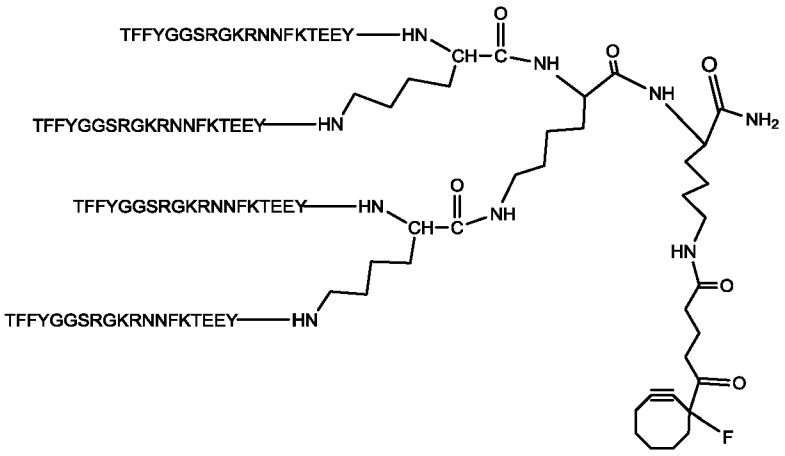


,

wherein Cys is cysteine, and Ahx is azidohexanoic acid;



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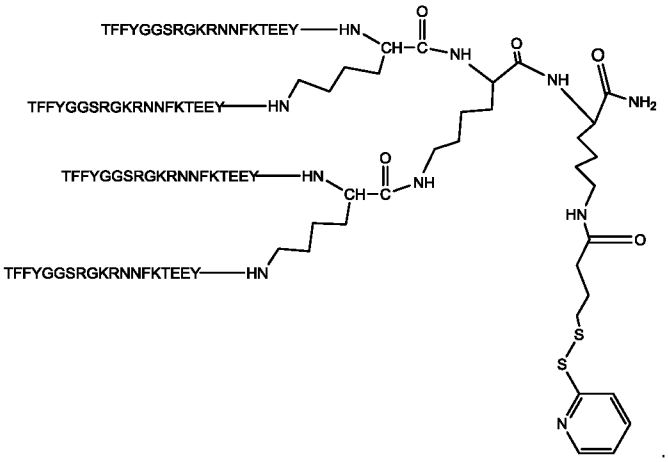

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The compound of the invention can enter endothelial cells or enter cells that express the LRP-1 receptor, for example, liver, kidney, and spleen cells. The compound of the invention can also cross the BBB.

The invention also features a method of synthesizing the compound which includes attaching at least two or more targeting peptides, via a linker, to a dendrimer to form a dendrimer-targeting peptide complex; attaching one or more first agents, via a linker, to the dendrimer-targeting peptide complex; and subsequently optionally attaching one or more second agents, via a linker, to this complex. Alternatively, the method can include first attaching at least one first agent to a dendrimer via a reactive group (e.g., a maleimide, a hydrazide, an azide, a haloacetamide, or an alkoxyamine), via a linker, to form a dendrimer-first agent complex; attaching at least two targeting peptides, via a linker, to the dendrimer-first agent complex; and subsequently optionally attaching one or more second agents, via a linker, to this complex.

The method can optionally include steps to attach linkers, for example attaching one or more linkers (e.g., pyridinedisulfide, thiosulfonate, vinylsulfonate, isocyanate, NHS ester, imidoester, diazine, hydrazine, thiol, carboxylic acid, a multi-peptide linker, acetylene, a cleavable linker, a non-cleavable linker, or a covalent bond) to the dendrimer prior to attachment of the targeting peptides; and/or attaching one or more linkers to said dendrimer-targeting peptide complex prior to attaching one or more first or second agents. The linkers can also be attached to a dendrimer prior to attachment of one or more first

or second agents or the linkers can be attached to the dendrimer-targeting peptide complex prior to attaching the targeting peptides.

Several methods can be used to functionalize the surface branch and attach targeting peptides to the functionalized surface branch. For example, one method involves reacting the dendrimer with *N*-succinimidyl 3-(2-pyridyldithio)-propionate followed by reacting with cysteine residue-containing targeting peptides. Alternatively, the targeting peptides can be attached by reacting a dendrimer with N-succinimidyl S-acetylthioacetate followed by reacting with a maleimide derivative of said targeting peptides.

The targeting peptides attached to the dendrimer can have an amino acid sequence at least 85% identical to a sequence selected from the group consisting of SEQ ID NOs:1-105 and 107-117 or a fragment thereof. For example, the targeting peptide can have an amino acid sequence selected from the group consisting of Angiopep-1 (SEQ ID NO:67), Angiopep-2 (An_2) (SEQ ID NO:97), cys-Angiopep-2 ($CysAn_2$) (SEQ ID NO:113), Angiopep-2-cys (SEQ ID NO:114), and reversed Angiopep-2 (SEQ ID NO:117). Alternatively, the targeting peptide can have an amino acid sequence selected from the group consisting of Angiopep-1 (SEQ ID NO:67), Angiopep-2 (An_2) (SEQ ID NO:97), cys-Angiopep-2 ($CysAn_2$) (SEQ ID NO:113), Angiopep-2-cys (SEQ ID NO:114), and reversed Angiopep-2 (SEQ ID NO:117).

The method includes attachment of a first agent, D to the dendrimer via a reactive group. The first agent can be selected from the group consisting of a protein, a peptide, a small molecule, a nucleic acid, a diagnostic agent, an imaging agent, and a therapeutic agent.

The method also includes attachment of an optional second agent, D' to the dendrimer or the targeting peptide via a reactive group. The second agent can be selected from the group consisting of a protein, a small molecule, a nucleic acid, a diagnostic agent, an imaging agent, and a therapeutic agent. The second agent, when present, can be attached to one or more of the A_m peptides, or is attached to one or more of the X_n^{th} branches. The first and second agents maybe identical or maybe different types of molecules. The addition of D and D' may optionally involve one or more linkers which are described above.

The method also includes synthesis of a pharmaceutically acceptable salt of the compound of the invention.

By "dendrimer" is meant a synthetically produced molecule with one or more branches radiating from a core moiety. The branches include functional groups for attaching one or more targeting peptides and/or one or more agents to the dendrimer. As used herein, the term dendrimer does not include the targeting peptide or the therapeutic agent. Exemplary dendrimers include poly(amidoamine) (PAMAM) and poly(propyleneamine) (POPAM).

By "core moiety" is meant a molecule with at least three functional groups arranged symmetrically or asymmetrically. One or more branch moieties, one or more targeting peptides, and one or more agents can be attached to the core moiety via the functional groups. Exemplary core moieties include propargylamine, ethylenediamine, triethanolamine, pentaerythritol, azido-propyl(alkyl)amine, hydroxyethyl(alkyl)amine, tetraphenyl methane, trimesoylchloride, diamino hexane, diaminobutane, cystamine, propylenediamine, lysine, and propyleneamine.

By "branch moiety" is meant a molecule that can be attached to a core moiety or another branch moiety and has at least three functional groups arranged symmetrically or asymmetrically (e.g., carboxyl or amine groups). Additional branch moieties or other molecules such as targeting peptides or agents can be attached via these functional groups. The branch moiety can be same as the core moiety molecule or a derivative of the core moiety or is entirely different from the core moiety.

By "surface branch" is meant the terminal branch moiety at the surface layer of the dendrimer. The surface branch has one or more functional groups (e.g., carboxyl or amine groups) to which a peptide or another molecule (e.g., a biomolecule or a linker) can be attached.

By "fragment" is meant a portion of a full-length amino acid (e.g., any sequence described herein). A fragment may retain at least one of the biological activities of the full length protein.

By "substantially identical" is meant a polypeptide with at least 35%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 85%, 90%, 95%, or even 99% identity to a reference amino acid. For polypeptides, the length of comparison sequences will generally be at least 4 (e.g., at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 50, or 100) amino acids. It is to be understood herein that gaps may be found between the amino acids of sequences that are identical or similar to amino acids of the original polypeptide. The gaps may include no amino acids, one or more amino acids that are not identical or similar to the original polypeptide. Percent identity may be determined, for example, with n algorithm GAP, BESTFIT, or FASTA in the Wisconsin Genetics Software Package Release 7.0, using default gap weights.

By "blood-brain barrier" (BBB) is meant the membrane structure that protects the brain from chemicals in the blood, while still allowing essential metabolic function. The BBB is composed of endothelial cells, which are packed very tightly in brain capillaries. The BBB includes the blood-retinal barrier.

By "targeting peptide" is meant a compound or molecule such as a polypeptide that can be transported into a particular cell type (e.g., liver, lungs, kidney, spleen, or muscle) or across the BBB. The peptide may be attached to (covalently or not) or conjugated to ann agent via a dendrimer and thereby may be able to transport the agent into a particular cell type or across the BBB. The targeting peptide may bind to receptors present on cancer cells or brain endothelial cells and thereby be transported into the cancer cell or across the BBB by transcytosis. The targeting peptide may be a molecule for which high levels of trans-endothelial transport may be obtained, without affecting the cell or BBB integrity. The targeting peptide may be a peptide and may be naturally occurring or produced by chemical synthesis or recombinant genetic technology.

By "therapeutic agent" is meant a molecule that is capable of being used in the treatment or prophylactic treatment of a disease or condition.

By "linkage" is meant a covalent bond or a cross-linking moiety that connects two molecules e.g., a dendrimer to targeting peptides or a dendrimer to the first or second agents. Exemplary linkages include a thioether linkage.

By "linker" is meant a molecule with one or more functional groups that can be used to connect a dendrimer to targeting peptides or a dendrimer to the first or second agents. Exemplary linkers include

pyridinedisulfide, thiosulfonate, vinylsulfonate, isocyanate, NHS ester, imidoester, diazine, hydrazine, thiol, carboxylic acid, a multi-peptide linker, and acetylene.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1A is a graph showing the transport of Angiopep-2 and an Angiopep-2 multimer conjugated to PAMAM into the brain. Figure 1B is a bar graph showing the distribution of Angiopep-2-PAMAM multimer in the brain, capillaries, and parenchyma.

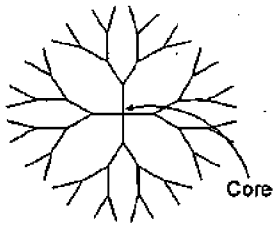
Figure 2 is an image of a gel showing migration of a radiolabeled cleavable An2-PAMAM dendrimer conjugate before and after treatment with beta-mercaptoethanol (BME). An2 is attached to the PAMAM dendrimer via a thiol ester which is cleaved by BME to release the radiolabeled Angiopep-2 peptide.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to dendrimers conjugated to targeting peptides that are able to cross the BBB or are able to enter particular cell types (e.g., liver, spleen, kidney, muscle, and ovary) with enhanced efficiency. The dendrimer-targeting peptide complex, when conjugated to one or more agents (e.g., a therapeutic agent, a diagnostic agent, an imaging agent, a small molecule, a protein, and a nucleic acid), can transport the agents across the BBB or into particular cell types (e.g., cells expressing LRP-1 receptor) with increased efficiency as compared to the agent conjugated directly to a monomeric targeting peptide. One advantage of the invention is that a dendrimer provides multiple sites for attachment of targeting peptides and agents and this helps increase transport efficiency across the BBB or into specific cell types. This increased efficiency in transport may allow for lower dosages of the agents as compared either to the unconjugated agent or to the agent conjugated to a monomeric form of the targeting peptide. This may be a helpful property in case of therapeutic and diagnostic agents. In other cases, by directing the agent more efficiently to its target tissue(s), the compounds of the invention may be administered in higher dosages than either the unconjugated agent or the agent conjugated to a monomeric form of the targeting peptide, as the greater targeting efficiency can reduce side effects. Compounds including such dendrimers and their use in diagnosis and treatment of diseases are described in detail below.

Dendrimers

A dendrimer is a branched macromolecule having a core moiety with at least three functional groups. A dendrimer can have multiple branch moieties that are attached to the core moiety, and the surface branch moieties can be functionalized for attachments of various molecules (e.g., targeting peptides). One advantage of using a dendrimer is the availability of multiple surface functionalities to which multiple molecules (e.g., targeting peptides) can be conjugated.



Dendrimer

The core moiety of a dendrimer can be any known in the art, including those selected from the group consisting of propargylamine, ethylenediamine, triethanolamine, pentaerythritol, azido-propyl(alkyl)amine, hydroxyethyl(alkyl)amine, tetraphenyl methane, trimesoylchloride, diamino hexane, diaminobutane, cystamine, and propylenediamine, in case of PAMAM dendrimers. The core moiety can also be propyleneime in which case the dendrimer is poly(propyleneamine) (POPAM). Alternatively, the core moiety can be lysine, in which case the dendrimer is poly-lysine. Typically core moieties can have 1 to 12 branches (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 branches). The core moieties are functionalized to form reactive groups (e.g., by reacting to methyl acrylate) for addition of the branch moieties. One or branch moieties can be attached to the core moiety via the functional groups. Targeting peptide and agents can also be attached to the core moiety with or without linkers via the functional groups.

The branch moieties form successive layers around the core moiety, and are also referred to as "generations" in the art. Each branch moiety attached to a branch of the core moiety can have 2 to 8 branches (2, 3, 4, 5, 6, 7, or 8 branches). The branch moieties can be the same as the core moieties, can be a derivative of the core moiety, or can be selected from the group consisting of propargylamine, ethylenediamine, triethanolamine, pentaerythritol, azido-propyl(alkyl)amine, hydroxyethyl(alkyl)amine, tetraphenyl methane, trimesoylchloride, diamino hexane, diaminobutane, cystamine, propylenediamine, proplyleneamine, and lysine.

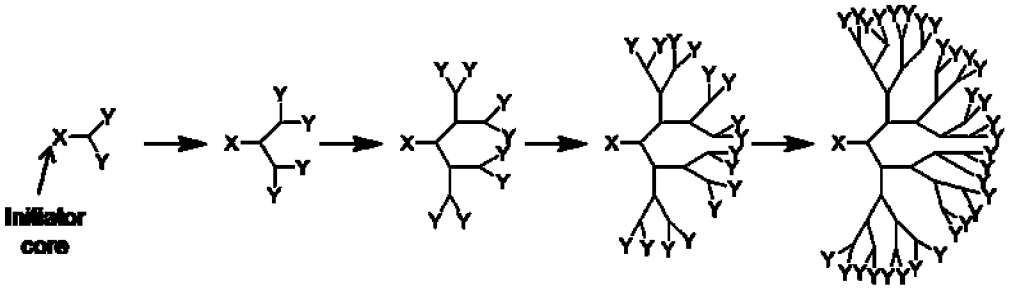
A dendrimer can have 2 to 10 layers (2, 3, 4, 5, 6, 7, 8, 9, or 10 layers) of branches, terminating in the outer most branch moieties, which are also referred to as surface branches. The surface branches can be functionalized for attachment of multiple chemical entities (e.g., targeting peptides). The number of surface branches is computed by the formula, $n = p(b)^l$, where n = the number of surface branches, b= the number of branches each branch moiety has, and l = the number of successive layers of branches of the dendrimer. Since a dendrimer will be attached to multiple targeting peptides and one or more agents, it is desirable that the dendrimer size be in a range to accommodate attachment of these cargoes. For example, a desirable dendrimer molecular weight is less than 500 kilodaltons (e.g., 10, 50, 100, 200, 300, or 500 kilodaltons).

PAMAM is perhaps the most well known dendrimer. The core of PAMAM is a diamine (commonly ethylenediamine), which is reacted with methyl acrylate, and then another ethylenediamine to make the generation-0 (G-0) PAMAM. Successive reactions create higher generations, which tend to have different properties. Lower generations are generally flexible molecules with no appreciable inner regions, while medium sized (G-3 or G-4) have internal space that is essentially separated from the outer

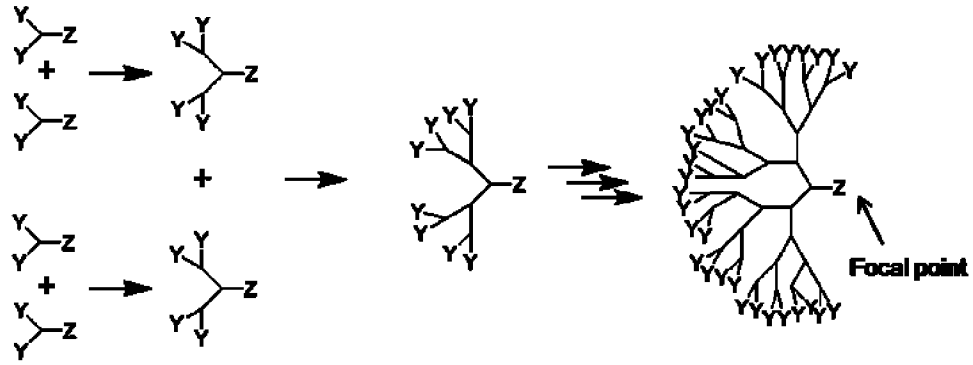
shell of the dendrimer. Very large (G-7 and greater) dendrimers are generally more like solid particles with very dense surfaces due to the structure of their outer shell.

Synthesis of dendrimers

Methods for synthesizing dendrimers are well known in the art, as described herein, and the branched portion of the dendrimer (the X_{core} and X_{branch} portions) can also be purchased from a commercial supplier with varying numbers of layers of branches. There are two commonly used methods of dendrimer synthesis: divergent synthesis and convergent synthesis. In divergent synthesis (shown below), the dendrimer is assembled from a multifunctional core, which is extended outward by a series of reactions, commonly a Michael reaction. Each step of the reaction is generally driven to full completion to prevent mistakes in the dendrimer, which can cause trailing generations (some branches are shorter than the others). Such impurities can impact the functionality and symmetry of the dendrimer, but are extremely difficult to purify out because the relative size difference between perfect and imperfect dendrimers is very small.



In convergent synthesis (shown below), dendrimers are built from small molecules that end up at the surface of the sphere, and reactions proceed inward, such that the inward most molecules that are attached last are attached to a core. This method makes it much easier to remove impurities and shorter branches along the way, so that the final dendrimer is more monodisperse. However dendrimers made this way are not as large as those made by divergent methods because crowding due to steric effects along the core is limiting.



Alternatively, dendrimers can also be synthesized by click chemistry, employing Diels-Alder reactions, thiol-yne reactions, and azide-alkyne reactions.

A dendrimer can be synthesized to have different functionalities in the core and the branches to control properties such as solubility, thermal stability, and attachment of compounds for particular applications. Synthetic processes can also precisely control the size, number of branches, numbers of layers of branches from the core, and the functionalities of the terminal branches for attachment of various reactive groups.

An exemplary synthesis of a dendrimer is shown in example 1, where a propargylamine core is reacted first with methyl acrylate followed by ethylenediamine to attach branches. Successive reactions of methyl acrylate and ethylenediamine result in attachment of further layers of ethylenediamine branches.

Conjugation of targeting peptides to surface branches of dendrimers

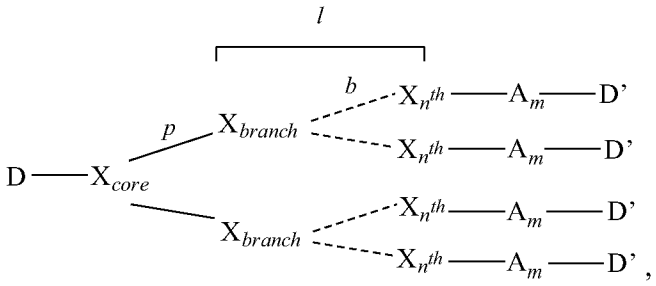
The surface branches of dendrimers can be functionalized for conjugation of targeting peptides derivatized with appropriate reactive groups. For example, the surface branches can be reacted with compounds, e.g., *N* -succinimidyl 3-(2-pyridyldithio) (SPDP) to generate a dendrimer-pyridyl-disulfide intermediate that can be then be reacted with a targeting peptides containing a cysteine residue. Alternatively, the surface branches of dendrimers can be reacted with N-succinimidyl S-acetylthioacetate (SATA) to form a dendrimer-sulfydryl intermediate that can be reacted with a maleimide derivatized targeting peptides. SATA is reactive towards amines and adds protected sulfhydryls groups), and BMOE (bis-maleimidoethane). Linkers can be used to conjugate targeting peptides to the surface functionalities of dendrimers and are described below.

Dendrimer Configurations

Each part of a given conjugate, including the cytotoxic agent, linker, and polypeptide, can be selected independently. That is, insofar as the interacting chemical substituents are compatible with one another, any of the linkers described herein can be used to conjugate any of the polypeptides and cytotoxic agents described. The conjugates can then be used to deliver the cytotoxic agents to a patient for treatment of a CNS cancer or other cancer. With the inclusion of a detectable marker, the present conjugates can also be used as imaging agents, providing the means to map the distribution of the targets to which the cytotoxic agents bind and/or the receptors for which the polypeptides have affinity.

