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POWER OF ATTORNEY OR REVOCATION OF POWER OF ATTORNEY WITH A NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS

Application Number	To Be Assigned
Filing Date	Concurrently Herewith
First Named Inventor	Michael David BENTLEY
Title	BRANCHED POLYMERS
Art Unit	To Be Assigned
Examiner Name	To Be Assigned
Attorney Docket Number	SHE0058.11

I hereby revoke all previous powers of attorney given in the above-identified application.

☐ A Power of Attorney is submitted herewith.

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OR

☒ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) (Form PTO/SB/96) submitted herewith or filed on _____.

SIGNATURE of Applicant or Assignee of Record

Signature	/Mark A. Wilson/	Date	December 8, 2010
Name	Mark A. Wilson	Telephone	(650) 631-3100
Title and Company	Vice President, Intellectual Property and Assistant Secretary		

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of _____ forms are submitted.

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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PTO/SB/96 (07-09)
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STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Nektar Therapeutics

Application No./Patent No.: To Be Assigned Filed/Issue Date: Concurrently Herewith

Titled: BRANCHED POLYMERS

Nektar Therapeutics , a corporation

(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.

states that it is:

1. [X] the assignee of the entire right, title, and interest in;

2. [ ] an assignee of less than the entire right, title, and interest in (The extent (by percentage) of its ownership interest is _____ %); or

3. [ ] the assignee of an undivided interest in the entirety of (a complete assignment from one of the joint inventors was made)

the patent application/patent identified above, by virtue of either:

A. [ ] An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy therefore is attached.

OR

B. [X] A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Inventors To: Nektar Therapeutics AL, Corporation

The document was recorded in the United States Patent and Trademark Office at
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[ ] Additional documents in the chain of title are listed on a supplemental sheet(s).

[ ] As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (*i.e.*, a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Mark A. Wilson/ December 8, 2010

Signature Date

Mark A. Wilson V.P., I.P. and Asst. Secretary

Printed or Typed Name Title

BRANCHED POLYMERS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation application of U.S. Patent Application Serial No. 11/336,695, filed January 20, 2006, which is a divisional application of U.S. Patent Application Serial No. 10/290,082, filed November 7, 2002, now U.S. Patent No. 7,026,440, which claims the benefit of priority under 35 U.S.C. §119(e) to U.S. Provisional Patent Application Serial No. 60/337,613, filed November 7, 2001, all of which are incorporated herein by reference in their entireties.

FIELD OF THE INVENTION

[0002] This invention relates to branched, reactive water soluble polymers useful for conjugating to biologically active molecules and to methods for making an utilizing such polymers.

BACKGROUND OF THE INVENTION

[0003] Covalent attachment of the hydrophilic polymer poly(ethylene glycol), abbreviated PEG, is a highly advantageous method of increasing water solubility and bioavailability and extending the circulation time of many biologically active molecules, particularly hydrophobic molecules. For example, it has been shown that the water-insoluble drug paclitaxel, when coupled to PEG, becomes water-soluble. Greenwald, *et al.*, *J. Org. Chem.*, 60:331-336 (1995). The total molecular weight of the polymer or polymers attached to the biologically active molecule must be sufficiently high to impart the advantageous characteristics typically associated with PEG polymer attachment, such as increased water solubility and circulating half life, while not adversely impacting the bioactivity of the parent molecule.

[0004] Proteins and other molecules often have a limited number of reactive sites available for polymer attachment. Often, the sites most suitable for modification via polymer attachment play a significant role in receptor binding, and are necessary for retention of the biologically activity of the molecule. As a result, indiscriminate attachment of polymer chains to

such reactive sites on a biologically active molecule often leads to a significant reduction or even total loss of biological activity of the polymer-modified molecule. To form conjugates having sufficient polymer molecular weight for imparting the desired advantages to a target molecule, prior art approaches have typically involved either (i) random attachment of numerous polymer arms to the molecule, thereby increasing the risk of a reduction or even total loss in bioactivity of the parent molecule, or (ii) attachment of one or two very long polymer chains. Unfortunately, the use of very high molecular weight linear polymer chains is problematic because of the difficulty and expense associated with their preparation, purification, and associated instability.

[0005] Branched polymers comprising a plurality of polymer arms attached to a central core and having a single reactive group for conjugation to a biologically active molecule have been described in U.S. Patent Nos. 5,643,575 and 5,932,462. Both patents describe branched polymers formed by covalent attachment of a water-soluble polymer such as an end-capped PEG to a central core molecule bearing amino groups, such as lysine or 1,3-diamino-2-propanol. Although these branched polymers are useful for attaching a high molecular weight polymer to a molecule at a single attachment site without using an extremely long polymer chain, the methods of forming the branched PEG molecules of the prior art is difficult and requires extensive purification of the PEG polymers prior to attachment to the core molecule and also purification/removal of partially pegylated polymer intermediates.

[0006] There remains a need in the art for new branched polymer reagents that provide the benefits associated with branched polymers (i.e., high overall molecular weight in a single non-linear polymer molecule), but are easier to synthesize or provide more flexibility in their design than prior art reagents.

SUMMARY OF THE INVENTION

[0007] The present invention is based upon the development of branched, reactive water-soluble polymers useful for conjugation to biologically active molecules in a manner that tends to avoid a significant reduction in the biological activity of the molecule while still providing the benefits of water-soluble polymer conjugation. The branched polymers of the invention can be readily synthesized from a number of aliphatic core structures that do not require the presence of activating groups suitable for coupling to an activated linear polymer, such as succinimidyl

carbonate end-capped poly(ethylene glycol) or the like, for building the branched water-soluble polymer. That is to say, the preparation of the polymers of the invention is not hampered by the need to utilize core structures having reactive functional groups necessary for coupling with the polymer arms, since the polymer portions of the molecule are generally built directly onto the core by polymerization of suitable monomer units.

[0008] In one aspect, the present invention provides a branched, reactive water-soluble polymer comprising at least two polymer arms, such as poly(ethylene glycol), attached to a central core molecule through heteroatom linkages such as ether linkages. The central core molecule is an aliphatic hydrocarbon having a length of at least three carbon atoms. The branched polymers of the invention are preferably although not necessarily monofunctional (i.e., having one reactive functional group suitable for covalent attachment to a biologically active agent), and the single functional group is preferably attached, optionally via an intervening linkage, to the aliphatic hydrocarbon core molecule.

[0009] Suitable polymers for use in preparing the branched polymer structures of the invention include poly(alkylene glycols), poly(oxyethylated polyol), poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxyalkylmethacrylamide), poly(hydroxyalkylmethacrylate), poly(saccharides), poly(α-hydroxy acid), poly(vinyl alcohol), polyphosphazene, polyoxazoline, poly(N-acryloylmorpholine), poly(acrylic acid), carboxymethyl cellulose, hyaluronic acid, hydroxypropylmethyl cellulose, and copolymers, terpolymers, and mixtures thereof. In one embodiment of the invention, the polymer is a poly(ethylene glycol).

[0010] In another aspect, the invention provides a biologically active conjugate comprising a biologically active molecule, such as a protein, covalently attached to a branched polymer as described above. The biologically active molecule is preferably attached to the branched polymer via a linkage formed by reaction of a reactive functional group on the branched polymer with a suitable functional group on the biologically active molecule.

[0011] In yet another aspect, the invention provides a method of preparing branched reactive polymers comprising poly(alkylene glycol) polymer arms. The method includes polymerization of alkylene oxide monomer units, such as ethylene oxide, onto an aliphatic hydrocarbon core structure bearing at least two nucleophilic groups (e.g., thiol, amino or hydroxyl groups). Preferably, the nucleophilic groups are identical such as in propane substituted with

hydroxyl groups at the 1- and 3-positions (1,3-propanediol) to, for example, favor polymerization rates that are comparable in each of the polymer arms. At least one reactive group suitable for further modification, typically in protected form such as a protected hydroxyl group, is also attached to the aliphatic hydrocarbon core, optionally via an intervening linkage. Following polymerization of the alkylene oxide monomer units onto the core molecule, and optional end-capping of the poly(alkylene glycol) polymer arms, the protecting group of the protected hydroxyl or other functional group is removed to provide a reactive group suitable for further modification, e.g., to form a branched polymer suitable for direct covalent attachment to a biologically active molecule to form a branched polymer conjugate.

DETAILED DESCRIPTION OF THE INVENTION

[0012] The present invention now will be described more fully hereinafter. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

[0013] I. Definitions

[0014] The following terms as used herein have the meanings indicated.

[0015] As used in the specification, and in the appended claims, the singular forms "a", "an", "the", include plural referents unless the context clearly dictates otherwise.

[0016] The terms "functional group", "active moiety", "reactive site", "chemically reactive group" and "chemically reactive moiety" are used in the art and herein to refer to distinct, definable portions or units of a molecule. The terms are somewhat synonymous in the chemical arts and are used herein to indicate the portions of molecules that perform some function or activity and are reactive with other molecules. The term "active," when used in conjunction with a functional group, is intended to include those functional groups that react readily with electrophilic or nucleophilic groups on other molecules, in contrast to those groups that require strong catalysts or highly impractical reaction conditions in order to react (i.e., "non-reactive" or "inert" groups). For example, as would be understood in the art, the term "active ester" would

include those esters that react readily with nucleophilic groups such as amines. Exemplary active esters include N-hydroxysuccinimidyl esters or 1-benzotriazolyl esters. Typically, an active ester will react with an amine in aqueous medium in a matter of minutes, whereas certain esters, such as methyl or ethyl esters, require a strong catalyst in order to react with a nucleophilic group. As used herein, the term "functional group" includes protected functional groups.

[0017] The term "protected functional group" or "protecting group" or "protective group" refers 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, T.W., *et al.*, PROTECTIVE GROUPS IN ORGANIC SYNTHESIS, 3rd ed., John Wiley & Sons, New York, NY (1999).

[0018] The term "linkage" or "linker" (L) is used herein to refer to an atom or a collection of atoms used to link, preferably by one or more covalent bonds, interconnecting moieties such as two polymer segments or a terminus of a polymer and a reactive functional group present on a bioactive agent. A linker of the invention may be hydrolytically stable or may include a physiologically hydrolyzable or enzymatically degradable linkage.

[0019] A "physiologically hydrolyzable" or "hydrolytically degradable" bond is a weak bond that reacts with water (i.e., is hydrolyzed) under physiological conditions. Preferred are bonds that have a hydrolysis half life at pH 8, 25 °C of less than about 30 minutes. 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 degradable linkages include but are not limited to carboxylate ester, phosphate ester, anhydrides, acetals, ketals, acyloxyalkyl ether, imines, orthoesters, peptides and oligonucleotides.

[0020] 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.

[0021] An "enzymatically unstable" or degradable linkage is a linkage that can be degraded by one or more enzymes.

[0022] The term "polymer backbone" refers to the covalently bonded chain of repeating monomer units that form the polymer. The terms polymer and polymer backbone are used herein interchangeably. For example, the polymer backbone of PEG is $-CH_2CH_2O-(CH_2CH_2O)_n-CH_2CH_2$ where n typically ranges from about 2 to about 4000. As would be understood, the polymer backbone may be covalently attached to terminal functional groups or pendant functionalized side chains spaced along the polymer backbone.

[0023] The term "reactive polymer" refers to a polymer bearing at least one reactive functional group.

[0024] Unless otherwise noted, molecular weight is expressed herein as number average molecular weight (M_n), which is defined as $\frac{\sum NiMi}{\sum Ni}$, wherein Ni is the number of polymer molecules (or the number of moles of those molecules) having molecular weight Mi.

[0025] The term "alkyl", "alkenyl", "alkynyl" and "alkylene" refers to hydrocarbon chains typically ranging from about 1 to about 12 carbon atoms in length, preferably 1 to about 6 atoms, and includes straight and branched chains. Unless otherwise noted, the preferred embodiment of any alkyl or alkylene referred to herein is C1-C6alkyl (e.g., methyl or ethyl).

[0026] "Cycloalkyl" refers to a saturated or unsaturated cyclic hydrocarbon chain, including bridged, fused, or spiro cyclic compounds, preferably comprising 3 to about 12 carbon atoms, more preferably 3 to about 8.

[0027] "Aryl" means one or more aromatic rings, each of 5 or 6 core carbon atoms. Multiple aryl rings 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.

[0028] "Heteroaryl" is an aryl group containing from one to four heteroatoms, preferably N, O, or S, or a combination thereof, which heteroaryl group is optionally substituted at carbon or nitrogen atom(s) with C1-6 alkyl, -CF_3, phenyl, benzyl, or thienyl, or a carbon atom in the heteroaryl group together with an oxygen atom form a carbonyl group, or which heteroaryl group is optionally fused with a phenyl ring. Heteroaryl rings may also be fused with one or more cyclic hydrocarbon, heterocyclic, aryl, or heteroaryl rings. Heteroaryl includes, but is not limited to, 5-membered heteroaryls having one hetero atom (e.g., thiophenes, pyrroles, furans); 5-membered heteroaryls having two heteroatoms in 1,2 or 1,3 positions (e.g., oxazoles, pyrazoles, imidazoles, thiazoles, purines); 5-membered heteroaryls having three heteroatoms (e.g., triazoles, thiadiazoles); 5-membered heteroaryls having 3 heteroatoms; 6-membered heteroaryls with one heteroatom (e.g., pyridine, quinoline, isoquinoline, phenanthrine, 5,6-cycloheptenopyridine); 6-membered heteroaryls with two heteroatoms (e.g., pyridazines, cinnolines, phthalazines, pyrazines, pyrimidines, quinazolines); 6-membered heteroaryls with three heteroatoms (e.g., 1,3,5-triazine); and 6-membered heteroaryls with four heteroatoms.

[0029] "Heterocycle" or "heterocyclic" means one or more rings of 5-12 atoms, preferably 5-7 atoms, with or without unsaturation or aromatic character and at least one ring atom which is not carbon. Preferred heteroatoms include sulfur, oxygen, and nitrogen. Multiple rings may be fused, as in quinoline or benzofuran.

