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(54) **Title:** POLYMER COMPRISING MULTIPLE FUNCTIONALIZED SIDECHAINS FOR BIOMOLECULE DELIVERY

(57) **Abstract:** Provided is a polymer comprising a hydrolysable polymer backbone, the polymer backbone comprising (i) monomer units with a side chain comprising a hydrophobic group; (ii) monomer units with a side chain comprising an oligoamine or polyamine; and (iii) monomer units with a side chain comprising a polyalkylene oxide, polyglycolic acid, polylactic acid, or combination thereof, as well as a composition comprising the polymer, and a method of delivering one or more biomolecules or synthetic variants thereof to a cell using the polymer and/or the composition.

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POLYMER COMPRISING MULTIPLE FUNCTIONALIZED SIDECHAINS FOR BIOMOLECULE DELIVERY

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This patent application claims priority to U.S. provisional patent application U.S. provisional patent application 62/853,658 filed May 28, 2019, and U.S. provisional application 62/907,458 filed September 27, 2019, the entire disclosures of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

[0002] Peptide, protein, and nucleic based technologies have countless applications to prevent, cure and treat diseases. However, the safe and effective delivery of large molecules (e.g., polypeptides and nucleic acids) to their target tissues remains problematic. Accordingly, there continues to be a need for new compositions and methods useful for delivering therapeutic molecules.

BRIEF SUMMARY OF THE INVENTION

[0003] Provided herein is a polymer comprising a hydrolysable polymer backbone, the polymer backbone comprising: (a) monomer units with a side chain comprising a hydrophobic group; (b) monomer units with a side chain comprising an oligoamine or polyamine; and (c) monomer units with a side chain comprising a polyalkylene oxide, polyglycolic acid, polylactic acid, or combination thereof.

[0004] Also provided herein is a composition comprising a first polymer and a second polymer, wherein the first polymer comprises a hydrolysable polymer backbone, the polymer backbone comprising: (a) monomer units with a side chain comprising a hydrophobic group; (b) monomer units with a side chain comprising an oligoamine or polyamine; and (c) monomer units with a side chain comprising a polyalkylene oxide, polyglycolic acid, polylactic acid, or combination thereof; and the second polymer comprises a hydrolysable polymer backbone, the hydrolysable polymer backbone comprising (a) monomer units with a side chain comprising a hydrophobic group, (ii) monomer units with a side chain comprising an oligoamine or polyamine, and, optionally, (iii) monomer units with a side chain comprising an ionizable group, optionally with a pKa less than 7.

[0005] The disclosure also provides methods of using the compositions described herein, for example, to deliver a nucleic acid or protein to a cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] FIG. 1 provides the amino acid sequence of Cas9 from *Streptococcus pyogene* (SEQ ID NO:1).

[0007] FIG. 2 provides the amino acid sequence of Cpf1 from *Francisella tularensis* subsp. Novicida U112 (SEQ ID NO:2).

[0008] FIG. 3 provides the sequence of AsCpf1 (SEQ ID NO: 19).

[0009] FIG. 4 provides the sequence of LbCpf1 (SEQ ID NO: 20).

[0010] FIGs. 5A-5C show microscopic images of nanoparticles formed from polymers of the invention formulated with mCherry mRNA, as described in Example 3.

[0011] FIGs. 6A and 6B show microscopic images of Hep3B cells transfected with nanoparticles formed from polymers of the invention formulated with mCherry mRNA, as described in Example 4.

[0012] FIGs. 7A and 7B show microscopic images of primary myoblasts transfected with nanoparticles formed from polymers of the invention formulated with mCherry mRNA, as described in Example 5.

[0013] FIG. 8 is a graph illustrating transfection of polymer nanoparticles containing mCherry into human primary neural progenitor cells (NPCs), as described in Example 6.

[0014] FIG. 9 is a graph illustrating transfection efficiency of polymer nanoparticles containing Cas9 RNP as a function of GFP knock-out, as described in Example 7.

[0015] FIG. 10 is a schematic illustration of a mouse Loxp-luciferase reporter function.

[0016] FIGs. 11A-11C show the bioluminescence imaging of luciferase expressing mice treated with compositions as described in Example 8.

[0017] FIGs. 12A and 12B are graphs illustrating transfection efficiency of polymer nanoparticles in human neural progenitor cells (NPCs) and Hep3B cells, respectively, as a function of RFP fluorescence, and as described in Example 10. The x-axis names the polymer mixed with H27N and mCherry RNA to form nanoparticles.

[0018] FIGs. 13A and 13B are graphs illustrating transfection efficiency of polymer nanoparticles in human neural progenitor cells (NPCs) and Hep3B cells, respectively, as a function of RFP fluorescence, and as described in Example 12. The x-axis names the polymer mixed with II-46 and mCherry RNA to form nanoparticles.

[0019] FIG. 14 is a schematic illustration of a mouse ai9 reporter function.

DETAILED DESCRIPTION OF THE INVENTION

Polymer

[0020] The invention provides a polymer comprising a hydrolysable polymer backbone, the polymer backbone comprising: (a) monomer units with a side chain comprising a hydrophobic group; (b) monomer units with a side chain comprising an oligoamine or polyamine; and (c) monomer units with a side chain comprising a polyalkylene oxide, polyglycolic acid, polylactic acid, or combination thereof.

[0021] As used herein, the phrase hydrolysable polymer backbone refers to a polymer backbone having bonds that are susceptible to cleavage under physiological conditions (e.g., physiological pH, physiological temperature, or in a given *in vivo* tissue such as blood, serum, etc. due to naturally occurring factors (e.g., enzymes). Generally, the hydrolysable polymer backbone comprises a polyamide, poly-N-alkylamide, polyester, polycarbonate, polycarbamate, or a combination thereof. In certain embodiments, the hydrolysable polymer backbone comprises a polyamide.

[0022] The monomer units with a side chain comprising a hydrophobic group, can comprise any hydrophobic group, and can be linked to the polymer backbone in any suitable manner, such as directly or via a linkage comprising, for instance, an ester, an amide, or an ether group, optionally further comprising an alkylene linker (e.g., a methylene or ethylene linker). Examples of hydrophobic groups include, for instance, a C_1-C_{12} (e.g., C_2-C_{12}, C_2-C_{10}, C_2-C_8, C_2-C_6, C_3-C_{12}, C_3-C_{10}, C_3-C_8, C_3-C_6, C_4-C_{12}, C_4-C_{10}, C_4-C_8, C_4-C_6, C_6-C_{12}, C_6-C_8, C_8-C_{12}, C_8-C_{10},) alkyl group, a C_2-C_{12} (e.g., C_2-C_6, C_3-C_{12}, C_3-$C1_0$, C_3-C_8, C_3-C_6, C_4-C_{12}, C_4-C_{10}, C_4-C_8, C_4-C_6, C_6-C_{12}, C_6-C_8, C_8-C_{12}, C_8-C_{10},) alkenyl group, or a C_3-C_{12} (C_3-$C1_0$, C_3-C_8, C_3-C_6, C_4-C_{12}, C_4-C_{10}, C_4-C_8, C_4-C_6, C_6-C_{12}, C_6-C_8, C_8-C_{12}, C_8-C_{10},) cycloalkyl group or cycloalkenyl group. In certain embodiments, the hydrophobic group comprises a C_4-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group. In some embodiments, the hydrophobic group comprises fewer than 8 carbons or fewer than 6 carbons. For example, the hydrophobic group can comprise a C_2-C_8 or C_2-C_6 (e.g., C_3-C_8 or C_3-C_6) alkyl group. The alkyl or alkenyl groups can be branched or straight-chain. The alkyl or alkenyl groups can be substituted, provided the substituent groups do not negate the hydrophobicity of the overall hydrophobic side-chain (e.g., does not render the side-chain hydrophilic). For instance, the alkyl or alkenyl group can be substituted with hydroxyl groups (e.g., 1 or 2 hydroxyl groups) or halogen groups (e.g., substituted with one or more fluorine, chlorine, bromine, or iodine atoms). In any of the foregoing embodiments, the hydrophobic group can be linked to the polymer backbone directly or via a linkage comprising, for instance, an ester, an amide, or an

ether group, optionally further comprising an alkylene linker (e.g., a methylene or ethylene linker).

[0023] The polymer also comprises monomer units with a side chain comprising an oligoamine or polyamine. As used herein, the term "oligoamine" refers to any chemical moiety having two or three amine groups, and the term "polyamine" refers to any chemical moiety having four or more (e.g., 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, etc.) amine groups. The amine groups can be primary amine groups, secondary amine groups, tertiary amine groups, or any combination thereof. In certain embodiments, the oligoamine or polyamine is of the formula:

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2_2$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2_2]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2_2]_2\}_2$,

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^4\text{-}R^5]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^4\text{-}R^5\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^4\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^5]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^5\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[N\{(CH_2)_{s1}\text{-}R^4\text{-}R^5\}—(CH_2)_{q1}—]_{r1}NR^2_2$;

$—(CH_2)_{p1}—[N\{(CH_2)_{s1}\text{-}R^5\}—(CH_2)_{q1}—]_{r1}NR^2_2$,

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH(CONH_2)\text{-}(CH_2)_{s1}\text{-}R^5$; or

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH(CONH_2)\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5$,

wherein p1 to p4, q1 to q6, r1 and r2, and s1 to s4 are each independently an integer of 1 to 5;

each instance of R^2 is independently hydrogen or a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl group, or R^2 is combined with a second R^2 so as to form a heterocyclic group;

each instance of R^4 is independently -C(O)O-, -C(O)NH-, -O-C(O)O-, or -S(O)(O)-; and

each instance of R^5 is independently an alkyl group, cycloalkyl group, alkenyl group, cycloalkenyl group, aryl group, heteroalkyl group, heterocyclic group, or combination thereof optionally comprising from 2 to 8 tertiary amines or a substituent comprising a tissue-specific or cell-specific targeting moiety.

[0024] In some embodiments, the oligoamine or polyamine is of the formula:

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2_2$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2_2]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}$; or

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2_2]_2\}_2$,

wherein p1 to p4, q1 to q6, and r1 and r2 are each independently an integer of 1 to 5 (e.g., 1, 2, or 3); and

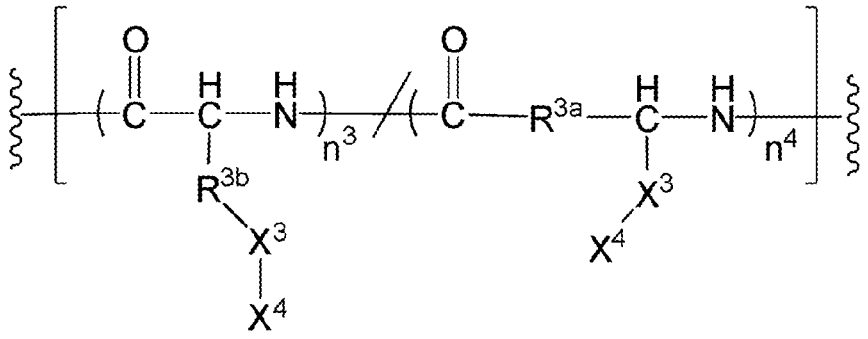
each instance of R^2 is independently hydrogen or a C_1-C_{12} (e.g., C_1-C_6, C_1-C_3, C_2, or C_1) alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, or R^2 is combined with a second R^2 so as to form a heterocyclic group. It is understood that the alkenyl groups must have at least 2 carbons (e.g., C_2-C_{12}, C_2-C_6, etc.) and the cycloalkyl and cycloalkenyl groups must have at least 3 carbons (e.g., C_3-C_{12}, C_3-C_6, etc.). In some embodiments, the polyamine is $—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2_2$, optionally wherein R^2 is independently hydrogen or a C_1-C_3 alkyl (e.g., methyl or ethyl).

[0025] Specific non-limiting examples of oligoamine or polyamine side chains include, for instance, -NH-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)$_2$; -N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)$_2$; -NH-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)$_2$; -N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)$_2$; -NH-CH_2-CH_2-N(CH_3)-CH_2-CH_2-NH(CH_3); -N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-NH(CH_3); -NH-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-NH(CH_3); -N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-NH(CH_3).

[0026] The polymer also comprises monomer units with a side chain comprising a polyalkylene oxide, polyglycolic acid, polylactic acid, or combination thereof, can be linked to the polymer backbone in any suitable manner, such as directly or via a linkage comprising, for instance, an ester, an amide, or an ether group, optionally further comprising an alkylene linker (e.g., a methylene or ethylene linker). In some embodiments, the side chain comprises at least one polyethylene glycol group (a.k.a. polyethylene oxide group) having a sum total of from 2 to 200 ethylene oxide units (e.g., from 2 to 150 units, from 2 to 100 units, from 2 to 50

units, from 10 to 200 units, from 10 to 150 units, from 10 to 100 units, from 10 to 50 units, from 25 to 200 units, from 25 to 150 units, from 25 to 100 units, from 25 to 50 units, from 50 to 200 units, from 50 to 150 units, or from 50 to 100 units). In some embodiments, the side chain comprises at least one polypropylene oxide group having a sum total of from 2 to 200 propylene oxide units (e.g., from 2 to 150 units, from 2 to 100 units, from 2 to 50 units, from 10 to 200 units, from 10 to 150 units, from 10 to 100 units, from 10 to 50 units, from 25 to 200 units, from 25 to 150 units, from 25 to 100 units, from 25 to 50 units, from 50 to 200 units, from 50 to 150 units, or from 50 to 100 units). In some embodiments, the side chain comprises at least one polylactic acid and/or polyglycolic acid group having a sum total of from 2 to 200 polylactic acid and/or polyglycolic acid units (e.g., from 2 to 150 units, from 2 to 100 units, from 2 to 50 units, from 10 to 200 units, from 10 to 150 units, from 10 to 100 units, from 10 to 50 units, from 25 to 200 units, from 25 to 150 units, from 25 to 100 units, from 25 to 50 units, from 50 to 200 units, from 50 to 150 units, or from 50 to 100 units). In certain embodiments, the side chain comprises at least one polyethylene glycol/polypropylene oxide group having a sum total of from 2 to 200 ethylene glycol and/or propylene oxide units and at least one polylactic acid and/or polyglycolic acid group having a sum total of from 2 to 200 polylactic acid and/or polyglycolic acid units. Without wishing to be bound by any particular theory, it is believed that the polyethylene glycol/polypropylene oxide group may enhance biodistribution and/or reduced toxicity when injected in vivo, and the polylactic acid and/or polyglycolic acid group may help to adjust the zeta potential of the positively charged nanoparticles (e.g., to negatively charged or neutral). In embodiments where the side chain comprises at least one polyethylene glycol/polypropylene oxide group having a sum total of from 2 to 200 ethylene glycol and/or propylene oxide units and at least one polylactic acid and/or polyglycolic acid group having a sum total of from 2 to 200 polylactic acid and/or polyglycolic acid units, the side chain can exist as any suitable structure type. For example, the side chain can comprise the ethylene glycol and/or propylene oxide units and the polylactic acid and/or polyglycolic acid units as an alternating polymer, random polymer, block polymer, graft polymer, linear polymer, branched polymer, cyclic polymer, or a combination thereof. In certain embodiments, the side chain comprises polyalkylene oxide units (e.g., ethylene oxide units, propylene oxide units, or both) and optionally further comprises polylactic acid or polyglycolic acid units. For example, the side chain of at least some of the monomers can be derived from PLURONIC® F65 or PLURONIC® F127.

[0027] For instance, the polymer (e.g., the first polymer) can comprise monomer units with a side chain comprising a polyalkylene oxide, polyglycolic acid, polylactic acid, or combination thereof, which comprise a structure of Formula 1:



wherein:

each of n^3 and n^4 is an integer from 0 to 1000, provided that the sum of $n^3 + n^4$ is greater than 1;

the symbol "/" indicates that the units separated thereby are linked randomly or in any order;

each instance of R^{3a} is independently a methylene or ethylene group;

each instance of R^{3b} is independently a methylene or ethylene group;

each X^3 independently is —C(O)O—, —C(O)NR^{13}—, —C(O)—, —S(O)(O)—, or a bond;

each instance of R^{13} is independently hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl group, any of which can be optionally substituted with one or more substituents;

each instance of X^4 comprises polyalkylene oxide, polyglycolic acid, polylactic acid, or a combination thereof;

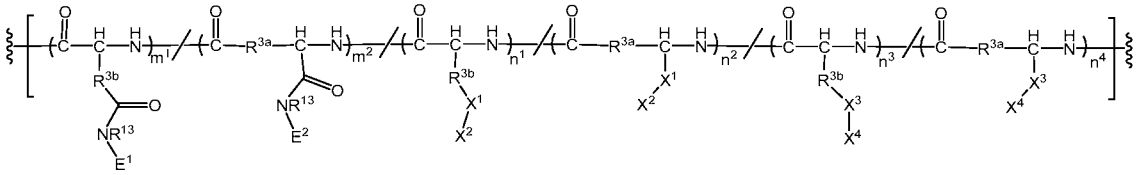
each instance of R^{18} is independently hydrogen or methyl; and

each instance of p is independently an integer from 2 to 200.

[0028] The different monomer units can be arranged in any order, including blocks of monomers or monomers randomly arranged throughout the polymer. Furthermore, the polymer can comprise any suitable number or amount (e.g., weight or number percent composition) of the monomer units with a side chain comprising a hydrophobic group, the monomer units with a side chain comprising an oligoamine or polyamine, and the monomer units with a side chain comprising a polyalkylene oxide, polyglycolic acid, polylactic acid, or combination thereof. In some embodiments, the polymer comprises about 1 to about 80 mol% (e.g., about 5 to about 80 mol%, about 10 to about 80 mol%, about 20 to about 80

mol%, about 40 to about 80 mol%, about 1 to about 60 mol%, about 1 to about 40 mol%, about 1 to about 20 mol%, or about 1 to about 10 mol%) of the monomer units having a hydrophobic group. In some embodiment, the polymer comprises about 1 to about 80 mol% (e.g., about 5 to about 80 mol%, about 10 to about 80 mol%, about 20 to about 80 mol%, about 40 to about 80 mol%, about 1 to about 60 mol%, about 1 to about 40 mol%, about 1 to about 20 mol%, or about 1 to about 10 mol%) of the monomer units having an oligoamine or polyamine. In some embodiments, the polymer comprises about 1 to about 80 mol% (e.g., about 5 to about 80 mol%, about 10 to about 80 mol%, about 20 to about 80 mol%, about 40 to about 80 mol%, about 1 to about 60 mol%, about 1 to about 40 mol%, about 1 to about 20 mol%, or about 1 to about 10 mol%) of the monomer units having a polyalkylene oxide, polyglycolic acid, polylactic acid, or combination thereof.

[0029] In some embodiments, the polymer has a polyamide backbone. For instance, the polymer can have the structure of Formula 2:



wherein

each of m^1 and m^2 is an integer from 0 to 1000, provided that the sum of $m^1 + m^2$ is greater than 1;

each of n^1 and n^2 is an integer from 0 to 1000, provided that the sum of $n^1 + n^2$ is greater than 1;

each of n^3 and n^4 is an integer from 0 to 1000, provided that the sum of $n^3 + n^4$ is greater than 1;

the symbol "/" indicates that the units separated thereby are linked randomly or in any order;

each instance of R^{3a} is independently a methylene or ethylene group;

each instance of R^{3b} is independently a methylene or ethylene group;

each X^1 independently is —C(O)O—, —C(O)NR^{13}—, —C(O)—, —S(O)(O)—, or a bond;

each instance of R^{13} is independently hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl group, any of which can be optionally substituted with one or more substituents;

each instance of X^2 is independently a C_1-C_{12} alkyl or heteroalkyl group, C_3-C_{12} cycloalkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkenyl group, aryl group, heterocyclic group, or combination thereof; any of which are optionally substituted with one or more substituents;

each X^3 independently is —C(O)O—, —C(O)NR^{13}—, —C(O)—, —S(O)(O)—, or a bond;

each instance of X^4 comprises polyalkylene oxide, polyglycolic acid, polylactic acid, or a combination thereof;

each of E^1 and E^2 are each independently a group of formula

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2{}_2]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}$; or

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2{}_2]_2\}_2$,

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^4\text{-}R^5]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^4\text{-}R^5\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^4\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^5]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^5\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[N\{(CH_2)_{s1}\text{-}R^4\text{-}R^5\}—(CH_2)_{q1}—]_{r1}NR^2{}_2$;

$—(CH_2)_{p1}—[N\{(CH_2)_{s1}\text{-}R^5\}—(CH_2)_{q1}—]_{r1}NR^2{}_2$,

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH(CONH_2)\text{-}(CH_2)_{s1}\text{-}R^5$; or

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH(CONH_2)\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5$,

wherein p1 to p4, q1 to q6, r1 and r2, and s1 to s4 are each independently an integer of 1 to 5;

each instance of R^2 is independently hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl

group, any of which are optionally substituted with one or more substituents, or R^2 is combined with a second R^2 so as to form a heterocyclic group;

each instance of R^4 is independently -C(O)O-, -C(O)NH-, -O-C(O)O-, or -S(O)(O)-; and

each instance of R^5 is independently a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl, aryl group, C_1-C_{12} heteroalkyl group, C_3-C_{12} heterocyclic group, or combination thereof optionally comprising from 2 to 8 tertiary amines or a substituent comprising a tissue-specific or cell-specific targeting moiety.

[0030] As used herein, "alkyl" or "alkylene" refers to a substituted or unsubstituted hydrocarbon chain. The alkyl group can have any number of carbon atoms (e.g., C_1-C_{100} alkyl, C_1-C_{50} alkyl, C_1-C_{12} alkyl, C_1-C_8 alkyl, C_1-C_6 alkyl, C_1-C_4 alkyl, C_1-C_2 alkyl, etc.). The alkyl or alkylene can be saturated, or can be unsaturated (e.g., to provide an alkenyl or alkynyl), and can be linear, branched, cyclic (e.g., cycloalkyl or cycloalkenyl), or a combination thereof. Cyclic groups can be monocyclic, fused to form bicyclic or tricyclic groups, linked by a bond, or spirocyclic. In some embodiments, the alkyl substituent can be interrupted by one or more heteroatoms (e.g., oxygen, nitrogen, and sulfur), thereby providing a heteroalkyl, heteroalkylene, or heterocyclyl (i.e., a heterocyclic group). In some embodiments, the alkyl is substituted with one or more substituents.

[0031] The term "aryl" refers to an aromatic ring system having any suitable number of ring atoms and any suitable number of rings. Aryl groups can include, for instance, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or 16 ring atoms, as well as from 6 to 10, 6 to 12, or 6 to 14 ring members. Aryl groups can be monocyclic, fused to form bicyclic or tricyclic groups, or linked by a bond to form a biaryl group. Representative aryl groups include phenyl, naphthyl and biphenyl. In some embodiments, the aryl group comprises an alkylene linking group so as to form an arylalkyl group (e.g., a benzyl group). Some aryl groups have from 6 to 12 ring members, such as phenyl, naphthyl or biphenyl. Other aryl groups have from 6 to 10 ring members, such as phenyl or naphthyl. In some embodiments, the aryl substituent can be interrupted by one or more heteroatoms (e.g., oxygen, nitrogen, and sulfur), thereby proving a heterocyclyl (i.e., a heterocyclic or heteroaryl group). In some embodiments, the aryl is substituted with one or more substituents.

[0032] The term "heterocyclyl," or "heterocyclic group" refers to a cyclic group, e.g., aromatic (e.g., heteroaryl) or non-aromatic where the cyclic group has one or more heteroatoms (e.g., oxygen, nitrogen, and sulfur). In some embodiments, the heterocyclyl or

heterocyclic group (i.e., cyclic group, e.g., aromatic (e.g., heteroaryl) or non-aromatic where the cyclic group has one or more heteroatoms) is substituted with one or more substituents.

[0033] As used herein, the term "substituted" can mean that one or more hydrogens on the designated atom or group (e.g., substituted alkyl group) are replaced with another group provided that the designated atom's normal valence is not exceeded. For example, when the substituent is oxo (i.e., =O), then two hydrogens on the atom are replaced. Substituent groups can include one or more of a hydroxyl, an amino (e.g., primary, secondary, or tertiary), an aldehyde, a carboxylic acid, an ester, an amide, a ketone, nitro, an urea, a guanidine, cyano, fluoroalkyl (e.g., trifluoromethane), halo (e.g., fluoro), aryl (e.g., phenyl), heterocyclyl or heterocyclic group (i.e., cyclic group, e.g., aromatic (e.g., heteroaryl) or non-aromatic where the cyclic group has one or more heteroatoms), oxo, or combinations thereof. Combinations of substituents and/or variables are permissible provided that the substitutions do not significantly adversely affect synthesis or use of the compound.

[0034] According to Formula 2, each of m^1, m^2, n^1, n^2, n^3, and n^4 is an integer from 0 to 1000 (e.g., 0 to 500, 0 to 200, 0 to 100, or 0 to 50), provided that the sum of $m^1 + m^2 + n^1 + n^2 + n^3 + n^4$ is greater than 5, such as 5-5000, 5-2000, 5-1000, 5-500, 5-100, or 5-50. In some embodiments, the sum of $m^1 + m^2 + n^1 + n^2 + n^3 + n^4$ is greater than 10 or greater than 20 (e.g., 10-5000, 10-2000, 10-1000, 10-500, 10-100, or 10-50; or 20-5000, 20-2000, 20-1000, 20-500, 20-100, or 20-50). In some embodiments, each of m^1 and m^2 is an integer from 0 to 1000 (e.g., 0 to 500, 0 to 200, 0 to 100, 0 to 50, or 0 to 25), provided that the sum of $m^1 + m^2$ is greater than 1 (e.g., 1-2000, 1-1000, 1-500, 1-200, 1-100, 1-50, or 1-25). In some embodiments, the sum of $m^1 + m^2$ is greater than 5 or greater than 10 (e.g., 5-2000, 5-1000, 5-500, 5-200, 5-100, 5-50, or 5-25; or 10-2000, 10-1000, 10-500, 10-200, 10-100, 10-50, or 10-25). In certain embodiments, each of n^1 and n^2 is an integer from 0 to 1000 (e.g., 0 to 500, 0 to 200, 0 to 100, 0 to 50, or 0 to 25), provided that the sum of $n^1 + n^2$ is greater than 1 (e.g., 1-2000, 1-1000, 1-500, 1-200, 1-100, 1-50, or 1-25). In some embodiments, the sum of $n^1 + n^2$ is greater than 5 or greater than 10 (e.g., 5-2000, 5-1000, 5-500, 5-200, 5-100, 5-50, or 5-25; or 10-2000, 10-1000, 10-500, 10-200, 10-100, 10-50, or 10-25). In some embodiments, each of n^3 and n^4 is an integer from 0 to 1000 (e.g., 0 to 500, 0 to 200, 0 to 100, 0 to 50, or 0 to 25), provided that the sum of $n^3 + n^4$ is greater than 1 (e.g., 1-2000, 1-1000, 1-500, 1-200, 1-100, 1-50, or 1-25). In some embodiments, the sum of $n^3 + n^4$ is greater than 5 or greater than 10 (e.g., 5-2000, 5-1000, 5-500, 5-200, 5-100, 5-50, or 5-25; or 10-2000, 10-1000, 10-500, 10-200, 10-100, 10-50, or 10-25).

[0035] In other words, the polymer comprises at least some n^3 and/or n^4 monomer units, i.e., at least some monomer units comprising X^4 groups, which comprise a polyalkylene oxide, polyglycolic acid, polylactic acid, or a combination thereof (referred to collectively as "X^4" monomers). The polymer also comprises at least some m^1 and/or m^2 monomeric units, i.e., monomer units comprising variables E^1 or E^2 comprising polyamine or oligoamine groups (referred to collectively as "E" monomers). In addition, the polymer comprises at least some monomeric units comprising groups n^1 and/or n^2 monomer units, i.e., monomer units comprising X^2 groups which include a hydrophobic group (referred to collectively as "X^2" monomers).

[0036] The polymer can comprise any suitable ratio of E monomers, X^2 monomers, and X^4 monomers. In some embodiments, the polymer comprises a number ratio of E monomers to the sum of X^2 and X^4 monomers (i.e., $(m^1 + m^2)/(n^1 + n^2+ n^3 + n^4)$) can be about 0.5 to about 500 (e.g., about 0.5 to about 250, about 0.5 to about 100, about 0.5 to about 50, about 0.5 to about 10, about 0.5 to about 5, about 0.5 to about 2, about 0.5 to about 1.5, about 0.5 to about 1, about 1 to about 500, about 5 to about 500, about 10 to about 500, about 50 to about 500, about 100 to about 500, or about 250 to about 500). Alternatively, or in addition, the ratio of X^2 monomers to the sum of X^4 monomers and E monomers (i.e., $(n^1 + n^2)/(m^1 + m^2+ n^3 + n^4)$) in some embodiments can be about 0.002 to about 500 (e.g., about 0.002 to about 250, about 0.002 to about 100, about 0.002 to about 50, about 0.002 to about 10, about 0.002 to about 5, about 0.002 to about 1, about 0.5 to about 250, about 0.5 to about 100, about 0.5 to about 50, about 0.5 to about 10, about 0.5 to about 5, about 0.5 to about 2, about 0.5 to about 1.5, about 0.5 to about 1, about 1 to about 500, about 5 to about 500, about 10 to about 500, about 50 to about 500, about 100 to about 500, or about 250 to about 500). Alternatively, or in addition, in some embodiments, the ratio of X^4 monomers to the sum of X^2 and E monomers (i.e., $(n^3 + n^4)/(m^1 + m^2+ n^1 + n^2)$) can be about 0.002 to about 500 (e.g., about 0.002 to about 250, about 0.002 to about 100, about 0.002 to about 50, about 0.002 to about 10, about 0.002 to about 5, about 0.002 to about 1, about 0.002 to about 0.5, about 0.002 to about 0.25), about 0.01 to about 500 (e.g., about 0.01 to about 250, about 0.01 to about 100, about 0.01 to about 50, about 0.01 to about 10, about 0.01 to about 5, about 0.01 to about 1, about 0.01 to about 0.5, about 0.01 to about 0.25), about 0.05 to about 500 (e.g., about 0.05 to about 250, about 0.05 to about 100, about 0.05 to about 50, about 0.05 to about 10, about 0.05 to about 5, about 0.05 to about 1, about 0.05 to about 0.5, about 0.05 to about 0.25), about 0.1 to about 500 (e.g., about 0.1 to about 250, about 0.1 to about 100, about 0.1 to about 50, about 0.1 to about 10, about 0.1 to about 5, about 0.1 to about 1, about 0.1 to

about 0.5, about 0.1 to about 0.25), about 0.5 to about 500 (e.g., about 0.5 to about 250, about 0.5 to about 100, about 0.5 to about 50, about 0.5 to about 10, about 0.5 to about 5, about 0.5 to about 1), about 1 to about 500 (e.g., about 1 to about 250, about 1 to about 100, about 1 to about 50, about 1 to about 10, about 1 to about 5, about 1 to about 2). The polymer can have any suitable type of structure. For example, the polymer can be an alternating polymer wherein the different monomers alternate according to some sequence or pattern; a block polymer wherein multiple monomers of a given type (e.g., "E" monomers, "X^2" monomers, or "X^4" monomers) are arranged in blocks; or random or grafted polymer wherein the various monomer types (e.g., "E" monomers, "X^2" monomers, or "X^4" monomers) are arranged in any order. The polymer can be structured as a linear polymer, branched polymer, cyclic polymer, or a combination thereof.

[0037] By way of further illustration, the monomers (which can be referred to by reference to their E^1, E^2, X^2, and X^4 side chains according to formula 2) can be arranged randomly or in any order. Thus, m^1, m^2, n^1, n^2, n^3, and n^4, merely denote the number of the respective monomers that appear in the chain overall, and do not describe or imply blocks of those monomers, although blocks or stretches of a given monomer might be present in some embodiments. For instance, the structure of Formula 2 can comprise the monomers in the order -E^1-E^2-X^2-X^4-, -E^2-E^1-X^2-X^4-, -E^1-X^2-E^2-X^4-, etc. Furthermore, the polymer can comprise blocks of E^1, E^2, X^2, and/or X^4 monomers in any order. In some embodiments, the E^1, E^2, X^2, and X^4 monomers are randomly distributed throughout the polymer. In other embodiments, the polymer comprises one or more segments in which the E^1, E^2, and X^2 monomers are randomly distributed, and a segment comprising a block of X^4 monomers. In some embodiments, the segment comprising a block of X^4 monomers can be sandwiched between segments comprising E^1, E^2, and X^2 monomers. In other embodiments, the segment comprising a block of X^4 monomers cap the polymer at one or both ends. In some embodiments, at least some portion (or the entirety) of the polymer (e.g., polyaspartamide) backbone will comprising monomers arranged in an alpha/beta configuration, such that monomers comprising R^{3a} and R^{3b} (referring to Formula 2) will alternate along the backbone.

[0038] In the polymer structures, R^{3a} and R^{3b} are each independently a methylene or ethylene group. In some embodiments, R^{3a} is an ethylene group and R^{3b} is a methylene group; or R^{3a} is a methylene group and R^{3b} is an ethylene group. In some embodiments, R^{3a} and R^{3b} are the same. In certain embodiments, R^{3a} and R^{3b} are both ethylene. In some embodiments, R^{3a} and R^{3b} are both methylene.

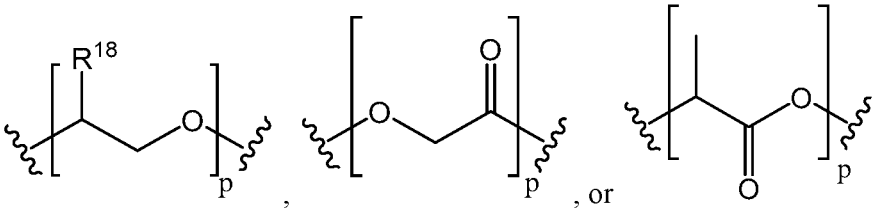
[0039] In the polymers described herein, each X^1 and X^3 group independently is —C(O)O—, —C(O)NR^{13}—, —C(O)—, —S(O)(O)—, or a bond. Each X^1 and X^3 group can be the same or different from one another. In some embodiments, X^1 and X^3 are the same. In some embodiments, X^1 and X^3 are —C(O)NR^{13}-. In some embodiments, X^1 and X^3 are —C(O)O—.

[0040] Each instance of R^{13} is independently hydrogen or a C_1-C_{12} (e.g., C_1-C_8, C_1-C_6, or C_1-C_3) alkyl group, C_2-C_{12} (e.g., C_2-C_8, C_2-C_6, or C_2-C_3) alkenyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkenyl group, aryl group, or heterocyclic group (e.g., 3-12, 3-10, 3-8, or 3-6 membered heterocyclic group comprising one, two, or three heteroatoms), any of which can be substituted with one or more substituents. In some embodiments, R^{13} is a C_1-C_{12} alkyl group (e.g., a C_1-C_{10} alkyl group; a C_1-C_8 alkyl group; a C_1-C_6 alkyl group; a C_1-C_4 alkyl group, a C_1-C_3 alkyl group, or a C_1 or C_2 alkyl group) which can be linear or branched. In some embodiments, R^{13} is a C_1-C_4 alkyl group, a C_1-C_3 alkyl group, or a C_1 or C_2 alkyl group. In certain embodiments, each R^{13} is methyl or hydrogen. In some embodiments, R^{13} is methyl; in other embodiments, R^{13} is hydrogen. Each R^{13} is independently chosen and can be the same or different; however, in some embodiments, each R^{13} is the same (e.g., all are methyl or all are hydrogen).

[0041] Each instance of X^2 is independently C_1-C_{12} (e.g., C_1-C_8, C_1-C_6, or C_1-C_3) alkyl group, C_2-C_{12} (e.g., C_2-C_8, C_2-C_6, or C_2-C_3) alkenyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkenyl group, aryl group, or heterocyclic group (e.g., 3-12, 3-10, 3-8, or 3-6 membered heterocyclic group comprising one, two, or three heteroatoms) or combination thereof, any of which can be substituted with one or more substituents. In some embodiments, X2 comprises a cyclic or fused ring hydrophobic group (e.g., cycloalkyl, cycloalkenyl, heterocyclyl, or aryl group having 4 to 12 members (e.g., C_4-C_{12}). In some embodiments, X^2 optionally can comprise one or more primary, secondary, or tertiary amines. In other embodiments, the alkyl or alkenyl group can be substituted with one or more hydroxyl groups (e.g., 1 or 2 hydroxyl groups) or halogen groups (e.g., substituted with one or more fluorine, chlorine, bromine, or iodine atoms). In embodiments, X^2 is hydrophobic, and any substituents should not negate the hydrophobicity of the X^2 group (e.g., does not render the group hydrophilic). Each X^2 is independently selected and, therefore, can be the same or different from one another. In some embodiments, one or more (or all) X^2 groups can be independently a C_2-C_{12} (e.g., C_3-C_{12}, C_3-C_8, C_3-C_6, C_4-C_{12}, C_4-C_6, C_6-C_{12}, or C_8-C_{12}) alkyl group or alkenyl group, or C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, C_4-C_{12}, C_4-C_6, C_6-C_{12}, or C_8-C_{12}) cycloalkenyl group, optionally substituted with

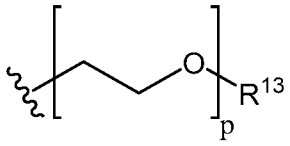
one or more hydroxyl groups (e.g., 1 or 2 hydroxyl groups) or halogen groups (e.g., substituted with one or more fluorine, chlorine, bromine, or iodine atoms). In other embodiments, one or more (or all) X^2 groups can be independently C_1-C_8 (e.g., C_1-C_6, C_1-C_4, C_1-C_3, C_2-C_8, C_2-C_6, C_3-C_8, or C_3-C_6) alkyl groups, optionally substituted with one or more hydroxyl groups (e.g., 1 or 2 hydroxyl groups) or halogen groups (e.g., substituted with one or more fluorine, chlorine, bromine, or iodine atoms). Any of the foregoing alkyl or alkenyl groups can be linear or branched.

[0042] Each instance of X^4 is a polyalkylene oxide, polyglycolic acid, polylactic acid, or a combination thereof. In some embodiments, each instance of X^4 comprises at least one polyethylene glycol group (a.k.a. polyethylene oxide group) having a sum total of from 2 to 200 ethylene oxide units (e.g., from 2 to 150 units, from 2 to 100 units, from 2 to 50 units, from 10 to 200 units, from 10 to 150 units, from 10 to 100 units, from 10 to 50 units, from 25 to 200 units, from 25 to 150 units, from 25 to 100 units, from 25 to 50 units, from 50 to 200 units, from 50 to 150 units, or from 50 to 100 units). In some embodiments, X^4 comprises at least one polypropylene oxide group having a sum total of from 2 to 200 propylene oxide units (e.g., from 2 to 150 units, from 2 to 100 units, from 2 to 50 units, from 10 to 200 units, from 10 to 150 units, from 10 to 100 units, from 10 to 50 units, from 25 to 200 units, from 25 to 150 units, from 25 to 100 units, from 25 to 50 units, from 50 to 200 units, from 50 to 150 units, or from 50 to 100 units). In some embodiments, X^4 comprises at least one polylactic acid and/or polyglycolic acid group having a sum total of from 2 to 200 polylactic acid and/or polyglycolic acid units (e.g., from 2 to 150 units, from 2 to 100 units, from 2 to 50 units, from 10 to 200 units, from 10 to 150 units, from 10 to 100 units, from 10 to 50 units, from 25 to 200 units, from 25 to 150 units, from 25 to 100 units, from 25 to 50 units, from 50 to 200 units, from 50 to 150 units, or from 50 to 100 units). In certain embodiments, X^4 comprises at least one polyethylene glycol/polypropylene oxide group having a sum total of from 2 to 200 ethylene glycol and/or propylene oxide units and at least one polylactic acid and/or polyglycolic acid group having a sum total of from 2 to 200 polylactic acid and/or polyglycolic acid units. In some embodiments, each instance of X^4 comprises



, , or , or some combination thereof, wherein p is independently an integer from 2 to 200 (e.g., from 2 to 150,

from 2 to 100, from 2 to 50, from 10 to 200, from 10 to 150, from 10 to 100, from 10 to 50, from 25 to 200, from 25 to 150, from 25 to 100, from 25 to 50, from 50 to 200, from 50 to 150, or from 50 to 100), and each instance of R^{18} is independently hydrogen or methyl. The X^4 group can further be terminated with an R^{13} group as defined herein. In some embodiments, X^4 is



wherein p and R^{13} are as previously defined.

[0043] In groups E^1 and E^2, integers p1 to p4 (i.e., p1, p2, p3, and p4), q1 to q6 (i.e., q1, q2, q3, q4, q5, and q6), r1, r2, and s1 to s4 (i.e., s1, s2, s3, and s4) are each independently an integer of 1 to 5 (e.g., 1, 2, 3, 4, or 5). In some embodiments, p1 to p4 (i.e., p1, p2, p3, and p4), q1 to q6 (i.e., q1, q2, q3, q4, q5, and q6), r1, r2, and/or s1 to s4 are each independently an integer of 1 to 3 (e.g., 1, 2, or 3). In certain embodiments, p1 to p4 (i.e., p1, p2, p3, and p4), q1 to q6 (i.e., q1, q2, q3, q4, q5, and q6), and/or s1 to s4 (i.e., s1, s2, s3, and s4) are each 2. In some embodiments, p1 to p4 (i.e., p1, p2, p3, and p4) and/or q1 to q6 (i.e., q1, q2, q3, q4, q5, and q6) are each 2, and r1, r2, and s1 to s4 (i.e., s1, s2, s3, and s4) are each 1.

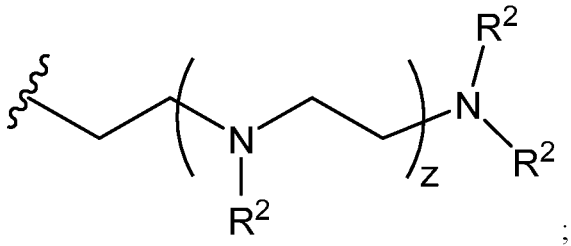
[0044] Each instance of R^2 can be hydrogen or a C_1-C_{12} (e.g., C_1-C_8, C_1-C_6, or C_1-C_3) alkyl group, C_2-C_{12} (e.g., C_2-C_8, C_2-C_6, or C_2-C_3) alkenyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkenyl group,, or R^2 is combined with a second R^2 so as to form a heterocyclic group. In some embodiments, R^2 is hydrogen or a C_1-C_{12} alkyl (e.g., a C_1-C_{10} alkyl group; a C_1-C_8 alkyl group; a C_1-C_6 alkyl group; a C_1-C_4 alkyl group, a C_1-C_3 alkyl group, or a C_1 or C_2 alkyl group) that can be linear or branched. In certain embodiments, R^2 is methyl. In other embodiments, R^2 can be hydrogen. Each R^2 is independently chosen and can be the same or different. In some embodiments, each R^2 in a given is the same (e.g., all methyl or all hydrogen).

[0045] Each instance of R^4 is independently —C(O)O—, —C(O)NH—, or —S(O)(O)—. In some embodiments, each instance of R^4 is independently —C(O)O— or —C(O)NH—. In certain embodiments, each instance of R^4 is —C(O)O—. In certain embodiments, each instance of R^4 is —C(O)NH—.

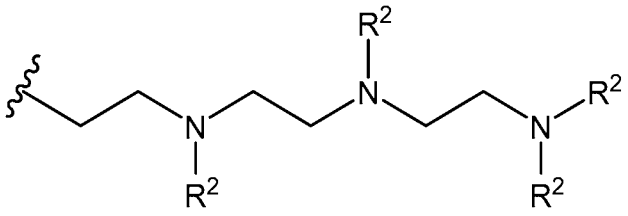
[0046] Each instance of R^5 is independently an alkyl group, cycloalkyl group, alkenyl group, cycloalkenyl group, aryl group, heteroalkyl group, heterocyclic group, or combination thereof, optionally comprising from 2 to 8 tertiary amines or a substituent comprising a

tissue-specific or cell-specific targeting moiety. R^5 can comprise from about 2 to about 50 carbon atoms (e.g., from about 2 to about 40 carbon atoms, from about 2 to about 30 carbon atoms, from about 2 to about 20 carbon atoms, from about 2 to about 16 carbon atoms, from about 2 to about 12 carbon atoms, from about 2 to about 10 carbon atoms, or from about 2 to about 8 carbon atoms). In some embodiments, R^5 is a heteroalkyl group comprising from 2 to 8 (i.e., 2, 3, 4, 5, 6, 7, or 8) tertiary amines. The tertiary amines can be part of the heteroalkyl backbone (i.e., the longest continuous chain of atoms in the heteroalkyl group, or a pendant substituent. Thus, for instance, the heteroalkyl group comprising the tertiary amines can provide an alkylamino group, amino alkyl group, alkylaminoalkyl group, aminoalkylamino group, or the like comprising 2 to 8 tertiary amines.

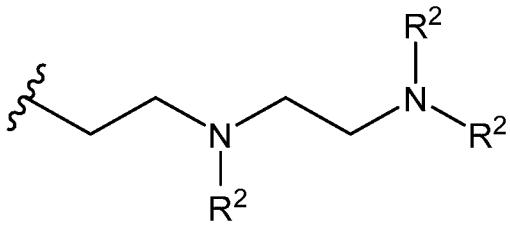
[0047] In some embodiments, each R^5 is independently selected from:



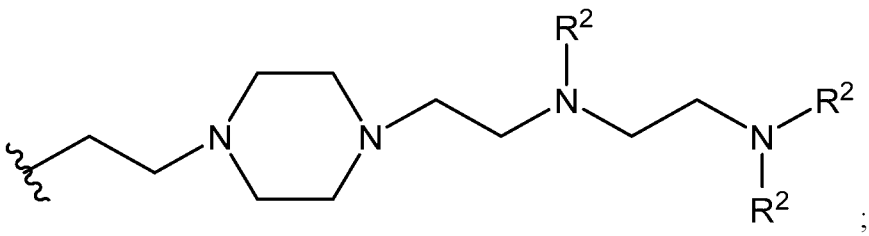
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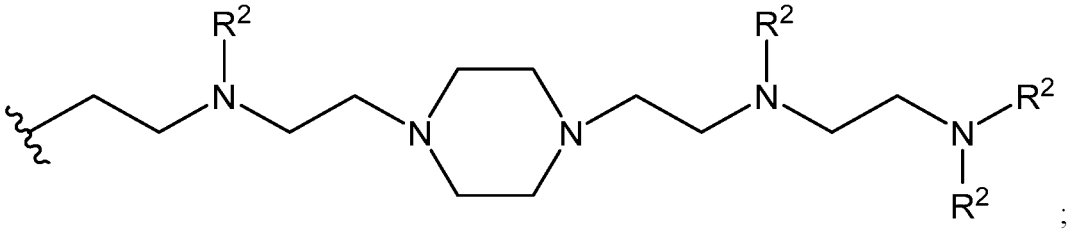
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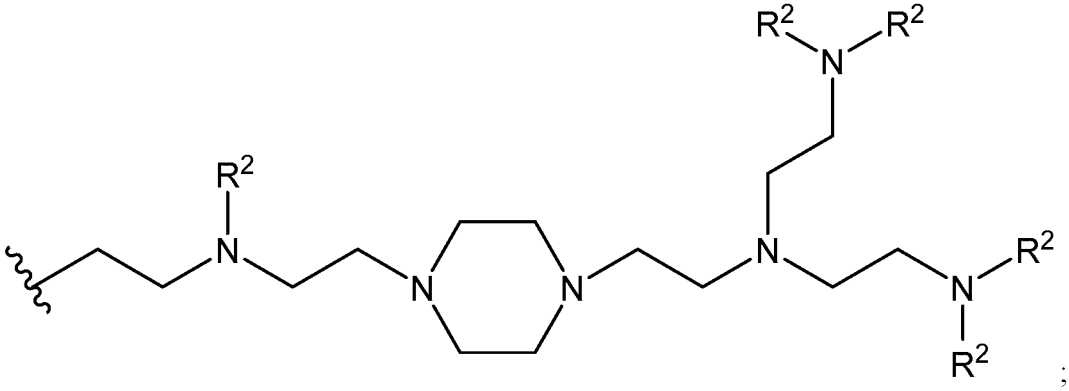


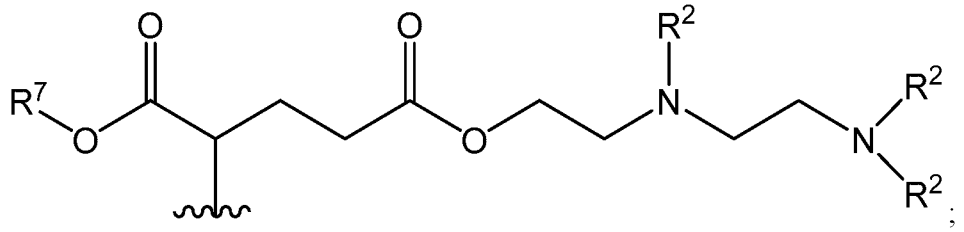
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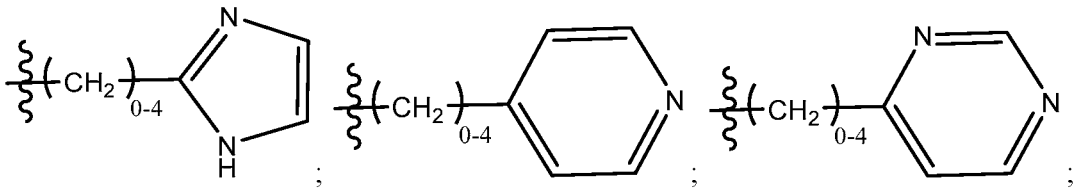


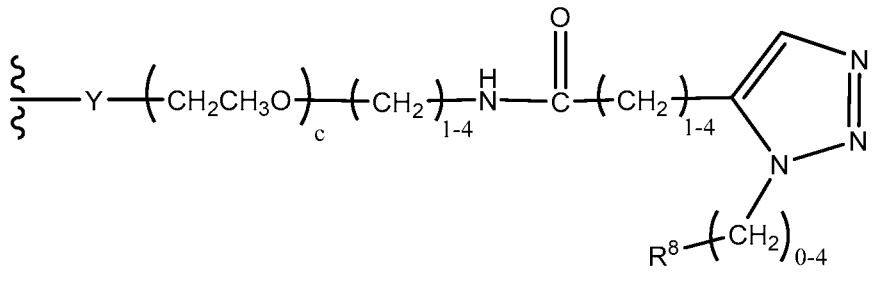
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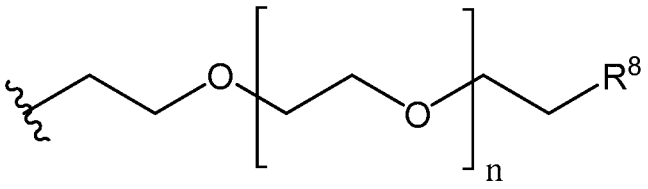

;


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;


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; and


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wherein

each instance of R^2 is as described above;

R^7 is a C_1-C_{50} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group optionally substituted with one or more amines;

z is an integer from 1 to 5;

c is an integer from 0 to 50;

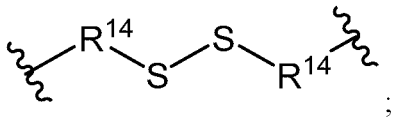
Y is optionally present and is a cleavable linker;

n is an integer from 0 to 50; and

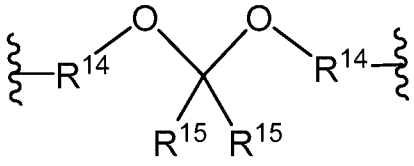
R^8 is a tissue-specific or cell-specific targeting moiety. C_1-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group.

[0048] R^7 can be a C_1-C_{50} (e.g., C_1-C_{40}, C_1-C_{30}, C_1-C_{20}, C_1-C_{10}, C_4-C_{12}, or C_6-C_8) alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group optionally substituted with one or more amines. In some embodiments, R^7 is a C_4-C_{12}, such as a C_6-C_8, alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group optionally substituted with one or more amines. In some embodiments, R^7 is substituted with one or more amines. In certain embodiments, R^7 is substituted with 2 to 8 (i.e., 2, 3, 4, 5, 6, 7, or 8) tertiary amines. The tertiary amines can be a part of the alkyl group (i.e., encompassed in the alkyl group backbone) or a pendant substituent.

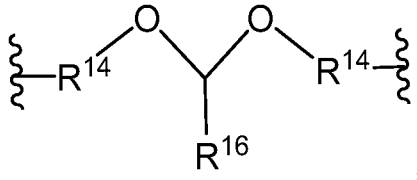
[0049] Each instance of Y is optionally present. As used herein, the phrase "optionally present" means that a substituent designated as optionally present can be present or not present, and when that substituent is not present, the adjoining substituents are bound directly to each other. When Y is present, Y is a cleavable linker. As used herein, the phrase "cleavable linker" refers to any chemical element that connects two species that can be cleaved as to separate the two species. For example, the cleavable linker can be cleaved by a hydrolytic process, photochemical process, radical process, enzymatic process, electrochemical process, or a combination thereof. Exemplary cleavable linkers include, but are not limited to:



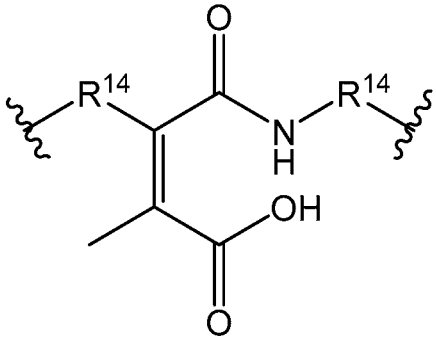
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; and



,

wherein each occasion of R^{14} independently is a C_1-C_4 alkyl group, each occasion of R^{15} independently is hydrogen, an aryl group, a heterocyclic group (e.g., aromatic or non-aromatic), a C_1-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, and R^{16} is a six-membered aromatic or heteroaromatic group optionally substituted with one or more $-OCH_3$, $-NHCH_3$, $-N(CH_3)_2$, $-SCH_3$, -OH, or a combination thereof.

[0050] Each of E^1 and E^2 can be any of the groups as previously defined. E^1 and E^2 can be the same or different. In some embodiments, E^1 and E^2 are the same. In some embodiments, E^1 and E^2 are independently of the formula:

$$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2 ;$$

$$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2{}_2]_2;$$

$$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}; \text{ or}$$

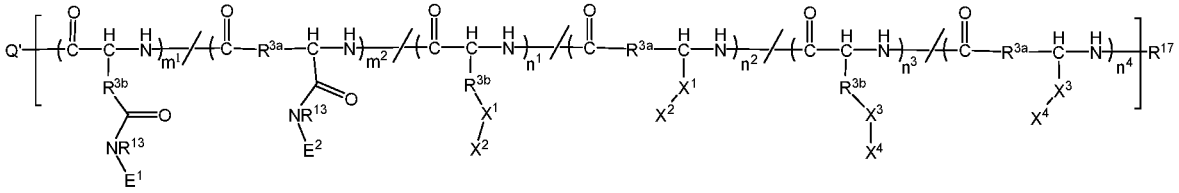
$$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2{}_2]_2\}_2,$$

wherein p1 to p4, q1 to q6, and r1 and r2 are each independently an integer of 1 to 5 (e.g., 1, 2, or 3); and each instance of R^2 is independently hydrogen or a C_1-C_{12} (e.g., C_1-C_6, C_1-C_3, C_2, or C_1) alkyl group, C_2-C_{12} (e.g., C_2-C_6, C_2-C_3, or C_2) alkenyl group, C_3-C_{12} (e.g., C_3-C_6, cycloalkyl or cycloalkenyl group, or R^2 is combined with a second R^2 so as to form a heterocyclic group. In some embodiments, E^1 and E^2 are $—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2$, optionally wherein R^2 is independently hydrogen or a C_1-C_3 alkyl (e.g., methyl or ethyl). In some embodiments, each nitrogen in group E^1 and E^2 containing an R^2 substituent is a tertiary amine, with the exception that the terminal amine can be a primary, secondary, or tertiary amine or, in some embodiments, a secondary or tertiary amine. By way of further illustration, each of E^1 and E^2 can be $–(CH_2)_2$-NR^2-$(CH_2)_2$-$NR^2{}_2$ or $–(CH_2)_2$-NR^2-$(CH_2)_2$-NHR^2, wherein each instance of R^2 is independently a hydrogen, alkyl group, alkenyl group,

cycloalkyl group, or cycloalkenyl group as described above, particularly an alkyl such as methyl or ethyl, optionally wherein each amine is a tertiary amine with the exception that the terminal amine is a secondary or tertiary amine. Specific non-limiting examples of groups E^1 and E^2 include, for instance, -CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)$_2$; -CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)$_2$; -CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)$_2$; -CH_2-CH_2-N(CH_3)-CH_2-CH_2-NH(CH_3); -CH_2-CH_2-N(CH_3)-CH_2-CH_2-NH(CH_3); -CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-NH(CH_3); -CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-NH(CH_3).

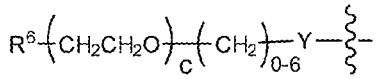
[0051] In some embodiments of the foregoing polymers, E^1 and E^2 are —$(CH_2)_{p1}$—(NR^2—$(CH_2)_{q1}$—)$_{r1}$$NR^2_2$, optionally wherein R^2 is independently hydrogen or a C_1-C_3 alkyl (e.g., methyl or ethyl), and p1, q1, and r1 are each independently an integer of 1 to 5 (e.g., 1, 2, or 3); for instance, E^1 and E^2 can be –$(CH_2)_2$-NR^2-$(CH_2)_2$-NR^2_2 or –$(CH_2)_2$-NR^2-$(CH_2)_2$-NHR^2; X^2 is a C_1-C_8 (e.g., C_1-C_6, C_1-C_4, C_1-C_3, C_2-C_8, C_2-C_6, C_3-C_8, or C_3-C_6) linear or branched alkyl group, optionally substituted with one or more hydroxyl groups (e.g., 1 or 2 hydroxyl groups) or halogen groups (e.g., substituted with one or more fluorine, chlorine, bromine, or iodine atoms); and X^4 is group comprising a polyalkylene oxide, polyglycolic acid, polylactic acid, or a combination thereof (e.g., a group comprising a polyalkylene oxide). Also, in such embodiments, R^{3a} and R^{3b} can both be methylene, X^1 and X^3 can both be —C(O)NR^{13}-, and each R^{13} can be methyl or hydrogen.

[0052] The polymer can have any suitable terminal groups. In some embodiments, the polymer has a structure of Formula 2A:



wherein

Q' is of formula:



c is an integer from 0 to 50 (e.g., 0), or c is an integer from 2 to 200 (e.g., 2 to 150, 2 to 100, 2 to 50, 10 to 200, 10 to 150, 10 to 100, 10 to 50, 25 to 200, 25 to 150, 25 to 100, 25 to 50, 50 to 200, 50 to 150, or 50 to 100);

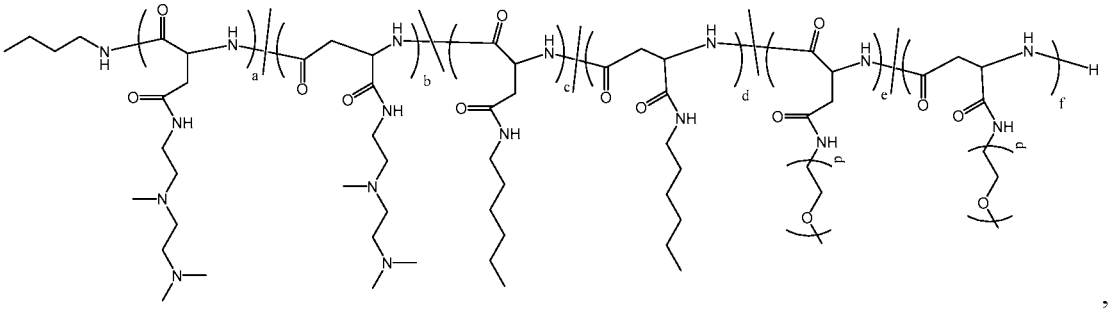
Y is optionally present and is a cleavable linker;

R^{17} is hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} (e.g., C_1-C_8, C_1-C_6, or C_1-C_3) alkyl or heteroalkyl group, C_2-C_{12} (e.g., C_2-C_8, C_2-C_6, or C_2-C_3) alkenyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkyl group, or C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkenyl group, optionally substituted with one or more substituents; and

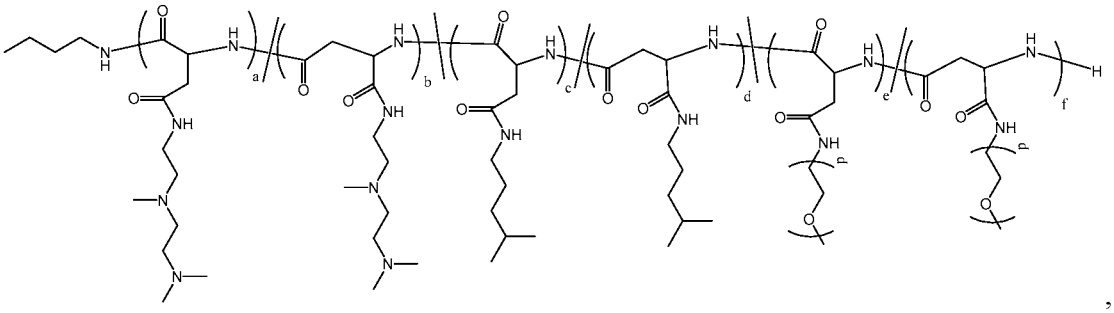
R^6 is hydrogen, an amino group, an aryl group, a heterocyclic group, a C_1-C_{12} (e.g., C_1-C_8, C_1-C_6, or C_1-C_3) alkyl or heteroalkyl group, a C_2-C_{12} (e.g., C_2-C_8, C_2-C_6, or C_2-C_3) alkenyl group, a C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkyl group, or a C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkenyl group, optionally substituted with one or more amines; or a tissue-specific or cell-specific targeting moiety; and

all other variables and substituents of Formula 2A are as defined with respect to Formula 2, including any and all embodiments thereof. In some embodiments, R^{17} and/or R^6 is a C_1-C_{12} alkyl (e.g., a C_1-C_{10} alkyl group; a C_1-C_8 alkyl group; a C_1-C_6 alkyl group; a C_1-C_4 alkyl group, a C_1-C_3 alkyl group, or a C_1 or C_2 alkyl group), which can be linear or branched, optionally substituted with one or more substituents. In certain embodiments, the heteroalkyl or alkyl group comprises or is substituted with one or more amines, for instance, from 2 to 8 (i.e., 2, 3, 4, 5, 6, 7, or 8) tertiary amines. The tertiary amines can be a part of the heteroalkyl backbone chain or pendant substituents.

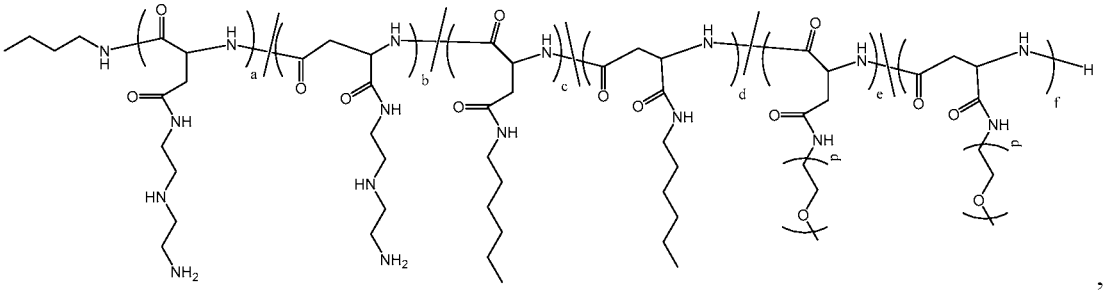
[0053] Non-limiting examples of the polymer provided herein include, for instance:



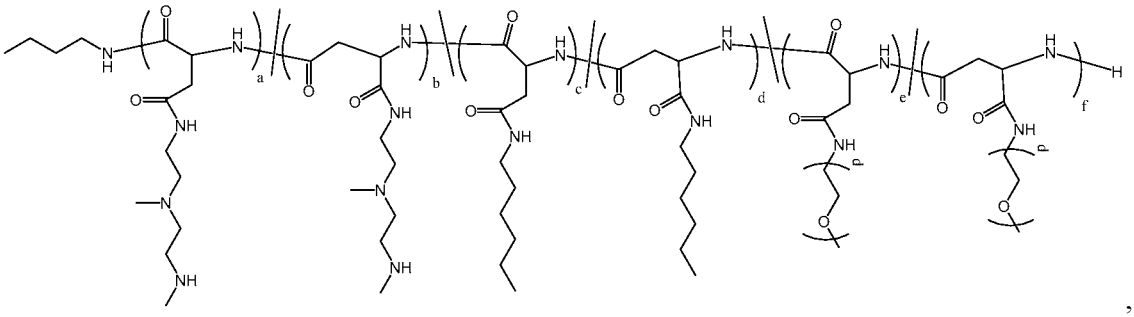
Polymer 72



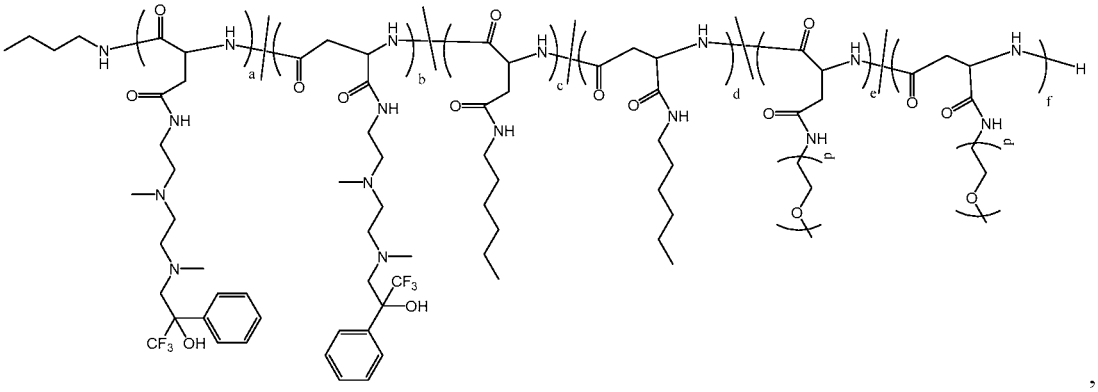
Polymer 73



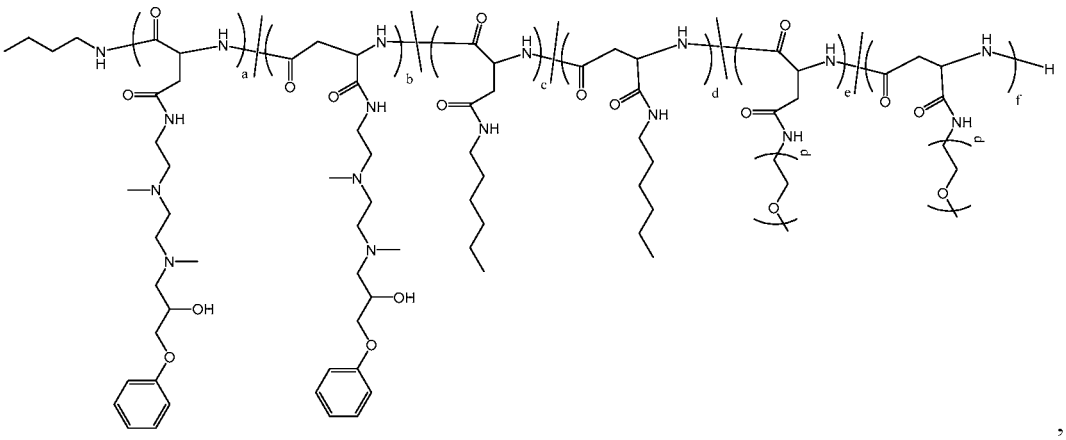
Polymer 74



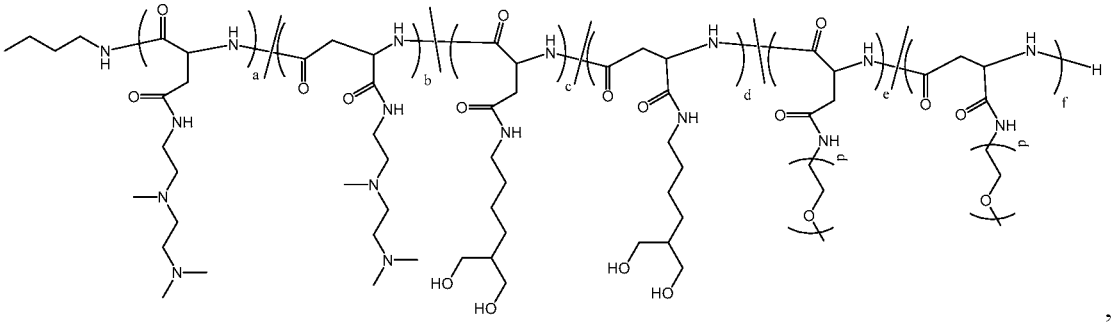
Polymer 75



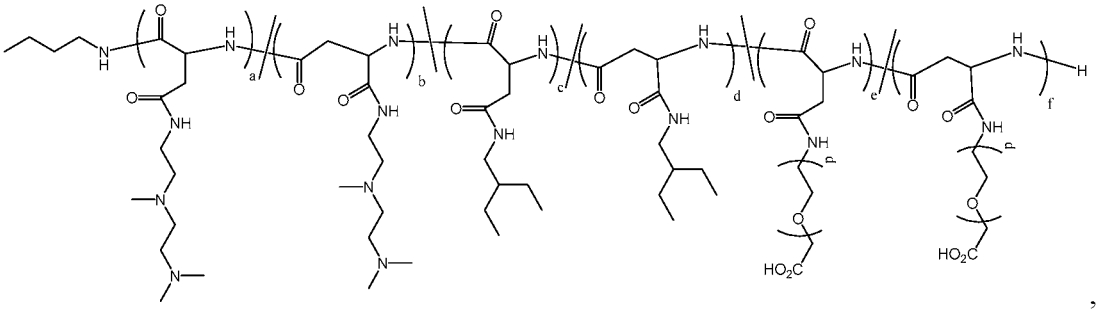
Polymer 76



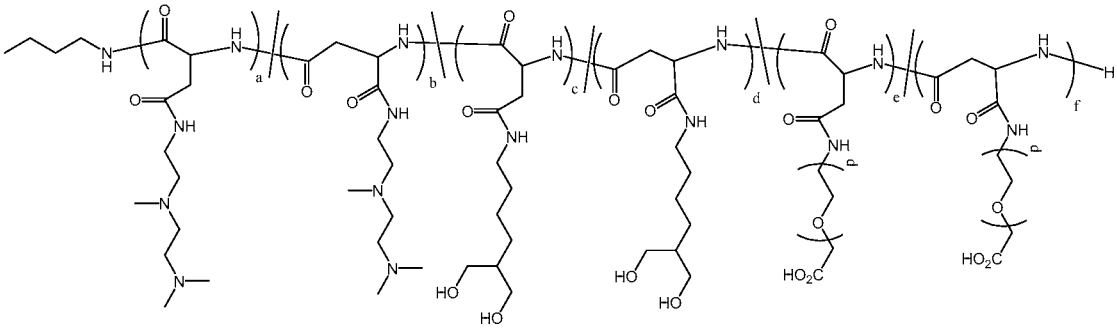
Polymer 77



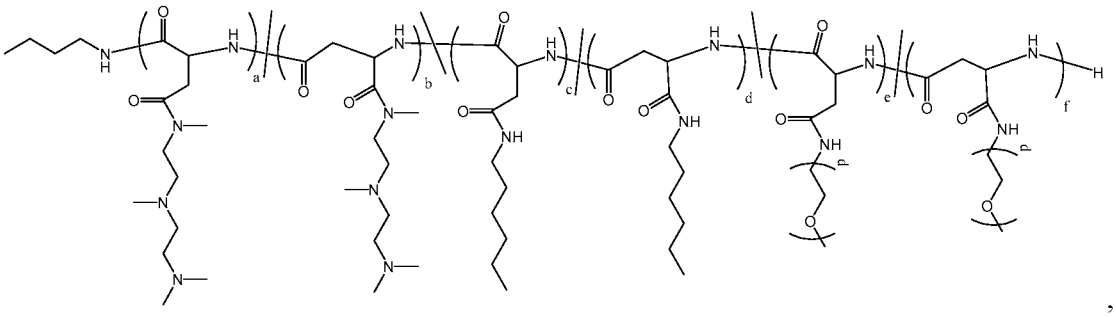
Polymer 78



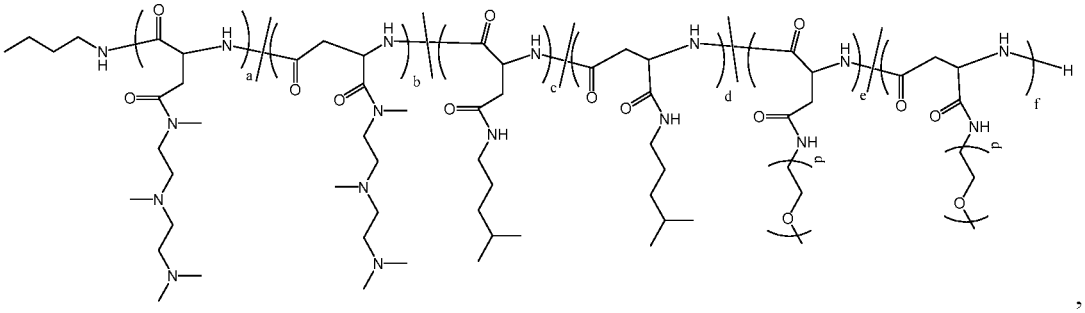
Polymer 79



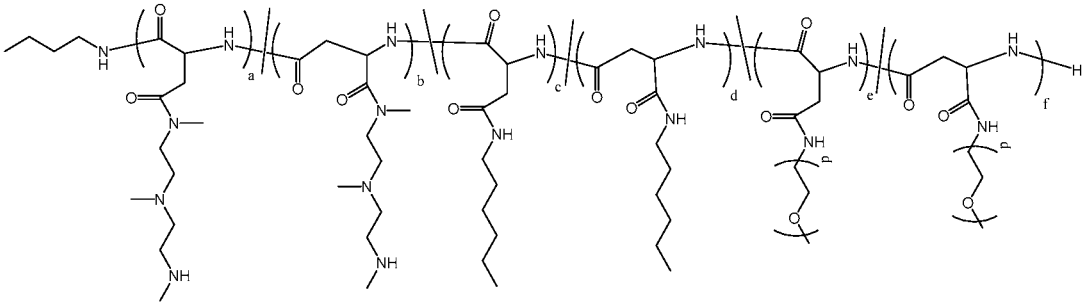
Polymer 80



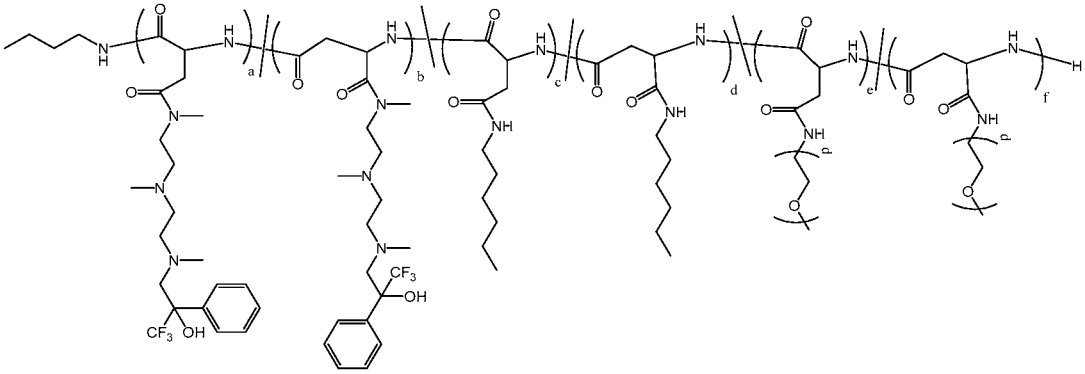
Polymer 81



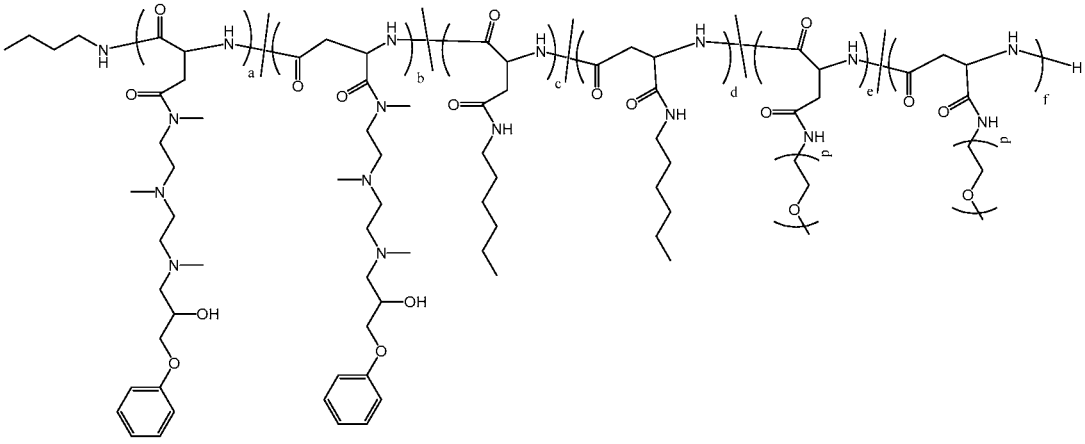
Polymer 82



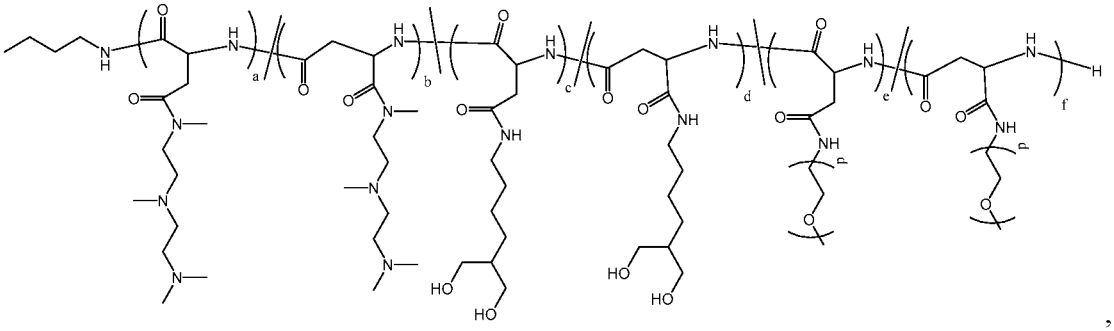
Polymer 83



Polymer 84

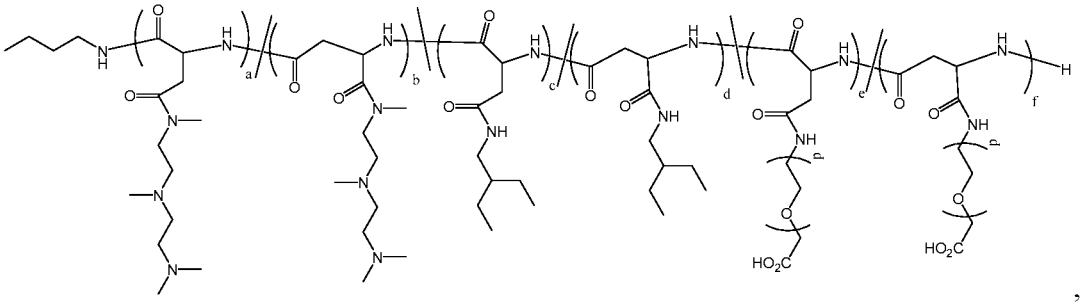


Polymer 85



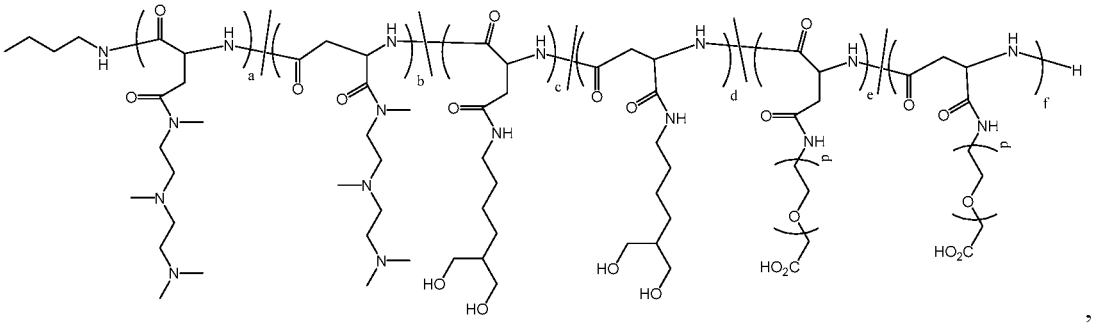
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Polymer 86



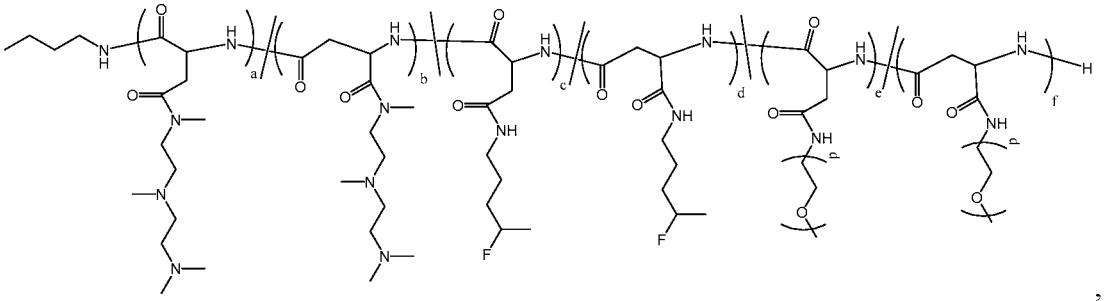
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Polymer 87



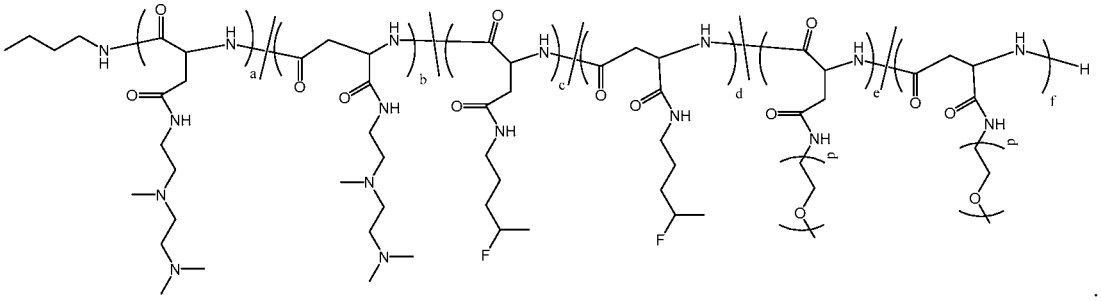
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Polymer 88



, or

Polymer 89

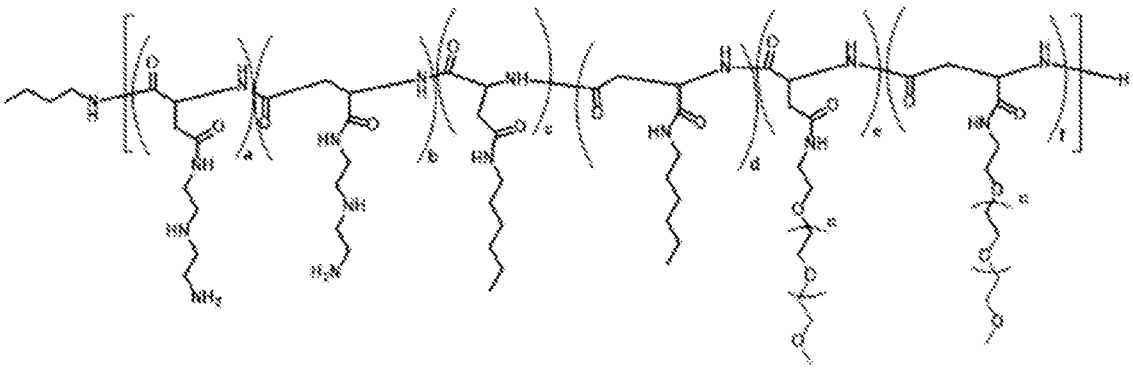


Polymer 90

[0054] The indication of the number of units ("a", "b", "c", "d", "e", and "f") in these examples of polymers does not imply a block co-polymer structure; rather, these numbers indicate the number of particular monomer units overall, which units can be arranged in any order, including blocks of monomers or monomers randomly arranged throughout the polymer. In some instances, this is additionally indicated by the "/" symbols in the formulas; however, the absence of a "/" should not be taken to mean that the polymers are joined in a particular order. In some embodiments of the foregoing polymers, the monomers designated by parenthesis and an integer ("a", "b", "c", "d", "e", and "f" as applicable) are randomly arranged or dispersed throughout the polymer.

[0055] In some embodiments of the foregoing polymers, (a+b) is from about 5 to about 65 (e.g., about 5 to about 50, about 5 to about 40, about 5 to about 30, about 5 to about 20, or about 5 to about 10), (c+d) is from about 2 to about 60 (e.g., about 2 to about 50, about 2 to about 40, about 2 to about 30, about 2 to about 20, or about 2 to about 10), (e+f) is from about 2 to about 60 (e.g., about 2 to about 50, about 2 to about 40, about 2 to about 30, about 2 to about 20, or about 2 to about 10), and each instance of p is independently an integer from 2 to 200 (e.g., 2 to 150, 2 to 100, 2 to 50, 6 to 36, 6 to 30, 6 to 24, 6 to 18, 8 to 36, 8 to 30, 8 to 26, 8 to 18, 10 to 200, 10 to 150, 10 to 100, 10 to 50, 25 to 200, 25 to 150, 25 to 100, 25 to 50, 50 to 200, 50 to 150, or 50 to 100).

[0056] In a particular embodiment, the polymer has the structure of polymer 72, 73, 74, or 75, or has the structure:



wherein n is 6-24, or more (e.g., 24-60 or 40-50), and a, b, c, d, e, and f are as described above.

[0057] Some of the above particular examples of polymers provided by the disclosure are depicted with specific terminal groups (e.g., alkylamino, hydrogen, etc.); however, any of the foregoing particular structures can comprise different terminal groups. For example, any of the foregoing structures comprise a group of R^1, R^6, or Q as described herein at either or both termini of the polymer backbone.

[0058] Typically, the polymer is cationic (i.e., positively charged at pH 7 and 23 °C). As used herein, "cationic" polymers refer to polymers having an overall net positive charge, whether the polymer comprises only cationic monomer units or a combination of cationic monomer units and non-ionic or anionic monomer units.

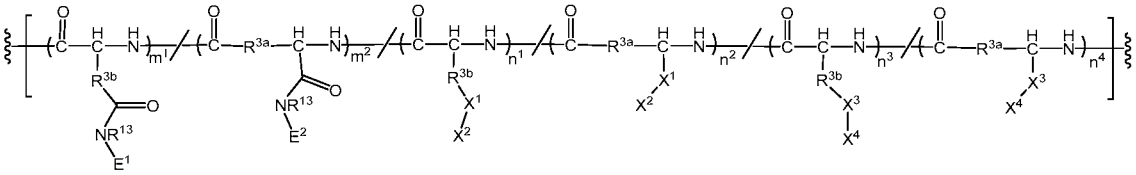
[0059] In certain embodiments, the polymer described herein has a weight average molecular weight of from about 5 kDa to about 2,000 kDa. The polymer can have a weight average molecular weight of about 2,000 kDa or less, for example, about 1,800 kDa or less, about 1,600 kDa or less, about 1,400 kDa or less, about 1,200 kDa or less, about 1,000 kDa or less, about 900 kDa, or less, about 800 kDa, or less, about 700 kDa or less, about 600 kDa or less, about 500 kDa or less, about 100 kDa or less, or about 50 kDa or less. Alternatively, or in addition, the polymer can have a weight average molecular weight of about 10 kDa or more, for example, about 50 kDa or more, about 100 kDa or more, about 200 kDa or more, about 300 kDa or more, or about 400 kDa or more. Thus, the polymer can have a weight average molecular weight bounded by any two of the aforementioned endpoints. For example, the polymer can have a weight average molecular weight of from about 10 kDa to about 50 kDa, from about, from about 10 kDa to about 100 kDa, from about 10 kDa to about 500 kDa, from about 50 kDa to about 500 kDa, from about 100 kDa to about 500 kDa, from about 200 kDa to about 500 kDa, from about 300 kDa to about 500 kDa, from about 400 kDa to about 500 kDa, from about 400 kDa to about 600 kDa, from about 400 kDa to about 700

kDa, from about 400 kDa to about 800 kDa, from about 400 kDa to about 900 kDa, from about 400 kDa to about 1,000 kDa, from about 400 kDa to about 1,200 kDa, from about 400 kDa to about 1,400 kDa, from about 400 kDa to about 1,600 kDa, from about 400 kDa to about 1,800 kDa, from about 400 kDa to about 2,000 kDa, from about 200 kDa to about 2,000 kDa, from about 500 kDa to about 2,000 kDa, or from about 800 kDa to about 2,000 kDa. The weight average molecular weight can be determined by any suitable technique. Generally, the weight average molecular weight is determined using size exclusion chromatography equipped with a column, selected from TSKgel Guard, GMPW, GMPW, G1000PW, and a Waters 2414 (Waters Corporation, Milford, Massachusetts) refractive index detector. Moreover, the weight average molecular weight is determined from calibration with polyethylene oxide/polyethylene glycol standards ranging from 150-875,000 Daltons.

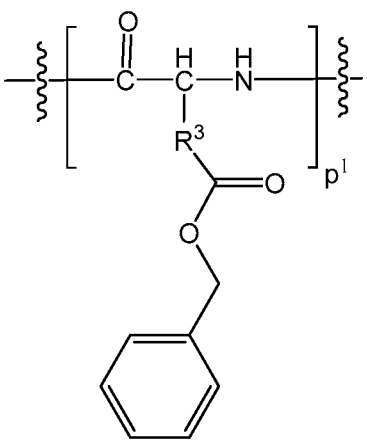
Method of Preparing Polymer

[0060] The polymer provided herein comprising a hydrolysable polymer backbone, which comprises: (a) monomer units with a side chain comprising a hydrophobic group; (b) monomer units with a side chain comprising an oligoamine or polyamine; and (c) monomer units with a side chain comprising a polyalkylene oxide, polyglycolic acid, polylactic acid, or combination thereof, can be prepared by any suitable method, examples of which are described herein and illustrated in the examples. In one aspect, the disclosure provides a method of providing such a polymer having monomers (a)-(c), above, by modifying the side chains of a suitable polymer, such as a polyamide, to include the desired side chains.

[0061] An aspect of the disclosure provides a method of preparing a polymer as described herein. In some embodiments, the method comprises preparing a polymer (e.g., a first polymer) comprising a structure of Formula 2:

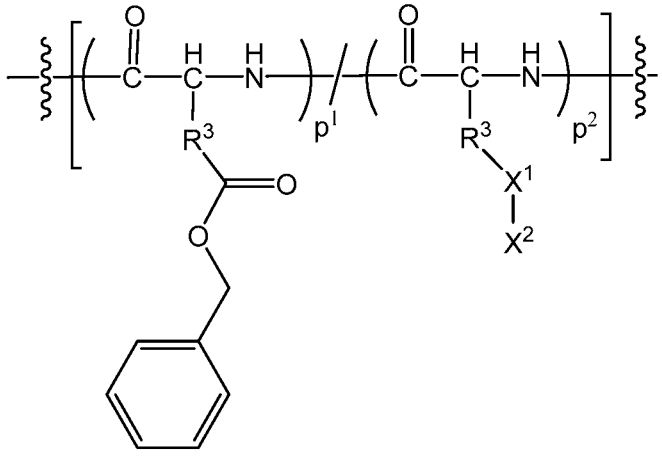


as described herein from a polymer comprising a structure of Formula 6 or Formula 7:



Formula 6

or



Formula 7

wherein,

p^1 is an integer from 1 to 2000 (e.g., from 1 to 1000, from 1 to 500, from 1 to 200, from 1 to 100, from 5 to 2000, from 5 to 1000, from 5 to 500, from 5 to 200, or from 5 to 100);

p^2 is an integer from 1 to 2000 (e.g., from 1 to 1000, from 1 to 500, from 1 to 200, from 1 to 100, from 2 to 2000, from 2 to 1000, from 2 to 500, from 2 to 200, or from 2 to 100);

each R^3 is independently a methylene or ethylene group;

and all other substituents and variables are as previously described with respect to Formulae 2 and 2A, including any and all embodiments thereof. According to one aspect of the method, the structure of Formula 6 can be combined (reacted) with (a) a compound of the formula $HNR^{13}E^1$ and/or $HNR^{13}E^2$; (b) a compound of formula H_2NX^4 or HOX^4; and (c) a compound of formula H_2NX^2 or HOX^2, simultaneously or in any sequential order, to a polymer comprising a structure of Formula 2. According to another aspect, the compound of Formula 7 (which already includes an X^2 group) can be combined (reacted) with (a) a compound of the formula $HNR^{13}E^1$ and/or $HNR^{13}E^2$ and (b) a compound of formula H_2NX^4 or HOX^4, simultaneously or in any sequential order, to a polymer comprising a structure of Formula 2.