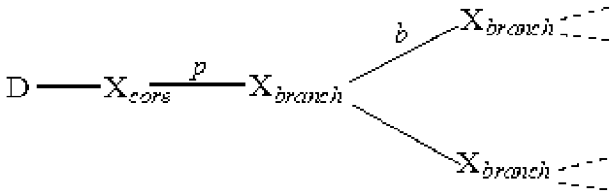
While specific configurations are discussed further below, we note that a given protein conjugate can include one or more polypeptide moieties relative to each cytotoxic agent (*e.g.*, 1-2 polypeptides relative to each cytotoxic agent within the conjugate) and one or more cytotoxic agents relative to the polypeptide (*e.g.*, 1-3 cytotoxic agents per polypeptide). As noted, a given protein conjugate is likely to include a single cytotoxic agent, but it may include two or more (*e.g.*, 2, 3, or 4) that are identical to one another or different from one another. Where different, the cytotoxic agents may specifically bind the same target or different targets. The component parts of the present conjugates can be configured in a variety of ways. Overall, the conjugate can assume an essentially linear form with a cytotoxic agent being linked to at least one polypeptide, which is in turn linked to at least one cytotoxic agent. Alternatively, the conjugate can have a branched configuration as seen in dendrimers, with one or more branches extending at some point from cytotoxic agent (D). Where the present conjugates include a branched

portion, we may refer to the conjugate as a "dendrimer conjugate" with the understanding that the inclusion of the cytotoxic agent does not allow for a fully symmetrical dendrimeric form. A dendrimeric conjugate can be structured as in Formula I:

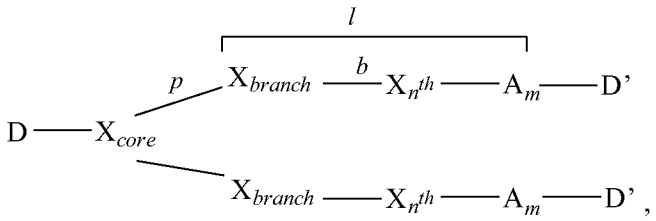


D is a cytotoxic agent that is linked to the core moiety of the dendrimer conjugate (X_{core}) either directly (*e.g.*, by way of a bond between the cytotoxic agent and X_{core}) or indirectly (*e.g.*, by way of a bifunctional linker that joins the cytotoxic agent moiety to X_{core}). As X_{core} and X_{branch} both join one part of a conjugate to another, we may also refer to either moiety more simply as a "linker". The complexity of the core moiety can vary, with the number of available extension points, *p*, varying from 2 to 6, inclusive. Each extension point *p* can terminate in (and be joined to) a branch moiety, X_{branch}, that, like X_{core}, varies in complexity with each X_{branch} having from 2 to 4 branches, *b*. X_n^{th} is one of *n* surface branches, and *l*, an integer from 1 to 5, inclusive, is the number of successive layers of X_{branch} moieties. Where *l* is 1, each X_{branch} is attached to X_{core}. Where *l* is more than 1, each X_{branch} distal to the first X_{branch} is attached to another X_{branch}. With regard to the surface branches, X_n^{th} is one of *n* surface branches of the dendrimer. $n = p(b^l)$, and *n* is typically ≤512 (*e.g.*, ≤500, ≤400, ≤300, ≤200, ≤50, ≤10, or ≤8 branches). To illustrate: where there are two extension points *p*, where *l* is 1, and where there are two branches *b* from each X_{branch}, X_n^{th} is 4; where there are three extension points *p*, where *l* is 1, and where there are three branches *b* from each X_{branch}, X_n^{th} is 9; and so forth. A_m is a polypeptide as described herein that is attached to a surface branch X_n^{th}. The number of polypeptides A_m is less than or equal to the number of surface branches, as each surface branch can be joined to a polypeptide, and some surface branches can be either free of any additional components or joined directly to a cytotoxic agent D' (*i.e.*, at some surface branches, the polypeptide represented by A_m is absent). The cytotoxic agent D' is attached to one or more A_m or, as noted, may replace one or more (but not all) A_m, attaching directly to one or more X_n^{th}. The number of D' in the dendrimer conjugate can be up to three times the number of polypeptides, as up to three cytotoxic agents can be joined to each polypeptide. The molecular weight of the dendrimer, excluding D, D' and A_m, is ≤ 500 kilodalton (*e.g.*, ≤ 500, ≤ 400, ≤ 300, ≤ 200, ≤ 100, ≤ 50, or ≤20 kilodaltons).

The linkers employed as X_{core} and X_{branch} can be the same or different, and one can make less complex dendrimer conjugates by employing a bifunctional linker as either X_{core} or X_{branch}. Where X_{core} is a bifunctional linker, *p* is 1 and the complexity that would have been generated by multiple extensions from X_{core} is missing. This arrangement is illustrated in the Formula below, with the remainder of the conjugate as described above.



In a variant of this configuration, X_{core} is absent, in which case the cytotoxic agent is joined directly to an X_{branch}. Where X_{branch} ,rather than X_{core}, is a bifunctional linker, b is 1, and the complexity that would have been generated by multiple extensions from X_{branch} is missing. This arrangement is illustrated in the Formula below, with the remainder of the conjugate as described above.



One advantage of the dendrimeric conjugate is the inclusion of multiple surface functionalities to which multiple polypeptides and/or cytotoxins can be conjugated. The ability to alter the complexity of the dendrimeric conjugate allows one to accommodate the various component parts of the protein conjugate. Where X_{core} and X_{branch} are both bifunctional linkers, the conjugate is linear, not dendrimeric.

Linkers

The targeting peptides may be conjugated through a variety of linking groups (linkers), e.g., sulfhydryl groups, amino groups (amines), or any appropriate reactive group. The linker can be a covalent bond. Homobifunctional and hetero-bifunctional cross-linkers (conjugation agents) are available from many commercial sources. Sites available for cross-linking may be found on the targeting peptides. The linker group may comprise a flexible arm, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 carbon atoms. Exemplary linkers include pyridinedisulfide, thiosulfonate, vinylsulfonate, isocyanate, imidoester, diazine, hydrazine, thiol, carboxylic acid, multi-peptide linkers, and acetylene. Alternatively other linkers than can be used include BS^3 [Bis(sulfosuccinimidyl)suberate] (which is a homobifunctional N-hydroxysuccinimide ester that targets accessible primary amines), NHS/EDC (N-hydroxysuccinimide and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (NHS/EDC allows for the conjugation of primary amine groups with carboxyl groups), sulfo-EMCS ([N-ε-maleimidocaproic acid]hydrazide (sulfo-EMCS are heterobifunctional reactive groups that are reactive toward sulfhydryl and amino groups), hydrazide (most proteins contain exposed carbohydrates and hydrazide is a useful reagent for linking carboxyl groups to primary amines).

To form covalent bonds, one can use as a chemically reactive group a wide variety of active carboxyl groups (e.g., esters) where the hydroxyl moiety is physiologically acceptable at the levels required to modify the peptide. Particular agents include N-hydroxysuccinimide (NHS), N-hydroxy-sulfosuccinimide (sulfo-NHS), maleimide-benzoyl-succinimide (MBS), gamma-maleimido-butyryloxy

succinimide ester (GMBS), maleimido propionic acid (MPA), maleimido hexanoic acid (MHA), and maleimido undecanoic acid (MUA).

Primary amines are the principal targets for NHS esters. Accessible α-amine groups present on the N-termini of proteins and the ε-amine of lysine react with NHS esters. Thus, compounds of the invention can include a linker having a NHS ester conjugated to an N-terminal amino of a peptide or to an ε-amine of lysine. An amide bond is formed when the NHS ester conjugation reaction reacts with primary amines releasing N-hydroxysuccinimide. These succinimide containing reactive groups are herein referred to as succinimidyl groups. In certain embodiments of the invention, the functional group on the protein will be a thiol group and the chemically reactive group will be a maleimido-containing group such as gamma-maleimide-butrylamide (GMBA or MPA). Such maleimide containing groups are referred to herein as maleido groups.

The maleimido group is most selective for sulfhydryl groups on peptides when the pH of the reaction mixture is 6.5-7.4. At pH 7.0, the rate of reaction of maleimido groups with sulfhydryls (e.g., thiol groups on proteins such as serum albumin or IgG) is 1000-fold faster than with amines. Thus, a stable thioether linkage between the maleimido group and the sulfhydryl can be formed. Accordingly, a compound of the invention can include a linker having a maleimido group conjugated to a sulfhydryl group of a targeting peptide.

Amine-to-amine linkers include NHS esters and imidoesters. Exemplary NHS esters are DSG (disuccinimidyl glutarate), DSS (disuccinimidyl suberate), BS^3 (bis[sulfosuccinimidyl] suberate), TSAT (*tris*-succinimidyl aminotriacetate), variants of bis-succinimide ester-activated compounds that include a polyethylene glycol spacer such as $BS(PEG)_n$ where n is 1-20 (e.g., $BS(PEG)_5$ and $BS(PEG)_9$), DSP (Dithiobis[succinimidyl propionate]), DTSSP (3,3'-dithiobis[sulfosuccinimidylpropionate]), DST (disuccinimidyl tartarate), BSOCOES (bis[2-(succinimidooxycarbonyloxy)ethyl]sulfone), EGS (ethylene glycol bis[succinimidylsuccinate]), and sulfo-EGS (ethylene glycol bis[sulfosuccinimidylsuccinate]). Imidoesters include DMA (dimethyl adipimidate•2 HCl), DMP (dimethyl pimelimidate•2 HCl), DMS (dimethyl suberimidate•2 HCl), and DTBP (dimethyl 3,3'-dithiobispropionimidate•2 HCl). Other amine-to-amine linkers include DFDNB (1,5-difluoro-2,4-dinitrobenzene) and THPP (β-[tris(hydroxymethyl) phosphino] propionic acid (betaine)).

The linker may be a sulfhydryl-to-sulfhydry linker. Such linkers include maleimides and pyridyldithiols. Exemplary maleimides include BMOE (bis-maleimidoethane), BMB (1,4-bismaleimidobutane), BMH (bismaleimidohexane), TMEA (*tris*[2-maleimidoethyl]amine), BM(PEG)2 1,8-bis-maleimidodiethyleneglycol) or $BM(PEG)_n$, where n is 1 to 20 (e.g., 2 or 3), BMDB (1,4 bismaleimidyl-2,3-dihydroxybutane), and DTME (dithio-bismaleimidoethane). Exemplary pyridyldithiols include DPDPB (1,4-di-[3'-(2'-pyridyldithio)-propionamido]butane). Other sulfhydryl linkers include HBVS (1,6-hexane-bis-vinylsulfone).

The linker may be an amine-to-sulfhydryl linker, which includes NHS ester/maliemide compounds. Examples of these compounds are AMAS (N-(α-maleimidoacetoxy)succinimide ester), BMPS (*N*-[β-maleimidopropyloxy]succinimide ester), GMBS (N-[γ-maleimidobutyryloxy]succinimide ester), sulfo-GMBS (N-[γ-maleimidobutyryloxy]sulfosuccinimide ester), MBS (*m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester), sulfo-MBS (*m*-maleimidobenzoyl-*N*-hydroxysulfosuccinimide ester), SMCC

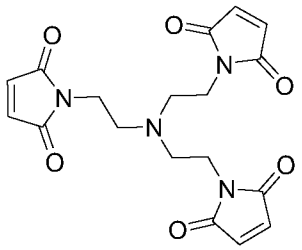
(succinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate), sulfo-SMCC (Sulfosuccinimidyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate), EMCS ([N-ε-maleimidocaproyloxy]succinimide ester), Sulfo-EMCS ([N-ε-maleimidocaproyloxy]sulfosuccinimide ester), SMPB (succinimidyl 4-[*p*-maleimidophenyl]butyrate), sulfo-SMPB (sulfosuccinimidyl 4-[*p*-maleimidophenyl]butyrate), SMPH (succinimidyl-6-[β-maleimidopropionamido]hexanoate), LC-SMCC (succinimidyl-4-[N-maleimidomethyl]cyclohexane-1-carboxy-[6-amidocaproate]), sulfo-KMUS (*N*-[κ-maleimidoundecanoyloxy]sulfosuccinimide ester), $SM(PEG)_n$ (succinimidyl-([*N*-maleimidopropionamido-polyethyleneglycol) ester), where n is 1 to 30 (e.g., 2, 4, 6, 8, 12, or 24), SPDP (*N*-succinimidyl 3-(2-pyridyldithio)-propionate), LC-SPDP (succinimidyl 6-(3-[2-pyridyldithio]-propionamido)hexanoate), sulfo-LC-SPDP (sulfosuccinimidyl 6-(3'-[2-pyridyldithio]-propionamido)hexanoate), SMPT (4-succinimidyloxycarbonyl-α-methyl-α-[2-pyridyldithio]toluene), Sulfo-LC-SMPT (4-sulfosuccinimidyl-6-[α-methyl-α-(2-pyridyldithio)toluamido]hexanoate), SIA (*N*-succinimidyl iodoacetate), SBAP (succinimidyl 3-[bromoacetamido]propionate), SIAB (*N*-succinimidyl[4-iodoacetyl]aminobenzoate), and sulfo-SIAB (*N*-sulfosuccinimidyl[4-iodoacetyl]aminobenzoate).

The linker can be an amino-to-nonselective linker. Examples of such linkers include NHS ester/aryl azide and NHS ester/diazirine linkers. NHS ester/aryl azide linkers include NHS-ASA (*N*-hydroxysuccinimidyl-4-azidosalicylic acid), ANB-NOS (*N*-5-azido-2-nitrobenzoyloxysuccinimide), sulfo-HSAB (*N*-hydroxysulfosuccinimidyl-4-azidobenzoate), sulfo-NHS-LC-ASA (sulfosuccinimidyl[4-azidosalicylamido]hexanoate), SANPAH (*N*-succinimidyl-6-(4'-azido-2'-nitrophenylamino)hexanoate), sulfo-SANPAH (*N*-sulfosuccinimidyl-6-(4'-azido-2'-nitrophenylamino)hexanoate), sulfo-SFAD (sulfosuccinimidyl-(perfluoroazidobenzamido)-ethyl-1,3'-dithioproprionate), sulfo-SAND (sulfosuccinimidyl-2-(*m*-azido-*o*-nitrobenzamido)-ethyl-1,3'-proprionate), and sulfo-SAED (sulfosuccinimidyl 2-[7-amino-4-methylcoumarin-3-acetamido]ethyl-1,3'dithiopropionate). NHS ester/diazirine linkers include SDA (succinimidyl 4,4'-azipentanoate), LC-SDA (succinimidyl 6-(4,4'-azipentanamido)hexanoate), SDAD (succinimidyl 2-([4,4'-azipentanamido]ethyl)-1,3'-dithioproprionate), sulfo-SDA (sulfosuccinimidyl 4,4'-azipentanoate), sulfo-LC-SDA (sulfosuccinimidyl 6-(4,4'-azipentanamido)hexanoate), and sulfo-SDAD (sulfosuccinimidyl 2-([4,4'-azipentanamido]ethyl)-1,3'-dithioproprionate).

Exemplary amine-to-carboxyl linkers include carbodiimide compounds (e.g., DCC (N,N-dicyclohexylcarbodimide) and EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide)). Exemplary sulfhydryl-to-nonselective linkers include pyridyldithiol/aryl azide compounds (e.g., APDP ((*N*-[4-(*p*-azidosalicylamido)butyl]-3'-(2'-pyridyldithio)propionamide)). Exemplary sulfhydryl-to-carbohydrate linkers include maleimide/hydrazide compounds (e.g., BMPH (*N*-[β-maleimidopropionic acid]hydrazide), EMCH ([*N*-ε-maleimidocaproic acid]hydrazide), MPBH 4-(4-*N*-maleimidophenyl)butyric acid hydrazide), and KMUH (*N*-[κ-maleimidoundecanoic acid]hydrazide)) and pyridyldithiol/hydrazide compounds (e.g., PDPH (3-(2-pyridyldithio)propionyl hydrazide)). Exemplary carbohydrate-to-nonselective linkers include hydrazide/aryl azide compounds (e.g., ABH (*p*-azidobenzoyl hydrazide)). Exemplary hydroxyl-to-sulfhydryl linkers include isocyanate/maleimide compounds (e.g., (*N*-[*p*-maleimidophenyl]isocyanate)). Exemplary amine-to-DNA linkers include NHS ester/psoralen compounds (e.g., SPB (succinimidyl-[4-(psoralen-8-yloxy)]-butyrate)).

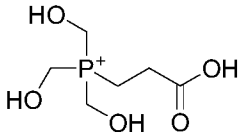
The linker can also be a trifunctional, tetrafunctional, or greater linking agent. Exemplary trifunctional linkers include TMEA, THPP, TSAT, LC-TSAT (*tris*-succinimidyl (6-aminocaproyl)aminotriacetate), *tris*-succinimidyl-1,3,5-benzenetricarboxylate, MDSI (maleimido-3,5-disuccinimidyl isophthalate), SDMB (succinimidyl-3,5-dimaleimidophenyl benzoate, Mal-4 (*tetrakis*-(3-maleimidopropyl)pentaerythritol, NHS-4 (*tetrakis*-(N-succinimidylcarboxypropyl)pentaerythritol)).

TMEA has the structure:



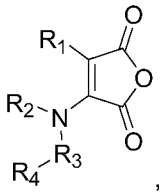
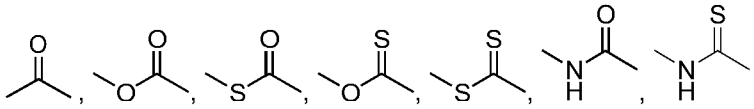
TMEA, through its maleimide groups, can react with sulfhydryl groups (e.g., through cysteine amino acid side chains).

THPP has the structure:



The hydroxyl groups and carboxy group of THPP can react with primary or secondary amines.

Linkers are also described in U.S. Patent No. 4,680,338 having the formula Y=C=N–Q–A–C(O)–Z, where Q is a homoaromatic or heteroaromatic ring system; A is a single bond or an unsubstituted or substituted divalent C_{1-30} bridging group, Y is O or S; and Z is Cl, Br, I, N_3, N-succinimidyloxy, imidazolyl, 1-benzotriazolyloxy, OAr where Ar is an electron-deficient activating aryl group, or OC(O)R where R is –A–Q–N=C=Y or C_4 -20 tertiary-alkyl.

Linkers are also described in U.S. Patent No. 5,306,809, which describes linkers having the formula  , where R_1 is H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{6-12} aryl or aralkyl or these coupled with a divalent organic –O–, –S–, or  , where R' is C_{1-6} alkyl, linking moiety; R_2 is H, C_{1-12} alkyl, C_{6-12} aryl, or C_{6-12} aralkyl, R_3 is  or another chemical structure which is able to delocalize the lone pair electrons of the adjacent nitrogen and R_4 is a pendant reactive group capable of linking R_3 to a peptide vector or to an agent.

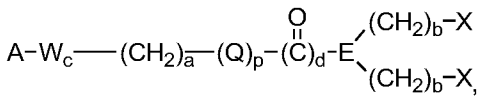
The linker may include at least one amino acid (e.g., a peptide of at least 2, 3, 4, 5, 6, 7, 10, 15, 20, 25, 40, or 50 amino acids). For example, the linker is a single amino acid (e.g., any naturally occurring amino acid such as Cys). A glycine-rich peptide such as a peptide having the sequence [Gly-ly-

Gly-Gly-Ser]$_n$ where n is 1, 2, 3, 4, 5 or 6, as described in U.S. Patent No. 7,271,149, or a serine-rich peptide linker is used, as described in U.S. Patent No. 5,525,491, can be used. Serine rich peptide linkers include those of the formula [X-X-X-X-Gly]$_y$, where up to two of the X are Thr, and the remaining X are Ser, and y is 1 to 5 (e.g., Ser-Ser-Ser-Ser-Gly, where y is greater than 1).

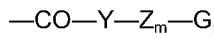
In some cases, the linker is a single amino acid (e.g., any amino acid, such as Gly or Cys). In some cases the linkers can be multi-amino acid or multi-peptide linkers. Amino acid linkers and multi-peptide linkers can be selected for flexibility (e.g., flexible or rigid) or may be selected on the basis of charge (e.g., positive, negative, or neutral). Flexible linkers typically include those with Gly resides (e.g., [Gly-Gly-Gly-Gly-Ser]$_n$ where n is 1, 2, 3, 4, 5 or 6). Other linkers include rigid linkers (e.g., PAPAP and (PT)$_n$P, where n is 2, 3, 4, 5, 6, or 7) and α-helical linkers (e.g., A(EAAAK)$_n$A, where n is 1, 2, 3, 4, or 5).

Examples of suitable amino acid linkers are succinic acid, Lys, Glu, and Asp, or a dipeptide such as Gly-Lys. When the linker is succinic acid, one carboxyl group thereof may form an amide bond with an amino group of the amino acid residue, and the other carboxyl group thereof may, for example, form an amide bond with an amino group of the peptide or substituent. When the linker is Lys, Glu, or Asp, the carboxyl group thereof may form an amide bond with an amino group of the amino acid residue, and the amino group thereof may, for example, form an amide bond with a carboxyl group of the substituent. When Lys is used as the linker, a further linker may be inserted between the ε-amino group of Lys and the substituent. The further linker may be succinic acid, which can form an amide bond with the ε- amino group of Lys and with an amino group present in the substituent. In one embodiment, the further linker is Glu or Asp (e.g., which forms an amide bond with the ε-amino group of Lys and another amide bond with a carboxyl group present in the substituent), that is, the substituent is a N^{ε}-acylated lysine residue.

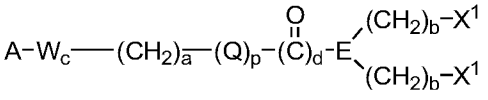
The peptide linker can also be a branched polypeptide. Exemplary branched peptide linkers are described in U.S. Patent No. 6,759,509. Such linkers include those of the formula:



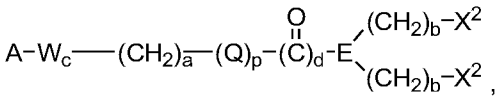
where A is a thiol acceptor; W is a bridging moiety; c is an integer of 0 to 1; a is an integer of 2 to 12; Q is O, NH, or N-lower alkyl; p is an integer of 0 or 1; d is an integer of 0 or 1; E is a polyvalent atom; each b is an integer of 1 to 10; each X is of the formula:



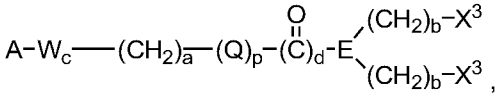
where Y is two amino acid residues in the L form; Z is one or two amino acid residues; m is an integer of 0 or 1; G is a self-immolative spacer; and n is a integer of 0 or 1; provided that when n is 0 then —Y—Z$_m$ is Ala-Leu-Ala-Leu or Gly-Phe-Leu-Gly; or each X is of the formula:



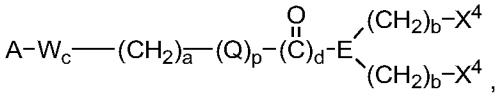
where each X^1 is of the formula —CO—Y—Z_m—G_n; and where Y, Z, Q, E, G, m, d, p, a, b, and n are as defined above; or each X^1 is of the formula:



where each X^2 is of the formula —CO—Y—Z_m—G_n; and where Y, Z, G, Q, E, m, d, p, a, b, and n are as defined above; or each X^2 is of the formula:

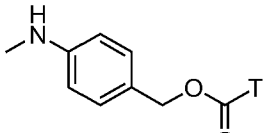


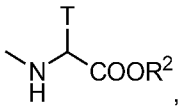
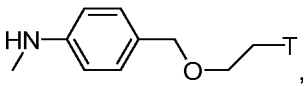
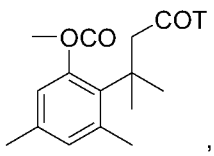
where each X^3 is of the formula —CO—Y—Z_m—G_n; and wherein Y, Z, G, Q, E, m, d, p, a, b, and n are as defined above; or each X^3 is of the formula



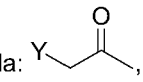
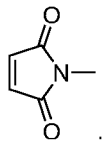
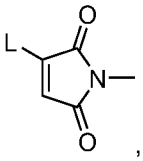
where each X^4 is of the formula —CO—Y—Z_m—G_n; and where Y, Z, G, Q, E, m, d, p, a, b, and n are as defined above.