[0030] "Heteroatom" means any non-carbon atom in a hydrocarbon analog compound. Examples include oxygen, sulfur, nitrogen, phosphorus, arsenic, silicon, selenium, tellurium, tin, and boron.

[0031] The term "drug", "biologically active molecule", "biologically active moiety" or "biologically active agent", when used herein means any substance which can affect any physical or biochemical properties of a biological organism, including but not limited to viruses, bacteria, fungi, plants, animals, and humans. In particular, as used herein, biologically active molecules include any substance intended for diagnosis, cure, mitigation, treatment, or prevention of disease in humans or other animals, or to otherwise enhance physical or mental well-being of humans or animals. Examples of biologically active molecules include, but are not limited to, peptides, proteins, enzymes, small molecule drugs, dyes, lipids, nucleosides, oligonucleotides, polynucleotides, nucleic acids, cells, viruses, liposomes, microparticles and micelles. Classes of

biologically active agents that are suitable for use with the invention include, but are not limited to, antibiotics, fungicides, anti-viral agents, anti-inflammatory agents, anti-tumor agents, cardiovascular agents, anti-anxiety agents, hormones, growth factors, steroidal agents, and the like.

[0032] "Polyolefinic alcohol" refers to a polymer comprising a polyolefin backbone, such as polyethylene, having multiple pendant hydroxyl groups attached to the polymer backbone. An exemplary polyolefinic alcohol is polyvinyl alcohol.

[0033] As used herein, "non-peptidic" refers to a polymer backbone substantially free of peptide linkages. However, the polymer backbone may include a minor number of peptide linkages spaced along the length of the backbone, such as, for example, no more than about 1 peptide linkage per about 50 monomer units.

[0034] By "residue" is meant the portion of a molecule remaining after reaction with one or more molecules. For example, a biologically active molecule residue in a branched polymer conjugate of the invention is the portion of a biologically active molecule remaining following covalent linkage to a branched polymer of the invention.

[0035] "Oligomer" refers to short monomer chains comprising 2 to about 10 monomer units, preferably 2 to about 5 monomer units.

[0036] The term "conjugate" is intended to refer to the entity formed as a result of covalent attachment of a molecule, e.g., a biologically active molecule, to a reactive polymer molecule, preferably a branched reactive polymer of the invention.

[0037] "Monofunctional" in the context of a polymer of the invention refers to a polymer possessing a single reactive functional group.

[0038] "Bifunctional" in the context of a polymer of the invention refers to a polymer possessing two reactive functional groups which may be the same or different.

[0039] "Multifunctional" in the context of a polymer of the invention means a polymer having 3 or more functional groups attached thereto, where the functional groups may be the same or different. Multifunctional polymers of the invention will typically comprise from about 3-100 functional groups, or from 3-50 functional groups, or from 3-25 functional groups, or from

3-15 functional groups, or from 3 to 10 functional groups, or will contain 3, 4, 5, 6, 7, 8, 9 or 10 functional groups attached to the polymer backbone.

[0040] II. Branched Reactive Polymers

[0041] In one aspect, the present invention provides branched reactive polymers comprising at least two polymer arms, such as PEG arms, attached to a central core through heteroatom linkages such as ether linkages. The central core molecule is an aliphatic hydrocarbon having a carbon chain length of at least three carbon atoms (i.e., propane, butane, pentane, and the like). Since the branched polymers of the invention combine at least two polymer arms in a single molecule, a polymer with sufficient molecular weight to impart beneficial properties to a biologically active molecule, such as increased water solubility, can be formed using shorter, easier to prepare polymer chains. The branched polymers of the invention are preferably monofunctional, meaning the polymer molecule contains only a single reactive site for conjugation to a biologically active molecule. Use of a monofunctional polymer eliminates the possibility of crosslinking with a biologically active molecule, such as a protein, which can lead to loss of activity.

[0042] As described in greater detail below, for branched polymers of the invention comprising poly(alkylene glycol) polymer arms, such as PEG arms, the branched polymers are advantageously synthesized by polymerizing alkylene oxide monomer units, such as ethylene oxide units, directly onto an aliphatic hydrocarbon core molecule substituted with two or more mucleophilic groups (e.g., thiol, amino or hydroxyl groups). In this manner, expensive and time-consuming polymer purification steps associated with prior art methods are avoided.

[0043] Typically, the total number average molecular weight of the branched reactive polymers of the invention will be about 500 to about 100,000 daltons (Da), preferably about 5,000 to about 60,000 Da, most preferably about 8,000 to about 40,000 Da. Each polymer arm of the branched polymer will typically have a molecular weight of about 250 Da to about 50,000 Da, more preferably about 2,500 to about 30,000 Da, and most preferably about 4,000 to about 20,000 Da. Branched polymers having a total number average molecular weight of about 500 Da, about 1,000 Da, about 2,000 Da, about 4,000 Da, about 5,000 Da, about 8,000 Da, about 10,000 Da, about 12,000 Da, about 15,000 Da, about 20,000, about 25,000 Da, and about 30,000 Da are particularly preferred.

[0044] A branched reactive polymer of the invention will typically comprise at least two water-soluble and non-peptidic polymer arms, such as poly(ethylene glycol) arms, covalently attached to an aliphatic hydrocarbon core structure bearing a single functional group. A generalized structure of the branched reactive polymers of the invention is shown below:

$$Y\text{-}(X)p\text{-}R(\text{-}X'\text{-}POLY)_q$$

Formula I

wherein:

R is an aliphatic hydrocarbon having a length of at least three carbon atoms;

each POLY is a water soluble and non-peptidic polymer, such as PEG;

X' is a heteroatom linkage, preferably -NH-, -O-, or -S-;

X is a linker;

p is 0 or 1;

q is 2 to about 10, preferably 2 to about 5 (e.g., 2, 3, 4, or 5); and

Y is a functional group.

[0045] The aliphatic hydrocarbon core, R, preferably comprises 3 to about 12 carbon atoms, more preferably 3 to about 7 carbon atoms, most preferably 3 to about 5 carbon atoms. Core structures of 3, 4, and 5 carbon atoms in length are particularly preferred. The aliphatic hydrocarbon core can be linear or branched and may include one or more heteroatoms in the hydrocarbon chain. In a preferred embodiment, the polymer arms, POLY, and the functional group, Y, are each attached to different carbon atoms of the core molecule. For example, in a three-carbon core embodiment, the POLY polymer arms are preferably attached at the 1- and 3-position and the Y functional group is preferably attached at the 2-position.

[0046] The branched polymers of the invention are preferably symmetrical, meaning the polymer arms are symmetrically located on the central core, R (e.g., at the 1- and 3- position of a three-carbon aliphatic core). A symmetrical arrangement lends itself to the preferential formation of only one polymer product having polymer arms of approximately the same number of subunits, since the initiation of the polymerization process should occur at approximately equal rates in equivalent arm positions extending from a symmetrical core.

[0047] A. Polymer Arms

[0048] In general, the water soluble and non-peptidic polymer portion of the branched polymer structure (i.e., POLY in Formula I above) should be non-toxic and biocompatible, meaning that the polymer is capable of coexistence with living tissues or organisms without causing harm. When referring to a branched reactive polymer, it is to be understood that the polymer can be any of a number of water soluble and non-peptidic polymers, such as those described herein as suitable for use in the present invention. Preferably, POLY as designated in Formula I above is poly(ethylene glycol) (PEG). The term PEG includes poly(ethylene glycol) in any of a number of geometries or forms, including linear forms (e.g., alkoxy PEG or bifunctional PEG), branched or multi-arm forms (e.g., forked PEG or PEG attached to a polyol core), pendant PEG, or PEG with degradable linkages therein, to be more fully described below. Preferred for forming the branched polymers of the invention are linear polymers such as linear forms of PEG.

[0049] In its simplest form, PEG has the formula

$$-CH_2CH_2O-(CH_2CH_2O)_n-CH_2CH_2-$$

Formula II

wherein n is from about 5 to about 1,200, typically from about 50 to about 700.

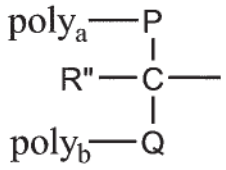
[0050] End-capped polymers, meaning polymers having at least one terminus capped with a relatively inert group (e.g., an alkoxy group), can be used as a polymer of the invention. For example, methoxy-PEG-OH, or mPEG in brief, is a form of PEG wherein one terminus of the polymer is a methoxy group, while the other terminus is a hydroxyl group that is subject to ready chemical modification. The structure of mPEG is given below.

$$CH_3O-(CH_2CH_2O)_n-CH_2CH_2-OH$$

Formula III

wherein n is as described above.

[0051] Multi-armed or branched PEG molecules, such as those described in U.S. Patent No. 5,932,462, which is incorporated by reference herein in its entirety, although less preferred, can also be used as the PEG polymer in the branched reactive polymers of the invention. For example, an exemplary branched PEG polymer can have the structure:



Formula IV

wherein:

$poly_a$ and $poly_b$ are PEG backbones, 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. As would be appreciated by one of skill in the art, use of branched polymers as the POLY polymer arms in the branched reactive polymers of the invention would result in a polymer having multiple branching points within the molecule. Such polymers, if utilized to prepare the branched structures of the invention, are attached to the aliphatic core structures provided herein not by polymerization but by covalent attachment.

[0052] Although less preferred due to its multifunctional nature, the PEG polymer may alternatively comprise a forked PEG. Generally speaking, a polymer having a forked structure is characterized as having a polymer chain attached to two or more active agents via covalent linkages extending from a hydrolytically stable branch point in the polymer. An example of a forked PEG is represented by -PEG-$YCHZ_2$, where Y is a linking group and each Z is an activated terminal group for covalent attachment to a biologically active agent. The Z group is linked to CH by a chain of atoms of defined length. International Application No. PCT/US99/05333, the contents of which are incorporated by reference herein, 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, an alkyl chain, ether linkage, ester linkage, amide linkage, or combinations thereof. In this embodiment of the invention, the resulting branched polymer is multifunctional, i.e., having reactive sites suitable for attachment to a biologically active molecule not only extending from the aliphatic core but also extending from the forked polymer arms(s). As in the above case, such forked polymers, if utilized to prepare the branched structures of the invention, are attached to the

aliphatic core structures provided herein not by polymerization but typically by covalent attachment.

[0053] Again, although less favored due to its multifunctional nature, the PEG polymer may comprise a pendant PEG molecule having reactive groups, such as carboxyl, covalently attached along the length of the PEG backbone rather than at the end of the PEG chain. The pendant reactive groups can be attached to the PEG backbone directly or through a linking moiety, such as an alkylene group.

[0054] In addition to the above-described forms of PEG, the polymer arms (POLY) can also be prepared with one or more weak or degradable linkages in the polymer backbone, including any of the above described polymers. For example, PEG can be prepared with ester linkages in the polymer backbone that are subject to hydrolysis. As shown below, this hydrolysis results in cleavage of the polymer into fragments of lower molecular weight

$$\text{-PEG-CO}_2\text{-PEG-} + \text{H}_2\text{O} \longrightarrow \text{-PEG-CO}_2\text{H} + \text{HO-PEG-}$$

[0055] 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., Polymer Preprints, 38(1):582-3 (1997), which is incorporated herein by reference.); 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; 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.

[0056] In one instance, the polymer arms having one or more hydrolyzable linkages contained therein are prepared in a two-step polymerization process which includes an intermediate step for inclusion of the desired hydrolyzable linkage. That is to say, polymerization of, e.g., ethylene oxide subunits, onto the central core is carried out to a certain desired chain length and the reactive polymer termini extending from the central core are then coupled to short

polymer chains suitably functionalized at one end to react with the hydroxyl groups of the intermediate polymer arms extending from the core to introduce the hydrolyzable linkages(s). Further polymerization of ethylene oxide subunits onto the polymer arms, now containing one or more hydrolyzable linkages, is then carried out to prepare polymer arms of a desired chain length.

[0057] It is understood by those skilled in the art that the term poly(ethylene glycol) or PEG represents or includes all the above forms of PEG.

[0058] Any of a variety of monofunctional, bifunctional or multifunctional polymers that are non-peptidic and water-soluble can also be used to form the branched polymers in accordance with the present invention. The polymer backbone can be linear, or can be in any of the above-described forms (e.g., branched, forked, and the like). Examples of suitable polymers include, but are not limited to, other poly(alkylene glycols), copolymers of ethylene glycol and propylene glycol, poly(olefinic alcohol), poly(vinylpyrrolidone), poly(hydroxyalkylmethacrylamide), poly(hydroxyalkylmethacrylate), poly(saccharides), poly(α-hydroxy acid), poly(acrylic acid), poly(vinyl alcohol), polyphosphazene, polyoxazoline, poly(N-acryloylmorpholine), such as described in U.S. Patent No. 5,629,384, which is incorporated by reference herein in its entirety, and copolymers, terpolymers, and mixtures thereof.

[0059] Typically, the two or more polymer arms of a branched polymer of the invention are the same. That is to say, most preferably, the polymer arms are each a poly(ethylene glycol) or each a polyolefinic alcohol, etc. Generally, not only are the polymer arms composed of the same type of subunits, but they also have identical geometries and similar molecular weights. That is to say, in a most preferred embodiment of the invention, the polymer arms extending from the aliphatic core are identical.

