WHAT IS CLAIMED IS:

1. An antibody conjugate comprising (1) an anti-VEGF-A antibody and (2) a phosphorylcholine containing polymer, wherein the polymer is covalently bonded to the antibody at a cysteine outside a variable region of the antibody wherein said cysteine has been added via recombinant DNA technology.

2. The antibody conjugate according to claim 1 wherein:

(a) the anti-VEGF-A antibody comprises a light chain and a heavy chain, said heavy chain comprising an Fc region;

(b) the arrangement in (a), wherein the cysteine is in the Fc region of the heavy chain;

(c) the arrangement in (b), wherein the anti-VEGF-A antibody is an immunoglobulin G (IgG)

(d) the arrangement in (b), wherein the anti-VEGF-A antibody heavy chain comprises CDR_H1:GYDFTHYGMN (SEQ ID NO: 9), CDR_H2: WINTYTGEPTYAADFKR (SEQ ID NO: 10), and CDR_H3: YPYYYGTSHWYFDV (SEQ ID NO: 11), and the anti-VEGF-A light chain comprises CDR_L1: SASQDISNYLN (SEQ ID NO: 12), CDR_L2: FTSSLHS (SEQ ID NO: 13), and CDR_L3: QQYSTVPWT (SEQ ID NO: 14);

(e) the arrangement in (c), wherein the anti-VEGF-A heavy chain isotype is human IgG1;

(f) the arrangement in (e), wherein the heavy chain constant domain of the anti-VEGF-A antibody has one or more mutations relative to the constant domain of human IgG1 to modulate effector function;

(g) the arrangement in (f), wherein the mutations are to one or more of the following amino acid positions (EU numbering): E233X, L234X, L235X, G236X, G237X, A327X, A330X, and P331X wherein X is any natural or unnatural amino acid;

(h) the arrangement in (g), wherein the mutations are selected from the group consisting of (EU numbering): E233P, L234V, L234A, L235A, G237A, A327G, A330S, and P331S;

(i) the arrangement in (h) comprising the following mutations: L234A, L235A, and G237A (EU numbering);

(j) the arrangement in (i), wherein the cysteine is in the anti-VEGF-A antibody heavy chain and is Q347C (EU numbering) or L443C (EU numbering);

(k) the arrangement in (j), w[RefreshLetterhead] - This method or property is not available because this command is not available for reading.herein the sequence of the anti-VEGF-A antibody heavy chain is SEQ ID NO. 1 and the sequence of the anti-VEGF-A light chain is SEQ ID NO. 2;

(l) the arrangement in (j), wherein the cysteine is L443C (EU numbering);

(m) the antibody conjugate is purified;

(n) the arrangement in (m), wherein the antibody conjugate is polydisperse in the polymer; or

(o) the arrangement in (n), wherein the polymer has a polydispersity value (PDI) of less than about 1.2; or