The branched linker may employ an intermediate self-immolative spacer moiety (G), which covalently links together the agent or peptide vector and the branched peptide linker. A self-immolative spacer can be a bifunctional chemical moiety capable of covalently linking together two chemical moieties and releasing one of said spaced chemical moieties from the tripartate molecule by means of enzymatic cleavage (e.g., any appropriate linker described herein. In certain embodiments, G is a self-immolative spacer moiety which spaces and covalently links together the agent or peptide vector and the peptide linker, where the spacer is linked to the peptide vector or agent via the T moiety (as used in the following formulas "T" represents a nucleophilic atom which is already contained in the agent or peptide vector),



and which may be represented by , where T is O, N or S; —HN—R^1—COT, where T is O, N or S, and R^1 is C_{1-5} alkyl;  , where T is O, N, or S, and R^2 is H or C_{1-5} alkyl;  , where T is O, N or S; or  , where T is O, N, or S. Preferred

Gs include PABC (p-aminobenzyl-carbamoyl), GABA (γ-aminobutyric acid), α,α-dimethyl GABA, and β,β-dimethyl GABA.

In the branched linker, the thiol acceptor "A" is linked to a peptide vector or agent by a sulfur atom derived from the peptide vector or agent. The thiol acceptor can be, for example, an α-substituted acetyl group. Such a group has the formula:  , where Y is a leaving group such as Cl, Br, I, mesylate, tosylate, and the like. If the thiol acceptor is an alpha-substituted acetyl group, the thiol adduct after linkage to the ligand forms the bond —S—CH_2 —. Preferably, the thiol acceptor is a Michael Addition acceptor. A representative Michael Addition acceptor of this invention has the formula  . After linkage the thiol group of the ligand, the Michael Addition acceptor becomes a Michael Addition adduct, e.g.,  , where L is an agent or peptide vector.

The bridging group "W" is a bifunctional chemical moiety capable of covalently linking together two spaced chemical moieties into a stable tripartate molecule. Examples of bridging groups are described in S. S. Wong, *Chemistry of Protein Conjugation and Crosslinking* . CRC Press, Florida, (1991); and G. E. Means and R. E. Feeney, *Bioconiugate Chemistry, vol.* 1, pp.2-12, (1990), the disclosures of which are incorporated herein by reference. W can covalently link the thiol acceptor to a keto moiety. An exemplary a bridging group has the formula —$(CH_2)_f$ —$(Z)_g$ —$(CH_2)_h$—, where f is 0 to 10; h is 0 to 10; g is 0 or 1, provided that when g is 0, then f+h is 1 to 10; Z is S, O, NH, SO_2, phenyl, naphthyl, a polyethylene glycol, a cycloaliphatic hydrocarbon ring containing 3 to 10 carbon atoms, or a heteroaromatic hydrocarbon ring containing 3 to 6 carbon atoms and 1 or 2 heteroatoms selected from O, N, or S. Preferred cycloaliphatic moieties include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like. Preferred heteroaromatic moieties include pyridyl, polyethlene glycol (1-20 repeating units), furanyl, pyranyl, pyrimidinyl, pyrazinyl, pyridazinyl, oxazinyl, pyrrolyl, thiazolyl, morpholinyl, and the like. In the bridging group, it is preferred that when g is 0, f+h is an integer of 2 to 6 (e.g., 2 to 4 such as 2). When g is 1, it is preferred that f is 0, 1 or 2; and that h is 0, 1 or 2. Preferred bridging groups coupled to thiol acceptors are shown in the Pierce Catalog, pp. E-12, E-13, E-14, E-15, E-16, and E-17 (1992).

The linker between a targeting peptide and the dendrimer can be a cleavable linker (e.g., a thiol ester linker) or a non-cleavable linker.

Targeting peptides

The targeting peptide of the invention can be attached to the dendrimer to form a dendrimer-targeting peptide complex. The targeting peptide may be a polypeptide substantially identical to any of the sequences in Table 1, or a fragment thereof. The targeting peptide may have a sequence of Angiopep-1 (SEQ ID NO:67), Angiopep-2 (SEQ ID NO:97), Angiopep-3 (SEQ ID NO:107), Angiopep-4a

(SEQ ID NO:108), Angiopep-4b (SEQ ID NO:109), Angiopep-5 (SEQ ID NO:110), Angiopep-6 (SEQ ID NO:111), Angiopep-7 (SEQ ID NO:112) or reversed Angiopep-2 (SEQ ID NO:117)). The targeting peptide or compound of the invention may be efficiently transported into a particular cell type (e.g., any one, two, three, four, or five of liver, lung, kidney, spleen, and muscle) or may cross the mammalian BBB efficiently (e.g., Angiopep-1, -2, -3, -4a, -4b, -5, and -6). The targeting peptide or compound will be able to enter a particular cell type (e.g., any one, two, three, four, or five of liver, lung, kidney, spleen, and muscle) but does not cross the BBB efficiently (e.g., a conjugate including Angiopep-7). The targeting peptide may be of any length, for example, at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 25, 35, 50, 75, 100, 200, or 500 amino acids, or any range between these numbers. The targeting peptide is 10 to 50 amino acids in length and may be produced by recombinant genetic technology or chemical synthesis.

Table 1: Exemplary Targeting peptides

SEQ ID NO:																		
1	T	F	V	Y	G	G	C	R	A	K	R	N	N	F	K	S	A	E	D
2	T	F	Q	Y	G	G	C	M	G	N	G	N	N	F	V	T	E	K	E
3	P	F	F	Y	G	G	C	G	G	N	R	N	N	F	D	T	E	E	Y
4	S	F	Y	Y	G	G	C	L	G	N	K	N	N	Y	L	R	E	E	E
5	T	F	F	Y	G	G	C	R	A	K	R	N	N	F	K	R	A	K	Y
6	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	R	A	K	Y
7	T	F	F	Y	G	G	C	R	A	K	K	N	N	Y	K	R	A	K	Y
8	T	F	F	Y	G	G	C	R	G	K	K	N	N	F	K	R	A	K	Y
9	T	F	Q	Y	G	G	C	R	A	K	R	N	N	F	K	R	A	K	Y
10	T	F	Q	Y	G	G	C	R	G	K	K	N	N	F	K	R	A	K	Y
11	T	F	F	Y	G	G	C	L	G	K	R	N	N	F	K	R	A	K	Y
12	T	F	F	Y	G	G	S	L	G	K	R	N	N	F	K	R	A	K	Y
13	P	F	F	Y	G	G	C	G	G	K	K	N	N	F	K	R	A	K	Y
14	T	F	F	Y	G	G	C	R	G	K	G	N	N	Y	K	R	A	K	Y
15	P	F	F	Y	G	G	C	R	G	K	R	N	N	F	L	R	A	K	Y
16	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	R	E	K	Y
17	P	F	F	Y	G	G	C	R	A	K	K	N	N	F	K	R	A	K	E
18	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	R	A	K	D
19	T	F	F	Y	G	G	C	R	A	K	R	N	N	F	D	R	A	K	Y
20	T	F	F	Y	G	G	C	R	G	K	K	N	N	F	K	R	A	E	Y
21	P	F	F	Y	G	G	C	G	A	N	R	N	N	F	K	R	A	K	Y
22	T	F	F	Y	G	G	C	G	G	K	K	N	N	F	K	T	A	K	Y
23	T	F	F	Y	G	G	C	R	G	N	R	N	N	F	L	R	A	K	Y
24	T	F	F	Y	G	G	C	R	G	N	R	N	N	F	K	T	A	K	Y
25	T	F	F	Y	G	G	S	R	G	N	R	N	N	F	K	T	A	K	Y
26	T	F	F	Y	G	G	C	L	G	N	G	N	N	F	K	R	A	K	Y
27	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	L	R	A	K	Y
28	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	K	T	A	K	Y
29	T	F	F	Y	G	G	C	R	G	N	G	N	N	F	K	S	A	K	Y
30	T	F	F	Y	G	G	C	R	G	K	K	N	N	F	D	R	E	K	Y
31	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	L	R	E	K	E
32	T	F	F	Y	G	G	C	R	G	K	G	N	N	F	D	R	A	K	Y
33	T	F	F	Y	G	G	S	R	G	K	G	N	N	F	D	R	A	K	Y
34	T	F	F	Y	G	G	C	R	G	N	G	N	N	F	V	T	A	K	Y
35	P	F	F	Y	G	G	C	G	G	K	G	N	N	Y	V	T	A	K	Y
36	T	F	F	Y	G	G	C	L	G	K	G	N	N	F	L	T	A	K	Y

37	S	F	F	Y	G	G	C	L	G	N	K	N	N	F	L	T	A	K	Y				
38	T	F	F	Y	G	G	C	G	G	N	K	N	N	F	V	R	E	K	Y				
39	T	F	F	Y	G	G	C	M	G	N	K	N	N	F	V	R	E	K	Y				
40	T	F	F	Y	G	G	S	M	G	N	K	N	N	F	V	R	E	K	Y				
41	P	F	F	Y	G	G	C	L	G	N	R	N	N	Y	V	R	E	K	Y				
42	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	V	R	E	K	Y				
43	T	F	F	Y	G	G	C	L	G	N	K	N	N	Y	V	R	E	K	Y				
44	T	F	F	Y	G	G	C	G	G	N	G	N	N	F	L	T	A	K	Y				
45	T	F	F	Y	G	G	C	R	G	N	R	N	N	F	L	T	A	E	Y				
46	T	F	F	Y	G	G	C	R	G	N	G	N	N	F	K	S	A	E	Y				
47	P	F	F	Y	G	G	C	L	G	N	K	N	N	F	K	T	A	E	Y				
48	T	F	F	Y	G	G	C	R	G	N	R	N	N	F	K	T	E	E	Y				
49	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	T	E	E	D				
50	P	F	F	Y	G	G	C	G	G	N	G	N	N	F	V	R	E	K	Y				
51	S	F	F	Y	G	G	C	M	G	N	G	N	N	F	V	R	E	K	Y				
52	P	F	F	Y	G	G	C	G	G	N	G	N	N	F	L	R	E	K	Y				
53	T	F	F	Y	G	G	C	L	G	N	G	N	N	F	V	R	E	K	Y				
54	S	F	F	Y	G	G	C	L	G	N	G	N	N	Y	L	R	E	K	Y				
55	T	F	F	Y	G	G	S	L	G	N	G	N	N	F	V	R	E	K	Y				
56	T	F	F	Y	G	G	C	R	G	N	G	N	N	F	V	T	A	E	Y				
57	T	F	F	Y	G	G	C	L	G	K	G	N	N	F	V	S	A	E	Y				
58	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	D	R	A	E	Y				
59	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	L	R	E	E	Y				
60	T	F	F	Y	G	G	C	L	G	N	K	N	N	Y	L	R	E	E	Y				
61	P	F	F	Y	G	G	C	G	G	N	R	N	N	Y	L	R	E	E	Y				
62	P	F	F	Y	G	G	S	G	G	N	R	N	N	Y	L	R	E	E	Y				
63	M	R	P	D	F	C	L	E	P	P	Y	T	G	P	C	V	A	R	I				
64	A	R	I	I	R	Y	F	Y	N	A	K	A	G	L	C	Q	T	F	V	Y	G		
65	Y	G	G	C	R	A	K	R	N	N	Y	K	S	A	E	D	C	M	R	T	C	G	
66	P	D	F	C	L	E	P	P	Y	T	G	P	C	V	A	R	I	I	R	Y	F	Y	
67	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	T	E	E	Y				
68	K	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	T	E	E	Y				
69	T	F	Y	Y	G	G	C	R	G	K	R	N	N	Y	K	T	E	E	Y				
70	T	F	F	Y	G	G	S	R	G	K	R	N	N	F	K	T	E	E	Y				
71	C	T	F	F	Y	G	C	C	R	G	K	R	N	N	F	K	T	E	E	Y			
72	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	T	E	E	Y	C			
73	C	T	F	F	Y	G	S	C	R	G	K	R	N	N	F	K	T	E	E	Y			
74	T	F	F	Y	G	G	S	R	G	K	R	N	N	F	K	T	E	E	Y	C			
75	P	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	T	E	E	Y				
76	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	T	K	E	Y				
77	T	F	F	Y	G	G	K	R	G	K	R	N	N	F	K	T	E	E	Y				
78	T	F	F	Y	G	G	C	R	G	K	R	N	N	F	K	T	K	R	Y				
79	T	F	F	Y	G	G	K	R	G	K	R	N	N	F	K	T	A	E	Y				
80	T	F	F	Y	G	G	K	R	G	K	R	N	N	F	K	T	A	G	Y				
81	T	F	F	Y	G	G	K	R	G	K	R	N	N	F	K	R	E	K	Y				
82	T	F	F	Y	G	G	K	R	G	K	R	N	N	F	K	R	A	K	Y				
83	T	F	F	Y	G	G	C	L	G	N	R	N	N	F	K	T	E	E	Y				
84	T	F	F	Y	G	C	G	R	G	K	R	N	N	F	K	T	E	E	Y				
85	T	F	F	Y	G	G	R	C	G	K	R	N	N	F	K	T	E	E	Y				
86	T	F	F	Y	G	G	C	L	G	N	G	N	N	F	D	T	E	E	E				
87	T	F	Q	Y	G	G	C	R	G	K	R	N	N	F	K	T	E	E	Y				
88	Y	N	K	E	F	G	T	F	N	T	K	G	C	E	R	G	Y	R	F				
89	R	F	K	Y	G	G	C	L	G	N	M	N	N	F	E	T	L	E	E				
90	R	F	K	Y	G	G	C	L	G	N	K	N	N	F	L	R	L	K	Y				
91	R	F	K	Y	G	G	C	L	G	N	K	N	N	Y	L	R	L	K	Y				
92	K	T	K	R	K	R	K	K	Q	R	V	K	I	A	Y	E	E	I	F	K	N	Y	
93	K	T	K	R	K	R	K	K	Q	R	V	K	I	A	Y								

94	R G G R L S Y S R R F S T S T G R
95	R R L S Y S R R R F
96	R Q I K I W F Q N R R M K W K K
97	T F F Y G G S R G K R N N F K T E E Y
98	M R P D F C L E P P Y T G P C V A R I
	I R Y F Y N A K A G L C Q T F V Y G G
	C R A K R N N F K S A E D C M R T C G G A
99	T F F Y G G C R G K R N N F K T K E Y
100	R F K Y G G C L G N K N N Y L R L K Y
101	T F F Y G G C R A K R N N F K R A K Y
102	N A K A G L C Q T F V Y G G C L A K R N N F
	E S A E D C M R T C G G A
103	Y G G C R A K R N N F K S A E D C M R T C G G A
104	G L C Q T F V Y G G C R A K R N N F K S A E
105	L C Q T F V Y G G C E A K R N N F K S A
107	T F F Y G G S R G K R N N F K T E E Y
108	R F F Y G G S R G K R N N F K T E E Y
109	R F F Y G G S R G K R N N F K T E E Y
110	R F F Y G G S R G K R N N F R T E E Y
111	T F F Y G G S R G K R N N F R T E E Y
112	T F F Y G G S R G R R N N F R T E E Y
113	C T F F Y G G S R G K R N N F K T E E Y
114	T F F Y G G S R G K R N N F K T E E Y C
115	C T F F Y G G S R G R R N N F R T E E Y
116	T F F Y G G S R G R R N N F R T E E Y C
117	Y E E T K F N N R K G R S G G Y F F T

Polypeptides Nos. 5, 67, 76, and 91, include the sequences of SEQ ID NOS:5, 67, 76, and 91, respectively, and are amidated at the C-terminus.

Polypeptides Nos. 107, 109, and 110 include the sequences of SEQ ID NOS:97, 109, and 110, respectively, and are acetylated at the N-terminus.

The targeting peptide may include an amino acid sequence having the formula:

X1-X2-X3-X4-X5-X6-X7-X8-X9-X10-X11-X12-X13-X14-X15-X16-X17-X18-X19,

where each of X1-X19 (e.g., X1-X6, X8, X9, X11-X14, and X16-X19) is, independently, any amino acid (e.g., a naturally occurring amino acid such as Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val) or absent and at least one (e.g., 2 or 3) of X1, X10, and X15 is arginine. X7 can be Ser or Cys; or X10 and X15 each are independently Arg or Lys. The residues from X1 through X19, inclusive, can be substantially identical to any of the amino acid sequences of any one of SEQ ID NOS:1-105 and 107-117 (e.g., Angiopep-1, Angiopep-2, Angiopep-3, Angiopep-4a, Angiopep-4b, Angiopep-5, Angiopep-6, Angiopep-7, and reversed Angiopep-2). At least one (e.g., 2, 3, 4, or 5) of the amino acids from X1-X19 is Arg. The polypeptide can have one or more additional cysteine residues at

the N-terminal of the polypeptide, the C-terminal of the polypeptide, or both. For example, the targeting peptide can have an amino acid sequence selected from the group consisting of cys-Angiopep-2 ($CysAn_2$) (SEQ ID NO:113), Angiopep-2-cys (SEQ ID NO:114).

The targeting peptide can be modified, e.g., amidated, acetylated, or both. Such modifications may be at the amino or carboxy terminus of the polypeptide. The peptide or polypeptide may also include peptidomimetics (e.g., those described herein) of any of the polypeptides described herein.

The targeting peptide can have an amino acid sequence described herein with at least one amino acid substitution (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 substitutions), insertion, or deletion or is substantially identical to an amino acid sequence described herein. The peptide or polypeptide may contain, for example, 1 to 12, 1 to 10, 1 to 5, or 1 to 3 amino acid substitutions, for example, 1 to 10 (e.g., to 9, 8, 7, 6, 5, 4, 3, 2) amino acid substitutions. The amino acid substitution(s) may be conservative or non-conservative. For example, the targeting peptide may have an arginine at one, two, or three of the positions corresponding to positions 1, 10, and 15 of the amino acid sequence of any of SEQ ID NO:1, Angiopep-1, Angiopep-2, Angiopep-3, Angiopep-4a, Angiopep-4b, Angiopep-5, Angiopep-6, Angiopep-7, and reversed Angiopep-2.

The invention also features fragments of these polypeptides (e.g., a functional fragment). Truncations of the polypeptide may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more amino acids from either the N-terminus of the polypeptide, the C-terminus of the polypeptide, or a combination thereof. Other fragments include sequences where internal portions of the polypeptide are deleted.

The targeting peptide of the invention can also have the formula:

Lys-Arg-X3-X4-X5-Lys (formula Ia),

where

X3 is Asn or Gln;

X4 is Asn or Gln; and

X5 is Phe, Tyr, or Trp.

The targeting peptide of the invention can also have the formula:

Z1-Lys-Arg-X3-X4-X5-Lys-Z2 (formula Ib),

where

X3 is Asn or Gln;

X4 is Asn or Gln;

X5 is Phe, Tyr, or Trp;

Z1 is absent, Cys, Gly, Cys-Gly, Arg-Gly, Cys-Arg-Gly, Ser-Arg-Gly, Cys-Ser-Arg-Gly, Gly-Ser-Arg-Gly, Cys-Gly-Ser-Arg-Gly, Gly-Gly-Ser-Arg-Gly, Cys-Gly-Gly-Ser-Arg-Gly, Tyr-Gly-Gly-Ser-Arg-Gly, Cys-Tyr-Gly-Gly-Ser-Arg-Gly, Phe-Tyr-Gly-Gly-Ser-Arg-Gly, Cys-Phe-Tyr-Gly-Gly-Ser-Arg-Gly, Phe-Phe-Tyr-Gly-Gly-Ser-Arg-Gly, Cys-Phe-Phe-Tyr-Gly-Gly-Ser-Arg-Gly, Thr-Phe-Phe-Tyr-Gly-Gly-Ser-Arg-Gly, or Cys-Thr-Phe-Phe-Tyr-Gly-Gly-Ser-Arg-Gly; and

Z2 is absent, Cys, Tyr, Tyr-Cys, Cys-Tyr, Thr-Glu-Glu-Tyr, or Thr-Glu-Glu-Tyr-Cys.

The targeting peptide of formulas (Ia) and (Ib) include the amino acid sequence Lys-Arg-Asn-Asn-Phe-Lys and conservative substitutions. Conservative substitutions and derivatives of amino acids and

peptides are well known in the art and can be determined by any useful methods (e.g., by using a substitution matrix or any other method described herein). A derivative of a targeting peptide includes a targeting moiety containing one or more conservative substitutions selected from the following groups or a subset of these groups: Ser, Thr, and Cys; Leu, Ile, and Val; Glu and Asp; Lys and Arg; Phe, Tyr, and Trp (e.g., Phe and Tyr); and Gln, Asn, Glu, Asp, and His (e.g., Gln and Asn). Conservative substitutions may also be determined by other methods, such as by the BLAST (Basic Local Alignment Search Tool) algorithm, the BLOSUM substitution matrix (e.g., BLOSUM 62 matrix), and PAM substitution matrix (e.g., PAM 250 matrix).

The targeting peptide can also include those having one or more D-amino acid substitutions, where one or more amino acid residues of formula (Ia) or (Ib) are substituted with a corresponding D-isomer. D-amino acid substitutions may provide peptides having increased resistance to cleavage by digestive enzymes (e.g., pepsin and/or trypsin). For example, one or more of amino acids in formula (Ia) or (Ib) having possible cleavage sites by pepsin or trypsin can be substituted with the D-isomer of that amino acid. Exemplary cleavage sites in formula (Ia) or (Ib) by pepsin and trypsin include the bond that is N-terminal or C-terminal to position 1 for Lys; position 2 for Arg; position 5 for X5 being Phe, Tyr, or Trp; and position 6 for Lys. Accordingly, the polypeptides of the invention also include those having one or more D-isomers for the amino acids recited at positions 1, 2, 5, and/or 6 of formula (Ia) or (Ib).

The targeting peptide of the invention can also have the formula:

X1-X2-Asn-Asn-X5-X6 (formula IIa),

where

X1 is Lys or D-Lys;

X2 is Arg or D-Arg;

X5 is Phe or D-Phe; and

X6 is Lys or D-Lys; and

where at least one of X1, X2, X5, or X6 is a D-amino acid.

