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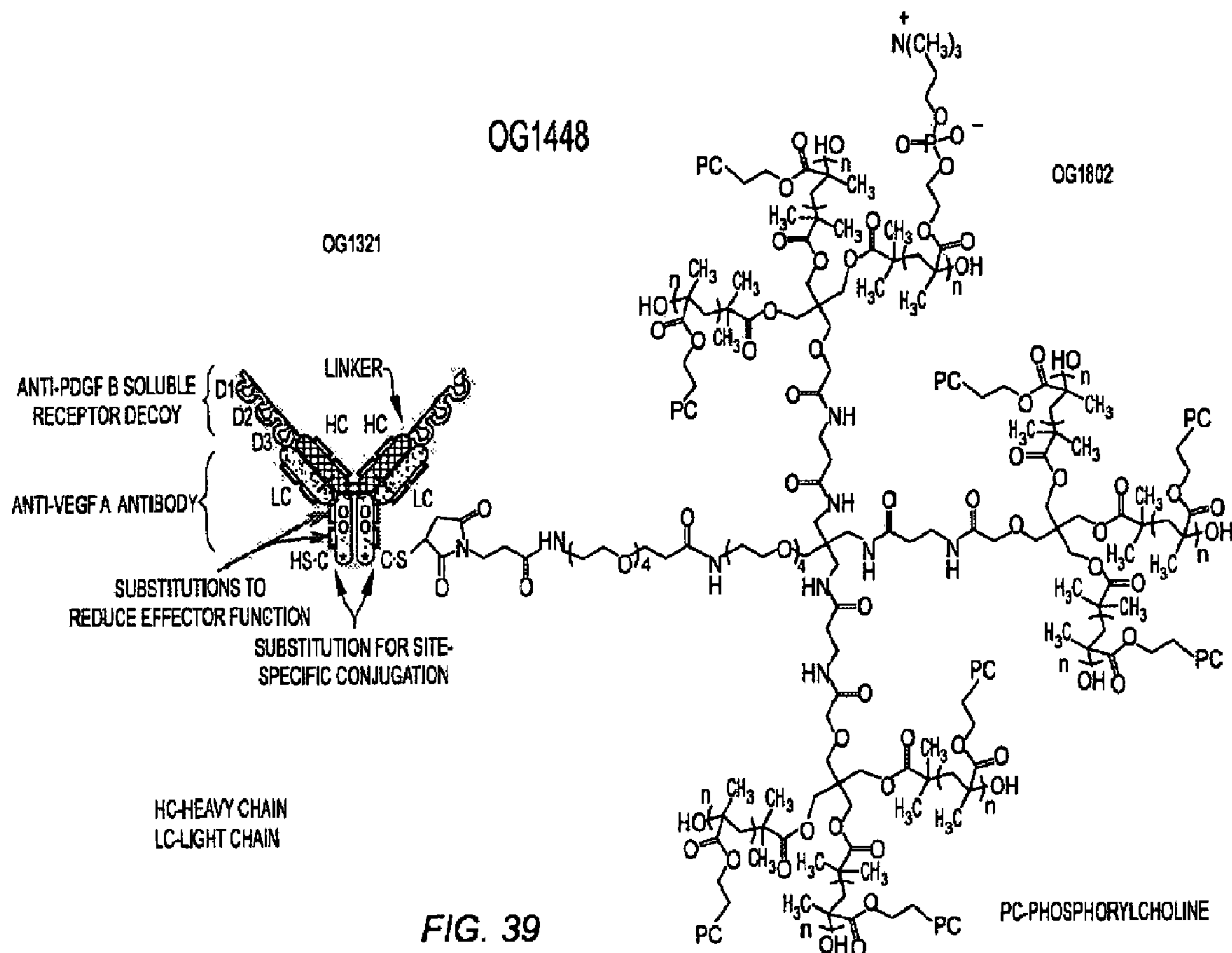
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(54) **Titre : DOUBLES ANTAGONISTES DE PDGF/VEGF**

(54) **Title: DUAL PDGF/VEGF ANTAGONISTS**



(57) **Abrégé/Abstract:**

The invention provides a dual VEGF/PDGF antagonist comprising a VEGF antagonist linked to a PDGF antagonist. The VEGF antagonist is an antibody to a VEGF or VEGFR or is a VEGFR extracellular trap segment (i.e., a segment from the extracellular

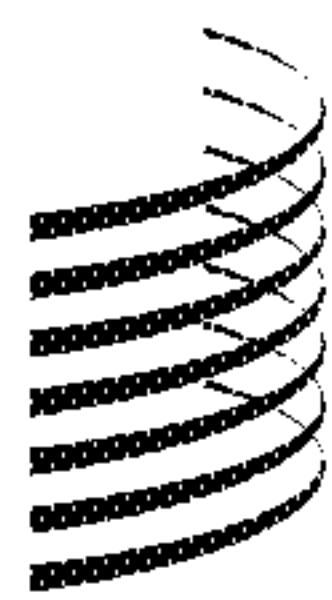


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(57) **Abrégé(suite)/Abstract(continued)**:

region of one or more VEGFR receptors that inhibits binding of at least one VEGFR to at least one VEGF). The PDGF antagonist is an antibody to a PDGF or PDGFR or is a PDGFR extracellular trap segment (i.e., segment from the extracellular region of one or more PDGFRs, which inhibits binding of at least one PDGFR and at least one PDGF). The dual antagonist is preferably conjugated to a half-life extending moiety, such as a HEMA-PC polymer. The dual antagonist is particularly useful for treating wet aged related macular degeneration.

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[Continued on next page]

(54) Title: DUAL PDGF/VEGF ANTAGONISTS

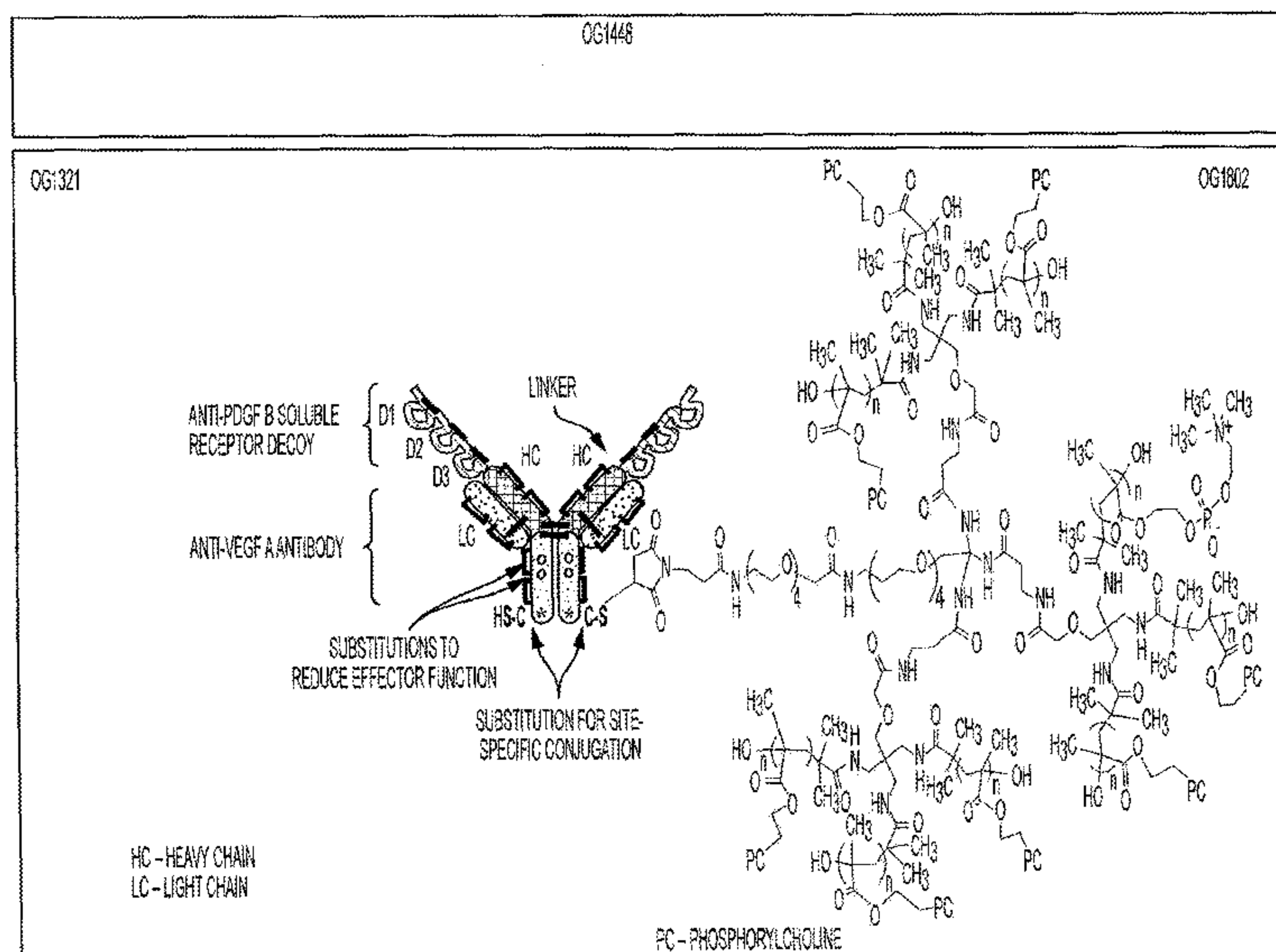


FIG. 39

(57) Abstract: The invention provides a dual VEGF/PDGF antagonist comprising a VEGF antagonist linked to a PDGF antagonist. The VEGF antagonist is an antibody to a VEGF or VEGFR or is a VEGFR extracellular trap segment (i.e., a segment from the extracellular region of one or more VEGFR receptors that inhibits binding of at least one VEGFR to at least one VEGF). The PDGF antagonist is an antibody to a PDGF or PDGFR or is a PDGFR extracellular trap segment (i.e., segment from the extracellular region of one or more PDGFRs, which inhibits binding of at least one PDGFR and at least one PDGF). The dual antagonist is preferably conjugated to a half-life extending moiety, such as a HEMA-PC polymer. The dual antagonist is particularly useful for treating wet aged related macular degeneration.

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DUAL PDGF/VEGF ANTAGONISTS

CROSS-REFERENCE TO RELATED APPLICATION

The present application is a non-provisional of 62/018,579 filed June 28, 2014, incorporated by reference in its entirety for all purposes.

BACKGROUND

[0001] Angiogenesis (the formation of blood vessels) occurs throughout an organism's development. Indeed, the first organ in an embryo is a blood vessel. Angiogenesis is also crucial for wound healing, restoring blood flow to damaged tissue. However, improper or dysregulated angiogenesis contributes to or causes many diseases including cancer, psoriasis, arthritis and blindness. Carmeliet P. 2003. Angiogenesis in health and disease. *Nature Med* 9(6):653-660.

[0002] Age related macular degeneration (AMD) is a leading cause of vision loss and blindness in the elderly. About ten million Americans are afflicted with AMD. The prevalence of AMD in the population increases steadily with age: at 40 years of age only about 2% of the population is affected by AMD but by the age of 80 it is about 25%. Friedman, D.S. et al. 2004. *Arch. Ophthalmol.* 122:564-572. There are generally two types of AMD: dry and wet.

[0003] Dry AMD is the most common form of the disease. In dry AMD, there is a depletion of the layer of the retinal pigment epithelial cells in the macula. Dry AMD is chronic and generally causes some loss of vision. In severe cases of dry AMD, patients can develop near total blindness. Wet AMD develops in some 10-15% of patients with dry AMD. Wet AMD is characterized by angiogenesis, specifically choroidal neovascularization (CNV). CNV is characterized by the presence of new immature blood vessels which grow towards the outer retina from the choroid. These immature blood vessels leak fluid below and in the retina, causing vision loss and blindness. Wet AMD blindness is typically acute.

[0004] Angiogenesis also plays a crucial role in cancer and tumor formation and maintenance. The recruitment of new blood vessels is an essential component of the metastatic pathway. For many tumors, the vascular density can provide a prognostic indicator of metastatic potential: highly vascular tumors have a higher incidence of metastasis than less vascular tumors.

[0005] Angiogenesis is the result of a complex interplay between growth factors, vascular endothelial cells, extracellular matrix molecules, chemokines and cell signaling molecules.

Factors identified as mediators of angiogenesis include: basic and acidic fibroblast growth factor, transforming growth factors α and β , platelet-derived growth factor (PDGF), angiogenin, platelet-derived endothelial cell growth factor, IL8, and vascular endothelial growth factor (VEGF). The role of VEGF in angiogenesis has been extensively reported on.

[0006] It has been shown that VEGF signaling presents a crucial rate limiting step in physiological angiogenesis. VEGF also plays a central role in pathological angiogenesis (e.g., tumor growth). Ferrara N and Davis-Smyth T. 1997. The biology of vascular endothelial growth factor. *Endocr. Rev.* 18: 4-25. VEGF is also known to induce vascular leakage. Bates DO and Curry FE. 1997. Vascular endothelial growth factor increases microvascular permeability via a Ca (2+)-dependent pathway. *Am J Physiol.* 273: H687-H694; Roberts WG and Palade GE. 1995. Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor. *J Cell Sci.* 108:2369-2379.

[0007] Anti-VEGF therapeutics have been successfully used to treat wet AMD and cancer. Genentech's anti-VEGF monoclonal antibody bevacizumab (Avastin®) received FDA approval in 2004 for the treatment of cancer. Anti-VEGF agents have been approved for the treatment of wet AMD. In 2004, the FDA approved Eyetech/Pfizer Macugen®. Genentech's Lucentis® was approved in 2006 for wet AMD. Bevacizumab is also used off label for the treatment of wet AMD. In 2011, Regeneron's Eylea® was approved for treatment of wet AMD.

[0008] Despite the success of anti-VEGF therapeutics, none of them causes regression in the pathological neovascular (NV) tissue. Hence, NV tissue remains despite continued anti-VEGF treatment and can prevent significant vision gain for treated patients. The NV tissue consists of endothelial cells, pericytes and inflammatory cells (i.e., occasional macrophages). The presence of pericytes on capillaries not only leads to NV support and stabilization but promotes endothelial cell survival through chemical signaling and physical interactions including pericyte production of VEGF. This endothelial survival signaling by integrated pericytes is critical and may explain the resistance of the NV tissue to VEGF withdrawal, i.e., lack of NV regression to monotherapy anti-VEGF treatment. In addition, over time the pathological NV tissue can lead to fibrosis and scarring.

[0009] Subretinal scarring develops in nearly half of treated eyes within two years of anti-VEGF therapy. Daniel E, Toth CA, Grunwald JE. 2014. Risk of scar in the comparison of age-related macular degeneration in clinical settings. *Retina* 32:1480-1485. Subretinal fibrosis

formation can cause permanent dysfunction of the macular system; it causes destruction of photoreceptors, retinal pigment epithelium and choroidal vessels. Ishikawa K, Ram K, Hinton DR. 2015. Molecular mechanisms of subretinal fibrosis in age-related macular degeneration. *Eye Res.* xxx:1-7. While anti-VEGF therapy generally stabilizes or improves visual acuity, scar formation has been identified as one of the causes of loss of visual acuity after treatment. Cohen SY, Oubraham H, Uzzan J, et al. 2012. Causes of unsuccessful ranibizumab treatment in exudative age-related macular degeneration in clinical settings. *Retina* 32:1480-1485.

[0010] PDGF has been reported to play a role in pericyte recruitment, maturation and resistance to anti-VEGF mediated regression. Corneal and choroidal neovascularization animal models have been reported to have demonstrated that administration of agents that block the PDGF-B/PDGFR- β interaction leads to pericyte stripping from the pathological neovasculature. Jo N, Mailhos C, Ju M, et al. 2006. Inhibition of Platelet-Derived Growth Factor B Signaling Enhances the Efficacy of Anti-Vascular Endothelial Growth Factor Therapy in Multiple Models of Ocular Neovascularization. *American J Path.* 168(6):2036-2053.

[0011] To target both pathways, clinical trials are currently underway in which patients receive two medications: Lucentis® (an anti-VEGF Fab) and Fovista™ a PEGYlated aptamer directed against PDGF by Ophthotech. Fovista is directed against only a single PDGF ligand: PDGF-BB. However, there are many other PDGF ligands: PDGF-AA, PDGF-CC and PDGF-DD. PDGF-DD, for example, has been shown to play a crucial role in ocular angiogenesis. Kumar A, Hou X, Chunsik L, et al. 2010. Platelet-derived Growth Factor-DD Targeting Arrests Pathological Angiogenesis by Modulating Glycogen Synthase Kinase-3 β Phosphorylation. *J Biol Chem* 285(20):15500-15510. Yet Fovista does not interact with PDGF-DD. There is a need in the art for broader based anti-PDGF therapies.

[0012] In addition, aptamer based therapeutics in general have poor pharmacokinetic properties in that aptamers are subject to renal filtration and to serum digestion. While these problems can be somewhat overcome with PEGylation, PEGylation tends to reduce binding to target. Aptamers typically bind with much lower affinity to targets than their antibody counterparts. PEGylation will tend to reduce binding even further. There is, thus, a need in the art for non-aptamer based anti-PDGF therapeutics.

[0013] Current clinical plans for Fovista double the number of injections patients must receive for treatment relative to the currently approved anti-VEGF therapies. Fovista is

formulated separately from the anti-VEGF agent so patients must be given two injections instead of one. Moreover the injections cannot be at the same time because of build-up in intraocular pressure caused by a single injection.

[0014] From the view point of both patients and treating physicians, intravitreal injections are not trivial. Many patients experience pain and discomfort from the injection and patient compliance is a serious issue. Common side effects of intravitreal injections include conjunctival hemorrhage, eye pain, vitreous floaters, increased intraocular pressure, and intraocular inflammation. Intravitreal injections are associated with relatively rare serious adverse events, including endophthalmitis, retinal detachment and traumatic cataracts.

[0015] There is thus a need in the art for therapies that do not increase the number of intravitreal injections that patients must endure. In addition, current anti-VEGF therapies often require once a month injections. There is also a need for therapies which are needed less frequently than once a month.

SUMMARY OF THE CLAIMED INVENTION

[0016] The invention provides a dual VEGF/PDGF antagonist comprising a VEGF antagonist linked to a PDGF antagonist, wherein the VEGF antagonist (a) is an antibody to a VEGF or VEGFR or (b) is a VEGFR extracellular trap segment and the PDGF antagonist (a) is an antibody to a PDGF or PDGFR or (b) is a PDGFR extracellular trap segment, provided that the VEGF and PDGF antagonists are not both antibodies. Optionally, the VEGF antagonist is an antibody comprising a heavy chain and a light chain and the PDGF antagonist is the PDGFR extracellular trap segment, and the heavy chain of the antibody is fused via a linker to the C-terminus of the PDGFR extracellular trap segment, and the light chain is complexed with the heavy chain. Optionally, the antibody is a Fab fragment. Optionally, the antibody is an intact antibody. Optionally, the PDGF antagonist is an extracellular trap segment of a PDGFR- α or PDGFR- β receptor and the VEGF antagonist is an antibody to a VEGF. Optionally, the PDGFR extracellular trap segment comprises one or more of domains D1-D5 of PDGFR- β . Optionally, the PDGFR extracellular trap segment comprises domains D1-D3 of PDGFR- β . Optionally, the PDGFR extracellular trap segment comprises amino acids 33 to 314 of SEQ ID NO. 11. Optionally, the VEGF antagonist comprises an anti-VEGF antibody. Optionally, the anti-VEGF antibody is an anti-VEGF-A antibody. Optionally, the PDGFR extracellular trap segment is

located C-terminal of the heavy or light chain. Optionally, the PDGFR extracellular trap segment is located N-terminal of the heavy or light chain.

[0017] Optionally, the dual VEGF/PDGF antagonist of further comprising a linker which is located between the PDGFR trap and the anti-VEGF antibody heavy chain. Optionally the linker is GGGGSGGGGS, GG, or GGGGSGGGGSGGGGSGGGGSG.

[0018] Optionally, the anti-VEGF antibody heavy chain comprises CDR_{H1}: GYDFTHYGMN, CDR_{H2}: WINTYTGEPTYAADFKR, and CDR_{H3}: YPYYYGTSHWYFDV. Optionally, the anti-VEGF light chain comprises CDR_{L1}: SASQDISNYLN, CDR_{L2}: FTSSLHS and CDR_{L3}: QQYSTVPWT.

[0019] Optionally, the anti-VEGF heavy chain isotype is IgG1 comprising a CH₁, hinge, CH₂ and CH₃ domains and the light chain isotype is kappa. Optionally the IgG1 constant domain has the sequence set forth in SEQ ID NO. 17 and the light chain constant region has the sequence set forth in SEQ ID NO. 18.

[0020] Optionally, the IgG1 constant domain has one or more mutations to reduce effector function. Optionally the mutations are to one or more of the following amino acid positions (EU numbering): E233, L234, L235, G236, G237, A327, A330, and P331. Optionally, the mutations are selected from the group consisting of: E233P, L234V, L234A, L235A, G237A, A327G, A330S and P331S. Optionally, mutations are L234A, L235A and G237A.

[0021] Optionally, the dual VEGF/PDGF antagonist comprises a heavy chain further comprising a cysteine residue added by recombinant DNA technology. Optionally, the cysteine residue is selected from the group consisting of (EU numbering) Q347C and L443C.

[0022] Optionally, the dual VEGF/PDGF antagonist has a heavy chain comprising the amino acid sequence of SEQ ID NO. 9 and the light chain has an amino acid sequence of SEQ ID NO. 10.

[0023] Optionally, the dual VEGF/PDGF antagonist comprises a PDGFR trap extracellular segment comprising one or more of domains D1-D5 of PDGFR- β . Optionally, the PDGFR trap extracellular segment comprises domains D1-D3 of PDGFR- β . Optionally, the PDGFR trap extracellular segment comprises amino acids 33 to 314 of SEQ ID NO. 11.

[0024] Optionally, the dual VEGF/PDGF antagonist comprises a VEGF antagonist, which is an anti-VEGF antibody. Optionally, the antibody is an anti-VEGF-A Fab fragment. Optionally, the PDGFR extracellular trap segment is located C-terminal of the Fab heavy or light chain.

Optionally, the PDGFR extracellular trap segment is located N-terminal of the Fab heavy or light chain.

[0025] Optionally, the dual VEGF/PDGF comprises a heavy chain comprising an anti-VEGF-A Fab fragment heavy chain and a light chain comprising an anti-VEGF-A light chain. Optionally, the dual antagonist further comprises a linker which is located between the PDGFR trap and the anti-VEGF Fab fragment heavy chain. Optionally, the linker is selected from group consisting of GGGGSGGGGS, GG, and GGGGSGGGGSGGGGSGGGGSG. Optionally, the anti-VEGF Fab fragment heavy chain comprises CDR_{H1}: GYDFTHYGMN, CDR_{H2}: WINTYTGEPTYAADFQR, and CDR_{H3}: YPYYGTSHWYFDV. Optionally, the anti-VEGF light chain comprises CDR_{L1}: SASQDISNYLN, CDR_{L2}: FTSSLHS and CDR_{L3}: QQYSTVPWT. Optionally, the anti-VEGF heavy chain isotype is IgG1 comprising a CH₁ domain and the light chain isotype is kappa.

[0026] Any of the dual VEGF/PDGF antagonists can further comprise a half-life extending moiety. Optionally, the half-life extending moiety comprises a polymer, which is PEG or a zwitterionic polymer. Optionally, the zwitterionic polymer comprises a monomer comprising phosphorylcholine. Optionally, the monomer comprises 2-(acryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate. Optionally, the monomer comprises 2-(methacryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate (HEMA-PC). Optionally, the polymer has 3 or more arms. Optionally, the polymer has 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 arms. Optionally, the polymer has 3, 6 or 9 arms. Optionally, the polymer has 9 arms. Optionally, the polymer portion of the conjugate has a peak molecular weight of between 300,000 and 1,750,000 Da. Optionally, the polymer portion of the conjugate has a peak molecular weight between 500,000 and 1,000,000 Da. Optionally, the polymer portion of the conjugate has a peak molecular weight between 600,000 to 800,000 Da. Optionally, the dual VEGF/PDGF antagonist is covalently bonded to the polymer. Optionally, the polymer is covalently bonded to at least one of an amino group, a hydroxyl group, a sulfhydryl group and a carboxyl group. Optionally, the sulfhydryl group is from a naturally occurring cysteine residue. Optionally, the sulfhydryl group is from a cysteine residue added by recombinant DNA technology. Optionally, the polymer is covalently bonded to the cysteine residue at position 741 of SEQ ID NO. 9.

[0027] Optionally, the VEGF antagonist comprises a VEGFR extracellular trap segment comprising one or more extracellular segments of VEGFR-1, VEGFR-2 and VEGFR-3 and the

PDGF antagonist is an anti-PDGF antibody. Optionally, the extracellular segment of VEGFR comprises one or more of domains D1-D7. Optionally, the extracellular segment comprises D2 from VEGFR-1 and D3 from VEGFR-2. Optionally, the D2 is N-terminal to the D3 and further comprises a linker between the domains. Optionally, the PDGF antagonist is an intact antibody. Optionally, the PDGF antagonist is a Fab fragment. Optionally, the anti-PDGFR antibody is humanized 2A1E2, HuM4Ts.22, humanized 1B3, humanized 2C5, anti-PDGF-BB, anti-PDGF-DD, anti-PDGF-BB or anti-PDGF-AB. Optionally, the heavy chain is IgG1 and the light chain is kappa. Optionally, the heavy chain sequence has a cysteine added via recombinant DNA technology the cysteine selected from the groups consisting of Q347C or a L443C. Optionally, the dual VEGF/PDGF antagonist further comprises a half-life extending moiety conjugated to the cysteine. Optionally, the dual VEGF/PDGF antagonist protein has a half-life extending moiety comprising a zwitterionic polymer, the polymer comprising one or more monomer units and wherein at least one monomer unit comprises a zwitterionic group, such as phosphorylcholine. Optionally, the monomer comprises 2-(acryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate. Optionally, the monomer comprises 2-(methacryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate (HEMA-PC). Optionally, the polymer has 3 or more arms. Optionally, the polymer has 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 arms. Optionally, the polymer has 3, 6 or 9 arms. Optionally, the polymer has 9 arms. Optionally, the polymer portion of the conjugate has a peak molecular weight of between 300,000 and 1,750,000 Da. Optionally, the polymer portion of the conjugate has a peak molecular weight between 500,000 and 1,000,000 Da. Optionally, the polymer portion of the conjugate has a peak molecular weight between 600,000 to 800,000 Da.

[0028] In some dual VEGF/PDGF antagonists the PDGF antagonist comprises a PDGF extracellular trap segment comprising one or more extracellular segments of a PDGFR selected from the group consisting of PDGFR- α and PDGFR- β and the VEGF antagonist is a VEGF extracellular trap segment comprising one or more extracellular segments of a VEGFR selected from the group consisting of VEGFR-1, VEGFR-2 and VEGFR-3. Optionally, the extracellular trap segment of VEGFR comprises one or more of domains D1-D7. Optionally, the extracellular trap segment comprises D2 from VEGFR-1 and D3 from VEGFR-2. Optionally, the D2 is N-terminal to the D3 and further comprises a linker between the domains. Optionally, the PDGFR trap comprises one or more of domains D1-D5 of PDGFR- β . Optionally, the PDGFR trap

comprises domains D1-D3 of PDGFR- β . Optionally, the PDGFR trap comprises amino acids 33 to 314 of SEQ ID NO. 11. Optionally, the dual VEGF/PDGF antagonist further comprises a linker sequence between the VEGF antagonist and the PDGF antagonist. Optionally, the dual VEGF/PDGF antagonist further comprises a half-life extending moiety. Optionally, the half-life extending moiety comprises a polymer selected from the group consisting of PEG and a zwitterionic polymer. Optionally, the half-life extending moiety comprises a zwitterionic polymer. Optionally, the zwitterionic polymer comprises a monomer comprising phosphorylcholine. Optionally, the monomer comprises 2-(acryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate. Optionally, the monomer comprises 2-(methacryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate (HEMA-PC). Optionally, the polymer has 3 or more arms. Optionally, the polymer has 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 arms. Optionally, the polymer has 3, 6 or 9 arms. Optionally, the polymer portion of the conjugate has a peak molecular weight of between 300,000 and 1,750,000 Da. Optionally, the polymer portion of the conjugate has a peak molecular weight between 500,000 and 1,000,000 Da. Optionally, the polymer portion of the conjugate has a peak molecular weight between 600,000 to 800,000 Da. Optionally, the polymer has 9 arms. Optionally, the dual VEGF/PDGF antagonist is covalently bonded to the polymer. Optionally, the polymer is covalently bonded to at least one of an amino group, a hydroxyl group, a sulfhydryl group and a carboxyl group. Optionally, the sulfhydryl group is from a naturally occurring cysteine residue. Optionally, the sulfhydryl group is from a cysteine residue added by recombinant DNA technology.

[0029] Any dual VEGF/PDGF antagonist as described above can be used in treatment or prophylaxis of disease, particularly a neovascular disorder, optionally an ocular neovascular disorder, such as wet age related macular degeneration.

BRIEF DESCRIPTION OF THE DRAWINGS

- [0030] FIG. 1: Protein Sequence of human PDGFR- β
- [0031] FIG. 2: Protein Sequence of VEGFR-1
- [0032] FIG. 3: Protein Sequence of VEGFR-2
- [0033] FIG. 4: Protein Sequence of VEGFR-3
- [0034] FIG. 5: bevacizumab sequence (DrugBank DB00112)
- [0035] FIG. 6: ranibizumab (published by Novartis).

- [0036] FIGs 7A, B: Protein Sequence of A. PDGFR β -GS10-anti-VEGF-A light chain and B. anti-VEGF-A heavy chain.
- [0037] FIGs. 8A, B: A. Protein Sequence of PDGFR β -GG-anti-VEGF-A light chain and B. anti-VEGF-A heavy chain.
- [0038] FIGs. 9A, B. Protein Sequence of A. PDGFR β -GS10-anti-VEGF-A heavy chain (wild type Fc) and B. anti-VEGF-A light chain.
- [0039] FIGs. 10A, B. Protein Sequence of A. PDGFR β -GG-anti-VEGF-A heavy chain (wild type Fc) and B. anti-VEGF-A light chain.
- [0040] FIGs. 11A, B. Protein Sequence of A. anti-VEGF-A heavy chain (wild type Fc)-GS21-PDGFR β and B. anti-VEGF-A light chain.
- [0041] FIGs. 12A, B. Protein Sequence of A. PDGFR- β -GS21-anti-VEGF-A heavy chain (Q347C) and B. anti-VEGF-A light chain (TAF347).
- [0042] FIGs. 13A, B. Protein Sequence of A. PDGFR- β -GS21-anti-VEGF-A heavy chain (L443C) and B. anti-VEGF-A light chain (TAF443).
- [0043] FIGs. 14A, B. Protein Sequence of A. PDGFR β -GS10-anti-VEGF-A light chain and B. anti-VEGF-A Fab.
- [0044] FIGs. 15A, B. Protein Sequence of A. PDGFR β -GG-anti-VEGF-A light chain and B. anti-VEGF-A Fab.
- [0045] FIGs. 16A, B. Protein Sequence of A. PDGFR β -GS10-anti-VEGF-A Fab and B. anti-VEGF-A light chain.
- [0046] FIGs. 17A, B. Protein Sequence of A. PDGFR β -GG-anti-VEGF-A Fab and B. anti-VEGF-A light chain.
- [0047] FIGs. 18A, B. Protein Sequence of A. anti-VEGF-A Fab-GS21-PDGFR β and B. anti-VEGF-A light chain.
- [0048] FIGs. 19A, B. Protein Sequence of A. PDGFR β -GS10-anti-VEGF-A Fab with certain mutations and B. anti-VEGF-A light chain.
- [0049] FIGs. 20A, B. Protein Sequence of A. PDGFR β -anti-VEGF-A heavy chain and B. anti-VEGF-A light chain (1a).
- [0050] FIGs. 21A, B. Protein Sequence of A. PDGFR- β (D2-D3)-anti-VEGF-A heavy chain and B. anti-VEGF-A light chain (1b).

- [0051] FIGs. 22A, B. Protein Sequence of A. PDGFR- β (D1-D3)-anti-VEGF-A Fab and B. anti-VEGF-A light chain (2b).
- [0052] FIGs. 23A, B. Protein Sequence of A. PDGFR- β (D2-D3)-6xGS-anti-VEGF-A Fab and B. anti-VEGF-A light chain (2b').
- [0053] FIGs. 24A, B. Protein sequence of A. PDGFR- β -6xGS-anti-VEGF-A Fab and B. anti-VEGF-A light chain.
- [0054] FIGs. 25A, B: Protein Sequence of A. anti-VEGF-A Fab-6xGS-PDGFR- β (D2-D3) and B. anti-VEGF-A light chain (3).
- [0055] FIG. 26 shows the chemical structure of OG1448.
- [0056] FIG. 27 shows Compound L.
- [0057] FIG. 28 shows Compound K.
- [0058] FIG. 29 shows the synthesis of OG1802 from R3707.
- [0059] FIG. 30 shows OG1786.
- [0060] FIG. 31 shows the synthesis of OG1546 from OG1550.
- [0061] FIG. 32 shows the synthesis of OG1784 from OG1546 and OG1563.
- [0062] FIG. 33 shows the synthesis of OG1405 from OG1784.
- [0063] FIG. 34 shows the synthesis of OG 1785 from OG1405.
- [0064] FIG. 35 shows the synthesis of OG1786 from OG1785.
- [0065] FIG. 36 shows OG1802.
- [0066] FIG. 37 shows a graph of percent Grade IV laser lesions.
- [0067] FIG. 38 shows Compound E.
- [0068] FIG. 39 depicts OG1448.
- [0069] FIG. 40 shows relative angiogenesis using OG1448, Avastin, and an anti-PDGF-BB antibody and various combinations thereof.
- [0070] FIG. 41 shows the % grade IV lesions compared to day in the CNV monkey model for the compounds indicated.
- [0071] FIG. 42 shows OG1448 ocular pharmacokinetics versus aflibercept and ranibizumab in the rabbit vitreous.

BRIEF DESCRIPTION OF SEQ ID NOs.

- [0072] SEQ ID NO. 1 is the protein sequence of PDGFR β -GS10-LightChain anti-VEGF-A (Bevacizumab).

- [0073] SEQ ID NO. 2 is the anti-VEGF-A Bevacizumab heavy chain.
- [0074] SEQ ID NO. 3 is protein sequence of PDGFR β -GG-Light Chain anti-VEGF-A (Bevacizumab).
- [0075] SEQ ID NO. 4 is PDGFR β -GS10-Heavy Chain-anti-VEGF-A (Bevacizumab).
- [0076] SEQ ID NO. 5 is the anti-VEGF-A Bevacizumab light chain.
- [0077] SEQ ID NO. 6 is PDGFR β -GG-Heavy Chain-anti-VEGF-A (Bevacizumab).
- [0078] SEQ ID NO. 7 is anti-VEGF-A Heavy Chain (Bevacizumab)-GS21-PDGFR β .
- [0079] SEQ ID NO.8 is the amino acid sequence of the heavy chain trap extracellular segment of TAF347: PDGFR- β trap-anti-VEGF-A heavy chain (Q347C).
- [0080] SEQ ID NO. 9 is the amino acid sequence of the heavy chain trap extracellular segment of TAF443: PDGFR- β trap-anti-VEGF-A heavy chain (L443C) and SEQ ID NO:10 is the amino acid sequence of the light chain of anti-VEGF-A.
- [0081] SEQ ID NO. 11 is human PDGFR- β .
- [0082] SEQ ID NO. 12 is the ranibizumab light chain.
- [0083] SEQ ID NO. 13 is the ranibizumab heavy chain.
- [0084] SEQ ID NO. 14 is human VEGFR-1.
- [0085] SEQ ID NO. 15 is human VEGFR-2.
- [0086] SEQ ID NO. 16 is human VEGFR-3.
- [0087] SEQ ID NO. 17 is a human IgG1 constant region.
- [0088] SEQ ID NO. 18 is a human kappa light constant region.
- [0089] SEQ ID NO. 19 is FIG 7. PDGFR-GS10-anti-VEGF-A light chain.
- [0090] SEQ ID NO. 20 is FIG 8. PDGFR-GG-anti-VEGF-A light chain.
- [0091] SEQ ID NO. 21 is a Bevacizumab Fab.
- [0092] SEQ ID NO. 22 is a PDGFR- β -GS10-anti-VEGF-A Fab.
- [0093] SEQ ID NO. 23 is a PDGFR- β -GG-anti-VEGF-A Fab.
- [0094] SEQ ID NO. 24 is an anti-VEGF-A Fab-GS21-PDGFR- β .
- [0095] SEQ ID NO. 25 is a PDGFR- β -GS10-anti-VEGF-A Fab with certain mutations.
- [0096] SEQ ID NO. 26 is a protein sequence of PDGFR β -anti-VEGF-A heavy chain (1a).
- [0097] SEQ ID NO. 27 is a protein sequence of PDGFR- β (D2-D3)-anti-VEGF-A heavy chain (1b).
- [0098] SEQ ID NO. 28 is a protein sequence of PDGFR- β (D2-D3)-anti-VEGF-A Fab (2b).

- [0099] SEQ ID NO. 29 is a protein sequence of PDGFR- β (D2-D3)-6xGS-anti-VEGF-A Fab.
- [00100] SEQ ID NO. 30 is a protein sequence of anti-VEGF-A Fab-6xGS-PDGFR- β (D2-D3).
- [00101] SEQ ID NO. 31 is a nucleic acid encoding a heavy chain anti-VEGF-PDGFR fusion.
- [00102] SEQ ID NO. 32 is a nucleic acid encoding a light chain anti-VEGF.
- [00103] GGGGS (SEQ ID NO. 37), GGGS (SEQ ID NO. 38), GGGS (SEQ ID NO. 39), GGGGSGGGGS (SEQ ID NO. 40) and GGGGSGGGGSGGGGSGGGGSG (SEQ ID NO. 41).
- [00104] Ranibizumab CDRs are: CDR_H1: GYDFTHYGMN, CDR_H2: WINTYTGEPTYAADFVKR, and CDR_H3: YPYYYGTSHWYFDV (SEQ ID NOS. 42-44), CDR_L1: SASQDISNYLN, CDR_L2: FTSSLHS and CDR_L3: QQYSTVPWT (SEQ ID NOS. 45-47). Bevacizumab CDR_H1 is GYTFTNYGMN (SEQ ID NO. 48) and CDR_H3 is YPHYYGSSHWYFDV (SEQ ID NO:49).

DEFINITIONS

- [00105] A “neovascular disorder” is a disorder or disease state characterized by altered, dysregulated or unregulated angiogenesis. Examples of neovascular disorders include neoplastic transformation (e.g. cancer) and ocular neovascular disorders including diabetic retinopathy and age-related macular degeneration.
- [00106] An “ocular neovascular” disorder is a disorder characterized by altered, dysregulated or unregulated angiogenesis in the eye of a patient. Such disorders include optic disc neovascularization, iris neovascularization, retinal neovascularization, choroidal neovascularization, corneal neovascularization, vitreal neovascularization, glaucoma, pannus, pterygium, macular edema, diabetic retinopathy, diabetic macular edema, vascular retinopathy, retinal degeneration, uveitis, inflammatory diseases of the retina, and proliferative vitreoretinopathy.
- [00107] A “polypeptide linker” is a polypeptide comprising two or more amino acid residues joined by peptide bonds that are used to link two polypeptides (e.g., a VH and VL domain or a VH domain and an extracellular trap segment). Examples of such linker polypeptides are well known in the art (see, e.g., Holliger P, Prospero T, Winter G. 1993. PNAS USA. 90:6444-6448; Poljak RJ. 1994. Production and Structure of Diabodies. Structure 2:1121-1123). Exemplary

linkers include G, GG, GGGGS, GGGS, and GGGES, and oligomers of such linkers (e.g., GGGGS GGGGS and GGGGS GGGGS GGGGS GGGGS G).

[00108] Dual antagonists or other biologics described herein are typically provided in isolated form. This means that an antagonist is typically at least 50% w/w pure of interfering proteins and other contaminants arising from its production or purification but does not exclude the possibility that the antagonist is combined with an excess of pharmaceutical acceptable excipient intended to facilitate its use. Sometimes antagonists are at least 60, 70, 80, 90, 95 or 99% w/w pure of interfering proteins and contaminants from production or purification. Often an antagonist is the predominant macromolecular species remaining after its purification.

[00109] The term antibody includes intact antibodies and binding fragments thereof. A binding fragment refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of binding fragments include Fv, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments. scFv antibodies are described in Houston JS. 1991. Methods in Enzymol. 203:46-96. In addition, antibody fragments comprise single chain polypeptides having the characteristics of a VH domain, namely being able to assemble together with a VL domain, or of a VL domain, namely being able to assemble together with a VH domain to a functional antigen binding site and thereby providing the antigen binding property of full length antibodies.

[00110] Specific binding of an antibody, extracellular trap segment or dual antagonist to its target antigen(s) means an affinity of at least 10^6 , 10^7 , 10^8 , 10^9 , or 10^{10} M⁻¹. Specific binding is detectably higher in magnitude and distinguishable from non-specific binding occurring to at least one unrelated target. Specific binding can be the result of formation of bonds between particular functional groups or particular spatial fit (e.g., lock and key type) whereas nonspecific binding is usually the result of van der Waals forces. Specific binding does not however necessarily imply that an antibody or fusion protein binds one and only one target.

[00111] A basic antibody structural unit is a tetramer of subunits. Each tetramer includes two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. This variable region is initially expressed linked to a cleavable signal peptide. The variable

region without the signal peptide is sometimes referred to as a mature variable region. Thus, for example, a light chain mature variable region means a light chain variable region without the light chain signal peptide. However, reference to a variable region does not mean that a signal sequence is necessarily present; and in fact signal sequences are cleaved once the antibodies or fusion proteins of the invention have been expressed and secreted. A pair of heavy and light chain variable regions defines a binding region of an antibody. The carboxy-terminal portion of the light and heavy chains respectively defines light and heavy chain constant regions. The heavy chain constant region is primarily responsible for effector function. In IgG antibodies, the heavy chain constant region is divided into CH1, hinge, CH2, and CH3 regions. The CH1 region binds to the light chain constant region by disulfide and noncovalent bonding. The hinge region provides flexibility between the binding and effector regions of an antibody and also provides sites for intermolecular disulfide bonding between the two heavy chain constant regions in a tetramer subunit. The CH2 and CH3 regions are the primary site of effector functions and FcR binding.

[00112] Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" segment of about 12 or more amino acids, with the heavy chain also including a "D" segment of about 10 or more amino acids. (See generally, *Fundamental Immunology* (Paul, W., ed., 2nd ed. Raven Press, N.Y., 1989), Ch. 7) (incorporated by reference in its entirety for all purposes).

[00113] The mature variable regions of each light/heavy chain pair form the antibody binding site. Thus, an intact antibody has two binding sites, i.e., is divalent. In natural antibodies, the binding sites are the same. However, bispecific antibodies can be made in which the two binding sites are different (see, e.g., Songsivilai S, Lachmann PC. 1990. Bispecific antibody: a tool for diagnosis and treatment of disease. *Clin Exp Immunol.* 79:315-321; Kostelny SA, Cole MS, Tso JY. 1992. Formation of bispecific antibody by the use of leucine zippers. *J Immunol.* 148: 1547-1553). The variable regions all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both

light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. For convenience, the variable heavy CDRs can be referred to as CDR_H1, CDR_H2 and CDR_H3; the variable light chain CDRs can be referred to as CDR_L1, CDR_L2 and CDR_L3. The assignment of amino acids to each domain is in accordance with the definitions of Kabat EA, et al. 1987 and 1991. Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, MD) or Chothia C, Lesk AM. 1987. Canonical Structures for the Hypervariable Regions of Immunoglobulins. J Mol Biol 196:901-917; Chothia C, et al. 1989. Conformations of Immunoglobulin Hypervariable Regions. Nature 342:877-883. Kabat also provides a widely used numbering convention (Kabat numbering) in which corresponding residues between different heavy chain variable regions or between different light chain variable regions are assigned the same number. Although Kabat numbering can be used for antibody constant regions, EU numbering is more commonly used, as is the case in this application. Although specific sequences are provided for exemplary dual antagonists, it will be appreciated that after expression of protein chains one to several amino acids at the amino or carboxy terminus of the light and/or heavy chain, particularly a heavy chain C-terminal lysine residue, may be missing or derivatized in a proportion or all of the molecules.

[00114] The term "epitope" refers to a site on an antigen to which an antibody or extracellular trap segment binds. An epitope on a protein can be formed from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of one or more proteins. Epitopes formed from contiguous amino acids (also known as linear epitopes) are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding (also known as conformational epitopes) are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., Epitope Mapping Protocols, in Methods in Molecular Biology, Vol. 66, Glenn E. Morris, Ed. (1996).

[00115] Antibodies that recognize the same or overlapping epitopes can be identified in a simple immunoassay showing the ability of one antibody to compete with the binding of another antibody to a target antigen. The epitope of an antibody can also be defined by X-ray crystallography of the antibody (or Fab fragment) bound to its antigen to identify contact residues.

[00116] Alternatively, two antibodies have the same epitope if all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[00117] Competition between antibodies is determined by an assay in which an antibody under test inhibits specific binding of a reference antibody to a common antigen (see, e.g., Junghans et al., Cancer Res. 50: 1495, 1990). A test antibody competes with a reference antibody if an excess of a test antibody (e.g., at least 2x, 5x, 10x, 20x or 100x) inhibits binding of the reference antibody by at least 50% but preferably 75%, 90% or 99% as measured in a competitive binding assay. Antibodies identified by competition assay (competing antibodies) include antibodies binding to the same epitope as the reference antibody and antibodies binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antibody for steric hindrance to occur.

[00118] The term "patient" includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment.

[00119] For purposes of classifying amino acids substitutions as conservative or nonconservative, amino acids are grouped as follows: Group I (hydrophobic side chains): met, ala, val, leu, ile; Group II (neutral hydrophilic side chains): cys, ser, thr; Group III (acidic side chains): asp, glu; Group IV (basic side chains): asn, gin, his, lys, arg; Group V (residues influencing chain orientation): gly, pro; and Group VI (aromatic side chains): trp, tyr, phe. Conservative substitutions involve substitutions between amino acids in the same class. Non-conservative substitutions constitute exchanging a member of one of these classes for a member of another.

[00120] Percentage sequence identities are determined with antibody sequences maximally aligned by the Kabat numbering convention for a variable region or EU numbering for a constant region. After alignment, if a subject antibody region (e.g., the entire mature variable region of a heavy or light chain) is being compared with the same region of a reference antibody, the percentage sequence identity between the subject and reference antibody regions is the number of positions occupied by the same amino acid in both the subject and reference antibody region divided by the total number of aligned positions of the two regions, with gaps not counted, multiplied by 100 to convert to percentage. Sequence identities of other sequences can be

determined by aligning sequences using algorithms, such as BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, WI, using default gap parameters, or by inspection, and the best alignment (*i.e.*, resulting in the highest percentage of sequence similarity over a comparison window).

Percentage of sequence identity is calculated by comparing two optimally aligned sequences over a window of comparison, determining the number of positions at which the identical residues occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

[00121] Compositions or methods "comprising" one or more recited elements may include other elements not specifically recited. For example, a composition that comprises antibody may contain the antibody alone or in combination with other ingredients.

[00122] The term "antibody-dependent cellular cytotoxicity", or ADCC, is a mechanism for inducing cell death that depends upon the interaction of antibody-coated target cells (*i.e.*, cells with bound antibody) with immune cells possessing lytic activity (also referred to as effector cells). Such effector cells include natural killer cells, monocytes/macrophages and neutrophils. ADCC is triggered by interactions between the Fc region of an antibody bound to a cell and Fc γ receptors, particularly Fc γ RI and Fc γ RIII, on immune effector cells such as neutrophils, macrophages and natural killer cells. The target cell is eliminated by phagocytosis or lysis, depending on the type of mediating effector cell. Death of the antibody-coated target cell occurs as a result of effector cell activity.

[00123] The term opsonization also known as "antibody-dependent cellular phagocytosis", or ADCP, refers to the process by which antibody-coated cells are internalized, either in whole or in part, by phagocytic immune cells (*e.g.*, macrophages, neutrophils and dendritic cells) that bind to an immunoglobulin Fc region.

[00124] The term "complement-dependent cytotoxicity" or CDC refers to a mechanism for inducing cell death in which an Fc effector domain(s) of a target-bound antibody activates a series of enzymatic reactions culminating in the formation of holes in the target cell membrane. Typically, antigen-antibody complexes such as those on antibody-coated target cells bind and activate complement component C1q which in turn activates the complement cascade leading to target cell death. Activation of complement may also result in deposition of complement

components on the target cell surface that facilitate ADCC by binding complement receptors (e.g., CR3) on leukocytes.

[00125] A humanized antibody is a genetically engineered antibody in which the CDRs from a non-human "donor" antibody are grafted into human "acceptor" antibody sequences (see, e.g., Queen, US 5,530,101 and 5,585,089; Winter, US 5,225,539, Carter, US 6,407,213, Adair, US 5,859,205 6,881,557, Foote, US 6,881,557). The acceptor antibody sequences can be, for example, a mature human antibody sequence, a composite of such sequences, a consensus sequence of human antibody sequences, or a germline region sequence. Thus, a humanized antibody is an antibody having some or all CDRs entirely or substantially from a donor antibody and variable region framework sequences and constant regions, if present, entirely or substantially from human antibody sequences. Similarly a humanized heavy chain has at least one, two and usually all three CDRs entirely or substantially from a donor antibody heavy chain, and a heavy chain variable region framework sequence and heavy chain constant region, if present, substantially from human heavy chain variable region framework and constant region sequences. Similarly a humanized light chain has at least one, two and usually all three CDRs entirely or substantially from a donor antibody light chain, and a light chain variable region framework sequence and light chain constant region, if present, substantially from human light chain variable region framework and constant region sequences. Other than nanobodies and dAbs, a humanized antibody comprises a humanized heavy chain and a humanized light chain. A CDR in a humanized antibody is substantially from a corresponding CDR in a non-human antibody when at least 85%, 90%, 95% or 100% of corresponding residues (as defined by Kabat) are identical between the respective CDRs. The variable region framework sequences of an antibody chain or the constant region of an antibody chain are substantially from a human variable region framework sequence or human constant region respectively when at least 85, 90, 95 or 100% of corresponding residues defined by Kabat are identical.

[00126] Although humanized antibodies often incorporate all six CDRs (preferably as defined by Kabat) from a mouse antibody, they can also be made with less than all CDRs (e.g., at least 3, 4, or 5 CDRs from a mouse antibody) (e.g., De Pascalis R, Iwahashi M, Tamura M, et al. 2002. Grafting "Abbreviated" Complementary-Determining Regions Containing Specificity-Determining Residues Essential for Ligand Contact to Engineer a Less Immunogenic Humanized Monoclonal Antibody. J Immunol. 169:3076-3084; Vajdos FF, Adams CW, Breece TN, Presta

LG, de Vos AM, Sidhu, SS. 2002. Comprehensive functional maps of the antigen-binding site of an anti-ErbB2 antibody obtained with shotgun scanning mutagenesis. *J Mol Biol.* 320: 415–428; Iwahashi M, Milenic DE, Padlan EA, et al. 1999. CDR substitutions of a humanized monoclonal antibody (CC49): Contributions of individual CDRs to antigen binding and immunogenicity. *Mol Immunol.* 36:1079-1091; Tamura M, Milenic DE, Iwahashi M, et al. 2000. Structural correlates of an anticarcinoma antibody: Identification of specificity-determining regions (SDRs) and development of a minimally immunogenic antibody variant by retention of SDRs only. *J Immunol.* 164:1432-1441).

[00127] A chimeric antibody is an antibody in which the mature variable regions of light and heavy chains of a non-human antibody (e.g., a mouse) are combined with human light and heavy chain constant regions. Such antibodies substantially or entirely retain the binding specificity of the mouse antibody, and are about two-thirds human sequence.

[00128] A veneered antibody is a type of humanized antibody that retains some and usually all of the CDRs and some of the non-human variable region framework residues of a non-human antibody but replaces other variable region framework residues that may contribute to B- or T-cell epitopes, for example exposed residues (Padlan EA. 1991. A possible procedure for reducing the immunogenicity of antibody variable domains while preserving their ligand-binding properties. *Mol Immunol.* 28:489-98) with residues from the corresponding positions of a human antibody sequence. The result is an antibody in which the CDRs are entirely or substantially from a non-human antibody and the variable region frameworks of the non-human antibody are made more human-like by the substitutions. A human antibody can be isolated from a human, or otherwise result from expression of human immunoglobulin genes (e.g., in a transgenic mouse, in vitro or by phage display). Methods for producing human antibodies include the trioma method of Östberg L, Pursch E. 1983. Human x (mouse x human) hybridomas stably producing human antibodies. *Hybridoma* 2:361-367; Östberg, U.S. Patent No. 4,634,664; and Engleman et al., US Patent 4,634,666, use of transgenic mice including human immunoglobulin genes (see, e.g., Lonberg et al., W093/12227 (1993); US 5,877,397, US 5,874,299, US 5,814,318, US 5,789,650, US 5,770,429, US 5,661,016, US 5,633,425, US 5,625,126, US 5,569,825, US 5,545,806, *Nature* 148, 1547-1553 (1994), *Nature Biotechnology* 14, 826 (1996), Kucherlapati, WO 91/10741 (1991) and phage display methods (see, .e.g. Dower et al., WO 91/17271 and

McCafferty et al., WO 92/01047, US 5,877,218, US 5,871,907, US 5,858,657, US 5,837,242, US 5,733,743 and US 5,565,332.

[00129] “Polymer” refers to a series of monomer groups linked together. A polymer is composed of multiple units of a single monomer (a homopolymer) or different monomers (a heteropolymer). High MW polymers are prepared from monomers that include, but are not limited to, acrylates, methacrylates, acrylamides, methacrylamides, styrenes, vinyl-pyridine, vinyl-pyrrolidone and vinyl esters such as vinyl acetate. Additional monomers are useful in the high MW polymers of the present invention. When two different monomers are used, the two monomers are called “comonomers,” meaning that the different monomers are copolymerized to form a single polymer. The polymer can be linear or branched. When the polymer is branched, each polymer chain is referred to as a “polymer arm.” The end of the polymer arm linked to the initiator moiety is the proximal end, and the growing-chain end of the polymer arm is the distal end. On the growing chain-end of the polymer arm, the polymer arm end group can be the radical scavenger, or another group.

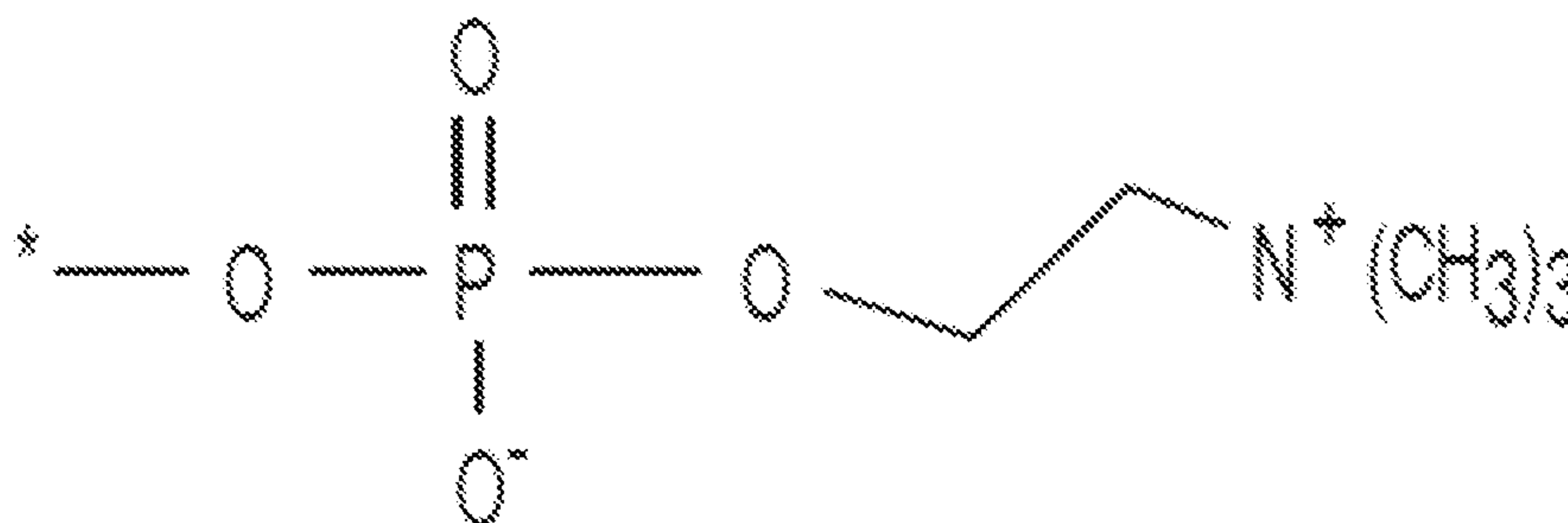
[00130] “Initiator” refers to a compound capable of initiating a polymerization using the monomers or comonomers of the present invention. The polymerization can be a conventional free radical polymerization or preferably a controlled/”living” radical polymerization, such as Atom Transfer Radical Polymerization (ATRP), Reversible Addition-Fragmentation-Termination (RAFT) polymerization or nitroxide mediated polymerization (NMP). The polymerization can be a “pseudo” controlled polymerization, such as degenerative transfer. When the initiator is suitable for ATRP, it contains a labile bond which can be homolytically cleaved to form an initiator fragment, I, being a radical capable of initiating a radical polymerization, and a radical scavenger, I’, which reacts with the radical of the growing polymer chain to reversibly terminate the polymerization. The radical scavenger I’ is typically a halogen, but can also be an organic moiety, such as a nitrile. In some embodiments of the present invention, the initiator contains one or more 2-bromoisobutyrate groups as sites for polymerization via ATRP.

[00131] A “chemical linker” refers to a chemical moiety that links two groups together, such as a half-life extending moiety and a protein. The linker can be cleavable or non-cleavable. Cleavable linkers can be hydrolyzable, enzymatically cleavable, pH sensitive, photolabile, or disulfide linkers, among others. Other linkers include homobifunctional and heterobifunctional

linkers. A “linking group” is a functional group capable of forming a covalent linkage consisting of one or more bonds to a bioactive agent. Non-limiting examples include those illustrated in Table 1 of WO2013059137 (incorporated by reference).

[00132] The term “reactive group” refers to a group that is capable of reacting with another chemical group to form a covalent bond, i.e. is covalently reactive under suitable reaction conditions, and generally represents a point of attachment for another substance. The reactive group is a moiety, such as maleimide or succinimidyl ester, is capable of chemically reacting with a functional group on a different moiety to form a covalent linkage. Reactive groups generally include nucleophiles, electrophiles and photoactivatable groups.

[00133] “Phosphorylcholine,” also denoted as “PC,” refers to the following:



where * denotes the point of attachment. The phosphorylcholine is a zwitterionic group and includes salts (such as inner salts), and protonated and deprotonated forms thereof.

[00134] “Phosphorylcholine containing polymer” is a polymer that contains phosphorylcholine. “Zwitterion containing polymer” refers to a polymer that contains a zwitterion.

[00135] Poly(acryloyloxyethyl phosphorylcholine) containing polymer refers to a polymer containing 2-(acryloyloxy)ethyl-2-(trimethylammonium)ethyl phosphate (HEA-PC shown below in Example 51) as monomer.

[00136] Poly(methacryloyloxyethyl phosphorylcholine) containing polymer refers to a polymer containing 2-(methacryloyloxy)ethyl-2-(trimethylammonium)ethyl phosphate (HEMA-PC) as monomer.