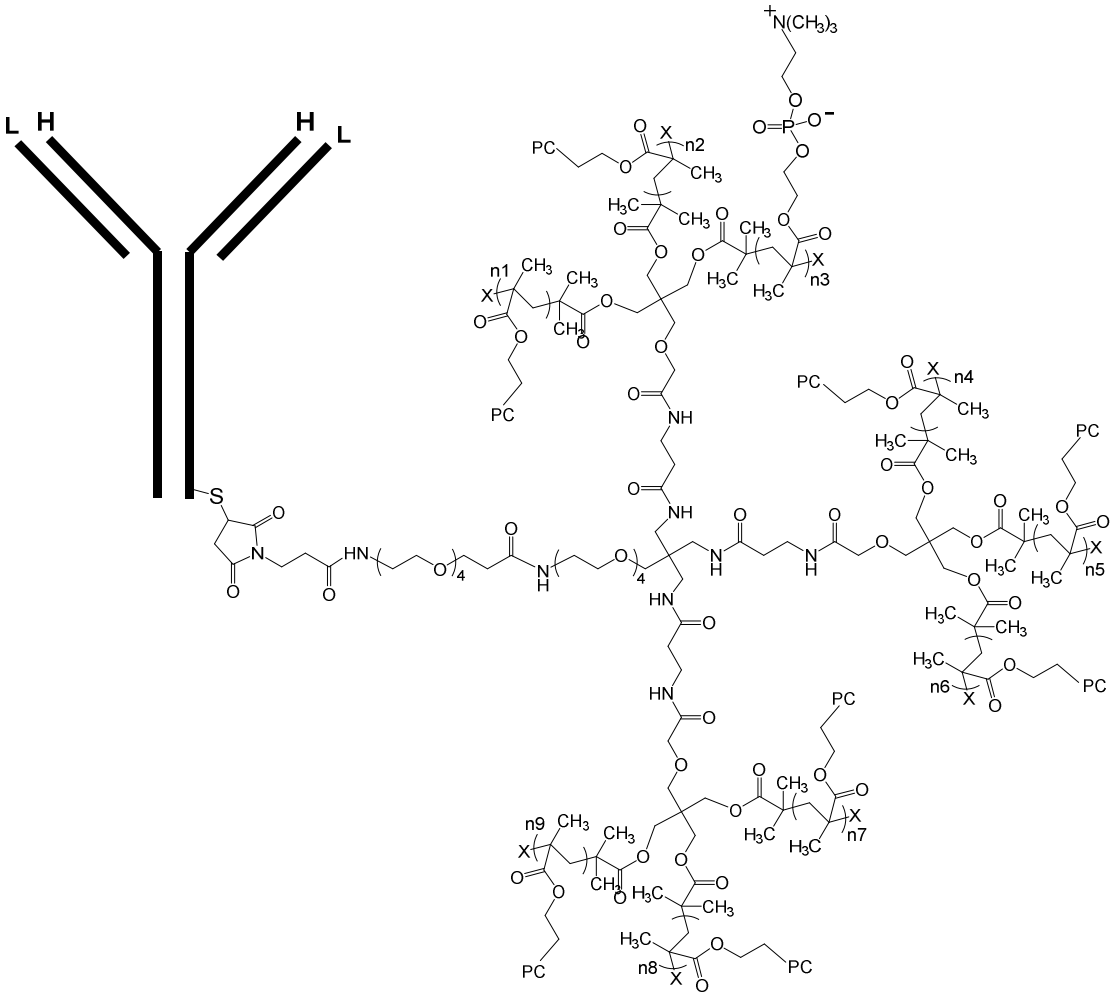
(p) wherein the anti-VEGF-A antibody is an anti-VEGF-A immunoglobulin G (IgG) and the phosphorylcholine containing polymer comprises MPC monomers, wherein the sequence of the anti-VEGF-A antibody heavy chain is SEQ ID NO: 1, and the sequence of the anti-VEGF-A antibody light chain is SEQ ID NO: 2, and wherein the antibody is bonded at C449 in SEQ ID NO. 1 to the polymer;

(q) the arrangement in (p), wherein

the polymer has 9 arms; and

the polymer has a molecular weight of between about 600,000 to about 900,000 Da; or

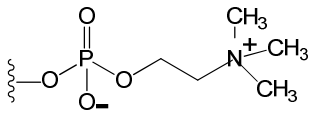
(r) the arrangement in (p), which has the following structure:



wherein:

each heavy chain of the anti-VEGF-A antibody is denoted by the letter H, and each light chain of the anti-VEGF-A antibody is denoted by the letter L;

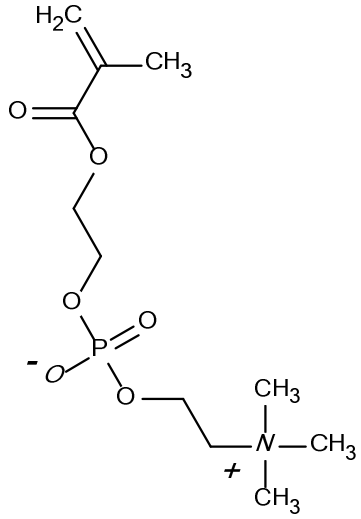
the polymer is bonded to the anti-VEGF-A antibody through the sulfhydryl of C443 (EU numbering), which bond is depicted on one of the heavy chains;

PC is , where the curvy line indicates the point of attachment to the rest of the polymer, where X=a) OR where R=H, methyl, ethyl, propyl, isopropyl, b) H, or c) any halide, including Br; or

n1, n2, n3, n4, n5, n6, n7, n8 and n9 are the same or different such that the sum of n1, n2, n3, n4, n5, n6, n6, n7, n8 and n9 is 2500 plus or minus 15%.

3. The antibody conjugate according to any one of claims 1-2,

(a) wherein the phosphorylcholine containing polymer comprises 2-(methacryloyloxyethyl)-2'-(trimethylammonium)ethyl phosphate (MPC) monomers as set forth below:



;

(b) the arrangement in (a), wherein the polymer has three or more arms or is synthesized with an initiator comprising 3 or more polymer initiation sites;

(c) the arrangement in (b), wherein the polymer has 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 arms or is synthesized with an initiator comprising 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 polymer initiation sites;

(d) the arrangement in (c), wherein the polymer has 2, 3, 6, or 9 arms or is synthesized with an initiator comprising 2, 3, 6, or 9 polymer initiation sites;

(e) the arrangement in (d), wherein the polymer has 9 arms or is synthesized with an initiator comprising 9 polymer initiation sites;

(f) the arrangement in (e), wherein the polymer has a molecular weight between about 300,000 and about 1,750,000 Da as measured by size exclusion chromatography – multi angle light scattering (hereinafter “SEC-MALS”);

(g) the arrangement in (f), wherein the polymer has a molecular weight between about 500,000 and about 1,000,000 Da; or

(h) the arrangement in (g), wherein the polymer has a molecular weight between about 750,000 to about 850,000 Da.