The targeting peptide of the invention can also have the formula:

X1-X2-Asn-Asn-X5-X6-X7 (formula IIb),

where

X1 is Lys or D-Lys;

X2 is Arg or D-Arg;

X5 is Phe or D-Phe;

X6 is Lys or D-Lys;

X7 is Tyr or D-Tyr; and

where at least one of X1, X2, X5, X6, or X7 is a D-amino acid.

The targeting peptide of the invention can also have the formula:

Z1-X1-X2-Asn-Asn-X5-X6-X7-Z2 (formula IIc),

where

X1 is Lys or D-Lys;

X2 is Arg or D-Arg;

X5 is Phe or D-Phe;

X6 is Lys or D-Lys;

X7 is Tyr or D-Tyr;

Z1 is absent, Cys, Gly, Cys-Gly, Arg-Gly, Cys-Arg-Gly, Ser-Arg-Gly, Cys-Ser-Arg-Gly, Gly-Ser-Arg-Gly, Cys-Gly-Ser-Arg-Gly, Gly-Gly-Ser-Arg-Gly, Cys-Gly-Gly-Ser-Arg-Gly, Tyr-Gly-Gly-Ser-Arg-Gly, Cys-Tyr-Gly-Gly-Ser-Arg-Gly, Phe-Tyr-Gly-Gly-Ser-Arg-Gly, Cys-Phe-Tyr-Gly-Gly-Ser-Arg-Gly, Phe-Phe-Tyr-Gly-Gly-Ser-Arg-Gly, Cys-Phe-Phe-Tyr-Gly-Gly-Ser-Arg-Gly, Thr-Phe-Phe-Tyr-Gly-Gly-Ser-Arg-Gly, or Cys-Thr-Phe-Phe-Tyr-Gly-Gly-Ser-Arg-Gly; and Z2 is absent, Cys, Tyr, Tyr-Cys, Cys-Tyr, Thr-Glu-Glu-Tyr, or Thr-Glu-Glu-Tyr-Cys; where at least one of X1, X2, X5, X6, or X7 is a D-amino acid; and where the polypeptide optionally includes one or more D-isomers of an amino acid recited in Z1 or Z2.

The targeting peptides of the invention include additions and deletions of amino acids to the formula of Lys-Arg-X3-X4-X5-Lys (formula Ia), where X3-X5 are as defined above; the formulae of X1-X2-Asn-Asn-X5-X6 and X1-X2-Asn-Asn-X5-X6-X7 (formulas IIa and IIb, respectively), where X1, X2, X5, X6, and X7 are as defined above; or the longer polypeptide of 3D-An2, as described herein. The deletions or additions can include any part of the formula of Lys-Arg-X3-X4-X5-Lys, X1-X2-Asn-Asn-X5-X6, X1-X2-Asn-Asn-X5-X6-X7, Lys-Arg-Asn-Asn-Phe-Lys, D-Lys-D-Arg-Asn-Asn-D-Phe-D-Lys, or D-Lys-D-Arg-Asn-Asn-D-Phe-D-Lys-D-Tyr, or of the longer sequence 3D-An2. Deletions or additions of 1, 2, 3, 4, or 5 amino acids may be made from the consensus sequence of the targeting moiety. Any useful substitutions, additions, and deletions can be made that does not destroy significantly the desired biological activity (e.g., ability to cross the BBB or agonist activity) of the targeting peptide. The modification may reduce (e.g., by at least 5%, 10%, 20%, 25%, 35%, 50%, 60%, 70%, 75%, 80%, 90%, or 95%), may have no effect, or may increase (e.g., by at least 5%, 10%, 25%, 50%, 100%, 200%, 500%, or 1000%) the biological activity of the consensus sequence or original polypeptide.

In particular, substitutions or additions of D-amino acids can be made within the targeting peptide. Such substitutions or additions may provide peptides having increased resistance to cleavage by enzymes, where one of more amino acids for cleavage sites can be substituted with its D-isomer. Exemplary enzymes include pepsin, trypsin, Arg-C proteinase, Asp-N endopeptidase, chymotrypsin, glutamyl endopeptidase, LysC lysyl endopeptidase, LysN peptidyl-Lys metalloendopeptidase, proteinase K, and thermolysin; and exemplary cleavage sites for these enzymes are described herein.

Furthermore, substitutions, additions and deletions may have or may optimize a characteristic of the targeting peptide, such as charge (e.g., positive or negative charge), hydrophilicity, hydrophobicity, in vivo stability, bioavailability, toxicity, immunological activity, immunological identity, and conjugation properties. For example, positive charge can be promoted by deleting one or more amino acids (e.g., from 1 to 3 amino acids) that are not basic/positively charged (as described below based on common side chain properties) or less positively charged (e.g., as determined by pKa). In another example, positive charge can be promoted by inserting one or more amino acids (e.g., from 1 to 3 amino acids) that are basic/positively charged or more positively charged (e.g., as determined by pKa).

Agents and conjugation of agents to dendrimer-targeting peptide complex

Any agent may be chemically conjugated to the dendrimer-targeting peptide complex. Such agents include small-molecule drugs, antibiotics, anticancer agents, an imaging agent (e.g., a radio

imaging agent), a diagnostic agent, a therapeutic agent, a reporter molecule, RNAi agents, and peptide and polypeptide therapeutics. The agents are conjugated via a functional group present on the dendrimer-targeting peptide complex. The functional group can include, without limitations, maleimide, a hydrazide, an azide, a haloacetamide, and an alkoxyamine. In addition, any of the linkers described above can also be used to conjugate an agent to the dendrimer-targeting peptide complex.

The compound of the invention can have a first agent attached to the dendrimer-targeting peptide complex at one location, for example a first agent can be attached to the core moiety. The compound of the invention can also have an optional second agent attached to the dendrimer-targeting peptide complex at a second location, for example one or more second agents attached to one or more targeting peptides or to one or more surface branches. A second agent may be attached to the targeting peptide or the surface branch via a linker. A second agent, when present, maybe the same as the agent or maybe a different type of agent compared to the first agent, e.g., the first agent is one cytotoxic agent and the second agent is a second cytotoxic agent, and the combination of cytotoxic agents are a combination therapy for a disease.

Manufacturing compounds of the invention

The invention features methods to synthesize the compounds that include a complex of dendrimer, targeting peptides and one or more agents. Dendrimers with a variety of core moieties and branch moieties, and with different numbers of surface branches and reactive groups are commercially available. The dendrimer can be conjugated to multiple Angiopep peptides via reactive groups on surface branches. For example, this can be done by reacting a dendrimer with *N* -Succinimidyl 3-(2-pyridyldithio)-propionate to form a dendrimer-pyridyl-disulfide intermediate; and then reacting the dendrimer-pyridyl-disulfide intermediate with targeting peptides containing cysteine residues to attach a targeting peptide to each of the surface branches. Alternatively, the dendrimer can be reacted with N-succinimidyl S-acetylthioacetate to form a dendrimer-sulfydryl intermediate followed by a reaction with a maleimide derivative of targeting peptides to form a dendrimer-targeting peptide complex.

The dendrimer-targeting peptide complex is then reacted with a first agent as described above and the resulting dendrimer-targeting peptide-first agent complex can be produced in a pharmaceutically acceptable form (e.g., a pharmaceutically acceptable salt).

Alternatively, the dendrimer can first be reacted with a first agent via a functional group (e.g., azide), and the surface branches of the resulting dendrimer-first agent complex can be functionalized to attach surface branches.

The method of manufacturing a compound of the invention may additionally involve attachment of any of the linkers described above to the dendrimer prior to attachment of the targeting peptides or the first agent.

The method of manufacturing a compound of the invention may optionally involve attachment of one or more second agents to the compound, at locations that may be different than the first agent. For example, one or more second agents can be attached to one or more of the targeting peptides. Alternatively, one or more second agents can be attached to one or more of the surface branches of the

dendrimer. At each of these locations, the attachment of a second agent can involve the use of a linker as described above.

Assays to determine accumulation of compound of the invention in tissues

Assays to determine accumulation of the compound of the invention in tissues may be performed to evaluate the transport capabilities of multiple targeting peptides attached to a dendrimer. Labeled compounds can be administered to an animal, and accumulation in different organs can be measured. For example, a dendrimer-targeting peptide complex conjugated to a detectable label (e.g., a near-IR fluorescence spectroscopy label such as Cy5.5) allows live *in vivo* visualization. Such a compound can be administered to an animal, and the presence of the polypeptide in an organ can be detected, thus allowing determination of the rate and amount of accumulation of the polypeptide in the desired organ. The compound can also be labeled with a radioactive isotope (e.g., ^{125}I). The compound is then administered to an animal. After a period of time, the animal is sacrificed and the organs are extracted. The amount of radioisotope in each organ can then be measured using any means known in the art. By comparing the amount of a labeled candidate compound in a particular organ relative to the amount of a labeled control compound, the ability of the candidate compound to access and accumulate in a particular tissue can be ascertained. In one aspect of the invention, the transport of multiple Angiopep peptides (e.g., Angiopep-2) across the BBB is compared to the transport of a single Angiopep peptide (e.g., Angiopep-2)

Administration and dosage of compound of the invention

The present invention also features pharmaceutical compositions that contain a therapeutically effective amount of a compound of the invention. The composition can be formulated for use in a variety of drug delivery systems. One or more physiologically acceptable excipients or carriers can also be included in the composition for proper formulation. Suitable formulations for use in the present invention are found in *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Philadelphia, PA, 17th ed., 1985. For a brief review of methods for drug delivery, see, e.g., Langer (*Science* 249:1527-1533, 1990).

The pharmaceutical compositions are intended for parenteral, intranasal, topical, oral, or local administration, such as by a transdermal means, for prophylactic and/or therapeutic treatment. The pharmaceutical compositions can be administered parenterally (e.g., by intravenous, intramuscular, or subcutaneous injection), or by oral ingestion, or by topical application or intraarticular injection at areas affected by the vascular or cancer condition. Additional routes of administration include intravascular, intra-arterial, intratumor, intraperitoneal, intraventricular, intraepidural, as well as nasal, ophthalmic, intrascleral, intraorbital, rectal, topical, or aerosol inhalation administration. Sustained release administration is also specifically included in the invention, by such means as depot injections or erodible implants or components. Thus, the invention provides compositions for parenteral administration that include the above mention agents dissolved or suspended in an acceptable carrier, preferably an aqueous carrier, e.g., water, buffered water, saline, PBS, and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions,

such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents, detergents and the like. The invention also provides compositions for oral delivery, which may contain inert ingredients such as binders or fillers for the formulation of a tablet, a capsule, and the like. Furthermore, this invention provides compositions for local administration, which may contain inert ingredients such as solvents or emulsifiers for the formulation of a cream, an ointment, and the like.

These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The pH of the preparations typically will be between 3 and 11, more preferably between 5 and 9 or between 6 and 8, and most preferably between 7 and 8, such as 7 to 7.5. The resulting compositions in solid form may be packaged in multiple single dose units, each containing a fixed amount of the above-mentioned agent or agents, such as in a sealed package of tablets or capsules. The composition in solid form can also be packaged in a container for a flexible quantity, such as in a squeezable tube designed for a topically applicable cream or ointment.

The compositions containing an effective amount can be administered for prophylactic or therapeutic treatments. Compositions of the invention can be administered to the subject (e.g., a human) in an amount sufficient to delay, reduce, or preferably prevent the onset of clinical disease. In therapeutic applications, compositions are administered to a subject (e.g., a human) already suffering from disease in an amount sufficient to cure or at least partially arrest the symptoms of the condition and its complications. An amount adequate to accomplish this purpose is defined as a "therapeutically effective amount," an amount of a compound sufficient to substantially improve some symptom associated with a disease or a medical condition. For example, in the treatment of a neurodegenerative disease (e.g., those described herein), an agent or compound that decreases, prevents, delays, suppresses, or arrests any symptom of the disease or condition would be therapeutically effective. A therapeutically effective amount of an agent or compound is not required to cure a disease or condition but will provide a treatment for a disease or condition such that the onset of the disease or condition is delayed, hindered, or prevented, or the disease or condition symptoms are ameliorated, or the term of the disease or condition is changed or, for example, is less severe or recovery is accelerated in an individual.

Amounts effective for this use may depend on the severity of the disease or condition and the weight and general state of the subject, but generally range from about 0.05 μg to about 1000 μg (e.g., 0.5-100 μg) of an equivalent amount of the agent per dose per subject. Suitable regimes for initial administration and booster administrations are typified by an initial administration followed by repeated doses at one or more hourly, daily, weekly, or monthly intervals by a subsequent administration. The total effective amount of an agent present in the compositions of the invention can be administered to a mammal as a single dose, either as a bolus or by infusion over a relatively short period of time, or can be administered using a fractionated treatment protocol, in which multiple doses are administered over a more prolonged period of time (e.g., a dose every 4-6, 8-12, 14-16, or 18-24 hours, or every 2-4 days, 1-2 weeks, once a month). Alternatively, continuous intravenous infusion sufficient to maintain therapeutically effective concentrations in the blood are contemplated.

The therapeutically effective amount of one or more agents present within the compositions of the invention and used in the methods of this invention applied to mammals (e.g., humans) can be determined by the ordinarily-skilled artisan with consideration of individual differences in age, weight, and the condition of the mammal. Because certain compounds of the invention exhibit an enhanced ability to cross the BBB, the dosage of the compounds of the invention can be lower than (e.g., less than or equal to about 90%, 75%, 50%, 40%, 30%, 20%, 15%, 12%, 10%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1% of) the equivalent dose of required for a therapeutic effect of the unconjugated agonist. The agents of the invention are administered to a subject (e.g. a mammal, such as a human) in an effective amount, which is an amount that produces a desirable result in a treated subject (e.g., preservation of neurons, new neuronal growth). Therapeutically effective amounts can also be determined empirically by those of skill in the art.

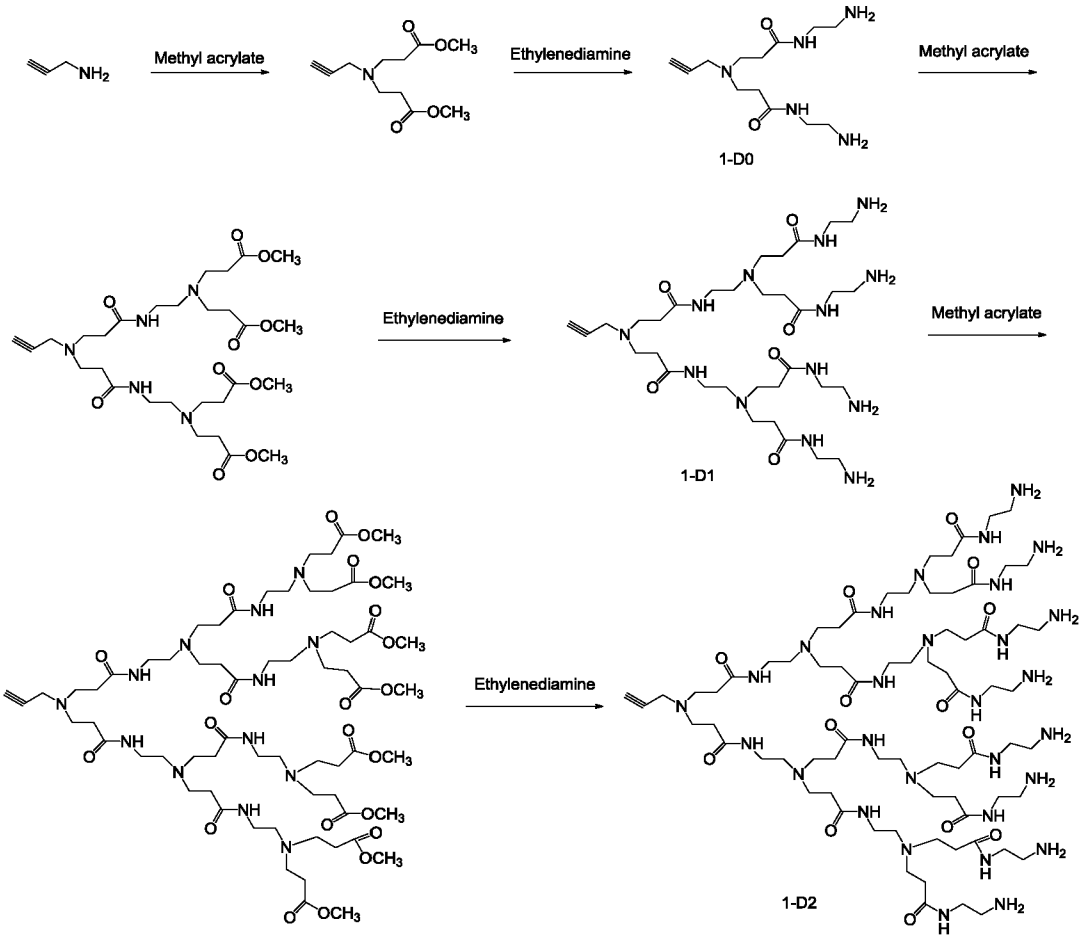
Single or multiple administrations of the compositions of the invention including an effective amount can be carried out with dose levels and pattern being selected by the treating physician. The dose and administration schedule can be determined and adjusted based on the severity of the disease or condition in the subject, which may be monitored throughout the course of treatment according to the methods commonly practiced by clinicians or those described herein.

The compounds of the present invention may be used in combination with either conventional methods of treatment or therapy or may be used separately from conventional methods of treatment or therapy.

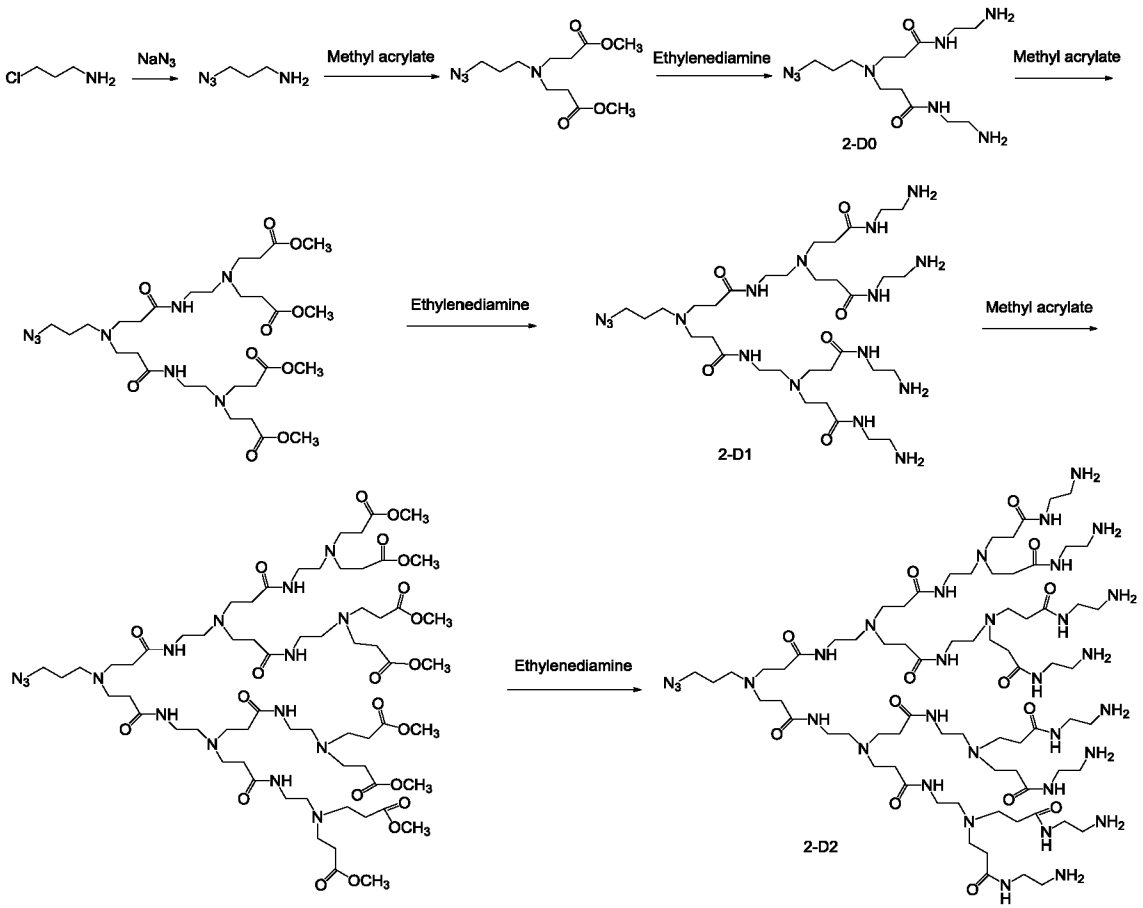
When the compounds of this invention are administered in combination therapies with other agents, they may be administered sequentially or concurrently to an individual. Alternatively, pharmaceutical compositions according to the present invention may be comprised of a combination of a compound of the present invention in association with a pharmaceutically acceptable excipient, as described herein, and another therapeutic or prophylactic agent known in the art.

EXAMPLES

Example 1: Synthesis of propagyl-PAMAM dendrons. Propagyl-PAMAM dendrons 1-D0, 1-D1, 1-D2 were prepared using a modified version (as shown in the schematic below) of the synthesis protocol described in Lee et al (*Macromolecules* 2006, 39, 2418-2422, incorporated herein by reference).



Example 2: Synthesis of azido-PAMAM dendrons. Azido-PAMAM dendrons 2-D0, 2-D1, 2-D2 were prepared using a modified version (as shown in the schematic below) of the synthesis protocol described in Lee et al (*Tetrahedron* 2006, 62, 9193-9200, incorporated herein by reference).