[00137] “Molecular weight” in the context of the polymer can be expressed as either a number average molecular weight, or a weight average molecular weight or a peak molecular

weight. Unless otherwise indicated, all references to molecular weight herein refer to the peak molecular weight. These molecular weight determinations, number average (M_n), weight average (M_w) and peak (M_p), can be measured using size exclusion chromatography or other liquid chromatography techniques. Other methods for measuring molecular weight values can also be used, such as the use of end-group analysis or the measurement of colligative properties (*e.g.*, freezing-point depression, boiling-point elevation, or osmotic pressure) to determine number average molecular weight, or the use of light scattering techniques, ultracentrifugation or viscometry to determine weight average molecular weight. In a preferred embodiment of the present invention, the molecular weight is measured by SEC-MALS (size exclusion chromatography – multi angle light scattering). The polymeric reagents of the invention are typically polydisperse (*i.e.*, number average molecular weight and weight average molecular weight of the polymers are not equal), preferably possessing low polydispersity values of, for example, less than about 1.5, as judged, for example, by the PDI value derived from the SEC-MALS measurement. In other embodiments, the polydispersities (PDI) are more preferably in the range of about 1.4 to about 1.2, still more preferably less than about 1.15, and still more preferably less than about 1.10, yet still more preferably less than about 1.05, and most preferably less than about 1.03.

[00138] The phrase “a” or “an” entity refers to one or more of that entity; for example, a compound refers to one or more compounds or at least one compound. As such, the terms “a” (or “an”), “one or more”, and “at least one” can be used interchangeably herein.

[00139] “About” means variation one might see in measurements taken among different instruments, samples, and sample preparations.

[00140] “Protected,” “protected form,” “protecting group” and “protective group” refer to the presence of a group (*i.e.*, the protecting group) that prevents or blocks reaction of a particular chemically reactive functional group in a molecule under certain reaction conditions. Protecting groups 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. Suitable protecting groups include those such as found in the treatise by Greene et al., “Protective Groups In Organic Synthesis,” 3rd Edition, John Wiley and Sons, Inc., New York, 1999.

[00141] “Alkyl” refers to a straight or branched, saturated, aliphatic radical having the number of carbon atoms indicated. For example, C₁-C₆ alkyl includes, but is not limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, hexyl, etc. Other alkyl groups include, but are not limited to heptyl, octyl, nonyl, decyl, etc. Alkyl can include any number of carbons, such as 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 2-3, 2-4, 2-5, 2-6, 3-4, 3-5, 3-6, 4-5, 4-6 and 5-6. The alkyl group is typically monovalent, but can be divalent, such as when the alkyl group links two moieties together.

[00142] The term “lower” referred to above and hereinafter in connection with organic radicals or compounds respectively defines a compound or radical which can be branched or unbranched with up to and including 7, preferably up to and including 4 and (as unbranched) one or two carbon atoms.

[00143] “Alkylene” refers to an alkyl group, as defined above, linking at least two other groups, *i.e.*, a divalent hydrocarbon radical. The two moieties linked to the alkylene can be linked to the same atom or different atoms of the alkylene. For instance, a straight chain alkylene can be the bivalent radical of -(CH₂)_n, where n is 1, 2, 3, 4, 5 or 6. Alkylene groups include, but are not limited to, methylene, ethylene, propylene, isopropylene, butylene, isobutylene, sec-butylene, pentylene and hexylene.

[00144] Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be a variety of groups selected from: -OR', =O, =NR', =N-OR', -NR'R'', -SR', -halogen, -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -CONR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)₂R', -NH-C(NH₂)=NH, -NR'C(NH₂)=NH, -NH-C(NH₂)=NR', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -CN and -NO₂ in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. R', R'' and R''' each independently refer to hydrogen, unsubstituted (C₁-C₈)alkyl and heteroalkyl, unsubstituted aryl, aryl substituted with 1-3 halogens, unsubstituted alkyl, alkoxy or thioalkoxy groups, or aryl-(C₁-C₄)alkyl groups. When R' and R'' are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 5-, 6-, or 7-membered ring. For example, -NR'R'' is meant to include 1-pyrrolidinyl and 4-morpholinyl. The term “alkyl” is include groups such as haloalkyl (*e.g.*, -CF₃ and -CH₂CF₃) and acyl

(*e.g.*, -C(O)CH₃, -C(O)CF₃, -C(O)CH₂OCH₃, and the like). Preferably, the substituted alkyl and heteroalkyl groups have from 1 to 4 substituents, more preferably 1, 2 or 3 substituents.

Exceptions are those perhalo alkyl groups (*e.g.*, pentafluoroethyl and the like) which are also preferred and contemplated by the present invention.

[00145] Substituents for the alkyl and heteroalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) can be one or more of a variety of groups selected from, but not limited to: -OR', =O, =NR', =N-OR', -NR'R'', -SR', -halogen, -SiR'R''R''', -OC(O)R', -C(O)R', -CO₂R', -CONR'R'', -OC(O)NR'R'', -NR''C(O)R', -NR'-C(O)NR''R''', -NR''C(O)₂R', -NR-C(NR'R''R''')=NR''', -NR-C(NR'R'')=NR''', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -NRSO₂R', -CN and -NO₂ in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. R', R'', R''' and R'''' each preferably independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, *e.g.*, aryl substituted with 1-3 halogens, substituted or unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. When a compound of the invention includes more than one R group, for example, each of the R groups is independently selected as are each R', R'', R''' and R'''' groups when more than one of these groups is present. When R' and R'' are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 5-, 6-, or 7-membered ring. For example, -NR'R'' is meant to include, but not be limited to, 1-pyrrolidinyl and 4-morpholinyl. From the above discussion of substituents, one of skill in the art will understand that the term "alkyl" is meant to include groups including carbon atoms bound to groups other than hydrogen groups, such as haloalkyl (*e.g.*, -CF₃ and -CH₂CF₃) and acyl (*e.g.*, -C(O)CH₃, -C(O)CF₃, -C(O)CH₂OCH₃, and the like).

[00146] "Alkoxy" refers to alkyl group having an oxygen atom that either connects the alkoxy group to the point of attachment or is linked to two carbons of the alkoxy group. Alkoxy groups include, for example, methoxy, ethoxy, propoxy, iso-propoxy, butoxy, 2-butoxy, iso-butoxy, sec-butoxy, tert-butoxy, pentoxy, hexoxy, etc. The alkoxy groups can be further substituted with a variety of substituents described within. For example, the alkoxy groups can be substituted with halogens to form a "halo-alkoxy" group.

[00147] “Carboxyalkyl” means an alkyl group (as defined herein) substituted with a carboxy group. The term “carboxycycloalkyl” means a cycloalkyl group (as defined herein) substituted with a carboxy group. The term alkoxyalkyl means an alkyl group (as defined herein) substituted with an alkoxy group. The term “carboxy” employed herein refers to carboxylic acids and their esters.

[00148] “Haloalkyl” refers to alkyl as defined above where some or all of the hydrogen atoms are substituted with halogen atoms. Halogen (halo) preferably represents chloro or fluoro, but may also be bromo or iodo. For example, haloalkyl includes trifluoromethyl, fluoromethyl, 1,2,3,4,5-pentafluoro-phenyl, etc. The term “perfluoro” defines a compound or radical which has all available hydrogens that are replaced with fluorine. For example, perfluorophenyl refers to 1,2,3,4,5-pentafluorophenyl, perfluoromethyl refers to 1,1,1-trifluoromethyl, and perfluoromethoxy refers to 1,1,1-trifluoromethoxy.

[00149] “Fluoro-substituted alkyl” refers to an alkyl group where one, some, or all hydrogen atoms have been replaced by fluorine.

[00150] “Cytokine” in the context of this invention is a member of a group of protein signaling molecules that may participate in cell-cell communication in immune and inflammatory responses. Cytokines are typically small, water-soluble glycoproteins that have a mass of about 8-35 kDa.

[00151] “Cycloalkyl” refers to a cyclic hydrocarbon group that contains from about 3 to 12, from 3 to 10, or from 3 to 7 endocyclic carbon atoms. Cycloalkyl groups include fused, bridged and spiro ring structures.

[00152] “Endocyclic” refers to an atom or group of atoms which comprise part of a cyclic ring structure.

[00153] “Exocyclic” refers to an atom or group of atoms which are attached but do not define the cyclic ring structure.

[00154] “Cyclic alkyl ether” refers to a 4 or 5 member cyclic alkyl group having 3 or 4 endocyclic carbon atoms and 1 endocyclic oxygen or sulfur atom (*e.g.*, oxetane, thietane, tetrahydrofuran, tetrahydrothiophene); or a 6 to 7 member cyclic alkyl group having 1 or 2 endocyclic oxygen or sulfur atoms (*e.g.*, tetrahydropyran, 1,3-dioxane, 1,4-dioxane, tetrahydrothiopyran, 1,3-dithiane, 1,4-dithiane, 1,4-oxathiane).

[00155] “Alkenyl” refers to either a straight chain or branched hydrocarbon of 2 to 6 carbon atoms, having at least one double bond. Examples of alkenyl groups include, but are not limited to, vinyl, propenyl, isopropenyl, 1-butenyl, 2-butenyl, isobutenyl, butadienyl, 1-pentenyl, 2-pentenyl, isopentenyl, 1,3-pentadienyl, 1,4-pentadienyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 1,3-hexadienyl, 1,4-hexadienyl, 1,5-hexadienyl, 2,4-hexadienyl, or 1,3,5-hexatrienyl. Alkenyl groups can also have from 2 to 3, 2 to 4, 2 to 5, 3 to 4, 3 to 5, 3 to 6, 4 to 5, 4 to 6 and 5 to 6 carbons. The alkenyl group is typically monovalent, but can be divalent, such as when the alkenyl group links two moieties together.

[00156] “Alkenylene” refers to an alkenyl group, as defined above, linking at least two other groups, *i.e.*, a divalent hydrocarbon radical. The two moieties linked to the alkenylene can be linked to the same atom or different atoms of the alkenylene. Alkenylene groups include, but are not limited to, ethenylene, propenylene, isopropenylene, butenylene, isobutenylene, sec-butenylene, pentenylene and hexenylene.

[00157] “Alkynyl” refers to either a straight chain or branched hydrocarbon of 2 to 6 carbon atoms, having at least one triple bond. Examples of alkynyl groups include, but are not limited to, acetylenyl, propynyl, 1-butyne, 2-butyne, isobutyne, sec-butyne, butadiynyl, 1-pentyne, 2-pentyne, isopentyne, 1,3-pentadiynyl, 1,4-pentadiynyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 1,3-hexadiynyl, 1,4-hexadiynyl, 1,5-hexadiynyl, 2,4-hexadiynyl, or 1,3,5-hexatriynyl. Alkynyl groups can also have from 2 to 3, 2 to 4, 2 to 5, 3 to 4, 3 to 5, 3 to 6, 4 to 5, 4 to 6 and 5 to 6 carbons. The alkynyl group is typically monovalent, but can be divalent, such as when the alkynyl group links two moieties together.

[00158] “Alkynylene” refers to an alkynyl group, as defined above, linking at least two other groups, *i.e.*, a divalent hydrocarbon radical. The two moieties linked to the alkynylene can be linked to the same atom or different atoms of the alkynylene. Alkynylene groups include, but are not limited to, ethynylene, propynylene, butynylene, sec-butyne, pentynylene and hexynylene.

[00159] “Cycloalkyl” refers to a saturated or partially unsaturated, monocyclic, fused bicyclic or bridged polycyclic ring assembly containing from 3 to 12 ring atoms, or the number of atoms indicated. Monocyclic rings include, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cyclooctyl. Bicyclic and polycyclic rings include, for example, norbornane,

decahydronaphthalene and adamantane. For example, C₃₋₈cycloalkyl includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclooctyl, and norbornane.

[00160] “Cycloalkylene” refers to a cycloalkyl group, as defined above, linking at least two other groups, *i.e.*, a divalent hydrocarbon radical. The two moieties linked to the cycloalkylene can be linked to the same atom or different atoms of the cycloalkylene. Cycloalkylene groups include, but are not limited to, cyclopropylene, cyclobutylene, cyclopentylene, cyclohexylene, and cyclooctylene.

[00161] “Heterocycloalkyl” refers to a ring system having from 3 ring members to about 20 ring members and from 1 to about 5 heteroatoms such as N, O and S. Additional heteroatoms can also be useful, including, but not limited to, B, Al, Si and P. The heteroatoms can also be oxidized, such as, but not limited to, -S(O)- and -S(O)₂-. For example, heterocycle includes, but is not limited to, tetrahydrofuranyl, tetrahydrothiophenyl, morpholino, pyrrolidinyl, pyrrolinyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperazinyl, piperidinyl, indolinyl, quinuclidinyl and 1,4-dioxa-8-aza-spiro[4.5]dec-8-yl.

[00162] “Heterocycloalkylene” refers to a heterocyclalkyl group, as defined above, linking at least two other groups. The two moieties linked to the heterocycloalkylene can be linked to the same atom or different atoms of the heterocycloalkylene.

[00163] “Aryl” refers to a monocyclic or fused bicyclic, tricyclic or greater, aromatic ring assembly containing 6 to 16 ring carbon atoms. For example, aryl may be phenyl, benzyl or naphthyl, preferably phenyl. “Arylene” means a divalent radical derived from an aryl group. Aryl groups can be mono-, di- or tri-substituted by one, two or three radicals selected from alkyl, alkoxy, aryl, hydroxy, halogen, cyano, amino, amino-alkyl, trifluoromethyl, alkylenedioxy and oxy-C₂-C₃-alkylene; all of which are optionally further substituted, for instance as hereinbefore defined; or 1- or 2-naphthyl; or 1- or 2-phenanthrenyl. Alkylenedioxy is a divalent substitute attached to two adjacent carbon atoms of phenyl, e.g. methylenedioxy or ethylenedioxy. Oxy-C₂-C₃-alkylene is also a divalent substituent attached to two adjacent carbon atoms of phenyl, e.g. oxyethylene or oxypropylene. An example for oxy- C₂-C₃-alkylene-phenyl is 2,3-dihydrobenzofuran-5-yl.

[00164] Preferred as aryl is naphthyl, phenyl or phenyl mono- or disubstituted by alkoxy, phenyl, halogen, alkyl or trifluoromethyl, especially phenyl or phenyl-mono- or disubstituted by alkoxy, halogen or trifluoromethyl, and in particular phenyl.

[00165] Examples of substituted phenyl groups as R are, *e.g.* 4-chlorophen-1-yl, 3,4-dichlorophen-1-yl, 4-methoxyphen-1-yl, 4-methylphen-1-yl, 4-aminomethylphen-1-yl, 4-methoxyethylaminomethylphen-1-yl, 4-hydroxyethylaminomethylphen-1-yl, 4-hydroxyethyl-(methyl)-aminomethylphen-1-yl, 3-aminomethylphen-1-yl, 4-N-acetylaminomethylphen-1-yl, 4-aminophen-1-yl, 3-aminophen-1-yl, 2-aminophen-1-yl, 4-phenyl-phen-1-yl, 4-(imidazol-1-yl)-phenyl, 4-(imidazol-1-ylmethyl)-phen-1-yl, 4-(morpholin-1-yl)-phen-1-yl, 4-(morpholin-1-ylmethyl)-phen-1-yl, 4-(2-methoxyethylaminomethyl)-phen-1-yl and 4-(pyrrolidin-1-ylmethyl)-phen-1-yl, 4-(thiophenyl)-phen-1-yl, 4-(3-thiophenyl)-phen-1-yl, 4-(4-methylpiperazin-1-yl)-phen-1-yl, and 4-(piperidinyl)-phenyl and 4-(pyridinyl)-phenyl optionally substituted in the heterocyclic ring.

[00166] “Arylene” refers to an aryl group, as defined above, linking at least two other groups. The two moieties linked to the arylene are linked to different atoms of the arylene. Arylene groups include, but are not limited to, phenylene.

[00167] “Arylene-oxy” refers to an arylene group, as defined above, where one of the moieties linked to the arylene is linked through an oxygen atom. Arylene-oxy groups include, but are not limited to, phenylene-oxy.

[00168] Similarly, substituents for the aryl and heteroaryl groups are varied and are selected from: -halogen, -OR', -OC(O)R', -NR'R'', -SR', -R', -CN, -NO₂, -CO₂R', -CONR'R'', -C(O)R', -OC(O)NR'R'', -NR''C(O)R', -NR''C(O)₂R', -NR'-C(O)NR''R''', -NH-C(NH₂)=NH, -NR'C(NH₂)=NH, -NH-C(NH₂)=NR', -S(O)R', -S(O)₂R', -S(O)₂NR'R'', -N₃, -CH(Ph)₂, perfluoro(C₁-C₄)alkoxy, and perfluoro(C₁-C₄)alkyl, in a number ranging from zero to the total number of open valences on the aromatic ring system; and where R', R'' and R''' are independently selected from hydrogen, (C₁-C₈)alkyl and heteroalkyl, unsubstituted aryl and heteroaryl, (unsubstituted aryl)-(C₁-C₄)alkyl, and (unsubstituted aryl)oxy-(C₁-C₄)alkyl.

[00169] Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -T-C(O)-(CH₂)_q-U-, wherein T and U are independently -NH-, -O-, -CH₂- or a single bond, and q is an integer of from 0 to 2.

Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -A-(CH₂)_r-B-, wherein A and B are independently -CH₂-, -O-, -NH-, -S-, -S(O)-, -S(O)₂-, -S(O)₂NR'- or a single bond, and r is an

integer of from 1 to 3. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula $-(CH_2)_s-X-(CH_2)_t-$, where s and t are independently integers of from 0 to 3, and X is $-O-$, $-NR'-$, $-S-$, $-S(O)-$, $-S(O)_2-$, or $-S(O)_2NR'-$. The substituent R' in $-NR'-$ and $-S(O)_2NR'-$ is selected from hydrogen or unsubstituted (C_1-C_6) alkyl.

[00170] “Heteroaryl” refers to a monocyclic or fused bicyclic or tricyclic aromatic ring assembly containing 5 to 16 ring atoms, where from 1 to 4 of the ring atoms are a heteroatom each N, O or S. For example, heteroaryl includes pyridyl, indolyl, indazolyl, quinoxaliny, quinoliny, isoquinoliny, benzothienyl, benzofuranyl, furanyl, pyrrolyl, thiazolyl, benzothiazolyl, oxazolyl, isoxazolyl, triazolyl, tetrazolyl, pyrazolyl, imidazolyl, thienyl, or any other radicals substituted, especially mono- or di-substituted, by e.g. alkyl, nitro or halogen. Pyridyl represents 2-, 3- or 4-pyridyl, advantageously 2- or 3-pyridyl. Thienyl represents 2- or 3-thienyl. Quinoliny represents preferably 2-, 3- or 4-quinoliny. Isoquinoliny represents preferably 1-, 3- or 4-isoquinoliny. Benzopyranyl, benzothiopyranyl represents preferably 3-benzopyranyl or 3-benzothiopyranyl, respectively. Thiazolyl represents preferably 2- or 4-thiazolyl, and most preferred 4-thiazolyl. Triazolyl is preferably 1-, 2- or 5-(1,2,4-triazolyl). Tetrazolyl is preferably 5-tetrazolyl.

[00171] Preferably, heteroaryl is pyridyl, indolyl, quinoliny, pyrrolyl, thiazolyl, isoxazolyl, triazolyl, tetrazolyl, pyrazolyl, imidazolyl, thienyl, furanyl, benzothiazolyl, benzofuranyl, isoquinoliny, benzothienyl, oxazolyl, indazolyl, or any of the radicals substituted, especially mono- or di-substituted.

[00172] The term “heteroalkyl” refers to an alkyl group having from 1 to 3 heteroatoms such as N, O and S. Additional heteroatoms can also be useful, including, but not limited to, B, Al, Si and P. The heteroatoms can also be oxidized, such as, but not limited to, $-S(O)-$ and $-S(O)_2-$. For example, heteroalkyl can include ethers, thioethers, alkyl-amines and alkyl-thiols.

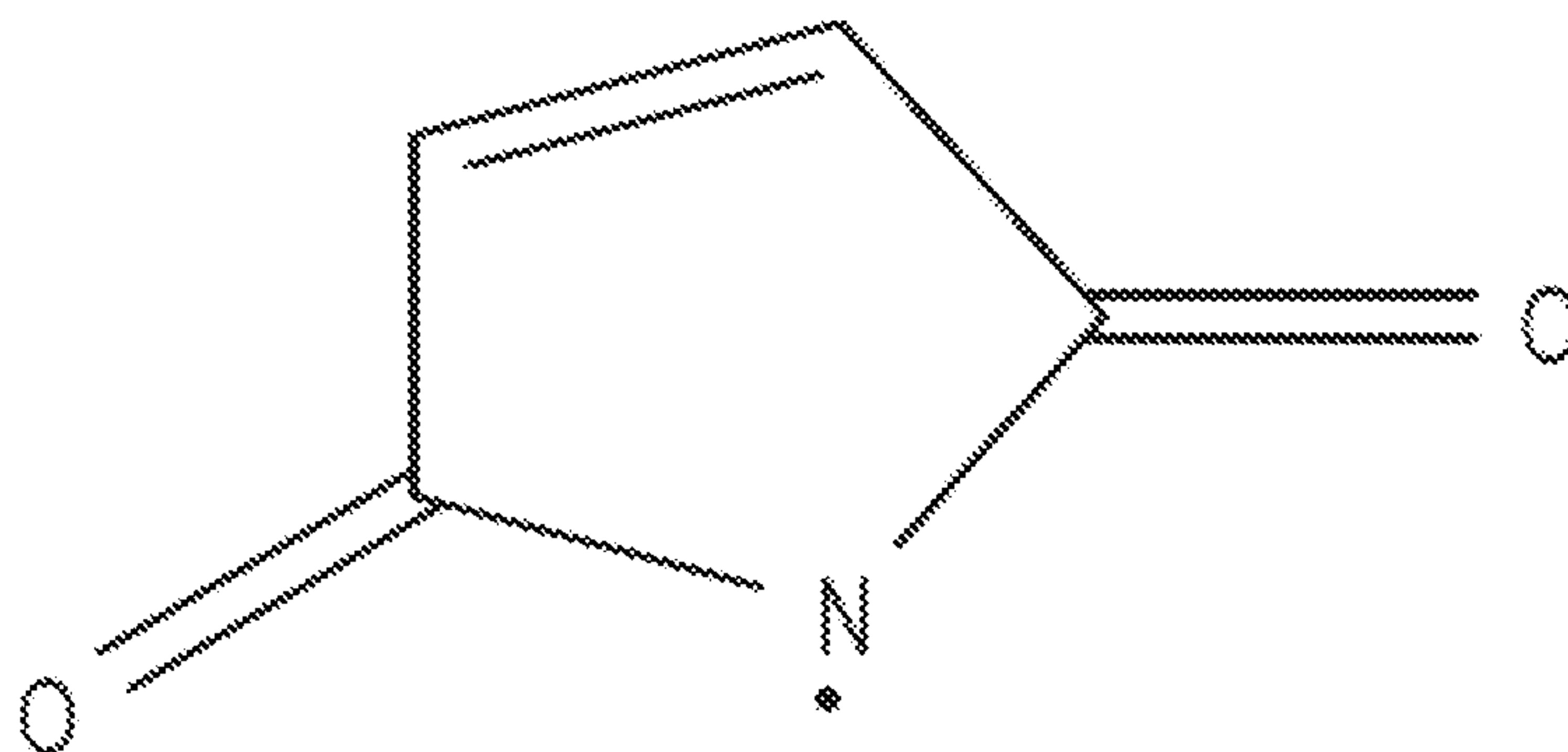
[00173] The term “heteroalkylene” refers to a heteroalkyl group, as defined above, linking at least two other groups. The two moieties linked to the heteroalkylene can be linked to the same atom or different atoms of the heteroalkylene.

[00174] “Electrophile” refers to an ion or atom or collection of atoms, which may be ionic, having an electrophilic center, *i.e.*, a center that is electron seeking, capable of reacting with a

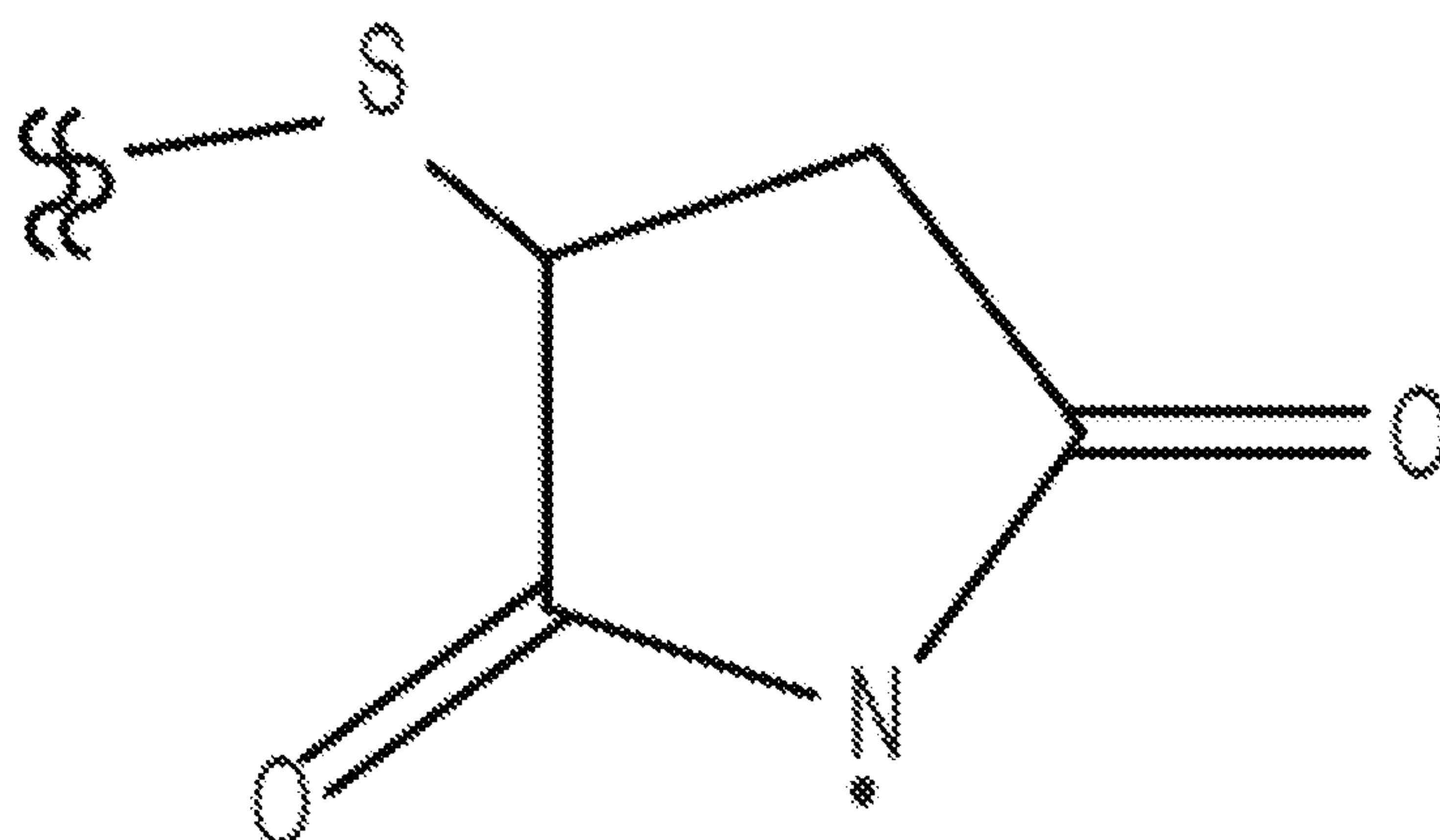
nucleophile. An electrophile (or electrophilic reagent) is a reagent that forms a bond to its reaction partner (the nucleophile) by accepting both bonding electrons from that reaction partner.

[00175] “Nucleophile” refers to an ion or atom or collection of atoms, which may be ionic, having a nucleophilic center, *i.e.*, a center that is seeking an electrophilic center or capable of reacting with an electrophile. A nucleophile (or nucleophilic reagent) is a reagent that forms a bond to its reaction partner (the electrophile) by donating both bonding electrons. A “nucleophilic group” refers to a nucleophile after it has reacted with a reactive group. Non limiting examples include amino, hydroxyl, alkoxy, haloalkoxy and the like.

[00176] “Maleimido” refers to a pyrrole-2,5-dione-1-yl group having the structure:



which upon reaction with a sulfhydryl (*e.g.*, a thio alkyl) forms an -S-maleimido group having the structure



where “•” indicates the point of attachment for the maleimido group and “S” indicates the point of attachment of the sulfur atom the thiol to the remainder of the original sulfhydryl bearing group.

[00177] For the purpose of this disclosure, “naturally occurring amino acids” found in proteins and polypeptides are L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamine, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, and or L-valine. “Non-naturally occurring amino acids” found in proteins are any amino acid other than those recited as naturally occurring amino acids. Non-naturally occurring amino acids include, without limitation, the D isomers of the naturally occurring amino acids, and mixtures of D and L isomers of the naturally occurring amino acids. Other amino acids, such as N-alpha-methyl amino acids (e.g. sarcosine), 4-hydroxyproline, desmosine, isodesmosine, 5-hydroxylysine, epsilon-N-methyllysine, 3-methylhistidine, although found in naturally occurring proteins, are considered to be non-naturally occurring amino acids found in proteins for the purpose of this disclosure as they are generally introduced by means other than ribosomal translation of mRNA.

[00178] “Linear” in reference to the geometry, architecture or overall structure of a polymer, refers to polymer having a single polymer arm.

[00179] “Branched,” in reference to the geometry, architecture or overall structure of a polymer, refers to a polymer having 2 or more polymer “arms” extending from a core structure contained within an initiator. The initiator may be employed in an atom transfer radical polymerization (ATRP) reaction. A branched polymer may possess 2 polymer chains (arms), 3 polymer arms, 4 polymer arms, 5 polymer arms, 6 polymer arms, 7 polymer arms, 8 polymer arms, 9 polymer arms or more. Each polymer arm extends from a polymer initiation site. Each polymer initiation site is capable of being a site for the growth of a polymer chain by the addition of monomers. For example and not by way of limitation, using ATRP, the site of polymer initiation on an initiator is typically an organic halide undergoing a reversible redox process catalyzed by a transition metal compound such as cuprous halide. Preferably, the halide is a bromine.

[00180] “Pharmaceutically acceptable excipient” refers to an excipient that can be included in the compositions of the invention and that causes no significant adverse toxicological effect on

the patient and is approved or approvable by the FDA for therapeutic use, particularly in humans. Non-limiting examples of pharmaceutically acceptable excipients include water, NaCl, normal saline solutions, lactated Ringer's, normal sucrose, normal glucose and the like.

[00181] Dual antagonists are administered in an effective regime meaning a dosage, route of administration and frequency of administration that delays the onset, reduces the severity, inhibits further deterioration, and/or ameliorates at least one sign or symptom of a disorder. If a patient is already suffering from a disorder, the regime can be referred to as a therapeutically effective regime. If the patient is at elevated risk of the disorder relative to the general population but is not yet experiencing symptoms, the regime can be referred to as a prophylactically effective regime. In some instances, therapeutic or prophylactic efficacy can be observed in an individual patient relative to historical controls or past experience in the same patient. In other instances, therapeutic or prophylactic efficacy can be demonstrated in a preclinical or clinical trial in a population of treated patients relative to a control population of untreated patients.

[00182] The "biological half-life" of a substance is a pharmacokinetic parameter which specifies the time required for one half of the substance to be removed from a tissue or an organism following introduction of the substance.

[00183] "HEMA-PC" is 2-(methacryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate.

[00184] "TAF" means a PDGFR β -GS10-anti-VEGF-A heavy chain/anti-VEGF-A light chain wherein amino acids 1-282 of the heavy chain correspond to amino acids 33 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1), fused as a single open reading frame via a glycine-serine linker (GGGGSGGGGS) linked to the N terminus of a bevacizumab heavy chain sequence having the following mutations in the variable region: T28D, N31H, H97Y, S100aT (Ferrara N, Damico L, Shams N, et al. 2006. Development of Ranibizumab, an anti-vascular endothelial growth factor antigen binding fragment, as therapy for neovascular age-related macular degeneration. *Retina* 26(8):859-870); and the following in the Fc region: L234A, L235A, and G237A (EU numbering) (Strohl WR. 2009. Optimization of Fc-mediated effector functions of monoclonal antibodies. *Curr Opin in Biotech.* 20: 685–691). The light chain is the bevacizumab light chain having an M4L mutation. TAF normally exists as a dimer having two heavy chains and two light chains. TAF may or may not have carbohydrate or other post-translational modifications after being expressed from cells. TAF is also sometimes called

TAFwt or TAFWT, which indicates that the molecule in question does not have either the Q347C or L443C mutations in the heavy chain (Fc region) as do TAF347 or TAF443, defined *infra*.

[00185] “TAF347” is the same as TAF except that it has the Q347C mutation.

[00186] “TAF443” is the same as TAF except that it has the L443C mutation. TAF443 is sometimes referred to herein as OG1321.

[00187] “OG1786” is a 9-arm initiator used for polymer synthesis with the structure shown in FIG. 35, which depicts that salt form of OG1786 with trifluoroacetic acid. OG1786 may be used in accordance with the present invention as other salts or as the free base.

[00188] “OG1801” is an approximately (+/- 15%) 750 kDa polymer (either by Mn or Mp) made using OG1786 as an initiator for ATRP synthesis using the monomer HEMA-PC.

[00189] “OG1802” is OG1801 with a maleimide functionality added and is shown in FIG. 36 wherein each of $n_1, n_2, n_3, n_4, n_5, n_6, n_7, n_8$ and n_9 is an integer (positive) (from 0 up to about 3000) such that the total molecular weight of the polymer is (Mw) $750,000 \pm 15\%$ daltons.

[00190] “OG1448” is TAF443 conjugated to the OG1802 biopolymer. .

DETAILED DESCRIPTION

I. GENERAL

[00191] The present invention provides a dual VEGF/PDGF antagonist comprising a VEGF antagonist linked to a PDGF antagonist. The VEGF antagonist is an antibody to a VEGF or VEGFR or is a VEGFR extracellular trap segment (i.e., a segment from the extracellular region of one or more VEGFR receptors that inhibits binding of at least one VEGFR to at least one VEGF). The PDGF antagonist is an antibody to a PDGF or PDGFR or is a PDGFR extracellular trap segment (i.e., segment from the extracellular region of one or more PDGFRs, which inhibits binding of at least one PDGFR and at least one PDGF). At least one of the antagonists is not an antibody, or put another way, at least one of the antagonists is an extracellular trap segment. Preferably, the dual antagonist includes an antibody antagonist and one extracellular trap segment antagonist. In such a dual antagonist the extracellular trap segment is preferably fused, optionally via a linker to the N-terminus of the antibody heavy chain. The antibody light chain is complexed with the antibody heavy chain in similar manner to that in a natural antibody. Such dual antagonists are preferably provided in the form of conjugates with a half-life extending

moiety conjugated to the dual antagonist. Preferably, a cysteine residue is used for conjugation which has been introduced into the antagonist. More preferably, the cysteine residue is at positions 347 or 443 of an IgG1 heavy chain. It is preferred that the half-life extending moiety is a zwitterionic polymer. Most preferably the zwitterionic polymer is a phosphorylcholine containing polymer.

[00192] Angiogenesis is the process by which new blood vessels are created and plays a crucial role in development (going from embryo to adult) and in wound healing (restoring blood flow to damaged or injured tissue). However, when angiogenesis is dysregulated, it contributes to the pathologies of many disorders, including cancer, psoriasis, arthritis and blindness.

Carmeliet P. 2003. Angiogenesis in health and disease. *Nature Med* 9(6):653-660.

[00193] Abnormal angiogenesis is associated with wet age related macular degeneration (a leading cause of blindness in the elderly) and with cancer. Angiogenesis is characterized by an increase in proliferating endothelial and stromal cells and vasculature with altered morphology. See, generally, Folkman J. 2007. Angiogenesis: an organizing principle for drug discovery?. *Nat Rev Drug* 6:273-286 and Baluk P, Hashizume H, McDonald DM. 2005. Cellular abnormalities of blood vessels as targets in cancer. *Curr Opin Genet Dev*. 15:102-111.

[00194] As mentioned above, neovascularization (NV) is a normal process occurring both in development and in wound healing but can become pathological when angiogenesis is dysregulated and occurs in tissues associated with tumors (cancer), avascular cornea or the subretinal space (wet AMD). The proliferation, invasion and migration of NV vessels is controlled by a complex interplay between growth factors, vascular endothelial cells, extracellular matrix molecules, chemokines and cell signaling molecules.

[00195] NV tissue is composed of endothelial cells (EC), pericytes and inflammatory cells (e.g. macrophages). Pericytes are derived via differentiation from mast cells. The process of neovascularization first involves the formation of angiogenic sprouts composed of EC from existing capillaries into the avascular space. VEGF signaling is understood to be the master switch for this NV process. In this regard, VEGF has been localized in the tip cell filopodia which leads the angiogenic sprout.

[00196] Following sprout formation, the newly formed vessels are coated by pericytes, leading to maturation of the NV. Pericyte coating of NV leads to stabilization and support of NV

both physically and through signaling, including pericyte production of VEGF. Armulik A, Abramsson A, Betsholtz C. 2005. Endothelial/Pericyte Interactions. *Circ Res.* 97:512-523.

[00197] Approved wet AMD therapies are all directed at the suppression of VEGF signaling. These therapies include pegaptanib (Macugen®), approved in 2004, Genentech's bevacizumab (Avastin®), approved in 2004 for cancer, used off label for AMD, Genentech's ranibizumab (Lucentis®), approved in 2006, and Regeneron's aflibercept (Eylea®) approved in 2011.

Pegaptanib is an aptamer based therapeutic, but with a limited market compared with protein based therapeutics likely due to the limited gains in visual acuity for patients. Bevacizumab is an anti-VEGFA IgG1 antibody approved for cancer treatment, but is widely used off label for treatment of AMD. Ranibizumab is a Fab which was affinity matured from bevacizumab and is approved for AMD. However, the market for Ranibizumab is substantially undercut by use of the much cheaper bevacizumab. Finally, aflibercept is a VEGF trap, employing a soluble receptor fragment decoy.

[00198] Anti-VEGF monotherapy has not lead to disease-modifying regression of pathological NV. Brown DM, Kaiser PK, Michels M, et al. 2006. ANCHOR Study Group. Ranibizumab versus verteporfin for neovascular age-related macular degeneration. *N Engl J Med* 355(14):1432-1444; Rosenfeld PJ, Brown DM, Heier JS, et al. 2006. MARINA study group. Ranibizumab for neovascular age-related macular degeneration. *N Engl J Med* 355(14):1419-1431; Regillo CD, Brown DM, Abraham P, et al. 2008. Randomized, double-masked, sham0controloed trial of ranibizumab for neovacular age-related macular degeneration: PIER study year 1. *Am J Ophthalmol.* 145:239-248. Instead the majority of the efficacy or therapeutic benefit of anti-VEGF therapies is due to their anti-permeability property. Zebrowski BK, Yano S, Liu W, et al. 1999. Vascular endothelial growth factor levels and induction of permeability in malignant pleural effusions. *Clin Cancer Res* 5:3364-3368.

[00199] Because conventional anti-VEGF therapies do not cause regression of pathological NV, visual acuity gains for many patients have been quite limited. Moreover, neovasculature can also lead to subretinal fibrosis which is a cause of blindness in wet AMD patients.

[00200] Subretinal scarring develops in nearly half of treated eyes within two years of anti-VEGF therapy. Daniel E, Toth CA, Grunwald JE. 2014. Risk of scar in the comparison of age-related macular degeneration in clinical settings. *Retina* 32:1480-1485. Subretinal fibrosis formation can cause permanent dysfunction of the macular system; it causes destruction of

photoreceptors, retinal pigment epithelium and choroidal vessels. Ishikawa K, Ram K, Hinton DR. 2015. Molecular mechanisms of subretinal fibrosis in age-related macular degeneration. *Eye Res.* Mar 13, 2015 Epub 1-7. Although anti-VEGF therapy generally stabilizes or improves visual acuity, scar formation has been identified as one of the causes of loss of visual acuity after treatment. Cohen SY, Oubraham H, Uzzan J, et al. 2012. Causes of unsuccessful ranibizumab treatment in exudative age-related macular degeneration in clinical settings. *Retina* 32:1480-1485.

[00201] Proangiogenic factors are generally upregulated in pathological angiogenesis, including two members of the vascular endothelial growth factor (VEGF) family: VEGF-A and placental growth factor (PGF). VEGF-A and PGF activate quiescent endothelial cells, promote cell proliferation and vascular permeability. VEGF-A has been identified as a major factor in vascular leak in wet AMD. Dvorak HF, Nagy JA, Feng D, Brown LF, Dvorak AM. 1999. Vascular permeability factor/vascular endothelial growth factor and the significance of microvascular hyperpermeability in angiogenesis. *Curr Top Microbiol Immunol.* 237:97-132.

[00202] Platelet derived growth factor “PDGF” signaling plays an important role in NV maturation and in particular to the coating of NV by pericytes. The coating of NV endothelial cells by pericytes begins with EC expression of the paracrine platelet-derived growth factor B, which forms the homodimer PDGF-BB. PDGF-BB is highly retained in the tip cells of the angiogenic sprouts by heparin sulfate proteoglycan. This PDGF-BB is then recognized by the pericyte bound receptor PDGFR- β , which initiates the proliferation and migration of pericytes along the growing neovascularization.

[00203] PDGF-DD had also been discovered to play a central role in pathological angiogenesis. Kumar A, Hou X, Chunsik L, et al. 2010. Platelet-derived Growth Factor-DD Targeting Arrests Pathological Angiogenesis by Modulating Glycogen Synthase Kinase-3 β Phosphorylation. *J Biol Chem* 285(20):15500-15510. PDGF-DD overexpression induces blood vessel maturation during angiogenesis. Kong D, Wang Z, Sarkar FH, et al. 2008. Platelet-Derived Growth Factor-D Overexpression Contributes to Epithelial-Mesenchymal Transition of PC3 Prostate Cancer Cells. *Stem Cells* 26:1425-1435. PDGF-DD is highly expressed in the eye. Ray S, Gao C, Wyatt K, et al. 2005. Platelet-derived Growth Factor D, Tissue-specific Expression in the Eye, and a Key Role in Control of Lens Epithelial Cell Proliferation. *J Biol Chem.* 280:8494-8502. Kumar et al. (2010) found that PDGF-DD expression was upregulated

during pathological angiogenesis and that inhibition of PDGF-DD signaling decreased choroidal and retinal neovascularization.

[00204] The term “PDGF” as used herein means any member of the class of growth factors that (i) bind to a PDGF receptor such as PDGFR- β , or PDGFR- α ; (ii) activates a tyrosine kinase activity associated with the PDGF receptor; and (iii) thereby affects angiogenesis or an angiogenic process. The term “PDGF” generally refers to those members of the class of growth factors that induce DNA synthesis and mitogenesis through the binding and activation of a platelet-derived growth factor cell surface receptor (i.e., PDGFR) on a responsive cell type. PDGFs effect specific biological effects including, for example: directed cell migration (chemotaxis) and cell activation; phospholipase activation; increased phosphatidylinositol turnover and prostaglandin metabolism; stimulation of both collagen and collagenase synthesis by responsive cells; alteration of cellular metabolic activities, including matrix synthesis, cytokine production, and lipoprotein uptake; induction, indirectly, of a proliferative response in cells lacking PDGF receptors; fibrosis and potent vasoconstrictor activity. The term “PDGF” is meant to include both a “PDGF” polypeptide and its corresponding “PDGF” encoding gene or nucleic acid.

[00205] The PDGF family consists of disulfide bonded homo-ffdimers of PDGF-A (Swiss Protein P04085), -B (P01127), -C (Q9NRA1) and -D (Q9GZP0) and the hetero dimer PDGF-AB. The various PDGF isoforms exert their effect by binding to α and β -tyrosine kinase receptors (PDGFR- α (P16234) and PDGFR- β (P09619) respectively). See generally United States Patent No. 5,872,218 which is incorporated herein by reference for all purposes. The α and β receptors are structurally similar: both have extracellular domains with five immunoglobulin (Ig) like domains and intracellular domains with a kinase function. PDGF binding occurs mainly through domains 2 and 3 of the receptors and causes dimerization of the receptors. Ig like domain 4 is involved in receptor dimerization. Receptor dimerization is a key component of PDGF signaling: receptor dimerization leads to receptor auto-phosphorylation. Auto-phosphorylation in turns causes a conformational change in the receptor and activates the receptor kinase. PDGF-A, -B, -C and -D bind to the two different receptors with different affinities and effects. PDGF-AA, -AB, -BB and -CC induce $\alpha\alpha$ receptor homodimers, PDGF-BB and -DD induced $\beta\beta$ homodimers and PDGF-AB, -BB, -CC and -DD produce $\alpha\beta$ receptor heterodimers.

[00206] In terms of function, PDGFR- α and PDGFR- β appear to have substantially different roles. PDGFR- α signaling is involved in gastrulation and in development of the cranial and cardiac neural crest, gonads, lung, intestine, skin, CNS and skeleton. PDGFR- β signaling is involved in blood vessel formation and early hematopoiesis. Andrae J, Radosa G, Betsholtz C. 2008. Role of platelet-derived growth factors in physiology and medicine. *Genes Develop* 22:1276-1312. In terms of interaction of the various PDGF ligands with the receptors, PDGF-AA and PDGF-CC exclusively bind to and interact with PDGFR- α . PDGF-BB and PDGF-AB bind with α and β receptors. PDGF-DD exclusively interacts with PDGFR- β . Raica M, Cimpean AM. 2010. Platelet-Derived Growth Factor (PDGF)/PDGF Receptors (PDGFR) Axis as Target for Antitumor and Antiangiogenic Therapy. *Pharmaceut*. 3:572-599.

[00207] Unless otherwise apparent from the context reference to a PDGF means any of PDGF-A, -B, -C and -D in any of the natural isoforms or natural variants or induced variants having at least 90, 95, 98 or 99% sequence identity to a natural form. Preferably, such PDGFs are human PDGFs. Likewise reference to a PDGFR means PDGFR-A (P16234) or PDGFR-B including any natural isoform or natural variant, or an induced variant having at least 90, 95, 98 or 99% or 100% sequence identity to a natural sequences.

[00208] The amino acid sequence of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1) is set forth in FIG. 1, which shows a full-length human PDGFR- β (including the leader sequence), a 1106 amino acid protein. Amino acids 1-32 are part of the leader peptide which is cleaved off in the mature protein. PDGFR- β has five extracellular Ig-like domains D1-D5. Williams AF, Barclay AN. 1988. The immunoglobulin superfamily—domains for cell surface recognition. *Annu Rev Immunol*. 6:381-405. The full-length extracellular region runs from about amino acid 33 to 532, the transmembrane domain from about residue 533 to 553 and the cytoplasmic domain from about residue 554 to 1106. The extracellular region includes five immunoglobulin-like domains, D1-D5. The D1 domain is typically considered to be from about amino acid 33 (Leu) to about 123 (Pro). In accordance with the present invention, D1 may also be from 33 to 122 (Val). D2 is typically considered to be from about 124 (Thr) to about 213 (Ser). In accordance with the present invention, D2 may be 129 (Pro) to 210 (Gln). D3 is typically considered to be from about amino acid 214 (Ile) to 314 (Gly). In accordance with the present invention, D3 may be from 214 (Ile) to 309 (Thr). D4 is typically considered to be from about amino acid 315 (Tyr) to 416 (Pro). D5 is typically considered to be from about amino acid 417 (Val) to 531 (Lys).

[00209] The exact boundaries of the D1-D5 domains can vary depending on how the analysis is done. Preferably, the boundaries vary by 9 amino acids or less. Typically they vary by 7 amino acids or less, more typically by 5 amino acids or less. Usually, boundary variance is 3 amino acids or less. Most typically the boundaries vary by only an amino acid. The essential characteristic of each domain is its ability to bind to its cognate ligands.

[00210] A “PDGF antagonist” or a molecule that “antagonizes PDGF” is an agent that reduces, or inhibits, either partially or fully, at least one activity of a PDGF including its ability to specifically bind to a PDGFR, and consequent cellular responses, such as proliferation. PDGF antagonists include antibodies that specifically bind to a PDGF or PDGFR and extracellular trap segments from a PDGFR.

[00211] One or more portions of a PDGFR- β extracellular receptor sequence can be used as an antagonist for PDGF-PDGFR- β signaling. The term extracellular trap segment refers to a full length extracellular region or any portion thereof, or combination of portions from different PDGF receptors that can antagonize PDGF-PDGFR-beta signaling. Such portions are typically used free of the transmembrane and intracellular sequence of the PDGFR and are consequently referred to as being soluble. The portions antagonize by acting as a trap or decoy for a cognate PDGF. PDGF binds to the soluble PDGFR- β segment trap and is unable to bind to the corresponding membrane bound receptor. Preferably, such traps include one or more of PDGFR- β domains D1-D5. Preferably, the trap contains at least one of D2 and D3. More preferably, the trap contains D1, D2 and D3. More preferably the trap is a contiguous segment corresponding to amino acids 33 to 314 of FIG. 8 which contains D1-D3. PDGFR-alpha likewise includes domains D1 through D5 and extracellular trap segments incorporating corresponding domains of PDGFR-alpha can likewise be used instead of PDGFR-beta.

[00212] Antibodies can also be used as antagonists of PDGFR- β , including antibodies which bind to the receptor (e.g., 2A1E2 [U.S. Pat. No. 7,060,271]; HuM4Ts.22 [U.S. Pat. No. 5,882,644]; or 1B3 or 2C5 [U.S. Pat. No. 7,740,850]), and anti-PDGF antibodies such as anti-PDGF BB, anti-PDGF-DD, anti-PDGF-BB and anti-PDGF-AB.

[00213] “VEGF” or “vascular endothelial growth factor” is a human vascular endothelial growth factor that affects angiogenesis or an angiogenic process. In particular, the term VEGF means any member of the class of growth factors that (i) bind to a VEGF receptor such as VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), or VEGFR-3 (FLT-4); (ii) activates a tyrosine kinase

activity associated with the VEGF receptor; and (iii) thereby affects angiogenesis or an angiogenic process.

[00214] The VEGF family of factors is made up of five related glycoproteins: VEGF-A (also known as VPE), -B, -C, -D and PGF (placental growth factor). Of these, VEGF-A is the most well studied and is the target of anti-angiogenic therapy. Ferrara et al, (2003) Nat. Med. 9:669-676. VEGF-A exists as a number of different isoforms which are generated both by alternative splicing and proteolysis: VEGF-A₂₀₆, VEGF-A₁₈₉, VEGF-A₁₆₅, and VEGF-A₁₂₁. The isoforms differ in their ability to bind heparin and non-signaling binding proteins called neuropilins. The isoforms are all biologically active as dimers.

[00215] The various effects of VEGF are mediated by the binding of a VEGF, e.g., VEGF-A (P15692), -B (P49766), -C (P49767) and -D (Q43915), to receptor tyrosine kinases (RTKs). The VEGF family receptors belong to class V RTKs and each carry seven Ig-like domains in the extracellular domain (ECD). In humans, VEGF binds to three types of RTKs: VEGFR-1 (Flt-1) (P17948), VEGFR-2 (KDR, Flk-1) (P935968) and VEGFR-3 (Flt-4) (P35916). A sequence of VEGFR-1 is shown in FIG. 2. Unless otherwise apparent from the context reference to a VEGF means any of VEGF-A, -B, -C, -D, and PGF, in any of the natural isoforms or natural variants or induced variants having at least 90, 95, 98 or 99% or 100% sequence identity to a natural form. Preferably, such VEGFs are human VEGFs. Likewise reference to a VEGFR means any of VEGFR-1, R-2 or R-3, including any natural isoform or natural variant, or an induced variant having at least 90, 95, 98 or 99% or 100% sequence identity to a natural sequences.

[00216] The extracellular region runs from about amino acid 27-758, the transmembrane domain from about amino acid 759 to 780 and the intracellular region from about 781-1338. The extracellular region includes seven immunoglobulin-like domains, D1-D7. Domain 1 of VEGFR-1 is from 32 (P) to 128 (I), Domain 2 from 134 (P) to 125 (Q), Domain 3 from 232 (V) to 331 (K), Domain 4 from 333 (F) to 428 (P), Domain 5 is from 431 (Y) to 553 (T), Domain 6 from 558 (G) to 656 (R) and Domain 7 from 662 (Y) to 751 (T). See generally U.S. Patent No. 8,273,353, incorporated herein by reference for all purposes. The exact boundaries of the domains D1-D7 of VEGFR-1 can vary depending on how the analysis is done. Preferably, the boundaries vary by 9 amino acids or less. Typically they vary by 7 amino acids or less, more typically by 5 amino acids or less. Usually, boundary variance is 3 amino acids or less. Most typically the boundaries vary by only an amino acid.

[00217] The protein sequence of VEGFR-2 is shown below in FIG. 3.

[00218] The extracellular region runs from about residues 20-764, the transmembrane domain from about residues 765-785 and the intracellular domain from about residues 786 to 1356. The extracellular region includes seven immunoglobulin-like domains, D1-D7. Domain 1 of VEGFR-2 is from 32 (P) to 118 (V), Domain 2 is from 124 (P) to 220 (G), Domain 3 is from 226 (V) to 327 (K), Domain 4 is from 329 (F) to 421 (P), Domain 5 is from 424 (G) to 548 (T), Domain 6 is from 553 (I) to 662 (L), and Domain 7 is from 668 (T) to 757 (A). See generally U.S. Patent No. 8,273,353, incorporated herein by reference for all purposes. The exact boundaries of the domains D1-D7 of VEGFR-2 can vary depending on how the analysis is done. Preferably, the boundaries vary by 9 amino acids or less. Typically they vary by 7 amino acids or less, more typically by 5 amino acids or less. Usually, boundary variance be by 3 amino acids or less. Most typically the boundaries vary by only an amino acid.

[00219] The protein sequence of VEGFR-3 is shown below in FIG. 4. The extracellular region runs from about residues 25-775, the transmembrane domain from about residues 776-796 and the intracellular domain from about residues 797-1363. The extracellular domain includes seven immunoglobulin-like domains D1-D7. Domain 1 of VEGFR-3 is from 30 (P) to 132 (V), Domain 2 is from 138 (P) to 226 (G), Domain 3 is from 232 (I) to 330 (N), Domain 4 is from 332 (F) to 422 (P), Domain 5 is from 425 (H) to 552 (T), Domain 6 is from 557 (G) to 673 (Q), and Domain 7 is from 679 (R) to 768 (S). See generally U.S. Patent No. 8,273,353, incorporated herein by reference for all purposes. The exact boundaries of the domains D1-D7 of VEGFR-3 can vary depending on how the analysis is done. Preferably, the boundaries vary by 9 amino acids or less. Typically they vary by 7 amino acids or less, more typically by 5 amino acids or less. Usually, boundary variance is 3 amino acids or less. Most typically the boundaries vary by only an amino acid.

[00220] VEGFR-2 is expressed predominately on vascular endothelial cells. VEGFR-1 is also expressed on the vascular endothelium, but in addition is also expressed by a number of other cell types: neutrophils, monocytes, macrophages, mural cells and endothelial progenitor cells. VEGFR-1 has a higher affinity for VEGF-A than does VEGFR-2. However, when VEGFR-1 is bound to VEGF-A in endothelial cells, VEGFR-1 exhibits only very weak tyrosine phosphorylation. Hence, it is believed that the effects of VEGF-A (including its various isoforms) on the vascular endothelium are mediated by the binding of VEGF-A to VEGFR-2.

[00221] PGF and VEGF-B bind only to VEGFR-1. PGF and VEGF-B have been implicated in pathogenic vascular remodeling. Carmeliet P, Moons L, Lutten A, et al. 2001. Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nat Med.* 7(5);575-583. VEGF-C and -D bind with high affinity to VEGFR-3, which is primarily found on lymphatic endothelial cells in the adult. VEGF-C and -D are thought to play a role in regard to Lymphangiogenesis.

[00222] A “VEGF antagonist” or a molecule that “antagonizes VEGF” is an agent that reduces, or inhibits, either partially or fully, an activity of a VEGF including its ability to specifically bind to its receptor a VEGFR and consequent cellular responses, such as angiogenesis and cellular proliferation. VEGF antagonists include antibodies specifically binding to a VEGF or a VEGFR or a VEGFR extracellular trap segment.

[00223] The term extracellular trap segment refers to a full length extracellular region or any portion thereof, or combination of portions from different VEGFR receptors that can antagonize signaling between at least one VEGF and VEGFR. Preferably, the extracellular trap segment includes at least one domain from one of VEGFR-1, -2 or -3 defined above, and more preferably at least two contiguous domains, such as D2 and D3. Optionally, an extracellular domain includes at least one domain as defined above from at least two different VEGFRs. A preferred extracellular domain comprises or consists essentially of D2 of VEGFR-1 and D3 of VEGFR-2.

[00224] VEGF antagonist therapies have been approved for the treatment of certain cancers and wet AMD. Bevacizumab (AVASTIN[®], Genentech/Roche) is a humanized mouse monoclonal antibody that binds to and neutralizes human VEGF, in particular to all isoforms of VEGF-A and to bioactive proteolytic fragments of VEGF-A. See, e.g., Ferrara N, Hillan KJ, Gerber HP, Novotny W. 2004. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nat Rev Drug Discov.* 3(5):391-400. Bevacizumab has been approved for the treatment of certain cancers. The protein sequence of the heavy and light chains of bevacizumab (DrugBank DB00112) is shown below in FIG. 5 with CDRs underlined (see also SEQ ID NOs. 2 and 5).

[00225] Bevacizumab variable light chain CDRs are CDR_{L1}: SASQDISNYLN, CDR_{L2}: FTSSLHS and CDR_{L3}: QQYSTVPWT. Bevacizumab variable heavy chain CDRs are CDR_{H1}:

GYTFTNYGMN, CDR_{H2}: WINTYTGEPTYAADFKR, and CDR_{H3}: YPHYYGSSHWYFDV. CDRs are defined by Kabat except CDR_{H1} is the composite Kabat/Chothia definition.

[00226] Another anti-VEGF molecule, derived from the same mouse monoclonal antibody as bevacizumab has been approved as a treatment for wet AMD: ranibizumab (LUCENTIS[®], Genentech/Roche). Ranibizumab is an antibody fragment or Fab. Ranibizumab was produced by affinity maturation of the variable heavy and light chains of bevacizumab. The sequence of the heavy and light chains of ranibizumab is shown below (as published by Novartis) in FIG. 6 (see also SEQ ID NOs. 12 and 13):

[00227] Ranibizumab variable light chain CDRs are CDR_{L1}: SASQDISNYLN, CDR_{L2}: FTSSLHS and CDR_{L3}: QQYSTVPWT. Ranibizumab variable heavy chain CDRs are CDR_{H1}: GYDFTHYGMN, CDR_{H2}: WINTYTGEPTYAADFKR, and CDR_{H3}: YPYYGTSHWYFDV.

[00228] Antibodies competing with bevacizumab for binding to VEGF-A or binding to the same epitope on VEGF-A as bevacizumab can also be used.

[00229] Another anti-VEGF therapy is a VEGF Trap. For example, aflibercept (Eylea[®], Regeneron), consists of the second Ig like domain of VEGFR-1 and the third Ig like domain of VEGFR-2 expressed as an in line fusion with the constant region (Fc) of human IgG1. Papadopoulos N, et al. 2012. Binding and neutralization of vascular endothelial growth factor (VEGF) and related ligands by VEGF Trap, ranibizumab and bevacizumab. Angiogenesis 15:171-185. In theory, aflibercept binds not only VEGF-A, but also VEGF-B and PGF thereby antagonizing their interaction with VEGFR-1.

[00230] In accordance with the present invention, a dual VEGF/PDGF antagonist is provided comprising a VEGF antagonist linked to a PDGF antagonist. The linkage preferably includes a fusion of protein chains to form a hybrid chain formed from components of both antagonists. Alternatively, the components can be joined by chemical cross linking. As an example, of linkage by fusion, if the dual antagonist is formed from an antibody and an extracellular trap segment, then a heavy or light chain of the antibody can be fused to the extracellular trap segment. Preferably, the extracellular trap segment is fused directly or indirectly via a linker to the N-terminus of the antibody heavy or light chain. Whichever chain is not fused to the extracellular trap segment can associate with the chain that is in similar fashion to heavy light chain association in a natural antibody. For example, an exemplary format has an extracellular trap segment fused to the N-terminus of an antibody heavy chain via a linker and the antibody

light chain complexed with the antibody heavy chain. The antibody in such a dual antagonist can be an intact antibody or any of the binding fragments described above, such as a Fab fragment. Preferably, in such dual antagonists, the VEGF antagonist is an antibody to VEGF-A, such as bevacizumab or ranibizumab, and the PDGF antagonist is an extracellular trap segment from PDGR-1.