[0060] B. Linker (X)

[0061] The branched polymers of the invention optionally include a linkage (i.e., X in Formula I) that joins a branching carbon of the aliphatic hydrocarbon central core molecule with the functional group, Y. The structure of the X linkage is typically determined by the structure of the aliphatic hydrocarbon core used to form the polymers of the invention and has an overall length of from 1 to about 40 atoms, preferably 1 to about 10 atoms, and most preferably 1 to about 5 atoms. Preferred linkages include heteroatoms such as -O- or -S-, -alkylene-, -O-

alkylene-O-, -alkylene-O-alkylene-, -aryl-O- (e.g., -phenylene-O-), -O-aryl- (e.g., -O-phenylene), $(\text{-O-alkylene-})_m$, and $(\text{-alkylene-O-})_m$, wherein m is 1-10, preferably 1-5 (e.g., 1, 2, 3, 4, or 5). The alkylene groups of the X linkage are preferably C1-C6 alkylene, more preferably C1-C3 alkylene, including methylene and ethylene.

[0062] In some instances, it may be advantageous to have a linker (i.e., X in Formula I) extending the point of covalent attachment of the biologically active agent away from the central aliphatic core. In such particular embodiments of the invention, the terminus for activation and subsequent attachment to an active agent is then at a primary rather than at a secondary carbon position, thereby increasing the ease of subsequent modifications due to the increased reactivity of a primary carbon in nucleophilic displacement reactions.

[0063] C. Functional Group (Y)

[0064] The Y functional group can be any functional group suitable for reaction with a functional group on a biologically active molecule or a functional group that is a precursor thereof. Examples of suitable functional groups include hydroxyl, active ester (e.g., N-hydroxysuccinimidyl ester and 1-benzotriazolyl ester), active carbonate (e.g., N-hydroxysuccinimidyl carbonate, 1-benzotriazolyl carbonate, p-nitrophenyl carbonate), acetal, aldehyde having a carbon length of 1 to 25 carbons (e.g., acetaldehyde, propionaldehyde, and butyraldehyde), aldehyde hydrate, alkenyl, acrylate, methacrylate, acrylamide, active sulfone, amine, hydrazide, thiol, alkanoic acids having a carbon length (including the carbonyl carbon) of 1 to about 25 carbon atoms (e.g., carboxylic acid, carboxymethyl, propanoic acid, and butanoic acid), acid halide, isocyanate, isothiocyanate, maleimide, vinylsulfone, dithiopyridine, vinylpyridine, iodoacetamide, epoxide, glyoxal, dione, mesylate, tosylate, and tresylate.

[0065] Exemplary functional groups are also described in the following references, all of which are incorporated by reference herein: N-succinimidyl carbonate (see e.g., U.S. Patent Nos. 5,281,698, 5,468,478), amine (see, e.g., Buckmann et al. Makromol.Chem. 182:1379 (1981), Zalipsky et al. Eur. Polym. J. 19:1177 (1983)), hydrazide (See, e.g., Andresz et al. Makromol. Chem. 179:301 (1978)), succinimidyl propionate and succinimidyl butanoate (see, e.g., Olson et al. in Poly(ethylene glycol) Chemistry & Biological Applications, pp 170-181, Harris & Zalipsky Eds., ACS, Washington, DC, 1997; see also U.S. Patent No. 5,672,662), succinimidyl succinate (See, e.g., Abuchowski et al. Cancer Biochem. Biophys. 7:175 (1984) and Joppich et al.,

Makromol. Chem. 180:1381 (1979), succinimidyl ester (see, e.g., U.S. Patent No. 4,670,417), benzotriazole carbonate (see, e.g., U.S. Patent No. 5,650,234), glycidyl ether (see, e.g., Pitha et al. Eur. J. Biochem. 94:11 (1979), Elling et al., Biotech. Appl. Biochem. 13:354 (1991), oxycarbonylimidazole (see, e.g., Beauchamp, et al., Anal. Biochem. 131:25 (1983), Tondelli et al. J. Controlled Release 1:251 (1985)), p-nitrophenyl carbonate (see, e.g., Veronese, et al., Appl. Biochem. Biotech., 11:141 (1985); and Sartore et al., Appl. Biochem. Biotech., 27:45 (1991)), aldehyde (see, e.g., Harris et al. J. Polym. Sci. Chem. Ed. 22:341 (1984), U.S. Patent No. 5,824,784, U.S. Patent 5,252,714), maleimide (see, e.g., Goodson et al. Bio/Technology 8:343 (1990), Romani et al. in Chemistry of Peptides and Proteins 2:29 (1984)), and Kogan, Synthetic Comm. 22:2417 (1992)), orthopyridyl-disulfide (see, e.g., Woghiren, et al. Bioconj. Chem. 4:314 (1993)), acrylol (see, e.g., Sawhney et al., Macromolecules, 26:581 (1993)), vinylsulfone (see, e.g., U.S. Patent No. 5,900,461).

[0066] In one embodiment of the invention, the Y functional group is a protected functional group, such as a protected hydroxyl group of formula -O-Gp, wherein Gp is a protecting group. The Gp protecting group can be any of various hydroxyl protecting groups known in the art, such as benzyl or other alkylaryl groups (e.g., groups having the formula $-CH_2-$Ar, wherein Ar is any aryl group), acetal, and dihydropyranyl. Other suitable protecting groups are described in Greene, T.W., *et al.*, PROTECTIVE GROUPS IN ORGANIC SYNTHESIS, 3rd ed., John Wiley & Sons, New York, NY (1999). As would be readily understood, the protecting group, Gp, can be readily displaced from the molecule to form a hydroxyl group, which can be further modified to form other functional groups using techniques known in the art.

[0067] D. Core Structures

[0068] The branched polymers of the invention are composed of an aliphatic hydrocarbon-based core (i.e., R in Formula I above) having a length of least three carbon atoms, preferably from 3 to 7 carbon atoms. That is to say, a central core structure will typically contain at its core a number of carbon atoms selected from the following: 3, 4, 5, 6, 7 or more carbon atoms. Preferred are core structures containing 3, 5 or 7 core carbons. Although the carbon atoms of the central core may have polymer arms extending from any of the aforementioned carbons, preferably but not necessarily, the overall branched polymer is symmetrical. That is to say, for a three-carbon core, the polymer arms preferably extend from positions 1 and 3, with a site suitable

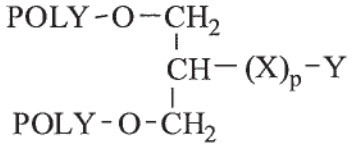
for covalent attachment to a biologically active molecule extending from the central carbon or the carbon at position 2. Similarly, for a five carbon core, the polymer arms extend from positions 1 and 5, with a site suitable for covalent attachment to a biologically active molecule extending from position 3, or polymer arms extending from positions 2 and 4, or, if a highly branched structure is desired, with polymer arms extending from each of positions 1, 2, 4, and 5. Exemplary three-carbon core structures possessing as the nucleophile an oxygen atom directly attached to carbons 1 and 3 are provided in the Examples. These examples demonstrate synthetic approaches for building core structures having a variety of (X)p groups. Preferably, the nucleophiles (heteroatoms) attached to the central core are the same, e.g., all oxygen, all nitrogen, etc.

[0069] Although less preferred, suitable for use in forming the branched polymers of the invention are unsymmetrical core structures such as those derived from 2-aminopentanedioic acid (glutamic acid), 2-aminosuccinic acid (aspartic acid), and the like. In utilizing core structures such as these, the terminal acid groups are typically activated for coupling with a reactive polymer to form the branched polymer core. Alternatively, the carboxylic acid groups are reduced with a reducing agent to form the corresponding diol, which then possesses sites suitable for building the polymer chains, for example, by a catalyzed reaction of the N-protected diol with an appropriate monomer subunit and subsequent polymerization thereof directly onto the central core.

[0070] E. Exemplary Branched Reactive Polymer Structures

[0071] More specific structural embodiments of the branched polymers of the invention will now be described, all of which are intended to be encompassed by the structure of Formula I above. The specific structures shown below are presented as exemplary structures only, and are not intended to limit the scope of the invention.

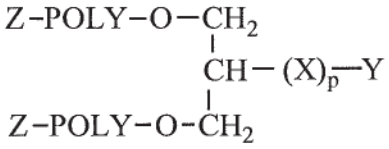
[0072] One embodiment of the invention having a three-carbon core has the structure:



Formula Ia

wherein POLY, X, p and Y are described above.

[0073] In a preferred embodiment of Formula Ia, the branched polymer of the invention has the structure:



Formula Ia_1

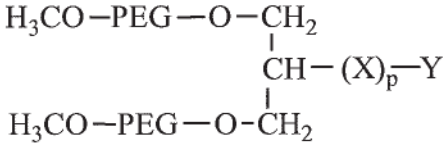
wherein:

Z is a capping group or a functional group; and

POLY, X, p, and Y are described above.

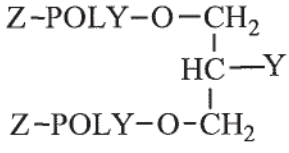
[0074] The Z group is preferably a relatively inert capping group, such as alkoxy (e.g. methoxy or ethoxy), alkyl, benzyl, aryl, or aryloxy (e.g. benzyloxy). Alternatively, the Z group is a functional group capable of readily reacting with a functional group on a biologically active molecule, such as any of the functional groups discussed above for the Y functional group.

[0075] In another embodiment of Formula Ia, each POLY is PEG end-capped with a methoxy as shown below:



Formula Ia_2

[0076] In yet another embodiment of Formula Ia, the X linkage is absent as shown below:

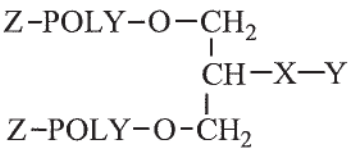


Formula Ia_3

wherein:

Z, POLY, and Y are defined above. Preferably, Z is methoxy and POLY is PEG.

[0077] In yet a further embodiment of Formula Ia, the X linkage is one of the specific linkages shown below:



Formula Ia_4

wherein:

X is $-CH_2CH_2-O-CH_2CH_2-$ or $-O-CH_2CH_2-$; and

Z, POLY and Y are defined above. Preferably, Z is methoxy and POLY is PEG.

[0078] As described above, other heteroatoms, such as -NH- or -S- could be used in place of the -O- linkages illustrated in Formulas Ia, Ia_1, Ia_2, Ia_3, and Ia_4 above.

[0079] F. Method of Forming Branched Reactive Polymers

[0080] The branched polymers of the invention are formed by attaching polymer arms to a heteroatom-substituted aliphatic hydrocarbon core molecule having at least three carbon atoms, such as propane, via heteroatom linkages (e.g., -NH-, -O-, or -S-). Although the polymer arms may be attached to the aliphatic hydrocarbon structure by simply reacting terminal functional groups on preformed purified polymers with reactive nucleophiles on the aliphatic hydrocarbon core without departing from the invention, for poly(alkylene glycol) polymers, it is preferable in many respects to directly polymerize alkylene oxide monomer units, such as ethylene oxide, propylene oxide or butylene oxide subunits, onto an aliphatic hydrocarbon core bearing at least two available hydroxyl groups (or other nucleophilic groups such as amino or thiol groups). As illustrated in the Examples, alkylene oxide units can be polymerized onto, for example, an alcohol molecule using a catalyzed reaction to form ether-linked polymer arms, preferably using base catalysis although other catalysts such as metal or acid catalysts could also be employed. By polymerizing the alkylene oxide directly onto a suitably functionalized aliphatic hydrocarbon core structure, the branched polymer can be formed without first forming and purifying high molecular weight polymers, which is technically challenging, expensive, and time-consuming.

[0081] The aliphatic hydrocarbon core molecule comprises two or more available nucleophilic groups, such as hydroxyl groups, depending on the number of polymer arms to be attached to the core molecule. In one particular embodiment, the aliphatic hydrocarbon has two hydroxyl groups. The core molecule also bears at least one protected functional group, such as a protected hydroxyl group (i.e., -O-Gp, where Gp is described above). Preferably, the aliphatic

hydrocarbon is 1,3-dihydroxy-2-substituted propane, wherein the protected hydroxyl group is attached at the 2-position, optionally via an intervening linkage (i.e., X in Formula I above). The presence of the protecting group prevents polymerization at that position, thereby ensuring that at least one side chain of the aliphatic hydrocarbon core will be available for further modification, for example, to a form suitable for covalent attachment to a biologically active molecule.

[0082] The generalized structure for the aliphatic hydrocarbon core molecule of the invention is shown below:

$$Y'(X)p\text{-}R(\text{-}Nu)_q$$

Formula V

wherein:

Y' is a protected functional group, such as a protected hydroxyl group, wherein the presence of the protecting group prevents polymerization at the Y' position on the aliphatic core,

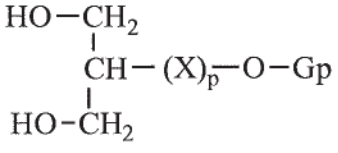
R:

Nu is a nucleophile, such as amino, thiol or hydroxyl; and

R, X, p and q are defined above.

[0083] Unlike certain prior art applications that utilize a polyol or polyamine core molecule to form a highly cross-linked hydrogel, the present invention utilizes a nucleophile-substituted aliphatic hydrocarbon core molecule to form a branched polymer suitable for covalent coupling to a biologically active molecule.

[0084] The generalized structure for a preferred hydroxyl-substituted three-carbon aliphatic hydrocarbon core structure is shown below:



Formula Va

wherein:

X, p, and Gp are defined above.

[0085] Exemplary core structures of Formula Va include 2-benzyloxy-1,3-propanediol, 2-benzyloxyethoxy-1,3-propanediol, and 2-benzyloxyethoxyethyl-1,3-propanediol. The core

structures of Formula V are either commercially available (See Examples 1-2) or can be prepared from commercially available reagents (See Examples 3-4).

[0086] Base-initiated polymerization of ethylene oxide onto a hydroxyl-substituted aliphatic hydrocarbon of Formula Va results in a branched polymer of Formula Ia where Y is -O-Gp and POLY is -PEG-OH. Thereafter, in order to form a monofunctional branched polymer, the terminal hydroxyl groups of the PEG polymer chains are preferably alkylated (e.g., methylated to form mPEG) by reaction with an alkylating agent, such as methyl toluenesulfonate.

