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(54) **POLYMER DERIVATIVES COMPRISING AN ACETAL OR KETAL BRANCHING POINT**

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(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The invention conjugates that can be prepared by contacting a polymer comprising an acetal or ketal branching point with a pharmacologically active agent under conditions suitable to form a covalent attachment between the polymer and the pharmacologically active agent.

2 Claims, No Drawings

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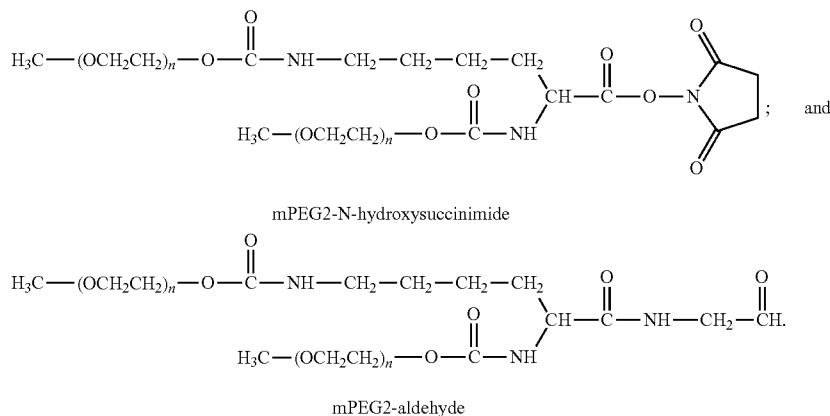
CROSS-REFERENCE TO RELATED APPLICATIONS

FIELD OF THE INVENTION

BACKGROUND OF THE INVENTION

Some success has been achieved in addressing the problems of delivering active agents via injection. For example, conjugating the active agent to a water-soluble polymer has resulted in polymer-active agent conjugates having reduced immunogenicity and antigenicity. In addition, these polymer-active agent conjugates often have greatly increased half-

The problem associated with inactive conjugates bearing relatively large polyethylene glycol moieties has been solved, in part, by using "branched" forms of a polymer derivative. Examples of branched versions of a polyethylene glycol derivative are shown below:



wherein n represents the number of times the ethylene oxide moiety is repeated.

Although solving some of the issues associated with using relatively large polymers, branched versions of polymer derivatives also have problems. For example, the increased structural complexity of branching often results in a concomitant increase in synthetic complexity. In addition, there will always be a need to provide ever more readily synthesized and/or purified polymer derivatives that can be conveniently used in conjugation reactions. Thus, the present invention seeks to solve these and other needs in the art by providing a branched polymer derivative whereby branching is effected through either an acetal or ketal moiety.

SUMMARY OF THE INVENTION

In one or more embodiments of the invention, a polymer is provided comprising a first water-soluble polymer segment, a second water-soluble polymer segment, a reactive group, and an acetal or ketal moiety. The acetal or ketal moiety provides the polymer with a branching moiety to attach each of the first and second water-soluble polymer segments as well as the reactive group. As will be shown in further detail below, the acetal or ketal moiety comprises two oxygen atoms, each of which is linked to a single carbon atom, herein referred to as a "linking carbon atom." Typically, the first and second water-soluble polymer segments are attached through these two oxygen atoms, one water-soluble polymer segment per oxygen atom. The reactive group is also attached to the linking carbon atom. Thus, the various elements are attached in such as way as to result in a branched structure.

Attachment of the first and second water-soluble polymer segments as well as the reactive group to the acetal or ketal moiety can be effected directly or indirectly. Direct attachment typically comprises a linkage—without any intervening atoms—between the acetal or ketal moiety and the first or second water-soluble polymer segment or the reactive group. Indirect attachment comprises attachment through one or more atoms of the first or second water-soluble polymer segment or reactive group and the acetal or ketal moiety. In some instances, both a direct and indirect attachments can be present within a single polymer.

Any water-soluble polymer segment and any reactive group can be used the polymer and the invention is not limited in this regard. It is preferred, however, that the water-soluble polymer segments comprise a poly(ethylene glycol). In addition, preferred reactive groups include nucleophiles and electrophiles commonly used in synthetic organic chemistry. Both water-soluble polymer segments and reactive groups suitable for use in connection with the present invention are discussed in more detail below.

In one or more embodiments, the invention provides a method for preparing the polymers described herein. Briefly, the method comprises a first step of providing an acetal- or ketal-containing precursor molecule having an attached (either directly or indirectly attached) carboxylic acid or a carboxylic acid derivative such as an ester. As will set be forth more clearly below, the precursor molecule comprises two ether moieties (required by all acetals or ketals, by definition) and an attached carboxylic acid or a carboxylic acid derivative such as an ester functionality. The precursor molecule is then contacted, under aqueous acid conditions, with an excess of water-soluble polymer segments having at least one terminal hydroxyl group to form a tri-substituted intermediate: a water-soluble polymer functionality substituted at each of two ether functionalities of the precursor molecule—and attached via an ether linkage—and a water-soluble polymer

functionality attached at the ester functionality of the precursor molecule—and attached via an ester linkage—. Next, the water-soluble polymer segment attached via an ester linkage to the tri-substituted intermediate is then cleaved, thereby resulting in the polymers of the present invention. Cleavage of the water-soluble polymer segment results in formation of carboxylic acid, a reactive group. This carboxylic acid, however, can optionally be further modified to provide a reactive group other than a carboxylic acid.

In one or more embodiments, the invention provides another method for preparing the polymers described. This particular method comprises the step of providing a hydroxy-terminated water-soluble polymer (e.g., a PEG alcohol), optionally having a spacer moiety (e.g., a C₂₋₈ linker). A composition comprising hydroxy-terminated water-soluble polymer optionally having a spacer moiety is then combined, under electrophilic addition conditions, with a composition comprising a water-soluble polymer bearing a vinyl ether. Exemplary electrophilic addition conditions include the presence of electrophile (e.g., bromosuccinate). The electrophile can be further modified using conventional techniques to provide a polymer bearing a functional group of interest.

In one or more embodiments, an active agent-polymer conjugate is provided wherein conjugate uses a polymer as described herein. In addition, the invention also provides a method for forming active-agent conjugates wherein the method comprises the step of contacting a polymer as described herein to a pharmacologically active agent under conditions suitable to form a covalent attachment between the polymer and the pharmacologically active agent.

In one or more embodiments, pharmaceutical compositions are provided comprising a polymer conjugate as described herein in combination with a pharmaceutically acceptable carrier. The pharmaceutical compositions encompass all types of formulations and in particular those that are suited for injection, e.g., powders that can be reconstituted as well as suspensions and solutions. Furthermore, the invention provides a method of treating a patient comprising the step of administering a polymer conjugate as described herein.

Among other things, the branching linkage provides a different rate of in vivo chain cleavage. Differential rates of in vivo chain cleavage advantageously provides the ability to customize clearance rates of the polymer and/or the active agent to which the reagent is attached.

DETAILED DESCRIPTION OF THE INVENTION

Before describing the present invention in detail, it is to be understood that this invention is not limited to the particular polymers, synthetic techniques, active agents, and the like as such may vary.

It must be noted that, as used in this specification and the claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to a "polymer" includes a single polymer as well as two or more of the same or different polymers, reference to a "conjugate" refers to a single conjugate as well as two or more of the same or different conjugates, reference to an "excipient" includes a single excipient as well as two or more of the same or different excipients, and the like.

In describing and claiming the present invention, the following terminology will be used in accordance with the definitions described below.

"PEG," "polyethylene glycol" and "poly(ethylene glycol)" as used herein, are meant to encompass any water-soluble poly(ethylene oxide). Typically, PEGs for use in accordance with the invention comprise the following structure "—O

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(CH₂CH₂O)_m—” where (m) is 2 to 4000. As used herein, PEG also includes “—CH₂CH₂—O(CH₂CH₂O)_m—CH₂CH₂—” and “—(CH₂CH₂O)_m—,” depending upon whether or not the terminal oxygens have been displaced. When the PEG further comprises a spacer moiety (to be described in greater detail below), the atoms comprising the spacer moiety, when covalently attached to a water-soluble polymer segment, do not result in the formation of an oxygen-oxygen bond (i.e., an “—O—O—” or peroxide linkage). Throughout the specification and claims, it should be remembered that the term “PEG” includes structures having various terminal or “end capping” groups and so forth. The term “PEG” also means a polymer that contains a majority, that is to say, greater than 50%, of —CH₂CH₂O— monomeric sub-units. With respect to specific forms, the PEG can take any number of a variety of molecular weights, as well as structures or geometries such as “branched,” “linear,” “forked,” “multifunctional,” and the like, to be described in greater detail below.

The terms “end-capped” or “terminally capped” are interchangeably used herein to refer to a terminal or endpoint of a polymer having an end-capping moiety. Typically, although not necessarily, the end-capping moiety comprises a hydroxy or C₁₋₂₀ alkoxy group. Thus, examples of end-capping moieties include alkoxy (e.g., methoxy, ethoxy and benzyloxy), as well as aryl, heteroaryl, cyclo, heterocyclo, and the like. In addition, saturated, unsaturated, substituted and unsubstituted forms of each of the foregoing are envisioned. Moreover, the end-capping group can also be a silane. The end-capping group can also advantageously comprise a detectable label. When the polymer has an end-capping group comprising a detectable label, the amount or location of the polymer and/or the moiety (e.g., active agent) or interest to which the polymer is coupled to can be determined by using a suitable detector. Such labels include, without limitation, fluorescers, chemiluminescers, moieties used in enzyme labeling, colorimetric (e.g., dyes), metal ions, radioactive moieties, and the like. Suitable detectors include photometers, films, spectrometers, and the like.

“Non-naturally occurring” with respect to a polymer or water-soluble polymer segment means a polymer that in its entirety is not found in nature. A non-naturally occurring polymer or water-soluble polymer segment may, however, contain one or more subunits or portions of a subunit that are naturally occurring, so long as the overall polymer structure is not found in nature.

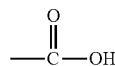
The term “water soluble” as in a “water-soluble polymer segment” and “water-soluble polymer” means any segment or polymer that is soluble in water at room temperature. Typically, a water-soluble polymer or segment will transmit at least about 75%, more preferably at least about 95% of light, transmitted by the same solution after filtering. On a weight basis, a water-soluble polymer or segment thereof will preferably be at least about 35% (by weight) soluble in water, more preferably at least about 50% (by weight) soluble in water, still more preferably about 70% (by weight) soluble in water, and still more preferably about 85% (by weight) soluble in water. It is most preferred, however, that the water-soluble polymer or segment is about 95% (by weight) soluble in water or completely soluble in water.

Molecular weight in the context of a water-soluble polymer of the invention, such as PEG, can be expressed as either a number average molecular weight or a weight average molecular weight. Unless otherwise indicated, all references to molecular weight herein refer to the weight average molecular weight. Both molecular weight determinations, number average and weight average, can be measured using

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gel permeation chromatography or other liquid chromatography techniques. Other methods for measuring molecular weight values can also be used, such as the use of end-group analysis or the measurement of colligative properties (e.g., freezing-point depression, boiling-point elevation, or osmotic pressure) to determine number average molecular weight or the use of light scattering techniques, ultracentrifugation or viscometry to determine weight average molecular weight. The polymers of the invention are typically polydisperse (i.e., number average molecular weight and weight average molecular weight of the polymers are not equal), possessing low polydispersity values of preferably less than about 1.2, more preferably less than about 1.15, still more preferably less than about 1.10, yet still more preferably less than about 1.05, and most preferably less than about 1.03.

As used herein, the term “carboxylic acid” is a moiety having a



functional group [also represented as a “—COOH” or —C(O)OH], as well as moieties that are derivatives of a carboxylic acid, such derivatives including, for example, protected carboxylic acids. Thus, unless the context clearly dictates otherwise, the term carboxylic acid includes not only the acid form, but corresponding esters and protected forms as well. Reference is again made to Greene et al., “PROTECTIVE GROUPS IN ORGANIC SYNTHESIS” 3rd Edition, John Wiley and Sons, Inc., New York, 1999.

The term “reactive” or “activated” when used in conjunction with a particular functional group, refers to a reactive functional group that reacts readily with an electrophile or a nucleophile on another molecule. This is in contrast to those groups that require strong catalysts or highly impractical reaction conditions in order to react (i.e., a “nonreactive” or “inert” group).

The terms “protected” or “protecting group” or “protective group” refer to the presence of a moiety (i.e., the protecting group) that prevents or blocks reaction of a particular chemically reactive functional group in a molecule under certain reaction conditions. The protecting group will vary depending upon the type of chemically reactive group being protected as well as the reaction conditions to be employed and the presence of additional reactive or protecting groups in the molecule, if any. Protecting groups known in the art can be found in Greene et al., *supra*.

As used herein, the term “functional group” or any synonym thereof is meant to encompass protected forms thereof.