[00231] In an alternative format, the VEGF antagonist and PDGF antagonist are both extracellular trap segments. The two segments can be fused in either orientation with respect to one another, directly or via a linker. That is the VEGFR extracellular trap region can be joined to the N-terminus or the C-terminus of the PDGFR extracellular trap region. The C-terminus of such a fusion protein can be linked to an Fc region of an antibody forming an Fc fusion proteins.

[00232] In preferred embodiments, the PDGFR is PDGFR- β and the extracellular trap segment comprises one or more of domains D1-D5 of PDGFR- β . More preferably, the extracellular trap segment comprises domains D1-D3 of PDGFR- β . Still more preferably, the extracellular trap segment comprises or consists of amino acids 33 to 314 of SEQ ID NO. 11. In preferred embodiments, the VEGF antagonist is an anti-VEGF antibody, preferably an anti-VEGF-A antibody.

[00233] In dual antagonists having antibody and extracellular trap components fused to one another, the respective components, typically the antibody heavy chain and the extracellular trap segment are separated by a linker sequence. The linker is preferably GGGGSGGGGS, GG, or GGGGSGGGGSGGGGSGGGGSG or an oligomers of any of these. More preferably, the linker is GGGGSGGGGS.

[00234] In accordance with an aspect of the present invention, the anti-VEGF-A antibody heavy chain has at least the following CDR sequences: CDR_{H1}: GYDFTHYGMN, CDR_{H2}: WINTYTGEPTYAADFQR, and CDR_{H3}: YPYYYGTSHWYFDV. Preferably, the anti-VEGF-A light chain has at least the following CDRs: CDR_{L1}: SASQDISNYLN, CDR_{L2}: FTSSLHS and CDR_{L3}: QQYSTVPWT. In the case of the anti-VEGF-A antibody heavy chain, it is preferred that its isotype is IgG1 and has a CH₁, hinge, CH₂ and CH₃ domains. It is also preferred that the light chain isotype is kappa. The constant region of the preferred IgG1 sequence is set forth in SEQ ID NO. 17. The sequence of the light chain constant region is preferably set forth in SEQ ID NO. 18.

[00235] The IgG1 domain of the anti-VEGF-A antibody preferably has one or more mutations to reduce or lower effector function. Preferred amino acids to use for effector function reducing mutations include (EU numbering) E233P, L234V, L235, G236, G237, delG236, D270A, K322A, A327G, P329A, A330, A330S, P331S, and P331A, in which the second mentioned amino acid is the mutation. Preferably, the mutations include one or more of the following: E233P, L234V, L234A, L235A, G237A, A327G, A330S and P331S (EU numbering). More preferably, the anti-VEGF-A heavy chain has the following mutations: L234A, L235A and G237A. The number of such mutations relative to a natural human IgG1 sequence is no more than 10, and preferably no more than 5, 4, 3, 2 or 1.

[00236] Alternatively, the IgG domain can be IgG2, IgG3 or IgG4, preferably, human IgG2, IgG3 or IgG4, or a composite in which a constant regions is formed from more than one of these isotypes (e.g., CH1 region from IgG2 or IgG4, hinge, CH2 and CH3 regions from IgG1). Such domains can contain mutations to reduce effector function at one or more of the EU position mentioned for IgG1. Human IgG2 and IgG4 have reduced effector functions relative to human IgG1 and IgG3.

[00237] The anti-VEGF-A heavy chain can also contain a cysteine residue added as a mutation by recombinant DNA technology which can be used to conjugate a half-life extending moiety. Preferably, the mutation is (EU numbering) Q347C and/or L443C. More preferably, the mutation is L443C. Preferably, the stoichiometry of dual antagonist to polymer is 1:1; in other words, a conjugate consists essentially of molecules each comprising one molecule of dual antagonist conjugated to one molecule of polymer.

[00238] A preferred dual antagonist including an antibody to VEGF-A and a PDGFR extracellular trap segment comprises a fusion protein of the antibody heavy chain and the PDGFR extracellular trap segment having the amino acid sequence of SEQ ID NO. 9 and the antibody light chain having the amino acid sequence of SEQ ID NO. 10, or variants thereof including sequences differing each of from SEQ ID NO: 9 and 10 by no more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acids.

[00239] In another aspect of the present invention, a dual VEGF/PDGF antagonist is presented having a PDGF antagonist constituting one or more segments of a PDGFR as described above and a VEGF antagonist constituting an anti-VEGF Fab fragment. For this aspect of the present invention, the PDGFR extracellular trap comprises one or more of domains

D1-D5 of PDGFR- β . More preferably, the PDGFR trap constitutes domains D1-D3 of PDGFR- β . More preferably, the PDGFR trap is amino acids 33 to 314 of SEQ ID NO. 11.

[00240] The PDGFR trap is preferably located C-terminal of the Fab heavy or light chain.

The PDGFR trap is also preferentially located N-terminal of the Fab heavy or light chain.

Preferably, the dual antagonist includes an anti-VEGF-A Fab fragment heavy chain fused via a linker to a PDGFR extracellular trap segment and an anti-VEGF-A light chain.

[00241] In another aspect of the invention, a dual VEGF/PDGF antagonist is presented wherein the extracellular trap segment binds to one or more of PDGF-AA, PDGF-BB, PDGF-AB, PDGF-CC and PDGF-DD. Preferably, the extracellular trap binds PDGF-AB, PDGF-BB and PDGF-DD. Still more preferably, the extracellular trap inhibits PDGF-AB, PDGF-BB and PDGF-DD from binding to any one of PDGFR- $\alpha\alpha$, PDGFR- $\alpha\beta$, and PDGFR- $\beta\beta$ receptors.

[00242] A linker is preferably located between the PDGFR trap and the anti-VEGF Fab fragment heavy chain. Preferably, the linker is selected from group consisting of GGGGSGGGGS, GG, and GGGGSGGGGSGGGGSGGGGSG, and oligomers of any of these. More preferably, the linker is GGGGSGGGGS.

[00243] The anti-VEGF Fab fragment heavy chain preferably has at least the following CDRs: CDR_{H1}: GYDFTHYGMN, CDR_{H2}: WINTYTGEPTYAADFQR, and CDR_{H3}: YPYYYGTSHWYFDV. The anti-VEGF-A light chain preferably has at least the following CDRs: CDR_{L1}: SASQDISNYLN, CDR_{L2}: FTSSLHS and CDR_{L3}: QQYSTVPWT.

[00244] A preferred anti-VEGF Fab fragment heavy chain isotype is IgG1 and comprises a CH₁ domain and the light chain isotype is kappa.

[00245] The dual VEGF/PDGF antagonist can have a half-life extending moiety attached. Preferably the half-life extending moiety is a zwitterionic polymer but PEG or other half-life extenders discussed below can alternatively be used. More preferably, the zwitterionic polymer is formed of monomers having a phosphorylcholine group. Preferably the monomer is 2-(acryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate. More preferably, the monomer is 2-(methacryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate (HEMA-PC).

[00246] A polymer conjugated to a dual antagonist preferably has at least 2 and more preferably 3 or more arms. Some polymers have 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 arms. Still more preferably the polymer has 3, 6 or 9 arms. Most preferably, the polymer has 9 arms. Preferably, the polymer peak molecular weight is between 300,000 and 1,750,000 Da. More

preferably, the polymer has a peak molecular weight between 500,000 and 1,000,000 Da. Still more preferably, the polymer has a peak molecular weight between 600,000 to 800,000 Da.

[00247] The polymer can be covalently bonded to the dual antagonist via conjugation.

Preferably, the polymer is conjugated to the dual VEGF/PDGF antagonist via a group such as an amino group, a hydroxyl group, a sulfhydryl group or a carboxyl group. The sulfhydryl group can be from a naturally occurring cysteine residue. The sulfhydryl group can also be from a cysteine residue added by recombinant DNA technology.

[00248] In a preferred aspect of the present invention, the polymer is conjugated to the cysteine residue at position 741 of SEQ ID NO. 9, or aligned position of any variants of SEQ ID NO. 9 disclosed herein.

[00249] In another aspect of the present invention, a dual VEGF/PDGF antagonist having a VEGFR trap containing one or more extracellular segments of a VEGFR, such as VEGFR-1, VEGFR-2 or VEGFR-3, fused to an anti-PDGF antibody or Fab fragment heavy or light chain and an anti-PDGF antibody or Fab fragment heavy or light chain not included in fusion.

[00250] In accordance with an aspect of the present invention, the extracellular segment of VEGFR is preferably one or more of domains D1-D7. More preferably, the extracellular segment comprises D2 from VEGFR-1 and D3 from VEGFR-2. Still more preferably, the D2 is N-terminal to the D3 and further comprises a linker between the domains.

[00251] In preferred embodiments of this aspect of the present invention, the PDGF antagonist is an antibody. More preferably, the antibody is selected from the group consisting of humanized 2A1E2, HuM4Ts.22, humanized 1B3, humanized 2C5, anti-PDGF-BB, anti-PDGF-DD, anti-PDGF-BB and anti-PDGF-AB. The PDGF antagonist is also preferably a Fab fragment.

[00252] In accordance with this aspect of the present invention, the antibody heavy chain is preferably IgG1, more preferably human IgG1 and the light chain is preferably kappa, human kappa. The heavy chain can have a cysteine added via recombinant DNA technology. Preferably, the cysteine is selected from the group consisting of Q347C and L443C. Preferably, there is a half-life extending moiety conjugated to the cysteine.

[00253] Preferably, the half-life extending moiety is a zwitterionic polymer having one or more monomer units and wherein at least one monomer unit has a zwitterionic group. Preferably, the zwitterionic group is phosphorylcholine. The monomer is preferably 2-

(acryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate. More preferably, the monomer is 2-(methacryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate (HEMA-PC).

[00254] In accordance with this aspect of the present invention, the polymer preferably has at least 2 and more preferably 3 or more arms. Some polymers have 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 arms. Still more preferably the polymer has 3, 6 or 9 arms. Most preferably, the polymer has 9 arms. In accordance with an aspect of the present invention, the polymer peak molecular weight of between 300,000 and 1,750,000 Da. More preferably, the polymer has a peak molecular weight between 500,000 and 1,000,000 Da. Still more preferably, the polymer has a peak molecular weight between 600,000 to 800,000 Da.

[00255] In accordance with an aspect of the present invention, the polymer is covalently bound to the polymer via conjugation. Preferably, the polymer is conjugated to the dual VEGF/PDGF antagonist via a group selected from the group consisting of an amino group, a hydroxyl group, a sulfhydryl group and a carboxyl group. Preferably, the sulfhydryl group is from a naturally occurring cysteine residue. In other preferred embodiments, the sulfhydryl group is from a cysteine residue added by recombinant DNA technology.

[00256] In preferred aspects of the present invention, the PDGF trap-VEGF trap is conjugated to a half-life extending moiety as discussed with other dual antagonists.

[00257] Preferably, the half-life extending moiety is a zwitterionic polymer having one or more monomer units and wherein at least one monomer unit has a zwitterionic group.

Preferably, the zwitterionic group is phosphorylcholine. The monomer is preferably 2-(acryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate. More preferably, the monomer is 2-(methacryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate (HEMA-PC).

[00258] In accordance with this aspect of the present invention, the polymer preferably has at least 2 and more preferably 3 or more arms. Some polymers have 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 arms. Still more preferably the polymer has 3, 6 or 9 arms. Most preferably, the polymer has 9 arms. In accordance with an aspect of the present invention, the polymer peak molecular weight of between 300,000 and 1,750,000 Da. More preferably, the polymer has a peak molecular weight between 500,000 and 1,000,000 Da. Still more preferably, the polymer has a peak molecular weight between 600,000 to 800,000 Da.

[00259] In accordance with an aspect of the present invention, the polymer is covalently bound to the polymer via conjugation. Preferably, the polymer is conjugated to the dual

VEGF/PDGF antagonist via a group such as an amino group, a hydroxyl group, a sulfhydryl group or a carboxyl group. In some conjugates, the sulfhydryl group is from a naturally occurring cysteine residue. In some conjugates, the sulfhydryl group is from a cysteine residue added by recombinant DNA technology.

[00260] Dual PDGF/VEGF antagonists can be produced by recombinant expression including (i) the production of recombinant DNA by genetic engineering, (ii) introducing recombinant DNA into prokaryotic or eukaryotic cells by, for example and without limitation, transfection, electroporation or microinjection, (iii) cultivating the transformed cells, (iv) expressing dual antagonists, *e.g.* constitutively or on induction, and (v) isolating the dual antagonist, *e.g.* from the culture medium or by harvesting the transformed cells, in order to (vi) obtain purified dual antagonist.

[00261] Dual antagonists can be produced by expression in a suitable prokaryotic or eukaryotic host system characterized by producing a pharmacologically acceptable dual antagonist molecule. Examples of eukaryotic cells are mammalian cells, such as CHO, COS, HEK 293, BHK, SK-Hip, and HepG2. Other suitable expression systems are prokaryotic (*e.g.*, *E. coli* with pET/BL21 expression system), yeast (*Saccharomyces cerevisiae* and/or *Pichia pastoris* systems), and insect cells.

[00262] A wide variety of vectors can be used for the preparation of the dual antagonist and are selected from eukaryotic and prokaryotic expression vectors. Examples of vectors for prokaryotic expression include plasmids such as, and without limitation, preset, pet, and pad, wherein the promoters used in prokaryotic expression vectors include one or more of, and without limitation, lac, trc, trp, recA, or araBAD. Examples of vectors for eukaryotic expression include: (i) for expression in yeast, vectors such as, and without limitation, pAO, pPIC, pYES, or pMET, using promoters such as, and without limitation, AOX1, GAP, GAL1, or AUG1; (ii) for expression in insect cells, vectors such as and without limitation, pMT, pAc5, pIB, pMIB, or pBAC, using promoters such as and without limitation PH, p10, MT, Ac5, OpIE2, gp64, or polh, and (iii) for expression in mammalian cells, vectors such as, and without limitation, pSVL, pCMV, pRc/RSV, pcDNA3, or pBPV, and vectors derived from, in one aspect, viral systems such as and without limitation vaccinia virus, adeno-associated viruses, herpes viruses, or retroviruses, using promoters such as and without limitation CMV, SV40, EF-1, UbC, RSV, ADV, BPV, and beta-actin.

[00263] The half-life of dual antagonists can be extended by attachment of a “half-life extending moieties” or “half-life extending groups,” which terms are herein used interchangeably to refer to one or more chemical groups attached to one or more amino acid side chain functionalities such as -SH, -OH, -COOH, -CONH₂, -NH₂, or one or more N- and/or O-glycan structures and that can increase *in vivo* circulatory half-life of proteins/peptides when conjugated to these proteins/peptides. Examples of half-life extending moieties include polymers described herein, particularly those of zwitterionic monomers, such as HEMA-phosphorylcholine, PEG, biocompatible fatty acids and derivatives thereof, Hydroxy Alkyl Starch (HAS) e.g. Hydroxy Ethyl Starch (HES), Poly Ethylene Glycol (PEG), Poly (Glyx-Sery) (HAP), Hyaluronic acid (HA), Heparosan polymers (HEP), Fleximers, Dextran, Poly-sialic acids (PSA), Fc domains, Transferrin, 25 Albumin, Elastin like (ELP) peptides, XTEN polymers, PAS polymers, PA polymers, Albumin binding peptides, CTP peptides, FcRn binding peptides and any combination thereof.

[00264] In one embodiment a half-life extending moiety can be conjugated to a dual antagonist via free amino groups of the protein using N-hydroxysuccinimide (NHS) esters. Reagents targeting conjugation to amine groups can randomly react to ϵ -amine group of lysines, α -amine group of N-terminal amino acids, and δ -amine group of histidines.

[00265] However, dual antagonists of the present have many amine groups available for polymer conjugation. Conjugation of polymers to free amino groups, thus, might negatively impact the ability of the dual antagonist proteins to bind to VEGF and/or PDGF.

[00266] In another embodiment, a half-life extending moiety is coupled to one or more free SH groups using any appropriate thiol-reactive chemistry including, without limitation, maleimide chemistry, or the coupling of polymer hydrazides or polymer amines to carbohydrate moieties of the dual antagonist after prior oxidation. The use of maleimide coupling is a particularly preferred embodiment of the present invention. Coupling preferably occurs at cysteines naturally present or introduced via genetic engineering.

[00267] Polymers are preferably covalently attached to cysteine residues introduced into dual antagonist by site directed mutagenesis. It is particularly preferred to employ cysteine residues in the Fc portion of the dual antagonist. For preferred sites to introduce cysteine residues into an Fc region see WO 2013/093809, US 7,521,541, WO 2008/020827, US 8,008,453, US 8,455,622 and US2012/0213705, incorporated herein by reference for all purposes. Particularly preferred

cysteine mutations are Q347C and L443C referring to the human IgG heavy chain by EU numbering.

[00268] The invention provides conjugates of dual antagonist and high MW polymers serving as half-life extenders. A preferred conjugate comprises a dual antagonist is coupled to a zwitterionic polymer wherein the polymer is formed from one or more monomer units and wherein at least one monomer unit has a zwitterionic group. Preferably, the zwitterionic group is phosphorylcholine.

[00269] Preferably, one of the monomer units is 2-(acryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate or 2-(methacryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate (HEMA-PC). In other preferred embodiments, polymer is synthesized from a single monomer which is preferably 2-(acryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate or 2-(methacryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate.

[00270] Some dual antagonist conjugates have 2 or more preferably 3 or more polymer arms wherein the monomer is HEMA-PC. Preferably, the conjugates have 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 polymer arms wherein the monomer is HEMA-PC. More preferably, the conjugates have 3, 6 or 9 arms. Most preferably, the conjugate has 9 arms.

[00271] Polymer-dual antagonist conjugates preferably have a polymer portion with a molecular weight of between 100,000 and 1,500,000 Da. More preferably the conjugate has a polymer portion with a molecular weight between 500,000 and 1,000,000 Da. Still more preferably the conjugate has a polymer portion with a molecular weight between 600,000 to 800,000 Da. Most preferably the conjugate has a polymer portion with a molecular weight between 600,000 and 850,000 Da and has 9 arms. When a molecular weight is given for a dual VEGF/PDGF antagonist conjugated to a polymer, the molecular weight will be the addition of the molecular weight of the protein, including any carbohydrate moieties associated therewith, and the molecular weight of the polymer.

[00272] In accordance with an aspect of the present invention, a dual VEGF/PDGF antagonist having a HEMA-PC polymer which has a molecular weight measured by Mw of between about 100 kDa and 1500 kDa. More preferably, the molecular weight of the polymer as measured by Mw is between about 500 kDa and 1000 kDa. Still more preferably, the molecular weight of the

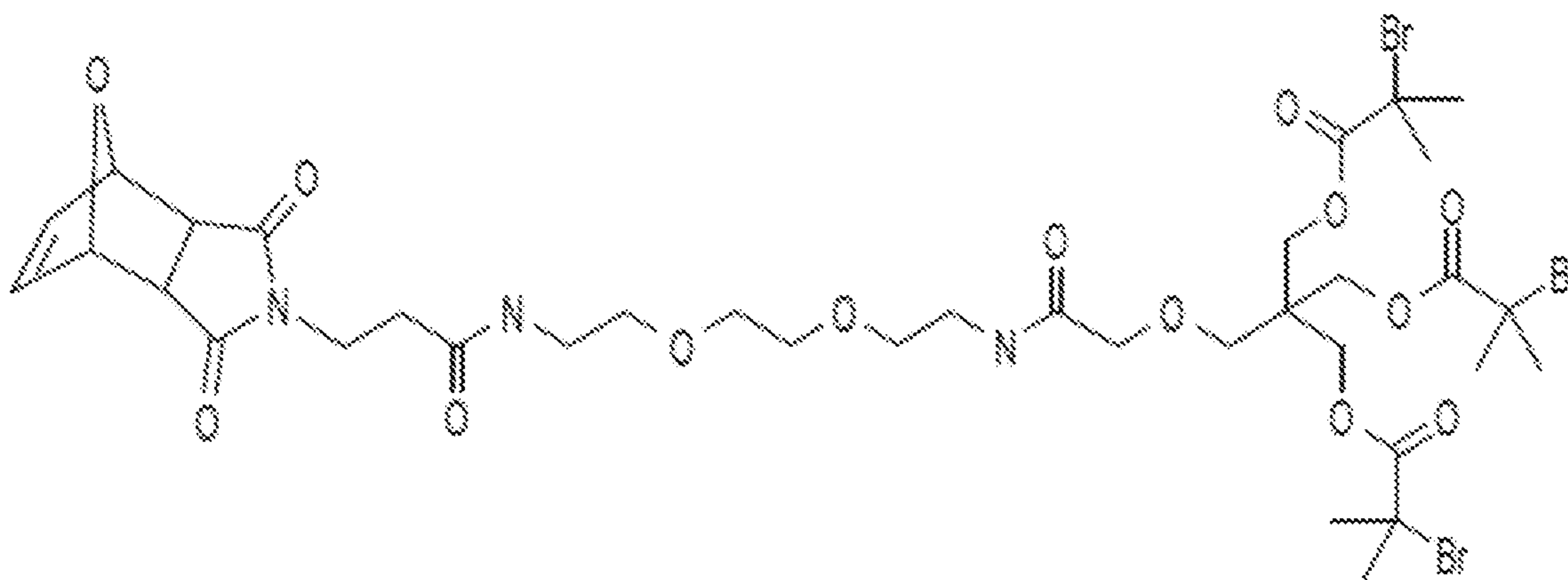
polymer as measured by Mw is between about 600 kDa to about 900 kDa. Most preferably, the polymer molecular weight as measured by Mw is 750 kDa plus or minus 15%.

[00273] In this aspect of the present invention, the polymer is preferably made from an initiator suitable for ATRP having one or more polymer initiation sites. Preferably, the polymer initiation site has a 2-bromoisobutyrate site. Preferably, the initiator has 3 or more polymer initiation sites. More preferably, the initiator has 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 polymer initiation sites. More preferably, the initiator has 3, 6 or 9 polymer initiation sites. Still more preferably, the initiator has 9 polymer initiation sites. Most preferably, the initiator is OG1786.

[00274] The invention provides methods for synthesizing a zwitterionic polymer-dual antagonist conjugate, the conjugate having one or more functional agents and one or more polymer arms wherein each of the polymer arms has one or more monomer units wherein at least one of the units has a zwitterion. The method can have the steps of

- a. providing an initiator having one or more sites for monomer polymerization and a first linker having an amine group wherein the initiator is a trifluoro acetic acid salt;
- b. providing one or more monomers suitable for polymerization wherein at least one of the monomers is zwitterionic;
- c. reacting the monomers with the initiator to form one or more polymer arms each corresponding to the sites for monomer polymerization to provide an initiator-polymer conjugate having the first linker with the amine group;
- d. providing a second linker having at least second and third reactive groups;
- e. coupling one of the second and third reactive groups of the second linker to the amine group of the first linker of the initiator-polymer conjugate to provide a linker-initiator-polymer conjugate having one or more reactive groups that were not used in the coupling step; and
- f. coupling one or more functional agents to one or more of the unreacted reactive groups of the linker-initiator-polymer moiety to provide the polymer-functional agent conjugate.

[00275] Prior to the instant invention, the initiator molecule or entity had to contain a deprotectable functional group that would allow coupling of the functional agent. An example of such an initiator having a protected maleimide is shown below:



[00276] After polymer synthesis, the protected maleimide is deprotected with heat to allow for generation of maleimide which could be used to couple functional agent. If one wanted to vary the nature of the chemical entity in between the maleimide and the polymer initiation site, one would have to synthesize an entire new initiator.

[00277] Each time the initiator is changed or altered in any way, a new scaled up synthesis procedure would have to be developed. Each change in the nature of the initiator molecule can have a wide range of effects on polymer synthesis. However, in accordance with the present invention, a method is presented where the conjugation group (e.g. maleimide) is added after polymer synthesis. This is sometimes referred to as a “snap-on strategy” or “universal polymer strategy. A single initiator moiety can be used for large scale polymer and bioconjugate discovery and development. Thus, conditions can be developed for scaled up optimal polymer synthesis. Such polymer can then be adapted to various types of functional agents by “snapping-on” various types of linkers and functional conjugation chemistries.

[00278] For example, if it is desired to conjugate a larger functional agent to a polymer of the instant invention such as an antibody or even a Fab fragment, a longer linker sequence can be snapped on to the polymer. In contrast, smaller functional agents may call for relatively shorter linker sequences.

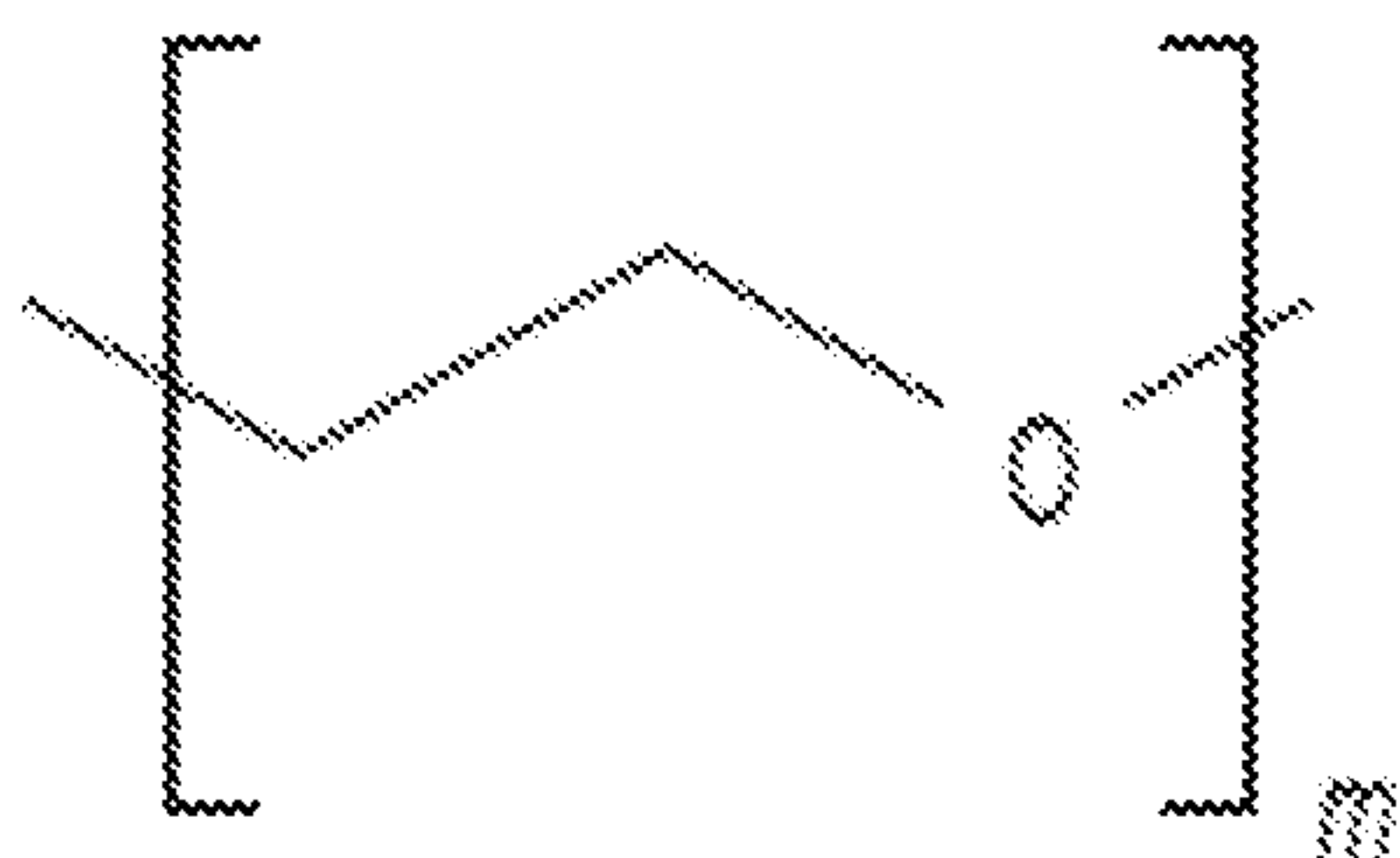
[00279] In preferred embodiments of the methods, the initiator has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 sites for polymer initiation. Preferably, the initiator has 3, 6 or 9 sites for polymer initiation.

[00280] In accordance with an aspect of the present invention, a second linker has second, third, fourth, fifth, and sixth reactive groups. More preferably, a second linker has just second and third reactive groups.

[00281] In accordance with an aspect of the present invention, each polymer arm has from about 20 to about 2000 monomer units. Preferably, each arm has from about 100 to 500 monomer units or from about 500 to 1000 monomer units or from about 1000 to 1500 monomer units or from about 1500 to 2000 monomer units.

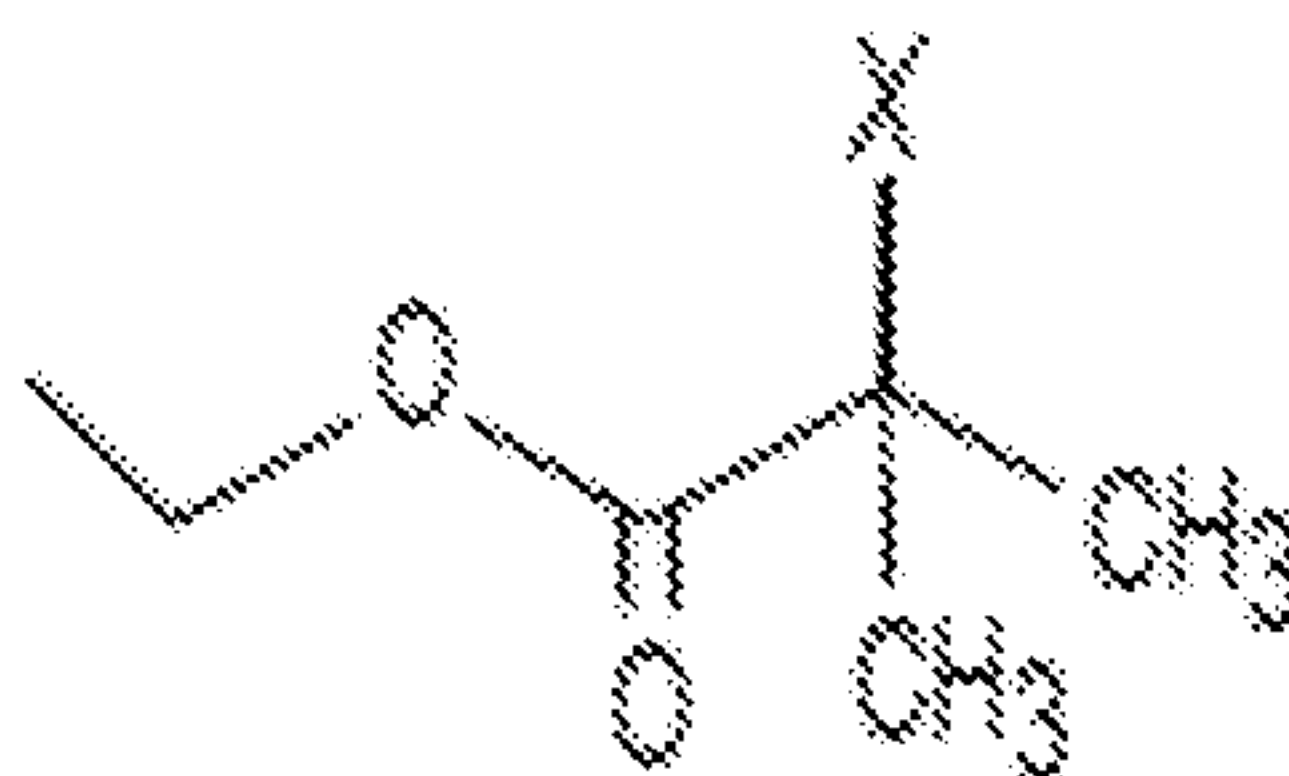
[00282] In accordance with an aspect of the present invention, the peak molecular weight of the polymer-functional agent conjugate is about 100,000 to 1,500,000 Da. Preferably, the peak molecular weight of the polymer-functional agent conjugate is about 200,000 to about 300,000 Da, about 400,000 to about 600,000 Da or about 650,000 to about 850,000 Da.

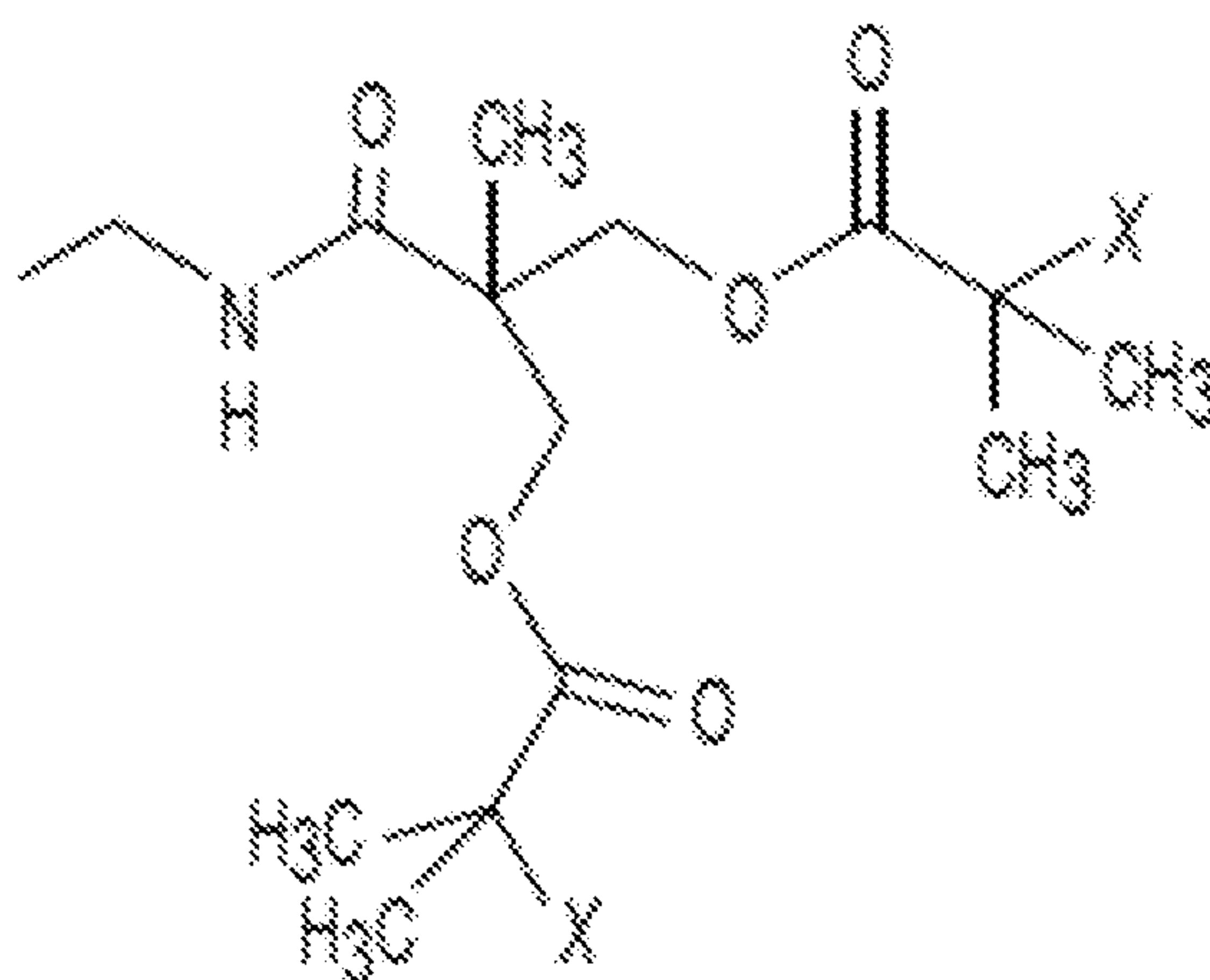
[00283] In accordance with another aspect of the present invention, the first linker is preferably alkyl, substituted alkyl, alkylene, alkoxy, carboxyalkyl, haloalkyl, cycloalkyl, cyclic alkyl ether, alkenyl, alkenylene, alkynyl, alkynylene, cycloalkylene, heterocycloalkyl, heterocycloalkylene, aryl, arylene, arylene-oxy, heteroaryl, amino, amido or any combination thereof. More preferably, the first linker has the formula:



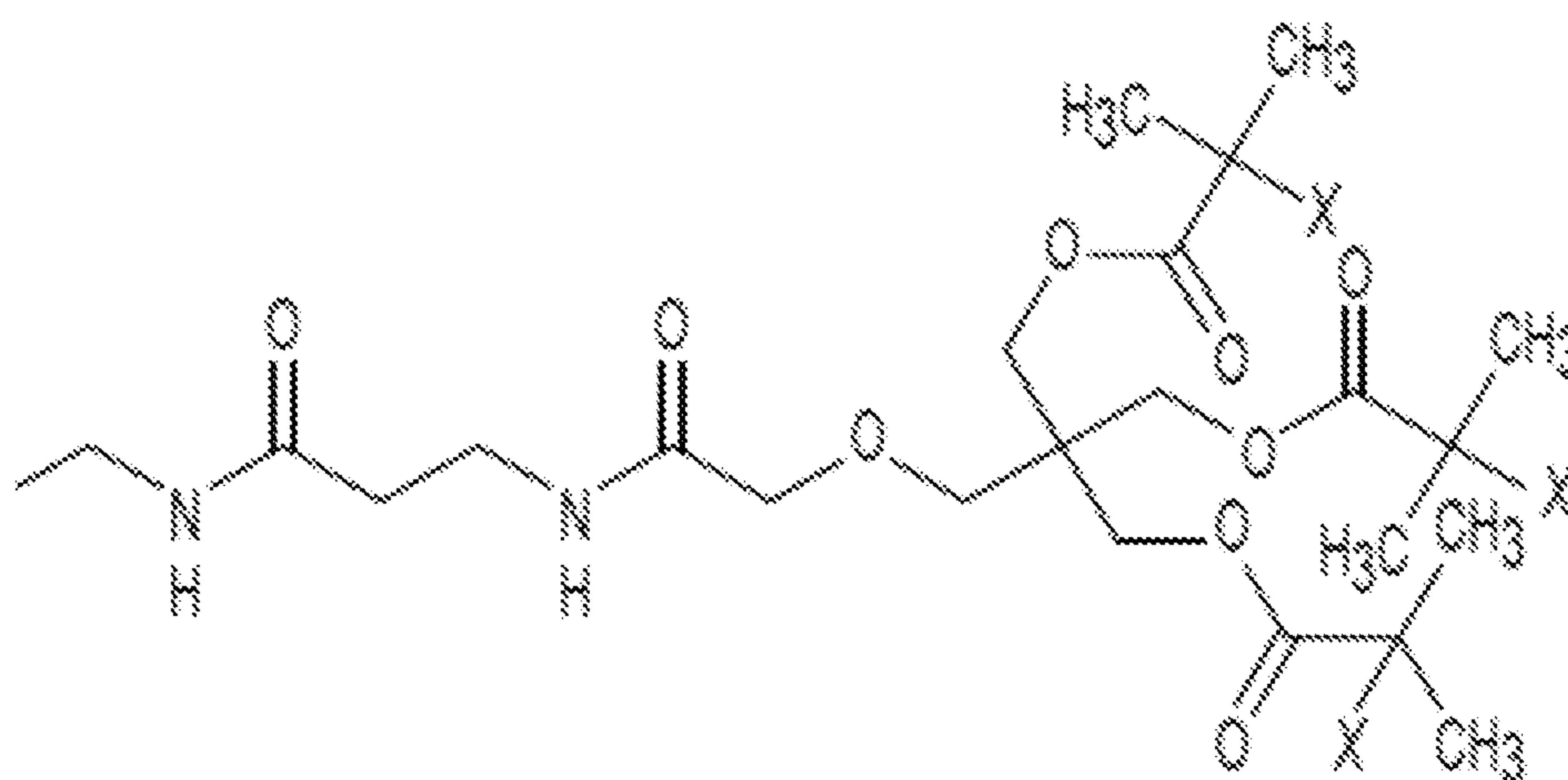
wherein m is 1 to 10. More preferably, the first linker has the above formula and m is 4.

[00284] In still other aspects of the present invention, the initiator preferably includes a structure selected from group consisting of



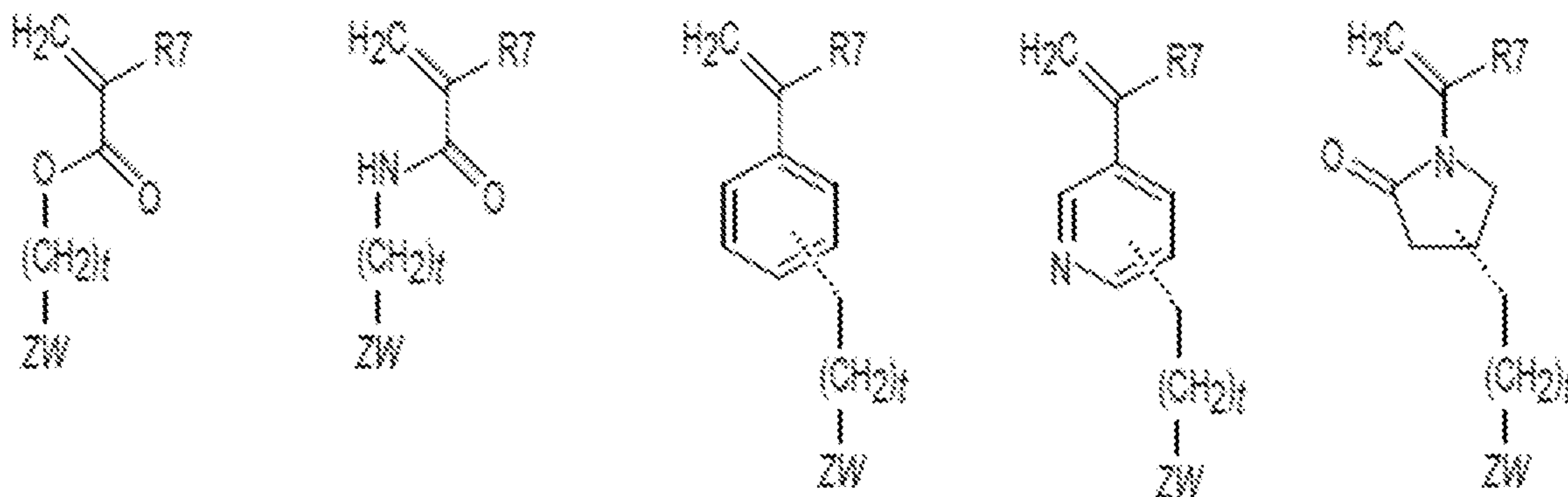


and



wherein X is selected from the group consisting of NCS, F, Cl, Br and I. More preferably, X is Br.

[00285] In preferred embodiments of the present invention, the monomer is selected from the group consisting of

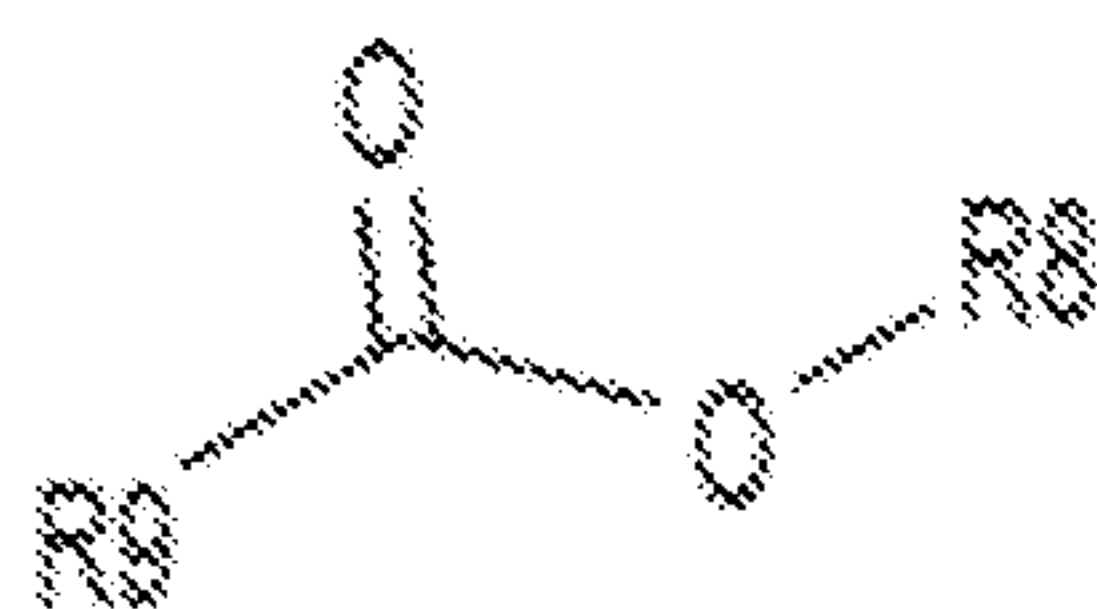


wherein R₇ is H or C₁₋₆ alkyl and t is 1 to 6.

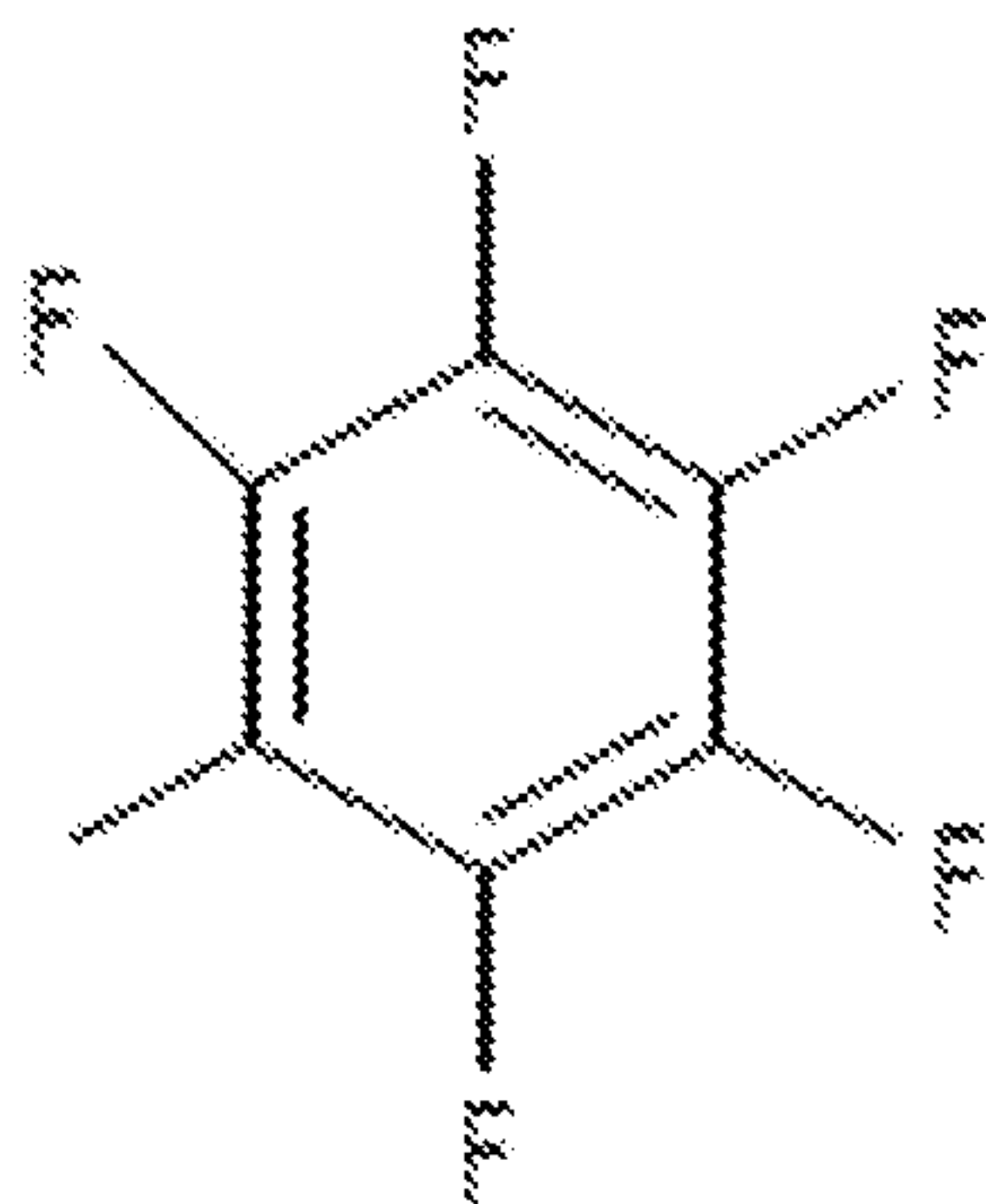
[00286] More preferably, the monomer is selected from the group consisting of 2-(methacryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate (HEMA-PC) and 2-(acryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate.

[00287] Most preferably, the monomer is 2-(methacryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate.

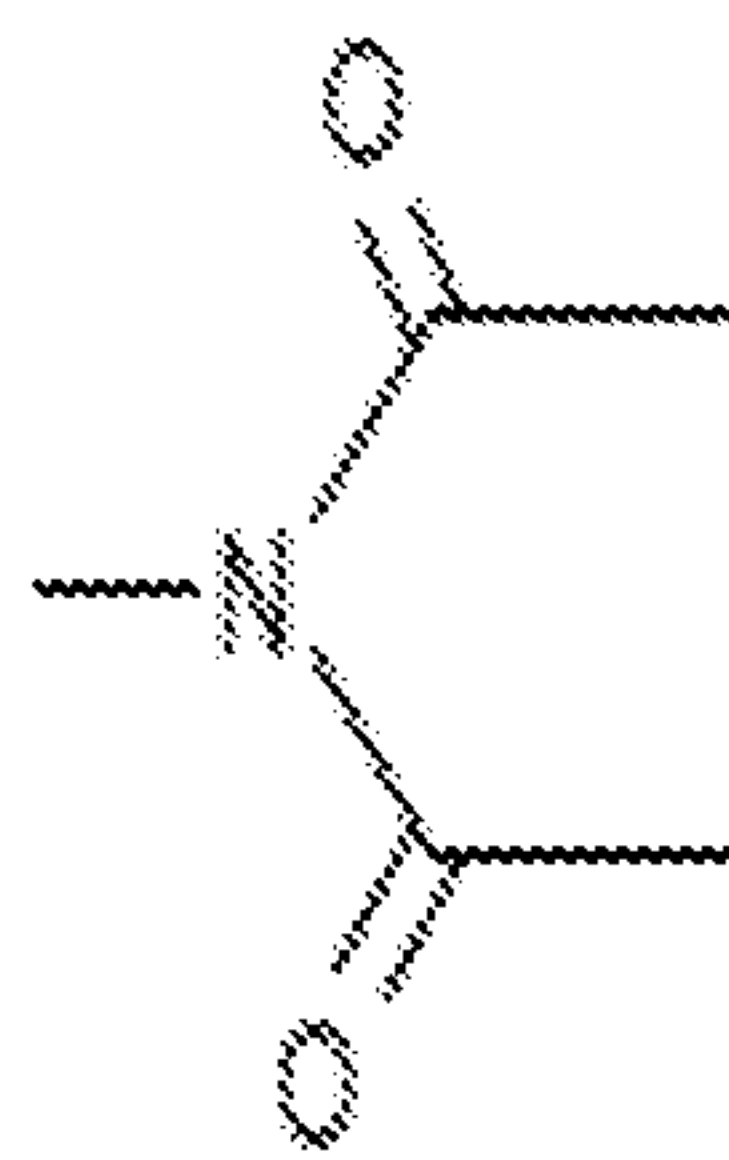
[00288] The second linker moiety preferably comprises an activated ester having the structure



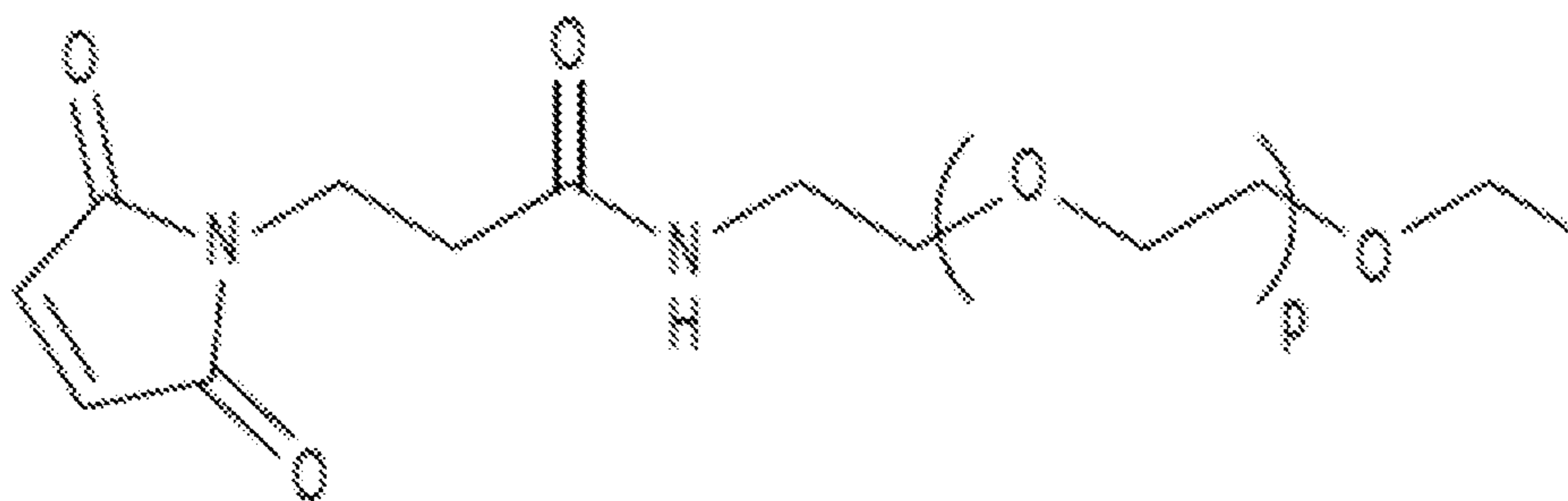
wherein R₈ is selected from the group consisting of



and

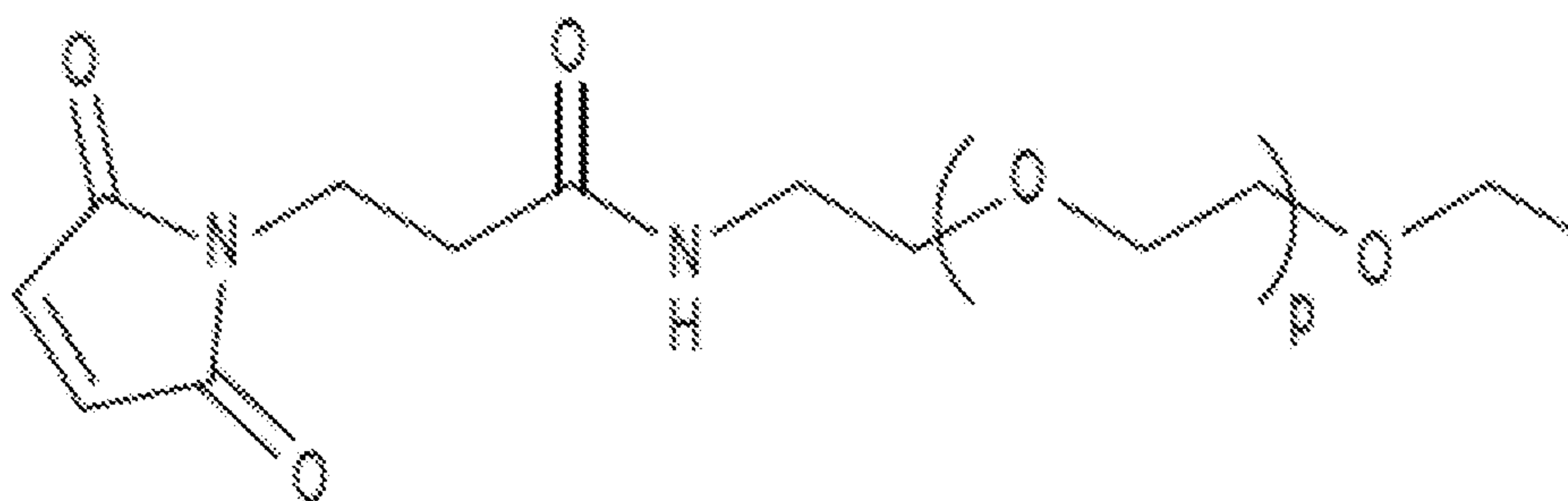


and R₉ is



wherein p is 1 to 12.

[00289] In more preferred embodiments of the present invention, the polymer has 9 arms, m of R2 is 2-4, R9 is



and p is 4 to 15. Still more preferably, m is 4 and p is 12.

[00290] When a polymer is to be conjugated via a cysteine (or other specified residue), the polymer can be linked directly or indirectly to the residue (e.g., with an intervening initiator, and or spacer or the like).

[00291] Dual antagonists can be incorporated into a pharmaceutical composition with a pharmaceutically acceptable excipient. Pharmaceutical compositions adapted for oral administration may be presented as discrete units such as capsules, as solutions, syrups or suspensions (in aqueous or non-aqueous liquids; or as edible foams or whips; or as emulsions). Suitable excipients for tablets or hard gelatine capsules include lactose, maize starch or derivatives thereof, stearic acid or salts thereof. Suitable excipients for use with soft gelatine capsules include for example vegetable oils, waxes, fats, semi-solid, or liquid polyols etc. For the preparation of solutions and syrups, excipients which may be used include for example water, polyols and sugars. For the preparation of suspensions oils (e.g. vegetable oils) may be used to provide oil-in-water or water in oil suspensions.

[00292] Pharmaceutical compositions can be adapted for nasal administration wherein the excipient is a solid include a coarse powder having a particle size for example in the range 20 to 500 microns which is administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable compositions wherein the excipient is a liquid, for administration as a nasal spray or as nasal drops, include aqueous or oil solutions of the active ingredient. Pharmaceutical compositions adapted for administration by inhalation include fine particle dusts or mists which may be generated by means of various types of metered dose pressurized aerosols, nebulizers or insufflators.

[00293] Pharmaceutical compositions adapted for parenteral administration include aqueous and non-aqueous sterile injection solution which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation substantially isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. Excipients which may be used for injectable solutions include water, alcohols, polyols, glycerine and vegetable oils, for example. The compositions may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carried, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

Pharmaceutical compositions can be substantially isotonic, implying an osmolality of about 250-400 mOsm/kg water.

[00294] The pharmaceutical compositions may contain preserving agents, solubilizing agents, stabilizing agents, wetting agents, emulsifiers, sweeteners, colorants, odorants, salts (substances of the present invention may themselves be provided in the form of a pharmaceutically acceptable salt), buffers, coating agents or antioxidants. They may also contain therapeutically active agents in addition to the substance of the present invention. The pharmaceutical compositions of the invention may be employed in combination with one or more pharmaceutically acceptable excipients. Such excipients may include, but are not limited to, saline, buffered saline (such as phosphate buffered saline), dextrose, liposomes, water, glycerol, ethanol and combinations thereof.

[00295] The dual antagonists and pharmaceutical compositions containing them may be administered in an effective regime for treating or prophylaxis of a patient's disease including, for instance, administration by oral, intravitreal, intravenous, subcutaneous, intramuscular, intraosseous, intranasal, topical, intraperitoneal, and intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration or routes among others. In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic or substantially isotonic.

[00296] For administration to mammals, and particularly humans, it is expected that the dosage of the active agent is from 0.01 mg/kg body weight, typically around 1 mg/kg. The physician can determine the actual dosage most suitable for an individual which depends on factors including the age, weight, sex and response of the individual, the disease or disorder being treated and the age and condition of the individual being treated. The above dosages are exemplary of the average case. There can, of course, be instances where higher or lower dosages are merited.