[0087] Following alkylation of the terminus of the PEG chains, the protecting group, Gp, can be displaced by hydrolysis or hydrogenolysis to produce a hydroxyl group. As would be understood, the hydroxyl group can then be modified or converted to other reactive groups as desired, such as the reactive groups listed above for the Y moiety of Formula I.

[0088] III. Biologically Active Conjugates of Branched Reactive Polymers

[0089] The present invention also includes biologically active conjugates comprising a biologically active molecule covalently attached to a branched polymer of the invention. As noted above, the branched polymers of the invention are preferably although not necessarily monofunctional (e.g., they may also be bifunctional or less preferably multifunctional), and the biologically active agent is preferably attached to the branched polymer via a linkage formed from reaction of the functional group on the branched polymer and a functional group on the biologically active agent.

[0090] The specific linkage will depend on the structure of the functional groups utilized, and will typically be governed by the functional groups contained in the biologically active molecule. For example, an amide linkage can be formed by reaction of a branched polymer bearing a carboxylic acid group, or an active ester thereof, in the presence of a coupling agent, such as DCC, DMAP, or HOBT, with a biologically active agent having an amine group. Alternatively, a sulfide linkage can be formed by reaction of a branched polymer bearing a thiol group with a biologically active agent bearing a hydroxyl group. In another embodiment, an amine linkage is formed by reaction of a branched polymer bearing an amino group with a biologically active molecule bearing a hydroxyl group. In yet another embodiment, a branched polymer bearing a carboxylic acid is reacted with a biologically active molecule bearing a

hydroxyl group in the presence of a coupling agent to form an ester linkage. The particular coupling chemistry employed will depend upon the structure of the biologically active agent, the potential presence of multiple functional groups within the biologically active molecule, the need for protection/deprotection steps, chemical stability of the molecule, and the like, and will be readily determined by one skilled in the art. Illustrative linking chemistry useful for preparing the branched polymer conjugates of the invention can be found, for example, in Wong, S.H., (1991), "*Chemistry of Protein Conjugation and Crosslinking*", CRC Press, Boca Raton, FL and in Brinkley, M. (1992) "*A Brief Survey of Methods for Preparing Protein Conjugates with Dyes, Haptens, and Crosslinking Reagents*", in *Bioconjug. Chem.*, 3, 2013.

[0091] The linkage (i.e., L_1 in Formula VI below) can be hydrolytically degradable so that the biologically active agent is released into circulation over time after administration to a patient. Exemplary hydrolytically degradable linkages include carboxylate ester, phosphate ester, anhydrides, acetals, ketals, acyloxyalkyl ether, imines, orthoesters, peptides and oligonucleotides. If desired, a hydrolytically stable linkage, such as amide, urethane (also known as carbamate), amine, thioether (also known as sulfide), and urea (also known as carbamide) linkages, can also be used without departing from the invention.

[0092] A generalized structure for a biologically active conjugate of the invention comprising a branched polymer of Formula I can be represented as shown below:

$$D\text{-}L_1\text{-}(X)p\text{-}R(\text{-}X'\text{-}POLY)_q$$

Formula VI

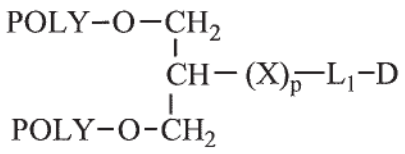
wherein:

D is a biologically active molecule, such as a peptide, protein, enzyme, small molecule drug, dye, lipid, nucleoside, nucleotide, oligonucleotide, polynucleotide, nucleic acid, polysaccharide, steroid, cell, virus, liposome, microparticle, micelle, fat, electrolyte and the like;

L_1 is a linkage resulting from the reaction of the functional group of the branched polymer (i.e., Y is Formula I) and a functional group on the biologically active molecule; and

POLY, X, X', q and p are defined above.

[0093] In one preferred embodiment, the biologically active conjugate has the structure:



Formula VIa

wherein:

D, L_1 POLY, X and p are defined above.

[0094] A biologically active agent for use in coupling to a branched polymer of the invention may be any one or more of the following. Suitable agents may 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, antimalarials, antiemetics, anepileptics, bronchodilators, cytokines, growth factors, anti-cancer agents, antithrombotic agents, antihypertensives, cardiovascular drugs, antiarrhythmics, antioxicants, 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, antienteritis agents, vaccines, antibodies, diagnostic agents, and contrasting agents.

[0095] Examples of active agents suitable for use in covalent attachment to a branched polymer of the invention include, but are not limited to, calcitonin, erythropoietin (EPO), Factor VIII, Factor IX, ceredase, cerezyme, cyclosporin, granulocyte colony stimulating factor (GCSF), thrombopoietin (TPO), alpha-1 proteinase inhibitor, elcatonin, granulocyte macrophage colony stimulating factor (GMCSF), growth hormone, human growth hormone (HGH), growth hormone releasing hormone (GHRH), heparin, low molecular weight heparin (LMWH), interferon alpha, interferon beta, interferon gamma, interleukin-1 receptor, interleukin-2, interleukin-1 receptor antagonist, interleukin-3, interleukin-4, interleukin-6, luteinizing hormone releasing hormone (LHRH), factor IX 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), insulin-like growth factor

(IGF), insulintropin, macrophage colony stimulating factor (M-CSF), nerve growth factor (NGF), tissue growth factors, keratinocyte growth factor (KGF), glial growth factor (GGF), tumor necrosis factor (TNF), endothelial growth factors, parathyroid hormone (PTH), glucagon-like peptide thymosin alpha 1, IIb/IIIa inhibitor, alpha-1 antitrypsin, phosphodiesterase (PDE) compounds, VLA-4 inhibitors, bisphosphonates, respiratory syncytial virus antibody, cystic fibrosis transmembrane regulator (CFTR) gene, deoxyreibonuclease (Dnase), bactericidal/permeability increasing protein (BPI), anti-CMV antibody, 13-cis retinoic acid, macrolides such as erythromycin, oleandomycin, troleandomycin, roxithromycin, clarithromycin, davercin, azithromycin, flurithromycin, dirithromycin, josamycin, spiromycin, midecamycin, leucomycin, miocamycin, rokitamycin, andazithromycin, and swinolide A; fluoroquinolones such as ciprofloxacin, ofloxacin, levofloxacin, trovafloxacin, alatrofloxacin, moxifloxicin, norfloxacin, enoxacin, grepafloxacin, gatifloxacin, lomefloxacin, sparfloxacin, temafloxacin, pefloxacin, amifloxacin, fleroxacin, tosufloxacin, prulifloxacin, irloxacin, pazufloxacin, clinafloxacin, and sitafloxacin, aminoglycosides such as gentamicin, netilmicin, paramecin, tobramycin, amikacin, kanamycin, neomycin, and streptomycin, vancomycin, teicoplanin, rampolanin, mideplanin, colistin, daptomycin, gramicidin, colistimethate, polymixins such as polymixin B, capreomycin, bacitracin, penems; penicillins including penicllinase-sensitive agents like penicillin G, penicillin V, penicllinase-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 ipiperacillin; cephalosporins like cefpodoxime, cefprozil, ceftbuten, ceftizoxime, ceftriaxone, cephalothin, cephapirin, cephalexin, cephradrine, cefoxitin, cefamandole, cefazolin, cephaloridine, cefaclor, cefadroxil, cephaloglycin, cefuroxime, ceforanide, cefotaxime, cefatrizine, cephacetrile, cefepime, cefixime, cefonicid, cefoperazone, cefotetan, cefinetazole, ceftazidime, loracarbef, and moxalactam, monobactams like aztreonam; and carbapenems such as imipenem, meropenem, pentamidine isethiouate, albuterol sulfate, lidocaine, metaproterenol sulfate, beclomethasone diprepionate, triamcinolone acetamide, budesonide acetonide, fluticasone, ipratropium bromide, flunisolide, cromolyn sodium, ergotamine tartrate and where applicable, analogues, agonists, antagonists, inhibitors, and pharmaceutically acceptable salt forms of the above. In reference to peptides and proteins, the invention is intended to encompass

synthetic, native, glycosylated, unglycosylated, pegylated forms, and biologically active fragments and analogs thereof.

[0096] IV. Examples

[0097] The following examples are given to illustrate the invention, but should not be considered in limitation of the invention. For example, although PEG is used in the examples to illustrate the invention, other polymers that are useful in the practice of the invention are encompassed by the invention as discussed above.

[0098] All PEG reagents referred to in the appended examples are available from Shearwater Corporation of Huntsville, AL. All other reagents are commercially available. All ^{1}HNMR data was generated by a 300 or 400 MHz NMR spectrometer manufactured by Bruker.

[0099] Examples 1-2 illustrate a method of forming a branched reactive polymer of the invention using a commercially available hydroxyl-substituted aliphatic hydrocarbon core molecule (2-benzyloxy-1,3-propanediol). In Example 1, the branched polymer is functionalized with an acetaldehyde diethyl acetal. In Example 2, the branched polymer is functionalized with a succinimidyl ester of butyric acid. Examples 3-4 illustrate a method of synthesizing two additional core molecules having intervening linkages between the aliphatic hydrocarbon core molecule and the protected hydroxyl side chain. Examples 5-6 illustrate PEGylation of an enzyme with a branched polymer of the invention to form a conjugate.

Example 1

Synthesis of 2-(1,3-di-mPEGoxy-2-propanoxy) acetaldehyde diethyl acetal

[0100] A. Synthesis of 1,3-diPEGoxy-2-benzyloxypropane [MW poly(ethylene glycol) (PEG) = 9 kDa]]

[0101] A 500 ml round bottom flask was charged with 250 ml of freshly distilled, dry THF containing 2-benzyloxy-1,3-propanediol (.84g, 4.59 mmole). Potassium naphthalenide was added (0.28 M, 16.4 ml) with continuous stirring under an inert atmosphere. The flask was then cooled to 0°C in an ice bath. Ethylene oxide (50.0 ml, 1.02 moles) was added via a cooled syringe. The reaction was allowed to warm to room temperature and was stirred for 72 hours.

The reaction was quenched by the addition of 5 ml of .2M acetic acid. The solvents were removed by rotary evaporation and the crude material redissolved in 100 ml of methylene chloride. The product was precipitated by the addition of 400 ml of diethyl ether and collected by filtration. The product was dried under vacuum.

[0102] Yield: 42 g. (93%). ^{1}H nmr (400 MHz DMSO-d_6), δ7.25-7.34, (m, 5H), 4.6(s, 2H), 3.2-3.8(m, 826H).

[0103] B. Methylation of 1,3-diPEGoxy-2-benzyloxypropane

[0104] 1,3-diPEGoxy-2-benzyloxypropane [MW poly(ethylene glycol) (PEG) = 9 kDa] (5.0g, 0.55 mmoles) from Step A was placed in a two-necked round bottom flask and dissolved in 150 ml of toluene. The flask was fitted with a septum and a Dean-Stark trap and the compound was azeotropically dried under an inert atmosphere. The trap was replaced with a reflux condenser and the temperature of the flask was kept at 45°C by placing the flask in a constant temperature oil bath. Methyl toluenesulfonate (1.62 ml, 5.4 mmoles) and 2.8 ml of potassium *t*-butoxide solution (1.0 M in THF) was added and the reaction stirred for 3 hours. Methyl toluenesulfonate (0.81 ml) and 1.4 ml of potassium *t*-butoxide solution were then added and the reaction was stirred for an additional 3 hours. The flask was removed from the oil bath and cooled to room temperature. The solution was transferred to a single-necked round bottom flask and the solvent was removed by rotary evaporation. The residue was dissolved in 5 ml of methylene chloride and precipitated by the addition of 50 ml of diethyl ether. The product was collected by filtration and dried under vacuum.

[0105] Yield: 4.2 g. ^{1}H Nmr (400 MHz DMSO-d_6), δ7.25-7.34(m, 5H) 4.6(s, 2H), 3.3-3.8(m, 826H), 3.24(s, 6H).

[0106] C. Debenzylation of 1,3-di-mPEGoxy-2-benzyloxypropane

[0107] 1,3-di-mPEGoxy-2-benzyloxypropane [MW poly(ethylene glycol) (PEG) = 9 kDa] (2.9 g, 0.32 mmoles) from Step B was dissolved in 100ml ethanol. $Pd(OH)_2/C$ (0.5 g) and cyclohexene (10 ml) was added and the mixture was refluxed for 4 hours. After cooling to room temperature, the mixture was filtered and the filtrate solvent removed by rotary evaporation. The crude residual material was dissolved in 5 ml of methylene chloride and precipitated by the

addition of 50 ml of diethyl ether. The product was collected by filtration and dried under vacuum.

[0108] ^{1}H Nmr (300 MHz, DMSO-d_6), δ 4.76(d, 1H), 3.3-3.8(m, 826H), 3.24(s, 6H).