[0062] In the compound of formula $HNR^{13}E^1$, $HNR^{13}E^2$, H_2NX^2, HOX^2, H_2NX^4, or HOX^4each instance of R^{13}, E^1, E^2, X^2, and X^4 is as previously described with respect to the polymers of Formulae 2 and 2A, including any and all embodiments thereof.

[0063] Thus, for example, each instance of R^{13} can be independently hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl

group, or C_3-C_{12} cycloalkenyl group, any of which can be optionally substituted with one or more substituents.

[0064] Similarly, E^1 and E^2 are as previously described with respect to the polymers of Formulae 2 and 2A. Thus, for instance, E^1 and E^2 are each independently a group of formula:

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2_2$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2_2]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}$; or

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2_2]_2\}_2$,

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^4\text{-}R^5]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^4\text{-}R^5\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^4\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^5]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^5\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[N\{(CH_2)_{s1}\text{-}R^4\text{-}R^5\}—(CH_2)_{q1}—]_{r1}NR^2_2$;

$—(CH_2)_{p1}—[N\{(CH_2)_{s1}\text{-}R^5\}—(CH_2)_{q1}—]_{r1}NR^2_2$,

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH(CONH_2)\text{-}(CH_2)_{s1}\text{-}R^5$; or

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH(CONH_2)\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5$,

wherein p1 to p4, q1 to q6, r1 and r2, and s1 to s4 are each independently an integer of 1 to 5;

each instance of R^2 is independently hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl group, any of which are optionally substituted with one or more substituents, or R^2 is combined with a second R^2 so as to form a heterocyclic group;

each instance of R^4 is independently -C(O)O-, -C(O)NH-, -O-C(O)O-, or -S(O)(O)-; and

each instance of R^5 is independently a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl, aryl group, C_1-C_{12} heteroalkyl group, C_3-C_{12}

heterocyclic group, or combination thereof optionally comprising from 2 to 8 tertiary amines or a substituent comprising a tissue-specific or cell-specific targeting moiety.

[0065] Group X^2 of the compound of formula H_2NX^2 or HOX^2 is as described with respect to Formulae 2 and 2A, including any and all embodiments thereof.

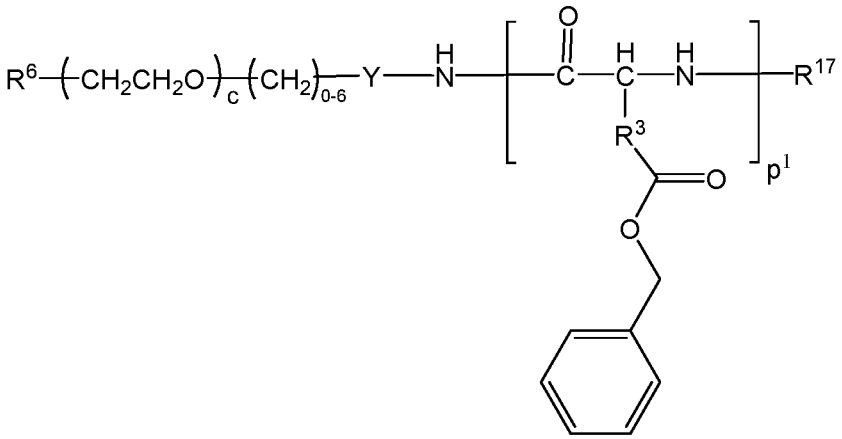
[0066] All other substituents and variables of Formulae 2, 6, and 7, are as described herein with respect to the polymers of the invention (e.g., Formulae 2 and 2A), including any and all embodiments thereof.

[0067] The compounds of formula $HNR^{13}E^1$ and/or $HNR^{13}E^2$, of formula H_2NX^2 and/or HOX^2, and/or of formula H_2NX^4 and/or HOX^4 can be added to the compound of formula 6 or 7 in any suitable manner and amount depending upon the desired degree of substitution. In some embodiments, about 1-400 equivalents (e.g., about 1-350, 1-300, 1-250, 1-200, 1-150, 1-100, 1-50, 10-400, 10-350, 10-300, 10-250, 10-200, 10-150, 10-100, 10-50, 20-400, 20-350, 20-300, 20-250, 20-200, 20-150, 20-100, 20-50, 30-400, 30-350, 30-300, 30-250, 30-200, 30-150, 30-100, 30-50, 40-400, 40-350, 40-300, 40-250, 40-200, 40-150, 40-100, 40-50, 50-400, 50-350, 50-300, 50-250, 50-200, 50-150, or 50-100 equivalents) of the compound of formula H_2NX^2 or HOX^2 is added to polymer comprising a structure of Formula 6. Also, in some embodiments, about 1-400 equivalents (e.g., about 1-350, 1-300, 1-250, 1-200, 1-150, 1-100, 1-50, 10-400, 10-350, 10-300, 10-250, 10-200, 10-150, 10-100, 10-50, 20-400, 20-350, 20-300, 20-250, 20-200, 20-150, 20-100, 20-50, 30-400, 30-350, 30-300, 30-250, 30-200, 30-150, 30-100, 30-50, 40-400, 40-350, 40-300, 40-250, 40-200, 40-150, 40-100, 40-50, 50-400, 50-350, 50-300, 50-250, 50-200, 50-150, or 50-100 equivalents) of the compound of formula $HNR^{13}E^1$ or $HNR^{13}E^2$ is added to the polymer comprising a structure of Formula 6 or Formula 7. Also, in some embodiments, about 1-400 equivalents (e.g., about 1-350, 1-300, 1-250, 1-200, 1-150, 1-100, 1-50, 10-400, 10-350, 10-300, 10-250, 10-200, 10-150, 10-100, 10-50, 20-400, 20-350, 20-300, 20-250, 20-200, 20-150, 20-100, 20-50, 30-400, 30-350, 30-300, 30-250, 30-200, 30-150, 30-100, 30-50, 40-400, 40-350, 40-300, 40-250, 40-200, 40-150, 40-100, 40-50, 50-400, 50-350, 50-300, 50-250, 50-200, 50-150, or 50-100 equivalents) of the compound of formula H_2NX^4 or HOX^4 is added to the polymer comprising a structure of Formula 6 or Formula 7.

[0068] In embodiments where the method comprises adding a compound of formula $HNR^{13}E^1$ and/or $HNR^{13}E^2$ and a compound of formula H_2NX^2 or HOX^2 to the polymer of Formula 6, the compound of formula $HNR^{13}E^1$ and/or $HNR^{13}E^2$ and the compound of formula H_2NX^2 and/or HOX^2 can be present in the reaction mixture in any suitable ratio. For example, the compound of formula $HNR^{13}E^1$ and/or $HNR^{13}E^2$ and the compound of formula

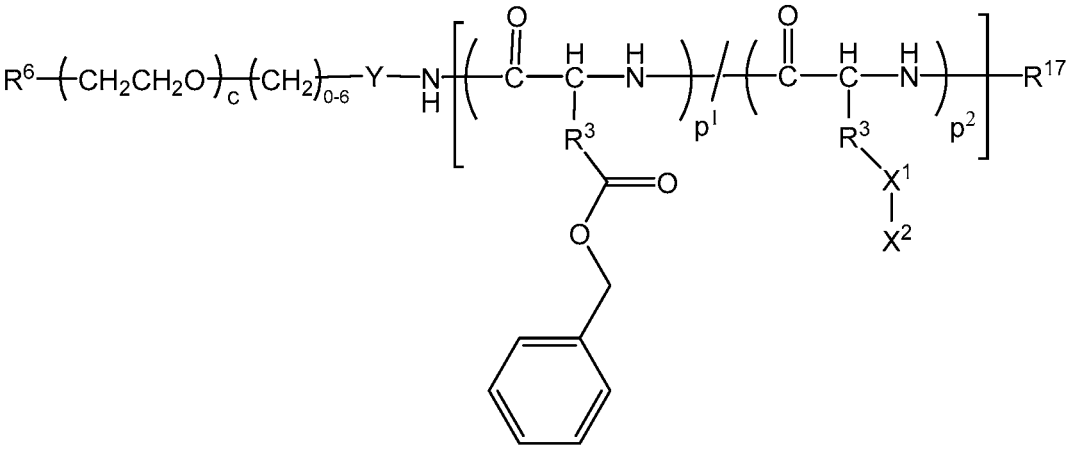
H_2NX^2 or HOX^2 can be present in a molar ratio of about 150:1 to about 1:150. In some embodiments, a ratio of about 150:1 to about 1:1, such as about 50:1 to about 1:1 (e.g., about 25:1 to about 1:1, about 10:1 to about 1:1, about 5:1 to about 1:1, or about 2.5:1 to about 1:1) is used. In other embodiments, the ratio is about 1:150 to about 1:1, such as about 1:50 to about 1:1 (e.g., about 1:25 to about 1:1, about 1:10 to about 1:1, about 1:5 to about 1:1, or about 1:2.5 to about 1:1). In still other embodiments, the ratio is about 1:10 to about 1:150, about 1:40 to about 1:150, or about 1:80 to about 1:150.

[0069] The polymer of Formula 6 or 7 can have any suitable terminal groups as desired in the resulting polymer of Formula 2 or 2A. In some embodiments, the polymer comprising a



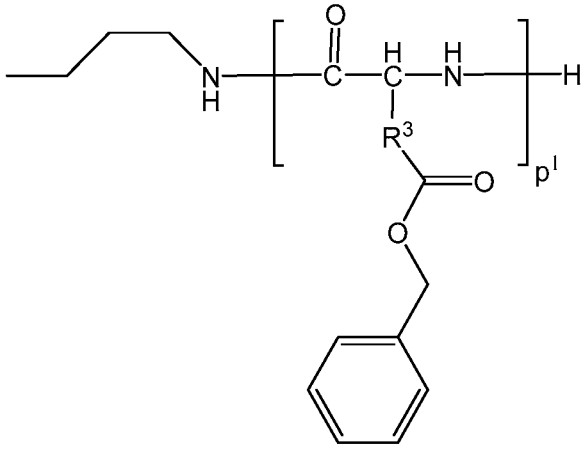
Formula 6A

or



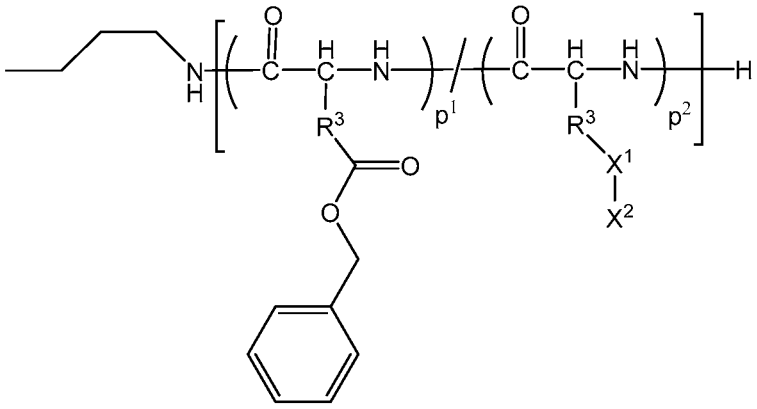
Formula 7A ;

wherein c, Y, R^{17}, and R^6 are as previously described with respect to the polymers of Formulae 2A, including any and all embodiments thereof; and p^1, p^2, R^3, X^1, and X^2, are as described above with respect to Formulae 6 and 7. In certain embodiments, c is 0, Y is not present, and R^6 and R^{17} are both H. For instance, the polymer of Formula 6 or 7 can be a polymer of Formula 6B or Formula 7B, respectively:



Formula 6B

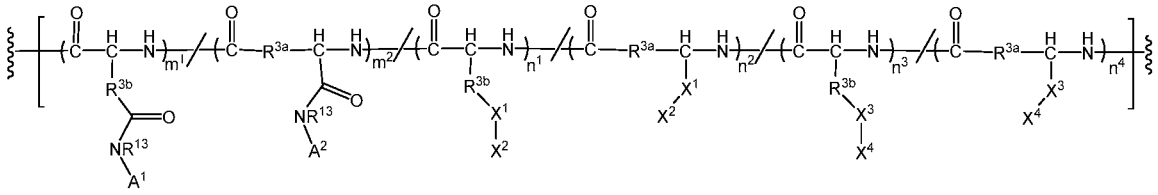
or



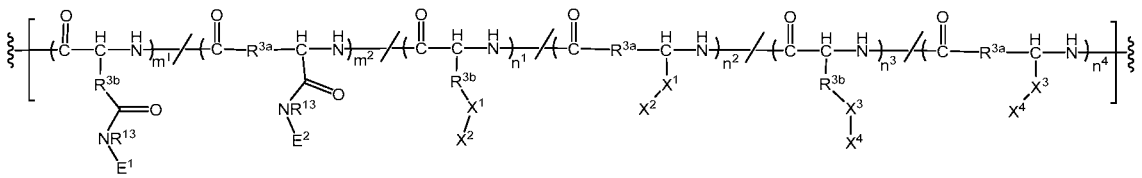
Formula 7B ;

wherein p^1, p^2, R^3, X^1, and X^2, are as described above with respect to Formulae 6, 6A, 7, and 7A.

[0070] In some embodiments, the method also provides a method of preparing a polymer comprising a structure of Formula 2 (e.g., polymer of Formula 2A), wherein at least a portion of the E^1 and E^2 have R^5 groups. The method comprises modifying at least a portion of groups A^1 and/or A^2 of a polymer comprising a structure of Formula 5:



to produce a polymer comprising a structure of Formula 2:



wherein each of A^1 and A^2 are each independently a group of formula

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2{}_2]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}$; or

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2{}_2]_2\}_2$,

each of E^1 and E^2 are each independently a group of formula

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2{}_2]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}$; or

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2{}_2]_2\}_2$,

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^4\text{-}R^5]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^4\text{-}R^5\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^4\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^5]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^5\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[N\{(CH_2)_{s1}\text{-}R^4\text{-}R^5\}—(CH_2)_{q1}—]_{r1}NR^2{}_2$;

$—(CH_2)_{p1}—[N\{(CH_2)_{s1}\text{-}R^5\}—(CH_2)_{q1}—]_{r1}NR^2{}_2$,

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH(CONH_2)\text{-}(CH_2)_{s1}\text{-}R^5$; or

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH(CONH_2)\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5$,

provided that at least a portion of E^1 and/or E^2 are selected from:

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^4\text{-}R^5]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^4\text{-}R^5\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^4\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}\text{-}CH_2\text{-}CHOH\text{-}R^5$;

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-CH_2-CHOH-R^5]$_2$}$_2$;

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-$(CH_2)_{s1}$-$R^5$;

—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—NR^2-$(CH_2)_{s2}$-R^5]$_2$;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—NR^2_2][—$(CH_2)_{q4}$—NR^2—]$_{r2}$$(CH_2)_{s3}$-$R^5$};

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-$(CH_2)_{s4}$-R^5]$_2$}$_2$;

—$(CH_2)_{p1}$—[N{$(CH_2)_{s1}$-R^4-R^5}—$(CH_2)_{q1}$—]$_{r1}$$NR^2_2$;

—$(CH_2)_{p1}$—[N{$(CH_2)_{s1}$-R^5}—$(CH_2)_{q1}$—]$_{r1}$$NR^2_2$,

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-CH($CONH_2$)-$(CH_2)_{s1}$-$R^5$; or

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-CH($CONH_2$)-$(CH_2)_{s1}$-$R^4$-$R^5$,

p1 to p4, q1 to q6, r1 and r2, and s1 to s4 are each independently an integer of 1 to 5;

each instance of R^2 is independently hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl group, any of which are optionally substituted with one or more substituents, or R^2 is combined with a second R^2 so as to form a heterocyclic group;

each instance of R^4 is independently -C(O)O-, -C(O)NH-, -O-C(O)O-, or -S(O)(O)-; and

each instance of R^5 is independently a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl, aryl group, C_1-C_{12} heteroalkyl group, C_3-C_{12} heterocyclic group, or combination thereof optionally comprising from 2 to 8 tertiary amines or a substituent comprising a tissue-specific or cell-specific targeting moiety.

[0071] All other substituents and variables of Formulae 2 and 5, are otherwise as described herein with respect to the other aspects of the disclosure, including any and all embodiments of the structures of Formulae 2 and 2A previously described herein. Thus, for instance:

each of m^1 and m^2 is an integer from 0 to 1000, provided that the sum of m^1 + m^2 is greater than 1;

each of n^1 and n^2 is an integer from 0 to 1000;

each of n^3 and n^4 is an integer from 0 to 1000, provided that the sum of n^3 + n^4 is greater than 1;

the symbol "/" indicates that the units separated thereby are linked randomly or in any order;

each instance of R^{3a} is independently a methylene or ethylene group;

each instance of R^{3b} is independently a methylene or ethylene group;

each X^1 independently is —C(O)O—, —C(O)NR^{13}—, —C(O)—, —S(O)(O)—, or a bond;

each instance of R^{13} is independently hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl group, any of which can be optionally substituted with one or more substituents;

each instance of X^2 is independently a C_1-C_{12} alkyl or heteroalkyl group, C_3-C_{12} cycloalkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkenyl group, aryl group, heterocyclic group, or combination thereof any of which are optionally substituted with one or more substituents;

each X^3 independently is —C(O)O—, —C(O)NR^{13}—, —C(O)—, —S(O)(O)—, or a bond;

each instance of X^4 is polyalkylene oxide, polyglycolic acid, polylactic acid, or a combination thereof.

[0072] The groups designated A^1 and/or A^2 of the polymer of Formula 5 can be modified by any suitable means. For example, the groups designated A^1 and/or A^2 can be modified by a Michael addition reaction, an epoxide opening, or a displacement reaction. In preferred embodiments, the groups designated A^1 and/or A^2 are modified by a Michael addition reaction.

[0073] In one embodiment, groups A^1 and/or A^2 of the polymer comprising a structure of Formula 5 are modified by a Michael addition reaction between the polymer comprising the structure of Formula 5 and α,β-unsaturated carbonyl compound. As used herein, the term "Michael addition" refers to a nucleophilic addition of a nucleophile of the polymer (e.g., a carbanion, an oxygen anion, a nitrogen anion, an oxygen atom, a nitrogen atom, or a combination thereof) to an α,β-unsaturated carbonyl compound. Accordingly, the Michael addition reaction is between the polymer comprising the structure of Formula 5 and an α,β-unsaturated carbonyl compound. In some embodiments, the nucleophile of the polymer is a nitrogen anion, a nitrogen atom, or a combination thereof.

[0074] The α,β-unsaturated carbonyl compound can be any α,β-unsaturated carbonyl compound capable of accepting a Michael addition from a nucleophile. In some embodiments, the α,β-unsaturated carbonyl compound is an acrylate, an acrylamide, a vinyl sulfone, or a combination thereof. Accordingly, the Michael addition reaction can be between the polymer comprising the structure of Formula 5 and an acrylate, an acrylamide, a vinyl sulfone, or a combination thereof. Thus, in some embodiments, the method comprises contacting the polymer comprising the structure of Formula 5 and an acrylate; contacting the

polymer comprising the structure of Formula 5 and an acrylamide; or contacting the polymer comprising the structure of Formula 5 and a vinyl sulfone.

[0075] In embodiments where the groups designated A^1 and/or A^2 are modified by a Michael addition reaction, they produce groups E^1 and/or E^2 having the formula:

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^4\text{-}R^5]_2$;

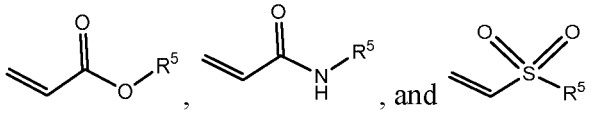
$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^4\text{-}R^5\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^4\text{-}R^5]_2\}_2$; or

$—(CH_2)_{p1}—[N\{(CH_2)_{s1}\text{-}R^4\text{-}R^5\}—(CH_2)_{q1}—]_{r1}NR^2{}_2$;

wherein p1 to p4, q1 to q6, r1 and r2, and s1 to s4, R^2, R^4, and R^5 are as previously defined.

[0076] Examples of acrylates, acrylamides, and vinyl sulfones suitable for use include an acrylate of the formula:



wherein R^5 is as described with respect to any of Formulae 2 or 2A.

[0077] In some embodiments, the Michael addition reaction is facilitated by an acid and/or base. The acid and/or base can be any suitable acid and/or base with any suitable pKa. The acid and/or base can be an organic acid (e.g., *p*-toluenesulfonic acid), organic base (e.g., triethylamine), inorganic acid (e.g., titanium tetrachloride), inorganic base (e.g., potassium carbonate), or a combination thereof.

[0078] In some embodiments, the Michael addition reaction is facilitated by an acid. The acid can be a Brønsted acid or a Lewis acid. In embodiments where the acid is a Brønsted acid, the acid can be a weak acid (i.e., a pKa of from about 4 to about 7) or a strong acid (i.e., a pKa of from about -2 to about 4). Typically, the acid is a weak acid. In certain embodiments, the acid is a Lewis acid. For example, the acid can be bis(trifluoromethanesulfon)imide or p-toluenesulfonic acid.

[0079] In some embodiments, the Michael addition reaction is facilitated by a base. The base can be a weak base (i.e., a pKa of from about 7 to about 12) or a strong base (i.e., a pKa of from about 12 to about 50). Typically, the base is a weak base. For example, the base can be triethylamine, diisopropylethylamine, pyridine, N-methyl morpholine, or N,N-dimethyl-piperazine, or derivatives thereof.

[0080] In some embodiments, the Michael addition reaction is performed in a solvent. The solvent can be any suitable solvent, or mixture of solvents, capable of solubilizing the

polymer and the α,β-unsaturated carbonyl compound to be reacted. For example, the solvent can include water, protic organic solvents, and/or aprotic organic solvents. An exemplary list of solvents includes water, dichloromethane, diethyl ether, dimethyl sulfoxide, acetonitrile, methanol, and ethanol.

[0081] In one embodiment, groups A^1 and/or A^2 of the polymer are modified by an epoxide opening reaction between the polymer and an epoxide compound. As used herein, the term "epoxide opening" refers to a nucleophilic addition of a nucleophile of the polymer (e.g., a carbanion, an oxygen anion, a nitrogen anion, an oxygen atom, a nitrogen atom, or a combination thereof) to an epoxide compound, thereby opening the epoxide. Accordingly, the epoxide opening reaction is between the polymer and an epoxide compound. In some embodiments, the nucleophile of the polymer is a nitrogen anion, a nitrogen atom, or a combination thereof.

[0082] In embodiments where the groups designated A^1 and/or A^2 are modified by an epoxide opening reaction, they produce groups E^1 and/or E^2 having the formula:

$$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5;$$

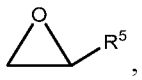
$$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5;$$

$$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}\text{-}CH_2\text{-}CHOH\text{-}R^5;$$

$$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5]_2\}_2;$$

wherein p1 to p4, q1 to q6, r1, r2, R^2, and R^5 are as previously defined.

[0083] Examples of epoxides suitable for use include epoxides of the formula:



,

wherein R^5 is as described with respect to any of Formulae 2 or 2A.

[0084] In some embodiments, the epoxide opening reaction is facilitated by an acid and/or base. The acid and/or base can be any suitable acid and/or base with any suitable pKa. The acid and/or base can be an organic acid (e.g., *p*-toluenesulfonic acid), organic base (e.g., triethylamine), inorganic acid (e.g., titanium tetrachloride), inorganic base (e.g., potassium carbonate), or a combination thereof.

[0085] In some embodiments, the epoxide opening reaction is facilitated by an acid. The acid can be a Brønsted acid or a Lewis acid. In embodiments where the acid is a Brønsted acid, the acid can be a weak acid (i.e., a pKa of from about 4 to about 7) or a strong acid (i.e., a pKa of from about -2 to about 4). Typically, the acid is a weak acid. In certain embodiments, the acid is a Lewis acid. For example, the acid can be bis(trifluoromethanesulfon)imide or p-toluenesulfonic acid.

[0086] In some embodiments, the epoxide opening reaction is facilitated by a base. The base can be a weak base (i.e., a pKa of from about 7 to about 12) or a strong base (i.e., a pKa of from about 12 to about 50). Typically, the base is a weak base. For example, the base can be triethylamine, diisopropylethylamine, pyridine, N-methyl morpholine, or N,N-dimethyl-piperazine, or derivatives thereof.

[0087] In some embodiments, the epoxide opening reaction is performed in a solvent. The solvent can be any suitable solvent, or mixture of solvents, capable of solubilizing the polymer and the epoxide compound to be reacted. For example, the solvent can include water, protic organic solvents, and/or aprotic organic solvents. An exemplary list of solvents includes water, dichloromethane, diethyl ether, dimethyl sulfoxide, acetonitrile, methanol, and ethanol.

[0088] In one embodiment, groups A^1 and/or A^2 of the polymer are modified by a displacement reaction between the polymer and a compound comprising a leaving group (e.g., chloride atom, bromide atom, iodide atom, tosylate, triflate, mesylate, etc.). As used herein, the term "displacement" refers to a nucleophilic addition of a nucleophile of the polymer (e.g., a carbanion, an oxygen anion, a nitrogen anion, an oxygen atom, a nitrogen atom, or a combination thereof) to a compound comprising a leaving group. Accordingly, the displacement reaction is between the polymer and a compound comprising a leaving group. In some embodiments, the nucleophile of the polymer is a nitrogen anion, a nitrogen atom, or a combination thereof.

[0089] In embodiments where the groups designated A^1 and/or A^2 are modified by a displacement reaction, they produce groups designated E^1 and/or E^2 of the formula:

$$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^5;$$

$$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^5]_2;$$

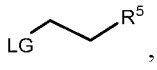
$$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^5\};$$

$$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^5]_2\}_2; \text{ or}$$

$$—(CH_2)_{p1}—[N\{(CH_2)_{s1}\text{-}R^5\}—(CH_2)_{q1}—]_{r1}NR^2{}_2,$$

wherein p1 to p4, q1 to q6, r1, r2, s1 to s4, R^2, and R^5 are as previously defined.

[0090] Examples of compounds containing a leaving group suitable for use include compound of formula:



,

wherein LG is a leaving group (e.g., chloride atom, bromide atom, iodide atom, tosylate, triflate, mesylate, etc.) and R^5 is as described with respect to any of Formulae 2 or 2A.

[0091] In some embodiments, the displacement reaction is facilitated by an acid and/or base. The acid and/or base can be any suitable acid and/or base with any suitable pKa. The acid and/or base can be an organic acid (e.g., *p*-toluenesulfonic acid), organic base (e.g., triethylamine), inorganic acid (e.g., titanium tetrachloride), inorganic base (e.g., potassium carbonate), or a combination thereof.

[0092] In some embodiments, the displacement reaction is facilitated by an acid. The acid can be a Brønsted acid or a Lewis acid. In embodiments where the acid is a Brønsted acid, the acid can be a weak acid (i.e., a pKa of from about 4 to about 7) or a strong acid (i.e., a pKa of from about -2 to about 4). Typically, the acid is a weak acid. In certain embodiments, the acid is a Lewis acid. For example, the acid can be bis(trifluoromethanesulfon)imide or p-toluenesulfonic acid.

[0093] In some embodiments, the displacement reaction is facilitated by a base. The base can be a weak base (i.e., a pKa of from about 7 to about 12) or a strong base (i.e., a pKa of from about 12 to about 50). Typically, the base is a weak base. For example, the base can be triethylamine, diisopropylethylamine, pyridine, N-methyl morpholine, or N,N-dimethyl-piperazine, or derivatives thereof.

[0094] In some embodiments, the displacement reaction is performed in a solvent. The solvent can be any suitable solvent, or mixture of solvents, capable of solubilizing the polymer and the compound comprising a leaving group to be reacted. For example, the solvent can include water, protic organic solvents, and/or aprotic organic solvents. An exemplary list of solvents includes water, dichloromethane, diethyl ether, dimethyl sulfoxide, acetonitrile, methanol, and ethanol.

[0095] In some embodiments, the method further comprises isolating the polymer comprising the structure of Formula 2. The polymer comprising the structure of Formula 2 can be isolated by any suitable means. For example, the polymer comprising the structure of Formula 2 can be isolated by extraction, dialysis, crystallization, recrystallization, column chromatography, filtration, or any combination thereof.

Polymer Composition

[0096] The polymer provided herein can be used for any purpose. However, it is believed the polymer is particularly useful for delivering one or more biomolecules or synthetic variants thereof, for example, nucleic acids and/or polypeptides (e.g., protein) to cells. Thus, provided herein is a composition comprising (a) a polymer as described herein comprising a hydrolysable polymer backbone, the hydrolysable polymer backbone

comprising (i) monomer units with a side chain comprising a polyamine or oligoamine; (ii) monomer units with a side chain comprising a hydrophobic group, and (iii) monomer units with a side chain comprising a polyalkylene oxide, a polylactic acid, a polyglycolic acid, or combination thereof; and (b) a compound to be delivered to a host or cell, such as one or more biomolecules or synthetic variants thereof. Exemplary biomolecules or synthetic variants thereof will be readily apparent from the disclosure provided herein.

[0097] In some embodiments, the composition comprises a nucleic acid. Any nucleic acid can be used. An exemplary list of nucleic acids includes guide and/or donor nucleic acids of CRISPR systems, siRNA, microRNA, interfering RNA or RNAi, dsRNA, mRNA, DNA vector, ribozymes, antisense polynucleotides, and DNA expression cassettes encoding siRNA, microRNA, dsRNA, ribozymes or antisense nucleic acids. SiRNA comprises a double stranded structure typically containing 15-50 base pairs and preferably 19-25 base pairs and having a nucleotide sequence identical or nearly identical to an expressed target gene or RNA within the cell. An siRNA may be composed of two annealed polynucleotides or a single polynucleotide that forms a hairpin structure. MicroRNAs (miRNAs) are small noncoding polynucleotides, about 22 nucleotides long, that direct destruction or translational repression of their mRNA targets. Antisense polynucleotides comprise sequence that is complimentary to a gene or mRNA. Antisense polynucleotides include, but are not limited to: morpholinos, 2′-O-methyl polynucleotides, DNA, RNA and the like. The polynucleotide-based expression inhibitor may be polymerized in vitro, recombinant, contain chimeric sequences, or derivatives of these groups. The polynucleotide-based expression inhibitor may contain ribonucleotides, deoxyribonucleotides, synthetic nucleotides, or any suitable combination such that the target RNA and/or gene is inhibited.

[0098] The composition also can comprise any protein for delivery, in addition to or instead of a nucleic acid. The polypeptide can be any suitable polypeptide. For example, the polypeptide can be a zinc finger nuclease, a transcription activator-like effector nuclease ("TALEN"), a recombinase, a deaminase, an endonuclease, or a combination thereof. In some embodiments, the polypeptide is an RNA-guided endonuclease (e.g., a Cas9 polypeptide, a Cpf1 polypeptide, or variants thereof) or a DNA recombinase (e.g., a Cre polypeptide).

[0099] It is believed the compositions provided herein are particularly useful for delivering one or more components of a CRISPR system. Thus, in some embodiments, the composition comprises a guide RNA, an RNA-guided endonuclease or nucleic acid encoding same, and/or a donor nucleic acid. The composition can comprise one, two, or all three

components together with the polymers described herein. Furthermore, the composition can comprise a plurality of guide RNAs, RNA-guided endonucleases or nucleic acids encoding same, and/or donor nucleic acids. For instance, multiple different guide RNAs for different target sites could be included, optionally with multiple different donor nucleic acids and even multiple different RNA guided endonucleases or nucleic acids encoding same.

[0100] Furthermore, the components of the CRISPR system can be combined with one another (when multiple components are present) and the polymers in any particular manner or order. In some embodiments, the guide RNA is complexed with an RNA endonuclease prior to combining with the polymers. In addition, or instead, the guide RNA can be linked (covalently or non-covalently) to a donor nucleic acid prior to combining with the polymers.

[0101] The compositions are not limited with respect to any particular CRISPR system (i.e., any particular guide RNA, RNA-guided endonuclease, or donor nucleic acid), many of which are known. Nevertheless, for the sake of further illustration, the components of some such systems are described below.

[0102] The polymer provided herein can be used in conjunction with additional polymers. In one embodiment, there is provided a composition comprising a first polymer and a second polymer. The first polymer is as described herein comprising a hydrolysable polymer backbone, the polymer backbone comprising: (a) monomer units with a side chain comprising a hydrophobic group; (b) monomer units with a side chain comprising an oligoamine or polyamine; and (c) monomer units with a side chain comprising a polyalkylene oxide, polyglycolic acid, polylactic acid, or combination thereof. All aspects and embodiments of the first polymer of the composition are as described above. The second polymer of the composition comprises a hydrolysable polymer backbone, the hydrolysable polymer backbone comprising (a) monomer units with a side chain comprising a hydrophobic group, (ii) monomer units with a side chain comprising an oligoamine or polyamine, and, optionally, (iii) monomer units with a side chain comprising an ionizable group, optionally with a pKa less than 7.

[0103] The composition can comprise any suitable amount of the first polymer and second polymer. For example, the composition can comprise a ratio (by weight) of the first polymer to the second polymer from about 1:99 to 99:1. In some embodiments, the composition comprises a ratio of the first polymer to the second polymer of about 1:1 to about 1:20 (e.g., about 1:1 to about 1:15, or about 1:1 to about 1:10) by weight. The relative amounts also can be expressed as percent composition by weight. In some embodiments, the composition comprises about 1 wt.% or more (e.g., about 5 wt.% or more, about 10 wt.% or

more, about 20 wt.% or more, about 30 wt.% or more, or about 40 wt.% or more) of the first polymer based on the total weight of the first and second polymers combined. Also, in some embodiments, the composition comprises about 60 wt.% or less (e.g., about 50 wt.% or less) of the first polymer based on the total weight of the first and second polymers combined. The foregoing percent compositions can also be stated as ranges. Thus, for instance, in some embodiments, the composition comprises from about 1 wt.% to about 60 wt.% (e.g., about 5 wt.% to about 60 wt.%, about 10 wt.% to about 60 wt.%, about 5 wt.% to about 50 wt.%, about 10 wt.% to about 50 wt.%, etc.) of the first polymer based on a sum total weight of the first polymer and the second polymer.

[0104] The composition comprising the first and, optionally second, polymer can further comprise any carrier suitable for administration to cells or hosts, such as a mammal or human, typically an aqueous carrier. The polymer(s) in the carrier form nanoparticles that partially or completely encapsulate the compound to be delivered when present.

[0105] The various elements of the polymer composition, including examples of the second polymer, nucleic acids, and polypeptide compounds, are described in greater detail below.

Second Polymer

[0106] The second polymer of the composition comprises a hydrolysable polymer backbone, the hydrolysable polymer backbone comprising (a) monomer units with a side chain comprising a hydrophobic group, (ii) monomer units with a side chain comprising an oligoamine or polyamine, and, optionally, (iii) monomer units with a side chain comprising an ionizable group, optionally with a pKa less than 7.

[0107] As discussed previously, a hydrolysable polymer backbone refers to a polymer backbone having bonds that are susceptible to cleavage under physiological conditions (e.g., physiological pH, physiological temperature, or in a given *in vivo* tissue such as blood, serum, etc. due to naturally occurring factors (e.g., enzymes). Generally, the hydrolysable polymer backbone comprises a polyamide, poly-N-alkylamide, polyester, polycarbonate, polycarbamate, or a combination thereof. In certain embodiments, the hydrolysable polymer backbone comprises a polyamide.

[0108] The monomer units with a side chain comprising a hydrophobic group, can comprise any hydrophobic group. Examples of hydrophobic groups include, for instance, a C_1-C_{12} (e.g., C_2-C_{12}, C_2-C_{10}, C_2-C_8, C_2-C_6, C_3-C_{12}, C_3-C_{10}, C_3-C_8, C_3-C_6, C_4-C_{12}, C_4-C_{10}, C_4-C_8, C_4-C_6, C_6-C_{12}, C_6-C_8, C_8-C_{12}, C_8-C_{10},) alkyl group, a C_2-C_{12} (e.g., C_2-C_6, C_3-C_{12}, C_3-C_{10},

C_3-C_8, C_3-C_6, C_4-C_{12}, C_4-C_{10}, C_4-C_8, C_4-C_6, C_6-C_{12}, C_6-C_8, C_8-C_{12}, C_8-C_{10},) alkenyl group, or a C_3-C_{12} (C_3-C_{10}, C_3-C_8, C_3-C_6, C_4-C_{12}, C_4-C_{10}, C_4-C_8, C_4-C_6, C_6-C_{12}, C_6-C_8, C_8-C_{12}, C_8-C_{10},) cycloalkyl group or cycloalkenyl group. In certain embodiments, the hydrophobic group comprises a C_4-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group. In some embodiments, the hydrophobic group comprises fewer than 8 carbons or fewer than 6 carbons. For example, the hydrophobic group can comprise a C_2-C_8 or C_2-C_6 (e.g., C_3-C_8 or C_3-C_6) alkyl group. The alkyl or alkenyl groups can be branched or straight-chain. In any of the foregoing embodiments, the hydrophobic group can be linked to the polymer backbone directly or via a linkage comprising, for instance, an ester, an amide, or an ether group, optionally further comprising an alkylene linker (e.g., a methylene or ethylene linker).

[0109] The second polymer also comprises monomer units with a side chain comprising an oligoamine or polyamine. As used herein, the term "oligoamine" refers to any chemical moiety having two or three amine groups, and the term "polyamine" refers to any chemical moiety having four or more (e.g., 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, etc.) amine groups. The amine groups can be primary amine groups, secondary amine groups, tertiary amine groups, or any combination thereof. In certain embodiments, the oligoamine or polyamine is of the formula:

$$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2_2\ ;$$

$$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2_2]_2;$$

$$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\};$$

$$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2_2]_2\}_2,$$

$$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5;$$

$$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^4\text{-}R^5]_2;$$

$$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^4\text{-}R^5\};$$

$$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^4\text{-}R^5]_2\}_2;$$

$$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5;$$

$$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5;$$

$$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}\text{-}CH_2\text{-}CHOH\text{-}R^5;$$

$$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5]_2\}_2;$$

$$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^5;$$

$$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^5]_2;$$

$$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^5\};$$

$$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^5]_2\}_2;$$

$$—(CH_2)_{p1}—[N\{(CH_2)_{s1}\text{-}R^4\text{-}R^5\}—(CH_2)_{q1}—]_{r1}NR^2_2;$$

$—(CH_2)_{p1}—[N\{(CH_2)_{s1}\text{-}R^5\}—(CH_2)_{q1}—]_{r1}NR^2{}_2$,

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH(CONH_2)\text{-}(CH_2)_{s1}\text{-}R^5$; or

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH(CONH_2)\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5$,

wherein p1 to p4, q1 to q6, r1 and r2, and s1 to s4 are each independently an integer of 1 to 5; each instance of R^2 is independently hydrogen or a C_1-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, or R^2 is combined with a second R^2 so as to form a heterocyclic group; each instance of R^4 is independently -C(O)O-, -C(O)NH-, -O-, -C-O-C(O)-C-O-, or -S(O)(O)-; and each instance of R^5 is independently an alkyl group, cycloalkyl group, alkenyl group, cycloalkenyl group, aryl group, heteroalkyl group, heterocyclic group, or combination thereof optionally comprising from 2 to 8 tertiary amines or a substituent comprising a tissue-specific or cell-specific targeting moiety.

[0110] In some embodiments, the oligoamine or polyamine is of the formula:

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2{}_2]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}$; or

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2{}_2]_2\}_2$,

wherein p1 to p4, q1 to q6, and r1 and r2 are each independently an integer of 1 to 5 (e.g., 1, 2, or 3); and each instance of R^2 is independently hydrogen or a C_1-C_{12} (e.g., C_1-C_6, C_1-C_3, C_2, or C_1) alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, or R^2 is combined with a second R^2 so as to form a heterocyclic group. It is understood that the alkenyl groups must have at least 2 carbons (e.g., C_2-C_{12}, C_2-C_6, etc.) and the cycloalkyl and cycloalkenyl groups must have at least 3 carbons (e.g., C_3-C_{12}, C_3-C_6, etc.). In some embodiments, the polyamine is $—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2$, optionally wherein R^2 is independently hydrogen or a C_1-C_3 alkyl (e.g., methyl or ethyl).

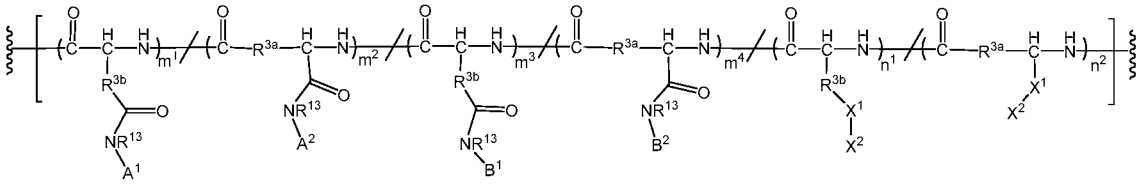
[0111] In some embodiments, the second polymer further comprises monomer units with a side chain comprising an ionizable group. As used herein, the phrase "ionizable group" refers to any chemical moiety with a substituent that can readily be converted into a charged species. For example, the ionizable group can be a group that is a proton-donor or proton-acceptor. The group can be protonated or deprotonated under physiological conditions. In certain embodiments, the ionizable group has a pKa less than 7 (in water at 25° C). For example, the ionizable group described herein can have a pKa of less than 6, a pKa of less than 5, a pKa of less than 4, a pKa of less than 3, a pKa of less than 2, or a pKa of less than 1. Alternatively, or additionally, the ionizable group described herein can have a pKa of greater than -2, a pKa of greater than -1, a pKa of greater than 0, a pKa of greater than 1, a pKa of

greater than 2, a pKa of greater than 3, a pKa of greater than 4, a pKa of greater than 5, or a pKa of greater than 6. Accordingly, the ionizable group described herein can have a pKa from -2 to 7, for example, a pKa from -1 to 7, a pKa from 0 to 7, a pKa from 1 to 7, a pKa from 2 to 7, a pKa from 3 to 7, a pKa from 4 to 7, a pKa from 5 to 7, a pKa from 6 to 7, a pKa from 0 to 6, a pKa from 2 to 6, a pKa from 4 to 6, a pKa from 0 to 5, a pKa from 2 to 5, or a pKa from 4 to 5. Examples of ionizable groups include, for instance, sulfonic acid, sulfonamide, carboxylic acid, thiol, phenol, amine salt, imide, and amide groups.

[0112] In some embodiments, the second polymer has an overall pKa of less than 7 (in water at 25° C). For example, the second polymer described herein can have a pKa of less than 6, a pKa of less than 5, a pKa of less than 4, a pKa of less than 3, a pKa of less than 2, or a pKa of less than 1. Alternatively, or additionally, the second polymer described herein can have a pKa of greater than -2, a pKa of greater than -1, a pKa of greater than 0, a pKa of greater than 1, a pKa of greater than 2, a pKa of greater than 3, a pKa of greater than 4, a pKa of greater than 5, or a pKa of greater than 6. Accordingly, the second polymer described herein can have a pKa from -2 to 7, for example, a pKa from -1 to 7, a pKa from 0 to 7, a pKa from 1 to 7, a pKa from 2 to 7, a pKa from 3 to 7, a pKa from 4 to 7, a pKa from 5 to 7, a pKa from 6 to 7, a pKa from 0 to 6, a pKa from 2 to 6, a pKa from 4 to 6, a pKa from 0 to 5, a pKa from 2 to 5, or a pKa from 4 to 5.

[0113] The second polymer can comprise any suitable number or amount (e.g., weight or number percent composition) of the monomer units with a side chain comprising a hydrophobic group, the monomer units with a side chain comprising an oligoamine or polyamine, and, when present, the monomer units with a side chain comprising an ionizable group. In some embodiments, the second polymer comprises about 1 to about 80 mol% (e.g., about 5 to about 80 mol%, about 10 to about 80 mol%, about 20 to about 80 mol%, about 40 to about 80 mol%, about 1 to about 60 mol%, about 1 to about 40 mol%, about 1 to about 20 mol%, or about 1 to about 10 mol%) of the monomer units having a hydrophobic group, about 1 to about 80 mol% (e.g., about 5 to about 80 mol%, about 10 to about 80 mol%, about 20 to about 80 mol%, about 40 to about 80 mol%, about 1 to about 60 mol%, about 1 to about 40 mol%, about 1 to about 20 mol%, or about 1 to about 10 mol%) of the monomer units having an oligoamine or polyamine, and 0 to about 80 mol% (e.g., about 5 to about 80 mol%, about 10 to about 80 mol%, about 20 to about 80 mol%, about 40 to about 80 mol%, about 1 to about 60 mol%, about 1 to about 40 mol%, about 1 to about 20 mol%, or about 1 to about 10 mol%) of the monomer units having an ionizable group.

[0114] The second polymer can comprise a structure of Formula 3:



wherein:

each of m^1, m^2, m^3, and m^4 is an integer from 0 to 1000, provided that the sum of $m^1 + m^2 + m^3 + m^4$ is greater than 5;

each of n^1 and n^2 is an integer from 0 to 1000, provided that the sum of $n^1 + n^2$ is greater than 2;

the symbol "/" indicates that the units separated thereby are linked randomly or in any order;

each instance of R^{3a} is independently a methylene or ethylene group;

each instance of R^{3b} is independently a methylene or ethylene group;

each X^1 independently is —C(O)O—, —C(O)NR^{13}—, —C(O)—, —S(O)(O)—, or a bond;

each instance of R^{13} is independently hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl group, any of which can be optionally substituted with one or more substituents;

each instance of X^2 is independently a C_1-C_{12} alkyl or heteroalkyl group, C_3-C_{12} cycloalkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkenyl group, aryl group, heterocyclic group, or combination thereof optionally comprising one or more primary, secondary, or tertiary amines; any of which are optionally substituted with one or more substituents;

A^1 and A^2 are each independently a group of formula

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}NR^2{}_2$;

—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—$NR^2{}_2$]$_2$;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—$NR^2{}_2$][—$(CH_2)_{q4}$—NR^2—]$_{r2}R^2$}; or

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—$NR^2{}_2$]$_2$}$_2$,

B^1 and B^2 are each independently

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}NR^2$-$(CH_2)_{s1}$-R^4-R^5;

—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—NR^2-$(CH_2)_{s2}$-R^4-R^5]$_2$;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—$NR^2{}_2$][—$(CH_2)_{q4}$—NR^2—]$_{r2}$$(CH_2)_{s3}$-$R^4$-$R^5$};

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-$(CH_2)_{s4}$-R^4-R^5]$_2$}$_2$;

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}NR^2$-CH_2-CHOH-R^5;

—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—NR^2-CH_2-CHOH-R^5;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—NR^2_2][—$(CH_2)_{q4}$—NR^2—]$_{r2}$-CH_2-CHOH-R^5;

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-CH_2-CHOH-R^5]$_2$}$_2$;

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-$(CH_2)_{s1}$-$R^5$;

—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—NR^2-$(CH_2)_{s2}$-R^5]$_2$;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—NR^2_2][—$(CH_2)_{q4}$—NR^2—]$_{r2}$$(CH_2)_{s3}$-$R^5$};

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-$(CH_2)_{s4}$-R^5]$_2$}$_2$;

—$(CH_2)_{p1}$—[N{$(CH_2)_{s1}$-R^4-R^5}—$(CH_2)_{q1}$—]$_{r1}$$NR^2_2$;

—$(CH_2)_{p1}$—[N{$(CH_2)_{s1}$-R^5}—$(CH_2)_{q1}$—]$_{r1}$$NR^2_2$,

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-CH($CONH_2$)-$(CH_2)_{s1}$-$R^5$; or

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-CH($CONH_2$)-$(CH_2)_{s1}$-$R^4$-$R^5$,

wherein p1 to p4, q1 to q6, r1 and r2, and s1 to s4 are each independently an integer of 1 to 5; each instance of R^2 is independently hydrogen or a C_1-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, or R^2 is combined with a second R^2 so as to form a heterocyclic group; each instance of R^4 is independently -C(O)O-, -C(O)NH-, -O-, -C-O-C(O)-C-O-, or S(O)(O)-; and each instance of R^5 is independently an alkyl group, cycloalkyl group, alkenyl group, cycloalkenyl group, aryl group, heteroalkyl group, heterocyclic group, or combination thereof optionally comprising from 2 to 8 tertiary amines or a substituent comprising a tissue-specific or cell-specific targeting moiety.

[0115] According to Formula 3, each of m^1, m^2, m^3, and m^4 is an integer from 0 to 1000 (e.g., 0 to 500, 0 to 200, 0 to 100, or 0 to 50), provided that the sum of $m^1 + m^2 + m^3 + m^4$ is greater than 5, such as 5-5000, 5-2000, 5-1000, 5-500, 5-100, or 5-50. In some embodiments, the sum of $m^1 + m^2 + m^3 + m^4$ is greater than 10 or greater than 20 (e.g., 10-5000, 10-2000, 10-1000, 10-500, 10-100, or 10-50; or 20-5000, 20-2000, 20-1000, 20-500, 20-100, or 20-50). Furthermore, each of n^1 and n^2 is an integer from 0 to 1000 (e.g., 0 to 500, 0 to 200, 0 to 100, 0 to 50, or 0 to 25), provided that the sum of $n^1 + n^2$ is greater than 2 (e.g., 2-2000, 2-1000, 2-500, 2-200, 2-100, 2-50, or 2-25). In some embodiments, the sum of $n^1 + n^2$ is greater than 5 or greater than 10 (e.g., 5-2000, 5-1000, 5-500, 5-200, 5-100, 5-50, or 5-25; or 10-2000, 10-1000, 10-500, 10-200, 10-100, 10-50, or 10-25). In other words, the second polymer comprises at least some monomeric units comprising groups A^1, A^2, B^1, and/or B^2, herein referred to collectively as the "A monomers" and "B monomers," respectively. Similarly, the second polymer comprises at least some monomeric units comprising groups X^1 and/or X^2, herein referred to collectively as the "X monomers." In some embodiments, m^1 and m^2 are zero, such that the second polymer comprises no A^1 or A^2 groups. In some

embodiments, m^3 and m^4 are zero, such that the second polymer comprises no B^1 or B^2 groups.

[0116] The second polymer can comprise any suitable ratio of A and B monomers to X monomers. In some embodiments, the second polymer comprises a ratio of A and B monomers to X monomers (e.g., the ratio of $(m^1+m^2+m^3+m^4)/(n^1+n^2)$) of about 25 or less, and, optionally, about 1 or more. For example, the ratio of A and B monomers to X monomers can be about 1 to about 25, from about 1 to about 20, from about 1 to about 10, from about 1 to about 5, from about 5 to about 25, from about 10 to about 25, or from about 15 to about 25.

[0117] In embodiments where the second polymer comprises both A monomers and B monomers, the second polymer can comprise any suitable ratio of A monomers to B monomers. In some embodiments, the ratio of A monomers to B monomers (e.g., $(m^1+m^2)/(m^3+m^4)$) can be about 20 or less (e.g., about 10 or less, about 5 or less, about 2 or less, or even about 1 or less). In some embodiments, the ratio of $(m^1+m^2)/(m^3+m^4)$ is about 0.2 or more, such as about 0.5 or more.

[0118] The second polymer can exist as any suitable structure type. For example, the second polymer can exist as an alternating polymer, random polymer, block polymer, graft polymer, linear polymer, branched polymer, cyclic polymer, or a combination thereof. In certain embodiments, the second polymer is a random polymer, block polymer, graft polymer, or a combination thereof.

[0119] Thus, in the structure of Formula 3, the monomers (which can be referred to by their respective side chains A^1, A^2, B^1, B^2, X^1, and X^2) can be arranged randomly or in any order. The integers m^1, m^2, m^3, m^4, n^1, and n^2 merely denote the number of the respective monomers that appear in the chain overall, and do not necessarily imply or represent any particular order or blocks of those monomers, although blocks or stretches of a given monomer might be present in some embodiments. For instance, the structure of Formula 3 can comprise the monomers in the order $-A^1-A^2-B^1-B^2-$, $-A^2-A^1-B^2-B^1-$, $-A^1-B^1-A^2-B^2-$, etc. Furthermore, the second polymer can comprise blocks of A and/or B polymers (e.g., $[A\ monomers]_{m1+m2}$-$[B\ monomers]_{m3+m4}$) in any order). The second polymer can comprise individual X monomers interspersed with the A and B monomers (e.g., -A-X-B-, -A-B-X-, -B-X-A, etc.), or the second polymer can be "capped" with one or more X monomers (e.g., a block of X monomers) at one or both ends of the polymer. Likewise, when the second polymer comprises blocks of A and/or B monomers, the second polymer can comprise blocks of X monomers interspersed between blocks of A and/or B monomers, or the second polymer

can be "capped" with one or more X monomers (e.g., a block of X monomers) at one or both ends of the polymer. In some embodiments, the polypeptide (e.g., polyaspartamide) backbone will be arranged in an alpha/beta configuration, such that the "1" and "2" monomers will alternate (e.g., -A^1-A^2-B^1-B^2-, -A^2-A^1-B^2-B^1-, -A^1-B^2-B^1-A^2-, -A^2-B^1-B^2-A^1-, -B^1-A^2-B^1-A^2-, etc.), wherein the second polymer is capped with X monomers or the X monomers are interspersed throughout. However, the "A" and "B" sidechains (e.g., A^1/A^2 and B^1/B^2) can be dispersed randomly throughout the polymer backbone.

[0120] In the polymer structures, R^{3a} and R^{3b} are each independently a methylene or ethylene group. In some embodiments, R^{3a} is an ethylene group and R^{3b} is a methylene group; or R^{3a} is a methylene group and R^{3b} is an ethylene group. In certain embodiments, R^{3a} and R^{3b} are each an ethylene group. In some embodiments, R^{3a} and R^{3b} are each a methylene group.

[0121] In the polymers described herein, each X^1 group independently is —C(O)O—, —C(O)NR^{13}—, —C(O)—, —S(O)(O)—, or a bond. Each X^1 group can be the same or different from one another. In some embodiments, X^1 is —C(O)NR^{13}—. In some embodiments, X^1 is —C(O)O—.

[0122] Each instance of R^{13} is independently hydrogen or a C_1-C_{12} (e.g., C_1-C_8, C_1-C_6, or C_1-C_3) alkyl group, C_2-C_{12} (e.g., C_2-C_8, C_2-C_6, or C_2-C_3) alkenyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkenyl group, aryl group, or heterocyclic group (e.g., 3-12, 3-10, 3-8, or 3-6 membered heterocyclic group comprising one, two, or three heteroatoms), any of which can be substituted with one or more substituents. In some embodiments, R^{13} is a C_1-C_{12} alkyl group (e.g., a C_1-C_{10} alkyl group; a C_1-C_8 alkyl group; a C_1-C_6 alkyl group; a C_1-C_4 alkyl group, a C_1-C_3 alkyl group, or a C_1 or C_2 alkyl group) which can be linear or branched. In certain embodiments, each R^{13} is methyl or hydrogen. In some embodiments, R^{13} is methyl; in other embodiments, R^{13} is hydrogen. Each R^{13} is independently chosen and can be the same or different; however, in some embodiments, each R^{13} is the same (e.g., all methyl or all hydrogen).

[0123] Each instance of X^2 is independently C_1-C_{12} (e.g., C_1-C_8, C_1-C_6, or C_1-C_3) alkyl group, C_2-C_{12} (e.g., C_2-C_8, C_2-C_6, or C_2-C_3) alkenyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkenyl group, aryl group, or heterocyclic group (e.g., 3-12, 3-10, 3-8, or 3-6 membered heterocyclic group comprising one, two, or three heteroatoms) or combination thereof, any of which can be substituted with one or more substituents. In some embodiments, X^2 optionally can comprise one or more primary, secondary, or tertiary amines. Accordingly, each X^2 is independently selected and,

therefore, can be the same or different from one another. In certain embodiments, each instance of X^2 is independently a C_1-C_{12} (e.g., C_1-C_8, C_1-C_6, or C_1-C_3) alkyl group, C_2-C_{12} (e.g., C_2-C_8, C_2-C_6, or C_2-C_3) alkenyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkenyl group, or combination thereof optionally comprising one or more primary, secondary, or tertiary amines. In some embodiments, one or more (or all) X^2 groups can be independently a C_2-C_{12} (e.g., C_3-C_{12}, C_3-C_8, C_3-C_6, C_4-C_{12}, C_4-C_6, C_6-C_{12}, or C_8-C_{12}) alkyl group or alkenyl group, or C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, C_4-C_{12}, C_4-C_6, C_6-C_{12}, or C_8-C_{12}) cycloalkenyl group. In other embodiments, one or more (or all) X^2 groups can be independently a C_1-C_8 (e.g., C_1-C_6, C_1-C_4, C_1-C_3, C_2-C_8, or C_2-C_6) alkyl groups. Any of the foregoing alkyl or alkenyl groups can be linear or branched.

[0124] Groups A^1 and A^2 are independently selected and, therefore, can be the same or different from one another. Similarly, groups B^1 and B^2 are independently selected and can be the same or different from one another. However, in some embodiments, A^1 and A^2 are the same and/or B^1 and B^2 are the same.

[0125] In groups A^1, A^2, B^1, and B^2, integers p1 to p4 (i.e., p1, p2, p3, and p4), q1 to q6 (i.e., q1, q2, q3, q4, q5, and q6), r1, r2, and s1 to s4 (i.e., s1, s2, s3, and s4) are each independently an integer of 1 to 5 (e.g., 1, 2, 3, 4, or 5). In some embodiments, p1 to p4 (i.e., p1, p2, p3, and p4), q1 to q6 (i.e., q1, q2, q3, q4, q5, and q6), r1, r2, and/or s1 to s4 are each independently an integer of 1 to 3 (e.g., 1, 2, or 3). In certain embodiments, p1 to p4 (i.e., p1, p2, p3, and p4), q1 to q6 (i.e., q1, q2, q3, q4, q5, and q6), and/or s1 to s4 (i.e., s1, s2, s3, and s4) are each 2. In some embodiments, p1 to p4 (i.e., p1, p2, p3, and p4) and/or q1 to q6 (i.e., q1, q2, q3, q4, q5, and q6) are each 2, and r1, r2, and s1 to s4 (i.e., s1, s2, s3, and s4) are each 1.

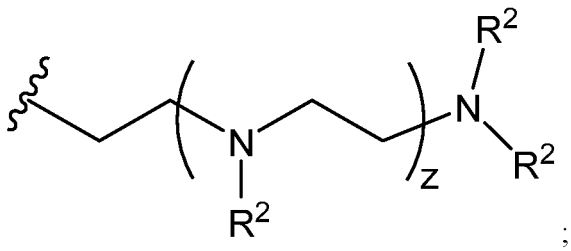
[0126] Each instance of R^2 can be hydrogen or a C_1-C_{12} (e.g., C_1-C_8, C_1-C_6, or C_1-C_3) alkyl group, C_2-C_{12} (e.g., C_2-C_8, C_2-C_6, or C_2-C_3) alkenyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkenyl group, or R^2 is combined with a second R^2 so as to form a heterocyclic group. In some embodiments, R^2 is hydrogen or a C_1-C_{12} alkyl (e.g., a C_1-C_{10} alkyl group; a C_1-C_8 alkyl group; a C_1-C_6 alkyl group; a C_1-C_4 alkyl group, a C_1-C_3 alkyl group, or a C_1 or C_2 alkyl group) that can be linear or branched. In certain embodiments, R^2 is methyl. In other embodiments, R^2 can be hydrogen. Each R^2 is independently chosen and can be the same or different. In some embodiments, each R^2 in a given is the same (e.g., all methyl or all hydrogen).

[0127] Each instance of R^4 is independently —C(O)O—, —C(O)NH—, or —S(O)(O)—. In some embodiments, each instance of R^4 is independently —C(O)O— or —C(O)NH—.

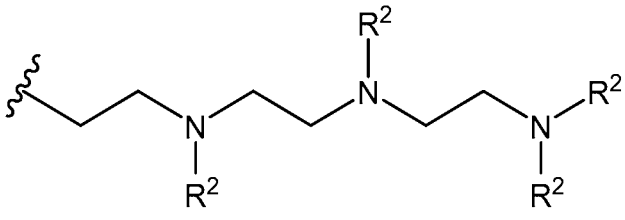
In certain embodiments, each instance of R^4 is —C(O)O—. In certain embodiments, each instance of R^4 is —C(O)NH—.

[0128] Each instance of R^5 is independently an alkyl group, cycloalkyl group, alkenyl group, cycloalkenyl group, aryl group, heteroalkyl group, heterocyclic group, or combination thereof, optionally comprising from 2 to 8 tertiary amines or a substituent comprising a tissue-specific or cell-specific targeting moiety. R^5 can comprise from about 2 to about 50 carbon atoms (e.g., from about 2 to about 40 carbon atoms, from about 2 to about 30 carbon atoms, from about 2 to about 20 carbon atoms, from about 2 to about 16 carbon atoms, from about 2 to about 12 carbon atoms, from about 2 to about 10 carbon atoms, or from about 2 to about 8 carbon atoms). In some embodiments, R^5 is a heteroalkyl group comprising from 2 to 8 (i.e., 2, 3, 4, 5, 6, 7, or 8) tertiary amines. The tertiary amines can be part of the heteroalkyl backbone (i.e., the longest continuous chain of atoms in the heteroalkyl group, or a pendant substituent. Thus, for instance, the heteroalkyl group comprising the tertiary amines can provide an alkylamino group, amino alkyl group, alkylaminoalkyl group, aminoalkylamino group, or the like comprising 2 to 8 tertiary amines.

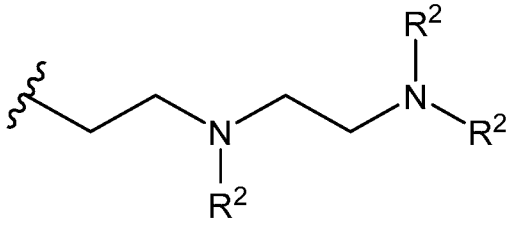
[0129] In some embodiments, each R^5 is independently selected from:



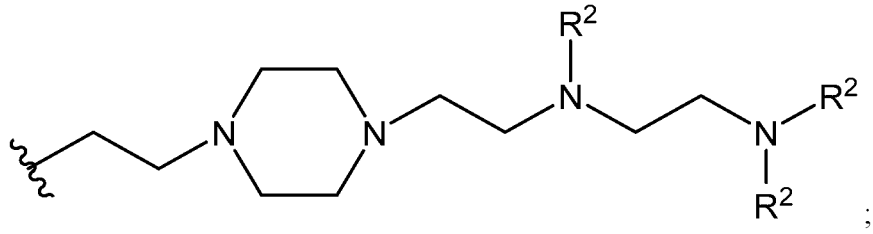
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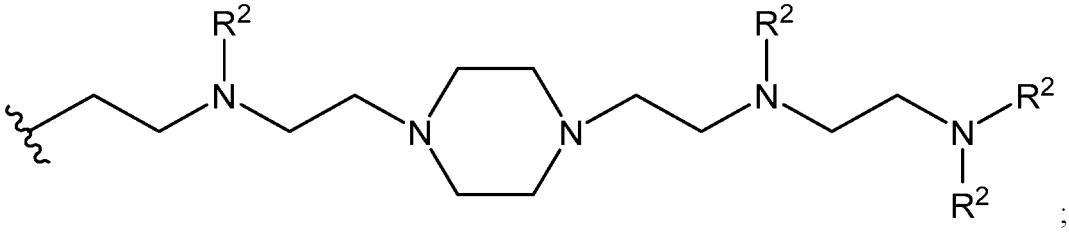


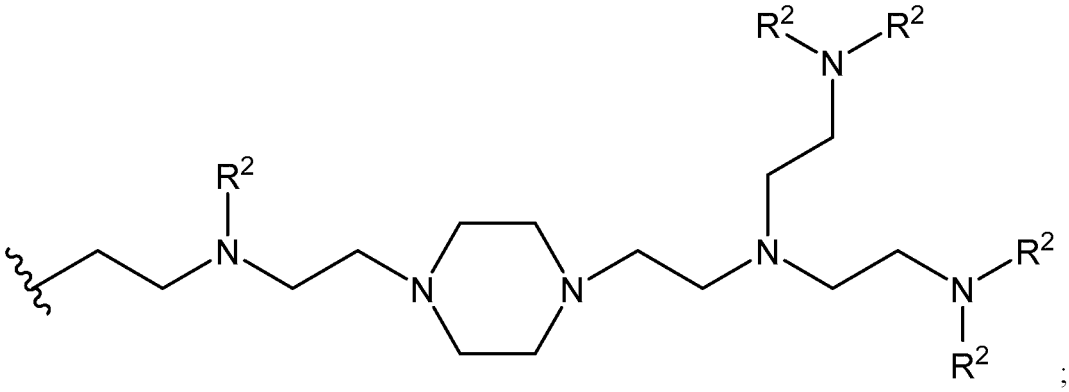
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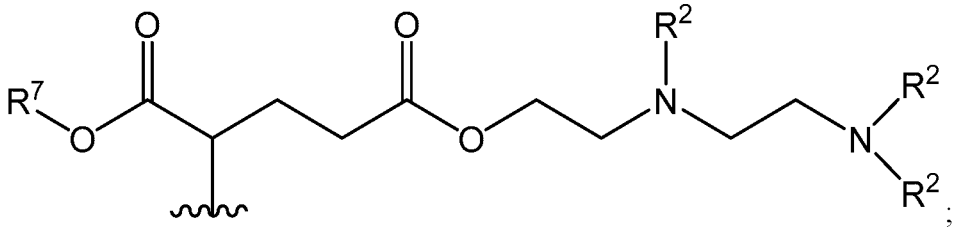


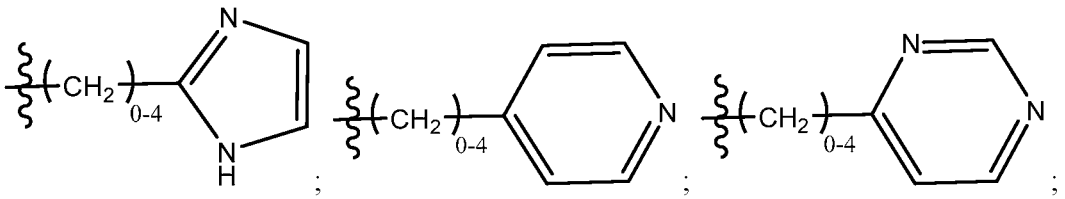
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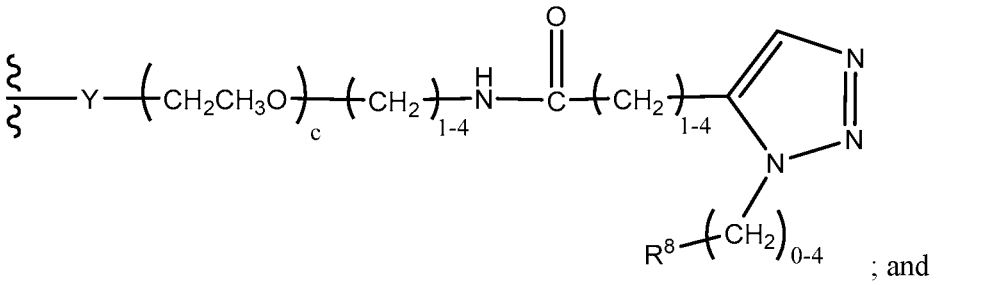

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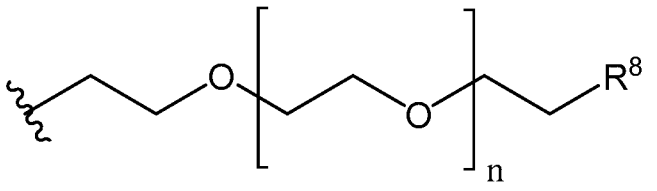

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; and



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wherein

each instance of R^2 is as described above;

R^7 is a C_1-C_{50} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group optionally substituted with one or more amines;

z is an integer from 1 to 5;

c is an integer from 0 to 50;

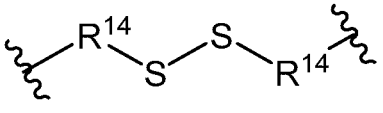
Y is optionally present and is a cleavable linker;

n is an integer from 0 to 50; and

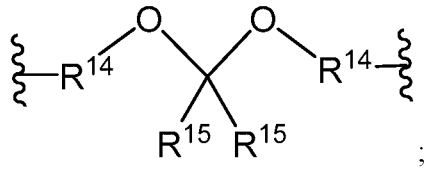
R^8 is a tissue-specific or cell-specific targeting moiety. C_1-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group.

[0130] R^7 can be a C_1-C_{50} (e.g., C_1-C_{40}, C_1-C_{30}, C_1-C_{20}, C_1-C_{10}, C_4-C_{12}, or C_6-C_8) alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group optionally substituted with one or more amines. In some embodiments, R^7 is a C_4-C_{12},, such as a C_6-C_8, alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group optionally substituted with one or more amines. In some embodiments, R^7 is substituted with one or more amines. In certain embodiments, R^7 is substituted with 2 to 8 (i.e., 2, 3, 4, 5, 6, 7, or 8) tertiary amines. The tertiary amines can be a part of the alkyl group (i.e., encompassed in the alkyl group backbone) or a pendant substituent.

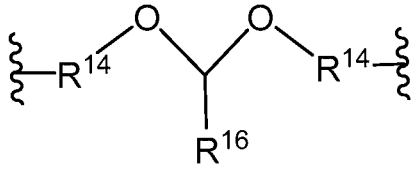
[0131] Each instance of Y is optionally present. As used herein, the phrase "optionally present" means that a substituent designated as optionally present can be present or not present, and when that substituent is not present, the adjoining substituents are bound directly to each other. When Y is present, Y is a cleavable linker. As used herein, the phrase "cleavable linker" refers to any chemical element that connects two species that can be cleaved as to separate the two species. For example, the cleavable linker can be cleaved by a hydrolytic process, photochemical process, radical process, enzymatic process, electrochemical process, or a combination thereof. Exemplary cleavable linkers include, but are not limited to:



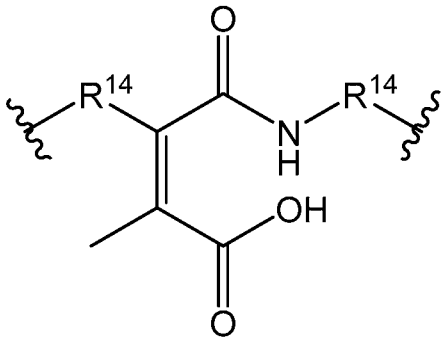
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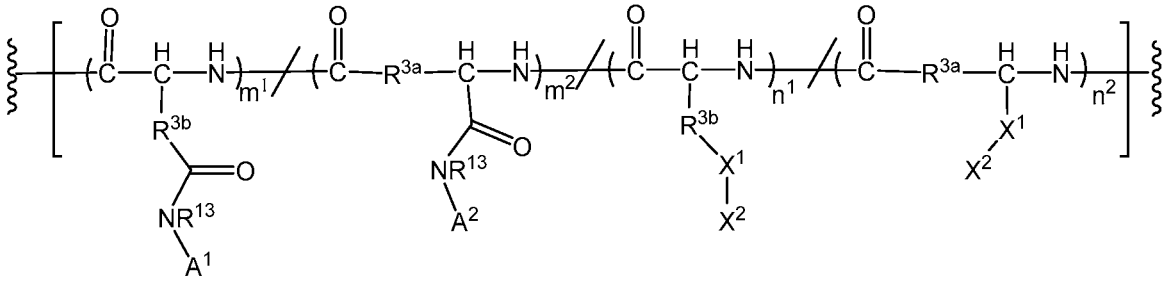
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wherein each occasion of R^{14} independently is a C_1-C_4 alkyl group, each occasion of R^{15} independently is hydrogen, an aryl group, a heterocyclic group (e.g., aromatic or non-aromatic), a C_1-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, and R^{16} is a six-membered aromatic or heteroaromatic group optionally substituted with one or more $-OCH_3$, $-NHCH_3$, $-N(CH_3)_2$, $-SCH_3$, -OH, or a combination thereof.

[0132] In some embodiments, each of A^1 and A^2 is independently a group of formula $—(CH_2)_{p1}—[NH—(CH_2)_{q1}—]_{r1}NH_2$ or $—(CH_2)_{p1}—[NH—(CH_2)_{q1}—]_{r1}NHCH_3$, or a group $–(CH_2)_2$-NH-$(CH_2)_2$-NH_2 or $–(CH_2)_2$-NH-$(CH_2)_2$-$NHCH_3$ or $–(CH_2)_2$-NH-$(CH_2)_2$-NH_2. In some embodiments, each of A^1 and A^2 is independently a group of formula $—(CH_2)_{p1}—[N(R^2))—(CH_2)_{q1}—]_{r1}N(R^2)_2$ or $—(CH_2)_{p1}—[N(R^2)—(CH_2)_{q1}—]_{r1}NH(R^2)$, wherein R^2 is a methyl or ethyl; or a group $–(CH_2)_2$-N(CH_3)-$(CH_2)_2$-NH_2, or $–(CH_2)_2$-N(CH_3)-$(CH_2)_2$-$NHCH_3$, or $–(CH_2)_2$-N(CH_3)-$(CH_2)_2$-$N(CH_3)_2$.

[0133] In addition, or alternatively, each of B^1 and B^2 is a group of formula $—(CH_2)_{p1}—[NH—(CH_2)_{q1}—]_{r1}$NH-$(CH_2)_2$-$R^4$-$R^5$, such as a group $–(CH_2)_2$-NH-$(CH_2)_2$-NH-$(CH_2)_2$-R^4-R^5, or a group $–(CH_2)_2$-NH-$(CH_2)_2$-NH-$(CH_2)_2$-C(O)-O-R^5, wherein R^4 and R^5 are as described above.

[0134] In some embodiments, the polymer comprising a structure of Formula 3 does not have any B monomers (e.g., m^3 and m^4 are both 0). Thus, the second polymer can comprise the structure of Formula 4:



Formula 4

wherein:

each of m^1 and m^2 is an integer from 0 to 1000 (e.g., 0 to 500, 0 to 200, 0 to 100, or 0 to 50), provided that the sum of $m^1 + m^2$ is greater than 5 (e.g., 5-2000, 5-1000, 5-500, 5-100, or 5-50). In some embodiments, the sum of $m^1 + m^2$ is greater than 10 or greater than 20 (e.g., 10-5000, 10-2000, 10-1000, 10-500, 10-100, or 10-50; or 20-5000, 20-2000, 20-1000, 20-500, 20-100, or 20-50). Furthermore, each of n^1 and n^2 is an integer from 0 to 1000 (e.g., 0 to 500, 0 to 200, 0 to 100, 0 to 50, or 0-25), provided that the sum of $n^1 + n^2$ is greater than 2 (e.g., 2-2000, 2-1000, 2-500, 2-200, 2-100, 2-50, or 2-25). In some embodiments, the sum of $n^1 + n^2$ is greater than 5 or greater than 10 (e.g., 5-2000, 5-1000, 5-500, 5-200, 5-100, 5-50, or 5-25; or 10-2000, 10-1000, 10-500, 10-200, 10-100, 10-50, or 10-25).

[0135] In some embodiments of the polymer comprising a structure of Formula 4, A^1 and A^2 are each independently a group of formula

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2_2$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2_2]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}$; or

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2_2]_2\}_2$,

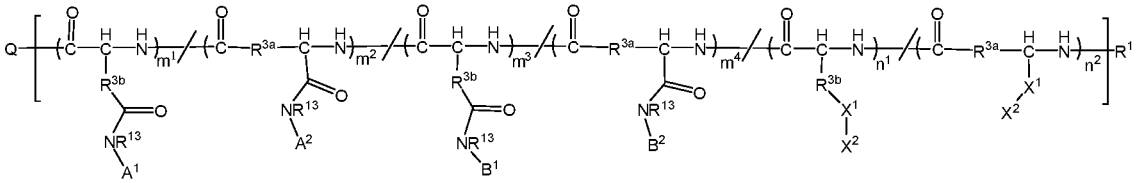
wherein p1 to p4, q1 to q6, and r1 and r2 are each independently an integer of 1 to 5 (e.g., an integer of 1-3); and each instance of R^2 is independently hydrogen or a C_1-C_{12} (e.g., C_1-C_8, C_1-C_6, or C_1-C_3) alkyl group, C_2-C_{12} (e.g., C_2-C_8, C_2-C_6, or C_2-C_3) alkenyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkenyl group. In some embodiments, each nitrogen in group A^1 and A^2 containing R^2 substituents is a tertiary amine, with the exception that the terminal amine can be a primary, secondary, or tertiary amine or, in some embodiments, a secondary or tertiary amine. By way of further illustration, each of A^1 and A^2 can be $–(CH_2)_2$-NR^2-$(CH_2)_2$-NR^2_2, wherein each instance of R^2 is independently a hydrogen, alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group as described above, particularly an alkyl such as methyl or ethyl,

optionally wherein each amine is a tertiary amine with the exception that the terminal amine is a secondary or tertiary amine.

[0136] Specific non-limiting examples of groups A^1 and A^2 include, for instance, -NH-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)$_2$; -N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)$_2$; -NH-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)$_2$; -N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)$_2$; -NH-CH_2-CH_2-N(CH_3)-CH_2-CH_2-NH(CH_3); -N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-NH(CH_3); -NH-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-NH(CH_3); -N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-N(CH_3)-CH_2-CH_2-NH(CH_3).

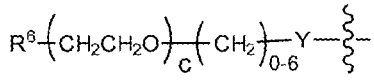
[0137] All other aspects of the polymer comprising a structure of Formula 4 are as described with respect to Formula 3, including all embodiments thereof with respect to substituents of Formula 4. Thus, for instance, in some embodiments of Formula 4, each instance of R^{13} can be any group as described with respect to Formula 3, including specific embodiments in which R^{13} is hydrogen or methyl, and each instance of R^{3a} and R^{3b} can be any group as described with respect to Formula 3, including embodiments wherein R^{3a} and R^3 are methylene or ethylene. Similarly, X^1 and X^2 can be any group as described with respect to Formula 3, including embodiments wherein X^1 is —C(O)NR^{13}— or —C(O)O— and/or one or more (or all) X^2 groups can be independently a C_1-C_8 (e.g., C_1-C_6, C_1-C_4, C_1-C_3, C_2-C_8, or C_2-C_6) alkyl group.

[0138] In some embodiments, the second polymer has structure of Formula 3A:



wherein

Q is of formula:



c is an integer from 0 to 50;

Y is optionally present and is a cleavable linker;

each of m^1, m^2, m^3, and m^4 is an integer from 0 to 1000, provided that the sum of m^1 + m^2 + m^3 + m^4 is greater than 5;

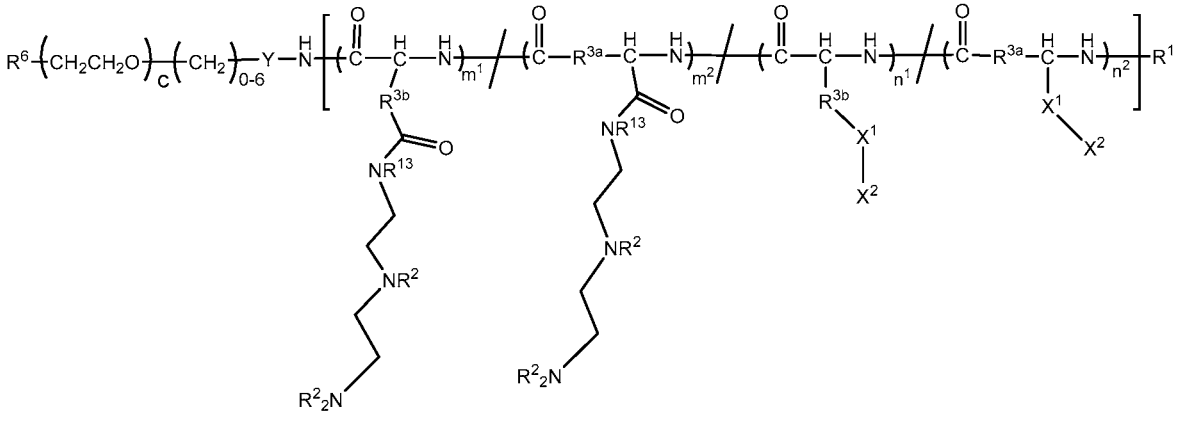
each of n^1 and n^2 is an integer from 0 to 1000, provided that the sum of n^1 + n^2 is greater than 2;

the symbol "/" indicates that the units separated thereby are linked randomly or in any order;

R^1 is hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} (e.g., C_1-C_8, C_1-C_6, or C_1-C_3) alkyl or heteroalkyl group, C_2-C_{12} (e.g., C_2-C_8, C_2-C_6, or C_2-C_3) alkenyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkyl group, or C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkenyl group, optionally substituted with one or more substituents; and

R^6 is hydrogen, an amino group, an aryl group, a heterocyclic group, a C_1-C_{12} (e.g., C_1-C_8, C_1-C_6, or C_1-C_3) alkyl or heteroalkyl group, a C_2-C_{12} (e.g., C_2-C_8, C_2-C_6, or C_2-C_3) alkenyl group, a C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkyl group, or a C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkenyl group, optionally substituted with one or more amines; or a tissue-specific or cell-specific targeting moiety. All other aspects of Formula 3A are as described with respect to Formula 3, above, including any and all embodiments thereof.

[0139] In some embodiments, the second polymer has structure of Formula 3B:



wherein

c is an integer from 0 to 50;

Y is optionally present and is a cleavable linker;

each of m^1 and m^2 is an integer from 0 to 1000, provided that the sum of $m^1 + m^2$ is greater than 5;

each of n^1 and n^2 is an integer from 0 to 1000, provided that the sum of $n^1 + n^2$ is greater than 2;

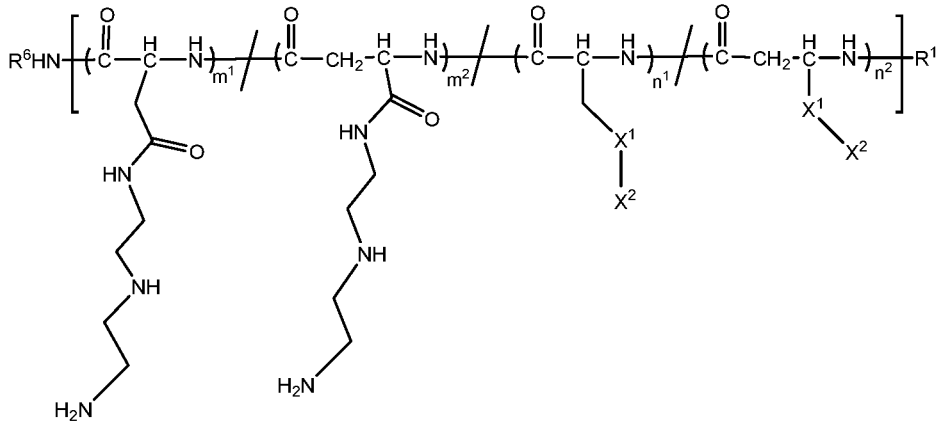
the symbol "/" indicates that the units separated thereby are linked randomly or in any order;

R^1 is hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} (e.g., C_1-C_8, C_1-C_6, or C_1-C_3) alkyl or heteroalkyl group, C_2-C_{12} (e.g., C_2-C_8, C_2-C_6, or C_2-C_3) alkenyl group, C_3-C_{12}

(e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkyl group, or C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkenyl group optionally substituted with one or more substituents; and

R^6 is hydrogen, an amino group, an aryl group, a heterocyclic group, a C_1-C_{12} (e.g., C_1-C_8, C_1-C_6, or C_1-C_3) alkyl or heteroalkyl group, a C_2-C_{12} (e.g., C_2-C_8, C_2-C_6, or C_2-C_3) alkenyl group, a C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkyl group, or a C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkenyl group optionally substituted with one or more amines; or a tissue-specific or cell-specific targeting moiety. All other aspects of Formula 3B are as described with respect to Formula 3 and Formula 4, including any and all embodiments thereof.

[0140] In some embodiments, the second polymer has structure of Formula 3C:



wherein

each of m^1 and m^2 is an integer from 0 to 1000, provided that the sum of $m^1 + m^2$ is greater than 5;

each of n^1 and n^2 is an integer from 0 to 1000, provided that the sum of $n^1 + n^2$ is greater than 2;

the symbol "/" indicates that the units separated thereby are linked randomly or in any order;

R^1 is hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} (e.g., C_1-C_8, C_1-C_6, or C_1-C_3) alkyl or heteroalkyl group, C_2-C_{12} (e.g., C_2-C_8, C_2-C_6, or C_2-C_3) alkenyl group, C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkyl group, or C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkenyl group optionally substituted with one or more substituents; and

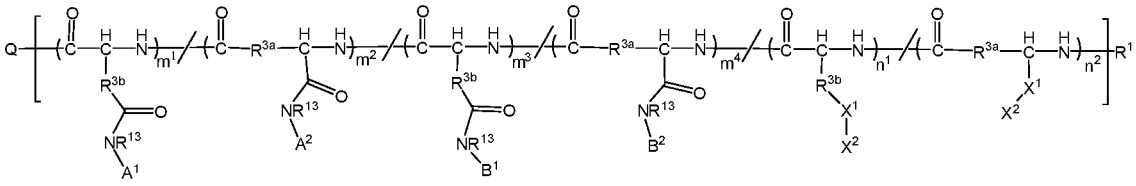
R^6 is hydrogen, an amino group, an aryl group, a heterocyclic group, a C_1-C_{12} (e.g., C_1-C_8, C_1-C_6, or C_1-C_3) alkyl or heteroalkyl group, a C_2-C_{12} (e.g., C_2-C_8, C_2-C_6, or C_2-C_3) alkenyl group, a C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkyl group, or a C_3-C_{12} (e.g., C_3-C_8, C_3-C_6, or C_3-C_5) cycloalkenyl group optionally substituted with one or more amines; or a

tissue-specific or cell-specific targeting moiety. All other aspects of Formula 3C are as described with respect to Formula 3 and Formula 4 including any and all embodiments thereof.

[0141] In some embodiments, R^1 and/or R^6 is a C_1-C_{12} alkyl (e.g., a C_1-C_{10} alkyl group; a C_1-C_8 alkyl group; a C_1-C_6 alkyl group; a C_1-C_4 alkyl group, a C_1-C_3 alkyl group, or a C_1 or C_2 alkyl group), which can be linear or branched, optionally substituted with one or more substituents. In certain embodiments, the heteroalkyl or alkyl group comprises or is substituted with one or more amines, for instance, from 2 to 8 (i.e., 2, 3, 4, 5, 6, 7, or 8) tertiary amines. The tertiary amines can be a part of the heteroalkyl backbone chain or pendant substituents.

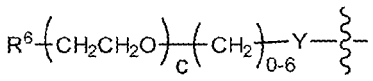
[0142] The second polymer can be any suitable polymer, provided the polymer comprises the foregoing polymer structure. In some embodiments, the second polymer is a block copolymer comprising a polymer block having the structure of Formula 3 and one or more other polymer blocks, such as a polyalkylene oxide, polylactic acid, or polyglycolic acid block. However, the second polymer of the composition need not comprise such additional polymer blocks. In some embodiments, the second polymer does not comprise polyalkylene oxide, polylactic acid, or polyglycolic acid in the side chains of the polymer. In some embodiments, the second polymer does not comprise polyalkylene oxide, polylactic acid, or polyglycolic acid in the backbone or at either terminus of the polymer. In some embodiments, the second polymer does not comprise polyalkylene oxide, polylactic acid, or polyglycolic acid at all. In still other embodiments, the second polymer does not comprise any additional polymer units other than as shown in the structure of Formula 3, which can comprise any suitable end groups. In certain embodiments, the polymer further comprises a substituent comprising a tissue-specific or cell-specific targeting moiety.

[0143] In some embodiments, the second polymer has structure of Formula 3A':



wherein

Q is of formula:

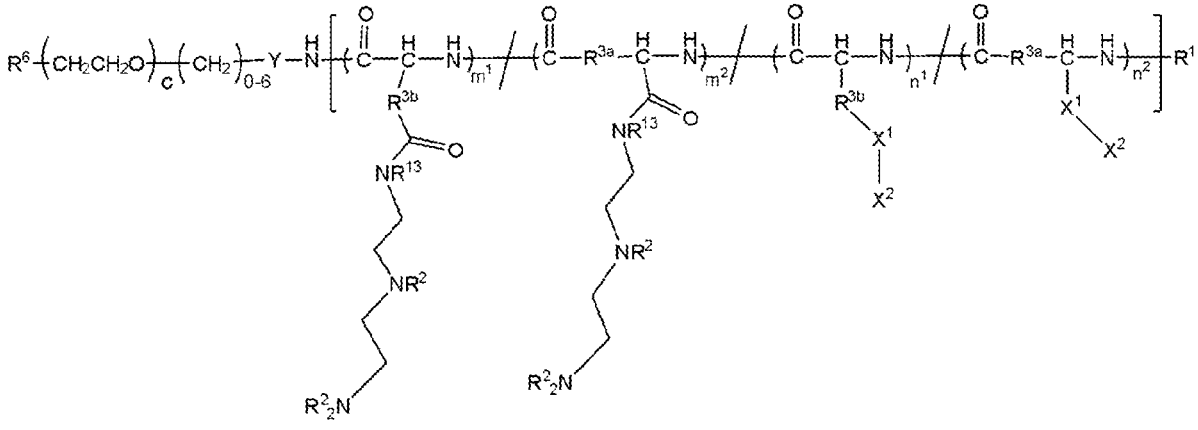


c is an integer from 2 to 200 (e.g., 2 to 150, 2 to 100, 2 to 50, 10 to 200, 10 to 150, 10 to 100, 10 to 50, 25 to 200, 25 to 150, 25 to 100, 25 to 50, 50 to 200, 50 to 150, or 50 to 100);

Y is optionally present and is a cleavable linker;

and all other substituents are as described with respect to Formulae 3 and 4, including any and all embodiments thereof.

[0144] In some embodiments, the second polymer has structure of Formula 3B':



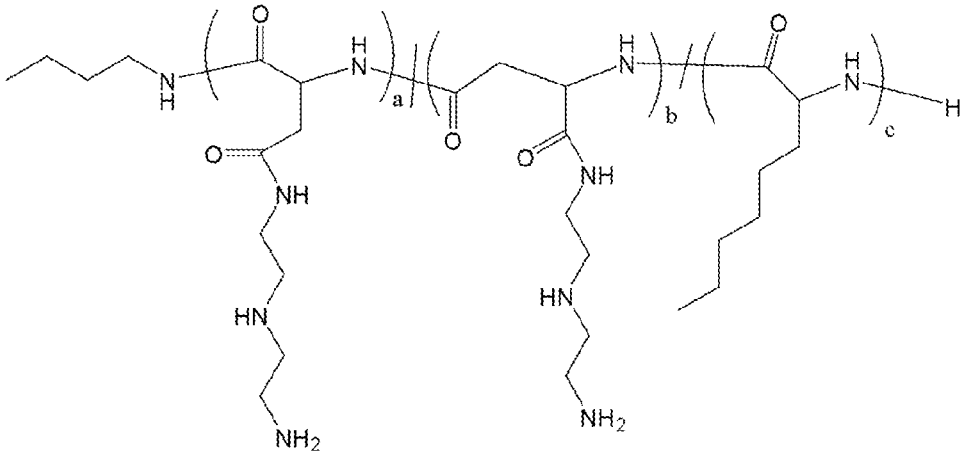
wherein

c is an integer from 2 to 200 (e.g., 2 to 150, 2 to 100, 2 to 50, 10 to 200, 10 to 150, 10 to 100, 10 to 50, 25 to 200, 25 to 150, 25 to 100, 25 to 50, 50 to 200, 50 to 150, or 50 to 100);

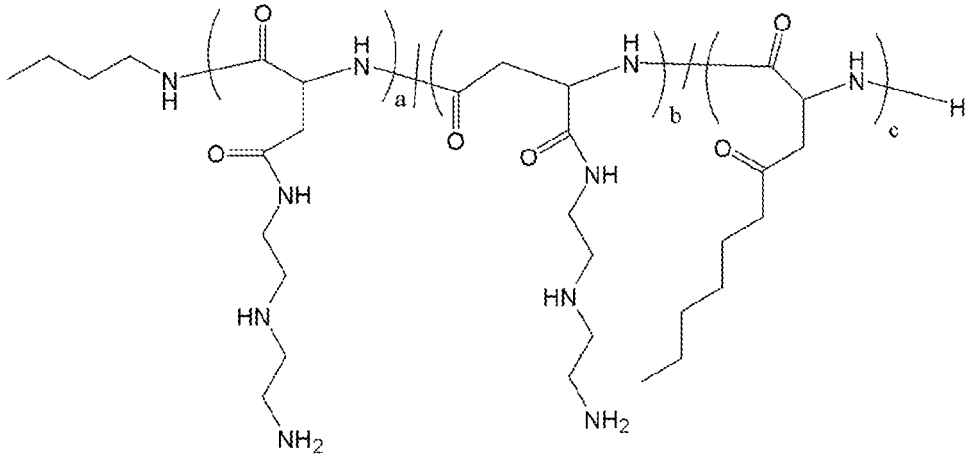
Y is optionally present and is a cleavable linker;

and all other substituents are as described with respect to Formulae 3 and 4, including any and all embodiments thereof.