[00297] This dosage may be repeated as often as appropriate (e.g., weekly, fortnightly, monthly, quarterly). If side effects develop the amount and/or frequency of the dosage can be reduced, in accordance with normal clinical practice. In one embodiment, the pharmaceutical composition may be administered once every one to thirty days.

[00298] The dual antagonists and pharmaceutical compositions of the invention can be employed alone or in conjunction with other compounds, such as therapeutic compounds or molecules, e.g. anti-inflammatory drugs, analgesics or antibiotics. Such administration with other compounds may be simultaneous, separate or sequential. The components may be prepared in the form of a kit which may comprise instructions as appropriate.

[00299] The dual antagonists and pharmaceutical compositions disclosed herein can be used for treatment or prophylaxis of disease, particularly the ocular diseases or conditions described herein. Although both antagonist modalities within the dual antagonist are believed to contribute to efficacy as discussed above and shown in Example 40 an understanding of mechanism is not required for practice of the invention. Preferably, a dual antagonist is more effective than an equimolar concentration of each antagonist administered alone, or a 1:1 combination of the antagonists administered as separate molecules.

[00300] So used, the conjugates are typically formulated for and administered by ocular, intraocular, and/or intravitreal injection, and/or juxtasceral injection, and/or subretinal injection and/or subtenon injection, and/or superchoroidal injection and/or topical administration in the form of eye drops and/or ointment. Such dual antagonists and compositions can be delivered by a variety of methods, e.g. intravitreally as a device and/or a depot that allows for slow release of the compound into the vitreous, including those described in references such as Intraocular Drug Delivery, Jaffe, Ashton, and Pearson, editors, Taylor & Francis (March 2006). In one example, a device may be in the form of a minipump and/or a matrix and/or a passive diffusion system and/or encapsulated cells that release the compound for a prolonged period of time (Intraocular Drug Delivery, Jaffe, Ashton, and Pearson, editors, Taylor & Francis (March 2006).

[00301] Formulations for ocular, intraocular or intravitreal administration can be prepared by methods and using ingredients known in the art. A main requirement for efficient treatment is proper penetration through the eye. Unlike diseases of the front of the eye, where drugs can be delivered topically, retinal diseases require a more site-specific approach. Eye drops and ointments rarely penetrate the back of the eye, and the blood-ocular barrier hinders penetration of systemically administered drugs into ocular tissue. Accordingly, usually the method of choice for drug delivery to treat retinal disease, such as AMD and CNV, is direct intravitreal injection. Intravitreal injections are usually repeated at intervals which depend on the patient's condition, and the properties and half-life of the drug delivered.

[00302] Therapeutic dual agonists and related conjugates according to the present invention generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle. Such compositions may also be supplied in the form of pre-filled syringes.

[00303] A "stable" formulation is one in which the protein or protein conjugated to a polymer of other half-life extending moiety therein essentially retains its physical stability and/or chemical stability and/or biological activity upon storage. By "stable" is also meant a formulation which exhibits little or no signs of instability, including aggregation and/or deamidation. For example, in accordance with an aspect of the present invention, the formulations provided by the present invention may remain stable for at least two year, when stored as indicated at a temperature of 5-8°C.

[00304] Various analytical techniques for measuring protein stability are available in the art and are reviewed in Peptide and Protein Drug Delivery, 247-301 (Vincent Lee ed., New York, N.Y., 1991) and Jones, 1993 Adv. Drug Delivery Rev. 10: 29-90, for examples. Stability can be measured at a selected temperature for a selected time period. Storage of stable formulations is preferably for at least 6 months, more preferably 12 months, more preferably 12-18 months, and more preferably for 2 or more years.

[00305] A protein, such as an antibody or fragment thereof, "retains its physical stability" in a pharmaceutical formulation if it shows no signs of aggregation, precipitation, deamidation and/or denaturation upon visual examination of color and/or clarity, or as measured by UV light scattering or by size exclusion chromatography.

[00306] A protein "retains its chemical stability" in a pharmaceutical formulation, if the chemical stability at a given time is such that the protein is considered to still retain its biological activity. Chemical stability can be assessed by detecting and quantifying chemically altered forms of the protein. Chemical alteration may involve size modification (e.g., clipping), which can be evaluated using size exclusion chromatography, SDS-PAGE and/or matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI/TOF MS), for examples. Other types of chemical alteration include charge alteration (e.g., occurring as a result of deamidation), which can be evaluated by ion-exchange chromatography, for example. An antibody "retains its biological activity" in a pharmaceutical formulation, if the biological activity of the antibody at a given time is within about 10% (within the errors of the assay) of the biological activity exhibited at the time the pharmaceutical formulation was prepared as determined in an antigen binding assay, for example.

[00307] A protein-polymer conjugate "retains its chemical stability" the chemical bond between the protein and the polymer is maintained intact, e.g., it is not hydrolyzed or otherwise disrupted. The protein part of the conjugate retains its chemical stability as described above.

[00308] By "isotonic" is meant that the formulation of interest has essentially the same osmotic pressure as human blood or the vitreous for intravitreal injections. Isotonic formulations will generally have an osmotic pressure from about 250 to 400 mOsm. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example.

[00309] As used herein, "buffer" refers to a buffered solution that resists changes in pH by the action of its acid-base conjugate components. The buffer of this invention has a pH in the range

of preferably from about 3.0 to about 8.0; for example from about 4.5 to 8; or about pH 6 to about 7.5; or about 6.0 to about 7.0, or about 6.5-7.0, or about pH 7.0 to about 7.5; or about 7.1 to about 7.4. A pH of any point in between the above ranges is also contemplated.

[00310] “PBS” phosphate buffered saline, Tris based buffers and histidine based buffers are particularly preferred buffers for the instantly invented dual antagonists. In the case of OG1448, PBS is particularly preferred. More preferably, in the case of OG1448, the PBS buffer has a pH of 7-8 and the concentration of OG1448 is from about 10 mg/ml to about 100 mg/ml. Still more preferably, the OG1448 is from about 25 to about 65 mg/ml and the pH is about 7.4. In the most preferred embodiments of the present invention, the concentration of OG1448 is 50 mg/ml to 60 mg/ml.

[00311] In preferred embodiments of the present invention, the PBS buffer is made up of at least Na_2HPO_4 , KH_2PO_4 and NaCl adjusted so as to provide the appropriate pH. In particularly preferred embodiments of the present invention, the buffer may contain other pharmaceutical excipients such as KCl and other salts, detergents and/or preservatives so as to provide a stable storage solution.

[00312] A "preservative" is a compound which can be included in the formulation to essentially reduce bacterial action therein, thus facilitating the production of a multi-use formulation, for example. Examples of potential preservatives include octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride (a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride. Other types of preservatives include aromatic alcohols such as phenol, butyl and benzyl alcohol, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol.

[00313] In accordance with an aspect of the present invention, formulations of dual PDGF/VEGF antagonists according to the present invention to be safe for human use or for animal testing must have sufficiently low levels of endotoxin. “Endotoxin” is lipopolysaccharide (LPS) derived from the cell membrane of Gram-negative bacteria. Endotoxin is composed of a hydrophilic polysaccharide moiety covalently linked to a hydrophobic lipid moiety (lipid A). Raetz CR, Ulevitch RJ, Wright SD, Sibley CH, Ding A, Nathan CF. 1991. Gram-negative endotoxin: an extraordinary lipid with profound effects on eukaryotic signal transduction. *FASEB J.* 5(12):2652-2660. Lipid A is responsible for most of the biological activities of

endotoxin, i.e., its toxicity. Endotoxins are shed in large amount upon bacterial cell death as well as during growth and division. They are highly heat-stable and are not destroyed under regular sterilizing conditions. Extreme treatments with heat or pH, e.g., 180-250°C and over 0.1 M of acid or base must be used (Petsch D, Anspach F. 2000. Endotoxin removal from protein solutions. J Biotechnol. 76: 97-119). Such conditions of course would be highly detrimental to biological drugs.

[00314] In the biotech and pharmaceutical industries, it is possible to find endotoxin during both production processes and in final products. As bacteria can grow in nutrient poor media, including water, saline and buffers, endotoxins are prevalent unless precautions are taken. Endotoxin injection into an animal or human causes a wide variety of pathophysiological effects, including endotoxin shock, tissue injury and even death. Ogikubo Y, Ogikubo Y, Norimatsu M, Noda K, Takahashi J, Inotsume M, Tsuchiya M, Tamura Y. 2004. Evaluation of the bacterial endotoxin test for quantifications of endotoxin contamination of porcine vaccines. Biologics 32:88-93.

[00315] Pyrogenic reactions and shock are induced in mammals upon intravenous injection of endotoxin at low concentrations (1 ng/mL) (Fiske JM, Ross A, VanDerMeid RK, McMichael JC, Arumugham. 2001. Method for reducing endotoxin in *Moraxella catarrhalis* UspA2 protein preparations. J Chrom B. 753:269-278). The maximum level of endotoxin for intravenous applications of pharmaceutical and biologic product is set to 5 endotoxin units (EU) per kg of body weight per hour by all pharmacopoeias (Daneshian M, Guenther A, Wendel A, Hartung T, Von Aulock S. 2006. In vitro pyrogen test for toxic or immunomodulatory drugs. J Immunol Method 313:169-175). EU is a measurement of the biological activity of an endotoxin. For example, 100 pg of the standard endotoxin EC-5 and 120 pg of endotoxin from *Escherichia coli* O111:B4 have activity of 1 EU (Hirayama C, Sakata M. 2002. Chromatographic removal of endotoxin from protein solutions by polymer particles. J Chrom B 781:419-432). Meeting this threshold level has always been a challenge in biological research and pharmaceutical industry (Berthold W, Walter J. 1994. Protein Purification: Aspects of Processes for Pharmaceutical Products. Biologics 22:135-150; Petsch D, Anspach FB. 2000. Endotoxin removal from protein solutions. J Biotech 76:97-119).

[00316] The presence of endotoxin in drugs to be delivered via intravitreal injection is of particular concern. Intravitreal injection of drug (penicillin) was first performed in 1945 by

Rycroft. Rycroft BW. 1945. Penicillin and the control of deep intra-ocular infection. *British J Ophthalmol* 29 (2): 57-87. The vitreous is a chamber where high level of drug can be introduced and maintained for relatively long periods of time. The concentration of drug that can be achieved via intravitreal injection far exceeds what can be generated by topical administration or by systemic administration (e.g. intravenous).

[00317] One of the most dangerous complications potentially arising from intravitreal injections is endophthalmitis. Endophthalmitis falls into two classes: infectious and sterile. Infectious endophthalmitis is generally caused by bacteria, fungi or parasites. The symptoms of infectious endophthalmitis include severe pain, loss of vision, and redness of the conjunctiva and the underlying episclera. Infectious endophthalmitis requires urgent diagnosis and treatment. Possible treatments include intravitreal injection of antibiotics and pars plana vitrectomy in some cases. Enucleation may be called for to remove a blind and painful eye. See, e.g., Christy NE, Sommer A. 1979. Antibiotic prophylaxis of postoperative endophthalmitis. *Ann Ophthalmol* 11 (8): 1261–1265.

[00318] Sterile endophthalmitis in contrast does not involve an infectious agent and can be defined as the acute intraocular inflammation of the vitreous cavity that resolves without the need of intravitreal antibiotics and/or vitreoretinal surgery. If a vitreous microbiological study has been done, it needs to be negative culture proven to sustain a diagnosis of sterile endophthalmitis. Marticorena J, Romano V, Gomez-Ulla F. 2012 “Sterile Endophthalmitis after Intravitreal Injections” *Med Inflam*. 928123.

[00319] It has been observed that intravitreal injection of biological drugs contaminated with endotoxin can result in sterile endophthalmitis. Marticorena, et al. Bevacizumab (Avastin) is approved by the Food and Drug Administration for the treatment of glioblastoma and of metastatic colorectal cancer, advanced nonsquamous non-small-cell lung cancer and metastatic kidney cancer. Bevacizumab is also widely used off label as a treatment for wet AMD. Bevacizumab comes from the manufacturer as a 100 mg/4 ml. This solution cannot be directly used for intravitreal injection and must be compounded by a pharmacist. Clusters of sterile endophthalmitis have been observed and are theorized to be caused by inadvertent contamination of bevacizumab by endotoxin by the compounding pharmacist.

[00320] Given the dire clinical results of intravitreal injection of endotoxin, the total amount of endotoxin that can be given to a patient via intravitreal dosing is highly limited. In accordance

with an aspect of the present invention, a solution having a dual VEGF/PDGF antagonist according to the present invention is provided having an endotoxin level that does not exceed 5.0 EU/ml. More preferably, the endotoxin level does not exceed 1.0 EU/ml. Still more preferably, the endotoxin level does not exceed 0.5 EU/ml. Still more preferably, the endotoxin level does not exceed 0.2 EU/ml. In still more preferred embodiments, the endotoxin level does not exceed 0.1, 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02 or 0.01 EU/ml.

[00321] Two commonly used FDA-approved tests for the presence of endotoxin are the rabbit pyrogen test and Limulus Amoebocyte Lysate (LAL) assay (Hoffman S, et al. 2005.

International validation of novel pyrogen tests based on human monocytoic cells J. Immunol. Methods 298:161-173; Ding JL, Ho BA. 2001. New era in pyrogen testing. Biotech. 19:277-281). The rabbit pyrogen test was developed in the 1920s and involves monitoring the temperature rise in a rabbit injected with a test solution. However, use of the rabbit pyrogen test has greatly diminished over the years due to expense and long turnaround time. Much more common is the LAL test. LAL is derived from the blood of a horseshoe crab and clots upon exposure to endotoxin.

[00322] One of the simplest LAL assays is the LAL gel-clot assay. Essentially, the LAL clotting assay is combined with a serial dilution of the sample in question. Formation of the gel is proportional to the amount of endotoxin in the sample. Serial dilutions are prepared from the sample and each dilution assayed for its ability to form LAL gel. At some point a negative reaction is contained. The amount of endotoxin in the original sample can be estimated from the dilution assay.

[00323] Other LAL tests have also been developed, including the turbidimetric LAL assay (Ong KG, Lelan JM, Zeng KF, Barrett G, Aourob M, Grimes CA. 2006. A rapid highly-sensitive endotoxin detection system. Biosensors and Bioelectronics 21:2270-2274) and the chromogenic LAL assay (Haishima Y, Hasegawa C, Yagami T, Tsuchiya T, Matsuda R, Hayashi Y. 2003. Estimation of uncertainty in kinetic-colorimetric assay of bacterial endotoxins. J Pharm Biomed Analysis. 32:495-503). The turbidimetric and chromogenic assays are much more sensitive and quantitative than the simple gel-clot dilution assay.

[00324] The present invention provides a method of reducing the amount of endotoxin in a composition having a dual VEGF/PDGF antagonist, the method having the steps of contacting the composition with an affinity chromatography resin that binds to the dual VEGF/PDGF

antagonist; eluting the dual VEGF/PDGF antagonist from the affinity chromatography resin to form an affinity chromatography eluent having the antagonist; contacting the affinity chromatography eluent with an ion-exchange resin that binds the dual VEGF/PDGF antagonist; and eluting the dual VEGF/PDGF antagonist from the ion-exchange resin, wherein the dual VEGF/PDGF antagonist eluted from the ion-exchange resin is substantially free from endotoxin.

[00325] The above method for reducing the amount of endotoxin, or other method or process recited herein, can be performed in the order described in the steps above or it can optionally be performed by varying the order of the steps or even repeating one or more of the steps. In one embodiment, the method of reducing the amount of endotoxin in a composition is performed in the order of the described steps. In some embodiments, the affinity chromatography resin contacting, washing and eluting steps are repeated in the same order more than one time before contacting the affinity chromatography eluent with the ion exchange resin. The method can also include a filtering step using, for example, a 0.1 micron, 0.22 micron, or 0.44 micron filter, that can be performed on either one or more of the eluents removed after each resin binding step.

[00326] In certain instances, the steps of contacting the composition with affinity chromatography resin, washing and eluting the antibody from the affinity chromatography resin can be repeated more than one time before contacting the first eluent with an ion-exchange resin. In one embodiment, the affinity chromatography resin comprises a recombinant Protein A ("rProteinA") resin. One example of a suitable recombinant Protein A resin is rProteinA Sepharose FF® resin (Amersham, Piscataway, N.J.). In another embodiment, a suitable affinity chromatography resin would comprise a protein G chromatography resin. In other embodiments, a suitable affinity chromatography resin comprises a mixed Protein A/Protein G resin. In other embodiments, a suitable affinity chromatography resin comprises a hydrophobic charge induction resin that comprises a 4-mercaptoethylpyridine ligand such as a MEP HyperCel® resin (BioSeptra, Cergy, Saint Christophe, France).

[00327] In some embodiments, it is preferred that the ion exchange resin comprises an anion-exchange resin. As will be known by the person skilled in the art, ion exchangers may be based on various materials with respect to the matrix as well as to the attached charged groups. For example, the following matrices may be used, in which the materials mentioned may be more or less cross-linked: MacroCap Q (GE Healthcare Biosciences, Piscataway, NJ), agarose based (such as Sepharose CL-6B®, Sepharose Fast Flow® and Sepharose High Performance ®),

cellulose based (such as DEAE Sephacel®), dextran based (such as Sephadex®), silica based and synthetic polymer based. For the anion exchange resin, the charged groups, which are covalently attached to the matrix, may, for example, be diethylaminoethyl, quaternary aminoethyl, and/ or quaternary ammonium. It is preferred that the anion-exchange resin comprises a quaternary amine group. An exemplarily anion-exchange resin that has a quaternary amine group for binding the anti-M-CSF antibody is a Q Sepharose® resin (Amersham, Piscataway, N.J.).

[00328] In other aspects, if the endotoxin levels are higher than desired after subjecting the composition to the aforementioned anion-exchange chromatography step, the composition may in the alternative be subjected to a cation exchange resin. In accordance with this aspect of the present invention, any endotoxin in the composition should have a differential binding to the ion-exchange resin than the protein in question to allow purification of the protein from the endotoxin. In this regard, endotoxin is negatively charged and will generally bind to an anion exchange resin. If both the protein and the endotoxin bind to the anion exchange resin, purification of one from the other may be effectuated by using a salt gradient to elute the two into different fractions. The relative binding of the protein to a particular resin may also be effected by changing the pH of the buffer relative to the pI of the protein. In a preferred aspect of the present invention, cation-exchange chromatography is the sole ion-exchange chromatography employed.

[00329] In accordance with another aspect of the present invention, if the endotoxin levels are too high after the anion exchange resin, the composition may be further subjected to a second ion-exchange step, for example, by contacting the compositions with a cation exchange resin and followed by a wash step, then elution from the ion-exchange resin. In preferred embodiments, the cation exchange resin comprises a sulfonic group for binding. Exemplary cation exchange resins are SP Sepharose® resin FF (Amersham, Piscataway, N.J.) Poros XS (CEX) (Life Technology, Grand Island, New York).

[00330] In accordance with an aspect of the invention, after the solution of dual PDGF/VEGF antagonist protein is produced having the specified level of endotoxin, there are a number of steps prior to final formulation of the protein. In some embodiments of the present invention, a half-life extending moiety is conjugated to the protein. The conjugate is then formulated into a final drug formulation which is injected into the patients. In some embodiments, the conjugate is

again purified on an ion-exchange resin which can preferably be a cation-exchange resin. In other embodiments, the protein is formulated. In all cases, normal laboratory procedures must be employed to prevent the introduction of endotoxin contaminants into the protein sample or into the protein-polymer conjugate.

EXAMPLES

[00331] Example 1. Protein Sequence of PDGFR β -GS10-anti-VEGF-A light chain /anti-VEGF-A heavy chain (wild type Fc).

[00332] A PDGFR- β trap-anti-VEGF-A light chain/anti-VEGF-A heavy chain was constructed having the sequence set forth below in FIGs. 7A, B. PDGFR-GS10-anti-VEGF-A light chain amino acids 1-282 correspond to 33 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1), followed by the linker sequence GGGGSGGGGS and the bevacizumab light chain sequence. Optionally, no linker need be used between the PDGFR- β segment and the anti-VEGF segment. Alternatively, the linker may be the GGGGS motif x1, x2 (as noted above), x3, x4, etc. such that the activity of the two proteins is optimized. Other linker motifs known to those of skill in the art may also be used in accordance with the present invention, including G, GG, GGGS and GGGES x1, x2, x3, x4, etc. The linker may be combinations of the above. The sequence of FIG. 7A is set forth in SEQ ID NO. 19. FIG. 7B shows the bevacizumab heavy chain sequence (SEQ ID NO. 2). The bevacizumab light chain optionally has an M4L mutation (Kabat numbering). The bevacizumab heavy chain optionally has one or more of the following mutations: T28D, N31H, H97Y, S100aT (Kabat numbering), L234A, L235A, G237A, Q347C and L443C EU numbering).

[00333] Example 2. Protein Sequence of PDGFR β -GG-anti-VEGF-A light chain /anti-VEGF-A heavy chain (wild type Fc).

[00334] Another PDGFR- β trap-anti-VEGF-A light chain/anti-VEGF-A heavy chain was constructed having the sequence set forth below in FIGs. 8A, B. FIG. 8A amino acids 1-282 correspond to 33 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1), followed by the linker sequence GG and the bevacizumab light chain sequence. Alternatively, the linker may be the GGGGS motif x1, x2, x3, x4, etc. such that the activity of the two proteins is optimized. Other linker motifs may also be used in accordance with the present invention, including G, GG

(as noted above), GGGS and GGGES x1, x2, x3, x4, etc. The linker may be combinations of the above. The protein sequence of FIG. 8A is set forth in SEQ ID NO. 3. FIG. 8B shows bevacizumab heavy chain sequence (SEQ ID NO. 2). The bevacizumab light chain of FIG. 8A optionally has an M4L mutation. The bevacizumab heavy chain optionally has one or more of the following mutations: T28D, N31H, H97Y, S100aT (Kabat numbering), L234A, L235A, G237A, Q347C and L443C (EU numbering).

[00335] EXAMPLE 3. Protein Sequence of PDGFR β -GS10-anti-VEGF-A heavy chain (wild type Fc)/anti-VEGF-A light chain.

[00336] Another PDGFR- β trap-anti-VEGF-A heavy chain (wild type Fc)/anti-VEGF-A light chain was constructed having the sequence set forth in FIGs. 9A, B. FIG. 9A amino acids 1-282 correspond to 33 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1), followed by the linker sequence GGGGSGGGGS and the bevacizumab heavy chain sequence, optionally having Q347C or L443C (EU numbering). Alternatively, the linker may be the GGGGS motif x1, x2 (as noted above), x3, x4, etc. such that the activity of the two proteins is optimized. Other linker motifs may also be used in accordance with the present invention, including G, GG, GGGS and GGGES x1, x2, x3, x4, etc. The linker may be combinations of the above. The protein sequence of FIG. 9A is set forth in SEQ ID NO. 4. The protein of FIG. 9B is the bevacizumab light chain sequence (SEQ ID NO. 5). The bevacizumab light chain optionally has an M4L mutation. The bevacizumab heavy chain optionally has one or more of the following mutations: T28D, N31H, H97Y, S100aT (Kabat numbering), L234A, L235A, G237A, Q347C and L443C (EU numbering).

[00337] EXAMPLE 4. Protein Sequence of PDGFR β -GG-anti-VEGF-A heavy chain (wild type Fc)/anti-VEGF-A light chain.

[00338] Another PDGFR- β trap-anti-VEGF-A heavy chain (wild type Fc)/anti-VEGF-A light chain was constructed having the sequence set forth below in FIGs. 10A, 10B. FIG. 10A amino acids 1-282 correspond to 33 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1), followed by the linker sequence GG and the bevacizumab heavy chain sequence, optionally having Q347C or L443C. Alternatively, the linker may be the GGGGS motif x1, x2, x3, x4, etc. such that the activity of the two proteins is optimized. Other linker motifs may also be used in accordance with the present invention, including G, GG (as noted above), GGGS and GGGES x1, x2, x3, x4, etc. The linker may be combinations of the above. The protein sequence of FIG.

10A is set forth in SEQ ID NO. 6. The protein of FIG. 10B is the bevacizumab light chain sequence (SEQ ID NO. 5). The bevacizumab light chain optionally has an M4L mutation. The bevacizumab heavy chain optionally has one or more of the following mutations: T28D, N31H, H97Y, S100aT (Kabat numbering), L234A, L235A, G237A, Q347C and L443C (EU numbering).

[00339] EXAMPLE 5. Protein Sequence of anti-VEGF-A heavy chain (wild type Fc)-GS21-PDGFR β /anti-VEGF-A light chain.

[00340] A PDGFR- β trap-anti-VEGF-A antibody construct was constructed with the anti-VEGF-A heavy chain being upstream or N-terminal to the PDGFR- β trap having the sequence set forth below in FIGs. 11A, B. FIG. 11A amino acids 1-451 correspond to the bevacizumab heavy chain sequence, optionally having Q347C or L443C, followed by linker sequence GGGGSGGGGSGGGGSGGGGSG. Alternatively, the linker may be the GGGGS motif x1, x2 (as noted above), x3, x4, etc. such that the activity of the two proteins is optimized. Other linker motifs also be used in accordance with the present invention, including G, GG, GGGS and GGGES x1, x2, x3, x4, etc. The linker may be combinations of the above. The linker is followed by amino acid sequences 33 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1). The protein sequence of FIG. 11A is set forth in SEQ ID NO. 7. FIG. 11B shows the bevacizumab light chain sequence (SEQ ID NO. 5). The bevacizumab light chain optionally has an M4L mutation. The bevacizumab heavy chain optionally has one or more of the following mutations: T28D, N31H, H97Y, S100aT (Kabat numbering), L234A, L235A, G237A, Q347C and L443C (EU numbering).

[00341] EXAMPLE 6. Protein Sequence of PDGFR β -GS10-anti-VEGF-A heavy chain (Q347C)/anti-VEGF-A light chain (TAF347).

[00342] Another PDGFR- β trap-anti-VEGF-A heavy chain (Q347C)/anti-VEGF-A light chain was constructed having the sequence set forth below in FIGs. 12A, B. FIG. 12A amino acids 1-282 correspond to 33 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1).

Immediately following the PDGFR sequence is a 10 amino acid linker GGGGSGGGGS.

Optionally, no linker need be used between the PDGFR- β segment and the anti-VEGF segment.

The linker may be combinations of the above. Joined to the carboxyl terminus of the serine residue of the linker is the bevacizumab heavy chain with the following amino acid: T28D, N31H, H97Y, S100aT (Kabat numbering), L234A, L235A, G237A and Q347C (EU numbering).

The protein sequence of FIG. 12A is set forth in SEQ ID NO. 8. The protein of FIG. 12B is ranibizumab light chain (bevacizumab w/ M4L) (SEQ ID NO. 12).

[00343] EXAMPLE 7. Protein Sequence of PDGFR β -GS10-anti-VEGF-A heavy chain (L443C)/anti-VEGF-A light chain.

[00344] Another PDGFR- β trap-anti-VEGF-A heavy chain (L443C)/anti-VEGF-A light chain was constructed having the sequence set forth below in FIGs. 13A, B. FIG. 13A amino acids 1-282 correspond to 33 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1). Immediately following the PDGFR sequence is a 10 amino acid linker GGGGSGGGGS. Joined to the carboxyl terminus of the serine residue of the linker is the bevacizumab heavy chain with the following amino acid: T28D, N31H, H97Y, S100aT (Kabat numbering), L234A, L235A, G237A and L443C (EU numbering). The TAF443 light chain is the same as bevacizumab except for a M4L change (Kabat numbering). The protein sequence of FIG. 13A is set forth in SEQ ID NO. 9. FIG. 13B shows the ranibizumab light chain (bevacizumab w/ M4L) (SEQ ID NO. 12).

[00345] Example 8. Protein Sequence of PDGFR β -GS10-anti-VEGF-A light chain /anti-VEGF-A Fab.

[00346] A PDGFR- β trap-anti-VEGF-A light chain/anti-VEGF-A Fab was constructed having the sequence set forth below in FIGs. 14A, B. FIG. 14A amino acids 1-282 correspond to 33 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1), followed by the linker sequence GGGGSGGGGS and the bevacizumab light chain sequence. Optionally, no linker need be used between the PDGFR- β segment and the anti-VEGF segment. Alternatively, the linker may be the GGGGS motif x1, x2 (as noted above), x3, x4, etc. such that the activity of the two proteins is optimized. Other linker motifs may also be used in accordance with the present invention, including G, GG, GGS and GGGS x1, x2, x3, x4, etc. The linker may be combinations of the above. The protein sequence of FIG. 14A is set forth in SEQ ID NO. 1. The protein of FIG. 14B is the bevacizumab Fab (SEQ ID NO. 21). The bevacizumab light chain of FIG. 14A optionally has an M4L mutation. The bevacizumab Fab of the second protein optionally has one or more of the following mutations: T28D, N31H, H97Y, and S100aT. The bevacizumab Fab of the second chain optionally has a cysteine moiety added to the C-terminus for conjugating a half-life extending moiety. Preferably, the cysteine moiety is added via SGGGC or CAA. Alternatively, SGGGC or CAA may be added to the C-terminus of the light chain.

[00347] Example 9. Protein Sequence of PDGFR β -GG-anti-VEGF-A light chain /anti-VEGF-A Fab.

[00348] A PDGFR- β trap-anti-VEGF-A light chain/anti-VEGF-A Fab was constructed having the sequence set forth below in FIGs 15A, B. FIG. 15A amino acids 1-282 correspond to 33 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1), followed by the linker sequence GG and the bevacizumab light chain sequence. Optionally, no linker need be used between the PDGFR- β segment and the anti-VEGF segment. Alternatively, the linker may be the GGGGS motif x1, x2, x3, x4, etc. such that the activity of the two proteins is optimized. Other linker motifs may also be used in accordance with the present invention, including G, GG (as above), GGGs and GGGES x1, x2, x3, x4, etc. The linker may be combinations of the above. The protein sequence of FIG. 15A is set forth in SEQ ID NO. 3. FIG. 15B shows the heavy chain of bevacizumab Fab (SEQ ID NO. 21). The bevacizumab light chain of FIG. 15A optionally has an M4L mutation (Kabat numbering). The bevacizumab Fab of FIG. 15B optionally has one or more of the following mutations: T28D, N31H, H97Y, and S100aT (Kabat numbering). The bevacizumab Fab of FIG. 15B optionally has a cysteine moiety added to the C-terminus for conjugating a half-life extending moiety. Preferably, the cysteine moiety is added via SGGGC or CAA. Alternatively, SGGGC or CAA may be added to the C-terminus of the light chain.

[00349] EXAMPLE 10. Protein Sequence of PDGFR β -GS10-anti-VEGF-A Fab/anti-VEGF-A light chain.

[00350] Another PDGFR- β trap-anti-VEGF-A Fab/anti-VEGF-A light chain was constructed having the sequence set forth below in FIGs. 16A, B. FIG. 16A amino acids 1-282 correspond to 33 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1), followed by the linker sequence GGGSGGGGS and the bevacizumab Fab sequence. Optionally, no linker need be used between the PDGFR- β segment and the anti-VEGF segment. Alternatively, the linker may be the GGGGS motif x1, x2 (as above), x3, x4, etc. such that the activity of the two proteins is optimized. Other linker motifs may also be used in accordance with the present invention, including G, GG, GGGs and GGGES x1, x2, x3, x4, etc. The linker may be combinations of the above. The protein sequence of FIG. 16A is set forth in SEQ ID NO. 22. FIG. 16B shows the bevacizumab light chain sequence (SEQ ID NO. 5). The bevacizumab light chain optionally has an M4L mutation. The bevacizumab heavy chain optionally has one or more of the following mutations: T28D, N31H, H97Y, and S100aT (Kabat numbering). The heavy chain optionally

has a cysteine moiety added to the C-terminus for conjugating a half-life extending moiety. Preferably, the cysteine moiety is added via SGGGC or CAA. Alternatively, SGGGC or CAA may be added to the C-terminus of the light chain.

[00351] EXAMPLE 11. Protein Sequence of PDGFR β -GG-anti-VEGF-A Fab/anti-VEGF-A light chain.

[00352] Another PDGFR- β trap-anti-VEGF-A Fab/anti-VEGF-A light chain was constructed having the sequence set forth below in FIGs. 17A, B. FIG. 17A amino acids 1-282 correspond to 33 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1), followed by the linker sequence GGGGSGGGGS and the bevacizumab Fab sequence. Optionally, no linker need be used between the PDGFR- β segment and the anti-VEGF segment. Alternatively, the linker may be the GGGGS motif x1, x2 (as above), x3, x4, etc. such that the activity of the two proteins is optimized. Other linker motifs may also be used in accordance with the present invention, including G, GG, GGGS and GGGES x1, x2, x3, x4, etc. The linker may be combinations of the above. The protein sequence of FIG. 17A is set forth in SEQ ID NO. 23. FIG. 17B shows the bevacizumab light chain sequence (SEQ ID NO. 5). The bevacizumab light chain optionally has an M4L mutation. The bevacizumab heavy chain optionally has one or more of the following mutations: T28D, N31H, H97Y, and S100aT (Kabat numbering). The bevacizumab Fab heavy chain optionally has a cysteine moiety added to the C-terminus for conjugating a half-life extending moiety. Preferably, the cysteine moiety is added via SGGGC or CAA. Alternatively, SGGGC or CAA may be added to the C-terminus of the light chain.

[00353] EXAMPLE 12. Protein Sequence of anti-VEGF-A Fab-GS21-PDGFR β /anti-VEGF-A light chain.

[00354] A PDGFR- β trap-anti-VEGF-A antibody construct was constructed with the anti-VEGF-A heavy chain being upstream or N-terminal to the PDGFR- β trap having the sequence set forth below in FIGs. 18A, B. FIG. 18A amino acids 1-231 correspond to the bevacizumab Fab followed by linker sequence GGGGSGGGGSGGGGSGGGGSG. Optionally, no linker need be used between the PDGFR- β segment and the anti-VEGF segment. Alternatively, the linker may be the GGGGS motif x1, x2, x3, x4, etc. such that the activity of the two proteins is optimized. Other linker motifs may also be used in accordance with the present invention, including G, GG, GGGS and GGGES x1, x2, x3, x4, etc. The linker may be combinations of the above. The linker is followed by amino acid sequences 33 to 314 of human PDGFR- β

(UniProtKB/Swiss-Prot: P09619.1). The protein sequence of FIG. 18A is set forth in SEQ ID NO. 24. FIG. 18B shows the bevacizumab light chain sequence (SEQ ID NO. 5). The bevacizumab light chain optionally has an M4L mutation. The bevacizumab heavy chain optionally has one or more of the following mutations: T28D, N31H, H97Y, and S100aT. The protein of FIG. 18A optionally has a cysteine moiety added to the C-terminus for conjugating a half-life extending moiety. Preferably, the cysteine moiety is added via SGGGC or CAA.

Alternatively, SGGGC or CAA may be added to the C-terminus of the light chain of FIG. 18B.

[00355] EXAMPLE 13. Protein Sequence of PDGFR β -GS10-anti-VEGF-A Fab/anti-VEGF-A light chain.

[00356] Another PDGFR- β trap-anti-VEGF-A Fab/anti-VEGF-A light chain was constructed having the sequence set forth below in FIGs. 19A, B. FIG. 19A amino acids 1-282 correspond to 33 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1). Immediately following the PDGFR sequence is a 10 amino acid linker GGGGSGGGGS. Optionally, no linker need be used between the PDGFR- β segment and the anti-VEGF segment. Alternatively, the linker may be the GGGGS motif x1, x2 (as above), x3, x4, etc. such that the activity of the two proteins is optimized. Other linker motifs may also be used in accordance with the present invention, including G, GG, GGS and GGGS x1, x2, x3, x4, etc. The linker may be combinations of the above. Joined to the carboxyl terminus of the serine residue of the linker is the bevacizumab Fab having the mutations T28D, N31H, H97Y, and S100aT (Kabat numbering). The protein sequence of FIG. 19A is set forth in SEQ ID NO. 25. The protein of FIG. 19B is the ranibizumab light chain (bevacizumab w/ M4L) (SEQ ID NO. 12). The protein of FIG. 19A optionally has a cysteine moiety added to the C-terminus for conjugating a half-life extending moiety. Preferably, the cysteine moiety is added via SGGGC or CAA. Alternatively, SGGGC or CAA may be added to the C-terminus of the light chain of FIG. 19B.

[00357] Example 14. Protein Sequence of PDGFR β -anti-VEGF-A Fab/anti-VEGF-A light chain (1a).

[00358] Another PDGFR- β trap-anti-VEGF-A Fab/anti-VEGF-A light chain was constructed having the sequence set forth in FIGs. 20A, B. FIG. 20A amino acids 1-283 correspond to 32 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1), followed by the bevacizumab heavy chain. The protein sequence of FIG. 20A is set forth in SEQ ID NO. 26. The protein of FIG. 20B is the bevacizumab light chain sequence (SEQ ID NO. 5). As set forth in this example,

no linker need be used between the PDGFR- β segment and the anti-VEGF segment.

Alternatively, the linker may be the GGGGS motif x1, x2, x3, x4, etc. such that the activity of the two proteins is optimized. Other linker motifs may also be used in accordance with the present invention, including G, GG, GGG and GGGES x1, x2, x3, x4, etc. The linker may be combinations of the above. The bevacizumab light chain optionally has an M4L mutation. The bevacizumab heavy chain optionally has one or more of the following mutations: T28D, N31H, H97Y, S100aT (Kabat numbering), Q347C and L443C (EU numbering).

[00359] Example 15. Protein Sequence of PDGFR- β (D2-D3)-anti-VEGF-A heavy chain/anti-VEGF-A light chain (1b).

[00360] Another PDGFR- β trap (D2-D3)-anti-VEGF-A heavy chain/anti-VEGF-A light chain was constructed having the sequence set forth below in FIG. 21A, B. FIG. 21A amino acids 1-190 correspond to 125 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1), followed by the bevacizumab heavy chain. The protein sequence of FIG. 21A is set forth in SEQ ID NO. 27. As set forth in this example, no linker need be used between the PDGFR- β segment and the anti-VEGF segment. Alternatively, the linker may be the GGGGS motif x1, x2, x3, x4, etc. such that the activity of the two proteins is optimized. Other linker motifs may also be used in accordance with the present invention, including G, GG, GGG and GGGES x1, x2, x3, x4, etc. The linker may be combinations of the above. FIG. 21B shows the bevacizumab light chain sequence (SEQ ID NO. 5). The bevacizumab light chain optionally has an M4L mutation. The bevacizumab heavy chain optionally has one or more of the following mutations: T28D, N31H, H97Y, S100aT (Kabat numbering), Q347C and L443C (Eu numbering).

[00361] Example 16. Protein Sequence of PDGFR- β (D2-D3)-anti-VEGF-A Fab/anti-VEGF-A light chain (2b).

[00362] Another PDGFR- β trap (D2-D3)-anti-VEGF-A Fab/anti-VEGF-A light chain was constructed having the sequence set forth in FIGs. 22A, B. FIG. 22A amino acids 1-190 correspond to 125 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1), followed by the bevacizumab Fab. As set forth in this example, no linker need be used between the PDGFR- β segment and the anti-VEGF segment. Alternatively, the linker may be the GGGGS motif x1, x2, x3, x4, etc. such that the activity of the two proteins is optimized. The GGGGS GGGGS linker is particularly preferred. Other linker motifs may also be used in accordance with the present invention, including G, GG, GGG and GGGES x1, x2, x3, x4, etc. The linker may be

combinations of the above. The sequence of FIG. 22A is set forth in SEQ ID NO. 28. FIG. 22B shows the bevacizumab light chain sequence (SEQ ID NO. 5). The bevacizumab light chain optionally has an M4L mutation. The bevacizumab heavy chain optionally has one or more of the following mutations: T28D, N31H, H97Y, and S100aT (Kabat numbering). The bevacizumab Fab of FIG. 22A optionally has a cysteine moiety added to the C-terminus for conjugating a half-life extending moiety. Preferably, the cysteine moiety is added via SGGGC or CAA. Alternatively, SGGGC or CAA may be added to the C-terminus of the light chain of FIG. 22B.

[00363] Example 17. Protein Sequence of PDGFR- β (D2-D3)-anti-VEGF-A Fab/anti-VEGF-A light chain (2b').

[00364] Another PDGFR- β trap (D2-D3)-6xGS-anti-VEGF-A Fab/anti-VEGF-A light chain was constructed having the sequence set forth below in FIGs. 23A, B. FIG. 23A amino acids 1-190 correspond to 125 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1), followed by the linker GGGSGGGSGGGSGGGSGGGSGGGSGGGSG and then by bevacizumab Fab. Optionally, no linker need be used between the PDGFR- β segment and the anti-VEGF segment. Alternatively, the linker may be the GGGGS motif x1, x2, x3, x4, etc. such that the activity of the two proteins is optimized. Other linker motifs known to those of skill in the art may also be used in accordance with the present invention, including G, GG, GGS and GGGS x1, x2, x3, x4, etc. The linker may be combinations of the above. The protein sequence of FIG. 23A is set forth in SEQ ID NO. 29. FIG. 23B shows the bevacizumab light chain sequence (SEQ ID NO. 5). The bevacizumab light chain optionally has an M4L mutation. The bevacizumab heavy chain optionally has one or more of the following mutations: T28D, N31H, H97Y, and S100aT (Kabat numbering). The bevacizumab Fab heavy chain optionally has a cysteine moiety added to the C-terminus for conjugating a half-life extending moiety. Preferably, the cysteine moiety is added via SGGGC or CAA. Alternatively, SGGGC or CAA may be added to the C-terminus of the light chain.

[00365] Example 18. Protein Sequence of PDGFR- β (D2-D3)-anti-VEGF-A Fab/anti-VEGF-A light chain (2b').

[00366] Another anti-VEGF-A Fab-6xGS-PDGFR- β trap (D2-D3)/anti-VEGF-A light chain was constructed having the sequence set forth below in FIGs. 24A, B. FIG. 24A amino acids 1-190 correspond to 125 to 314 of human PDGFR- β (UniProtKB/Swiss-Prot: P09619.1), followed

[00369] **Example 20.** Production of dual PDGFR/VEGF antagonist protein.

[00370] The TAF443 heavy and light chains were cloned into expression plasmids and transfected into CHO cells. Cells were grown up in appropriate media and harvested. TAF443 was purified as follows. 10L culture medium from CHO cells expressing SEQ ID NOS. 31 and 32 were adjusted with 5% (v/v) 1.1 M HEPES, 0.22 M EDTA, pH 6.7 or 10% 0.55 M Hepes, 0.11M EDTA, 5.5% Triton X-100, pH 6.7, and loaded onto a 167/400ml Protein A column (2-run) packed with Mab Select Sure resin equilibrated in 50mM Tris, 150mM NaCl, pH 7.5 (5-CV). The column was washed with 50mM Tris, 150mM NaCl, pH 7.5 (2-CV), 50mM Tris, 0.5M CaCl₂, pH 7.5 (5-CV), and then 10mM Tris, 10mM NaCl, pH 7.5 (3-CV) before the protein was eluted using 150mM Glycine, 40mM NaCl, pH 3.5 (4-CV). Fractions were pooled, adjusted to pH 3.5 using 2M Glycine, pH 2.7, and then neutralized to pH 7 using 2M HEPES, pH 8.0. The Protein A pool was loaded onto a 274ml TMAE column equilibrated in 50 mM Hepes, 65 mM NaCl, pH 7.0 (5-CV). The column was washed with 50 mM Hepes, 65 mM NaCl, pH 7.0 (3-CV), and then eluted with 50 mM Tris, 200 mM NaCl, pH 7.5 (5-CV). The elution fractions were pooled and buffer exchanged in a 1150 mL Sephadex G-25 Coarse column equilibrated with PBS-CMF, pH 7.2. The pool was filtered, concentrated to >5mg/ml via 30k MWCO VivaFlow200. The concentrated protein was filtered through a 0.22um filter, and then characterized by SDS-PAGE, analytical SEC, O.D.280/320, end toxin LAL assay, Protein A ELISA, IEF, and Freeze/Thaw Analysis.

[00371] The table below summarizes the properties of an example batch of purified TAF443.

TAF443 Purified Lot Characteristics	
Concentration (UV)	5.69 mg/ml
Purity (SEC)	98.6%
MW (SDS-PAGE)	~200 kDa (NR)
pI (IEF)	4.2-4.5
Endotoxin (LAL)	0.1 EU/mg
Protein A (Elisa)	<10 ng/ml
Final Yield	~700 mg/L (CM)

[00372] **Example 21.** TAF bi-functional molecule stability at high concentration in representative formulations

[00373] The TAF bi-functionals were concentrated to 50-85 mg/ml in a series of standard formulation buffers ranging from pH 4.5 to 7.5, in the presence of excipients such as sucrose. Aliquots of these samples were stored at room temperature (RT) and 4°C over a period of 6 weeks, and sampled at time zero and after each subsequent week to measure the percentage of aggregated material by analytical SEC. The effect of pH on aggregation of TAF443 can be seen in the following table.

% Aggregates Observed in TAF Solution at various pHs over Time				
	Tris pH 7.5	His pH 6.0	His pH 5.5	Lac pH 4.5
Time 0	<1	<1	<1	<2
Day 4	<1	<1	<1	~3
Week 1	<1	<1	<1	~4
Week 2	<1	<1	~2	~6
Week 4	<1	<1	~3	~10
Week 6	<1	<1	~3	~10

[00374] **Example 22.** Transfection of Constructs into CHO Cells

[00375] DNA constructs for TAFwt, TAF443 and TAF347 were transfected into CHO-K1 SV SSI: 3 pools/construct. The normal 3 weeks of recovery was observed in most of the cell lines. However, TAFwt and TAF347 cell lines lagged approximately 1 week behind the other cell lines. Once the pools were established, day 4 for most and day 3 for TAFwt and TAF347, conditioned media samples were run on Octet. 3-day conditioned media for TAFwt and TAF347 showed about 7 mg/ml by Octet. 4-day conditioned media showed about 21 mg/ml for TAF443. Small differences were observed between pools and the pools were used to make pools of pools which were carried forward for protein generation.

[00376] **Example 23.** SEC-MALS of Proteins

[00377] The PDGFR segment of TAF has 7 putative glycosylation sites. The protein appears to be heavily glycosylated from SEC-MALS measurements:

Construct	Protein (kDa)	Sugar (kDa)	Total (kDa)
TAFwt	184	63	247
TAF334	182	62	244

TAF443	187	63	250
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[00378] The samples run on SEC-MALS were all greater than 98% pure. The molecular weights measured were reasonable. Some high molecular weight material was observed, probably a tri- to pentamer (data not shown).

[00379] **Example 24.** Thermal Stability of TAF Proteins

[00380] Thermal stability profiles were run of TAFwt, TAF443 and TAF347 in PBS, pH 7.2. Each protein had three peaks (data not shown). The relative positions of the peaks are set forth in the table below:

Sample	T _m 1(°C)	T _m 2(°C)	T _m 3(°C)
TAFwt	58.1±0.1	71.9±0.1	83.2±0.1
TAF347	58.2±0.1	71.9±0.1	81.7±0.1
TAF443	58.2±0.1	71.9±0.1	84.4±0.1

The stabilities of the proteins over the temperature range are very similar. It is noted however that there are some small changes in T_m3. T_m3 likely corresponds to the CH3 domain of the antibody domain of the three TAF proteins and the changes reflect the Cys mutations. The low overall stability of the TAF proteins is likely due to unfolding of the PDGFR segment of the proteins.

[00381] **Example 25.** TAF Forced Aggregation

[00382] The percentage of aggregates in a solution of the three TAF proteins as a function of heat was examined (data not shown). Solutions of each of the proteins (TAFwt, TAF347 and TAF443) started to show aggregates starting around 54°C. The percentage of aggregates for each of the proteins increased sharply as the temperature was increased. At 64°C, roughly 40% of each of the TAF proteins constituted aggregates. It is noted that the aggregation starts to occur at the lowest T_m, seemingly corresponding to the unfolding of the PDGFR portion of the protein.

[00383] **Example 26.** TAF443 Thermal Stability as a Function of pH

[00384] The thermal stability of TAF443 was examined at various pHs as set forth in the table below. In non PBS buffers, 4 thermal denaturation peaks are seen:

Buffer	T _m 1(°C)	T _m 2(°C)	T _m 3(°C)	T _m 4(°C)
Tris pH 7.5	57	67	74	85.9
His pH 6.0	53.3	62.9	75.4	84.9
Succinate	55.9	66.7	74.9	85.8
Lucentis buffer, pH 4.8	53.9	61.3	75.1	82.9
PBS pH 7.2	58.2	71.9		84.4

[00385] As can be seen, there is a weak pH dependence. Notably, the T_m2 and T_m3 domain (presumably C_H2, F_{ab}) overlap in PBS, but not in other buffers.

[00386] **Example 27.** Affinity of Dual PDGF/VEGF Antagonist Proteins and Conjugates to Targets

[00387] Surface plasmon resonance (SPR) was used to characterize the binding kinetics of recombinant human PDGF-BB (PeproTech, 100-14B) to TAF-WT, TAF-347, TAF-443, TAF443-6A250K, and TAF443-3A250K dual PDGF/VEGF antagonist variants. Initially, an anti-human IgG antibody (GE Healthcare, BR-1008-39) was covalently amine coupled onto all four flow cells of a CM5 carboxymethylated dextran coated sensorchip to a density of about 10,000 resonance units (RUs) following the manufacturer's protocol. Each PDGF/VEGF variant was captured to a level of approximately 150 RUs. The running and sample buffer for the PDGF analysis was HBS-EP+ 300mM NaCl (10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) pH 7.4, 300 mM NaCl, 3mM ethylenediaminetetraacetic acid (EDTA), 0.05% (v/v) Tween-20). A 2-fold serial dilution series of PDGF-BB ranging in concentration from 1nM to 0.125nM was injected at a flow rate of 100 µL/minute for a 110 second association with dissociations that varied from 300 to 2700 seconds. The surface was then regenerated with a 30 second pulse of 3M MgCl₂, a 30 second pulse of an ionic regeneration buffer (0.46M KSCN, 1.83 M MgCl₂, 0.92 M urea, and 1.83 M guanidine-HCl pH7.4, Andersson et al., Analytical Chemistry, 1999) and then equilibrated with a 30 second pulse of HBS-EP+ 300mM NaCl running buffer.

[00388] Similarly, SPR was used to determine the binding affinities of recombinant human VEGF121 (PeproTech, 100-20A) against the TAF-WT, TAF-347, and TAF-443 dual

PDGF/VEGF antagonist variants. The running and sample buffer for the VEGF analysis was HBS-EP+ with a final concentration of 150mM NaCl. A 2-fold dilution series of VEGF121 ranging in concentration from 100nM to 12.5nM was injected at a flow rate of 50 uL/minute for about a 50 second association with dissociations that varied from 300 to 3600 seconds. The surface was then regenerated with a 30 second pulse of 3M MgCl₂, a 30 second pulse of ionic regeneration buffer (0.46M KSCN, 1.83 M MgCl₂, 0.92 M urea, and 1.83 M guanidine-HCl pH7.4, Andersson et al., Analytical Chemistry,1999), and then equilibrated with a 30 second pulse of HBS-EP+ 150mM NaCl running buffer.

[00389] All SPR assays were performed at 25°C with a data collection rate of 1Hz using a Biacore T200 instrument (GE Healthcare). The resulting PDGF and VEGF sensorgrams were double referenced using both a control surface and buffer injections. The rate constants were determined by fitting the data to a 1:1 Langmuir model with Biacore T200 evaluation software v2.0 and the equation $K_D = k_d/k_a$.

Biacore Affinity to PDGF-BB							
Analyte	Ligand	k_a (1/Ms)	k_d (1/s)	$t_{1/2}$ (min)	Rmax (RU)	Chi2/Rmax	KD (pM)*
PDGF-A*	TAF-WT	7.97E+07	8.01E-05	144.28	15.16	0.16%	1.01
PDGF-B*	TAF-WT	8.01E+07	9.19E-05	125.68	15.15	0.20%	1.15
PDGF-C*	TAF-WT	8.65E+07	1.03E-04	111.94	15.32	0.15%	1.19
						AVG +/- STDEV	1.1 ± 0.1
PDGF-A*	TAF-347C	4.15E+07	8.41E-05	137.33	13.99	0.86%	2.03
PDGF-B*	TAF-347C	5.82E+07	7.87E-05	146.79	13.08	1.05%	1.35
						AVG +/- STDEV	1.7±0.3
PDGF-A*	TAF-443C	3.22E+07	4.96E-05	233.15	13.15	0.81%	1.54
PDGF-B*	TAF-443C	5.62E+07	8.76E-05	131.89	12.19	0.96%	1.56
						AVG +/- STDEV	1.55±0.01
PDGF-A*	R3643-6A (TAF443-6A250K)	7.60E+07	9.46E-05	122.09	8.11	0.41%	1.25
PDGF-B*	R3643-6A (TAF443-	5.62E+07	5.85E-05	197.55	8.33	0.30%	1.04

Biacore Affinity to PDGF-BB							
Analyte	Ligand	ka (1/Ms)	kd(1/s)	t1/2 (min)	Rmax (RU)	Chi2/Rmax	KD (pM)*
	6A250K)						
PDGF-C*	R3643-6A (TAF443-6A250K)	3.48E+07	5.13E-05	225.41	8.45	0.80%	1.47
						AVG +/- STDEV	1.3 ± 0.2
PDGF-A*	R3644-3A (TAF443-3A250K)	5.76E+07	5.92E-05	195.04	8.15	0.31%	1.03
PDGF-B*	R3644-3A (TAF443-3A250K)	2.86E+07	4.96E-05	233.05	8.52	0.60%	1.73
PDGF-C*	R3644-3A (TAF443-3A250K)	4.71E+07	7.52E-05	153.56	8.15	0.49%	1.60
						AVG +/- STDEV	1.5 ± 0.4

*A, B and C refer to separate runs or measurements concerning the same analyte PDGF-BB

Biacore Affinity to VEGF121							
Analyte*	Ligand	ka(1/Ms)	kd (1/s)	T1/2 (min)	Rmax (RU)	Chi2/Rmax	KD (pM)
VEGF-A	TAF-WT	1.14E+05	2.01E-05	573.89	29.4	0.17%	176.60
VEGF-B	TAF-WT	6.85E+05	5.89E-05	196.00	13.90	0.16%	86.03
VEGF-C	TAF-WT	1.40E+05	2.96E-05	390.68	27.36	0.17%	212.00
VEGF-D	TAF-WT	1.55E+05	2.69E-05	429.78	24.92	0.23%	173.00
AVG/STD DEV							161.96±53.46
VEGF-A	TAF347	1.37E+05	3.83E-05	301.55	26.57	0.87%	280.30
VEGF-B	TAF347	1.42E+05	2.59E-05	446.21	24.56	0.35%	182.40
AVG							231.35±48.95

VEGF-A	TAF443	1.46E+05	3.20E-05	361.01	25.87	0.75%	219.30
VEGF-B	TAF443	1.35E+05	3.10E-05	372.18	25.47	0,31%	229.70
AVG 224.5±5.2							
*A, B, C, D, refer to different runs of the same analyte (VEGF121).							

[00390] Example 28. Decapping of TAF443 Prior to Maleimide Conjugation

[00391] The TAF443 Cysteine residue is typically “capped” or oxidized by chemicals in the cell culture media and is not available for conjugation. In this regard, purified TAF443 (OG1321) is subjected to a decapping (i.e. reducing) procedure to remove the cap and enable the free (i.e. those not involved in Cys-Cys disulfide bonds) cysteine residue to be conjugated to the maleimide functionality of a polymer. Decapping is done by mixing TAF protein with a 30x molar excess for 1 hour at 25°C of the reducing agent TCEP (3,3',3''-Phosphanetriyltripropanoic acid). The reduction reaction with TCEP is monitored by SDS-PAGE. Undenatured TAF runs as a single band at about 250 kDa (about 40 kDa of this weight is carbohydrate). When fully denatured the single 250 kDa band is converted into bands corresponding to the light and heavy chains. Following denaturation, the TAF protein was washed by UFdF using a Pellion XL Ultrafiltration Cassette with 20mM Tris pH7.5, 150mM NaCl, 0.5mM TCEP buffer to remove the cap. The TCEP reagent was then removed in the same UFdF setup with 20mM Tris pH7.5, 150mM NaCl. Reduced TAF was allowed to refold using air (ambient Oxygen) which was again followed by SDS-PAGE as an assay.

[00392] A detailed procedure for decapping is as follows:

500mg of OG1321 was thawed from -80°C at 4°C overnight, and warmed up in the 25°C water bath before mixing with TCEP at 30x molar excess. The reaction was incubated in the 25°C water bath for 1 hour. Samples were taken out at 15, 30, and 60 minutes to run on SDS-PAGE in order to evaluate the reduction completeness. A UFdF cassette with 10kD MWCO was used to carry out buffer exchange. First buffer exchange step was done with 20mM Tris pH7.5, 100mM NaCl, 0.5mM TCEP for ~100x to thoroughly remove the cap. A second buffer exchange step was done with 20mM Tris pH7.5, 100mM NaCl for ~1000x for TCEP remove prior to air refolding. The final TCEP concentration in the sample was ~0.5µM. Samples were taken out from both buffer exchange steps for both SDS-PAGE and SEC analyses to evaluate the protein reoxidation status and protein aggregation. After the second buffer exchange step, the OG1321

was concentrated to ~2mg/ml, 0.22µm filtered, and allowed to re-oxidize with air at 4°C.

Samples were taken out for SDS-PAGE and SEC analyses at different time points to evaluate the re-oxidation status. Re-oxidized OG1321 was 0.22µm filtered and further concentrated.

Continued to concentrate the sample with VIVACELL 100 30k MWCO spin concentrators to 4-6mg/ml and sterile filtered the sample. Quantified by OD280.

[00393] Example 29. Conjugation of TAF443 to Biopolymer.

[00394] TAF443 which is also called OG1321 was conjugated to polymer OG1802 (see below) after decapping using a 15x excess of polymer in pH 7.5 Tris buffer to produce OG1448, shown in FIG. 26, showing the chemical structure of OG1448 which is TAF443 conjugated to biopolymer OG1802. TAF443 is on the extreme right hand of the molecule shown in the figure, conjugated via the cysteine 443 residue to the 5 member ring. Conjugation was monitored by SDS-PAGE and driven to near completion. Conjugate was purified via anion exchange chromatography and buffer exchanged into the formulation buffer by UF/DF.

[00395] In general, there are three steps involved in the synthesis of OG1448 from components OG1802 and OG1321. Step A: OG1321 much be reduced or decapped to free up the sulfhydryl groups at cysteine position 443. Although the cysteine position at 443 of the heavy chain of TAF is not believed to be involved in cysteine-cysteine disulfide pairing, this cysteine is typically capped by components of the media and absent reduction is not available to react with maleimide. Step B: reduced TAF is then conjugated to OG1802. Step C: conjugated TAF (OG1448) is then separated from unconjugated TAF and polymer via chromatography.