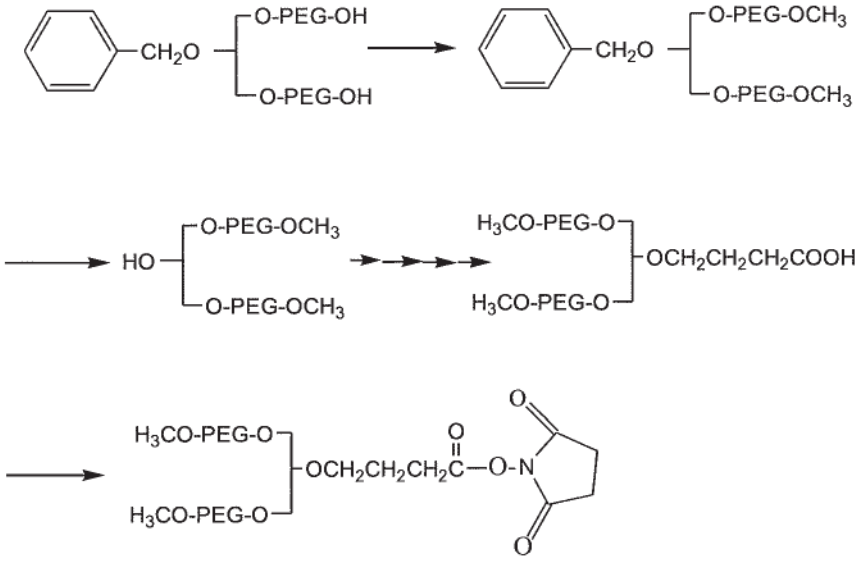
[0109] D. Synthesis of 2-(1,3-di-mPEGoxy-2-propanoxy) acetaldehyde diethyl acetal

[0110] To 2-hydroxy-1,3-di-mPEGoxypropane from Step C (M.W. 9000 Da, 4.5 g, 0.0005 moles) in dioxane (250 ml) is added sodium hydroxide (0.20 g, 0.005 moles) and chloroacetaldehyde diethyl acetal (0.38 g, 0.0025 moles) and the mixture is refluxed 24 h with vigorous stirring. The solution is concentrated to about 150 ml, cooled, and filtered. The filtrate is evaporated to dryness, dissolved in 100 ml of water, and extracted with methylene chloride (3 x 75 ml). The combined extracts are dried over sodium sulfate, concentrated, and the product precipitated by addition of 300 ml of ether. The precipitated product is collected by filtration and dried under vacuum.

[0111] This reaction demonstrates conversion of the 2-benzyloxypropane protecting group to a protected form of an aldehyde (acetaldehyde diethyl acetal) suitable for covalent coupling with amino groups on a protein or other biologically active agent.

Example 2

Synthesis of 2-(1,3-di-mPEGoxy-2-propanoxy) succinimidyl butyrate (mPEG2-SBA) (20 kDa)



[0112] A. Methylation of 1,3-diPEGoxy-2-benzyloxypropane

[0113] 20 g of 1,3-diPEGoxy-2-benzyloxypropane (20 kDa) prepared as described in Step A of Example 1 and 0.01 g of BHT(2,6-Di-tert-butyl-4-methylphenol) were dissolved in 400 ml of toluene. The resulting solution was azeotropically dried by distillation under reduced pressure. The residue was redissolved in 700 mL of anhydrous toluene and 14 mL of potassium tert-butoxide solution (1.0 M solution in tert-butanol) and 3.0 ml of methyl-toluene sulfonate were added separately. The reaction mixture was stirred overnight at 45 °C under nitrogen.

[0114] The insoluble material was filtered and filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in 700 ml of deionized water and saturated with NaCl. The pH of the solution was adjusted to 7.5 and it was then extracted with dichloromethane (300 ml x 2). The extracted dichloromethane was dried over Na_2SO_4, filtered, evaporated and precipitated with Et_2O (500 mL). The methylated product was collected by vacuum filtration and dried under vacuum overnight.

[0115] Yield: 18.5 g ^{1}H nmr (DMSO-d6): δ 7.33 ppm (mult. -$OCH_2C_6\underline{H}_5$), δ 4.61ppm (s. -$OC\underline{H}_2C_6H_5$), δ 4.31ppm (t, -$OCH_2C\underline{H}_2OMs$), 3.5ppm (br.mult., PEG), δ 3.24 ppm(s, $C\underline{H}_3$OPEG-).

[0116] B. Debenzylation of 1,3-di-mPEGoxy-2-benzyloxypropane

[0117] 18.0 g of 1,3-di-mPEGoxy-2-benzyloxypropane (20kDa) from Step A was dissolved in 225 ml of 5mM phosphate buffer (pH 7.2) and 1.13 g of 10% Pd on charcoal was added. The suspension was hydrogenated 20 hours under 40 psi of hydrogen.

[0118] The suspension was filtered to remove catalyst and the filtrate was saturated with NaCl and the pH of the solution was adjusted to 3.0. The solution was extracted with dichloromethane (300 ml x 2) and the combined extracts were dried over Na_2SO_4, filtered, evaporated and precipitated with Et_2O (500 mL). The product was collected by vacuum filtration and dried in vacuum overnight.

[0119] Yield: 13.2 g; ^{1}H nmr (DMSO-d6): δ 4.76 ppm (d. $\underline{H}$O-CH-); δ3.5ppm (br.mult., PEG), δ 3.24 ppm(s, $C\underline{H}_3$OPEG-).

[0120] C. Synthesis of 2-(1,3-di-mPEGoxy-2-propanoxy) butyric acid

[0121] 2.5 g of 2-hydroxy-1,3-di-mPEGoxypropane (20 kDa) from Step B was dissolved in 30 ml of toluene and the resulting solution was azeotropically dried by distillation under reduced pressure. The residue was redissolved in 30 mL of anhydrous toluene and 1 mL of potassium tert-butoxide solution (1.0M solution in tert-butanol), 2.5 mg of BHT, and 0.25 g of 1-(3-bromopropyl)-4-methyl-2,6,7- trioxabicyclo[2,2,2,]octane were added. The reaction mixture was stirred overnight at 65 °C under nitrogen.

[0122] The solvent was evaporated to dryness under reduced pressure, the residue dried under vacuum for 2 hours, and finally redissolved in 60 ml of deionized water. The pH of the solution was adjusted to 2.0 with 10 % H_3PO_4. After stirring at pH 2.0 for 15 min., the pH of the solution was adjusted to 12.0 with 1.0 N NaOH and stirred at pH 12.0 for 2 hours. The hydrolyzed solution was saturated with NaCl and the pH adjusted to 3.0 with 10% H_3PO_4. The solution was extracted with dichloromethane (100 ml x 2) and the combined extracts were dried over Na_2SO_4, filtered, evaporated and precipitated with Et_2O (100 mL). The product was collected by vacuum filtration and dried in vacuum overnight.

[0123] Yield: 2.3 g GPC: 79 %.

[0124] D. Purification of 2-(1,3-di-mPEGoxy-2-propanoxy) butyric acid

[0125] The crude 2-(1,3-di-mPEGoxy-2-propanoxy) butyric acid from Step C was purified by DEAE sepharose FF ion exchange column (100 mL). After purification, the yield was 1.55 g.

[0126] ^{1}H nmr (DMSO-d6): δ3.5ppm (br.mult., PEG), δ 3.24 ppm(s, $C\underline{H}_3OPEG$-), δ 2.23 ppm(t, -$OCH_2CH_2C\underline{H}_2COOH$), δ 1.70 ppm (mult. -$OCH_2C\underline{H}_2CH_2COOH$).

[0127] E. Synthesis of 2-(1,3-di-mPEGoxy-2-propanoxy) succinimidyl butyrate (20 kDa)

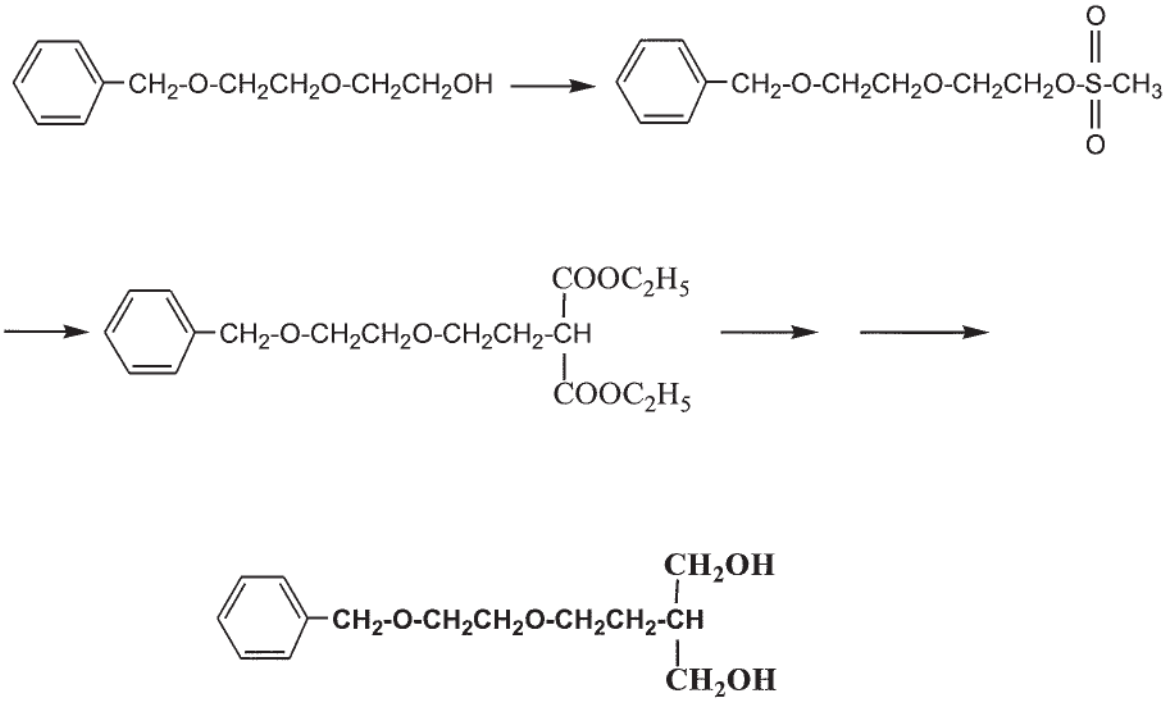
[0128] 1.5 g 2-(1,3-di-mPEGoxy-2-propanoxy) butyric acid from Step D was dissolved in 20 ml of anhydrous dichloromethane under nitrogen. N-hydroxysuccinimide (0.0132 g) was first added to the solution followed by 0.0234 g of dicyclohexylcarbodiimide. The solution was stirred overnight at room temperature under nitrogen. The product was filtered, concentrated under

vacuum, precipitated into a mixture of IPA and Et_2O (1:1), collected by filtration and dried under vacuum.

[0129] Yield: 1.2 g; ^{1}H nmr (DMSO-d6): δ3.5ppm (br.mult., PEG), δ 3.24 ppm(s, $C\underline{H}_3OPEG$-), δ 2.80 ppm(s, -NHS), δ 2.70 ppm (t. $-OCH_2CH_2C\underline{H}_2COONHS$), δ 1.81 ppm (mult. $-OCH_2C\underline{H}_2CH_2COONHS$).

Example 3

Synthesis of (2'-benzyloxyethoxy)ethyl-1,3-propanediol (BEEP) – An Illustrative Aliphatic Hydrocarbon Core Molecule Suitable for Use in Preparing a Branched Polymer



[0130] A. Synthesis of di(ethylene glycol) monobenzyl ether methanesulfonate

[0131] Di(ethylene glycol) monobenzyl ether (15 g) in 200 ml of toluene was dried by azeotropic distillation and the residue was redissolved in 400 ml of anhydrous toluene and 100 ml of anhydrous dichloromethane. To the solution was added 11.5 ml of dry triethylamine and 6.23 ml of methanesulfonyl chloride dropwise at 0-5 °C. The reaction mixture was stirred at room temperature under nitrogen overnight and the reaction was quenched by adding 5 ml absolute

ethanol. The insoluble material was filtered off and filtrate was evaporated to dryness. The residue was redissolved in 200 ml of anhydrous toluene and insoluble material was filtered off. The filtrate was evaporated to dryness and residue was dried under vacuum overnight.

[0132] Yield: 22 g ^{1}H nmr (DMSO-d6): δ 7.33 ppm (mult. -$OCH_2C_6H_5$), δ 4.49ppm (s. -$OCH_2C_6H_5$), δ 4.31ppm (t, $OCH_2CH_2OSO_2$-CH_3), δ 3.69ppm (t, $OCH_2CH_2OSO_2$-CH_3), δ 3.59ppm (mult., -OCH_2CH_2O-), δ 3.24 ppm(s, $OCH_2CH_2OSO_2$-CH_3).

[0133] B. Synthesis of C_6H_5-CH_2O-$CH_2CH_2OCH_2CH_2$-$CH(COOC_2H_5)_2$

[0134] Diethyl malonate (17.5 g) in 100 ml of 1,4-dioxane was added dropwise NaH (3.6 g) in 150 ml of 1,4-dioxane under nitrogen. Di(ethylene glycol) monobenzyl ether methanesulfonate (10 g) from Step A in 600 ml of 1,4-dioxane was added to the above diethyl malonate solution. After refluxing the mixture for 4 hours, the reaction solution was filtered and evaporated to dryness. The residue was dried in vacuum overnight.

[0135] The remaining diethyl malonate was distilled off under reduced pressure. After distillation, the residue was purified by flash chromatography on a silica gel column eluted with hexane followed by dichloromethane. The combined dichloromethane extracts were evaporated to dryness and the product dried under vacuum overnight.

[0136] Yield: 6 g. ^{1}H nmr (DMSO-d6): δ 7.33 ppm (mult. -$OCH_2C_6H_5$), δ 4.48ppm (s. -$OCH_2C_6H_5$), δ 4.10ppm (mult. OCH_2CH_3), δ 3.51ppm (mult., -OCH_2CH_2O-CH_2CH_2-, -$CH(CO_2$-$C_2H_5)_2$), δ 2.01ppm (mult.-OCH_2CH_2-$CH(CO_2$-$C_2H_5)_2$), δ1.16ppm (t, -OCH_2CH_3).