4. A pharmaceutical composition comprising:

(a) the antibody conjugate according to any one of claims 1-3 in a liquid solution; or

(b) the antibody conjugate according to any one of claims 1-3 and a pharmaceutically acceptable carrier.

5. An anti-VEGF-A antibody which comprises a heavy chain and a light chain, wherein:

(a) the heavy chain comprises CDR_H1: GYDFTHYGMN (SEQ ID NO: 9), CDR_H2: WINTYTGEPTYAADFKR (SEQ ID NO: 10), and CDR_H3: YPYYYGTSHWYFDV (SEQ ID NO: 11) and the light chain comprises CDR_L1: SASQDISNYLN (SEQ ID NO: 12), CDR_L2: FTSSLHS (SEQ ID NO: 13), and CDR_L3: QQYSTVPWT (SEQ ID NO: 14); and the heavy chain isotype is IgG1, wherein the IgG1 constant domain comprises one or more of the following mutations to modulate effector function (EU numbering): E233P, L234V, L234A, L235A, G237A, A327G, A330S, and P331S;

(b) the arrangement in (a), comprising the following mutations (EU numbering): L234A, L235A, and G237A;

(c) the arrangement in (a), wherein the sequence of the anti-VEGF-A heavy chain is SEQ ID NO. 1 and sequence of the anti-VEGF-A light chain is SEQ ID NO. 2; or

(d) the arrangement in (a), which is an IgG.

6. A method for treatment or prophylaxis of an ocular disease comprising administering the antibody conjugate according to any one of claims 1-3, or the pharmaceutical composition according to claim 4.

7. The method for treatment or prophylaxis according to claim 6,

(a) wherein the ocular disease is selected from the group consisting of diabetic retinopathy, choroidal neovascularization (CNV), age-related macular degeneration (AMD),

diabetic macular edema (DME), pathological myopia, von Hippel-Lindau disease, histoplasmosis of the eye, central retinal vein occlusion (CRVO), branched central retinal vein occlusion (BRVO), corneal neovascularization, retinal neovascularization, retinopathy of prematurity (ROP), subconjunctival hemorrhage, and hypertensive retinopathy; or

(b) wherein the disease is diabetic retinopathy.

8. A method of making an antibody conjugate comprising an anti-VEGF-A antibody conjugated to a phosphorylcholine containing polymer, the method comprising the step of:

conjugating an anti-VEGF-A antibody to a phosphorylcholine containing polymer;

wherein the anti-VEGF-A antibody comprises a cysteine residue added via recombinant DNA technology and wherein the cysteine is outside a variable region of the antibody;

wherein the phosphorylcholine containing polymer comprises a sulfhydryl specific reacting group selected from the group consisting of a maleimide, a vinylsulfone, an orthopyridyl-disulfide, and an iodoacetamide; and

wherein the sulfhydryl specific reacting group on the phosphorylcholine containing polymer reacts with the cysteine residue on the anti-VEGF-A antibody to make the antibody conjugate.

9. The method according to claim 8,

(a) wherein the anti-VEGF-A antibody is an immunoglobulin G (IgG) and the cysteine is in the Fc region of the antibody;

(b) wherein the anti-VEGF-A antibody comprises a light chain and a heavy chain, wherein the anti-VEGF-A antibody heavy chain comprises CDR_H1: GYDFTHYGMN (SEQ ID NO: 9), CDR_H2: WINTYTGEPTYAADFKR (SEQ ID NO: 10), and CDR_H3: YPYYYGTSHWYFDV (SEQ ID NO: 11), and the anti-VEGF-A antibody light chain comprises CDR_L1: SASQDISNYLN (SEQ ID NO: 12), CDR_L2: FTSSLHS (SEQ ID NO: 13), and CDR_L3: QQYSTVPWT (SEQ ID NO: 14);

(c) the method in (b), wherein the anti-VEGF-A antibody heavy chain isotype is IgG1;

(d) the method in (c), wherein the anti-VEGF-A antibody constant domain has one or more mutations relative to an IgG1 constant domain to modulate effector function;