The term “spacer” or “spacer moiety” is used herein to refer to an atom or a collection of atoms optionally used to link one moiety to another, such as a water-soluble polymer segment to a functional group such as an acetal or ketal. The spacer moieties of the invention may be hydrolytically stable or may include a physiologically hydrolyzable or enzymatically degradable linkage.

“Alkyl” refers to a hydrocarbon chain, typically ranging from about 1 to 20 atoms in length. Such hydrocarbon chains are preferably but not necessarily saturated and may be branched or straight chain, although typically straight chain is preferred. Exemplary alkyl groups include ethyl, propyl, butyl, pentyl, 1-methylbutyl, 1-ethylpropyl, 3-methylpentyl, and the like. As used herein, “alkyl” includes cycloalkyl when three or more carbon atoms are referenced.

"Lower alkyl" refers to an alkyl group containing from 1 to 6 carbon atoms, and may be straight chain or branched, as exemplified by methyl, ethyl, n-butyl, iso-butyl, and tert-butyl.

"Cycloalkyl" refers to a saturated or unsaturated cyclic hydrocarbon chain, including bridged, fused, or spiro cyclic compounds, preferably made up of 3 to about 12 carbon atoms, more preferably 3 to about 8.

"Non-interfering substituents" are those groups that, when present in a molecule, are typically non-reactive with other functional groups contained within the molecule.

The term "substituted" as in, for example, "substituted alkyl," refers to a moiety (e.g., an alkyl group) substituted with one or more non-interfering substituents, such as, but not limited to: C₃-C₈ cycloalkyl, e.g., cyclopropyl, cyclobutyl, and the like; halo, e.g., fluoro, chloro, bromo, and iodo; cyano; alkoxy, lower phenyl (e.g., 0-2 substituted phenyl); substituted phenyl; and the like. "Substituted aryl" is aryl having one or more non-interfering groups as a substituent. For substitutions on a phenyl ring, the substituents may be in any orientation (i.e., ortho, meta, or para).

"Alkoxy" refers to an —O—R group, wherein R is alkyl or substituted alkyl, preferably C₁-C₂₀ alkyl (e.g., methoxy, ethoxy, propoxy, benzyl, etc.), preferably C₁-C₇.

As used herein, "alkenyl" refers to a branched or unbranched hydrocarbon group of 1 to 15 atoms in length, containing at least one double bond, such as ethenyl, n-propenyl, isopropenyl, n-butenyl, isobutenyl, octenyl, decenyl, tetradecenyl, and the like.

The term "alkynyl" as used herein refers to a branched or unbranched hydrocarbon group of 2 to 15 atoms in length, containing at least one triple bond, e.g., ethynyl, n-butylnyl, isopentylnyl, octynyl, decynyl, and so forth.

"Aryl" means one or more aromatic rings, each of 5 or 6 core carbon atoms. Aryl includes multiple aryl rings that may be fused, as in naphthyl or unfused, as in biphenyl. Aryl rings may also be fused or unfused with one or more cyclic hydrocarbon, heteroaryl, or heterocyclic rings. As used herein, "aryl" includes heteroaryl.

"Heteroaryl" is an aryl group containing from one to four heteroatoms, preferably N, O, or S, or a combination thereof. Heteroaryl rings may also be fused with one or more cyclic hydrocarbon, heterocyclic, aryl, or heteroaryl rings.

"Heterocycle" or "heterocyclic" means one or more rings of 5-12 atoms, preferably 5-7 atoms, with or without unsaturation or aromatic character and having at least one ring atom which is not a carbon. Preferred heteroatoms include sulfur, oxygen, and nitrogen.

"Substituted heteroaryl" is heteroaryl having one or more non-interfering groups as substituents.

"Substituted heterocycle" is a heterocycle having one or more side chains formed from non-interfering substituents.

"Electrophile" refers to an ion or atom or collection of atoms, that may be ionic, having an electrophilic center, i.e., a center that is electron seeking, capable of reacting with a nucleophile.

"Nucleophile" refers to an ion or atom or collection of atoms that may be ionic having a nucleophilic center, i.e., a center that is seeking an electrophilic center or with an electrophile.

A "physiologically cleavable" or "hydrolyzable" or "degradable" bond is a relatively weak bond that reacts with water (i.e., is hydrolyzed) under physiological conditions. The tendency of a bond to hydrolyze in water will depend not only on the general type of linkage connecting two central atoms but also on the substituents attached to these central atoms. Appropriate hydrolytically unstable or weak linkages

include, but are not limited to, carboxylate ester, phosphate ester, anhydrides, acetals, ketals, acyloxyalkyl ether, imines, ortho esters, peptides and oligonucleotides.

An "enzymatically degradable linkage" means a linkage that is subject to degradation by one or more enzymes.

A "hydrolytically stable" linkage or bond refers to a chemical bond, typically a covalent bond, that is substantially stable in water, that is to say, does not undergo hydrolysis under physiological conditions to any appreciable extent over an extended period of time. Examples of hydrolytically stable linkages include but are not limited to the following: carbon-carbon bonds (e.g., in aliphatic chains), ethers, amides, urethanes, and the like. Generally, a hydrolytically stable linkage is one that exhibits a rate of hydrolysis of less than about 1-2% per day under physiological conditions. Hydrolysis rates of representative chemical bonds can be found in most standard chemistry textbooks.

The terms "active agent," "biologically active agent" and "pharmacologically active agent" are used interchangeably herein and are defined to include any agent, drug, compound, composition of matter or mixture that provides some pharmacologic, often beneficial, effect that can be demonstrated in-vivo or in vitro. This includes foods, food supplements, nutrients, nutraceuticals, drugs, proteins, vaccines, antibodies, vitamins, and other beneficial agents. As used herein, these terms further include any physiologically or pharmacologically active substance that produces a localized or systemic effect in a patient.

"Pharmaceutically acceptable excipient" or "pharmaceutically acceptable carrier" refers to an excipient that can be included in the compositions of the invention and that causes no significant adverse toxicological effects to the patient.

"Pharmacologically effective amount," "physiologically effective amount," and "therapeutically effective amount" are used interchangeably herein to mean the amount of a polymer-active agent conjugate—typically present in a pharmaceutical preparation—that is needed to provide a desired level of active agent and/or conjugate in the bloodstream or in a target tissue. The exact amount will depend upon numerous factors, e.g., the particular active agent, the components and physical characteristics of the pharmaceutical preparation, intended patient population, patient considerations, and the like, and can readily be determined by one of ordinary skill in the art, based upon the information provided herein and available in the relevant literature.

"Multifunctional" in the context of a polymer of the invention means a polymer having 3 or more functional groups contained therein, where the functional groups may be the same or different. Multifunctional polymers of the invention will typically contain from about 3-100 functional groups, e.g., from 3-50 functional groups, from 3-25 functional groups, from 3-15 functional groups, from 3 to 10 functional groups (i.e., 3, 4, 5, 6, 7, 8, 9 or 10 functional groups within the polymer. A "difunctional" polymer means a polymer having two functional groups contained therein, either the same (i.e., homodifunctional) or different (i.e., heterodifunctional).

"Branched," in reference to the geometry or overall structure of a polymer, refers to a polymer having 2 or more polymer "arms." A branched polymer may possess 2 polymer arms, 3 polymer arms, 4 polymer arms, 6 polymer arms, 8 polymer arms or more. One particular type of highly branched polymer is a dendritic polymer or dendrimer, which, for the purposes of the invention, is considered to possess a structure distinct from that of a branched polymer.

A "dendrimer" or dendritic polymer is a globular, size monodisperse polymer in which all bonds emerge radially from a central focal point or core with a regular branching

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pattern and with repeat units that each contribute a branch point. Dendrimers exhibit certain dendritic state properties such as core encapsulation, making them unique from other types of polymers.

A basic or acidic reactant described herein includes neutral, charged, and any corresponding salt forms thereof.

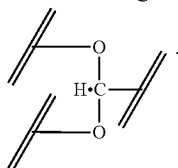
The term "patient," refers to a living organism suffering from or prone to a condition that can be prevented or treated by administration of a conjugate as provided herein, and includes both humans and animals.

"Optional" and "optionally" mean that the subsequently described circumstance may or may not occur, so that the description includes instances where the circumstance occurs and instances where it does not.

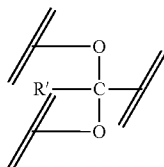
As used herein, the "halo" designator (e.g., fluoro, chloro, iodo, bromo, and so forth) is generally used when the halogen is attached to a molecule, while the suffix "ide" (e.g., fluoride, chloride, iodide, bromide, and so forth) is used when the ionic form is used when the halogen exists in its independent ionic form (e.g., such as when a leaving group leaves a molecule).

In the context of the present discussion, it should be recognized that the definition of a variable provided with respect to one structure or formula is applicable to the same variable repeated in a different structure, unless the context dictates otherwise. Thus, for example, the definition of "POLY," "a spacer moiety," "(z)," and so forth with respect to a polymer can be equally applicable to a water-soluble polymer conjugate provided herein.

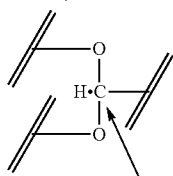
Turning to the first embodiment of the invention, then a polymer is provided comprising, among other things, an acetal or ketal moiety. As used herein, an acetal moiety comprises the following structure:



Moreover, the structure of a ketal moiety for purposes of the present disclosure comprises:



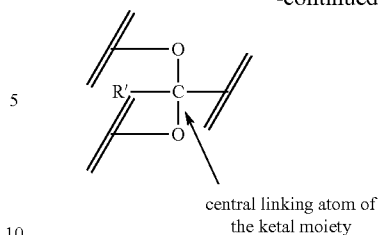
wherein R' is an organic radical. Both the acetal and ketal moieties comprise as central linking carbon that is covalently bonded to two oxygen atoms. The central carbon atom for each moiety is identified below.



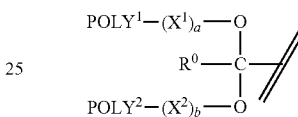
central linking atom of
the acetal moiety

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-continued



In addition to an acetal or ketal moiety, the polymer of the invention also comprises a first water-soluble polymer segment and a second water-soluble polymer segment. Each water-soluble polymer segment is attached, either directly or through a spacer moiety, to one of two oxygen atoms attached to the linking carbon atom in the acetal or ketal moiety, one water-soluble polymer segment per oxygen atom. Thus, the first and second water-soluble polymer segments are attached to the acetal and ketal moiety as shown below.



wherein:

- POLY¹ is a first water-soluble polymer segment;
- POLY² is a second water-soluble polymer segment;
- each of (a) and (b) is independently either zero or one;
- X¹, when present, is a first spacer moiety;
- X², when present, is a second spacer moiety; and
- when the polymer comprises an acetal moiety, R⁰ is H, and when the polymer comprises a ketal moiety, R⁰ is an organic radical.

Each of the first and second water-soluble polymer segments can comprise any polymer so long as the polymer is water-soluble. Moreover, a water-soluble polymer segment as used herein is typically non-peptidic. Although preferably a poly(ethylene glycol), a water-soluble polymer segment for use herein can be, for example, other water-soluble polymers such as other poly(alkylene glycols), such as poly(propylene glycol) ("PPG"), copolymers of ethylene glycol and propylene glycol and the like, poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxyalkylmethacrylamide), poly(hydroxyalkylmethacrylate), poly(saccharides), poly(α-hydroxy acid), polyvinyl alcohol, polyphosphazene, polyoxazoline, poly(N-acryloylmorpholine), such as described in U.S. Pat. No. 5,629,384. The polymer segments can be homopolymers, copolymers, terpolymers, nonrandom block, and random block polymers of any of the foregoing. In addition, a water-soluble polymer segment is often linear, but can be in other forms (e.g., branched, forked, and the like), as will be described in further detail below. In the context of being present within an overall structure, a water-soluble polymer segment has from 1 to about 300 termini.

Each water-soluble polymer segment in the overall structure can be the same or different. It is preferred, however, that all water-soluble polymer segments in the overall structure are of the same type. For example, it is preferred that all water-soluble polymer segments within a given structure are each a poly(ethylene glycol).

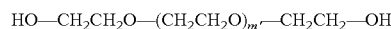
Although the weight average molecular weight of any individual water-soluble polymer segment can vary, the weight average molecular weight will typically be in one or more of

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the following ranges: about 100 Daltons to about 100,000 Daltons; from about 500 Daltons to about 80,000 Daltons; from about 1,000 Daltons to about 50,000 Daltons; from about 2,000 Daltons to about 25,000 Daltons; from about 5,000 Daltons to about 20,000 Daltons. Exemplary weight average molecular weights for the water-soluble polymer segment include about 1,000 Daltons, about 5,000 Daltons, about 10,000 Daltons, about 15,000 Daltons, about 20,000 Daltons, about 25,000 Daltons, and about 30,000 Daltons.