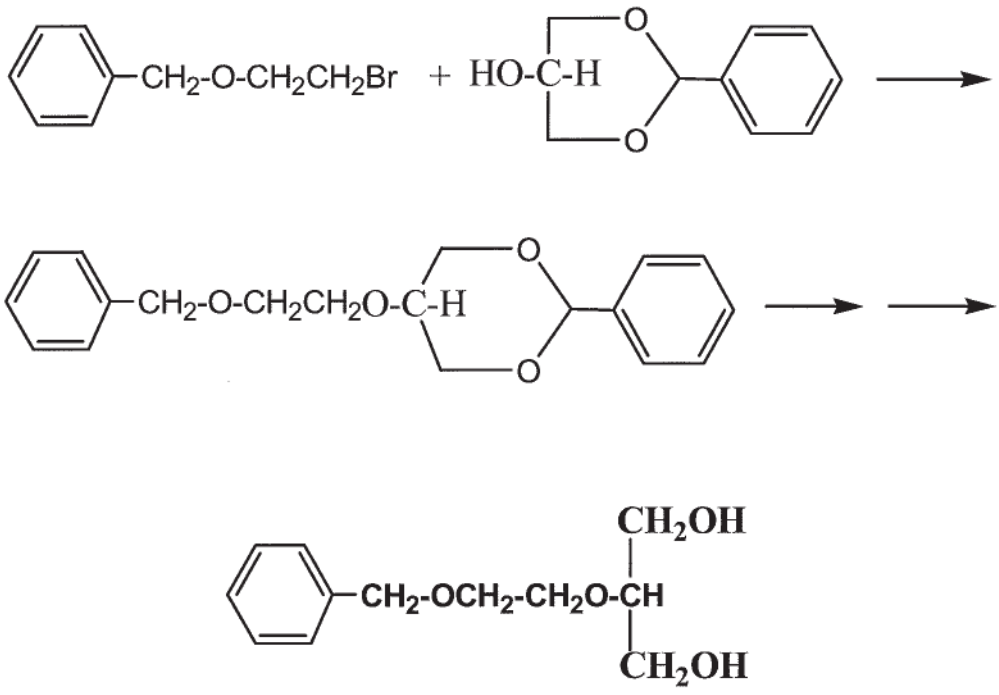
[0137] C. Synthesis of C_6H_5-CH_2O-$CH_2CH_2OCH_2CH_2$-$CH(CH_2OH)_2$

[0138] C_6H_5-CH_2O-$CH_2CH_2OCH_2CH_2$-$CH(COOC_2H_5)_2$ (5 g) from Step B was dissolved in 200 ml of toluene and 29.5 ml of $LiAlH_4$ (1 M in THF) was added at 0-5 °C. After stirring overnight at room temperature, 1 ml of water was added followed by 1.0 ml of 15 % NaOH and 3.0 ml of water. The insoluble material was filtered and the filtrate was evaporated to dryness. The product was purified by flash chromatography on a silica gel column eluted with ethyl acetate. Combined fractions were evaporated to dryness. The final product was dried under vacuum overnight.

[0139] Yield: 1.5 g ^{1}H nmr ($CDCl_3$): δ 7.29 ppm (mult. -$OCH_2C_6\underline{H}_5$), δ 4.55ppm (s. -$OC\underline{H}_2C_6H_5$), δ 3.61ppm (mult.,$C_6H_5CH_2$-$O\underline{CH_2CH_2}O$-$\underline{CH_2}CH_2$-, -$CH(\underline{CH_2}OH)_2$, δ 1.81ppm (mult.-OCH_2CH_2-$\underline{CH}(CH_2OH)_2$), δ1.65ppm (mult.-$OCH_2\underline{CH_2}$-$CH(CH_2OH)_2$).

Example 4

Synthesis of (2'-benzyloxyethoxy)-1,3-propanediol – An Exemplary Aliphatic Hydrocarbon Core Molecule Useful for Preparing a Branched Polymer



[0140] A mixture of 2 g of cis-1,3-O-benzylideneglycerol, 3.51 ml of benzyl 2-bromoethyl ether,1.25 g of KOH powder and 30 ml of toluene was stirred under reflux for about 20 hours. After cooling to room temperature, the insoluble material was removed by filtration and the filtrate concentrated. The residue was distilled at 140 °C under reduced pressure to remove benzyl 2-bromoethyl ether. After distillation, the residue dissolved in 20 ml of methanol containing 2 ml of conc.HCl and refluxed for 4 hours. 100 ml of water was added and the pH was adjusted to 5-6 with solid NaOH. NaCl was added to ~ 10 % and the product was extracted with dichloromethane (50 ml x 3). The combined dichloromethane extracts were dried over Na_2SO_4,

filtered and evaporated. The residue was dried under vacuum and the product was purified by chromatography on a silica gel column (80 g) eluted with ethyl acetate. The combined fractions were evaporated and dried under vacuum.

[0141] Yield: ~ 0.9 g ^{1}H nmr (DMSO-d6): δ 7.31 ppm (mult. -$OCH_2C_6\underline{H}_5$), δ 4.48ppm (s. -$OC\underline{H}_2C_6H_5$), δ 4.43 ppm (s.br. -$CH(CH_2O\underline{H})_2$).), δ 3.67ppm (t., -$OCH_2\underline{CH}_2O$-$CH(CH_2OH)_2$), δ 3.54ppm (t., -$O\underline{CH}_2CH_2O$-$CH(CH_2OH)_2$), δ3.41 ppm (mult.-OCH_2CH_2O-$CH(C\underline{H}_2OH)_2$), δ3.29 ppm (mult.-OCH_2CH_2O-$C\underline{H}(CH_2OH)_2$).

Example 5

PEGylation of Lysozyme with Branched PEG Polymer

[0142] Lysozyme (0.0021g, Sigma)) was dissolved in 1 ml of 50 mM sodium phosphate buffer (pH 7.5) in a 2ml vial. MPEG2 (20 kDa)-SBA (0.006g, 2 fold molar excess to the lysozyme) from Example 2 was added and the reaction vial was shaken at room temperature for 18 h.

[0143] The MALDI-TOF spectrum of the crude reaction mixture showed lysozyme, PEG2 (20 kDa)-butanoic acid, and mono-, and di-pegylated lysozyme at masses of 14,028Da, 21,810Da, 35,612Da, and 57,783Da, respectively to be present. SDS-PAGE (10% Tris-HCL gel) displayed six bands indicating tetra-, tri-, di-, mono-pegylated lysozyme (meaning polymer-modified forms of the enzyme having 4, 3, 2, and 1 of the branched polymers of the invention covalently attached thereto, respectively), PEG2 (20K)-butanoic acid, and unpegylated lysozyme.

[0144] Example 5 demonstrates the utility of the polymers of the invention in forming conjugates having an amide linkage coupling the branched polymer structure with a biological agent.

Example 6

PEGylation of Lysozyme with Branched PEG Polymer

[0145] 2.2mg, 1.9mg, and 2.1mg of lysozyme (Sigma) were dissolved in 1 ml of 50 mM sodium phosphate buffer of pH 5.5, 6.5, and 7.6, respectively. 1.5 mg of di-mPEG 2kDa-butyraldehyde (5 fold molar excess relative to the lysozyme) and 0.1 mg of $NaCNBH_3$ (10 fold

molar excess relative to the lysozyme) were added to the lysozyme solution of pH 5.5. 1.3mg and 1.5 mg of MPEG 2kDa-butyraldehyde were added to the lysozyme solution of pH 6.5 and 7.5, respectively, followed by the addition of 0.08mg and 0.09mg of $NaCNBH_3$, respectively. The three reaction vials were shaken at room temperature for 6 h.

[0146] Samples from the reactions of pH 5.5 and 6.5 showed the presence of lysozyme, mono-, and di-pegylated lysozyme by MALDI-TOF. Samples from the reaction conducted at pH 7.5 indicated the presence of di-pegylated lysozyme only by MALDI-TOF. After 24 h all reaction product mixtures contained lysozyme, mono-, and di-pegylated lysozyme, and, tri-pegylated lysozyme.

[0147] Three samples withdrawn from the pH 5.5, 6.5, and 7.5 reactions after 6 hours were spotted on a 15% Tris-HCl gel. Each sample showed three visible bands corresponding to di-, mono-, and native lysozyme. Three samples withdrawn from the reactions of pH 5.5, 6.5, and 7.5 after 24 hours showed four visible bands, which indicated tri-, di-, mono-, and unpegylated lysozyme.

[0148] Example 6 demonstrates the utility of the polymers of the invention in forming conjugates wherein a biologically active agent is covalently coupled to the branched polymer via a secondary amine linkage generated by reductive amination of the corresponding Schiff base.

WHAT IS CLAIMED IS:

1. A branched reactive polymer having the structure:

$$Y\text{-}(X)p\text{-}R(\text{-}X'\text{-}POLY)_q$$

wherein:

Y is a functional group;

R is an aliphatic hydrocarbon having a length of at least three carbon atoms;

X' is -O-;

X is a linker of 1 to 10 atoms in length;

P is 0 or 1;

q is 2 to about 10; and

each POLY is a water soluble and non-peptidic polymer that terminates with a hydroxyl or methoxy group,

and further wherein the branched polymer has a molecular weight of about 12,000 Da to about 100,000 Da.

2. The reactive polymer of Claim 1, wherein each water soluble and non-peptidic polymer is a PEG.

3. The reactive polymer of Claim 2, wherein each water soluble and non-peptidic polymer is a PEG that terminates with a hydroxyl group.

4. The reactive polymer of Claim 2, wherein each water soluble and non-peptidic polymer is a PEG that terminates with a methoxy group.

5. The reactive polymer of Claim 1, wherein Y selected from the group consisting of hydroxyl, active ester, active carbonate, acetal, aldehyde, aldehyde hydrate, alkenyl, acrylate, methacrylate, acrylamide, active sulfone, amine, hydrazide, thiol, alkanoic acid, acid halide, isocyanate, isothiocyanate, maleimide, vinylsulfone, dithiopyridine, vinylpyridine, iodoacetamide, epoxide, glyoxal, dione, mesylate, tosylate, and tresylate.

6. The reactive polymer of Claim 1, wherein p is 1 and X is selected from the group consisting of a heteroatom, -alkylene-, -O-alkylene-O-, -alkylene-O-alkylene-, -aryl-O-, -O-aryl-, $(\text{-O-alkylene-})_m$, and $(\text{-alkylene-O-})_m$, wherein m is 1-10.

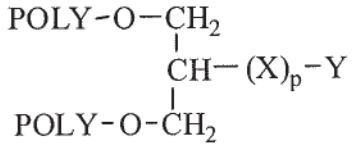
7. The reactive polymer of Claim 1, wherein p is 0 and Y is hydroxyl.

8. The reactive polymer of Claim 1, wherein the branched polymer has a molecular weight of about 20,000 Da.

9. The reactive polymer of Claim 1, wherein the branched polymer has a molecular weight of about 40,000 Da.

10. The reactive polymer of Claim 1, wherein the branched polymer has a molecular weight of about 60,000 Da.

11. The reactive polymer of Claim 1, having the structure:



BRANCHED POLYMERS

ABSTRACT OF THE DISCLOSURE

The present invention is directed to branched reactive water-soluble polymers comprising at least two polymer arms, such as poly(ethylene glycol), attached to a central aliphatic hydrocarbon core molecule through ether linkages. The branched polymers bear at least one functional group for reacting with a biologically active agent to form a biologically active conjugate. The functional group of the branched polymer can be directly attached to the aliphatic hydrocarbon core or via an intervening linkage, such as a heteroatom, -alkylene-, -O-alkylene-O-, -alkylene-O-alkylene-, -aryl-O-, -O-aryl-, $(-O\text{-alkylene-})_m$, or $(-\text{alkylene-O-})_m$ linkage, wherein m is 1-10.

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12/963,170	12/08/2010	Michael David Bentley	SHE0058.11	2323

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EXAMINER	
KRISHNAN, GANAPATHY	
ART UNIT	PAPER NUMBER
1623	

MAIL DATE	DELIVERY MODE
01/04/2012	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	12/963,170	BENTLEY ET AL.	
	Examiner	Art Unit	
	GANAPATHY KRISHNAN	1623	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *08 December 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

5)☒ Claim(s) *1-11* is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6)☐ Claim(s) _____ is/are allowed.

7)☒ Claim(s) *1-11* is/are rejected.

8)☐ Claim(s) _____ is/are objected to.

9)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

12)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *03/09/11*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Claims 1-11 are pending in the instant application and have been examined on the merits herein.

Priority

This application is a continuation of 11/336,695 filed 1/20/2006, now US 7,872,072, which is a divisional of 10/290,082 filed 11/07/2002, now US 7,026,440. The parent applications 11/336,695 and 60/337,613 to which priority is claimed is seen to provide adequate support under 35 U.S.C. 112 for claims 1-11 of this application.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 5 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 5 recites the term active. It is not clear what "active" means.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-3, 6 and 11 are rejected under 35 U.S.C. 102(b) as being anticipated by Shimomura et al (EP 0899310).

Shimomura et al teach polymers having structures 13 and 15 (page 38). Formula 13 of Shimomura has a functional group, which is an amine (Y in instant claim 1, 11), has an oxygen (hetero atom linker X' and X and p = 1 in claim 1), has a 3-carbon aliphatic chain (R in instant claim 1 wherein q = 2-10) and has polyethylene oxide chains (polymer arms, POLY as in claim 2) attached to the hydrocarbon chain. The polyethylene oxide (polymer arms) chains are also symmetrically located on the aliphatic hydrocarbon (part of the limitation of claim 11). Structure 15 (page 38) of Shimomura has a O-CH2-A and the moiety A has a terminal hydroxyl group (limitation of claim 3). The middle carbon in the 3-carbon chain of structure 13 of Shimomura has an oxygen (a hetero atom, X in claim 6). The structure also has (O-CH2-CH2-O) and (-CH2-CH2-O) groups attached to the hydrocarbon chain. This meets the limitations of claims 6 for X being heteroatoms, O-alkylene-O, alkylene-O-alkylene-O and alkylene-O-alkylene, groups. Structure 13 and 15 of Shimomura et al also meet the limitations of claim 11 since the middle carbon of the hydrocarbon chain has an oxygen that acts as a linker (X in instant claim 11). This oxygen has an amino methyl O-alkylene group attached to it (Y in claim 11, Y is just defined as a functional group).

Claims 1-3 and 8 are rejected under 35 U.S.C. 102(b) as being anticipated by Taylor et al (US 5684096).