These three general steps are broken down into seven smaller steps in the following table:

General Step	Description	IPC Assays	Target Range
A	Step 1: To reduce OG1321 using <i>tris</i> (2-carboxyethyl) phosphine (TCEP). 30x molar of TCEP at 25°C for 1 hour.	Non-reducing SDS-PAGE	>95% reduction
	Step 2: To remove TCEP reducing agent and cap groups using UF/DF. First, wash with 0.5 mM TCEP in Tris pH 7.5 for a 100-fold volume exchange factor; followed by a 2 nd wash with Tris buffer pH 7.5 for 1,000 fold volume exchange factor to remove the TCEP, targeting final TCEP level lower than 0.5 µM.	Non-reducing SDS-PAGE.	Band shift upon removal of the reducing agent.
	Step 3: To refold protein to ensure the native disulfide pairs are fully oxidized	Non-reducing SDS-PAGE	Band upshift upon oxidation of

General Step	Description	IPC Assays	Target Range
	while the internal cysteine residues remain reduced.		the native disulfide pairs.
		UV/Vis for protein	Final protein concentration at 6-8 mg/ml.
B	Step 4: To conjugate OG1321 protein to OG1802 biopolymer. Conjugate by mixing the oxidized OG1321 with OG1802. The process requires 15x molar of biopolymer to decapped protein and constant mixing. Low temperature at 2-8°C for 20 hours and overlay the reaction with nitrogen gas to minimize oxidation.	Non-reducing SDS-PAGE	<20% full length band remains.
		Analytical AE-HPLC	<20% unreacted protein at OD 280 nm.
C	Step 5: To separate OG1448 conjugate from the unreacted OG1321 protein, unreacted OG1802 biopolymer, protein aggregates and other process contaminants. Purify OG1448 using MacroCap Q (AEX). The chromatography is performed at pH 7.5 in 20mM Tris buffer and eluted using a NaCl gradient. A pool is made by combining fractions.	Analytical AE-HPLC for unreacted polymer and unreacted protein	<5% unreacted protein at OD 180 nm: <15% unreacted polymer at OD 220 nm.
		Non-reducing SDS PAGE	<5% unreacted protein
	Step 6: To concentrate the OG1448 and to exchange the chromatography buffers for the formulation buffers. The pooled fractions from the previous step are diafiltered and then concentrated by UF/DF to achieve the target OG1448.	UV/Vis for protein concentration	OG1448 at 50 mg/ml
	Step 7: To remove bioburden from the final product and to dispense into storage containers. The UF/DF final pool is 0.2 µm filtered into sterile containers, and pH and conductivity of the final filtrate is established. The drug substance is stored at -20°C.	UV/Vis for protein concentration	OG1448 at 50 mg/ml
		pH, Conductivity	pH 7.2-7.5

[00396] Example 30. Purification of OG1448 via Anion Exchange (Macrocap Q)

After conjugation of TAF443 to OG1802 as described above, OG1448 was purified as follows: After conjugation of TAF443 to OG1802 as described above, OG1448 was purified as follows: 2 x 400ml of Macrocap Q columns were packed according to ~3 : 1 ratio of resin : conjugate. The columns were flushed with 5M of NaCl and equilibrated with 20mM Tris pH7.5,

20mM NaCl (equilibration buffer) by syphoning. The conjugation reaction mixture was diluted with 20mM Tris pH7.5 and loaded on the columns. The columns were then chased with the equilibration buffer, and washed with 20mM Tris pH7.5, 50mM NaCl (Wash 1) and then 20mM Tris pH7.5, 100mM NaCl (Wash 2). Elution was done with 20mM Tris pH7.5, with step gradient of 150mM, 200mM, 220mM, 250mM, 300mM, and 500mM NaCl. All the column flow-through, washes, and elution were collected in clean bottles for SDS-PAGE and AEX analyses. Elution fractions containing the conjugate were pooled and concentrated using Pellicon XL TFF cassette with 30kD MWCO and PES membrane. The concentrated pool was then buffer exchanged against 1xPBS pH7.4 buffer for ~100x using the same TFF cassette and transferred to the VIVACELL 100 spin concentrators to further concentrate until the targeted concentration (~30mg/ml) was achieved. The final conjugate was filtered through a 0.2µm PES syringe filter for lot release.

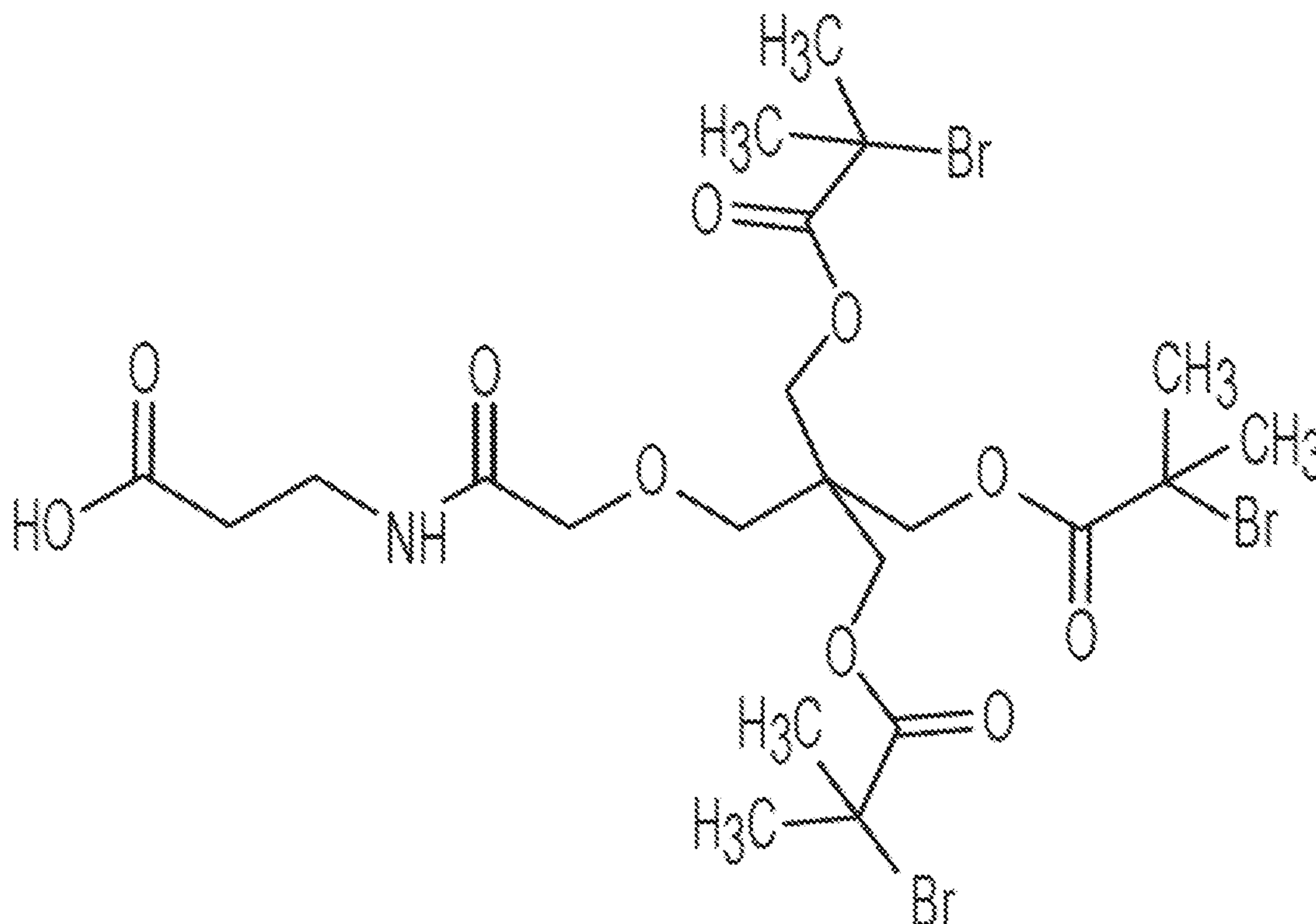
Example 31. Reduction of Bacterial Endotoxin

To reduce levels of endotoxin in the final protein (OG1321) or conjugate (OG1448), purification procedures may be employed for either protein or conjugate which utilize cation exchanges in place of anion exchanges. For example, in the above procedure for purifying OG1321, the anion exchange TMAE resin is employed. In place of TMAE resin, the cation exchange resin CEX may be used. However, in order to use CEX residue the pH of the solution containing the protein in question must be reduced to below the protein's pI. For OG1321, the pH of the protein solution after the protein A column, is reduced to pH 3.5. The OG1321 is bound to the Poros XS column at pH5. Then, Porox XS (CEX) can be used to bind and elute the OG1321.

[00397] Example 32. Route 1 Synthesis of OG1802.

A first route for the synthesis of OG1802 is as follows. First, TFA/amine salt initiator (Compound L) having the structure shown in FIG. 27 was synthesized as follows.

[00398] First, Compound K, having the structure shown in FIG. 28 was synthesized as follows. Into a 200 mL round bottom flask under nitrogen was placed Compound J (OG1563) (1.9 g, 2.67 mmol, 3.3 equiv)



Compound J.

and Compound E (0.525 g, 0.81 mmol, 1.0 equiv) (see FIG. 38) followed by dimethylformamide (10 mL) then diisopropylethylamine (2.5 mL, 14.6 mmol, 18 equiv). The flask was cooled to 0°C using an ice bath. To this was added propylphosphonic anhydride solution (50 wt. % in ethyl acetate, 2.5 mL, 4.04 mmol, 5 equiv) over ~6 minutes.

[00399] The reaction was warmed to room temperature and stirred for 15 minutes. The reaction was quenched by adding water (20 mL), saturated aqueous sodium bicarbonate (20 mL) and ethyl acetate (100 mL). The organic layer was separated and the aqueous layer extracted with ethyl acetate (75 mL). The combined organic layers were washed with saturated aqueous sodium bicarbonate (30 mL), 0.5 M aqueous citric acid (40 mL), water (25 mL), and saturated aqueous sodium chloride (40 mL), then dried (sodium sulfate), filtered and concentrated under vacuum. The residue which was used without further purification resulted in 2.0 g (0.80 mmol, 99%) of Compound K.

¹H NMR (400 MHz DMSO-d₆): = 1.36 (s, 9H, OCCH₃), 1.90 (s, 54H, CC(CH₃)₂Br), 2.31 (t, J = 7.2 Hz, 6H, CCH₂CH₂NH), 2.98 (d, J = 5.6 Hz, 6H, CCH₂NH), 3.04 (q, J = 6.0 Hz, 2H, OCH₂CH₂NH), 3.18 (s, 2H, OCH₂C), 3.3-3.37 (m, 8H, CH₂), 3.47-3.55 (m, 12H, CH₂), 3.58 (s, 6H, OCH₂C), 3.87 (s, 6H, O=CCH₂O), 4.27 (s, 18H, CCH₂OC=O), 6.74 (br t, 1H, CH₂NHC=O), 7.69 (t, J = 6.8 Hz, 3H, CH₂NHC=O), 7.84 (t, J = 6.0 Hz, 3H, CH₂NHC=O).
 LC-MS (ES, m/z): [(M+2H-boc)/2]⁺ Calcd for (C₈₄H₁₃₆Br₉N₇O₃₃+2H-Boc)/2 = 1196.6; Found 1196.6.

[00400] Next Compound L (FIG. 27) was synthesized as follows: into a 100 mL round bottom under nitrogen was added Compound K (2.0 g, 0.8 mmol), dichloromethane (10 mL) followed by trifluoroacetic acid (5 mL). The reaction was stirred at room temperature for 30 minutes. The reaction was concentrated under a vacuum. The reaction was diluted using dichloromethane (10 mL) and concentrated under a vacuum. The residue was dissolved using acetonitrile (10 mL), filtered through a syringe filter (Acrodisc CR25, PN 4225T) and loaded onto a preparatory HPLC column and eluted with 60% acetonitrile in water (with 0.1% trifluoroacetic acid) up to 98% acetonitrile (with 0.1% trifluoroacetic acid). The tubes containing product were pooled, concentrated under vacuum, frozen and placed on a lyophilizer. This resulted in 990 mgs (0.4 mmol, 50% over 2 steps) Compound L as a white powder.

¹H NMR (400 MHz DMSO-d₆): = 1.90 (s, 54H, CC(CH₃)₂Br), 2.31 (t, J = 7.2 Hz, 6H, CCH₂CH₂NH), 2.97-3.0 (m, 8H, CCH₂NH and OCH₂CH₂NH), 3.17 (s, 2H, OCH₂C), 3.3 (q, 6H, CH₂CH₂NHC=O), 3.4-3.59 (m, 20H, CH₂), 3.87 (s, 6H, O=CCH₂O), 4.27 (s, 18H, CCH₂OC=O), 7.69-7.84 (m, 9H, both CH₂NHC=O and NH₃⁺).

LC-MS (ES, m/z): [(M+2H)/2]⁺ Calcd for (C₈₄H₁₃₆Br₉N₇O₃₃+2H)/2 = 1196.6; Found 1197.4.

[00401] Next, compound L was used as an initiator to synthesize MPC polymer. Initiator is typically prepared as a stock solution in DMF of about 100 mg/mL. The initiator and the ligand (2,2'-bipyridyl) were introduced into a Schlenk tube. The resultant solution was cooled to -78°C using a dry ice/acetone mixture, and was degassed under vacuum for 10min. The tube was refilled under Argon and the catalyst (CuBr unless otherwise indicated), kept under Argon, was introduced into the Schlenk tube (the Molar ratio of atom bromine on the initiator/catalyst (CuBr)/ligand was kept at 1/1/2). The solution became dark brown immediately. The Schlenk tube was sealed and immediately purged by applying a short cycle vacuum/Argon. A solution of HEMA-PC was prepared by mixing a defined quantity of monomer, prepared in a glovebox kept

under nitrogen, with 200 proof degassed ethanol. The monomer solution was added drop wise into the Schlenk tube (via cannula) (and homogenized by light stirring). The temperature was maintained at -78°C. A thorough vacuum was applied to the reaction mixture for at least 10 to 15 min. until bubbling from the solution ceased. The tube was then refilled with Argon and warmed to room temperature. The solution was stirred, and as the polymerization proceeded, the solution became viscous. After 3 to 8 hours or just left overnight, the reaction was quenched by direct exposure to air in order to oxidize Cu (I) to Cu (II), the mixture became blue-green in color, and was passed through a silica column in order to remove the copper catalyst. The collected solution was concentrated by rotary evaporation and the resulting mixture was either precipitated with tetrahydrofuran or dialyzed against water followed by freeze drying to yield a free-flowing white powder. The table below sets forth polymer data for polymer employing compound L as an initiator.

Theor. MW (kDa)	Polymer ID No.	Initiator	Mn(kDa)	Mp(kDa)	PDI
500	130	L	490	530	1.1
750	150	L	645	750	1.1

[00402] Next, the maleimide Mal-PEG4-PFP ester was snapped on (as set forth in FIG. 29) to the 750 kDa polymer referred to above to provide OG1802. Into a 20 mL vial was placed Polymer R3707 (750 kDa polymer made using L as initiator, 515mg) and dissolved using ethanol (4.0mL) after stirring for 40 minutes. To this was added a 1% solution of 4-methylmorpholine in acetonitrile (22uL). In a separate vial was dissolved Mal-PEG4-PFP (1.97mg) in acetonitrile (1.0mL) and this solution was added to the polymer solution over ~2 minute at room temperature and the resulting solution was stirred for overnight. The reaction was diluted with 0.1% aqueous trifluoroacetic acid (2 mL) (pH ~5) followed by water (~12 mL), filtered through a syringe filter (Acrodisc Supor, PN 4612) and placed evenly into 3 Amicon centrifuge membrane dialysis tubes (30,000 mwco). The tubes were diluted and mixed with water (~5 mL each), placed into centrifuge (rpm 3200) for 25 minutes. The filtrate is removed for analysis while the retentate is diluted and mixed with water (~10 mL/tube). The centrifuge procedure repeated 5 more times, after which the retentate is removed and placed into a

vial. The Amicon membrane tubes were rinsed with water (2 x ~2 mL each tube) and this combined with the retentate. The retentate solution was filtered through a syringe filter (Acrodisc Supor, PN 4612), frozen and placed on a lyophilizer. This resulted in 485 mgs as a white powder.

[00403] Example 33. Biacore Binding Studies of TAF (OG1448 and OG1321)

[00404] The binding affinity of OG1448 (and OG1321) to its intended targets was evaluated via Biacore assay. Binding studies were performed at 25°C and 37°C using BioRad Proteon XPR36 and Biacore 2000 optical biosensors equipped with GLM (Proteon) and CM4 (Biacore) sensor chips and equilibrated with running buffer (10 mM HEPES, 150 mM NaCl, 0.005% Tween-20, 0.2 mg/ml BSA). OG1448, OG1321, bevacizumab, aflibercept and anti-PDGF were immobilized to the sensor surface via amine-coupling.

[00405] Binding of the coupled proteins to the ligands was determined by standard methodology. For example, rhVEGFA-165 was tested for binding in a three-fold dilution series starting at 52 nM. rhVEGFA-165 was injected across the surface for five minutes and then the dissociation phase was monitored for > 1000 seconds as the surfaces were washed with buffer. The rhVEGFA-165/OG1448 complex appeared quite stable, as indicated by the apparently flat response during the wash phase (>300 seconds) (data not shown). The dissociation phase for the 52 nM rhVEGFA-165 was monitored for more than 2 hours. No decrease in the binding response over time was observed.

[00406] Similarly, rhPDGF-BB was tested for binding in a three-fold series starting at 11.4 nM. For the rhPDGF-BB/OG1448 interactions, the rate constants were too fast to be reported with confidence because of mass transport effects. The following K_D constants were observed:

K_D (pM)					
	OG1321	OG1448	Bevacizumab	Aflibercept	Anti-PDGF
rhVEGFA-165 (25°C)	9.8±0.1	5.1±0.1	9.6±0.8	1.56±0.2	
rhPDGF-BB (25°C)	14±3	17±2			107±3

Example 34. TAF – a Competitive Inhibitor of rhVEGFA-165 Binding to rhVEGFR

As a measure of its potential potency on anti-VEGF activity, binding activity of TAF (OG1448 and OG1321) to VEGFA-165 was evaluated in a competitive binding assay where TAF, at different concentrations, was competing with immobilized rhVEGFR for binding of rhVEGF. rhVEGFA-165 bound by the immobilized VEGFR was determined by ELISA (data not shown).

Human VEGFR1/Fc was coated onto the bottom of 96-well ELISA plates at 1.0 µg/mL. Various concentrations of TAF (OG1448 and OG1321), ranging from 0.39 to 200 nM, were incubated with 0.1 nM of biotinylated VEGFA-165 for 30 min before adding to the ELISA plates. Biotinylated-rhVEGFA-165 bound to VEGFR1 was detected by streptavidin-HRP and followed by development with HRP substrates. Ranibizumab (Lucentis) and bevacizumab (Avastin) were similarly tested for competitive binding inhibition of VEGFA-165 to VEGFR1.

OG1321, OG1448, ranibizumab and bevacizumab showed similar IC₅₀s in inhibiting the binding of VEGF-165 to rhVEGFR suggesting similar potential potency in anti-VEGF activity. These results suggest that TAF (both OG1448 and OG1321) can be as potent as the approved agents ranibizumab and bevacizumab, hence, suitable for treating neovascular (i.e., wet) AMD.

IC ₅₀ (nM)				
	OG1321	OG1448	Ranibizumab	Bevacizumab
Competitive binding to rhVEGFA-165 (vs VEGFR)	12.5±1.2*	8.5±1.1*	12.5±1.2*	10.7±0.9*
* Mean and SD of at least three trials.				

[00407] Example 35. OG1448 - a Competitive Inhibitor of rhVEGFA-165 Binding to rhVEGFR in the Presence of rhPDGF-BB

[00408] To evaluate whether OG1448 can bind rhVEGFA-165 in the presence of rhPDGF-BB, i.e., whether rhPDGF-BB binding to the receptor decoy of TAF inhibits the ability of TAF

to bind to rhVEGFA-165, a similar binding study to Example 27 was conducted but in the presence of various concentrations of rhPDGF-BB.

[00409] Human VEGFR1/Fc was coated onto the bottom of 96 well ELISA plates at 1.0 $\mu\text{g/mL}$. Various concentrations of OG1448 were incubated with 0.1 nM of rhVEGFA-165 plus rhPDGF-BB at 0.4, 1.2 and 2.0 nM, respectively, for 30 minutes before adding to the ELISA plates. rhVEGFA-165 binding to rhVEGFR1 was detected by biotinylated anti-VEGFA antibody, 0.4 $\mu\text{g/mL}$, followed with streptavidin HRP and HRP substrate. OG1448 was found to have an IC₅₀ (nM) of 10.1. This is quite comparable to the IC₅₀ observed without rhPDGF-BB from example 28. The value for OG1321 was not determined in this assay but is expected to be similar to OG1448.

[00410] Example 36. TAF -a Competitive Inhibitor of rhPDGF-BB Binding to rhPDGFR

[00411] As a measure of its potential potency of anti-PDGF activity, the binding activity of TAF (OG1448 and OG1321) to rhPDGF-BB was evaluated in a competitive binding assay where TAF, at different concentrations, was competing with immobilized PDGFR for binding of rhPDGFBB. rhPDGF-BB bound to immobilized PDGFR was determined by ELISA assay.

[00412] Human PDGFR/Fc was coated onto the bottom of 96-well ELISA plates at 0.4 $\mu\text{g/mL}$. Various concentrations of OG1448 and OG1321, ranging from 1 pM to 20 nM, were incubated with 0.2 nM of rhPDGF-BB for 30 minutes before adding to the ELISA plates. rhPDGF-BB bound to rhPDGFR was detected by biotinylated anti-PDGFBB antibody, 0.4 $\mu\text{g/mL}$, followed with streptavidin-HRP and HRP substrate.

[00413] OG1448, OG1321 and a reference anti-PDGF antibody showed similar IC₅₀s in inhibiting rhPDGFBB binding to PDGFR, as shown in the following table, suggesting highly potent anti-PDGF activity.

IC ₅₀ (pM)			
Competitive Binding	OG1321	OG1448	Anti-PDGFBB
to rhPDGFBB (vs rhPDGFR)	46 $\pm$ 21*	54 $\pm$ 21	66
* Mean and SD of at least 3 trials.			

[00414] Example 37. OG1448 - a Competitive Inhibitor of rhPDGF-BB Binding to rhPDGFR in the Presence of rhVEGFA-165

[00415] To evaluate whether OG1448 can bind rhPDGF-BB in the presence of rhVEGFA-165, a similar competitive inhibition of binding study towards PDGF (as Example 29) was performed in the presence and absence of rhVEGFA-165.

[00416] Human PDGFRb/Fc was coated onto the bottom of 96-well ELISA plates at 0.4 $\mu\text{g/mL}$. Various concentrations of OG1448 were incubated with 0.2 nM of PDGFBb and with 0.2 nM of PDGFRb plus rhVEGFA-165 at 0.2 nM, 0.6 nM and 1.0 nM, respectively, for 30 minutes before adding to the ELISA plates. PDGF-BB bind to PDGFRb was detected by biotinylated anti-PEGFBB antibody, 0.4 $\mu\text{g/mL}$, followed by streptavidin HRP and HRP substrate. The IC₅₀ (pM) in the presence of rhVEGFA-165 (25) was comparable to the figure derived in Example 29. The figure for OG1321 in the presence of rhVEGFA-165 was not determined but is expected to be similar.

[00417] **Example 38.** Inhibition of VEGF-induced Proliferation of Primary Human Retinal Microvascular Endothelial Cells (HRMVEC)

[00418] Endothelial cell proliferation is a crucial step in angiogenesis and hence in the pathogenesis of neovascular AMD. The ability of OG1448 to antagonize the proliferating action of VEGF on primary human retinal microvascular endothelial cells can be a measure of its bioactivity in treating neovascular AMD.

[00419] HRMVECs were stimulated with 1.3 nM of rhVEGF165-A for 3 days in the presence of various concentrations of TAF (OG1448 and OG1321) and reference drugs. Cell proliferation was measured by WST-1 cell proliferation detection reagent. Results are shown in the table below:

IC ₅₀ (nM)					
	OG1321	OG1448	Ranibizumab	Bevacizumab	Aflibercept
Inhibition of VEGF induced proliferation of HRMVECs	0.43±0.05*	0.49±0.05*	0.98±1.21*	0.81±0.32*	0.55±0.08*
* Mean and SD of at least 3 trials.					

[00420] OG1448 and OG1321 demonstrated an IC₅₀ in this assay comparable to other approved anti-VEGF therapies. These data show that TAF (both OG1448 and OG1321) has at least comparable potency to inhibit VEGF-mediated retinal microvascular endothelial cell proliferation activity as ranibizumab, bevacizumab and aflibercept.

[00421] **Example 39.** Inhibition of PDGF-Induced Proliferation of Primary Human Brain Vascular Pericytes (HBVP)

[00422] Pericyte migration and proliferation are crucial events in angiogenesis and hence play important roles in the pathogenesis of neovascular AMD. The ability of TAF (OG1448 and OG1321) to antagonize the proliferating action of PDGF on human brain pericytes can be a measure of its effectiveness in treating neovascular AMD.

[00423] HBVPs were stimulated with 2.0 nM of PDGFBB for 3 days in the presence of various concentrations of TAF (OG1449 and OG1321) and a reference anti-PDGF-BB antibody (R&D Systems, Catalog # AB-220-NA). Cell proliferation was measured by WST-1 cell proliferation detection reagent.

IC ₅₀ (nM)			
	OG1321	OG1448	Anti-PDGF
Inhibition of PDGF induced proliferation of HPVPs	5.0±2.0*	2.9±1.4	5.4
* Mean and SD of at least 3 trials.			

From the various experiments above comparing OG1321 (TAF443) to OG1448 (TAF443 polymer conjugate), it can be seen that conjugation to the HEMA-PC biopolymer does not negatively impact protein activity.

[00424] OG1448 and OG1321 show a comparable IC₅₀ to the anti-PDGF antibody.

[00425] **Example 40.** Inhibition of Sprouting in Co-culture of Human Retinal Microvascular Endothelial Cells (HRMVEC) and Human Mesenchymal Pericytes (HMPs)

[00426] To mimic in vivo conditions where endothelial cells and pericytes coexist in blood vessels and proliferate and migrate together during angiogenesis, events crucial in neovascular

AMD, a three dimensional co-culture of HRMVECs and HMPs was established with the goal of evaluating the ability of OG1448 to inhibit angiogenesis in this complex model.

[00427] Vehicle, Avastin, an anti-PDGF-BB antibody (same as above), Avastin in combination with the anti-PDGF-BB antibody and OG1448 were added to the co-cultures on day 7. On day 14, immunohistochemical staining of CD31 (endothelial cells) and α SMA (pericytes) was used to quantify the lengths of sprouts emanating from established endothelial cell spheroids as compared across the experimental groups.

[00428] OG1448 was more effective in inhibiting endothelial/pericyte sprouting in HRMVEC-HMP co-culture than Avastin alone or anti-PDGF alone at two different concentrations. Moreover, OG1448 was also more effective in inhibiting sprouting than a combination of Avastin and the anti-PDGF-BB antibody. This demonstrates that OG1448 is synergistic relative to Avastin and an anti-PDGF-BB antibody. The results are shown in the table below and in FIG. 40.

Drug	Mean Total Sprout Length (pix)	S.D. (pix)	Relative Angiogenesis %	S.D. %
Vehicle	6999	1266	100	18
Avastin-5nM	4700	722	67	10
Avastin-25nM	3763	909	54	13
Anti-PDGF-5nM	4924	884	70	13
Anti-PDGF-25nM	4461	1051	64	15
Avastin + anti-PDGF-5nM	5197	948	74	14
Avastin + anti-PDGF-25nM	4287	822	61	12
OG1448-5nM	3584	478	51	7
OG1448-25nM	2933	360	42	5

[00429] **Example 41.** Efficacy of OG1448 on Inhibition of Laser-Induced Choroidal Neovascularization in Cynomolgus Monkeys

[00430] The in vivo efficacy of OG1448 was evaluated using the laser-induced choroidal neovascularization (CNV) model in cynomolgus monkeys, a well-recognized primate model of CNV. See, e.g., Nork TM, Dubielzig RR, Christian BJ, et al. 2011. Prevention of experimental choroidal neovascularization and resolution of active lesions by VEGF trap in nonhuman primates. *Arch Ophthalmol.* 129: 1042-1052; Lloyd RL, Harris J, Wadhwa S, Chambers W. 2008. Food and Drug Administration approval process for ophthalmic drugs in the U.S. *Curr Opin Ophthalmol.* 19:190-194, both of which are hereby incorporated by reference. In this model, laser lesions are placed in the chorioretinal complex in the macula of the monkey eye with evidence of Bruch's membrane breakage. Choroidal neovascularization is developed in two to three weeks. At various time points, fluorescein angiography is used to evaluate the clinically relevant lesions (Grade IV) which show fluorescein leakage beyond the primary lesion. This CNV model has been used extensively for the study of CNV lesions and used as a benchmark for all currently approved treatment for neovascular AMD. In this model, all approved anti-VEGF agents for neovascular AMD are effective in inhibiting the leakage from the clinically relevant Grade IV lesions. The study was conducted at Covance, Madison, WI.

[00431] In summary, a dose-related response to a single intravitreal injection of OG1448 at 0.5 or 2.4 mg/eye (calculated based on protein content) was observed in the animals in which CNV lesions were allowed to develop for 14 days before treatment and evaluated at subsequent time points using fluorescein angiography focusing on the clinically relevant Grade IV lesions on the retina/choroid. At 0.5 mg/eye, the beneficial effect on the Grade IV lesions was noticeable ($p=0.019$; generalized estimating equation [GEE] model; 0.5 mg treatment Group 7 vs PBS injected placebo Group 5). At 2.4 mg/eye OG1448, a dose (in molar equivalence) within the therapeutic dose of bevacizumab or aflibercept, was highly effective (75% reduction in Grade IV-CNV like lesions on Day 43 from Day 15 versus 27% reduction in the PBS-treated group) ($p=0.0007$; GEE model; 2.4 mg treatment Group 9 vs PBS injected placebo Group 5) in ameliorating the leakage of Grade IV- CNV lesions.

[00432] OG1448 shows effectiveness in inhibiting the leakage from the clinically relevant Grade IV lesion in this benchmark CNV model.

[00433] The groups and study design are shown in the following table. The study included groups for tolerability (Groups 1 thorough 4) however for purposes of this patent application

only the groups for pharmacological activity and a control group treated with phosphate buffered saline (PBS) injection are shown.

Group	No. of Females	Dose Route	Dose Level			Dose Concentration (mg/ml)
			mg/left eye/dose	mg/right eye/dose	mg/kg/dose	
5	6	Intravitreal ^a	0	0	NA	0
6	6	Intravitreal ^a	0.24	0.24	NA	5.9
7	6	Intravitreal ^b	0.51	0.51	NA	10.2
9	6	Intravitreal ^b	2.40	2.40	NA	26.6
NA = not applicable a = at days 1, 15, and 29 (a total of 3 doses); laser on day 8 of the dosing phase. b = once; laser treatment on 15 days prior to injection						

[00434] Two treatment regimens were evaluated. In the prevention regimen, OG1448 was given intravitreally three times bilaterally at 0.24 mg/eye/dose (dose content was based on protein content; Group 6) or PBS (Group 5) on days 1, 15 and 29 with laser treatment on day 8 of the dosing phase. Fluorescein angiograms on days 15, 21, 30, 37 and 43 of laser treatment (days 22, 28, 37, 44 and 50 of the dosing phase) were used for evaluation of the clinically relevant Grade IV lesions.

[00435] In the treatment regimen (Groups 7 [0.5 mg], 8 [0.5 mg] and 9 [2.4 mg], OG1448 was administered intravitreally to both eyes of 6 animals at doses of 0.5 mg (Groups 7 and 9) or 2.4 mg/eye (Group 9) 15 days after laser induction when CNV lesions were established. Fluorescein angiograms obtained at Days 15, 21, 30, 37 and 43 of laser treatment were used for evaluation of the clinically relevant Grade IV lesions.

[00436] Using generalized estimating equation (Gee) models (Halekoh, U & Yan J (2006) The R Package geepack for Generalized Estimating Equations Journal of Statistical Software 15, 2, pp1-11), a dose-related response to OG1448 was observed in the intervention regimen. At 0.5 mg/eye, the effect was notable as shown by the difference in the percent change in Grade IV lesions as compared to the vehicle control (0.5 mg treatment Group 7 vs PBS injected placebo Group 5; p=0.019, GEE). With 2.4 mg/eye OG1448 (a dose in molar equivalence within the therapeutic dose of bevacizumab or aflibercept) a 75% reduction in percent change in Grade IV lesions (2.4 mg treatment Group 9 vs PBS injected placebo Group 5; p=0.0007, GEE) was observed on day 43 as compared to a 27% reduction in CNV in the PBS control group. The data from the various experiments in the monkey CNV model are shown in FIG. 41.

[00437] OG1448 shows dose dependent effectiveness in inhibiting the leakage from the clinically relevant Grade IV lesion in this CNV model. These results are consistent with the studies described above showing activity of OG1448 against VEGF-mediated angiogenic activities.

[00438] **Example 42.** Tissue Distribution and Pharmacokinetics

[00439] A tissue distribution and pharmacokinetic study using ^{125}I -OG1448 was conducted using male New Zealand Red White F1 Cross pigmented rabbits. In summary, this study showed a vitreal half-life of 16.1 days for OG1448 in rabbits, approximately three times that reported for aflibercept (4.5 days) and 5 times that of ranibizumab (2.9 days) (Bakri SJ, Snyder MR, Reid JM et al. 2007. Pharmacokinetics of Intravitreal Ranibizumab [Lucentis].

Ophthalmology 114:2179-2182) with little plasma exposure (approximately 0.2% of that of vitreous exposure) and a plasma half-life of 6.5 days (aflibercept reported 6.5 days) (Struble C, Koehler-Stec E, Zimmer E, and Tu W. 2008. Pharmacokinetics and ocular tissue penetration of VEGF Trap after intravitreal injections in rabbits. EVER; Portorz, Slovenia).

[00440] The purpose of this study was to assess the ocular distribution and pharmacokinetics of non-radiolabeled test articles and radiolabeled test articles following an intravitreal or intravenous dose administration to male New Zealand Red White F1 rabbits. Treatment groups and the study design are shown in the table below:

Groups and Study Design (Covance study)					
Group	# of males	Dose Route	Test Article	Dose (mg)	Sample Collected
1	14	IVT	^{125}I -OG1448	0.25/eye (OU)	Blood, ocular tissues
2	2	IV	^{125}I -OG1448	0.25/animal	Blood
3	6	IVT	OG1448	0.25 (OD)	Blood, whole eyes for histology
4	6	IVT	OG1448	0.25 (OD)	Blood, vitreous humor
IVT: intravitreal; IV: intravenous; OU: Both eyes; OD: right eye					

[00441] PK parameters were obtained based on radioanalysis. Clearance profiles from vitreous, retina and choroid were similar to one another. This pattern is consistent with other established CNV treatments such as ranibizumab or aflibercept. Set forth in the table below are pharmacokinetic parameters in different ocular tissues after single bilateral intravitreal injection of 0.25 mg ¹²⁵I-OG1448.

Matrix	C _{MAX} (NG Eq./G)	T _{1/2} (day)	AUC _{0-∞} (Day*NG EQ./G)	Exposure as % of vitreous exposure
Plasma	494	6.48	3,790	0.189
Aqueous humor	5,250	11.6	68,800	3.423
Choroid-RPE	4,170	32.8	134,000	6.667
Iris-ciliary body	12,100	42.6	235,000	11.692
Retina	13,500	30.4	309,000	15.373
Vitreous humor	112,000	16.1	2,010,000	100.00

[00442] The ocular tissue half-life of various VEGF inhibitors is compared with OG1448 in the table below and in FIG. 42, which suggests that OG1448 can stay above a pharmaceutically active minimal inhibitory concentration of 0.1 µg/ml for greater than 90 days, as opposed to 30 days for Lucentis and 50 days for Eylea:

Ocular Tissue Elimination Half Life (Days)			
	Vitreous	Retina	Choroid
Pegaptinib ¹	3.5	--	--
Ranibizumab ¹	2.9	2.9	--
Aflibercept ¹	4.5	5.5	4.8
OG1448 ²	16.1	30.5	32.9

¹Based on publicly available data from 28-day rabbit studies: Drolet DW, Nelson J, Tucker CE, et al. 2000. Pharmacokinetics and safety of an anti-vascular endothelial growth factor Aptamer (NX 1828) following injection into the vitreous humor of rhesus monkeys. Pharm Res. 17:1503-1510; Gaudreault J, Fei D, Beyer JC et al. 2007. Pharmacokinetics and retinal distribution of ranibizumab, a humanized antibody fragment directed against VEGF-A, following intravitreal administration in rabbits. Retina 27:859-870; Bakri (2007), supra; Struble 2008, supra.

²Based on intravitreal injection of 250 µg in the rabbit eye.

[00443] The study showed a vitreal half-life of 16.1 days for OG1448 in rabbits, approximately three times the 4.5 day vitreal half-life reported for aflibercept and five times the vitreal half-life of ranibizumab (2.9 days) (Bakri 2007, supra) with low plasma exposure (approximately 0.2% of that of vitreous exposure); the plasma exposure is consistent to that of aflibercept (Sinapis CI, Routsias JG, Sinapis AI, et al. 2011. Pharmacokinetics of intravitreal bevacizumab [Avastin®] in rabbits. *Clinical Ophthalmology* 5:697-704). Similar to the reported data for ranibizumab and aflibercept, the vitreal, retinal and choroidal clearance profiles are similar to one another.

[00444] **Example 43. Toxicology**

[00445] Two pilot non-GLP single dose ocular and systemic tolerability studies on OG1448 were conducted at Covance: (i) a single dose 57-day intravitreal or intravenous tolerability study in pigmented rabbits and (ii) a single dose tolerability study after intravitreal (58-day study) or intravenous (28-day study) administration in cynomolgus monkeys.

[00446] In brief, single dose intravitreal injection of 0.25 mg OG1448/dose/eye in rabbits was initially well tolerated but was associated with persistent anterior (mild to moderate conjunctival hyperemia, mild to moderate aqueous flare and cells) and posterior segment (mild to severe white vitreous cells, mild to moderate vitreous haze and presence of vitreous floaters, and multifocal grey-white subretinal inflammatory foci) inflammation which developed approximately two weeks postdose (or later). This inflammatory response improved with immune-suppressive and anti-inflammatory therapy. The time of onset post-dose and response to treatment are consistent with an immune-mediated response typical for intraocularly administered humanized biopharmaceuticals in animals.

[00447] In contrast, a single intravitreal dose at 0.24 or 1.4 mg OG1448/dose/eye was well tolerated in cynomolgus monkeys with no adverse finding or evidence of immune reactions ophthalmologically, clinically, and histopathologically.

[00448] In the efficacy study (discussed above), intravitreal injections of 0.24 mg/eye/dose for three times at 14 days apart or a single injection of 0.5 mg/eye/dose were well tolerated with at least 40 days of follow-up as shown on ocular examinations. No immune-related reactions were noted in the eyes of treated animals.

[00449] These studies demonstrate that OG1448 is well tolerated when administered intravitreally or intravenously at the doses evaluated.

[00450] Example 44. Single-Dose Tolerance in Cynomolgus Monkeys

[00451] The purpose of this part of the study was to evaluate tolerability of OG1448 after intravitreal or intravenous administration in cynomolgus monkeys.

[00452] Ocular and systemic tolerability groups and study design are shown in the table below:

Group and Study Design						
Group	No. of females	Dose Route	Dose Level ^{a,b}			Dose Concentration (mg/ml)
			µg/left eye/dose	mg/right eye/dose	mg/Kg/dose	
1	3	IVT	0	0.236	NA	5.9
2	3	IVT	0	1.36	NA	27.2
3	2	IV	NA	NA	0.235	9.4
4	2	IV	NA	NA	1.41	9.4
IVT=intravitreal; IV=intravenous; NA=not applicable a. The right eye of animals in Groups 1 and 2 received the test article via intravitreal injection. Animals in Groups 3 and 4 received the test article via colus intravenous injection. b. The left eye of animals in Groups 1 and 2 animals received vehicle control only (phosphate buffered saline, pH 7.4).						

[00453] Ocular examinations by board certified veterinary ophthalmologists were performed across all four groups predose and (i) for intravitreal groups: on days 3, 8, 15, 29, 43 and 57, and (ii) for intravenous groups: on days 3, 8, 15, and 29. Animals were followed with clinical observations and clinical pathology on days 3, 8, 15, 29, 43 and 57 when applicable. Anatomic pathology was also performed – macroscopic observation during necropsy for all animals, and microscopic evaluations for ocular tissues for groups 1 and 2 (day 57) and for a standard list of systemic organs for groups 3 and 4 (day 29).

[00454] There were no adverse or toxicologically meaningful findings in any group. There were no findings in clinical observations and body weight in any group. There were no OG1448-related macroscopic or microscopic findings from anatomic pathology for any group (ocular tissues for intravitreally injected groups and standard list of organs/tissues for intravenously injected groups).

[00455] Ophthalmic findings for intravitreal administration groups were limited to injection-related events such as mild to moderate and transient presence of aqueous and/or vitreous cells and scars at the site of aqueous humor sampling.

[00456] **Example 45.** Synthesis of Polymer OG1786

[00457] OG1786 is the nine-arm initiator for polymer synthesis used as a precursor in the synthesis of OG1802. Each arm is terminated with a 2-bromoisobutyrate which is capable of initiating polymerization under ATRP. OG1786 is a salt of trifluoro acetic acid (TFA) as shown in FIG. 30. OG1786 is prepared as follows. First, OG1550 is reacted with TFA (trifluoro acetic acid) to produce OG1546 as depicted in FIG. 31.

[00458] In a 1L round bottom flask equipped with a magnetic stir bar and an addition funnel was added OG1550 (14.8 g), methyl tert-butyl ether (MTBE) (350 ml) and water (30 ml). The mixture was stirred to dissolve the OG1550, then cooled in an ice bath. To this mixture was added a solution of trifluoroacetic acid (4.9 ml) in water (90 ml) dropwise over 90 minutes. After addition is complete the mixture was stirred an additional 15 minutes then removed from the ice bath and allowed to warm to room temperature. The mixture was stirred (after removal from the ice bath) for a further 4-5 hours, until tlc showed ~5% starting material remaining, and the pH of the aqueous was between 3 and 4 (pH paper).

[00459] The mixture was partitioned. The MTBE layer was washed with water (30 ml). Combine aqueous layers then the aqueous extracted with MTBE (150 ml). This second MTBE phase was washed with water (30 ml). The combined aqueous layers were washed with a third portion of MTBE (100 ml). The third MBTE phase was washed with water (25 ml). The aqueous layers were again combined (~250 ml, pH ~4, by pH paper).

[00460] The product was collected by lyophilization. 11.5 g white solid was obtained. This material is extremely hygroscopic, so best handled under nitrogen. The product was confirmed by LCMS.

[00461] The prepared OG1546 was then reacted with OG1563 to yield OG1784 (as depicted in FIG. 32).

[00462] In a 250 ml flask under nitrogen equipped with a stir bar was added OG1546 (hygroscopic, 9.0 g), followed by N,N-dimethylformamide (110 ml). The mixture was stirred at room temperature until all OG1546 dissolved (about 15 minutes), then OG1563 (29.9 g) was added, and the mixture stirred a further 3 minutes until the OG1563 had also been dissolved. The resulting solution was cooled in an ice bath, and N,N-diisopropylethylamine (37.6 ml) was added over 3 minutes, followed by propylphosphonic anhydride (T3P), 50% in ethyl acetate (34.5 ml) dropwise over 5 minutes (T3P addition is exothermic). After T3P addition was

complete, the flask was removed from the cooling bath and allowed to reach room temperature. Samples were then taken at 5 minute intervals for LCMS analysis. The reaction showed very light yellow/tan color.

[00463] After 20 minutes the reaction was cooled again in an ice bath and 5 ml water added. The mixture was then removed from the cooling bath and a further 50 ml water portion added, followed by 50 ml 0.5 M citric acid then isopropylacetate (300 ml). The mixture was partitioned. The aqueous phase (~300 ml) was extracted with additional isopropyl acetate (150 ml). The aqueous phase was AQ1 for HPLC test. The combined organics were washed with aqueous citric acid (115 ml, 65 mM, which was the mixture of 15 ml of 0.5 M citric acid plus 100 ml water), and the aqueous phase was AQ2 (pH~3). The organic phase was washed with water/saturated sodium chloride (100 ml/25 ml), and the aqueous phase was AQ3 (pH~3). The organic phase was finally washed with saturated sodium chloride (100 ml), and the aqueous phase was AQ4. None of the AQ fractions contained any significant product (data not provided). The organic phase confirmed the product via LCMS. The product was dried over sodium sulfate (80 g), filtered and rinsed with isopropyl acetate (75 ml), and concentrated on a rotary evaporator to a tan oil (33.2 g). The crude was stored overnight under nitrogen.

[00464] The next day the crude was allowed to come to room temperature, then dissolved in acetonitrile/water (46 ml/12 ml) and filtered using an HPLC filter disk (Cole-Parmer PTFE 0.2 μ m, product number 02915-20). The filtrate was split into three equal portions and purified in three runs.

[00465] Loaded onto a RediSep Rf Gold C18 column (275 g, SN 69-2203-339, Lot# 24126-611Y) equilibrated with 50% acetonitrile/water. The material was eluted at 100 ml/min using the following gradient (solvent A: water, solvent B: acetonitrile). All the relevant fractions were checked by HPLC. The fractions adjudged to be pure enough were pooled (from all three runs) and concentrated (bath temperature kept at about 20°C) on rotovap, then partitioned between dichloromethane (100 ml) and water (5 ml)/saturated sodium chloride (25 ml). The aqueous was extracted twice more with dichloromethane (2 x 30 ml). The combined organics were dried over sodium sulfate (35 g), filtered, rinsed with DCM (30 ml), and concentrated. The product and purity were confirmed by LCMS methods.

OG1784 lot	R5172	R5228
OG1546 used	5.3 g	9.0 g

OG1563 used	17.6 g	29.9 g
Isolated yield	53%	58%
Purity (a/a 210 nm)	99.3%	100.0%

[00466] Next OG1405 was prepared from OG1784 as depicted in FIG. 33. In a 500 ml round bottom flask equipped with a magnetic stir bar was added OG1784 (20.9 g), followed by dichloromethane (50 ml) then trifluoroacetic acid (20 ml). The mixture was stirred at room temperature and HPLC analysis showed complete deprotection in 23 minutes. The mixture was concentrated on a rotary evaporator, redissolved in dichloromethane (25 ml) and re-concentrated, then redissolved in acetonitrile (25 ml) and re-concentrated. The product was confirmed by LCMS. The material from above (OG1405, 34.5 g, assume 21.0 g as quantitative yield) was used as a crude oil in the next step. No purification is needed.

Next, OG1405 was reacted with OG1402 to prepare OG1785 as set forth in FIG. 34. In a 500 ml flask under nitrogen equipped with a stir bar was placed OG1402 (5.5 g), followed by acetonitrile (70 ml), then N,N-diisopropylethylamine (26.3 ml) and T3P solution (see above) (7.9 ml). The solution was stirred at room temperature for 30 minutes, then cooled in an ice water bath and a solution of OG1405 (crude oil from above, 34.5 g) in acetonitrile (70 ml) added. The mixture was warmed to room temperature. After 20 minutes the reaction was cooled in an ice water bath and quenched with water (5 ml). The mixture was then concentrated under vacuum using a rotary evaporator to half volume. Samples were taken for LCMS.

[00467] More water (50 ml), followed by 0.5 M citric acid (75 ml) and isopropyl acetate (175 ml) was added. The mixture was partitioned in 5 minutes. The aqueous was extracted with additional isopropyl acetate (50mL). The combined organics were washed with aqueous citric acid (0.13 M, 30 ml, consist of 10 ml of 0.5 M citric acid and 20 ml water). The organics were then washed with the mixture of saturated sodium chloride (25 ml) and water (25 ml), then finally washed with the saturated sodium chloride (25 ml). They were then dried over sodium sulfate (124 g), filtered and rinsed with isopropyl acetate (30 ml) and concentrated under rotary evaporator to a tan oil (27.3 g). Samples were taken for LCMS analysis.

[00468] The oil was dissolved in acetonitrile/water (3:1, 15 ml/5ml), filtered through an HPLC filter disk (Cole-Parmer PTFE membrane 0.2 μ m, product number 02915-20) and split into three equal portions, each of which were individually purified as follows.

[00469] Portions were loaded onto Redi-Sep Gold C18 column (275 g, SN – 69-2203-339, Lot 241234-611W) equilibrated at 50% solvent B (acetonitrile)/50% solvent A (water). The material was then purified by reverse phase HPLC with a solvent A: water/solvent B: acetonitrile gradient. Appropriate fractions were pooled and partitioned between dichloromethane (150 ml) and water (5 ml)/saturated sodium chloride (25 ml). The aqueous was extracted twice with dichloromethane (2 x 50 ml). Combined organics were dried over sodium sulfate (60g), filtered and rinsed with dichloromethane (40 ml) and concentrated. Structure and purity were confirmed by various analytics including LCMS: OG1785 was isolated as a foamy solid (R5329, 19.0 g, 83% yield, 95.1% purity (a/a 210 nm), stored under nitrogen at 4°C.

[00470] Next, the tert-butyloxycarbonyl protecting group on OG1785 was removed using trifluoroacetic acid (TFA) to produce OG1786 as depicted in FIG. 35.

[00471] **Example 46.** Synthesis of Polymer 1801

[00472] Compound OG1802 is conjugated to a sulfhydryl group of TAF443 to produce OG1448. Polymer OG1801 is made first from the initiator OG1786. OG1801 has an amine functionality, which is more stable (than maleimide) during polymer synthesis. To synthesize polymer OG1801, a modified version of ATRP is used wherein the copper species (Cu(I)) is generated in situ by adding metallic copper to Cu (II). Starting materials and reagents needed in the reaction are calculated based on batch input of the monomer (HEMA-PC) OG47, as well as the targeted molecular weight (MW).

[00473] Weighed 50 g monomer OG47 in glove box and added 200 mL of degassed EtOH to dissolve the monomer at room temperature; sampled for monomer concentration test. Weighed Cu (II), Bpy, Cu(0) in a 500 mL flask; purged with Argon, while adding monomer solution to the flask; sealed the flask with stopper and vacuumed for 25 min until no bubbles. The reaction changed color gradually from light green to dark green, then to light brown; weighed ~200 mg of initiator OG1786 in glove box, and dissolved in ~2000 uL of DMF under room temperature to make 100 mg/mL stock solution; Sampled for initiator concentration and purity test; Added the initiator solution to the flask under Argon. The reaction solution became dark brown and started thickening over time; Sealed the system and let the reaction occur over 2 days.

[00474] OG1801 was then prepared for addition of the maleimide and catalyst (copper) was removed as follows: A prepacked RediSep® Rf normal phase silica column is used to remove the catalyst. The size of the column is chosen based on the copper amount in the reaction

mixture. For instance, a 330 g column (Cat. # 69-2203-330, Column size 330g, CV=443 mL) was used for a 50 g batch of OG1801. Teflon tubing is used for all the connection as EtOH is the elute solvent.

[00475] After copper removal, transferred all the fractions to a round bottom flask in batches, and evaporated the EtOH by rotary evaporator at 45-50 °C at reduced pressure to dryness. In this step, EtOH volume collected from condensation was monitored to make sure EtOH removal was > 90%. The polymer was dissolved in 250 mL of WFI and filtered using a 0.2 um filter. It resulted in a clear to light yellow polymer solution at ~150 mg/mL. The solution could be stored at 2-8 °C up to 3 month before use.

[00476] **Example 47.** Synthesis of Polymer OG1802

[00477] Starting materials and reagents needed in the reaction is calculated based on batch input of OG1801. The linker is 3-maleimidopropionic acid, NHS ester. Added 30 ml of 0.5 M sodium phosphate (in WFI, pH8) to 50 g polymer solution (~150 mg/mL). Let stir for 1 min; pH was 8.0 by pH paper. Weighed 204.8 mg of linker and dissolved in DMF 4.1 mL to make 50 mg/mL stock sln; Added linker solution dropwise 815 uL per minute to the polymer sln with strong stirring. Took 5 min to added 4095 uL of linker solution. Reacted at room temperature for 30 min. Quenched reaction with 20 mL of 5% acetic acid to achieve a final pH of 5. Filtered the solution using 1L vacuum filter (0.2 um).

[00478] OG1802 is then purified as follows: Milipore cross flow cassettes was used for polymer purification in aqueous system. Started with concentrating the polymer solution to 250 mL (~ 200 mg/mL). Added the fresh WFI from reservoir, and adjusted the flow rate of the fresh WFI feed to the same as the permeate (~ 2mL/min). The UF/DF was set up at 2-8 °C overnight. Typically 2.5 L of WFI was used (10x volume ratio to the polymer solution). A sample of retentate was collected for purity test. The targeted purity was > 98%. Filtered the polymer solution by 0.2 µM 1L filter bottle. The polymer solution could be stored at 2-8 °C for up to 3 month before conjugation.

[00479] **Example 48.** Formulations of OG1448; Injectability

27.2 mg/ml and 44.5 mg/ml solutions of OG1448 were prepared using 1.7 mM KH₂PO₄; 5 mM Na₂HPO₄; 150 mM NaCl in sterile water for injection. The OG1448 conjugate was concentrated by a Millipore Pellicon XL TFF cartridge (catalog# PXB030A50, EMD Millipore), 30kD MWCO or VIVACELL 100 spin concentrator (catalog# VC1022, Sartorius), 30kD MWCO,

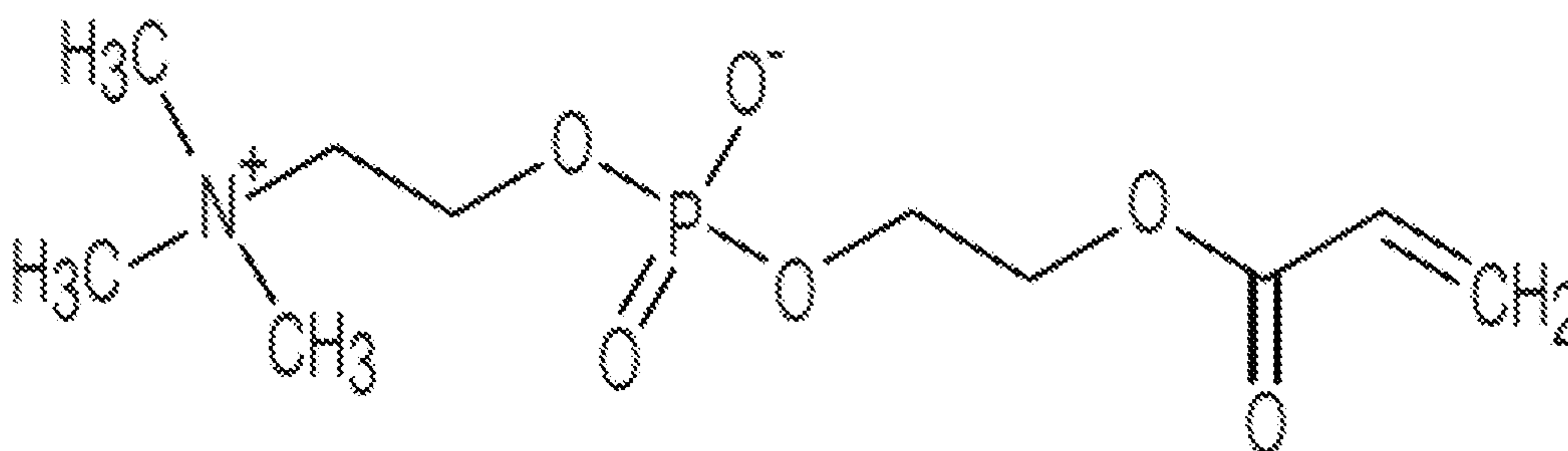
depending on the volume. The 27.2 mg/ml solution of TAF was injected intravitreally into the monkeys for the efficacy experiments described above through a 30 gauge (G) ½ inch needle. Excessive pressure was not required to push the OG1448 through the needle. The 44.5 mg/ml solution was tested for injectability in the laboratory and was also capable of being pushed through the needle without excessive pressure by a female operator.

[00480] Example 49. Storage Stability

[00481] An ongoing stability study was conducted using OG1448 reference lot R5606 at 44.5 mg/ml in PBS at pH 7.4 (as described above). Three temperatures were chosen for the study: room temperature (RT), 4°C and -20°C. Sampling frequency is at 0, 14, 28, 91, 181 and 362 days. Samples were evaluated by SDS-PAGE and analytical AE-HPLC for unreacted and sequestered protein, and potential aggregates. It was observed (data not shown) that OG1448 demonstrates less than 5% protein impurity by AE-HPLC at all three temperatures up to six months, which is similar to the level at time 0. This study is ongoing.

[00482] Example 50. Alternative Phosphorylcholine Polymers

[00483] A HEA-PC polymer was synthesized as described below. HEA-PC (2-(acryloyloxy)ethyl-2-(trimethylammonium)ethyl phosphate), which is an acrylate as opposed to the methacrylate HEMA-PC described above, has the following structure:



HEA-PC

HEA-PC was polymerized to the initiator shown in Example 23 as compound L.

Reactant	Name	Amount	MW
Initiator	Compound L (see above)	1.65 mg	2505.5
Monomer	HEA-PC	0.461 g	281.24
Catalyst	Cu (I) Bromide	1.2 mg	143.45

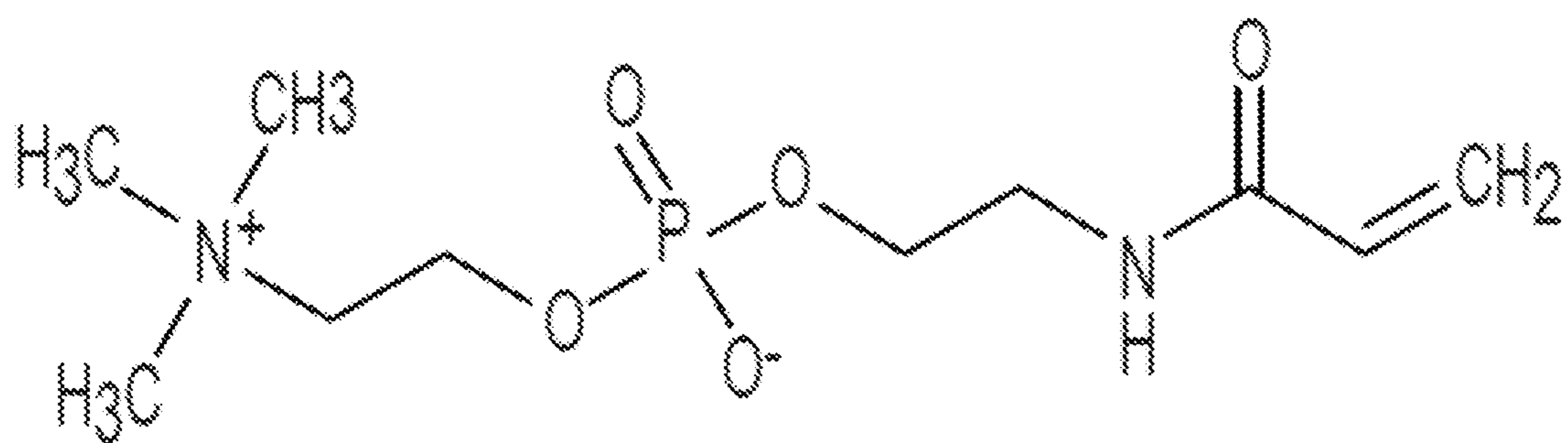
Ligand	Tris [2-(dimethylamino)ethyl]amine (Me6TREN)	2.73 mg	230.39
Solvent A	N,N-Dimethylformamide (DMF)	21.85 µl	73.09
Solvent B	Water	0.7 ml	18.02
Solvent C	Methanol	0.7 ml	32.04

[00484] Prepared a stock solution of initiator at 200mg/mL by dissolving 2.2 mg of initiator in 11 µl of dry DMF and a 200mg/ml solution of ligand by dissolving 4.6 mg of Me6TREN in 23 µL of dry DMF. Dispense 8.25 µl of the stock solution of initiator and 13.6 µl of the ligand into a tube. Degas at -78°C for 5 mn then refill with Argon and add 1.2 mg of CuBr. Degas and refill with Argon. Add a stock solution of HEA-PC in methanol (weigh out 0.461 g of HEA-PC and dissolve it in 0.5 mL of methanol) to the solution inside the reactor at -78°C. Rinse the vial with 200 µl of methanol and add it inside the reactor at -78°C and then 0.5 mL of distilled water then another 200 µl of water. Degas thoroughly until no bubbling is seen and all heterogeneity disappears (solid particulates dissolve or disappear). Refill with 4 psi of Argon and let the reaction to proceed at RT for an hour. The reaction was already viscous. The reaction was allowed to proceed for about one hour. A solution of bipyridine in methanol (5mg in 0.5uL) was added. Another 2-3 ml of methanol was added and the catalyst was allowed to oxidize overnight at 4°C. Conversion determined by ¹H NMR was estimated to be 94%.