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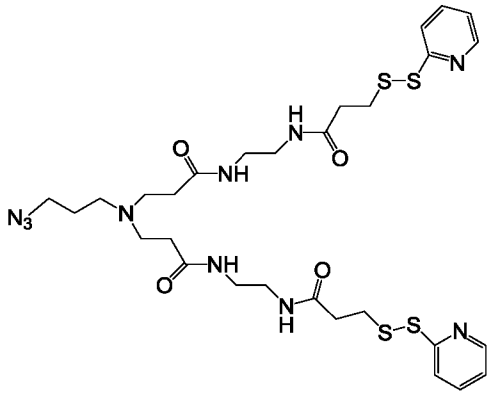
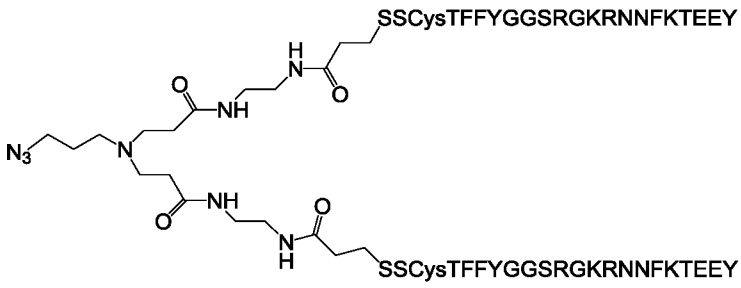
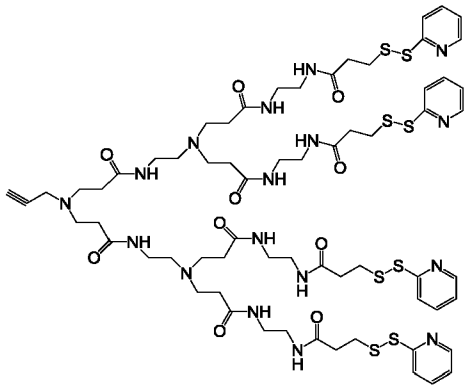
Example 3: Synthesis of azido-PAMAM(0)-(SSPy)$_2$. Azido-PAMAM **2-D0** (66 mg, 0.2 mmol) was dissolved in DMF (1.3 ml). SPDP (125 mg, 0.4 mmol) was then added. The mixture was stirred at room temperature for 1 h, diluted with 0.1% TFA in water (20 ml). The resulting solution was directly loaded on to a phenyl 42 ml column. Preparative HPLC purification (8% ACN/H_2O to 40% ACN/H_2O with 0.05% TFA) produced 65 mg of pure azido-PAMAM(0)-SSPy, 45%. UPLC purity, 95%. HRMS (Microtof, ESI) m/z, calcd. for $C_{29}H_{42}N_{10}O_4S_4$, 722.2273, found 723.2184 (M+1).



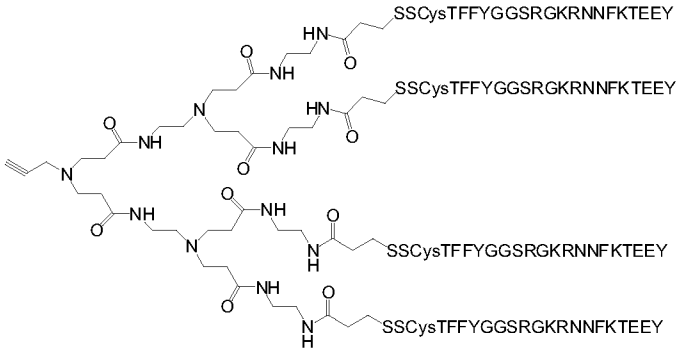
Example 4: Synthesis of azido-PAMAM(0)-(SSCysAn2)$_2$. A mixture of azido-PAMAM(0)-SSPy (40 mg, 55.5 µmol), An2Cys (240 mg, 80 µmol) and $NaHCO_3$ (30 mg, 0.35 mmol) in DMSO (1.5 ml) and DMF (1.5 ml) was stirred at room temperature under argon for 1 h. After cooling to 0 °C, the reaction mixture was diluted with 0.1% TFA in water (30 ml), and directly loaded to a phenyl 42 ml column for purification (8% ACN/H_2O to 40% ACN/H_2O with 0.05% TFA). Pure product azido-PAMAM(0)-(SSAn2)$_2$ (127 mg, 43%) was obtained as a colorless power after lyophilization. UPLC purity, 95%. HRMS (Microtof, ESI) m/z, calcd. for $C_{233}H_{340}N_{68}O_{68}S_4$, 5308.4187, found 1327.8346 (4+), 1062.6763 (5+), 885.5691 (6+).



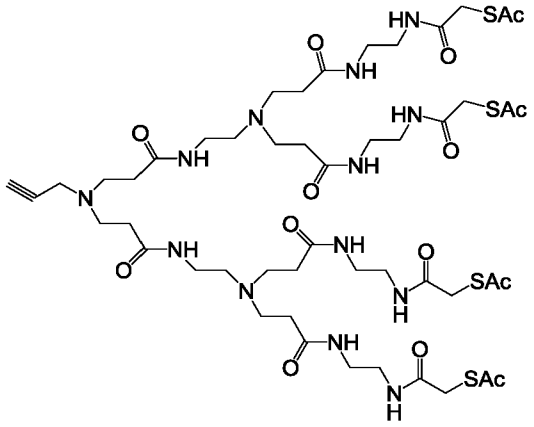
Example 5: Synthesis of propagyl-PAMAM(1)-(SSPy)$_4$. Propagyl-PAMAM **1-D1** (45 mg, 0.06 mmol) was dissolved in DMF (1.0 ml). SPDP (75 mg, 0.24 mmol) was then added. The mixture was stirred at room temperature for 1 h, diluted with 0.1% TFA in water (20 ml). The resulting solution was directly loaded to a phenyl 42 ml column for HPLC purification (8% ACN/H_2O to 40% ACN/H_2O with 0.05% TFA) resulting in a pure product of propagyl-PAMAM(1)-(SSPy)$_4$ 50 mg, 55%. UPLC purity, 95%. HRMS (Microtof, ESI) m/z, calcd. for $C_{65}H_{93}N_{17}O_{10}S_8$, 1527.5057, found 1528.4723 (M+1).



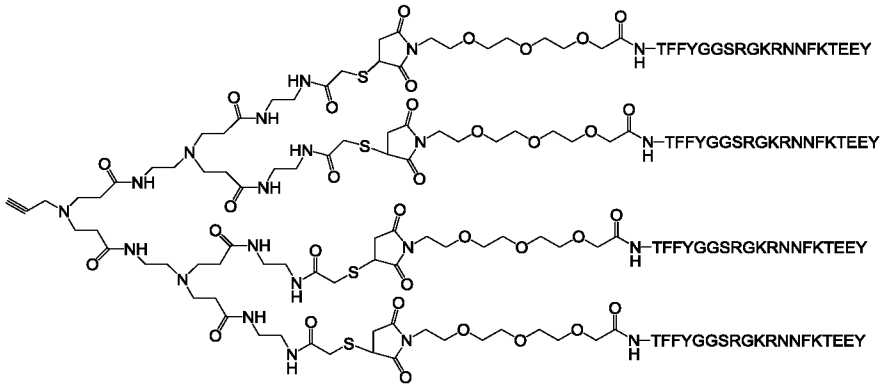
Example 6: Synthesis of Propagyl-PAMAM(1)-(SSCysAn2)$_4$. A mixture of propagyl-PAMAM(1)-(SSPy)$_4$ (20 mg, 13 µmol), An2Cys (125 mg, 52 µmol) and $NaHCO_3$ (12 mg, 0.14 mmol) in DMSO (0.8 ml) and DMF (0.8 ml) was stirred at room temperature under argon for 2.5 h. After cooling to 0 °C, the reaction mixture was diluted with 0.1% TFA in water (15 ml) and directly loaded to a phenyl 42 ml column for purification (8% ACN/H_2O to 40% ACN/H_2O with 0.05% TFA). Pure product propagyl-PAMAM(1)-(SSCysAn2)$_4$ (91 mg, 65%) was obtained as a colorless power after lyophilization. UPLC purity, 95%. HRMS (Microtof, ESI) m/z, calcd. for $C_{473}H_{689}N_{133}O_{138}S_8$, 10700.8918, found 2675.6306 (4+), 2140.7530 (5+), 1529.4280 (7+).



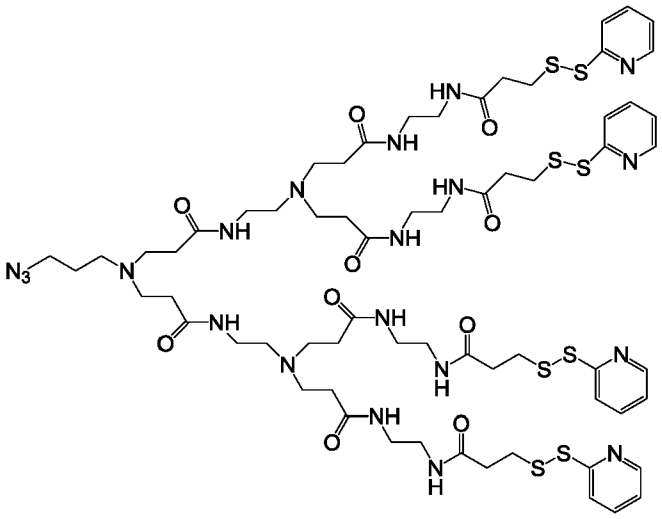
Example 7: Synthesis of propagyl-PAMAM(1)-(SAc)$_4$. Propagyl-PAMAM **1-D1** (111 mg, 0.15 mmol) was dissolved in DMF (2.0 ml). SATA (139 mg, 0.6 mmol) in DMSO (1 ml) was then added. The mixture was stirred at room temperature for 1.5 h, diluted with 0.1% TFA in water (40 ml). The resulting solution was directly loaded to a phenyl 42 ml column for HPLC purification (4% ACN/H_2O to 30% ACN/H_2O with 0.05% TFA) resulting in pure product of propagyl-PAMAM(1)-(Sac)$_4$ 72 mg, 40%. UPLC purity, 95%. HRMS (Microtof, ESI) m/z, calcd. for $C_{49}H_{81}N_{13}O_{14}S_4$, 1203.4909, found 1204.4896 (M+1).



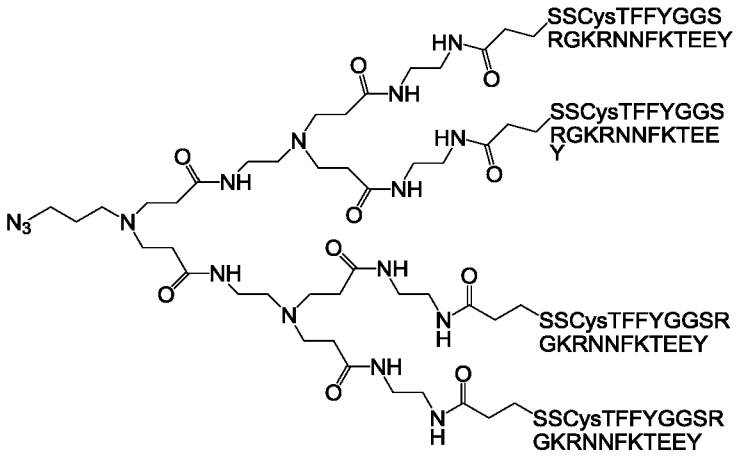
Example 8: Synthesis of propagyl-PAMAM(1)-(SMal-An2)$_4$. Into a solution of propagyl-PAMAM(1)-(Sac)$_4$ (17.4 mg, 14.4 µmol) in phosphate buffer pH 7.2 (1 ml) $NH_2OH.HCl$ (0.5 M, 0.07 ml) was added. The mixture was stirred at room temperature for 2 h. Next, maleimide-An2 (78 mg, 66 µmol) in DMSO (1.5 ml) was added and the reaction mixture was stirred at room temperature overnight. After cooling to 0 °C, the reaction mixture was diluted with 0.1% TFA in water (40 ml), and directly loaded to a C4 24 ml column for purification (4% ACN/H_2O to 40% ACN/H_2O with 0.05% TFA). Pure product of propagyl-PAMAM(1)-(SMal-An2)$_4$ (33 mg, 21%) was obtained as a colorless power after lyophilization. UPLC purity, 95%. HRMS (Microtof, ESI) m/z, calcd. for $C_{505}H_{729}N_{133}O_{158}S_4$, 11317.2149, found 2830.8163 (4+), 2264.8163 (5+), 1887.5322 (6+).



Example 9: Synthesis of azido-PAMAM(1)-(SSPy)$_4$. Azido-PAMAM **2-D1** (86 mg, 0.11 mmol) was dissolved in DMF (2.0 ml). SPDP (137 mg, 0.44 mmol) in DMSO (1 ml) was then added. The mixture was stirred at room temperature for 1 h, diluted with 0.1% TFA in water (30 ml). The resulting solution was directly loaded to a phenyl 42 ml column for HPLC purification (8% ACN/H_2O to 40% ACN/H_2O with 0.05% TFA) resulting in a pure product of azido-PAMAM(1)-SSPy 85 mg, 49%. UPLC purity, 95%. HRMS (Microtof, ESI) m/z, calcd. for $C_{65}H_{96}N_{20}O_{10}S_8$, 1572.5384, found 1573.4874 (M+1).

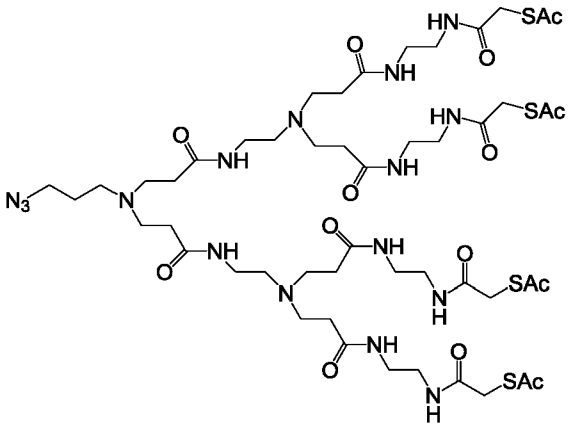


Example 10: Synthesis of azido-PAMAM(1)-(SSCysAn2)$_4$. A mixture of azido-PAMAM(1)-(SSPy)$_4$ (30 mg, 19 µmol), An2Cys (183 mg, 76 µmol) and $NaHCO_3$ (18 mg, 0.14 mmol) in DMSO (1.2 ml) and DMF (1.2 ml) was stirred at room temperature under argon for 3 h. After cooling to 0 °C, the reaction mixture was diluted with 0.1% TFA in water (25 ml), and directly loaded to a C4 24 ml column for purification (8% ACN/H_2O to 40% ACN/H_2O with 0.05% TFA). Pure product of azido-PAMAM(1)-(SSCysAn2)$_4$ (88 mg, 43%) was obtained as a colorless power after lyophilization. UPLC purity, 95%. HRMS (Microtof, ESI) m/z, calcd. for $C_{473}H_{692}N_{136}O_{138}S_8$, 10745.9245, found 1791.6586 (6+), 1535.8257 (7+), 1343.9730 (8+), 1075.3835 (10+).

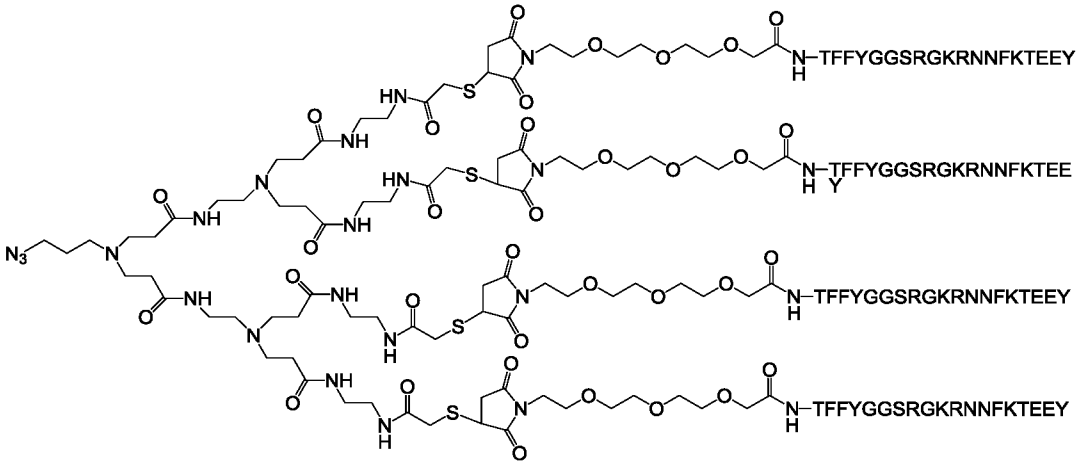


Example 11: Synthesis of azido-PAMAM(1)-(SAc)$_4$. Azido-PAMAM **2-D1** (102 mg, 0.13 mmol) was dissolved in DMF (2.0 ml). SATA (120 mg, 0.52 mmol) in DMSO (1.5 ml) was then added. The mixture was stirred at room temperature for 1.5 h, diluted with 0.1% TFA in water (30 ml). The resulting solution was directly loaded to a phenyl 42 ml column for HPLC purification (4% ACN/H_2O to 30%

ACN/H_2O with 0.05% TFA) yielding a pure product of azido-PAMAM(1)-$(Sac)_4$ 57 mg, 35%. UPLC purity, 95%. HRMS (Microtof, ESI) m/z, calcd. for $C_{49}H_{80}N_{16}O_{14}S_4$, 1248.5236, found 1249.4825 (M+1).

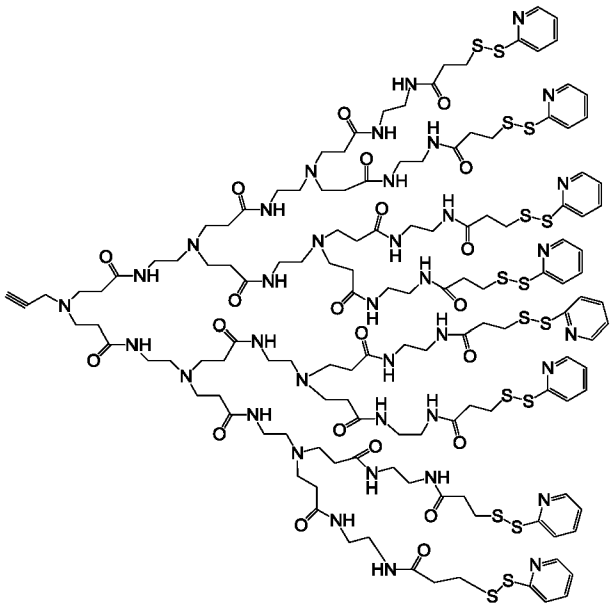


Example 12: Synthesis of azido-PAMAM(1)-(SMal-An2)$_4$. Into a solution of azido-PAMAM(1)-$(Sac)_4$ (30 mg, 24 µmol) in phosphate buffer pH 7.2 (1.5 ml) $NH_2OH.HCl$ (0.5 M, 0.3 ml) was added. The mixture was stirred at room temperature for 2 h. Maleomide-An2 (160 mg, 65 µmol) in DMSO (2 ml) was added and the reaction mixture was stirred at room another 2 h. After cooling to 0 °C, the reaction mixture was diluted with 0.1% TFA in water (30 ml), and directly loaded to a C4 24 ml column for purification (4% ACN/H_2O to 40% ACN/H_2O with 0.05% TFA). Pure product of azidol-PAMAM(1)-(SMal-An2)$_4$ (54 mg, 20%) was obtained as a colorless power after lyophilization. UPLC purity, 95%. HRMS (Microtof, ESI) m/z, calcd. for $C_{505}H_{728}N_{136}O_{158}S_4$, 11358.2163, found 2841.9829 (4+), 2273.7718 (5+), 1894.9193 (6+), 1421.5047 (8+).

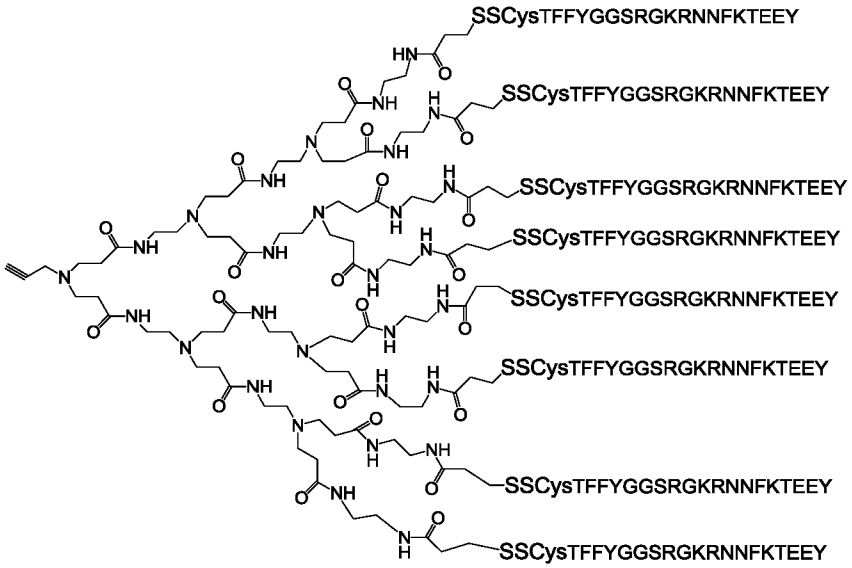


Example 13: Synthesis of propagyl-PAMAM(2)-(SSPy)$_8$. Propagyl-PAMAM **1-D2** (84 mg, 0.05 mmol) was dissolved in DMF (1.2 ml). SPDP (129 mg, 0.41 mmol) in DMSO (8 ml) was then added. The mixture was stirred at room temperature for 1 h, diluted with 0.1% TFA in water (20 ml). The resulting solution was directly loaded to a phenyl 42 ml column for HPLC purification (8% ACN/H_2O to 40% ACN/H_2O with 0.05% TFA) yielding a pure product of propagyl-PAMAM(2)-$(SSPy)_8$ 36 mg, 22%. %.

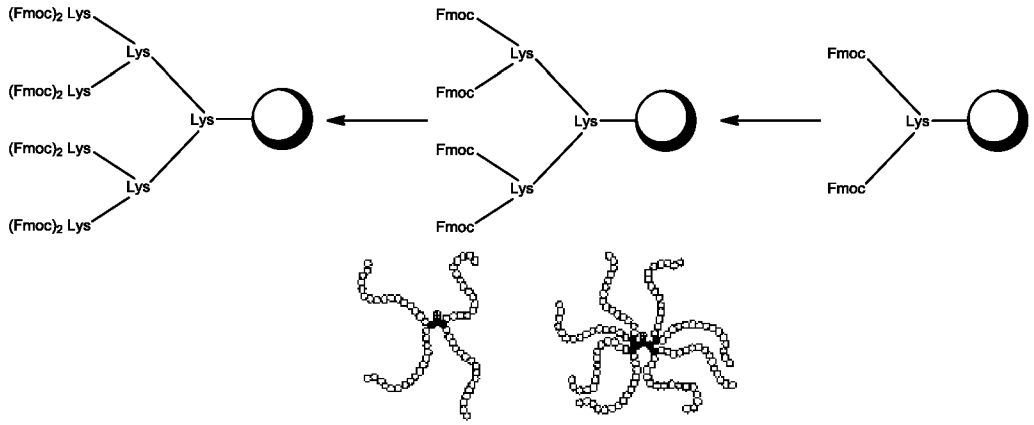
UPLC purity, 95%. HRMS (Microtof, ESI) m/z, calcd. for $C_{137}H_{201}N_{37}O_{22}S_{16}$, 3229.1312, found 1616.5492 (2+), 1077.7144 (3+).