Taylor et al disclose a polyfunctional polyalkylene glycol (see structure at col. 5, lines 20-25) where n is in the range of 1 to 200. This structure has a polymer arm (POLY) with each polymer arm attached to the three-carbon backbone (this 3 carbon backbone is R in instant claim 1) via oxygen atoms (heteroatom linkage). This structure meets the limitations of instant claim 1 for p=0, i.e., no linker group X in the structure in instant claim 1. This means that the functional group is directly attached to the carbon backbone. Taylor's structure has an ethyl group (alkyl functional group, since instant claim 1 does not define the functional group Y). The structure meets the limitations of instant claim 1 (X' is oxygen, heteroatom linkage and q is 3). The formula of Taylor has a PEG arm that terminates with an OH (limitations of claims 2-3). In Taylor's formula n can be 200. This gives a molecular weight of about 26,000 for the polymer (limitations of claim 1 and 8).

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., In re Berg, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); In re Goodman, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); In re Longi, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); In re Van Ornum, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and In re Thorington, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned

with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 1-11 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-19 of U.S. Patent No. 7,026,440 (‘440). Although the conflicting claims are not identical, they are not patentably distinct from each other because:

Instant Claim 1 is drawn to a branched polymer having an aliphatic chain of at least three carbon atoms that is substituted with a linker having a functional group and a no-peptidic polymer. Dependent claims 2-11 recite further limitations regarding the specific non-peptidic polymer, the functional groups, the linker and molecular weight.

Claims 1-19 of ‘440 are also drawn to a branched polymer that is similar to the instant polymer.

Claims 1-19 differ from the instant claims in that the instant polymer has Y in the formula as any functional group, X’ can be only oxygen atom and X is any linker of 1 to 10 atoms in length whereas in the polymer of '440 Y is a functional group that is reactive with an electrophilic or nucleophilic group, X' is any heteroatom and X is a linker. ‘440 defines the linker to be one that has an overall length of 1 to about 40 atoms, preferably about 1 to 10 atoms including some specific linkers (col. 10, lines 29-50). The same definitions also hold good for the instant polymer (page 14, paragraph 0061). However, it would have been obvious to one of

ordinary skill in the art at the time the invention was made that the substitutions in the instant polymer could successfully employed in the polymer of '440.

In the instant case '440 teaches structural limitations which are seen applicable to the polymer in the instant claims since the instant claims recite broader limitations. Although the claims of '440 employ a specific heteroatom, namely oxygen for the linker X', one of ordinary skill in the art would readily recognize that the substitutions taught by '440 could be employed in polymer with a reasonable expectation of success. The use of known substitutions to effectuate the same type of modifications in polymers taught in the prior art is not seen to render the instantly claimed polymer unobvious over the art. Once the general substitution pattern has been shown to be old, the burden is on the applicant to present reason or authority for believing that a group on polymer would affect and thus alter the nature of the product or the operability of the process and thus the unobviousness of the method of producing it.

With respect to the non-statutory double patenting rejection(s) made in this Office action, note as follows. The use of the terminology "defined in 35 U.S.C. §154 to §156 and §173" in a terminal disclaimer can result in the terminal disclaimer being found improper. To address this, note that a proper terminal disclaimer need only disclaim the patent's remaining "full statutory term" as defined in 35 U.S.C., without specifying 35 U.S.C. 154 and 173. This is so, because the "full statutory term" inherently is a statutorily defined item.

Accordingly, the following language would be deemed acceptable:

The owner*, _____, of _____ percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of any patent granted on pending reference Application Number _____, filed on _____, and as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and any patent granted on the reference application are commonly owned. This

agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term of any patent granted on said reference application, "as the term of any patent granted on said reference application may be shortened by any terminal disclaimer filed prior to the grant of any patent on the pending reference application," in the event that: any such patent: granted on the pending reference application: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as shortened by any terminal disclaimer filed prior to its grant.

Note: the above language corresponds to PTO/SB/25 (07-09) (reproduced at page 1400-120 in Revision 7 (July 2008) of the 8th edition of the MPEP), but the reference to 35 U.S.C. 154 and 173 has been deleted.

Conclusion

Claims 1-11 are rejected

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ganapathy Krishnan whose telephone number is 571-272-0654. The examiner can normally be reached on 9.00am-5pm; Mon-Fri. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Shaojia A. Jiang can be reached on 571-272-0627. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Ganapathy Krishnan/

Examiner, Art Unit 1623.

/SHAOJIA ANNA JIANG/

Supervisory Patent Examiner, Art Unit 1623

Notice of References Cited	Application/Control No. 12/963,170	Applicant(s)/Patent Under Reexamination BENTLEY ET AL.	
	Examiner GANAPATHY KRISHNAN	Art Unit 1623	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-5,684,096	11-1997	Taylor et al.	525/523
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N	EP 0 899 310 A1	03-1999	Europe	Shimomura et al	C09D 11/00
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

PATENT
Docket No. SHE0058.11

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Michael David BENTLEY et al.		Examiner:	Ganapathy KRISHNAN
Serial No.:	12/963,170	Art Unit:	1623
Filed:	December 8, 2010	Confirmation No.:	2323
Title:	**BRANCHED POLYMERS**		

REPLY UNDER 37 C.F.R. §1.111

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Sir:

This Reply is in response to the Office Action mailed January 4, 2012, received in connection with the above-identified patent application. As this Reply is being filed within the three-month shortened statutory period, no extension fees are due.

No fees other than those that may be identified in the papers accompanying the present Reply are believed to be due; however, the Commissioner is hereby authorized to charge any additional fees to Deposit Account No. 50-0348.

Amendments to the Specification begin on page 2.

Amendments to the claims begin on page 3. This listing of claims replaces all prior versions and listings of claims in the application.

Remarks begin on page 5.

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AMENDMENTS

In the specification:

On page 1 of the specification, please amend paragraph **[0001]** as follows:

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation application of U.S. Patent Application Serial No. 11/336,695, filed January 20, 2006, now U.S. Patent No. 7,872,072, which is a divisional application of U.S. Patent Application Serial No. 10/290,082, filed November 7, 2002, now U.S. Patent No. 7,026,440, which claims the benefit of priority under 35 U.S.C. §119(e) to U.S. Provisional Patent Application Serial No. 60/337,613, filed November 7, 2001, all of which are incorporated herein by reference in their entireties.

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Listing of Claims

1. (Currently Amended) A branched reactive polymer having the structure:

$$Y\text{-}(X)p\text{-}R(\text{-}X'\text{-}POLY)_q$$

wherein:

Y is a functional group reactive with an electrophilic or nucleophilic group;

R is an aliphatic hydrocarbon having a length of at least three carbon atoms;

X' is -O-;

X is a linker of 1 to 10 atoms in length;

P is 0 or 1;

q is 2 to about 10; and

each POLY is a water soluble and non-peptidic polymer that terminates with a hydroxyl or methoxy group,

and further wherein the branched polymer has a molecular weight of about 12,000 Da to about 100,000 Da.

2. (Original) The reactive polymer of Claim 1, wherein each water soluble and non-peptidic polymer is a PEG.

3. (Original) The reactive polymer of Claim 2, wherein each water soluble and non-peptidic polymer is a PEG that terminates with a hydroxyl group.

4. (Original) The reactive polymer of Claim 2, wherein each water soluble and non-peptidic polymer is a PEG that terminates with a methoxy group.

5. (Original) The reactive polymer of Claim 1, wherein Y selected from the group consisting of hydroxyl, active ester, active carbonate, acetal, aldehyde, aldehyde hydrate, alkenyl, acrylate, methacrylate, acrylamide, active sulfone, amine, hydrazide, thiol, alkanoic acid, acid halide, isocyanate, isothiocyanate, maleimide, vinylsulfone, dithiopyridine, vinylpyridine, iodoacetamide, epoxide, glyoxal, dione, mesylate, tosylate, and tresylate.

6. (Original) The reactive polymer of Claim 1, wherein p is 1 and X is selected from the group consisting of a heteroatom, -alkylene-, -O-alkylene-O-, -alkylene-O-alkylene-, -aryl-O-, -O-aryl-, $(\text{-O-alkylene-})_m$, and $(\text{-alkylene-O-})_m$, wherein m is 1-10.

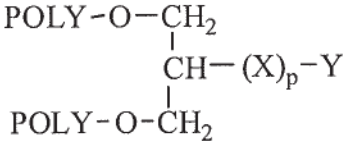
7. (Original) The reactive polymer of Claim 1, wherein p is 0 and Y is hydroxyl.

8. (Original) The reactive polymer of Claim 1, wherein the branched polymer has a molecular weight of about 20,000 Da.

9. (Original) The reactive polymer of Claim 1, wherein the branched polymer has a molecular weight of about 40,000 Da.

10. (Original) The reactive polymer of Claim 1, wherein the branched polymer has a molecular weight of about 60,000 Da.

11. (Original) The reactive polymer of Claim 1, having the structure:



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REMARKS

I. Introductory Comments

In the Office Action under reply, the Patent Office has rejected the claims as follows: under 35 U.S.C. §112, second paragraph, as allegedly being indefinite (claim 5); under 35 U.S.C. §102(b), as allegedly being anticipated by Shimomura et al. (EP 0899310) (claims 1-3, 6 and 11); under 35 U.S.C. §102(b), as allegedly being anticipated by Taylor et al. (U.S. Patent No. 5,684,096) (claims 1-3 and 8); and under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1-19 of U.S. Patent No. 7,026,440 (claims 1-11).

II. Amendments to the Specification

Paragraph **[0001]** of the Specification -- the Cross-Reference to Related Applications -- has been updated to reflect issuance of a patent based on U.S. Patent Application No. 11/336,695.

III. Status of the Claims

Claims 1-11 were previously pending. Claim 1 has been amended. Thus, upon entry of the changes to the claims, claims 1-11 will be pending and under consideration.

Support for the amendment to claim 1 may be found at least, for example, at paragraph [0016] of the application as-filed. As support for the amendment is found in the application as-filed, no new matter is introduced by the entry of the above-identified amendment. This amendment is made to expedite prosecution and not in acquiescence to any claim rejection.

IV. The Rejection Under 35 U.S.C. §112, Second Paragraph

The Patent Office has rejected claim 5 under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. Particularly, the Office asserts that the term "active" recited in claim 5 is unclear, as it is allegedly "not clear what 'active' means." Applicants respectively traverse this rejection.

As recited by the Office, 35 U.S.C. § 112, second paragraph states:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

The MPEP provides further guidance regarding the examination requirements for this statutory requirement. For example:

> The essential inquiry pertaining to this requirement is whether the claims set out and circumscribe a particular subject matter with a reasonable degree of clarity and particularity. Definiteness of claim language must be analyzed, not in a vacuum, but in light of:
> (A) The content of the particular application disclosure;
> (B) The teachings of the prior art; and
> (C) The claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made.
> MPEP § 2173.02

Further, as stated by the Federal Circuit:

> [t]he test for definiteness under 35 U.S.C. 112, second paragraph, is whether "those skilled in the art would understand what is claimed when the claim is read in light of the specification." *Orthokinetics, Inc. v. Safety Travel Chairs, Inc.*, 806 F.2d 1565, 1576, 1 USPQ2d 1081, 1088 (Fed. Cir. 1986).
> MPEP § 2173.02.

In view of the standards set forth above, pending claim 5 meets the requirements of 35 U.S.C. § 112. In that claim, the Office has rejected the use of the term "active," for example in the terms "*active* ester" and "*active* carbonate." Applicants assert those terms satisfy the requirements of 35 U.S.C. § 112, second paragraph, particularly when read by one of skill in the art in view of the teachings in the art. For example, such teachings include the Shearwater Polymers, Inc. Catalog from July 1997 (at least pages 16-23, and 27) listed on the PTO Form SB/08 submitted in this application and initialed by the Examiner on December 30, 2011. While not being limited to those examples, this document provides an example indicating that the terms "*active* ester" and "*active* carbonate," are understood by those of skill in the art.

Further, the disclosure of the present application provides additional guidance to one of skill in the art. For example, paragraph [0016] of the as-filed application states:

> The term "active," when used in conjunction with a functional group, is intended to include those functional groups that react readily with electrophilic or nucleophilic groups on other molecules, in contrast to those groups that require strong catalysts or highly impractical reaction conditions in order to react.

By way of a non-limiting example, paragraph [0016] of the as-filed application further states:

> the term "active ester" would include those esters that react readily with nucleophilic groups such as amines. Exemplary active esters include N-hydroxysuccinimidyl esters or 1-benzotriazolyl esters. Typically, an active ester will react with an amine in aqueous medium in a matter of minutes, whereas certain esters, such as methyl or ethyl esters, require a strong catalyst in order to react with a nucleophilic group.

Further, paragraph [0064] of the present application provides non-limiting examples of active carbonates (e.g., N-hydroxysuccinimidyl carbonate, 1-benzotriazolyl carbonate, p-nitrophenyl carbonate).

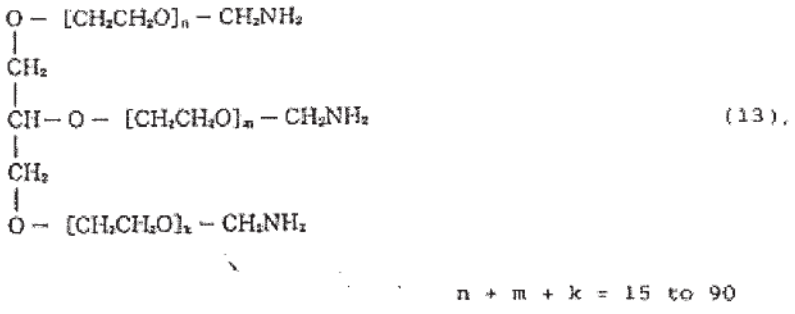
In view of the knowledge of one of skill in the art, the teachings of the prior art, and the present disclosure, the use of the term "active" in claim 5 satisfies the requirements of 35 U.S.C. §112, second paragraph. The use of the term "active" in that claim provides, at the very least, the "reasonable degree of particularity and distinctness." MPEP § 2173.02. As such, Applicants respectfully request that the present rejection be withdrawn.