[00485] The next day the polymer was dialyzed and subjected SEC/MALS analysis using Shodex SB806M_HQ column (7.8x300mm) in 1x PBS pH 7.4 at 1ml/min, giving a PDI of 1.157, Mn of 723.5 kDa, Mp of 820.4 kDa and Mw of 837.2 kDa (before dialysis PDI is 1.12, Mn = 695 kDa, Mp = 778 kDa). Next a maleimide functionality was added to the polymer so that it could be conjugate to a protein, including TAF443.

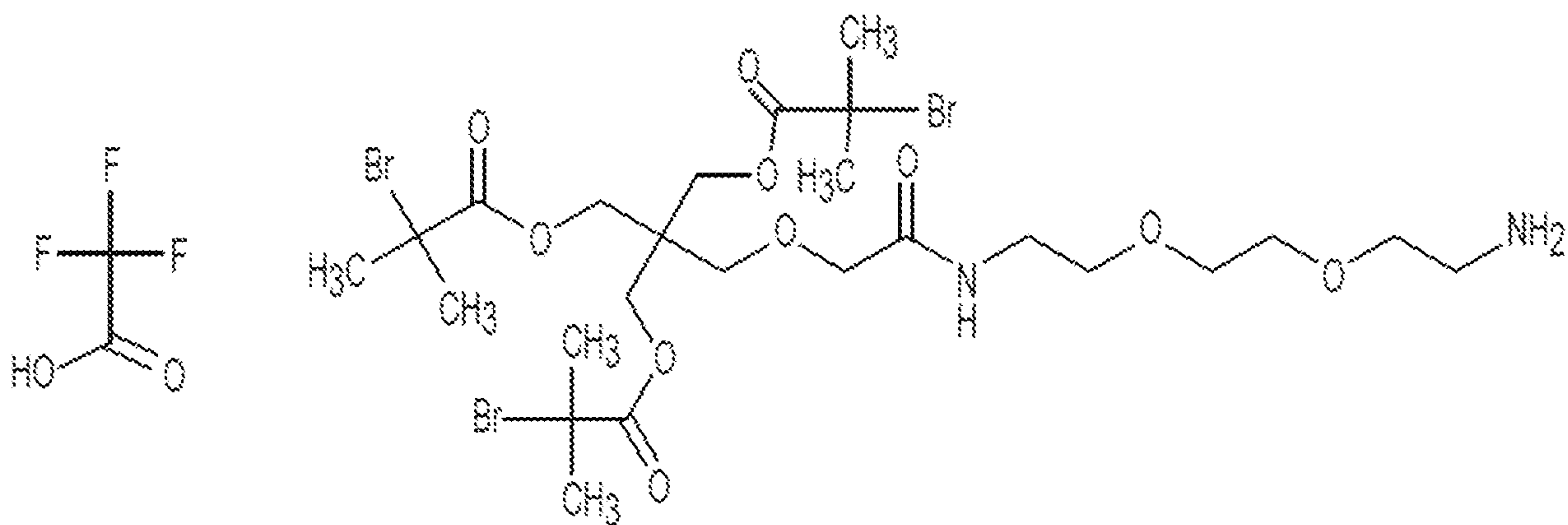
[00486] Next, the maleimide Mal-PEG4-PFP (see Example 23 above) ester was snapped on to the HEA-PC polymer as shown in Example 23. The resulting maleimide functionalized HEA-PC polymer can then be conjugated to sulfhydryl groups as discussed herein for HEMA-PC polymers.

[00487] An acrylamide PC polymer was also made using the monomer 2-(acrylamyl)ethyl-2-(trimethylammonium)ethyl phosphate (Am-PC), having the following structure:



Am-PC

[00488] The Am-PC was used for polymerization employing a 3 arm initiator (a TFA salt) having the structure:



[00489] The synthesis of the Am-PC polymer was conducted as follows:

Reactant	Name/Identity	Amount	MW
Initiator	3-arm initiator (see above)	2.2 mg	885.35
Monomer	Am-PC	0.5 g	280.26
Catalyst (I)	Copper (I) Bromide	1 mg	143.45
Catalyst (II)	Copper (II) Bromide	0.2 mg	223.35
Ligand	Tris[2-(dimethylamino)ethyl]amine	3.94 mg	230.39

	(Me6TREN)		
Solvent A	N,N-Dimethylformamide (DMF)	31.7 μ l	73.09
Solvent B	Water	1 ml	18.02
Solvent C	Methanol	1 ml	32.04

[00490] A stock solution of ligand at 200mg/mL was prepared by dissolving 9 mg of Me6TREN in 45uL of dry DMF. Add 19.7uL of the stock solution to a reaction vessel. Prepare a stock solution of initiator at 200mg/mL by dissolving 6.5 mg of material in 32.5uL of DMF. Add 11uL of the initiator stock solution to the ligand from above. Degas for 5 mn. Add 1 mg of CuBr. Prepared a stock solution of CuBr₂ at 200mg/mL by dissolving 4mg CuBr₂ in 20 μ L of DMF. Add 0.5g of monomer (AmPC) to 1 mL of methanol (slow dissolution/viscous solution), followed by 1uL of the stock solution of CuBr₂. Add the monomer solution dropwise to the reaction mixture above. Rinse with 1 mL of water. Degas the reaction mixture thoroughly (freeze-thaw). Let the reaction proceed for 24 hours.

[00491] Afterwards the Am-PC polymer may be dialyzed. The molecular weight of the above polymer was determined by SEC/MALS: Mn is 215kDa, Mp: 250kDa, PDI is 1.17. Conversion was estimated by ¹H NMR to be 94%. A maleimide functionality can be added to the Am-PC polymer as discussed above for HEMA-PC and HEA-PC. Maleimide functionalized Am-PC polymer can be conjugated to a protein, such as TAF443, as described above.

[00492] **Example 51.** Reverse Ellman's Assay for Calculating Free Maleimide in a Compound

[00493] After addition of the maleimide functionality to polymer OG1801 to form OG1802 (see above), an Ellman's assay is used to determine the amount of functional maleimide (i.e. conjugatable) in a sample. Thiol converts Ellman's reagent (DTNB) to TNB- then to TNB₂- in water at neutral and alkaline pH, which gives off a yellow color (measured at 412nm). A standard curve is established with cysteine. Since the maleimide reacts with thiol, this assay actually measures the thiol (cysteine) left. The inhibition is calculated as the (original thiol – thiol left after maleimide polymer addition)/ (original thiol) and is expressed as a percentage.

[00494] Reagents Employed in Assay: A standard curve was prepared using the cysteine from 62.5 μ M to 2 μ M. Polymer stock solutions were prepared by dissolving the powder in 1xPBS pH7.4 (reaction buffer) and mixing thoroughly. An equal molar of polymer and cysteine

solutions were mixed and allowed to react at 27°C for 30 minutes. The 150µM of DTNB solution was added into the cysteine standards and polymer/cysteine reactions and the color was developed at 27°C for 5 minutes. OD at 412nm was read on the Spectramax plate reader and percent inhibition was calculated with the Softmax Pro software and the cysteine standard curve.

[00495] All patent filings, websites, other publications, accession numbers and the like cited above or below are incorporated by reference in their entirety for all purposes to the same extent as if each individual item were specifically and individually indicated to be so incorporated by reference. If different versions of a sequence are associated with an accession number at different times, the version associated with the accession number at the effective filing date of this application is meant. The effective filing date means the earlier of the actual filing date or filing date of a priority application referring to the accession number if applicable. Likewise if different versions of a publication, website or the like are published at different times, the version most recently published at the effective filing date of the application is meant unless otherwise indicated. Any feature, step, element, embodiment, or aspect of the invention can be used in combination with any other unless specifically indicated otherwise. Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

SEQUENCES

SEQ ID NO. 1

1 LVVTPPGPEL VLNVSSTFVL TCSGSAPVVW ERMSQEPPQE MAKAQDGTFS SVLTLTNLTG
 LDTGEYFCTH NDSRGLETDE
 81 RKRLYIFVPD PTVGFLPNDA EELFIFLTEI TEITIPCRVT DPQLVVTLHE KKG DVALPVP
 YDHQRGFSGI FEDRSYICKT
 161 TIGDREVDSD AYYVYRLQVS SINVS VNAVQ TVVRQGENIT LMCIVIGNEV VNFEW TYPRK
 ESGRLVEPVT DFLLDMPYHI
 241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVVE SGGGGSGGG GS DIQMTQSP
 SSL SASVGDR VTITCSASQD
 321 ISNYLNWYQQ KPGKAPKVL I YFTSSLHSGV PSRFSGSGSG TDFTLTISSL QPEDFATYYC
 QQYSTVPWTF GQGTKVEIKR
 401 TVAAPSVFIF PPSDEQLKSG TASVCLLNN FYPREAKVQW KVDNALQSGN SQESVTEQDS
 K DSTYLSST LTL SKADYEK
 481 HKVYACEVTH QGLSSPVTKS FNRGEC

SEQ ID NO. 2

1 EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW INTYTGEPTY
 AADFKRRTF SLDTSKSTAY
 81 LQMNSLRAED TAVYYCAKYP HYYGSSHWF DVWGQGTILVT VSSASTKGPS VFPLAPSSKS
 TSGGTAALGC LVKDYFPEPV
 161 TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKK
 VEPKSCDKTH TCPPCPAPEL
 241 LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
 QYNSTYRVVS VLTVLHQDWL
 321 NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP
 SDIAVEWESN GPENNYKTT
 401 PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK

SEQ ID NO. 3

1 LVVTPPGPEL VLNVSSTFVL TCSGSAPVVW ERMSQEPPQE MAKAQDGTFS SVLTLTNLTG
 LDTGEYFCTH NDSRGLETDE
 81 RKRLYIFVPD PTVGFLPNDA EELFIFLTEI TEITIPCRVT DPQLVVTLHE KKG DVALPVP
 YDHQRGFSGI FEDRSYICKT
 161 TIGDREVDSD AYYVYRLQVS SINVS VNAVQ TVVRQGENIT LMCIVIGNEV VNFEW TYPRK
 ESGRLVEPVT DFLLDMPYHI
 241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVVE SGGGDIQMTQ SPSSLSASVG
 DRVTITCSAS QDISNYLNWY
 321 QQKPGKAPKV LIYFTSSLHS GVPSRFSGSG SGTDFTLTIS SLQPEDFATY YCQQYSTVPW
 TFGQGTKVEI KRTVAAPSVF
 401 IFPPSDEQLK SGTASVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYLS
 STLTL SKADY EKHKVYACEV
 481 THQGLSSPVT KSFNRGEC

SEQ ID NO. 4

1 LVVTPPGPEL VLNVSSTFVL TCSGSAPVVW ERMSQEPPQE MAKAQDGTFS SVLTLTNLTG
 LDTGEYFCTH NDSRGLETDE

81 RKRLYIFVPD PTVGFLPND A EELFIFLTEI TEITIPCRVT DPQLVVTLHE KKG DVALPVP
 YDHQRGFSGI FEDRSYICKT
 161 TIGDREVDSD AYYVYRLQVS SINVS VNAVQ TVVRQGENIT LMCIVIGNEV VNFEW TYPRK
 ESGRLVEPVT DFLLDMPYHI
 241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVVE SGGGGGSGGG GSEVQLVESG
 GGLVQPGGSL RLSCAASGYT
 321 FTNYGMNWVR QAPGKGLEWV GWINTYTGEP TYAADFKRRF TFSLDTSKST AYLQMNSLRA
 EDTAVYYCAK YPHYYGSSHW
 401 YFDVWGQGT L VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA
 LTSGVHTFPA VLQSSGLYSL
 481 SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPCPAP ELLGGPSVFL
 FPPKPKDTLM ISRTPEVTCV
 561 VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
 VSNKALPAPI EKTISKAKGQ
 641 PREPQVYTL P PSREEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG
 SFFLYSKLTV DKSRWQQGNV
 721 FSCSVMHEAL HNHYTQKSLS LSPGK

SEQ ID NO. 5

1 DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP GKAPKVL IYF TSSLHSGVPS
 RFSGSGSGTD FTLTISSLQP
 81 EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY
 PREAKVQWKV DNALQSGNSQ
 161 ESVTEQDSKD STYLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC

SEQ ID NO. 6

1 LVVTPPGPEL VLNVSSTFVL TCSGSAPVWV ERMSQEPPQE MAKAQDGTFS SVLTLTNLTG
 LDTGEYFCTH NDSRGLETDE
 81 RKRLYIFVPD PTVGFLPND A EELFIFLTEI TEITIPCRVT DPQLVVTLHE KKG DVALPVP
 YDHQRGFSGI FEDRSYICKT
 161 TIGDREVDSD AYYVYRLQVS SINVS VNAVQ TVVRQGENIT LMCIVIGNEV VNFEW TYPRK
 ESGRLVEPVT DFLLDMPYHI
 241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVVE SGGGEVQLVE SGGGLVQPGG
 SLRLSCAASG YTFTNYGMNW
 321 VRQAPGKGLE WVGWINTYTG EPTYAADFKR RFTFSLDTSK STAYLQMNSL RAEDTAVYYC
 AKYPHYYGSS HWYFDVWGQG
 401 TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF
 PAVLQSSGLY SLSSVTVPS
 481 SSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKHTCPPCP APPELLGGPSV FLFPPKPKDT
 LMISRTPEVT CVVVDVSHED
 561 PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
 PIEKTISKAK GQPREPQVYT
 641 LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDS DGSFFLYSKL
 TVDKSRWQQG NVFSCSVMHE
 721 ALHNHYTQKS LSLSPGK

SEQ ID NO. 7

1 EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW INTYTGEPTY
 AADFKRRFTF SLDTSKSTAY
 81 LQMNSLRAED TAVYYCAKYP HYYGSSHWYF DVWGQGTLVT VSSASTKGPS VFPLAPSSKS
 TSGGTAALGC LVKDYFPEPV
 161 TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKK
 VEPKSCDKTH TCPPCPAPEL
 241 LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
 QYNSTYRVVS VLTVLHQDWL
 321 NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP
 SDIAVEWESN GQPENNYKTT
 401 PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGGGGGSGGG
 GSGGGGSGGG GSGLVVTPPG
 481 PELVLNVSS FVLTCGSAP VVWERMSQEP PQEMAKAQDG TFSSVLTLTN LTGLDTGEYF
 CTHNDSRGLE TDERKRLYIF
 561 VPDPTVGFLP NDAEELFIFL TEITEITIPC RVTDPQLVVT LHEKKGDVAL PVPYDHQGRF
 SGIFEDRSYI CKTTIGDREV
 641 DSDAYYVYRL QVSSINVSVN AVQTVVRQGE NITLMCIVIG NEVVNFEWY PRKESGRLVE
 PVTDFLLDMP YHIRSILHIP
 721 SAELEDSGTY TCNVTESVND HQDEKAINIT VVESG

SEQ ID NO. 8

1 LVVTPPGPEL VLNVSSTFVL TCSGSAPVVW ERMSQEPPQE MAKAQDGTFS SVLTLTNLTG
 LDTGEYFCTH NDSRGLETDE
 81 RKRLYIFVPD PTVGFLPNDA EELFIFLTEI TEITIPCRVT DPQLVVTLHE KKG DVALPVP
 YDHQRGFSGI FEDRSYICKT
 161 TIGDREVDSD AYYVYRLQVS SINVS VNAVQ TVVRQGENIT LMCIVIGNEV VNF EWYPRK
 ESGRLVEPVT DFLLDMPYHI
 241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVVE SGGGGGSGGG GSEVQLVESG
 GGLVQPGGSL RLSCAASGYD
 321 FTHYGMNWVR QAPGKLEWV GWINTYTGEPT YAAADFKRRF TFSLDTSKST AYLQMNSLRA
 EDTAVYYCAK YPYYGTSHW
 401 YFDVWGQGTI VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA
 LTSGVHTFPA VLQSSGLYSL
 481 SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPCPAP EAAGAPSVFL
 FPPKPKDTLM ISRTPEVTCV
 561 VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
 VSNKALPAPI EKTISKAKGQ
 641 PREPCVYTL PPSREEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG
 SFFLYSKLTV DKSRWQQGNV
 721 FSCSVMHEAL HNHYTQKSLS LSPGK

SEQ ID NO. 9

1 LVVTPPGPEL VLNVSSTFVL TCSGSAPVVW ERMSQEPPQE MAKAQDGTFS SVLTLTNLTG
 LDTGEYFCTH NDSRGLETDE
 81 RKRLYIFVPD PTVGFLPNDA EELFIFLTEI TEITIPCRVT DPQLVVTLHE KKG DVALPVP
 YDHQRGFSGI FEDRSYICKT
 161 TIGDREVDSD AYYVYRLQVS SINVS VNAVQ TVVRQGENIT LMCIVIGNEV VNF EWYPRK
 ESGRLVEPVT DFLLDMPYHI
 241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVVE SGGGGGSGGG GSEVQLVESG
 GGLVQPGGSL RLSCAASGYD
 321 FTHYGMNWVR QAPGKLEWV GWINTYTGEPT YAAADFKRRF TFSLDTSKST AYLQMNSLRA
 EDTAVYYCAK YPYYGTSHW

WO 2015/200905

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401 YFDVWGQGTL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA
 LTSGVHTFPA VLQSSGLYSL
 481 SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPCPAP EAAGAPSVFL
 FPPKPKDTLM ISRTPEVTCV
 561 VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
 VSNKALPAPI EKTISKAKGQ
 641 PREPQVYTLP PSREEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG
 SFFLYSKLTV DKSRWQQGNV
 721 FSCSVMHEAL HNHYTQKSLS CSPGK

SEQ ID NO. 10

1 DIQLTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP GKAPKVLIYF TSSLHSGVPS
 RFSGSGSGTD FTLTISSLQP
 81 EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY
 PREAKVQWKV DNALQSGNSQ
 161 ESVTEQDSKD STYLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEN

SEQ ID NO. 11

1 MRLPGAMPAL ALKGELLLLS LLLLLEPQIS QGLVVTTPGP ELVLNVSTF VLTCSGSAPV
 VWERMSQEPQ QEMAKAQDGT
 81 FSSVLTLTNL TGLDTGEYFC THNDSRGLET DERKRLYIFV PDPTVGFLPN DAEELFIFLT
 EITEITIPCR VTDPQLVVTL
 161 HEKKGDAVALP VPYDHQRGFS GIFEDRSYIC KTTIGDREVD SDAYYVYRLQ VSSINVSUNA
 VQTVVRQGEN ITLMCIVIGN
 241 EVVNFETYP RKESGRLVEP VTDFLLDMPY HIRSILHIPS AELEDSTYT CNVTESVNDH
 QDEKAINITV VESGYVRLLG
 321 EVGTLQFAEL HRSRTLQVVF EAYPPPTVLW FKDNRTLGD SAGEIALSTR NVSETRYVSE
 LTLVRVKVAE AGHYTMRAFH
 401 EDAEVQLSFQ LQINVPVRVL ELSESHPDG EQTVRCRGRG MPQPNIWSA CRDLKRCPRE
 LPPTLLGNSS EESQLETNV
 481 TYWEEEQEFE VVSTLRLQHV DRPLSVRCTL RNAVGQDTQE VIVVPHSLPF KVVVISAILA
 LVVLTIIISLI ILIMLWQKKP
 561 RYEIRWKVIE SVSSDGHEYI YVDPMQLPYD STWELPRDQL VLGRTLGSAG FGQVVEATAH
 GLSHSQATMK VAVKMLKSTA
 641 RSSEKQALMS ELKIMSHLGP HLNVVNLLGA CTKGGPIYII TEYCRYGDLV DYLRNKHTE
 LQHHSDKRRP PSAELYSNAL
 721 PVGLPLPSHV SLTGESDGGY MDMSKDESVD YVPMLDMKGD VKYADIENN YMAPYDNYVP
 SAPERTCRAT LINESPVLSY
 801 MDLVGFSYQV ANGMEFLASK NCVHRDLAAR NVLICEGKLV KICDFGLARD IMRDSNYISK
 GSTFLPLKWM APESIFNSLY
 881 TTLSDVWSFG ILLWEIFTLG GTPYPELPMN EQFYNAIKRG YRMAQPAHAS DEIYEIMQKC
 WEEKFEIRPP FSQVLVLLER
 961 LLGEGYKKKY QQVDEEFLRS DHPAILRSQA RLPGFHGLRS PLDTSSVLYT AVQPNEGDN
 YIIPLPDPKP EVADEGPLEG
 1041 SPSLASSTLN EVNTSSTISC DSPLEPQDEP EPEPQLELQV EPEPELEQLP DSGCPAPRAE
 AEDSFL

SEQ ID NO. 12

1 DIQLTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP GKAPKVLIYF TSSLHSGVPS
 RFSGSGSGTD FTLTISSLQP
 81 EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY
 PREAKVQWKV DNALQSGNSQ

161 ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC

SEQ ID NO. 13

1 EVQLVESGGG LVQPGGSLRL SCAASGYDFT HYGMNWVRQA PGKGLEWVGW INTYTGEPTY
AADFKRRFTF SLDTSKSTAY
81 LQMNSLRAED TAVYYCAKYP YYYGTSHWYF DVWGQGTLVT VSSASTKGPS VFPLAPSSKS
TSGGTAALGC LVKDYFPEPV
161 TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKK
VEPKSCDKTH L

SEQ ID NO. 14

1 MVSYWDTGVL LCALLSCLLL TGSSSGSKLK DPESLKGTO HIMQAGQTLH LQCRGEAAHK
WSLPEMVSKE SERLSITKSA
81 CGRNGKQFCS TLTLNTAQAN HTGFYSCYKL AVPTSKKKET ESAIYIFISD TGRPFVEMYS
EIPETIHMTE GRELVIPCRV
161 TSPNITVTLK KFPLDTLIPD GKRIIWDNRK GFIIISNATYK EIGLLTCEAT VNGHLYKTNV
LTHRQNTNII DVQISTPRPV
241 KLLRGHTLVN NCTATTPLNT RVQMTWSYPD EKNKRASVRR RIDQSNRSHN IFYSVLTIDK
MQNKDKGLYT CRVRSGPSFK
321 SVNTSVHIYD KAFITVKHRK QQVLETVAGK RSYRLSMKVK AFPSPEVVWL KDGLPATEKS
ARYLTRGYSL IIKDVTEEDA
401 GNYTILLSIK QSNVFNKLTN TLIVNVKPQI YEKAVSSFPD PALYPLGSRQ ILTCTAYGIP
QPTIKWFVHP CNHNHSEARC
481 DFCSNNEESF ILDADSNMGN RIESITQDMA IIEGKNKMAS TLVVADSRIS GIYICIASNK
VGTVGRNISF YITDVPNGFH
561 VNLEKMPTEG EDLKLSTVN KFLYRDVTWI LLRTVNNRTM HYSISKQKMA ITKEHSITLN
LTMNVSLQD SGTYACRARN
641 VYTGEELQK KEITIRDQEA PYLLRNLSNH TVAISSTTL DCHANGVPEP QITWFKNNHK
IQQEPGIILG PGSSTLFIER
721 VTEDEGEVYH CKATNQKGSV ESSAYLTVQG TSDKSNLELI TLTCTCVAAT LFWLLLTLEI
RKMKRSSSEI KTDYLSIIMD
801 PDEVPLDEQC ERLPYDASKW EFARERLKLK KSLGRGAFGK VVQASAFGIK KSPTCRTLAV
KMLKEGATAS EYKALMTELK
881 ILTHIGHHLN VVNLGACTK QGGPLMVIVE YCKYGNLSNY LKSKRDLFFL NKDAALHMEP
KKEKMEPGLE QGKKPRLDV
961 TSSESFASSG FQEDKSLSDV EEEEDSDGFY KEPITMEDLI SYSFQVARGM EFLSSRKCIH
RDLAARNILL SENNVVKICD
1041 FGLARDIYKN PDYVRKGDTR LPLKWMAPES IFDKIYSTKS DVWSYGVLLW EIFSLGGSPY
PGVQMDDEFC SRLREGMRMR
1121 APEYSTPEIY QIMLDCWHRD PKERPRFAEL VEKLGDLLQA NVQQDGKDYI PINAILTGNS
GFTYSTPAFS EDDFKESISA
1201 PKFNSGSSDD VRYVNAFKFM SLERIKTFEE LLPNATSMFD DYQGDSTLL ASPMLKRFTW
TDSKPKASLK IDLRVTSKSK
1281 ESGLSDVSRP SFCHSSCGHV SEGKRRFTYD HAELERKIAC CSPPPDYNV VLYSTPPI

SEQ ID NO. 15

1 MQSKVLLAVA LWLCVETRAA SVGLPSVSLD LPRLSIQKDI LTIKANTTLQ ITCRGQRDLN
WLWPNNQSGS EQRVEVTECS
81 DGLFCKTLTI PKVIGNDTGA YKCFYRETDL ASVIYVYVQD YRSPFIASVS DQHGCVYITE
NKNKTVVIPCLGSGISNLNVS
161 LCARYPEKRF VPDGNRISWD SKKGFTIPSY MISYAGMVFC EAKINDESYQ SIMYIVVVVG
YRIYDVVLSP SHGIELSVGE

241 KLVNCTART ELNVGIDFNW EYPSSKHQHK KLVNRDLKTQ SGSEMKKFLS TLTIDGVTRS
 DQGLYTCAAS SGLMTKKNST
 321 FVRVHEKPFV AFGSGMESLV EATVGERVRI PAKYLGYPPE EIKWYKNGIP LESNHTIKAG
 HVLTIMEVSE RDTGNYTVIL
 401 TNPISKEKQS HVVSLVVYVP PQIGEKSLIS PVDSYQYGTT QTLTCTVYAI PPPHHIHWYW
 QLEEECANEP SQAVSVTNPY
 481 PCEEWRSEVED FQGGNKIEVN KNQFALIEGK NKTVSTLVIQ AANVSALYKC EAVNKVGRGE
 RVISFHVTRG PEITLQPDQM
 561 PTEQESVSLW CTADRSTFEN LTWYKLGPPQ LPIHVGELPT PVCKNLDLW KLNATMFSNS
 TNDILIMELK NASLQDQGDY
 641 VCLAQDRKTK KRHCVRQLT VLERVAPTIT GNLENQTTSI GESIEVSCTA SGNPPPQIMW
 FKDNELTVED SGIVLKDGNR
 721 NLTIRRVKE DEGLYTCQAC SVLGCAKVEA FFIIEGAQEK TNLEIIILVG TAVIAMFFWL
 LLVILRTVK RANGGELKTG
 801 YLSIVMDPDE LPLDEHCERL PYDASKWEFP RDRLKLGKPL GRGAFGQVIE ADAFGIDKTA
 TCRTVAVKML KEGATHSEHR
 881 ALMSELKILI HIGHHLNVVN LLGACTKPGG PLMVIVEFCK FGNLSTYLRK KRNEFVPYKT
 KGARFRQKDK YVGAIPVDLK
 961 RRLDSITSSQ SSASSGFVEE KSLSDVEEEE APEDLYKDFL TLEHLICYSF QVAKGMEFLA
 SRKCIHRDLA ARNILLSEKN
 1041 VVKICDFGLA RDIYKDPDYV RKGDARLPLK WMAPETIFDR VYTIQSDVWS FGVLLWEIFS
 LGASPYPGVK IDEEFCRRLK
 1121 EGTRMRAPDY TTPEMYQTML DCWHGEPSSQ PTFSELVEHL GNLLQANAQQ DGKDYIVLPI
 SETLSMEEDS GLSLPTSPVS
 1201 CMEEEEEVCDP KFHYDNTAGI SQYLQNSKRK SRPVSVKTFE DIPLEEPEVK VIPDDNQTD
 GMVLASEELK TLEDRTKLSP
 1281 SFGGMVPSKS RESVASEGSN QTSQYQSGYH SDDTDTTVYS SEEAELLKLI EIGVQTGSTA
 QILQPDSTT LSSPPV

SEQ ID NO. 16

1 MQRGAALCLR LWLCLGLLDG LVSGYSMTTP TLNITEESHV IDTGDSLSSIS CRGQHPLEWA
 WPGAQEAPAT GDKDSEDGTV
 81 VRDCEGTDAR PYCKVLLHE VHANDTGSYV CYYKYIKARI EGTTAASSYV FVRDFEQPFI
 NKPDILLVNR KDAMWVPCLV
 161 SIPGLNVTLR SQSSVLWPDG QEVVWDDRRG MLVSTPLLHD ALYLQCETTW GDQDFLSNPF
 LVHITGNELY DIQLLPRKSL
 241 ELLVGEKLV NCTVWAEFNS GVTFDWDYPG KQAERGKWVP ERRSQQTHTE LSSILTIHNV
 SQHDLGSYVC KANNGIQRFR
 321 ESTEVIVHEN PFISVEWLKG PILEATAGDE LVKLPVKLAA YPPPEFQWYK DGKALSGRHS
 PHALVLKEVT EASTGTYTLA
 401 LWNSAAGLRR NISLELVNV PPQIHEKEAS SPSIYSRHSR QALTCTAYGV PLPLSIQWHW
 RPWTPCKMFA QRSLRRRQQQ
 481 DLMPQCRDWR AVTTQDAVNP IESLDTWTEF VEGKNKTVSK LVIQNANVSA MYKCVVSNKV
 GQDERLIIFY VTTIPDGFTI
 561 ESKPSEELLE GQPVLLSCQA DSYKYEHLRW YRLNLSTLHD AHGNPLLLDC KNVHLFATPL
 AASLEEVAPG ARHATLSLSI
 641 PRVAPEHEGH YVCEVQDRRS HDKHCHKKYL SVQALEAPRL TQNLTDLLVN VSDSLEMQCL
 VAGAHAPSIV WYKDERLLEE
 721 KSGVDLADSN QKLSIQRVRE EDAGRYLCSV CNAKGCVNSS ASVAVEGSED KGSMEIVILV
 GTGVIAVFFW VLLLLIFCNM
 801 RRPAAHDIKT GYLSIIMDPG EVPLEEQCEY LSYDASQWEF PRERLHLGRV LGYGAFGKVV
 EASAFGIHKG SSCDTVAVKM
 881 LKEGATASEH RALMSELKIL IHIGHHLNVV NLLGACTKPQ GPLMVIVEFC KYGNLSNFLR
 AKRDAFSPCA EKSPEQRGRF
 961 RAMVELARLD RRRPGSSDRV LFARFSKTEG GARRASPDQE AEDLWLSPLT MEDLVCYSFQ
 VARGMEFLAS RKCIHRDLAA

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1041 RNILLSESDV VKICDFGLAR DIYKDPDYVR KGSARLPLKW MAPESIFDKV YTTQSDVWSF
 GVLLWEIFSL GASPYPGVQI
 1121 NEEFCQRLRD GTRMRAPELA TPAIRRIMLN CWSGDPKARP AFSELVEILG DLLQGRGLQE
 EEEVCMAPRS SQSSEEGSFS
 1201 QVSTMALHIA QADAEDSPPS LQRHSLAARY YNWVSFPGCL ARGAE TRGSS RMKTFEEFPM
 TPTTYKGSVD NQTDSGMVLA
 1281 SEEFEQIESR HRQESGFSCK GPGQNVAVTR AHPDSQGRRR RPERGARGGQ VFYNSEYGEL
 SEPSEEDHCS PSARVTFFTD
 1361 NSY

SEQ ID NO. 17

1 ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS
 GLYSLSSVVT VPSSSLGTQT
 81 YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PSVFLEPPKP KDTLMISRTP
 EVTCVVVDVS HEDPEVKFNW
 161 YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
 KAKGQPREPQ VYTLPPSRDE
 241 LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW
 QQGNVFSCSV MHEALHNHYT
 321 QKSLSLSPGK

SEQ ID NO. 18

1 TVAAPSVFIF PPSDEQLKSG TASVVCLLNN FYPREAKVQW KVDNALQSGN SQESVTEQDS
 KDSTYSLSST LTLISKADYEK
 81 HKVYACEVTH QGLSSPVTKS FNRGEC

SEQ ID NO. 19

1 LVVTPPGPEL VLNVSSTFVL TCSGSAPVVW ERMSQEPPQE MAKAQDGTFS SVLTLTNLTG
 LDTGEYFCTH NDSRGLETDE
 81 RKRLYIFVPD PTVGFLPNDA EELFIFLTEI TEITIPCRVT DPQLVVTLHE KKG DVALPVP
 YDHQRGFSGI FEDRSYICKT
 161 TIGDREVDSD AYYVYRLQVS SINVS VNAVQ TVVRQGENIT LMCIVIGNEV VNFEW TYPRK
 ESGRLVEPVT DFLLDMPYHI
 241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVVE SGGGGGSGGG GS DIQMTQSP
 SSLASV GDR VTITCSASQD
 321 ISNYLNWYQQ KPGKAPKVL I YFTSSLHSGV PSRFSGSGSG TDFTLTISSL QPEDFATYYC
 QQYSTVPWTF GQGTKVEIKR
 401 TVAAPSVFIF PPSDEQLKSG TASVVCLLNN FYPREAKVQW KVDNALQSGN SQESVTEQDS
 KDSTYSLSST LTLISKADYEK
 481 HKVYACEVTH QGLSSPVTKS FNRGEC

SEQ ID NO. 21

1 EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW INTYTGEPTY
 AADFKRRFTF SLDTSKSTAY
 81 LQMNSLRAED TAVYYCAKYP HYYGSSHWFY DVWGQGTLVT VSSASTKGPS VFPLAPSSKS
 TSGGTAALGC LVKDYFPEPV
 161 TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKK
 VEPKSCDKTH T

SEQ ID NO. 22

WO 2015/200905

PCT/US2015/038203

1 LVVTPPGPEL VLNVSSTFVL TCSGSAPVWV ERMSQEPPQE MAKAQDGTFS SVLTLTNLTG
 LDTGEYFCTH NDSRGLETDE
 81 RKRLYIFVPD PTVGFLPNDA EELFIFLTEI TEITIPCRVT DPQLVVTLHE KKG DVALPVP
 YDHQRGFSGI FEDRSYICKT
 161 TIGDREVDSD AYYVYRLQVS SINVS VNAVQ TVVRQGENIT LMCIVIGNEV VNFEW TYPRK
 ESGRLVEPVT DFLLDMPYHI
 241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVVE SGGGGGSGGG GSEVQLVESG
 GGLVQPGGSL RLSCAASGYT
 321 FTNYGMNWVR QAPGKGLEWV GWINTYTGEF TYAADFKRRF TFSLDTSKST AYLQMNSLRA
 EDTAVYYCAK YPHYYGSSHW
 401 YFDVWGQGTI VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA
 LTSGVHTFPA VLQSSGLYSL
 481 SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THT

SEQ ID NO. 23

1 LVVTPPGPEL VLNVSSTFVL TCSGSAPVWV ERMSQEPPQE MAKAQDGTFS SVLTLTNLTG
 LDTGEYFCTH NDSRGLETDE
 81 RKRLYIFVPD PTVGFLPNDA EELFIFLTEI TEITIPCRVT DPQLVVTLHE KKG DVALPVP
 YDHQRGFSGI FEDRSYICKT
 161 TIGDREVDSD AYYVYRLQVS SINVS VNAVQ TVVRQGENIT LMCIVIGNEV VNFEW TYPRK
 ESGRLVEPVT DFLLDMPYHI
 241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVVE SGGGEVQLVE SGGGLVQPGG
 SLRLSCAASG YTFTNYGMNW
 321 VRQAPGKGLE WVGWINTYTG EPTYAADFKR RFTFSLDSK STAYLQMNSL RAEDTAVYYC
 AKYPHYYGSS HWYFDVWGQG
 401 TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF
 PAVLQSSGLY SLSSVVTVPS
 481 SSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKHT

SEQ ID NO. 24

1 EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW INTYTGEPTY
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 81 LQMNSLRAED TAVYYCAKYP HYYGSSHWYF DVWGQGTILV VSSASTKGPS VFPLAPSSKS
 TSGGTAALGC LVKDYFPEPV
 161 TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKK
 VEPKSCDKTH TGGGGGSGGG
 241 GSGGGGSGGG GSGLVVTPPG PELVLNVSST FVLTCGSAP VVWERMSQEP PQEMAKAQDG
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 321 CTHNDSRGLE TDERKRLYIF VPDPTVGFLP NDAEELFIFL TEITEITIPC RVTDPQLVVT
 LHEKKGDVAL PVPYDHQRGF
 401 SGIFEDRSYI CKTTIGDREV DSDAYYVYRL QVSSINVS VN AVQTVVRQGE NITLMCIVIG
 NEVVNFEW TY PRKESGRLVE
 481 PVTDFLLDMP YHIRSILHIP SAELEDSGTY TCNVTESVND HQDEKAINIT VVESG

SEQ ID NO. 25

1 LVVTPPGPEL VLNVSSTFVL TCSGSAPVWV ERMSQEPPQE MAKAQDGTFS SVLTLTNLTG
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 81 RKRLYIFVPD PTVGFLPNDA EELFIFLTEI TEITIPCRVT DPQLVVTLHE KKG DVALPVP
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161 TIGDREVDSD AYYVYRLQVS SINVSVNAVQ TVVRQGENIT LMCIVIGNEV VNFEWYPRK
 ESGRLVEPVT DFLLDMPYHI
 241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVVE SGGGGSGGG GSEVQLVESG
 GGLVQPGGSL RLSCAASGYD
 321 FTHYGMNWVR QAPGKGLEWV GWINTYTGEPT TYAADFKRRF TFSLDTSKST AYLQMNSLRA
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 401 YFDVWGQGITL VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA
 LTSGVHTFPA VLQSSGLYSL
 481 SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THL

SEQ ID NO. 26

1 LVVTPPGPE LVLNVSSTFV LTCSGSAPVV WERMSQEPPQ EMAKAQDGTF SSVLTLTNLT
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 80 ERKRLYIFVP DPTVGFLPND AEELFIFLTE ITEITIPCRV TDPQLVVTILH EKKGDVALPV
 PYDHQRGFSG IFEDRSYICK
 160 TTIGDREVDS DAYYVYRLQV SSINVSVNAV QTVVRQGENI TLMCIVIGNE VVNFEWYPR
 KESGRLVEPV TDFLLDMPYH
 240 IRSILHIPSA ELEDSTYTC NVTESVNDHQ DEKAINITVV ESSEVQLVES GGGLVQPGGS
 LRLSCAASGY TFTNYGMNWV
 320 RQAPGKGLEW VGWINTYTGE PTYAADFRR FTFSLDTSKS TAYLQMNSLR AEDTAVYYCA
 KYPHYGGSSH WYFDVWGQGT
 400 LVTVSSASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP
 AVLQSSGLYS LSSVVTVPSS
 480 SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL
 MISRTPEVTC VVVDVSHEDP
 560 EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNNALPAP
 IEKTISKAKG QPREPQVYTL
 640 PPSREEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSG GSFFLYSKLT
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 720 LHNHYTQKSL SLSPGK

SEQ ID NO. 27

1 VGFLPNDAEE LFIFLTEITE ITIPCRVTDP QLVVTLHEKK GDVALPVPYD HQRGFSGIFE
 DRSYICKTTI GDREVDSDAY
 81 YVYRLQVSSI NVSVNAVQTV VRQGENITLM CIVIGNEVVN FEWYPRKES GRLVEPVTDF
 LLDMPYHIRS ILHIPSAELE
 161 DSGTYTCNVT ESVDNDHQDEK AINITVVEVG EVQLVESGGG LVQPGGSLRL SCAASGYTFT
 NYGMNWVRQA PGKGLEWVGW
 241 INTYTGEPTY AADFRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP HYYGSSHWFY
 DVWGQGITLVT VSSASTKGPS
 321 VFPLAPSSKS TSGGTAAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS
 VVTVPSSSLG TQTYICNVNH
 401 KPSNTKVDK VEPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV
 DVSHEDPEVK FNWYVDGVEV
 481 HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
 EPQVYTLPPS REEMTKNQVS
 561 LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSGDSF FLYSKLTVDK SRWQQGNVFS
 CSVMHEALHN HYTQKSLSL
 641 PGK

SEQ ID NO. 28

1 VGFLPNDAEE LFIFLTEITE ITIPCRVTD P QLVVTLHEKK GDVALPVPYD HQRGFSGIFE
 DRSYICKTTI GDREVDSDAY
 81 YVYRLQVSSI NVSVNAVQTV VRQGENITLM CIVIGNEVVN FEWTYPRKES GRLVEPVTDF
 LLDMPYHIRS ILHIPSAELE
 161 DSGTYTCNVT ESVNDHQDEK AINITVVESG EVQLVESGGG LVQPGGSLRL SCAASGYTFT
 NYGMNWVRQA PGKGLEWVGW
 241 INTYTGEPTY AADFKRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP HYYGSSHWFYF
 DVWGQGTILVT VSSASTKGPS
 321 VFPLAPSSKS TSGGTAALGC LVKDYPPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS
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 401 KPSNTKVDKK VEPKSCDKTH T

SEQ ID NO. 29

1 VGFLPNDAEE LFIFLTEITE ITIPCRVTD P QLVVTLHEKK GDVALPVPYD HQRGFSGIFE
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 81 YVYRLQVSSI NVSVNAVQTV VRQGENITLM CIVIGNEVVN FEWTYPRKES GRLVEPVTDF
 LLDMPYHIRS ILHIPSAELE
 161 DSGTYTCNVT ESVNDHQDEK AINITVVESG GGGSGGGGSG GGGSGGGGSG GGGSGGGGSE
 VQLVESGGGL VQPGGSLRLS
 241 CAASGYTFTN YGMNWVRQAP GKGLEWVGWI NTYTGEPTYA ADFKRRFTFS LDTSKSTAYL
 QMNSLRAEDT AVYYCAKYPH
 321 YYGSSHWFYF DVWGQGTILVTV SSASTKGPSV FPLAPSSKST SGGTAALGCL VKDYFPEPVT
 VSWNSGALTS GVHTFPAVLQ
 401 SSGLYSLSSV VTVPSSSLGT QTYICNVNHK PSNTKVDKKV EPKSCDKTHT

SEQ ID NO. 30

1 EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW INTYTGEPTY
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 81 LQMNSLRAED TAVYYCAKYP HYYGSSHWFYF DVWGQGTILVT VSSASTKGPS VFPLAPSSKS
 TSGGTAALGC LVKDYPPEPV
 161 TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH KPSNTKVDKK
 VEPKSCDKTH TGGGSGGGGS
 241 GGGSGGGGGS GGGSGGGGGS VGFLPNDAEE LFIFLTEITE ITIPCRVTD P QLVVTLHEKK
 GDVALPVPYD HQRGFSGIFE
 321 DRSYICKTTI GDREVDSDAY YVYRLQVSSI NVSVNAVQTV VRQGENITLM CIVIGNEVVN
 FEWTYPRKES GRLVEPVTDF
 401 LLDMPYHIRS ILHIPSAELE DSGTYTCNVT ESVNDHQDEK AINITVVESG

SEQ ID NO:31 Nucleic acid encoding heavy chain anti-VEGF-PDGFR fusion

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SEQ ID NO:32: Light chain encoding anti-VEGF light chain
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gaagtgacccaccagggcctcagctccccagtgaccaagtcttcaaccggggcgagtgctagta
aTCTAGAGGGCCCGTTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTG
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TAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGG
GCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTA
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GATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTTCGCCCTTTGACGTT
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TCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATT
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TGGGGTTTCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGAGATTTTCGATTCCACCGCC
GCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCG
CGGGGATCTCATGCTGGAGTTCTTCGCCCACCCCAACTTGTTTTATTGCAGCTTATAATGGTTACA
AATAAAGCAATAGCATCACAAATTTACAAATAAAGCATTTTTTTTCACTGCATTCTAGTTGTGGT
TTGTCCAAACTCATCAATGTATCTTATCATGTCTGTATACCGTCGACCTCTAGCTAGAGCTTGGC
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GGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTTCGGTGTAGGTGCTTCGCT
CCAAGCTGGGCTGTGTGCACGAACCCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTAT
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TAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACA
CTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGT
AGCTCTTGATCCGGCAAACAAACACCGCTGGTAGCGGTTTTTTTTTGTTTGCAAGCAGCAGATTAC
GCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGA
ACGAAAACCTCACGTTAAGGGATTTTGGTTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTT

TTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTA
CCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCT
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ATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGC
CGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGAAG
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GTTGAATACTCATACTCTTCCTTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATG
AGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCGCGCACATTTCCCCG
AAAAGTGCCACCTGACGTC

What is claimed:

1. A dual VEGF/PDGF antagonist comprising a VEGF antagonist linked to a PDGF antagonist, wherein the VEGF antagonist (a) is an antibody to a VEGF or VEGFR or (b) is a VEGFR extracellular trap segment and the PDGF antagonist (a) is an antibody to a PDGF or PDGFR or (b) is a PDGFR extracellular trap segment, provided that the VEGF and PDGF antagonists are not both antibodies.
2. The dual antagonist of claim 1, wherein the VEGF antagonist is an antibody comprising a heavy chain and a light chain and the PDGF antagonist is the PDGFR extracellular trap segment, and the heavy chain of the antibody is fused via a linker to the C-terminus of the PDGFR extracellular trap segment, and the light chain is complexed with the heavy chain.
3. The dual antagonist of claim 1 or 2, wherein the antibody is a Fab fragment.
4. The dual antagonist of claim 1, wherein the antibody is an intact antibody.
5. The dual antagonist of any preceding claim 1 wherein the PDGF antagonist is an extracellular trap segment of a PDGFR- α or PDGFR- β receptor and the VEGF antagonist as an antibody to a VEGF.
6. A dual VEGF/PDGF antagonist of any preceding claim wherein the PDGFR extracellular trap segment comprises one or more of domains D1-D5 of PDGFR- β
7. A dual VEGF/PDGF antagonist of claim 6 wherein the PDGFR extracellular trap segment comprises domains D1-D3 of PDGFR- β .
8. A dual VEGF/PDGF antagonist of claim 7 wherein the PDGFR extracellular trap segment comprises amino acids 33 to 314 of SEQ ID NO. 11.
9. A dual VEGF/PDGF antagonist of claim 5-8 wherein the VEGF antagonist comprises an anti-VEGF antibody.

10. A dual VEGF/PDGF antagonist of claim 9, wherein the anti-VEGF antibody is an anti-VEGF-A antibody.

11. A dual VEGF/PDGF antagonist of any of claims 6-10 wherein the PDGFR extracellular trap segment is located C-terminal of the heavy or light chain.

12. A dual VEGF/PDGF antagonist of any of claims 6-10 wherein the PDGFR extracellular trap segment is located N-terminal of the heavy or light chain.

13. A dual VEGF/PDGF antagonist of claim 12 further comprising a linker which is located between the PDGFR trap and the anti-VEGF antibody heavy chain.

14. A dual VEGF/PDGF antagonist of claim 13 wherein the linker is GGGGSGGGGS, GG, or GGGGSGGGGSGGGGSGGGGSG.

15. A dual VEGF/PDGF antagonist of claim 14 wherein the linker is GGGGSGGGGS.

16. A dual VEGF/PDGF antagonist of claim 15 wherein the anti-VEGF antibody heavy chain comprises CDR_H1: GYDFTHYGMN, CDR_H2: WINTYTGEPTYAADFQR, and CDR_H3: YPYYYGTSHWYFDV.

17. A dual VEGF/PDGF antagonist of claim 16 wherein the anti-VEGF light chain comprises CDR_L1: SASQDISNYLN, CDR_L2: FTSSLHS and CDR_L3: QQYSTVPWT.

18. A dual VEGF/PDGF antagonist of claim 17 wherein the anti-VEGF heavy chain isotype is IgG1 comprising a CH₁, hinge, CH₂ and CH₃ domains and the light chain isotype is kappa.

19. A dual VEGF/PDGF antagonist of claim 18 wherein the IgG1 constant domain has the sequence set forth in SEQ ID NO. 17 and the light chain constant region has the sequence set forth in SEQ ID NO. 18.

20. A dual VEGF/PDGF antagonist of claim 19 wherein the IgG1 constant domain has one or more mutations to reduce effector function.

21. A dual VEGF/PDGF antagonist of claim 20 wherein the mutations are to one or more of the following amino acid positions (EU numbering): E233, L234, L235, G236, G237, A327, A330, and P331.

22. A dual VEGF/PDGF antagonist of claim 20 wherein the mutations are selected from the group consisting of: E233P, L234V, L234A, L235A, G237A, A327G, A330S and P331S (EU numbering).

23. A dual VEGF/PDGF antagonist of claim 22 having the following mutations: L234A, L235A and G237A.

24. A dual VEGF/PDGF antagonist of claim 23 wherein the heavy chain further comprises a cysteine residue added by recombinant DNA technology.

25. A dual VEGF/PDGF antagonist of claim 24 wherein the cysteine residue is selected from the group consisting of (EU numbering) Q347C and L443C.

26. A dual VEGF/PDGF antagonist of claim 25 wherein the cysteine residue is L443C.

27. A dual VEGF/PDGF antagonist of claim 26 wherein the heavy chain trap fusion to the extracellular trap segment has an amino acid sequence of SEQ ID NO. 9 and the light chain has an amino acid sequence of SEQ ID NO. 10.

28. A dual VEGF/PDGF antagonist of claim 3 wherein the PDGFR trap comprises one or more of domains D1-D5 of PDGFR- β .

29. A dual VEGF/PDGF antagonist of claim 28 wherein the PDGFR trap comprises domains D1-D3 of PDGFR- β .

30. A dual VEGF/PDGF antagonist of claim 29 wherein the PDGFR trap comprises amino acids 33 to 314 of SEQ ID NO. 11.

31. A dual VEGF/PDGF antagonist of claim 30 wherein the VEGF antagonist comprises an anti-VEGF antibody.

32. A dual VEGF/PDGF antagonist of claim 31 wherein the anti-VEGF Fab fragment comprises an anti-VEGF-A Fab fragment.

33. A dual VEGF/PDGF antagonist of claim 32 wherein the PDGFR trap is located C-terminal of the Fab heavy or light chain.

34. A dual VEGF/PDGF antagonist of claim 33 wherein the PDGFR trap is located N-terminal of the Fab heavy or light chain.

35. A dual VEGF/PDGF antagonist of claim 34 wherein the heavy chain fusion to the trap extracellular segment comprises an anti-VEGF-A Fab fragment heavy chain and the light chain comprises an anti-VEGF-A light chain.

36. A dual VEGF/PDGF antagonist of claim 35 further comprising a linker which is located between the PDGFR trap and the anti-VEGF Fab fragment heavy chain.

37. A dual VEGF/PDGF antagonist of claim 36 wherein the linker is selected from group consisting of GGGGSGGGGS, GG, and GGGGSGGGGSGGGGSGGGGSG.

38. A dual VEGF/PDGF antagonist of claim 37 wherein the linker is GGGGSGGGGS.

39. A dual VEGF/PDGF antagonist of claim 38 wherein the anti-VEGF Fab fragment heavy chain comprises CDR_H1: GYDFTHYGMN, CDR_H2: WINTYTGEPTYAADFQR, and CDR_H3: YPYYGTSHWYFDV.

40. A dual VEGF/PDGF antagonist of claim 39 wherein the anti-VEGF light chain comprises CDR_L1: SASQDISNYLN, CDR_L2: FTSSLHS and CDR_L3: QQYSTVPWT.

41. A dual VEGF/PDGF antagonist of claim 40 wherein the anti-VEGF heavy chain isotype is IgG1 comprising a CH₁ domain and the light chain isotype is kappa.

42. A dual VEGF/PDGF antagonist of any of claims 1-41 further comprising a half-life extending moiety.

43. A dual VEGF/PDGF antagonist of claim 42 wherein the half-life extending moiety is a polymer.

44. A dual VEGF/PDGF antagonist of claim 43 wherein the polymer is selected from the group consisting of PEG and a zwitterionic polymer.

45. A dual VEGF/PDGF antagonist of claim 44 wherein the polymer comprises a zwitterionic polymer.

46. A dual VEGF/PDGF antagonist of claim 45 wherein the zwitterionic polymer comprises a monomer comprising phosphorylcholine.

47. A dual VEGF/PDGF antagonist of claim 46 wherein the monomer comprises 2-(acryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate.

48. A dual VEGF/PDGF antagonist of claim 46 wherein the monomer comprises 2-(methacryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate (HEMA-PC).

49. A dual VEGF/PDGF antagonist of claim 48 wherein the polymer has 3 or more arms.

50. A dual VEGF/PDGF antagonist of claim 49 wherein the polymer has 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 arms.

51. A dual VEGF/PDGF antagonist of claim 50 wherein the polymer has 3, 6 or 9 arms.

52. A dual VEGF/PDGF antagonist of claim 51 wherein the polymer has 9 arms.

53. A dual VEGF/PDGF antagonist of claim 49 wherein the polymer portion of the conjugate has a peak molecular weight of between 300,000 and 1,750,000 Da.

54. A dual VEGF/PDGF antagonist of claim 53 wherein the polymer portion of the conjugate has a peak molecular weight between 500,000 and 1,000,000 Da.

55. A dual VEGF/PDGF antagonist of claim 54 wherein the polymer portion of the conjugate has a peak molecular weight between 600,000 to 800,000 Da.

56. A dual VEGF/PDGF antagonist of claim 55 wherein the polymer has 9 arms.
57. A dual VEGF/PDGF antagonist of any of claims 43 to 56 wherein the dual VEGF/PDGF antagonist is covalently bonded to the polymer.
58. A dual VEGF/PDGF antagonist of claim 57 wherein the polymer is covalently bonded to a moiety selected from the group consisting of an amino group, a hydroxyl group, a sulfhydryl group and a carboxyl group.
59. A dual VEGF/PDGF antagonist of claim 58 wherein the polymer is covalently bonded to a sulfhydryl group.
60. A dual VEGF/PDGF antagonist of claim 59 wherein the sulfhydryl group is from a naturally occurring cysteine residue.
61. A dual VEGF/PDGF antagonist of claim 59 wherein the sulfhydryl group is from a cysteine residue added by recombinant DNA technology.
62. A dual VEGF/PDGF antagonist of claim 61 wherein the polymer is covalently bonded to the cysteine residue at position 731 of SEQ ID NO. 9.
63. A dual VEGF/PDGF antagonist of claim 1 wherein the VEGF antagonist comprises a VEGFR extracellular trap segment comprising one or more extracellular segments of VEGFR-1, VEGFR-2 and VEGFR-3 and the PDGF antagonist is an anti-PDGF antibody.
64. A dual VEGF/PDGF antagonist of claim 63 wherein the extracellular segment of VEGFR comprises one or more of domains D1-D7.
65. A dual VEGF/PDGF antagonist of claim 63 wherein the extracellular segment comprises D2 from VEGFR-1 and D3 from VEGFR-2.
66. A dual VEGF/PDGF antagonist of claim 65 wherein the D2 is N-terminal to the D3 and further comprises a linker between the domains.

67. A dual VEGF/PDGF antagonist of claim 63 wherein the PDGF antagonist is an intact antibody.

68. A dual VEGF/PDGF antagonist of claim 63 wherein the PDGF antagonist is a Fab fragment.

69. A dual VEGF/PDGF antagonist of claim 67 wherein the anti-PDGFR antibody is humanized 2A1E2, HuM4Ts.22, humanized 1B3, humanized 2C5, anti-PDGF-BB, anti-PDGF-DD, anti-PDGF-BB or anti-PDGF-AB.

70. A dual VEGF/PDGF antagonist protein of claim 69 wherein the heavy chain is IgG1 and the light chain is kappa.

71. A dual VEGF/PDGF antagonist of claim 70 wherein the heavy chain has a cysteine selected from the group consisting of Q347C or a L443C.

72. A dual VEGF/PDGF antagonist of claim 71 further comprising a half-life extending moiety conjugated to the cysteine.

73. A dual VEGF/PDGF antagonist protein of claim 72 wherein the half-life extending moiety comprises a zwitterionic polymer, the polymer comprising one or more monomer units and wherein at least one monomer unit comprises a zwitterionic group.

74. A dual VEGF/PDGF antagonist of claim 73 wherein the zwitterionic group comprises phosphorylcholine.

75. A dual VEGF/PDGF antagonist of claim 74 wherein the monomer comprises 2-(acryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate.

76. A dual VEGF/PDGF antagonist of claim 74 wherein the monomer comprises 2-(methacryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate (HEMA-PC).

77. A dual VEGF/PDGF antagonist of claim 76 wherein the polymer has 3 or more arms.

78. A dual VEGF/PDGF antagonist of claim 77 wherein the polymer has 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 arms.

79. A dual VEGF/PDGF antagonist of claim 78 wherein the polymer has 3, 6 or 9 arms.

80. A dual VEGF/PDGF antagonist of claim 79 wherein the polymer has 9 arms.

81. A dual VEGF/PDGF antagonist of claim 80 wherein the polymer portion of the conjugate has a peak molecular weight of between 300,000 and 1,750,000 Da.

82. A dual VEGF/PDGF antagonist of claim 81 wherein the polymer portion of the conjugate has a peak molecular weight between 500,000 and 1,000,000 Da.

83. A dual VEGF/PDGF antagonist of claim 82 wherein the polymer portion of the conjugate has a peak molecular weight between 600,000 to 800,000 Da.

84. A dual VEGF/PDGF antagonist of claim 1 wherein the PDGF antagonists comprises is a PDGF extracellular trap segment comprising one or more extracellular segments of a PDGFR selected from the group consisting of PDGFR- α and PDGFR- β and the VEGF antagonist is a VEGF extracellular trap segment comprising one or more extracellular segments of a VEGFR selected from the group consisting of VEGFR-1, VEGFR-2 and VEGFR-3.

85. A dual VEGF/PDGF antagonist of claim 84 wherein the extracellular trap segment of VEGFR comprises one or more of domains D1-D7.

86. A dual VEGF/PDGF antagonist of claim 85 wherein the extracellular trap segment comprises D2 from VEGFR-1 and D3 from VEGFR-2.

87. A dual VEGF/PDGF antagonist of claim 86 wherein the D2 is N-terminal to the D3 and further comprises a linker between the domains.

88. A dual VEGF/PDGF antagonist of claim 84 wherein the PDGFR trap comprises one or more of domains D1-D5 of PDGFR- β .

89. A dual VEGF/PDGF antagonist of claim 88 wherein the PDGFR trap comprises domains D1-D3 of PDGFR- β .

90. A dual VEGF/PDGF antagonist of claim 89 wherein the PDGFR trap comprises amino acids 33 to 314 of SEQ ID NO. 11.

91. A dual VEGF/PDGF antagonist of claim 90 further comprising a linker sequence between the VEGF antagonist and the PDGF antagonist.

92. A dual VEGF/PDGF antagonist of any of claims 84-91 further comprising a half-life extending moiety.

93. A dual VEGF/PDGF antagonist of claim 92 wherein the half-life extending moiety comprises a polymer selected from the group consisting of PEG and a zwitterionic polymer.

94. A dual VEGF/PDGF antagonist of claim 93 wherein the half-life extending moiety comprises a zwitterionic polymer.

95. A dual VEGF/PDGF antagonist of claim 94 wherein the zwitterionic polymer comprises a monomer comprising phosphorylcholine.

96. A dual VEGF/PDGF antagonist of claim 95 wherein the monomer comprises 2-(acryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate.

97. A dual VEGF/PDGF antagonist of claim 95 wherein the monomer comprises 2-(methacryloyloxyethyl)-2'-(trimethylammoniummethyl) phosphate (HEMA-PC).

98. A dual VEGF/PDGF antagonist of claim 97 wherein the polymer has 3 or more arms.

99. A dual VEGF/PDGF antagonist of claim 98 wherein the polymer has 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 arms.

100. A dual VEGF/PDGF antagonist of claim 99 wherein the polymer has 3, 6 or 9 arms.

101. A dual VEGF/PDGF antagonist of claim 100 wherein the polymer has 9 arms.

102. A dual VEGF/PDGF antagonist of claim 99 wherein the polymer portion of the conjugate has a peak molecular weight of between 300,000 and 1,750,000 Da.

103. A dual VEGF/PDGF antagonist of claim 102 wherein the polymer portion of the conjugate has a peak molecular weight between 500,000 and 1,000,000 Da.

104. A dual VEGF/PDGF antagonist of claim 103 wherein the polymer portion of the conjugate has a peak molecular weight between 600,000 to 800,000 Da.

105. A dual VEGF/PDGF antagonist of claim 104 wherein the polymer has 9 arms.

106. A dual VEGF/PDGF antagonist according to claim 5 wherein the extracellular trap segment binds to one or more of PDGF-AA, PDGF-BB, PDGF-AB, PDGF-CC and PDGF-DD.