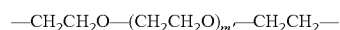
Each water-soluble polymer segment is typically biocompatible and non-immunogenic. With respect to biocompatibility, a substance is considered biocompatible if the beneficial effects associated with use of the substance alone or with another substance (e.g., an active agent) in connection with living tissues (e.g., administration to a patient) outweighs any deleterious effects as evaluated by a clinician, e.g., a physician. With respect to non-immunogenicity, a substance is considered non-immunogenic if use of the substance alone or with another substance in connection with living tissues does not produce an immune response (e.g., the formation of antibodies) or, if an immune response is produced, that such a response is not deemed clinically significant or important as evaluated by a clinician. It is particularly preferred that the polymers and water-soluble polymer segments, described herein as well as conjugates of active agents and the polymers are biocompatible and non-immunogenic.

In one form useful in the present invention, free or non-bound PEG is a linear polymer terminated at each end with hydroxyl groups:



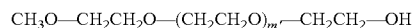
wherein (m') typically ranges from zero to about 4,000, preferably from about 20 to about 1,000.

The above polymer, alpha-, omega-dihydroxypoly(ethylene glycol), can be represented in brief form as HO-PEG-OH where it is understood that the -PEG- symbol can represent the following structural unit:



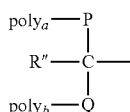
where (m') is as defined as above.

Another type of free or nonbound PEG useful in the present invention is methoxy-PEG-OH, or mPEG in brief, in which one terminus is the relatively inert methoxy group, while the other terminus is a hydroxyl group. The structure of mPEG is given below.



where (m') is as described above.

Multi-armed or branched PEG molecules, such as those described in U.S. Pat. No. 5,932,462, can also be used as the PEG polymer. For example, PEG can have the structure:



wherein:

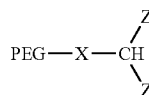
poly_a and poly_b are PEG backbones (either the same or different), such as methoxy poly(ethylene glycol);

R'' is a nonreactive moiety, such as H, methyl or a PEG backbone; and

P and Q are nonreactive linkages. In a preferred embodiment, the branched PEG polymer is methoxy poly(ethylene glycol) disubstituted lysine.

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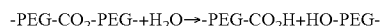
In addition, the PEG can comprise a forked PEG. An example of a free or nonbound forked PEG is represented by the following structure:



wherein: X is a spacer moiety and each Z is an activated terminal group linked to CH by a chain of atoms of defined length. International Application No. PCT/US99/05333, discloses various forked PEG structures capable of use in the present invention. The chain of atoms linking the Z functional groups to the branching carbon atom serve as a tethering group and may comprise, for example, alkyl chains, ether chains, ester chains, amide chains and combinations thereof.

The PEG polymer may comprise a pendant PEG molecule having reactive groups, such as carboxyl, covalently attached along the length of the PEG rather than at the end of the PEG chain. The pendant reactive groups can be attached to the PEG directly or through a spacer moiety, such as an alkylene group.

In addition to the above-described forms of PEG, the polymer can also be prepared with one or more weak or degradable linkages in the polymer, including any of the above described polymers. For example, PEG can be prepared with ester linkages in the polymer that are subject to hydrolysis. As shown below, this hydrolysis results in cleavage of the polymer into fragments of lower molecular weight:



Other hydrolytically degradable linkages, useful as a degradable linkage within a polymer backbone, include carbonate linkages; imine linkages resulting, for example, from reaction of an amine and an aldehyde (see, e.g., Ouchi et al. (1997) *Polymer Preprints* 38(1):582-3); phosphate ester linkages formed, for example, by reacting an alcohol with a phosphate group; hydrazone linkages which are typically formed by reaction of a hydrazide and an aldehyde; acetal linkages that are typically formed by reaction between an aldehyde and an alcohol; ortho ester linkages that are, for example, formed by reaction between a formate and an alcohol; amide linkages formed by an amine group, e.g., at an end of a polymer such as PEG, and a carboxyl group of another PEG chain; urethane linkages formed from reaction of, e.g., a PEG with a terminal isocyanate group and a PEG alcohol; peptide linkages formed by an amine group, e.g., at an end of a polymer such as PEG, and a carboxyl group of a peptide; and oligonucleotide linkages formed by, for example, a phosphoramidite group, e.g., at the end of a polymer, and a 5' hydroxyl group of an oligonucleotide.

It is understood by those of ordinary skill in the art that the term poly(ethylene glycol) or PEG represents or includes all the above forms of PEG.

Those of ordinary skill in the art will recognize that the foregoing discussion concerning substantially water-soluble polymers is by no means exhaustive and is merely illustrative, and that all polymeric materials having the qualities described above are contemplated. As used herein, the "term water-soluble polymer" generally refers to an entire molecule, which can comprise functional groups such as hydroxyl groups, thiol groups, ortho ester functionalities and so forth. The term water-soluble polymer segment is generally reserved for use in discussing specific molecular struc-

POLY¹, POLY², (a), (b), X¹ (when present), X² (when present), and R⁰ are as previously defined;

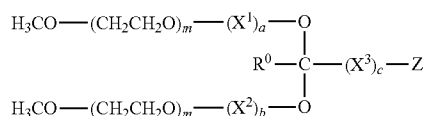
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X^3 , when present, is a third spacer moiety
(c) is either zero or one; and
Z is a reactive group.

R^0 is either H (thereby resulting in an acetal-containing moiety) or an organic radical (thereby resulting in a ketal-containing moiety). Nonlimiting examples of suitable organic radicals include those selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, and substituted aryl. It is particularly, preferred, however, that R^0 is either H or a lower alkyl.

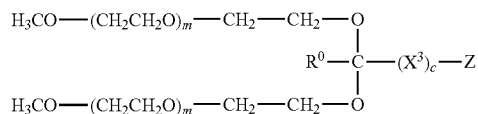
The third spacer moiety, X^3 , when present, can be any atom or series of atoms connecting one part of a molecule to another. Although the third spacer moiety is typically different from the first and/or second spacer moieties (when present), the third spacer moiety can be the same as another spacer moiety present in the polymer. The third spacer moiety can be selected from one of the group of spacer moieties provided above with respect to first and second spacer moieties. The third spacer moiety is absent when (c) is defined as zero and is present when (c) is defined as one. As it is preferred that a third spacer moiety is present in the “nonringed” structures, (c) is preferably defined as one in the above formula.

A preferred structure wherein each of $POLY^1$ and $POLY^2$ is defined as an mPEG comprises:



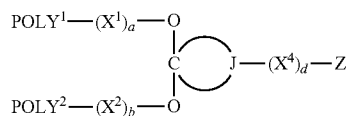
wherein each (a), (b), (c), X^1 (when present), X^2 (when present), X^3 (when present), R^0 , and Z are as previously defined, and m is from 2 to about 4000. PEG versions other than those that are end capped with methoxy (e.g., end capped with a hydroxyl or other alkoxy) are also envisioned.

Further substituting an ethylene for each of X^1 and X^2 and defining each of (a) and (b) as one results in a polymer comprising the following structure:



wherein each of (m), (c), X^3 (when present), R^0 and Z are as previously defined, and m is from 2 to about 4000. Again, PEGs having end capping groups other than methoxy can be used.

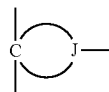
With respect to “ringed” versions then, the polymer will comprise the following structure when the reactive moiety along with a third optional spacer moiety of the polymer are taken into account:



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wherein:

$POLY^1$, $POLY^2$, (a), (b), X^1 (when present), and X^2 (when present) are as previously defined;



represents a ring comprising three to eight atoms, wherein J is N or C(R^2) wherein R^2 is H or an organic radical selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, and substituted aryl;

X^4 , when present, is a fourth spacer moiety

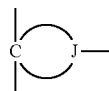
(d) is either zero or one; and

Z is a reactive group.

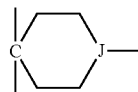
The fourth spacer moiety, X^4 , when present, can be any atom or series of atoms connecting one part of a molecule to another. Although the fourth spacer moiety is typically different from the first, second and/or third spacer moieties (when present), the fourth spacer moiety can be the same as another spacer moiety present in the polymer. The fourth spacer moiety can be selected from one of the group of spacer moieties provided above with respect to first and second spacer moieties. The fourth spacer moiety is absent when (d) is defined as zero and is present when (d) is defined as one. As it is preferred that the fourth spacer moiety is present “ringed” in the structures, (d) is preferably defined as one in the above formula.

The ring optionally comprises one or more branches, wherein an organic radical (e.g., a lower alkyl) is attached to one or more of the atoms comprising the ring. The ring can also be polycyclic in nature. Heterocycles are also envisioned wherein the ringed structure comprises atoms other than carbon, such as nitrogen or oxygen. In addition, the ring can be saturated or unsaturated. For unsaturated versions, the ring can be aromatic or nonaromatic.

The ring comprises from three to eight atoms, inclusive of both the carbon atom and the atom represented by the “J” variable depicted in the

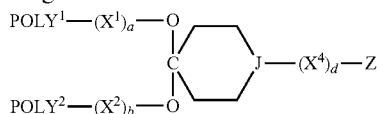


structure. Preferably, the ring is comprised of six atoms exclusive of any atom or atoms that are attached to an atom that forms the ring core. When a polymer of the invention includes a six-membered ring in its structure, the six-membered ring will preferably have the following structure:



wherein J is as previously defined.

Thus, a polymer of the invention that includes a ringed six-membered ringed structure will preferably comprise the following structure:

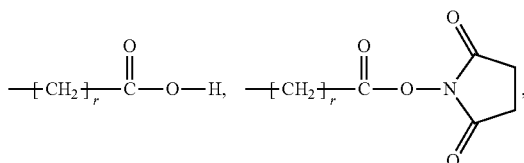


wherein $POLY^1$, $POLY^2$, (a), (b), (d), X^1 (when present), X^2 (when present), X^4 (when present), J and Z are as previously defined.

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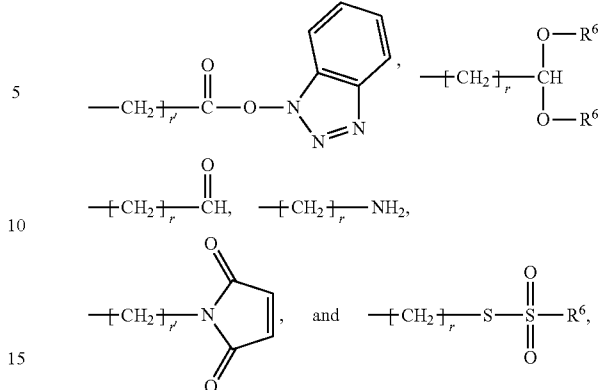
It is particular preferred that J is N or C—H. With respect to a six-membered ring then, a piperidinyl derivative results when J is N, while a cyclohexyl moiety results when J is C—H.

Regardless of whether a ringed structure is present, the polymer will comprise a reactive moiety designated as “Z” throughout the formulae. Preferred reactive moieties are selected from the group of electrophiles and nucleophiles. Specific examples of preferred reactive groups include carboxylic acid, ester, succinimide, and maleimide. Illustrative examples of Q and Z combinations include



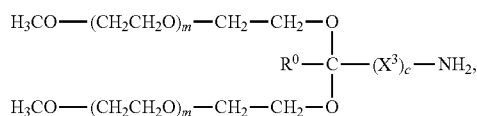
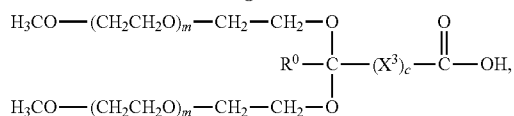
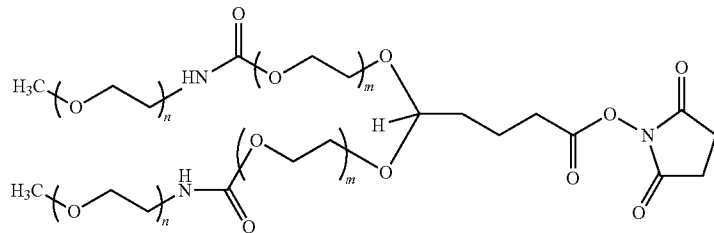
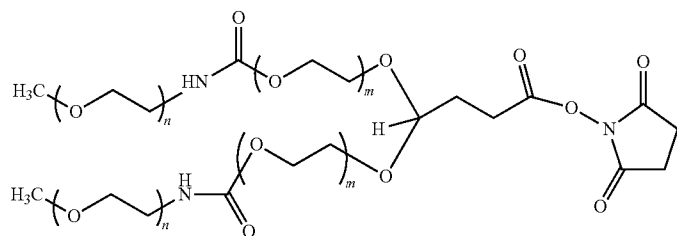
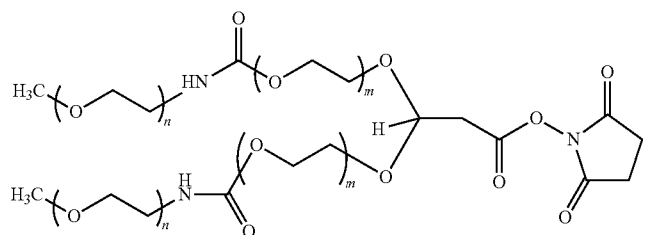
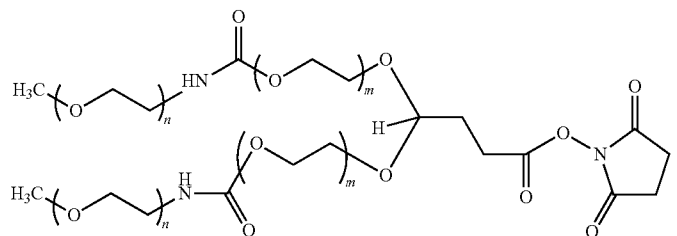
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wherein r is 1-5, r' is 0-5, and R⁶ is aryl or alkyl.