Example 14: Synthesis of propagyl-PAMAM(2)-(SSCysAn2)$_8$. A mixture of Propagyl-PAMAM(2)-(SSPy)$_8$ (35 mg, 11 µmol), An2Cys (200 mg, 88 µmol) and $NaHCO_3$ (22 mg, 0.26 mmol) in DMSO (2 ml) and DMF (2 ml) was stirred at room temperature under argon for 2 h. After cooling to 0 °C, the reaction mixture was diluted with 0.1% TFA in water (25 ml), and directly loaded to a C4 24 ml column for purification (8% ACN/H_2O to 40% ACN/H_2O with 0.05% TFA). Pure product of propagyl-PAMAM(2)-(SSCysAn2)$_8$ (70 mg, 30%) was obtained as a colorless powder after lyophilization. UPLC purity, 95%. HRMS (Microtof, ESI) m/z, calcd. for $C_{953}H_{1393}N_{269}O_{278}S_{19}$, 21575.2346, found 2397.8071 (9+), 2158.0533 (10+), 1962.0438 (11+).

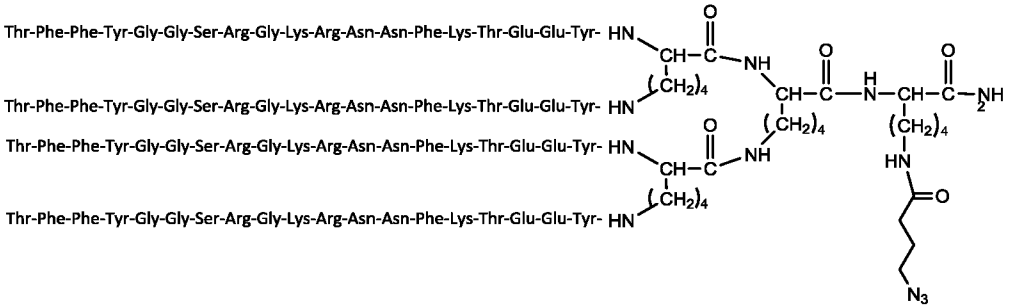


Example 15: Synthesis of peptide dendrimer based on a branched lysine core. A solid-phase approach was used to synthesize a dendrimer based on a branched lysine core. A Lys residue with both the α- and ε-amino groups blocked with the same labile protecting group (Fmoc) was coupled to a linker that was previously attached to resin (as shown in the schematic below). When deprotected, this Lys coupling was repeated once or twice so that either four or eight amino groups were available for further coupling reactions. Optionally three residues of Gly per chain can be added to provide a flexible link between the poly-Lysine core of the matrix and the peptides to be attached.

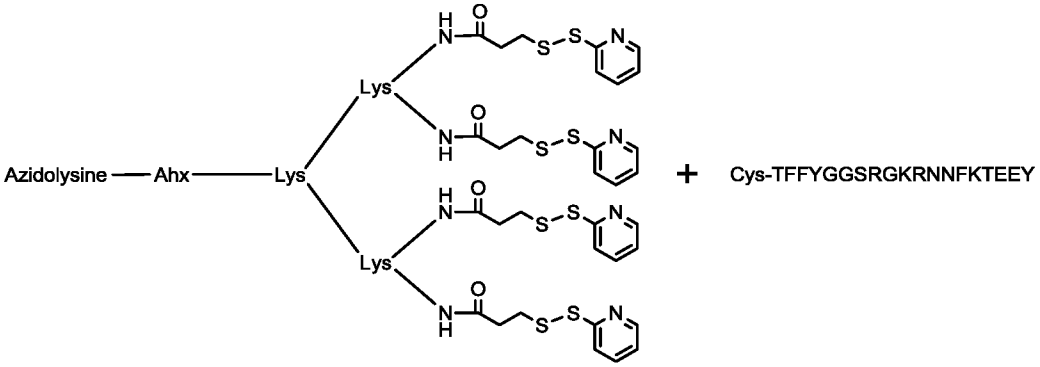


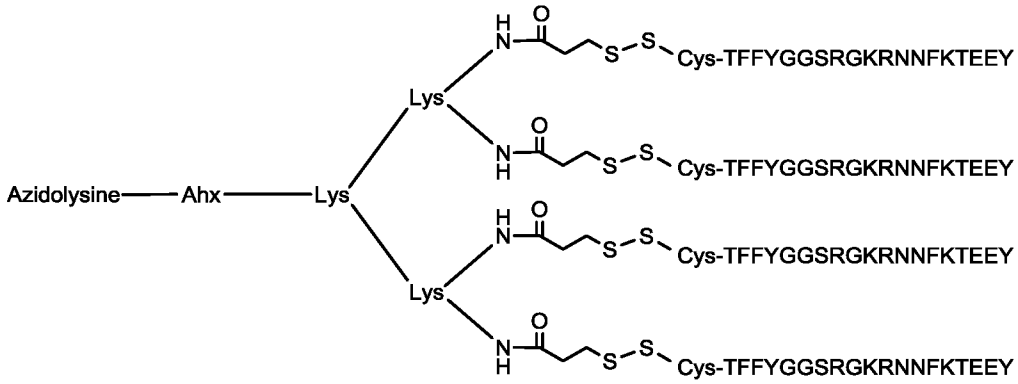
Synthesis and cleavage of poly-lysines-AN2 multimers analogs. All of the peptides were assembled on rink amide methylbenzhydrylamine resin using manual solid-phase peptide synthesis with HCTU - (2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate) activation procedure for Fmoc (*N*-(9-fluorenyl)methoxycarbonyl) chemistry. Cleavage of peptides from the resin was achieved by treatment with trifluoroacetic acid, triisopropylsilane and water as scavengers (95:2:3 trifluoroacetic acid: triisopropylsilane:water). The reaction was allowed to proceed at room temperature (20–23 °C) for 2.0 h. The trifluoroacetic acid was then evaporated, and the peptide was precipitated with ice-cold ether, filtered, dissolved in 20% acetic acid/80% H_2O and lyophilized. Crude peptides were purified by reversed phase-HPLC on a C4 column using a gradient of 20–80% B in 45min, (Buffer A: H_2O/0.1% trifluoroacetic acid; Buffer B: 40% CH_3CN/60% H_2O/0.1% trifluoroacetic acid) with the eluant monitored at 229. Waters Acquity UPLC with Bruker Q-TOF mass spectroscopy detection confirmed the molecular mass and purity of the fractions collected, those displaying the correct purity and molecular mass of desired peptide were pooled and lyophilized.

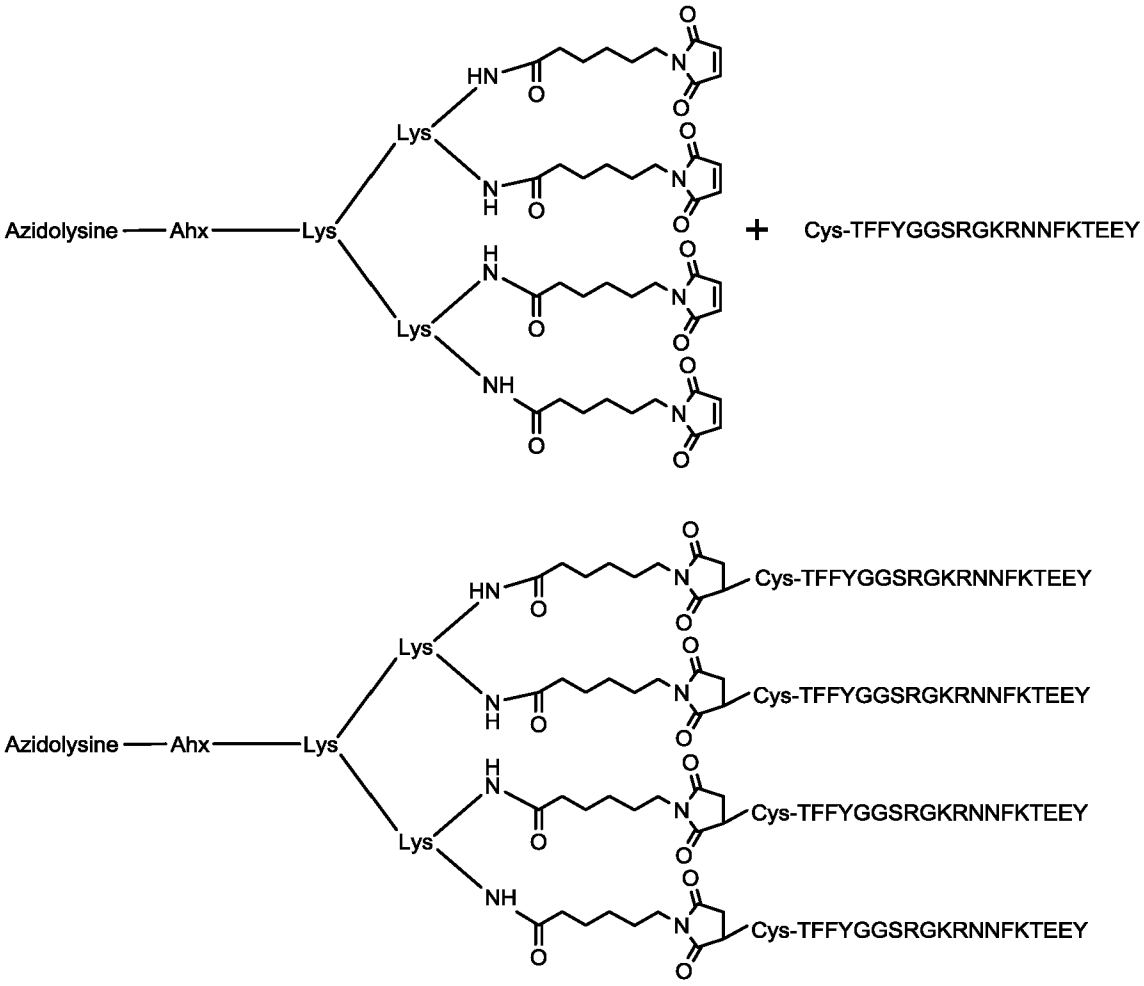
In order to overcome contamination of the dendrimer mixture by byproducts containing amino acids side-chains protecting groups (Pbf group, Trt group, or tBu groups) the synthesis and deprotection steps were optimized so that the crude, lyophilized product was as homogeneous as possible. The use of fully protected Angiopep-2 fragments obtained from a 2-chlorotrityl chloride resin was also successful at achieving overall better quality products without deletions.



Alternatively, a pure peptide can be conjugated to a poly-Lysine core on a resin matrix for later purification. For example, cysteine containing Angiopep-2 was coupled to maleimide or SPDP activated poly-lysine resulting in a product with reduced byproduct contaminants. This was mainly because the Angiopep-2 monomers were purified before the conjugation step.



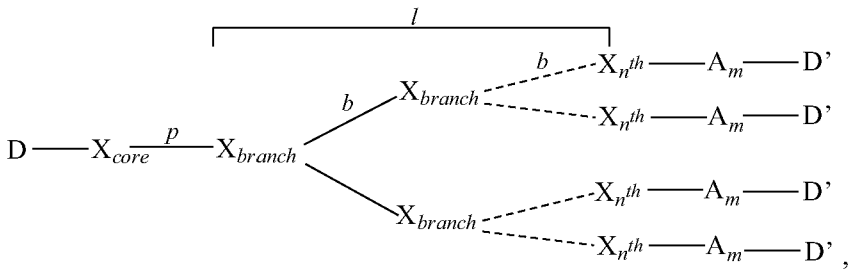



Azidolysine
Ahx
Lys
Lys
Lys
HN
NH
NH
Cys-TFFYGGSRGKRNNFKTEEY
Cys-TFFYGGSRGKRNNFKTEEY
Cys-TFFYGGSRGKRNNFKTEEY
Cys-TFFYGGSRGKRNNFKTEEY
Cys-TFFYGGSRGKRNNFKTEEY

5

CLAIMS

1. A compound comprising the formula:



wherein

D is a first agent;

X_{core} is a core moiety of a dendrimer with *p* number of branches, wherein *p* is an integer from 1 to 12;

X_{branch} is a branch moiety of said dendrimer, wherein each X_{branch} is attached to a branch of X_{core} or to a branch of another X_{branch}, and wherein each X_{branch} has *b* branches, and wherein *b* is an integer from 2 to 8;

l is the number of successive layers of X_{branch} branches of said dendrimer and is an integer from 2 to 10;

X_n^{th} is one of *n* surface branches of said dendrimer and is attached to a *b* branch of a X_{branch} moiety, wherein $n = p(b)^l$, and wherein *n* is ≤ 512;

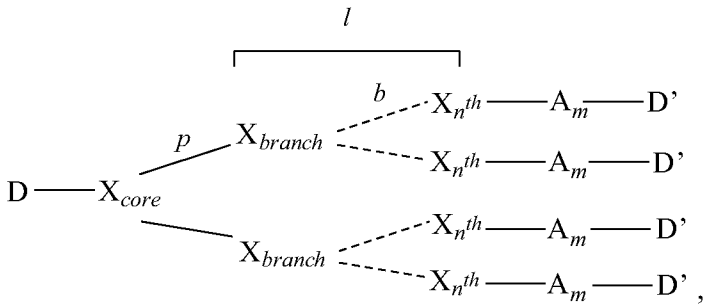
A_m is a targeting peptide attached to an X_n^{th} and comprises an amino acid sequence substantially identical to a sequence selected from the group consisting of SEQ ID NOs:1-105 and 107-117, or a functional fragment thereof;

m is a positive integer ≤ *n*;

D' is a second agent that is optionally present, and either is attached to one or more A_m or replaces one or more A_m and is attached directly to one or more X_n^{th}, wherein the number of D' in said compound is ≤ *n*; and

the molecular weight of said dendrimer, excluding D, D' and A_m, is ≤ 500 kilodaltons.

2. The compound of claim 1, comprising the formula:



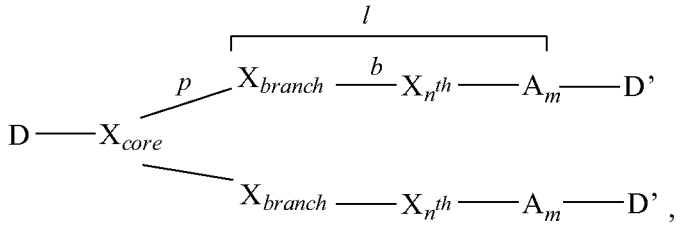
wherein

p is an integer between 2 and 6;

b is an integer from 2 to 4; and

l is an integer from 2 to 5.

3. A compound comprising the formula:



wherein

D is a first agent;

X_{core} is a core moiety of a dendrimer with *p* number of branches, wherein *p* is an integer from 2 to 6; and wherein said core moiety is selected from the group consisting of propargylamine, ethylenediamine, triethanolamine, pentaerythritol, azido-propyl(alkyl)amine, hydroxyethyl(alkyl)amine, tetraphenyl methane, trimesoylchloride, diamino hexane, diaminobutane, cystamine, propylenediamine, lysine, and propyleneamine;

X_{branch} is a branch moiety of said dendrimer, wherein each X_{branch}, is attached to a branch of X_{core} or to a branch of another X_{branch}, and wherein each X_{branch} has *b* branches, and wherein *b* is an integer from 1 to 4;

l is the number of successive layers of X_{branch} branches of said dendrimer and is an integer from 0 to 4;

X_n^{th} is one of *n* surface branches of said dendrimer and is attached to a *b* branch of a X_{branch} moiety, wherein $n = p(b)^l$, and wherein *n* is ≤ 256;

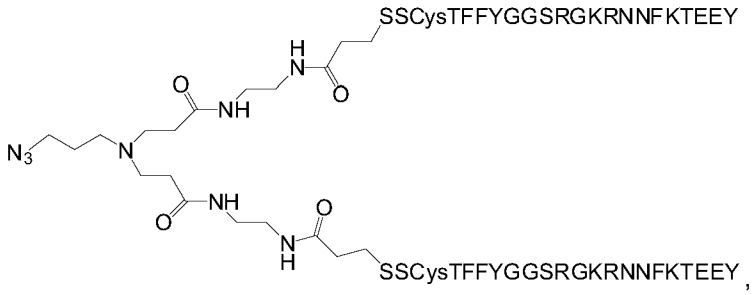
A_m is a targeting peptide attached to an X_n^{th} and comprises an amino acid sequence substantially identical to a sequence selected from the group consisting of SEQ ID NOs:1-105 and 107-117, or a functional fragment thereof;

m is a positive integer ≤ *n*;

D' is a second agent that is optionally present, and either is attached to one or more A_m or replaces one or more A_m and is attached directly to one or more X_n^{th}, wherein the number of D' in said compound is ≤ *n*; and

the molecular weight of said dendrimer, excluding D, D' and A_m, is ≤ 500 kilodaltons.

4. The compound of claim 3 comprising:



wherein Cys is cysteine and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine

5. The compound of any one of claims 1 to 4, wherein said *n* is ≤ 128.

6. The compound of claim 5, wherein said *n* is ≤ 64.

7. The compound of claim 6, wherein said *n* is ≤ 32.

8. The compound of any one of claims 1 to 7, wherein said molecular weight of said dendrimer is ≤ 100 kilodaltons.

9. The compound of any one of claims 1 to 8, wherein said molecular weight of said dendrimer is ≤ 50 kilodaltons.

10. The compound of any one of claims 1 to 9, wherein said molecular weight of said dendrimer is ≤ 25 kilodaltons.

11. The compound of any one of claims 1 to 10, wherein said core moiety is selected from the group consisting of propargylamine, ethylenediamine, triethanolamine, pentaerythritol, azido-propyl(alkyl)amine, hydroxyethyl(alkyl)amine, tetraphenyl methane, trimesoylchloride, diamino hexane, diaminobutane, cystamine, and propylenediamine.

12. The compound of any one of claims 1 to 11, wherein said dendrimer is poly(amido amine) (PAMAM).

13. The compound of any one of claims 1 to 10, wherein said core moiety is lysine or a lysine analogue.

14. The compound of claim 13, wherein said dendrimer is polylysine.

15. The compound of any one of claims 1 to 10, wherein said core moiety is propylamine.

16. The compound of claim 15, wherein said dendrimer is poly(propyleneimine) (POPAM).

17. The compound of any one of claims 1 to 16, wherein said branch moiety is selected from the group consisting of propargylamine, ethylenediamine, triethanolamine, pentaerythritol, propylamine, propyleneimine, azido-propyl(alkyl)amine, hydroxyethyl(alkyl)amine, tetraphenyl methane, trimesoylchloride, diamino hexane, diaminobutane, cystamine, propylenediamine, and lysine.

18. The compound of any one of claims 1 to 17, wherein said branch moiety is a derivative of any one of propargylamine, ethylenediamine, triethanolamine, pentaerythritol, propylamine, propyleneimine, azido-propyl(alkyl)amine, hydroxyethyl(alkyl)amine, tetraphenyl methane, trimesoylchloride, diamino hexane, diaminobutane, cystamine, propylenediamine, and lysine.

19. The compound of any one of claims 1 to 18, wherein at least two said surface branches of said dendrimer has a targeting peptide attached.

20. The compound of any one of claims 1 to 19, wherein more than two surface branches of said dendrimer have a targeting peptide attached.

21. The compound of claim 20, wherein all of said surface branches have a targeting peptide attached.

22. The compound of any one of claims 1 to 21, wherein said targeting peptide is attached to said surface branch via a linker.

23. The compound of claim 22, wherein said linker is selected from a group consisting of pyridinedisulfide, thiosulfonate, vinylsulfonate, isocyanate, NHS ester, imidoester, diazine, hydrazine, thiol, carboxylic acid, a multi-peptide linker, and acetylene.

24. The compound of claim 22, wherein said linker is a covalent bond.

25. The compound of any one of claims 1 to 24, wherein said targeting peptide is attached to said surface branch by a cleavable linkage.

26. The compound of any one of claims 1 to 24, wherein said targeting peptide is attached to said surface branch by a non-cleavable linkage.

27. The compound of claim 25, wherein said linkage is an ester linkage.

28. The compound of claim 26, wherein said linkage is a maleimide linkage.

29. The compound of any one of claims 1 to 28, wherein at least one of said targeting peptides attached to said dendrimer comprises an amino acid sequence at least 85% identical to a sequence selected from the group consisting of SEQ ID NOs:1-105 and 107-117 or a fragment thereof.

30. The compound of claim 29, wherein at least one of said targeting peptides attached to said dendrimer comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:1-105 and 107-117 or a fragment thereof.

31. The compound of claim 30, wherein all of said targeting peptides attached to said dendrimer comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:1-105 and 107-117 or a fragment thereof.

32. The compound of any one of claims 1 to 31, wherein at least one of said targeting peptides attached to said dendrimer comprises an amino acid sequence selected from the group consisting of Angiopep-1 (SEQ ID NO:67), Angiopep-2 (An_2) (SEQ ID NO:97), cys-Angiopep-2 ($CysAn_2$) (SEQ ID NO:113), Angiopep-2-cys (SEQ ID NO:114), and reversed Angiopep-2 (SEQ ID NO:117).

33. The compound of claim 32, wherein all of said targeting peptides comprises an amino acid sequence selected from the group consisting of Angiopep-1 (SEQ ID NO:67), Angiopep-2 (An_2) (SEQ ID NO:97), cys-Angiopep-2 ($CysAn_2$) (SEQ ID NO:113), Angiopep-2-cys (SEQ ID NO:114), and reversed Angiopep-2 (SEQ ID NO:117).

34. The compound of any one of claims 1 to 33, wherein said targeting peptide is Angiopep-2 (An_2) (SEQ ID NO:97).

35. The compound of any one of claims 1 to 34, wherein said targeting peptide is cys-Angiopep-2 ($CysAn_2$) (SEQ ID NO:113).

36. The compound of any one of claims 1 to 35, wherein said first agent is attached to said dendrimer by a reactive group.

37. The compound of any one of claims 1 to 36, wherein one or more said second agent is optional, and when present, is attached to one or more said A_m by a reactive group.