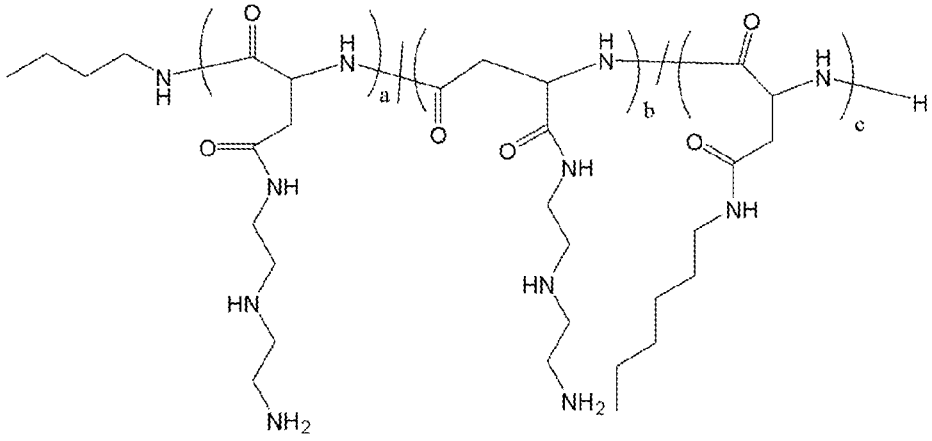
[0145] Non-limiting examples of the second polymers provided herein include, for instance:



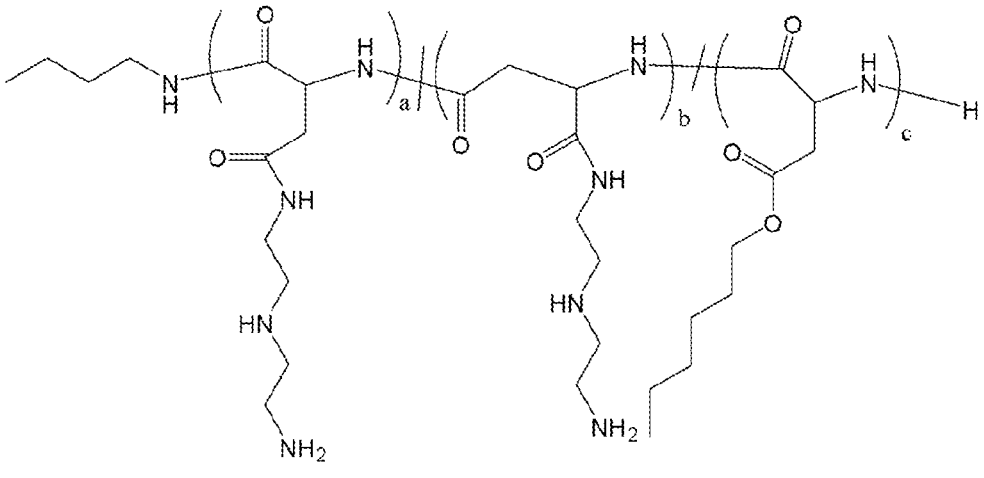
Polymer 1



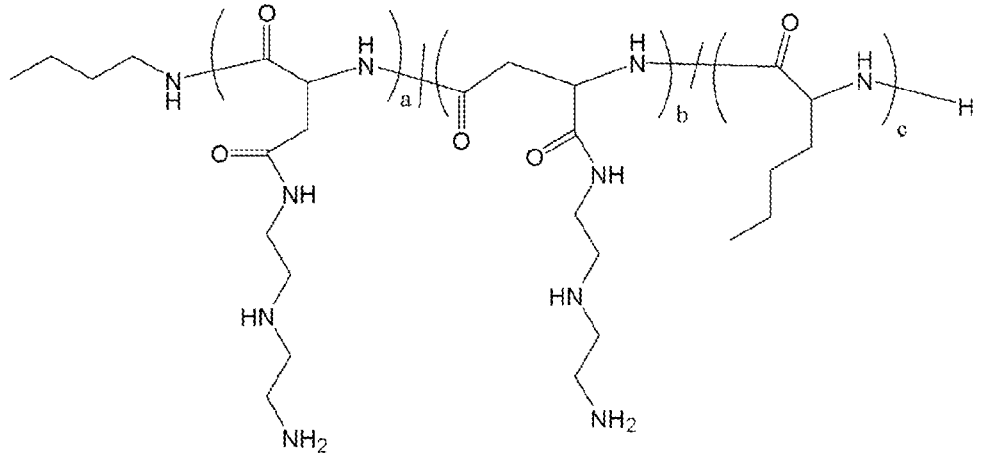
Polymer 2



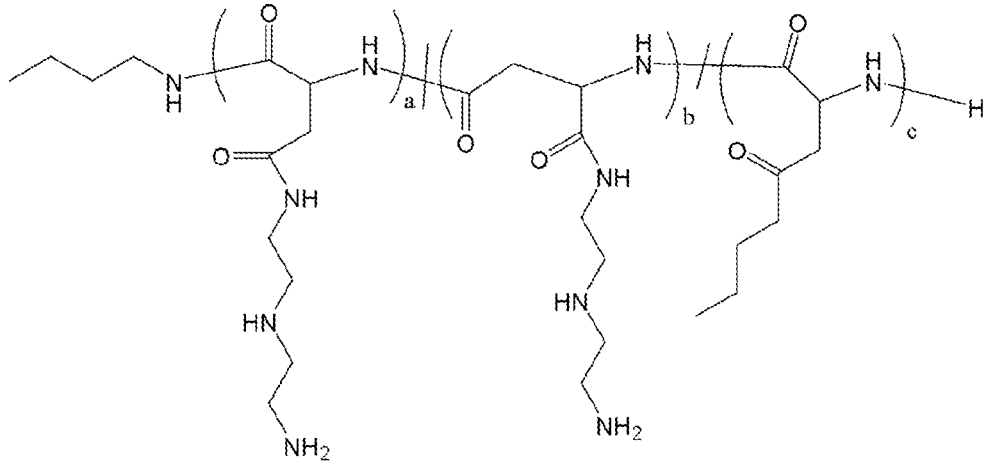
Polymer 3



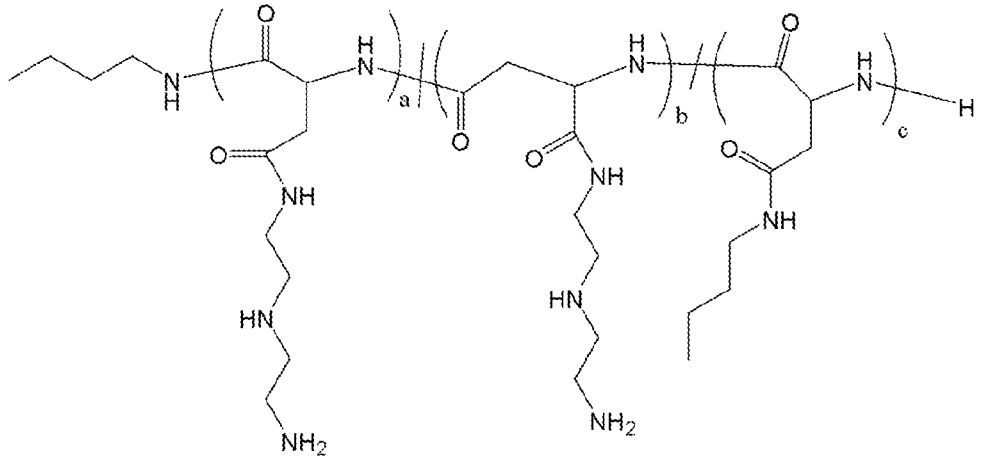
Polymer 4



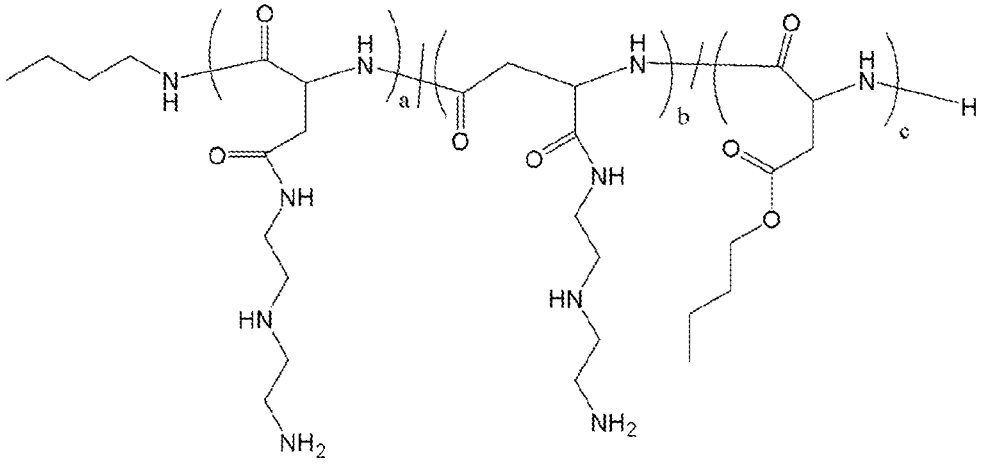
Polymer 5



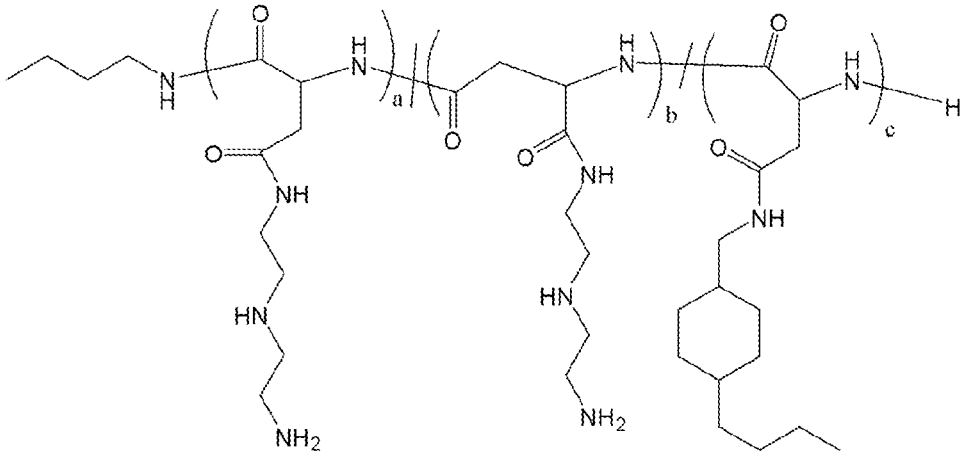
Polymer 6



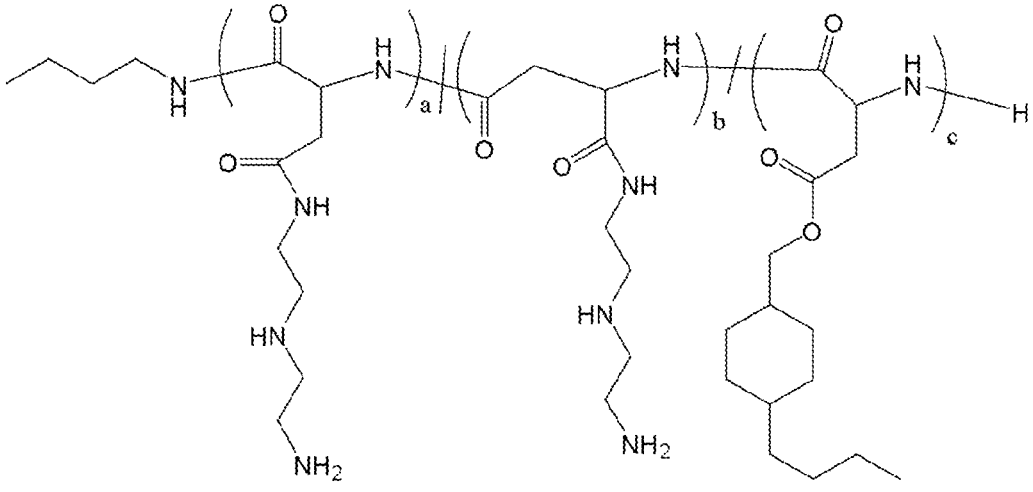
Polymer 7



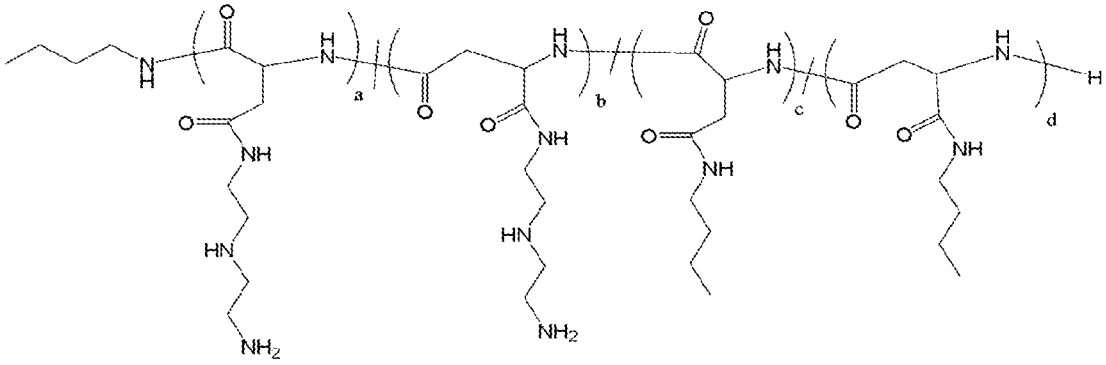
Polymer 8



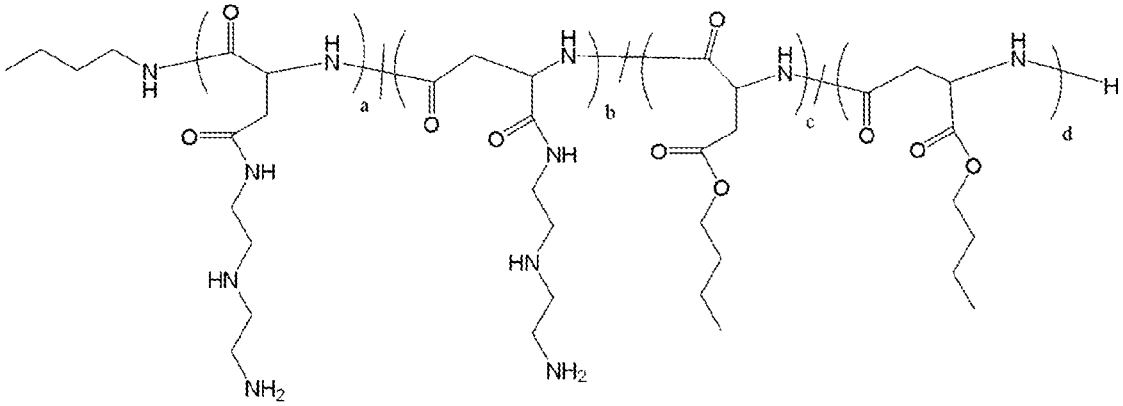
Polymer 9



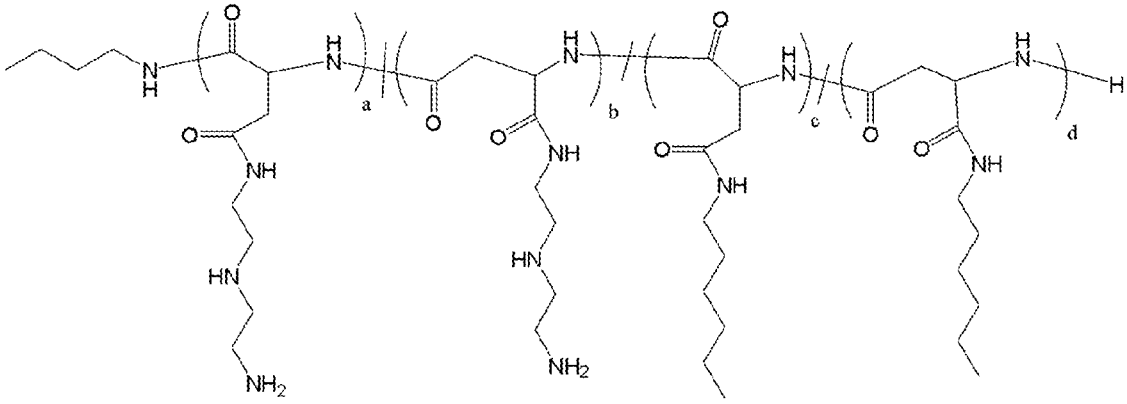
Polymer 10



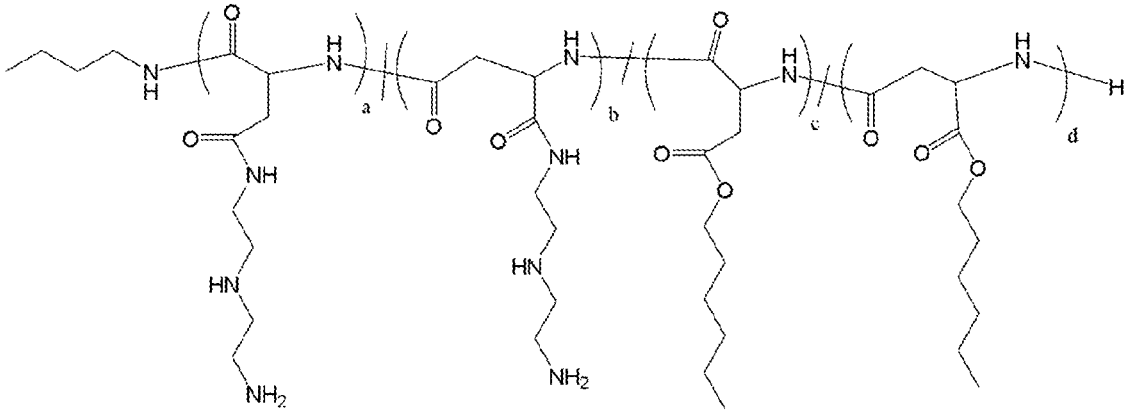
Polymer 11



Polymer 12

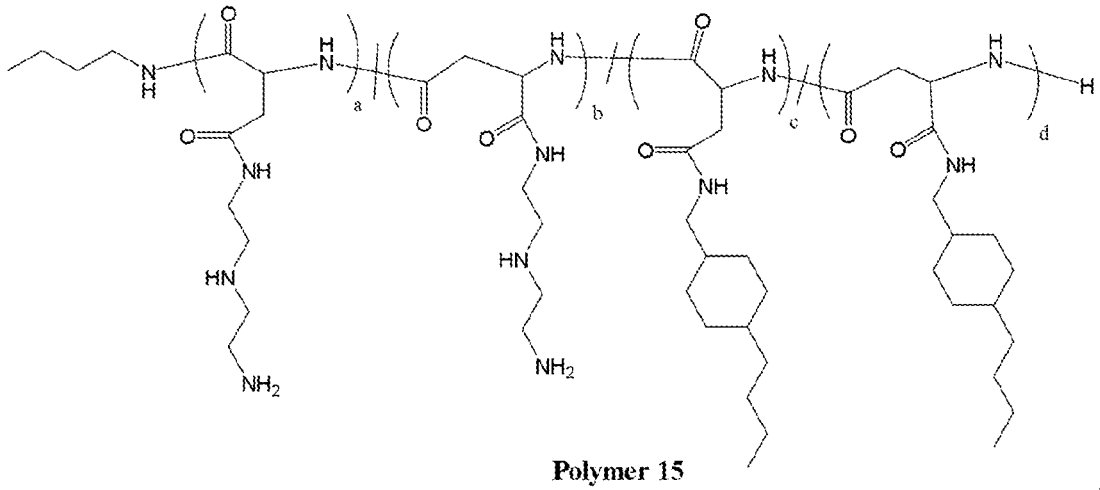


Polymer 13



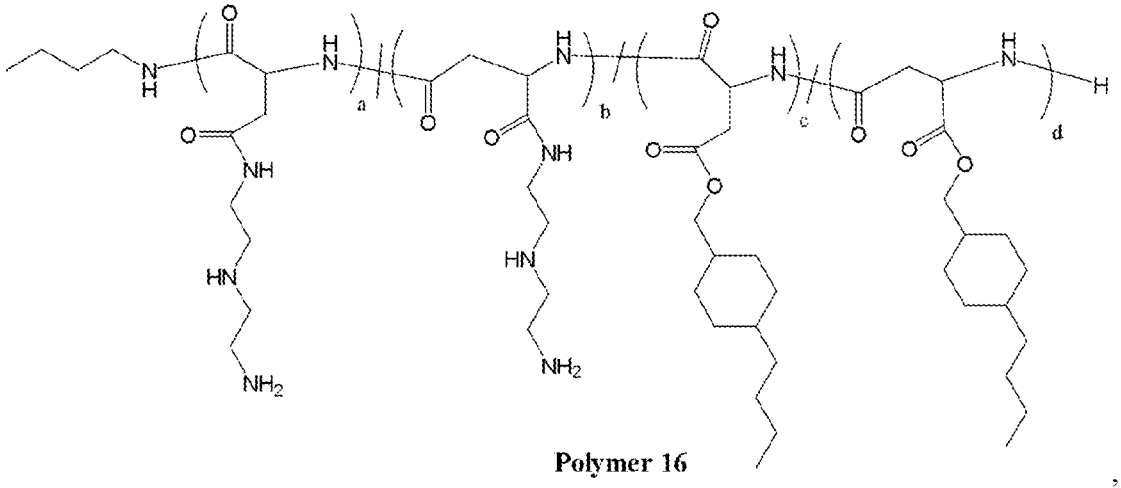
Polymer 14

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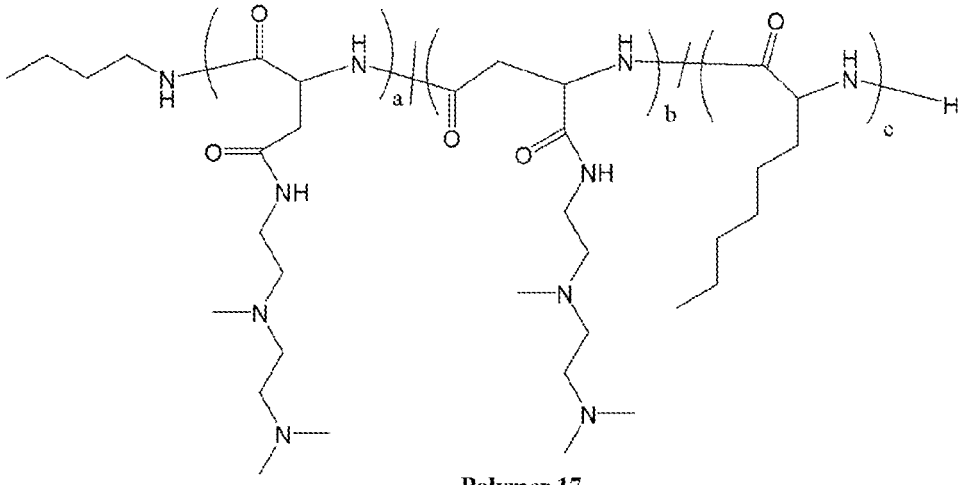
Polymer 15

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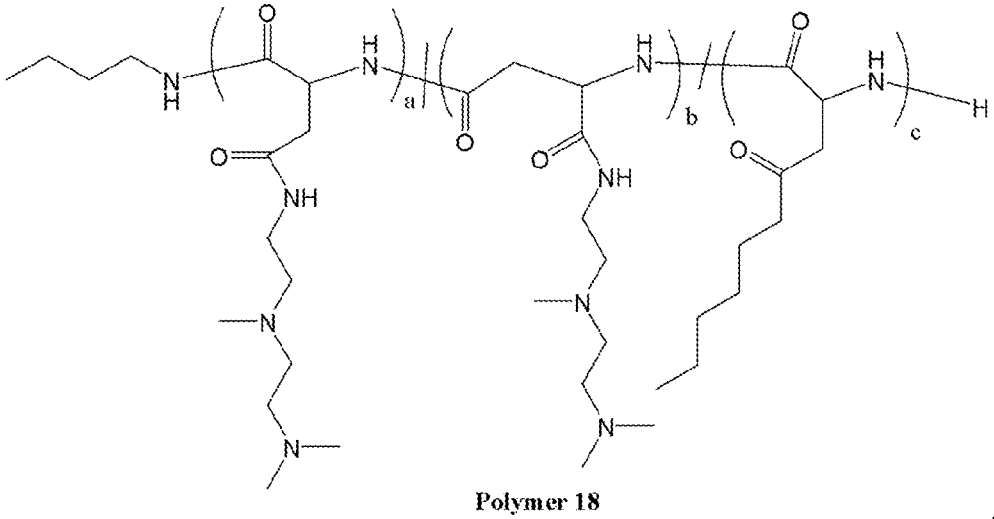


Polymer 16

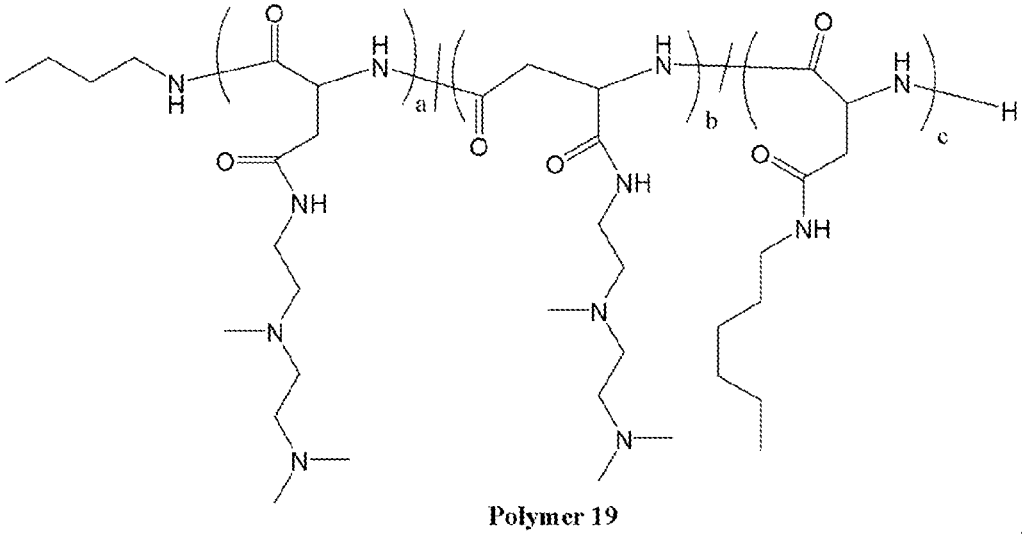
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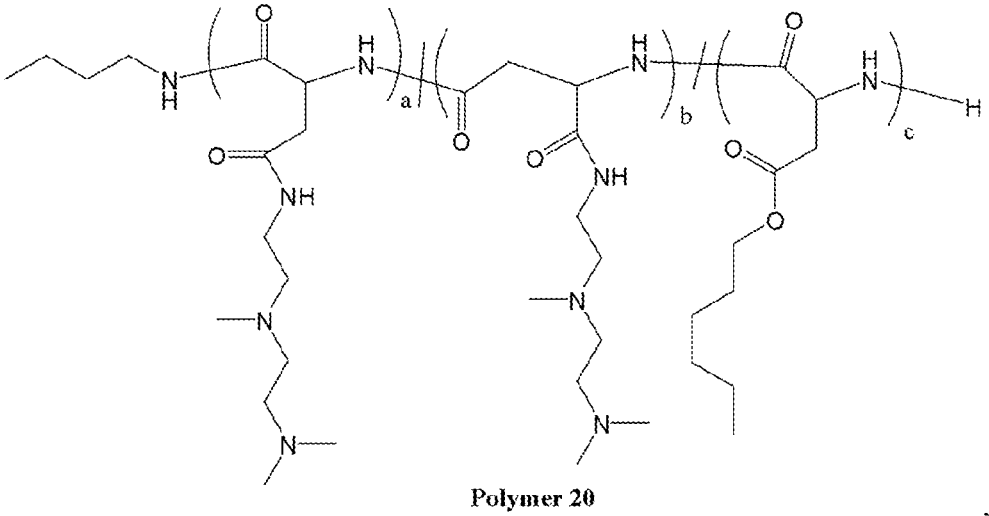
Polymer 17



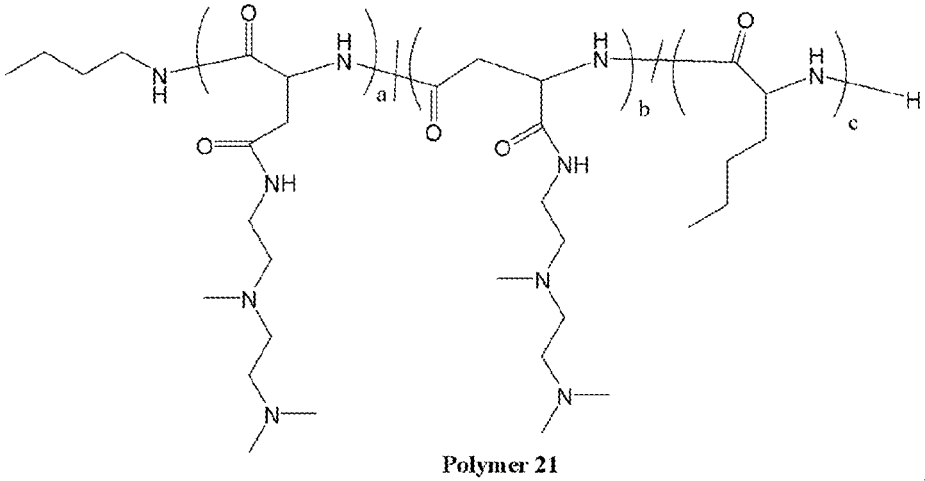
Polymer 18



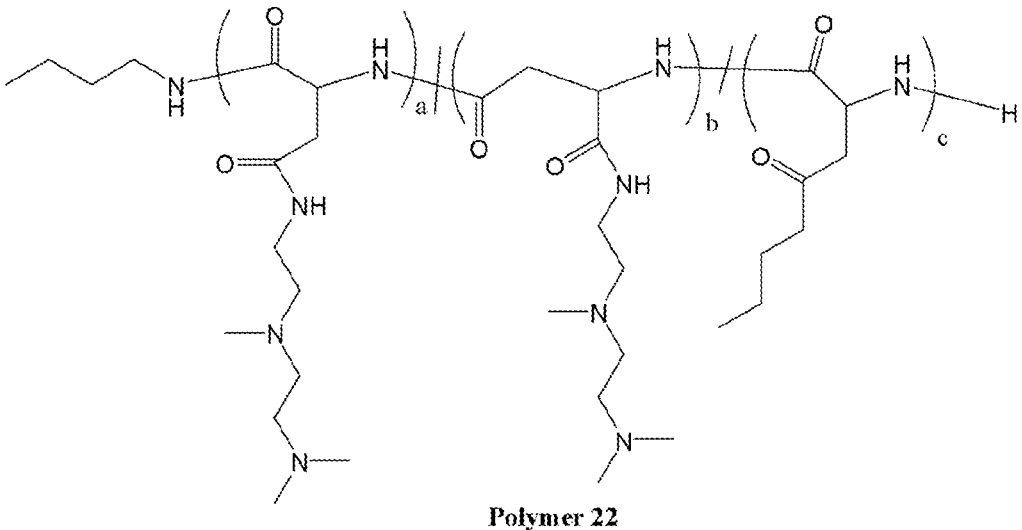
Polymer 19



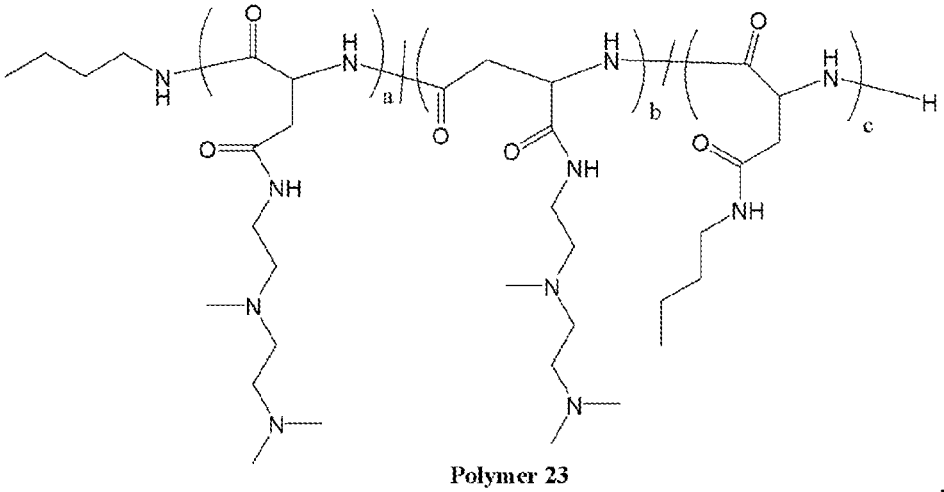
Polymer 20



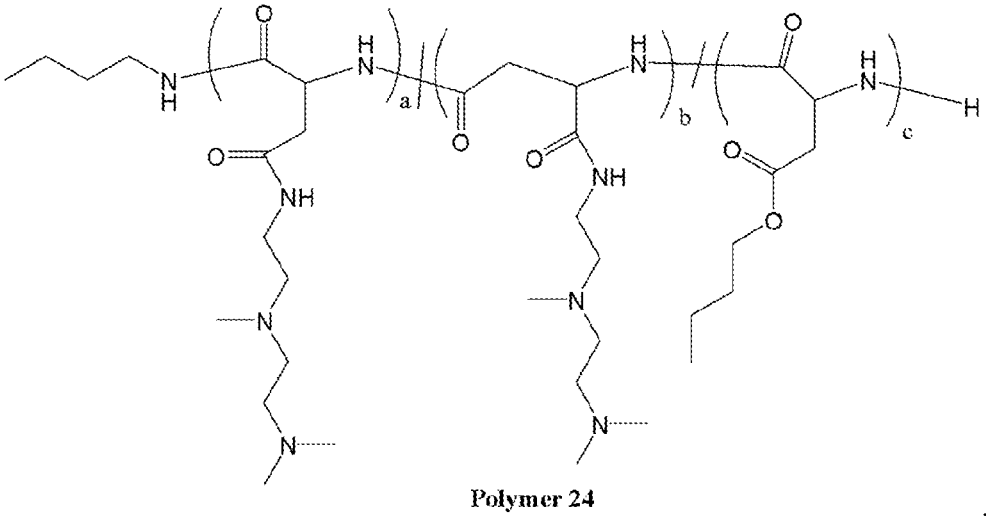
Polymer 21



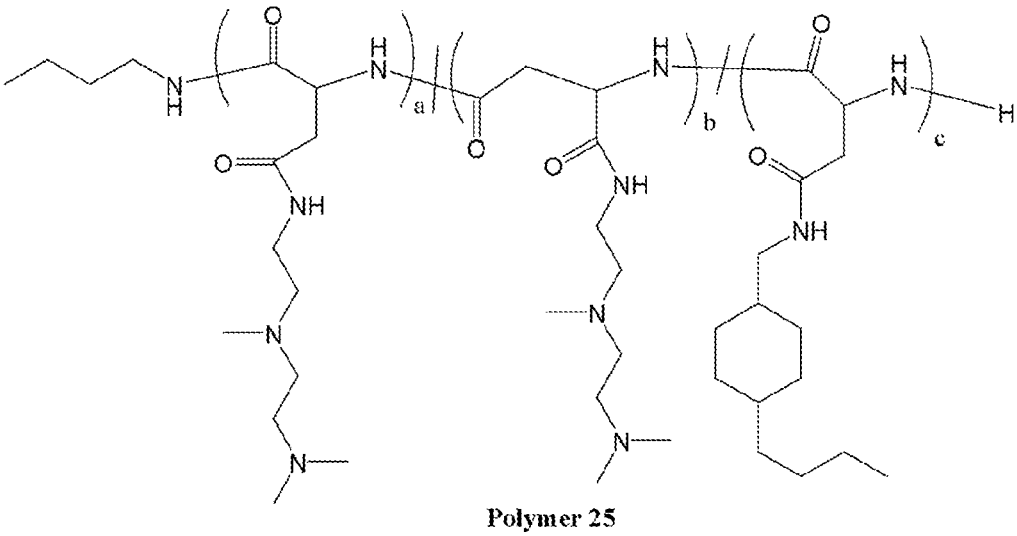
Polymer 22



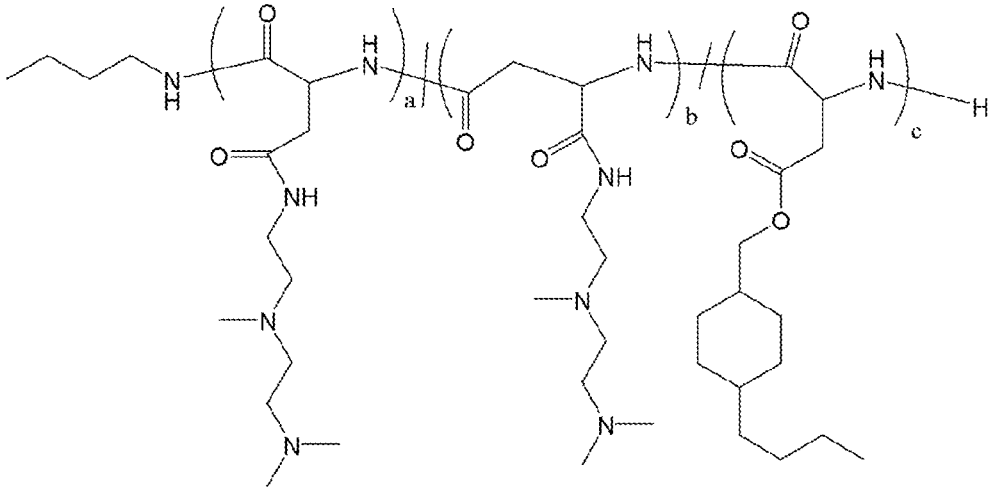
Polymer 23



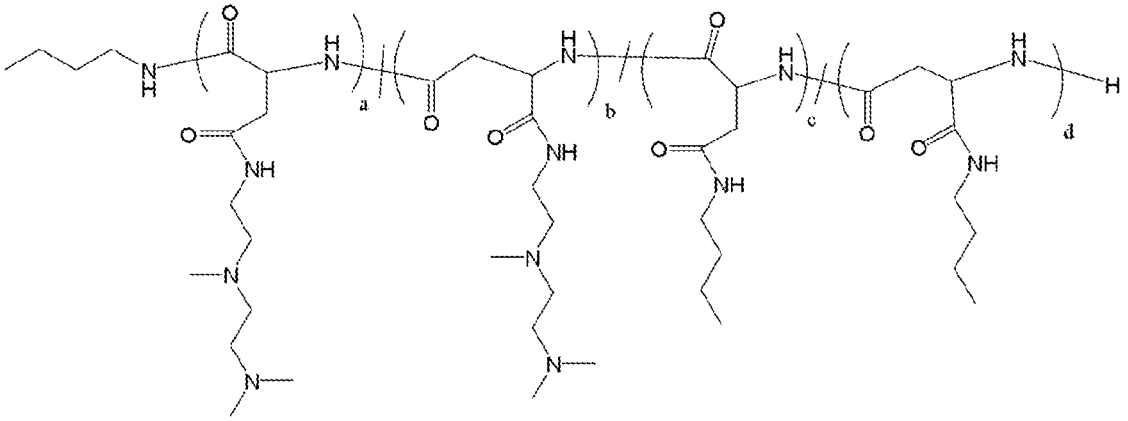
Polymer 24



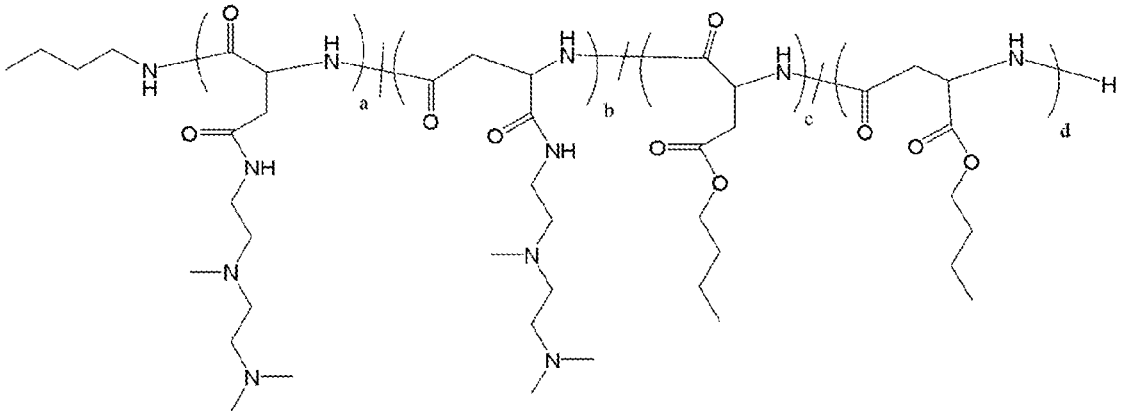
Polymer 25



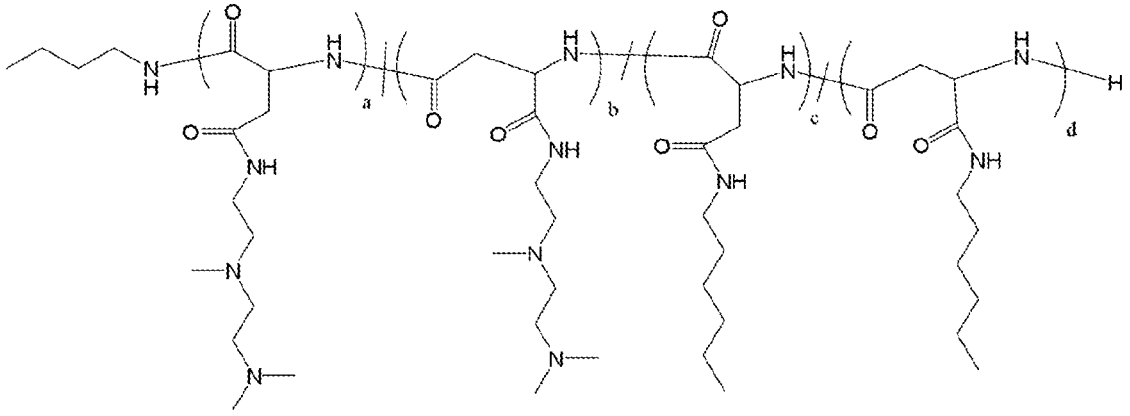
Polymer 26



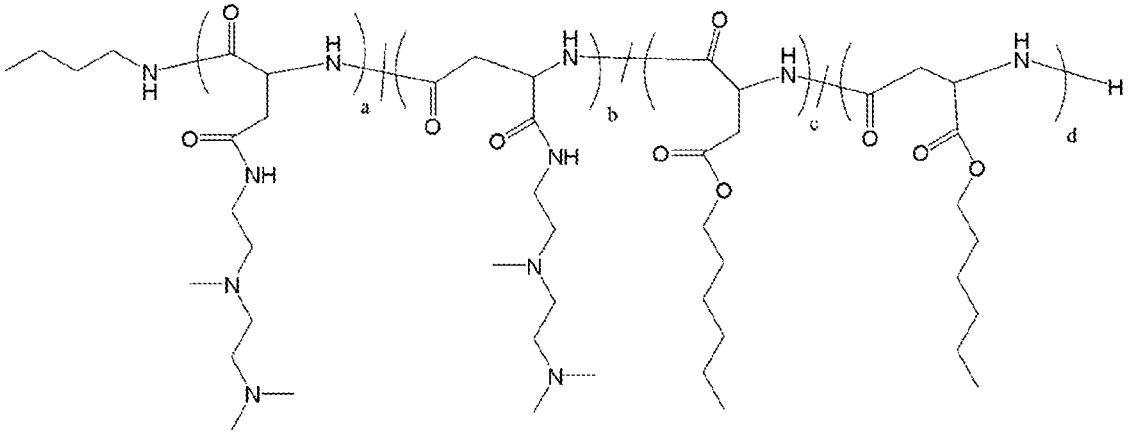
Polymer 27



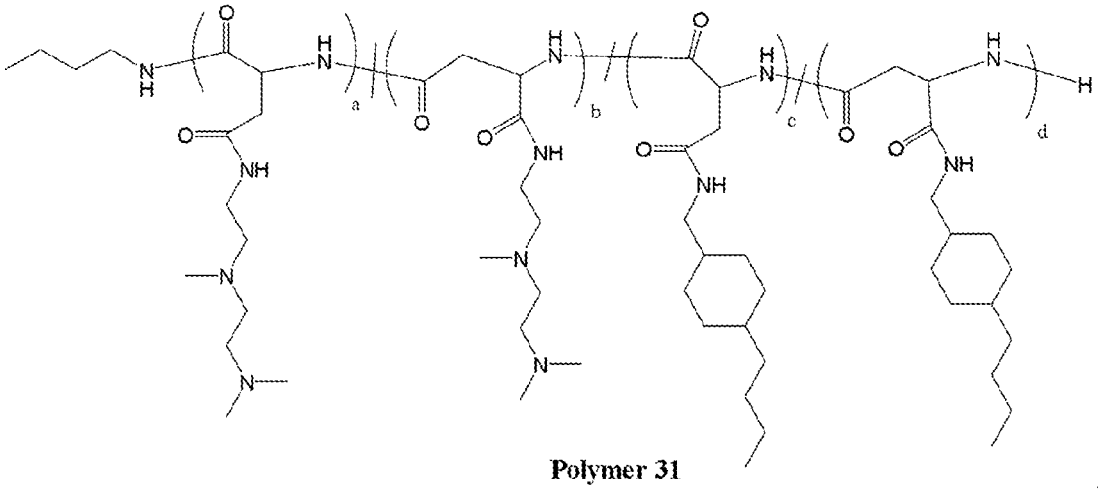
Polymer 28



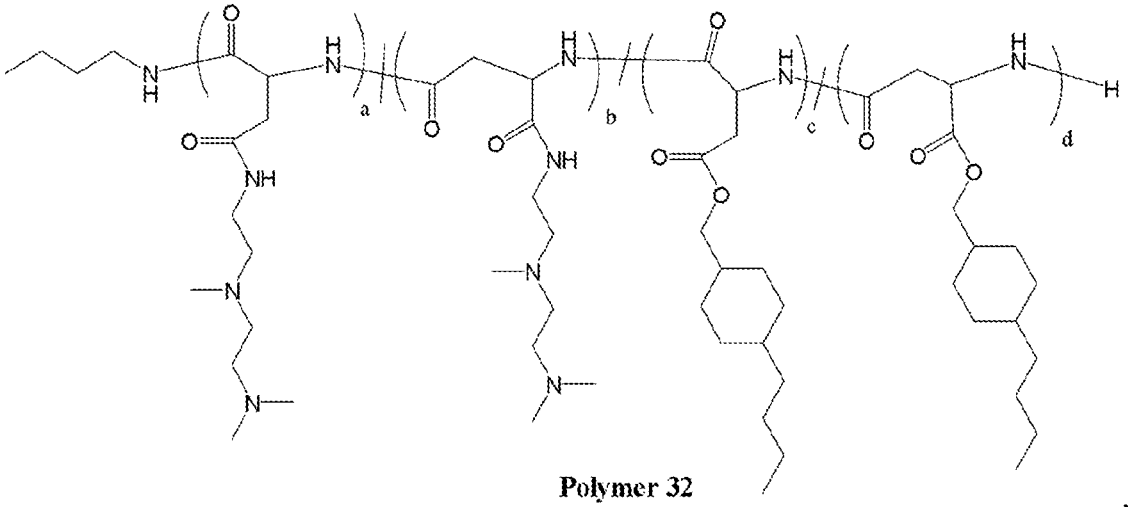
Polymer 29



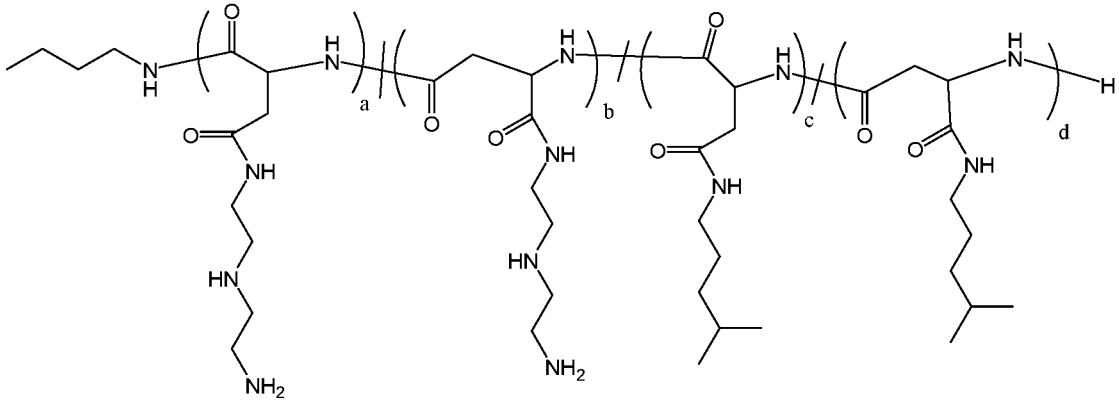
Polymer 30



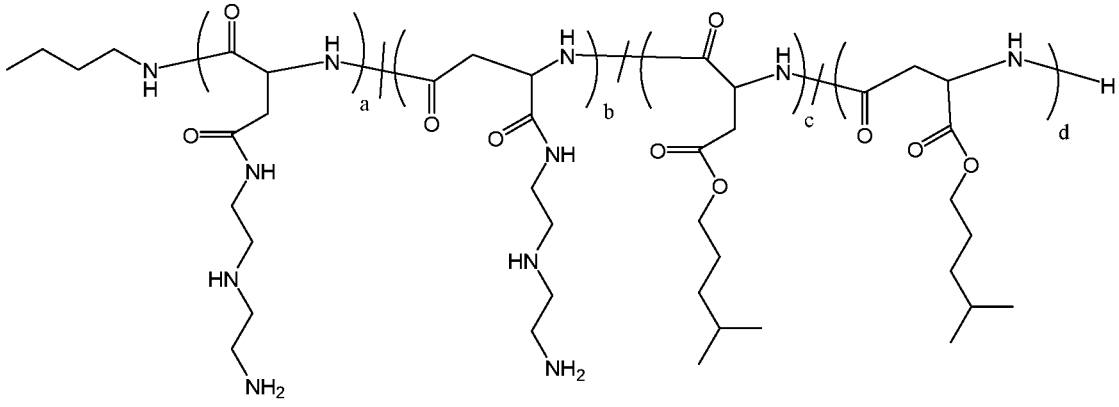
Polymer 31



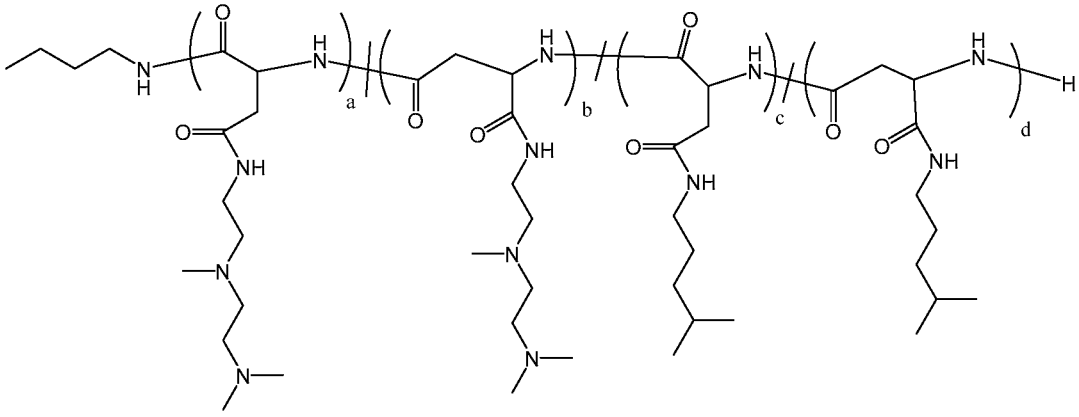
Polymer 32



Polymer 33

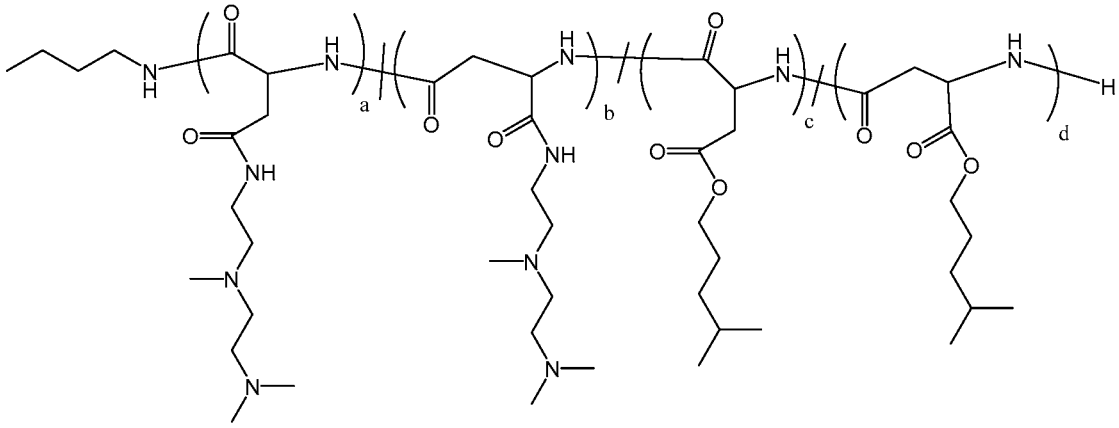


Polymer 34



Polymer 35

, or

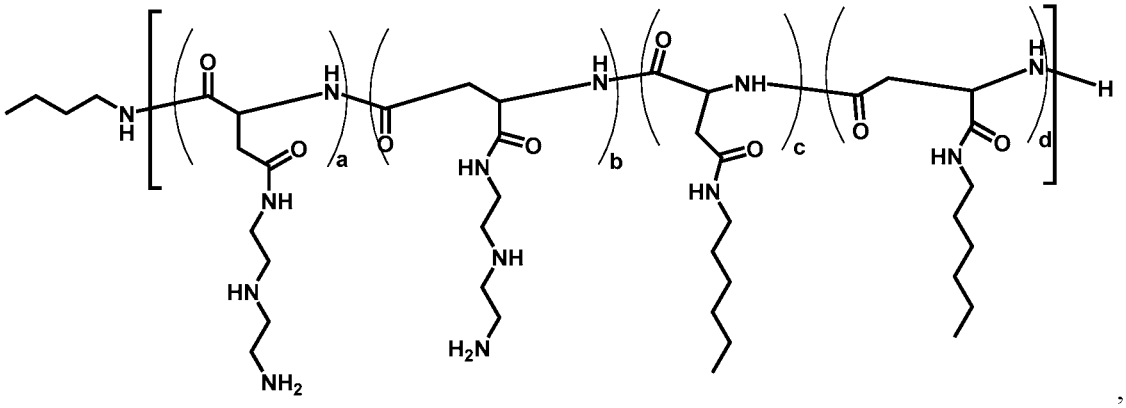


Polymer 36

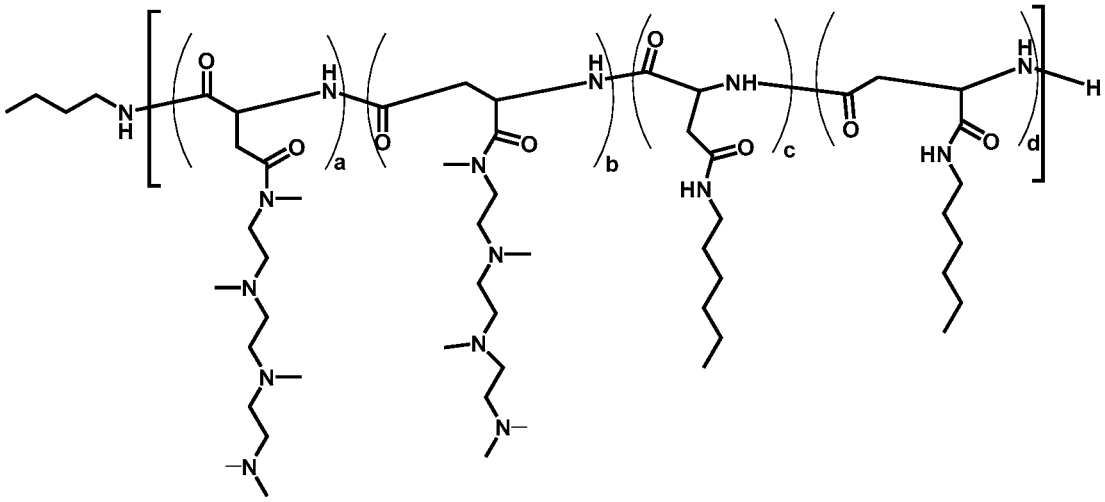
,

wherein (a+b) is from about 5 to about 65 (e.g., about 5 to about 50, about 5 to about 40, about 5 to about 30, about 5 to about 20, or about 5 to about 10) and (c+d) is from about 2 to about 60 (e.g., about 2 to about 50, about 2 to about 40, about 2 to about 30, about 2 to about 20, or about 2 to about 10). In other embodiments, (a+b) is about 45 and (c+d) is about 20. Again, the indication of the number of units ("a", "b", "c", and "d") in these exemplary polymers does not imply a block co-polymer structure; rather, these numbers indicate the number of units overall, which units can be randomly arranged as indicated by the "/" symbols in the formulas.

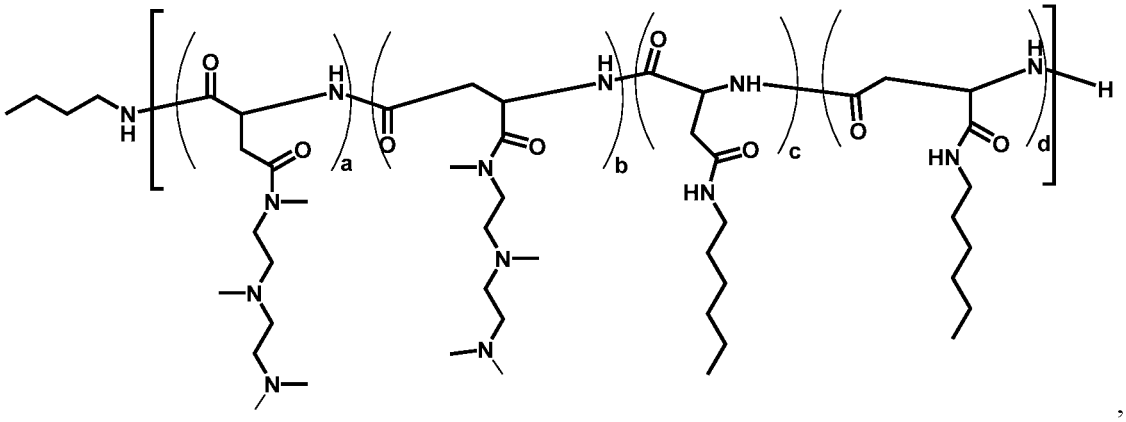
[0146] Additional specific examples of second polymers provided by the present disclosure further include the following:



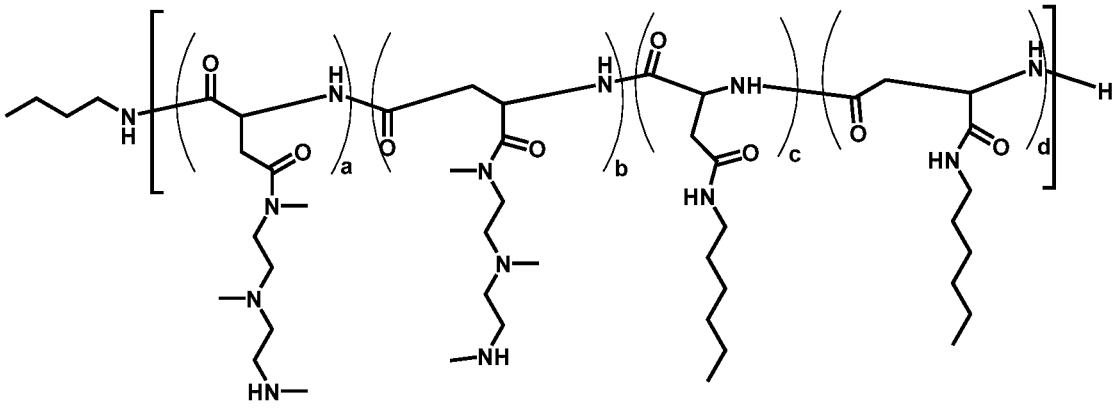
Polymer 37



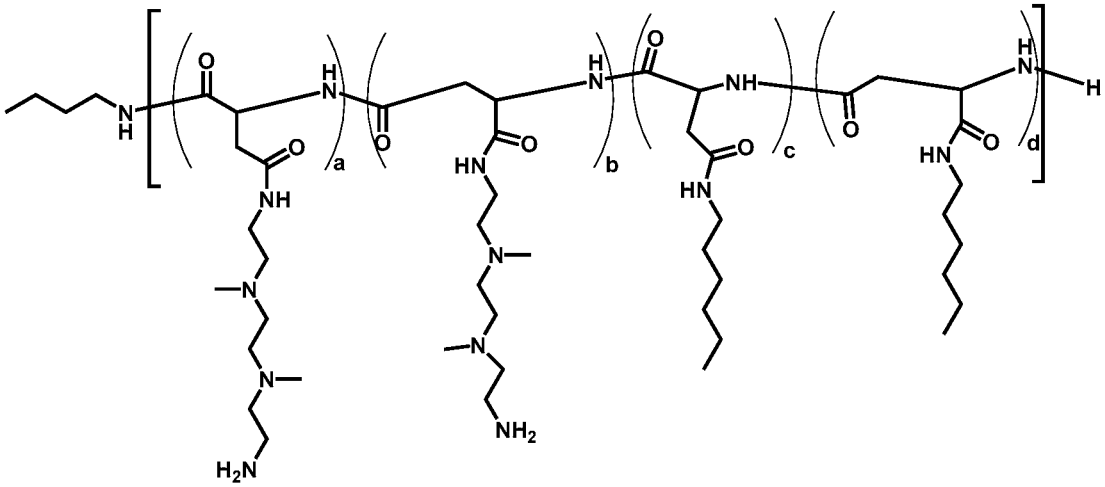
Polymer 38



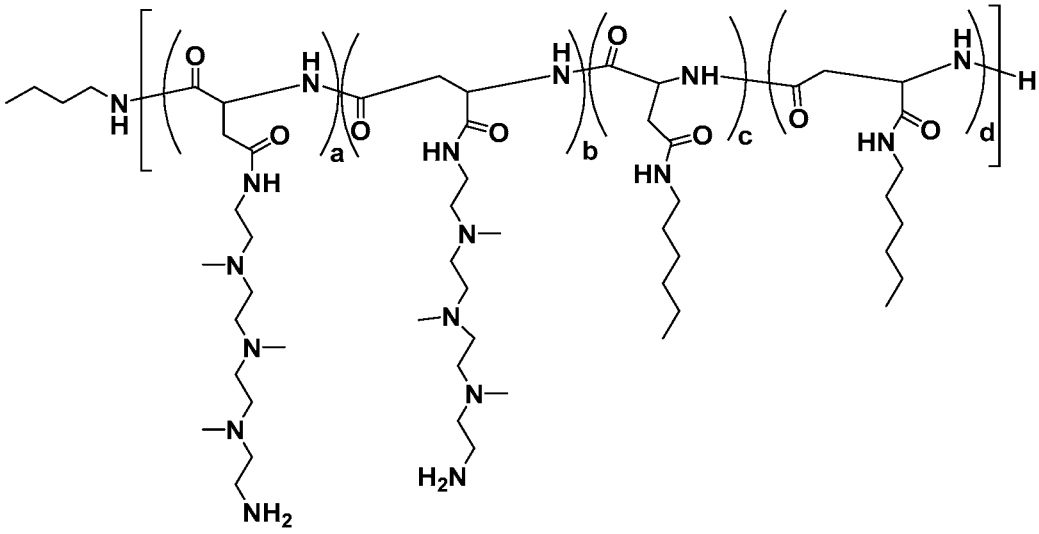
Polymer 39



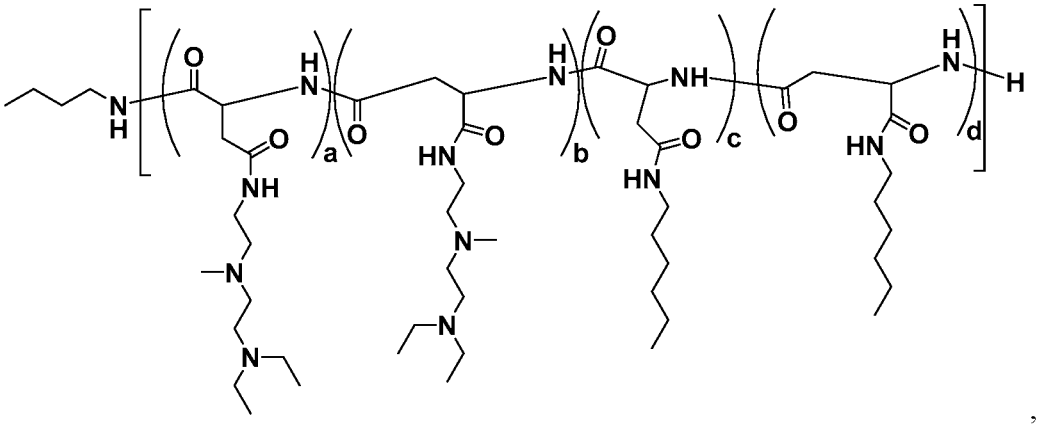
Polymer 40



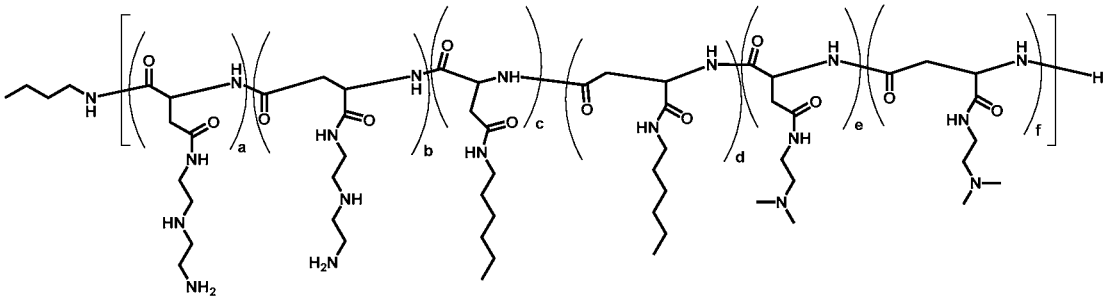
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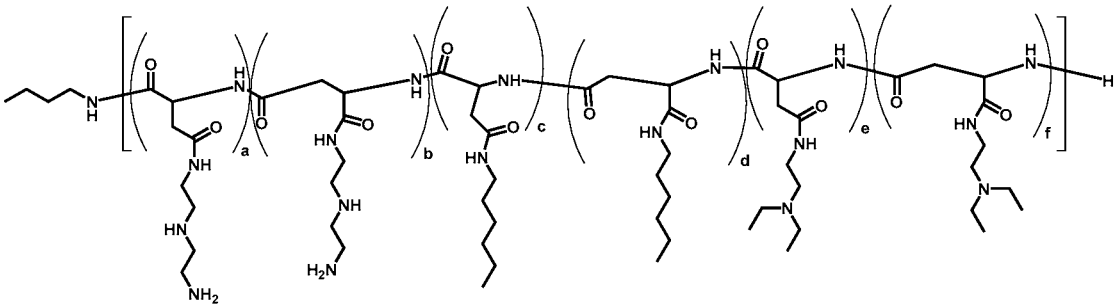
Polymer 42



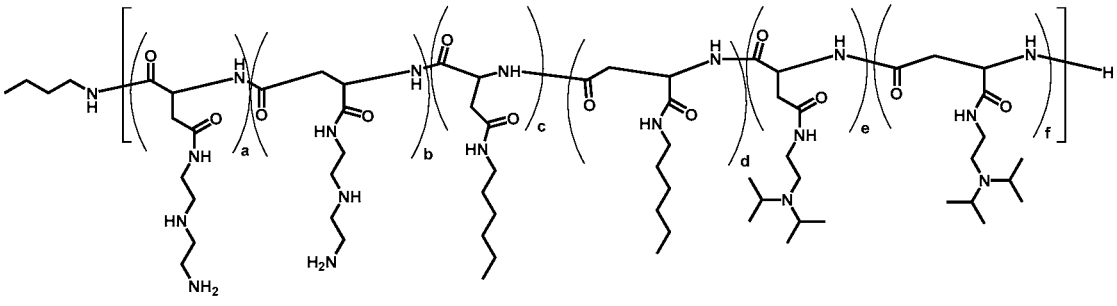
Polymer 43



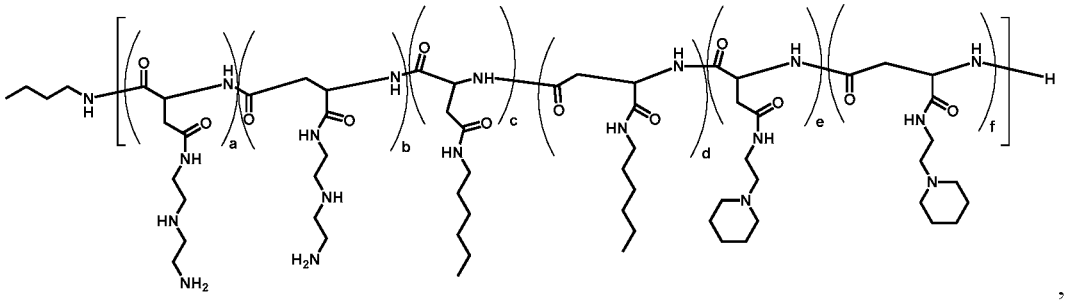
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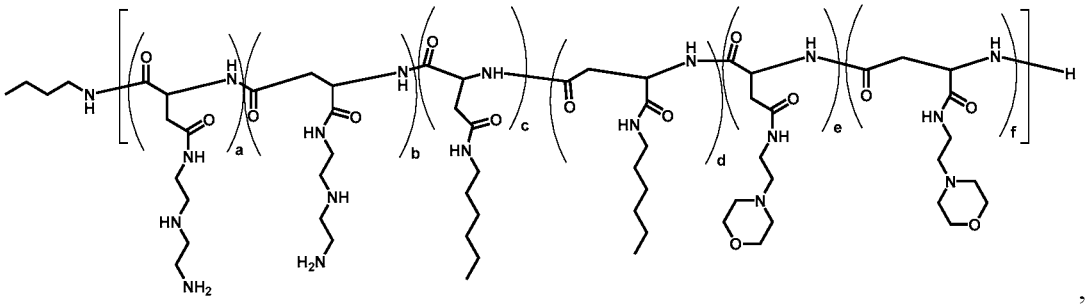
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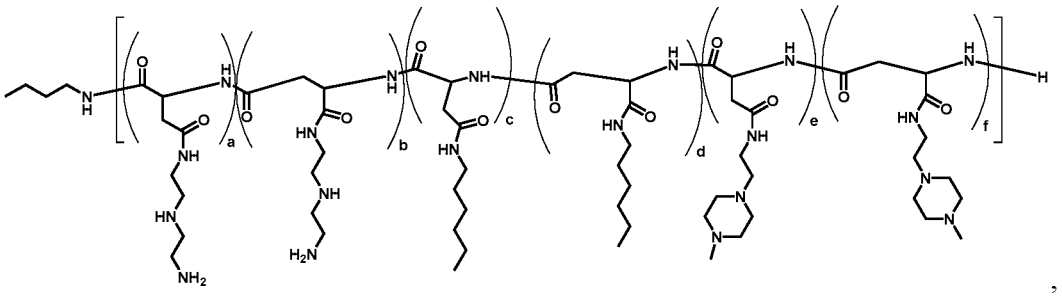
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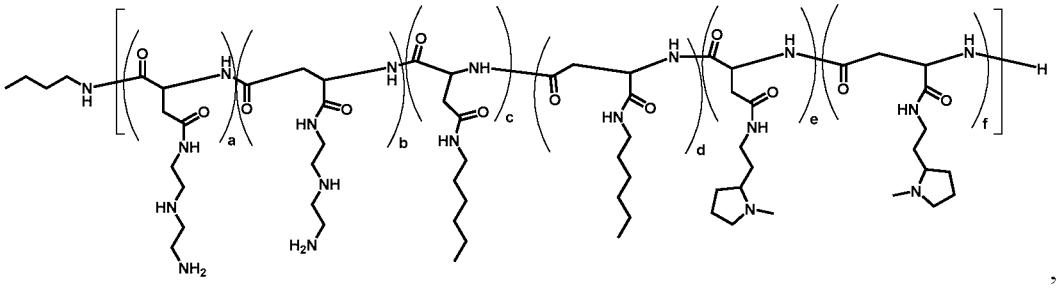
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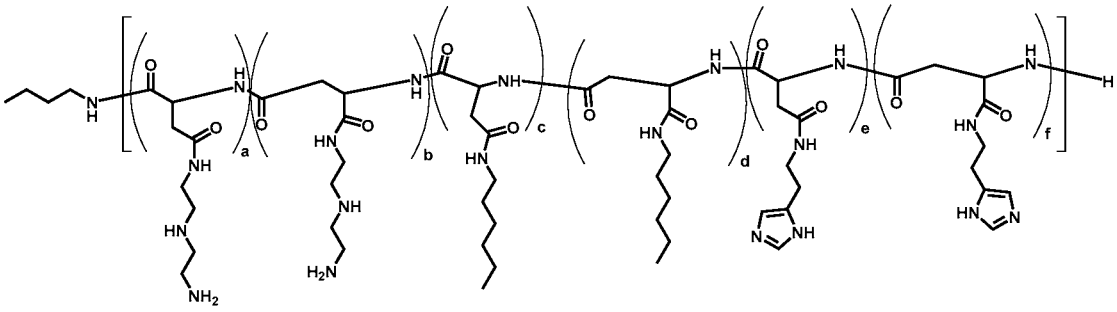
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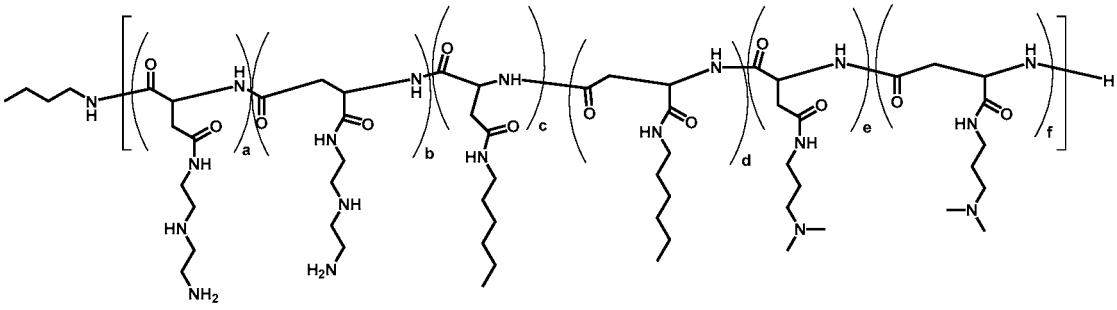
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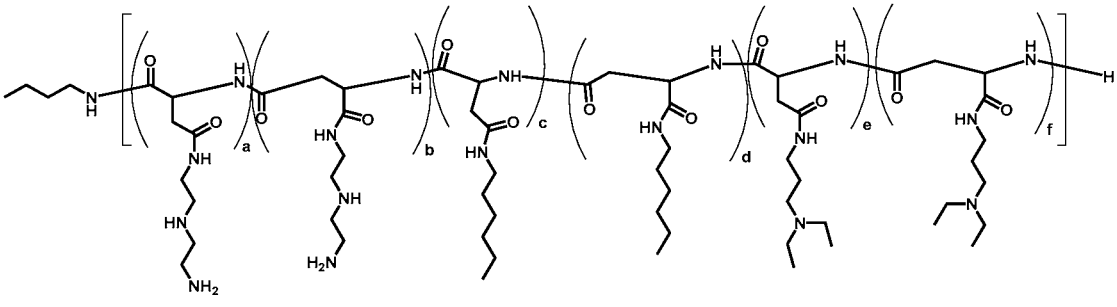
Polymer 50



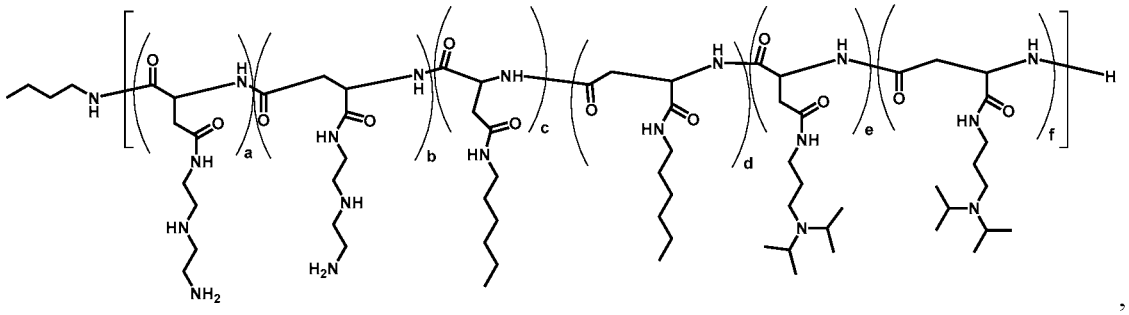
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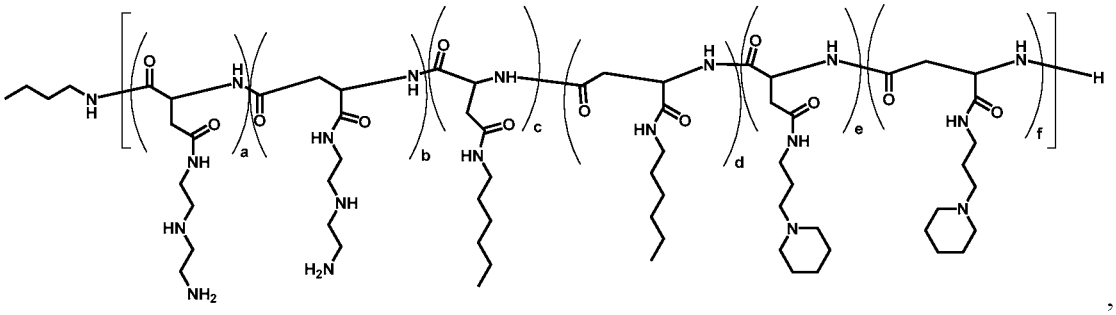
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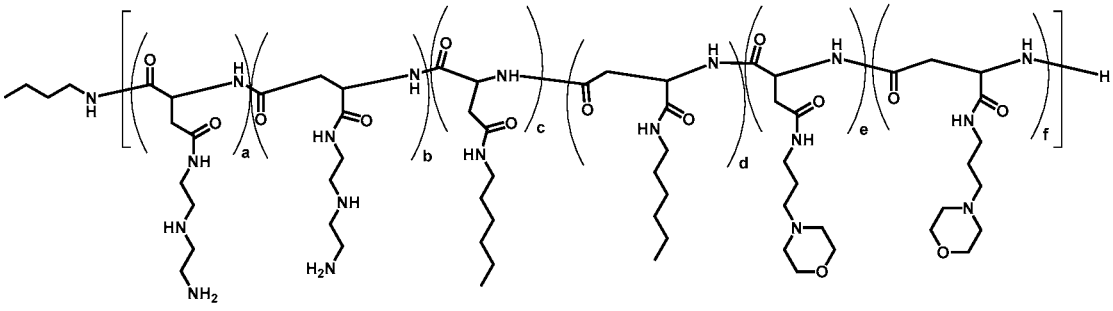
Polymer 53



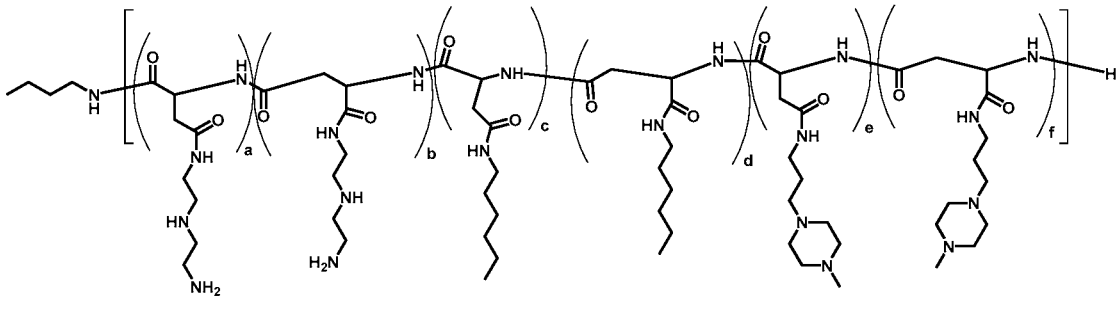
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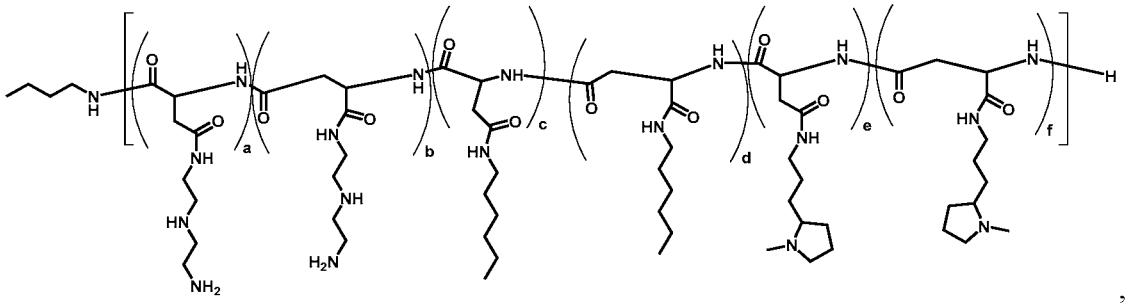
Polymer 55



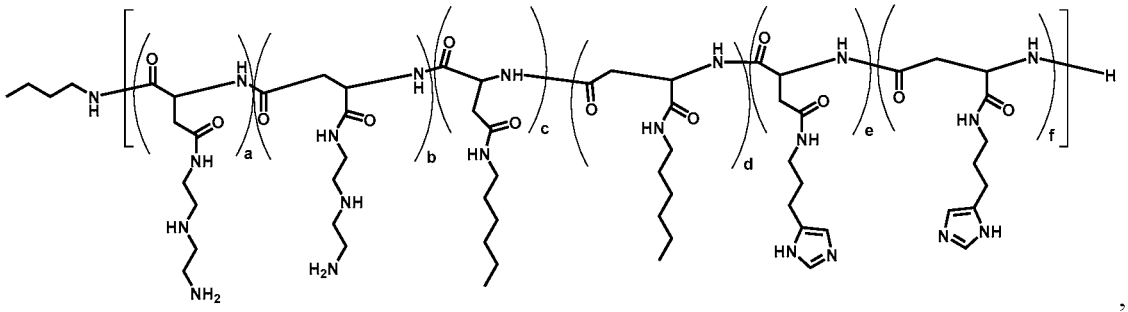
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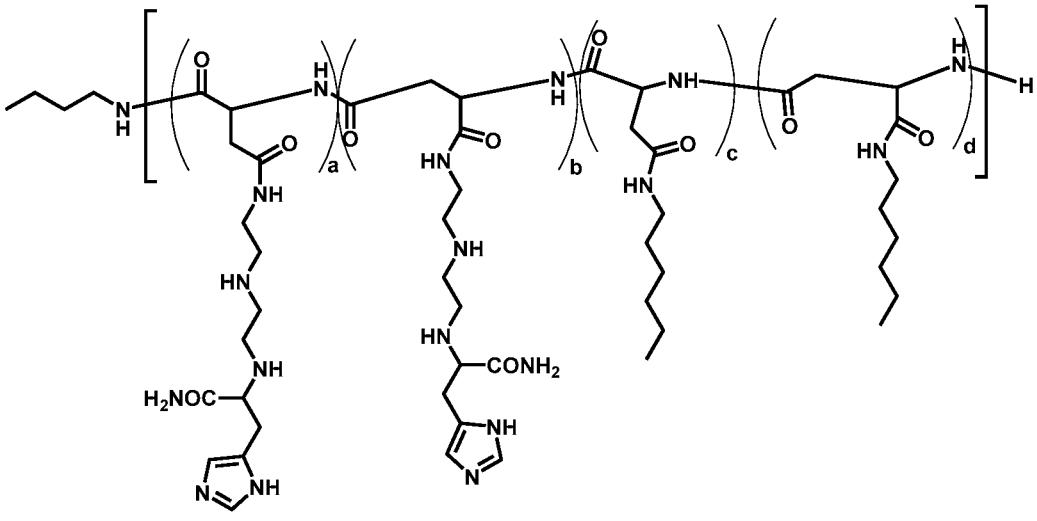
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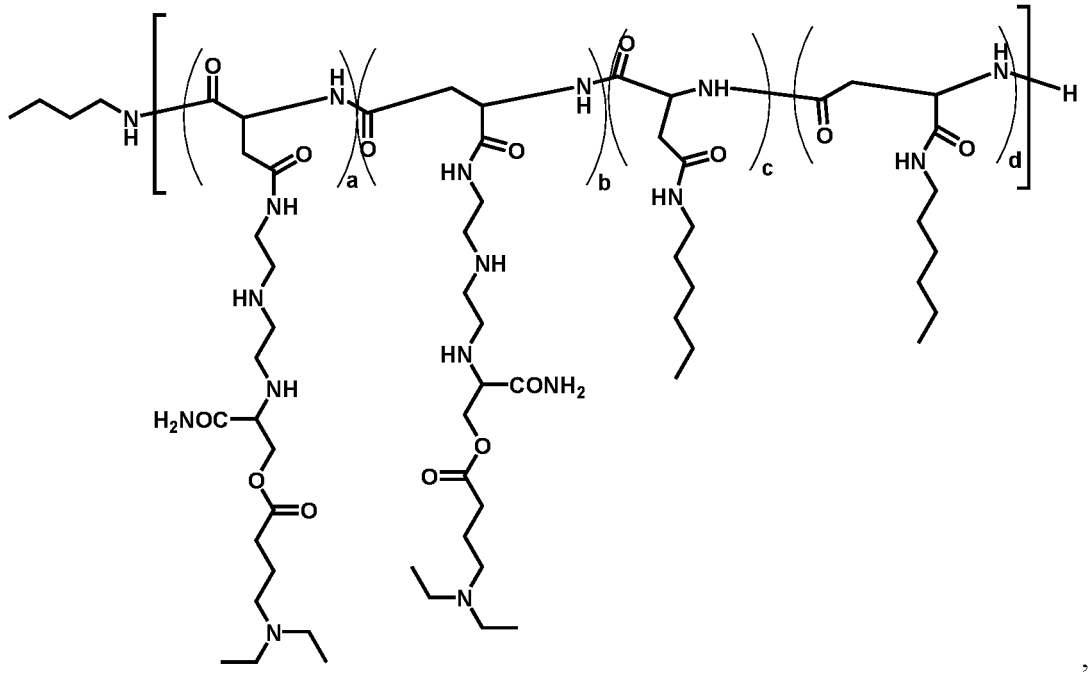
Polymer 58



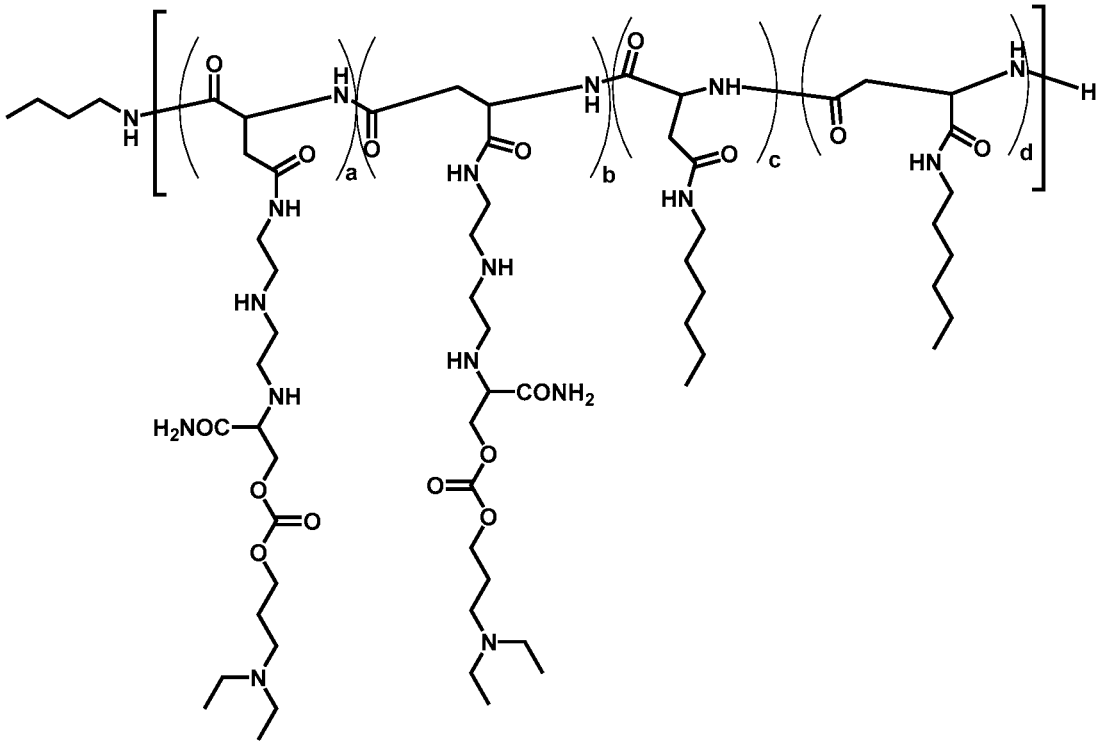
Polymer 59



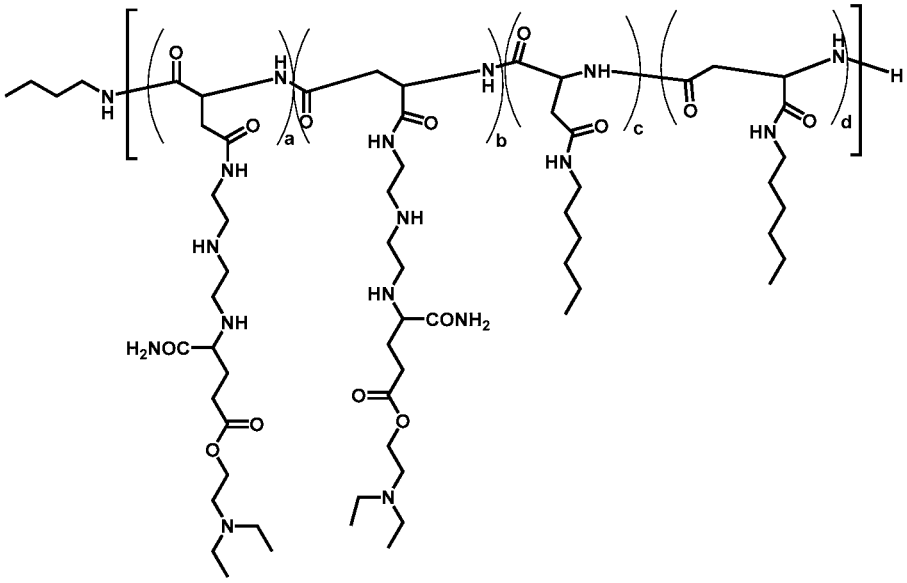
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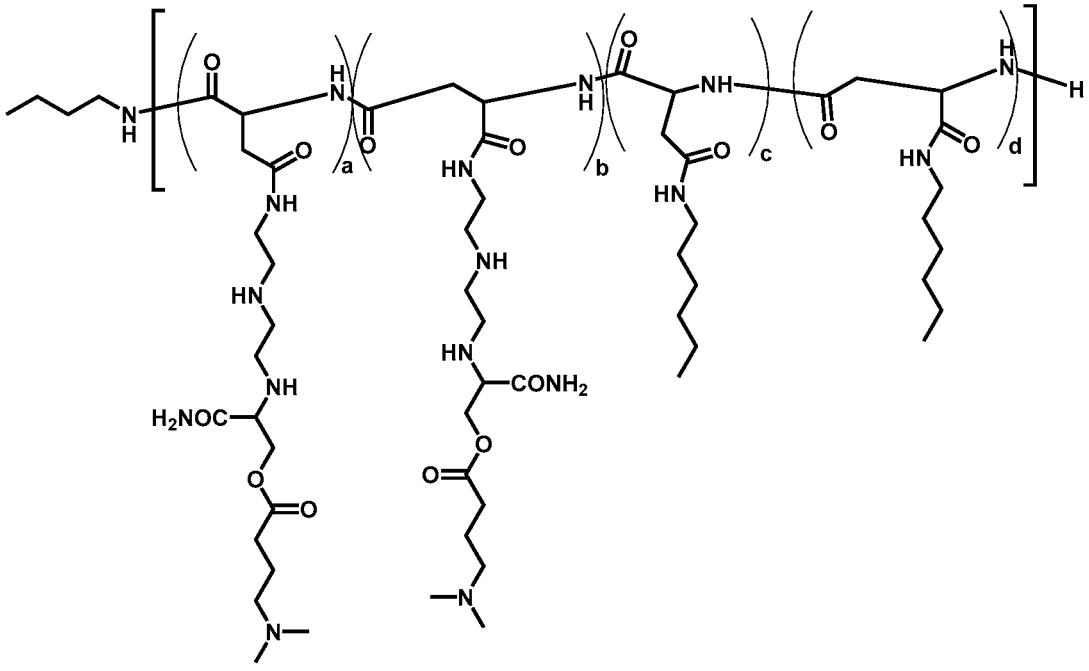
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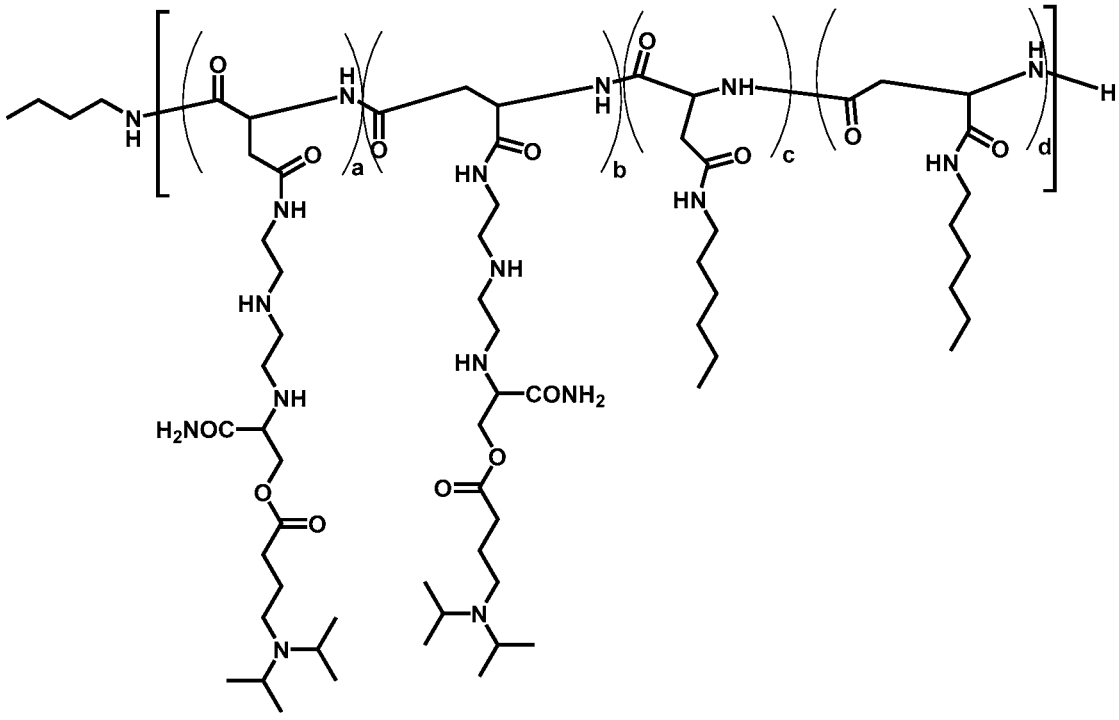
Polymer 62