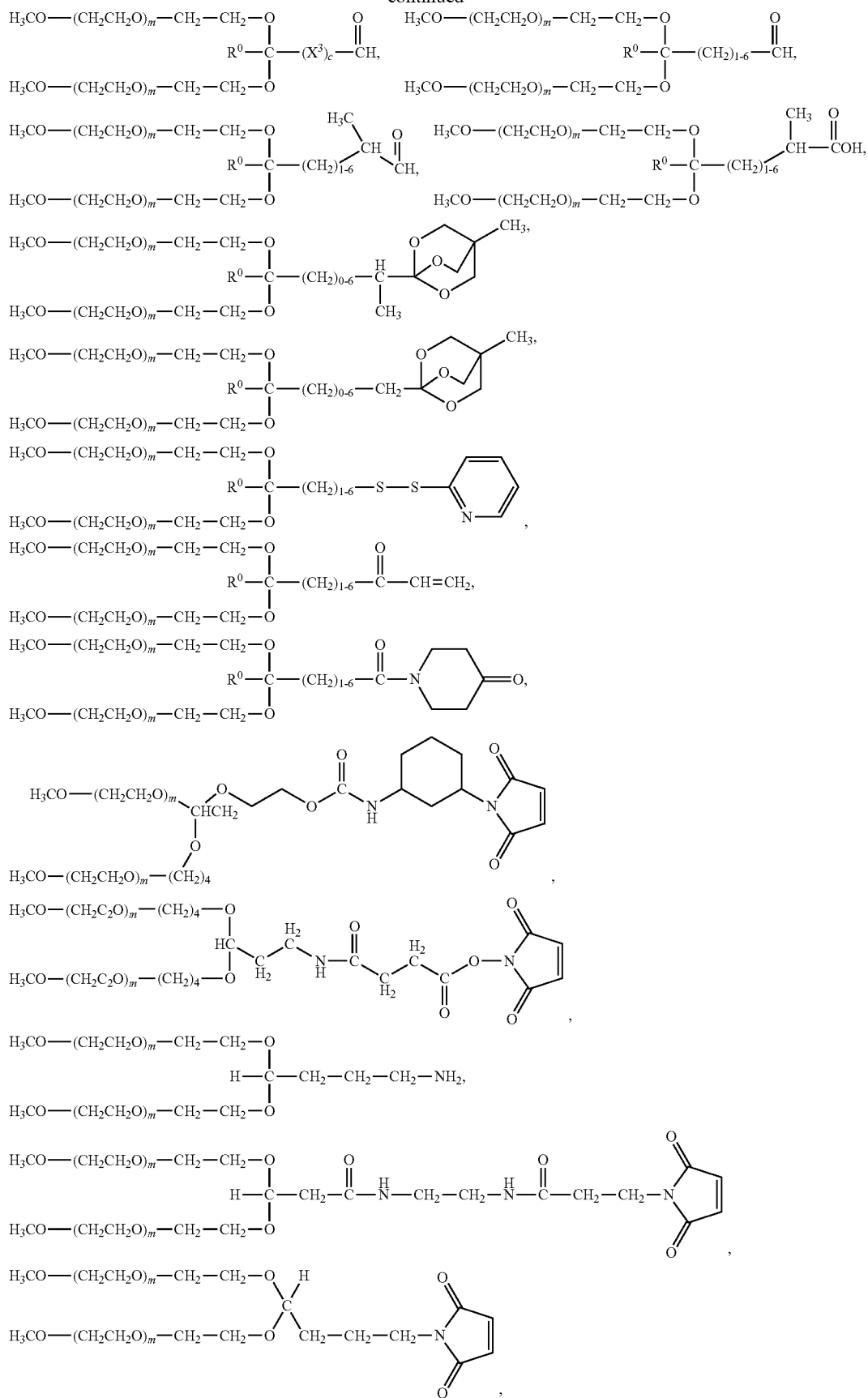
Thus, the polymers of the invention comprise many forms. Exemplary nonringed versions of the polymers of the present invention include the following:

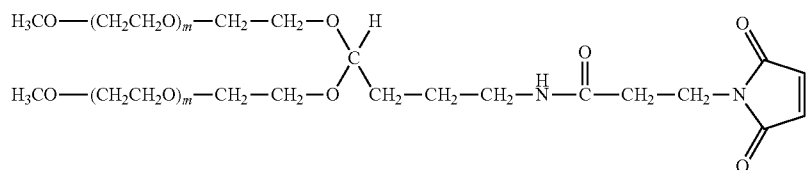


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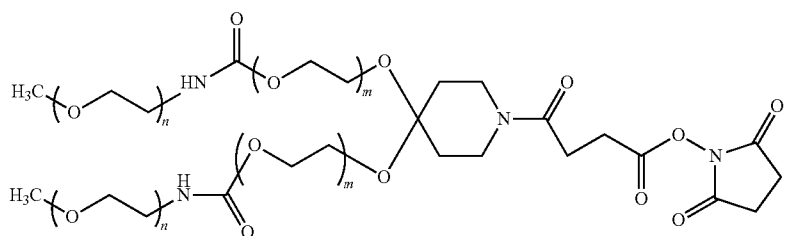
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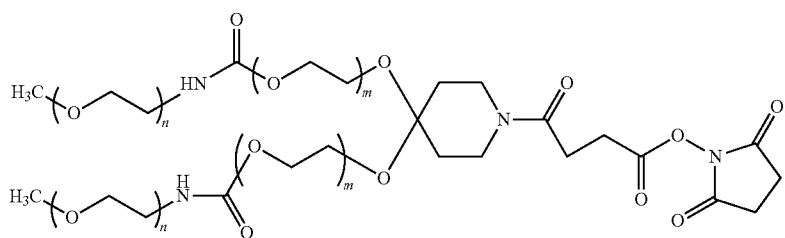
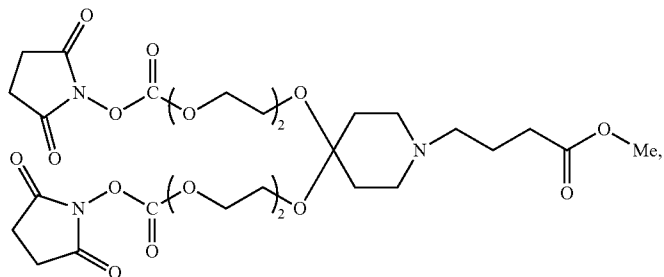
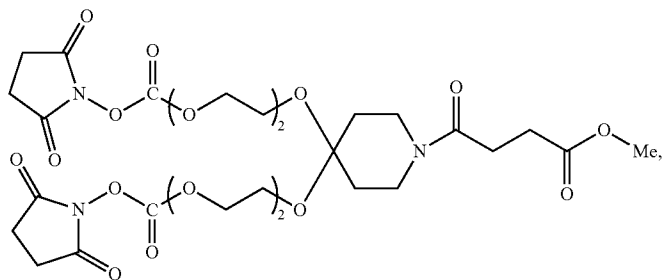
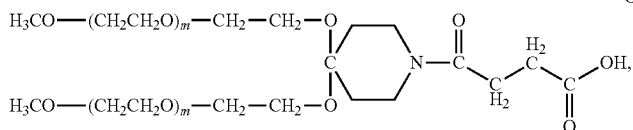
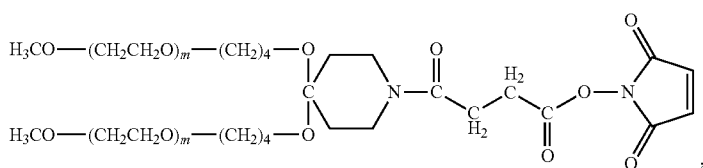
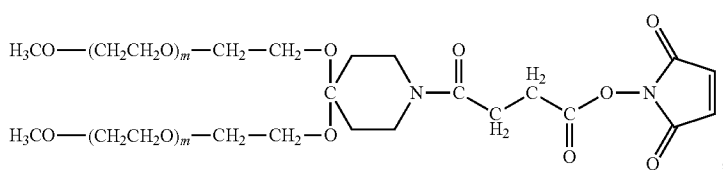
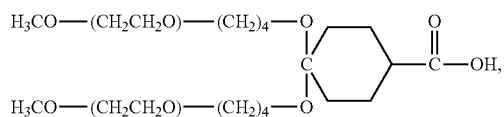
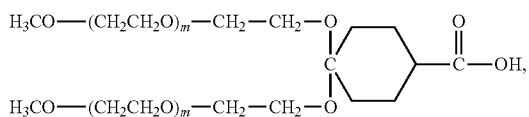
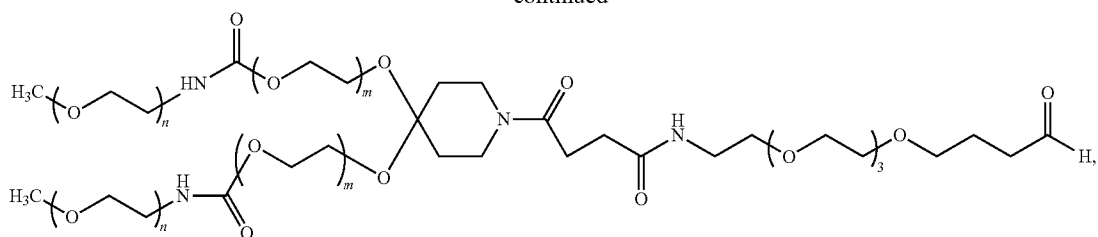
Exemplary ringed versions of the polymers of the present invention include the following:

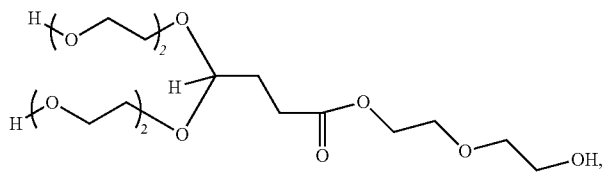
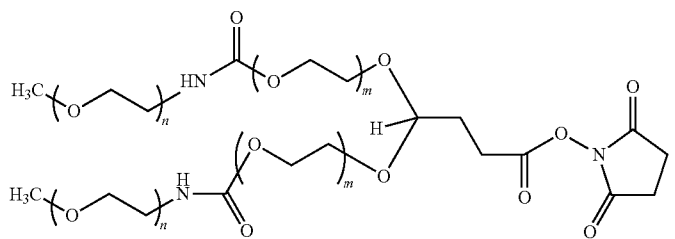
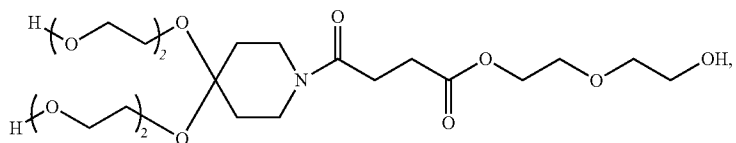
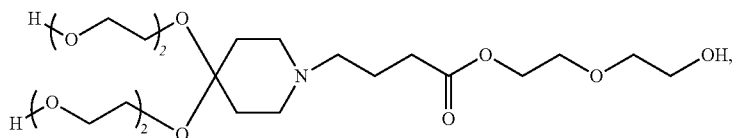
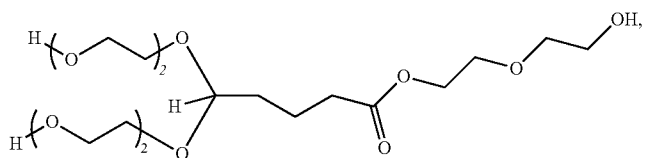
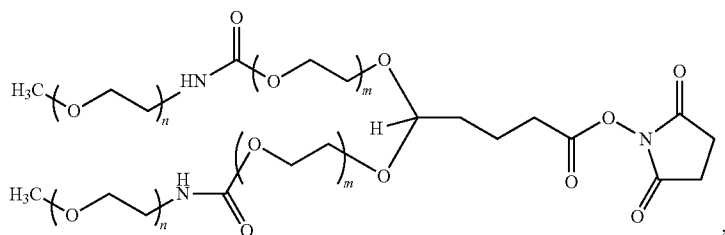
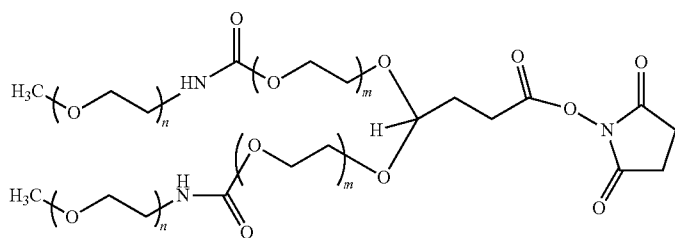