107. A dual VEGF/PDGF antagonist according to claim 107 wherein the extracellular trap binds PDGF-AB, PDGF-BB and PDGF-DD.

108. A dual VEGF/PDGF antagonist according to claim 108 wherein the extracellular trap inhibits PDGF-AB, PDGF-BB and PDGF-DD from binding to any one of PDGFR- $\alpha\alpha$, PDGFR- $\alpha\beta$, and PDGFR- $\beta\beta$ receptors.

109. A method of purifying a dual VEGF/PDGF antagonist comprising:

contacting the dual VEGF/PDGF antagonist with an affinity chromatography residue that binds to the dual VEGF/PDGF antagonist;

eluting the dual VEGF/PDGF antagonist from the affinity chromatography resin to form an affinity chromatography eluent comprising the dual VEGF/PDGF antagonist;

contacting the affinity chromatography eluent with an ion-exchange resin that binds the dual VEGF/PDGF antagonist; and

eluting the dual VEGF/PDGF antagonist from the ion exchange resin.

110. A method according to claim 110 wherein the ion exchange resin is an anion exchange resin

111. A method according to claim 110 wherein the ion exchange resin is a cation exchange resin.

112. A method according to any of claims 110, 111 and 112 wherein the affinity chromatography resin is a Protein A column.

113. A liquid pharmaceutical composition comprising:

a pharmaceutically acceptable excipient; and at least one dual VEGF/PDGF antagonist wherein the composition has less than 0.2 EU/ml.

114. A liquid pharmaceutical composition according to claim 114 wherein the composition has less than 0.1 EU/ml.

115. A liquid pharmaceutical composition according to claim 115 wherein the composition has less than 0.05 EU/ml.

116. A dual VEGF/PDGF antagonist according to claim 48 wherein the polymer has a molecular weight measured by Mw of between about 100 kDa and 1500 kDa.

117. A dual VEGF/PDGF antagonist according to claim 116 wherein the polymer is made from an initiator suitable for ATRP having one or more polymer initiation sites.

118. A dual VEGF/PDGF antagonist according to claim 117 wherein the initiator has 3 or more polymer initiation sites.

119. A dual VEGF/PDGF antagonist according to claim 118 wherein the initiator has 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 polymer initiation sites.

120. A dual VEGF/PDGF antagonist according to claim 119 wherein the initiator has 3, 6 or 9 polymer initiation sites.

121. A dual VEGF/PDGF antagonist according to claim 120 wherein the initiator has 9 polymer initiation sites.

122. A dual VEGF/PDGF antagonist according to claim 121 wherein the initiator is OG1786.

123. A dual VEGF/PDGF antagonist according to claim 116 wherein the polymer has a molecular weight measured by Mw of between about 500 kDa and 1000 kDa.

124. A dual VEGF/PDGF antagonist according to claim 123 wherein the polymer is made from an initiator suitable for ATRP having one or more polymer initiation sites.

125. A dual VEGF/PDGF antagonist according to claim 124 wherein the initiator has 3 or more polymer initiation sites.

126. A dual VEGF/PDGF antagonist according to claim 125 wherein the initiator has 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 polymer initiation sites.

127. A dual VEGF/PDGF antagonist according to claim 126 wherein the initiator has 3, 6 or 9 polymer initiation sites.

128. A dual VEGF/PDGF antagonist according to claim 127 wherein the initiator has 9 polymer initiation sites.

129. A dual VEGF/PDGF antagonist according to claim 128 wherein the initiator is OG1786.

130. A dual VEGF/PDGF antagonist according to claim 125 wherein the polymer has a molecular weight measured by Mw of between about 600 kDa to about 900 kDa.

131. A dual VEGF/PDGF antagonist according to claim 130 wherein the polymer is made from an initiator suitable for ATRP having one or more polymer initiation sites.

132. A dual VEGF/PDGF antagonist according to claim 131 wherein the initiator has 3 or more polymer initiation sites.

133. A dual VEGF/PDGF antagonist according to claim 132 where in the imitator has 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 polymer initiation sites.

134. A dual VEGF/PDGF antagonist according to claim 133 wherein the initiator has 3, 6 or 9 polymer initiation sites.

135. A dual VEGF/PDGF antagonist according to claim 134 wherein the initiator has 9 polymer initiation sites.

136. A dual VEGF/PDGF antagonist according to claim 135 wherein the initiator is OG1786.

137. A dual VEGF/PDGF antagonist according to claim 116 wherein the polymer has a molecular weight measured by Mw of 750 kDa plus or minus 15%.

138. A dual VEGF/PDGF antagonist according to claim 137 wherein the polymer is made from an initiator suitable for ATRP having one or more polymer initiation sites.

139. A dual VEGF/PDGF antagonist according to claim 140 wherein the initiator has 3 or more polymer initiation sites.

140. A dual VEGF/PDGF antagonist according to claim 139 where in the imitator has 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 polymer initiation sites.

141. A dual VEGF/PDGF antagonist according to claim 140 wherein the initiator has 3, 6 or 9 polymer initiation sites.

142. A dual VEGF/PDGF antagonist according to claim 141 wherein the initiator has 9 polymer initiation sites.

143. A dual VEGF/PDGF antagonist according to claim 142 wherein the initiator is OG1786.

144. A dual VEGF/PDGF antagonist as described in any preceding claim for treatment or prophylaxis of disease.

145. A dual VEGF/PDGF antagonist as described in any preceding claim for treatment or prophylaxis of a neovascular disorder, optionally an ocular neovascular disorder, such as wet age related macular degeneration.

1 MRLPGAMPAL ALKGELLLLS LLLLLLEPQIS QGLVVTTPGP ELVLNVSSTF VLTCSGSAPV
 61 VWERMSQEPP QEMAKAQDGT FSSVLTLTNL TGLDTGEYFC THNDSRGLET DERKRLYIFV
 121 PDPTVGFLPN DAEELFIPLT EITEITIPCR VTDPLVVTL HEKKGVALP VPDHQRGFS
 181 GIFEDRSYIC KTTIGDREVD SDAYVYRLQ VSSINVSVA VQTVVROGEN ITLMCIVIGN
 241 EVVNFETYP RKESGRIVEP VTDFLLDMPY HIRSILHIPS AELEDSTYT CNVTESVNDH
 301 QDEKAINITV VESGYVRLG EVGTLQFAEL HRSRTLQVVF EAYPPPTVLW FKDNRTLGD
 361 SAGEIALSTR NVSETRYVSE LTLVRVKVAE AGHYTMRAFH EDAEVQLSFQ LQINVPVRVL
 421 ELSESHPDG EQTVRCRGRG MPQPNIIWSA CRDLKRCPRE LPPTLLGNSS EESOLETNV
 481 TYWEEQEFV VVSTLRLQHV DRPLSVRCTL RNAVGQDTQE VIVVPHSLPF KVVVISAILA
 541 LVVLTIIISLI ILIMLWQKP RYEIRWKVIE SVSSDGHEYI YVDPMLPYD STWELPRDQ
 601 VLGRTLGSAG FGQVVEATAH GLSHSQATMK VAVKMLKSTA RSSEKQALMS ELKIMSHLGP
 661 HLNVVNLLGA CTGGPIYII TEYCRYGDLV DYLRNKHTE LQHHSDKRRP PSAELYSNAL
 721 PVGLPLPSHV SLTGESDGGY MDMSKDESVD YVPLDMKGD VKYADIESSN YMAPYDNYVP
 781 SAPERTCRAT LINEPVLSY MDLVGFSYQV ANGMEFLASK NCVHRDLAAR NVLICEGKLV
 841 KICDFGLARD IMRDSNYISK GSTFLPLKWM APESIFNSLY TTLSDVWSFG ILLWEIFTLG
 901 GTPYPELPMN EQFYNAIKRG YRMAQPAHAS DEIYEIMQKC WEEKFEIRPP FSQVLVLLER
 961 LLGEGYKKKY QQVDEEFLRS DHPAILRSQA RLPGFHGLRS PLDTSSVLYT AVQPNEGDN
 1021 YIIPLDPKP EVADEGPLEG SPSLASSTLN EVNTSSTISC DSPLEPQDEP EPEPQLELQV
 1081 EPEPELEQLP DSGCPAPRAE AEDSFL (SEQ. ID No.: 33)

FIG. 1

1 MVSYNWDTGVL LCALLSCILL TGSSSGSKLK DPESLKGTO HIMQAGQTLH LQCRGEAAHK WSLPEMVSKE SERLSITKSA
 81 CGRNGKQFCS TLTINTAQAN HTGPFYSCKYL AVPTSKKKEF ESAIYIFISD TGRPFVEMYS EPEIIHMT ERELVIPCRV
 161 TSPNITVTIK KFPLDTLIPD GKRIWDSRK GFIIISNATYK EIGLLTCEAT VNGHLYKTNV LTHRQNTNII DVQISTPRPV
 241 KLLRGHTLV L NCTATTPLNT RVQNTWSYPD EKNKRASVR RRDQSNSHAN IFYSLVTIDK MONKDKGLYT CRVRSRGPSEK
 321 SVNTSVHIYD KAFITVKHRK QOVLETVAGK RSYRLSMKVK AFPSPEVWL KDGLPATEKS ARYLTRGYSL IIKDVTEEDA
 401 GNYTILLSIK QSNVFNLT TLIWVVKPQI YEKAVSSFPD PALYPLGSRQ ILTCTAYGIP OPTIKWFWHP CNHNSHSEARC
 481 DFCSNNEESF ILDADSNMGN RIESITQMA IIEGKNKMAS TLWADSRIS GIYICIASNK VGTVGRNISE YITDVPNGPH
 561 VNLEKMPTEG EDLKLCTVN KFLYRDVTWI LLRTVNNRTM HYSISKQKMA ITKEHSITLN LTIMNVSLQD SGTYACRARN
 641 VYTGEIILQK KEITIRDQEA PYLLRNLSH TVAISSSTL DCHANGVPEP QITWFKNNHK IOQEPGIILG PGSSTLFIER
 721 VTEDEGVYH CKATNQKGSV ESSAYLTVQG TSDKSNLELI TLTCTCVAAT LFWLLITLFI RKMKRSSSEI KTDYLSIIMD
 801 PDEVPLDEQC ERLPYDASKW EFARERLKLK KSLGRGAFGK VVQASAFGK KSPTCRTVAV KMLKEGATAS EYKALMTELK
 881 ILTHIGHHLN VVNLIGACTK QGGPLMVIVE YCKYGNLSNY LKSKRDLFFL NKDAALHMEP KKEKMEPGLE QGKKPRLDSV
 961 TSSESFASSG FQEDKSLSDV EEEEDSDGFY KEPITMEDLI SYSEQVARGM EFLSSRKCIH RDLAARNILL SENNVVKICD
 1041 FGLARDIYKN PDYVRKGDTR LPLKNWAPES IFDKIYSTKS DVWSYGVLLW EIFSLGGSPY PGVQWDEDFC SRLREGMRMR
 1121 APEYSTPEIY QIMLDCWHRD PKERPRFAEL VEKLGDLLOA NVQODGKDYI PINAILTGNS GFTYSTPAFS EDDFFKESISA
 1201 PKPNSGSSDD VRYVNAFKFM SLERIKTFEE LLPNATSMED DYQGDSSILL ASPMLKRFTW TDSKPKASLK IDLRVTSKSK
 1281 ESGLSDVSRP SFCHSSCGHV SEGKRRFTYD HAELEKRIAC CSPPPDYNV VLYSTPPI (SEQ. ID NO.:34)

FIG. 2

1 MQSKVLLAVA LMLCVETRAA SVGLPSVS LD LPRLSIQKDI LTIKANTTLQ ITCRGQDLD WLPNNOQSG EQRVEVTECS
 81 DELPCKTLTI PKVIGNDTGA YKCFYREYDL ASVIYVYVQD YRSPFIASVS DOHGVVYITE NKNKTVVIPC LGSISNLNVS
 161 LCARYPEKRF VPDGNRISWD SKKGFTIPSY MisyAGWFC EAKINDESYQ SIMYIVVVG YRIYDVVLSP SHGIELSVGE
 241 KLVNCTART ELNVGIDFNW EYPSSKHQHK KLVNRDLKTQ SGSEMKKELS TLTIDGVTRS DQGLYTCAAS SGLMTKKNST
 321 FVRVHEKPFV AFGSMESLV EATVGERVRI PAKYLGYPPI EIKWYKNGIP LESNHTIKAG HVLTIMEVSE RDTGNYTVIL
 401 TNPISEKQOS HVVSLVVVVP PQIGEKSLIS PVDSYQYGT QTLTCTVYAI PPPHHIHWY QLEEECANEP SQAVSVTNPY
 481 PCEWRSVED FQGNKIEVN KNQFALIECK NKTVSTLVIQ AANVSALYKC EAVNKVGRGE RVISEHVTRG PEITLQPDNQ
 561 PTEQESVSLW CTADRSTFEN LTWYKLGQPQ LPIHVGE LPT PVCKNLDTLW KLNATMFNS TNDILIMELK NASLODQGDY
 641 VCLAQDRKTK KRHCVVVRQLT VLERVAPTIT GNLENQTTSI GESIEVSC TA SGNPPQIMW FKDNETLVED SGIVLKDGNR
 721 NLTIRVRKE DEGLYTCQAC SVLGCAKVEA PFIIEGAQEK TNLEIILVG TAVIAMFFWL LLVILRTVK RANGGELKYG
 801 YLSIVMDPDE LPLDEHCERL PYDASKWEFP RDRKLKCKPL GRGAFQVIE ADAFGIDKTA TCRTVAUVKML KEGATHSEHR
 881 ALMSELKILI HIGHHLNVVN LIGACTKPGG PLMVIVEFCK FGNLSTYLS KRNEFVVPYKT KGARFRQCKD YVGAIPVDLK
 961 RRLDSITSSQ SSASSGFVEE KSLSDVEEEE APEDLYKDFL TLEHLICYSF QVAKGMEFLA SRKCIHRDLA ARNILLSEKN
 1041 VVKICDFGLA RDIYKDPDYV RKGDA RLPLK WMAPETIFDR VYTIQSDVMS FGVLLWEIFS LGASPYPGVK IDEEFCRRLK
 1121 EGTRMRAPDY TTPEMYQTML DCWHGEPsQR PTFSELVEHL GNLLQANAQQ DGKDYIVLPI SETLSMEEDS GLSLPTSPVS
 1201 CMEEEEVCDP KFYDNTAGI SQYLQNSKRK SRPVSVKTFE DIPLEEPVK VIPDDNQTDs GNVLASEELK TLEDRTKLSP
 1281 SFGGMVPSKS RESVASEGSN QTSGYQSGYH SDDTDTTVYS SEEAELKLI EIGVQTGSTA QILQPDsGTT LSSPPV (SEQ. ID No.: 35)

FIG. 3

1 MQRGAALCLR LNLCLGLLDG LVSGYSMTTPP TLNITEESHV IDTGDSLSIS CRGQHLEWA WPGAQEAPAT GDKDSEDTCV
81 VRDCEGTDAR PYCKVLLLHE VHNDTGSYV CYYKIYKARI EGTAAASSYV FVRDFEQPFI NKPDILLVNR KDAMWVPCLV
161 SIPGLNVTLR SQSSVLWPDG QEVVWDDRRG MLVSTPLIHD ALYLQCETTW GDQDFLSNPF LVHITGNELY DIQLPRKSL
241 ELLVGEKLV NCTVWAEFNS GVTFDNDYPG QOERCKWVP ERRSQQTHTE LSSILTIHNV SOHDLGSYVC KANNGIQRF
321 ESTEVIVHEN PFISVENLKG PILEATAGDE LVKLPVKLAA YPPPEQWYK DGKALSGRHS PHALVLKEVT EASTGTYTLA
401 LWNAAGLRR NISLELVNV PPQIHEKEAS SPSIYSRHSR QALTCTAYGV PLPLSIQWHW RPWTPCKMFA QRSLSRRRQOQ
481 DLMPOCRDWR AVTTQDAVNP IESLDTWTEF VEGKNKTVSK LVIQNANVSA MYKCVVSNKV GQDERLIIFY VTTIPDGFTI
561 ESKPSEELLE GOPVLLSCQA DSYKVEHLRW YRLNLSTLHD AHGNPLLLDC KNVHLFATPL AASLEEVAPG ARHATLSLSI
641 PRVAPEHEGH YVCEVQDRRS HDKCHCKYL SVOALEAPRL TQNLFDLLN VSDSLEMQL VAGAHAPSIV WYKDERLLEE
721 KSGVDLADSN QKLSIQRVRE EDAGRYLCV CNAKGCVNSS ASVAVEGSED KGSMEIVILV GTGVIAVFFW VLLLLIFCNM
801 RRPAAHADIKT GYLSIINDPG EVPLEEQCEY LSYDASQWEP PRERLHLGRV LGYGAFGKVV EASAFGIHKG SSCDTVAVKM
881 LKEGATASEH RALMSELKIL IHIGNHLNVV NLLGACTKPQ GPLMVTIVEFC KYGNLSNFLR AKRDAFSPCA EKSPEQGRF
961 RAMVELARLD RRRPGSSDRV LEARFSKTEG GARRASPDQE AEDLWLSPLT MEDLVCYSFQ VARGMEFLAS RACIHRDLAA
1041 RNILLSESDV VKICDFGLAR DIYKDPDYVR KGSARLPLKW MAPESIFDKV YTTQSDVWSF GVLLWEIFSL GASPYPGVQI
1121 NEEFQORLDR GTRMRAPELA TPAIRRIMLN CWSGDPKARP AFSELVEILG DLLQGRGLQE EEEVCMAPRS SQSSEEGSFS
1201 QVSTMALHIA QADAEDSPPS LQHSIAARY YNWVSFPGCL ARGAETRGSS RMKTFEEFPM TPTTYKGSVD NQTDSCNVLA
1281 SEEFQIESR HRQESGFSCX GPGQNVAVTR AHPDSQGRRR RPERGARGGQ VFYNSEYGEL SEPSEEDHCS PSARVTFTTD
1361 NSY (SEQ. ID No.: 36)

FIG. 4

A. LIGHT CHAIN

1 DIQMTQSPSS LSASVGDRVIT ITCASQDIS NYLNWYQOKP GKAPKVLIIYF TSSLHSGVPS RFSGSGSGTD FTLTISSLPQ
81 EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFFP SDEQLKSGTA SVVCLLNIFY PREAKVQWKV DNALQSGNSQ
161 ESVTEQDSKD STYSLSSLT LSKADYEKKH VYACEVTHQG LSSPVTKSFN RGEN (SEQ. ID No.: 5)

B. HEAVY CHAIN

1 EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW INTYTGEPTY AAEKRRFTF SLDTSKSTAY
81 LQMSLRAED TAVYYCAKYP HYGSSHWF DVWGQGLTVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFFEPV
161 TVSWNSGALT SGVHTFPAVL QSSGLYSLS VVTPSSSLG TQTYICNVNH KPSNTKVDKK VEPKSCDKTH TCPPCPAPEL
241 LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL
321 NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFPY SDIAVEWESN GQENNYKTT
401 PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTKSLSLS PGK (SEQ. ID No.: 2)

FIG. 5

A. Light Chain Sequence

1 DIQMTQSPSS LSASVGDRVIT ITCASQDIS NYLNWYQOKP GKAPKVLIIYF TSSLHSGVPS RFSGSGSGTD FTLTISSLPQ
81 EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFFP SDEQLKSGTA SVVCLLNIFY PREAKVQWKV DNALQSGNSQ
161 ESVTEQDSKD STYSLSSLT LSKADYEKKH VYACEVTHQG LSSPVTKSFN RGEN (SEQ. ID No.: 12)

B. Heavy Chain Sequence

1 EVQLVESGGG LVQPGGSLRL SCAASGYDFT NYGMNWVRQA PGKGLEWVGW INTYTGEPTY AAEKRRFTF SLDTSKSTAY
81 LQMSLRAED TAVYYCAKYP YYIGTSHWF DVWGQGLTVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFFEPV
161 TVSWNSGALT SGVHTFPAVL QSSGLYSLS VVTPSSSLG TQTYICNVNH KPSNTKVDKK VEPKSCDKTH L (SEQ. ID No.: 13)

FIG. 6

PDGFR-GS10-ANTI-VEGF-A LIGHT CHAIN:

1 LVVTPPGPEL VLVNSSTFVL TCSGAPVW ERMSQEPPOE MAKAOQGTF S VLTLTNLTG LDTGEYFCTH NDSRGLETDE
81 RKRLYIFVPD PTVGFLPND A EELFIFLTEI TEITIPCRVT DPQLVVTLHE KKG DVALPVP YDHQGFSGI FEDRSYICKT
161 TIGDREVDSD AYYVYRLQVS SINVSNAVQ TVVRQGENIT LMCIVIGNEV VNFETYP RK ESGRLVEPVT DFLDMPYHI
241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVVE SGGGGSGGG GSDIQMTQSP SLSASVGDR VTITCSASQD
321 ISNYLNWYQQ KPGKAPKVL I YFTSSLHSGV PSRPSGSGG TDFTLFISSL QPEDFATYYC QQYSTVPWTF GQGTKVEIKR
401 TVAAPSVFIF PPSDEQLKSG TASVVCLLN FYPREAKVQW KVDNALQSGN SQESVTEQDS KDSTYSLSST LTLSKADYEK
481 HKVIACEVTH QGLSSPVTKS FNRGEC (SEQ. ID No.: 19)

FIG. 7A

ANTI-VEGF-A HEAVY CHAIN (WILD TYPE Fc):

1 EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PKGLEWVGW INTYTGEPTY AADFKRRFTF SLDTSKSTAY
81 LQMNLSRAED TAVYYCAKYP HYYGSSHWF DVWGQGLT VT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV
161 TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTPSSSLG TQYICNVNH KPSNTKVDKK VEPKSCDKTH TCPPCPAPEL
241 LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNNYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDNL
321 NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNOVS LTCLVKGFYP SDIAVENWESN GQPENNYKTT
401 PPVLDSGSGF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PKG (SEQ. ID No.: 2)

FIG. 7B

PDGFR-GG-ANTI-VEGF-A LIGHT CHAIN:

1 LVVTPPGPEL VLNVSSTFVL TCSGSAPVVW ERMSQEPPOE MAKAQDGTFS SVLTNLNLTG LDTGEYFCTH NDSRGLETDE
81 RKRLYIFVPD PTVGFLPND^A EELFIFL^{TEI} TELTIPCRVT DPQLVVTLHE KKG^DVALPVP YDHQGFESGI FEDRSYICKT
161 TIGDREVDSD AYYVYRLQVS SINVS^VNAVQ TVVRQENIT LMCIVIGNEV VNEFWTYPRK ESGRLVEPV^T DFLLDMPYHI
241 RSILHIPSAE LEDSGTYTCN VTESVNDHOD EKAINITVVE SGGDIQNTQ SPSSLSASVG DRVTITCSAS ODISNYLNWY
321 QOKPGKAPKV LIYFTSSLHS GVPSEFSGSG SGTDFTLTIS SLOPE^DFATY YCQOYSTVPW TFGQGT^KVEI KRTVAAPS^VF
401 IFPPSDEQLK SGTASVVCLL NNFPYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV
481 THQGLSSPVT KSENRGEC (SEQ. ID No.: 3)

FIG. 8A

ANTI-VEGF-A HEAVY CHAIN (WILD TYPE Fc):

1 EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW INTYTGEPTY AADFKRRR^{FTF} SLDTSKSTAY
81 LQMN^SLRAED TAVYICAKYP HYGSSHWYF DVWGO^GTLVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV
161 TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTPPSSSLG TQTYICNVNH KPSNTKVDK^K VEPKSCDKTH TCP^{PC}PAPEL
241 LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHODWL
321 NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQENNYK^{TT}
401 PPVLDS^DGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTKSLSL^S PKG (SEQ. ID No.: 2)

FIG. 8B

PDGFR-GS10-ANTI-VEGF-A HEAVY CHAIN (WILD TYPE Fc):

1 LVVTPGPPEL VLNVSSTFVL TCSGSAPVWV ERMSQEPPOE MAKQDGTFS SVLTLTNLTG LDTGEYFCTH NDSRGLETDE
81 RKRLYIFVPD PTVGFLPND A EELFIELTEI TEITIPCRVT DPQLVVTLHE KKGDVALPVP YDHQRCFSGI FEDRSYICKT
161 TIGDREVDSD AYYVYRLQVS SINVSNAVQ TVVRQGENIT LNCIVIGNEV VNFENTYPRK ESGRLVEPVT DFLDMPYHI
241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVE SCGGGGSGGG GSEVQLVESG GGLVQPGGSL RLSCAASGYT
321 FTNYGMNWVR QAPGKGLEWV GWINTYTGEPT YAADFRRF TFSLDTSKST AYLOMNSLRA EDTAVVYCAK YPHYYGSSHW
401 YEDVWGQGT L VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
481 SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV
561 VVDVSHEDPE VKFNWYVDGV EVHNATKPR EEQYNSTYRV VSVLTVLHQD WLNKKEYKCK VSNKALPAPI ERTISKAKGQ
641 PREPQVYTL P PSREEMTKNQ VSLTCLVKGF YPSDIAVENE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQOGNV
721 FSCSVNHEAL HNHVYQKSL S LSPGK (SEQ. ID No.: 4)

FIG. 9A

ANTI-VEGF LIGHT CHAIN:

1 DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQKP GKAPKVL IYF TSSLHSGVPS RFSGSGSGTD FTLTISSLQP
81 EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSCTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ
161 ESVTEQDSKD STYSLSSLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN R GEC (SEQ. ID No.: 5)

FIG. 9B

PDGFR-GG-ANTI-VEGF-A HEAVY CHAIN (WILD TYPE Fc):

1 LVVTPGPPEL VLVNSTFVL TCGSAPVW ERMSQEPPOE MAKAQDGTF SVLTFLNLTG LDTGEYFCTH NDSRGLETDE
81 RKRLYIFVPD PTVGFLPND EELFIFLTEI TEITPCRVIT DPQLVVTLHE KKGDVALPVP YDHQRGFSGI FEDRSYICKT
161 TIGDREVDSD AYYVYRLQVS SINVSUNAVQ TVVROGENIT LMCIVIGNEV VNFETYPYRK ESGRLVEPVT DFLLDMPYHI
241 RSILHIPS AE LEDSGTYTCN VTESVNDHQD EKAINITVVE SGGGLVQPGG SLRLSCAASG YTFNNGMNV
321 VRQAPGKGLE WVGWINITYTG EPTYAADFKR RFTFSLDTSK STAYLQMNLS RAEDTAVYYC AKYPHYYGSS HWYFDVWGQG
401 TLVTVSSAST KGPSVFFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPS
481 SSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKTHTCPPCP APELLGGSV FLFPPKPKDT LMISRTPEVT CWVVDVSHED
561 PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYTT
641 LPPSREEMTK NOVSLTCLVK GFYPSDIAVE WESNGOPENN YKTTTPPVLD DGSFFLYSKL TVDKSRWQOG NVFSCSVNHE
721 ALHNHYTQKS LSLSPGK (SEQ. ID No.: 6)

FIG. 10A

ANTI-VEGF LIGHT CHAIN:

1 DIQMTQSPSS LSASVGDRVIT TCSASQDIS NYLNWYQQKPK GKAPKVLIVF TSSLHSGVPS RFSGSGSGTD FTLTISSLPQ
81 EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLINNFY PREAKVQWKV DNALQSGNSQ
161 ESVTEQDSKD STYSLSSLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGE C (SEQ. ID No.: 5)

FIG. 10B

ANTI-VEGF-A HEAVY CHAIN (WILD TYPE Fc)-GS21-PDGFR-β TRAP:

1 EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW INTYTGEPTY AADFRRFTF SLDTSKSTAY
81 LQMNSLRAED TAVYYCAKYP HYGSSHWF DWGQGLTIVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV
161 TVSWNSGALT SGVHTFPAVL QSSGLYSLS VVTVPSSLG TQYICNVNH KPSNTKVDKK VEPKSCDKTH TCPPCPAPEL
241 LGGPSVFLFP PKPKDTLMIS RTPETCVV DVSHEDPEVK FNNYVDGVEV HNAKTKPREE QYNSTYRVVS VLTVLHQDWL
321 NGKEYCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS REEMTKNOVS LTCLVKGFYP SDIAVEWESN GOPENNYKTT
401 PPVLDSDGSF FLYSKLTVDX SRWQGNVFS CSVMHEALHN HYTKLSLSL PGGGGSGGG GSGLVVTPPG
481 PELVLNVST FVLTCGSAP VVWRMSQEP PQEMAKAQDG TFSSVLTLTN LTGLDTGEYF CTHNDSRGLE TDERKRLYIF
561 VPDPTVGFPL NDAEELFIFL TEITEITPC RVTDPQLVVT LHEKKGVAL PVPYDHQGF SGIFEDRSYI CKTTIGDREV
641 DSDAYVYRL QVSSINSVN AVQTVVRQGE NITLMCIVIG NEVNFEWTY PRKESGRIVE PVTDFLLDMP YHIRSILHIP
721 SAELEDSTGY TCNVTESVND HQDEKAINIT VVSG (SEQ. ID No.: 7)

FIG. 11A

ANTI-VEGF LIGHT CHAIN:

1 DIQMTQSPSS LSASVGRVT ITCASQDIS NYLNWYQQK GKAPKVLIVT TSSLHSGVPS RFSGSGGTD FTLTISSLQP
81 EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY PREAKVQWKV DNALQSGNSQ
161 ESVTEQDSKD STYSLSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEK (SEQ. ID No.: 5)

FIG. 11B

PDGFR-β-GS10-ANTI-VEGF-A HEAVY CHAIN (Q347C):

1 LVVTPPGPEL VLNVSSTFVL TCSGSAPVWV ERMSQEPPOE MAKAOQGTFE SVLTILTNLTG LDTGEYFCTH NDSRGLETDE
81 RKRLYIFVPD PTVGFLPDA EELFIFLITEI TEITIPCRVT DPQLVVTLHE KKGDVALLPVP YDHQGFSGI FEDRSYICKT
161 TIGDREVDSD AYYVYRLQVS SINVSNAVQ TVVRQGENIT LMCIVIGNEV VNFENTYPRK ESGRLVEPVT DFLIDMPYHI
241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVE SGGGGSGGG GSEVOLVESG GGLVOPGGSL RLSCAASGYD
321 FTHYGMNWR QAPGKGLEWV GWINTYTGEY TYAADFKRRF TFSLDTSKST AYLQMNLSRA EDTAVYYCAK YPYYYGTSHW
401 YFDVWGQGTI VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LFSGVHTFPA VLQSSGLYSL
481 SSVVTVPPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THTCPPCPAP EAAGAPSVFL FPPKPKDTLM ISRTPEVTCV
561 VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLIHQD WLNKGKEYCK VSNKALPAPI EKTISKAKGQ
641 PREPCVYTL PPSREEMTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SPFLYSKLTIV DKSRWQQGNV
721 FSCSVMEAL HNHYTQKSL SLPCK (SEQ. ID No.: 8)

FIG. 12A

ANTI-VEGF LIGHT CHAIN:

1 DIQLTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQKP GKAPKVLIVF TSSLHSGVPS RFSGSGSGTD FTLTISSLOP
81 EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ
161 ESVTEQDSKD STYSLSSLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEK (SEQ. ID No.: 12)

FIG. 12B

PDGFR-GS10-ANTI-VEGF-A HEAVY CHAIN (L443C):

1 LVTTPGPEL VLVSSSTFVL TCSSGAPVWV ERMSQEPPOE MAKQDGTFS SVLTLTNLTG LDTGEYFCTH NDSRGLETDE
81 RKRLYIFVPD PTVGFLPND A EELFIFLTEI TEITIPCRVT DPQLVVTLHE KKGDAVALPV YDEHQRGFSGI FEDRSYICKT
161 TIGDREVDSD AYYVVRLOVS SINVSNAVQ TVVRQGENIT LMCIVIGNEV VNFETTYPRK ESGRLVEPVT DFLDMPYHI
241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVE SGGGGGGGG GSEVQLVESG GGLVQPGSL RLSCAASGYD
321 FTHYGMNWR QAPCKGLEWV GWINTYTGEF TYAADFKRRF TFSLDTSKST AYLOMNSLRA EDTAVYICAK YPYYYGTSHW
401 YFDVWGQGT L VTVSSASTKG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSNNSGA LTSGVHTFPA VLQSSGLYSL
481 SSVTVTPSSS LGTQTYICNV NEKPSNTKVD KKVEPKSCDK THTCPPCPAP EAAGAPSVFL FPPKPKDTLM ISRTPEVTCV
561 VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLVNGKEYCK VSNKALPAPI EKTISKAKGQ
641 PREPQVYTL P PSREMTKNQ VSLTCLVKGF YPSDIAVEWE SNGOPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
721 FSCSVNHEAL HNHYTQKSL S CSPGK (SEQ. ID No.: 9)

FIG. 13A

ANTI-VEGF LIGHT CHAIN:

1 DIQLTQSPSS LSASVGDRVT ITCSASQDIS NYLWVYQKP GKAPKVLIF TSSLHSGVPS RFGSGSGTD FTLTISLQ
81 EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY PREAKVQWV DNALQSGNSQ
161 ESVTEQDSKD STYLSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGE C (SEQ. ID No.: 12)

FIG. 13B

PDGFR-GS10-ANTI-VEGF-A LIGHT CHAIN:

1 LVVTPPGPEL VLNVSSTFVL TCSGAPVWV ERMSQEPPOE MAKAOQGTF S VLTLTNLTG LDTGEYFCTH NDSRGLETDE
81 RXRLYIFVPD PTVGELPND A EELFIFLFEI TEITIPCRVT DPQLVVTLHE KKGDVALPVP YDHQGFSGI FEDRSYICKT
161 TIGDREVDSD AYYVYRLQVS SINVSNAVQ TVVRQGENIT LMCIVIGNEV VNFETYP RK ESGRLVEPVT DFLDMPYHI
241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVE SGGGGSGGG GSDIQMTQSP SLSASVGDR VTITCSASQD
321 ISNVLNWIQQ KPGKAPKVL I YFTSSLHSGV PSRFGSGSG TDETLTISL QPEDFATYYC QQYSTVPWTF GQGTKVEIKR
401 TVAAPSVFIF PPSDEQLKSG TASVVCLLN FYPREAKVQW KVDNALQSGN SQESVTEQDS KDSTYSLSST LTLSKADYEX
481 HKVYACEVTH QGLSSPVTKS ENRGEC (SEQ. ID No.: 1)

FIG. 14A

ANTI-VEGF-A FAB:

1 EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWRQA PGKGLEWVGW INTYTGEPTY AADFRRRFTF SLDTSKSTAY
81 LQMNSLRAED TAVYCAKYP HYIGSSHWF DVWGQGLVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYPPEPV
161 TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTFPSSLG TQTYICNVNH KPSNTKVDKK VEPKSCDKTH T (SEQ. ID No.: 21)

FIG. 14B

PDGFR-GG-ANTI-VEGF-A LIGHT CHAIN:

1 LVVTPPGPEL VLVSSTFVL TCSGSAFVW ERMSQEPPOE MAKAOQGTFE SVLTILNLTG LDTGEYFCTH NDSRGLETDE
81 RKRLYIFVPD PTVGFPLPDA EELFIFLFEI TEITIPCRVT DPQLVVTLHE KKGDVALLPV YDHQGFSGI FEDRSYICKT
161 TIGDREVDSD AYYVYRLQVS SINVSNAVQ TVVRQENIT LMCIVIGNEV VNFETYPYRK ESGRLVEPVT DFLIDMPYHI
241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVE SGGDIQMTQ SPSSLSASVG DRVITITCSAS QDISNYLNWY
321 QOKPGKAPKV LIYFTSSLHS GVPSRFSCSG SGTDFLTIS SLOPEDFATY YCQOYSTVPW TFGQGTKEI KRTVAAPSVF
401 IFPPSDEQLK SGTASVVCLL NNFYPREKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLISKADY EKHKVYACEV
481 THQGLSSPVT KSFNRGEC (SEQ. ID No.: 3)

FIG. 15A

ANTI-VEGF-A FAB:

1 EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNVVRQA PGKGLEWVGW INTYTGEPTY AAEFKRRFTF SLDTSKSTAY
81 LQMNSLRAED TAVYCAKYP HYYGSSHWF DVWGQGLVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV
161 TVSWNSGALT SGVHTFPAYL QSSGLYSLSS VVTPSSSLG TQTYICNVNH KPSNTKVDKK VEPKSCDKTH T (SEQ. ID No.: 21)

FIG. 15B

PDGFR-GS10-ANTI-VEGF-A FAB:

1 LVVTPPGPEL VLVNSSTFVL TCSGSAFVW ERMSQEPPOE MAKAQDGTFS SVLTLTNLTG LDTGEYFCTH NDSRGLETDE
81 RKRLYIFVPD PTVGFLPND A EELFIFLFEI TEITIPCRVT DPQLVVTLHE KKGDVALPVP YDHQGFESGI FEDRSYICKT
161 TIGDREVDSD AYYVYRLQVS SINVSNAVQ TVVRQGENIT LMCIVIGNEV VNFENTYPRK ESGRLVEPVT DFLLDMPYHI
241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITWVE SGGGSGSGG GSEVOLVESG GGLVQPGGSL RLSCAASGYT
321 FTNYGMNVR QAPCKGLENV GWINTYTGEPTAADEKRRF TFSLDTSKST AYLQMNLSRA EDTAVYYCAK YPHYGSSH
401 YFDVWGQGL VTVSSASTKG PSVFFLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
481 SSVVTVPPSS LGTQTYICNV NHKPSNTKVD KKVEPKCDK THT (SEQ. ID No.: 22)

FIG. 16A

ANTI-VEGF LIGHT CHAIN:

1 DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQK GKAPKVLIIY TSSLHSGVPS RFSGSGSGTD FTLTISSLOP
81 EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY PREAKVQWKV DNALQSGNSQ
161 ESVTEQDSKD STYSLSSLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGE C (SEQ. ID No.: 5)

FIG. 16B

PDGFR-GG-ANTI-VEGF-A FAB:

1 LVTTPGPEL VLVNSTFVL TCSGAPVW ERMSQEPPOE MAKAOQGTFE SVLTLTNLTG LDTGEYFCTH NDSRGLETDE
81 RKRLYIEVPD PTVGFLPND A BELFIFLTEL TEITIPCRVT DPQLVVTLHE KKGDAVALPV YDHQGFSGI FEDRSYICKT
161 TIGDREVDSD AYYVYRLQVS SINVSNAVQ TVVRQGENIT LMCIVIGNEV VNFETYPK ESGRLVEPVT DFLDMPYHI
241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVE SGGELVQPGG SLRLSCAASG YFTTNYGMNW
321 VRQAPGKGLE WVGWINTYTG EPTYAADEKR RFTFSLDTSK STAYLQMNLS RAEDTAVYYC AKYPHYGSS HWYFDVWGOG
401 TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVPS
481 SSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKTHT (SEQ. ID No.: 23)

FIG. 17A

ANTI-VEGF LIGHT CHAIN:

1 DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQKP GKAPKVLIFY TSSLHSGVPS RFSGSGSGTD FTLTSSLP
81 EDFATYYCQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY PREAKVQWKV DNALQSGNSQ
161 ESVTEQDSKD STYSLSSLT LT LSKADYERHK VYACEVTHQG LSSPVTKSFN RGE C (SEQ. ID No.: 5)

FIG. 17B

ANTI-VEGF-A FAB-GS21-PDGFR-β TRAP:

1 EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKLEWVGW INTYTGEPTY AADFKRRFTF SLDTSKSTAY
81 LQMSLRAED TAVYYCAKYP HYGSSHWYF DWNGQGLVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV
161 TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSLG TQYIICNVNH KPSNTKVDKK VEPKSCDKTH TCGGGSGGG
241 GSGGGSGGG GSGLVVTTPG PELVLNVST FVLTCGSAP VVWERNSEEP PQEMAKAQDG TFSSVLTILTN LTGLDTGEYF
321 CTHNDSRGL E TDERKRLYIF VPDPTVGFLP NDAEELFIFL TEITEITIPC RVTDPQLVVT LHEKKGDVAL PVPYDHQGE
401 SGIFEDRSYI CKTTIGDREV DSDAYVYVRL QVSSINVSVN AVQTVVRQGE NITLMCIVIG NEVVNFEWTY PRKESGRIVE
481 PVTDFLLDMP YHIRSILHIP SAELEDSTGY TCNVTESVND HODEKAINIT VESG (SEQ. ID No.: 24)

FIG. 18A

ANTI-VEGF LIGHT CHAIN:

1 DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP GKAPKVLIIYF TSSLHSGVPS RFSGSGSGTD FTLTISSLOP
81 EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY PREAKVQWKV DNALQSGNSQ
161 ESVTEQDSKD STYSLSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEK (SEQ. ID No.: 5)

FIG. 18B

PDGFR-β-GS10-ANTI-VEGF-A FAB:

1 LVVTPGPPEL VLVSSTFVL TCSGAPVWV ERMQEPPOE MAKADGTFS SVLTILNLTG LDTGEVFTCH NDSRGLETDE
81 RKRLYIFVPD PTVGFLPND A BELFIFLTEI TEITIPCRVT DPQLVVTLHE KKGVALPVP YDHQRGFSCI FEDRSYICKT
161 TIGDREVDSD AYYVYRLQVS SINVSNAVQ TVVROGENIT LMCIVIGNEV VNFWTYPRK ESGRLVEPVT DFLLDMPYHI
241 RSILHIPSAE LEDSGTYTCN VTESVNDHQD EKAINITVVE SGGGSGGGG GSEVOLVESG GGLVQPGGSL RLSCAASGYD
321 FTHYGMNWR QAPKGLEWV GWINTYTGER TYAADFKRRF TFSLDTSKST AYLOMNSLRA EDTAVYYCAK YPYYVYGTSHW
401 YFDVNGQGT L VTVSSASTRG PSVFPLAPSS KSTSGGTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA VLQSSGLYSL
481 SSVVTVPPSS LGTQTYICNV NHKPSNTKVD KKVEPKSCDK THL (SEQ. ID No.: 25)

FIG. 19A

ANTI-VEGF LIGHT CHAIN:

1 DIQLTQSPSS LSASVGDRVT ITCASQDIS NYLNWYQQK GKAPKVL IYF TSSLHSGVPS RFSGSGSGTD FTLTISSLOP
81 EDFATYYCQQ YSTVPWTFQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY PREAKVQWKV DNALQSGNSQ
161 ESVTEQDSKD STYSLSSLT LSKADYEKHK VYACEVTHQ LSSPVTKSFN RGE C (SEQ. ID No.: 12)

FIG. 19B

PDGFR-β-ANTI-VEGF-A HEAVY CHAIN:

1 LVTTPGPE LVLNVSSFEV LTCGSA PVV WERNSQEP PQ EMAKAODGTF SSVLTLTNLT GLDTGEYFCT HND SRGLETD
80 ERKRLYIFVP DPTVGFPLND AEELFIFLTE ITEITPCRV TDPQLVVT LH EKKGDVALPV PYDHQGFSG IFEDRSYICK
160 TTIGDREVDS DAYVYRLQV SSINVSNAV QTVVROGENI TLMCIVIGNE VVNFEWTPR KESGRLVEPV TDFLLDMPYH
240 IRSILHIPSA ELED SGTYTC NVTESVNDHQ DEKAINITVV ESGEVOLVES GGGLVQPGGS LRLSCAASGY TFTNYGMNWV
320 ROAPGKGLEW VGHINTYTGE PTYAADFRRR FTFSLDTSKS TAYLQMNSLR AEDTAVYYCA KYPHYYGSSH WYFDVWGQGT
400 LVTVSSASTK GPSVFFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS
480 SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSHEDP
560 EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC KVS NKALPAP IEKTISKAKG QPREPQVYTL
640 PPSREEMTKN QVSLTCLVKG FYP SDI AVEW ESNQO PENNY KTT PVPVLDSD GSFFLYSKLT VDKSRWQQGN VFSCSVMEHA
720 LHNHYTQKSL SLSPGK (SEQ. ID No.: 26)

FIG. 20A

ANTI-VEGF-A LIGHT CHAIN

1 DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQKP GKAPKVL IYF TSSLHSGVPS RPSGSGSGTD FTLTISSLPQ
81 EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFI FPP SDEQLKSGTA SVVCLLNIFY PREAKVQWKV DNALQSGNSQ
161 ESYTEQDSKD STYSLSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN R GEC (SEQ. ID No.: 5)

FIG. 20B

PDGFR-β-ANTI-VEGF-A HEAVY CHAIN:

1 VGFLPNDAAE LFIFLITEITE ITIPCRVTDP QLVVTLHEKK GDVALPVYPD HORGFSGIPE DRSYICKTTI GDREVDSDAY
81 YVYRLQVSSI NVSVNAVQTV VRQGENITLM CIVIGNEVNV FENTYPRKES GRLEVPVDF LLDMPYHIRS ILHIPSAELE
161 DSGTYTCNVT ESVNDHQDEK AINITVVESE EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNHWROA PCKGLEHWGM
241 INTYTGEPTY AADFRRRFTF SLDTSKSTAY LQMNSLRAED TAVYYCAKYP HYYGSSHWF DVWGQGTLVV VSSASTKGPS
321 VFPLAPSSKS TSGGTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH
401 KPSNTKVDKK VEPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNNWYVDGVEV
481 HNAKTKPREE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS REENTKNQVS
561 LTCLVKGFYP SDIAVEWESN GPENNYKTT PPVLDSDGSF FLYSKLTVDK SRWQQGNVPS CSVMHEALHN HYTQKSLSL
641 PGK (SEQ. ID No.: 27)

FIG. 21A

ANTI-VEGF-A LIGHT CHAIN

1 DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQK GKAPKVLIF TSSLHSGVPS RFSGSGSGTD FTLTISSLQP
81 EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLINNFY PREAKVQWKV DNALQSGNSQ
161 ESVTEQDSKD STYSLSSITL LSKADYEKHK VIACEVTHQG LSSPVTKSFN RGEK (SEQ. ID No.: 5)

FIG. 21B

PDGFR-β-ANTI-VEGF-A FAB:

1 VGFLPNDAAE LFIFLTEITE ITIPCRVTD P QLVVTLHEKK GDVALPVPYD HQRGFGIFE DRSYICKTTI GDREVDSDAY
81 YVYRLQVSSI NVSVNAVQTV VRQGENITLM CIVIGNEVYN FEWTPRKES GRLEVPVTD F LLDMPYHIRS ILHIPSAELE
161 DSGTYTCNVT ESVNDHDEK AINITVVEG EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWRQA PCKGLEWVGW
241 INTYTGEPTY AADFKRRTF SLDTSKSTAY LQNSLRAED TAVYCAKYP HYGSSHWF DVWGQGLVT VSSASTKGPS
321 VFPLAPSSKS TSGGTAALGC LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTPSSSLG TQTYICNVNH
401 KPSNTKVDKK VEPKCDKTH T (SEQ. ID No.: 28)

FIG. 22A

ANTI-VEGF-A LIGHT CHAIN

1 DIQMTQSPSS LSASVGDRVT ITCSAQDIS NYLNWYQQK GKAPKVLIF TSSLHSGVPS RFSGSGGTD PTLTISSLQ P
81 EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNPFY PREAKVQWKV DNALQSGNSQ
161 ESVTEQDSKD STYSLSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEN (SEQ. ID No.: 5)

FIG. 22B

PDGFR- β -ANTI-VEGF-A FAB:
1 VGFLPNDAAE LFIFLTEITE ITIPCRVTD P QLVVTLHEKK GDVALPVYPD HORGFSGIFE DRSYICKTTI GDREVDSDAY
81 YVYRLQVSSI NVSVNAVQTV VROGENITLM CIVIGNEVVN FEWTPRKES GRIVEPVTDF LLDMPYHIRS ILHIPSAELE
161 DSGTYTCNVT ESVNDHODEK AINITVVEG GGGGGGGSG GGGGGGGSE VQLVESGGGL VQPGGSLRLS
241 CAASGYTFN YGMNWRQAP GKGLEWVGI NTYTGEPTYA ADEKRRFTFS LDTSKSTAYL QMNSLRAEDT AVYYCAKYPH
321 YYGSSHWYFD VNGQGLTVV SSASTKGPSV FPLAPSSKST SGGTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ
401 SSGLYSLSSV VTVPSSSLGT QTYICNVNHH PSNTKVDKKV EPKCDKTHT (SEQ. ID No.: 29)

FIG. 23A

ANTI-VEGF-A LIGHT CHAIN
1 DIQWTQSPSS LSASVGDRVT ITCASQDIS NYLNWYQKP GKAPKVLIFY TSSLHSGVPS RFGSGSGTD FTLTISSLQP
81 EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY PREAKVQMKV DNALQSGNSQ
161 ESVTEQDSKD STYSLSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEN (SEQ. ID No.: 5)

FIG. 23B

PDGFR- β -6XGS-ANTI-VEGF-A FAB:

1 VGFLPNDAAE LFIFLTEITE ITIPCRVTD P QLVVTLHEKK GDVALPVPYD HQRGSGIFE DRSYICKTTI GDREVDSDAY
81 YVYRLQVSSI NVSVNAVQTV VROGENITIM CIVIGNEVVN FEWTPRKES GRIVEPTDF LLDMPYHIRS ILHIPSAELE
161 DSGTYTCNVT ESVDHQDEK AINITVVEG GCGSGGGSG GCGSGGGSE VQLVESGGGL VQPGGSLRLS
241 CAASGYTFN YGMNWRQAP GKGLEWVGI NITYTGEPTYA ADFKRRFTFS LDTSKSTAYL QMNSLRAEDT AVYYCAKYPH
321 YGSSHWYFD VWGQGLTVV SSASTKCPV FFLAPSSKST SGGTAALGCL VKDYFFPEPT VSWNSGALTS GVHTFPAVLQ
401 SSGLYSLSSV VTPSSSLGT QTYICNVNHK PSNTKVDKKV EPKSCDKTHT (SEQ. ID No.: 29)

FIG. 24A

ANTI-VEGF-A LIGHT CHAIN

1 DIQMTQSPSS LSASVGRVT ITCSASQDIS NYLNWYQQK GKAPKVLIF TSSLHSGVPS RFSGSGGTD FTLTISSLQ P
81 EDFATYICQQ YSTVPWTFQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY PREAKVQWKV DNALQSGNSQ
161 ESVTEQDSKD STYLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGE C (SEQ. ID No.: 5)

FIG. 24B

ANTI-VEGF-A FAB-6XGS-PDGFR-β

1 EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW INTYTGEPTY AADFRRRTF SLDTSKSTAY
81 LQMNSLRAED TAVYYCAKYP HYYGSSHWF DVWGQGTILVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV
161 TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTFPSSLG TQTYICNVNH KPSNTKVDKK VEPKSCDKTH TGGSGGGGS
241 GGGSGGGGS GGGSGGGGS VGFLPNDAAE LFIFLTEITE ITIPCRVTDQ QLIVTLHEKK GDVALPVPYD HQGFSGIFE
321 DRSYICKTTI GDREVDSDAY YVYRLQVSSI NVSVNAVQTV VRQENITLM CIVIGNEVNV FENTYPRKES GRLVEPVTDF
401 LLDMPYHIRS ILHIPSAELE DSGTYTCNVT ESNVDHQDEK AINITVVESSG (SEQ. ID No.: 30)

FIG. 25A

ANTI-VEGF-A LIGHT CHAIN

1 DIQMTQSPSS LSASVGDRVT ITCASQDIS NYLNWYQQK GKAPKVLIF TSSLHSGVPS RFSGSGSGTD FTLTISSLP
81 EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY PREAKVQWKV DNALQSGNSQ
161 ESVTEQDSKD STYSLSSLT LSKADYEKKH VYACEVTHQG LSSPVTKSFN RGEK (SEQ. ID No.: 5)

FIG. 25B

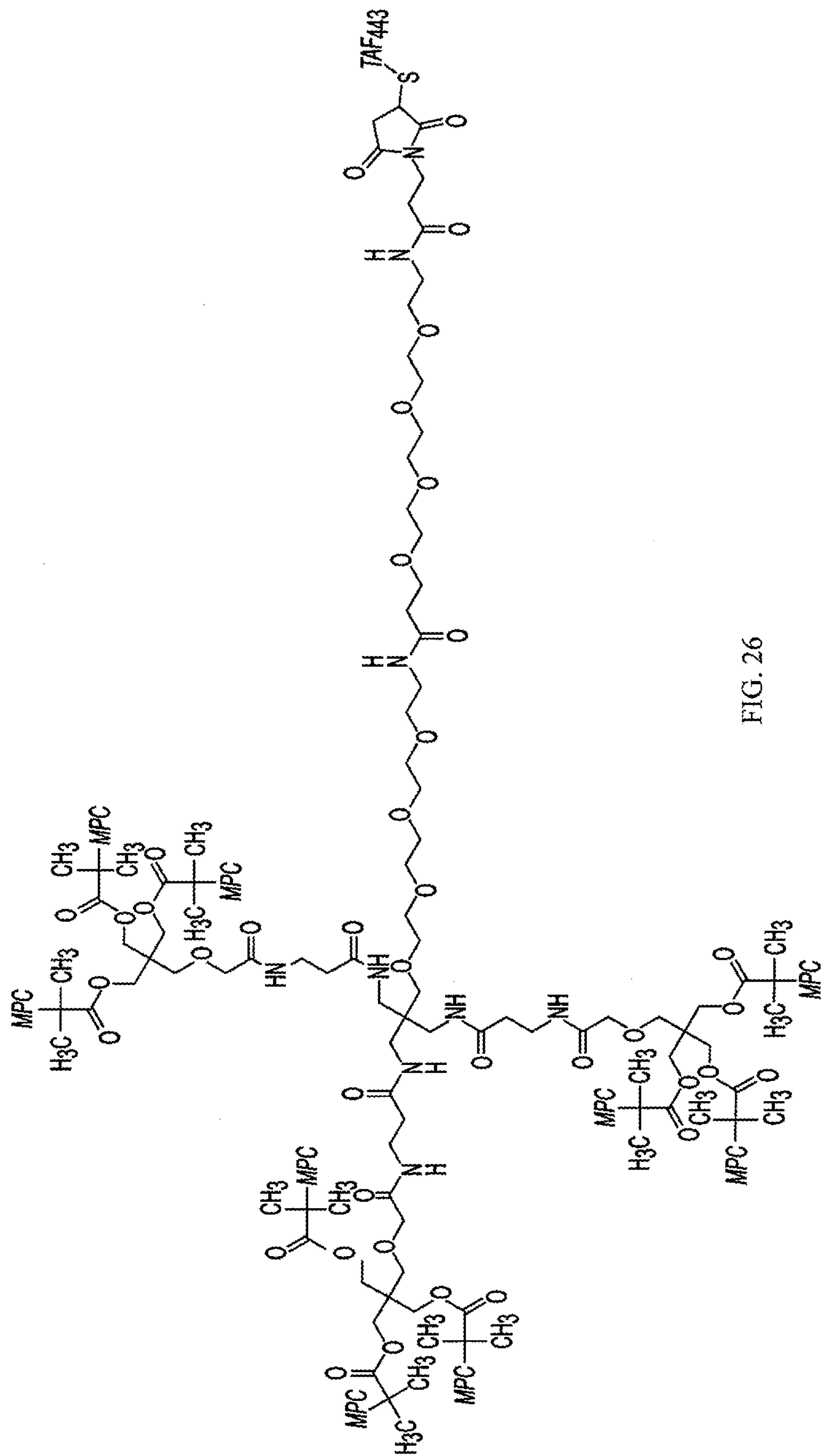
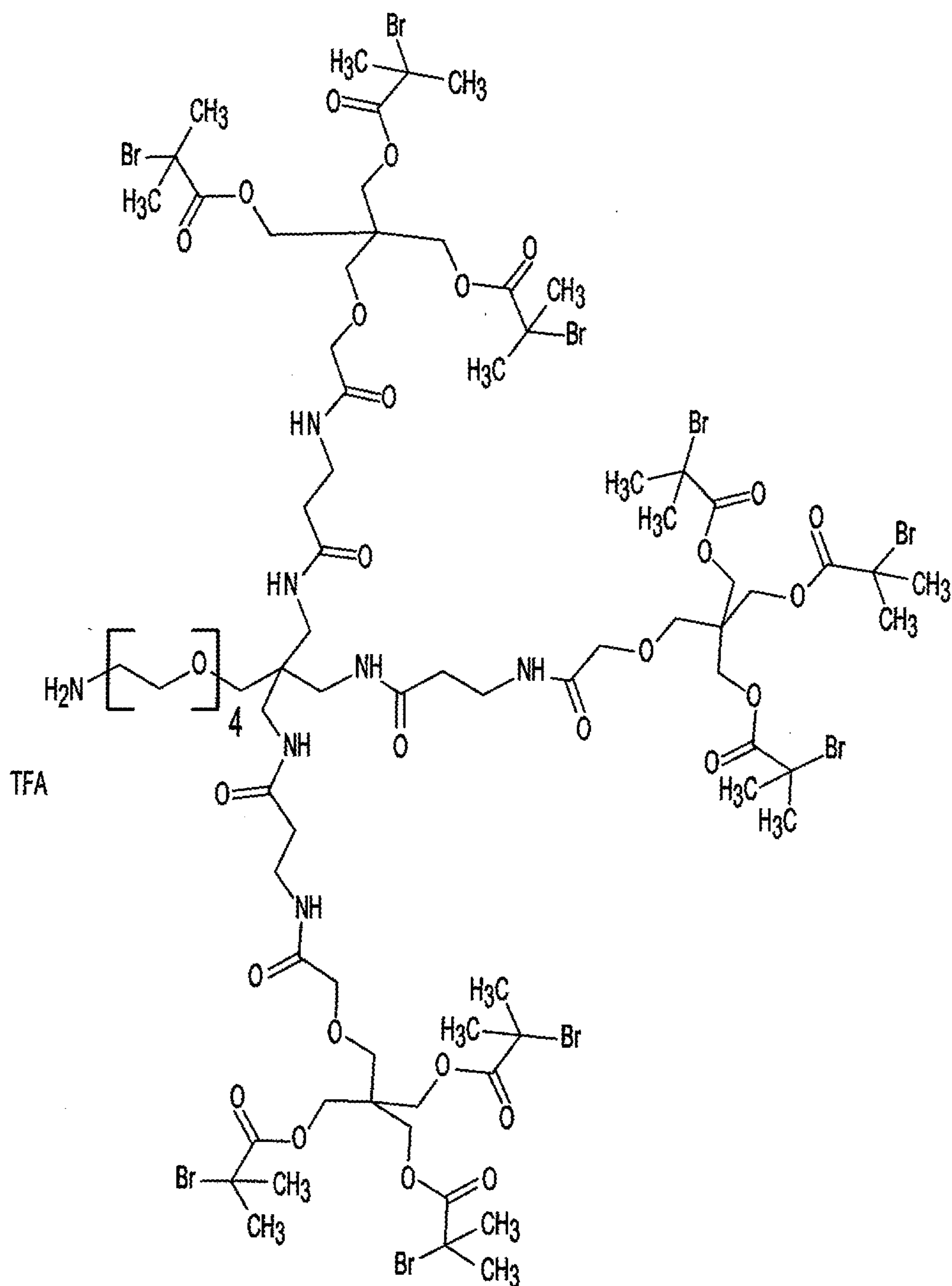
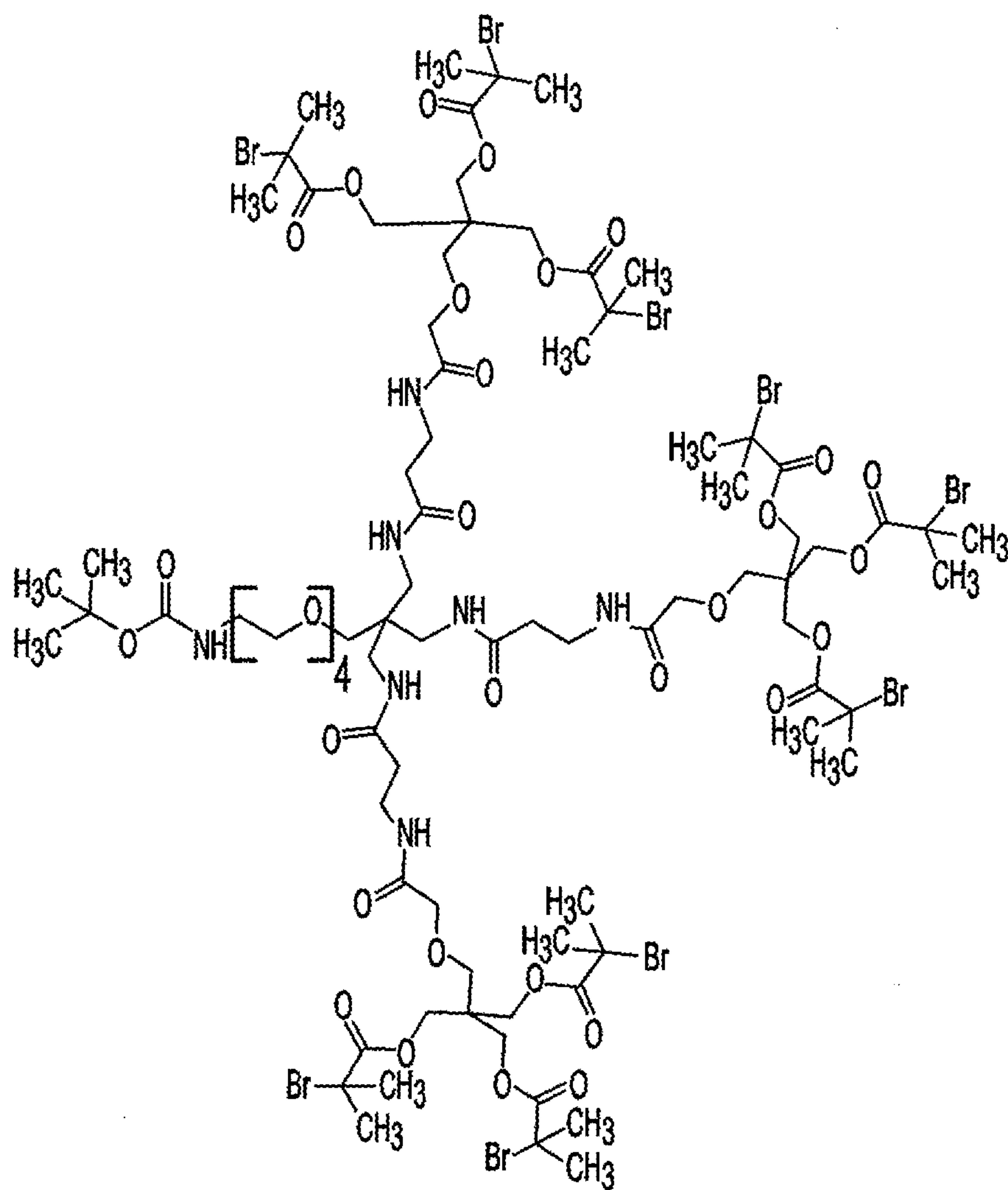