Polymer 63

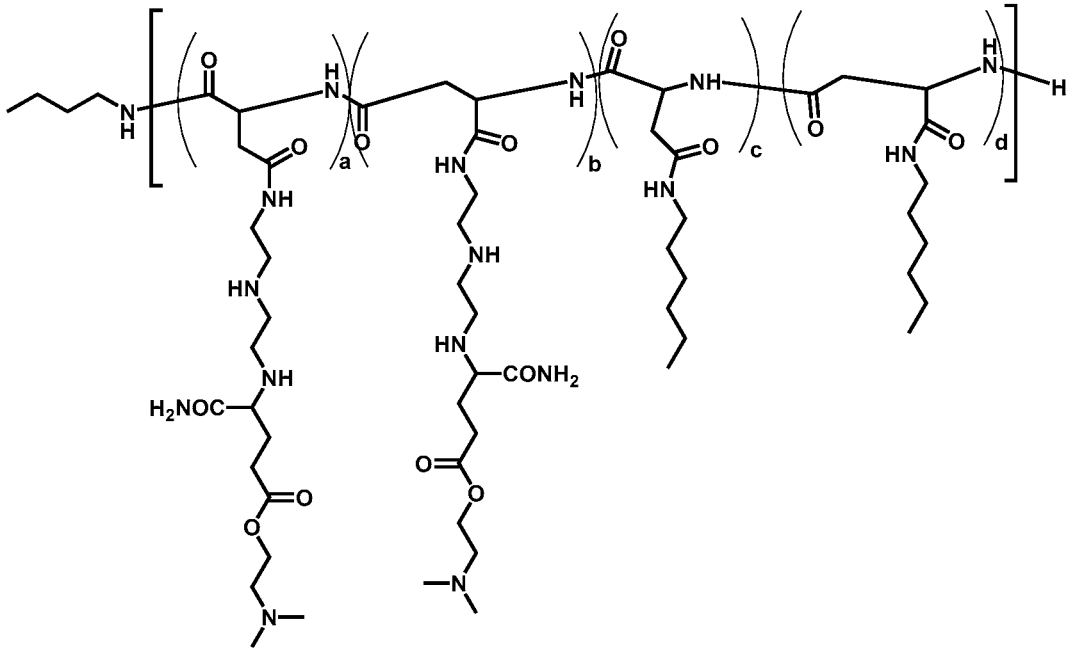


Polymer 64



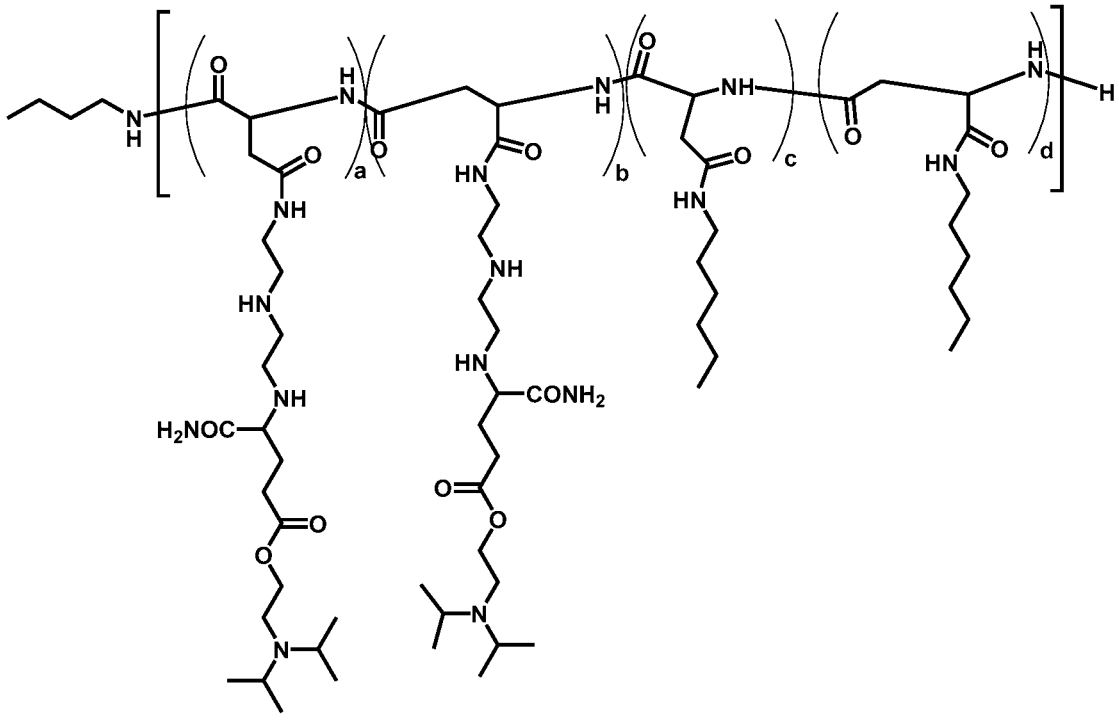
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Polymer 65



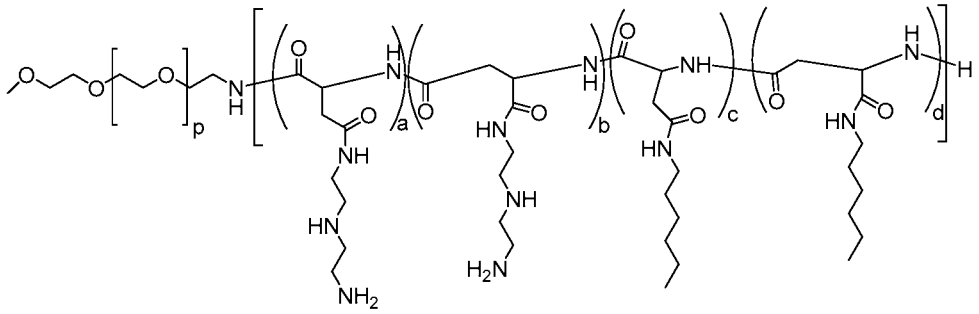
, or

Polymer 66

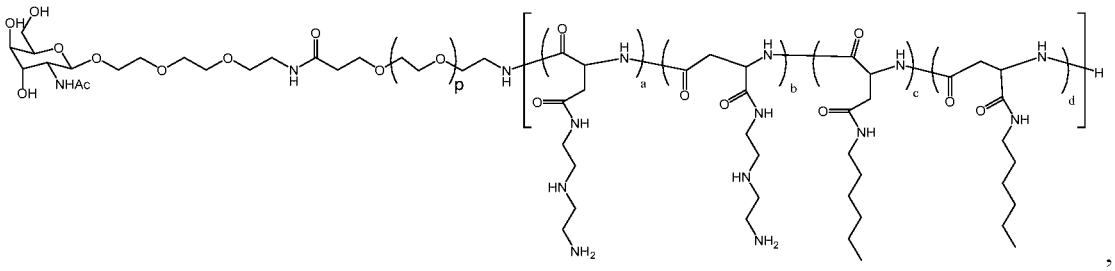


Polymer 67

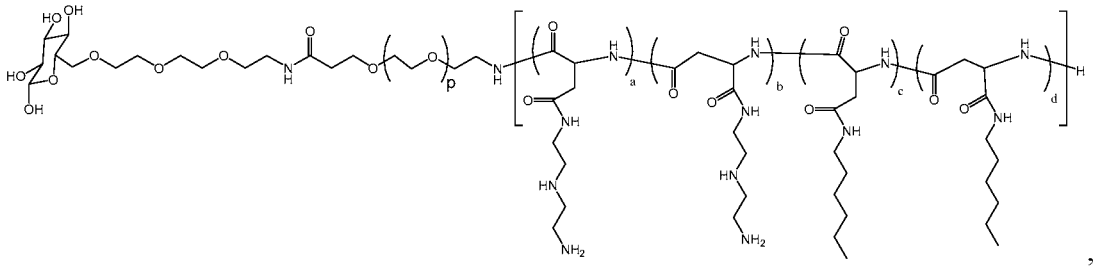
[0147] Further examples of polymers provided herein comprising PEG terminal groups are as follows:



Polymer 68

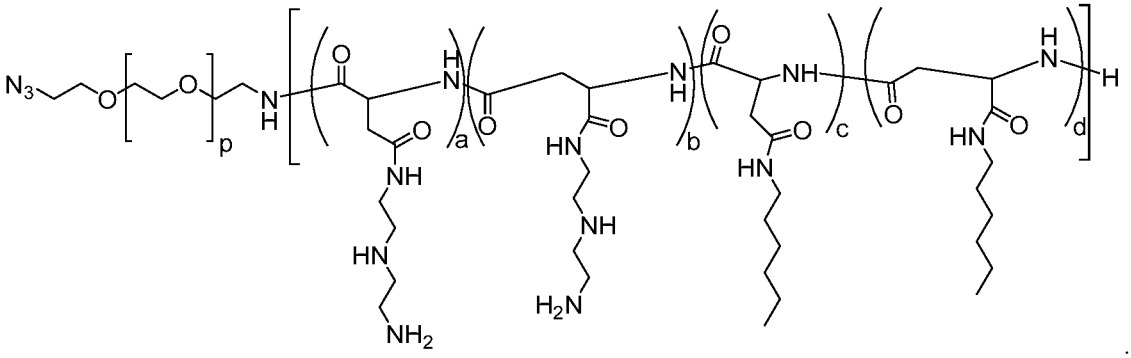


Polymer 69



, or

Polymer 70



Polymer 71

[0148] The indication of the number of units ("a", "b", "c", and "d") in these exemplary polymers does not imply a block co-polymer structure; rather, these numbers indicate the number of particular monomer units overall, which units can be arranged in any order, including blocks of monomers or monomers randomly arranged throughout the polymer. In some instances, but not all, this is additionally indicated by the "/" symbols in the formulas; however, the absence of a "/" should not be taken to mean that the polymers are joined in a particular order. In some embodiments of the foregoing Polymers 1-69, the monomers designated by parenthesis and an integer ("a", "b", "c", or "d") are randomly arranged or dispersed throughout the polymer.

[0149] In any of the foregoing second polymers, (a+b) is from about 5 to about 65 (e.g., about 5 to about 50, about 5 to about 40, about 5 to about 30, about 5 to about 20, or about 5 to about 10) and (c+d) is from about 2 to about 60 (e.g., about 2 to about 50, about 2 to about 40, about 2 to about 30, about 2 to about 20, or about 2 to about 10). In certain embodiments, (a+b) is about 55 and (c+d) is about 10. In other embodiments, (a+b) is about 45 and (c+d) is about 20. In certain embodiments, (a+b+c+d) is about 10-500, such as about 10-400, about 10-200, or about 10-100 (e.g., about 25-100 or about 50-75).

[0150] The second polymer can contain any suitable proportion of (a+b) to (c+d). In other embodiments, (a+b) ranges from 10-95% (e.g., 10-75%, 10-65%, 10-50%, 20-95%, 20-

75%, 20-65%, 20-50%, 30-95%, 30-75%, 30-65%, or 30-50%) of the total number of polymer units (a+b+c+d). In other embodiments, (c+d) ranges from 5-90% of the total number of polymer units (a+b+c+d) (e.g., 5-75%, 5-65%, 5-50%, 5-40%, 5-30%, 10-90%, 10-75%, 10-65%, 10-50%, 10-40%, or 10-30%). In still other embodiments, the ratio of (a+b):(c+d) can be about 1 to about 25, from about 1 to about 20, from about 1 to about 10, from about 1 to about 5, from about 5 to about 25, from about 10 to about 25, or from about 15 to about 25.

[0151] Certain of the above second polymers comprise monomers with ionizable side chains "e" and "f," in which case a, b, c, and d are as described above, and (e+f) is from about 2 to about 60 (e.g., about 2 to about 50, about 2 to about 40, about 2 to about 30, about 2 to about 20, or about 2 to about 10). In addition, each instance of p is independently an integer from 2 to 200 (e.g., 2 to 150, 2 to 100, 2 to 50, 6 to 36, 6 to 30, 6 to 24, 6 to 18, 10 to 200, 10 to 150, 10 to 100, 10 to 50, 25 to 200, 25 to 150, 25 to 100, 25 to 50, 50 to 200, 50 to 150, or 50 to 100). Furthermore, (a+b+c+d+e+f) is about 10-500, such as about 10-400, about 10-200, or about 10-100 (e.g., about 25-100 or about 50-75). Again, the indication of the number of units ("a", "b", "c", "d," "e," and "f") in these exemplary second polymers does not imply a block co-polymer structure; rather, these numbers indicate the number of units overall, which units can be randomly arranged. In some embodiments, the second polymer has the structure of polymer 29, 37, 39, or 40, above, wherein a, b, c, and d are as described herein.

[0152] Some of the above particular examples of second polymers provided by the disclosure are depicted with specific terminal groups (e.g., alkylamino, hydrogen, or PEG); however, any of the foregoing particular structures can comprise different terminal groups. For example, any of the foregoing structures comprise a group of R^1, R^6, or Q as described herein at either or both termini of the polymer backbone.

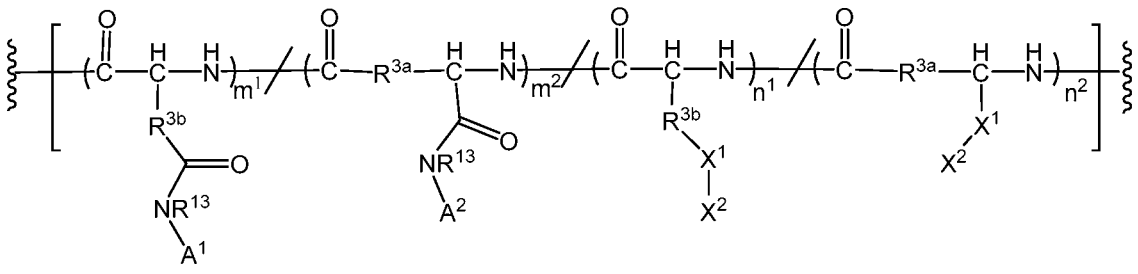
[0153] Typically, the second polymer is cationic (i.e., positively charged at pH 7 and 23 °C). As used herein, "cationic" polymers refer to polymers having an overall net positive charge, whether the polymer comprises only cationic monomer units or a combination of cationic monomer units and non-ionic or anionic monomer units.

[0154] In certain embodiments, the second polymer has a weight average molecular weight of from about 5 kDa to about 2,000 kDa. The second polymer can have a weight average molecular weight of about 2,000 kDa or less, for example, about 1,800 kDa or less, about 1,600 kDa or less, about 1,400 kDa or less, about 1,200 kDa or less, about 1,000 kDa or less, about 900 kDa, or less, about 800 kDa, or less, about 700 kDa or less, about 600 kDa

or less, about 500 kDa or less, about 100 kDa or less, or about 50 kDa or less. Alternatively, or in addition, the second polymer can have a weight average molecular weight of about 10 kDa or more, for example, about 50 kDa or more, about 100 kDa or more, about 200 kDa or more, about 300 kDa or more, or about 400 kDa or more. Thus, the second polymer can have a weight average molecular weight bounded by any two of the aforementioned endpoints. For example, the second polymer can have a weight average molecular weight of from about 10 kDa to about 50 kDa, from about 10 kDa to about 100 kDa, from about 10 kDa to about 500 kDa, from about 50 kDa to about 500 kDa, from about 100 kDa to about 500 kDa, from about 200 kDa to about 500 kDa, from about 300 kDa to about 500 kDa, from about 400 kDa to about 500 kDa, from about 400 kDa to about 600 kDa, from about 400 kDa to about 700 kDa, from about 400 kDa to about 800 kDa, from about 400 kDa to about 900 kDa, from about 400 kDa to about 1,000 kDa, from about 400 kDa to about 1,200 kDa, from about 400 kDa to about 1,400 kDa, from about 400 kDa to about 1,600 kDa, from about 400 kDa to about 1,800 kDa, from about 400 kDa to about 2,000 kDa, from about 200 kDa to about 2,000 kDa, from about 500 kDa to about 2,000 kDa, or from about 800 kDa to about 2,000 kDa. The weight average molecular weight can be determined by any suitable technique. Generally, the weight average molecular weight is determined using size exclusion chromatography equipped with a column, selected from TSKgel Guard, GMPW, GMPW, G1000PW, and a Waters 2414 (Waters Corporation, Milford, Massachusetts) refractive index detector. Moreover, the weight average molecular weight is determined from calibration with polyethylene oxide/polyethylene glycol standards ranging from 150-875,000 Daltons.

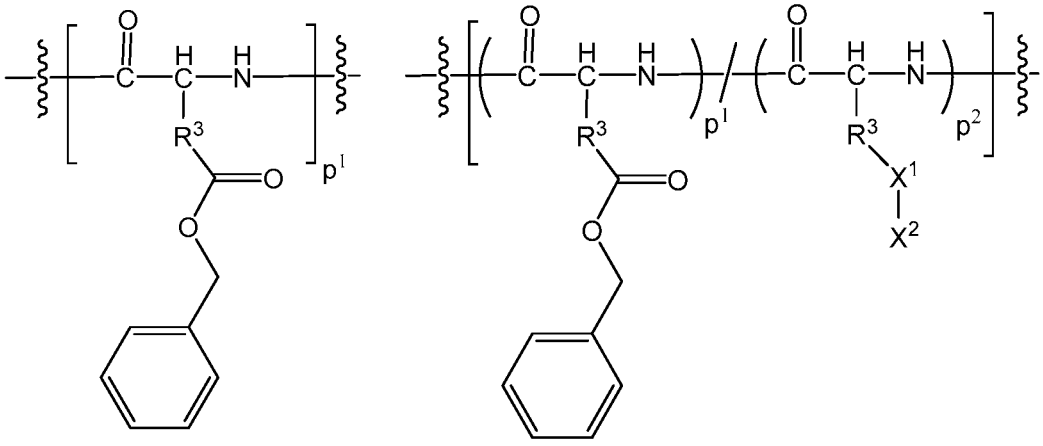
Method of Preparing Second Polymer

[0155] The second polymer can be provided by any suitable method. One example of such a method is provided herein and illustrated in the examples. In some embodiments, the method comprises preparing a polymer of Formula 4:



Formula 4

as described herein from a polymer comprising a structure of Formula 8 or Formula 9:



Formula 8 or Formula 9

wherein,

p^1 is an integer from 1 to 2000 (e.g., from 1 to 1000, from 1 to 500, from 1 to 200, from 1 to 100, from 5 to 2000, from 5 to 1000, from 5 to 500, from 5 to 200, or from 5 to 100);

p^2 is an integer from 1 to 2000 (e.g., from 1 to 1000, from 1 to 500, from 1 to 200, from 1 to 100, from 2 to 2000, from 2 to 1000, from 2 to 500, from 2 to 200, or from 2 to 100);

each R^3 is independently a methylene or ethylene group;

and X^1 and X^2 are as previously described with respect to Formulae 3, 3A-3C, and 4. Thus, for instance, each X^1 independently is —C(O)O—, —C(O)NR^{13}—, —C(O)—, —S(O)(O)—, or a bond; and each instance of X^2 is independently a C_1-C_{12} alkyl or heteroalkyl group, C_3-C_{12} cycloalkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkenyl group, aryl group, heterocyclic group, or combination thereof optionally substituted with one or more substituents. All other embodiments of X^1 and X^2 as previously described with respect to Formulae 3, 3A-3C, and 4 also apply to X^1 and X^2 of Formulae 8 and 9.

[0156] The method comprises combining the structure of Formula 8 or Formula 9 with a compound of formula HNR^{13}A^1 and/or HNR^{13}A^2, and optionally a compound of formula H_2NX^2 or HOX^2. More specifically, the structure of Formula 8 can be combined (reacted) with (a) a compound of formula HNR^{13}A^1 and/or HNR^{13}A^2, and (b) a compound of formula H_2NX^2 or HOX^2, simultaneously or sequentially in any order, to provide the compound of Formula 4. Similarly, the compound of Formula 9, which already includes an X^2 group, can be combined (reacted) with a compound of formula HNR^{13}A^1 and/or HNR^{13}A^2 to provide the compound of Formula 4.

[0157] In the compound of $HNR^{13}A^1$ and/or $HNR^{13}A^2$, each instance of R^{13} is as previously described with respect to the polymers of Formulae 3, 3A-3C, and 4, including any and all embodiments thereof. Thus, for instance, each instance of R^{13} can be independently hydrogen, an aryl group, a heterocyclic group, an alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, any of which can be optionally substituted with one or more substituents.

[0158] Similarly, A^1 and A^2 are as previously described with respect to the polymers of Formulae 3, 3A-3C, and 4. Thus, for instance, A^1 and A^2 are each independently a group of formula

$$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2 ;$$

$$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2{}_2]_2;$$

$$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}; \text{ or}$$

$$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2{}_2]_2\}_2,$$

wherein p1 to p4, q1 to q6, and r1 and r2 are each independently an integer of 1 to 5; and each instance of R^2 is independently hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, or a C_1-C_{12} linear or branched alkyl group optionally substituted with one or more substituents, or R^2 is combined with a second R^2 so as to form a heterocyclic group. In some embodiments, A^1 and A^2 are the same.

[0159] Group X^2 of the compound of formula H_2NX^2 or HOX^2 is as described with respect to Formulae 3, 3A-3C, and 4, including any and all embodiments thereof.

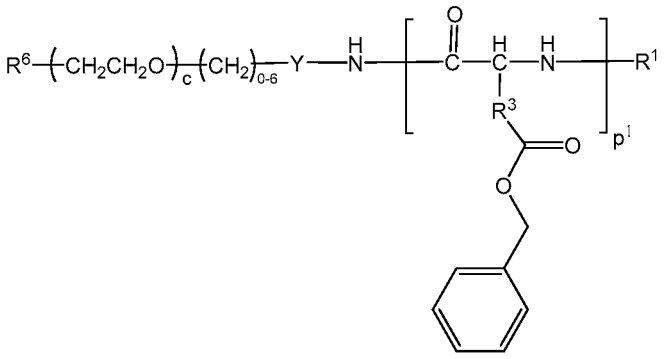
[0160] All other substituents and aspects of Formulae 8, 9, and 4, are as described herein with respect to the polymers of the invention (e.g., Formulae 3, 3A, 3B, 3C, and 4), including any and all embodiments thereof.

[0161] The compounds of $HNR^{13}A^1$ and/or $HNR^{13}A^2$ and of formula H_2NX^2 or HOX^2 can be added to the compound of formula 8 or 9 in any suitable manner and amount depending upon the desired degree of substitution. In some embodiments, about 1-400 equivalents (e.g., about 1-350, 1-300, 1-250, 1-200, 1-150, 1-100, 1-50, 10-400, 10-350, 10-300, 10-250, 10-200, 10-150, 10-100, 10-50, 20-400, 20-350, 20-300, 20-250, 20-200, 20-150, 20-100, 20-50, 30-400, 30-350, 30-300, 30-250, 30-200, 30-150, 30-100, 30-50, 40-400, 40-350, 40-300, 40-250, 40-200, 40-150, 40-100, 40-50, 50-400, 50-350, 50-300, 50-250, 50-200, 50-150, or 50-100 equivalents) of the compound of formula H_2NX^2 or HOX^2 is added to polymer of Formula 8. Also, in some embodiments, about 1-400 equivalents (e.g., about 1-350, 1-300, 1-250, 1-200, 1-150, 1-100, 1-50, 10-400, 10-350, 10-300, 10-250, 10-200, 10-

150, 10-100, 10-50, 20-400, 20-350, 20-300, 20-250, 20-200, 20-150, 20-100, 20-50, 30-400, 30-350, 30-300, 30-250, 30-200, 30-150, 30-100, 30-50, 40-400, 40-350, 40-300, 40-250, 40-200, 40-150, 40-100, 40-50, 50-400, 50-350, 50-300, 50-250, 50-200, 50-150, or 50-100 equivalents) of the compound of formula $HNR^{13}A^1$ and/or $HNR^{13}A^2$ is added to the polymer of Formula 8 or Formula 9.

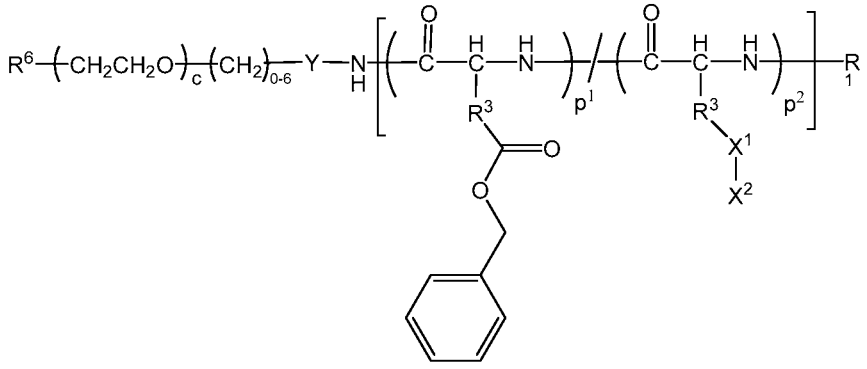
[0162] In embodiments where the method comprises adding a compound of formula $HNR^{13}A^1$ and/or $HNR^{13}A^2$ and a compound of formula H_2NX^2 or HOX^2 to the polymer of Formula 8, the compound of formula $HNR^{13}A^1$ and/or $HNR^{13}A^2$ and the compound of formula H_2NX^2 or HOX^2 can be present in the reaction mixture in any suitable ratio. For example, the compound of formula $HNR^{13}A^1$ and/or $HNR^{13}A^2$ and the compound of formula H_2NX^2 or HOX^2 can be present in a molar ratio of about 150:1 to about 1:150. In some embodiments, a ratio of about 150:1 to about 1:1, such as about 50:1 to about 1:1 (e.g., about 25:1 to about 1:1, about 10:1 to about 1:1, about 5:1 to about 1:1, or about 2.5:1 to about 1:1) is used. In other embodiments, the ratio is about 1:150 to about 1:1, such as about 1:50 to about 1:1 (e.g., about 1:25 to about 1:1, about 1:10 to about 1:1, about 1:5 to about 1:1, or about 1:2.5 to about 1:1). In still other embodiments, the ratio is about 1:10 to about 1:150, about 1:40 to about 1:150, or about 1:80 to about 1:150.

[0163] In some embodiments, the polymer comprising a structure of Formula 8 or Formula 9 is a polymer of Formula 8A or Formula 9A, respectively:



Formula 8A

or



Formula 9A ;

wherein c, Y, R^1, and R^6 are as previously described with respect to the polymers of Formulae 3A and 3B, including any and all embodiments thereof; and p^1, p^2, R^3, X^1, and X^2, are as described above with respect to Formulae 8 and 9. Thus, for instance:

p^1 is an integer from 1 to 2000;

p^2 is an integer from 1 to 2000;

each R^3 is independently a methylene or ethylene group;

each X^1 independently is —C(O)O—, —C(O)NR^{13}—, —C(O)—, —S(O)(O)—, or a bond;

each instance of X^2 is independently a C_1-C_{12} alkyl or heteroalkyl group, C_3-C_{12} cycloalkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkenyl group, aryl group, heterocyclic group, or combination thereof optionally substituted with one or more substituents, or any other embodiments of X^1 and X^2 as previously described with respect to Formulae 3,3A-3C, 4, 8, and 9;

the symbol "/" indicates that the units separated thereby are linked randomly or in any order;

c is an integer from 0 to 50;

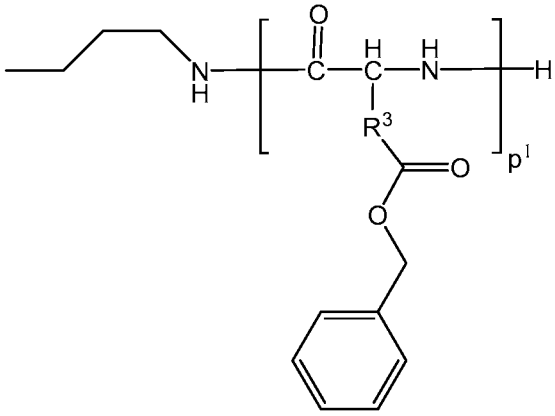
Y is optionally present and is a cleavable linker;

R^1 is hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, or a C_1-C_{12} linear or branched alkyl group optionally substituted with one or more substituents; and

R^6 is hydrogen, an amino group, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, a C_1-C_{12} heteroalkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, a C_1-C_{12} linear or branched alkyl group optionally substituted with one or more amines; or a tissue-specific or cell-specific targeting moiety. All aspects of Formulae 8A and 9A are

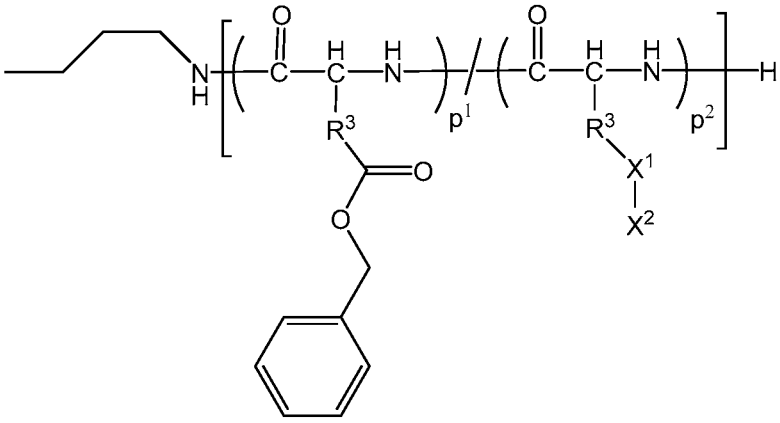
otherwise as described herein with respect to the polymers of the invention (e.g., Formulae 3, 3A, 3B, 3C, 4, 8, and 9).

[0164] In certain embodiments, the polymer comprising a structure of Formula 8 or Formula 9 is a polymer of Formula 8B or Formula 9B, respectively:



Formula 8B

or



Formula 9B ;

wherein p^1, p^2, R^3, X^1, and X^2, are as described above with respect to Formulae 8, 8A, 9, and 9A. Thus, for instance,

p^1 is an integer from 1 to 2000;

p^2 is an integer from 1 to 2000;

each R^3 is independently a methylene or ethylene group;

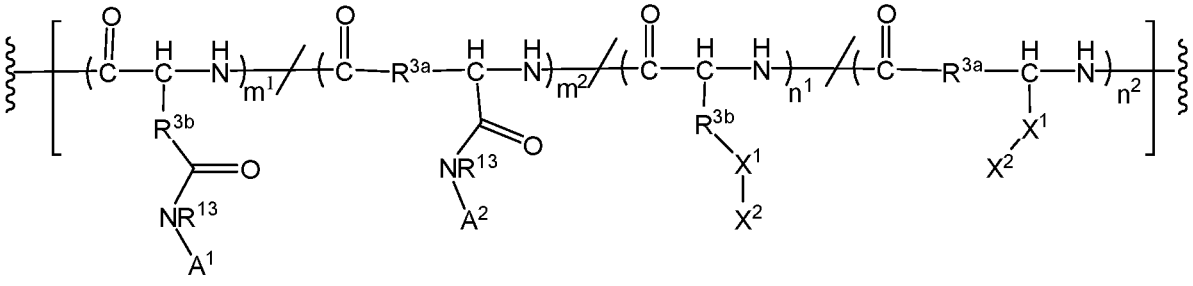
each X^1 independently is —C(O)O—, —C(O)NR^{13}—, —C(O)—, —S(O)(O)—, or a bond;

each instance of X^2 is independently a C_1-C_{12} alkyl or heteroalkyl group, C_3-C_{12} cycloalkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkenyl group, aryl group, heterocyclic group, or combination thereof optionally substituted with one or more substituents, or any

other embodiments of X^1 and X^2 as previously described with respect to Formulae 3, 3A-3C, 4, 8, and 9; and

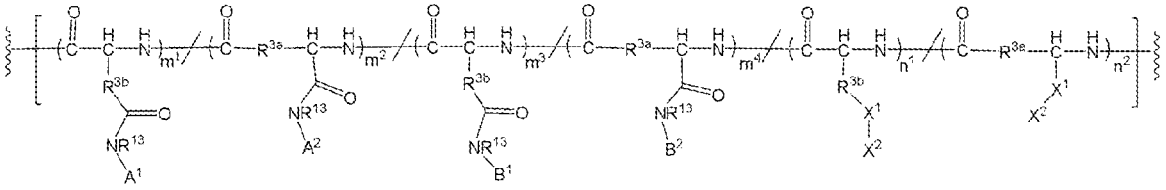
the symbol "/" indicates that the units separated thereby are linked randomly or in any order. All aspects of Formulae 8B and 9B, are otherwise as described herein with respect to the polymers of the invention (e.g., Formulae 3A-3C, 4, 8, 8A, 9 and 9A).

[0165] In some embodiments, the method also provides a method of preparing a second polymer comprising a structure of Formula 3, which comprises modifying at least a portion of groups A^1 and/or A^2 of a polymer comprising a structure of Formula 4:



Formula 4

to produce a polymer comprising a structure of Formula 3:



All aspects of the polymers of Formula 3 and 4 are as previously disclosed herein. Thus, for instance:

each of m^1, m^2, m^3, and m^4 is an integer from 0 to 1000, provided that the sum of $m^1 + m^2 + m^3 + m^4$ is greater than 5;

each of n^1 and n^2 is an integer from 0 to 1000, provided that the sum of $n^1 + n^2$ is greater than 2;

the symbol "/" indicates that the units separated thereby are linked randomly or in any order;

each instance of R^{3a} is independently a methylene or ethylene group;

each instance of R^{3b} is independently a methylene or ethylene group;

each X^1 independently is —C(O)O—, —C(O)NR^{13}—, —C(O)—, —S(O)(O)—, or a bond;

each instance of R^{13} is independently hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl group, any of which can be optionally substituted with one or more substituents;

each instance of X^2 is independently a C_1-C_{12} alkyl or heteroalkyl group, C_3-C_{12} cycloalkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkenyl group, aryl group, heterocyclic group, or combination thereof optionally comprising one or more primary, secondary, or tertiary amines; any of which are optionally substituted with one or more substituents;

A^1 and A^2 are each independently a group of formula

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2{}_2]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}$; or

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2{}_2]_2\}_2$,

B^1 and B^2 are each independently

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^4\text{-}R^5]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^4\text{-}R^5\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^4\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^5]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^5\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[N\{(CH_2)_{s1}\text{-}R^4\text{-}R^5\}—(CH_2)_{q1}—]_{r1}NR^2{}_2$;

$—(CH_2)_{p1}—[N\{(CH_2)_{s1}\text{-}R^5\}—(CH_2)_{q1}—]_{r1}NR^2{}_2$,

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH(CONH_2)\text{-}(CH_2)_{s1}\text{-}R^5$; or

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH(CONH_2)\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5$,

wherein p1 to p4, q1 to q6, r1 and r2, and s1 to s4 are each independently an integer of 1 to 5; each instance of R^2 is independently hydrogen or a C_1-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, or R^2 is combined with a second R^2 so as to form a heterocyclic group; each instance of R^4 is independently -C(O)O-, -C(O)NH-, -O-C(O)O-, or -S(O)(O)-; and each instance of R^5 is independently an alkyl group, cycloalkyl group, alkenyl

group, cycloalkenyl group, aryl group, heteroalkyl group, heterocyclic group, or combination thereof optionally comprising from 2 to 8 tertiary amines or a substituent comprising a tissue-specific or cell-specific targeting moiety. All aspects of Formulae 3, 3A-3C, and 4, are otherwise as described herein with respect to the polymers of the invention, including any and all embodiments of the structures of Formulae 3, 3A-3C, and 4 described herein.

[0166] The polymer comprising a structure of Formula 3 or Formula 4 can be any second polymer described herein, including Formulae 3A, 3B, and 3C, as well as any and all embodiments thereof as described with respect to the second polymer of the invention.

[0167] The groups designated A^1 and/or A^2 of the polymer of Formula 4 can be modified by any suitable means to produce groups designated B^1 and/or B^2. For example, the groups designated A^1 and/or A^2 can be modified by a Michael addition reaction, an epoxide opening, or a displacement reaction. In preferred embodiments, the groups designated A^1 and/or A^2 are modified by a Michael addition reaction.

[0168] In one embodiment, groups A^1 and/or A^2 of the polymer comprising a structure of Formula 4 are modified by a Michael addition reaction between the polymer comprising the structure of Formula 4 and α,β-unsaturated carbonyl compound. As used herein, the term "Michael addition" refers to a nucleophilic addition of a nucleophile of the polymer (e.g., a carbanion, an oxygen anion, a nitrogen anion, an oxygen atom, a nitrogen atom, or a combination thereof) to an α,β-unsaturated carbonyl compound. Accordingly, the Michael addition reaction is between the polymer comprising the structure of Formula 4 and an α,β-unsaturated carbonyl compound. In some embodiments, the nucleophile of the polymer is a nitrogen anion, a nitrogen atom, or a combination thereof.

[0169] The α,β-unsaturated carbonyl compound can be any α,β-unsaturated carbonyl compound capable of accepting a Michael addition from a nucleophile. In some embodiments, the α,β-unsaturated carbonyl compound is an acrylate, an acrylamide, a vinyl sulfone, or a combination thereof. Accordingly, the Michael addition reaction can be between the polymer comprising the structure of Formula 4 and an acrylate, an acrylamide, a vinyl sulfone, or a combination thereof. Thus, in some embodiments, the method comprises contacting the polymer comprising the structure of Formula 4 and an acrylate; contacting the polymer comprising the structure of Formula 4 and an acrylamide; or contacting the polymer comprising the structure of Formula 4 and a vinyl sulfone.

[0170] In embodiments where the groups designated A^1 and/or A^2 are modified by a Michael addition reaction, they produce groups designated B^1 and/or B^2 of the formula:

$$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5;$$

—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—NR^2-$(CH_2)_{s2}$-R^4-R^5]$_2$;

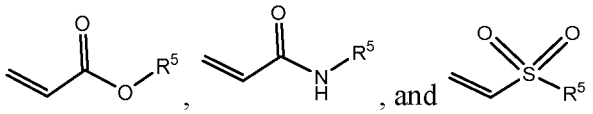
—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—NR^2_2][—$(CH_2)_{q4}$—NR^2—]$_{r2}$$(CH_2)_{s3}$-$R^4$-$R^5$};

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-$(CH_2)_{s4}$-R^4-R^5]$_2$}$_2$; or

—$(CH_2)_{p1}$—[N{$(CH_2)_{s1}$-R^4-R^5}—$(CH_2)_{q1}$—]$_{r1}$$NR^2_2$;

wherein p1 to p4, q1 to q6, r1 and r2, and s1 to s4 are each independently an integer of 1 to 5; each instance of R^2 is independently hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, or a C_1-C_{12} linear or branched alkyl group optionally substituted with one or more substituents, or R^2 is combined with a second R^2 so as to form a heterocyclic group; each instance of R^4 is independently -C(O)O-, -C(O)NH-, -O-C(O)O-, or -S(O)(O)-; and each instance of R^5 is independently an alkyl group, cycloalkyl group, alkenyl group, cycloalkenyl group, aryl group, heteroalkyl group, heterocyclic group, or combination thereof optionally comprising from 2 to 8 tertiary amines or a substituent comprising a tissue-specific or cell-specific targeting moiety.

[0171] Examples of acrylates, acrylamides, and vinyl sulfones suitable for use include an acrylate of the formula:



, , and

wherein R^5 is as described with respect to any of Formulae 3, 3A, 3B, or 3C.

[0172] In some embodiments, the Michael addition reaction is facilitated by an acid and/or base. The acid and/or base can be any suitable acid and/or base with any suitable pKa. The acid and/or base can be an organic acid (e.g., *p*-toluenesulfonic acid), organic base (e.g., triethylamine), inorganic acid (e.g., titanium tetrachloride), inorganic base (e.g., potassium carbonate), or a combination thereof.

[0173] In some embodiments, the Michael addition reaction is facilitated by an acid. The acid can be a Brønsted acid or a Lewis acid. In embodiments where the acid is a Brønsted acid, the acid can be a weak acid (i.e., a pKa of from about 4 to about 7) or a strong acid (i.e., a pKa of from about -2 to about 4). Typically, the acid is a weak acid. In certain embodiments, the acid is a Lewis acid. For example, the acid can be bis(trifluoromethanesulfon)imide or p-toluenesulfonic acid.

[0174] In some embodiments, the Michael addition reaction is facilitated by a base. The base can be a weak base (i.e., a pKa of from about 7 to about 12) or a strong base (i.e., a pKa of from about 12 to about 50). Typically, the base is a weak base. For example, the base can

be triethylamine, diisopropylethylamine, pyridine, N-methyl morpholine, or N,N-dimethyl-piperazine, or derivatives thereof.

[0175] In some embodiments, the Michael addition reaction is performed in a solvent. The solvent can be any suitable solvent, or mixture of solvents, capable of solubilizing the polymer and the α,β-unsaturated carbonyl compound to be reacted. For example, the solvent can include water, protic organic solvents, and/or aprotic organic solvents. An exemplary list of solvents includes water, dichloromethane, diethyl ether, dimethyl sulfoxide, acetonitrile, methanol, and ethanol.

[0176] In one embodiment, groups A^1 and/or A^2 of the polymer are modified by an epoxide opening reaction between the polymer and an epoxide compound. As used herein, the term "epoxide opening" refers to a nucleophilic addition of a nucleophile of the polymer (e.g., a carbanion, an oxygen anion, a nitrogen anion, an oxygen atom, a nitrogen atom, or a combination thereof) to an epoxide compound, thereby opening the epoxide. Accordingly, the epoxide opening reaction is between the polymer and an epoxide compound. In some embodiments, the nucleophile of the polymer is a nitrogen anion, a nitrogen atom, or a combination thereof.

[0177] In embodiments where the groups designated A^1 and/or A^2 are modified by an epoxide opening reaction, they produce groups designated B^1 and/or B^2 of the formula:

$$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5;$$

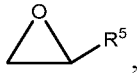
$$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5;$$

$$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}\text{-}CH_2\text{-}CHOH\text{-}R^5;$$

$$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5]_2\}_2;$$

wherein p1 to p4, q1 to q6, and r1 and r2 are each independently an integer of 1 to 5; each instance of R^2 is independently hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, or a C_1-C_{12} linear or branched alkyl group optionally substituted with one or more substituents, or R^2 is combined with a second R^2 so as to form a heterocyclic group; and each instance of R^5 is independently an alkyl group, cycloalkyl group, alkenyl group, cycloalkenyl group, aryl group, heteroalkyl group, heterocyclic group, or combination thereof optionally comprising from 2 to 8 tertiary amines or a substituent comprising a tissue-specific or cell-specific targeting moiety.

[0178] Examples of epoxides suitable for use include epoxides of the formula:



,

wherein R^5 is as described with respect to any of Formulae 3, 3A, 3B, or 3C.

[0179] In some embodiments, the epoxide opening reaction is facilitated by an acid and/or base. The acid and/or base can be any suitable acid and/or base with any suitable pKa. The acid and/or base can be an organic acid (e.g., *p*-toluenesulfonic acid), organic base (e.g., triethylamine), inorganic acid (e.g., titanium tetrachloride), inorganic base (e.g., potassium carbonate), or a combination thereof.

[0180] In some embodiments, the epoxide opening reaction is facilitated by an acid. The acid can be a Brønsted acid or a Lewis acid. In embodiments where the acid is a Brønsted acid, the acid can be a weak acid (i.e., a pKa of from about 4 to about 7) or a strong acid (i.e., a pKa of from about -2 to about 4). Typically, the acid is a weak acid. In certain embodiments, the acid is a Lewis acid. For example, the acid can be bis(trifluoromethanesulfon)imide or p-toluenesulfonic acid.

[0181] In some embodiments, the epoxide opening reaction is facilitated by a base. The base can be a weak base (i.e., a pKa of from about 7 to about 12) or a strong base (i.e., a pKa of from about 12 to about 50). Typically, the base is a weak base. For example, the base can be triethylamine, diisopropylethylamine, pyridine, N-methyl morpholine, or N,N-dimethyl-piperazine, or derivatives thereof.

[0182] In some embodiments, the epoxide opening reaction is performed in a solvent. The solvent can be any suitable solvent, or mixture of solvents, capable of solubilizing the polymer and the epoxide compound to be reacted. For example, the solvent can include water, protic organic solvents, and/or aprotic organic solvents. An exemplary list of solvents includes water, dichloromethane, diethyl ether, dimethyl sulfoxide, acetonitrile, methanol, and ethanol.

[0183] In one embodiment, groups A^1 and/or A^2 of the polymer are modified by a displacement reaction between the polymer and a compound comprising a leaving group (e.g., chloride atom, bromide atom, iodide atom, tosylate, triflate, mesylate, etc.). As used herein, the term "displacement" refers to a nucleophilic addition of a nucleophile of the polymer (e.g., a carbanion, an oxygen anion, a nitrogen anion, an oxygen atom, a nitrogen atom, or a combination thereof) to a compound comprising a leaving group. Accordingly, the displacement reaction is between the polymer and a compound comprising a leaving group. In some embodiments, the nucleophile of the polymer is a nitrogen anion, a nitrogen atom, or a combination thereof.

[0184] In embodiments where the groups designated A^1 and/or A^2 are modified by a displacement reaction, they produce groups designated B^1 and/or B^2 of the formula:

$$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^5;$$

—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—NR^2-$(CH_2)_{s2}$-R^5]$_2$;

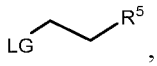
—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—NR^2_2][—$(CH_2)_{q4}$—NR^2—]$_{r2}$$(CH_2)_{s3}$-$R^5$};

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-$(CH_2)_{s4}$-R^5]$_2$}$_2$; or

—$(CH_2)_{p1}$—[N{$(CH_2)_{s1}$-R^5}—$(CH_2)_{q1}$—]$_{r1}$$NR^2_2$,

wherein p1 to p4, q1 to q6, r1 and r2, and s1 to s4 are each independently an integer of 1 to 5; each instance of R^2 is independently hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, or a C_1-C_{12} linear or branched alkyl group optionally substituted with one or more substituents, or R^2 is combined with a second R^2 so as to form a heterocyclic group; and each instance of R^5 is independently an alkyl group, cycloalkyl group, alkenyl group, cycloalkenyl group, aryl group, heteroalkyl group, heterocyclic group, or combination thereof optionally comprising from 2 to 8 tertiary amines or a substituent comprising a tissue-specific or cell-specific targeting moiety.

[0185] Examples of compounds containing a leaving group suitable for use include compound of formula:



,

wherein LG is a leaving group (e.g., chloride atom, bromide atom, iodide atom, tosylate, triflate, mesylate, etc.) and R^5 is as described with respect to any of Formulae 3, 3A, 3B, or 3C.

[0186] In some embodiments, the displacement reaction is facilitated by an acid and/or base. The acid and/or base can be any suitable acid and/or base with any suitable pKa. The acid and/or base can be an organic acid (e.g., *p*-toluenesulfonic acid), organic base (e.g., triethylamine), inorganic acid (e.g., titanium tetrachloride), inorganic base (e.g., potassium carbonate), or a combination thereof.

[0187] In some embodiments, the displacement reaction is facilitated by an acid. The acid can be a Brønsted acid or a Lewis acid. In embodiments where the acid is a Brønsted acid, the acid can be a weak acid (i.e., a pKa of from about 4 to about 7) or a strong acid (i.e., a pKa of from about -2 to about 4). Typically, the acid is a weak acid. In certain embodiments, the acid is a Lewis acid. For example, the acid can be bis(trifluoromethanesulfon)imide or p-toluenesulfonic acid.

[0188] In some embodiments, the displacement reaction is facilitated by a base. The base can be a weak base (i.e., a pKa of from about 7 to about 12) or a strong base (i.e., a pKa of from about 12 to about 50). Typically, the base is a weak base. For example, the base can be

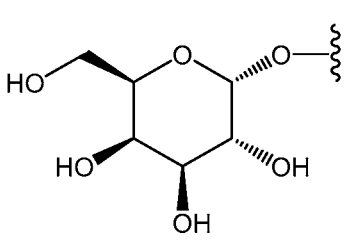
triethylamine, diisopropylethylamine, pyridine, N-methyl morpholine, or N,N-dimethyl-piperazine, or derivatives thereof.

[0189] In some embodiments, the displacement reaction is performed in a solvent. The solvent can be any suitable solvent, or mixture of solvents, capable of solubilizing the polymer and the compound comprising a leaving group to be reacted. For example, the solvent can include water, protic organic solvents, and/or aprotic organic solvents. An exemplary list of solvents includes water, dichloromethane, diethyl ether, dimethyl sulfoxide, acetonitrile, methanol, and ethanol.

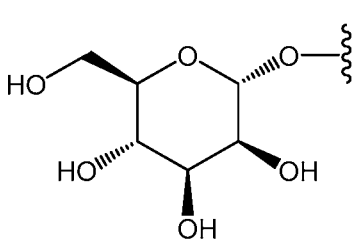
[0190] In some embodiments, the method further comprises isolating the polymer comprising the structure of Formula 3. The polymer comprising the structure of Formula 3 can be isolated by any suitable means. For example, the polymer comprising the structure of Formula 3 can be isolated by extraction, dialysis, crystallization, recrystallization, column chromatography, filtration, or any combination thereof. Any of the forgoing first or second polymers can comprise a tissue-specific or cell-specific targeting moiety at a position indicated in the described formulas, or the polymers can be otherwise modified to include a tissue-specific or cell-specific targeting moiety. For example, the moiety can be added to a terminus of the polymer, or a terminal amine of groups E^1, E^2, A^1, A^2, B^1, and/or B^2 can be modified (e.g., by a Michael addition reaction, an epoxide opening, a displacement reaction, or any other suitable technique) to attach the tissue-specific or cell-specific targeting moiety. The tissue-specific or cell-specific targeting moiety can be any small molecule, protein (e.g., antibody or antigen), amino acid sequence, sugar, oligonucleotide, metal-based nanoparticle, or combination thereof, capable of recognizing (e.g., specifically binding) a given target tissue or cell (e.g., specifically binding a particular ligand, receptor, or other protein or molecule that allows the targeting moiety to discriminate between the target tissue or cell and other non-target tissues or cells). In some embodiments, the tissue-specific or cell-specific targeting moiety is a receptor for a ligand. In some embodiments, the tissue-specific or cell-specific targeting moiety is a ligand for a receptor.

[0191] The tissue-specific or cell-specific targeting moiety can be used to target any desired tissue or cell type. In some embodiments, the tissue-specific or cell-specific targeting moiety localizes the polymer to tissues of the peripheral nervous system, the central nervous system, liver, muscle (e.g., cardiac muscle), lung, bone (e.g., hematopoietic cells), or the eye of the subject. In certain embodiments, the tissue-specific or cell-specific targeting moiety localizes the polymer to tumor cells. For example, the tissue-specific or cell-specific targeting moiety can be a sugar that binds to a receptor on a specific tissue or cell.

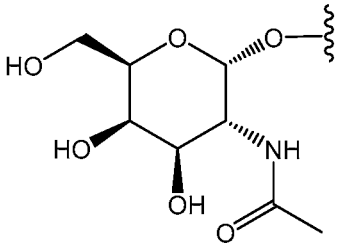
[0192] In some embodiments, the tissue-specific or cell-specific targeting moiety is:



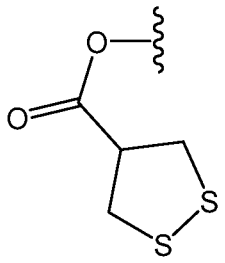
alpha-d-galactose ;



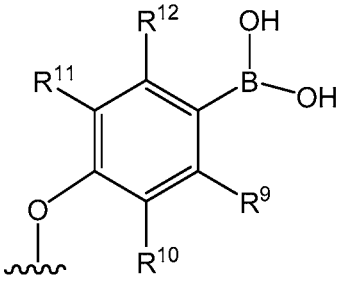
alpha-d-mannose ;



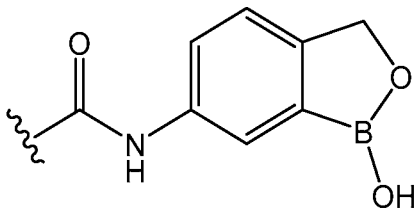
N-acetylgalactosamine ;



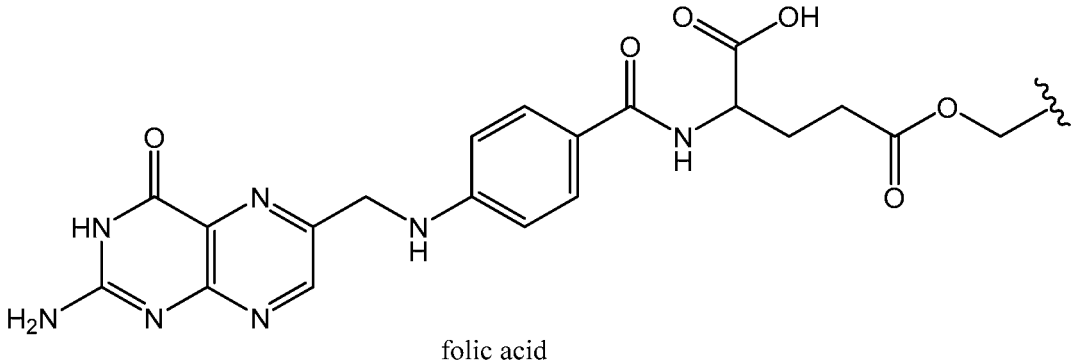
Asparagusic acid ;



Phenylboronic acid ;



; or



folic acid ,

wherein each of R^9, R^{10}, R^{11}, and R^{12} is independently hydrogen, halogen, C_1-C_4 alkyl, or C_1-C_4 alkoxy, optionally substituted with one or more amino groups. The specified tissue-specific or cell-specific targeting moieties can be chosen to localize the polymer to a tissue described herein. For example, alpha-d-mannose can be used to localize the polymer to the peripheral nervous system, the central nervous system, or immune cells, alpha-d-galactose and N-acetylgalactosamine can be used to localize the polymer to liver hepatocytes, and folic acid can be used to localize the polymer to tumor cells.

Donor Nucleic Acid

[0193] The donor nucleic acid (or "donor sequence" or "donor polynucleotide" or "donor DNA") is a nucleic acid sequence to be inserted at the cleavage site induced by an RNA-directed endonuclease (e.g., a Cas9 polypeptide or a Cpf1 polypeptide). The donor

polynucleotide will contain sufficient homology to a target genomic sequence at the cleavage site, e.g. 70%, 80%, 85%, 90%, 95%, or 100% homology with the nucleotide sequences flanking the cleavage site, e.g. within about 50 bases or less of the cleavage site, e.g. within about 30 bases, within about 15 bases, within about 10 bases, within about 5 bases, or immediately flanking the cleavage site, to support homology-directed repair between it and the genomic sequence to which it bears homology. Approximately 25, 50, 100, or 200 nucleotides, or more than 200 nucleotides, of sequence homology between a donor and a genomic sequence (or any integral value between 10 and 200 nucleotides, or more) will support homology-directed repair. Donor sequences can be of any length, e.g. 10 nucleotides or more, 50 nucleotides or more, 100 nucleotides or more, 250 nucleotides or more, 500 nucleotides or more, 1000 nucleotides or more, 5000 nucleotides or more, etc.

[0194] The donor sequence is typically not identical to the genomic sequence that it replaces. Rather, the donor sequence may contain one or more single base changes, insertions, deletions, inversions or rearrangements with respect to the genomic sequence, so long as sufficient homology is present to support homology-directed repair. In some embodiments, the donor sequence comprises a non-homologous sequence flanked by two regions of homology, such that homology-directed repair between the target DNA region and the two flanking sequences results in insertion of the non-homologous sequence at the target region. Donor sequences may also comprise a vector backbone containing sequences that are not homologous to the DNA region of interest and that are not intended for insertion into the DNA region of interest. Generally, the homologous region(s) of a donor sequence will have at least 50% sequence identity to a genomic sequence with which recombination is desired. In certain embodiments, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 99.9% sequence identity is present. Any value between 1% and 100% sequence identity can be present, depending upon the length of the donor polynucleotide.

[0195] The donor sequence may comprise certain sequence differences as compared to the genomic sequence, e.g. restriction sites, nucleotide polymorphisms, selectable markers (e.g., drug resistance genes, fluorescent proteins, enzymes etc.), etc., which may be used to assess for successful insertion of the donor sequence at the cleavage site or in some embodiments may be used for other purposes (e.g., to signify expression at the targeted genomic locus). In some embodiments, if located in a coding region, such nucleotide sequence differences will not change the amino acid sequence, or will make silent amino acid changes (i.e., changes which do not affect the structure or function of the protein). Alternatively, these sequences differences may include flanking recombination sequences

such as FLPs, loxP sequences, or the like, that can be activated at a later time for removal of the marker sequence.

[0196] The donor sequence may be provided to the cell as single-stranded DNA, single-stranded RNA, double-stranded DNA, or double-stranded RNA. It may be introduced into a cell in linear or circular form. If introduced in linear form, the ends of the donor sequence may be protected (e.g., from exonucleolytic degradation) by methods known to those of skill in the art. For example, one or more dideoxynucleotide residues are added to the 3' terminus of a linear molecule and/or self-complementary oligonucleotides are ligated to one or both ends. See, for example, Chang et al. (1987) Proc. Natl. Acad Sci USA 84:4959-4963; Nehls et al. (1996) Science 272:886-889. Amplification procedures such as rolling circle amplification can also be advantageously employed, as exemplified herein. Additional methods for protecting exogenous polynucleotides from degradation include, but are not limited to, addition of terminal amino group(s) and the use of modified internucleotide linkages such as, for example, phosphorothioates, phosphoramidates, and O-methyl ribose or deoxyribose residues.

[0197] As an alternative to protecting the termini of a linear donor sequence, additional lengths of sequence may be included outside of the regions of homology that can be degraded without impacting recombination. A donor sequence can be introduced into a cell as part of a vector molecule having additional sequences such as, for example, replication origins, promoters and genes encoding antibiotic resistance. Moreover, donor sequences can be introduced as naked nucleic acid, as nucleic acid complexed with an agent such as a liposome or polymer, or can be delivered by viruses (e.g., adenovirus, AAV), as described herein for nucleic acids encoding a Cas9 guide RNA and/or a Cas9 fusion polypeptide and/or donor polynucleotide.

Guide Nucleic Acid

[0198] In some embodiments, the composition comprises guide nucleic acid. Guide nucleic acids suitable for inclusion in a composition of the present disclosure include single-molecule guide RNAs ("single-guide RNA" / "sgRNA") and dual-molecule guide nucleic acids ("dual-guide RNA" / "dgRNA").

[0199] A guide nucleic acid (e.g., guide RNA) suitable for inclusion in a complex of the present disclosure directs the activities of an RNA-guided endonuclease (e.g., a Cas9 or Cpf1 polypeptide) to a specific target sequence within a target nucleic acid. A guide nucleic acid (e.g., guide RNA) comprises: a first segment (also referred to herein as a "nucleic acid

targeting segment", or simply a "targeting segment"); and a second segment (also referred to herein as a "protein-binding segment"). The terms "first" and "second" do not imply the order in which the segments occur in the guide RNA. The order of the elements relative to one another depends upon the particular RNA-guided polypeptide to be used. For instance, guide RNA for Cas9 typically has the protein-binding segment located 3' of the targeting segment, whereas guide RNA for Cpf1 typically has the protein-binding segment located 5' of the targeting segment.

[0200] The guide RNA may be introduced into a cell in linear or circular form. If introduced in linear form, the ends of the guide RNA may be protected (e.g., from exonucleolytic degradation) by methods known to those of skill in the art. Amplification procedures such as rolling circle amplification can also be advantageously employed, as exemplified herein.

First segment: targeting segment

[0201] The first segment of a guide nucleic acid (*e.g.*, guide RNA) includes a nucleotide sequence that is complementary to a sequence (a target site) in a target nucleic acid. In other words, the targeting segment of a guide nucleic acid (*e.g.*, guide RNA) can interact with a target nucleic acid (e.g., an RNA, a DNA, a double-stranded DNA) in a sequence-specific manner via hybridization (*i.e.*, base pairing). As such, the nucleotide sequence of the targeting segment may vary and can determine the location within the target nucleic acid that the guide nucleic acid (*e.g.*, guide RNA) and the target nucleic acid will interact. The targeting segment of a guide nucleic acid (*e.g.*, guide RNA) can be modified (*e.g.*, by genetic engineering) to hybridize to any desired sequence (target site) within a target nucleic acid.

[0202] The targeting segment can have a length of from 12 nucleotides to 100 nucleotides. The nucleotide sequence (the targeting sequence, also referred to as a guide sequence) of the targeting segment that is complementary to a nucleotide sequence (target site) of the target nucleic acid can have a length of 12 nt or more. For example, the targeting sequence of the targeting segment that is complementary to a target site of the target nucleic acid can have a length of 12 nt or more, 15 nt or more, 17 nt or more, 18 nt or more, 19 nt or more, 20 nt or more, 25 nt or more, 30 nt or more, 35 nt or more or 40 nt.

[0203] The percent complementarity between the targeting sequence (i.e., guide sequence) of the targeting segment and the target site of the target nucleic acid can be 60% or more (e.g., 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 97% or more, 98% or more, 99% or more, or 100%). In some

embodiments, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the seven contiguous 5'-most nucleotides of the target site of the target nucleic acid. In some embodiments, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 60% or more over 20 contiguous nucleotides. In some embodiments, the percent complementarity between the targeting sequence of the targeting segment and the target site of the target nucleic acid is 100% over the seventeen, eighteen, nineteen or twenty contiguous 5'-most nucleotides of the target site of the target nucleic acid and as low as 0% or more over the remainder. In such a case, the targeting sequence can be considered to be 17, 18, 19 or 20 nucleotides in length, respectively.

Second segment: protein-binding segment

[0204] The protein-binding segment of a guide nucleic acid (*e.g.*, guide RNA) interacts with (binds) an RNA-guided endonuclease. The guide nucleic acid (*e.g.*, guide RNA) guides the bound endonuclease to a specific nucleotide sequence within target nucleic acid (the target site) via the above mentioned targeting segment/targeting sequence/guide sequence. The protein-binding segment of a guide nucleic acid (e.g., guide RNA) comprises two stretches of nucleotides that are complementary to one another. The complementary nucleotides of the protein-binding segment hybridize to form a double stranded RNA duplex (dsRNA).

Single and dual guide nucleic acids

[0205] A dual guide nucleic acid (e.g., guide RNA) comprises two separate nucleic acid molecules. Each of the two molecules of a subject dual guide nucleic acid (e.g., guide RNA) comprises a stretch of nucleotides that are complementary to one another such that the complementary nucleotides of the two molecules hybridize to form the double stranded RNA duplex of the protein-binding segment.

[0206] In some embodiments, the duplex-forming segment of the activator is 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more identical or 100% identical to one of the activator (tracrRNA) molecules set forth in International Patent Application Nos. PCT/US2016/052690 and PCT/US2017/062617, or a complement thereof, over a stretch of 8 or more contiguous nucleotides (e.g., 8 or more contiguous nucleotides, 10 or more contiguous nucleotides, 12 or more contiguous nucleotides, 15 or more contiguous nucleotides, or 20 or more contiguous nucleotides).

[0207] In some embodiments, the duplex-forming segment of the targeter is 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more identical or 100% identical to one of the targeter (crRNA) sequences set forth in International Patent Application Nos. PCT/US2016/052690 and PCT/US2017/062617, or a complement thereof, over a stretch of 8 or more contiguous nucleotides (e.g., 8 or more contiguous nucleotides, 10 or more contiguous nucleotides, 12 or more contiguous nucleotides, 15 or more contiguous nucleotides, or 20 or more contiguous nucleotides).

[0208] A dual guide nucleic acid (*e.g.*, guide RNA) can be designed to allow for controlled (*i.e.*, conditional) binding of a targeter with an activator. Because a dual guide nucleic acid (*e.g.*, guide RNA) is not functional unless both the activator and the targeter are bound in a functional complex with Cas9, a dual guide nucleic acid (*e.g.*, guide RNA) can be inducible (*e.g.*, drug inducible) by rendering the binding between the activator and the targeter to be inducible. As one non-limiting example, RNA aptamers can be used to regulate (i.e., control) the binding of the activator with the targeter. Accordingly, the activator and/or the targeter can include an RNA aptamer sequence.

[0209] Aptamers (e.g., RNA aptamers) are known in the art and are generally a synthetic version of a riboswitch. The terms "RNA aptamer" and "riboswitch" are used interchangeably herein to encompass both synthetic and natural nucleic acid sequences that provide for inducible regulation of the structure (and therefore the availability of specific sequences) of the nucleic acid molecule (e.g., RNA, DNA/RNA hybrid, etc.) of which they are part. RNA aptamers usually comprise a sequence that folds into a particular structure (e.g., a hairpin), which specifically binds a particular drug (e.g., a small molecule). Binding of the drug causes a structural change in the folding of the RNA, which changes a feature of the nucleic acid of which the aptamer is a part. As non-limiting examples: (i) an activator with an aptamer may not be able to bind to the cognate targeter unless the aptamer is bound by the appropriate drug; (ii) a targeter with an aptamer may not be able to bind to the cognate activator unless the aptamer is bound by the appropriate drug; and (iii) a targeter and an activator, each comprising a different aptamer that binds a different drug, may not be able to bind to each other unless both drugs are present. As illustrated by these examples, a dual guide nucleic acid (e.g., guide RNA) can be designed to be inducible.

[0210] Examples of aptamers and riboswitches can be found, for example, in: Nakamura et al., Genes Cells. 2012 May;17(5):344-64; Vavalle et al., Future Cardiol. 2012 May;8(3):371-82; Citartan et al., Biosens Bioelectron. 2012 Apr 15;34(1):1-11; and

Liberman et al., Wiley Interdiscip Rev RNA. 2012 May-Jun;3(3):369-84; all of which are herein incorporated by reference in their entirety.

[0211] Non-limiting examples of nucleotide sequences that can be included in a dual guide nucleic acid (e.g., guide RNA) included in International Patent Application Nos. PCT/US2016/052690 and PCT/US2017/062617, or complements thereof that can hybridize to form a protein binding segment.

[0212] A subject single guide nucleic acid (e.g., guide RNA) comprises two stretches of nucleotides (much like a "targeter" and an "activator" of a dual guide nucleic acid) that are complementary to one another, hybridize to form the double stranded RNA duplex (dsRNA duplex) of the protein-binding segment (thus resulting in a stem-loop structure), and are covalently linked by intervening nucleotides ("linkers" or "linker nucleotides"). Thus, a subject single guide nucleic acid (e.g., a single guide RNA) can comprise a targeter and an activator, each having a duplex-forming segment, where the duplex-forming segments of the targeter and the activator hybridize with one another to form a dsRNA duplex. The targeter and the activator can be covalently linked via the 3' end of the targeter and the 5' end of the activator. Alternatively, targeter and the activator can be covalently linked via the 5' end of the targeter and the 3' end of the activator.

[0213] The linker of a single guide nucleic acid can have a length of from 3 nucleotides to 100 nucleotides. In some embodiments, the linker of a single guide nucleic acid (e.g., guide RNA) is 4 nt.

[0214] An exemplary single guide nucleic acid (e.g., guide RNA) comprises two complementary stretches of nucleotides that hybridize to form a dsRNA duplex. In some embodiments, one of the two complementary stretches of nucleotides of the single guide nucleic acid (e.g., guide RNA) (or the DNA encoding the stretch) is 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more identical or 100% identical to one of the activator (tracrRNA) molecules set forth in International Patent Application Nos. PCT/US2016/052690 and PCT/US2017/062617, or a complement thereof, over a stretch of 8 or more contiguous nucleotides (e.g., 8 or more contiguous nucleotides, 10 or more contiguous nucleotides, 12 or more contiguous nucleotides, 15 or more contiguous nucleotides, or 20 or more contiguous nucleotides).

[0215] In some embodiments, one of the two complementary stretches of nucleotides of the single guide nucleic acid (e.g., guide RNA) (or the DNA encoding the stretch) is 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more identical or 100% identical to one of the targeter (crRNA) sequences set forth in International Patent Application Nos.

PCT/US2016/052690 and PCT/US2017/062617, or a complement thereof, over a stretch of 8 or more contiguous nucleotides (e.g., 8 or more contiguous nucleotides, 10 or more contiguous nucleotides, 12 or more contiguous nucleotides, 15 or more contiguous nucleotides, or 20 or more contiguous nucleotides).

[0216] In some embodiments, one of the two complementary stretches of nucleotides of the single guide nucleic acid (e.g., guide RNA) (or the DNA encoding the stretch) is 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more identical or 100% identical to one of the targeter (crRNA) sequences or activator (tracrRNA) sequences set forth in International Patent Application Nos. PCT/US2016/052690 and PCT/US2017/062617, or a complement thereof, over a stretch of 8 or more contiguous nucleotides (e.g., 8 or more contiguous nucleotides, 10 or more contiguous nucleotides, 12 or more contiguous nucleotides, 15 or more contiguous nucleotides, or 20 or more contiguous nucleotides).

[0217] Appropriate cognate pairs of targeters and activators can be routinely determined by taking into account the species name and base-pairing (for the dsRNA duplex of the protein-binding domain) Any activator/targeter pair can be used as part of dual guide nucleic acid (e.g., guide RNA) or as part of a single guide nucleic acid (e.g., guide RNA).

[0218] In some embodiments, an activator (e.g., a trRNA, trRNA-like molecule, etc.) of a dual guide nucleic acid (e.g., guide RNA) (e.g., a dual guide RNA) or a single guide nucleic acid (e.g., guide RNA) (e.g., a single guide RNA) includes a stretch of nucleotides with 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% or more, or 100% sequence identity with an activator (tracrRNA) molecule set forth in International Patent Application Nos. PCT/US2016/052690 and PCT/US2017/062617, or a complement thereof.

[0219] In some embodiments, an activator (e.g., a trRNA, trRNA-like molecule, etc.) of a dual guide nucleic acid (e.g., a dual guide RNA) or a single guide nucleic acid (e.g., a single guide RNA) includes 30 or more nucleotides (nt) (e.g., 40 or more, 50 or more, 60 or more, 70 or more, 75 or more nt). In some embodiments, an activator (e.g., a trRNA, trRNA-like molecule, etc.) of a dual guide nucleic acid (e.g., a dual guide RNA) or a single guide nucleic acid (e.g., a single guide RNA) has a length in a range of from 30 to 200 nucleotides (nt).

[0220] The protein-binding segment can have a length of from 10 nucleotides to 100 nucleotides.

[0221] Also with regard to both a subject single guide nucleic acid (e.g., single guide RNA) and to a subject dual guide nucleic acid (e.g., dual guide RNA), the dsRNA duplex of the protein-binding segment can have a length from 6 base pairs (bp) to 50bp. The percent complementarity between the nucleotide sequences that hybridize to form the dsRNA duplex

of the protein-binding segment can be 60% or more. For example, the percent complementarity between the nucleotide sequences that hybridize to form the dsRNA duplex of the protein-binding segment can be 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, or 99% or more (e.g., in some embodiments, there are some nucleotides that do not hybridize and therefore create a bulge within the dsRNA duplex. In some embodiments, the percent complementarity between the nucleotide sequences that hybridize to form the dsRNA duplex of the protein-binding segment is 100%.

Hybrid guide nucleic acids

[0222] In some embodiments, a guide nucleic acid is two RNA molecules (dual guide RNA). In some embodiments, a guide nucleic acid is one RNA molecule (single guide RNA). In some embodiments, a guide nucleic acid is a DNA/RNA hybrid molecule. In such embodiments, the protein-binding segment of the guide nucleic acid is RNA and forms an RNA duplex. Thus, the duplex-forming segments of the activator and the targeter is RNA. However, the targeting segment of a guide nucleic acid can be DNA. Thus, if a DNA/RNA hybrid guide nucleic acid is a dual guide nucleic acid, the "targeter" molecule and be a hybrid molecule (e.g., the targeting segment can be DNA and the duplex-forming segment can be RNA). In such embodiments, the duplex-forming segment of the "activator" molecule can be RNA (e.g., in order to form an RNA-duplex with the duplex-forming segment of the targeter molecule), while nucleotides of the "activator" molecule that are outside of the duplex-forming segment can be DNA (in which case the activator molecule is a hybrid DNA/RNA molecule) or can be RNA (in which case the activator molecule is RNA). If a DNA/RNA hybrid guide nucleic acid is a single guide nucleic acid, then the targeting segment can be DNA, the duplex-forming segments (which make up the protein-binding segment of the single guide nucleic acid) can be RNA, and nucleotides outside of the targeting and duplex-forming segments can be RNA or DNA.

[0223] A DNA/RNA hybrid guide nucleic can be useful in some embodiments, for example, when a target nucleic acid is an RNA. Cas9 normally associates with a guide RNA that hybridizes with a target DNA, thus forming a DNA-RNA duplex at the target site. Therefore, when the target nucleic acid is an RNA, it is sometimes advantageous to recapitulate a DNA-RNA duplex at the target site by using a targeting segment (of the guide nucleic acid) that is DNA instead of RNA. However, because the protein-binding segment of a guide nucleic acid is an RNA-duplex, the targeter molecule is DNA in the targeting

segment and RNA in the duplex-forming segment. Hybrid guide nucleic acids can bias Cas9 binding to single stranded target nucleic acids relative to double stranded target nucleic acids.

Exemplary guide nucleic acids

[0224] Any guide nucleic acid can be used. Many different types of guide nucleic acids are known in the art. The guide nucleic selected will be appropriately paired to the particular CRISPR system being used (e.g., the particular RNA guided endonuclease being used). Thus, the guide nucleic acid can be, for instance, a guide nucleic acid corresponding to any RNA guided endonuclease described herein or known in the art. Guide nucleic acids and RNA guided endonucleases are described, for example, in International Patent Application Nos. PCT/US2016/052690 and PCT/US2017/062617

[0225] In some embodiments, a suitable guide nucleic acid includes two separate RNA polynucleotide molecules. In some embodiments, the first of the two separate RNA polynucleotide molecules (the activator) comprises a nucleotide sequence having 60% or more (e.g., 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, 99% or more, or 100%) nucleotide sequence identity over a stretch of 8 or more contiguous nucleotides (e.g., 8 or more contiguous nucleotides, 10 or more contiguous nucleotides, 12 or more contiguous nucleotides, 15 or more contiguous nucleotides, or 20 or more contiguous nucleotides) to any one of the nucleotide sequences set forth in International Patent Application Nos. PCT/US2016/052690 and PCT/US2017/062617, or a complement thereof. In some embodiments, the second of the two separate RNA polynucleotide molecules (the targeter) comprises a nucleotide sequence having 60% or more (e.g., 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, 99% or more, or 100%) nucleotide sequence identity over a stretch of 8 or more contiguous nucleotides (e.g., 8 or more contiguous nucleotides, 10 or more contiguous nucleotides, 12 or more contiguous nucleotides, 15 or more contiguous nucleotides, or 20 or more contiguous nucleotides) to any one of the nucleotide sequences set forth in International Patent Application Nos. PCT/US2016/052690 and PCT/US2017/062617, or a complement thereof.

[0226] In some embodiments, a suitable guide nucleic acid is a single RNA polynucleotide and comprises first and second nucleotide sequence having 60% or more (e.g., 65% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 98% or more, 99% or more, or 100%) nucleotide sequence identity over a stretch of 8 or more contiguous nucleotides (e.g., 8 or more contiguous nucleotides, 10 or more

contiguous nucleotides, 12 or more contiguous nucleotides, 15 or more contiguous nucleotides, or 20 or more contiguous nucleotides) to any one of the nucleotide sequences set forth in International Patent Application Nos. PCT/US2016/052690 and PCT/US2017/062617, or complements thereof.

[0227] In some embodiments, the guide RNA is a Cpf1 and/or Cas9 guide RNA. A Cpf1 and/or Cas9 guide RNA can have a total length of from 30 nucleotides (nt) to 100 nt, e.g., from 30 nt to 40 nt, from 40 nt to 45 nt, from 45 nt to 50 nt, from 50 nt to 60 nt, from 60 nt to 70 nt, from 70 nt to 80 nt, from 80 nt to 90 nt, or from 90 nt to 100 nt. In some embodiments, a Cpf1 and/or Cas9 guide RNA has a total length of 35 nt, 36 nt, 37 nt, 38 nt, 39 nt, 40 nt, 41 nt, 42 nt, 43 nt, 44 nt, 45 nt, 46 nt, 47 nt, 48 nt, 49 nt, or 50 nt. A Cpf1 and/or Cas9 guide RNA can include a target nucleic acid-binding segment and a duplex-forming segment.

[0228] The target nucleic acid-binding segment of a Cpf1 and/or Cas9 guide RNA can have a length of from 15 nt to 30 nt, e.g., 15 nt, 16 nt, 17 nt, 18 nt, 19 nt, 20 nt, 21 nt, 22 nt, 23 nt, 24 nt, 25 nt, 26 nt, 27 nt, 28 nt, 29 nt, or 30 nt. In some embodiments, the target nucleic acid-binding segment has a length of 23 nt. In some embodiments, the target nucleic acid-binding segment has a length of 24 nt. In some embodiments, the target nucleic acid-binding segment has a length of 25 nt.

[0229] The target nucleic acid-binding segment of a Cpf1 and/or Cas9 guide RNA can have 100% complementarity with a corresponding length of target nucleic acid sequence. The targeting segment can have less than 100% complementarity with a corresponding length of target nucleic acid sequence. For example, the target nucleic acid binding segment of a Cpf1 and/or Cas9 guide RNA can have 1, 2, 3, 4, or 5 nucleotides that are not complementary to the target nucleic acid sequence. For example, in some embodiments, where a target nucleic acid-binding segment has a length of 25 nucleotides, and the target nucleic acid sequence has a length of 25 nucleotides, in some embodiments, the target nucleic acid-binding segment has 100% complementarity to the target nucleic acid sequence. As another example, in some embodiments, where a target nucleic acid-binding segment has a length of 25 nucleotides, and the target nucleic acid sequence has a length of 25 nucleotides, in some embodiments, the target nucleic acid-binding segment has 1 non-complementary nucleotide and 24 complementary nucleotides with the target nucleic acid sequence.

[0230] The duplex-forming segment of a Cpf1 and/or Cas9 guide RNA can have a length of from 15 nt to 25 nt, e.g., 15 nt, 16 nt, 17 nt, 18 nt, 19 nt, 20 nt, 21 nt, 22 nt, 23 nt, 24 nt, or 25 nt.

[0231] In some embodiments, the duplex-forming segment of a Cpf1 guide RNA can comprise the nucleotide sequence 5'-AAUUUCUACUGUUGUAGAU-3'.

Additional Elements

[0232] In some embodiments, a guide nucleic acid (e.g., guide RNA) includes an additional segment or segments (in some embodiments at the 5' end, in some embodiments the 3' end, in some embodiments at either the 5' or 3' end, in some embodiments embedded within the sequence (i.e., not at the 5' and/or 3' end), in some embodiments at both the 5' end and the 3' end, in some embodiments embedded and at the 5' end and/or the 3' end, etc.). For example, a suitable additional segment can include a 5' cap (e.g., a 7-methylguanylate cap (m^7G)); a 3' polyadenylated tail (i.e., a 3' poly(A) tail); a ribozyme sequence (e.g. to allow for self-cleavage of a guide nucleic acid or component of a guide nucleic acid, e.g., a targeter, an activator, etc.); a riboswitch sequence (e.g., to allow for regulated stability and/or regulated accessibility by proteins and protein complexes); a sequence that forms a dsRNA duplex (i.e., a hairpin)); a sequence that targets an RNA to a subcellular location (e.g., nucleus, mitochondria, chloroplasts, and the like); a modification or sequence that provides for tracking (e.g., a label such as a fluorescent molecule (i.e., fluorescent dye), a sequence or other moiety that facilitates fluorescent detection; a sequence or other modification that provides a binding site for proteins (e.g., proteins that act on DNA, including transcriptional activators, transcriptional repressors, DNA methyltransferases, DNA demethylases, histone acetyltransferases, histone deacetylases, proteins that bind RNA (e.g., RNA aptamers), labeled proteins, fluorescently labeled proteins, and the like); a modification or sequence that provides for increased, decreased, and/or controllable stability; and combinations thereof.

RNA-Guided Endonuclease

[0233] In addition to, or instead of, a guide nucleic acid, the composition can comprise an RNA-guided endonuclease protein or nucleic acid (e.g., mRNA or vector) encoding same. Any RNA-guided endonuclease can be used. The selection of the RNA guided endonuclease used will depend, at least in part, to the intended end-use of the CRISPR system employed.

[0234] In some embodiments, the polypeptide is a Cas 9 polypeptide. Suitable Cas9 polypeptides for inclusion in a composition of the present disclosure include a naturally-occurring Cas9 polypeptide (*e.g.*, naturally occurs in bacterial and/or archaeal cells), or a non- naturally-occurring Cas9 polypeptide (*e.g.*, the Cas9 polypeptide is a variant Cas9 polypeptide, a chimeric polypeptide as discussed below, and the like), as described below. In

some embodiments, one skilled in the art can appreciate that the Cas9 polypeptide disclosed herein can be any variant derived or isolated from any source. In other embodiments, the Cas9 peptide of the present disclosure can include one or more of the mutations described in the literature, including but not limited to the functional mutations described in: Fonfara et al. Nucleic Acids Res. 2014 Feb;42(4):2577-90; Nishimasu H. et al. Cell. 2014 Feb 27;156(5):935-49; Jinek M. et al. Science. 2012 337:816-21; and Jinek M. et al. Science. 2014 Mar 14;343(6176); see also U.S. Pat. App. No. 13/842,859, filed March 15, 2013, which is hereby incorporated by reference; further, see U.S. Pat. Nos. 8,697,359; 8,771,945; 8,795,965; 8,865,406; 8,871,445; 8,889,356; 8,895,308; 8,906,616; 8,932,814; 8,945,839; 8,993,233; and 8,999,641, which are all hereby incorporated by reference. Thus, in some embodiments, the systems and methods disclosed herein can be used with the wild type Cas9 protein having double-stranded nuclease activity, Cas9 mutants that act as single stranded nickases, or other mutants with modified nuclease activity. As such, a Cas9 polypeptide that is suitable for inclusion in a composition of the present disclosure can be an enzymatically active Cas9 polypeptide, *e.g.*, can make single- or double-stranded breaks in a target nucleic acid, or alternatively can have reduced enzymatic activity compared to a wild-type Cas9 polypeptide.

[0235] Naturally occurring Cas9 polypeptides bind a guide nucleic acid, are thereby directed to a specific sequence within a target nucleic acid (a target site), and cleave the target nucleic acid (e.g., cleave dsDNA to generate a double strand break, cleave ssDNA, cleave ssRNA, etc.). A subject Cas9 polypeptide comprises two portions, an RNA-binding portion and an activity portion. The RNA-binding portion interacts with a subject guide nucleic acid, and an activity portion exhibits site-directed enzymatic activity (*e.g.*, nuclease activity, activity for DNA and/or RNA methylation, activity for DNA and/or RNA cleavage, activity for histone acetylation, activity for histone methylation, activity for RNA modification, activity for RNA-binding, activity for RNA splicing etc. In some embodiments the activity portion exhibits reduced nuclease activity relative to the corresponding portion of a wild type Cas9 polypeptide. In some embodiments, the activity portion is enzymatically inactive.

[0236] Assays to determine whether a protein has an RNA-binding portion that interacts with a subject guide nucleic acid can be any convenient binding assay that tests for binding between a protein and a nucleic acid. Exemplary binding assays include binding assays (e.g., gel shift assays) that involve adding a guide nucleic acid and a Cas9 polypeptide to a target nucleic acid.

[0237] Assays to determine whether a protein has an activity portion (e.g., to determine if the polypeptide has nuclease activity that cleave a target nucleic acid) can be any convenient nucleic acid cleavage assay that tests for nucleic acid cleavage. Exemplary cleavage assays that include adding a guide nucleic acid and a Cas9 polypeptide to a target nucleic acid.

[0238] In some embodiments, a suitable Cas9 polypeptide for inclusion in a composition of the present disclosure has enzymatic activity that modifies target nucleic acid (e.g., nuclease activity, methyltransferase activity, demethylase activity, DNA repair activity, DNA damage activity, deamination activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, integrase activity, transposase activity, recombinase activity, polymerase activity, ligase activity, helicase activity, photolyase activity or glycosylase activity).

[0239] In other embodiments, a suitable Cas9 polypeptide for inclusion in a composition of the present disclosure has enzymatic activity that modifies a polypeptide (e.g., a histone) associated with target nucleic acid (e.g., methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity or demyristoylation activity).

[0240] Many Cas9 orthologues from a wide variety of species have been identified and in some embodiments, the proteins share only a few identical amino acids. All identified Cas9 orthologues have the same domain architecture with a central HNH endonuclease domain and a split RuvC/RNaseH domain. Cas9 proteins share 4 key motifs with a conserved architecture. Motifs 1, 2, and 4 are RuvC like motifs while motif 3 is an HNH-motif.

[0241] In some embodiments, a suitable Cas9 polypeptide comprises an amino acid sequence having 4 motifs, each of motifs 1-4 having 60% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 99% or more or 100% amino acid sequence identity to the Cas9 amino acid sequence depicted in FIG. 1 (SEQ ID NO:1); or alternatively to motifs 1-4 of the Cas9 amino acid sequence depicted in Table 1 below; or alternatively to amino acids 7-166 or 731-1003 of the Cas9 amino acid sequence depicted in FIG. 1 (SEQ ID NO:1)

[0242] In some embodiments, a Cas9 polypeptide comprises an amino acid sequence having 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, or 98%, amino acid sequence identity to the amino acid sequence depicted in FIG. 1 and set forth in SEQ ID NO:1; and comprises amino acid substitutions of N497, R661, Q695, and Q926 relative to the

amino acid sequence set forth in SEQ ID NO:1; or comprises an amino acid substitution of K855 relative to the amino acid sequence set forth in SEQ ID NO:1; or comprises amino acid substitutions of K810, K1003, and R1060 relative to the amino acid sequence set forth in SEQ ID NO:1; or comprises amino acid substitutions of K848, K1003, and R1060 relative to the amino acid sequence set forth in SEQ ID NO:1.

[0243] As used herein, the term "Cas9 polypeptide" encompasses the term "variant Cas9 polypeptide"; and the term "variant Cas9 polypeptide" encompasses the term "chimeric Cas9 polypeptide."

Variant Cas9 polypeptides

[0244] A suitable Cas9 polypeptides for inclusion in a composition of the present disclosure includes a variant Cas9 polypeptide. A variant Cas9 polypeptide has an amino acid sequence that is different by one amino acid (e.g., has a deletion, insertion, substitution, fusion) (i.e., different by at least one amino acid) when compared to the amino acid sequence of a wild type Cas9 polypeptide (e.g., a naturally occurring Cas9 polypeptide, as described above). In some instances, the variant Cas9 polypeptide has an amino acid change (e.g., deletion, insertion, or substitution) that reduces the nuclease activity of the Cas9 polypeptide. For example, in some instances, the variant Cas9 polypeptide has less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, or less than 1% of the nuclease activity of the corresponding wild-type Cas9 polypeptide. In some embodiments, the variant Cas9 polypeptide has no substantial nuclease activity. When a Cas9 polypeptide is a variant Cas9 polypeptide that has no substantial nuclease activity, it can be referred to as "dCas9."

[0245] In some embodiments, a variant Cas9 polypeptide has reduced nuclease activity. For example, a variant Cas9 polypeptide suitable for use in a binding method of the present disclosure exhibits less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 1%, or less than about 0.1%, of the endonuclease activity of a wild-type Cas9 polypeptide, *e.g.*, a wild-type Cas9 polypeptide comprising an amino acid sequence as depicted in FIG. 1 (SEQ ID NO:1).

[0246] In some embodiments, a variant Cas9 polypeptide can cleave the complementary strand of a target nucleic acid but has reduced ability to cleave the non-complementary strand of a double stranded target nucleic acid. For example, the variant Cas9 polypeptide can have a mutation (amino acid substitution) that reduces the function of the RuvC domain (e.g., "domain 1" of FIG. 1). As a non-limiting example, in some embodiments, a variant Cas9

polypeptide has a D10A mutation (e.g., aspartate to alanine at an amino acid position corresponding to position 10 of SEQ ID NO:1) and can therefore cleave the complementary strand of a double stranded target nucleic acid but has reduced ability to cleave the non-complementary strand of a double stranded target nucleic acid (thus resulting in a single strand break (SSB) instead of a double strand break (DSB) when the variant Cas9 polypeptide cleaves a double stranded target nucleic acid) (see, for example, Jinek et al., Science. 2012 Aug 17;337(6096):816-21).