(e) the method in (d), wherein the mutations are to one or more of the following amino acid positions (EU numbering): E233X, L234X, L235X, G236X, G237X, G236X, D270X, K322X, A327X, P329X, A330X, A330X, P331X, and P331X, wherein X is any natural or non-natural amino acid;

(f) the method in (e), wherein the mutations are selected from the group consisting of (EU numbering) E233P, L234V, L234A, L235A, G237A, A327G, A330S, and P331S;

(g) the method in (f), wherein the mutations are (EU numbering) L234A, L235A, and G237A;

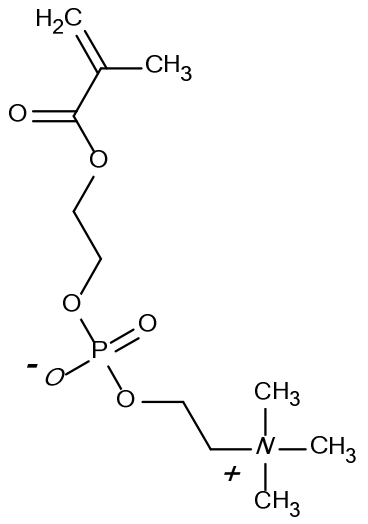
(h) the method in (g), wherein the cysteine residue added by recombinant DNA technology is selected from the group consisting of Q347C (EU numbering) and L443C (EU numbering);

(i) the method in (h), wherein the cysteine residue added by recombinant DNA technology is L443C (EU numbering);

(j) the method in (h), wherein the sequence of the anti-VEGF-A antibody heavy chain is SEQ ID NO. 1 and sequence of the anti-VEGF-A antibody light chain is SEQ ID NO. 2;

(k) wherein the sulfhydral specific reacting group is maleimide.;

(l) the method in (k), wherein wherein the phosphorylcholine containing polymer comprises 2-(methacryloyloxyethyl)-2'-(trimethylammonium)ethyl phosphate (MPC) monomers as set forth below:



;

(m) the method in (l), wherein the polymer has three or more arms or is synthesized with an initiator comprising 3 polymer initiation sites;

(n) the method in (m), wherein the polymer has 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 arms or is synthesized with an initiator comprising 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 polymer initiation sites;

(o) the method in (n), wherein the polymer has 2, 3, 6, or 9 arms or is synthesized with an initiator comprising 2, 3, 6, or, 9, polymer initiation sites.

(p) the method in (o), wherein the polymer has 9 arms or is synthesized with an initiator comprising 9 polymer initiation sites;

(q) the method in (p), wherein the polymer has a molecular weight between about 300,000 and 1,750,000 Da;

(r) the method in (q), wherein the polymer has a molecular weight between about 600,000 and 1,000,000 Da.;

(s) the method in (r), wherein the polymer has a molecular weight of between about 600,000 to 850,000 Da;

(t) further comprising the step of contacting the anti-VEGF-A antibody with a thiol reductant under conditions that produce a reduced cysteine sulfhydryl group to produce a reduced anti-VEGF-A antibody in which all cysteine residues are reduced;

(u) the method of (t), wherein the thiol reductant is selected from the group consisting of Tris[2-carboxyethyl]phosphine hydrochloride (TCEP), dithiothreitol (DTT), dithioerythritol (DTE), sodium borohydride ($NaBH_4$), sodium cyanoborohydride ($NaCNBH_3$), β-mercaptoethanol (BME), cysteine hydrochloride, and cysteine;

(v) the method of (u), wherein the thiol reductant is TCEP;

(w) the method of (v), wherein the concentration of the thiol reductant is between 1 and 100 fold molar excess relative to the concentration of the anti-VEGF-A antibody;

(x) the method of (w), wherein the concentration of the thiol reductant is between 20 to 50 fold molar excess relative to the concentration of the anti-VEGF-A antibody;

(y) the method of (w), further comprising the steps of removing the thiol reductant from the reduced anti-VEGF-A antibody, and treating the reduced anti-VEGF-A antibody with an oxidizing agent;

(z) the method of (x), wherein the oxidizing agent is air, aqueous $CuSO_4$, or dehydroascorbic acid (DHAA);

(aa) further comprising the step of purifying the antibody conjugate;

(bb) the method of (aa), wherein the antibody conjugate is purified using a technique selected from the group consisting of ion exchange chromatography, hydrophobic interaction chromatography, size exclusion chromatography, affinity chromatography, and combinations thereof.

(cc) the method of (bb), wherein the purified antibody conjugate retains at least 20% biological activity relative to an unconjugated anti-VEGF-A antibody.