FIG. 26



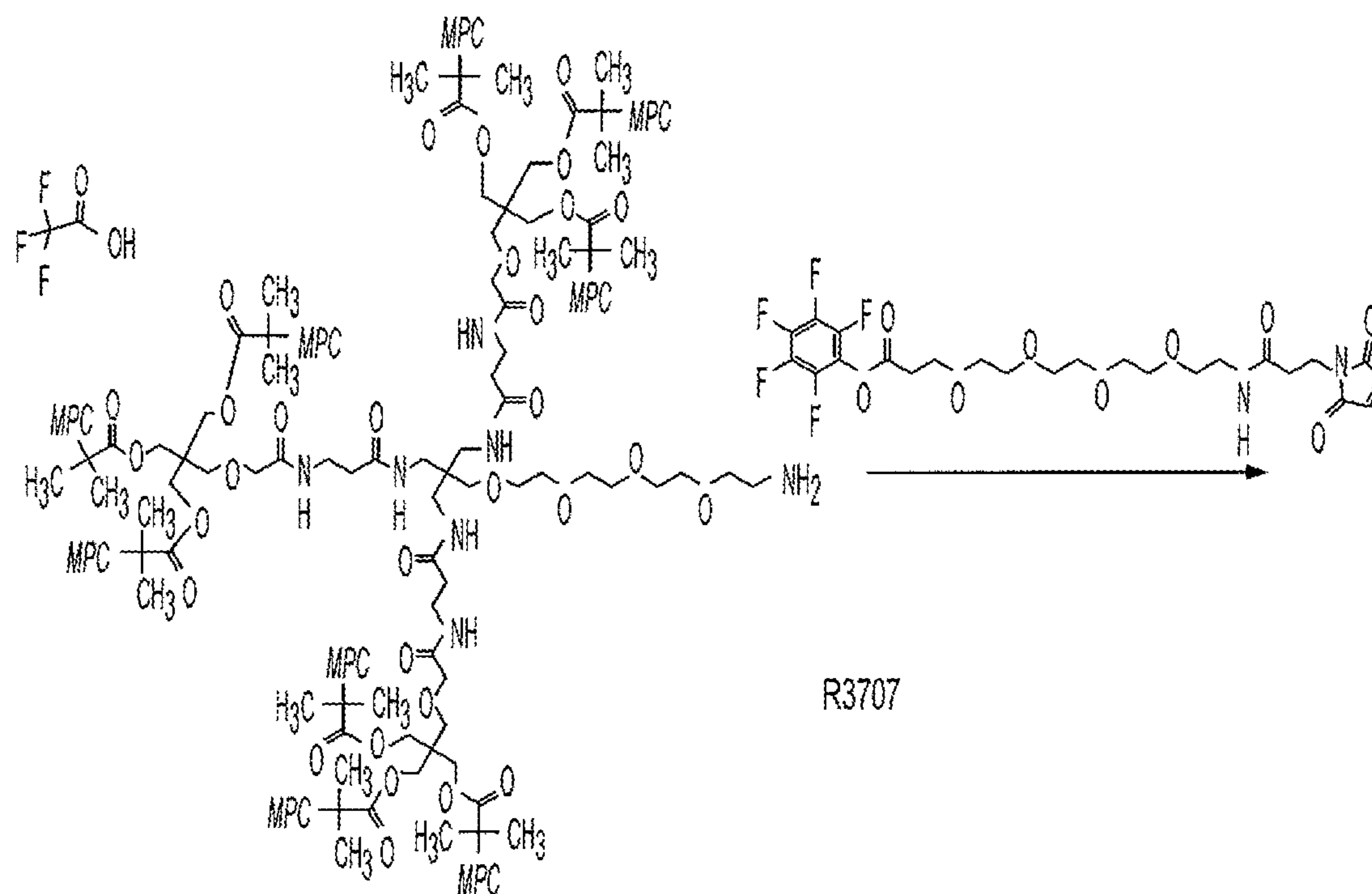
COMPOUND L

FIG. 27

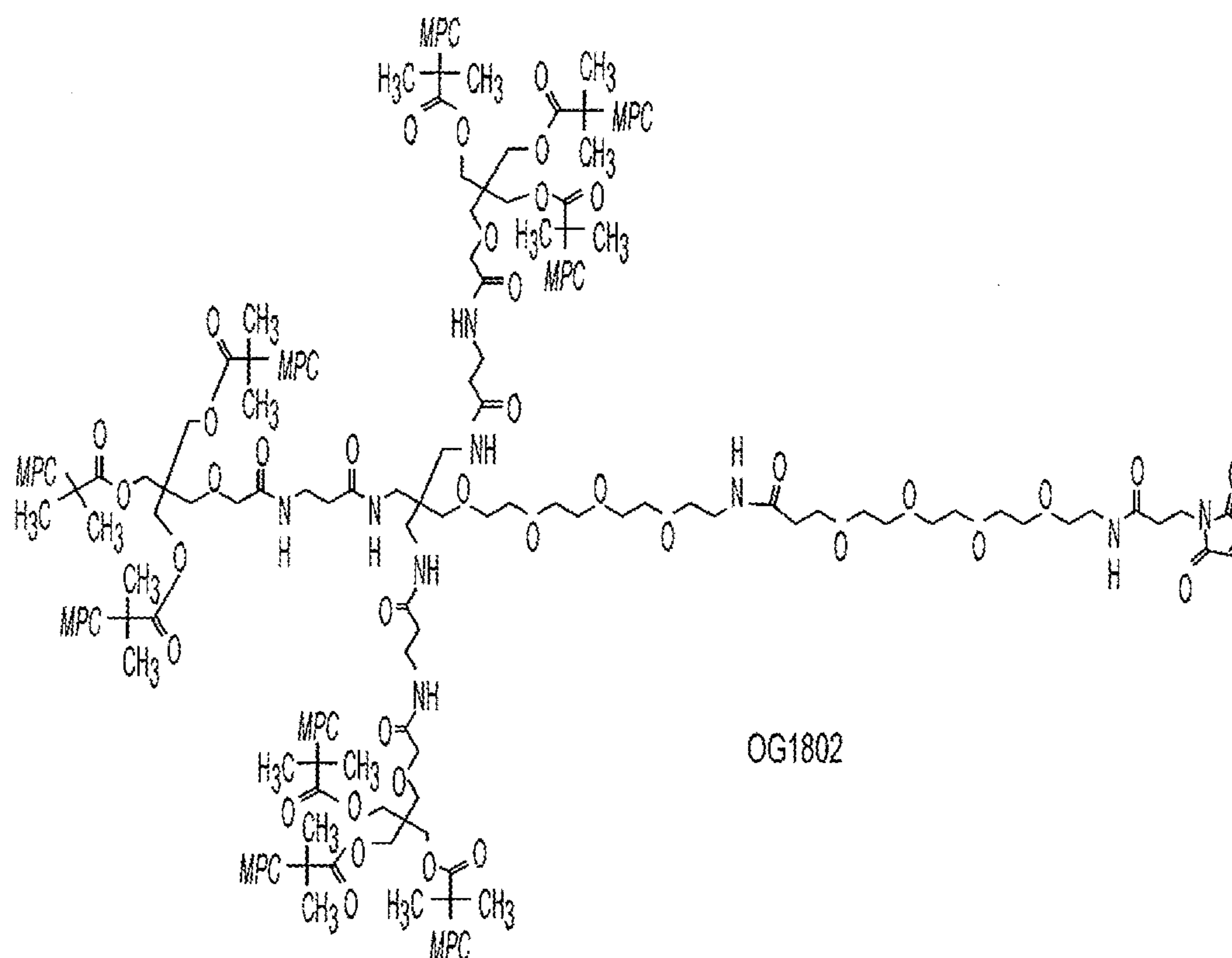


COMPOUND K

FIG. 28

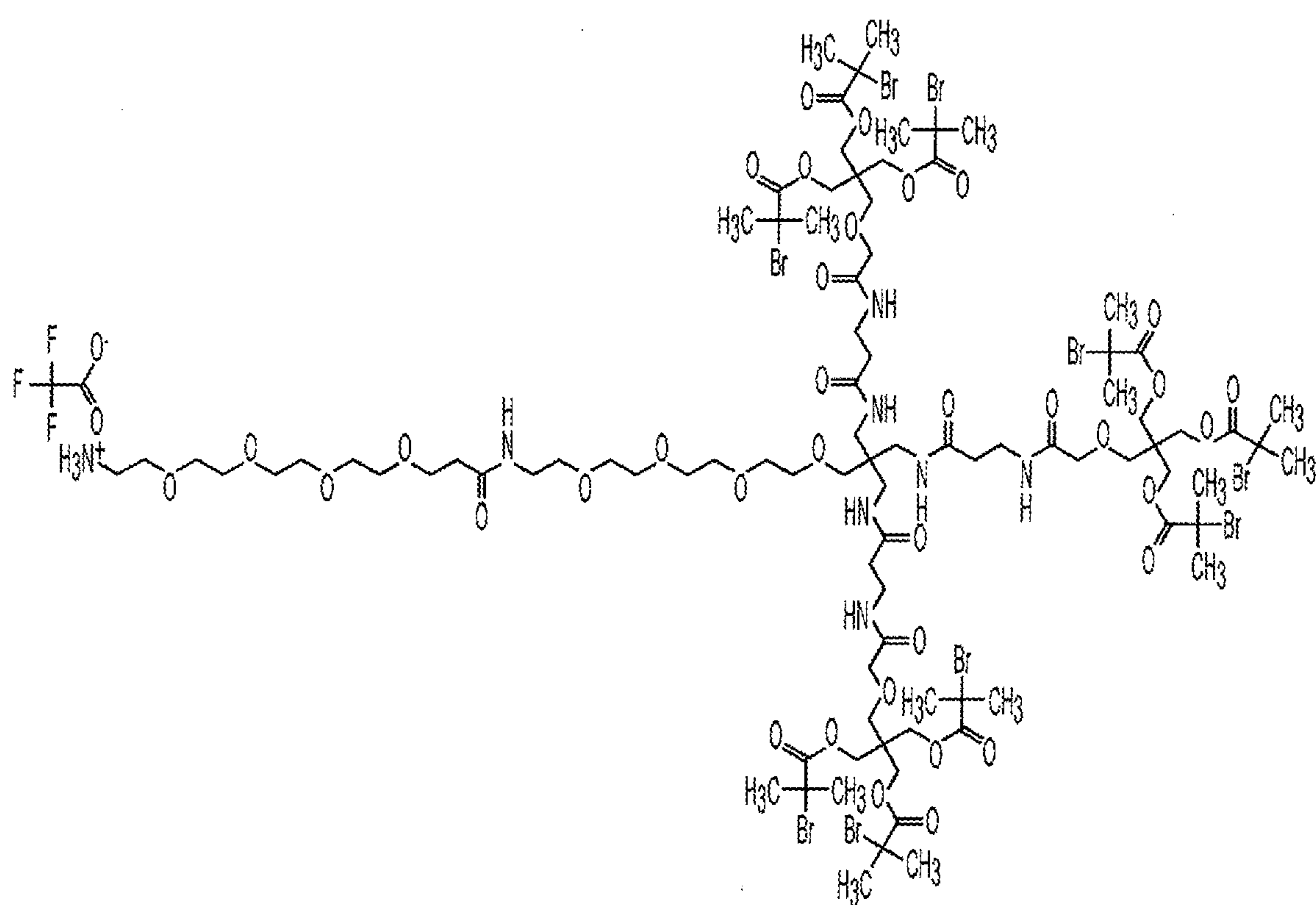


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FIG. 29



OG1786

FIG. 30

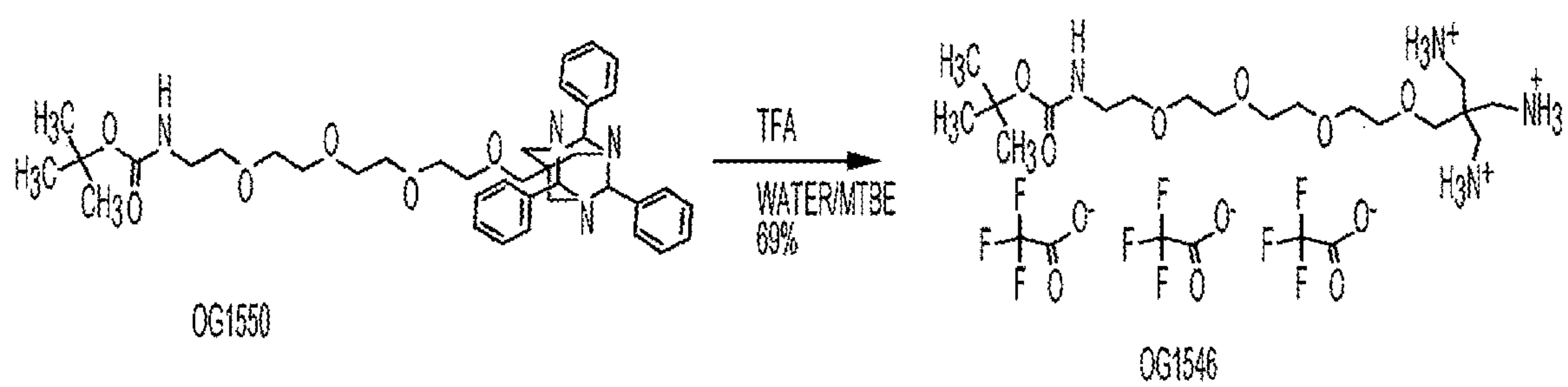


FIG. 31

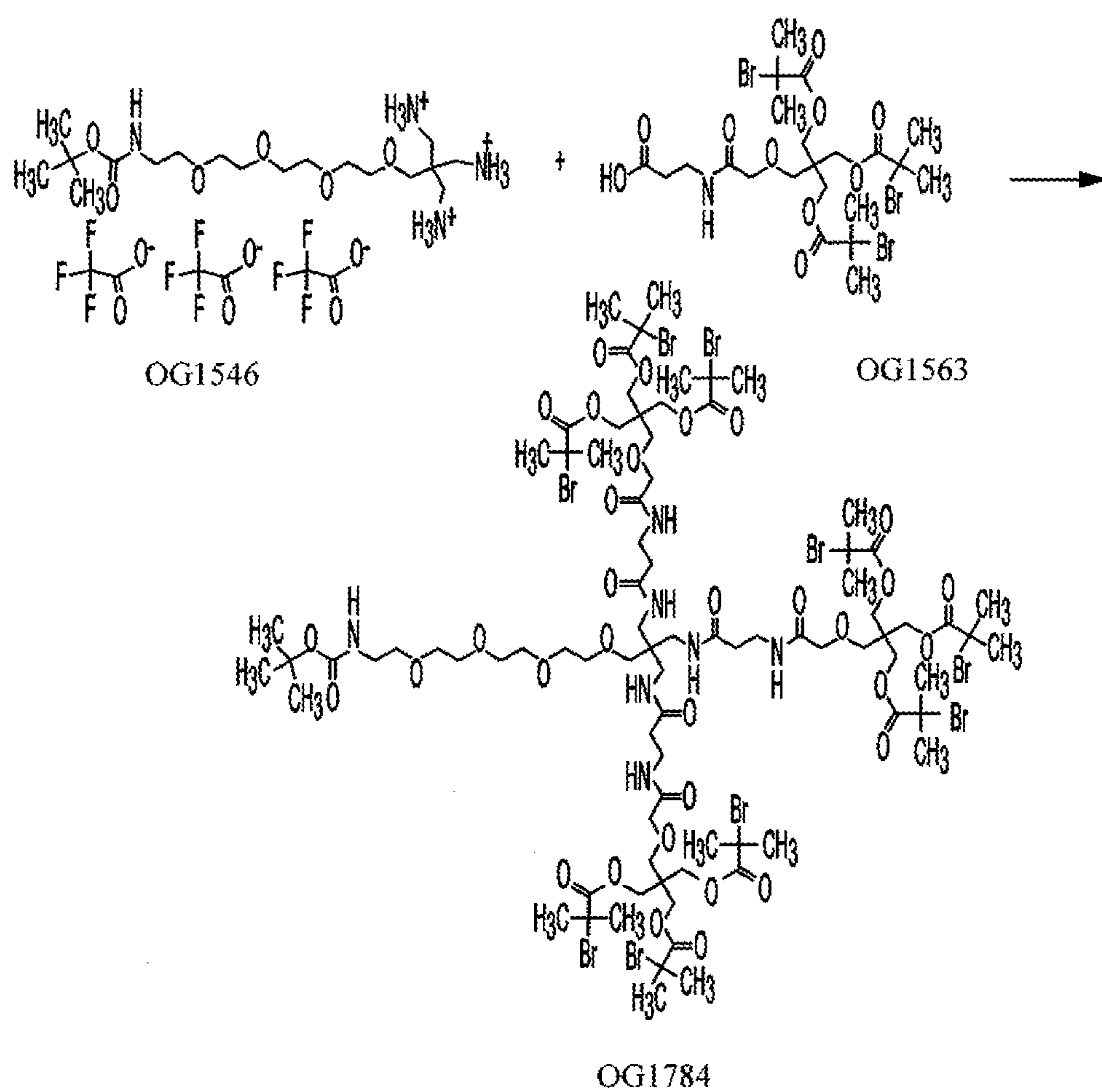


FIG. 32

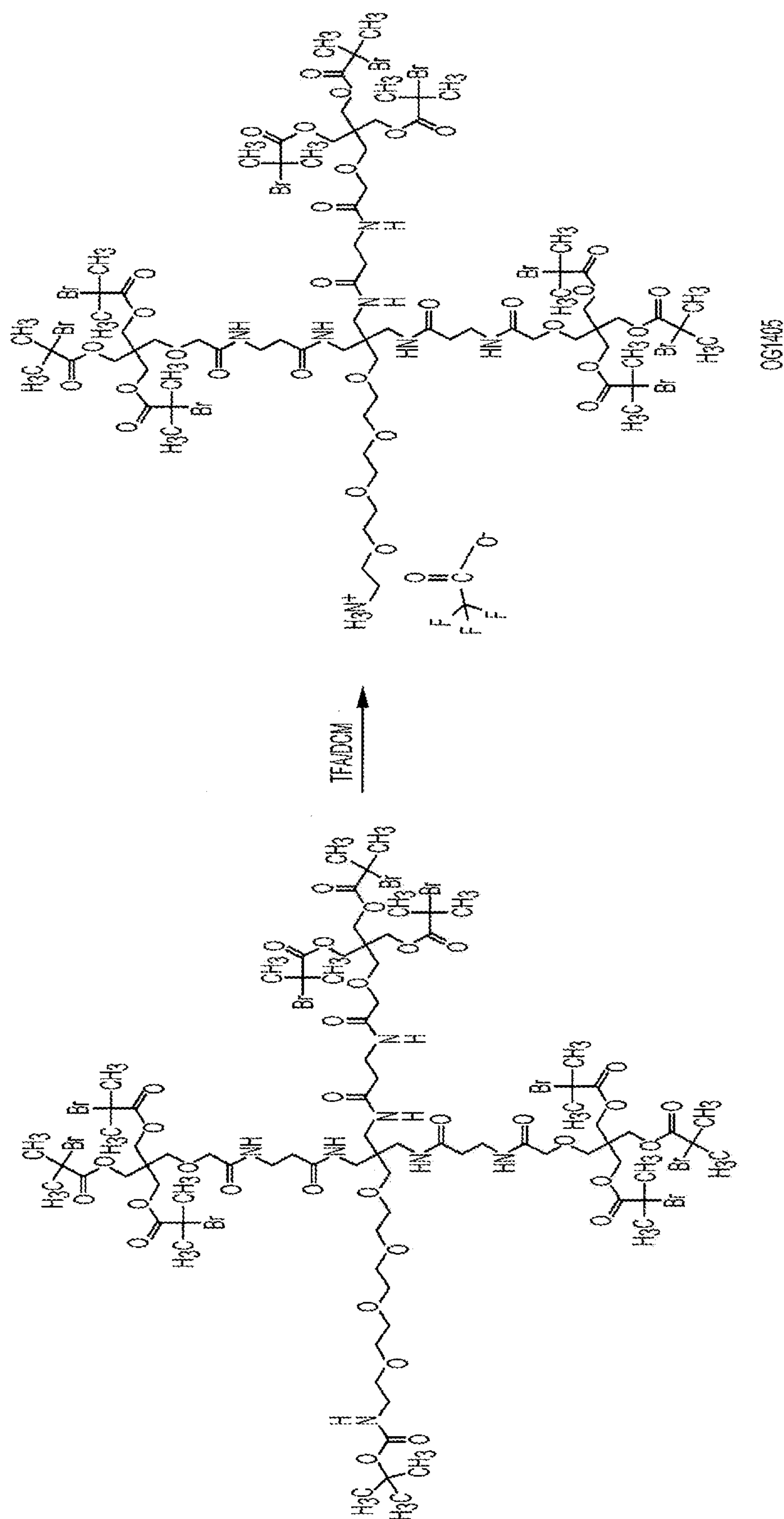


FIG. 33

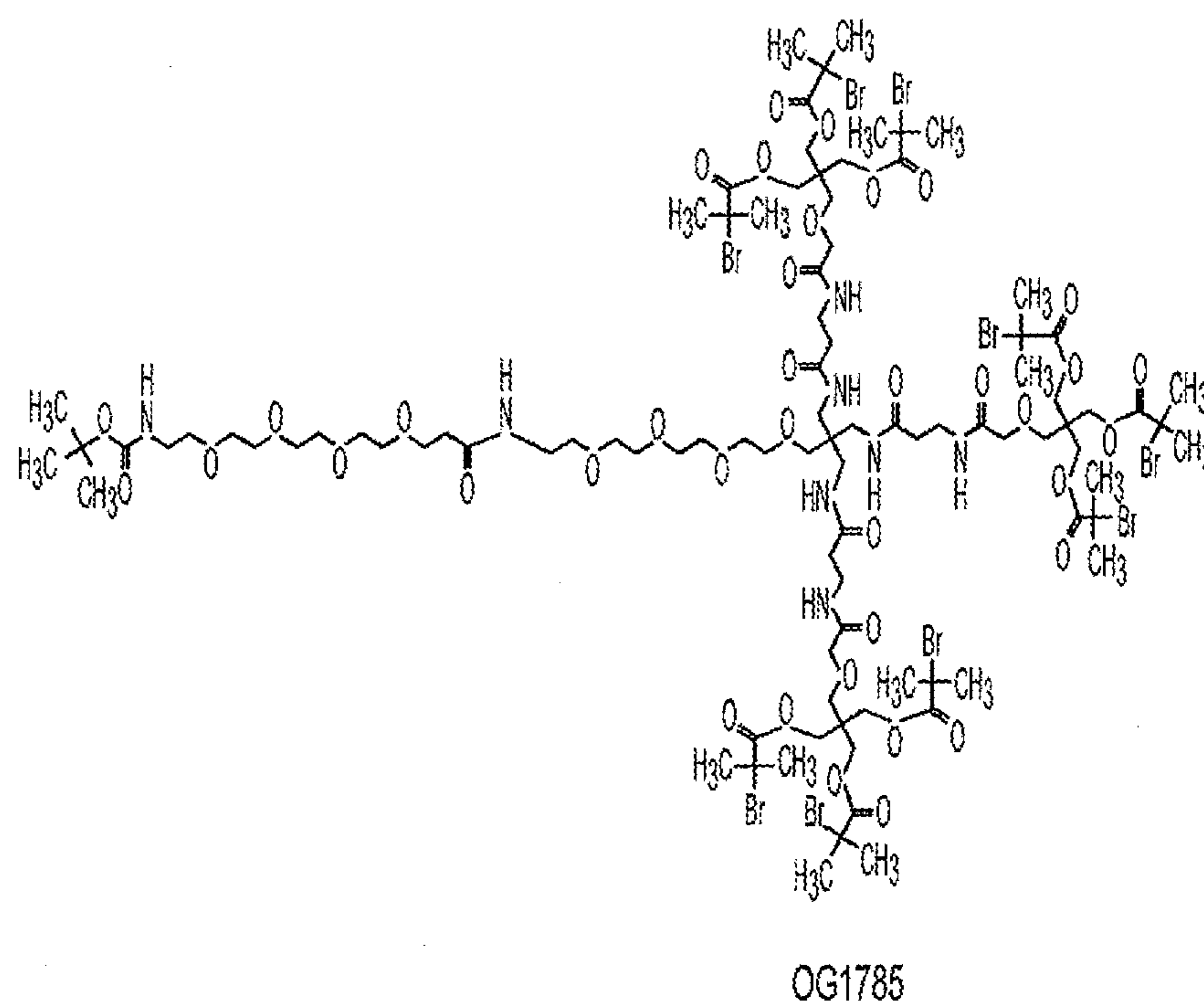
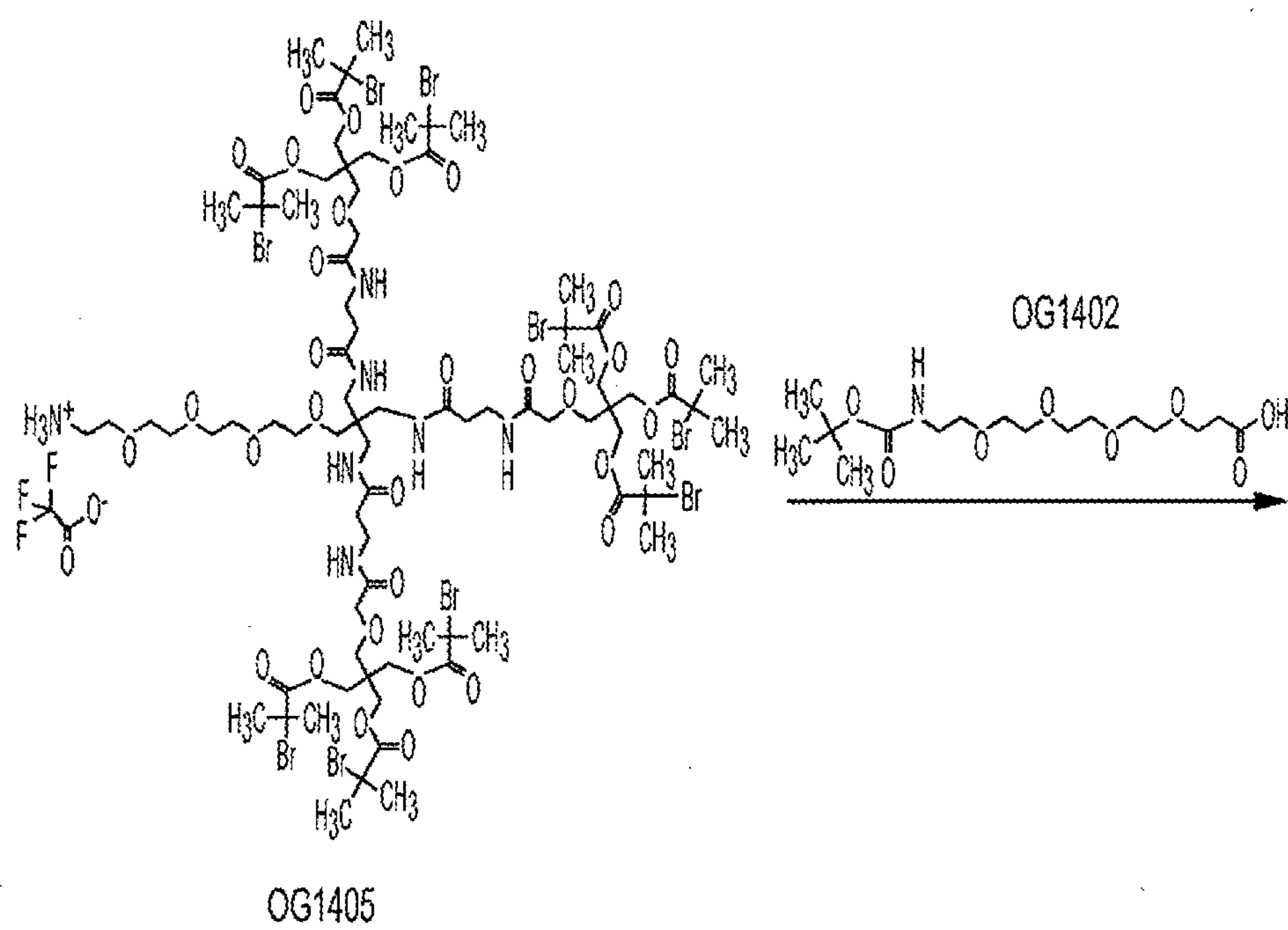


FIG. 34

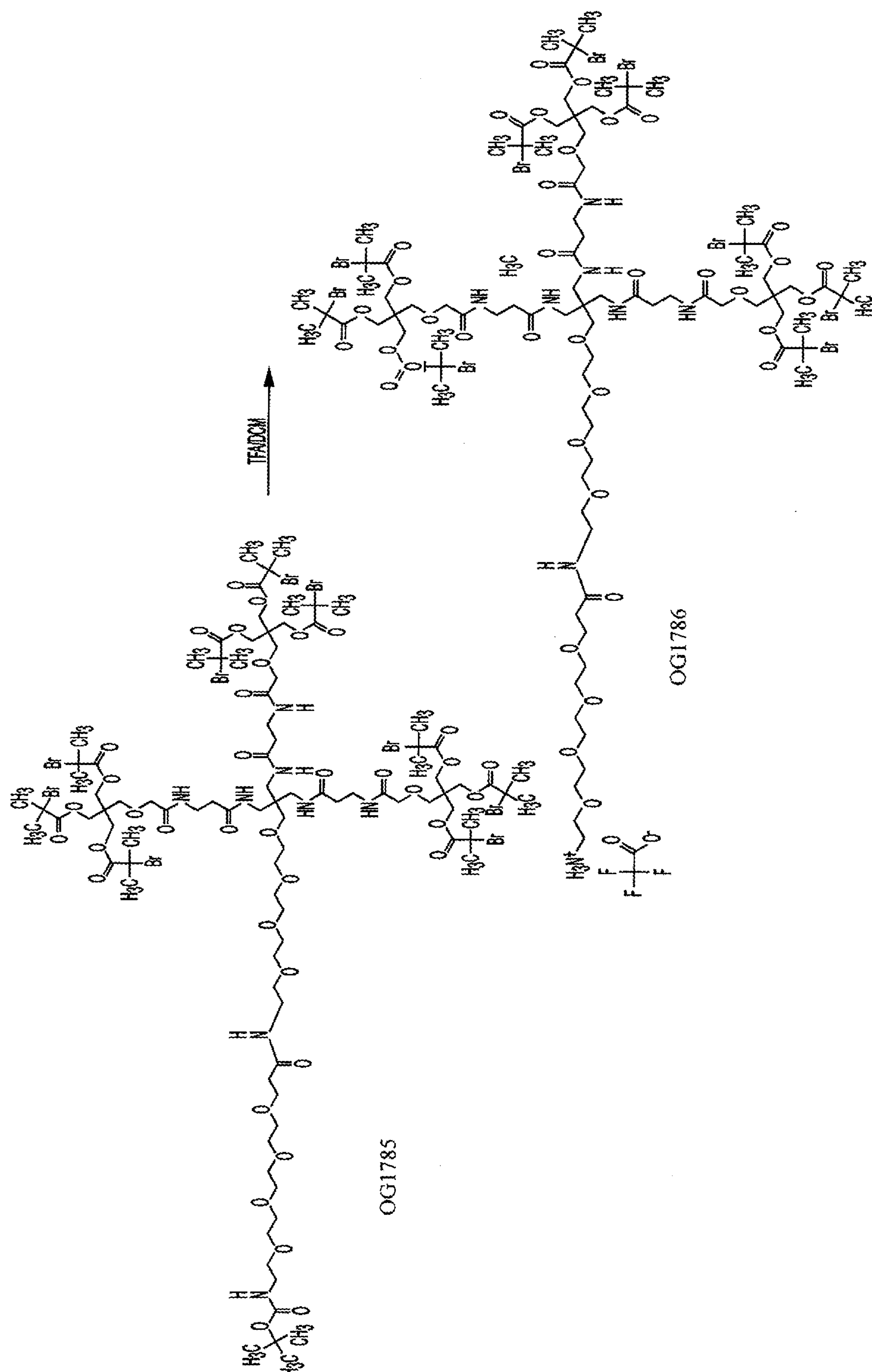


FIG. 35

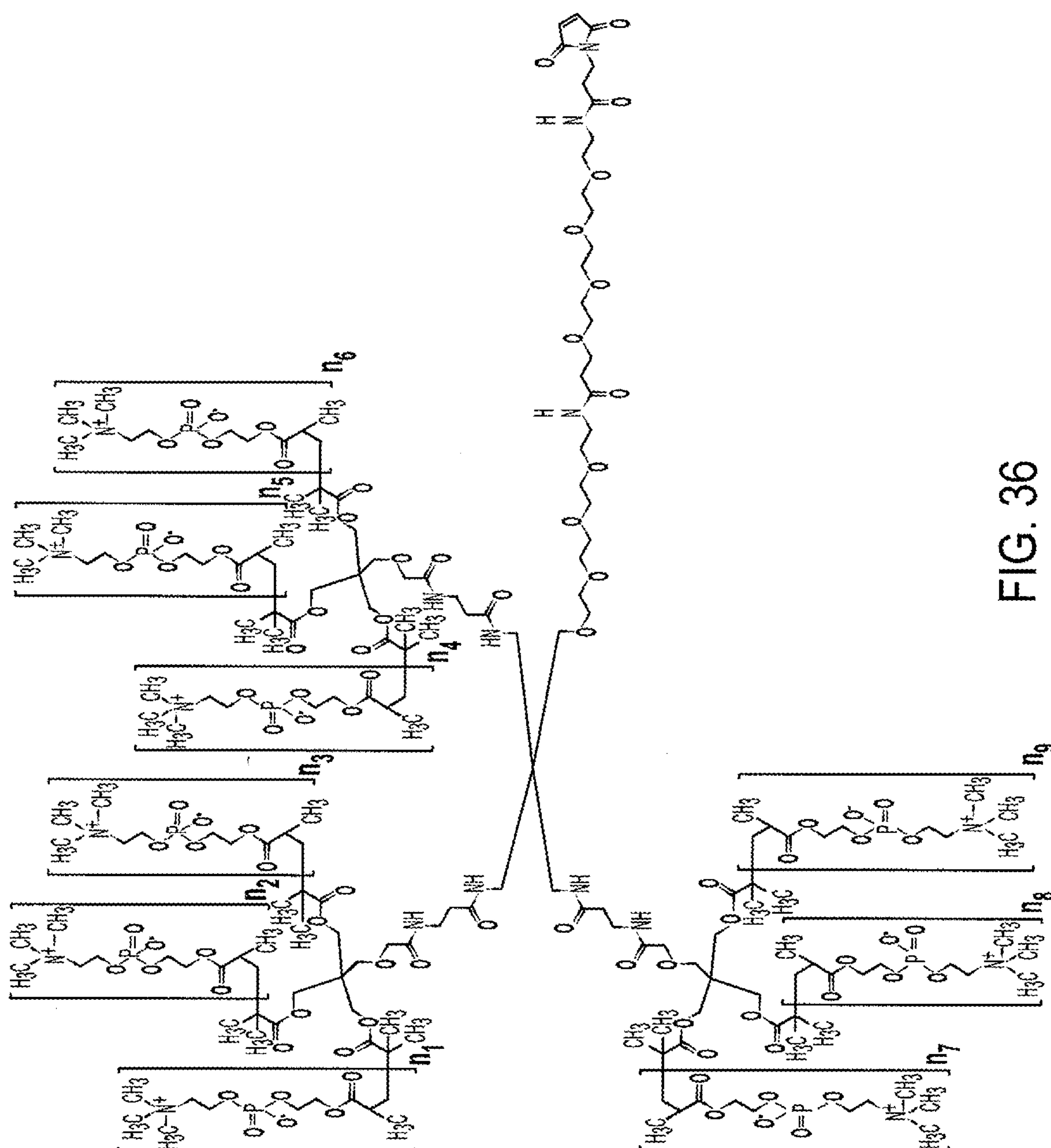


FIG. 36

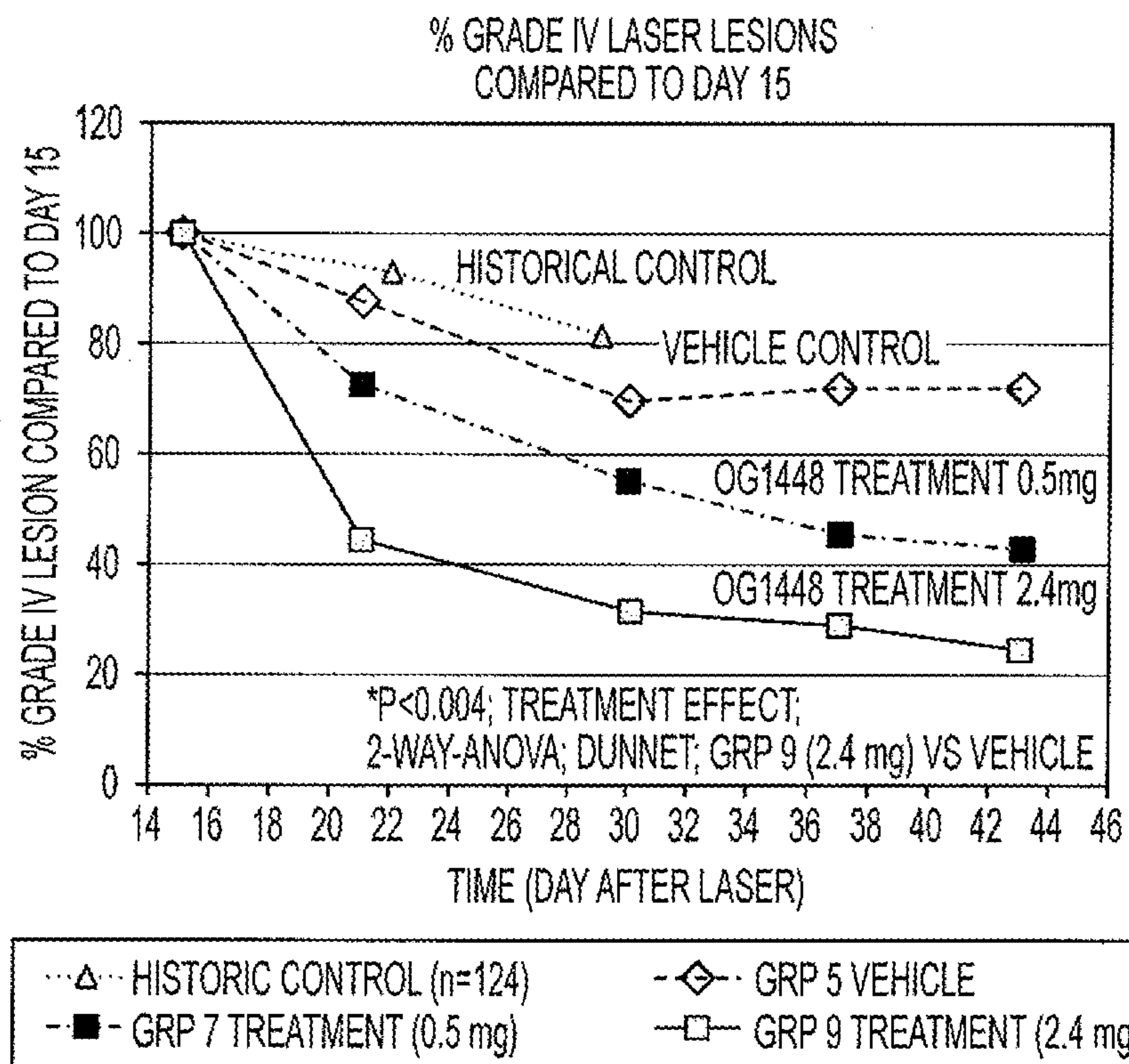


FIG. 37

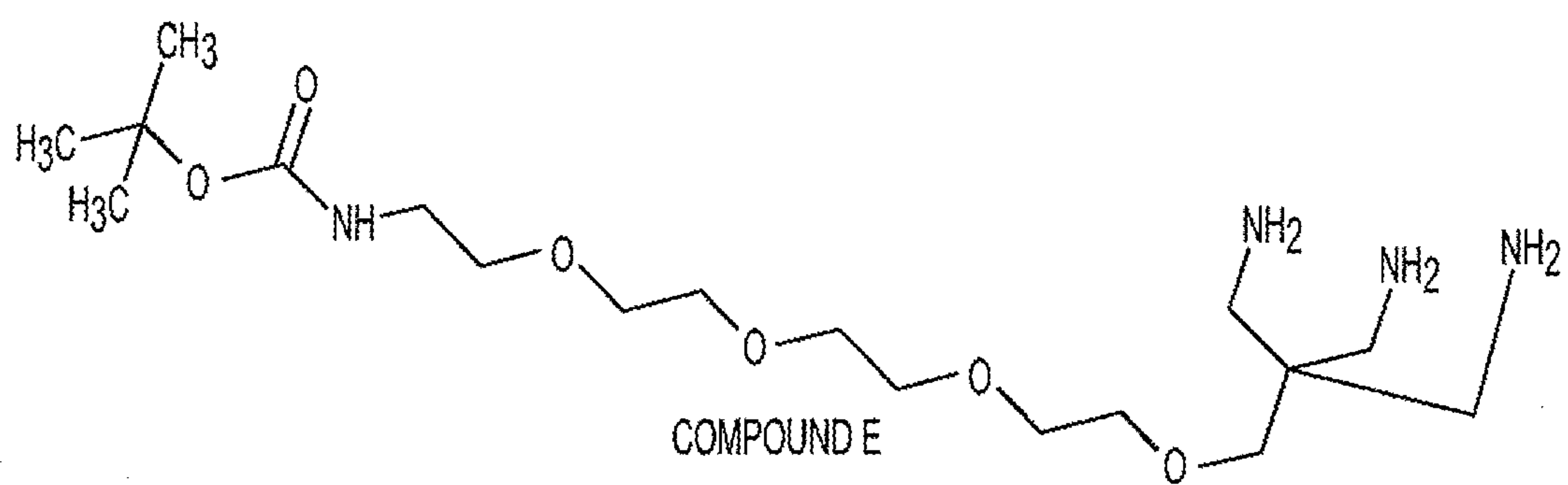


FIG. 38

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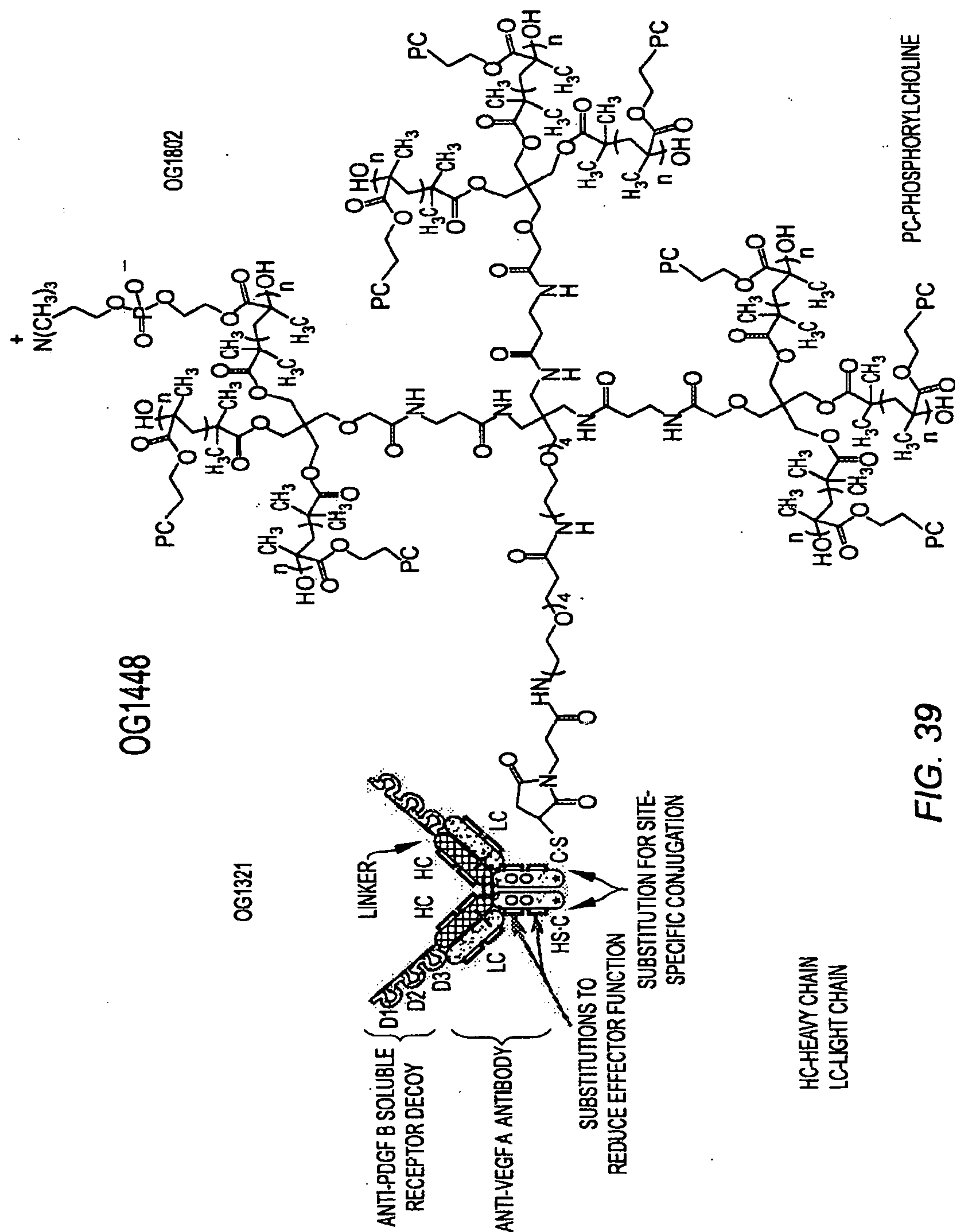


FIG. 39

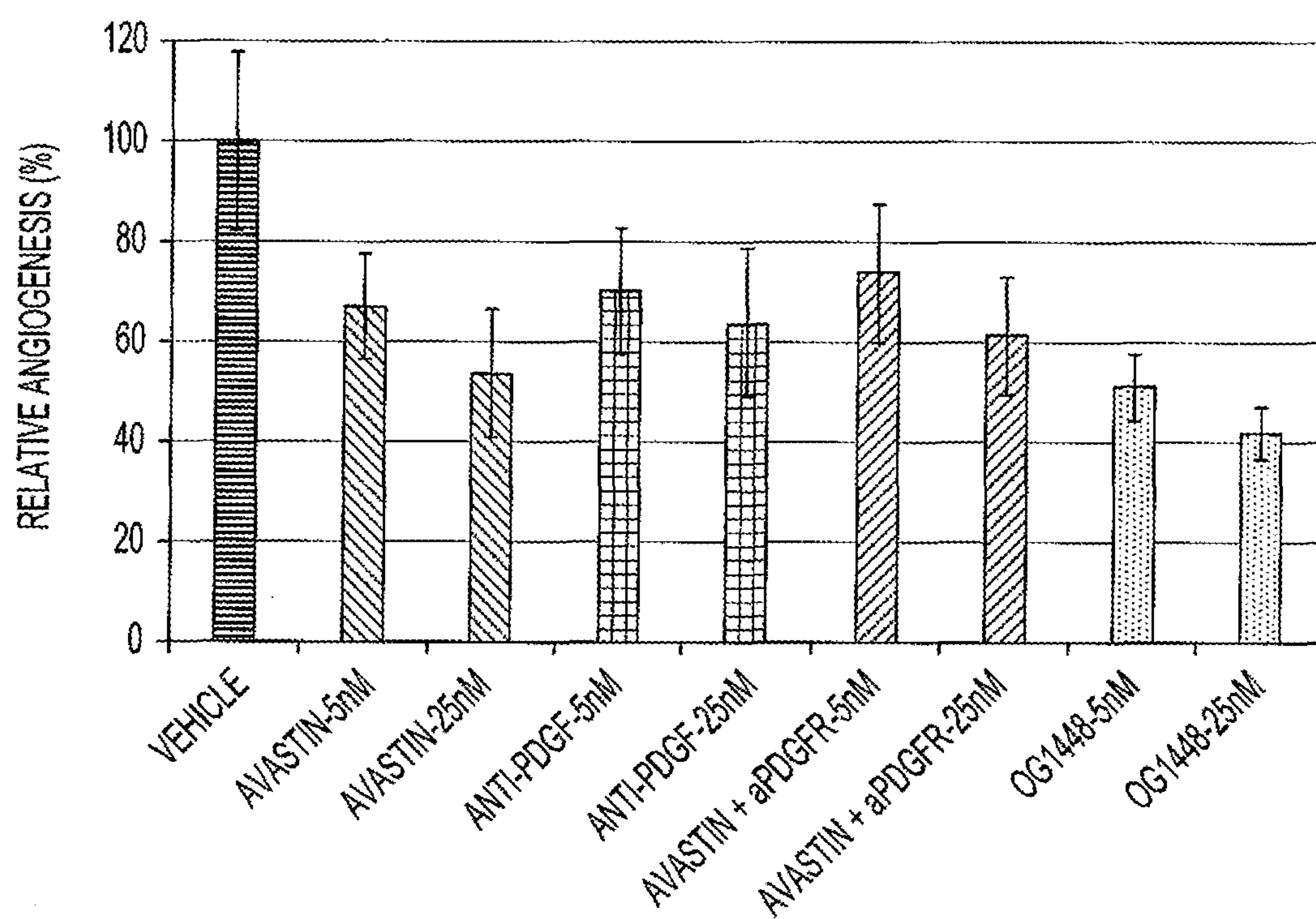


FIG. 40

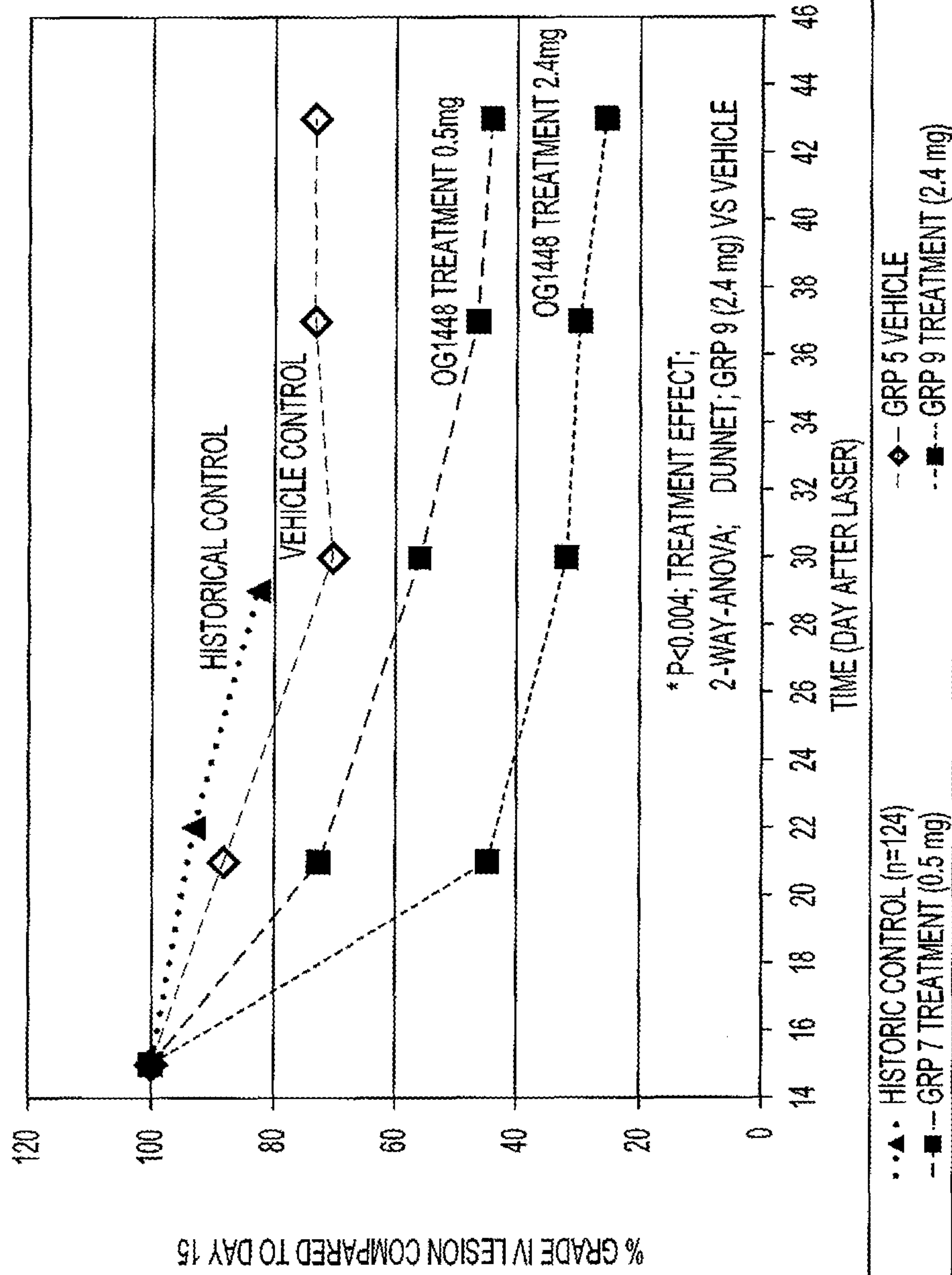


FIG. 41

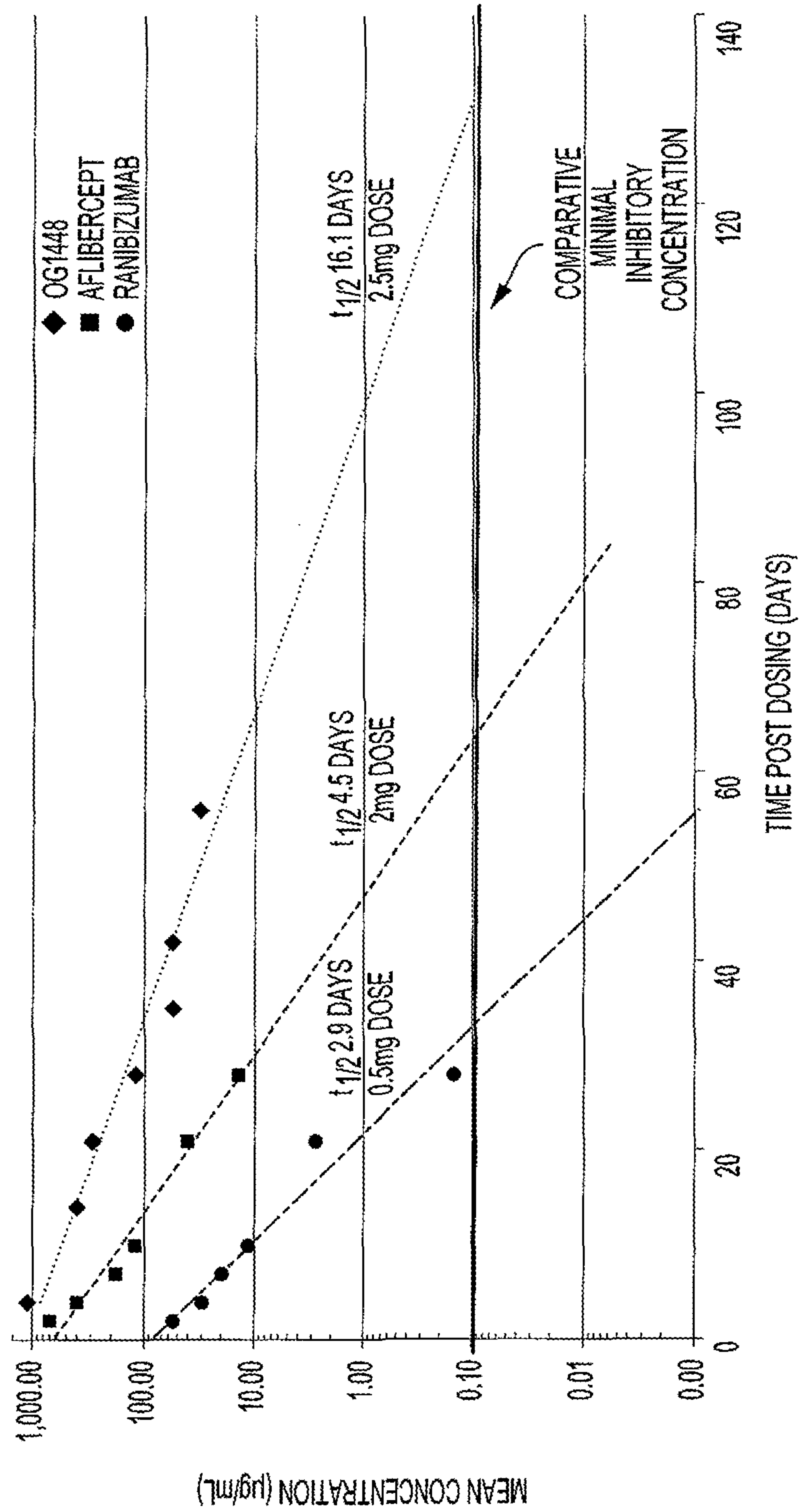


FIG. 42

FIG. 39

OG1802

ANTI-VEGFA ANTIBODY

SUBSTITUTIONS TO UCE EFFECTOR FUNCTION

SUBSTITUTION FOR SITE-SPECIFIC CONJUGATION

HC-HEAVY CHAIN
LC-LIGHT CHAIN

PC-PHOSPHORYLCHOLINE