V. The First Rejection Under 35 U.S.C. §102(b)

The Patent Office has rejected claims 1-3, 6 and 11 under 35 U.S.C. §102, as allegedly being anticipated by Shimomura et al. (EP 0899310). Particularly, the Office cites the "polymers having structures 13 and 15." As the present claims are novel over Shimomura, Applicants respectfully traverse this rejection.

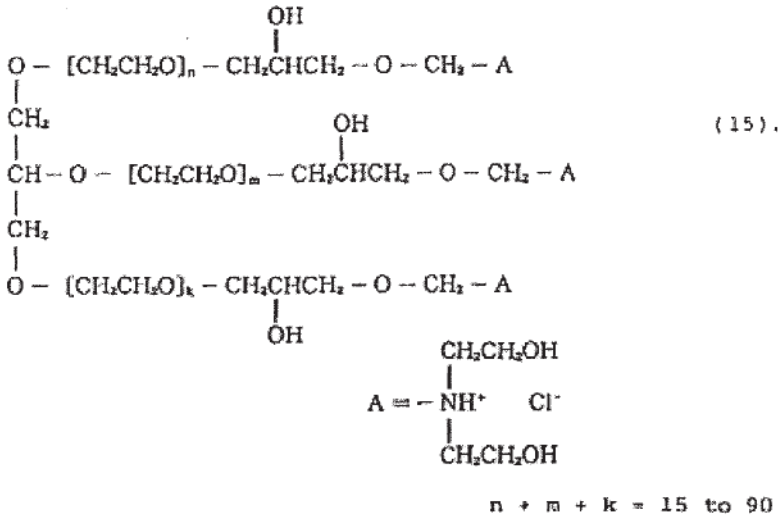
"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). MPEP § 2131. As the Office has not satisfied its burden that each and every element of the present claims is described in Shimomura, the present rejection should be withdrawn.

For example, the Office cites structure 13 and 15 for allegedly impacting the novelty of the present claims. Those structures are depicted below.



(13),

n + m + k = 15 to 90



(15),

n + m + k = 15 to 90

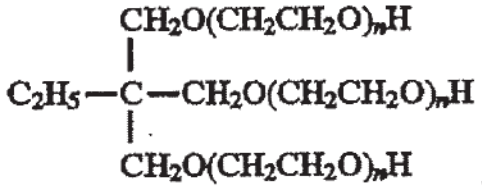
Based on the depicted structures, it is clear that the Office has not fully considered each element of the pending claims, as the referenced compounds do not disclose each and every element of the pending claims. For example, the sole independent claim (claim 1) recites the limitation that "the branched polymer has a molecular weight of about 12,000 Da to about 100,000 Da." As disclosed in both compound 13 and 15 of Shimomura, the value of n + m + k can at most be 90. Even when that sum is 90, the molecular weight of those compounds does not fall within the range of the pending claims. For example, for compound 13 the molecular weight would be about 4153 Daltons and for compound 15 the molecular weight would be about 4733 Daltons (when n + m + k = 90). As such, the Office has not satisfied its initial burden of demonstrating that each and every element of the present claims is found in Shimomura. Applicants in no way acquiesce to the Office's identification of any other limitations of the pending claims in

Shimomura and reserve the right to present arguments against this rejection should the Office maintain the present rejection.

In view of the above, Applicants respectfully request that the rejection under 35 U.S.C. §102 based Shimomura be withdrawn.

VI. The Second Rejection Under 35 U.S.C. § 102(b)

The Patent Office has rejected claims 1-3 and 8 under 35 U.S.C. §102(b), as allegedly being anticipated by Taylor et al. (U.S. Patent No. 5,684,096). Particularly the Office has cited Taylor for its disclosure of a compound of the formula:



The Office has taken the position that the structure recited above, teaches each and every element of the pending claims. Applicants respectfully traverse the rejection.

Currently pending claim 1 recite a variable "Y" which "is a functional group reactive with an electrophilic or nucleophilic group." In the present rejection, the Office asserts that the group C_2H_5 meets the limitation for Y. However, since a C_2H_5 group is not recognized as a functional group "reactive with an electrophilic or nucleophilic group" it does not meet that limitation recited in the present claims. As such, for at least this reason each and every limitation of the present claims is not found in Taylor. As such, the present rejection under §102(b) should be withdrawn.

VII. The Obviousness-type Double Patenting Rejection

Claims 1-11 are rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being unpatentable over claims 1-19 of U.S. Patent No. 7,026,440.

Applicants respectfully request that this rejection be held in abeyance until the Office has withdrawn all other rejections in this application. At that time, Applicants will address the provisional double patenting rejection.

VIII. Conclusion:

In view of the foregoing, Applicants submit that the all of pending claims satisfy the requirements of patentability and are therefore in condition for allowance. Consequently, a prompt mailing of a Notice of Allowance is earnestly solicited.

If a telephone conference would expedite the prosecution of the subject application, the Examiner is requested to call the undersigned at (415) 482-5575.

Respectfully submitted on behalf of
Nektar Therapeutics,

Date: 4/4/2012 By: _____
Timothy A. Marquart, Reg. No. 63,700

CORRESPONDENCE ADDRESS
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San Francisco, California 94158
Telephone: (415) 482-5300
Facsimile: (415) 339-5323

PATENT

Docket No. SHE0058.11

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Michael David BENTLEY et al.		Examiner:	Ganapathy KRISHNAN
Serial No.:	12/963,170	Art Unit:	1623
Filed:	December 8, 2010	Confirmation No.:	2323
Title:	**BRANCHED POLYMERS**		

TERMINAL DISCLAIMER

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Sir:

The owner, Nektar Therapeutics, of 100 percent interest in the instant application by virtue of an Assignment recorded in the U.S. Patent and Trademark Office at Reel 013871, Frame 0011, on March 24, 2003; and by virtue of an Assignment recorded in the U.S. Patent and Trademark Office at Reel 023196, Frame 0394, on August 31, 2009, hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term, as presently shortened by any terminal disclaimer, of United States Patent No. 7,026,440, issued on April 11, 2006. Nektar Therapeutics is also the owner of 100 percent interest in United States Patent No. 7,026,440, issued on April 11, 2006, by virtue of an Assignment recorded in the U.S. Patent and Trademark Office at Reel 013871, Frame 0011, on March 24, 2003; and by virtue of an Assignment recorded in the U.S. Patent and Trademark Office at Reel 023196, Frame 0394, on August 31, 2009.

The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the above-listed patents are commonly

owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the above-listed patents, as presently shortened by any terminal disclaimer, in the event that said patent later: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 C.F.R. §1.321, has all claims cancelled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

AUTHORITY OF UNDERSIGNED

The undersigned is an attorney of record.

FEE PAYMENT

The Commissioner is hereby authorized and requested to charge the Terminal Disclaimer fee of $160.00, in accordance with 37 C.F.R. §1.20(d), to Deposit Account No. 50-0348. The Commissioner is also authorized and requested to charge any additional fee(s) to Deposit Account No. 50-0348.

Respectfully submitted on behalf of
Nektar Therapeutics,

Date: 5/3/12 By: _____
Timothy A. Marquart, Reg. No. 63,700

CORRESPONDENCE ADDRESS:
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UNITED STATES PATENT AND TRADEMARK OFFICE

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www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/963,170	12/08/2010	Michael David Bentley	SHE0058.11	2323

21968 7590 05/24/2012

NEKTAR THERAPEUTICS
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EXAMINER	
KRISHNAN, GANAPATHY	

ART UNIT	PAPER NUMBER
1623	

DATE MAILED: 05/24/2012

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 0 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 0 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

PTOL-85 (Rev. 02/11)

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Examiner-Initiated Interview Summary	Application No.	Applicant(s)	
	12/963,170	BENTLEY ET AL.	
	Examiner	Art Unit	
	GANAPATHY KRISHNAN	1623	

All participants (applicant, applicant's representative, PTO personnel):

(1) *GANAPATHY KRISHNAN*. (3)_____.

(2) *Mr. Timothy Marquart*. (4)_____.

Date of Interview: *03 May 2012*.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☐112 ☐102 ☐103 ☒Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: *None*.

Identification of prior art discussed: *None*.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

Informed the applicant that inserting the limitation of claim 2 into claim 1 would distinguish the pending claims over the prior art of record and filing of a Terminal Disclaimer would overcome the double patenting rejection of record, which is the only remaining issue. Applicants agreed and authorized an Examiner's amendment and will also be filing an e-TD..

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/Ganapathy Krishnan/ Examiner, Art Unit 1623	

Notice of Allowability	Application No.	Applicant(s)	
	12/963,170	BENTLEY ET AL.	
	Examiner	Art Unit	
	GANAPATHY KRISHNAN	1623	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *04 April 2012*.
2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.
3. ☒ The allowed claim(s) is/are *1-11*.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.
6. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
7. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date 04/04/2012
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application
6. ☒ Interview Summary (PTO-413), Paper No./Mail Date *03 May 2012* .
7. ☐ Examiner's Amendment/Comment
8. ☒ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

/SHAOJIA ANNA JIANG/
Supervisory Patent Examiner, Art Unit 1623

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Mr. Tim Marquart on 15 May 2012.

In the Amendments to the Claims of 04 April 2012:

In claim 1, line 10, the term "polyethylene glycol (PEG)" has been inserted after the terms "non-peptidic".

Claim 2 has been **cancelled**.

In claims 3 and 4, line 1, the recitation "Claim 2" has been has been replaced by the recitation "Claim 1".

REASONS FOR ALLOWANCE

The following is an examiner's statement of reasons for allowance:

The rejection of Claim 5 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention, recitation of the term "active" has been withdrawn in view of applicants remarks and the guidance provided in the specification, which would be well understood by one of ordinary skill in the art.

The rejection of Claims 1-3, 6 and 11 under 35 U.S.C. 102(b) as being anticipated by Shimomura et al (EP 0899310) has been overcome in view of applicants arguments. Structures 13 ad 15 disclosed by Shimomura have the X'-POLY moiety which is attached to a three carbon aliphatic hydrocarbon chain. But the two structures do not have the Y moiety (support seen for amendment done to the limitation of Y) and the structures of Shimomura do not also have the recited molecular weight as recited in claim 1. Therefore, Shimomura's compounds do not teach each and every limitation of instant claim 1 and also do not meet the limitations of dependent claims 2, 3, 6 and 11.

The rejection of Claims 1-3 and 8 under 35 U.S.C. 102(b) as being anticipated by Taylor et al (US 5684096) has been withdrawn. Taylor's compound has an ethyl group attached to the three carbon aliphatic chain, which is not a functional group reactive with an electrophilic or nucleophilic group as defined for the moiety Y in instant formula. The structural formula disclosed by Taylor at col. 2, line 20, the three carbon alkyl chain on the left that bears an OH group (nucleophilic group) has polymeric groups (POLY) attached to the end carbons. The polymeric group on the left is a polyalkylene oxide chain having propyl and butyl groups. The polymeric group on the right is a hydroxy and bis-phenol A substituted propyloxy group. This compound of Taylor is not seen to read on the formula recited in instant claim 1 and is not seen to render the instant formula obvious either.

The terminal disclaimer filed on 03 May 2012, disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of U.S. 7,026,440 patent has been reviewed and is accepted. The terminal disclaimer has been recorded. Therefore, the obviousness-type double patenting rejection of Claims 1-11 on the ground of

nonstatutory obviousness-type double patenting as being unpatentable over claims 1-19 of U.S. Patent No. 7,026,440 ('440) of record in the Office Action dated 04 January 2012 is withdrawn.

Instant claims 1-11 are therefore allowable.

Any comments considered necessary by the applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance".

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ganapathy Krishnan whose telephone number is 571-272-0654. The examiner can normally be reached on 9.00am-5pm; Mon-Fri. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Shaojia A. Jiang can be reached on 571-272-0627. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Ganapathy Krishnan/
Examiner, Art Unit 1623.

/SHAOJIA ANNA JIANG/
Supervisory Patent Examiner
Art Unit 1623

PATENT

Docket No. SHE0058.11

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Michael David BENTLEY et al.		Examiner:	Ganapathy KRISHNAN
Serial No.:	12/963,170	Art Unit:	1623
Filed:	December 8, 2010	Confirmation No.:	2323
Title:	**BRANCHED POLYMERS**		

APPLICANTS' SUBSTANCE OF EXAMINER INITIATED INTERVIEW

On May 3, 2012, Examiner Krishnan contacted Applicants' representative, Timothy Marquart, by telephone to discuss claims 1 to 3 of the above-referenced application. During that telephone conference, the Examiner proposed amendments to independent claim 1 and dependent claims 2 and 3. Particularly the Examiner proposed the inclusion of "polyethylene glycol PEG" after "non-peptidic" in claim 1. While the Examiner-Initiated Interview Summary (May 24, 2012) states that this amendment "would distinguish the pending claims over the prior art of record," Applicants agreed to the suggested amendment as a means to advance prosecution and not in acquiescence to the basis any of the rejections of record. In other words, Applicants agree to enter the amendments, but do not agree with the Examiner's position regarding the prior art. Applicants maintain that the arguments on record regarding the prior art provide sufficient basis for overcoming those rejections.

No fees other than those that may be identified in the papers accompanying the present Reply are believed to be due; however, the Commissioner is hereby authorized to charge any additional fees to Deposit Account No. 50-0348.

If a telephone conference would expedite the prosecution of the subject application, the Examiner is requested to call the undersigned at (415) 482-5575.

Respectfully submitted on behalf of
Nektar Therapeutics,

Date: August 24, 2012 By: /Timothy A. Marquart/
Timothy A. Marquart, Reg. No. 63,700

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