(dd) the method of (cc), wherein the purified antibody conjugate retains at least 50% biological activity relative to an unconjugated anti-VEGF-A antibody;

(ee) the method of (dd), wherein the purified antibody conjugate retains at least 90% biological activity relative to an unconjugated anti-VEGF-A antibody;

(ff) the method of (dd), wherein the purified antibody conjugate has an increased half-life relative to an unconjugated anti-VEGF-A antibody;

(gg) the method of (ff), wherein the purified antibody conjugate has at least a 1.5 fold increase in half-life relative to an unconjugated anti-VEGF-A antibody;

(hh) wherein the method further comprises the step of polymerizing a free radically polymerizable phosphorylcholine containing monomer in a polymerization medium to provide the phosphorylcholine containing polymer, the medium comprising:

the radically polymerizable phosphorylcholine containing monomer;

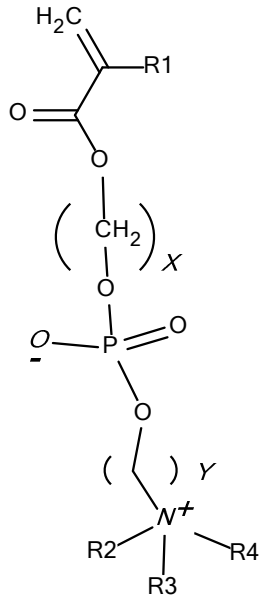
a transition metal catalyst $M_t^{(q-1)+}$ wherein M_t is a transition metal, q is the maximum oxidation state of the metal and q-1 is the oxidation state of the metal, wherein the metal can act as a catalyst, wherein the transition metal catalyst is supplied as a salt of the form $Mt^{(q-1)+}X'_{(q-1)}$, wherein X' is a counterion or group, or wherein the transition metal catalyst is supplied in situ by providing the inactive metal salt at its highest oxidation state $M_t^{q+}X'_q$ together with a

reducing agent that is capable of reducing the transition metal from the oxidized inactive state to the reduced active state;

a ligand; and

an initiator;

(ii) the method of (hh), wherein the radically polymerizable phosphorylcholine containing monomer is



wherein R1 is H or C_{1-6} alkyl;

R2, R3, R4 are each methyl; and

X and Y are each 2;

(jj) the method of (ii), wherein Mt is selected from the group consisting of Cu, Fe, Ru, Cr, Mo, W, Mn, Rh, Re, Co, V, Zn, Au, and Ag;

(kk) the method of (jj), wherein the metal catalyst is supplied as a salt of the form $Mt^{(q-1)+}X'_{(q-1)}$;

(ll) the method of (kk), wherein $M_t^{(q-1)+}$ is selected from the group consisting of Cu^{1+}, Fe^{2+}, Ru^{2+}, Cr^{2+}, Mo^{2+}, W^{2+}, Mn^{3+}, Rh^{3+}, Re^{2+}, Co^{+}, V^{2+}, Zn^{+}, Au^{+}, and Ag^{+} and X' is selected from the group consisting of halogen, C_{1-6} alkoxy, $(SO_4)_{1/2}$, $(PO_4)_{1/3}$, $(R7PO_4)_{1/2}$, $(R7_2PO_4)$, triflate, hexafluorophosphate, methanesulfonate, arylsulfonate, CN and $R7CO_2$, where R7 is H

or a straight or branched C_{1-6} alkyl group which may be substituted from 1 to 5 times with a halogen;

(mm) the method of (ll), wherein $M_t^{(q-1)+}$ is Cu^{1+} and X' is Br;

(nn) the method of (ii), wherein $M_t^{(q-1)+}$ is supplied in situ;

(oo) the method of (nn), wherein $M_t^{q+}X_q$ is $CuBr_2$;

(pp) the method of (ii), wherein the reducing agent is an inorganic compound;

(qq) the method of (pp), wherein the inorganic compound is selected from the group consisting of a sulfur compound of a low oxidation level, sodium hydrogen sulfite, an inorganic salt comprising a metal ion, a metal, hydrazine hydrate, and derivatives of such compounds;

(rr) the method of (qq), wherein the reducing agent is a metal;

(ss) the method of (rr), wherein the reducing agent is Cu^0;

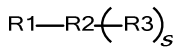
(tt) the method of (ii), wherein the reducing agent is an organic compound;

(uu) the method of (tt), wherein the organic compound is selected from the group consisting of alkylthiols, mercaptoethanol, or carbonyl compounds that can be easily enolized, ascorbic acid, acetyl acetonate, camphosulfonic acid, hydroxy acetone, reducing sugars, monosaccharides, glucose, aldehydes, and derivatives of such organic compounds;