[0247] In some embodiments, a variant Cas9 polypeptide can cleave the non-complementary strand of a double stranded target nucleic acid but has reduced ability to cleave the complementary strand of the target nucleic acid. For example, the variant Cas9 polypeptide can have a mutation (amino acid substitution) that reduces the function of the HNH domain (RuvC/HNH/RuvC domain motifs, "domain 2" of FIG. 1). As a non-limiting example, in some embodiments, the variant Cas9 polypeptide can have an H840A mutation (e.g., histidine to alanine at an amino acid position corresponding to position 840 of SEQ ID NO:1) (FIG. 1) and can therefore cleave the non-complementary strand of the target nucleic acid but has reduced ability to cleave the complementary strand of the target nucleic acid (thus resulting in a SSB instead of a DSB when the variant Cas9 polypeptide cleaves a double stranded target nucleic acid). Such a Cas9 polypeptide has a reduced ability to cleave a target nucleic acid (e.g., a single stranded target nucleic acid) but retains the ability to bind a target nucleic acid (e.g., a single-stranded or a double-stranded target nucleic acid).

[0248] In some embodiments, a variant Cas9 polypeptide has a reduced ability to cleave both the complementary and the non-complementary strands of a double stranded target nucleic acid. As a non-limiting example, in some embodiments, the variant Cas9 polypeptide harbors both the D10A and the H840A mutations (e.g., mutations in both the RuvC domain and the HNH domain) such that the polypeptide has a reduced ability to cleave both the complementary and the non-complementary strands of a double stranded target nucleic acid. Such a Cas9 polypeptide has a reduced ability to cleave a target nucleic acid (e.g., a single-stranded target nucleic acid or a double-stranded target nucleic acid) but retains the ability to bind a target nucleic acid (e.g., a single stranded target nucleic acid or a double-stranded target nucleic acid).

[0249] As another non-limiting example, in some embodiments, the variant Cas9 polypeptide harbors W476A and W1126A mutations such that the polypeptide has a reduced ability to cleave a target nucleic acid. Such a Cas9 polypeptide has a reduced ability to cleave a target nucleic acid but retains the ability to bind a target nucleic acid.

[0250] As another non-limiting example, in some embodiments, the variant Cas9 polypeptide harbors P475A, W476A, N477A, D1125A, W1126A, and D1127A mutations such that the polypeptide has a reduced ability to cleave a target nucleic acid. Such a Cas9 polypeptide has a reduced ability to cleave a target nucleic acid but retains the ability to bind a target nucleic acid.

[0251] As another non-limiting example, in some embodiments, the variant Cas9 polypeptide harbors H840A, W476A, and W1126A, mutations such that the polypeptide has a reduced ability to cleave a target nucleic acid. Such a Cas9 polypeptide has a reduced ability to cleave a target nucleic acid but retains the ability to bind a target nucleic acid.

[0252] As another non-limiting example, in some embodiments, the variant Cas9 polypeptide harbors H840A, D10A, W476A, and W1126A, mutations such that the polypeptide has a reduced ability to cleave a target nucleic acid. Such a Cas9 polypeptide has a reduced ability to cleave a target nucleic acid but retains the ability to bind a target nucleic acid.

[0253] As another non-limiting example, in some embodiments, the variant Cas9 polypeptide harbors, H840A, P475A, W476A, N477A, D1125A, W1126A, and D1127A mutations such that the polypeptide has a reduced ability to cleave a target nucleic acid. Such a Cas9 polypeptide has a reduced ability to cleave a target nucleic acid but retains the ability to bind a target nucleic acid.

[0254] As another non-limiting example, in some embodiments, the variant Cas9 polypeptide harbors D10A, H840A, P475A, W476A, N477A, D1125A, W1126A, and D1127A mutations such that the polypeptide has a reduced ability to cleave a target nucleic acid. Such a Cas9 polypeptide has a reduced ability to cleave a target nucleic acid but retains the ability to bind a target nucleic acid.

[0255] Other residues can be mutated to achieve the above effects (i.e. inactivate one or the other nuclease portions). As non-limiting examples, residues D10, G12, G17, E762, H840, N854, N863, H982, H983, A984, D986, and/or A987 can be altered (i.e., substituted) (see **Table 1** for more information regarding the conservation of Cas9 amino acid residues). Also, mutations other than alanine substitutions are suitable.

[0256] In some embodiments, a variant Cas9 polypeptide that has reduced catalytic activity (e.g., when a Cas9 protein has a D10, G12, G17, E762, H840, N854, N863, H982, H983, A984, D986, and/or a A987 mutation, e.g., D10A, G12A, G17A, E762A, H840A, N854A, N863A, H982A, H983A, A984A, and/or D986A), the variant Cas9 polypeptide can still bind to target nucleic acid in a site-specific manner (because it is still guided to a target

nucleic acid sequence by a guide nucleic acid) as long as it retains the ability to interact with the guide nucleic acid.

Table 1. Table 1 lists 4 motifs that are present in Cas9 sequences from various species. The amino acids listed here are from the Cas9 from *S. pyogenes* (SEQ ID NO: 1).

Motif	Motif	Amino acids (residue #s)	Highly conserved
1	RuvC	IGLDIGTNSVGWAVI (7-21) (SEQ ID NO:3)	D10, G12, G17
2	RuvC	IVIEMARE (759-766) (SEQ ID NO:4)	E762
3	HNH-motif	DVDHIVPQSFLKDDSIDNKVLTRSDKN (837-863) (SEQ ID NO:5)	H840, N854, N863
4	RuvC	HHAHDAYL (982-989) (SEQ ID NO:6)	H982, H983, A984, D986, A987

[0257] In addition to the above, a variant Cas9 protein can have the same parameters for sequence identity as described above for Cas9 polypeptides. Thus, in some embodiments, a suitable variant Cas9 polypeptide comprises an amino acid sequence having 4 motifs, each of motifs 1-4 having 60% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 99% or more or 100% amino acid sequence identity of the Cas9 amino acid sequence depicted in FIG. 1 (SEQ ID NO:1), or alternatively to motifs 1-4 (motifs 1-4 of SEQ ID NO:1 are SEQ ID NOs: 3-6, respectively, as depicted in Table 1); or alternatively to amino acids 7-166 or 731-1003 of the Cas9 amino acid sequence depicted in FIG. 1 (SEQ ID NO:1). Any Cas9 protein as defined above can be used as a Cas9 polypeptide, or as part of a chimeric Cas9 polypeptide, in a composition of the present disclosure, including those specifically referenced in International Patent Application Nos. PCT/US2016/052690 and PCT/US2017/062617.

[0258] In some embodiments, a suitable variant Cas9 polypeptide comprises an amino acid sequence having 60% or more, 70% or more, 75% or more, 80% or more, 85% or more, 90% or more, 95% or more, 99% or more, or 100% amino acid sequence identity to the Cas9 amino acid sequence depicted in FIG. 1 (SEQ ID NO:1). Any Cas9 protein as defined above can be used as a variant Cas9 polypeptide or as part of a chimeric variant Cas9 polypeptide in a composition of the present disclosure, including those specifically referenced in International Patent Application Nos. PCT/US2016/052690 and PCT/US2017/062617.

Chimeric polypeptides (fusion polypeptides)

[0259] In some embodiments, a variant Cas9 polypeptide is a chimeric Cas9 polypeptide (also referred to herein as a fusion polypeptide, e.g., a "Cas9 fusion polypeptide"). A Cas9 fusion polypeptide can bind and/or modify a target nucleic acid (e.g., cleave, methylate, demethylate, etc.) and/or a polypeptide associated with target nucleic acid (e.g., methylation, acetylation, etc., of, for example, a histone tail).

[0260] A Cas9 fusion polypeptide is a variant Cas9 polypeptide by virtue of differing in sequence from a wild type Cas9 polypeptide (e.g., a naturally occurring Cas9 polypeptide). A Cas9 fusion polypeptide is a Cas9 polypeptide (e.g., a wild type Cas9 polypeptide, a variant Cas9 polypeptide, a variant Cas9 polypeptide with reduced nuclease activity (as described above), and the like) fused to a covalently linked heterologous polypeptide (also referred to as a "fusion partner"). In some embodiments, a Cas9 fusion polypeptide is a variant Cas9 polypeptide with reduced nuclease activity (e.g., dCas9) fused to a covalently linked heterologous polypeptide. In some embodiments, the heterologous polypeptide exhibits (and therefore provides for) an activity (e.g., an enzymatic activity) that will also be exhibited by the Cas9 fusion polypeptide (e.g., methyltransferase activity, acetyltransferase activity, kinase activity, ubiquitinating activity, etc.). In some such embodiments, a method of binding, e.g., where the Cas9 polypeptide is a variant Cas9 polypeptide having a fusion partner (i.e., having a heterologous polypeptide) with an activity (e.g., an enzymatic activity) that modifies the target nucleic acid, the method can also be considered to be a method of modifying the target nucleic acid. In some embodiments, a method of binding a target nucleic acid (e.g., a single stranded target nucleic acid) can result in modification of the target nucleic acid. Thus, in some embodiments, a method of binding a target nucleic acid (e.g., a single stranded target nucleic acid) can be a method of modifying the target nucleic acid.

[0261] In some embodiments, the heterologous sequence provides for subcellular localization, i.e., the heterologous sequence is a subcellular localization sequence (e.g., a nuclear localization signal (NLS) for targeting to the nucleus, a sequence to keep the fusion protein out of the nucleus, e.g., a nuclear export sequence (NES), a sequence to keep the fusion protein retained in the cytoplasm, a mitochondrial localization signal for targeting to the mitochondria, a chloroplast localization signal for targeting to a chloroplast, an endoplasmic reticulum (ER) retention signal, and the like). In some embodiments, a variant Cas9 does not include a NLS so that the protein is not targeted to the nucleus (which can be advantageous, e.g., when the target nucleic acid is an RNA that is present in the cytosol). In some embodiments, the heterologous sequence can provide a tag (i.e., the heterologous

sequence is a detectable label) for ease of tracking and/or purification (e.g., a fluorescent protein, e.g., green fluorescent protein (GFP), YFP, RFP, CFP, mCherry, tdTomato, and the like; a histidine tag, e.g., a 6XHis tag; a hemagglutinin (HA) tag; a FLAG tag; a Myc tag; and the like). In some embodiments, the heterologous sequence can provide for increased or decreased stability (i.e., the heterologous sequence is a stability control peptide, e.g., a degron, which in some embodiments is controllable (e.g., a temperature sensitive or drug controllable degron sequence, see below). In some embodiments, the heterologous sequence can provide for increased or decreased transcription from the target nucleic acid (i.e., the heterologous sequence is a transcription modulation sequence, e.g., a transcription factor/activator or a fragment thereof, a protein or fragment thereof that recruits a transcription factor/activator, a transcription repressor or a fragment thereof, a protein or fragment thereof that recruits a transcription repressor, a small molecule/drug-responsive transcription regulator, etc.). In some embodiments, the heterologous sequence can provide a binding domain (i.e., the heterologous sequence is a protein binding sequence, e.g., to provide the ability of a Cas9 fusion polypeptide to bind to another protein of interest, e.g., a DNA or histone modifying protein, a transcription factor or transcription repressor, a recruiting protein, an RNA modification enzyme, an RNA-binding protein, a translation initiation factor, an RNA splicing factor, etc.). A heterologous nucleic acid sequence may be linked to another nucleic acid sequence (e.g., by genetic engineering) to generate a chimeric nucleotide sequence encoding a chimeric polypeptide.

[0262] A subject Cas9 fusion polypeptide (Cas9 fusion protein) can have multiple (1 or more, 2 or more, 3 or more, etc.) fusion partners in any combination of the above. As an illustrative example, a Cas9 fusion protein can have a heterologous sequence that provides an activity (e.g., for transcription modulation, target modification, modification of a protein associated with a target nucleic acid, etc.) and can also have a subcellular localization sequence. In some embodiments, such a Cas9 fusion protein might also have a tag for ease of tracking and/or purification (e.g., green fluorescent protein (GFP), YFP, RFP, CFP, mCherry, tdTomato, and the like; a histidine tag, e.g., a 6XHis tag; a hemagglutinin (HA) tag; a FLAG tag; a Myc tag; and the like). As another illustrative example, a Cas9 protein can have one or more NLSs (e.g., two or more, three or more, four or more, five or more, 1, 2, 3, 4, or 5 NLSs). In some embodiments a fusion partner (or multiple fusion partners) (e.g., an NLS, a tag, a fusion partner providing an activity, etc.) is located at or near the C-terminus of Cas9. In some embodiments a fusion partner (or multiple fusion partners) (e.g., an NLS, a tag, a fusion partner providing an activity, etc.) is located at the N-terminus of Cas9. In some

embodiments a Cas9 has a fusion partner (or multiple fusion partners)(e.g., an NLS, a tag, a fusion partner providing an activity, etc.) at both the N-terminus and C-terminus.

[0263] Suitable fusion partners that provide for increased or decreased stability include, but are not limited to degron sequences. Degrons are readily understood by one of ordinary skill in the art to be amino acid sequences that control the stability of the protein of which they are part. For example, the stability of a protein comprising a degron sequence is controlled in part by the degron sequence. In some embodiments, a suitable degron is constitutive such that the degron exerts its influence on protein stability independent of experimental control (i.e., the degron is not drug inducible, temperature inducible, etc.) In some embodiments, the degron provides the variant Cas9 polypeptide with controllable stability such that the variant Cas9 polypeptide can be turned "on" (i.e., stable) or "off" (i.e., unstable, degraded) depending on the desired conditions. For example, if the degron is a temperature sensitive degron, the variant Cas9 polypeptide may be functional (i.e., "on", stable) below a threshold temperature (e.g., 42°C, 41°C, 40°C, 39°C, 38°C, 37°C, 36°C, 35°C, 34°C, 33°C, 32°C, 31°C, 30°C, etc.) but non-functional (i.e., "off", degraded) above the threshold temperature. As another example, if the degron is a drug inducible degron, the presence or absence of drug can switch the protein from an "off" (i.e., unstable) state to an "on" (i.e., stable) state or vice versa. An exemplary drug inducible degron is derived from the FKBP12 protein. The stability of the degron is controlled by the presence or absence of a small molecule that binds to the degron.

[0264] Examples of suitable degrons include, but are not limited to those degrons controlled by Shield-1, DHFR, auxins, and/or temperature. Non-limiting examples of suitable degrons are known in the art (e.g., Dohmen et al., Science, 1994. 263(5151): p. 1273-1276: Heat-inducible degron: a method for constructing temperature-sensitive mutants; Schoeber et al., Am J Physiol Renal Physiol. 2009 Jan;296(1):F204-11 : Conditional fast expression and function of multimeric TRPV5 channels using Shield-1 ; Chu et al., Bioorg Med Chem Lett. 2008 Nov 15;18(22):5941-4: Recent progress with FKBP-derived destabilizing domains; Kanemaki, Pflugers Arch. 2012 Dec 28: Frontiers of protein expression control with conditional degrons; Yang et al., Mol Cell. 2012 Nov 30;48(4):487-8: Titivated for destruction: the methyl degron; Barbour et al., Biosci Rep. 2013 Jan 18;33(1).: Characterization of the bipartite degron that regulates ubiquitin-independent degradation of thymidylate synthase; and Greussing et al., J Vis Exp. 2012 Nov 10;(69): Monitoring of ubiquitin-proteasome activity in living cells using a Degron (dgn)-destabilized green

fluorescent protein (GFP)-based reporter protein; all of which are hereby incorporated in their entirety by reference).

[0265] Exemplary degron sequences have been well-characterized and tested in both cells and animals. Thus, fusing Cas9 (e.g., wild type Cas9; variant Cas9; variant Cas9 with reduced nuclease activity, e.g., dCas9; and the like) to a degron sequence produces a "tunable" and "inducible" Cas9 polypeptide. Any of the fusion partners described herein can be used in any desirable combination. As one non-limiting example to illustrate this point, a Cas9 fusion protein (i.e., a chimeric Cas9 polypeptide) can comprise a YFP sequence for detection, a degron sequence for stability, and transcription activator sequence to increase transcription of the target nucleic acid. A suitable reporter protein for use as a fusion partner for a Cas9 polypeptide (e.g., wild type Cas9, variant Cas9, variant Cas9 with reduced nuclease function, etc.), includes, but is not limited to, the following exemplary proteins (or functional fragment thereof): his3, β-galactosidase, a fluorescent protein (e.g., GFP, RFP, YFP, cherry, tomato, etc., and various derivatives thereof), luciferase, β- glucuronidase, and alkaline phosphatase. Furthermore, the number of fusion partners that can be used in a Cas9 fusion protein is unlimited. In some embodiments, a Cas9 fusion protein comprises one or more (e.g. two or more, three or more, four or more, or five or more) heterologous sequences.

[0266] Suitable fusion partners include, but are not limited to, a polypeptide that provides for methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity, or demyristoylation activity, any of which can be directed at modifying nucleic acid directly (e.g., methylation of DNA or RNA) or at modifying a nucleic acid-associated polypeptide (e.g., a histone, a DNA binding protein, and RNA binding protein, and the like). Further suitable fusion partners include, but are not limited to boundary elements (e.g., CTCF), proteins and fragments thereof that provide periphery recruitment (e.g., Lamin A, Lamin B, etc.), and protein docking elements (e.g., FKBP/FRB, Pil1/Aby1, etc.).

[0267] Examples of various additional suitable fusion partners (or fragments thereof) for a subject variant Cas9 polypeptide include, but are not limited to those described in the PCT patent applications: WO2010/075303, WO2012/068627, and WO2013/155555 which are hereby incorporated by reference in their entirety.

[0268] Suitable fusion partners include, but are not limited to, a polypeptide that provides an activity that indirectly increases transcription by acting directly on the target nucleic acid

or on a polypeptide (e.g., a histone, a DNA-binding protein, an RNA-binding protein, an RNA editing protein, etc.) associated with the target nucleic acid. Suitable fusion partners include, but are not limited to, a polypeptide that provides for methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity, or demyristoylation activity.

[0269] Additional suitable fusion partners include, but are not limited to, a polypeptide that directly provides for increased transcription and/or translation of a target nucleic acid (e.g., a transcription activator or a fragment thereof, a protein or fragment thereof that recruits a transcription activator, a small molecule/drug-responsive transcription and/or translation regulator, a translation-regulating protein, etc.).

[0270] Non-limiting examples of fusion partners to accomplish increased or decreased transcription include transcription activator and transcription repressor domains (e.g., the Krüppel associated box (KRAB or SKD); the Mad mSIN3 interaction domain (SID); the ERF repressor domain (ERD), etc.). In some such embodiments, a Cas9 fusion protein is targeted by the guide nucleic acid to a specific location (i.e., sequence) in the target nucleic acid and exerts locus-specific regulation such as blocking RNA polymerase binding to a promoter (which selectively inhibits transcription activator function), and/or modifying the local chromatin status (e.g., when a fusion sequence is used that modifies the target nucleic acid or modifies a polypeptide associated with the target nucleic acid). In some embodiments, the changes are transient (e.g., transcription repression or activation). In some embodiments, the changes are inheritable (e.g., when epigenetic modifications are made to the target nucleic acid or to proteins associated with the target nucleic acid, e.g., nucleosomal histones).

[0271] Non-limiting examples of fusion partners for use when targeting ssRNA target nucleic acids include (but are not limited to): splicing factors (e.g., RS domains); protein translation components (e.g., translation initiation, elongation, and/or release factors; e.g., eIF4G); RNA methylases; RNA editing enzymes (e.g., RNA deaminases, e.g., adenosine deaminase acting on RNA (ADAR), including A to I and/or C to U editing enzymes); heliembodiments; RNA-binding proteins; and the like. It is understood that a fusion partner can include the entire protein or in some embodiments can include a fragment of the protein (e.g., a functional domain).

[0272] In some embodiments, the heterologous sequence can be fused to the C-terminus of the Cas9 polypeptide. In some embodiments, the heterologous sequence can be fused to

the N-terminus of the Cas9 polypeptide. In some embodiments, the heterologous sequence can be fused to an internal portion (i.e., a portion other than the N- or C-terminus) of the Cas9 polypeptide.

[0273] In addition the fusion partner of a chimeric Cas9 polypeptide can be any domain capable of interacting with ssRNA (which, for the purposes of this disclosure, includes intramolecular and/or intermolecular secondary structures, e.g., double-stranded RNA duplexes such as hairpins, stem-loops, etc.), whether transiently or irreversibly, directly or indirectly, including but not limited to an effector domain selected from the group comprising; Endonucleases (for example RNase I, the CRR22 DYW domain, Dicer, and PIN (PilT N-terminus) domains from proteins such as SMG5 and SMG6); proteins and protein domains responsible for stimulating RNA cleavage (for example CPSF, CstF, CFIm and CFIIm); Exonucleases (for example XRN-1 or Exonuclease T) ; Deadenylases (for example HNT3); proteins and protein domains responsible for nonsense mediated RNA decay (for example UPF1, UPF2, UPF3, UPF3b, RNP S1, Y14, DEK, REF2, and SRm160); proteins and protein domains responsible for stabilizing RNA (for example PABP) ; proteins and protein domains responsible for repressing translation (for example Ago2 and Ago4); proteins and protein domains responsible for stimulating translation (for example Staufen); proteins and protein domains responsible for (e.g., capable of) modulating translation (e.g., translation factors such as initiation factors, elongation factors, release factors, etc., e.g., eIF4G); proteins and protein domains responsible for polyadenylation of RNA (for example PAP1, GLD-2, and Star- PAP); proteins and protein domains responsible for polyuridinylation of RNA (for example CI D1 and terminal uridylate transferase) ; proteins and protein domains responsible for RNA localization (for example from IMP1, ZBP1, She2p, She3p, and Bicaudal-D); proteins and protein domains responsible for nuclear retention of RNA (for example Rrp6); proteins and protein domains responsible for nuclear export of RNA (for example TAP, NXF1, THO, TREX, REF, and Aly); proteins and protein domains responsible for repression of RNA splicing (for example PTB, Sam68, and hnRNP A1); proteins and protein domains responsible for stimulation of RNA splicing (for example Serine/Arginine-rich (SR) domains); proteins and protein domains responsible for reducing the efficiency of transcription (for example FUS (TLS)); and proteins and protein domains responsible for stimulating transcription (for example CDK7 and HIV Tat). Alternatively, the effector domain may be selected from the group comprising Endonucleases; proteins and protein domains capable of stimulating RNA cleavage; Exonucleases; Deadenylases; proteins and protein domains having nonsense mediated RNA decay activity; proteins and protein

domains capable of stabilizing RNA; proteins and protein domains capable of repressing translation; proteins and protein domains capable of stimulating translation; proteins and protein domains capable of modulating translation (e.g., translation factors such as initiation factors, elongation factors, release factors, etc., e.g., eIF4G); proteins and protein domains capable of polyadenylation of RNA; proteins and protein domains capable of polyuridinylation of RNA; proteins and protein domains having RNA localization activity; proteins and protein domains capable of nuclear retention of RNA; proteins and protein domains having RNA nuclear export activity; proteins and protein domains capable of repression of RNA splicing; proteins and protein domains capable of stimulation of RNA splicing; proteins and protein domains capable of reducing the efficiency of transcription; and proteins and protein domains capable of stimulating transcription. Another suitable fusion partner is a PUF RNA-binding domain, which is described in more detail in WO2012068627.

[0274] Some RNA splicing factors that can be used (in whole or as fragments thereof) as fusion partners for a Cas9 polypeptide have modular organization, with separate sequence-specific RNA binding modules and splicing effector domains. For example, members of the Serine/ Arginine-rich (SR) protein family contain N-terminal RNA recognition motifs (RRMs) that bind to exonic splicing enhancers (ESEs) in pre-mRNAs and C-terminal RS domains that promote exon inclusion. As another example, the hnRNP protein hnRNP Al binds to exonic splicing silencers (ESSs) through its RRM domains and inhibits exon inclusion through a C-terminal Glycine-rich domain. Some splicing factors can regulate alternative use of splice site (ss) by binding to regulatory sequences between the two alternative sites. For example, ASF/SF2 can recognize ESEs and promote the use of intron proximal sites, whereas hnRNP Al can bind to ESSs and shift splicing towards the use of intron distal sites. One application for such factors is to generate ESFs that modulate alternative splicing of endogenous genes, particularly disease associated genes. For example, Bcl-x pre-mRNA produces two splicing isoforms with two alternative 5' splice sites to encode proteins of opposite functions. The long splicing isoform Bcl-xL is a potent apoptosis inhibitor expressed in long-lived postmitotic cells and is up-regulated in many cancer cells, protecting cells against apoptotic signals. The short isoform Bcl-xS is a pro-apoptotic isoform and expressed at high levels in cells with a high turnover rate (e.g., developing lymphocytes). The ratio of the two Bcl-x splicing isoforms is regulated by multiple cώ-elements that are located in either the core exon region or the exon extension region (i.e., between the two alternative 5' splice sites). For more examples, see WO2010075303.

[0275] In some embodiments, a Cas9 polypeptide (e.g., a wild type Cas9, a variant Cas9, a variant Cas9 with reduced nuclease activity, etc.) can be linked to a fusion partner via a peptide spacer.

[0276] In some embodiments, a Cas9 polypeptide comprises a "Protein Transduction Domain" or PTD (also known as a CPP – cell penetrating peptide), which may refer to a polypeptide, polynucleotide, carbohydrate, or organic or inorganic compound that facilitates traversing a lipid bilayer, micelle, cell membrane, organelle membrane, or vesicle membrane. A PTD attached to another molecule, which can range from a small polar molecule to a large macromolecule and/or a nanoparticle, facilitates the molecule traversing a membrane, for example going from extracellular space to intracellular space, or cytosol to within an organelle. In some embodiments, a PTD attached to another molecule facilitates entry of the molecule into the nucleus (e.g., in some embodiments, a PTD includes a nuclear localization signal (NLS)). In some embodiments, a Cas9 polypeptide comprises two or more NLSs, e.g., two or more NLSs in tandem. In some embodiments, a PTD is covalently linked to the amino terminus of a Cas9 polypeptide. In some embodiments, a PTD is covalently linked to the carboxyl terminus of a Cas9 polypeptide. In some embodiments, a PTD is covalently linked to the amino terminus and to the carboxyl terminus of a Cas9 polypeptide. In some embodiments, a PTD is covalently linked to a nucleic acid (e.g., a guide nucleic acid, a polynucleotide encoding a guide nucleic acid, a polynucleotide encoding a Cas9 polypeptide, etc.). Exemplary PTDs include but are not limited to a minimal undecapeptide protein transduction domain (corresponding to residues 47-57 of HIV-1 TAT comprising YGRKKRRQRRR; SEQ ID NO:7); a polyarginine sequence comprising a number of arginines sufficient to direct entry into a cell (e.g., 3, 4, 5, 6, 7, 8, 9, 10, or 10-50 arginines); a VP22 domain (Zender et al. (2002) *Cancer Gene Ther.* 9(6):489-96); an Drosophila Antennapedia protein transduction domain (Noguchi et al. (2003) *Diabetes* 52(7):1732-1737); a truncated human calcitonin peptide (Trehin et al. (2004) *Pharm. Research* 21:1248-1256); polylysine (Wender et al. (2000) *Proc. Natl. Acad. Sci. USA* 97:13003-13008); RRQRRTSKLMKR (SEQ ID NO:8); Transportan GWTLNSAGYLLGKINLKALAALAKKIL (SEQ ID NO:9); KALAWEAKLAKALAKALAKHLAKALAKALKCEA (SEQ ID NO:10); and RQIKIWFQNRRMKWKK (SEQ ID NO:11). Exemplary PTDs include but are not limited to, YGRKKRRQRRR (SEQ ID NO:12), RKKRRQRRR (SEQ ID NO:13); an arginine homopolymer of from 3 arginine residues to 50 arginine residues; Exemplary PTD domain amino acid sequences include, but are not limited to, any of the following: YGRKKRRQRRR

(SEQ ID NO:14); RKKRRQRR (SEQ ID NO:15); YARAAARQARA (SEQ ID NO:16); THRLPRRRRRR (SEQ ID NO:17); and GGRRARRRRRR (SEQ ID NO:18). In some embodiments, the PTD is an activatable CPP (ACPP) (Aguilera et al. (2009) *Integr Biol (Camb)* June; 1(5-6): 371-381). ACPPs comprise a polycationic CPP (e.g., Arg9 or "R9") connected via a cleavable linker to a matching polyanion (e.g., Glu9 or "E9"), which reduces the net charge to nearly zero and thereby inhibits adhesion and uptake into cells. Upon cleavage of the linker, the polyanion is released, locally unmasking the polyarginine and its inherent adhesiveness, thus "activating" the ACPP to traverse the membrane.

[0277] In some embodiments, the composition can comprise a Cpf1 RNA-guided endonuclease, an example of which is provided in FIGs. 2, 3, or 4. Another name for the Cpf1 RNA-guided endonuclease is Cas12a. The Cpf1 CRISPR systems of the present disclosure comprise i) a single endonuclease protein, and ii) a crRNA, wherein a portion of the 3' end of crRNA contains the guide sequence complementary to a target nucleic acid. In this system, the Cpf1 nuclease is directly recruited to the target DNA by the crRNA. In some embodiments, guide sequences for Cpf1 must be at least 12nt, 13nt, 14nt, 15nt, or 16nt in order to achieve detectable DNA cleavage, and a minimum of 14nt, 15nt, 16nt, 17nt, or 18nt to achieve efficient DNA cleavage.

[0278] The Cpf1 systems of the present disclosure differ from Cas9 in a variety of ways. First, unlike Cas9, Cpf1 does not require a separate tracrRNA for cleavage. In some embodiments, Cpf1 crRNAs can be as short as about 42-44 bases long—of which 23-25 nt is guide sequence and 19 nt is the constitutive direct repeat sequence. In contrast, the combined Cas9 tracrRNA and crRNA synthetic sequences can be about 100 bases long.

[0279] Second, Cpf1 prefers a "TTN" PAM motif that is located 5′ upstream of its target. This is in contrast to the "NGG" PAM motifs located on the 3' of the target DNA for Cas9 systems. In some embodiments, the uracil base immediately preceding the guide sequence cannot be substituted (Zetsche, B. et al. 2015. "Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System" Cell 163, 759-771, which is hereby incorporated by reference in its entirety for all purposes).

[0280] Third, the cut sites for Cpf1 are staggered by about 3-5 bases, which create "sticky ends" (Kim et al., 2016. "Genome-wide analysis reveals specificities of Cpf1 endonucleases in human cells" published online June 06, 2016). These sticky ends with 3-5 bp overhangs are thought to facilitate NHEJ-mediated-ligation, and improve gene editing of DNA fragments with matching ends. The cut sites are in the 3′ end of the target DNA, distal to the 5′ end where the PAM is. The cut positions usually follow the 18th base on the non-

hybridized strand and the corresponding 23rd base on the complementary strand hybridized to the crRNA.

[0281] Fourth, in Cpf1 complexes, the "seed" region is located within the first 5 nt of the guide sequence. Cpf1 crRNA seed regions are highly sensitive to mutations, and even single base substitutions in this region can drastically reduce cleavage activity (see Zetsche B. et al. 2015 "Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System" Cell 163, 759-771). Critically, unlike the Cas9 CRISPR target, the cleavage sites and the seed region of Cpf1 systems do not overlap. Additional guidance on designing Cpf1 crRNA targeting oligos is available on (Zetsche B. et al. 2015. "Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System" Cell 163, 759-771).

[0282] Persons skilled in the art will appreciate that the Cpf1 disclosed herein can be any variant derived or isolated from any source, many of which are known in the art. For example, in some embodiments, the Cpf1 peptide of the present disclosure can include FnCPF1 (e.g., SEQ ID NO: 2) set forth in FIG. 2, AsCpf1 (e.g., Fig. 3), LbCpf1 (e.g., Fig. 4) or any other of the many known Cpf1 proteins from various other microorganism species, and synthetic variants thereof.

[0283] In some embodiments, the composition comprises a Cpf1 polypeptide. In some embodiments, the Cpf1 polypeptide is enzymatically active, e.g., the Cpf1 polypeptide, when bound to a guide RNA, cleaves a target nucleic acid. In some embodiments, the Cpf1 polypeptide exhibits reduced enzymatic activity relative to a wild-type Cpf1 polypeptide (e.g., relative to a Cpf1 polypeptide comprising the amino acid sequence depicted in FIGs. 2, 3, or 4), and retains DNA binding activity.

[0284] In some embodiments, a Cpf1 polypeptide comprises an amino acid sequence having at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 90%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIGs. 2, 3, or 4. In some embodiments, a Cpf1 polypeptide comprises an amino acid sequence having at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 90%, or 100%, amino acid sequence identity to a contiguous stretch of from 100 amino acids to 200 amino acids (aa), from 200 aa to 400 aa, from 400 aa to 600 aa, from 600 aa to 800 aa, from 800 aa to 1000 aa, from 1000 aa to 1100 aa, from 1100 aa to 1200 aa, or from 1200 aa to 1300 aa, of the amino acid sequence depicted in FIGs. 2, 3, or 4.

[0285] In some embodiments, a Cpf1 polypeptide comprises an amino acid sequence having at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 90%, or 100%, amino acid sequence identity to the RuvCI domain of a Cpf1 polypeptide of the amino acid sequence depicted in FIGs. 2, 3, or 4. In some embodiments, a Cpf1 polypeptide comprises an amino acid sequence having at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 90%, or 100%, amino acid sequence identity to the RuvCII domain of a Cpf1 polypeptide of the amino acid sequence depicted in FIGs. 2, 3, or 4. In some embodiments, a Cpf1 polypeptide comprises an amino acid sequence having at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 90%, or 100%, amino acid sequence identity to the RuvCIII domain of a Cpf1 polypeptide of the amino acid sequence depicted in FIGs. 2, 3, or 4.

[0286] In some embodiments, the Cpf1 polypeptide exhibits reduced enzymatic activity relative to a wild-type Cpf1 polypeptide (e.g., relative to a Cpf1 polypeptide comprising the amino acid sequence depicted in FIGs. 2, 3, or 4), and retains DNA binding activity. In some embodiments, a Cpf1 polypeptide comprises an amino acid sequence having at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 90%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIGs. 2, 3, or 4; and comprises an amino acid substitution (e.g., a D→A substitution) at an amino acid residue corresponding to amino acid 917 of the amino acid sequence depicted in FIGs. 2, 3, or 4. In some embodiments, a Cpf1 polypeptide comprises an amino acid sequence having at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 90%, or 100%, amino acid sequence identity to the amino acid sequence depicted in FIGs. 2, 3, or 4; and comprises an amino acid substitution (e.g., an E→A substitution) at an amino acid residue corresponding to amino acid 1006 of the amino acid sequence depicted in FIGs. 2, 3, or 4. In some embodiments, a Cpf1 polypeptide comprises an amino acid sequence having at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 90%, or 100%, amino acid sequence identity to the amino acid sequence

depicted in FIGs. 2, 3, or 4; and comprises an amino acid substitution (e.g., a D→A substitution) at an amino acid residue corresponding to amino acid 1255 of the amino acid sequence depicted in FIGs. 2, 3, or 4.

[0287] In some embodiments, the Cpf1 polypeptide is a fusion polypeptide, e.g., where a Cpf1 fusion polypeptide comprises: a) a Cpf1 polypeptide; and b) a heterologous fusion partner. In some embodiments, the heterologous fusion partner is fused to the N-terminus of the Cpf1 polypeptide. In some embodiments, the heterologous fusion partner is fused to the C-terminus of the Cpf1 polypeptide. In some embodiments, the heterologous fusion partner is fused to both the N-terminus and the C-terminus of the Cpf1 polypeptide. In some embodiments, the heterologous fusion partner is inserted internally within the Cpf1 polypeptide.

[0288] Suitable heterologous fusion partners include NLS, epitope tags, fluorescent polypeptides, and the like.

Linked Guide RNA and Donor Nucleic Acid

[0289] In one aspect, the invention provides a complex comprising a CRISPR system comprising an RNA-guided endonuclease (*e.g.* a Cas9 or Cpf1 polypeptide), a guide RNA and a donor polynucleotide, wherein the guide RNA and the donor polynucleotide are linked. As exemplified herein, the guide RNA and donor polynucleotide can be either covalently or non-covalently linked. In one embodiment, the guide RNA and donor polynucleotide are chemically ligated. In another embodiment, the guide RNA and donor polynucleotide are enzymatically ligated. In one embodiment, the guide RNA and donor polynucleotide hybridize to each other. In another embodiment, the guide RNA and donor polynucleotide both hybridize to a bridge sequence. Any number of such hybridization schemes are possible.

Deaminase

[0290] In some embodiments, the complex or composition further comprises a deaminase (e.g., an adenine base editor). As used herein, the term "deaminase" or "deaminase domain" refers to an enzyme that catalyzes the removal of an amine group from a molecule, or deamination. In some embodiments, the deaminase is a cytidine deaminase, catalyzing the hydrolytic deamination of cytidine or deoxycytidine to uridine or deoxyuridine, respectively. In some embodiments, the deaminase is a cytosine deaminase, catalyzing the hydrolytic deamination of cytosine to uracil (e.g., in RNA) or thymine (e.g., in DNA).

[0291] In some embodiments, the deaminase is an adenosine deaminase, which catalyzes the hydrolytic deamination of adenine or adenosine. In some embodiments, the deaminase or deaminase domain is an adenosine deaminase, catalyzing the hydrolytic deamination of adenosine or deoxyadenosine to inosine or deoxyinosine, respectively. In some embodiments, the adenosine deaminase catalyzes the hydrolytic deamination of adenine or adenosine in deoxyribonucleic acid (DNA). The adenosine deaminases (e.g. engineered adenosine deaminases, evolved adenosine deaminases) provided herein may be from any organism, such as a bacterium. In some embodiments, the deaminase or deaminase domain is a variant of a naturally-occurring deaminase from an organism, such as a human, chimpanzee, gorilla, monkey, cow, dog, rat, or mouse.

[0292] In some embodiments, the deaminase or deaminase domain does not occur in nature. For example, in some embodiments, the deaminase or deaminase domain is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75% at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to a naturally-occurring deaminase. In some embodiments, the adenosine deaminase is from a bacterium, such as, *E. coli, S. aureus, S. typhi, S. putrefaciens, H. influenzae*, or *C. crescentus*. In some embodiments, the adenosine deaminase is a TadA deaminase. In some embodiments, the TadA deaminase is an *E. coli* TadA deaminase (ecTadA). In some embodiments, the TadA deaminase is a truncated *E. coli* TadA deaminase. For example, the truncated ecTadA may be missing one or more N-terminal amino acids relative to a full-length ecTadA. In some embodiments, the truncated ecTadA may be missing 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 N-terminal amino acid residues relative to the full length ecTadA. In some embodiments, the truncated ecTadA may be missing 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 6, 17, 18, 19, or 20 C-terminal amino acid residues relative to the full length ecTadA. In some embodiments, the ecTadA deaminase does not comprise an N-terminal methionine. In some embodiments, the deaminase is APOBEC1 or a variant thereof.

[0293] The deaminase can be used in conjugation with any of the other CRISPR elements described herein (i.e., as a composition), or the deaminase can be fused to any of the other CRISPR elements (e.g., Cas9 or Cpf1) described herein (i.e., as a complex). In certain embodiments, the deaminase is fused to Cas9, Cpf1, or a variant thereof.

Other Components

[0294] The composition can further comprise any other components typically used in nucleic acid or protein delivery formulations. For instance, the composition can further comprise lipids, lipoproteins (e.g., cholesterol and derivatives), phospholipids, polymers or other components of liposomal or micellar delivery vehicles. The composition also can comprise solvent or carrier suitable for administration to cells or hosts, such as a mammal or human.

[0295] In some embodiments, the composition further comprises one or more surfactants and cryoprotectants. The surfactant can be a non-ionic surfactant and/or a zwitterionic surfactant. A list of exemplary surfactants includes, but is not limited to: the polyoxyethylene sorbitan esters surfactants (commonly referred to as the Tweens), especially polysorbate 20 and polysorbate 80; copolymers of ethylene oxide (EO), propylene oxide (PO), and/or butylene oxide (BO), sold under the DOWFAX™ tradename, such as linear EO/PO block copolymers; octoxynols, which can vary in the number of repeating ethoxy (oxy-1,2-ethanediyl) groups, with octoxynol-9 (Triton X-100, or t-octylphenoxypolyethoxyethanol) being of particular interest; (octylphenoxy)polyethoxyethanol (IGEPAL CA-6301NP-40); phospholipids such as phosphatidylcholine (lecithin); polyoxyethylene fatty ethers derived from lauryl, cetyl, stearyl and oleyl alcohols (known as Brij surfactants), such as triethyleneglycol monolauryl ether (Brij 30); polyoxyethylene-9-lauryl ether, and sorbitan esters (commonly known as the Spans), such as sorbitan trioleate (Span 85) and sorbitan monolaurate. In some embodiments, the surfactant is an anticoagulant (e.g., heparin or the like). In some embodiments, the composition further comprises one or more pharmaceutically acceptable carriers (e.g., an aqueous carrier, such as water) and/or excipients, including buffers, antioxidants, pH adjusting agents, chelating agents, osmotic agents, cryo- or lyoprotectants and the like).

[0296] In some instances, a component (e.g., a nucleic acid component (e.g., a guide nucleic acid, etc.); a protein component (e.g., a Cas9 or Cpf1 polypeptide, a variant Cas9 or Cpf1 polypeptide); and the like) includes a label moiety. The terms "label", "detectable label", or "label moiety" as used herein refer to any moiety that provides for signal detection and may vary widely depending on the particular nature of the assay. Label moieties of interest include both directly detectable labels (direct labels)(e.g., a fluorescent label) and indirectly detectable labels (indirect labels)(e.g., a binding pair member). A fluorescent label can be any fluorescent label (e.g., a fluorescent dye (e.g., fluorescein, Texas red, rhodamine, ALEXAFLUOR® labels, and the like), a fluorescent protein (e.g., green fluorescent protein

(GFP), enhanced GFP (EGFP), yellow fluorescent protein (YFP), red fluorescent protein (RFP), cyan fluorescent protein (CFP), cherry, tomato, tangerine, and any fluorescent derivative thereof), etc.). Suitable detectable (directly or indirectly) label moieties for use in the methods include any moiety that is detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical, chemical, or other means. For example, suitable indirect labels include biotin (a binding pair member), which can be bound by streptavidin (which can itself be directly or indirectly labeled). Labels can also include: a radiolabel (a direct label)(e.g., ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P); an enzyme (an indirect label)(e.g., peroxidase, alkaline phosphatase, galactosidase, luciferase, glucose oxidase, and the like); a fluorescent protein (a direct label)(e.g., green fluorescent protein, red fluorescent protein, yellow fluorescent protein, and any convenient derivatives thereof); a metal label (a direct label); a colorimetric label; a binding pair member; and the like. By "partner of a binding pair" or "binding pair member" is meant one of a first and a second moiety, wherein the first and the second moiety have a specific binding affinity for each other. Suitable binding pairs include, but are not limited to: antigen/antibodies (for example, digoxigenin/anti-digoxigenin, dinitrophenyl (DNP)/anti-DNP, dansyl-X-anti-dansyl, fluorescein/anti-fluorescein, lucifer yellow/anti-lucifer yellow, and rhodamine anti-rhodamine), biotin/avidin (or biotin/streptavidin) and calmodulin binding protein (CBP)/calmodulin. Any binding pair member can be suitable for use as an indirectly detectable label moiety.

[0297] Any given component, or combination of components can be unlabeled, or can be detectably labeled with a label moiety. In some embodiments, when two or more components are labeled, they can be labeled with label moieties that are distinguishable from one another.

Encapsulation and Nanoparticles

[0298] In some embodiments of the composition, the one or more polymers (e.g., first polymer and, optionally, second polymer of the composition described herein) form nanoparticles in a carrier solution (e.g., an aqueous solution, such as water). Furthermore, when an agent for deliver is present with the polymer(s), the polymer(s)combines with the agent to be delivered (e.g., nucleic acid and/or polypeptide) and partially or completely encapsulates the agent to be delivered. The composition can, thus, provide a nanoparticle comprising the one or more of the polymers and nucleic acid and/or polypeptide.

[0299] The nanoparticles can have any suitable size. In some embodiments, the nanoparticles have a particle diameter of about 20 nm to about 800 nm (e.g., about 40 nm to

about 800 nm, about 100 nm to about 800 nm, about 200 nm to about 800 nm, about 20 nm to about 400 nm, about 40 nm to about 400 nm, about 100 nm to about 400 nm, about 200 nm to about 400 nm, about 100 nm to about 800 nm, about 100 nm to about 400 nm, or about 100 nm to about 200 nm).

[0300] The nanoparticles can have any suitable surface charge. For instance, the zeta potential of the nanoparticles can be about -5 mV to about +40 mV (e.g., about -5 mV to about +30 mV, about -5 mV to about +20 mV, about -5 mV to about +10 mV, about -5 mV to about +5 mV, about 0 mV to about +40 mV, about 0 mV to about +30 mV, about 0 mV to about +20 mV, about 0 mV to about +10 mV, or about 0 mV to about +5 mV).

[0301] In some embodiments, the composition can comprise a core nanoparticle in addition to the one or more of the polymers described herein and the nucleic acid or polypeptide. Any suitable nanoparticle can be used, including metal (e.g., gold) nanoparticles or polymer nanoparticles. However, a core nanoparticle is not required and, in some embodiments, the composition is free of a core nanoparticle.

[0302] The one or more of the polymers described herein and the nucleic acid (e.g., guide RNA, donor polynucleotide, or both) or polypeptide can be conjugated directly or indirectly to a surface of a core nanoparticle if desired. For example, the one or more of the polymers described herein and the nucleic acid (e.g., guide RNA, donor polynucleotide, or both) or polypeptide can be conjugated directly to the surface of a nanoparticle or indirectly through an intervening linker.

[0303] Any type of molecule can be used as a linker. For example, a linker can be an aliphatic chain including at least two carbon atoms (e.g., 3, 4, 5, 6, 7, 8, 9, 10 or more carbon atoms), and can be substituted with one or more functional groups including ketone, ether, ester, amide, alcohol, amine, urea, thiourea, sulfoxide, sulfone, sulfonamide, and disulfide functionalities. In embodiments where the nanoparticle includes gold, a linker can be any thiol-containing molecule. Reaction of a thiol group with the gold results in a covalent sulfide (-S-) bond. Linker design and synthesis are well known in the art.

[0304] In some embodiments, the nucleic acid conjugated to the nanoparticle is a linker nucleic acid that serves to non-covalently bind one or more elements described herein (e.g., a Cas9 polypeptide, and a guide RNA, a donor polynucleotide, and a Cpf1 polypeptide) to the nanoparticle-nucleic acid conjugate. For instance, the linker nucleic acid can have a sequence that hybridizes to the guide RNA or donor polynucleotide.

[0305] The nucleic acid conjugated to the nanoparticle can have any suitable length. When the nucleic acid is a guide RNA or donor polynucleotide, the length will be as suitable

for such molecules, as discussed herein and known in the art. If the nucleic acid is a linker nucleic acid, it can have any suitable length for a linker, for instance, a length of from 10 nucleotides (nt) to 1000 nt, e.g., from about 1 nt to about 25 nt, from about 25 nt to about 50 nt, from about 50 nt to about 100 nt, from about 100 nt to about 250 nt, from about 250 nt to about 500 nt, or from about 500 nt to about 1000 nt. In some instances, the nucleic acid conjugated to the nanoparticle (e.g., a colloidal metal (e.g., gold) nanoparticle; a nanoparticle comprising a biocompatible polymer) nanoparticle can have a length of greater than 1000 nt.

[0306] When the nucleic acid linked (e.g., covalently linked; non-covalently linked) to a nanoparticle comprises a nucleotide sequence that hybridizes to at least a portion of the guide RNA or donor polynucleotide present in a complex of the present disclosure, it has a region with sequence identity to a region of the complement of the guide RNA or donor polynucleotide sequence sufficient to facilitate hybridization. In some embodiments, a nucleic acid linked to a nanoparticle in a complex of the present disclosure has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, nucleotide sequence identity to a complement of from 10 to 50 nucleotides (e.g., from 10 nucleotides (nt) to 15 nt, from 15 nt to 20 nt, from 20 nt to 25 nt, from 25 nt to 30 nt, from 30 nt to 40 nt, or from 40 nt to 50 nt) of a guide RNA or donor polynucleotide present in the complex.

[0307] In some embodiments, a nucleic acid linked (e.g., covalently linked; non-covalently linked) to a nanoparticle is a donor polynucleotide, or has the same or substantially the same nucleotide sequence as a donor polynucleotide. In some embodiments, a nucleic acid linked (e.g., covalently linked; non-covalently linked) to a nanoparticle comprises a nucleotide sequence that is complementary to a donor DNA template.

Method of Use

[0308] Also provided herein is a method of delivering a nucleic acid and/or polypeptide to a cell, wherein the cell can be *in vitro* or *in vivo*. The method comprises administering a composition as described herein comprising the first polymer and, optionally, second polymer along with an agent to be delivered (e.g., a nucleic acid and/or polypeptide), to the cell or to a subject containing the cell. The method can be used with respect to any type of cell or subject, but is particularly useful for mammalian cells (e.g., human cells). In some embodiments, the one or more of the polymers comprises a targeting agent, such that nucleic acid and/or polypeptide is delivered predominantly or exclusively to target cells or tissues

(e.g., cells or tissues of the peripheral nervous system, the central nervous system, the eye of the subject, liver, muscle, lung, bone (e.g., hematopoietic cells), or tumor cells or tissues).

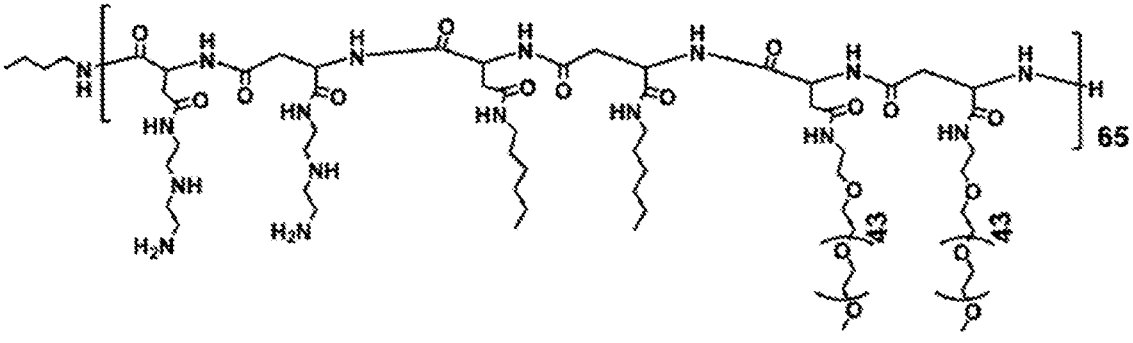
[0309] When used to deliver a protein or nucleic acid to a cell in a subject (i.e., *in vivo*), it is desirable that the polymer(s) are stable in serum. Stability in serum can be assessed as a function of the efficiency by which the polymers deliver a protein or nucleic acid payload to a cell in serum (e.g., *in vitro* or *in vivo)*. Thus, in some embodiments, the polymer(s) deliver a given protein or nucleic acid to a cell in serum with an efficiency greater than pAsp[DET] under the same conditions.

[0310] When used with a composition comprising one or more components of a CRISPR system, the method may be employed to edit a target nucleic acid or gene. In some embodiments, a method of modifying a target nucleic acid comprises homology-directed repair (HDR). In some embodiments, use of a complex of the present disclosure to carry out HDR provides an efficiency of HDR of at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, or more than 25%. In some embodiments, a method of modifying a target nucleic acid comprises non-homologous end joining (NHEJ). In some embodiments, use of a complex of the present disclosure to carry out HDR provides an efficiency of NHEJ of at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, or more than 25%.

[0311] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

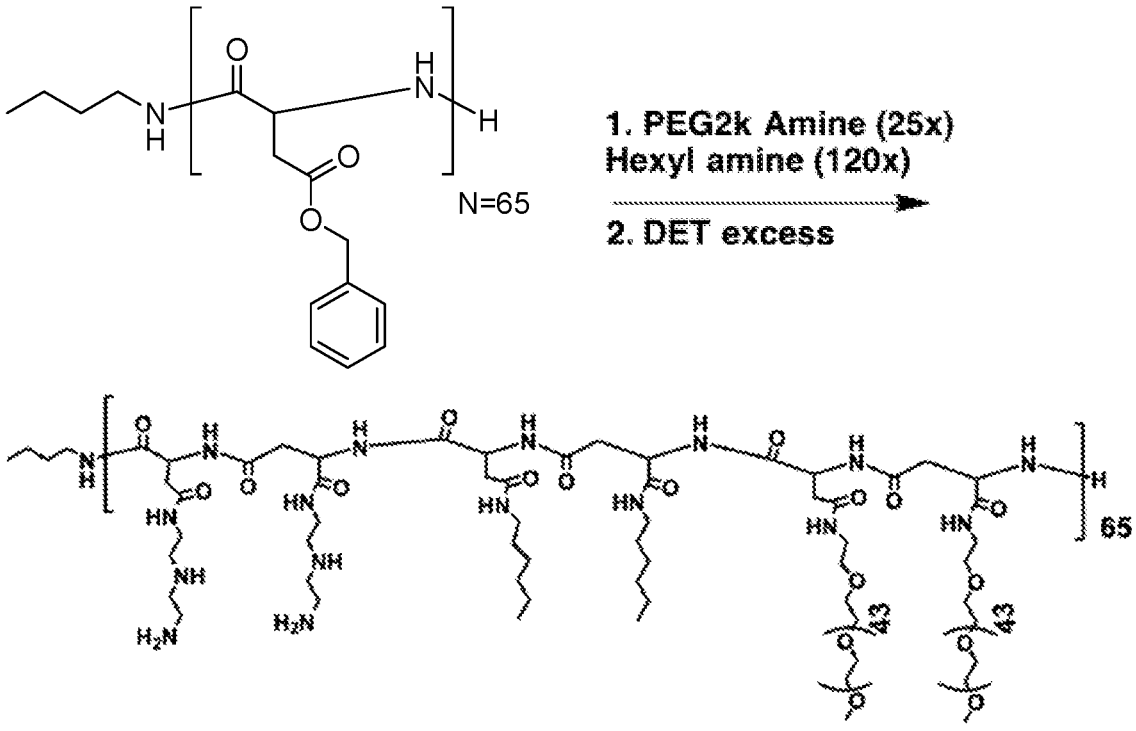
[0312] Polymer P2K25 was prepared and used in Examples 3-8, 10, 12, 15, and 16 provided herein:



(P2K25)

[0313] P2K25 was prepared by modifying PBLA with a PEGylated amine, hexyl amine, and *N*-(2-aminoethyl)ethane-1,2-diamine ("DET"), as in Scheme 1.

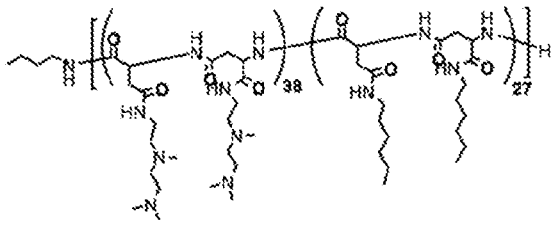
Scheme 1.



[0314] More particularly, lyophilized PBLA (50 mg, 0.0037 mMol; degree of polymerization ("DP") 65) was placed into a flask and dissolved in tetrahydrofuran/N-methyl-2-pyrrolidine (1 mL each). To the clear solution was added n-hexylamine (58.8 μL, 0.44 mMol, 120 equivalents) and PEGylated amine (PEG2K amine; weight average MW = 2000; number of ethylene oxide units (n) = 43-46 (approx.)) (25 equivalents), and the clear reaction mixture was stirred for 24 hours at room temperature. After approximately 24 hours, diethylenetriamine (excess equivalents to benzyl group of PBLA segment, 1.0 g) was added to the clear mixture under mild anhydrous conditions. After approximately 18 hours at room temperature, the reaction mixture was precipitated into diethyl ether (10 – 12X volume, 35 mL). The precipitate was then centrifuged, and washed twice with diethyl ether. The polymer was dissolved in 1 M HCl (3 mL) and dialyzed in an excess of deionized water in a 3.5 – 5 KD cut-off membrane. When the pH of the solution was between 5 – 6, the dialysis was stopped, and the solution was lyophilized, to give the polymer product.

EXAMPLE 2

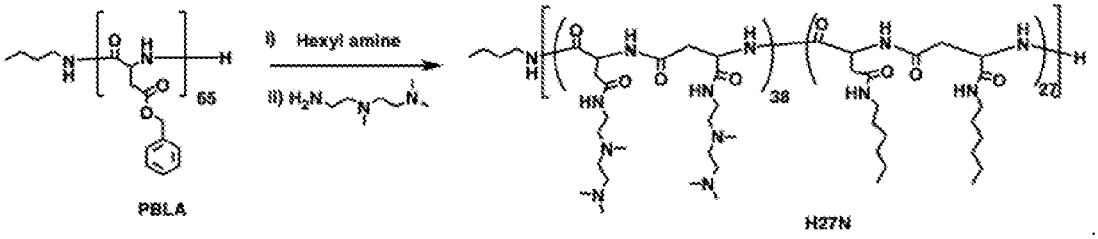
[0315] Polymer H27N was prepared and used in Examples 3-8, 10, and 14-20 provided herein:



(H27N; bracketing does not imply block copolymer structure).

[0316] H27N was prepared by modifying PBLA with N^1-(2-aminoethyl)-N^1,N^2,N^2-trimethylethane-1,2-diamine and hexylamine, as shown in Scheme 2.

Scheme 2.



[0317] Lyophilized PBLA (50 mg, 0.0037 mmol; degree of polymerization ("DP") 65) was placed into a flask and dissolved in tetrahydrofuran/N-methyl-2-pyrrolidine (1 mL each). To the clear solution was added n-hexylamine (160 equivalents), and the clear reaction mixture was stirred for 24 hours at room temperature. After approximately 24 hours, N^1-(2-aminoethyl)-N^1,N^2,N^2-trimethylethane-1,2-diamine (50 equivalents to benzyl group of PBLA segment) was added to the clear mixture under mild anhydrous conditions. After approximately 18 hours at room temperature, the reaction mixture was precipitated into diethyl ether (10 – 12X volume, 35 mL). The precipitate was then centrifuged, and washed twice with diethyl ether. The polymer was dissolved in 1 M HCl (3 mL) and dialyzed in an excess of deionized water in a 3.5 – 5 KD cut-off membrane. When the pH of the solution was between 5 – 6, the dialysis was stopped, and the solution was lyophilized, to give the polymer product.

EXAMPLE 3

[0318] The following example shows the stability of the polymer compositions provided herein as demonstrated by degree of aggregation of the nanoparticles.

[0319] Three compositions were prepared containing (i) a 60:40 ratio of H27N:P2K25 and no mCherry mRNA ("polymer only" control), (ii) a 60:40 ratio of H27N:P2K25 and mCherry mRNA, and (iii) a 100:0 ratio of H27N:P2K25 (i.e., only H27N polymer) and

mCherry mRNA (all ratios based on weight). The resulting nanoparticles were incubated in artificial cerebrospinal fluid (aCSF) for one hour at room temperature. aCSF can be a useful tool to gauge stability in CSF as aCSF has a similar ion concentration to CSF, which is a path that nanoparticle travels to reach CNS after intrathecal (IT) injection. After one hour, the degree of aggregation was viewed using a microscope. Microscope images are set forth in FIGs. 5A-5C.

[0320] Analysis of the microscope images demonstrated that nanoparticles of composition (iii) containing only H27N polymer and mCherry mRNA, without P2K25, resulted in significantly more aggregation than composition (ii) containing a 60:40 ratio of H27N:P2K25 polymer and mCherry mRNA. Similarly, minimal aggregation of the control "polymer only" sample was observed. These results show that the mixture of H27N and P2K25 polymers increases the stability of the nanoparticle.

EXAMPLE 4

[0321] The following example illustrates the use of polymer nanoparticles to deliver mCherry to Hep3B cells.

[0322] P2K25 (Example 1) was combined with H27N (Example 2) in a weight ratio of 40:60, and the resulting mixture was combined with mCherry mRNA to form nanoparticles. Hep3B cells were transfected with the resulting nanoparticles. After 48 hours, the presence of mCherry expression was viewed using a microscope. Microscope images are set forth in FIGs. 6A (Negative Control in Normal Field and Bright Field) and 6B (mRNA Nanoparticle in Normal Field and Bright Field).

[0323] The microscopic analysis showed that nanoparticles containing P2K25 and H27N effectively delivered mCherry mRNA to Hep3B cells. The results demonstrate that nanoparticles containing P2K25 and H27N are stable and effective at delivering a payload.

EXAMPLE 5

[0324] The following example illustrates the use of polymer nanoparticles to deliver mCherry to primary myoblasts.

[0325] P2K25 (as shown in Example 1) was combined with H27N (as shown in Example 2) in a weight ratio of 40:60 (P2K25:H27N), and the resulting mixture was combined with (i) mCherry mRNA to form nanoparticles, and heparin and (ii) mCherry mRNA to form nanoparticles (without heparin). Mouse primary myoblasts were transfected with the resulting mixtures (i.e., (i) and (ii)). 48 hours after transfection, the presence of mCherry expression was viewed using a microscope. Microscope images are set forth in FIGs. 7A

(mRNA Nanoparticle with heparin in Normal Field and Bright Field) and 7B (mRNA Nanoparticle in Normal Field and Bright Field).

[0326] Microscopic analysis showed that nanoparticles containing P2K25 and H27N without heparin effectively delivered mCherry mRNA to mouse primary myoblasts. As expected, the mRNA nanoparticle with heparin was unable to effectively deliver mCherry mRNA to mouse primary myoblasts, as heparin dissociates the nanoparticle such that no transfection can be observed.

EXAMPLE 6

[0327] The following example illustrates the use of polymer nanoparticles to deliver mCherry to human primary neural progenitor cells (NPCs).

[0328] P2K25 (as shown in Example 1) was combined with H27N (as shown in Example 2) in weight ratios of 10:90, 20:80, and 40:60 (P2K25:H27N), and the resulting mixture was combined with mCherry mRNA to form nanoparticles. The resulting nanoparticles were incubated in artificial cerebrospinal fluid (aCSF) for 30 minutes at 37 °C before adding to NPCs in culture medium with defined growth factors and without serum. Flow cytometry was conducted two days after the transfection and the percentage of mCherry+ cells was measured. The results are plotted in FIG. 8.

[0329] The results show that nanoparticles containing P2K25 and H27N effectively delivered mCherry mRNA even after incubating in aCSF. Such results demonstrate that nanoparticles containing P2K25 and H27N are stable and effective at delivering a payload.

EXAMPLE 7

[0330] The following example illustrates the use of polymer nanoparticles to deliver Cas9 RNP to GFP expressing HEK293T cells (GFP-HEK cells).

[0331] P2K25 (as shown in Example 1) was combined with H27N (as shown in Example 2) in a weight ratio of 50:50, and the resulting mixture was combined with Cas9 RNP to form nanoparticles with a lower (a) and higher (b) loading level of Cas9 RNPs were prepared with 1:1 or 2:1 molar ratio of gRNA to Cas9. The resulting nanoparticles were incubated with GFP-HEK cells in 10% FBS containing DMEM culture medium. The ability of the nanoparticles to knock out GFP was measured and the results are plotted in FIG. 9.

[0332] FIG. 9 shows that nanoparticles containing P2K25 and H27N effectively delivered Cas9 RNP into GFP-HEK cells and can induce efficient gene editing.

EXAMPLE 8

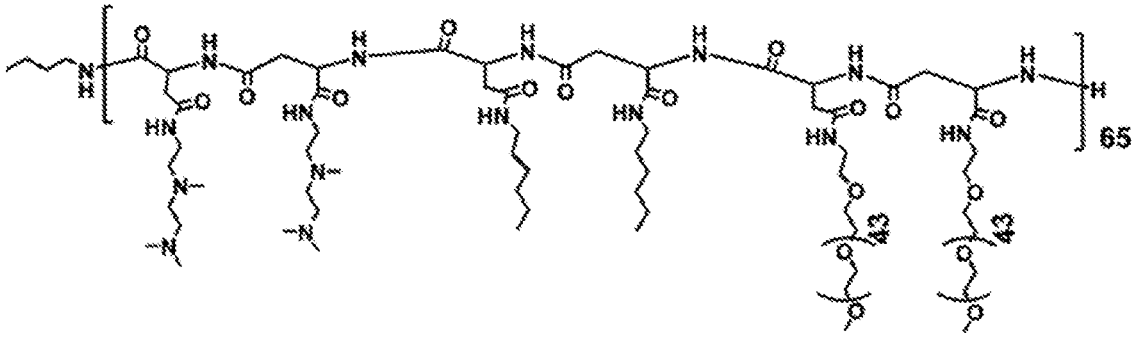
[0333] The following example illustrates the use of polymers of the invention to deliver Cre mRNA to mice as exhibited by Loxp-luciferase mice.

[0334] Loxp-luciferase mice having the reporter sequence set forth in FIG. 10 were treated with one of two nanoparticle compositions: (i) nanoparticles formulated with a 100:0 ratio of H27N:P2K25 and Cre mRNA ("mRNA + 1st Polymer"), or (ii) nanoparticles formulated with a 50:50 weight ratio of H27N:P2K25 and Cre mRNA ("mRNA + 2nd Generation Polymer"). The control represents an untreated mouse. Administration was via intrathecal (IT) injection. Cre mRNA delivery was assessed via bioluminescence, and the resulting images are set forth in FIGs. 11A-11C.

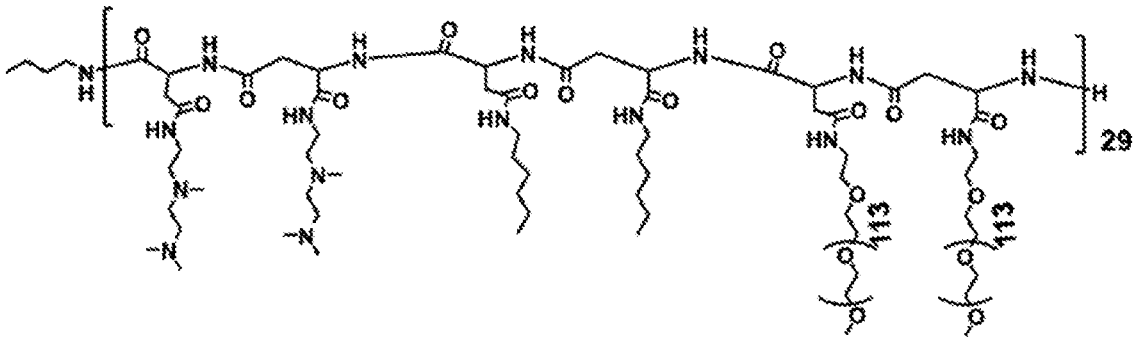
[0335] The mice treated with nanoparticle composition (ii) containing a 50:50 weight ratio of H27N:P2K25 and Cre mRNA ("mRNA + 2nd Generation Polymer") showed significantly more delivery of Cre mRNA into brain as compared to mice treated with the nanoparticles containing only N27N polymer and Cre mRNA. Further sectional analysis of the brain treated with H27N:P2K25 and Cre mRNA ("mRNA + 2nd Generation Polymer") showed distribution of Cre mRNA throughout the brain. No signs of toxicity or inflammation were observed.

EXAMPLE 9

[0336] Polymers II-41-3R, II-43-6, II-43-9, and II-43-10 were prepared and used in Examples 10 and 12 provided herein:



(II-41-3R)



(II-43-6)

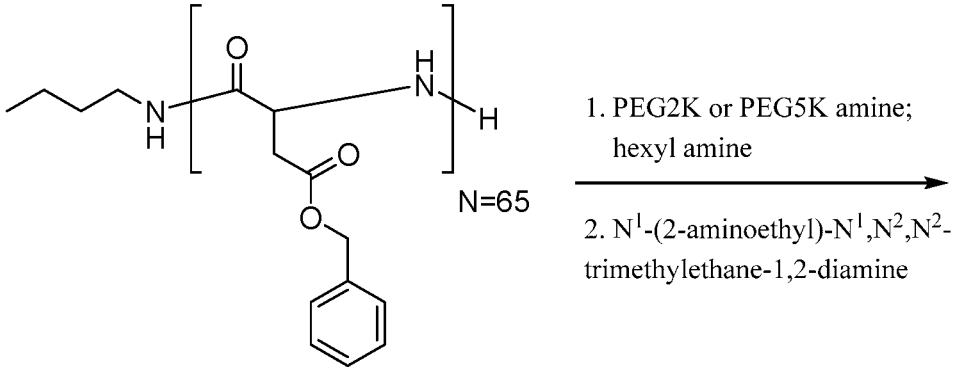
(II-43-9)

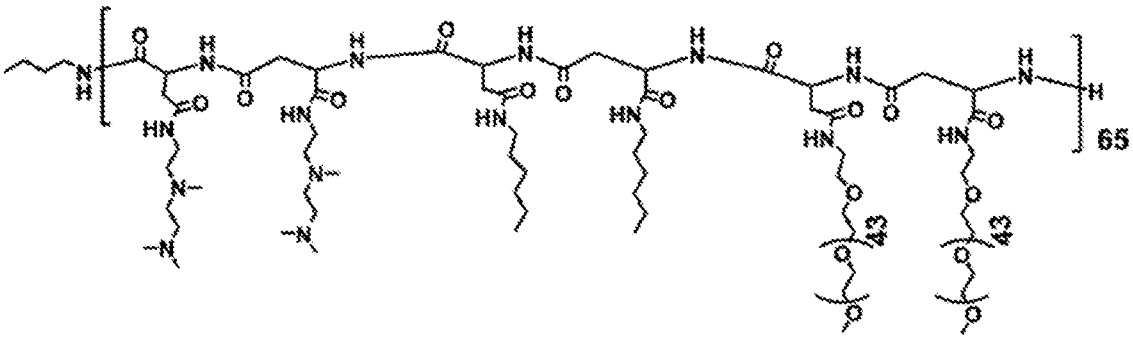
(II-43-10)

In the above structures, the numerals "65" and "29" outside of the brackets denotes the overall degree of polymerization in the polyamide backbone, and does not indicate that the bracketed area is a repeating unit. II-43-6, II-43-9, and II-43-10 have the same polymer structure but differ by the degree (i.e., relative amount) of side chain modification.

[0337] II-41-3R, II-43-6, II-43-9, and II-43-10 were prepared by modifying PBLA with a PEGylated amine, hexyl amine, and N^1-(2-aminoethyl)-N^1,N^2,N^2-trimethylethane-1,2-diamine, as illustrated in Scheme 3.

Scheme 3

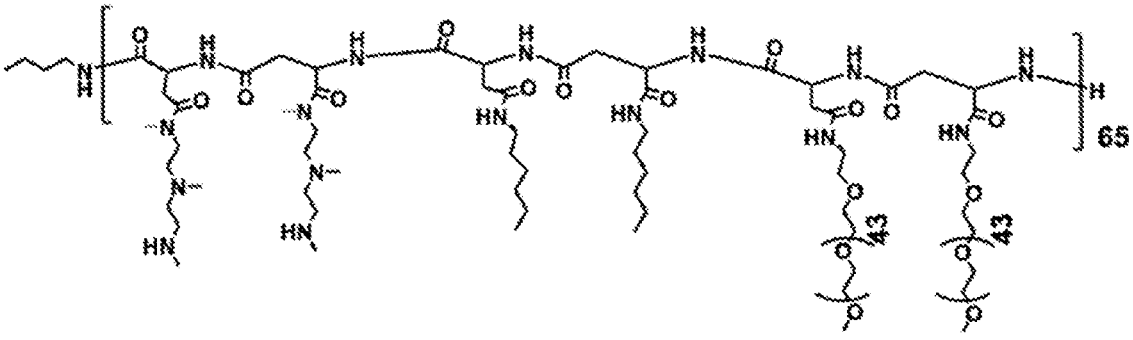




wherein "65" denotes the overall degree of polymerization in the polyamide backbone, and does not indicate that the bracketed area is a repeating unit. PEG2K and PEG5K refer to PEG polymer with weight-average molecular weights of 2000 and 5000 daltons, respectively, with approximately 43 and 113 ethylene oxide units, respectively.

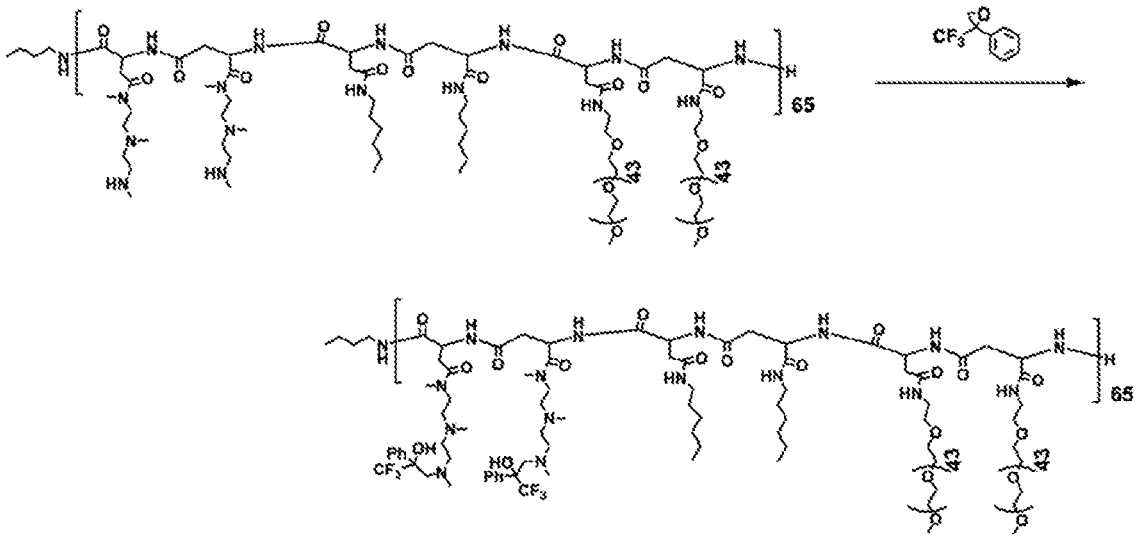
[0338] An exemplary procedure is as follows. Lyophilized PBLA (50 mg, 0.0037 mMol) was placed into a flask and dissolved in tetrahydrofuran/N-methyl-2-pyrrolidine (1 mL each). To the clear solution was added n-hexylamine (58.8 uL, 0.44 mMol, 120 equivalents) and PEG2K amine (25 equivalents), and the clear reaction mixture was stirred for 24 hours at room temperature. After approximately 24 hours, N^1-(2-aminoethyl)-N^1,N^2,N^2-trimethylethane-1,2-diamine (excess equivalents to benzyl group of PBLA segment) was added to the clear mixture under mild anhydrous conditions. After approximately 18 hours at room temperature, the reaction mixture was precipitated into diethyl ether (10 – 12X volume, 35 mL). The precipitate was then centrifuged, and washed twice with diethyl ether. The polymer was dissolved in 1 M HCl (3 mL) and dialyzed in an excess of deionized water in a 3.5 – 5 KD cut-off membrane. When the pH of the solution was between 5 – 6, the dialysis was stopped, and the solution was lyophilized, to give the polymer product.

[0339] Using a similar synthetic protocol, Polymers P2K-TD, P2K-B3, and P2K-B12 were prepared and used in Examples 10 and 12 provided herein:



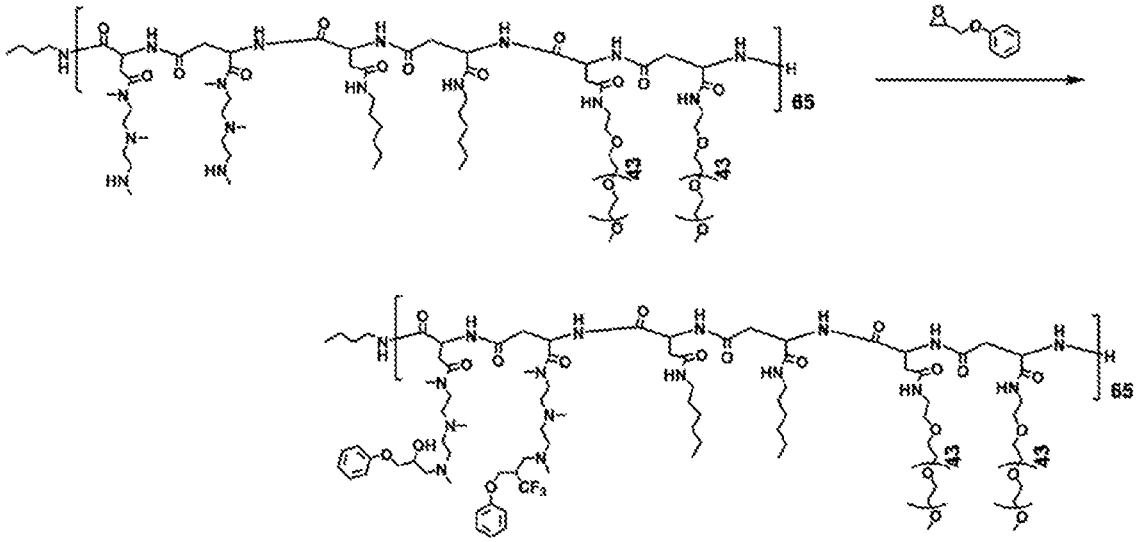
(P2K-TD)

Scheme 3A:



(P2K-B3)

Scheme 3B:



(P2K-B12)

[0340] Polymer P2K-TD was prepared following a similar synthesis procedure of P2K25 (e.g., by modifying PBLA with a PEGylated amine, hexyl amine, and N^1,N^2-dimethyl-N^1-(2-(methylamino)ethyl)ethane-1,2-diamine). P2K-B3, and P2K-B12 were prepared by modifying P2kTD using appropriate epoxide fragments.

EXAMPLE 10

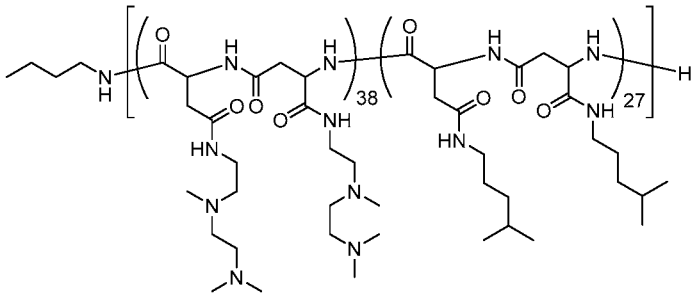
[0341] The following example illustrates the use of polymer nanoparticles to deliver mCherry to human primary neural progenitor cells (NPCs) and Hep3B cells.

[0342] P2K25, II-41-3R, II-43-6, II-43-9, II-43-10, P2K-TD, P2K-B3, and P2K-B12 (as shown in Examples 1 and 9) were combined with H27N (as shown in Example 2) in weight ratios of 10:90 (black bar in figures), 20:80 (purple bar in figures), and 40:60 (blue bar in figures) (all weight ratios expressed as Polymer X:H27N, wherein Polymer X is P2K25, II-41-3R, II-43-6, II-43-9, II-43-10, P2K-TD, P2K-B3, or P2K-B12), and the resulting mixture was combined with mCherry mRNA to form nanoparticles. The resulting nanoparticles were added to Hep3B cells and NPCs in culture medium with defined growth factors and without serum. Flow cytometry was conducted two days after the transfection and the percentage of mCherry+ cells was measured. The results are plotted in FIGs. 12A (NPC) and 12B (Hep3B), wherein the x-axis names the polymer mixed with H27N and mCherry RNA to form nanoparticles.

[0343] The results show that nanoparticles containing P2K25, II-41-3R, II-43-6, II-43-9, II-43-10, P2K-TD, P2K-B3, or P2K-B12 and H27N effectively delivered mCherry mRNA to Hep3B cells and NPCs. Such results demonstrate that nanoparticles containing P2K25, II-41-3R, II-43-6, II-43-9, II-43-10, P2K-TD, P2K-B3, or P2K-B12 and H27N are stable and effective at delivering a payload.

EXAMPLE 11

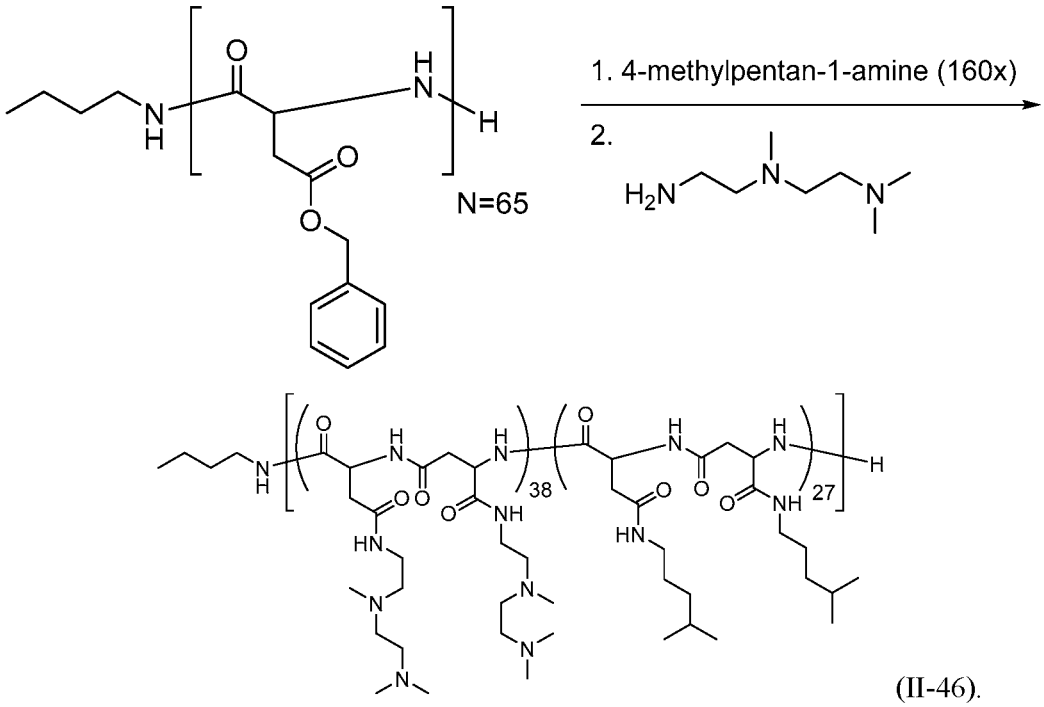
[0344] Polymer II-46 was prepared and used in Example 12 provided herein:



(II-46)

II-46 was prepared by modifying PBLA with N^1-(2-aminoethyl)-N^1,N^2,N^2-trimethylethane-1,2-diamine and 4-methylpentan-1-amine. An exemplary procedure is provided in Scheme 4.

Scheme 4



(II-46).

[0345] Lyophilized PBLA (50 mg, 0.0037 mMol) was placed into a flask and dissolved in tetrahydrofuran/N-methyl-2-pyrrolidine (1 mL each). To the clear solution was added n-4-methylpentan-1-amine (160 equivalents), and the clear reaction mixture was stirred for 24 hours at room temperature. After approximately 24 hours, N^1-(2-aminoethyl)-N^1,N^2,N^2-trimethylethane-1,2-diamine (50 equivalents to benzyl group of PBLA segment) was added to the clear mixture under mild anhydrous conditions. After approximately 18 hours at room temperature, the reaction mixture was precipitated into diethyl ether (10 – 12X volume, 35 mL). The precipitate was then centrifuged, and washed twice with diethyl ether. The polymer was dissolved in 1 M HCl (3 mL) and dialyzed in an excess of deionized water in a 3.5 – 5 KD cut-off membrane. When the pH of the solution was between 5 – 6, the dialysis was stopped, and the solution was lyophilized, to give the polymer product.

EXAMPLE 12

[0346] The following example illustrates the use of polymer nanoparticles to deliver mCherry to human primary neural progenitor cells (NPCs) and Hep3B cells.

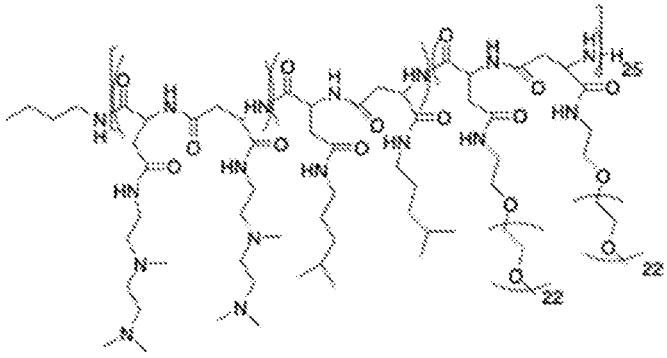
[0347] P2K25, II-41-3R, II-43-6, II-43-9, II-43-10, P2K-TD, P2K-B3, and P2K-B12 (as shown in Examples 1 and 9) were combined with II-46 (as shown in Example 11) the resulting mixture was combined with mCherry mRNA to form nanoparticles. The resulting nanoparticles were added to Hep3B cells and NPCs in culture medium with defined growth factors and without serum. Flow cytometry was conducted two days after the transfection and the percentage of mCherry+ cells was measured. The results are plotted in FIGs. 13A (NPC)

and 13B (Hep3B), wherein the x-axis names the polymer that was mixed with II-46 and mCherry RNA to form nanoparticles.

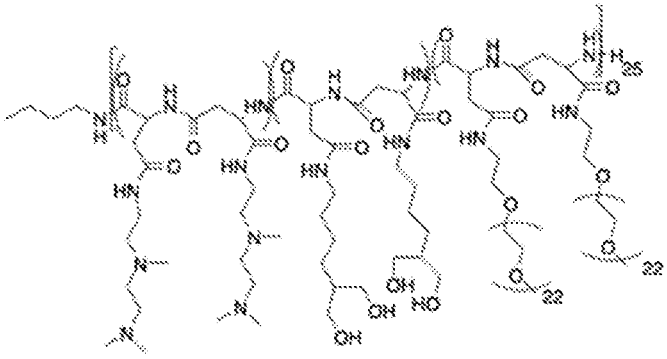
[0348] The results show that nanoparticles containing P2K25, II-41-3R, II-43-6, II-43-9, II-43-10, P2K-TD, P2K-B3, or P2K-B12 and II-46 effectively delivered mCherry mRNA to Hep3B cells and NPCs. Such results demonstrate that nanoparticles containing P2K25, II-41-3R, II-43-6, II-43-9, II-43-10, P2K-TD, P2K-B3, or P2K-B12 and II-46 are stable and effective at delivering a payload.

EXAMPLE 13

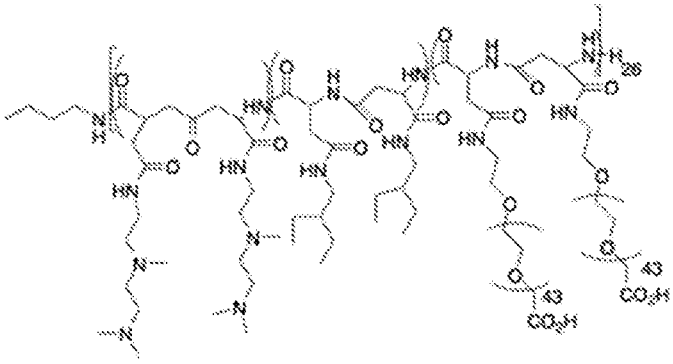
[0349] Polymers II-66, II-67, II-68, II-72, and II-75 were prepared and used in Example 14 provided herein:



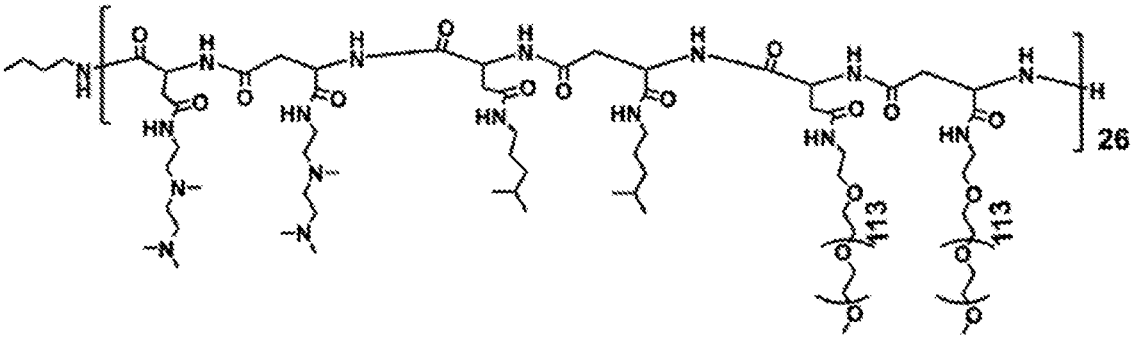
II-66-2, II-66-3, and II-66-4



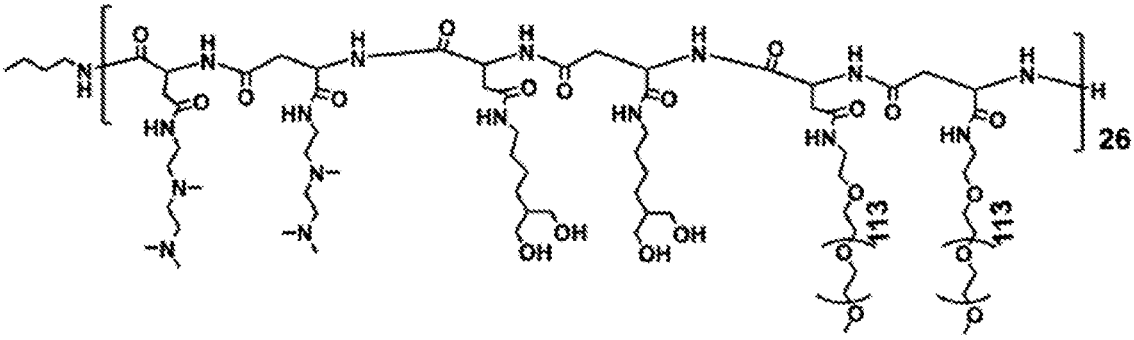
II-67-1, II-67-2, and II-67-3



II-68-1, II-68-2, II-68-3, II-68-4, II-68-5, II-68-6, and II-68-7



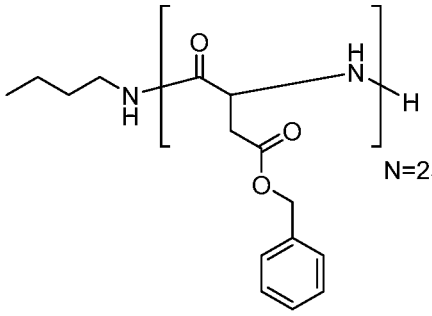
II-72-1, II-72-2, II-72-5, and II-72-6



II-75-2

[0350] Polymers II-66, II-67, II-68, II-72, and II-75 were prepared by modifying PBLA with a PEGylated amine, alkyl amine (or hydroxylated alkyl amine), and N^1-(2-aminoethyl)-N^1,N^2,N^2-trimethylethane-1,2-diamine. An exemplary procedure is provided in Scheme 5.

Scheme 5



1. PEG1K, PEG2K, or PEG5K Amine;
alkyl amine (or hydroxylated alkyl amine)

2. N^1-(2-aminoethyl)-N^1,N^2,N^2-trimethylethane-1,2-diamine

II-66
II-67
II-68
II-72
II-75

[0351] An exemplary procedure is as follows. Lyophilized PBLA (50 mg, 0.0037 mmol; degree of polymerization ("DP") 25) was placed into a flask and dissolved in tetrahydrofuran/N-methyl-2-pyrrolidine (1 mL each). To the clear solution was added 4-methylpentan-1-amine (0.44 mmol, 120 equivalents) and PEG1K amine (25 equivalents), and the clear reaction mixture was stirred for 24 hours at room temperature. After approximately 24 hours, N^1-(2-aminoethyl)-N^1,N^2,N^2-trimethylethane-1,2-diamine (excess equivalents to benzyl group of PBLA segment) was added to the clear mixture under mild anhydrous conditions. After approximately 18 hours at room temperature, the reaction mixture was precipitated into diethyl ether (10 – 12X volume, 35 mL). The precipitate was then centrifuged, and washed twice with diethyl ether. The polymer was dissolved in 1 M HCl (3 mL) and dialyzed in an excess of deionized water in a 3.5 – 5 KD cut-off membrane. When the pH of the solution was between 5 and 6, the dialysis was stopped, and the solution was lyophilized, to give the polymer product. The same procedure was used with different amounts of PEG1K amine, PEG2K amine, and PEG5K amine as summarized in Table 2. The resulting degree of sidechain modification for each polymer was quantified by nuclear magnetic resonance (NMR) spectroscopy, and is summarized in Table 2.

Table 2. Table 2 lists the degree of polymerization for the starting polymer, the reaction conditions for sidechain modification, and the resulting proportion of side chains after modification for each of polymers II-66, II-67, II-68, II-72, and II-75.