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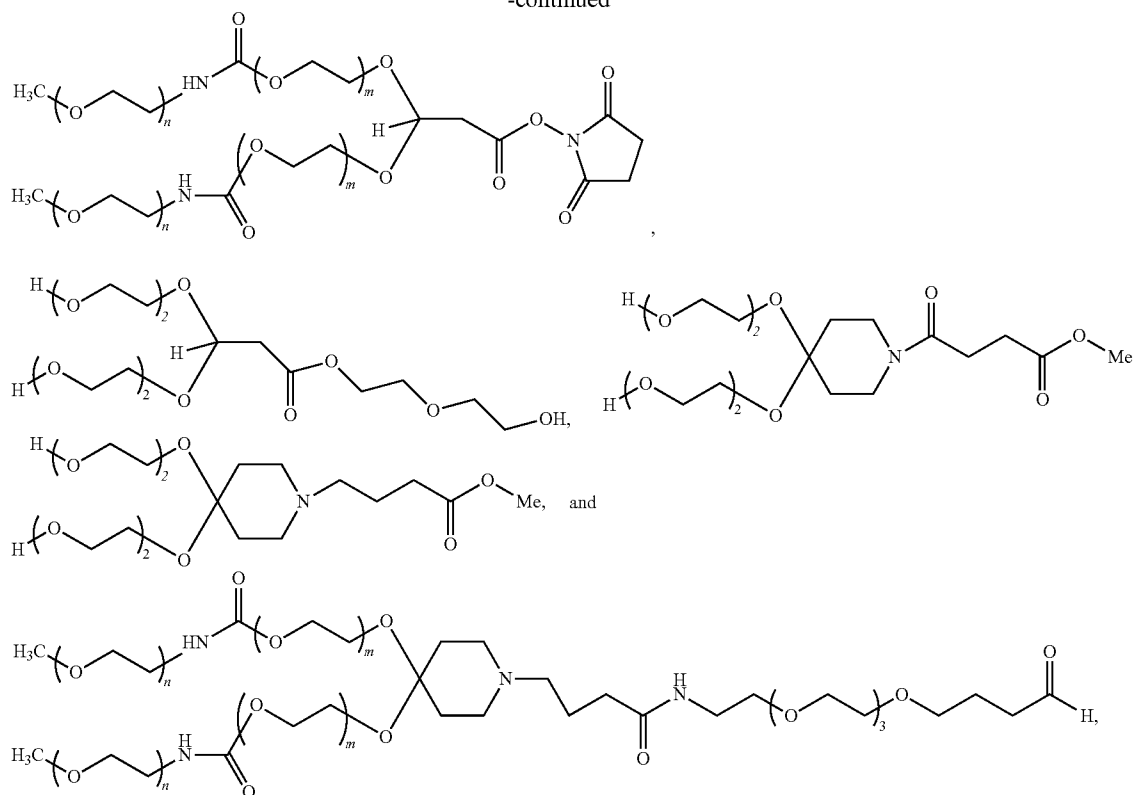




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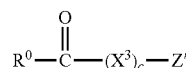


wherein each (m) is as previously defined, each (n) is from 2 to 4000, and Me is methyl (i.e., —CH₃). Furthermore, with respect to the above structures, PEGs having end capping groups other than methoxy can be used and smaller ethylene oxide monomers [e.g., (OCH₂CH₂)₂ and (OCH₂CH₂)₃] can range from one to twenty (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20). The above structures can be used as shown or can serve as useful intermediates in the preparation of more complex polymeric.

The polymers of the invention can be prepared in any number of ways. Consequently, the polymers provided herein are not limited to the specific technique or approach used in their preparation. Preferred approaches, however, will be discussed in detail below.

In one approach for providing the polymers of the invention, a method is provided comprising the steps of (i) adding, under hydrating conditions, an excess of water-soluble polymer segments having at least one terminal hydroxyl group to an aldehyde- or ketone-containing precursor molecule having a reactive group or a protected form thereof to form the corresponding multi-substituted acetal- or ketal-containing moiety, and (ii) removing any water-soluble polymer segments directly attached to the reactive group or the protected form thereof. Optionally, the method further comprises the step of removing the protective group (when present) using techniques known to those of ordinary skill in the art and/or the step of modifying the reactive group to form a different reactive group

For some “nonringed” versions of the polymer, the aldehyde- or ketone-containing precursor molecule having a reactive group of a protected form thereof will comprise the following structure:



wherein:

R⁰ is either H (thereby resulting in an aldehyde-containing precursor molecule) or an organic radical (thereby resulting in a ketone-containing precursor molecule);

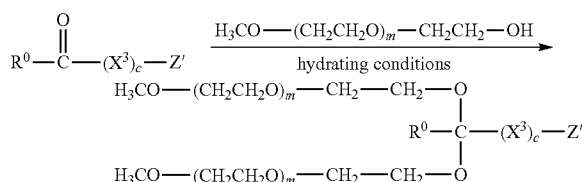
(c) is either zero or one;

(X³), when present, is a third spacer moiety; and

Z' is a reactive group a protected form thereof.

Nonlimiting examples of suitable organic radicals for use with the above structure include those selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, and substituted aryl. It is particularly, preferred, however, that R⁰ is either H or a lower alkyl.

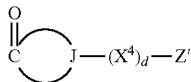
When employing this precursor molecule, a preferred reaction scheme is schematically provided below.



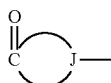
wherein R⁰, X³ (when present), (c), Z' are each as previously defined and (m) is from 2 to about 4000.

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For some “ringed” versions of the polymer, the aldehyde- or ketone-containing precursor molecule having a reactive group of a protected form thereof will comprise the following structure:



wherein:



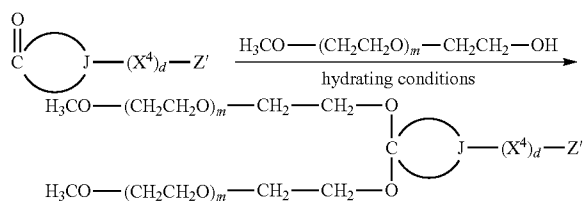
represents a ring comprising three to eight atoms, wherein J is N or C(R²), wherein R² is H or an organic radical selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, and substituted aryl;

(d) is either zero or one;

(X⁴), when present, is a fourth spacer moiety; and

Z' is a reactive group a protected form thereof.

Employing this precursor molecule, a preferred reaction approach is schematically provided below.



wherein X⁴ (when present), (c), Z' are each as previously defined and (m) is from 2 to about 4000.

While not wishing to be bound by theory, the hydrating conditions enable a water molecule to add across the carbonyl group (i.e., C=O) of the aldehyde or ketone in the precursor molecule, thereby forming the corresponding gem-diol (also known as a hydrate) intermediate. While those of ordinary skill in the art will appreciate suitable hydrating conditions for carrying out the method, it is preferred that acid or basic conditions are provided. It is most preferred, however, to provide acidic conditions.

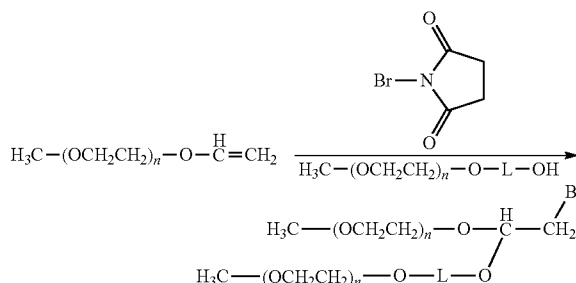
In certain instances, the polymer segment may attach itself to the reactive group (e.g., carboxylic acid) or protected form thereof (the corresponding methyl ester). In any event, the undesired polymer segment is linked to the rest of the molecule via an ester bond. Removal of the undesired polymer is typically carried out by performing a hydrolysis step. It is preferred that a base-promoted hydrolysis step is used. For example, the ester bearing the undesired polymer segment is treated with any aqueous base, such as lithium hydroxide (LiOH), sodium hydroxide (NaOH), potassium hydroxide (KOH), rubidium hydroxide (RuOH), cesium hydroxide [Cs

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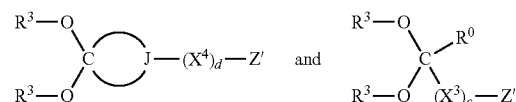
(OH)₂], strontium hydroxide [Sr(OH)₂], barium hydroxide [Ba(OH)₂], ammonium hydroxide (NH₄OH), magnesium hydroxide [Mg(OH)₂], calcium hydroxide [Ca(OH)₂], sodium acetate (NaCH₃CO₂), potassium acetate (KCH₃CO₂), sodium carbonate (Na₂CO₃), potassium carbonate (K₂CO₃), lithium carbonate (Li₂CO₃), sodium phosphate (Na₃PO₄), potassium phosphate (K₃PO₄), sodium borate (Na₃BO₄), potassium borate (Li₃PO₄), and so forth. This treatment removes the undesired polymer and results in carboxylic acid.

Another approach for preparing the polymers of the invention involves the use of a hydroxy-terminated water-soluble polymer (e.g., a PEG alcohol), optionally having a spacer moiety (e.g., a C₂₋₈ linker). A composition comprising hydroxy-terminated water-soluble polymer optionally having a spacer moiety is then combined, under electrophilic addition conditions, with a composition comprising a water-soluble polymer bearing a vinyl ether. Exemplary electrophilic addition conditions include the presence of electrophile (e.g., a halosuccinate such as bromosuccinate). The electrophile (e.g., halo) can be removed and/or further modified using conventional techniques to provide a polymer bearing a functional group of interest.

Schematically, the reaction proceeds as shown below.



In another approach for providing a polymer of the invention, an acetal- or ketal-precursor molecule bearing a reactive group (or protected form thereof) is provided. Exemplary acetal- or ketal-precursor molecule bearing a reactive group are encompassed by the following structures:



wherein R³ is an organic radical (non water-soluble polymeric) and each of R⁰, J, X³, X⁴, (c), (d), and Z' are as previously defined. A preferred reactive group is nitrile (i.e., —C≡N).

The acetal- or ketal-precursor molecule bearing a reactive group (or protected form thereof) is then contacted, under acid aqueous conditions, with an excess of water-soluble polymer, each segment having at least one terminal hydroxyl group to form a bis-substituted intermediate. With respect to the structure provided above, a water-soluble polymer replaces each R³, following this step. Optionally, the reactive group can be modified to form other reactive groups using techniques known to those of ordinary skill in the art. For example, a nitrile can be reduced provide the corresponding amine (e.g., —C≡N to form —CH₂—NH₂). Exemplary