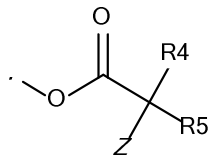
(vv) the method of (ii), wherein the ligand is selected from the group consisting of 2,2'-bipyridine, 4,4'-Di-5-nonyl-2,2'-bipyridine, 4,4-dinonyl-2,2'-dipyridyl, 4,4',4''-tris(5-nonyl)-2,2':6',2''-terpyridine, N,N,N',N',N''-Pentamethyldiethylenetriamine, 1,1,4,7,10,10-Hexamethyltriethylenetetramine, Tris(2-dimethylaminoethyl)amine**,** N,N-bis(2-pyridylmethyl)octadecylamine, N,N,N',N'-tetra[(2-pyridal)methyl]ethylenediamine, tris[(2-pyridyl)methyl]amine**,** tris(2-aminoethyl)amine, tris(2-bis(3-butoxy-3-oxopropyl)aminoethyl)amine, tris(2-bis(3-(2-ethylhexoxy)-3-oxopropyl)aminoethyl)amine, and Tris(2-bis(3-dodecoxy-3-oxopropyl)aminoethyl)amine;

(ww) the method of (vv), wherein the ligand is 2,2'-bipyridine;

(xx) the method of (ii), wherein wherein the initiator has the structure:



wherein R1 is a nucleophilic reactive group, R2 comprises a linker, and R3 comprises a polymer synthesis initiator moiety having the structure



wherein R4 and R5 and are the same or different and are selected from the group consisting of alkyl, substituted alkyl, alkylene, alkoxy, carboxyalkyl, haloalkyl, cycloalkyl, cyclic alkyl ether, alkenyl, alkenylene, alkynyl, alkynylene, cycloalkylene, heterocycloalkyl, heterocycloalkylene, aryl, arylene, arylene-oxy, heteroaryl, amino, amido, and any combination thereof;

Z is a halogen or CN; and s is an integer between 1 and 20;

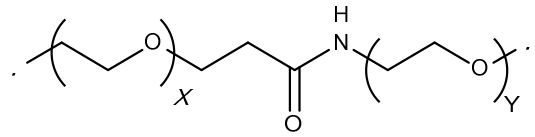
(yy) the method of (xx), wherein Z is Br and R4 and R5 are each methyl;

(zz) the method of (yy), wherein R1 is selected from the group consisting of NH_2-, OH-, and SH-;

(aaa) the method of (zz), wherein R1 is NH_2-;

(bbb) the method of (aaa), wherein R2 is alkyl, substituted alkyl, alkylene, alkoxy, carboxyalkyl, haloalkyl, cycloalkyl, cyclic alkyl ether, alkenyl, alkenylene, alkynyl, alkynylene, cycloalkylene, heterocycloalkyl, heterocycloalkylene, aryl, arylene, arylene-oxy, heteroaryl, amino, amido, and any combination thereof.

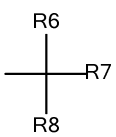
(ccc) the method of (bbb), wherein R2 is



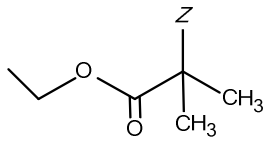
wherein X and Y are the same or different and are integers from 1-20;

(ddd) the method of (ccc), wherein X and Y are each 4;

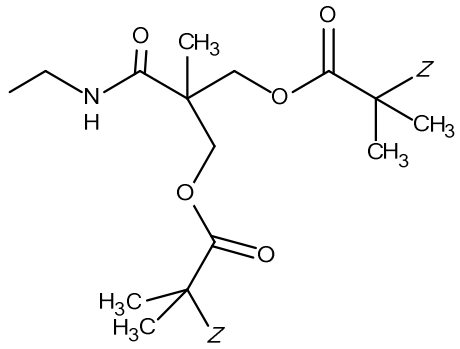
(eee) the method of (ddd), wherein R3 further comprises



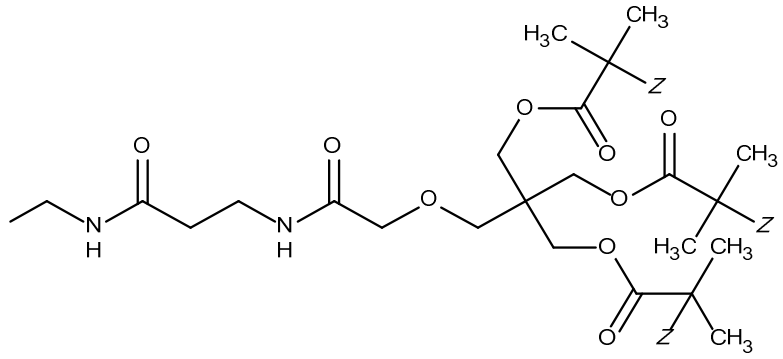
wherein R6, R7 and R8 are the same or different and are selected from the group consisting of