PBLA DP	Polymer	PEG	Triamine	(Hydroxy) Alkyl Amine Block	Reaction Conditions
25	II-66-2	3	21	1	10x P1K-Amine
25	II-66-3	3	20	2	15x P1K-Amine
25	II-66-4	3	21	1	125x P1K-Amine
25	II-67-1	2	13	10	10x P1K-Amine
25	II-67-2	3	12	10	15x P1K-Amine
25	II-67-3	3	13	9	125x P1K-Amine
26	II-68-1	4	10	12	5x P2K-Amine
26	II-68-2	8	9	9	10x P2K-Amine
26	II-68-3	11	9	6	15x P2K-Amine
26	II-68-4	14	8	4	25x P2K-Amine
26	II-68-5	7	13	6	10x P2K-Amine
26	II-68-6	8	16	2	10x P2K-Amine
26	II-68-7	10	15	1	10x P2K-Amine
26	II-72-1	5	8	13	5x P5K-Amine
26	II-72-2	10	8	8	10x P5K-Amine
26	II-72-5	10	8	8	10x P5K-Amine
26	II-72-6	12	10	4	10x P5K-Amine
26	II-75-2	7	14	5	10x P5K-Amine

EXAMPLE 14

[0352] The following example illustrates the ability of Polymers II-66 and II-67 to deliver Cas9 RNP to Hep3B cells and HEK293T cells.

[0353] Hep3B cells were seeded 50,000 cells/well in culture medium composed of Dulbecco's Modified Eagle Medium (DMEM) and 10% fetal bovine serum (FBS) to form 40 pmol Cas9 RNP. sgRNA targeting SERPINA1 gene was prepared and Cas9 protein was added slowly and mixed thoroughly by pipetting. Separately, compositions containing Polymers II-66 and II-67 were prepared. Nanoparticles were formed by mixing the resulting compositions with sgRNA. The resulting nanoparticle was treated in Hep3B cells and flow cytometry was used to quantify RFP+ cells 24 hours after transfection.

[0354] Similarly, nanoparticles were formed by mixing the II-66 and II-67 compositions with RFP mRNA, and the resulting nanoparticle was treated in HEK293T cells and flow cytometry was used to quantify RFP+ cells 24 hours after transfection. The results are set forth in Table 3.

Table 3

Polymer	HEP3B RFP+ (%)	HEK293T RFP+ (%)
II-66-2	0	1.4
II-66-3	0.5	2
II-66-4	0.7	1.5
II-67-1	0.8	4.1
II-67-2	29.4	12.9
II-67-3	34.3	12.3
H27N	32	12.3
Control	0.4	1.7

[0355] As the results show, polymer II-67, which contains a terminal diol moiety, had RFP activity comparable to the H27N positive control polymer for HEP3B cells (RFP = ~29 for II-67 versus RFP = ~32 for H27N). In addition, polymer II-67 had RFP activity comparable to the H27N positive control polymer for HEK293T cells (RFP = ~12 for II-67 versus RFP = ~12 for H27N).

EXAMPLE 15

[0356] The following example illustrates the use of polymers of the invention to deliver Cre mRNA to mice as exhibited by ai9 mice.

[0357] ai9 mice having the reporter sequence set forth in FIG. 14 were treated with nanoparticles formulated with a 50:50 weight ratio of H27N:P2K25 and Cre mRNA ("H27N + 50% P2K25"). An untreated mouse served as a control. The nanoparticle properties summarized in Table 4.

Table 4

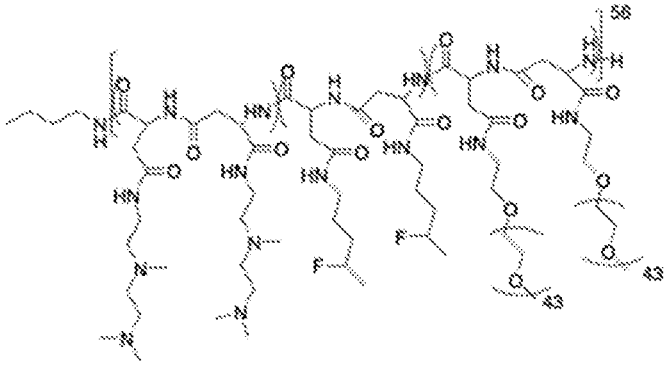
	Pre-Lyophilization			Post-Lyophilization		
Particle	Diameter (nm)	Zeta-Potential	PDI	Diameter (nm)	Zeta-Potential	PDI
H27N + 50% P2K25	70 nm	~ +5 mV	0.39	69 nm	~ +2 mV	0.33

[0358] The resulting nanoparticle formulation was administered to mice via intrathecal (IT) injection. Ten days after treatment, the mice were sacrificed via CO_2 asphyxiation and perfused through the left ventricle with 1% heparinized saline followed by PBS to remove blood. The brain and spinal cord were then harvested. The mouse brains were sectioned at 100 μm thickness in the coronal plane and every other section was collected and imaged.

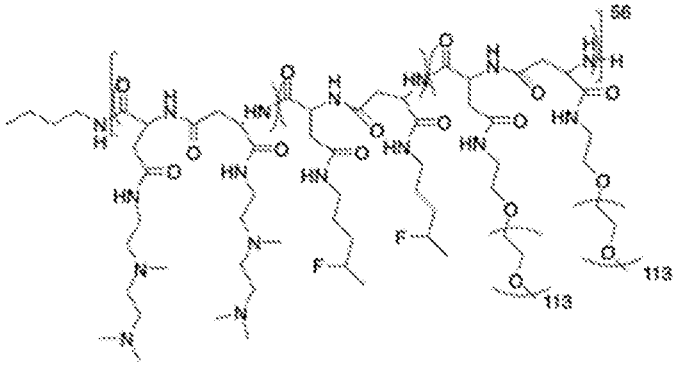
[0359] *In vivo* Cre mRNA delivery was assessed for the rostral and caudal sections of the brain via bioluminescence. Analysis of the bioluminescence showed that the nanoparticle composition delivered Cre mRNA to the caudal sections of the brain stem and cerebellum (i.e., the areas of the brain that are surrounded by cerebrospinal fluid (CSF)).

EXAMPLE 16

[0360] 2K-PEG-Fluoro-Polymer and 5K-PEG-Fluoro Polymer were prepared using the procedure described in this example.



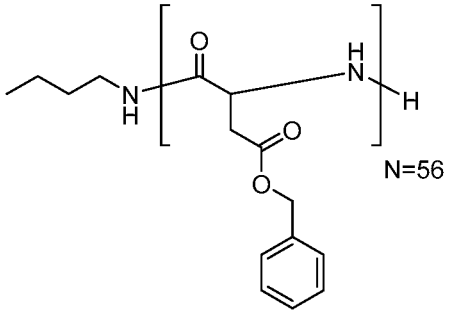
2K-PEG-Fluoro-Polymer



5K-PEG-Fluoro-Polymer

[0361] 2K-PEG-Fluoro-Polymer and 5K-PEG-Fluoro Polymer were prepared by modifying PBLA with a PEGylated amine, fluoroalkyl amine, and N^1-(2-aminoethyl)-N^1,N^2,N^2-trimethylethane-1,2-diamine. An exemplary procedure is provided in Scheme 6.

Scheme 6



1. PEG2K or PEG5K Amine; fluoroalkyl amine → 2K-PEG-Fluoro-Polymer

2. N^1-(2-aminoethyl)-N^1,N^2,N^2-trimethylethane-1,2-diamine → 5K-PEG-Fluoro-Polymer

[0362] An exemplary procedure is as follows. Lyophilized PBLA (50 mg, 0.0037 mmol; degree of polymerization ("DP") 56) was placed into a flask and dissolved in tetrahydrofuran/N-methyl-2-pyrrolidine (1 mL each). To the clear solution was added 4-fluoropentan-1-amine (0.44 mmol, 120 equivalents) and PEG2K amine (25 equivalents), and the clear reaction mixture was stirred for 24 hours at room temperature. After approximately 24 hours, N^1-(2-aminoethyl)-N^1,N^2,N^2-trimethylethane-1,2-diamine (excess equivalents to benzyl group of PBLA segment) was added to the clear mixture under mild anhydrous conditions. After approximately 18 hours at room temperature, the reaction mixture was precipitated into diethyl ether (10 – 12X volume, 35 mL). The precipitate was then centrifuged, and washed twice with diethyl ether. The polymer was dissolved in 1 M HCl (3 mL) and dialyzed in an excess of deionized water in a 3.5 – 5 KD cut-off membrane. When the pH of the solution was between 5 and 6, the dialysis was stopped, and the solution was lyophilized, to give the polymer product. The same procedure was used with PEG5K amine for the preparation of 5K-PEG-Fluoro Polymer.

[0363] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments can become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0364] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise,

between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0365] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0366] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a complex" includes a plurality of such complexes and reference to "the Cas9 polypeptide" includes reference to one or more Cas9 polypeptides and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

[0367] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0368] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior

invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

CLAIM(S):

1. A polymer comprising a hydrolysable polymer backbone, the polymer backbone comprising:

(i) monomer units with a side chain comprising a hydrophobic group;

(ii) monomer units with a side chain comprising an oligoamine or polyamine; and

(iii) monomer units with a side chain comprising a polyalkylene oxide, polyglycolic acid, polylactic acid, or combination thereof.

2. The polymer of claim 1, wherein the hydrophobic group comprises an alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, optionally substituted with one or more substituents, optionally wherein the substituents are hydroxyl or halogen groups.

3. The polymer of claim 1, wherein the hydrophobic group comprises a C_3-C_{12} linear or branched alkyl group, optionally a C_3-C_6 linear or branched alkyl group, optionally substituted with one or more substituents, optionally wherein the substituents are hydroxyl or halogen group.

4. The polymer of any of claims 1-3, wherein the oligoamine or polyamine is a group of the formula:

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2{}_2]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2{}_2]_2\}_2$,

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^4\text{-}R^5]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^4\text{-}R^5\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^4\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^5]_2$;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—NR^2_2][—$(CH_2)_{q4}$—NR^2—]$_{r2}$$(CH_2)_{s3}$-$R^5$};

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-$(CH_2)_{s4}$-R^5]$_2$}$_2$;

—$(CH_2)_{p1}$—[N{$(CH_2)_{s1}$-R^4-R^5}—$(CH_2)_{q1}$—]$_{r1}$$NR^2_2$;

—$(CH_2)_{p1}$—[N{$(CH_2)_{s1}$-R^5}—$(CH_2)_{q1}$—]$_{r1}$$NR^2_2$,

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-CH($CONH_2$)-$(CH_2)_{s1}$-$R^5$; or

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-CH($CONH_2$)-$(CH_2)_{s1}$-$R^4$-$R^5$,

wherein p1 to p4, q1 to q6, r1 and r2, and s1 to s4 are each independently an integer of 1 to 5;

each instance of R^2 is independently hydrogen or a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12}cycloalkyl group, or C_3-C_{12}cycloalkenyl group, or R^2 is combined with a second R^2 so as to form a heterocyclic group;

each instance of R^4 is independently -C(O)O-, -C(O)NH-, -O-C(O)O-, or -S(O)(O)-; and

each instance of R^5 is independently an alkyl group, cycloalkyl group, alkenyl group, cycloalkenyl group, aryl group, heteroalkyl group, heterocyclic group, or combination thereof optionally comprising from 2 to 8 tertiary amines or a substituent comprising a tissue-specific or cell-specific targeting moiety.

5. The polymer of any one of claims 1-4, wherein the polyamine comprises

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2_2$;

—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—NR^2_2]$_2$;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—NR^2_2][—$(CH_2)_{q4}$—NR^2—]r_2R^2}; or

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2_2]$_2$}$_2$;

and each R^2 is independently hydrogen or a C_1-C_3 alkyl group.

6. The polymer of claim 5, wherein the polyamine comprises

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2_2$.

7. The polymer of any one of claims 1-6, wherein the hydrolysable polymer backbone comprises about 1 to about 80 mol% of the monomer units having a hydrophobic group, about 1 to about 80 mol% of the monomer units having an oligoamine or polyamine,

and 1 to about 80 mol% of the monomer units having a polyalkylene oxide, polyglycolic acid, polylactic acid, or combination thereof.

8. The polymer of any one of claims 1-7, wherein the hydrolysable polymer backbone comprises a polyamide, poly-N-alkylamide, polyester, polycarbonate, polycarbamate, or a combination thereof.

9. The polymer of claim 8, wherein the hydrolysable polymer backbone comprises a polyamide.

10. The polymer of any one of claims 1-9, wherein the polymer comprises at least one polyethylene glycol group having from 2 to 200 ethylene glycol units.

11. The polymer of any one of claims 1-9, wherein the polymer comprises at least one polyethylene glycol group having from 2 to 50 ethylene glycol units.

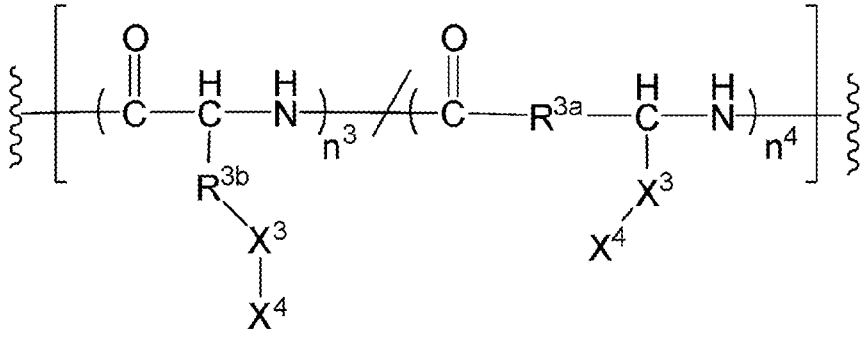
12. The polymer of any one of claims 1-11, wherein the polymer comprises at least one polyglycolic acid group having from 2 to 200 polyglycolic acid units.

13. The polymer of any one of claims 1-11, wherein the polymer comprises at least one polyglycolic acid group having from 2 to 50 polyglycolic acid units.

14. The polymer of any one of claims 1-13, wherein the polymer comprises at least one polylactic acid group having from 2 to 200 polylactic acid units.

15. The polymer of any one of claims 1-13, wherein the polymer comprises at least one polylactic acid group having from 2 to 50 polylactic acid units.

16. The polymer of any one of claims 1-15, wherein the monomer units with a side chain comprising a polyalkylene oxide, polyglycolic acid, polylactic acid, or combination thereof comprise a structure of Formula 1:



wherein:

each of n^3 and n^4 is an integer from 0 to 1000, provided that the sum of $n^3 + n^4$ is greater than 1;

the symbol "/" indicates that the units separated thereby are linked randomly or in any order;

each instance of R^{3a} is independently a methylene or ethylene group;

each instance of R^{3b} is independently a methylene or ethylene group;

each X^3 independently is —C(O)O—, —C(O)NR^{13}—, —C(O)—, —S(O)(O)—, or a bond;

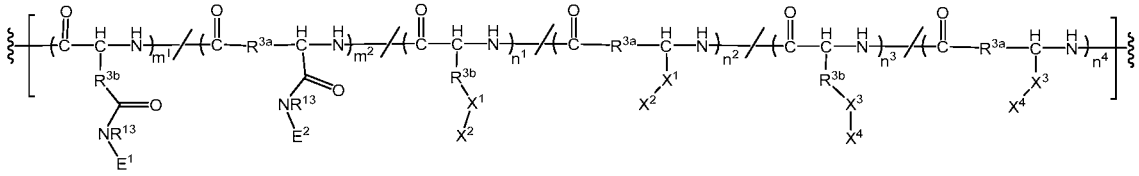
each instance of R^{13} is independently hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl group, any of which can be optionally substituted with one or more substituents;

each instance of X^4 comprises a polyalkylene oxide, polyglycolic acid, polylactic acid, or a combination thereof;

each instance of R^{18} is independently hydrogen or methyl; and

each instance of p is independently an integer from 2 to 200.

17. The polymer of any one of claims 1-15, the polymer comprising a structure of Formula 2:



wherein:

each of m^1 and m^2 is an integer from 0 to 1000, provided that the sum of $m^1 + m^2$ is greater than 1;

each of n^1 and n^2 is an integer from 0 to 1000; provided that the sum of $n^3 + n^4$ is greater than 1;

each of n^3 and n^4 is an integer from 0 to 1000, provided that the sum of $n^3 + n^4$ is greater than 1;

the symbol "/" indicates that the units separated thereby are linked randomly or in any order;

each instance of R^{3a} is independently a methylene or ethylene group;

each instance of R^{3b} is independently a methylene or ethylene group;

each X^1 independently is —C(O)O—, —C(O)NR^{13}—, —C(O)—, —S(O)(O)—, or a bond;

each instance of R^{13} is independently hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl group, any of which can be optionally substituted with one or more substituents;

each instance of X^2 is independently a C_1-C_{12} alkyl or heteroalkyl group, C_3-C_{12} cycloalkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkenyl group, aryl group, heterocyclic group, or combination thereof; any of which are optionally substituted with one or more substituents;

each X^3 independently is —C(O)O—, —C(O)NR^{13}—, —C(O)—, —S(O)(O)—, or a bond;

each instance of X^4 comprises a polyalkylene oxide, polyglycolic acid, polylactic acid, or a combination thereof;

E^1 and E^2 are each independently a group of formula

$$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2;$$

$$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2{}_2]_2;$$

$$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\};$$

$$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2{}_2]_2\}_2,$$

$$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5;$$

$$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^4\text{-}R^5]_2;$$

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—NR^2_2][—$(CH_2)_{q4}$—NR^2—]$_{r2}$$(CH_2)_{s3}$-$R^4$-$R^5$};

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-$(CH_2)_{s4}$-R^4-R^5]$_2$}$_2$;

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-$CH_2$-CHOH-$R^5$;

—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—NR^2-CH_2-CHOH-R^5;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—NR^2_2][—$(CH_2)_{q4}$—NR^2—]$_{r2}$-CH_2-CHOH-R^5;

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-CH_2-CHOH-R^5]$_2$}$_2$;

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-$(CH_2)_{s1}$-$R^5$;

—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—NR^2-$(CH_2)_{s2}$-R^5]$_2$;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—NR^2_2][—$(CH_2)_{q4}$—NR^2—]$_{r2}$$(CH_2)_{s3}$-$R^5$};

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-$(CH_2)_{s4}$-R^5]$_2$}$_2$;

—$(CH_2)_{p1}$—[N{$(CH_2)_{s1}$-R^4-R^5}—$(CH_2)_{q1}$—]$_{r1}$$NR^2_2$;

—$(CH_2)_{p1}$—[N{$(CH_2)_{s1}$-R^5}—$(CH_2)_{q1}$—]$_{r1}$$NR^2_2$,

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-CH($CONH_2$)-$(CH_2)_{s1}$-$R^5$; or

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-CH($CONH_2$)-$(CH_2)_{s1}$-$R^4$-$R^5$,

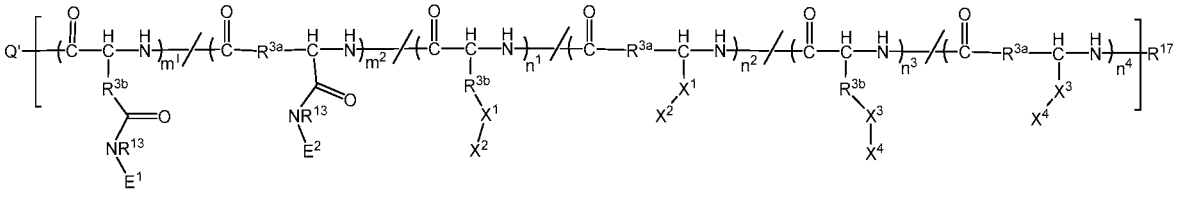
wherein p1 to p4, q1 to q6, r1 and r2, and s1 to s4 are each independently an integer of 1 to 5;

each instance of R^2 is independently hydrogen or a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12}cycloalkyl group, or C_3-C_{12}cycloalkenyl group, or R^2 is combined with a second R^2 so as to form a heterocyclic group;

each instance of R^4 is independently -C(O)O-, -C(O)NH-, -O-C(O)O-, or -S(O)(O)-; and

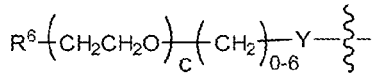
each instance of R^5 is independently an alkyl group, cycloalkyl group, alkenyl group, cycloalkenyl group, aryl group, heteroalkyl group, heterocyclic group, or combination thereof optionally comprising from 2 to 8 tertiary amines or a substituent comprising a tissue-specific or cell-specific targeting moiety.

18. The polymer of claim 17, the polymer having a structure of Formula 2A:



wherein

Q' is of formula:



c is an integer from 0 to 50;

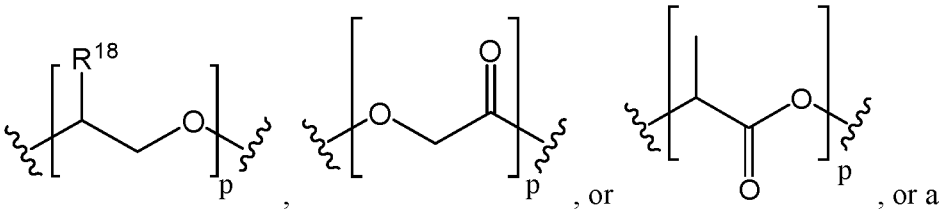
Y is optionally present and is a cleavable linker;

R^{17} is hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, or a C_1-C_{12} linear or branched alkyl group optionally substituted with one or more substituents;

R^6 is hydrogen, an amino group, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, a C_1-C_{12} heteroalkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, a C_1-C_{12} linear or branched alkyl group optionally substituted with one or more amines; or a tissue-specific or cell-specific targeting moiety; and

m^1, m^2, n^1, n^2, n^3, n^4, R^{3a}, R^{3b}, R^{13}, X^1, X^2, X^3, X^4, E^1, and E^2, are as defined in claim 17.

19. The polymer of any one of claims 16-18, wherein each instance of X^4 comprises



, or a combination thereof,

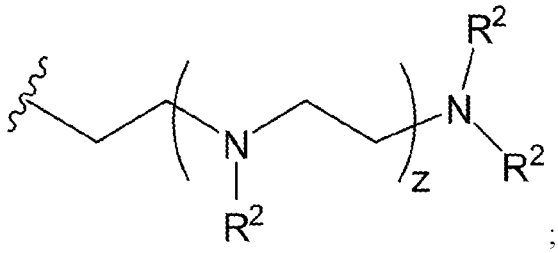
wherein

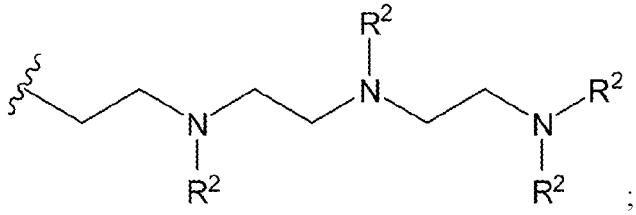
each instance of R^{18} is independently hydrogen or methyl; and

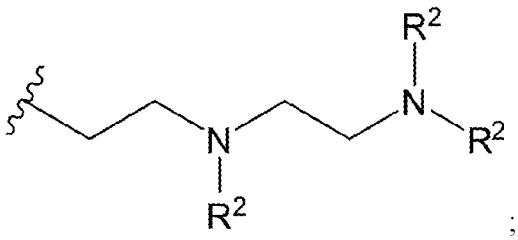
each instance of p is independently an integer from 2 to 200.

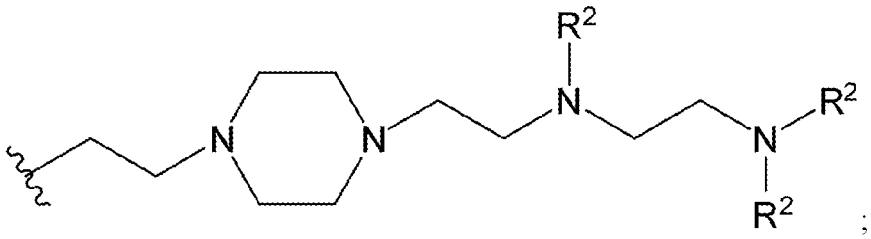
20. The polymer of any one of claims 17-19, wherein each of E^1 and E^2 is a group of formula $–(CH_2)_2-NR^2-(CH_2)_2-NR^2_2$ or $–(CH_2)_2-NR^2-(CH_2)_2-NHR^2$, wherein R^2 is hydrogen, methyl, or ethyl.

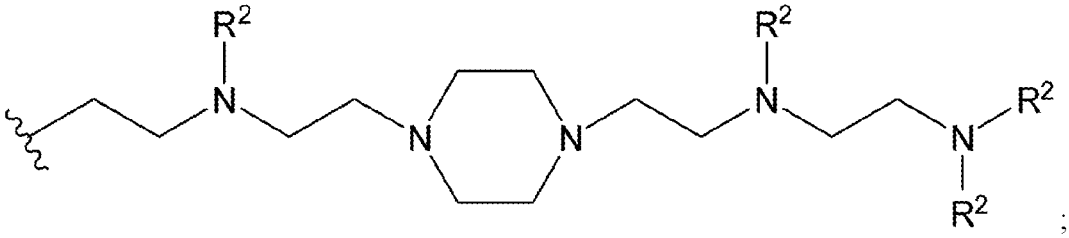
21. The polymer of any one of claims 17-19, wherein each R^5 is independently:

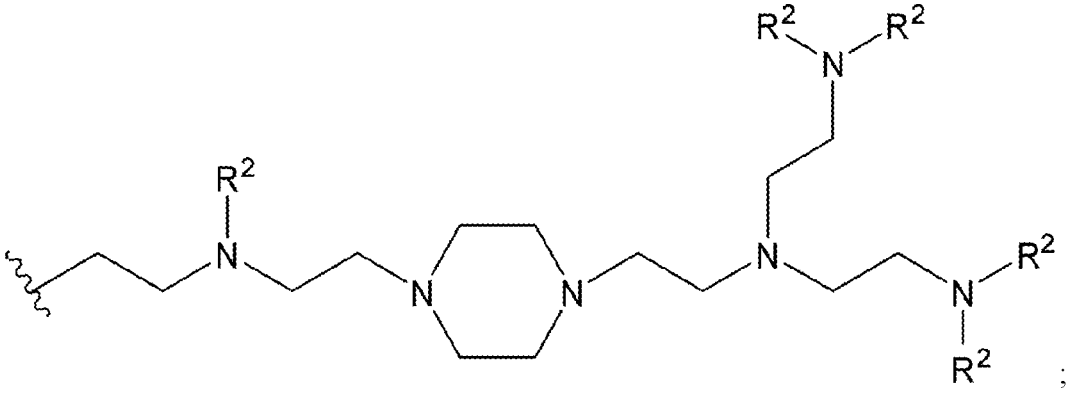

;


;

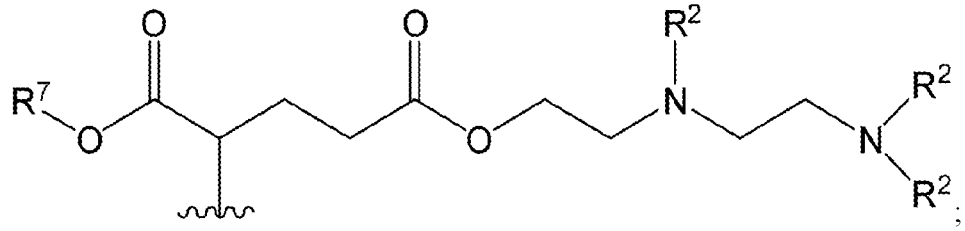

;


;

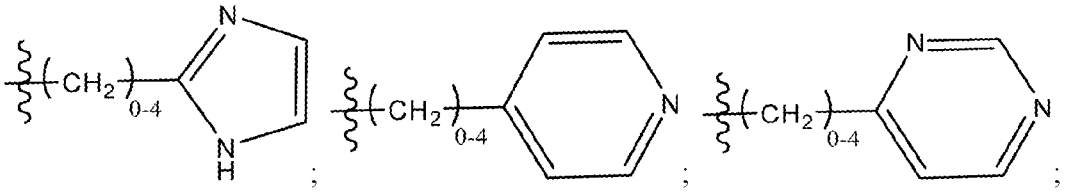

;



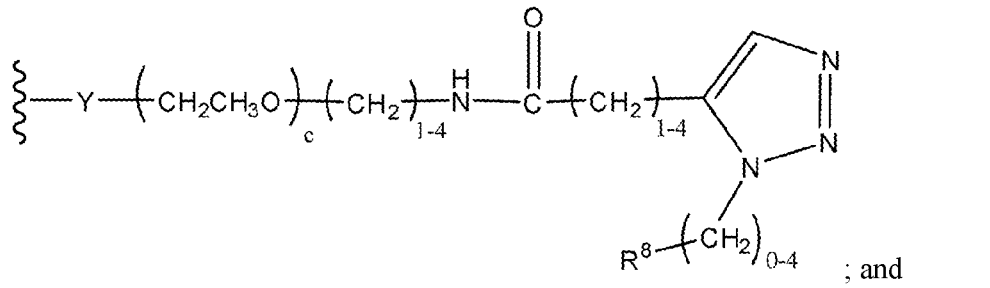
;



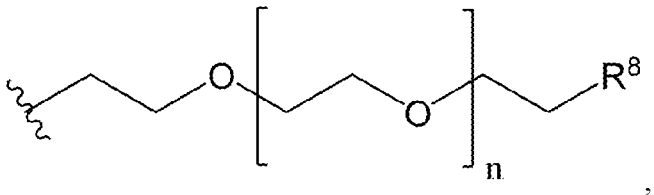
;



; ; ;



; and



,

wherein

each instance of R^2 is independently hydrogen or a C_1-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, or R^2 is combined with a second R^2 so as to form a heterocyclic group;

R^7 is a C_1-C_{50} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group optionally substituted with one or more amines;

z is an integer from 1 to 5;

c is an integer from 0 to 50;

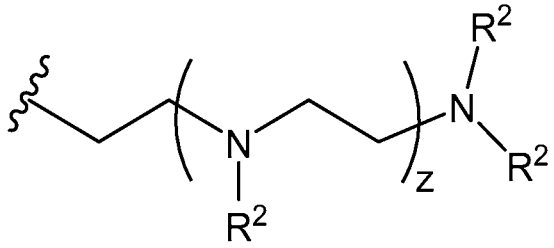
Y is optionally present and is a cleavable linker;

n is an integer from 0 to 50; and

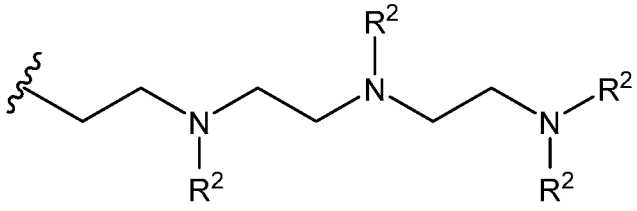
R^8 is a tissue-specific or cell-specific targeting moiety.

22. The polymer of any one of claims 17-19 or 21, wherein R^4 is –C(O)-O-.

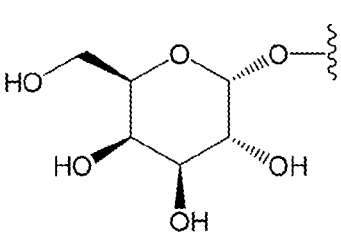
23. The polymer of any one of claims 17-19, 21, or 22, wherein R^5 is



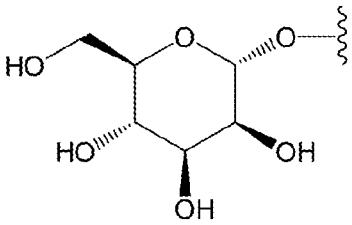
or



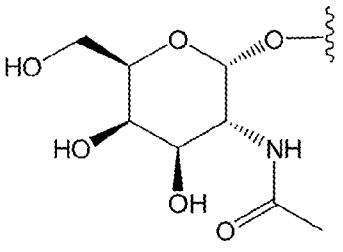
24. The polymer of any one of claims 17-19 or 21-23, wherein the tissue-specific or cell-specific targeting moiety is:



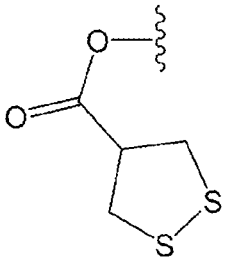
alpha-d-galactose ;



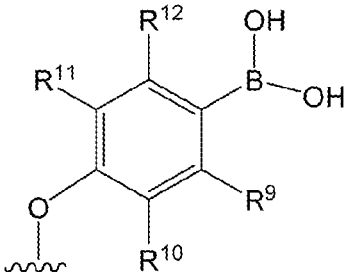
alpha-d-mannose ;



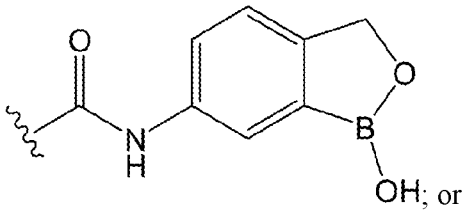
N-acetylgalactosamine ;



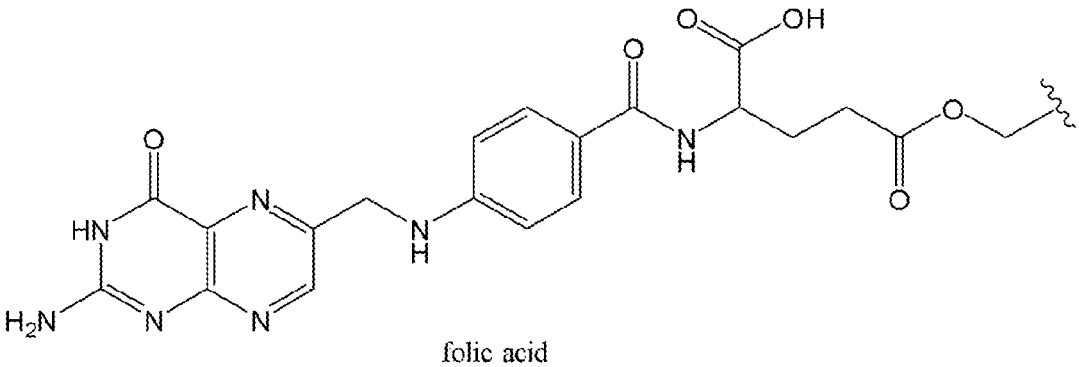
Asparagusic acid ;



Phenylboronic acid ;



OH; or



folic acid ,

wherein each of R^9, R^{10}, R^{11}, and R^{12} is independently hydrogen, halogen, C_1-C_4 alkyl, or C_1-C_4 alkoxy, optionally substituted with one or more amino groups.

25. The polymer of any one of claims 17-24, wherein Q' is —NHR^6.

26. The polymer of any one of claims 17-25, wherein R^{17} is hydrogen.

27. The polymer of claim 17, wherein:

each of m^1 and m^2 is an integer from 5 to 100;

each of n^1 and n^2 is an integer from 5 to 100;

each of n^3 and n^4 is an integer from 5 to 100;

each instance of R^{3a} and R^{3b} is methylene;

each X^1 and X^3 independently is —C(O)O—, —C(O)NR^{13}—, or —C(O)—, or a bond;

each instance of R^{13} is hydrogen or a C_1-C_3 alkyl;

each instance of X^2 is independently a C_3-C_{12} linear or branched alkyl or C_4-C_{12} cyclic or fused ring hydrophobic group, any of which is optionally substituted with one or more hydroxyl groups or halogen groups;

each instance of X^4 is polyalkylene oxide, polyglycolic acid, polylactic acid, or a combination thereof;

E^1 and E^2 are each independently a group of formula

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2$;

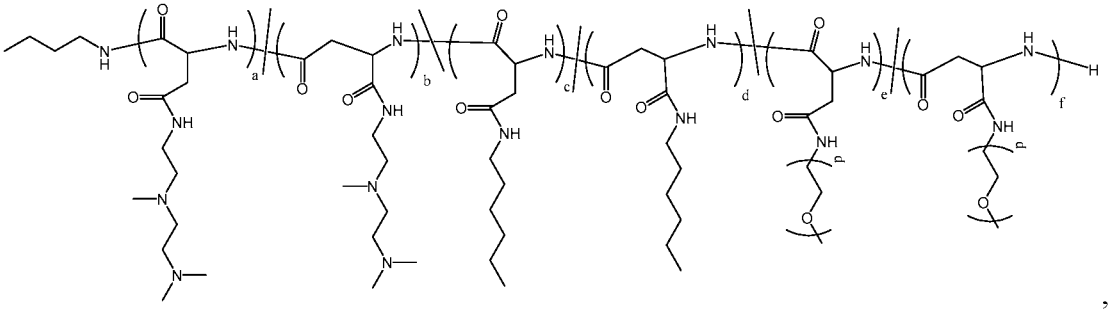
$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2{}_2]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2{}_2]_2\}_2$,

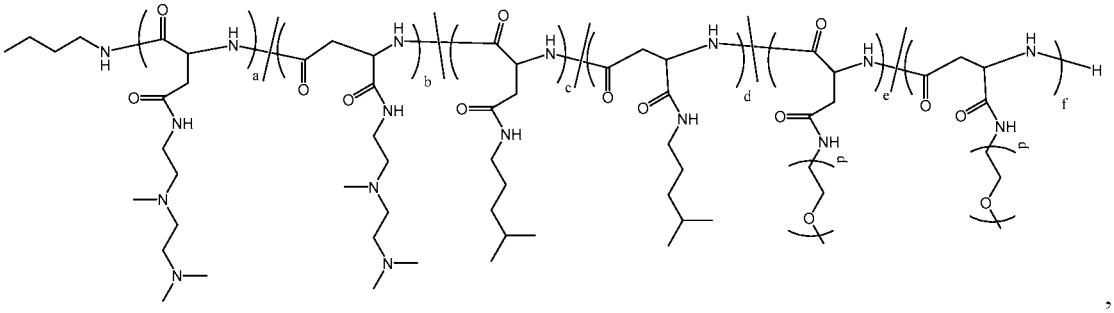
wherein p1 to p4, q1 to q6, and r1 and r2, are each independently an integer of 1 to 5; and each instance of R^2 is independently hydrogen or a C_1-C_3 alkyl group.

28. The polymer of claim 1, the polymer having the formula:

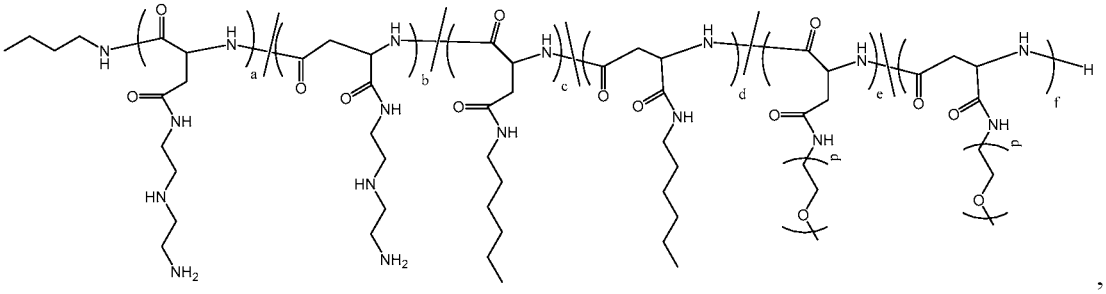


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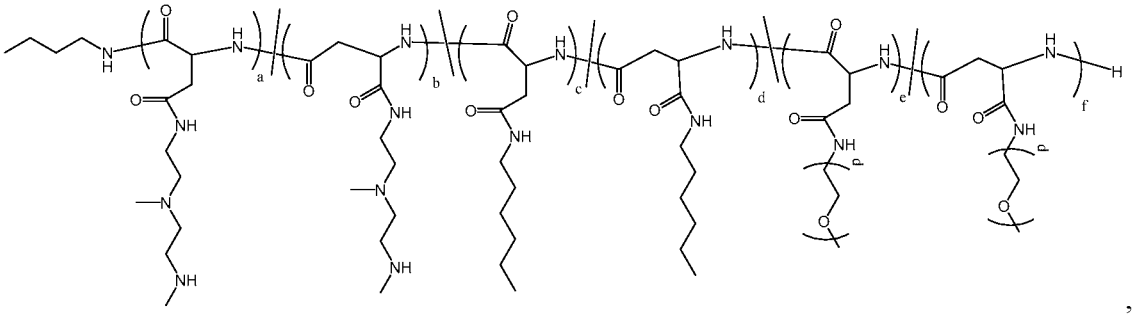
Polymer 72



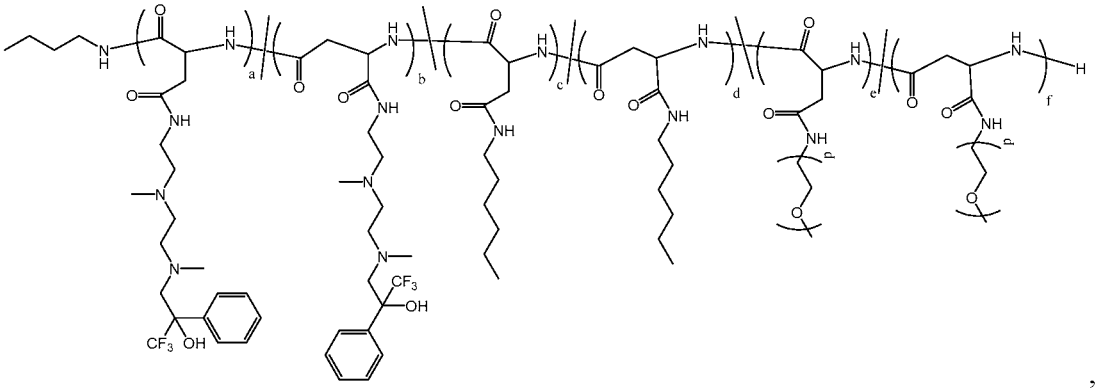
Polymer 73



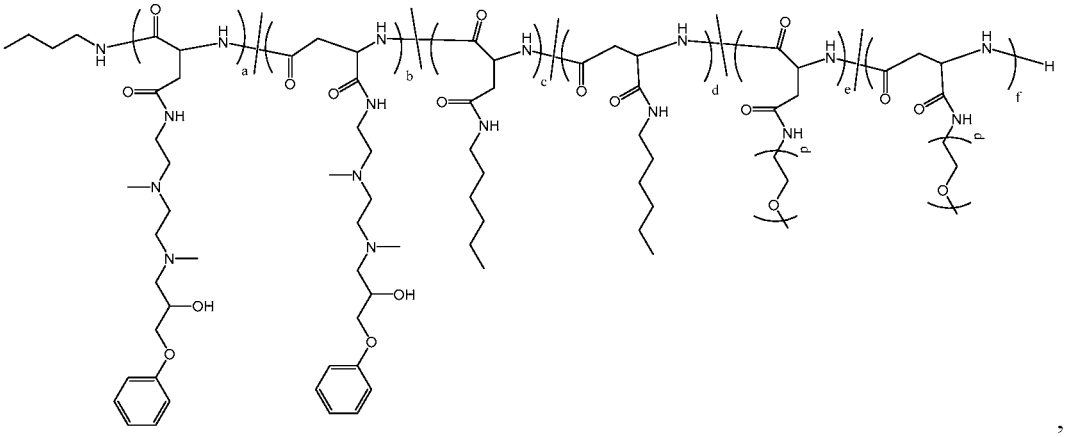
Polymer 74



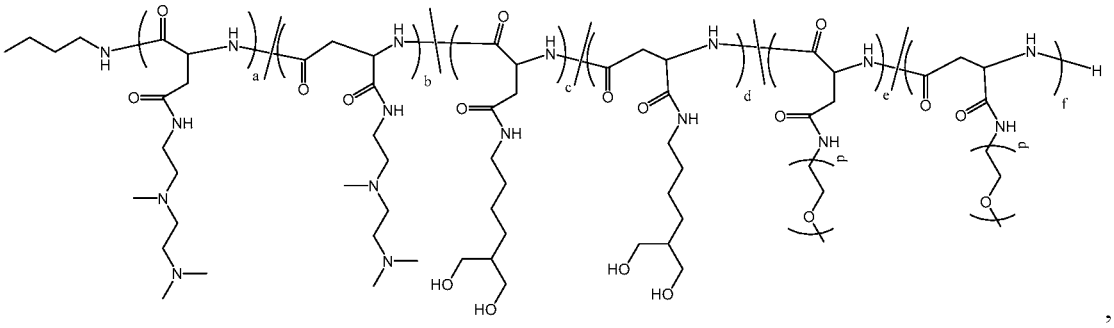
Polymer 75



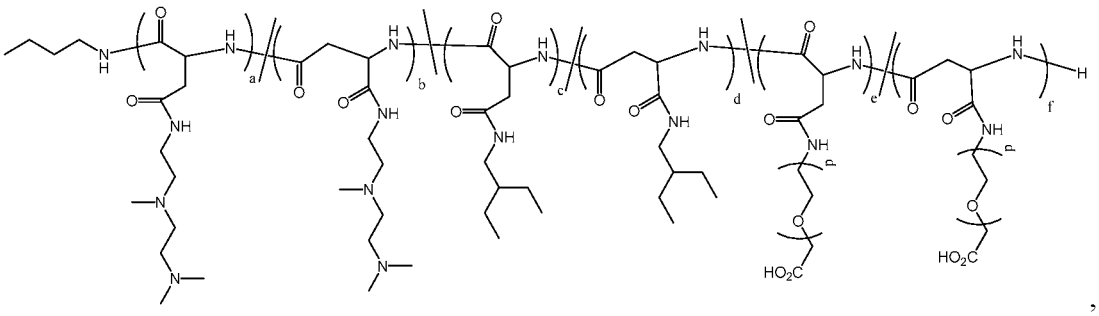
Polymer 76



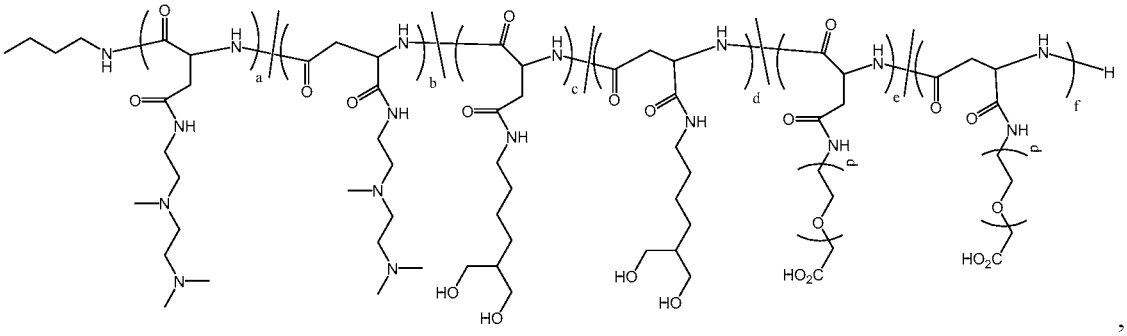
Polymer 77



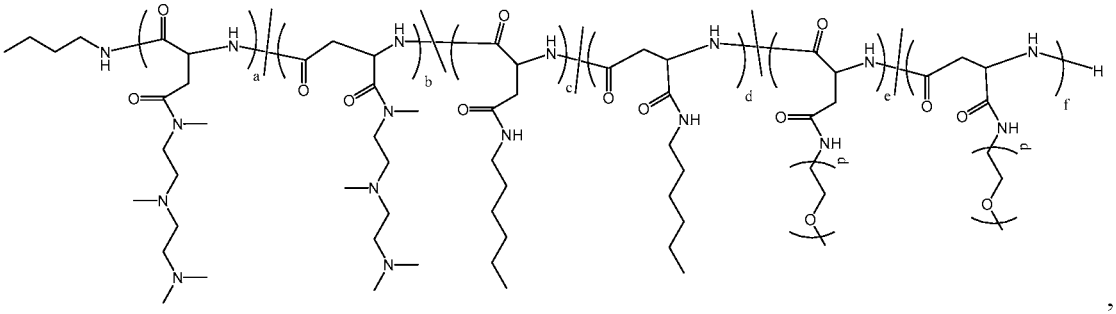
Polymer 78



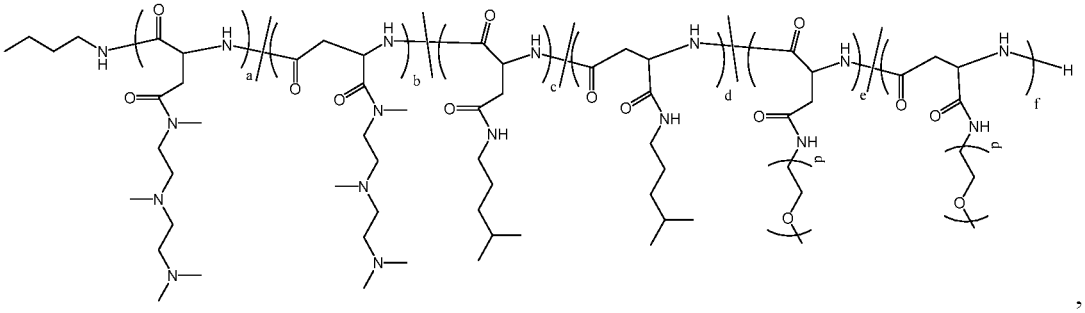
Polymer 79



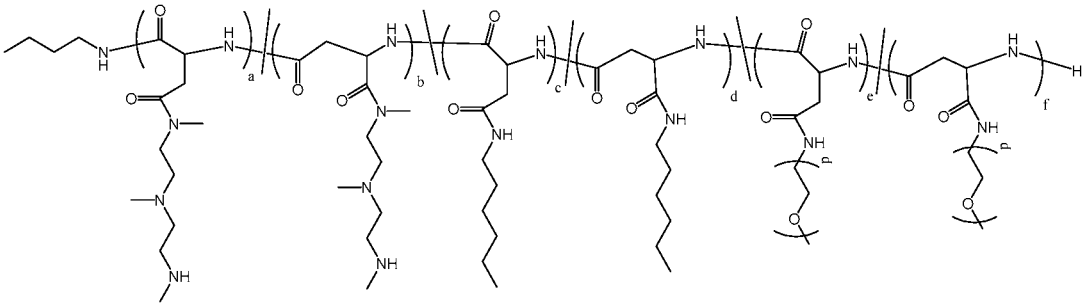
Polymer 80



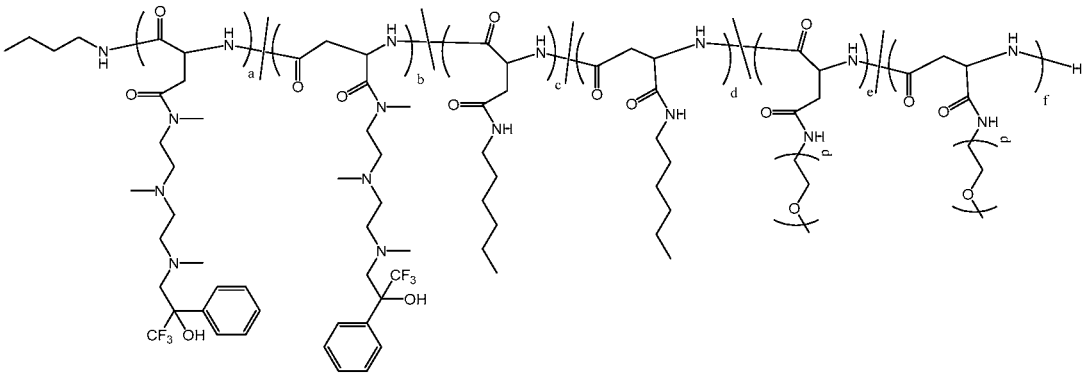
Polymer 81



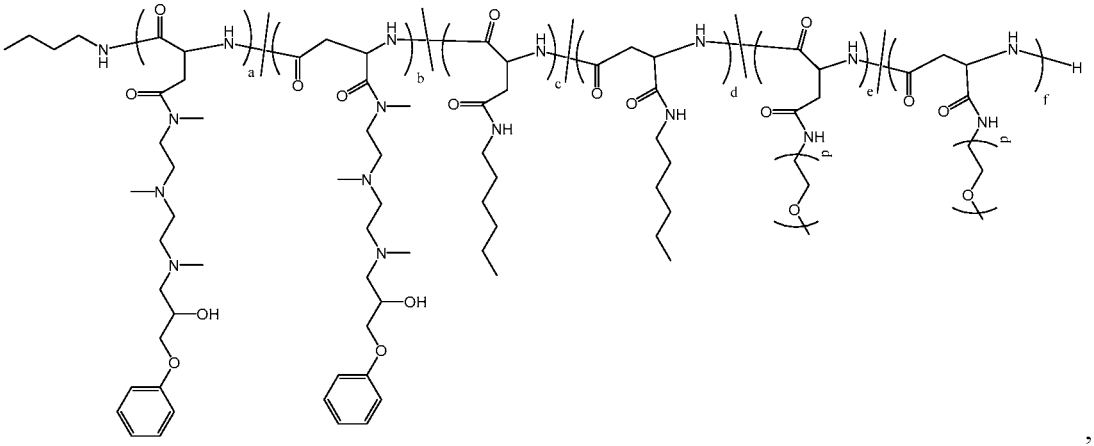
Polymer 82



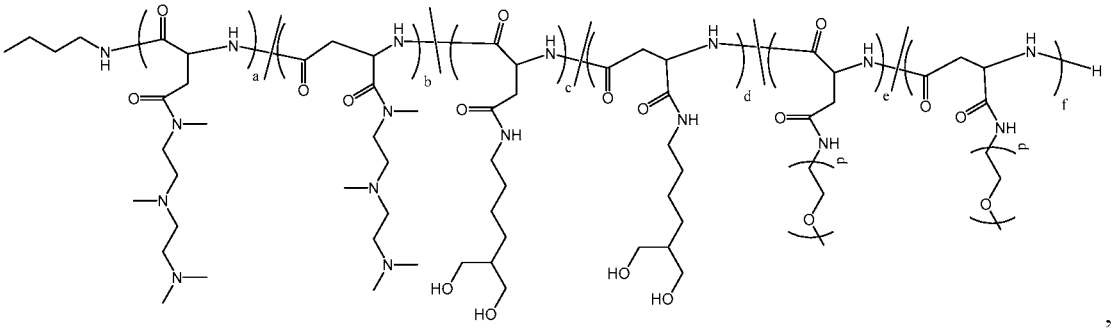
Polymer 83



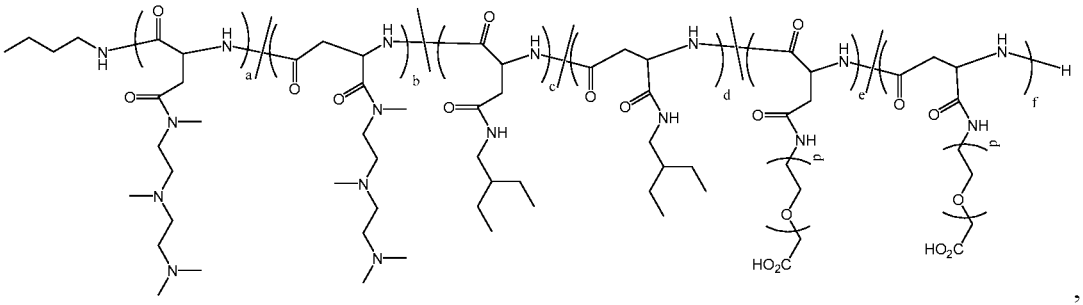
Polymer 84



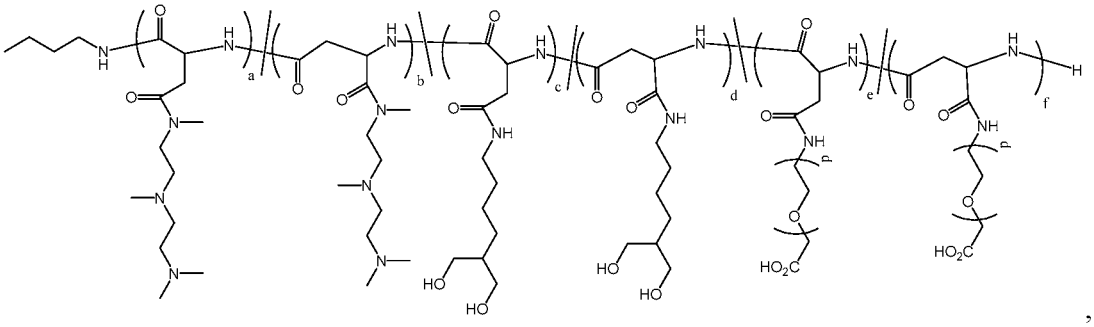
Polymer 85



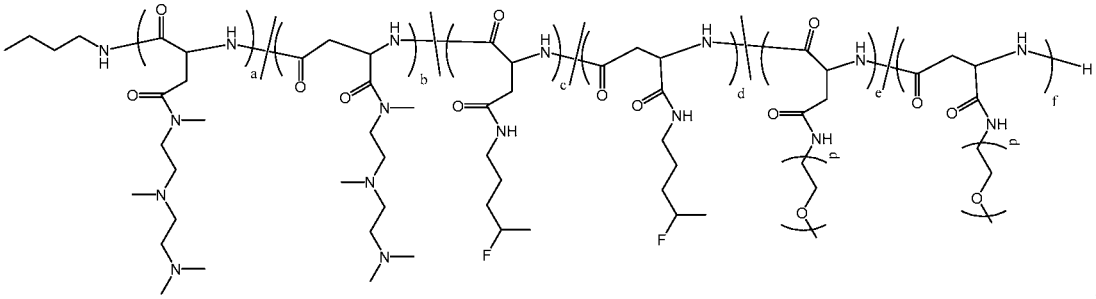
Polymer 86



Polymer 87

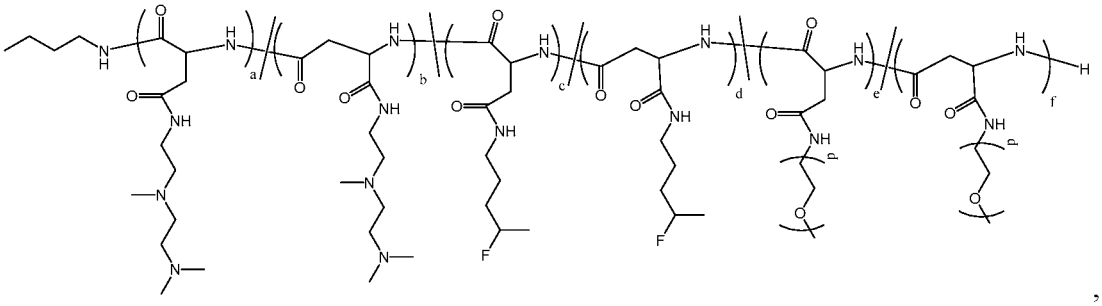


Polymer 88



, or

Polymer 89



,

Polymer 90

wherein (a+b) is from about 5 to about 65, (c+d) is from about 2 to about 60, (e+f) is from about 2 to about 60, and each instance of p is independently an integer from 2 to 200 or 6 to 30.

29. The polymer of any one of claims 1-28, wherein the polymer has a weight average molecular weight of from about 5 kDa to about 2,000 kDa.

30. The polymer of claim 29, wherein the polymer has a weight average molecular weight of from about 10 kDa to about 500 kDa.

31. A composition comprising a first polymer according to any one of claims 1-30 and optionally a pharmaceutically acceptable excipient.

32. The composition of claim 31, wherein the composition comprises a non-ionic surfactant and/or a zwitterionic surfactant.

33. The composition of claim 31 or claim 32, wherein the composition further comprises one or more biomolecules or synthetic variants thereof.

34. The composition of claim 33, wherein the composition comprises a nucleic acid and/or polypeptide.

35. The composition of claim 34, wherein the composition comprises a guide nucleic acid and/or donor nucleic acid.

36. The composition of any one of claims 33-35, wherein the composition comprises an endonuclease.

37. The composition of claim 36, wherein the composition comprises an RNA-guided endonuclease or nucleic acid encoding same.

38. The composition of claim 37, wherein the RNA-guided endonuclease is Cas9, Cpf1, or a combination thereof.

39. The composition of any one of claims 33-38, wherein the composition comprises a DNA recombinase.

40. The composition of claim 39, wherein the DNA recombinase is Cre recombinase.

41. The composition of any one of claims 33-40, wherein the composition comprises a zinc finger nuclease.

42. The composition of any one of claims 33-41, wherein the composition comprises a transcription activator-like effector nuclease.

43. The composition of any one of claims 34-42, wherein the composition comprises a nanoparticle comprising the polymer of any of claims 1-30 and the nucleic acid or polypeptide.

44. The composition of any one of claims 31-43, wherein the composition further comprises a second polymer comprising a hydrolysable polymer backbone, the polymer backbone comprising:

(i) monomer units with a side chain comprising a hydrophobic group;

(ii) monomer units with a side chain comprising an oligoamine or polyamine; and optionally

(iii) monomer units with a side chain comprising an ionizable group, optionally with a pKa less than 7.

45. The composition of claim 44, wherein the hydrophobic group of the second polymer comprises an alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group.

46. The composition of claim 45, wherein the hydrophobic group of the second polymer comprises a C_3-C_{12} linear or branched alkyl group, optionally a C_3-C_6 linear or branched alkyl group.

47. The composition of any one of claims 44-46, wherein the oligoamine or polyamine of the second polymer comprises a group of the formula:

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2_2$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2_2]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2_2]_2\}_2$,

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^4\text{-}R^5]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^4\text{-}R^5\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^4\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^5]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^5\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[N\{(CH_2)_{s1}\text{-}R^4\text{-}R^5\}—(CH_2)_{q1}—]_{r1}NR^2_2$;

$—(CH_2)_{p1}—[N\{(CH_2)_{s1}\text{-}R^5\}—(CH_2)_{q1}—]_{r1}NR^2_2$,

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH(CONH_2)\text{-}(CH_2)_{s1}\text{-}R^5$; or

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH(CONH_2)\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5$,

wherein p1 to p4, q1 to q6, r1 and r2, and s1 to s4 are each independently an integer of 1 to 5; each instance of R^2 is independently hydrogen or a C_1-C_{12} alkyl group, alkenyl

group, cycloalkyl group, or cycloalkenyl group, or R^2 is combined with a second R^2 so as to form a heterocyclic group; each instance of is independently -C(O)O-, -C(O)NH-, or -S(O)(O)-; and each instance of R^5 is independently an alkyl group, cycloalkyl group, alkenyl group, cycloalkenyl group, aryl group, heteroalkyl group, heterocyclic group, or combination thereof optionally comprising from 2 to 8 tertiary amines or a substituent comprising a tissue-specific or cell-specific targeting moiety.

48. The composition of any one of claims 44-47, wherein the polyamine of the second polymer comprises

$$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2\ ;$$

$$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2{}_2]_2;$$

$$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}; \text{ or}$$

$$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2{}_2]_2\}_2;$$

and each R^2 is independently hydrogen or a C_1-C_3 alkyl group;

optionally wherein the polyamine comprises

$$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2.$$

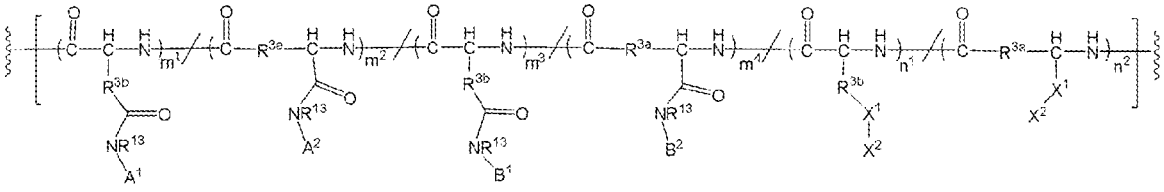
49. The composition of any one of claims 44-48, wherein the hydrolysable polymer backbone of the second polymer comprises about 1 to about 80 mol% of the monomer units having a hydrophobic group, about 1 to about 80 mol% of the monomer units having an oligoamine or polyamine, and 0 to about 80 mol% of the monomer units having an ionizable group.

50. The composition of any one of claims 44-49, wherein the hydrolysable polymer backbone of the second polymer comprises monomer units with a side chain comprising an ionizable group with a pKa less than 7.

51. The composition of any one of claims 44-50, wherein the hydrolysable polymer backbone of the second polymer comprises a polyamide, poly-N-alkylamide, polyester, polycarbonate, polycarbamate, or a combination thereof.

52. The composition of claim 51, wherein the hydrolysable polymer backbone of the second polymer comprises a polyamide.

53. The composition of claim 44, the second polymer comprising a structure of Formula 3:



wherein:

each of m^1, m^2, m^3, and m^4 is an integer from 0 to 1000, provided that the sum of $m^1 + m^2 + m^3 + m^4$ is greater than 5;

each of n^1 and n^2 is an integer from 0 to 1000, provided that the sum of $n^1 + n^2$ is greater than 2;

the symbol "/" indicates that the units separated thereby are linked randomly or in any order;

each instance of R^{3a} is independently a methylene or ethylene group;

each instance of R^{3b} is independently a methylene or ethylene group;

each X^1 independently is —C(O)O—, —C(O)NR^{13}—, —C(O)—, —S(O)(O)—, or a bond;

each instance of R^{13} is independently hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl group, any of which can be optionally substituted with one or more substituents;

each instance of X^2 is independently a C_1-C_{12} alkyl or heteroalkyl group, C_3-C_{12} cycloalkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkenyl group, aryl group, heterocyclic group, or combination thereof optionally comprising one or more primary, secondary, or tertiary amines; any of which are optionally substituted with one or more substituents;

A^1 and A^2 are each independently a group of formula

$$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2 ;$$

$$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2{}_2]_2;$$

$$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}; \text{ or}$$

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—$NR^2{}_2$]$_2$}$_2$,

B^1 and B^2 are each independently

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-$(CH_2)_{s1}$-$R^4$-$R^5$;

—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—NR^2-$(CH_2)_{s2}$-R^4-R^5]$_2$;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—$NR^2{}_2$][—$(CH_2)_{q4}$—NR^2—]$_{r2}$$(CH_2)_{s3}$-$R^4$-$R^5$};

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-$(CH_2)_{s4}$-R^4-R^5]$_2$}$_2$;

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-$CH_2$-CHOH-$R^5$;

—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—NR^2-CH_2-CHOH-R^5;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—$NR^2{}_2$][—$(CH_2)_{q4}$—NR^2—]$_{r2}$-CH_2-CHOH-R^5;

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-CH_2-CHOH-R^5]$_2$}$_2$;

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-$(CH_2)_{s1}$-$R^5$;

—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—NR^2-$(CH_2)_{s2}$-R^5]$_2$;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—$NR^2{}_2$][—$(CH_2)_{q4}$—NR^2—]$_{r2}$$(CH_2)_{s3}$-$R^5$};

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-$(CH_2)_{s4}$-R^5]$_2$}$_2$;

—$(CH_2)_{p1}$—[N{$(CH_2)_{s1}$-R^4-R^5}—$(CH_2)_{q1}$—]$_{r1}$$NR^2{}_2$;

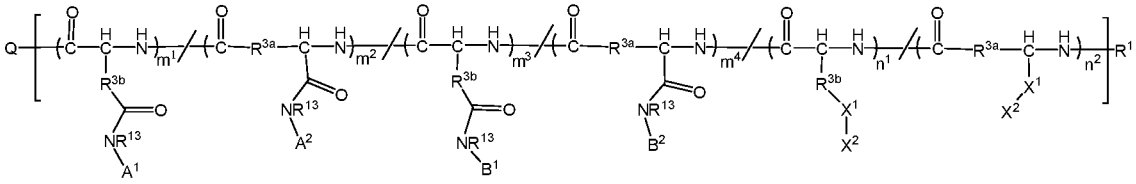
—$(CH_2)_{p1}$—[N{$(CH_2)_{s1}$-R^5}—$(CH_2)_{q1}$—]$_{r1}$$NR^2{}_2$,

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-CH($CONH_2$)-$(CH_2)_{s1}$-$R^5$; or

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-CH($CONH_2$)-$(CH_2)_{s1}$-$R^4$-$R^5$,

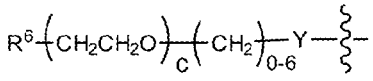
wherein p1 to p4, q1 to q6, r1 and r2, and s1 to s4 are each independently an integer of 1 to 5; each instance of R^2 is independently hydrogen or a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12}cycloalkyl group, or C_3-C_{12}cycloalkenyl group, or R^2 is combined with a second R^2 so as to form a heterocyclic group; each instance of R^4 is independently -C(O)O-, -C(O)NH-, -O-C(O)O-, or -S(O)(O)-; and each instance of R^5 is independently an alkyl group, cycloalkyl group, alkenyl group, cycloalkenyl group, aryl group, heteroalkyl group, heterocyclic group, or combination thereof optionally comprising from 2 to 8 tertiary amines or a substituent comprising a tissue-specific or cell-specific targeting moiety.

54. The composition of claim 53, the second polymer having the structure of Formula 3A:



wherein

Q is of formula:



c is an integer from 0 to 50;

Y is optionally present and is a cleavable linker;

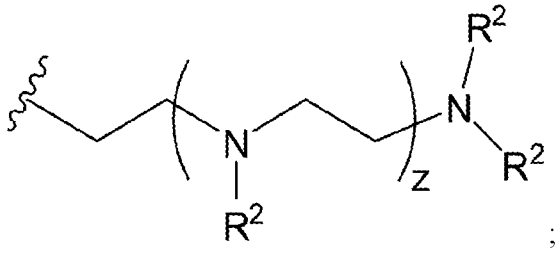
R^1 is hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, or a C_1-C_{12} linear or branched alkyl group optionally substituted with one or more substituents;

R^6 is hydrogen, an amino group, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, a C_1-C_{12} heteroalkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, a C_1-C_{12} linear or branched alkyl group optionally substituted with one or more amines; or a tissue-specific or cell-specific targeting moiety;

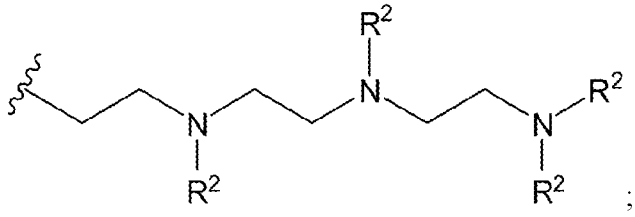
and m^1, m^2, m^3, m^4, n^1, n^2, R^{3a}, R^{3b} , R^{13}, X^1, X^2, A^1, A^2, B^1, and B^2 are as defined in claim 49.

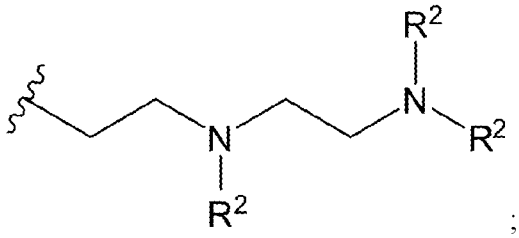
55. The composition of claim 53 or claim 54, wherein each of B^1 and B^2 of the second polymer is a group of formula $–(CH_2)_2$-NH-$(CH_2)_2$-NH-$(CH_2)_2$-R^4-R^5.

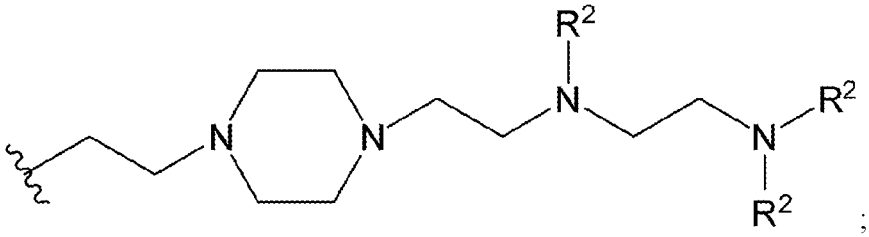
56. The composition of any of claims 53-55, wherein each R^5 of the second polymer is independently:

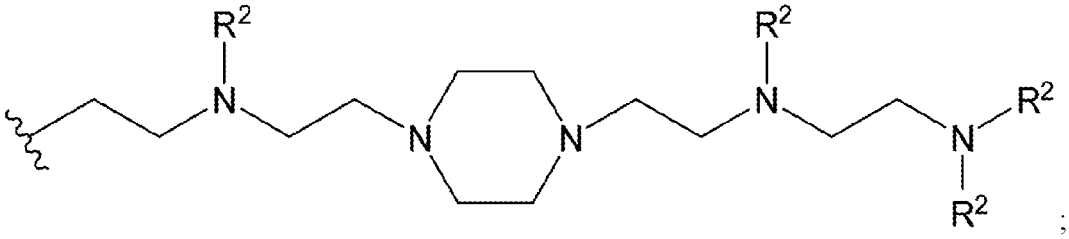


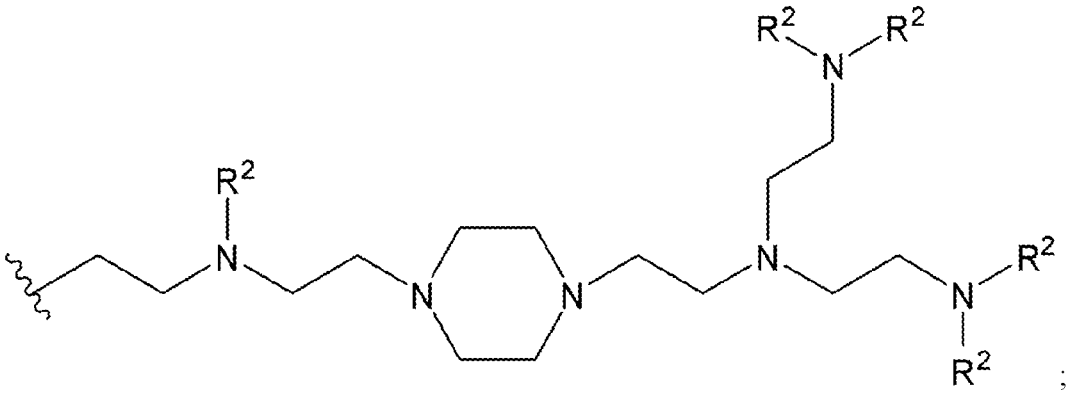
;

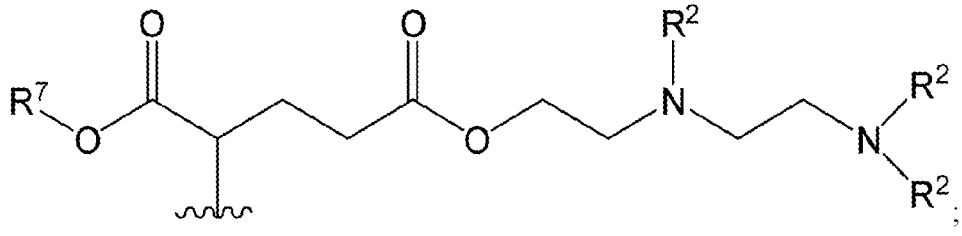


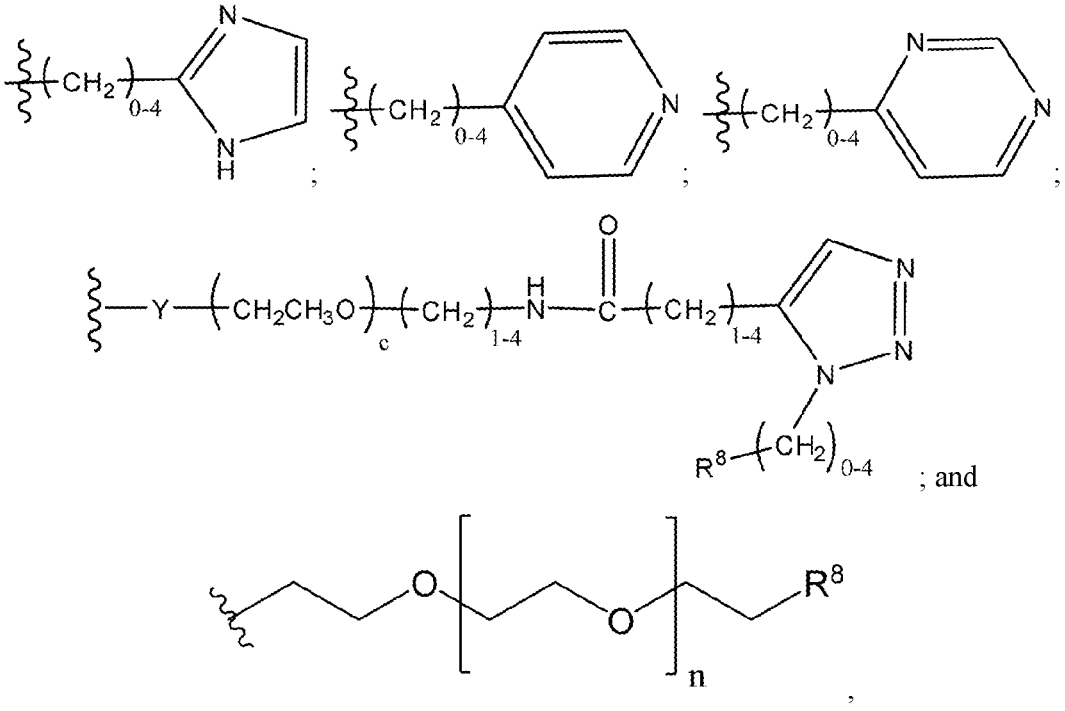












; and

,

wherein

each instance of R^2 is independently hydrogen or a C_1-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, or R^2 is combined with a second R^2 so as to form a heterocyclic group;

R^7 is a C_1-C_{50} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group optionally substituted with one or more amines;

z is an integer from 1 to 5;

c is an integer from 0 to 50;

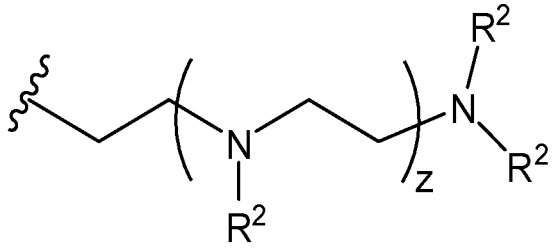
Y is optionally present and is a cleavable linker;

n is an integer from 0 to 50; and

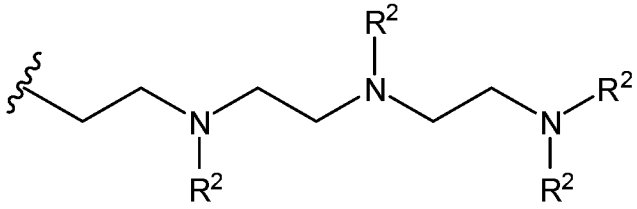
R^8 is a tissue-specific or cell-specific targeting moiety.

57. The composition of any of claims 53-56, wherein R^4 of the second polymer is –C(O)-O-.

58. The composition of any of claims 53-57, wherein R^5 of the second polymer is

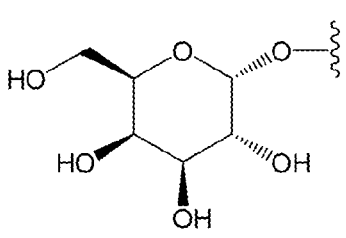


or

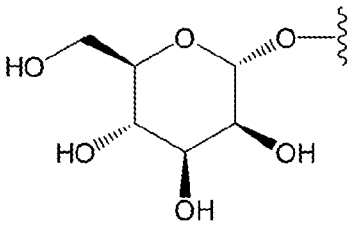


59. The composition of any of claims 53-58, wherein the ratio of $(m^1+m^2+m^3+m^4)/(n^1+n^2)$ of the second polymer is about 25 or less, and, optionally, about 1 or more.

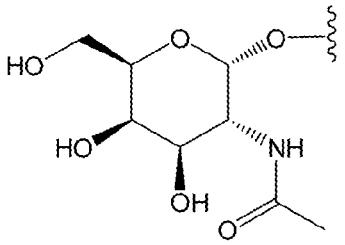
60. The composition of any of claims 53-59, wherein the tissue-specific or cell-specific targeting moiety of the second polymer is:



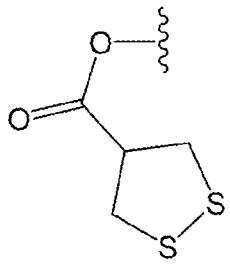
alpha-d-galactose ;



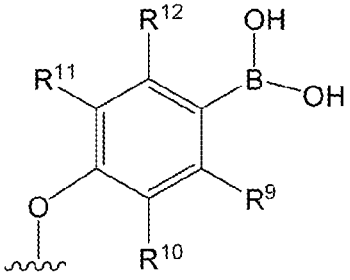
alpha-d-mannose ;



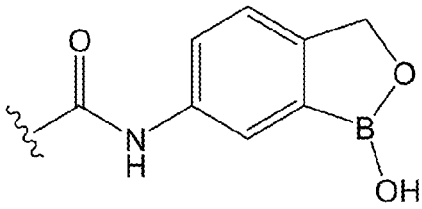
N-acetylgalactosamine ;



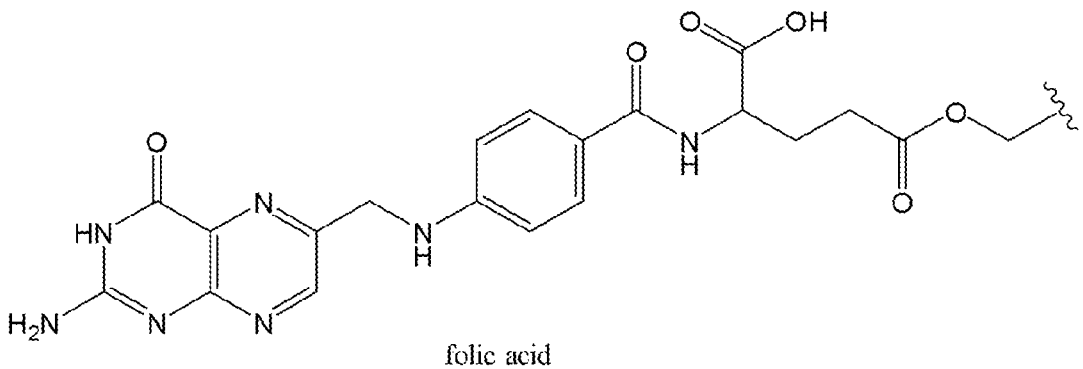
Asparagusic acid ;



Phenylboronic acid ;



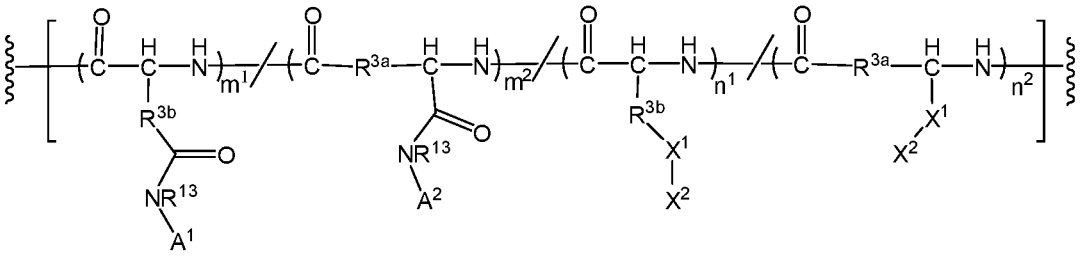
OH; or



folic acid

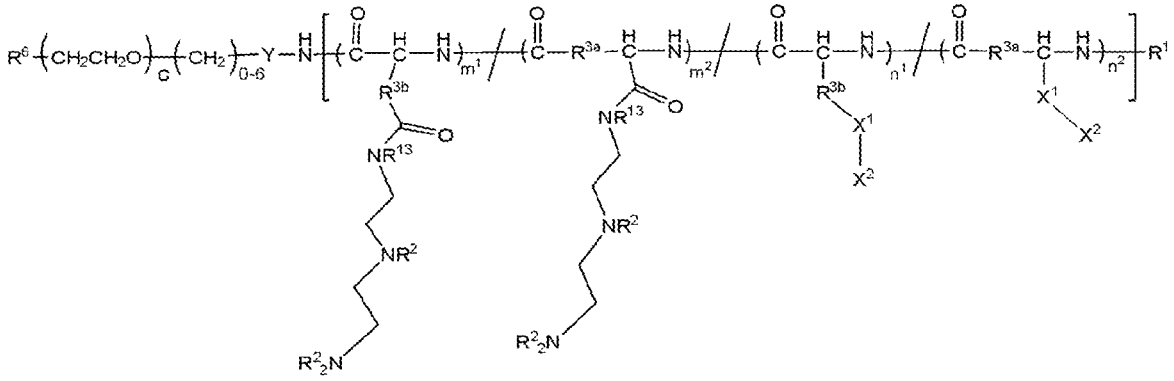
wherein each of R^9, R^{10}, R^{11}, and R^{12} is independently hydrogen, halogen, C_1-C_4 alkyl, or C_1-C_4 alkoxy, optionally substituted with one or more amino groups.

61. The composition of claim 53, the second polymer having the structure of Formula 4:



wherein m^1, m^2, n^1, n^2, R^{3a}, R^{3b}, R^{13}, X^1, X^2, A^1, and A^2 are as defined in claim 49.

62. The composition of claim 53, the second polymer having the structure of Formula 3B:



wherein

c is an integer from 0 to 50;

Y is optionally present and is a cleavable linker;

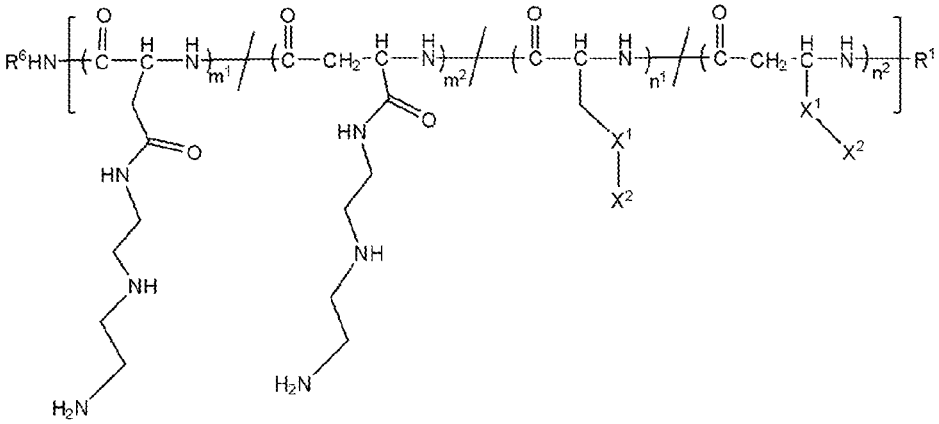
R^1 is hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl group, any of which are optionally substituted with one or more substituents;

each instance of R^2 is independently hydrogen or a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl group; and

R^6 is hydrogen, an amino group, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, a C_1-C_{12} heteroalkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl group, optionally substituted with one or more amines; or a tissue-specific or cell-specific targeting moiety;

and m^1, m^2, n^1, n^2, R^{3a}, R^{3b}, R^{13}, X^1, X^2, A^1, and A^2 are as defined in claim 49.

63. The composition of claim 53, the second polymer having the structure of Formula 3C:



wherein

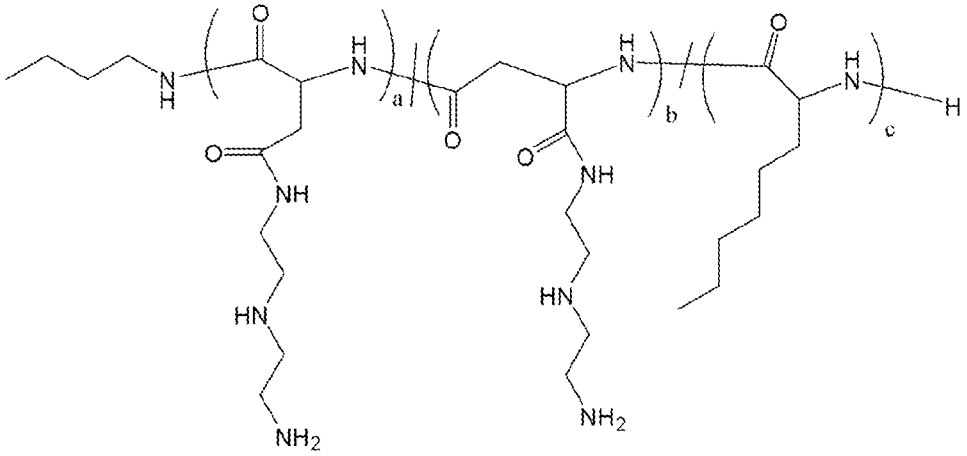
R^1 is hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12} cycloalkenyl group, any of which are optionally substituted with one or more substituents; and

R^6 is hydrogen, an amino group, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, a C_1-C_{12} heteroalkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkyl group, or C_3-C_{12}

cycloalkenyl group, any of which are optionally substituted with one or more amines; or a tissue-specific or cell-specific targeting moiety;

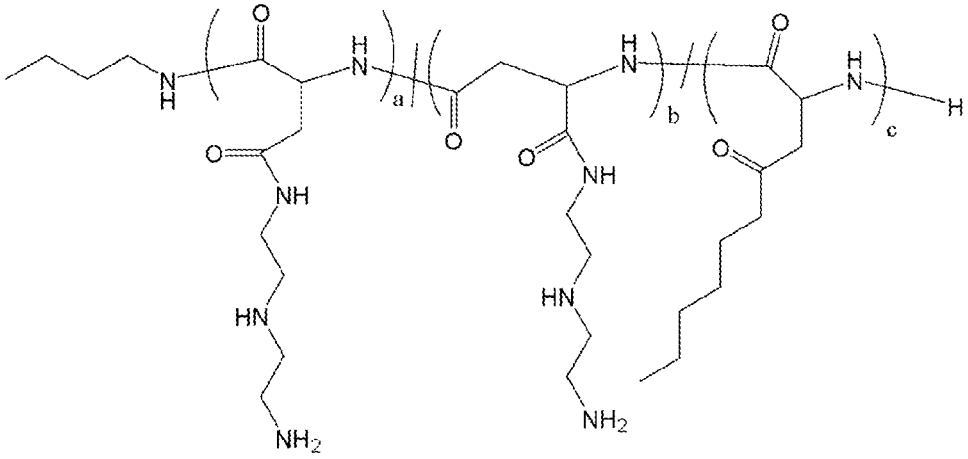
wherein m^1, m^2, n^1, n^2, R^{3a}, R^{3b}, R^{13}, X^1, X^2, A^1, and A^2 are as defined in 49.