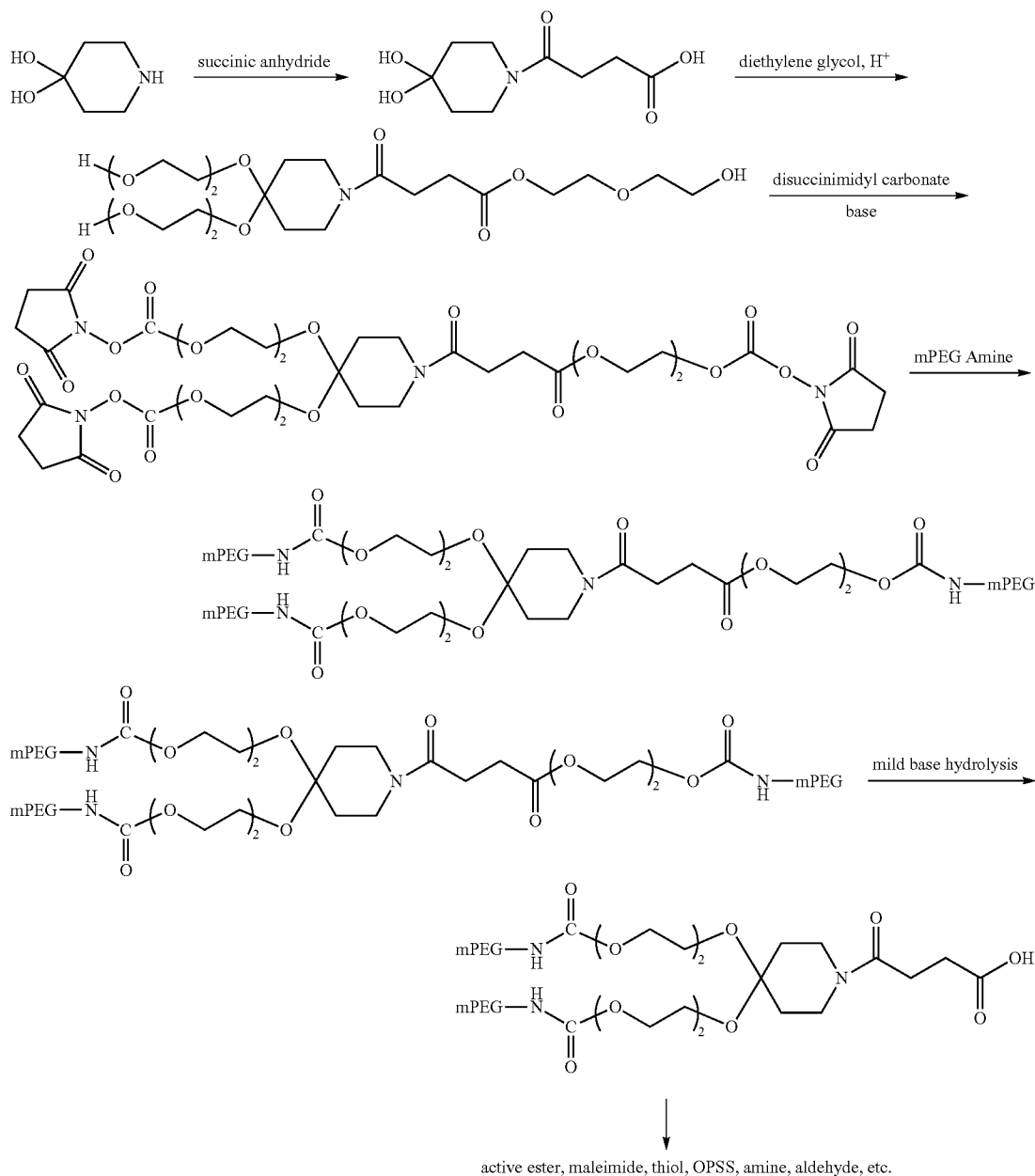
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reducing conditions include exposure to a reducing agent and exposure to a hydrogen atmosphere in the presence of a suitable metal catalyst.

In still another approach for providing a polymer of the invention, a geminal diol bearing a nucleophilic group (e.g., amine) is reacted with an acid anhydride (e.g., succinic anhydride) to form a geminal diol bearing a carboxylic acid. The geminal diol bearing a carboxylic acid is reacted with a glycol [e.g., a poly(ethylene glycol) such as diethylene glycol], typically in an acidic environment, which results in a polyol intermediate. The polyol intermediate, in turn, is reacted with

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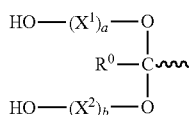
polymers can be selectively cleaved (although not wishing to be bound by theory, due to the different reactivity introduced by the acid anhydride) by hydrolysis using a base to result in a carboxylic acid-terminated polymeric species. The carboxylic acid-terminated polymeric species can optionally be further reacted to form active esters, maleimides, thiols, activated thiols (orthopyridyl disulfide or "OPSS"), amines, aldehydes, and so forth. A schematic of this approach is provided below.



an activated carbonate (e.g., disuccinimidyl carbonate), typically in a basic environment, to form a poly(activated carbonate) intermediate. The poly(activated carbonate) intermediate is then reacted with an amine-terminated polymer (e.g., PEG-amine), wherein a multi-polymeric species results. One of the

In still another approach for providing a polymer of the invention, an ethoxylation reaction. The ethoxylation approach typically involves an alkoxide-containing precursor molecule, often starting in the form of an unionized diol or a moiety having the following structure

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wherein (a), (b), X^1 (when present), X^2 (when present), and R^0 are as previously defined. In the above structure, the linking carbon is, for example: (a) attached to $-(\text{X}^3)_c-\text{Z}$, wherein X^3 , when present, (c) and Z are as previously defined; (b) part of a ringed structure including $\text{J}-(\text{X}^4)_d-\text{Z}$; or (c) a structure that is or can be modified to provide a functional group. These and other molecules suited for use as precursor molecules can be obtained commercially (either as shown above or in their corresponding alcohol forms) and/or may be synthesized using conventional techniques.

If the hydroxyl groups associated with the precursor molecule are not in ionized form (i.e., the hydrogen atom is attached to the hydroxyoxygen), an added step of removing the hydrogen (by, for example, treating the alcohol with a deprotonating base) is required in order to yield alkoxide initiator sites. In addition, thiolates (i.e., $-\text{S}^-$) can be used in the place of alkoxide moieties. In such a case then, the corresponding thiol or dithiol can be placed in a based to provide the necessary ionic site suited for polymerization.

Having provided a precursor molecule with an initiator site (e.g., an anionic site) suited for polymerization, the next step in this approach comprises the step of contacting the initiator site of the precursor molecule with a reactive monomer capable of polymerizing, to thereby initiate polymerization of the reactive monomer onto the precursor molecule. Any reactive monomer can be used to "grow" the polymer chain(s) so long as the resulting polymer chain is water soluble. It is particularly preferred, however, that the reactive monomer is ethylene oxide, thereby providing poly(ethylene oxide) chain(s). These and other polymerization techniques are known to those of ordinary skill in the art and are referenced in, for example, Odian, Chap. 7, Principles of Polymerization, 3rd Ed., McGraw-Hill, 1991.

Once polymerization is initiated, additional reactive monomers are added to the precursor molecule to form one or more polymer chains. The addition of the reactive monomers effec-

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medium will halt the growth of the polymer chain(s). In addition, adding a specific weight or amount of the reactive monomer and allowing the polymerization to proceed until all reactive monomer is exhausted results in a polymer chain having a certain and determinable molecular weight. The result is a polymeric reagent comprising a functional group or protected form thereof.

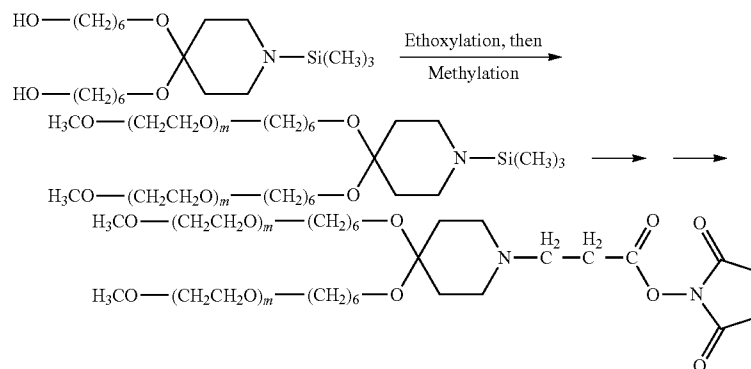
Optionally and prior to carrying out an end-capping step, an electrophilically reactive polymer (either hydrophobic or hydrophilic) can be added to the polymer chain(s).

An added benefit of the ethoxylation route is that it allows for capping the living polymer end (i.e., the terminal of the polymer where additional monomers can be added) with various moieties including other polymers. This allows for the generation of polymers with different properties. Thus, it is possible to cap the already formed water-soluble polymer segment with a hydrophobic polymer and generate a final polymer having hydrophilic and hydrophobic regions. More importantly for many applications, it is possible to add an electrophilically reactive PEG derivative, thereby providing chain extension while, at the same time, adding a hydrolytically cleavable unit. The latter point is important with drug delivery of active agents, such as pharmacologically active proteins, which may have undesirable clearance profiles from the body. A generalized example of this chain extending capping comprises reacting a monosubstituted PEG derivative that gives a carbonate, urethane or similar functional group linkage with the living polymer. Attachment of the polymer, however, must not result in a polymer conjugate that is neither water-soluble nor immunogenic.

Having formed the water-soluble polymer segments in the polymer, an end-capping group optionally can be added using conventional techniques. For example, an alkyl halide (e.g., methyl halide or methyl p-toluenesulfonate) can be reacted with the exposed terminal (the terminal or termini distal to the linking carbon) of the polymer chain. In addition, the one or more polymer chains can be capped with an additional polymer.

Optionally, when the polymer bears a protected form a functional group, the functional group can be removed using art known methods. Again, reference is made to Greene et al. *supra.* for methods of removing other protecting groups.

An ethoxylation-based approach to providing the polymers of the invention is schematically provided below:



tively allows the one or more polymer chains to "grow." Growth of the polymer chain(s) continues until a desired molecular weight is achieved, at which time the reaction is terminated. Termination can occur through any of a number of art known methods. For example, neutralizing the reaction

For any given polymer, the reactive group method advantageously provides the ability to further transform the polymer (either prior or subsequent to any deprotection step) so that it bears a specific reactive group. Thus, using techniques well known in the art, the polymer can be functionalized to

include a reactive group (e.g., active ester, thiol, maleimide, aldehyde, ketone, and so forth).

For example, when the polymer bears a carboxylic acid as the reactive group, the corresponding ester can be formed using conventional techniques. For example, the carboxylic acid can undergo acid-catalyzed condensation with an alcohol, thereby providing the corresponding ester. One approach to accomplish this is to use the method commonly referred to as a Fischer esterification reaction. Other techniques for forming a desired ester are known by those of ordinary skill in the art.

In addition, polymers bearing a carboxylic acid can be modified to form useful reactive groups other than esters. For example, the carboxylic acid can be further derivatized to form acyl halides, acyl pseudohalides, such as acyl cyanide, acyl isocyanate, and acyl azide, neutral salts, such as alkali metal or alkaline-earth metal salts (e.g. calcium, sodium, and barium salts), esters, anhydrides, amides, imides, hydrazides, and the like. In a preferred embodiment, the carboxylic acid is esterified to form an N-succinimidyl ester, o-, m-, or p-nitrophenyl ester, 1-benzotriazolyl ester, imidazolyl ester, or N-sulfosuccinimidyl ester. For example, the carboxylic acid can be converted into the corresponding N-succinimidyl ester by reacting the carboxylic acid with dicyclohexyl carbodiimide (DCC) or diisopropyl carbodiimide (DIC) in the presence of a base.

The steps of the method take place in an appropriate solvent. One of ordinary skill in the art can determine whether any specific solvent is appropriate for any given reaction. Typically, however, the solvent is preferably a nonpolar solvent or a polar aprotic solvent. Nonlimiting examples of nonpolar solvents include benzene, xylene, dioxane, tetrahydrofuran (THF), t-butyl alcohol and toluene. Particularly preferred nonpolar solvents include toluene, xylene, dioxane, tetrahydrofuran, and t-butyl alcohol. Exemplary polar aprotic solvents include, but are not limited to, DMSO (dimethyl sulfoxide), HMPA (hexamethylphosphoramide), DMF (dimethylformamide), DMA (dimethylacetamide), NMP (N-methylpyrrolidinone).

The method of preparing the polymers optionally comprises an additional step of isolating the polymer once it is formed. Known methods can be used to isolate the polymer, but it is particularly preferred to use chromatography, e.g., size exclusion chromatography. Alternately or in addition, the method includes the step of purifying the polymer once it is formed. Again, standard art-known purification methods can be used to purify the polymer.

The polymers of the invention can be stored under an inert atmosphere, such as under argon or under nitrogen. In this way, potentially degradative processes associated with, for example, atmospheric oxygen, are reduced or avoided entirely. In some cases, to avoid oxidative degradation, antioxidants, such as butylated hydroxyl toluene (BHT), can be added to the final product prior to storage. In addition, it is preferred to minimize the amount of moisture associated with the storage conditions to reduce potentially damaging reactions associated with water. Moreover, it is preferred to keep the storage conditions dark in order to prevent certain degradative processes that involve light. Thus, preferred storage conditions include one or more of the following: storage under dry argon or another dry inert gas; storage at temperatures below about -15°C .; storage in the absence of light; and storage with a suitable amount (e.g., about 50 to about 500 parts per million) of an antioxidant such as BHT.

The above-described polymers are useful for conjugation to biologically active agents or surfaces comprising at least one group suitable for reaction with the reactive group on the

polymer. For example, amino groups (e.g., primary amines), hydrazines, hydrazides, and alcohols on an active agent or surface will react with a carboxylic acid group on the polymer. In addition, a more "activated" version of the carboxylic acid of the polymer can be prepared in order to enhance reactivity to the biologically active agent or surface. Methods for activating carboxylic acids are known in the art and include, for example, dissolving the water-soluble polymer bearing a terminal carboxylic acid in methylene chloride and subsequently adding N-hydroxysuccinimide and N,N-dicyclohexylcarbodiimide (DCC) to form an activated N-succinimidyl ester version of the carboxylic acid. Other approaches for activating a carboxylic acid are known to those of ordinary skill in the art.

An active agent-polymer conjugate comprises (i) a first water-soluble polymer segment that is covalently attached, either directly or through one or more atoms, to a first oxygen atom that is covalently attached to a linking carbon atom; (ii) a second water-soluble polymer segment is covalently attached, either directly or through a second spacer moiety, to a second oxygen atom that is covalently attached to the linking carbon atom; and (iii) a pharmacologically active agent that is covalently attached, either directly or through one or more atoms, to the linking carbon.