,



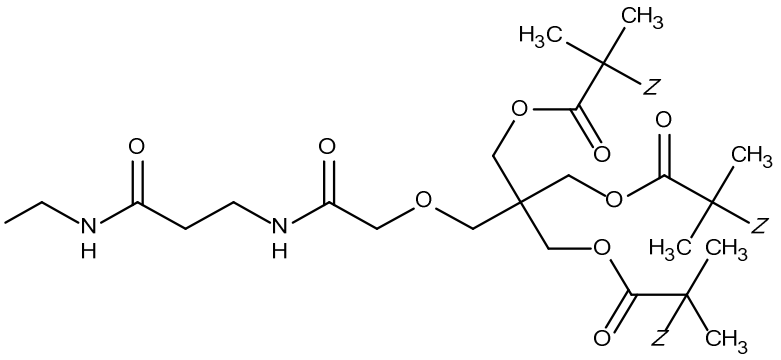
and



wherein Z is NCS, F, Cl, Br or I;

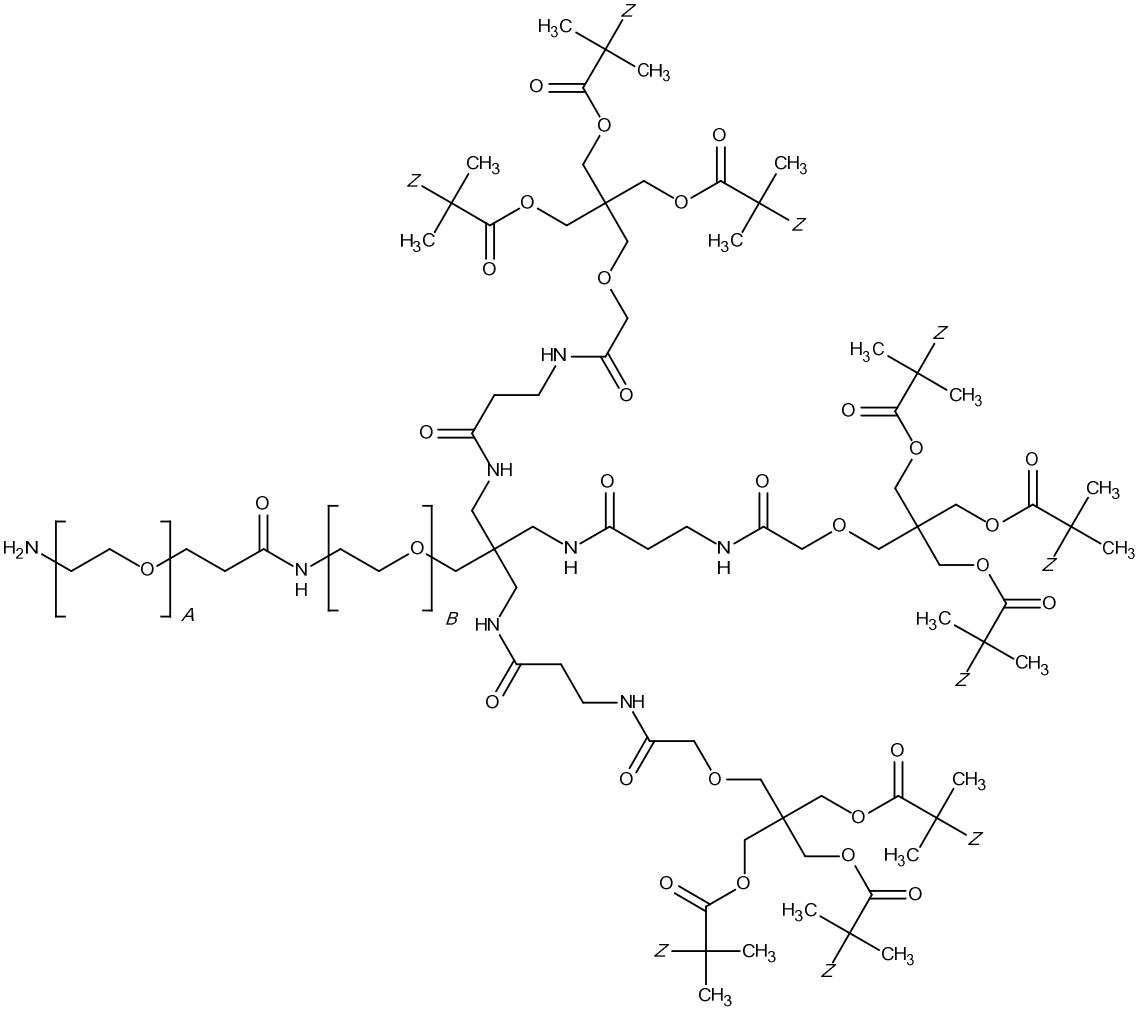
(fff) the method of (eee), wherein Z is Br;