64. The composition of claim 44, the second polymer having the formula:



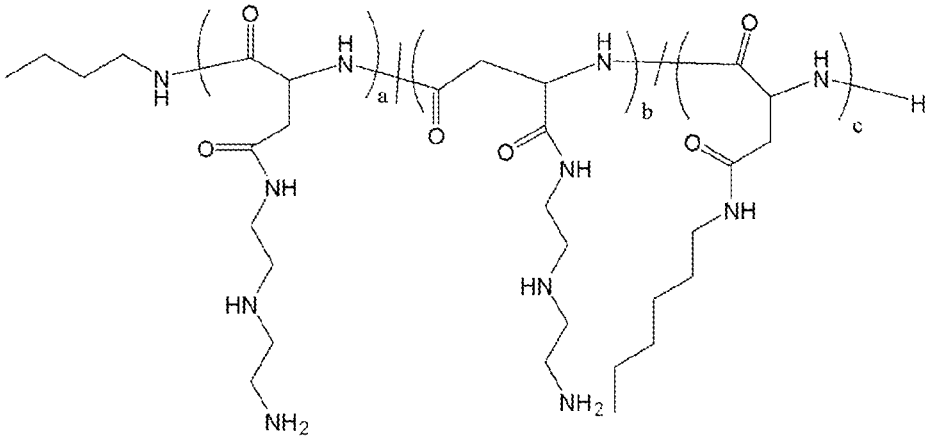
Polymer 1

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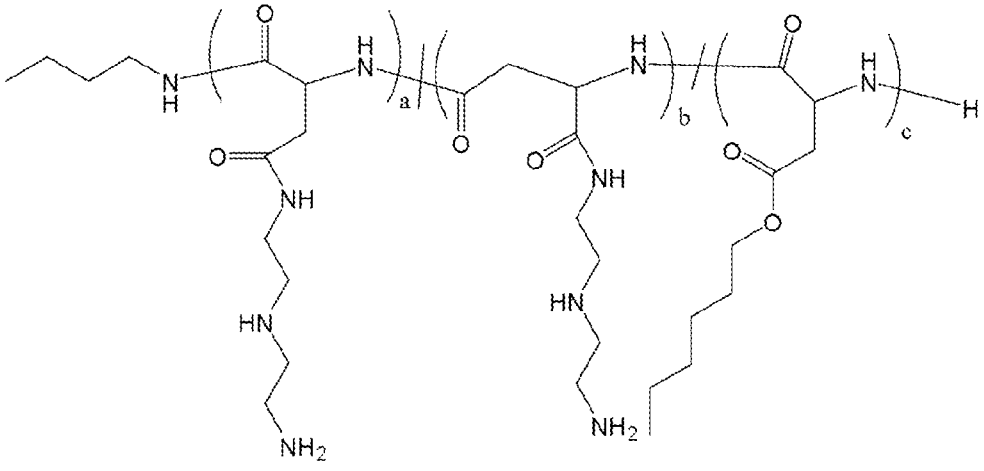


Polymer 2

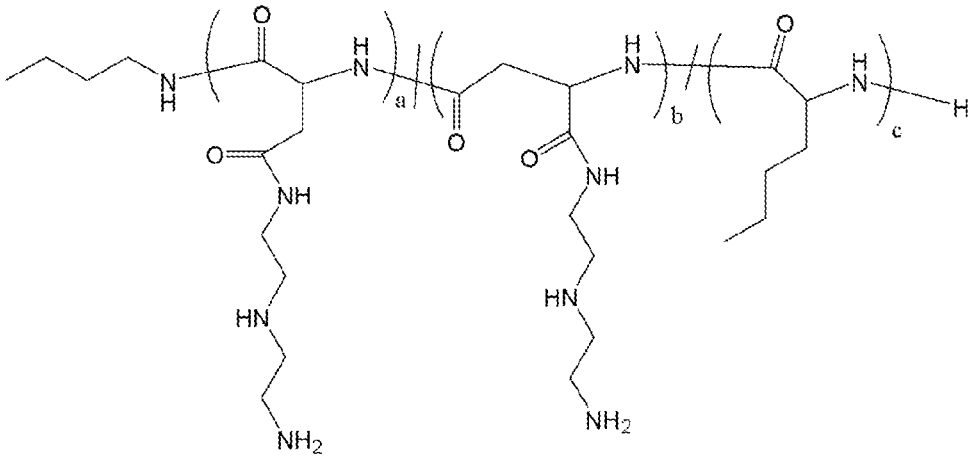
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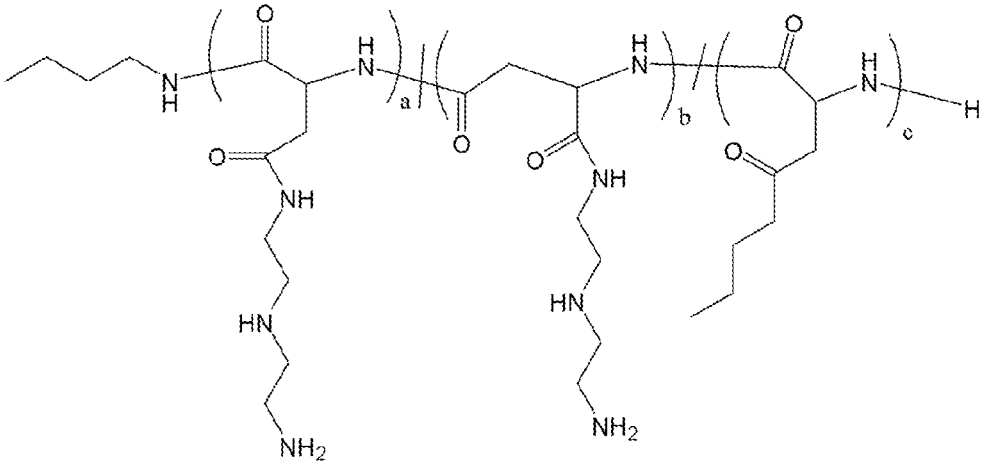
Polymer 3



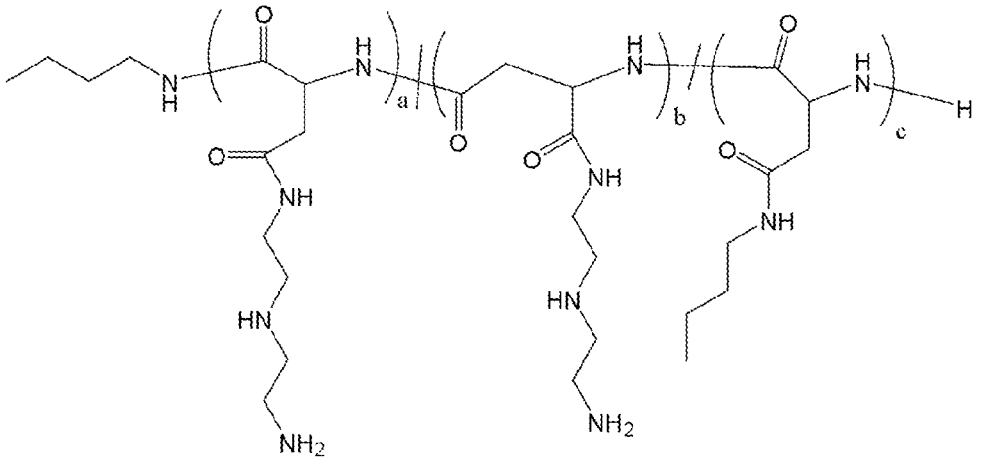
Polymer 4



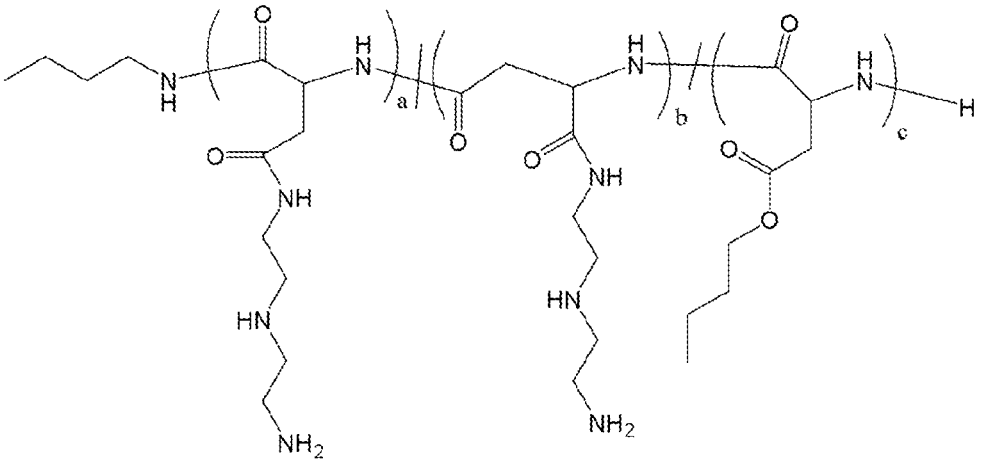
Polymer 5



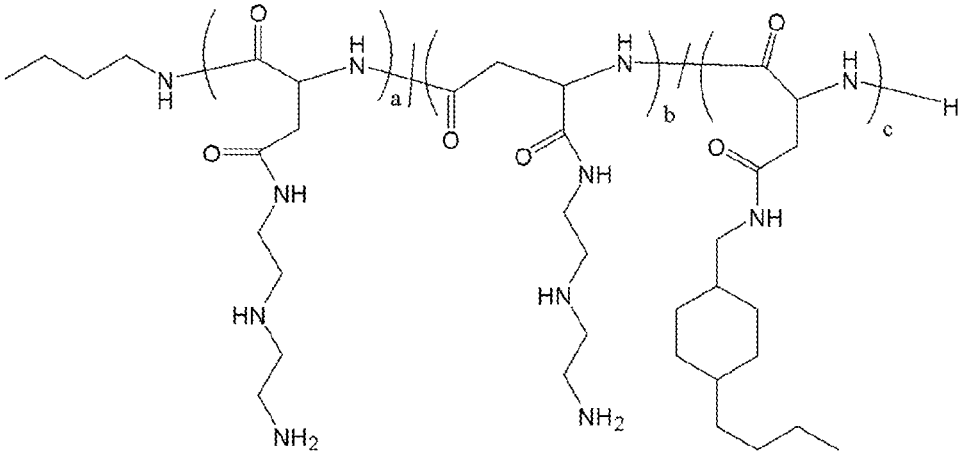
Polymer 6



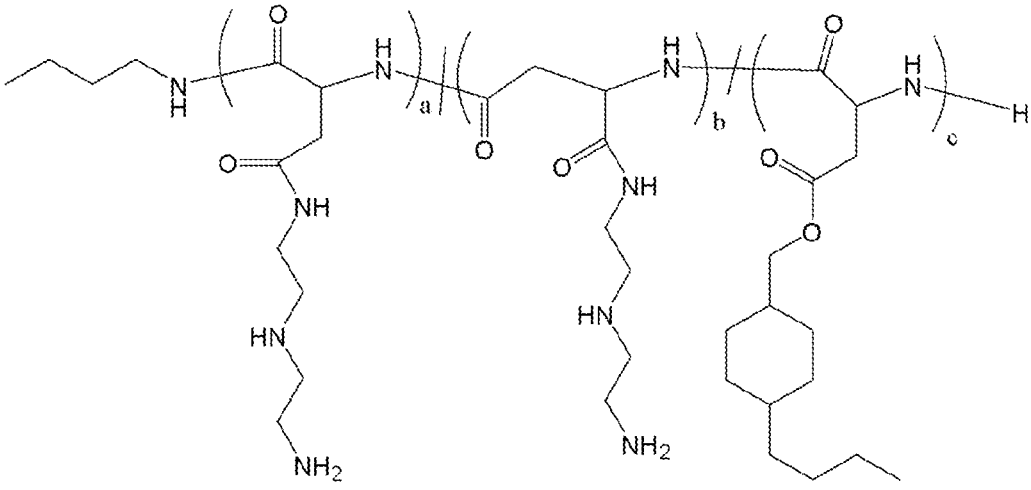
Polymer 7



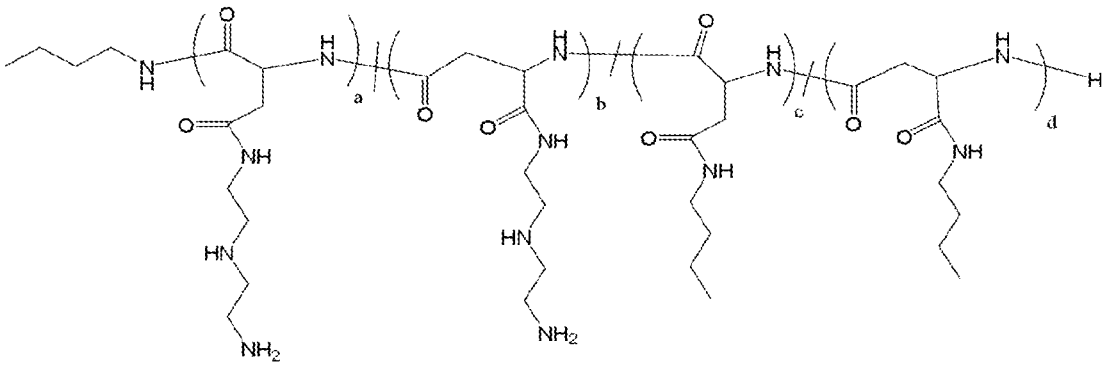
Polymer 8



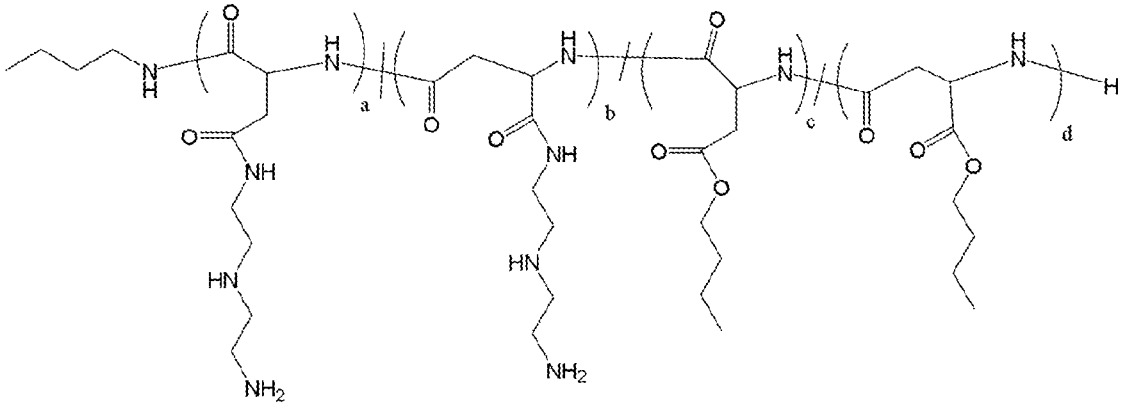
Polymer 9



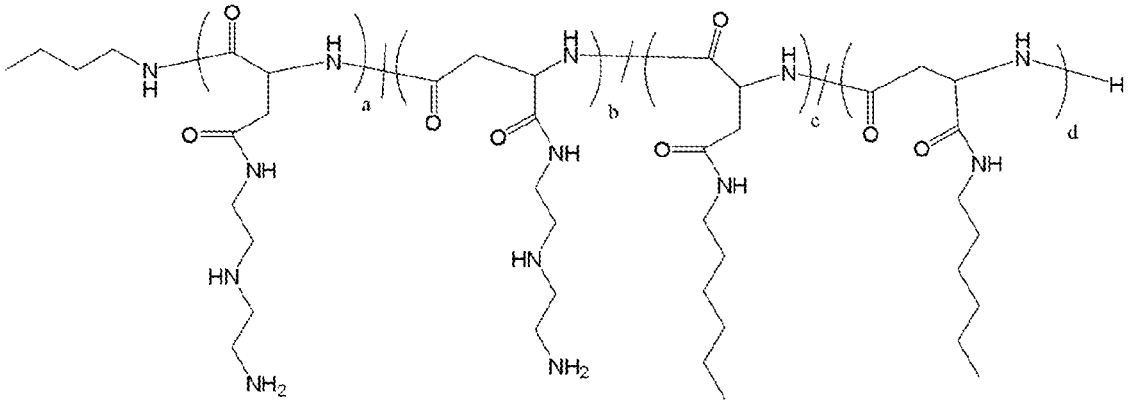
Polymer 10



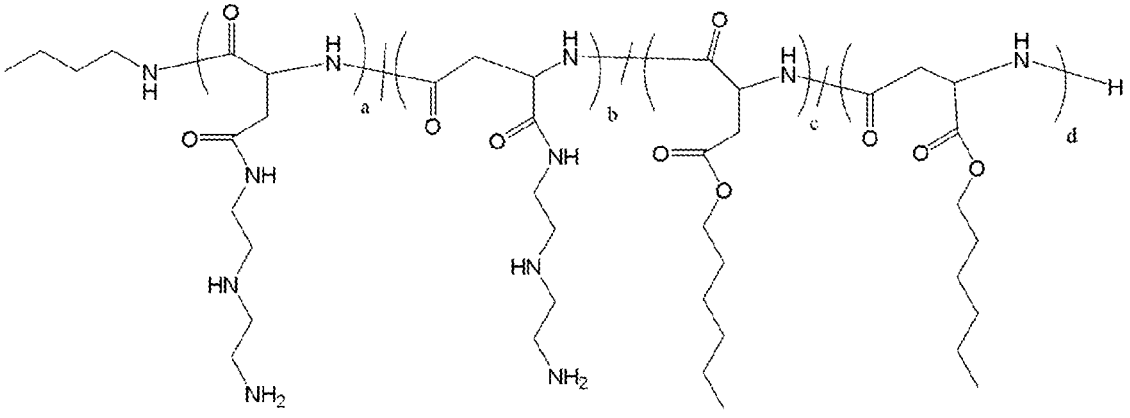
Polymer 11



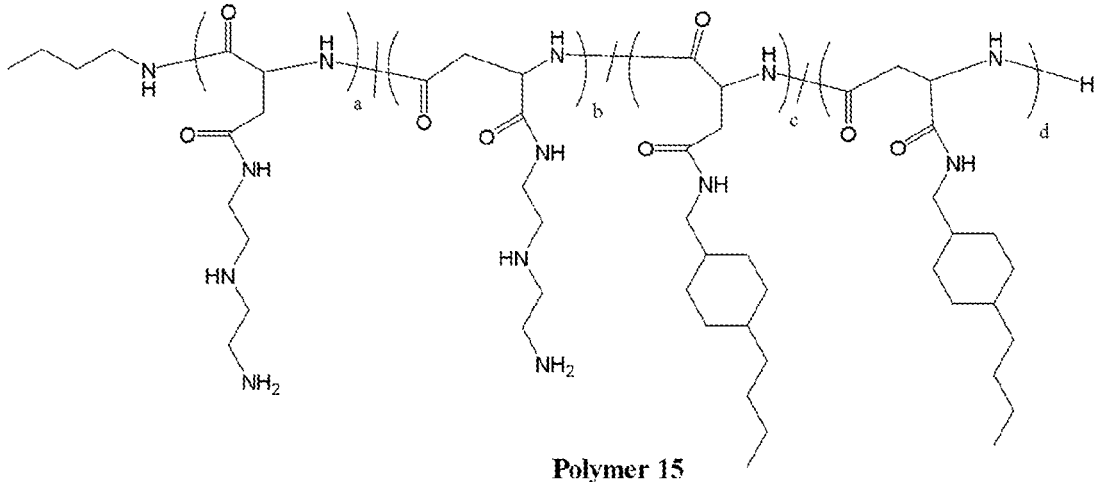
Polymer 12



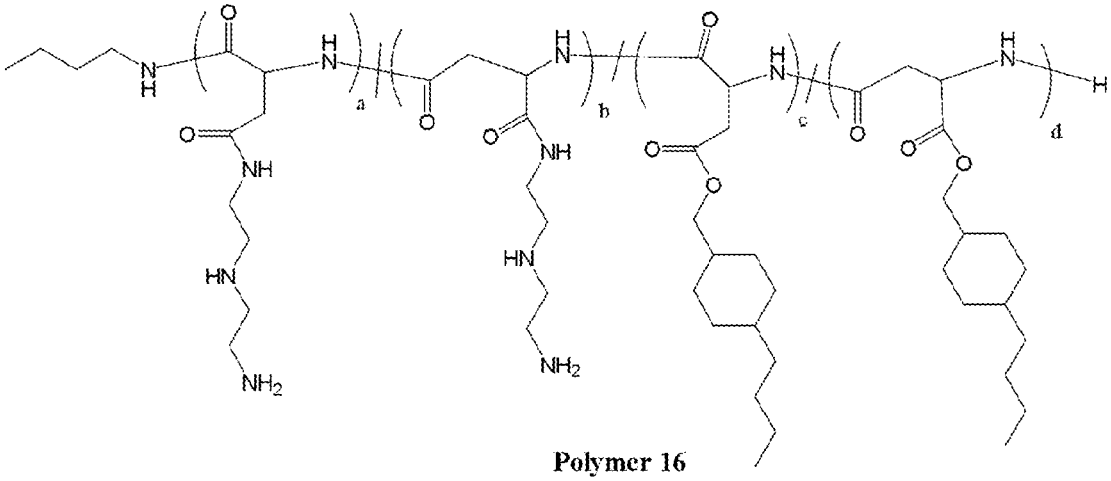
Polymer 13



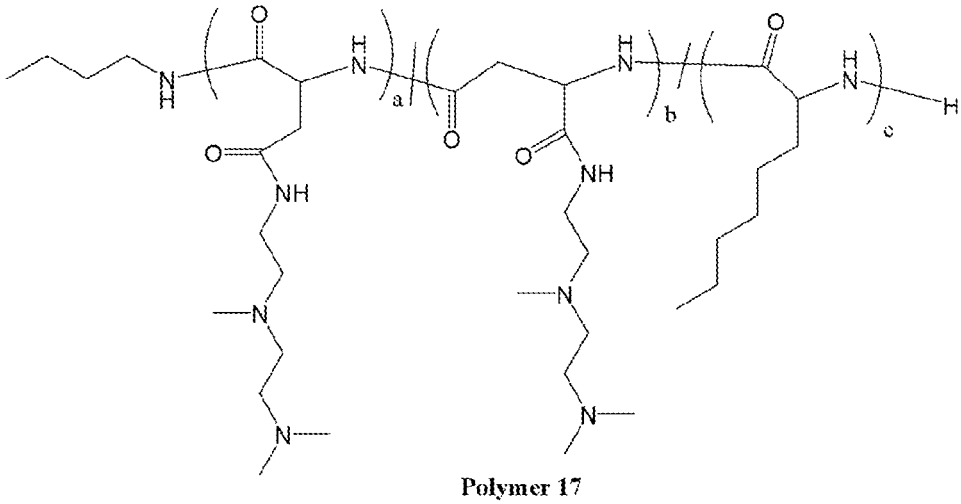
Polymer 14



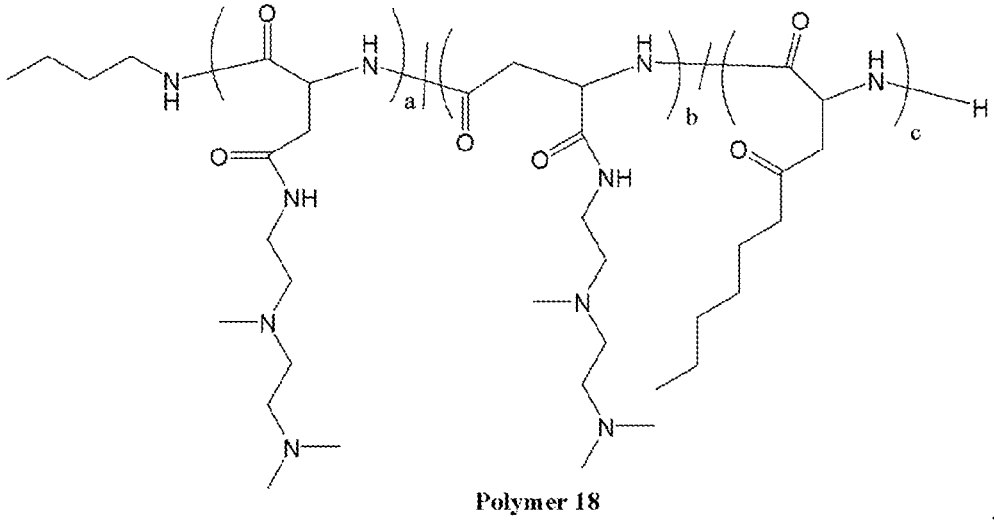
Polymer 15



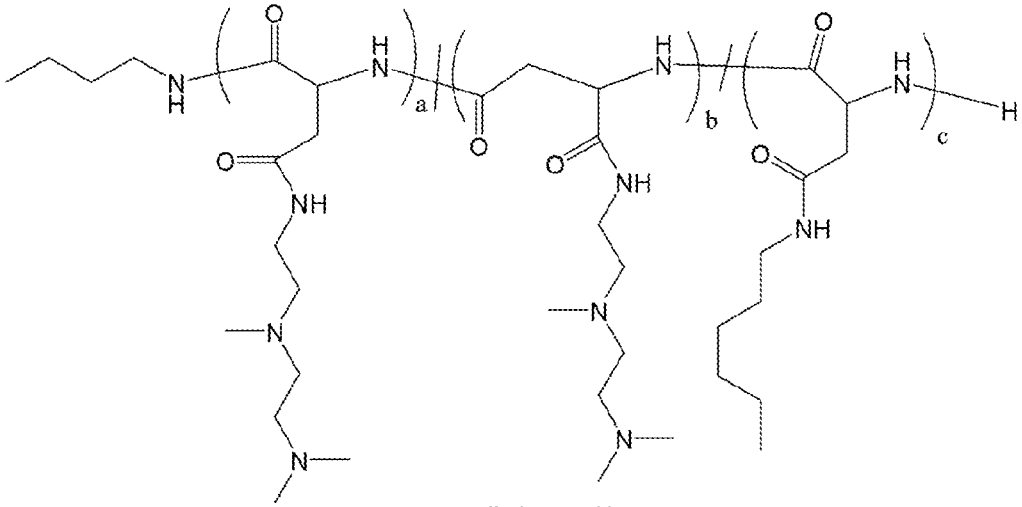
Polymer 16



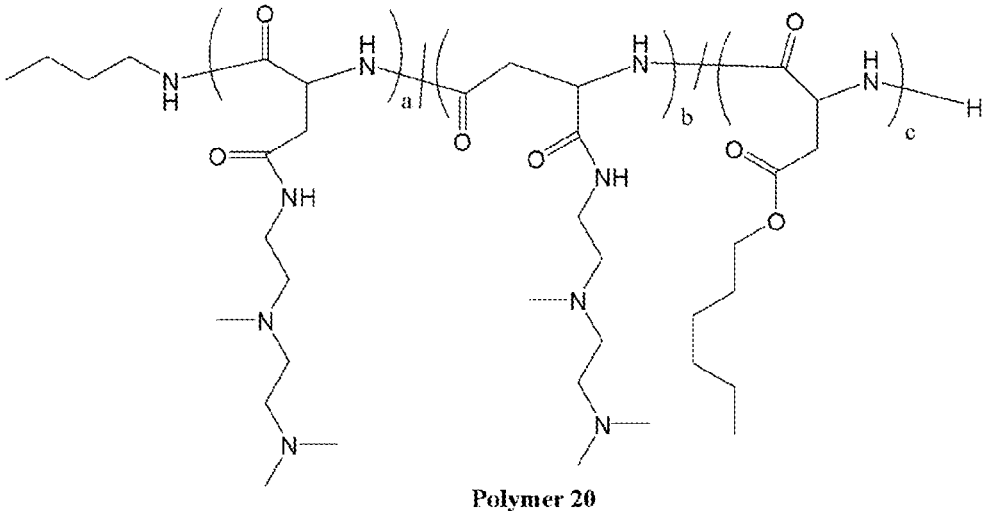
Polymer 17



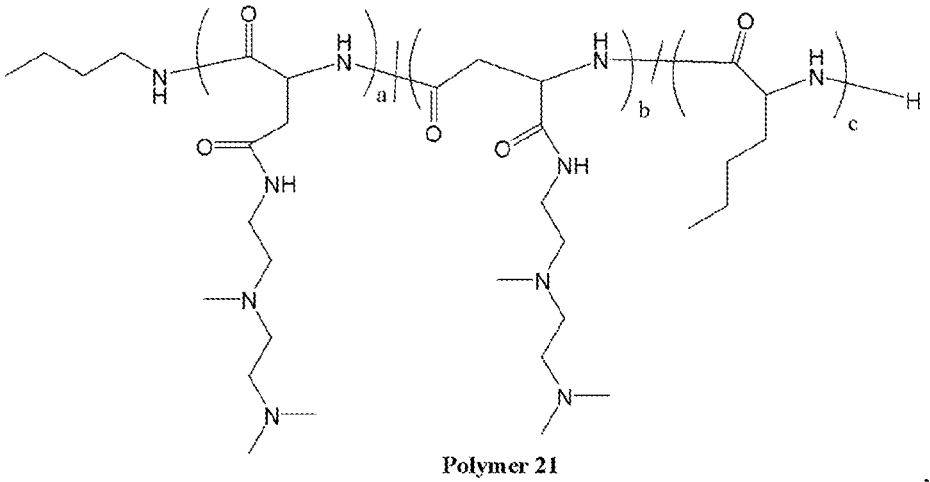
Polymer 18



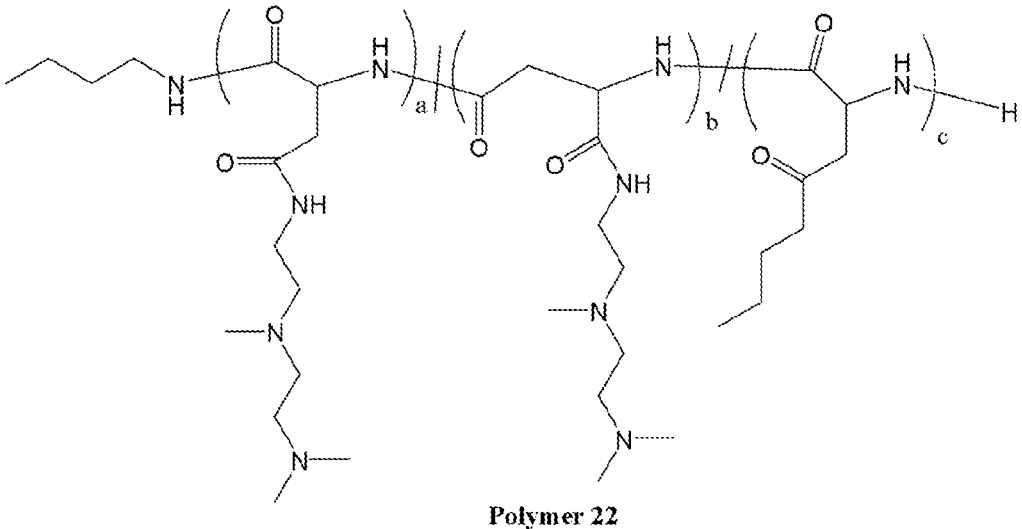
Polymer 19



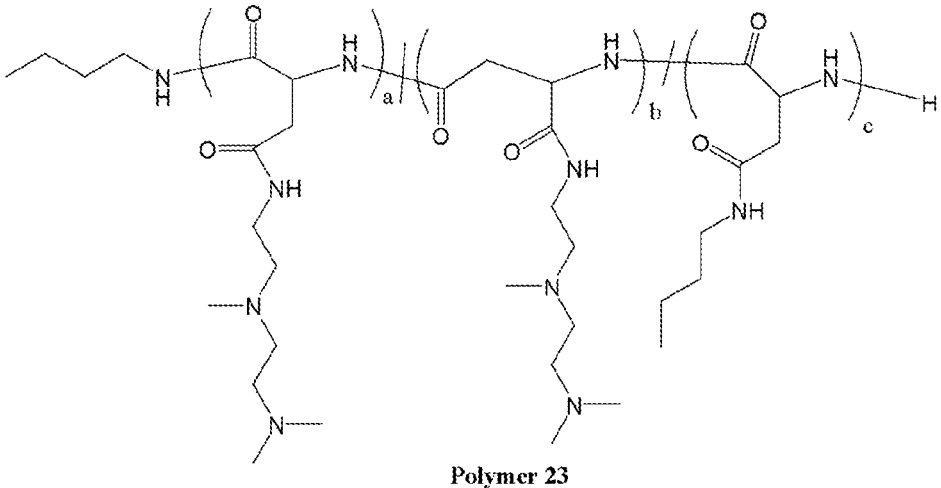
Polymer 20



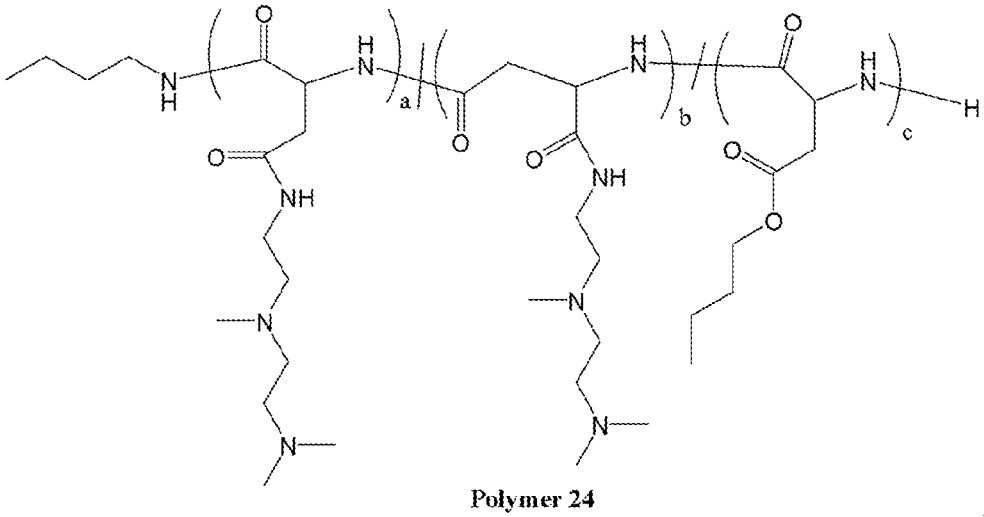
Polymer 21



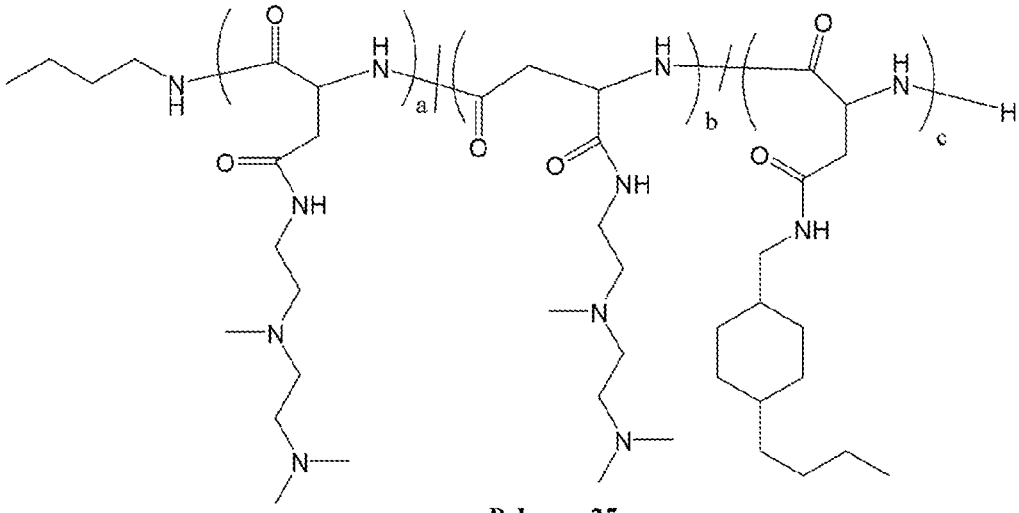
Polymer 22



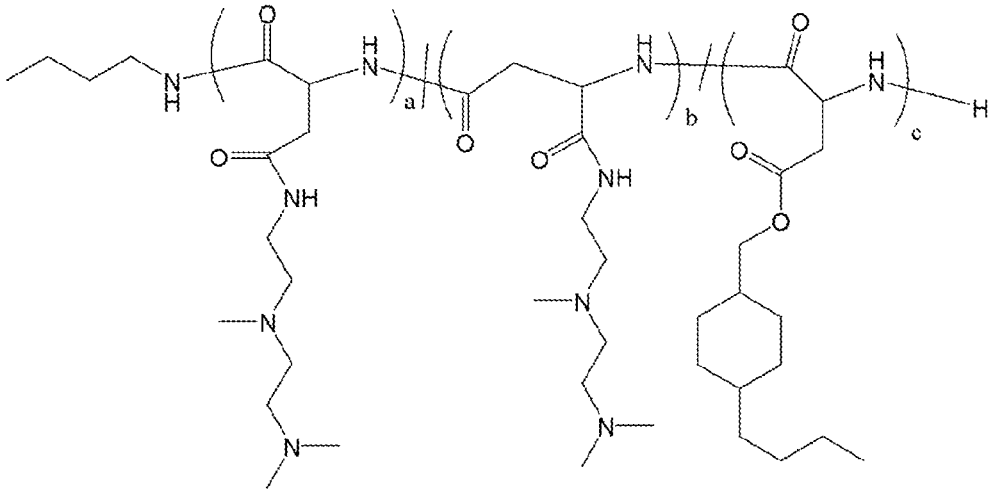
Polymer 23



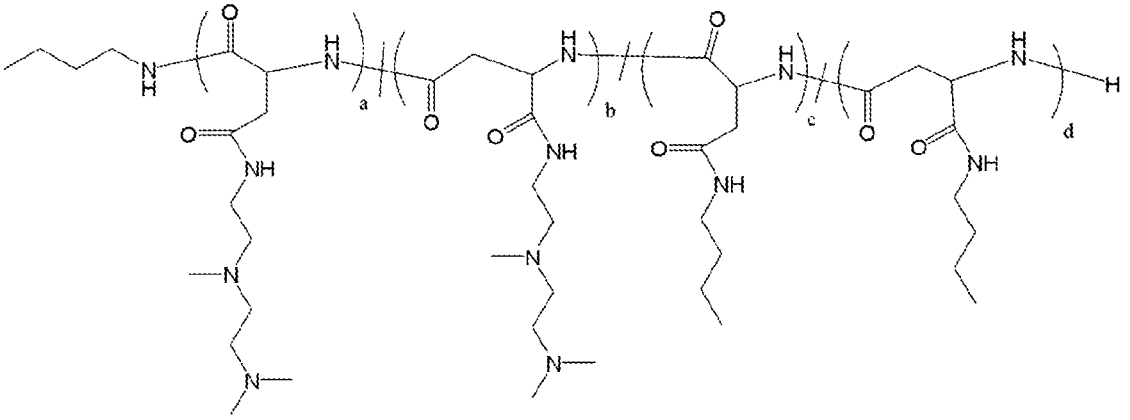
Polymer 24



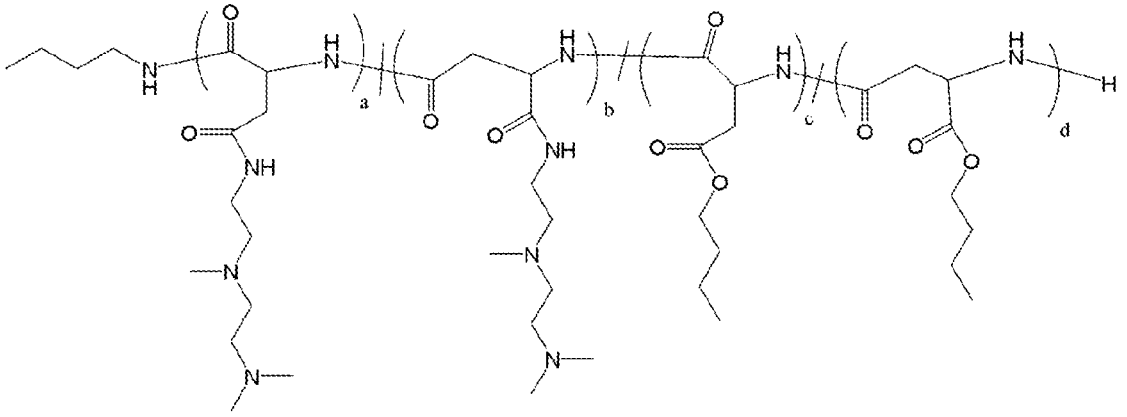
Polymer 25



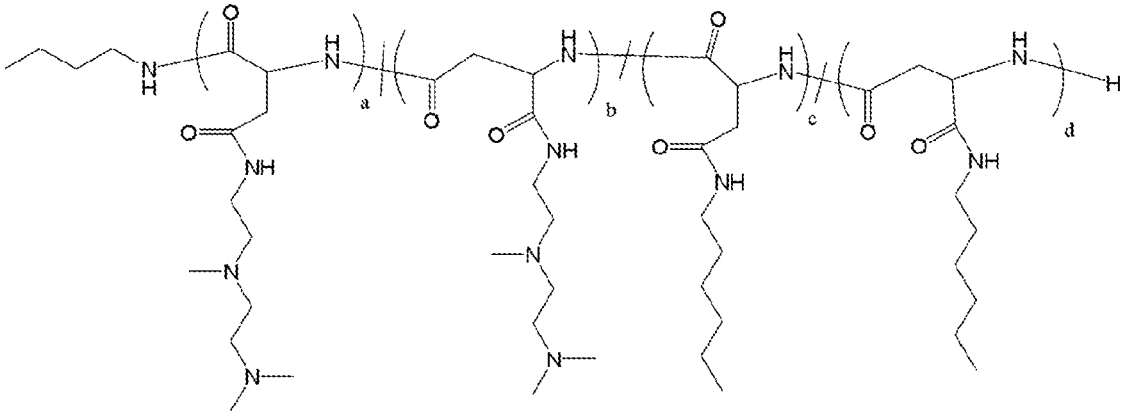
Polymer 26



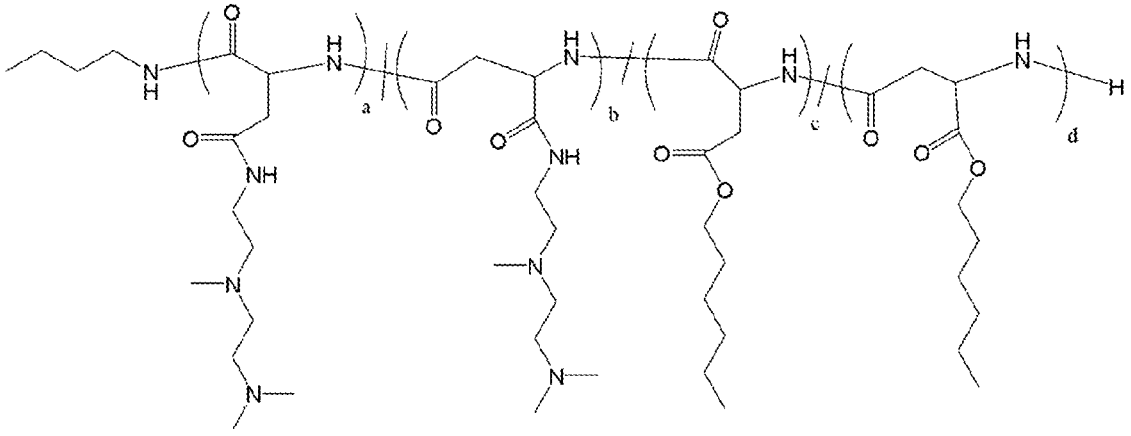
Polymer 27



Polymer 28

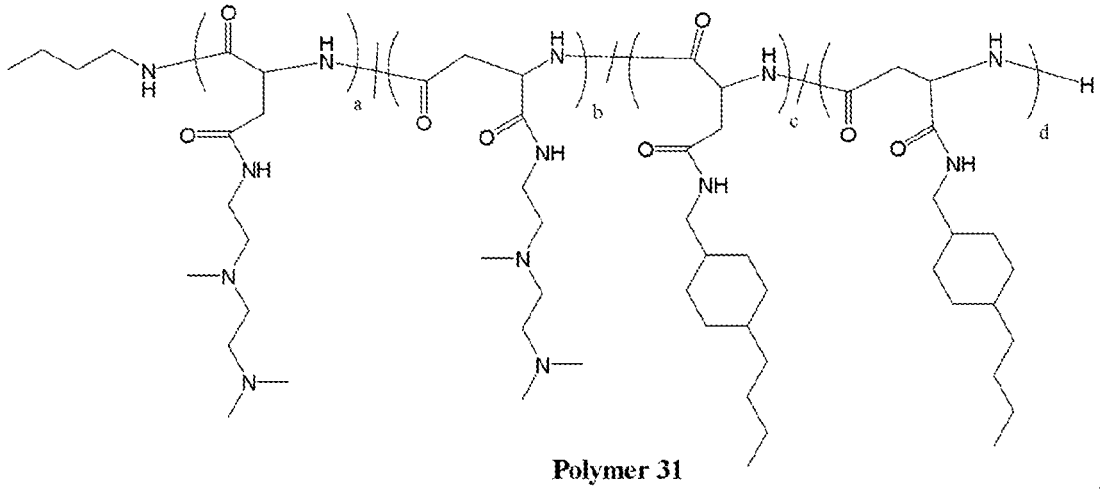


Polymer 29



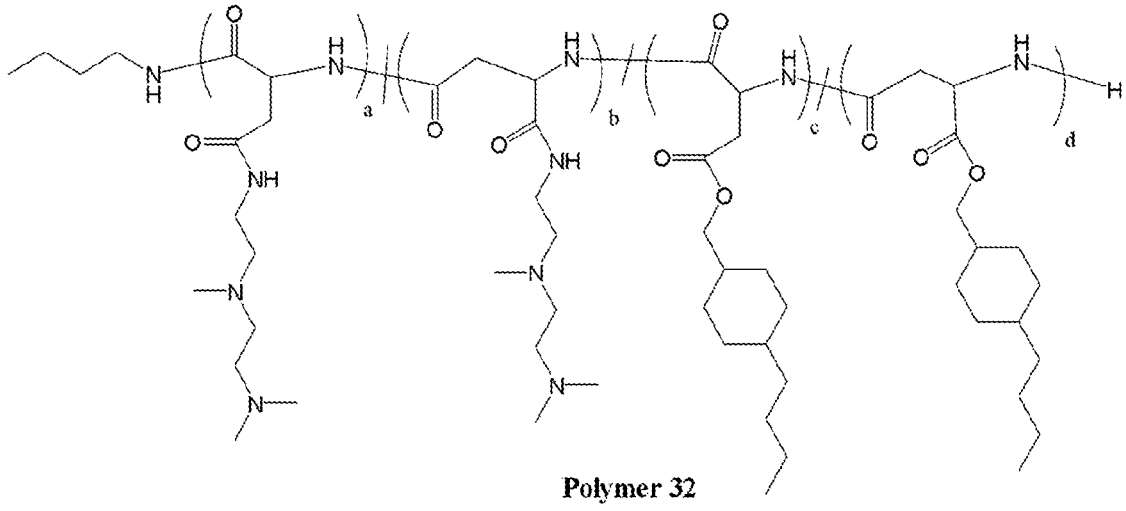
Polymer 30

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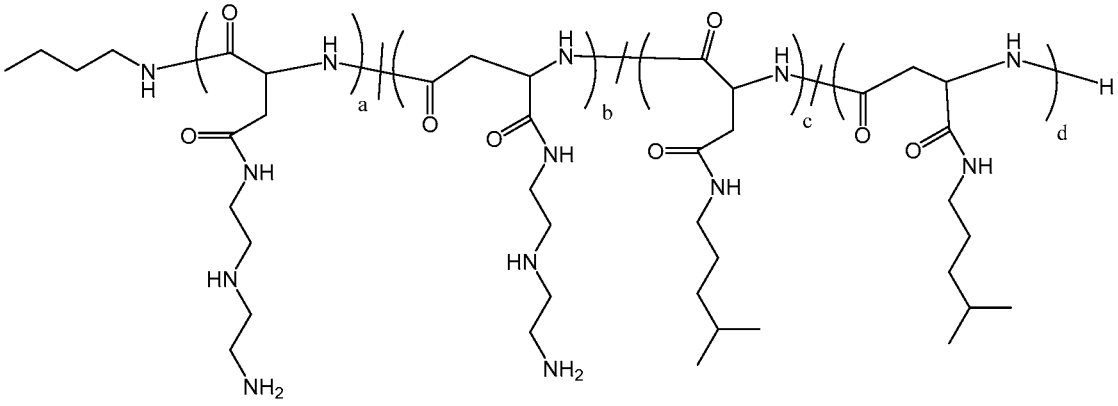
Polymer 31

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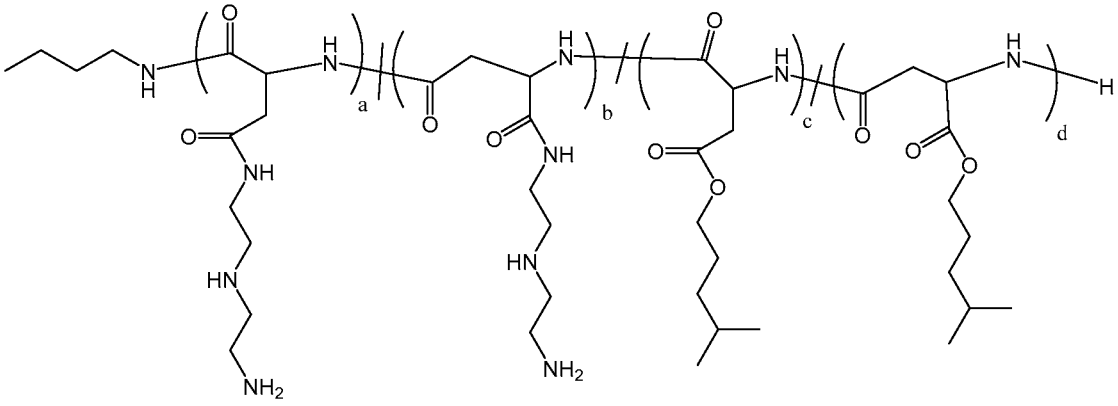
Polymer 32

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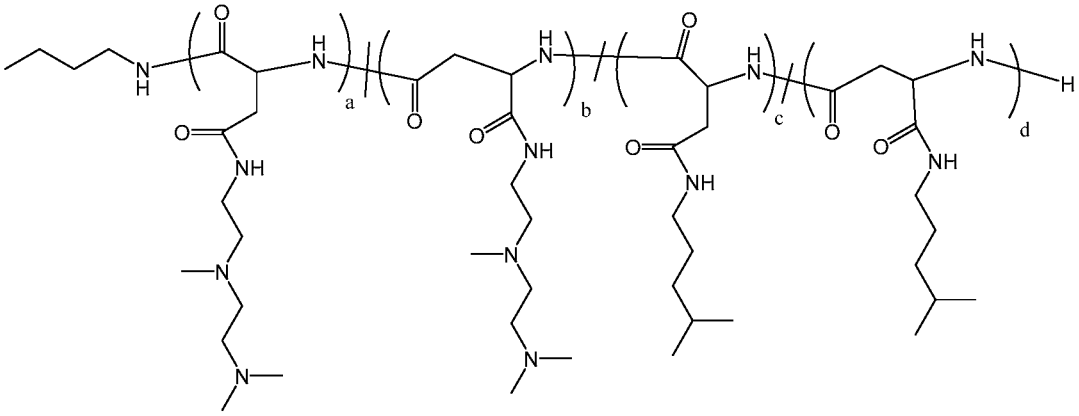
Polymer 33

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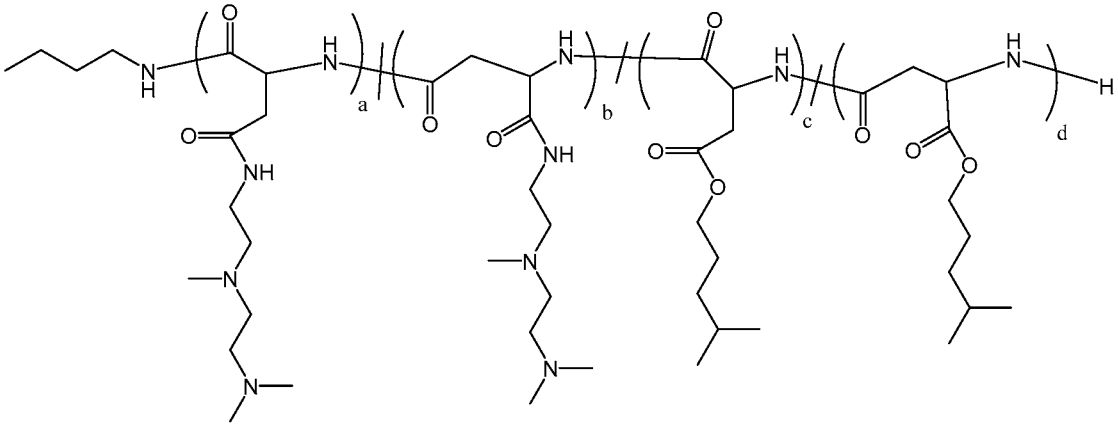
Polymer 34

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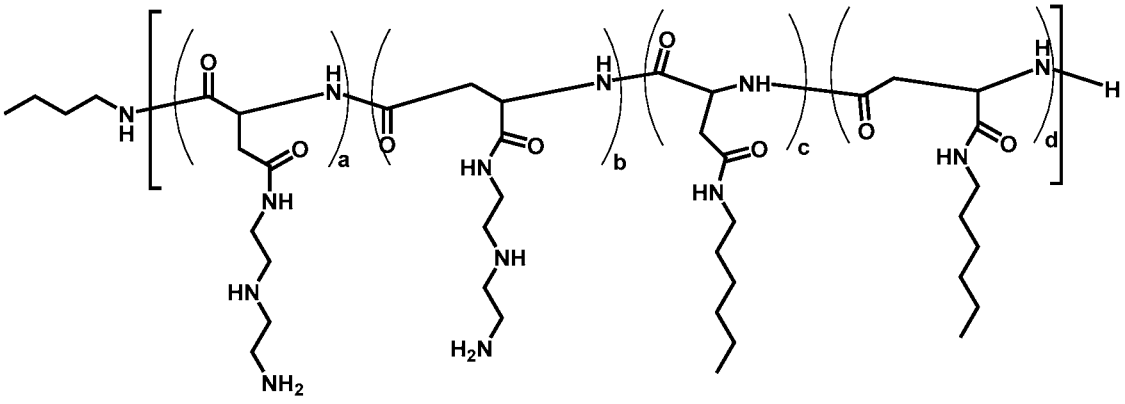


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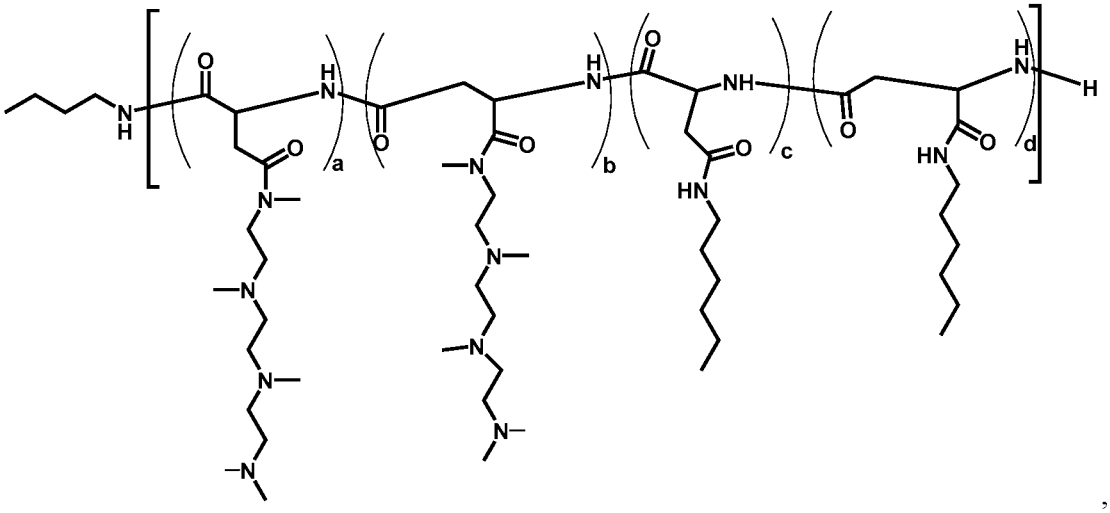
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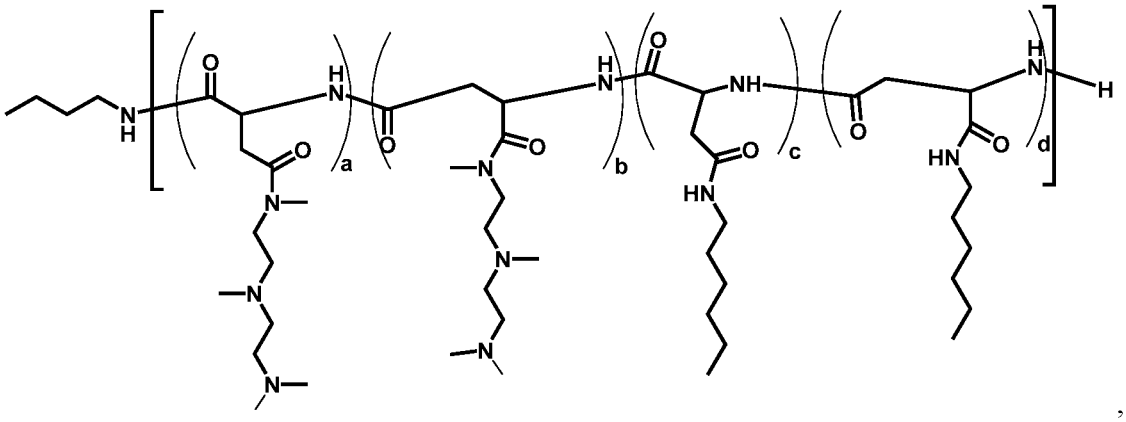
Polymer 36



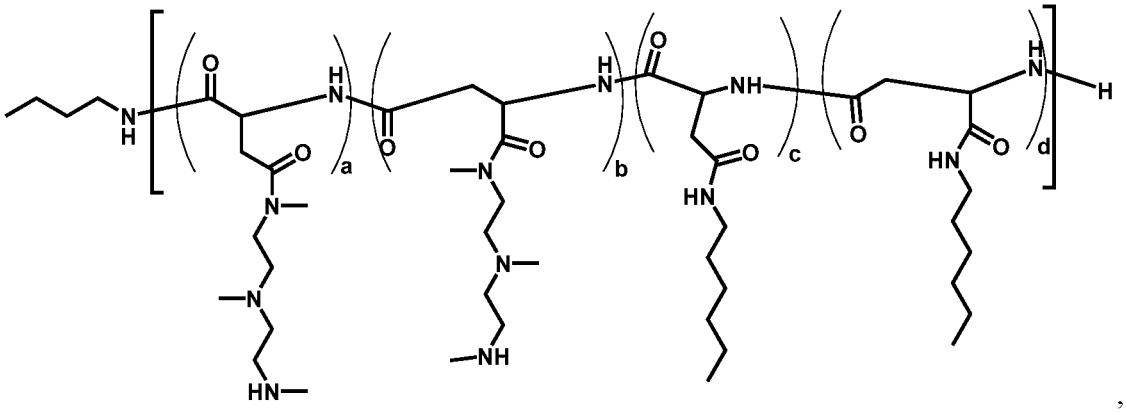
Polymer 37



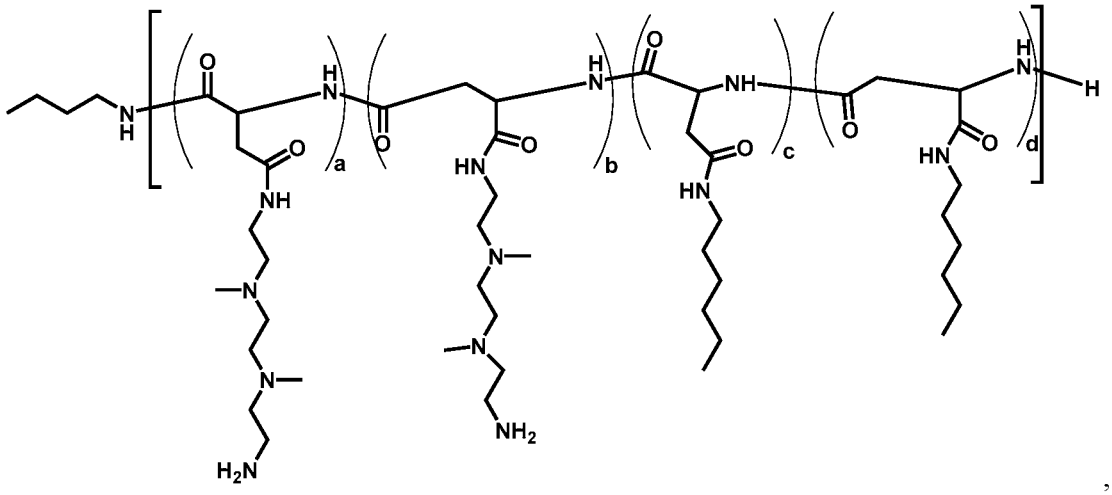
Polymer 38



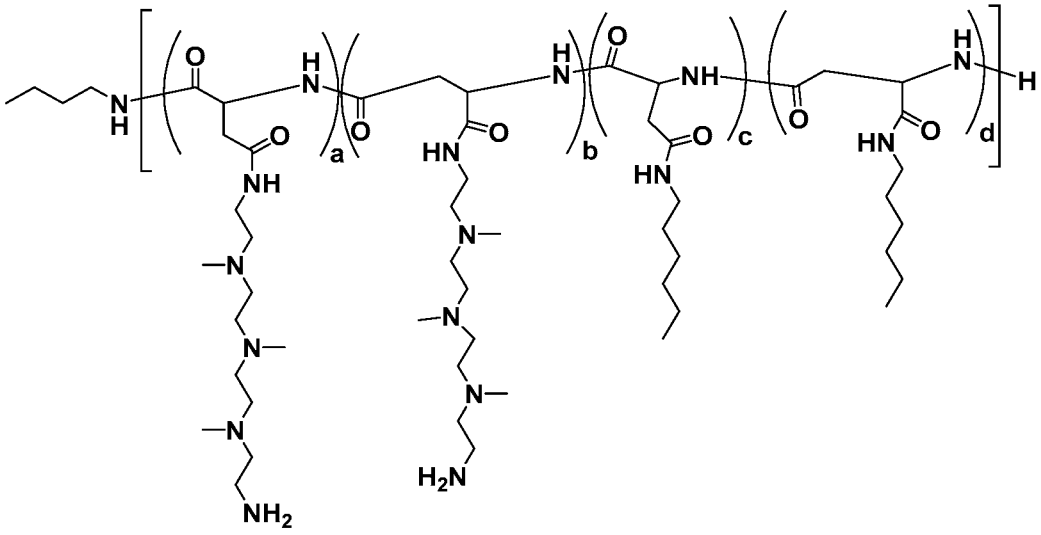
Polymer 39



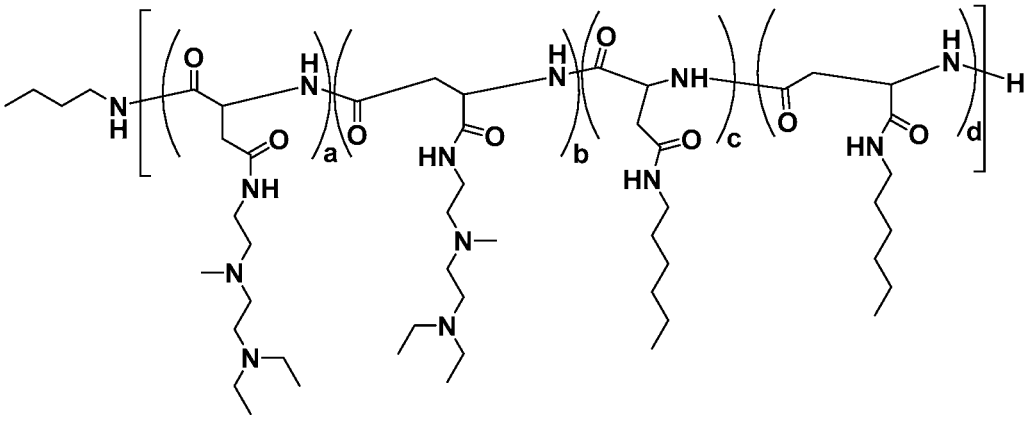
Polymer 40



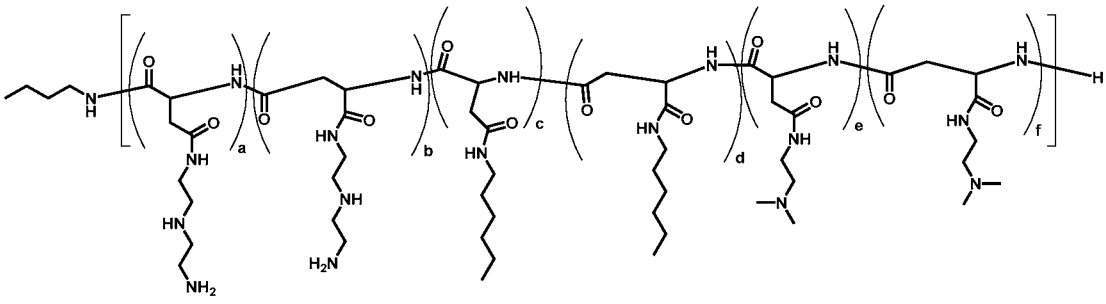
Polymer 41



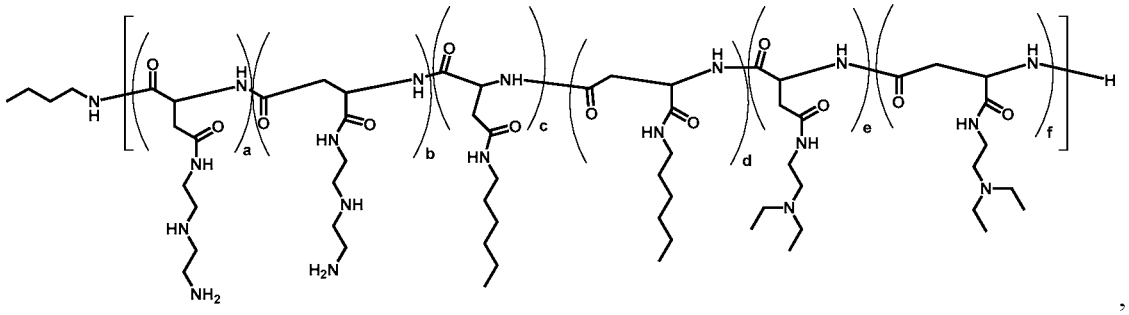
Polymer 42



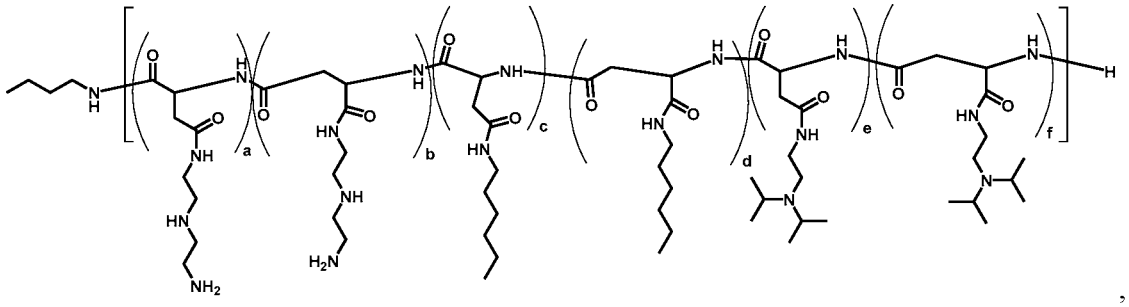
Polymer 43



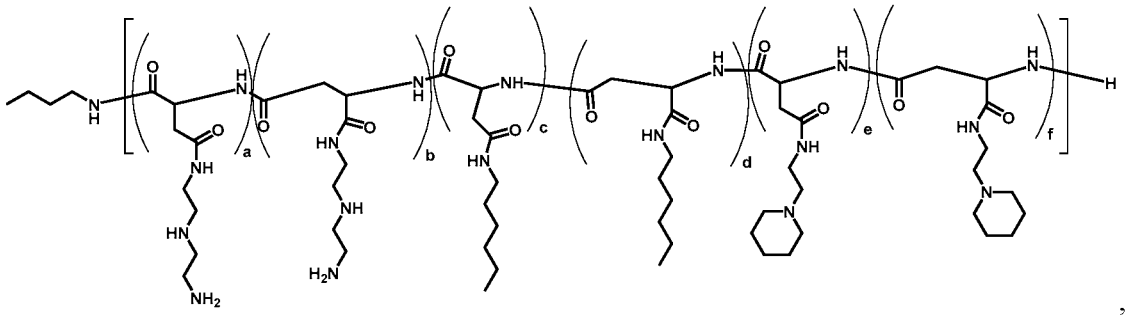
Polymer 44



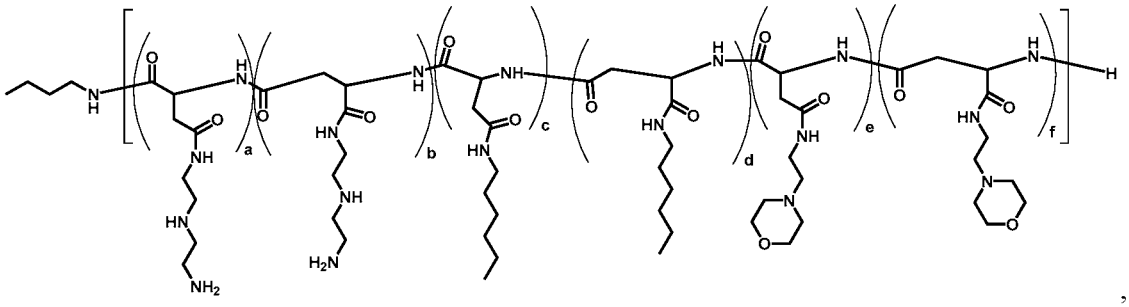
Polymer 45



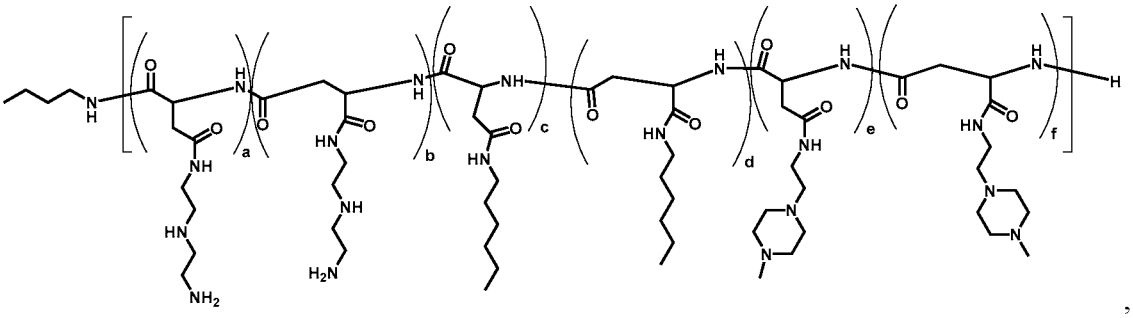
Polymer 46



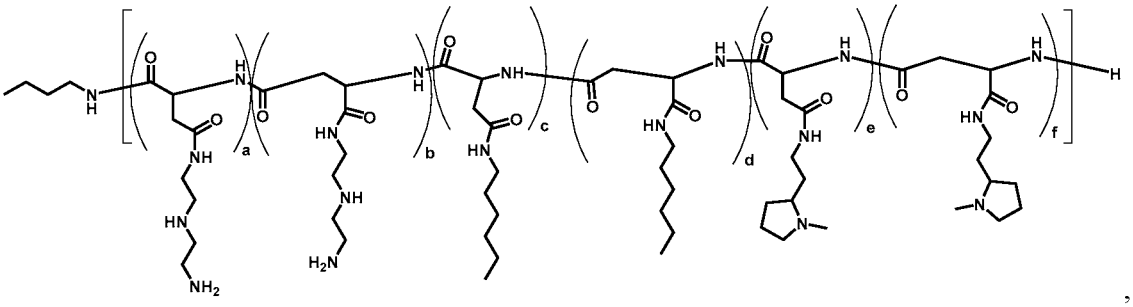
Polymer 47



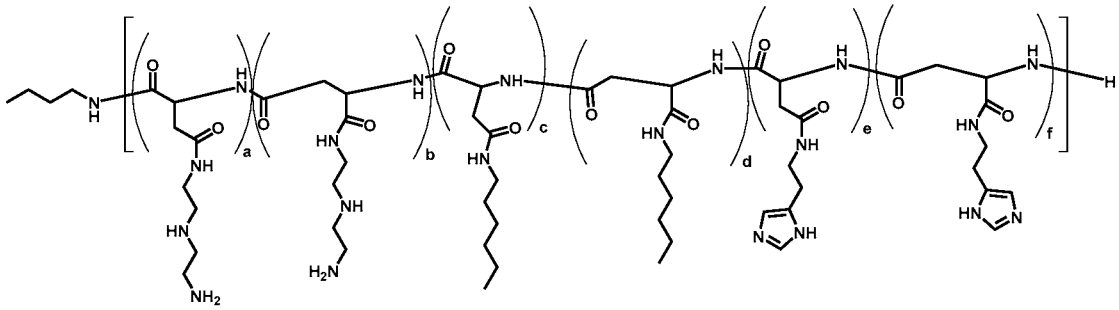
Polymer 48



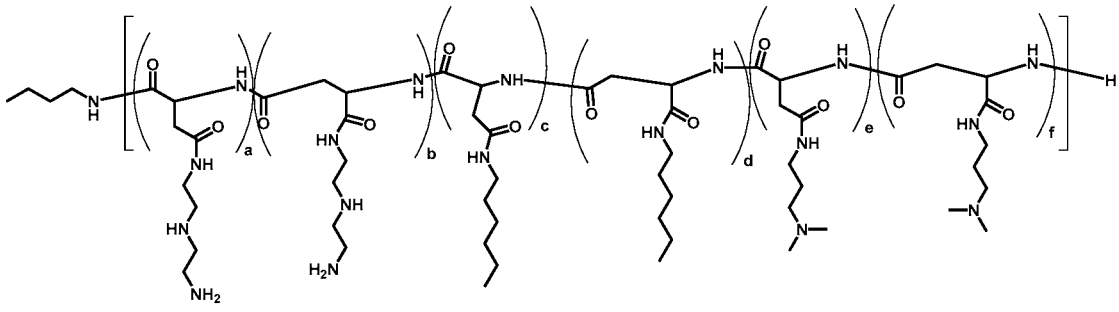
Polymer 49



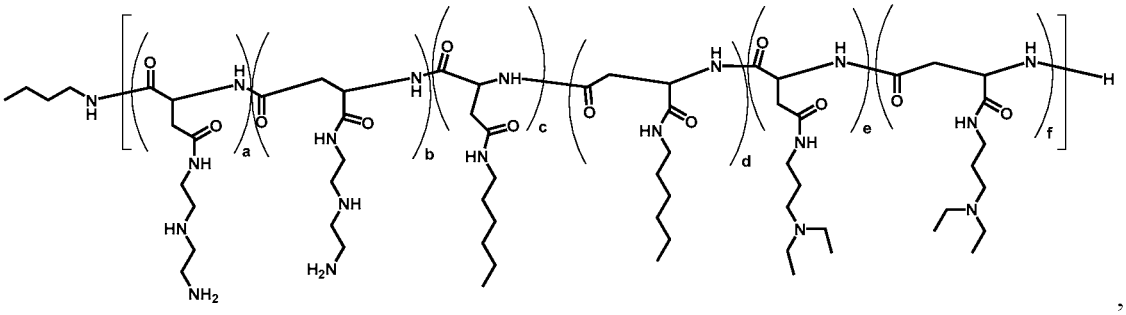
Polymer 50



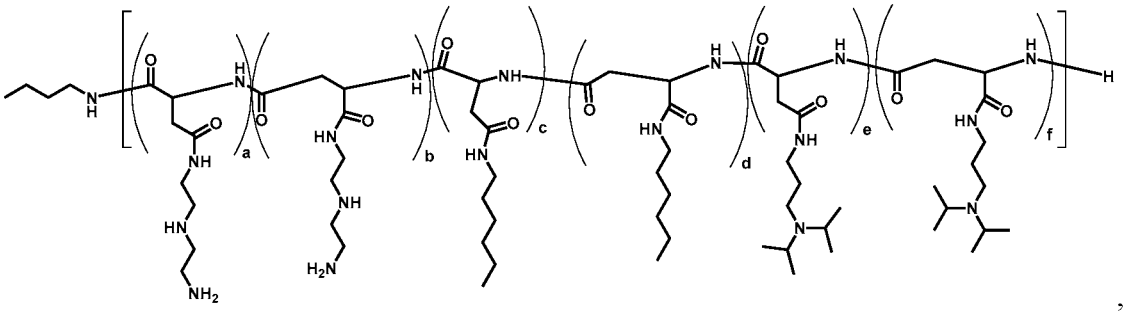
Polymer 51



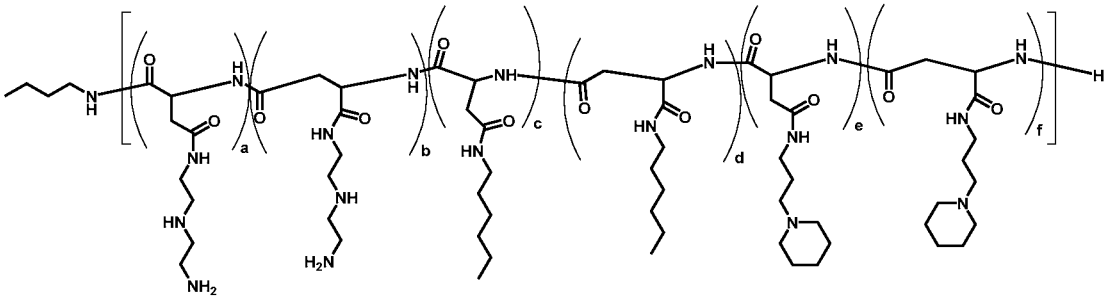
Polymer 52



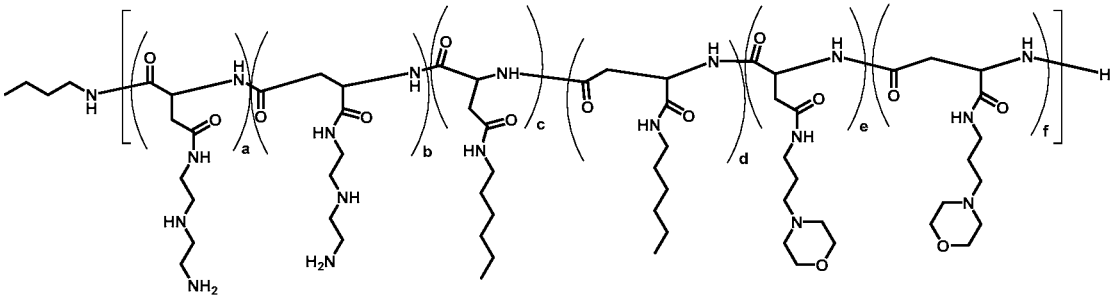
Polymer 53



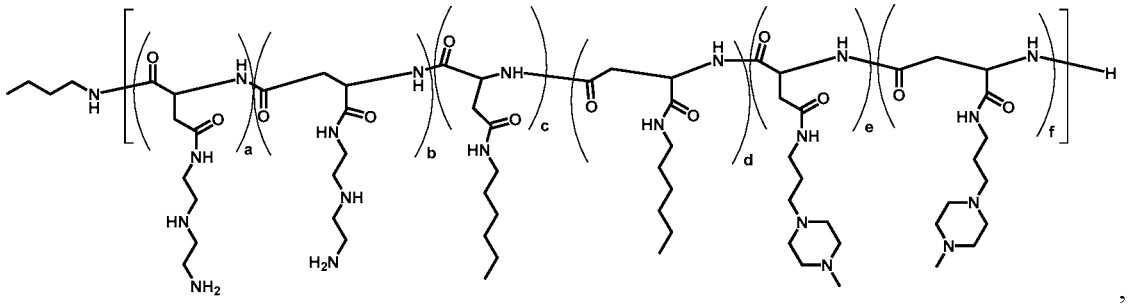
Polymer 54



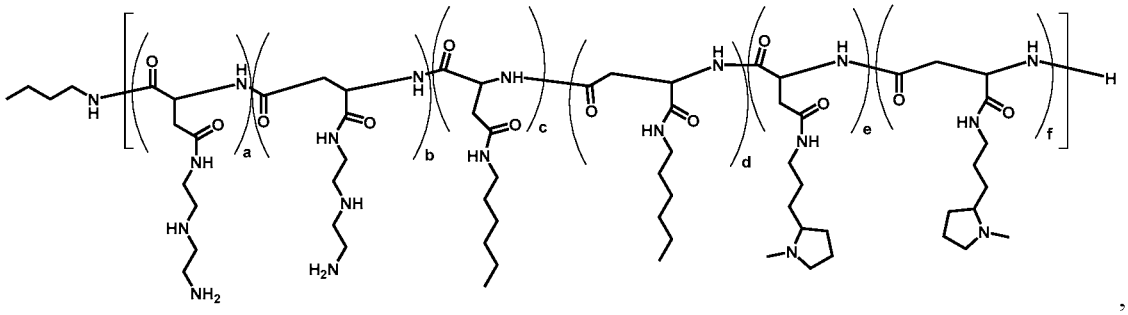
Polymer 55



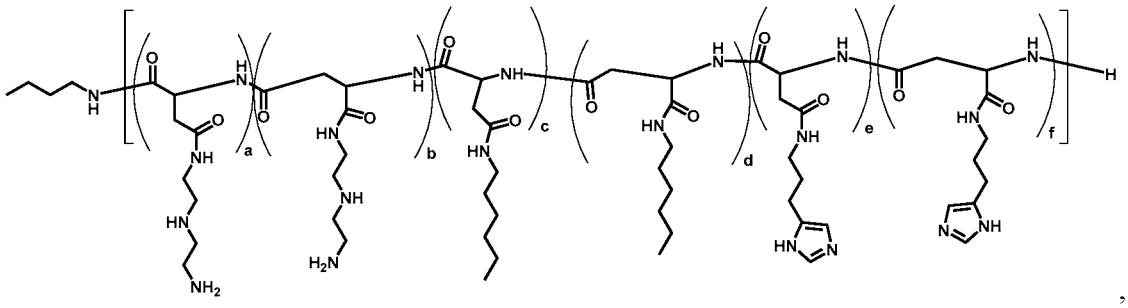
Polymer 56



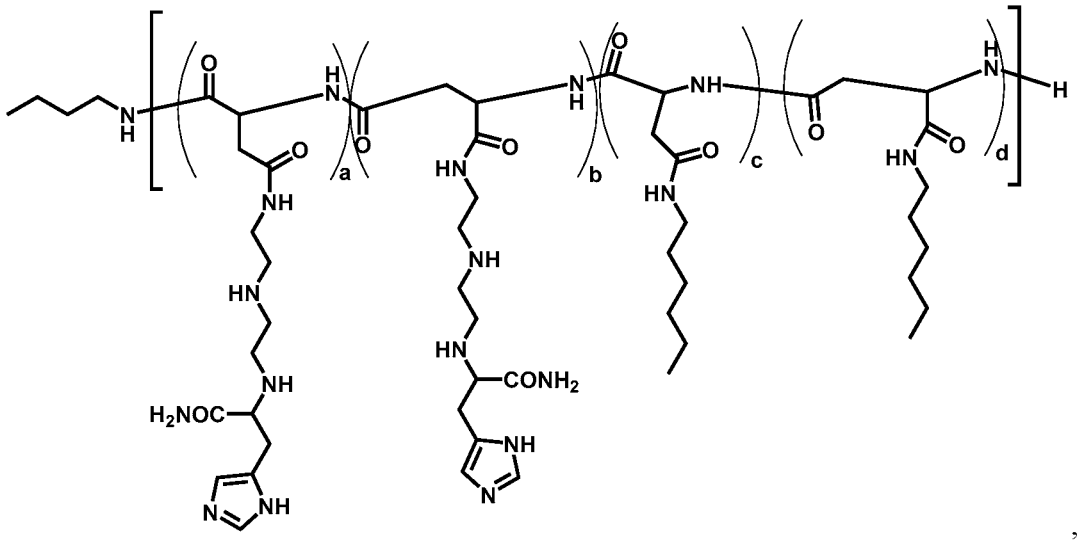
Polymer 57



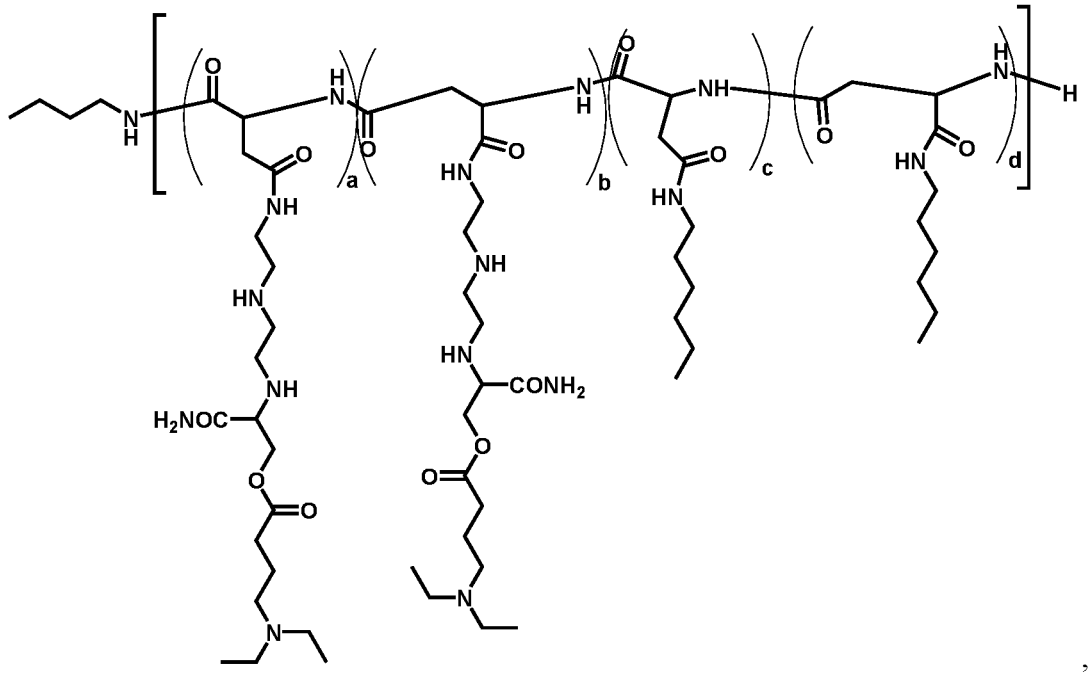
Polymer 58



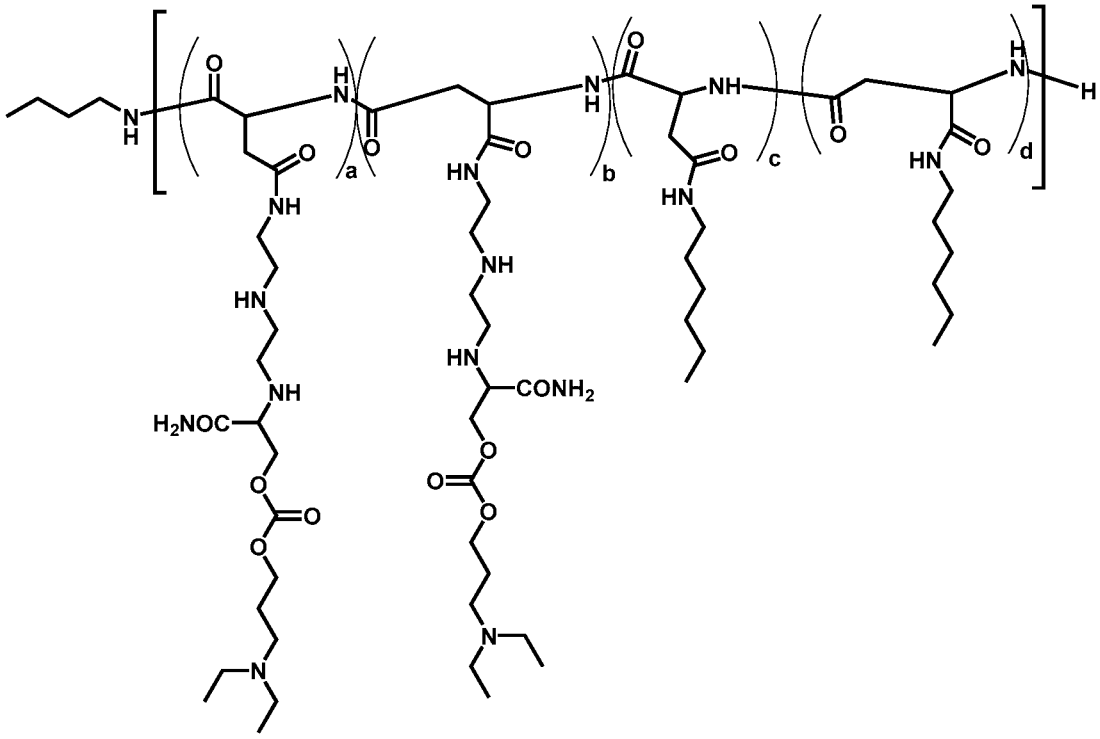
Polymer 59



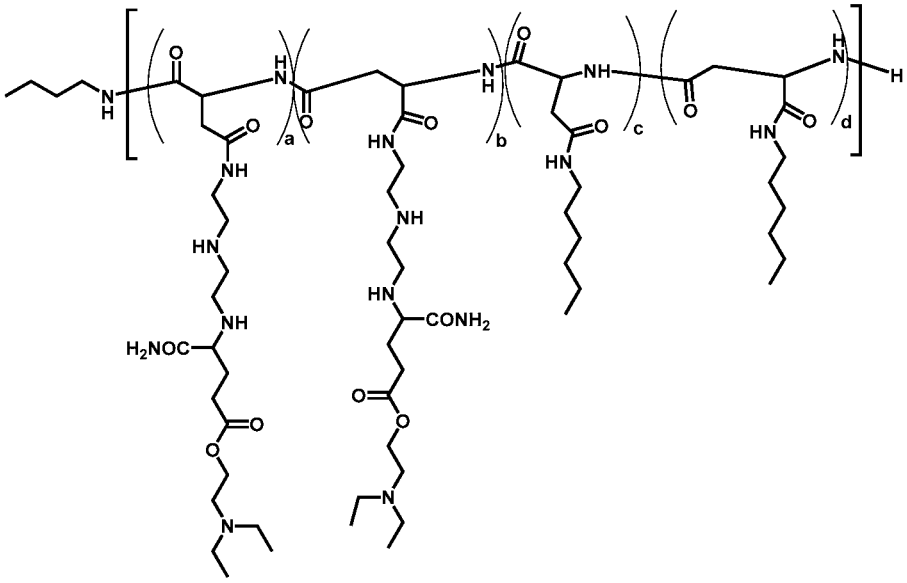
Polymer 60



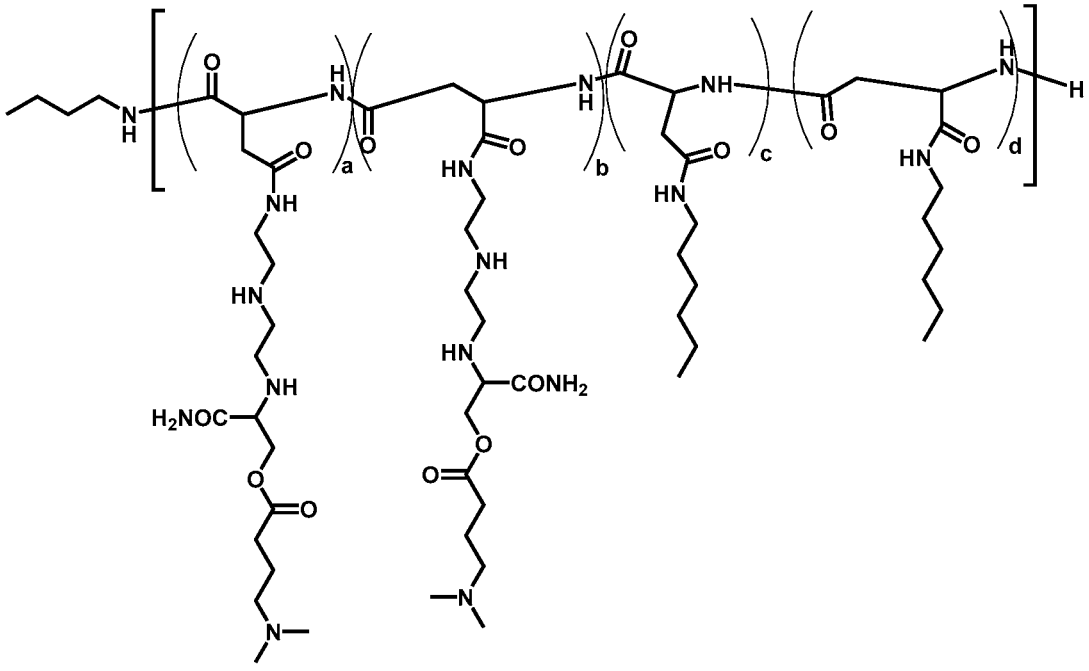
Polymer 61



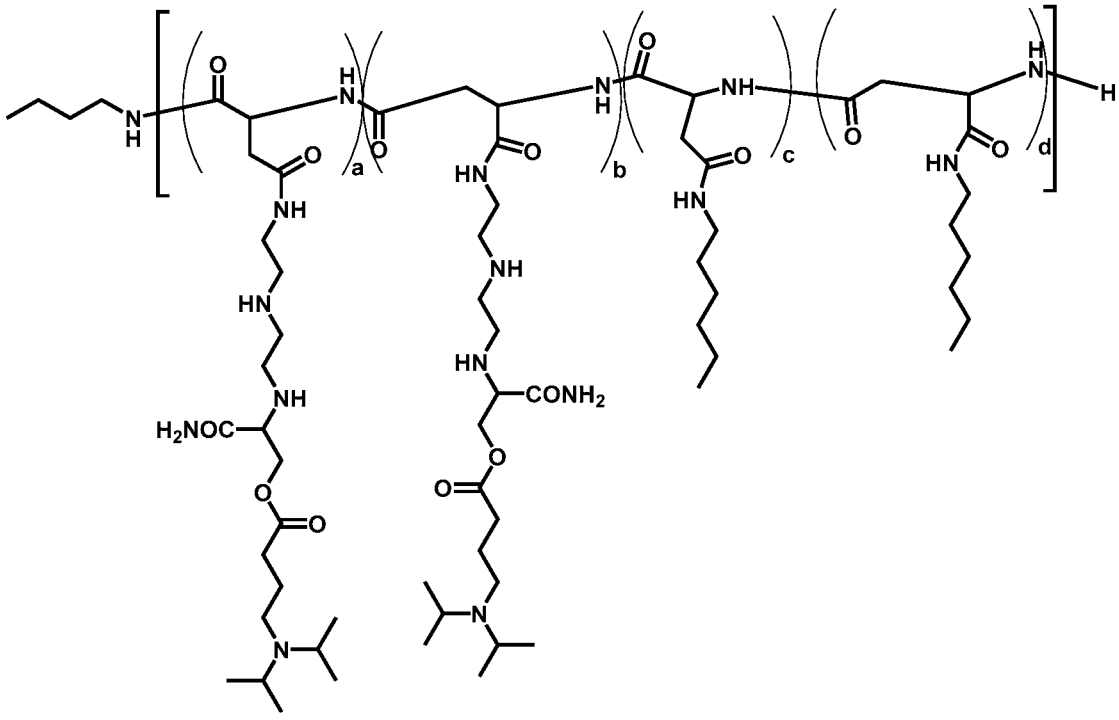
Polymer 62



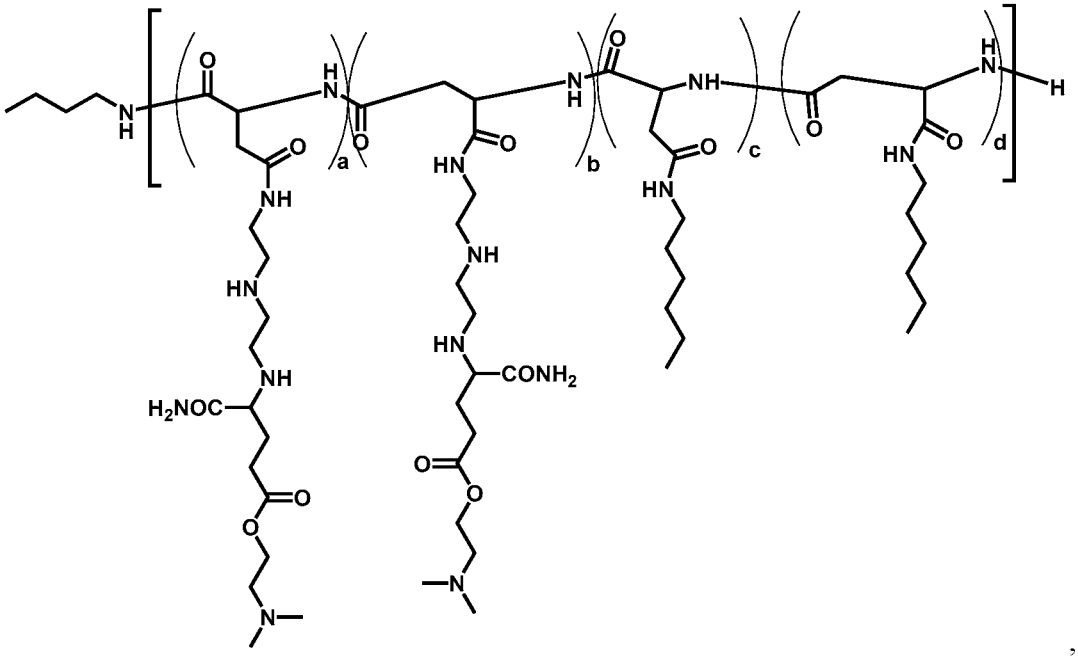
Polymer 63



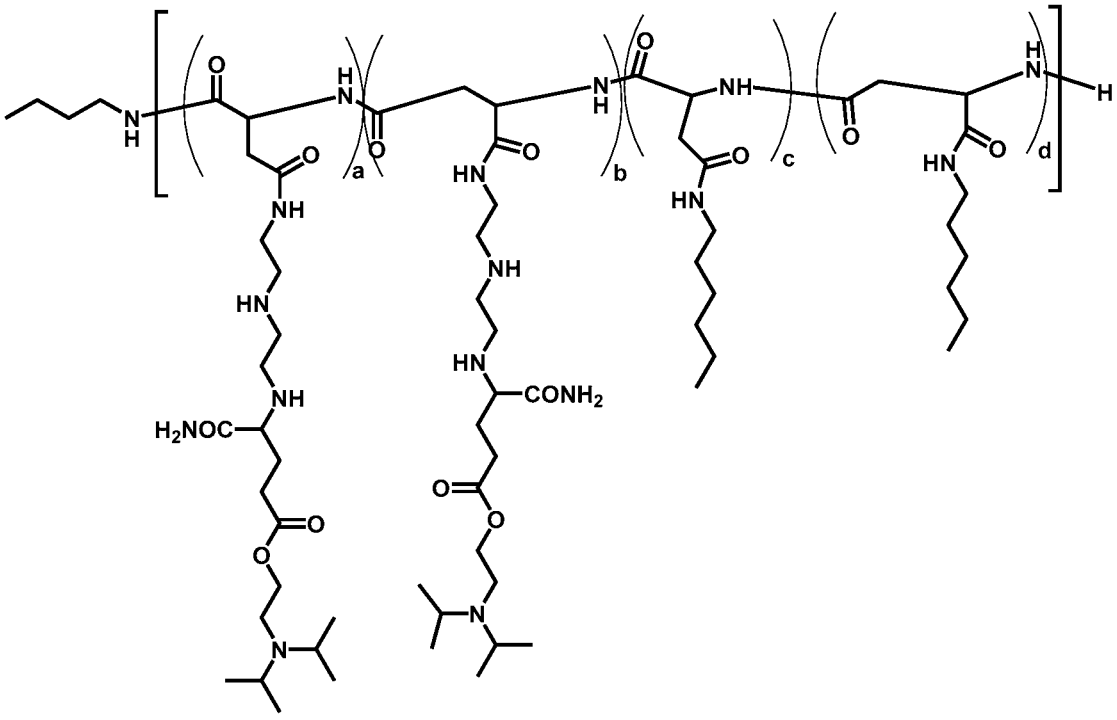
Polymer 64



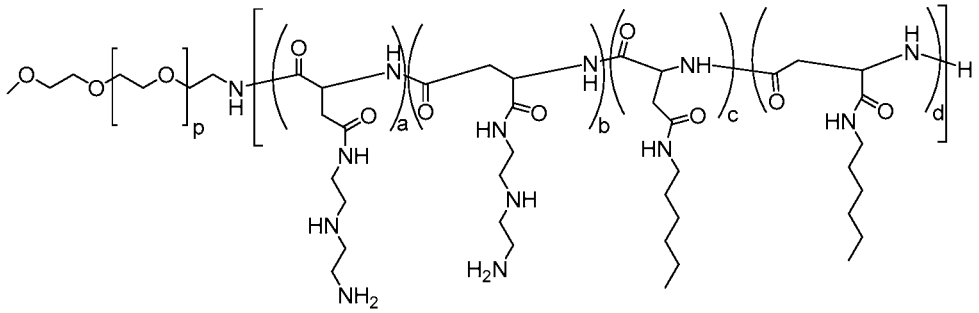
Polymer 65



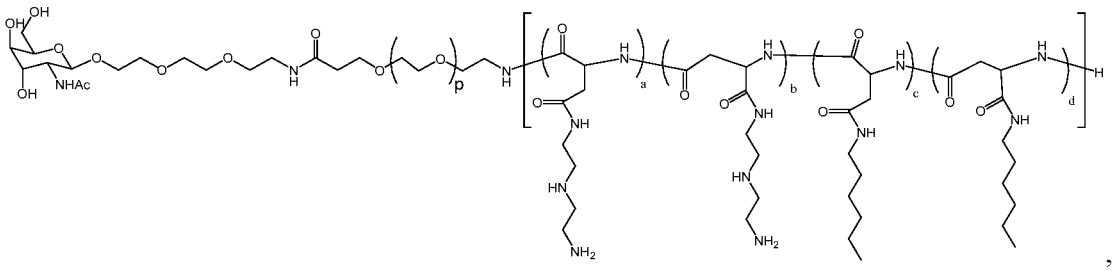
Polymer 66



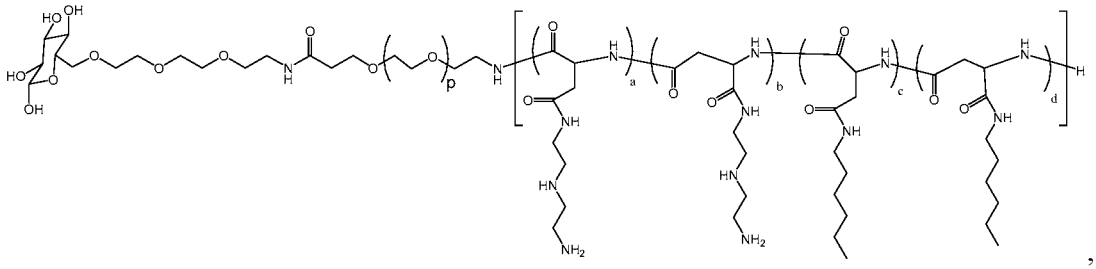
Polymer 67



Polymer 68

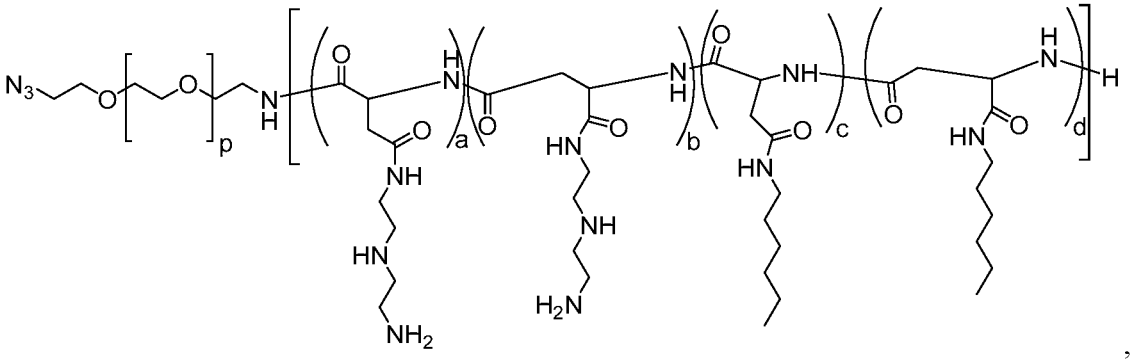


Polymer 69



, or

Polymer 70



,

Polymer 71

wherein (a+b) is from about 5 to about 65, (c+d) is from about 2 to about 60, and (e+f) is from about 2 to about 60.