The invention also provides for a method of preparing a conjugate comprising the step of contacting a polymer of the invention with a pharmacologically active agent under conditions suitable to form a covalent attachment between the polymer and the pharmacologically active agent. Typically, the polymer is added to the active agent or surface at an equimolar amount (with respect to the desired number of groups suitable for reaction with the reactive group) or at a molar excess. For example, the polymer can be added to the target active agent at a molar ratio of about 1:1 (polymer: active agent), 1.5:1, 2:1, 3:1, 4:1, 5:1, 6:1, 8:1, or 10:1. The conjugation reaction is allowed to proceed until substantially no further conjugation occurs, which can generally be determined by monitoring the progress of the reaction over time. Progress of the reaction can be monitored by withdrawing aliquots from the reaction mixture at various time points and analyzing the reaction mixture by SDS-PAGE or MALDI-TOF mass spectrometry or any other suitable analytical method. Once a plateau is reached with respect to the amount of conjugate formed or the amount of unconjugated polymer remaining, the reaction is assumed to be complete. Typically, the conjugation reaction takes anywhere from minutes to several hours (e.g., from 5 minutes to 24 hours or more). The resulting product mixture is preferably, but not necessarily purified, to separate out excess reagents, unconjugated reactants (e.g., active agent) undesired multi-conjugated species, and free or unreacted polymer. The resulting conjugates can then be further characterized using analytical methods such as MALDI, capillary electrophoresis, gel electrophoresis, and/or chromatography.

With respect to polymer-active agent conjugates, the conjugates can be purified to obtain/isolate different conjugated species. Alternatively, and more preferably for lower molecular weight (e.g., less than about 20 kiloDaltons, more preferably less than about 10 kiloDaltons) polymers, the product mixture can be purified to obtain the distribution of water-soluble polymer segments per active agent. For example, the product mixture can be purified to obtain an average of anywhere from one to five PEGs per active agent (e.g., protein), typically an average of about three PEGs per active agent (e.g., protein). The strategy for purification of the final conjugate reaction mixture will depend upon a number of factors, including, for example, the molecular weight of the polymer

employed, the particular active agent, the desired dosing regimen, and the residual activity and in vivo properties of the individual conjugate(s).

If desired, conjugates having different molecular weights can be isolated using gel filtration chromatography. That is to say, gel filtration chromatography is used to fractionate differently numbered polymer-to-active agent ratios (e.g., 1-mer, 2-mer, 3-mer, and so forth, wherein "1-mer" indicates 1 polymer to active agent, "2-mer" indicates two polymers to active agent, and so on) on the basis of their differing molecular weights (where the difference corresponds essentially to the average molecular weight of the water-soluble polymer segments). For example, in an exemplary reaction where a 100 kDa protein is randomly conjugated to a PEG alkanolic acid having a molecular weight of about 20 kDa, the resulting reaction mixture will likely contain unmodified protein (MW 100 kDa), mono-pegylated protein (MW 120 kDa), di-pegylated protein (MW 140 kDa), and so forth. While this approach can be used to separate PEG and other polymer conjugates having different molecular weights, this approach is generally ineffective for separating positional isomers having different polymer attachment sites within the protein. For example, gel filtration chromatography can be used to separate from each other mixtures of PEG 1-mers, 2-mers, 3-mers, and so forth, although each of the recovered PEG-mer compositions may contain PEGs attached to different reactive amino groups (e.g., lysine residues) within the active agent.

Gel filtration columns suitable for carrying out this type of separation include Superdex™ and Sephadex™ columns available from Amersham Biosciences (Piscataway, N.J.). Selection of a particular column will depend upon the desired fractionation range desired. Elution is generally carried out using a suitable buffer, such as phosphate, acetate, or the like. The collected fractions may be analyzed by a number of different methods, for example, (i) optical density (OD) at 280 nm for protein content, (ii) bovine serum albumin (BSA) protein analysis, (iii) iodine testing for PEG content [Sims et al. (1980) *Anal. Biochem.* 107:60-63], and (iv) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), followed by staining with barium iodide.

Separation of positional isomers is carried out by reverse phase chromatography using a reverse phase-high performance liquid chromatography (RP-HPLC) C18 column (Amersham Biosciences or Vydac) or by ion exchange chromatography using an ion exchange column, e.g., a Sepharose™ ion exchange column available from Amersham Biosciences. Either approach can be used to separate polymer-active agent isomers having the same molecular weight (positional isomers).

Following conjugation, and optionally additional separation steps, the conjugate mixture can be concentrated, sterile filtered, and stored at low a temperature, typically from about -20° C. to about -80° C. Alternatively, the conjugate may be lyophilized, either with or without residual buffer and stored as a lyophilized powder. In some instances, it is preferable to exchange a buffer used for conjugation, such as sodium acetate, for a volatile buffer such as ammonium carbonate or ammonium acetate, that can be readily removed during lyophilization, so that the lyophilized powder is absent residual buffer. Alternatively, a buffer exchange step may be used using a formulation buffer, so that the lyophilized conjugate is in a form suitable for reconstitution into a formulation buffer and ultimately for administration to a mammal.

The polymers of the invention can be attached, either covalently or noncovalently, to a number of entities including films, chemical separation and purification surfaces, solid supports, metal surfaces such as gold, titanium, tantalum,

niobium, aluminum, steel, and their oxides, silicon oxide, macromolecules (e.g., proteins, polypeptides, and so forth), and small molecules. Additionally, the polymers can also be used in biochemical sensors, bioelectronic switches, and gates. The polymers can also be employed as carriers for peptide synthesis, for the preparation of polymer-coated surfaces and polymer grafts, to prepare polymer-ligand conjugates for affinity partitioning, to prepare cross-linked or non-cross-linked hydrogels, and to prepare polymer-cofactor adducts for bioreactors.

A biologically active agent for use in coupling to a polymer as presented herein may be any one or more of the following. Suitable agents can be selected from, for example, hypnotics and sedatives, psychic energizers, tranquilizers, respiratory drugs, anticonvulsants, muscle relaxants, antiparkinson agents (dopamine antagonists), analgesics, anti-inflammatories, antianxiety drugs (anxiolytics), appetite suppressants, antimigraine agents, muscle contractants, anti-infectives (antibiotics, antivirals, antifungals, vaccines) antiarthritics, anti-malarials, antiemetics, anepileptics, bronchodilators, cytokines, growth factors, anti-cancer agents, antithrombotic agents, antihypertensives, cardiovascular drugs, antiarrhythmics, antioxidants, anti-asthma agents, hormonal agents including contraceptives, sympathomimetics, diuretics, lipid regulating agents, antiandrogenic agents, antiparasitics, anticoagulants, neoplastics, antineoplastics, hypoglycemics, nutritional agents and supplements, growth supplements, anti-enteritis agents, vaccines, antibodies, diagnostic agents, and contrasting agents.

More particularly, the active agent may fall into one of a number of structural classes, including but not limited to small molecules (preferably insoluble small molecules), peptides, polypeptides, proteins, polysaccharides, steroids, nucleotides, oligonucleotides, polynucleotides, fats, electrolytes, and the like. Preferably, an active agent for coupling to a polymer as described herein possesses a native amino group, or alternatively, is modified to contain at least one reactive amino group suitable for conjugating to a polymer described herein.

Specific examples of active agents suitable for covalent attachment include but are not limited to asparaginase, amdoxovir (DAPD), antide, becaplermin, calcitonins, cyanovirin, denileukin difitox, erythropoietin (EPO), EPO agonists (e.g., peptides from about 10-40 amino acids in length and comprising a particular core sequence as described in WO 96/40749), dornase alpha, erythropoiesis stimulating protein (NESP), coagulation factors such as Factor V, Factor VII, Factor VIIa, Factor VIII, Factor IX, Factor X, Factor XII, Factor XIII, von Willebrand factor; ceredase, cerezyme, alpha-glucosidase, collagen, cyclosporin, alpha defensins, beta defensins, exedin-4, granulocyte colony stimulating factor (GCSF), thrombopoietin (TPO), alpha-1 proteinase inhibitor, elcatonin, granulocyte macrophage colony stimulating factor (GM-CSF), fibrinogen, filgrastim, growth hormones human growth hormone (hGH), growth hormone releasing hormone (GHRH), GRO-beta, GRO-beta antibody, bone morphogenic proteins such as bone morphogenic protein-2, bone morphogenic protein-6, OP-1; acidic fibroblast growth factor, basic fibroblast growth factor, CD-40 ligand, heparin, human serum albumin, low molecular weight heparin (LMWH), interferons such as interferon alpha, interferon beta, interferon gamma, interferon omega, interferon tau, consensus interferon; interleukins and interleukin receptors such as interleukin-1 receptor, interleukin-2, interleukin-2 fusion proteins, interleukin-1 receptor antagonist, interleukin-3, interleukin-4, interleukin-4 receptor, interleukin-6, interleukin-8, interleukin-12, interleukin-13 receptor, inter-

leukin-17 receptor; interleukin 18, interleukin 18 receptor, lactoferrin and lactoferrin fragments, luteinizing hormone releasing hormone (LHRH), insulin, pro-insulin, insulin analogues (e.g., mono-acylated insulin as described in U.S. Pat. No. 5,922,675), amylin, C-peptide, somatostatin, somatostatin analogs including octreotide, vasopressin, follicle stimulating hormone (FSH), influenza vaccine, insulin-like growth factor (IGF), insulintropin, macrophage colony stimulating factor (M-CSF), plasminogen activators such as alteplase, urokinase, reteplase, streptokinase, pamiteplase, lanoteplase, and tenecteplase; nerve growth factor (NGF), osteoprotegerin, platelet-derived growth factor, tissue growth factors, transforming growth factor-1, vascular endothelial growth factor, leukemia inhibiting factor, keratinocyte growth factor (KGF), glial growth factor (GGF), T Cell receptors, CD molecules/antigens, tumor necrosis factor (TNF), monocyte chemoattractant protein-1, endothelial growth factors, parathyroid hormone (PTH), glucagon-like peptide, somatotropin, thymosin alpha 1, rasburicase, thymosin alpha 1 Iib/IIa inhibitor, thymosin beta 10, thymosin beta 9, thymosin beta 4, alpha-1 antitrypsin, phosphodiesterase (PDE) compounds, VLA-4 (very late antigen-4), VLA-4 inhibitors, bisphosphonates, respiratory syncytial virus antibody, cystic fibrosis transmembrane regulator (CFTR) gene, deoxyribonuclease (Dnase), bactericidal/permeability increasing protein (BPI), and anti-CMV antibody. Exemplary monoclonal antibodies include etanercept (a dimeric fusion protein consisting of the extracellular ligand-binding portion of the human 75 kD TNF receptor linked to the Fc portion of IgG1), abciximab, adalimumab, afelimomab, alemtuzumab, antibody to B-lymphocyte (lymphostat-B™), atlizumab, basiliximab, bevacizumab, bciromab, CAT-213 or bertilimumab, CDP-571, CDP-870, cetuximab, clenoliximab, daclizumab, eculizumab, edrecolomab, efalizumab, epratuzumab, fontolizumab, gavilimumab, gemtuzumab ozogamicin, ibritumomab tiuxetan, infliximab, inolimumab, keliximab, labetuzumab, lerdelimumab, radiolabeled lym-1, metelimumab, mepolizumab, mitumomab, muromonad-CD3, nebacumab, natalizumab, odulimumab, omalizumab, oregovomab, palivizumab, pemtumorab, pexelizumab, rituximab satumomab pendetide, sevirumab, sipilizumab, tositumomab and I¹³¹ tositumomab, olizumab, trastuzumab, tuvirumab, and visilizumab.