38. The compound of any one of claims 1 to 37, wherein one or more said second agent is optional, and when present, is attached to one or more said X_n^{th} by a reactive group.

39. The compound of any one of claims 36 to 38, wherein said reactive group is selected from a group consisting of a maleimide, a hydrazide, an azide, a haloacetamide, and an alkoxyamine.

40. The compound of claim 39, wherein said reactive group is an azide group.

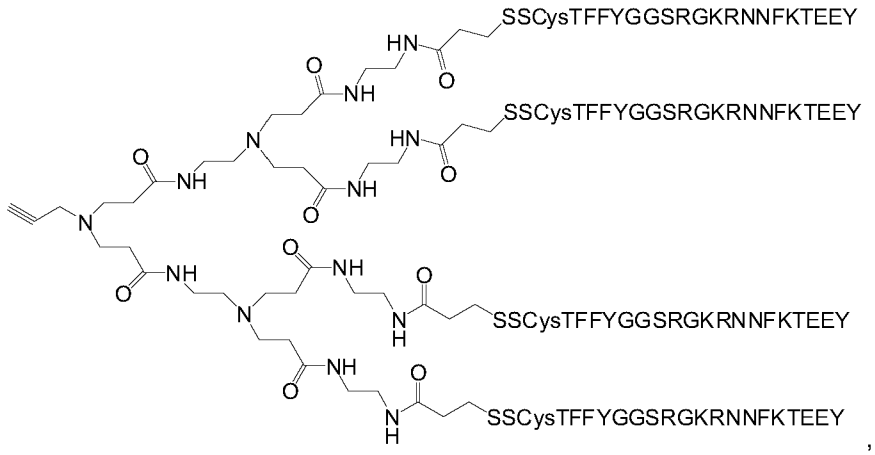
41. The compound of claim 39, wherein said reactive group is a maleimide group.

42. The compound of any one of claims 1 to 36, wherein said dendrimer is attached to said reactive group by a linker.

43. The compound of claim 37 or 38, wherein said reactive group is attached to said A_m or said X_n^{th} by a linker.

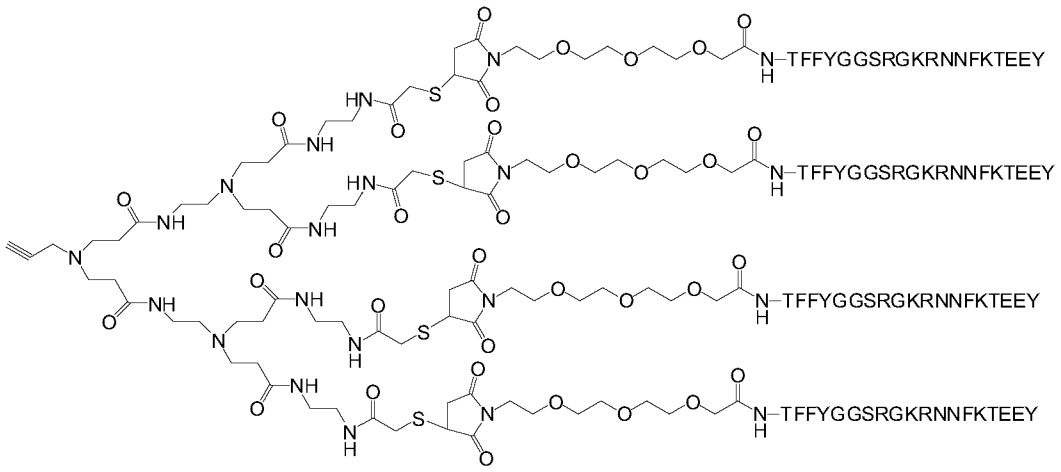
44. The compound of claim 42 or 43, wherein said linker is selected from a group consisting of pyridinedisulfide, thiosulfonate, vinylsulfonate, isocyanate, NHS ester, imidoester, diazine, hydrazine, thiol, carboxylic acid, a multi-peptide linker, acetylene, and a covalent bond.

45. The compound of claim 1 having the formula:

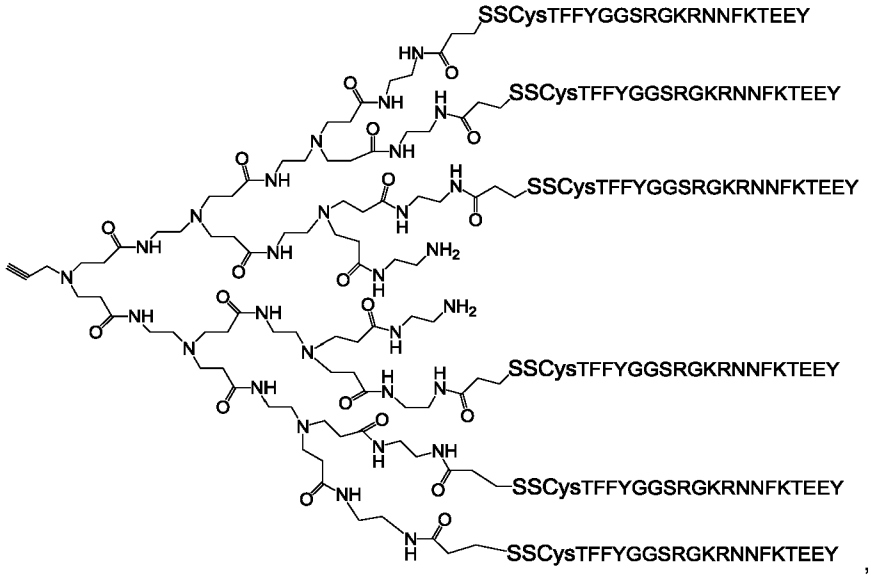


wherein Cys is cysteine and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine.

46. The compound of claim 1 having the formula:



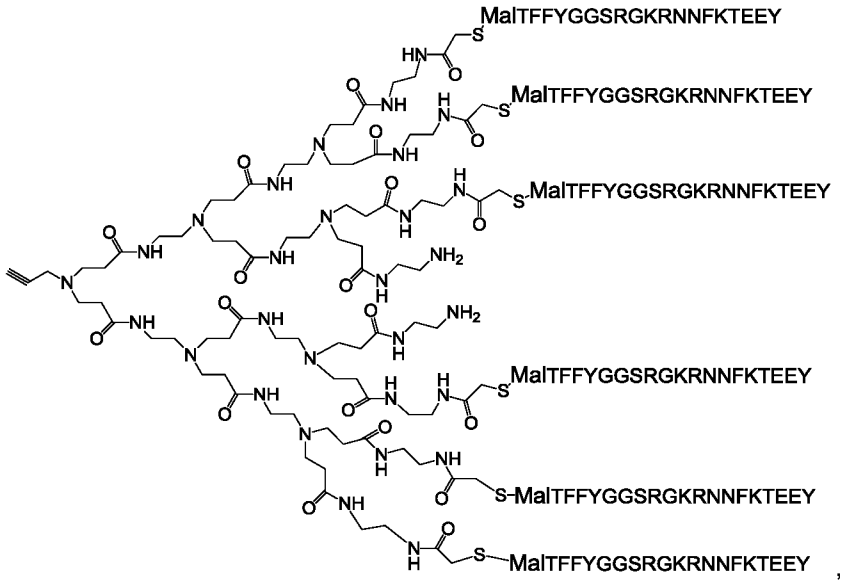
47. The compound of claim 1 having the formula:



,

wherein Cys is cysteine and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine.

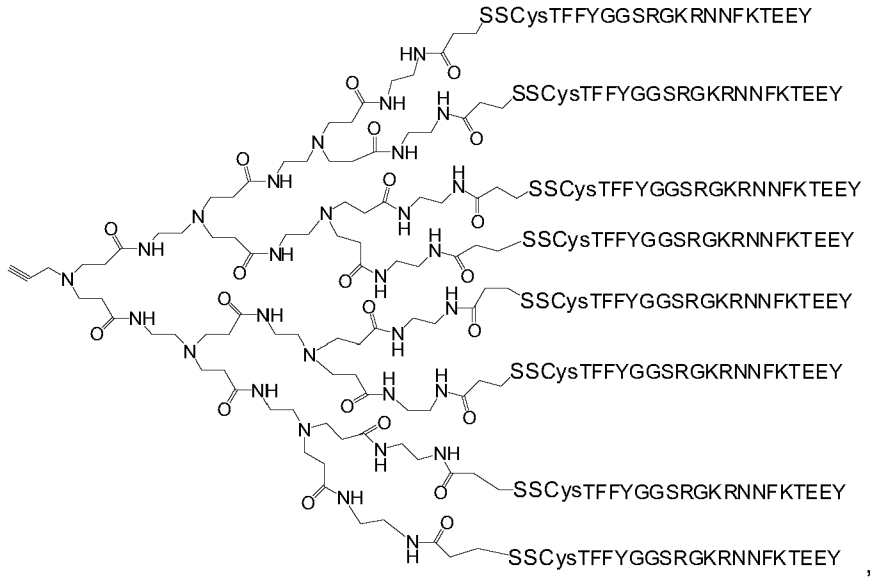
48. The compound of claim 1 having the formula:



,

wherein Mal is maleimide.

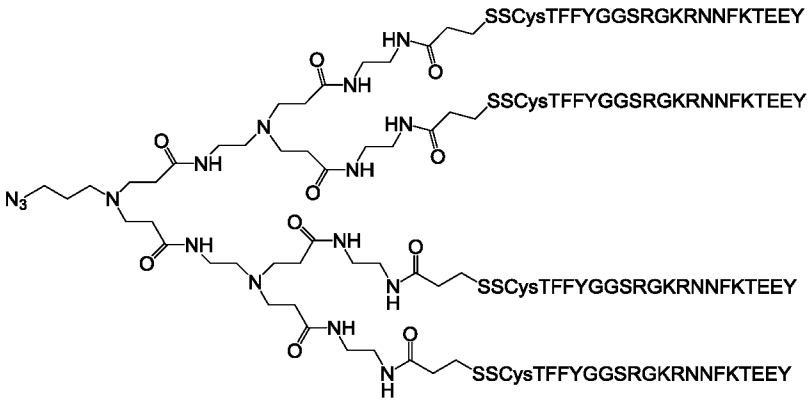
49. The compound of claim 1 having the formula:



,

wherein Cys is cysteine and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine.

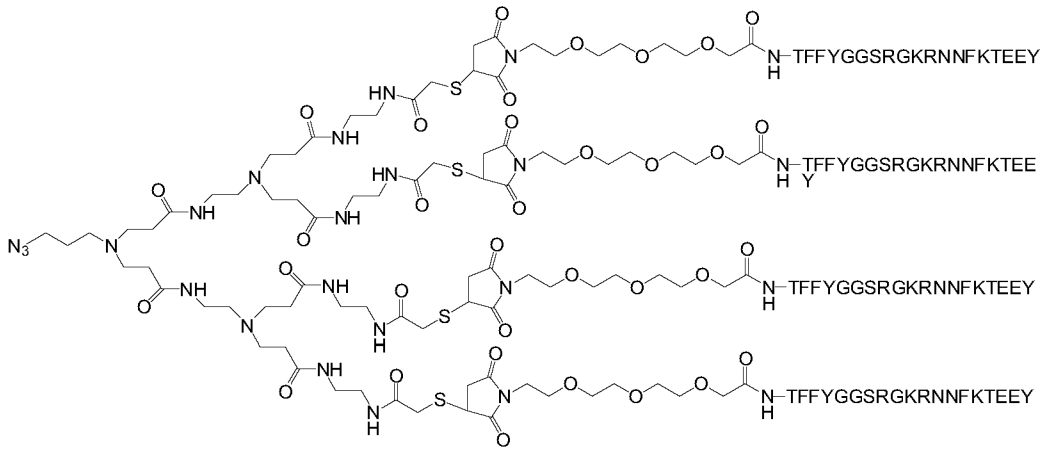
50. The compound of claim 1 having the formula:



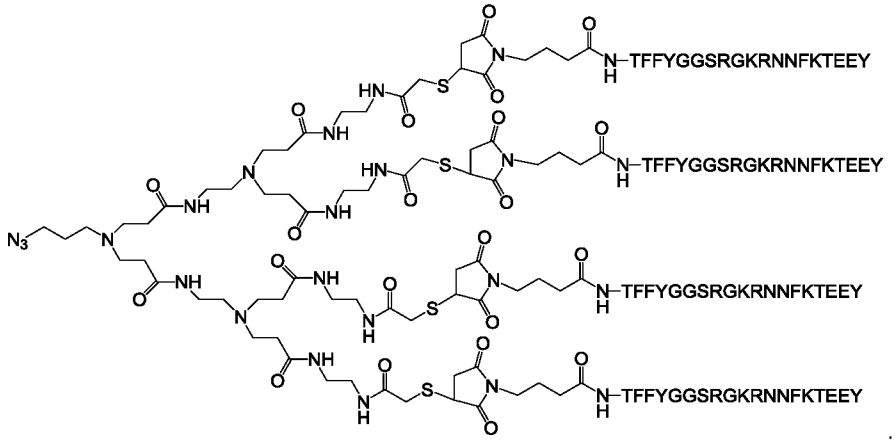
,

wherein Cys is cysteine and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine.

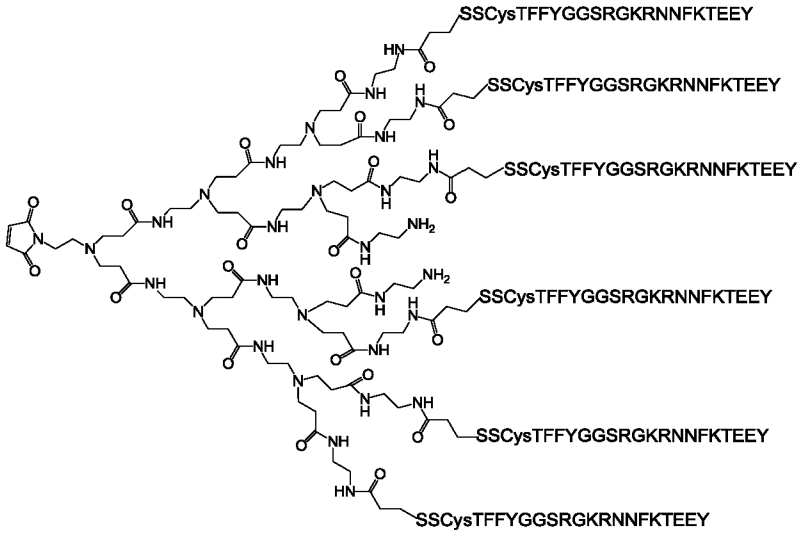
51. The compound of claim 1 having the formula:



52. The compound of claim 1 having the formula:

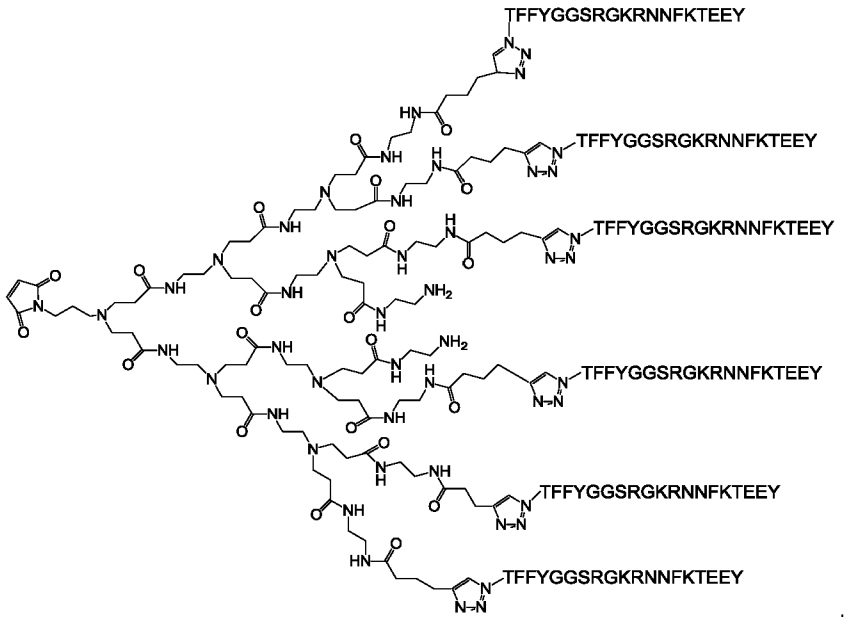


53. The compound of claim 1 having the formula:

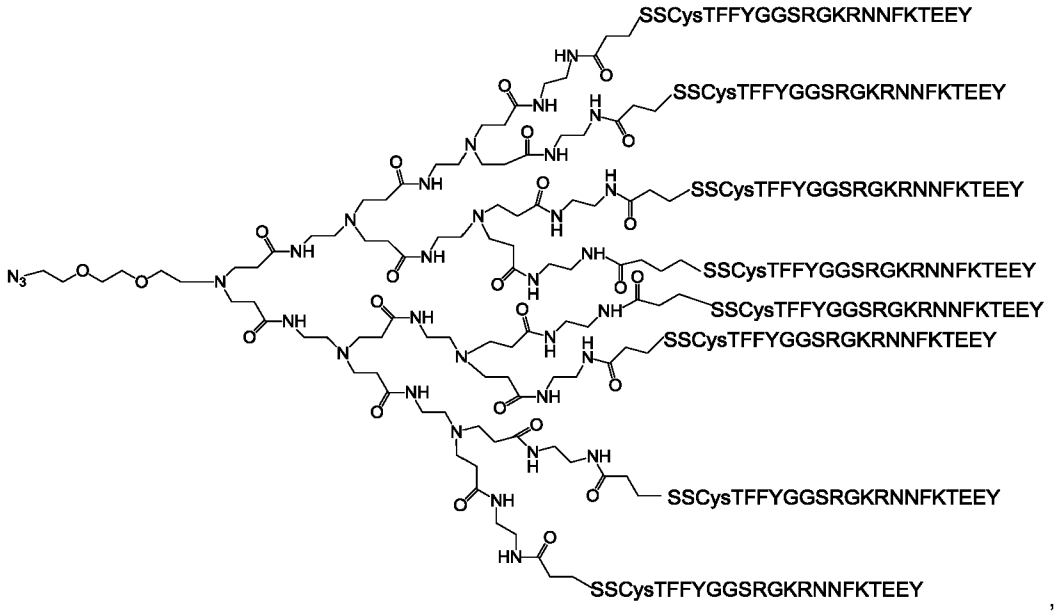


wherein Cys is cysteine and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine.

54. The compound of claim 1 having the formula:

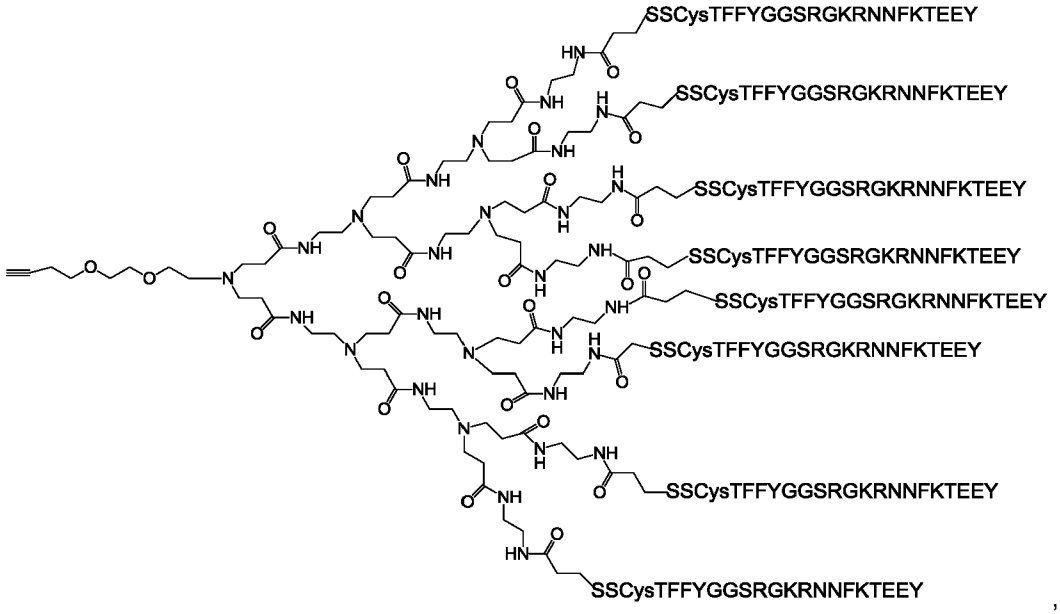


55. The compound of claim 1 having the formula:



wherein Cys is cysteine and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine.

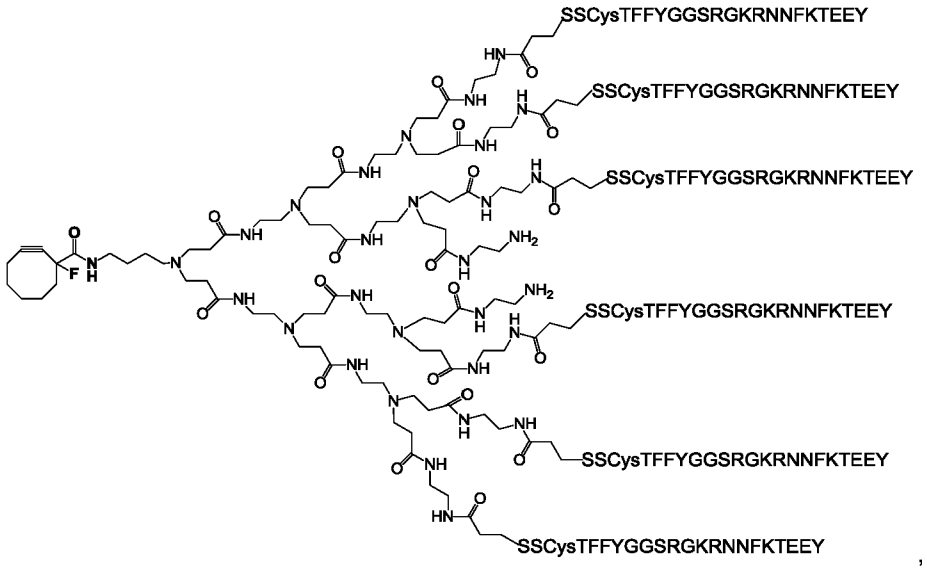
56. The compound of claim 1 having the formula:



,

wherein Cys is cysteine and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine.