(ggg) the method of (eee), wherein R6, R7 and R8 are each



;

(hhh) the method of (ggg), wherein the initiator has the structure

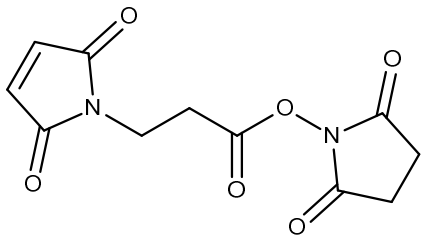


wherein A and B are the same or different and are integers from 2 to 12 and Z is Br’

(iii) the method of (hhh), wherein A and B are each 4;

(jjj) the method of (iii), further comprising the step of reacting the polymer with a maleimide reagent to provide a polymer having a terminal maleimide; or

(kkk) the method of (jjj), wherein the maleimide compound is



.

10. An antibody comprising:

a heavy chain amino acid variable region that comprises SEQ ID NO 1; and

a light chain amino acid variable region that comprises SEQ ID NO. 2.

11. The antibody of claim 10, wherein

(a) the antibody is a human IgG1, and wherein a heavy chain constant domain comprises one or more mutations that reduce an immune-mediated effector function;

(b) wherein the antibody is further conjugated to a polymer to form a bioconjugate, and wherein the bioconjugate has a molecular weight between about 350,000 and 1,900,000 Daltons; or

(c) the arrangement of (b), wherein the PolyDispersity Index (PDI) is equal to or less than 1.5.

12. An antibody that binds to VEGF-A, the antibody comprising:

a CDR_H1 that is the CDR_H1 in SEQ ID NO: 1;

a CDR_H2 that is the CDR_H2 in SEQ ID NO: 1;

a CDR_H3 that is the CDR_H3 in SEQ ID NO: 1;

a CDR_L1 that is the CDR_L1 in SEQ ID NO: 2;

a CDR_L2 that is the CDR_L2 in SEQ ID NO: 2;

a CDR_L3 that is the CDR_L3 in SEQ ID NO: 2;

at least one of the following mutations (EU numbering): L234A, L235A, and G237A; and

at least one of the following mutations(EU numbering): Q347C or L443C.

13. The antibody of claim 12, wherein the antibody comprises all three of the following mutations (EU numbering) L234A, L235A, and G237A, and wherein the antibody comprises L443C (EU numbering).

14. A process for preparing a conjugated protein, the process comprising:

reducing one or more cysteines in a protein to form a decapped protein in a solution;

reoxidizing the decapped protein to restore at least one disulfide linkage in the reduced protein while ensuring that an engineered cysteine residue in the protein remains in a free thiol form to thereby form a reoxidized decapped protein in the solution;

adding at least one excipient to the solution, wherein the excipient reduces a polymer induced protein precipitation;

adding a polymer to the solution; and

conjugating the polymer to the reoxidized decapped protein at the engineered cysteine residue to form a conjugated protein.

15. The process of claim 14, wherein

(a) the protein is an antibody, an antibody protein fusion or a binding fragment thereof;

(b) the process of (a), wherein the excipient is an acid or a base;

(c) the process of (b), wherein the excipient is selected from the group consisting of at least one of: a detergent, a sugar, and a charged amino acid;

(d) wherein reaction of a polymer with the reduced protein occurs under aqueous conditions between pH 6.0 to pH 8.5.;

(e) wherein an amount of the reduced protein is less than an amount of the polymer;

(f) wherein the polymer is conjugated to the protein at 2-37 degrees Celsius;

(g) further comprising the process of contacting a solution comprising the conjugated protein to an ion exchange medium or hydrophobic interaction chromatography or affinity chromatography medium;

(h) the process of (g), wherein the ion exchange medium or hydrophobic interaction chromatography or affinity chromatography medium separates the conjugated protein from the free polymer and from the reoxidized decapped protein;

(i) wherein the polymer comprises a zwitterion;

(j) wherein the polymer comprises a phosphorylcholine; or

(k) wherein the polymer comprises a PEG linker bridging a center of a polymer branching point to the maleimide functional group.

16. An anti-VEGF antibody conjugate that is capable of blocking at least 90% of an interaction between a VEGF ligand and a VEGF-receptor.

17. An anti-VEGF antibody conjugate that blocks at least 95% of an interaction between a VEGF ligand and a VEGF-receptor.

18. An anti-VEGF antibody that blocks at least 90% of an interaction between a VEGF ligand and a VEGF-receptor.