Additional agents suitable for covalent attachment include, but are not limited to, adefovir, alosectron, amifostine, amiodarone, aminocaproic acid, aminohippurate sodium, aminoglutethimide, aminolevulinic acid, aminosalicic acid, amsacrine, anagrelide, anastrozole, aripiprazole, asparaginase, anthracyclines, bexarotene, bicalutamide, bleomycin, buserelin, busulfan, cabergoline, capecitabine, carboplatin, carmustine, chlorambucin, cilastatin sodium, cisplatin, cladribine, clodronate, cyclophosphamide, cyproterone, cytarabine, camptothecins, 13-cis retinoic acid, all trans retinoic acid; dacarbazine, dactinomycin, daunorubicin, deferoxamine, dexamethasone, diclofenac, diethylstilbestrol, docetaxel, doxorubicin, dutasteride, epirubicin, estramustine, etoposide, exemestane, ezetimibe, fexofenadine, fludarabine, fludrocortisone, fluorouracil, fluoxymesterone, flutamide, fondaparinux, fulvestrant, gamma-hydroxybutyrate, gemcitabine, epinephrine, L-Dopa, hydroxyurea, idarubicin, ifosfamide, imatinib, irinotecan, itraconazole, goserelin, letrozole, leucovorin, levamisole, lisinopril, lovathyroxine sodium, lomustine, mechlorethamine, medroxyprogesterone, megestrol, melphalan, mercaptopurine, metaraminol bitartrate, methotrexate, metoclopramide, mexiletine, mitomycin, mitotane, mitoxantrone, naloxone, nicotine, nilutamide, nitisinone, octreotide, oxaliplatin, pamidronate, pentostatin,

pirarubicin, porfimer, prednisone, procarbazine, prochlorperazine, ondansetron, oxaliplatin, raltitrexed, sirolimus, streptozocin, tacrolimus, pimecrolimus, tamoxifen, tegaserod, temozolomide, teniposide, testosterone, tetrahydrocannabinol, thalidomide, thioguanine, thiotepa, topotecan, treprostinil, tretinoin, valdecoxib, celecoxib, rofecoxib, valrubicin, vinblastine, vincristine, vindesine, vinorelbine, voriconazole, dolasetron, granisetron; formoterol, fluticasone, leuprolide, midazolam, alprazolam, amphotericin B, podophylotoxins, nucleoside antivirals, aroyl hydrazones, sumatriptan; macrolides such as erythromycin, oleandomycin, troleandomycin, roxithromycin, clarithromycin, davericin, azithromycin, flurithromycin, dirithromycin, josamycin, spirocycin, midecamycin, loratadine, desloratadine, leucomycin, miocamycin, rokitamycin, and azithromycin, and swinolide A; fluoroquinolones such as ciprofloxacin, ofloxacin, levofloxacin, trovafloxacin, alatrofloxacin, moxifloxacin, norfloxacin, enoxacin, grepafloxacin, gatifloxacin, lomefloxacin, sparfloxacin, temafloxacin, pefloxacin, amifloxacin, fleroxacin, tosufloxacin, prulifloxacin, irloxacin, pazufloxacin, cinafloxacin, and sitafloxacin; aminoglycosides such as gentamicin, netilmicin, paramecin, tobramycin, amikacin, kanamycin, neomycin, and streptomycin, vancomycin, teicoplanin, rampolanin, mideplanin, colistin, daptomycin, gramicidin, colistimethate; polymyxins such as polymixin B, capreomycin, bacitracin, penems; penicillins including penicillinase-sensitive agents like penicillin G, penicillin V; penicillinase-resistant agents like methicillin, oxacillin, cloxacillin, dicloxacillin, floxacillin, nafcillin; gram negative microorganism active agents like ampicillin, amoxicillin, and hetacillin, cillin, and galampicillin; antipseudomonal penicillins like carbenicillin, ticarcillin, azlocillin, mezlocillin, and piperacillin; cephalosporins like cefpodoxime, cefprozil, ceftibuten, ceftizoxime, ceftioxone, cephalothin, cephradine, cephalixin, cephradine, cefoxitin, cefamandole, cefazolin, cephaloridine, cefaclor, cefadroxil, cephaloglycin, cefuroxime, ceforanide, cefotaxime, cefatrizine, cephacetrile, cefepime, cefixime, cefonicid, cefoperazone, cefotetan, cefinotazole, ceftazidime, loracarbef, and moxalactam, monobactams like aztreonam; and carbapenems such as imipenem, meropenem, and ertapenem, pentamidine isethionate, albuterol sulfate, lidocaine, metaproterenol sulfate, beclomethasone dipropionate, triamcinolone acetamide, budesonide acetamide, fluticasone, ipratropium bromide, flunisolide, cromolyn sodium, and ergotamine tartrate; taxanes such as paclitaxel; SN-38, and typhostines.

Preferred small molecules for coupling to a polymer as described herein are those having at least one naturally occurring amino group. Preferred molecules such as these include aminohippurate sodium, amphotericin B, doxorubicin, aminocaproic acid, aminolevulinic acid, aminosalicic acid, metaraminol bitartrate, pamidronate disodium, daunorubicin, levothyroxine sodium, lisinopril, cilastatin sodium, mexiletine, cephalixin, deferoxamine, and amifostine.

Preferred peptides or proteins for coupling to a polymer as described herein include EPO, IFN- α , IFN- β , consensus IFN, Factor VIII, Factor IX, GCSF, GM-CSF, hGH, insulin, FSH, and PTH.

The above exemplary biologically active agents are meant to encompass, where applicable, analogues, agonists, antagonists, inhibitors, isomers, and pharmaceutically acceptable salt forms thereof. In reference to peptides and proteins, the invention is intended to encompass synthetic, recombinant, native, glycosylated, and non-glycosylated forms, as well as biologically active fragments thereof.

The present invention also includes pharmaceutical preparations comprising a conjugate as provided herein in combi-

nation with a pharmaceutical excipient. Generally, the conjugate itself will be in a solid form (e.g., a precipitate), which can be combined with a suitable pharmaceutical excipient that can be in either solid or liquid form.

Exemplary excipients include, without limitation, those selected from the group consisting of carbohydrates, inorganic salts, antimicrobial agents, antioxidants, surfactants, buffers, acids, bases, and combinations thereof.

A carbohydrate such as a sugar, a derivatized sugar such as an alditol, aldonic acid, an esterified sugar, and/or a sugar polymer may be present as an excipient. Specific carbohydrate excipients include, for example: monosaccharides, such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol, sorbitol (glucitol), pyranosyl sorbitol, myoinositol, and the like.

The excipient can also include an inorganic salt or buffer such as citric acid, sodium chloride, potassium chloride, sodium sulfate, potassium nitrate, sodium phosphate monobasic, sodium phosphate dibasic, and combinations thereof.

The preparation may also include an antimicrobial agent for preventing or deterring microbial growth. Nonlimiting examples of antimicrobial agents suitable for the present invention include benzalkonium chloride, benzethonium chloride, benzyl alcohol, cetylpyridinium chloride, chlorobutanol, phenol, phenylethyl alcohol, phenylmercuric nitrate, thimersol, and combinations thereof.

An antioxidant can be present in the preparation as well. Antioxidants are used to prevent oxidation, thereby preventing the deterioration of the conjugate or other components of the preparation. Suitable antioxidants for use in the present invention include, for example, ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, hypophosphorous acid, monothioglycerol, propyl gallate, sodium bisulfite, sodium formaldehyde sulfoxylate, sodium metabisulfite, and combinations thereof.

A surfactant may be present as an excipient. Exemplary surfactants include: polysorbates, such as "Tween 20" and "Tween 80," and pluronics such as F68 and F88 (both of which are available from BASF, Mount Olive, N.J.); sorbitan esters; lipids, such as phospholipids such as lecithin and other phosphatidylcholines, phosphatidylethanolamines (although preferably not in liposomal form), fatty acids and fatty esters; steroids, such as cholesterol; and chelating agents, such as EDTA, zinc and other such suitable cations.

Acids or bases may be present as an excipient in the preparation. Nonlimiting examples of acids that can be used include those acids selected from the group consisting of hydrochloric acid, acetic acid, phosphoric acid, citric acid, malic acid, lactic acid, formic acid, trichloroacetic acid, nitric acid, perchloric acid, phosphoric acid, sulfuric acid, fumaric acid, and combinations thereof. Examples of suitable bases include, without limitation, bases selected from the group consisting of sodium hydroxide, sodium acetate, ammonium hydroxide, potassium hydroxide, ammonium acetate, potassium acetate, sodium phosphate, potassium phosphate, sodium citrate, sodium formate, sodium sulfate, potassium sulfate, potassium fumarate, and combinations thereof.

The pharmaceutical preparations encompass all types of formulations and in particular those that are suited for injection, e.g., powders that can be reconstituted as well as suspensions and solutions. The amount of the conjugate (i.e., the conjugate formed between the active agent and the polymer

described herein) in the composition will vary depending on a number of factors, but will optimally be a therapeutically effective dose when the composition is stored in a unit dose container (e.g., a vial). In addition, the pharmaceutical preparation can be housed in a syringe. A therapeutically effective dose can be determined experimentally by repeated administration of increasing amounts of the conjugate in order to determine which amount produces a clinically desired endpoint.

The amount of any individual excipient in the composition will vary depending on the activity of the excipient and particular needs of the composition. Typically, the optimal amount of any individual excipient is determined through routine experimentation, i.e., by preparing compositions containing varying amounts of the excipient (ranging from low to high), examining the stability and other parameters, and then determining the range at which optimal performance is attained with no significant adverse effects.

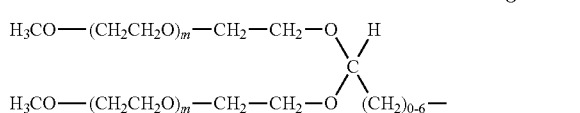
Generally, however, the excipient will be present in the composition in an amount of about 1% to about 99% by weight, preferably from about 5%-98% by weight, more preferably from about 15-95% by weight of the excipient, with concentrations less than 30% by weight most preferred.

These foregoing pharmaceutical excipients along with other excipients are described in "Remington: The Science & Practice of Pharmacy", 19th ed., Williams & Williams, (1995), the "Physician's Desk Reference", 52nd ed., Medical Economics, Montvale, N.J. (1998), and Kibbe, A. H., Handbook of Pharmaceutical Excipients, 3rd Edition, American Pharmaceutical Association, Washington, D.C., 2000.

The pharmaceutical preparations of the present invention are typically, although not necessarily, administered via injection and are therefore generally liquid solutions or suspensions immediately prior to administration. The pharmaceutical preparation can also take other forms such as syrups, creams, ointments, tablets, powders, and the like. Other modes of administration are also included, such as pulmonary, rectal, transdermal, transmucosal, oral, intrathecal, subcutaneous, intra-arterial, and so forth.

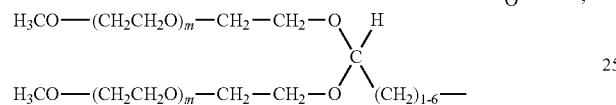
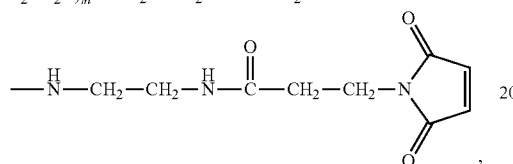
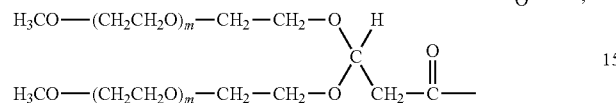
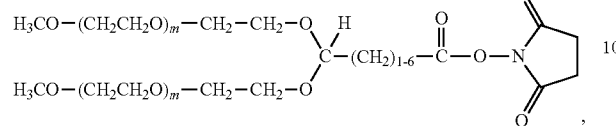
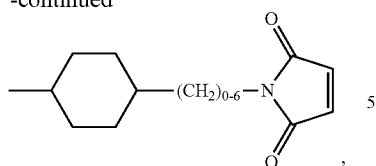
As previously described, the conjugates can be administered injected parenterally by intravenous injection, or less preferably by intramuscular or by subcutaneous injection. Suitable formulation types for parenteral administration include ready-for-injection solutions, dry powders for combination with a solvent prior to use, suspensions ready for injection, dry insoluble compositions for combination with a vehicle prior to use, and emulsions and liquid concentrates for dilution prior to administration, among others.

The invention also provides a method for administering a conjugate as provided herein to a patient suffering from a condition that is responsive to treatment with conjugate. The method comprises administering, generally via injection, a therapeutically effective amount of the conjugate (preferably provided as part of a pharmaceutical preparation). The method of administering may be used to treat any condition that can be remedied or prevented by administration of the particular conjugate. Those of ordinary skill in the art appreciate which conditions a specific conjugate can effectively treat. The actual dose to be administered will vary depending upon the age, weight, and general condition of the subject as well as the severity of the condition being treated, the judgment of the health care professional, and conjugate being administered. Therapeutically effective amounts are known to those skilled in the art and/or are described in the pertinent reference texts and literature. Generally, a therapeutically effective amount will range from about 0.001 mg to 100 mg,

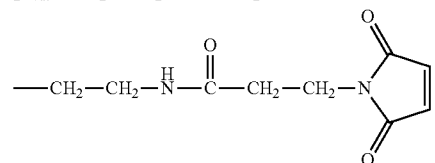
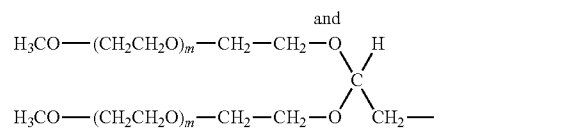
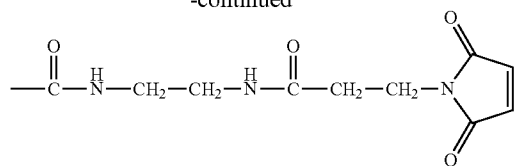


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wherein each (m) is from 2 to 4,000 and R^o is H or an organic radical.

2. The conjugate of claim 1, wherein the pharmacologically active agent is selected from the group consisting of peptides, polypeptides and proteins.

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