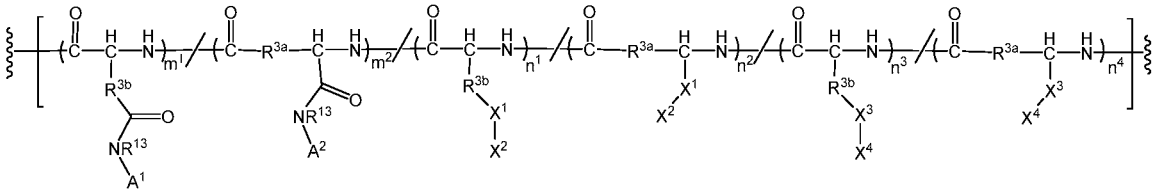
65. The composition of any one of claims 44-64, wherein the composition comprises about 1 wt.% to about 60 wt.% of the first polymer based on a sum total weight of the first polymer and the second polymer.

66. The composition of claim 65, wherein the composition comprises about 5 wt.% to about 50 wt.% of the first polymer based on a sum total weight of the first polymer and the second polymer.

67. The composition of claim 66, wherein the composition comprises about 20 wt.% to about 40 wt.% of the first polymer based on a sum total weight of the first polymer and the second polymer.

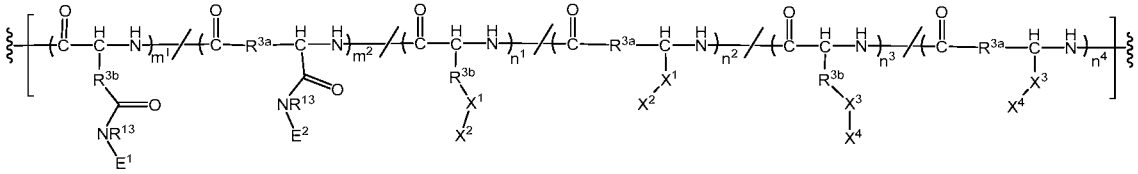
68. A method of preparing a polymer comprising a structure of Formula 2 according to claim 17, the method comprising:

(a) providing a polymer comprising a structure of Formula 5:



and

(b) modifying a portion of groups A^1 and/or A^2 of the polymer comprising a structure of Formula 5 to provide the polymer comprising a structure of Formula 2:



wherein

each of A^1 and A^2 are each independently a group of formula

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2{}_2]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}$; or

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2{}_2]_2\}_2$;

each of E^1 and E^2 are each independently a group of formula

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2{}_2]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}R^2\}$; or

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2{}_2]_2\}_2$,

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^4\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}(CH_2)_{s2}\text{-}R^4\text{-}R^5]_2$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}(CH_2)_{s3}\text{-}R^4\text{-}R^5\}$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}(CH_2)_{s4}\text{-}R^4\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p3}—N\{[—(CH_2)_{q3}—NR^2{}_2][—(CH_2)_{q4}—NR^2—]_{r2}\text{-}CH_2\text{-}CHOH\text{-}R^5$;

$—(CH_2)_{p4}—N\{—(CH_2)_{q5}—N[—(CH_2)_{q6}—NR^2\text{-}CH_2\text{-}CHOH\text{-}R^5]_2\}_2$;

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2\text{-}(CH_2)_{s1}\text{-}R^5$;

—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—NR^2-$(CH_2)_{s2}$-R^5]$_2$;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—NR^2_2][—$(CH_2)_{q4}$—NR^2—]$_{r2}$$(CH_2)_{s3}$-$R^5$};

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-$(CH_2)_{s4}$-R^5]$_2$}$_2$;

—$(CH_2)_{p1}$—[N{$(CH_2)_{s1}$-R^4-R^5}—$(CH_2)_{q1}$—]$_{r1}$$NR^2_2$;

—$(CH_2)_{p1}$—[N{$(CH_2)_{s1}$-R^5}—$(CH_2)_{q1}$—]$_{r1}$$NR^2_2$,

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-CH($CONH_2$)-$(CH_2)_{s1}$-$R^5$; or

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-CH($CONH_2$)-$(CH_2)_{s1}$-$R^4$-$R^5$,

provided that at least a portion of E^1 and/or E^2 are selected from:

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-$(CH_2)_{s1}$-$R^4$-$R^5$;

—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—NR^2-$(CH_2)_{s2}$-R^4-R^5]$_2$;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—NR^2_2][—$(CH_2)_{q4}$—NR^2—]$_{r2}$$(CH_2)_{s3}$-$R^4$-$R^5$};

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-$(CH_2)_{s4}$-R^4-R^5]$_2$}$_2$;

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-$CH_2$-CHOH-$R^5$;

—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—NR^2-CH_2-CHOH-R^5;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—NR^2_2][—$(CH_2)_{q4}$—NR^2—]$_{r2}$-CH_2-CHOH-R^5;

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-CH_2-CHOH-R^5]$_2$}$_2$;

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-$(CH_2)_{s1}$-$R^5$;

—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—NR^2-$(CH_2)_{s2}$-R^5]$_2$;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—NR^2_2][—$(CH_2)_{q4}$—NR^2—]$_{r2}$$(CH_2)_{s3}$-$R^5$};

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-$(CH_2)_{s4}$-R^5]$_2$}$_2$;

—$(CH_2)_{p1}$—[N{$(CH_2)_{s1}$-R^4-R^5}—$(CH_2)_{q1}$—]$_{r1}$$NR^2_2$;

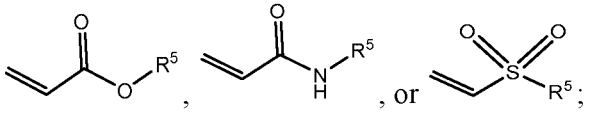
—$(CH_2)_{p1}$—[N{$(CH_2)_{s1}$-R^5}—$(CH_2)_{q1}$—]$_{r1}$$NR^2_2$,

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-CH($CONH_2$)-$(CH_2)_{s1}$-$R^5$; or

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-CH($CONH_2$)-$(CH_2)_{s1}$-$R^4$-$R^5$,

and m^1, m^2, n^1, n^2, n^3, n^4, R^{3a}, R^{3b}, R^{13}, X^1, X^2, X^3, X^4, E^1, and E^2 of Formulae 2 and 5 are as defined in claim 17.

69. The method of claim 68, wherein: modifying a portion of groups A^1 and/or A^2 of the polymer comprising a structure of Formula 5 comprises reacting a portion of groups with a compound having the structure:



to provide the polymer comprising a structure of Formula 2 wherein at least a portion of the E^1 and/or E^2 groups are:

—$(CH_2)_{p1}$—[NR2—(CH2)q1—]r1NR2-(CH2)s1-R4-R5;

—(CH2)p2—N[—(CH2)q2—NR2-(CH2)s2-R4-R5]2;

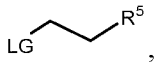
—(CH2)p3—N{[—(CH2)q3—NR22][—(CH2)q4—NR2—]r2(CH2)s3-R4-R5};

—(CH2)p4—N{—(CH2)q5—N[—(CH2)q6—NR2-(CH2)s4-R4-R5]2}2;

—(CH2)p1—[N{(CH2)s1-R4-R5}—(CH2)q1—]r1NR22; or

—(CH2)p1—[NR2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-CH($CONH_2$)-$(CH_2)_{s1}$-$R^4$-$R^5$;

or wherein: modifying a portion of groups A^1 and/or A^2 of the polymer comprising a structure of Formula 5 comprises reacting a portion of groups with a compound having the structure:



,

to provide the polymer comprising a structure of Formula 2 wherein at least a portion of the E^1 and/or E^2 groups are:

—$(CH_2)_{p1}$—[NR^2—$(CH_2)_{q1}$—]$_{r1}$$NR^2$-$(CH_2)_{s1}$-$R^5$;

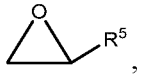
—$(CH_2)_{p2}$—N[—$(CH_2)_{q2}$—NR^2-$(CH_2)_{s2}$-R^5]$_2$;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—$NR^2{}_2$][—$(CH_2)_{q4}$—NR^2—]$_{r2}$$(CH_2)_{s3}$-$R^5$};

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2-$(CH_2)_{s4}$-R^5]$_2$}$_2$; or

—$(CH_2)_{p1}$—[N{$(CH_2)_{s1}$-R^5}—$(CH_2)_{q1}$—]$_{r1}$$NR^2{}_2$.

70. The method of claim 68, wherein: modifying a portion of groups A^1 and/or A^2 of the polymer comprising a structure of Formula 5 comprises reacting a portion of groups with a compound having the structure:



,

to provide the polymer comprising a structure of Formula 2 wherein at least a portion of the E^1 and/or E^2 groups are:

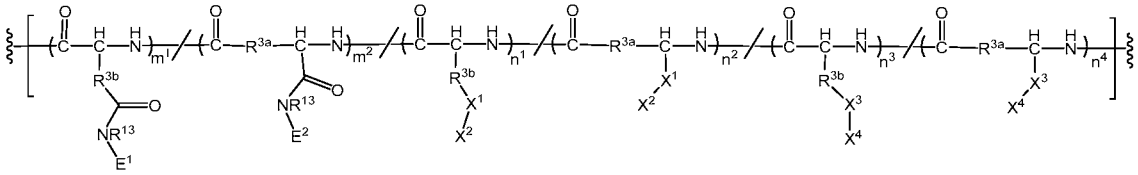
—(CH_2)p1—[NR2—(CH2)q1—]r1NR2-CH2-CHOH-R5;

—(CH2)p2—N[—(CH2)q2—NR2-CH2-CHOH-R5;

—(CH2)p3—N{[—(CH2)q3—NR22][—(CH2)q4—NR2—]r2-CH2-CHOH-R5; or

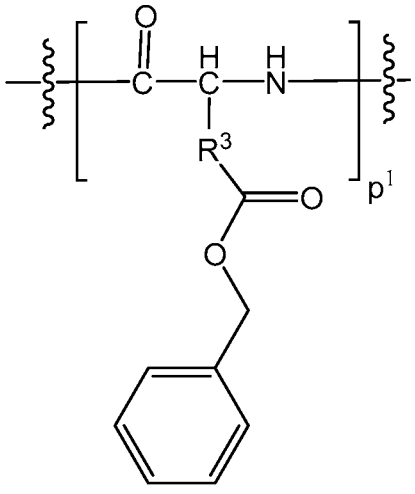
—(CH2)p4—N{—(CH2)q5—N[—$(CH_2)_{q6}$—NR^2-CH_2-CHOH-R^5]$_2$}$_2$.

71. A method of preparing a polymer comprising a structure of Formula 2:



the method comprising:

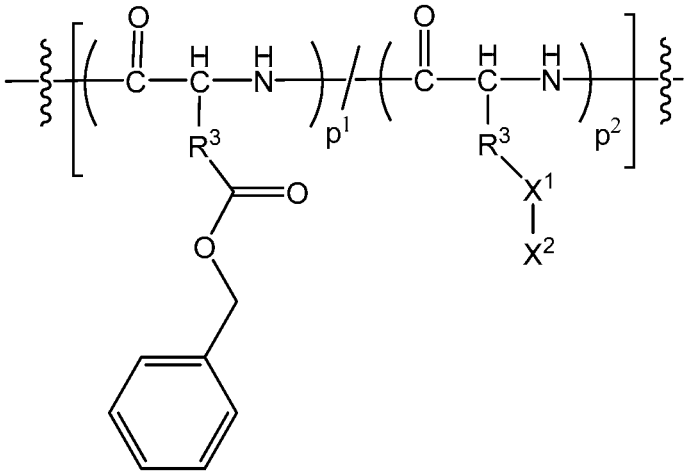
(I) reacting a polymer comprising a structure of Formula 6:



with (a) a compound of the formula $HNR^{13}E^1$ and/or $HNR^{13}E^2$; (b) a compound of formula H_2NX^4 or HOX^4; and (c) a compound of formula H_2NX^2 or HOX^2, simultaneously or in any sequential order;

or

(II) reacting a polymer comprising a structure of Formula 7:



with (a) a compound of the formula $HNR^{13}E^1$ and/or $HNR^{13}E^2$, and (b) a compound of formula H_2NX^4 or HOX^4, simultaneously or in any sequential order;

wherein,

p^1 is an integer from 1 to 2000;

p^2 is an integer from 1 to 2000;

each R^3 is independently a methylene or ethylene group; and

and m^1, m^2, n^1, n^2, n^3, n^4, R^{3a}, R^{3b}, R^{13}, X^1, X^2, X^3, X^4, E^1, and E^2 are as defined in claim 17,

optionally wherein

each of m^1 and m^2 is an integer from 5 to 100;

each of n^1 and n^2 is an integer from 5 to 100;

each of n^3 and n^4 is an integer from 5 to 100;

each instance of R^{3a} and R^{3b} is methylene;

each X^1 and X^3 independently is —C(O)O—, —C(O)NR^{13}—, or —C(O)—, or a bond;

each instance of R^{13} is hydrogen or a C_1-C_3 alkyl;

each instance of X^2 is independently a C_1-C_{12} linear or branched alkyl;

each instance of X^4 is polyalkylene oxide, polyglycolic acid, polylactic acid, or a combination thereof; and/or

E^1 and E^2 are each independently a group of formula

$—(CH_2)_{p1}—[NR^2—(CH_2)_{q1}—]_{r1}NR^2{}_2$;

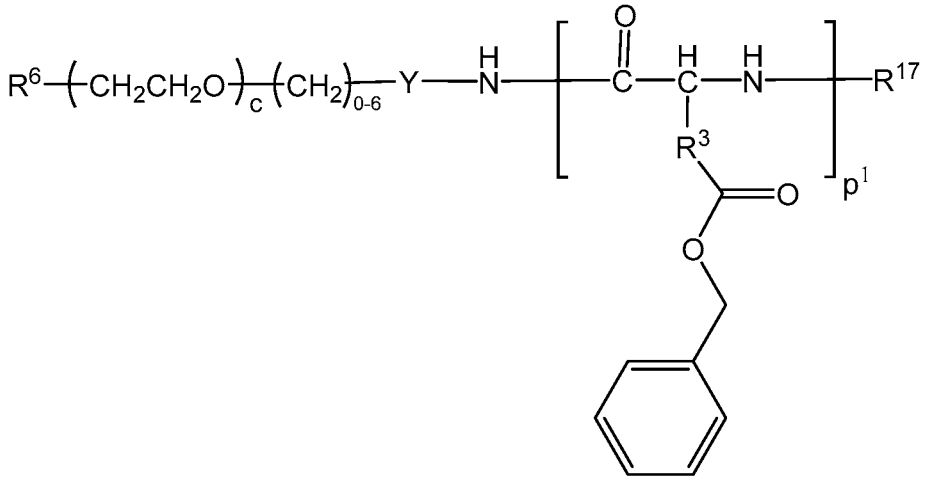
$—(CH_2)_{p2}—N[—(CH_2)_{q2}—NR^2{}_2]_2$;

—$(CH_2)_{p3}$—N{[—$(CH_2)_{q3}$—NR^2_2][—$(CH_2)_{q4}$—NR^2—]$_{r2}R^2$};

—$(CH_2)_{p4}$—N{—$(CH_2)_{q5}$—N[—$(CH_2)_{q6}$—NR^2_2]$_2$}$_2$,

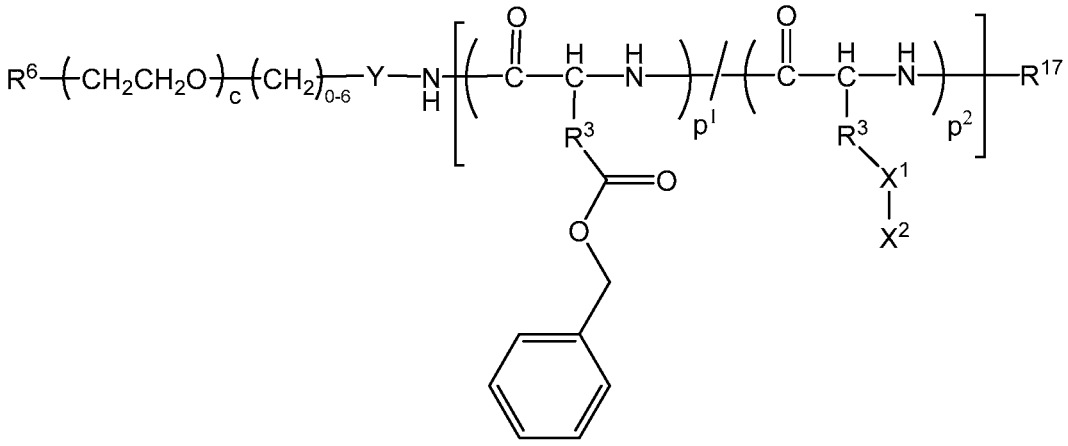
wherein p1 to p4, q1 to q6, and r1 and r2, are each independently an integer of 1 to 5; and each instance of R^2 is independently hydrogen or a C_1-C_3 alkyl group.

72. The method of claim 71, wherein the polymer comprising the structure of Formula 6 or Formula 7 is a polymer of Formula 6A or Formula 7A, respectively:



Formula 6A

or



Formula 7A

;

wherein,

p^1 is an integer from 1 to 2000;

p^2 is an integer from 1 to 2000;

each R^3 is independently a methylene or ethylene group;

each X^1 independently is —C(O)O—, —C(O)NR^{13}—, —C(O)—, —S(O)(O)—, or a bond;

each instance of X^2 is independently a C_1-C_{12} alkyl or heteroalkyl group, C_3-C_{12} cycloalkyl group, C_2-C_{12} alkenyl group, C_3-C_{12} cycloalkenyl group, aryl group, heterocyclic group, or combination thereof, any of which can be substituted with one or more substituents;

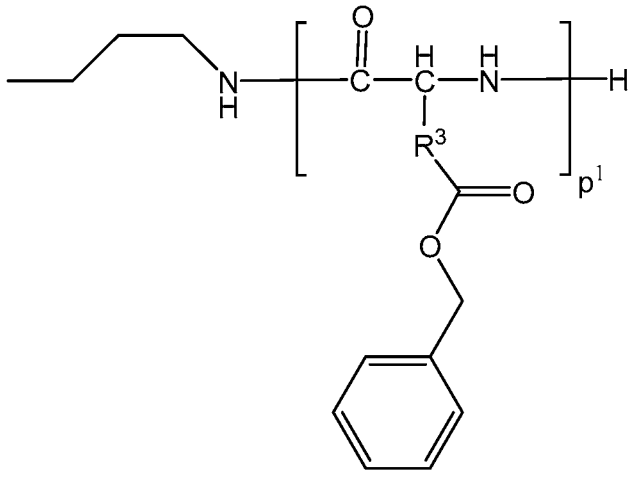
c is an integer from 0 to 50;

Y is optionally present and is a cleavable linker;

R^{17} is hydrogen, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, or a C_1-C_{12} linear or branched alkyl group optionally substituted with one or more substituents; and

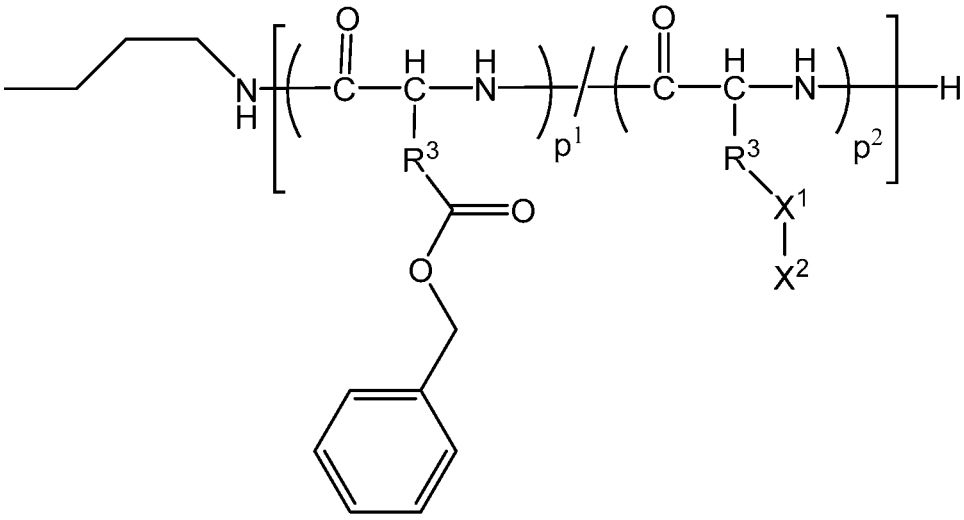
R^6 is hydrogen, an amino group, an aryl group, a heterocyclic group, a C_1-C_{12} alkyl group, a C_1-C_{12} heteroalkyl group, alkenyl group, cycloalkyl group, or cycloalkenyl group, a C_1-C_{12} linear or branched alkyl group optionally substituted with one or more amines; or a tissue-specific or cell-specific targeting moiety.

73. The method of claim 72, wherein the polymer comprising the structure of Formula 7 or Formula 7 is a polymer of Formula 6B or Formula 7B, respectively:



Formula 6B

or



Formula 7B ;

wherein R^3, p^1, p^2, X^1, and X^2 are as defined in claim 72.

74. The method of claim 71, wherein the method the method comprises reacting a polymer comprising a structure of Formula 6 with (a) a compound of the formula $HNR^{13}E^1$ and/or $HNR^{13}E^2$; (b) a compound of formula H_2NX^4 or HOX^4; and (c) a compound of formula H_2NX^2 or HOX^2, simultaneously or in any sequential order, and wherein (a) and (c) are present in a molar ratio of about 1:10 to about 1:150, optionally about 1:40 to about 1:150, or about 1:80 to about 1:150.

75. A method of delivering a nucleic acid and/or polypeptide to a cell, the method comprising administering the composition of any one of claims 34-67 to the cell.

76. The method of claim 75, wherein the cell is in a subject and the composition of any one of claims 34-67 is administered to the subject.

77. The method of claim 75 or 76, wherein the first polymer and/or the second polymer comprises a tissue-specific targeting moiety that localizes the polymer to tissues of the peripheral nervous system, the central nervous system, liver, muscle, lung, bone, or the eye of the subject.

78. The method of claim 77, wherein the first polymer and/or the second polymer comprises a targeting moiety that preferentially binds to tumor cells.

79. The method of any of claims 75-78, wherein the composition comprises one or more of an RNA guided endonuclease or nucleic acid encoding same, a guide nucleic acid, and a donor nucleic acid, and the composition facilitates editing of a target gene in the cell.

80. The method of any of claims 75-79, wherein the cell is in a host, optionally a human, and the composition is delivered to the cell by administering the composition to the host.

81. Use of a composition of any one of claims 34-67 for delivering a nucleic acid or protein to a cell.

82. The use of claim 81, wherein the composition is for delivering a nucleic acid or protein to a cell in accordance with the method of any of claims 76-80.

83. The polymer of claim 27, wherein

X^1 and X^3 are both—C(O)NR^{13}-, wherein R^{13} is methyl or hydrogen;

X^2 is a C_3-C_8, optionally C_3-C_6, linear or branched alkyl group;

X^4 is group comprising a polyalkylene oxide, polyglycolic acid, polylactic acid, or a combination thereof; and

E^1 and E^2 are —$(CH_2)_{p1}$—$(NR^2$—$(CH_2)_{q1}$—$)_{r1}NR^2_2$, wherein R^2 is independently hydrogen or a C_1-C_3 alkyl (e.g., methyl or ethyl), and p1, q1, and r1 are each independently an integer of 1, 2, or 3; optionally wherein E^1 and E^2 are be –$(CH_2)_2$-NR^2-$(CH_2)_2$-NR^2_2 or –$(CH_2)_2$-NR^2-$(CH_2)_2$-NHR^2.

84. The composition of any of claims 44-67, or method of any of claims 75-82, wherein the first polymer is a polymer of claim 27, and the second polymer is a polymer of claim 61;

or the first polymer is a polymer of claim 82 and the second polymer is a polymer of claim 62;

or the first polymer is a polymer of claim 28 and the second polymer is a polymer of claim 64;

or the first polymer is polymer 72, 73, 74, or 75 and the second polymer is polymer 29, 37, 39, or 40.

Streptococcus pyogenes Cas9
GenBank AKP81606

```
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 121 nivdevayhe kyptiyhlrk klvdstdkad lrliylalah mikfrghfli egdlnpdnsd
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Motif 1
Domain 1
Motif 2
Motif 3
Domain 2
Motif 4

FIG. 1

Francisella tularensis subsp. novicida U112 Cpf1

```
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 601 enstlangwd knkepdntai lfikddkyyl gvmnkknnki fddkaikenk gegykkivyk
 661 llpqankmlp kvffsaksik fynpsedilr irnhsthtkn qspqkgyekf efniedcrkf
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FIG. 2

AsCpf1

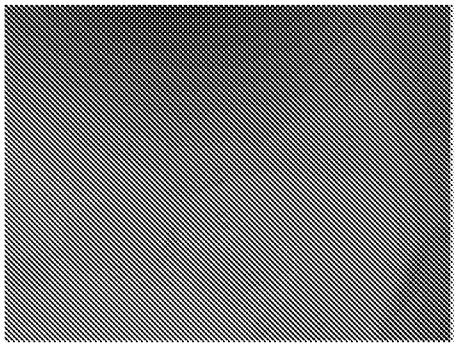
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FIG. 3

LbCpf1

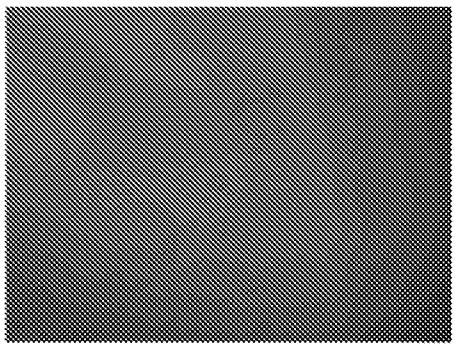
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FIG. 4



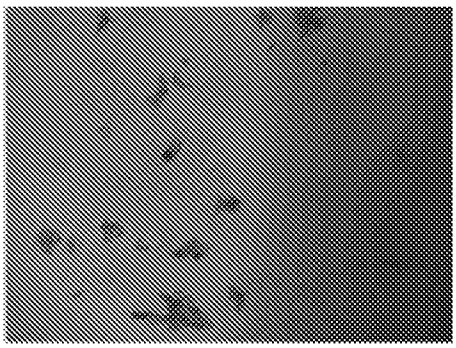
Polymer Only

FIG. 5A



H27N + 40% P2K25

FIG. 5B



H27N

FIG. 5C

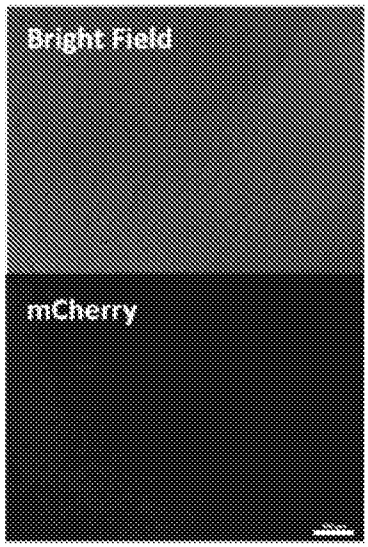


FIG. 6A

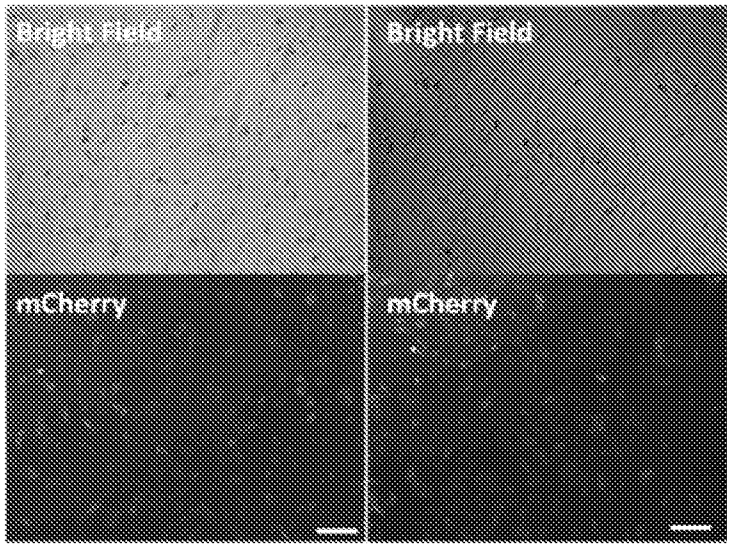


FIG. 6B

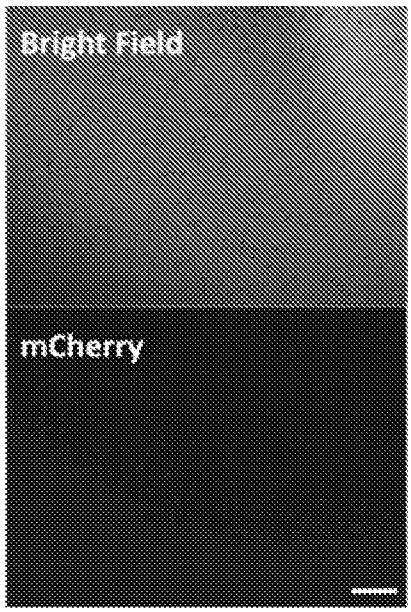


FIG. 7A

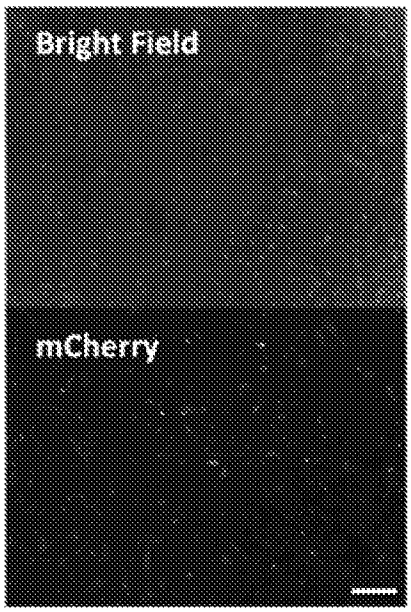


FIG. 7B

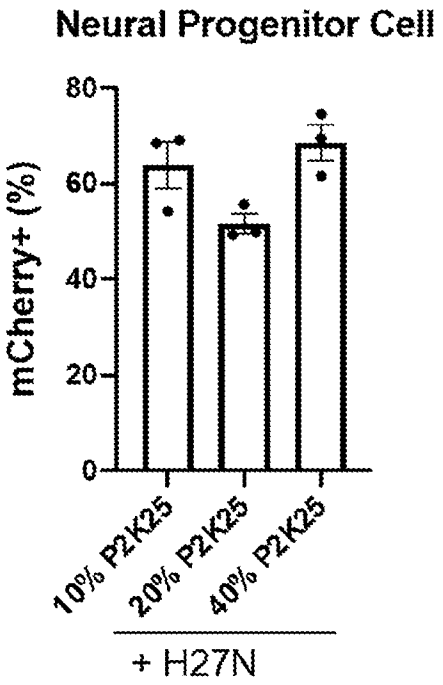

Neural Progenitor Cell
mCherry+ (%)
80
60
40
20
0
10% P2K25
20% P2K25
40% P2K25
+ H27N

FIG. 8

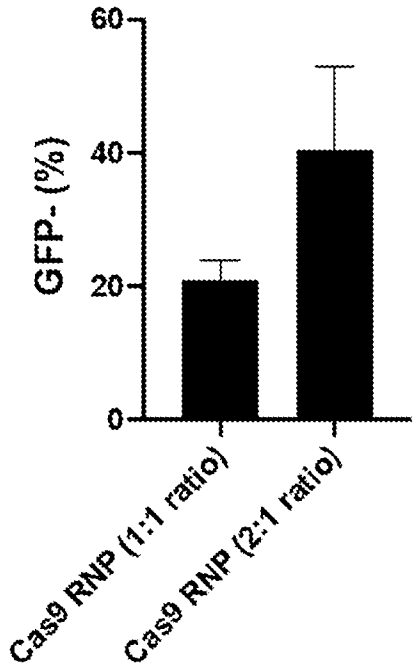

GFP- (%)
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40
20
0
Cas9 RNP (1:1 ratio)
Cas9 RNP (2:1 ratio)

FIG. 9



FIG. 10



FIG. 11A



FIG. 11B

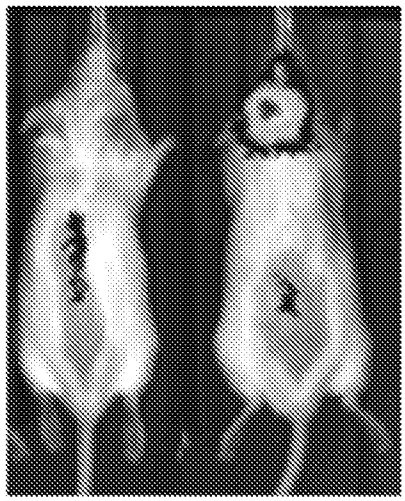


FIG. 11C

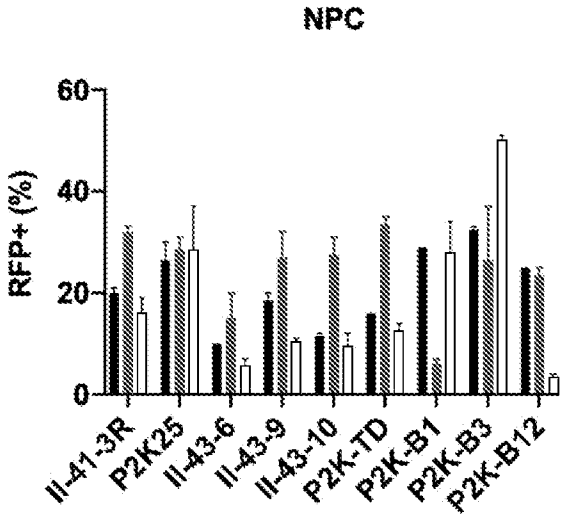


FIG. 12A

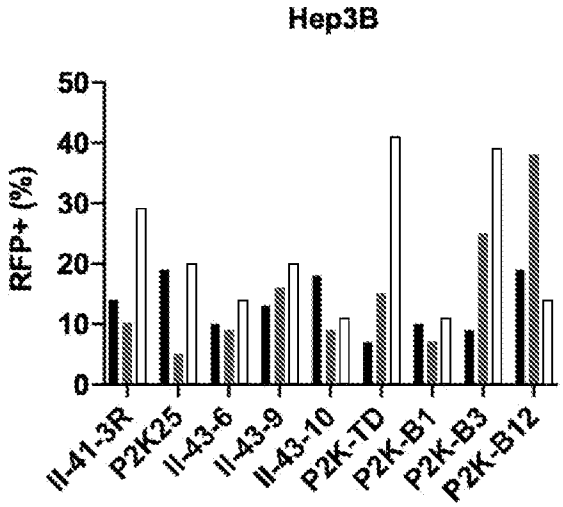


FIG. 12B

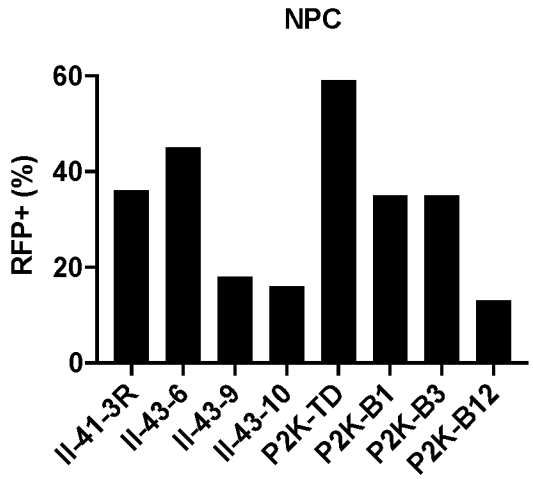


FIG. 13A

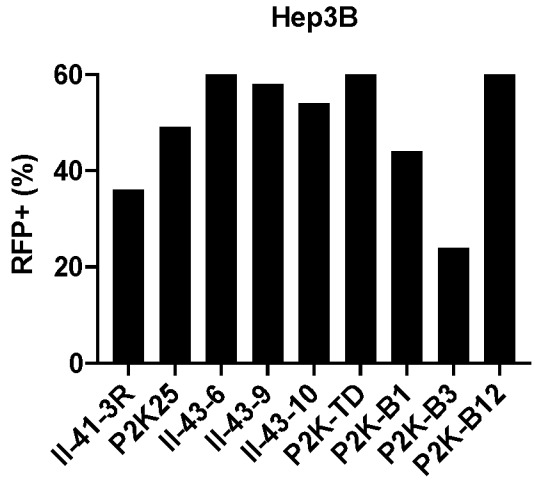


FIG. 13B



FIG. 14

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20 25 30

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35 40 45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
50 55 60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys

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65                  70                  75                  80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160

Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Ser Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
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Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
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Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
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            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
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Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

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            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590

Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720
```

```
His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815

Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
```

```
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020

Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140
```

```
Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230

Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Gln His Lys
    1250                1255                1260

His Tyr  Leu Asp Glu Ile Ile  Glu Gln Ile Ser Glu  Phe Ser Lys
    1265                1270                1275

Arg Val  Ile Leu Ala Asp Ala  Asn Leu Asp Lys Val  Leu Ser Ala
    1280                1285                1290

Tyr Asn  Lys His Arg Asp Lys  Pro Ile Arg Glu Gln  Ala Glu Asn
    1295                1300                1305

Ile Ile  His Leu Phe Thr Leu  Thr Asn Leu Gly Ala  Pro Ala Ala
    1310                1315                1320

Phe Lys  Tyr Phe Asp Thr Thr  Ile Asp Arg Lys Arg  Tyr Thr Ser
    1325                1330                1335

Thr Lys  Glu Val Leu Asp Ala  Thr Leu Ile His Gln  Ser Ile Thr
```

```
    1340                1345                1350

Gly Leu  Tyr Glu Thr Arg Ile  Asp Leu Ser Gln Leu  Gly Gly Asp
    1355                1360                1365
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<212> PRT
<213> Francisella tularensis

<400> 2

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Met Asp Lys Lys Tyr Ser Ile Gly Leu Asp Ile Gly Thr Asn Ser Val
1               5                   10                  15

Gly Trp Ala Val Ile Thr Asp Asp Tyr Lys Val Pro Ser Lys Lys Phe
            20                  25                  30

Lys Val Leu Gly Asn Thr Asp Arg His Ser Ile Lys Lys Asn Leu Ile
        35                  40                  45

Gly Ala Leu Leu Phe Asp Ser Gly Glu Thr Ala Glu Ala Thr Arg Leu
    50                  55                  60

Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
65                  70                  75                  80

Tyr Leu Gln Glu Ile Phe Ser Asn Glu Met Ala Lys Val Asp Asp Ser
                85                  90                  95

Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
            100                 105                 110

His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
        115                 120                 125

His Glu Lys Tyr Pro Thr Ile Tyr His Leu Arg Lys Lys Leu Val Asp
    130                 135                 140

Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
145                 150                 155                 160
```

```
Met Ile Lys Phe Arg Gly His Phe Leu Ile Glu Gly Asp Leu Asn Pro
                165                 170                 175

Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
            180                 185                 190

Asn Gln Leu Phe Glu Glu Asn Pro Ile Asn Ala Ser Gly Val Asp Ala
        195                 200                 205

Lys Ala Ile Leu Ser Ala Arg Leu Ser Lys Ser Arg Arg Leu Glu Asn
    210                 215                 220

Leu Ile Ala Gln Leu Pro Gly Glu Lys Lys Asn Ser Leu Phe Gly Asn
225                 230                 235                 240

Leu Ile Ala Leu Ser Leu Gly Leu Thr Pro Asn Phe Lys Ser Asn Phe
                245                 250                 255

Asp Leu Ala Glu Asp Ala Lys Leu Gln Leu Ser Lys Asp Thr Tyr Asp
            260                 265                 270

Asp Asp Leu Asp Asn Leu Leu Ala Gln Ile Gly Asp Gln Tyr Ala Asp
        275                 280                 285

Leu Phe Leu Ala Ala Lys Asn Leu Ser Asp Ala Ile Leu Leu Ser Asp
    290                 295                 300

Ile Leu Arg Val Asn Thr Glu Ile Thr Lys Ala Pro Leu Ser Ala Ser
305                 310                 315                 320

Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
                325                 330                 335

Ala Leu Val Arg Gln Gln Leu Pro Glu Lys Tyr Lys Glu Ile Phe Phe
            340                 345                 350

Asp Gln Ser Lys Asn Gly Tyr Ala Gly Tyr Ile Asp Gly Gly Ala Ser
        355                 360                 365

Gln Glu Glu Phe Tyr Lys Phe Ile Lys Pro Ile Leu Glu Lys Met Asp
    370                 375                 380
```

```
Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
385                 390                 395                 400

Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
                405                 410                 415

Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
            420                 425                 430

Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
        435                 440                 445

Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
    450                 455                 460

Met Thr Arg Lys Ser Glu Glu Thr Ile Thr Pro Trp Asn Phe Glu Glu
465                 470                 475                 480

Val Val Asp Lys Gly Ala Ser Ala Gln Ser Phe Ile Glu Arg Met Thr
                485                 490                 495

Asn Phe Asp Lys Asn Leu Pro Asn Glu Lys Val Leu Pro Lys His Ser
            500                 505                 510

Leu Leu Tyr Glu Tyr Phe Thr Val Tyr Asn Glu Leu Thr Lys Val Lys
        515                 520                 525

Tyr Val Thr Glu Gly Met Arg Lys Pro Ala Phe Leu Ser Gly Glu Gln
    530                 535                 540

Lys Lys Ala Ile Val Asp Leu Leu Phe Lys Thr Asn Arg Lys Val Thr
545                 550                 555                 560

Val Lys Gln Leu Lys Glu Asp Tyr Phe Lys Lys Ile Glu Cys Phe Asp
                565                 570                 575

Ser Val Glu Ile Ser Gly Val Glu Asp Arg Phe Asn Ala Ser Leu Gly
            580                 585                 590
```

```
Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
        595                 600                 605

Asn Glu Glu Asn Glu Asp Ile Leu Glu Asp Ile Val Leu Thr Leu Thr
    610                 615                 620

Leu Phe Glu Asp Arg Glu Met Ile Glu Glu Arg Leu Lys Thr Tyr Ala
625                 630                 635                 640

His Leu Phe Asp Asp Lys Val Met Lys Gln Leu Lys Arg Arg Arg Tyr
                645                 650                 655

Thr Gly Trp Gly Arg Leu Ser Arg Lys Leu Ile Asn Gly Ile Arg Asp
            660                 665                 670

Lys Gln Ser Gly Lys Thr Ile Leu Asp Phe Leu Lys Ser Asp Gly Phe
        675                 680                 685

Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe
    690                 695                 700

Lys Glu Asp Ile Gln Lys Ala Gln Val Ser Gly Gln Gly Asp Ser Leu
705                 710                 715                 720

His Glu His Ile Ala Asn Leu Ala Gly Ser Pro Ala Ile Lys Lys Gly
                725                 730                 735

Ile Leu Gln Thr Val Lys Val Val Asp Glu Leu Val Lys Val Met Gly
            740                 745                 750

Arg His Lys Pro Glu Asn Ile Val Ile Glu Met Ala Arg Glu Asn Gln
        755                 760                 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
    770                 775                 780

Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
785                 790                 795                 800

Val Glu Asn Thr Gln Leu Gln Asn Glu Lys Leu Tyr Leu Tyr Tyr Leu
                805                 810                 815
```

```
Gln Asn Gly Arg Asp Met Tyr Val Asp Gln Glu Leu Asp Ile Asn Arg
            820                 825                 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
        835                 840                 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
    850                 855                 860

Gly Lys Ser Asp Asn Val Pro Ser Glu Glu Val Val Lys Lys Met Lys
865                 870                 875                 880

Asn Tyr Trp Arg Gln Leu Leu Asn Ala Lys Leu Ile Thr Gln Arg Lys
                885                 890                 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
            900                 905                 910

Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr
        915                 920                 925

Lys His Val Ala Gln Ile Leu Asp Ser Arg Met Asn Thr Lys Tyr Asp
    930                 935                 940

Glu Asn Asp Lys Leu Ile Arg Glu Val Lys Val Ile Thr Leu Lys Ser
945                 950                 955                 960

Lys Leu Val Ser Asp Phe Arg Lys Asp Phe Gln Phe Tyr Lys Val Arg
                965                 970                 975

Glu Ile Asn Asn Tyr His His Ala His Asp Ala Tyr Leu Asn Ala Val
            980                 985                 990

Val Gly Thr Ala Leu Ile Lys Lys  Tyr Pro Lys Leu Glu  Ser Glu Phe
        995                 1000                1005

Val Tyr  Gly Asp Tyr Lys Val  Tyr Asp Val Arg Lys  Met Ile Ala
    1010                1015                1020
```

```
Lys Ser  Glu Gln Glu Ile Gly  Lys Ala Thr Ala Lys  Tyr Phe Phe
    1025                1030                1035

Tyr Ser  Asn Ile Met Asn Phe  Phe Lys Thr Glu Ile  Thr Leu Ala
    1040                1045                1050

Asn Gly  Glu Ile Arg Lys Arg  Pro Leu Ile Glu Thr  Asn Gly Glu
    1055                1060                1065

Thr Gly  Glu Ile Val Trp Asp  Lys Gly Arg Asp Phe  Ala Thr Val
    1070                1075                1080

Arg Lys  Val Leu Ser Met Pro  Gln Val Asn Ile Val  Lys Lys Thr
    1085                1090                1095

Glu Val  Gln Thr Gly Gly Phe  Ser Lys Glu Ser Ile  Leu Pro Lys
    1100                1105                1110

Arg Asn  Ser Asp Lys Leu Ile  Ala Arg Lys Lys Asp  Trp Asp Pro
    1115                1120                1125

Lys Lys  Tyr Gly Gly Phe Asp  Ser Pro Thr Val Ala  Tyr Ser Val
    1130                1135                1140

Leu Val  Val Ala Lys Val Glu  Lys Gly Lys Ser Lys  Lys Leu Lys
    1145                1150                1155

Ser Val  Lys Glu Leu Leu Gly  Ile Thr Ile Met Glu  Arg Ser Ser
    1160                1165                1170

Phe Glu  Lys Asn Pro Ile Asp  Phe Leu Glu Ala Lys  Gly Tyr Lys
    1175                1180                1185

Glu Val  Lys Lys Asp Leu Ile  Ile Lys Leu Pro Lys  Tyr Ser Leu
    1190                1195                1200

Phe Glu  Leu Glu Asn Gly Arg  Lys Arg Met Leu Ala  Ser Ala Gly
    1205                1210                1215

Glu Leu  Gln Lys Gly Asn Glu  Leu Ala Leu Pro Ser  Lys Tyr Val
    1220                1225                1230
```

```
Asn Phe  Leu Tyr Leu Ala Ser  His Tyr Glu Lys Leu  Lys Gly Ser
    1235                1240                1245

Pro Glu  Asp Asn Glu Gln Lys  Gln Leu Phe Val Glu  Met Ser Ile
    1250                1255                1260

Tyr Gln  Glu Phe Val Asn Lys  Tyr Ser Leu Ser Lys  Thr Leu Arg
    1265                1270                1275

Phe Glu  Leu Ile Pro Gln Gly  Lys Thr Leu Glu Asn  Ile Lys Ala
    1280                1285                1290

Arg Gly  Leu Ile Leu Asp Asp  Glu Lys Arg Ala Lys  Asp Tyr Lys
    1295                1300                1305

Lys Ala  Lys Gln Ile Ile Asp  Lys Tyr His Gln Phe  Phe Ile Glu
    1310                1315                1320

Glu Ile  Leu Ser Ser Val Cys  Ile Ser Glu Asp Leu  Leu Gln Asn
    1325                1330                1335

Tyr Ser  Asp Val Tyr Phe Lys  Leu Lys Lys Ser Asp  Asp Asp Asn
    1340                1345                1350

Leu Gln  Lys Asp Phe Lys Ser  Ala Lys Asp Thr Ile  Lys Lys Gln
    1355                1360                1365

Ile Ser  Glu Tyr Ile Lys Asp  Ser Glu Lys Phe Lys  Asn Leu Phe
    1370                1375                1380

Asn Gln  Asn Leu Ile Asp Ala  Lys Lys Gly Gln Glu  Ser Asp Leu
    1385                1390                1395

Ile Leu  Trp Leu Lys Gln Ser  Lys Asp Asn Gly Ile  Glu Leu Phe
    1400                1405                1410

Lys Ala  Asn Ser Asp Ile Thr  Asp Ile Asp Glu Ala  Leu Glu Ile
    1415                1420                1425
```

```
Ile Lys  Ser Phe Lys Gly Trp  Thr Thr Tyr Phe Lys  Gly Phe His
    1430                1435                1440

Glu Asn  Arg Lys Asn Val Tyr  Ser Ser Asn Asp Ile  Pro Thr Ser
    1445                1450                1455

Ile Ile  Tyr Arg Ile Val Asp  Asp Asn Leu Pro Lys  Phe Leu Glu
    1460                1465                1470

Asn Lys  Ala Lys Tyr Glu Ser  Leu Lys Asp Lys Ala  Pro Glu Ala
    1475                1480                1485

Ile Asn  Tyr Glu Gln Ile Lys  Lys Asp Leu Ala Glu  Glu Leu Thr
    1490                1495                1500

Phe Asp  Ile Asp Tyr Lys Thr  Ser Glu Val Asn Gln  Arg Val Phe
    1505                1510                1515

Ser Leu  Asp Glu Val Phe Glu  Ile Ala Asn Phe Asn  Asn Tyr Leu
    1520                1525                1530

Asn Gln  Ser Gly Ile Thr Lys  Phe Asn Thr Ile Ile  Gly Gly Lys
    1535                1540                1545

Phe Val  Asn Gly Glu Asn Thr  Lys Arg Lys Gly Ile  Asn Glu Tyr
    1550                1555                1560

Ile Asn  Leu Tyr Ser Gln Gln  Ile Asn Asp Lys Thr  Leu Lys Lys
    1565                1570                1575

Tyr Lys  Met Ser Val Leu Phe  Lys Gln Ile Leu Ser  Asp Thr Glu
    1580                1585                1590

Ser Lys  Ser Phe Val Ile Asp  Lys Leu Glu Asp Asp  Ser Asp Val
    1595                1600                1605

Val Thr  Thr Met Gln Ser Phe  Tyr Glu Gln Ile Ala  Ala Phe Lys
    1610                1615                1620

Thr Val  Glu Glu Lys Ser Ile  Lys Glu Thr Leu Ser  Leu Leu Phe
    1625                1630                1635
```

```
Asp Asp  Leu Lys Ala Gln Lys  Leu Asp Leu Ser Lys  Ile Tyr Phe
    1640                1645                1650

Lys Asn  Asp Lys Ser Leu Thr  Asp Leu Ser Gln Gln  Val Phe Asp
    1655                1660                1665

Asp Tyr  Ser Val Ile Gly Thr  Ala Val Leu Glu Tyr  Ile Thr Gln
    1670                1675                1680

Gln Ile  Ala Pro Lys Asn Leu  Asp Asn Pro Ser Lys  Lys Glu Gln
    1685                1690                1695

Glu Leu  Ile Ala Lys Lys Thr  Glu Lys Ala Lys Tyr  Leu Ser Leu
    1700                1705                1710

Glu Thr  Ile Lys Leu Ala Leu  Glu Glu Phe Asn Lys  His Arg Asp
    1715                1720                1725

Ile Asp  Lys Gln Cys Arg Phe  Glu Glu Ile Leu Ala  Asn Phe Ala
    1730                1735                1740

Ala Ile  Pro Met Ile Phe Asp  Glu Ile Ala Gln Asn  Lys Asp Asn
    1745                1750                1755

Leu Ala  Gln Ile Ser Ile Lys  Tyr Gln Asn Gln Gly  Lys Lys Asp
    1760                1765                1770

Leu Leu  Gln Ala Ser Ala Glu  Asp Asp Val Lys Ala  Ile Lys Asp
    1775                1780                1785

Leu Leu  Asp Gln Thr Asn Asn  Leu Leu His Lys Leu  Lys Ile Phe
    1790                1795                1800

His Ile  Ser Gln Ser Glu Asp  Lys Ala Asn Ile Leu  Asp Lys Asp
    1805                1810                1815

Glu His  Phe Tyr Leu Val Phe  Glu Glu Cys Tyr Phe  Glu Leu Ala
    1820                1825                1830
```

```
Asn Ile  Val Pro Leu Tyr Asn  Lys Ile Arg Asn Tyr  Ile Thr Gln
    1835                1840                1845

Lys Pro  Tyr Ser Asp Glu Lys  Phe Lys Leu Asn Phe  Glu Asn Ser
    1850                1855                1860

Thr Leu  Ala Asn Gly Trp Asp  Lys Asn Lys Glu Pro  Asp Asn Thr
    1865                1870                1875

Ala Ile  Leu Phe Ile Lys Asp  Asp Lys Tyr Tyr Leu  Gly Val Met
    1880                1885                1890

Asn Lys  Lys Asn Asn Lys Ile  Phe Asp Asp Lys Ala  Ile Lys Glu
    1895                1900                1905

Asn Lys  Gly Glu Gly Tyr Lys  Lys Ile Val Tyr Lys  Leu Leu Pro
    1910                1915                1920

Gly Ala  Asn Lys Met Leu Pro  Lys Val Phe Phe Ser  Ala Lys Ser
    1925                1930                1935

Ile Lys  Phe Tyr Asn Pro Ser  Glu Asp Ile Leu Arg  Ile Arg Asn
    1940                1945                1950

His Ser  Thr His Thr Lys Asn  Gly Ser Pro Gln Lys  Gly Tyr Glu
    1955                1960                1965

Lys Phe  Glu Phe Asn Ile Glu  Asp Cys Arg Lys Phe  Ile Asp Phe
    1970                1975                1980

Tyr Lys  Gln Ser Ile Ser Lys  His Pro Glu Trp Lys  Asp Phe Gly
    1985                1990                1995

Phe Arg  Phe Ser Asp Thr Gln  Arg Tyr Asn Ser Ile  Asp Glu Phe
    2000                2005                2010

Tyr Arg  Glu Val Glu Asn Gln  Gly Tyr Lys Leu Thr  Phe Glu Asn
    2015                2020                2025

Ile Ser  Glu Ser Tyr Ile Asp  Ser Val Val Asn Gln  Gly Lys Leu
    2030                2035                2040
```

```
Tyr Leu  Phe Gln Ile Tyr Asn  Lys Asp Phe Ser Ala  Tyr Ser Lys
    2045                2050                2055

Gly Arg  Pro Asn Leu His Thr  Leu Tyr Trp Lys Ala  Leu Phe Asp
    2060                2065                2070

Glu Arg  Asn Leu Gln Asp Val  Val Tyr Lys Leu Asn  Gly Glu Ala
    2075                2080                2085

Glu Leu  Phe Tyr Arg Lys Gln  Ser Ile Pro Lys Lys  Ile Thr His
    2090                2095                2100

Pro Ala  Lys Glu Ala Ile Ala  Asn Lys Asn Lys Asp  Asn Pro Lys
    2105                2110                2115

Lys Glu  Ser Val Phe Glu Tyr  Asp Leu Ile Lys Asp  Lys Arg Phe
    2120                2125                2130

Thr Glu  Asp Lys Phe Phe Phe  His Cys Pro Ile Thr  Ile Asn Phe
    2135                2140                2145

Lys Ser  Ser Gly Ala Asn Lys  Phe Asn Asp Glu Ile  Asn Leu Leu
    2150                2155                2160

Leu Lys  Glu Lys Ala Asn Asp  Val His Ile Leu Ser  Ile Asp Arg
    2165                2170                2175

Gly Glu  Arg His Leu Ala Tyr  Tyr Thr Leu Val Asp  Gly Lys Gly
    2180                2185                2190

Asn Ile  Ile Lys Gln Asp Thr  Phe Asn Ile Ile Gly  Asn Asp Arg
    2195                2200                2205

Met Lys  Thr Asn Tyr His Asp  Lys Leu Ala Ala Ile  Glu Lys Asp
    2210                2215                2220

Arg Asp  Ser Ala Arg Lys Asp  Trp Lys Lys Ile Asn  Asn Ile Lys
    2225                2230                2235
```

```
Glu Met  Lys Glu Gly Tyr Leu  Ser Gln Val Val His  Glu Ile Ala
    2240                2245                2250

Lys Leu  Val Ile Glu Tyr Asn  Ala Ile Val Val Phe  Glu Asp Leu
    2255                2260                2265

Asn Phe  Gly Phe Lys Arg Gly  Arg Phe Lys Val Glu  Lys Gln Val
    2270                2275                2280

Tyr Gln  Lys Leu Glu Lys Met  Leu Ile Glu Lys Leu  Asn Tyr Leu
    2285                2290                2295

Val Phe  Lys Asp Asn Glu Phe  Asp Lys Thr Gly Gly  Val Leu Arg
    2300                2305                2310

Ala Tyr  Gln Leu Thr Ala Pro  Phe Glu Thr Phe Lys  Lys Met Gly
    2315                2320                2325

Lys Gln  Thr Gly Ile Ile Tyr  Tyr Val Pro Ala Gly  Phe Thr Ser
    2330                2335                2340

Lys Ile  Cys Pro Val Thr Gly  Phe Val Asn Gln Leu  Tyr Pro Lys
    2345                2350                2355

Tyr Glu  Ser Val Ser Lys Ser  Gln Glu Phe Phe Ser  Lys Phe Asp
    2360                2365                2370

Lys Ile  Cys Tyr Asn Leu Asp  Lys Gly Tyr Phe Glu  Phe Ser Phe
    2375                2380                2385

Asp Tyr  Lys Asn Phe Gly Asp  Lys Ala Ala Lys Gly  Lys Trp Thr
    2390                2395                2400

Ile Ala  Ser Phe Gly Ser Arg  Leu Ile Asn Phe Arg  Asn Ser Asp
    2405                2410                2415

Lys Asn  His Asn Trp Asp Thr  Arg Glu Val Tyr Pro  Thr Lys Glu
    2420                2425                2430

Leu Glu  Lys Leu Leu Lys Asp  Tyr Ser Ile Glu Tyr  Gly His Gly
    2435                2440                2445
```

Glu Cys Ile Lys Ala Ala Ile Cys Gly Glu Ser Asp Lys Lys Phe
2450 2455 2460

Phe Ala Lys Leu Thr Ser Val Leu Asn Thr Ile Leu Gln Met Arg
2465 2470 2475

Asn Ser Lys Thr Gly Thr Glu Leu Asp Tyr Leu Ile Ser Pro Val
2480 2485 2490

Ala Asp Val Asn Gly Asn Phe Phe Asp Ser Arg Gln Ala Pro Lys
2495 2500 2505

Asn Met Pro Gln Asp Ala Asp Ala Asn Gly Ala Tyr His Ile Gly
2510 2515 2520

Leu Lys Gly Leu Met Leu Leu Gly Arg Ile Lys Asn Asn Gln Glu
2525 2530 2535

Gly Lys Lys Leu Asn Leu Val Ile Lys Asn Glu Glu Tyr Phe Glu
2540 2545 2550

Phe Val Gln Asn Arg Asn Asn
2555 2560

<210> 3
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<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 3

Ile Gly Leu Asp Ile Gly Thr Asn Ser Val Gly Trp Ala Val Ile
1 5 10 15

<210> 4
<211> 8
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic Sequence

<400> 4

Ile Val Ile Glu Met Ala Arg Glu
1   5

<210> 5
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 5

Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys Asp Asp Ser Ile
1   5   10   15

Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn
            20   25

<210> 6
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 6

His His Ala His Asp Ala Tyr Leu
1   5

<210> 7
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 7

Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg
1   5   10

<210> 8
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 8

Arg Arg Gln Arg Arg Thr Ser Lys Leu Met Lys Arg
1 5 10

<210> 9
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 9

Gly Trp Thr Leu Asn Ser Ala Gly Tyr Leu Leu Gly Lys Ile Asn Leu
1 5 10 15

Lys Ala Leu Ala Ala Leu Ala Lys Lys Ile Leu
20 25

<210> 10
<211> 33
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 10

Lys Ala Leu Ala Trp Glu Ala Lys Leu Ala Lys Ala Leu Ala Lys Ala
1 5 10 15

Leu Ala Lys His Leu Ala Lys Ala Leu Ala Lys Ala Leu Lys Cys Glu
20 25 30

Ala

<210> 11
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 11

Arg Gln Ile Lys Ile Trp Phe Gln Asn Arg Arg Met Lys Trp Lys Lys
1 5 10 15

<210> 12
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 12

Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg
1 5 10

<210> 13
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 13

Arg Lys Lys Arg Arg Gln Arg Arg Arg
1 5

<210> 14
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 14

Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg
1 5 10

<210> 15
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 15

Arg Lys Lys Arg Arg Gln Arg Arg
1 5

<210> 16
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 16

Tyr Ala Arg Ala Ala Ala Arg Gln Ala Arg Ala
1 5 10

<210> 17
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 17

Thr His Arg Leu Pro Arg Arg Arg Arg Arg Arg
1 5 10

<210> 18
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 18

Gly Gly Arg Arg Ala Arg Arg Arg Arg Arg Arg
1 5 10

<210> 19
<211> 1307
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic Sequence

<400> 19

Met Thr Gln Phe Glu Gly Phe Thr Asn Leu Tyr Gln Val Ser Lys Thr
1 5 10 15

Leu Arg Phe Glu Leu Ile Pro Gln Gly Lys Thr Leu Lys His Ile Gln
20 25 30

Glu Gln Gly Phe Ile Glu Glu Asp Lys Ala Arg Asn Asp His Tyr Lys
35 40 45

Glu Leu Lys Pro Ile Ile Asp Arg Ile Tyr Lys Thr Tyr Ala Asp Gln
50 55 60

Cys Leu Gln Leu Val Gln Leu Asp Trp Glu Asn Leu Ser Ala Ala Ile
65 70 75 80

Asp Ser Tyr Arg Lys Glu Lys Thr Glu Glu Thr Arg Asn Ala Leu Ile
85 90 95

Glu Glu Gln Ala Thr Tyr Arg Asn Ala Ile His Asp Tyr Phe Ile Gly
100 105 110

Arg Thr Asp Asn Leu Thr Asp Ala Ile Asn Lys Arg His Ala Glu Ile
115 120 125

Tyr Lys Gly Leu Phe Lys Ala Glu Leu Phe Asn Gly Lys Val Leu Lys
130 135 140

Gln Leu Gly Thr Val Thr Thr Thr Glu His Glu Asn Ala Leu Leu Arg
145 150 155 160

```
Ser Phe Asp Lys Phe Thr Thr Tyr Phe Ser Gly Phe Tyr Glu Asn Arg
                165                 170                 175

Lys Asn Val Phe Ser Ala Glu Asp Ile Ser Thr Ala Ile Pro His Arg
            180                 185                 190

Ile Val Gln Asp Asn Phe Pro Lys Phe Lys Glu Asn Cys His Ile Phe
        195                 200                 205

Thr Arg Leu Ile Thr Ala Val Pro Ser Leu Arg Glu His Phe Glu Asn
    210                 215                 220

Val Lys Lys Ala Ile Gly Ile Phe Val Ser Thr Ser Ile Glu Glu Val
225                 230                 235                 240

Phe Ser Phe Pro Phe Tyr Asn Gln Leu Leu Thr Gln Thr Gln Ile Asp
                245                 250                 255

Leu Tyr Asn Gln Leu Leu Gly Gly Ile Ser Arg Glu Ala Gly Thr Glu
            260                 265                 270

Lys Ile Lys Gly Leu Asn Glu Val Leu Asn Leu Ala Ile Gln Lys Asn
        275                 280                 285

Asp Glu Thr Ala His Ile Ile Ala Ser Leu Pro His Arg Phe Ile Pro
    290                 295                 300

Leu Phe Lys Gln Ile Leu Ser Asp Arg Asn Thr Leu Ser Phe Ile Leu
305                 310                 315                 320

Glu Glu Phe Lys Ser Asp Glu Glu Val Ile Gln Ser Phe Cys Lys Tyr
                325                 330                 335

Lys Thr Leu Leu Arg Asn Glu Asn Val Leu Glu Thr Ala Glu Ala Leu
            340                 345                 350

Phe Asn Glu Leu Asn Ser Ile Asp Leu Thr His Ile Phe Ile Ser His
        355                 360                 365

Lys Lys Leu Glu Thr Ile Ser Ser Ala Leu Cys Asp His Trp Asp Thr
    370                 375                 380
```

```
Leu Arg Asn Ala Leu Tyr Glu Arg Arg Ile Ser Glu Leu Thr Gly Lys
385                 390                 395                 400

Ile Thr Lys Ser Ala Lys Glu Lys Val Gln Arg Ser Leu Lys His Glu
                405                 410                 415

Asp Ile Asn Leu Gln Glu Ile Ile Ser Ala Ala Gly Lys Glu Leu Ser
            420                 425                 430

Glu Ala Phe Lys Gln Lys Thr Ser Glu Ile Leu Ser His Ala His Ala
        435                 440                 445

Ala Leu Asp Gln Pro Leu Pro Thr Thr Leu Lys Lys Gln Glu Glu Lys
    450                 455                 460

Glu Ile Leu Lys Ser Gln Leu Asp Ser Leu Leu Gly Leu Tyr His Leu
465                 470                 475                 480

Leu Asp Trp Phe Ala Val Asp Glu Ser Asn Glu Val Asp Pro Glu Phe
                485                 490                 495

Ser Ala Arg Leu Thr Gly Ile Lys Leu Glu Met Glu Pro Ser Leu Ser
            500                 505                 510

Phe Tyr Asn Lys Ala Arg Asn Tyr Ala Thr Lys Lys Pro Tyr Ser Val
        515                 520                 525

Glu Lys Phe Lys Leu Asn Phe Gln Met Pro Thr Leu Ala Ser Gly Trp
    530                 535                 540

Asp Val Asn Lys Glu Lys Asn Asn Gly Ala Ile Leu Phe Val Lys Asn
545                 550                 555                 560

Gly Leu Tyr Tyr Leu Gly Ile Met Pro Lys Gln Lys Gly Arg Tyr Lys
                565                 570                 575

Ala Leu Ser Phe Glu Pro Thr Glu Lys Thr Ser Glu Gly Phe Asp Lys
            580                 585                 590
```

```
Met Tyr Tyr Asp Tyr Phe Pro Asp Ala Ala Lys Met Ile Pro Lys Cys
        595                 600                 605

Ser Thr Gln Leu Lys Ala Val Thr Ala His Phe Gln Thr His Thr Thr
    610                 615                 620

Pro Ile Leu Leu Ser Asn Asn Phe Ile Glu Pro Leu Glu Ile Thr Lys
625                 630                 635                 640

Glu Ile Tyr Asp Leu Asn Asn Pro Glu Lys Glu Pro Lys Lys Phe Gln
                645                 650                 655

Thr Ala Tyr Ala Lys Lys Thr Gly Asp Gln Lys Gly Tyr Arg Glu Ala
            660                 665                 670

Leu Cys Lys Trp Ile Asp Phe Thr Arg Asp Phe Leu Ser Lys Tyr Thr
        675                 680                 685

Lys Thr Thr Ser Ile Asp Leu Ser Ser Leu Arg Pro Ser Ser Gln Tyr
    690                 695                 700

Lys Asp Leu Gly Glu Tyr Tyr Ala Glu Leu Asn Pro Leu Leu Tyr His
705                 710                 715                 720

Ile Ser Phe Gln Arg Ile Ala Glu Lys Glu Ile Met Asp Ala Val Glu
                725                 730                 735

Thr Gly Lys Leu Tyr Leu Phe Gln Ile Tyr Asn Lys Asp Phe Ala Lys
            740                 745                 750

Gly His His Gly Lys Pro Asn Leu His Thr Leu Tyr Trp Thr Gly Leu
        755                 760                 765

Phe Ser Pro Glu Asn Leu Ala Lys Thr Ser Ile Lys Leu Asn Gly Gln
    770                 775                 780

Ala Glu Leu Phe Tyr Arg Pro Lys Ser Arg Met Lys Arg Met Ala His
785                 790                 795                 800

Arg Leu Gly Glu Lys Met Leu Asn Lys Lys Leu Lys Asp Gln Lys Thr
                805                 810                 815
```

```
Pro Ile Pro Asp Thr Leu Tyr Gln Glu Leu Tyr Asp Tyr Val Asn His
            820                 825                 830

Arg Leu Ser His Asp Leu Ser Asp Glu Ala Arg Ala Leu Leu Pro Asn
        835                 840                 845

Val Ile Thr Lys Glu Val Ser His Glu Ile Ile Lys Asp Arg Arg Phe
    850                 855                 860

Thr Ser Asp Lys Phe Phe Phe His Val Pro Ile Thr Leu Asn Tyr Gln
865                 870                 875                 880

Ala Ala Asn Ser Pro Ser Lys Phe Asn Gln Arg Val Asn Ala Tyr Leu
                885                 890                 895

Lys Glu His Pro Glu Thr Pro Ile Ile Gly Ile Asp Arg Gly Glu Arg
            900                 905                 910

Asn Leu Ile Tyr Ile Thr Val Ile Asp Ser Thr Gly Lys Ile Leu Glu
        915                 920                 925

Gln Arg Ser Leu Asn Thr Ile Gln Gln Phe Asp Tyr Gln Lys Lys Leu
    930                 935                 940

Asp Asn Arg Glu Lys Glu Arg Val Ala Ala Arg Gln Ala Trp Ser Val
945                 950                 955                 960

Val Gly Thr Ile Lys Asp Leu Lys Gln Gly Tyr Leu Ser Gln Val Ile
                965                 970                 975

His Glu Ile Val Asp Leu Met Ile His Tyr Gln Ala Val Val Val Leu
            980                 985                 990

Glu Asn Leu Asn Phe Gly Phe Lys  Ser Lys Arg Thr Gly  Ile Ala Glu
        995                 1000                1005

Lys Ala  Val Tyr Gln Gln Phe  Glu Lys Met Leu Ile  Asp Lys Leu
    1010                1015                1020
```

```
Asn Cys  Leu Val Leu Lys Asp  Tyr Pro Ala Glu Lys  Val Gly Gly
    1025                1030                1035

Val Leu  Asn Pro Tyr Gln Leu  Thr Asp Gln Phe Thr  Ser Phe Ala
    1040                1045                1050

Lys Met  Gly Thr Gln Ser Gly  Phe Leu Phe Tyr Val  Pro Ala Pro
    1055                1060                1065

Tyr Thr  Ser Lys Ile Asp Pro  Leu Thr Gly Phe Val  Asp Pro Phe
    1070                1075                1080

Val Trp  Lys Thr Ile Lys Asn  His Glu Ser Arg Lys  His Phe Leu
    1085                1090                1095

Glu Gly  Phe Asp Phe Leu His  Tyr Asp Val Lys Thr  Gly Asp Phe
    1100                1105                1110

Ile Leu  His Phe Lys Met Asn  Arg Asn Leu Ser Phe  Gln Arg Gly
    1115                1120                1125

Leu Pro  Gly Phe Met Pro Ala  Trp Asp Ile Val Phe  Glu Lys Asn
    1130                1135                1140

Glu Thr  Gln Phe Asp Ala Lys  Gly Thr Pro Phe Ile  Ala Gly Lys
    1145                1150                1155

Arg Ile  Val Pro Val Ile Glu  Asn His Arg Phe Thr  Gly Arg Tyr
    1160                1165                1170

Arg Asp  Leu Tyr Pro Ala Asn  Glu Leu Ile Ala Leu  Leu Glu Glu
    1175                1180                1185

Lys Gly  Ile Val Phe Arg Asp  Gly Ser Asn Ile Leu  Pro Lys Leu
    1190                1195                1200

Leu Glu  Asn Asp Asp Ser His  Ala Ile Asp Thr Met  Val Ala Leu
    1205                1210                1215

Ile Arg  Ser Val Leu Gln Met  Arg Asn Ser Asn Ala  Ala Thr Gly
    1220                1225                1230
```

Glu Asp Tyr Ile Asn Ser Pro Val Arg Asp Leu Asn Gly Val Cys
    1235   1240   1245

Phe Asp Ser Arg Phe Gln Asn Pro Glu Trp Pro Met Asp Ala Asp
    1250   1255   1260

Ala Asn Gly Ala Tyr His Ile Ala Leu Lys Gly Gln Leu Leu Leu
    1265   1270   1275

Asn His Leu Lys Glu Ser Lys Asp Leu Lys Leu Gln Asn Gly Ile
    1280   1285   1290

Ser Asn Gln Asp Trp Leu Ala Tyr Ile Gln Glu Leu Arg Asn
    1295   1300   1305

<210> 20
<211> 1228
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic

<400> 20

Ala Ala Ser Lys Leu Glu Lys Phe Thr Asn Cys Tyr Ser Leu Ser Lys
1   5   10   15

Thr Leu Arg Phe Lys Ala Ile Pro Val Gly Lys Thr Gln Glu Asn Ile
            20   25   30

Asp Asn Lys Arg Leu Leu Val Glu Asp Glu Lys Arg Ala Glu Asp Tyr
        35   40   45

Lys Gly Val Lys Lys Leu Leu Asp Arg Tyr Tyr Leu Ser Phe Ile Asn
    50   55   60

Asp Val Leu His Ser Ile Lys Leu Lys Asn Leu Asn Asn Tyr Ile Ser
65   70   75   80

Leu Phe Arg Lys Lys Thr Arg Thr Glu Lys Glu Asn Lys Glu Leu Glu
                85   90   95

```
Asn Leu Glu Ile Asn Leu Arg Lys Glu Ile Ala Lys Ala Phe Lys Gly
            100                 105                 110

Ala Ala Gly Tyr Lys Ser Leu Phe Lys Lys Asp Ile Ile Glu Thr Ile
        115                 120                 125

Leu Pro Glu Ala Ala Asp Asp Lys Asp Glu Ile Ala Leu Val Asn Ser
    130                 135                 140

Phe Asn Gly Phe Thr Thr Ala Phe Thr Gly Phe Phe Asp Asn Arg Glu
145                 150                 155                 160

Asn Met Phe Ser Glu Glu Ala Lys Ser Thr Ser Ile Ala Phe Arg Cys
                165                 170                 175

Ile Asn Glu Asn Leu Thr Arg Tyr Ile Ser Asn Met Asp Ile Phe Glu
            180                 185                 190

Lys Val Asp Ala Ile Phe Asp Lys His Glu Val Gln Glu Ile Lys Glu
        195                 200                 205

Lys Ile Leu Asn Ser Asp Tyr Asp Val Glu Asp Phe Phe Glu Gly Glu
    210                 215                 220

Phe Phe Asn Phe Val Leu Thr Gln Glu Gly Ile Asp Val Tyr Asn Ala
225                 230                 235                 240

Ile Ile Gly Gly Phe Val Thr Glu Ser Gly Glu Lys Ile Lys Gly Leu
                245                 250                 255

Asn Glu Tyr Ile Asn Leu Tyr Asn Ala Lys Thr Lys Gln Ala Leu Pro
            260                 265                 270

Lys Phe Lys Pro Leu Tyr Lys Gln Val Leu Ser Asp Arg Glu Ser Leu
        275                 280                 285

Ser Phe Tyr Gly Glu Gly Tyr Thr Ser Asp Glu Glu Val Leu Glu Val
    290                 295                 300
```

```
Phe Arg Asn Thr Leu Asn Lys Asn Ser Glu Ile Phe Ser Ser Ile Lys
305                 310                 315                 320

Lys Leu Glu Lys Leu Phe Lys Asn Phe Asp Glu Tyr Ser Ser Ala Gly
                325                 330                 335

Ile Phe Val Lys Asn Gly Pro Ala Ile Ser Thr Ile Ser Lys Asp Ile
            340                 345                 350

Phe Gly Glu Trp Asn Leu Ile Arg Asp Lys Trp Asn Ala Glu Tyr Asp
        355                 360                 365

Asp Ile His Leu Lys Lys Lys Ala Val Val Thr Glu Lys Tyr Glu Asp
    370                 375                 380

Asp Arg Arg Lys Ser Phe Lys Lys Ile Gly Ser Phe Ser Leu Glu Gln
385                 390                 395                 400

Leu Gln Glu Tyr Ala Asp Ala Asp Leu Ser Val Val Glu Lys Leu Lys
                405                 410                 415

Glu Ile Ile Ile Gln Lys Val Asp Glu Ile Tyr Lys Val Tyr Gly Ser
            420                 425                 430

Ser Glu Lys Leu Phe Asp Ala Asp Phe Val Leu Glu Lys Ser Leu Lys
        435                 440                 445

Lys Asn Asp Ala Val Val Ala Ile Met Lys Asp Leu Leu Asp Ser Val
    450                 455                 460

Lys Ser Phe Glu Asn Tyr Ile Lys Ala Phe Phe Gly Glu Gly Lys Glu
465                 470                 475                 480

Thr Asn Arg Asp Glu Ser Phe Tyr Gly Asp Phe Val Leu Ala Tyr Asp
                485                 490                 495

Ile Leu Leu Lys Val Asp His Ile Tyr Asp Ala Ile Arg Asn Tyr Val
            500                 505                 510

Thr Gln Lys Pro Tyr Ser Lys Asp Lys Phe Lys Leu Tyr Phe Gln Asn
        515                 520                 525
```

```
Pro Gln Phe Met Gly Gly Trp Asp Lys Asp Lys Glu Thr Asp Tyr Arg
    530                 535                 540

Ala Thr Ile Leu Arg Tyr Gly Ser Lys Tyr Tyr Leu Ala Ile Met Asp
545                 550                 555                 560

Lys Lys Tyr Ala Lys Cys Leu Gln Lys Ile Asp Lys Asp Asp Val Asn
                565                 570                 575

Gly Asn Tyr Glu Lys Ile Asn Tyr Lys Leu Leu Pro Gly Pro Asn Lys
            580                 585                 590

Met Leu Pro Lys Val Phe Phe Ser Lys Lys Trp Met Ala Tyr Tyr Asn
        595                 600                 605

Pro Ser Glu Asp Ile Gln Lys Ile Tyr Lys Asn Gly Thr Phe Lys Lys
    610                 615                 620

Gly Asp Met Phe Asn Leu Asn Asp Cys His Lys Leu Ile Asp Phe Phe
625                 630                 635                 640

Lys Asp Ser Ile Ser Arg Tyr Pro Lys Trp Ser Asn Ala Tyr Asp Phe
                645                 650                 655

Asn Phe Ser Glu Thr Glu Lys Tyr Lys Asp Ile Ala Gly Phe Tyr Arg
            660                 665                 670

Glu Val Glu Glu Gln Gly Tyr Lys Val Ser Phe Glu Ser Ala Ser Lys
        675                 680                 685

Lys Glu Val Asp Lys Leu Val Glu Glu Gly Lys Leu Tyr Met Phe Gln
    690                 695                 700

Ile Tyr Asn Lys Asp Phe Ser Asp Lys Ser His Gly Thr Pro Asn Leu
705                 710                 715                 720

His Thr Met Tyr Phe Lys Leu Leu Phe Asp Glu Asn Asn His Gly Gln
                725                 730                 735
```

```
Ile Arg Leu Ser Gly Gly Ala Glu Leu Phe Met Arg Arg Ala Ser Leu
            740                 745                 750

Lys Lys Glu Glu Leu Val Val His Pro Ala Asn Ser Pro Ile Ala Asn
        755                 760                 765

Lys Asn Pro Asp Asn Pro Lys Lys Thr Thr Thr Leu Ser Tyr Asp Val
    770                 775                 780

Tyr Lys Asp Lys Arg Phe Ser Glu Asp Gln Tyr Glu Leu His Ile Pro
785                 790                 795                 800

Ile Ala Ile Asn Lys Cys Pro Lys Asn Ile Phe Lys Ile Asn Thr Glu
                805                 810                 815

Val Arg Val Leu Leu Lys His Asp Asp Asn Pro Tyr Val Ile Gly Ile
            820                 825                 830

Asp Arg Gly Glu Arg Asn Leu Leu Tyr Ile Val Val Val Asp Gly Lys
        835                 840                 845

Gly Asn Ile Val Glu Gln Tyr Ser Leu Asn Glu Ile Ile Asn Asn Phe
    850                 855                 860

Asn Gly Ile Arg Ile Lys Thr Asp Tyr His Ser Leu Leu Asp Lys Lys
865                 870                 875                 880

Glu Lys Glu Arg Phe Glu Ala Arg Gln Asn Trp Thr Ser Ile Glu Asn
                885                 890                 895

Ile Lys Glu Leu Lys Ala Gly Tyr Ile Ser Gln Val Val His Lys Ile
            900                 905                 910

Cys Glu Leu Val Glu Lys Tyr Asp Ala Val Ile Ala Leu Glu Asp Leu
        915                 920                 925

Asn Ser Gly Phe Lys Asn Ser Arg Val Lys Val Glu Lys Gln Val Tyr
    930                 935                 940

Gln Lys Phe Glu Lys Met Leu Ile Asp Lys Leu Asn Tyr Met Val Asp
945                 950                 955                 960
```

```
Lys Lys Ser Asn Pro Cys Ala Thr Gly Gly Ala Leu Lys Gly Tyr Gln
               965                 970                 975

Ile Thr Asn Lys Phe Glu Ser Phe Lys Ser Met Ser Thr Gln Asn Gly
            980                 985                 990

Phe Ile Phe Tyr Ile Pro Ala Trp  Leu Thr Ser Lys Ile  Asp Pro Ser
        995                 1000                1005

Thr Gly  Phe Val Asn Leu Leu  Lys Thr Lys Tyr Thr  Ser Ile Ala
    1010                1015                1020

Asp Ser  Lys Lys Phe Ile Ser  Ser Phe Asp Arg Ile  Met Tyr Val
    1025                1030                1035

Pro Glu  Glu Asp Leu Phe Glu  Phe Ala Leu Asp Tyr  Lys Asn Phe
    1040                1045                1050

Ser Arg  Thr Asp Ala Asp Tyr  Ile Lys Lys Trp Lys  Leu Tyr Ser
    1055                1060                1065

Tyr Gly  Asn Arg Ile Arg Ile  Phe Ala Ala Ala Lys  Lys Asn Asn
    1070                1075                1080

Val Phe  Ala Trp Glu Glu Val  Cys Leu Thr Ser Ala  Tyr Lys Glu
    1085                1090                1095

Leu Phe  Asn Lys Tyr Gly Ile  Asn Tyr Gln Gln Gly  Asp Ile Arg
    1100                1105                1110

Ala Leu  Leu Cys Glu Gln Ser  Asp Lys Ala Phe Tyr  Ser Ser Phe
    1115                1120                1125

Met Ala  Leu Met Ser Leu Met  Leu Gln Met Arg Asn  Ser Ile Thr
    1130                1135                1140

Gly Arg  Thr Asp Val Asp Phe  Leu Ile Ser Pro Val  Lys Asn Ser
    1145                1150                1155
```

```
Asp Gly  Ile Phe Tyr Asp Ser  Arg Asn Tyr Glu Ala  Gln Glu Asn
    1160                 1165                 1170

Ala Ile  Leu Pro Lys Asn Ala  Asp Ala Asn Gly Ala  Tyr Asn Ile
    1175                 1180                 1185

Ala Arg  Lys Val Leu Trp Ala  Ile Gly Gln Phe Lys  Lys Ala Glu
    1190                 1195                 1200

Asp Glu  Lys Leu Asp Lys Val  Lys Ile Ala Ile Ser  Asn Lys Glu
    1205                 1210                 1215

Trp Leu  Glu Tyr Ala Gln Thr  Ser Val Lys
    1220                 1225
```