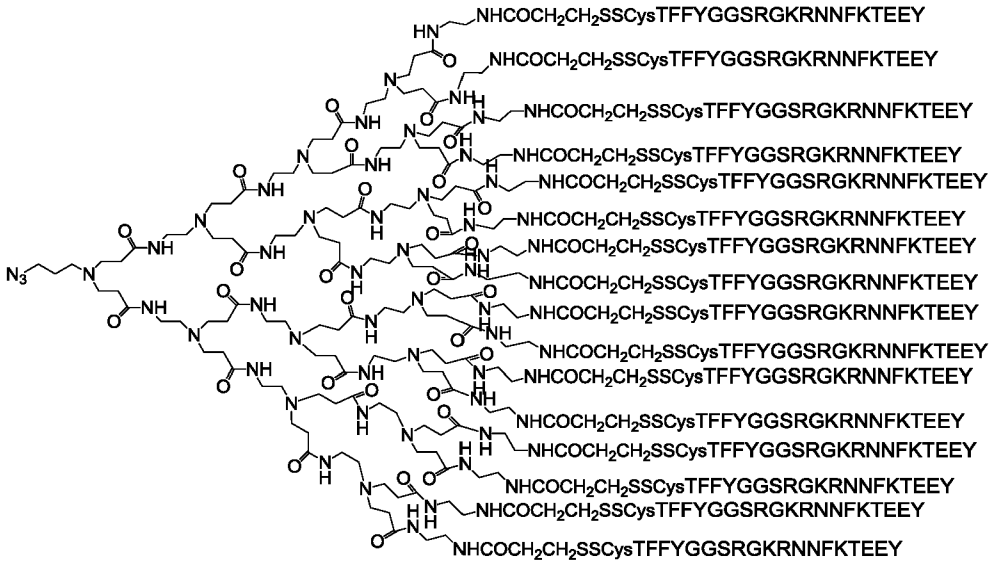
57. The compound of claim 1 having the formula:



,

wherein Cys is cysteine and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine.

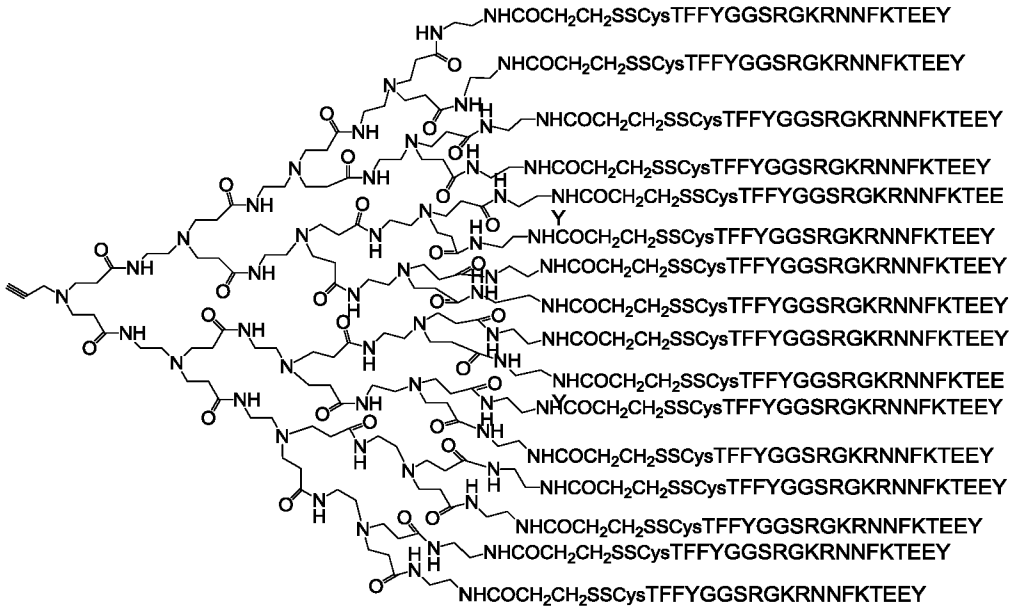
58. The compound of claim 1 having the formula:



,

wherein Cys is cysteine and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine.

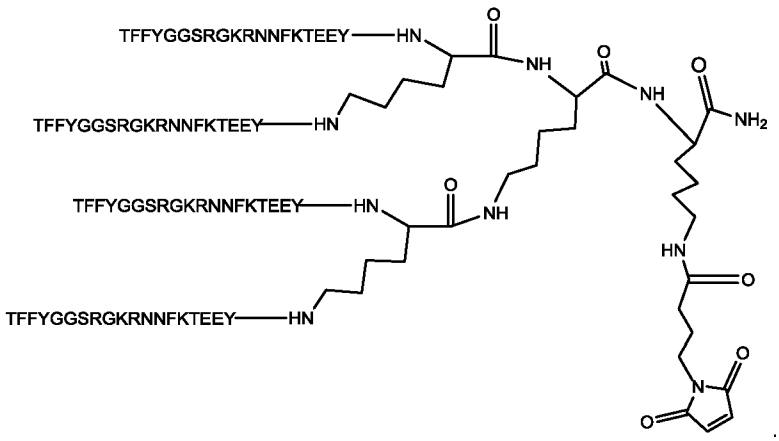
59. The compound of claim 1 having the formula:



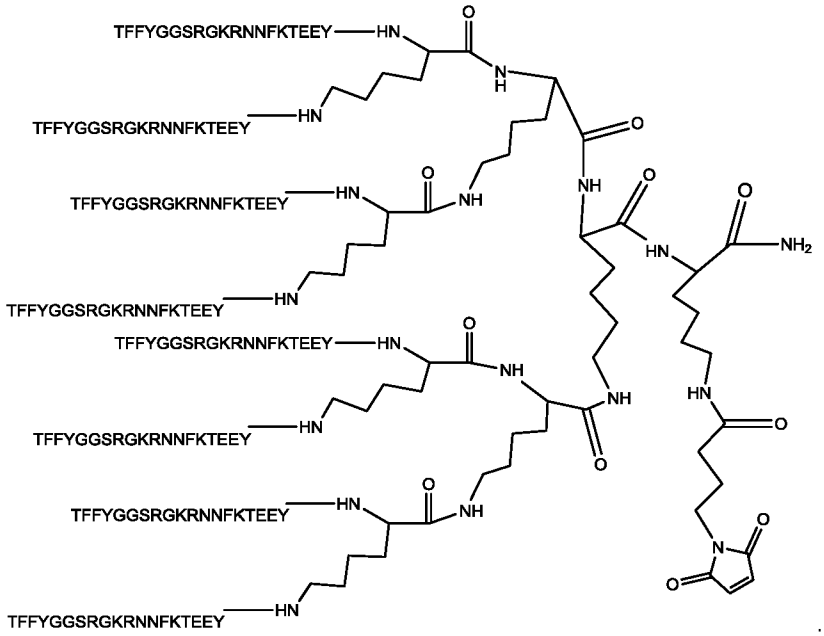
,

wherein Cys is cysteine and the "SS" adjacent to Cys represents a disulfide bond, including the sulfur atom of the cysteine.

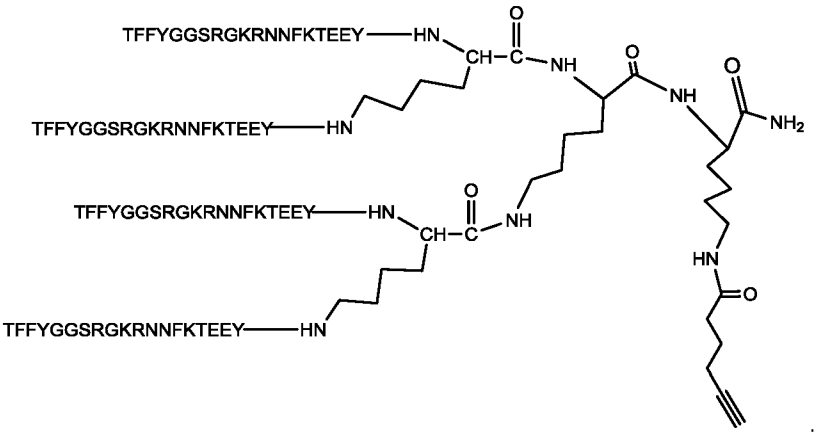
60. The compound of claim 1 having the formula:



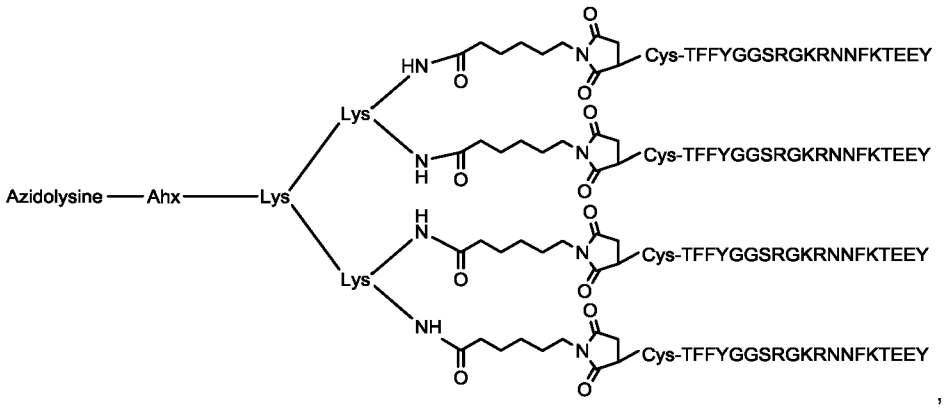
61. The compound of claim 1 having the formula:



62. The compound of claim 1 having the formula:

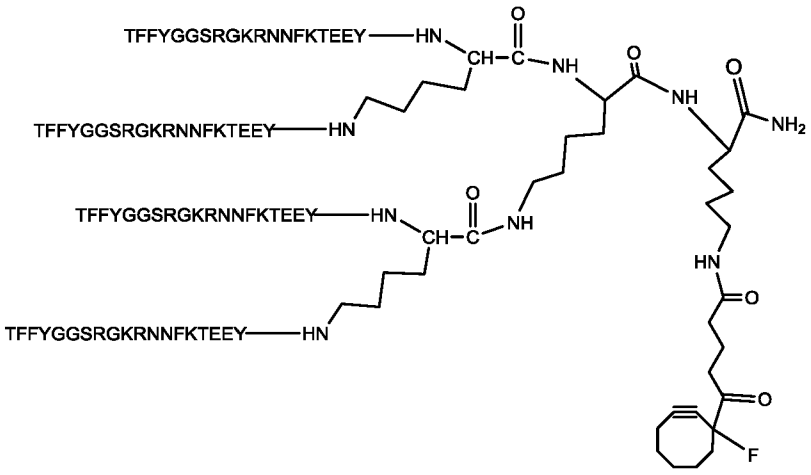


63. The compound of claim 1 having the formula:

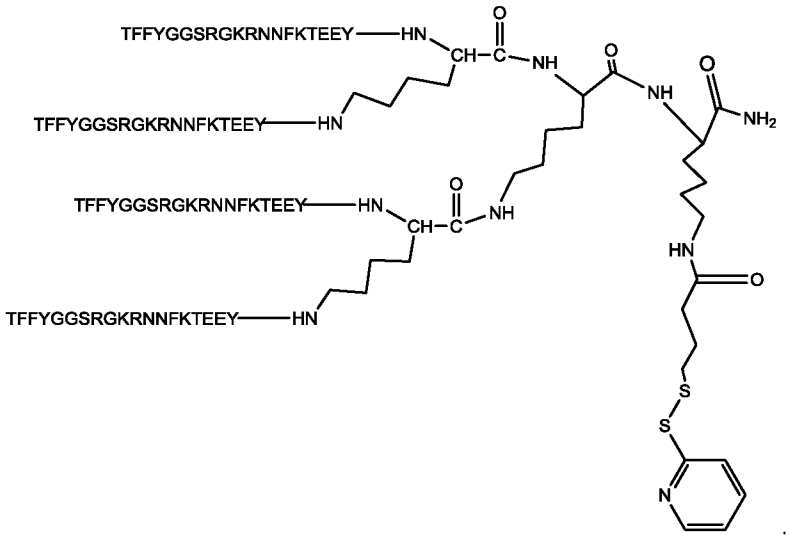


wherein Cys is cysteine, and Ahx is azidohexanoic acid.

64. The compound of claim 1 having the formula:



65. The compound of claim 1 having the formula:



66. The compound of any one of claims 1 to 65, wherein said first agent is selected from the group consisting of a protein, a small molecule, a nucleic acid, a diagnostic agent, an imaging agent, and a therapeutic agent.

67. The compound of any one of claims 1 to 66, wherein said second agent is selected from the group consisting of a protein, a small molecule, a nucleic acid, a diagnostic agent, an imaging agent, and a therapeutic agent.

68. The compound of any one of claims 1 to 67, wherein said first agent and said second agent are different.

69. The compound of any one of claims 1 to 67, wherein said first agent and said second agent are the same.

70. The compound of any one of claims 1 to 69, wherein said compound is able to enter endothelial cells or enter cells that express the LRP-1 receptor.

71. The compound of claim 70, wherein said cells expressing LRP-1 receptor are selected from the group consisting of liver, kidney, and spleen cells.

72. The compound of any one of claims 1 to 71, wherein said compound is able to cross the blood brain barrier.

73. A method of synthesizing a compound of any one of claims 1 to 72 comprising:

(a) attaching at least two targeting peptides to a dendrimer, via a linker, to form a dendrimer-targeting peptide complex;

(b) attaching one or more first agents, via a linker, to said dendrimer-targeting peptide complex; and

(c) optionally attaching one or more second agents, via a linker, to the complex formed in step (b).

74. A method of synthesizing a compound of any one of claims 1 to 72 comprising:

(a) attaching at least one first agent to a dendrimer, via a linker, to form a dendrimer-first agent complex;

(b) attaching at least two targeting peptides, via a linker, to said dendrimer-first agent complex; and

(c) optionally attaching one or more second agents, via a linker, to the complex formed in step (b).

75. The method of claim 73 or 74, wherein each of said targeting peptides is attached to a surface branch of said dendrimer.

76. The method of any one of claims 73 to 75, wherein said first agent is attached to the core moiety of said dendrimer.

77. The method of any one of claims 73 to 76, wherein said second agent is attached to at least one of said targeting peptides or to at least one of said surface branches.

78. The method of any one of claims 73 to 77, wherein said linker is selected from a group consisting of pyridinedisulfide, thiosulfonate, vinylsulfonate, isocyanate, NHS ester, imidoester, diazine, hydrazine, thiol, carboxylic acid, a multi-peptide linker, acetylene, a cleavable linker, and a non-cleavable linker.

79. The method of any one of claims 73 to 77, wherein said linker is a covalent bond.

80. The method of any one of claims 73 to 79, wherein one or more linkers are attached to said surface branch of said dendrimer prior to attachment of said targeting peptide to said linkers.

81. The method of any one of claims 73 to 80, wherein one or more linkers are attached to the core moiety of said dendrimer prior to attachment of said first agent to said linkers.

82. The method of any one of claims 73 to 81, wherein one or more linkers are attached to said targeting peptide prior to attaching said second agent to said linkers.

83. The method of any one of claims 73 to 82 wherein a linker is attached to said surface branch prior to attaching said second agent to said linker.

84. The method of any one of claims 73 to 83, wherein said attaching of said targeting peptide comprises reacting said dendrimer with *N*-succinimidyl 3-(2-pyridyldithio)-propionate; followed by reacting the resulting product with a cysteine residue-containing targeting peptide.

85. The method of any one of claims 73 to 83, wherein said attaching of said targeting peptide comprises reacting said dendrimer with *N*-succinimidyl S-acetylthioacetate; followed by reacting the resulting product with a maleimide derivative of said targeting peptide.

86. The method of claim 85, wherein said maleimide derivative of said targeting peptide comprises an amino acid sequence selected from the group consisting of Angiopep-1 (SEQ ID NO:67), Angiopep-2 (An_2) (SEQ ID NO:97), and reversed Angiopep-2 (SEQ ID NO:117).

87. The method of any one of claims 73 to 86, wherein said targeting peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:1-105 and 107-117 or a fragment thereof.

88. The method of claim 87, wherein said targeting peptide comprises an amino acid sequence selected from the group consisting of Angiopep-1 (SEQ ID NO:67), Angiopep-2 (An_2) (SEQ ID NO:97), and reversed Angiopep-2 (SEQ ID NO:117).

89. The method of claim 87, wherein said targeting peptide comprises an amino acid sequence selected from the group consisting of cys-Angiopep-2 ($CysAn_2$) (SEQ ID NO:113), Angiopep-2-cys (SEQ ID NO:114).

90. The method of any one of claims 73 to 89, wherein more than two targeting peptides are attached to said dendrimer.

91. The method of any one of claims 73 to 90, wherein said first agent is attached to the core moiety of said dendrimer via a reactive group.

92. The method of any one of claims 73 to 91, wherein one or more said second agent is attached to one or more said targeting peptides by a reactive group.

93. The method of any one of claims 73 to 92, wherein one or more said second agent is attached to one or more said surface branches of said dendrimer by a reactive group.

94. The method of any one of claims 73 to 93, wherein said reactive group is selected from a group consisting of a maleimide, a hydrazide, an azide, a haloacetamide, and an alkoxyamine.

95. The method of claim 94, wherein said reactive group is an azide group.

96. The method of claim 94, wherein said reactive group is a maleimide group.

97. The method of any one of claims 73 to 92, wherein said dendrimer is separated from said reactive group by a linker

98. The method of claim 93 or 94, wherein said reactive group is separated from said surface branch or said targeting peptide by a linker.

99. The method of claim 91, wherein said reactive group is present on a linker attached to said core moiety.

100. The method of any one of claims 97 to 99, wherein said linker is selected from a group consisting of pyridinedisulfide, thiosulfonate, vinylsulfonate, isocyanate, NHS ester, imidoester, diazine, hydrazine, thiol, carboxylic acid, a multi-peptide linker, acetylene, a cleavable linker, and a non-cleavable linker.

101. The method of any one of claims 73 to 100, wherein said first agent is selected from the group consisting of a protein, a small molecule, a nucleic acid, a diagnostic agent, an imaging agent, and a therapeutic agent.

102. The method of any one of claims 73 to 101, wherein said second agent is selected from the group consisting of a protein, a small molecule, a nucleic acid, a diagnostic agent, an imaging agent, and a therapeutic agent.

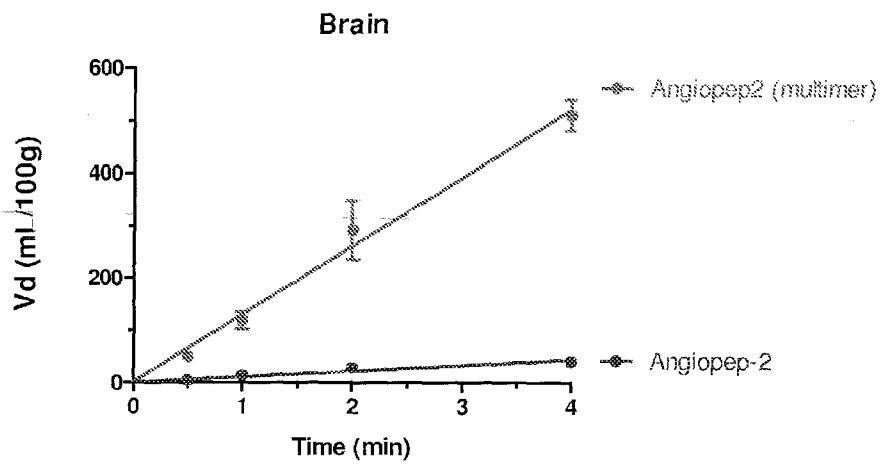
103. The method of any one of claims 73 to 102, wherein said first agent and said second agent are different.

104. The method of any one of claims 73 to 102, wherein said first agent and said second agent are identical.

105. The method of any one of claims 73 to 104, wherein said method further comprises synthesizing a pharmaceutically acceptable salt of a compound of any one of claims 1 to 72.

Figure 1

A



Kin (Brain):
An2 (multimer): 2.2 x 10^{-2} ml/s/g
Angiopep-2: 1.7 x 10^{-3} ml/s/g

B

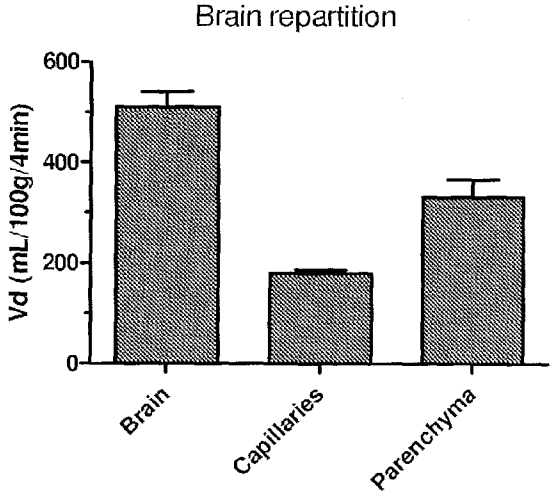
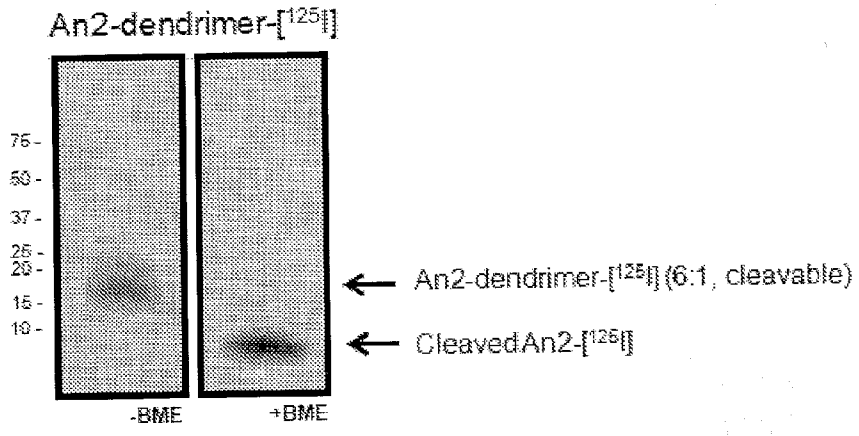


Figure 2


An2-dendrimer-[125I]
75 -
50 -
37 -
25 -
20 -
15 -
10 -
-BME
+BME
An2-dendrimer-[125I] (6:1, cleavable)
Cleaved An2-[125I]